BB 101
Gene regulation and Cell Communication
Tutorial 5
08.02.2024

Complementary DNA (cDNA) Synthesis

1. mRNA & Reverse transcriptase added

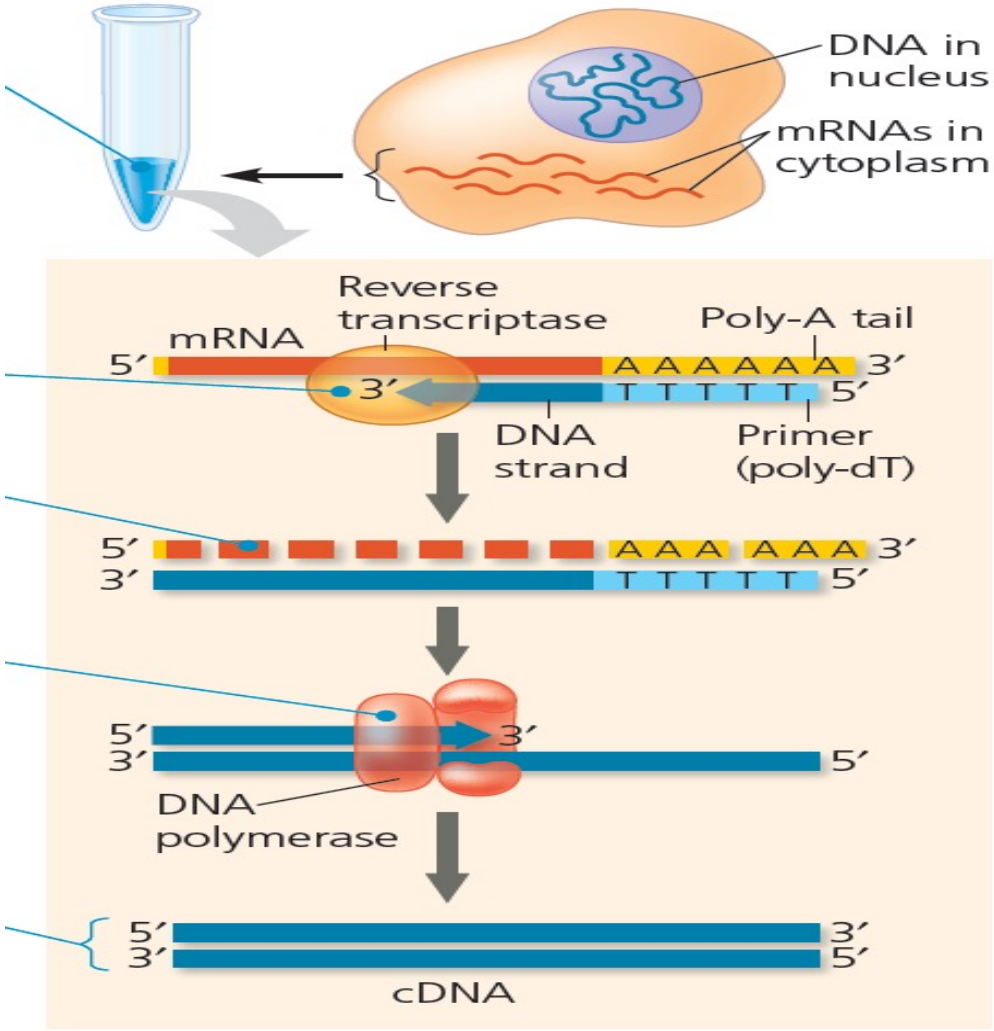
3' end of mRNA has a stretch of adenine - poly-A tail, which allows use of a short complementary strand of poly-dT primer for synthesis of DNA strand

2. RT makes first DNA strand (mRNA template; poly-dT DNA primer)

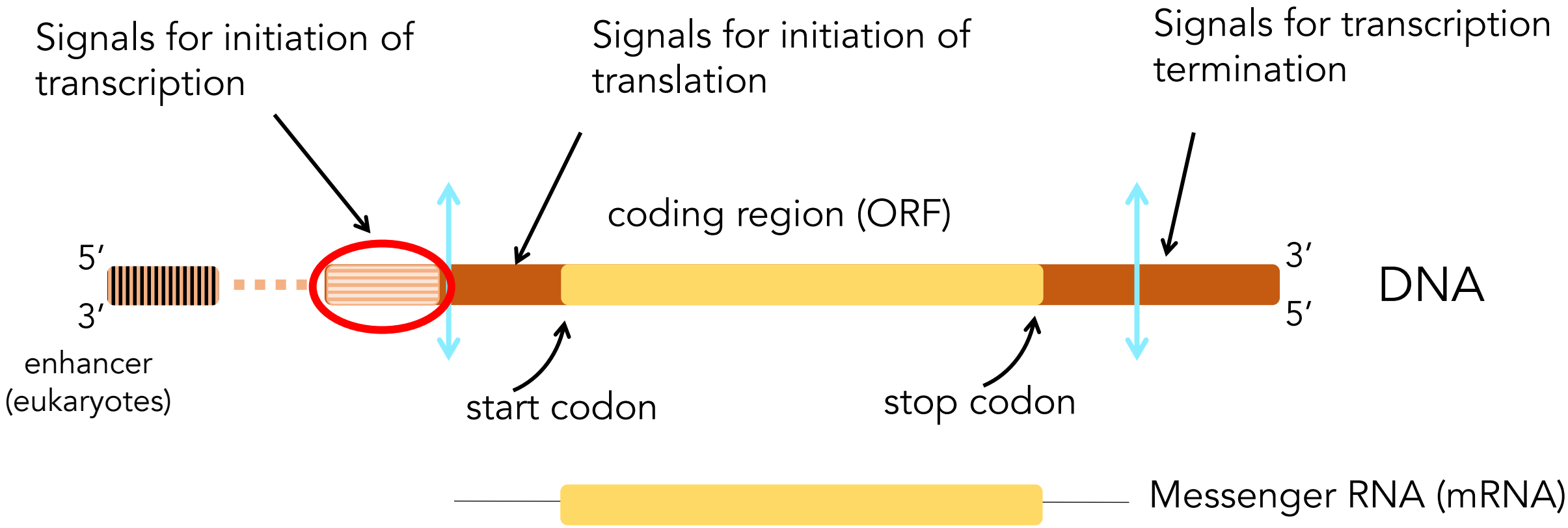
3. mRNA degraded by enzyme

4. DNA polymerase synthesizes 2nd DNA strand

5. cDNA is synthesized which carries complete coding sequence of gene but no introns

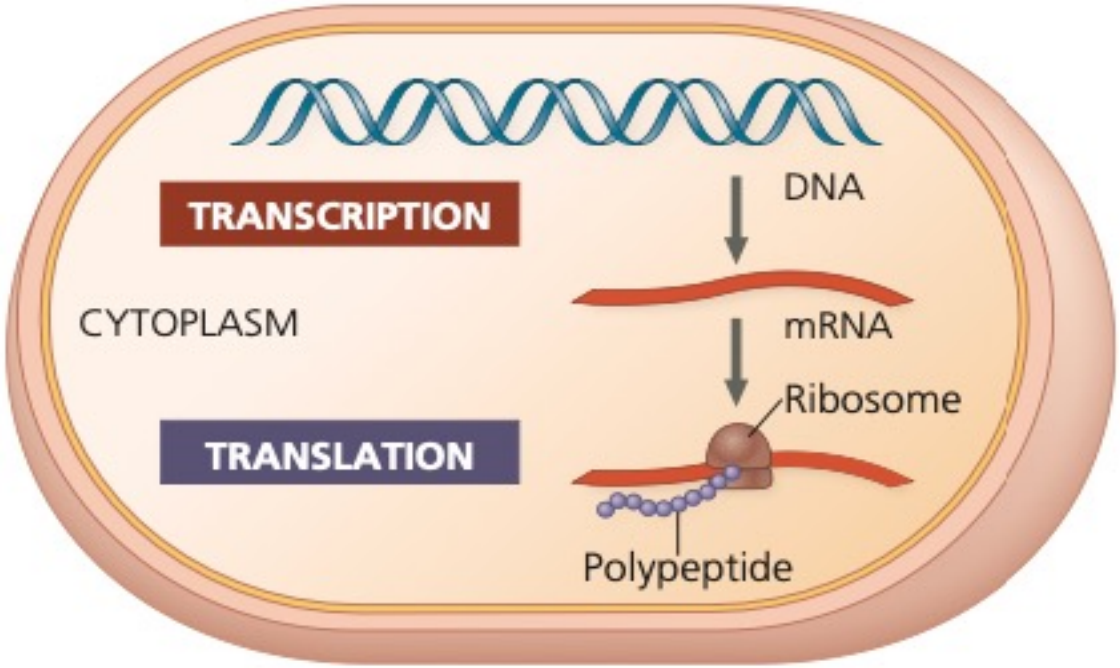


Gene: stretch of DNA that encodes a protein



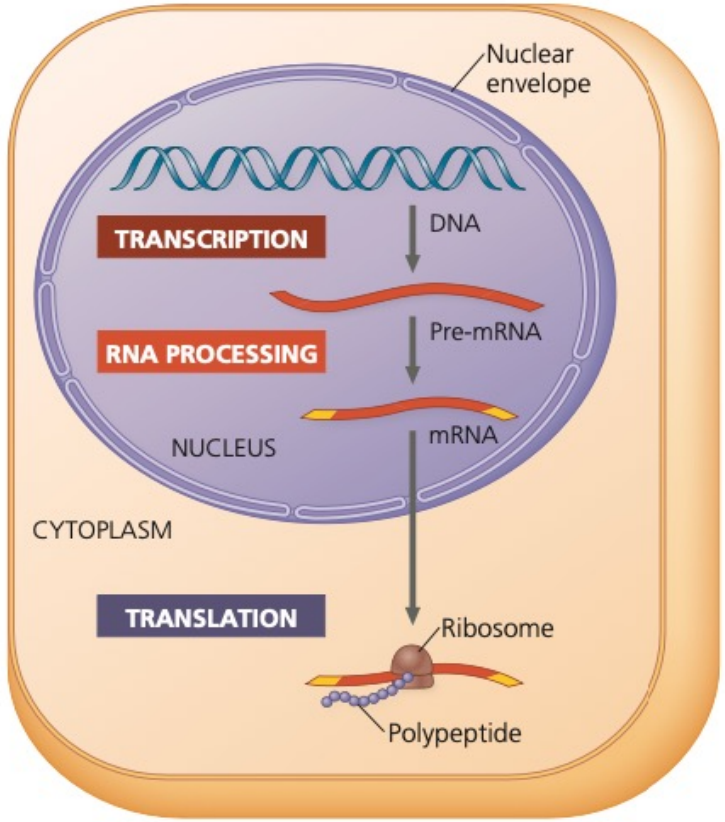
A gene includes the entire sequence represented in mRNA

Comparison of Transcription & Translation in Prokaryotes & Eukaryotes



Bacteria do not have nuclei, their DNA is not separated by nuclear membranes from ribosomes and the other protein-synthesizing equipment.

