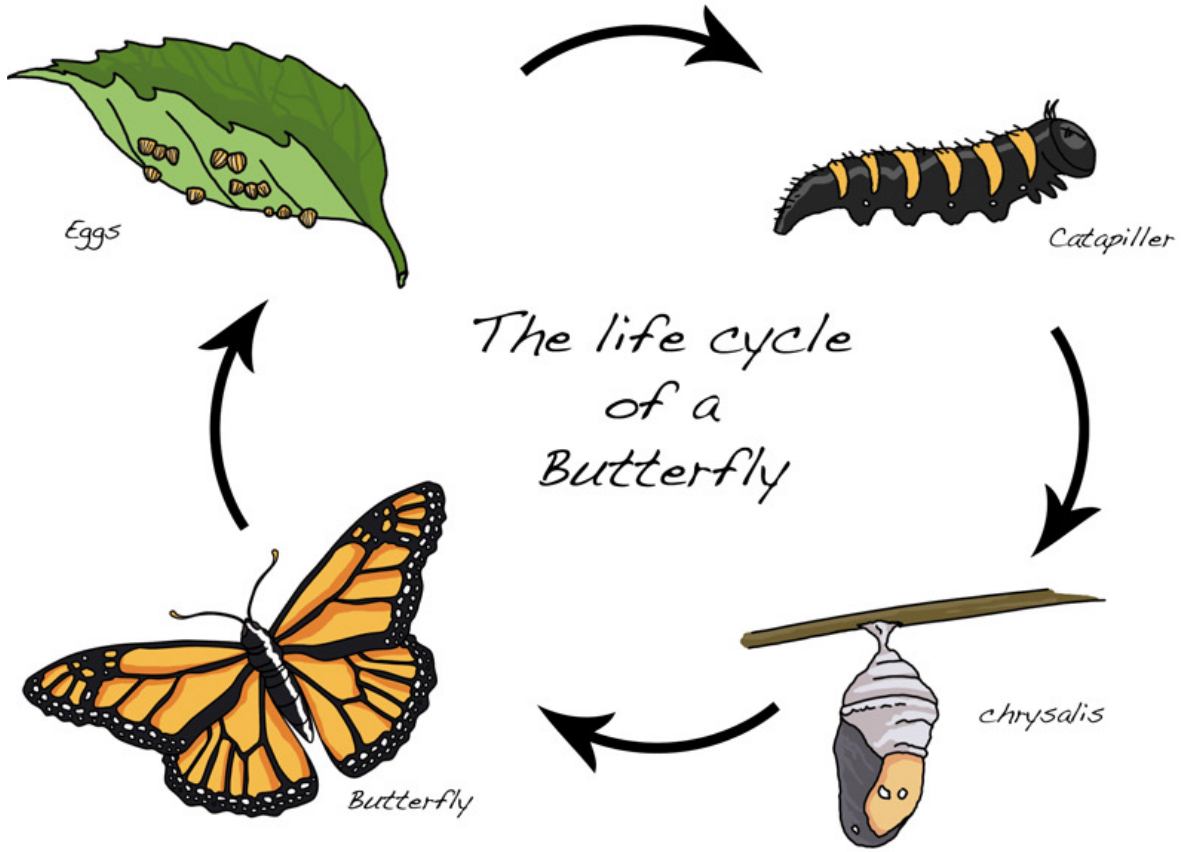
Does having something means using it ALWAYS?



Does DNA stored in a cell remain the same or change in different stages?

https://lovecraftianscience.files.wordpress.com/2013/12/mac_life_cycle_butterfly_shawnfischer_com.jpg

Mobile apps and features

Do we use all apps always?

Do we use all features always?

http://mobile.itechind.com/

Development of a multi-cellular organism from a zygote

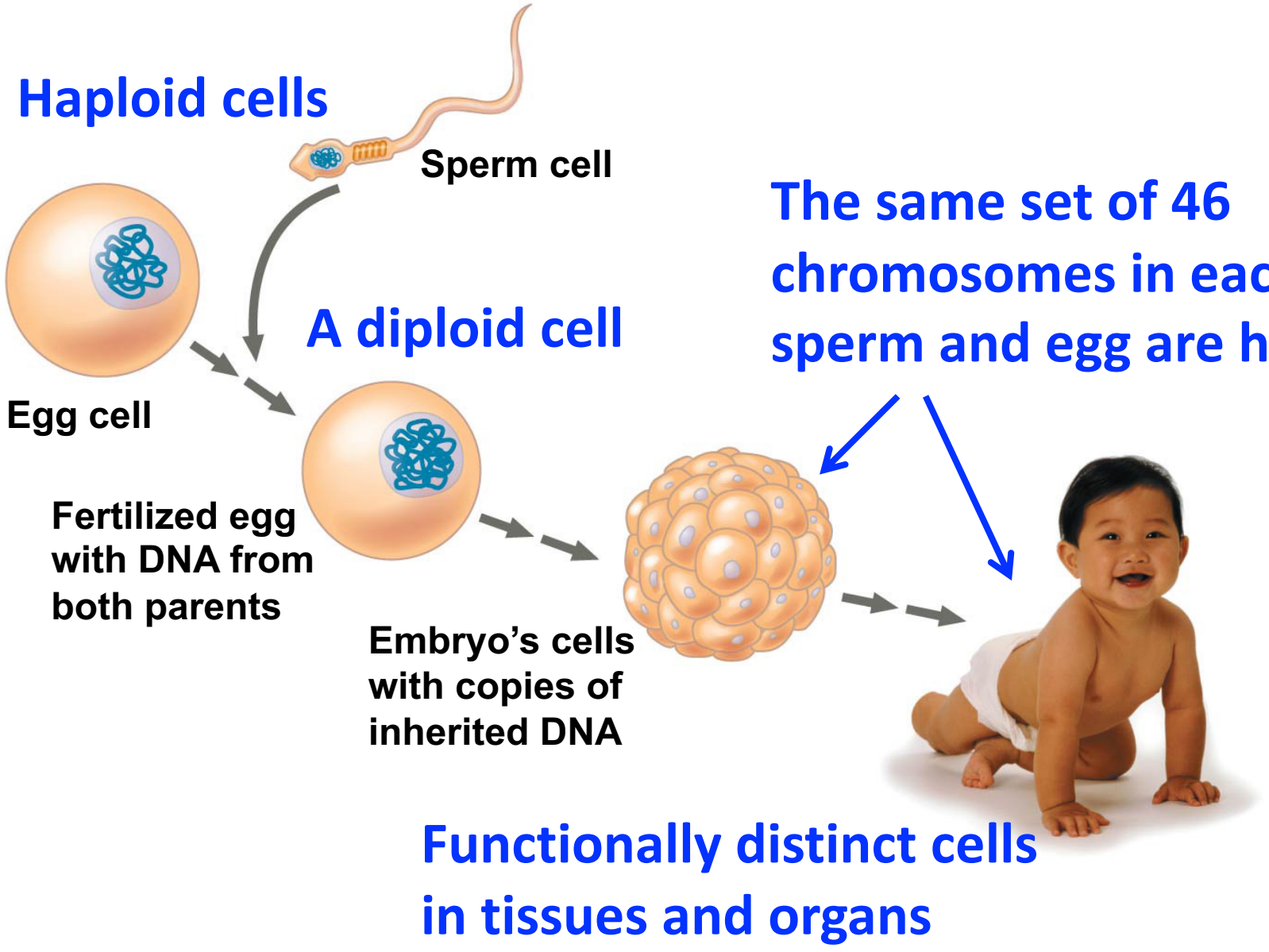
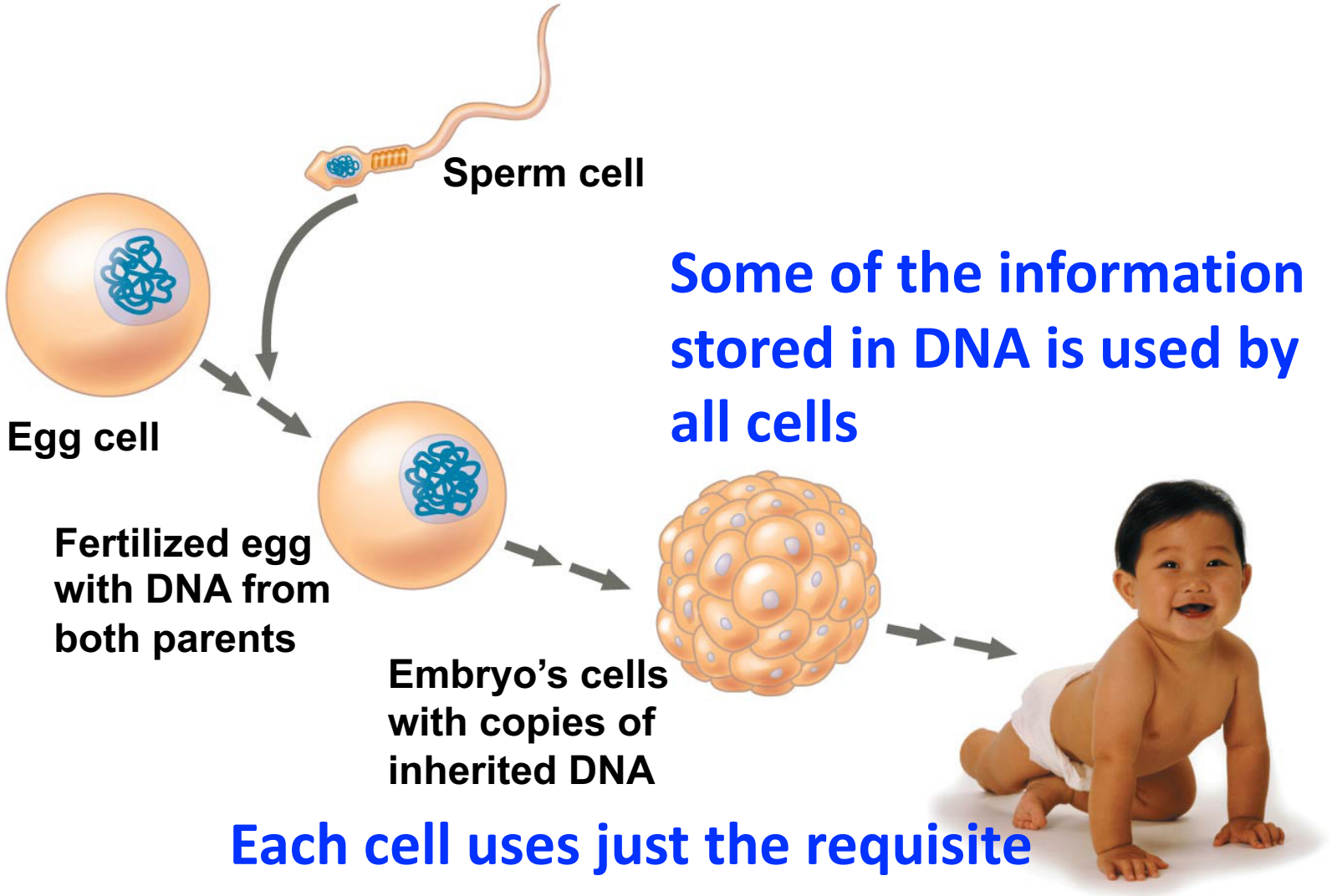


Figure 1.10 in Campbell Biology by Reece et al., (10th edition)

Development of a multi-cellular organism from a zygote



Some of the information stored in DNA is used by all cells

Each cell uses just the requisite information; rest of the information is not meant to be used!

Figure 1.10 in Campbell Biology by Reece et al., (10th edition)

Library collection and pattern of usage

Do all students of IIT Bombay read all the books in IIT Library?

Image courtesy:
Sri Manju Naika
Central Library
IIT Bombay

Library collection and pattern of usage

General books	All patrons (i.e., users) of a library
Specialized books	Only specialists

Is mother Nature dumb?

- Cells of human body are constantly re-generated – old cells are replaced by new ones of the same type
- Frequency of such replacements varies with cell type
- Each such replacement cycle involves making a copy of all 46 chromosomes
- If a cell already knows what its “specialization” is, then why store and copy information that it never (?) uses?

Why and how?

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

Answer: No idea…

We don't even know if information is NEVER used!

One can speculate and come up with possible answers

Why and how?

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

Answer: No idea…

We don‘t even know if information is NEVER used, for that matter!

One can speculate and come up with possible answers

Nothing in biology makes sense except in the light of evolution

Remember, there are trade-offs in evolution

Theodosius Grigorievich Dobzhansky
(https://en.wikipedia.org/wiki/Theodosius_Dobzhansky)

Why and how?

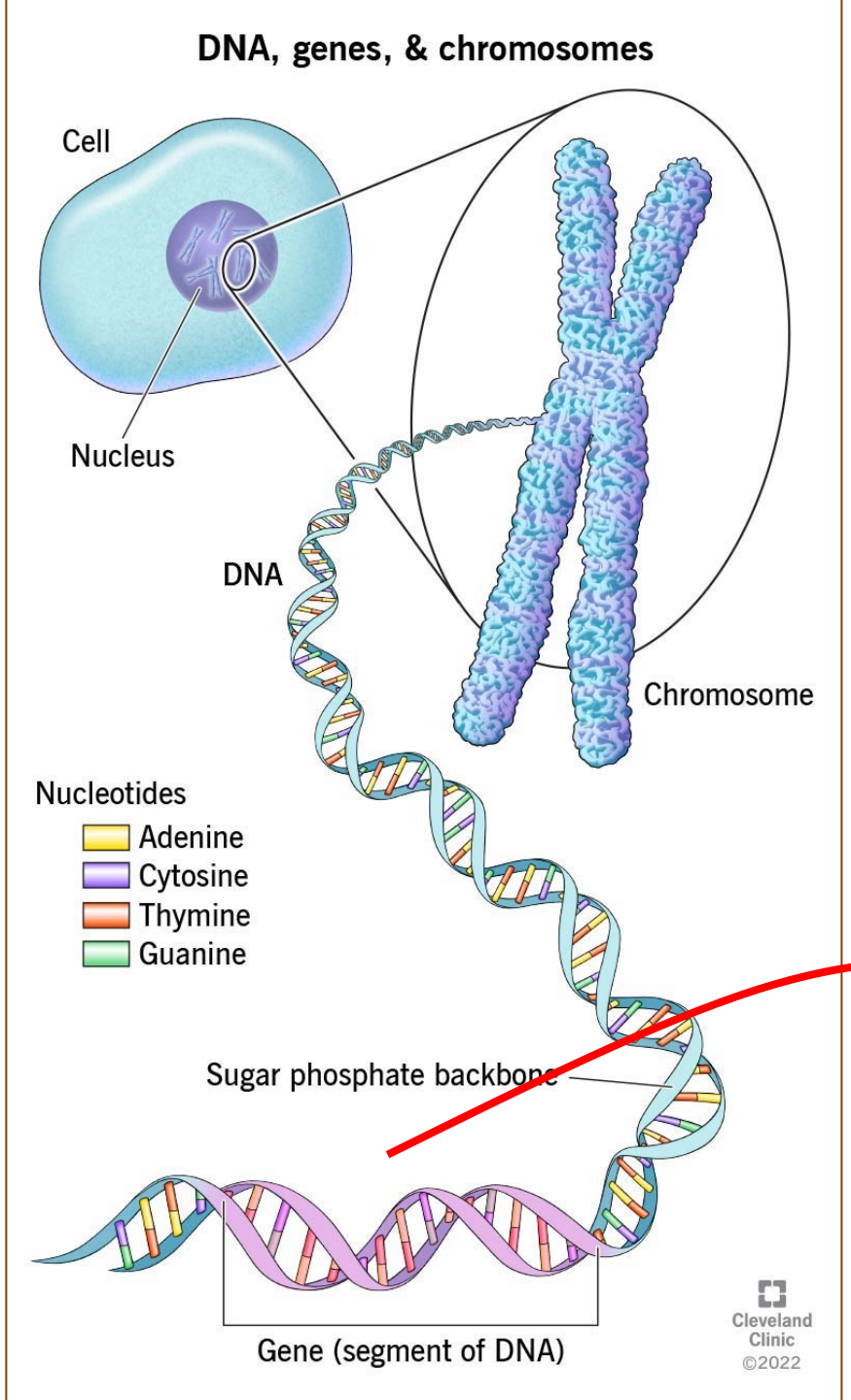
Question: WHY does a cell contain information that it never uses?

