Chapter 3 Signaling changes in the environment

a.a. 2020-21

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 mainly 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).

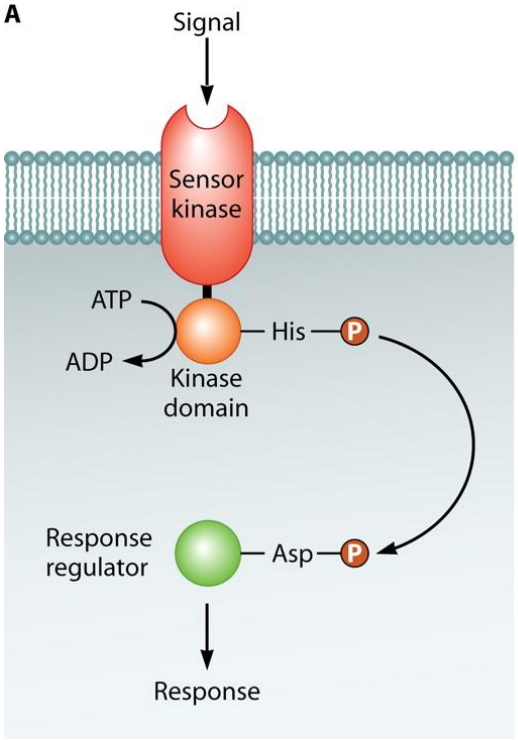
Two-component regulatory systems (TCS)

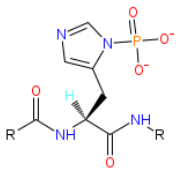
The main signal-transducing system largely used in bacteria is mediated by a **phosphotransfer signaling system** named **two-component system (TCS).**
It occurs also in archea, fungi, and plants.

TCSs are linear signal transduction systems in which there is not amplification of the signal (phosphotransfer).

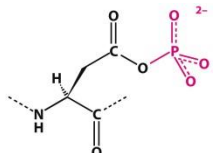
I component is a **sensor protein** (sensor kinase) that "senses" a specific environmental stimulus changing its conformation.

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





Phosphohistidine

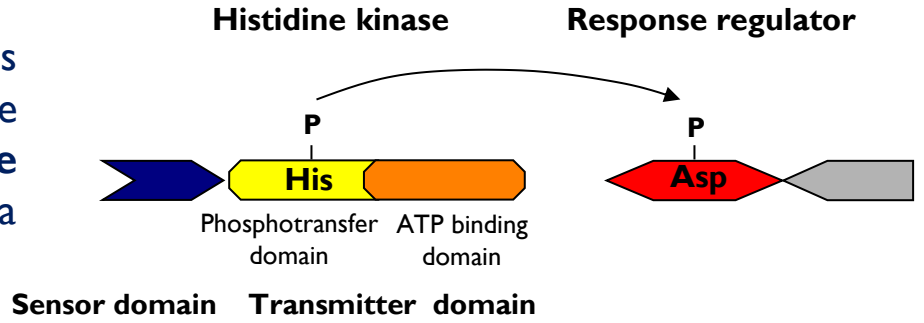


Phosphoaspartate

Sensor proteins: histidine kinases (HK)

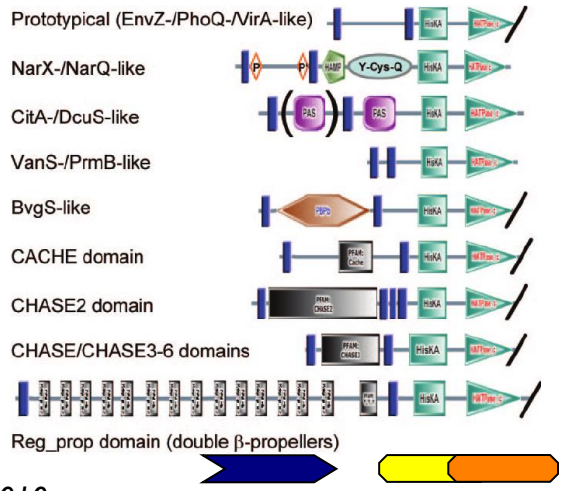
Sensor proteins are membrane-bound **histidine kinases (HK)** having two domains:
a N-terminal variable in sequence **sensor domain** (input domain) that is used to sense extracellular signals.

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



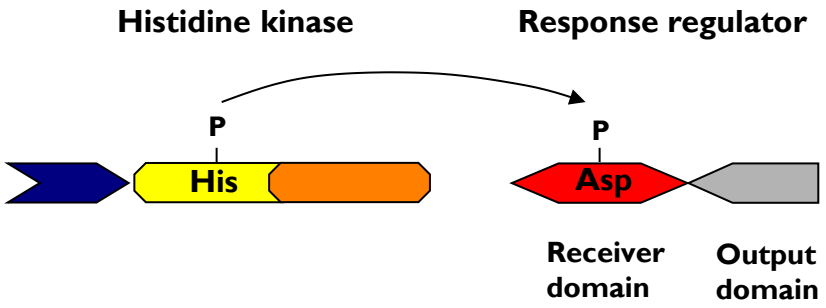
Canonical HKs function as **homodimers**: autophosphorylation is a bimolecular event. In many cases the ligand is unknown

The **transmitter domains** (green colour) contain conserved sequence motifs common to most sensor proteins. Most sensors are periplasmic-sensing HK (A), others show sensing mechanisms linked to the transmembrane regions or are cytoplasmic-sensing HKs



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 **HK** catalyzes the transfer of the phosphoryl group from the phospho-His residue to a conserved **aspartate** residue in the of the associated **receiver domain** of the **response regulator** (RR). This domain contains conserved sequence motifs common to most receiver proteins.

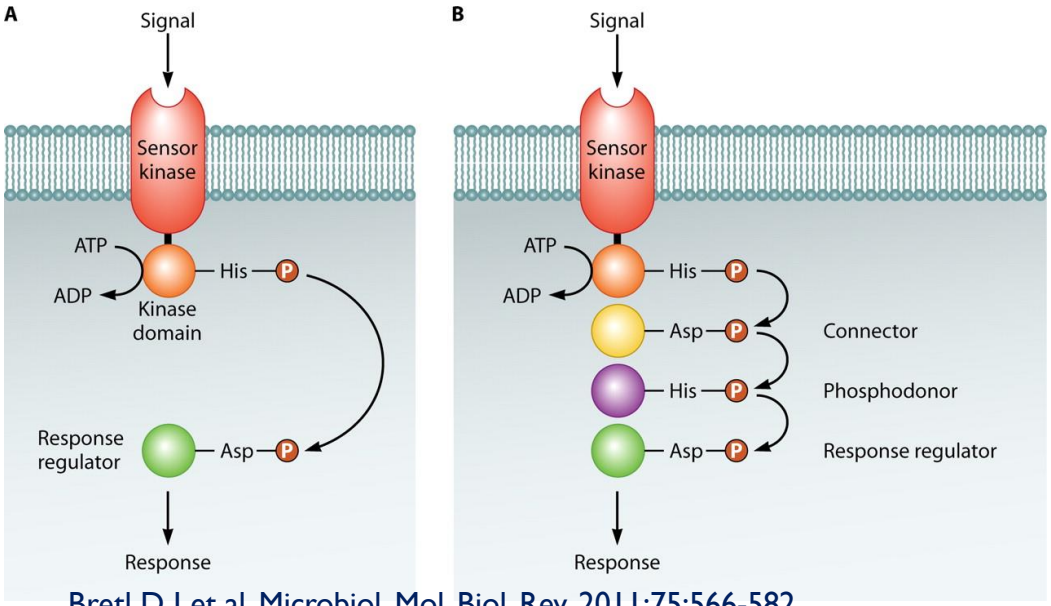
Its phosphorylation changes the conformation of RR which results in modulation of the function of the linked **output domain.** Hydrolysis of the phosphoryl group in the RR by phosphatase that dephosphorylates the RR (often due to HKs) resets the system.

Output domains (regulatory domain) are usually **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.

