Welcome back!!

- Find your nametag and a seat

Welcome back!!

- Find your nametag and a seat
- Turn to a partner and tell them about a place that you most want to travel to

Class 3: Bacterial genetics, movement, and communication

March 23

Last time

- The growth phases of bacteria in a batch culture

Last time

- The growth phases of bacteria in a batch culture
- Biofilm formation

Last time

- The growth phases of bacteria in a batch culture
- Biofilm formation

What do these two topics have in common? What drives bacterial growth and biofilm development?

Today: Bacterial genetics!

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Plus two more applications of genetics: movement and cell communication

What does a eukaryotic genome look like?

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Multiple linear chromosomes - diploid

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Protein-coding regions but also a lot of non-protein-coding regions

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DNA coiled around histones

How is a prokaryotic genome different?

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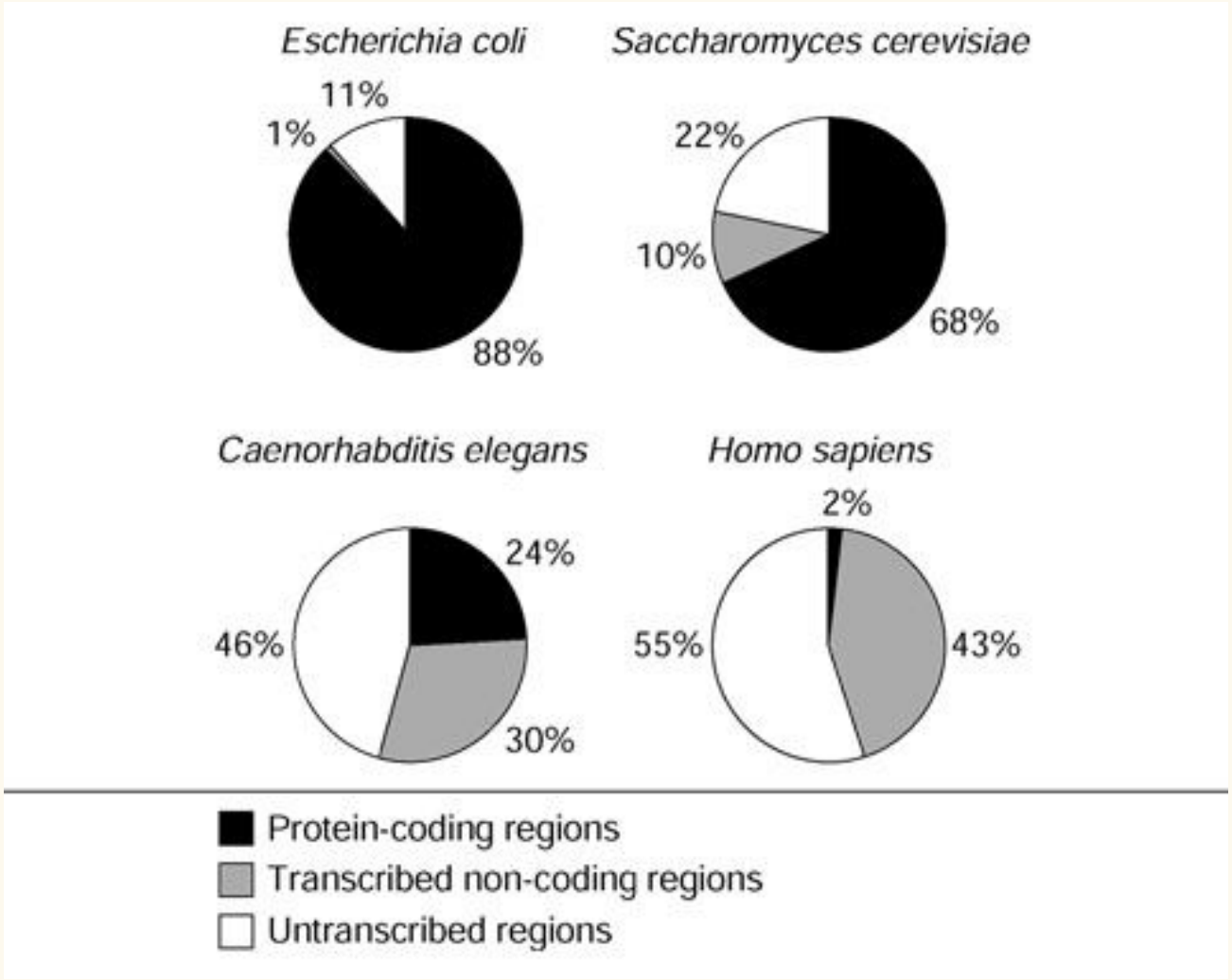
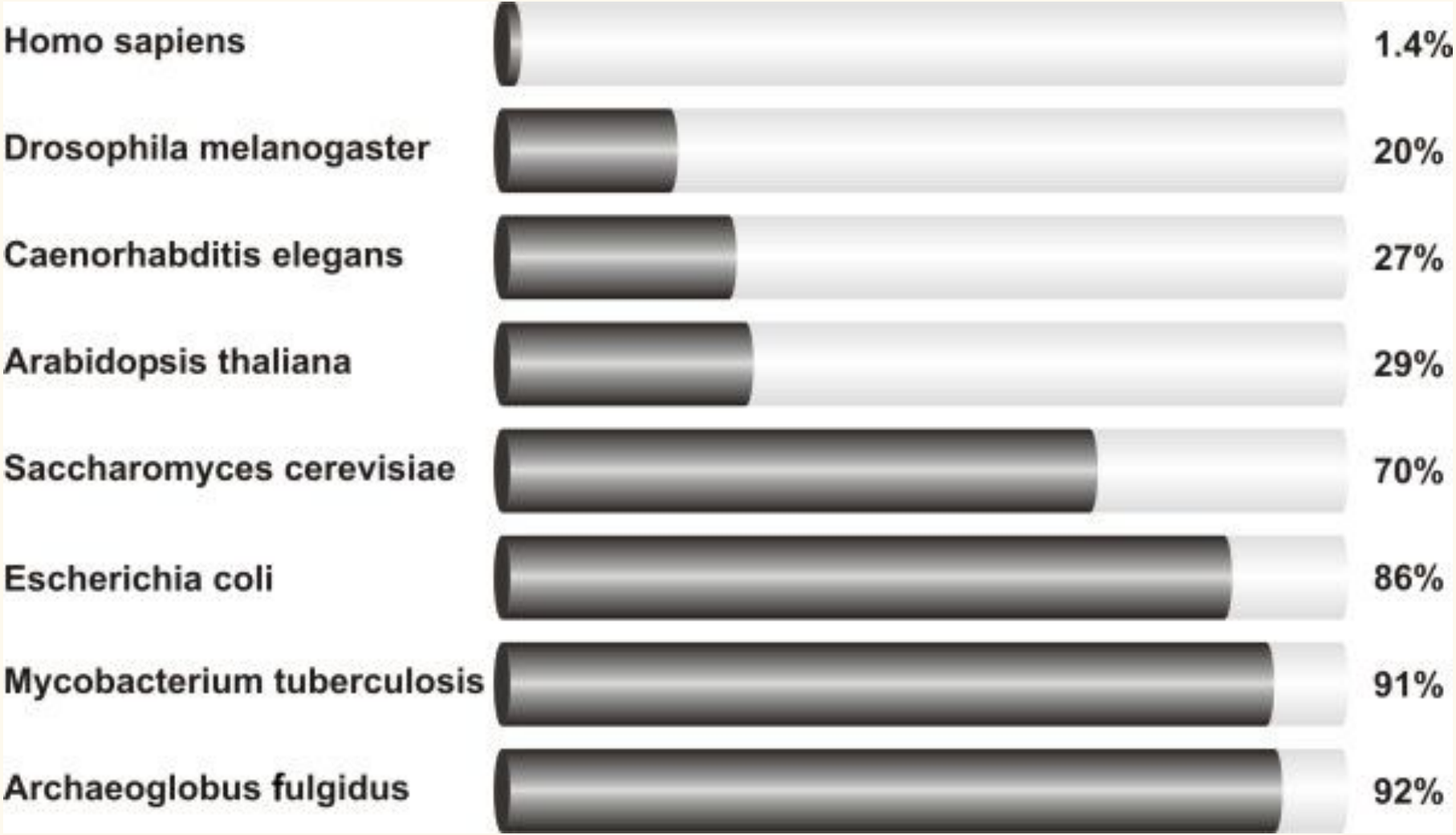
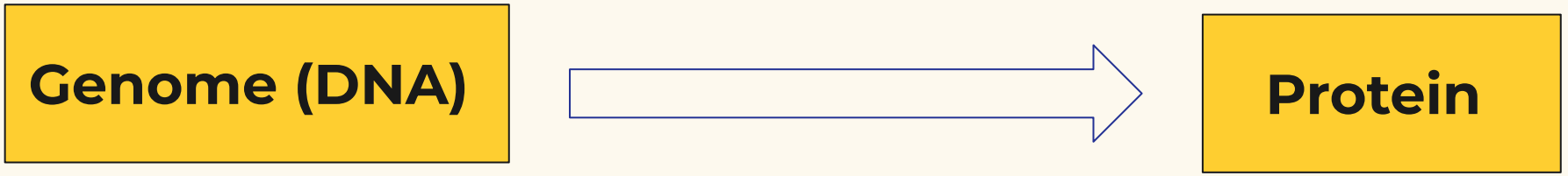
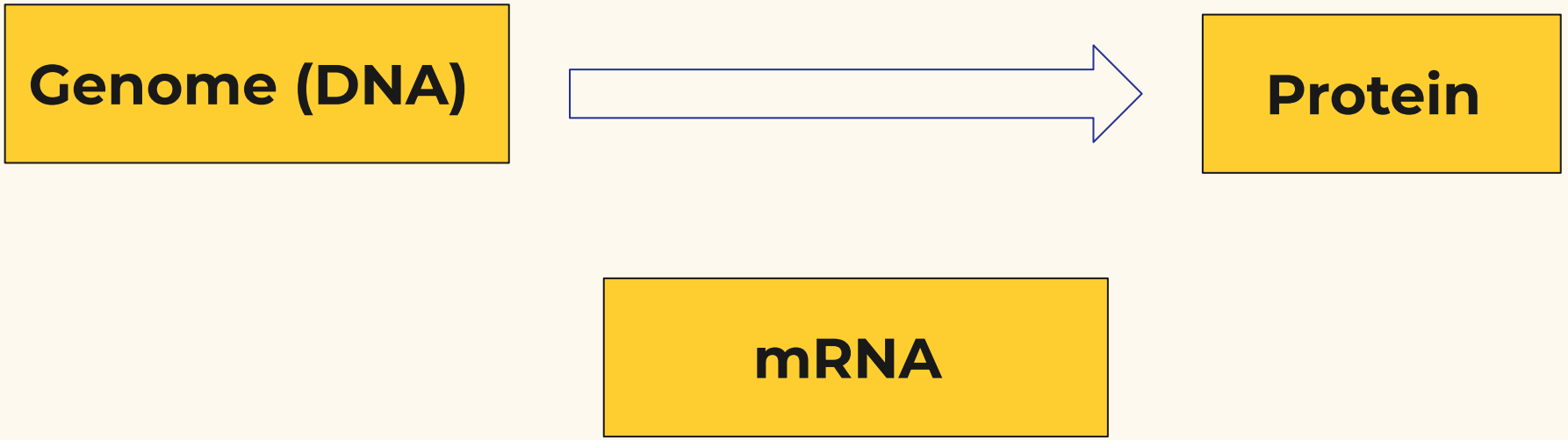
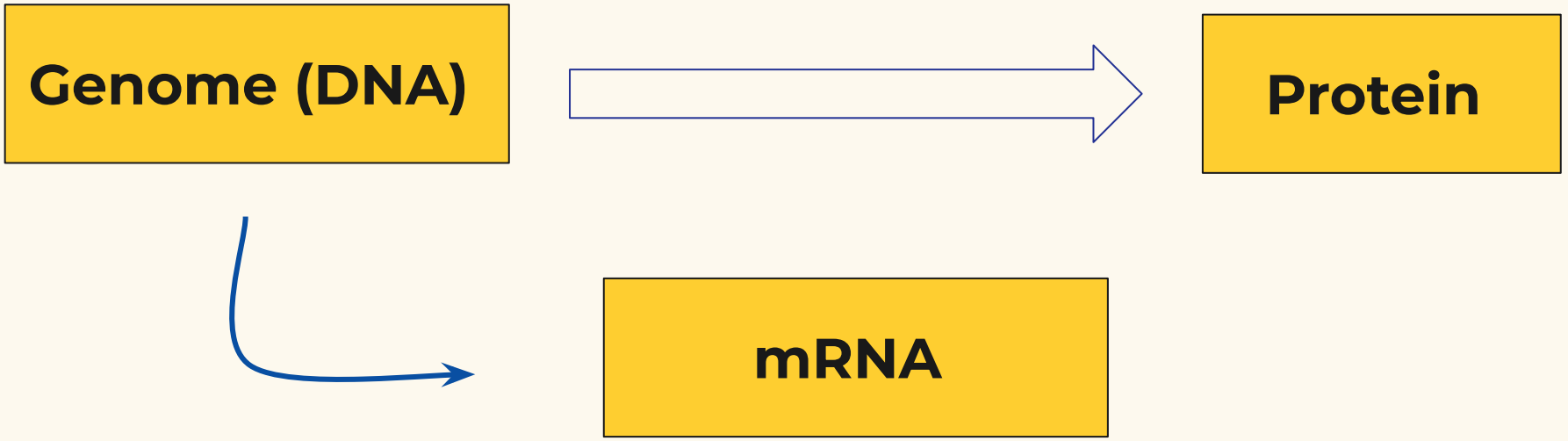
No histones- DNA forms a supercoil


Escherichia coli
11%
1%
88%
Saccharomyces cerevisiae
22%
10%
68%
Caenorhabditis elegans
24%
46%
30%
Homo sapiens
2%
55%
43%
Protein-coding regions
Transcribed non-coding regions
Untranscribed regions


Homo sapiens
1.4%
Drosophila melanogaster
20%
Caenorhabditis elegans
27%
Arabidopsis thaliana
29%
Saccharomyces cerevisiae
70%
Escherichia coli
86%
Mycobacterium tuberculosis
91%
Archaeoglobus fulgidus
92%


