Chapter 16

Regulation of Gene Expression in Prokaryotes

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Prokaryotic Genes and Operons

- Genes involved in “related functions” often clustered together and expressed as a unit on a single mRNA
  - Operon
  - Polycistronic mRNA

Gene Expression Must Respond to Environmental Conditions

- Some regulatory proteins present at 5-10 copies per cell, some enzymes for glycolysis present at 100,000 copies/cell
- Genes whose products are presently unneeded or at acceptable levels are turned off
- Mechanisms to achieve proper mix of gene expression are varied
  - Whatever works best strategy…

Gene Expression

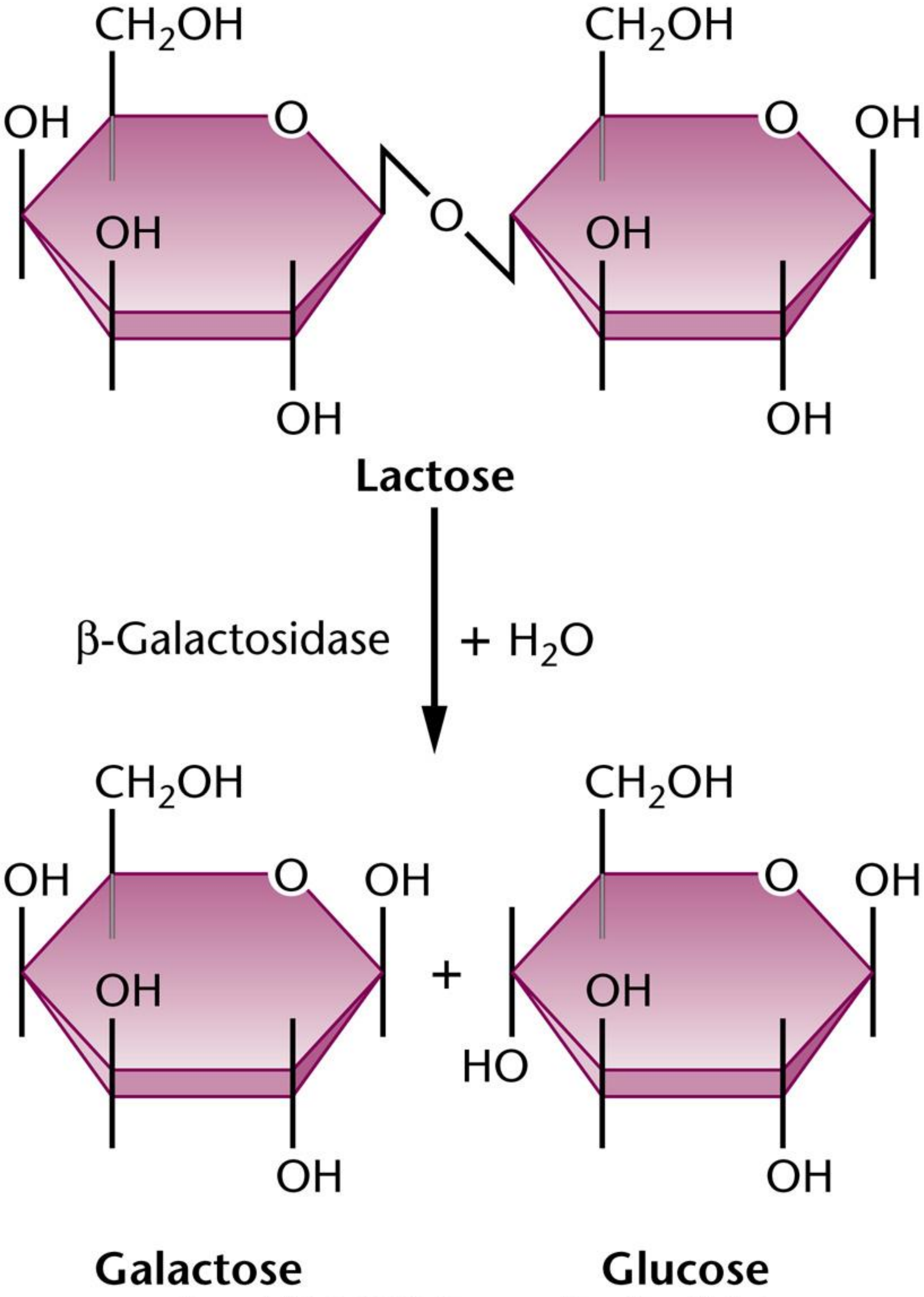
- Constitutive
  - Expressed constantly at a set rate
    - But rate can vary widely
- Positive vs. Negative control
  - Activators vs. repressors
- Inducible
  - Expression blocked by a repressor that can be inactivated by a small molecule called an inducer
- Repressible
  - Expression can be blocked by a repressor (aporepressor) that must be bound by a small molecule corepressor in order to be active

Lactose Metabolism in *E. coli*

- Jacob and Monod (1946) studied as model system, many others followed…
- Enzyme β-galactosidase only expressed when lactose present in the medium
  - Enzyme said to be expressed in inducible fashion with lactose as inducer
- Identified cis-acting elements (operator, promoter) and trans-acting factors

Lactose Hydrolysis

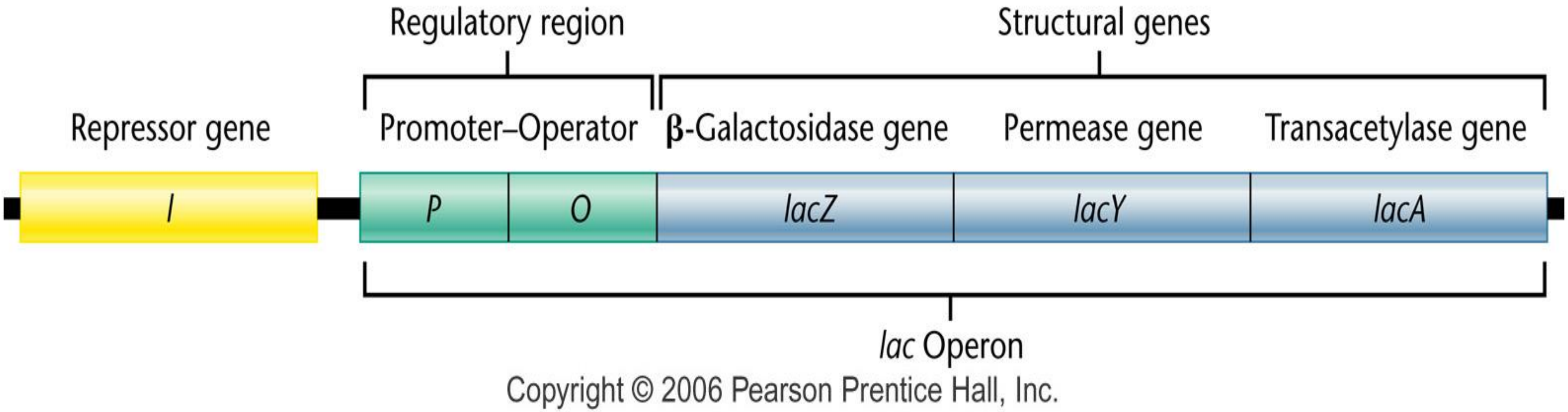
- Enzyme encoded by *lacZ* gene
- Glucose and galactose products
- Enzyme cleaves broad range of β-galactosides
  - Including synthetic analogs such as ONPG and X-gal



Structural Genes of *lac* Operon

- Structural genes encode the primary structure of the enzymes/proteins
  - For *lac* operon these are *lacZ*, *lacY* and *lacA*
  - Enzymes encoded are β-galactosidase, lactose permease and transacetylase, respectively
  - Lederberg mapped *lacZ*, *lacY* and *lacA* mutants to show that genes were very closely linked in order ZYA
    - Genes are also coordinately regulated

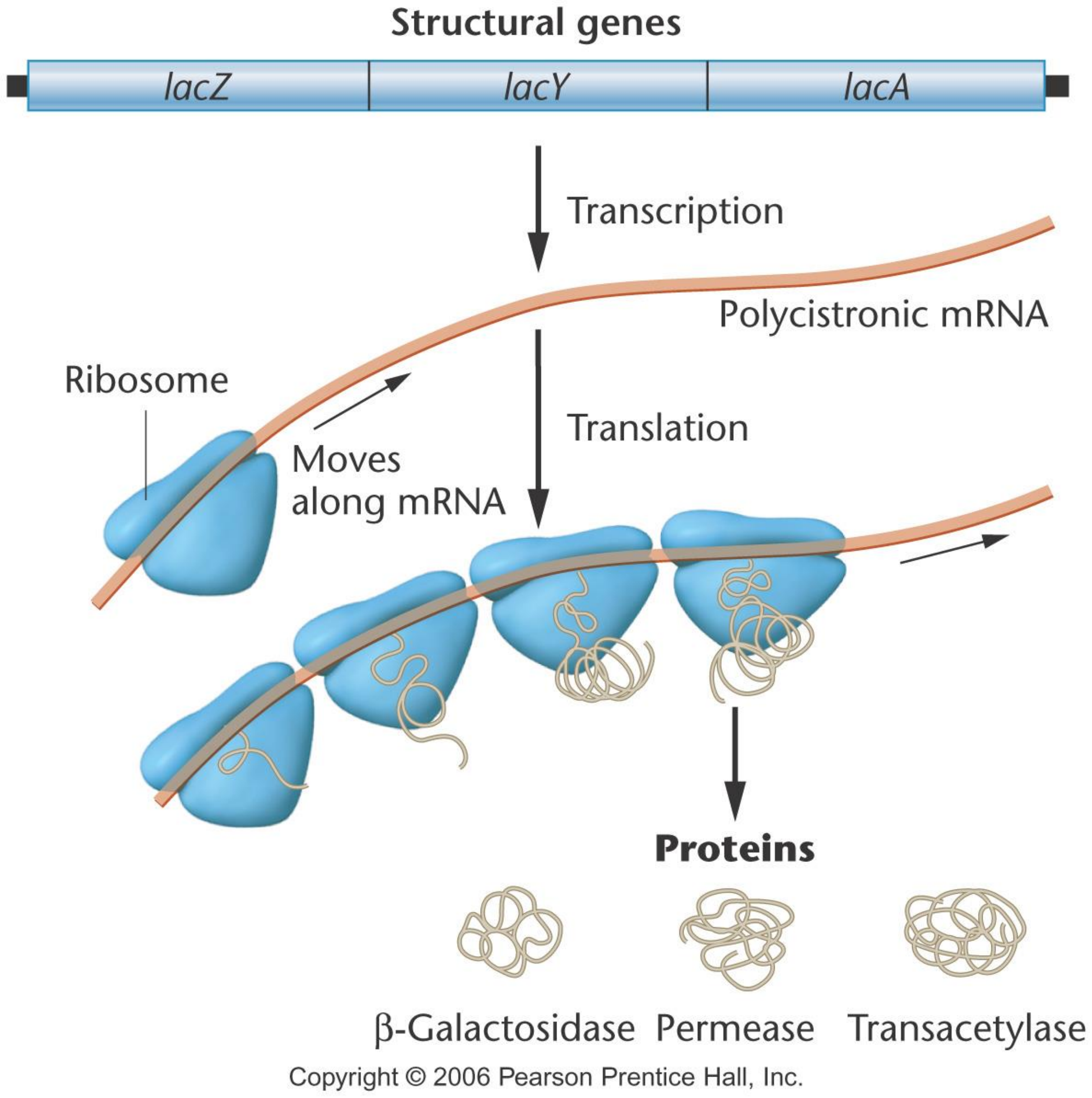
E. Coli lac Operon



- *lacI* has a constitutive promoter and is expressed separately from the *lac* operon
  - Encodes *lac* repressor
  - Low level of expression

