Regulation of Gene Expression

Chapter 9

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Regulation

- *Gene expression*: transcription of gene into mRNA followed by translation of mRNA into a protein
- Most proteins are enzymes that carry out biochemical reactions essential for cell growth
- Microbial genomes encode many more proteins than are present at any one time
- Regulation is important in all cells and helps conserve energy and resources

Enzyme Regulation

- Constitutive enzymes
  - Enzymes needed at the same level all of the time
- Regulated enzymes
  - Enzymes needed under some conditions but not others
    - Lac Operon
      - Enzymes are made to break down lactose only if lactose is present

2 Types of Regulation

- Regulation of enzyme activity
  - After the protein is synthesized
  - Posttranslational control
  - Very rapid process (seconds)

- Regulation of amount of enzyme made
  - Transcriptional control = is RNA made?
  - Translational control = is protein made?
  - Slower process (minutes)

An Overview of Mechanisms of Regulation

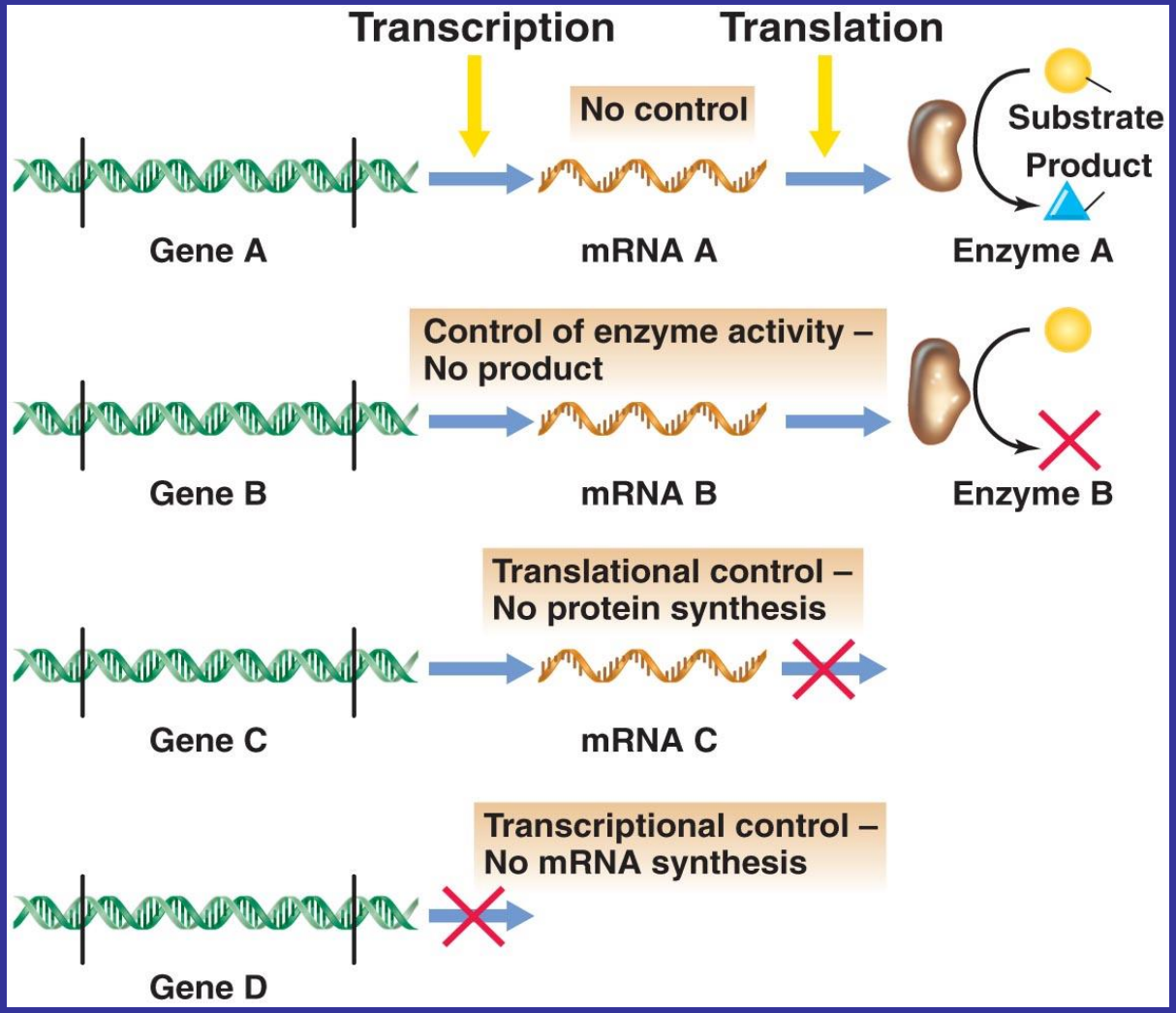
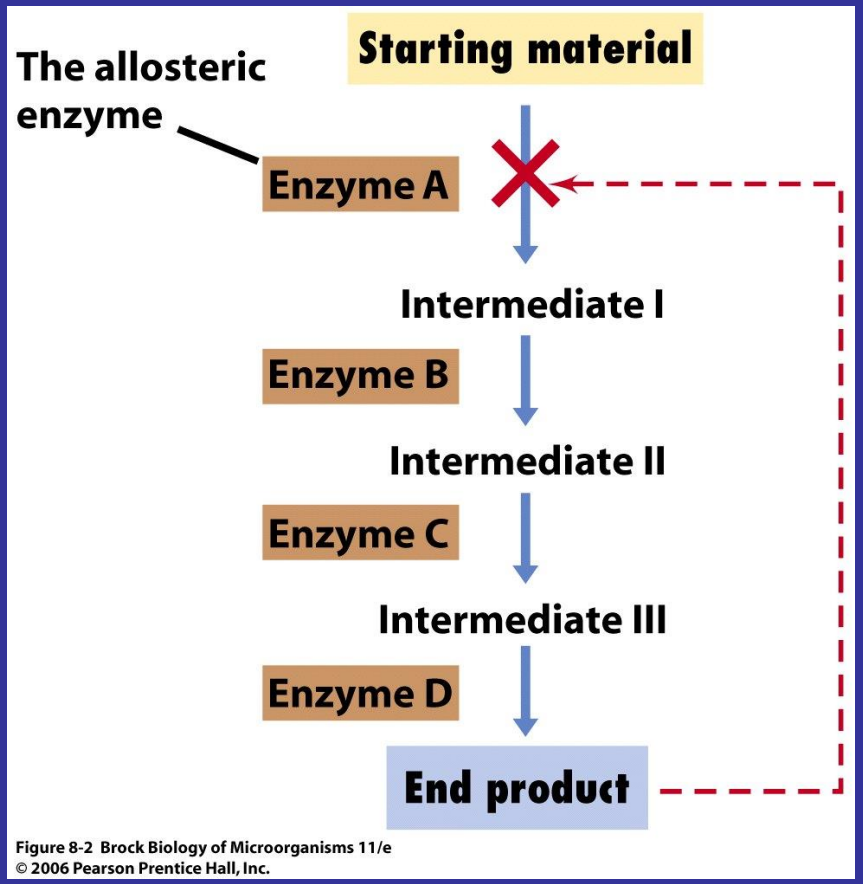


