1720–1726 *Nucleic Acids Research, 1997, Vol. 25, No. 9*

Leader peptides of inducible chloramphenicol resistance genes from Gram-positive and Gram-negative bacteria bind to yeast and Archaea large subunit rRNA

Robert Harrod and Paul S. Lovett*

Department of Biological Sciences, University of Maryland–Baltimore County, Baltimore, MD 21250, USA

Received January 30, 1997; Revised and Accepted March 5, 1997

ABSTRACT

catA86 **is the second gene in a constitutively transcribed, two-gene operon cloned from** ***Bacillus pumilus*****. The region that intervenes between the upstream gene, termed the leader, and the** ***catA86*** **coding sequence contains a pair of inverted repeat sequences which cause sequestration of the** ***catA86*** **ribosome binding site in mRNA secondary structure. As a consequence, the** ***catA86*** **coding sequence is untranslatable in the absence of inducer. Translation of the** ***catA86*** **coding sequence is induced by chloramphenicol in Gram-positives and induction requires a function of the leader coding sequence. The leader-encoded peptide has been proposed to instruct its translating ribosome to pause at leader codon 6, enabling chloramphenicol to stall the ribosome at that site. Ribosome stalling causes destabilization of the RNA secondary structure, exposing the** ***catA86*** **ribosome binding site, allowing activation of its translation. A comparable mechanism of induction by chloramphenicol has been proposed for the regulated** ***cmlA*** **gene from Gram-negative bacteria. The** ***catA86*** **and** ***cmlA*** **leader-encoded peptides are** ***in vitro*** **inhibitors of peptidyl transferase, which is thought to be the basis for selection of the site of ribosome stalling. Both leader-encoded peptides have been shown to alter the secondary structure of** ***Escherichia coli*** **23S rRNA** ***in vitro*****. All peptide-induced changes in rRNA conformation are within domains IV and V, which contains the peptidyl transferase center. Here we demonstrate that the leader peptides alter the conformation of domains IV and V of large subunit rRNA from yeast and a representative of the Archaea. The rRNA target for binding the leader peptides is therefore conserved across kingdoms.**

INTRODUCTION

The basis for the modulated expression of many bacterial and eukaryotic genes involves the control of mRNA translation, frequently as a complement to transcriptional control. A novel *cis*-acting form of translational control has recently been suggested as the basis for the regulation of several bacterial and eukaryotic genes, which relies on principles unlike those seen in other examples of translational regulation (1–4). Genes such as *cat* and *cmlA*, which specify inducible chloramphenicol resistance in *Bacillus subtilis* and *Escherichia coli* respectively, and eukaryotic genes, which include the *arg-2* gene of *Neurospora crassa*, the CPA1 gene of *Saccharomyces cerevisiae*, gp48 of cytomegalovirus, the human β_2-adrenergic receptor gene and several other examples (1), appear to be controlled by a fundamentally similar mechanism. In each, the regulated gene is the downstream gene in a two-gene operon and it is the act of translation of the upstream gene that can activate or depress translation of the downstream gene. The role of the upstream gene appears to reside in its peptide product. Current evidence indicates that the amino acid sequence of the peptide product of the upstream gene enables it to interact *in cis* with its translating ribosome (1–5). This interaction, either alone or in conjunction with a small effector molecule, interferes with a normal catalytic function of the ribosome, preventing its release from the mRNA.

We have studied the regulation of two classes of chloramphenicol-inducible chloramphenicol resistance genes: the *cat* genes in Gram-positive bacteria and the *cmlA* gene in Gram-negatives. Both classes are induced by chloramphenicol through the translation attenuation mechanism (6). In this form of translational control, a single transcript spans a short upstream leader coding sequence (the upstream gene) and a downstream chloramphenicol resistance coding sequence (the downstream gene). The region in the mRNA that intervenes between the two coding sequences contains a secondary structure domain that sequesters the ribosome binding site (RBS) for the (downstream) chloramphenicol resistance coding sequence. As a consequence, the chloramphenicol resistance coding sequence cannot be translated because its RBS is unavailable for translation initiation. It has been shown that during induction of *catA86* by chloramphenicol, the RNA secondary structure domain becomes destabilized (7), and this is also presumed to be the basis for induction of the *cmlA* gene (8).

Activation of translation of the *catA86* gene in *B.subtilis* can be achieved by stalling a ribosome, through selective amino acid deprivation, at a specific codon within the leader coding sequence

*To whom correspondence should be addressed. Tel: +1 410 455 2249; Fax: +1 410 455 3875; Email: lovett@umbc.edu