lac Operon Expression

- *lac* operon encodes polycistronic mRNA giving rise to 3 different enzymes



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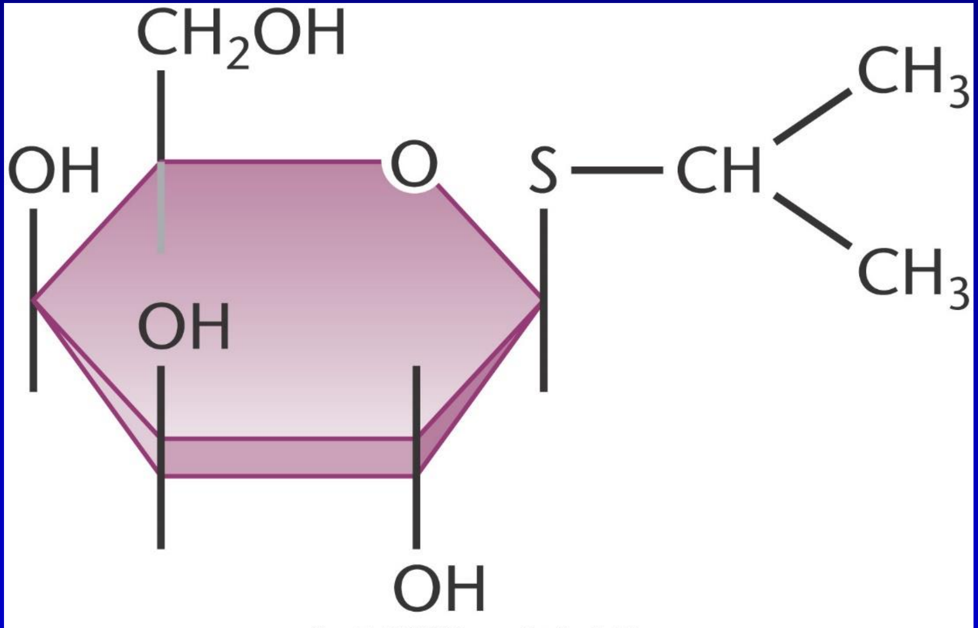
Gratuitous Inducers

- Lactose is normal inducer (actually allolactose for the pure at heart)
  - But other β-galactosides also work
- Isopropylthiogalactoside (IPTG) also acts as an inducer but is not metabolized
  - Shows induction does not involve interaction with the actual enzyme being synthesized

Isopropylthiogalactoside

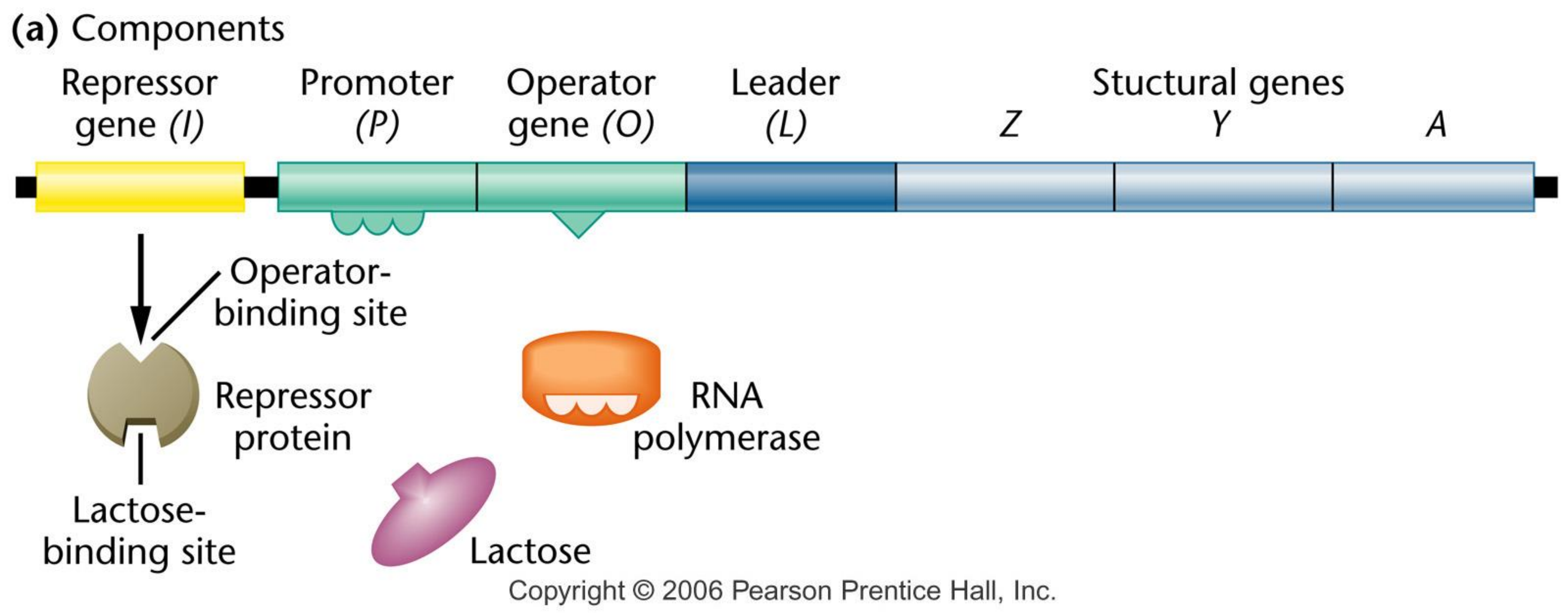
- IPTG
- Gratuitous inducer
- Not metabolized
  - Level remains constant once added



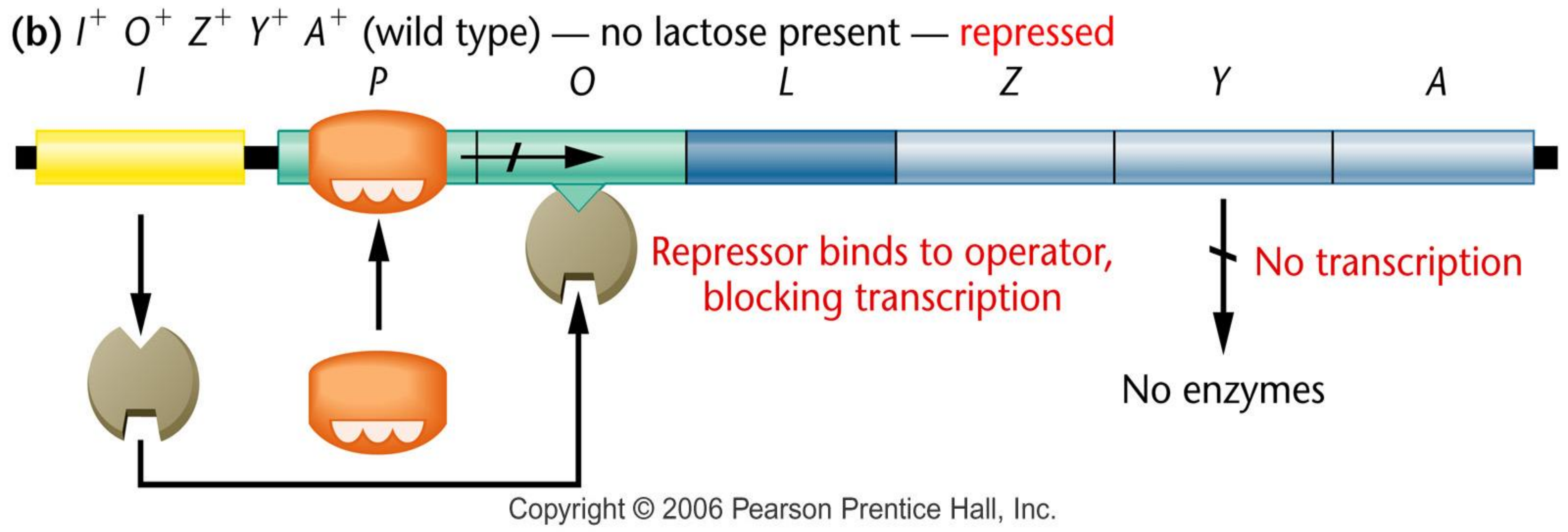
Operon Model

- 1960, Jacob and Monod
- Group of genes regulated/expressed as a unit
- Structural genes
  - *lacZYA*
- Promoter and operator
- *lacI* gene produces allosteric repressor
  - Conformational shape upon binding lactose
  - LacI binds to operator in absence of lactose
  - binding to lactose induces conformational change and prevents interaction with operator

