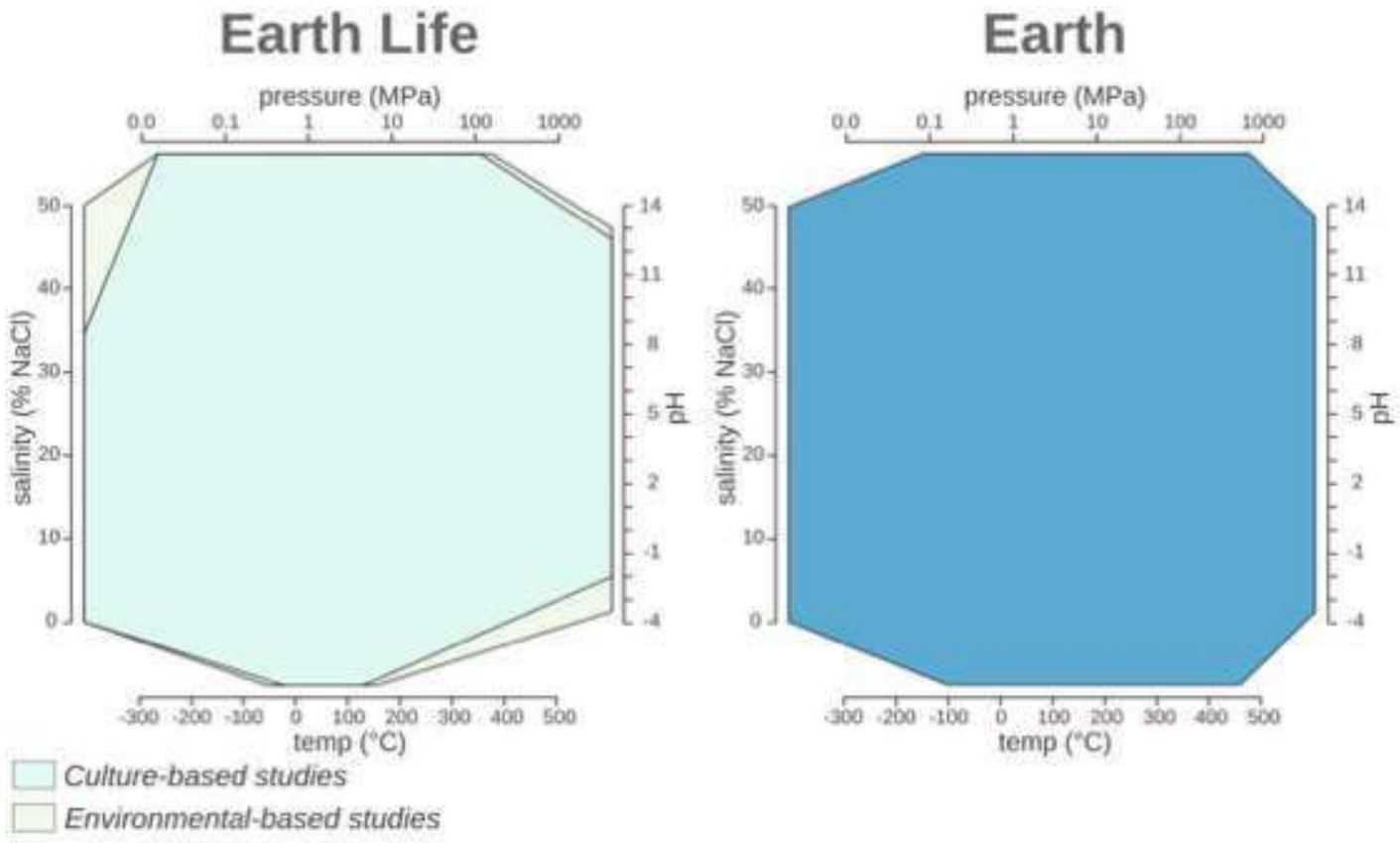
# L06b

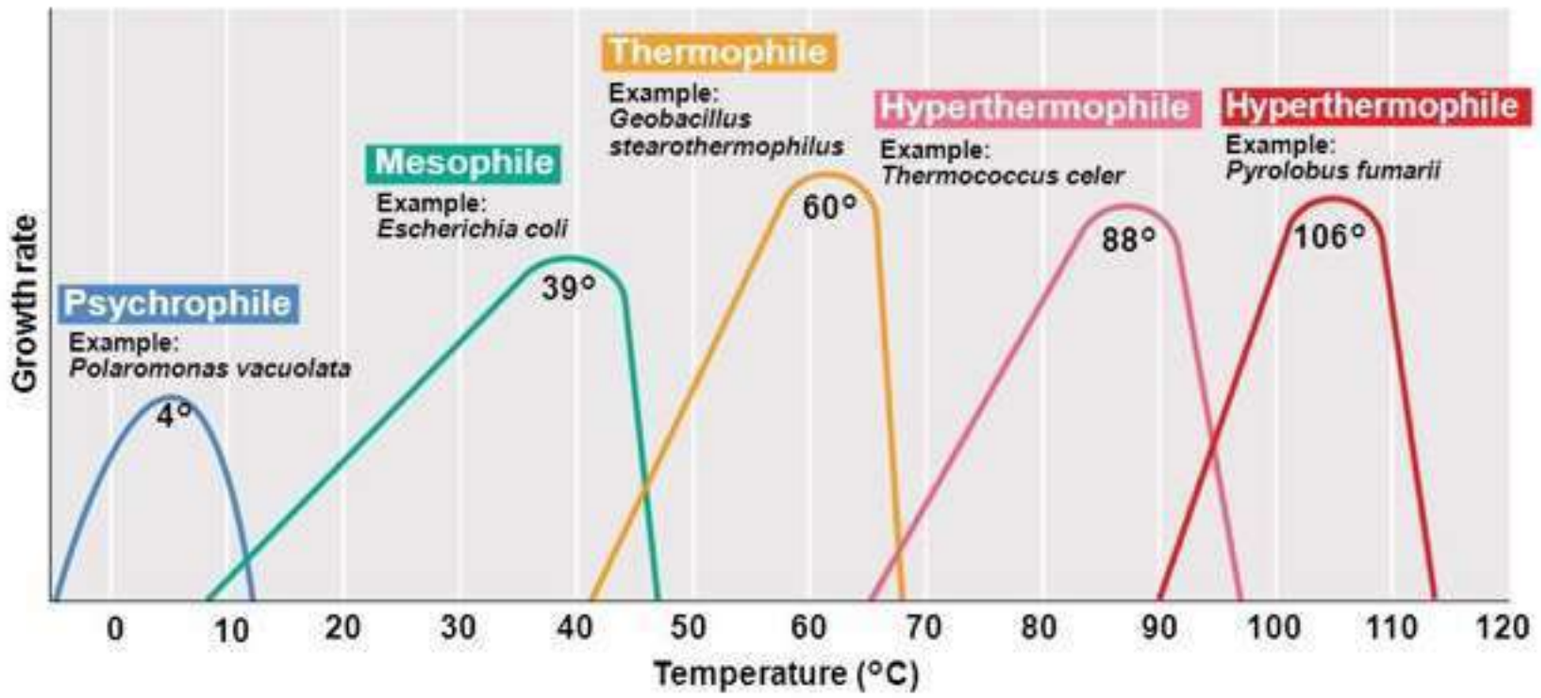
# Recap L06a

# Wide ranges of Pressure, Temperature, pH and salinity on Earth

- No such stable environment
- Dial cycle
- Chemical warfare: antibiotics, secondary metabolites
- Microbial competitions
- Predators: grazers and viruses
- Optimal conditions in a variable narrow range
- Outside from this conditions there is stress



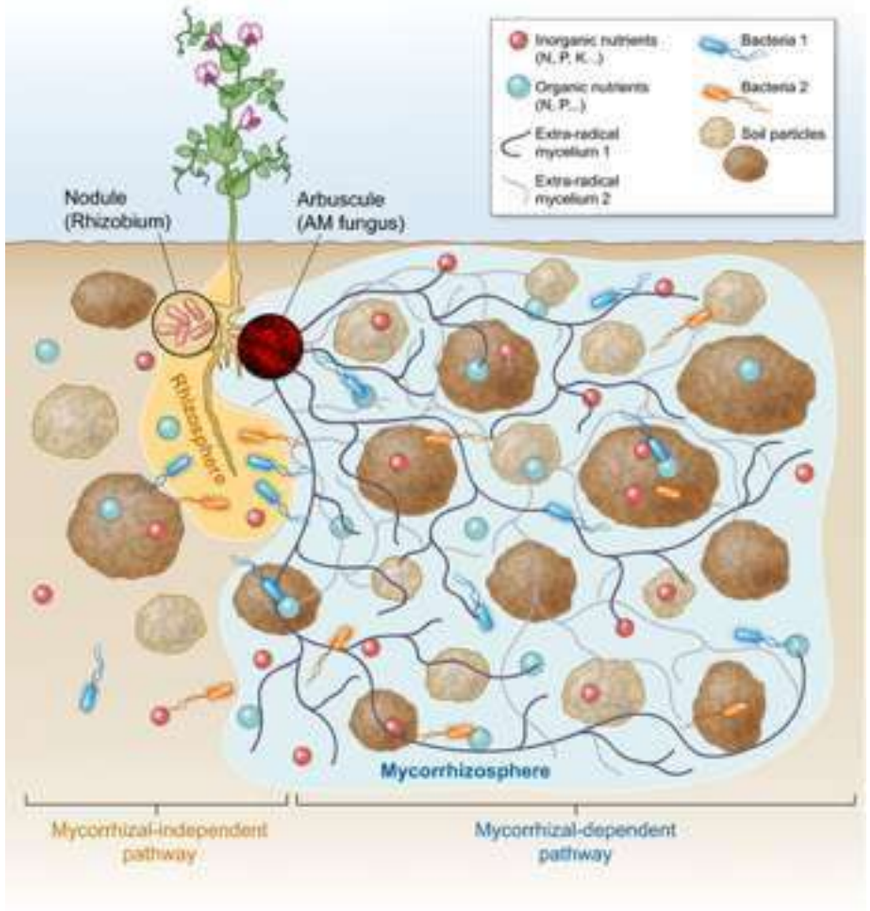

Merino et al. 2019



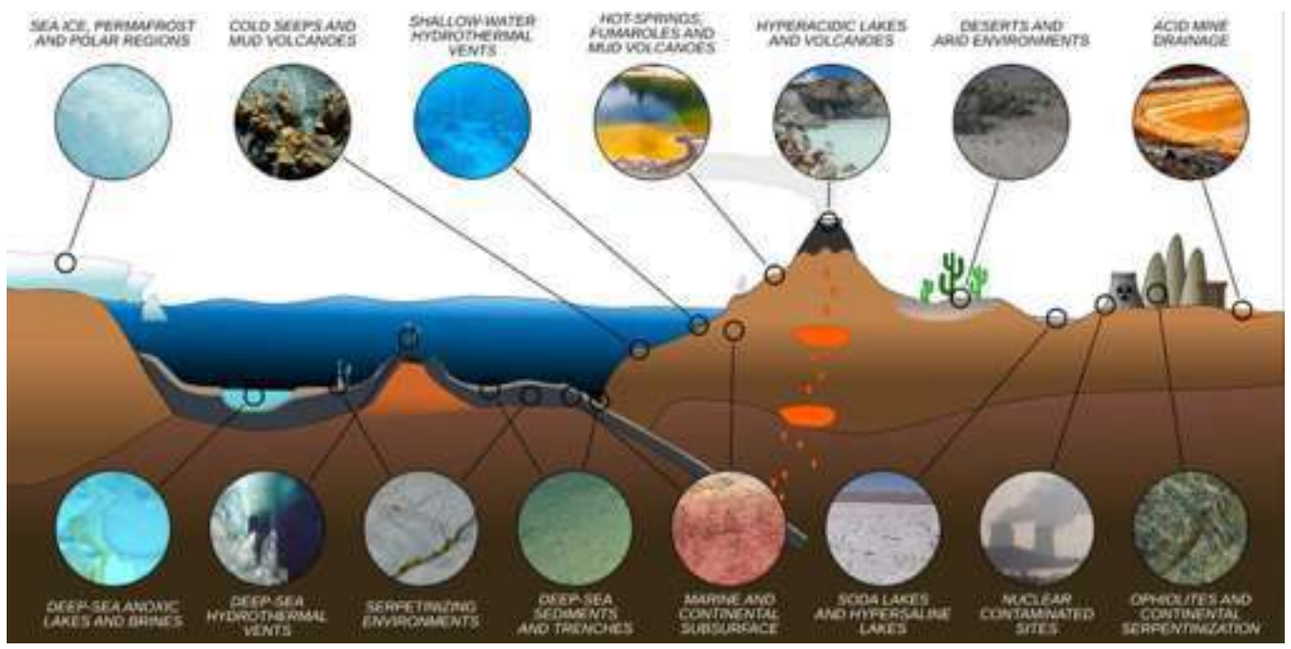

Madigan et al. 2020

# Microbial environments, I

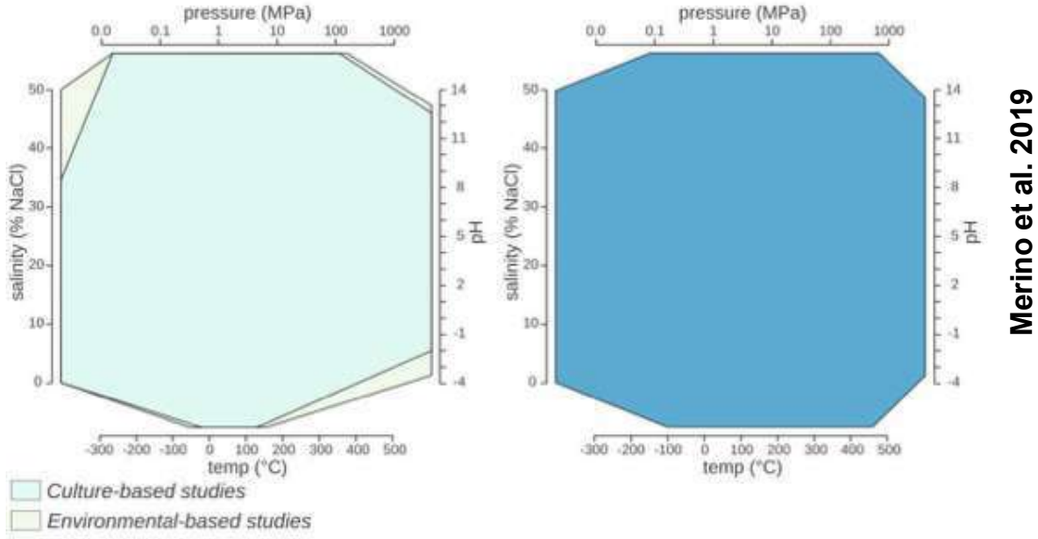
- Temperature
- pH  Light/
- Dark
- Humidity
- Pressure
- Radiations (not on Earth)
- Viscosity (low Reynolds number)




Wipf et al. 2019



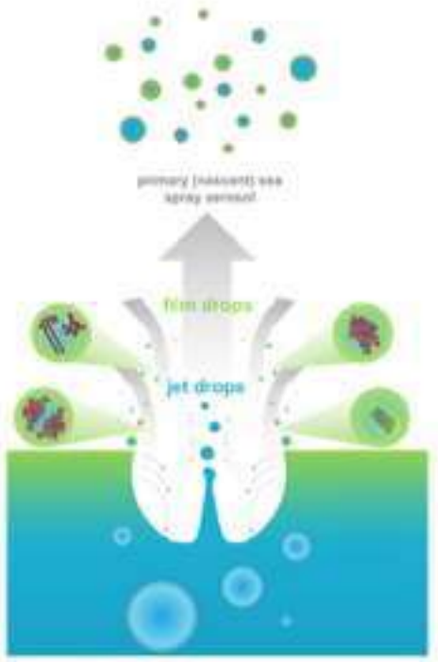

Merino et al. 2019

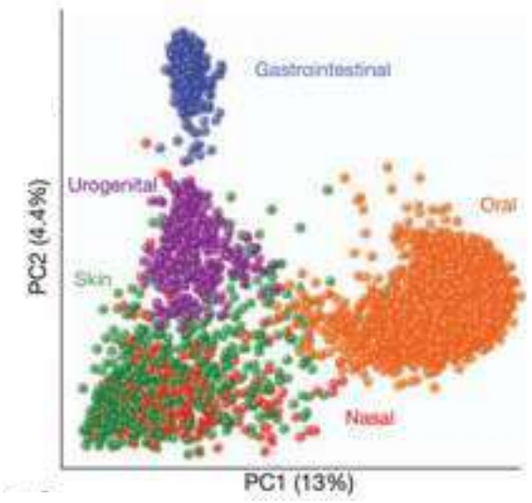



Merino et al. 2019

# Microbial environments, II

https://youtu.be/i-icXZ2tMRM



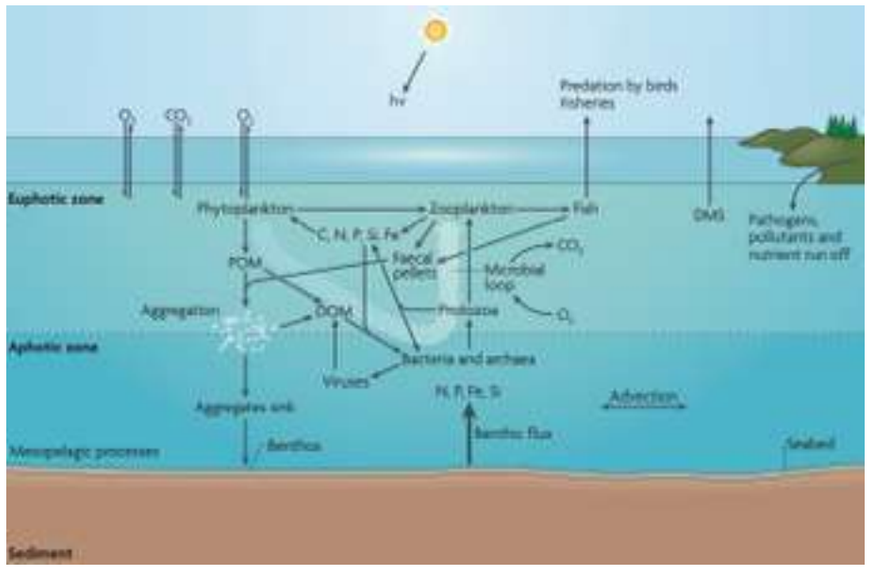

HMPC 2012

- Ionic strength/Salinity
- State of water (vapor, liquid, solid)
- Organic matter concentration
- Oxygen and other redox active molecules
- 3D structure in space and time
- Other microorganisms and their biology
- Humans and their defense

