# 212 SM L06a

# Microbial behaviour=gene expression=adaptive strategies to persist

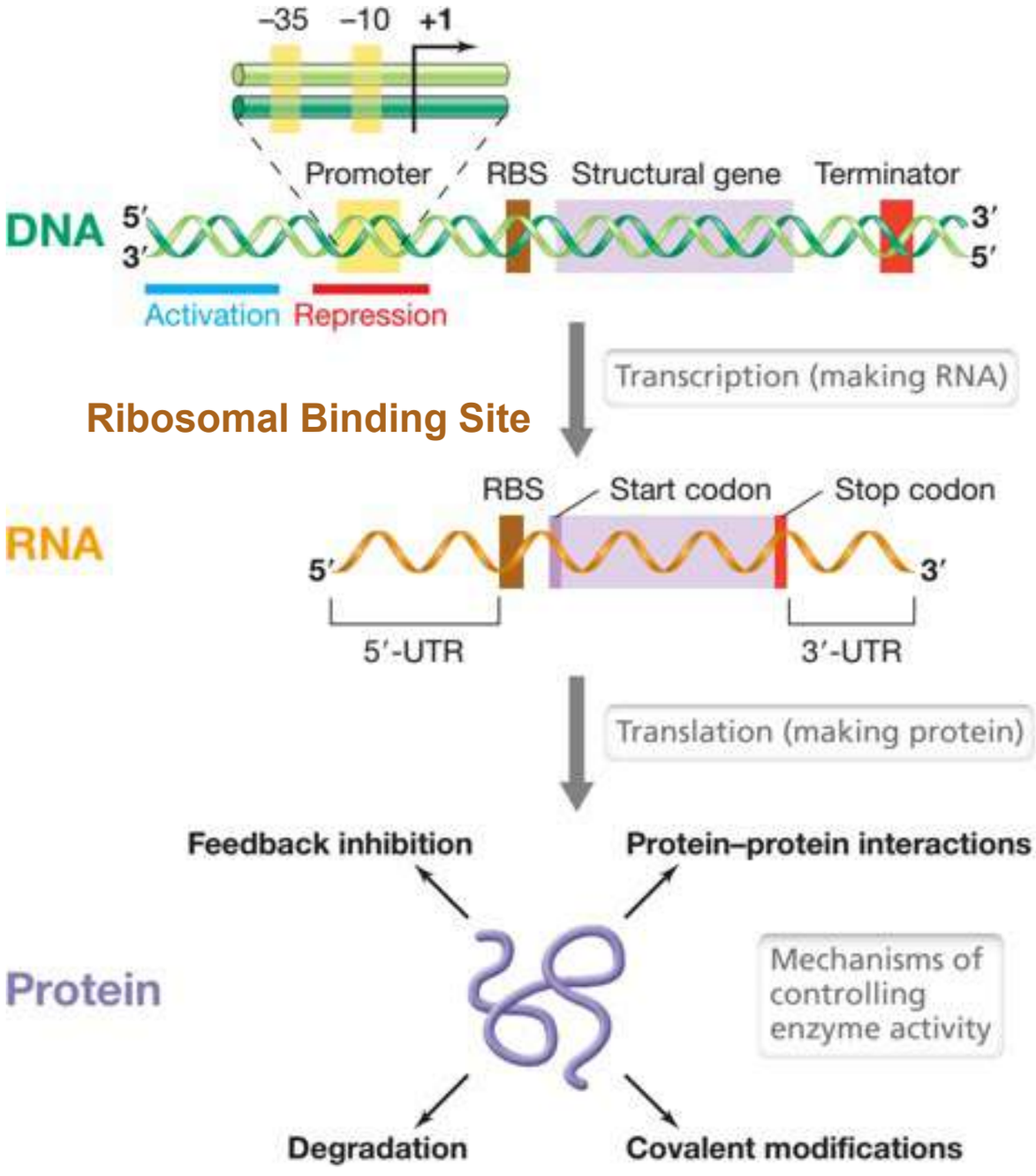
# Microbial Regulatory Systems

- Regulatory system **couples growth with available resources**
- Some proteins and RNAs are needed in the cell at about the same level under all growth conditions: **constitutive expression**
- 2 major approaches to regulate protein function:

  A. Control protein **amount**

  B. Control protein **activity**

- *Amount of protein synthesized can be **regulated** at either the level of transcription, by varying the amount of mRNA made, at the level of translation, by translating or not translating the mRNA—> **gene expression***
- After the protein has been synthesized, **post-translational regulatory** processes

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# Gene regulation within environmental context

- Microbes need to **adapt** to **changes** in environmental conditions in order to **survive**

- **Adaptation** requires to quickly express the **genes** necessary to **cope** with specific **environmental stimuli** and **maximize energy saving** in any conditions

- rRNAs, tRNAs, ribosomal proteins, RNA polymerases genes are essential —> always expressed —> **constitutive** expression

- Other genes whose activity is **regulated** (i.e. **activation**, **repression**) according to the need of the microbe in a **coordinate** fashion —> **OPERON** indicates a **cluster of genes** with **related functions** and regulated in a **coordinated** manner

# RNA synthesis: Transcription_recap

- RNA polymerase (multicomplex enzyme)
- **σ recognizes the appropriate site on DNA** for transcription to begin (σ dissociates from holoenzyme once a short sequence of RNA has been formed)
- **Several σ**, most used $\sigma^{70}$
- **Several promoters** w. 2 highly conserved regions
- Upstream the transcription start site:

**A. 10 bases upstream, the -10 region, or Pribnow box; consensus sequence of TATAAT**

**B. 35 bases upstream consensus sequence is TTGACA, -35 region**

**TABLE 4.3 Sigma factors in *Escherichia coli***

| *Name*[a] | *Upstream recognition sequence*[b] | *Function* |
|---|---|---|
| $\sigma^{70}$ RpoD | TTGACA | For most genes, major sigma factor for normal growth |
| $\sigma^{54}$ RpoN | TTGGCACA | Nitrogen assimilation |
| $\sigma^{38}$ RpoS | CCGGCG | Stationary phase, plus oxidative and osmotic stress |
| $\sigma^{32}$ RpoH | TNTCNCCTTGAA | Heat shock response |
| $\sigma^{28}$ FliA | TAAA | For genes involved in flagella synthesis |
| $\sigma^{24}$ RpoE | GAACTT | Response to misfolded proteins in periplasm |
| $\sigma^{19}$ FecI | AAGGAAAAT | For certain genes in iron transport |

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**Jacob F, Perrin D, Sanchez C, Monod J (1960) L'operon: Groupe de genes a l'expression coordonne par un operateur. C R Acad Sci 245: 1727–729**

**Jacob F, Monod J (1961) On the regulation of gene activity. In: Cold Spring Harbor Symposium Quantitative Biology 26, pp 193–211**

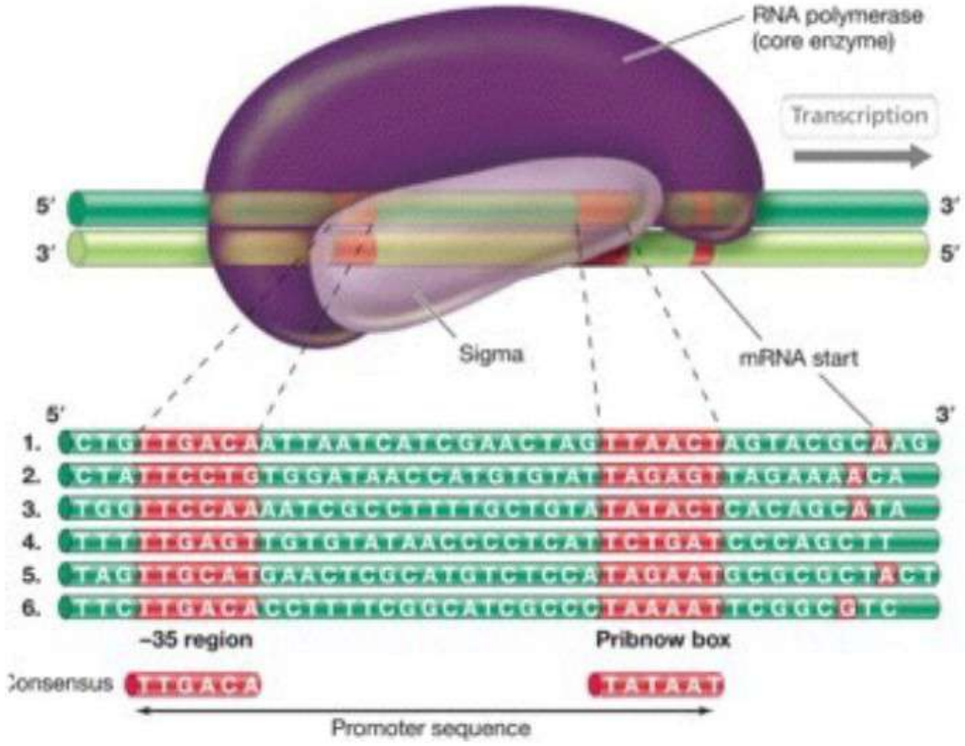
## OPERON STRUCTURE: PROG: promoter, repressor, operator and genes

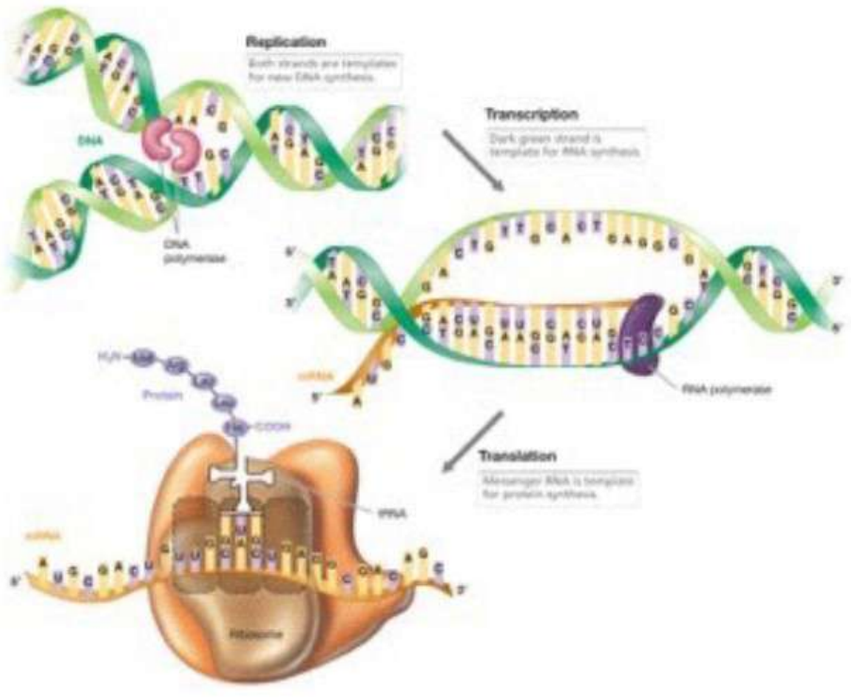


**https://youtu.be/10YWgqmAEsQ**

# RNA synthesis: Transcription_recap II

- Transcription begins at a unique base just downstream from -35 and the Pribnow box
- **Sigma recognizes the promoter sequences on the 5′—>3′ (dark green) strand of DNA**
- **RNA polymerase** core enzyme will actually transcribe the light green strand (that runs 3′—>5′) b/c **core enzyme synthesizes 5′—>3′ direction**

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# The Nobel Prize in Physiology or Medicine 1965

Photo from the Nobel Foundation archive.

## François Jacob

Prize share: 1/3

Photo from the Nobel Foundation archive.

## André Lwoff

Prize share: 1/3

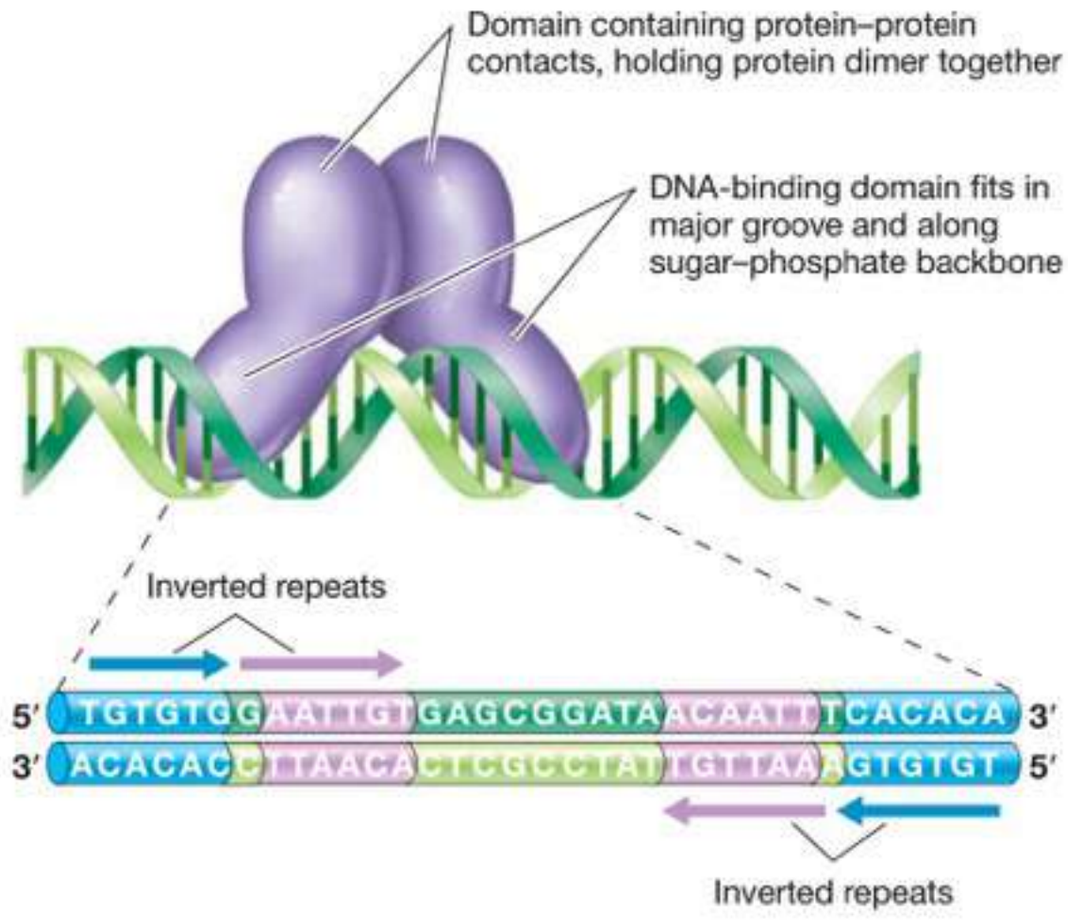
Photo from the Nobel Foundation archive.

## Jacques Monod

Prize share: 1/3

# DNA-Protein Interaction: Regulation

- For a gene to be transcribed: **RNA pol & σ must recognize a specific promoter site on the DNA**
- **Regulatory proteins influence protein binding** to specific DNA sites —> gene expression by turning transcription on or off
- **Protein-nucleic acid interactions are central to replication, transcription, translation, their regulation**
- **DNA-binding proteins are often homodimeric, 2 identical polypeptide subunits w. domains** (= regions of the protein with a specific structure and function)

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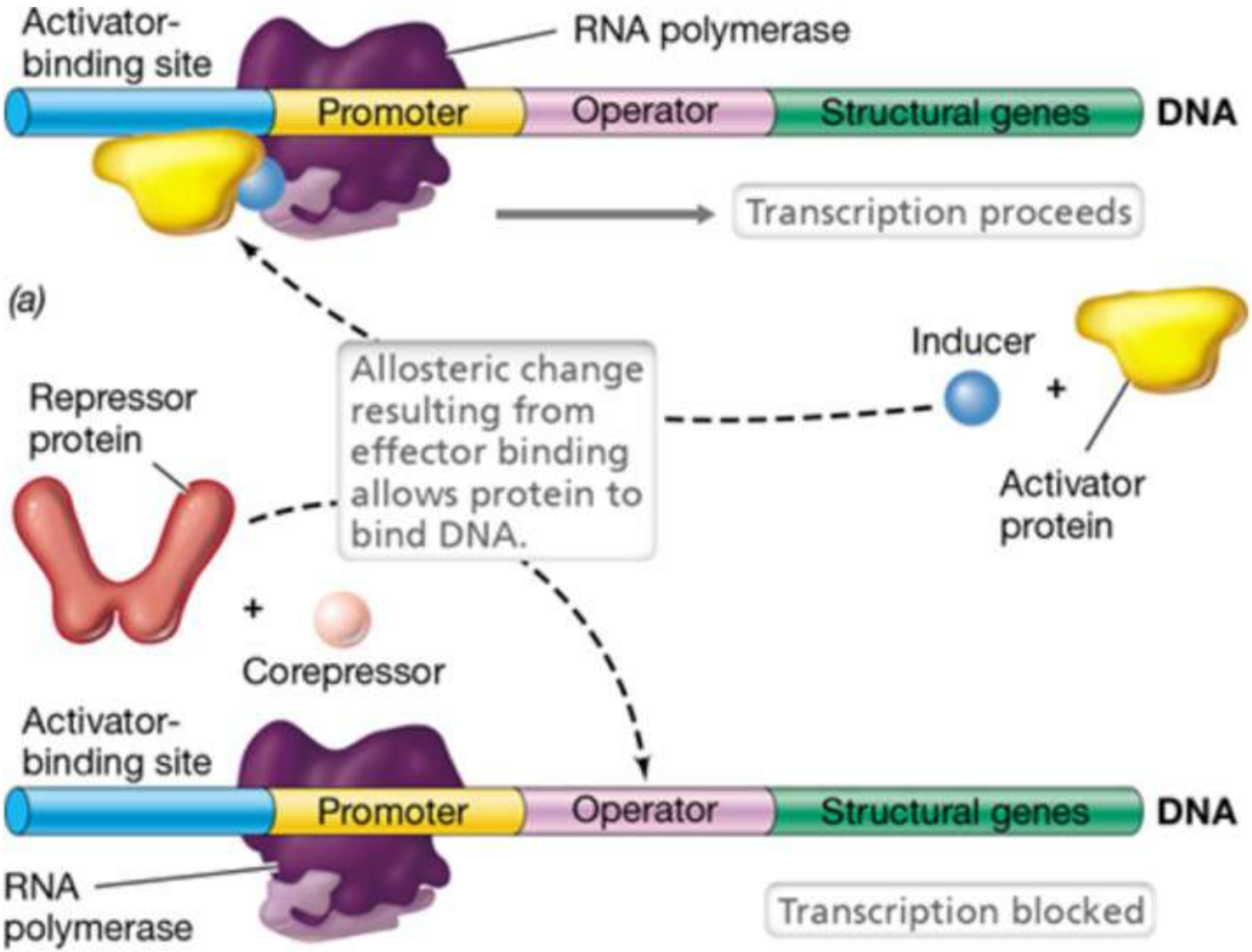
# Transcription factors: DNA-protein interactions

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Binding of effector molecules to activator and repressor proteins results in an allosteric change that affects the DNA-binding ability of the transcription factors. *(a)* Binding of an activator to the DNA results in recruiting RNA polymerase and turning transcription on. *(b)* Binding of a repressor protein to the operator region of the DNA results in blocking RNA polymerase and turning transcription off.

