BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biomedical science

Course Title : Molecular Biology

Course Code : BM35C5

Unit-V

TOPIC: Riboswitch

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riboswitches

- RNA elements
- Binding to small metabolite/ligand- bringing conformational changes.
- Locates in the 5' untranslated region.
- Regulate gene expression
- Very sensitive and ligand specific.
- The PreQ1 riboswitch is the smallest known naturally occurring riboswitch with just 34 nucleotides required for substrate binding.

Classification and nomenclature

- Families and classes

-type of ligand they bind

-their secondory structure.

- Named after ligand they bind.
- 10-12 classes

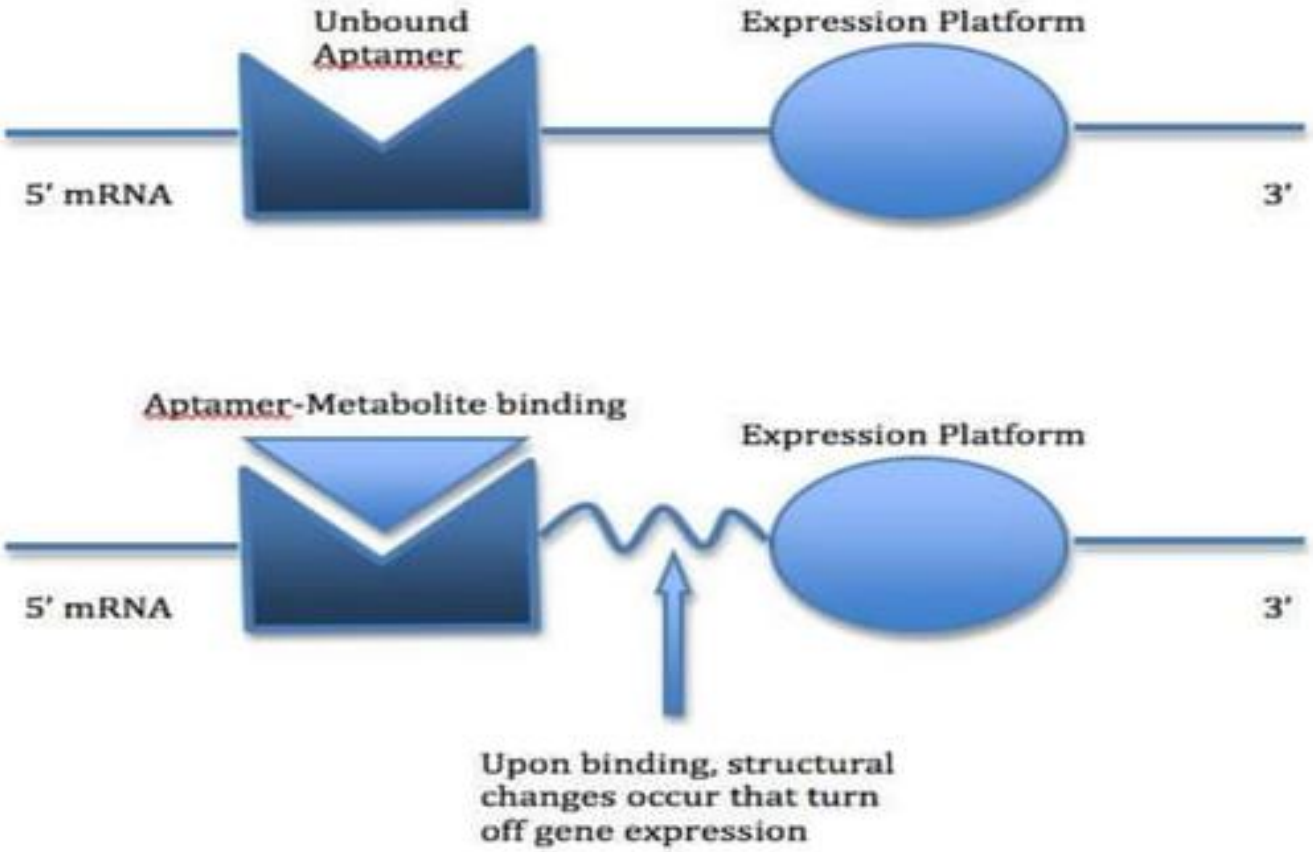
Examples..

