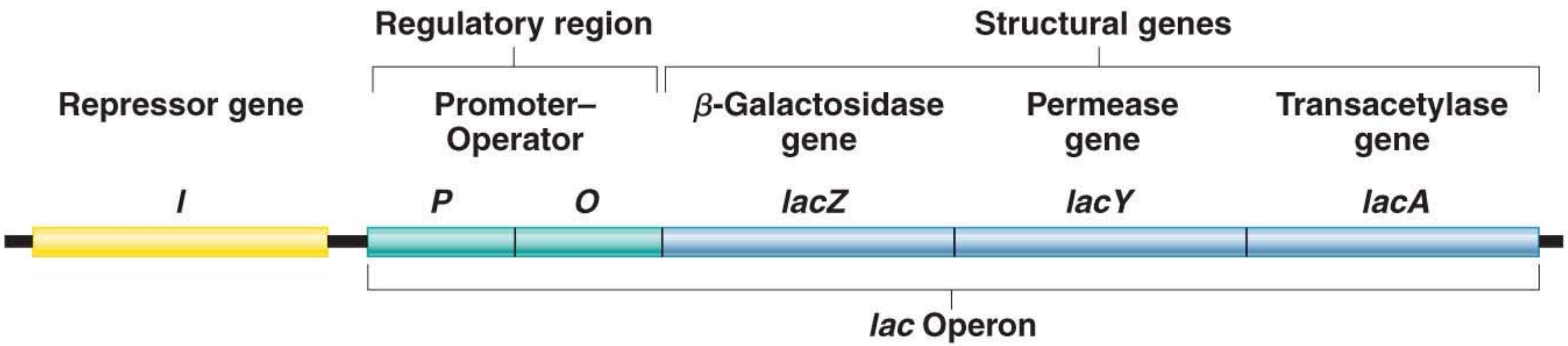
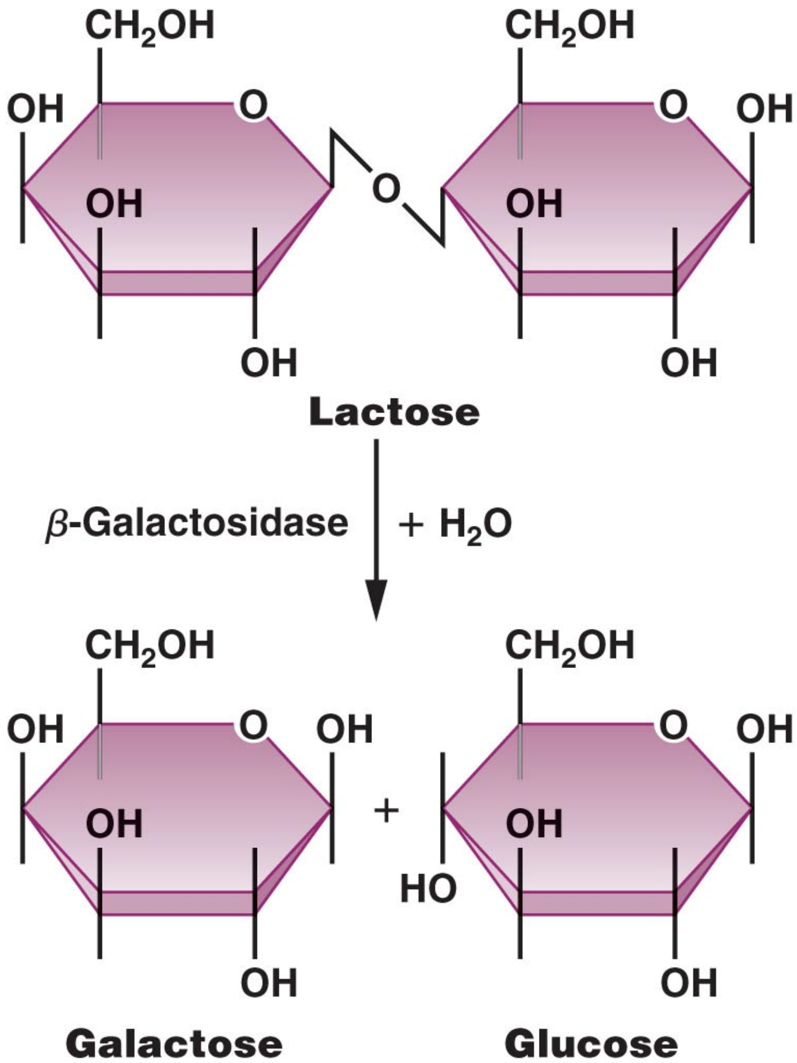
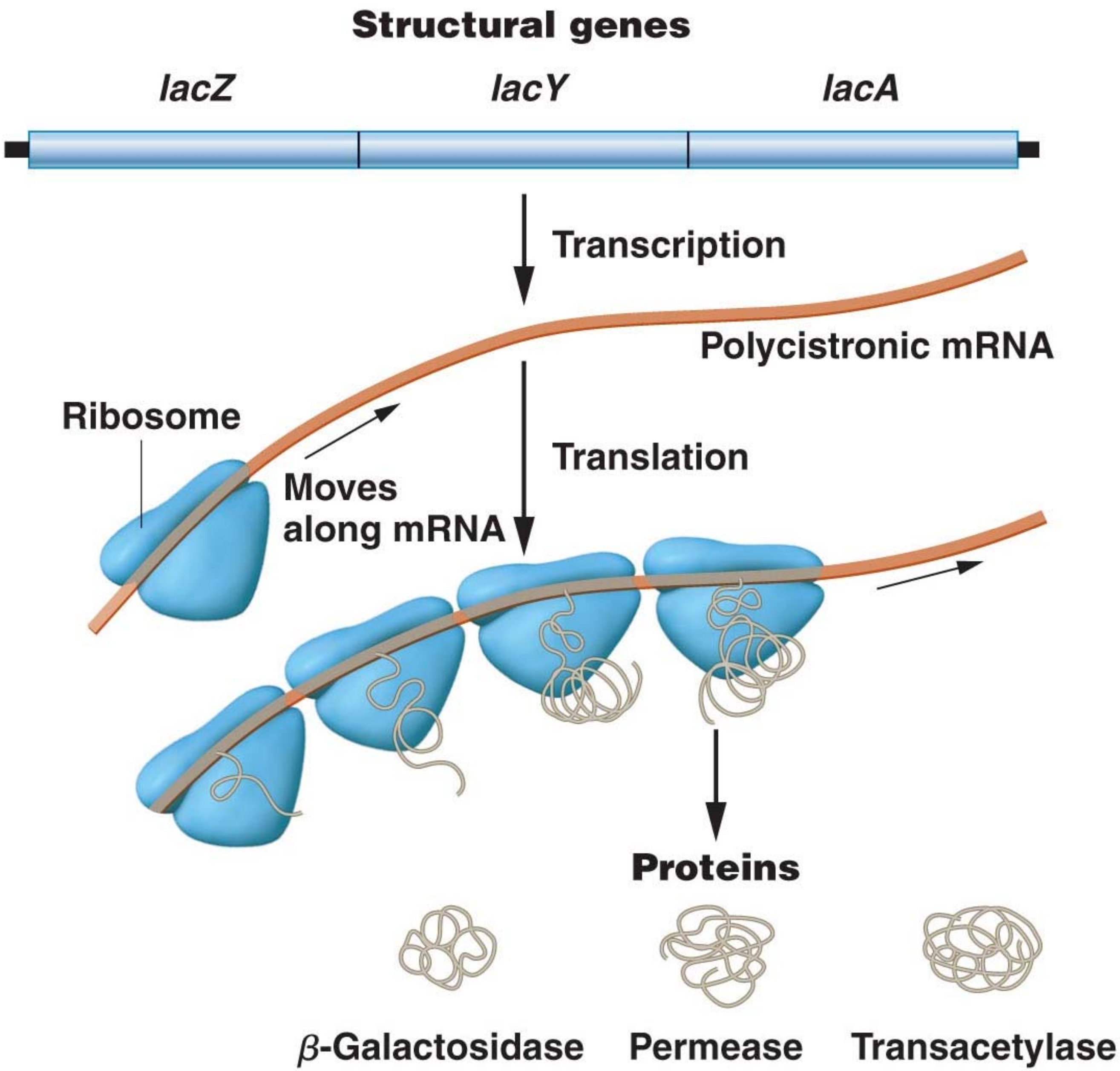
Chapter 16 Regulation of Gene Expression in Prokaryotes

