Lecture 12

Regulation of Gene Expression
Negative regulation - Lac Operon
Attenuation - Tryptophan Operon

Acknowledgement: Leninger Chapter 28

Objectives

Emergence of Life
Fundamental units of life
Cellular assemblies
Protein Folding
Protein Synthesis
Gene Regulation

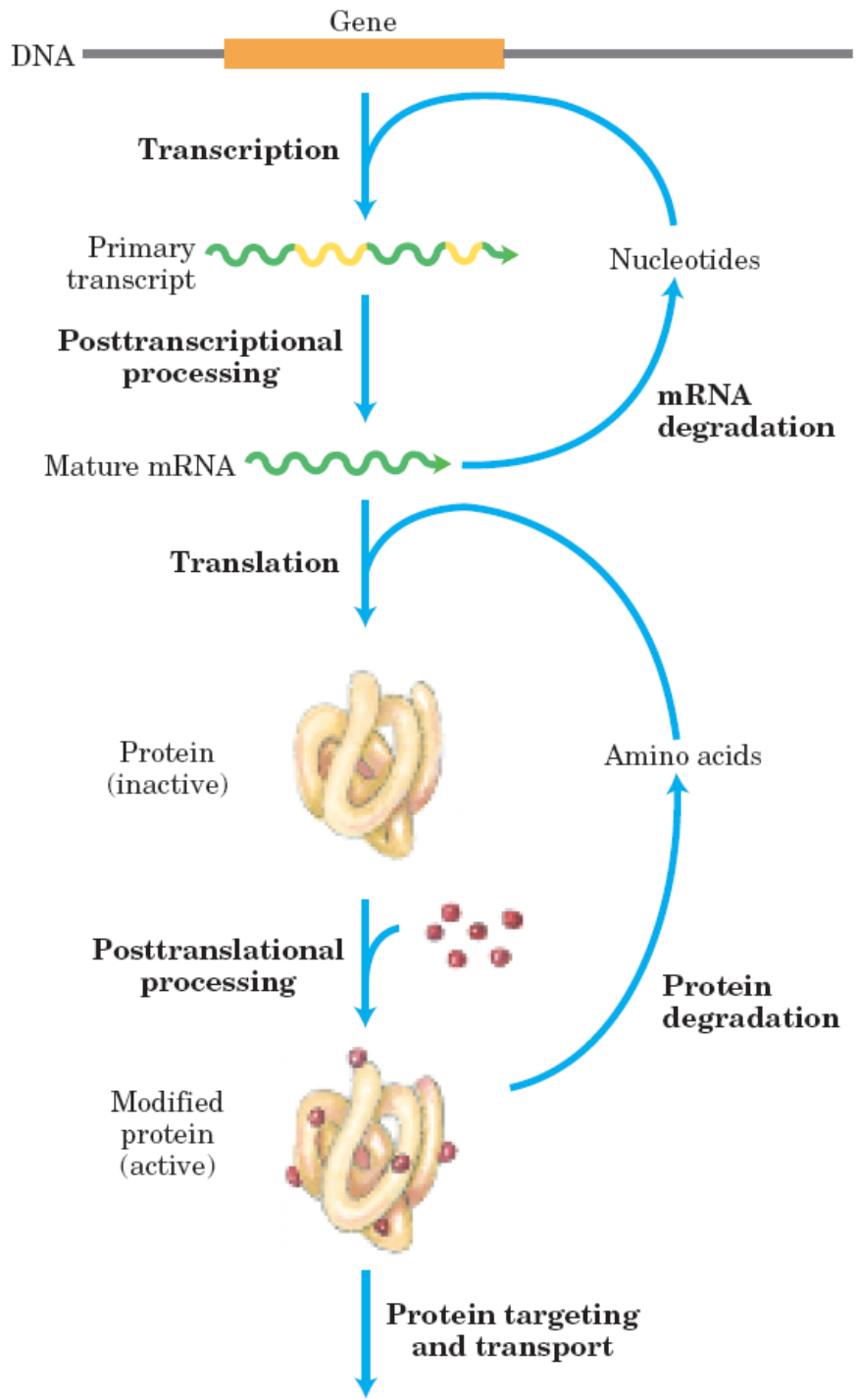
1. Understanding gene regulation
   a) Operons and regulons
2. Negative and positive regulation
3. Lac operon
4. Attenuation regulation – Tryp operon

Genes are expressed when required

- Some proteins are expressed abundantly such as elongation factors and rubisco
- Others such as DNA repair enzymes are synthesized very few in number
- Requirements of gene products varies in the cell-type and in its life cycle
  - Ribosomes are synthesized rapidly during the exponential growth phase of the cell

What factors determine the cellular concentration of proteins

1. Synthesis of the primary RNA transcript (transcription)
2. Posttranscriptional modification of mRNA
3. Messenger RNA degradation
4. Protein synthesis (translation)
5. Posttranslational modification of proteins
6. Protein targeting and transport
7. Protein degradation


Gene
DNA
Transcription
Primary transcript
Nucleotides
Posttranscriptional processing
mRNA degradation
Mature mRNA
Translation
Protein (inactive)
Amino acids
Posttranslational processing
Protein degradation
Modified protein (active)
Protein targeting and transport

