Review Warm-Up

1. What is the Central Dogma?

2. How does prokaryotic DNA compare to eukaryotic DNA?

3. How is DNA organized in eukaryotic cells?

Ch. 15 Warm-Up

1. Draw and label the 3 parts of an operon.
2. Contrast inducible vs. repressible operons.
3. How does DNA methylation and histone acetylation affect gene expression?

Ch. 15 Warm-Up

1. Compare DNA methylation and histone acetylation.
2. What is the role of activators vs. repressors? Where do they bind to?
3. List the components found in a eukaryotic transcription initiation complex.
4. What is the function of miRNAs and siRNAs?

Regulation of Gene Expression

Chapter 15

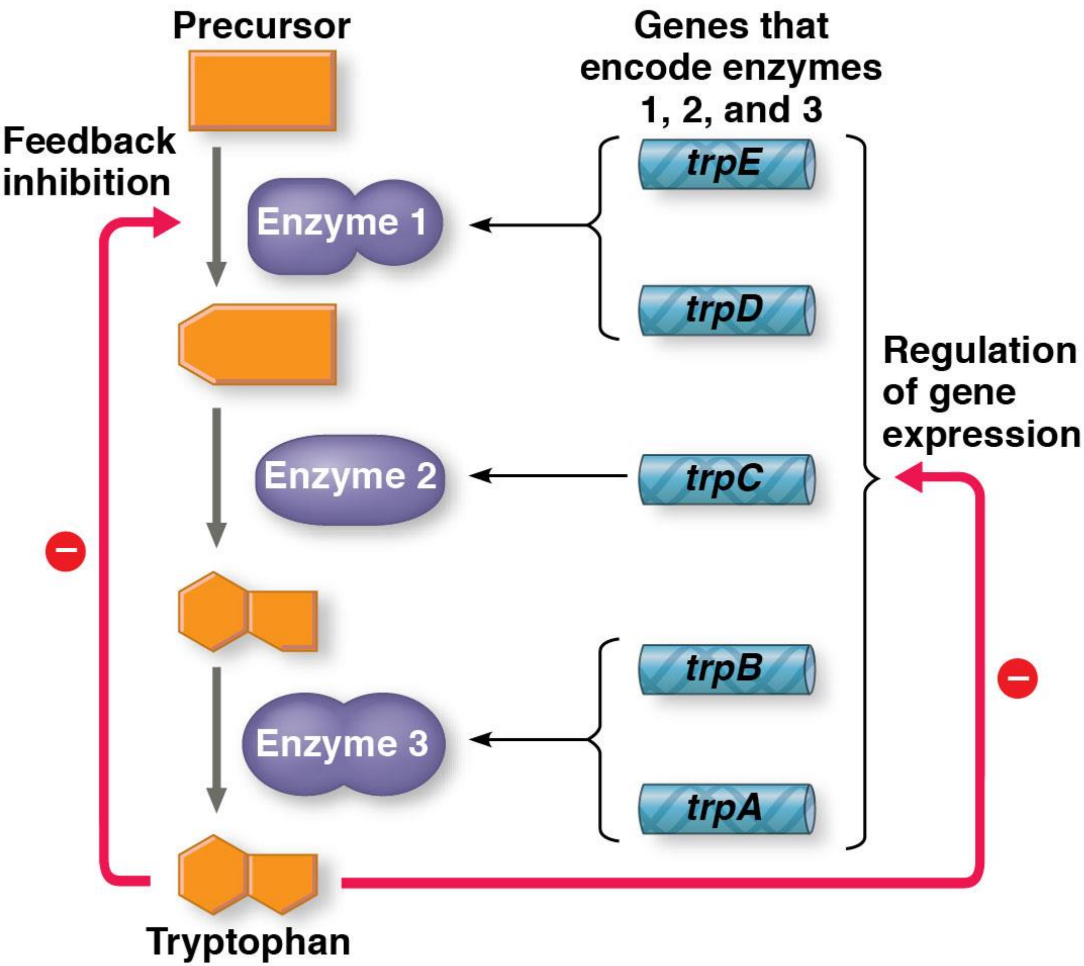
What you must know:

- Genes can be activated by *inducer* molecules, or they can be inhibited by the presence of a *repressor* as they interact with regulatory proteins or sequences.
- A *regulatory gene* is a sequence of DNA that codes for a regulatory protein such as a repressor protein.
- How the components of an operon function to regulate gene expression in both repressible and inducible operons.
- How positive and negative control function in gene expression.
- The impact of DNA methylation and histone acetylation on gene expression.
- The role of microRNAs in control of cellular functions.

Regulation of Gene Expression by Bacteria

- Transcription

Regulation of metabolic pathways

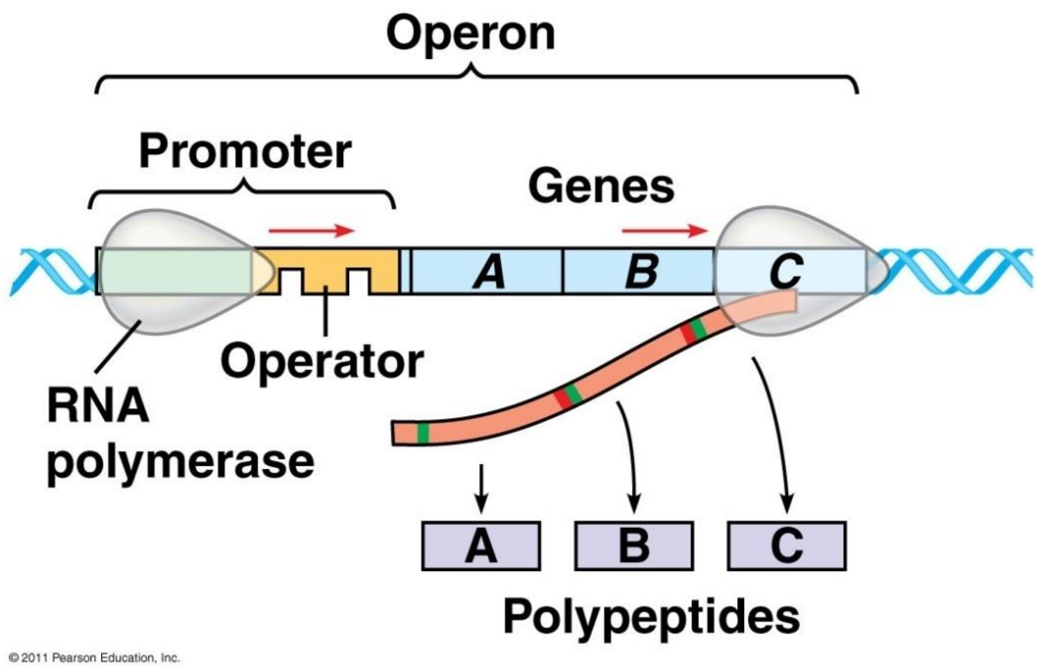


(a) Regulation of enzyme activity

(b) Regulation of enzyme production

Bacterial control of gene expression

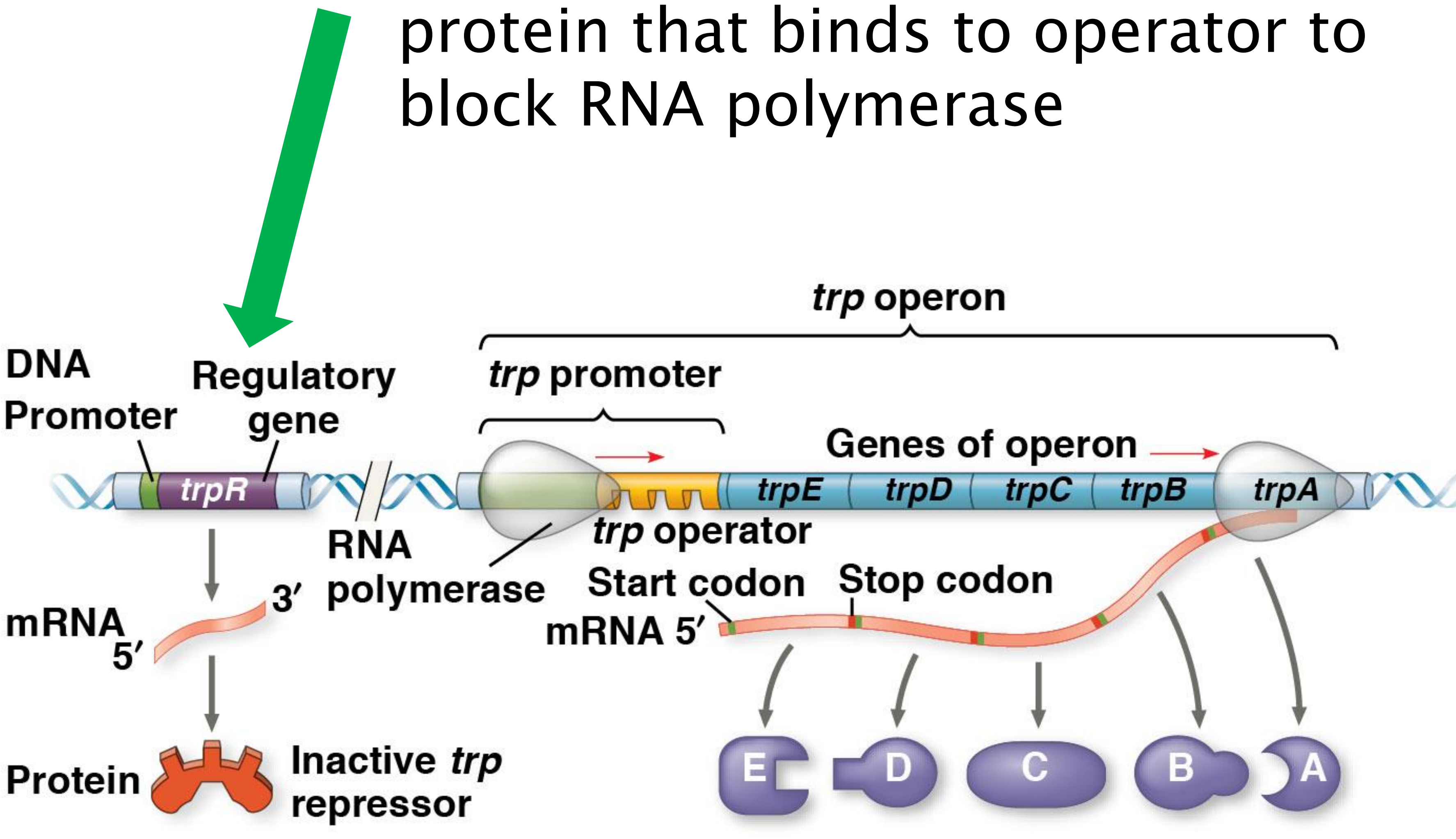
Operon: cluster of related genes with on/off switch



