Supporting Information

Saad et al. 10.1073/pnas.1304307110

SI Materials and Methods

Materials. All primers were from Sigma. All enzymes were purchased from Fermentas, with the exception of the restriction enzymes (New England Biolabs) and T7 RNA polymerase that was prepared as previously described (1). *Clostridium acetobutylicum* (*Cac*) genomic DNA from American Type Culture Collection and the pLacZFT plasmid were gifts from Peter Dürre (Ülm University, Ülm, Germany). The analytical size-exclusion chromatography column was from Amersham. *Escherichia coli* RNAP was from USB Chemicals. *Escherichia coli* M5154 *lacZ* mutant strain was from *E. coli* Genetic Stock Center (Yale University). The preparation of plasmid DNA was done using the GenElute HP plasmid Maxiprep Kit from Sigma-Aldrich. RNA elution was done using CHROMA SPIN-30 columns (Clontech Laboratories.). The [γ-^{32}P]ATP was purchased from Hartmann Analytic.

Chemical Modification of WT and n−2 Mutant NT-Boxes. The in vitro transcribed NT-box and $tRNA^{Asn}$ were denatured separately at 60 °C for 10 min in the presence of 20 mM magnesium acetate and 50 mM potassium acetate, followed by slow cooling to room temperature. A total of 15 pmoles of NT-box were mixed with an excess of $tRNA^{Asn}$ (75, 150, 225 pmoles), and the mixed reactions were incubated for 20 min at room temperature in the presence of the modification buffer [140 mM Hepes-KOH, pH 7.8, 20 mM $Mg(OAc)_2$, 540 mM KOAc]. After addition of 1 mM DTT, the RNA samples were modified by DMS (1:5 dilution), kethoxal (KE), or carbodiimidemetho-*p*-toluenesulfonate (CMCT) at 37 °C for 8 min for DMS and at 30 °C for KE and CMCT, for 15 and 30 min, respectively. The reactions were stopped by adding 0.25 M β-mercaptoethanol, 3 M sodium acetate, pH 6.0, in the presence of 0.5 M boric acid and 0.3 M sodium acetate, pH 6.0, respectively. Modified RNAs were precipitated with ethanol and resuspended in 1× Tris–EDTA buffer (10 mM Tris-acetate, pH 7.5, 0.1 mM EDTA). In the case of samples modified with KE, 50 mM boric acid was added in the Tris–EDTA buffer at a final concentration of 1 pmol/μL.

β-Gal Activity Test. To assay the tRNA-dependent antitermination in vivo, two different *E. coli* strains were used: the M5154 *lacZ* strain [F^- *ΔlacZ39*, λ^-, *trpA49*(Am), *recA11*, *relA1*, *rpsL150* (strR), *spoT1*] and the BL21-AI [F^- *ompT* $hsdS_B(r_B^-\ m_B^-)$ *gal dcm araB::T7RNAP-tetA*] that contains a plasmid encoding the T7 RNAP. The NT-box mutants tested in the *ΔlacZ39* strain. For this reason, the strain was cotransformed with the placZFT–$NT\text{-}box^{WT}$ or the placZFT–$NT\text{-}box^{mutant(s)}$ constructs and the pKK223-3 plasmid containing the *Cac* $tRNA^{Asn}$ gene. Five different tRNAs, controlled by a T7 promoter, have been individually cloned into the BL21 ai strain: *Cac* $tRNA^{Asn}$, *Cac* $tRNA^{Asp}$, *Cac* $tRNA^{Ser}$, *Saccharomyces cerevisiae* (*Sce*) $tRNA^{Asp(C36U)}$, and $tRNA^{Glu}$. For construct selection, the strains were grown on agar plates supplemented with clarithromycin (5 μg/mL), ampicillin (100 μg/mL), streptomycin (100 μg/mL), and tetracycline (20 μg/mL) when necessary. The growth conditions used for β-gal measurements were as described previously (2, 3), using the spectrofluorometer from Glomax Multi Detection System.

More precisely, all cultures were grown in minimal M9 medium at 37 °C. After 4 h of growth, cultures were taken for cell-density (absorbance at 595 nm) and β-gal activity measurements. All measurements were carried out in triplicate, and all experiments were performed in duplicate. Absorbance at 560 nm was measured to remove any false-positive signals from cell debris pellet. In the BL21 ai strains, the tRNA expressions were induced with IPTG, and a negative control transformant has been used to eliminate the endogenous β-gal activity and the background antitermination of the NT-box by the endogenous $tRNA^{Asn}$ of *E. coli*. This strain was transformed only with the $NT\text{-}box^{WT}$–placZFT plasmid. The endogenous specific activity was subtracted from all the specific activities obtained with WT NT-box assayed with various tRNA.

1. Becker HD, Giegé R, Kern D (1996) Identity of prokaryotic and eukaryotic $tRNA^{(Asp)}$ for aminoacylation by aspartyl-tRNA synthetase from *Thermus thermophilus*. *Biochemistry* 35(23):7447–7458.
2. Henkin TM (2009) Riboswitches: Methods and protocols. *Springer Protocols: in Molecular Biology*, ed Serganov A (Humana, Totowa, NJ), Vol 540, pp 281–290.
3. Saad NY, et al. (2012) Riboswitch (T-box)-mediated control of tRNA-dependent amidation in Clostridium acetobutylicum rationalizes gene and pathway redundancy for asparagine and asparaginyl-trnaasn synthesis. *J Biol Chem* 287(24):20382–20394.