Gene regulation

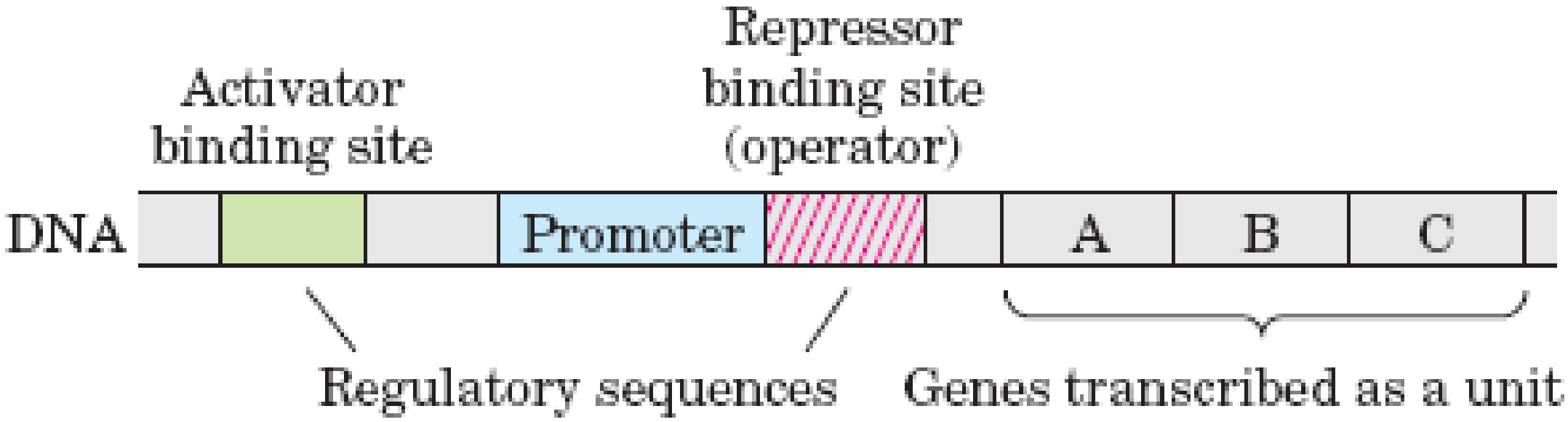
House Keeping Genes

- Constitutive gene expression

Regulated Genes

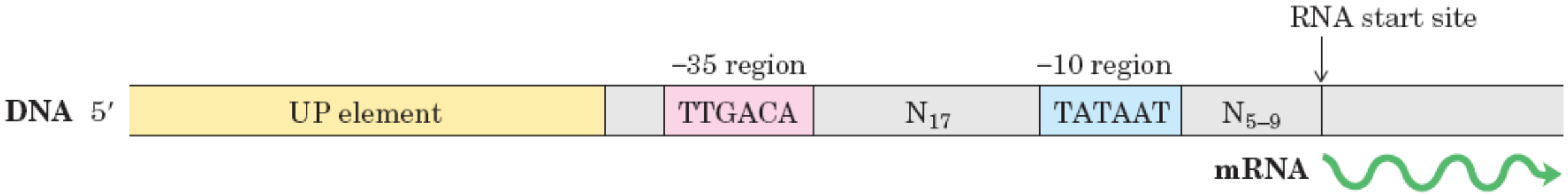
- Inducible gene expression
- Repressible gene expression

Representative Prokaryotic Operon



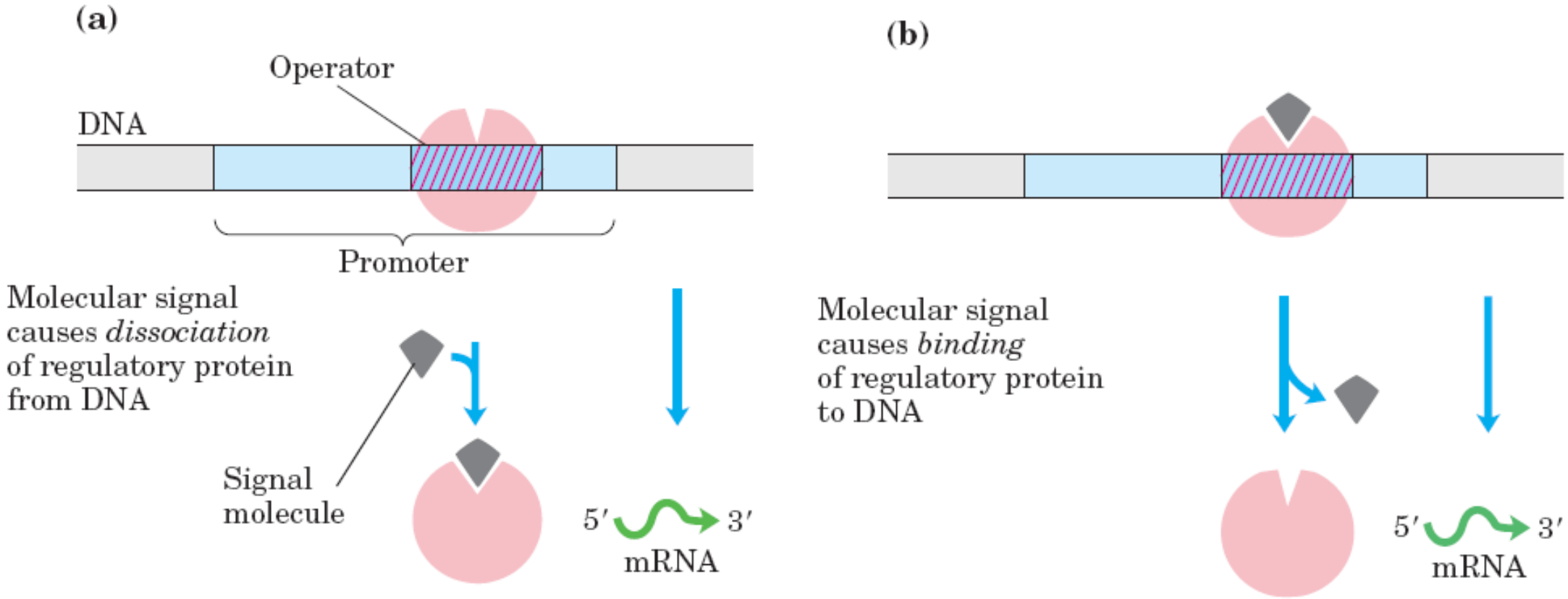
- Genes A, B, and C are transcribed on one polycistronic mRNA. Typical regulatory sequences include binding sites for proteins that either activate or repress transcription from the promoter