Three Parts:

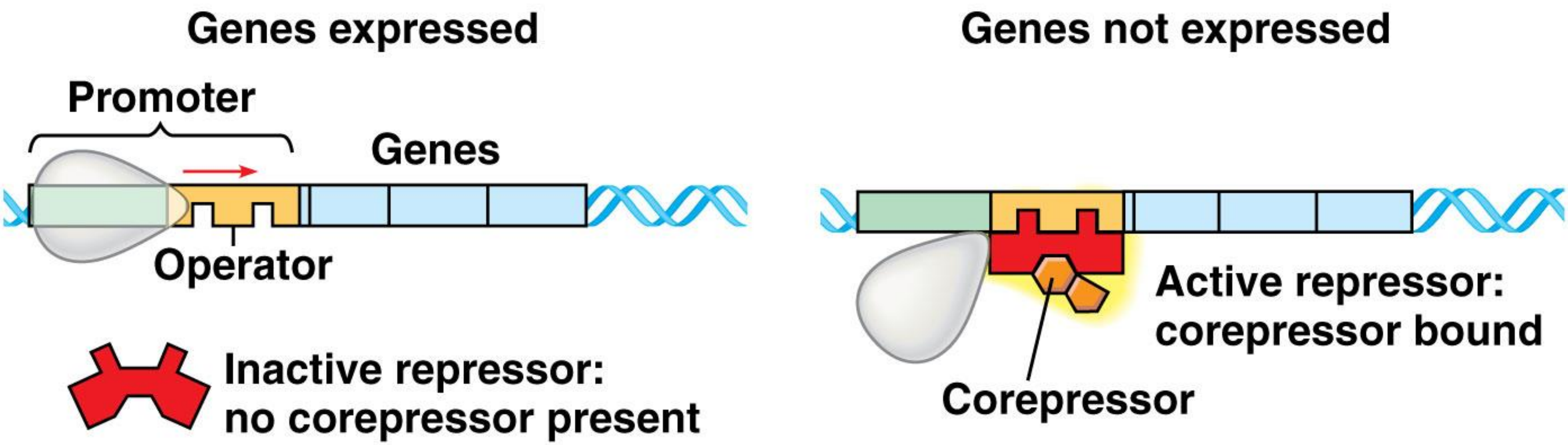
1. Promoter – where RNA polymerase attaches
2. Operator – "on/off", controls access of RNA poly
3. Genes – code for related enzymes in a pathway

Regulatory gene: produces **repressor** protein that binds to operator to block RNA polymerase

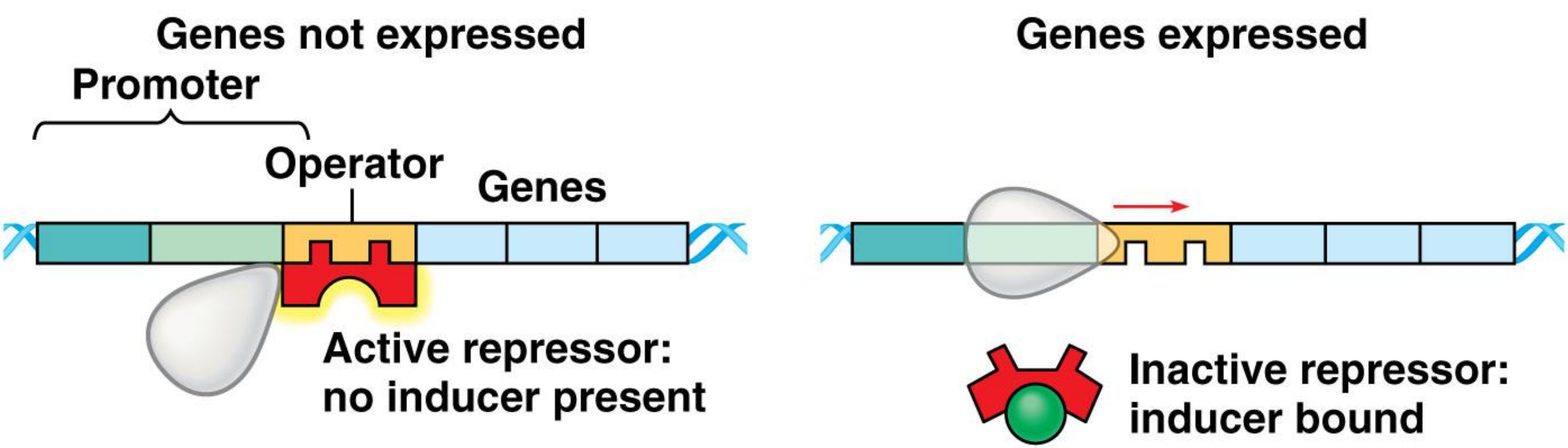


(a) Tryptophan absent, repressor inactive, operon on

Repressible Operon (ON → OFF)

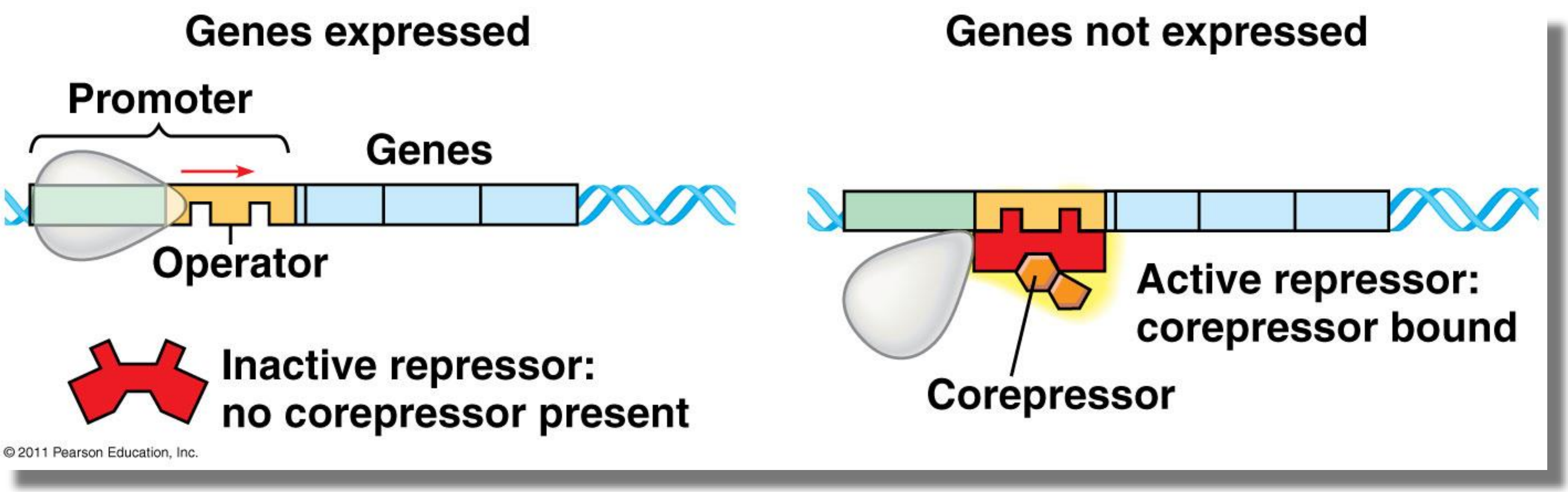


Inducible Operon (OFF → ON)