16.1 Prokaryotes Regulate Gene Expressions in Response to Environmental Conditions

16.2 Lactose Metabolism in *E. coli* Is Regulated by an Inducible System

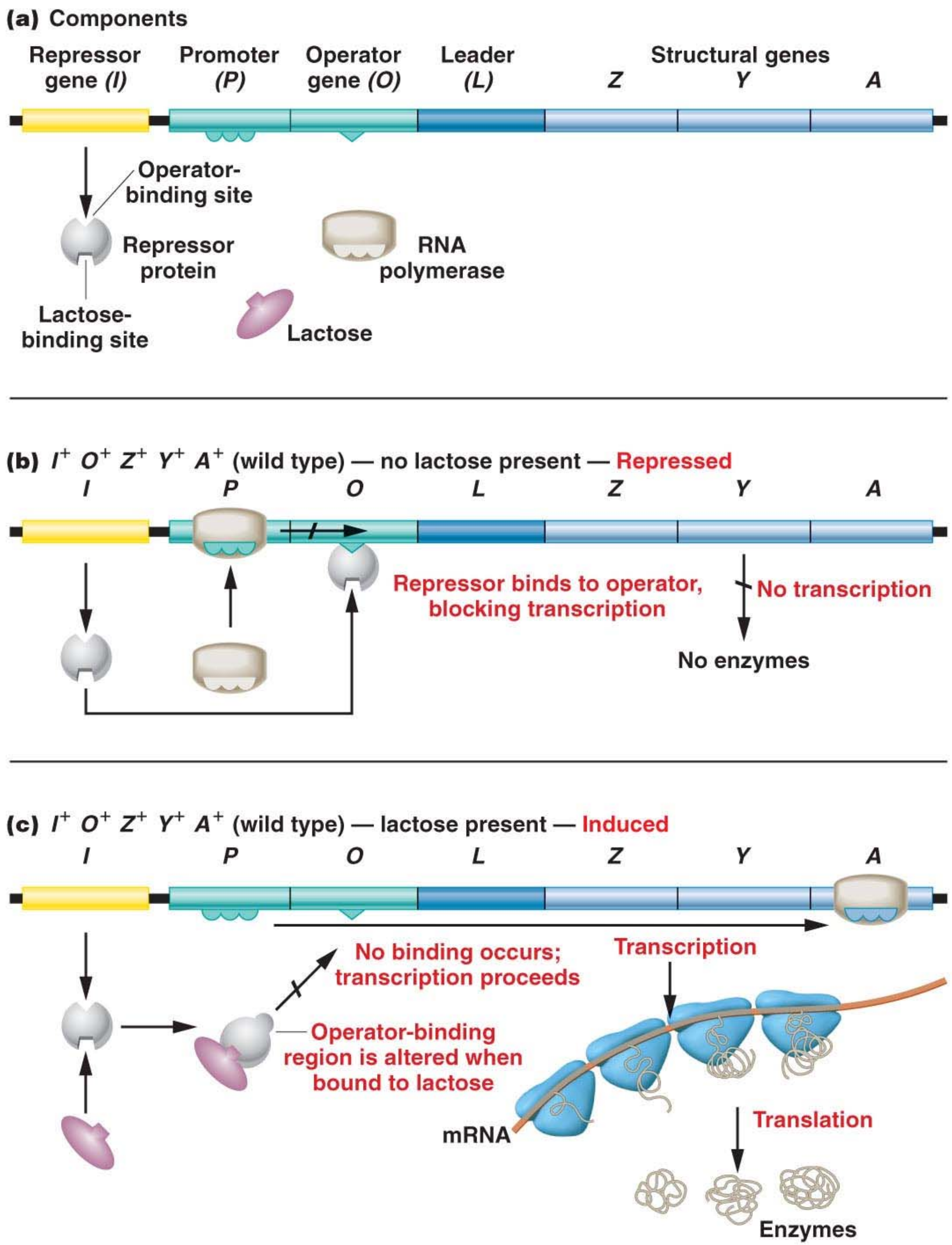



CH2OH
OH
O
OH
O
CH2OH
O
OH
OH
OH
OH
Lactose
β-Galactosidase
+ H2O
CH2OH
OH
O
OH
OH
OH
OH
+
CH2OH
O
OH
OH
HO
OH
Galactose
Glucose

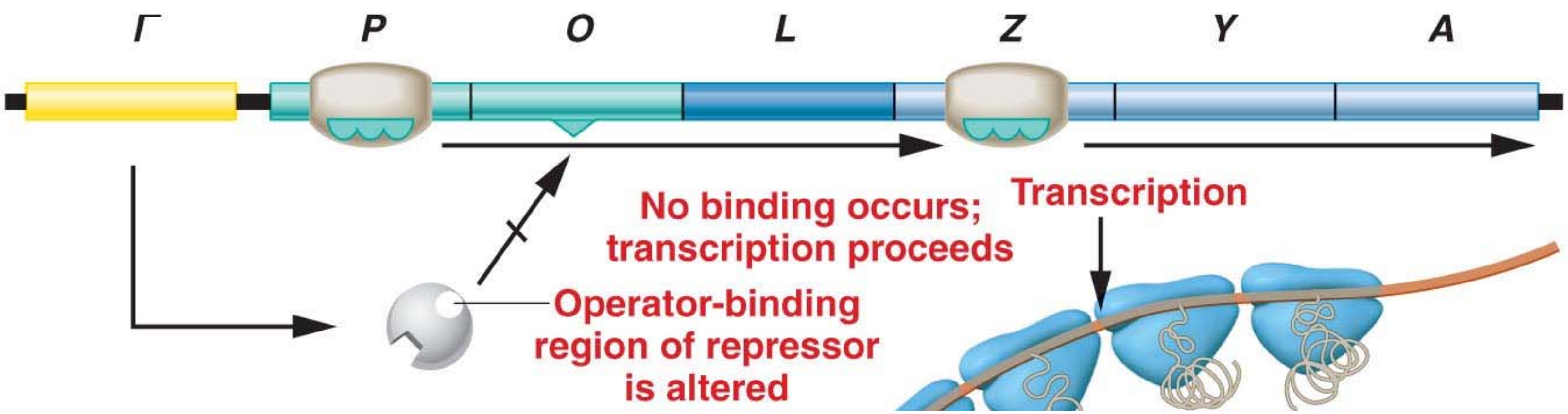


Structural genes
lacZ
lacY
lacA
Transcription
Polycistronic mRNA
Ribosome
Moves
along mRNA
Translation
Proteins
β-Galactosidase
Permease
Transacetylase

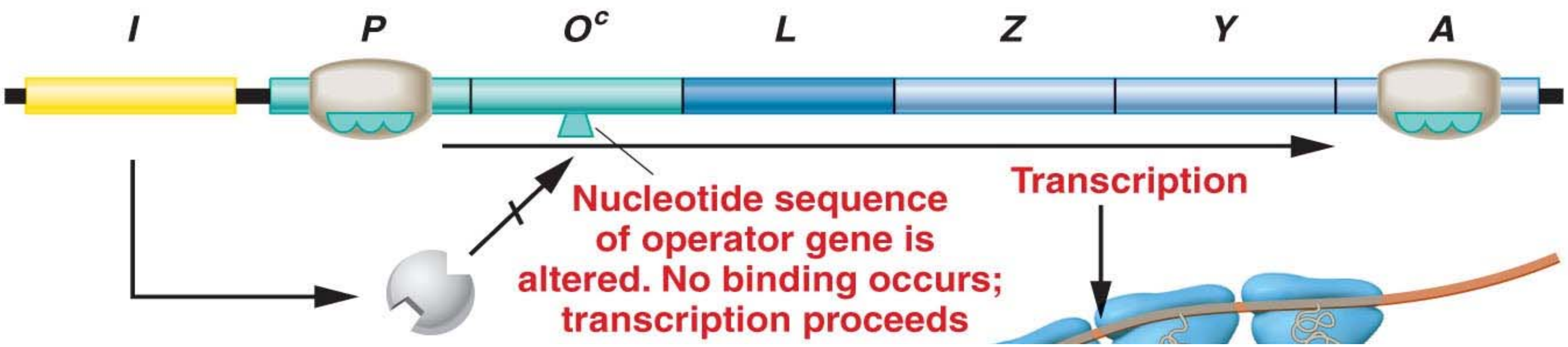
The Operon Model: Negative Control



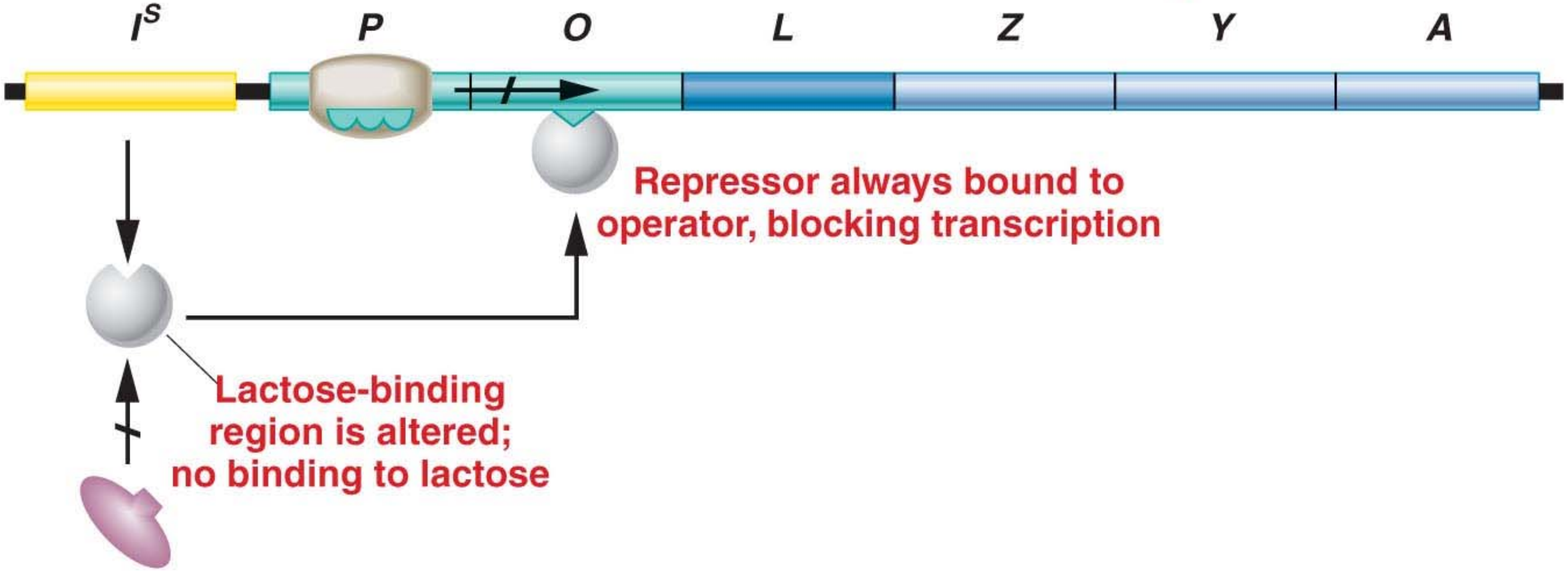
(a) $I^- O^+ Z^+ Y^+ A^+$ **(mutant repressor gene) — no lactose present — Constitutive**