2020 CENTER FOR AEROSOL IMPACTS ON CHEMISTRY OF THE ENVIRONMENT

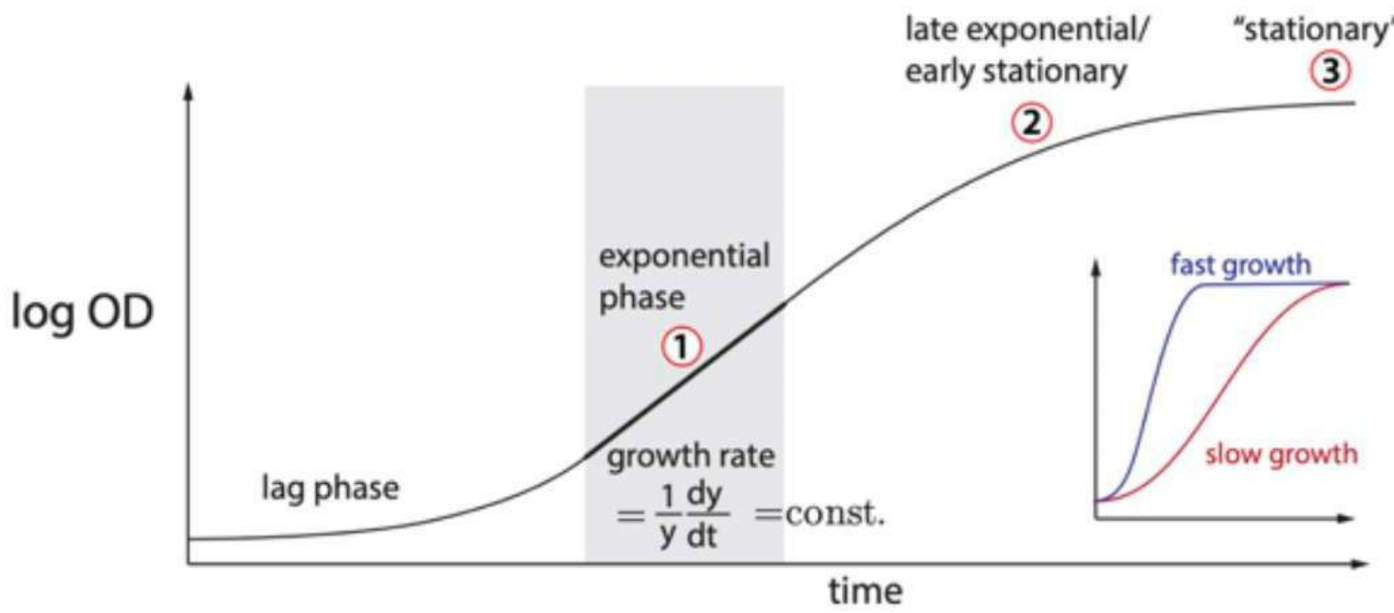
Azam & Malfatti 2007

# Specific adaptation to grow in the microenvironment



# 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**

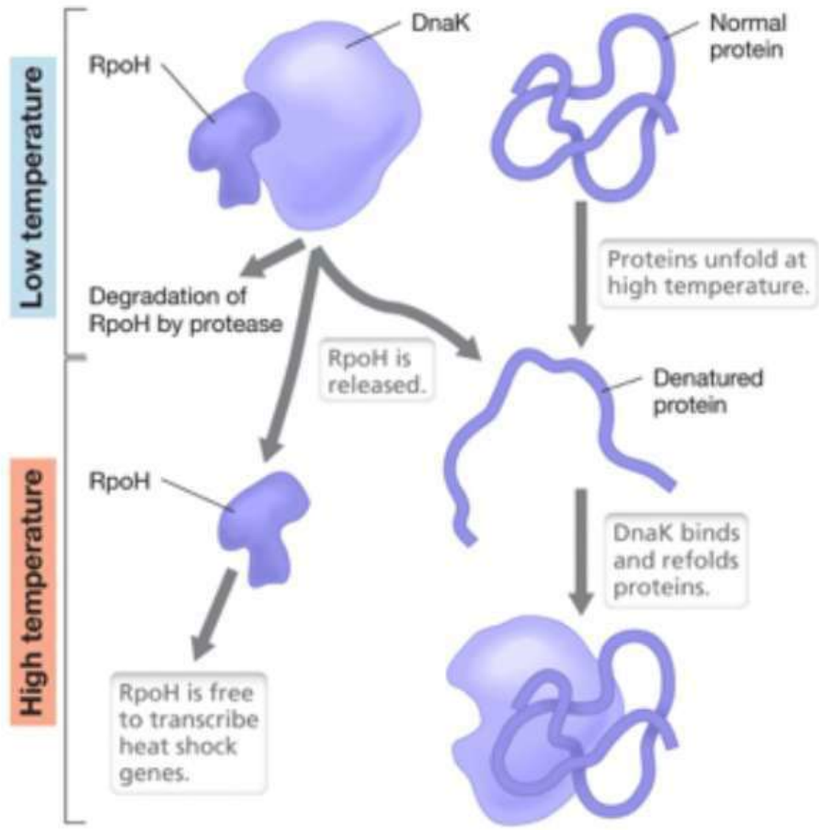
**TABLE 4.3 Sigma factors in *Escherichia coli***

| 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 |

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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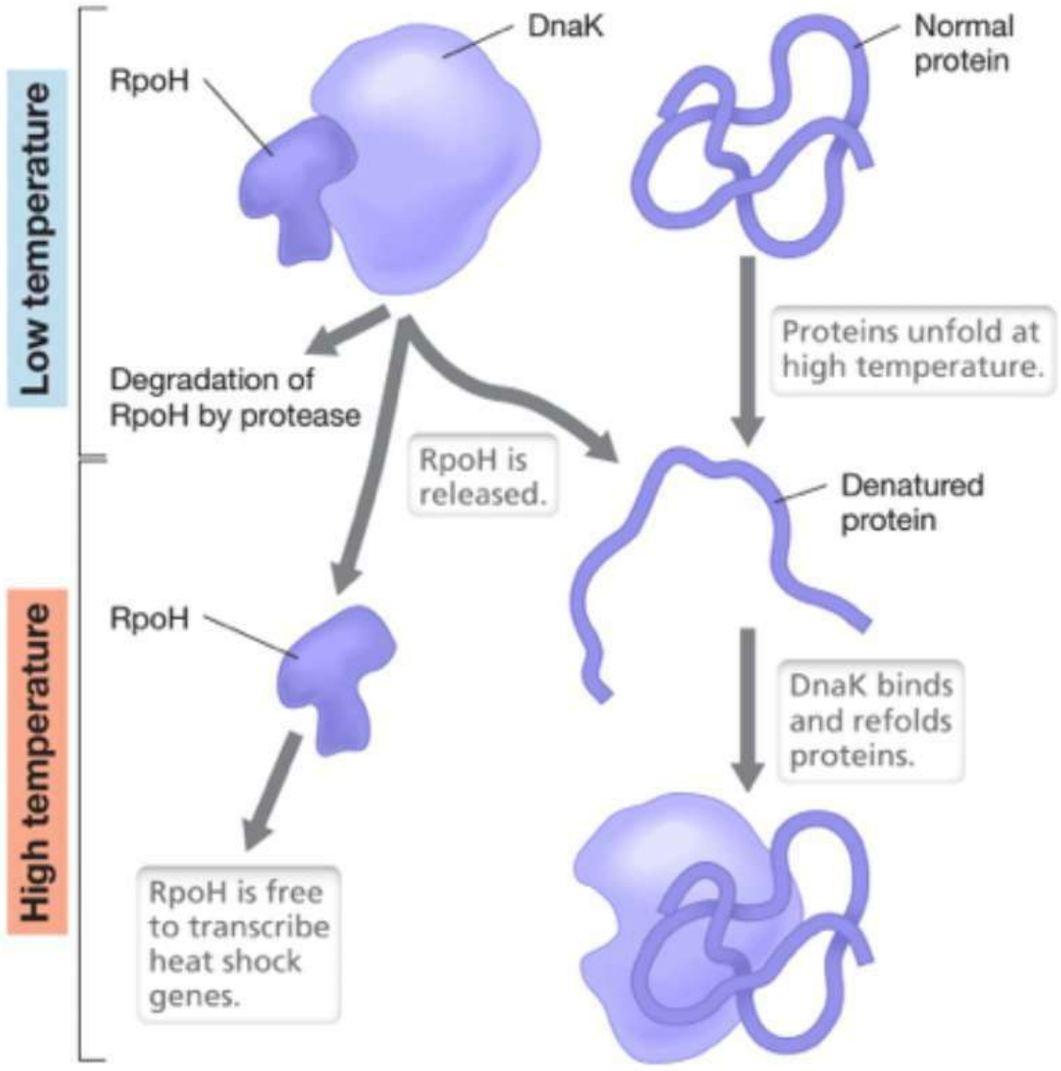
# Heat Shock Proteins, II

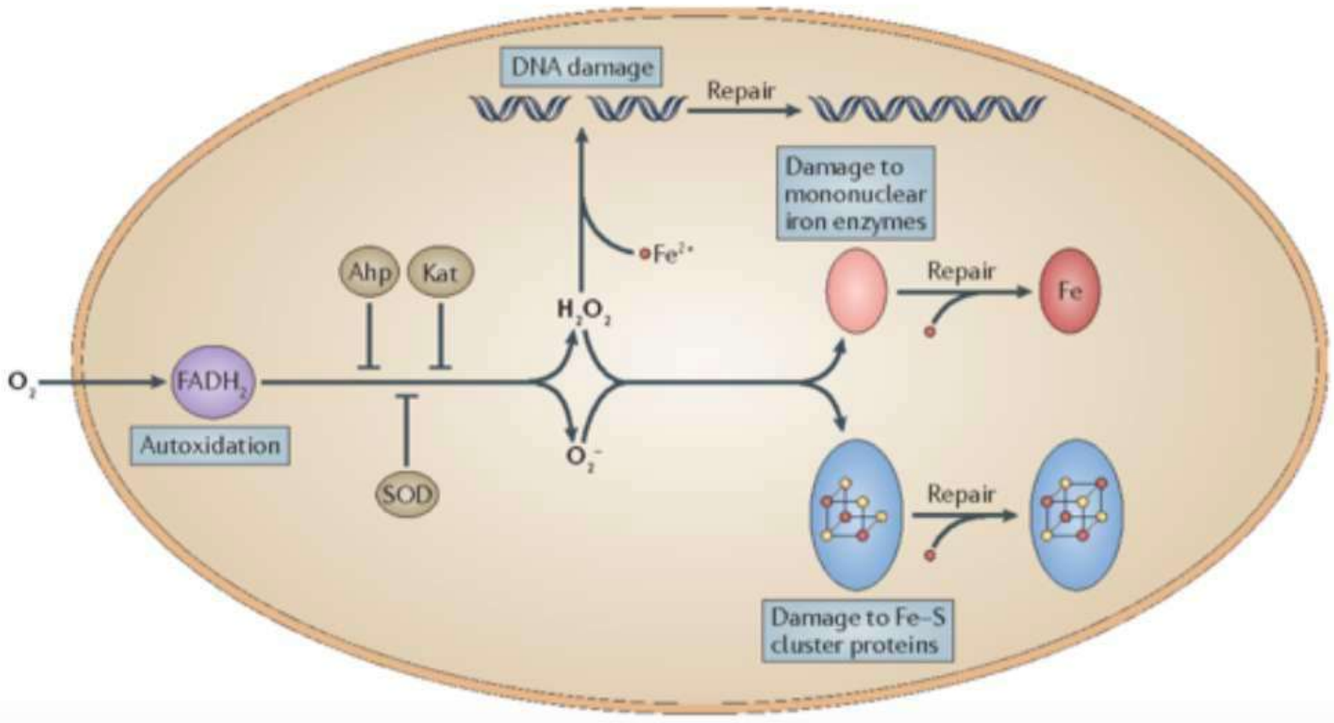
- In *E. coli*, the **heat shock response** is controlled by the **alternative sigma factor RpoH** (s32)
- RpoH controls expression of heat shock proteins, is **normally degraded** within a minute or two of synthesis
- When cells suffer a **heat shock**, **degradation of RpoH is inhibited** —> level >>
- 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




Madigan et al. 2020

# Oxidative Stress

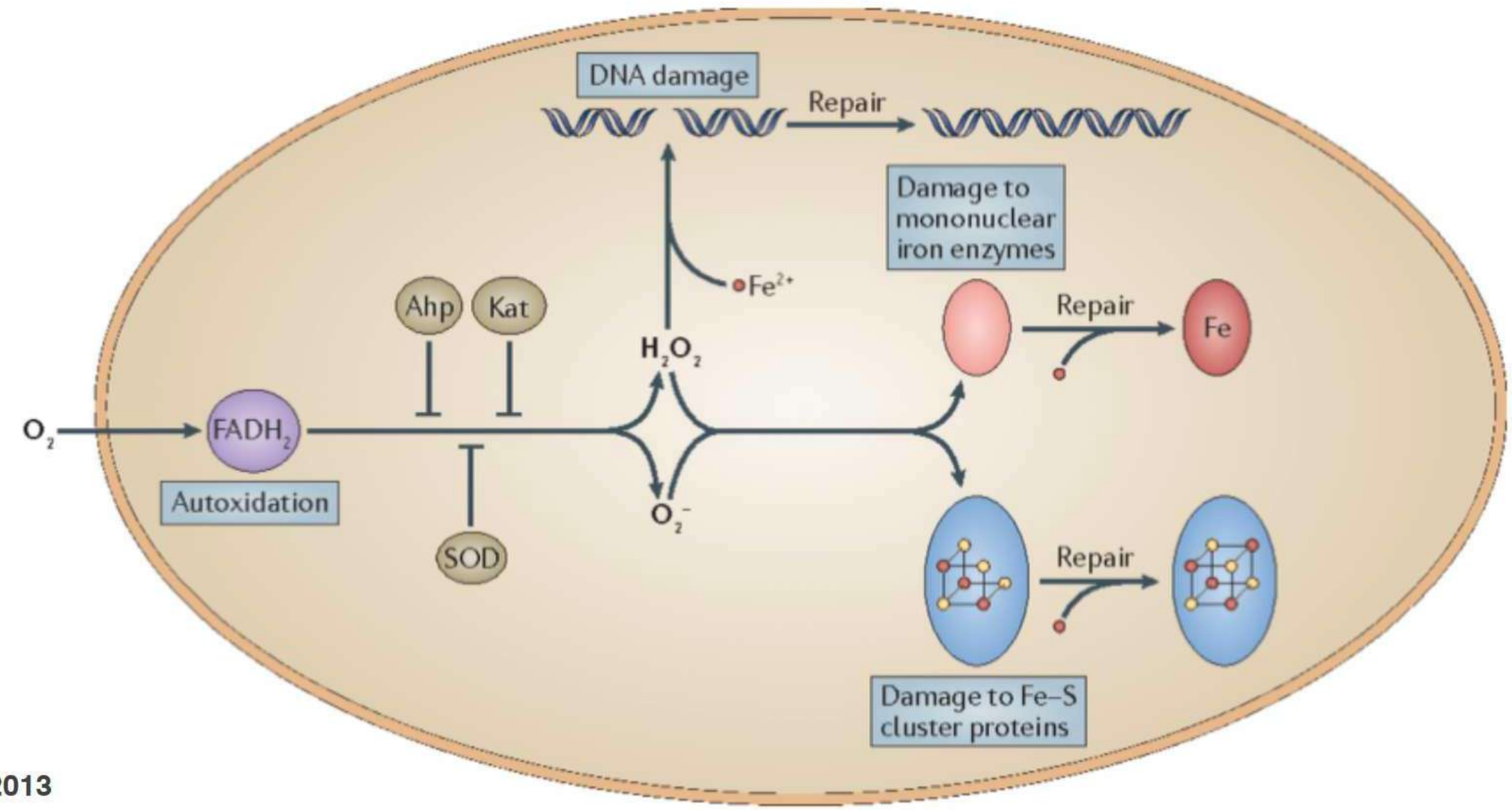
- **Oxidative damage** can have a devastating effect on the **structure and activity of proteins** (covalent modification), including DNA, membrane lipids
- The **sulfur-containing amino acids** cysteine and methionine are particularly **susceptible** to reactive oxygen species (ROS) and reactive chlorine 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

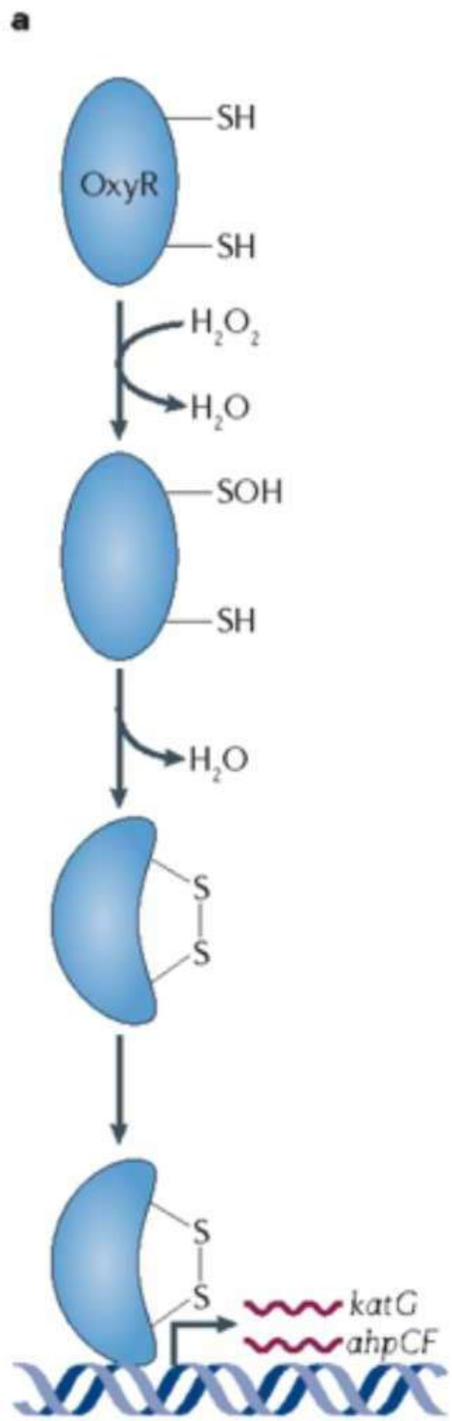
- Production of **catalases** (Kats), **peroxidases** (Ahp) and **superoxide dismutases** (SOD), which are enzymes that react with harmful oxidants and convert them to harmless products by neutralizing them before they cause damage to cellular components