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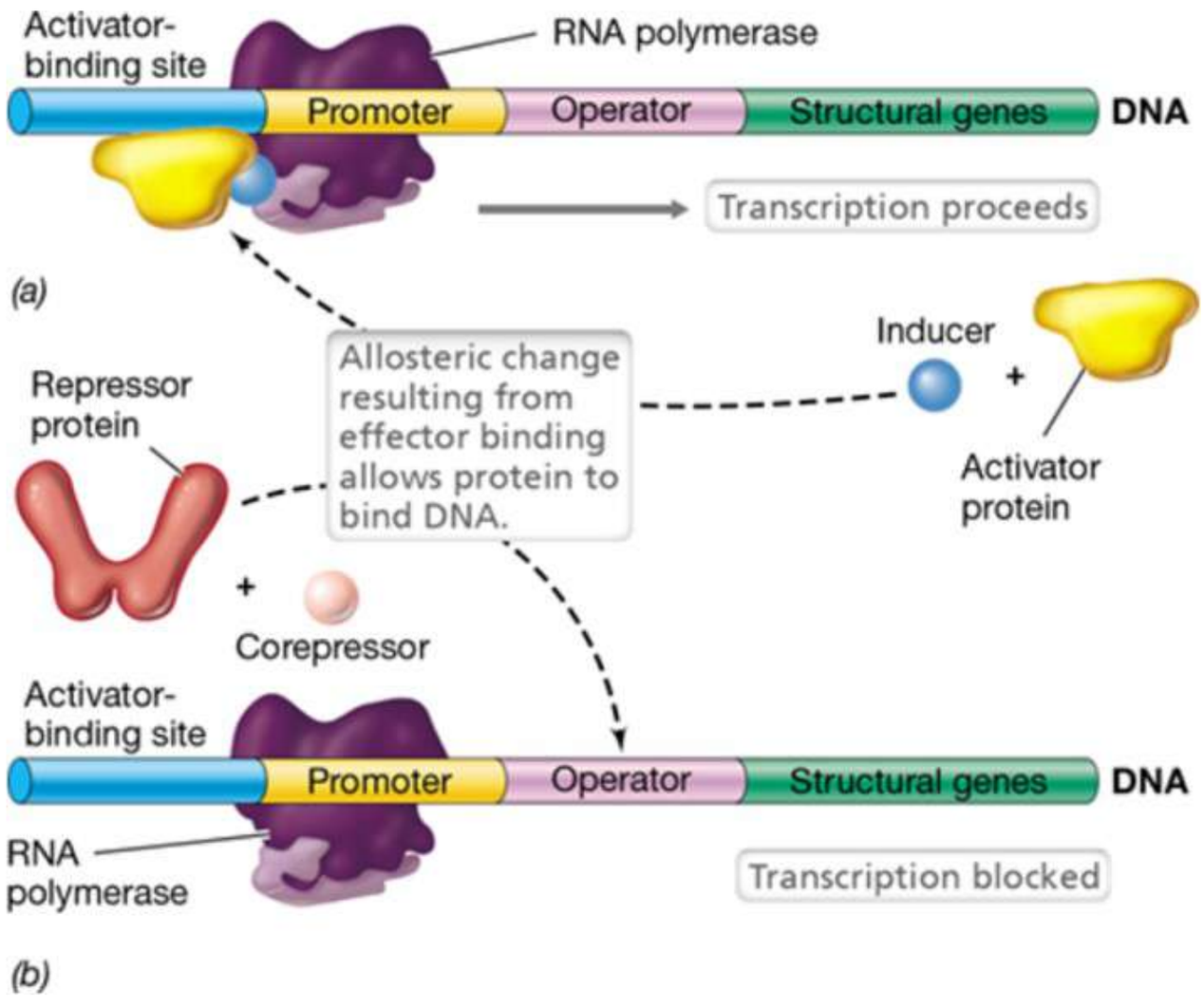
# OPERON STRUCTURE

**Promoter: RNA-σ binding site** (Promoter sequences are DNA sequences that define where transcription of a gene by **RNA polymerase** begins)

**Repressor, a protein (and co-repressor)**

**Operator: Repressor binding site**

**Genes**




## Positive control

Activator proteins help RNA polymerase to recognise promoter site

## Negative control

Change in 3D structure of repressor favours (REPRESSION) or prevent (INDUCTION) binding to operator

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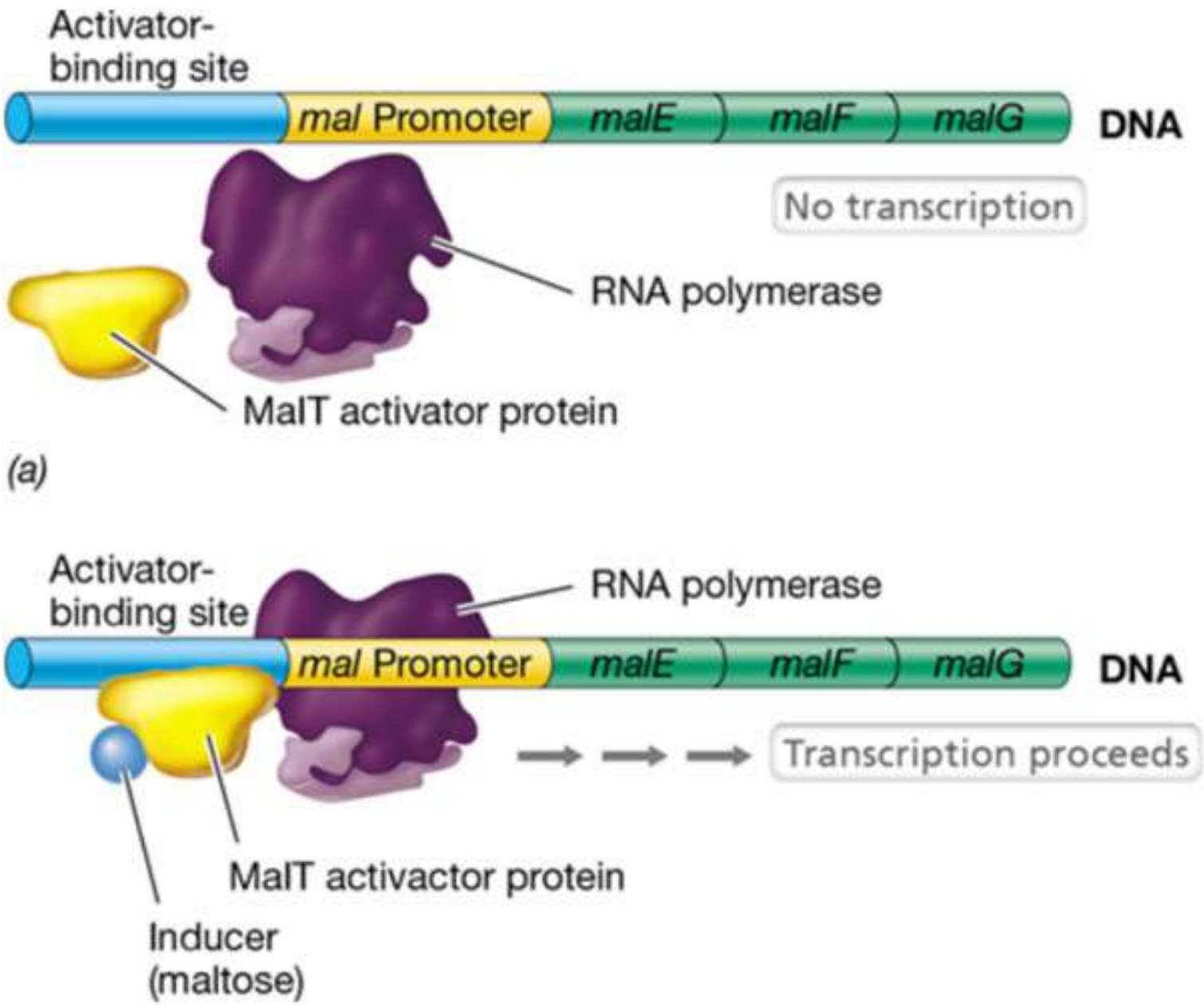
# POSITIVE CONTROL

# Figure 7.9 Positive control of enzyme induction in the maltose operon.




- Enzymes for maltose (disaccaride) catabolism in *E. coli* are synthesized only after maltose addition to medium
- Maltose activator protein cannot bind to DNA unless it first binds maltose (inducer)
- When maltose activator protein binds to DNA, it allows RNA polymerase to begin transcription

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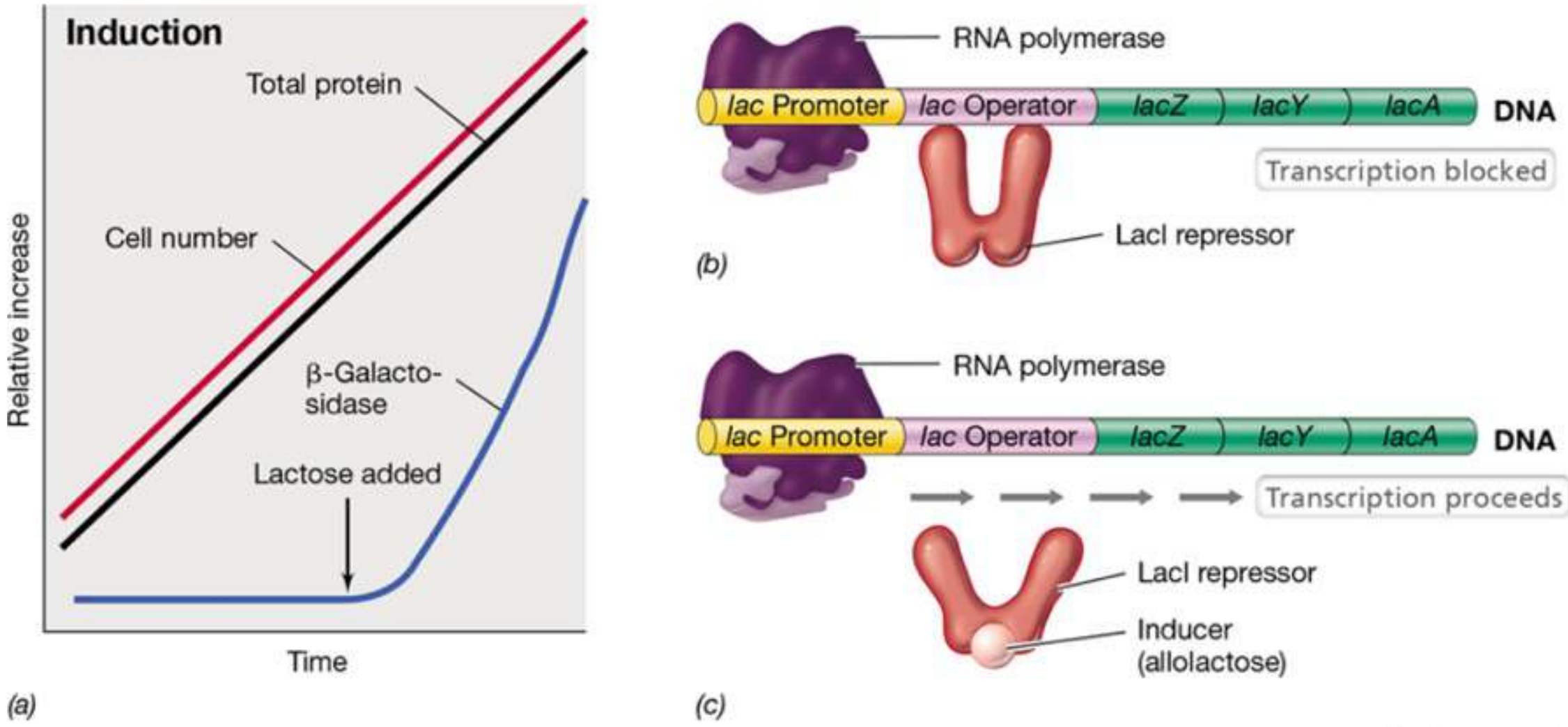
# NEGATIVE CONTROL

# Figure 7.6 Enzyme induction and expression of the lactose operon.

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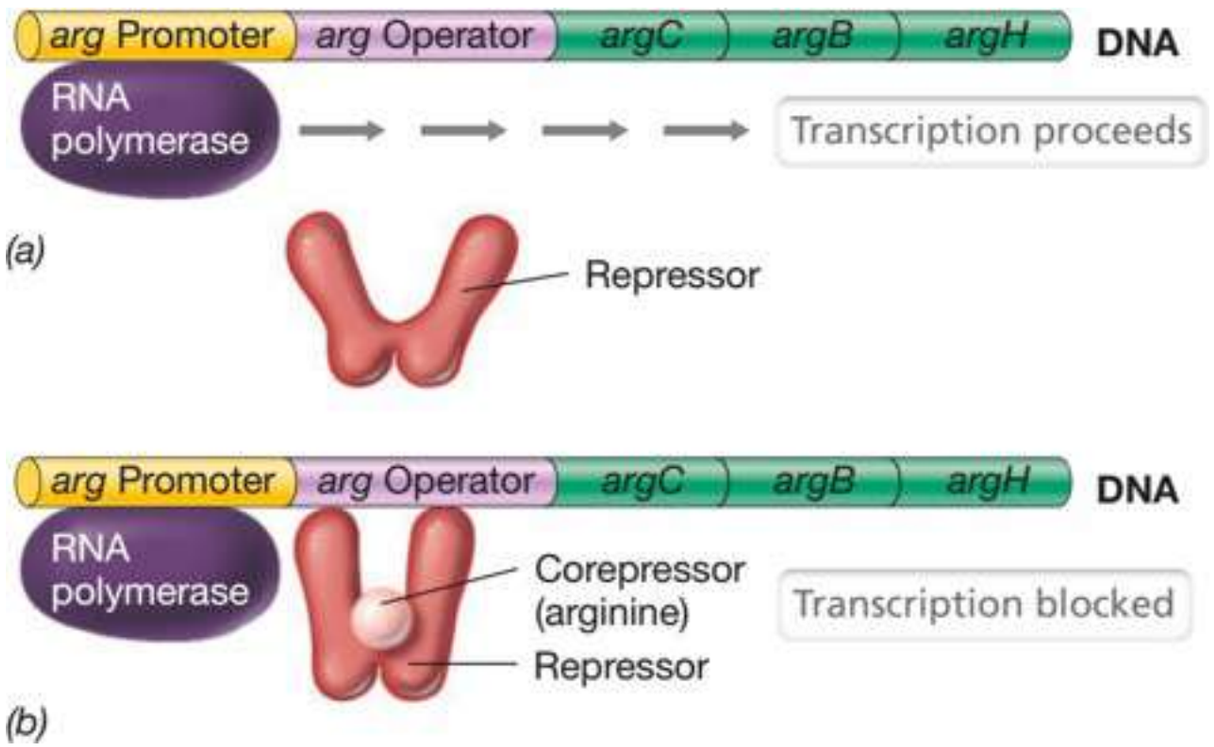
# Enzyme repression of arginine biosynthetic pathway

- In *E. coli,* enzymes for **Arg** synthesis are made **only when Arg is absent**—> an excess of Arg decreases synthesis of these enzymes: **enzyme repression**
- *Final product of a particular biosynthetic pathway represses the enzymes of the pathway —> organism does not waste energy and nutrients synthesizing unneeded enzymes*
- A substance that represses enzyme synthesis is called a **corepressor, Arg (effector)**
- **Repressor protein is allosteric** —> its **conformation is altered when effector binds to it**




- By binding its effector, **repressor** protein is activated —> bind to a specific region **Operator (near the promoter of the gene)**

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# Global Networks

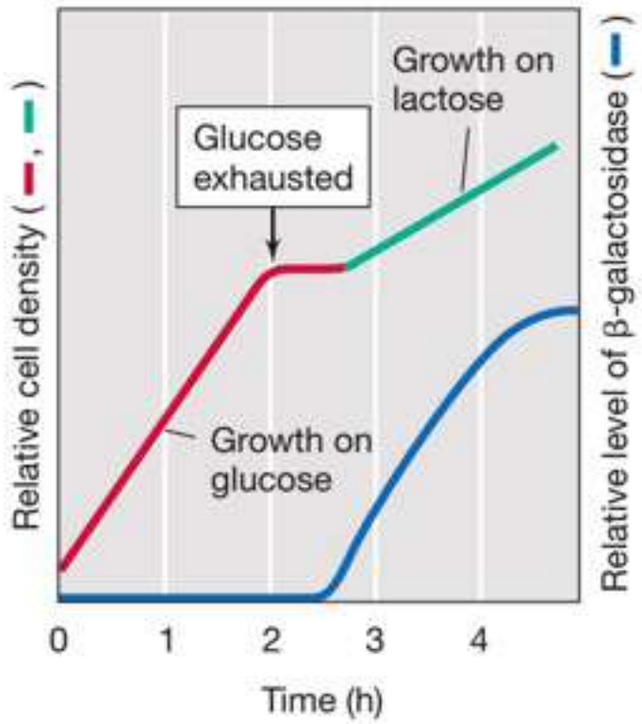
- When **more than one operon** is **under the control of a single regulatory protein**, these operons are collectively called a **regulon**
- **Global control systems regulate many genes** comprising **more** than one **regulon**
- Global control networks may include activators, repressors, signal molecules, two-component regulatory systems, regulatory RNA & alternative sigma factors