The lack of compartmentalization allows translation of an mRNA to begin while its transcription is still in progress

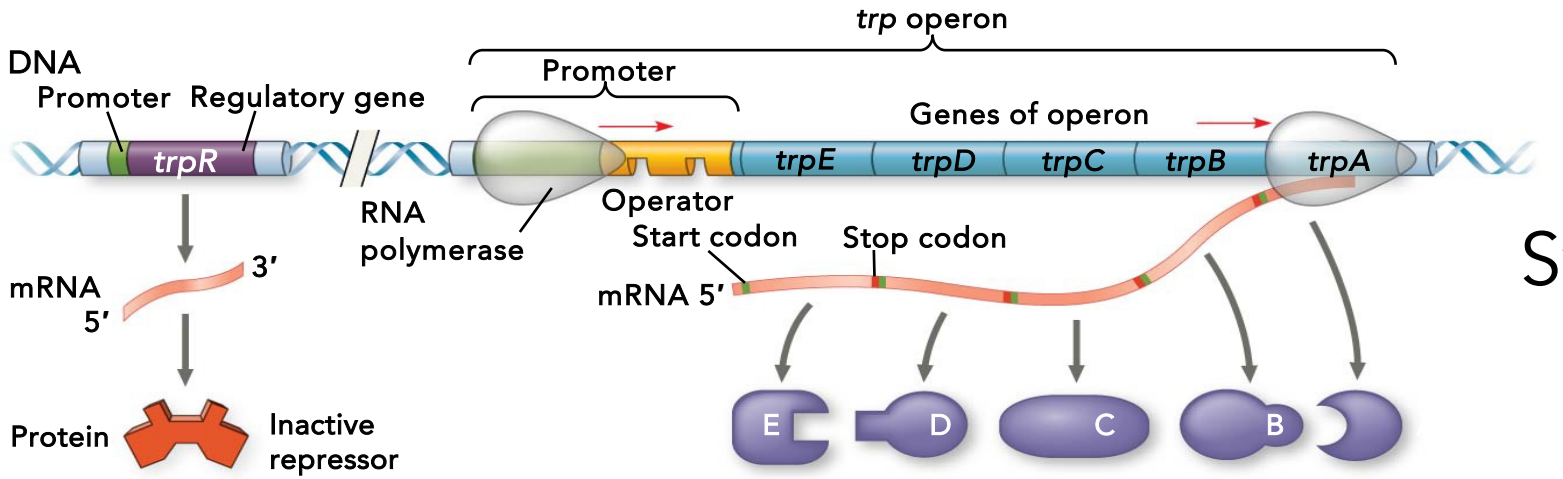


In a eukaryotic cell, by contrast, the nuclear envelope separates transcription from translation in space and time

Gene Regulation

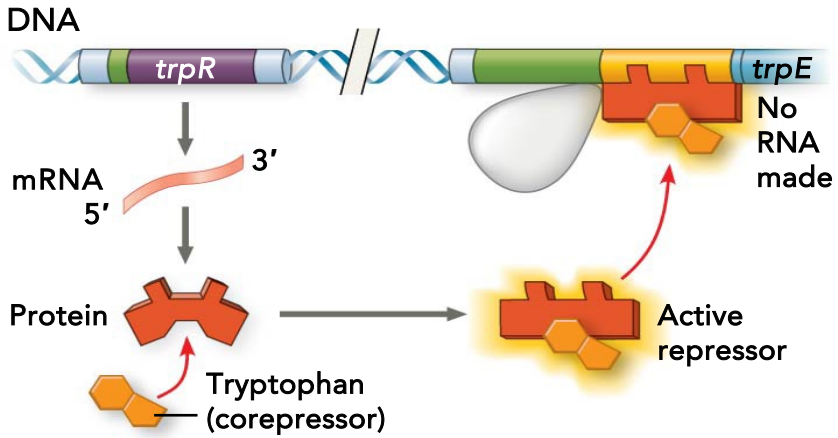
A hallmark of prokaryotic and eukaryotic cells alike—from a bacterium to the cells of a fish - is their intricate and precise regulation of gene expression.

An Overview of trp operon in E. coli: regulated synthesis of repressible enzymes



(a) Tryptophan absent, repressor inactive, operon on

Switch is ON



(b) Tryptophan present, repressor active, operon off

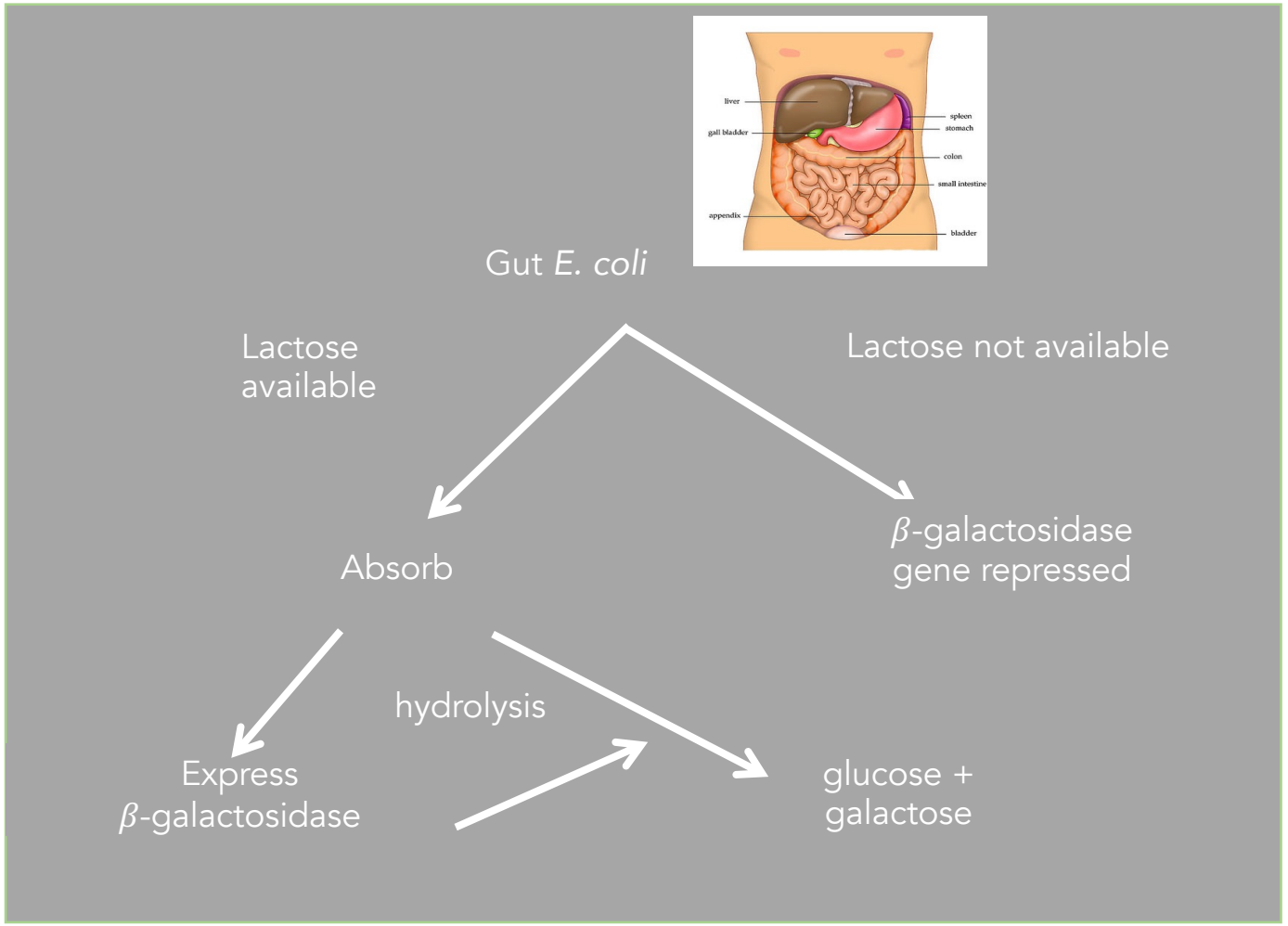
Switch is OFF

Inducible Operons

An inducible operon is usually off but can be stimulated (induced) when a specific small molecule interacts with a regulatory protein. Example of an inducible operon is the lac operon (lac for lactose).