**Ilmay, 2013**

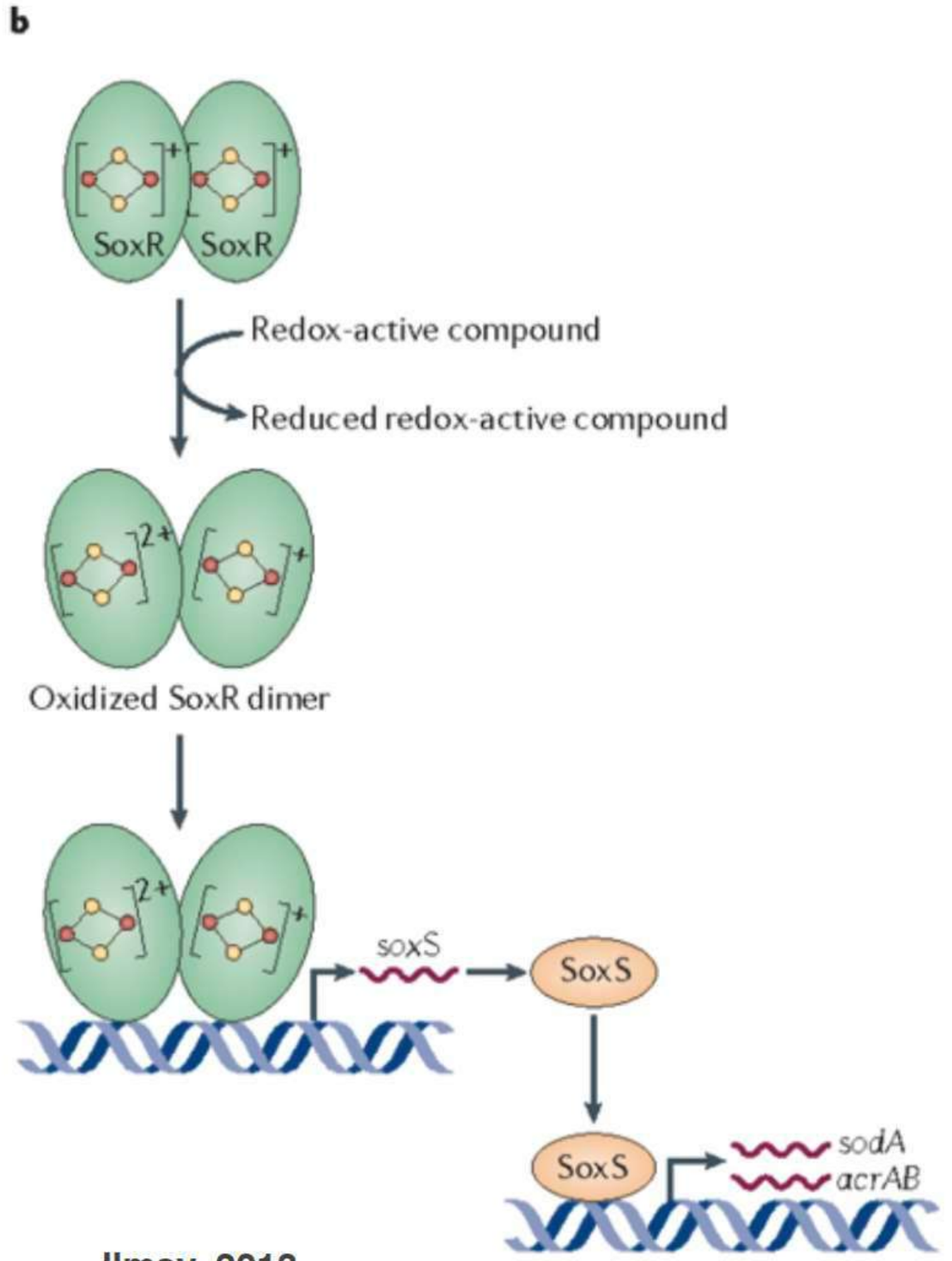
# Oxidative Stress- response I

- 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)**

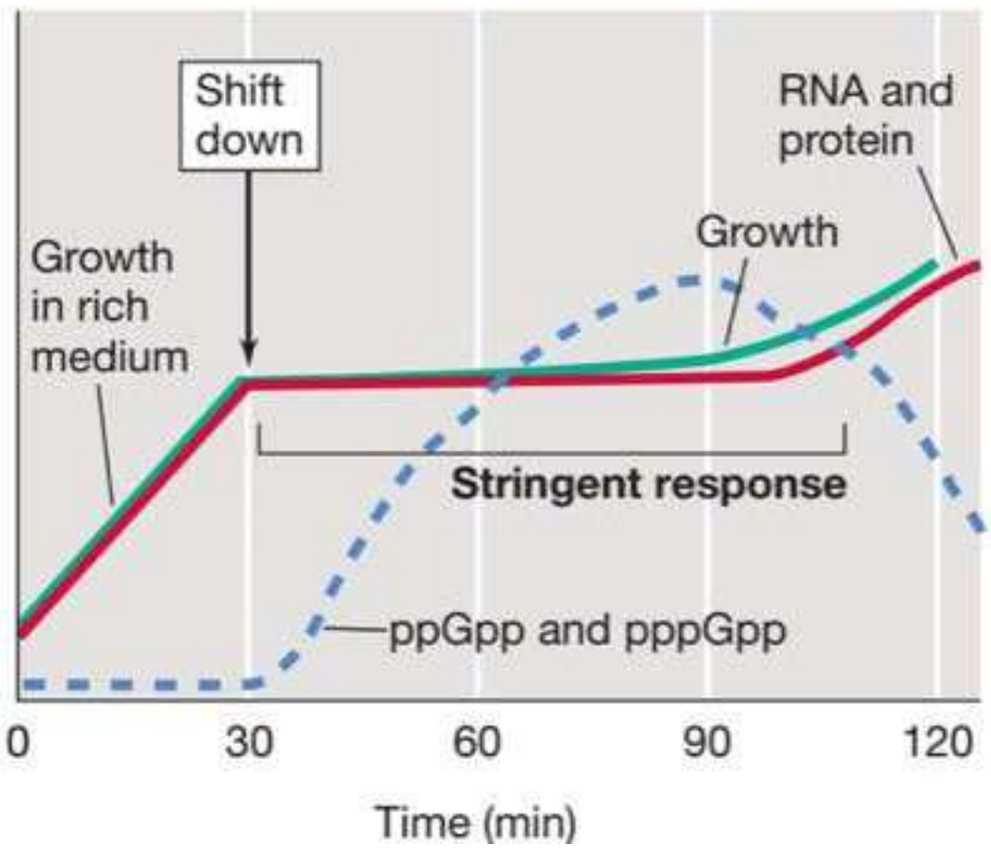
- Stringent response triggering leads to a **shutdown of macromolecule synthesis and activation of stress survival pathways** to improve the cell's ability to compete in nature

- 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

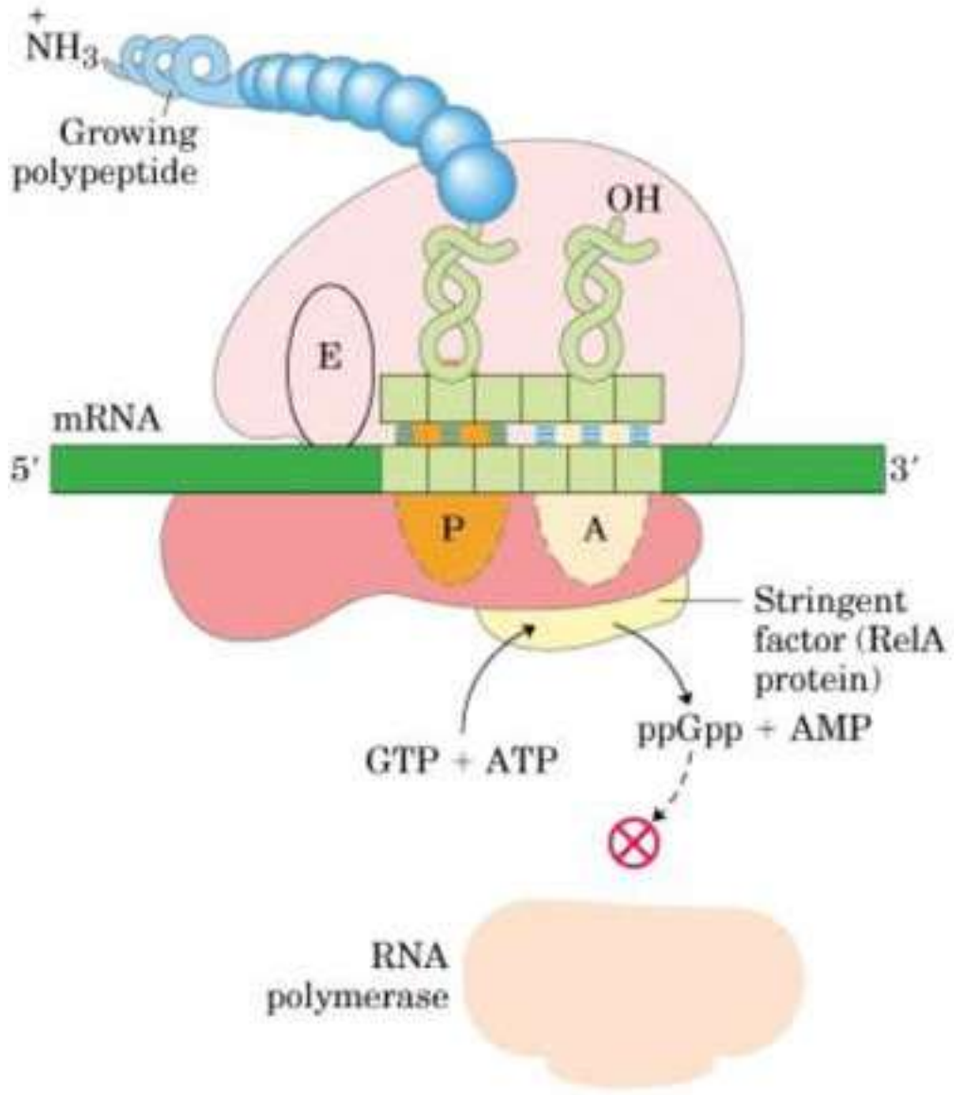
- Amino acid shift down: rRNA, tRNA syntheses cease almost immediately – > no new ribosomes are produced
- Protein and DNA synthesis are also curtailed
- Biosynthesis of **new aa is activated**
- **New proteins** must be made to synthesize amino acids no longer available in thevenvironment **from existing 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




Madigan et al. 2020

# Stringent Response, III

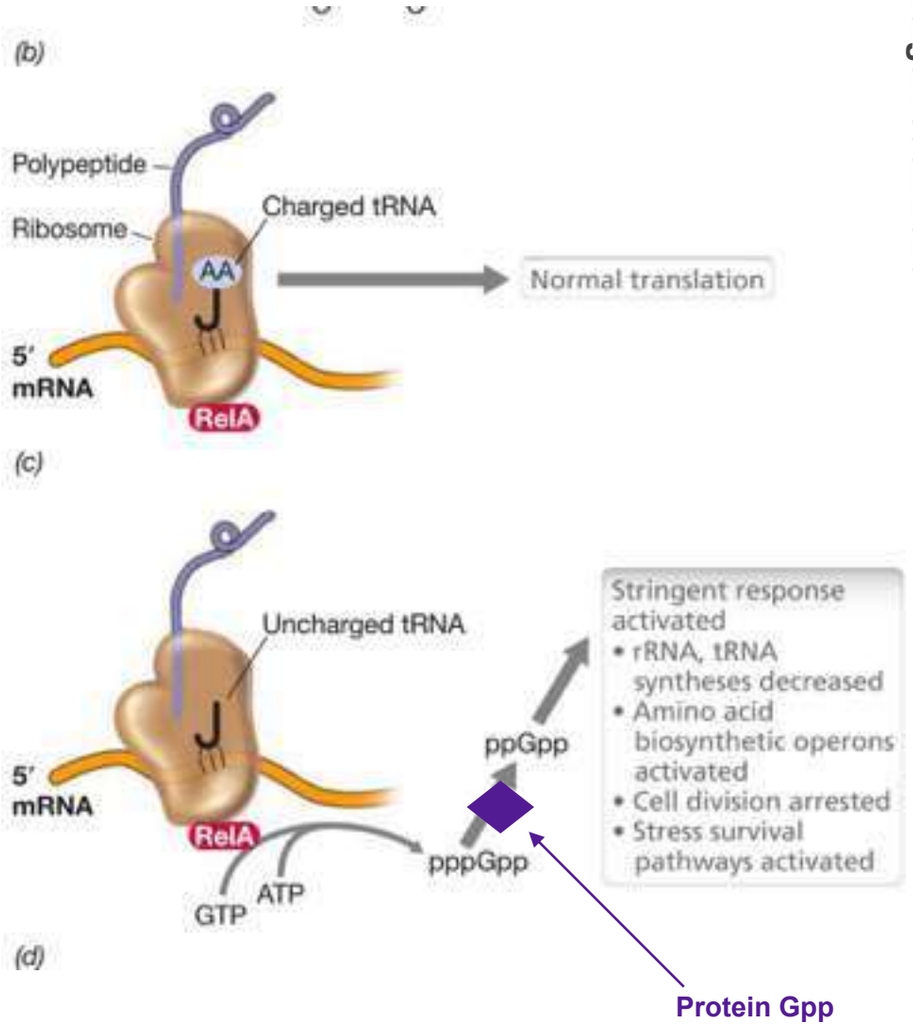
- Stringent response is **triggered by mixture** ((p)ppGpp) of 2 regulatory nucleotides: guanosine tetraphosphate (ppGpp) & guanosine pentaphosphate (pppGpp)
- pppGpp & ppGpp are **alarmones** (*E.coli*) rapidly accumulate **during stress or shift down** (aa starvation)
- Alarmones are **synthesized 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

# 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:**

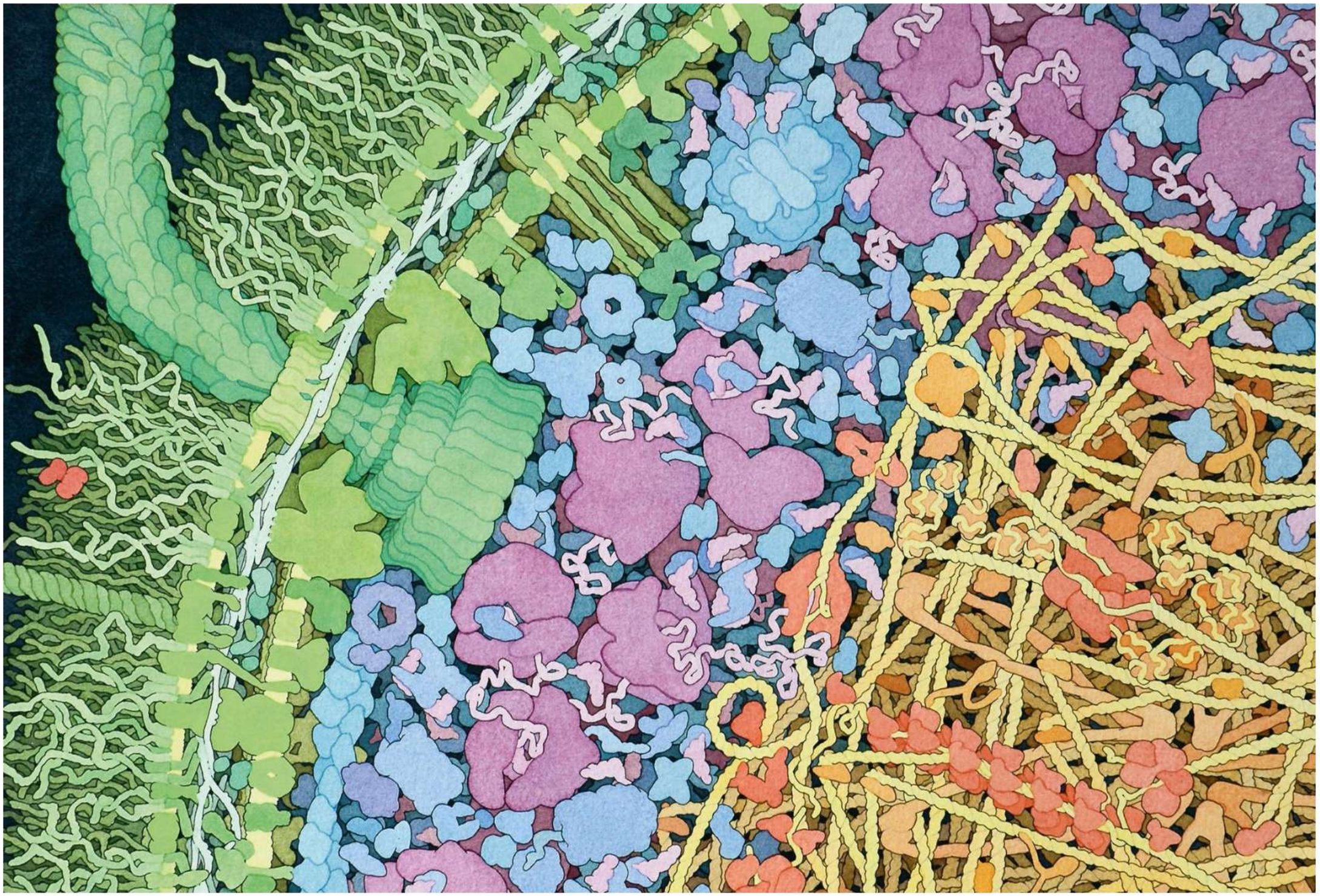
**(1) very important**

**(2) possible in many different ways**

**(3) a major genetic investment**

**(4) allow strategies for conserve resources and maximize progeny**

# Surviving…navigating in the microenvironment



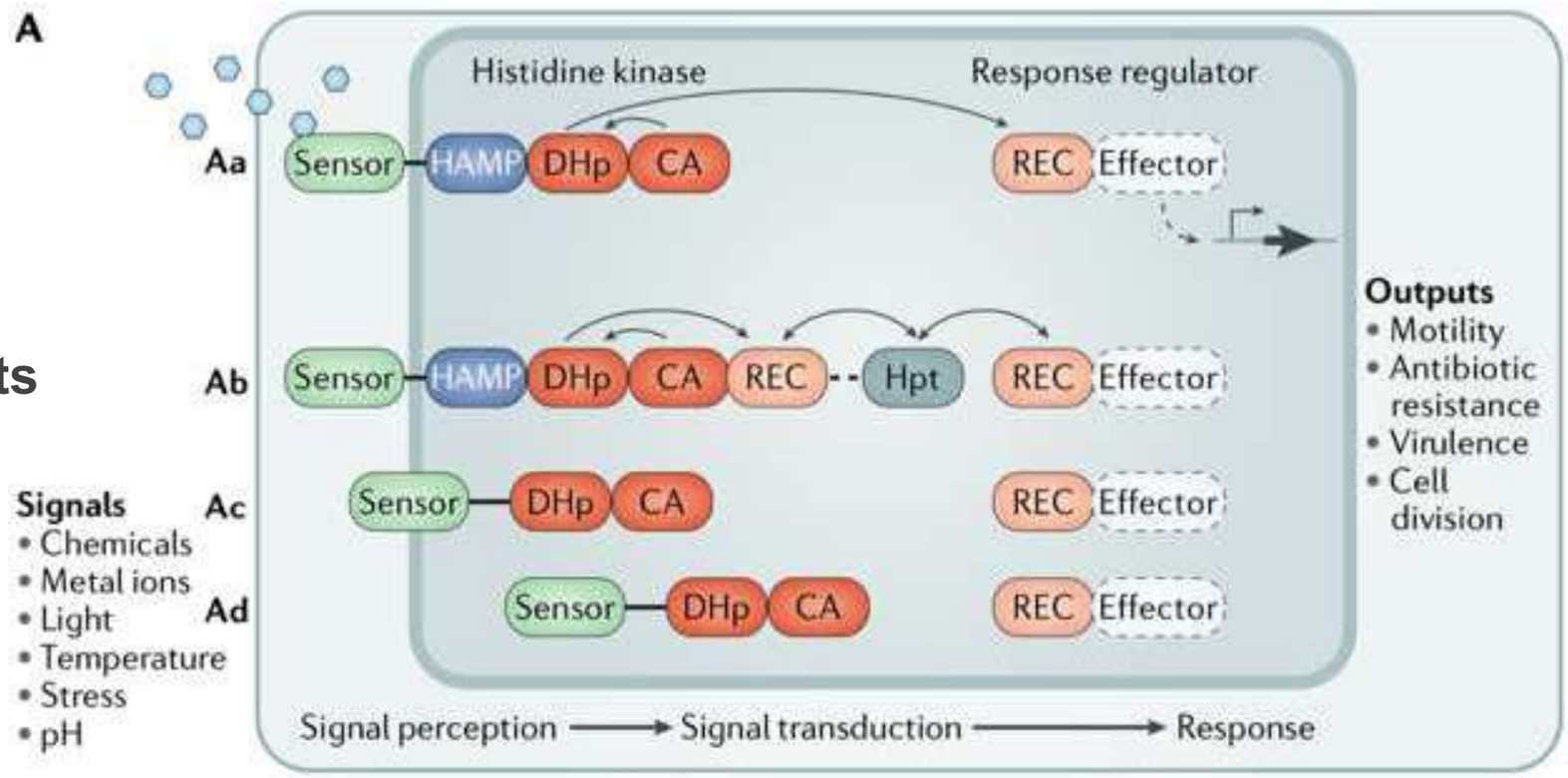
David S. Goodsell

# Sensing and Signal Transduction

- 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:**

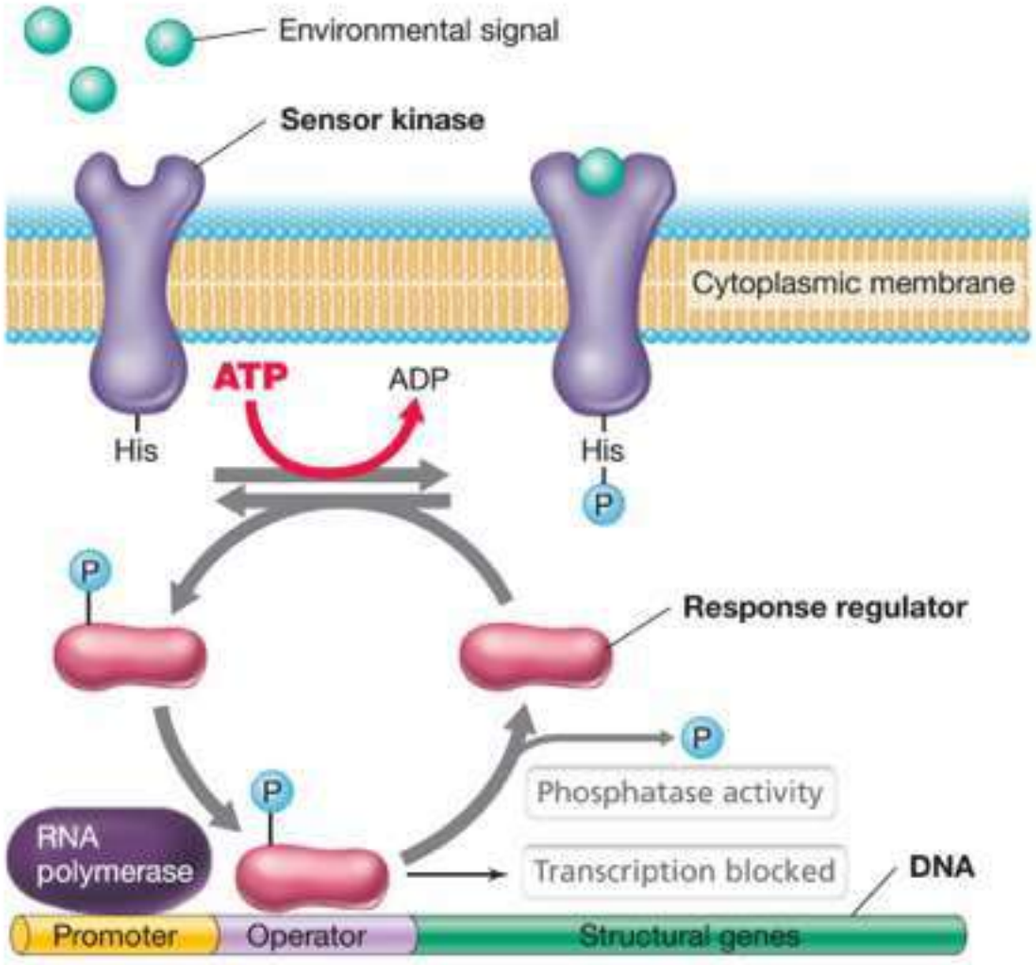
A. **External signal not transmitted directly** to regulatory protein
B. External signal detected by **surface** sensing system
C. Surface sensing system **transmits signal to regulatory machinery**




