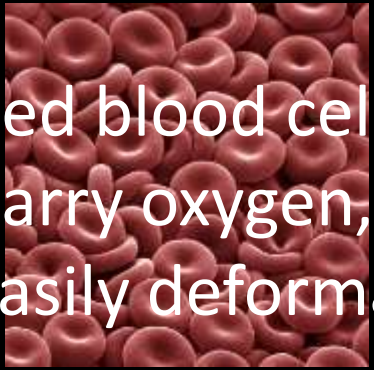
Class 6: Regulation of gene expression

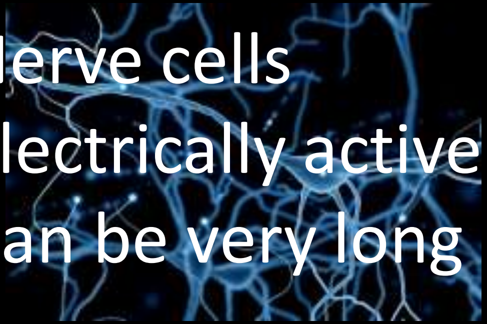
- Gene expression
- Switching a gene "ON" and "OFF"
- Metabolic pathways: two levels of regulation
  - Feedback inhibition and regulation of gene expression
- Concept of operon in prokaryotes
  - Features of *trp* operon
  - Features of *lac* operon

Different cells have different phenotypes

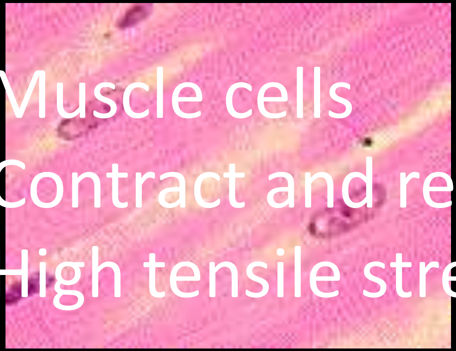


Red blood cells
Carry oxygen,
Easily deformable

$\sim 10^{13}$ cells



Nerve cells
Electrically active
Can be very long



Muscle cells
Contract and relax
High tensile strength

Respiratory cells
Exchange gases efficiently
Soft and spongy

But, one set of chromosomes is present in all cells

$\sim 10^{13}$ cells: all having the same genome

A part of the information stored in DNA is used by all cells

A part of the information is used only by specific types of cells…
Other cells never use this information!

Only a part of the information is used by each cell…

Each cell transcribes into RNA only a subset of the genes in the genome.

Why should each specialized cell copy all 46 chromosomes every time it divides?

Figure 1.6

How and why?

Question: HOW does a cell decode only the relevant information contained in chromosomes?

Answer: By **regulating** the transcription of genes (i.e., flow of information)

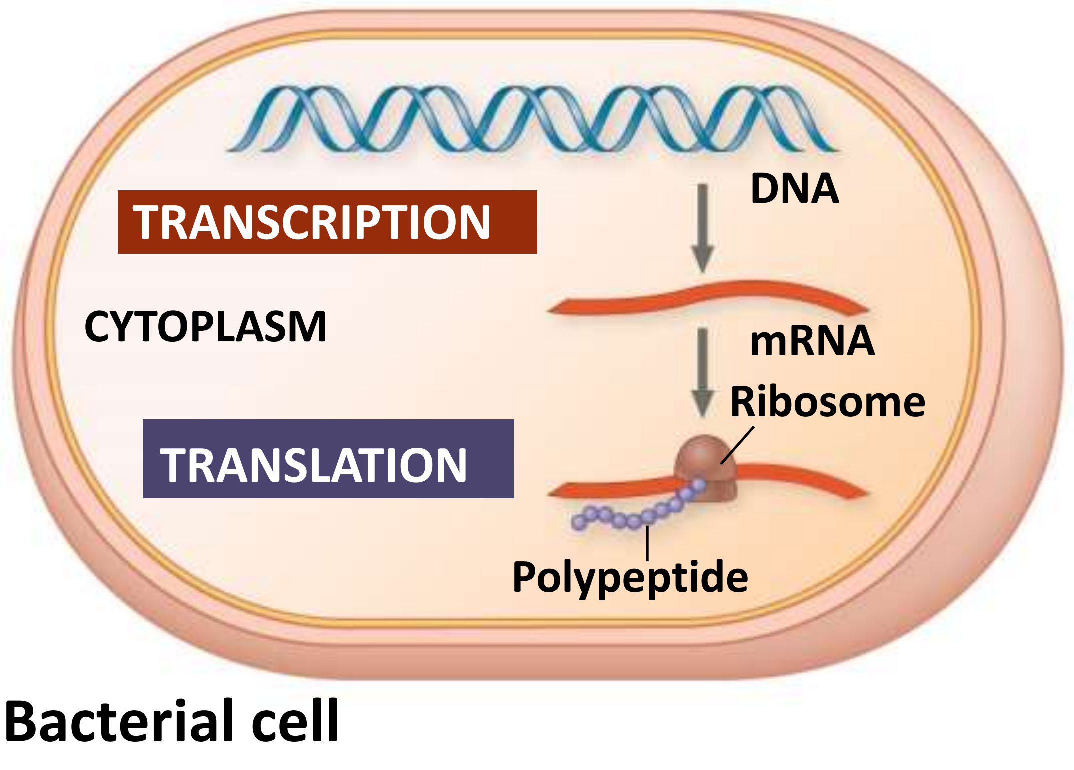
Question: WHY does a cell contain information that it never uses?

Answer: No idea…

One can speculate and come up with possible answers

Gene expression

Biosynthesis of the polypeptide (protein) encoded by a gene



"Gene expression" includes transcription and translation

Figure 17.3

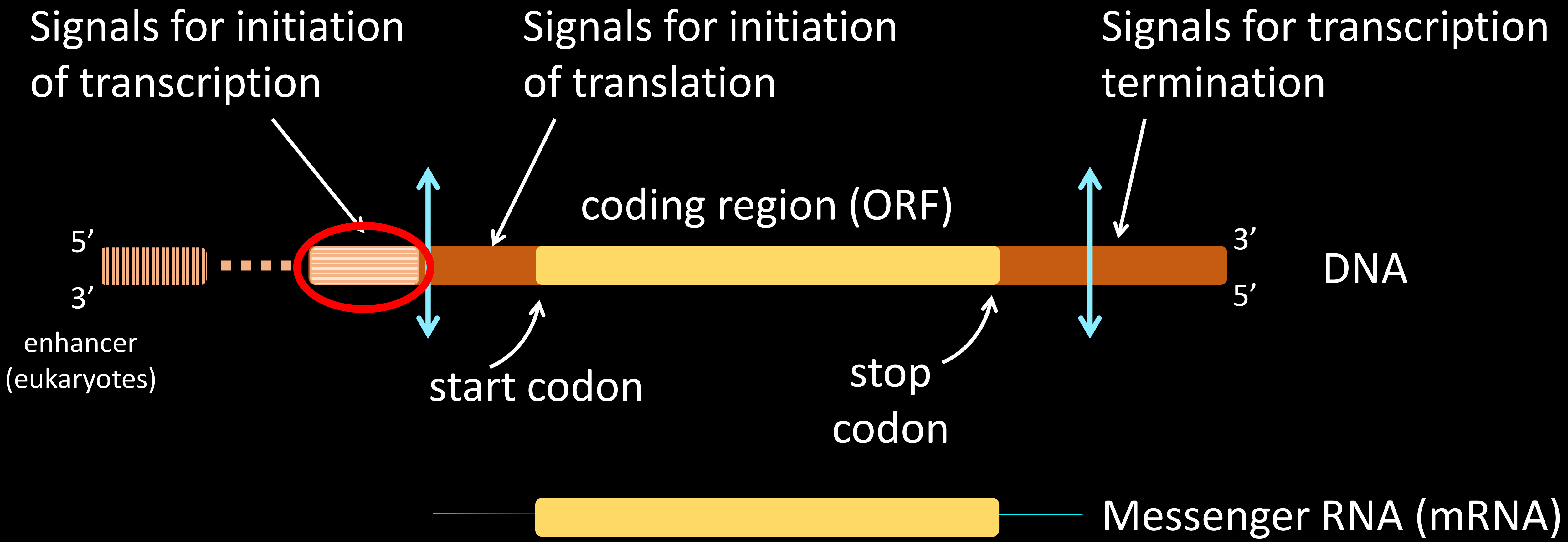
Open reading frame (ORF)

...CCTTTGGG	ATG	GAA	GCA	ACA	AAA	CCT	CAA	GAA	AGA	AAA	AGG	ACT	ATT
	M	E	A	T	K	P	Q	E	R	K	R	T	I

TTG	AGA	AGA	GTA	GCT	GTG	GTT	GTA	AAG	CAG	TAC	CCT	ACC	CTG	ATT	GTA
L	R	R	V	A	V	V	V	K	Q	Y	P	T	L	I	V
GGG	TCT	CAT	TTT	CTC	ACC	GAT	ATT	AGT	CCT	ACA	CCA	ATT	GAA	GTG	AAA
G	S	H	F	L	T	D	I	S	P	T	P	I	E	V	K
TTT	TGC	AGA	TGT	GCC	TAT	GAG	CAC	AAA	CTT	CTG	TGG	CAA	ATG	CCA	ATT
F	C	R	C	A	Y	E	H	K	L	L	W	Q	M	P	I
TTG	TTT	AAT	AAT	GTA	CCT	ATT	CCT	TCA	GAA	AGA	TGA	TACCCCA...			
L	F	N	N	V	P	I	P	S	E	R	-				

The primary structure of a protein is its amino acid sequence. Likewise, the primary structure of a DNA/RNA is its nucleotide sequence. Shown above is a fragment of DNA represented by its nucleotide sequence. Note that it is a linear polymer wherein nucleotides are covalently liked to each other. In the above representation, a blank space is included before/after every codon merely to facilitate reading / show clearly which trinucleotide is forming a codon. As you know, if the reading frame changes, a different set of three nucleotides will form a codon.

