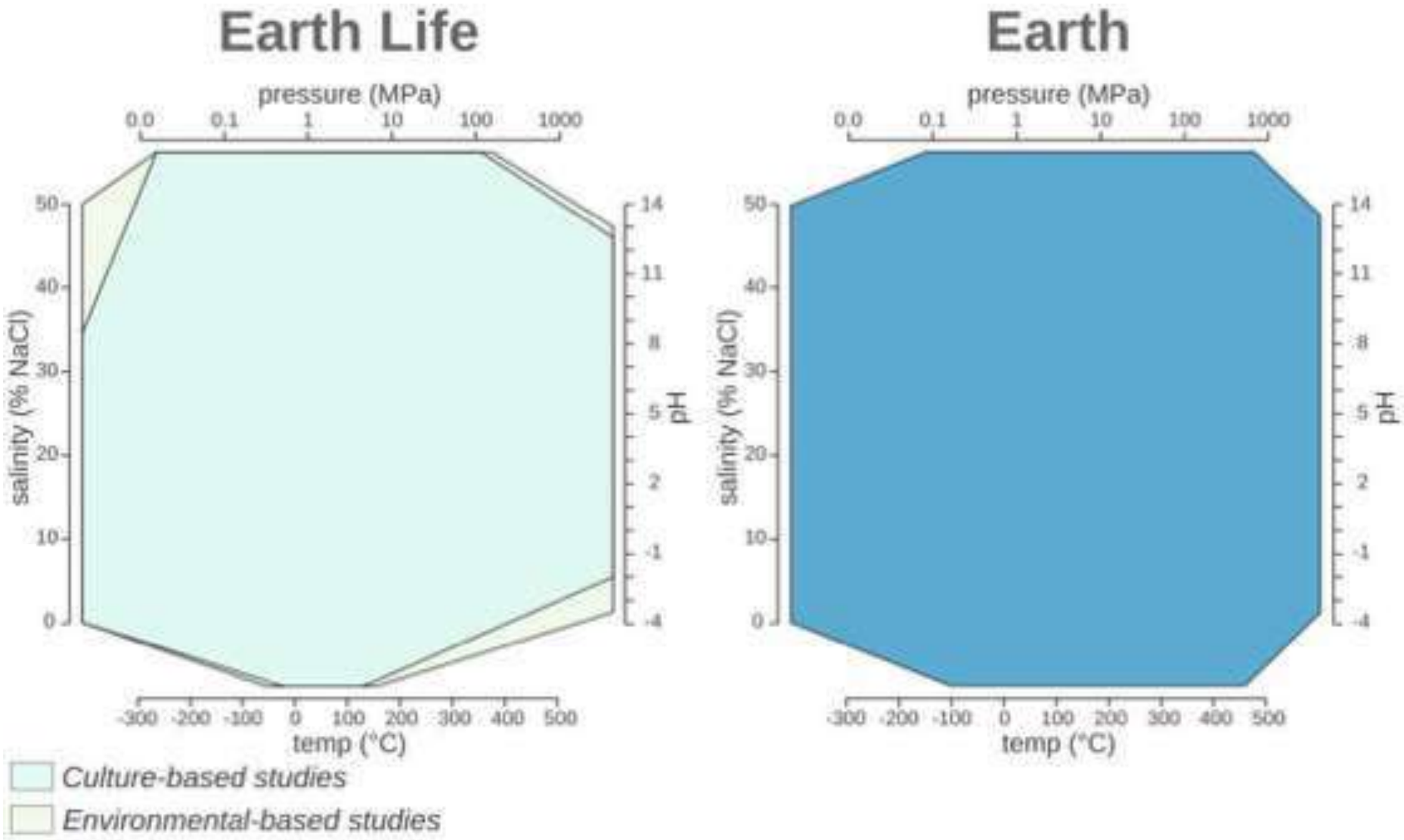
L06b

Recap L06a

No such stable and homogeneous environment at the microscale for microbes

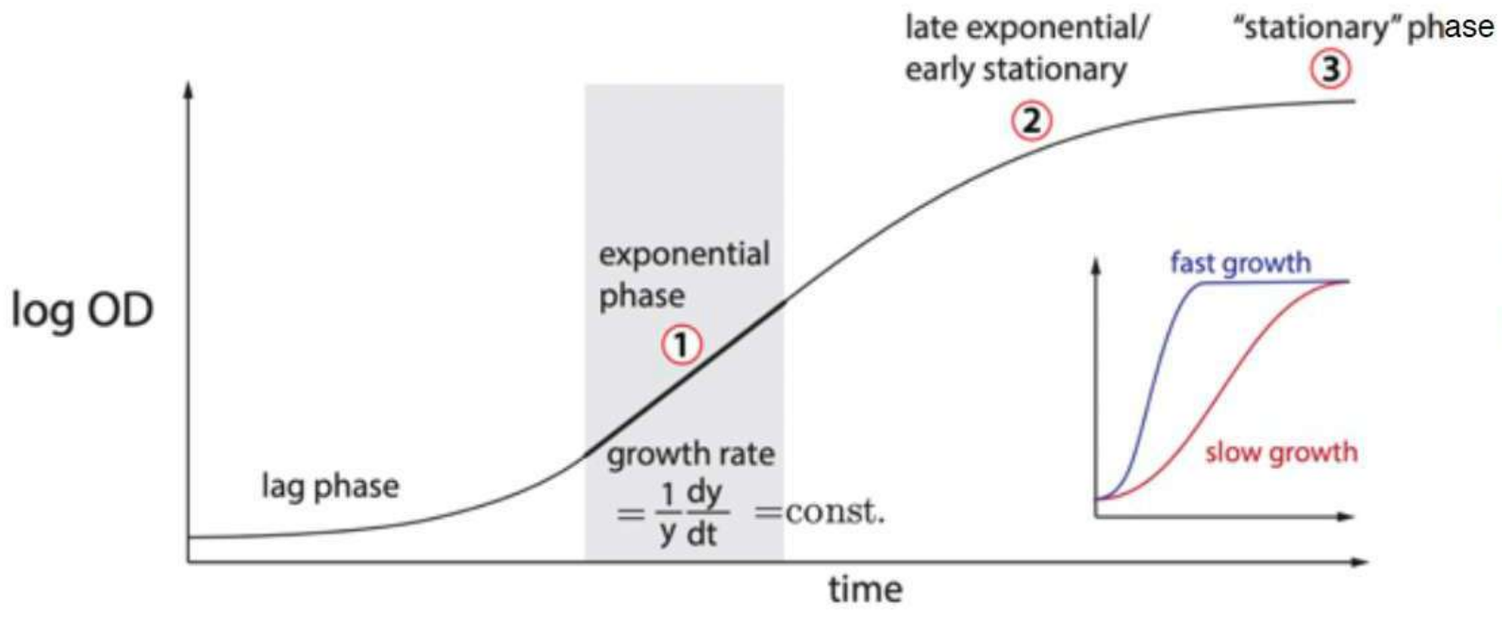
Merino et al. 2019



- How to deal with stress ?
- How to deal with heat shock ?
- How to deal with oxidative stress ?
- How to deal with nutrient deprivation ?

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**

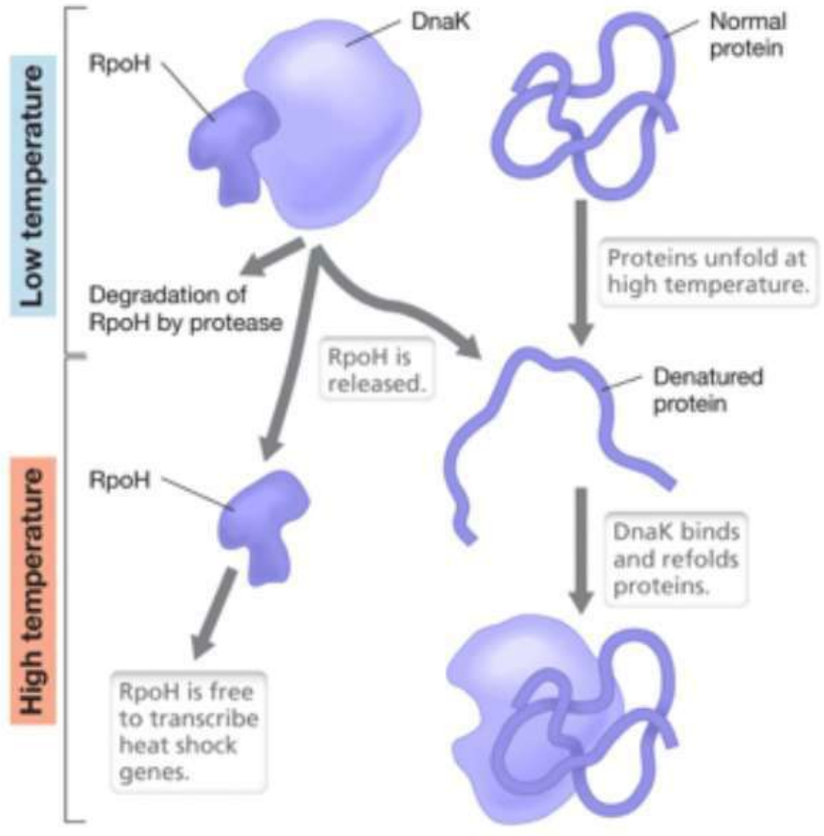
TABLE 4.3 Sigma factors in *Escherichia coli*

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

Madigan et al. 2020

Heat Shock Proteins, I

- Some **proteins are less stable at elevated temperatures** and tend to unfold (denature)
- **Improperly folded** proteins are **recognized by protease** —> degraded
- **Heat stress triggers synthesis of heat shock proteins** —> counteracting cell damage, assisting cell recovering from stress
- Heat shock proteins are induced by stress factors: **chemicals-ethanol- or exposure to high doses of ultraviolet (UV) radiation**
- Hsp70 protein of *E. coli* is **DnaK, which prevents aggregation of newly synthesized proteins and stabilizes unfolded proteins**
- Hsp60 and Hsp10 families in *E. coli* are the proteins **GroEL and GroES —> molecular chaperones that catalyze correct refolding of misfolded proteins**
- Another class of heat shock proteins includes various proteases that degrade denatured or irreversibly aggregated proteins



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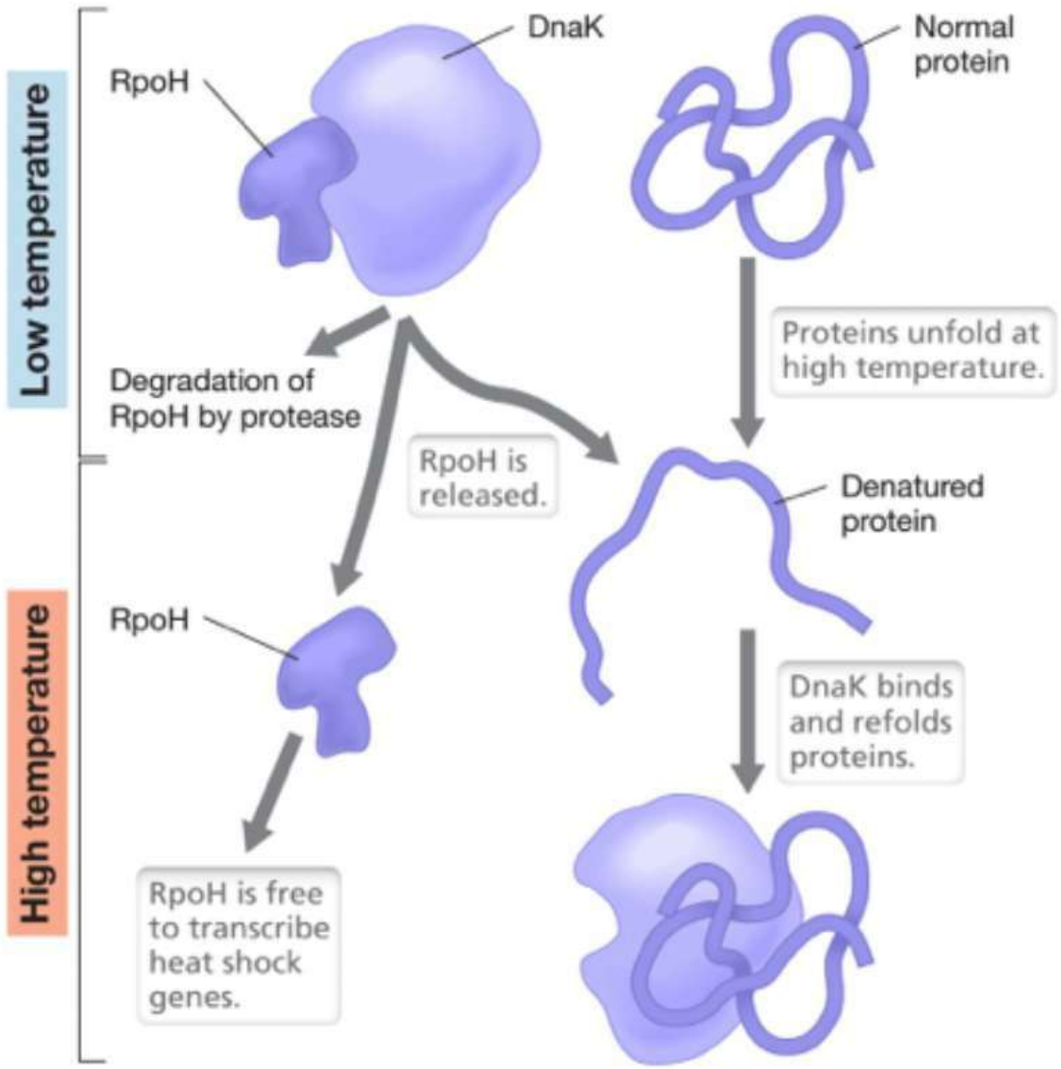
Heat Shock Proteins, II

- In *E. coli*, the **heat shock response** is controlled by the **alternative sigma factor RpoH** (s32)
- RpoH controls expression of heat shock proteins, is **normally degraded** within a minute or two of synthesis
- When cells suffer a **heat shock**, **degradation of RpoH is inhibited** —> level >>
- RpoH degradation rate depends on level of free **DnaK, inactivator of RpoH**
- If **heat begins** to unfold proteins, **DnaK binds preferentially to unfolded proteins** and so is **no longer free to degrade RpoH**
- **Heat shock proteins perform vital functions** in the cell, there is always a **low level** of these proteins present, even under optimal conditions