**Jacob-Dubuisson et al., 2018**

# Sensing and Signal Transduction

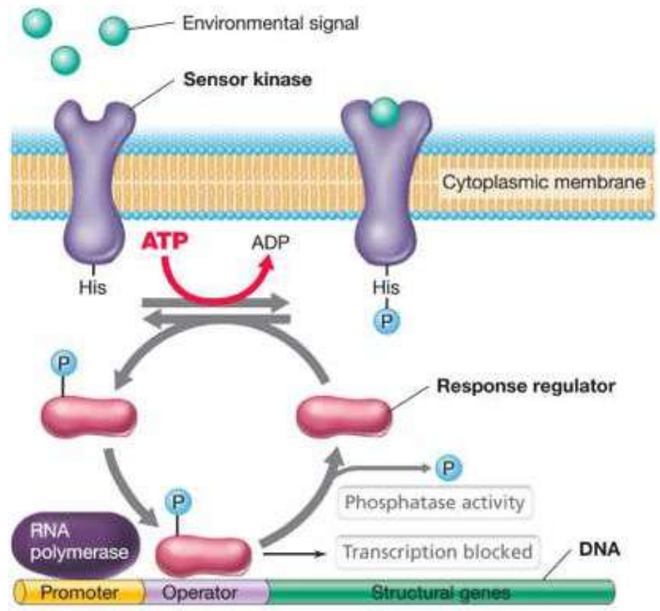
- Signal transduction systems contain two parts, they are called **two-component regulatory systems**
- Specific **sensor kinase** protein usually located in the cytoplasmic membrane, and a **response regulator** protein, present in the cytoplasm
- A kinase is an enzyme that **phosphorylates** compounds,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

- 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

**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**

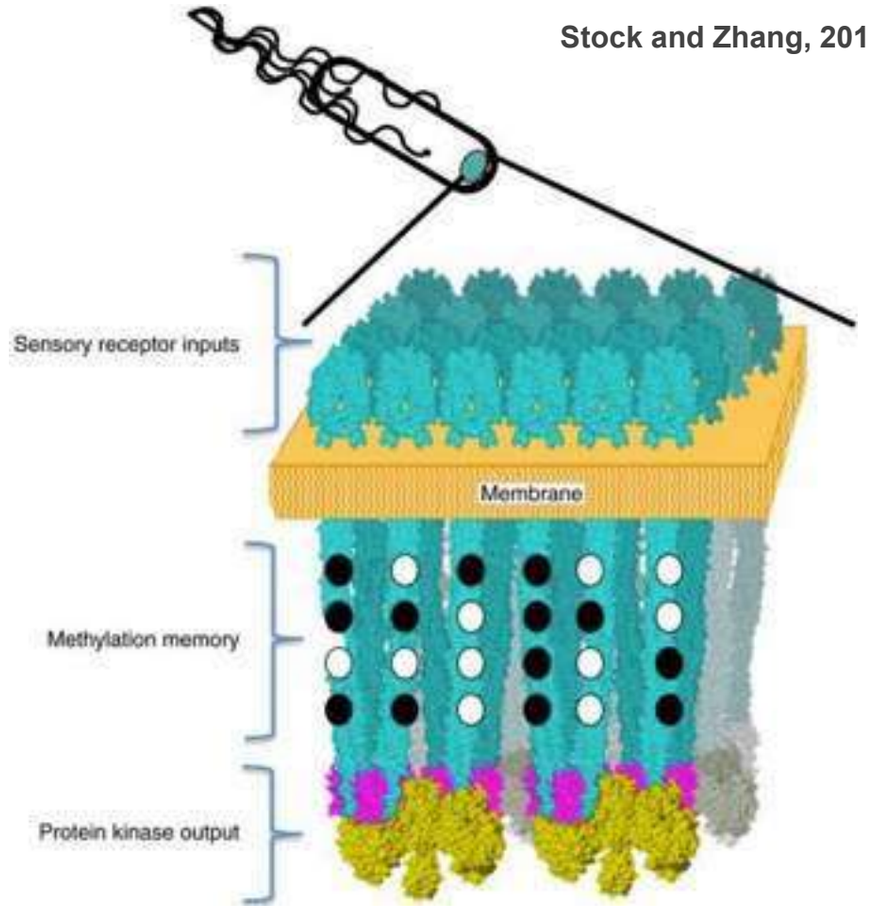
# Chemotaxis, I

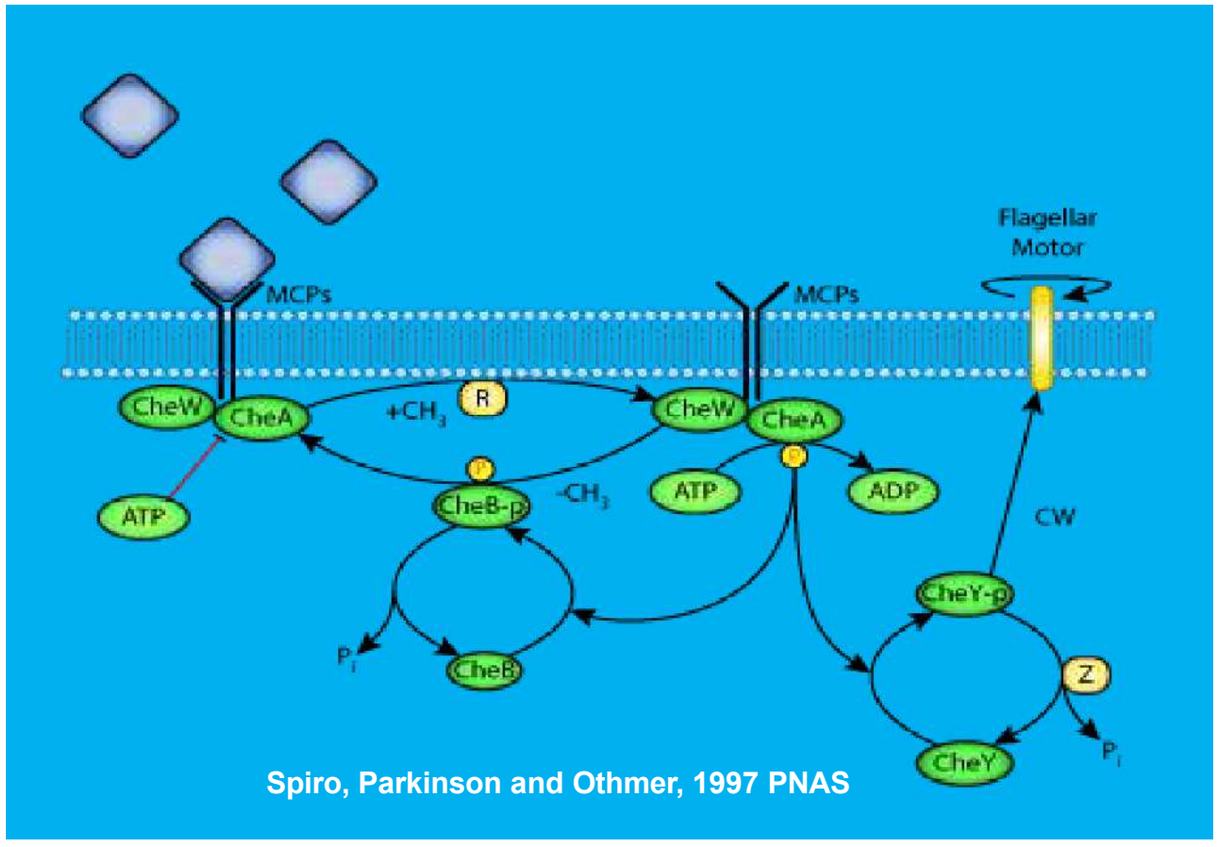
- **Chemokinesis: random movements, in absence of a concentration gradient of chemoattractant**
- **Chemotaxis: directional movement along a + gradient of chemoattractant**