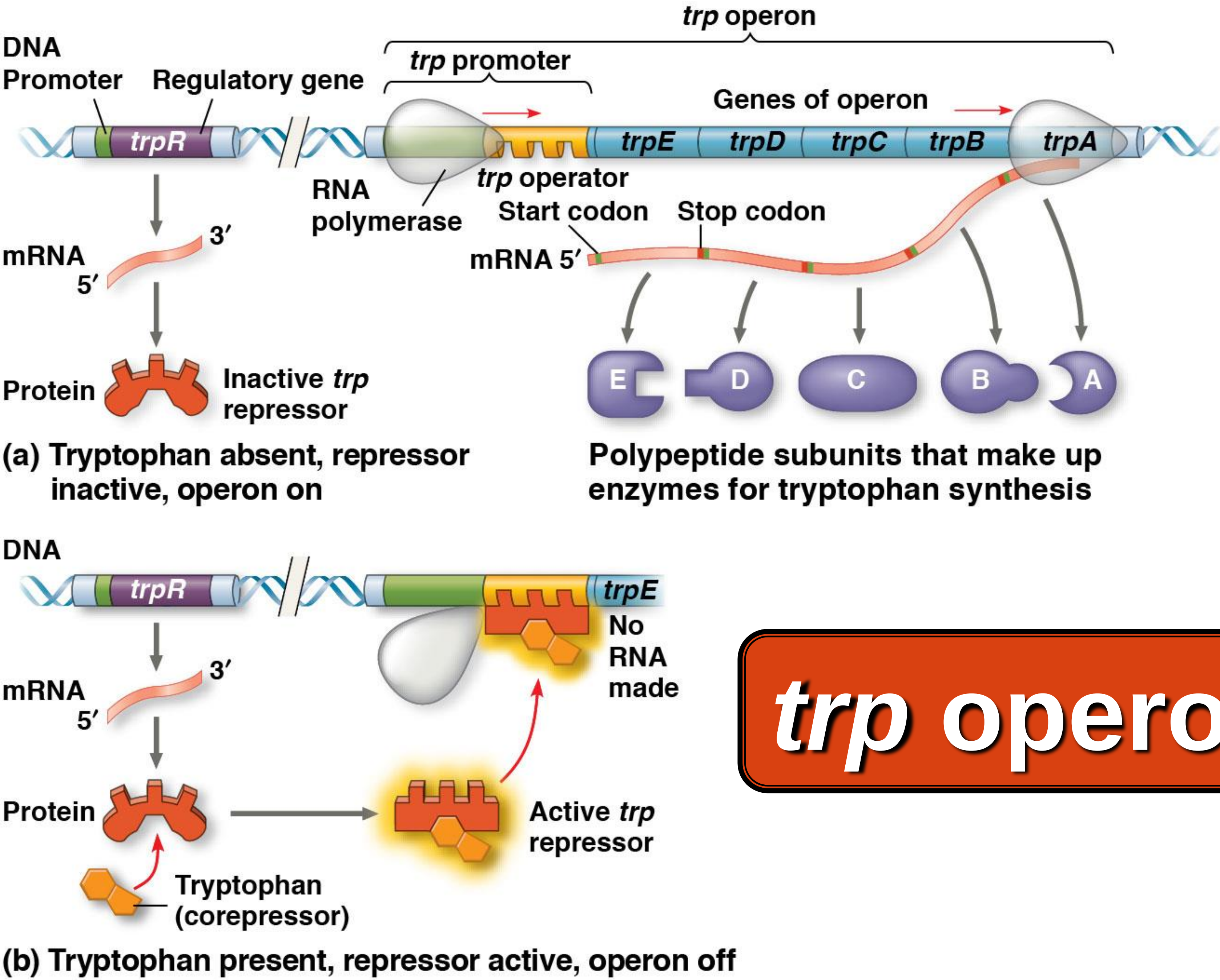


Repressible Operon

- Normally ON
- Anabolic (build organic molecules)
- Organic molecule product acts as <u>**corepressor**</u> → binds to repressor to **activate** it
- Operon is turned OFF
- Eg. ***trp*** **operon**



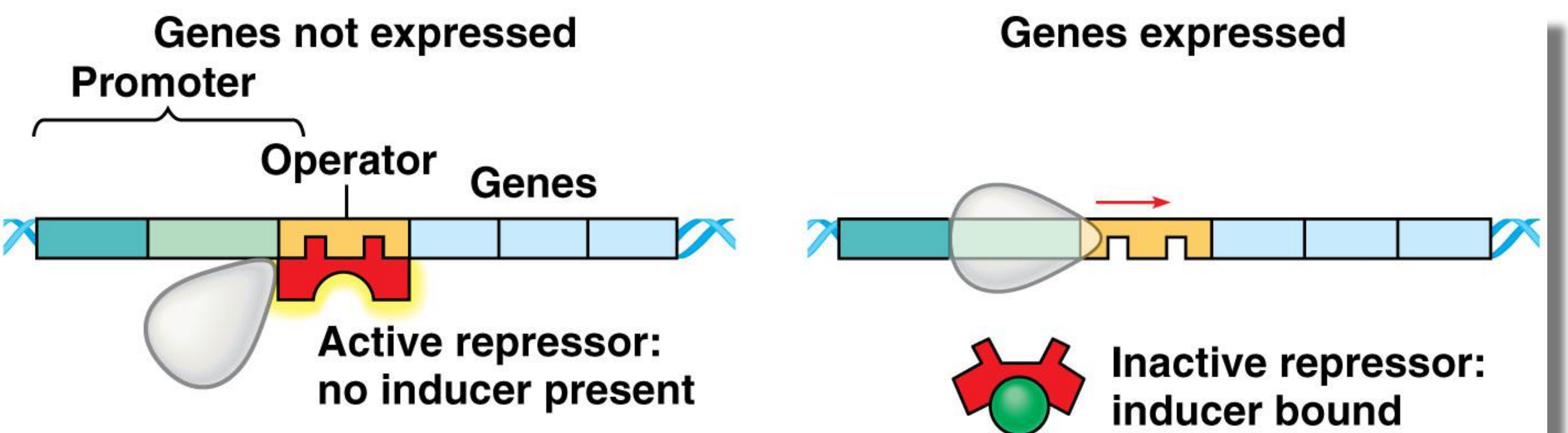
trp operon



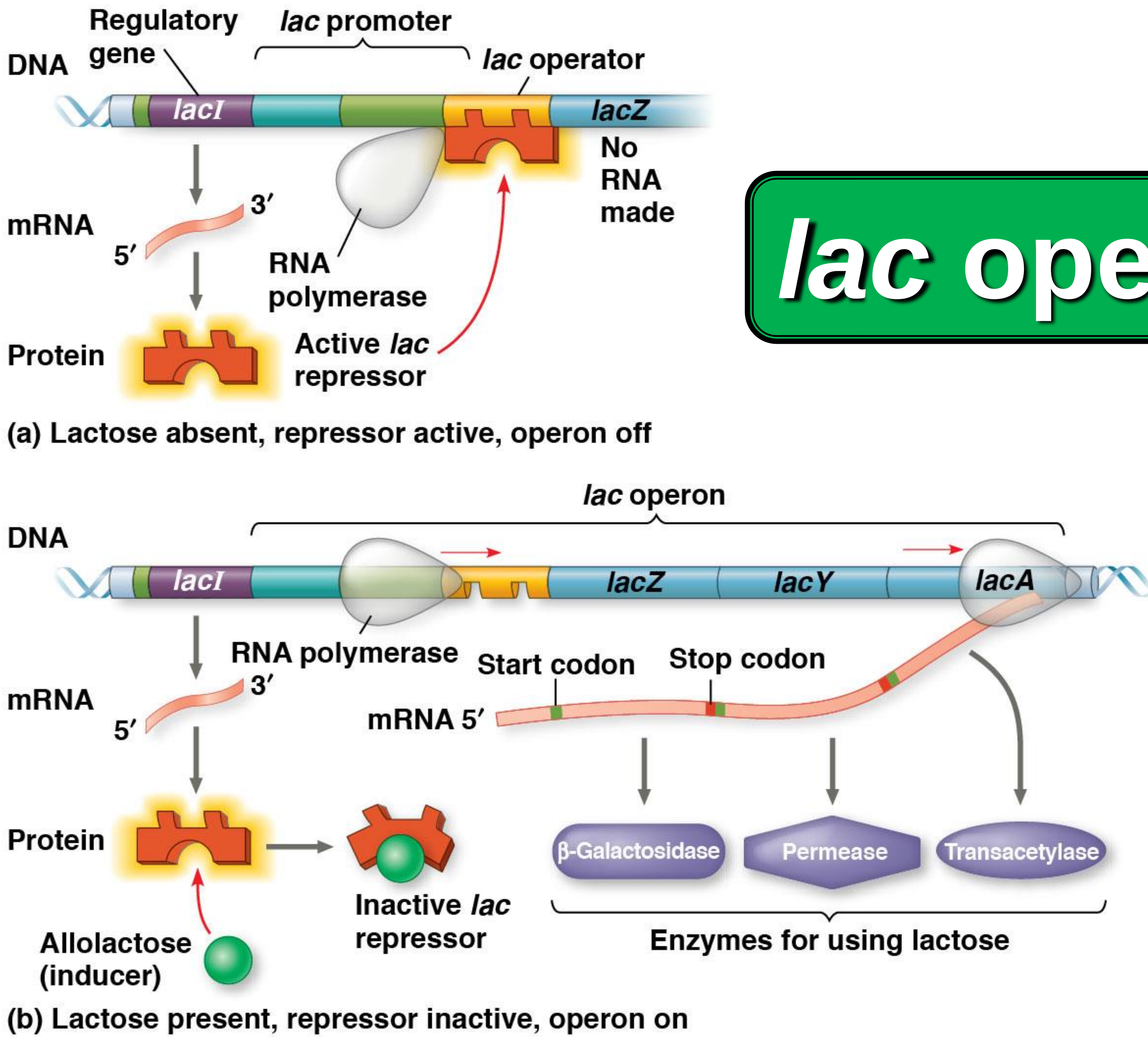
trp operon
DNA
Promoter
Regulatory gene
trp promoter
Genes of operon
trpR
trpE
trpD
trpC
trpB
trpA
RNA polymerase
trp operator
Start codon
Stop codon
mRNA 5′
3′
mRNA
5′
Protein
Inactive trp repressor
E
D
C
B
A
(a) Tryptophan absent, repressor inactive, operon on
Polypeptide subunits that make up enzymes for tryptophan synthesis
DNA
trpR
trpE
No RNA made
3′
mRNA
5′
Protein
Active trp repressor
Tryptophan (corepressor)
(b) Tryptophan present, repressor active, operon off