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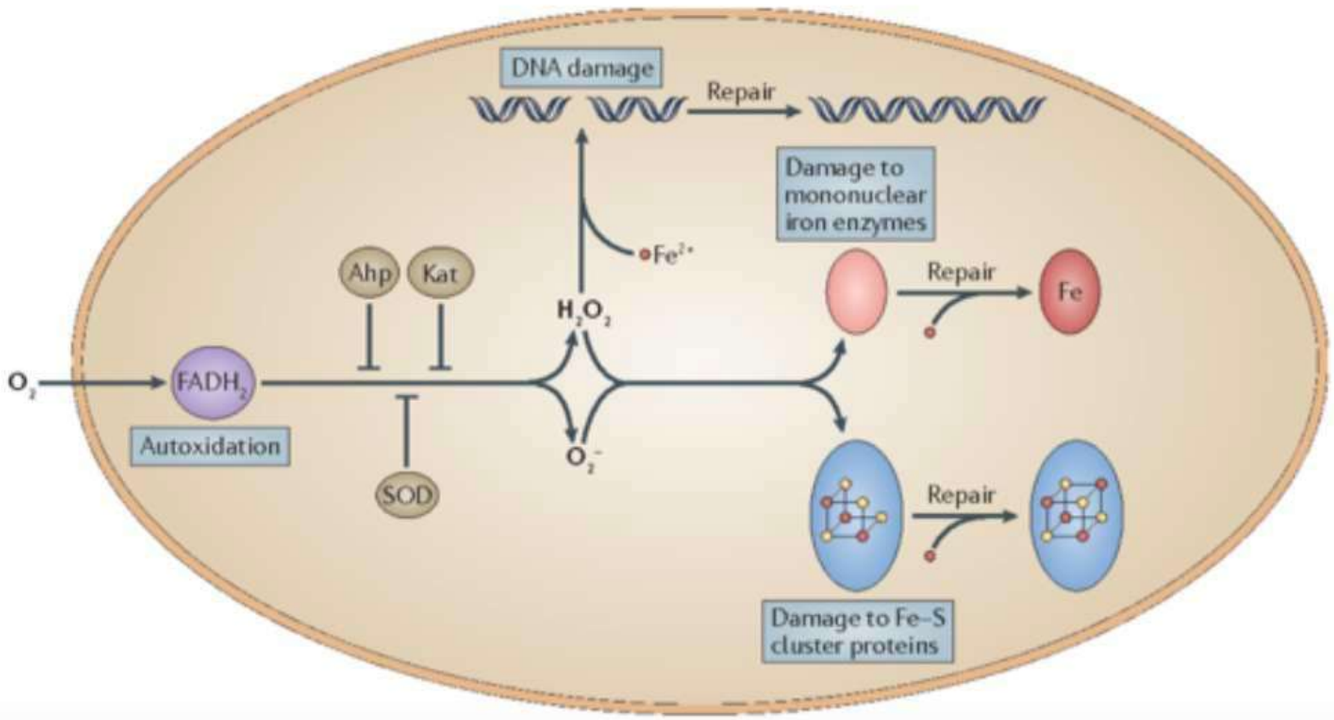
Oxidative Stress

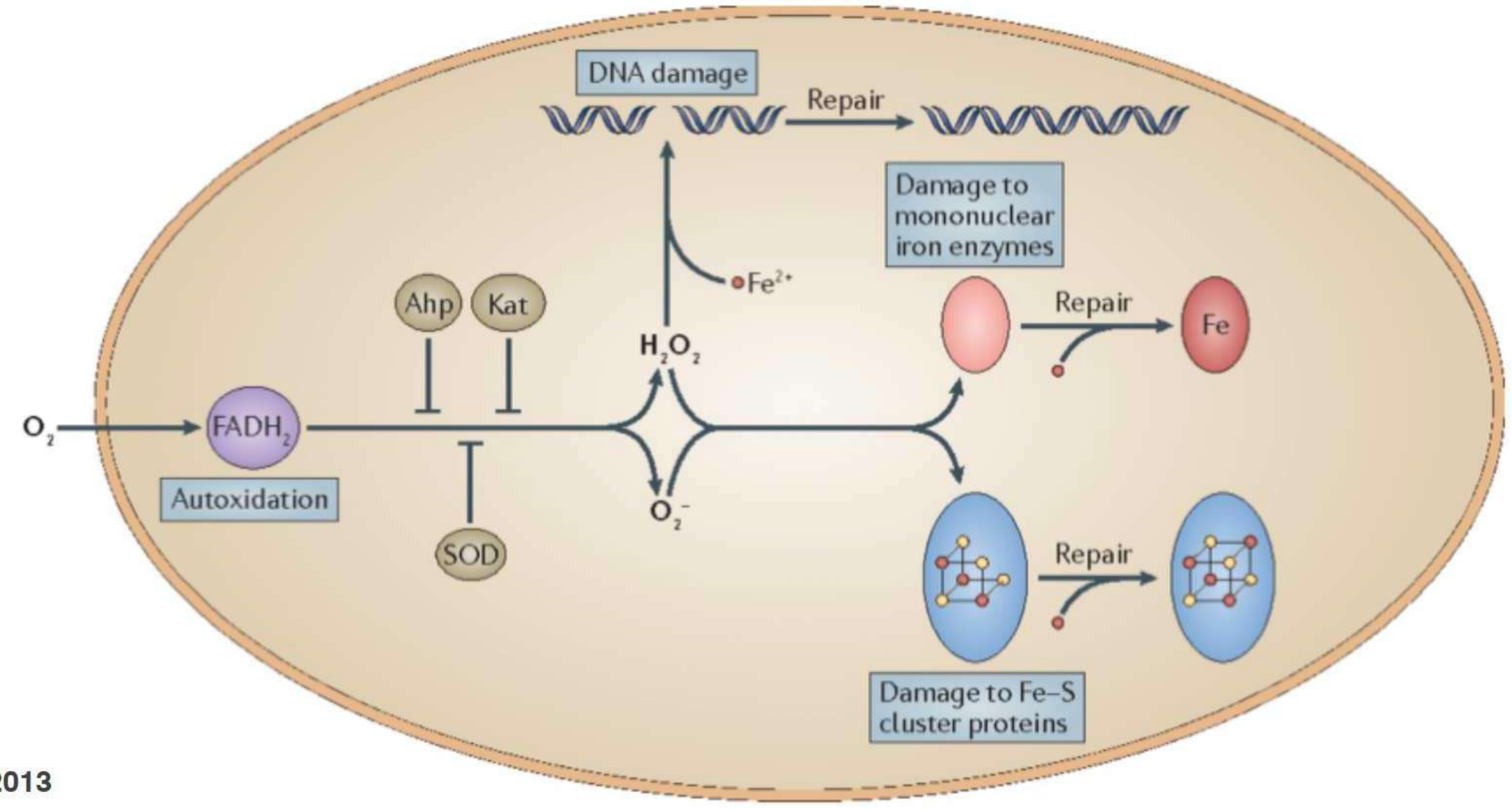
- **Oxidative damage** can have a devastating effect on the **structure and activity of proteins** (covalent modification), including DNA, membrane lipids
- The **sulfur-containing amino acids** cysteine and methionine are particularly **susceptible** to reactive oxygen species (ROS) and reactive chlorine species (RCS) and proteins with Fe^{2+}
- **Cellular pool of Fe^{2+}** interacts w. DNA (loosly associated w. biomolecules), proteins in damage and repair
- The gain of single electrons by oxygen (O_2) generates partially reduced reactive oxygen species (ROS), including superoxide anions ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$)
- In aerobic bacteria, ROS can form endogenously: reaction between O_2 acquires e^-, such as metal centers, ($FADH_2$ cofactors and quinones) part of the ETC
- Reactive nitrogen species (RNS) and reactive chlorine species (RCS) arise in environments that are hostile to bacteria



Ilmay, 2013

Oxidative Stress

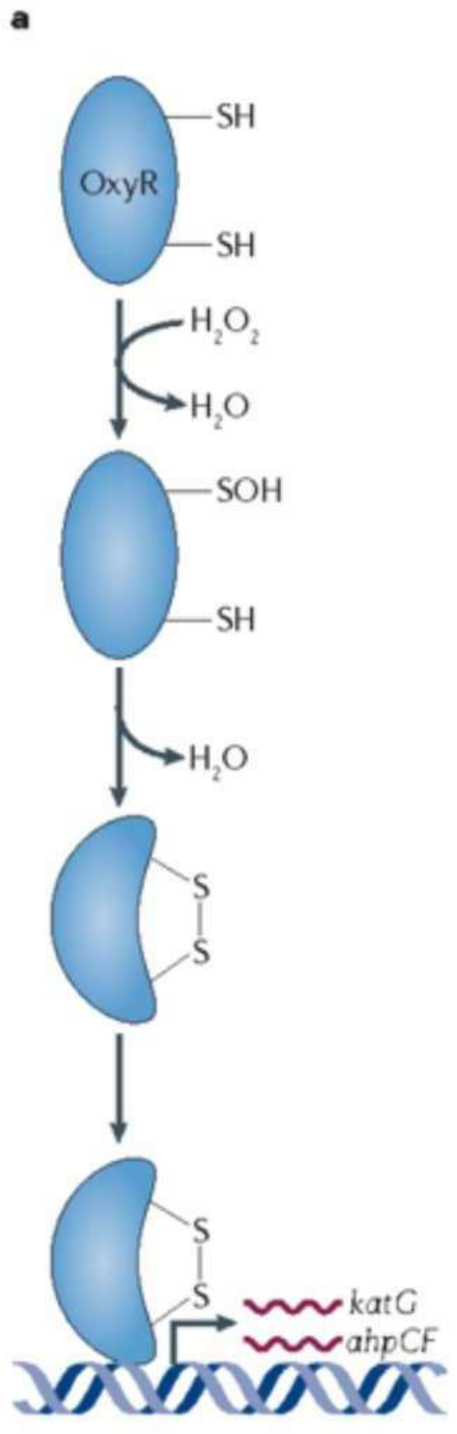
- Production of **catalases** (Kats), **peroxidases** (Ahp) and **superoxide dismutases** (SOD), which are enzymes that react with harmful oxidants and convert them to harmless products by neutralizing them before they cause damage to cellular components



Ilmay, 2013

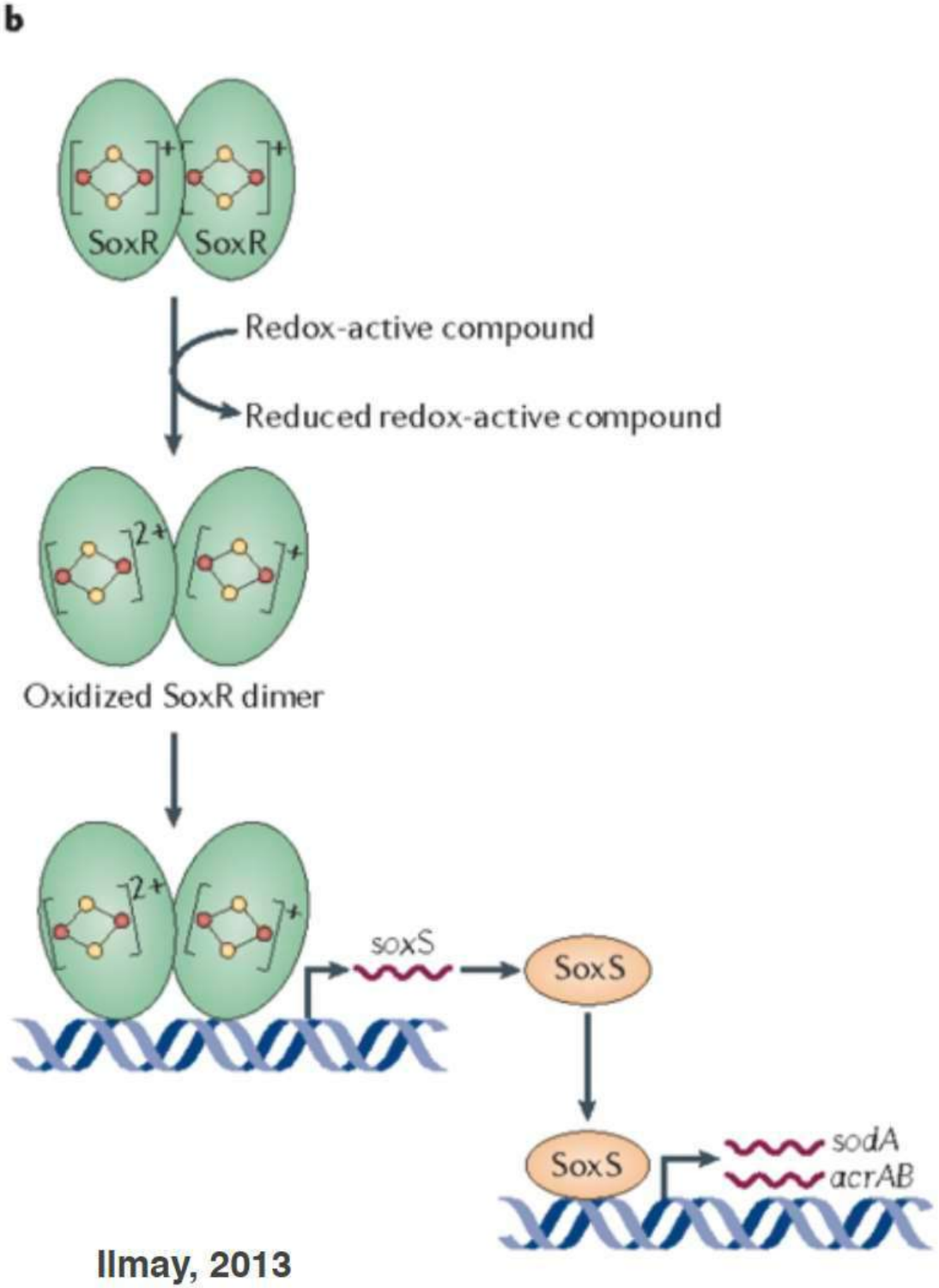
Oxidative Stress- response I

- Activation of redox-sensitive transcriptional regulators in *E.coli*
- Under these conditions, the induction of **OxyR**- and **SoxRS-directed defence regulons** is essential for cell recovery
- 2 defence systems: 1. **OxyR** system, responds to hydrogen peroxide (**H_2O_2**) 2. **SoxRS** system, responds to **redox-active compounds**
- **Transcription factor OxyR** detects modest increments in intracellular H_2O_2 —> activates several responses that help preserve the activities of Fe–S and mononuclear metalloenzymes
- Activates gene expression of **catalases** (Kats), **peroxidases** (Ahp)



Oxidative Stress- response II

- SoxRS system detects redox-active compounds that are released by plants and some bacteria —> generate toxic doses of O_2^-
- SoxRS system acts primarily to minimize the amounts inside the cell
- **SoxR is a homodimeric transcription factor**, and each monomer contains a [2Fe–2S] cluster
- Oxidized SoxR by redox-active compounds produced by bacterial competitors or plants (phenazines or quinones)—> **stimulates** transcription of soxS gene —> **SoxS** a secondary transcription factor that goes on to **activate expression** —> superoxide dismutase, multidrug efflux pump, other genes