RNA polymerase

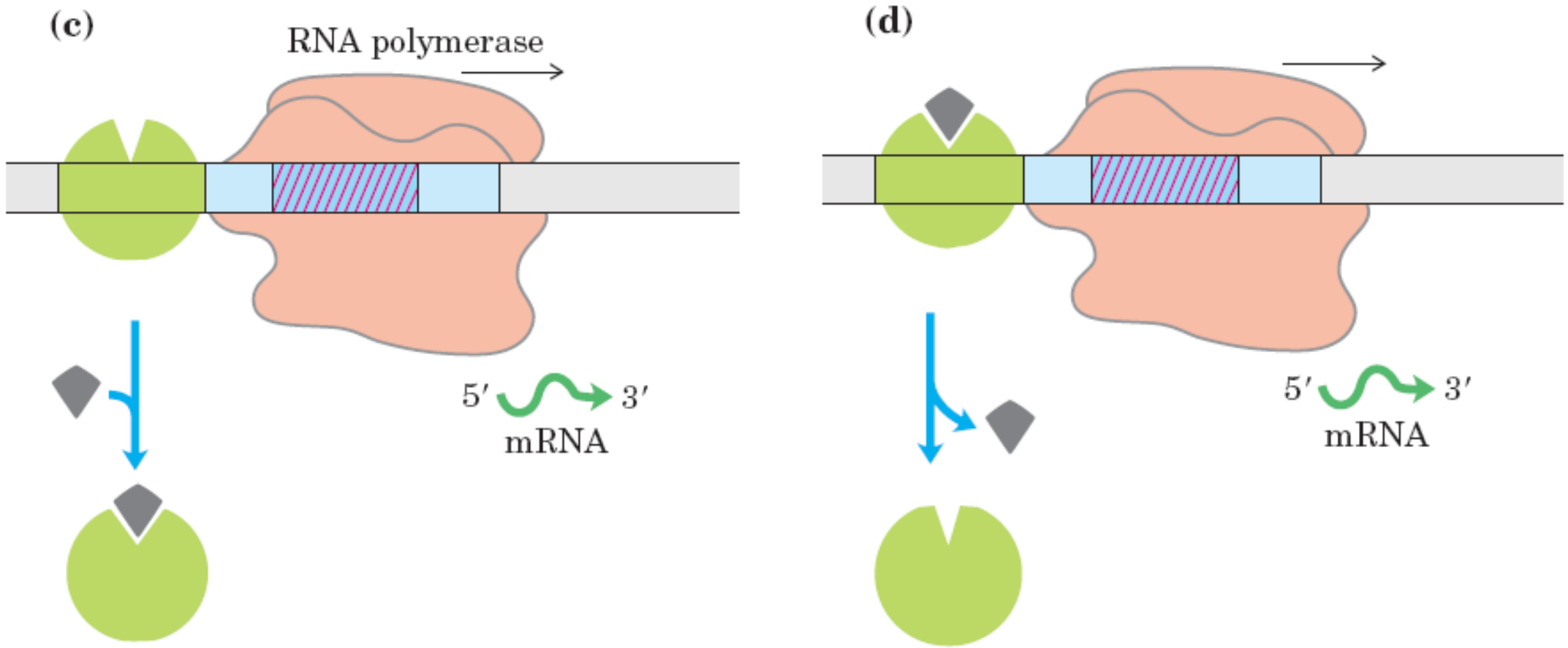


- RNA polymerases bind to DNA and initiate transcription at promoters, sites generally found near points at which RNA synthesis begins on the DNA template
- The regulation of transcription initiation often entails changes in how RNA polymerase interacts with a promoter