Inducible Operon

- Normally OFF
- Catabolic (break down food for energy)
- Repressor is active → **inducer** binds to and **inactivates** repressor
- Operon is turned ON
- Eg. ***lac* operon**



lac operon

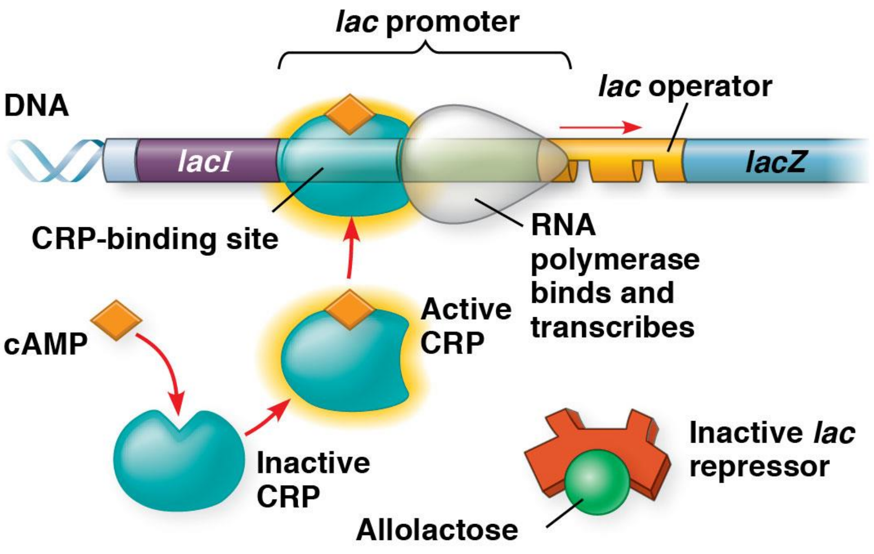

DNA
Regulatory gene
lac promoter
lac operator
lacI
lacZ
No RNA made
mRNA
3′
5′
RNA polymerase
Protein
Active lac repressor
(a) Lactose absent, repressor active, operon off
lac operon
DNA
lacI
lacZ
lacY
lacA
RNA polymerase
Start codon
Stop codon
mRNA
3′
5′
mRNA 5′
Protein
Inactive lac repressor
β-Galactosidase
Permease
Transacetylase
Enzymes for using lactose
Allolactose (inducer)
(b) Lactose present, repressor inactive, operon on

Gene Regulation: Positive vs. Negative Control

- ***Negative control***: operons are switched off by active form of repressor protein
  - Eg. *trp* operon, *lac* operon
- ***Positive control***: regulatory protein interacts directly with genome to increase transcription
  - Eg. cAMP & CRP

cAMP + CRP = Positive Control

- cAMP: accumulates when glucose is scarce
- cAMP binds to CRP (cAMP receptor protein)
- Active CRP → binds to DNA upstream of promoter, ↑ affinity of RNA polymerase to promoter, ↑ transcription



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

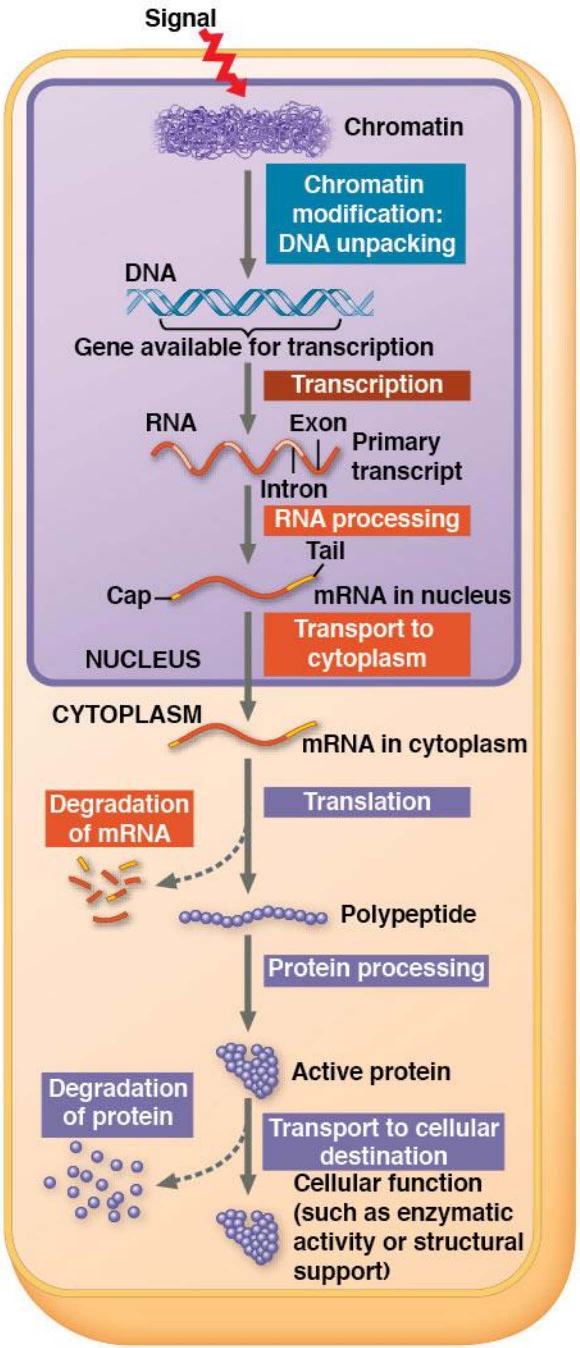
Gene Regulation and the Order of the Operon

- Amoeba Sisters Video

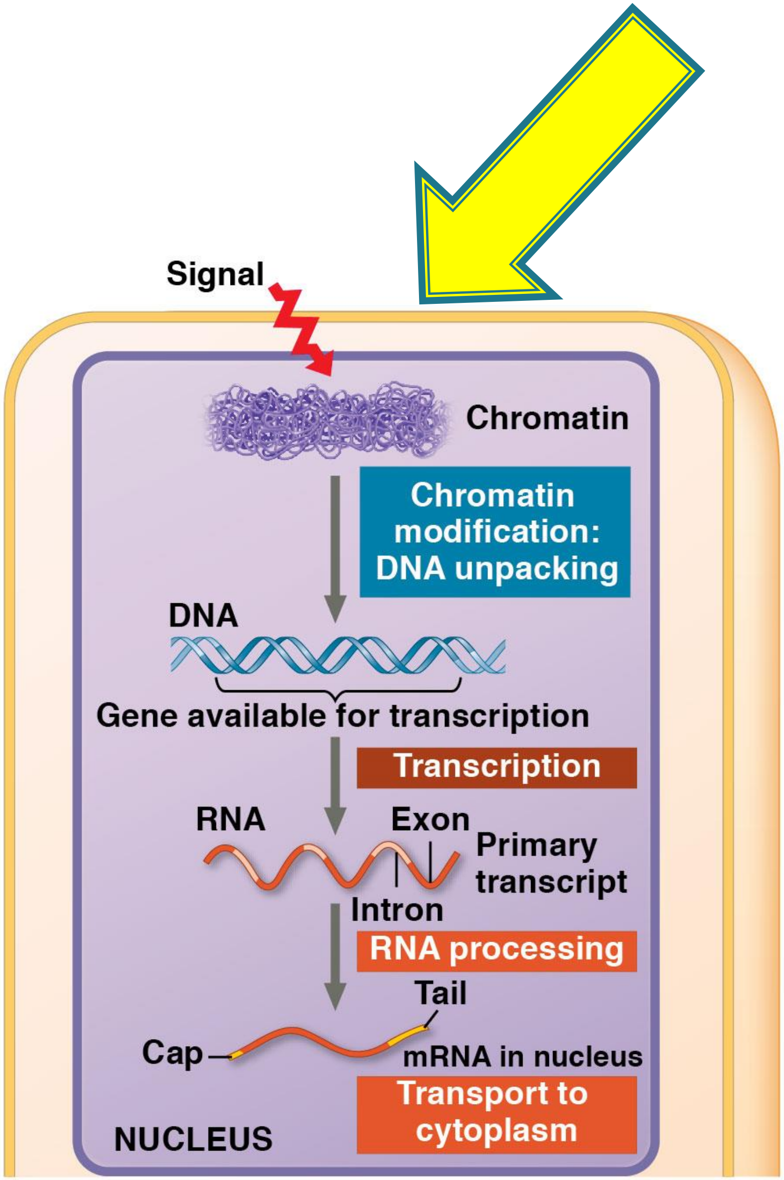
Regulation of Gene Expression by Eukaryotes

- Many stages

- Typical human cell: only 20% of genes expressed at any given time
- Different cell types (with identical genomes) turn on different genes to carry out specific functions
- Differences between cell types is due to **differential gene expression**