The lac Operon

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

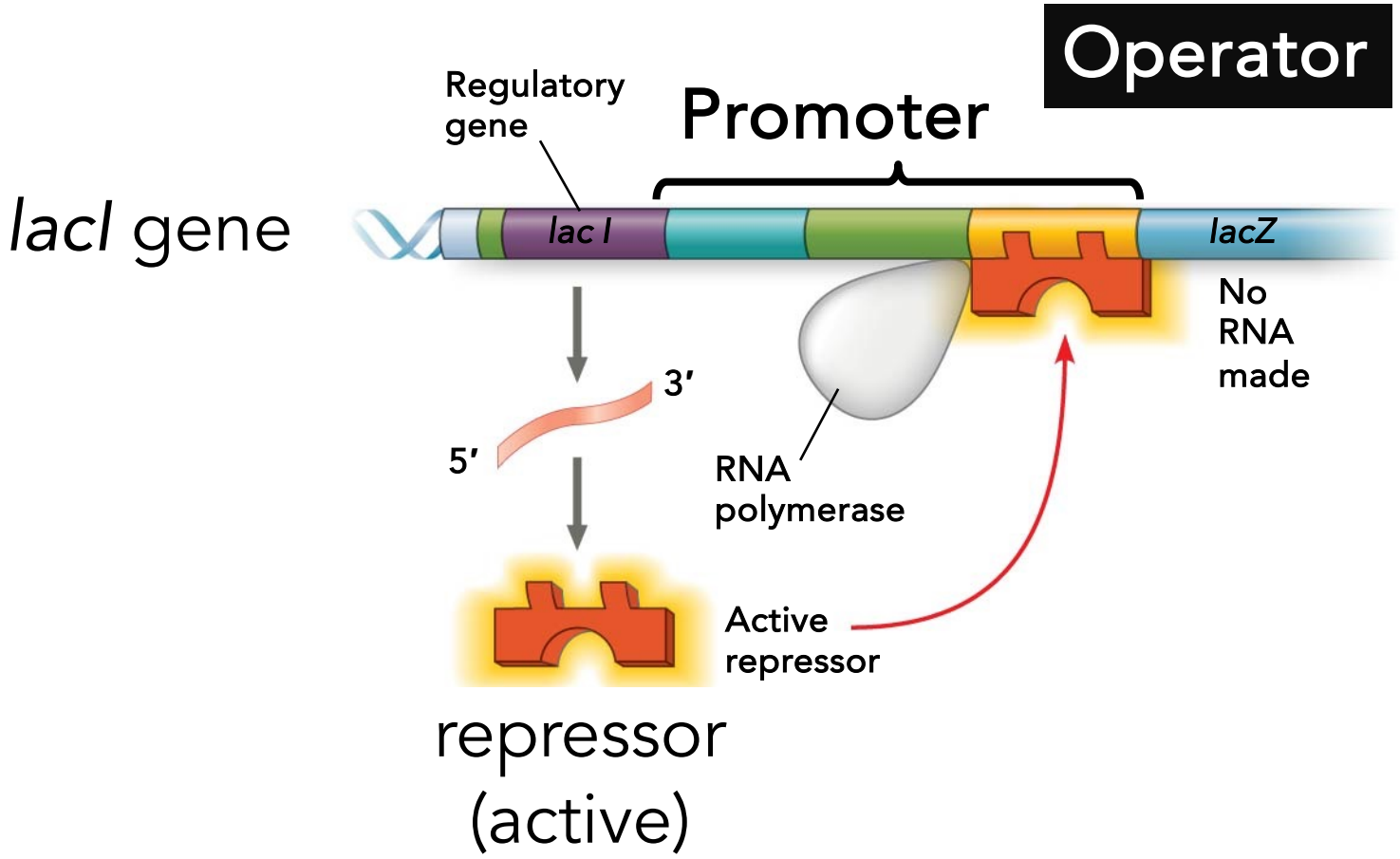


Induction of the lac operon

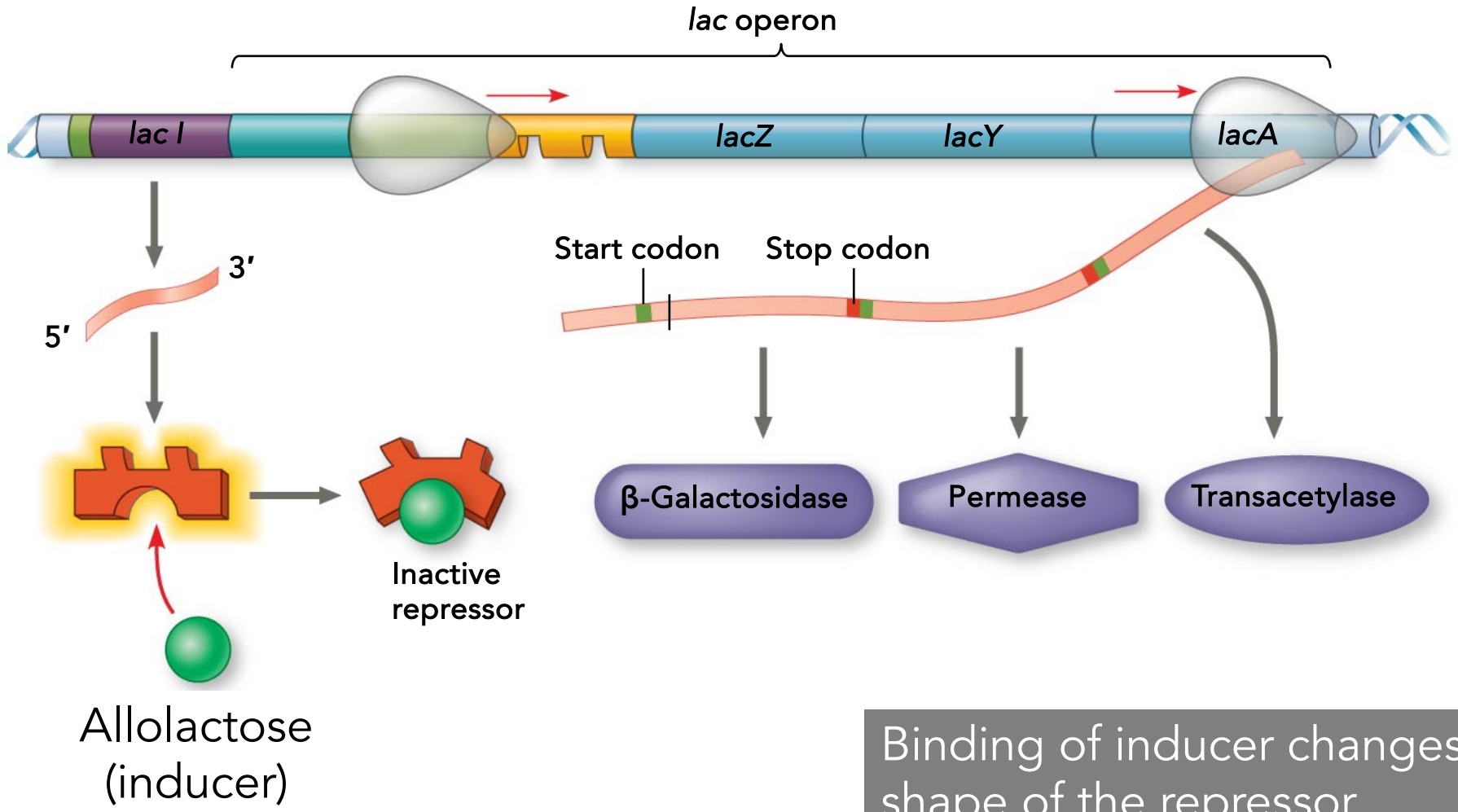
Lactose absent	Only a few molecules of β-galactosidase
Lactose present	1000-fold increase in the number of β-galactosidase Time taken: about 15 minutes

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

Induction of the lac operon



Induction of the lac operon



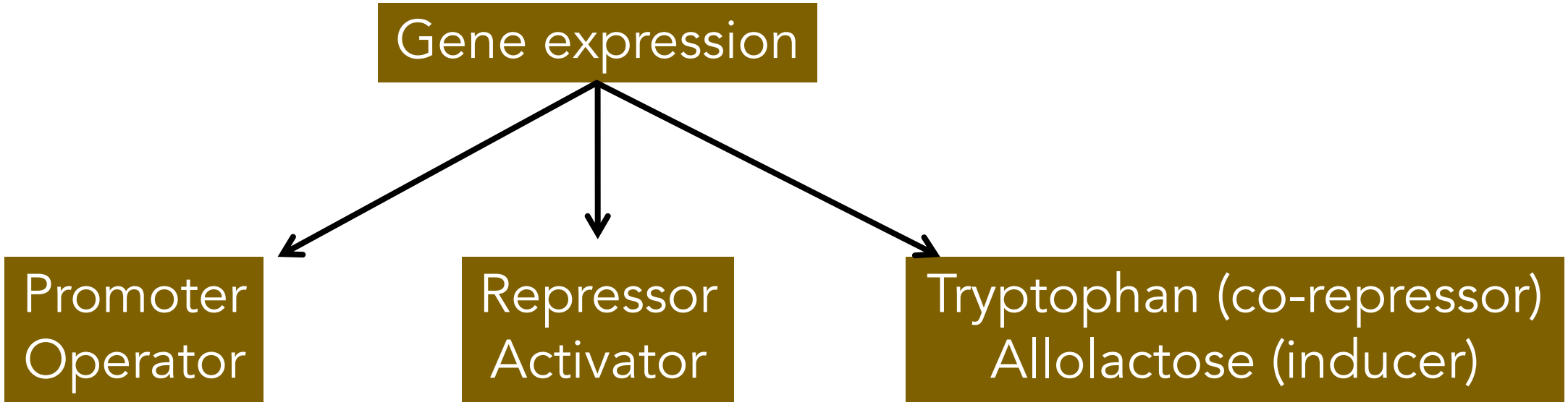
Binding of inducer changes the shape of the repressor

Summary: 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

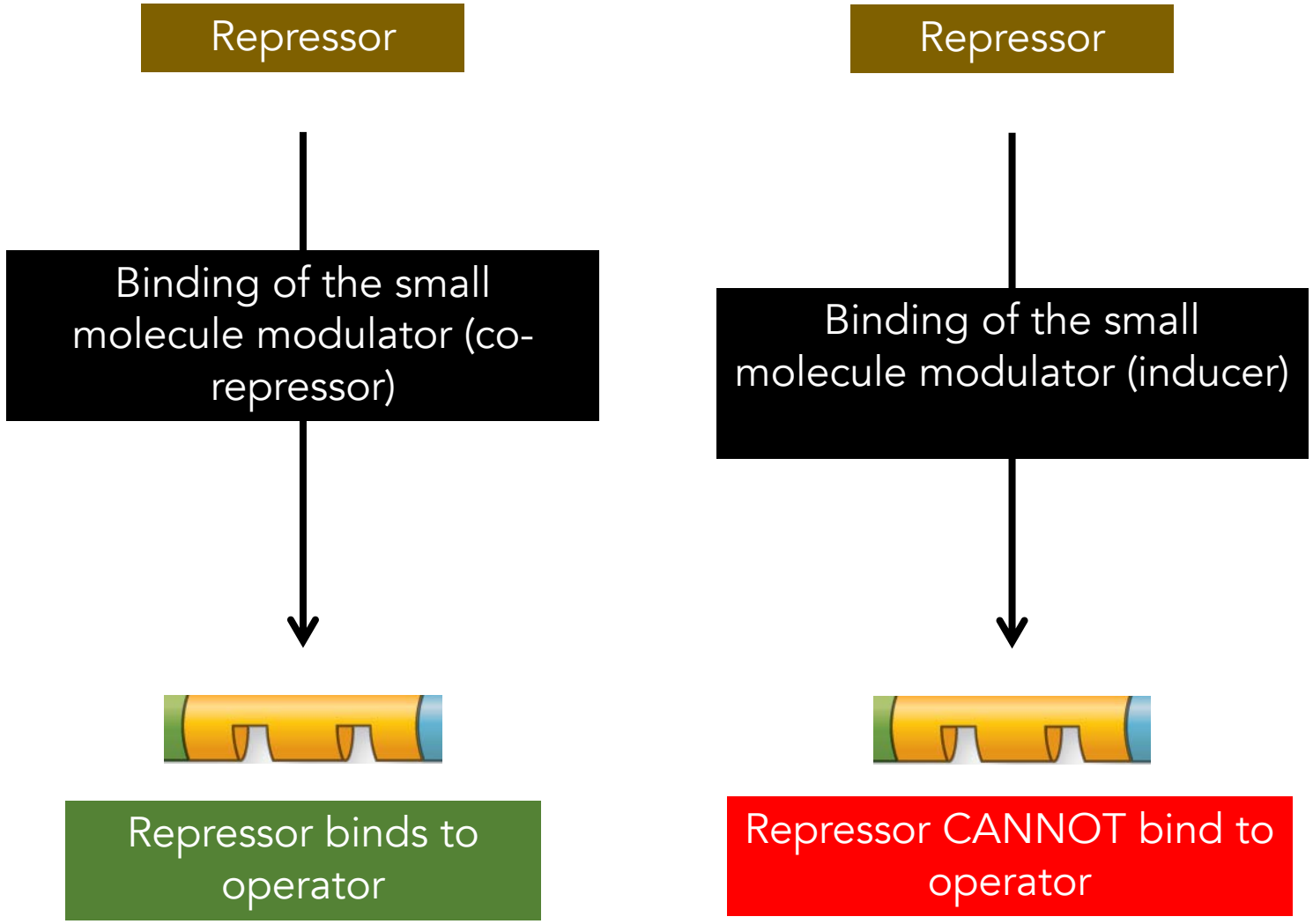
Negative regulation: binding of repressor shuts down gene expression

Summary: trp and lac operons



Summary: trp and lac operons

These are examples of genetic switches!

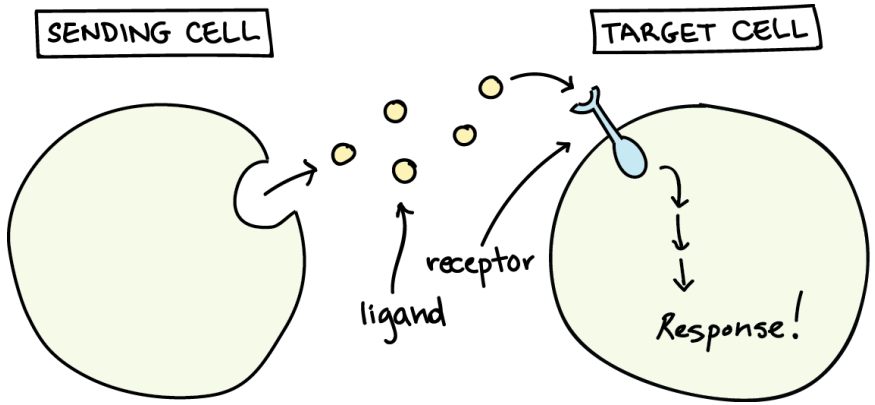


Cell Communication

- Cell communication is the ability of the cells to communicate with adjacent cells within an organism (cell signaling)
- All cells receive and respond to signals from their surroundings.
- Signaling by extracellular signal molecules usually involves:
  - Synthesis and release of the signal molecule by signaling cell
  - Transport of the signal molecule to the target cell
  - Binding of the signal by specific receptor leading to activation
  - Initiation of signal transduction pathways