lac Operon Expression

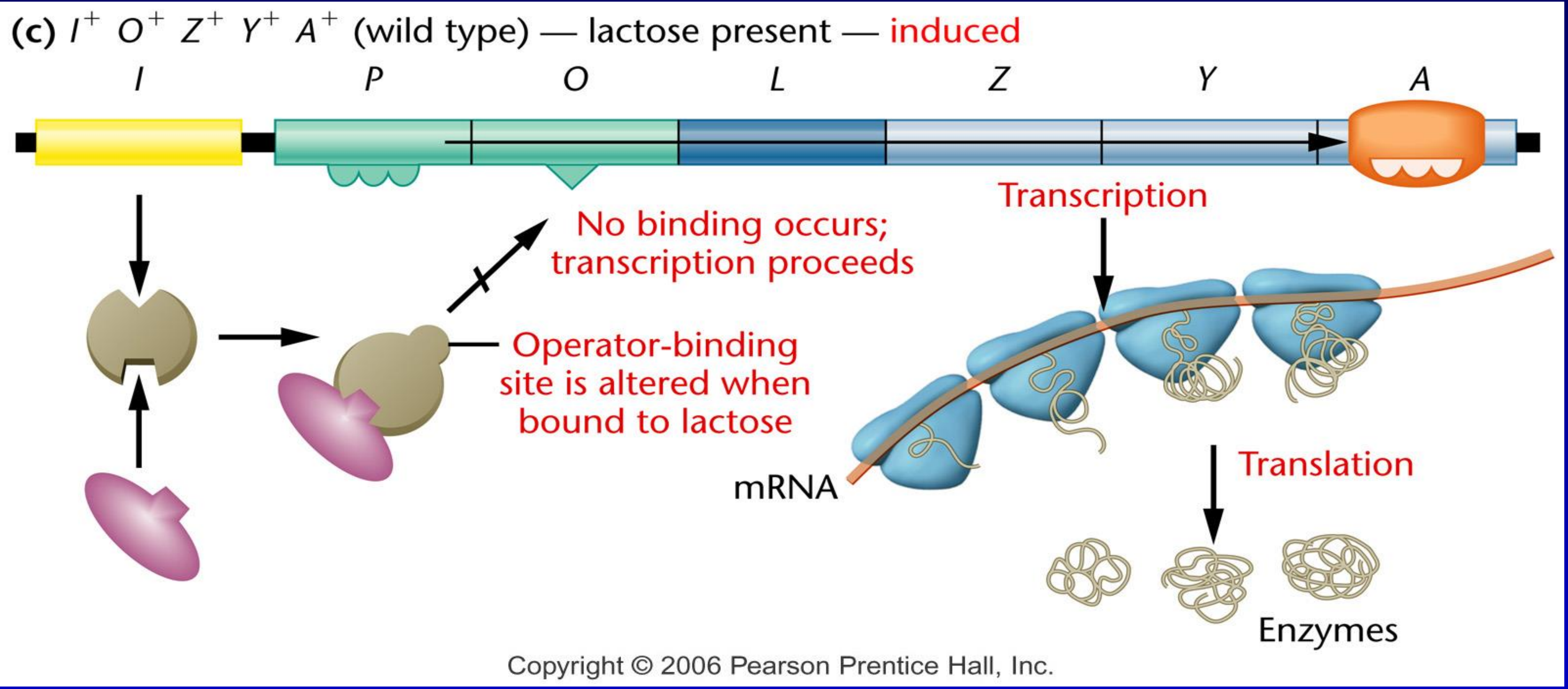


lac Operon Expression



- In absence of inducer, repressor tetramer binds to operator and blocks RNAP from binding to promoter

lac Operon Expression

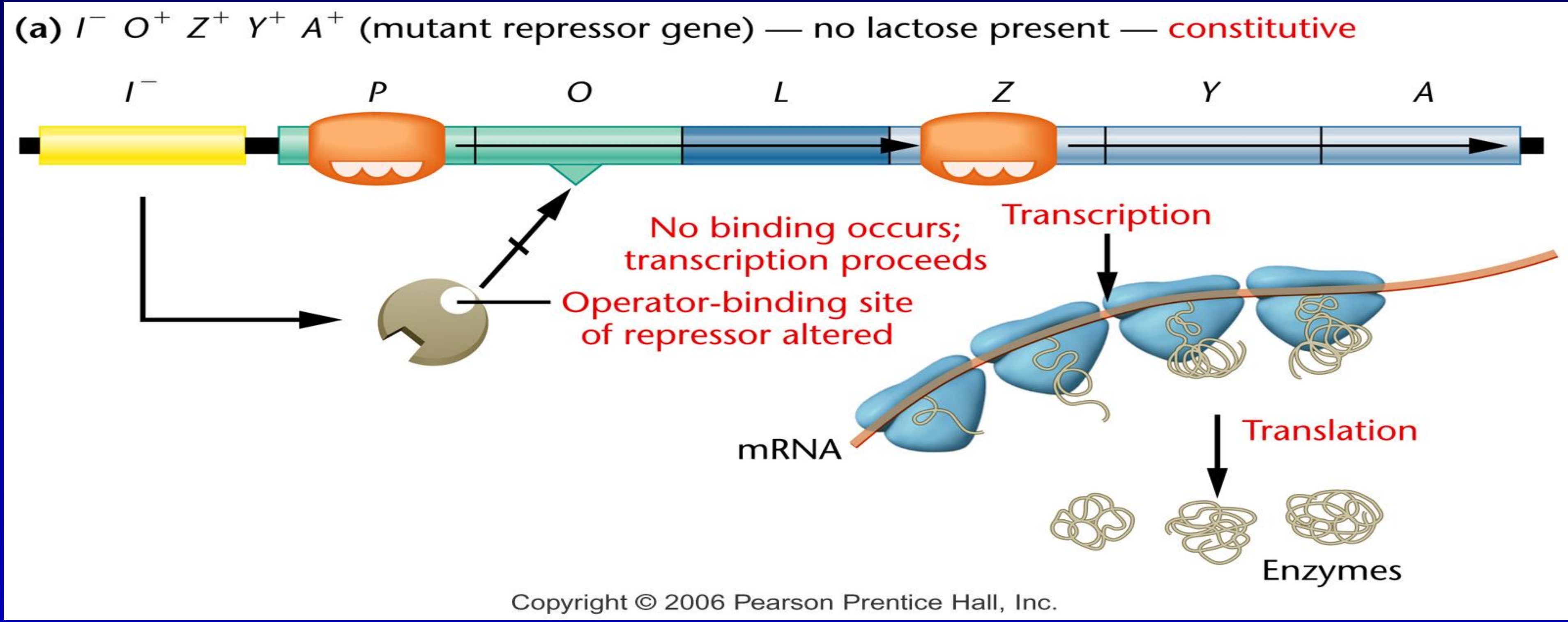


- Binding of inducer to repressor causes conformational change in protein, preventing interaction with operator
- RNA polymerase binds to promoter and expresses operon

lac Regulatory Mutations

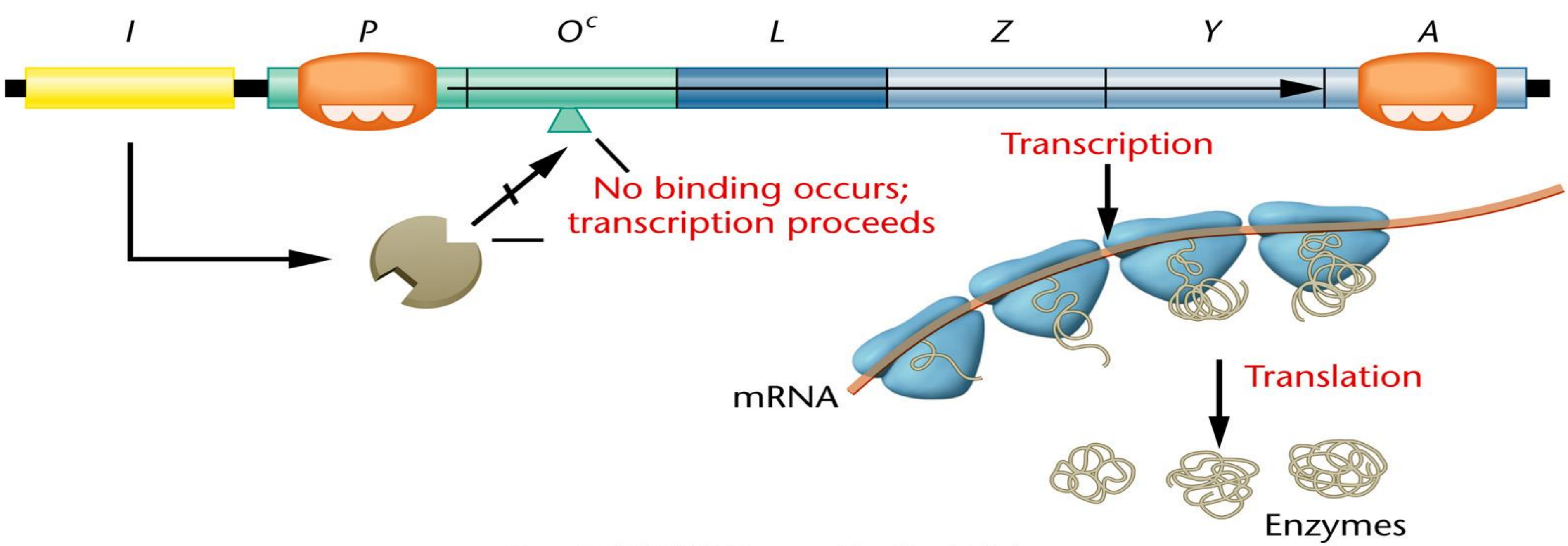
- Constitutive mutants
  - Lactose repressor gene (*lacI*) mutants (*lacI*$^-$)
  - Operator region mutants (O^c)
- Various combinations of these and other mutations studied using F’ plasmids encoding all or portion of *lac* operon
  - Merozygotes
- I^S mutants
  - Mutation eliminates induced binding site
- I^Q mutants
  - High level (quantity) expression

Constitutive Mutations

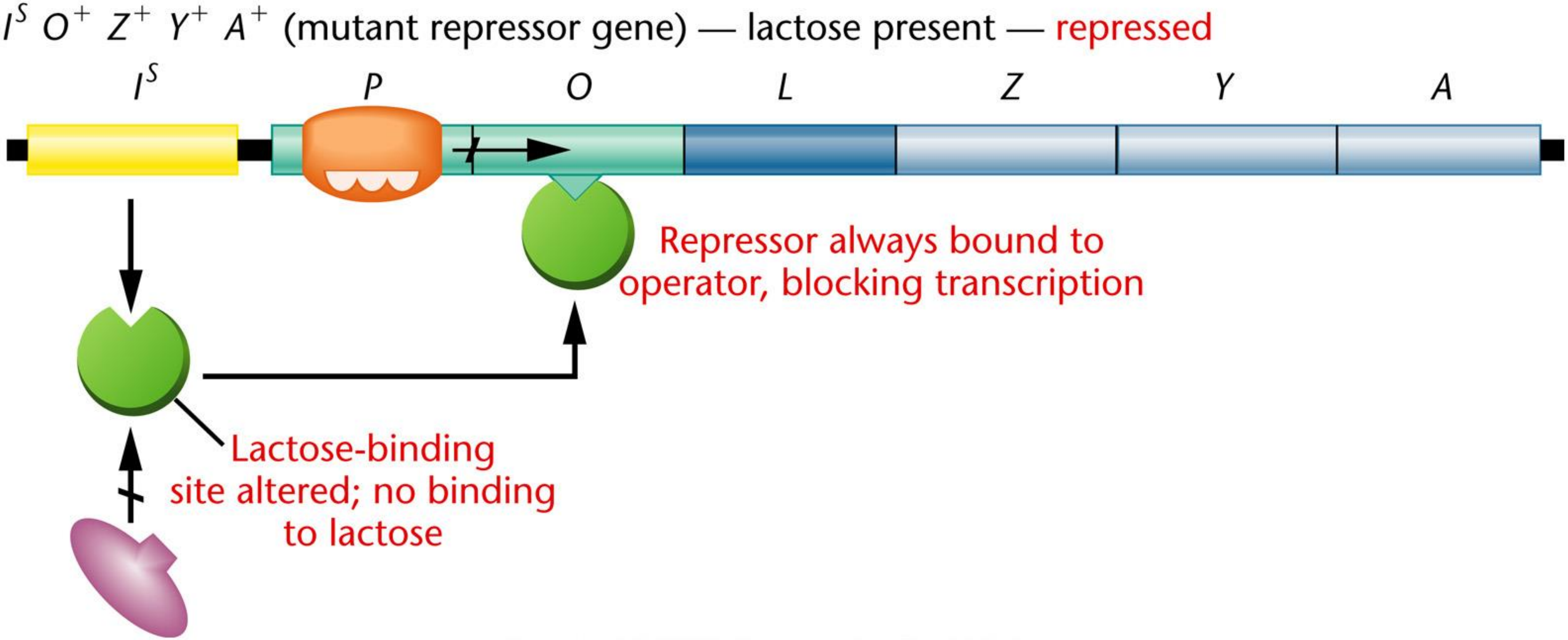

(a) $I^- \; O^+ \; Z^+ \; Y^+ \; A^+$ (mutant repressor gene) — no lactose present — constitutive

