Riboswitches

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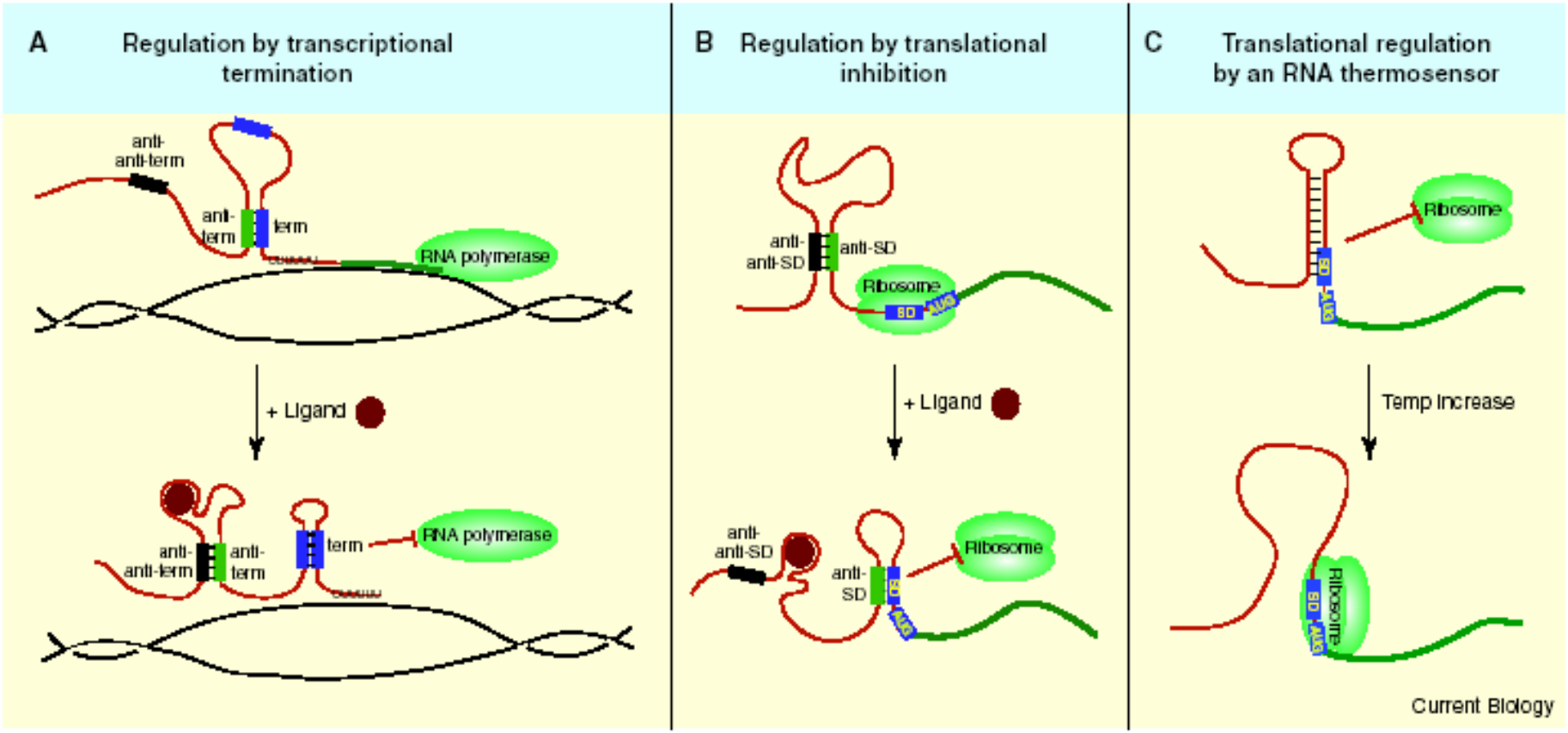
Small molecules regulate their own biosynthesis
by interacting directly with an mRNA of its pathway.

From the Ron Breaker lab: Thiamin, Cobalamin and FMN

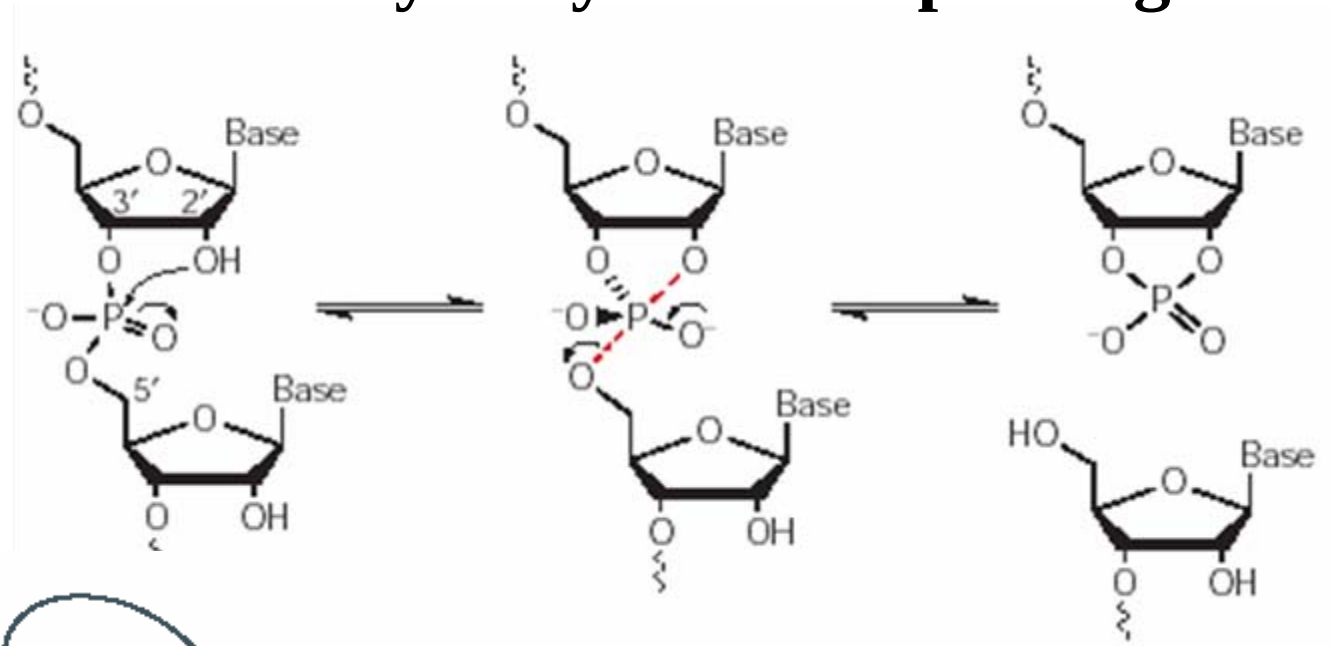
Winkler at al. Nature 2002, vol 419, 31. Thiamin