Signal molecule

Receptor

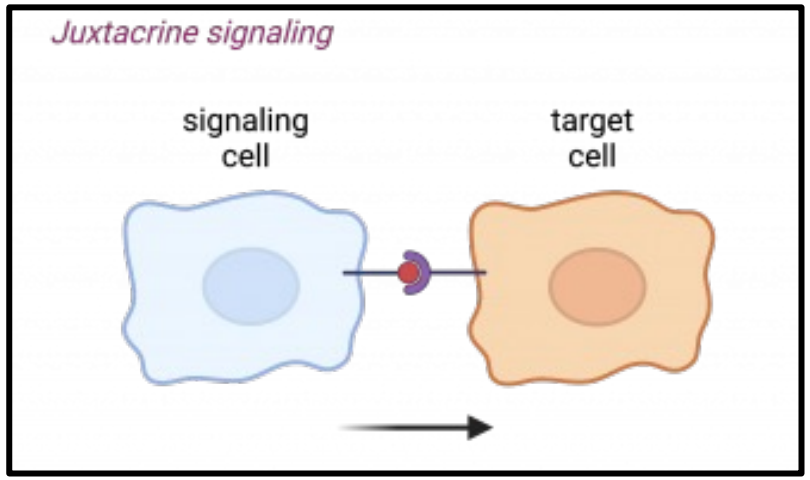


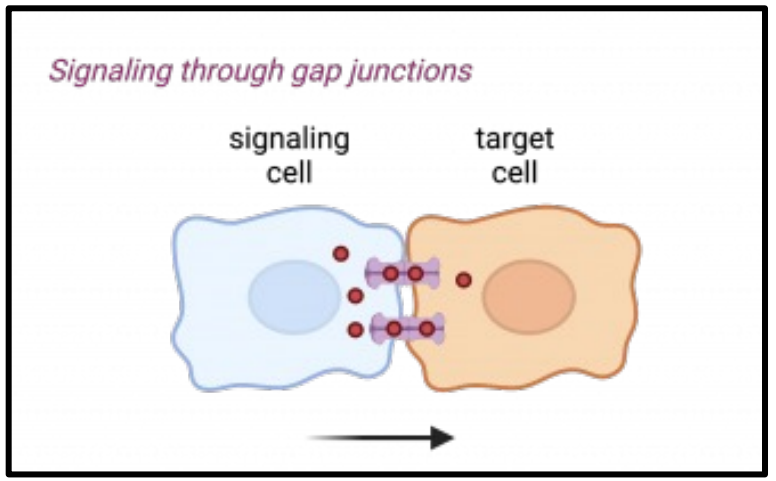
Types of signaling

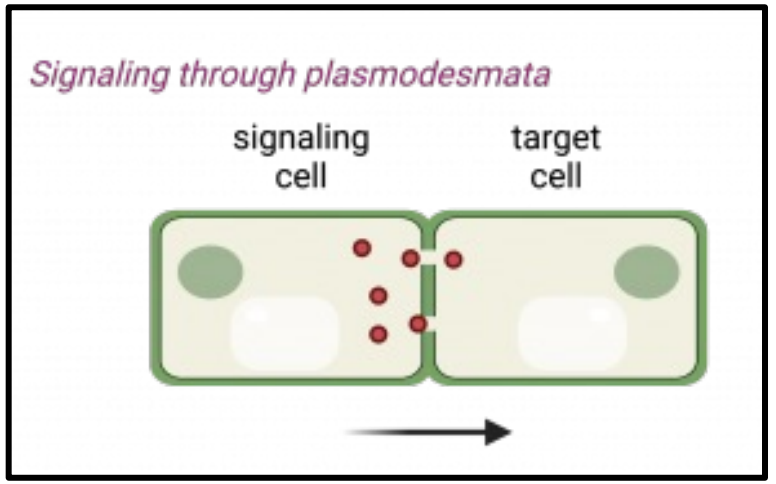
- Direct signaling- E.g.→ Gap junction
- Autocrine signaling- E.g.→ Leptin
- Paracrine signaling- E.g.→ Estrogen
- Endocrine signaling- E.g.→ Insulin

Direct Signaling: between the cells that are in direct contact with each other

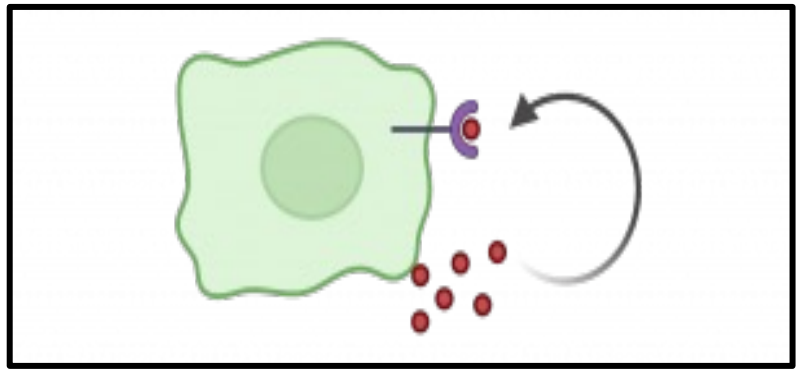
Example: cell-cell communication (juxtacrine signaling), cell-junction communication (gap junctions)







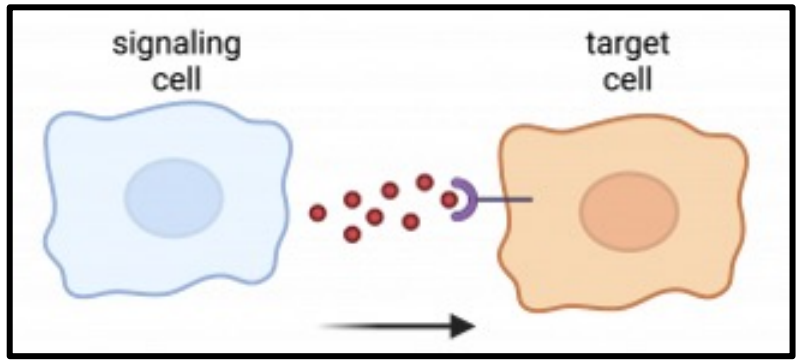
Types of signaling



A cell targets itself

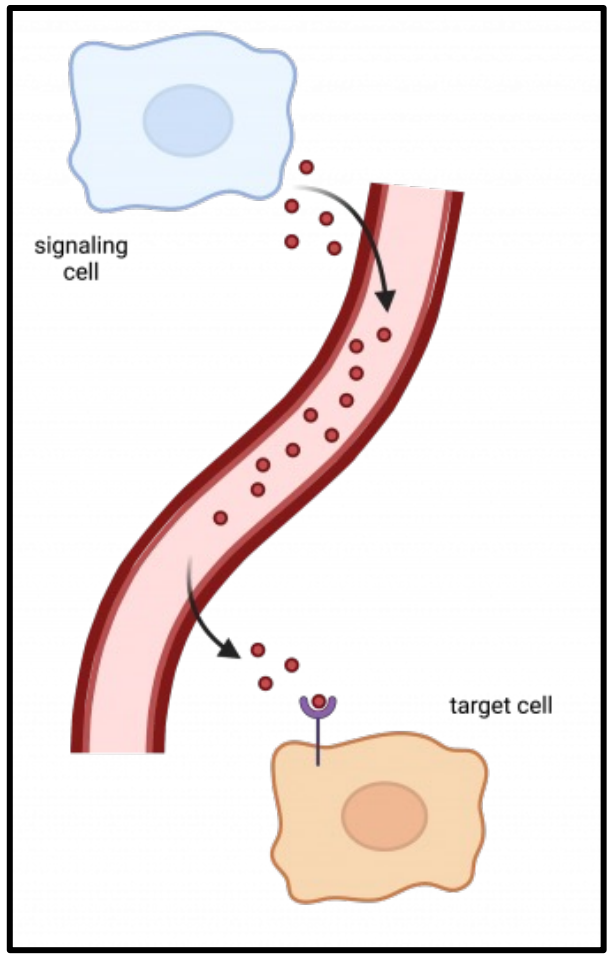
Autocrine

Endocrine



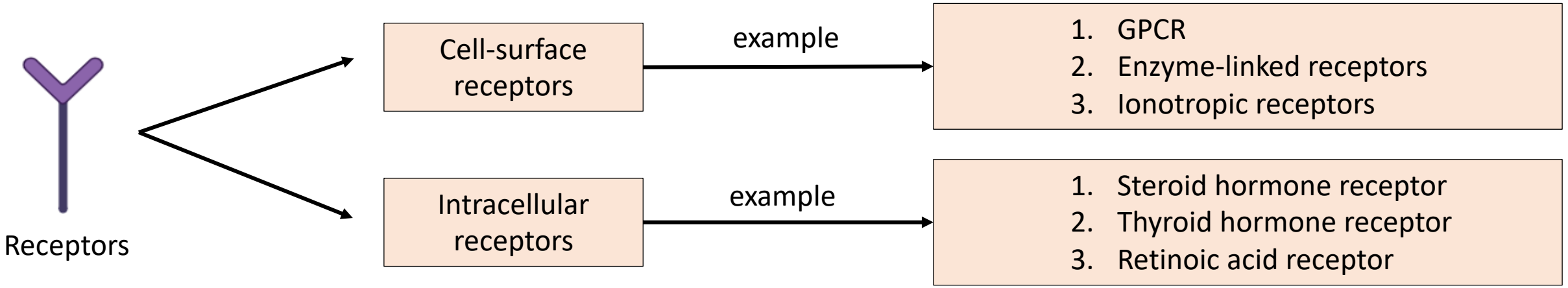
A cell targets a nearby cell

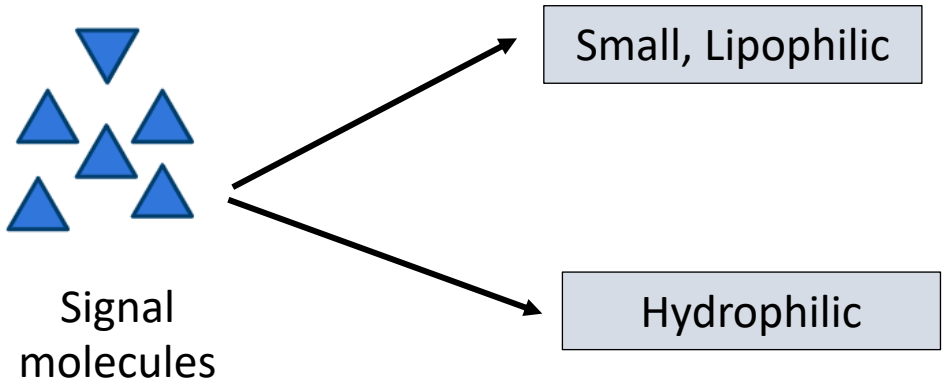
Paracrine



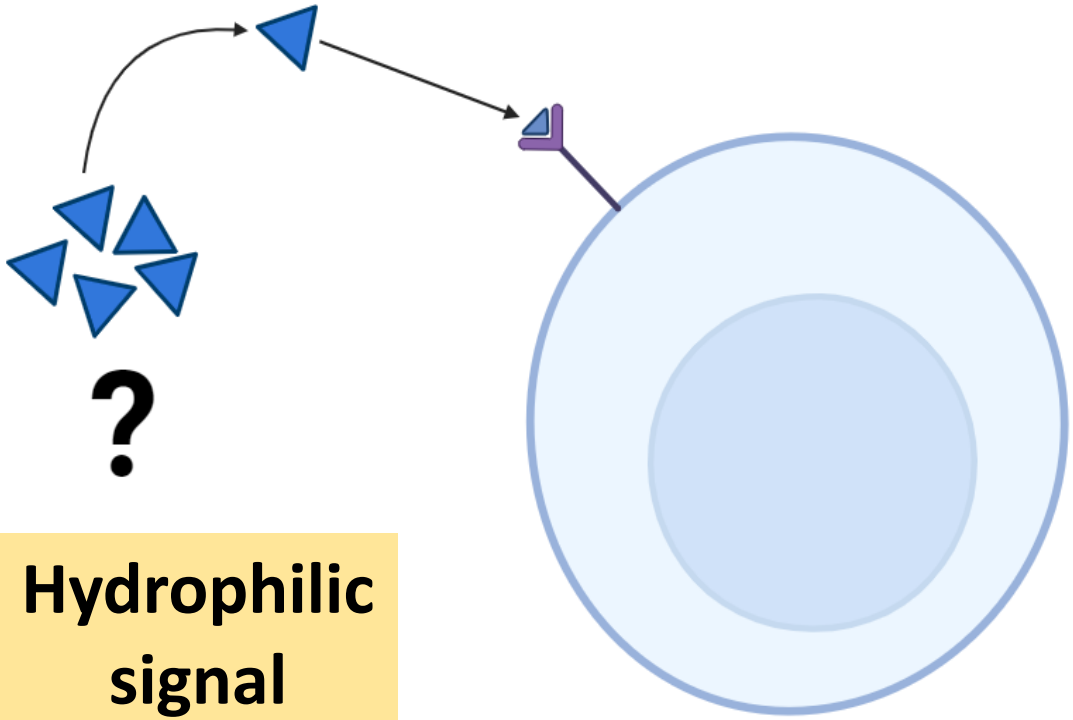
A cell targets a distant cell through bloodstream