Answer: No idea…
We don‘t even know if information is NEVER used, for that matter!
One can speculate and come up with possible answers

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

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

Gene expression



Biosynthesis of the polypeptide (protein) encoded by a gene

messenger RNA → Protein

https://my.clevelandclinic.org/health/body/23064-dna-genes--chromosomes

Slide from Lecture 5

BB101 Biology
March – June 2023
Lecture 05

Central dogma

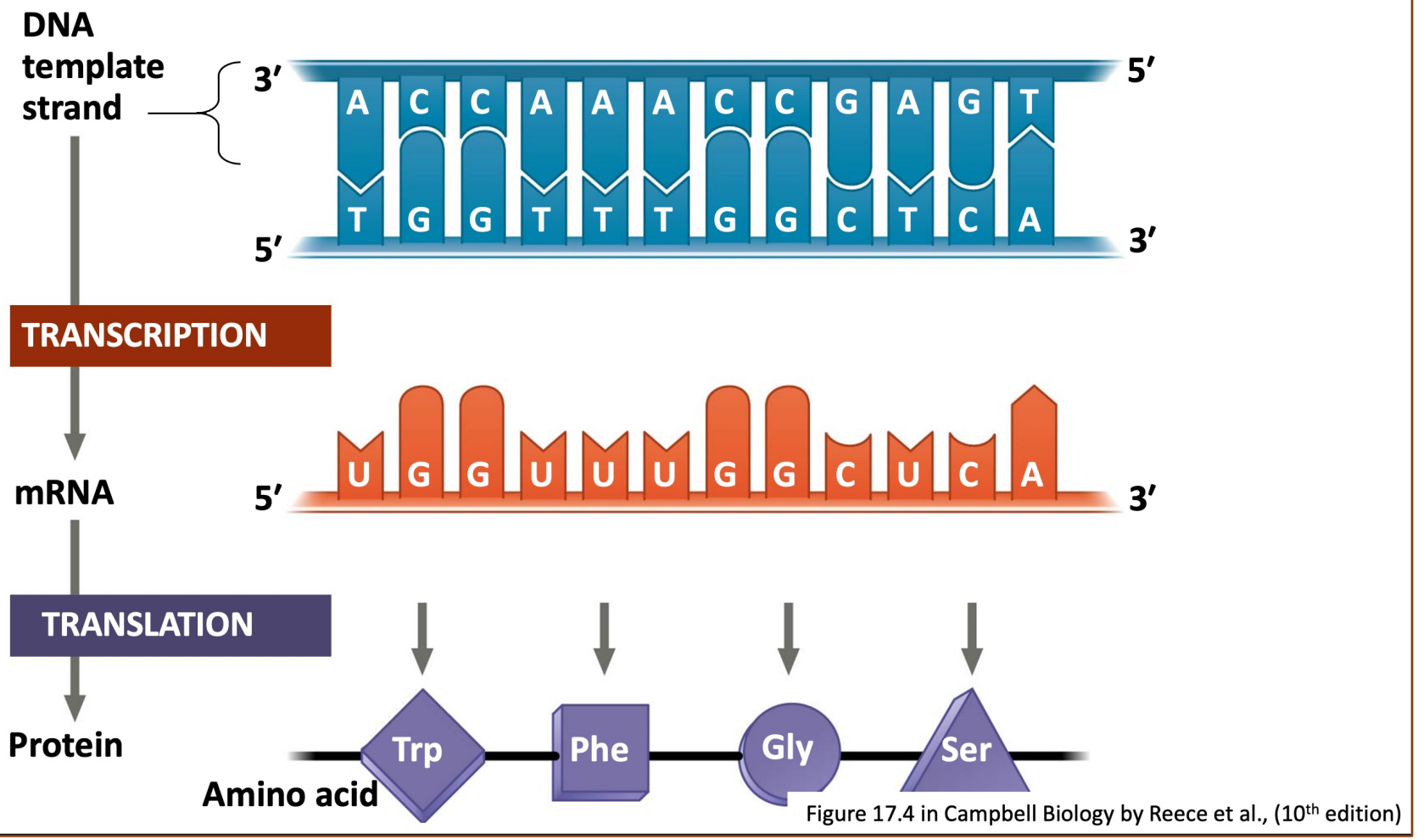


Figure 17.4 in Campbell Biology by Reece et al., (10th edition)

Today's topics

- Gene expression – promoter
- Metabolic pathways: two levels of regulation
  - Feedback inhibition and regulation of gene expression
- Concept of operon
  - Features of *trp* operon
  - Features of *lac* operon
- Negative versus positive gene regulation
  - cAMP and catabolite activator protein (CAP)
- Gene expression in a population

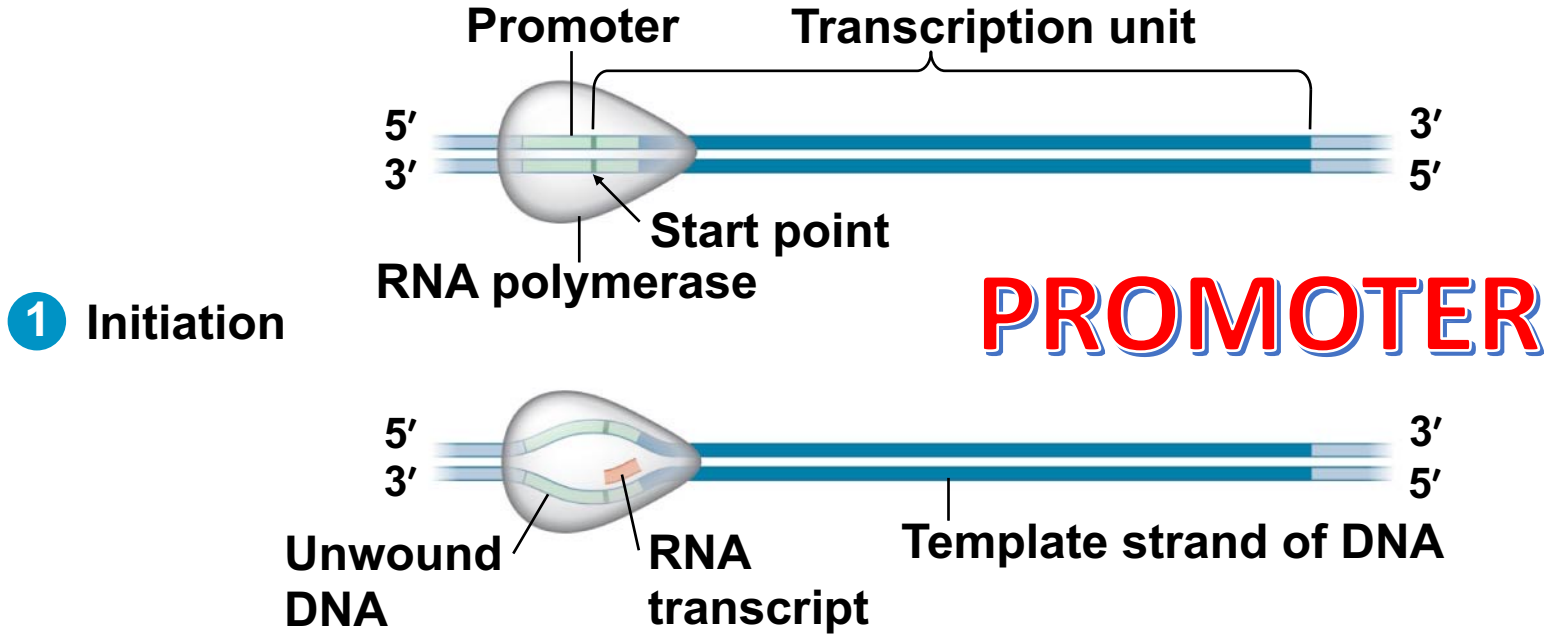
Promoter

Protein data bank accession id: 2KEI.pdb

- 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

DNA binding domain of the repressor
It is a homodimer
The two monomers are shown in different shades

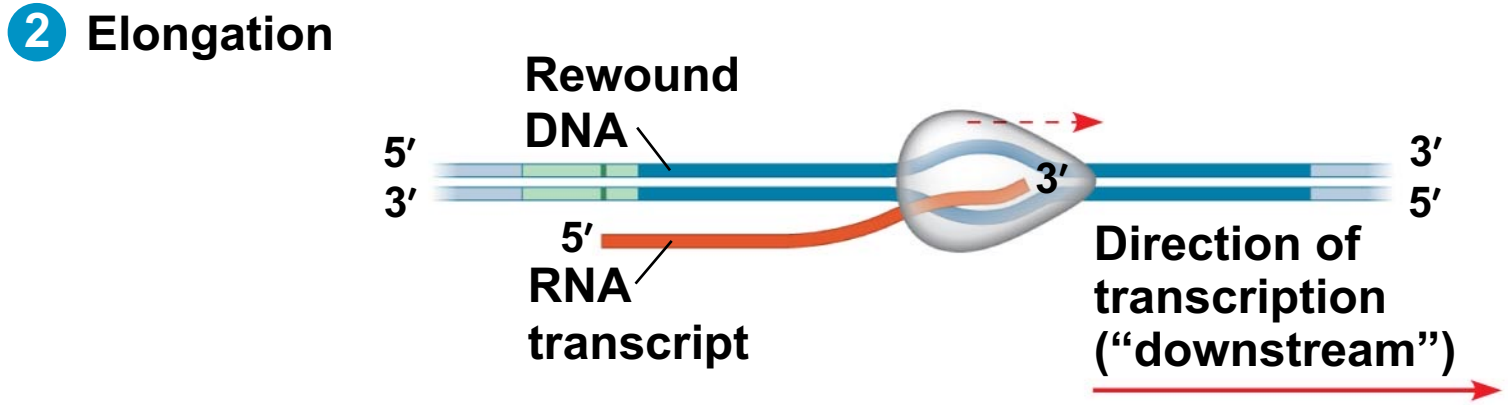
Transcription. Step 1 of 3



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

Figure 17.7 in Campbell Biology by Reece et al., (10th edition)

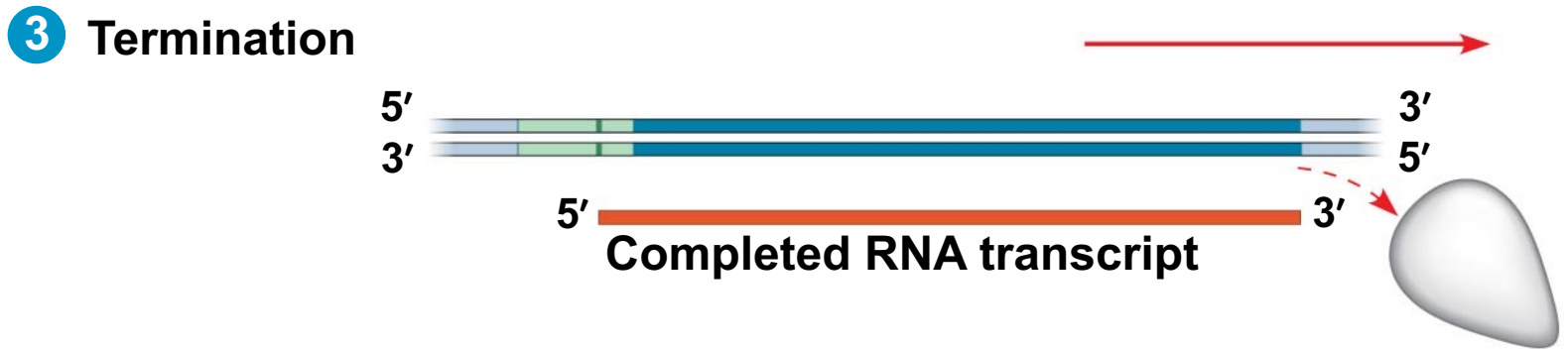
Transcription. Step 2 of 3



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

Figure 17.7 in Campbell Biology by Reece et al., (10th edition)

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 in Campbell Biology by Reece et al., (10th edition)

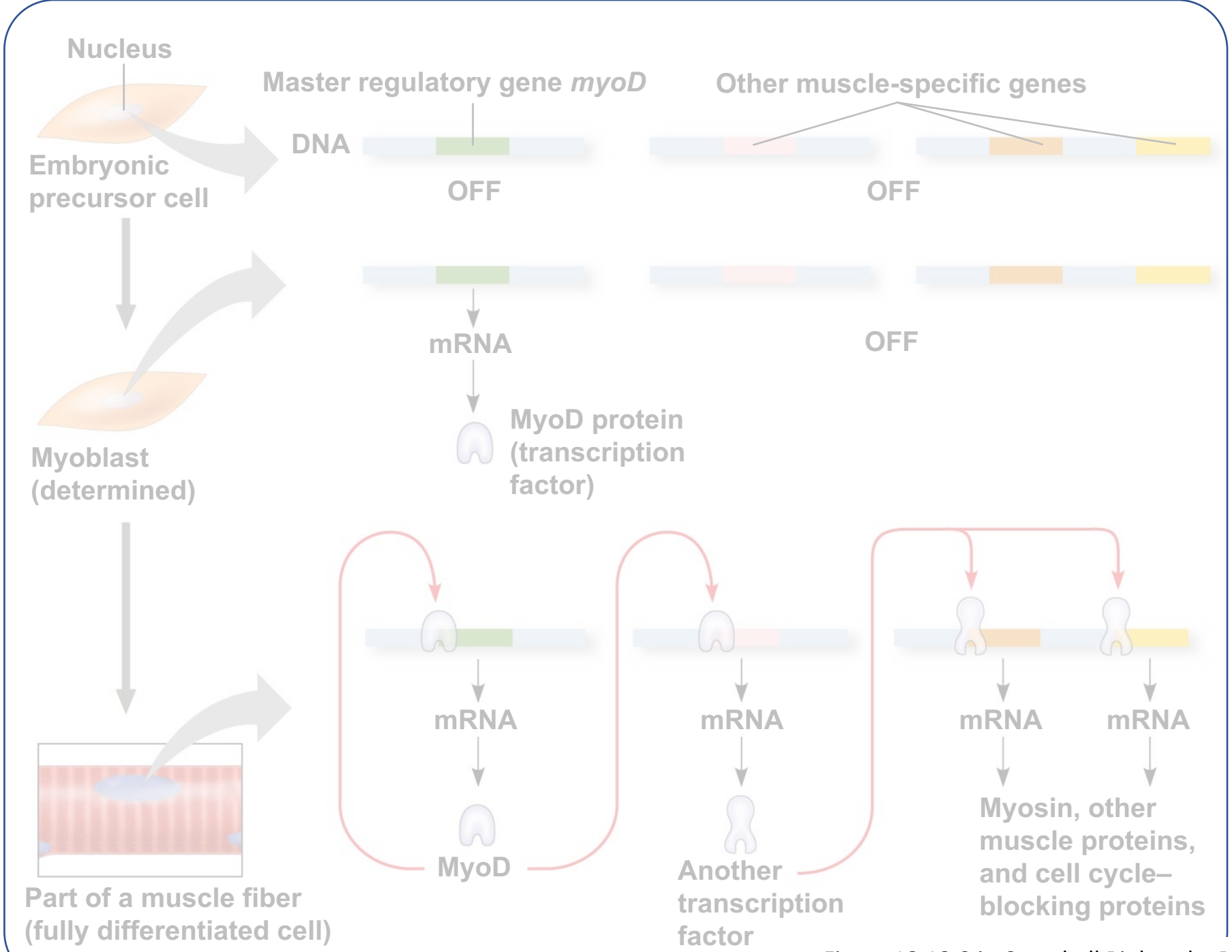


Figure 18.18-3 in Campbell Biology by Reece et al., (10th edition)

