Regulation of Bacterial Gene Expression by Riboswitches

Introduction

- Microorganisms experience a wide variety of fluctuating conditions caused by:
  - Changes in their surroundings
  - Shifting metabolic demands

- Cells must be able to:
  - Quantify these changes
  - Concordantly alter expression of gene subsets in a measured manner.

Regulation of gene expression

- Levels of gene expression regulation
  - Transcription
  - Translation
- Regulatory protein factors
- **Regulatory RNA factors**

RNA Factors

- Regulatory RNA structures → often used for post-transcriptional control of essential genes in bacteria.
  - **Trans-acting RNA elements**
    - Small RNA
    - Riboregulator
  - **Cis-acting RNA elements** → located within the non-coding portions of mRNAs

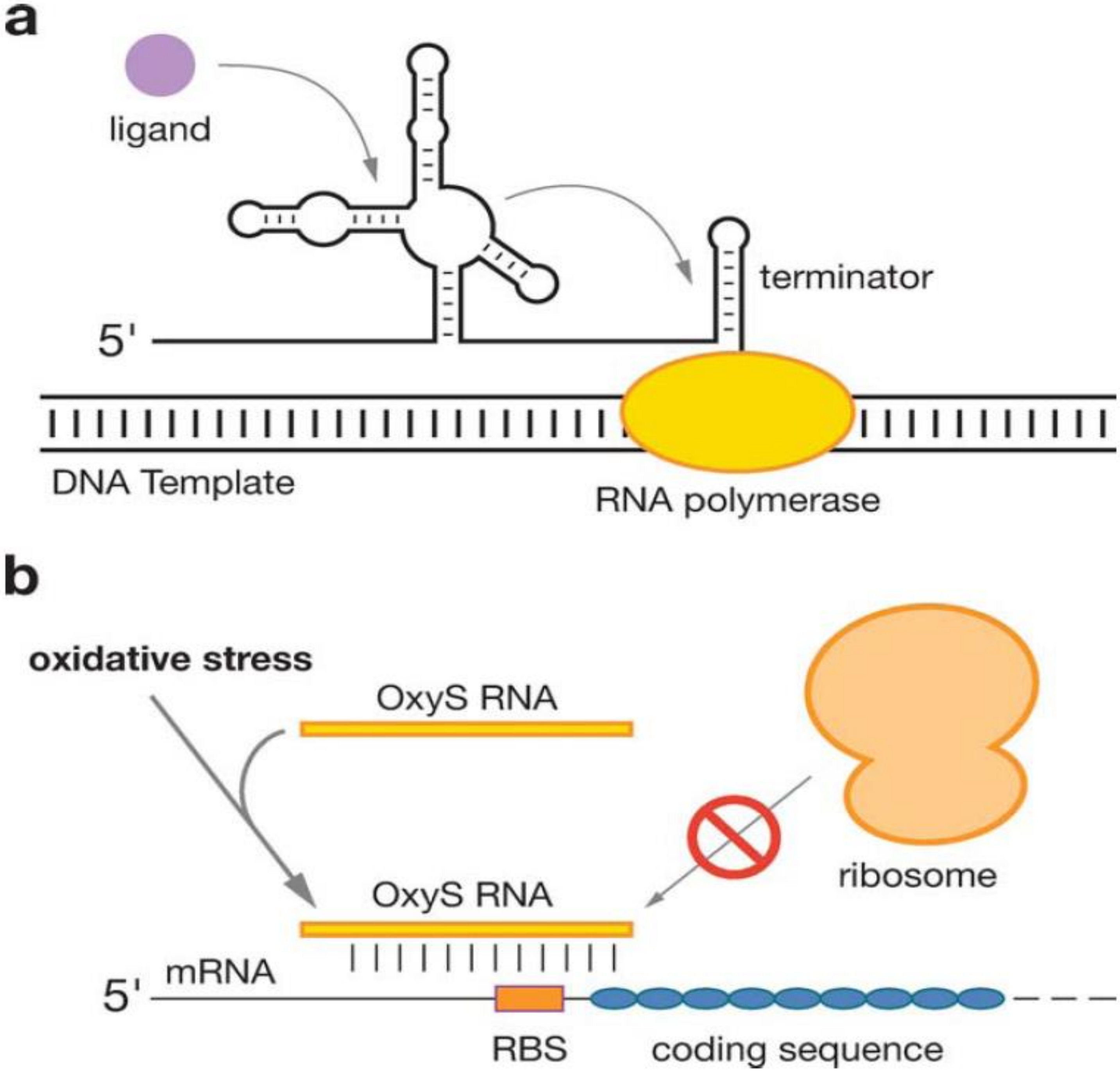


Figure 1

Examples of gene expression regulation by *cis*- and *trans*-acting RNAs. (*a*) A SAM-dependent riboswitch located within the 5′ UTR of an mRNA acts in *cis* to inhibit transcription of the full-length mRNA upon ligand binding (16, 52, 108). (*b*) During conditions of oxidative stress the OxyS riboregulator, a *trans*-acting RNA, interacts with *fhlA* transcripts and inhibits translation initiation (2). RBS, ribosome-binding site.