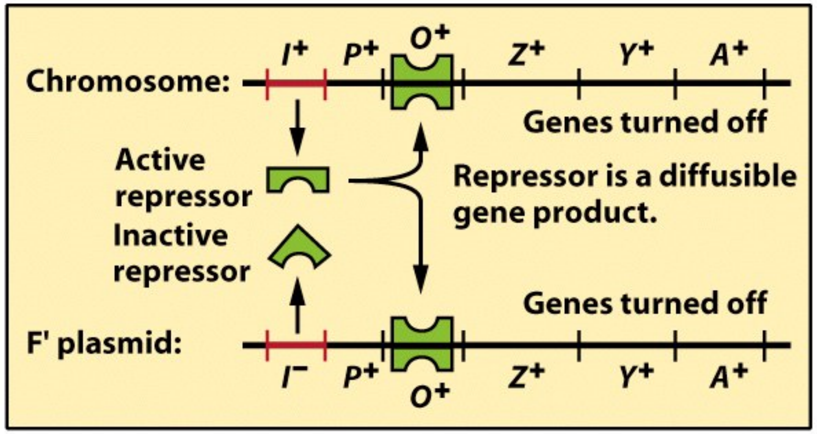
(b) $I^+ O^c Z^+ Y^+ A^+$ **(mutant operator gene) — no lactose present — Constitutive**



I^S O^+ Z^+ Y^+ A^+ (mutant repressor gene) — lactose present — Repressed



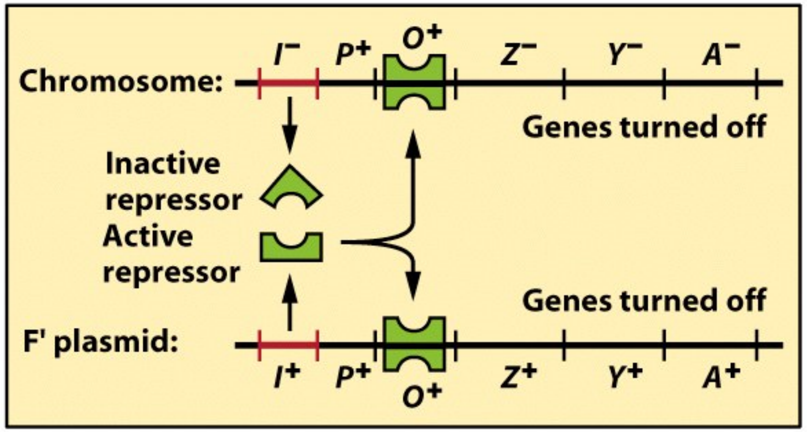
E. coli partial diploid



Inducible synthesis of *lac* operon gene products because the wild-type ($lacI^+$) repressor binds to the *lac* operators on both chromosomes

(a) Dominance of $lacI^+$ over $lacI^-$.

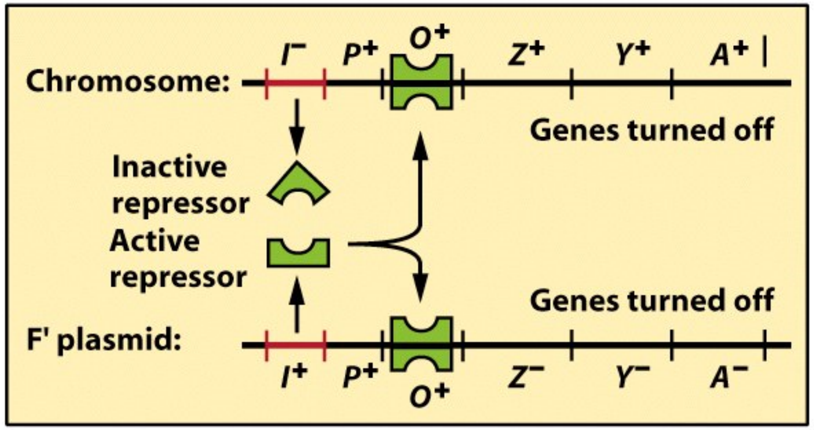
E. coli partial diploid



Inducible synthesis of the *lac* operon gene products

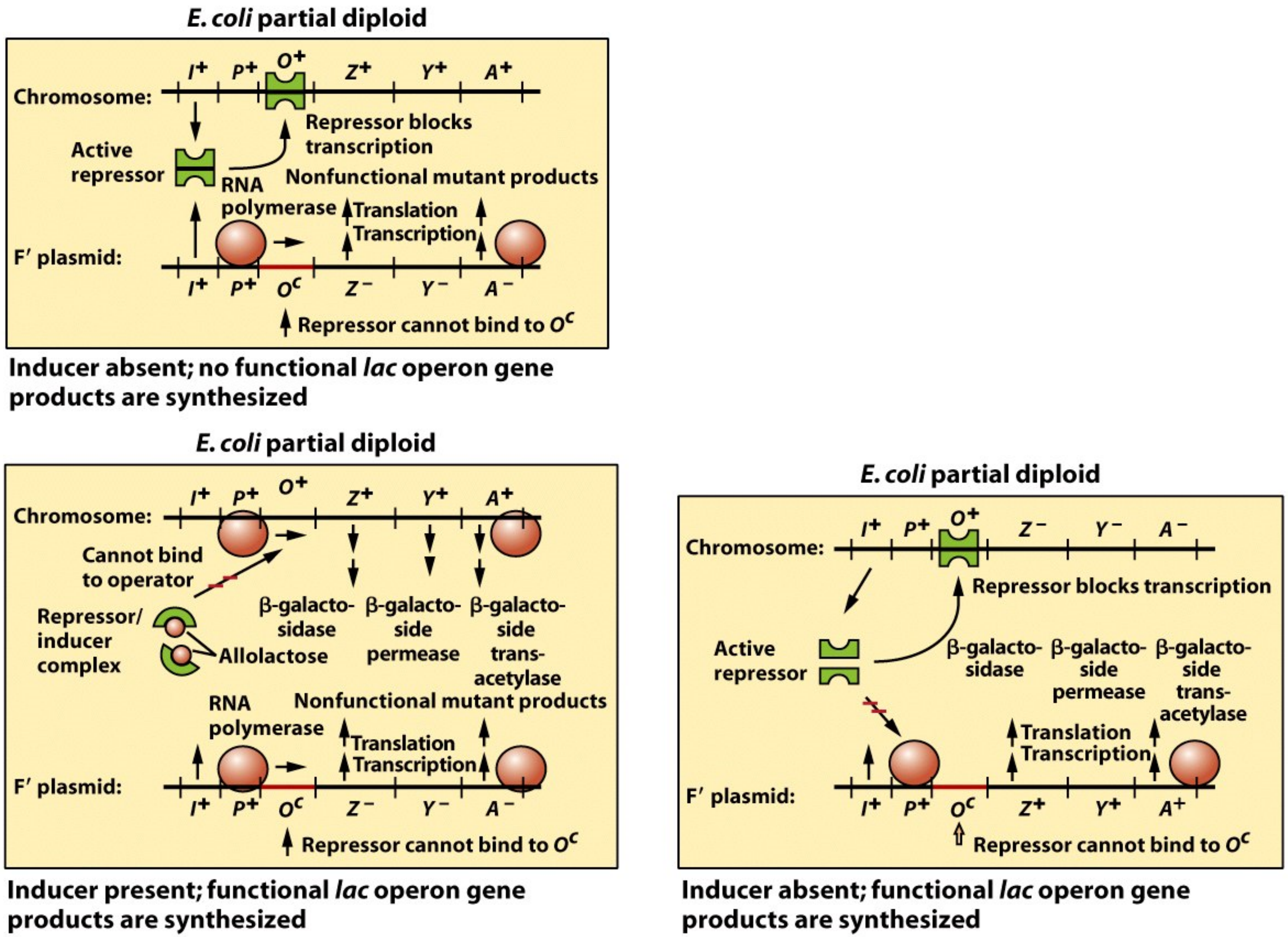
(b) *cis* dominance of $lacI^+$: I^+ located *cis* to Z^+, Y^+ and A^+.

E. coli partial diploid



Inducible synthesis of the *lac* operon gene products

(c) *trans* dominance of $lacI^+$: I^+ located *trans* to Z^+, Y^+ and A^+.