Phosphorelay systems are variants of TCS in bacteria

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

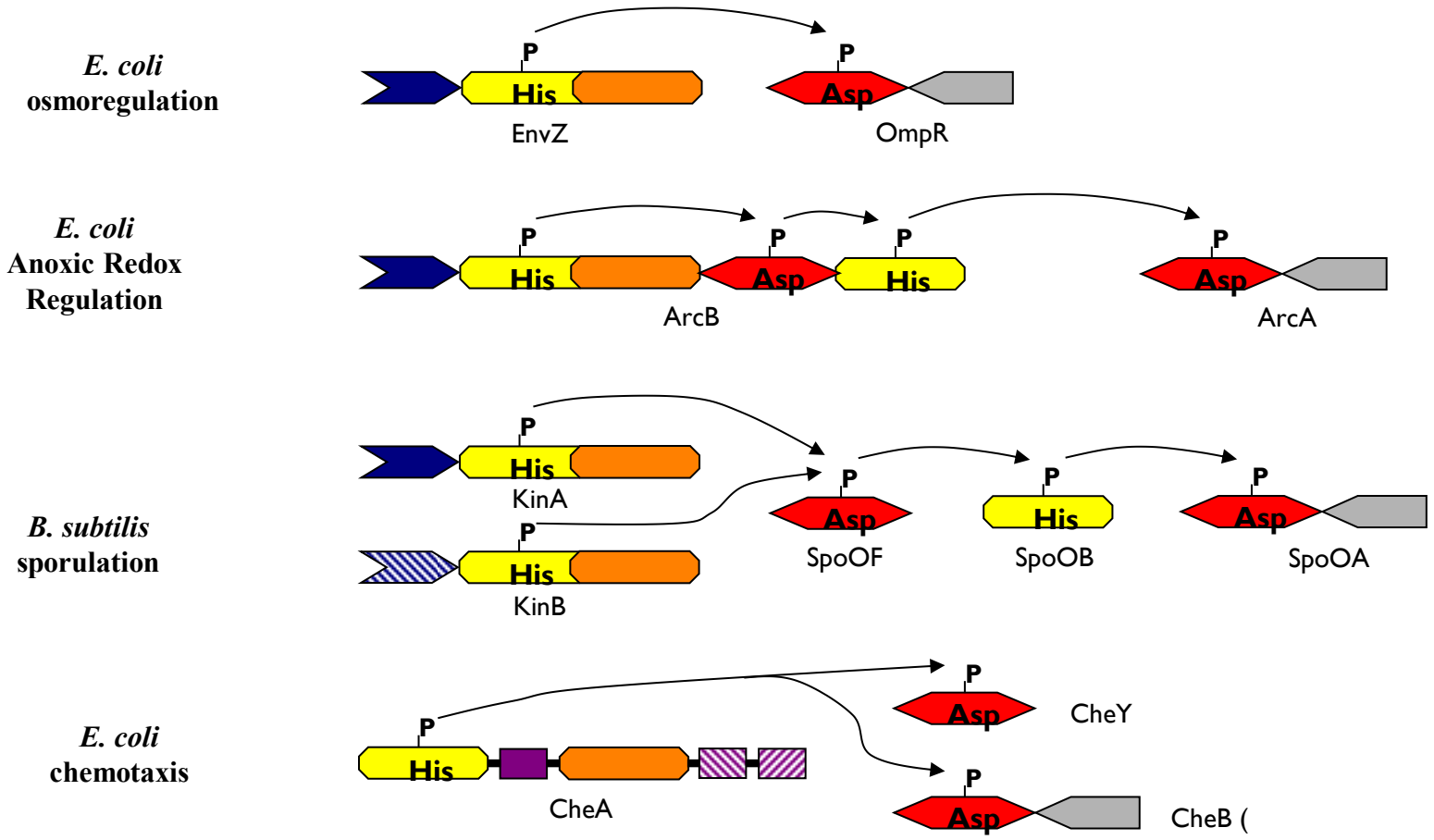
A phosphorelay contains, additional elements (**phosphotransfer proteins)** which modulate the levels of the active form of response regulators by affecting the various possible steps that determine their phosphorylation state or their activity.
Additional elements include intermediate response regulator (**connector**) lacking an output domain and His-containing phosphotransfer protein (**phosphodonor**).



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

In some phosphorelays, the phosphotransfer protein and/or the intermediate response regulator is fused with the sensor kinase in a single polypeptide giving hybrid HK. Cross-talk between different two-component signaling pathways at the level of phosphorylation is rare

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*

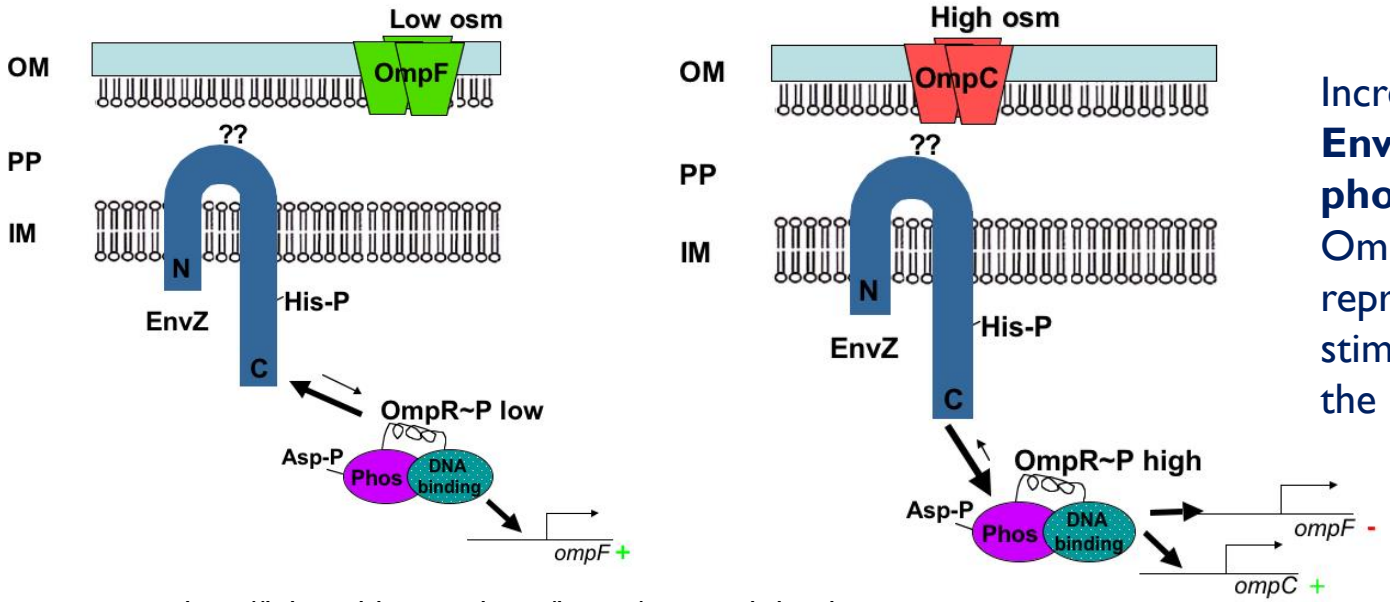
Table 4.22. Examples of two-component signal transduction systems in *Escherichia coli*

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+}

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.

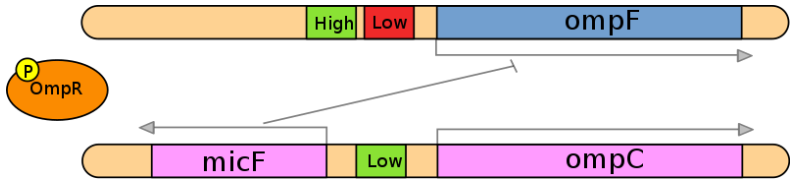


http://labs.mbi.nus.edu.sg/bpast/research.html

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

Osmolarity Changes and Porin Regulation

Low osmolarity response: the amount of the phosphorylated form of OmpR in cells is relatively small. In this situation, the OmpR-P complex binds cooperatively to high affinity activator sites of ompF gene and upregulates its transcription. Activator sites of ompC have low relative affinity for OmpR-P complex and transcription levels of ompC is less as compared to ompF.