Figure 9.1

Postranslational regulation or regulation of activity

- Feedback inhibition
  - Pathways with many intermediates
  - The end product of a pathway feeds back and inhibits the activity of the first step in a pathway
  - If there are enough end products available, more does not need to be made
  - By inhibiting the enzymes in the pathway, the end product will not be made



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DNA-Binding Proteins

- Recall that mRNA transcripts generally have a short half-life
  - Prevents the production of unneeded proteins
- Regulation of transcription typically requires proteins that can bind to DNA
- Small molecules influence the binding of regulatory proteins to DNA
  - Proteins actually regulate transcription

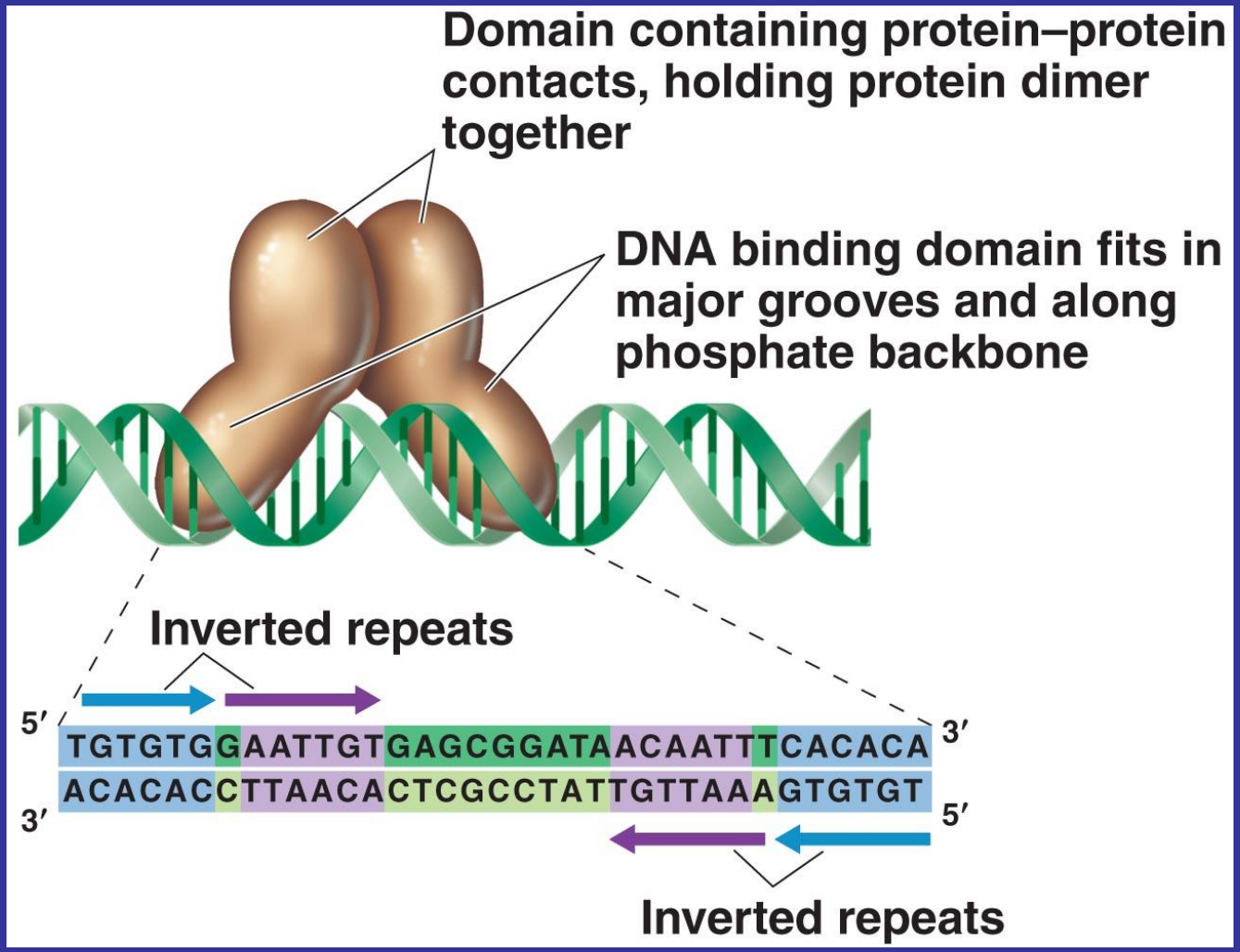
DNA-Binding Proteins

- Most *DNA-binding proteins* interact with DNA in a sequence-specific manner
- Specificity provided by interactions between amino acid side chains and chemical groups on the bases and sugar-phosphate backbone of DNA
- Major groove of DNA is the main site of protein binding
- Inverted repeats frequently are binding site for regulatory proteins

Transcriptional Control: Amount of enzyme made

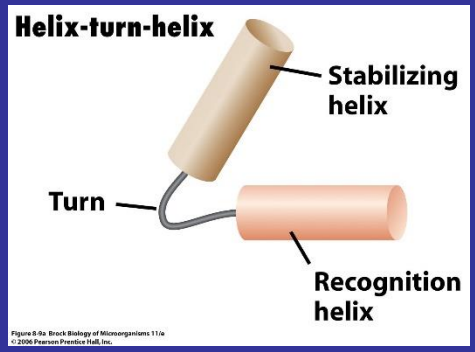
- Transcriptional regulators/proteins must interact with DNA
- Block or activate transcription; RNA polymerase must bind to a promoter
- Nonspecific interactions
  - Example: Histones in Archaea and Eukarya
  - Positively charge proteins that interact with the DNA (negatively charged)
  - Prevents RNA polymerase and protein factors from interacting with the DNA
- Sequence specific interactions
  - Proteins bind DNA at a specific motif (ex - inverted repeat)
    - Major Groove
  - DNA binding proteins typically homodimeric – composed of two identical polypeptides
  - Protein dimers interact with inverted repeats on DNA
    - Each of the polypeptides binds to one inverted repeat