RNA Factors

- *Cis*-acting regulatory RNAs:
  - Short RNA sequences
  - Most of them located within the 5`-UTRs of transcripts
  - Intricately folded RNAs
  - Carry high-affinity receptors for effector molecules

RNA Factors

- *Cis*-acting RNAs in *B. subtilis* → greater than 4% of its genes are regulated

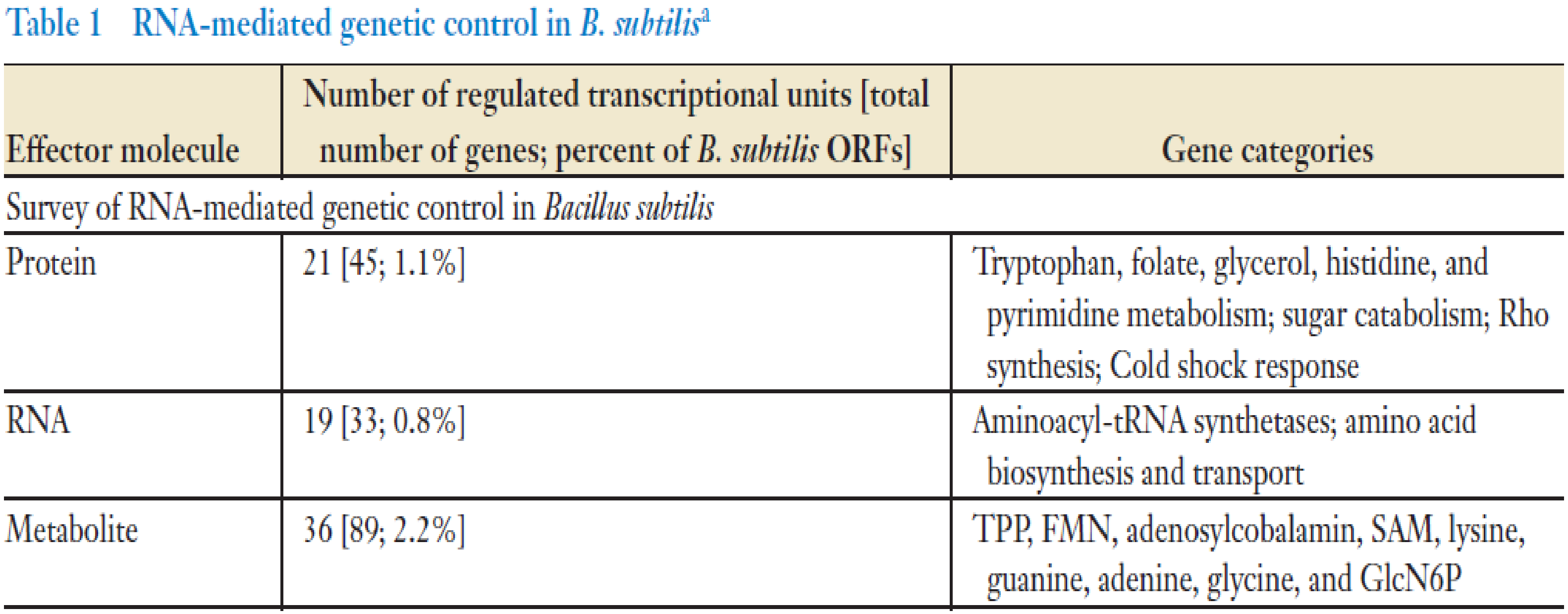


Table 1 RNA-mediated genetic control in *B. subtilis*[a]

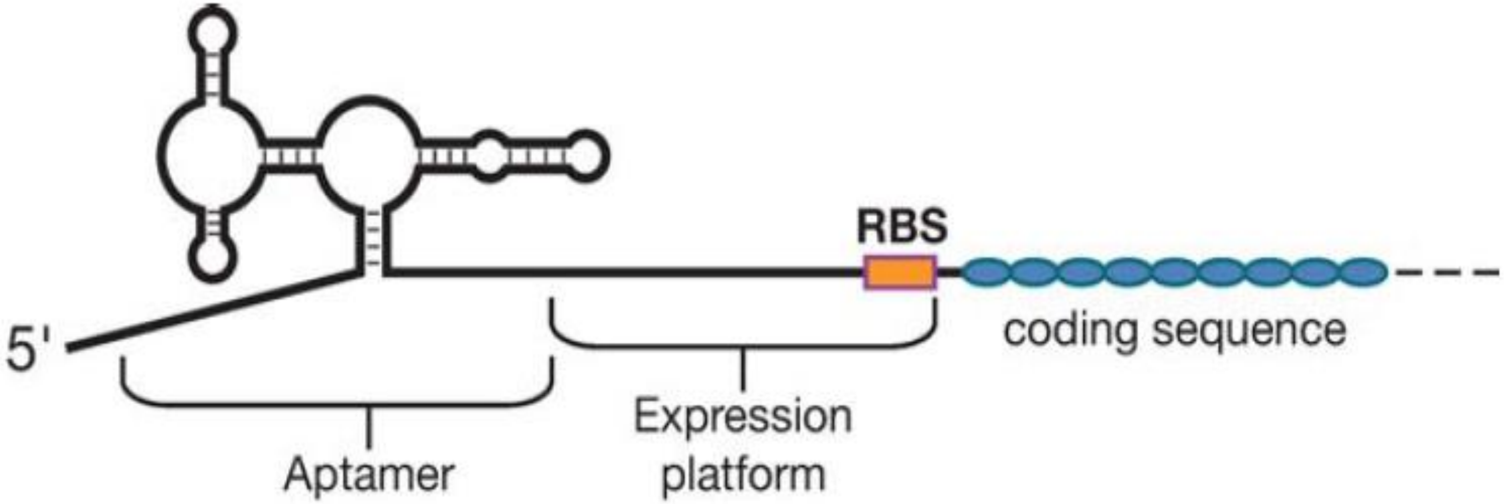
Effector molecule	Number of regulated transcriptional units [total number of genes; percent of *B. subtilis* ORFs]	Gene categories
Survey of RNA-mediated genetic control in *Bacillus subtilis*		
Protein	21 [45; 1.1%]	Tryptophan, folate, glycerol, histidine, and pyrimidine metabolism; sugar catabolism; Rho synthesis; Cold shock response
RNA	19 [33; 0.8%]	Aminoacyl-tRNA synthetases; amino acid biosynthesis and transport
Metabolite	36 [89; 2.2%]	TPP, FMN, adenosylcobalamin, SAM, lysine, guanine, adenine, glycine, and GlcN6P

