Chapter 5 Signaling changes in the environment

a.a. 2018-19

Signal transduction in bacteria

To survive, bacteria have to regulate nutrient acquisition and to adapt to physical or chemical aspects of the environment (pH, osmolarity, light, temperature, immune system).

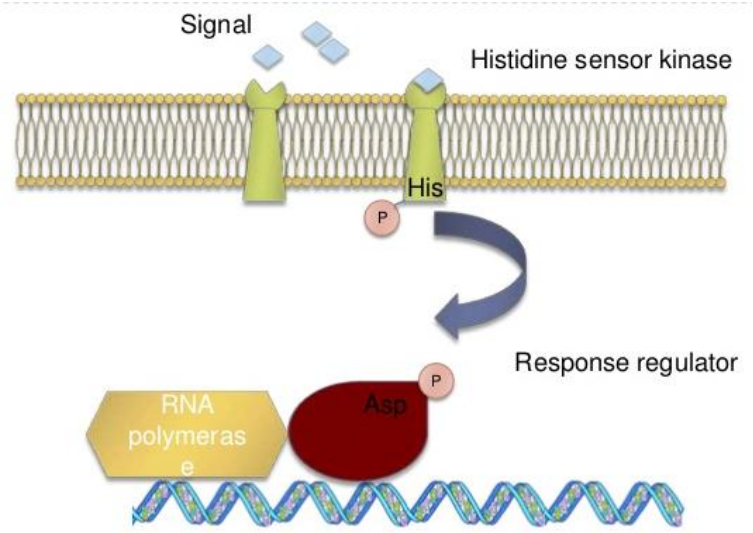
In order to respond to elicit appropriate adaptive responses to changing environmental conditions, cells must be able to transmit the information from the cell surface (site of sensing) to the cytoplasm (site of cellular response).

Intracellular signalling in many bacterial species to response to a variety of environmental stimuli occurs by:

- **phosphotransfer signaling systems** (Two-component regulatory system)
- Second messengers: bis-(3′-5′)-cyclic dimeric guanosine monophosphate (**c-di-GMP**)
- Alternative sigma factors (extracytoplasmic function (ECF) sigma factors).

Two-component regulatory systems (TCS)

The main signal-transducing system largely used in bacteria is mediated by a **phosphotransfer signaling systems** named **two-component system (TCS).**
It occurs also in archea, fungi, and plants.



TCS is a linear signal transduction system in which there is not amplification of the signal (phosphotransfer).

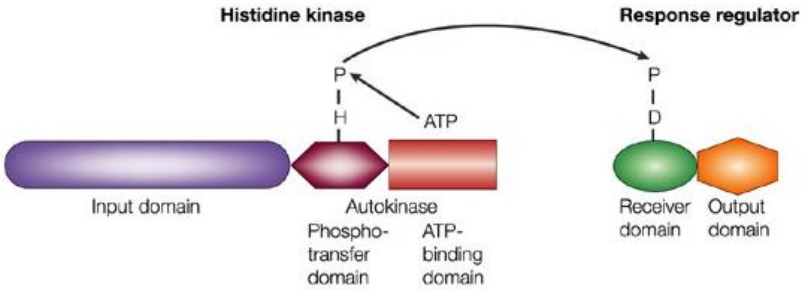
I component is a **sensor protein** (sensor kinase) that "senses" a specific environmental stimulus changing its conformation.

II component (cognate) corresponding to a cytoplasmic **response regulator** that mediates the cellular response, mostly through differential expression of target genes.

Sensor proteins: histidine kinases (HK)

Sensor proteins are membrane-bound **histidine kinases (HK)** having two domains:
a N-terminal variable in sequence **sensor domain** (input domain) that is used to sense extracellular signals.
A C-terminal **transmitter (kinase domain)** with autokinase function.

The sensor domain, sensing signals, changes conformation and transfers it via transmembrane domain to the autokinase domain which results in phosphorylation of a specific **histidine residue** (using ATP as P donor) in the phosphotransfer subdomain of the autokinase.



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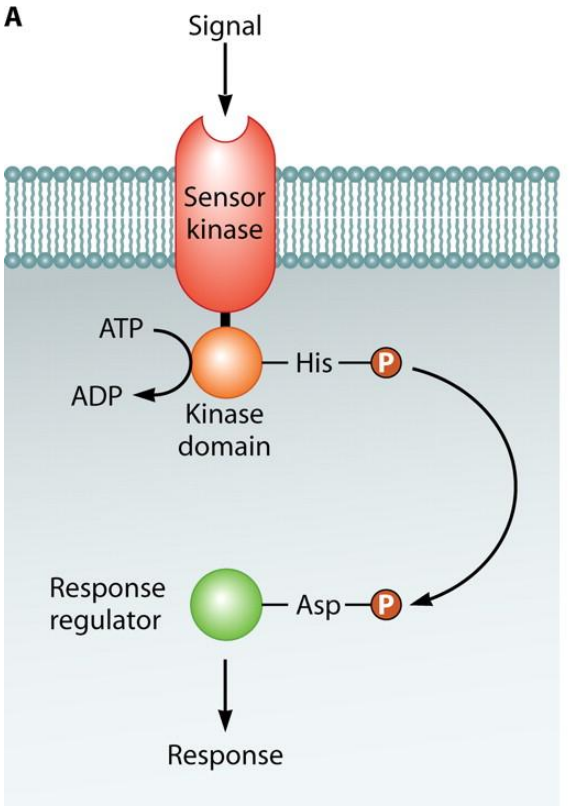
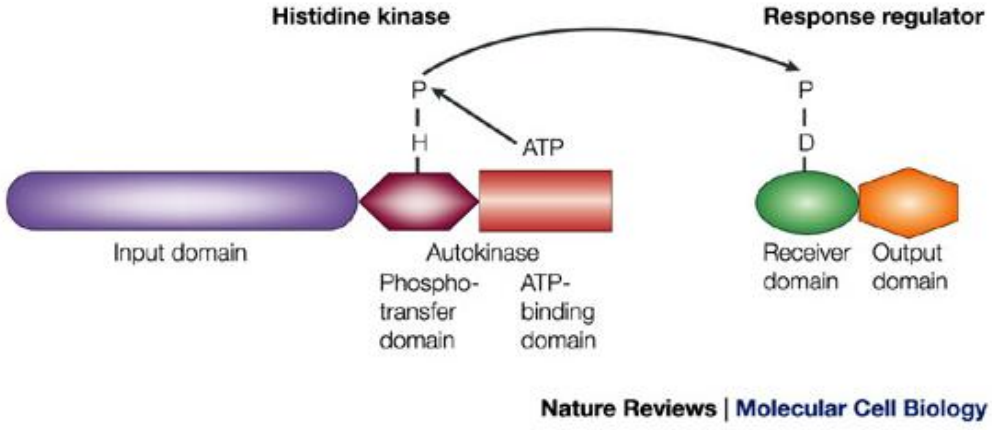


Transfer of a phosphoryl group to the response regulator

Response regulator proteins are cytoplasmic proteins having 1) an N-terminal conserved **receiver domain** containing the Asp residue, 2) a C-terminal variable **output domain** that mediate specific biological activities.



The **response regulator** catalyzes the transfer of the phosphoryl group from the phospho-His residue of the associated HK to a conserved **aspartate** residue in the **receiver domain** of the **response regulator** (RR).

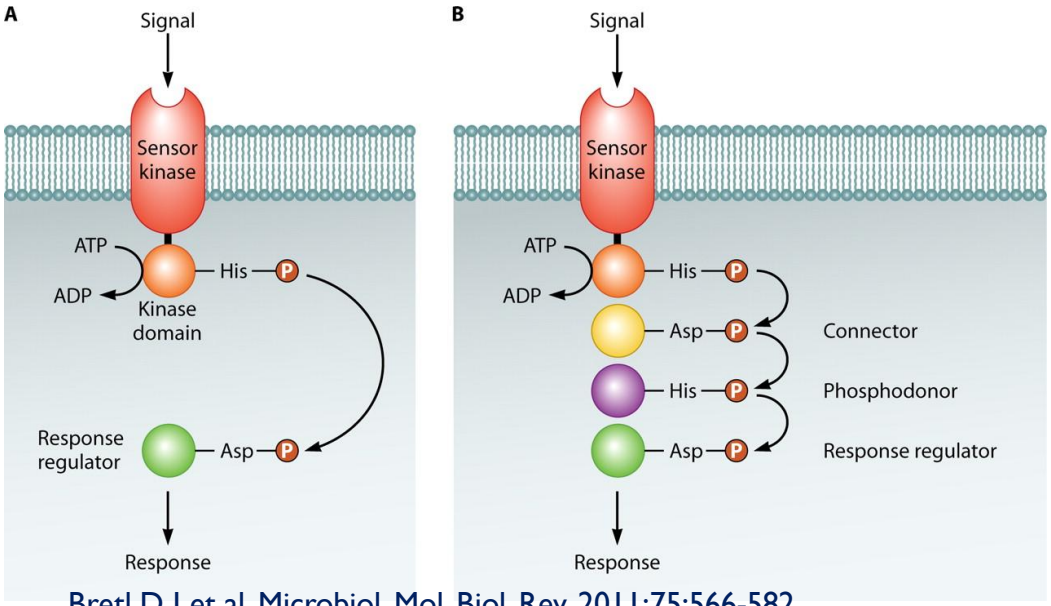
Its phosphorylation changes the conformation of RR which results in modulation of the function of the linked **output domain.**

Hydrolysis of the phosphoryl group in the RR by phosphatase that dephosphorylates the RR resets the system.

Phosphorelay systems are variants of TCS in bacteria

Several TCS can be more complex involving extra receivers and phosphotransferase domains (**phosphorelay**).

A phosphorelay contains, additional elements (**phosphotransfer proteins)** which modulate the levels of the active form of response regulators by affecting the various possible steps that determine their phosphorylation state or their activity.



Bretl D J et al. Microbiol. Mol. Biol. Rev. 2011;75:566-582

Additional elements: an intermediate response regulator (connector) lacking an output domain and a His-containing phosphotransfer protein (p donor). In some phosphorelays, the phosphotransfer protein and/or the intermediate response regulator is fused with the sensor kinase in a single polypeptide (hybrid HK)

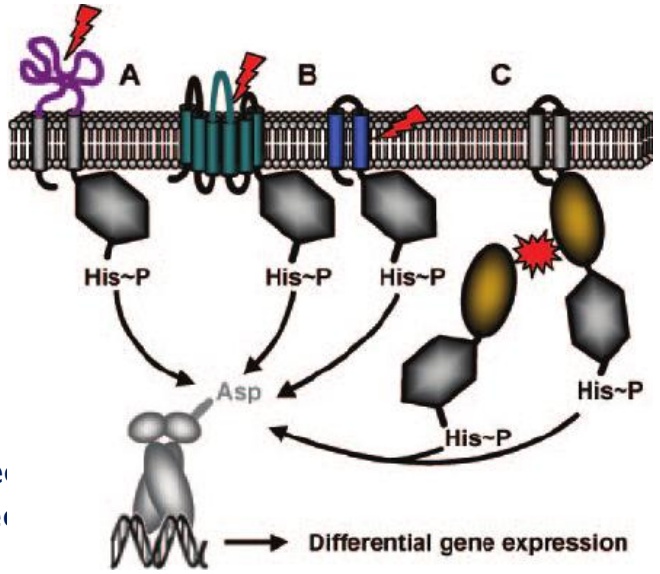
Different architecture of histidine kinases in TCS

Most sensors are periplasmic-sensing HK (A), others show sensing mechanisms linked to the transmembrane regions (B) or are cytoplasmic-sensing HKs (C).