Gene: stretch of DNA that encodes a protein



In this class, we are interested in this portion of the gene

Gene: stretch of DNA that encodes a protein

A gene includes the entire sequence represented in mRNA

start codon

stop codon

$$n_{nt} = 3 \times n_{aa} + 3$$

no. of nucleotides in an ORF

no. of amino acids in the protein

Human genome has ~ 30,000 gene
Primate genomes have ~ 30, 000 genes

Is gene part of DNA? Or, is DNA part of a gene?

"DNA" can mean two different things, depending upon the context

(1) "*DNA of an organism*" means the entire "*genetic content*" of the organism

In this context, DNA contains genes; in fact, many genes

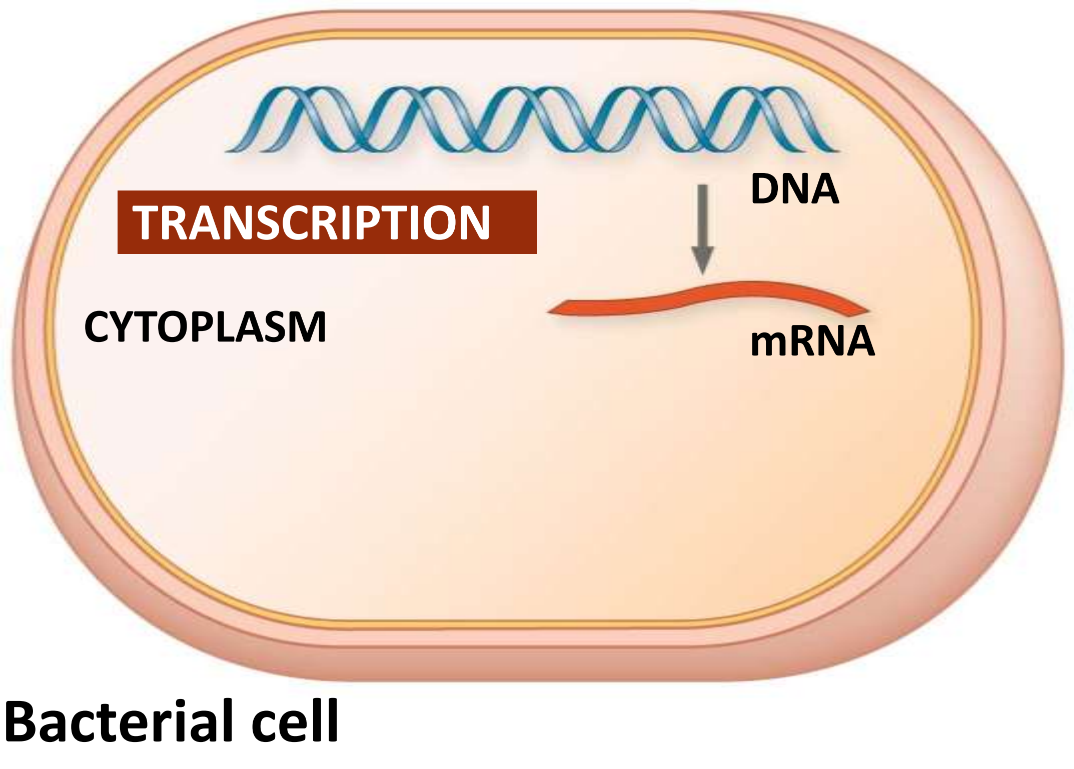
(2) DNA can also mean the two strands of a double helix

In this context, genes are made of DNA

Figure 16.1
Biology. A global approach

Transcription in prokaryotes

Transcription: synthesis of RNA using information in DNA

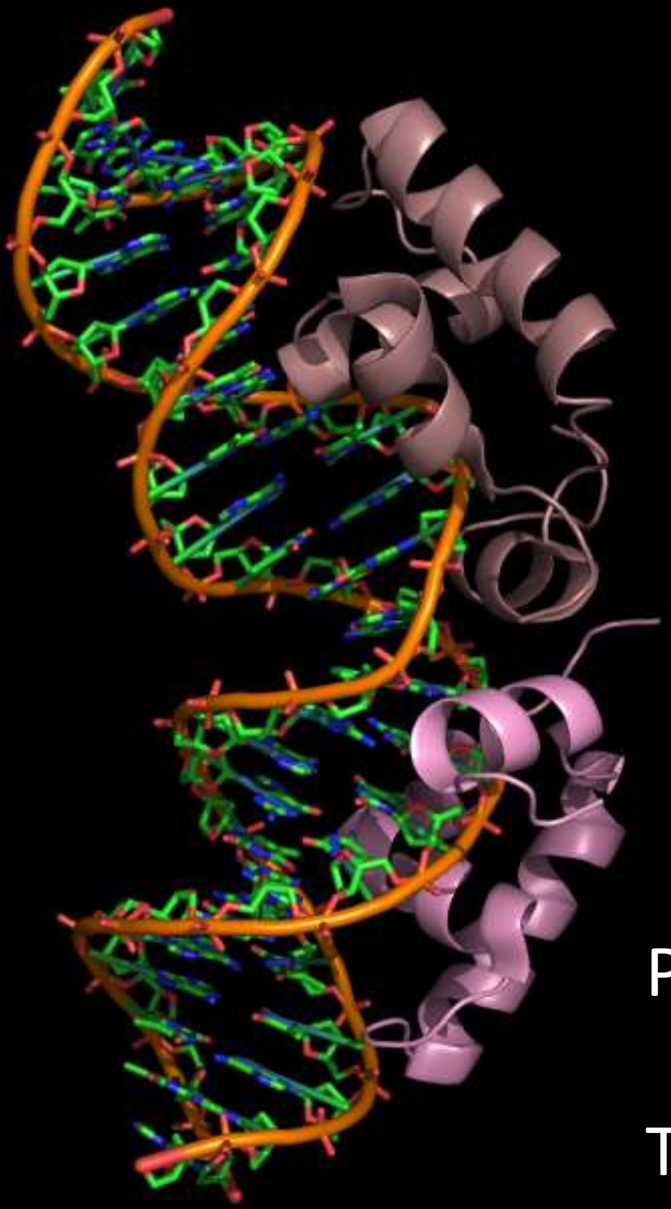


RNA: bridge between genes and encoded proteins

Transcription produces messenger RNA (mRNA)

Figure 17.3

Where does transcription start? Promoter

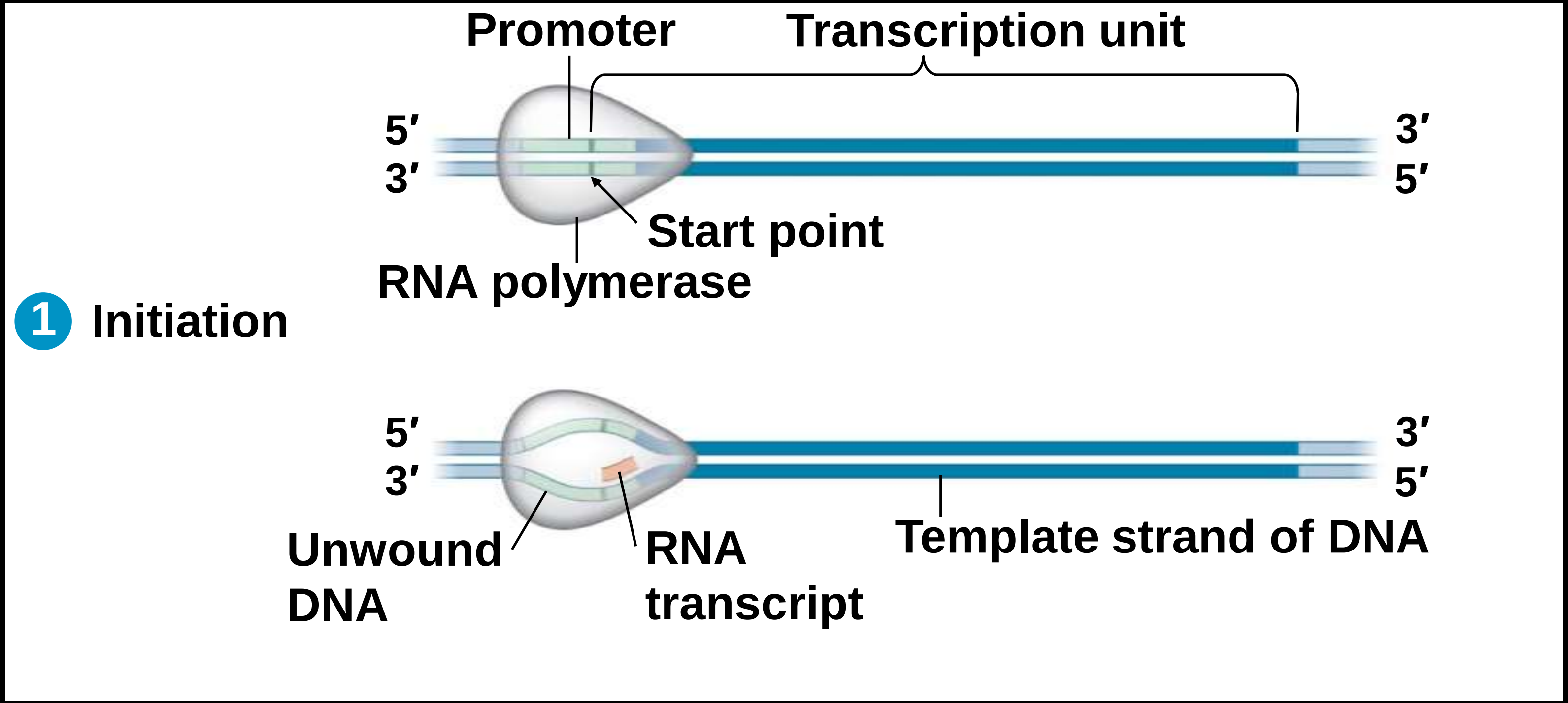


- Promoter is a region or segment of DNA
- The nucleotide sequence is what characterizes a promoter
- In terms of structure, promoter also has double helical structure

