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The GA motif: An RNA element common to bacterial antitermination systems, rRNA, and eukaryotic RNAs

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ABSTRACT

Two different transcription termination control mechanisms, the T box and S box systems, are used to regulate transcription of many bacterial aminoacyl-tRNA synthetase, amino acid biosynthesis, and amino acid transport genes. Both of these regulatory mechanisms involve an untranslated mRNA leader region capable of adopting alternate structural conformations that result in transcription termination or transcription elongation into the downstream region. Comparative analyses revealed a small RNA secondary structural element, designated the GA motif, that is highly conserved in both T box and S box leader sequences. The motif consists of two short helices separated by an asymmetric internal loop, with highly conserved GA dinucleotide sequences on either side of the internal loop. Site-directed mutagenesis of this motif in model T and S box leader sequences indicated that it is essential for transcriptional regulation in both systems. This motif is similar to the binding site of yeast ribosomal protein L30, the Snu13p binding sites found in U4 snRNA and box C/D snoRNAs, and two elements in 23S rRNA.

Keywords: antitermination; box C/D snoRNAs; ribosomal protein L30; secondary structural motif; U4 snRNA

INTRODUCTION

The T box and S box transcription termination control systems are widely used in Gram-positive bacteria for control of gene expression (Henkin, 2000). The T box regulatory system controls many different aminoacyl-tRNA synthetase, amino acid biosynthesis, and amino acid transporter genes; each transcriptional unit regulated by this mechanism responds individually to the charging ratio of the cognate tRNA (Grundy & Henkin, 1993; Grundy et al., 1994). In contrast, the S box system is dedicated to genes involved in methionine biosynthesis, all of which respond in concert to limitation for methionine (Grundy & Henkin, 1998). There are several genes controlled by the T box system in one organism and by the S box system in another (Grundy & Henkin, in press), suggesting that the two systems represent alternate mechanisms for solving the same regulatory problem.

Genes regulated by either the T or S box mechanisms contain mRNA leader regions with the capacity to fold into mutually exclusive terminator and antiterminator structures, so that transcription of the downstream coding region(s) is controlled by the folding of the leader RNA. Each family of leader RNAs is characterized by a complex array of primary sequence and structural features that have been identified by conservation, covariance, and mutational analyses (Grundy & Henkin, 1993, 1998; Rollins et al., 1997). A major difference between the two systems is that whereas disruption of conserved features in the T box leaders (with the exception of the terminator itself) results in loss of expression, consistent with a requirement for binding of uncharged tRNA to drive antitermination (Grundy et al., 1994), mutations in the S box leaders (other than disruption of the antiterminator) result in constitutive expression, suggesting that in this case some negative-acting factor is required to promote termination (Grundy & Henkin, 1998). It therefore appears that the two termination control systems operate differently at the molecular level.

In this article, we report the identification of a motif conserved in all of the S box leaders as well as in most of the T box leaders. This motif, found at the base of Stem I in the T box leaders (Fig. 1A) and in helix 2 of the S box leaders (Fig. 1B), consists of two short heli-

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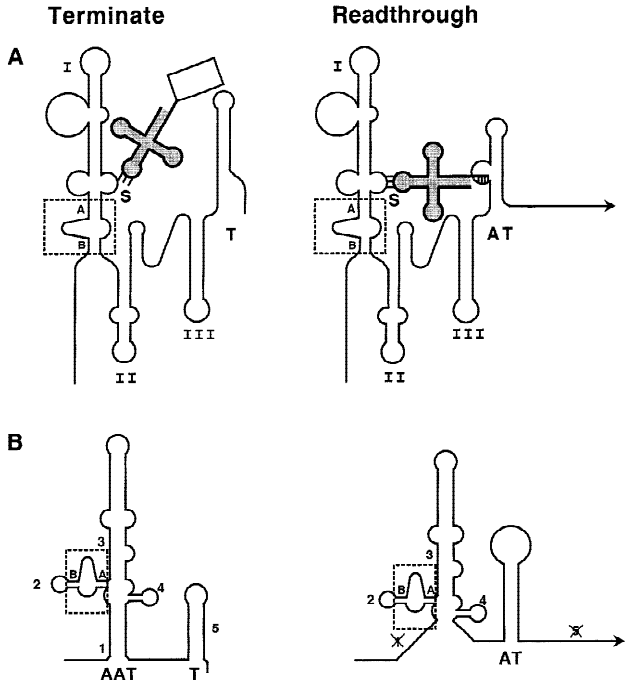