Metabolite- sensing RNAs (Riboswitches)

Overview

- Riboswitches:
  - First in 1970s → discovered several metabolic riboswitches for lysine
  - Cis-acting RNAs
  - Have complex sequence and structural features
  - Usually reside in the non-coding regions of mRNAs
  - They directly sense small molecule metabolite concentrations and control gene expression (genes contributing in metabolite production).
  - Widespread throughout bacteria

Riboswitch Organization



~ 70-200 nt

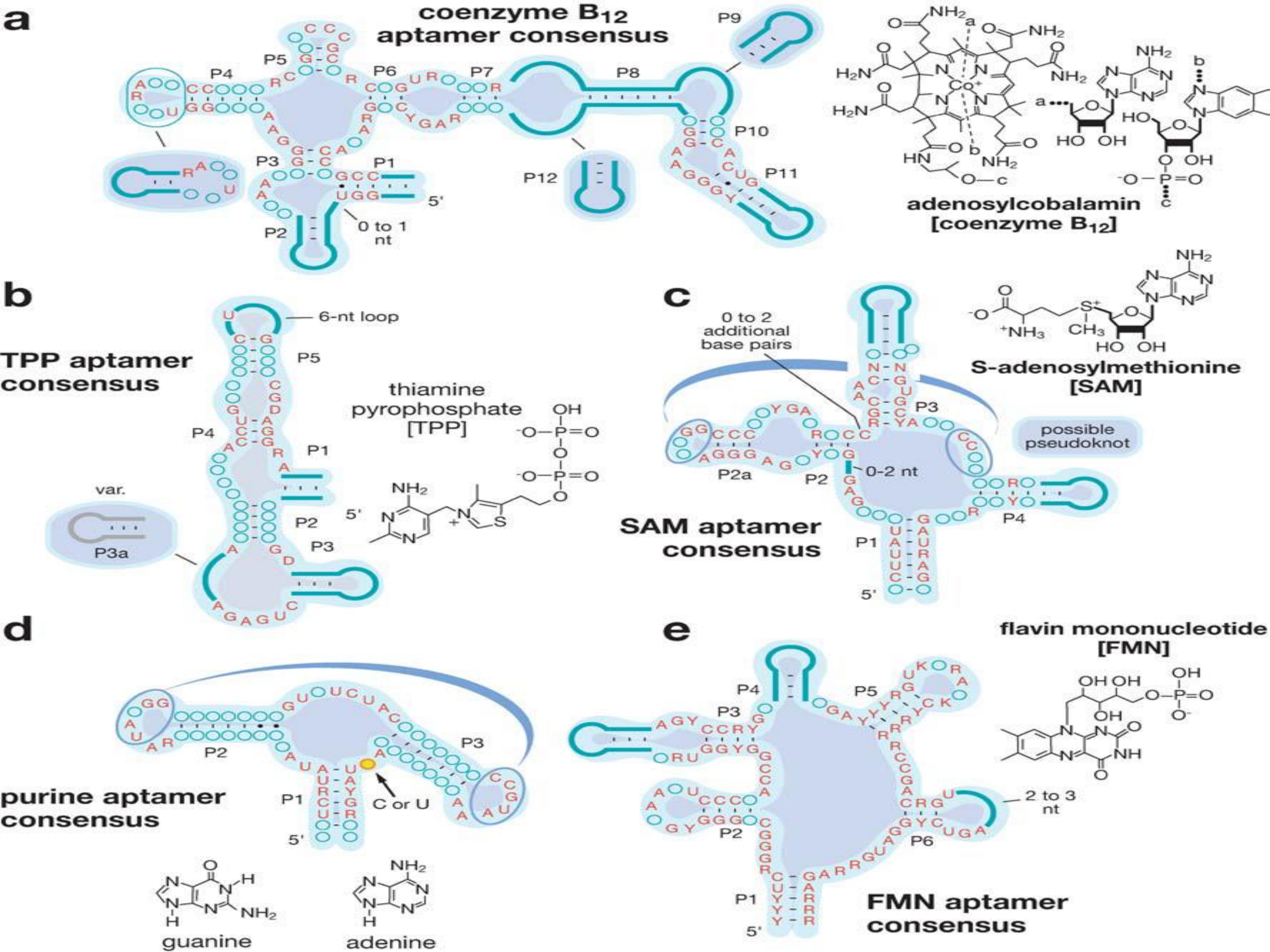
Highly conserved in sequence and structure