Protein complex bound to DNA at the promoter

The two units of the protein are shown in different shades

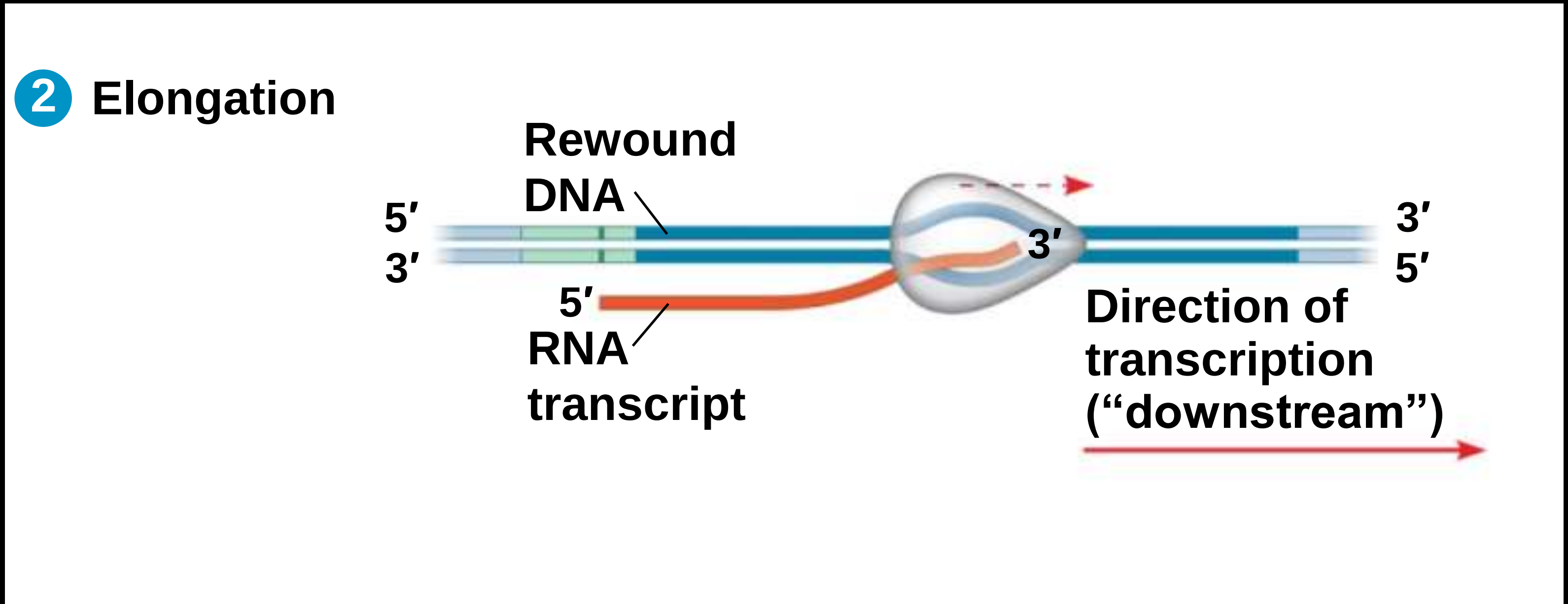
Transcription. Step 1 of 3



- RNA polymerase binds to the promoter
- DNA unwinds
- Synthesis of the RNA transcript begins

Figure 17.7

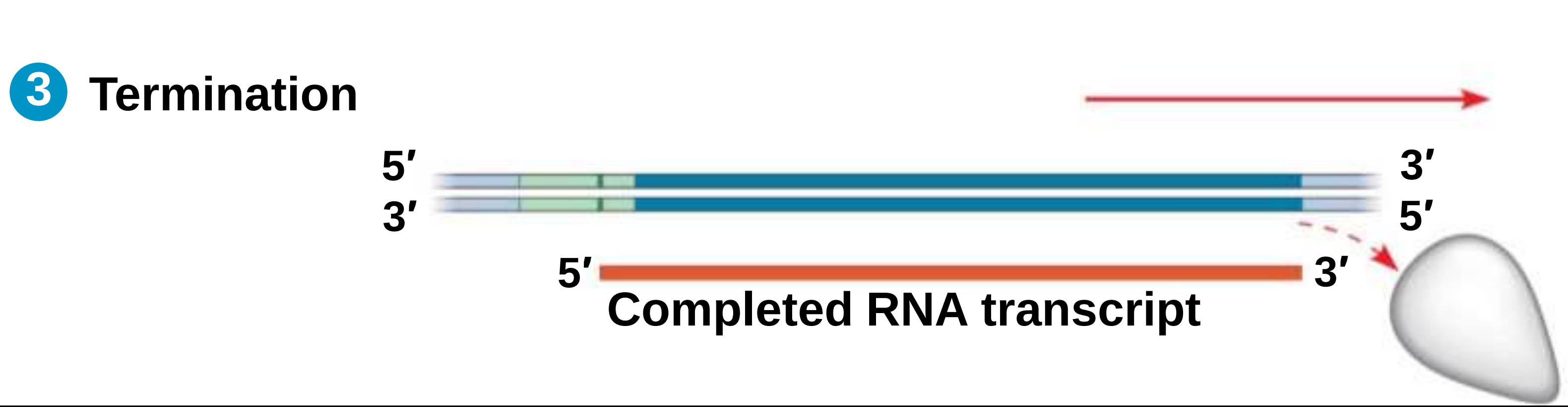
Transcription. Step 2 of 3



- RNA polymerase moves downstream
- DNA keeps unwinding
- RNA transcript grows
- Unwound DNA, after the message is transcribed, winds back

Figure 17.7

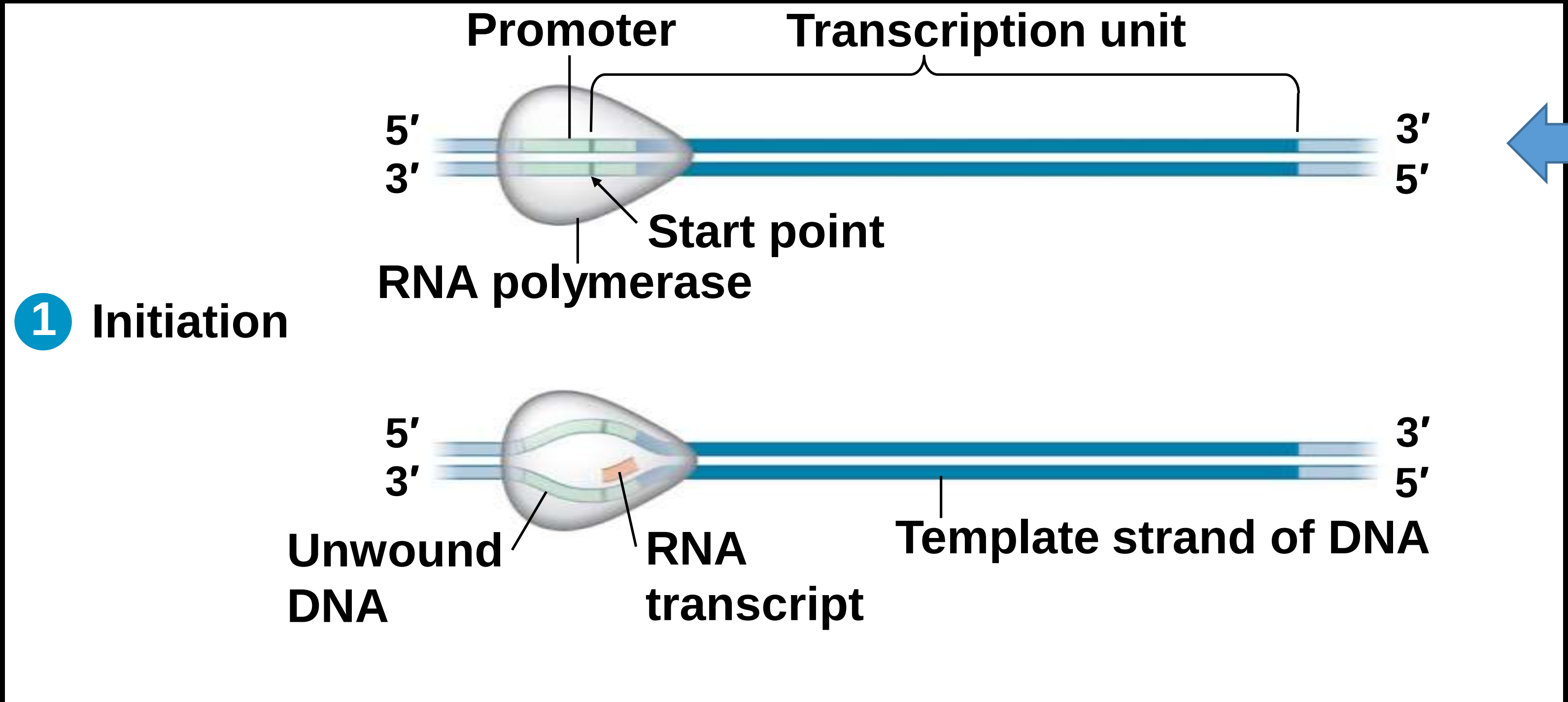
Transcription. Step 3 of 3



- RNA polymerase reaches the termination site
- Synthesis of the RNA transcript ends
- RNA polymerase “falls off”
- Completed RNA transcript is also released

Figure 17.7

How is transcription regulated?