Today's topics

- Gene expression – promoter
- Metabolic pathways: two levels of regulation
  - Feedback inhibition and regulation of gene expression
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  - Features of *lac* operon
- Negative versus positive gene regulation
  - cAMP and catabolite activator protein (CAP)
- Gene expression in a population

Inflow versus utilization

Open the tap to fill in the bucket

Keep using the water

Suppose, the amount of water used
is less than
the amount of water flowing in to the bucket
(normalized to unit time)

bucket will fill and water overflows

Feedback response

Inflow of cold water = outflow of hot water

Temperature of water reaches a preset value

↓

cut off power supply

This is reversible

Previous slide: focus ➛ amount of water flowing

This slide: focus ➛ temperature of water

Regulating product formation

- Natural selection has favored bacteria that produce only the products needed by that cell
- A cell can regulate the production of tryptophan by
  (A) feedback inhibition and
  (B) regulating gene expression

Slide from Lecture 5

Growth media

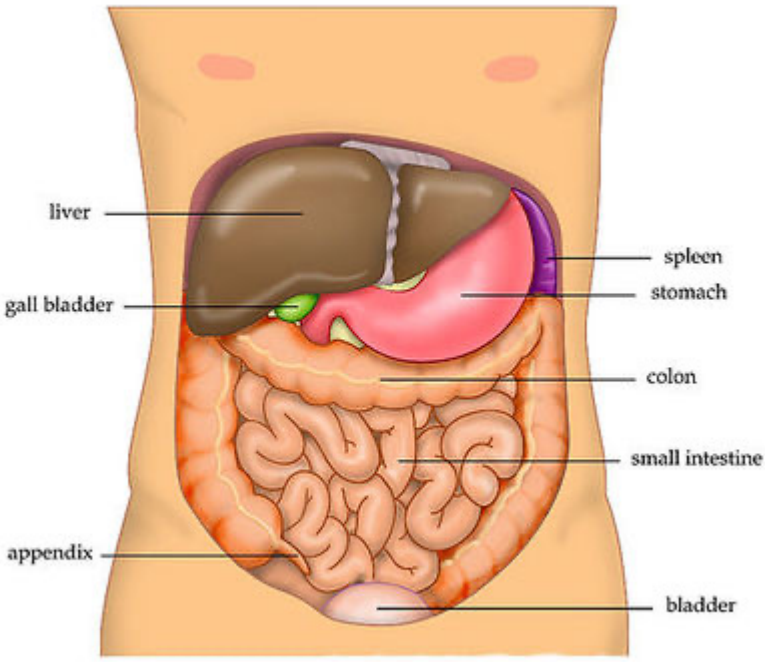
Minimal medium consists of
- inorganic salts,
- glucose and
- biotin (a vitamin)

in agar, a support medium

Complete medium consists of
- minimal media,
- all 20 amino acids and
- a few other nutrients

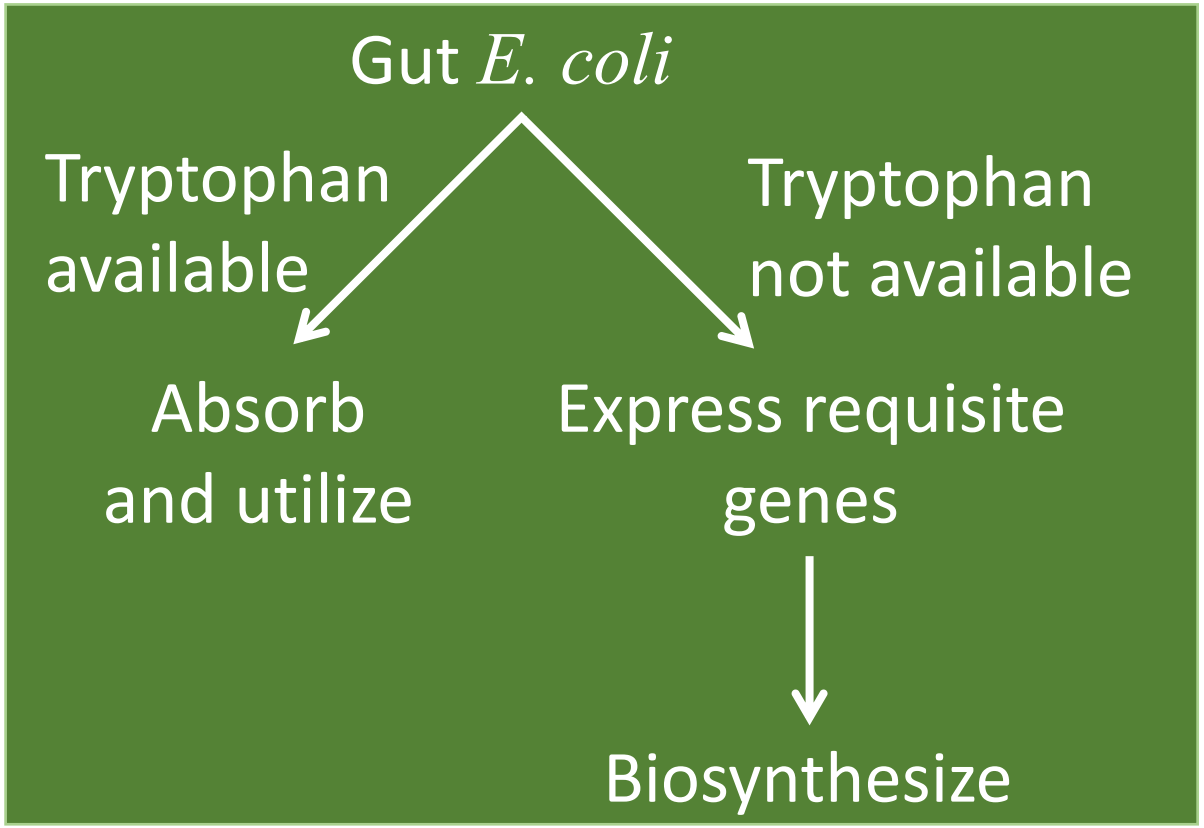
in agar, a support medium

Responding to environmental cues



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

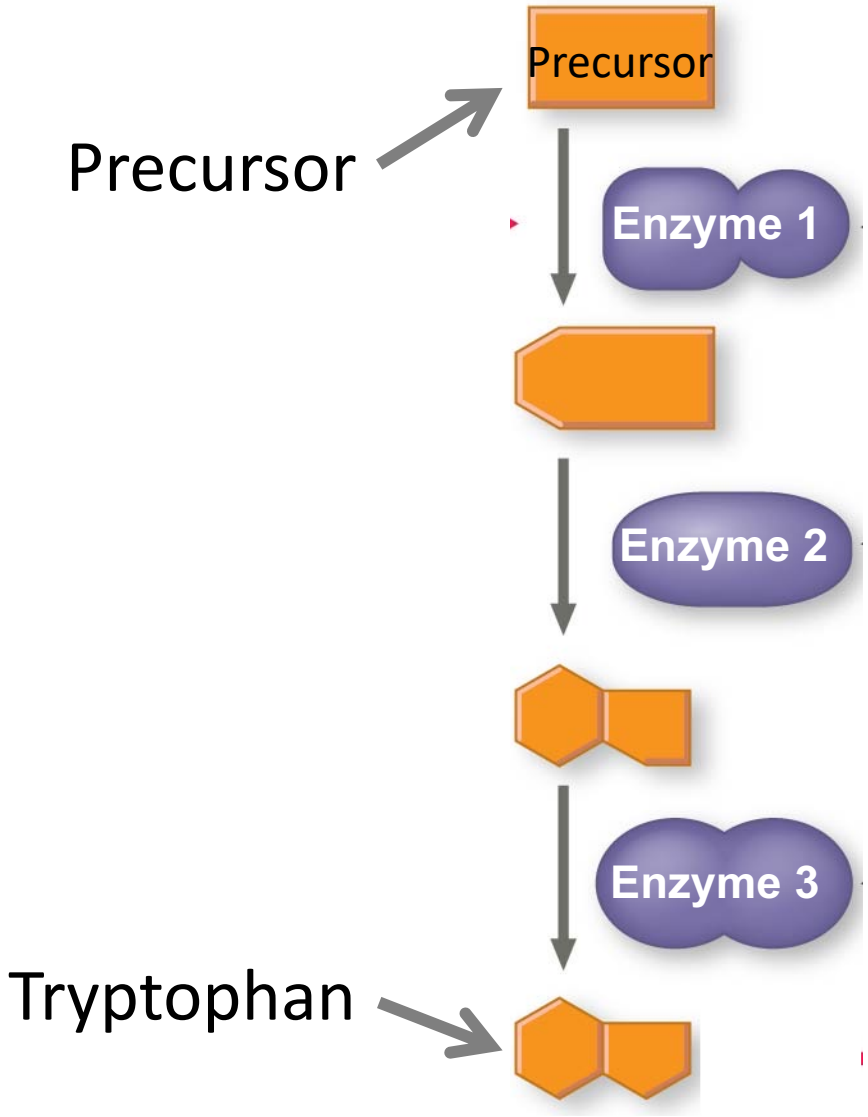
- *E. coli* inhabits human gut
- Tryptophan is an amino acid found in proteins



Regulating product formation

- Natural selection has favored *E. coli* that produce only products needed by that cell
- *E. coli* can regulate the production of tryptophan by
  (A) feedback inhibition and
  (B) regulating gene expression

Tryptophan biosynthesis pathway



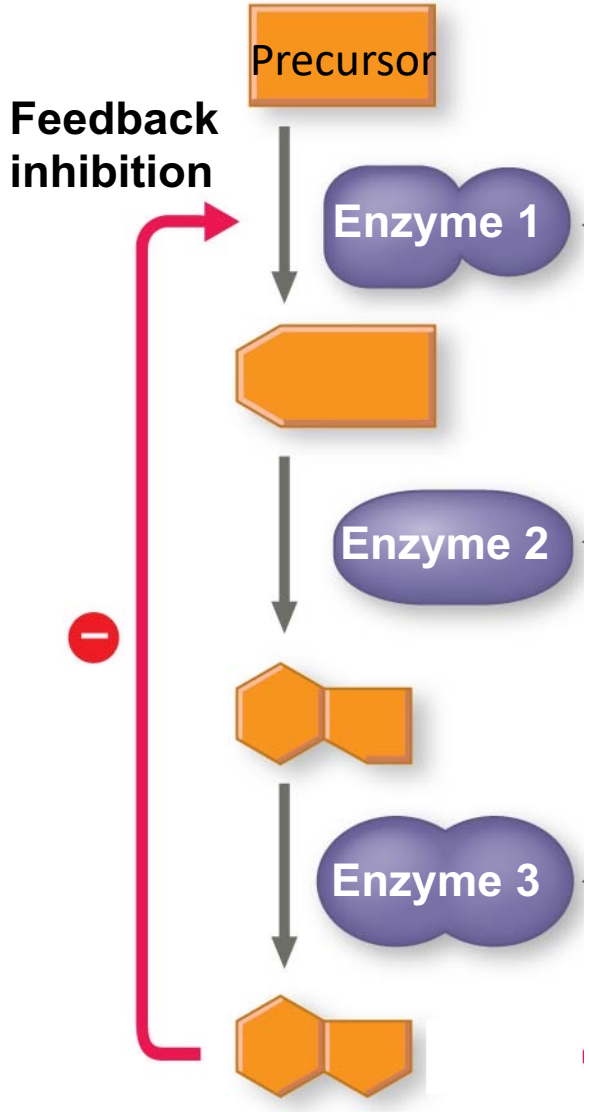
Mode of regulation #1:
Feedback inhibition

Figure 18.2 in Campbell Biology by Reece et al., (10th edition)

Feedback inhibition

Allosteric inhibitor

Regulation of enzyme activity

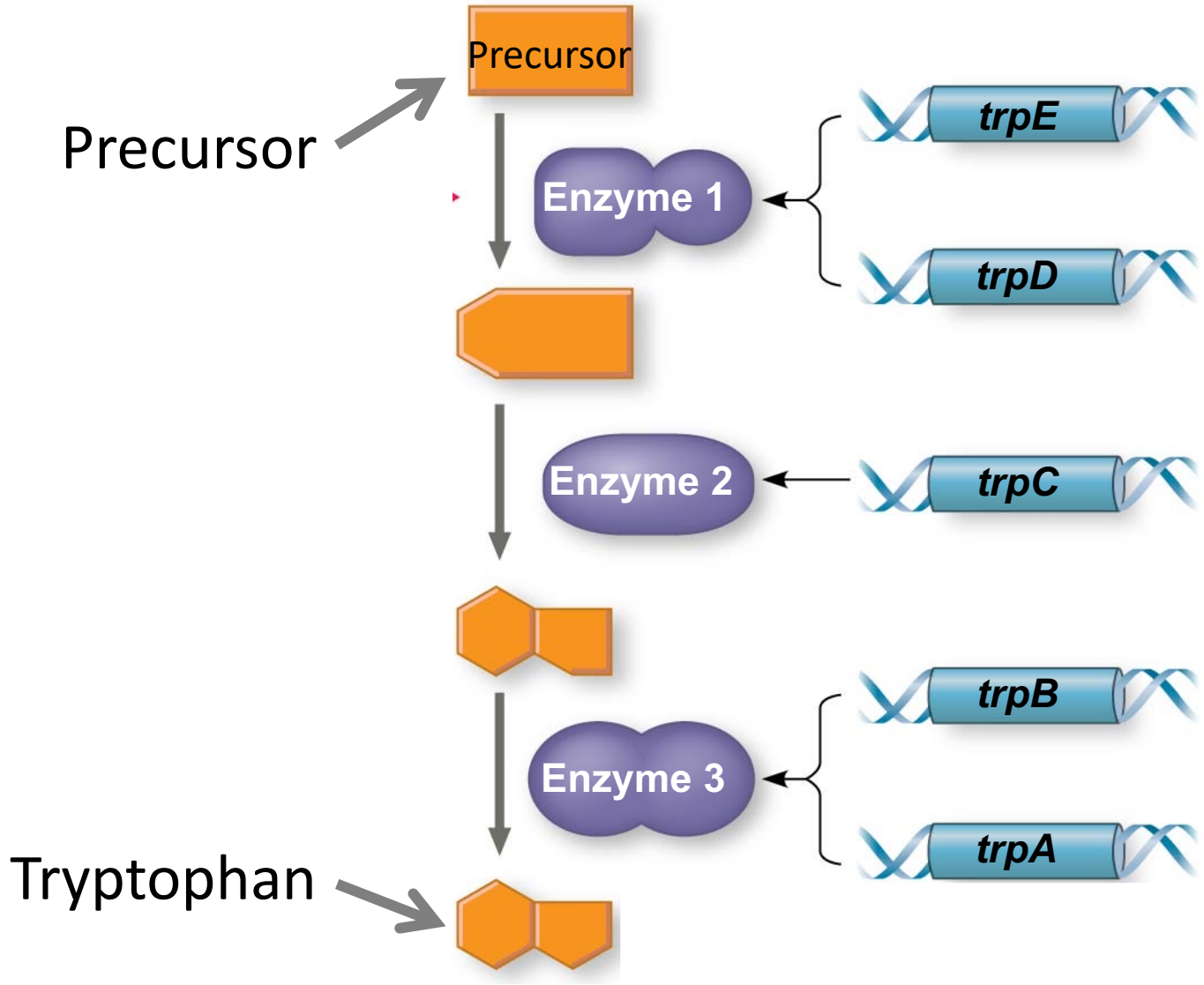


End product of the metabolic pathway inhibits the enzyme that catalyzes the first reaction in the pathway

