Gene Regulation

Conducting the Genetic Orchestra

- Prokaryotes and eukaryotes alter gene expression in response to their changing environment
- In multicellular eukaryotes, gene expression regulates development and is responsible for differences in cell types
- RNA molecules play many roles in regulating gene expression in eukaryotes

Bacteria often respond to environmental change by regulating transcription

- Natural selection favors bacteria that produce only the products needed by that cell
- A cell can regulate the production of enzymes by feedback inhibition or by gene regulation
- Gene expression in **bacteria** is controlled by the **operon model**

Prokaryotic Regulation: The Operon Model

- **Operon** consist of three components
  - **Promoter**
    - DNA sequence where RNA polymerase first attaches
  - **Operator**
    - DNA sequence where active repressor binds
  - **Structural Genes**
    - One to several genes coding for enzymes of a metabolic pathway
    - Translated simultaneously as a block

Operons: The Basic Concept

- A group of functionally related genes can be controlled by a single on-off "switch"
- Regulatory "switch" is a segment of DNA called an **operator** usually found inside the promoter
- An **operon** is the entire stretch of DNA that includes the operator, the promoter, and the genes that they control

- Operon can be switched off by a **repressor**
- Repressor prevents gene transcription by binding to the operator and blocking RNA polymerase
- Repressor can be in an active or inactive form, depending on the presence of other molecules
- A **corepressor** is a molecule that cooperates with a repressor to switch an operon off
- Repressor is the product of a separate **regulatory gene**

Repressible and Inducible Operons: Two Types of Negative Gene Regulation

- A repressible operon is usually on; binding of repressor to the operator shuts off transcription
- The *trp* operon is a repressible operon
- An inducible operon is one that is usually off; an **inducer inactivates the repressor** and turns on transcription (*lac* operon)

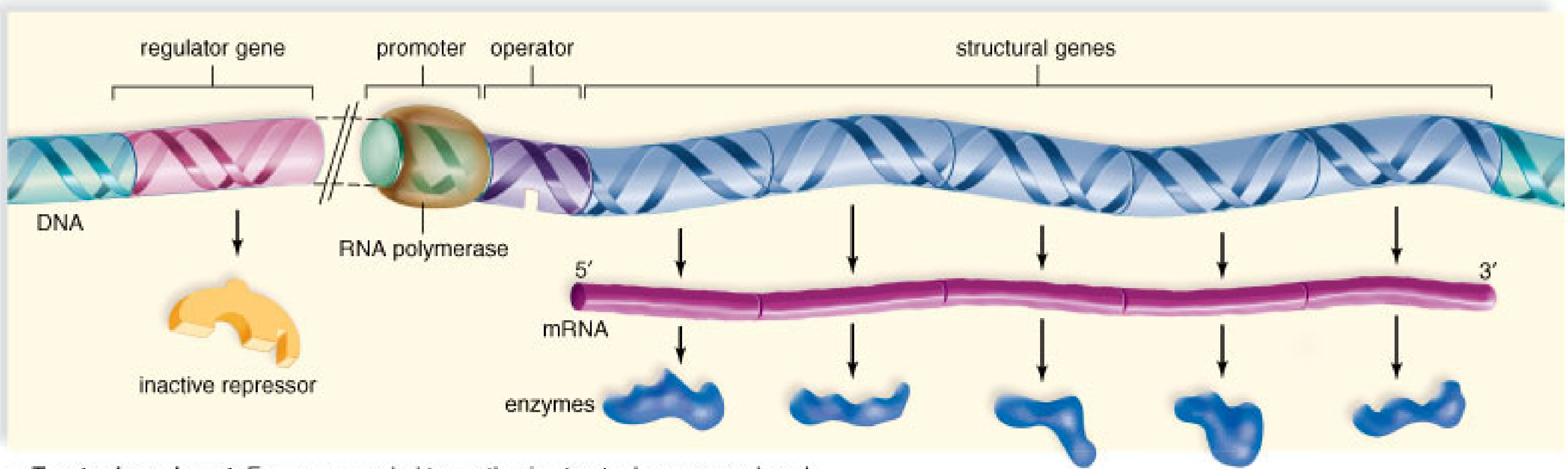
E. coli regulates tryptophan synthesis

- Normally the *trp* operon is on and the genes for tryptophan synthesis are transcribed
- When tryptophan is present, it binds to the *trp* repressor, which turns the operon off
- Repressor is active only in the presence of its corepressor tryptophan
- So, the *trp* operon is turned off (repressed) if tryptophan levels are high-FEEDBACK INHIBITION

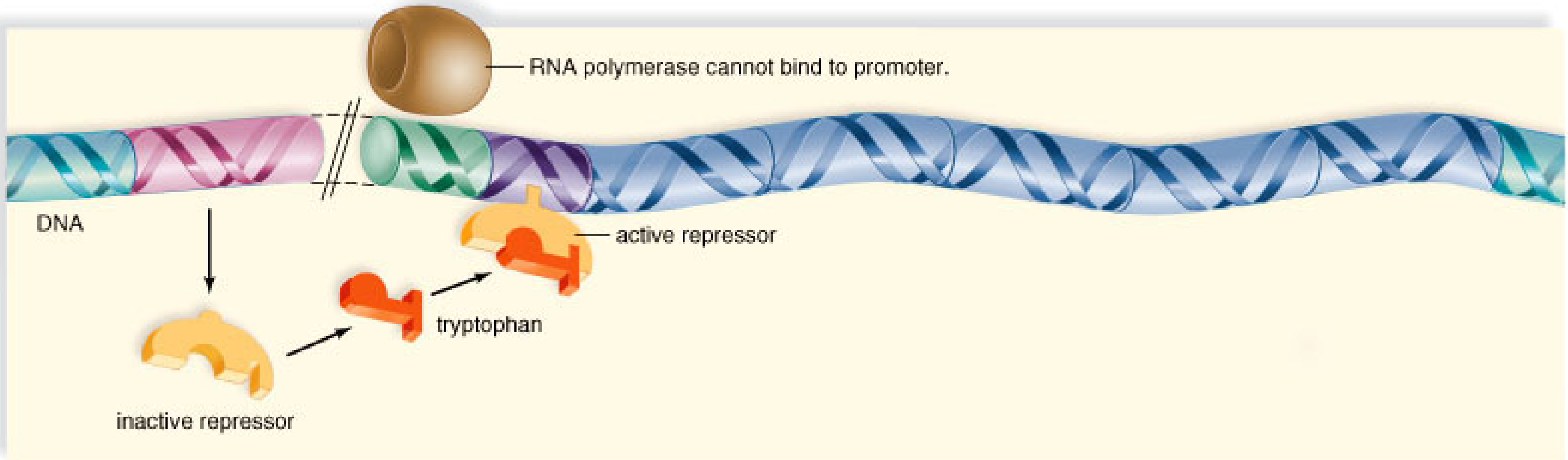
Repressible Operons: The trp Operon

- **Regulator gene** codes for a **repressor**
- If tryptophan (an amino acid) is absent:
  - Repressor can't attach to operator (expression is "on")
  - RNA polymerase binds to promoter
  - Enzymes for synthesis of tryptophan are produced
- If tryptophan is present:
  - Combines with repressor as corepressor
  - Repressor becomes functional
  - Blocks synthesis of enzymes and tryptophan