Constitutive Mutations

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



I^S Dominant Mutations



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

Repressor always bound to operator, blocking transcription

Lactose-binding site altered; no binding to lactose

Summary

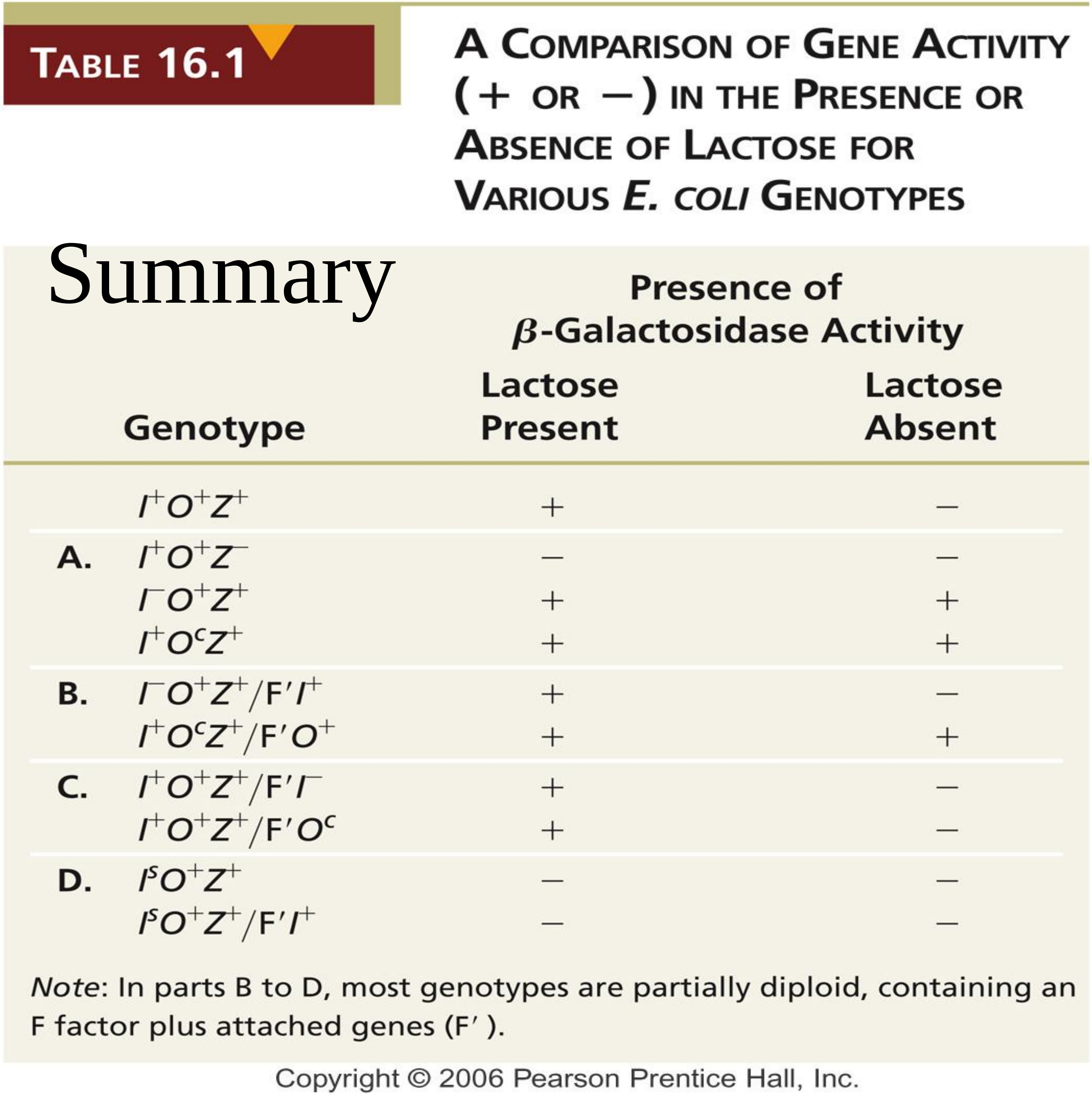


TABLE 16.1 A COMPARISON OF GENE ACTIVITY (+ OR −) IN THE PRESENCE OR ABSENCE OF LACTOSE FOR VARIOUS *E. COLI* GENOTYPES

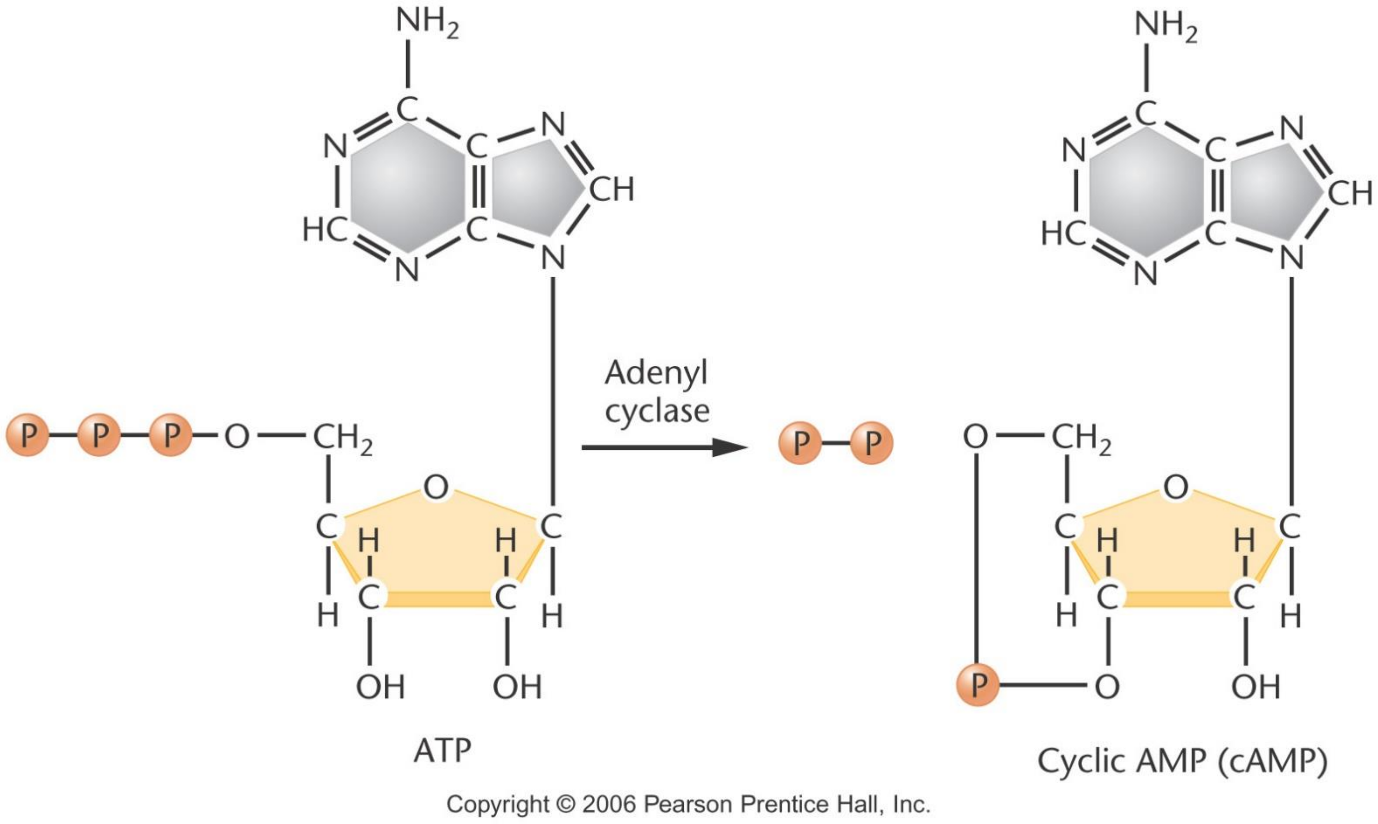
	Genotype	Presence of β-Galactosidase Activity	
		Lactose Present	Lactose Absent
	$I^+O^+Z^+$	+	−
A.	$I^+O^+Z^-$	−	−
	$I^-O^+Z^+$	+	+
	$I^+O^cZ^+$	+	+
B.	$I^-O^+Z^+/F'I^+$	+	−
	$I^+O^cZ^+/F'O^+$	+	+
C.	$I^+O^+Z^+/F'I^-$	+	−
	$I^+O^+Z^+/F'O^c$	+	−
D.	$I^sO^+Z^+$	−	−
	$I^sO^+Z^+/F'I^+$	−	−

Note: In parts B to D, most genotypes are partially diploid, containing an F factor plus attached genes (F′).