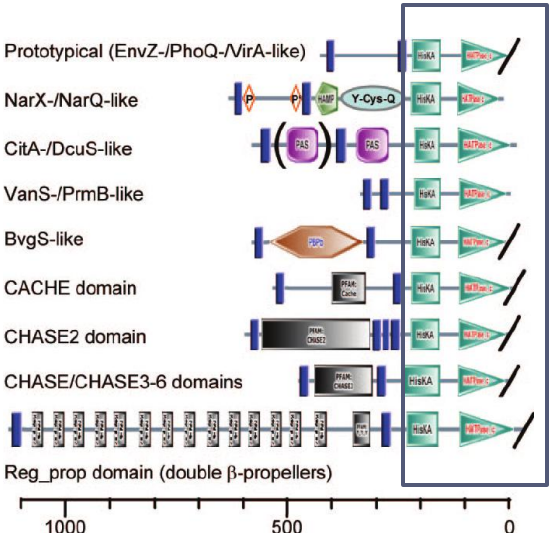
Often HKs function as **homodimers**: autophosphorylation is a bimolecular event.

In many cases the ligand or stimulus is unknown

Little or no sequence similarity.



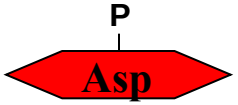
The parts of the proteins involve stimulus perception are highlighte color and are the variable parts.



Domain architecture of **periplasmic-sensing** histidine kinases.

The **transmitter domains** (green colour) contain conserved sequence motifs common to most sensor proteins.

Different types of response regulators (RR)



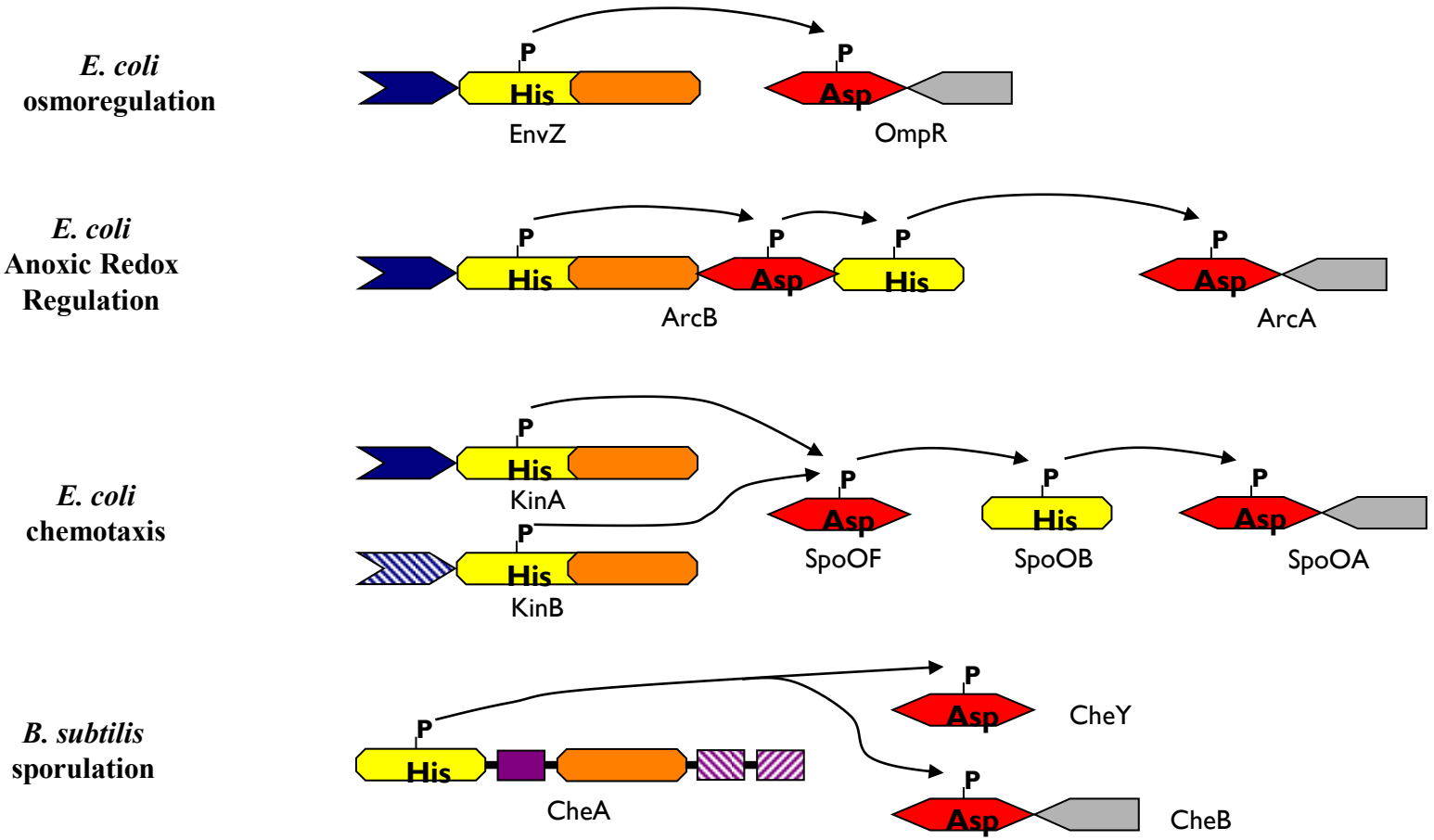
RR: N-terminal Receiver domains: Catalyze the transfer of phosphryl group from phospho-HK to conserved aspartic acid: phosphorylation results in conformational change of response regulator. They contain conserved sequence motifs common to most receiver proteins.

- Cross-talk between different two-component signaling pathways at the level of phosphorylation is rare

Output domains (regulatory domain): are often **transcriptional regulators**: the binding of the phosphorylated RR changes the transcription of regulons encoding proteins that can help le cell to respond to the original stress condition. These RRs contain **DNA-binding output domains** with different variants of helix-turn-helix DNA-binding structural motifs.

Other RR types exert regulation at post-translational levels by enzymatic activity or protein-protein interactions.

Modular organization of TCS



Response regulators (RRs) within two-component signal transduction systems control a variety of cellular processes, such as oxigen metabolism, chemotaxis, sporulation, and osmoregulation.

Genomic distribution of TCS

Usually the TCS proteins are coded by transcriptionally co-regulated genes (operon).
Their number vary among different species

- E. coli: 30 HKs (5 hybrids) and 32 RRs
- Synechocystis sp: 80
- Mycoplasma sp: 0
- Bacillus subtilis: 70
- Haemophilus influenza: 9
- Helicobacter pylori: 11

The **number** of **TCS** genes within an organism **varies** greatly and a strong relationship between bacterial ecological niche and the complexity of the organism

Two-component signal transduction system in *E. coli*

Table 4.22. Examples of two-component signal transduction systems in *Escherichia coli*

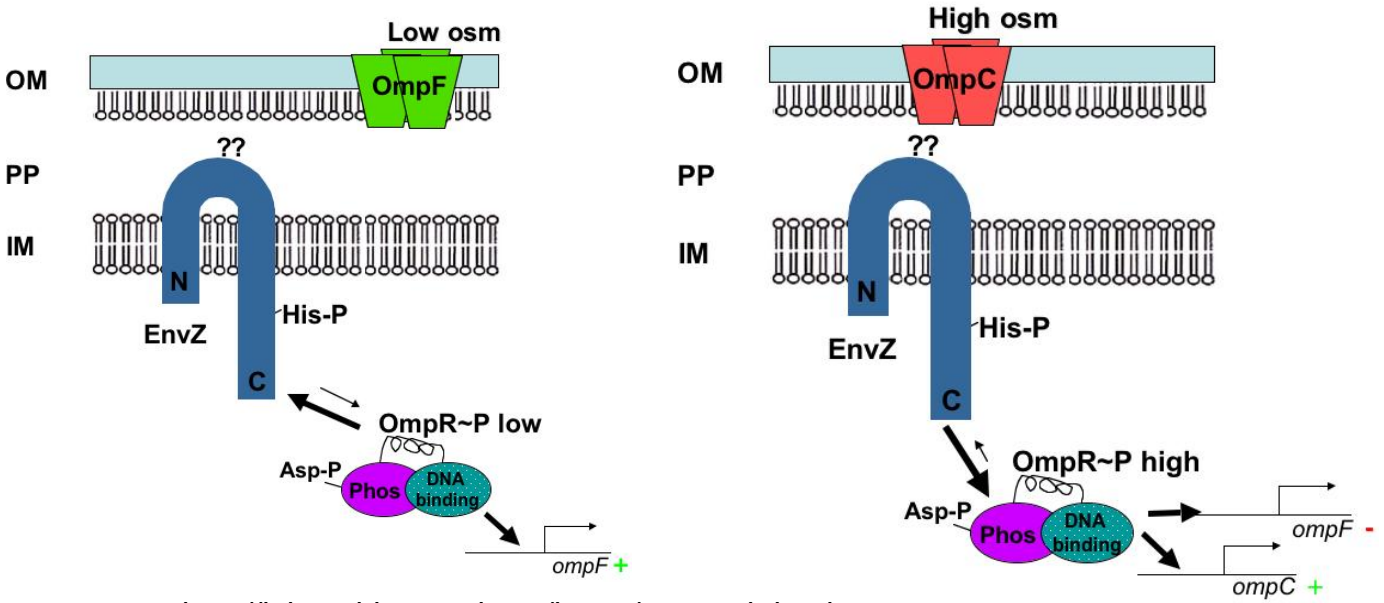
Sensor	Response regulator	Signal	Response
NtrB	NtrC	Concentration of nitrogen-containing compounds in the environment	Transcription of genes involved in uptake and metabolism of nitrogenous compounds such as glutamine, arginine, histidine, nitrate and nitrite
PhoR	PhoB	Concentration of phosphate in the environment	Phosphate regulation
EnvZ	OmpR	Osmolarity of environment	Osmoregulation, regulation of porin expression
EvgS	EvgA	Low temperature, Mg^{2+}, nicotinic acid	Regulation of OmpC expression
PhoQ	PhoP	Concentration of Mg^{2+} in environment	Transcription of loci essential for growth at low concentrations of Mg^{2+}

Bacteria contain varying number of TCSs : *E.coli* has nearly 30 TCS.