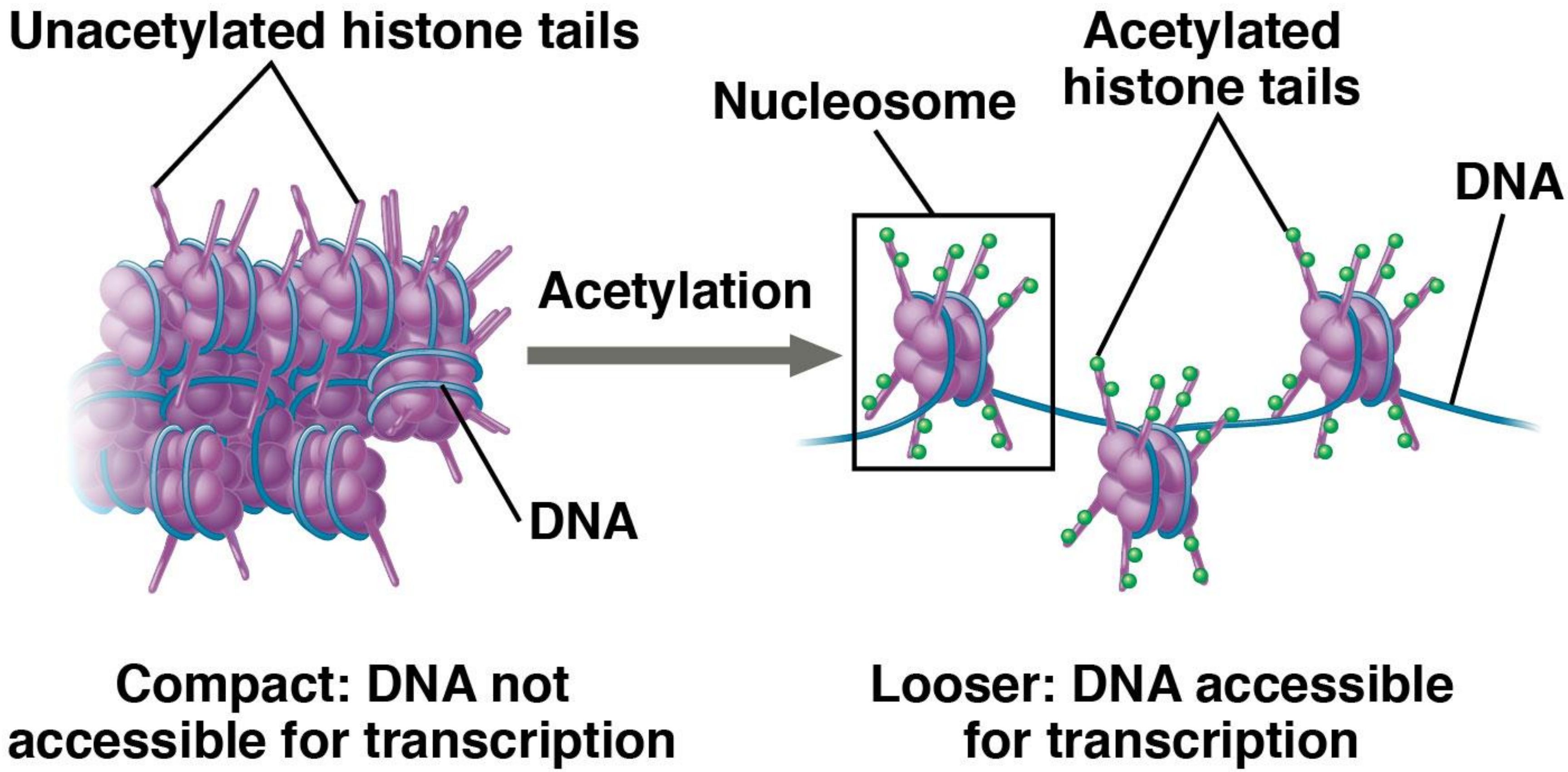


Eukaryotic gene expression regulated at different stages



Chromatin Structure:

- Tightly bound DNA → less accessible for transcription
- DNA methylation: methyl groups added to DNA; tightly packed;
  ↓ transcription
- Histone acetylation: acetyl groups added to histones; loosened;
  ↑ transcription

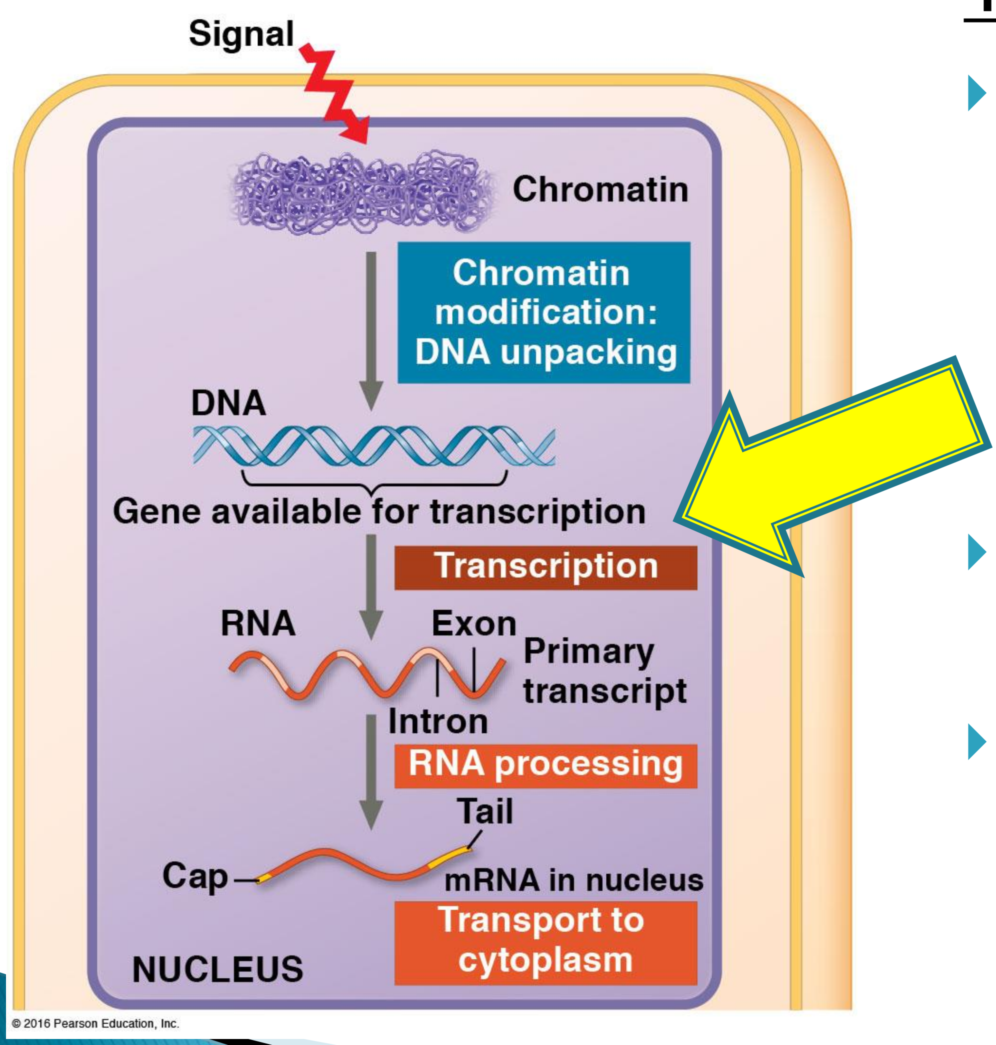

Unacetylated histone tails
Acetylation
Nucleosome
Acetylated histone tails
DNA
DNA
Compact: DNA not accessible for transcription
Looser: DNA accessible for transcription

Epigenetic Inheritance

- Modifications on chromatin can be passed on to future generations
- Unlike DNA mutations, these changes to chromatin can be reversed (de-methylation of DNA)
- Explains differences between identical twins
  - Eg. DNA methylation (gene silencing), histone acetylation, X chromosome inactivation, heterochromatin (silent chromatin)