Sequence specific interactions of protein with DNA



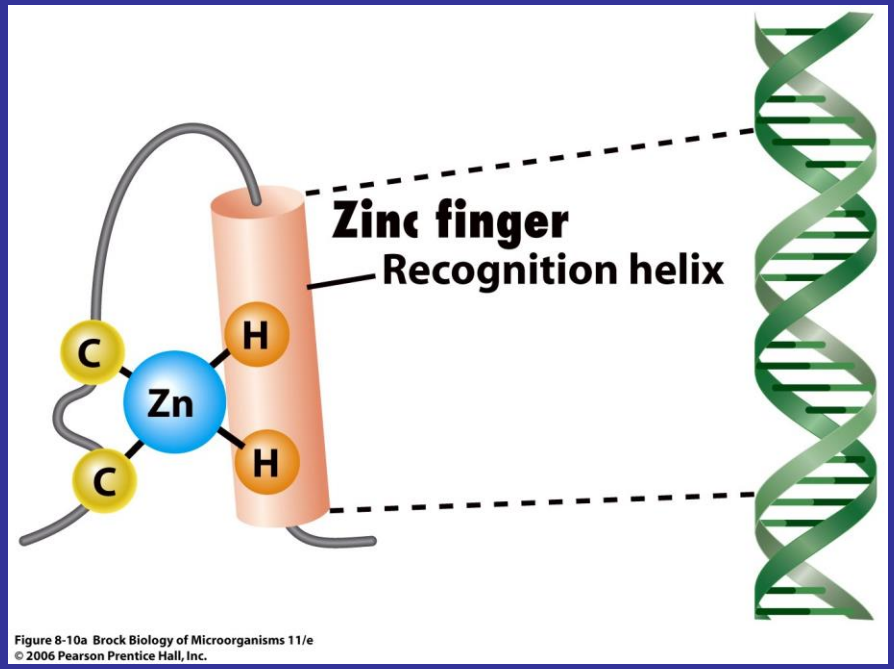
Structure of DNA-Binding Proteins

- Helix turn Helix
- 2 α-helices connected by a short chain of amino acids or turns;
    - First helix is the recognition helix
        - – Interacts with the major groove of the DNA
    - Second helix is the stabilizing helix
- Many different DNA-binding proteins from *Bacteria* contain helix-turn-helix
- The first amino acid is typically a glycine that functions to turn the protein
- Example = lac repressor



Structure of DNA-Binding Proteins

- Zinc Finger Motif
  - Protein structure that binds a zinc ion
  - Interacts with 2 cysteine and 2 histidine residues
  - α-helix (recognition domain) binds to the DNA at the major groove



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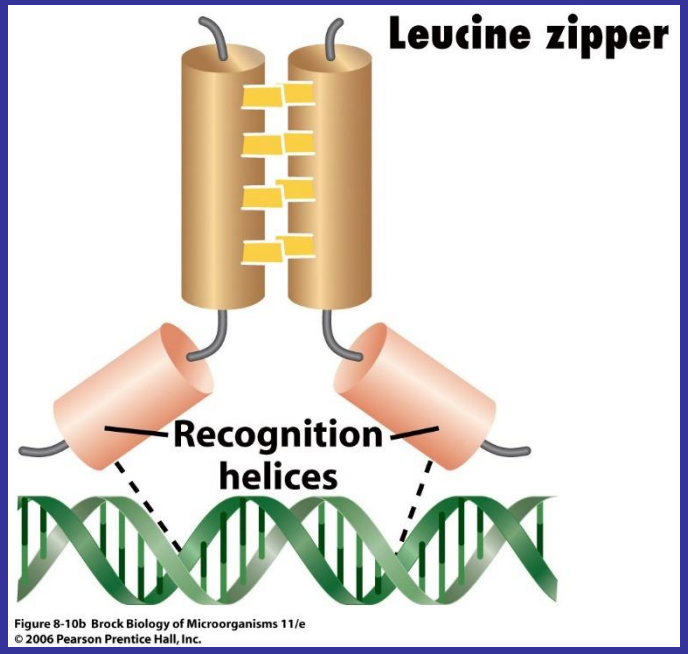
Leucine Zipper

- Leucine Zipper
  - Two recognition helices and stabilizing leucine zipper
  - Leu every 7th aa
  - Leucine does not interact with the DNA



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DNA-Binding Proteins

- Multiple outcomes after DNA binding are possible
  1) DNA-binding protein may catalyze a specific reaction on the DNA molecule (i.e., transcription by RNA polymerase)
  2) The binding event can block transcription (*negative regulation*)
  3) The binding event can activate transcription (*positive regulation*)

Negative control of transcription

- Negative control
  - A regulatory mechanism that stops transcription
- Enzyme repression
  - Preventing the synthesis of an enzyme in response to a signal
  - If the product is present, the enzymes that synthesize this product will not be made
  - Specific process – inhibit synthesis of one enzyme used in biosynthesis; other enzymes unaffected
    - Enzymes affected by any repression make up a small fraction of total proteins in the cell

