Riboswitches

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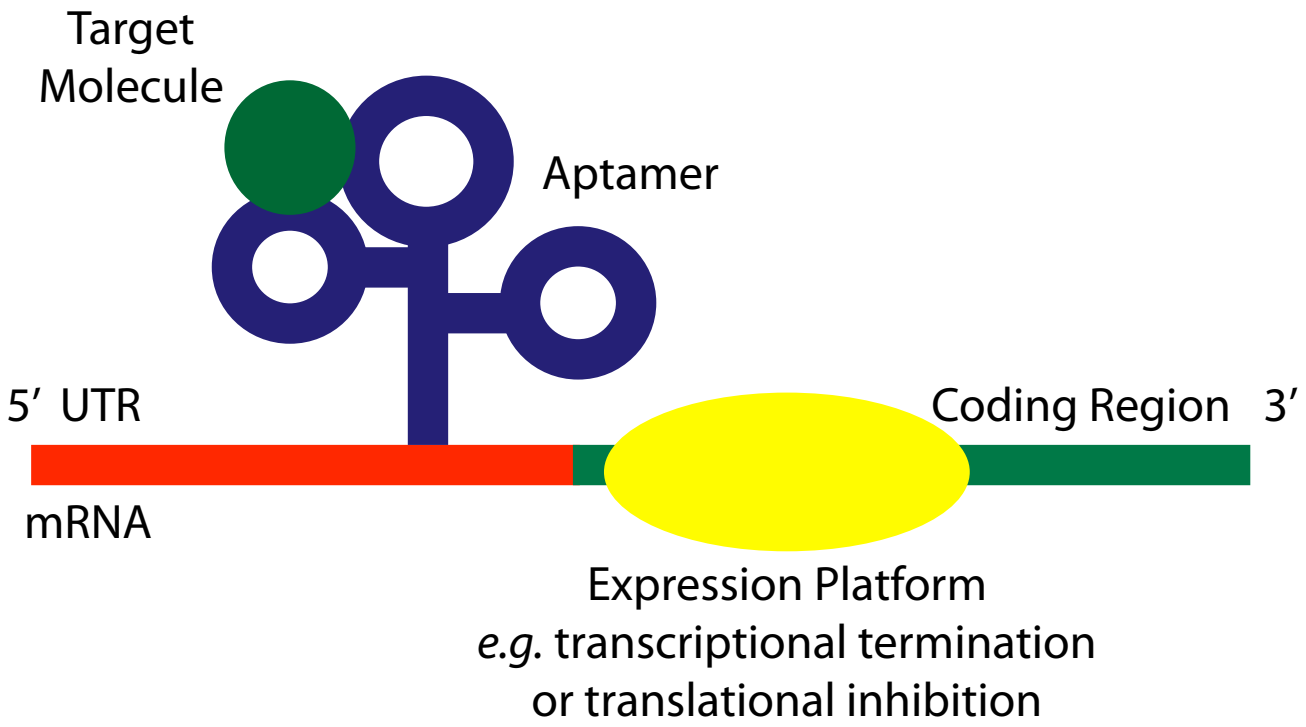
Discovery

- **1998:** Tina Henkin and coworkers observe a conserved sequence in the 5' UTR of the S-box gene family involving the biosynthesis of MET and CYS
  - conformational change when the 5' UTR bound a negative regulatory factor.
- **1999:** Ron Breaker works on synthetic RNAs, called aptamers, which bind to small molecules. He's surprised that they hadn't been observed *in vivo*.
- **2002:** Breaker demonstrates that mRNAs can bind metabolites directly in the absence of proteins. He coins the term 'riboswitch'.
  - first natural riboswitches found in genes involved in B1 and B12 biosynthesis.
- Several gene regulation 'mysteries' can now be explained by riboswitches.
- More than 200 unique riboswitches that have been categorized into about 9-12 classes
- Most riboswitches studied have been in bacteria, however recent studies have found some in eukaryotes.