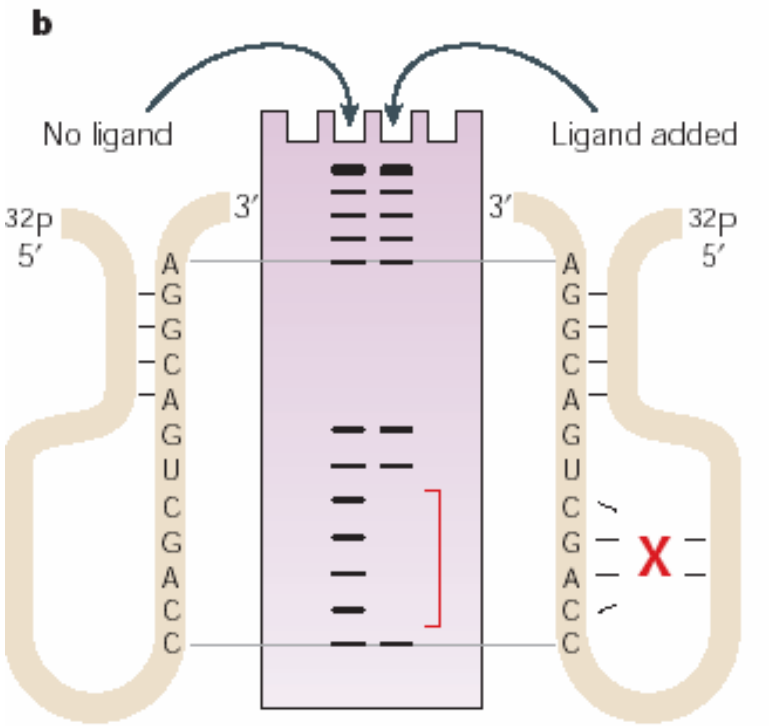
Strategies for gene regulation by RNA sensors

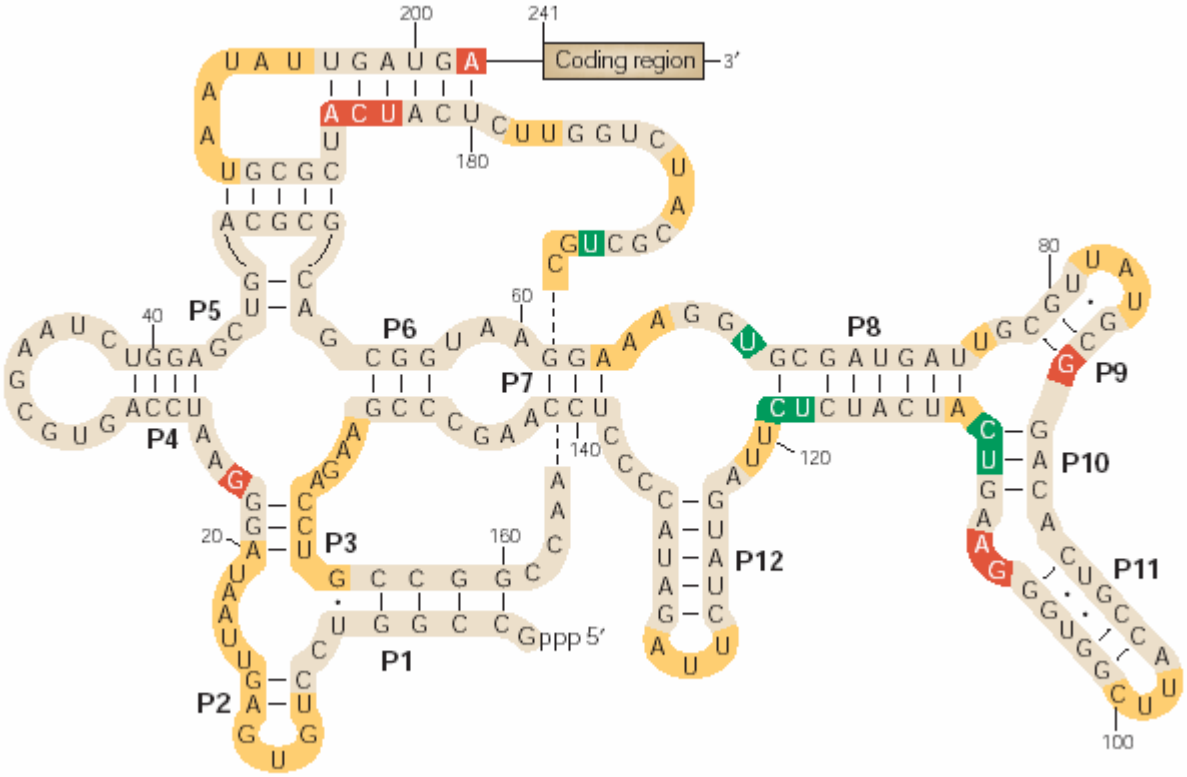


Small molecule-regulated riboswitches have been linked to both transcriptional attenuation (A) and translational inhibition (B) mechanisms, while characterized thermosensors use a translational inhibition strategy (C). (A) Transcriptional termination mechanism. DNA is depicted as a black double helix, the nascent mRNA transcript as a colored line (red is untranslated leader, green is coding sequence), and RNA polymerase as a green oval. (Top) In the absence of the relevant metabolite, part of the terminator (two blue boxes) is bound by an anti-terminator (green box) and is non-functional. Transcription proceeds past the poly-uridine stretch following the terminator sequence and extends into coding sequence. (Bottom) In the presence of the relevant metabolite (red circle), ligandbound RNA adopts an alternative conformation in which the anti-terminator is bound by an anti-anti-terminator (black box). This allows formation of the structurally distinctive terminator hairpin, which induces release of RNA polymerase following the poly-uridine tract. (B) Translational inhibition mechanism. The mRNA is depicted with the colored line; untranslated leader in red and the coding region in green. (Top) In the absence of cofactor-binding, the anti-Shine-Dalgarno (anti-SD) ribosome binding sequence is sequestered by an anti-anti-SD sequence; this conformation allows ribosome (double green oval) binding to the SD box and translation to occur. (Bottom) Cofactor binding (red circle) restructures the RNA so that the anti-SD sequence is allowed to pair with the SD box, thus inhibiting translation. Note that variations on both mechanisms exist, as may dual regulatory mechanisms involving both transcriptional attenuation and translational inhibition (see text). (C) A thermosensor from *L. monocytogenes prfA*. (Top) A stem structure adjacent to the SD box prevents translation at lower temperatures. (Bottom) The increase in ambient temperature following host infection melts this structure, allowing the ribosome to access the SD box and translate *prfA*.