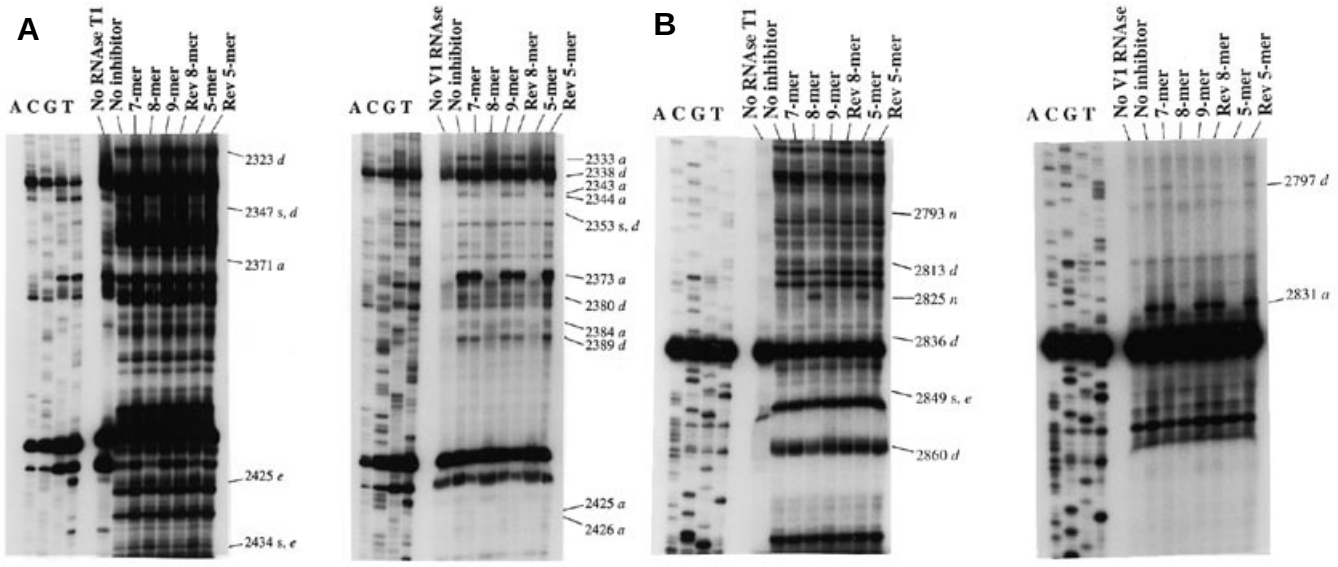

Figure 1. Footprinting the 5mer and 8mer peptides on yeast large subunit rRNA using endonuclease probes. Experimental procedures are described in Materials and Methods. The sequence of the peptides used are: 7mer, MSTSKNA; 8mer, MSTSKNAD; 9mer, MSTSKNADK; Rev 8mer, DANKSTSM; 5mer, MVKTD; Rev 5mer, DTKVM. Of these peptides, only MSTSKNAD and MVKTD were inhibitory to peptidyl transferase and altered the nuclease susceptibility of domains IV and V of *E.coli* 23S rRNA (17). Nucleotides that show a change in susceptibility to the nucleases due to the presence of the inhibitor peptides are numbered according to their location on the yeast rRNA map (22). The letter following each numbered nucleotide refers to the type of change observed: a, absence of a cleavage site in rRNA exposed to the inhibitor 5mer and 8mer peptides; e, enhanced cleavage due to the inhibitor 5mer and 8mer peptides; n, new site of cleavage due to the inhibitor 5mer and 8mer peptides; d, decrease in the intensity of cleavage due to the 5mer and 8mer inhibitor peptides as judged by visual inspection; s, a site of termination due to secondary structure. All changes noted were consistently observed in independent experiments. A few changes due to the inhibitor peptides are not designated because these were observed in some but not all experiments. In viewing these data, one is comparing the cleavage patterns observed in lanes with the inhibitor 5mer and 8mer peptides with the other lanes which contain no peptide or peptides which are not inhibitory for peptidyl transferase. (**A**) Probing the left half of domain V and the right half of domain IV using T1 (left panel) or V1 (right panel) endonuclease. The DNA primer was L369 (see Fig. 2). (**B**) Probing the right half of domain V using T1 (left panel) or V1 (right panel) endonuclease. The DNA primer was L371 (see Fig. 2).

(9). Significant translational activation of *cat* results only when a ribosome is stalled with its amino acyl site at leader codon 6 (9). This places the stalled ribosome adjacent to the secondary structure, but at a location that does not interfere with the entrance of a second ribosome at the *cat* RBS, designated RBS-C. It is therefore presumed that chloramphenicol induces *cat* translation only when it stalls a ribosome at leader codon 6.

Chloramphenicol is an inhibitor of peptidyl transferase, the ribosome activity that forms peptide bonds. However, the antibiotic does not act only on ribosomes translating a particular coding sequence. Genetic evidence has shown that leader codons 1–5 determine the site specificity of ribosome stalling during *cat* induction by chloramphenicol (10–12). Recently it was found that addition of a synthetic peptide (MVKTD) to ribosomes, a peptide which corresponds to the product of leader codons 1–5, inhibits ribosomal peptidyl transferase (13,14). The reverse sequence (DTKVM) is not inhibitory, nor are C- or N-terminal truncations of MVKTD. Amino acid substitutions in the 5mer peptide that correspond to missense mutations known to block *cat* induction *in vivo* prevent the 5mer peptide from inhibiting peptidyl transferase. Comparable results have been obtained using the N-terminal 8mer peptide (MSTSKNAD) encoded by the leader for the *cmlA* gene (15). It is inferred that during leader translation *in vivo*, a ribosome will pause at the induction site due to activity of the leader peptide.

Peptidyl transferase activity appears to require participation of 23S (large subunit) rRNA sequences (16). *In vitro* studies demonstrated that synthetic *cat* leader 5mer peptide (MVKTD) and synthetic *cmlA* leader 8mer peptide (MSTSKNAD) bind to extracted or *in vitro* transcribed *E.coli* 23S rRNA and alter conformation of the rRNA uniquely in the vicinity of the peptidyl transferase center (17). Here we demonstrate that both peptides bind to large subunit rRNA extracted from yeast and a representative of the Archaea. Thus, the rRNA target for the inhibitor peptides is conserved across kingdoms.