FIGURE 1. Model for T box and S box leader RNAs. **A:** T box model. Roman numerals indicate major conserved secondary structural features. The cloverleaf structure represents tRNA, charged (box), or uncharged. Uncharged tRNA is postulated to interact with the leader mRNA by codon:anticodon pairing at the specifier sequence (S) in the leader and by pairing of the acceptor end of the tRNA with the side bulge of the antiterminator structure (AT), which is stabilized by interaction with the tRNA. When levels of the cognate amino acid are high, most of the cognate tRNAs are charged and unable to interact with the leader mRNA to stimulate readthrough; the terminator structure (T) forms and transcription terminates. A decrease in the charging ratio of the cognate tRNA promotes readthrough. **B:** S box model. Numbers represent conserved secondary structural features. When methionine levels are high, the anti-antiterminator (AAT, helix 1) is stabilized, preventing formation of the antiterminator (AT) and allowing for formation of the terminator (T, helix 5). When methionine levels are low, the AAT is destabilized, allowing formation of the AT and preventing termination. The dashed boxes in both leader structures indicate the position of the GA motif. Helices A and B of the GA motif are labeled.

ces separated by an asymmetric internal loop, with highly conserved GA dinucleotide sequences positioned on opposite sides of the internal loop. Disruption of this element in the *Bacillus subtilis tyrS* T box leader resulted in loss of antitermination, and similar mutations in the *yitJ* S box leader resulted in constitutive expression, indicating that the motif is important in both regulatory systems. We also note that this motif is similar to the well-characterized yeast ribosomal protein L30 binding site, as well as the yeast Snu13p binding sites in U4 snRNA and box C/D snoRNAs, and that two related elements are present in 23S rRNA. These comparisons suggest that this motif has been utilized in a variety of different RNAs, and may provide insight into structural and functional roles for this element in the T box and S box systems.

RESULTS AND DISCUSSION

Identification of a conserved RNA element in T box and S box leader sequences

T box leaders

Database analyses have identified approximately 200 putative T box leader sequences in a variety of microorganisms (Table 1; SM Rollins, FJ Grundy, TM Henkin, in prep.). The leader sequences are highly conserved in structural arrangement and placement of conserved primary sequence elements. A model of the leader region of the *Bacillus subtilis tyrS* gene, encoding tyrosyl-tRNA synthetase, is shown in Figure 1A. The structural arrangement of the region at the base of Stem I varies somewhat, with the arrangement shown for *tyrS* found in 140 of 198 T box leader sequences analyzed (71%; Table 1). Based on these 140 sequences, a consensus pattern was derived (Fig. 2A). Due to the highly conserved GA dinucleotide sequences we refer to this RNA element as the "GA motif."

The T box leader GA motif contains two short helices separated by an asymmetric internal loop. Helix A averages 5 bp in length. The base pair at the internal loop-proximal end of helix A (N24:N107) is generally a weak or mismatched pair. Helix B averages 3 bp in length. At the internal loop-proximal end of helix B is a conserved C18:G110 pair (87% conserved; *tyrS* contains U18:A110 at this position; Fig. 2B). The two helices exhibit further conservation at the primary sequence level, with positions 103–106 and 111 conserved as

TABLE 1. Distribution of T box and S box GA motifs.

Genus	Number of T box leaders identified[a]	T box leaders with GA motif	Number of S box leaders identified[a]	S box leaders with GA motif
Bacillus	77	72	31	31
Carboxydothermus	4	4	2	2
Chlorobium	0	0	1	1
Clostridium	43	41	6	6
Corynebacterium	2	1	0	0
Deinococcus	3	2	0	0
Enterococcus	17	12	0	0
Geobacter	1	1	2	2
Lactobacillus	3	2	0	0
Lactococcus	2	0	0	0
Mycobacterium	2	2	0	0
Staphylococcus	12	0	7	7
Streptococcus	30	1	0	0
Streptomyces	1	1	0	0
Symbiobacterium	1	1	0	0

[a]Complete and unfinished genomes were searched for conserved elements in the T box and S box leaders, and structural models were derived by analogy with the established T box and S box leader models (SM Rollins, FJ Grundy, TM Henkin, in prep; FJ Grundy, BA Murphy, TM Henkin, in prep.). Because unfinished genomes were included, this listing does not represent a complete set.