Observation

- Adding glucose to lactose medium reduces *lac* operon expression by cells dramatically
- Another level of expression
  - Cells must use most efficient source of carbon/energy
  - Glucose is most efficient because all necessary enzymes expressed constitutively

cAMP Synthesis

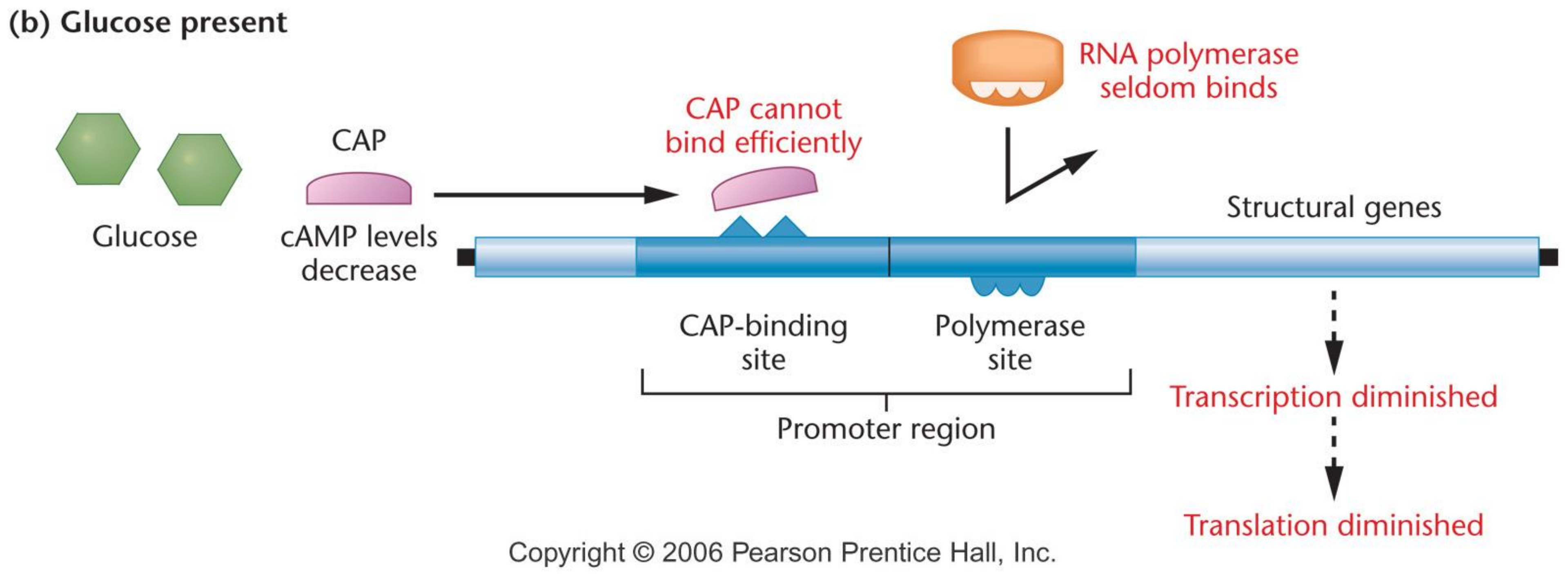


- cAMP means something to virtually all cells
- For *E. coli* it means “I’m hungry”
- Synthesized from ATP by adenylate cyclase

Catabolite Repression

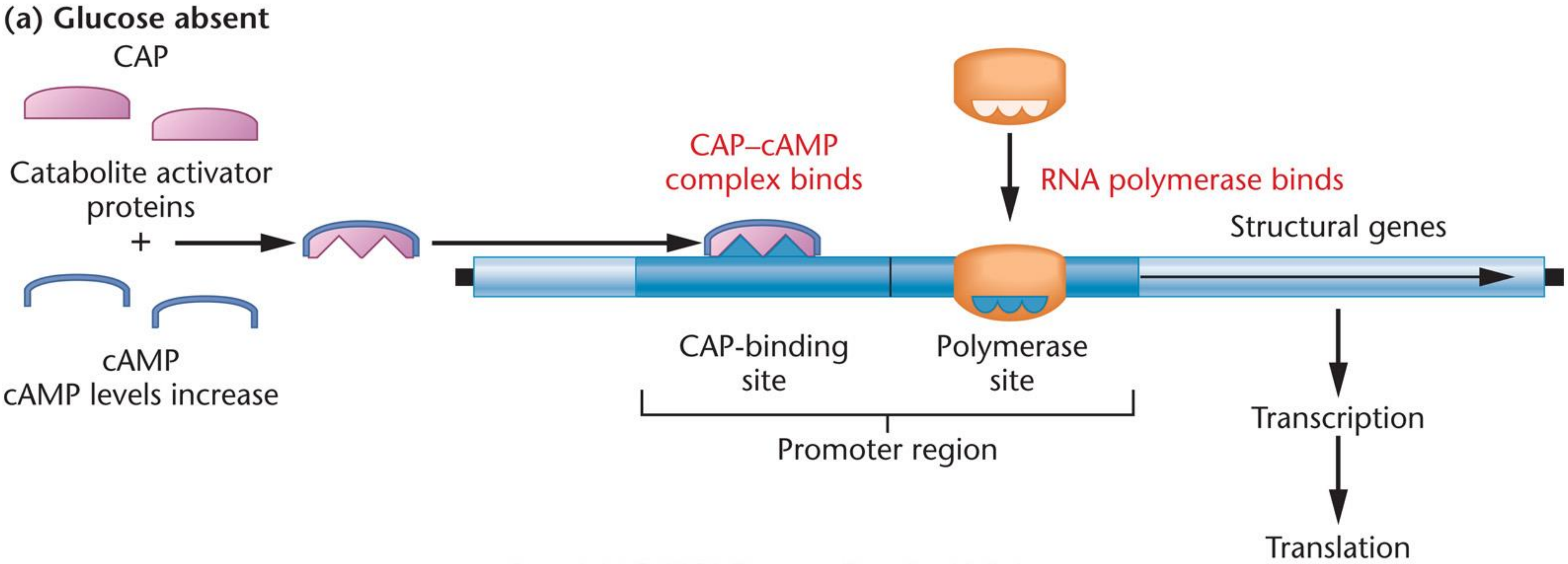
- Catabolite activator protein (CAP, also called catabolite regulatory protein or CRP)
- Positive control of catabolic operons
  - Allosteric
  - Regulated by cAMP
  - ATP up, cAMP down; ATP down, cAMP up
- cAMP:CAP binds to promoter
  - Converts relatively weak promoter into one of the strongest in the *E. coli* system

“Glucose Repression”



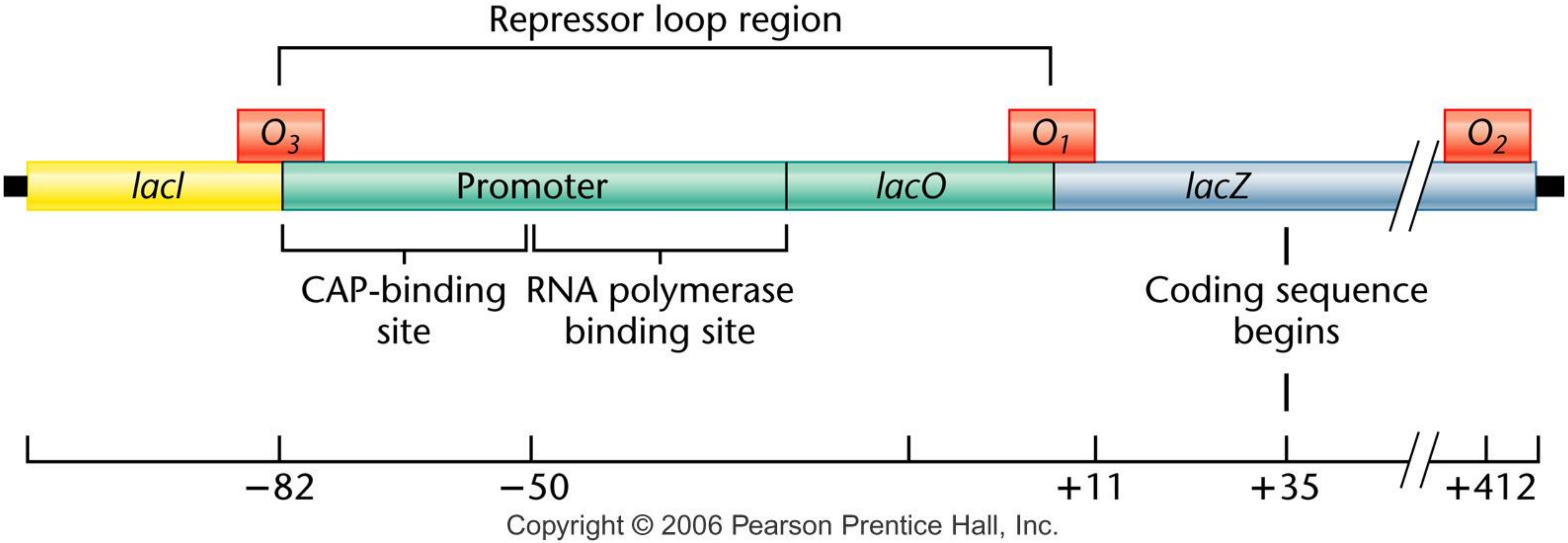
- CAP in incorrect conformation to bind promoter when not bound with cAMP