Ilmay, 2013

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

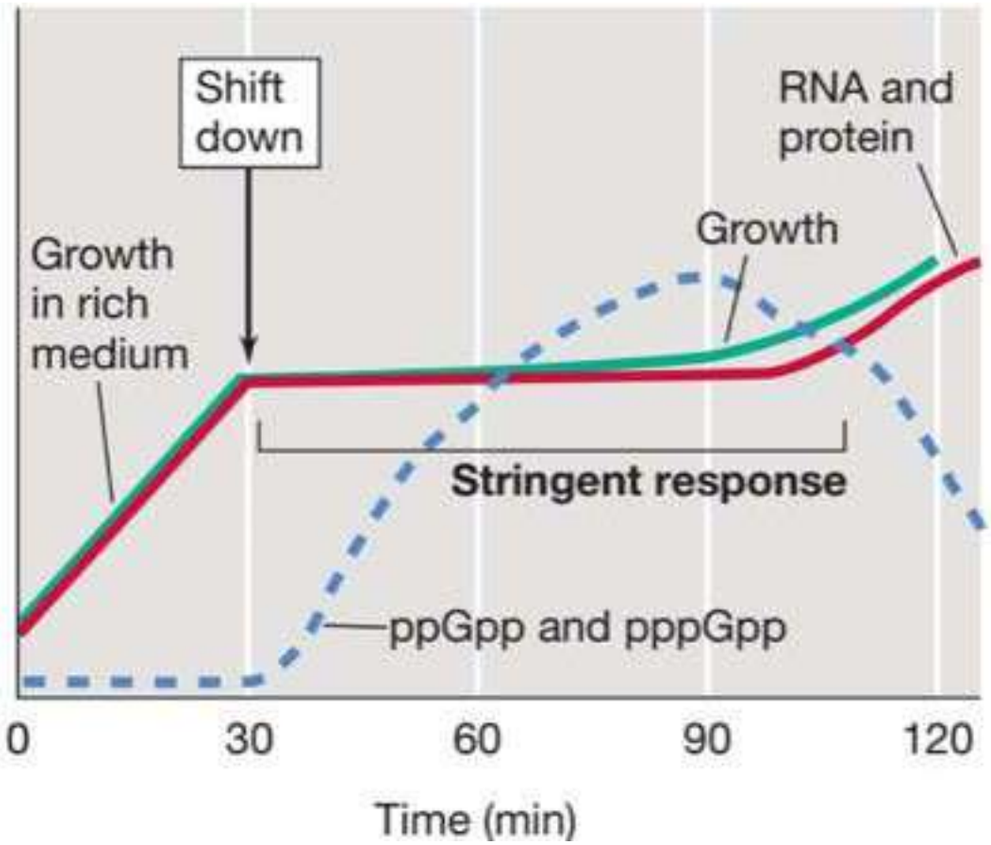
Amino acid shift down: rRNA, tRNA syntheses cease almost immediately –
> no new ribosomes are produced

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



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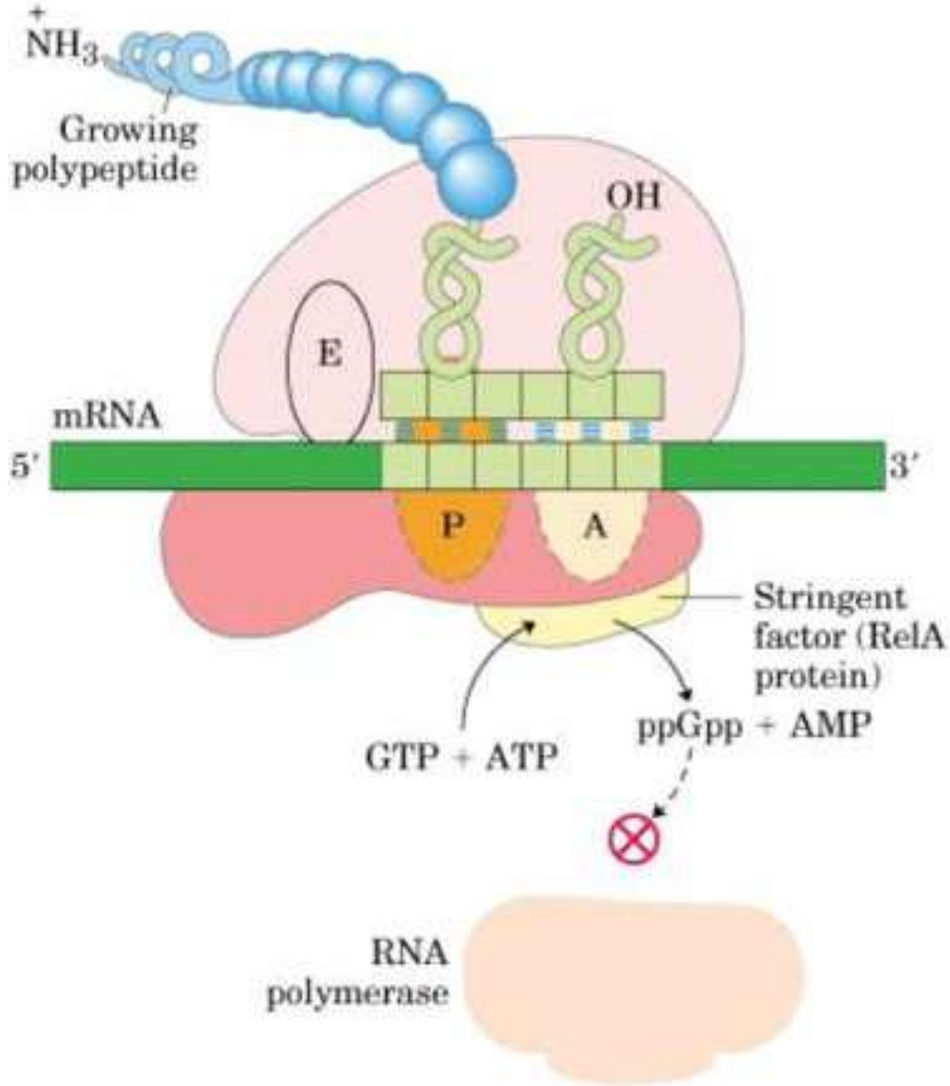
Stringent Response, III

- 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

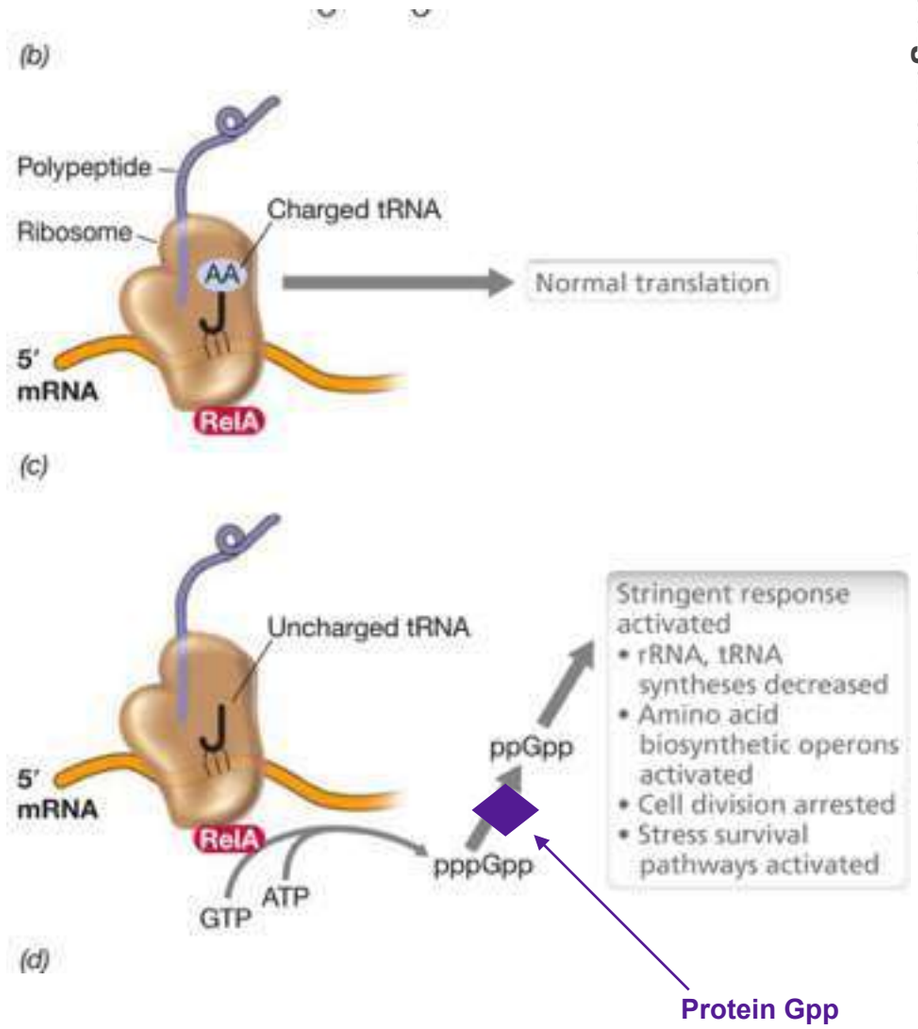
Activation both the **stress response pathways and biosynthetic operons for certain aa**

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**



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Post-Translational Regulation

- **Phosphorylation and methylation**: two-component regulatory systems, chemotaxis
- Biosynthetic enzymes can also **be regulated by the attachment of other small molecules**, such as the nucleotides adenosine monophosphate (AMP), adenosine diphosphate (ADP), and uridine monophosphate (UMP)
- **Enzymes are regulated by covalent modification**, due to **attachment or removal of a small molecule** or from **enzyme** that subsequently **affects its activity**
- **PII proteins** are a widespread family of signal-transducing proteins
- PII play role in nitrogen metabolism —> modifications range from uridylylation (addition of a UMP group), adenylylation (addition of AMP), phosphorylation (in some cyanobacteria)
- Proteins known as **anti-sigma factors** can also **bind to sigma factors —> inactivation**
- Anti-sigma factor, in stress response, in endospore formation

- **Regulating the synthesis and activities of a cell's RNAs and proteins is:**

(1) very important

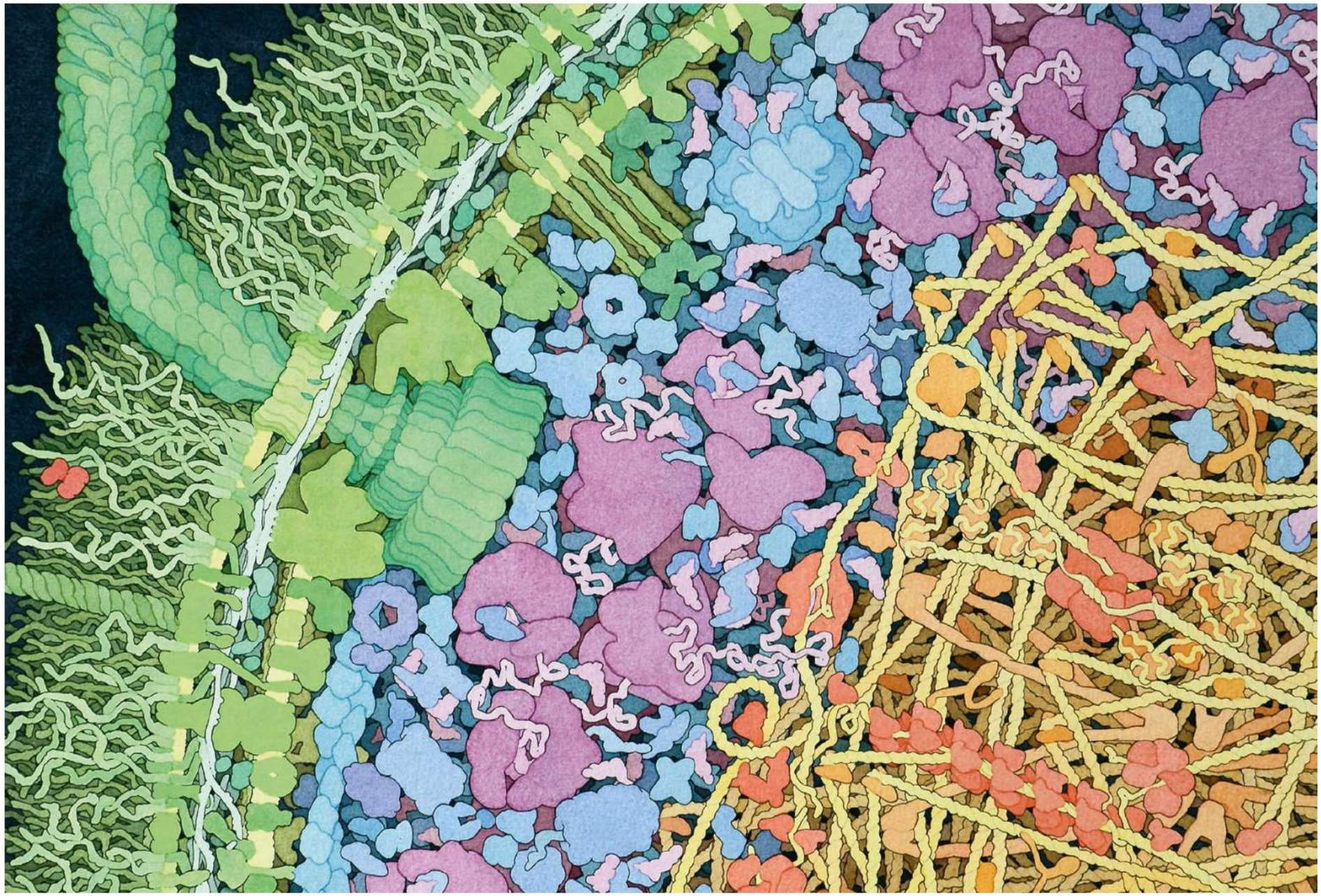
(2) possible in many different ways

(3) a major genetic investment

(4) allow strategies for conserve resources and maximize progeny

- How to sense the environment ?