**TABLE 6.2 Examples of global control systems known in *Escherichia coli*[a]**

| *System* | *Signal* | *Primary activity of regulatory protein* | *Number of genes regulated* |
|---|---|---|---|
| Aerobic respiration | Presence of $O_2$ | Repressor (ArcA) | > 50 |
| Anaerobic respiration | Lack of $O_2$ | Activator (FNR) | > 70 |
| Catabolite repression | Cyclic AMP level | Activator (CRP) | > 300 |
| Heat shock | Temperature | Alternative sigma factors (RpoH and RpoE) | 36 |
| Nitrogen utilization | $NH_3$ limitation | Activator (NRI)/alternative sigma factor (RpoN) | > 12 |
| Oxidative stress | Oxidizing agents | Activator (OxyR) | > 30 |
| SOS response | Damaged DNA | Repressor (LexA) | > 20 |
| General stress response | Stress conditions | Alternative sigma factor (RpoS) | > 400 |

# Catabolite repression, I

- An organism needs to **regulate many unrelated genes simultaneously** in response to a change in its environment
- Global control systems: **regulatory mechanisms responding to environmental signals by regulating the transcription of many different genes**
- In a complex environment, the **presence of a favored carbon** source represses the induction of pathways that catabolize other carbon sources
- **Catabolite repression** ensures that the organism uses the **best carbon and energy source first** (e.g. glucose)
- Catabolic operons: **lac, malt, genes for the synthesis of flagella (bc if bacteria have a good carbon source available, no need to swim around)**
- One consequence of catabolite repression —> 2 exponential growth phases: **diauxic growth**
- If two usable energy sources are available, the cells first consume the better energy source

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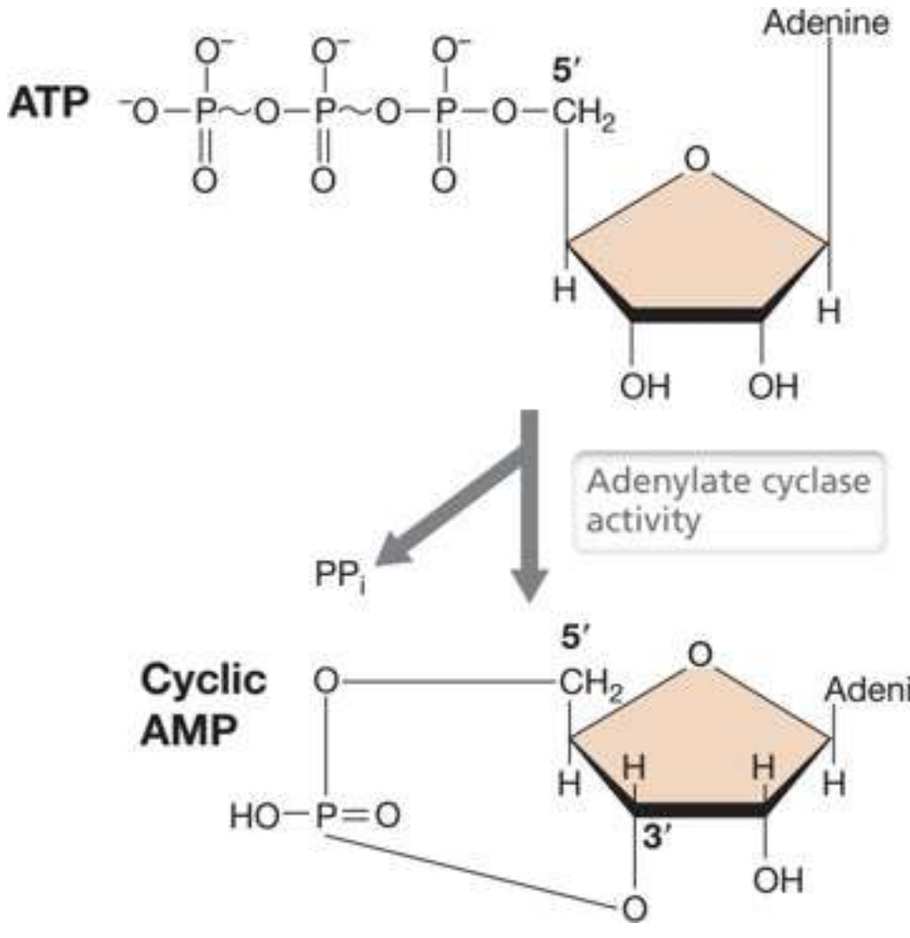
# Catabolite repression, II

- **Catabolite repression** relies on an **activator protein** (**positive control**): cyclic AMP receptor protein (CRP) a dimer
- A gene that encodes a **catabolite-repressible enzyme is expressed only if CRP binds to DNA promoter region —> allowing RNA polymerase binding to promoter**
- **Effector** is **cAMP** derived from a nucleic acid precursor, it is a **regulatory nucleotide**
- **Cyclic di-GMP** (biofilm formation)
- **Guanosine tetraphosphate (ppGpp**, stringent response)
- Cyclic AMP is synthesized from ATP by an enzyme called adenylate cyclase
- **Glucose inhibits cyclic AMP synthesis and stimulates cyclic AMP transport out of the cell**
- Direct cause of catabolite repression is low level of cyclic AMP

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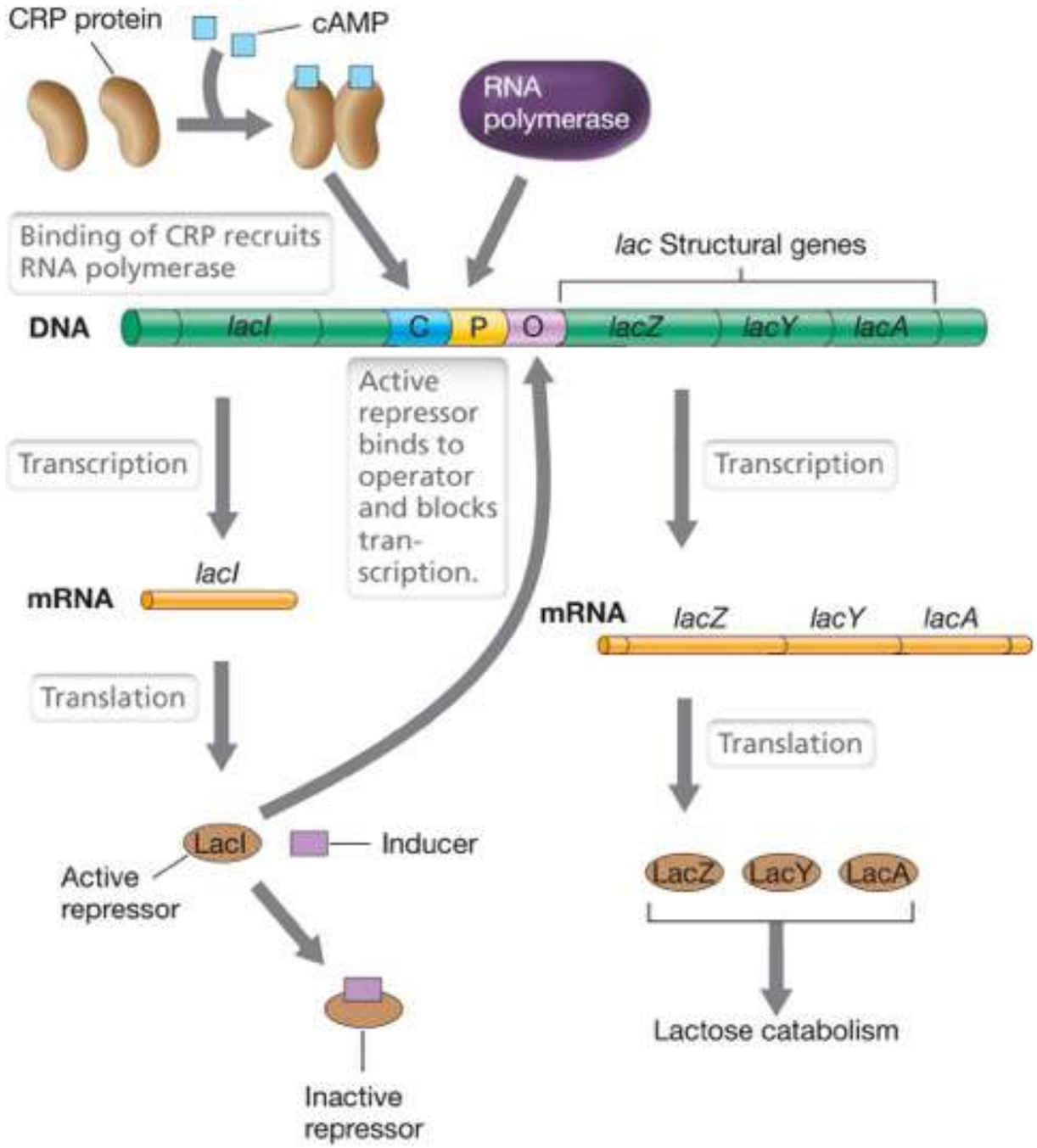
# Catabolite repression, III

For **lac** genes to be **transcribed**:

**(1)Level of cyclic AMP must be high enough for the CRP protein to bind to the CRP-binding site** (positive control)

(2)Lactose or another suitable inducer must be present so that the lactose repressor (LacI protein) does not block transcription by binding to the operator (negative control)

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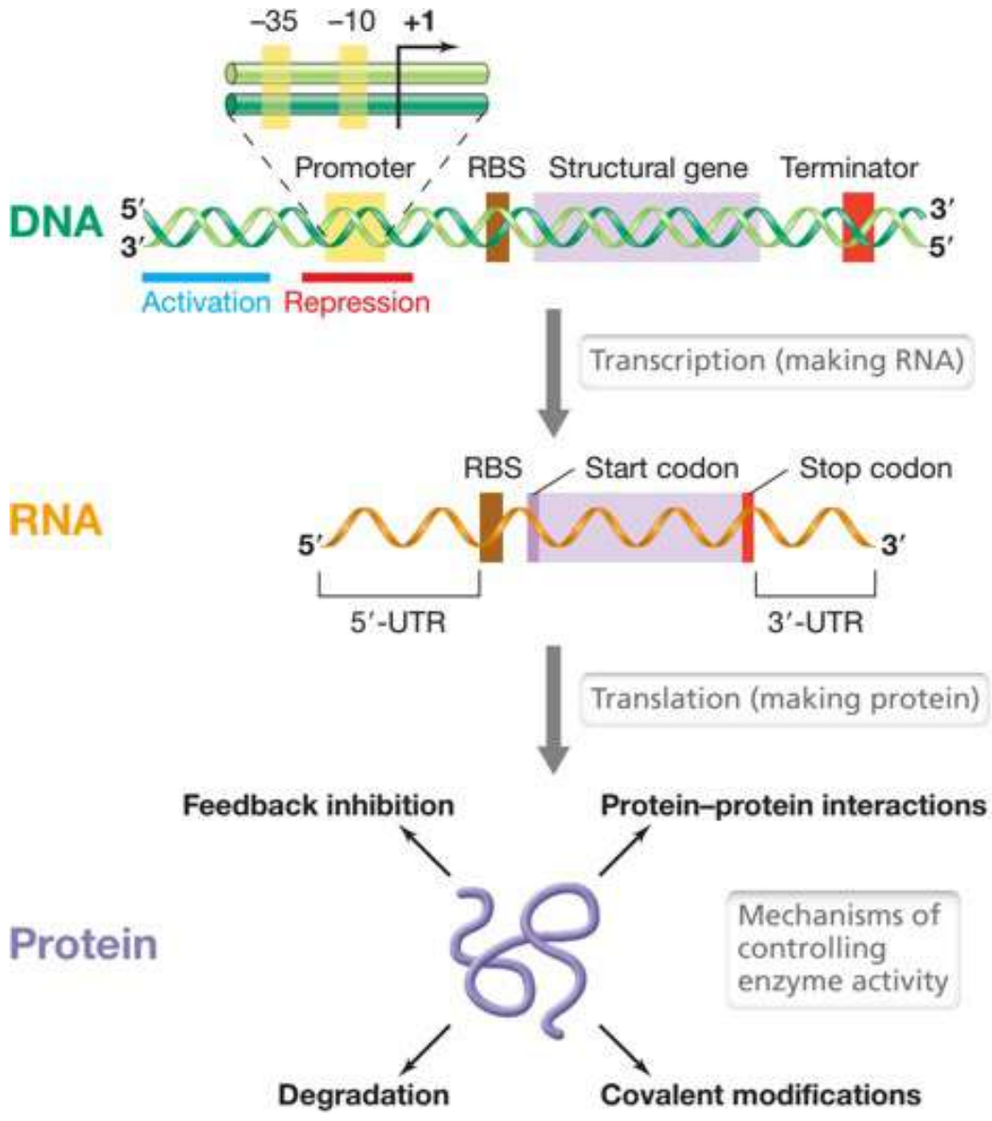
# RNA-Based Regulation, I

- RNA can regulate gene expression at the level of transcription & of translation
- RNA molecules that are **not translated to give proteins are known as noncoding RNA (ncRNA)**: **rRNA, tRNA, RNA present in the signal recognition particle** that catalyzes some types of protein secretion
- Small RNAs (sRNAs) that range from **40–400 nucleotides long** and regulate gene expression are **widely distributed**
- **sRNA binds to other RNAs or to small molecules**—> control of gene expression

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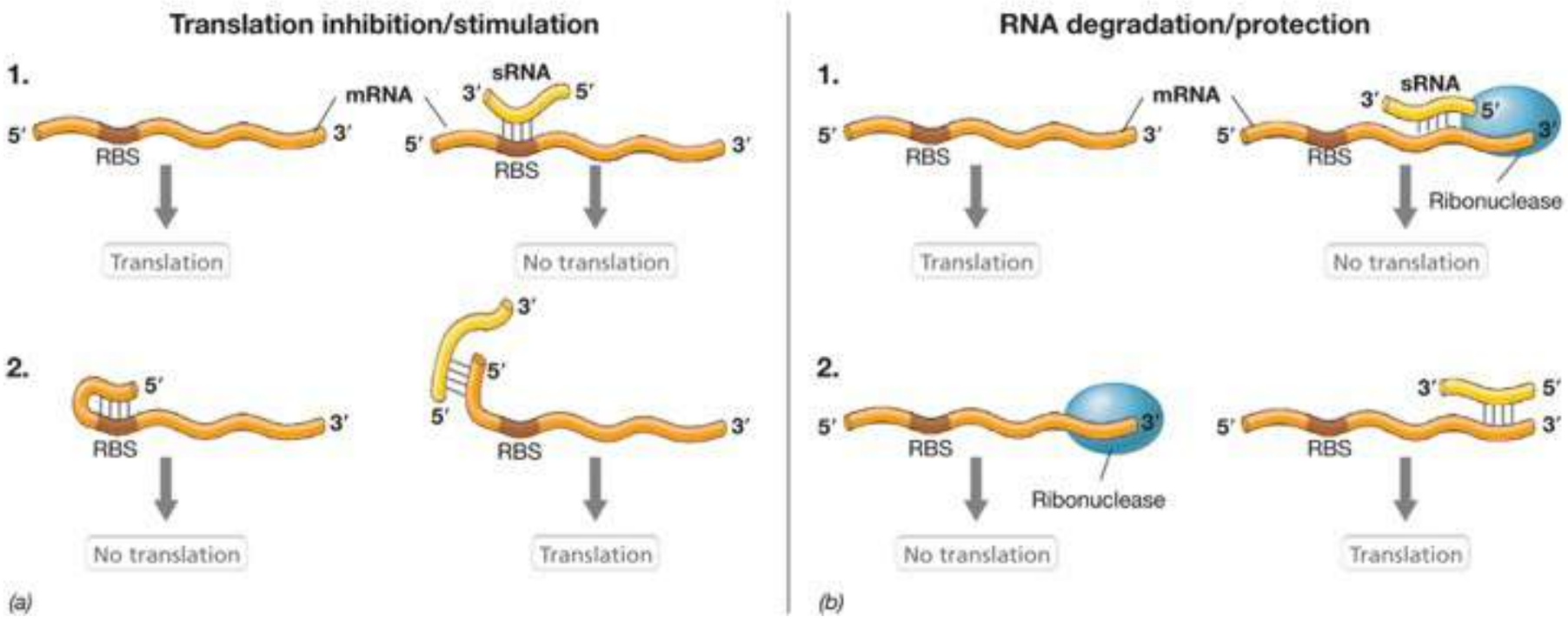
# RNA-Based Regulation, II

- Small RNAs (sRNAs) exert their **effects by base-pairing** directly to other RNA molecules, usually mRNAs, which have regions of complementary sequence
- **Binding** immediately **modulates** rate of target mRNA translation b/c ribosome cannot translate double-stranded RNA
- **sRNAs** provide additional mechanism to **regulate protein synthesis** once its corresponding **mRNA** has **already** been **transcribed**
- **sRNA interaction** affect **mRNA stability** —> binding of sRNA to its target can: either 3. increase or 4. decrease degradation of the transcript by bacterial ribonucleases —> modulating protein expression