Regulation by CAP



- CAP in correct conformation to bind promoter when bound with cAMP

Auxiliary Operator Regions



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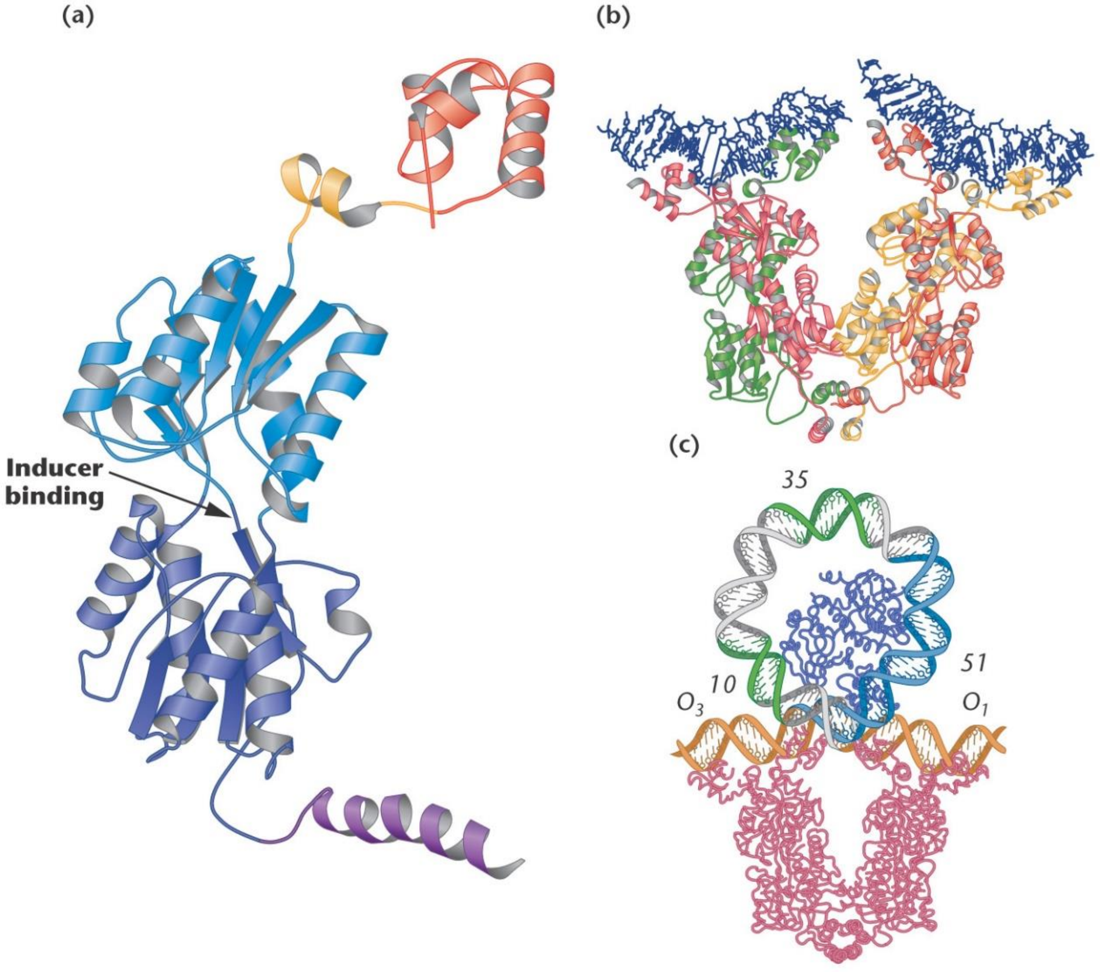
- Two additional repressor binding sequences located
- Maximum repression when all bound
- Binding O1 and O3 by a repressor tetramer (two DNA binding sites) causes looping of DNA similar to that found in eukaryotes

lac Repressor and the Operator

- Crystal structure studies



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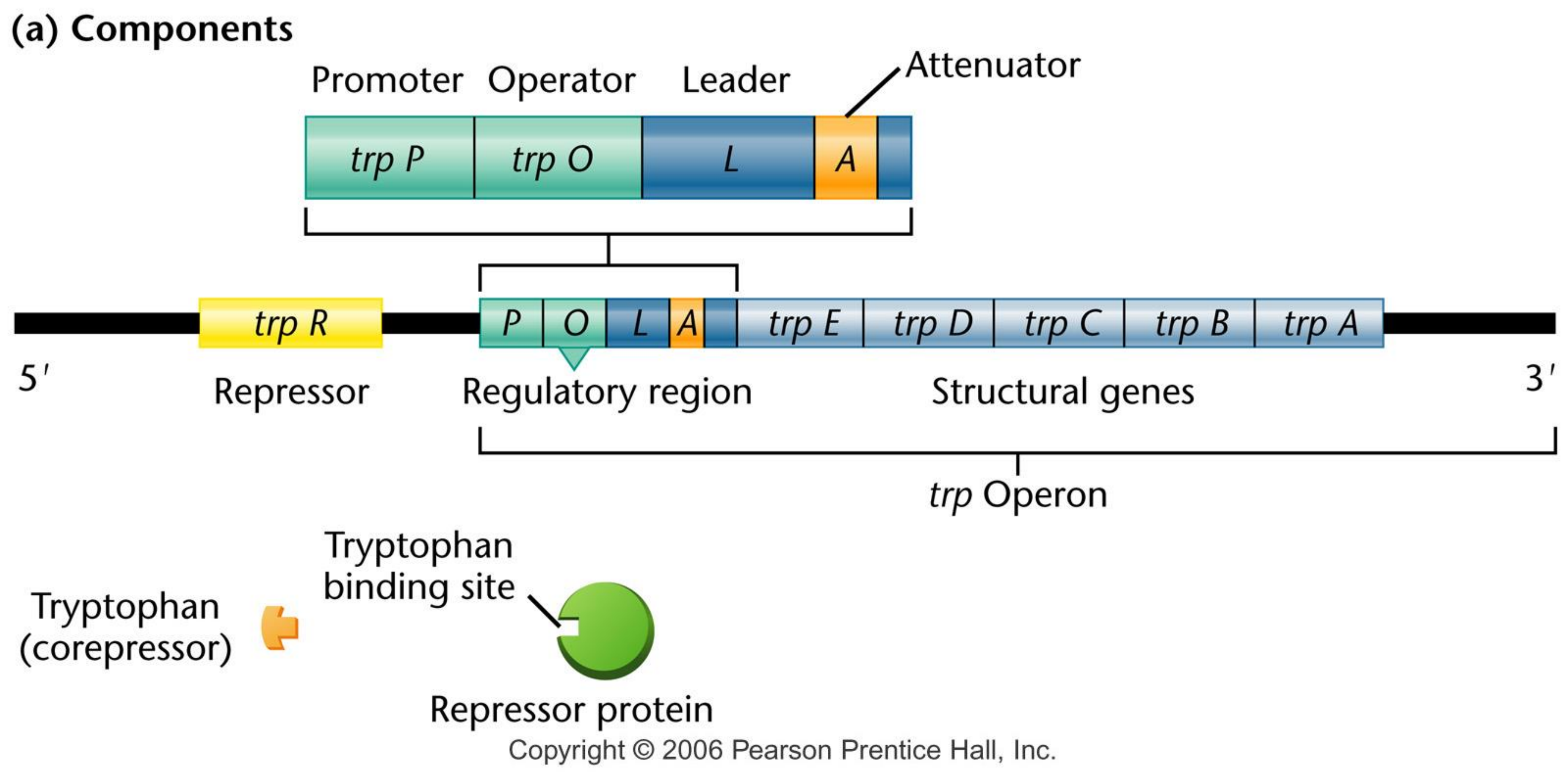
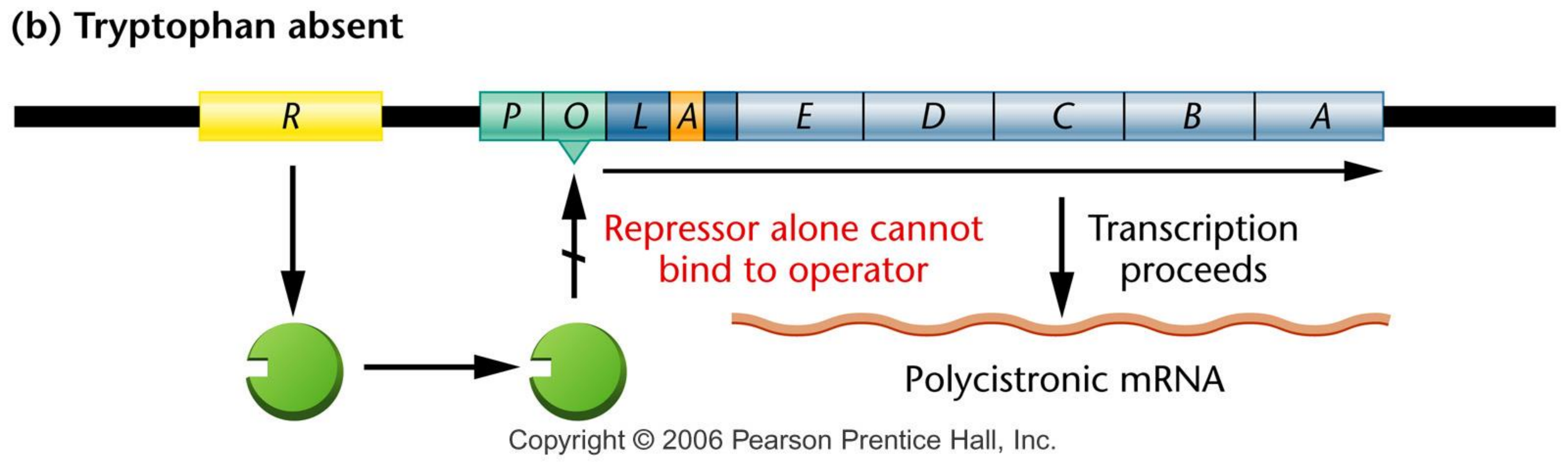
E. coli trp Operon

- Encodes five polypeptides required for tryptophan biosynthesis
  - Anabolic operon
    - *trpEDCBA*
  - Has repressor like *lac* operon but…
    - Called aporepressor (inactive by itself)
    - Tryptophan is the corepressor
    - Repressor active only when bound with tryptophan
  - Knock out *trpR* gene and operon still repressed in presence of tryptophan