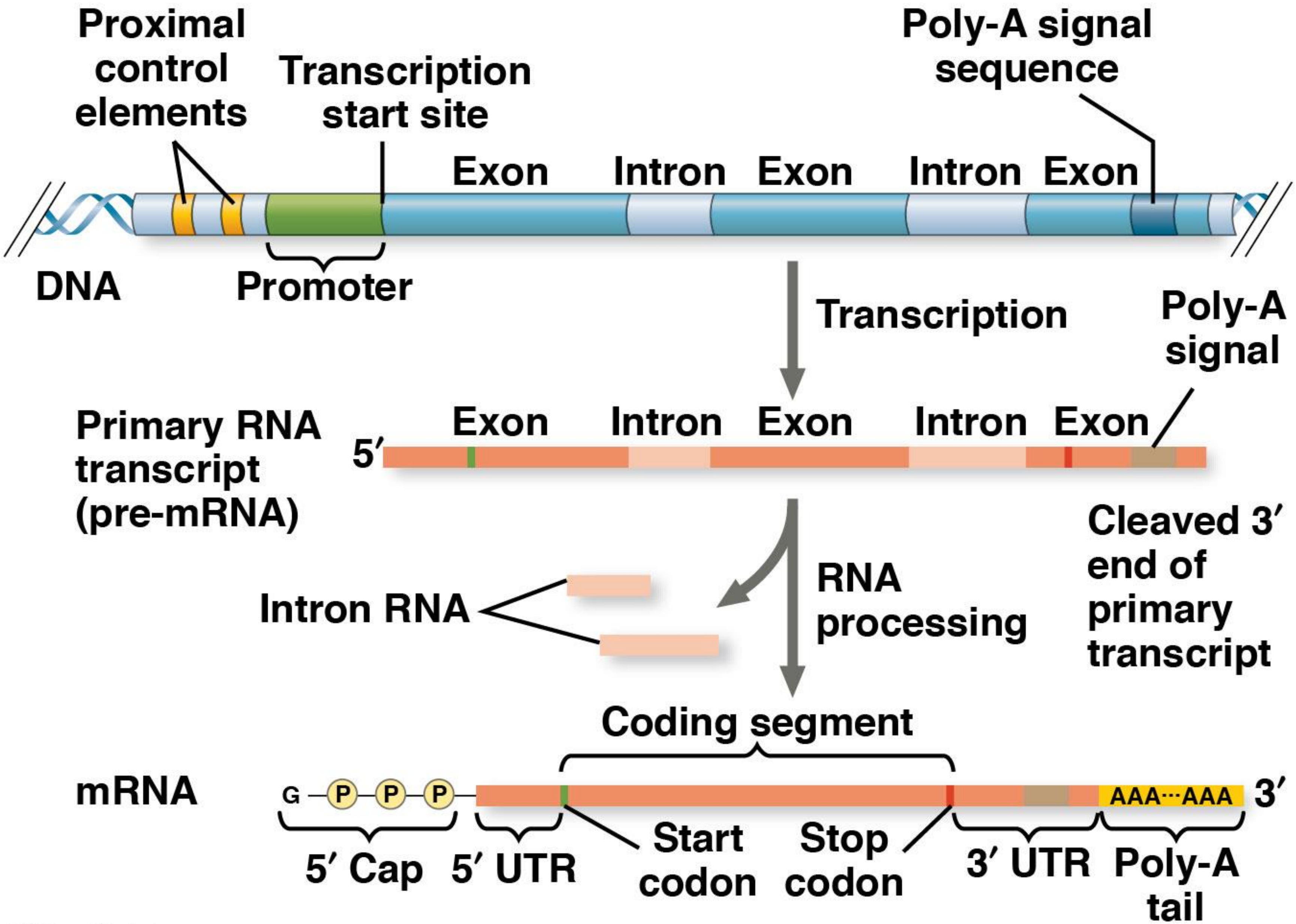
Video: The Epigenome at a Glance

- Genetic Science Learning Center



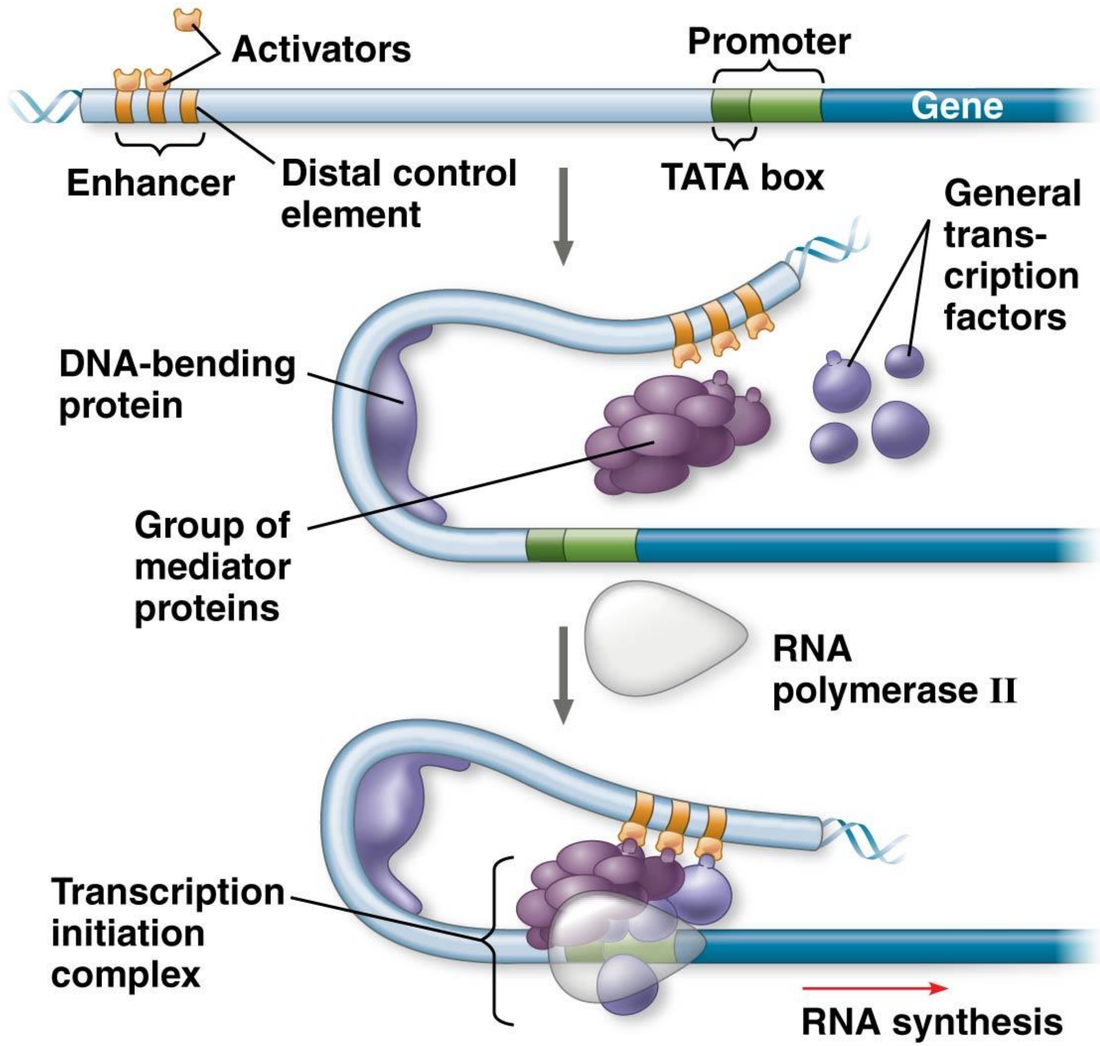
Transcription Initiation:

- Specific transcription factors (activators or repressors) bind to **control elements (enhancer region)**
- **Activators**: increase transcription
- **Repressors**: decrease transcription

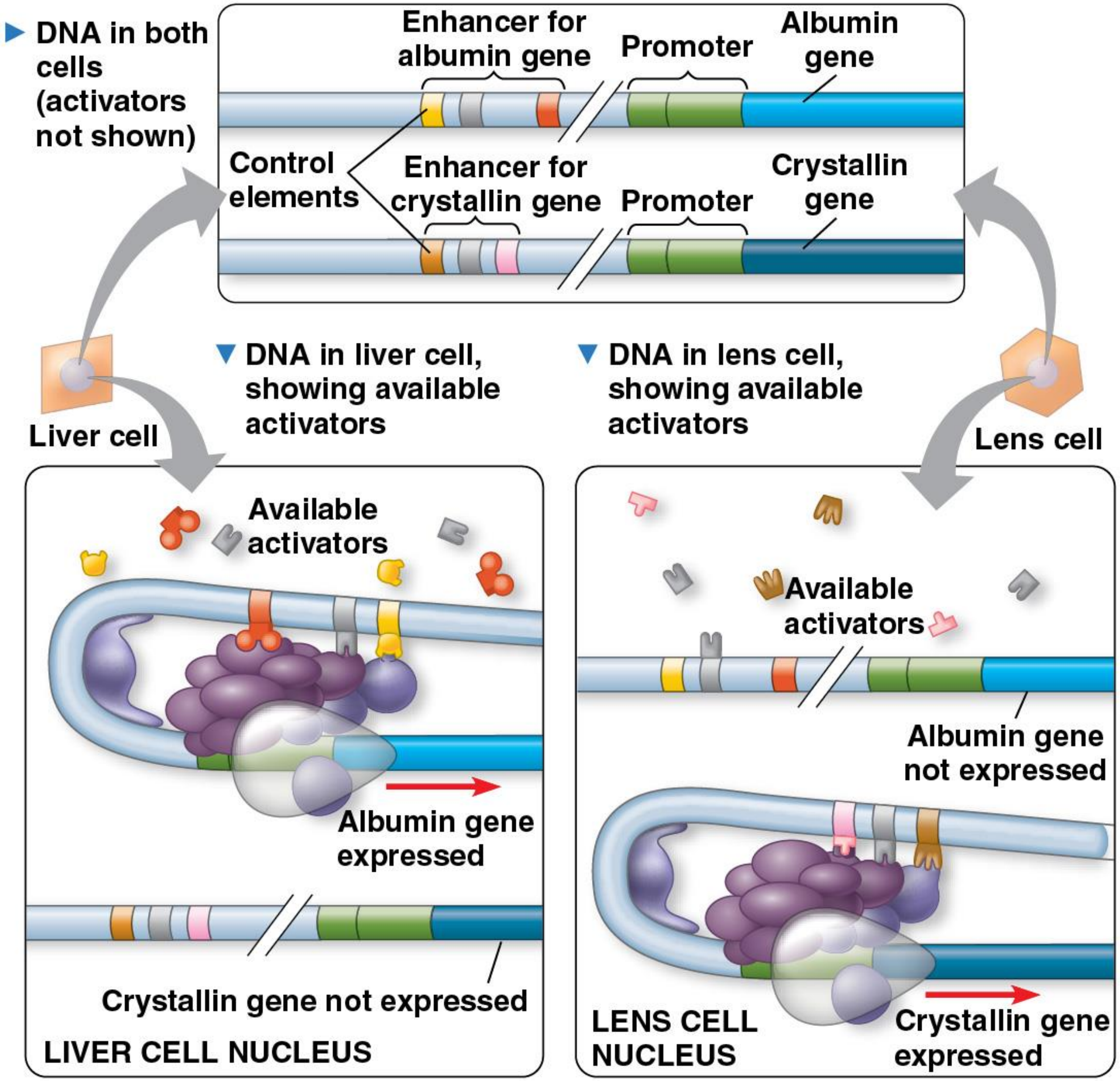

Proximal control elements
Transcription start site
Poly-A signal sequence
Exon
Intron
Exon
Intron
Exon
DNA
Promoter
Transcription
Poly-A signal
Primary RNA transcript (pre-mRNA)
5′
Exon
Intron
Exon
Intron
Exon
Cleaved 3′ end of primary transcript
RNA processing
Intron RNA
Coding segment
mRNA
G
P
P
P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