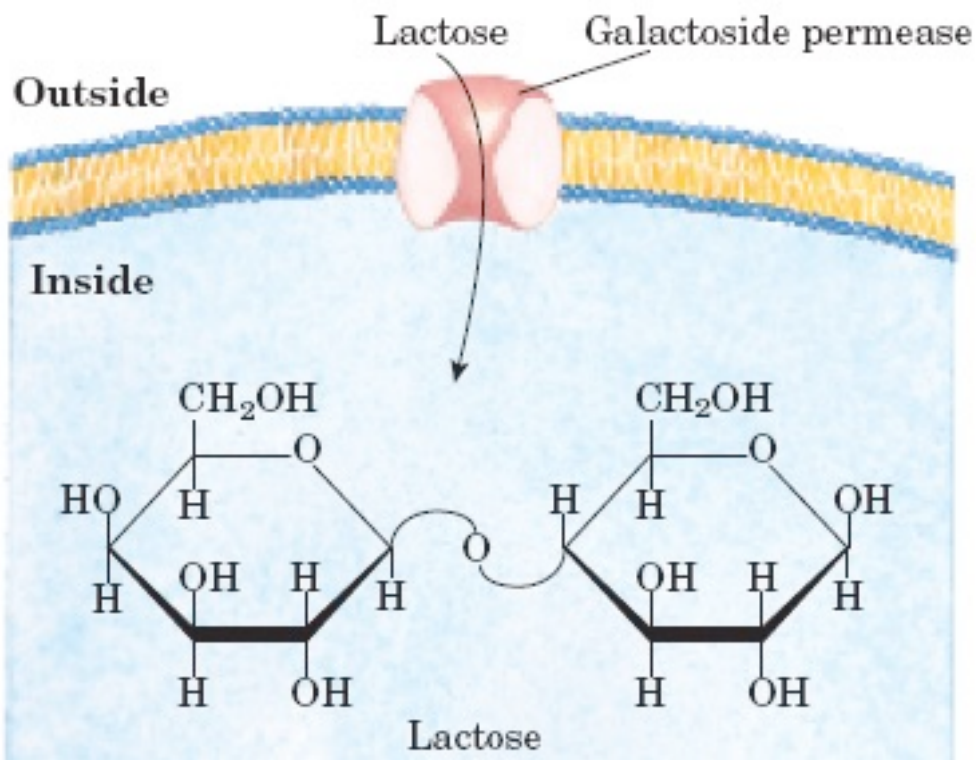
Negative Regulation of Gene Expression

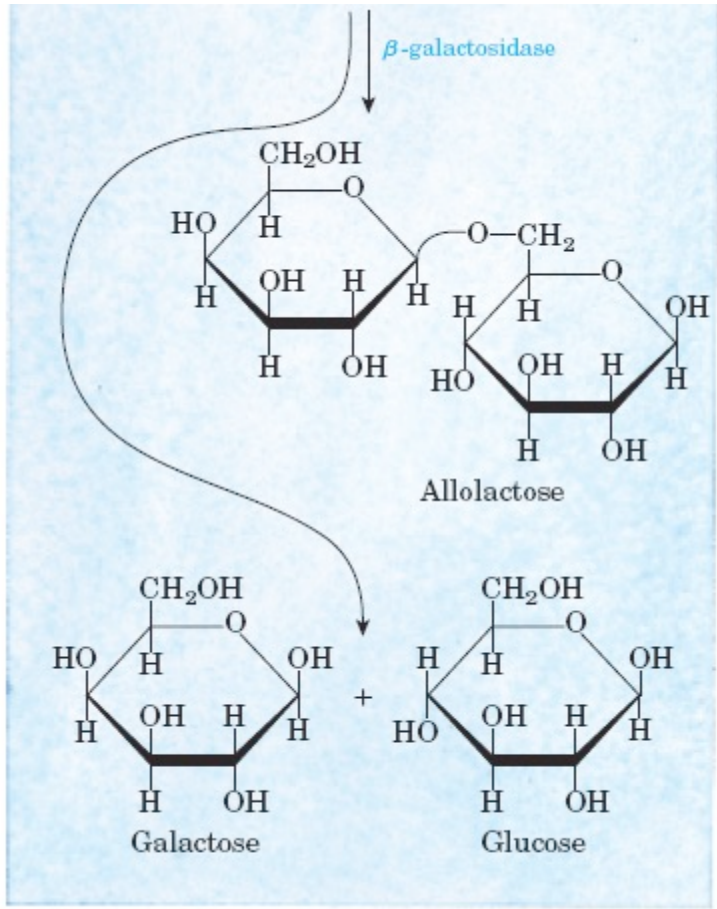


Positive Regulation of Gene Expression

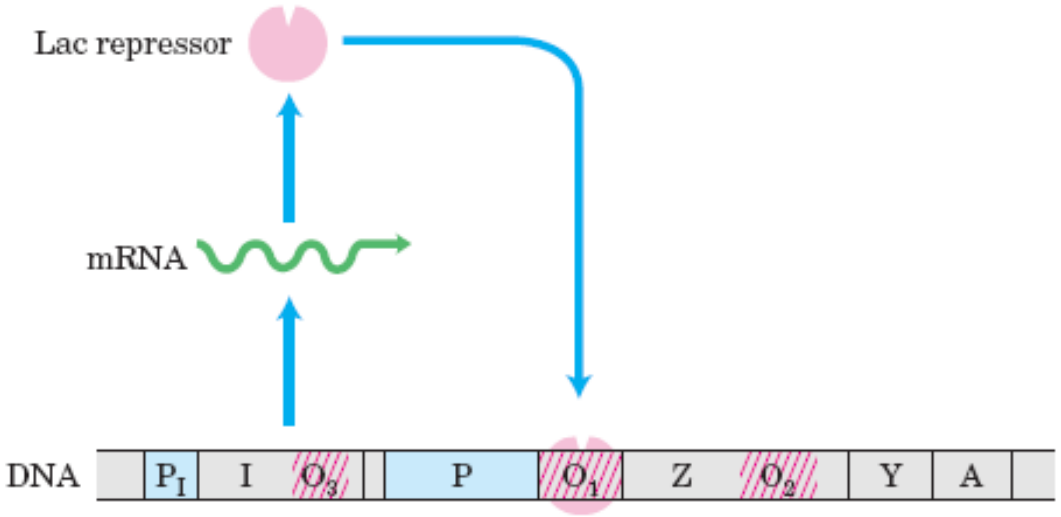


Lactose metabolism in *E. coli*