The trp Operon

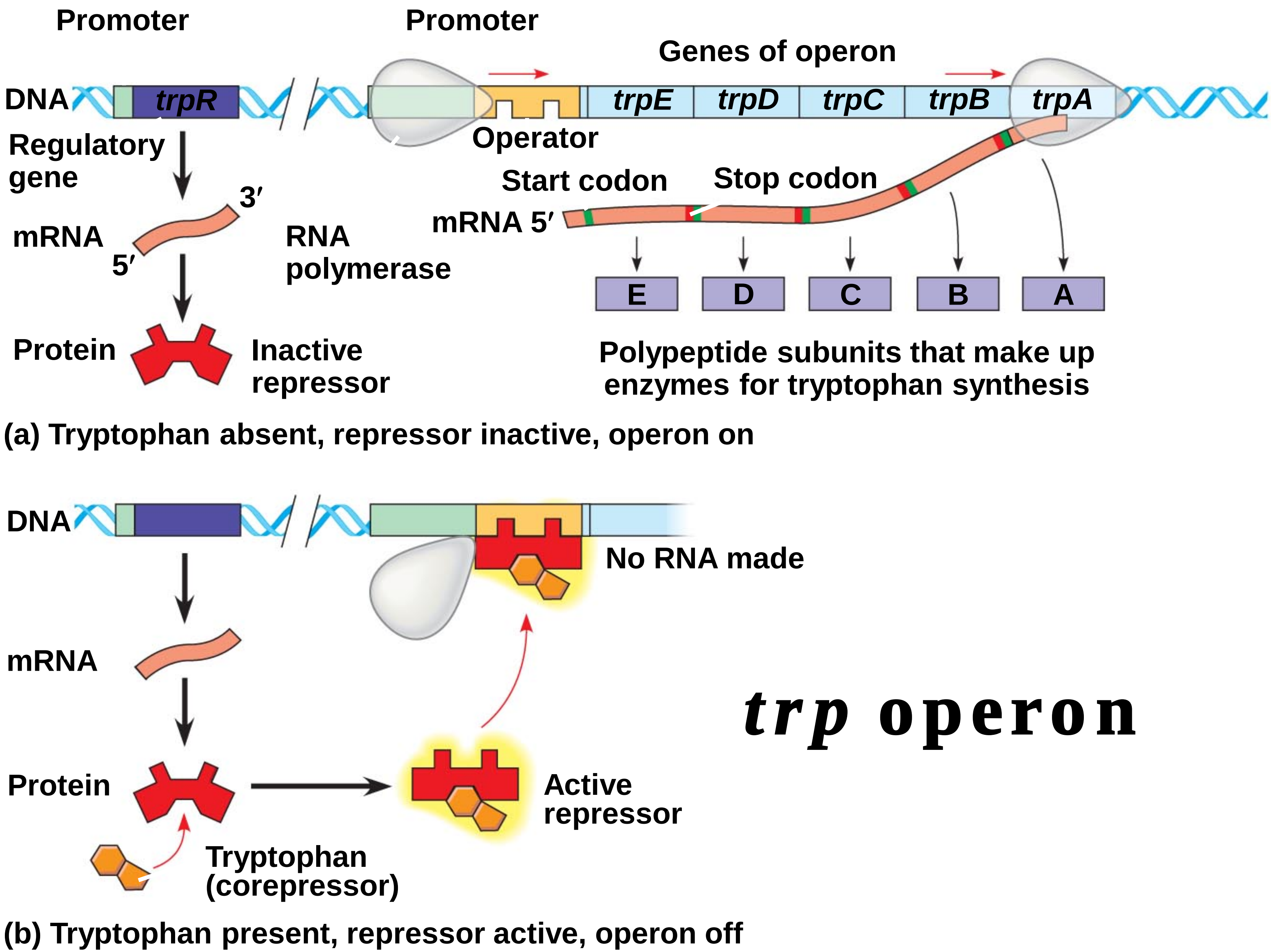


a. **Tryptophan absent.** Enzymes needed to synthesize tryptophan are produced.



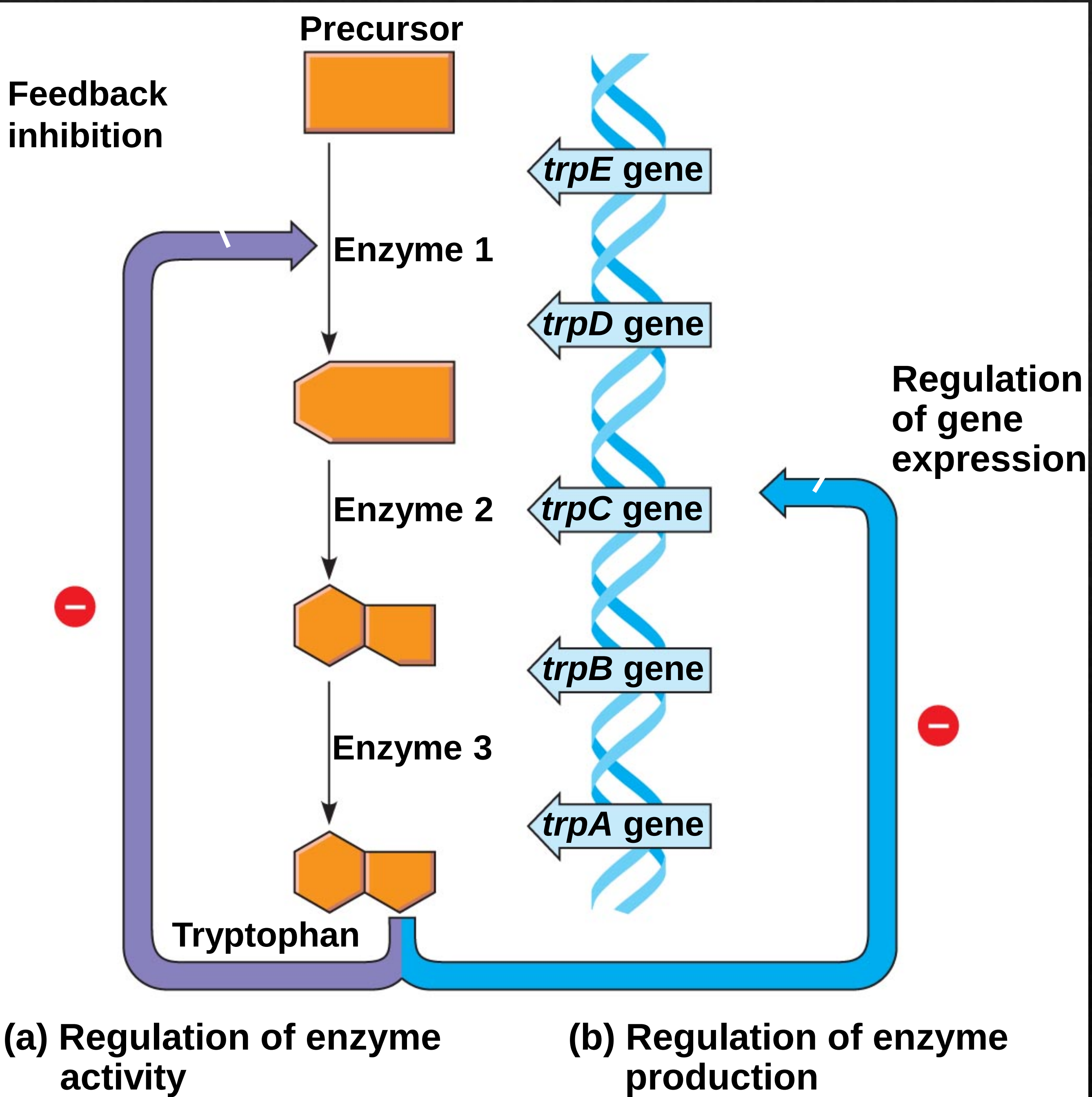
b. **Tryptophan present.** Presence of tryptophan prevents production of enzymes used to synthesize tryptophan.

Fig. 18-3



trp operon

Fig. 18-2



Precursor
Feedback inhibition
Enzyme 1
Enzyme 2
Enzyme 3
Tryptophan
trpE gene
trpD gene
trpC gene
trpB gene
trpA gene
Regulation of gene expression
(a) Regulation of enzyme activity
(b) Regulation of enzyme production

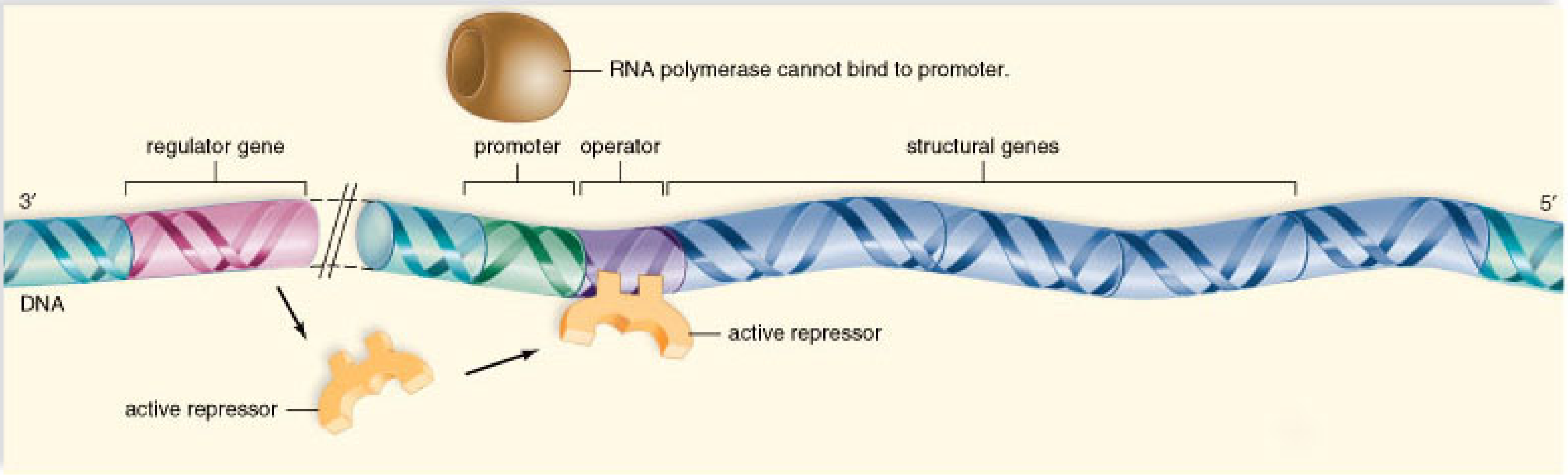
lac operon is an inducible operon

- The *lac* operon is an inducible operon and contains genes that code for enzymes used in the hydrolysis and metabolism of lactose
- By itself, the *lac* repressor is active and switches the *lac* operon off
- An **inducer** inactivates the repressor to turn the *lac* operon on