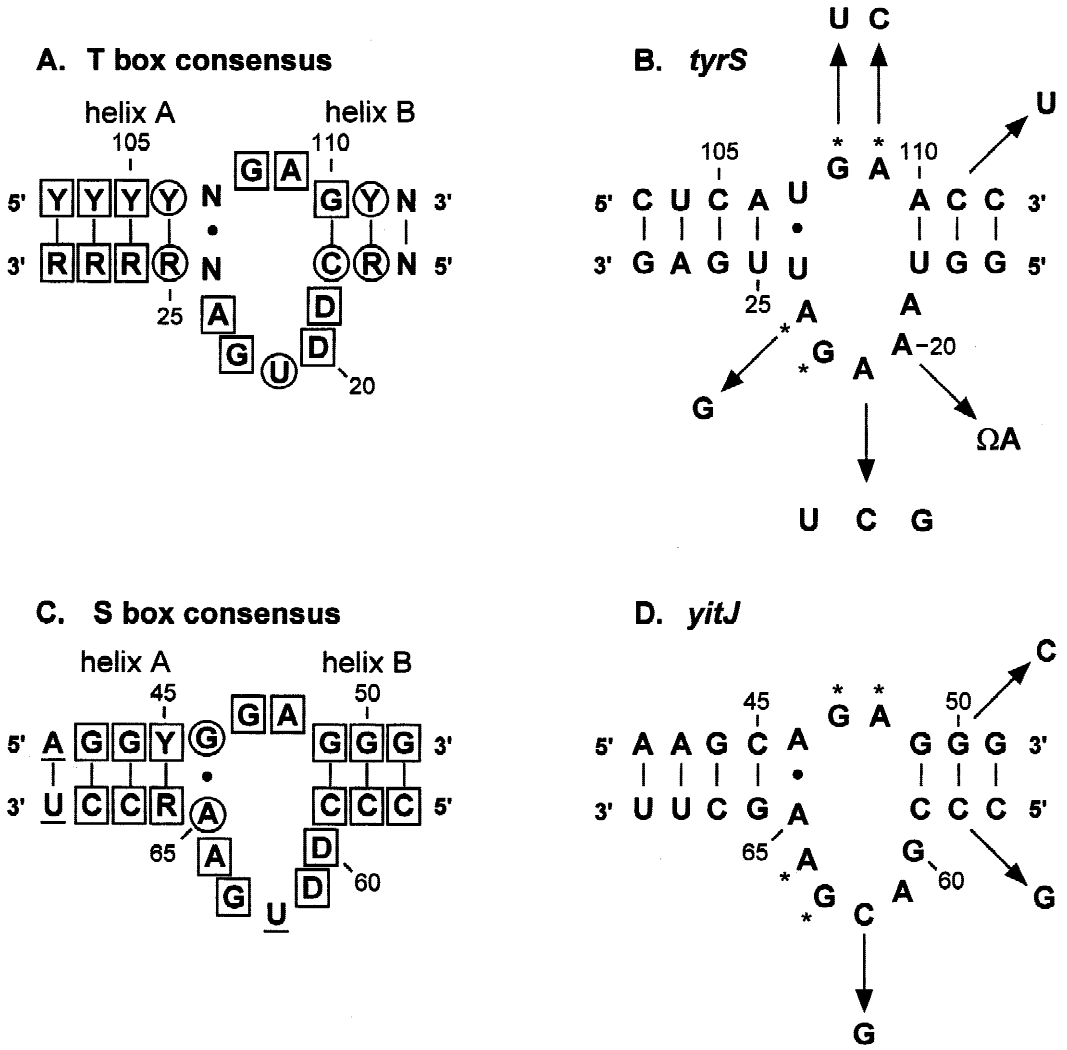

FIGURE 2. GA motif sequences and consensus patterns. **A:** Consensus pattern from 140 T box leader sequences. Positions are numbered relative to the *tyrS* transcription start-point, and match previous structural models (Rollins et al., 1997). **B:** *B. subtilis tyrS* leader. Arrows indicate mutational alterations. **C:** Consensus pattern from 49 S box leader sequences. Positions are numbered according to the intact *yitJ* leader (Grundy & Henkin, 1998). **D:** *B. subtilis yitJ* sequence. Arrows indicate mutations introduced. Boxes indicate >90% conservation, circles indicate >80% conservation, underlined positions indicate >70% conservation. Asterisks indicate conserved GA dinucleotides. R = G, A; Y = C, U; D = G, A, U; N = any nucleotide.