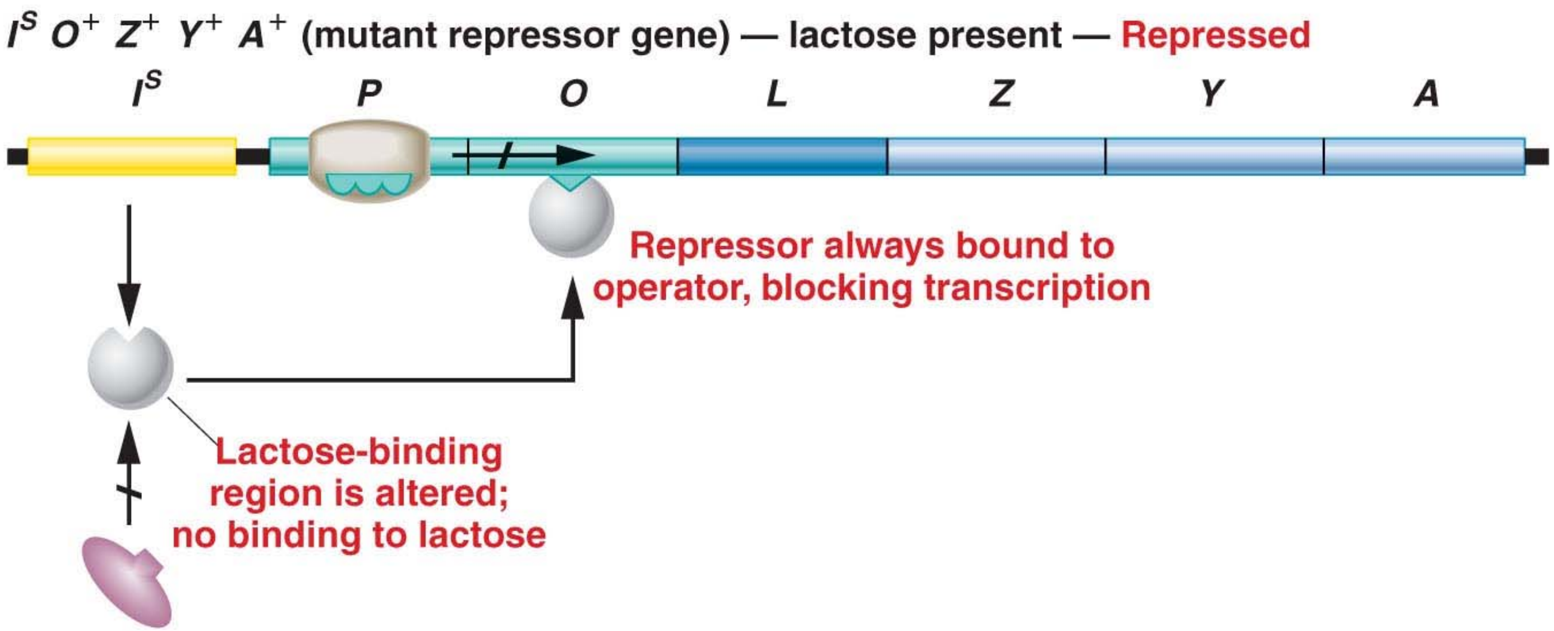


(a) **Inducible synthesis of the *lac* operon gene products in an F′ $I^+ P^+ O^C Z^- Y^- A^- / I^+ P^+ O^+ Z^+ Y^+ A^+$ bacterium.**

(b) **Constitutive synthesis of the *lac* operon gene products in an F′ $I^+ P^+ O^C Z^+ Y^+ A^+ / I^+ P^+ O^+ Z^- Y^- A^-$ bacterium.**

Using F' plasmids to analyze lac operon mutants

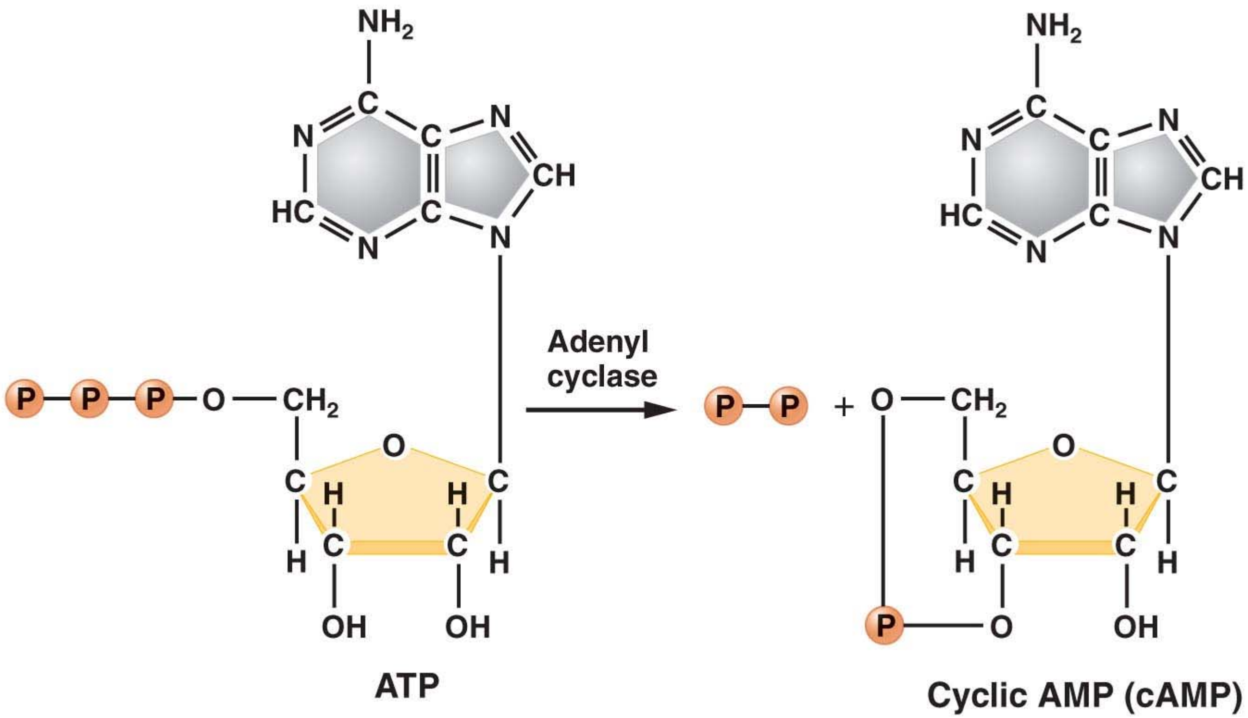
- Mate a I^S O^+ Z^+ Y^+ A^+ bacteria to wildtype.
- The result is I^S O^+ Z^+ Y^+ A^+/ F' I^+ O^+ Z^+ Y^+ A^+
- How does this change the phenotype?