Specific Repression

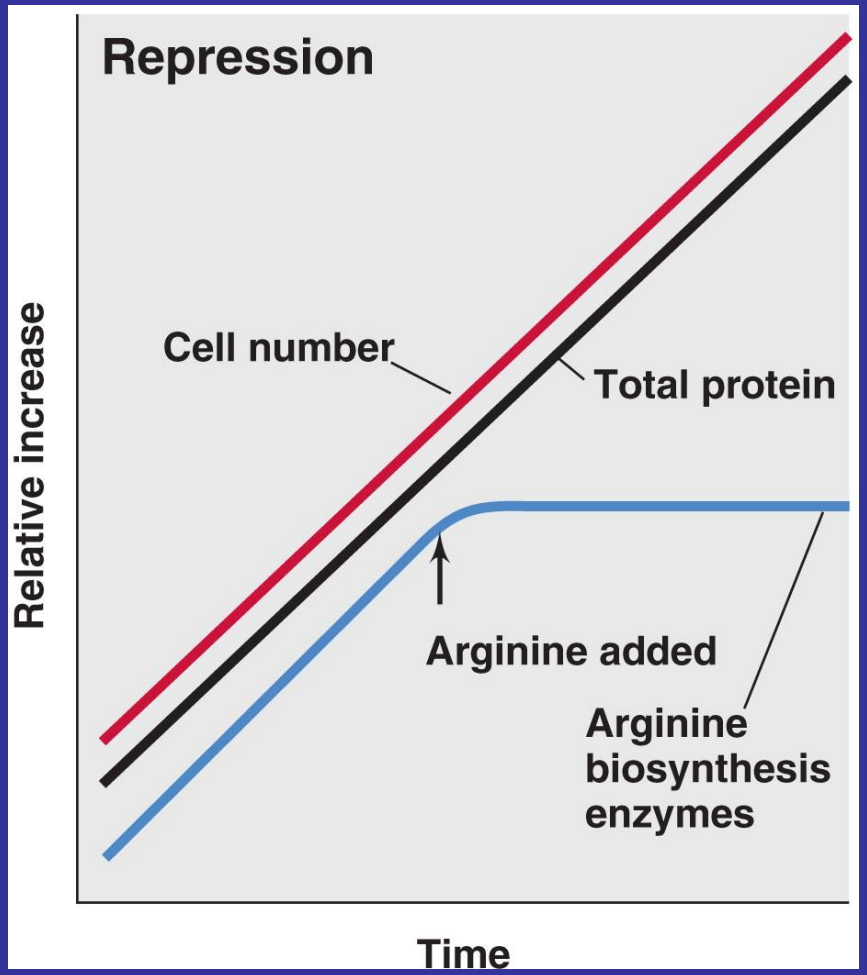


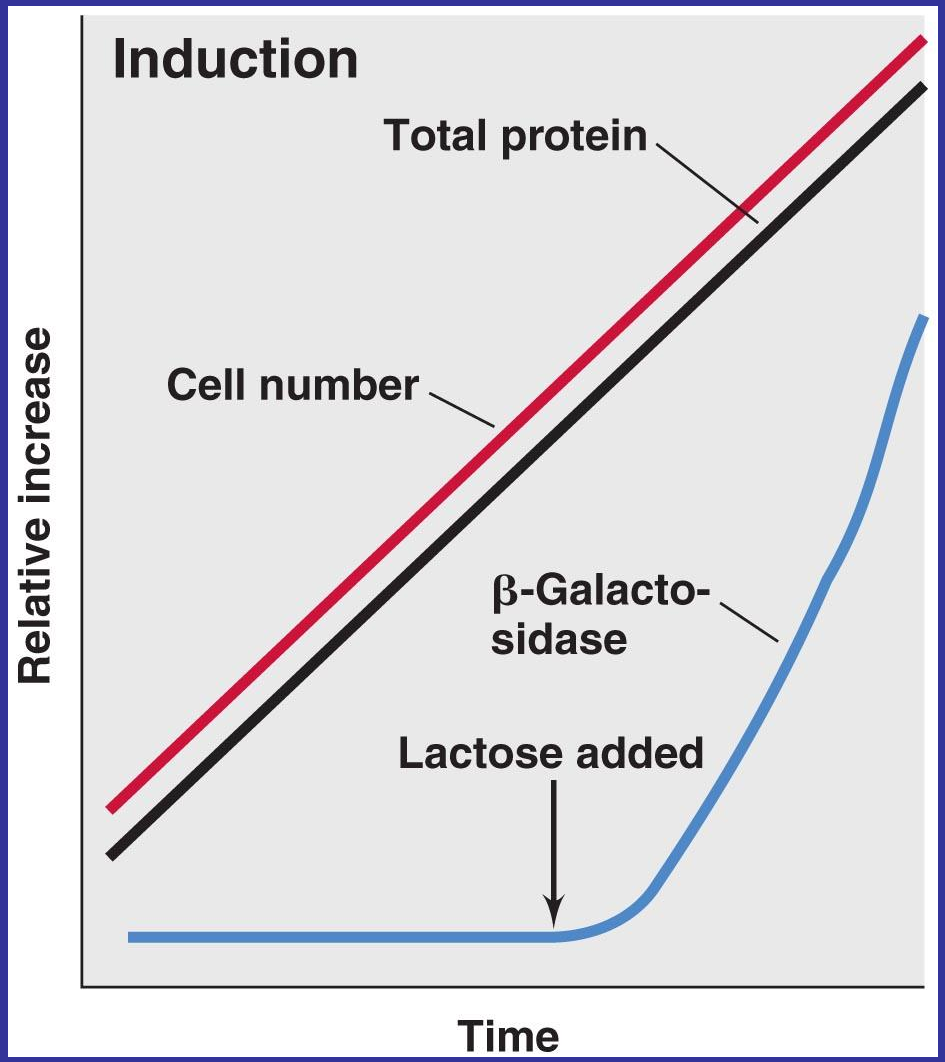
Figure 9.5

Only enzymes involved in arg synthesis are affected

Enzyme induction

- Induction: production of an enzyme in response to a signal
- An enzyme is made only when its substrate is present
  - Enzymes are synthesized only when they are needed (no wasted energy)
- Typical with catabolic enzymes
  - Example: β galactosidase used to metabolize lactose
- Only enzymes involved in Lactose utilization are affected. Lac operon is inducible

Specific Induction



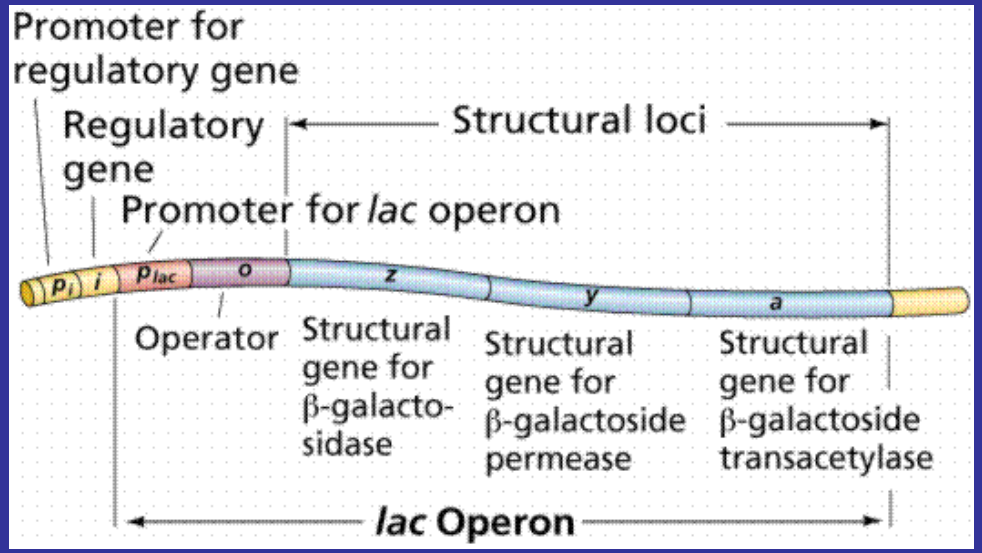
Only enzymes involved in Lactose utilization are affected.

Terms

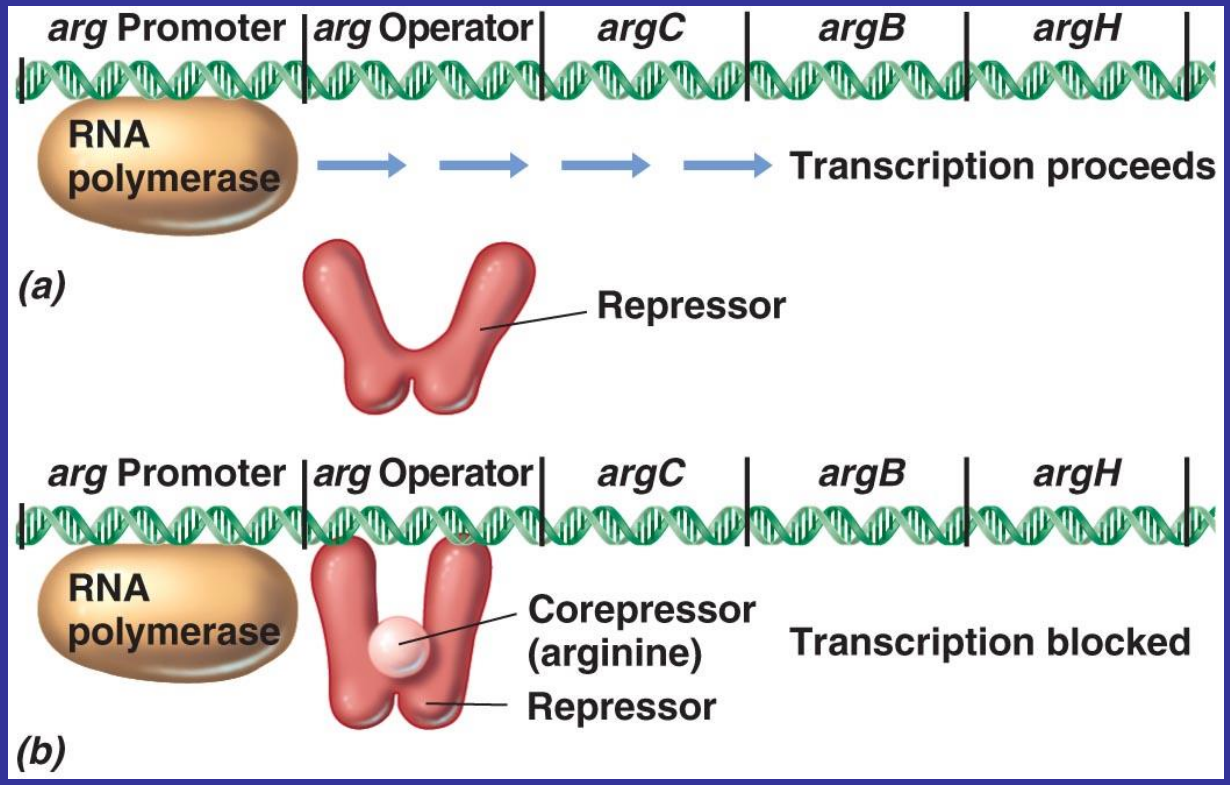
- Inducer – a substance that initiates enzyme induction; lactose
- Corepressor – a substance that represses enzyme synthesis; arginine
- Both are called effectors