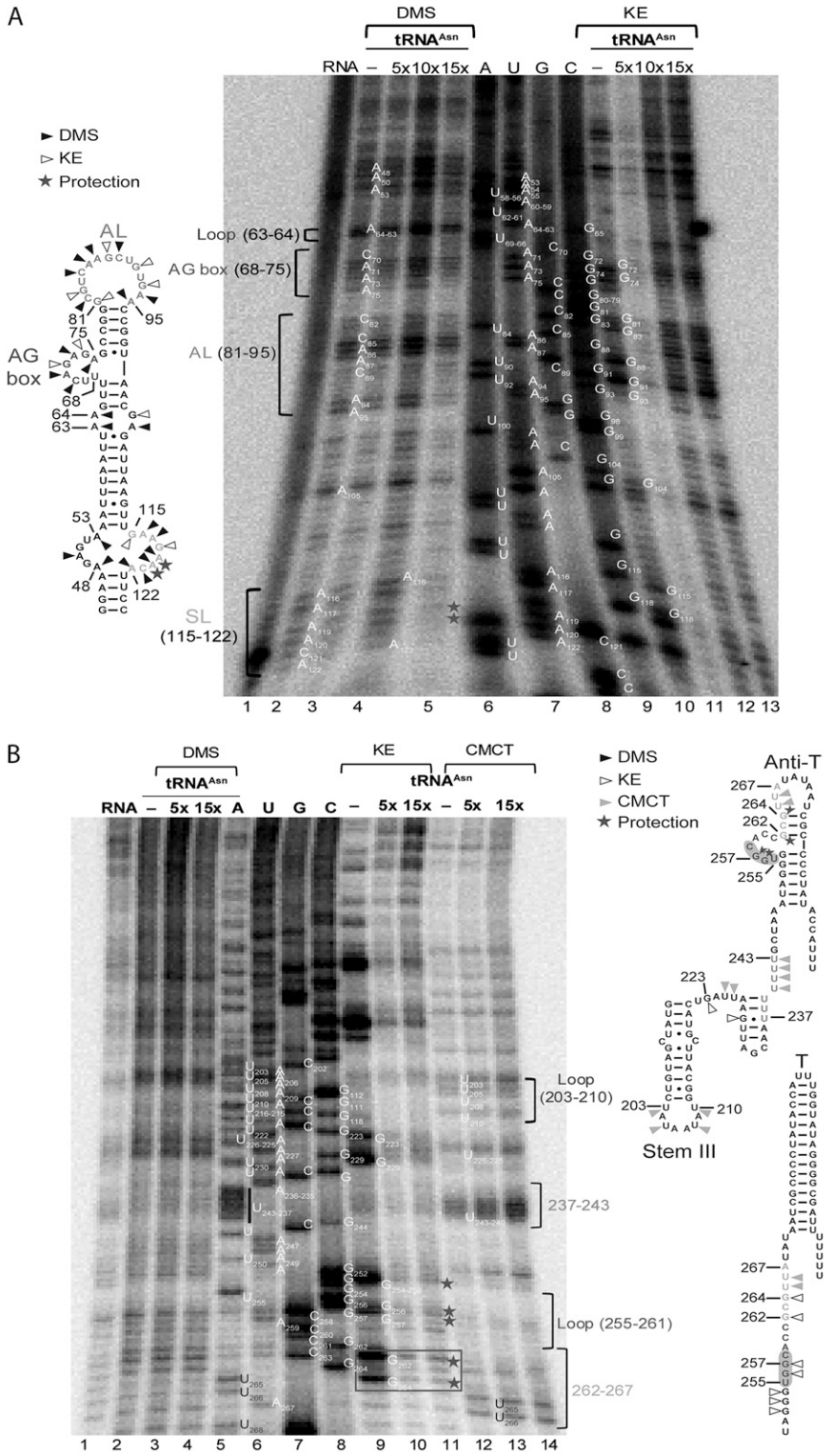


Fig. S1. Chemical probing of (*A*) stem I including the specifier loop (SL) and (*B*) the terminator (marked as "T")/antiterminator (Anti) hairpins was performed using DMS, KE, and CMCT chemical probes. The nucleotides that were accessible for chemical modification are marked with triangles. Protection (star) of certain nucleotides at the SL and the T-box bulge from modification is observed when the tRNA binds the T-box.