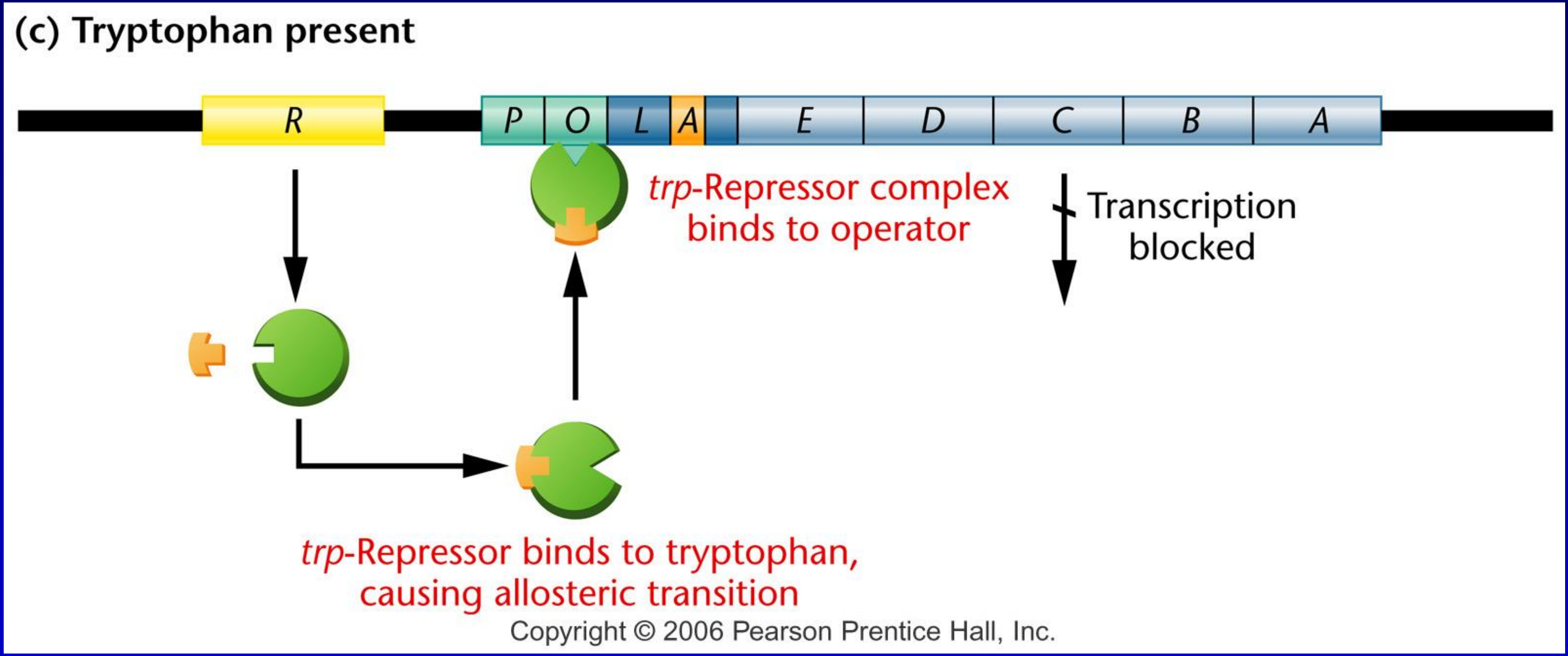
E. Coli trp Operon



E. Coli trp Operon: No Trp Present



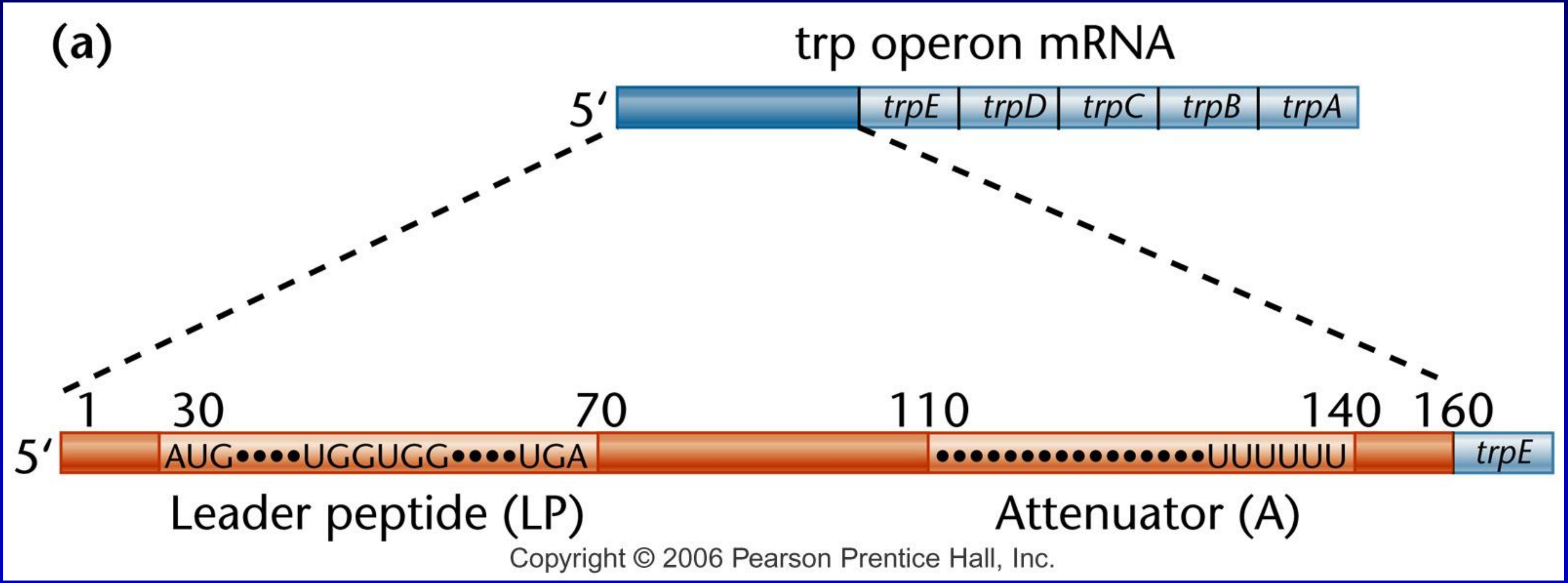
E. Coli trp Operon: Trp Present



The Rest of the Story…

- Attenuation
  - Leader sequence on mRNA before reaching coding for TrpE
    - Seems to encode short polypeptide with two consecutive trp codons
  - Leader can form either of two secondary structures via hydrogen bonding
    - One has a single stem and loop structure (antiterminator)
    - Other has two stem and loops and one is an intrinsic transcriptional terminator
  - Ribosomes stalled on the two trp codons (uncharged tRNAs) prevent terminator structure from forming

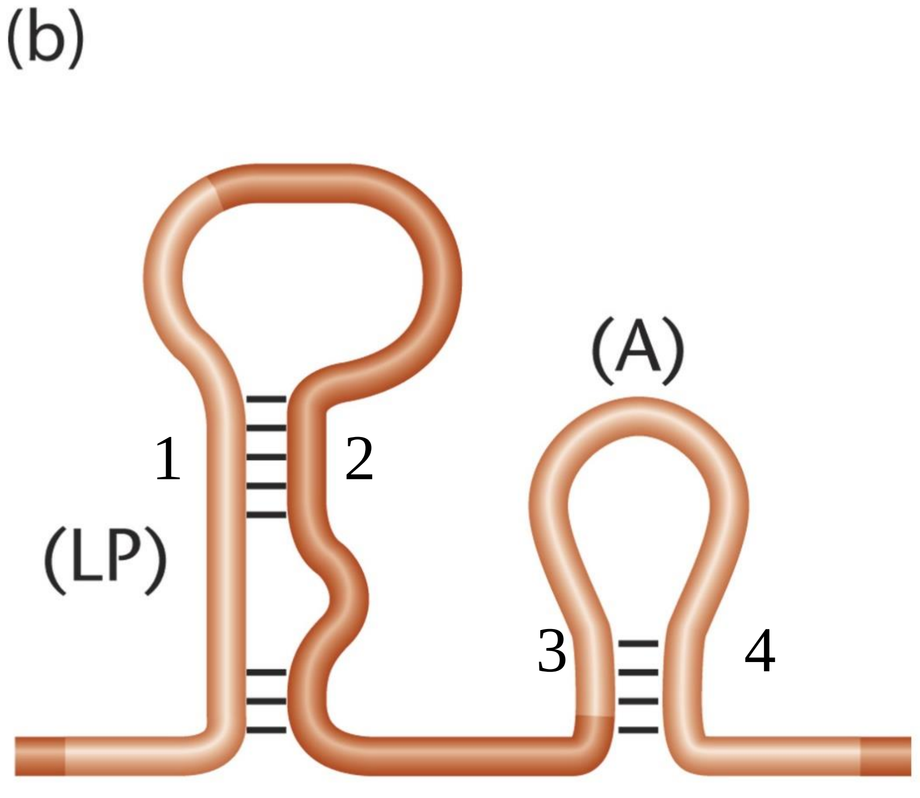
E. Coli trp Operon: Attenuator



- UGG is the codon for tryptophan

E. coli trp Operon: Terminator Hairpin

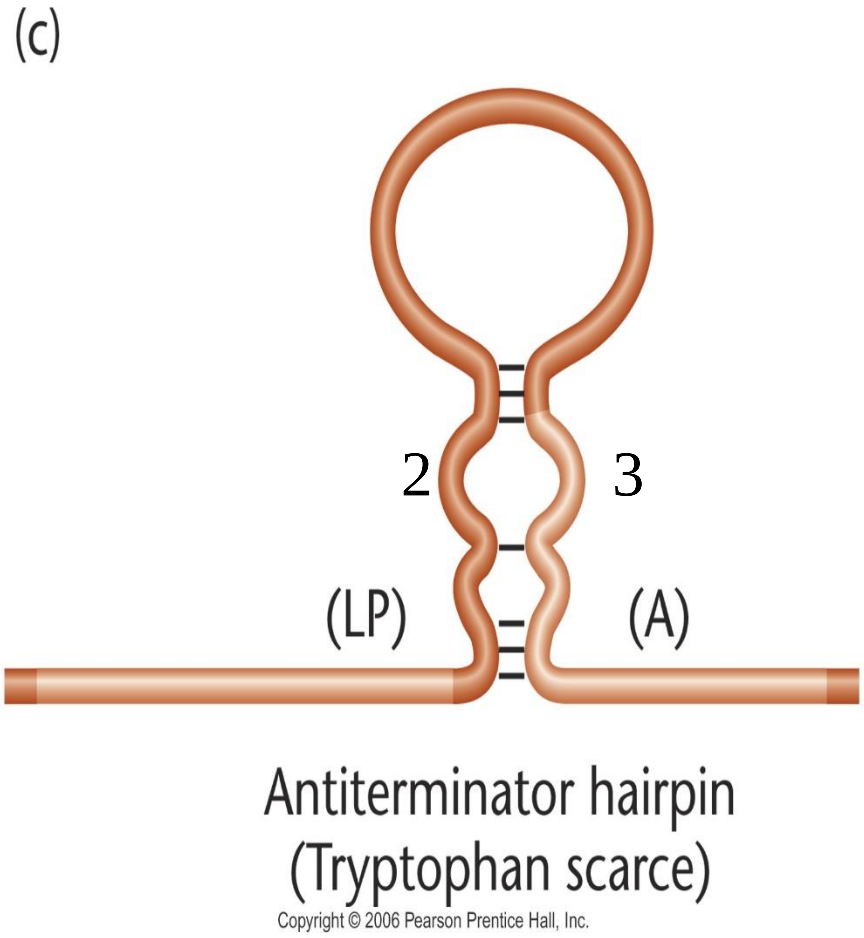
- No ribosome present or ribosome moves through region 1 and into region 2
- Region 1 pairs with 2 (or both are covered by ribosome and 2 is unavailable to pair with 3)
- Region 3 pairs with 4
  - G:C stem with string of U's following
  - Terminator has formed and transcription ceases



Terminator hairpin (Tryptophan abundant)

E. coli trp Operon: Antiterminator Hairpin

- Ribosome stalls on region 1 waiting for charged tryptophan tRNAs (2)
- Region 2 pairs with 3
- Region 3:4 structure does not form
  - No terminator
  - Transcription continues