Genome (DNA)
Protein


Genome (DNA)
Protein
mRNA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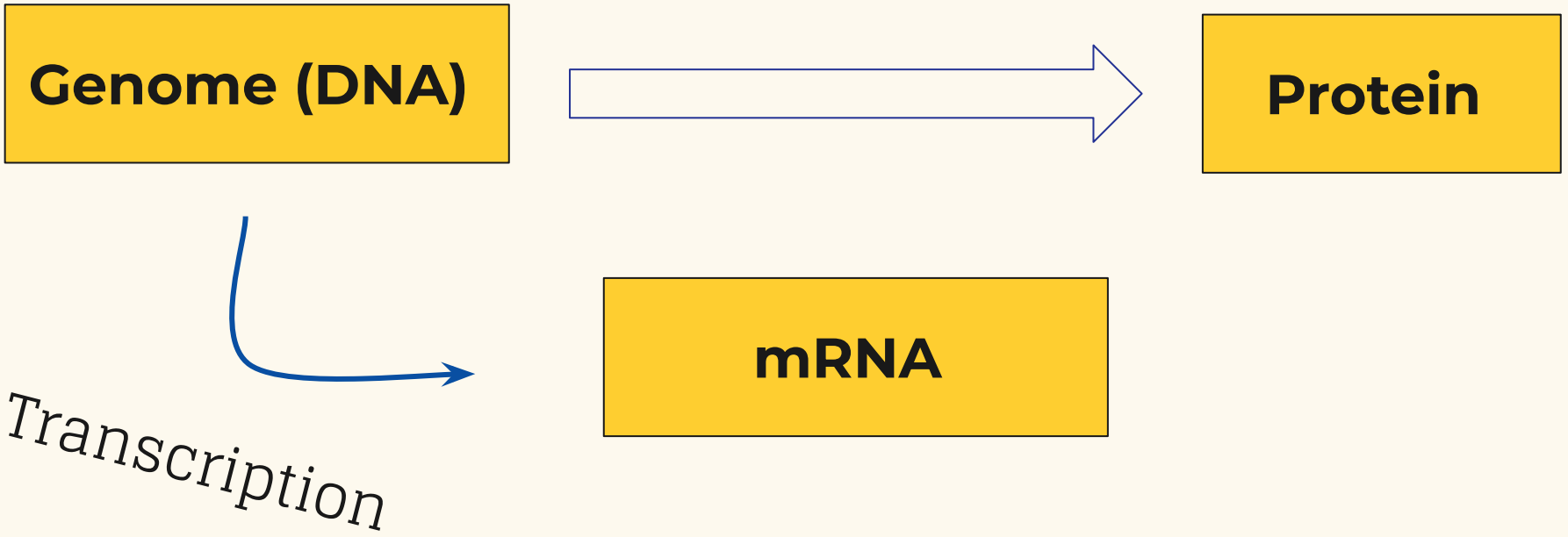
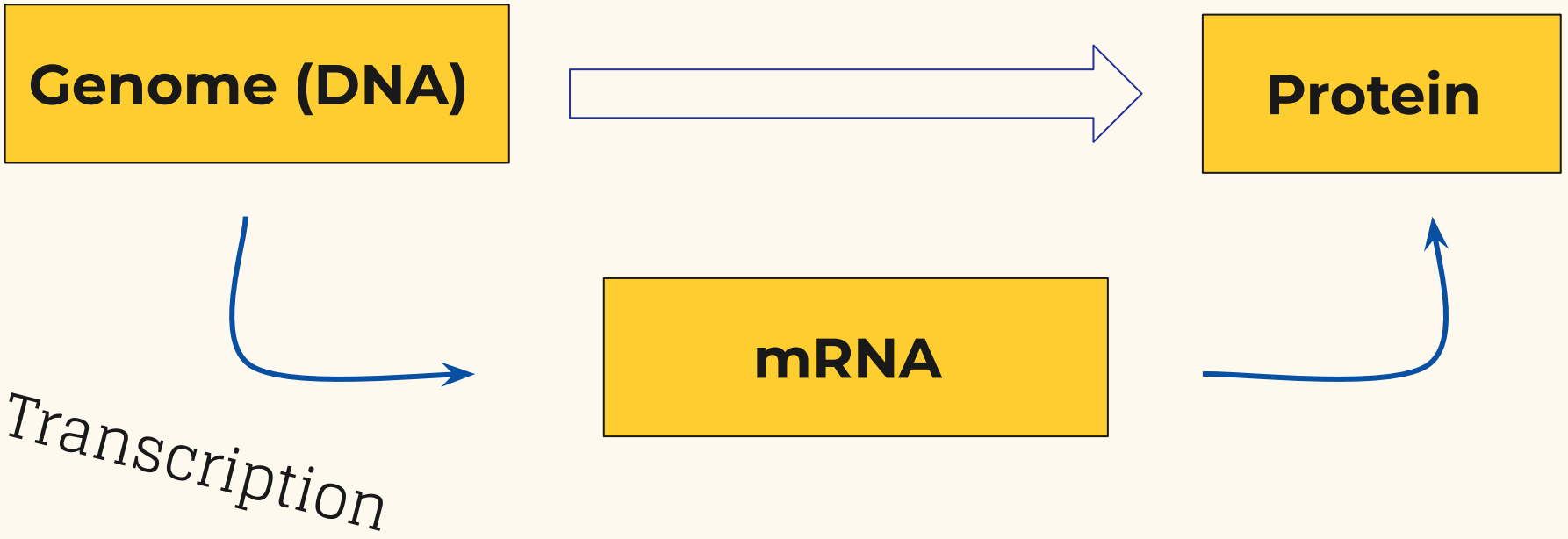

Genome (DNA)
Protein
mRNA
Transcription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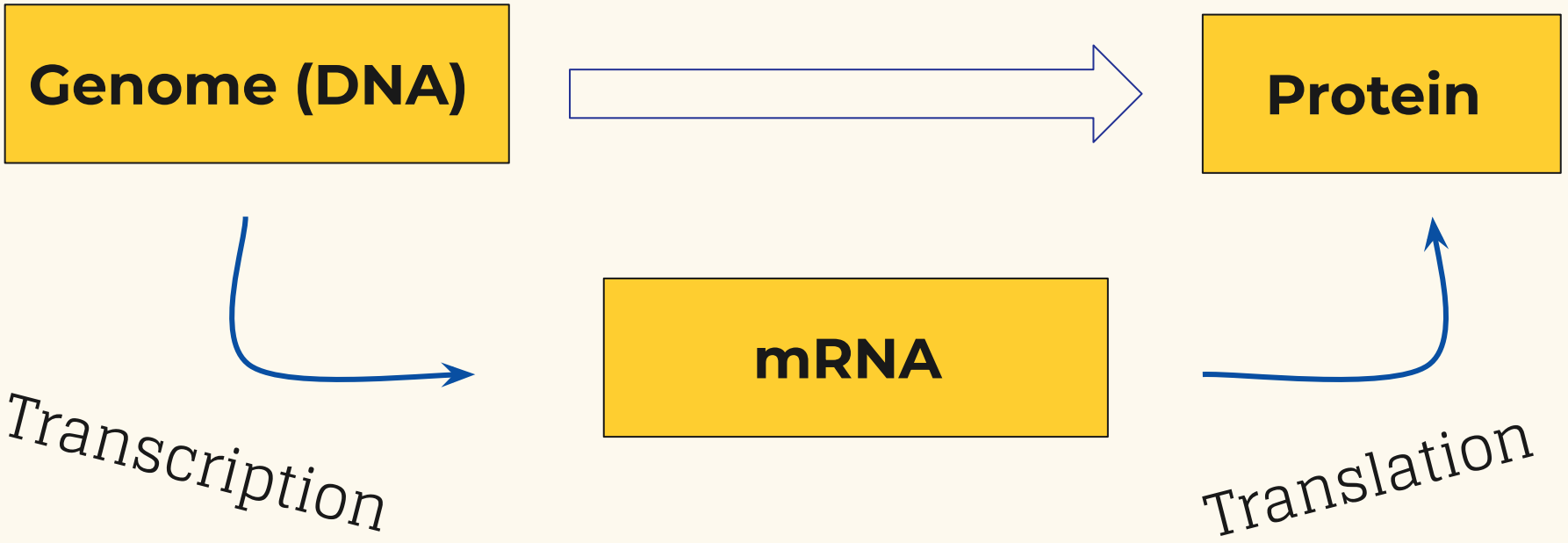
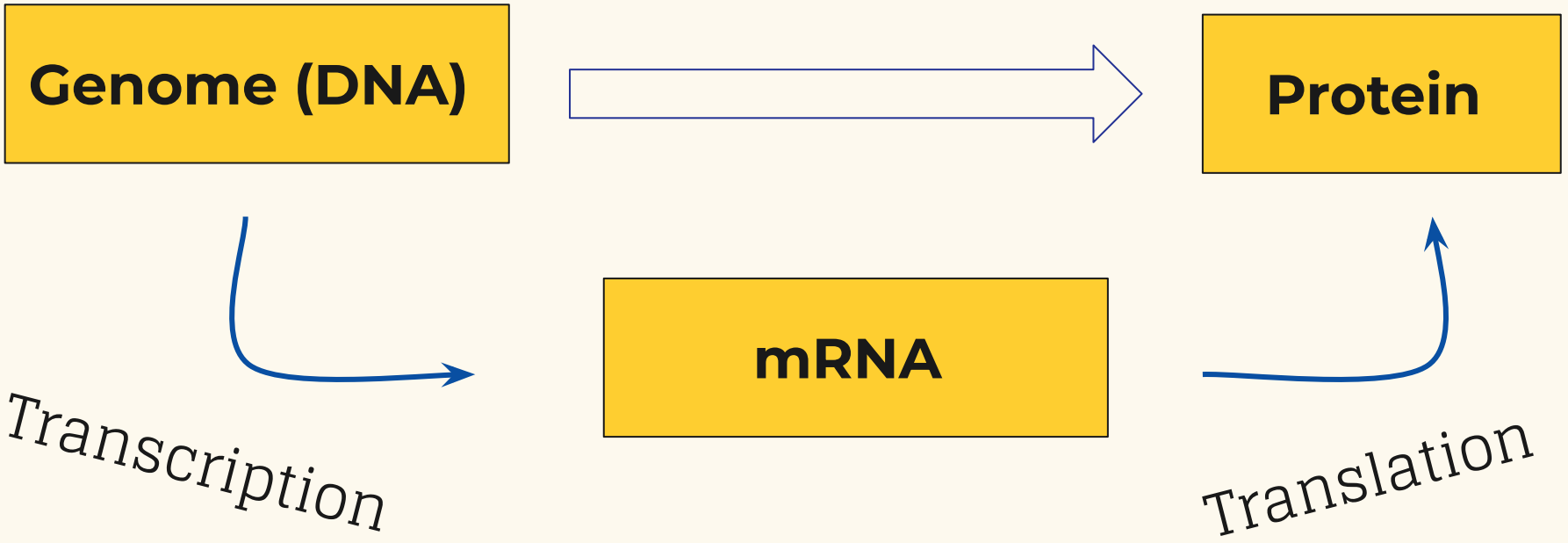

Genome (DNA)
Protein
mRNA
Transcription
Translation

The central dogma



Think, pair, share

Rate of transcription in eukaryotes vs. prokaryotes

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Rate of transcription in eukaryotes vs. prokaryotes

Which will be faster? Which has more regulation?

Think, pair, share

Rate of transcription in eukaryotes vs. prokaryotes

Eukaryotes: regulatory elements like introns and histones

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Rate of transcription in eukaryotes vs. prokaryotes

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Transcription and translation occur in different locations

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Rate of transcription in eukaryotes vs. prokaryotes

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SLOW

Think, pair, share

Rate of transcription in eukaryotes vs. prokaryotes

Prokaryotes: fewer steps involved to bypass regulation

Think, pair, share

Rate of transcription in eukaryotes vs. prokaryotes

Prokaryotes: fewer steps involved to bypass regulation
Transcription and translation can happen at the same time, in the same place

FAST

So how do prokaryotes regulate gene expression?

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RNA polymerase: the protein in charge of transcription

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Sigma (σ) factor: a secret, behind-the-scenes friend that helps guide RNA polymerase into place

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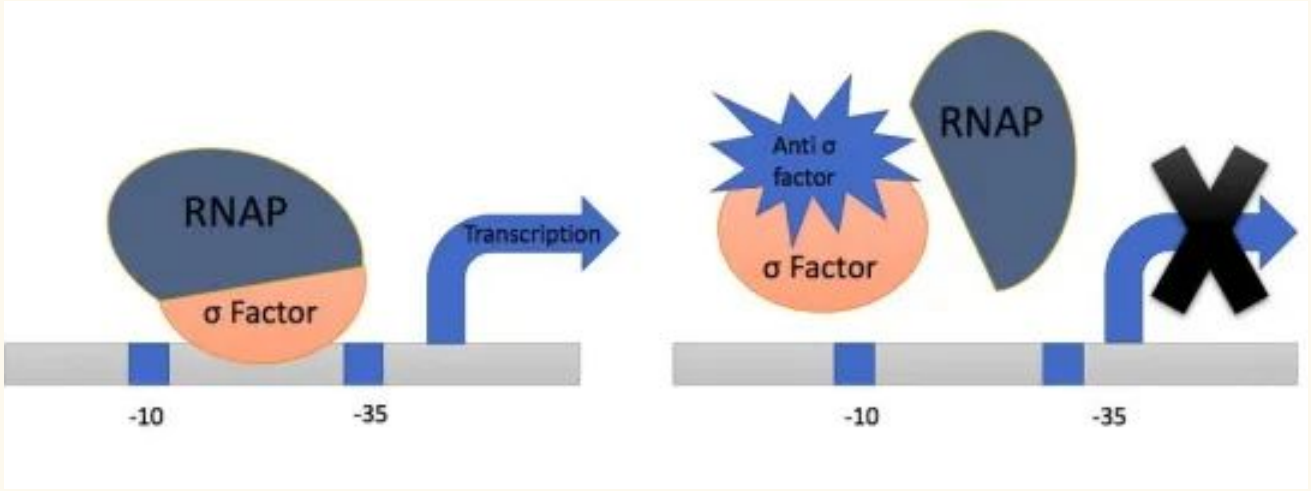

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Different sigma factors are used in different conditions