Osmolarity Changes and Porin Regulation

E. coli **EnvZ** and **OmpR** proteins are involved in sensing **osmolarity changes** in the environment. OmpR regulates two genes (*ompF* and *ompC*) encoding two different porins. OmpF porin predominates in low-osmolarity condition and its pore diameter is 1.5 nm. OmpC protein predominates in high-osmolarity condition and diameter of its pore is 1 nm.

Increased osmolarity activates **EnvZ** so that it increase phosphorylation of OmpR. High level of P-OmpR represses *ompF and* stimulates *ompC* that become the major porin.

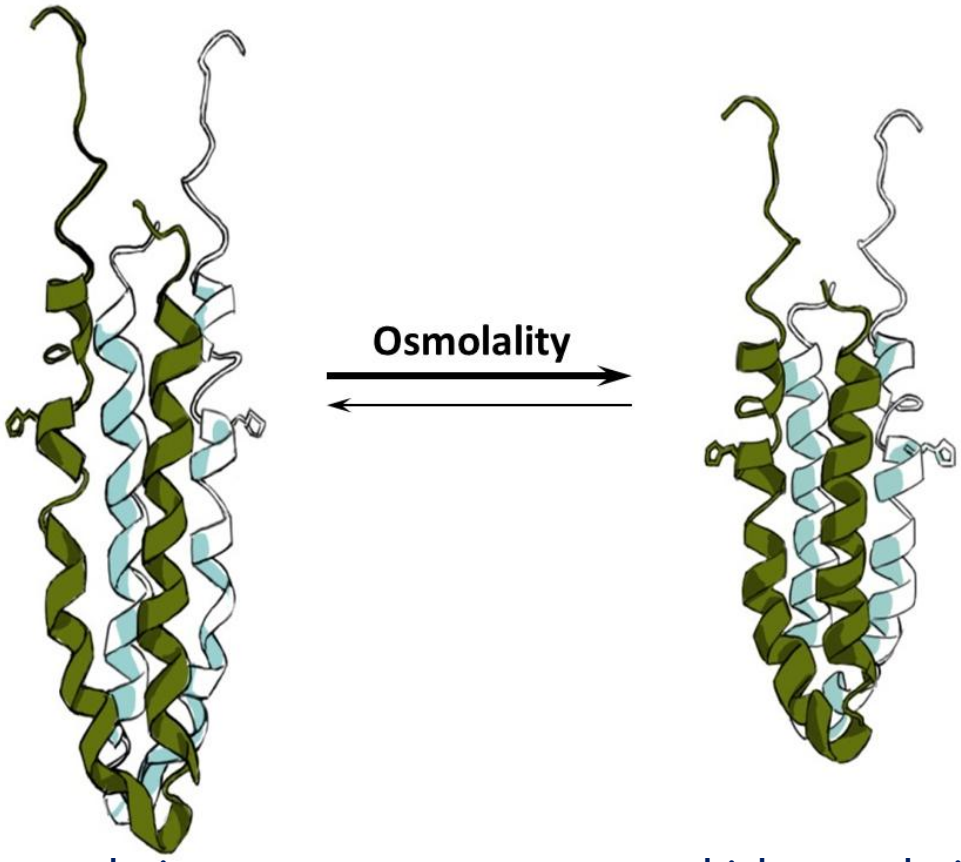


The change do not modify the intracellular osmotic pressure; smaller OmpC porin let to introduce less toxic compounds in high osmolarity environment (intestine) in comparison with other environment al low osmolarity

http://labs.mbi.nus.edu.sg/bpast/research.html

The Mechanism of Osmosensing by EnvZ

Coil to helix transition is the mechanism of osmosensing by the EnvZ histidine kinase



Low osmolarity

high osmolarity

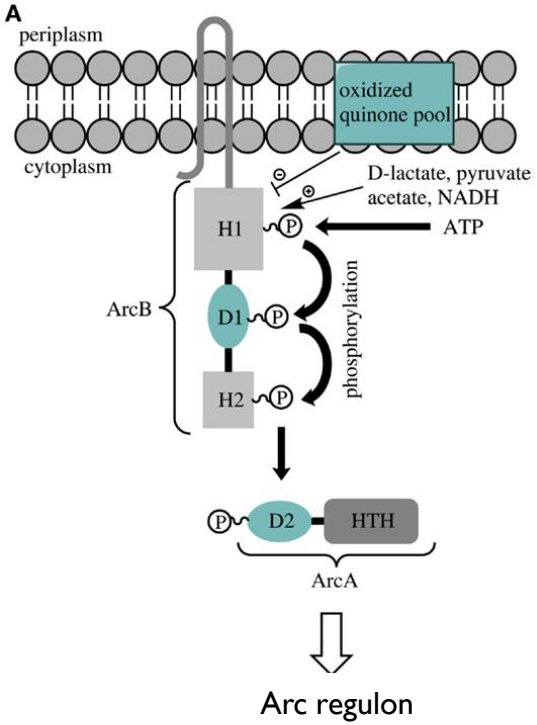
At high osmolarity, this peptide shows increased hydrogen bonding leading to helix stabilization and increased autophosphorylation.

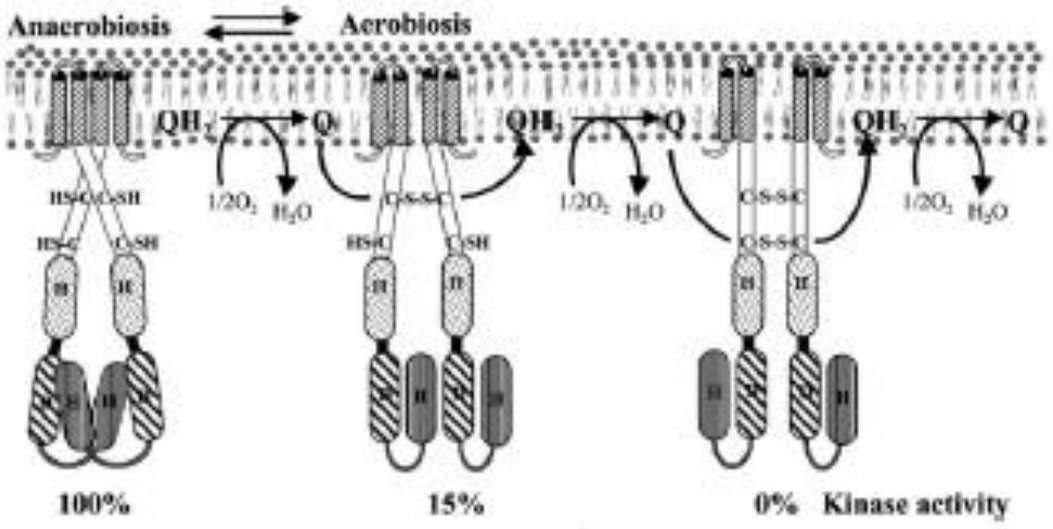
http://labs.mbi.nus.edu.sg/bpast/research.html

Redox regulation of ArcB sensor TK in *E. coli*.

Arc system regulates transcription under anaerobic conditions: it **down regulates** (through the ArcA RR) **oxigen metabolism** and activate anaerobic pathways.

the ArcB (HK) is phosphorylated when oxygen tension is low.





In presence of oxygen, the oxidized ubiquinone (Q) oxidize ArcB linker domain reducing (%) its phoshorylation state.

Arc regulon: comprises genes contributing to respiration and those involved in fermentation

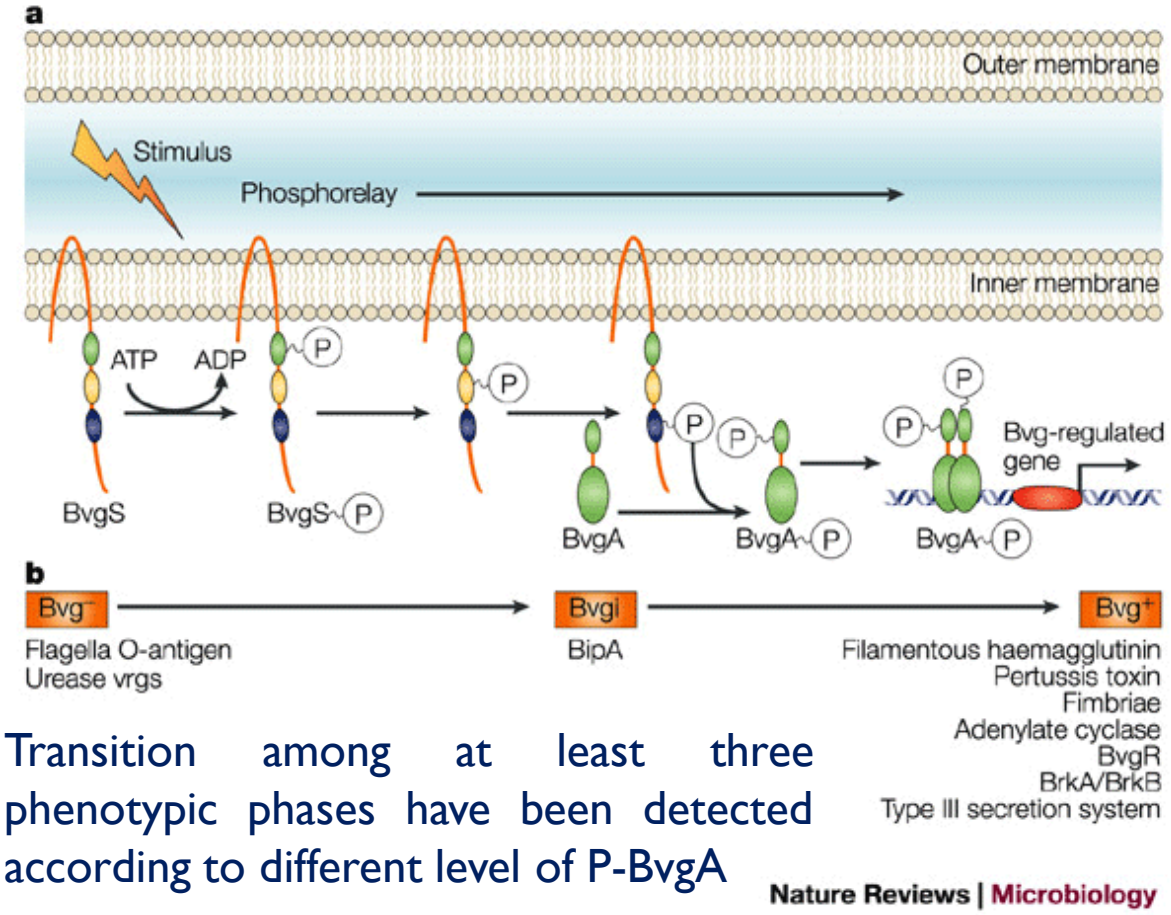
Two-component regulatory system used in the control of virulence gene expression

Table 4.23. Examples of two-component regulatory systems used in the control of virulence gene expression

Organism	Signal	Sensor	Response regulator	Response
Salmonella typhimurium	Mg^{2+}	PhoQ	PhoP	Ability to survive low pH and withstand antibacterial peptides
Salmonella typhimurium	Low pH within macrophage phagosome	EnvZ	OmpR	Activates a type III secretion system
Bordetella pertussis	Temperature, Mg^{2+}, nicotinic acid, SO_4^{2-}	BvgS	BvgA	Synthesis of filamentous haemagglutinin, pertussis toxin and adenylyl cyclase toxin
Enterococcus faecalis	Vancomycin	VanS	VanR	Enzymes required for vancomycin resistance
Shigella flexneri	Osmolarity	EnvZ	OmpR	Porin expression
Pseudomonas aeruginosa	Osmolarity	AlgR2	AlgR1	Alginate synthesis
Klebsiella pneumoniae	N_2	NtrC	NtrA	Urease production
Vibrio cholerae	pH, osmolarity, temperature	ToxS	ToxR	Synthesis of toxin and pili

The BvgAS TCS of *Bordetella pertussis* is switched on by a temperature sensor

BvgAS TCS of *B. pertussis* is a complex phosphorelay which is activated at 37°C. At T = 30°C no expression of virulence factors, toxins and adhesion structures are produced.