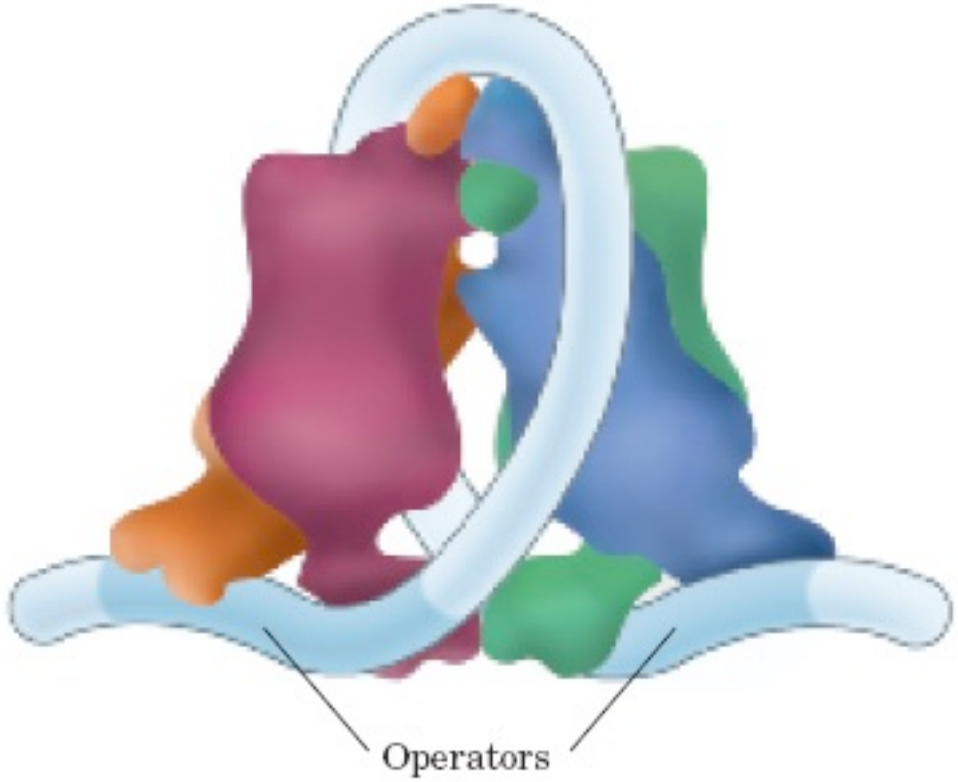




The Lac Operon

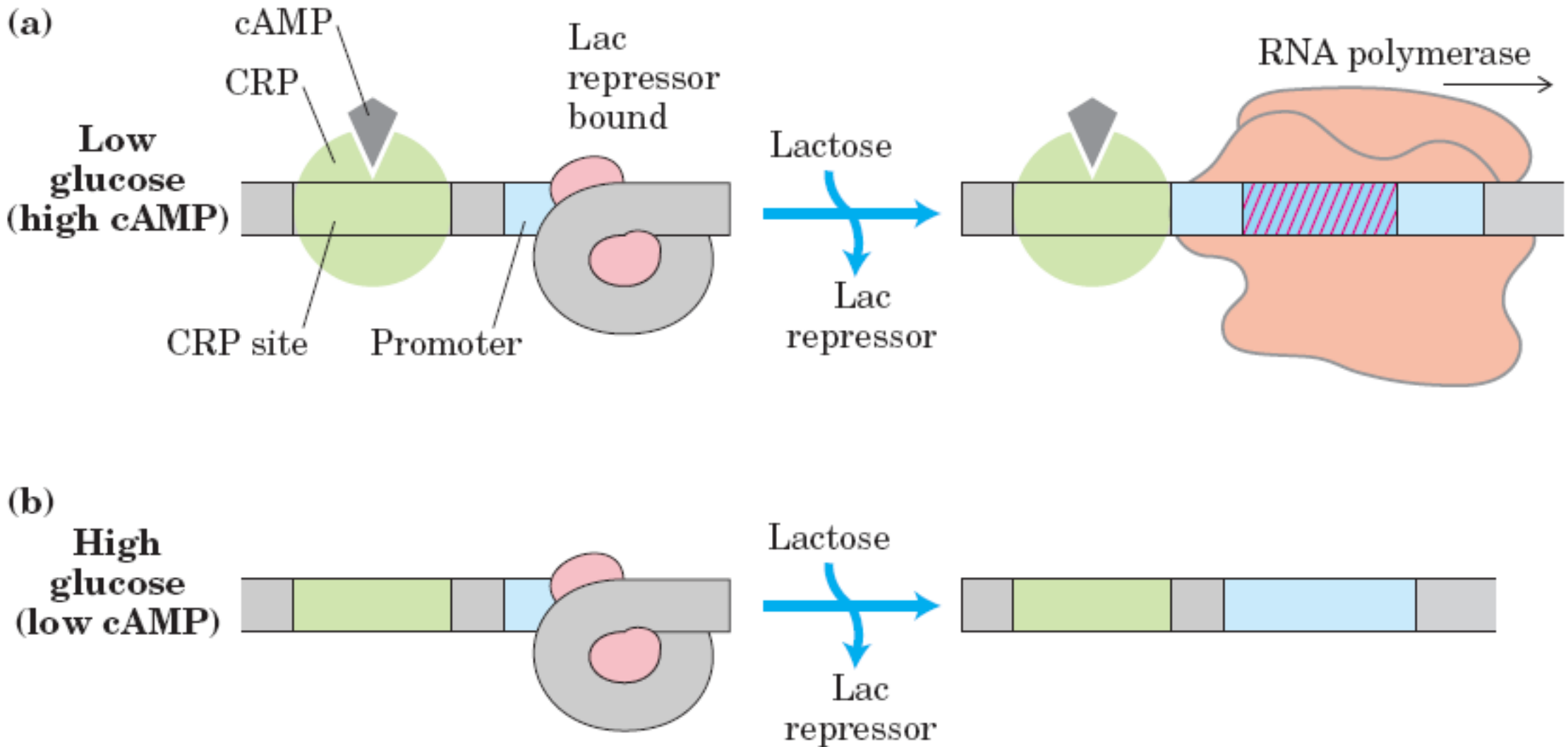


- The I gene encodes the Lac repressor. The lac Z, Y, and A genes encode beta-galactosidase, galactoside permease, and thiogalactoside transacetylase, respectively