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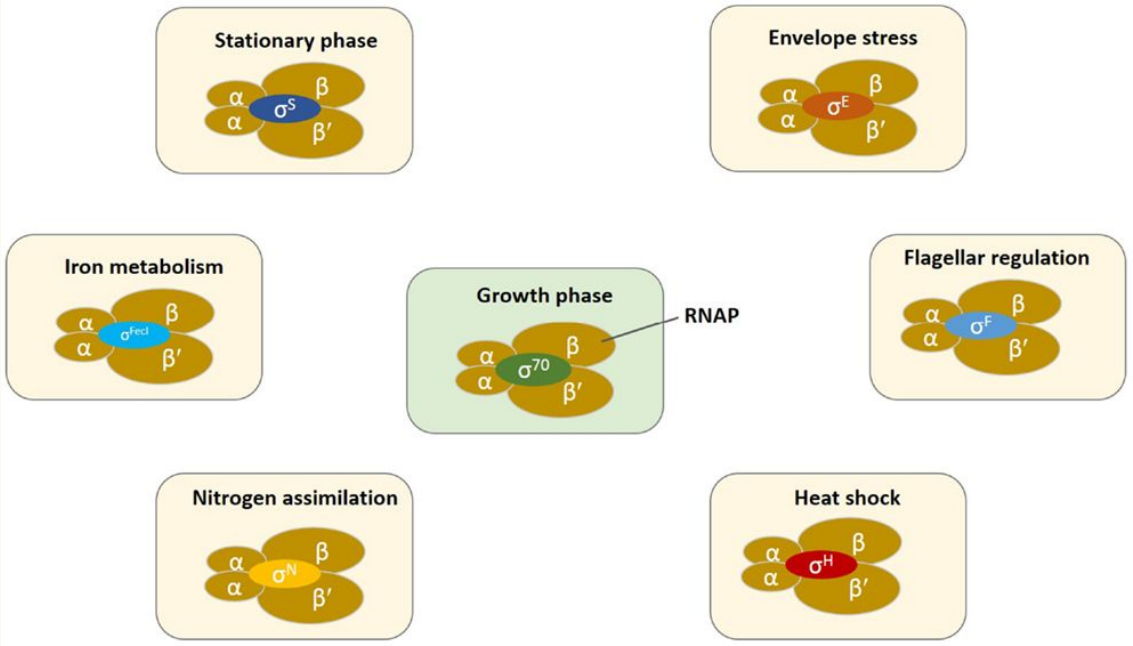

Gene structure

Sigma factor binds to the *promoter* region of a gene

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Or genes, plural

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- **Operon**: a unit of linked genes that share regulatory regions (promoter and operator regions)

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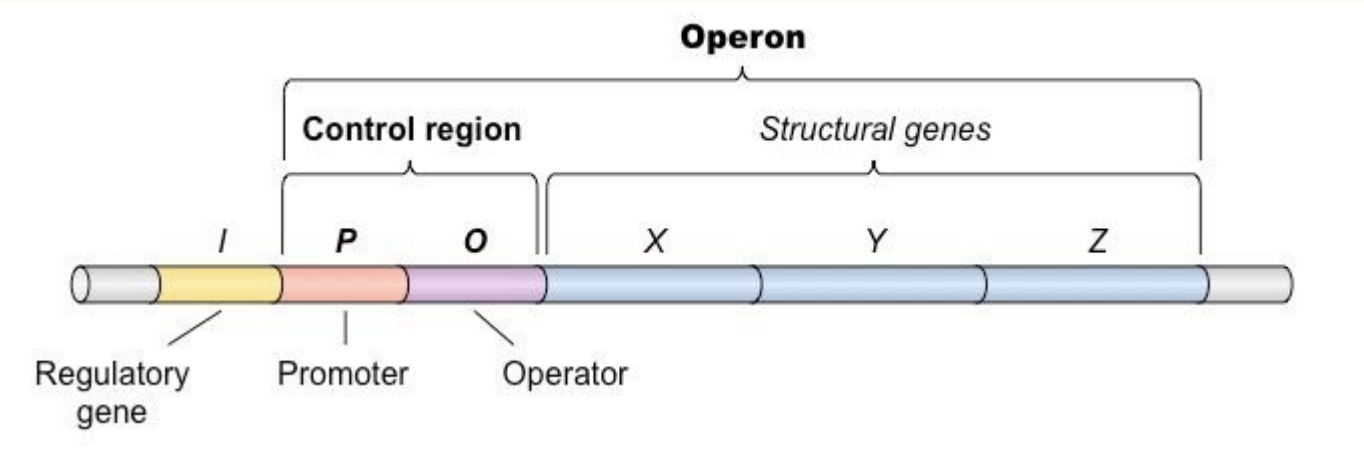
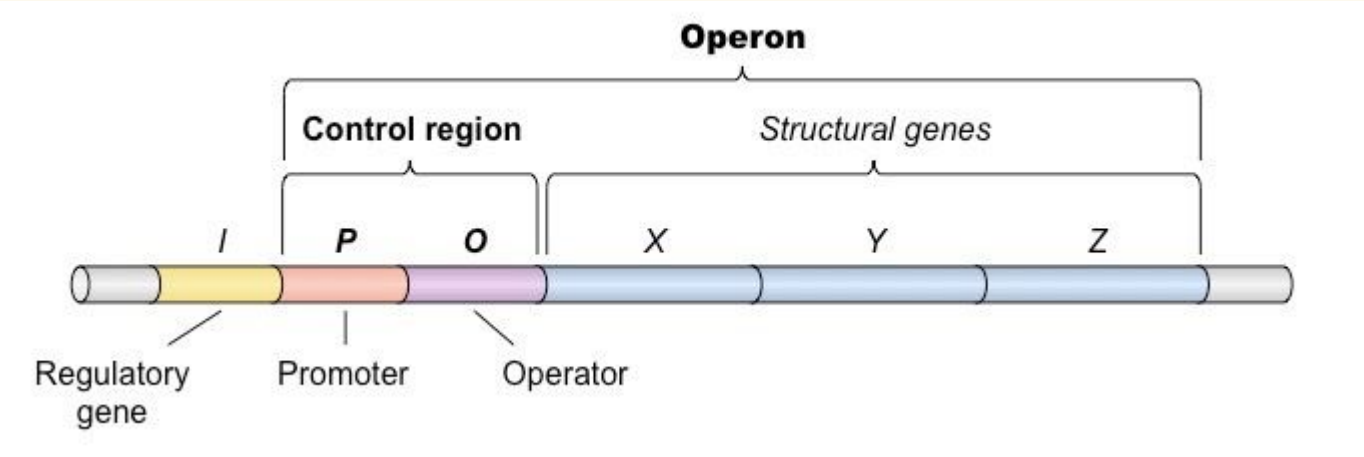


Gene structure

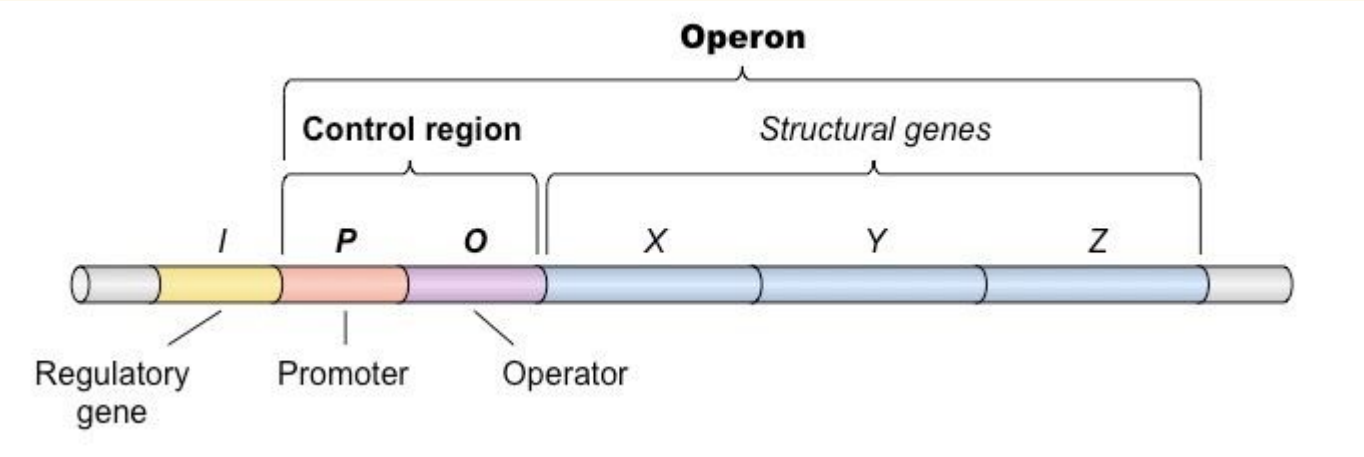
Structural genes: encode for a protein that does actual work in the cell



Gene structure

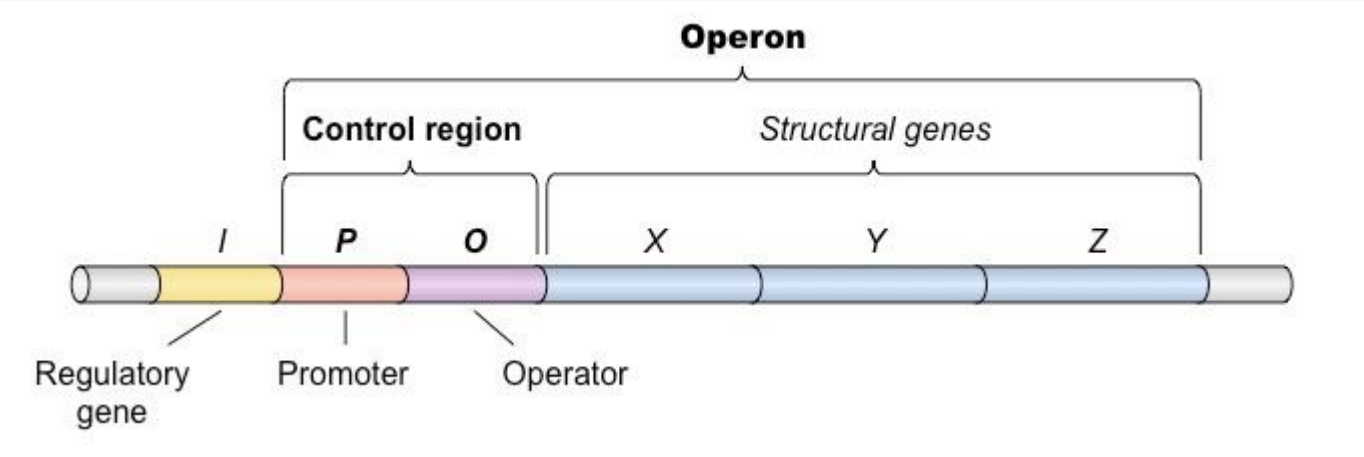
Structural genes: encode for a protein that does actual work in the cell

Are X, Y, and Z going to be related genes? Why is this useful?



Gene structure

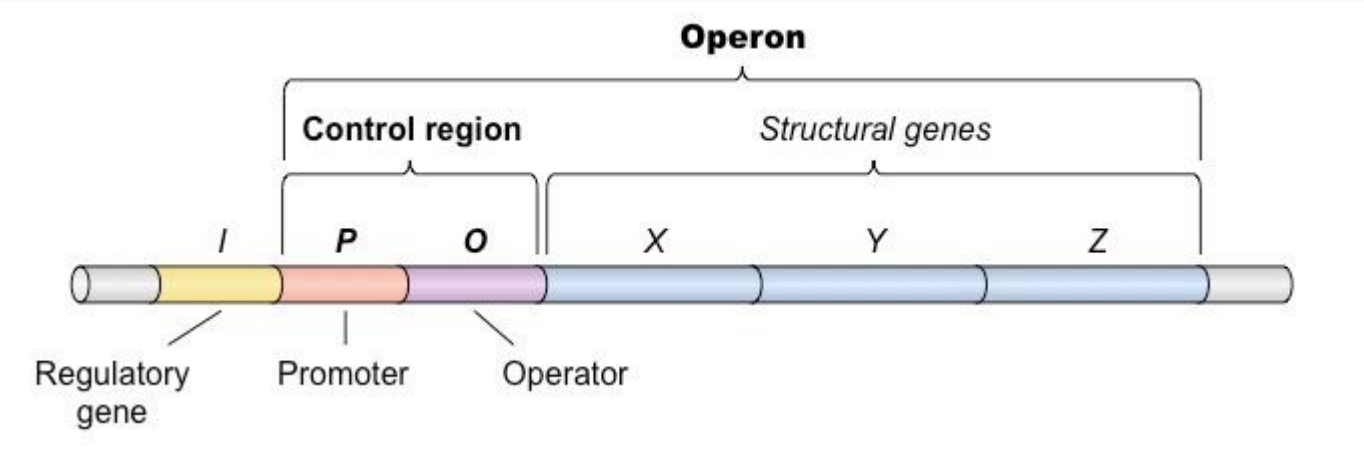
Operator: where an inhibitory protein may bind

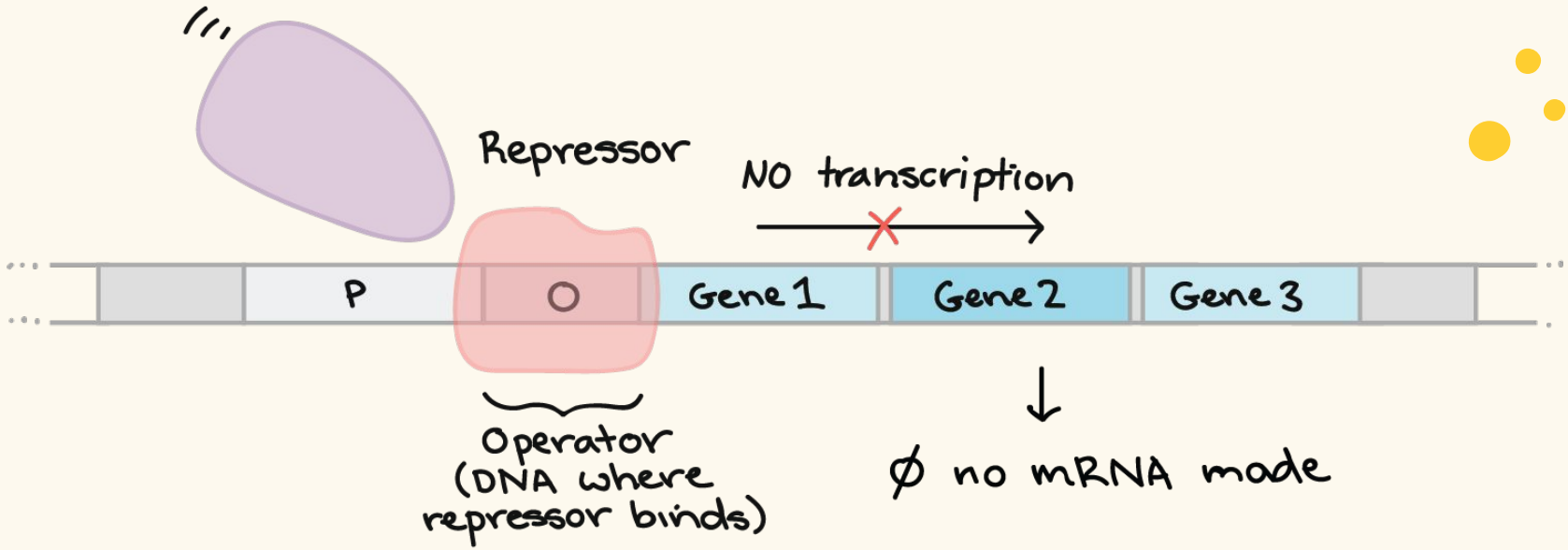


Gene structure

Operator: where an inhibitory protein may bind

Why does it make sense for an operator to be located further upstream (closer to the structural genes) than the promoter?