Operon Model

- Cluster of genes all transcribed in a single unit
  - Promoter: transcription initiation, where RNA polymerase binds
  - Operator: stop or go signal; where repressor binds
  - Genes are located downstream
- **Effectors** control whether regulator/repressor will bind or not; if repressor binds operator, RNAP won't bind promoter



Repression of genes that code for Arginine Synthesis enzymes

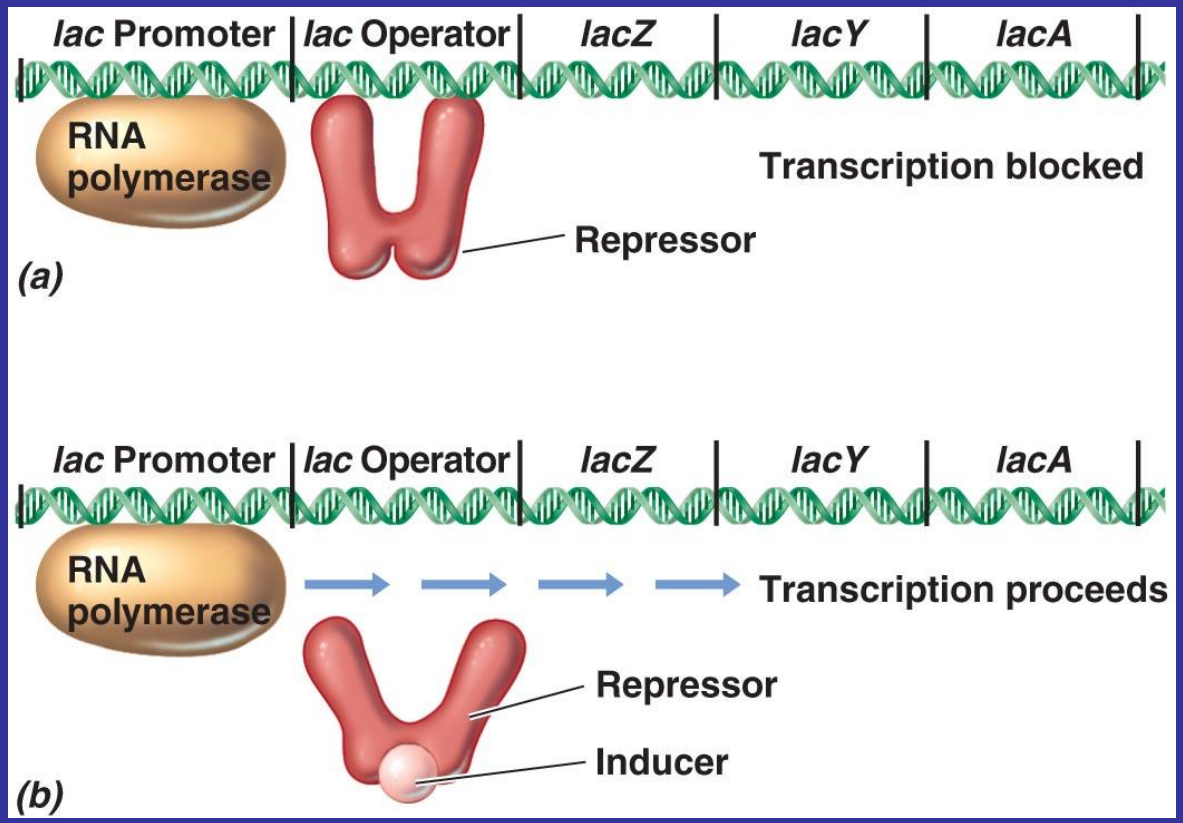


- When arg is needed, the cell trc and trl the genes the code for arg synthesis enzymes→Lack of repression.
- When arg is in excess, it acts as a co-repressor and repression occurs.

Parts of the Lac Operon

- RNA polymerase
  - The enzyme which makes the RNA (transcription)
- Promoter:
  - A region on the DNA where the RNA polymerase can attach and initiate transcription
- Operator:
  - A region on the DNA next to the promoter where the repressor binds
  - The repressor binds to the operator when lactose is absent
  - The repressor cannot bind to the operator when lactose is present, when RNA polymerase binds to the promoter