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

Stock and Zhang, 2013



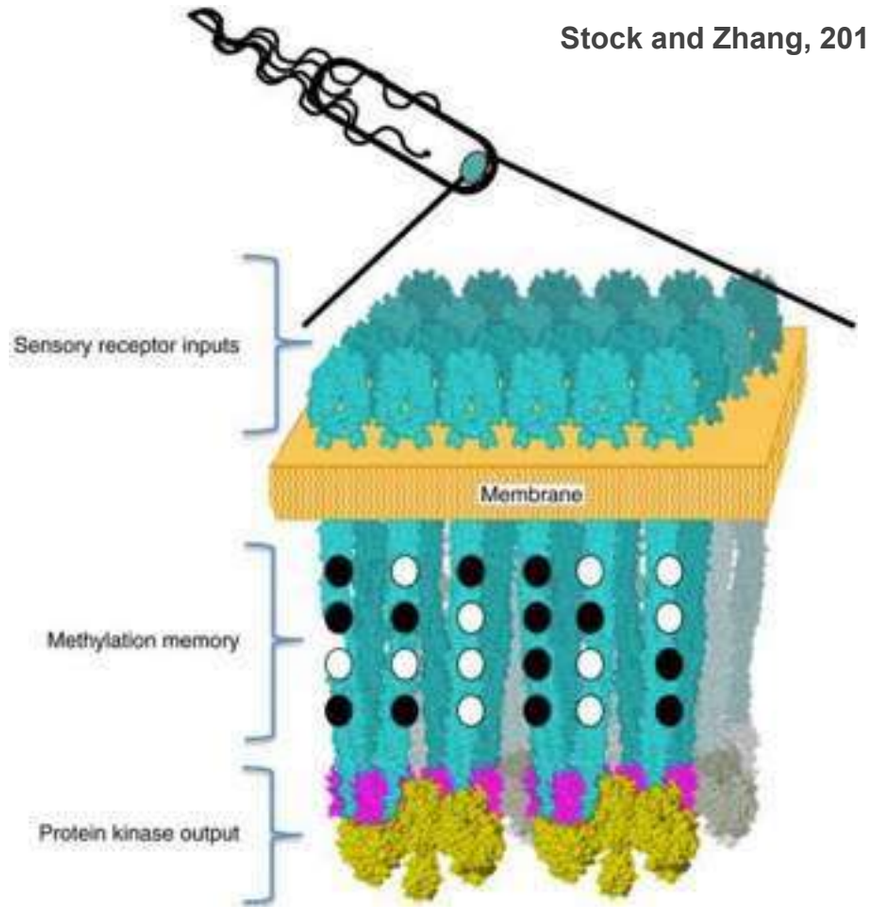



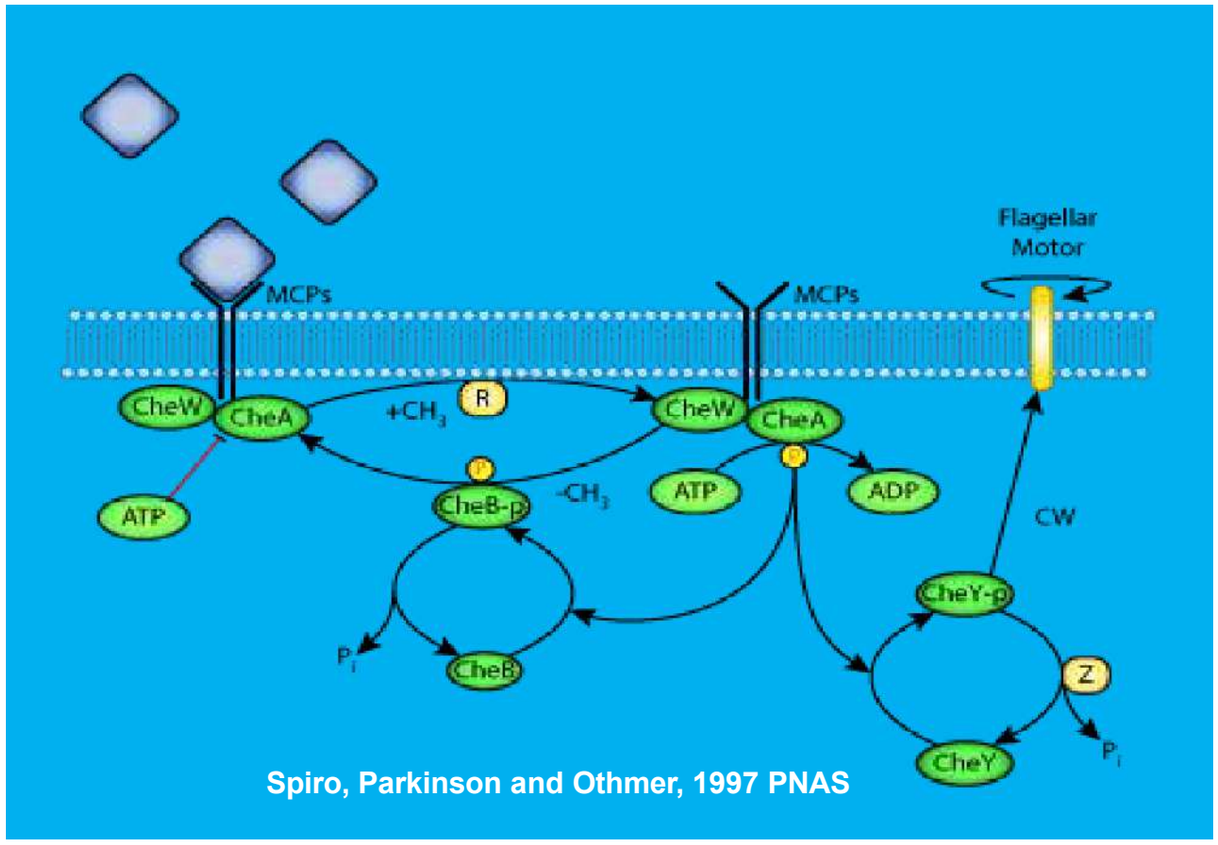

Spiro, Parkinson and Othmer, 1997 PNAS

# Chemotaxis, III

- 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**

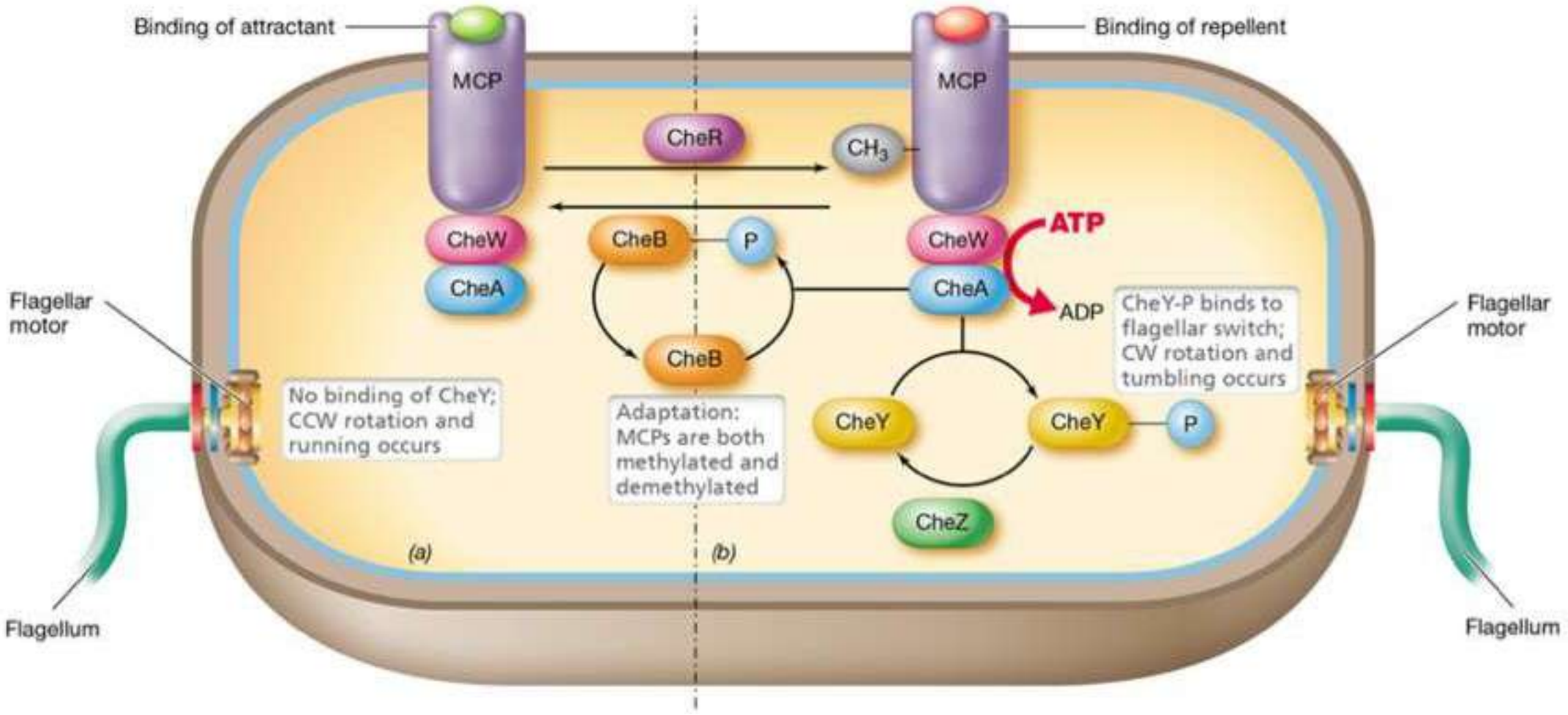
Stock and Zhang, 2013




Spiro, Parkinson and Othmer, 1997 PNAS

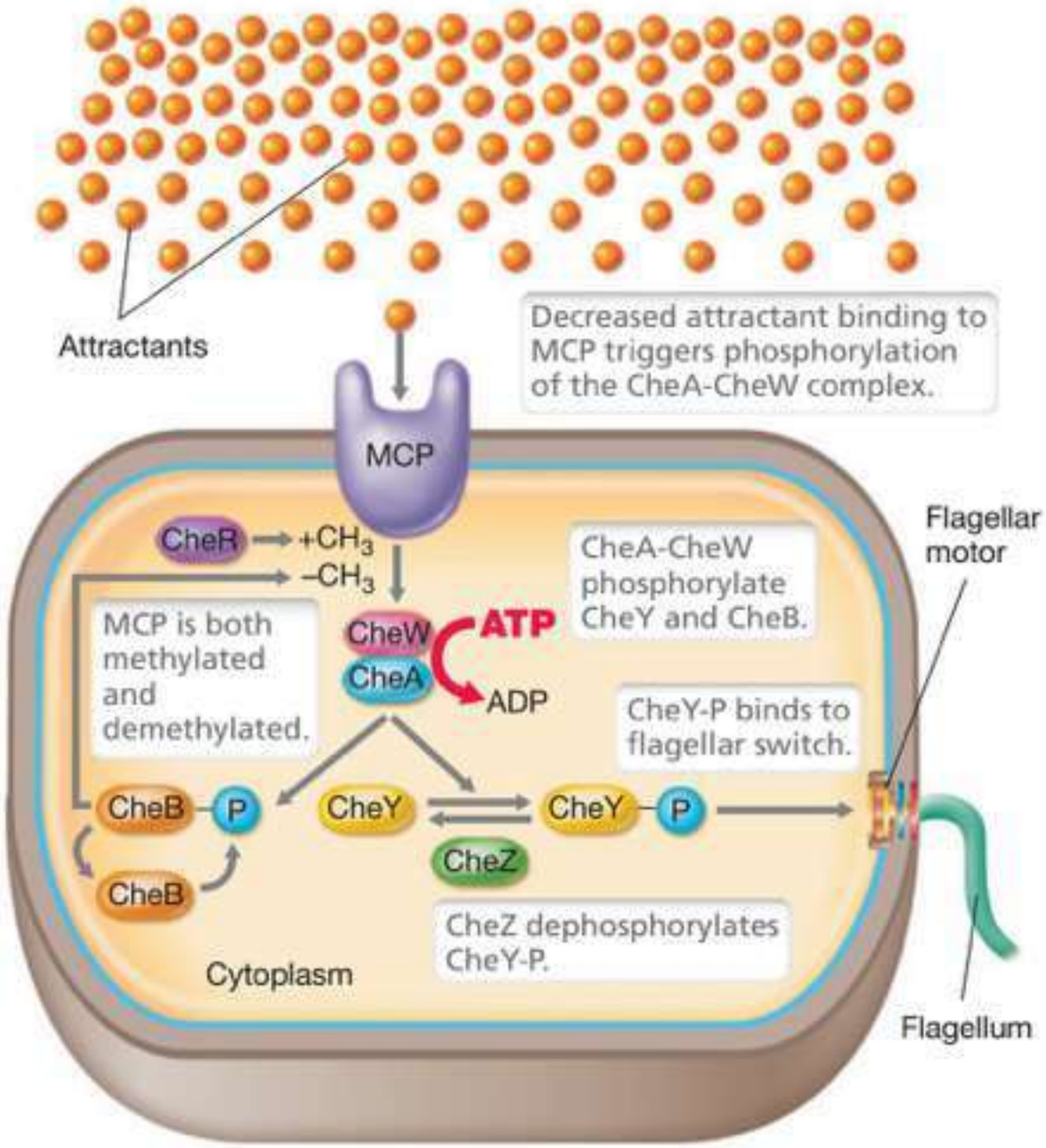
# Chemotaxis architecture




Madigan et al. 2020

# Chemotaxis, IV

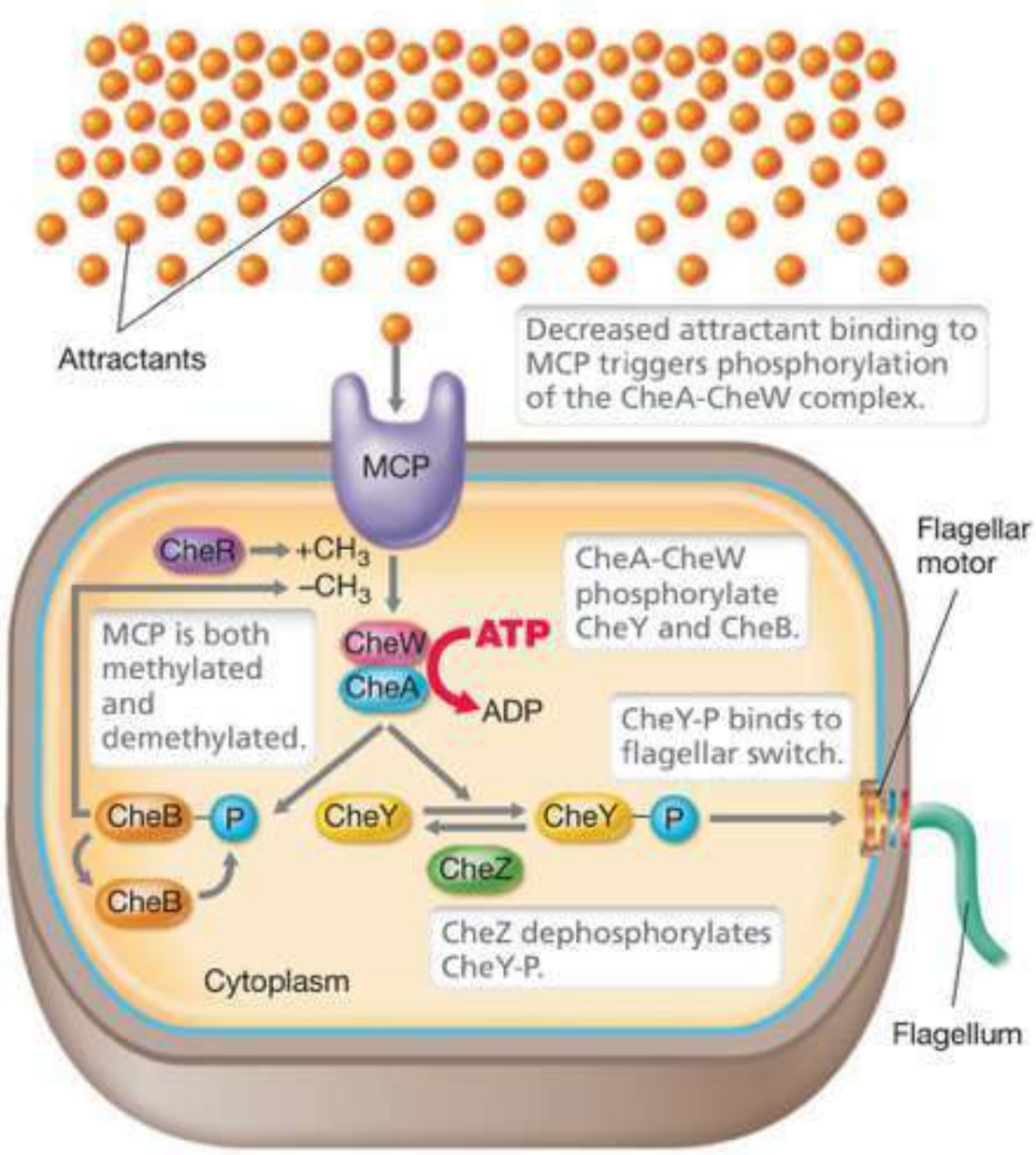
- MCPs (**methyl-accepting chemotaxis proteins)** make contact with the cytoplasmic proteins **CheA and CheW**
- **CheA is the sensor kinase** for chemotaxis
- When MCP binds chemical – > **changes conformation with CheW – > autophosphorylation of CheA —> CheA-P**
- **Increase in attractant** concentration **decreases** the **rate of autophosphorylation**
- **Decrease in attractant / increase in repellent increases** the rate of **autophosphorylation**




Madigan et al. 2020

# 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**




**Madigan et al. 2020**

# Proton motive force or Na motive force

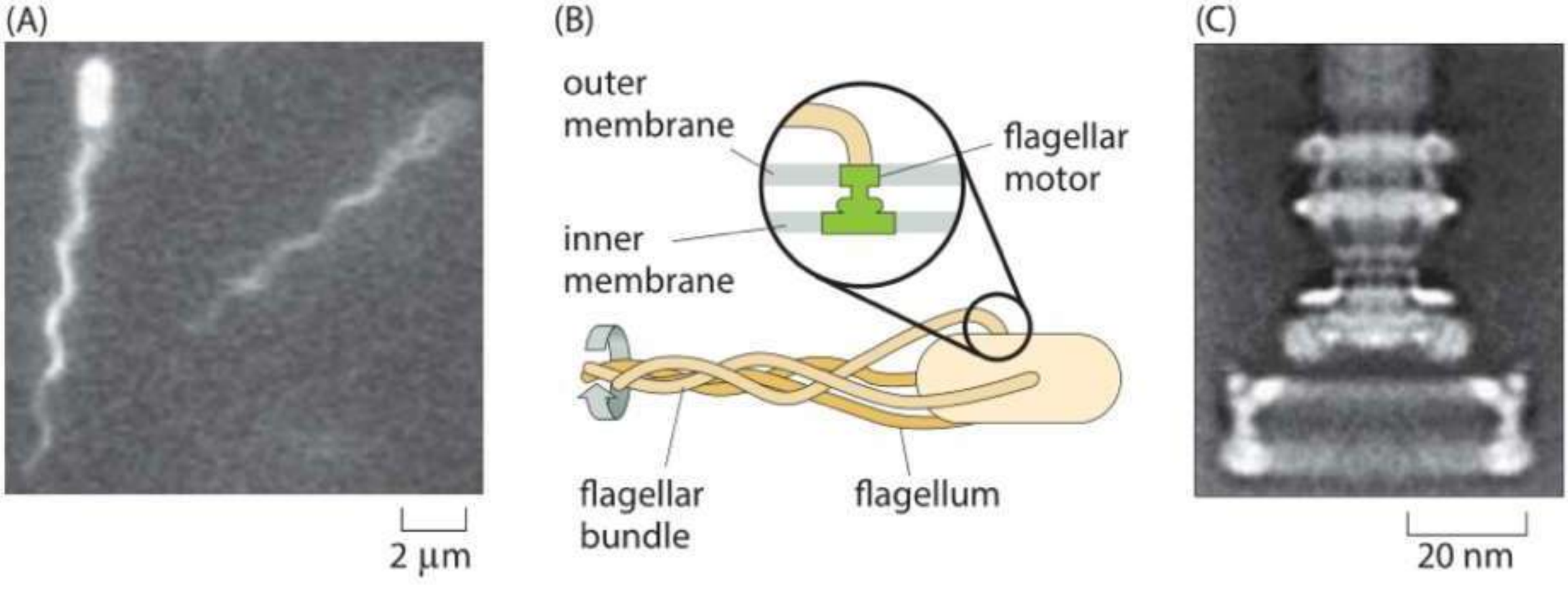
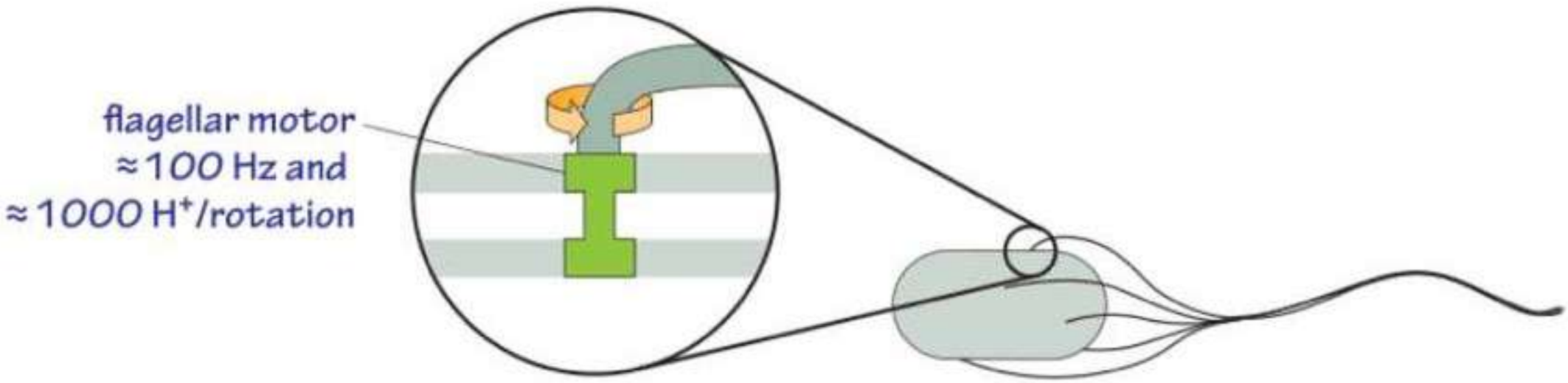



Figure 1: Flagellar-based motility in E. coli. (A) Two E. coli cells and their bundle of fluorescently labeled flagella. (B) Schematic of the bundling of flagella that drives bacterial motility. The inset shows how the rotary motor is embedded in the cell membrane. (C) Electron microscopy image of the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

**biobythenumbers.org**

What is the energy demand for flagella based rotation?




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

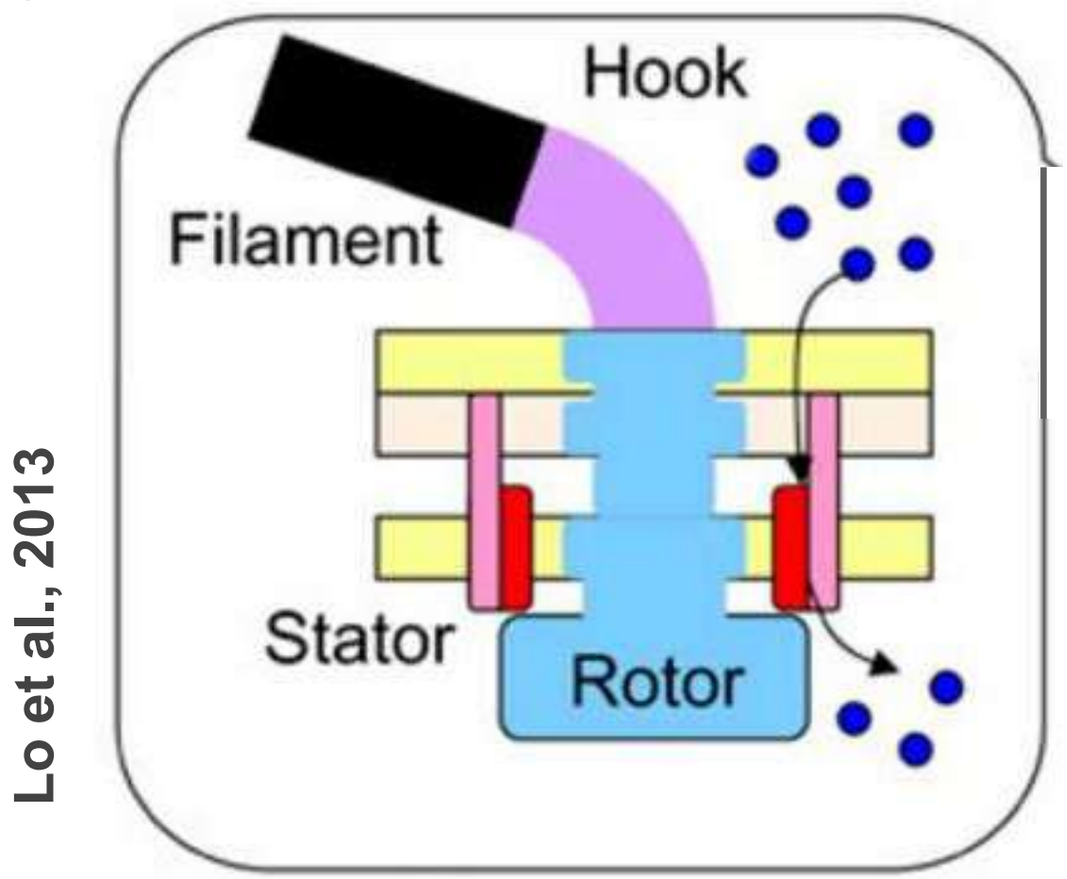
$$\text{proton motive force} \approx 150 \text{ mV} \rightarrow \text{energy per proton} \approx 0.15 \text{ V} \times 1.6 \times 10^{-19} \text{ J/V} \approx 0.2 \times 10^{-19} \text{ J}$$

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

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.

**biobythenumbers.org**

# Bacterial flagellar motor (BFM) is an ion-driven rotary motor

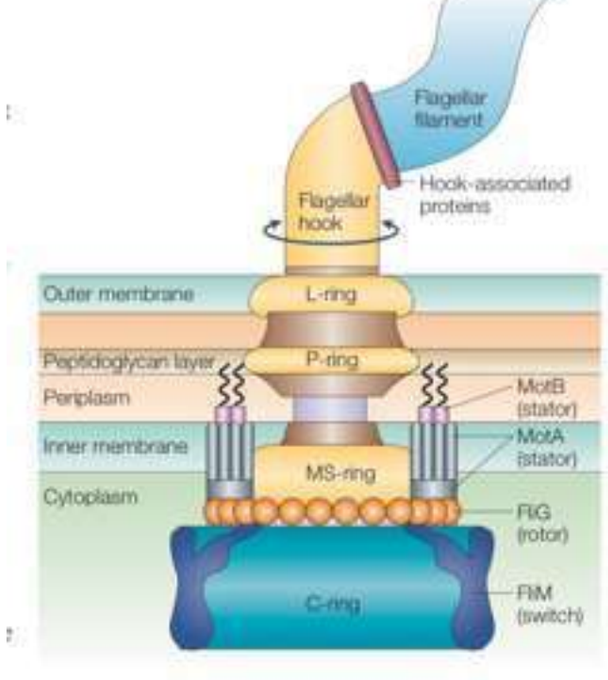



Lo et al., 2013

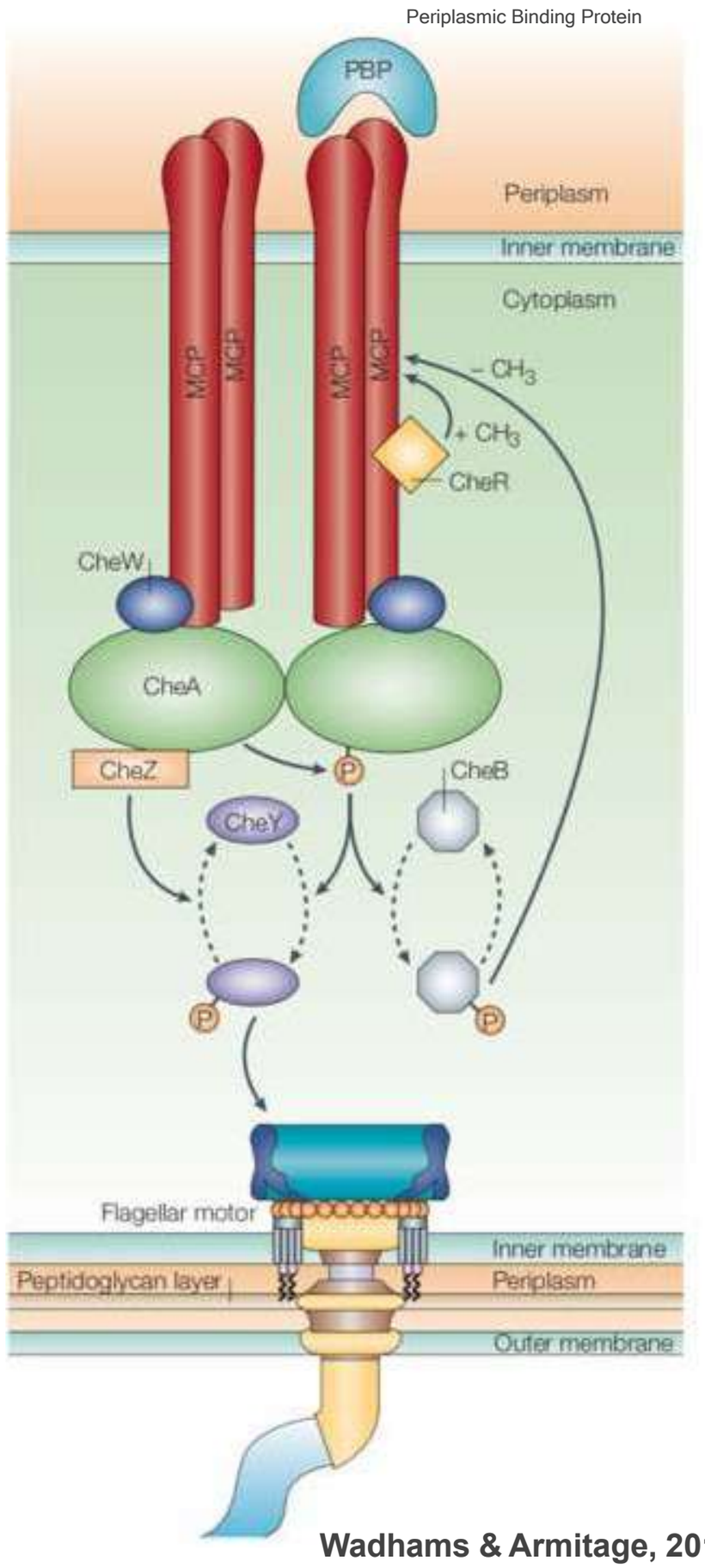
- Ion Motive Force (H+) supports ATP synthesis: forced rotation of F1 part of F1FO ATP-synthase
- F1 is mechanically coupled to and rotated by FO, which like the bacterial flagellar motor (BFM) is an ion-driven rotary motor
- BFM is a rotary molecular machine that propels many species of swimming bacteria.
- Rotation of extracellular helical flagellar filaments at hundreds of revolutions per second (Hz)
- Torque is generated by interactions between stator complexes (containing the proteins MotA and MotB in E. coli) and the rotor protein FliG