“Go” or “no go”

- A major strategy for regulating transcription is to either make mRNA or not
- This decision is made at the step of initiation

Class 6: Learning objectives

- Gene expression
- Metabolic pathways: two levels of regulation
  - Feedback inhibition and regulation of gene expression
- Concept of operon
  - Features of *trp* operon
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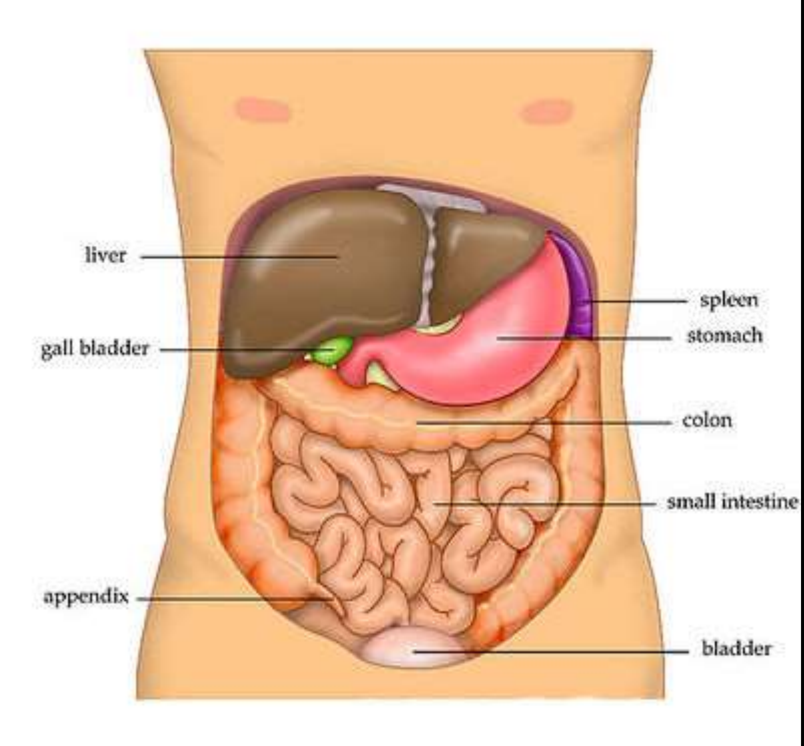
Responding to environmental cues



https://microbewiki.kenyon.edu/index.php/Metabolic_disorders_associated_with_the_human_gut_microbiota

- *E. coli* inhabits human gut
- Tryptophan is an amino acid found in proteins

Gut *E. coli*

Tryptophan available → Absorb and utilize

Tryptophan not available → Express requisite genes → Biosynthesize

Regulating product formation

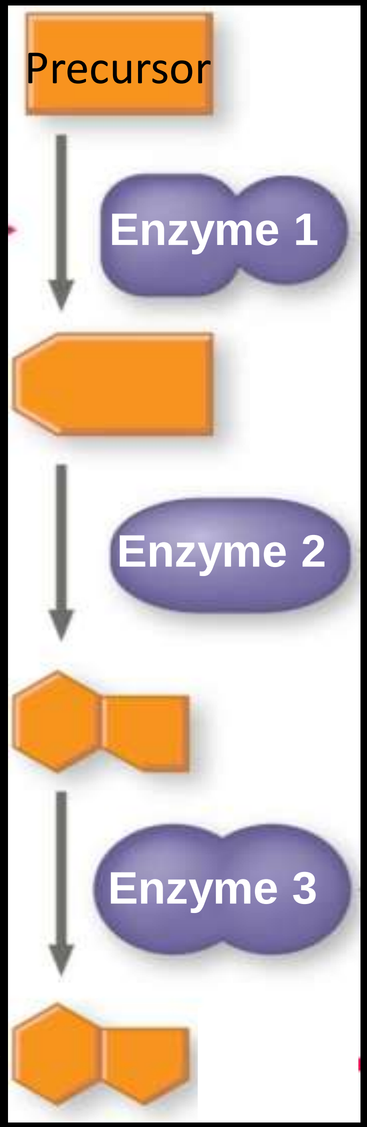
- *E. coli* produce only proteins needed by that cell

(this is a similar idea to the expression of muscle genes only in muscle cells)

- *E. coli* can regulate the production of tryptophan by
  (A) feedback inhibition and
  (B) regulating gene expression

Tryptophan biosynthesis pathway

Precursor



Tryptophan

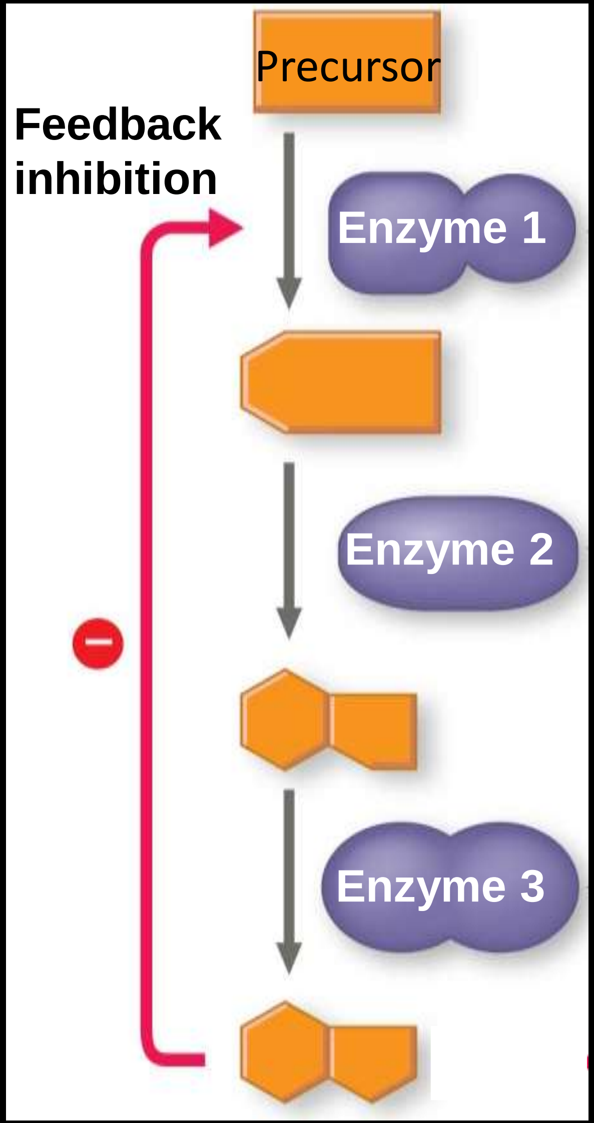
Mode of regulation #1:
Feedback inhibition

Where have you seen something similar?
Arginine biosynthesis pathway

Figure 18.2

Feedback inhibition

Regulation of enzyme activity



Tryptophan

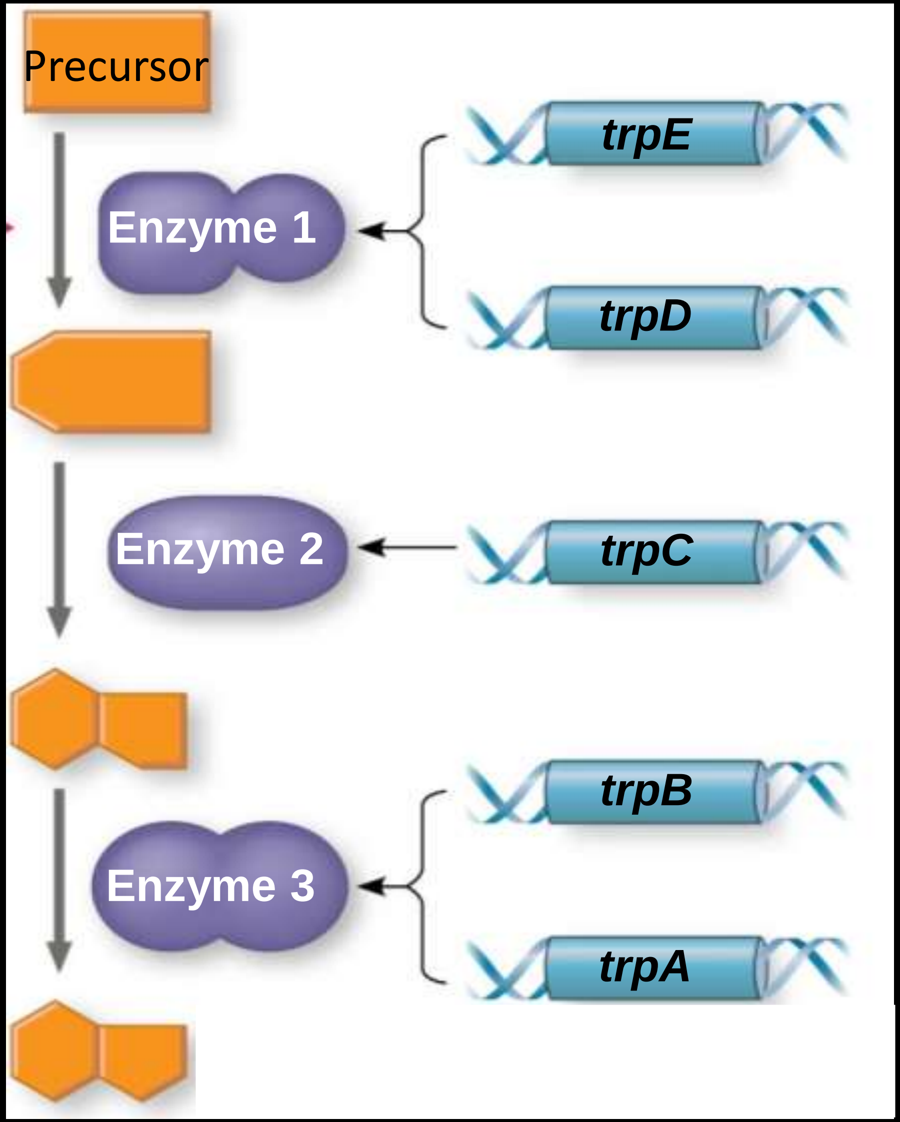
End product of the metabolic pathway inhibits the enzyme that catalyzes the first reaction in the pathway