Fast
as well as
reversible
response

Figure 18.2 in Campbell Biology by Reece et al., (10th edition)

Genes encoding tryptophan biosynthesizing enzymes

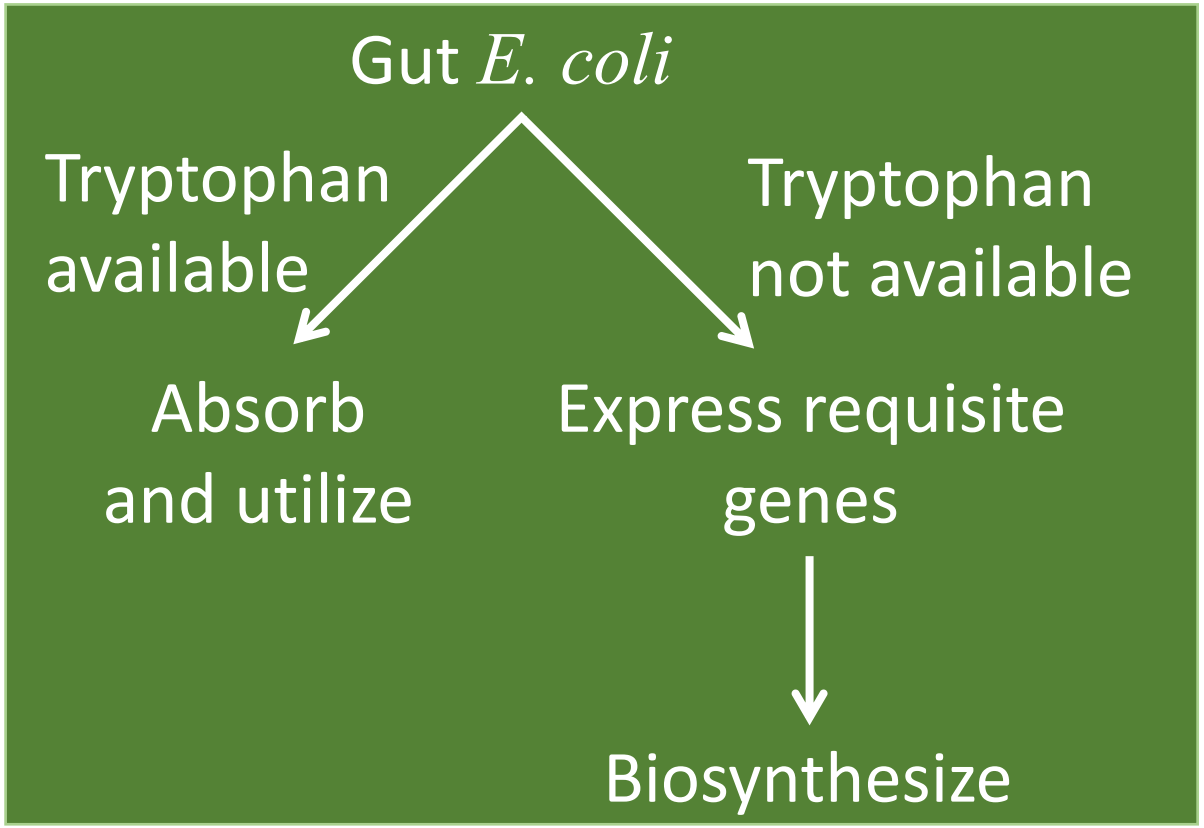


Mode of regulation #2: Regulation of gene expression

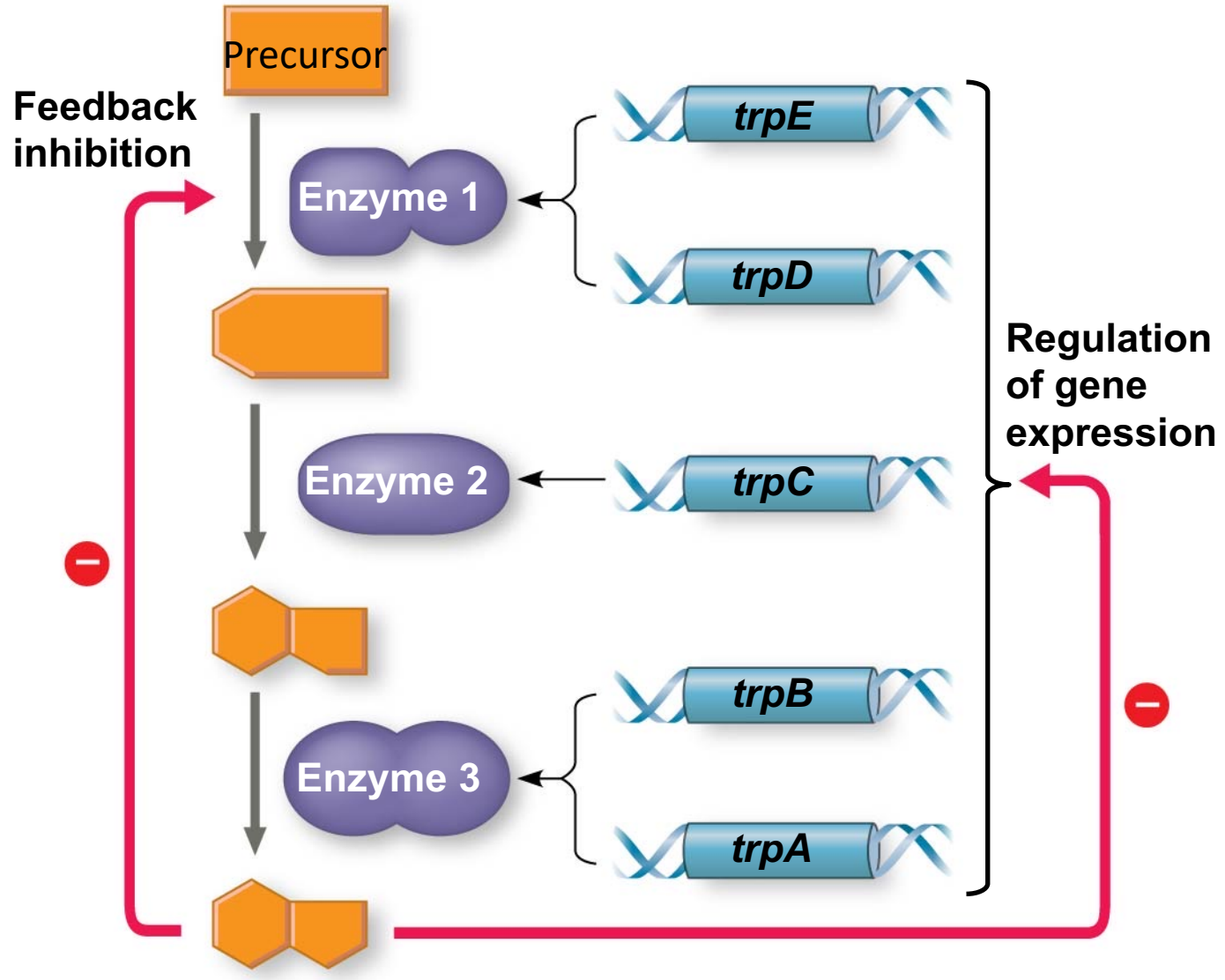
Figure 18.2 in Campbell Biology by Reece et al., (10th edition)

Coordinated switching on-off

- Suppose tryptophan is to be synthesized
- Switch on the expression of all five genes
- When sufficient tryptophan is made, turn off all five genes



Regulation of a metabolic pathway



Regulation of enzyme activity

Regulation of enzyme production

HOW?

Figure 18.2 in Campbell Biology by Reece et al., (10th edition)

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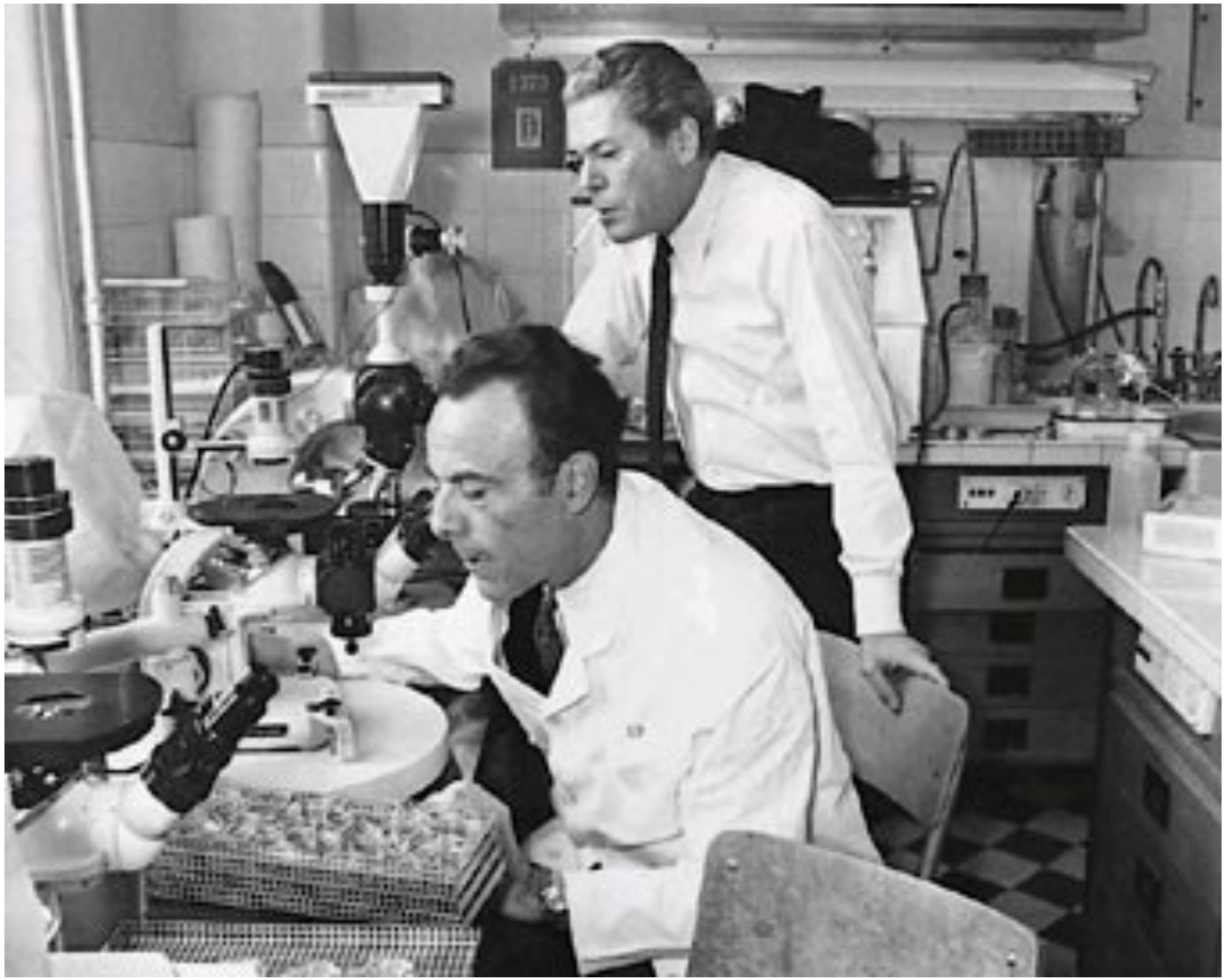
- Gene expression in a population

Operon

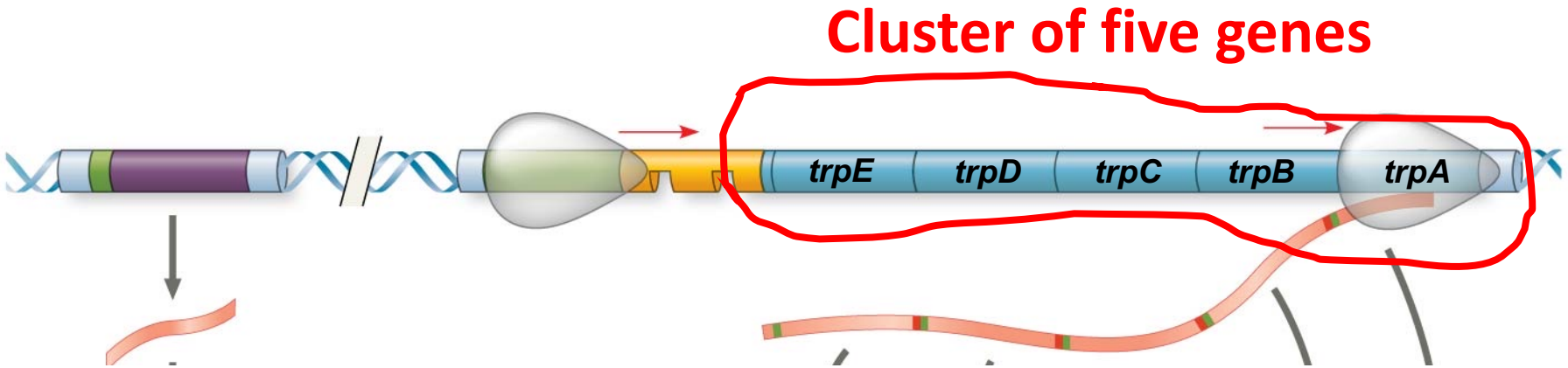
1965 Nobel Prize in Physiology or Medicine