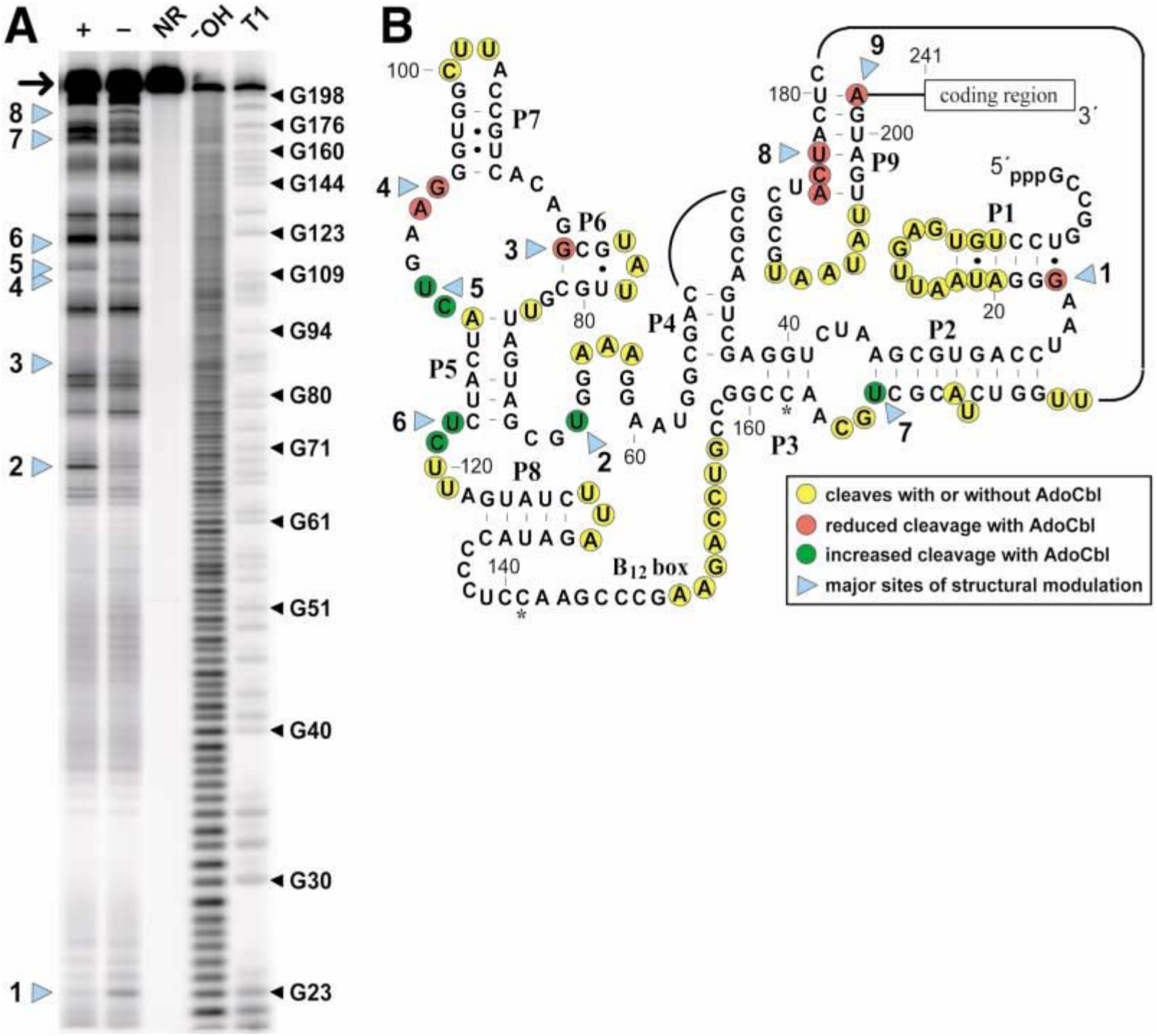
RNA analysis by “in line” probing

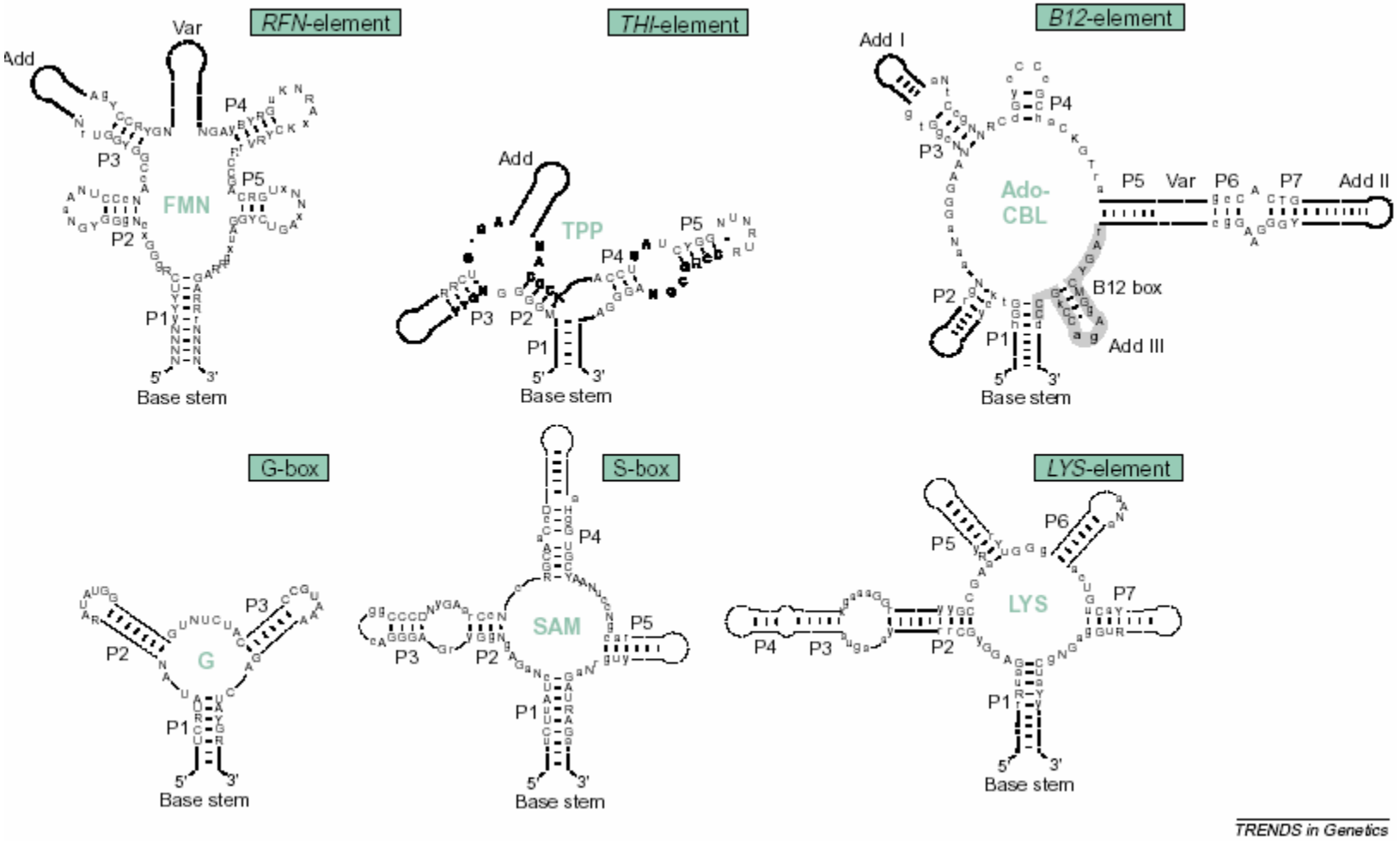






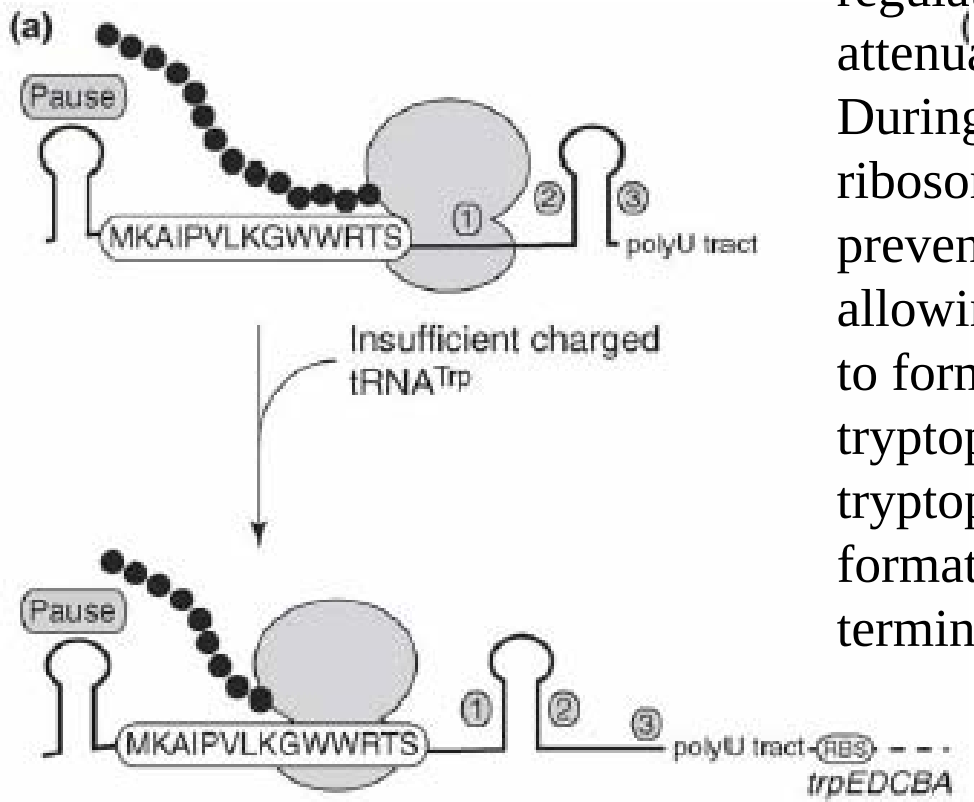
Structural model and allosteric changes of a coenzyme-B12 riboswitch. The secondary-structure model for the aptamer domain (nucleotides 1–202) of the coenzyme-B12 riboswitch that precedes the *btuB* coding region of *Escherichia coli*. Inline probing assays have shown that many nucleotides in the loops and bulges are floppy and undergo relatively rapid spontaneous cleavage (yellow) regardless of whether coenzyme B12 is present. However, nucleotides at nine locations experience a decrease (red) or an increase (green) in the spontaneous cleavage rate.


A
+
–
NR
-OH
T1
G198
G176
G160
G144
G123
G109
G94
G80
G71
G61
G51
G40
G30
G23
B
P1
P2
P3
P4
P5
P6
P7
P8
P9
coding region
5′ pppG
3′
B12 box
cleaves with or without AdoCbl
reduced cleavage with AdoCbl
increased cleavage with AdoCbl
major sites of structural modulation



Riboswitch structures. The structures of riboswitches contain conserved base-paired regions and invariant (uppercase) and highly conserved (lowercase) positions, although some parts of the structures are variable (Var) or facultative (Add). The conserved helices are numbered independently P1 through P7, P1 being the base stem; regions identified in early experiments (thi-element and B12-element) are highlighted in bold and in gray, respectively. Abbreviations: Ado-CBL, adenysylcobalamin, FMN, flavin mononucleotide; G, guanine; LYS, lysine; SAM, S-adenosylmethionine; TPP, thiamin pyrophosphate

Tryptophan riboswitch



The E. coli trpEDCBA operon is regulated by ribosome-mediated attenuation [44].
During conditions of high tryptophan, ribosomes translate a leader peptide and prevent formation of 1+2, thereby allowing 2+3 (terminator)
to form. During conditions of low tryptophan, ribosomes pause at two tryptophan codons, thus allowing for formation of 1+2 and preventing terminator formation.