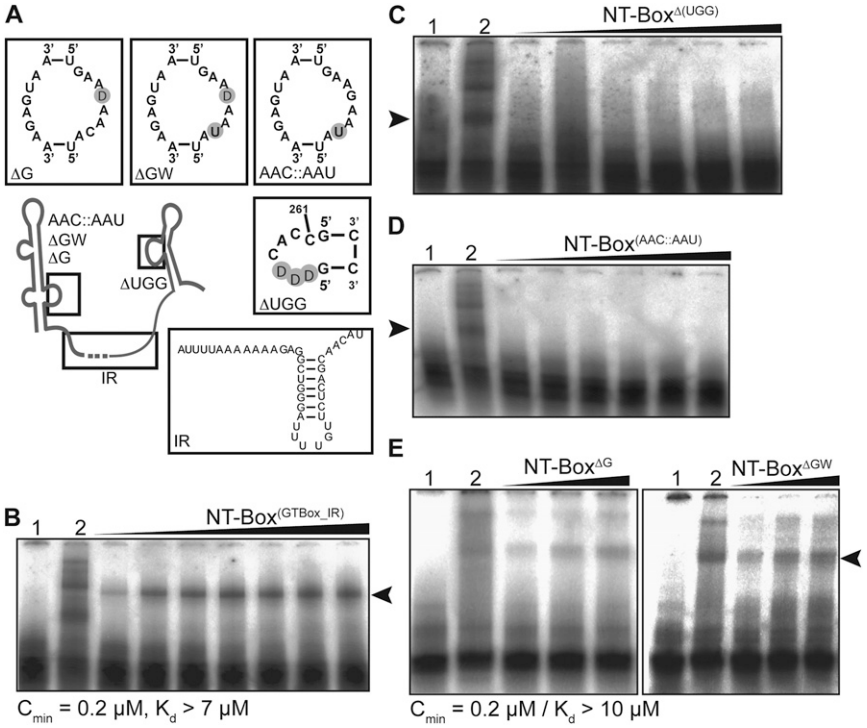


Fig. S2. Specific binding of *Cac* tRNA^{Asn} to NT-box mutants monitored by EMSA. (*A*) The mutated SLs for NT-box$^{\Delta G}$, NT-box$^{(\Delta GW)}$, NT-box$^{(AAC::AAU)}$, NT-box$^{\Delta(UGG)}$ and NT-box$^{(GTBox_IR)}$ are shown. (*B*) EMSA using NT-box$^{(GTBox_IR)}$. The tRNA^{Asn} was capable of binding the mutant. The complex (shifted bands) is gradually visualized with the increase of NT-box concentration (0.2, 0.5, 1, 2, 5, 10, and 15 μM). (*C* and *D*) EMSA using, respectively, NT-box$^{\Delta(UGG)}$ and NT-box$^{(AAC::AAU)}$. No binding with tRNA^{Asn} was observed. The increasing mutant concentrations are 0.2, 0.5, 1, 2, 5, and 15 μM. (*E*) EMSA using NT-box$^{\Delta G}$ and NT-box$^{(\Delta GW)}$. The size of mutant SL is 7 nt, and binding with tRNA^{Asn} is detected. The complex is gradually visualized with the increase of NT-box concentration (0.2, 2, and 5 μM). The tRNA^{Asn} was labeled at its 5′ extremity with [γ-^{32}P] and used at a constant concentration. Lane 1, *Cac* tRNA^{Asn}; lane 2, NT-box^{WT}·*Cac* tRNA^{Asn}. The shifted bands are indicated by arrows.