MATERIALS AND METHODS

Organisms and extraction of rRNA

Saccharomyces cerevisiae FY250 (*trp1 his3 ura3 leu2*) was grown at 30°C in YPD medium, consisting of 1% yeast extract, 2% peptone and 2% glucose. *Halobacterium halobium* strain R1 was grown at 37°C in a medium consisting of 0.3% yeast extract, 0.5% tryptone, 0.2% sodium chloride, 0.2% potassium chloride and 25% sodium chloride (18). Cells (2 l) were grown to late log phase (100 Klett U, filter 66), harvested by centrifugation and resuspended in 100 ml suspension buffer (20 mM Tris–HCl, pH 7.6, 10 mM magnesium chloride, 100 mM ammonium chloride and 6 mM β-2-mercaptoethanol). Yeast cells were washed twice with cold suspension buffer (17) and disrupted in a French pressure cell (15 000 p.s.i.). *Halobacterium halobium* cells began lysis upon resuspension and were therefore immediately disrupted as described above. Debris was removed from the lysates by centrifugation at 30 000 *g* for 15 min. The supernatant fractions were centrifuged through a 1.1 M sucrose cushion in suspension buffer. The ribosome pellet was rinsed with suspension buffer, resuspended and centrifuged at 105 000 *g*. Pellets were resuspended

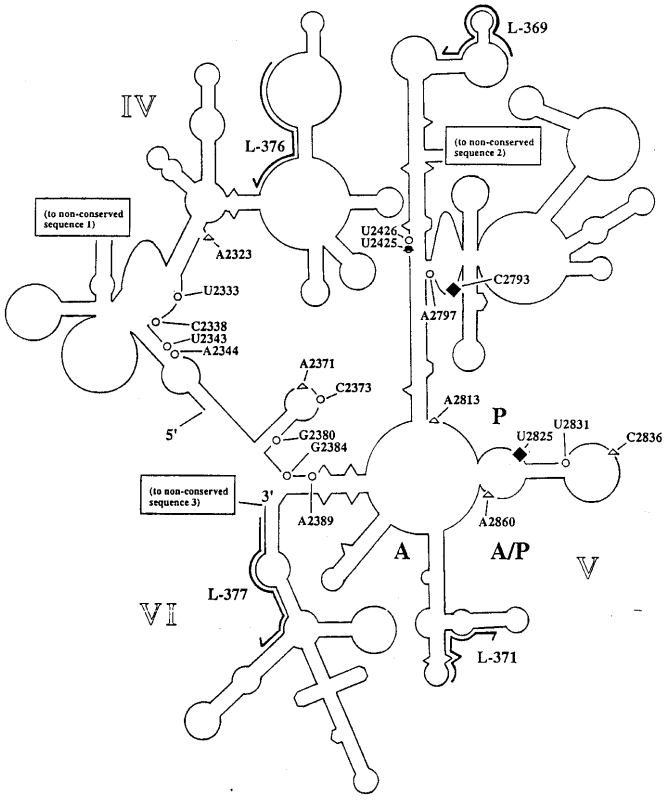

Figure 2. Sites of peptide-induced alterations of nuclease susceptibility in yeast large subunit rRNA. Large subunit rRNA consists of six domains. Peptide-induced alterations were observed only in domains IV and V. Each site of a peptide-induced altered response is designated with a letter and number. The letter corresponds to the nucleotide at the site of alteration and the number refers to the specific nucleotide in yeast rRNA. Symbols designate the type of response: open circle, loss of V1 susceptibility; open triangle, loss of a T1 cleavage site; solid diamond, new site of T1 cleavage. Four primers were used to analyze this region of the rRNA by reverse transcriptase. Primer L-369 is complementary to nt 2461–2478. Primer L-371 is complementary to nt 2902–2917. Primer L-376 is complementary to nt 2192–2210. Primer L-377 is complementary to nt 3129–3146. Boxes show the non-conserved sequences (so-called expansion sequences) present in yeast rRNA that appear unrelated to the 'core' rRNA found in eubacteria or Archaea. Large P and A refer to the peptidyl and aminoacyl sites. The symbol at U2425 is an open circle (designates loss of V1 cleavage) and a solid diamond (designates acquisition of a new T1 cleavage site).

in standard ribosome buffer (17) and clarified by centrifugation at 30 000 *g*. The ribosomes were dispensed in aliquots which were frozen at –80°C. An aliquot was extracted with phenol and precipitated with cold ethanol immediately prior to use according to the method of Noller *et al.* (16).

Peptide synthesis

Peptides were synthesized on an ABI model 432 peptide synthesizer and were purified by reverse phase HPLC as previously described (13).

Nuclease probing of peptide effects on rRNA

rRNA was incubated with peptides as previously described (17), using peptide:large subunit rRNA ratios of 6000:1. Preincubation was for 10 min at 0°C, after which time T1 or V1 ribonucleases was added to a final reaction volume of 20 µl (17). Digestion was at 0°C for 10 min and was terminated by addition of 100 µl phenol and 100 µl water. Samples were centrifuged and re-extracted with chloroform. RNA was precipitated in 3 vol cold ethanol + 0.3 M sodium acetate. The precipitate was pelleted, washed with 100 µl 70% ethanol, pelleted and resuspended in 5 µl water. This RNA served as template for primer extension analysis by AMV reverse transcriptase as previously described (17,19).