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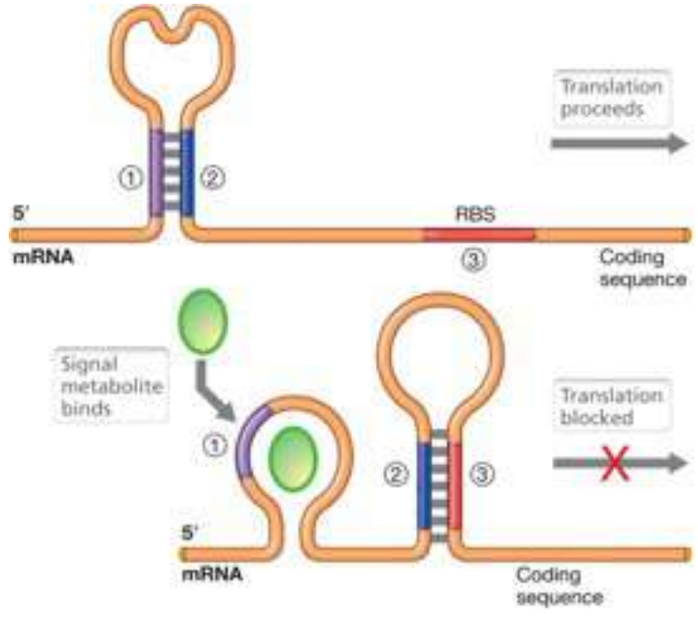
# Riboswitches

- **RNA** can specifically recognize and **bind other molecules** e.g. low-molecular-weight metabolites
- Binding due to RNA folding into a specific **3D structure** that recognizes target molecule
- **Catalytically active RNAs are called ribozymes**
- **Riboswitches: RNA molecules resemble repressors and activators in binding small metabolites and regulating gene expression**




- In **riboswitch** (no regulatory protein exert control) after synthesized mRNA control translation —> **metabolite binds directly to mRNA**
- Riboswitch **mRNAs** contain regions **upstream of their coding** sequences that can fold into specific 3D structures that bind small molecules: **recognition domains, "switch"** exist as 2 alternative secondary structures, one with the small molecule bound and the other without
- Riboswitches control synthesis of enzymes in **biosynthetic pathways**
- **Primitive mechanism of metabolic control:** RNA life forms could have controlled other RNAs synthesis

Riboswitches are intergrated in the in a specific pathway

**TABLE 6.3 Riboswitches in biosynthetic pathways of *Escherichia coli***

| *Type* | *Example of biosynthetic pathway* |
|---|---|
| Vitamins | Cobalamin ($B_{12}$), tetrahydrofolate (folic acid), thiamine |
| Amino acids | Glutamine, glycine, lysine, methionine |
| Nitrogen bases of nucleic acids | Adenine, guanine (purine bases) |
| Others | Flavin mononucleotide (FMN), *S*-adenosylmethionine (SAM), glucosamine 6-phosphate (peptidoglycan precursor), cyclic di-GMP (biofilm signaling molecule) |

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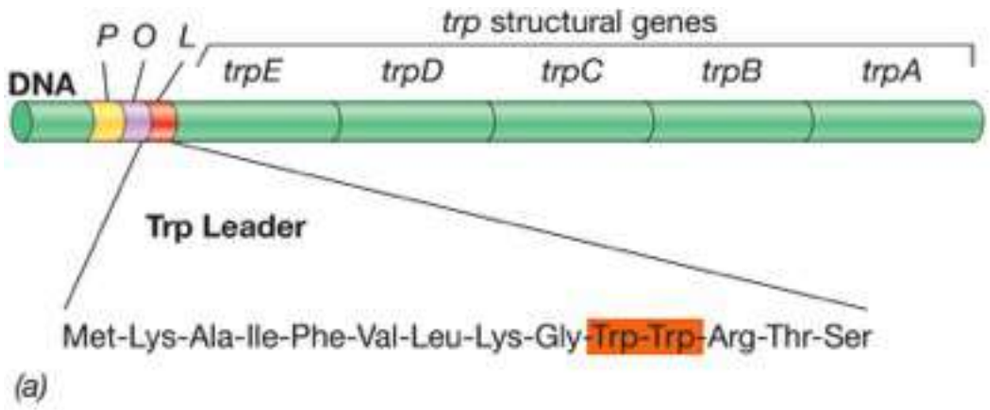
# Attenuation

- Attenuation is a form of **transcriptional control —> prematurely terminating mRNA synthesis**
- Control is exerted after the initiation of transcription but before its completion
- **Number of completed transcripts** from an operon is **reduced**, even though the **number of initiated transcripts is not**
- First part of mRNA to be made, peptide **leader**, can fold into **2 alternative secondary** structures: one structure allows continued synthesis vs other secondary structure causes premature termination
- **mRNA folding** depends either on events **at the ribosome or on the activity of regulatory proteins**
- **In attenuation control: transcription rate is influenced by translation rate**

# Tryptophan operon

- Trp operon contains structural genes for five proteins of **Trp biosynthetic pathway** plus, promoter (P), operator (O) and regulatory sequences at the beginning of the operon: **leader sequence (L) encoding for short leader peptide**
- Transcription of the entire trp operon is under **negative control**
- **Leader peptide sequence contains tandem trp codons near its terminus and functions as an attenuator**

- If **Trp >>** many charged Trp-tRNAs—> **leader peptide** is **synthesized** —> **termination** of transcription
- If **Trp << T**rp-rich leader peptide is not synthesized —> the rest of the **operon is transcribed**
- <u>Transcription and translation are simultaneous processes</u>
- Transcription is **attenuated b/c mRNA folds into a unique stem–loop that inhibits RNA polymerase**
- Stem–loop structure forms b/c two stretches of nucleotides near each other are complementary—> bases pair

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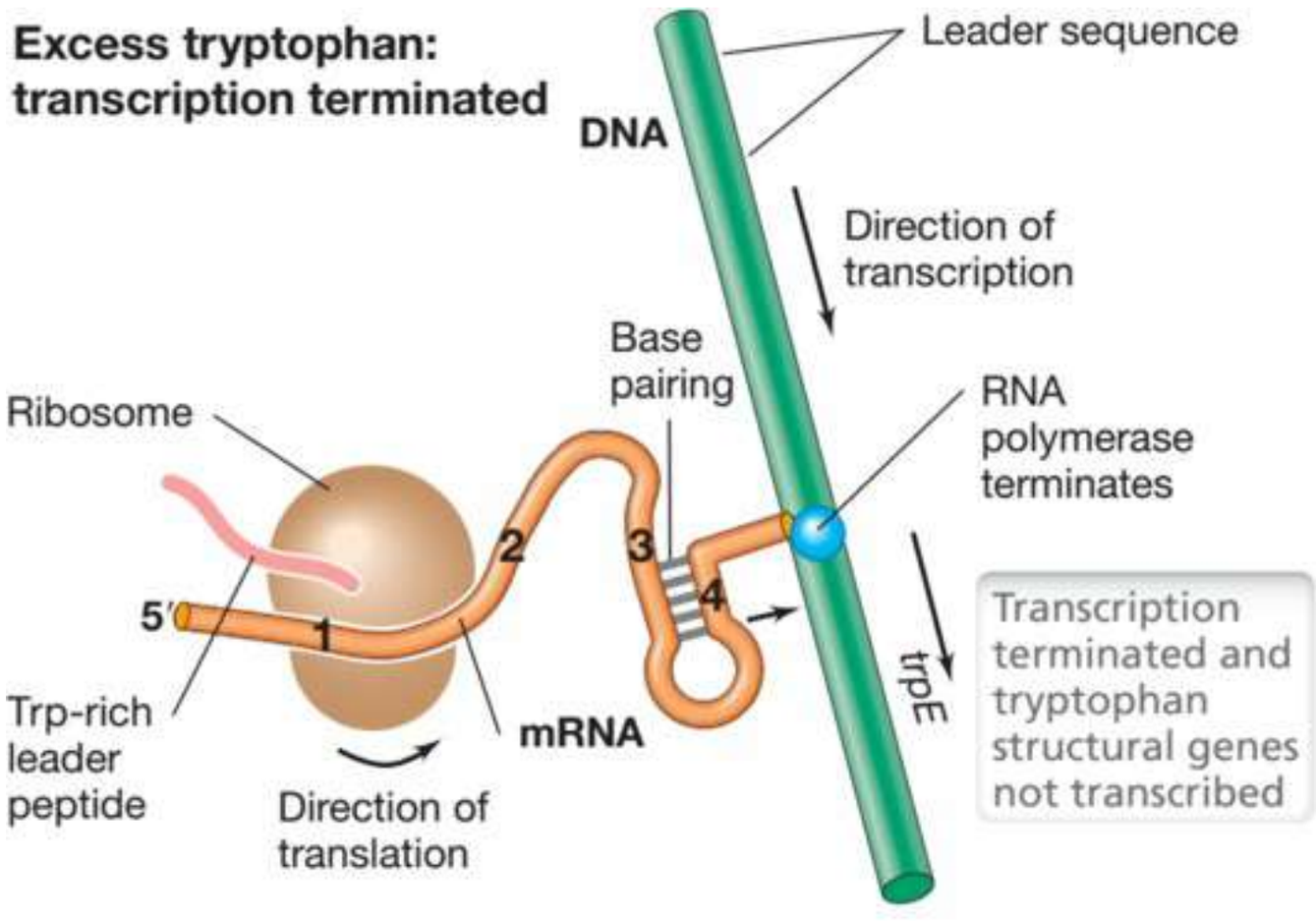
# Concentration-Time coupling, I

- Trp is >>
- Ribosome **translates the leader** sequence (1, 2) —> stop codon
- Remainder of the leader sequence then forms a stem–loop on mRNA (3:4)
- **Transcription —> termination**

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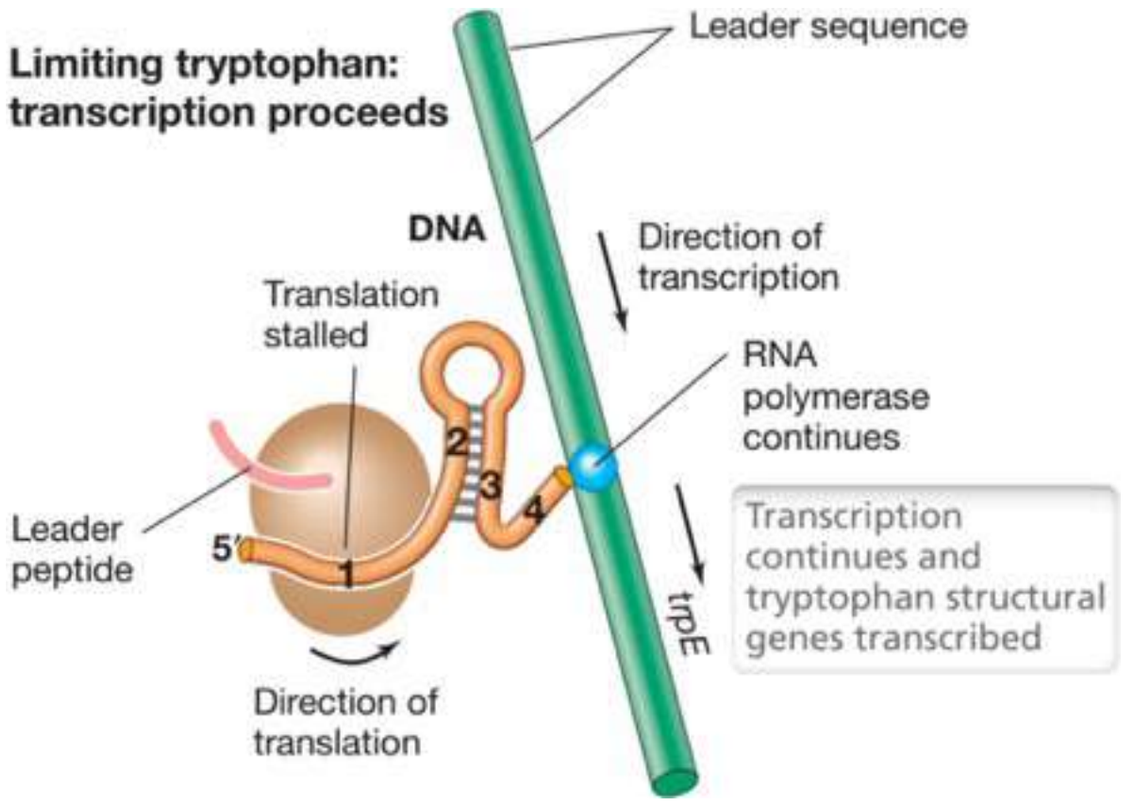
# Concentration-Time coupling, II

- Trp is limiting, <<<
- During leader transcription, ribosome **pauses** at a trp codon because of a shortage of charged tryptophan tRNAs
- The presence of the **stalled ribosome** at this position allows a stem–loop to form (2:3) that differs from the terminator stem–loop
- **2:3 stem– loop prevents formation of terminator 3:4 stem–loop**
- **RNA polymerase to move past the termination site and begin transcription of trp structural genes**

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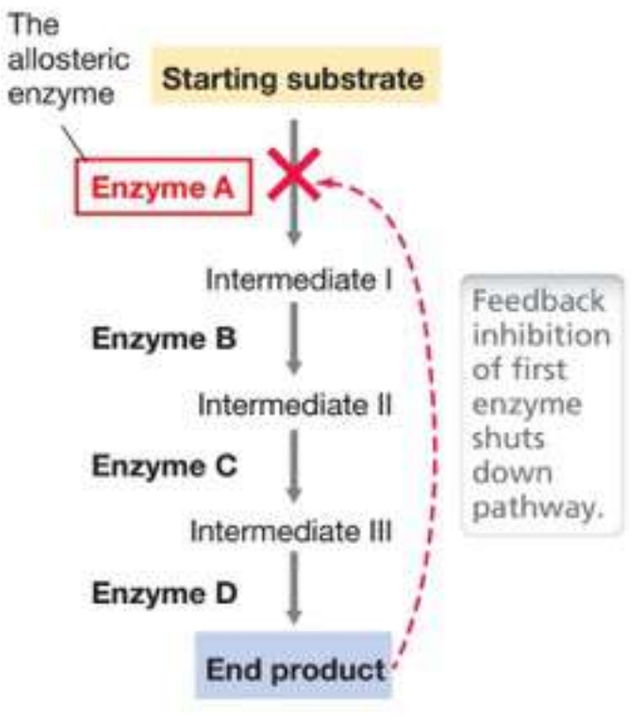
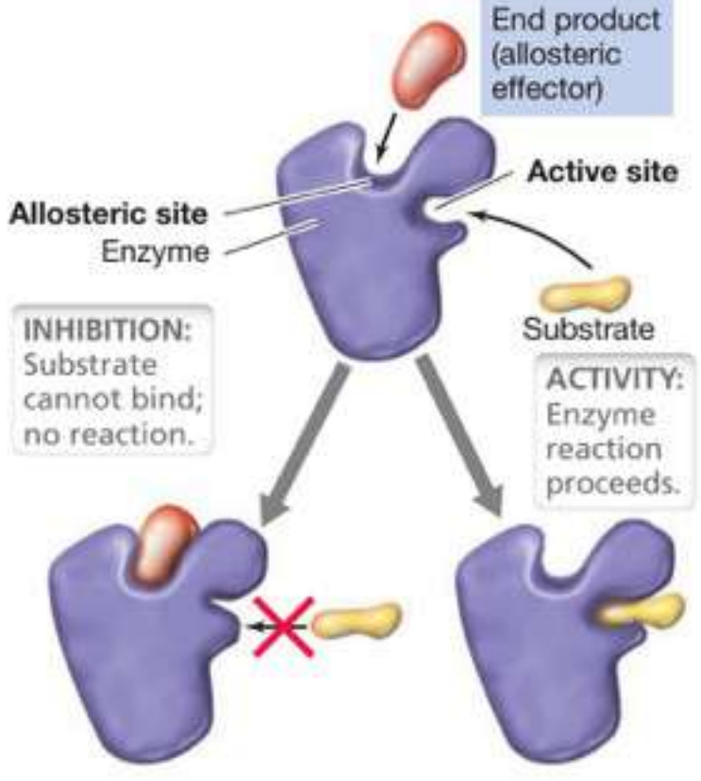
# Enzyme Regulation

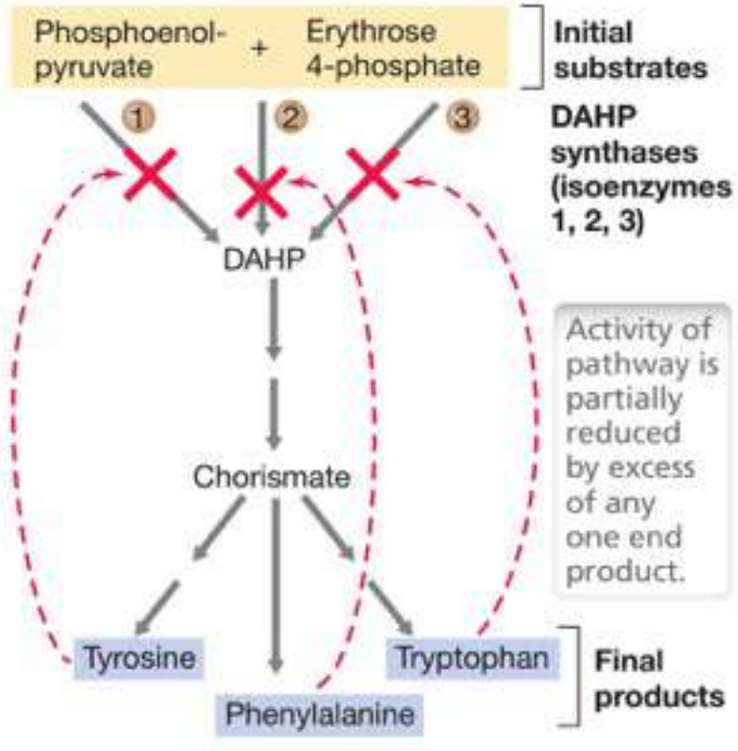
- Cellular mechanisms control enzyme activity already present in the cell through processes such as feedback inhibition and post-translational regulation
- **Feedback Inhibition**: **temporarily shuts off the reactions in an entire biosynthetic pathway** b/c excess of the end product of the pathway inhibits activity of an early (typically the first) enzyme of the pathway
- **Isoenzymes are different proteins** that **catalyze the same reaction** but are subject to **different regulatory controls**