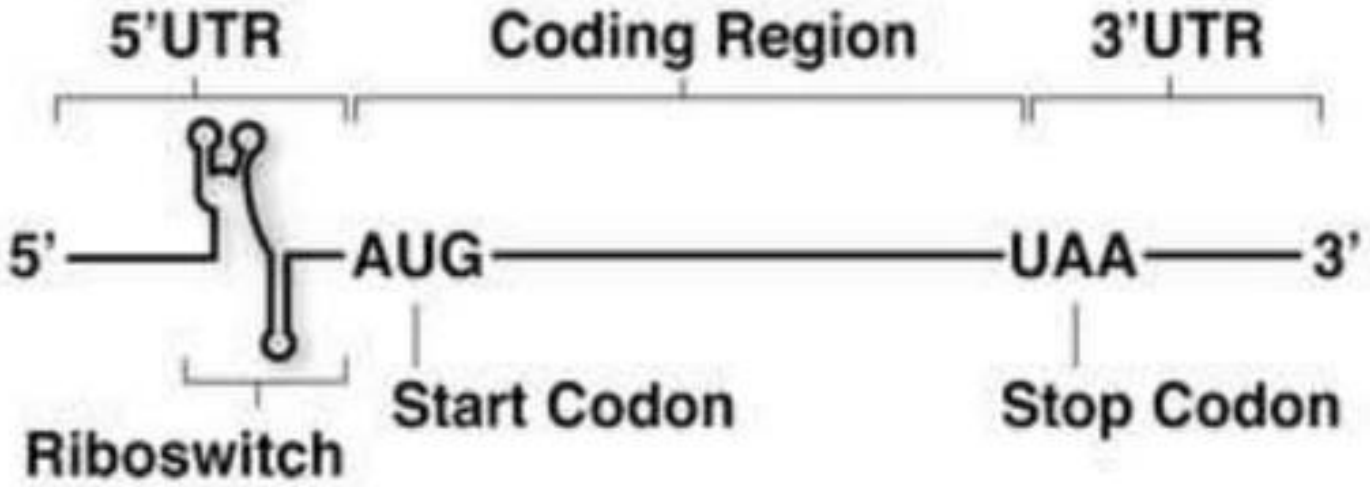
- TPP riboswitch : this riboswitch binds TPP (thiamin pyrophosphate in order to regulate the transport and synthesis of thiamin as well as other metabolites with similar properties.
- Lysine riboswitch : binds to lysine and regulates its biosynthesis, catabolism, and transport.
- Glycine riboswitch : this riboswitch regulates glycine metabolism. This is the only riboswitch known currently to be able to perform cooperative binding.
- FMN riboswitch : this riboswitch binds FMN (flavin mononucleotide) in order to regulate the transport and synthesis of riboflavin.
- Purine riboswitch : binds purines to regulate its transport and metabolism. Different forms of this riboswitch are able to bind either guanine or adenine depending on the pyrimidine in the riboswitch.
- Cobalamin riboswitch : this riboswitch binds adenosylcobalamin, the coenzyme form of B12 vitamin, in order to moderate the synthesis and transport of cobalamin and other similar metabolites.
- as well as many others such as SAM riboswitch, PreQ1 riboswitch, SAH riboswitch, glmS riboswitch, and cyclic di-GMP riboswitch