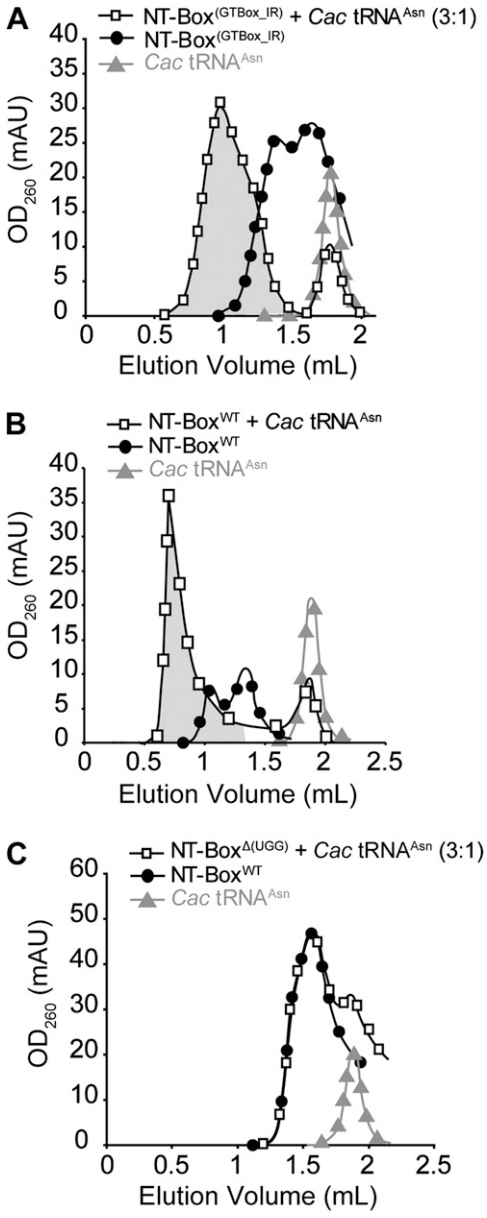


Fig. S3. Characteristic chromatograms of monitoring by size-exclusion chromatography the specificity of binding of uncharged *Cac* $tRNA^{Asn}$ to NT-$box^{(GTBox\_IR)}$, NT-box^{WT}, and NT-$box^{\Delta(UGG)}$. (*A*) The elution profile of the *Cac* $tRNA^{Asn}$, the NT-$box^{(GTBox\_IR)}$, and the formed complex are shown. To isolate this complex, 15 μM of NT-$box^{(GTBox\_IR)}$ and 5 μM of *Cac* $tRNA^{Asn}$ were used. We can clearly see the shift of the complex profiles (filled with gray color) compared with those of the free partners. (*B*) Isolated NT-box^{WT}·*Cac* $tRNA^{Asn}$ complex by size-exclusion chromatography. The elution profile of the *Cac* $tRNA^{Asn}$, the NT-box^{WT}, and the formed complex are shown. Note that the *Cac* $tRNA^{Asn}$ was eluted as a single peak with an elution volume (V_e) of 1.8 mL. The NT-box^{WT} elution profile shows two peaks with *Ve* of 1 mL and 1.4 mL, indicating the presence of two conformations for the unbound NT-box^{WT}. When both the NT-box^{WT} and *Cac* $tRNA^{Asn}$ were mixed for binding at equimolar concentrations (5 μM each), the elution profile showed an elution peak at V_e 0.75 mL The nonsymmetrical shape of the elution peak indicates the presence of a dynamic complex. (*C*) A total of 15 μM of the NT-$box^{\Delta(UGG)}$ and 5 μM of the *Cac* $tRNA^{Asn}$ were used. With these partners, no complex has been detected: the elution profile of the RNA mix is separated into two elution profiles that were superimposed on the elution profile of the NT-$box^{\Delta(UGG)}$ and the *Cac* $tRNA^{Asn}$. All elutions were measured with the elution volume in milliliters, and the RNAs were detected by using the optical density in mAU at 260 nm.

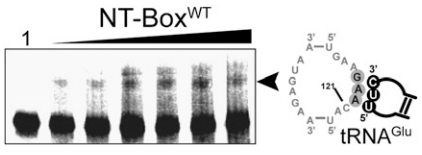


Fig. S4. Specific binding of uncharged *Sce* $tRNA^{Glu}$ to NT-box^{WT} monitored by EMSA with the same conditions as in Fig. 3. The increase of NT-box concentration was as follows: 0.2, 0.5, 1, 2, 5, and 15 μM. Lane 1, *Sce* $tRNA^{Glu}$ 5′-[^{32}P] without NT-box^{WT}.

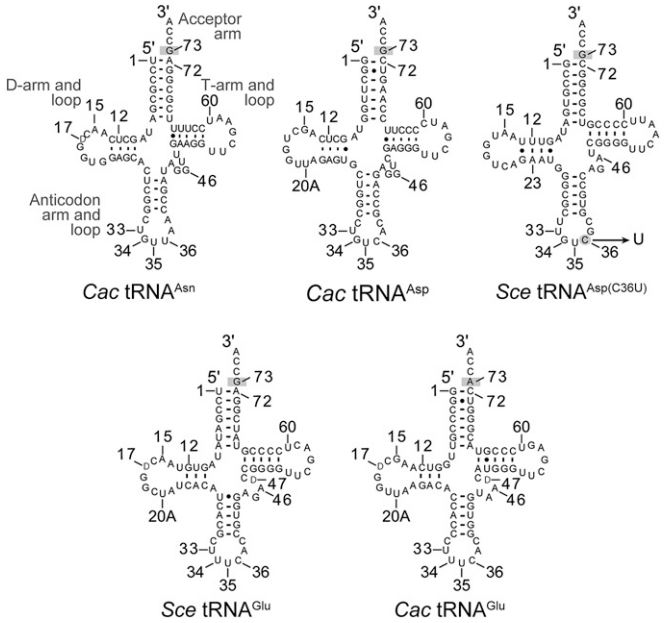


Fig. S5. Secondary structures of *Cac* tRNA^{Asn}, *Cac* tRNA^{Asp}, *Cac* tRNA^{Glu}, *Sce* tRNA^{Glu}, and *Sce* tRNA$^{Asp(C36U)}$. The discriminator base of all tRNAs is highlighted in a gray box.