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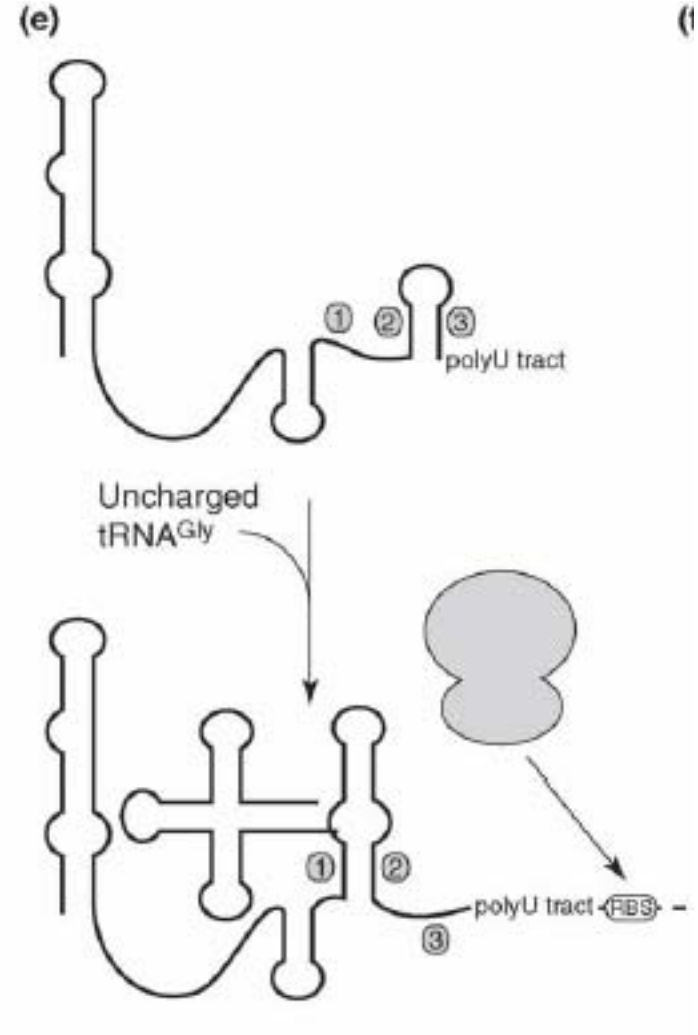
Gly-tRNA riboswitch



The B. subtilis glyQS RNA binds to uncharged tRNAGly during conditions of limited glycine, thereby stimulating formation of 1+2 and preventing formation of a terminator (2+3) [40]

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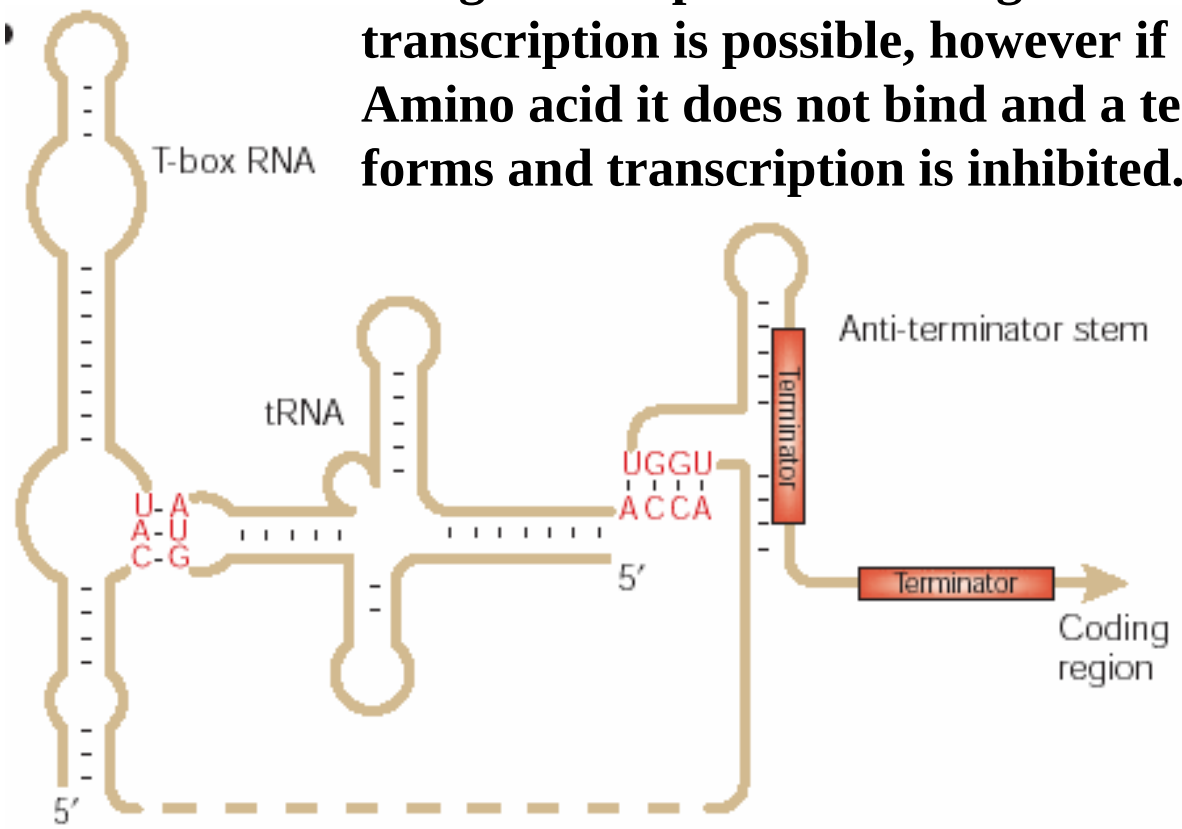
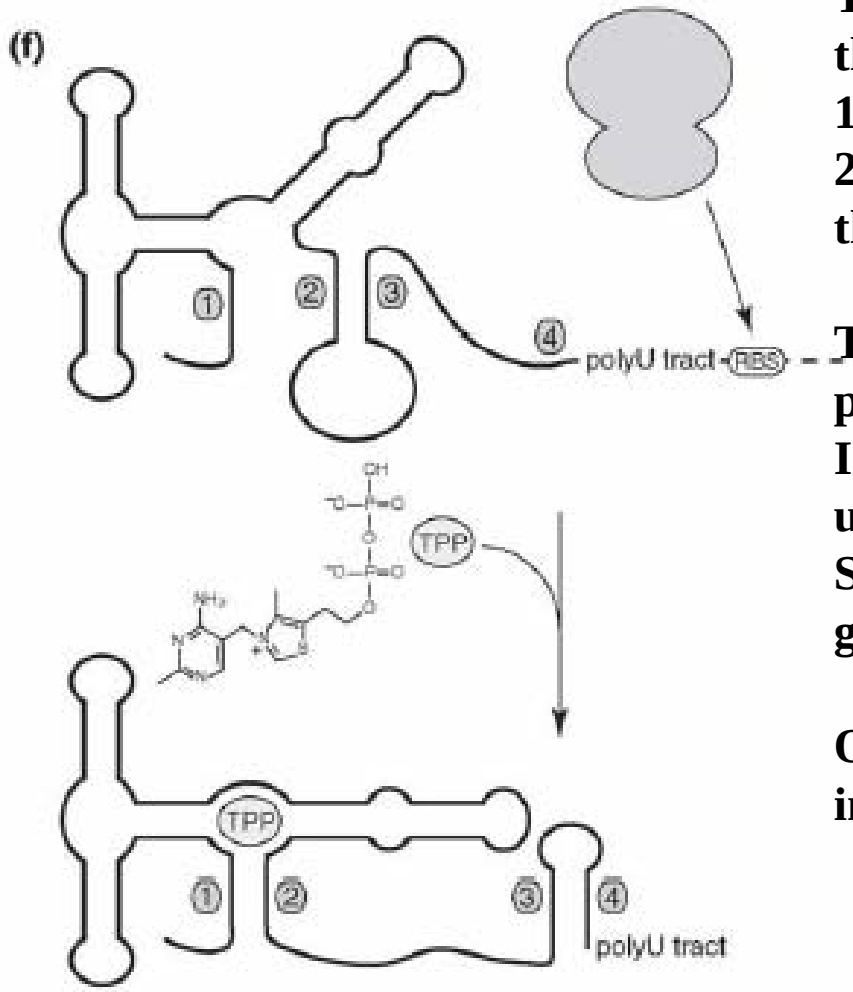
T-box RNAs are normally found in the 5' UTR of genes that encode AMINOACYL-tRNA SYNTHETASES **or related amino-acid-biosynthesis genes of Grampositive organisms. Each folds into a structure that selectively recognizes a specific uncharged tRNA**76**. If bound transcription is possible, however if charged with an Amino acid it does not bind and a termination stem forms and transcription is inhibited.**