STRUCTURE

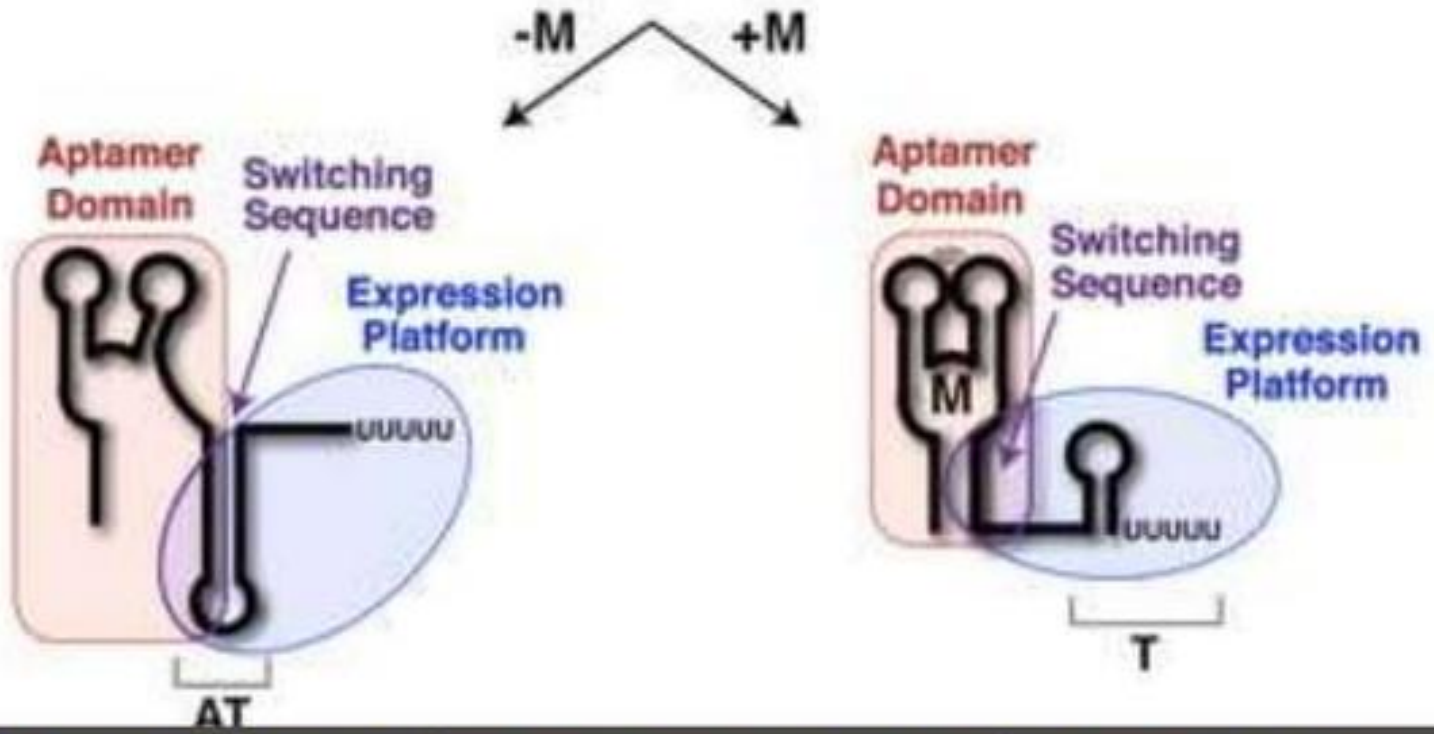
- Two domains
- Aptamer-(Ligand binding domain)
- Ligand recognation and binding.
- Highly conserved.
- Expression platform.
- Less conserved
- Adopts two mutually exclusive conformation.
- Shine-dalgano sequence locates in this domain.

structure


Unbound
Aptamer
Expression Platform
5' mRNA
3'
Aptamer-Metabolite binding
Expression Platform
5' mRNA
3'
Upon binding, structural
changes occur that turn
off gene expression



A typical bacterial mRNA transcript controlled by a riboregulatory element such as a riboswitch is composed of three sections: the 5' untranslated region (5' UTR), the protein-coding region beginning with the start codon (AUG) and ending with a stop codon (UAA), and the 3' untranslated region (3' UTR).