# 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

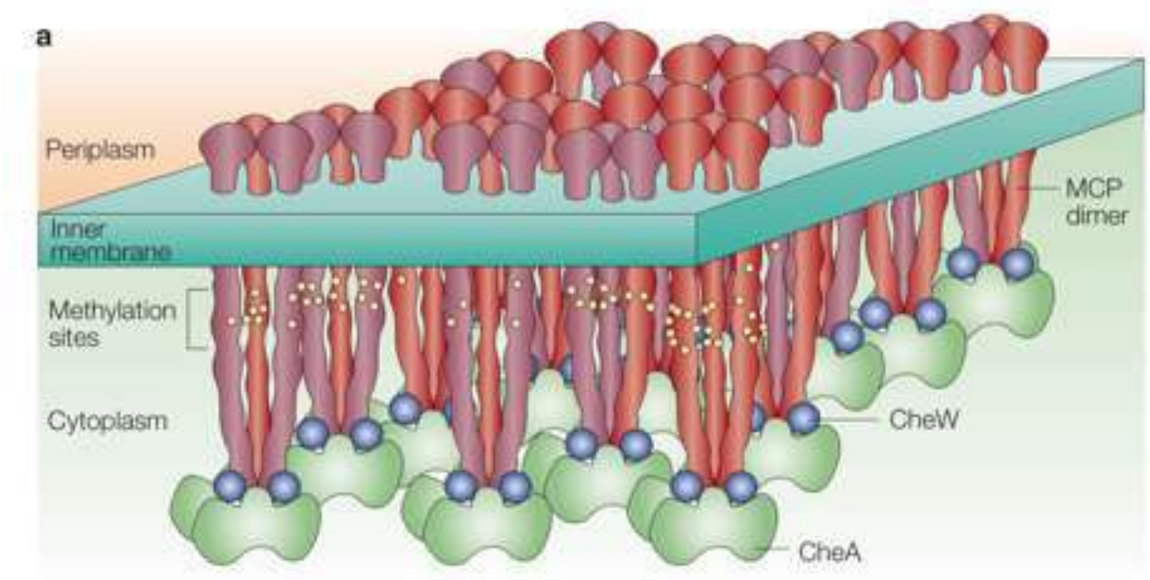



Wadhams & Armitage, 2014

# 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**

- 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 the level of attractant remains high but constant–> tumble & CheB –> CheB-P demethylate MCPs

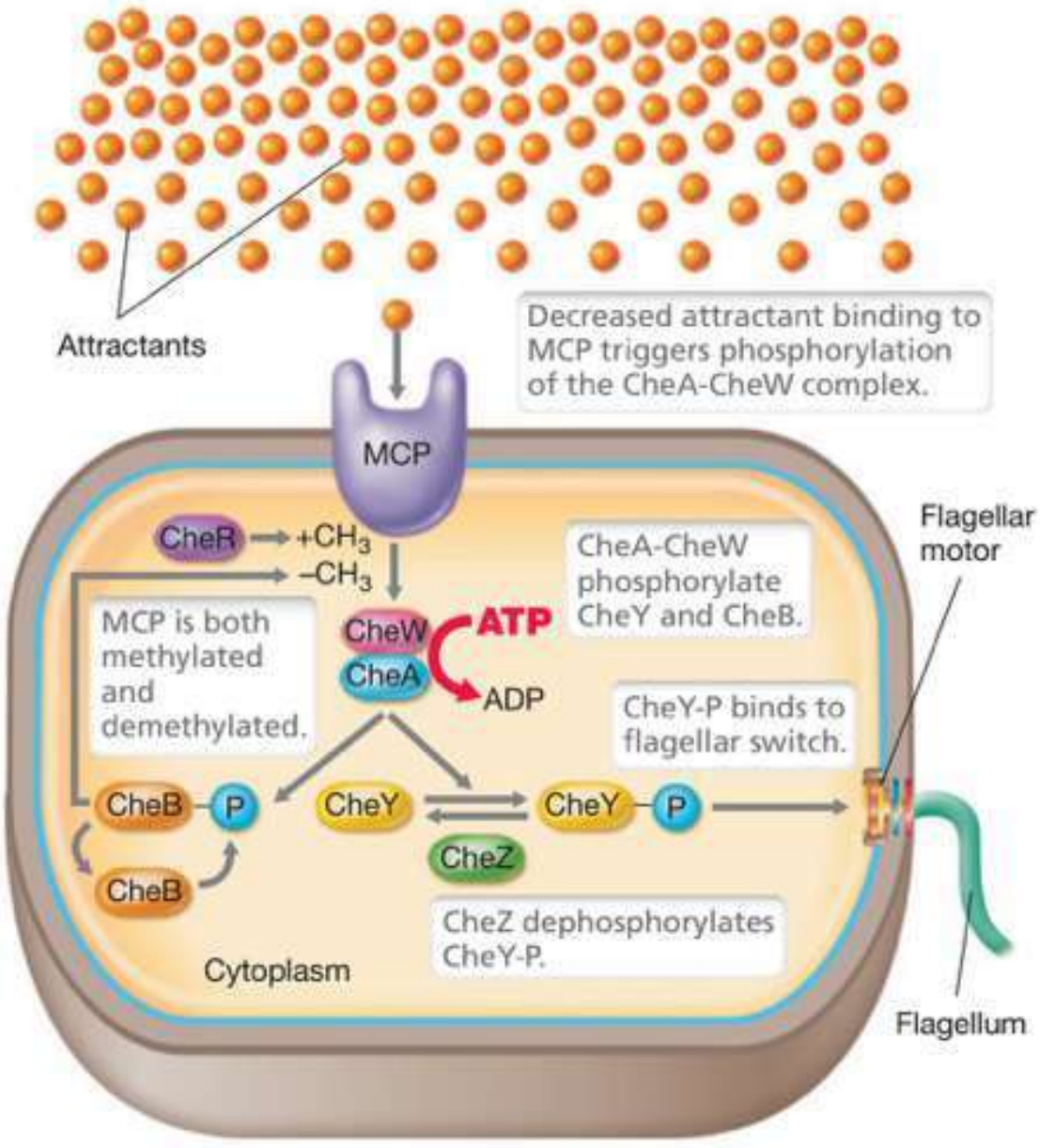



**Wadhams & Armitage, 2014**

# 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**




Madigan et al. 2020

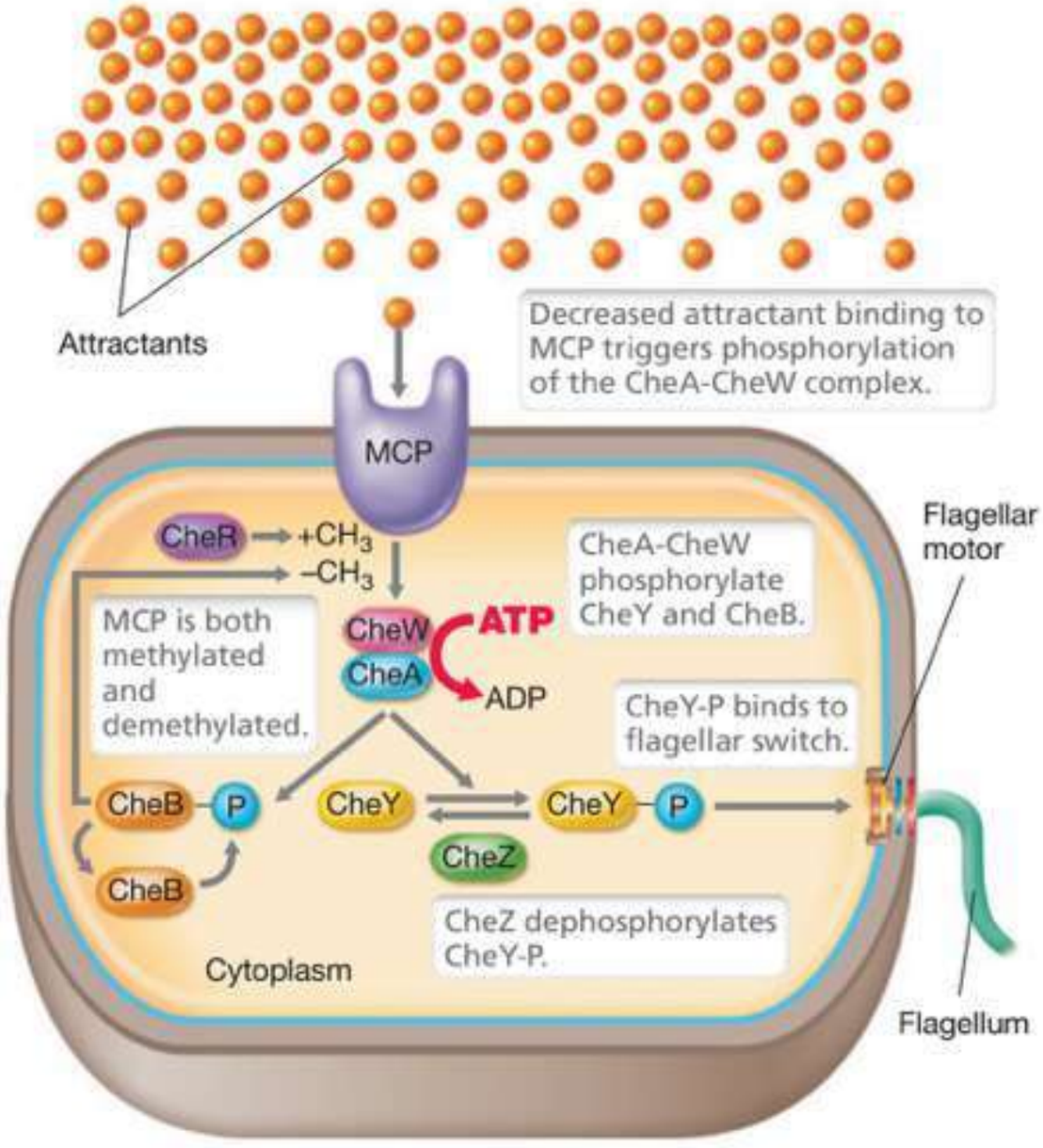
# Chemotaxis, IX

- **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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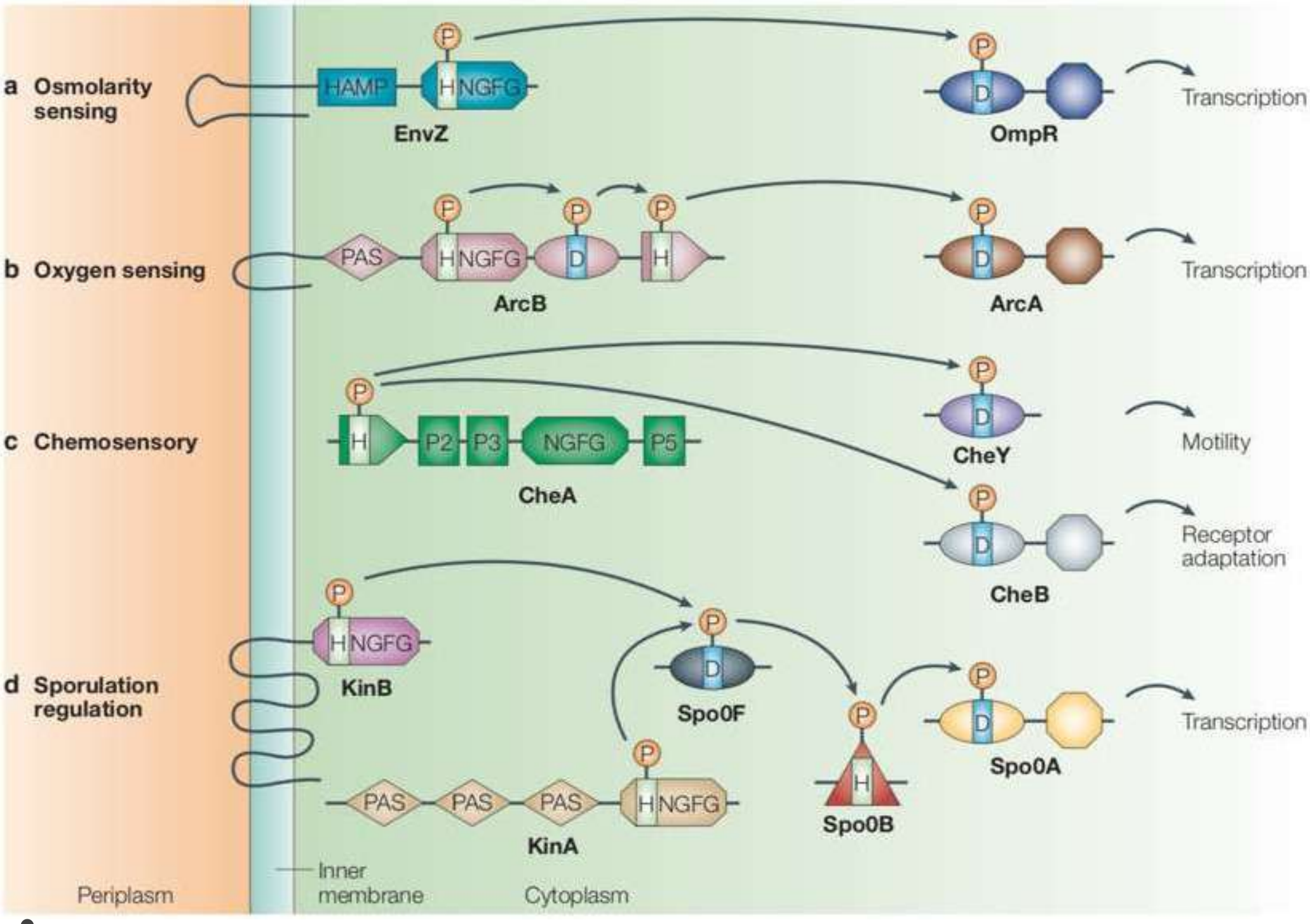
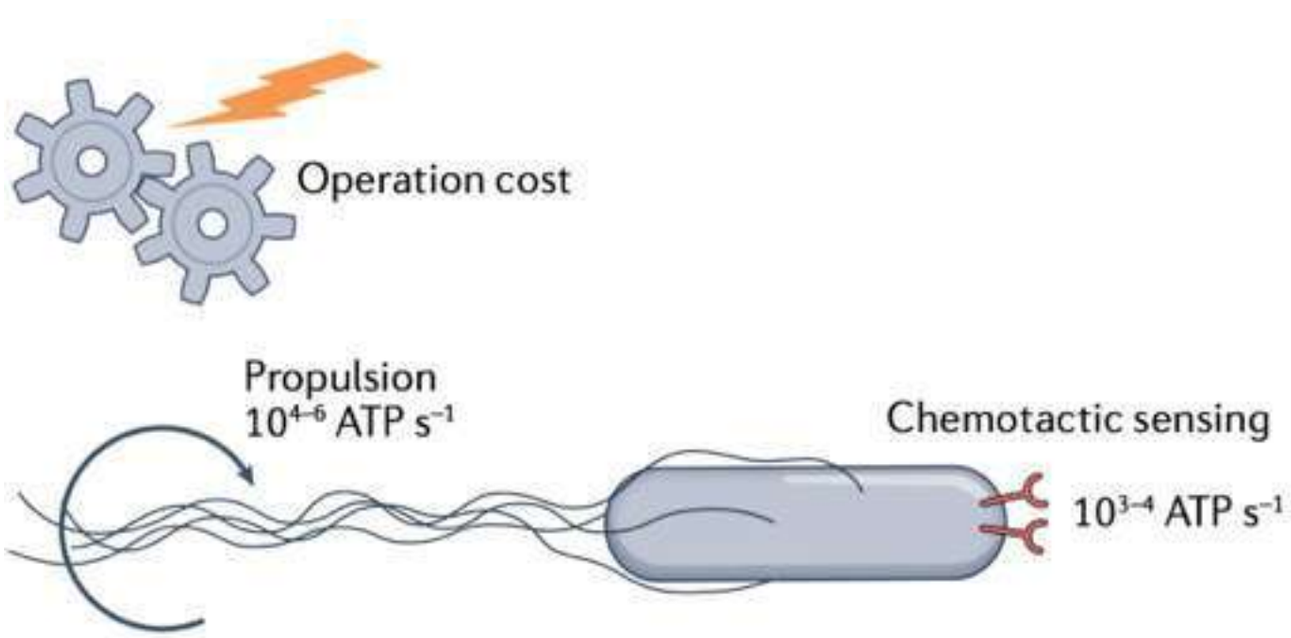
# Chemotaxis, X