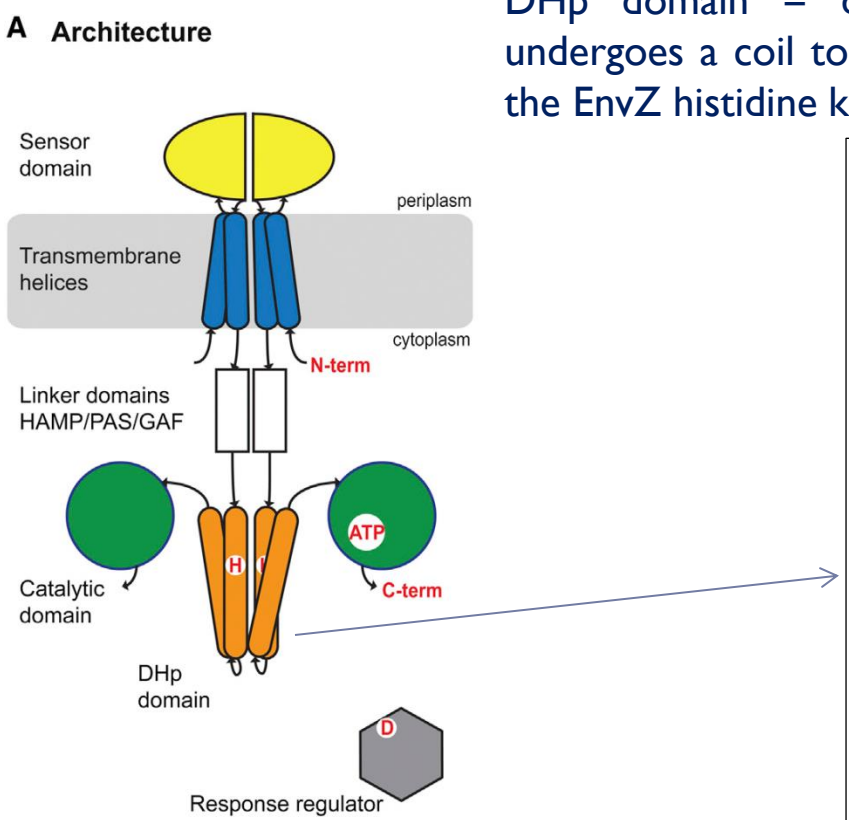


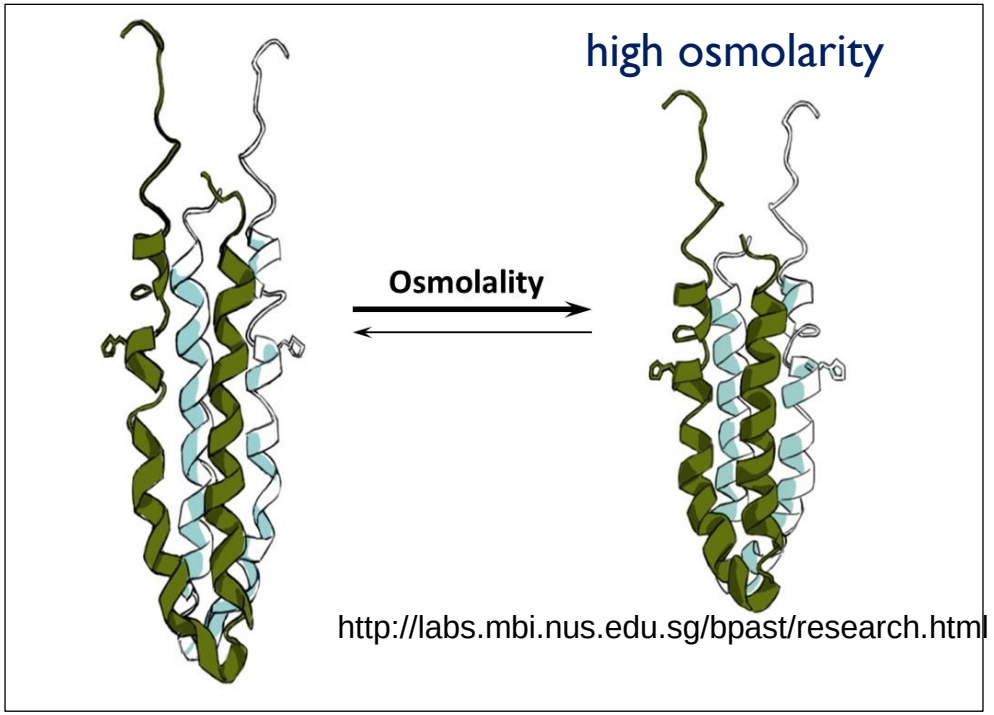
High osmolarity response: the number of OmpR-P protein molecules increases, it binds not only to the high affinity binding sites upstream of the ompF promoter but also to the low affinity-binding site resulting in repression of ompF gene. Further, OmpR-P binds to the low affinity activator sites upstream of the OmpC promoters, and as a result ompC gene expression is stimulated and more OmpC porin protein is expressed.
Also, transcription of micF antisense mRNA is initiated at high medium osmolarity. micF binds to complementary sequence of ompF mRNA to block its translation.

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.

The Mechanism of Osmosensing by EnvZ

DHp domain = dimerization and histidine phosphotransfer domain undergoes a coil to helix transition is the mechanism of osmosensing by the EnvZ histidine kinase



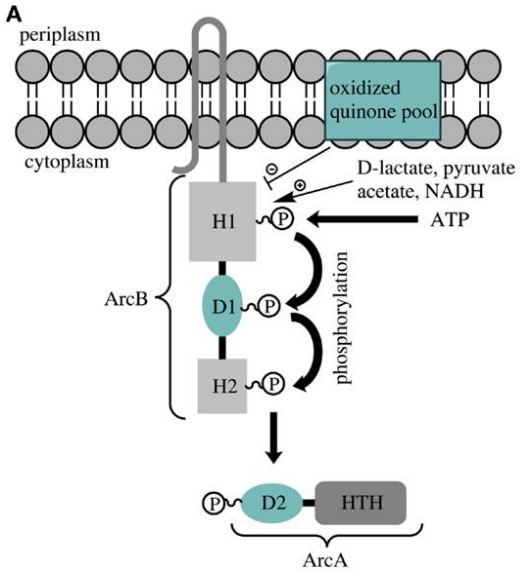


Structure 23, June 2, 2015 ª2015 Elsevier Ltd

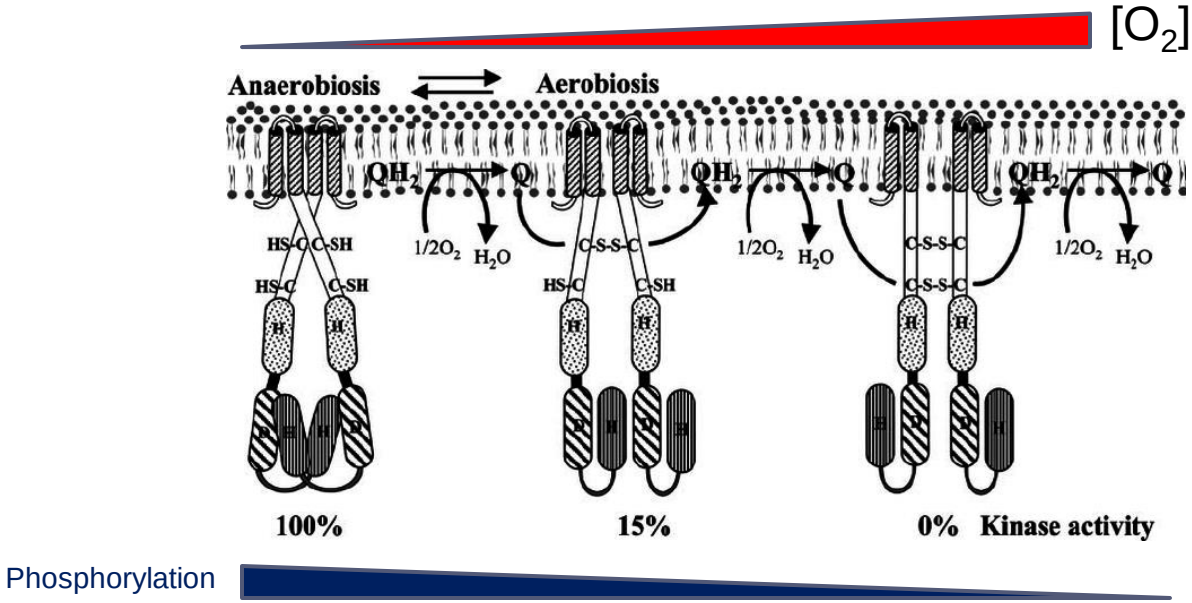
At high osmolarity, the histidine-containing helix (shown with the His side-chain sticking out peptide) shows increased hydrogen bonding leading to helix stabilization and increased autophosphorylation.

Redox regulation of ArcB sensor HK 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.