RESULTS

Footprinting the peptide on yeast large subunit rRNA using T1 and V1 endoribonucleases

T1 endonuclease cleaves RNA immediately 3′ of G residues at single-stranded sites (20). V1 nuclease cleaves RNA at various nucleotides that are duplexed (21). Hence, these enzymes have been used in determinations of the secondary structure of rRNA (19). To examine peptide effects on the conformation of yeast rRNA, total RNA was extracted from 80S ribosomes and mixed with peptides at peptide:large subunit rRNA ratios of ~6000:1. The peptide–RNA complex was digested with T1 or V1 endonuclease using enzyme concentrations empirically found to cleave each RNA molecule only a few times. Sites of cleavage were determined using specific DNA oligomers as primers for reverse transcriptase. Each cleavage site is detected as a site of termination of DNA synthesis by reverse transcriptase (19).

Primers for reverse transcriptase were chosen that allowed us to screen ~90% of the large subunit RNA for changes in termination of DNA synthesis (22). Peptide-induced changes in nuclease susceptibility were detected in only two regions of the rRNA, domains IV and V. The results of the footprint experiments are shown in Figure 1 and a sketch of the region of rRNA affected by the peptide is shown in Figure 2. No changes in enzymatic cleavage were observed using control peptides, such as reversemers or the *cmlA* leader 7mer and 9mer, which are not inhibitory for peptidyl transferase.

Footprinting the peptide on large subunit rRNA from *Halobacterium* using T1 and V1 endoribonucleases

Large subunit rRNA from *H.halobium* (18) was preincubated with the various peptides and probed with T1 and V1 endonucleases. DNA oligomers that prime synthesis in each of the six rRNA domains were separately used to assess peptide effects on susceptibility to the enzymes (Fig. 3). Peptide-induced changes in RNA susceptibility to the enzymes were observed only in rRNA domains IV and V (Fig. 4). Control peptides that do not inhibit peptidyl transferase, such as the reversemers, did not detectably alter the conformation of the rRNA (Fig. 3).

Comparison of the results obtained with *E.coli* rRNA with those of *Saccharomyces* and *Halobacterium*

Table 1 summarizes the individual rRNA nucleotides in *E.coli*, *Saccharomyces* and *Halobacterium* whose susceptibility to T1 or V1 nucleases becomes altered in the presence of the 5mer and 8mer peptides. An effect observed at a specific nucleotide in the rRNA of one organism typically did not show a peptide-altered response to the nuclease probe at the corresponding nucleotide of the other organisms, although generally a nearby nucleotide was affected. By presenting the data in this fashion, *patterns* of susceptibility become evident. The pattern is more apparent when the changes in the rRNAs of the three organisms are placed on a

Table 1. Comparison of sites in large subunit rRNA from *E.coli*, *S.cerevisiae* and *H.halobium* whose susceptibility to T1 or V1 nuclease is altered by MVKTD and MSTSKNAD[a]

E. coli	*S. cerevisiae*	*H. halobium*	*E. coli*	*S. cerevisiae*	*H. halobium*
U1955 △	**U**2297	**U**1981	**G2061** △	**G**2402	**G2087** ○
G1973	**G**2315	**G1999** ○	C2084	U**2425** ○ ▲	G2110
G1980	G2322	G**2006** ○	U2085	U**2426** ○	C2111
A1981 △	**A2323** △	**A2007** ▲	C**2416** △	A2781	G2435
G**1989** ◆	U2019	A2015	A2418	G2783	C**2437** ○
U1991	**U2333** ○	**U**2017	U2423	A2788	U**2442** ▲
U1993 △	**U**2335	**U**2019	G2428	C**2793** ◆	A2447
C1996	C**2338** ○	C2022	**A2430** ▲	**A**2795	**A**2449
C2001	U**2343** ○	U**2027** ▲	A2432	A**2797** ○	A2451
G2002	A**2344** ○	U2028	**U2438** ▲	**U**2803	**U**2457
A**2005** ◆	A2347	G2031	A2448	A**2813** △	A2467
A2013 △	**A**2355	**A**2039	C**2456** ◆	C2821	G2473
G2018	A2360	U**2044** ▲	A2459	G2824	C**2477** ▲
A**2019** △	C2361	A2045	**U**2460	**U2825** ◆	**U**2479
U2022	G2363	U**2048** ○	C2466	U**2831** ○	G2485
A**2030** △	A**2371** △	C2056	**A**2469	**A**2834	**A2488** ○
G2032	C**2373** ○	G2058	A2471	C**2836** △	A2490
A**2033** △	G2374	A2059	U**2473** ▲	U2837	C2492
U2039	G**2380** ○	G2065	**C**2475	**C**2840	**C2494** ○
G2040	G2381	A**2066** ▲	G2488	C2853	U**2507** ◆
U**2041** △	C2382	G**2067** ○	G2495	A**2860** △	C2513
A2042	A2383	G**2068** ○	C**2496** ◆	U2861	U2514
C2043	G**2384** ○	C2069	**C**2498	**C**2863	**C2516** ○
G2048	A**2389** ○	A2074	**A2503** ◆	**A**2868	**A**2521
C**2050** △	C2391	G2076	**U2506** ◆	**U**2871	**U**2524
A2054	G2395	A**2080** ○	G**2509** ○	G2874	G2527
G**2057** △	A2398	C2083	C**2510** ◆	C2875	U2528
A2059	**A**2400	**A2085** ○			