Receptors & Signal molecules

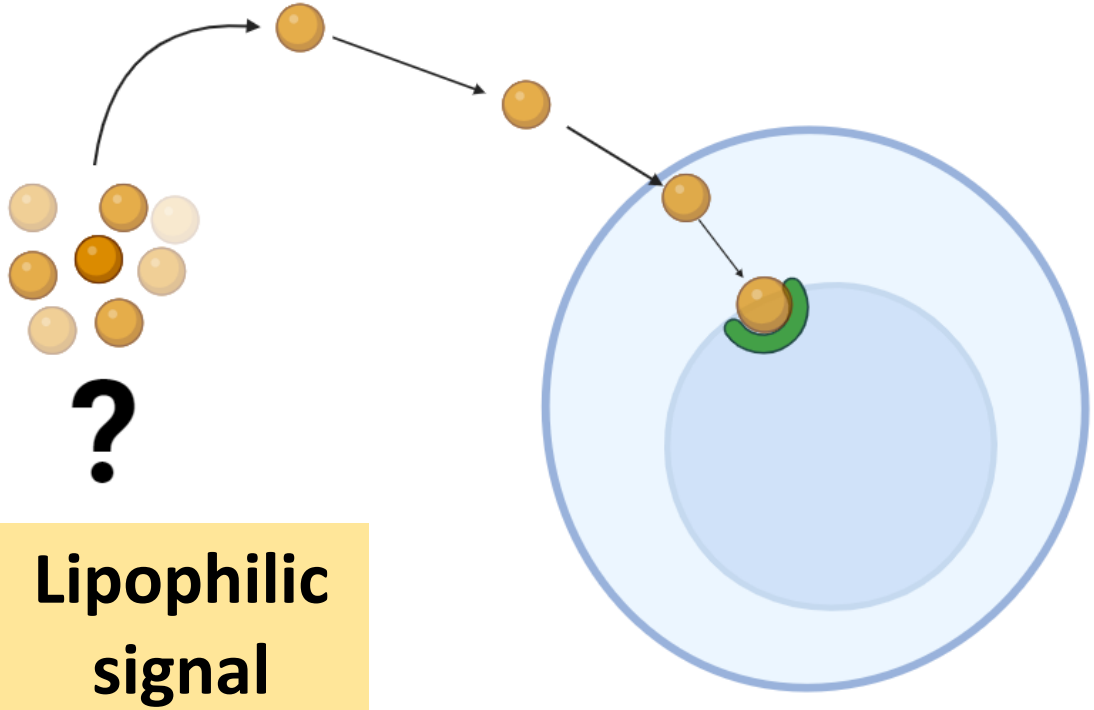




Signal molecule: which path to follow??



Hydrophilic signal

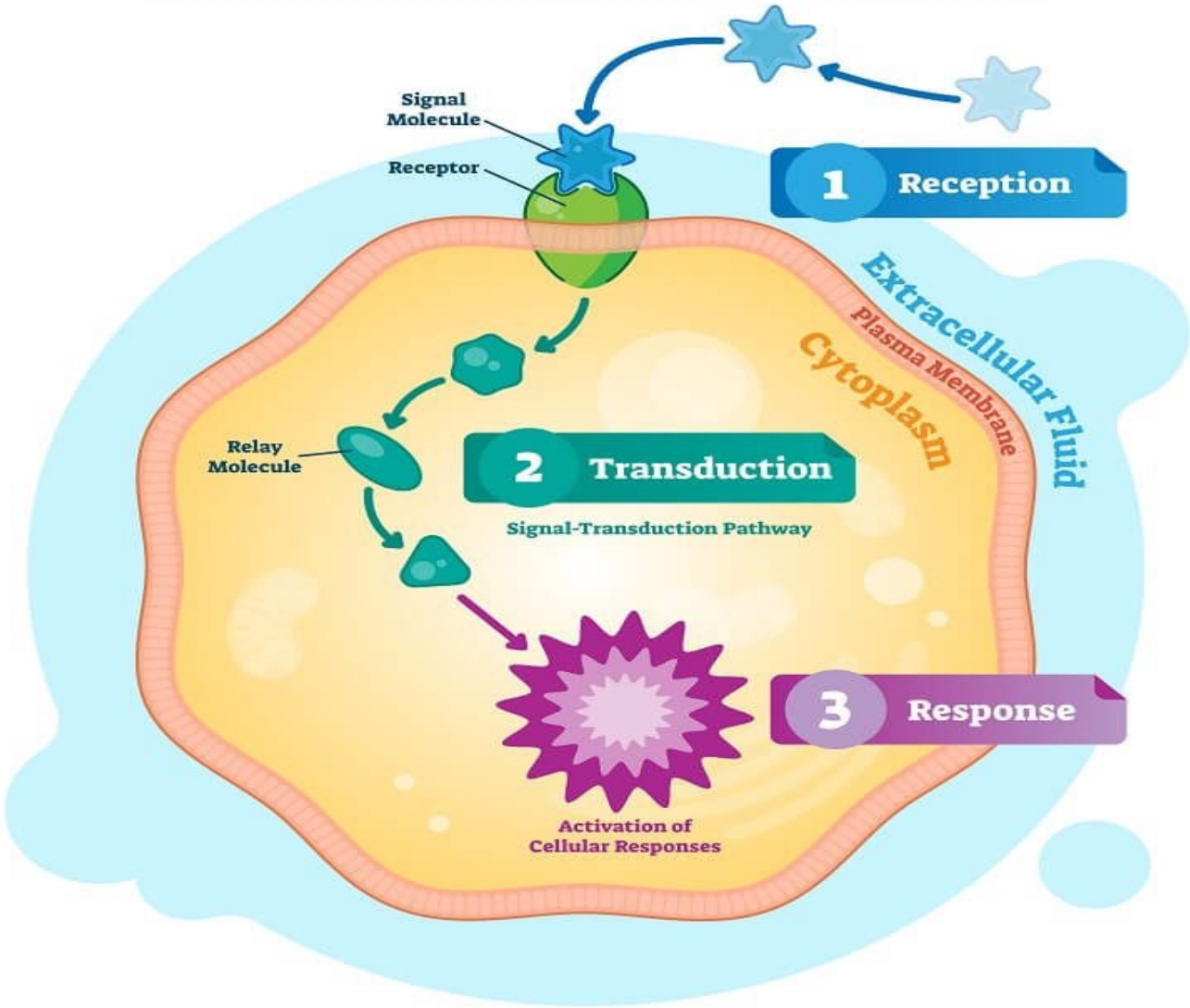


Lipophilic signal

Signal Transduction

- **Signal transduction**: the transmission of molecular signals from a cell's exterior to its interior.

- Steps involved:
  1. **Reception:**
     - Ligand binding; cell contact
  2. **Signal Transduction:**
     - Transfer of signal to the cell interior
     - Modulate the activity of Protein Kinases and phosphatases
  3. **Response:**
     - Phosphorylate on the state of the target
     - Modulation of effector activity



https://biologydictionary.net/cell-signaling/

Quorum (Ko-rum)

The minimum number of members of a society/group that must be present in a meeting to make the proceedings of that meeting valid.

Bacteria are capable of quorum sensing! Regulate gene expression depending upon cell-population density

How do the bacteria “know” the population density of their surroundings?

Luminescence and bioluminescence

Quorum-sensing is most extensively studied in the bacteria *Vibrio fischeri*

Luminescence: emission of light by a substance that is not heated

Lux: unit of luminescence

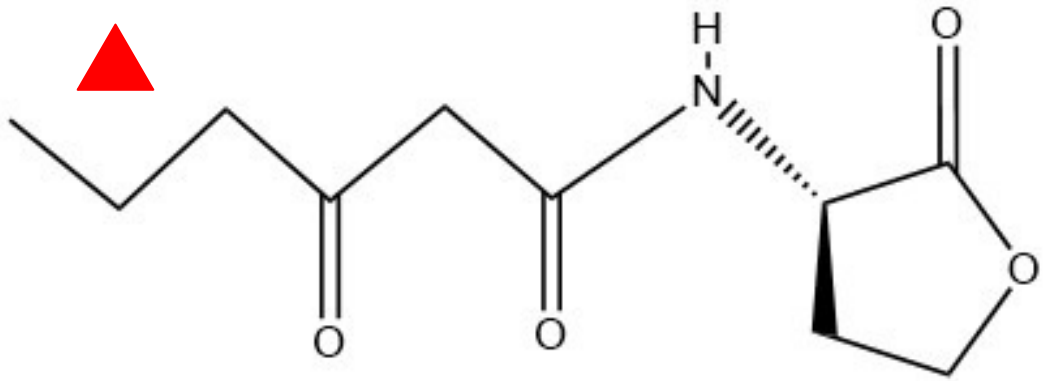
Genes involved in bioluminescence are named as lux genes in *V. fischeri*

Gene names: italics + all letters in lower case
Encoded proteins: normal font + first letter in upper case

Quorum sensing in Vibrio fischeri

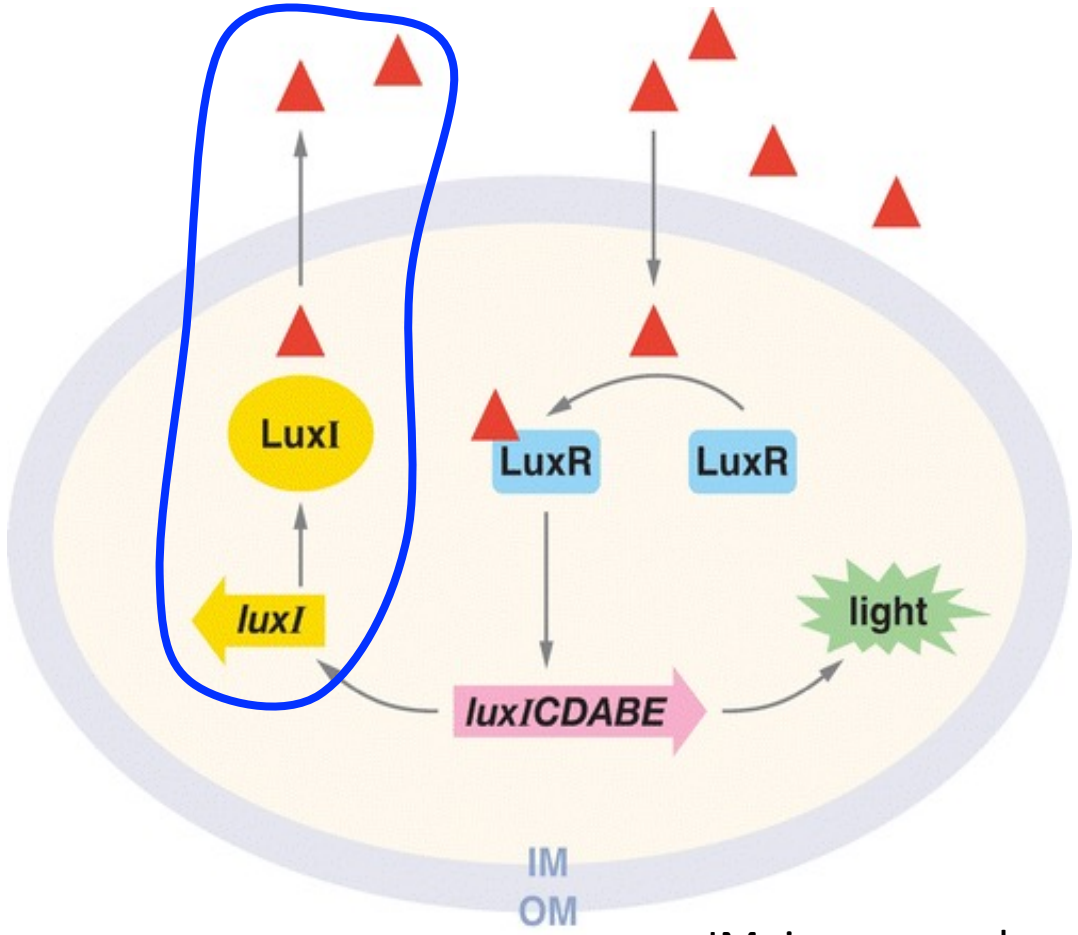
luxI: basal level of expression

LuxI: is an enzyme responsible for the synthesis of “auto-inducer”