Arc regulon



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. Under anaerobic conditions, ArcA-P directly represses the operons encoding enzymes of the TCA cycle, and for the β-oxidation of fatty acids

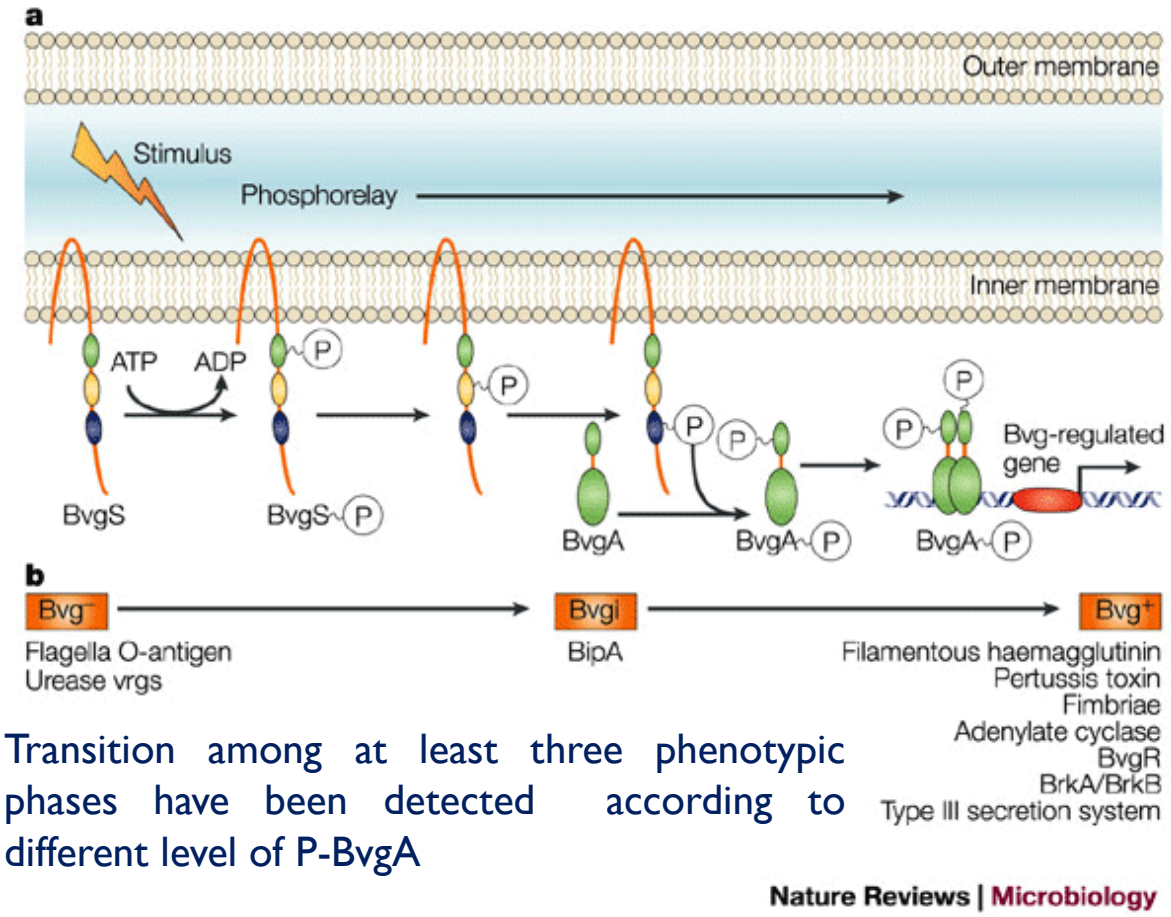
Two-component regulatory system used in the control of virulence gene expression

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

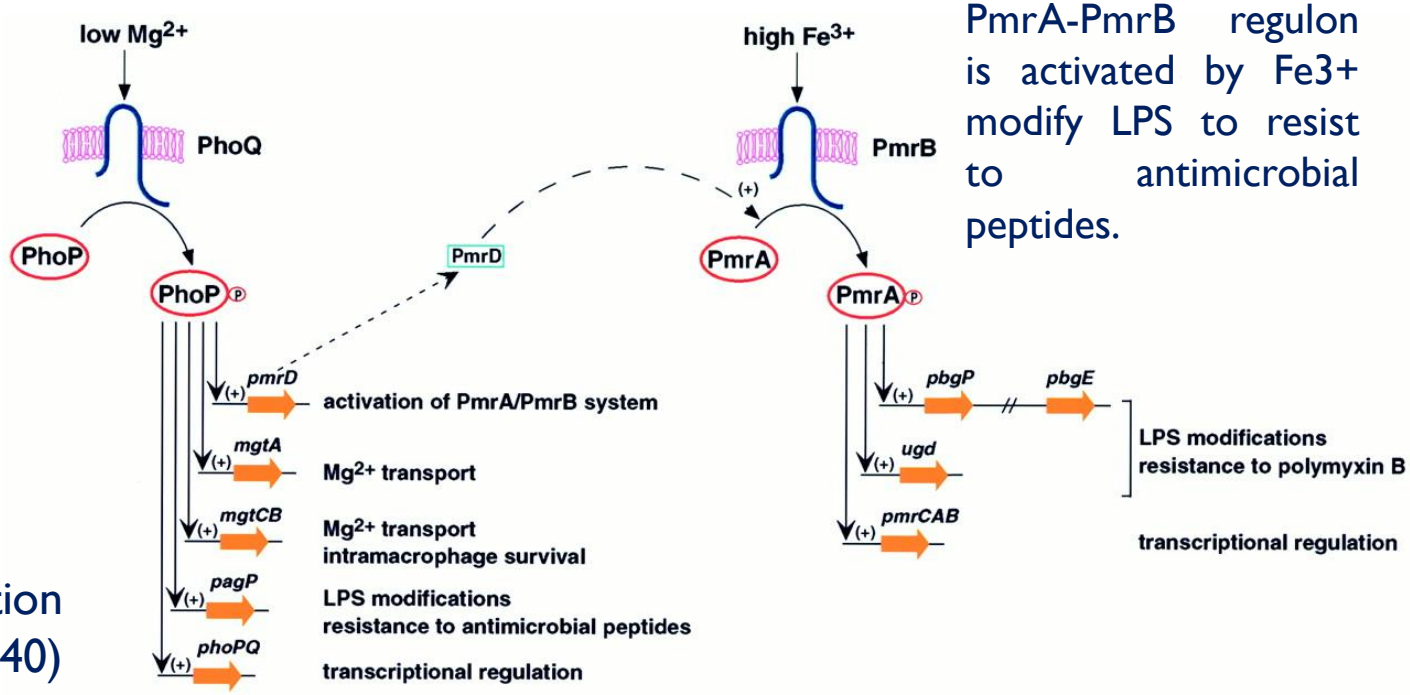
Several virulence genes (pertussin toxin) are activated by Bvg system, and different phenotype phases appear (Bvg-, Bvgi, Bvg+).

The expression of different classes of virulence genes reflects slight differences in the growth conditions within distinct host niches colonized by the bacteria, such as in the nasal cavity, larynx and trachea.

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.

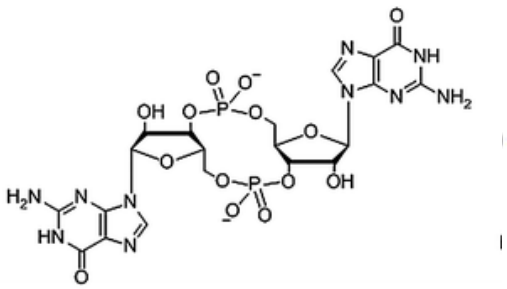


PmrA-PmrB regulon is activated by Fe3+ modify LPS to resist to antimicrobial peptides.

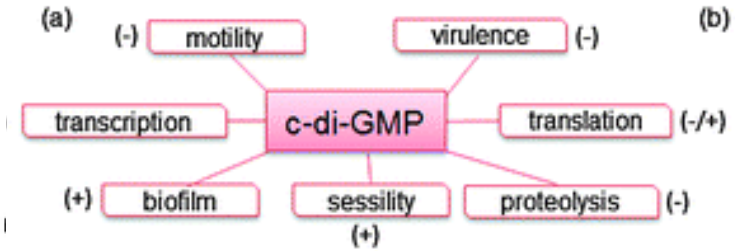
P-PhoP activates transcription of a number of genes (>40) that promote **survival of *Salmonella enterica* in the host** macrophages by direct and indirect effects.