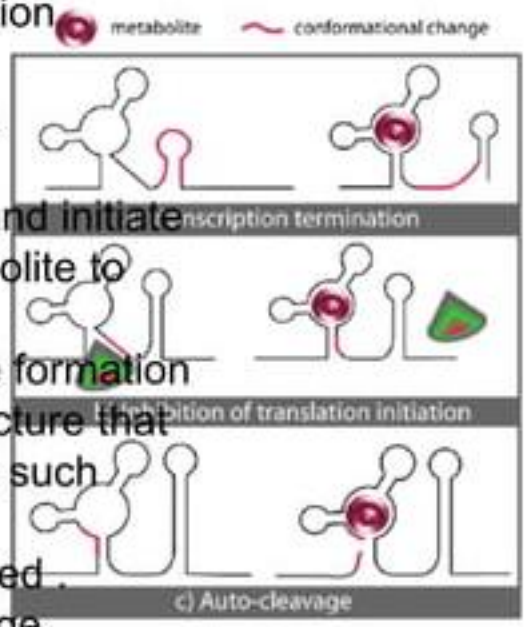
A riboswitch can adopt different secondary structures to effect gene regulation depending on whether ligand is bound. This schematic is an example of a riboswitch that controls transcription. When metabolite is not bound (-M), the expression platform incorporates the switching sequence into an antiterminator stem-loop (AT) and transcription proceeds through the coding region of the mRNA. When metabolite binds (+M), the switching sequence is incorporated into the aptamer domain, and the expression platform folds into a terminator stem-loop (T), causing transcription to abort.

Mechanism of action..

THREE MECHANISMS,,

A}The terminator loop reduces the stability of either the mRNA:RNA polymerase interaction and/or of the DNA:RNA hybrid causing the RNA polymerase to dissociate, terminating transcription prematurely

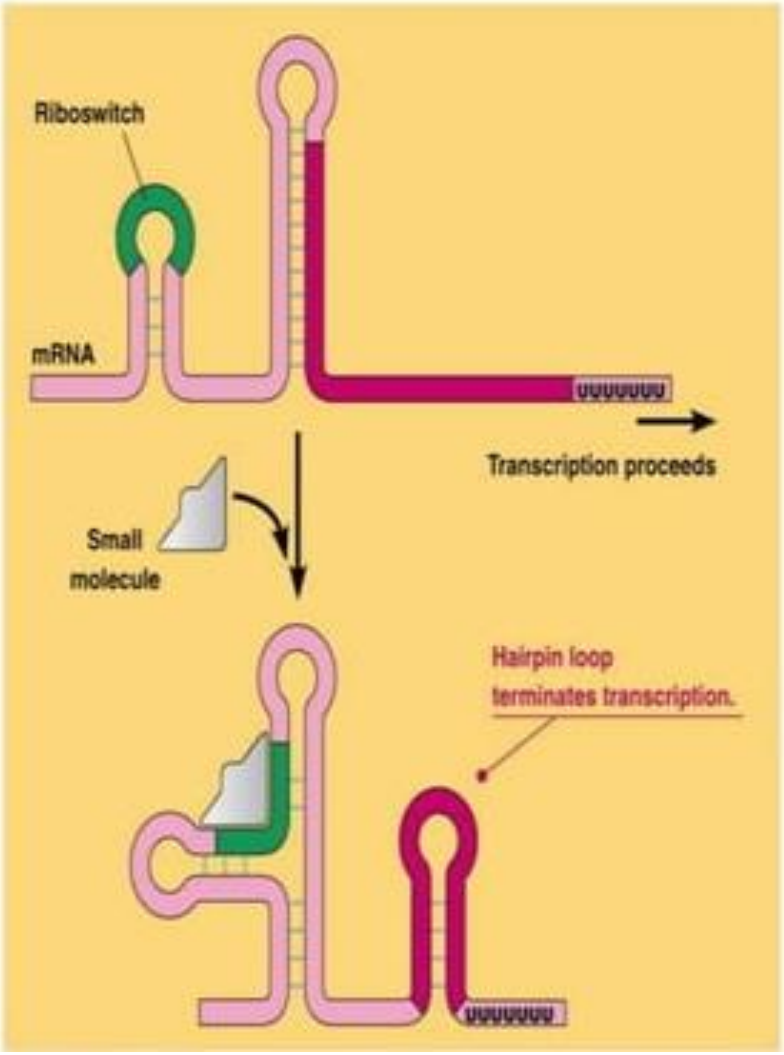
B}When no metabolite is bound, the Shine-Dalgarno (SD) site is exposed- ribosome can bind and initiate translation. Binding of the metabolite to the 5' leader region of the mRNA induces the formation of an SD:anti-SD stem-loop structure that masks the ribosome binding site such that initial step of translation,, is not achieved .