3-Oxo hexanoyl homoserine lactone

Auto-inducer (AI) diffuses out of the cell



Waters CM, Bassler BL. 2005.
Annu. Rev. Cell Dev. Biol. 21:319–46

IM: inner membrane
OM: outer membrane

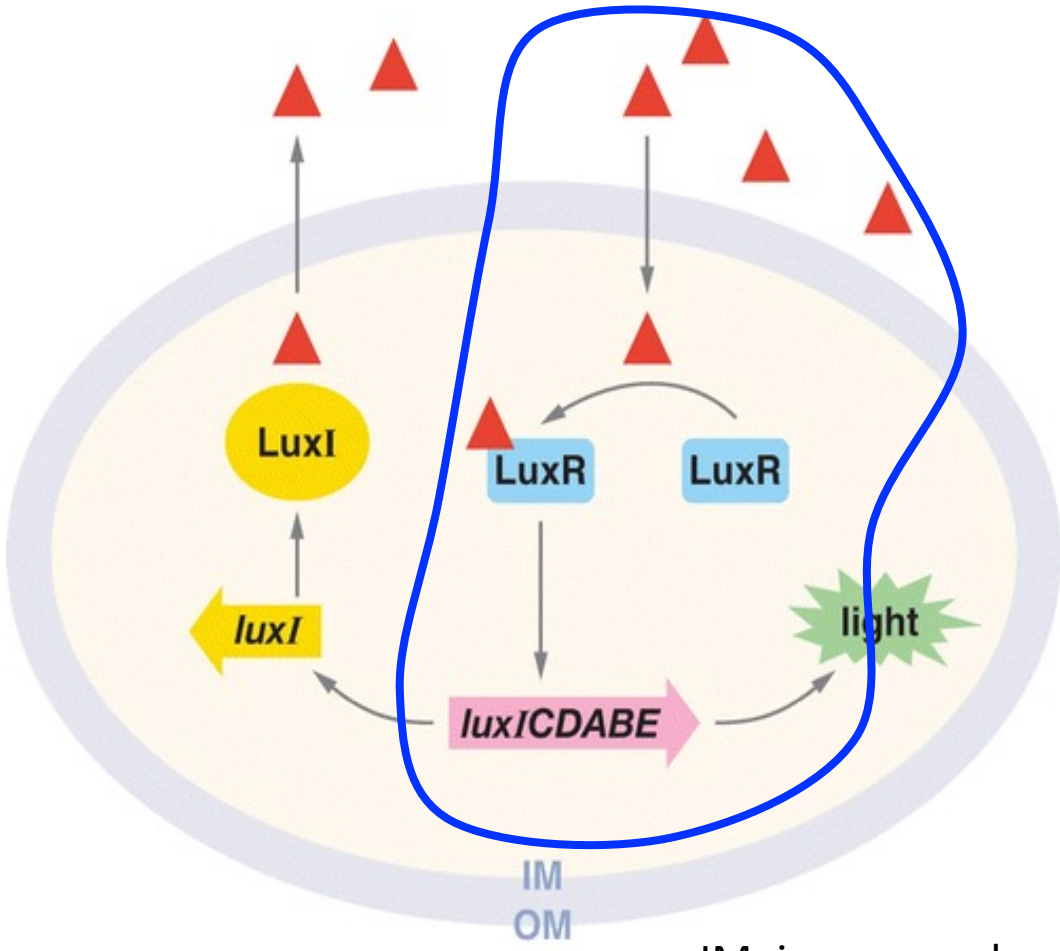
22

Quorum sensing in *Vibrio fischeri*

If quorum is met i.e., the number of bacteria reaches a certain critical level, then $[AI]_{extracellular}$ reaches a critical level

AI diffuses back into the cell and binds to LuxR, its cytosolic receptor

The LuxR-AI complex activates the transcription of the *lux* operon (*luxI* and *luxC* + *luxD* + *luxA* + *luxB* + *luxE*) resulting in **LIGHT!**



Waters CM, Bassler BL. 2005.
Annu. Rev. Cell Dev. Biol. 21:319–46

IM: inner membrane
OM: outer membrane

luxA, *luxB*: α, β subunits of luciferase
luxC, *luxD*, *luxE*: synthesis + recycling of luciferase substrate

Luminescence in *Vibrio fisheri*: purpose?

Luminescence is an energy-consuming process

- For the generation of light
- For the biosynthesis of the relevant proteins / associated molecules

Inference:

- Luminescence ought to be beneficial
- Why retain this phenotype if there is no advantage (growth / survival)?

Absence of luminescence in seawater suggests that the luminescent phenotype is produced under some other condition(s)

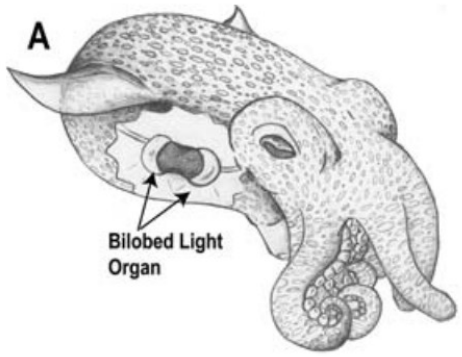
What are these other conditions?

J. Molec. Microbiol. Biotechnol. (1999) 1:5

Squid – bacteria (Vibrio fischeri) symbiosis

- Hawaiian bobtailed squid
- Luminescence in the light organ by symbiont *V. fischeri*
- Matches light intensity of the light organ to that of background light (moonlight / starlight), helps the squid to “cloak” and catch its prey
- Bioluminescence also controls circadian rhythms of the squid

What do the bacteria get out of this symbiosis? Nutrients from the light organ.

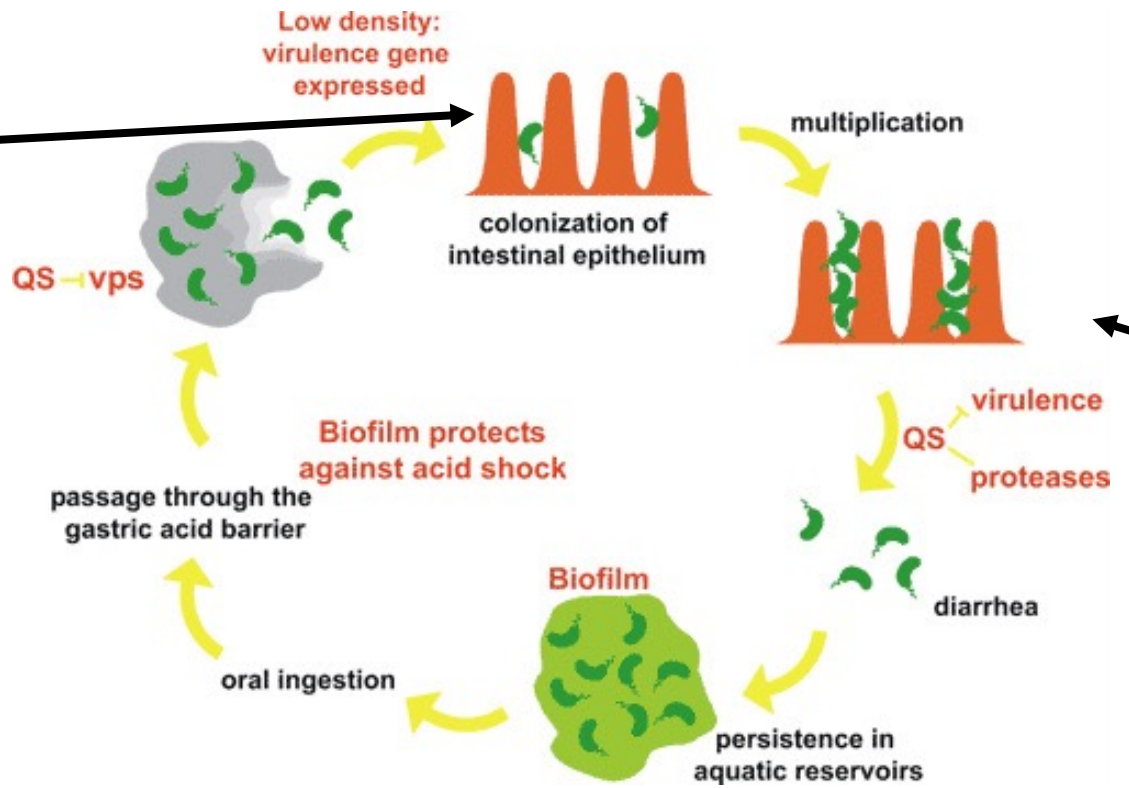


http://nishsymbiosislab.com/wp-content/uploads/2017/07/2-2004-JonesNish.pdf https://multimediaquest.c

Vibrio cholerae: causes diarrhea

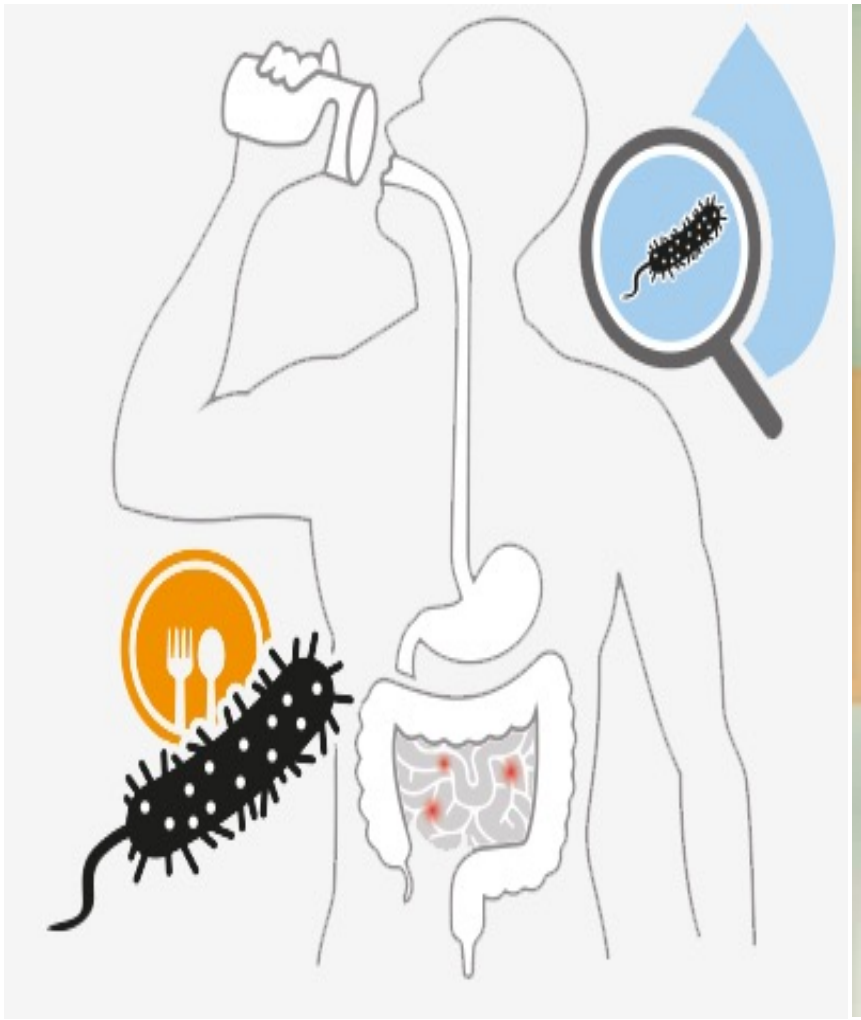
Quorum Sensing (QS) at different parts of the pathogenic cycle

Low density of bacteria:
Virulence genes expressed



High density of bacteria: Protease genes expressed, leading to diarrhea so that the bacteria can escape from the human host

As cells communicate for the "common good", we can study cooperation/cheating/etc.