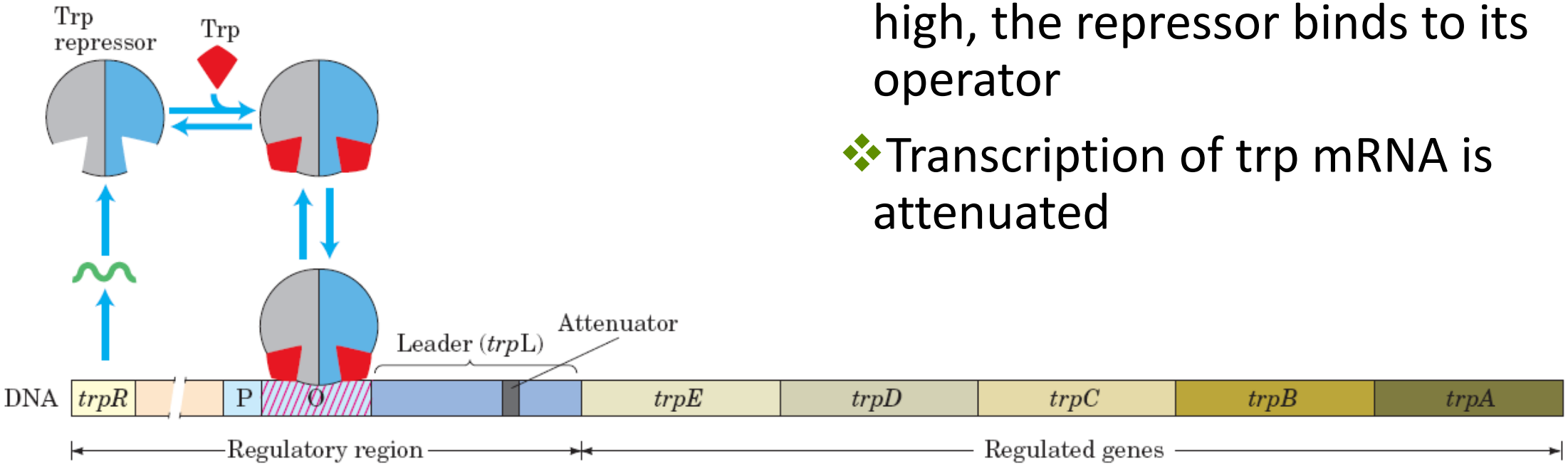


- O_1 is the main operator for the lac operon
- The Lac repressor binds to the main operator and O_2 or O_3, apparently forming a loop in the DNA that might wrap around the repressor