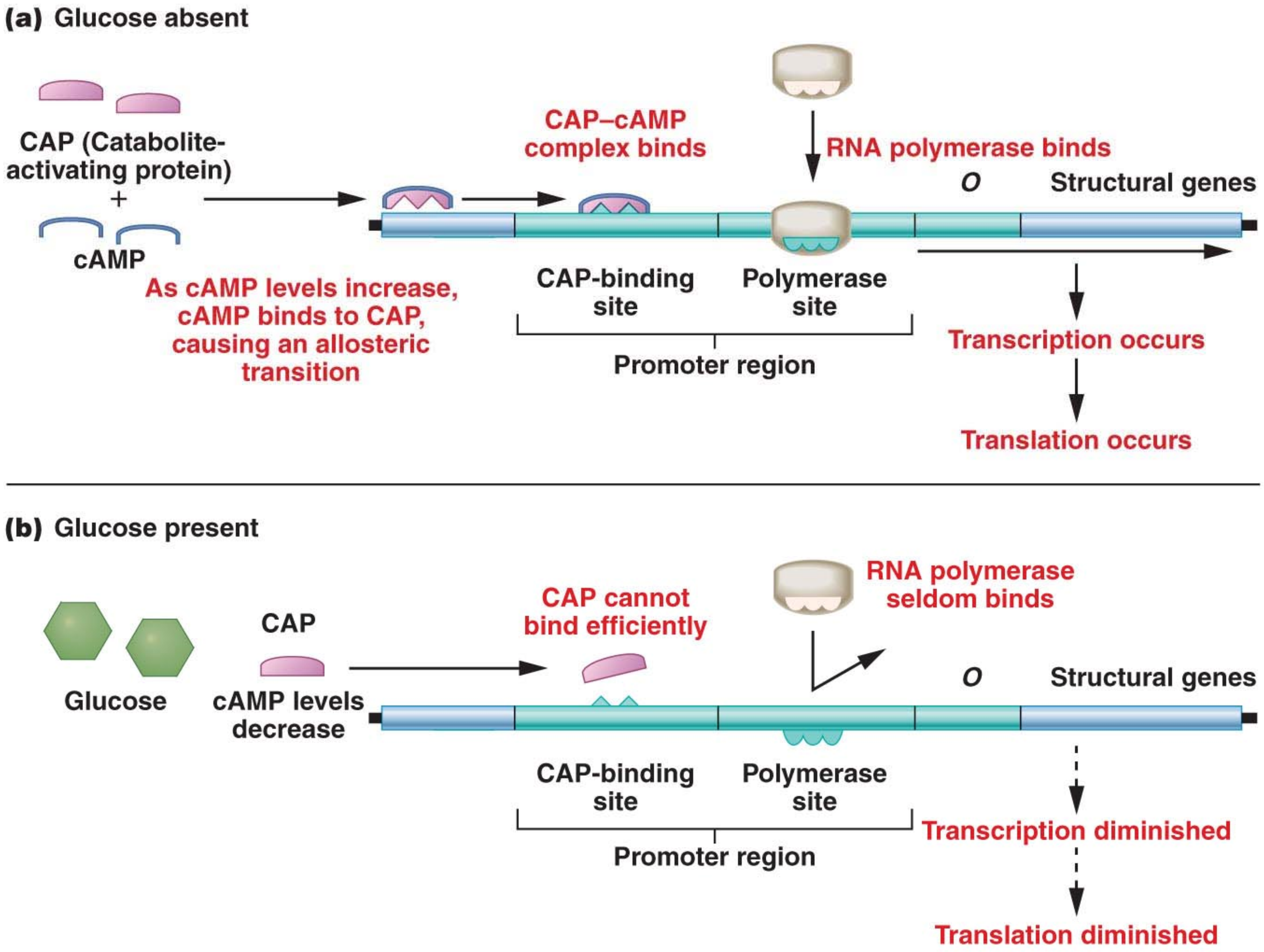


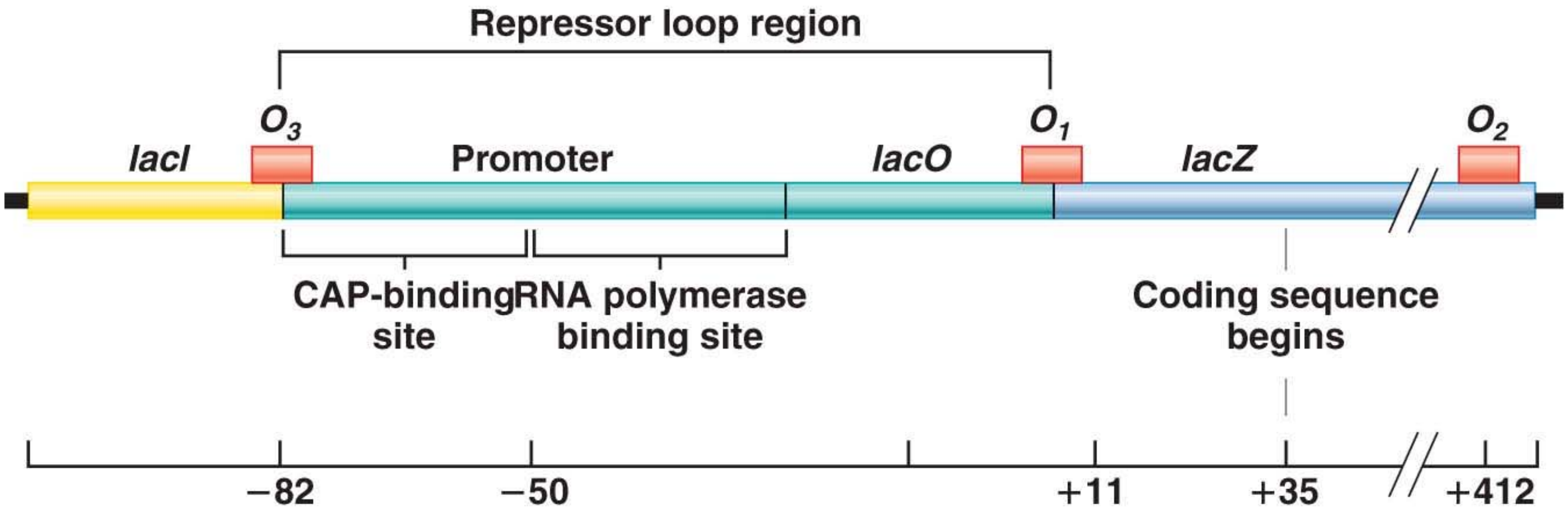
Genetic Proof of the Operon Model

	Genotype	Presence of β-Galactosidase Activity	
		Lactose Present	Lactose Absent
	$I^+O^+Z^+$	+	–
	A. $I^+O^+Z^-$	–	–
constitutive →	$I^-O^+Z^+$	+	+
→	$I^+O^CZ^+$	+	+
trans element →	**B.** $I^-O^+Z^+/F'I^+$	+	–
cis element →	$I^+O^CZ^+/F'O^+$	+	+
	C. $I^+O^+Z^+/F'I^-$	+	–
	$I^+O^+Z^+/F'O^C$	+	–
	D. $I^SO^+Z^+$	–	–
	$I^SO^+Z^+/F'I^+$	–	–

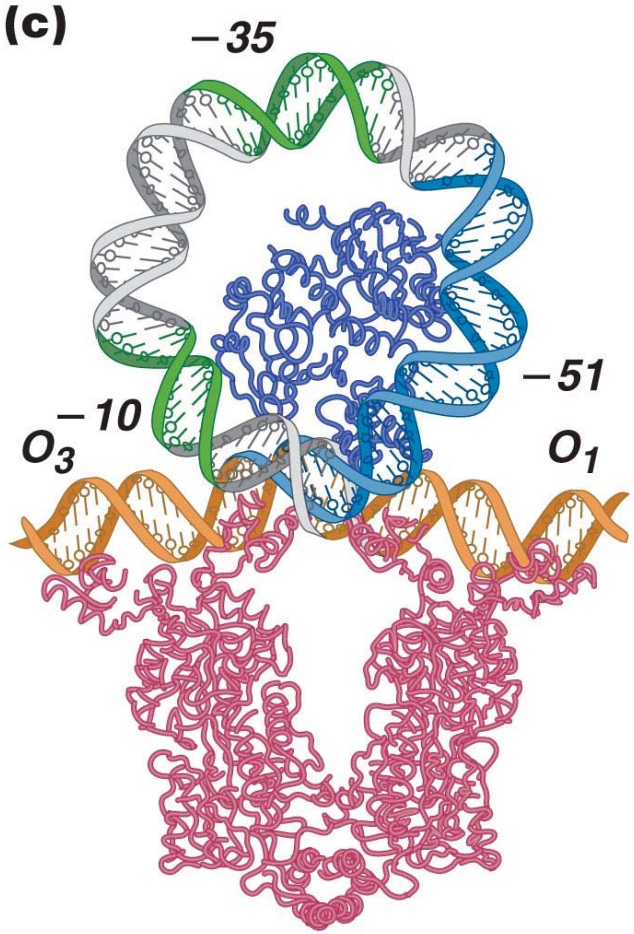
16.3 The Catabolite-Activating Protein (CAP) Exerts Positive Control over the *lac* Operon



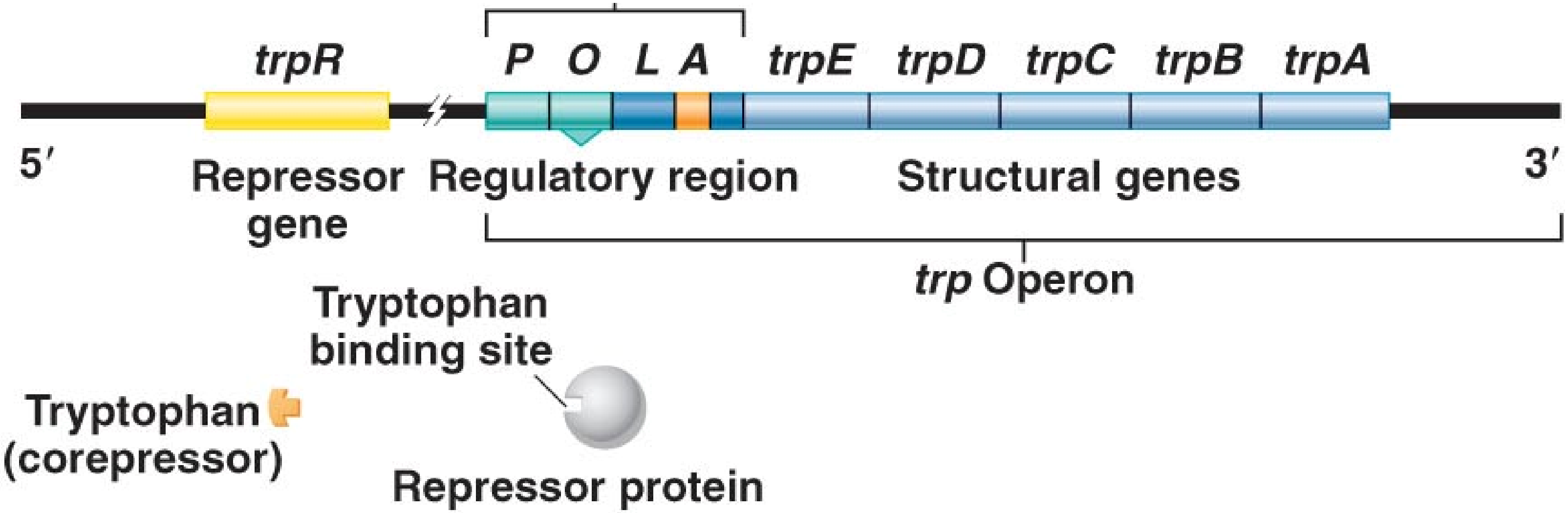

(a) Glucose absent
CAP (Catabolite-activating protein)
+
cAMP
As cAMP levels increase, cAMP binds to CAP, causing an allosteric transition
CAP–cAMP complex binds
RNA polymerase binds
O
Structural genes
CAP-binding site
Polymerase site
Promoter region
Transcription occurs
Translation occurs
(b) Glucose present
Glucose
CAP
cAMP levels decrease
CAP cannot bind efficiently
RNA polymerase seldom binds
O
Structural genes
CAP-binding site
Polymerase site
Promoter region
Transcription diminished
Translation diminished