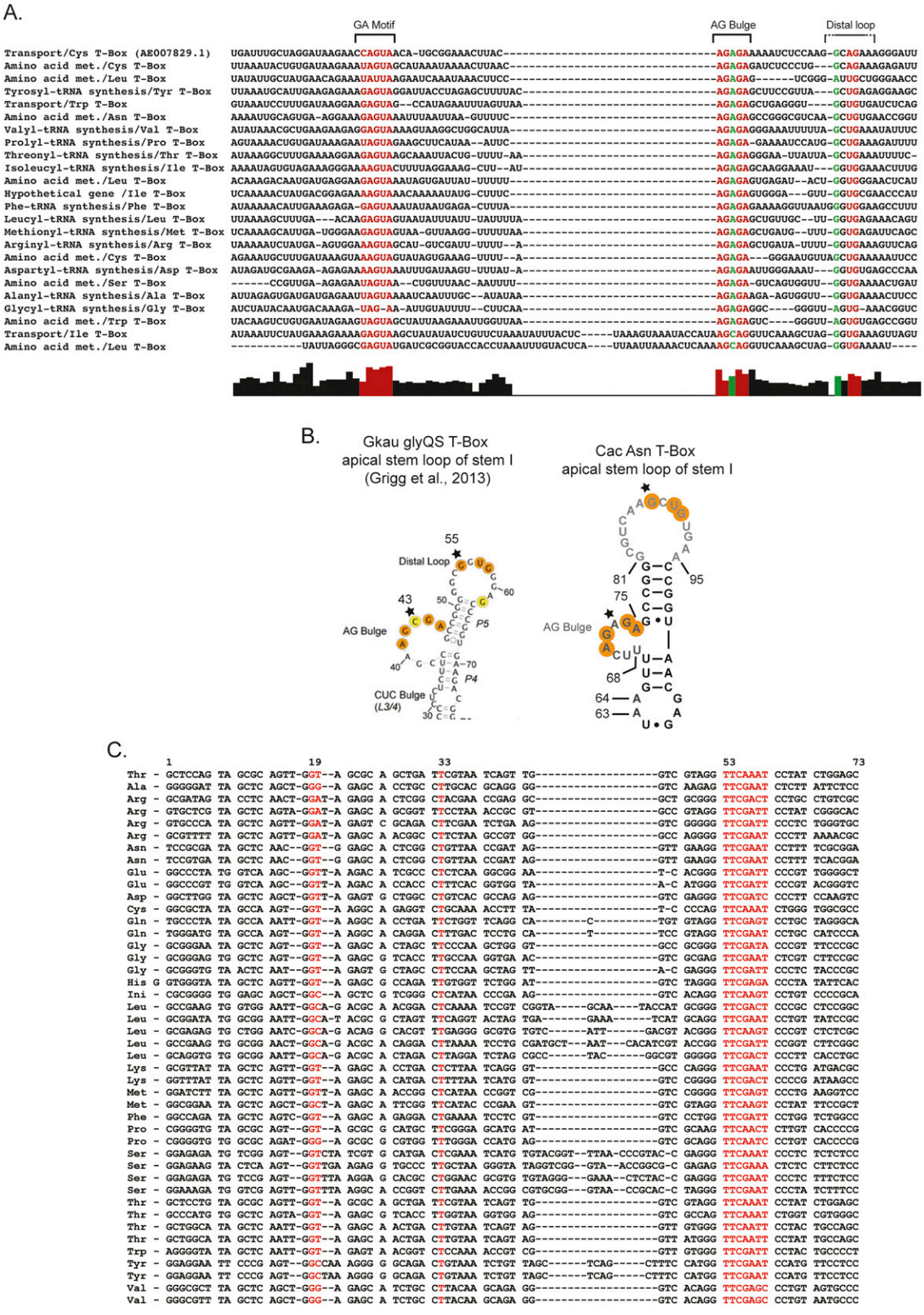


Fig. S6. (*A*) Multiple sequence alignment of all annotated *Cac* T-box apical loops and of all *Cac* tRNAs (*C*). (*B*) Predicted secondary structures of the apical stem loop I of GKau glyQS T-box (1) and *Cac* NT-box. (*C*) Multiple sequence alignment of the *Cac* tRNAs. Positions 18 and 19, the conserved U33 and the T-loop nucleotides are indicated in red letters.

1. Grigg JC, et al. (2013) T box RNA decodes both the information content and geometry of tRNA to affect gene expression. *Proc Natl Acad Sci USA* 110(18):7240–7245.

T-Box specifier stem-loop	Downstream gene/operon	Putative multiple codons (PMC)	Metabolic link between amino acids coded by PMCs

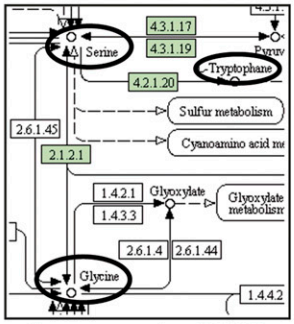
A. *S. pneumoniae 70585*

T-Box15:))))).....9 nts.....)))) GUAGUGAAAGUGGGCUGA

Tryptophan operon (*trpEGDCFBA*)

AGU: Ser*/UGG: Trp*/ GGG: Gly*

→ A metabolic link between Ser (AGU), Trp (UGG) and Gly (GGG) exist



S. pneumoniae 70585 metabolic pathway

B. *B. subtilis*

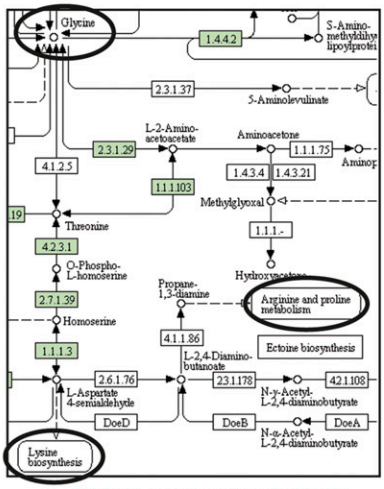
T-Box37:))))))).....9 nts.....))))) CACGCAACGAAAGGCAUUCU

glyQS (Glycyl-tRNA synthetase alpha and beta subunits)