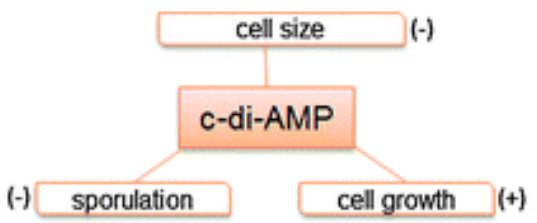
Among them, *pmrD*, mediates the PhoP/Q-dependent regulation of the PmrA-PmrB regulon

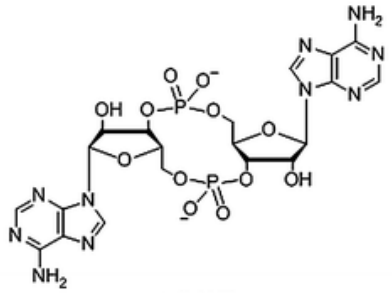
Nucleotide second messengers found in bacteria



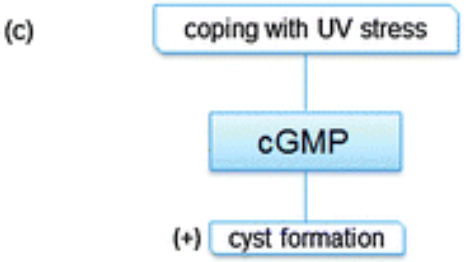
c-di-GMP

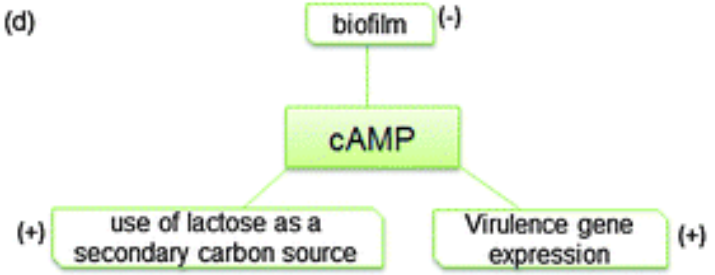






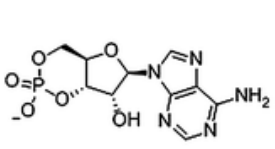
c-di-AMP



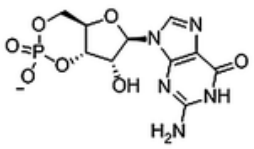


(catabolite repression)

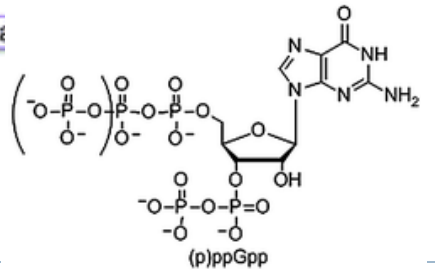
(stringent response)



cAMP



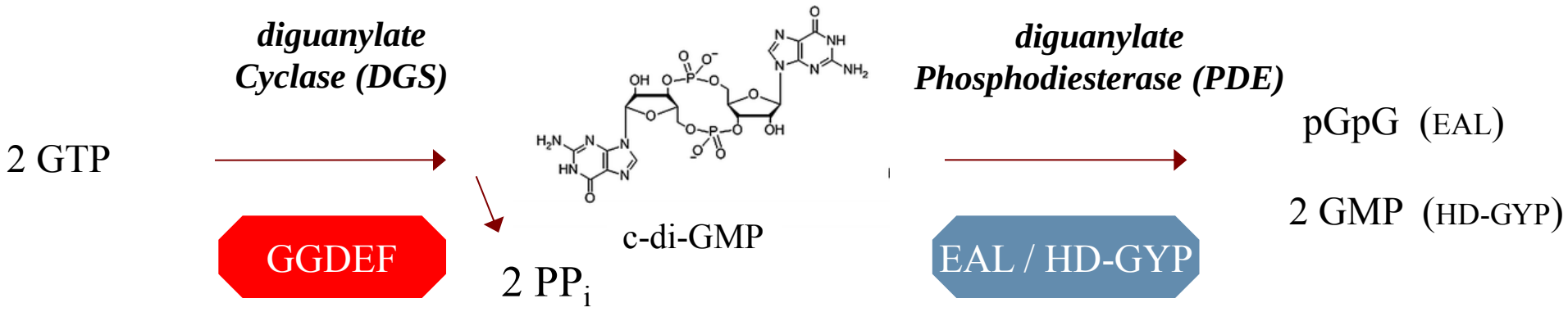
cGMP



(p)ppGpp

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 domains in proteins which 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) which provide selective signal degradation.

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

An important advantage of a second messenger systems is that its output is modulated by enzymatic synthesis and degradation, which is more rapid than the transduction of sensory information through gene transcription and translation.

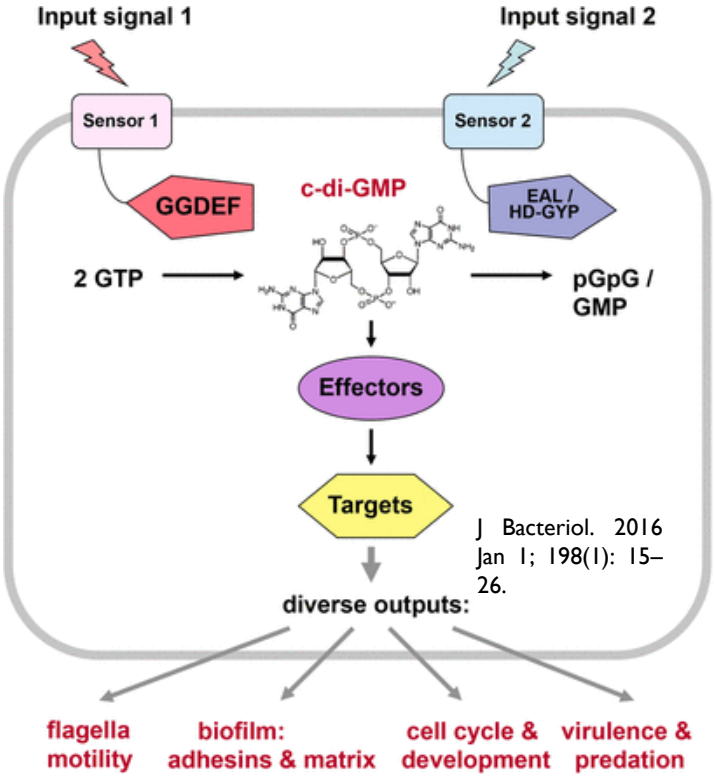
General scheme for c-di-GMP signaling

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 and enzymes containing a c-di-GMP binding site (PilZ domain) domains (allosteric modulators), transcription factors or repressors, riboswitches (regulate transcription/translation).

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

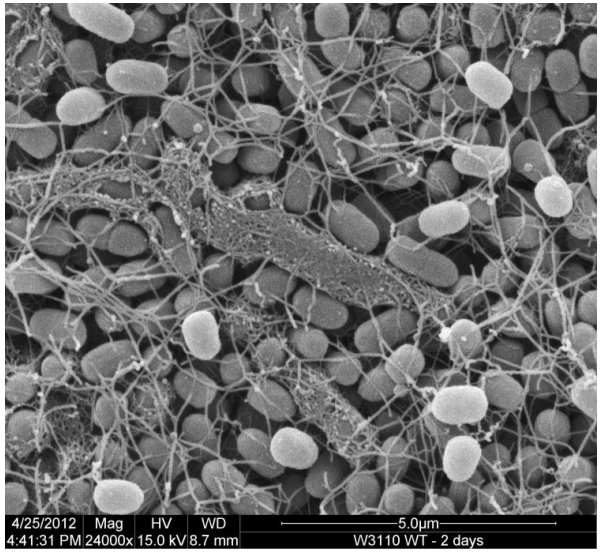


J Bacteriol. 2016 Jan 1; 198(1): 15–26.