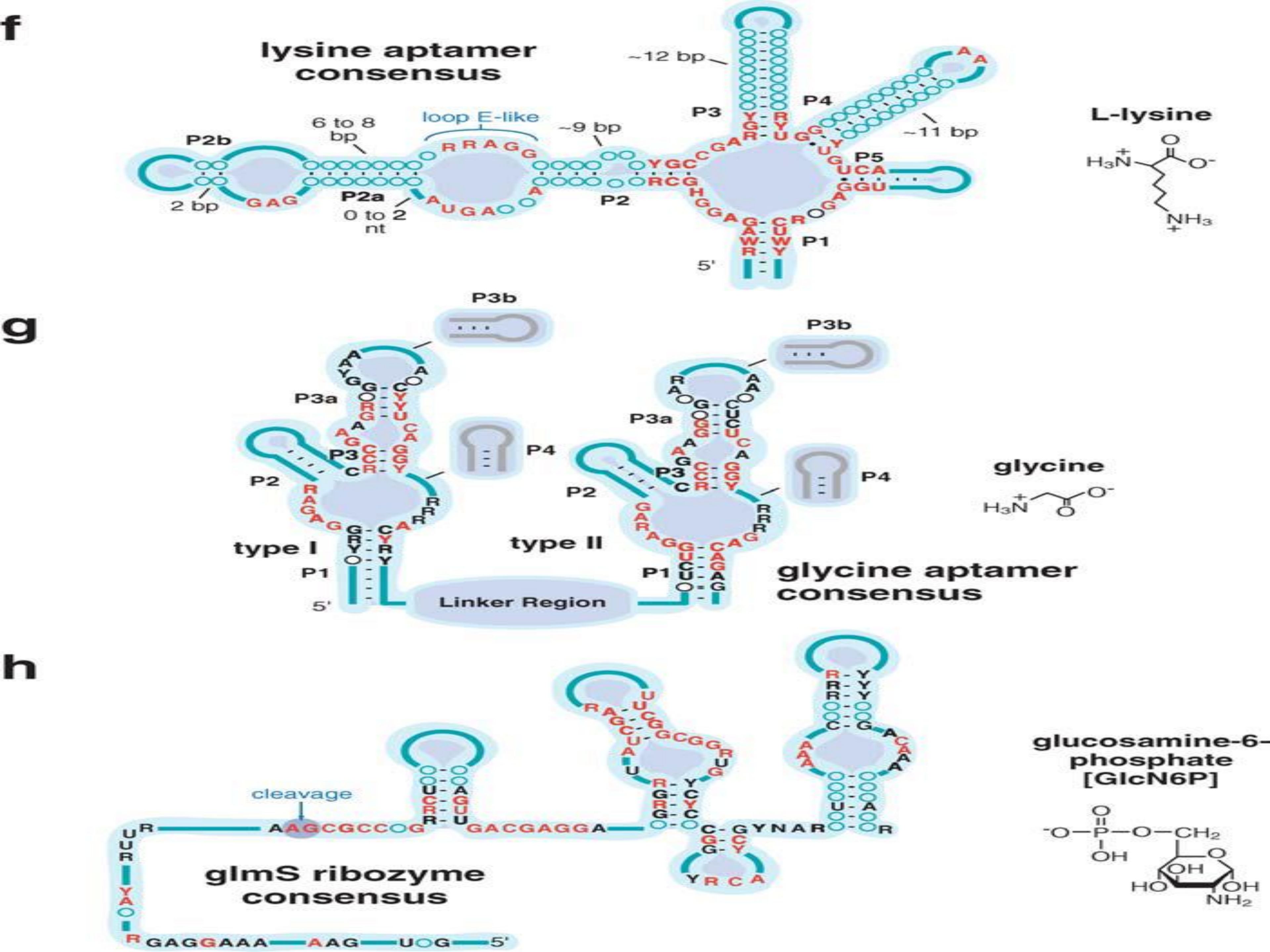
More diverse in sequence and size

Is functional domain

Riboswitches Classes

- To date, reports of nine separate riboswitch classes have been made:
  - Purine-specific riboswitches → Guanine and adenine riboswitches
  - FMN riboswitch → Flavin mononucleotide riboswitches
  - Coenzyme B12 riboswitch → Adenosylcobalamin-specific riboswitches
  - **TPP riboswitch → Thiamin Pyrophosphate riboswitches**
  - SAM riboswitch → S-adenosylmethionine riboswitches
  - Lysine riboswitch
  - GlcN6P riboswitch → Glucosamine-6-phosphate riboswitches
  - Glycine riboswitch
  - Orphan riboswitch candidates


a
coenzyme B_{12} aptamer consensus
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
5'
0 to 1 nt
adenosylcobalamin [coenzyme B_{12}]
b
TPP aptamer consensus
6-nt loop
P1
P2
P3
P3a
P4
P5
var.
5'
thiamine pyrophosphate [TPP]
c
SAM aptamer consensus
0 to 2 additional base pairs
0-2 nt
P1
P2
P2a
P3
P4
5'
possible pseudoknot
S-adenosylmethionine [SAM]
d
purine aptamer consensus
P1
P2
P3
5'
C or U
guanine
adenine
e
FMN aptamer consensus
P1
P2
P3
P4
P5
P6
5'
2 to 3 nt
flavin mononucleotide [FMN]


f
lysine aptamer consensus
~12 bp
P3
P4
~11 bp
P5
P2b
6 to 8 bp
loop E-like
~9 bp
2 bp
P2a
0 to 2 nt
P2
P1
5'
L-lysine
g
P3b
P3a
P4
P3
P2
type I
type II
P1
5'
Linker Region
glycine aptamer consensus
glycine
h
cleavage
glmS ribozyme consensus
5'
glucosamine-6-phosphate [GlcN6P]

Orphan Riboswitch Candidates

- They carry large aptamer domains
- Larger genetic targets →Associated with similar classes of genes
- Exhibit significant evolutionary conservation
- Exceedingly widespread in bacteria