Biogenesis & Function

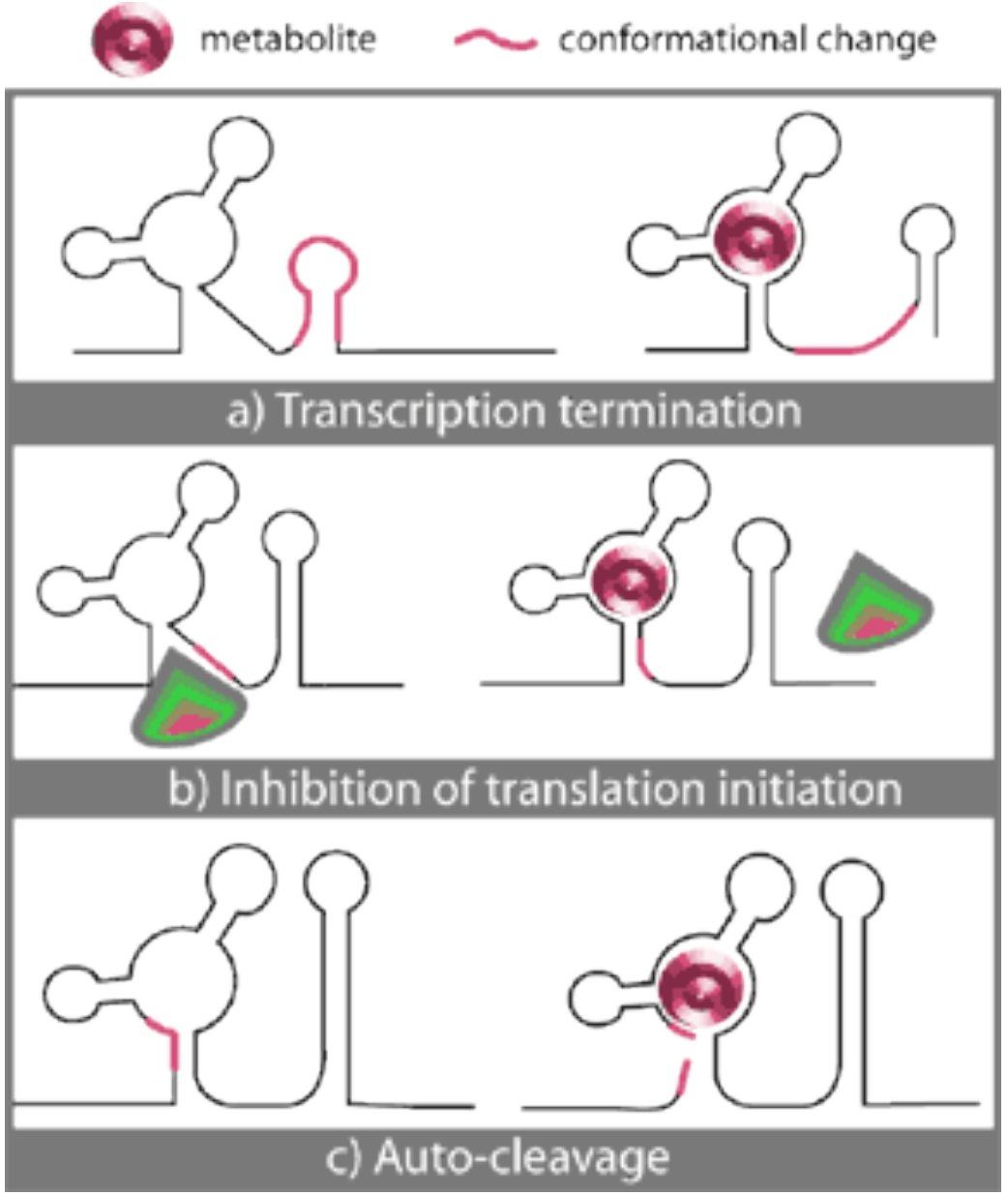
- Sequences of RNA located in the 5' untranslated region of mRNAs
- Bound *directly by small ligands*
  - vitamins, such as riboflavin, thiamine and cobalamin
  - amino acids, such as methionine and lysine
  - purine nucleotides (adenine, guanine)
- Binding affects the secondary structure of mRNA containing the riboswitch, exerting a regulatory function