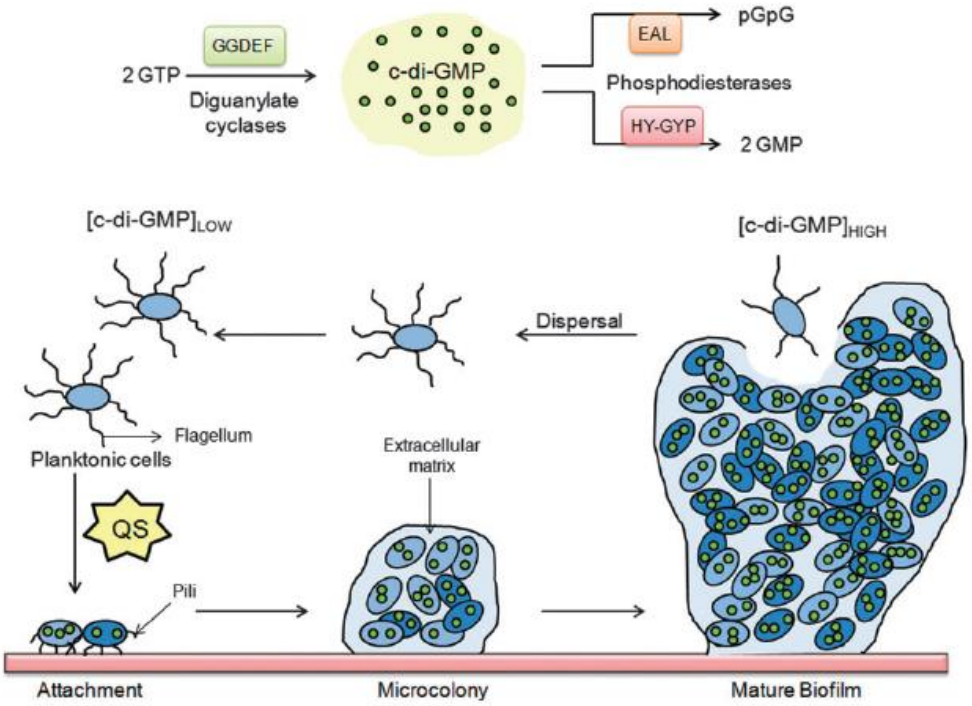
Production, degradation, mechanism of action, and physiological target processes of the second messenger c-di-GMP.

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.



Heterogeneous extracellular matrix production at the surface of a macrocolony biofilm of *E. coli* K-12. Only a subset of cells (arranged in small chains) are surrounded by a matrix of amyloid fibers, whose synthesis depends on high cellular levels of c-di-GMP. J Bacteriol. 2016 Jan 1; 198(1): 15–26.



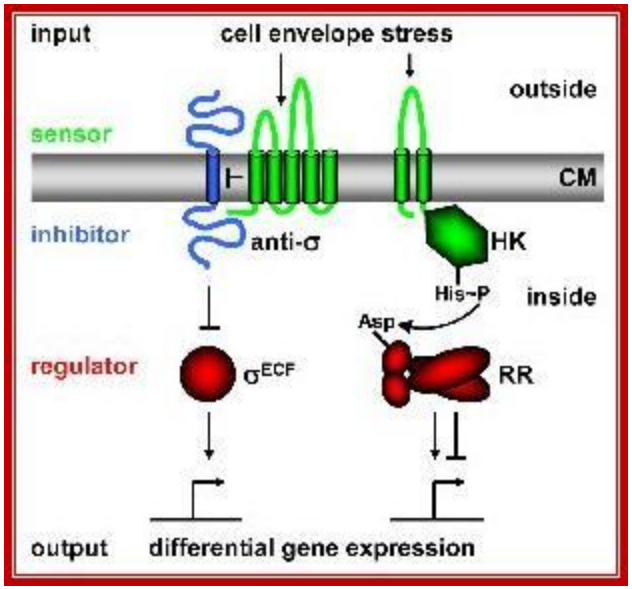
High level of c-di-GMP generally downregulates either the production or activity of flagella and activates the synthesis of adhesins and extracellular matrix components that are important for biofilm formation.

Alternative Sigma factors

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

Binding of σ to RNA polymerase, allows 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
(other examples: σ^{32} heat shock, σ^{s} stationary phase etc).

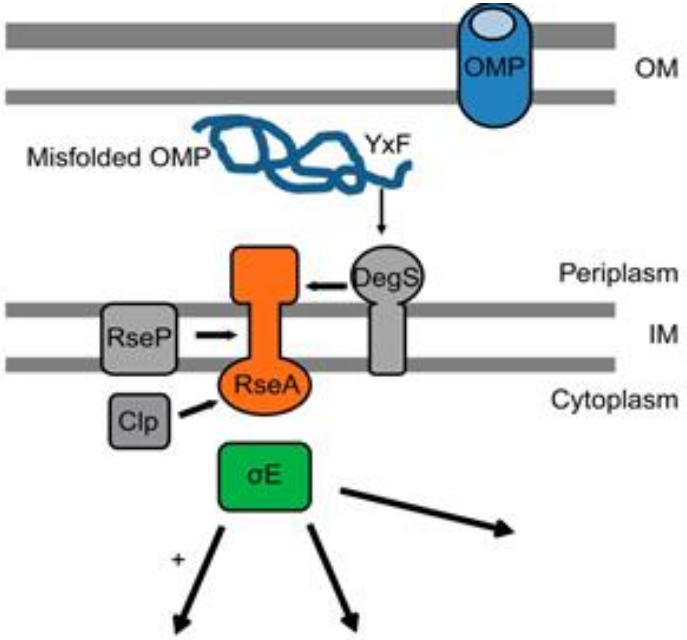
The extracytoplasmic function (ECF) sigma factors (σ^{E} in E. coli) are a unique class of alternative sigma factors that also regulate gene expression **in response to extracellular signals** (cell envelope stress).



Two mechanisms of differential gene expression in response to external inputs

The sigma factors σ^E stress response pathway

σ^E has been well studied for its role in combating extracytoplasmic stress (cell envelope stress)., e.g. damage of envelope. **σ^E** is inhibited being sequestered by the membrane protein anti-sigma factor RseA.

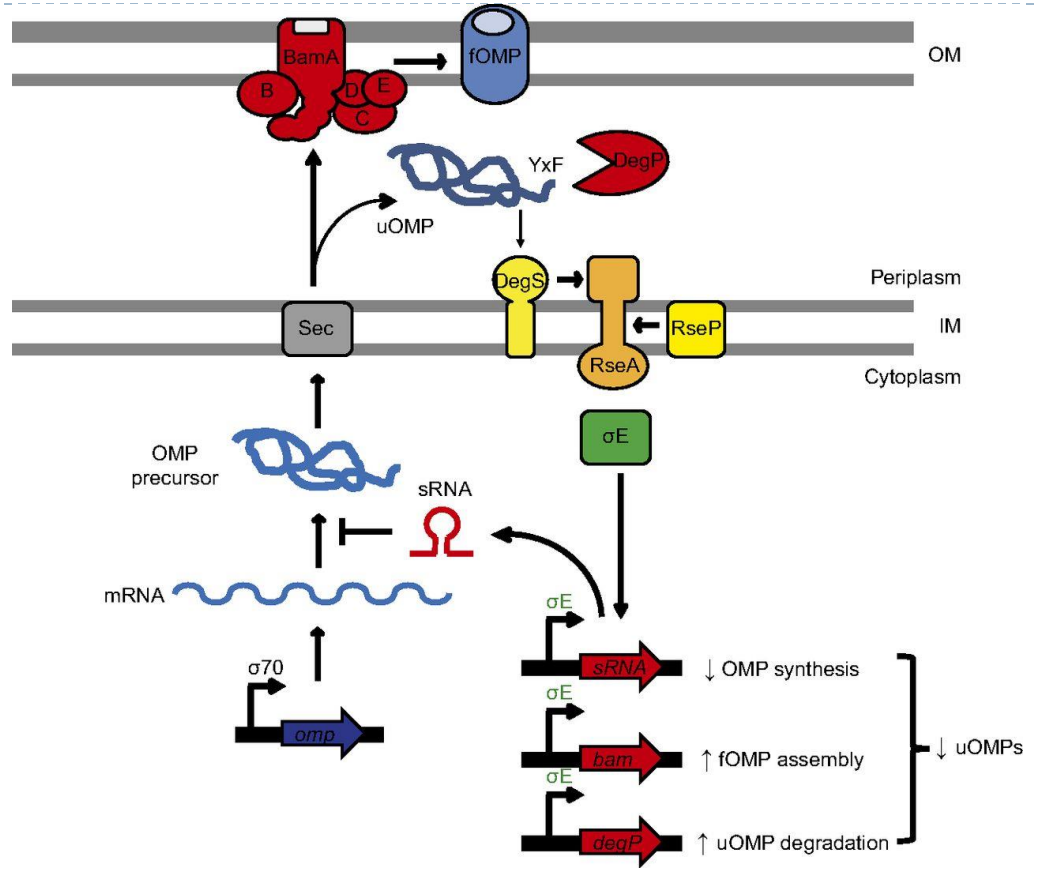


σE-regulated genes

When a cell envelope stress is present, the OMP assembly pathway may be altered. Misfolded or unfolded OMPs activates a membrane protease (DegS), which initiates to degrade the anti-sigma factor RseA together other membrane (RseP) and cytoplasmic (Clp) proteases.
σE is then released in the cytoplasm and can direct RNA polymerase to transcribe a specific subset of stress genes: the well-characterized **σE regulon**