[a]The correspondence between rRNA nucleotides in yeast, Archaea and bacteria is from Egebjerg *et al*. (23). Symbols represent the following changes in ribonuclease cleavage: (i) open triangle, protection from ribonuclease T1; (ii) filled triangle, enhanced susceptibility to T1 digestion; (iii) filled diamond, a new site of T1 cleavage; (iv) open circle, protection from V1 digestion. The nucleotide positions of peptide-induced alterations of nuclease cleavage are enlarged and in bold. Absolutely conserved nucleotides are enlarged and in bold. Thus, when the letter and number of a nucleotide are in bold, this indicates an absolutely conserved nucleotide whose susceptibility to a nuclease is altered by the peptides. Nucleotide sequence information for *E.coli* 23S rRNA, *H.halobium* 23S rRNA and *S.cerevisiae* 26S rRNA was taken from Egebjerg *et al*. (23), Mankin and Kagramanova (18) and Hogan *et al*. (22).

common (*E.coli*) secondary structure map for 23S domains IV–VI (23; Fig. 5).

A likely explanation for the observed differences in nuclease susceptibility of the three rRNAs, with or without added peptide, resides in the differences in the primary nucleotide sequence and the resulting differences in secondary structure (24). The primary sequence of large subunit rRNA differs, somewhat to substantially, among members of the three kingdoms depending on the particular region of large subunit rRNA that is examined; the peptidyl transferase center, comprising domain V, shows the highest conservation of primary sequence. We suggest that the differences in the primary sequence of the rRNAs, and the resulting differences in secondary structure, cause differences in susceptibility to the nuclease probes.

DISCUSSION

The model suggested to explain translation attenuation regulation of *catA86* and *cmlA* proposes that a ribosome translating the leader becomes stalled at a specific codon when the inducing antibiotic chloramphenicol is also present (6). Chloramphenicol is an inhibitor of translation in bacteria, presumably by interfering with peptidyl transferase. However, the antibiotic is not known to block translation at specific nucleotide sequences in mRNA nor at specific amino acid sequences in the nascent peptide. Stall site selection must therefore be a function of the leader. Genetic evidence suggested that induction might depend on the amino acid sequence of the leader peptide specified by codons upstream of the site of ribosome stalling (9–11,25). Our recent results strongly support the idea that the nascent leader peptide is the selector of the site of stalling through a *cis*-effect on its translating ribosome (13–15).

Peptidyl transferase inhibition

Peptidyl transferase activity is intimately associated with large subunit rRNA. It remains unknown if the catalytic activity is due to the RNA alone or to RNA maintained in proper conformation

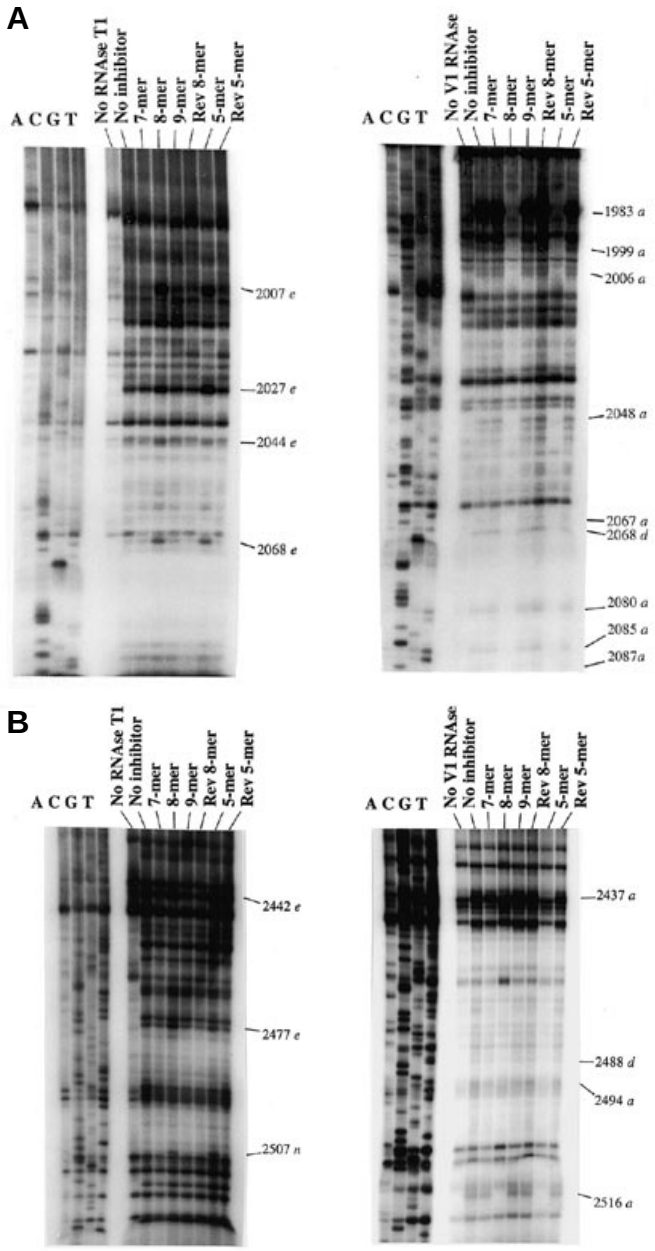

Figure 3. Footprinting the 5mer and 8mer peptides on *Halobacterium* large subunit rRNA using endonuclease probes. Peptides used and the designation of symbols are as in Figure 1. (**A**) Probing the left half of domain V and the right half of domain IV using T1 (left panel) or V1 (right panel) endonuclease. The DNA primer was HH23 V/IV (see Fig. 4). (**B**) Probing the right half of domain V using T1 (left panel) or V1 (right panel) endonuclease. The DNA primer was HH23V (see Fig. 4).