(a) **Feedback inhibition**




(b) **Allosteric inhibition**




(c) **Isoenzyme inhibition**



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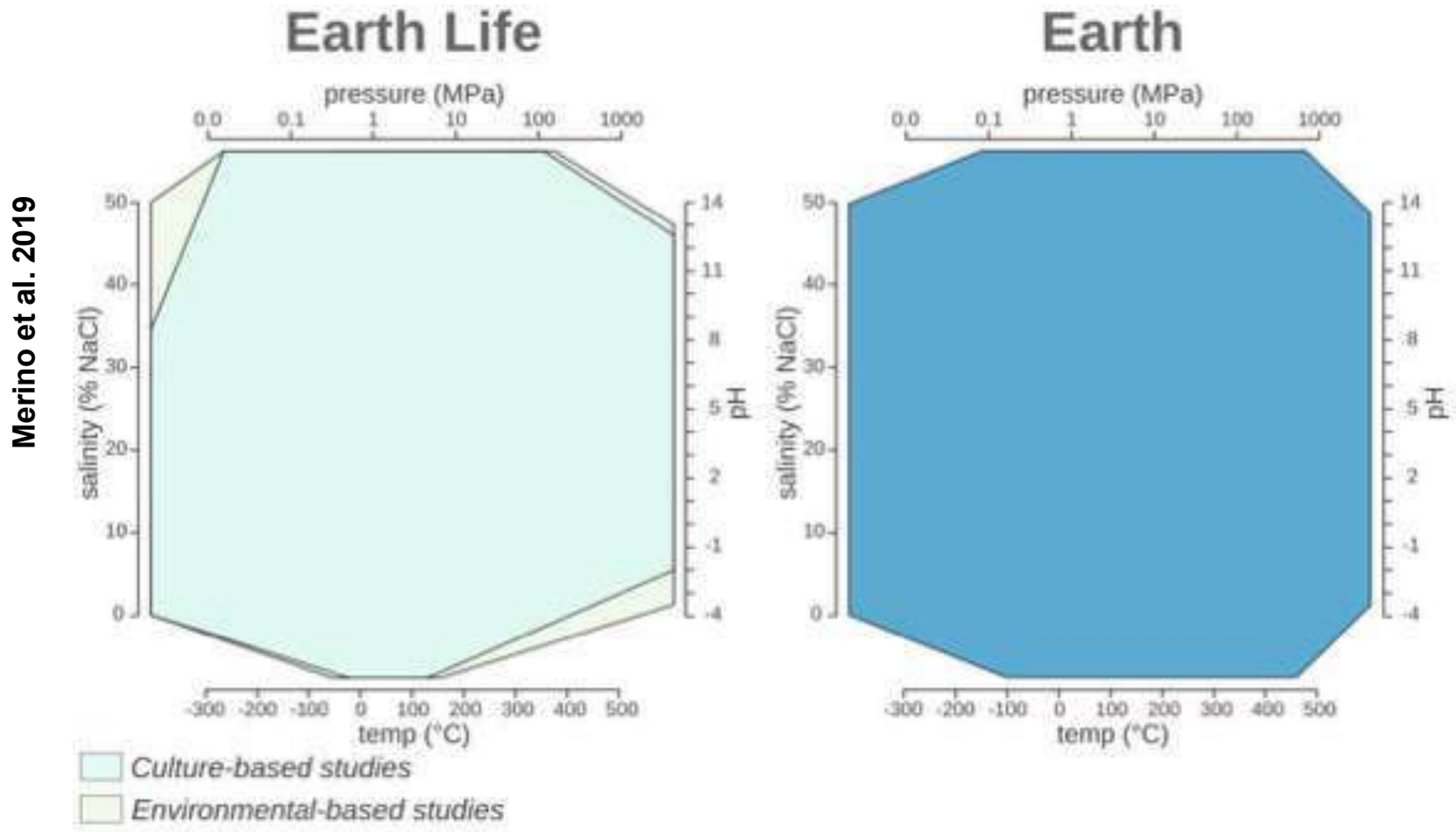
# Gene expression in the context of environmental changes




Merino et al. 2019

No stable environment

Dial cycle

Hydrodynamic flow

Chemical warfare:  antibiotics, secondary metabolites

Microbial competitions

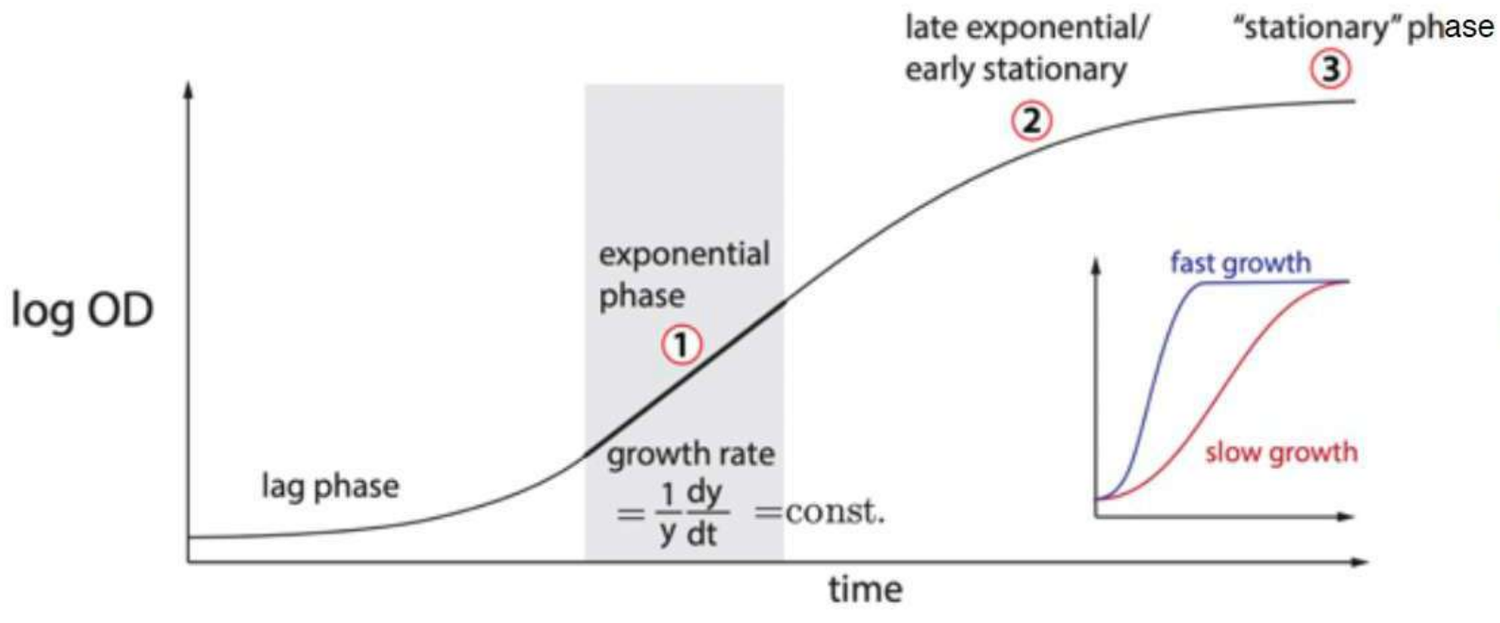
Predators: grazers, other microbes and viruses

Optimal conditions in a variable narrow range

Outside from these conditions there is stress

# General Stress Response, I

- In nature microorganisms must survive **under nutrient-limited conditions**, exposure to environmental **stressors** (e.g. extreme pH, oxidative stress)
- Gram + —> **sporulation** to withstand harsh conditions
- **General stress response controlled** by the **alternative sigma factor RpoS (sigma σS or σ 38)**
- B/c **RpoS (stationary phase sigma factor)** is highly expressed during transition from exponential to stationary phase




**Limiting nutrients**
**Waste accumulations**
**Competition**

# General Stress Response, II

- RpoS regulon comprises > 400 genes associated w. **nutrient limitation, resistance to DNA damage, biofilm formation, responses to osmotic, oxidative, acid stresses**
- **RpoS not only senses environmental changes** but also **relays signals to other regulators**
- ***E. coli* genes recognize by RpoS** are dinB- encodes DNA polymerase IV of **SOS repair system** and catalase genes necessary for **combating reactive oxygen species**
- **RpoS protein is susceptible to degradation during non stressful condition**

**TABLE 4.3 Sigma factors in *Escherichia coli***

| Name[a] | Upstream recognition sequence[b] | Function |
|---|---|---|
| $\sigma^{70}$ RpoD | TTGACA | For most genes, major sigma factor for normal growth |
| $\sigma^{54}$ RpoN | TTGGCACA | Nitrogen assimilation |
| $\sigma^{38}$ RpoS | CCGGCG | Stationary phase, plus oxidative and osmotic stress |
| $\sigma^{32}$ RpoH | TNTCNCCTTGAA | Heat shock response |
| $\sigma^{28}$ FliA | TAAA | For genes involved in flagella synthesis |
| $\sigma^{24}$ RpoE | GAACTT | Response to misfolded proteins in periplasm |
| $\sigma^{19}$ FecI | AAGGAAAAT | For certain genes in iron transport |

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# Stringent Response, I

**Stringent Response: regulatory mechanism** used by bacteria **to survive nutrient deprivation, environmental stresses, and antibiotic exposure (global control)**

Stringent response triggering leads to a **shutdown of macromolecule synthesis and activation of stress survival pathways** to improve the cell's ability to compete in nature

Nutrient levels for microbes in nature can change significantly and rapidly "**shift down**" or "**shift up**"

**Repression of the transcription of stable RNA species**, like tRNA and rRNA, and the **up-regulation of transcription** of genes coding the **enzymes involved in amino acid biosynthesis** are some of the effects during stringent conditions

# Stringent Response, II

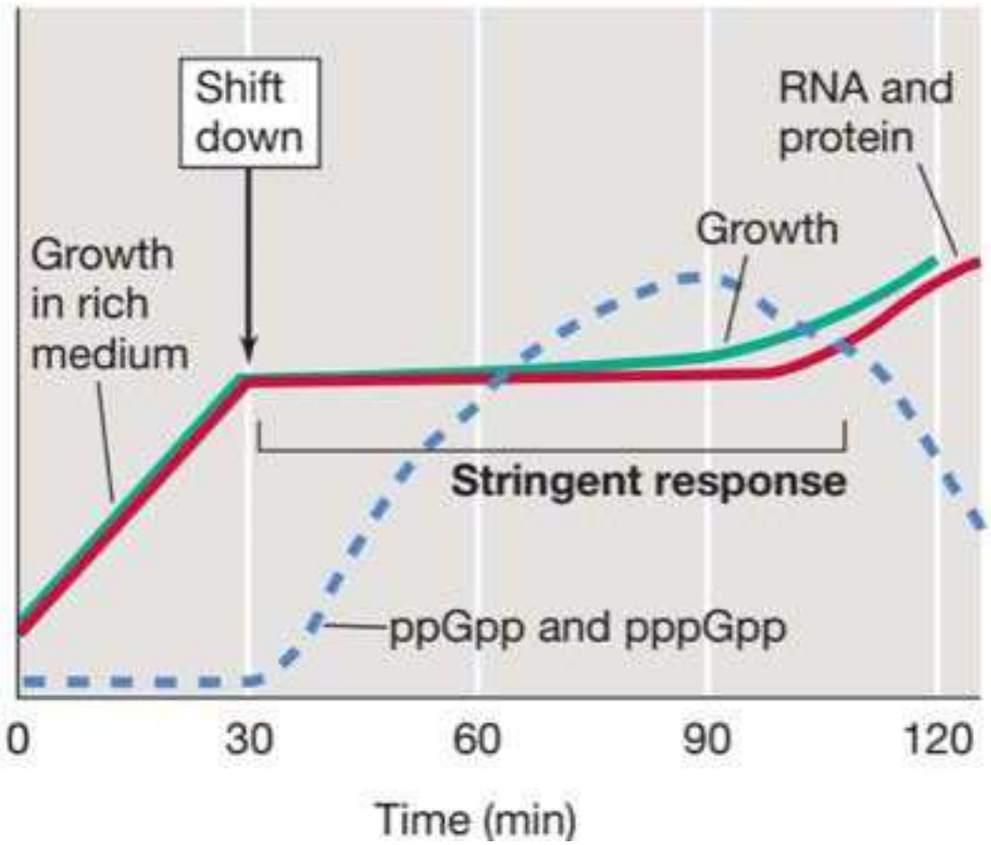
Amino acid shift down: rRNA, tRNA syntheses cease almost immediately –> no new ribosomes are produced

Protein and DNA synthesis are also curtailed

Biosynthesis of **new aa is activated**

**New proteins** must be made to synthesize amino acids no longer available in thevenvironment **from existing ribosome**

**After a while**, rRNA synthesis (i.e. the production of new ribosomes) begins but at **a new rate** commensurate w. cell's reduced growth rate




Madigan et al. 2020

# Stringent Response, III

Stringent response is **triggered by mixture** ((p)ppGpp) of 2 regulatory nucleotides: guanosine tetraphosphate (ppGpp) & guanosine pentaphosphate (pppGpp)

pppGpp & ppGpp are **alarmones** (*E.coli*) rapidly accumulate **during stress or shift down** (aa starvation)

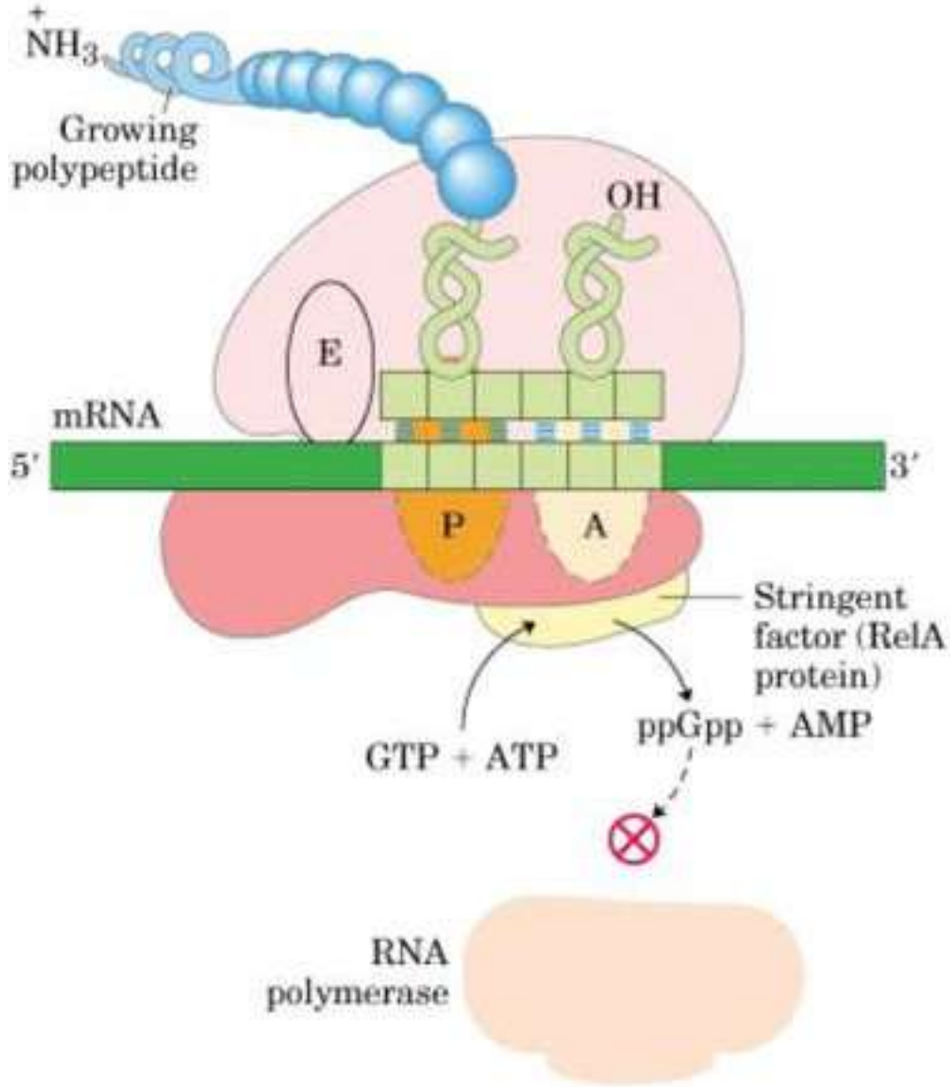
Alarmones are **synthesized by protein RelA**, using ATP as a P donor

**Stringent factor (SF), RelA (synthetase - hydrolase)**

RelA adds 2-P from ATP to GTP or GDP –> pppGpp or ppGpp; **RelA associates w. 50S ribosome subunit is activated by a signal from the ribosome during aa limitation (stalled ribosome)**

When cell growth is limited by aa shortage –> **pool of uncharged tRNAs >> relative to charged tRNAs**

An **uncharged tRNA** is inserted into the ribosome instead of a charged tRNA during protein synthesis –> **ribosome stalls** –> (p)ppGpp synthesis by RelA




Lehninger Principles of Biochemistry (4th Ed.)