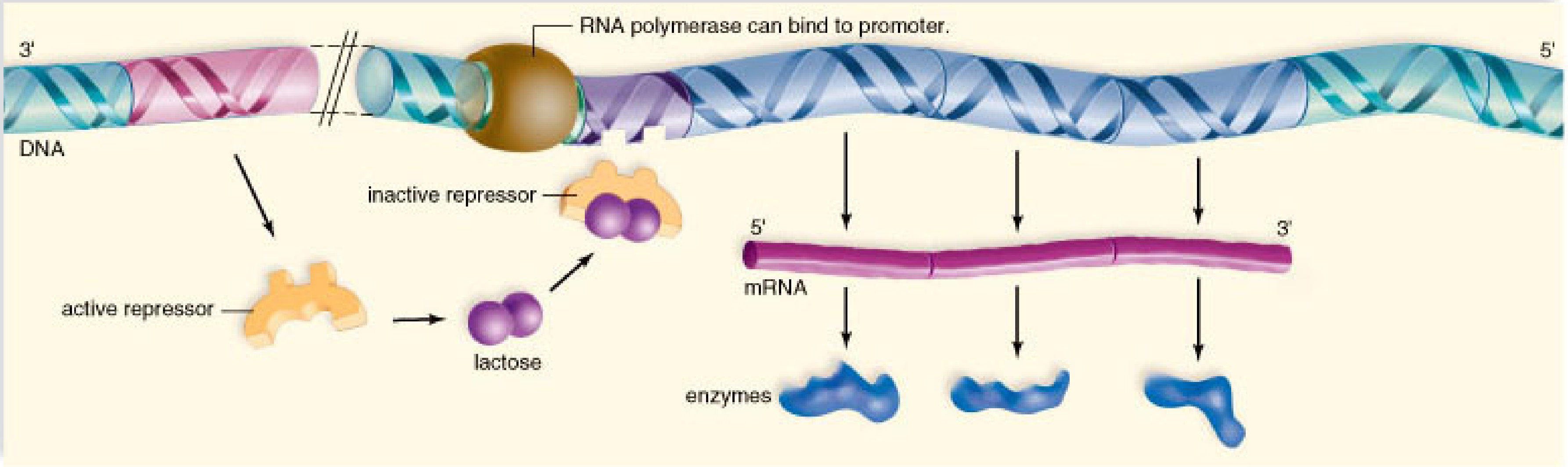
Inducible Operons: The *lac* Operon

- Regulator codes for a repressor
- If lactose (a sugar that can be used for food) is absent:
  - Repressor attaches to the operator
  - Expression is normally "off"
- If lactose is present:
  - It combines with repressor and prevents its binding to operator
  - RNA polymerase binds to the promoter
  - The three enzymes necessary for lactose catabolism are produced

The lac Operon



a. **Lactose absent.** Enzymes needed to take up and use lactose are not produced.

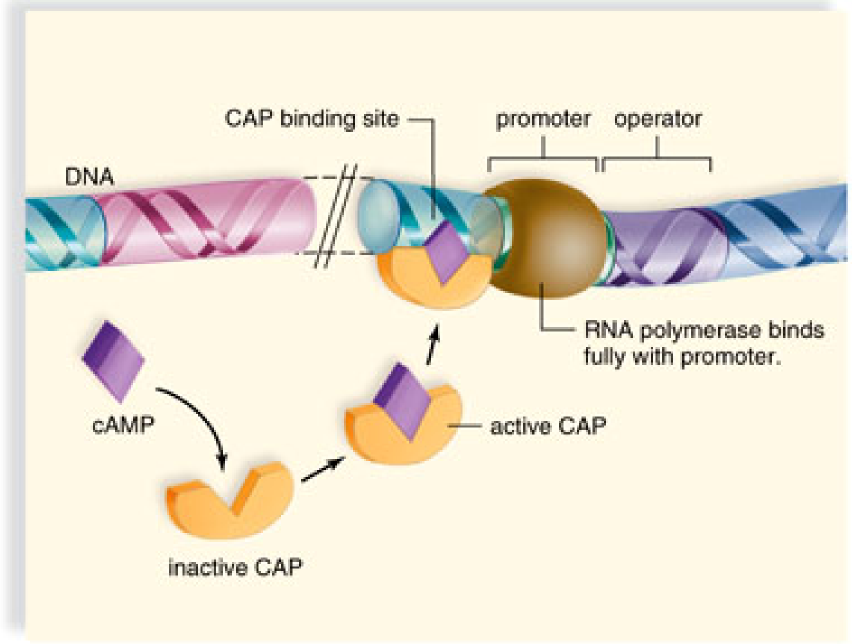


b. **Lactose present.** Enzymes needed to take up and use lactose are produced only when lactose is present.

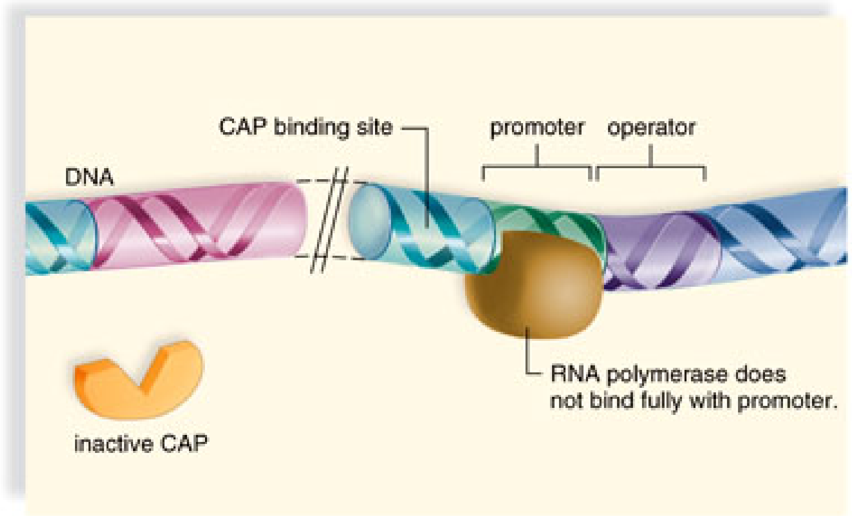
Positive Gene Regulation

- Some operons use positive control through an activator (stimulatory protein), i.e. (CAP), an **activator** of transcription
- When glucose (a preferred food source of *E. coli*) is scarce, CAP is activated by binding with **cyclic AMP**
- Activated CAP binds to the promoter of the *lac* operon stimulating transcription
- When glucose levels increase, CAP detaches from the *lac* operon, and transcription returns normal
- CAP regulates other operons that encode catabolic enzymes