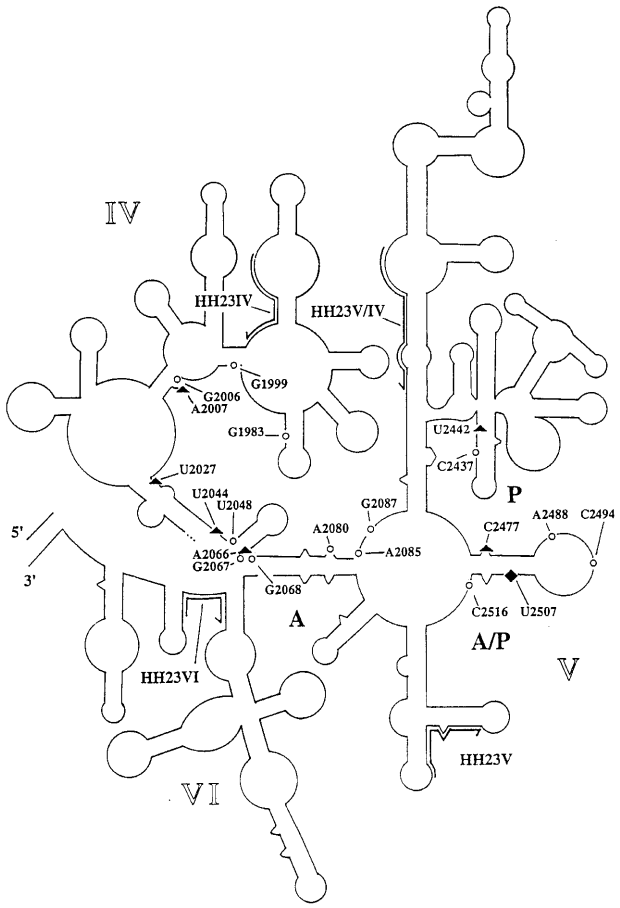

Figure 4. Sites of peptide-induced alterations of nuclease susceptibility in *H.halobium* large subunit rRNA. Peptide-induced alterations in secondary structure were observed only in domains IV and V. Nomenclature and symbols are as in Figure 2. Primers used to analyze the sites of reverse transcriptase termination were: HH23IV (complements nt 1876–1893), HH23V/IV (complements nt 2554–2571) and HH23VI (complements nt 2801–2819).

by protein or, conversely, if the RNA confers correct structure to catalytic ribosomal proteins (26). The *cat* and *cmlA* leader peptides, which are inhibitory for peptidyl transferase, bind to large subunit rRNA and modify the secondary structure of domains at the peptidyl transferase center. These findings are consistent with the previous connection between rRNA and peptidyl transferase activity (16). It is also interesting that the peptidyl transferase of *S.cerevisiae* cytoplasmic ribosomes is insensitive to chloramphenicol but is inhibited by the leader peptides, whereas *H.halobium* and *E.coli* peptidyl transferase activity is inhibited by the antibiotic and by the leader peptides (14). Susceptibility of peptidyl transferase to the leader peptides therefore appears independent of susceptibility to chloramphenicol.

Large subunit rRNAs from yeast, Archaea and bacteria do not show identical leader peptide-induced alterations of RNA conformation, although in each case the RNA domains affected by the peptides are the same. Indeed, the regions within domains IV and V that show an altered conformation in response to the peptides are very similar (Fig. 5). Consequently, we presume that within the rRNA there exists a conserved structure or sequence that serves as the peptide binding target. Binding produces a change in conformation that is determined by the initial secondary structure within each of the rRNAs tested. This reasoning argues that conformational alterations caused by peptide binding might, and perhaps should, differ somewhat among the three rRNAs examined.

Our results with rRNAs from the three organisms do not necessarily identify the target for peptide binding, but rather the effects produced on the rRNA as a consequence of binding a peptidyl transferase inhibitor peptide. It remains unknown whether the basis for peptidyl transferase inhibition is due only to