pyrimidines and positions 17 and 25–28 conserved as purines. Between helices A and B is an asymmetric internal loop with a conserved 5 + 2 arrangement. Two oppositely oriented GA dinucleotide sequences within this loop are almost completely conserved. Positions 19–21 of the 5-nt side of the loop exhibit some bias, with the preferred sequence pattern D19, D20, U21.

The 58 sequences lacking a recognizable GA motif were analyzed for the possibility of an alternative structure, and most leaders in this group were found to contain an extended helix without an internal loop. There were no obvious trends in the distribution of the GA motif in leaders from genes of different amino acid classes; however, there is a nonrandom distribution of this motif in different organisms, with a high representation (94%) in organisms in the *Bacillus-Clostridium* group, whereas the motif was very rare in leaders from *Staphylococcus* and *Streptococcus* (2%; Table 1).

S box leaders

Forty-nine S box leader sequences have been identified so far in *Bacillus*, *Clostridium*, *Carboxydothermus*, *Staphylococcus*, *Geobacter*, and *Chlorobium* (Table 1; FJ Grundy, BA Murphy, TM Henkin, in prep.); all of these organisms, with the exception of *Chlorobium*, also contain T box leaders. A model for the leader of the *B. subtilis yitJ* gene, encoding methylene tetrahydrofolate reductase, is shown in Figure 1B. A secondary struc-

tural motif similar to the T box GA motif was found in helix 2 of every S box leader (Table 1); the consensus pattern for this element (Fig. 2C) is very similar to that of the T box leaders in overall arrangement, the placement of two GA dinucleotide sequences, a weak or mismatched pair at the internal loop-proximal end of helix A, and the conservation of a C:G pair at the internal loop-proximal end of helix B. Within the internal loop, the S box leader positions D60, D61, and U62 are conserved similarly to the equivalent positions in the T box leader motif (D19, D20, U21). There is a higher degree of primary sequence conservation within the two helices of the S box leader GA motif, with positions 43–44 and 49–51 conserved as G, positions 57–59 and 67–68 conserved as C, position 45 conserved as a pyrimidine, and 66 conserved as a purine. It therefore appears that the T and S box leader GA motifs exhibit some differences in primary sequence conservation, although they are very similar overall.

Another difference between the S box and T box leaders is the arrangement of the motif within the overall RNA structure. In the S box leaders, the internal-loop-distal side of helix B is capped by a terminal loop. In contrast, the T box leader GA motif is at the base of a long stem-loop structure that contains several other conserved internal loops, and helix A abuts another highly conserved internal loop. This suggests that the GA motif may act as an autonomous structural unit that self-folds irrespective of sequences beyond either helix A or B.

Mutagenesis of the T and S box GA motifs

The tyrS GA motif is essential for antitermination

Site-directed mutagenesis of the *tyrS* leader (Fig. 2B) was used to test whether conservation of the T box leader GA motif reflects a requirement for function. These mutations were introduced into a *tyrS-lacZ* transcriptional fusion, which included the *tyrS* promoter, leader, and leader region terminator fused to a *lacZ* reporter, and the fusion was integrated in single copy into the chromosome of a strain auxotrophic for tyrosine by recombination into the specialized transducing phage SPβ (Grundy & Henkin, 1993). Expression of the *tyrS-lacZ* fusions was tested during growth in excess or limiting tyrosine. Limitation for tyrosine decreases the charging ratio of $tRNA^{Tyr}$, which leads to an increase in antitermination and increased expression of the *lacZ* reporter (Grundy & Henkin, 1993; data not shown).