Transition among at least three phenotypic phases have been detected according to different level of P-BvgA

BvgS Sensor protein
BvgA Response regulator
Stimulus: changing T

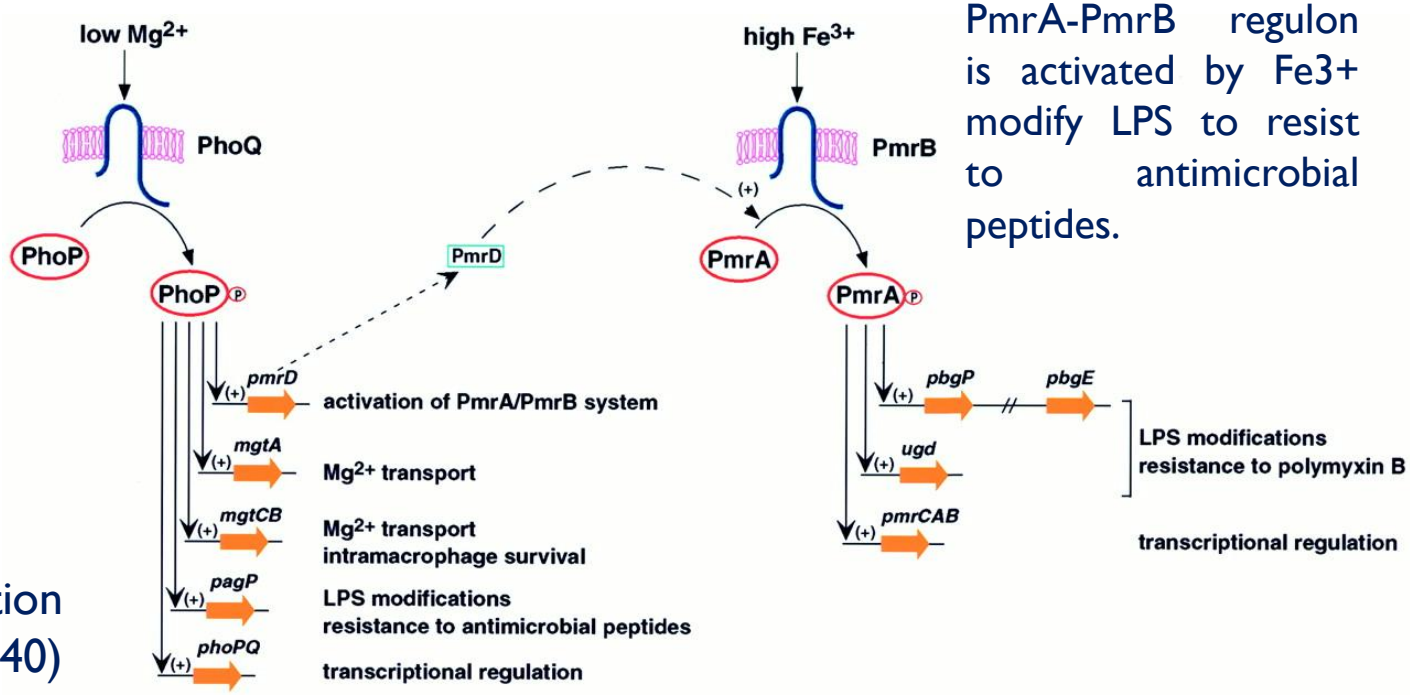
Several virulence genes (pertussin toxin) are activated by Bvg system, and different phenotype phases appear (Bvg-, Bvgi, Bvg+).

Bacteria do not synthesize molecules that they do not use normally in not in particular conditions

Salmonella enterica PhoP-PhoQ TCS

PhoP-PhoQ TCS is required for survival of *S. enterica* within macrophages in animal hosts. The TCS is regulated by **magnesium concentration**.

mM concentrations of Mg2+ repress the PhoP-PhoQ system.
Low concentrations of Mg2+ modifies PhoQ and promotes its autophosphorylation, with subsequent activation of PhoP.

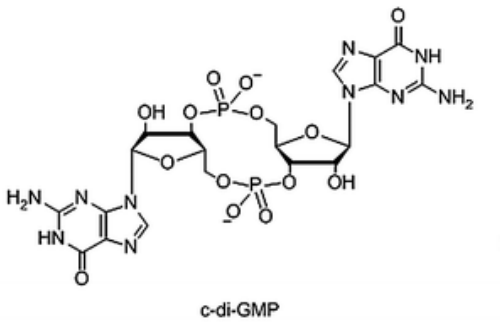


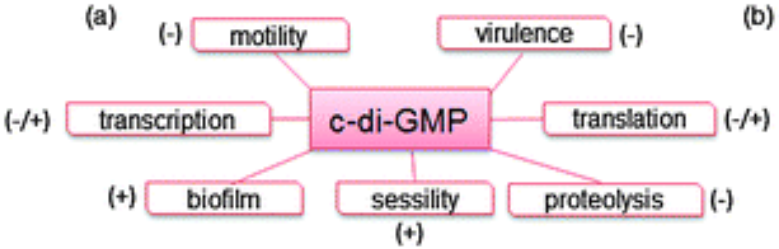
PmrA-PmrB regulon is activated by Fe3+ modify LPS to resist to antimicrobial peptides.

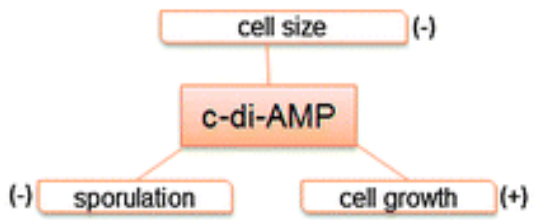
P-PhoP activates transcription of a number of genes (>40) that promote **survival of *Salmonella enterica* in the host** by direct and indirect effects.

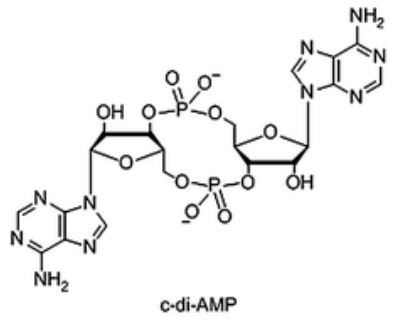
Among them, *pmrD*, mediates the PhoP/Q-dependent regulation of the PmrA-PmrB regulon

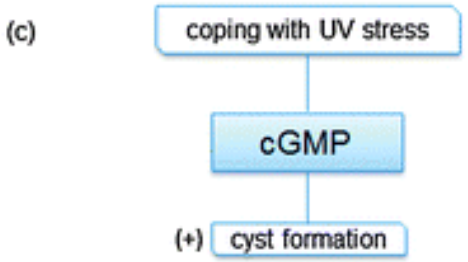
Nucleotide second messengers found in bacteria

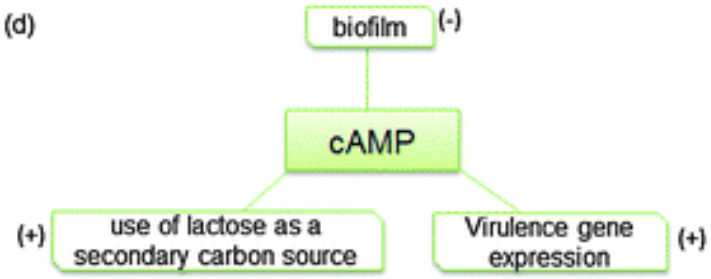


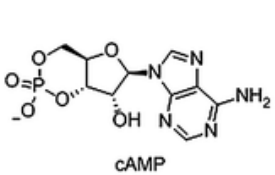


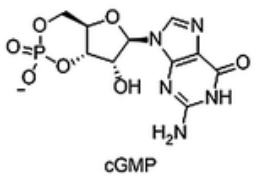


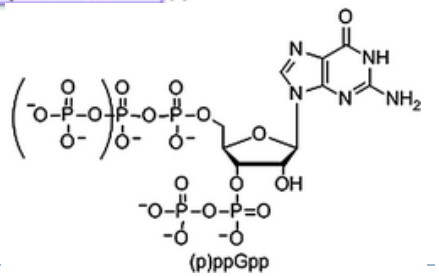






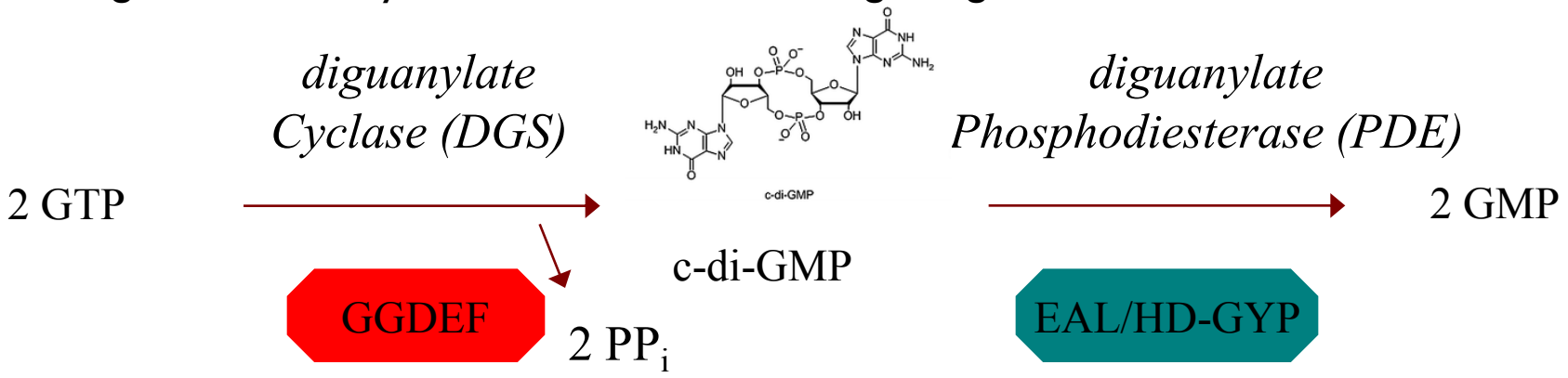






Cyclic-di-GMP-mediated regulation in bacteria

The second messenger bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) has emerged as a broadly conserved intracellular signaling molecule.



c-di-GMP is generated from two GTP molecules by GGDEF domain-containing **diguanylate cyclases.**

Phosphodiesterases containing either EAL or HD-GYP protein domains provide selective signal degradation.