Repressor
NO transcription
P
O
Gene 1
Gene 2
Gene 3
Operator
(DNA where
repressor binds)
∅ no mRNA made

A specific example: the *lac* operon

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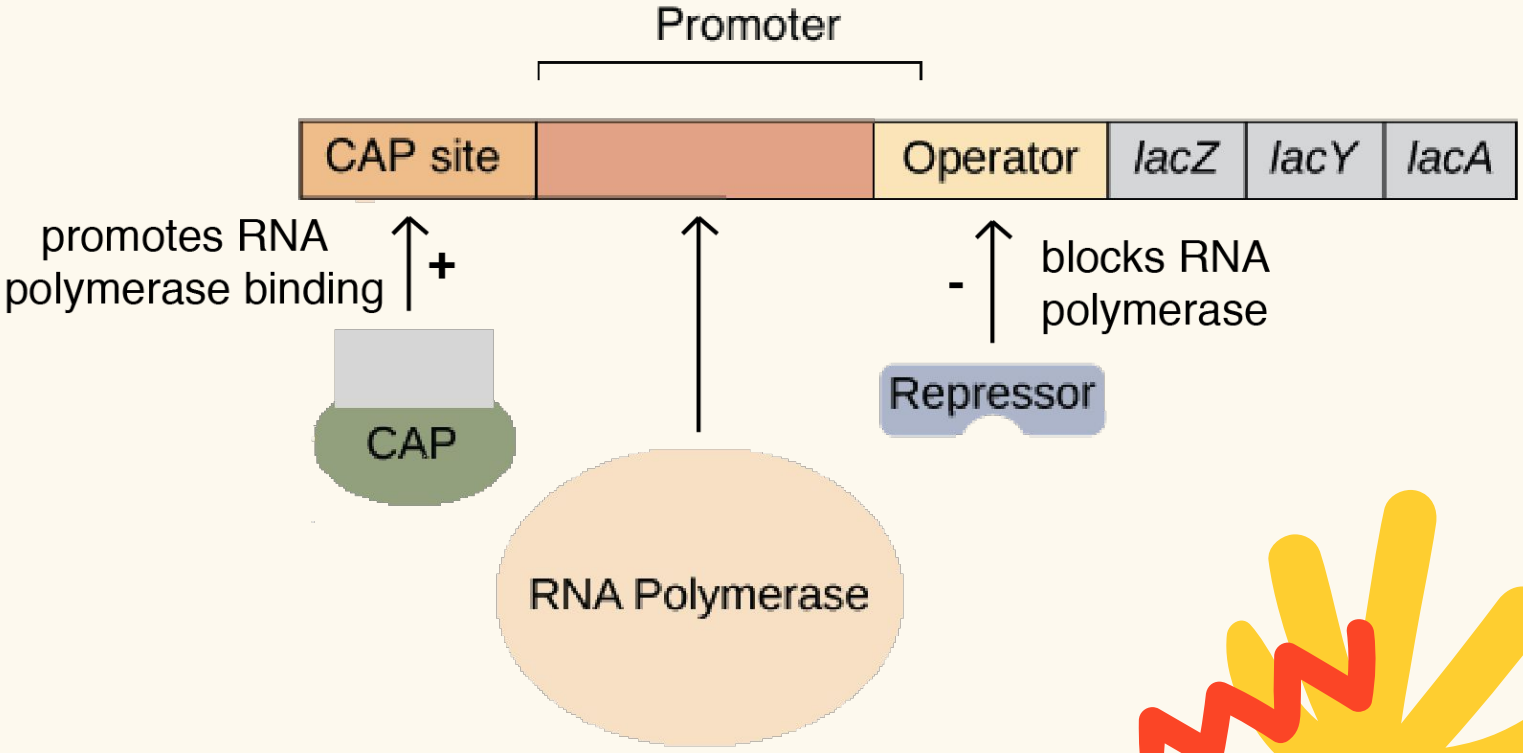

A specific example: the *lac* operon

Inducible gene: default off

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(As compared to **repressible genes**)

A specific example: the *lac* operon

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In most conditions: has a **repressor** bound to the operator site

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Prevents RNA polymerase from transcribing the gene

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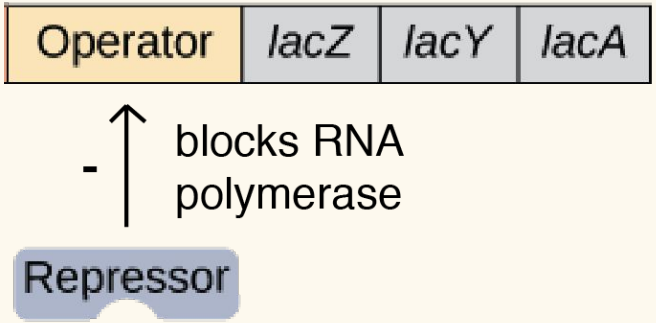
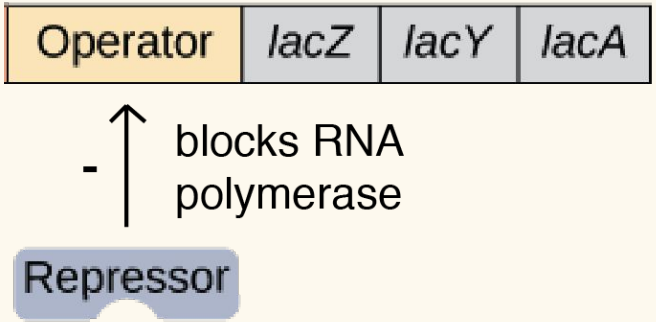


A specific example: the *lac* operon

Lactose: can break down into a form called **allolactose**



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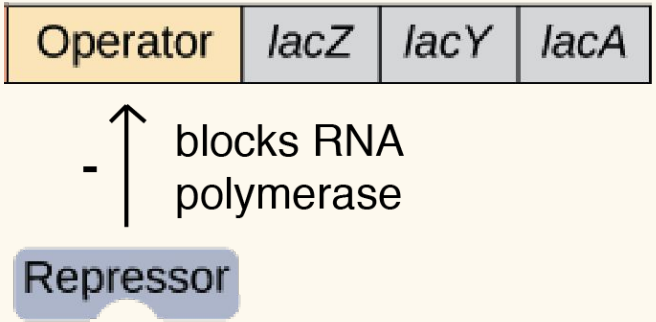
Lactose: can break down into a form called **allolactose**

Allolactose binds to the repressor, causing a **conformational change** that releases it from the operator



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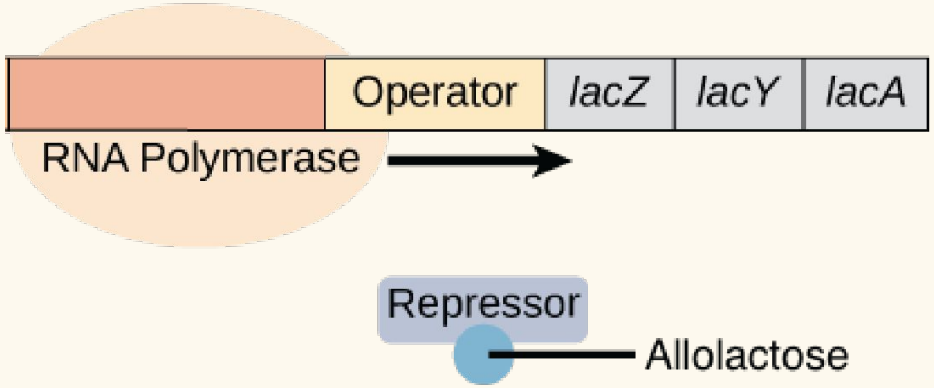

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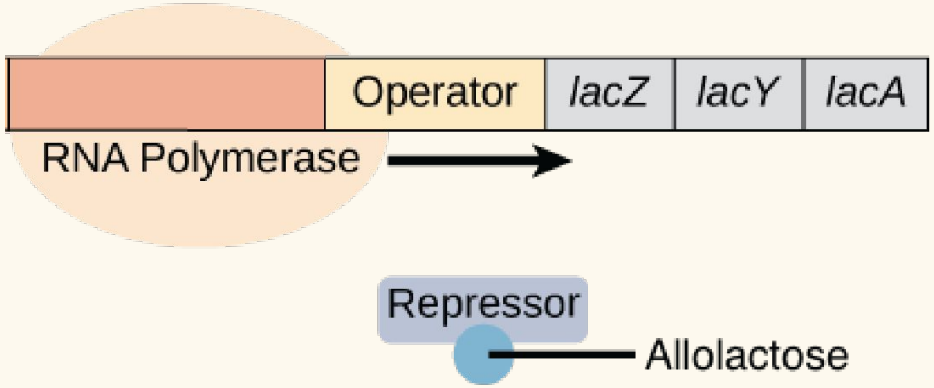
RNA polymerase is free to transcribe the structural genes



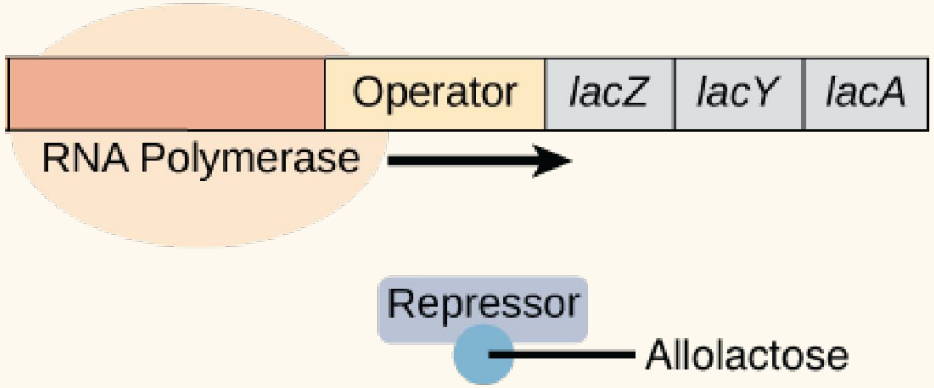
Think, pair, share

Brainstorm some metaphors that you could use to explain the *lac* operon and inducible genes.

(What are some activities or things that you do that are “default off”?)

A specific example: the *lac* operon

So the structural genes are activated when lactose is present, and they help to break down lactose and use it for energy



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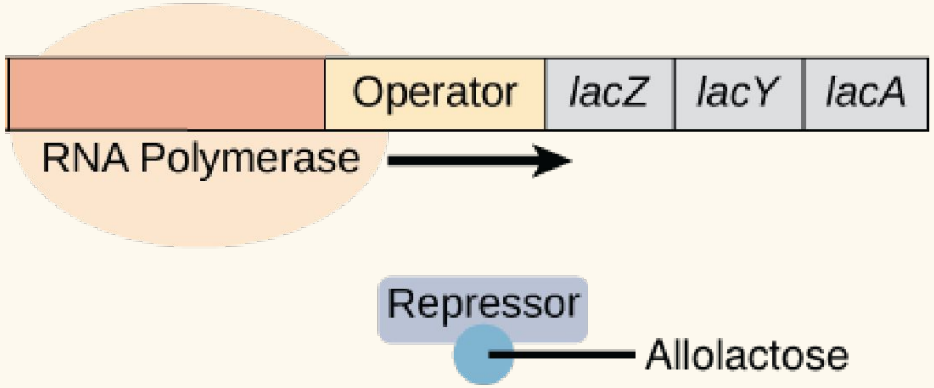
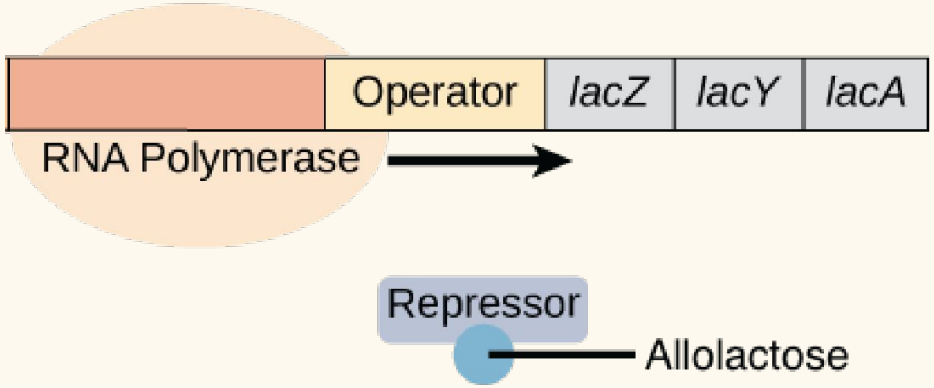
Preferred energy source??



A specific example: the *lac* operon

When glucose (the preferred energy source) is abundant: *lac* activity is off



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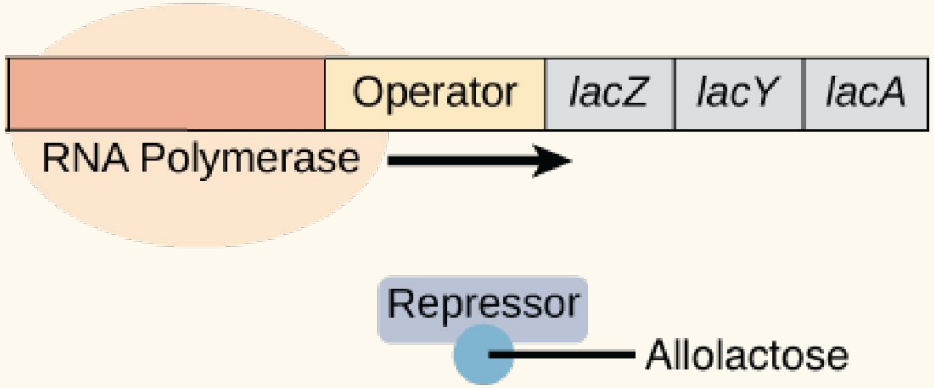
How does the cell know when glucose is low enough for *lac* transcription to become necessary?