Thiamine pyrophosphate (TPP) riboswitch



The B. subtilis tenA mRNA interacts with thiamine pyrophosphate (TPP) to stabilize 1+2 and 3+4. During conditions of low TPP, 2+3 is promoted, allowing transcription of the downstream genes.

This riboswitch is also found in fungi and plants, however at different positions, eg in INTRONS that reside in the 5' or 3' untranslated (UTR) regions of the gene. Seem to be important for the splicing of genes.

Open question: How many riboswitches are in higher eukaryotic organisms?

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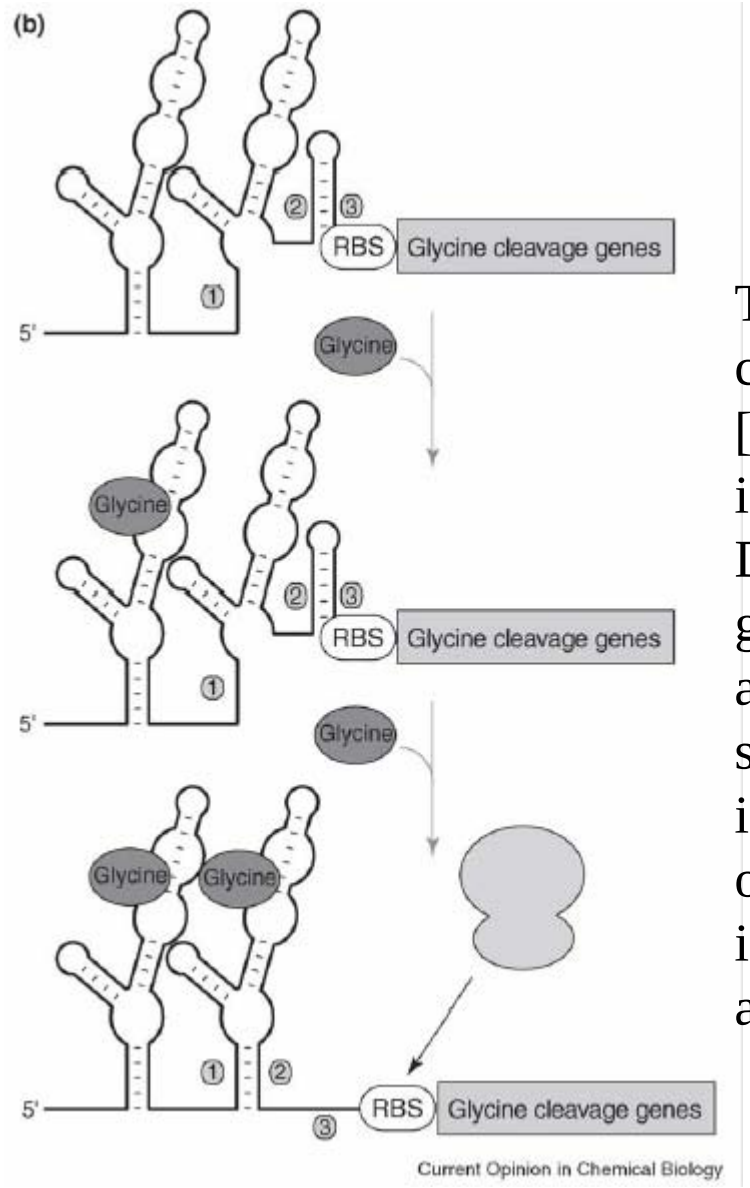

The Vibrio cholerae VC1422 50-UTR contains two nearly identical domains [17]. In the absence of glycine, 2+3 helix is formed, which inhibits ribosome access. During conditions of glycine excess, glycine binds to the first domain and increases the affinity of glycine for the second domain through cooperative interactions. Once both aptamers are occupied, the 1+2 helix is stimulated, thereby disrupting 2+3 and allowing ribosome access.