Nearly all bacteria have GGDEF, EAL, and HD-GYP domain proteins, with many of them encoding multiples of these enzymes

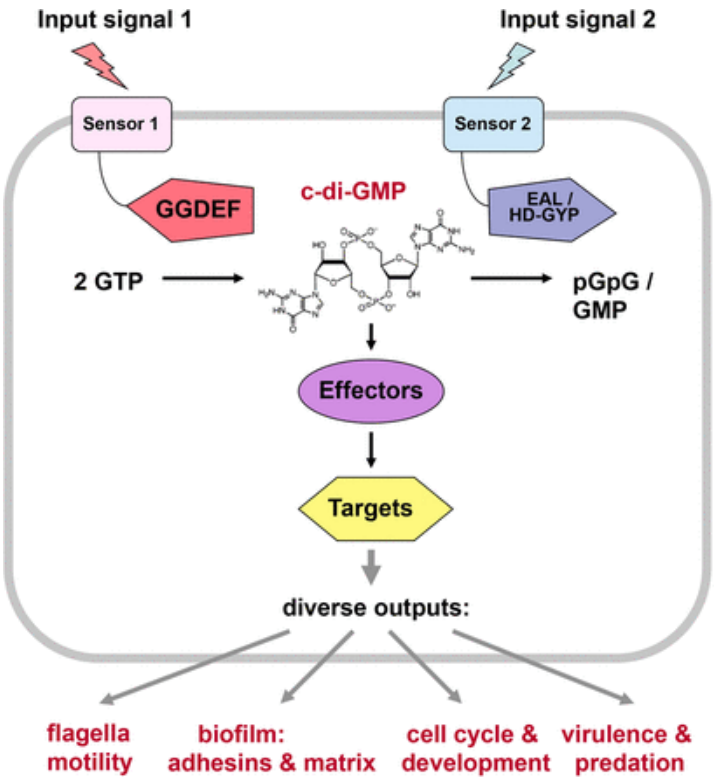
GGDEF domain proteins were unequivocally demonstrated to have **diguanylate cyclase** (DGC) activity, EAL and HD-GYP domain proteins were recognized as two distinct families of c-di-GMP-specific phosphodiesterases (PDEs)

General scheme for c-di-GMP signaling

The activities of a majority of GGDEF/EAL/HD-GYP domain proteins are controlled by N-terminal sensor domains, which are often integrated in the cytoplasmic membrane and may contain periplasmic loops that bind small ligands.

C-di-GMP signaling displays a high diversity concerning the effector components that bind the second messenger and mediate downstream effects.

Many types of c-di-GMP receptors have been found including: effectors proteins (as PilZ domain proteins), enzymes (allosteric modulators), transcription factors or repressors, riboswitches (regulate transcription/translation).



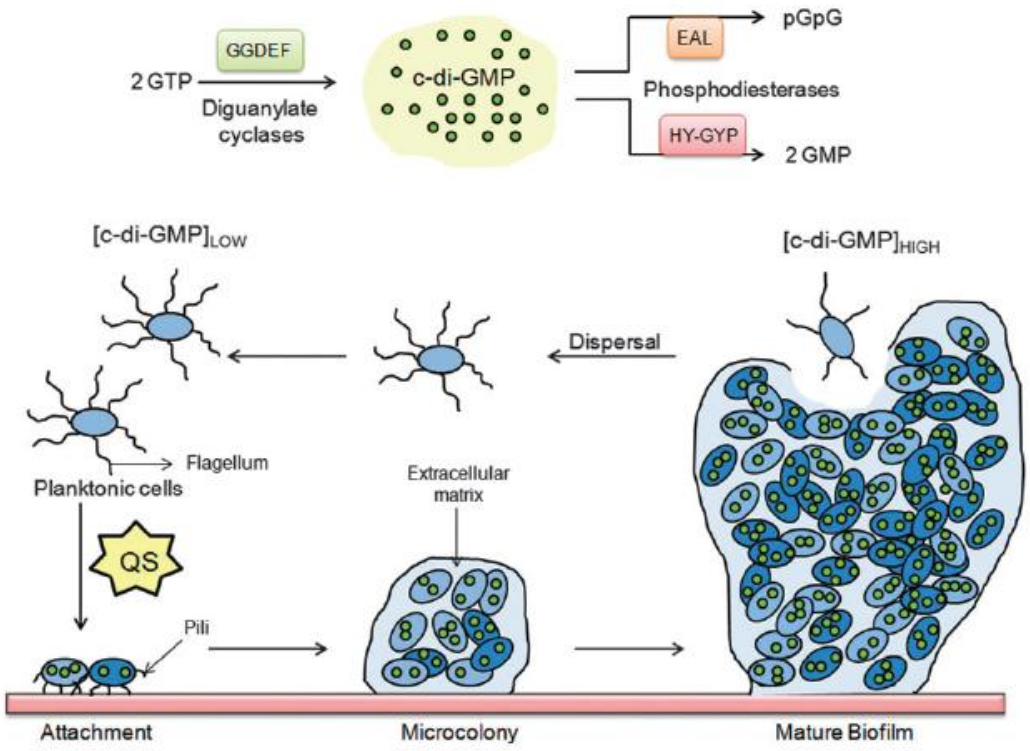
Production, degradation, mechanism of action, and physiological target processes of the second messenger c-di-GMP.

Puzzling questions of specificity of signaling in cells that might contain dozens of different enzymes that make and break c-di-GMP

C-di-GMP regulate several processes that control biofilm maturation pathways

Transition between two mutually exclusive lifestyles, motile single cells and sedentary multicellular communities (biofilms) is mainly regulated by the level of the secondary messenger c-di-GMP.

High level of c-di-GMP generally downregulates either the production or activity of flagella and activates the synthesis of adhesins and extracellular matrix components that are important for biofilm formation.



Alternative Sigma factors

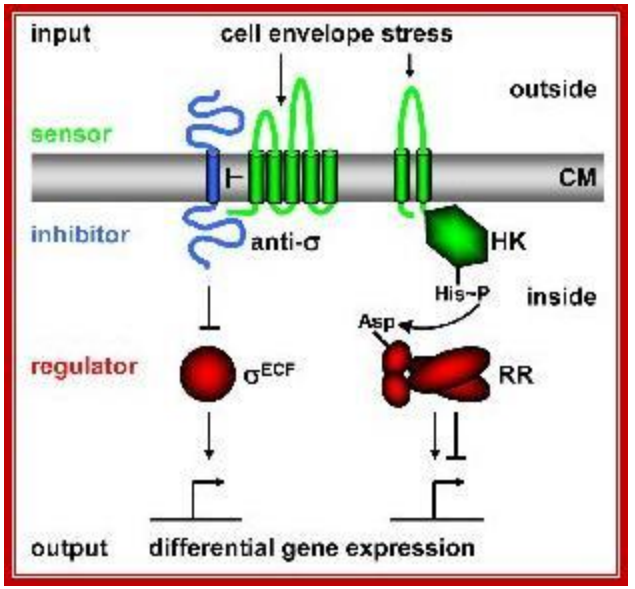
Alternative sigma factors are another common mechanism by which bacteria can alter their gene expression in response to a stimulus.

Bind RNA polymerase, allow recognition of alternative promoter sequences

σ^{70} (70 kDa)= major sigma factor in *E. coli*: housekeeping genes

At least 8 alternative sigma factors:

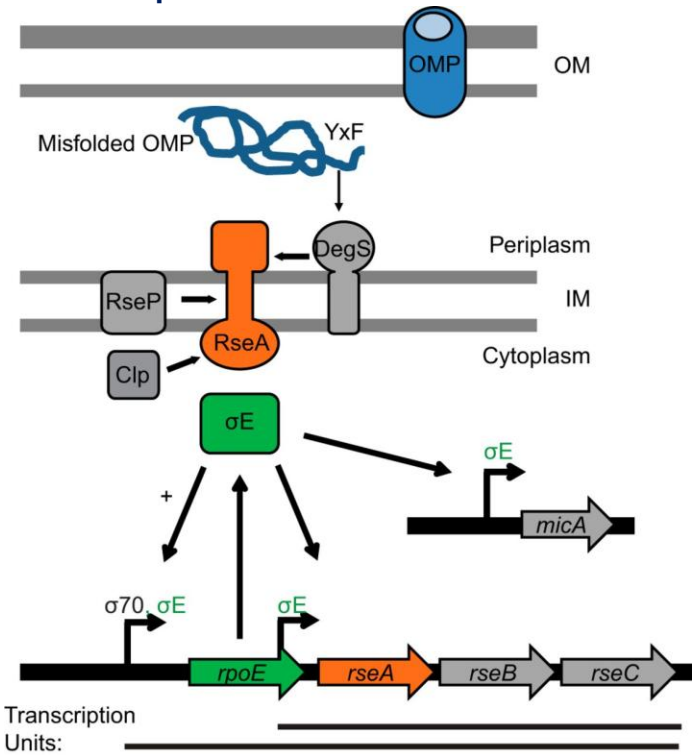
σ^{E} or extracytoplasmic function (ECF) protein



The extracytoplasmic function (ECF) sigma factors (σ^{E} in E. coli) are a unique class of alternative sigma factors that also regulate gene expression in response to extracellular signals (cell envelope stress).

The sigma factors σE stress response pathway

σ^E has been well studied for its role in combating extracytoplasmic stress. **Unfolded OMPs** activate a protease which cleaves the anti-sigma factor (RseA).



RseA (anti-sigma factor): IM protein which binds and inhibits σE. When the OMP assembly pathway is compromised, OMPs misfold in the periplasm and activates membrane protease (DegS) that initiates the proteolytic pathway, and RseA is further degraded by other membrane (RseP) and cytoplasmic (Clp) proteases. σE is released and activates expression from σE-dependent promoters.

σE is encoded by the *rpoE* gene, which is transcribed with *rseABC* from a σ70 promoter.