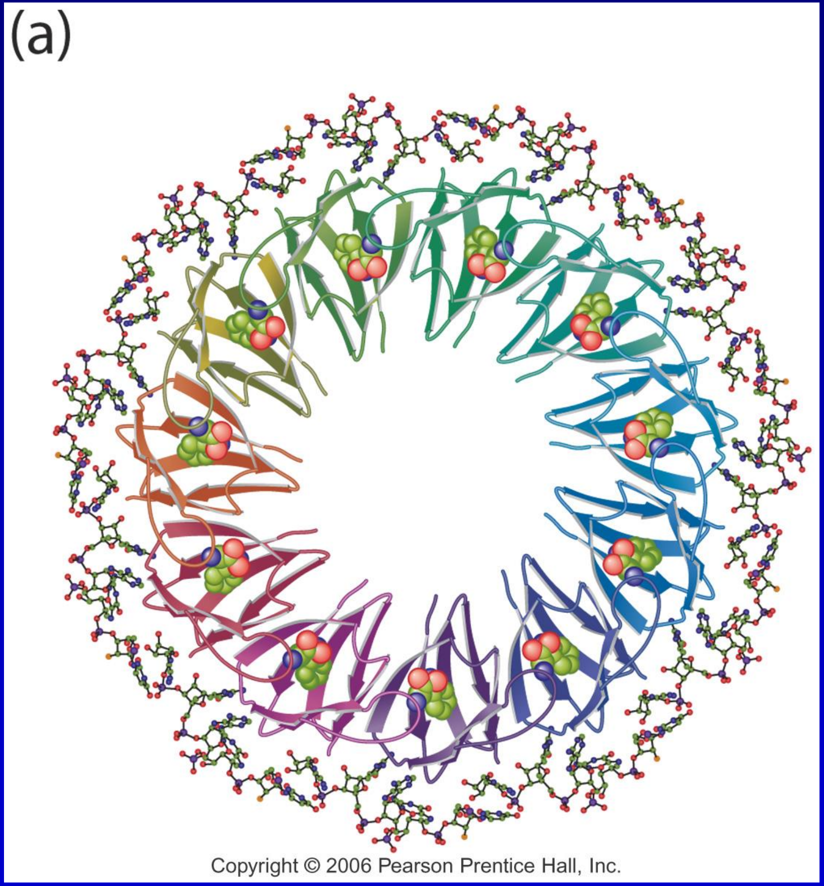
Antiterminator hairpin
(Tryptophan scarce)

B. subtilis trp operon

- Gram positive *B. subtilis* uses only attenuation to regulate *trp* operon
  - But does not involve translational stalling
- Trp RNA-binding attenuation protein (TRAP)
  - Binds to tryptophan and forms 11 subunit TRAP complex
  - GAG or UAG triplets in leader bound (one per subunit)
    - Creates RNA belt around TRAP and prevents antiterminator from forming (terminator forms)
    - Transcription terminated

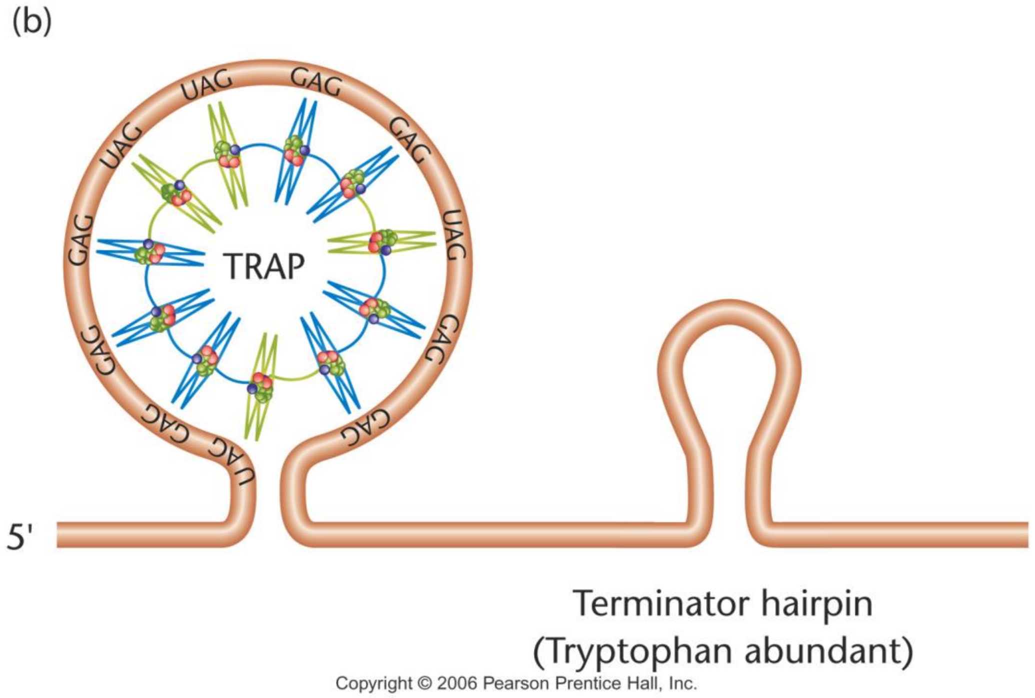
B. subtilis: *trp* Operon: TRAP

- 11-mer of TRAP binds mRNA leader triplets to form RNA belt around the complex



B. subtilis: *trp* Operon: Terminator Formation

- Binding of TRAP to the leader region of mRNA causes intrinsic terminator to form
  - Terminating transcription

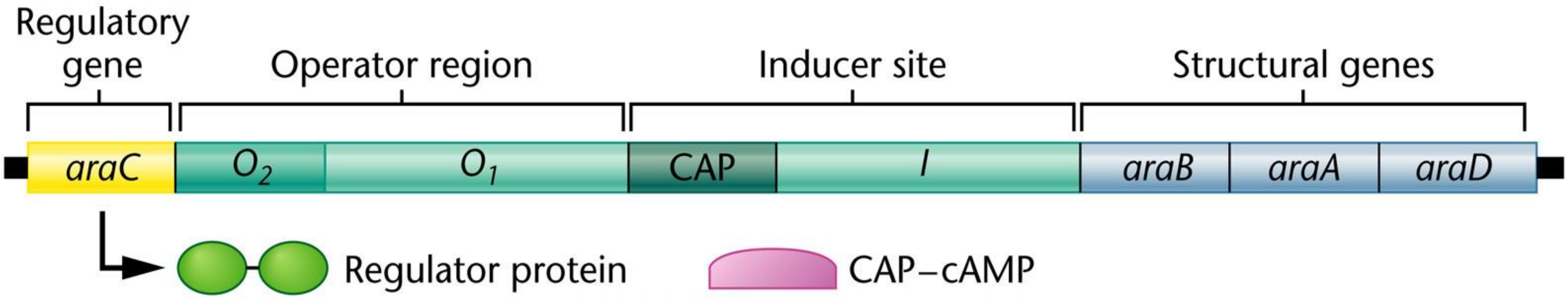


E. coli ara Operon

- Arabinose metabolism
  - Three structural genes (*araBAD*)
  - Regulatory gene (*araC*)
    - Encoded protein acts as activator and repressor for operon
  - Regulatory regions (*araI* and *araO*$_2$)
- AraC binds to region *araI* and this induces operon
  - Requires arabinose and cAMP bound to protein
  - In absence of arabinose two AraC dimers bind cooperatively to *araI* and *araO*$_2$, producing a loop and inhibiting transcription of operon

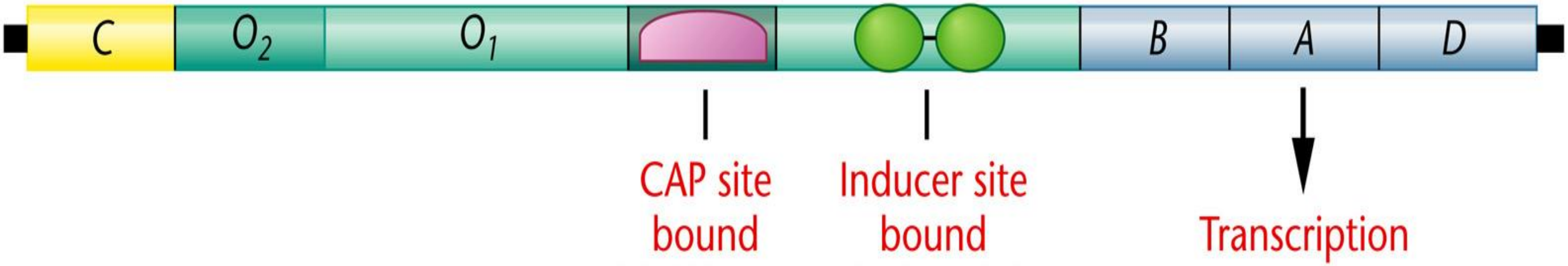
E. Coli ara Operon

(a) Components of *ara* operon



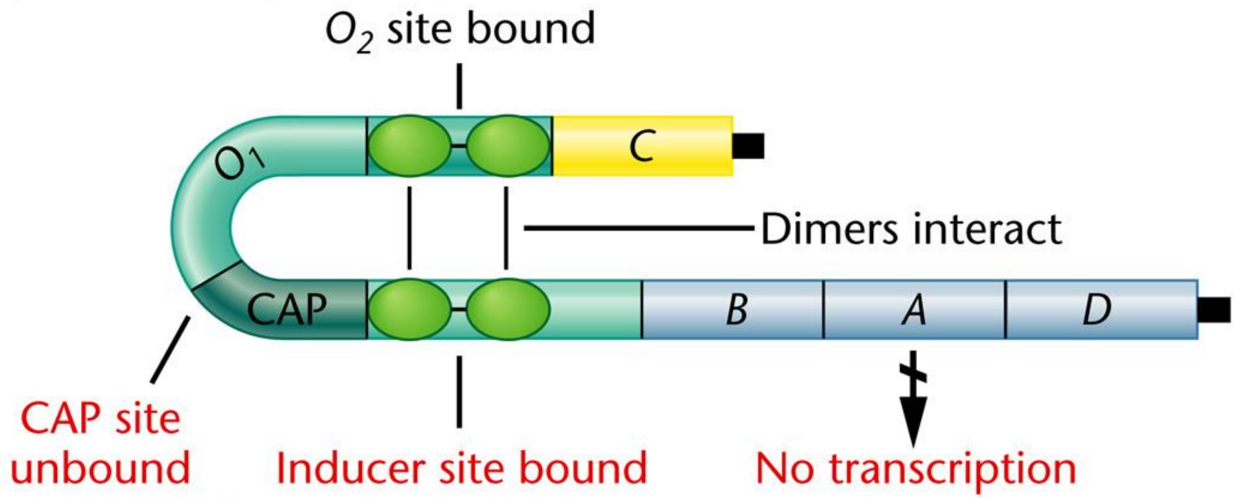
E. Coli ara Operon: Arabinose Present

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



E. coli ara Operon: Arabinose Absent

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



- Interaction between dimers bound to I and O_2 sites creates DNA loop and blocks transcription