Surviving…navigating in the microenvironment



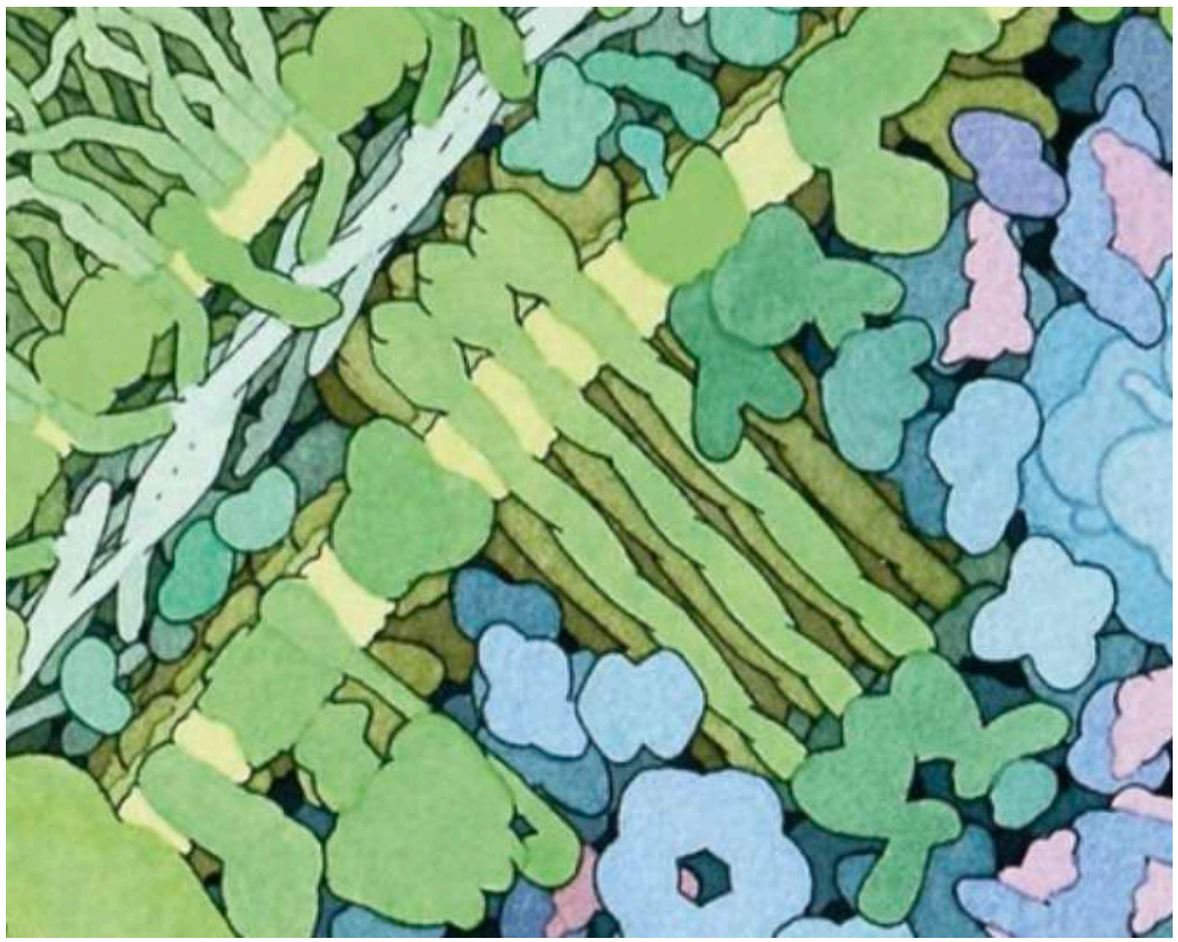
David S. Goodsell

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**

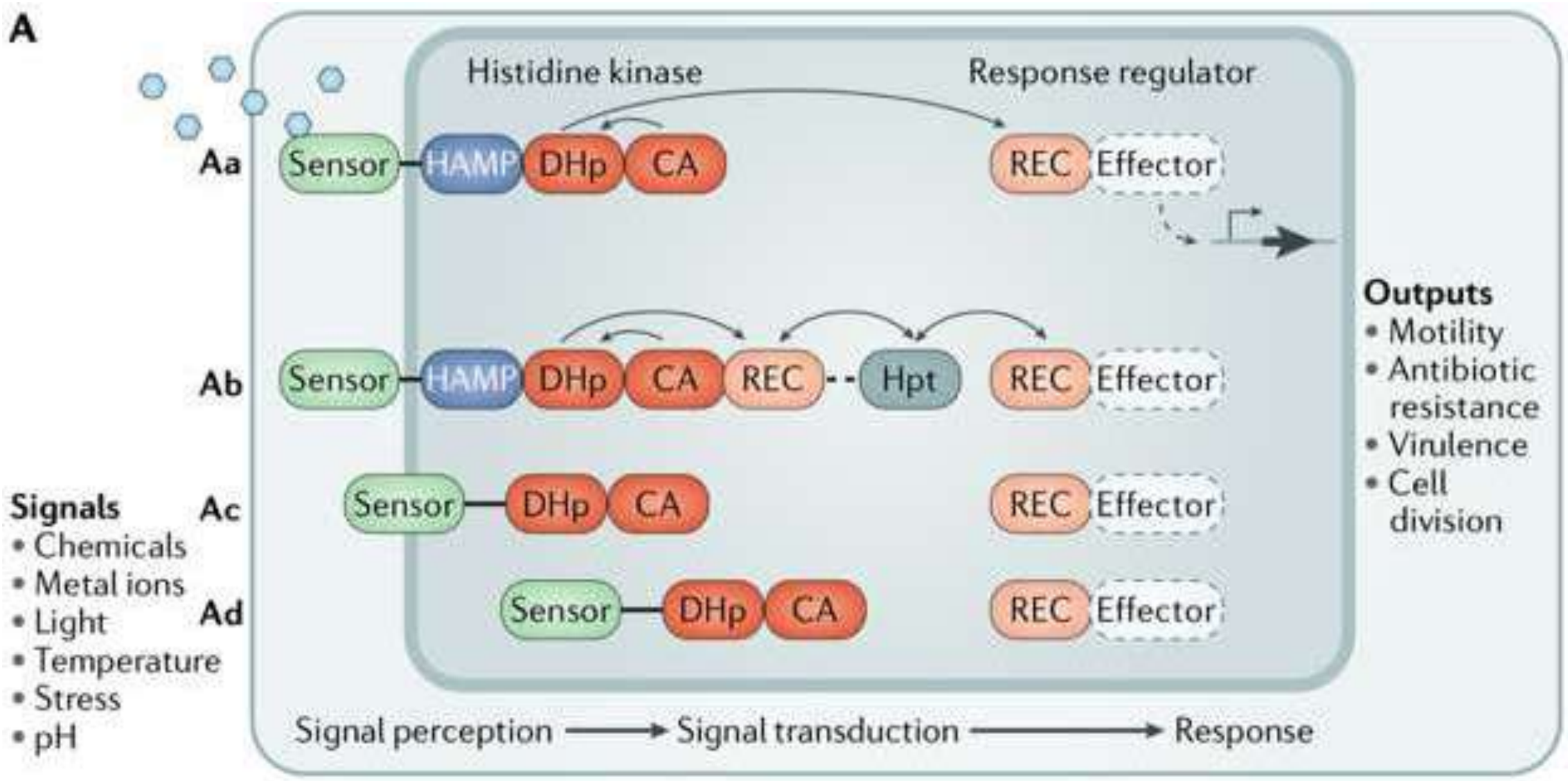
Signal transduction:

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**



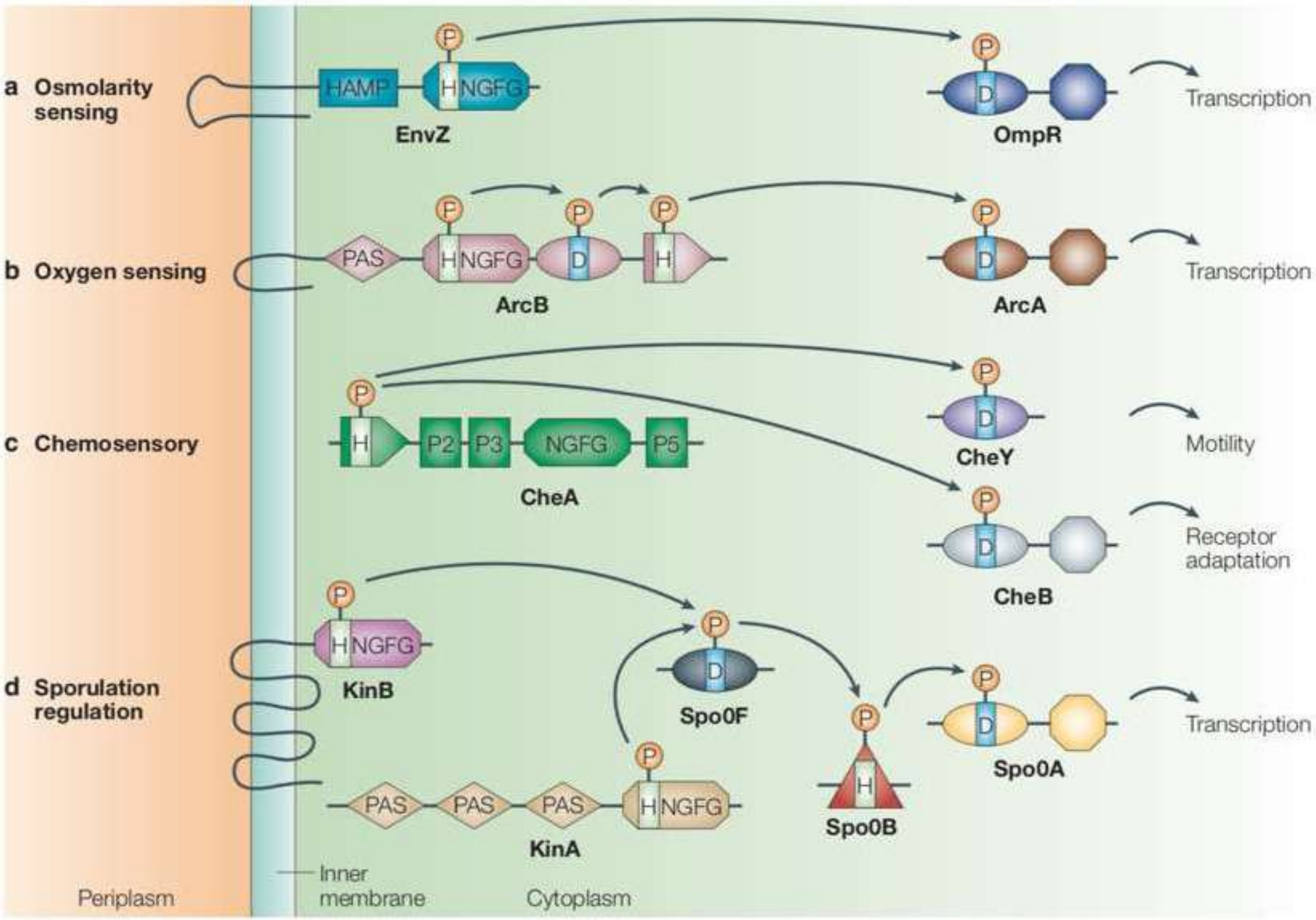
David S. Goodsell

Sensing and Signal Transduction II



Jacob-Dubuisson et al., 2018

Two-component systems

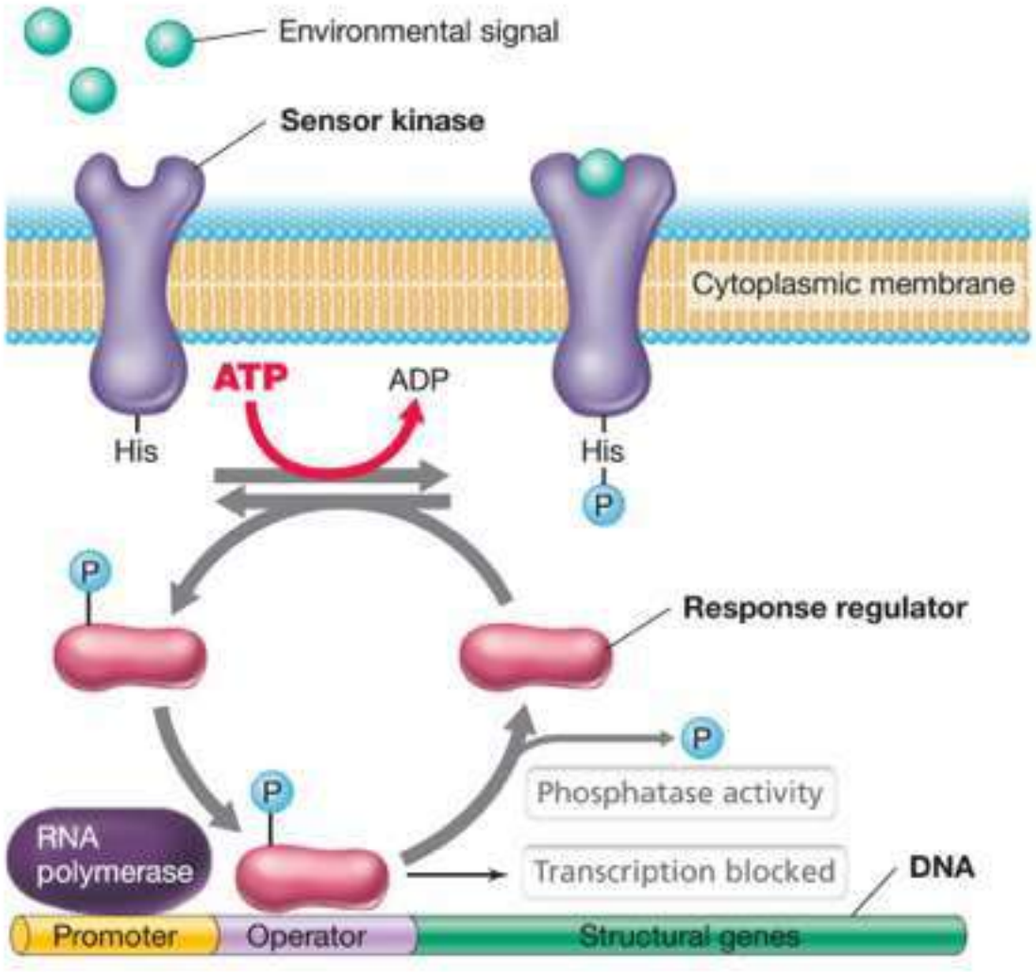


- Phototaxis

Wadhams & Armitage, 2014

Sensing and Signal Transduction III

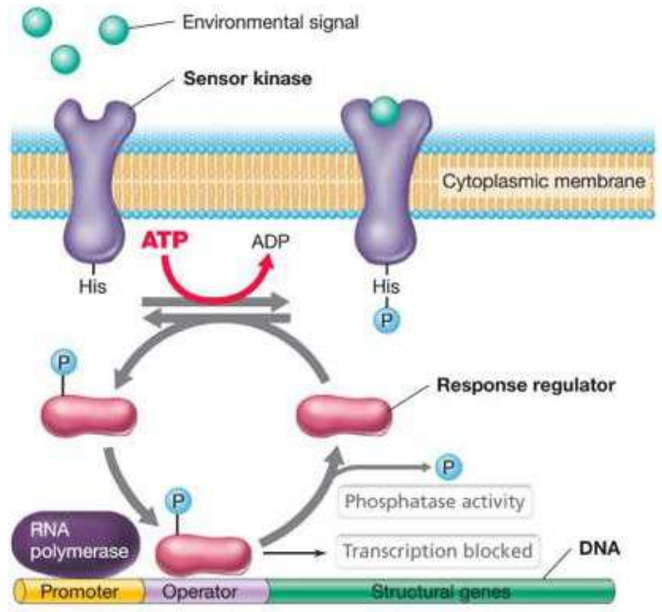
- Signal transduction systems contain two parts, they are called **two-component regulatory systems**
- Specific **sensor kinase** protein usually located in the cytoplasmic membrane, and a **response regulator** protein, present in the cytoplasm
- A kinase is an enzyme that **phosphorylates** compounds, 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 IV