Five mutations predicted to disrupt the overall structural integrity of the motif were examined. Insertion of an A at position 20 (ΩA20) was predicted to alter the 5 + 2 configuration of the internal loop to a 6 + 2 arrangement. This insertion led to a 23-fold decrease in expression during growth in excess tyrosine (Table 2); however, expression was induced 9.5-fold by tyrosine limitation (as compared to 16-fold for the wild-type fusion), indicating that the mutation did not completely block the ability to respond to increased availability of uncharged $tRNA^{Tyr}$. The C111U mutation alters G17:C111 of helix B to a G:U pair, which is predicted to destabilize helix B. This mutation resulted in a 6-fold drop in expression during growth in excess tyrosine, but induction in response to increased levels of uncharged $tRNA^{Tyr}$ was normal (17-fold; Rollins et al., 1997), indicating a much less severe defect than the alteration of the 5 + 2 structural arrangement. The A23G mutation, which targeted one of the conserved A residues in the internal loop, led to a 500-fold decrease in *tyrS-lacZ* expression during growth under inducing conditions, indicating that $tRNA^{Tyr}$-directed antitermination was completely blocked. A severe defect in antitermination was also observed when A109 was replaced with a C. The G108U mutation, which also targets one of the GA dinucleotides, significantly lowered *tyrS-lacZ* expression but still allowed 7.5-fold induction. These results are consistent with the predictions that the conserved GA residues, the 5 + 2 structural arrangement, and the integrity of helix B are required features of the *tyrS* GA motif, and demonstrate that this motif is functionally important for *tyrS* antitermination.

Phylogenetic analysis of the T box leader sequences demonstrated a strong preference (80%) for a U at position 21, with 14% A, and only 4% C and 2% G. To test whether this position is important for *tyrS-lacZ* expression, *tyrS* A21 was mutated to U, C, or G. The A21U and A21C mutations both conferred a small decrease in expression (Table 2), in contrast to the

TABLE 2. Effect of GA motif mutations on expression of *tyrS-lacZ* transcriptional fusions

	β-galactosidase activity[a]		
tyrS-lacZ fusion	Uninduced	Induced	Induction ratio[b]
Wild-type	15 ± 1.1	240 ± 11	16
ΩA20	0.65 ± 0.045	6.2 ± 0.59	9.5
C111U	2.3 ± 0.1	39 ± 2.0	17
A23G	0.25 ± 0.05	0.45 ± 0.04	1.8
A109C	0.13 ± 0.01	0.13 ± 0.01	1.0
G108U	0.59 ± 0.03	4.4 ± 0.2	7.5
A21U	8.3 ± 0.77	120 ± 6.0	14
A21C	7.2 ± 0.15	80 ± 4.2	11
A21G	0.41 ± 0.09	2.7 ± 0.58	6.6

[a]Fusions were integrated into the chromosome of strain K8Y, a tyrosine auxotroph, and cells were grown in the presence of excess tyrosine (uninduced) or limiting tyrosine (induced). Values are given in Miller units (Miller, 1972). All experiments were performed in duplicate at least three times.

[b]The induction ratio is the level of β-galactosidase activity obtained during growth under tyrosine-limiting conditions relative to the level of β-galactosidase activity during growth in excess tyrosine.

phylogenetic predictions. The A21G mutation, however, resulted in a severe defect in antitermination, with a 90-fold drop in the level of expression during growth under inducing conditions, indicating that nucleotide identity at this position is important for *tyrS* antitermination.