Transcription Initiation Complex

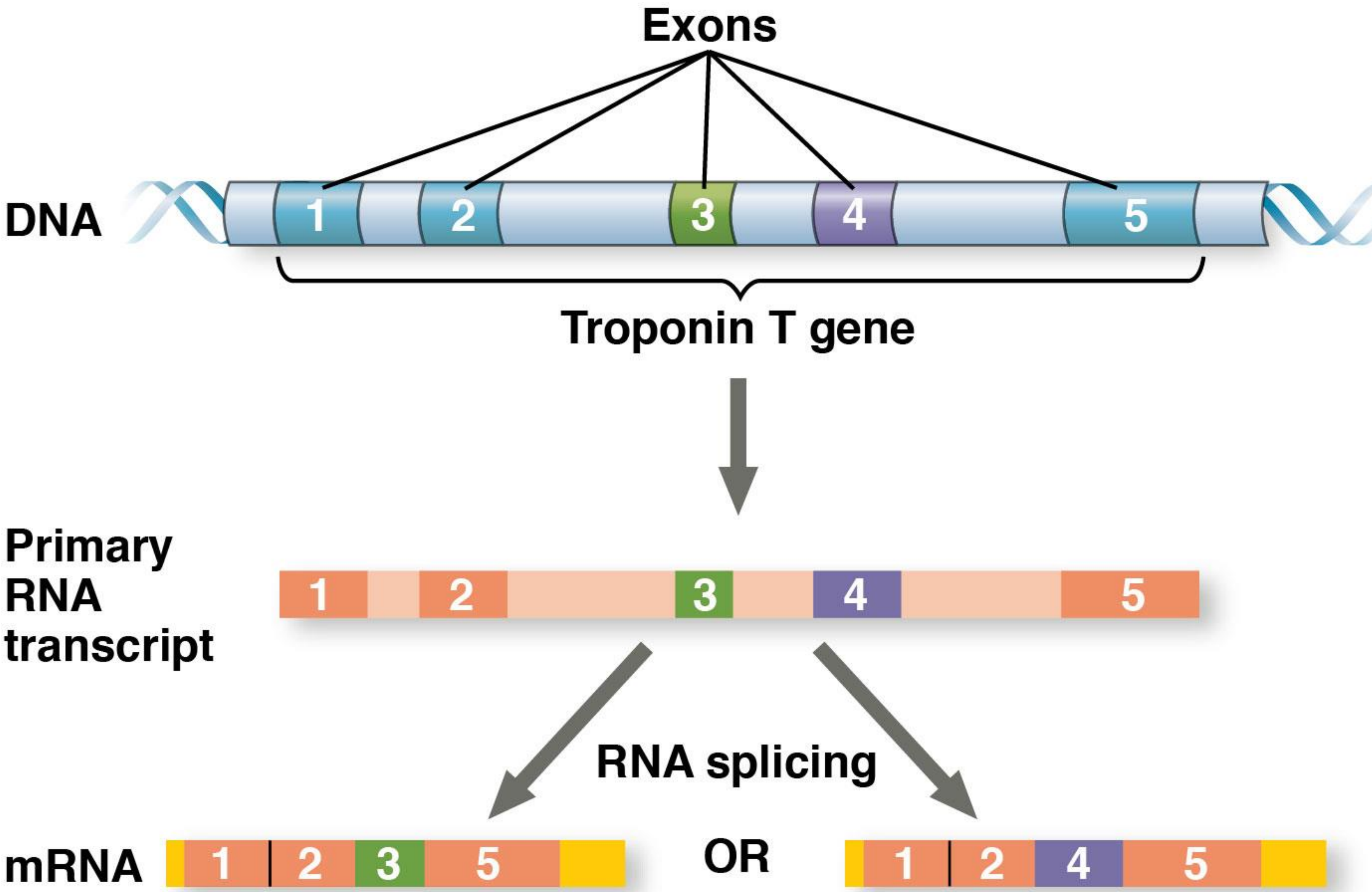
Activators or Repressors bind to enhancer regions + other proteins + RNA polymerase