# Stringent Response, IV

Protein Gpp converts pppGpp –> ppGpp

**ppGpp** inhibits rRNA and tRNA synthesis by **binding to RNA polymerase** and preventing initiation of transcription of genes

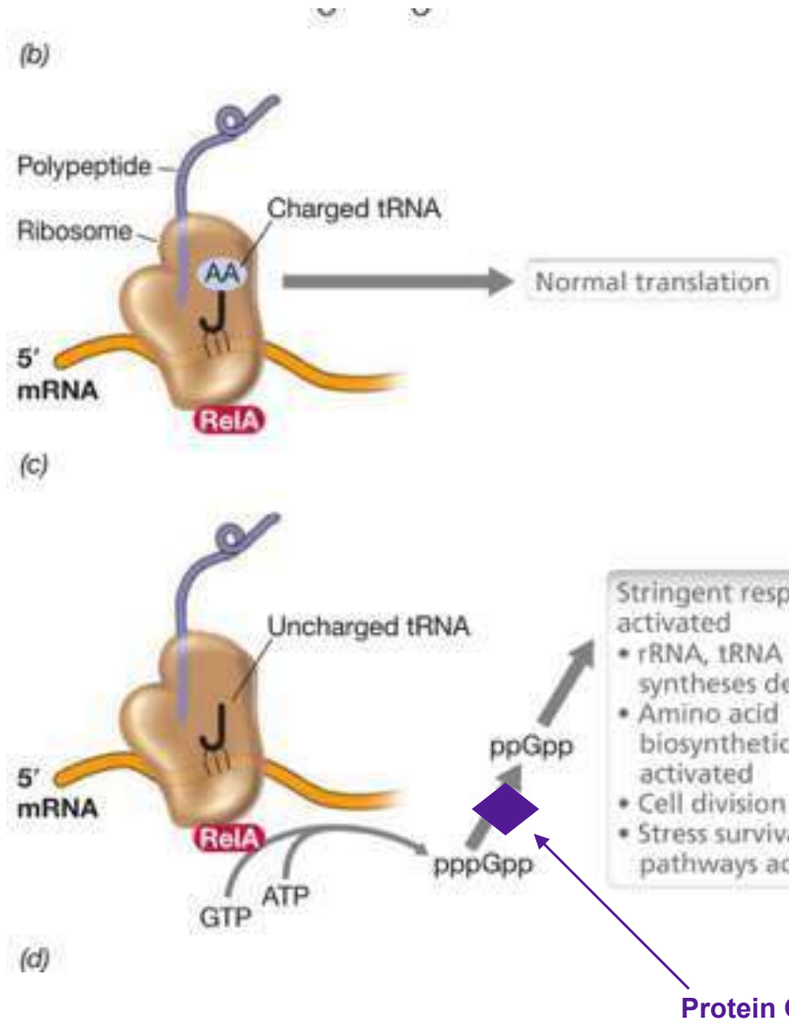
Activation both the **stress response pathways and biosynthetic operons for certain aa**

Inhibition of new DNA synthesis, cell division & slows down synthesis of cell envelope components (i.e. membrane lipids)

**SpoT triggers the stringent response**, synthesizes (p)ppGpp in response to certain stresses or when nutrient deprivation is detected

**SpoT can either make (p)ppGpp or degrade it**

Stringent response results not **only from the absence of precursors for protein synthesis**, but also from the **lack of energy for biosynthesis**




Madigan et al. 2020

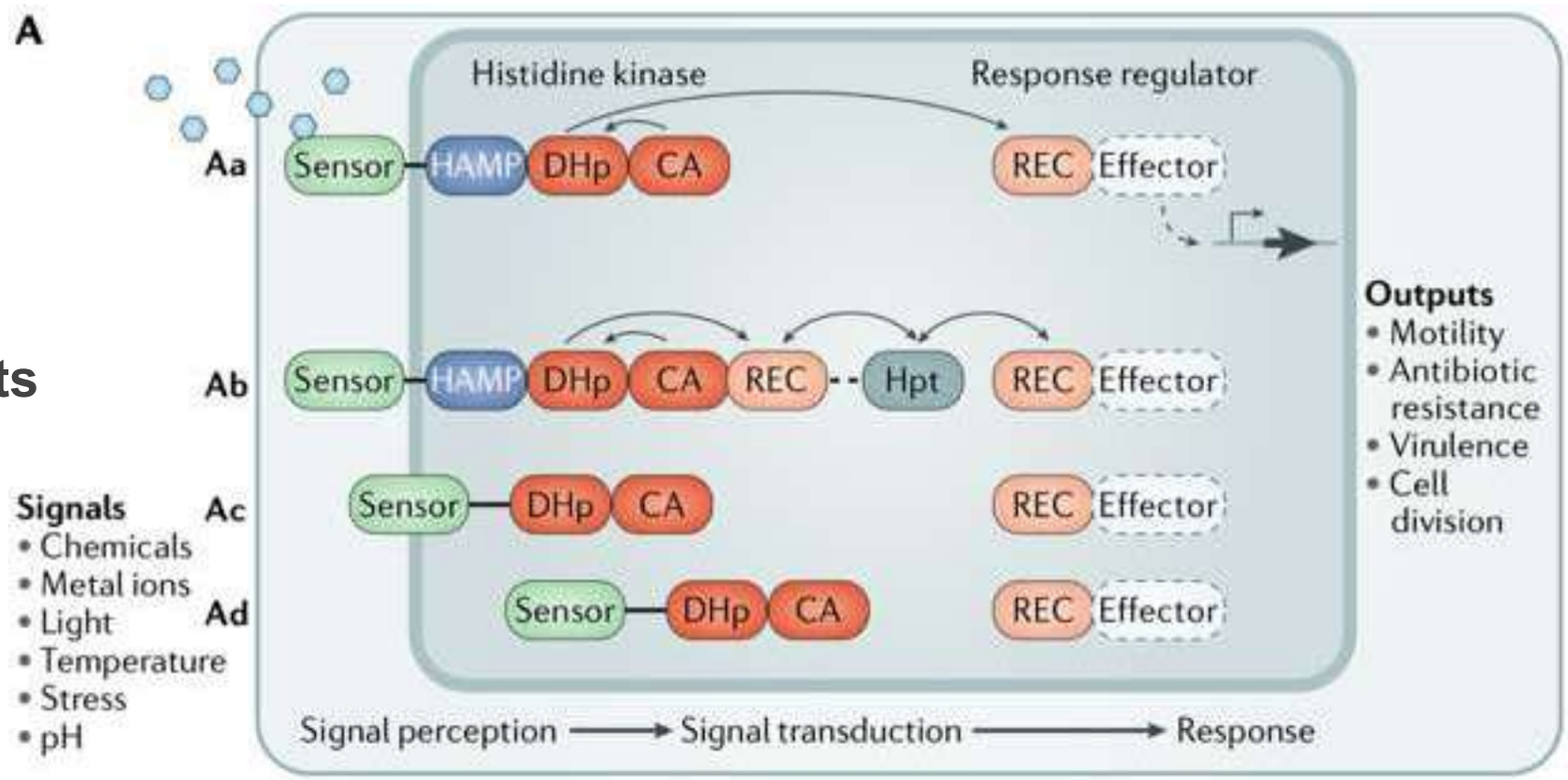
# Sensing and Signal Transduction, I

Cells regulate cell metabolism in response to many different environmental changes (e.g. temperature, pH, oxygen, nutrient availability, cell number)

Mechanisms exist by which cells **receive signals from the environment and transmit them to the specific target to be regulated**

**Signal transduction:**

A. **External signal not transmitted directly** to regulatory protein
B. External signal detected by **surface** sensing system
C. Surface sensing system **transmits signal to regulatory machinery**



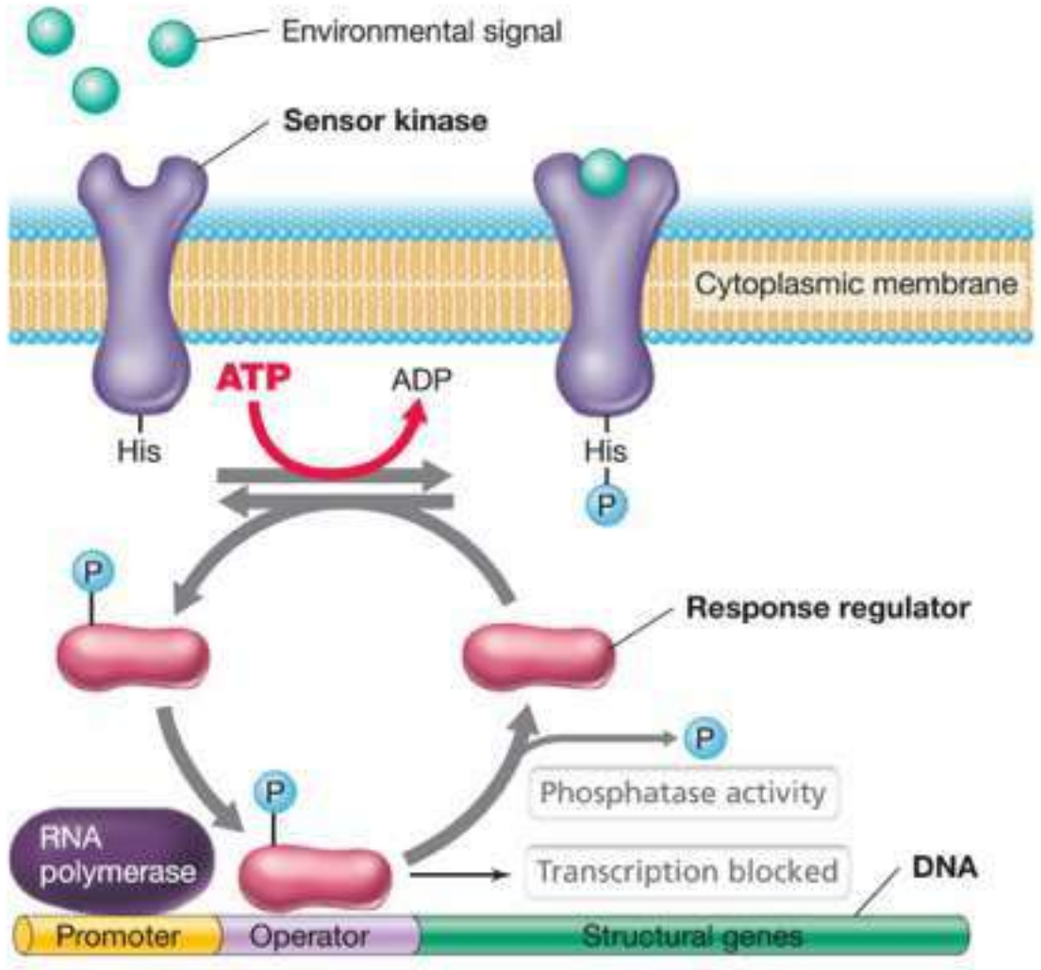

Jacob-Dubuisson et al., 2018

# Sensing and Signal Transduction, II

Signal transduction systems contain two parts, they are called **two-component regulatory systems**

Specific **sensor kinase** protein usually located in the cytoplasmic membrane, and a **response regulator** protein, present in the cytoplasm

A kinase is an enzyme that **phosphorylates** compounds, typically using phosphate (P) from ATP, **autophosphorylation** at a specific histidine residue on the protein (histidine kinases)



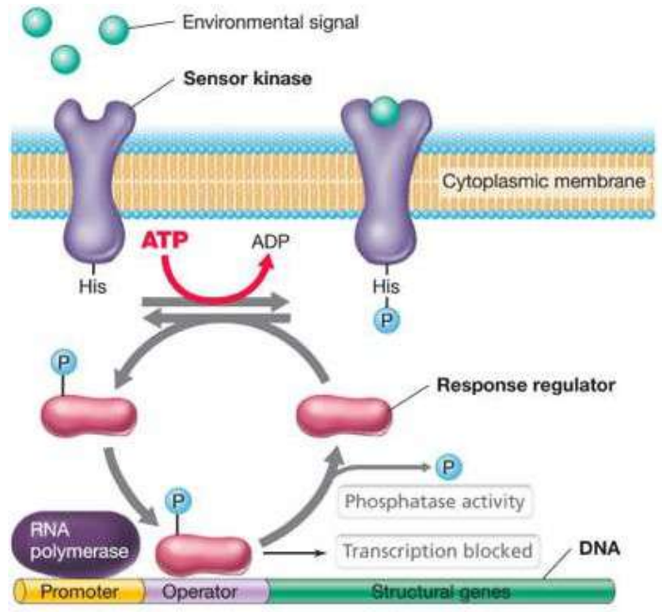

Madigan et al. 2020

# Sensing and Signal Transduction, III

P is then **transferred** from the sensor to another protein inside the cell, the response regulator: **a DNA-binding protein that regulates transcription in either a positive or a negative fashion**

A **feedback loop** completes regulatory circuit and terminate the response, resetting the system for another cycle: **phosphatase**, an enzyme that **removes the phosphate from the response regulator** at a **constant rate**

**Phosphatase activity is typically slower than phosphorylation**




Two-component systems are rare or **absent in parasite** Archaea, Bacteria

**Madigan et al. 2020**

**TABLE 6.1 Examples of two-component systems that regulate transcription in *Escherichia coli***

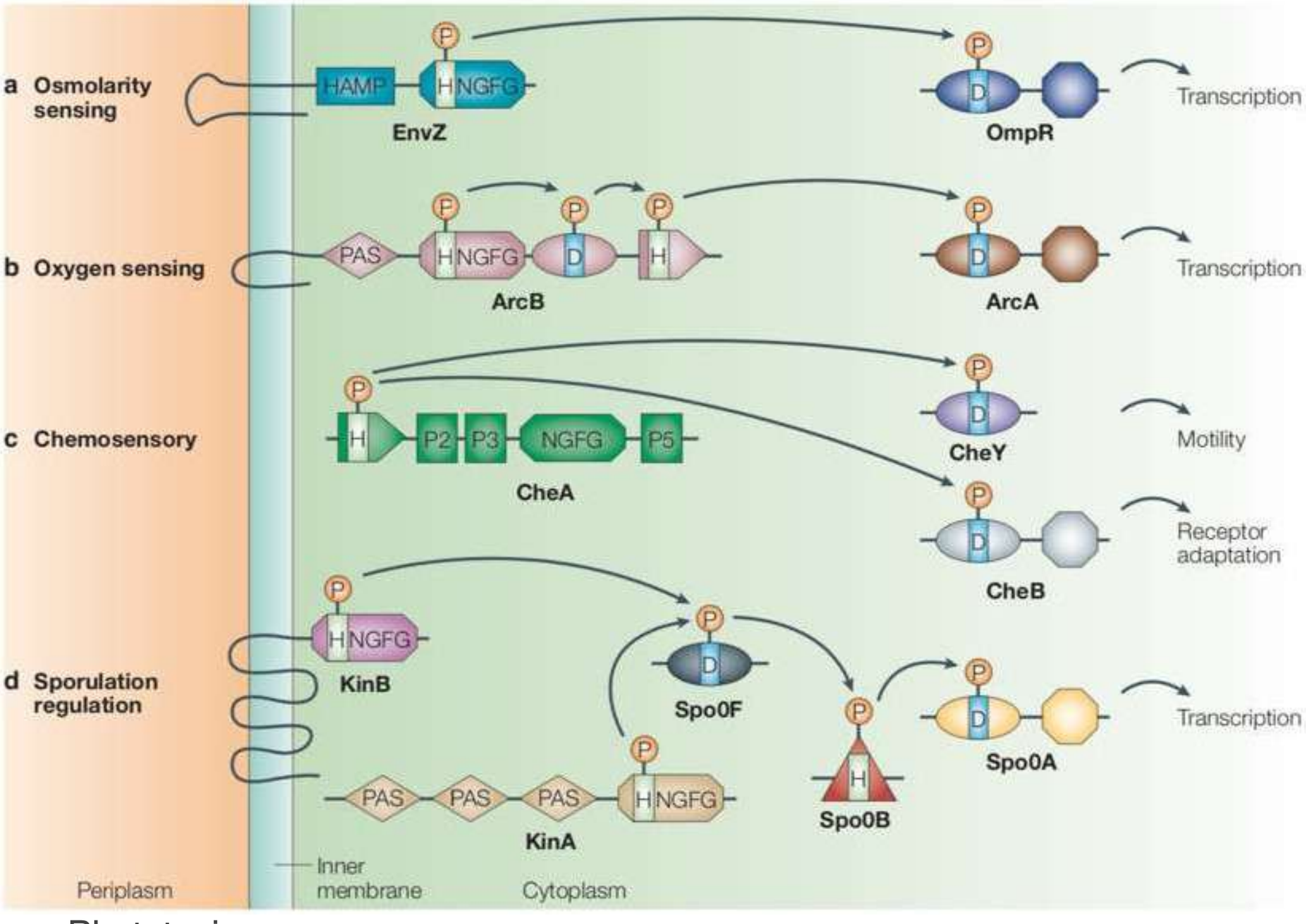
| *System* | *Environmental signal* | *Sensor kinase* | *Response regulator* | *Primary activity of response regulator*[a] |
|---|---|---|---|---|
| Arc system | Oxygen | ArcB | ArcA | Repressor/activator |
| Nitrate and nitrite respiration (Nar) | Nitrate and nitrite | NarX | NarL | Activator/repressor |
| | | NarQ | NarP | Activator/repressor |
| Nitrogen utilization (Ntr) | Shortage of organic nitrogen | NRII (= GlnL) | NRI (= GlnG) | Activator of promoters requiring RpoN/$\sigma^{54}$ |
| Pho regulon | Inorganic phosphate | PhoR | PhoB | Activator/repressor |
| Porin regulation | Osmotic pressure | EnvZ | OmpR | Activator/repressor |

[a]Note that many response regulator proteins act as both activators and repressors depending on the genes being regulated. Although ArcA can function as either an activator or a repressor, it functions as a repressor on most operons that it regulates.