Fast
as well as
reversible
response

Figure 18.2

Regulate the genes encoding tryptophan biosynthesizing enzymes

Precursor



Tryptophan

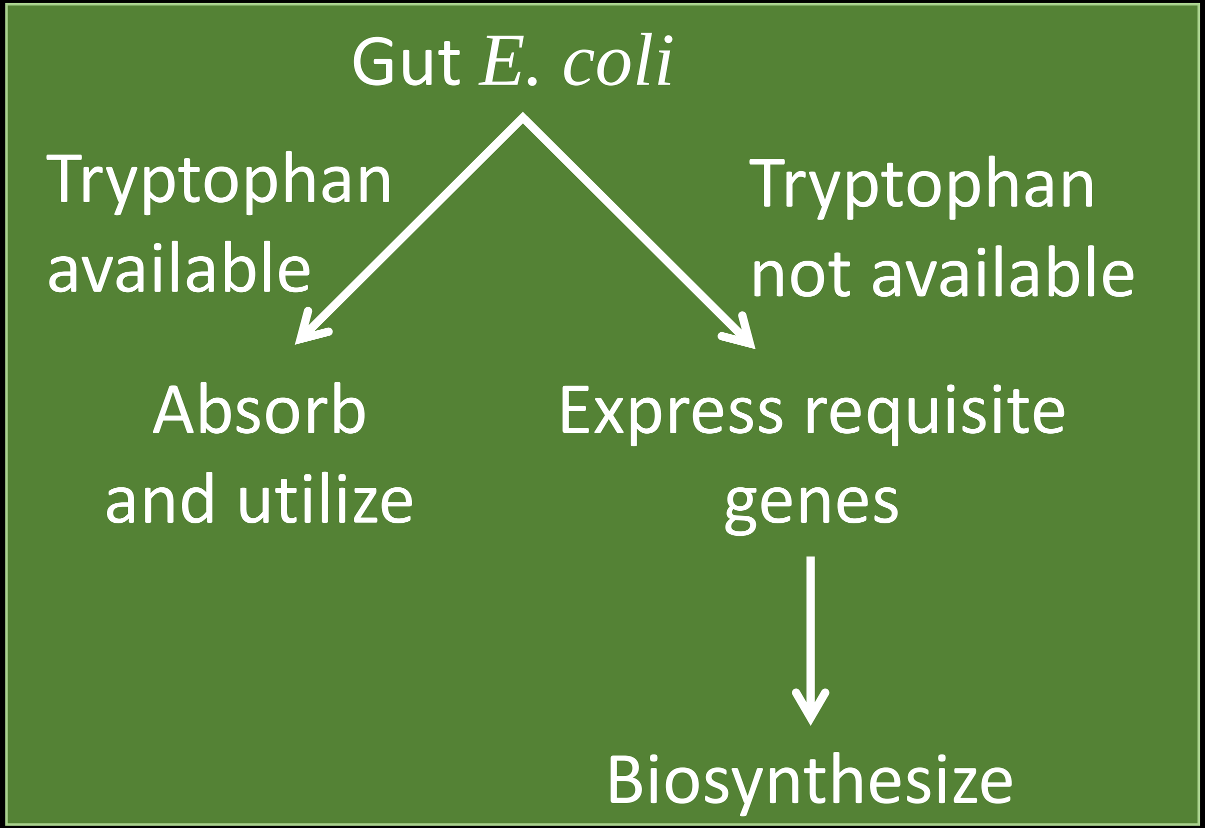
Mode of regulation #2: Regulation of gene expression

What could be the benefits/disadvantages of the two modes of regulation?

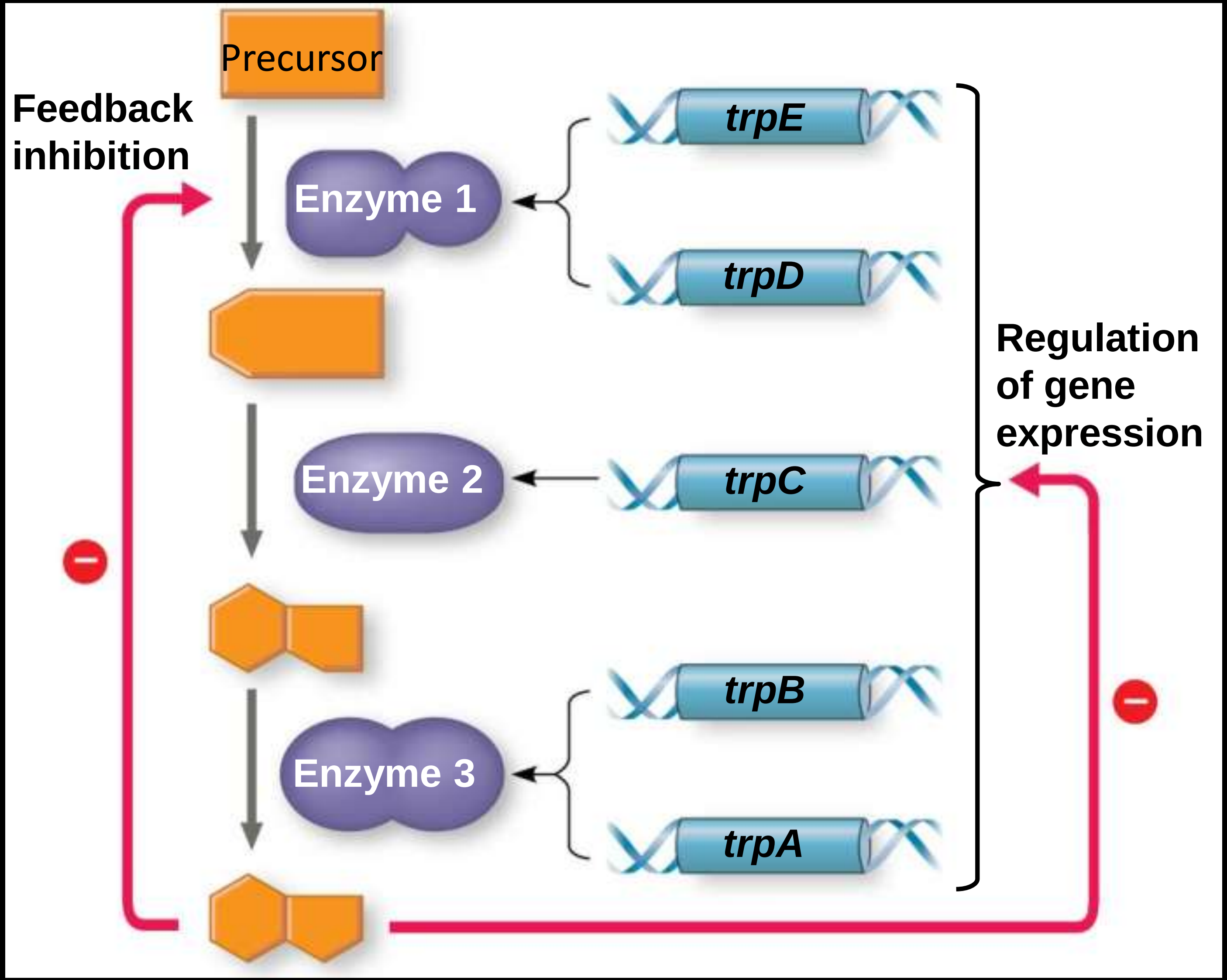
Figure 18.2

Coordinated switching on-off

- Suppose tryptophan is to be synthesized
- Switch on the expression of all five genes (trp A, B, C, D, E)
- When sufficient tryptophan is made, turn off all five genes



Regulation of a metabolic pathway



Regulation of enzyme activity

Regulation of enzyme production

Tryptophan

HOW?

Figure 18.2

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Operons were studied by Jacob & Monod

1965 Nobel Prize in Physiology or Medicine

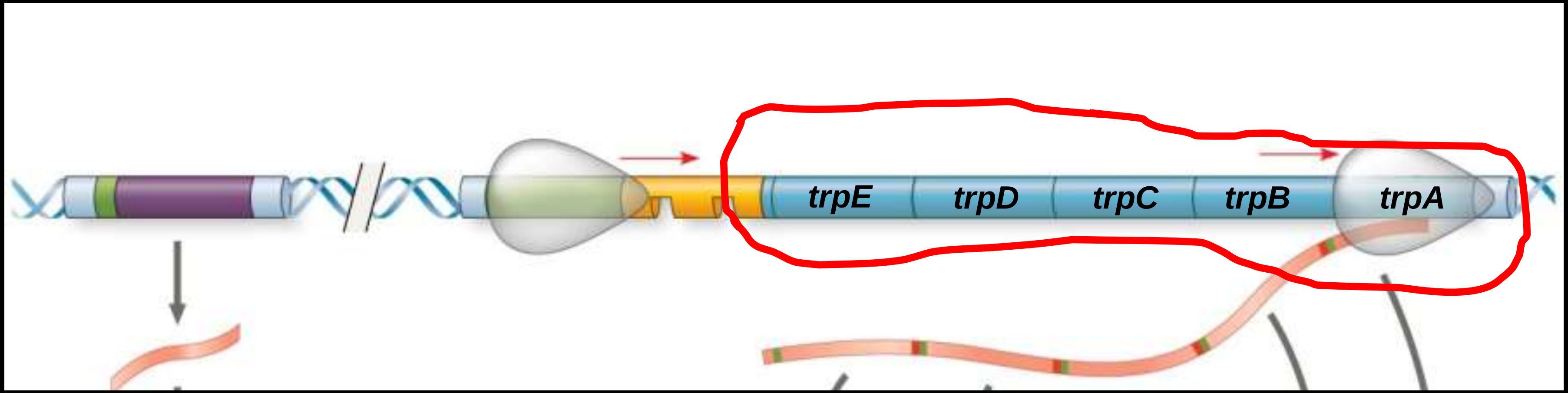
Jacques Monod

Francois Jacob

We will be looking at regulation of gene expression in bacteria, but the basic principles are true for humans too