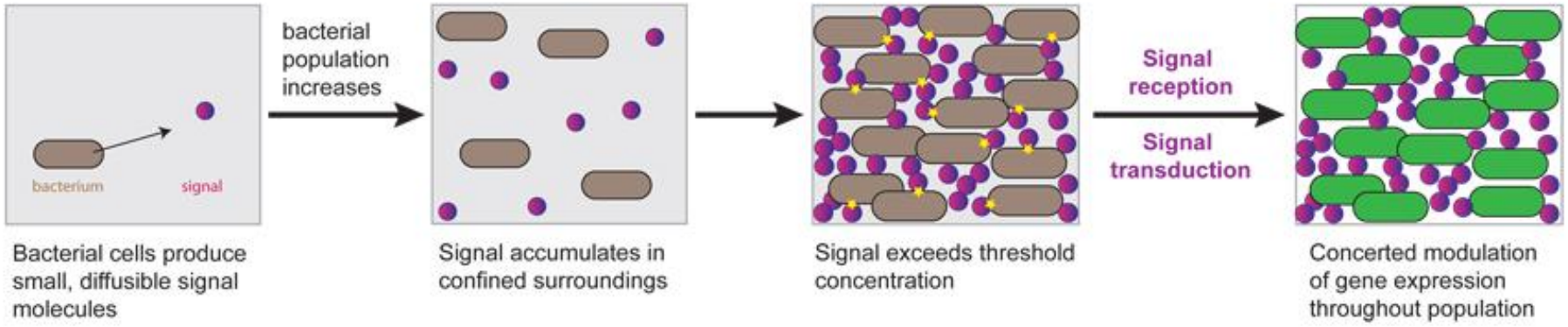
Anna Konovalova et al. J. Bacteriol. 2016;198:2345-2351

σE regulon: genes for translational downregulation of OMP synthesis, upregulation of the OM biogenesis machines and periplasmic chaperones to promote OMP folding as well as periplasmic proteases to degrade misfolded OMPs (positive-feedback loop).

Cell-to-cell signaling : Quorum sensing (QS)

Quorum sensing is a form of cell-to-cell signaling mechanism that enables the bacteria to collectively control gene expression. It is a form of **intercellular communication**. Bacteria normally release **autoinducers,** small, diffusible, signaling molecules in the extracellular medium. They uses autoinducers "to sense" the cell density of a population.

Schematic representation of bacterial gene regulation by 'quorum sensing':



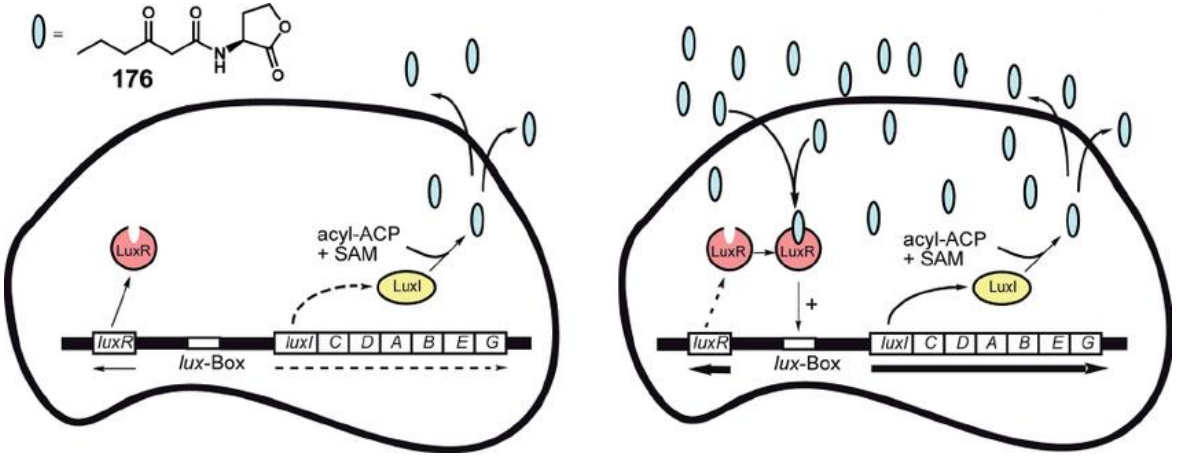
When concentration of these **signaling molecules** exceed a particular threshold value (**quorum**), these molecules activate transcription of a set of genes that are the same in all the bacterial population.

Quorum sensing allows the individual bacteria in that group to benefit from the activity of the entire group by concerted modulation of gene expression.

Quorum sensing in *Vibrio fischeri*

QS was discovered in marine bacterium ***Vibrio fischeri*** as mechanism of regulation of bioluminescence production**.** The LuxI/LuxR-type system is the model for Gram- bacteria quorum sensing.

LuxI is constitutively express at low level. Its product (autoinducer synthase, is responsible for the production of the autoinducer **AHLs** (acylACP +SAM). At high cell density, AHL is recognized by **LuxR** an autoinducer-receptor/DNA binding transcriptional activator. Upon the binding of AHL, LuxR positively regulates the luciferase operon.



The activation of **luxCDABEG** operon leads to the production of **luciferase** and its substrate which in turn generate bioluminescence.

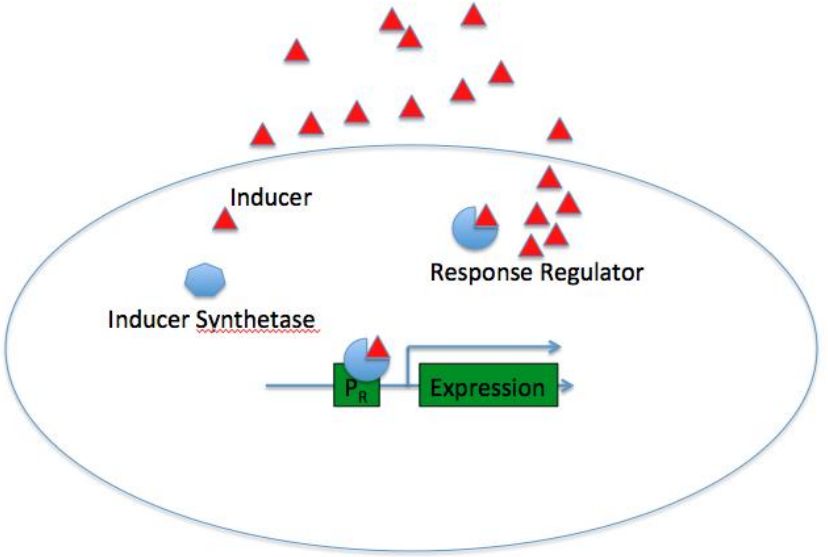
AHL = Acyl-homoserine lactones

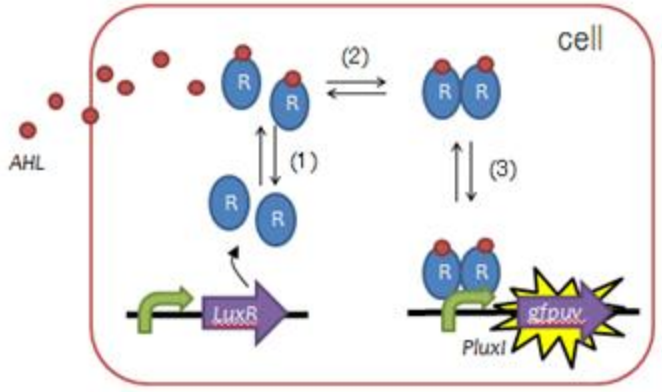
Quorum sensing in Gram-negative bacteria

A large number of gram-negative proteobacteria possesses LuxIR-type proteins and communicate with AHL signals, basically consisting of AHL synthase (homologous to **LuxI**) and a transcriptional activator (Homologous to **LuxR)** .

The LuxI-like proteins catalyze the formation of species-specific AHL signals. (intraspecies communication).
Extreme specificity exists between LuxR-like proteins bind their cognate autoinducers.

The LuxR-autoinducer complexes bind at target gene promoters and activate transcription.