Orphan riboswitch candidates

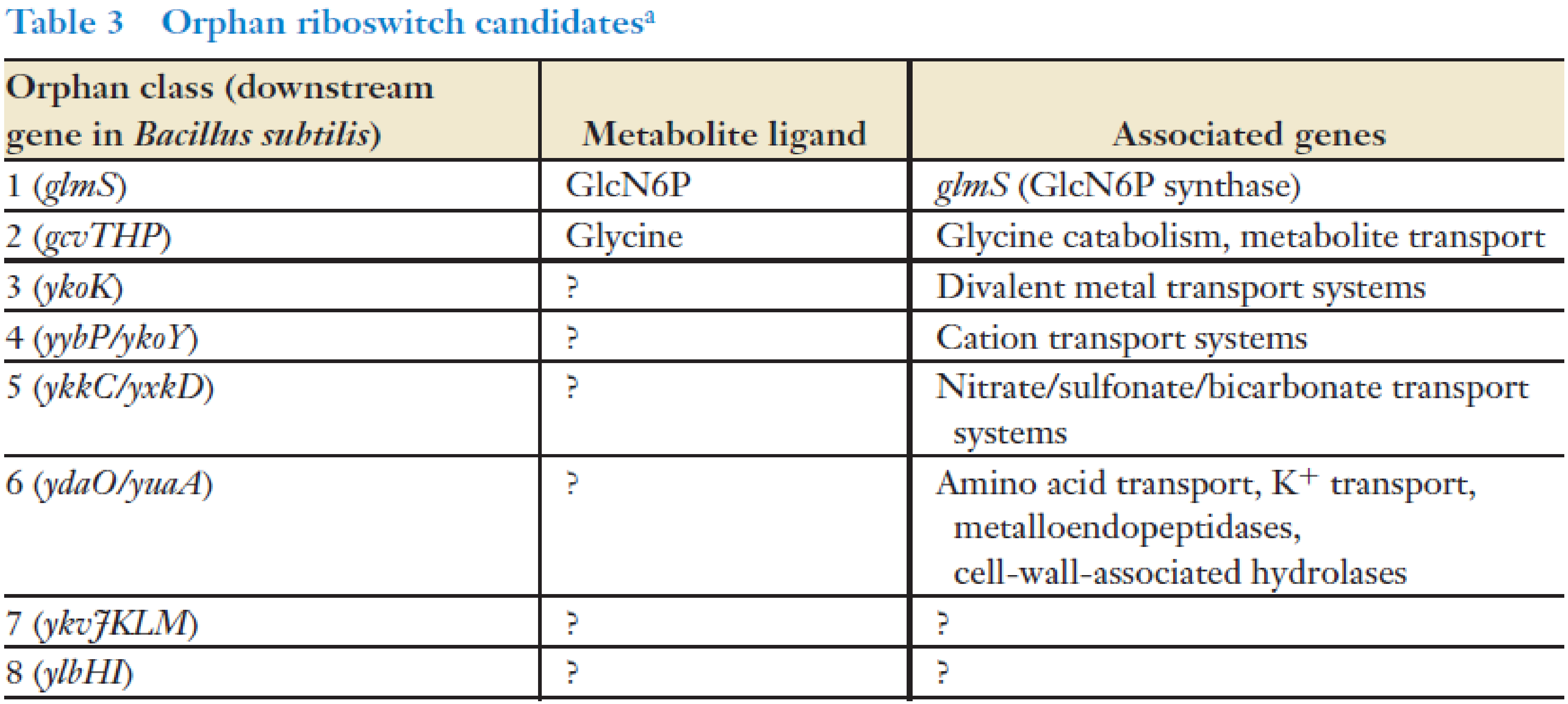
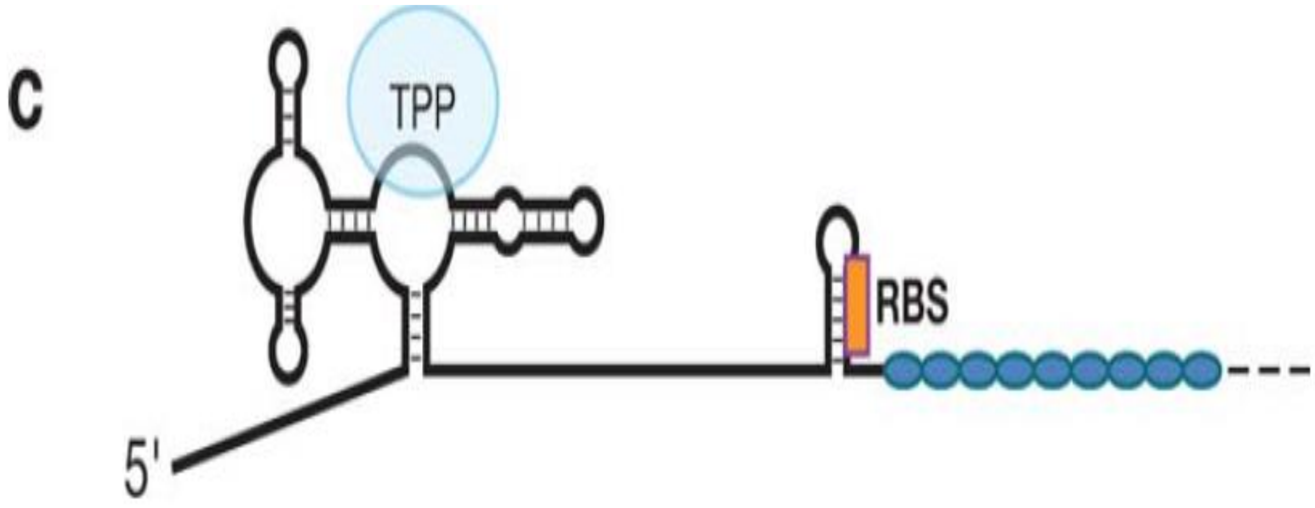


Table 3 Orphan riboswitch candidates[a]

Orphan class (downstream gene in *Bacillus subtilis*)	Metabolite ligand	Associated genes
1 (*glmS*)	GlcN6P	*glmS* (GlcN6P synthase)
2 (*gcvTHP*)	Glycine	Glycine catabolism, metabolite transport
3 (*ykoK*)	?	Divalent metal transport systems
4 (*yybP/ykoY*)	?	Cation transport systems
5 (*ykkC/yxkD*)	?	Nitrate/sulfonate/bicarbonate transport systems
6 (*ydaO/yuaA*)	?	Amino acid transport, K^+ transport, metalloendopeptidases, cell-wall-associated hydrolases
7 (*ykvJKLM*)	?	?
8 (*ylbHI*)	?	?