Clustering of the five genes

Cluster of five genes whose protein products are tryptophan biosynthesis enzymes



These five genes are clustered together on the bacterial chromosome

Together, these five genes constitute a SINGLE transcription unit

A single promoter serves all five genes

The promoter drives the synthesis of one RNA with five ORFs

Figure 18.3

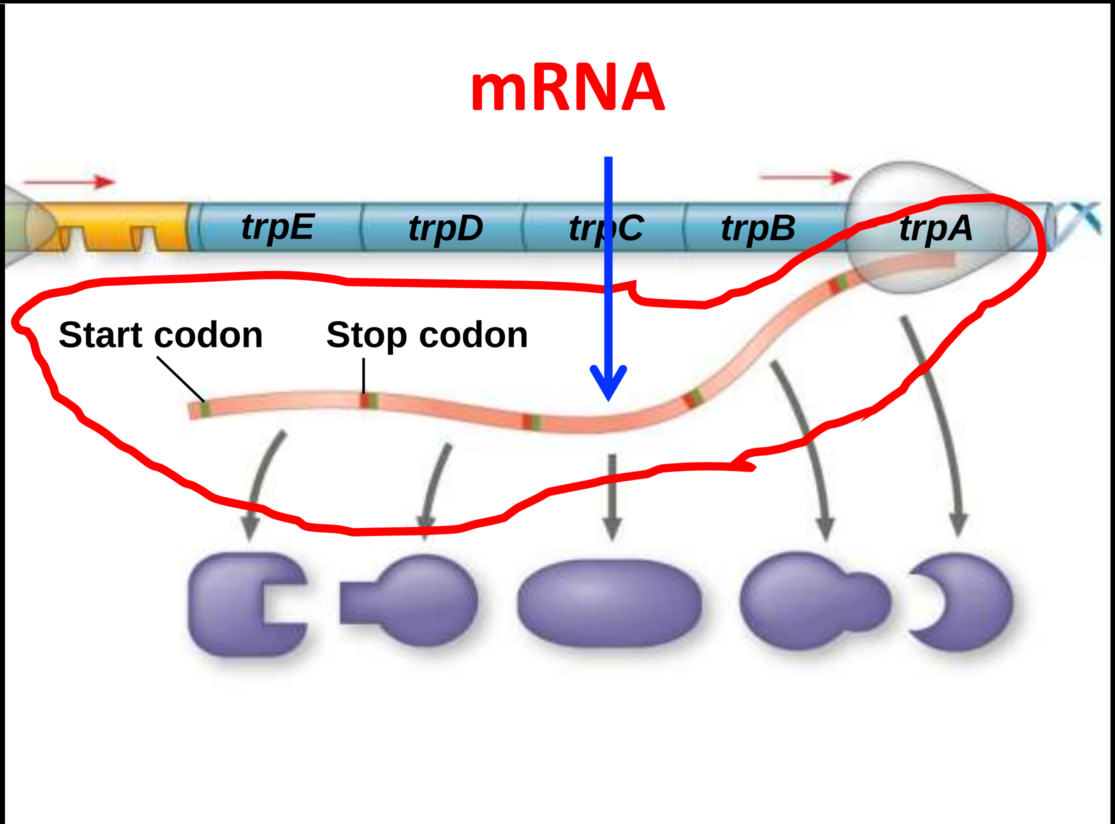
Coupled expression of genes in bacteria
Transcription linked to translation

Transcription results in a single mRNA

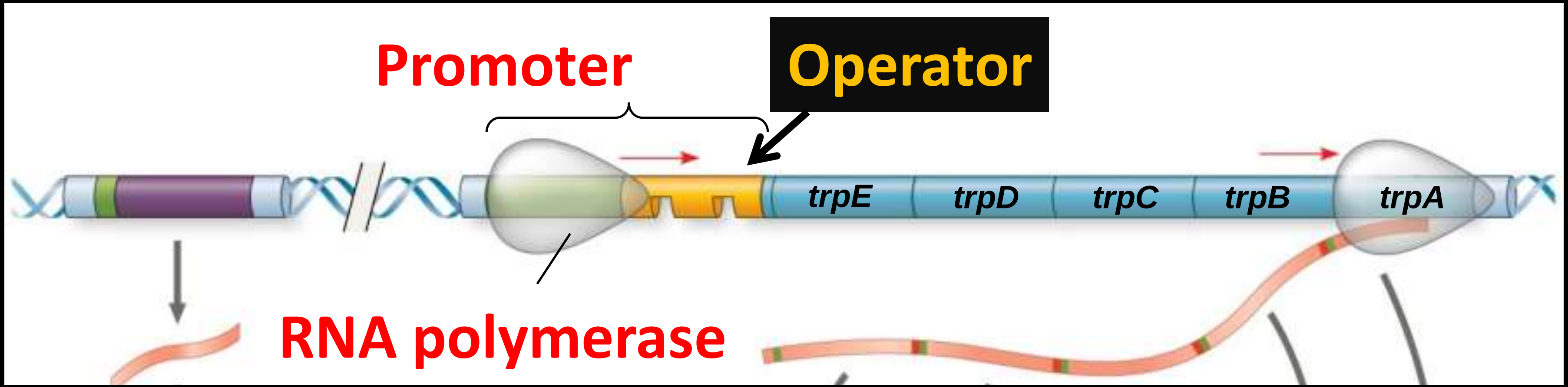
The entire mRNA is translated

Five separate polypeptide chains are formed

Separate start and stop codons for each polypeptide chain



Operator: the single on-off switch



Operator is a stretch of DNA sequence

It is located between the promoter and the *trp* genes

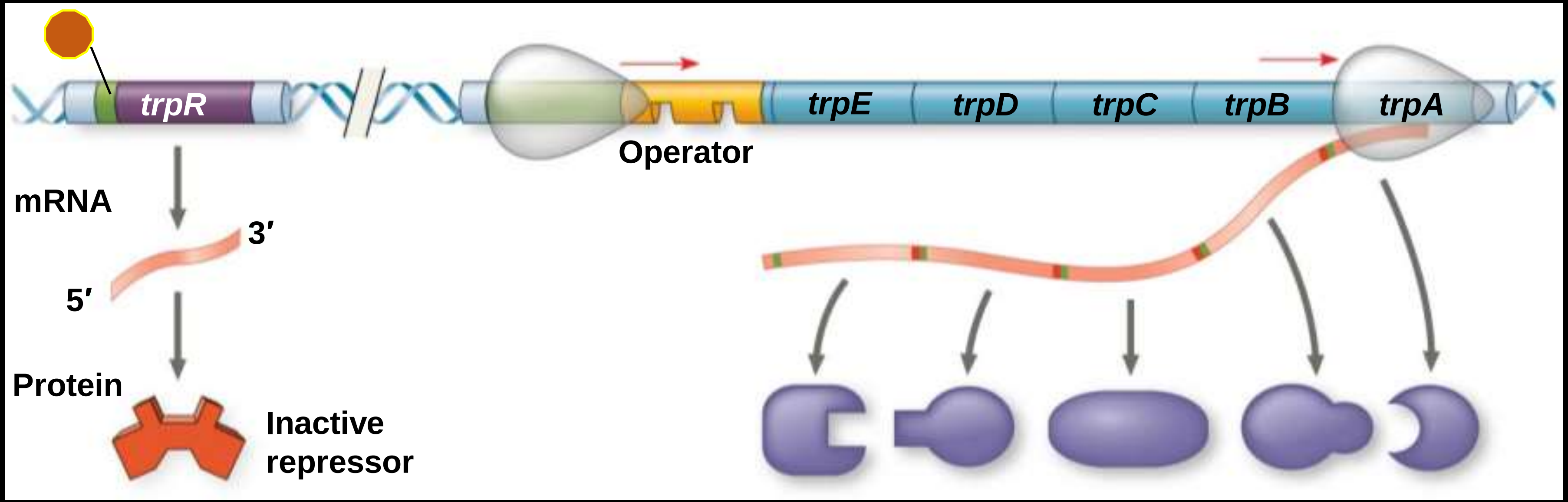
Promoter + operator + genes = operon

Role of operator in regulating gene expression?

Figure 18.3

Concept of repressor

trpR: Gene encoding the repressor; it is a regulatory gene



trp repressor
(inactive)

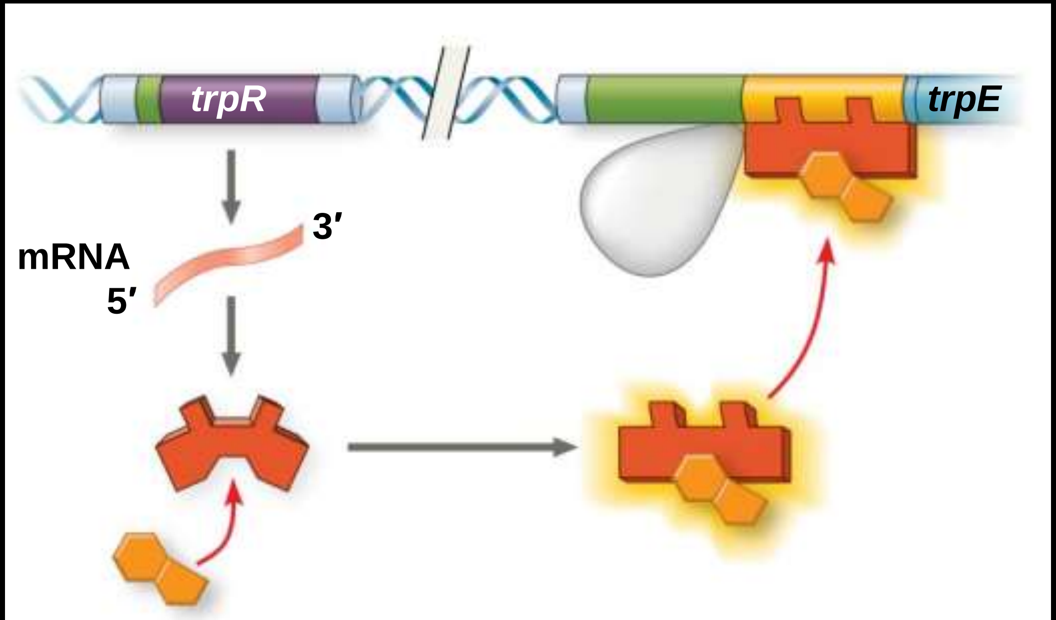
Promoter for *trpR*

This is the situation for “no tryptophan”

How does the repressor work?