Action of CAP

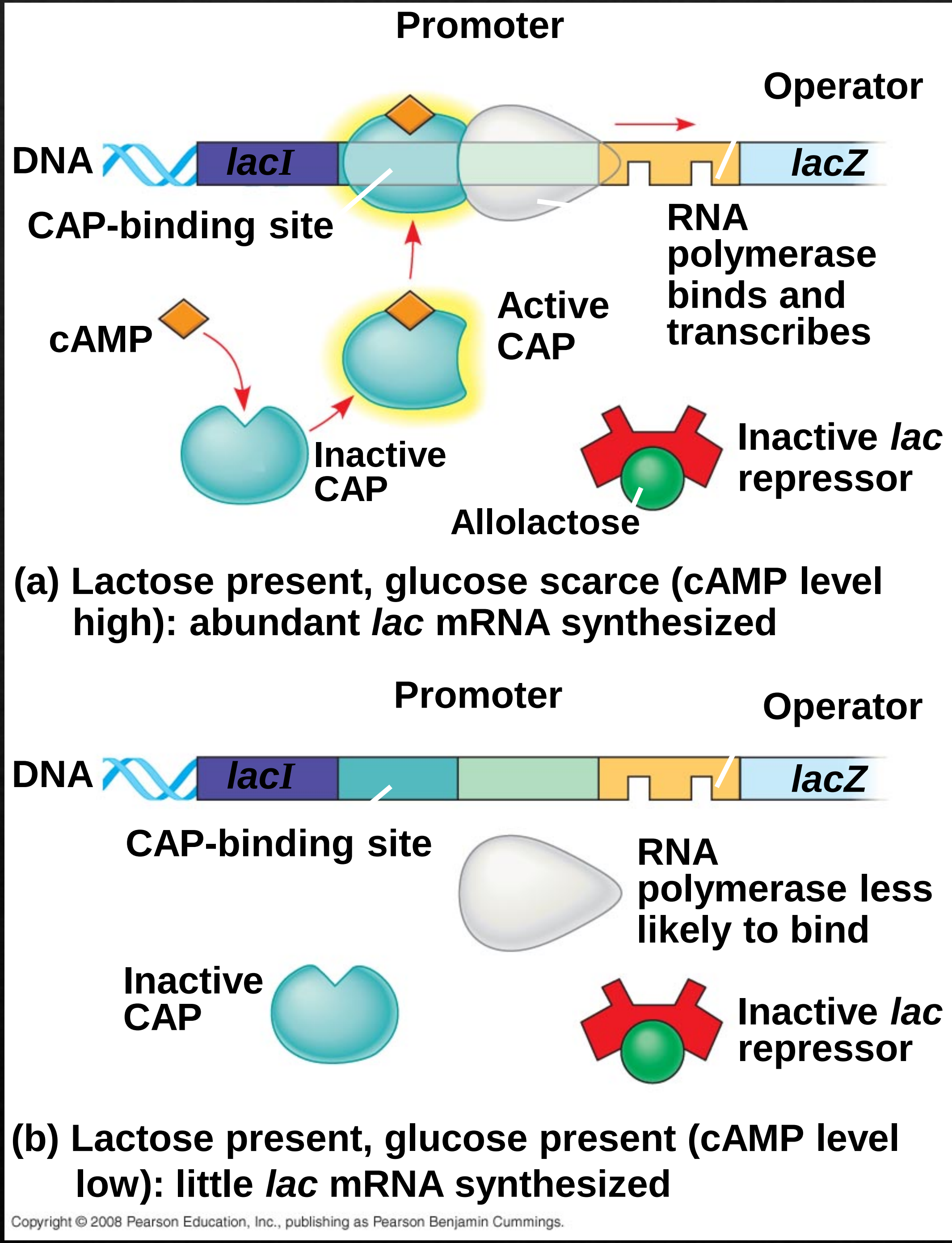


a. **Lactose present, glucose absent (cAMP level high)**



b. **Lactose present, glucose present (cAMP level low)**

Fig. 18-5


Promoter
Operator
DNA
lacI
lacZ
CAP-binding site
RNA polymerase binds and transcribes
cAMP
Active CAP
Inactive CAP
Inactive lac repressor
Allolactose
(a) Lactose present, glucose scarce (cAMP level high): abundant lac mRNA synthesized
Promoter
Operator
DNA
lacI
lacZ
CAP-binding site
RNA polymerase less likely to bind
Inactive CAP
Inactive lac repressor
(b) Lactose present, glucose present (cAMP level low): little lac mRNA synthesized
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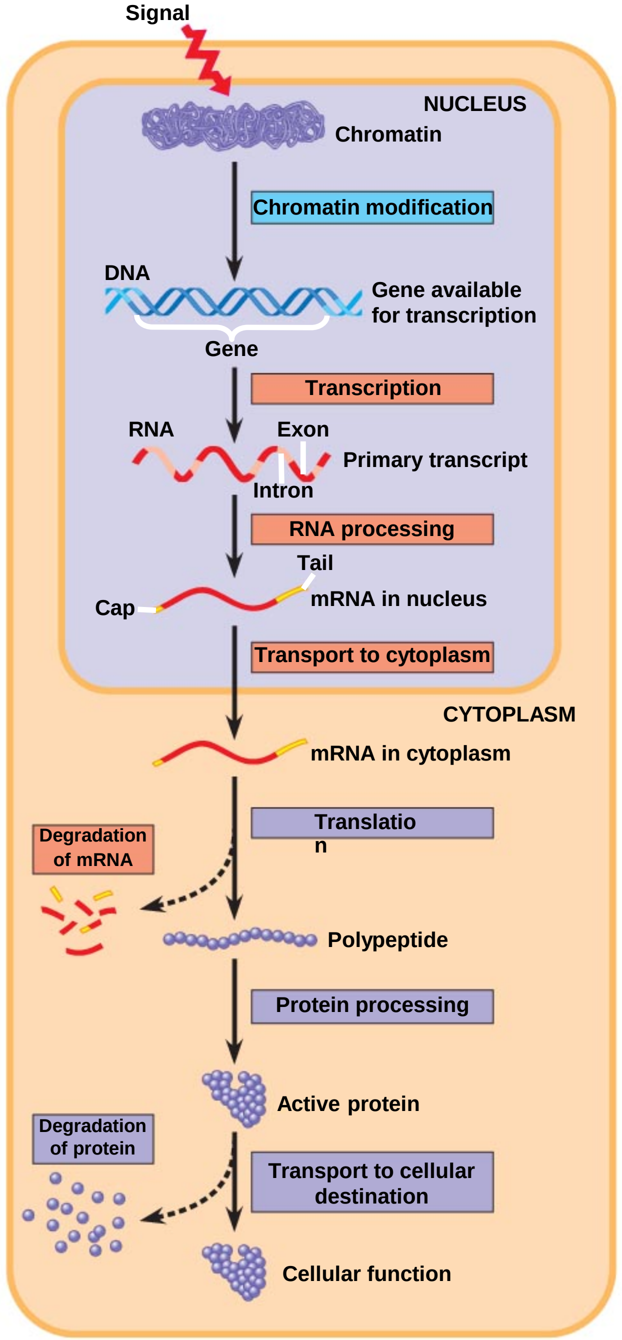
Eukaryotic gene expression can be regulated at any stage

- All organisms must regulate which genes are expressed at any given time
- In multicellular organisms, regulation of gene expression is essential for cell specialization

Differential Gene Expression

- Cells in an organism are genetically identical
- Differences between cell types result from **differential gene expression**, the expression of different genes by cells with the same genome
- Errors in gene expression can lead to diseases including cancer
- Gene expression is regulated at many stages

Fig. 18-6



Signal
NUCLEUS
Chromatin
Chromatin modification
DNA
Gene available for transcription
Gene
Transcription
RNA
Exon
Primary transcript
Intron
RNA processing
Tail
Cap
mRNA in nucleus
Transport to cytoplasm
CYTOPLASM
mRNA in cytoplasm
Degradation of mRNA
Translatio n
Polypeptide
Protein processing
Active protein
Degradation of protein
Transport to cellular destination
Cellular function