Riboswitch Genetic Control Mechanisms



- Typically turn off gene expression in response to the small molecule
- But some turn it on

Riboswitch Genetic Control Mechanisms

- **Transcription Attenuation**
- **Control of Translation Initiation**

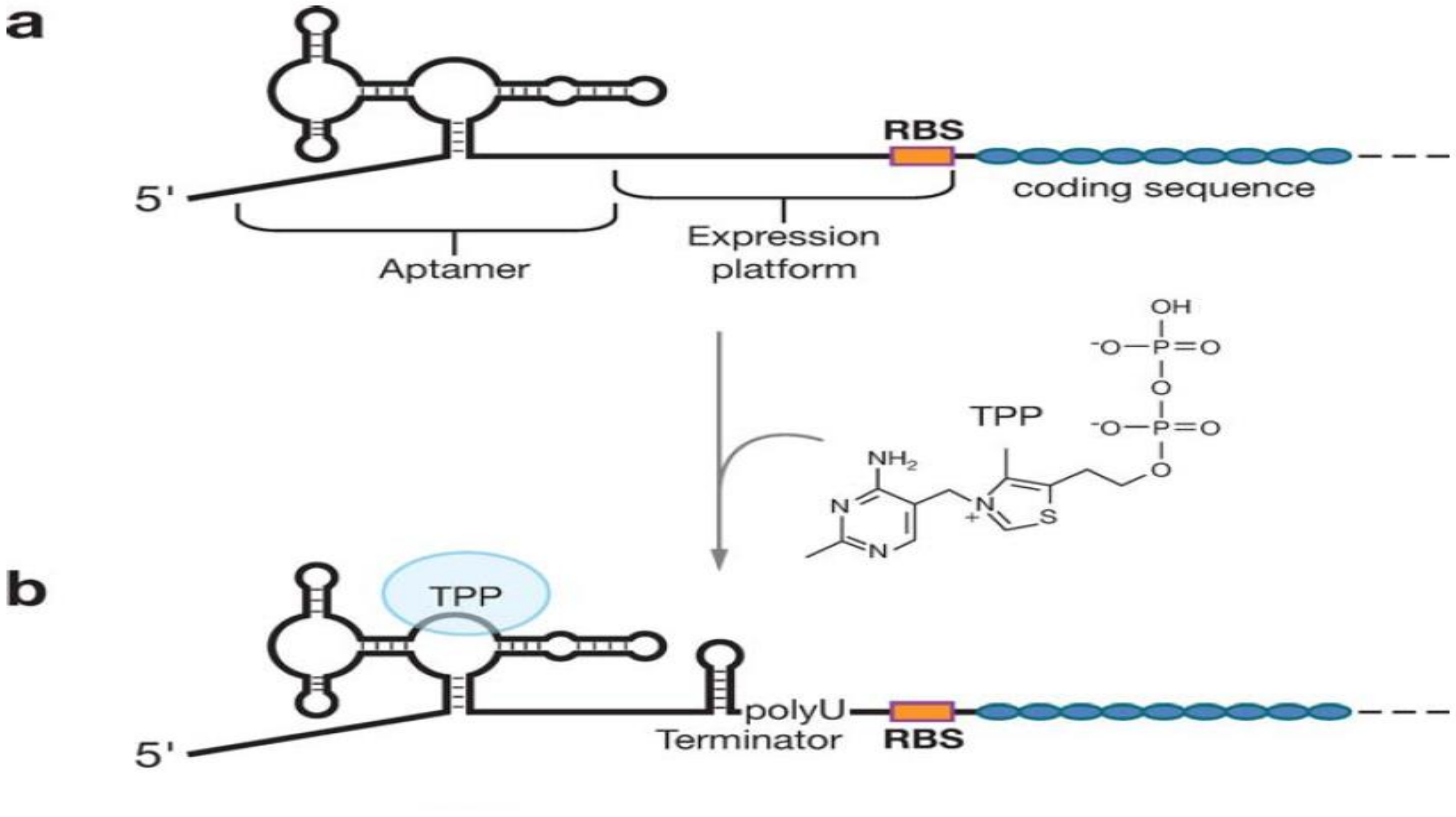
Two predominant mechanisms

- **Control of mRNA Processing**
- **mRNA cleavage by ribozyme activity**
- **Control of Anti-sense RNA (asRNA) production**