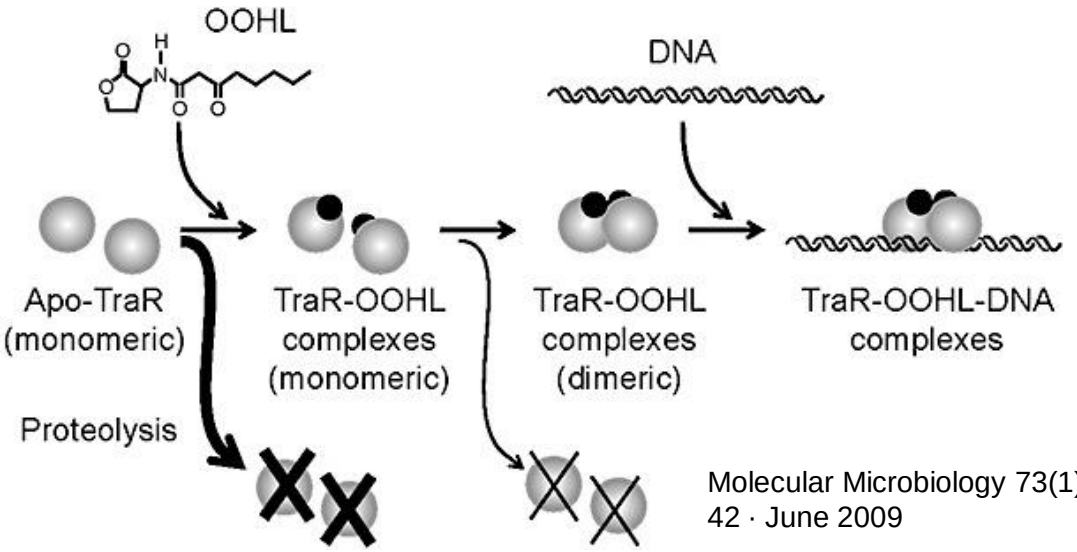




The LuxR-autoinducer complexes

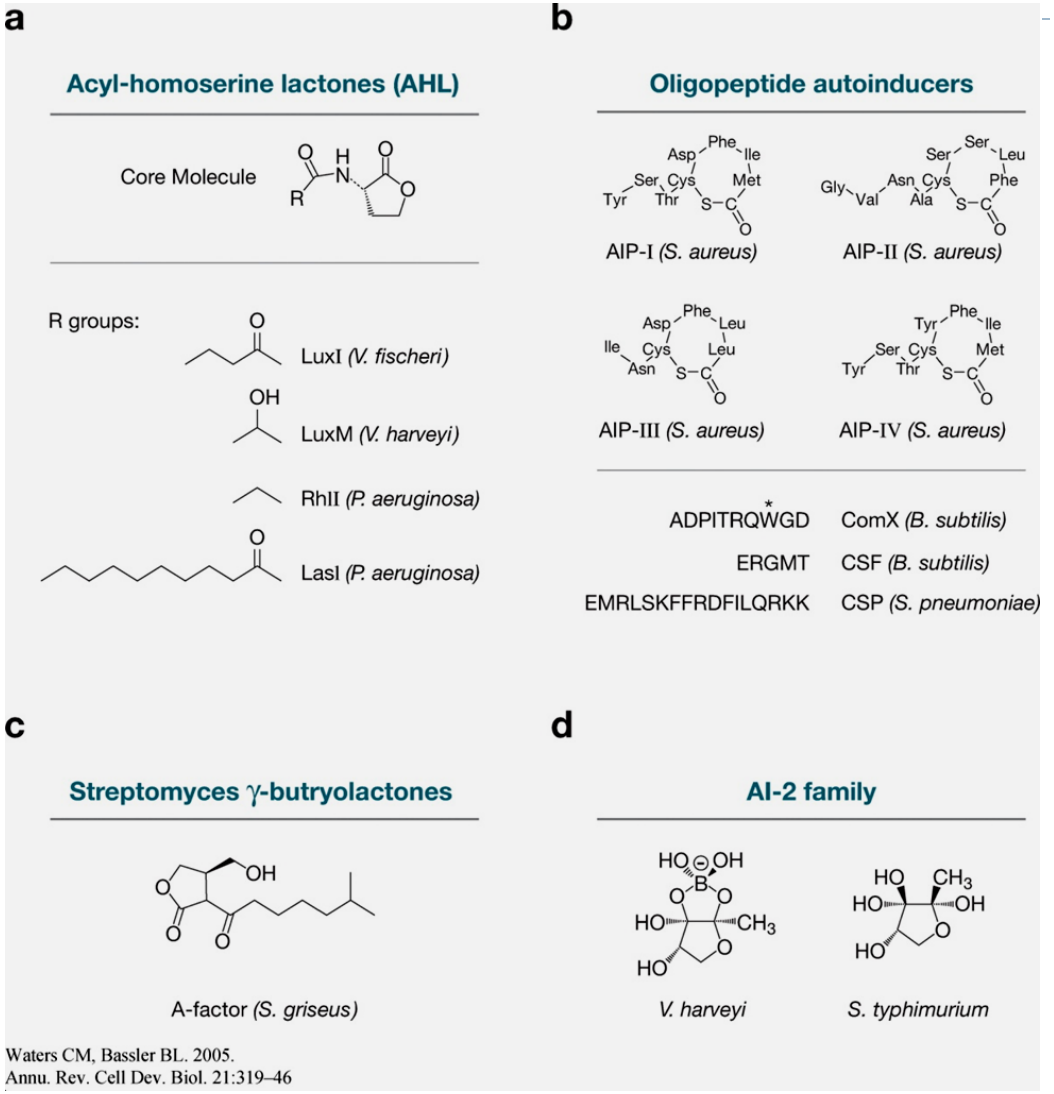
LuxR homologous (traR in the example): has a **C-terminal domain (DBD)** containing a **HTH motif** that can bind to the target promoter, and a **N-terminal domain (LBD)** that prevents the binding until the autoinducer is present and bound to LuxR.

Functional complex of quorum-sensing transcriptional regulator TraR from *A. tumefaciens,* a member of LuxR family. The pheromone OOHL indirectly affects gene activation by increasing stability of TraR and the formation of functional dimers that are predisposed to decode specific TraR-binding sites and activate transcription.



Molecular Microbiology 73(1):32-42 · June 2009

Different class of quorum sensing autoinducers



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

A) **Acyl-homoserine lactones (AHLs)** (AI-1) mediate QS in Gram- bacteria.
Most AHLs are species-specific, and differ each other in the length or modifications of the acyl side chain.
Able to diffuse through membranes.

B) Gram-positive bacteria use posttranslationally-modified cyclized **autoinducing peptides (AIPs)** as autoinducers. AIPs do not cross the membranes.

C) Streptomyces use γ–butyrolactones structurally related to AHLs

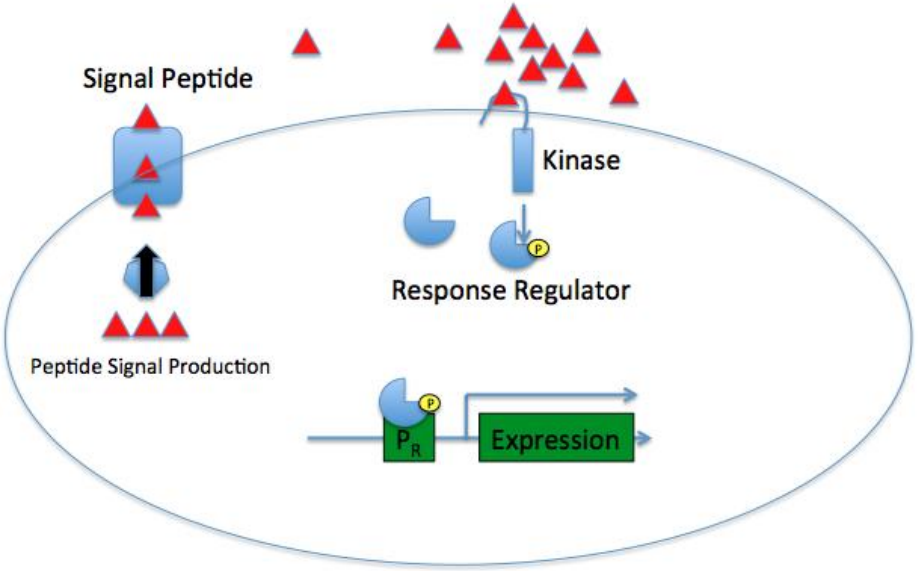
D) Autoinducer 2 (AI-2), such as **furanosyl borate diester**, let bacteria to have an **intergenic communication**. Receptor is a HK of a TCS.

Quorum sensing in Gram-positive bacteria: autoinducing peptides (AIPs)

Gram-positive bacteria use different signals: autoinducing peptides, **AIPs** are small peptides, inducers of QS gene expression in Gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus*

A specific precursor peptide is produced. The precursor peptide is modified, processed, and secreted as a mature **AIP** by an ATP-binding cassette (ABC) transporter. It is then recognized by a specific membrane-bound sensor protein belonging to a TCS.

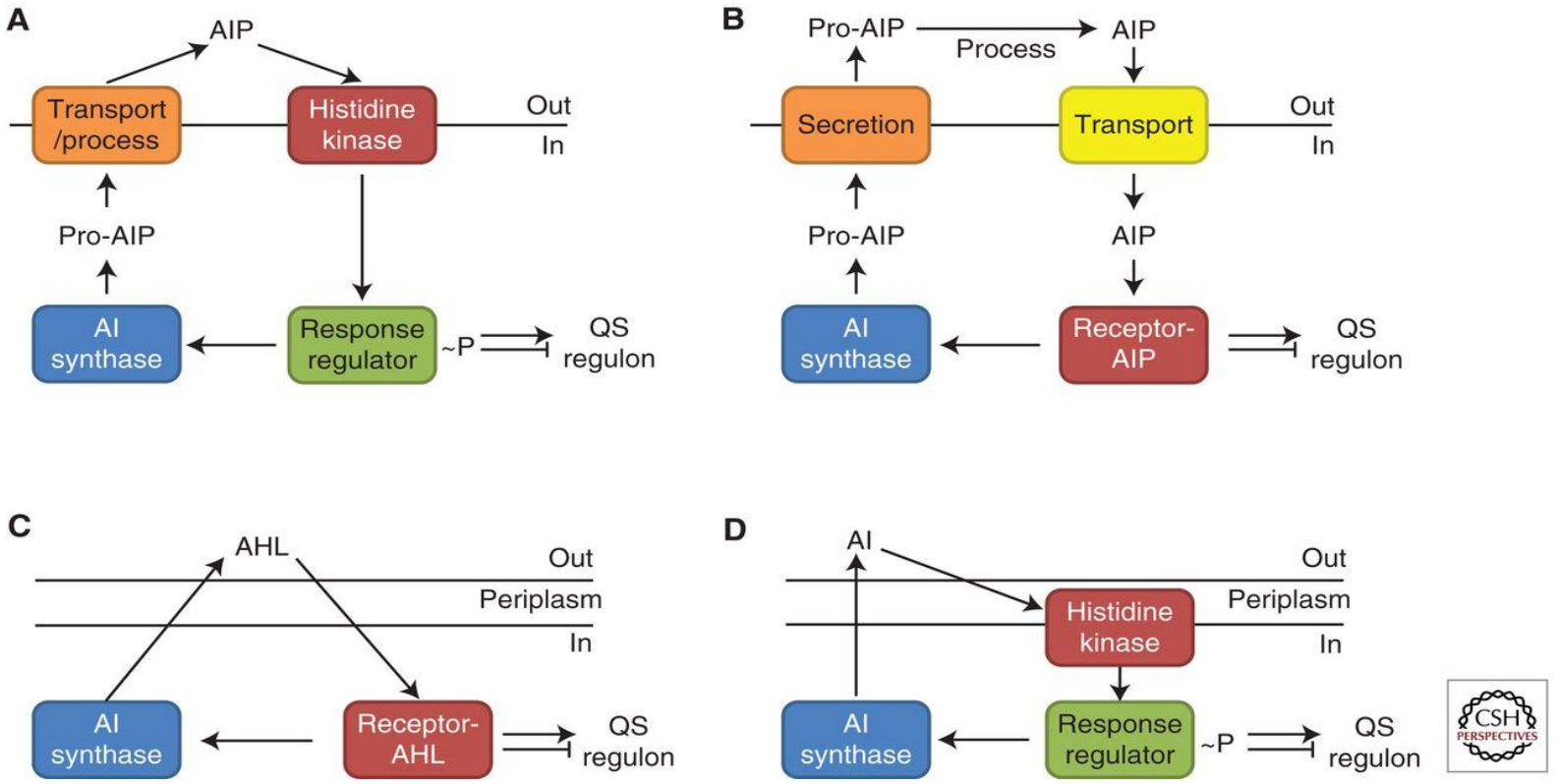
The genes encoding the peptide precursor, the ABC transporter and the TCS receptor are usually transcriptionally linked.



It regulates genes for DNA competence and sporulation.

AIPs from different Staphylococcal species may interfere with the signalling from each other.

Canonical bacterial quorum-sensing (QS) circuits



Steven T. Rutherford, and Bonnie L. Bassler Cold Spring Harb Perspect Med 2012;2:a012427

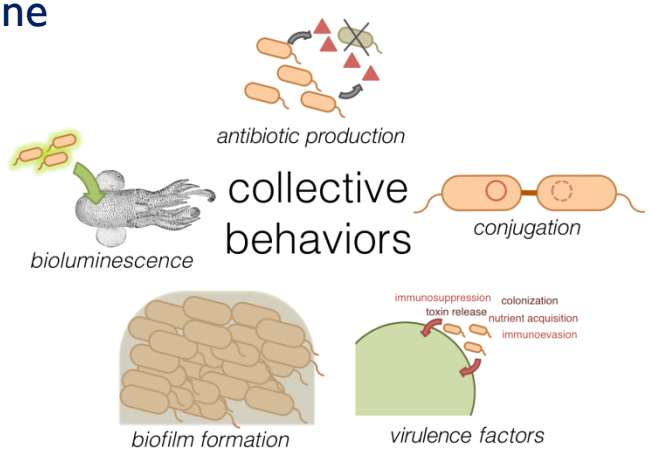
Autoinducing peptide (AIP) QS in Gram-positive bacteria by (*A*) two-component signaling, or (*B*) an AIP-binding transcription factor.
Small molecule QS in Gram-negative bacteria by (*C*) a LuxI/LuxR-type system, or(*D*) two-component signaling.