GGC: Gly

→ The overlapping codons that we could choose if T-Box37 contains more than one codon are: AAG coding for Lys and AGG coding for Arg

→ No metabolic link between Gly (GGC), Lys (AAG) and Arg (AGG)



B. subtilis metabolic pathway

Fig. S7. Association of metabolic pathways under the control of SLs bearing two or more codons in T-boxes. Each putative overlapping codon encodes for an amino acid. We check whether all or some of the amino acids encoded by overlapping codons are metabolically related by looking at the Kyoto Encyclopedia of Genes and Genomes database for amino acid metabolic pathways (www.genome.jp/kegg/pathway.html#amino). The SLs of two T-boxes belonging to two different bacterial species are shown (T-box15 from *Streptococcus pneumoniae 70585* and T-box37 from *Bacillus subtilis*). T-box15 represents T-boxes with a number of putative multiple codons (PMCs) ≥ 1 (PMC equal to three), as Ser, Trp, and Gly (marked with a circle on the shown pathway) are metabolically related (*A*). T-box37 represents T-boxes with a number of PMCs equal to one, as no metabolic connection can be found between amino acids coded by overlapping codons (*B*). The metabolic connection between two amino acids can be dissociated when at least one enzyme does not exist to catalyze the transformation reaction between both amino acids. In the shown metabolic pathways, enzymes in green rectangles exist in the corresponding bacterial species, and those in white rectangles do not exist in the corresponding bacterial species.

Table S1. Presentation of the specifier stem-loop domain of T-boxes regulating genes or operons involved in amino acid biosynthesis or transport

Species/T-box	T-box specifier stem-loop	Downstream gene/operon	Domain of action	PMCs
Clostridium difficile 630				
			aa metabolism	
T-box1	8 nt)))))...<u>...</u>..)))) GGAUU**GAAAA<u>CUA</u>**ACCUA	Threonine synthase (*thrC*)	Gly, Ser and Thr (cdf00260)	AAC: Asn/ACU: Thr*
T-box2	7 nt)))))...<u>...</u>)))) GUUCU**GAA<u>CUUG</u>**UCUA	Leucine operon (*leuACDB*)	Val, Leu and Ile (cdf00290)	CUU: Leu*/UUG: Leu*
T-box3	8 nt)))))...<u>...</u>..)))) GGAUU**GAAAA<u>CUA</u>**ACCUA	Homoserine dehydrogenase (*hom2*)	Gly, Ser and Thr (cdf00260) Lys (cdf00300)	AAA:Lys*/AAC: Asn/ACU: Thr*
T-box4	8 nt)))))....<u>...</u>)))) UUAGU**GAAAA<u>GAA</u>**CUUU	Argininosuccinate synthase	Ala, Asp and Glu (cdf00250) Arg and Pro (cdf00330)	AGA: Arg*/GAA: Glu*
			Amino acid transport	
T-box5	8 nt)))))...<u>...</u>.)))) UAUGU**GAA<u>GAG</u>AG**CUCU	Amino acid ABC transporter	Amino acid transport	GAG: Glu/AGA: Arg
Staphylococcus aureus MSSA 476				
			aa metabolism	
T-box6	8 nt)))))....<u>...</u>)))) CUUGU**GAA<u>UGGA</u>**CUUU	Tryptophan operon (*trpEGDCFBA*)	Gly, Ser and Thr (sas00260) Phe, Tyr and Trp (sas00400)	UGG: Trp*/GGA: Gly*UGG: Trp*/GGG: Gly*
T-box7	9 nt)))))....<u>...</u>)))) UUAAU**GAAA<u>UUGGG</u>**UUUU			
T-box8	8 nt)))))...<u>...</u>.)))) UGUUU**GAAAA<u>UGG</u>**CCUU	Cys/Met metabolism PLP-dependent enzyme	Cys and Met (sas00270)	AAU: Asn/AUG: Met*
T-box9	7 nt)))))...<u>...</u>.)))) UUAAU**GAA<u>UGCA</u>**CCUU	Serine acetyltransferase–cysteinyl-tRNA synthetase (*cysS*) operon	Cys and Met (sas00270) Cysteinyl-tRNA biosynthesis (sas00970)	AUG: Met*/UGC: Cys*
Bacillus anthracis Ames Ancestor				
			aa metabolism	
T-box10	8 nt)))))...<u>...</u>.)))) UCGAC**GA<u>ACA</u>UCG**CCCU	*ilvBHCleuABCD* operon	Val, Leu and Ile (ban00290)	ACA: Thr/AUC: Ile*
T-box11	7 nt)))))......)))) AUAUC**GAA<u>CCUA</u>**UCCC	Pyrroline-5-carboxylate reductase	Arg and Pro (ban00330)	CCU: Pro*
T-box12	9 nt)))))....<u>...</u>)))) AGCAU**GAA<u>UUACAG</u>**CCUU	*aroFhisCtyrAaroA* operon	Phe, Tyr and Trp (ban00400) His (ban00340)	UAC: Tyr*/CAG: Gln
Listeria innocua				
			aa metabolism	
T-box13	7 nt)))))...<u>...</u>)))) GAUGU**GAA<u>UGGA</u>**CUUC	Tryptophan operon (*trpEGDCFBA*)	Gly, Ser and Thr (lin00260) Phe, Tyr and Trp (lin00400)	UGG: Trp*/GGA: Gly* UGG: Trp*/GGG: Gly*
T-box14	8 nt)))))...<u>...</u>.)))) AAUAG**GAA<u>UGGGC</u>**CUUG			
S. pneumoniae 70585				
			aa metabolism	
T-box15	9 nt)))))....<u>...</u>.)))) GUAGU**GAA<u>AGUGGG</u>**CUGA	Tryptophan operon (*trpEGDCFBA*)	Gly, Ser and Thr (sne00260) Phe, Tyr and Trp (sne00400)	AGU: Ser*/UGG: Trp*/GGG: Gly*
Lactococcus lactis subsp. *lactis Il1403*				
			aa metabolism	
T-box16	7 nt)))))...<u>...</u>)))) AGAGC**GAA<u>UGGG</u>**CUGA	Tryptophan operon (*trpEGDCFBA*)	Gly, Ser and Thr (sne00260) Phe, Tyr and Trp (sne00400)	UGG: Trp*/GGG: Gly*