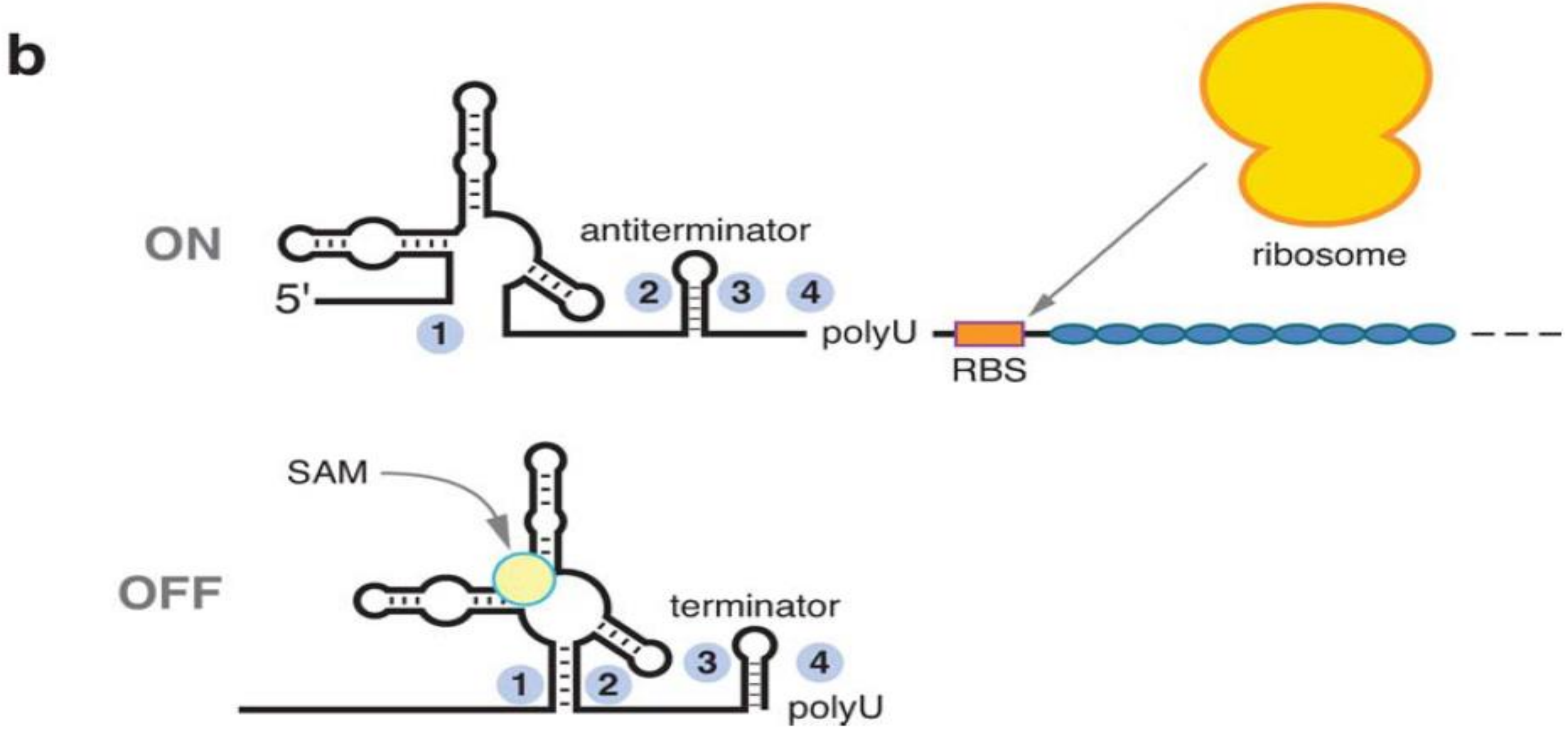
Transcription Attenuation

- Intrinsic transcription terminators:
  - GC-rich stem-loops
  - Poly-uridyl tract → five to nine nucleotides
- These structural elements destabilize elongation complexes, resulting in cessation of transcription.
- RNA elements that exhibit the structural features of known intrinsic terminator stems reside downstream of many riboswitch aptamers