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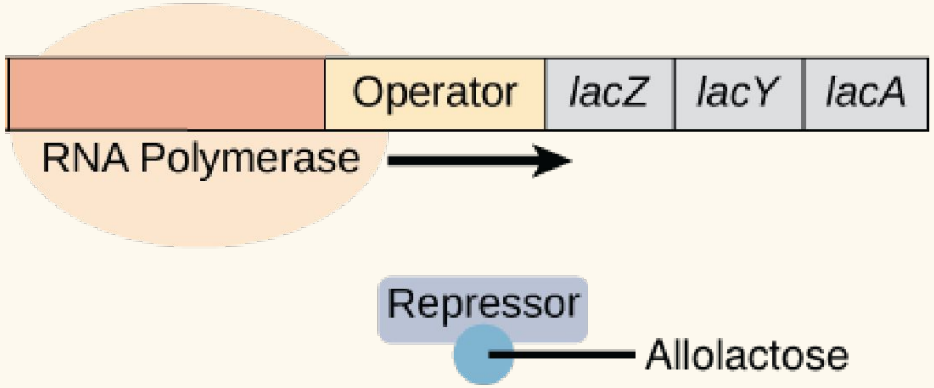

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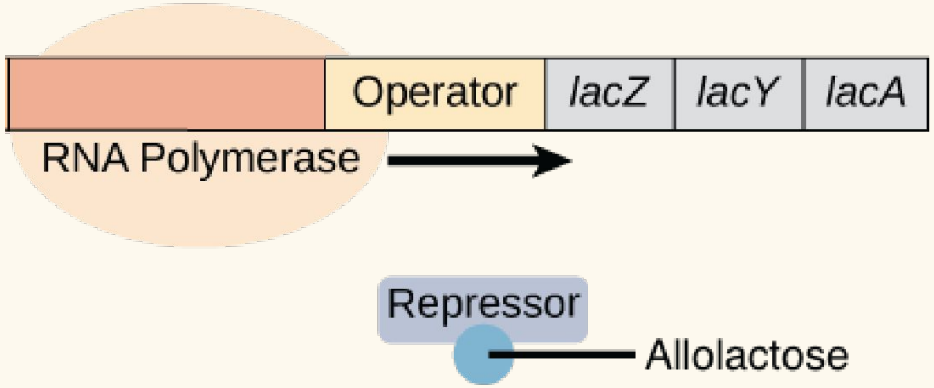
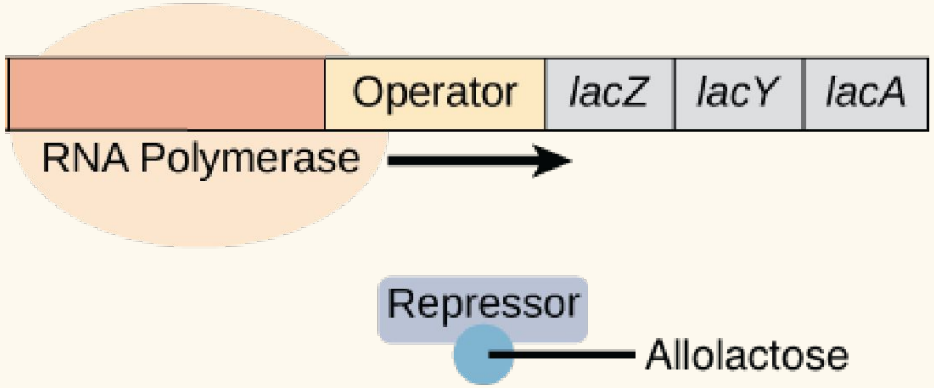


A specific example: the *lac* operon

cAMP: the hunger molecule



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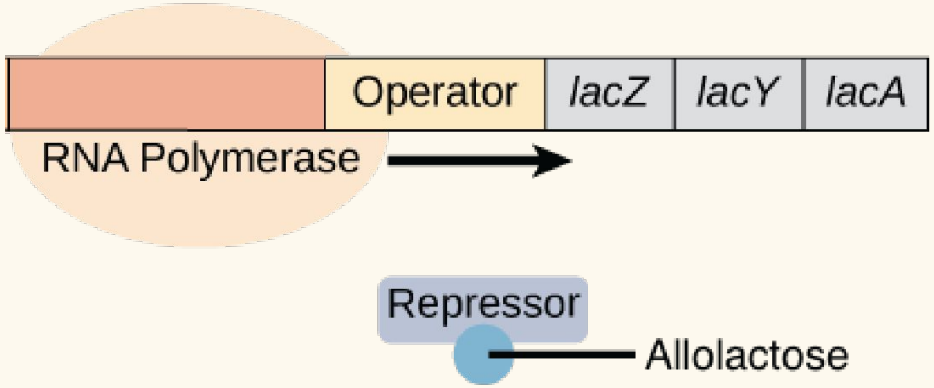
Only made when there's low amounts of glucose



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cAMP: the hunger molecule
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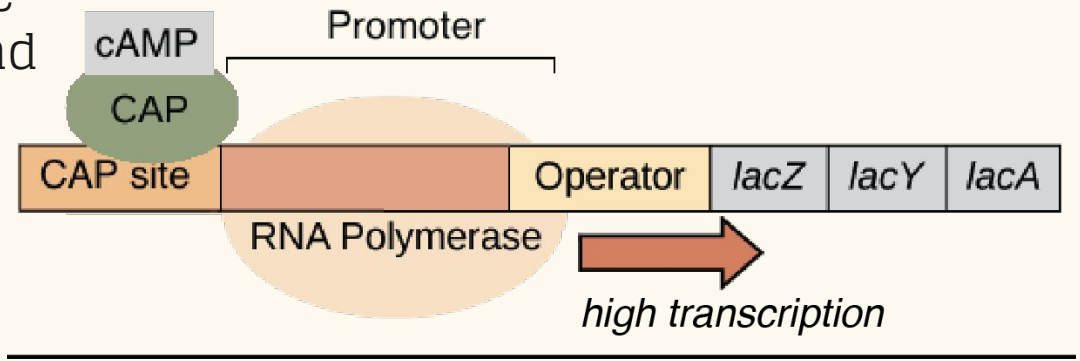
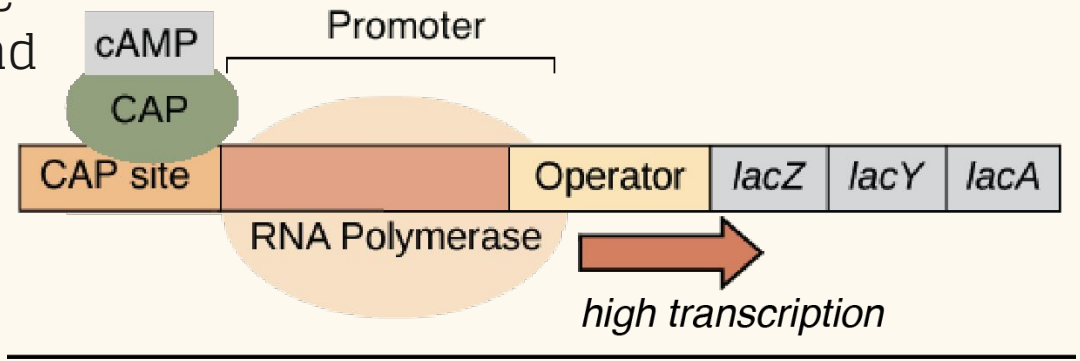
Binds to CAP, which can bind to the DNA



A specific example: the *lac* operon

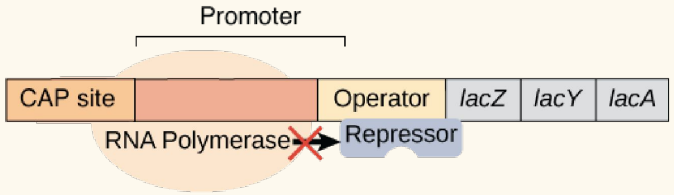
cAMP: the hunger molecule
    Only made when there's low amounts of glucose
Binds to CAP, which can bind to the DNA
Helps improve the affinity of RNA polymerase for the promoter region



A specific example: the *lac* operon

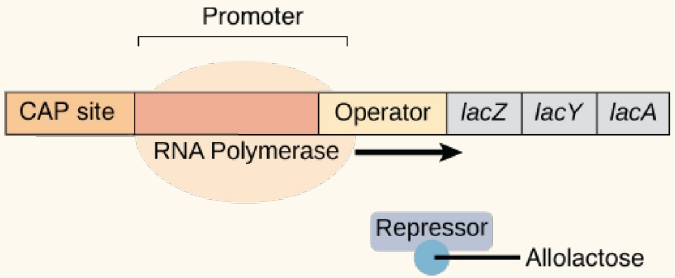
No lactose:

When lactose is absent, the *lac* repressor binds tightly to the operator. It gets in RNA polymerase's way, preventing transcription.



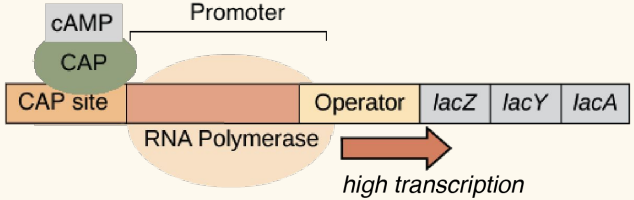
With lactose:

Allolactose (rearranged lactose) binds to the *lac* repressor and makes it let go of the operator. RNA polymerase can now transcribe the operon.



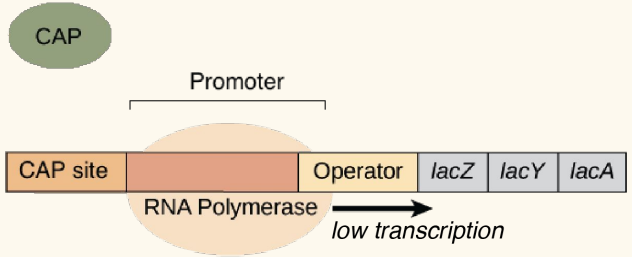
Low glucose:

When glucose levels are low, cAMP is produced. The cAMP attaches to CAP, allowing it to bind DNA. CAP helps RNA polymerase bind to the promoter, resulting in high levels of transcription.



High glucose:

When glucose levels are high, no cAMP is made. CAP cannot bind DNA without cAMP, so transcription occurs only at a low level.



Think, pair, share

Low glucose
Lactose available

High glucose
Lactose unavailable

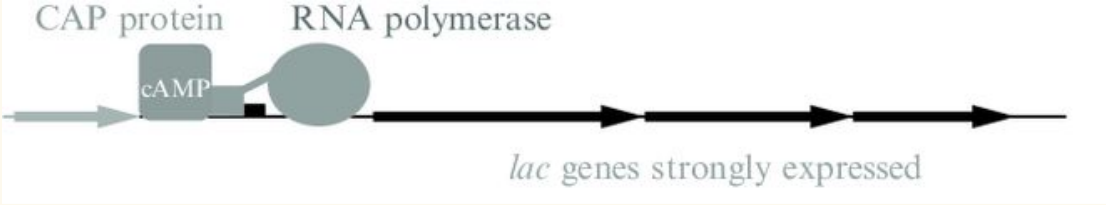
Low glucose
Lactose unavailable

High glucose
Lactose available

What does *lac* transcription look like in each of these four conditions?

Think, pair, share

Low glucose
Lactose available



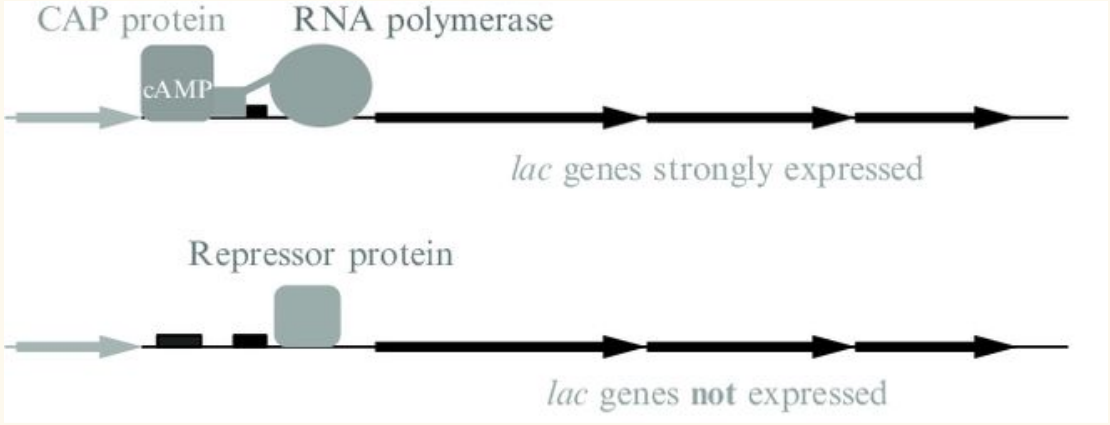
High glucose
Lactose unavailable

Low glucose
Lactose unavailable

High glucose
Lactose available

Think, pair, share

Low glucose
Lactose available



High glucose
Lactose unavailable

Low glucose
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Think, pair, share