- 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/σ^{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

Table 1

TCSs contributing to bacterial virulence regulation

Organism	TCS	Presumptive stimulus	Regulation of, or effect of inactivation	Reference
S. enterica	PhoP-PhoQ	Mg^{2+}/Ca^{2+}	Mg^{2+} uptake, modification of LPS, resistance to antimicrobial peptides, *pmrD*, transcriptional regulator genes *ssrB*, *hilA*, *slyA*, other virulence related genes post-transcriptional regulation of SsrA	[8,22]
	PmrA-PmrB	Fe^{3+}	Lipid A modification	[58]
	RcsC-YojN-RcsB	Desiccation, osmotic shock, growth on solid surfaces; specific *in vivo* stimulus unknown	Colonic acid capsule synthesis, *ftsA*, *osmC*, motility and chemotaxis genes, *fhlDC*, *tviA*, *rprA*	[15]
	OmpR-EnvZ	Osmolarity	Porin genes, *ssrB-ssrA*, stationary phase acid response	[23,59]
	SsrB-SsrA	ND	SPI-2 TTSS and effector genes	[60]
	SirA-BarA	ND	*csrB*, *hilD*	[27,28]
Shigella flexneri	OmpR-EnvZ		Invasion genes	[61]
S. sonnei	CpxR-CpxA	pH?	Virulence regulator gene *virF*	[62]
Vibrio cholerae	ArcA-ArcB		Virulence regulator gene *toxT*	[63]
Helicobacter pylori	FlgR-FlgS	ND	Flagellar genes	[64]
	ArsR-ArsS	Low pH	Urease and other acid-resistance genes	[65]
Campylobacter jejuni	DccR-DccS	ND	Colonization defect	[66]
Legionella pneumophila	CpxR-CpxA	ND	*icmR* and other *icm-dot* genes, no effect on intracellular replication in amoeba and human macrophages	[67]
	LetA-LetS	ND	Growth defect in amoeba, but not in human macrophages	[68]
Yersinia pseudo-tuberculosis	PhoP	ND	Virulence attenuation, reduced survival in macrophages	[69]
Pseudomonas aeruginosa	AlgR-FimS	ND	Alginate biosynthesis, twitching motility	[70]
	AlgB-KinB	ND	Alginate biosynthesis	[71]
	RocA1-RocS1 (SadR-SadS)	ND	Fimbrial genes, biofilm maturation	[72,73]
	PprB-PrpA	ND	Virulence genes and cell motility, QS signal production	[74]
	RtsM (RetS)	ND	TTSS and effector genes	[75,76]
Brucella abortus	BvrR-BvrS	ND	*omp* genes, virulence attenuation, reduced invasiveness in macrophages and HeLa cells	[77,78]
Neisseria meningitidis	MisR-MisS	ND	Composition of LOS inner core	[79]
B. pertussis	BvgA-BvgS	Temperature, redox state of quinones, SO_4^{2-}, nicotinic acid	Toxin and adhesin expression, biofilm formation	[35,80]

Beier & Gross 2006

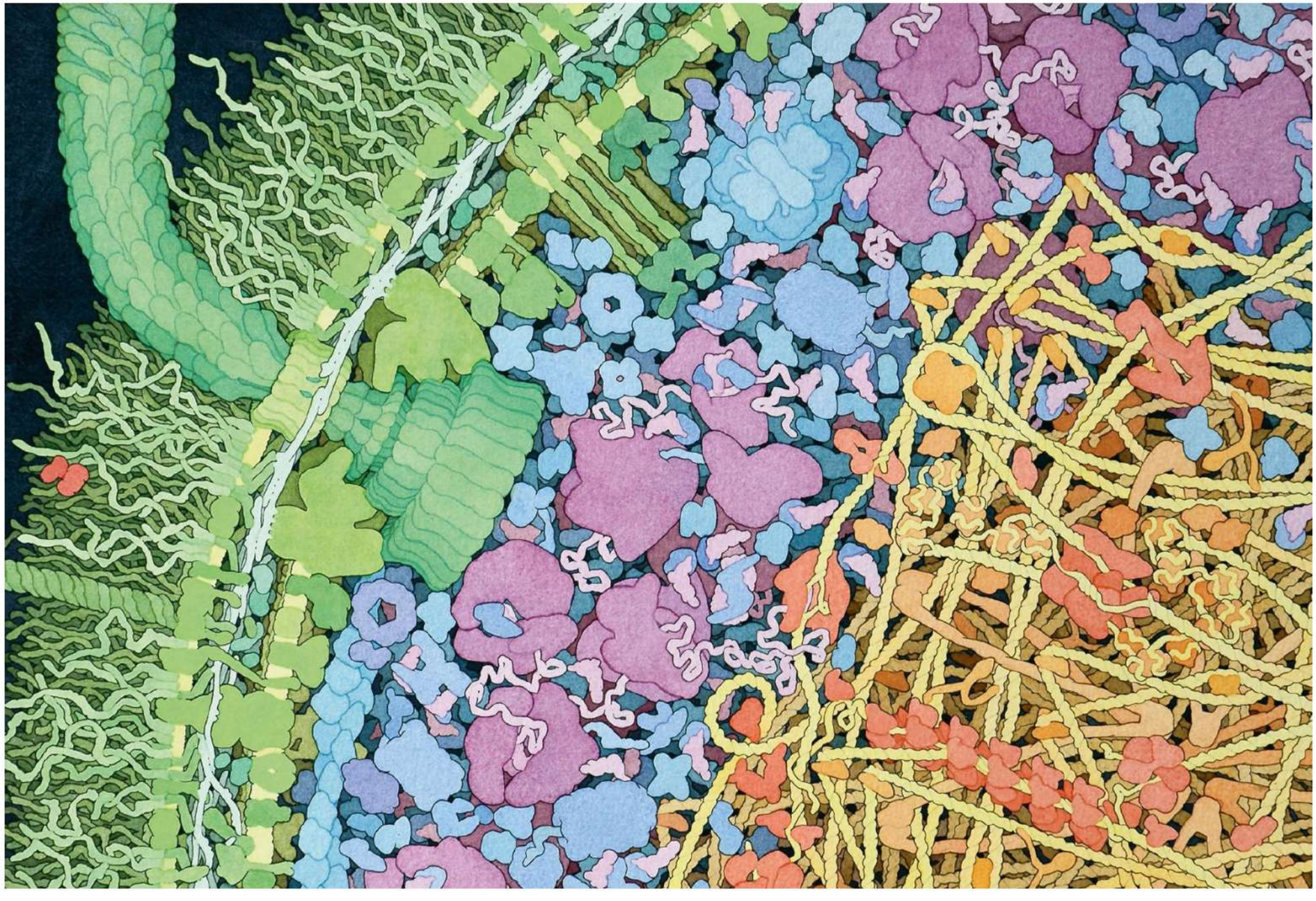
Listeria monocytogenes	DegU	ND	Virulence attenuation	[81]
	VirR-VirS	ND	Virulence attenuation	[82]
	AgrA-AgrC	ND	Virulence attenuation	[83]
	LisR-LisK	ND	Virulence attenuation	[84]
Mycobacterium tuberculosis	DevR-DevS	ND	Virulence attenuation	[85]
	MprA-MprB	ND	Virulence attenuation	[86]
	RegX3-SenX3	ND	Virulence attenuation	[87]
	PrrA-PrrB	ND	Intracellular growth defect during the early stages of macrophage infection	[88]
Streptococcus pneumoniae	CiaR-CiaH	ND	Virulence relevant gene *htrA*	[89]
	RR04-HK04	ND	Virulence genes *psaB*, *psaC*, *psaA*	[90]
	RR06-HK06	ND	Virulence gene *cbpA*	[91]
	RitR	ND	Iron homeostasis	[92]
	MicA-MicB	Oxygen?	Virulence attenuation	[93]
Streptococcus pyogenes	CsrR-CsrS (CovR-CovS)	Mg^{2+}	Capsule synthesis, virulence genes *ska*, *sagA*	[94,95]
Streptococcus agalactiae	CsrR-CsrS (CovR-CovS)	ND	Virulence attenuation	[96,97]
S. mutans	SMRR11-SMHK11	ND	Biofilm formation and acid resistance	[98]
Staphylococcus aureus	AgrA-AgrC	AIP	Regulatory RNA III	reviewed in [4]
	SrrA-SsrB	Oxygen?	Exoprotein genes, RNA III	[99]
	SaeR-SaeS	ND	Exoprotein genes	[100]
	ArlR-ArlS	ND	Exoprotein genes	[101]
	LytR-LytS	ND	Holin-like genes *lrgA*, *lrgB*	[102]
Clostridium perfringens	VirR-VirS	ND	Toxin (*pfoA*, *cpb2*) and adhesion genes (*cna*)	[103]

ND, not determined.

Beier & Gross 2006

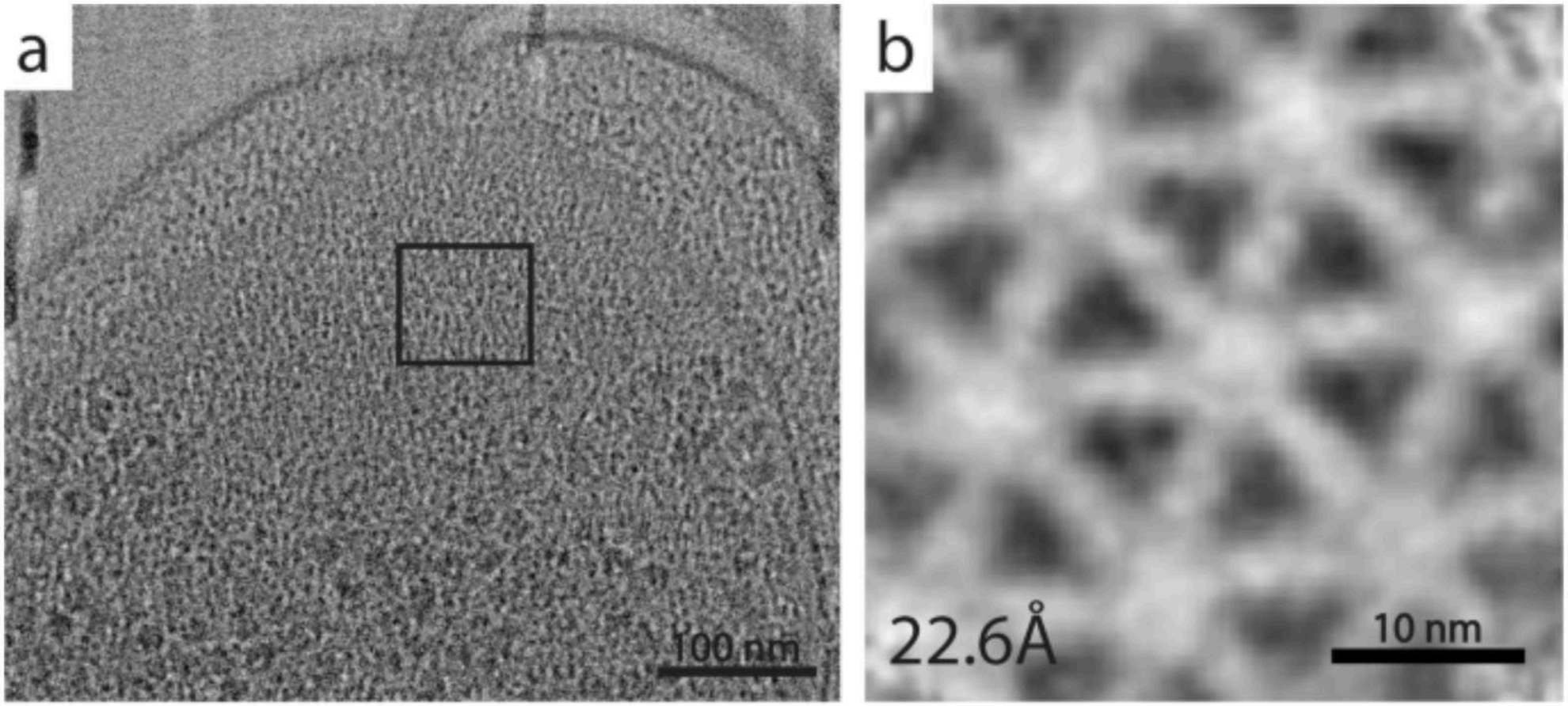
- How to coordinate motion and sensing ?