Table S1. Cont.

Species/T-box	T-box specifier stem-loop	Downstream gene/operon	Domain of action	PMCs
Listeria monocytogenes EGD-e				
			aa metabolism	
T-box17	8 nt)))))....<u>...</u>.)))) UUCAG**GAA<u>UGGG</u>C**CUUG	Tryptophan operon (*trpEGDCFBA*)	Gly, Ser and Thr (sne00260) Phe, Tyr and Trp (sne00400)	UGG: Trp*/GGG: Gly* UGG: Trp*/GGA: Gly*
T-box18	7 nt)))))...<u>...</u>.)))) GAUGU**GAA<u>UGG</u>A**CUUC			

This table shows T-boxes controlling genes or operons involved in amino acid (aa) metabolism or transport. T-box sequences from different bacterial species (first column) were extracted from the Rfam seed (accession no. RF00230) and arbitrary numbered (second column). The specifier loop (SL) nucleotides are displayed in bold (third column) while other nucleotides belong to the stems flanking the SL (brackets indicate that the nucleotide below belongs to a flanking stem, while dots indicate that the nucleotides bellows are part of the SL). The SL codons are underlined or marked by lines and the number of specifier loop nucleotides (nts) are indicated. The identity of the downstream genes putatively regulated by the corresponding T-boxes is mentioned (fourth column). The fifth column indicates the metabolic pathway to which each gene or operon participates; the accession number for each pathway is displayed into parentheses. The last column shows the putative multiple codons (specificity/sequence) that could regulate each T-box. When putative multiple codons are present, we found that they encode amino acids that are metabolically related in the considered bacterial species (www.genome.jp/kegg/pathway.html#amino).
*Amino acid is either the product or the substrate of the metabolic pathway transcriptionally controlled by the T-box.

Table S2. Presentation of the specifier stem-loop domain of T-boxes regulating genes or operons involved in aminoacyl-tRNA synthesis

Species/T-box	T-box specifier stem-loop	Downstream gene/operon	PMCs
C. difficile 630			
T-box19	8 nt)))))))........)))) UCUAUUU**GAAAACUA**CCUU	*thrS* (Threonyl-tRNA synthetase)	ACU: Thr
T-box20	8 nt)))))))........)))) GUCAUGA**GAAGUUCC**CUCU	*pheST* (Phenylalanyl-tRNA synthetase α- and β- subunits)	UUC: Phe
T-box21	7 nt)))))))........)))) GAUAUUU**GAAGCAG**CCUU	*alaS* (Alanyl-tRNA synthetase)	GCA: Ala
T-box22	7 nt)))))))........)))) GGUUUUU**GAAUCUA**CUCU	*serS1* (Seryl-tRNA synthetase)	UCU: Ser
T-box23	7 nt)))))))........)))) UUAGUUU**GAAUACA**UCUC	*tyrR* (Tyrosyl-tRNA synthetase)	UAC: Tyr
T-box24	7 nt)))))))........)))) AGUCUUU**GAACCUG**UCUU	*proS* (Prolyl-tRNA synthetase)	CCU: Pro
S. aureus MSSA 476			
T-box25	6 nt)))))))......)))) UAAAGUC**UUUUCA**CCUU	*pheST* (Phenylalanyl-tRNA synthetase α- and β-subunits)	UUC: Phe
T-box26	8 nt)))))))........)))) CAUUAGU**GAACGCUA**CUUU	*alaS* (Alanyl-tRNA synthetase)	GCU: Ala
T-box27	8 nt)))))))........)))) AUUAGAU**GAUUACUA**CUUC	*thrS* (Threonyl-tRNA synthetase)	ACU: Thr
T-box28	7 nt)))))))........)))) UAUAUAU**GAAUCUA**CCUA	*serS* (Seryl-tRNA synthetase)	UCU: Ser
T-box29	7 nt)))))))........)))) AUAAUAU**GAAUACA**CCUU	*tyrS* (Tyrosyl-tRNA synthetase)	UAC: Tyr
T-box30	7 nt)))))))........)))) UGUAUGU**UAUAUCA**CUGG	*IleS* (Isoleucyl-tRNA synthetase)	AUC: Ile
L. lactis subsp. *lactis Il1403*			
T-box31	7 nt)))))))........)))) GCAAAGU**GAAUGGG**CUUG	*trpS* (Tryptophanyl-tRNA synthetase)	UGG: Trp
T-box32	8 nt)))))))........)))) ACUUUAU**GAAGGUUG**CGUC	*valS* (Valyl-tRNA synthetase)	GUU: Val
T-box33	9 nt)))))))..........)))) AAAUAAU**GACUUUCUA**CUUC	*serS* (Seryl-tRNA synthetase)	UCU: Ser
T-box34	8 nt)))))))........)))) UGCAAUU**GAAGUUCA**CCUU	*pheST* (Phenylalanyl-tRNA synthetase α- and β-subunits)	UUC: Phe
L. monocytogenes EGD-e			
T-box35	7 nt)))))))........)))) UCUGGAU**GAACUUA**CCAU	*leuS* (Leucyl-tRNA synthetase)	CUU: Leu
B. subtilis			
T-box36	5 nt))))))).....)))) UCGCUU**UACCG**CCUU	*thrS* (Threonyl-tRNA synthetase)	ACC: Thr
T-box37	9 nt)))))))..........)))) CACGCAA**CGAAAGGCA**UUCU	*glyQS* (Glycyl-Trna synthetase α- and β-subunits)	GGC: Gly

