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T box transcription antitermination riboswitch: Influence of nucleotide sequence and orientation on tRNA binding by the antiterminator element

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Abstract

Many bacteria utilize riboswitch transcription regulation to monitor and appropriately respond to cellular levels of important metabolites or effector molecules. The T box transcription antitermination riboswitch responds to cognate uncharged tRNA by specifically stabilizing an antiterminator element in the 5'-untranslated mRNA leader region and precluding formation of a thermodynamically more stable terminator element. Stabilization occurs when the tRNA acceptor end base pairs with the first four nucleotides in the seven nucleotide bulge of the highly conserved antiterminator element. The significance of the conservation of the antiterminator bulge nucleotides that do not base pair with the tRNA is unknown, but they are required for optimal function. In vitro selection was used to determine if the isolated antiterminator bulge context alone dictates the mode in which the tRNA acceptor end binds the bulge nucleotides. No sequence conservation beyond complementarity was observed and the location was not constrained to the first four bases of the bulge. The results indicate that formation of a structure that recognizes the tRNA acceptor end in isolation is not the determinant driving force for the high phylogenetic sequence conservation observed within the antiterminator bulge. Additional factors or T box leader features more likely influenced the phylogenetic sequence conservation.

Keywords

T box; transcription antitermination; in vitro selection; binding; riboswitch; RNA

INTRODUCTION

Riboswitch regulation of transcription is extensively employed by bacteria to monitor and appropriately respond to cellular levels of important metabolites or other effector molecules [1-3]. In T box riboswitch genes, the specific binding of uncharged cognate tRNA to the nascent 5'-untranslated leader mRNA transcript is essential for regulation of gene expression [4,5]. The T box genes are found in many Gram-positive bacteria and typically encode aminoacyl-tRNA

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synthetases and other amino acid related genes such as amino acid biosynthesis and transport genes [4-6]. Each example possesses a set of conserved mRNA primary sequence and structural elements located upstream of the translation start codon which play a vital role in the binding of tRNA to control transcription antitermination [7-9]. The leader region folds into a structure selectively recognizing a specific cognate tRNA through the formation of Watson-Crick base pairs between the anticodon of the tRNA and a trinucleotide sequence (termed the “Specifier Sequence”, Figure 1a) in the specifier loop [4,10,11]. Another important interaction is the base pairing between the uncharged acceptor end of the tRNA with four conserved nucleotides at the 5'-end of a bulge in the antiterminator element. The base pairing stabilizes the otherwise unstable antiterminator element preventing the formation of a more thermodynamically stable terminator element (Figure 1a inset, [7-9]). When the antiterminator is not stabilized, the terminator forms and transcription is terminated [7-9]. The T box mechanism can function in vitro in the absence of additional cofactors [12], but more structurally complex leader sequences require a partially purified protein fraction for reconstituted activity [13]. The protein is believed to stabilize the specifier domain containing the Specifier Sequence [13].

The 14 nucleotide highly conserved T box sequence forms the 5' side of the antiterminator element including the seven nucleotide bulge (e.g., 5'-UGGNACC-3') [6,7]. The first three nucleotides of the bulge are absolutely conserved and base pair with the 5'-CCA-3' acceptor end nucleotides of uncharged tRNA. The N at the variable base position of the bulge covaries with the discriminator base at position 73 of the tRNA. The other nucleotides in the bulge have been shown to have sequence conservation patterns ranging from absolute (e.g., A223 in *B. subtilis tyrS* leader corresponding to position 10 in antiterminator model AM1A, Figure 1b) to moderately conserved [6,7], but the actual roles they play to facilitate antitermination are not known.

Structural studies of antiterminator model RNA AM1A indicated that the nucleotides at the 3'-end of the bulge in the antiterminator may facilitate the pre-selection of a set of conformations at the 5'-end of the bulge for tRNA to sample during binding [14]. Mutation of the non-base pairing nucleotides at the 3'-end of the bulge reduces tRNA-mediated antitermination efficiency in vivo [7], tRNA binding in vitro [15] and alters the antiterminator structure in solution [14,15]. Previous studies with T box antiterminator model RNAs highlighted a propensity for the bulge of the antiterminator to bind RNA other than the tRNA acceptor end. One model of the *B. subtilis tyrS* antiterminator readily formed homo-dimer kissing bulges [15] while in vitro selection of antiterminator binding tRNA containing randomized loops yielded tRNA loop-antiterminator RNA bulge complexes [16]. While some of the highly conserved antiterminator sequence in helix A1 has been found to facilitate Mg²⁺ binding [17], the reason for the high sequence conservation of the bulge nucleotides which do not appear to base pair with tRNA is not known. Possible reasons include formation of a functional receptor for tRNA to bind via tertiary-structure capture or for additional stabilization of the resulting complex. In addition, the absolute localization of the tRNA binding site to the first four nucleotides of the bulge could be dictated by the overall context of the leader region or by a more localized, isolated positioning requirement for optimally base pairing the four acceptor end nucleotides with the bulge nucleotides.