Jacques Monod

Francois Jacob

Clustering of the five genes



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

Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

Clustering of the five genes

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

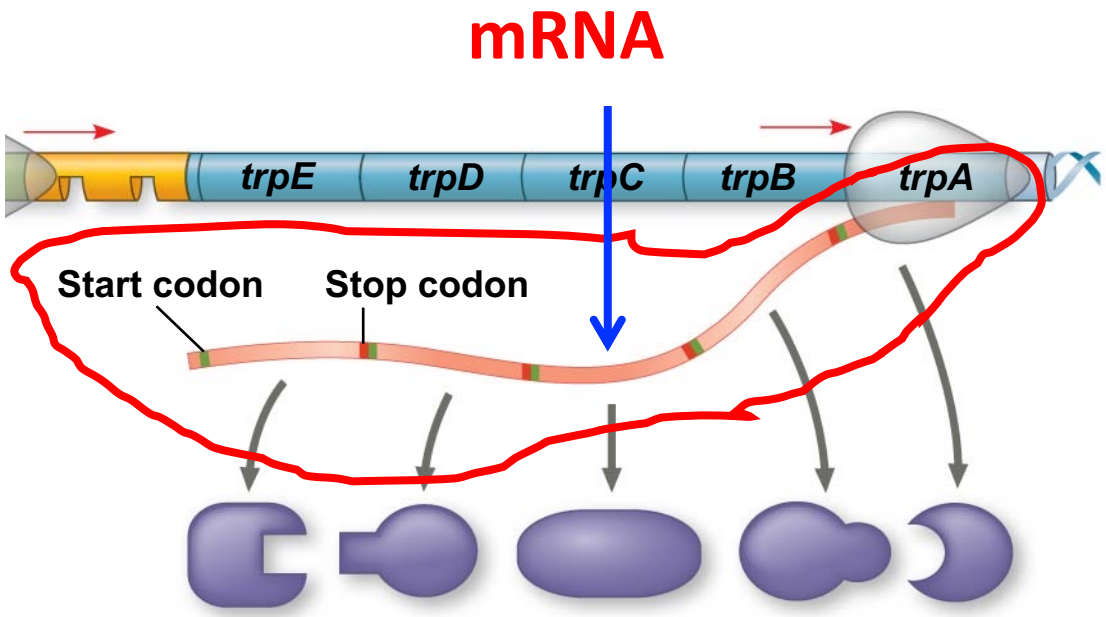


Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

Coupled expression of genes

Transcription results in a single mRNA

The entire mRNA is translated

Five separate polypeptide chain are formed

Separate start and stop codons for each polypeptide chain

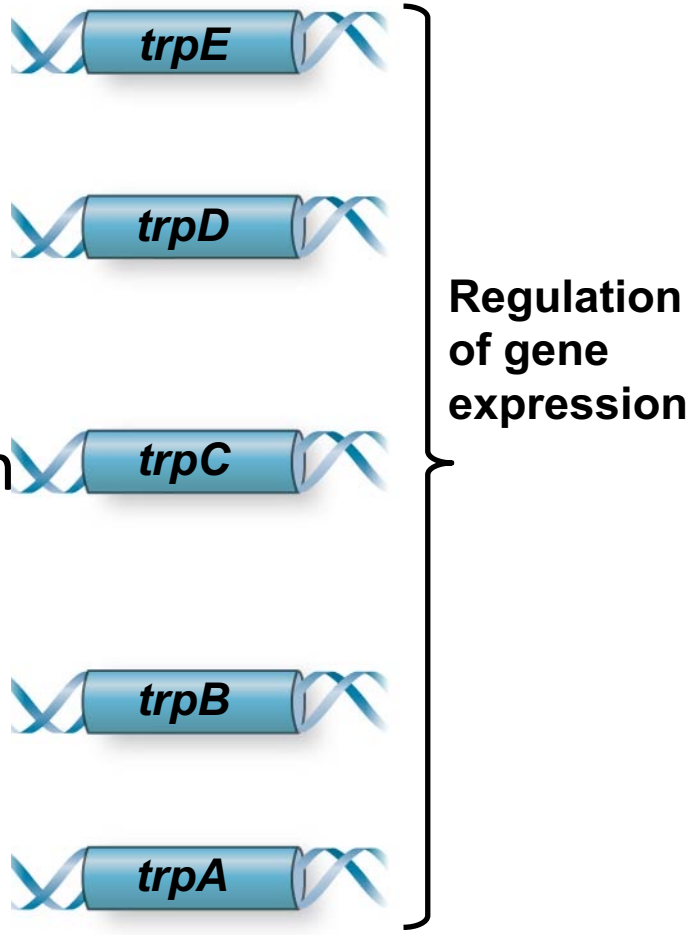
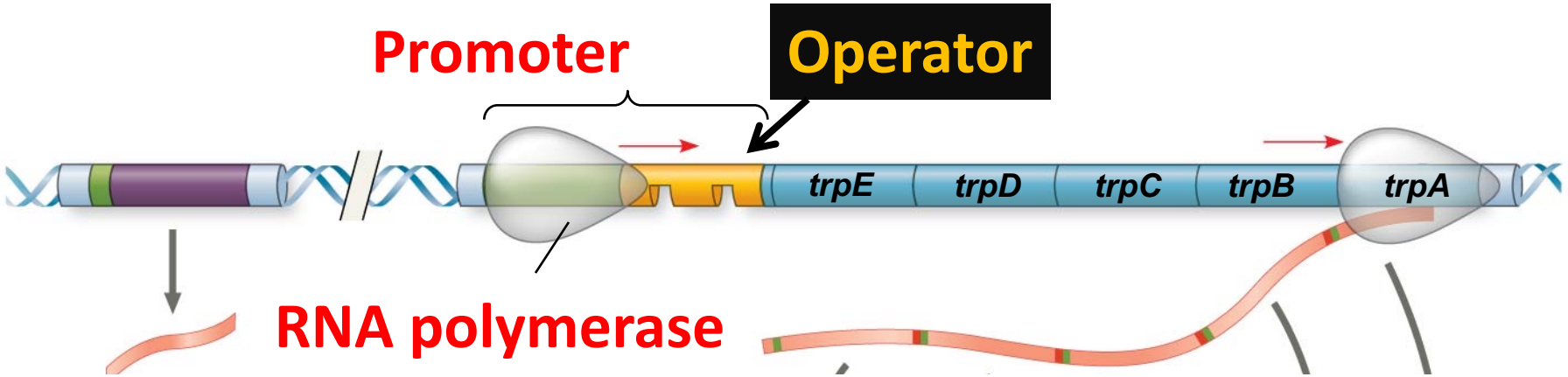


Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

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

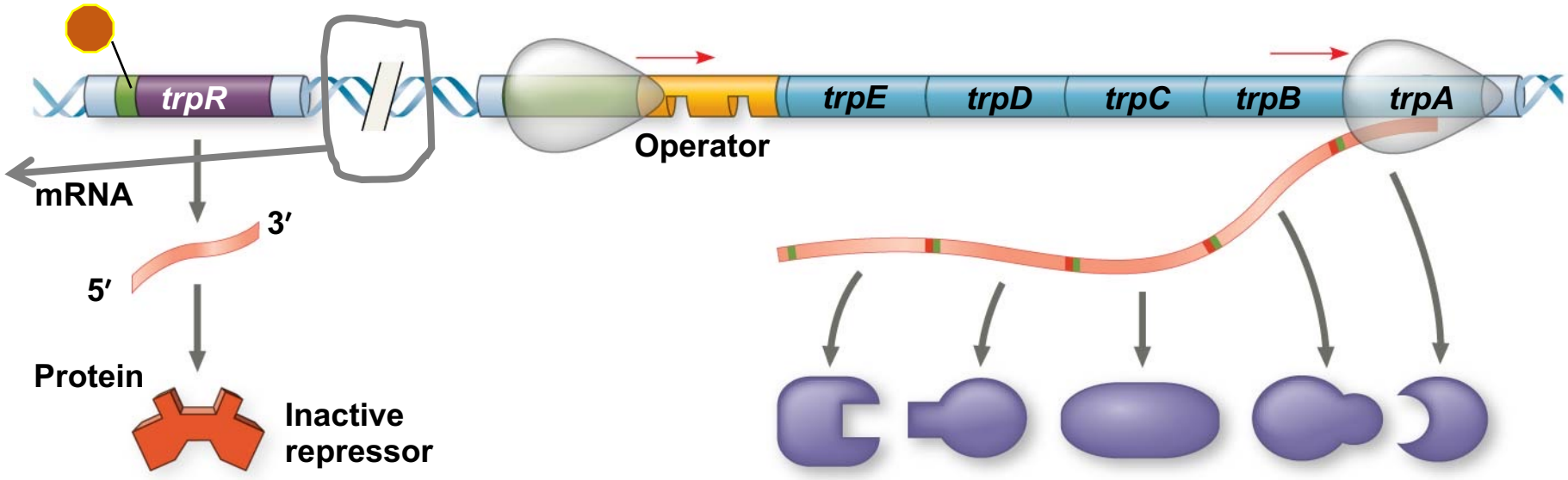
Role of operator in regulating gene expression?

Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

Concept of a repressor

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

Repressor gene is NOT contiguous with *trp* genes



trp repressor (inactive)

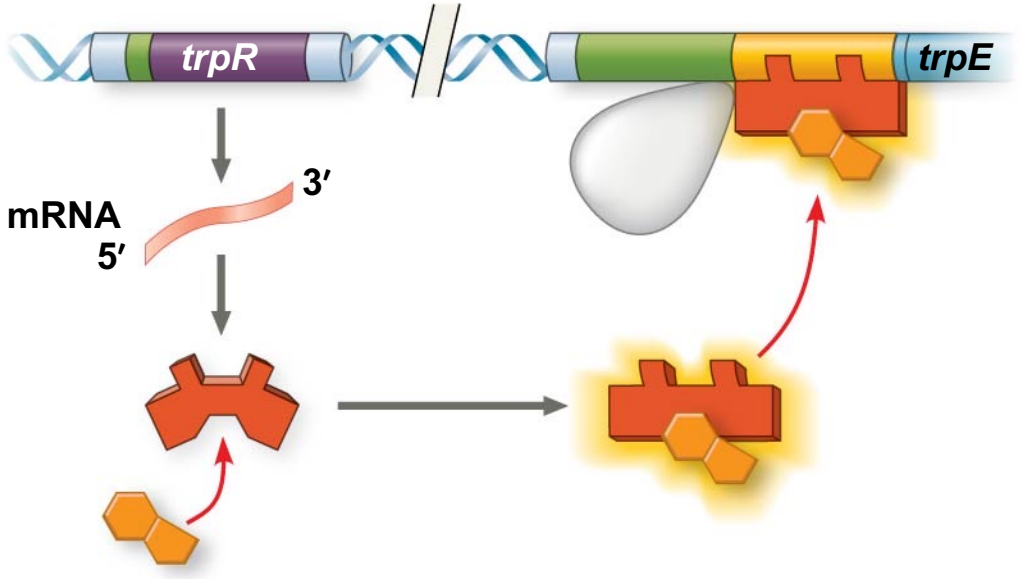
Promoter for *trpR*

Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

How does a repressor work?

trpR: Gene encoding the *trp* repressor

Only tryptophan-bound repressor can bind to the operator region



Trp repressor (inactive)

Tryptophan (co-repressor)

Trp repressor (active)

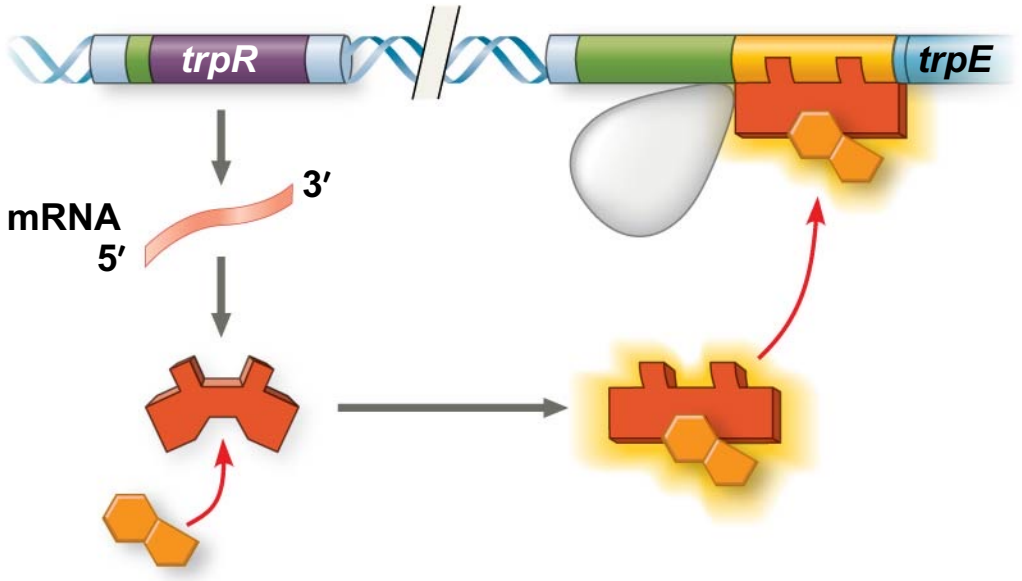
RNA polymerase is stalled if the operator region is bound by the repressor

Binding of tryptophan changes the shape of the repressor

Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

How does a repressor work?

Trp repressor is specific to *trp* operator



Binding of Trp repressor to the operator is reversible

Trp repressor (inactive)

tryptophan available

tryptophan not available

Trp repressor (active)

Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

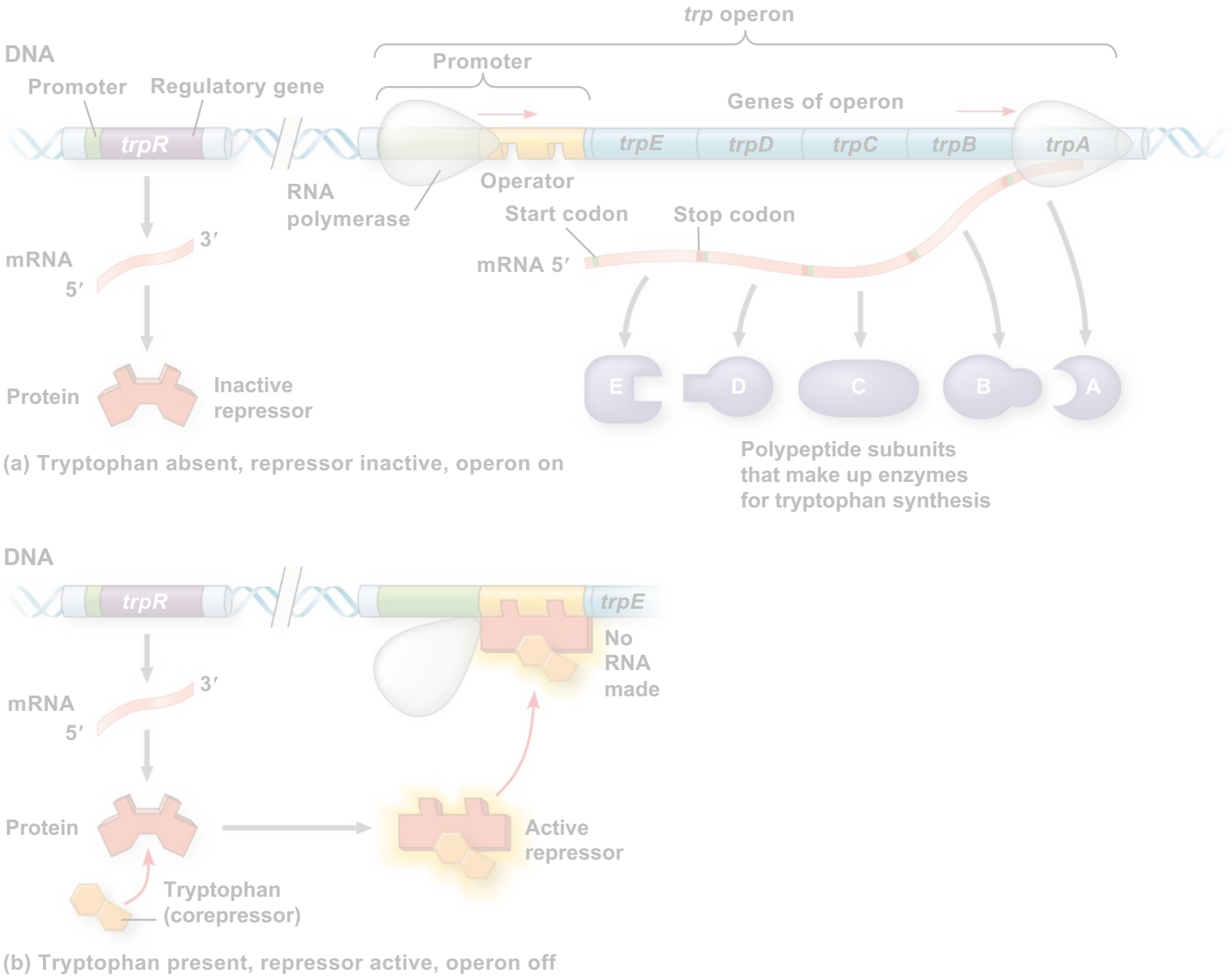


Figure 18.3 in Campbell Biology by Reece et al., (10th edition)