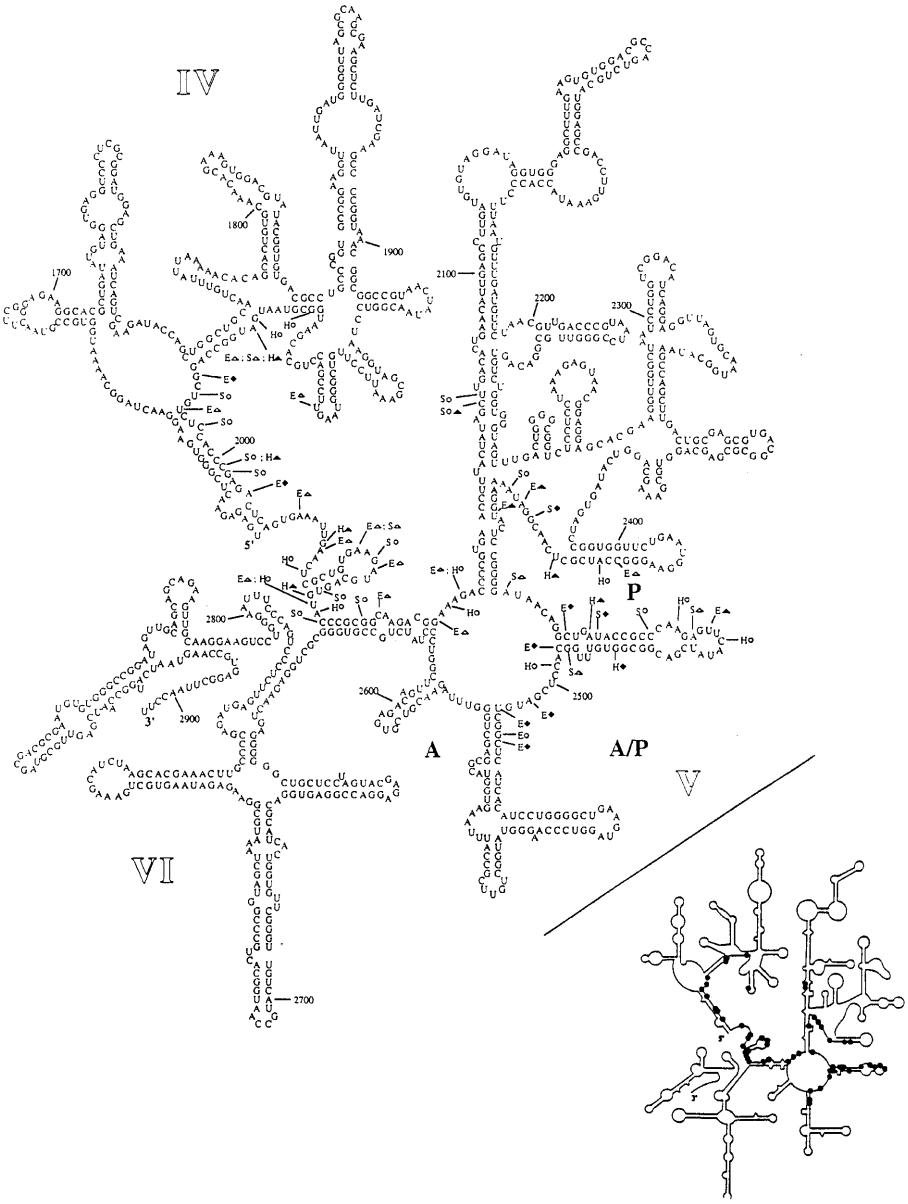

Figure 5. Summary of all peptide-induced changes in rRNA susceptibility to T1 and V1 ribonucleases placed on the map of *E.coli* 23S rRNA domains IV–VI. Using *E.coli* 23S rRNA domains IV–VI, we can place all peptide-induced changes in conformation of *E.coli*, yeast and Archaea rRNA on this common representation of the peptidyl transferase center. In one representation, the actual sequence of rRNA nucleotides is shown, with E, S and H noting the nucleotides altered in *E.coli*, *Saccharomyces* and *Halobacterium*. In the other representation, the sites of the changes are depicted as solid circles. All data shown are taken from Table 1.

binding of an inhibitor peptide to its rRNA target or to the resulting conformational changes that occur in the rRNA.

Observations that place the nascent peptide at the peptidyl transferase center

Nucleotides in domain IV of 23S rRNA can be crosslinked to nucleotides in domain V (27). This observation suggests that in the three-dimensional model of the rRNA, domain IV may fold onto domain V, forming a channel through which the nascent peptide passes (28). The peptide-induced conformational alterations are in the rRNA domains that likely form the walls of such a peptide transit channel. Furthermore, the peptide-induced conformational alterations are observed at those regions to which a nascent peptide can be crosslinked concurrent with its synthesis (29). Our footprinting data are therefore consistent both with a model for rRNA tertiary structure and with those nucleotides that can be contacted by a peptide as it passes from the P site through the ribosome during translation.

Peptide inhibitors of peptidyl transferase also inhibit translation termination

In addition to its role as an inhibitor of peptidyl transferase, the *catA86* 5mer peptide is an inhibitor of translation termination (12,30). Tate *et al.* (31) have suggested that peptidyl transferase and translation termination might be two different functions of the same enzyme. If this idea were correct, it would provide an

explanation for the bifunctional inhibitory activity of the leader peptides.

The translation of several eukaryotic genes is inhibited by short, sequence-specific, upstream open reading frames (uORFs) (2–4). It has been proposed that the uORFs may encode peptides that interact *in cis* with their translating ribosomes and prevent ribosome release from the mRNA at the uORF stop codon; specifically, the peptides are suggested to inhibit translation termination. A ribosome 'stalled' in this manner could serve as a block to ribosome scanning to downstream ORFs on the same transcript. Given the similarity between these observations and those we have made with the *catA86* and *cmlA* leader peptides, we suspect that the mechanism of action of the *cis*-acting eukaryotic translational repressors may parallel that of bacterial leader peptides.