Generalized Riboswitch



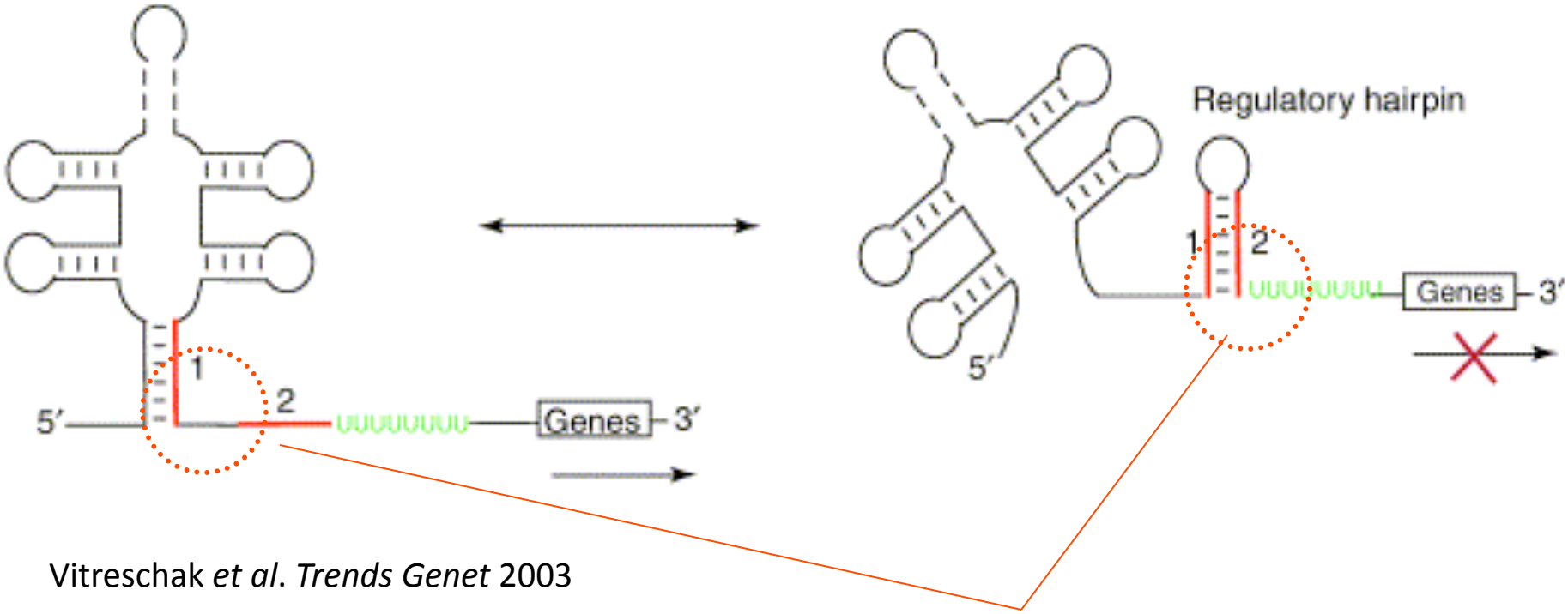
Mechanism

- Riboswitches form 3D conformations capable of specifically binding a small ligand
- Binding of the ligand stabilizes one particular conformation
  - If no ligand is bound, a different conformation becomes more energetically favorable