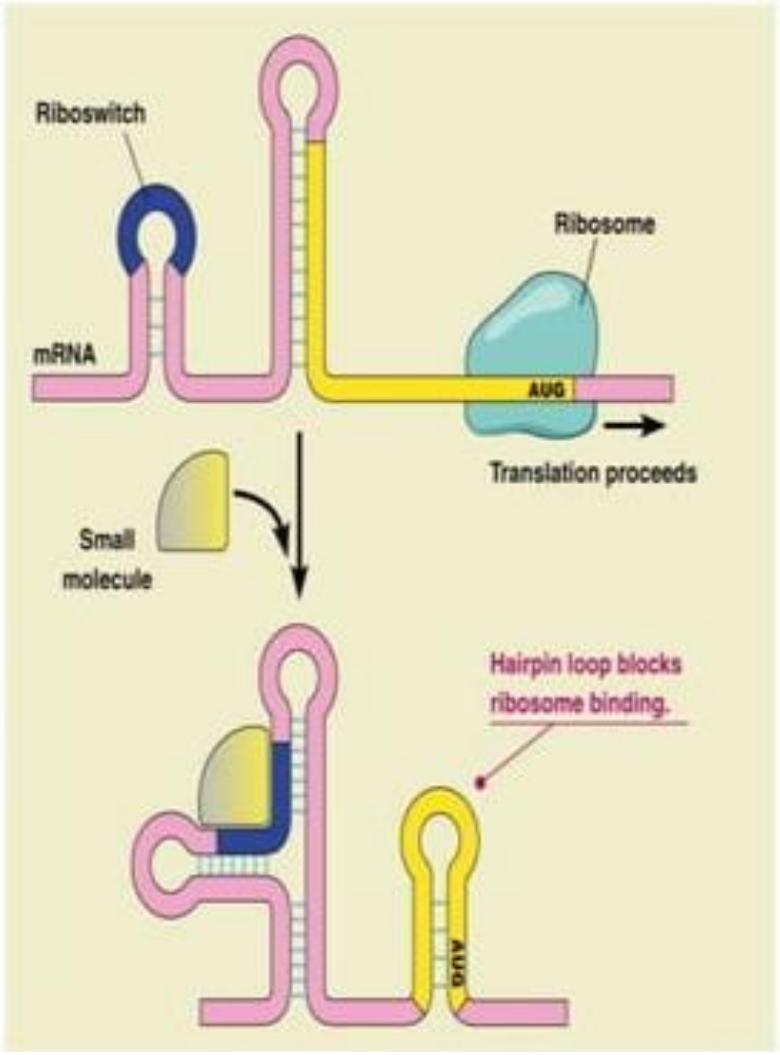


C} that the conformational change induced by the binding of the ligand to the riboswitch brings adjacent nucleotides in line with each other in an orientation that favours cleavage-ribozyme action eg. *Glms* box.

