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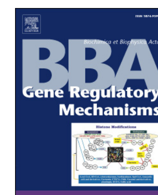

Review

The T box riboswitch: A novel regulatory RNA that utilizes tRNA as its ligand☆

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ABSTRACT

The T box riboswitch is a *cis*-acting regulatory RNA that controls expression of amino acid-related genes in response to the aminoacylation state of a specific tRNA. Multiple genes in the same organism can utilize this mechanism, with each gene responding independently to its cognate tRNA. The uncharged tRNA interacts directly with the regulatory RNA element, and this interaction promotes readthrough of an intrinsic transcriptional termination site upstream of the regulated coding sequence. A second class of T box elements uses a similar tRNA-dependent response to regulate translation initiation. This review will describe the current state of our knowledge about this regulatory system. This article is part of a Special Issue entitled: Riboswitches.

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1. Introduction

The T box regulatory system was one of the first regulatory mechanisms identified on the basis of the presence of conserved elements in the leader region of a gene or operon, between the promoter and the start of the first regulated coding sequence. Comparative sequence analysis revealed a complex set of conserved features, including the presence of a triplet sequence that was postulated to specify binding of the cognate tRNA [1]. The demonstration that a specific uncharged tRNA stimulates antitermination, that the tRNA acts in the absence of translation *in vivo* and *in vitro*, and that no other cellular factors are required for the tRNA–leader RNA interaction [1–4], indicated that this system can be considered a member of the riboswitch family, albeit one that utilizes a ligand other than a cellular metabolite [5]. This review will describe the identification and characterization of this unique class of regulatory elements, and will discuss the interesting variations on a theme that are emerging.

2. Identification of the T box system

The T box system was first identified by characterization of a single gene, the *Bacillus subtilis tyrS* gene, which encodes tyrosyl-tRNA synthetase (TyrRS) [6]. The levels of TyrRS activity in *B. subtilis* had been shown to increase when cells were grown under tyrosine depletion conditions [7], and this effect was shown to occur at the level of readthrough of an intrinsic transcriptional terminator located in the *tyrS* leader region [6]. Conservation of the leader region terminator in multiple *tyrS* genes in *Bacillus* species, coupled with the identification of a short conserved sequence upstream of the terminator, designated the T box sequence, suggested a conserved regulatory mechanism. Mutation of the conserved sequence resulted in loss of readthrough of the terminator, indicating an important role for this sequence [6].

Identification of the T box sequence in additional amino acid-related genes in *B. subtilis*, including multiple aminoacyl-tRNA synthetase genes and the *ilv-leu* branched chain amino acid biosynthesis operon, suggested that this regulatory mechanism extends beyond *tyrS* genes [6]. This was confirmed when a complex pattern of conserved primary sequence elements (in addition to the T box sequence) and structural elements (in addition to the intrinsic terminator) was identified in the leader regions of all of these genes (Fig. 1) [1,8].

Since it was likely that genes of different amino acid classes responded specifically to limitation for the cognate amino acid, the conservation of this overall pattern led to the question of how individual genes in this group could respond individually to the availability of the appropriate amino acid. This was resolved by identification of a triplet sequence embedded within the structural pattern that corresponded to a codon matching the amino acid specificity of the downstream gene [1]. Mutation of this triplet, termed the “Specifier Sequence,” in *tyrS* from a UAC tyrosine codon to a UUC phenylalanine codon was sufficient to block induction of the expression of a *tyrS-lacZ* reporter gene in response

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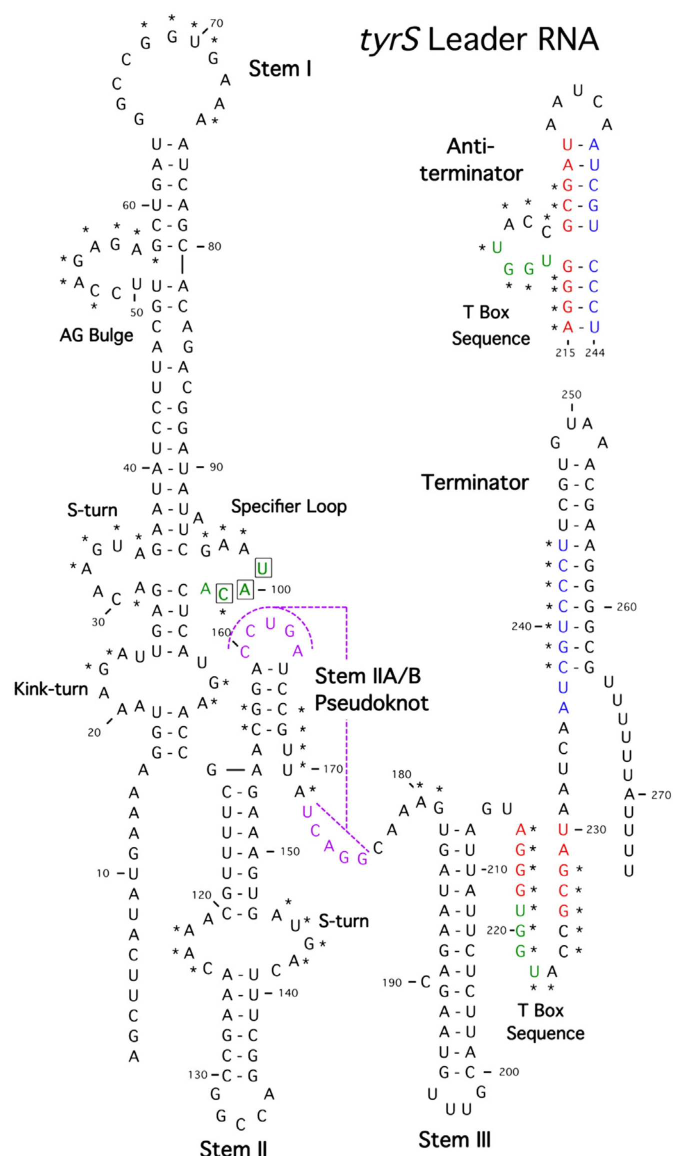