CHOLERA DISEASE

How does Cholera toxin causes diarrhea??

A-subunit of cholera toxin ribosylates the alpha subunit of the Gs protein

Leads to activation of adenylate cyclase

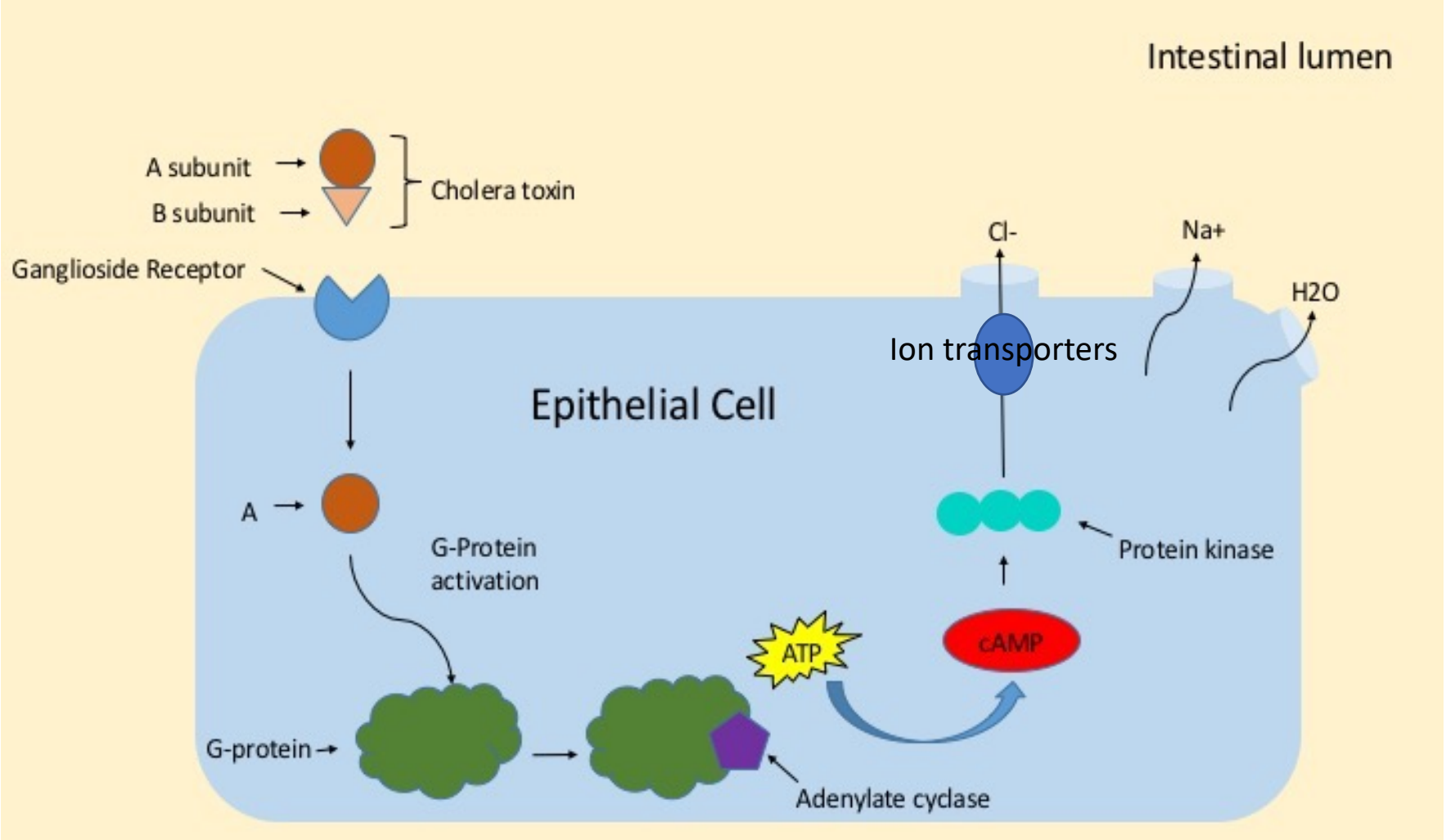
Results in increased levels of cAMP

This leads to the activation of PKA

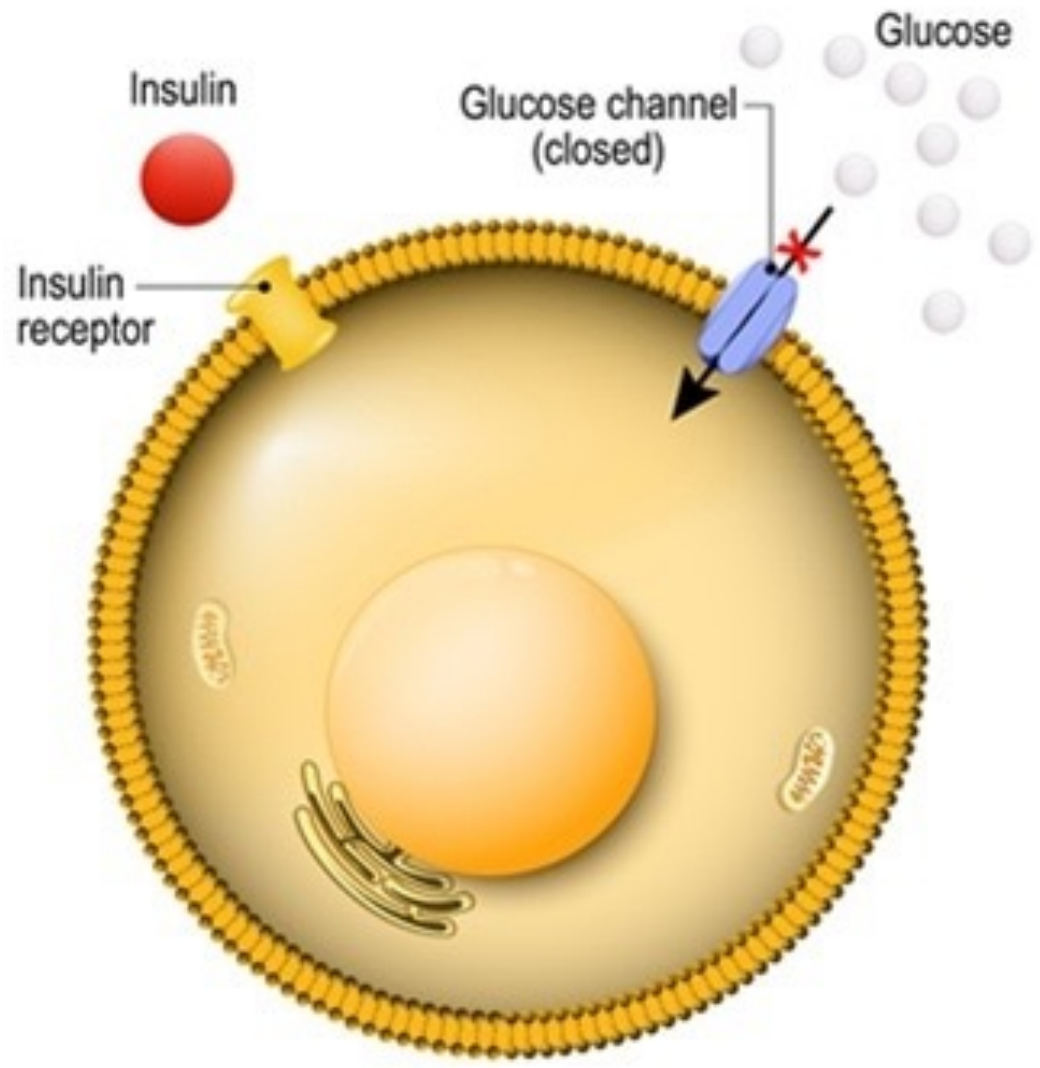
PKA phosphorylates CFTR and Na-H exchanger

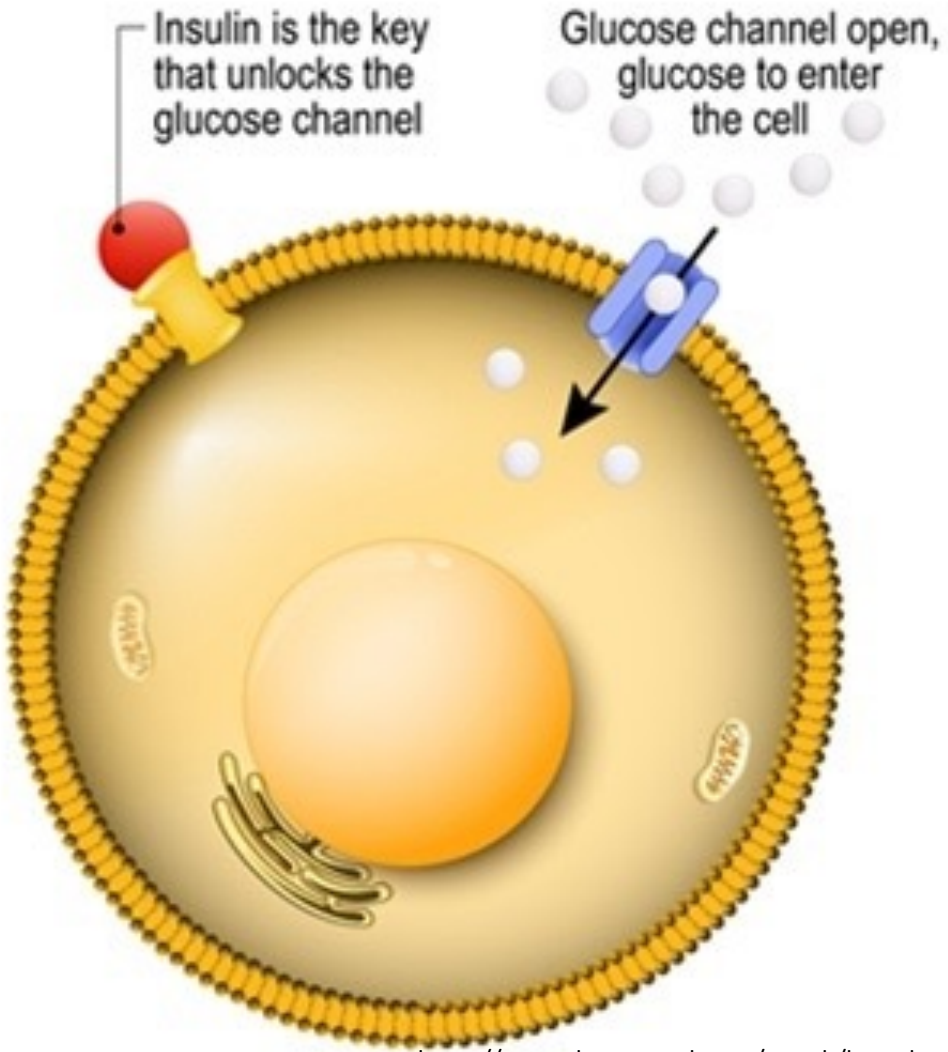
Causes efflux of chloride and Na and H2O