Fig. 18-6a

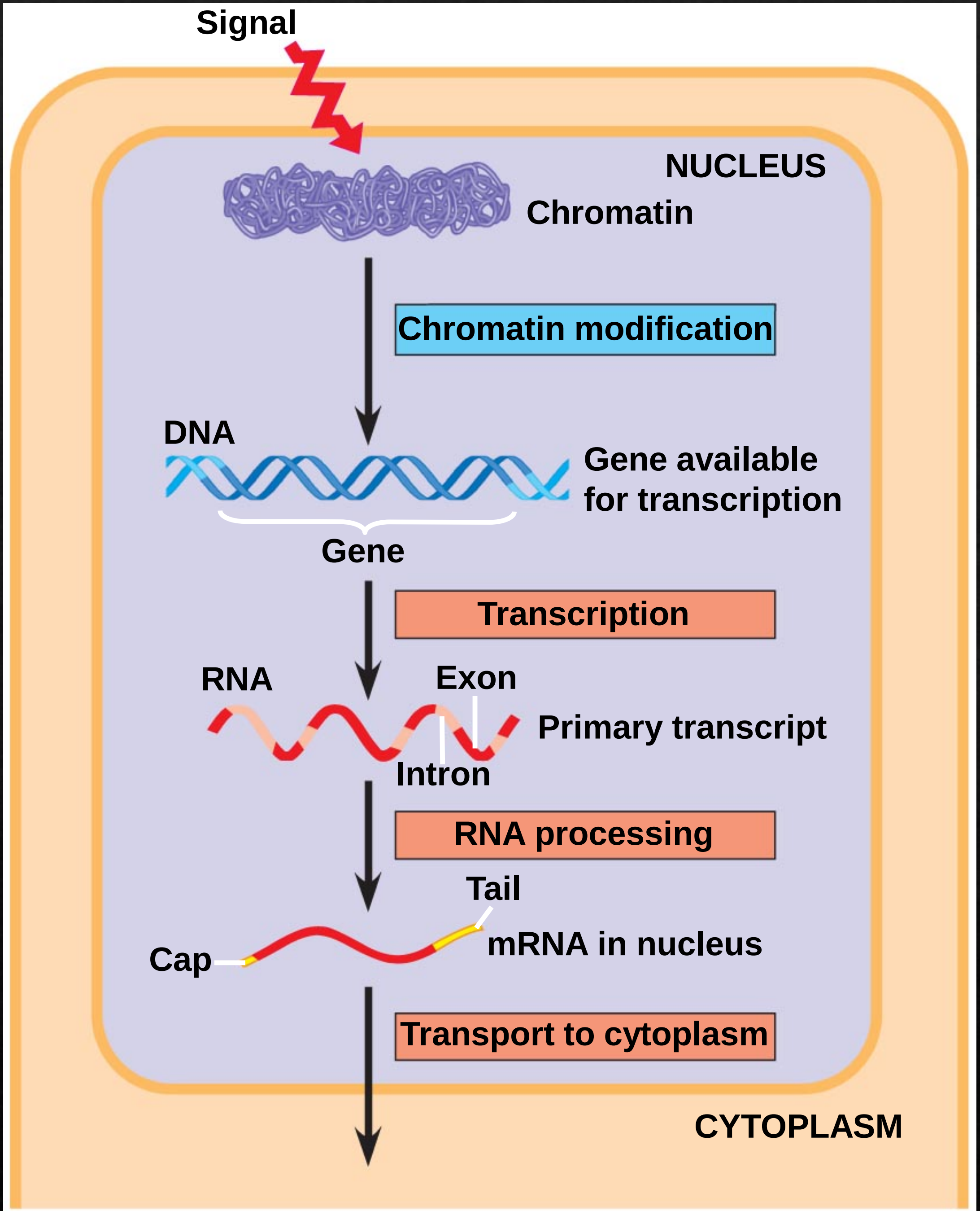
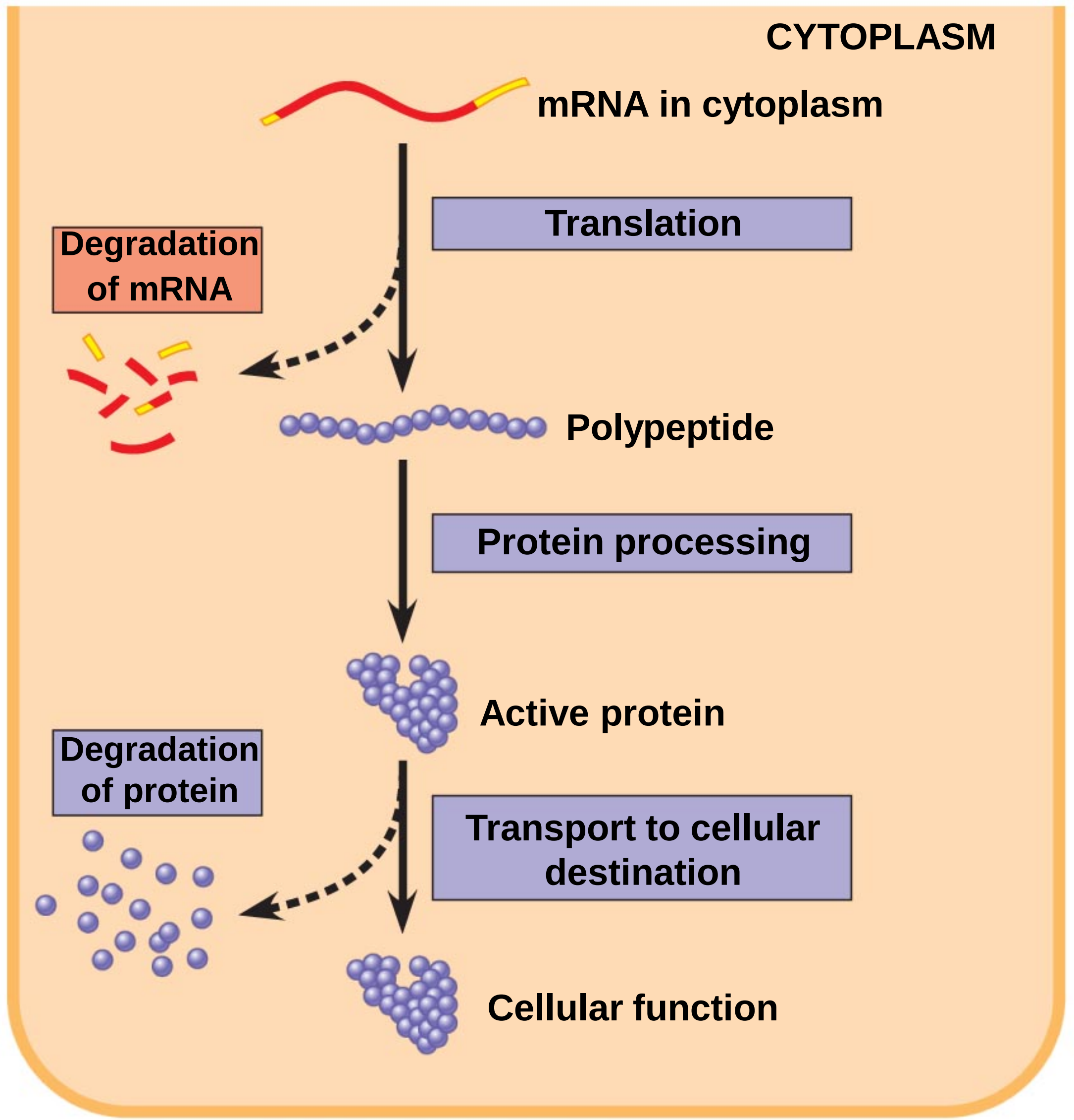
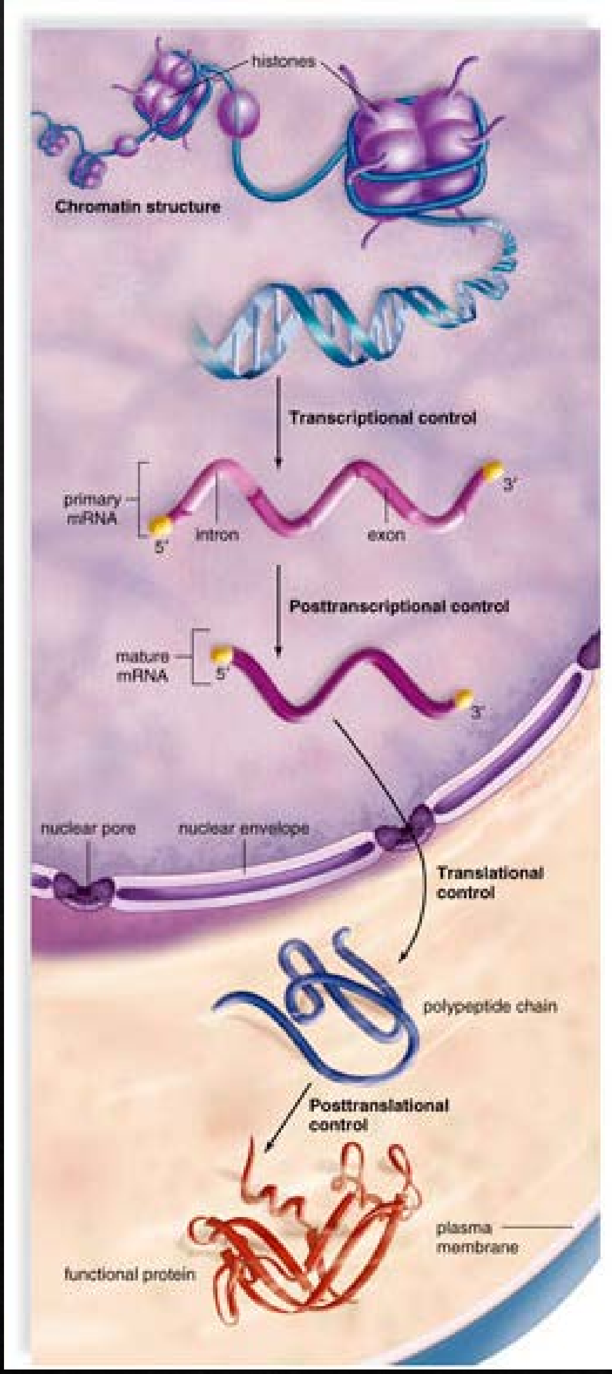

Signal
NUCLEUS
Chromatin
Chromatin modification
DNA
Gene available for transcription
Gene
Transcription
RNA
Exon
Primary transcript
Intron
RNA processing
Tail
Cap
mRNA in nucleus
Transport to cytoplasm
CYTOPLASM

Fig. 18-6b


CYTOPLASM
mRNA in cytoplasm
Translation
Degradation of mRNA
Polypeptide
Protein processing
Active protein
Degradation of protein
Transport to cellular destination
Cellular function