Today's topics

- Gene expression – promoter
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  - Feedback inhibition and regulation of gene expression
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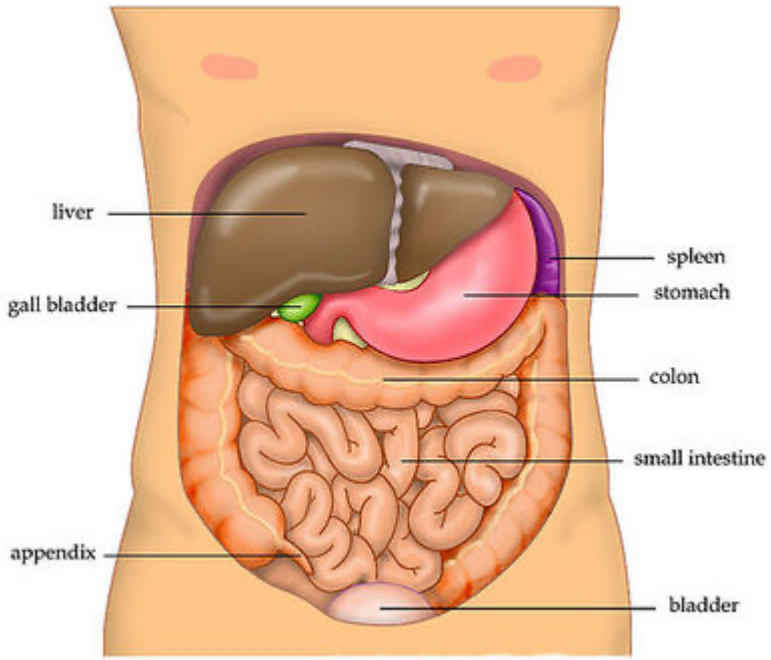
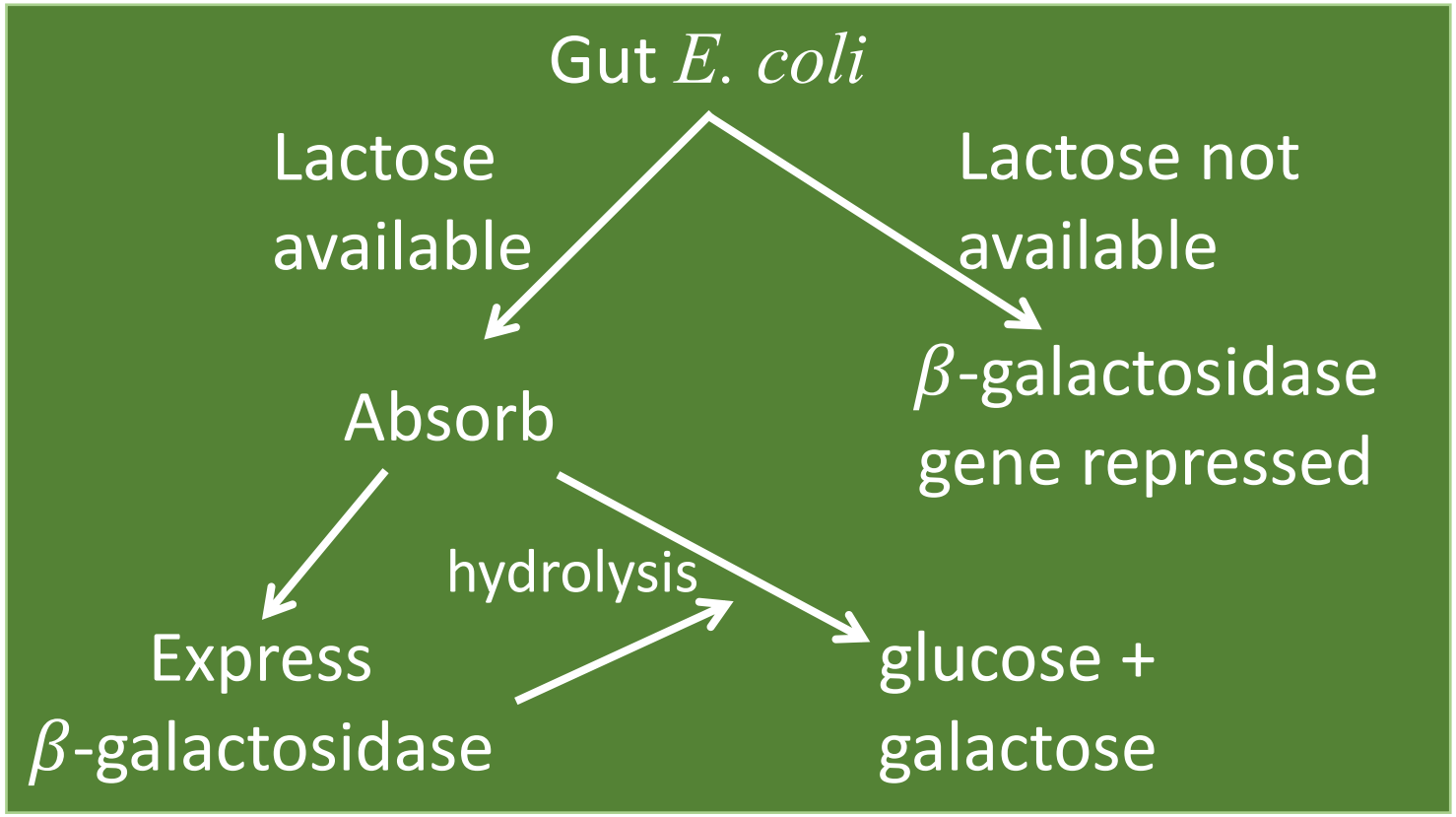
- Gene expression in a population

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



Induction of the *lac* operon

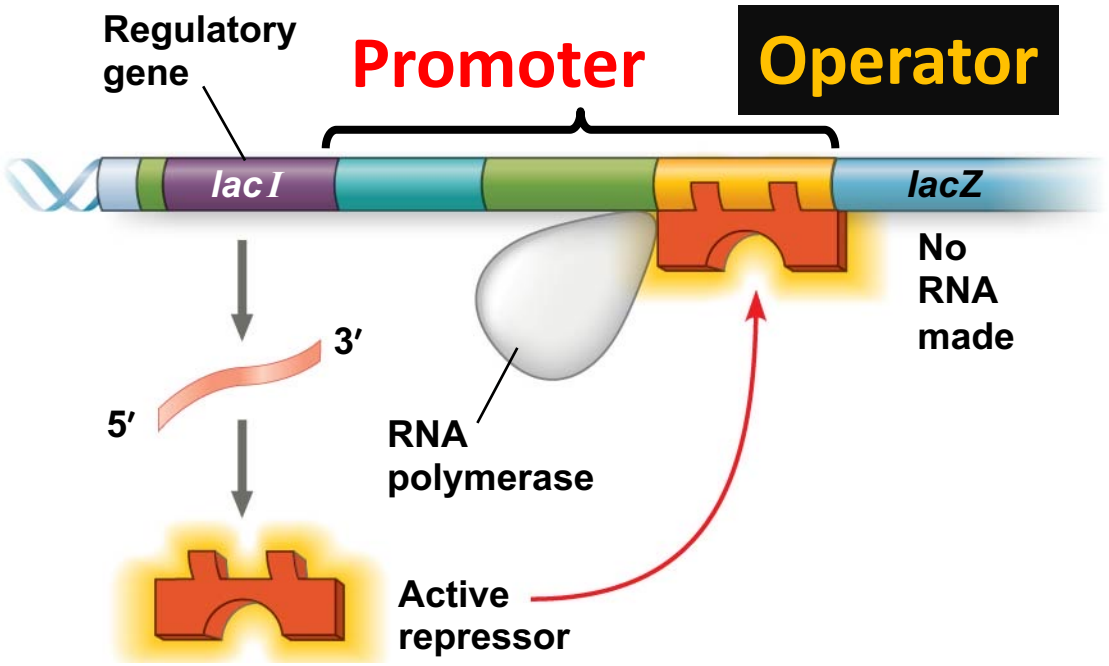
Lactose absent	Only a few molecules of β-galactosidase
Lactose present	1000-fold increase in the number of β-galactosidase Time taken: about 15 minutes

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

Induction of the *lac* operon

regulatory gene

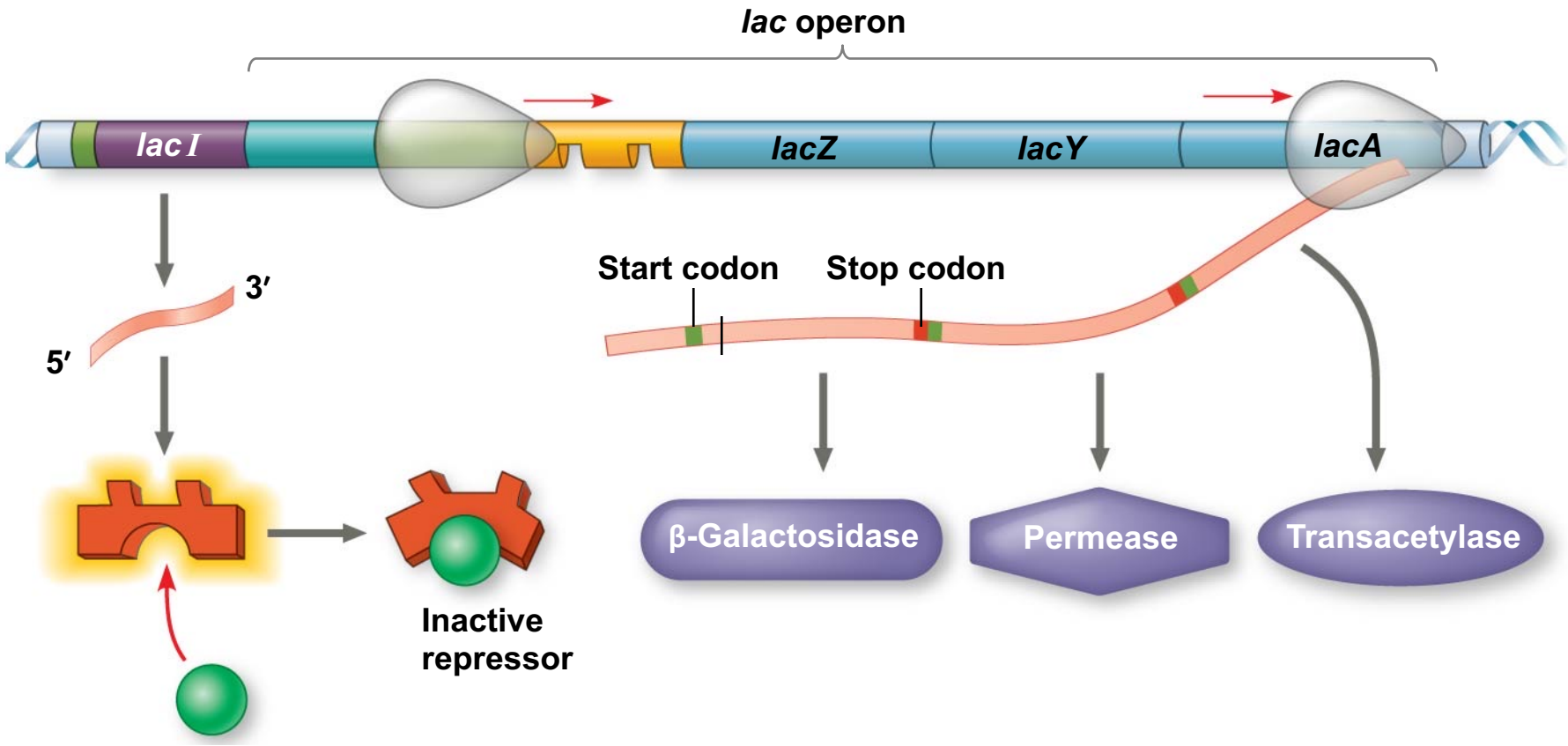
lacI gene



repressor (active)

Figure 18.4 in Campbell Biology by Reece et al., (10th edition)

Induction of the *lac* operon



Binding of inducer changes the shape of the repressor

Figure 18.4 in Campbell Biology by Reece et al., (10th edition)

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

Today's topics

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- Gene expression in a population

Nutrient requirements of bacteria

Two important requirements: Source of carbon
Source of energy

Source of energy: sunlight
Source of carbon: atmospheric CO_2

Source of energy: glucose
Source of carbon: glucose

Even disaccharides such as lactose and maltose are used as carbon and energy sources

Preferential utilization of glucose

Contains both glucose and lactose

- Glucose is used preferentially
- Lactose is used only when glucose is exhausted

Escherichia coli (*E. coli*)

Image courtesy:
Vishakha and Amrita

Utilizing carbon sources

- Glucose is metabolized by glycolysis (*glyco* + *lysis*)
- Glycolysis is a metabolic pathway
- Each reaction in the pathway is catalyzed by an enzyme
- Each enzyme is encoded by one or more genes

- Distinct pathways (set of reactions) metabolize other sources of carbon e.g., lactose or maltose

Utilization of lactose

Genes required for the utilization of lactose

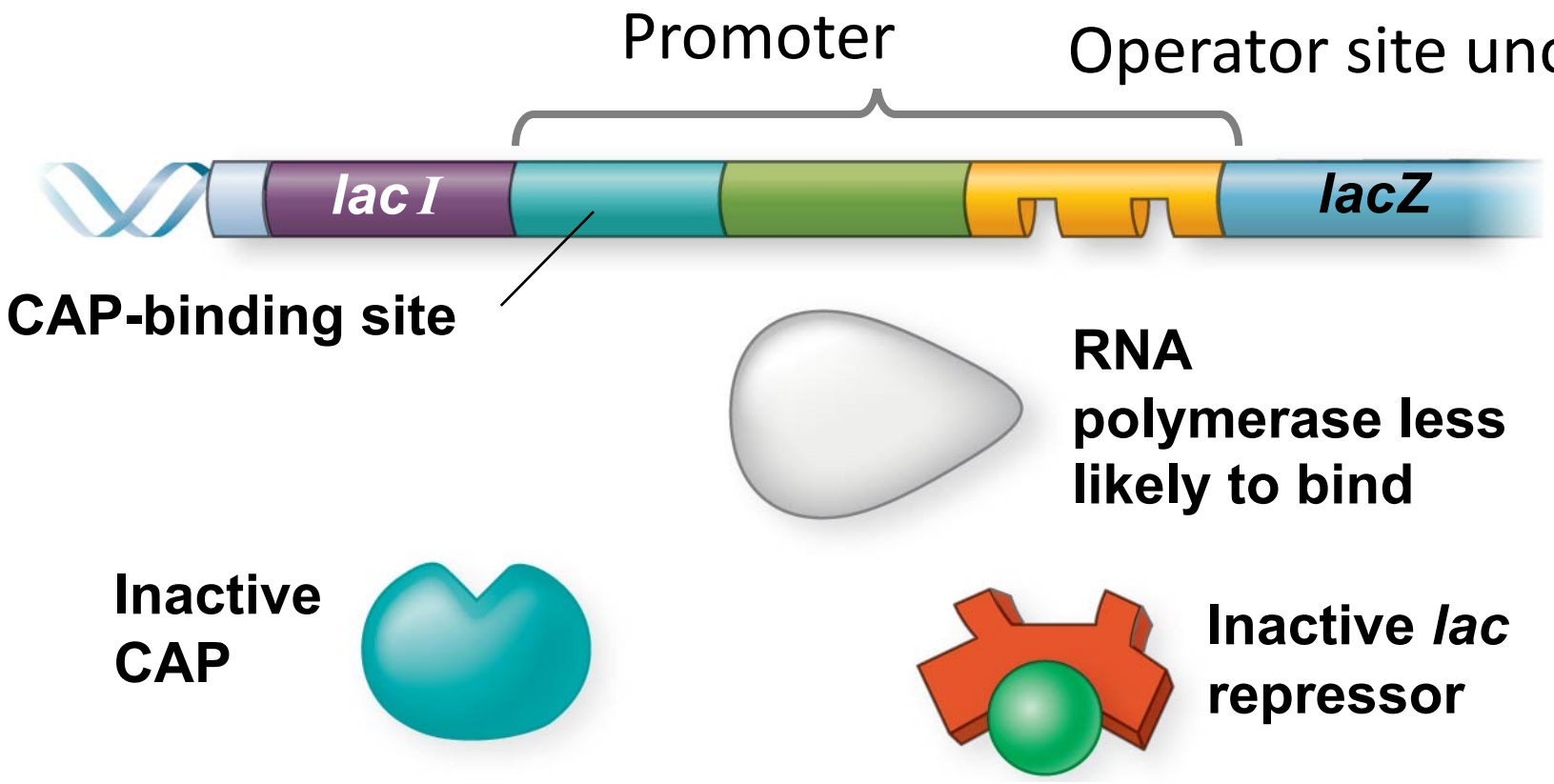
expressed only when bacteria sense that

glucose in the medium is exhausted
AND
lactose is present

catabolite activator protein (CAP)