Since isolated antiterminator model RNA selected for the tertiary fold of tRNA from a pool of loop-randomized tRNA [16], the distinct possibility exists that there are fundamental tRNA-antiterminator RNA interactions which dictated the observed phylogenetic sequence conservation of the antiterminator. In this study, in vitro selection [18] was utilized to test the hypothesis that there are fundamental sequence and orientation requirements within the bulge nucleotides of antiterminator RNA that are critical for tRNA acceptor end binding. This information is essential for determining if the interaction of the tRNA acceptor end with the bulge nucleotides (in the absence of the leader context) accounts for the observed, non-base

pairing, phylogenetic sequence conservation. While crystal structures have been determined for riboswitches which respond to small molecule metabolites [19-25], no such global structural detail exists for the tRNA sensing T box riboswitch. Determining whether or not the antiterminator bulge context alone dictates the mode in which the tRNA acceptor end binds the bulge nucleotides will contribute to elucidating a detailed mechanistic understanding of this biologically significant riboswitch.

MATERIALS & METHODS

Oligonucleotides

Synthetic single-stranded DNAs were purchased from IDT, Inc. Antiterminator RNAs were prepared via in vitro transcription using T7 RNA polymerase [26]. In the case of tRNA-UCCA (Figure 1c, [15]), a commercial T7 RNA polymerase was used (Ampliscribe, Epicentre Technology). All nucleic acids were purified on a 20% denaturing polyacrylamide gel (acrylamide/bisacrylamide 19:1). Purified aminopurine RNAs (Figure 1d) were obtained from Dharmacon Research Inc. All RNAs were dialyzed into 10 mM sodium phosphate buffer, pH 6.5, 0.01 mM EDTA and renatured by heating to 90 °C then cooling to room temperature prior to use.

Pool preparation

The pool of antiterminator sequences was derived from the 29 nt antiterminator model AM1A that was based on the *B. subtilis* *tyrS* leader antiterminator [15]. The AM1A antiterminator model RNA is fully functional in vivo [7]. The bulge nucleotides were randomized and 9 extra bases were added at the 5'-end for optimal reverse transcription during the selection cycles (Figure 1e). The resulting DNA pool sequence was 5'-GGT ATT AAG GAG GGN NNN NNN GCG CTT CGG CGT CCC TC-3'. The 5' and 3' primers used for the amplification of the pool were 5'-TAA TAC GAC TCA CTA TAG GTA TTA AGG AGG G-3' and 5'-GAG GGA CGC CGA AGC GC-3', respectively, with the T7 promoter sequence underlined. The initial DNA pool was generated from a synthetic ssDNA template by elongation of the primer complementary to the 5' fixed region with the Klenow fragment of DNA polymerase I (Roche) [27] and purified by non-denaturing polyacrylamide gel (acrylamide/bisacrylamide 29:1). Based on the starting amount of purified DNA, the complexity of the initial randomized pool of antiterminator was approximately 9×10^{14} molecules.

In vitro selection

Functional antiterminator sequences were selected for binding tRNA using the criterion of a gel mobility shift (Figure 2a, [15]). The initial pool and the first five cycles of randomized RNAs was 5'-end labeled with $\gamma^{32}\text{P}$ -ATP using KinaseMax (Ambion) according to the manufacturer's protocol. The labeled RNA was gel purified and ethanol precipitated. The binding reaction mixtures (10 μL) contained labeled antiterminator (100 – 200 pmol) and tRNA-UCCA. The concentration of tRNA-UCCA was reduced for each cycle from 200 μM in the first cycle to 30 μM in the final cycle. The antiterminator RNAs and tRNA-UCCA were renatured separately prior to mixing. Reaction mixtures were incubated at 4 °C for 30 - 40 min before loading on the gel. The samples were run on 20% TBE polyacrylamide gels (Invitrogen). The running buffer was 0.5 $\times$ TBE, 50 mM NaCl, 5 mM MgCl_2 (where 1 $\times$ TBE is 50 mM Tris-borate pH 8.3 and 1 mM EDTA). The binding buffer contained running buffer with 10% glycerol. The gels were run at 100 V for 4-5 h at 4 °C. Bands were visualized via autoradiography and bands corresponding to the tRNA-antiterminator RNA complex were excised from the gel. For cycles six to eight where no radioactivity was used, the location of the band to excise was determined based on a labeled reference sample. The RNA complex was eluted and ethanol precipitated with 20 μg of glycogen as a carrier.