Eukaryotic Regulation

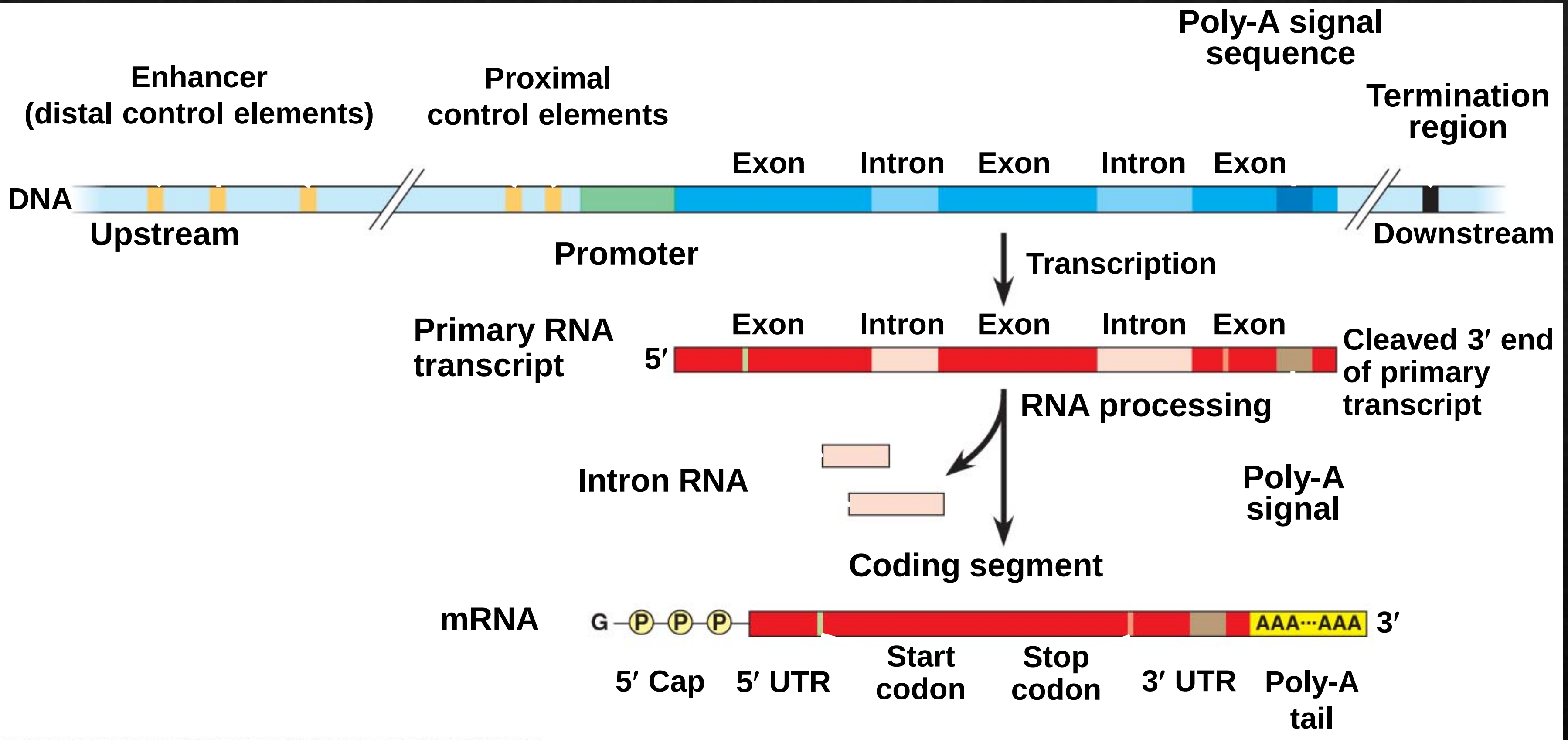
- A variety of mechanisms
- **Five primary levels of control**:
  - **Nuclear levels**
    - **Chromatin Packing**
    - **Transcriptional Control**
    - **Post-transcriptional Control**
  - **Cytoplasmic levels**
    - **Translational Control**
    - **Post-translational Control**



Organization of a Typical Eukaryotic Gene

- Most eukaryotic genes have **control elements**, segments of noncoding DNA that help regulate transcription by binding certain proteins
- Control elements and the proteins they bind are critical to the precise regulation of gene expression in different cell types

Fig. 18-8-3


Enhancer
(distal control elements)
Proximal
control elements
Poly-A signal
sequence
Termination
region
Exon
Intron
Exon
Intron
Exon
DNA
Upstream
Promoter
Downstream
Transcription
Primary RNA
transcript
5′
Exon
Intron
Exon
Intron
Exon
Cleaved 3′ end
of primary
transcript
RNA processing
Intron RNA
Poly-A
signal
Coding segment
mRNA
G
P
P
P
AAA···AAA
3′
5′ Cap
5′ UTR
Start
codon
Stop
codon
3′ UTR
Poly-A
tail

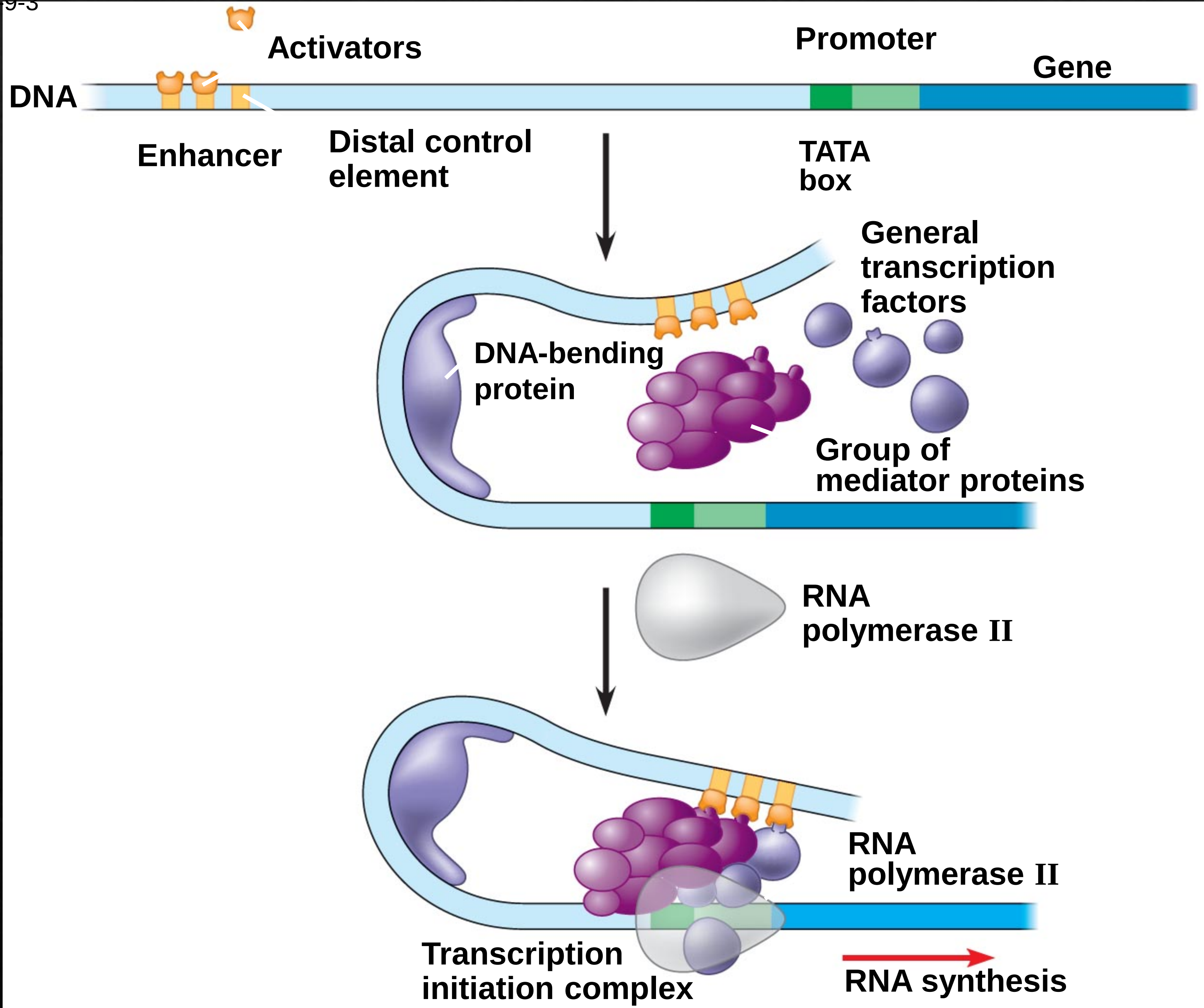
Transcriptional Control

- Transcription controlled by proteins called **transcription factors**
  - Bind to enhancer DNA
  - Regions of DNA where factors that regulate transcription can also bind
  - Always present in cell, but most have to be activated before they bind to DNA
- **Regulation of Transcription Initiation**
  - Chromatin-modifying enzymes provide initial control of gene expression by making a region of DNA more or less available to transcription machinery

Roles of Transcription Factors

- To initiate transcription, eukaryotic RNA polymerase needs the help of transcription factors
- General transcription factors are essential for transcription of all protein-coding genes
- In eukaryotes, high levels of transcription of some genes use control elements interacting with specific transcription factors
- **Proximal** control elements are located close to the promoter
- **Distal** control elements, groups of which are called **enhancers**, may be far away from a gene or even located in an intron