cyclic AMP

Lactose present, but glucose ALSO is present

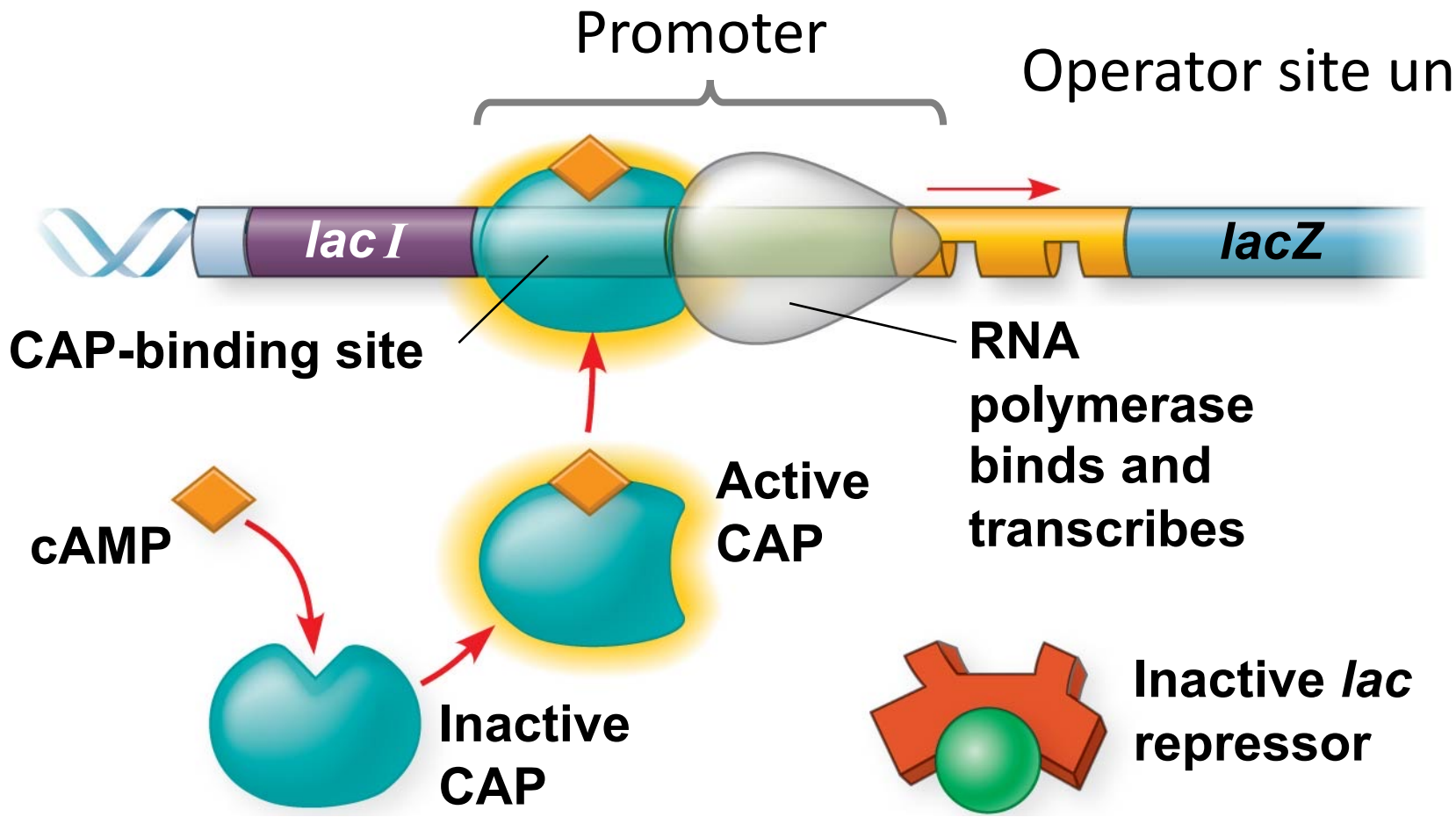


lac operon genes: transcribed in low levels

Lactose present
Repressor inactive

Figure 18.5 in Campbell Biology by Reece et al., (10th edition)

Lactose present, but glucose is NOT present



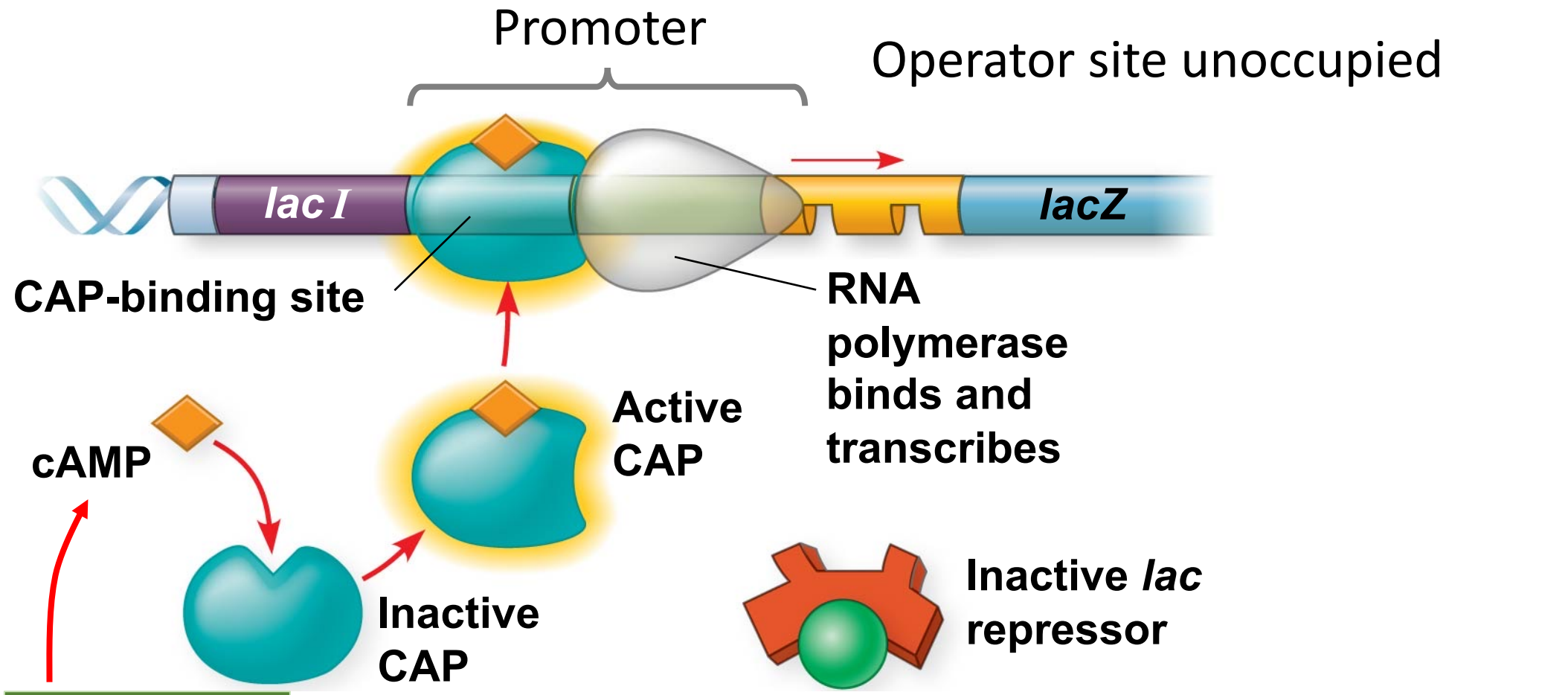
lac operon genes: transcribed

No glucose ➛ cAMP accumulates

Lactose present
Repressor inactive

Figure 18.5 in Campbell Biology by Reece et al., (10th edition)

Lactose present, but glucose is NOT present

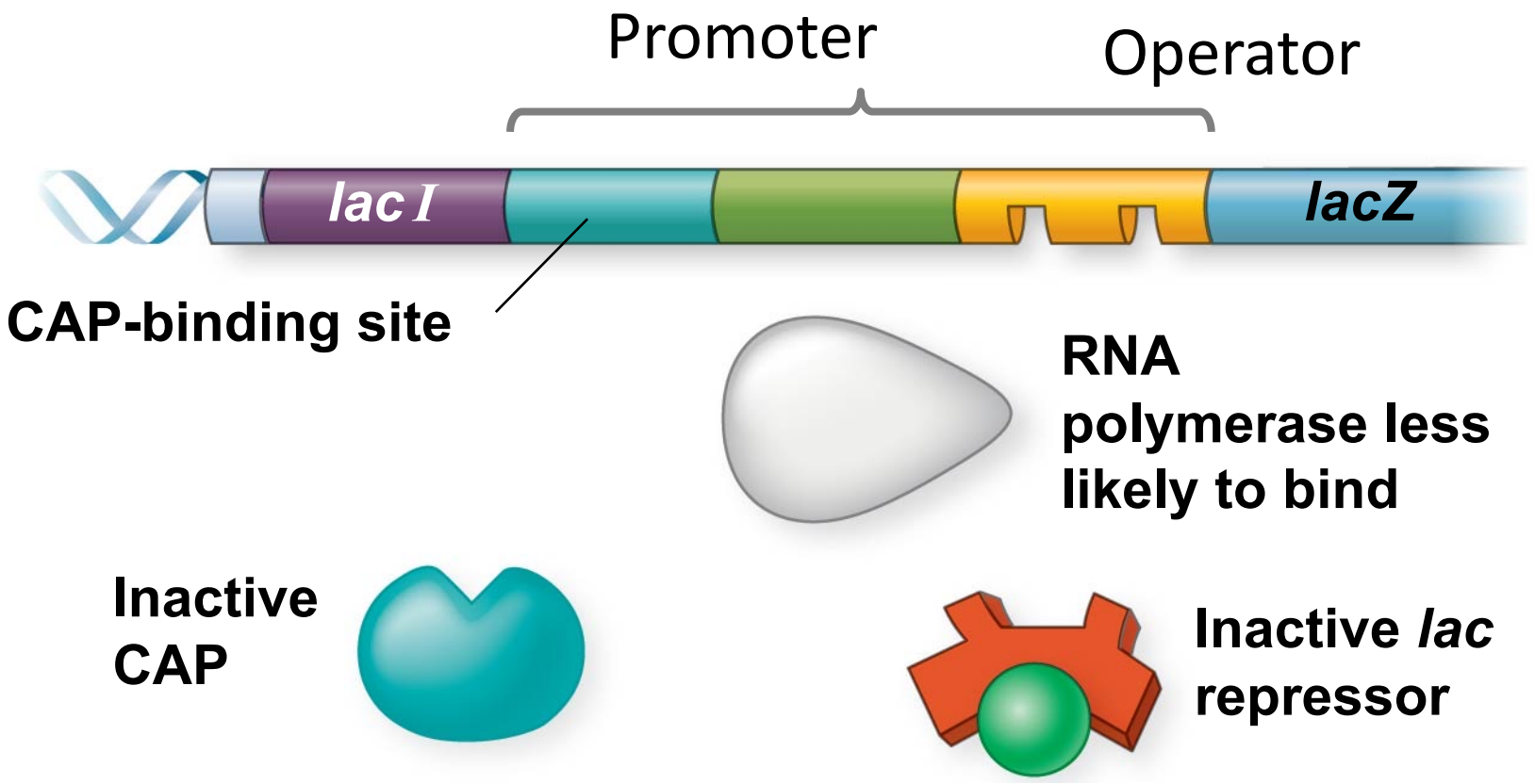


Secondary messenger

Example of positive gene regulation

Figure 18.5 in Campbell Biology by Reece et al., (10th edition)

lac operon: under dual control

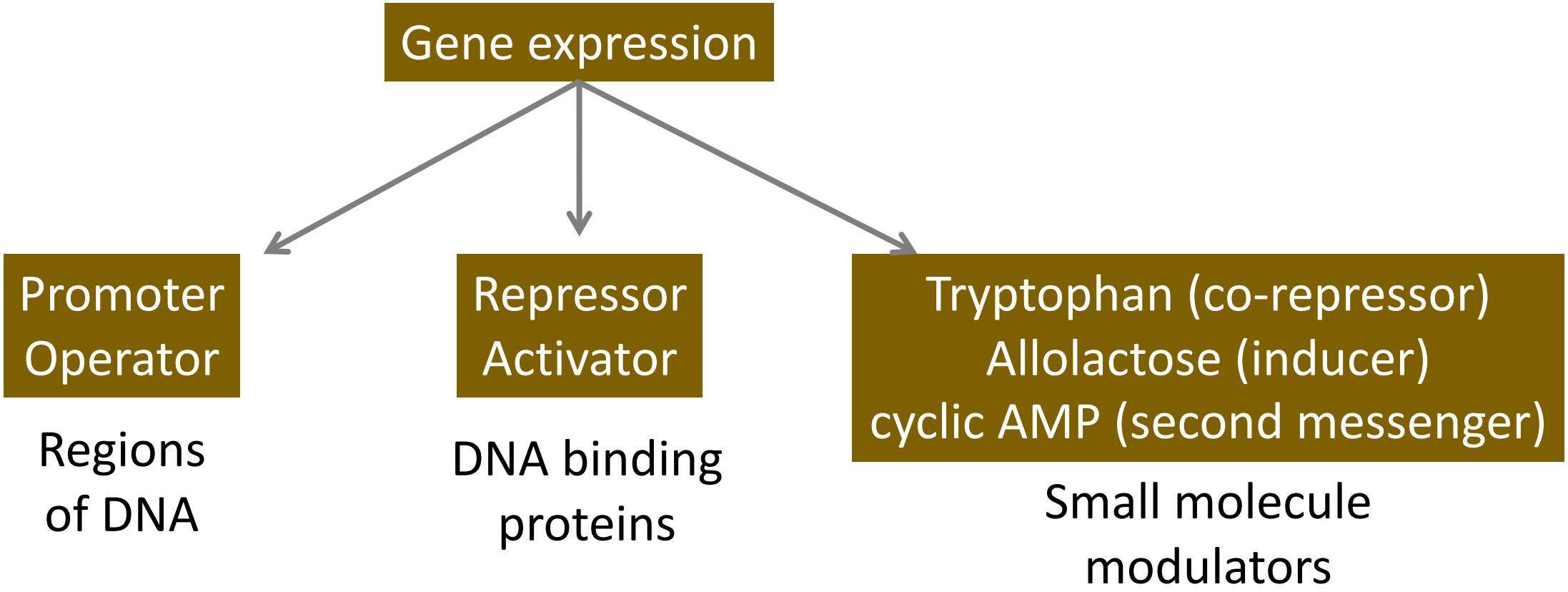


Positive regulation: by catabolite activator protein (CAP)

Negative regulation: by *lac* repressor

Figure 18.5 in Campbell Biology by Reece et al., (10th edition)

Summary: *trp* and *lac* operons



Summary: *trp* and *lac* operons

Negative regulation

Binding of repressor prevents transcription

Positive regulation

Binding of catabolite activator protein permits transcription

Summary: *trp* and *lac* operons

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

CAP

Binding of the small molecule modulator (secondary messenger)

CAP binds to promoter

Gene expression: key determinants and outcome

Concentrations	Binding events
Glucose (inside and outside the cell)	cAMP to CAP
Lactose (inside and outside the cell)	cAMP-CAP to promoter
mRNAs (biosynthesis, degradation)	Inducer to repressor
Proteins (repressor, CAP, RNA polymerase)	Inducer-repressor to its binding site
Small molecules (cyclic AMP, inducer)	RNA polymerase to promoter

Outcome: gene expression – utilization of lactose

Today's topics

- Gene expression – promoter
- Metabolic pathways: two levels of regulation
  - Feedback inhibition and regulation of gene expression
- Concept of operon
  - Features of *trp* operon
  - Features of *lac* operon
- Negative versus positive gene regulation
  - cAMP and catabolite activator protein (CAP)
- Gene expression in a population

Is expression of *lac* operon concurrent in all cells?