Only tryptophan-bound repressor can bind to the operator region

Binding of tryptophan changes the shape of the repressor



Trp repressor (inactive)

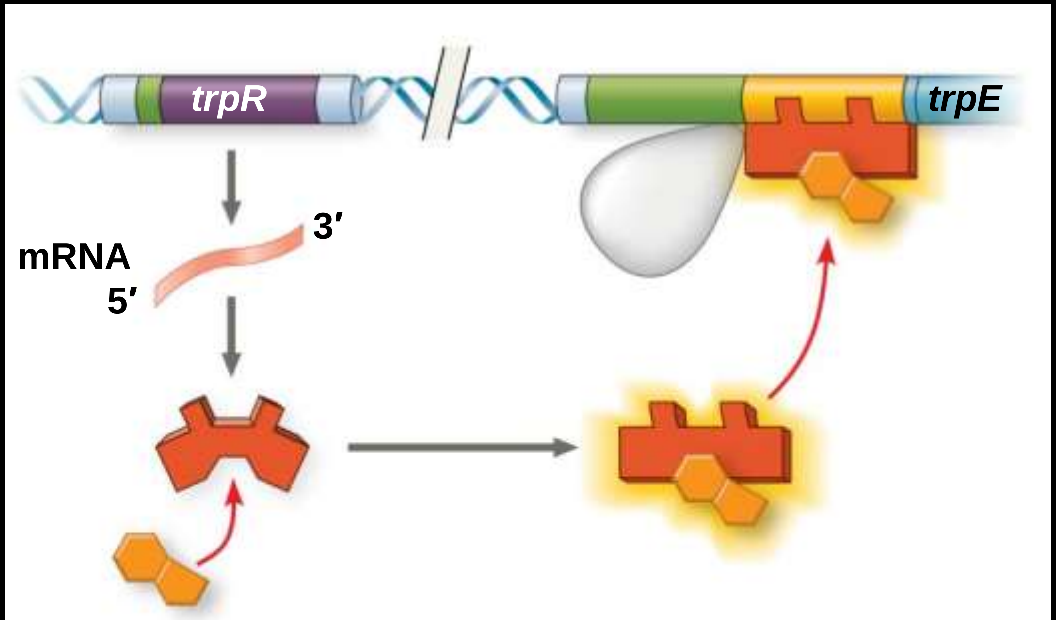
RNA polymerase is stalled if the operator region is bound by the repressor

Tryptophan (co-repressor)

Trp repressor (active)

How does the repressor work?

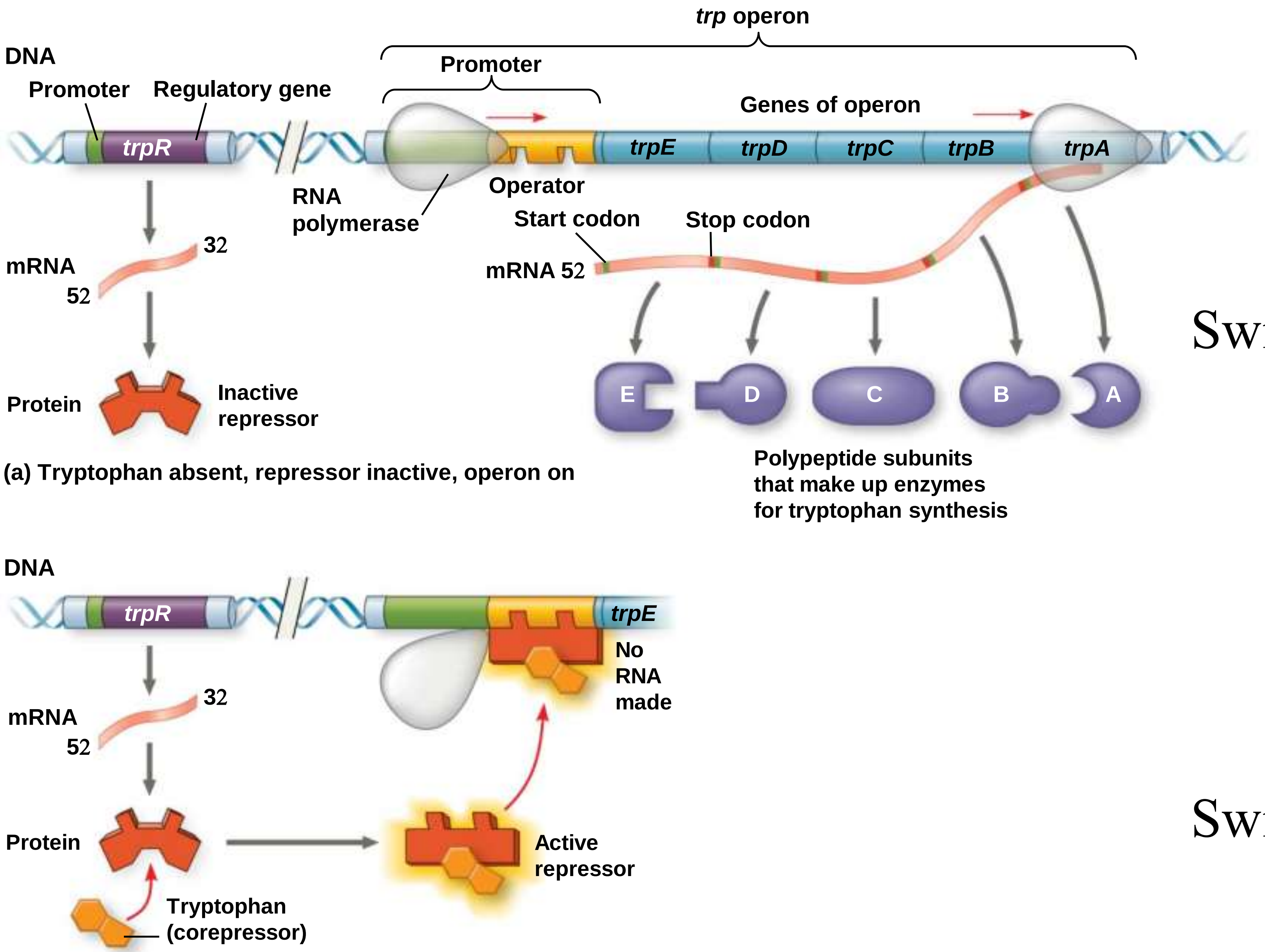
Trp repressor is specific to *trp* operator



Binding of Trp repressor to the operator is reversible

Trp repressor (inactive) $\xrightarrow{\text{tryptophan available}}$ / $\xleftarrow[\text{tryptophan not available}]{}$ Trp repressor (active)

Figure 18.3



(a) Tryptophan absent, repressor inactive, operon on

(b) Tryptophan present, repressor active, operon off

Switch is ON

Switch is OFF

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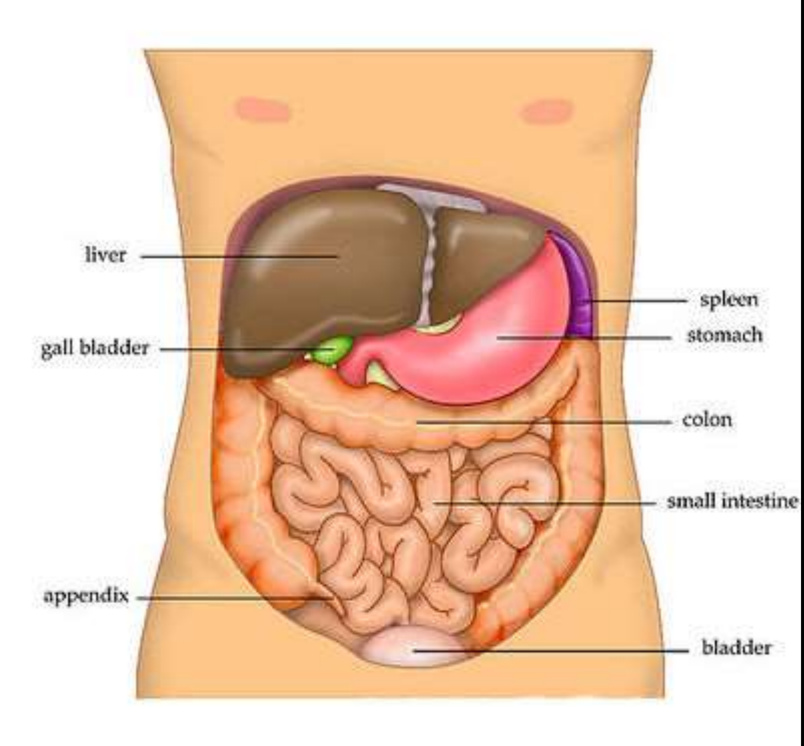
The *lac* operon



https://microbewiki.kenyon.edu/index.php/Metabolic_disorders_associated_with_the_human_gut_microbiota

- *E. coli* inhabits human gut
- Disaccharide lactose is available from milk

Gut *E. coli*

Lactose available

Lactose not available

Absorb

β-galactosidase gene repressed

hydrolysis

Express β-galactosidase

glucose + galactose

Induction of the *lac* operon

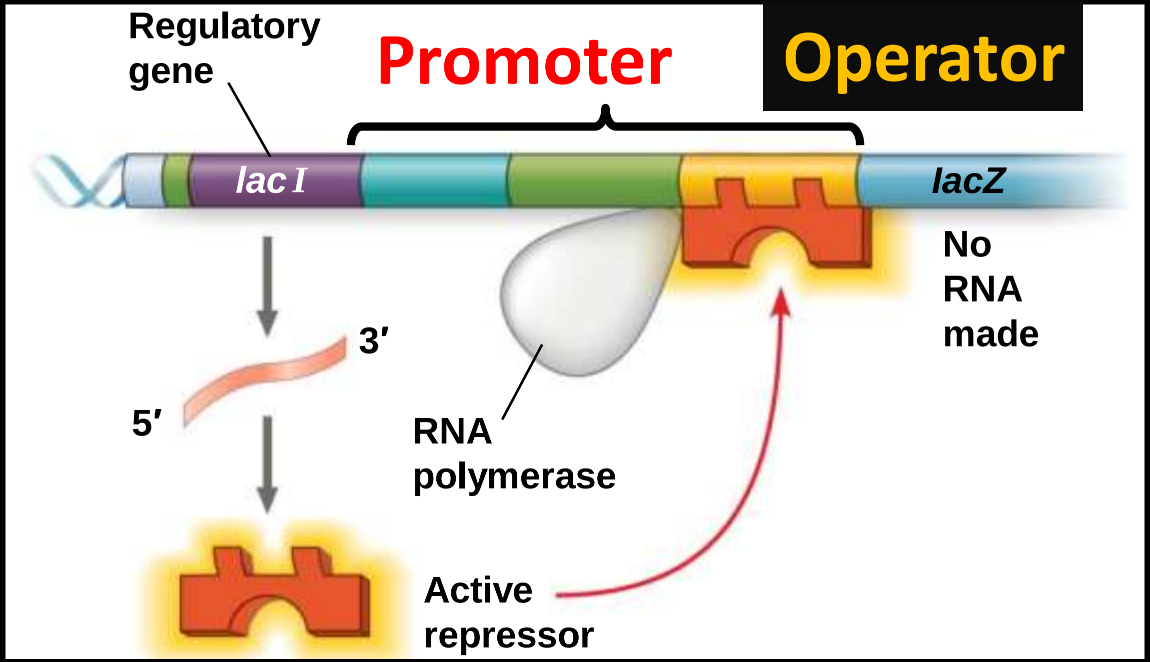
Lactose absent	Only a few molecules of β-galactosidase
Lactose present	1000-fold increase in the number of β-galactosidase Time taken: about 15 minutes

Is the *lac* operon similar to the *trp* operon?