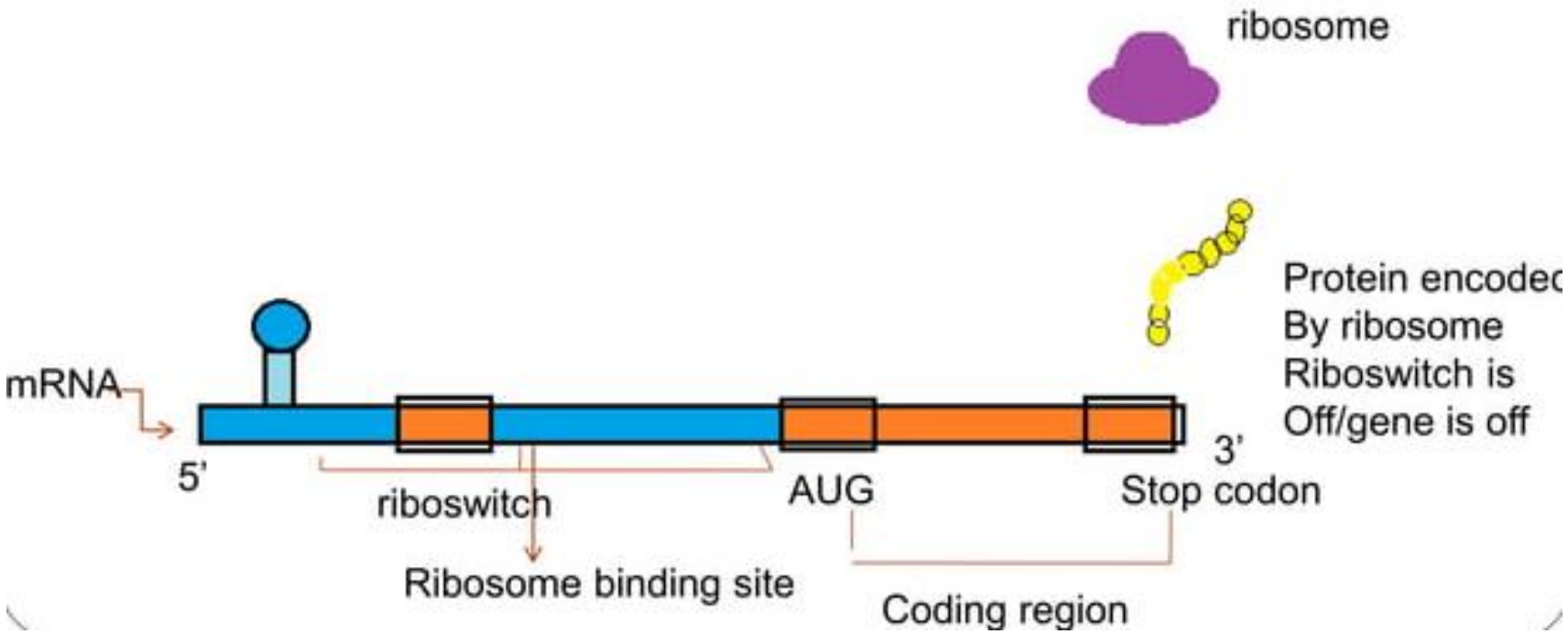
(a) Transcription termination. Binding of a small molecule to a riboswitch in the leader sequence of some mRNAs triggers the formation of a hairpin loop that terminates transcription. FMN binding to the leader sequence of the mRNA transcribed from the *rib* operon of *B. subtilis* works in this way.

(b) Translation initiation. In other mRNAs, a small molecule binding to a riboswitch triggers formation of a hairpin loop containing the site where ribosomes normally bind, thereby interfering with translation initiation. In *E. coli*, this type of control is used by FMN to inhibit translation of mRNAs coding for enzymes involved in FMN synthesis.