Colette Davis, Science Creative Quarterly, Sept 07

Mechanism:
Transcriptional Inhibition

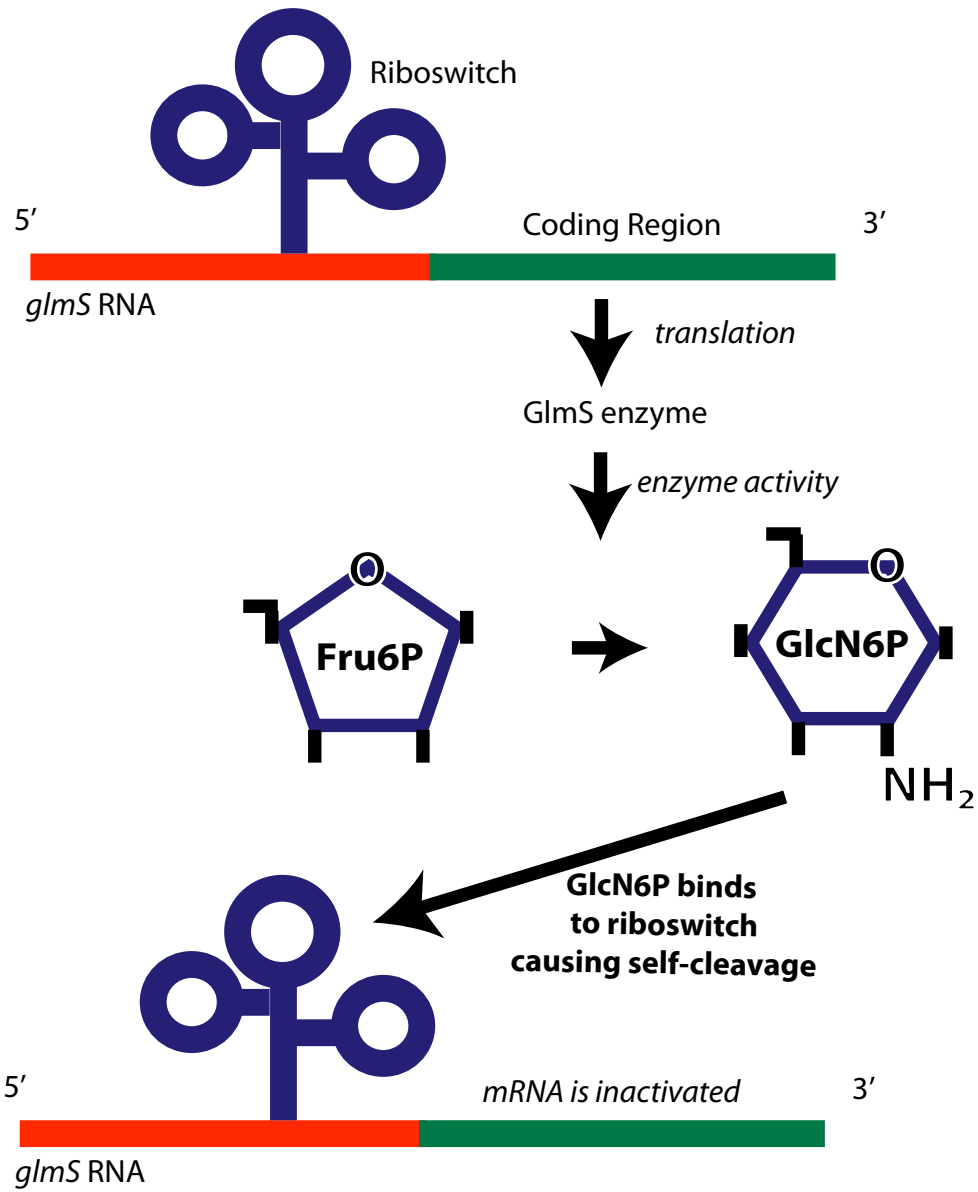


Vitreschak *et al*. *Trends Genet* 2003

Note the different secondary structures formed by sequences 1 and 2. When base-paired they become part of a transcription terminator structure!