Hence causing severe diarrhea



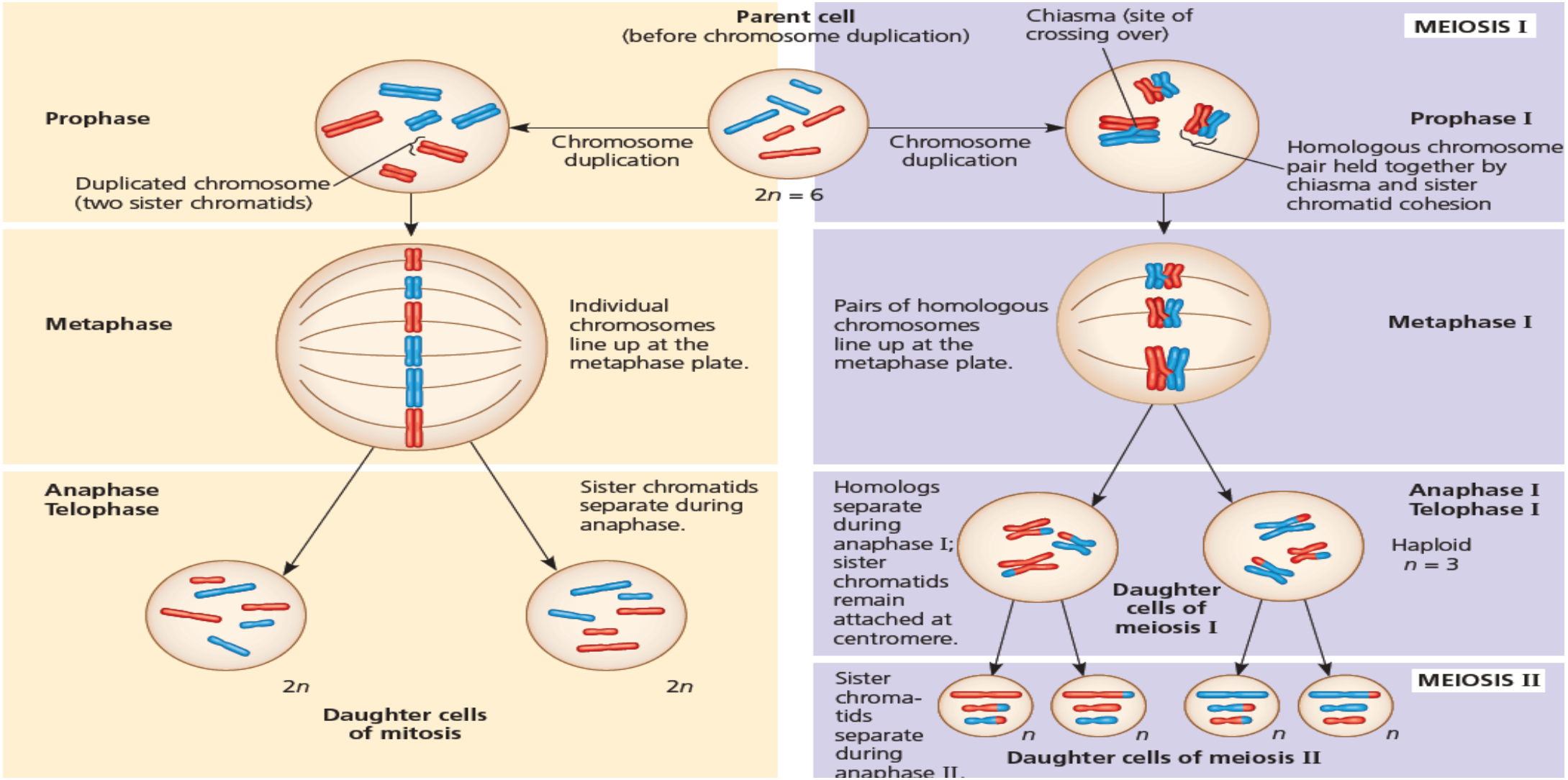
How does Insulin work?





https://www.shutterstock.com/search/how-does-insulin-work

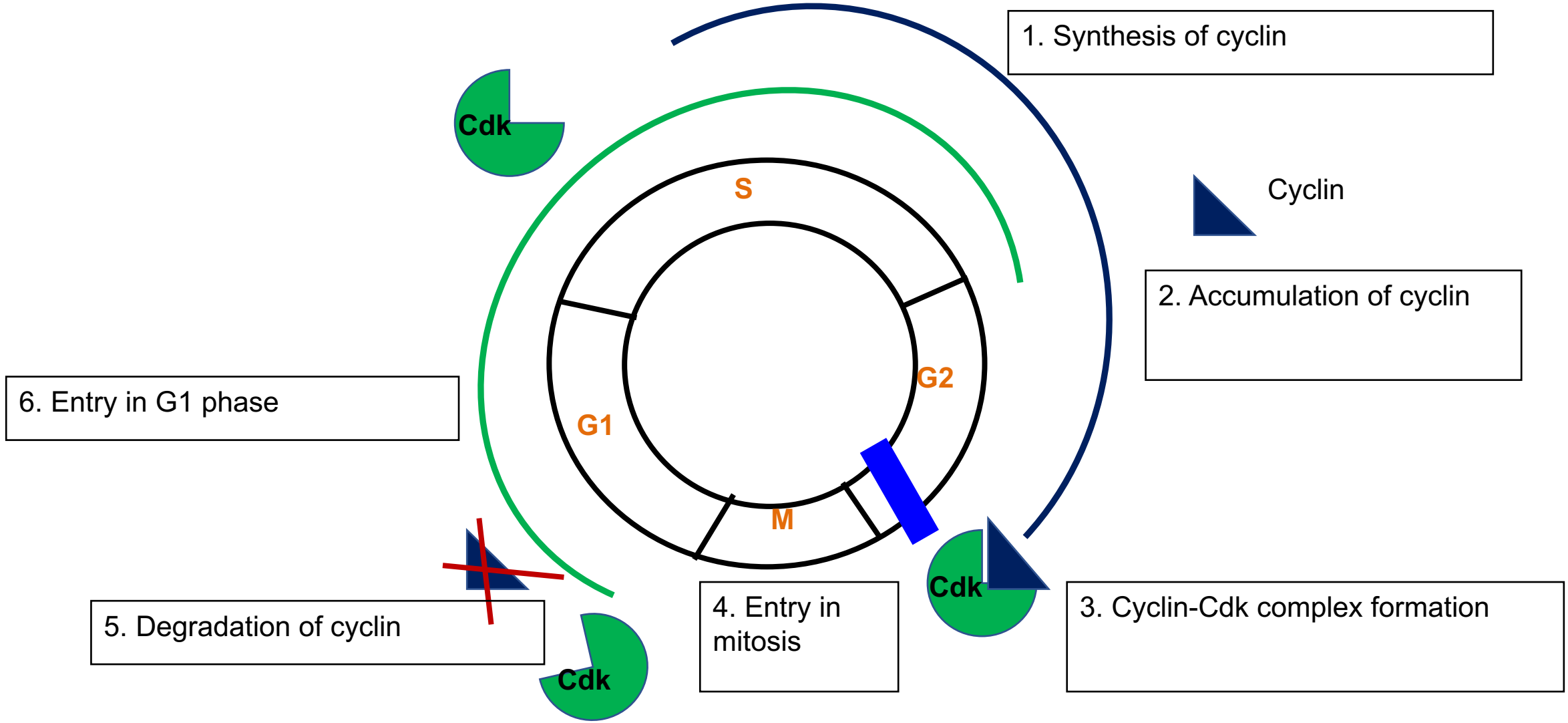
Cell Division: Mitosis vs. Meiosis



A comparison of mitosis and meiosis

Property	**Mitosis** (occurs in both diploid and haploid cells)	**Meiosis** (can only occur in diploid cells)
DNA replication	Occurs during interphase before mitosis begins	Occurs during interphase before meiosis I begins
Number of divisions	One, including prophase, prometaphase, metaphase, anaphase, and telophase	Two, each including prophase, metaphase, anaphase, and telophase
Synapsis of homologous chromosomes	Does not occur	Occurs during prophase I along with crossing over between nonsister chromatids; resulting chiasmata hold pairs together due to sister chromatid cohesion
Number of daughter cells and genetic composition	Two, each genetically identical to the parent cell, with the same number of chromosomes	Four, each haploid (*n*); genetically different from the parent cell and from each other
Role in the animal or plant body	Enables multicellular animal or plant (gametophyte or sporophyte) to arise from a single cell; produces cells for growth, repair, and, in some species, asexual reproduction; produces gametes in the gametophyte plant	Produces gametes (in animals) or spores (in the sporophyte plant); reduces number of chromosome sets by half and introduces genetic variability among the gametes or spores

Regulation of Cell Cycle by Cyclins and Cyclin Dependent Kinases



Cell cycle and its checkpoints

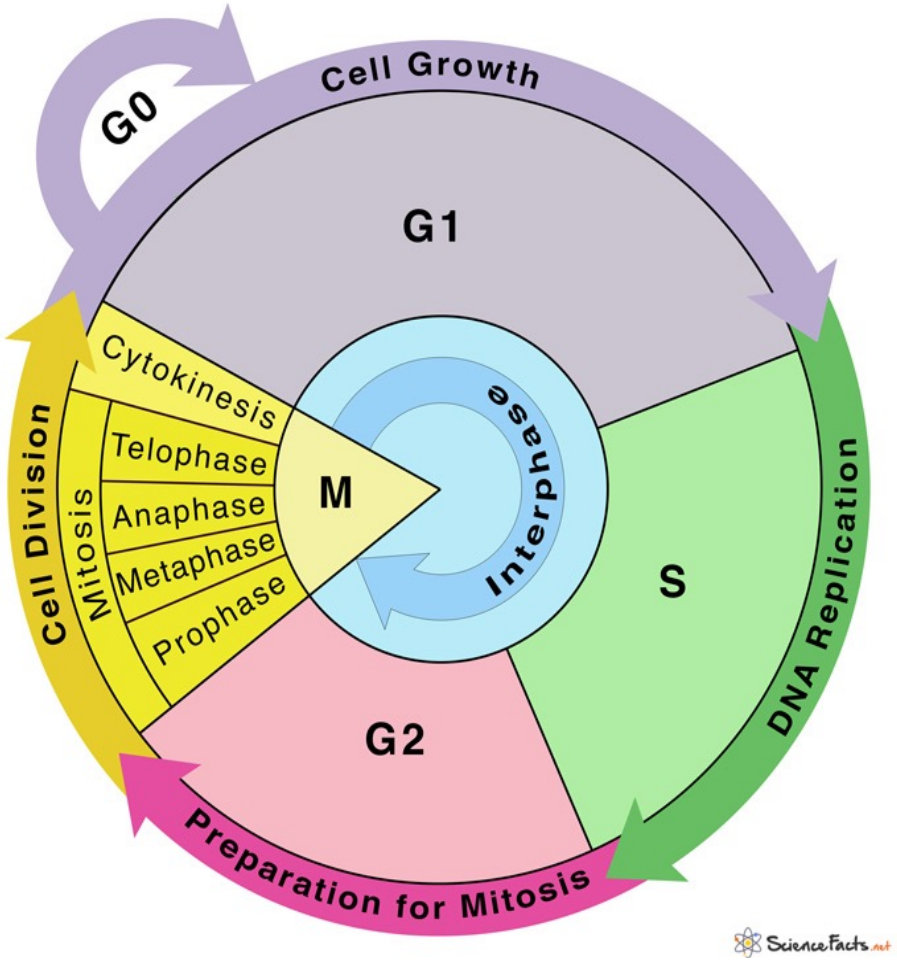


Fig.: Cell cycle

Important checkpoints:

1. G1 Checkpoint- Nutrients, growth factors, DNA damage
2. G2 Checkpoint- Cell size, DNA replication
3. M Checkpoint- Chromosome spindle attachment

Source: https://www.sciencefacts.net/cell-cycle.html