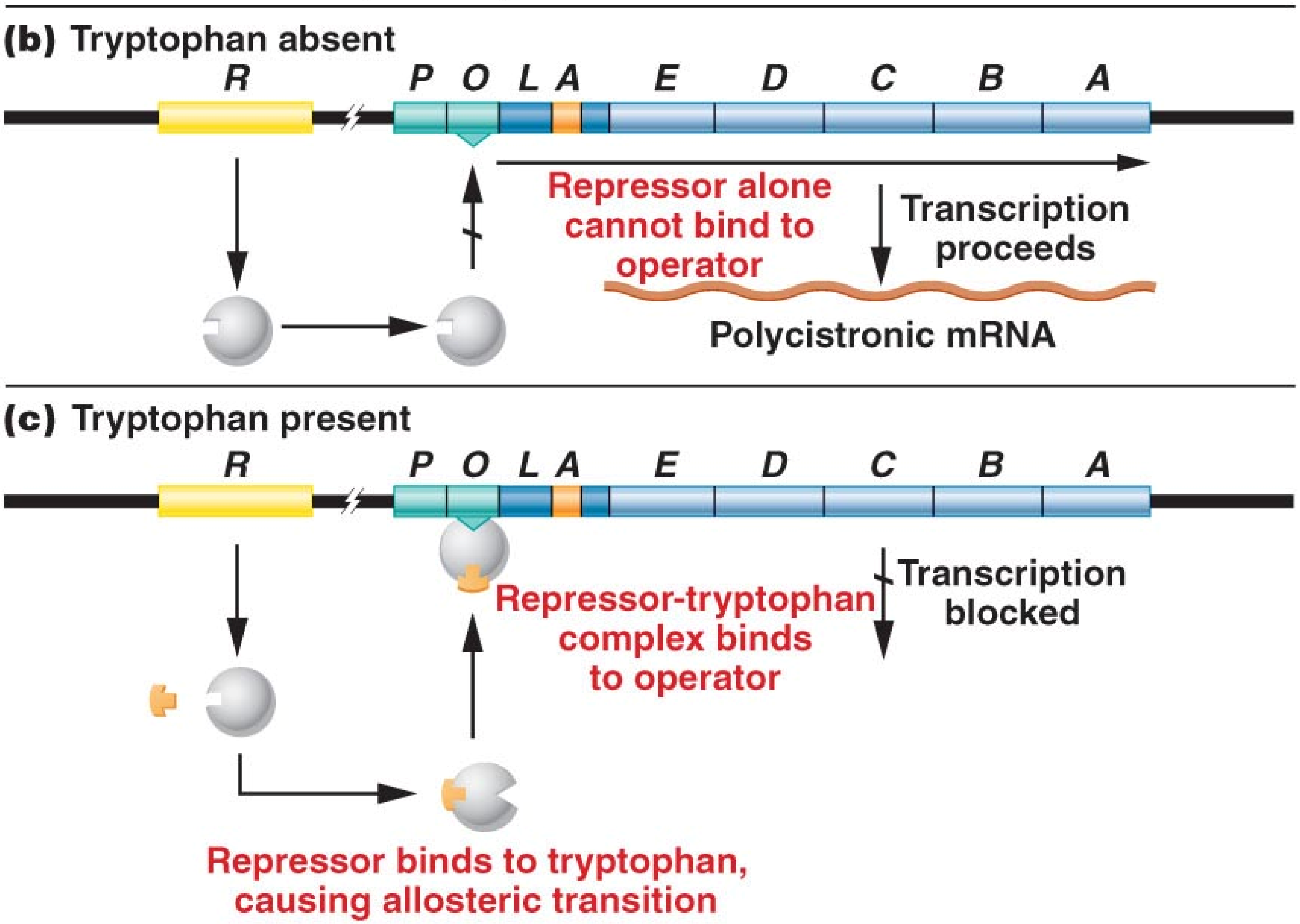

Repressor loop region
O_3
O_1
O_2
lacI
Promoter
lacO
lacZ
CAP-binding site
RNA polymerase binding site
Coding sequence begins
−82
−50
+11
+35
+412

16.4 Crystal Structure Analysis of Repressor Complexes Has Confirmed the Operon Model

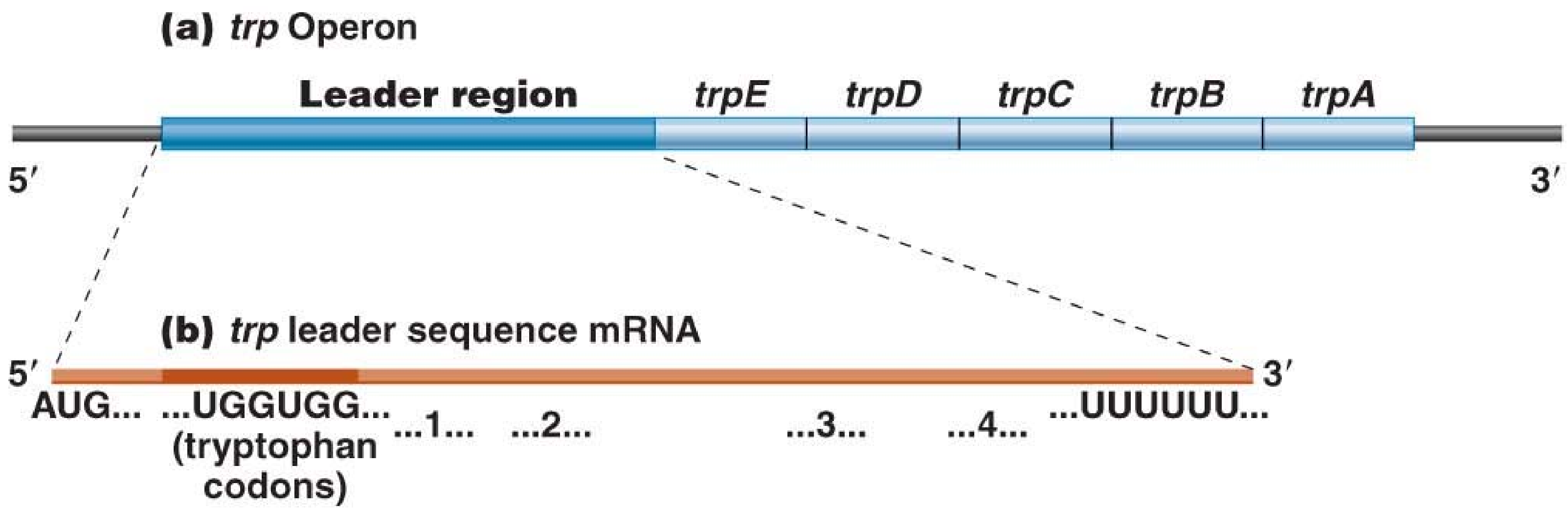


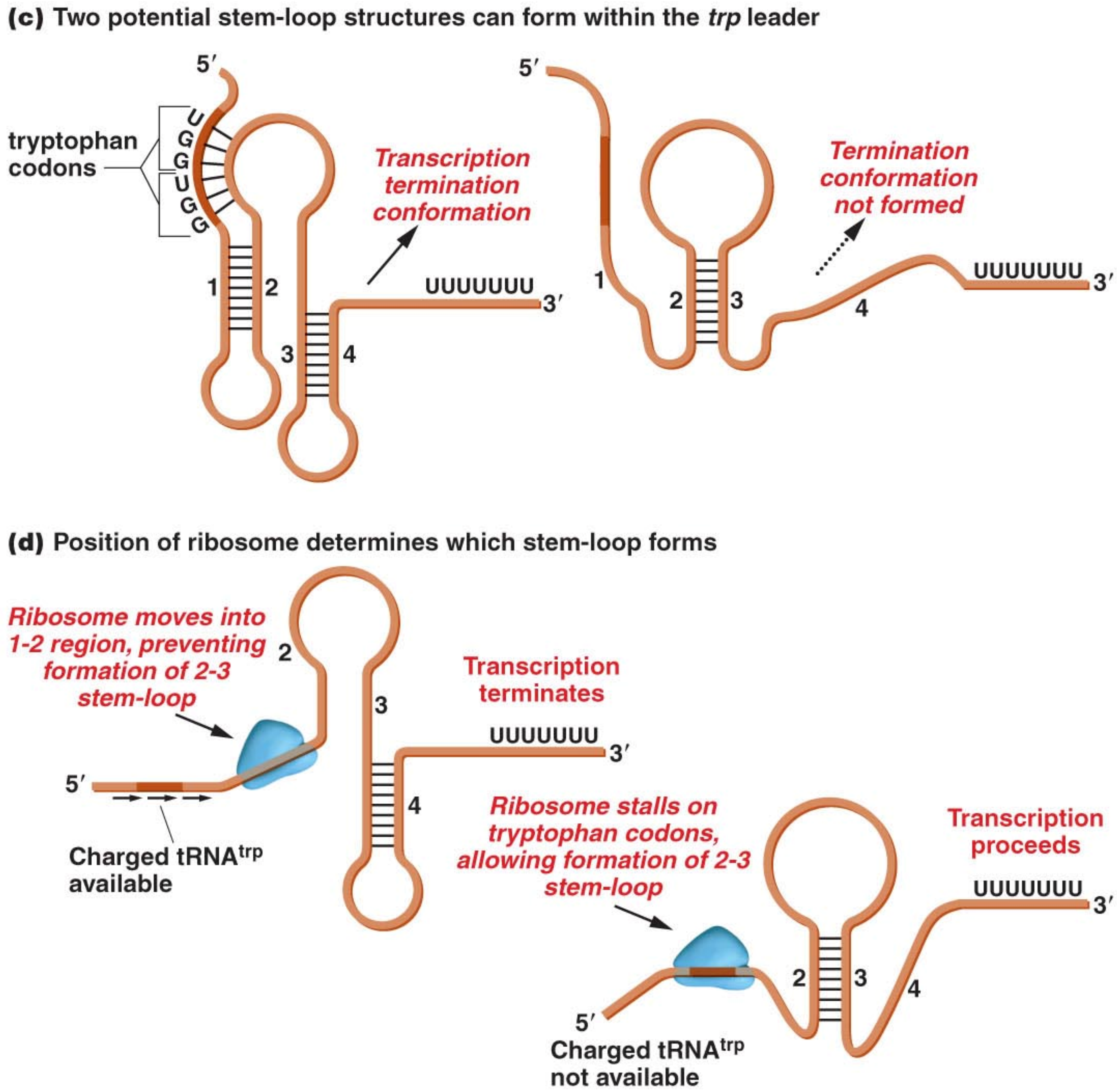
16.5 The Tryptophan (*trp*) Operon in *E. coli* Is a Repressible Gene System