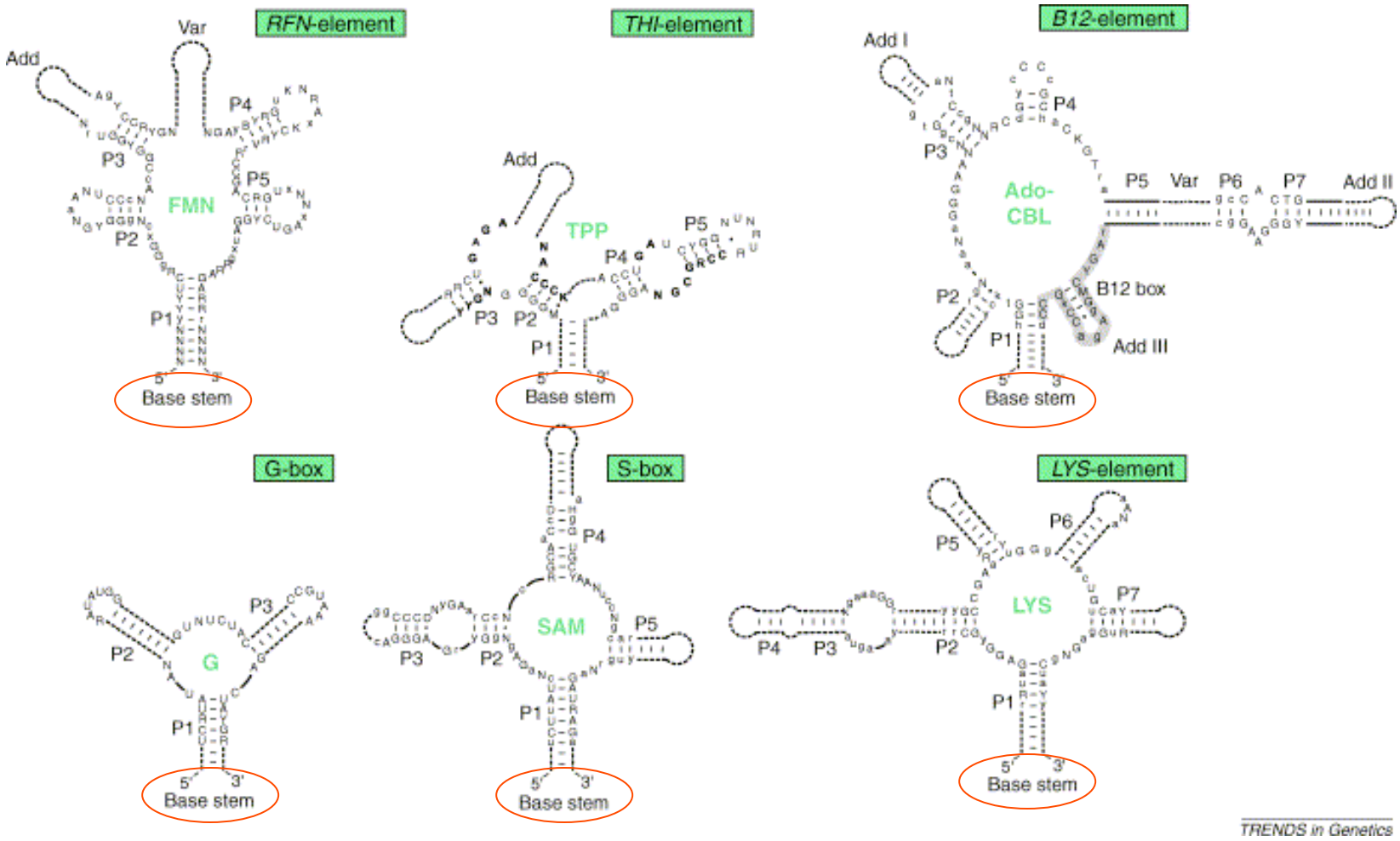
Mechanism: *glmS* Riboswitch

- Self-cleaving riboswitch regulating production of glucosamine-6-phosphate (GlcN6P).
- GlcN6P binds and activates the ability to cleave the RNA in an intramolecular reaction.
- Winkler *et al.* 2004



Riboswitch Structure Types

- Type I riboswitches are comprised of a preorganized tertiary complex with a single catalytic pocket.
  - Purine riboswitches, the *glmS* riboswitch, and the SAM-II riboswitch
- Type II riboswitches are made up of a two party catalytic pocket whose tertiary structure is induced by ligand binding.
  - TPP riboswitch, the SAM-I riboswitch, and the M-box magnesium riboswitch (Montagne & Batey 2008)



Some examples of riboswitch secondary structure

All known riboswitches fold into compact RNA secondary structures with a **base stem, a central multi-loop and several branching hairpins**

Nomenclature

- Riboswitches have no official nomenclature scheme.
- They are named after the metabolite or catalytic elements they bind.
- For example, the riboswitch that binds purines is called the 'Purine Riboswitch’.

Group	Members	Natural Ligand	Size (nt)	Distribution
Coenzymes	TPP (also THI-box)	TPP, thiamine pyrophosphate	100	Bacteria, archaea, eukaryotes
	FMN (also RFN-element)	FMN, flavin mononucleotide	120	Bacteria
	AdoCbl (also B_{12}-element)	AdoCbl, adenosylcobalamin	200	Bacteria
	SAM-I (also called S-box)	SAM, S-adenosylmethionine	105	Mostly Gram+ bacteria
	SAM-II	SAM, S-adenosylmethionine	60	α- and β-proteobacteria
	SAM-III (S_{MK})	SAM, S-adenosylmethionine	80	Gram– bacteria
Amino Acids	Lysine (also L-box)	Lysine	175	γ-proteobacteria, *Thermotogales*, *Firmicutes*
	Glycine (I+II)	Glycine	110	Bacteria
Nucleobases	Guanine (also G-box)	Guanine, hypoxanthine	70	Gram+ bacteria
	Adenine	Adenine	70	Bacteria
	$preQ_1$	preQ1, pre-quenosine-1	35	Bacteria
Self-cleaving mRNA	*glmS*	GlcN6P, glucosamine-6-phosphate	170	Gram+ bacteria

Adapted from Serganov & Patel *Nat Rev Genet* 2007

Evolution & Conservation

- Riboswitches may be one of the oldest of genetic control systems. (Vitreschak *et al*. 2004)
- Aptamer is highly conserved in even distantly related organisms
- Sequence and structure are far more varied in the expression platform.
- RNA world hypothesis, an ancient world based on RNA existed before DNA-based organisms. (Gesteland *et al.* 2006)

Synthetic Biology Tool

- Designer riboswitches could be customized to affect a gene's expression level.
- **2007:** Christina Smolke develops an entirely modular RNA-based gene-regulatory device.
  - library of aptamers developed around the hammerhead ribozyme (Win & Smolke 2007)
  - provides a universal system in which the ligand, aptamer, and expression platform are customizable.

Role in disease

- Lysine analogs that bind to lysine riboswitches have antibacterial activity (Blount *et al*. 2007)
- Engineered control of gene expression could be applied to gene therapy (Barrick & Breaker 2007)
  - riboswitch could function as a highly specific sensor for a benign drug-like molecule.

Bioinformatic Tools

- mfold: software for folding nucleic acids
- riboswitch finder
- riboswitch explorer
- Rfam: RNA families database of alignments and CMs
- BLISS (Breaker Lab Intergenic Sequence Server)

Thank You!

Questions?