Escherichia coli (*E. coli*)

Initially, contains both glucose and lactose

Glucose is utilized preferentially

What happens as $[Glc]_{out}$ keeps decreasing?

When will utilization of lactose start?

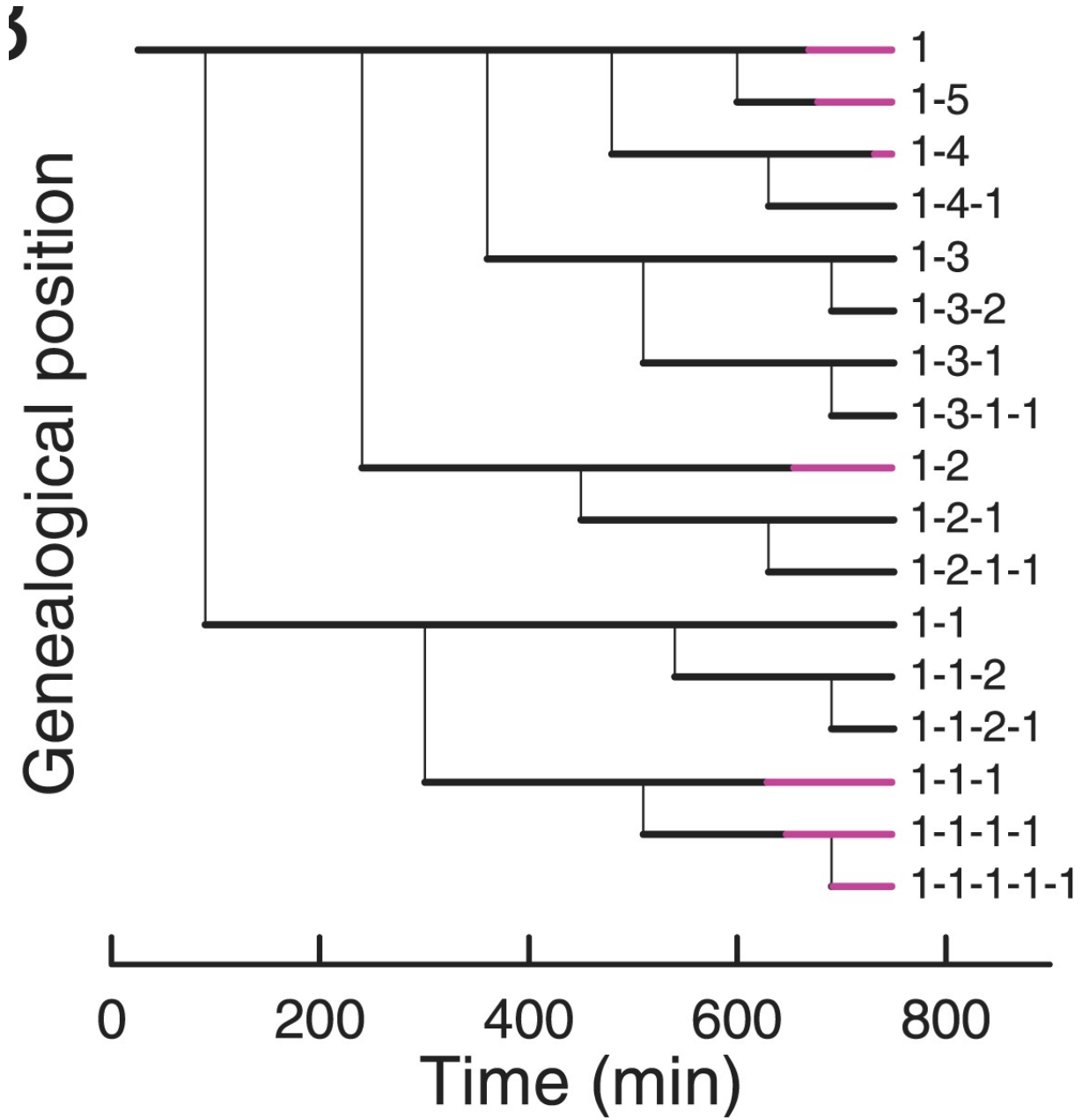
Will all cells in the medium switch in one go?

Image courtesy:
Vishakha and Amrita

Gene expression in a population

Play the video of yeast cell growth

Gene expression in a population



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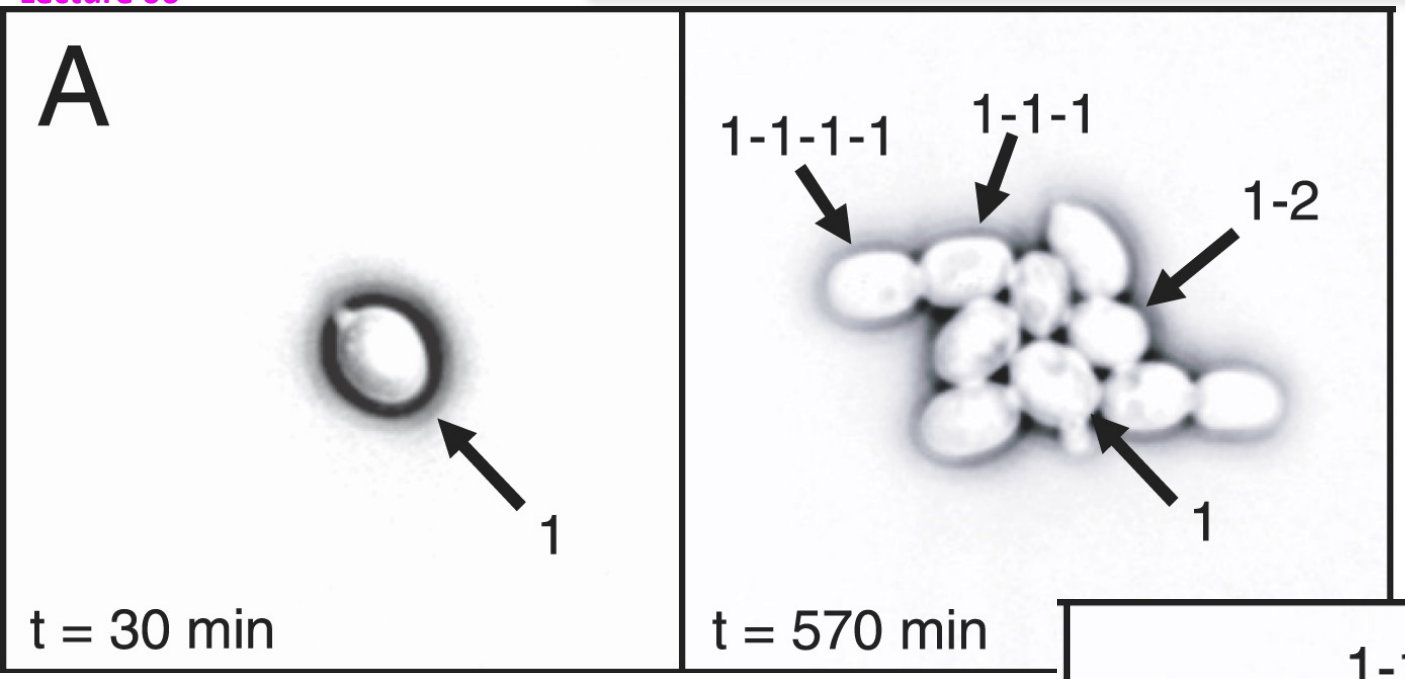
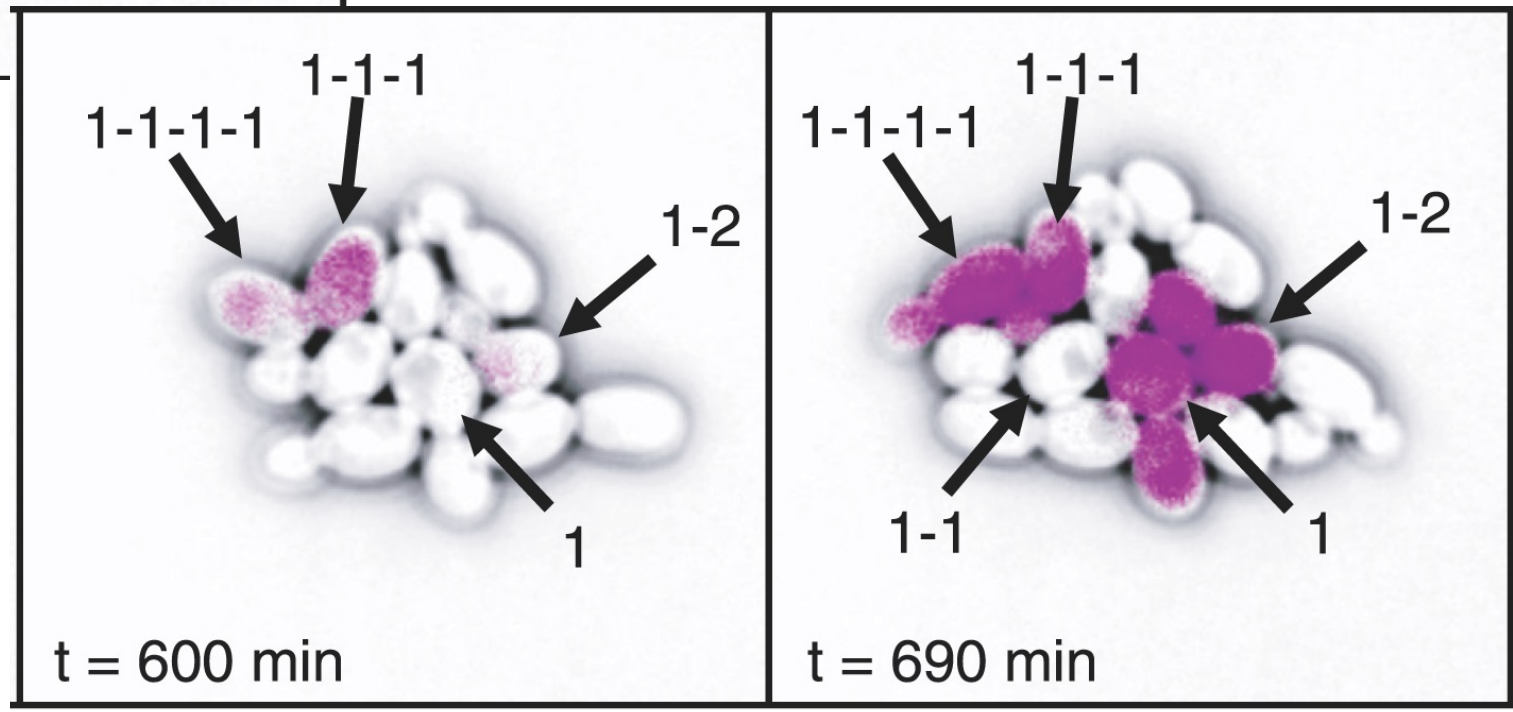
Gene expression in a population



Expression of galactose utilization genes in the yeast *Saccharymyces cerevisiae*



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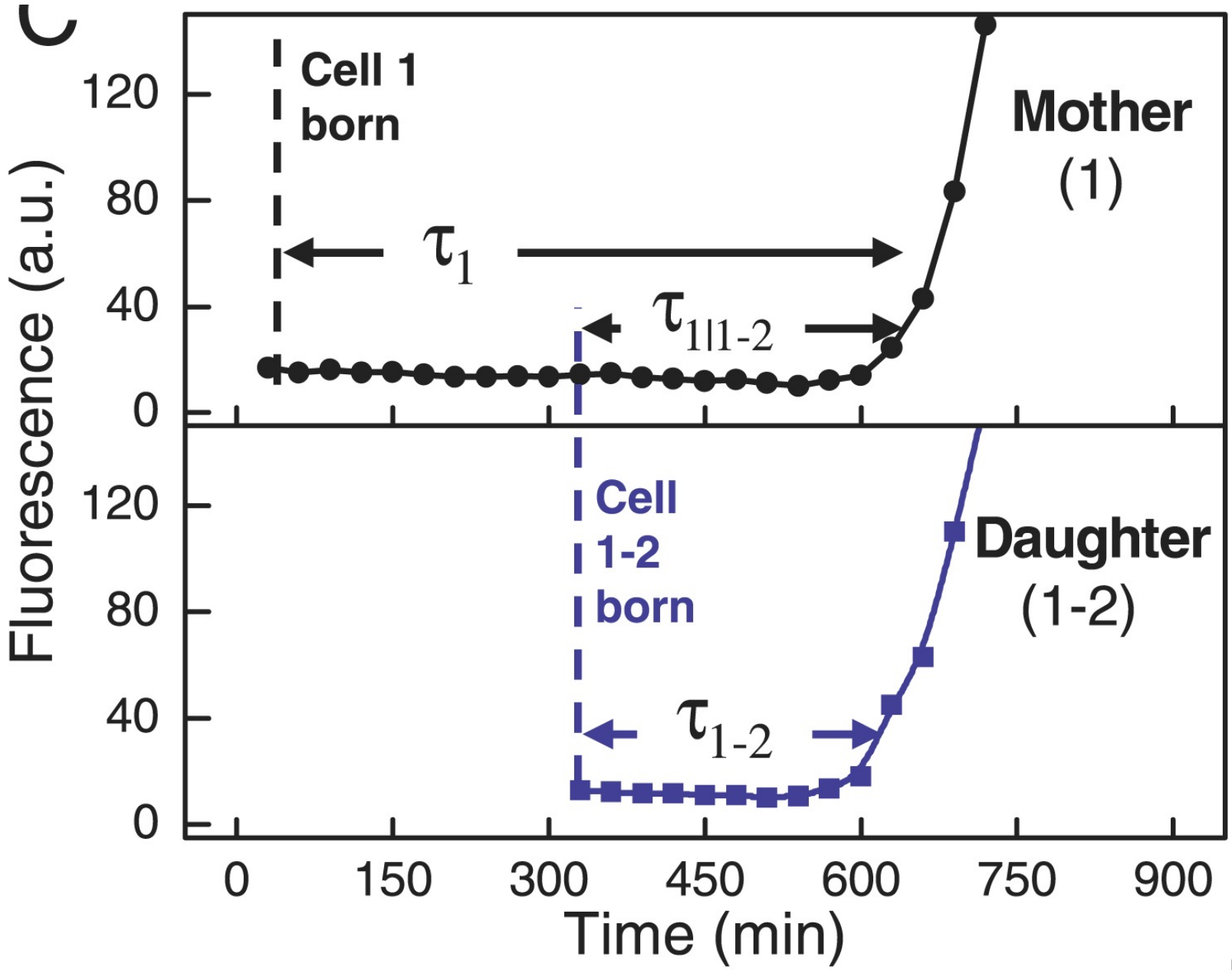
Gene expression in a population



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Boeing 737-800 cockpit

http://www.aviafilm
free-airplane-pictur

Glossary

Promoter: region of DNA where RNA polymerase / CAP binds
Operator: region of DNA where repressor or inducer binds

Repressor: DNA binding protein – binds to the operator and prevents gene expression
Inducer: DNA binding protein – binds to the operator and induces gene expression
Catabolite activator protein – binds to the promoter region and activates gene expression by facilitating the binding of RNA polymerase

Tryptophan, allolactose, cAMP: small molecule modulators