Induction of genes that code for Lac Utilization

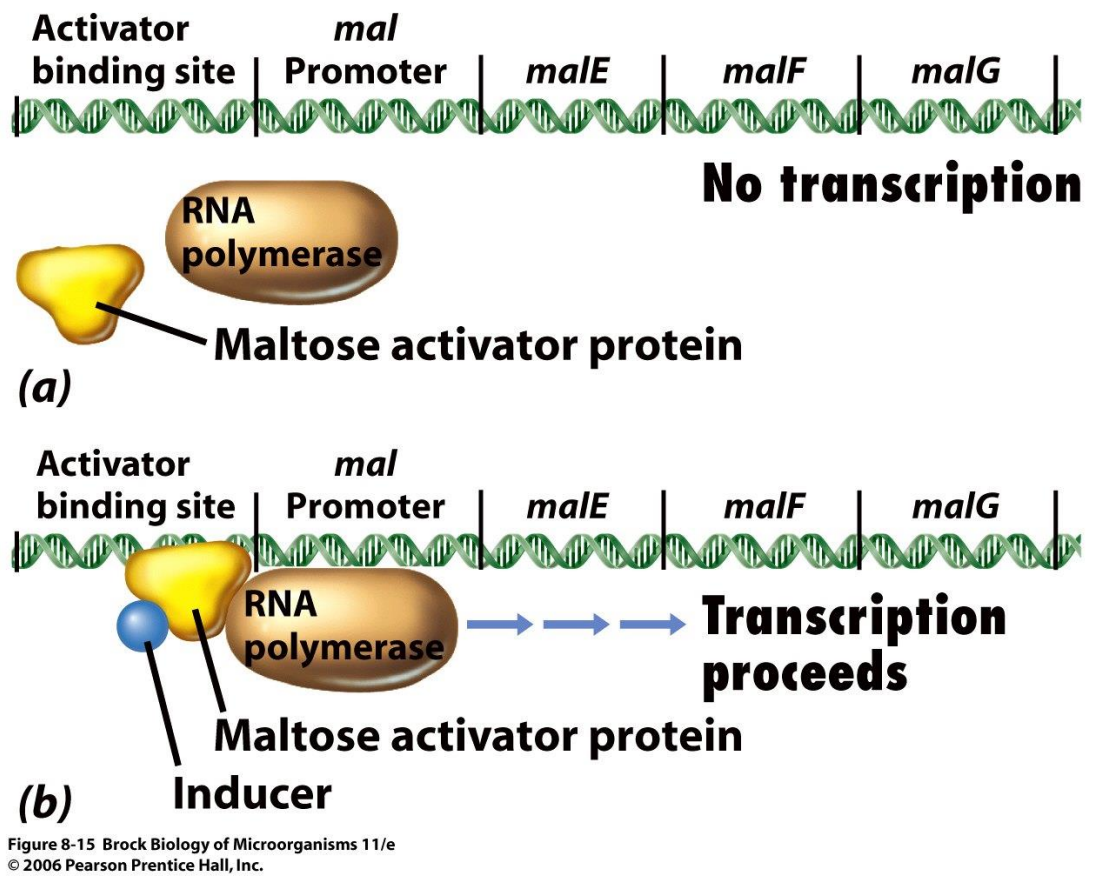


- When Lac is not present in media, the genes that code for Lac enzymes are repressed normally
- When Lac is present, it acts as an inducer and the cell makes the genes that code for Lac utilization enzymes→Induction.

Positive Transcriptional Control Mechanism

- Regulator
  - Activator promotes binding of RNA polymerase to DNA at promoter region
  - Binds to activator binding site (not repressor site)
- Activator proteins bind specifically to certain DNA sequence
  - Called *activator binding site*, not operator
- Maltose catabolism in *E. coli*
  - Maltose activator protein cannot bind to DNA unless it first binds maltose

Positive Control (Maltose Utilization)



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- When maltose is not present the activator is inactive.

- When maltose is present, it acts as an inducer and an activator protein binds and activates transcription.

Positive Control

- Operons versus Regulons
  - Genes for maltose are spread out over the chromosome in several operons
    - Each operon has an activator-binding site
    - Multiple operons controlled by the same regulatory protein are called a *regulon*

Global Regulation

- *Global control systems*: regulate expression of many different genes simultaneously
- Catabolite repression is an example of global control
  - Synthesis of a variety of unrelated enzymes are repressed when cells are grown in a medium containing glucose
  - Glucose = better carbon and energy source
  - Example: Lac Operon is under the control of catabolite repression

Diauxic growth

- Two exponential growth phases
- Two energy sources are available, then the organism grows first on the better energy source
- Then growth resumes on the other energy source
- Glucose initially inhibits β-galactosidase and lactose utilization

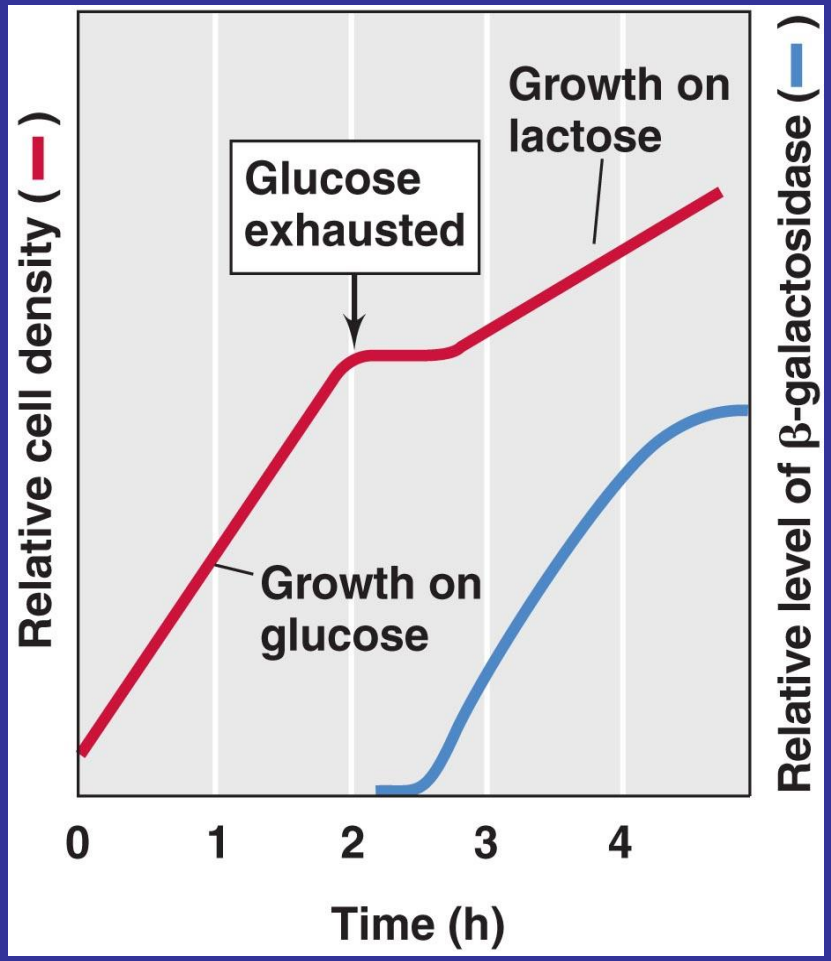


Figure 9.17

Lac Operon as Example of Catabolic Repression

- Glucose/Lactose media
  - When lactose is present it binds to the repressor and induces transcription
  - But only if CAP protein also binds to the DNA (Catabolic activator protein)

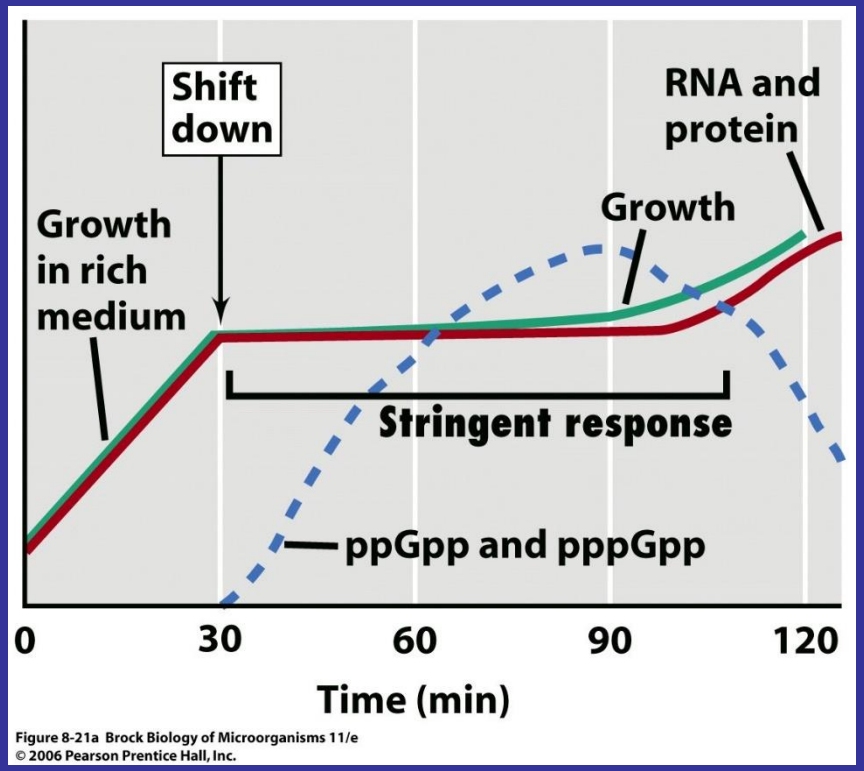
http://highered.mcgraw-hill.com/olc/dl/120080/bio27.swf