Induction of the *lac* operon

regulatory gene

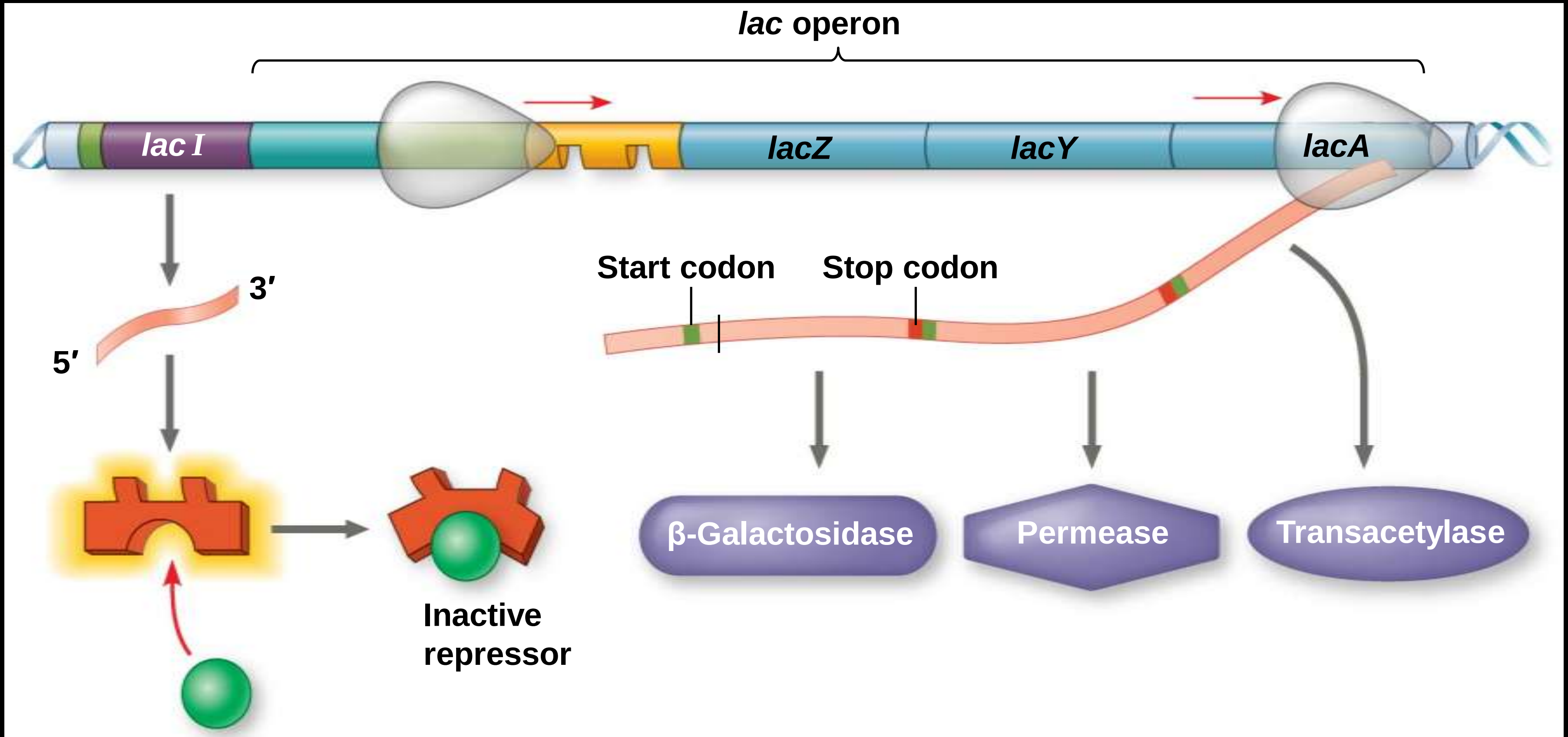
lacI gene



repressor
(active)

Figure 18.4

Induction of the *lac* operon



Allolactose (inducer)

Binding of inducer changes the shape of the repressor

Figure 18.4

trp and *lac* operons – two designs

***trp* operon**	***lac* operon**
Repressible operon	Inducible operon
“usually” on	“usually” off
Repressor inactive by itself	Repressor active by itself
Tryptophan: co-repressor	Lactose: inducer
Typical: biosynthetic pathway enzymes	Typical: degradation pathway enzymes
Regulation: negative	Regulation: negative

Negative regulation: binding of repressor shuts down gene expression

Summary: *trp* and *lac* operons

Gene expression

Promoter
Operator

Regions
of DNA

Repressor
Activator

DNA binding
proteins

Tryptophan (co-repressor)
Allolactose (inducer)

Small molecule
modulators

Summary: *trp* and *lac* operons

These are examples of genetic switches!

Repressor

Binding of the small molecule modulator (co-repressor)

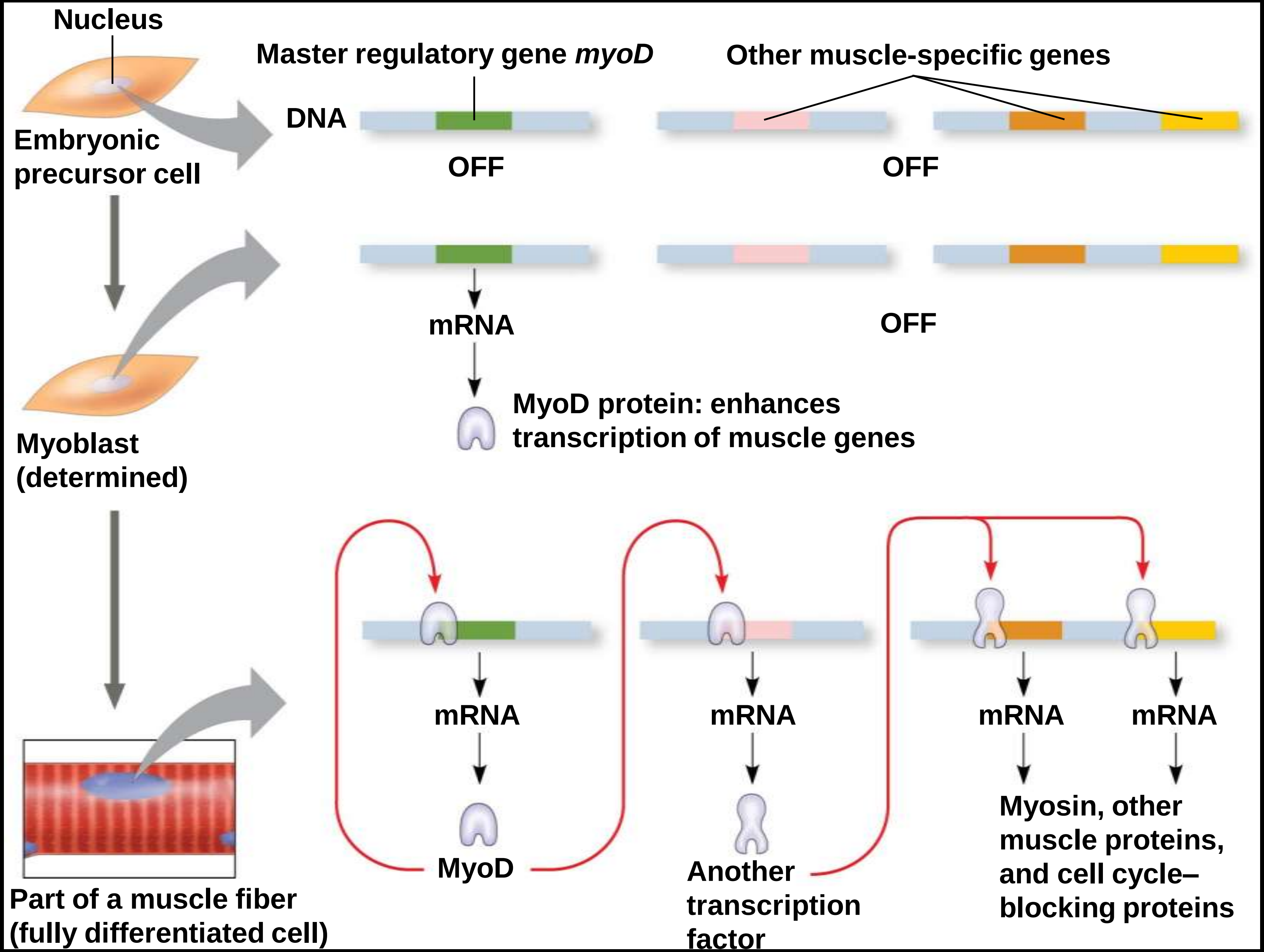
Repressor binds to operator

Repressor

Binding of the small molecule modulator (inducer)

Repressor CANNOT bind to operator

How do muscle cells get their phenotype?



Boeing 737-800 cockpit control room with many switches: cells are more complex

http://www.aviafilms.com/free-airplane-pictures.php

There is evidence that monkeys and humans differ not due to proteins, but how much protein is being made (regulation of gene expression)

Two selfies