Site-directed mutagenesis of the yitJ GA motif

Point mutations were introduced into a *yitJ-lacZ* transcriptional fusion, containing the *yitJ* promoter, leader, and leader region terminator fused to a *lacZ* reporter fragment, to determine whether the GA motif is important for the S box regulatory system. Mutants were grown under conditions of excess or limiting methionine; expression of *yitJ*, which encodes an enzyme required for methionine biosynthesis, is repressed at the level of transcription termination during growth in the presence of methionine (Grundy & Henkin, 1998). The G50C mutation (Fig. 2D), which disrupts the C58:G50 pair (corresponding to R17:Y111 in the T box consensus), resulted in a 290-fold increase in *yitJ-lacZ* expression during growth in methionine, indicating that repression of *yitJ* expression had been severely compromised (Table 3). A similar phenotype was observed for the C58G mutant, in which the C58:G50 pair is changed to a G:G pair. A C58G/G50C double mutant was constructed to test whether restoration of pairing would restore regulation, despite the alteration in primary sequence. There was a significant increase in methionine repression in the double mutant as compared to either single mutant (Table 3), but expression during growth in methionine remained 21-fold higher than that of the wild-type fusion. These results indicate that the integrity of helix B is essential for *yitJ* regulation, and that the primary sequence in this helix is also important for function. The position in the S box leaders analogous to *tyrS* A21 (C62 in *yitJ*) exhibits a pattern of conservation similar to that of the T box leaders, although in this case, C is more highly represented (74% U, 14% C, 12% A, 0% G). The C62G mutation was introduced into the *yitJ* leader to test whether this allele, equivalent to the A21G mutation in *tyrS*, would have a similar deleterious effect. This mutation had no significant effect on *yitJ-lacZ* expression (Table 3), indicating that the specific requirements at this position differ for the S box and T box leaders, despite the similarities in phylogenetic conservation.

The point mutations introduced into *yitJ* and *tyrS* were not predicted to result in major alterations of the predicted secondary structures for the leader RNAs.

TABLE 3. Effect of GA motif mutations on expression of *yitJ-lacZ* transcriptional fusions

	β-galactosidase activity[a]		
yitJ-lacZ fusion	Uninduced	Induced	Induction ratio[b]
Wild-type	0.25 ± 0.02	310 ± 20	1200
G50C	72 ± 3.5	130 ± 11	1.8
C58G	90 ± 8.8	110 ± 10	1.2
G50C/C58G	5.3 ± 0.42	220 ± 21	42
C62G	0.65 ± 0.07	440 ± 42	680

[a]Fusions were integrated into the chromosome of strain BR151, a methionine auxotroph, and cells were grown in the presence of excess or limiting methionine. Values are given in Miller units (Miller, 1972). All experiments were performed in duplicate at least three times.

[b]The induction ratio is the level of β-galactosidase activity obtained during growth under methionine-limiting conditions relative to the level of β-galactosidase activity during growth in excess methionine.

Similarity of the T box and S box leader GA motifs to RNA elements bound by yeast proteins Snu13p and L30, and two elements in 23S rRNA

The removal of introns from nuclear pre-mRNA, catalyzed by the spliceosome, requires several ribonucleoprotein units (U1, U2, U5, and U4/U6 snRNPs) as well as many non-snRNP factors (Burge et al., 1999). An RNA element similar to the GA motif in U4 snRNA (Fig. 3A) has been shown to interact with human protein 15.5 kD and the yeast ortholog Snu13p (Nottrott et al., 1999; Watkins et al., 2000; Vidovic et al., 2000). The Snu13p and 15.5 kD proteins also interact with box C/D snoRNAs, which are important for eukaryal and archaeal rRNA modification and processing (Venema & Tollervey, 1999; Weinstein & Steitz, 1999; Gaspin et al., 2000), at an RNA element similar to the GA motif (Fig. 3B; Watkins et al., 2000).

The T box and S box leader GA motif consensus structures are also very similar to an RNA structure found in the 5′ region of yeast *RPL30* pre-mRNA and mRNA (Eng & Warner, 1991; Vilardell & Warner, 1994; Fig. 3C). Ribosomal protein L30 (formerly designated L32) binds to this RNA structure to negatively autoregulate both splicing and translation of the *RPL30* transcript (Dabeva & Warner, 1993; Li et al., 1996; Vilardell et al., 2000).

The T box, S box, *RPL30*, U4 snRNA, and box C/D snoRNA elements are similar in overall structure (two short helices separated by an asymmetric interior loop with a 5 + 2 arrangement), placement of the two GA dinucleotide sequences, location of a weak or mismatched pair (U60:G10 in L30 pre-mRNA; U24:U107 in *tyrS*), and conservation of a C:G pair at the internal loop-proximal end of helix B. RNAs with a terminal loop on either side of the GA motif could bind L30 in vitro (Li & White, 1997); this is consistent with the different arrangements of the T box and S box leader motifs. These observations suggest that the RNA elements found in *RPL30*, box C/D snoRNAs, and U4 snRNA comprise a common RNA secondary structural class. Our results