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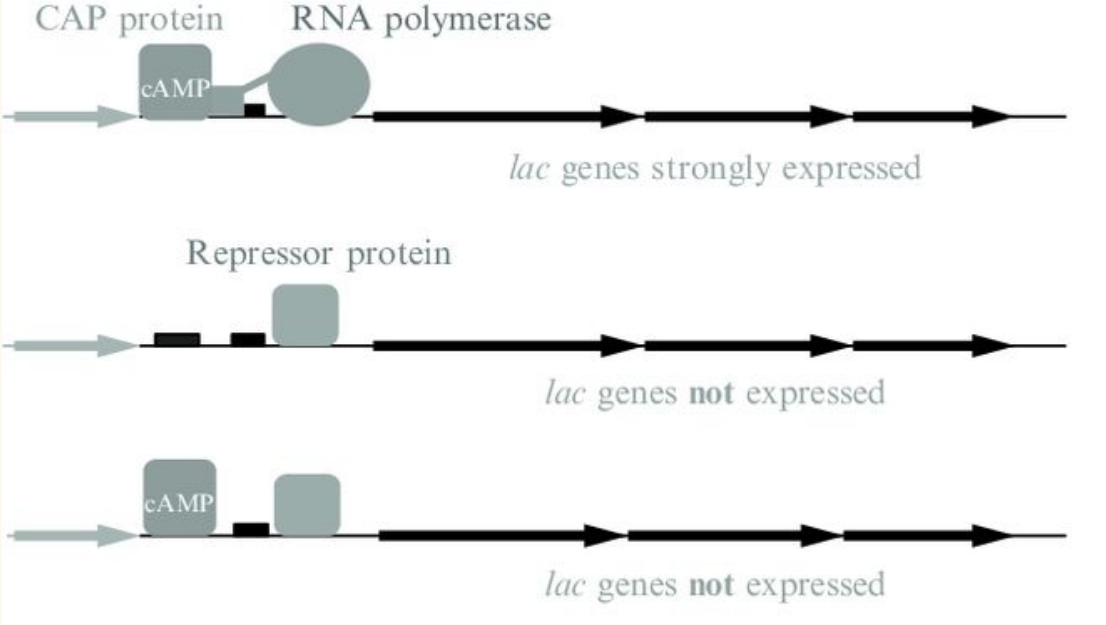
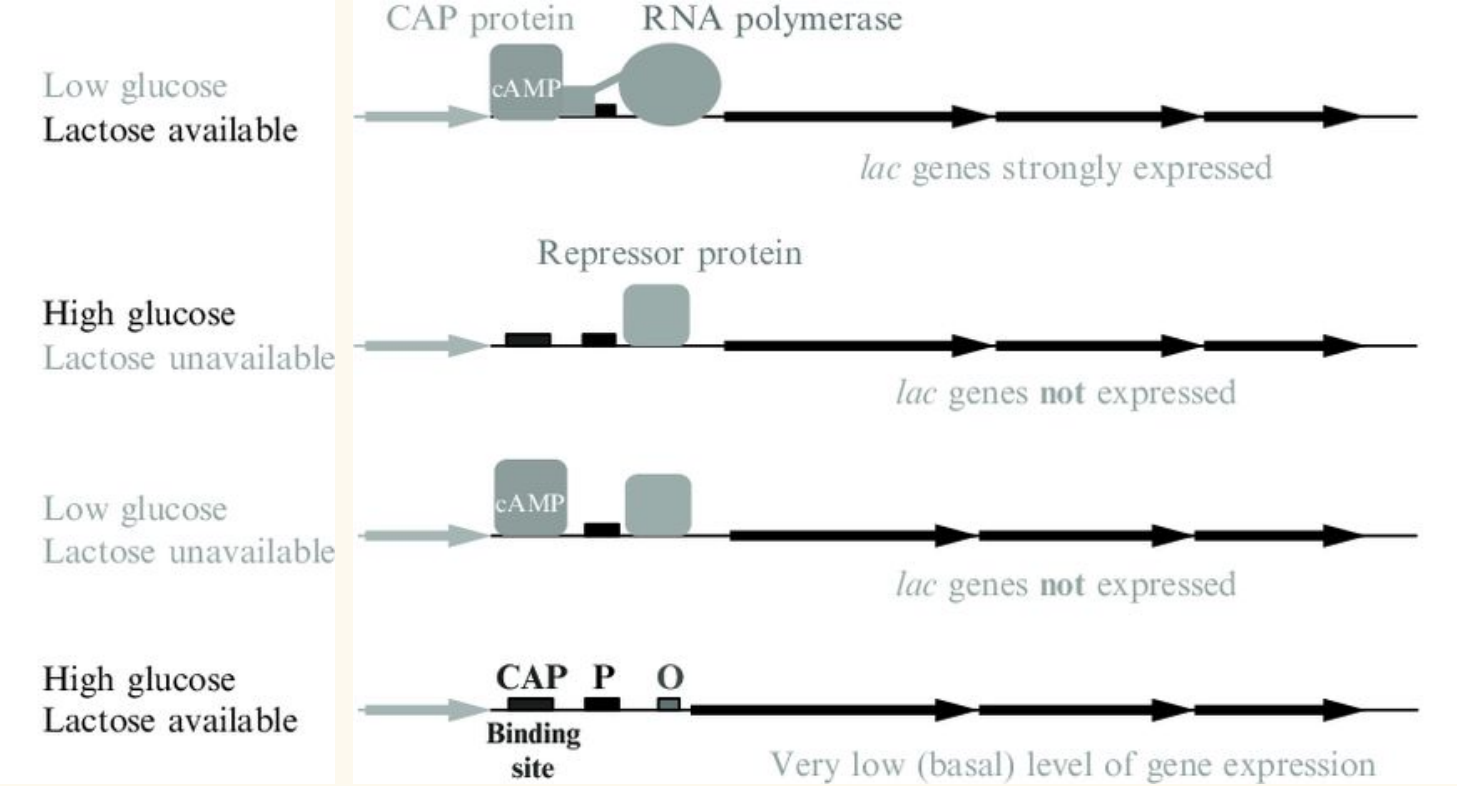


Think, pair, share



A different type of regulation: *trp* operon

Quorum sensing

A way to regulate genes beyond individual cells

How quorum sensing works

Cells secrete basal amounts of **AHL**, a signaling molecule, no matter where they are

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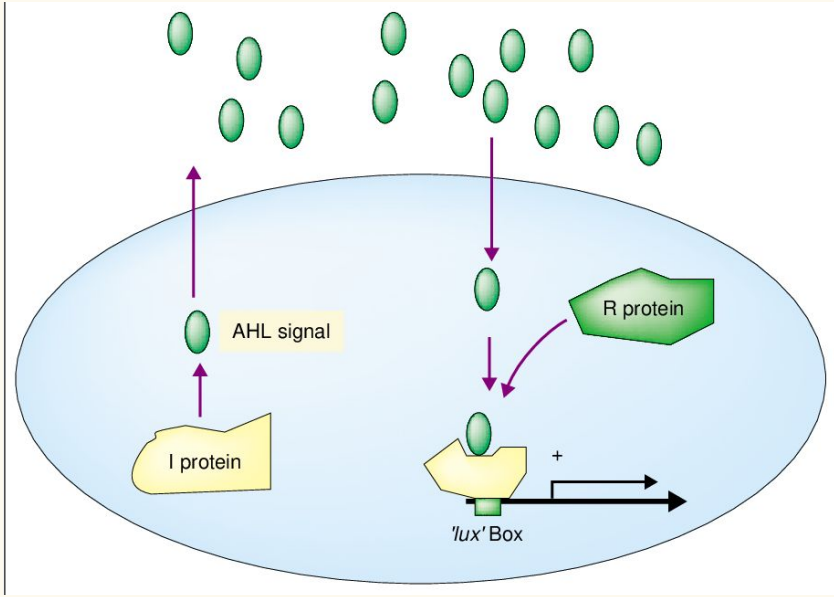

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Receiving AHL causes cells to secrete even more AHL – **positive feedback loop**

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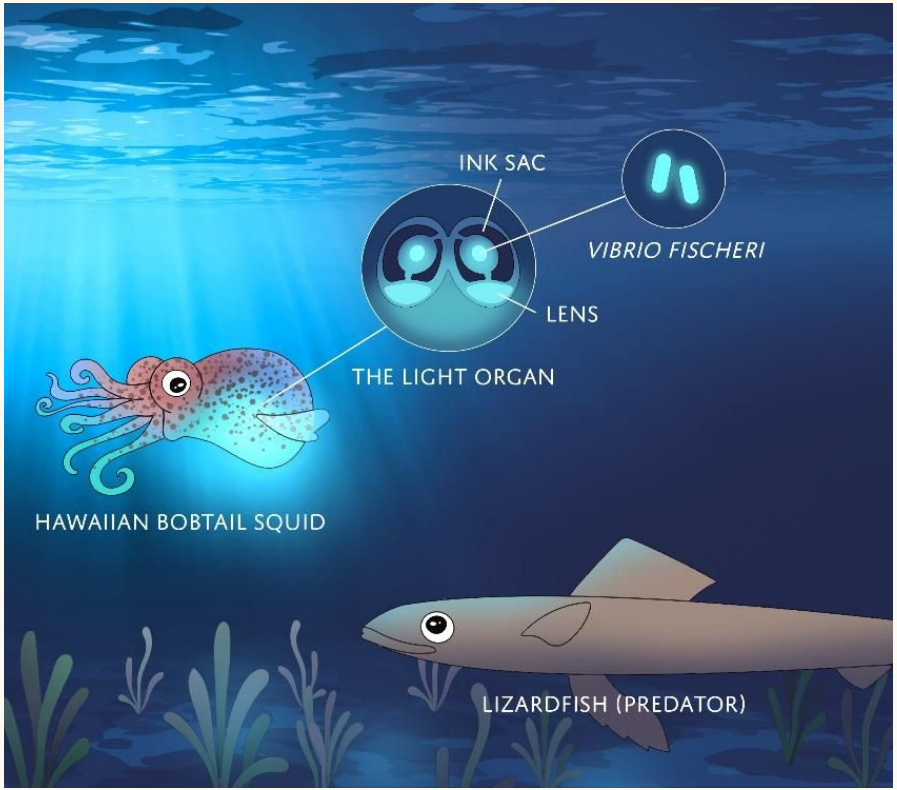
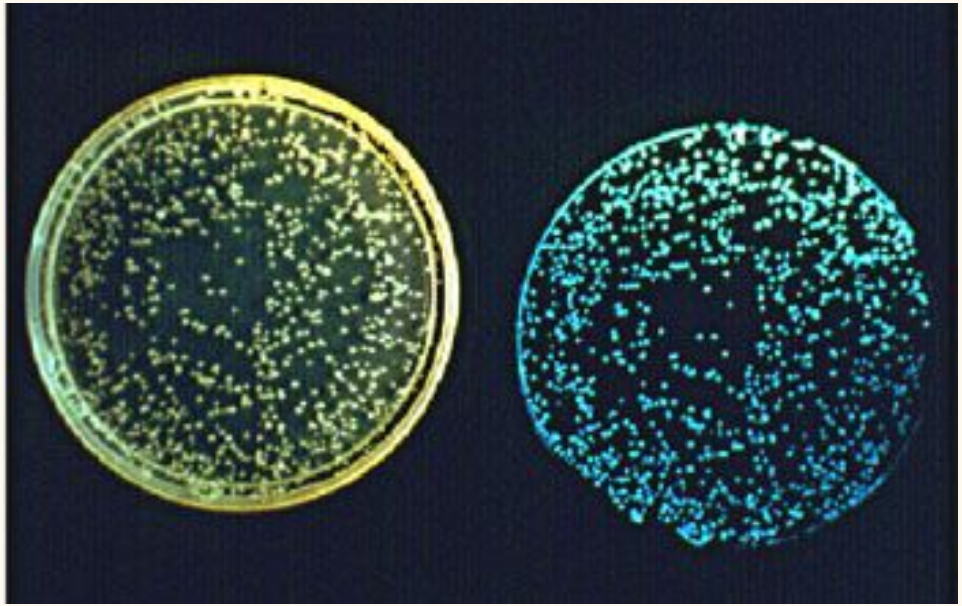
So a higher cell density leads to more AHL being produced and received

Vibrio fischeri





Vibrio fischeri

A bacterial species that can **bioluminesce** and lives in **symbiosis** with bobtail squids

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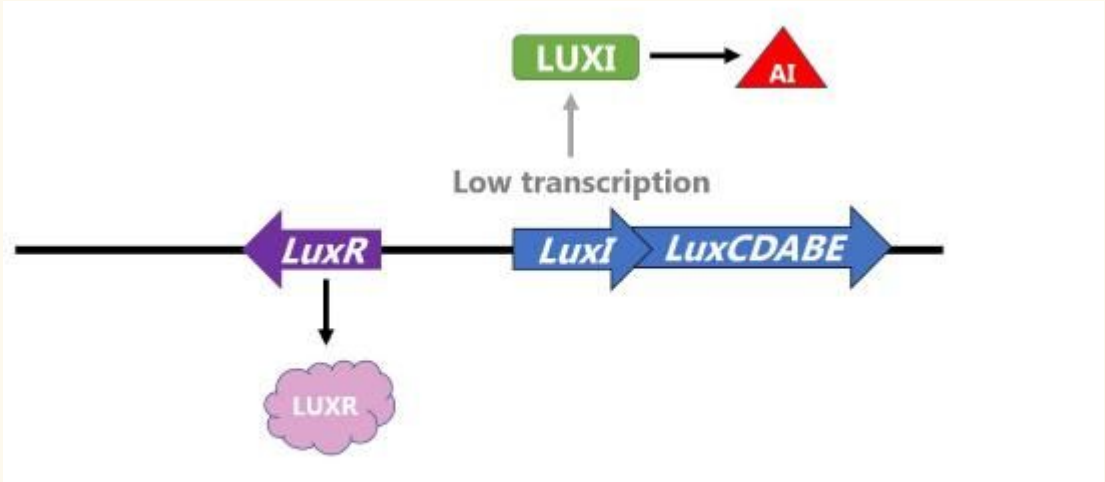
Lux operon:



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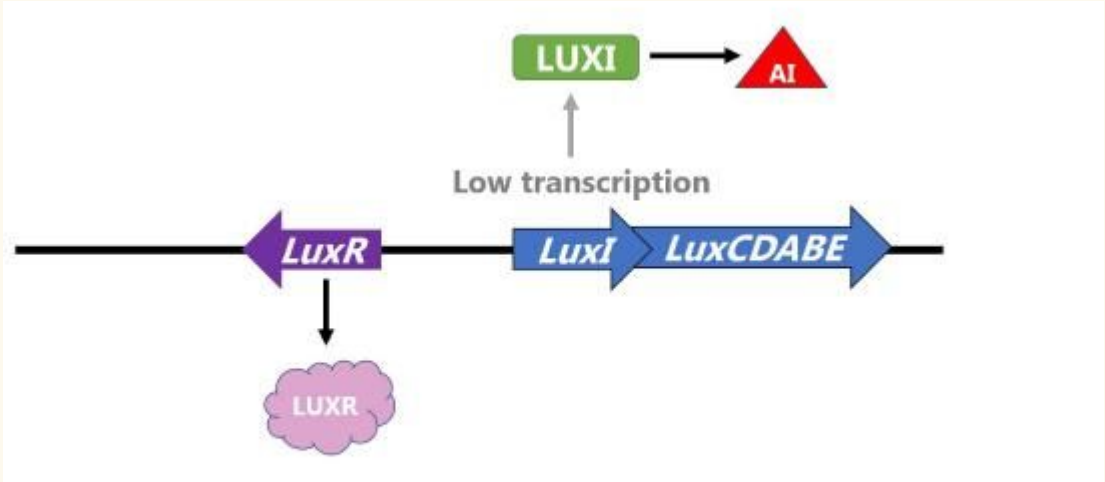

LuxI: produces AHL (**autoinducer**)

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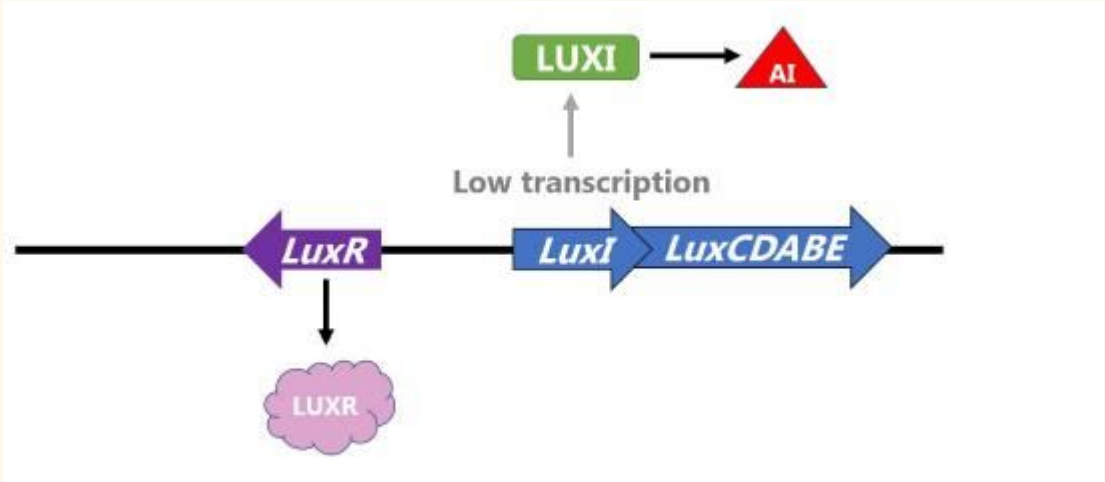