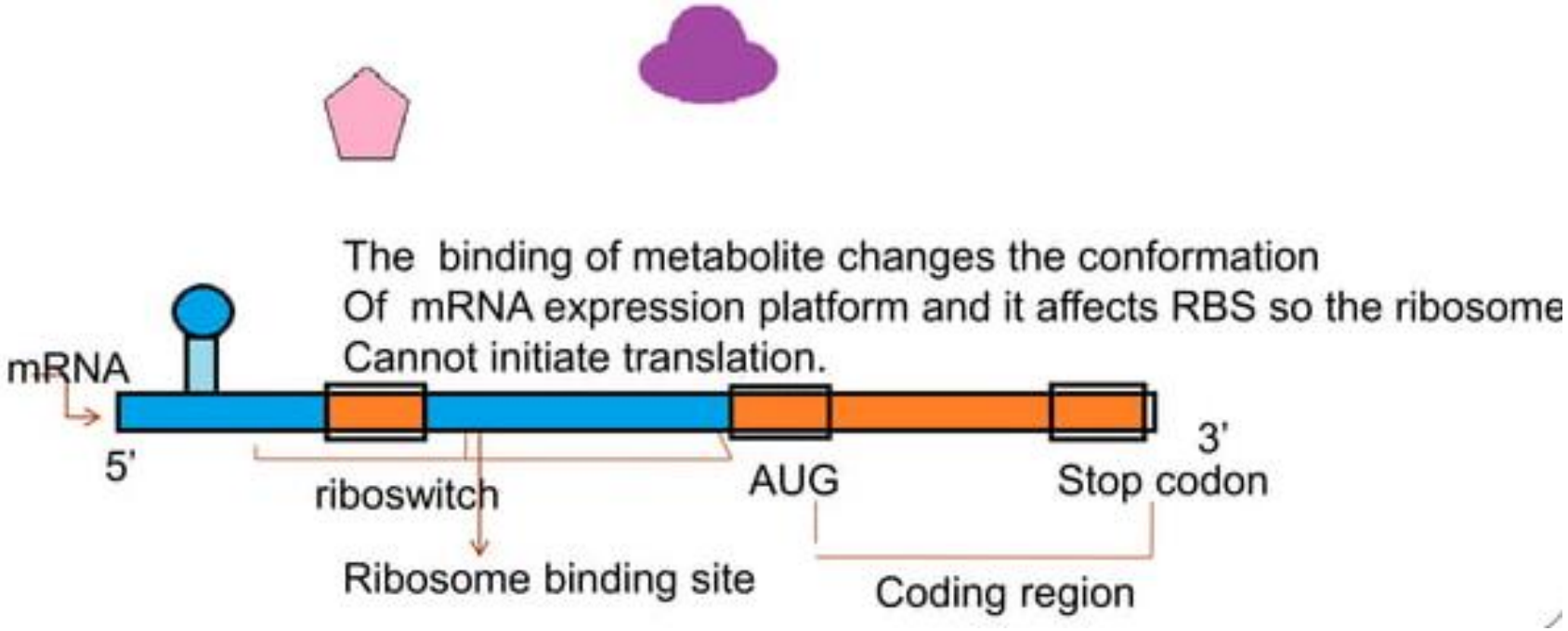




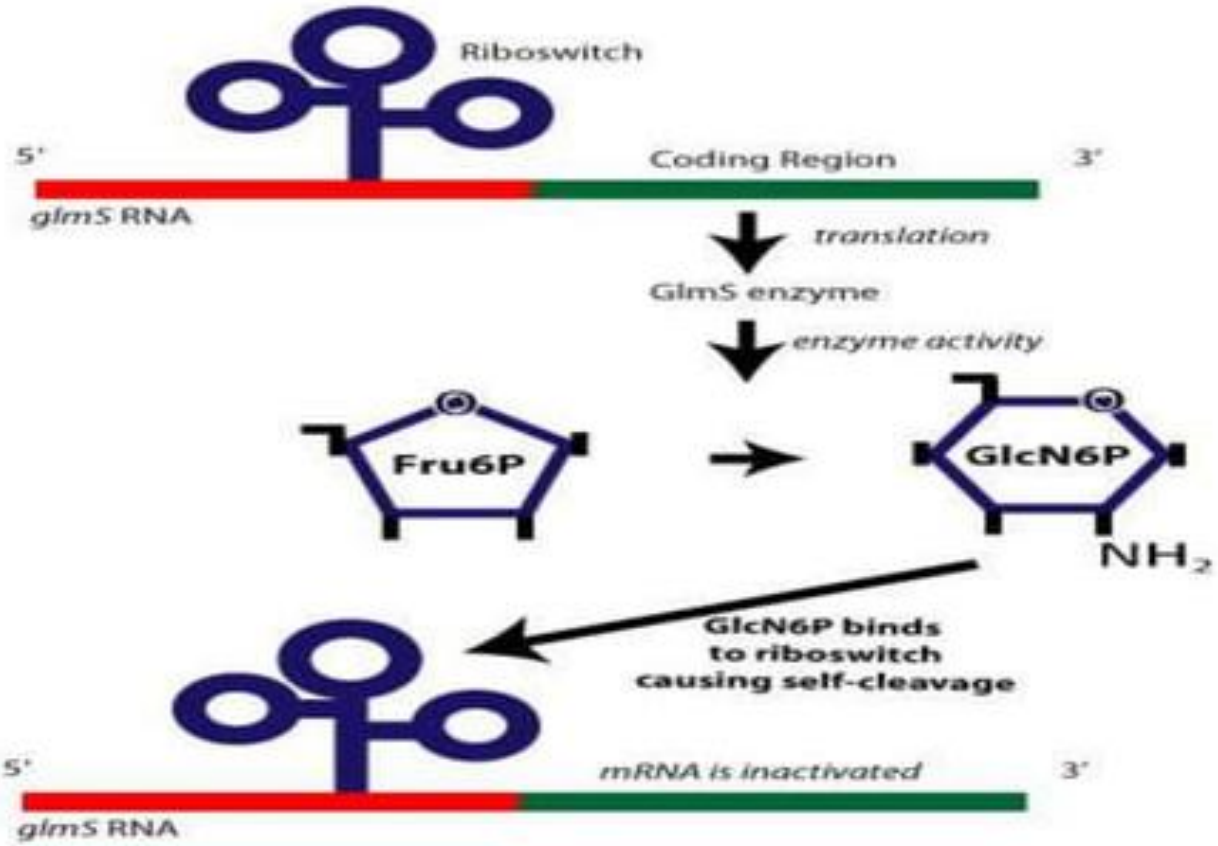
REPRESSION OF TRANLATION INITIATION



Riboswitch is on..in presence of metabolite



Auto-cleavage



Other methods of action

- All of the riboswitch effects described above have related to repression of gene function upon binding of a specific metabolite involved somehow in that gene's function.
- It has, however, also been suggested that the same mechanisms could be involved in positive gene regulation as well
- In these cases, binding of a metabolite would release the terminator hairpin or liberate the SD-site, permitting full transcription or translation, respectively. Although this type of control has not yet been observed in natural systems, there is no reason to rule it out.

Identifying riboswitches

- ***sequence analysis*** -riboswitch elements are well-conserved across genera. Two databases are available : Breaker Lab Intergenic Sequence Server (BLISS) and the Riboswitch Finder based on known riboswitch sequences and scan entries for related motifs,able to predict the structure of the putative riboswitches, can evaluate the statistical probability of a positive identification, and have a low false-positive rate
- ***lacZ* fusions**- gene of interest is amplfied and ligated in lacZ gene. B-galactosidase expression is under the control of riboswitch . Induceres are added and the effect can be measured with ß-galactosidase enzyme assays to detect the levels of LacZ in the cell
- ***in-line probing and equilibrium dialysis***

applications

- Synthetic analogs of riboswitch ligands could be engineered to shut off central metabolic pathways, arresting the growth of the bacteria-less toxic as RNA is targeted instead of protein.
- Can be used in the synthetic aptamers that led to their discovery: as molecular chemosensors for measuring chemical composition or biochemical secretions
- using riboswitch fusions to trans-genes as a means to regulate gene inserts through small molecule inducers . This could have widespread applications in genetic research, and even in medicine and gene therapy.
- use of riboswitches in taxonomic studies-Though regions of riboswitches are well-conserved, there are distinct variable regions that have been indicated as being dependant on taxonomy.

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Thank you