(b) Tryptophan absent
R
P O L A
E D C B A
Repressor alone cannot bind to operator
Transcription proceeds
Polycistronic mRNA
(c) Tryptophan present
R
P O L A
E D C B A
Repressor-tryptophan complex binds to operator
Transcription blocked
Repressor binds to tryptophan, causing allosteric transition

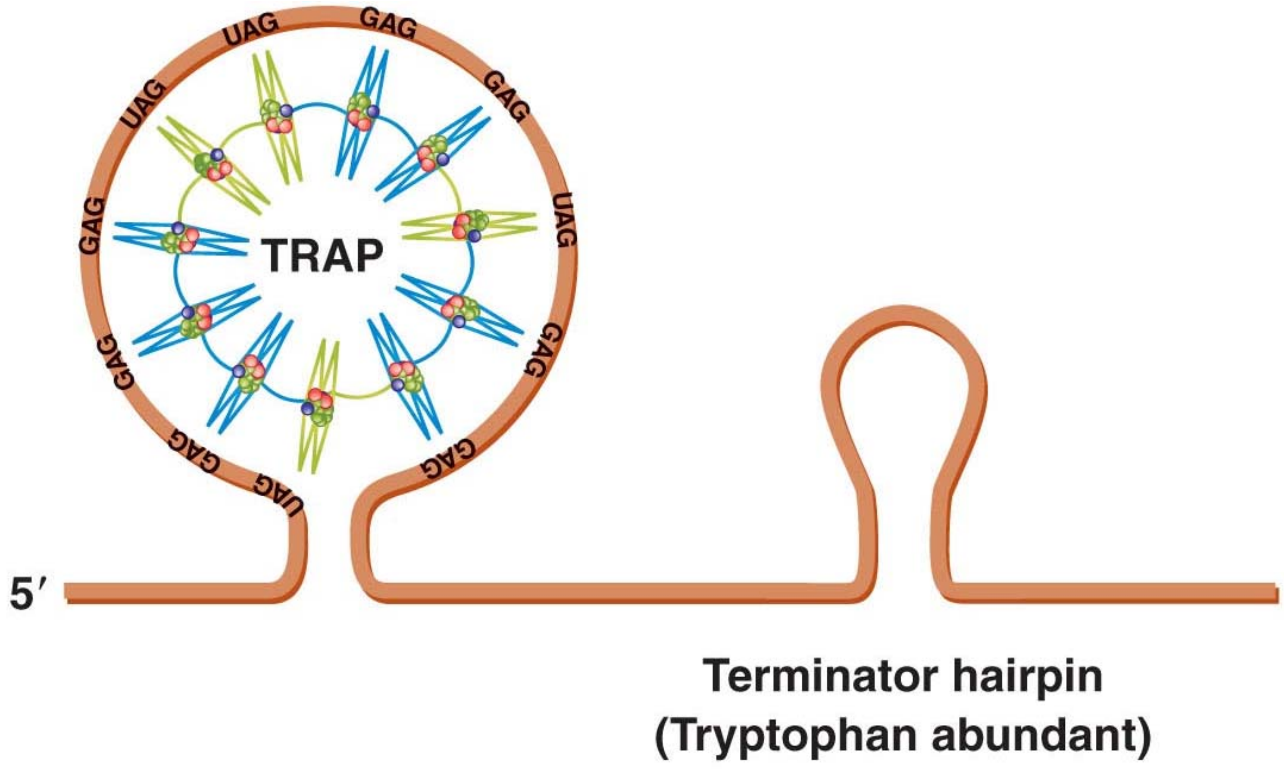
16.6 Attenuation Is a Critical Process during the Regulation of the *trp* Operon in *E. coli*



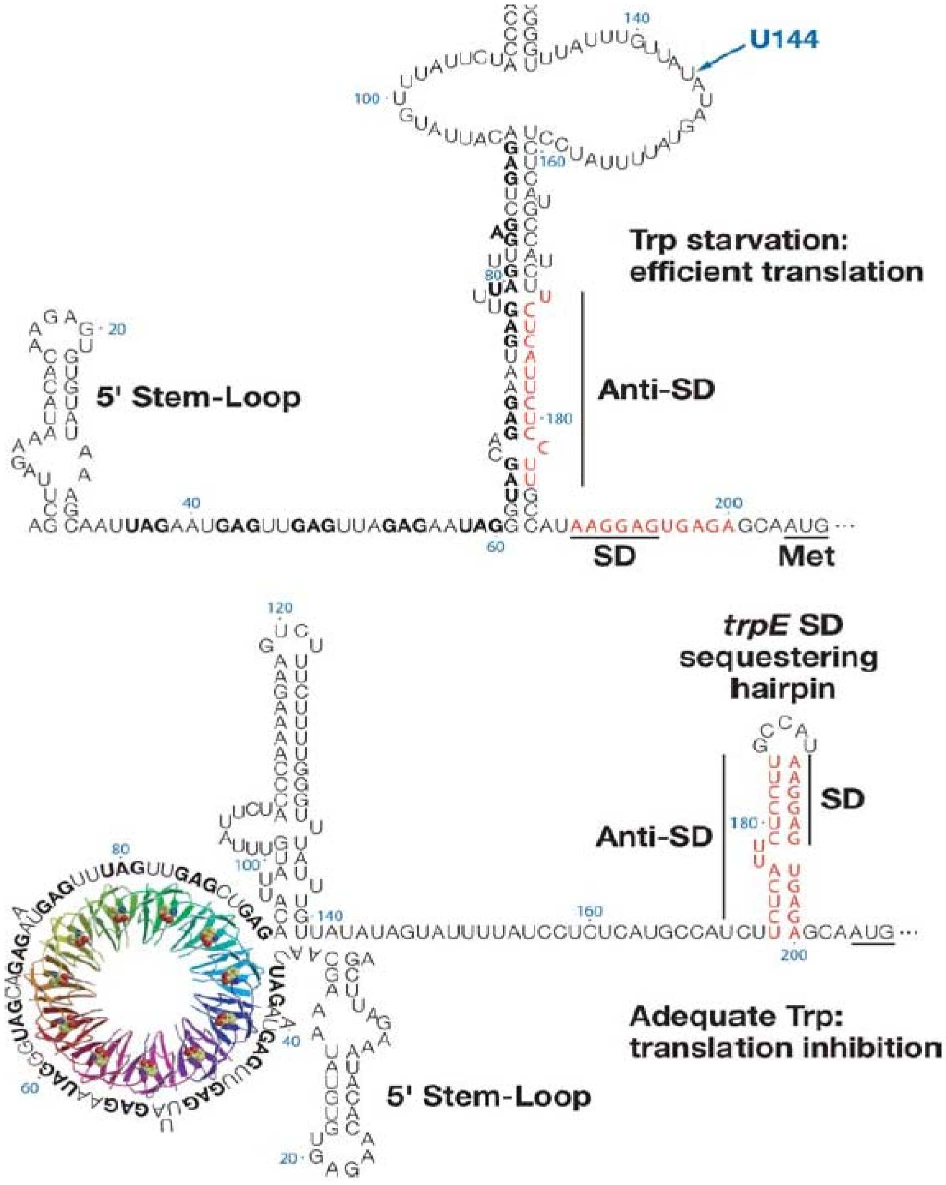

(c) Two potential stem-loop structures can form within the trp leader
5′
tryptophan codons
U G G U G G
1
2
3
4
Transcription termination conformation
UUUUUUU
3′
5′
1
2
3
4
Termination conformation not formed
UUUUUUU
3′
(d) Position of ribosome determines which stem-loop forms
Ribosome moves into 1-2 region, preventing formation of 2-3 stem-loop
2
3
4
Transcription terminates
UUUUUUU
3′
5′
Charged tRNAtrp available
Ribosome stalls on tryptophan codons, allowing formation of 2-3 stem-loop
Transcription proceeds
UUUUUUU
3′
2
3
4
5′
Charged tRNAtrp not available

TRAP and AT (anti-TRAP) Proteins Govern Attenuation in *B. subtilis*

The interaction of a tryptophan-bound TRAP (trp RNA-binding attenuation protein) with the leader sequence of the trp operon induces the formation of the terminator hairpin, attenuating expression of the trp operon



TRAP binding to *trp* operon readthrough transcripts promotes formation of the *trpE* Shine–Dalgarno (SD) sequestering hairpin; formation of this structure inhibits TrpE synthesis by preventing ribosome binding