Flagellum and two-component system coupling



David S. Goodsell

***Vibrio cholerae and its chemotaxis array*.**



Depelteau et al., 2022

https://www.cellstructureatlas.org/

https://www.pnas.org/doi/10.1073/pnas.1812871115

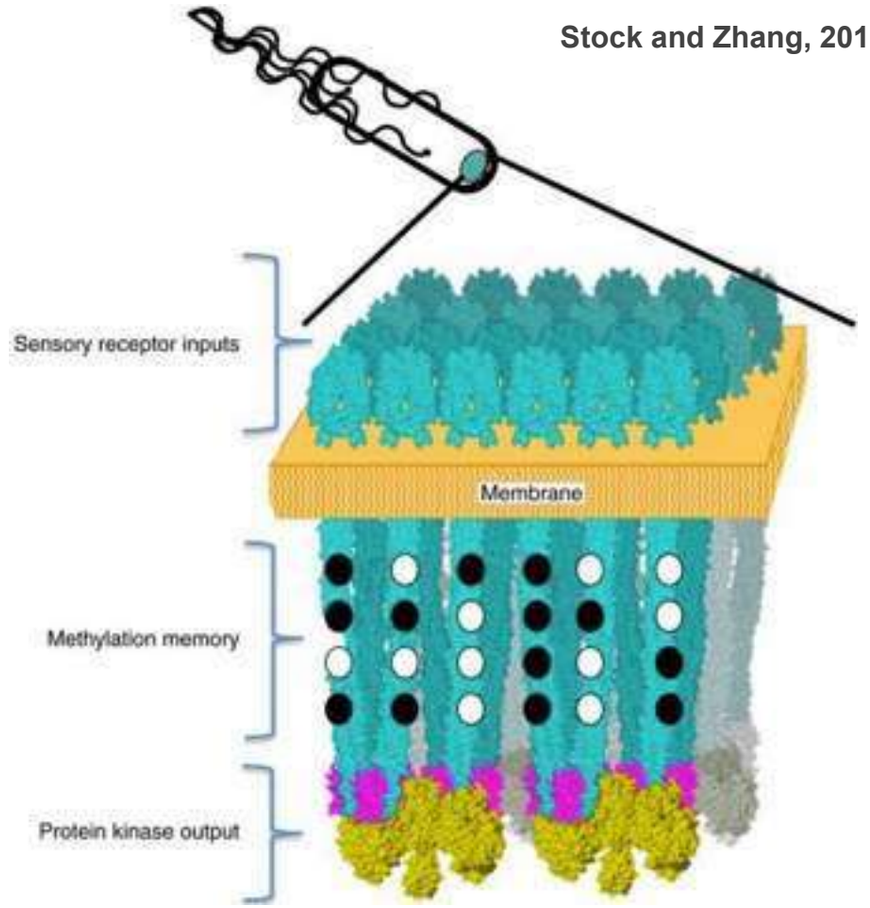
Chemotaxis, I

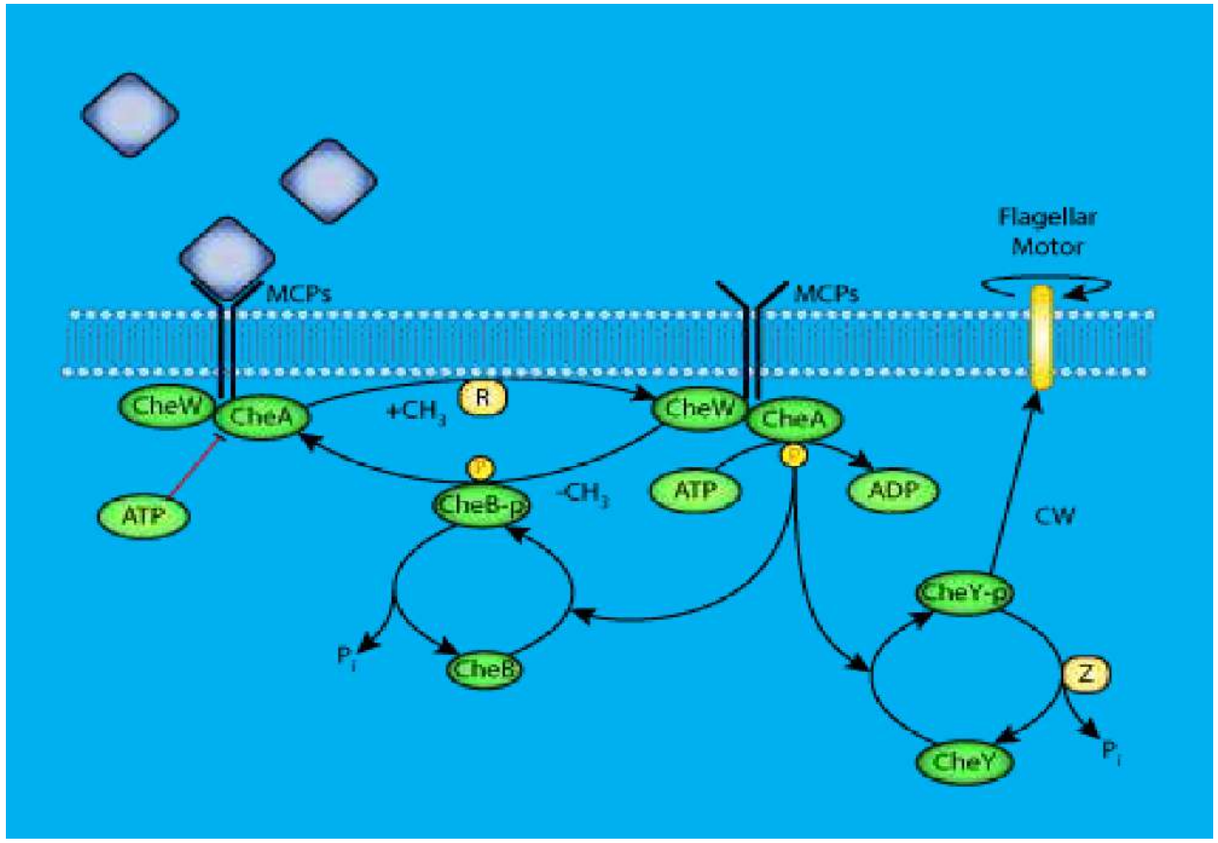
- **Chemokinesis: random movements, in absence of a concentration gradient of chemoattractant**
- **Chemotaxis: directional movement along a + gradient of chemoattractant**

Chemotaxis, II

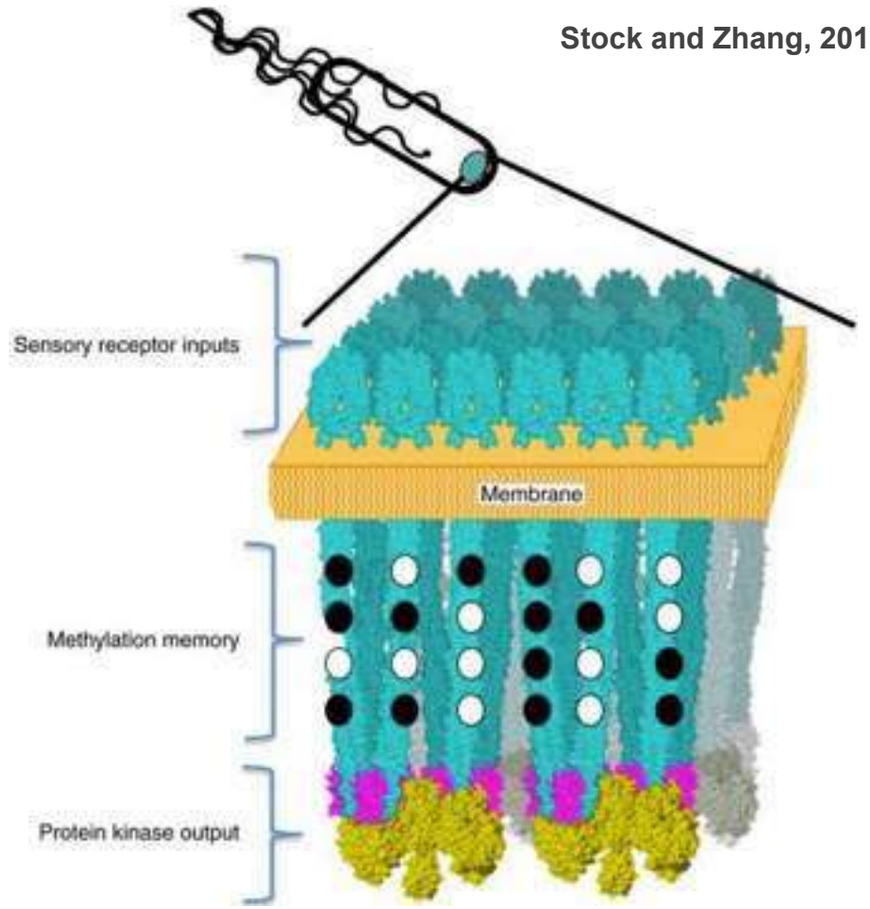
- Cells are too small to sense spatial gradients of a chemical, but they can respond to **temporal gradients**
- **Sensing the change in concentration** of a chemical (attractant or repellent) over time rather than the absolute concentration of the chemical stimulus – > **signal-to-noise ratio**
- Two-component system modulates activities in pre-existing proteins: flagellum machinery
- **Nanobrain**, chemoreceptor clusters

Stock and Zhang, 2013





Chemotaxis, III



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

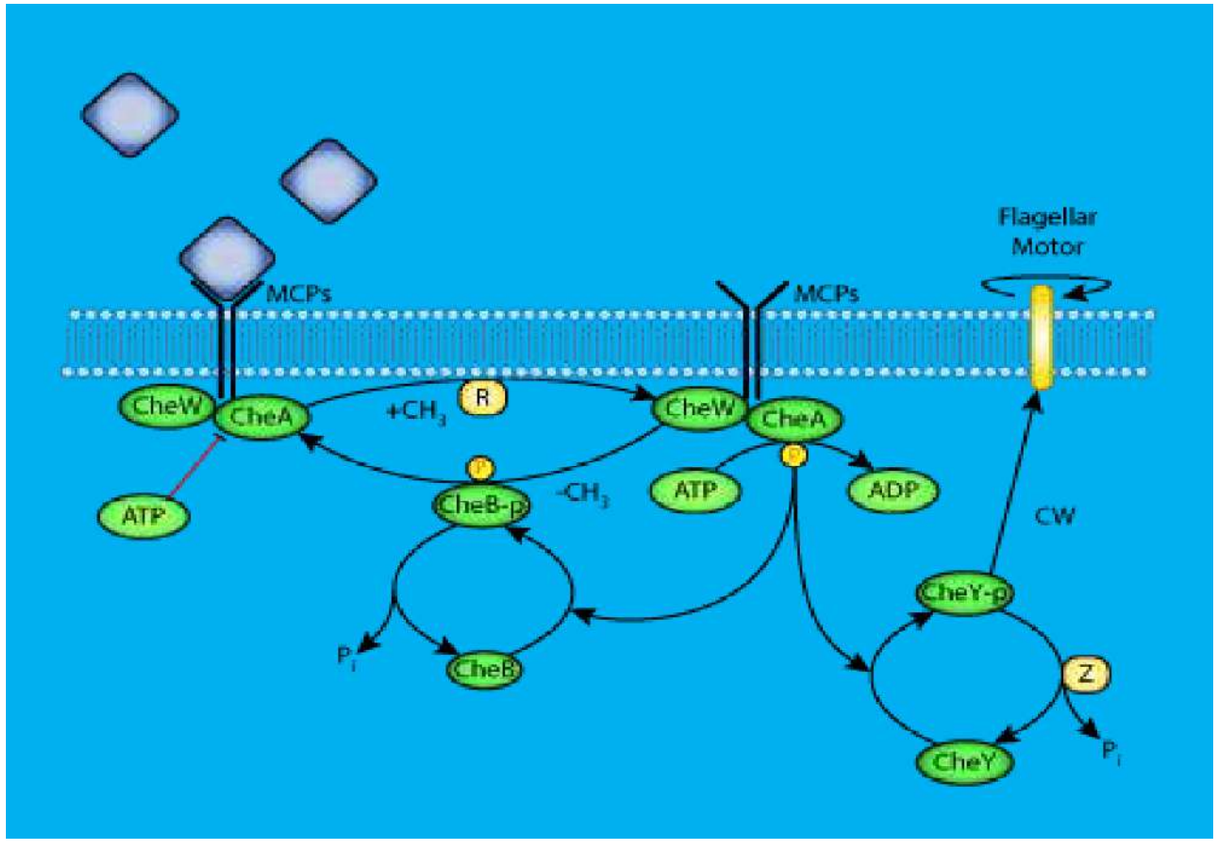
- MCPs allow the cell to monitor the concentration of various substances over time

- Diverse MCPs for **diverse compounds**

- *E. coli* Tar MCP senses attractants Asp, maltose & repellents Co, Ni

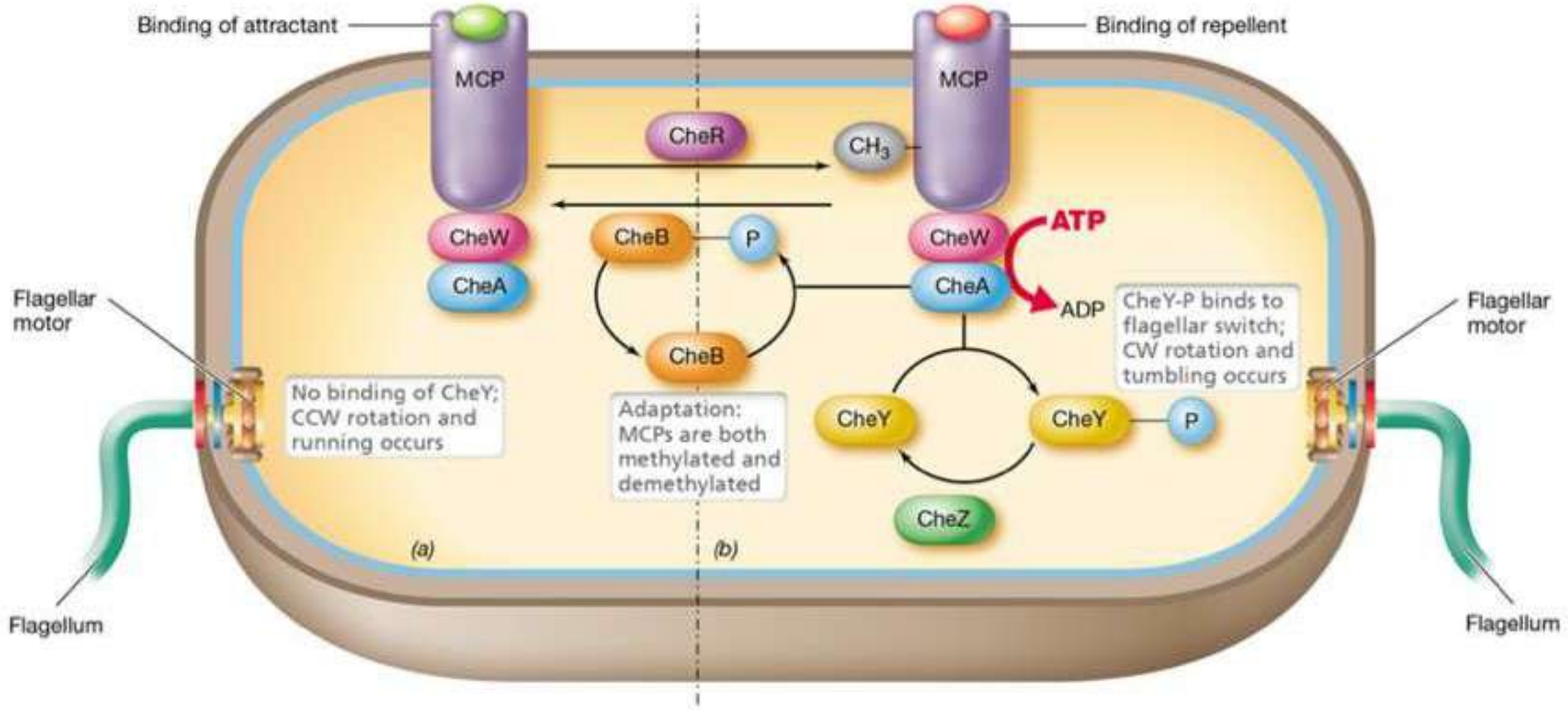
- **MCPs bind attractants or repellents** directly or in some cases indirectly through interactions with periplasmic binding proteins