Fig. 18-9-3



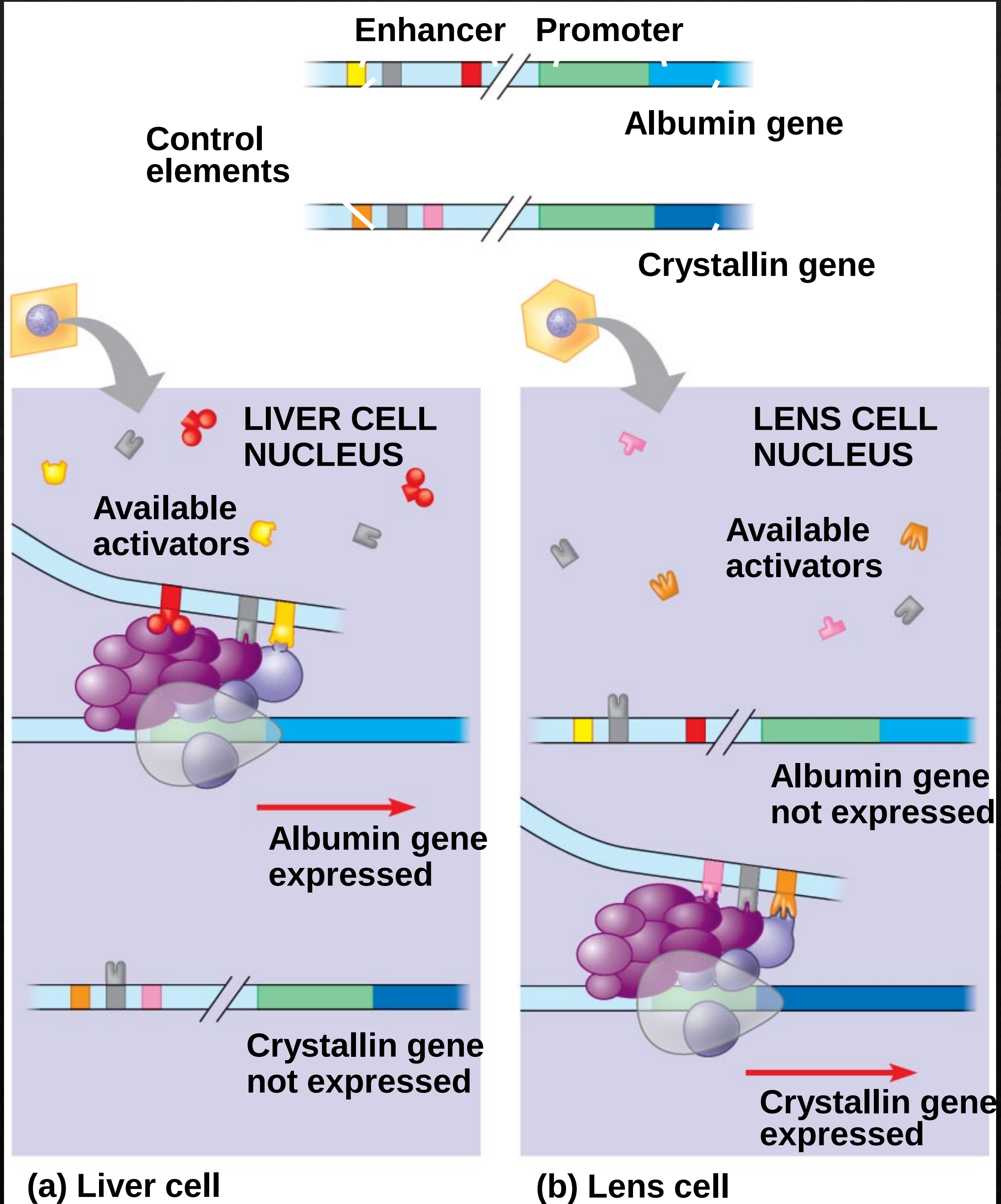
Activators
Promoter
Gene
DNA
Enhancer
Distal control element
TATA box
General transcription factors
DNA-bending protein
Group of mediator proteins
RNA polymerase II
RNA polymerase II
Transcription initiation complex
RNA synthesis

- Some transcription factors function as repressors, inhibiting expression of a particular gene

- Some activators and repressors act indirectly by influencing chromatin structure to promote or silence transcription

Combinatorial Control of Gene Activation

- A particular combination of control elements can activate transcription only when the appropriate activator proteins are present



- Unlike genes of prokaryotic operon, each coordinately controlled eukaryotic gene has its own promoter and control elements

- Genes can be scattered over different chromosomes, but each has the same combination of control elements

- Copies of the activators recognize specific control elements and promote simultaneous transcription of the genes

Post-transcriptional Control

- Post-transcriptional control operates on primary mRNA transcript
- Given a specific primary transcript:
  - Excision of introns can vary
  - Splicing of exons can vary
  - Determines the type of mature transcript that leaves the nucleus
- Can also control speed of mRNA transport from nucleus to cytoplasm
  - Will affect the number of transcripts arriving at rough ER and, therefore, amount of gene product made each time

Post-Transcriptional Regulation Mechanisms

- Transcription alone does not account for gene expression
- Regulatory mechanisms can operate at various stages after transcription
- Such mechanisms allow a cell to fine-tune gene expression rapidly in response to environmental changes

1. RNA Processing

- **Alternative RNA splicing**, different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and which as introns

2. mRNA Degradation

- Life span of mRNA molecules in cytoplasm is key to determining protein synthesis
- Eukaryotic mRNA is more long lived than prokaryotic mRNA
- mRNA life span is determined by sequences in leader and trailer regions