Amplified DNA was prepared from the recovered RNAs using reverse transcription followed by the polymerase chain reaction (RT-PCR). RNAs were reverse-transcribed in a 20 μ L reaction mixture containing 50 mM Tris (pH 8.3), 50 mM KCl, 5 mM MgCl₂, 5 mM DTT, 2.5 μ M 3' primer, 0.5 mM dNTP mix and 10 U reverse transcriptase (Promega). The dNTP mix and enzyme were added after an annealing step (2 min at 90 °C followed by incubation at room temperature for 10 min). Reverse transcription was performed at 42 °C for 1 h. For amplification by polymerase chain reaction (PCR) *Ex Taq* DNA Polymerase (Takara) was used. A 20 μ L cDNA reaction mixture (after reverse-transcription) was diluted in 80 μ L of a mixture of PCR supplied buffer, 2.5 unit of *Ex Taq* DNA polymerase (Takara) and 0.4 μ M each of 5' and 3' primers. The reaction mixture was cycled at 94 °C for 1.15 min, 50 °C for 1.15 min and 72 °C for 1.15 min for 8-10 cycles. The PCR product was visualized using ethidium bromide on 3% agarose gels. The PCR product was ethanol precipitated followed by in vitro transcription using T7 Ampliscribe FLASH (Epicentre Technologies) at 37 °C for 3 h. To remove remaining PCR product, the mixture was incubated with 1 μ L DNase at 37 °C for 30 min. The RNA was gel purified, extracted by crush and soak elution, ethanol precipitated and used for the next cycle of selection and amplification.

Cloning and sequencing

After six to eight identical cycles of transcription and RT-PCR, the DNA was directly cloned using the TOPO TA Cloning Kit (Invitrogen). DNA was isolated and purified from individual colonies using QIAprep Spin Miniprep Kit (Qiagen). Plasmids were sequenced at the Plan-Microbe Genomics Facility of The Ohio State University.

Fluorescence anisotropy

Fluorescence anisotropy studies were carried out using a JY Horiba SPEX Fluoromax-3 equipped with a temperature-controlled sample holder. A microhelix tRNA acceptor end analog previously shown to bind AM1A in a functionally relevant manner [15,28] was fluorescently labeled by substituting adenine with 2-aminopurine at the third nucleotide to obtain 3-AP-mh-UCCA and 3-AP-mh-ACCA. Fluorescence anisotropy measurements were obtained by titrating antiterminator RNAs into a 400 μ L solution of labeled microhelix, 50 mM sodium phosphate pH 6.5, 0.01 mM EDTA, 50 mM NaCl and 15 mM MgCl₂, at 4 °C with an equilibration time of 5 min between reads. After each 2 μ L antiterminator addition, the mixture was gently stirred to maintain a homogeneous solution. The fluorescently labeled microhelix was excited at 310 nm and the anisotropy measured at 370 nm. The K_d values were determined by analyzing an average of triplicate anisotropy binding isotherms using Prism (GraphPad) (Supplementary Material). An adjusted single-site binding equation was used to accommodate the non-zero initial value for the anisotropy, $y = [(B_{\max} - B_0) * X / (K_d + X)] + B_0$ where B_0 is the initial anisotropy of 3-AP-mhUCCA with no antiterminator added and $B_{\max}$ is the maximal anisotropy value. $B_{\max}$, B_0 and K_d were fit simultaneously. In each case where binding was observed, the data best fit single-site binding compared to a linear fit. No binding was observed in control experiments consisting of addition of antiterminator RNA to 2-aminopurine ribonucleoside (Berry and Associates) or addition of buffer alone to labeled microhelix (data not shown).