- *E. coli:* thousands of **MCPs** are often **clustered, forming chemoreceptors**



Chemotaxis architecture

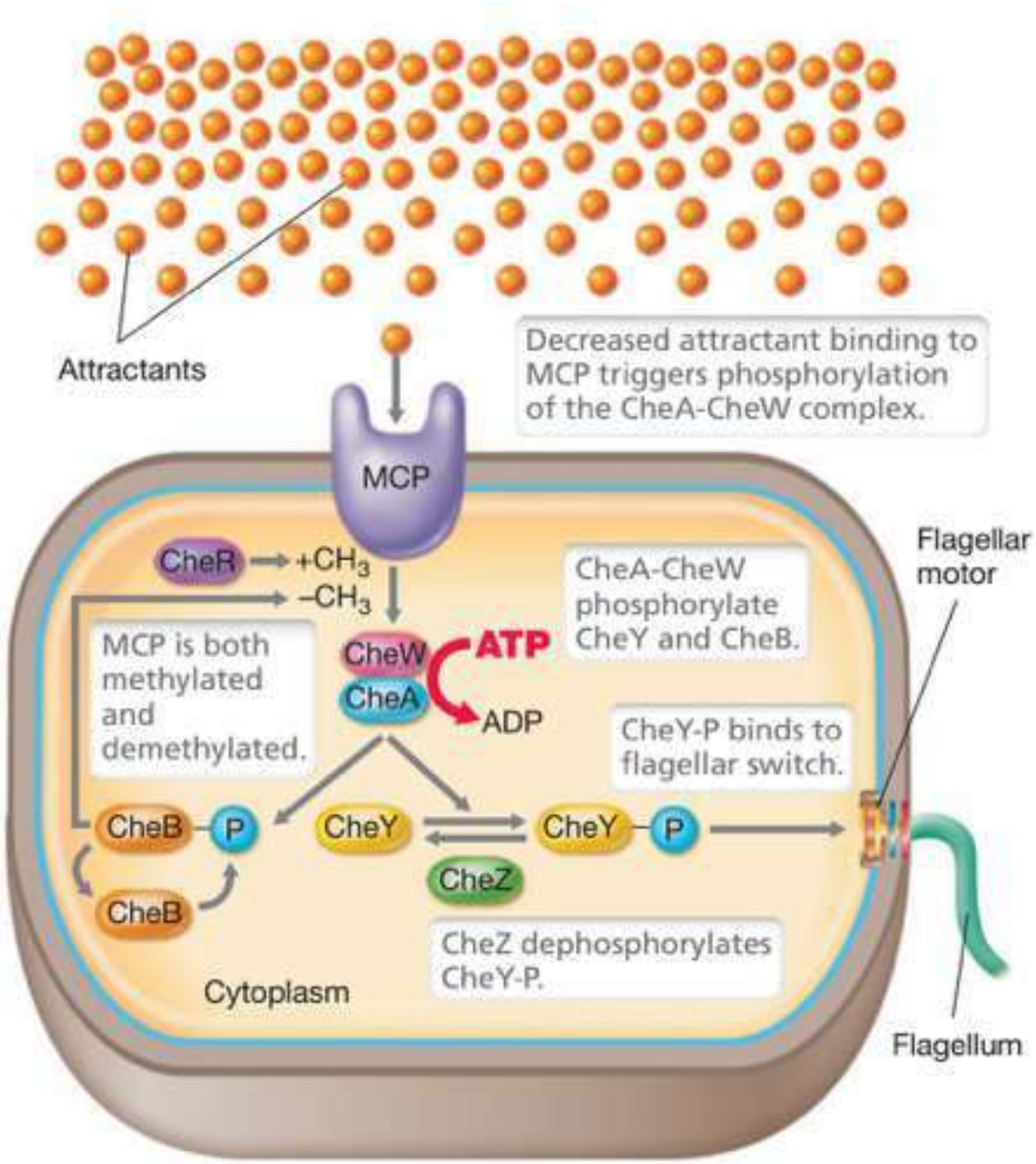
MCP (methyl-accepting chemotaxis proteins)



Madigan et al. 2020

Chemotaxis, IV

- 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



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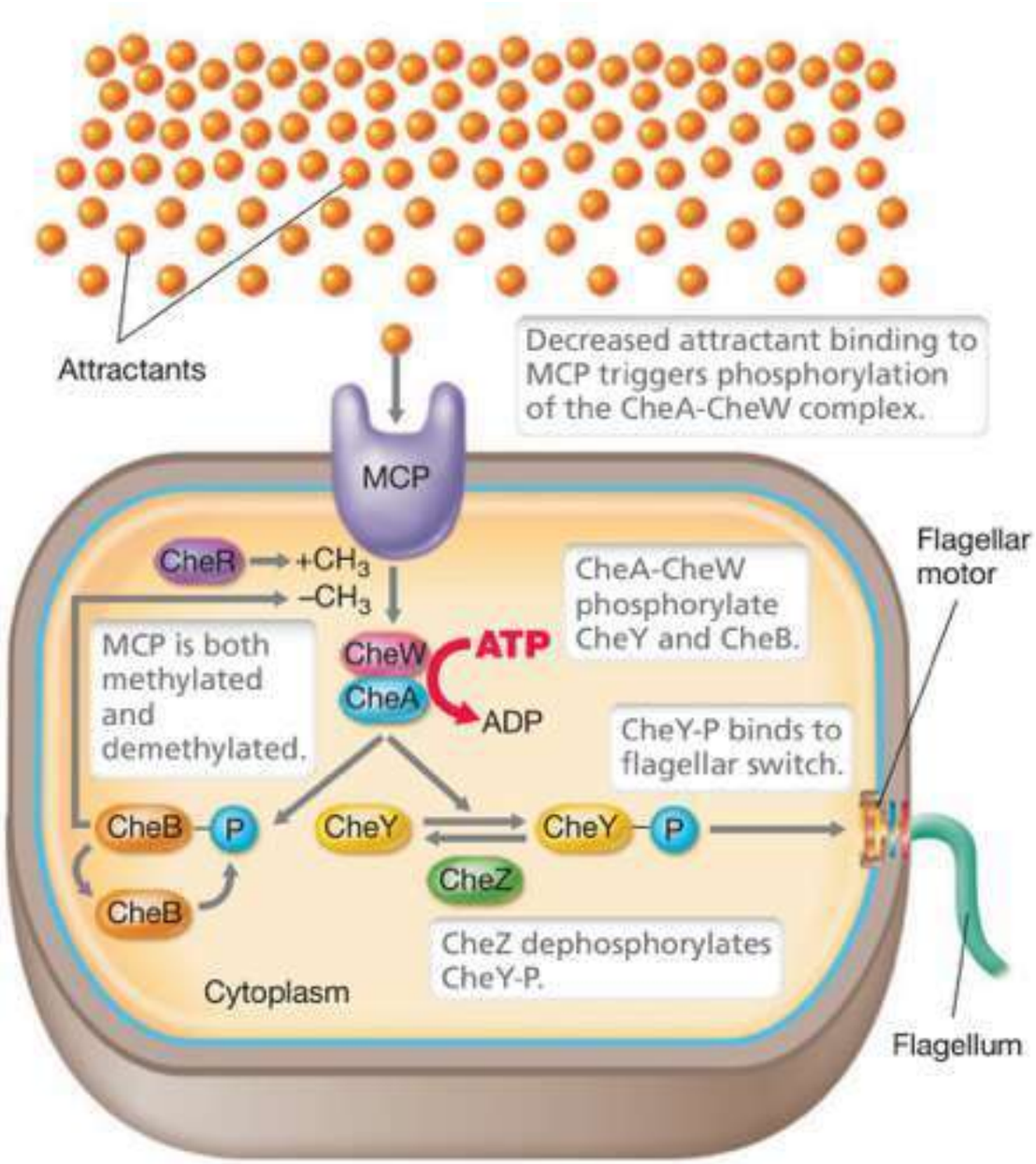
Chemotaxis, V

- 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**



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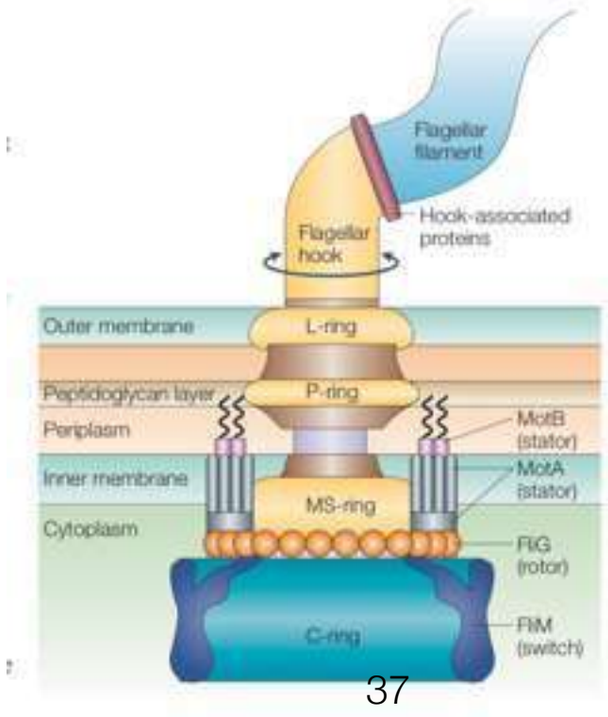
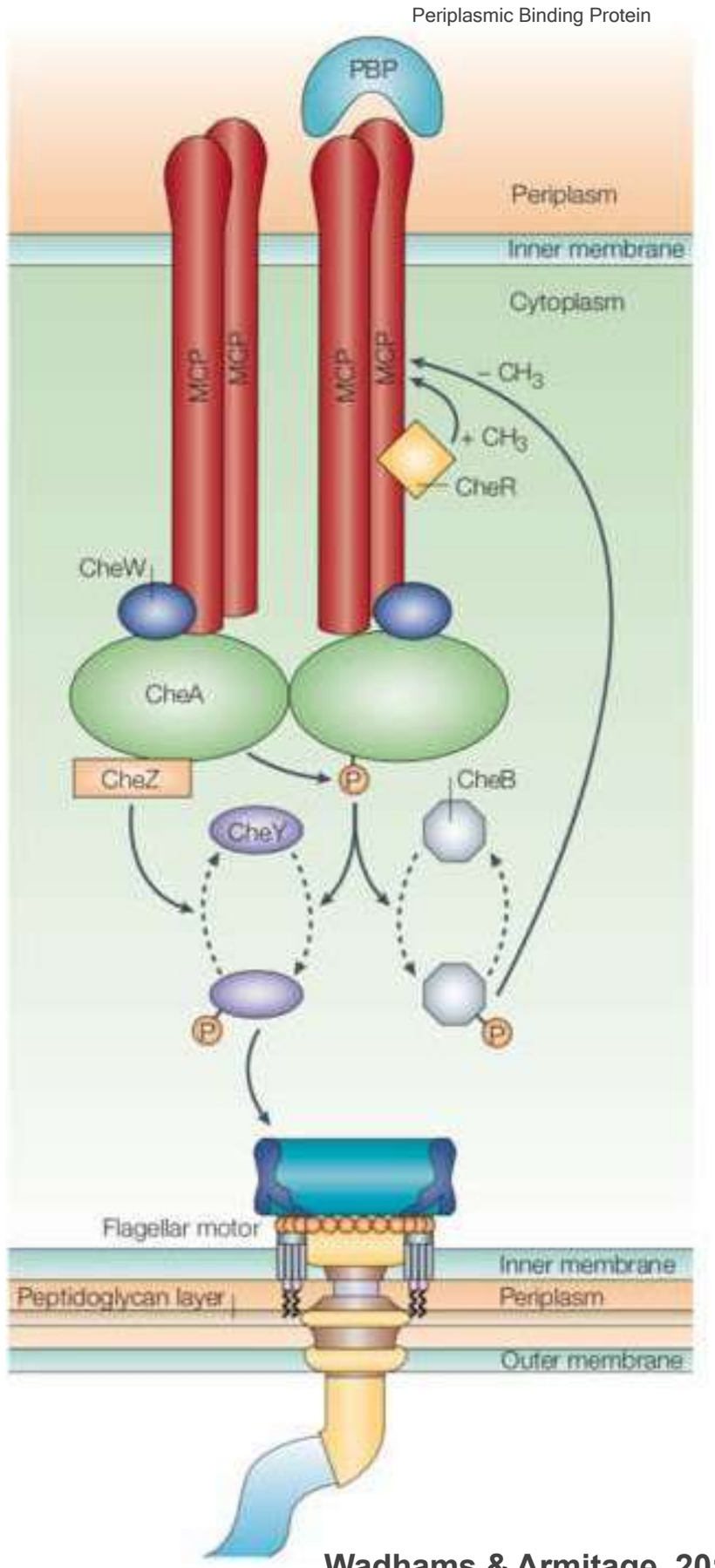
Chemotaxis, VI

- **CheA is the sensor kinase** for chemotaxis
- Once **CheY is phosphorylated**, it interacts with the flagellar motor (**switch protein FliM)** to induce **clockwise** flagellar rotation –> **tumbling**
- **Unphosphorylated, CheY** cannot bind to the flagellar motor –> running
- **CheZ, dephosphorylates CheY —> running**
- Either an increase in repellents or a decrease in attractants leads to an increase of CheY-P –> tumbling
- By contrast, if the cell is swimming toward attractants, the **lower** level of CheY-P — > **running**
- **The flagellar motor is composed of a rotor and multiple stator units**
- **Each stator unit acts as a transmembrane ion channel to conduct cations and applies force on the rotor**