Catabolic Repression Mechanism

- When glucose runs out, cAMP is produced
  - cAMP - effector
- cAMP binds to CAP and CAP binds to DNA
  - CAP (regulator) activates the transcription of the lac genes
- When glucose is available, catabolic repression will prevent organism from producing lactose catabolism genes
  - cAMP synthesis is inhibited by presence of glucose
  - Even if repressor does not bind to the operator

Stringent response

- Switch from rich to a simplier media
- Protein synthesis decreases and will eventually increase again but at a lower level



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Riboswitches

- RNA domains in an mRNA molecule that can bind small molecules to control translation of mRNA
  - Located at 5′ end of mRNA
  - Binding results from folding of RNA into a 3-D structure
  - Similar to a protein recognizing a substrate
  - Riboswitch control is analogous to negative control
  - Found in some bacteria, fungi, and plants

Regulation by Riboswitch

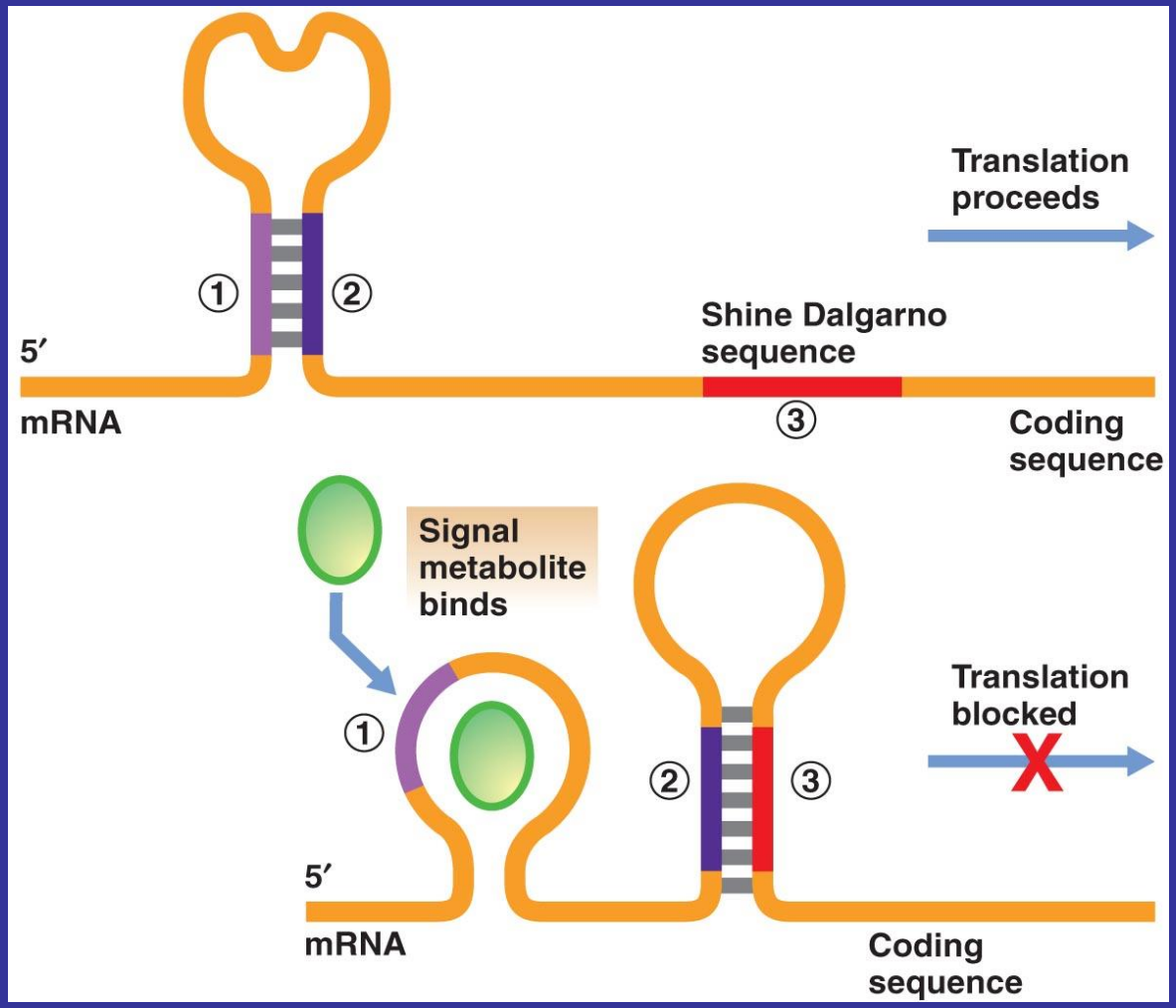

Figure 9.25

Tryptophan operon

- Regulated by two control mechanisms
  - Repressor binding to the operator
  - Attenuation: premature termination of transcription

Trp repressor

- Animation

- When tryptophan levels are low, repressor does not bind to operator and tryptophan can be made

- When tryptophan levels are high, tryptophan binds to the repressor and activates it and it binds to the operator (trp = corepressor)

In prokaryotes, transcription and translation are physically coupled

- Premature termination of transcription results in incomplete mRNA
  - Synthesis of leader sequence = premature termination

- Attenuation occurs due to coupling of transcription and translation

Attenuation: Tryptophan Operon

- Inverted repeat in mRNA of trp operon
  - Stem loop forms
  - 2 conformations: 3,4 stem loop and 2,3 stem loop
  - 3,4 stem loop = transcription pause and reach termination signal
  - 2,3 stem loop = do not have a termination signal
  - Animation

When are trp genes produced?