Quorum sensing-controlled processes

Most quorum-sensing-controlled processes are unproductive when undertaken by an individual bacterium acting alone but become beneficial when carried out simultaneously by a large number of cells. Thus, quorum sensing confuses the distinction between prokaryotes and eukaryotes because it enables bacteria to have collective behaviors and act as multicellular organisms.

- **Bioluminescence:** (symbiosis) It occurs in various marine bacteria such as *Vibrio fischeri.*

- **Biofilm formation:** It is compact mass of differentiated microbial cells, enclosed in a matrix of polysaccharides. Quorum sensing is responsible for development of thick layered biofilm.



- **Competence** It is ability to take up exogenous DNA QS Increase competence for genetic transformation in *Bacillus subtilis*

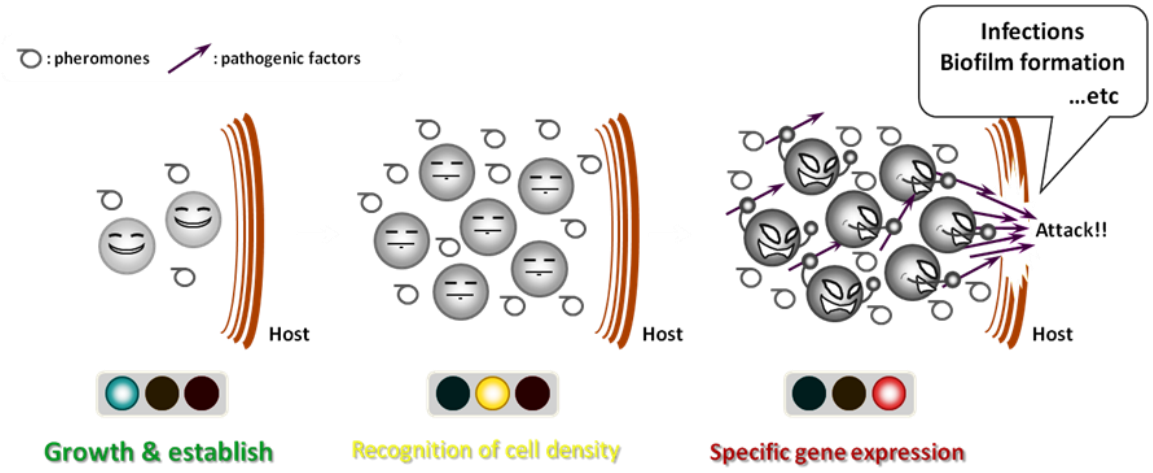
- **Sporulation** QS upregulates spore-forming genes in *Bacillus subtilis and other Gram+ bacteria*

- **Virulence gene expression** QS upregulates virulence gene expression.

Quorum sensing in bacterial pathogenesis

- A number of Gram-negative pathogenic bacteria employ AHLs as virulence determinants, for example, *P. aeruginosa, Yersinia* spp. *V. cholerae* and *Burkholderia cepacia* but also gram-positive such as *Staphylococcus aureus*.
- In Several QS system mutant in synthesis of autoinducer molecules, shows heavy reduction in virulence

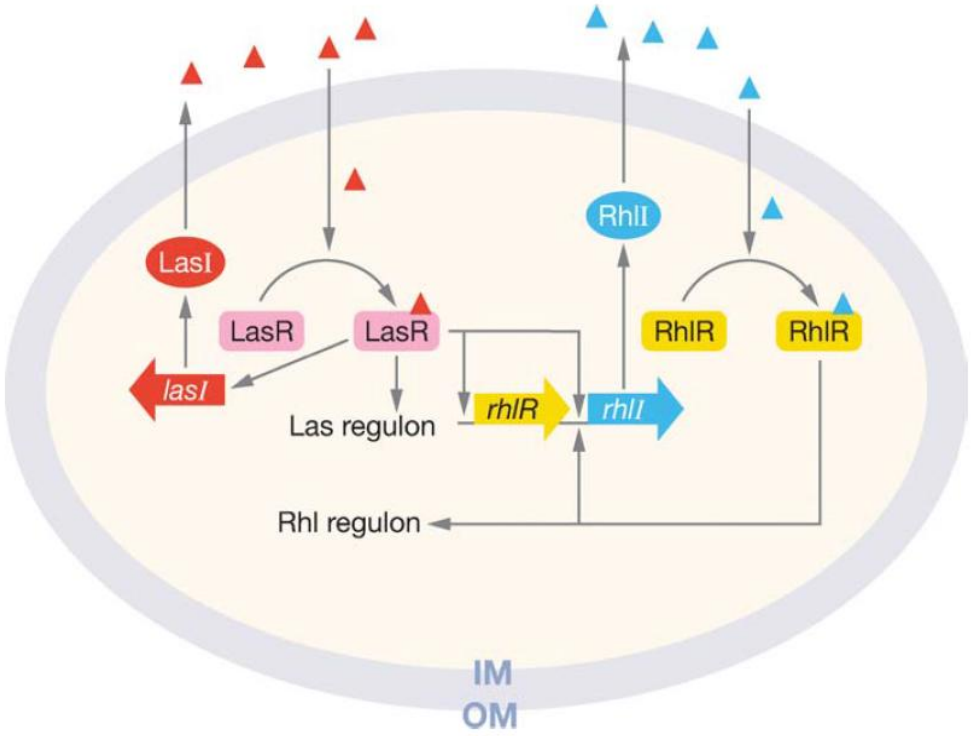
- Activation of virulence genes at low population density would result in the generation of host defence responses, thus providing the host an early lead over the invading bacteria.
- In order to guarantee their survival, certain pathogens evade the synthesis of virulence factors until their number makes certain their success in the infection process (to overpower the host defence mechanisms).



Cell density dependent gene expression in quorum sensing (e.g. virulence expression)

Quorum-Sensing Circuits P. Aeruginosa are Arranged in Series

Expression of many of the extracellular factors *P. aeruginosa* are cell-density dependent. Two QS systems, the LuxI/LuxR homologs **las** and **rhl are** essential for chronic *P. aeruginosa* respiratory infection because they controls adhesion, biofilm formation, and virulence factor expression.



LasI, produces an AHL autoinducer (3OC12-hsl)
RhlI produces the AHL C4-hsl

A number of virulence genes are regulated by either the **las** QS system or the **rhl** QS system or both.
The LasR-autoinducer complex also activates the expression of rhlR and rhlI encoding a second quorum-sensing circuit.

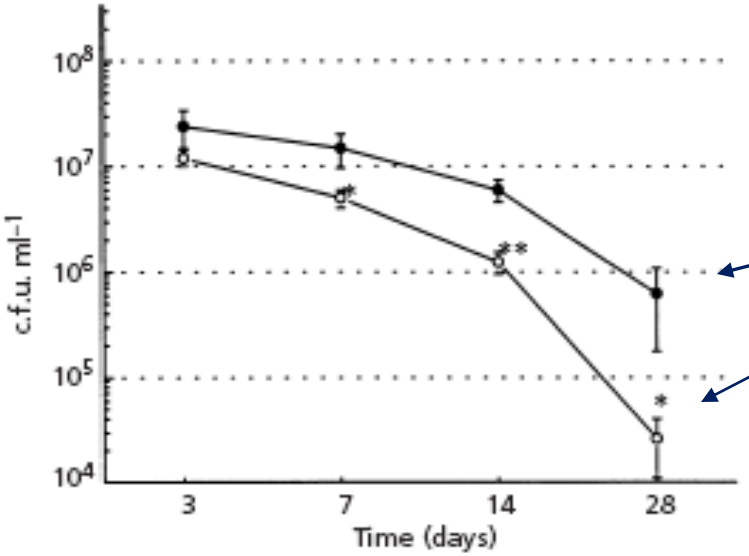
A hierarchical circuity network indeed produces a temporally ordered sequence of gene expression (multiple virulence traits including exoproteases and exotoxins production, siderophores, several secondary metabolites and biofilm formation) that may be critical for the ordering of early and late events in a successful infection

Quorum sensing Las and rhl have a role in virulence

Genetically engineered *las* and *rhl* system mutants have been shown to **have significantly reduced virulence** in several animal models of infection

These studies support a central role for AHL quorum sensing in *P. aeruginosa* disease.

lasI mutant was found to produce a thinner biofilm, more susceptible to disruption by detergents.
These findings, have led to considerable interest in the development of quorum-sensing inhibitors as a means to prevent or treat biofilm-associated infections.



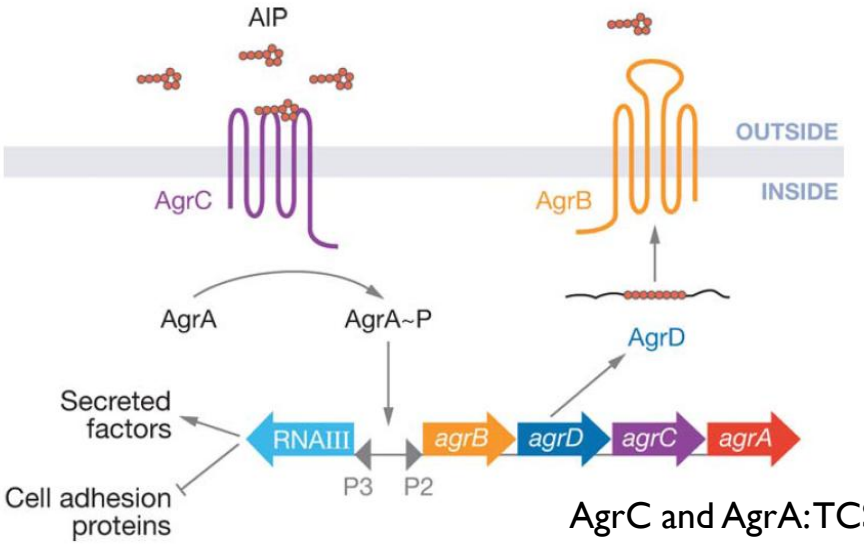
Wu et al., 2001

In an in-vivo study, using two strains P. aeruginosa; PAO1 (wt, virulent), and PAOR (lasI and rhlI double mutant, avirulent), it was seen that rats infected with PAOR are much immunologically active and the number of P. aeruginosa also reduced.

The Agr system of *S. aureus*

The virulence of *S. aureus* is dependent on **the temporal expression of a diverse array of virulence factors.** The genetic basis for this temporal gene expression depends on the QS system **Agr.**
In early stages of *S. aureus* infection, surface proteins involved in attachment predominate. Once a high cell density is achieved at the infection site, expression of *S. aureus* surface proteins is decreased and secreted proteins (proteases, lipases, collagenases, exotoxins) necessarily for invasion and dissemination are preferentially expressed.

The *agr* locus produce 2 divergent transcripts: an operon encoding, *agrB, agrD, agrC and agrA* genes and **RNAIII**, a regulatory RNA which is responsible for posttranscriptional regulation of multiple virulence factors.
When RNAIII concentration increases with cell density, it suppress production of cell wall-associated cell adhesion proteins and enhance that of secreted exoproteins.



AgrC and AgrA: TCS
AgrD: autoinducing peptide
AgrB: protein export system