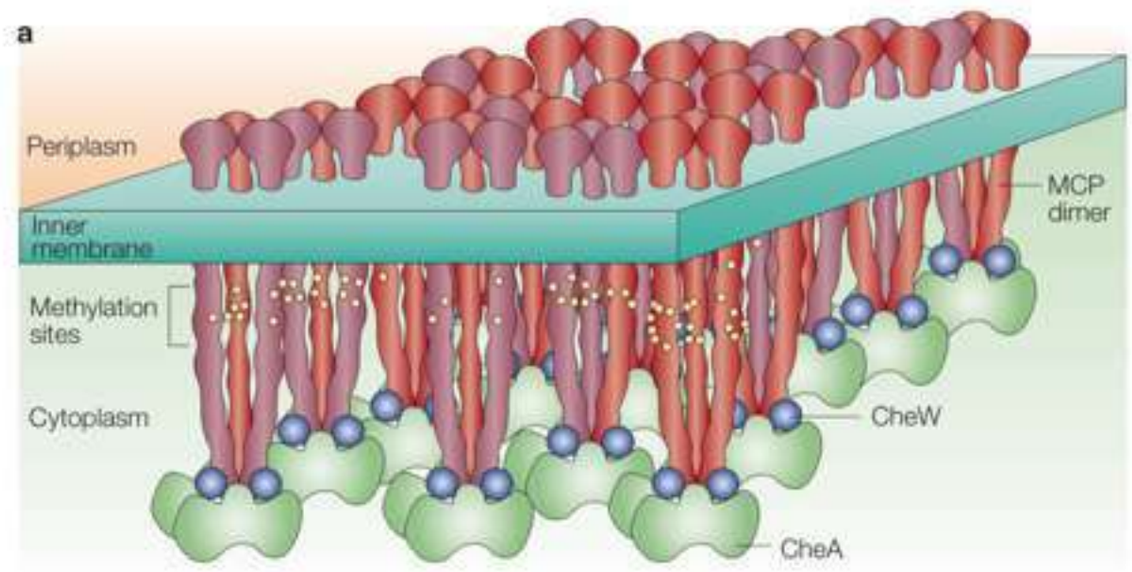
Wadhams & Armitage, 2014



Wadhams & Armitage, 2014

Chemotaxis, VII

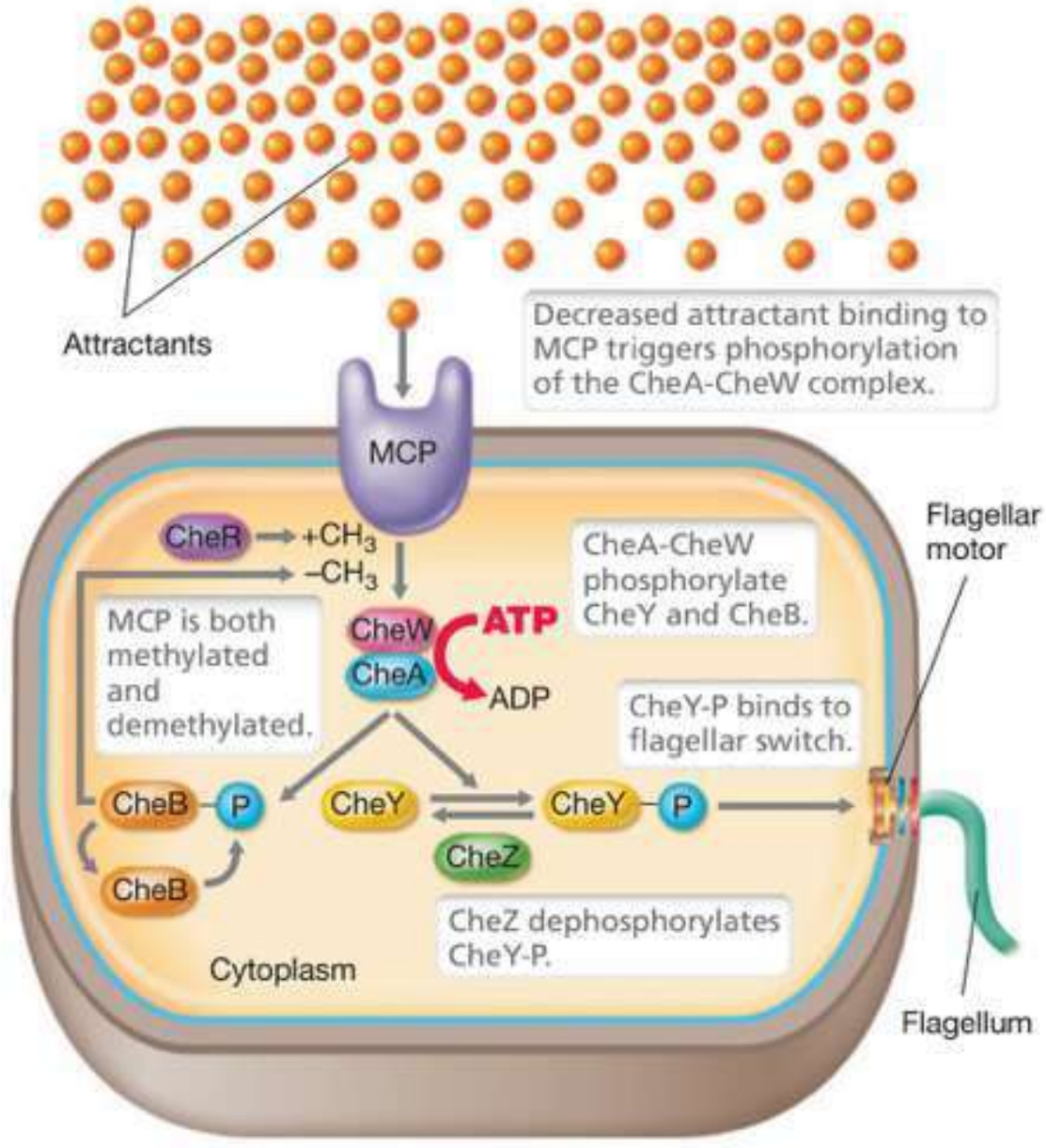
- **Adaptation**: **resetting of sensory system** to await further signals after finishing responding to stimulus
- **MCPs are fully methylated – > no longer respond to attractants**, more sensitive to repellents
- **MCPs are unmethylated —> respond strongly to attractants**, insensitive to repellents
- Varying the methylation level thus allows adaptation to sensory signals
- **Methylation by CheR & demethylation CheB-P**



Wadhams & Armitage, 2014

Chemotaxis, VIII

- 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**



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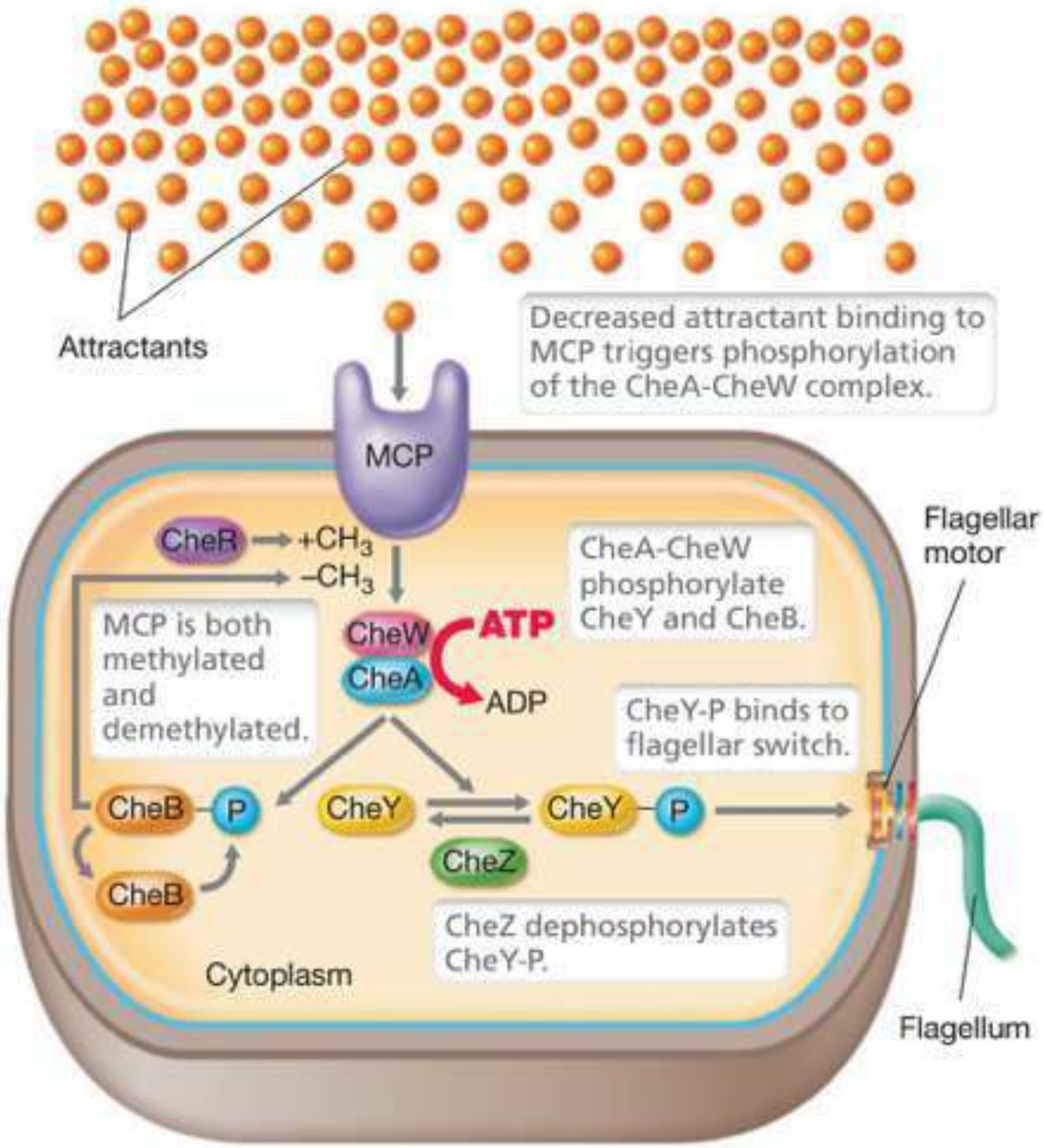
Chemotaxis, IX

- **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**



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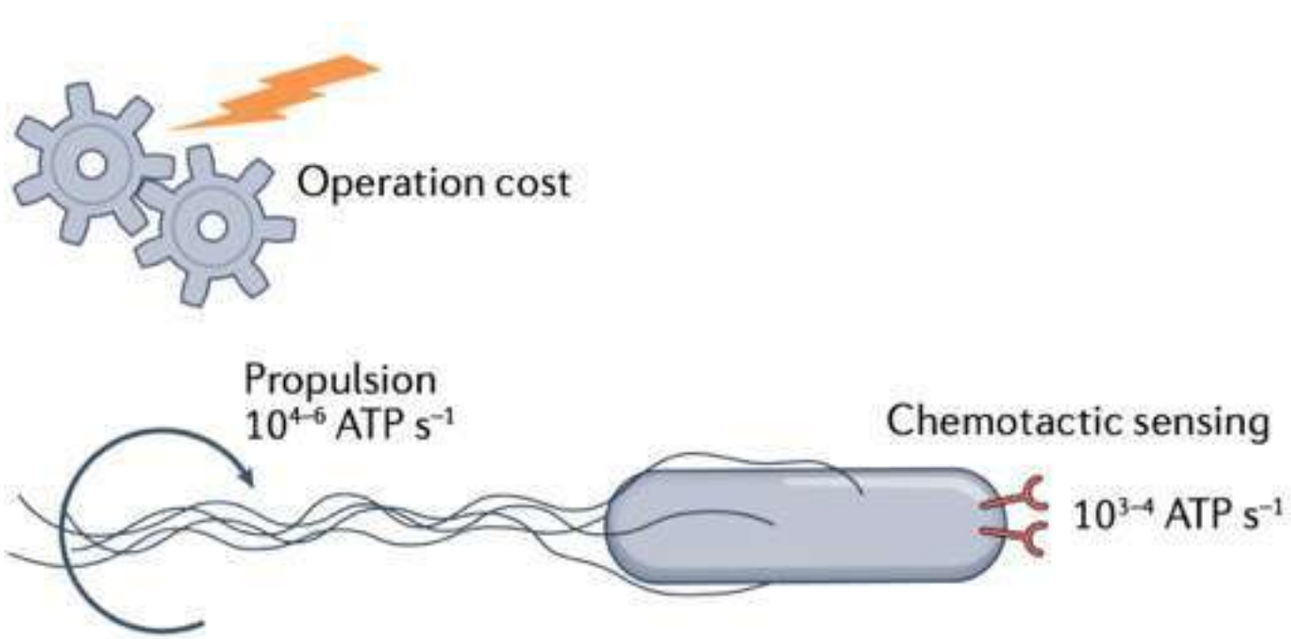
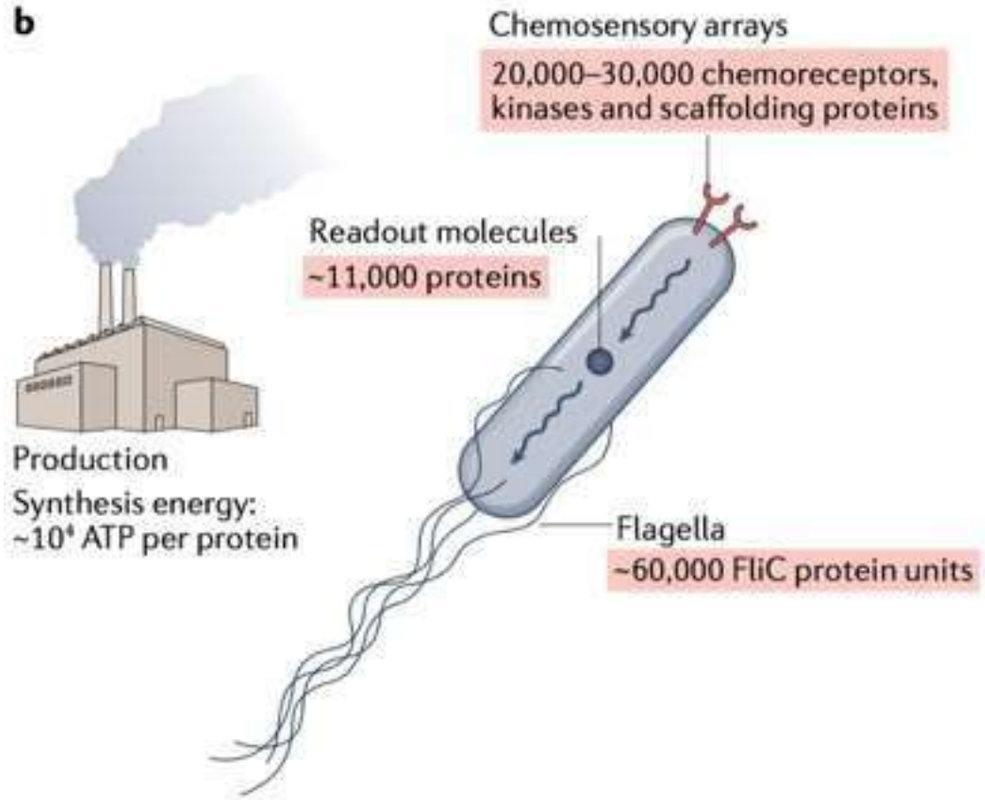
Relative cost of bacterial chemotaxis





- **Metabolism** fuels chemotaxis
- **Informed foraging** and **cue-based navigation**
- **Increase growth rate in a better environment**

Keegstra et al., 2022