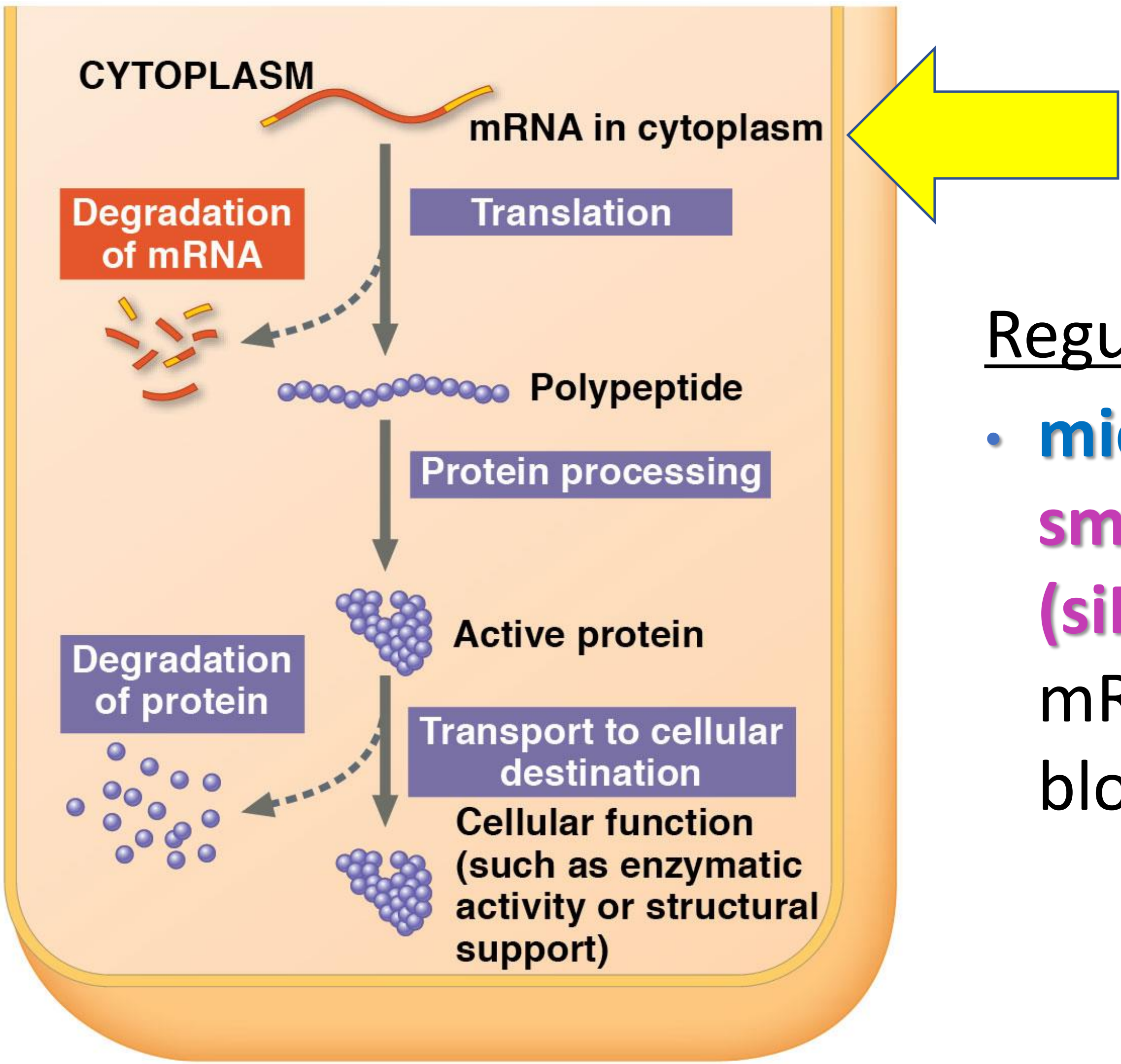


Cell type-specific transcription



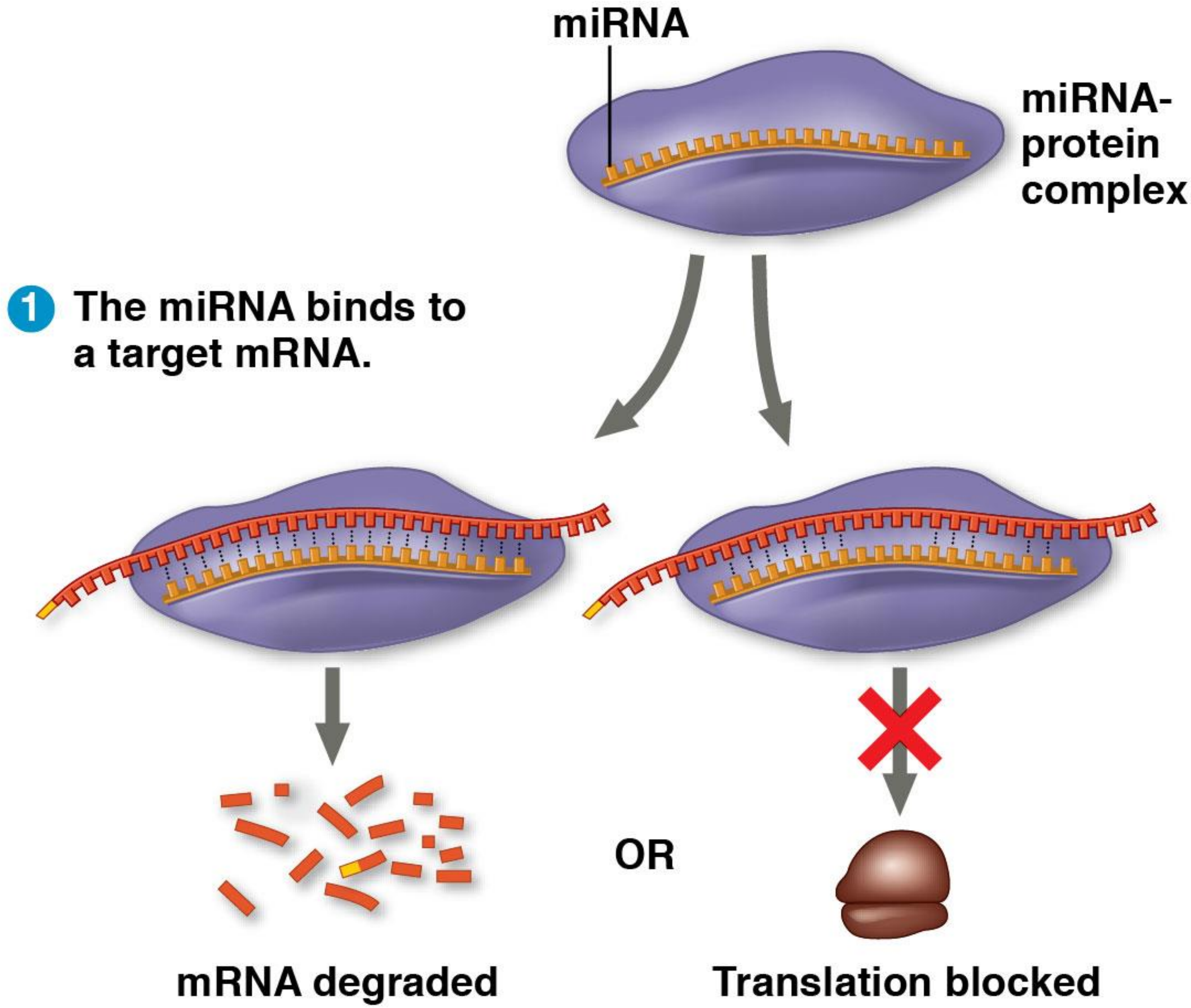
Alternative RNA Splicing





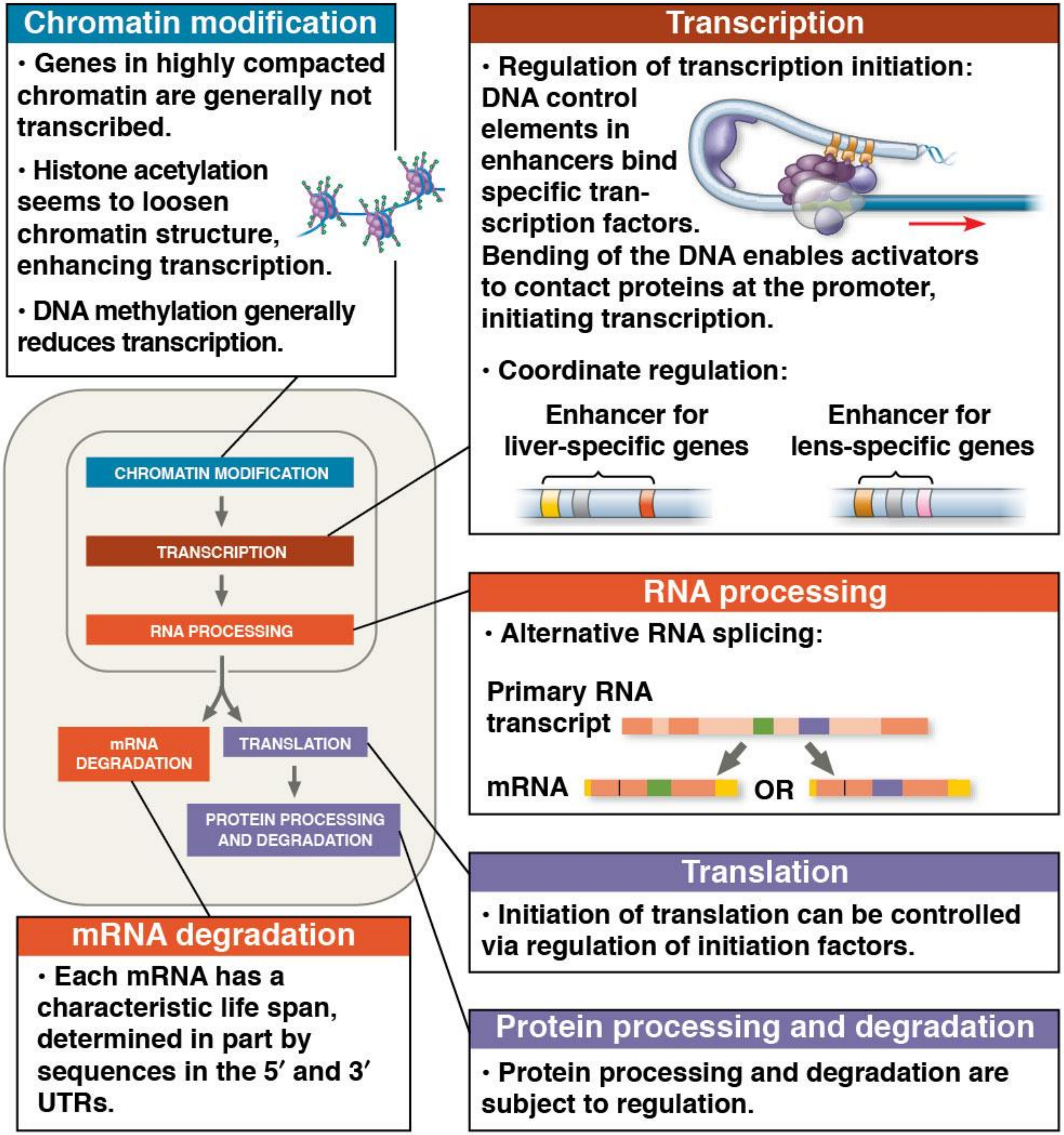
Regulation of mRNA:

- **micro RNAs (miRNAs)** and **small interfering RNAs (siRNAs)** can bind to mRNA and degrade it or block translation



2 If bases are completely complementary, mRNA is degraded. If match is less complete, translation is blocked.

Summary of Eukaryotic Gene Expression


Chromatin modification
• Genes in highly compacted chromatin are generally not transcribed.
• Histone acetylation seems to loosen chromatin structure, enhancing transcription.
• DNA methylation generally reduces transcription.
Transcription
• Regulation of transcription initiation: DNA control elements in enhancers bind specific transcription factors.
Bending of the DNA enables activators to contact proteins at the promoter, initiating transcription.
• Coordinate regulation:
Enhancer for liver-specific genes
Enhancer for lens-specific genes
CHROMATIN MODIFICATION
TRANSCRIPTION
RNA PROCESSING
mRNA DEGRADATION
TRANSLATION
PROTEIN PROCESSING AND DEGRADATION
RNA processing
• Alternative RNA splicing:
Primary RNA transcript
mRNA
OR
Translation
• Initiation of translation can be controlled via regulation of initiation factors.
mRNA degradation
• Each mRNA has a characteristic life span, determined in part by sequences in the 5′ and 3′ UTRs.
Protein processing and degradation
• Protein processing and degradation are subject to regulation.

Video: The Epigenetics of Identical Twins

- Genetic Science Learning Center

RNAi: Slicing, Dicing, and Serving Your Cells

- TED-Ed Video Clip

Researchers can monitor expression of specific genes

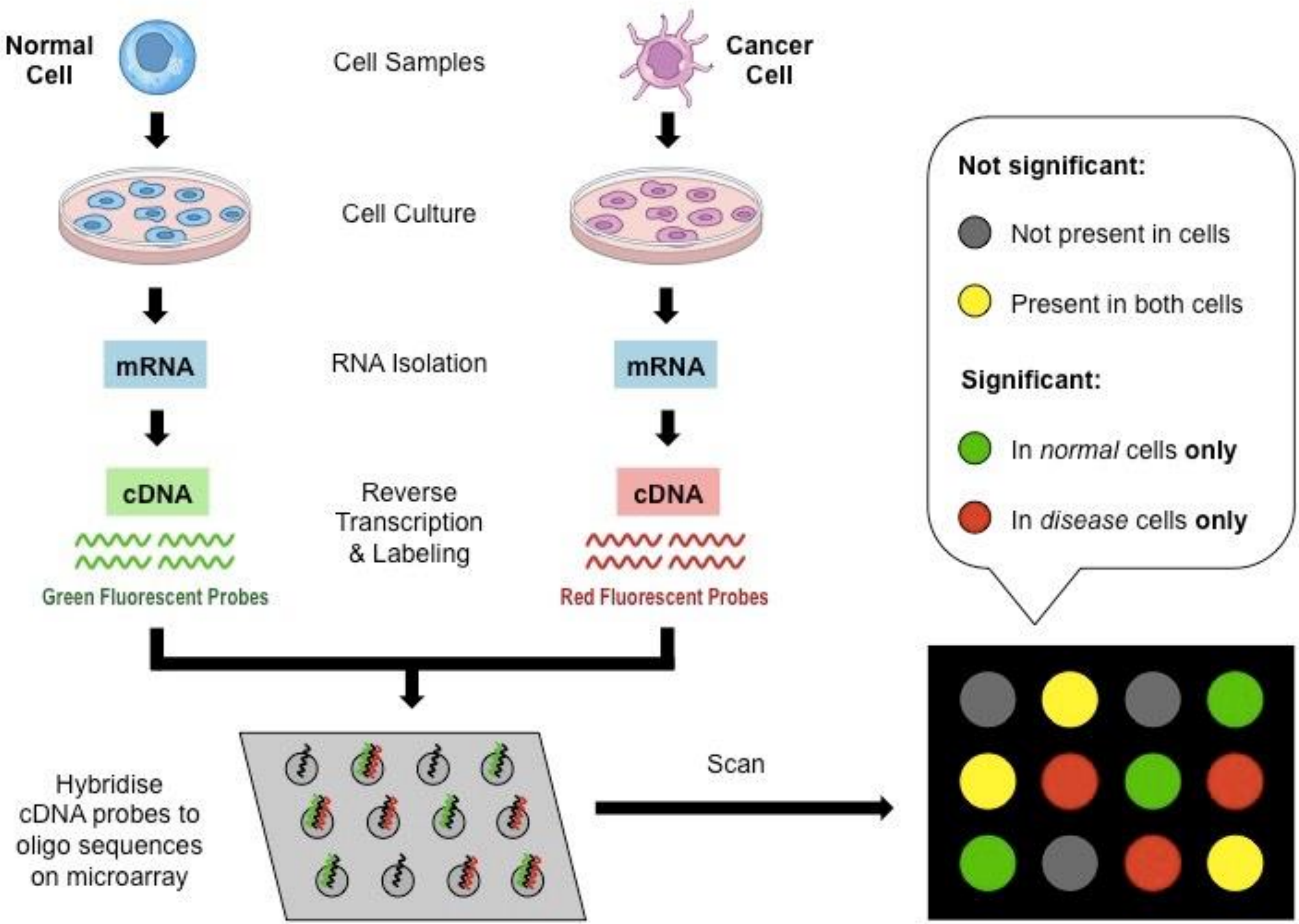
- Chapter 15.4

DNA Microarrays

Used to identify which genes are "on" in a cell → analyze expression of thousands of genes on chip

How it works:

1. Small amounts of single-stranded DNA (ssDNA) fragments representing different genes are fixed to a glass slide in a tight grid (*DNA chip*)
2. Tested cells: mRNA isolated and used to make cDNA using *reverse transcriptase*
3. cDNA bonds to ssDNA → indicates which genes are "on" in the cell


Normal Cell
Cell Samples
Cancer Cell
Cell Culture
mRNA
RNA Isolation
mRNA
cDNA
Reverse Transcription & Labeling
cDNA
Green Fluorescent Probes
Red Fluorescent Probes
Not significant:
Not present in cells
Present in both cells
Significant:
In *normal* cells **only**
In *disease* cells **only**
Hybridise cDNA probes to oligo sequences on microarray
Scan

Example: Identify differences in gene expression between breast cancer tissue vs. noncancerous breast tissue