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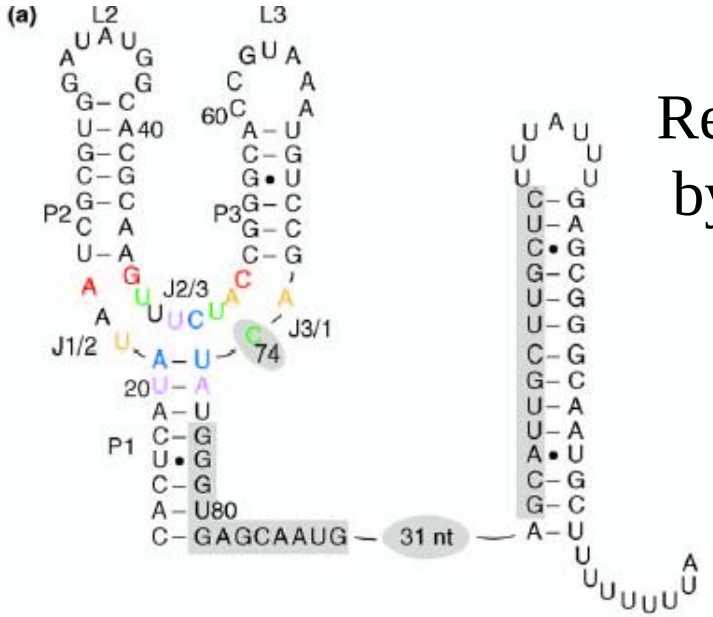
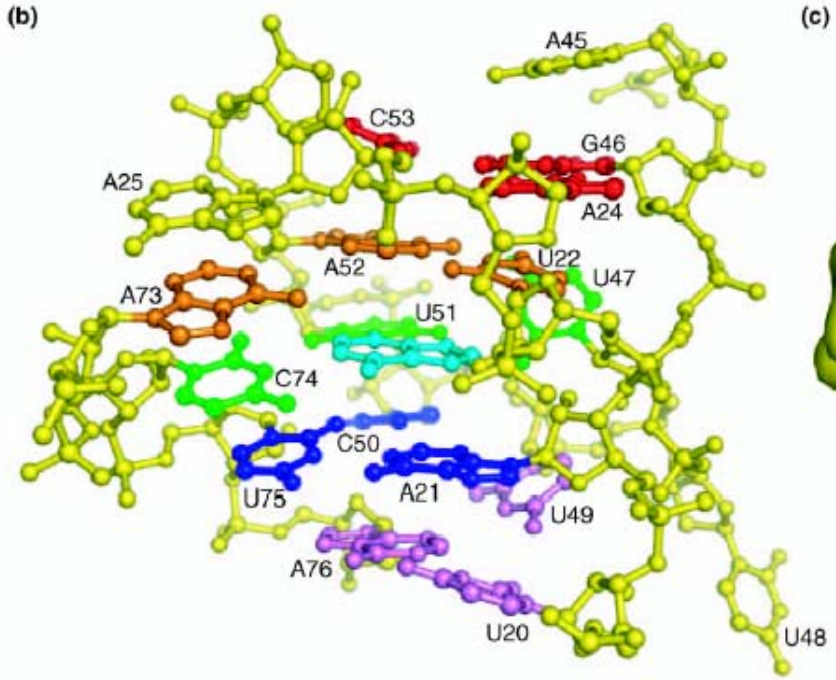
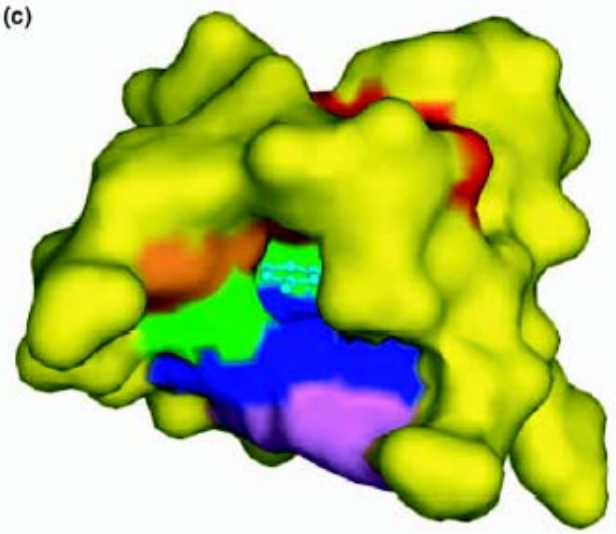
Regulation of B. subtilis xpt-pbuX mRNAs by a guanine-sensing riboswitch.







