RIBOSWITCH.

What is it?
Ron Breaker (Yale) coined the name to describe the properties of an RNA sequence/structure that controlled transcription or translation through binding to a ligand and subsequently altering its conformation.

As a mechanism to regulate translation initiation, in the ON conformation, the riboswitch structure allows access to the Ribosome Binding Site (RBS). In the OFF conformation, when the metabolite (here, SAM) is bound, the RBS is part of a hairpin and not accessible to the ribosome. (Winkler & Breaker, 2005. AnnRevMicro 59:487-517).
As a transcriptional regulator, when the riboswitch is in the ON conformation, the mRNA is transcribed. In the OFF conformation, an alternative stemloop forms and with the downstream (U)n tract, forms an intrinsic (rho-independent) transcriptional terminator.

The conformational change from ON to OFF is triggered by binding of a small molecule. Typically, it’s the metabolite regulated by the operon.
More riboswitch facts:
1. There are currently eleven classes of riboswitches in the 5’ UTR of bacterial mRNAs.
2. TPP (thiamine pyrophosphate)-sensing riboswitches are found in bacteria, fungi, and plants.
3. Riboswitches are typically located immediately adjacent to genes that transport or synthesize the metabolite they recognize.

These are some aptamer domains.
More pertinent riboswitch facts:
1. Gram positive bacteria prefer transcription termination mechanisms.
2. Not all attenuation is through the intrinsic terminator (e.g. E. coli thiC riboswitch)
3. Gram-negative bacteria preferentially use riboswitches that sequester the Shine-Delgarno sequence
4. Affinities for ligands vary from nM to μM.
Purine riboswitch

TPP riboswitch

SAM riboswitch

glmS ribozyme
In-line probing is used by the Breaker group to characterize the structures of riboswitches with and without their ligands. The principle: spontaneous cleavage of the phosphodiester backbone in the presence of Mg$^{2+}$. These are internal transesterification reactions that involve the 2’ OH and Mg$^{2+}$. This reaction requires a specific geometry (SN$_2$-inline displacement reaction) that does not correspond to typical conformations in structured regions of an RNA (especially duplex regions). However, flexible regions of the RNA can access this geometry, at least transiently with some probability. To enhance the efficiency of the transesterification reaction and thus backbone cleavage, the reactions contain 20 mM Mg$^{2+}$ and are run at room temperature for 40 hours.

Why room temperature for 40 hours?
An mRNA structure that controls gene expression by binding FMN.

The *RFN* element is a highly conserved domain that is found frequently in the 5′-untranslated regions of prokaryotic mRNAs that encode for flavin mononucleotide (FMN) biosynthesis and transport proteins. We report that this domain serves as the receptor for a metabolite-dependent riboswitch that directly binds FMN in the absence of proteins. Our results also indicate that in *Bacillus subtilis*, the riboswitch most likely controls gene expression by causing premature transcription termination of the *ribdeaht* operon and precluding access to the ribosome-binding site of *ypaA* mRNA. Sequence and structural analyses indicate that the *RFN* element is a natural FMN-binding aptamer, the allosteric character of which is harnessed to control gene expression.
≈1 nM 5′32P-labeled RNA was incubated for ≈40 h at 25°C in 20 mM MgCl2/50 mM Tris·HCl (pH 8.3 at 25°C)/100 mM KCl in the presence or absence of added ligand (FMN, FAD, or riboflavin) at concentrations that are indicated for each experiment.
Nucleotides that become less reactive when FMN is bound.
Binding is specific to FMN.

A) Chemical structures of riboflavin, FMN, and FAD.

B) Region around 165 ribD with different symbols indicating regions 1, 2, 3, 4, and 5.

C) Fraction cleaved (normalized) as a function of log c (FMN, M) and log c (effector, M) for 165 ribD with different effectors (FMN, FAD, riboflavin).

Graphs show the relationship between concentration and fraction cleaved for different effectors.
The Speed of RNA Transcription and Metabolite Binding Kinetics Operate an FMN Riboswitch

Transcribe 5' to 3'

DNA

RNA
NusA is a protein that binds to bacterial RNAP, with the result that transcription is slower.
This riboswitch is not at thermodynamic equilibrium at the time the choice is made to transcribe or terminate. Therefore, this decision is kinetically driven.

Solid lines show fast or likely transitions. Dashed lines are unlikely or slow transitions. FMN dissociation is slow compared to switching time.
The Purine riboswitch operates in two modes.

**add riboswitch** Translation activation (thermodynamic regulation / post-transcriptional binding)

Shine-Delgarno (GAA) and AUG start are paired in a stem. Adenine binding shifts the equilibrium.

**pbuE riboswitch** Transcription antitermination (kinetic regulation / co-transcriptional binding)

Low Adenine concentration leads to OFF state. High concentration can co-transcriptionally bind leading to the ON state.
The purine riboswitch is found in many prokaryotes.

Fig. 1. Secondary structure and positional entropy plot of the aptamer domain of purine riboswitches. (a) The conserved secondary structure of the aptamer domain is depicted such that each nucleotide position is represented as a colored dot with Watson–Crick base-pairing interactions shown as a bar/line connecting the interacting nucleotides. Nucleotide coloring corresponds to their degree of conservation, where a value of 1 denotes universal conservation (adapted from the Rfam database, accession number RF00167). (b) A sequence logo representing the nucleotide conservation as well as information content at each position in our structure-based alignment of 302 sequences. For each position, the height of each nucleotide symbol represents its frequency at that position, while the overall height represents the information content at that position.

The purine riboswitch

Ions in the crystal structure. What do they do?
A spectroscopic method to study the ligand binding process

Examine adenine binding by the purine riboswitch. Replace a single nucleotide with the fluorescent nucleotide 2-aminopurine (2AP).

When the riboswitch undergoes a conformational change, 2AP fluorescence could increase or decrease (or not change), reporting on the timescale of the binding and also the folding pathway.

What do you think you would observe if adenine were added first, then Mg$^{2+}$?

Folding of a riboswitch.

Transcription termination efficiency (TE) in solution and under load.

A. From in vitro transcription assays, analyzed by gel electrophoresis. T termination, R is full length transcript (mean, SEM).

B. TE as a function of tension applied to the RNA. Data from single-molecule recordings.

Data without adenine were fit by a model of intrinsic termination.

Equilibrium unfolding forces for adenine-bound aptamer and terminator hairpin are shaded orange and gray, respectively.
A. At 8.1 pN, the aptamer rarely forms, so there is no adenine dependence.
B. At 5.8 pN with 300 μM adenine, run-through traces are fit by a simple folding model, consistent with aptamer formation.
C. At 5.8 pN without adenine, termination is common. Run-through traces are not consistent with aptamer formation. With adenine, run-through traces predominate, following aptamer folding.
The lock and key paradigm of substrate binding clearly fails for these riboswitches. Instead, there is a coupled binding/folding that needs a new formalism to describe it. There are two popular models that you’ll find in the literature: Induced Fit, and Conformational Selection. Are they mutually exclusive mechanisms, or are they the same mechanism with different names, or can both be present in the same system?

**FIGURE 5.** Two models for molecular recognition: induced fit and conformational selection. In conformational selection, the binding-competent conformation (C2) is pre-existing in solution before the addition of ligand (L). In induced fit, initial binding contacts (C1*L) between ligand and receptor induce conformational rearrangements to achieve the conformation C2*L of the complex.