- Excess Trp
  - Leader sequence transcribed and translated fully
  - Leader polypeptide attached to ribosome
  - Causes 3,4 stem loop conformation, which signals transcription pause site and transcription terminated
- Trp concentration low
  - Leader sequence transcribed and translated until trp-rich region is reached→a pause occurs
  - Pause causes 2,3 stem loop conformation, which is not a termination signal and transcription of trp genes continues

Mechanism of Attenuation

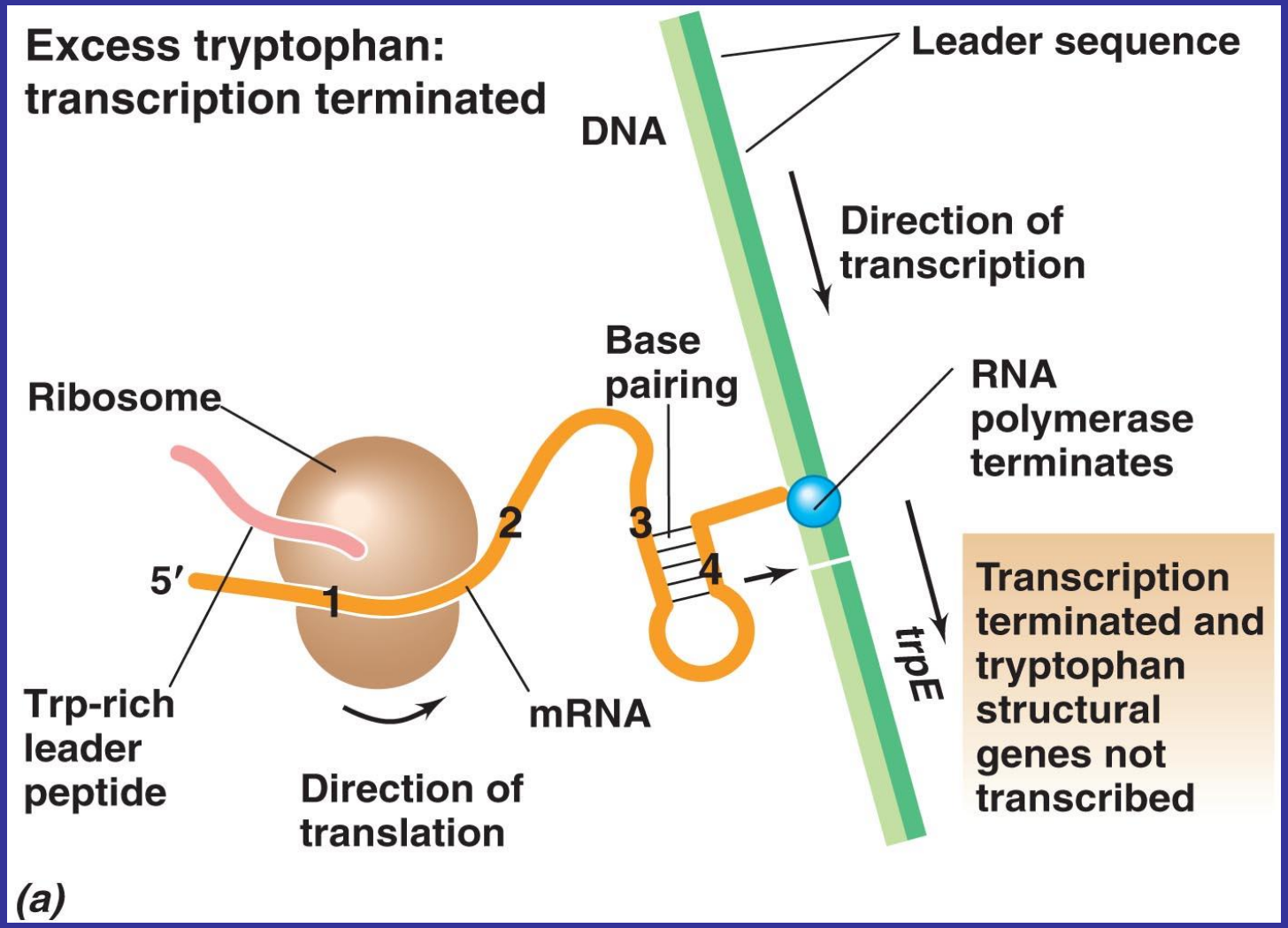

Figure 9.27

Mechanism of Attenuation

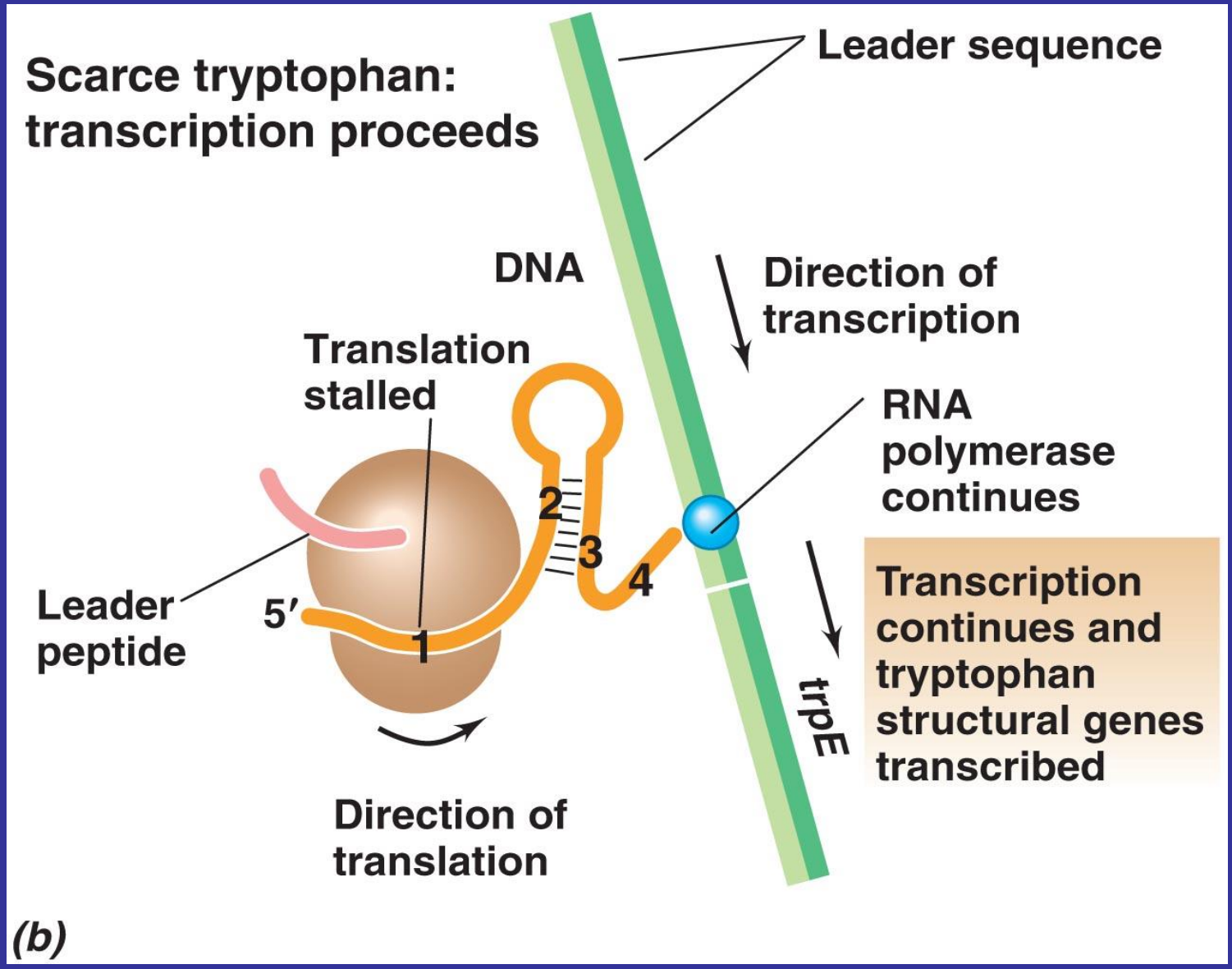

Figure 9.27