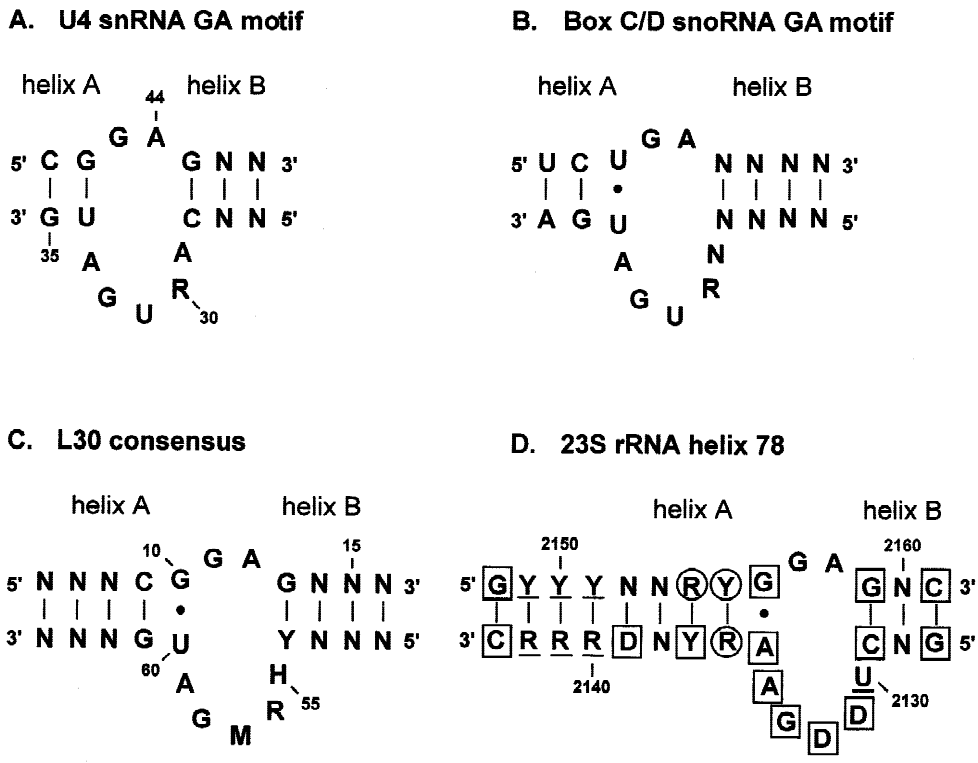

FIGURE 3. GA motifs in yeast U4 snRNAs, box C/D snoRNAs, *RPL30* RNA, and 23S rRNA. **A:** U4 snRNA GA motif (Nottrott et al., 1999). **B:** Box C/D snoRNA GA motif (Watkins et al., 2000). **C:** *RPL30* RNA elements required for binding of ribosomal protein L30, as demonstrated by in vitro selection studies (Li & White, 1997). **D:** 23S rRNA helix 78 consensus pattern, derived from 34 eubacterial sequences (http://www.rna.icmb.utexas.edu). **E:** 23S rRNA helix 7 consensus pattern, derived from 27 eubacterial sequences. Positions that are 90%, 80%, and 70% conserved are indicated by boxes, circles, or underlining, respectively. Asterisks indicate conserved GA dinucleotides. R = G, A; Y = C, U; D = G, A, U; H = A, C, U; M = A, C; N = any nucleotide.

indicate that this motif also extends into bacterial RNAs of unrelated function.

Because a number of RNA structural motifs found in diverse RNAs are also present in rRNA, we searched 16S and 23S rRNA sequences for elements resembling the GA motif. Two such elements were found, one in helix 78 of 23S domain V and the other in helix 7 of 23S domain I (Fig. 3D,E). Helix 7 of 23S rRNA domain I is more variable than the helix 78 region, and a number of bacterial 23S rRNAs contain an alternative RNA element in helix 7 (data not shown), suggesting that the GA motif in helix 7 can be substituted by a different RNA element. The role of the GA motif in 23S rRNA remains to be elucidated.