16.7 Riboswitches Utilize Metabolite-sensing RNAs to Regulate Gene Expression

A **Known Riboswitch Ligands**

Coenzymes	Nucleotide Derivatives	Amino Acids	Sugars	Ions
AdoCbl	Guanine	Glycine	GlcN6P	Mg^{2+}
TPP	Adenine	Lysine		
FMN	$PreQ_1$	Glutamine		
THF	2'-dG			
SAM	* c-di-GMP			
SAH				
MoCo				
WCo				

(shaded) = Contains RNA components or is directly derived from RNA

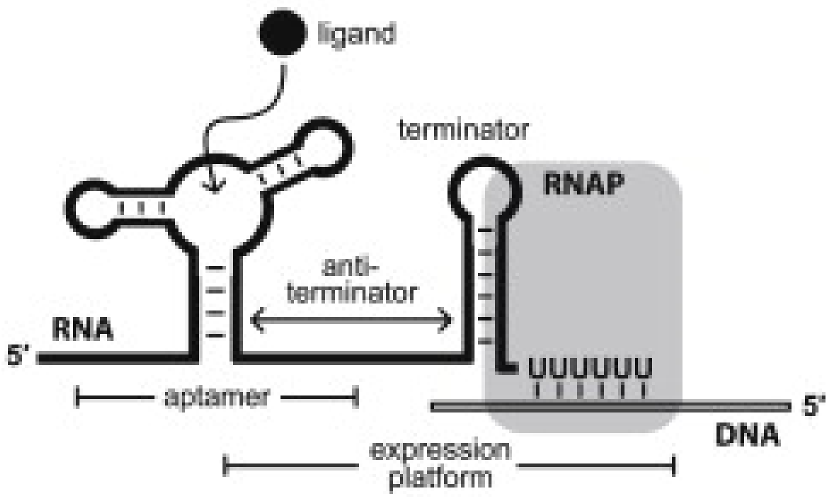
* = Not universal

(boxed) = Vitamin derivatives

Gene Control Mechanisms

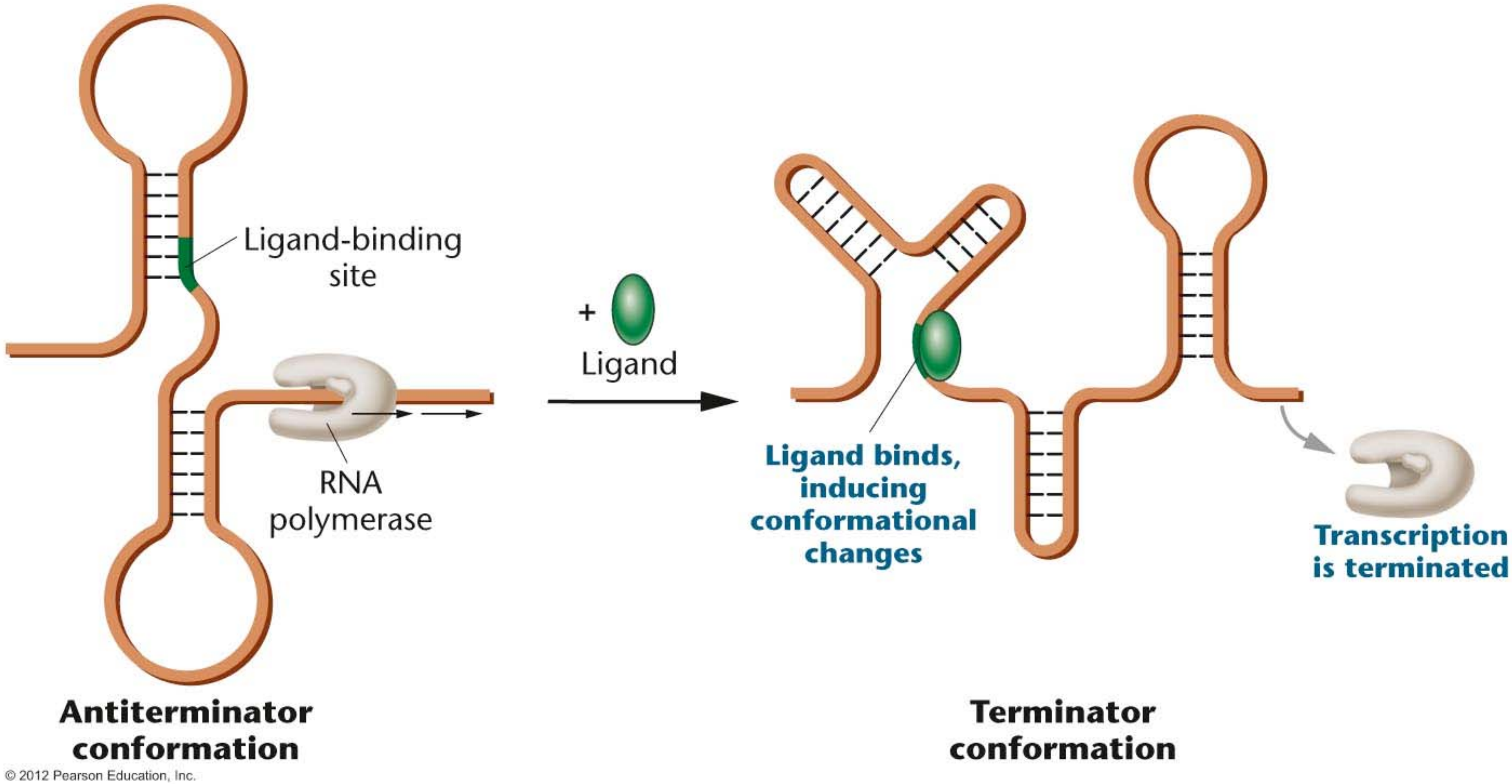
- transcription termination
- translation initiation
- ribozyme self-destruction
- transcriptional interference
- trans-acting via base pairing
- mRNA splicing control

B **Transcription Termination**



State 1: For an "OFF" switch, ligand binding stabilizes aptamer structure, permitting terminator to form and transcription to cease.

State 2: Absense of ligand permits an antiterminator to form and transcription to proceed.


Ligand-binding site
RNA polymerase
+
Ligand
Ligand binds, inducing conformational changes
Transcription is terminated
Antiterminator conformation
Terminator conformation
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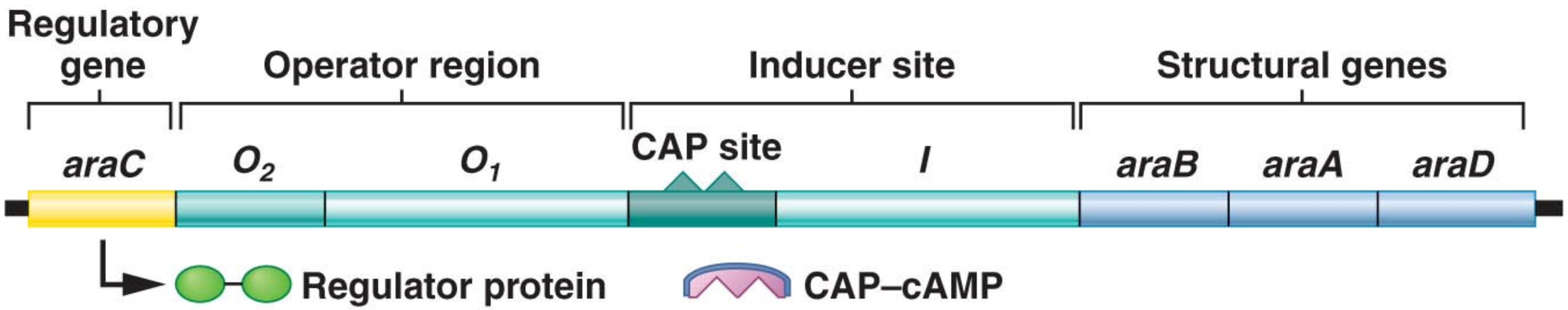
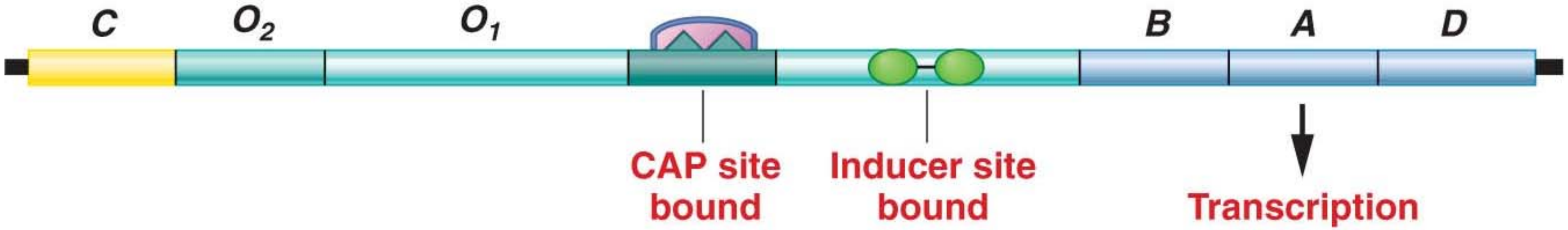
16.8 The *ara* Operon Is Controlled by a Regulator Protein That Exerts Both Positive and Negative Control



(b) Arabinose present; operon is induced – positive regulation



(c) Arabinose absent; operon is repressed – negative regulation