Enzymatic probing of antiterminator RNAs

RNAs were 5'-end-labeled as described above. Following ethanol precipitation the dried pellets were reconstituted in 10 mM sodium phosphate buffer, pH 6.5 and renatured. The probing reaction mixture (10 μ L) contained 1.0 μ L of 10 $\times$ RNA structure buffer (where 1 $\times$ is 10 mM Tris pH 7.0, 0.1 M KCl, 10 mM MgCl₂), 10 mM sodium phosphate buffer, pH 6.5, 1.0 μ L of 3.0 μ g/ml yeast RNA, and 1.0 μ L of the T1 or V1 enzyme solution (Ambion) with the control reactions containing no enzymes. The reactions were incubated at room temperature

for 15 min and then stopped by adding an equal volume of $2 \times$ gel loading buffer without dye. The samples were heated to 90 °C for 1.5 min and loaded on 20% polyacrylamide gel. Gels were run at 1800 V and 30 W for 3.5 h in $1 \times$ TBE, visualized via autoradiography and quantified using Nucleo Vision (NucleoTech).

RESULTS

Design and optimization of the antiterminator library

The in vitro selection library was designed based on the in vivo functional T box antiterminator model RNA AM1A (Figure 1b, [7, 15]) where the seven bases that form the bulge were randomized. For optimal results during the RT-PCR process, nine additional nucleotides (5'-GGUAUUAAG-3') were introduced at the 5'-end of the antiterminator RNA (Figure 1e). RNA sequences were selected by gel mobility shift (Figure 2a) using tRNA-UCCA (Figure 1c). This tRNA is the equivalent of *B. subtilis* tRNA^{Tyr}(A73U) and binds antiterminator model RNA in a functionally relevant manner [15, 28]. The selection technique and antiterminator model RNA were specifically chosen to remove the influence of long-range interactions with the leader region in order to definitively investigate whether or not there are fundamental, localized RNA-RNA recognition features between tRNA acceptor-end nucleotides and bulge nucleotides that facilitate binding. The larger context of an RNA motif can often significantly affect its function [29, 30]. These studies were specifically designed to remove these potential influences to see if the phylogenetic sequence conservation was dictated by localized, fundamental RNA-RNA interactions.

In vitro selection of antiterminator RNA

In the first five rounds of in vitro selection with the target tRNA-UCCA the percentage of the tRNA•antiterminator binding increased from 4% to 22% (data not shown). Subsequent cycles were run without 5'-end labeling and the PCR product obtained after cycles 6-8 was directly cloned and sequenced. After the 6th cycle, seven clones were selected for sequencing (data not shown). Four had a consensus sequence of 5'-AGGU(A)-3', two had complementarity to the T- or anticodon-loops of tRNA-UCCA (i.e., kissing bulge-loop complementarity) and only one (14%) had the consensus sequence of 5'-UGG(G)-3' observed in later cycles. After the 8th cycle, 20 clones were sequenced (Figure 2b) and 55% of the selected sequences had the consensus sequence 5'-UGG(G)-3' (Group I) that is fully complementary to the tRNA acceptor end. Significantly, none of the sequences from the 8th cycle were complementary to any of the tRNA loops. Within Group I, S2, S3 and S4 had the consensus sequence located at the 3'-end of the bulge; S2 was chosen for further study. S7, with the full consensus sequence located in the middle of the bulge, was also investigated further. S10 only had three of the consensus sequence nucleotides and therefore was not studied further. Both sequences from Group II (S12 and S13) were also investigated further.

In the presence of tRNA, a gel mobility shift was observed for sequences investigated in Group I (e.g., S7 and S2, Supplementary Material). In order to directly compare the in vitro selected bulge sequences with the functionally relevant model RNA AM1A, the nine base extension required in the selection experiments was deleted (designated by "Δ") to return to the model antiterminator size of 29 nucleotides (Figure 3). A concentration dependent shift in the native gel migration of the antiterminator-tRNA complex was observed for some sequences (e.g., ΔS7, Supplementary Material), thus prohibiting an accurate assessment of the binding constant using gel mobility shift assays. A similar effect was observed in the gel mobility shift of AM1A with model tRNA [15]. Consequently, a solution-based fluorescence assay was used to assess the relative affinities (see below).

Selected antiterminator secondary structures

Enzymatic probing with RNases T1 and V1 was utilized to compare the secondary structural features of the selected antiterminator RNAs investigated to the functional model AM1A in isolation. Based on the probing data, all the sequences investigated folded into the predicted [31] bulge secondary structure analogous to AM1A except for $\Delta S13$ (Supplementary material). For $\Delta S13$, enzymatic probing confirmed that the most stable secondary structure was a hairpin with no bulge. In addition, a qualitative comparison of the enzymatic probing data indicated that the four nucleotides complementary to the tRNA acceptor end in $\Delta S7$ (U8G9G10G11) were more single stranded in nature (less stacked) than those of $\Delta S2$ (U10G11G12G13) consistent with the observed enhanced model tRNA affinity of $\Delta S7$ compared to $\Delta S2$ (see below). Flexibility in the antiterminator region that ultimately base pairs with the tRNA acceptor end nucleotides has been proposed to facilitate binding [14].