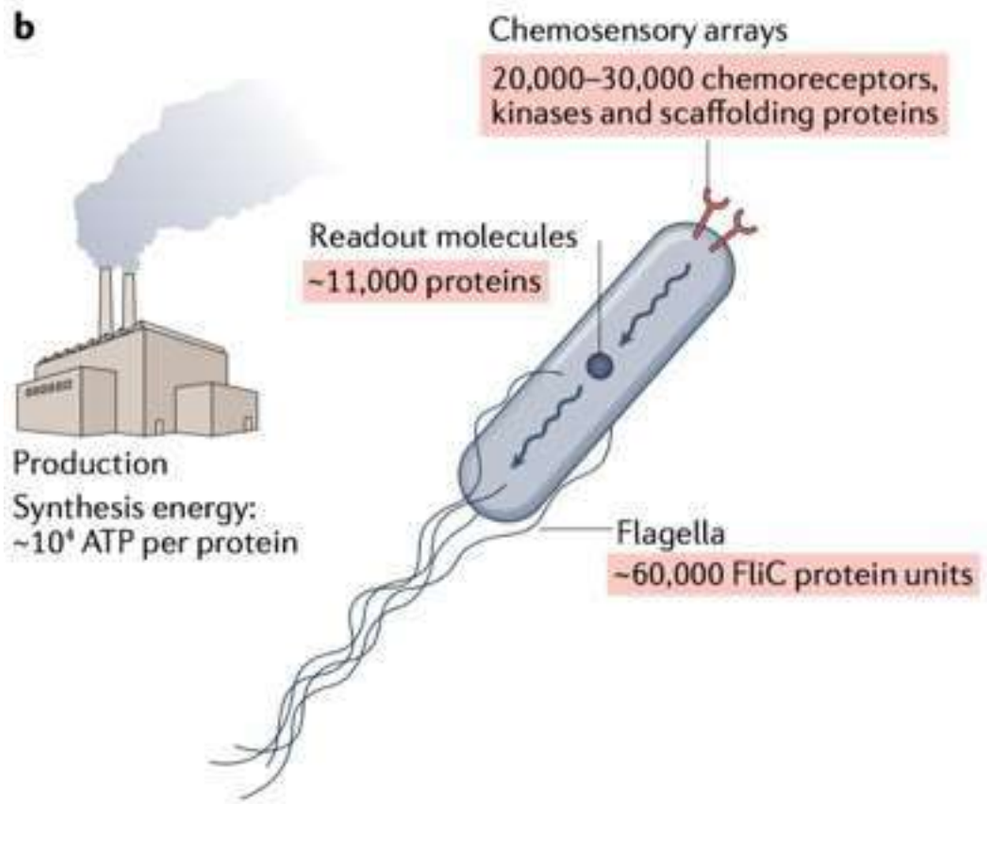



- Phototaxis

**Wadhams & Armitage, 2014**

# Relative  cost of bacterial chemotaxis



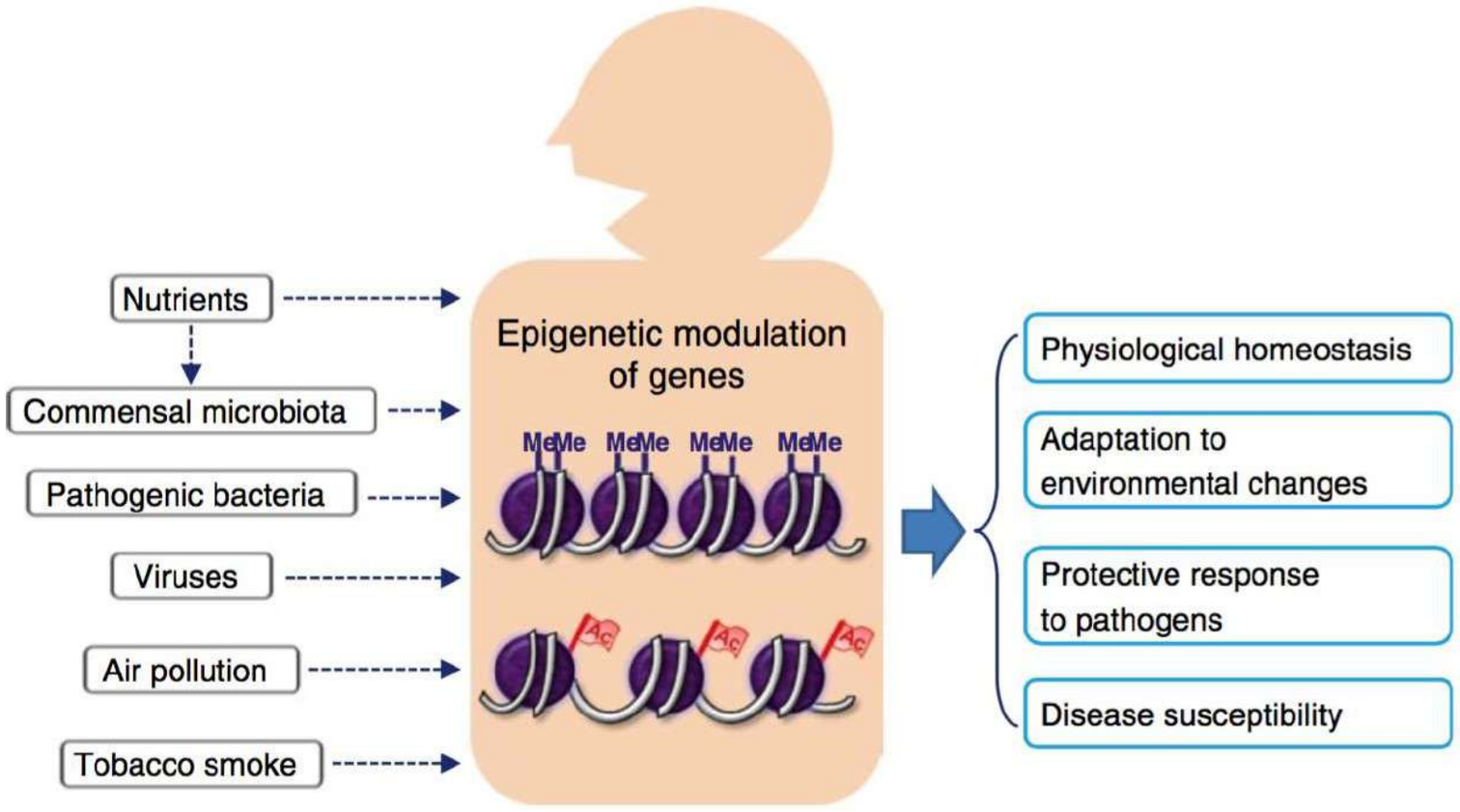




- **Metabolism** fuels chemotaxis
- **Informed foraging** and **cue-based navigation**
- **Increase growth rate in a better environment**

**Keegstra et al., 2022**

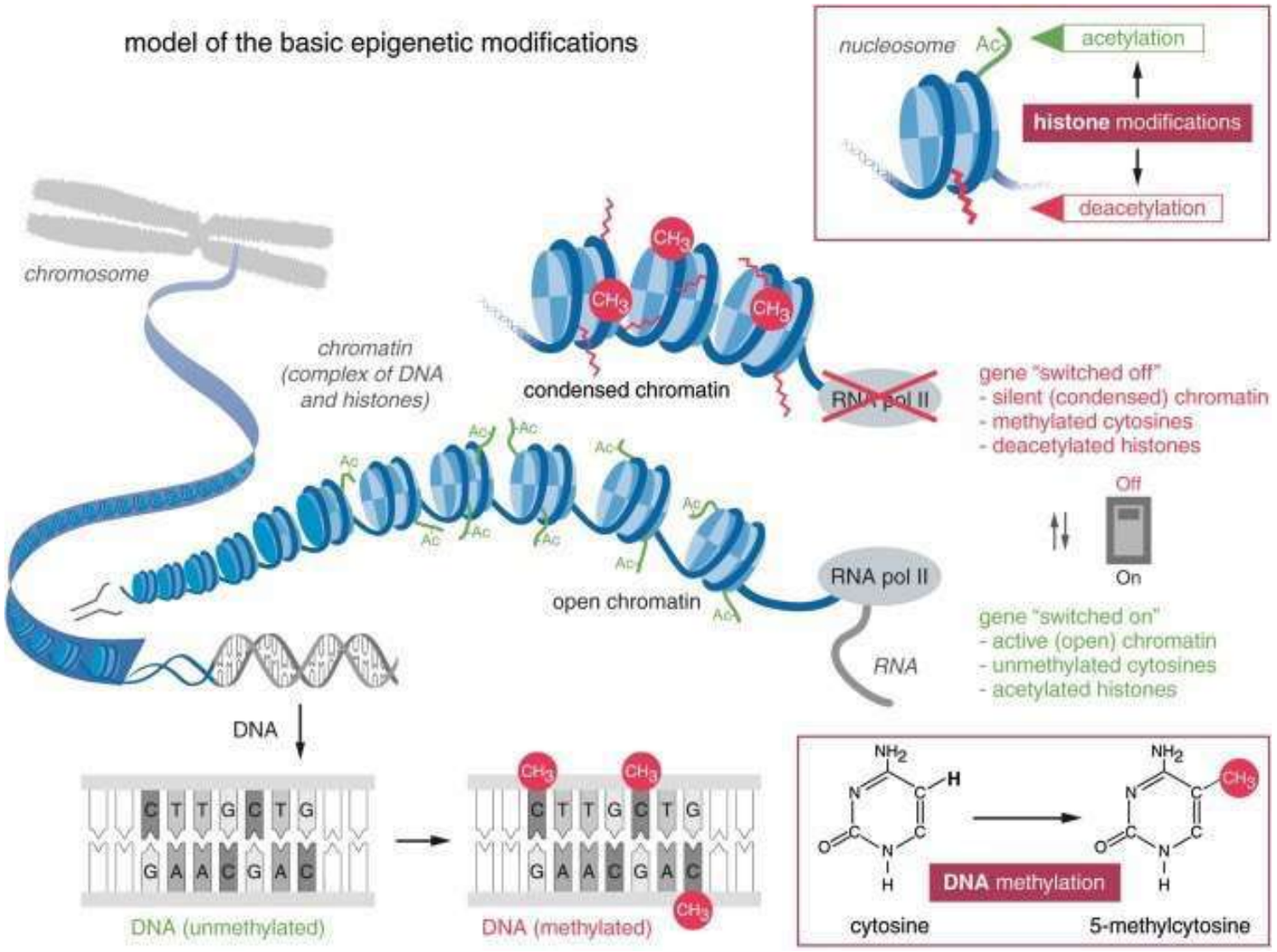
# Epigenetics

- The word “epigenetics” was originally coined by Conrad Waddington in 1942, referring to how genotypes give rise to phenotypes during development
- Now we refer as the study of **phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression** that are **not dependent on changes to DNA sequence** (Deans and Maggert 2015)
- In Humans, gene expression is regulated prior to transcriptional initiation by the **chemical modification of DNA or the histone proteins** that together form chromatin




**Takahashi, 2014**

# Epigenetic modifications of chromatin by DNA methylation and histone acetylation




**Vilcinskas, 2015**

- Methyl group transfer to cytosine –> 5-methylcytosine (m5C) pairs with guanosine
- m5C has different interactions with regulatory proteins
- **Chromatin structure** depends on net **charge** of core **histones**
- **Acetyl groups** promoting formation of *open* and **accessible** euchromatin vs **deacetylation** promoting the formation of *compact* and **inaccessible** heterochromatin

# aDNA: Reconstructing ancient genomes and epigenomes

Orlando et al., 2015

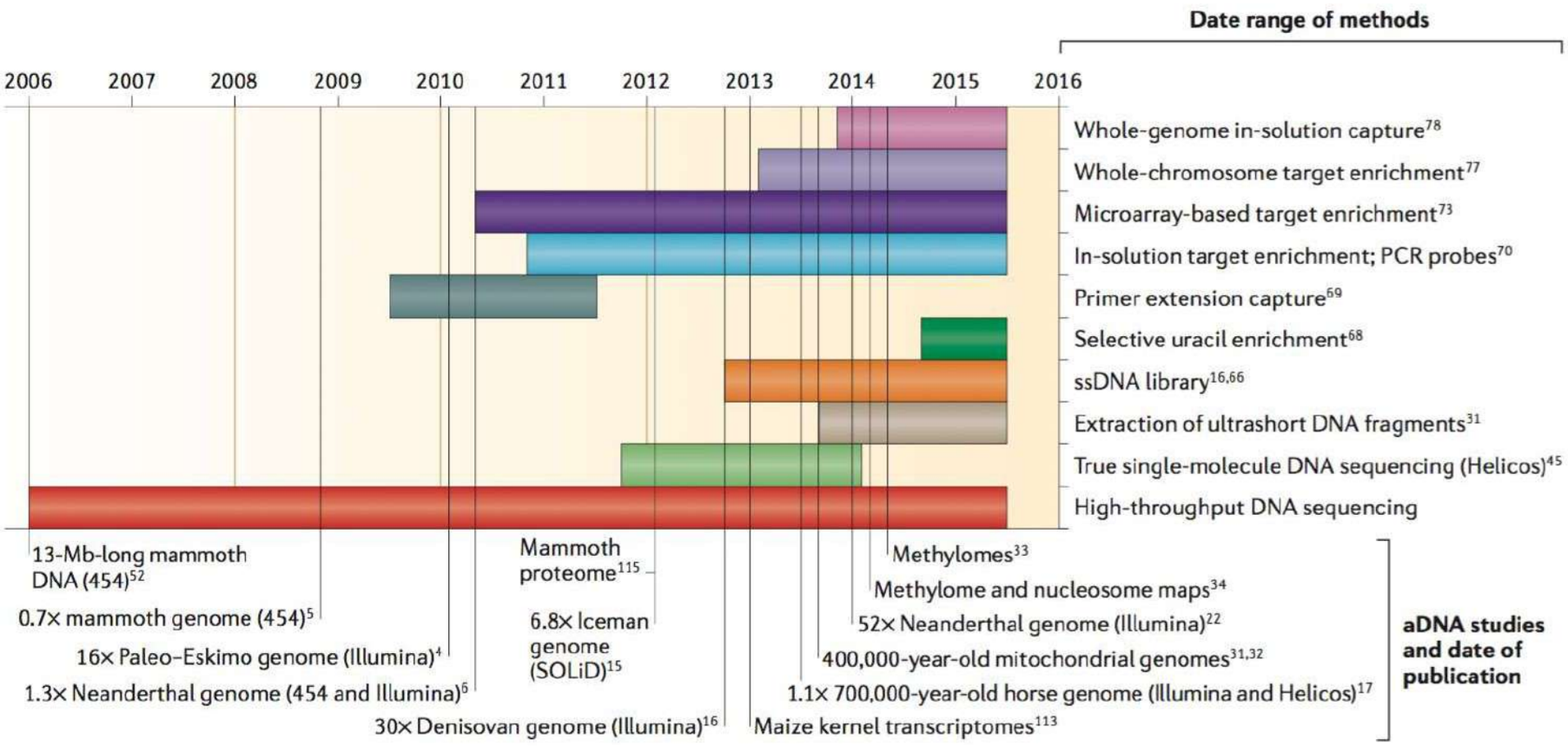
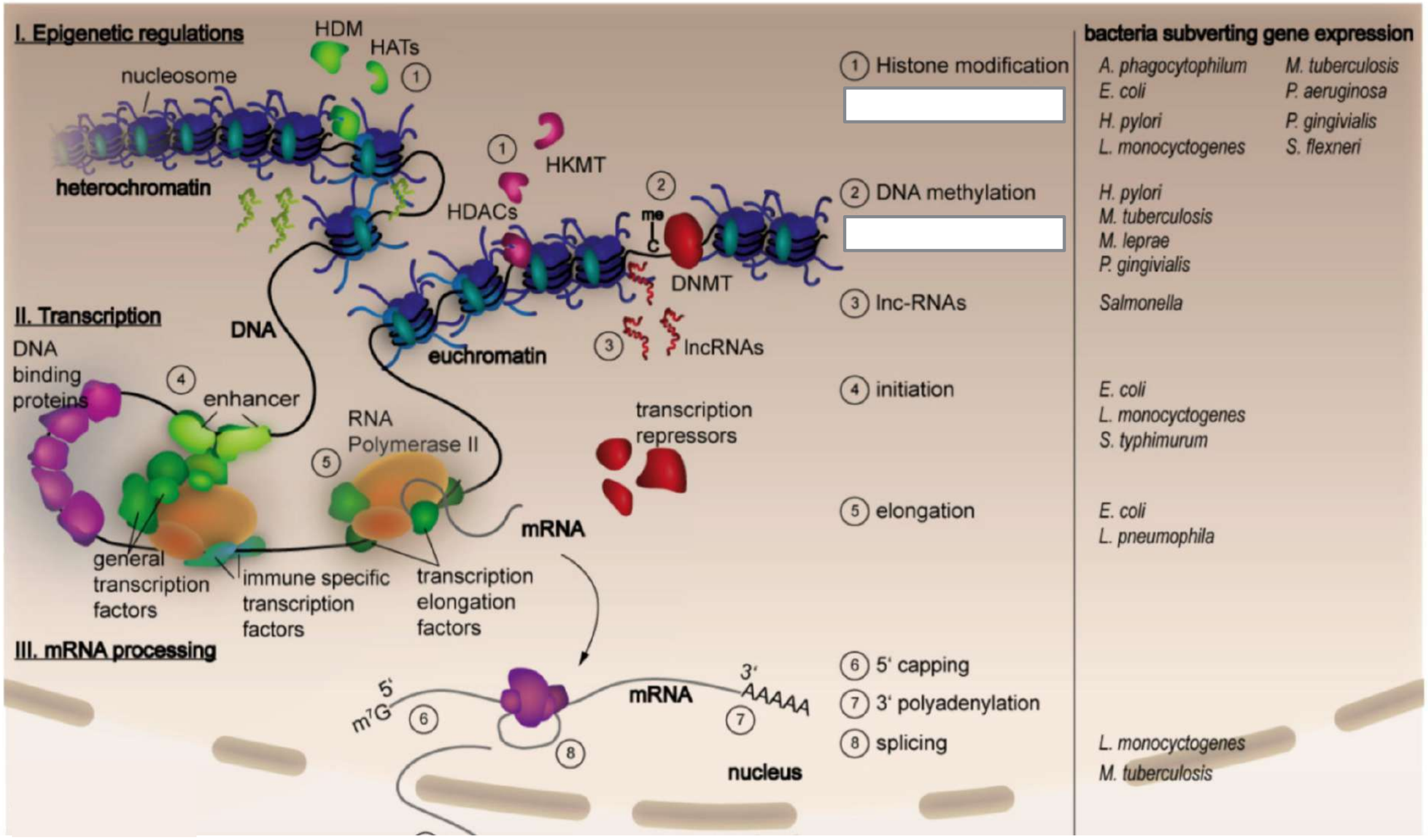



Figure 1 | **Major advances in ancient genomics.** The major methodological advances described in this Review are presented with respect to milestones in paleogenomics, including whole-genome sequencing and the characterization of transcriptomes, epigenomes and proteomes. Average genome fold-coverage (×) and sequencing platforms are indicated where applicable. aDNA, ancient DNA; ssDNA, single-stranded DNA.