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

Activation of σ^E regulon in *E. coli*



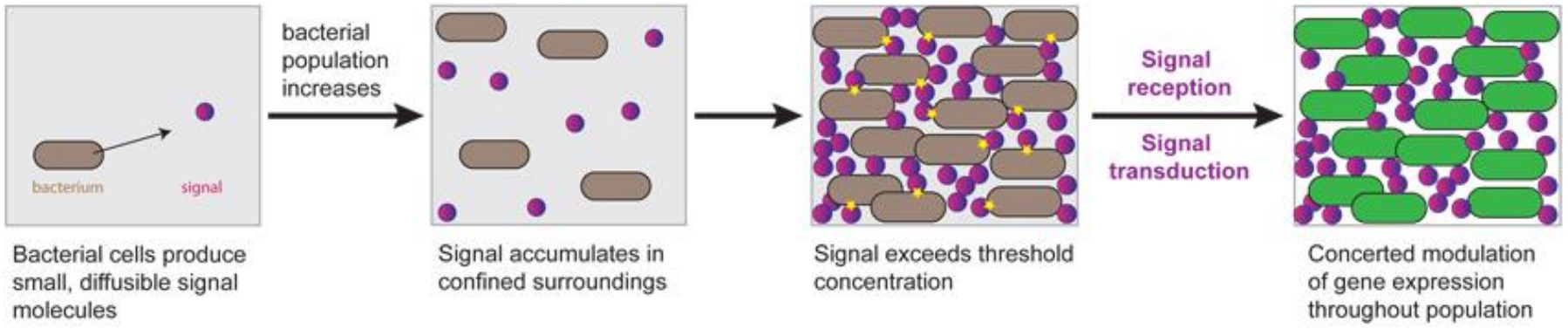
σE regulon: unregulation of : i) genes for translational downregulation of OMP synthesis (sRNA such as micA),
ii) Genes for upregulation of genes for OM biogenesis machines, for periplasmic chaperones to promote OMP folding
iii) Genes for periplasmic proteases (DegP) to degrade misfolded OMPs.

Anna Konovalova et al. PNAS July 10, 2018 115 (28) E6614-E6621;

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':



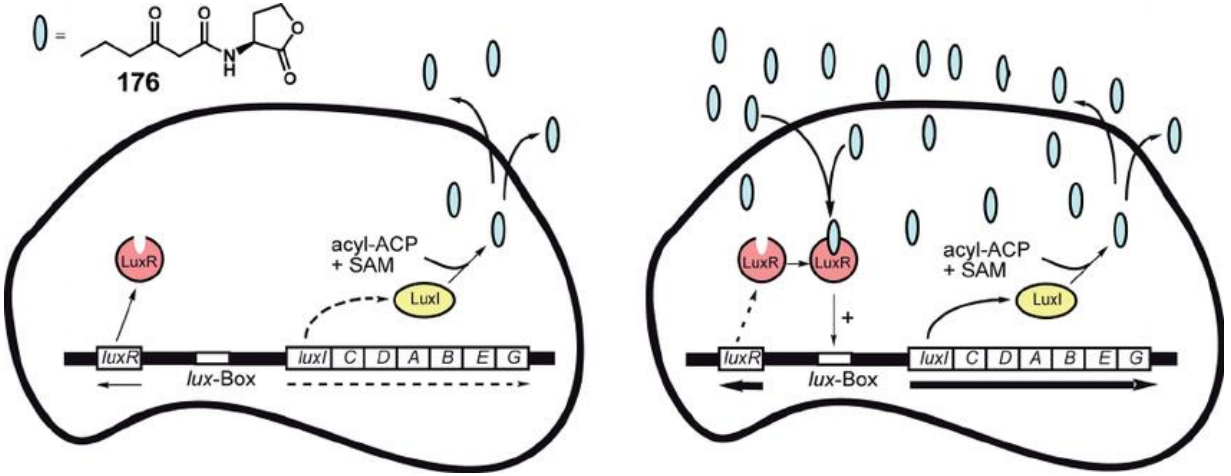
When concentration of these **signaling molecules** exceed a particular threshold value (**quorum**), these molecules are detected by surrounding cells that activate transcription of a set of genes, that are the same in all the bacterial population.
Quorum sensing allows the individual bacteria in that 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.

Hawaiian squid *Euprymna scolopes.*

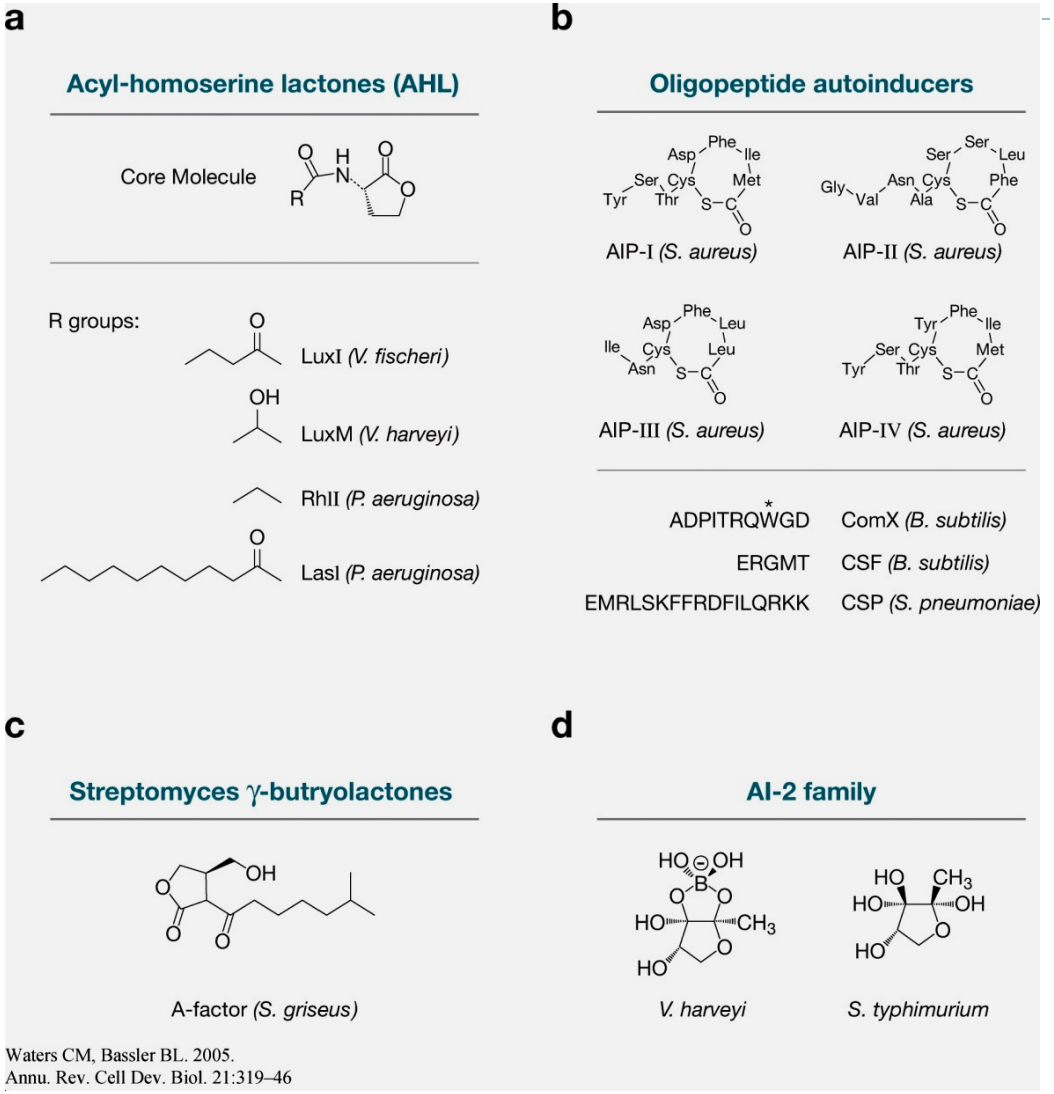
LuxI is constitutively expressed at low level. Its product, the **autoinducer synthase**, is responsible for the production of the autoinducer **AHLs (**acyl Homoserine lactones**).** At high cell density, AHL binds to **LuxR** an autoinducer-receptor/DNA binding transcriptional activator. Upon the binding 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. There is also a positive feedback mechanism that induces luxR gene

AHLs are synthesized by acylACP (acylcarrier protein,) + SAM (S-adenosyl-L-methionine.) by LuxI product.