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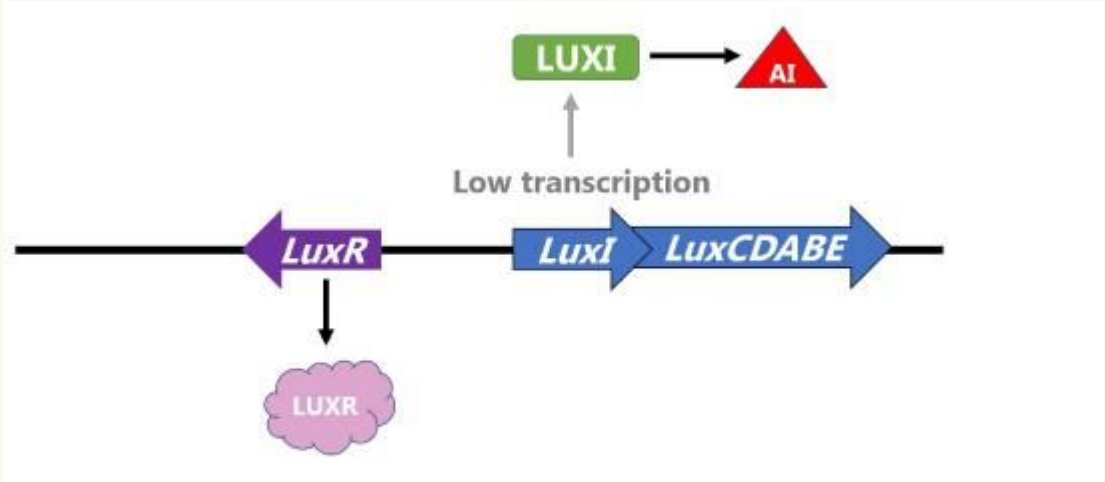

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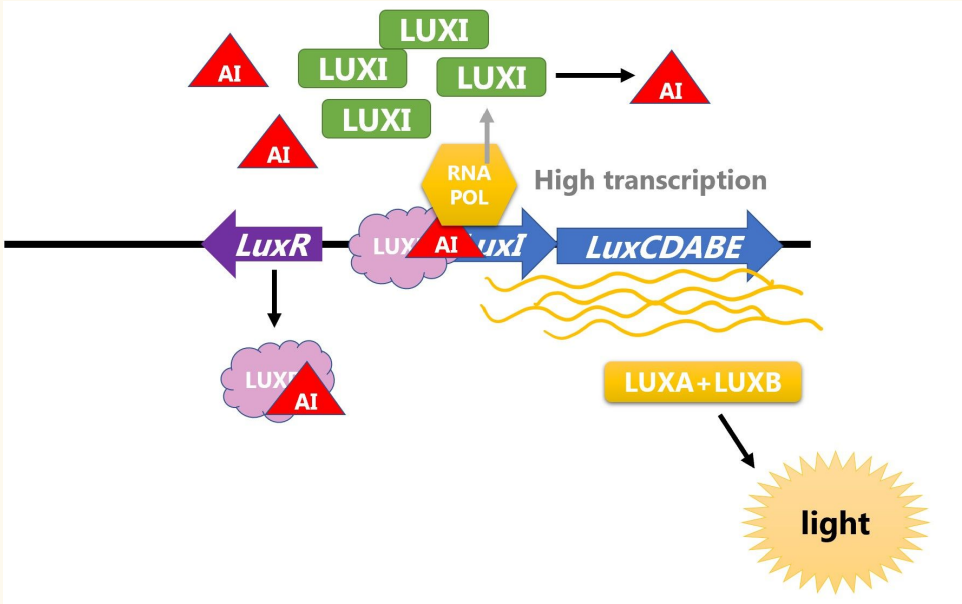

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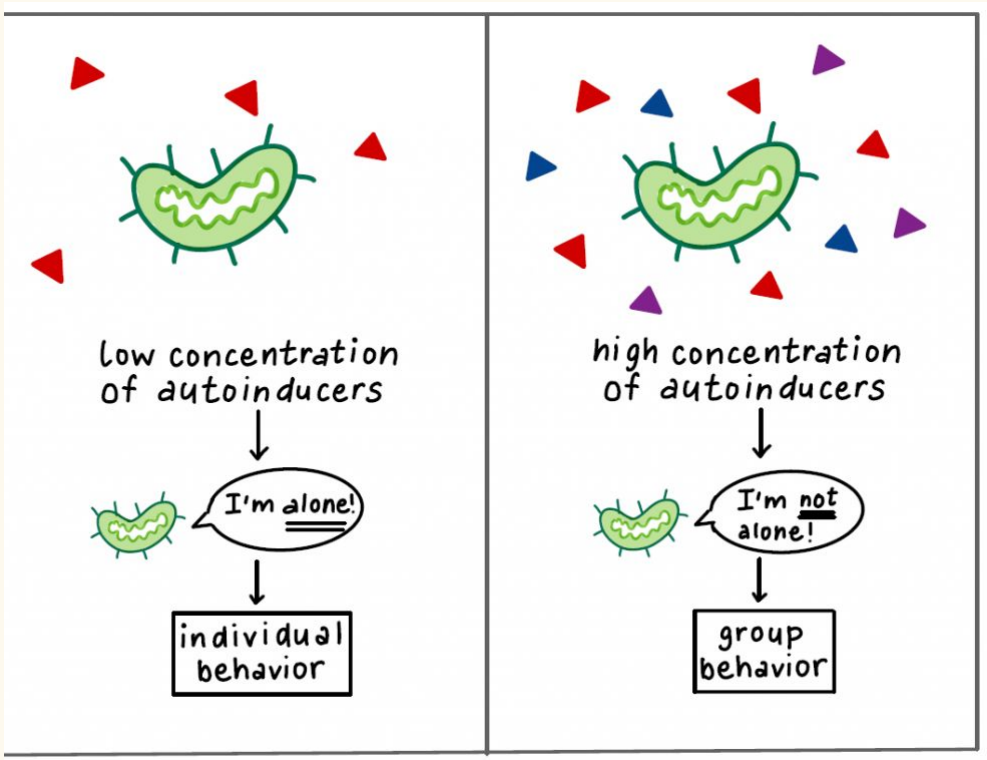
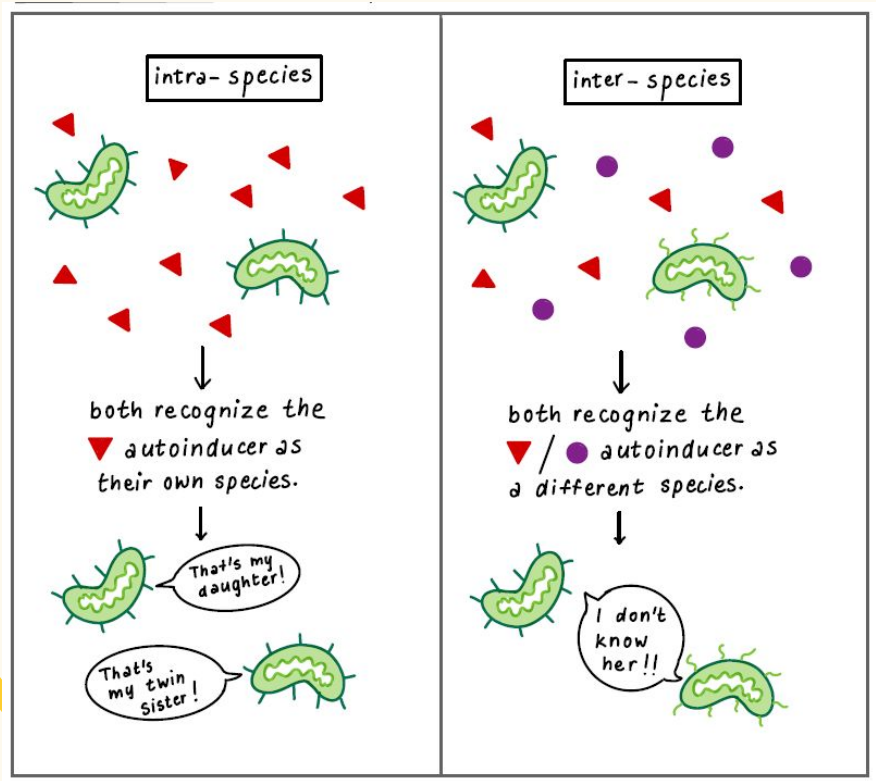
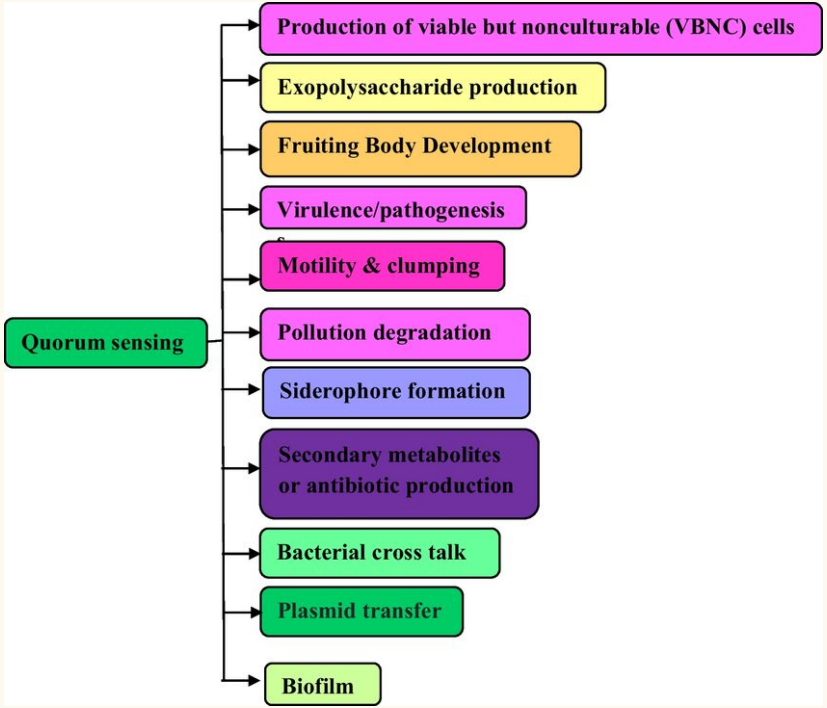
Summary


low concentration of autoinducers
I'm alone!
individual behavior
high concentration of autoinducers
I'm not alone!
group behavior


intra- species
both recognize the ▼ autoinducer as their own species.
That's my daughter!
That's my twin sister!
inter- species
both recognize the ▼ / ● autoinducer as a different species.
I don't know her!!

Summary

Microrganism	Major Signal Molecules	Regulatory System	Group-Derived Benefits
Bacillus subtlis	ComX CSF (PhrC) PhrA,-E, -F, -K, -H	ComP/ComA Rap proteins	Competence, sporulation, biofilm formation, antibiotic production,
Myxococcus xanthus	A-signal C-signal	SasSRN	Fruiting body formation or sporulation
Pseudomonas aeruginosa	3O-C12-HSL C4-HSL	LasI/LasR RhlI/RhlR OscR (orphan)	Structured biofilm formation, virulence factors
Staphylococcus aureus	AIP-I, AIP-II, AIP-II, AIP-IV	AgrC/AgrA	Biofilm formation, virulence factors
Streptococcus mutans	CSP (ComC) XIP (ComS)	ComD/ComE ComR	Bacteriocins, biofilm formation, competence
Streptococcus pneumoniae	CSPs	ComD/ComE	Competence, fratricide, biofilm formation, virulence



Think, pair, share

Why is quorum sensing so important?

i.e. why wouldn’t a population want to start the process of bioluminescence or biofilm formation without a certain population density present?

Chemotaxis

Chemotaxis

Any guesses?

Chemotaxis

Any guesses?

Chemotaxis

Any guesses?

Chemotaxis

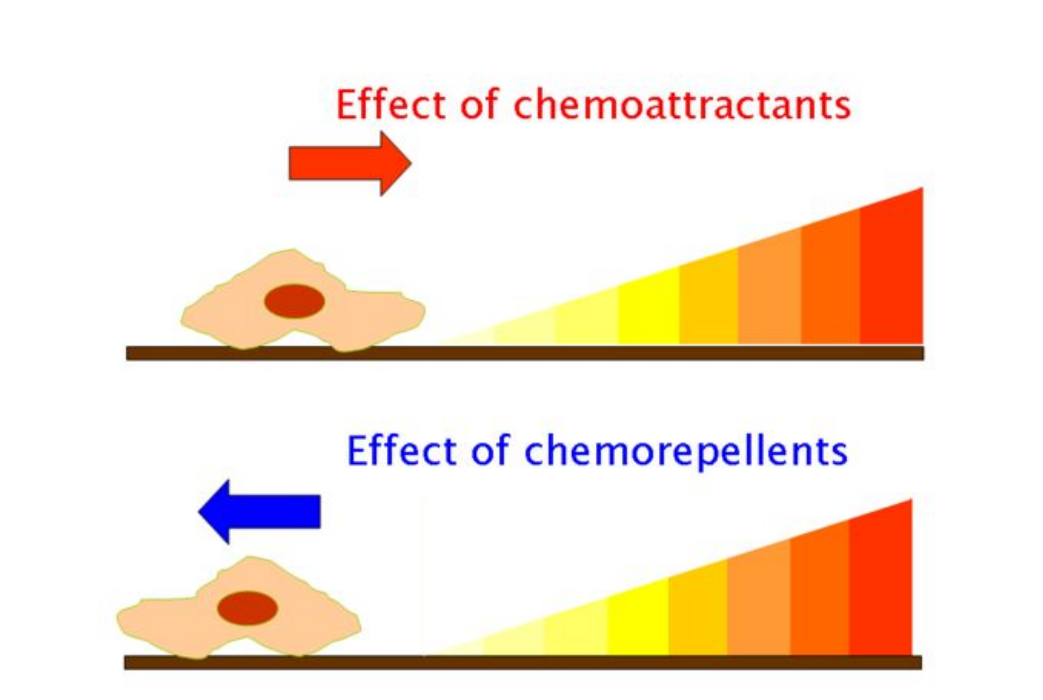
Movement in response to a chemical gradient

Chemotaxis

Why would a cell want to move?