**Madigan et al. 2020**

# Sensing and Signal Transduction, IV




Phototaxis

**Wadhams & Armitage, 2014**

# Navigating the microenvironment

## Chemotaxis, I

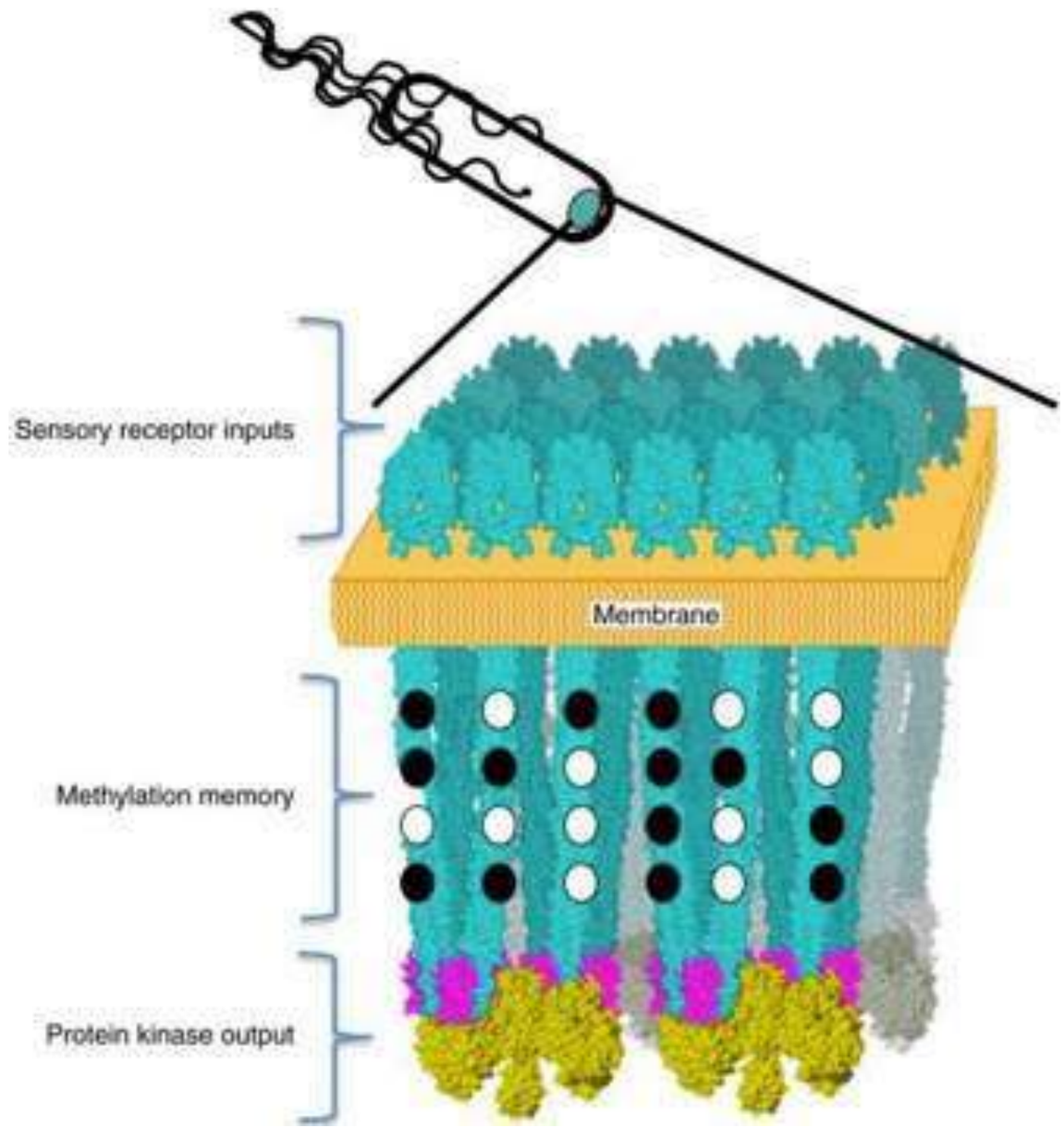
- Chemokinesis: random movements, in absence of a concentration gradient of chemoattractant
- Chemotaxis: directional movement along a + gradient of chemoattractant

# Chemotaxis, II

Stock and Zhang, 2013

Cells are too small to sense spatial gradients of a chemical, but they can respond to **temporal gradients**

**Sensing the change in concentration** of a chemical (attractant or repellent) over time rather than the absolute concentration of the chemical stimulus –> **signal-to-noise ratio**




Two-component system modulates activities in pre-existing proteins: flagellum machinery

**MCP (methyl-accepting chemotaxis proteins): Several sensory** proteins reside in the cytoplasmic membrane and sense attractants or repellents

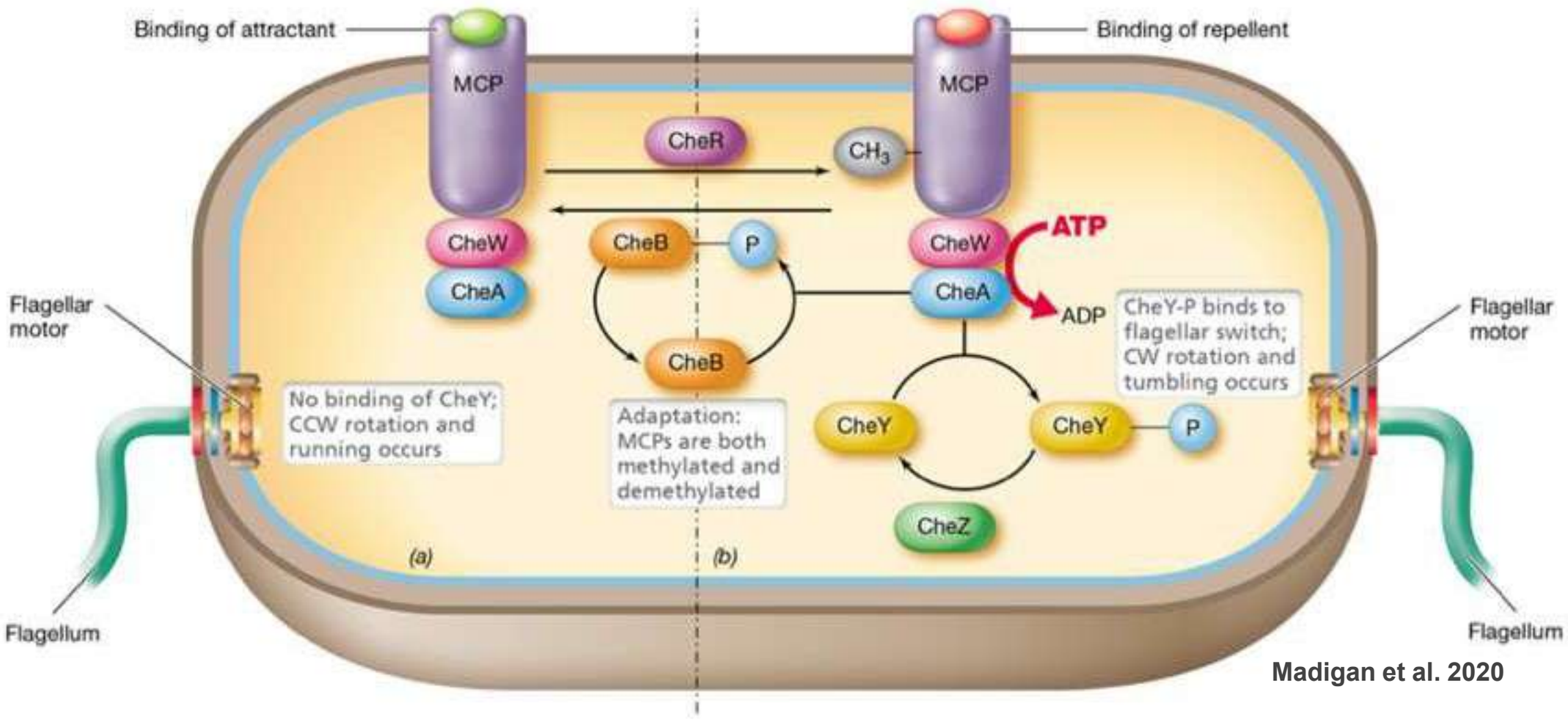
**Nanobrain**, chemoreceptor clusters formed by MCPs

# Chemotaxis, III

MCPs allow the cell to monitor the concentration of various substances over time

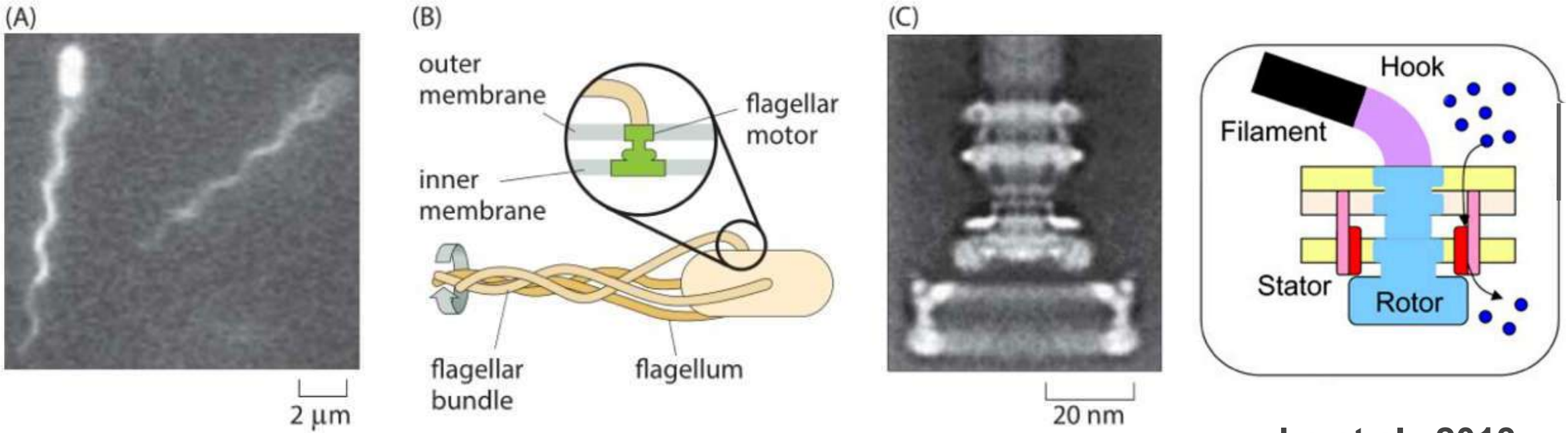
Diverse MCPs for **diverse compounds**

**MCPs bind attractants or repellents** directly or in some cases indirectly through interactions with periplasmic binding proteins




Madigan et al. 2020

# $H^+$ motive force or $Na^+$ motive force

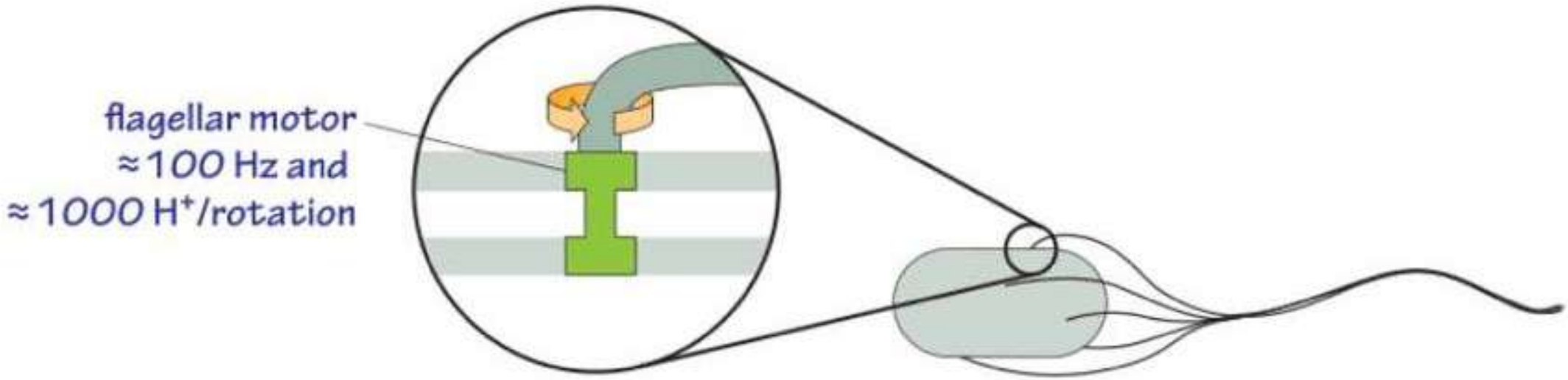



Lo et al., 2013

Figure 1: Flagellar-based motility in E. coli. (A) Two E. coli cells and their bundle of fluorescently labeled flagella. (B) Schematic of the bundling of flagella that drives bacterial motility. The inset shows how the rotary motor is embedded in the cell membrane. (C) Electron microscopy image of the rotary motor. (C adapted from H. C. Berg, Phys. Today, 53:24, 2000.)

**biobythenumbers.org**

## What is the energy demand for flagella based rotation?



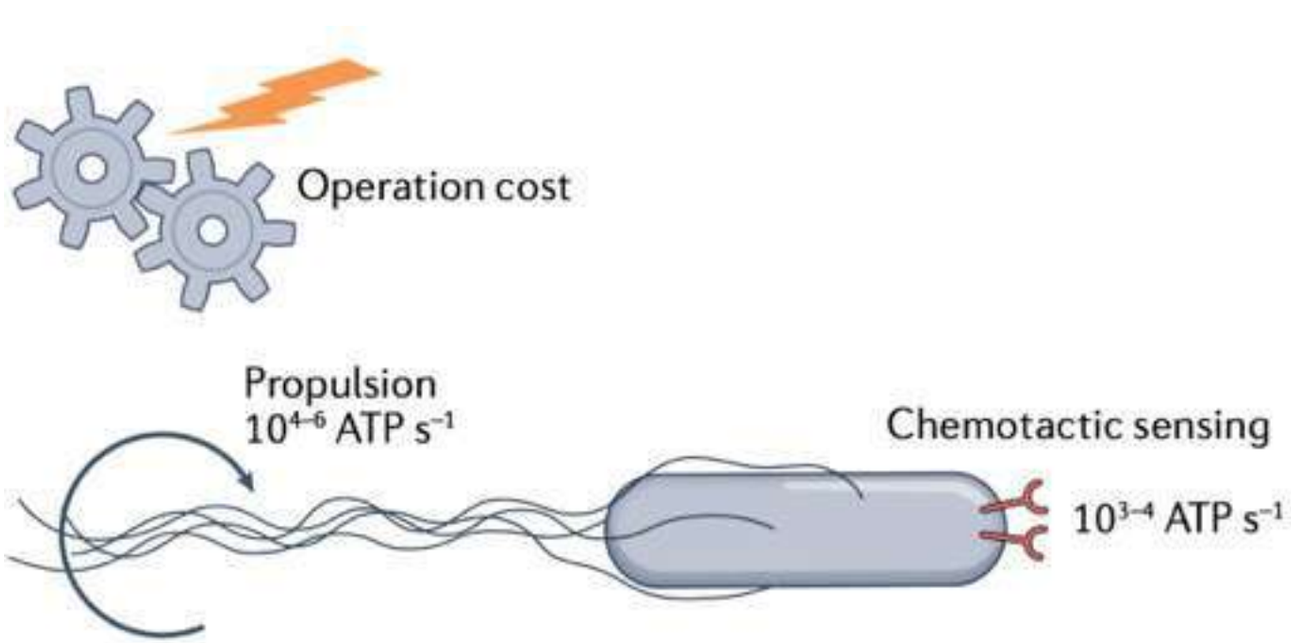

$$\text{protons needed} \approx 1000 \frac{H^+}{\text{rotation} \times \text{flagella}} \times 100 \frac{\text{rotations}}{s} \times 4 \text{ flagella} \approx 4 \times 10^5 \frac{H^+}{s}$$

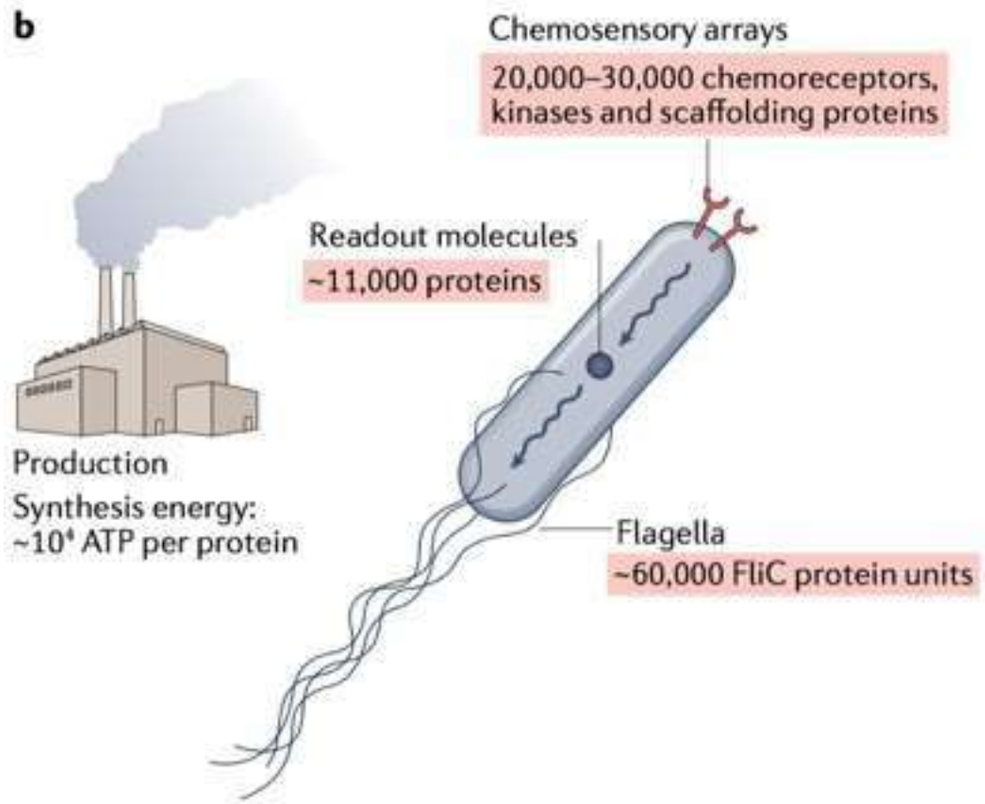
$$\text{proton motive force} \approx 150 \text{ mV} \rightarrow \text{energy per proton} \approx 0.15 \text{ V} \times 1.6 \times 10^{-19} \text{ J/V} \approx 0.2 \times 10^{-19} \text{ J}$$

$$\text{power expended} \approx 4 \times 10^5 \frac{H^+}{s} \times 0.2 \times 10^{-19} \frac{J}{H^+} \approx 10^{-14} \text{ W} \approx 10 \text{ W/kg cells}$$

Figure 2: Back of the envelope calculation showing the energy requirements for bacterial motility. For slow growing or stationary phase bacteria the power expended can be a non-negligible fraction of their overall energy budget.