Processing of mRNA Transcripts

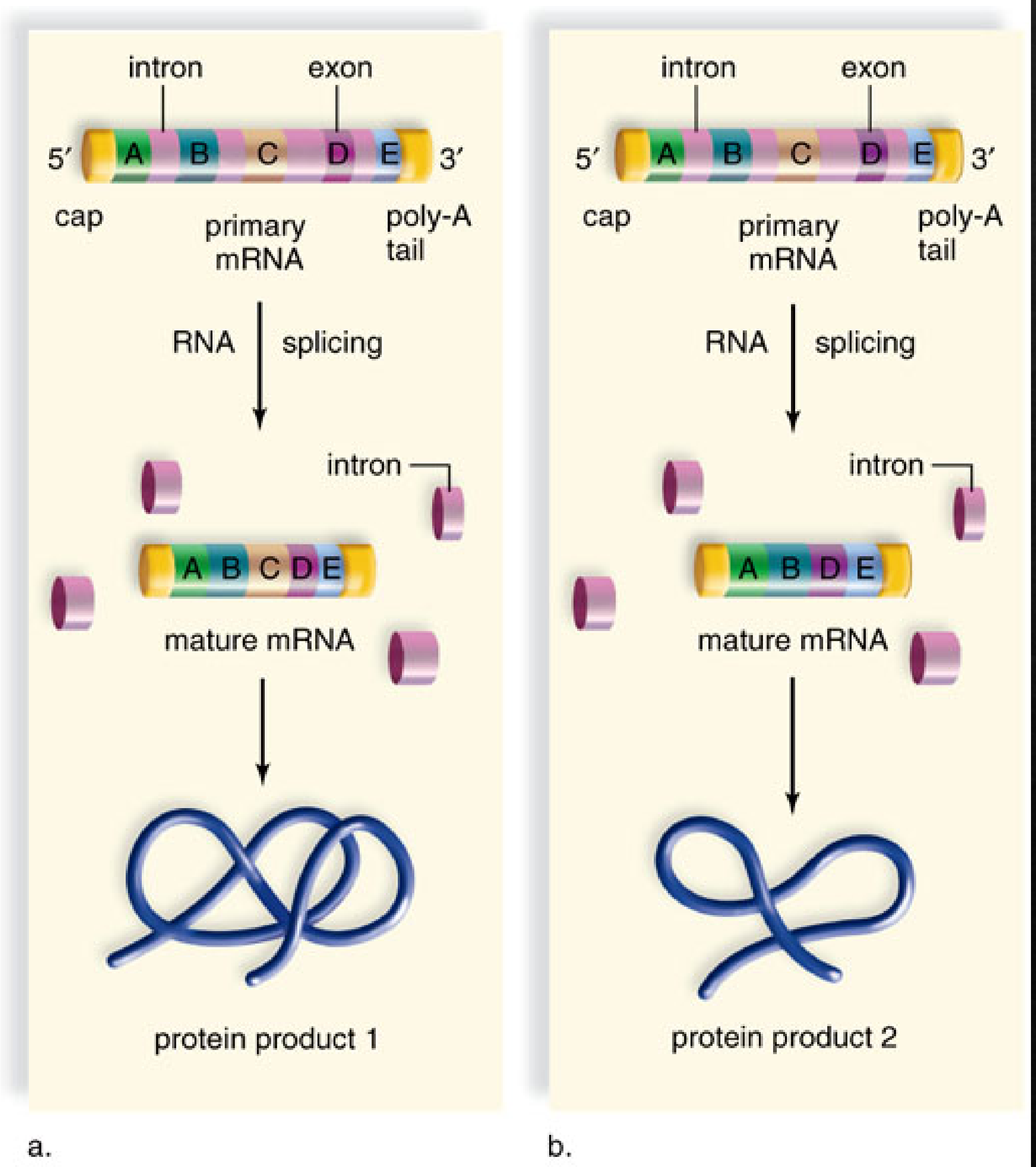
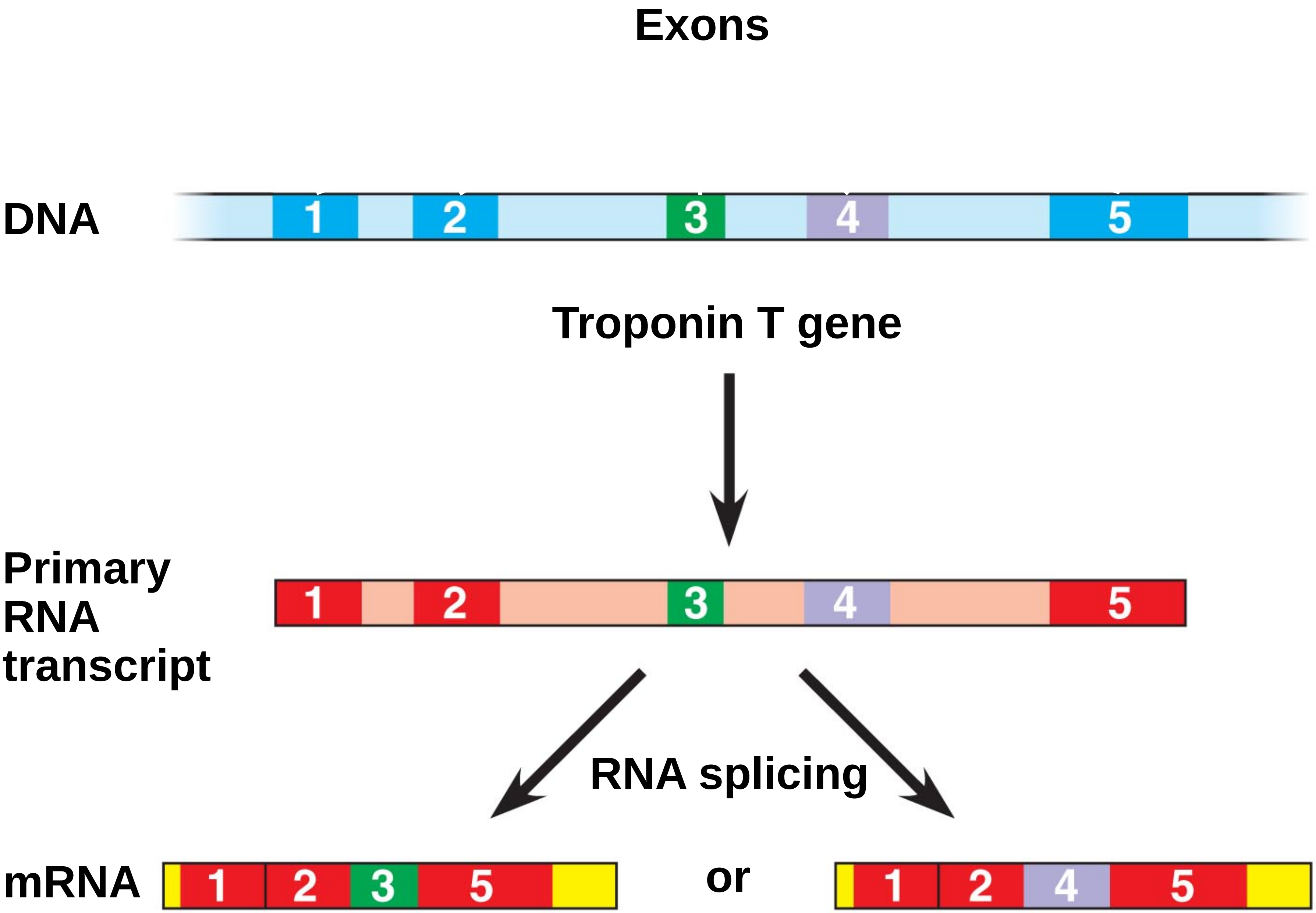


Fig. 18-11



Exons
DNA
1
2
3
4
5
Troponin T gene
Primary RNA transcript
1
2
3
4
5
RNA splicing
mRNA
1
2
3
5
or
1
2
4
5

Translational Control

- **Translational Control** - Determines how much mRNA is translated into a protein product
  - Presence of 5′ cap
  - Length of poly-A tail on 3′ end
- **Post-translational Control** - Affects the activity of a protein product
  - Activation
  - Degradation rate

Initiation of Translation

- Initiation of translation can be blocked by regulatory proteins that bind to sequences or structures of mRNA

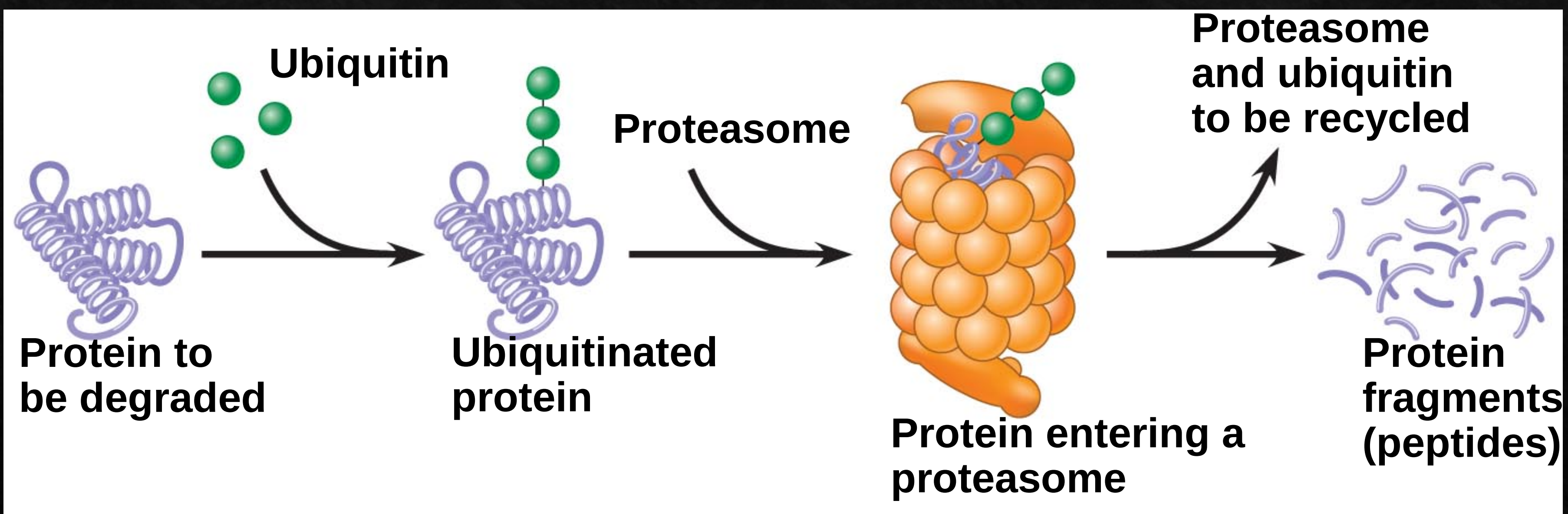
- Translation of all mRNAs in a cell can be regulated simultaneously. For example, initiation factors are simultaneously activated in an egg following fertilization

Fig. 18-12

Protein Processing and Degradation

After translation, various types of processing, including cleavage and the addition of chemical groups can occur

Proteasomes are giant protein complexes that bind protein molecules and degrade them



Noncoding RNAs play multiple roles in controlling gene expression

- Only a small fraction of DNA codes for proteins, rRNA, and tRNA
- A significant amount of the genome can be transcribed into noncoding RNAs
- Noncoding RNAs regulate gene expression at two points: mRNA translation and chromatin configuration

Effects on mRNAs by MicroRNAs and Small Interfering RNAs

- **MicroRNAs (miRNAs)**- small single-stranded RNA molecules that can bind to mRNA
- Can degrade mRNA or block its translation

- Inhibition of gene expression by RNA molecules is called **RNA interference (RNAi)**

- RNAi is caused by **small interfering RNAs (siRNAs)**

- siRNAs and miRNAs are similar but form from different RNA precursors

Chromatin Remodeling and Small RNA Silencing of Transcription

- siRNAs play a role in heterochromatin formation and can block large regions of the chromosome or transcription of specific genes

DNA Methylation

- Addition of methyl groups to certain bases in DNA, leads to reduced transcription in some species
- DNA methylation can cause long-term inactivation of genes in cellular differentiation

Epigenetic Inheritance

- **Genomic imprinting**, methylation regulates expression of either maternal or paternal alleles of certain genes at start of development

- Although the chromatin modifications just discussed do not alter DNA sequence, they may be passed to future generations of cells

- Inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called **epigenetic inheritance**