Energetically costly and risky

Chemotaxis



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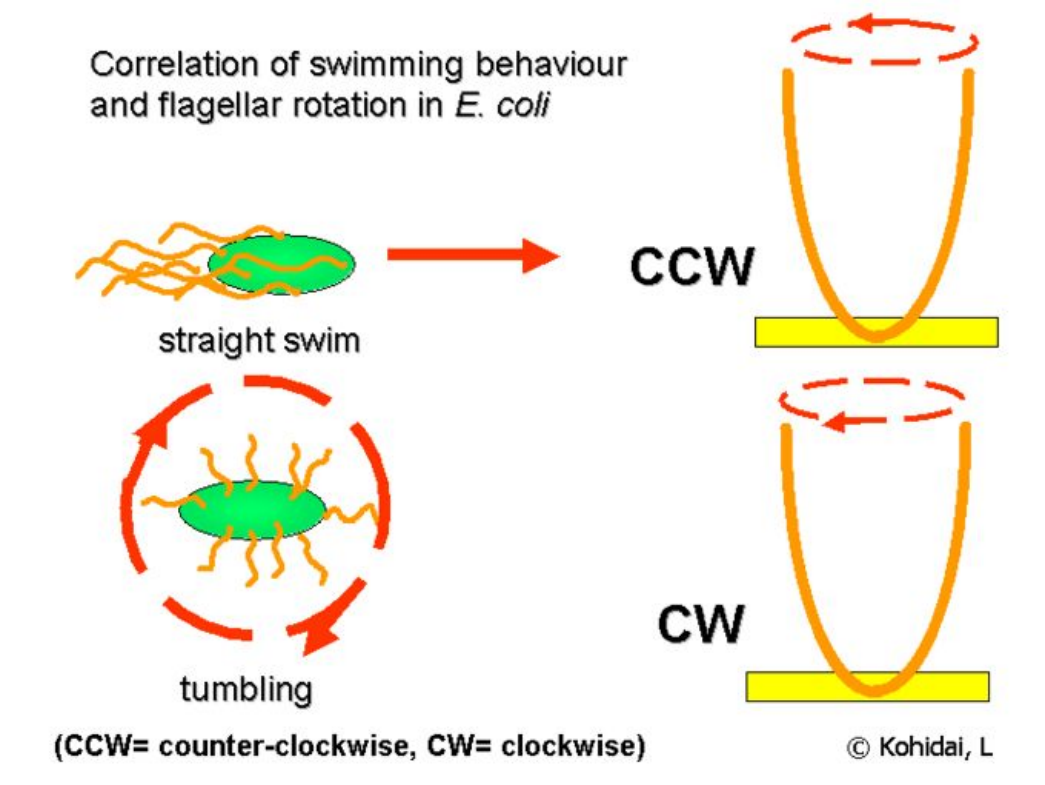
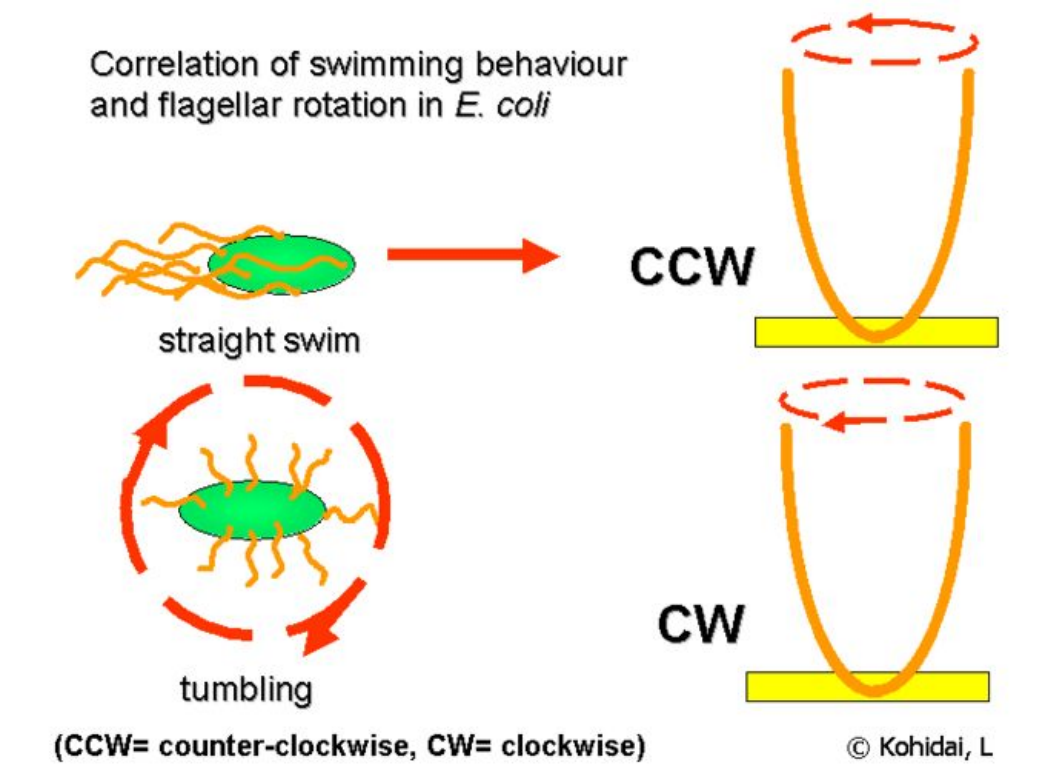


Requires flagella

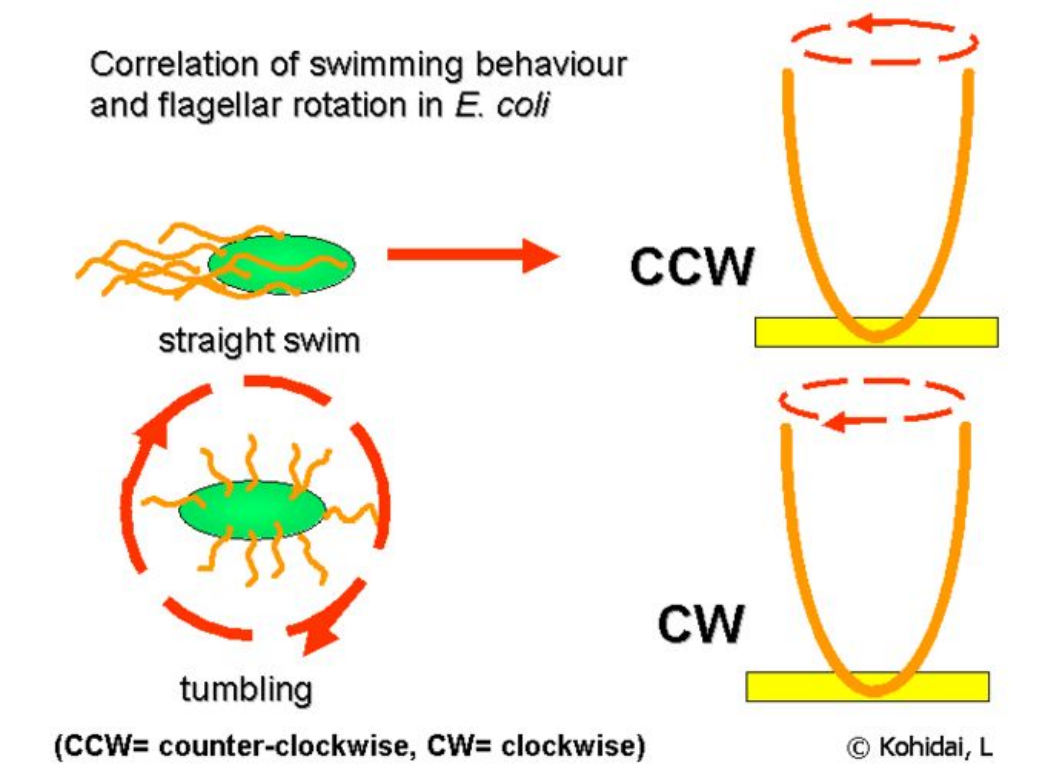
Chemotaxis



Requires flagella

Runs and tumbles

Chemotaxis

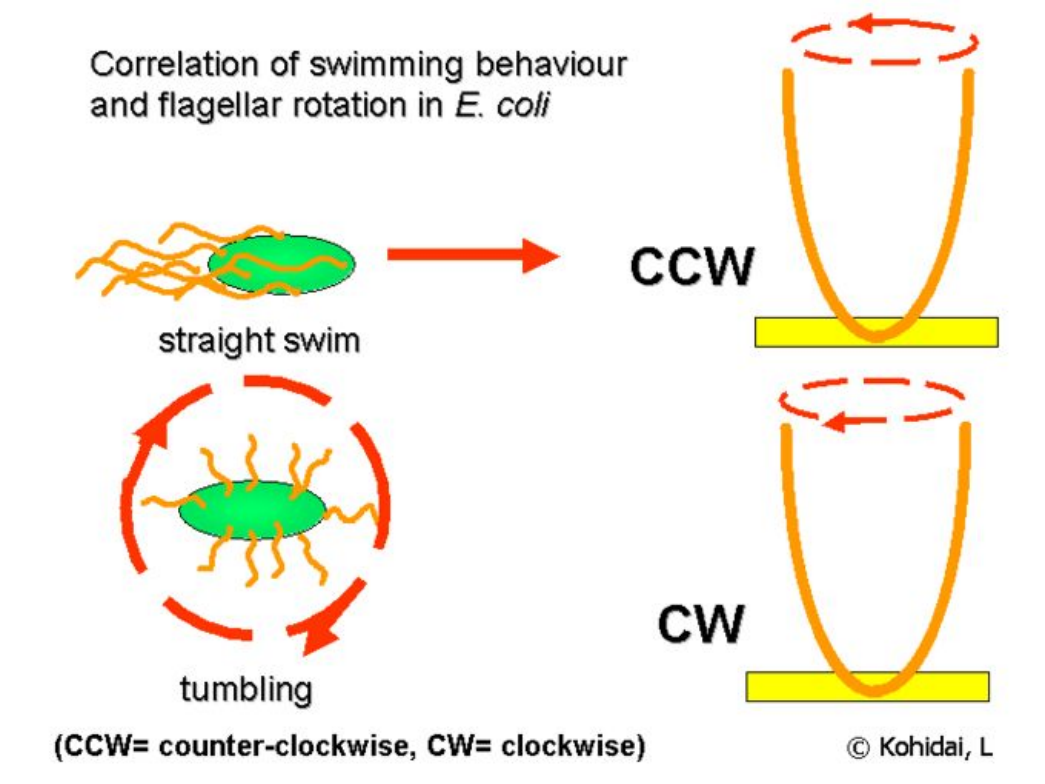


Requires flagella

Runs and tumbles

Going towards an attractant: longer runs and fewer tumbles

Chemotaxis



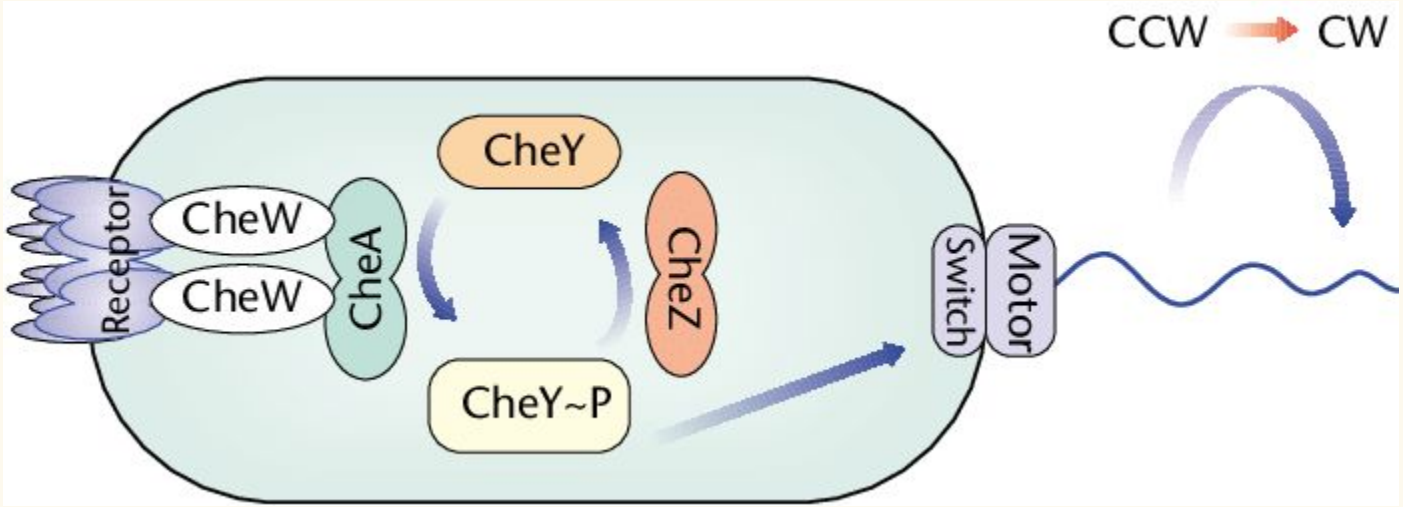
Requires flagella

Runs and tumbles

Going towards an attractant: longer runs and fewer tumbles

Going towards a repellant or away from an attractant: shorter runs and more tumbles

Chemotaxis



Assessment

You have isolated a population of *Vibrio fischeri* (the glowing bacteria that live in squids) and want to take advantage their quorum-sensing abilities. Edit the Lux operon so that a medium population density is required to produce protein P, but an extremely low and an extremely population density produce protein L, and inhibit transcription of protein P.

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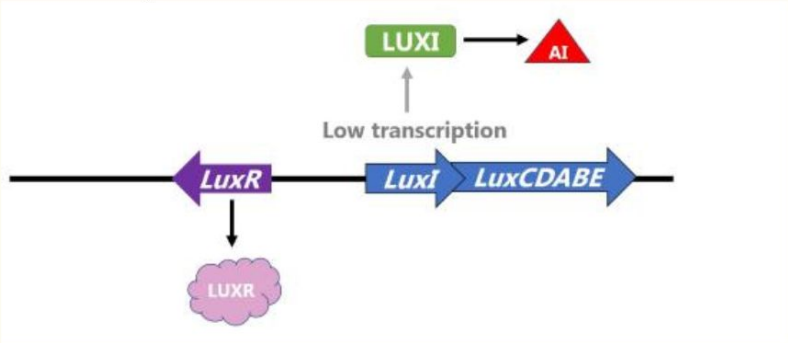

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