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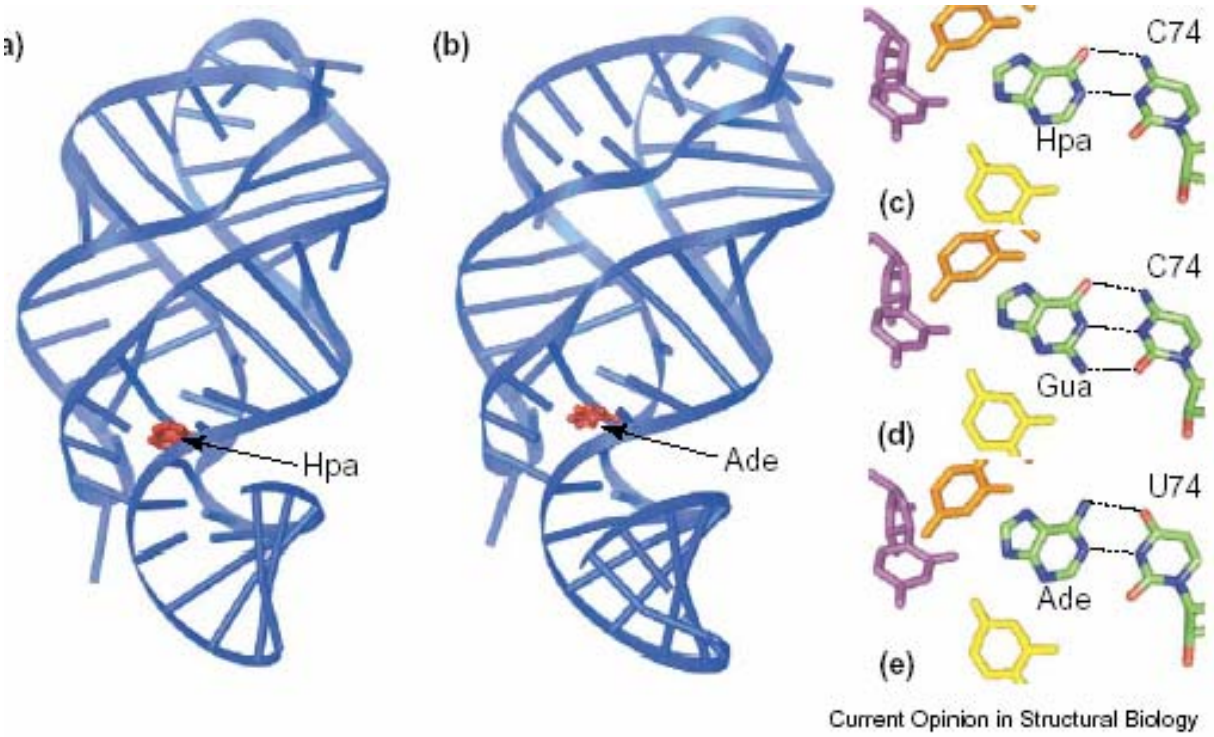
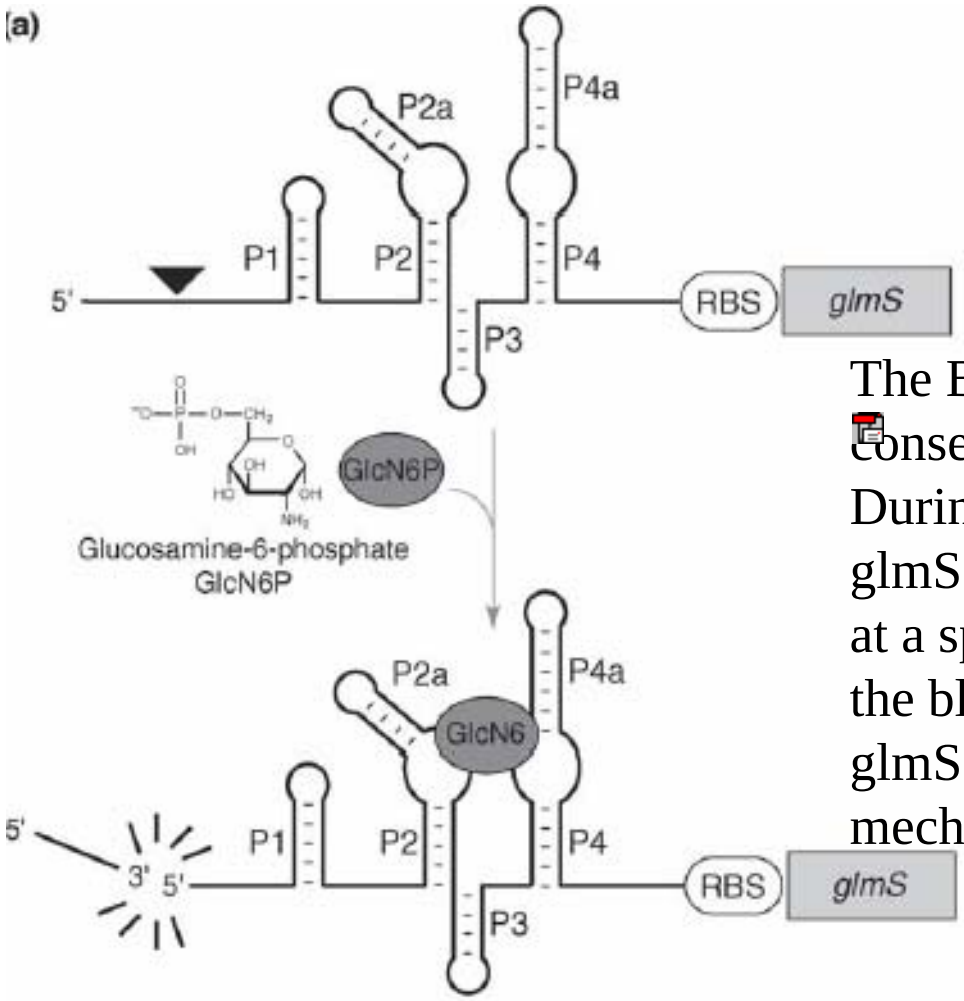
Regulation of B. subtilis xpt-pbuX mRNAs by a guanine-sensing riboswitch.
(a) Secondary structure of the B. subtilis xpt-pbuX aptamer and terminator [36]. The pyrimidine 74 specificity determinant is indicated by a shaded oval. The anti-terminator helix is indicated by grey shaded boxes. Residues involved in formation of the stacked array of triples are color coordinated, each color indicating a different triple.
(b) Tertiary architecture of hypoxanthine-bound xpt aptamer ligand binding pocket. Hypoxanthine is shown in cyan. The color scheme corresponds with the secondary structure diagram in (a).
(c) Surface representation of the binding pocket as shown in (b).

Structural features of the purine-responsive riboswitch aptamers.



Structures of (a) the guanine riboswitch aptamer (xpt-pbuX) and (b) the adenine riboswitch aptamer (add) reveal a similar tertiary fold, despite only 59% sequence identity. Bound metabolites, hypoxanthine (Hpa) and adenine (Ade), respectively, are shown in red. Discrimination at the binding site of the guanine aptamer (c,d) and the adenine aptamer (e) results from the identity of nucleotide 74 (using the numbering system of the B. subtilis construct), which makes Watson–Crick hydrogen bonds to the appropriate metabolite. Nucleotides U22, U47 and U51 are colored orange, magenta and yellow, respectively. The oxygen and nitrogen atoms of the metabolite and nucleotide 74 are colored red and blue, respectively.

Riboswitch coupled with a ribozym reaction



The B. subtilis glmS riboswitch is conserved upstream of the glmS gene [11]. During conditions of excess GlcN6P, the glmS 50-UTR is stimulated to self-cleave at a specific site at its 50-end (indicated by the black arrow). Cleavage leads to glmS repression through an unknown mechanism.

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