# Relative cost of bacterial chemotaxis




Keegstra et al., 2022

**Metabolism** fuels chemotaxis

**Informed foraging** and **cue-based navigation**

**Increase growth rate in a better environment**

# P- circuit, I

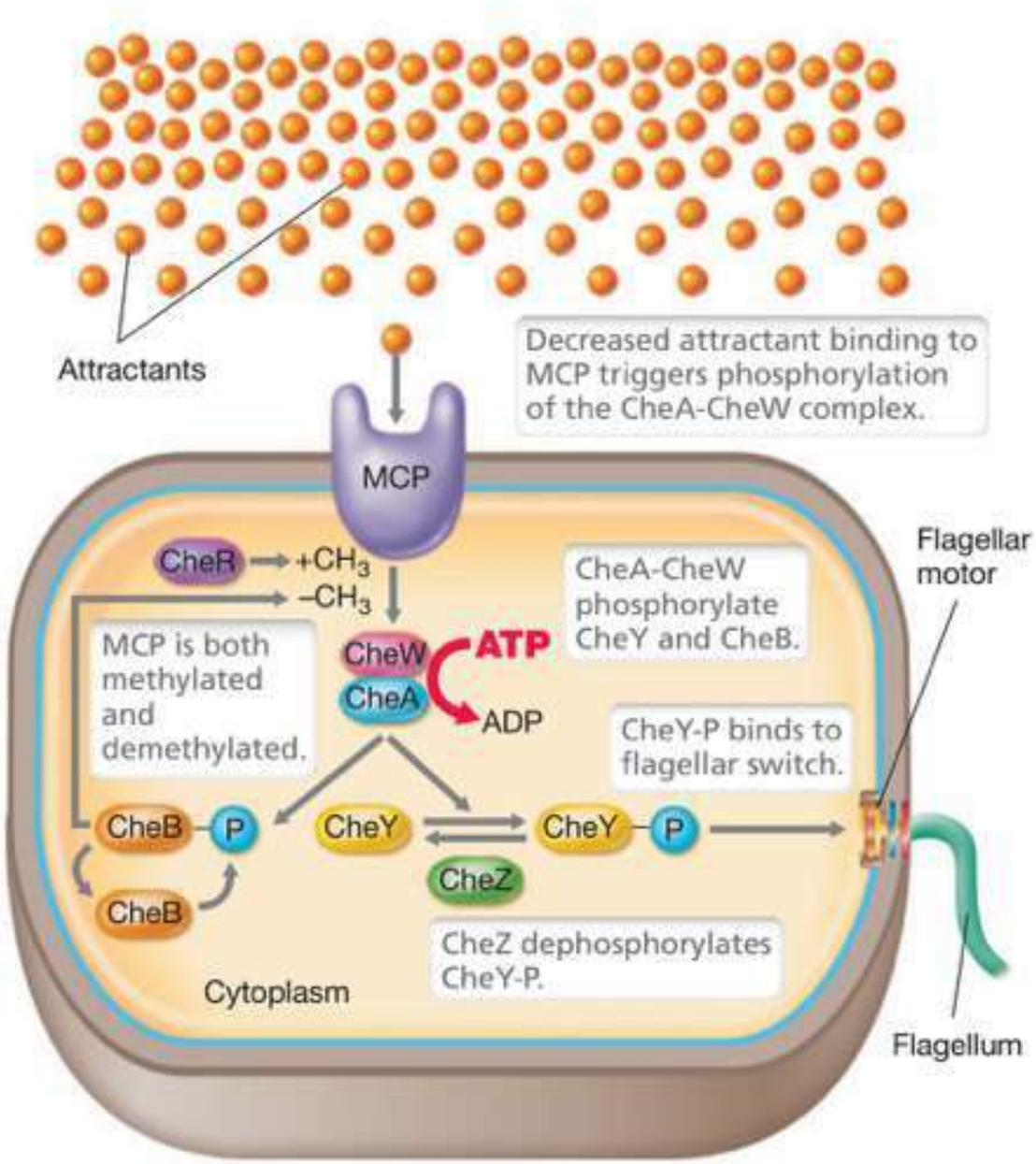
MCPs (**methyl-accepting chemotaxis proteins)** make contact with the cytoplasmic proteins **CheA and CheW**

**CheA is the sensor kinase** for chemotaxis

When MCP binds chemical – > **changes conformation with CheW** – > **autophosphorylation of CheA —> CheA-P**

**Increase in attractant** concentration **decreases** the **rate of autophosphorylation**

**Decrease in attractant / increase in repellent increases** the rate of **autophosphorylation**




Madigan et al. 2020

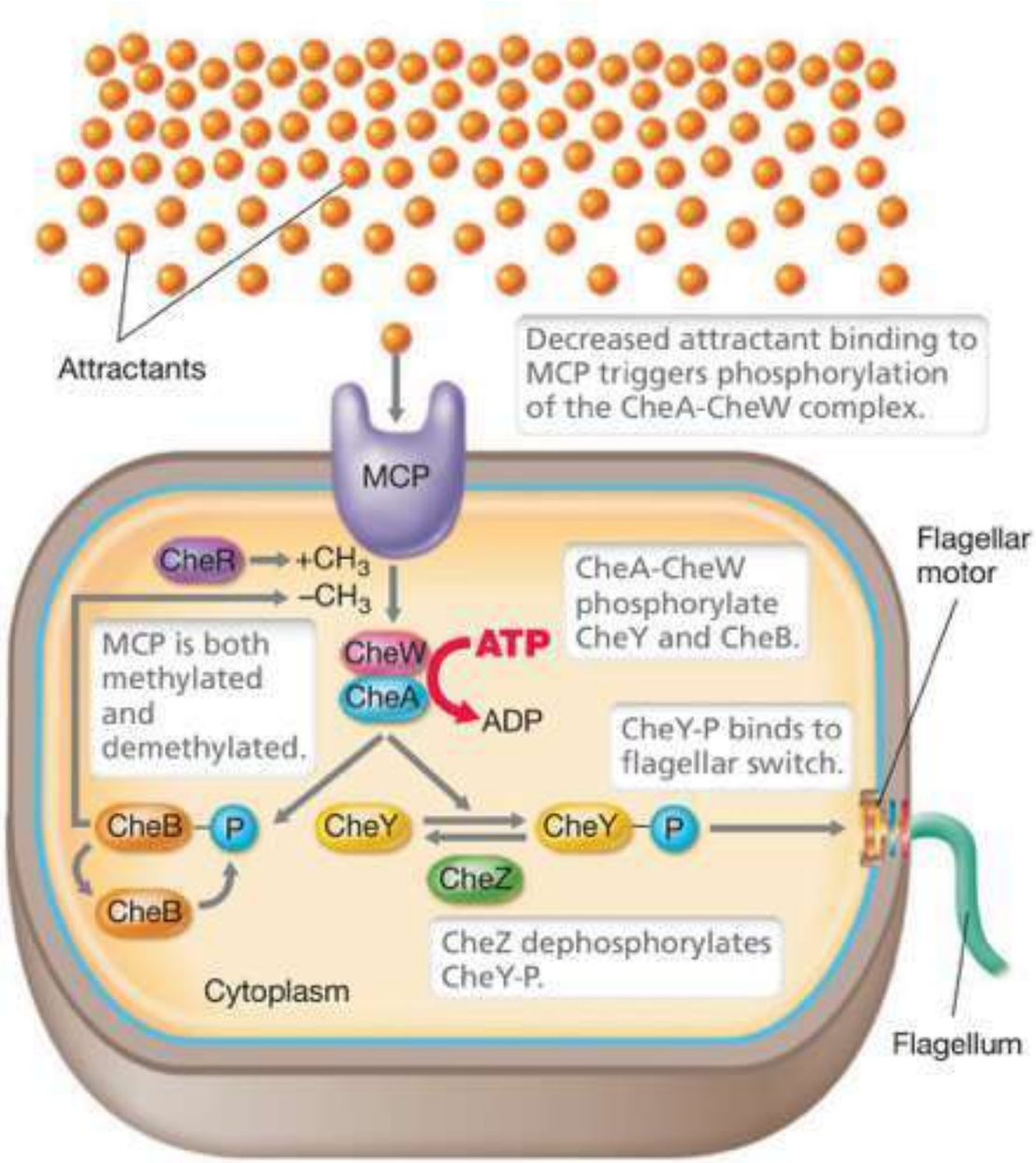
# P- circuit, II

CheA-P passes P- to **CheY** (forming CheY-P) **response regulator** controls flagellar rotation

CheA-P can also transfer P- to **CheB -plays role in adaptation**

**Counterclockwise** rotation cell will continue to move in a run (**swim smoothly**) —> *no CheY binding*

**Clockwise** rotation cell will **tumble** (move randomly) – > **CheY-P binding**

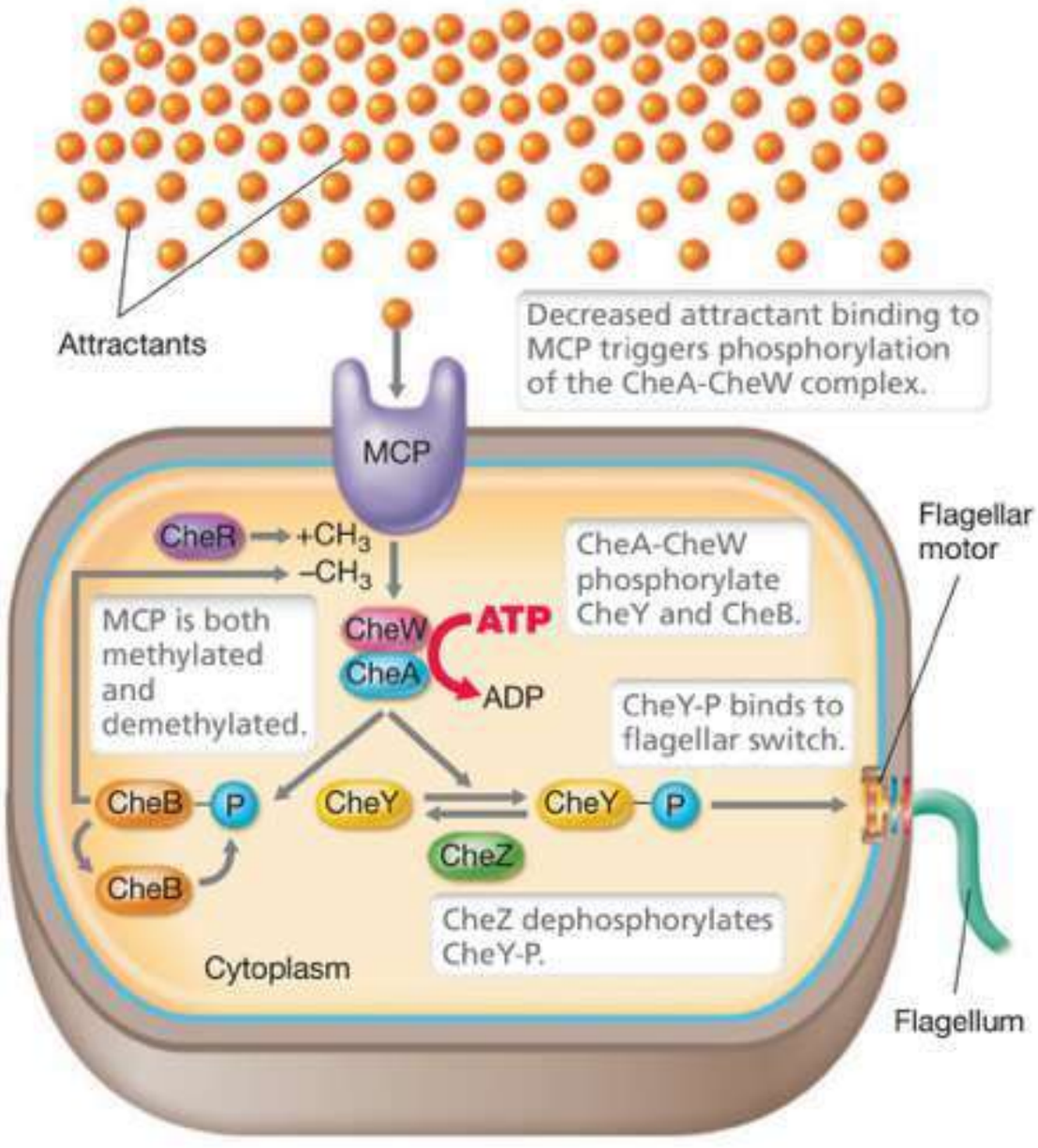



Madigan et al. 2020

# $CH_3$- circuit, I

If **attractant** level is **high** –> **CheA autophosphorylation** rate is **low** –> unphosphorylated CheY & CheB (smoothly)–> **MCPs methylation increases**

MCPs **no** longer **respond to attractant** when **fully methylated** –> **if attractant level** remains **high** but constant–> **tumble** & CheB –> **CheB-P demethylate MCPs**




Madigan et al. 2020

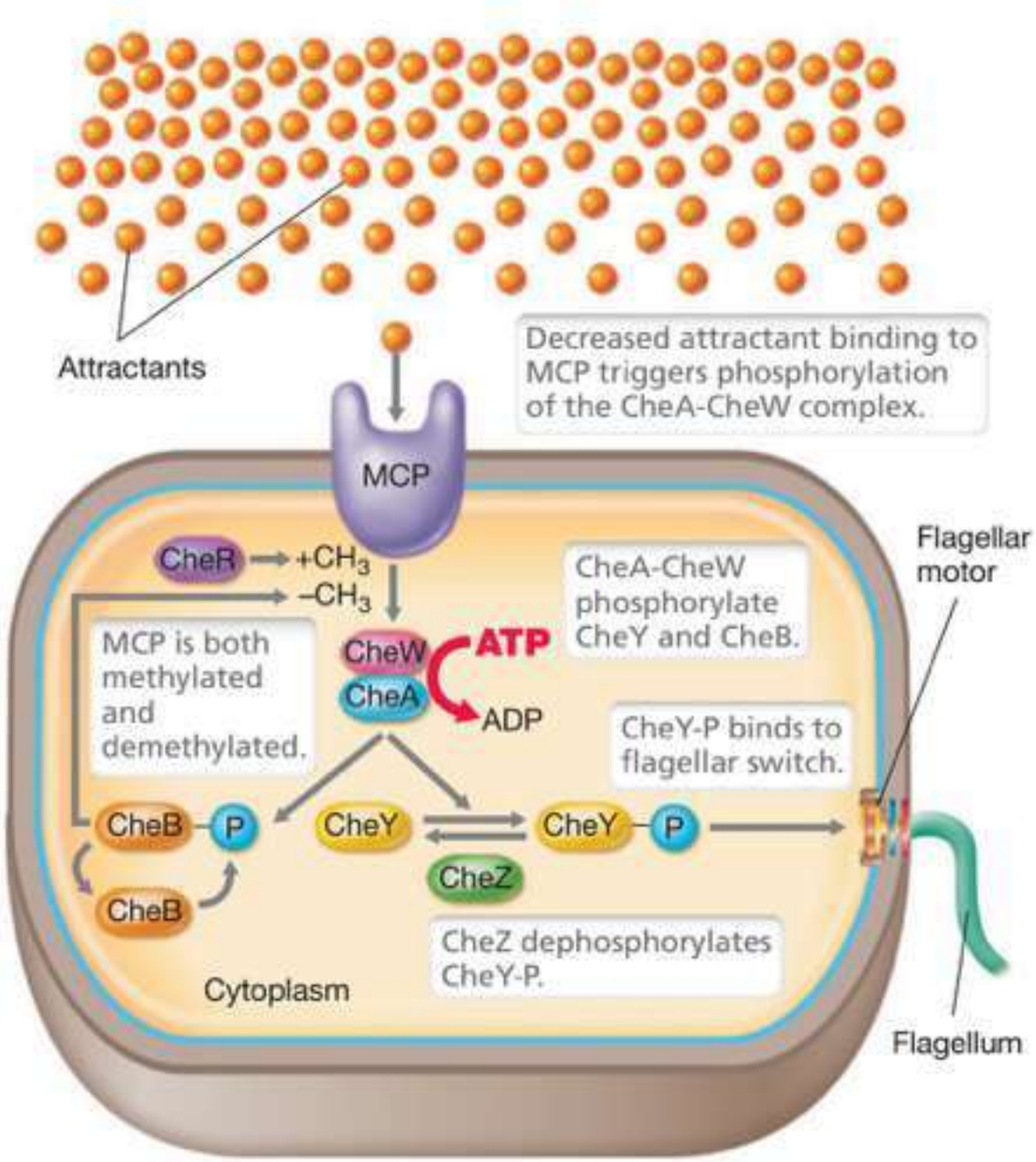
# $CH_3$- circuit, II

**Resetting receptors** –> respond to further **increases or decreases in level of attractants**

Cell **stops swimming** if the **attractant** concentration is **constant** –> continues to swim if even higher levels of attractant are encountered (opposite for repellents)

**Fully methylated MCPs** respond to repellent increase –> **tumbling**

**Cell moves off in a random direction while MCPs are slowly demethylated**




Madigan et al. 2020