The Lac Operon

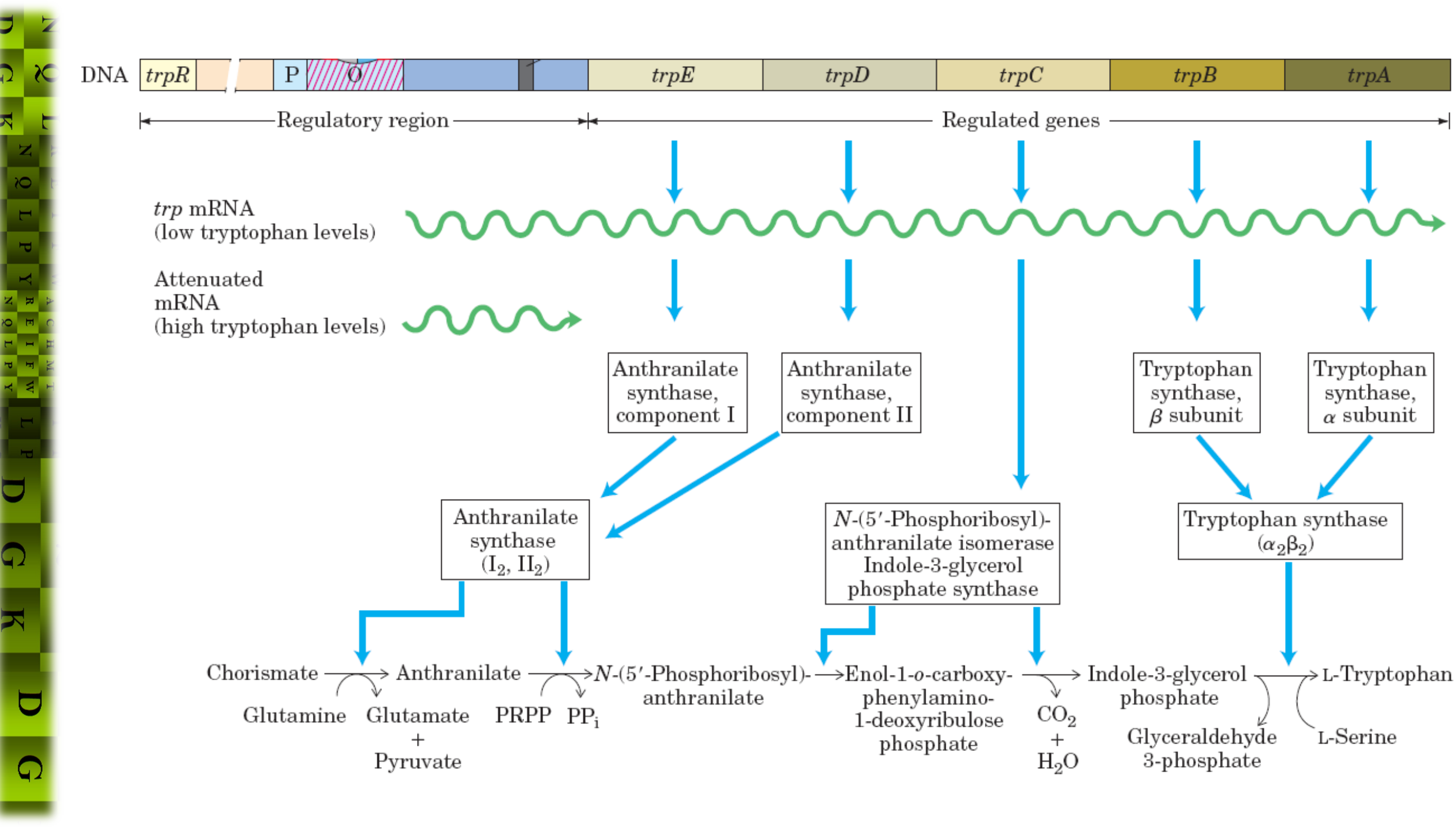


The Trp Operon

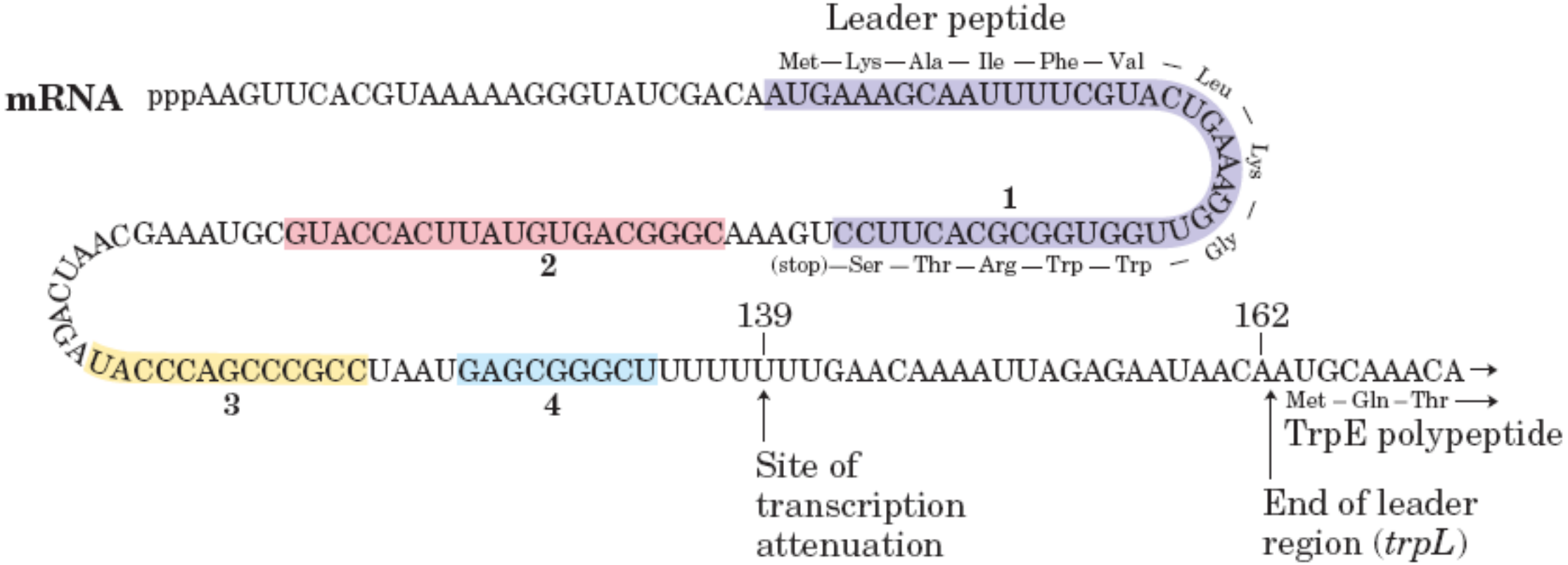


This operon is regulated by two mechanisms:

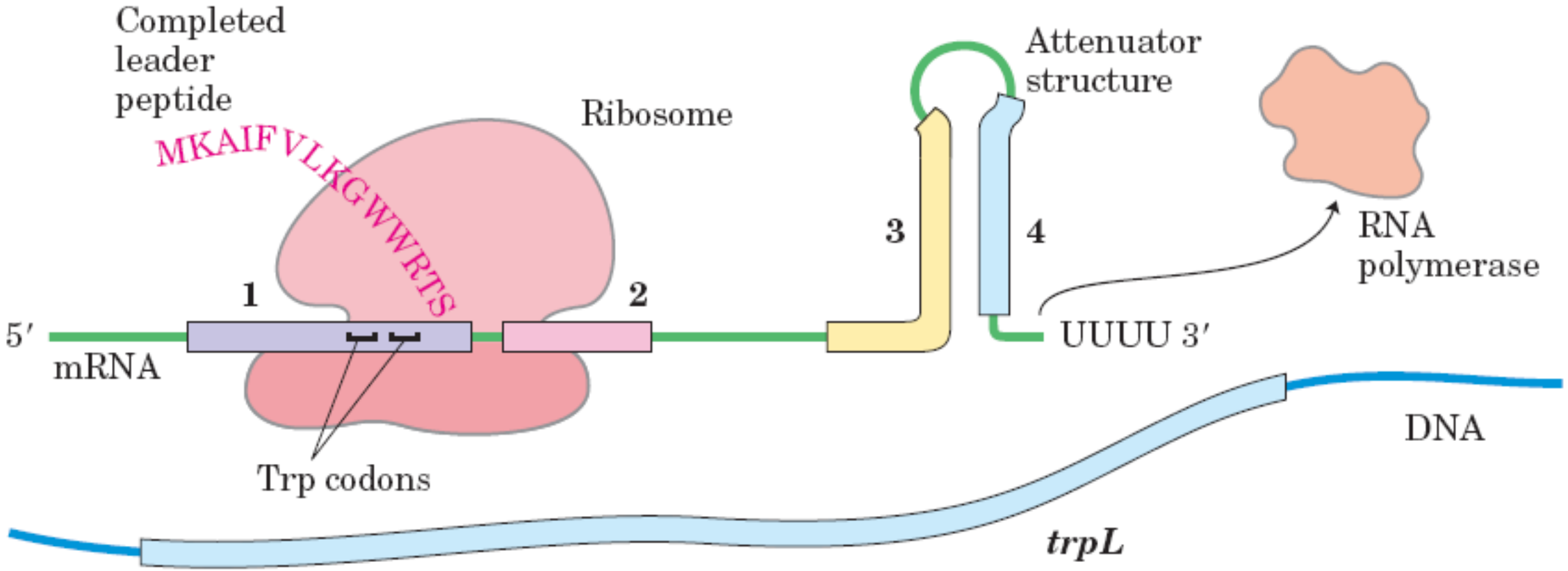
- When tryptophan levels are high, the repressor binds to its operator
- Transcription of trp mRNA is attenuated