- **Typical ancient DNA molecules:** diverse range of degradation reactions affect DNA post-mortem and result in extensive fragmentation (preferentially at purine nucleotides) and base modifications
- Most common base modification identified in high-throughput sequencing data sets is deamination of cytosines into uracils (red), or thymines (blue) when cytosines were methylated (mC) –>deaminations occur much faster at overhanging ends
- Other modification: abasic sites and single-strand breaks

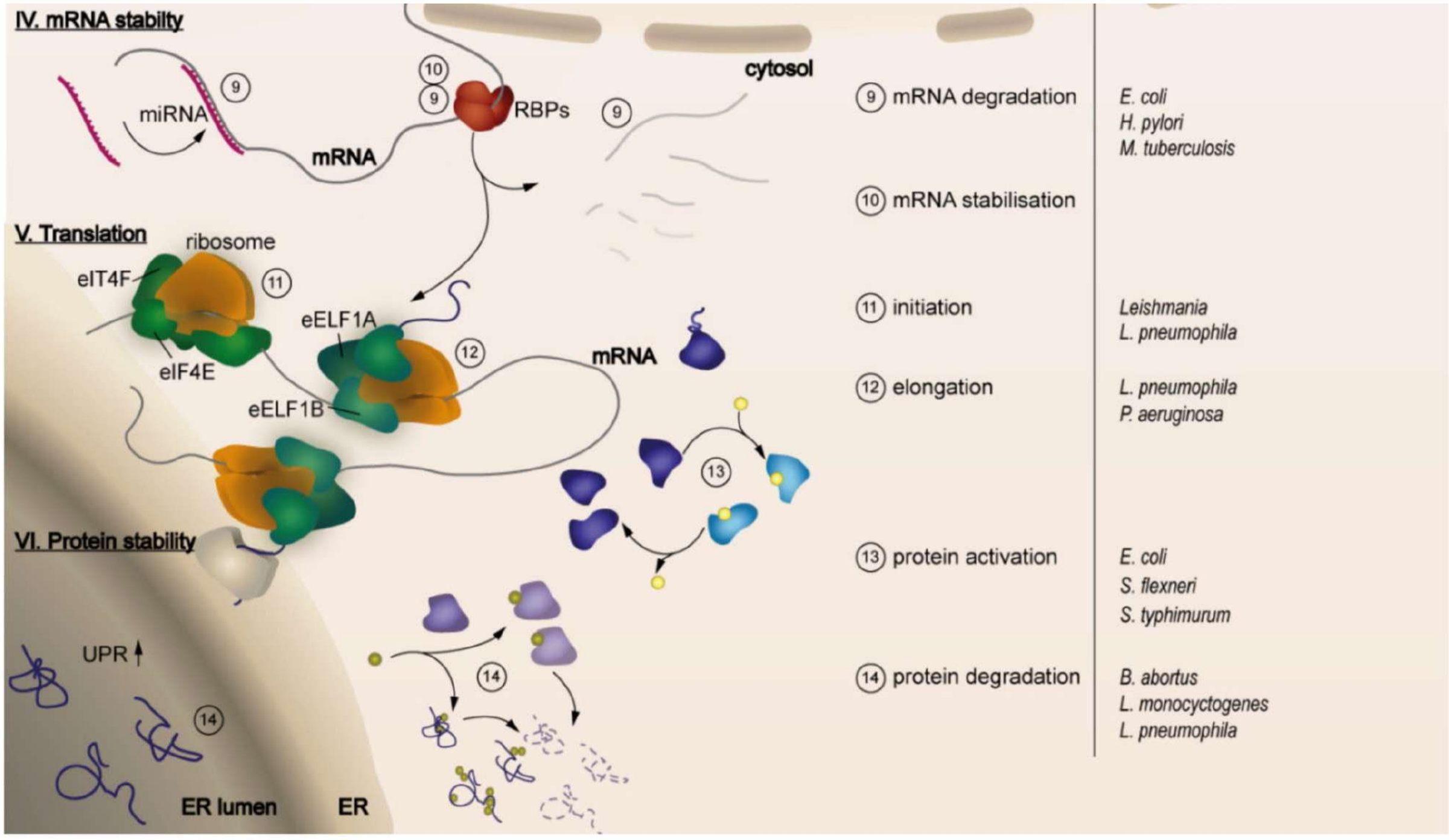
# Bacteria manipulate host gene expression during infection, I




Denzner et al., 2020

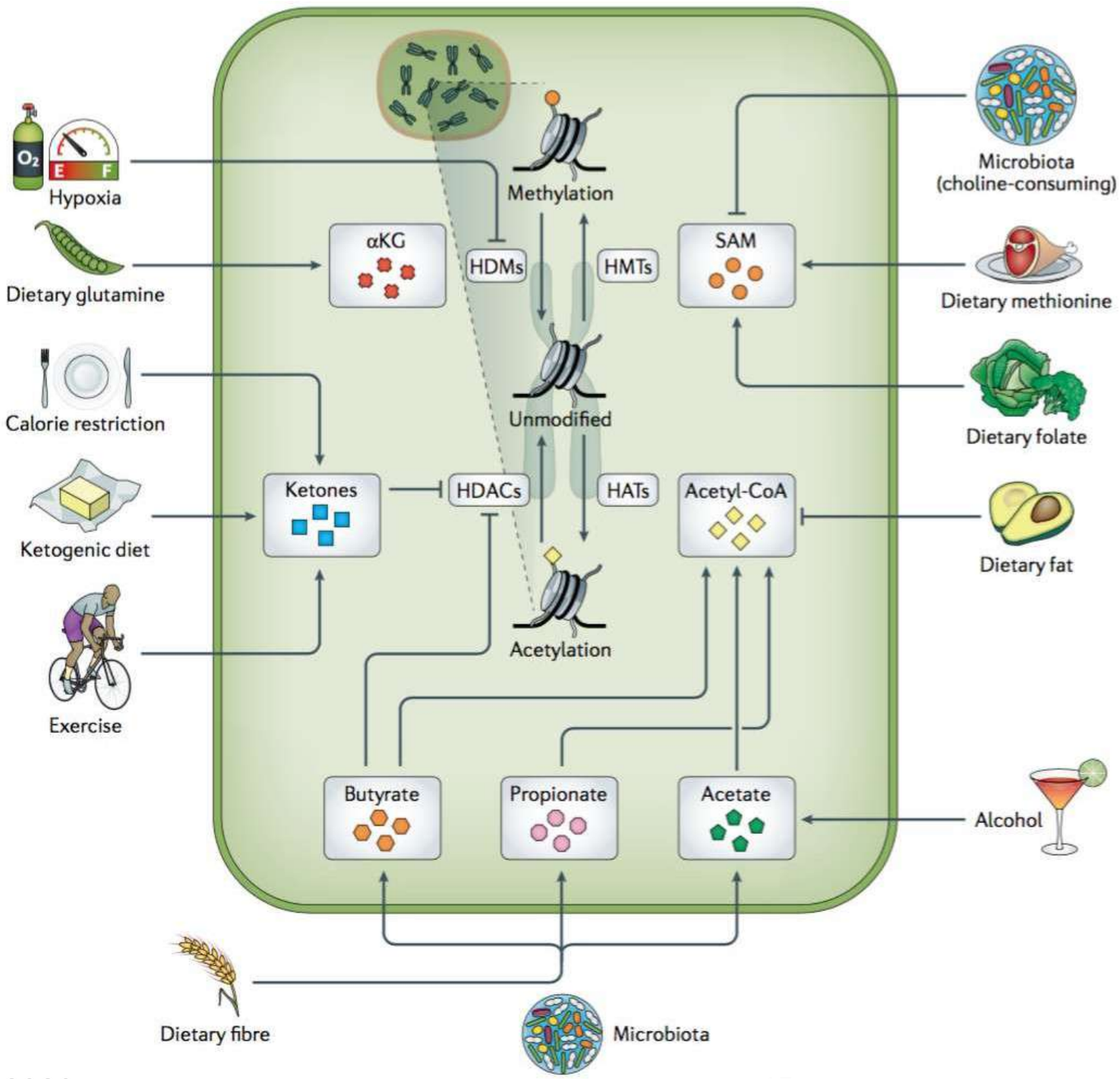
Bacteria evolved many strategies to survive and persist within host cells

# Bacteria manipulate host gene expression during infection, II




Denzner et al., 2020

# Influences of environmental factors on histone acetylation and methylation via micro biome




*S*-adenosylmethionine (**SAM**) and acetyl-CoA, that are used by histone methyltransferases (HMTs) and histone acetyltransferases (HATs)

The activity of histone demethylases (**HDMs**) is supported by α-ketoglutarate (αKG), which can be derived from dietary glutamine, and is inhibited by the limited oxygen availability during hypoxia

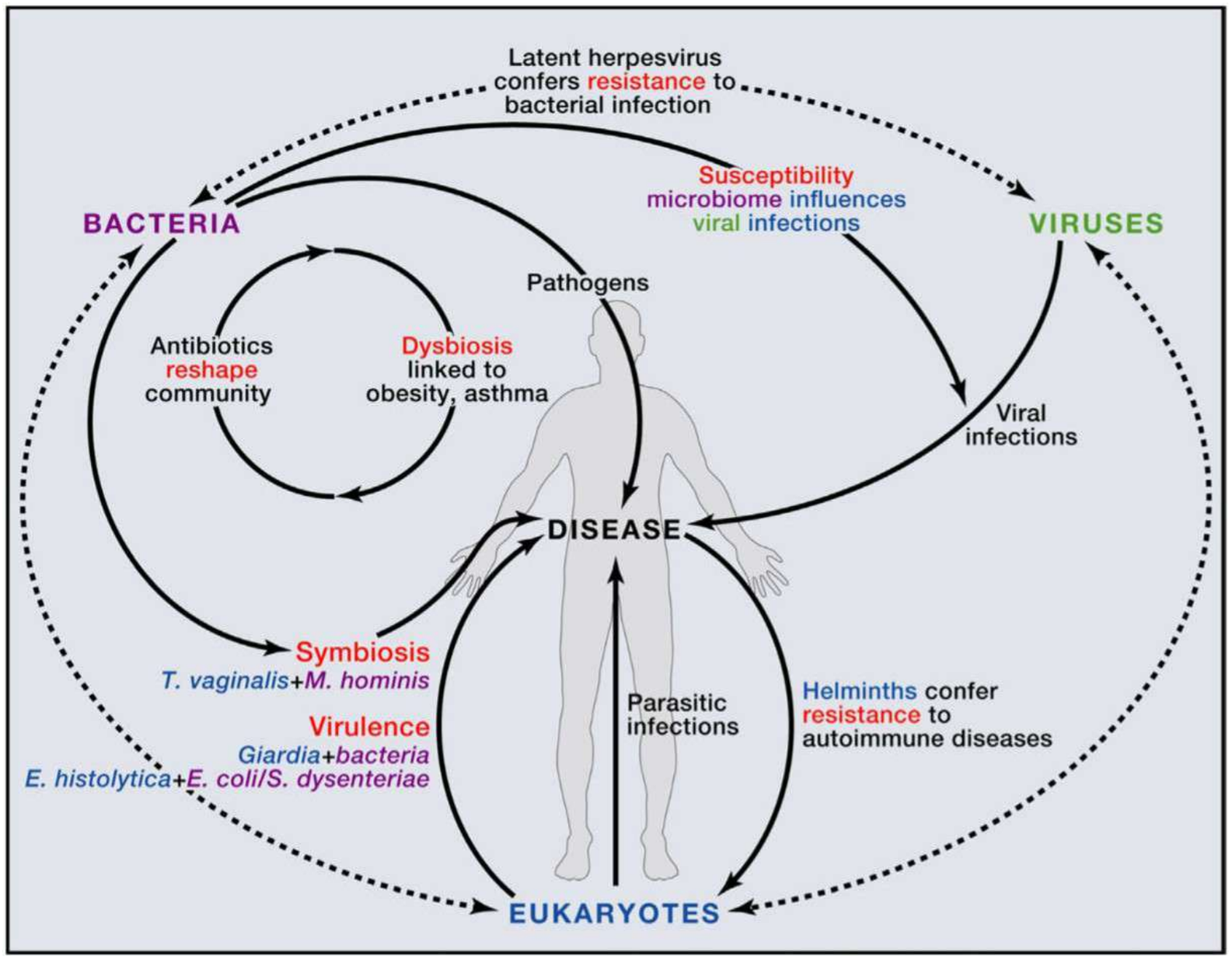
Ketone bodies and short-chain fatty acids (SCFAs) such as acetate, propionate and **butyrate** can provide **acyl-CoA** precursors for histone acylation, while also directly inhibiting the activity of histone deacetylases (HDACs)

**Dai et al., 2020**

## Box 1 | The epigenomes of eukaryotes and bacteria

- In eukaryotes, epigenetic modification of the genome involves DNA methylation[3] and histone modification[4]. Bacteria lack histones, and epigenetic control relies on DNA methylation only[6].
- In eukaryotes, de novo and maintenance forms of DNA methylation are performed by separate enzymes[2]. Bacterial DNA methyltransferases have both de novo and maintenance activities[37].
- In eukaryotes, two main mechanisms exist to erase DNA methylation marks: active demethylation by dedicated proteins (Tet enzymes), and passive demethylation by the hindrance of DNA methylase activity upon DNA replication[35]. In bacteria, DNA demethylation is usually passive[66], and the relevance of active demethylation by DNA repair remains to be evaluated[82].
- In both bacteria and eukaryotes, transcriptional repression by DNA methylation is common[3,6]. Transcriptional activation of bacterial genes under DNA methylation control often involves demethylation (partial or complete, single- or double-stranded) of promoters or regulatory regions[57,72,89,90,94,158].
- The methylated base typically involved in the control of eukaryotic transcription is $C^5$-methyl-cytosine[3], whereas in bacteria it is often $N^6$-methyl-adenine[7,14]. However, direct control of bacterial transcription by $C^5$-methyl-cytosine has been demonstrated recently[126]. Transcriptional control by $N^4$-methyl-cytosine may also exist[130].
- In multicellular eukaryotes, the DNA methylation pattern of the genome is reprogrammed during gametogenesis and during early embryonic development[2]. In bacteria, reprogramming does not occur, and the DNA methylation pattern can be transmitted unaltered across generations. However, the acquisition and loss of DNA methyltransferase genes[41] and recombinational shuffling of DNA methyltransferase domains[27,33,143] can produce novel methylation patterns in bacterial genomes.
- In both bacteria and eukaryotes, DNA methylation controls the formation of phenotypic variants of genetically identical cells. However, DNA methylation-dependent formation of bacterial cell lineages can show programmed reversion (phase variation)[15,27,93,111].

# Why is important to consider epigenetics?




To fully understand the microbial interactions in human health and disease —> new medicine and societal norms

To fully understand the microbial interactions at the microscale in our world —> modeling and protecting environment

**Watson & Søreide, 2017**

# Bacteria, small Eukaryotes and Viruses influencing host via epigenetic attack

| Microbe | Factor | Effect on the host |
|---|---|---|
| *Listeria monocytogenes* | LntA | Inhibition of binding of a chromatin silencing complex to the promoters of interferon-stimulated genes |
| | | Increase in IL-8 gene expression by inducing histone modifications through activation of MAPK signaling pathway |
| | LLO | Dephosphorylation of histone H3 through induction of $K^+$ efflux |
| *Chlamydia trachomatis* | NUE | Methylation of histones |
| *Legionella pneumophila* | RomA | Methylation of histones (H3K14 trimethylation) |
| | Flagellin | Increase in IL-8 gene expression by inducing histone modifications through activation of a signaling cascade |
| *Helicobacter pylori* | | Silencing selected promoters by inducing DNA methylation |
| | | Induction of histone modifications |
| | | Regulation of miRNA expression |
| *Bacteroides vulgatus* | | Induction of histone modifications through a signaling cascade |
| *Wolbachia* | | Interference with genetic imprinting by altering methylation patterns |
| *Bifidobacterium breve, Lactobacillus rhamnosus GG* | | Decrease in LPS-induced IL-17 and IL-23 production by suppressing histone acetylation |
| *Porphyromonas gingivalis* | | Reactivate latent HIV-1 integrated in the host genome as proviral DNA copies by butyrate-meditated HDAC inhibition |
| Influenza virus | NS1 | Suppression of antiviral protein production by hijacking a transcription elongation factor through a region similar to H3 histone tail |
| Epstein–Barr virus | LMP1 | Silencing of E-cadherin promoter by upregulating Dnmt1, 3A, 3B through the JNK-AP-1 pathway |
| Human adenovirus | E1A | Up-regulation of Dnmt1 by activation of E2F |
| | | Activation of Dnmt1 by associating with Dnmt1 |
| Hepatitis B virus | pX (HBx) | Silencing of tumor suppressor genes by up-regulating Dnmt1 through the cyclin D1-CDK4/6-pRb-E2F1 and p38 MAPK pathways |
| HIV | Early expressed proteins | Silencing of IFN-γ promoter by up-regulating Dnmt1 through the AP-1 pathway |

Takahashi, 2014

# Definitions, I

- **Epigenome**: complete record of all chemical modifications to DNA
- Epigenome with the epitranscriptome (chemical modifications of RNA) and epiproteome (chemical modifications of proteins), makes up the **epi-ome**
- **Methylome**: complete record of all methyl modifications to either DNA, RNA, or proteins in a particular cell or organism

# Definitions, II

- **DNA methyltransferase (MT-ases)**: family of enzymes that catalyze the **transfer** of a **methyl group** from an S-adenosyl-Lmethionine (AdoMet) donor to DNA
- **Restriction-modification (R-M) systems** almost ubiquitous in prokaryotes
- R-M consist of a **DNA methyltransferase** that methylates a specific target sequence in the host genome and a **cognate restriction endonuclease** that cleaves unmethylated or inappropriately methylated targets from exogenous DNA

# A second derivative in epigenetics: phase variation

MTases can generate **phenotypic lineages**, which enables **division of labour** in a community or prepares the community for future changes in the environment (bet-hedging)

**Bistability**: the state existing in a **clonal population with different phenotypes**

Due to genetic rearrangements, DNA MTase can generate a **distinct methylation pattern in genome —>** which results in **different gene expression profiles** and produces **lineages with different** (virulence) capacities

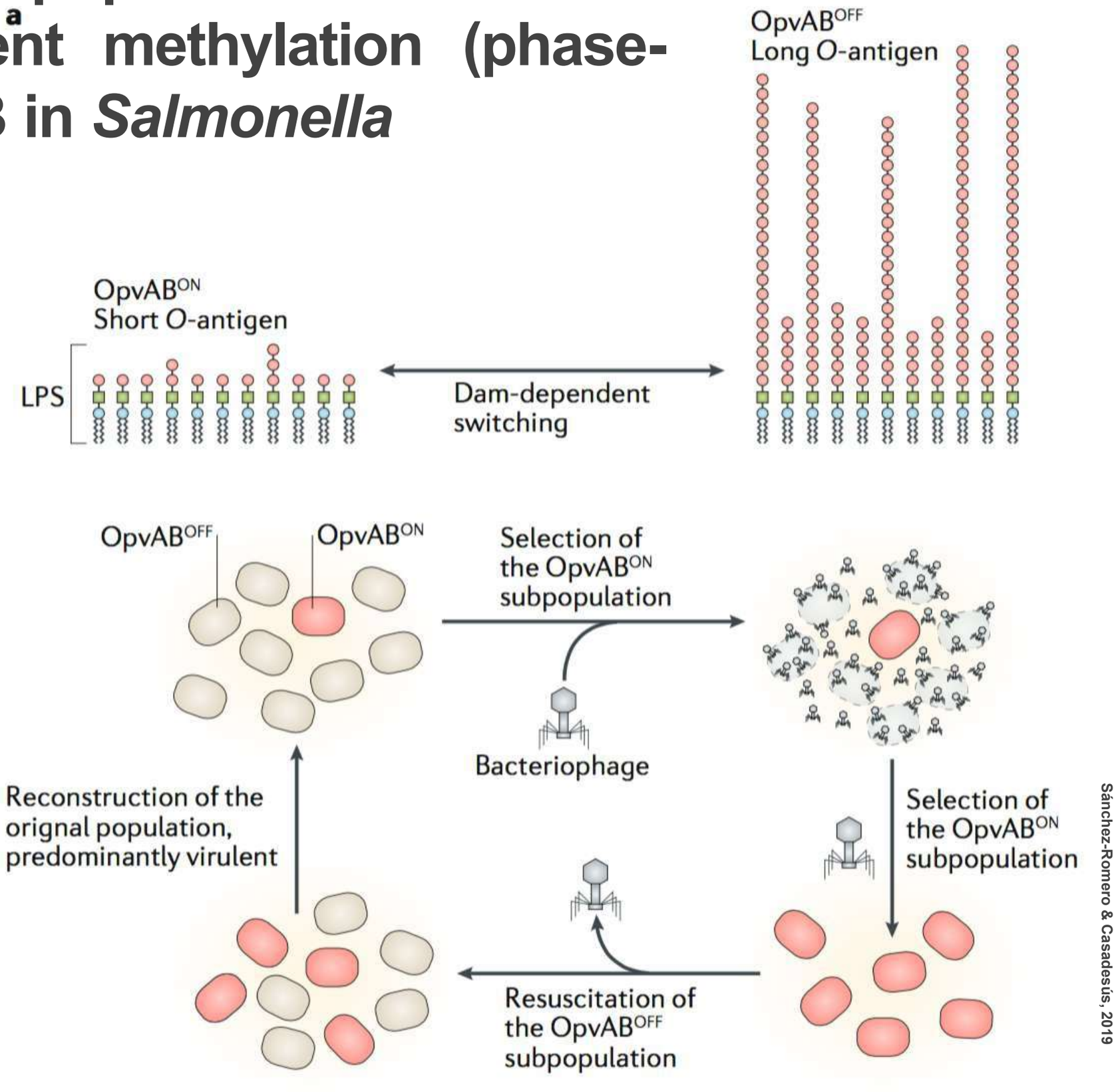
# Formation of subpopulations controlled by Dam-dependent[a] methylation (phase-variation): OpvAB in *Salmonella*

Shortening of the O-antigen renders the

OpvABON lineage avirulent but resistant to bacteriophages

When the phage challenge ceases,

OpvABOFF cells produced by phase variation will survive, and virulence will be regained




Sánchez-Romero & Casadesús, 2019