Model tRNA affinity

A fluorescence-based solution assay was used to quantify relative tRNA affinity. The tRNA acceptor stem microhelix model mh-UCCA [15] with a 2-aminopurine fluorescent base analog [32-35], 3-(2-aminopurine)-mh-UCCA (3-AP-mh-UCCA), was used to determine the model tRNA affinity by monitoring the change in fluorescence anisotropy [36] upon formation of the antiterminator•tRNA complex. The mh-UCCA model tRNA binds antiterminator model RNA in a functionally relevant manner, but with a lower affinity than the full tRNA [28]. The K_d values are summarized in Table 1.

The highest affinity observed was between the functionally relevant AM1A and the fully complementary 3-AP-mh-UCCA. The 3-AP-mh-ACCA (containing a mismatched discriminator base) did not bind AM1A indicating that the aminopurine labeled microhelix binds antiterminator model RNA in a functionally relevant manner. Similar specificity was observed previously in gel mobility shift experiments with microhelix model tRNA [15].

For Group I sequences, the enhanced affinity of $\Delta S7$ compared to $\Delta S2$ is consistent with greater accessibility of the complementary nucleotides. $\Delta S2$ and $\Delta S7$ both have a four-nucleotide anti-parallel base pair complementarity with the acceptor end of the tRNA (including a U•G base pair with the tRNA discriminator base). For $\Delta S2$ to fully bind the model tRNA acceptor end, however, the antiterminator G13•U24 base pair would have to be disrupted. In the case of $\Delta S7$, the location of the four complementary nucleotides in the center of the bulge leads to no competing base pair within the antiterminator that would interfere with tRNA base pairing.

For Group II sequences, the lack of binding observed with $\Delta S13$ is consistent with its stem-loop rather than bulge secondary structure. The preferential binding of $\Delta S12$ with 3-AP-mh-ACCA instead of with 3-AP-mh-UCCA is likely due to anti-parallel binding of the first three bases of the tRNA model acceptor end (5'-ACC-3') to the complementary sequence in the bulge (5'-G8G9U10-3'). In comparison, 3-AP-mh-UCCA has anti-parallel complementarity to only two nucleotides in the bulge of $\Delta S12$ (5'-G8G9-3'). It is intriguing that the consensus sequence (5'-AGGU-3') of the Group II selected sequences is for parallel complementarity to the 5'-UCCA-3' tRNA acceptor end sequence. While parallel base pairing has been observed in RNA and DNA, the stability of the resulting duplex is significantly reduced compared to anti-parallel base pairing [37-40]. The lack of observed binding between $\Delta S12$ and 3-AP-mh-UCCA indicates that parallel binding is not occurring to any significant extent in the fluorescence anisotropy monitored model system.

DISCUSSION

In this study, in vitro selection [18] of randomized T box antiterminator RNA was used to determine structural recognition features governing the binding of tRNA by the antiterminator bulge nucleotides in the absence of in vivo phylogenetic selection pressures. Most examples of in vitro selection of randomized bulges have involved small bulges such as randomization of the bulge in HIV-1 TAR RNA or the isolation of a bulge-containing aptamer to a protein or small molecule ligand from a large randomized RNA [41,42]. Few studies have been reported that investigate the selection of RNA sequences that bind tRNA or that involve randomization of larger bulges. Of the tRNA aptamers that have been identified only kissing loop-loop interactions have been found [43]. Significantly, none of the final sequences from the selection of randomized antiterminator RNAs were complementary to any of the tRNA loops indicating that constraining the randomized nucleotides to a bulge location (rather than a loop) appears to favor specific tRNA acceptor end base pairing.