DNA
trpR
P
O
trpE
trpD
trpC
trpB
trpA
Regulatory region
Regulated genes
trp mRNA
(low tryptophan levels)
Attenuated
mRNA
(high tryptophan levels)
Anthranilate synthase, component I
Anthranilate synthase, component II
Tryptophan synthase, β subunit
Tryptophan synthase, α subunit
Anthranilate synthase (I2, II2)
N-(5′-Phosphoribosyl)-anthranilate isomerase
Indole-3-glycerol phosphate synthase
Tryptophan synthase (α2β2)
Chorismate
Anthranilate
N-(5′-Phosphoribosyl)-anthranilate
Enol-1-o-carboxy-phenylamino-1-deoxyribulose phosphate
Indole-3-glycerol phosphate
L-Tryptophan
Glutamine
Glutamate + Pyruvate
PRPP
PPi
CO2 + H2O
Glyceraldehyde 3-phosphate
L-Serine

The Trp mRNA Sequence

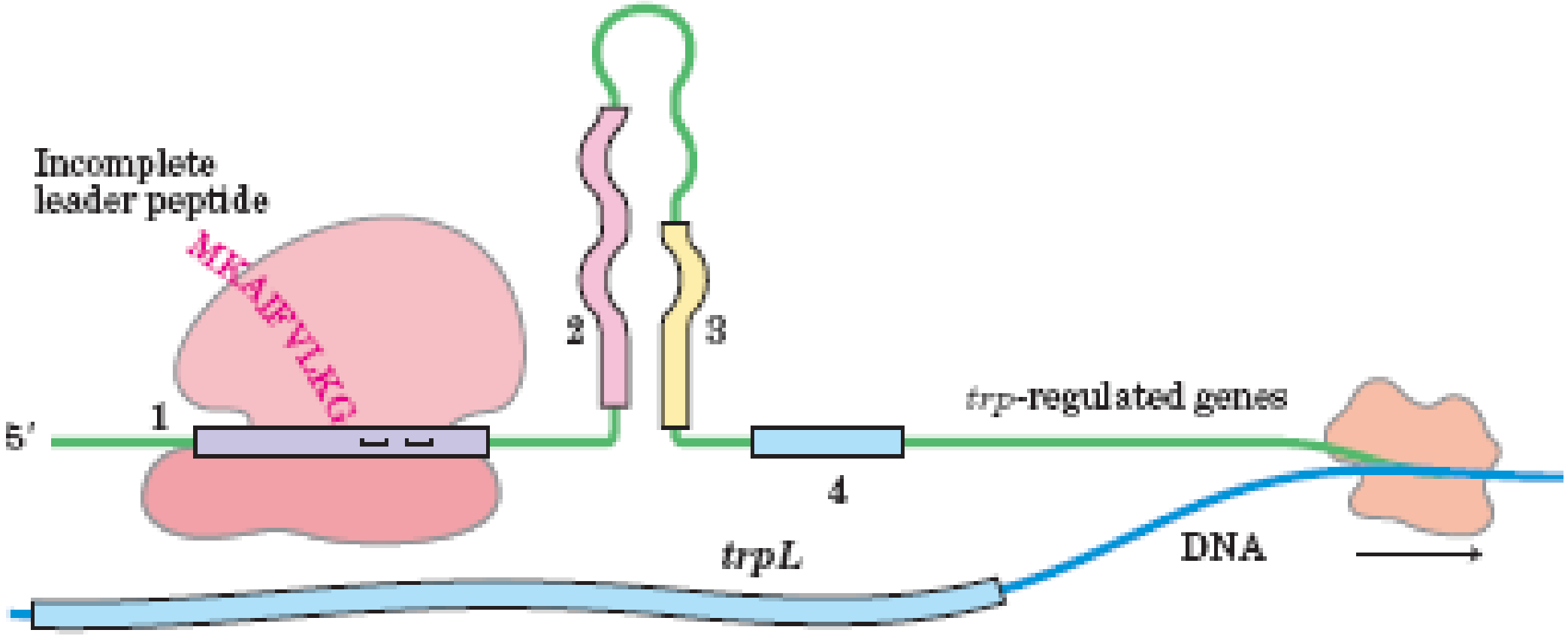


What happens at high Tryptophan levels



- When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4

What happens at low Tryptophan levels



- When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.