ACKNOWLEDGEMENTS

We thank Alexander Mankin for *H.halobium*, Steve Swift for *S.cerevisiae* and Beth Rogers for comments on the manuscript. Portions of this investigation were supported by grant GM-42925 from the National Institutes of Health.

REFERENCES

1 Lovett,P.S. and Rogers,E.J. (1996) *Microbiol. Rev.*, **60**, 366–385.
2 Morris,D.R. (1997) In Morris,D.R. and Harford,J. (eds), *Post-transcriptional Gene Regulation.* John Wiley & Sons, New York, NY, pp. 165–180.
3 Geballe,A.P. (1996) In Hershey,J., Matthews,M. and Sonenberg,N. (eds), *Translational Control.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 173–197.
4 Geballe,A.P. and Morris,D.R. (1994) *Trends Biochem. Sci.*, **19**, 159–164.
5 Lovett,P.S. (1994) *J. Bacteriol.*, **176**, 6415–6417.
6 Lovett,P.S. (1990) *J. Bacteriol.*, **172**, 1–6.
7 Harrod,R., Gu,Z. and Lovett,P.S. (1994) *Gene*, **140**, 79–83.
8 Stokes,H.W. and Hall,R.M. (1991) *Plasmid*, **26**, 10–19.
9 Alexieva,Z., Duvall,E.J., Ambulos,N.P.,Jr, Kim,U.J. and Lovett,P.S. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 3057–3061.
10 Mulbry,W., Ambulos,N.P.,Jr and Lovett,P.S. (1989) *J. Bacteriol.*, **171**, 5322–5324.
11 Rogers,E.J., Kim,U.J., Ambulos,N.P. Jr and Lovett,P.S. (1990) *J. Bacteriol.*, **172**, 110–115.
12 Rogers,E.J. and Lovett,P.S. (1994) *Mol. Microbiol.*, **12**, 181–186.
13 Gu,Z., Rogers,E.J. and Lovett,P.S. (1993) *J. Bacteriol.*, **173**, 5309–5313.
14 Gu,Z., Harrod,R., Rogers,E.J. and Lovett,P.S. (1994) *J. Bacteriol.*, **176**, 6238–6244.
15 Gu,Z., Harrod,R., Rogers,E.J. and Lovett,P.S. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 5612–5616.
16 Noller,H.F., Hoffarth,V. and Zimniak,L. (1992) *Science*, **256**, 1416–1419.
17 Harrod,R. and Lovett,P.S. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 8650–8654.
18 Mankin,A.S. and Kagramanova,V.K. (1986) *Mol. Gen. Genet.*, **202**, 152–161.
19 Stern,S., Moazed,D. and Noller,H.F. (1988) *Methods Enzymol.*, **164**, 481–489.
20 Wrede,P., Wurst,R., Vornakis,J. and Rich,A. (1979) *J. Biol. Chem.*, **254**, 9608–9616.
21 Lowman,J.B. and Draper,D.E. (1986) *J. Biol. Chem.*, **261**, 5396–5403.
22 Hogan,J.J., Gutell,R.R. and Noller,H.F. (1984) *Biochemistry*, **23**, 3330–3335.
23 Egebjerg,J., Larsen,N. and Garrett,R. (1990) In Hill,W., Dahlberg,A., Garrett,R.A., Moore,P.B., Schlessinger,D. and Warner,J.R. (eds), *The Ribosome: Structure, Function, and Evolution.* American Society for Microbiology, Washington, DC, pp. 168–179.
24 Leffers,H., Kjems,J., Ostergaard,L., Larsen,N. and Garrett,R.A. (1987) *J. Mol. Biol.*, **195**, 43–61.
25 Kim,U.J., Ambulos,N.P.,Jr, Duvall,E.J., Lorton,M.A. and Lovett,P.S. (1988) *J. Bacteriol.*, **170**, 2933–2938.
26 Noller,H.F. (1993) *J. Bacteriol.*, **175**, 5297–5300.
27 Zimmermann,R.A., Thomas,C.L. and Wower,J. (1990) In Hill,W.E., Dahlberg,A., Garrett,R.A., Moore,P.B., Schlessinger,D. and Warner,J.R. (eds), *The Ribosome: Structure, Function and Evolution.* American Society for Microbiology, Washington, DC, pp. 331–347.
28 Ryabova,L.A., Selivanova,O.M., Baranov,V.I., Vasiliev,V.D. and Spirin,A.S. (1988) *FEBS Lett.*, **226**, 255–260.
29 Stade,K., Junke,N. and Brimacombe,R. (1995) *Nucleic Acids Res.*, **23**, 2371–2380.
30 Moffat,J.G., Tate,W.P. and Lovett,P.S. (1994) *J. Bacteriol.*, **176**, 7115–7117.
31 Tate,W.P., Adamski,F.M., Brown,C.M., Dalphin,M.E., Gray,J.P., Horsfield,J.A., McCaughan,K.K., Moffat,J.G., Powell,R.J., Timms,K.M. and Trotman,C.N.A. (1993) In Nierhaus,K.H., Franceschi,F., Subramanian,A.R., Erdmann,V.A. and Wittmann-Leibold,B. (eds), *The Translational Apparatus: Structure, Function, Regulation, and Evolution.* Plenum Press, New York, NY, pp. 253–262.