The fact that $\Delta S7$ bound the microhelix model tRNA with only 2-4 fold weaker affinity than AM1A indicates that tRNA binding alone does not require that the complementarity be constrained to the first four nucleotides of the bulge. Consequently, the absolute conservation of the tRNA acceptor end binding site to the first four nucleotides in the 5'-end of the antiterminator bulge in known T box systems [6,7,44] is not due to constraints imposed at the fundamental RNA-RNA level of recognition between the tRNA acceptor end and bulge nucleotides. Instead, the conserved binding site location is likely due to orientation constraints imposed by the larger context (or additional cofactors) of the RNA leader region. This interpretation is consistent with in vivo transcription antitermination studies of tRNA with extended acceptor stems which showed the importance of correct presentation of the acceptor end nucleotides in the context of tRNA bound to the full leader [45].

The lack of observed 3-AP-mh-UCCA binding with $\Delta S2$ indicates that having the complementary sequence completely within the context of the single-stranded bulge nucleotides is critical for efficient tRNA acceptor end binding. As indicated previously, the microhelix model tRNA is functionally relevant, but has an overall lower affinity than tRNA for antiterminator RNA binding. This reduced affinity has been proposed to be due to tRNA more optimally presenting the acceptor end nucleotides to the antiterminator receptor for binding [28]. Since $\Delta S2$ bound tRNA-UCCA in the gel mobility shift experiment (Supplementary Material), but not in the 3-AP-mh-UCCA binding assay, the optimal presentation of the acceptor end nucleotides must be important for competing with the G13•U24 base pair in $\Delta S2$ to form a base pair with the discriminator base in the microhelix model tRNA.

In vitro selection was used to determine if there are sequence and orientation requirements within the bulge nucleotides of antiterminator RNA that are critical for tRNA acceptor end binding. The localized context of bulge nucleotides presented selective pressure for specifically binding the tRNA acceptor end rather than a tRNA loop. More significantly, while anti-parallel base pair accessibility was important, no trend was observed for the complementary sequence existing only at the 5'-end of the bulge, nor any absolute conservation of the A corresponding to A10 in AM1A (position A223 in *B. subtilis tyrS* leader RNA). These two features are absolutely conserved in vivo [6,7]. While additional factors may play a role in optimal antitermination in vivo, the results indicate that the high phylogenetic sequence conservation in the bulge of the T box antiterminator element is not dependent on any key structural constraints imposed by the fundamental RNA-RNA interaction of the tRNA acceptor end base pairing with the antiterminator bulge in isolation but rather is more likely due to constraints imposed by the full context of the leader-tRNA interaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

T box riboswitch and model RNAs. a) Schematic of T box untranscribed leader mRNA recognition of cognate uncharged tRNA [4,5]. The tRNA binds the leader region via base pairing of the anticodon with the Specifier Sequence and base pairing of the uncharged tRNA acceptor end nucleotides with the first 4 nucleotides in the antiterminator bulge. In the absence of tRNA binding, as transcription continues, a more stable stem-loop terminator element is formed and transcription is terminated (inset). The 5' side of the terminator stem is comprised of nucleotides at the 3' end of the antiterminator making the two elements mutually exclusive (bold line). b) T box antiterminator model RNA AM1A (positions that base pair with tRNA shown in grey); positions 2-15 of AM1A correspond to the highly conserved 14 nucleotide T box sequence, c) tRNA-UCCA, d) 3-AP-mh-UCCA model tRNA (discriminator base change corresponding to the single mismatch model 3-AP-mh-ACCA indicated by an arrow), e) randomized antiterminator RNA used for selection.

Figure 2.

In vitro selection and selected sequences a) In vitro selection strategy, b) Cloned in vitro selected sequences from cycle 8 shown 5'-3'. Randomized (or original for AM1A) bulge nucleotide positions shown in black, non-randomized helical and 5'-extension (see text) regions shown in gray. Consensus sequence alignments are highlighted in green with number of duplicate sequences indicated in parentheses.

Figure 3.

Selected sequences assayed for model tRNA binding. Nucleotides with anti-parallel complementarity to tRNA acceptor end shown in grey. Sequences correspond to those in Figure 2, but with the nine-nucleotide primer site sequence used for the in vitro selection deleted (designated by “Δ”, see text).

Table 1

K_d values (μM) of selected antiterminator RNAs binding microhelix RNA^a

RNA	3-AP-mh-UCCA	3-AP-mh-ACCA
AM1A	66 ± 10	-- ^b
ΔS2	-- ^b	-- ^b
ΔS7	282 ± 142	-- ^b
ΔS12	-- ^b	126 ± 12
ΔS13	-- ^b	-- ^b

^a All $R^2 > 0.9$

^b $R^2 \ll 0.9$ and/or error $> K_d$. No single-site binding or $K_d \gg 150 \mu\text{M}$