Fig. 1. Structural model of the *B. subtilis tyrS* leader RNA. The sequence is shown from the transcription initiation site through the termination site. The terminator form is shown, with the antiterminator form above the terminator helix. Structural domains and conserved sequence elements are labeled. Highly conserved residues are marked with asterisks. The pseudoknot pairing is shown in purple. Sequences on the 5′ side of the terminator (blue) pair with a portion of the T box sequence (red) to form the antiterminator. The residues that interact with $tRNA^{Tyr}$ (UAC Specifier Sequence and UGGU in antiterminator bulge) are shown in green. Modified from [5].

to tyrosine limitation, and to promote induction in response to limitation for phenylalanine.

The positive role of tRNA in this response was demonstrated by introduction of nonsense mutations into the position of the Specifier Sequence, which resulted in loss of expression (because a normal cell lacks tRNAs with the matching anticodon); suppression of the nonsense mutations by the corresponding nonsense suppressor tRNAs provided positive proof that tRNA is the effector [1,2]. tRNA mutants that are unchargeable *in vivo* conferred expression during growth in a rich medium, which demonstrated that uncharged tRNA is the key effector.

The basic properties of the *B. subtilis tyrS* regulatory system were shown to apply to a number of other genes in this family, including the *B. subtilis thrS*, *thrZ*, *ilv-leu* and *valS* genes [9–11], as well as genes from related Gram-positive organisms [12–15]. Each gene that was analyzed was shown to be induced in response to limitation for the cognate amino acid, and in the case of the *B. subtilis ilv-leu* operon, a mutation predicted to result in reduced aminoacylation *in vivo* resulted in increased expression in the absence of amino acid limitation [16], adding further support to the model that uncharged tRNA is the signal to which the system responds.

3. Structural features of T box leader RNAs

Leader RNAs in the T box family were identified initially by simple searches for conservation of the highly conserved T box element [1,2], and subsequently by bioinformatic analyses that relied on a combination of conserved features [17–19]. These studies revealed that the majority of T box family RNAs contained a full complement of the conserved features that were initially described [1], as shown in Fig. 1. These include a complex Stem I structure with a predicted kink-turn motif at the base, an S turn in the Specifier Loop region, an extended domain above the Specifier Loop that included conserved elements in the terminal loop and AG bulge [20,21]. Stem I is immediately followed by a second helix that most commonly contains an internal bulge with another predicted S turn motif, followed by a complex Stem IIA/B pseudoknot element [21]. A linker region is then followed by another helix (Stem III) that precedes the competing antiterminator and terminator elements. Mutational analysis revealed the importance of these conserved features, as even single nucleotide substitutions of conserved elements were often sufficient to disrupt tRNA-dependent antitermination *in vivo* [20–22].

Analysis of additional genomic sequences revealed subsets in which individual features exhibited specific patterns of variability; in many cases, the variants correlate with amino acid class, suggesting a basis either in evolutionary history or in tRNA recognition specificity [19]. These variants include the absence of the Stem II and Stem IIA/B pseudoknot domains (glycyl genes and some alanyl genes), absence of the S turn element in the Specifier Loop (threonyl genes), and absence of the region above the Specifier Loop (some isoleucyl genes). In each case, examples of these variant classes have been shown to be functional *in vivo* or *in vitro* (see below; [3, 23, Sherwood, Grundy and Henkin, unpublished; Liu, Grundy and Henkin, unpublished]). The ability of natural variants to function in the absence of elements that are highly sensitive to mutation in T box RNAs that contain these elements indicates that these variants must have evolved to compensate for the absence of these elements, through other structural changes or through other interactions with their cognate tRNAs.

4. tRNA features required for recognition *in vivo*

Initial studies of tRNA requirements for antitermination *in vivo* focused on expression of Specifier Sequence mutants of T box leader RNAs in response to limitation for the amino acid specified by the codon that was introduced. As described above, the initial switch of the UAC tyrosine codon in the *B. subtilis tyrS* gene to a UUC phenylalanine codon resulted in induction in response to limitation for phenylalanine [1]; however, the level of expression was much lower than that observed for the wild-type construct in response to limitation for tyrosine. This pattern was observed for a number of Specifier Sequence mutations, even in conjunction with mutations in the antiterminator that allowed pairing with the tRNA discriminator base (the residue upstream of the terminal CCA) [24]. Similar results were observed for other T box family genes [25]. These results suggested that tRNA features in addition to the anticodon and discriminator base are important for proper recognition of the tRNA. A detailed mutational analysis further supported the importance of the entire tRNA structure [26]; however, this study was limited by the requirement for *in vivo* expression of tRNA variants.

5. Biochemical analyses of the T box riboswitch

While *in vivo* studies could show that the cognate uncharged tRNA is necessary for antitermination, demonstration that tRNA is sufficient to induce antitermination required *in vitro* analyses with

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