CONCLUSIONS

The importance of the GA motif in the T box and S box leaders is supported by both phylogenetic and mutational analyses. It has not yet been determined what, if any, protein binds to the T box and S box GA motifs. Yeast ribosomal protein L30 and the Snu13p/15 kD proteins are members of a family of RNA binding proteins (Koonin et al., 1994; Nottrott et al., 1999). There is no protein homologous to yeast L30 in eubacterial ribosomes, but two *B. subtilis* ORFs of unknown function, designated *ylxQ* and *ybxF*, encode proteins that are members of the family of RNA binding proteins that includes L30 and Snu13p (Koonin et al., 1994). The

binding of ribosomal protein L30 to its RNA target stabilizes a bend of 130 deg between helices A and B (Mao et al., 1999). A similar bend in the S box and T box leader RNAs could be important for folding of the RNA into an active conformation. Based on covariance, a point of tertiary interaction was proposed between residues in the terminal loop of helix B and in an unpaired region between helices 3 and 4 of S box leader sequences (Grundy & Henkin, 1998), and mutations that eliminate covariation resulted in loss of regulation (FJ Grundy & TM Henkin, unpubl.). It is therefore tempting to speculate that the GA motif (perhaps with a protein partner) assists in forming this tertiary interaction through the introduction of a bend between helices A and B. This step could be important for proper folding of the RNA, or as a precursor to protein interaction. The GA motif in T box leader sequences is immediately adjacent to the internal loop containing the specifier sequence, which interacts with the anticodon of the cognate tRNA (Grundy & Henkin, 1993; Grundy et al., 1994, 1997). It is possible that the T box GA motif may arrange structural elements to facilitate tRNA interaction with the specifier sequence. The GA motif therefore appears to be a structural unit found in a variety of contexts, in both eukaryal and eubacterial RNAs.

MATERIAL AND METHODS

Bacterial strains and growth conditions

Escherichia coli strain DH5α [ϕ80d*lacZ*ΔM15 *endA1 recA1 supE44 hsdR17* ($r_k^- m_k^+$) *thi-1 gyrA96 relA1* Δ(*lacZYA-argF*)U169; Bethesda Research Laboratories] was used for the propagation of plasmid DNA. *E. coli* strains were grown on LB medium (Miller, 1972). *Bacillus subtilis* strains TH217 (*tyrA1 trpC2 thr-5*) and BR151 (*lys-3 metB10 trpC2*) were grown on tryptose blood agar base medium (TBAB; Difco) or 2XYT broth. Chloramphenicol (5 μg/mL) was included for strains containing the SPβ prophage. Amino acid starvation experiments were conducted in Spizizen's minimal medium (Anagnostopoulos & Spizizen, 1961) with glucose as the carbon source and the appropriate amino acids (50 μg/mL for amino acid excess conditions; 5 μg/mL for amino acid limitation conditions).

Site-directed mutagenesis

The constructions of *tyrS-lacZ* and *yitJ-lacZ* transcriptional fusions in plasmid pFG328, and introduction of the fusions into the *B. subtilis* chromosome by recombination into specialized transducing phage SPβ, have been described previously (Grundy & Henkin, 1993, 1998); *tyrS-lacZ* fusions were tested in strain K8Y (tyrosine auxotroph) and *yitJ-lacZ* fusions were tested in strain BR151 (methionine auxotroph). All site-directed mutations were introduced into this plasmid using the QuikChange site-directed mutagenesis kit (Stratagene), or by PCR using oligonucleotides containing sequence alterations. Oligonucleotides were purchased from Integrated DNA Technologies. All mutations were verified by dideoxynucleotide sequencing using Sequenase DNA polymerase (US Biochemicals) on plasmid templates prepared on Wizard columns (Promega). Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs.

β-Galactosidase assays

Cells were grown to midexponential phase under amino acid excess conditions in Spizizen minimal medium. The cultures were split, and the cells were pelleted by centrifugation and resuspended in minimal medium containing excess or limiting amino acids. Cells were collected following 3.5 h of growth. β-galactosidase activity was assayed by using toluene permeabilization of the cells as described by Miller (1972). The β-galactosidase enzyme assays were terminated at varying times, depending on activity levels, up to a maximum of 24 h. A strain containing a promoterless copy of the *lacZ* gene integrated in single copy on the chromosome conferred a background level of activity of less than 0.1 Miller units.

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