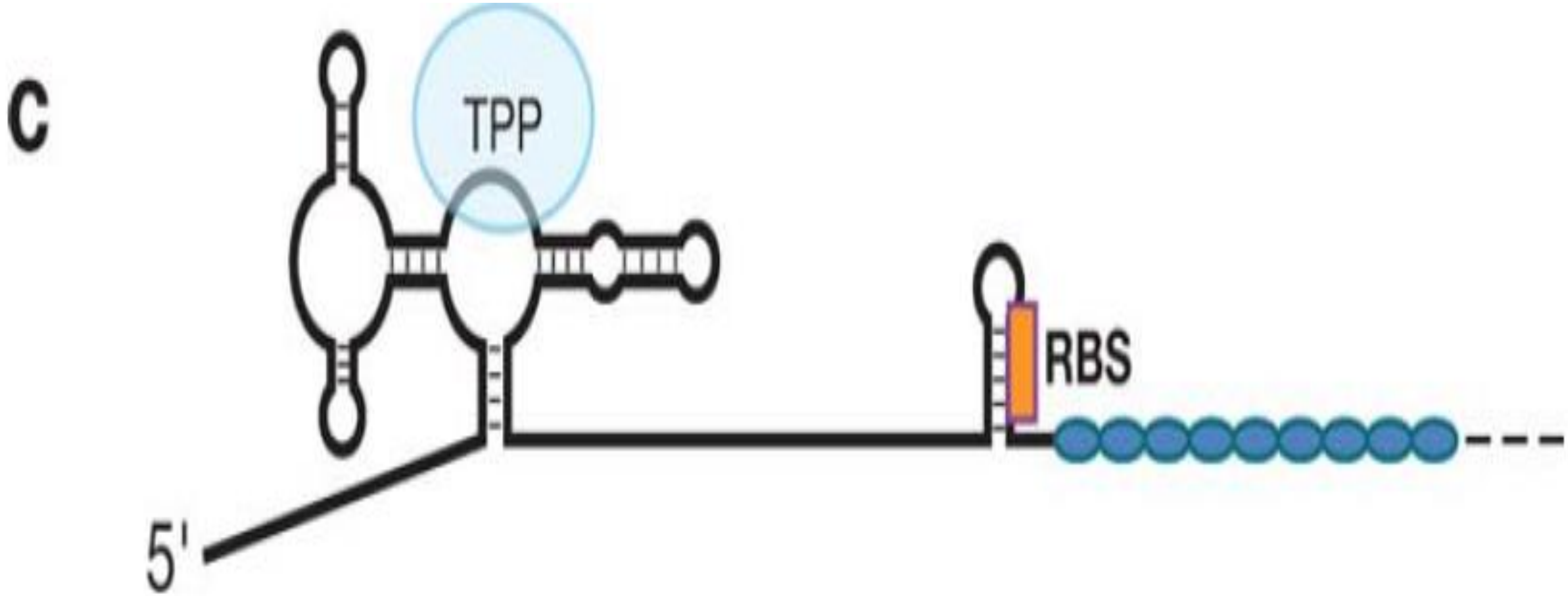
Transcription Attenuation



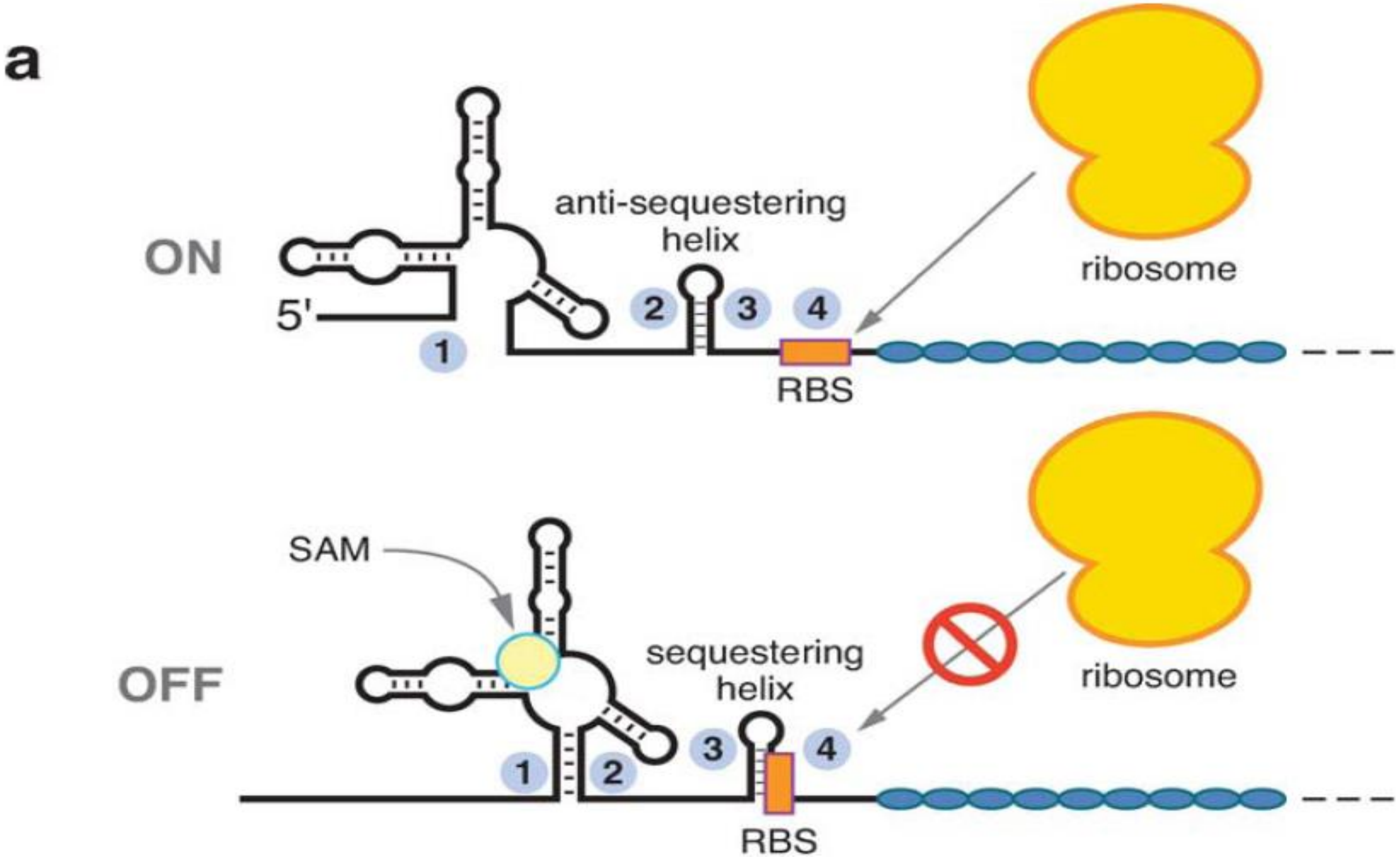
Transcription Attenuation



Control of Translation Initiation



Control of Translation Initiation



Control of mRNA Processing

Riboswitches Applications

- Molecular engineering of RNA-based genetic elements
- Riboswitches as drug targets

Molecular Engineering of RNA-Based Genetic Elements

- The gene control systems most widely used by genetic engineers:
  - Regulatory protein
  - Whose function is modified by a chemical effector that must be permeable to the cellular host.
- Disadvantages:
  - Their use is also limited to hosts that produce appropriate levels of the regulatory protein
  - In addition, each regulatory protein is responsive to a single chemical
    - ✓ Therefore a variety of protein factors and genetic elements are needed

Molecular Engineering of RNA-Based Genetic Elements

- The harnessing of natural or engineered riboswitches:
  - Expand the collection of gene control systems
  - That could integrate with their transgenic constructs.
- Advantages of riboswitches than protein-based systems:
  - Simpler and more versatile architecture for expanding gene control capabilities

Molecular Engineering of RNA-Based Genetic Elements

- The use of natural riboswitches:
  - Is complicated
  - Because they typically sense fundamental metabolites whose concentrations might be difficult to control at will.
- RNA engineers:
  - Might be able to coopt the mechanisms used by natural riboswitches to create a collection of RNA elements that respond to a diversity of chemical effectors.

Riboswitches as Drug Targets

- Riboswitches:
  - Control many fundamental genes and metabolic pathways
  - Therefore, they are potential targets for antimicrobial agents

Riboswitches as Drug Targets

- Riboswitches offer an advantage:
  - They naturally bind to small molecules
  - Drug-like compounds that compete with metabolite binding could be identified

Riboswitches as Drug Targets

- e. g., Antimicrobial compound AEC:
  - Directly binding to lysine riboswitches
  - Down-regulating the expression of lysine biosynthesis genes
- Such efforts to develop riboswitches as targets for drug action are still in their infancy.