Table S2. Cont.

Species/T-box	T-box specifier stem-loop	Downstream gene/operon	PMCs
T-box38	7 nt)))))))........)))) AUAUAUC**GAAUACA**CUCA	*tyrS* (Tyrosyl-tRNA synthetase)	UAC: Tyr
S. coelicolor A3(2)			
T-box39	8 nt)))))))........)))) GCGACGU**GAAGAUCA**CCCC	*IleS* (Isoleucyl-tRNA synthetase)	AUC: Ile
Bacillus thuringiensis serovar konkukian str. 97–27			
T-box40	8 nt)))))))....___.)))) UAUAUGU**GAAGAACA**CCUU	*asnC* (Asparaginyl-tRNA synthetase)	GAA: Glu/AAC: Asn*
C. acetobutylicum ATCC 824			
T-box41	8 nt)))))))....___.)))) UUAAGUU**GAAGAACA**UUCC	*aspS2ogatCABo* operon (tRNAdependent synthesis of Asn and Gln)	GAA: Glu/AAC: Asn*
B. anthracis Ames Ancestor			
T-box42	8 nt)))))))........)))) UAUGUUA**GAAGGUAG**CCCU	*valS* (Valyl-tRNA synthetase)	GUA: Val
T-box43	9 nt)))))........)))) UGAUU**GAAUGACAC**UCUA	*hisSaspS* operon (aminoacyl-tRNA biosynthesis (ban00970))	GAC: Asp*/CAC: His*
L. innocua			
T-box44	8 nt)))))))........)))) AAUGUCU**GAAGGUAG**CCUU	*valS* (Valyl-tRNA synthetase)	GUA: Val
T-box45	8 nt)))))))........)))) AGUAGAC**GAAUUACA**UCCC	*tyrS* (Tyrosyl-tRNA synthetase)	UAC: Tyr
T-box46	10 nt)))))...___....)))) UUUCC**GAAAAGACAC**UCUC	*hisSaspS* operon (aminoacyl-tRNA biosynthesis (lin00970)	AAA: Lys/AAG: Lys GAC: Asp*/CAC: His*

This table shows T-boxes controlling genes or operons involved in aminoacyl-tRNA formation. T-box sequences from different bacterial species (first column) were extracted from the Rfam seed (accession no. RF00230) and arbitrary numbered (second column). The specifier loop (SL) nucleotides are displayed in bold (third column) while other nucleotides belong to the stems flanking the SL (brackets indicate that the nucleotide below belongs to a flanking stem, while dots indicate that the nucleotides bellows are part of the SL). The SL codons are underlined or marked by lines and the number of specifier loop nucleotides (nts) are indicated. The identity of the downstream genes putatively regulated by the corresponding T-boxes is mentioned (fourth column). The fifth column indicates which aminoacyl-tRNA synthetase or aminoacyl-tRNA–forming enzymes are controlled by the T-box; the accession numbers are displayed into parentheses. The last column shows the codon or putative multiple codons (specificity/sequence) that could regulate each T-box.

*Amino acids are those which are substrates of the aminoacyl-tRNA synthetase or aminoacyl-tRNA–forming enzyme regulated by the T-box. *hisSaspS* operon encodes histidyl- and aspartyl-tRNA synthetase, respectively. The SL of T-box46 contains four PMCs (AAA and AAG for Lys, GAC for Asp, and CAC for His). The presence of Lys codon makes sense, as Lys and Asp are metabolically related; thus, $tRNA^{Lys}$ could also trigger *hisSaspS* T-box antitermination.