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.
AHLs can diffuse through membranes.

B) Gram-positive bacteria use posttranslationally-modified cyclized **autoinducing peptides (AIPs)** as autoinducers. AIPs do not cross the membranes. Receptor is a HK of a TCS.

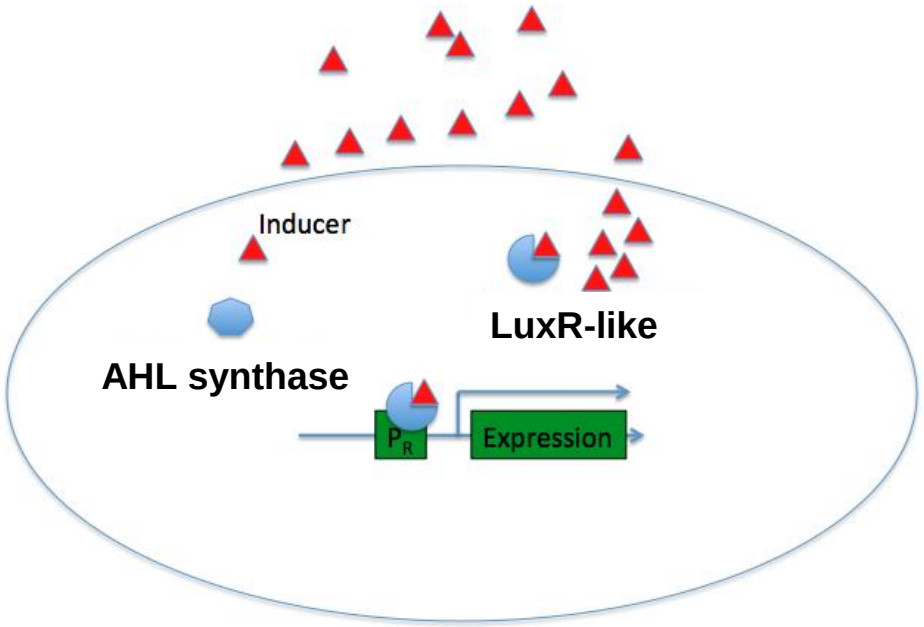
C) Streptomyces use γ–butyrolactones structurally related to AHLs

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-negative bacteria

A large number of gram-negative bacteria (more than 100) possesses **LuxR-type** transcriptional activator (Homologous to **LuxR)** and communicate with AHL signals, basically consisting of **AHL synthase** (homologous to **LuxI**).

The LuxI-like proteins catalyze the formation of species-specific AHL signals. (intraspecies communication).



Extreme specificity exists between each LuxR-like protein and its cognate AHL autoinducer. The LuxR-autoinducer complexes bind at target gene promoters and activate transcription.

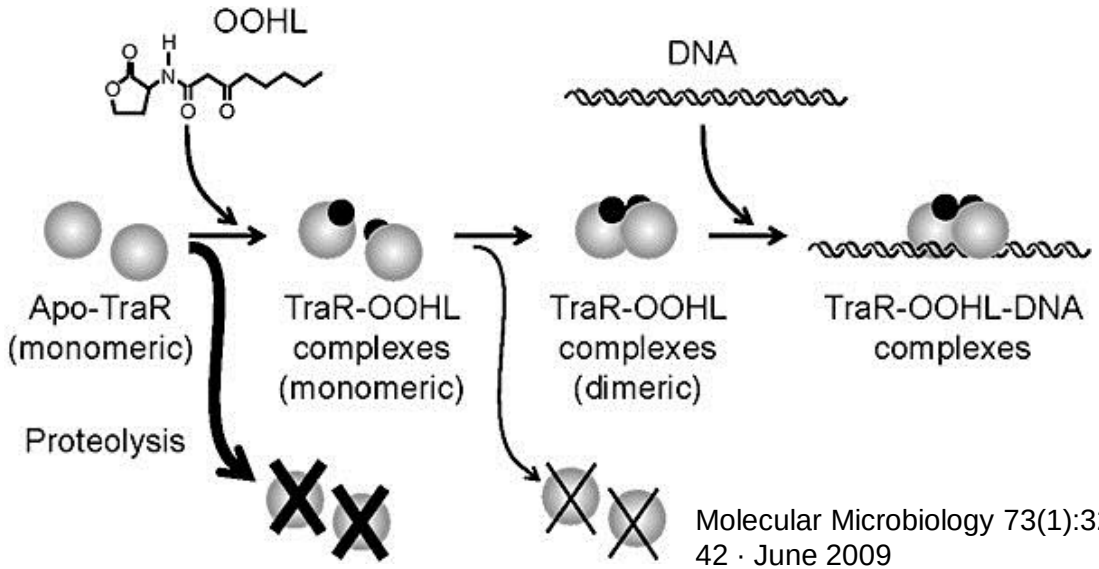
The LuxR-autoinducer complexes

LuxR homologous (traR in the example): has a **C-terminal domain (DBD)** containing a **HTH motif (**helix-turn-helix**)** that can bind to the target promoter, and a **N-terminal domain (LBD)** that prevents the binding until the autoinducer is not present and bound to LuxR.

Functional complex of quorum-sensing transcriptional regulator TraR (a member of LuxR family).

and its AHL has been described in *Agrobacterium tumefaciens*.

The autoinducer OOHL indirectly affects gene activation by **increasing stability of TraR** and by inducing 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

OOHL (N-3-oxooctanoyl- L-homoserine lactone)

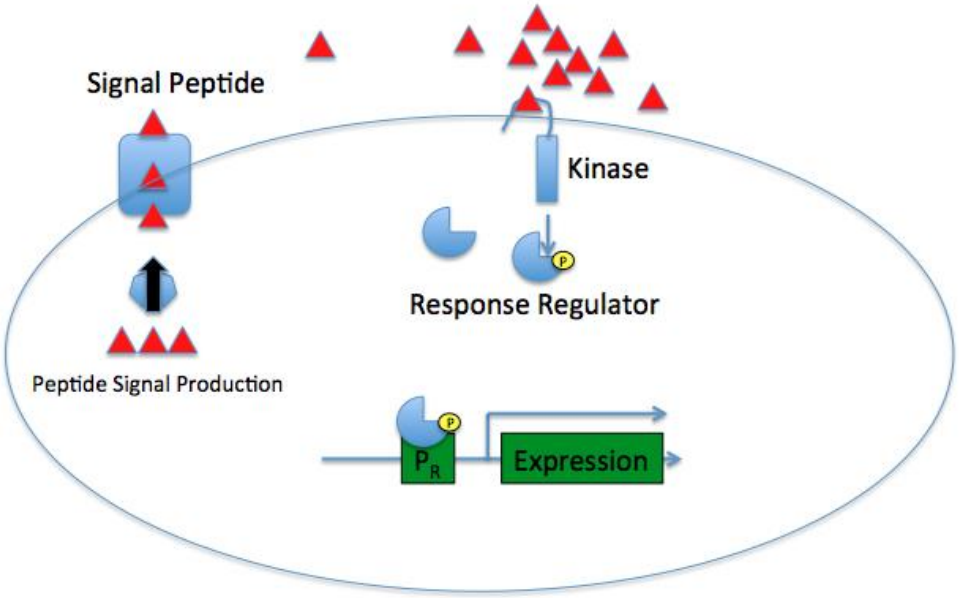
Quorum sensing in Gram-positive bacteria: autoinducing peptides (AIPs)

Gram-positive such as *Bacillus subtilis, Staphylococcus aureus* use small peptides as signals: autoinducing peptides, **AIPs**.

These peptides are synthesized by ribosomes as **precursor peptides** and undergo posttranslational modifications during excretion to become activated and stabilized.

In general AIP are secreted by an **ATP-binding cassette (ABC) transporter.** As the population density increases, the AIPs accumulate in the environment.

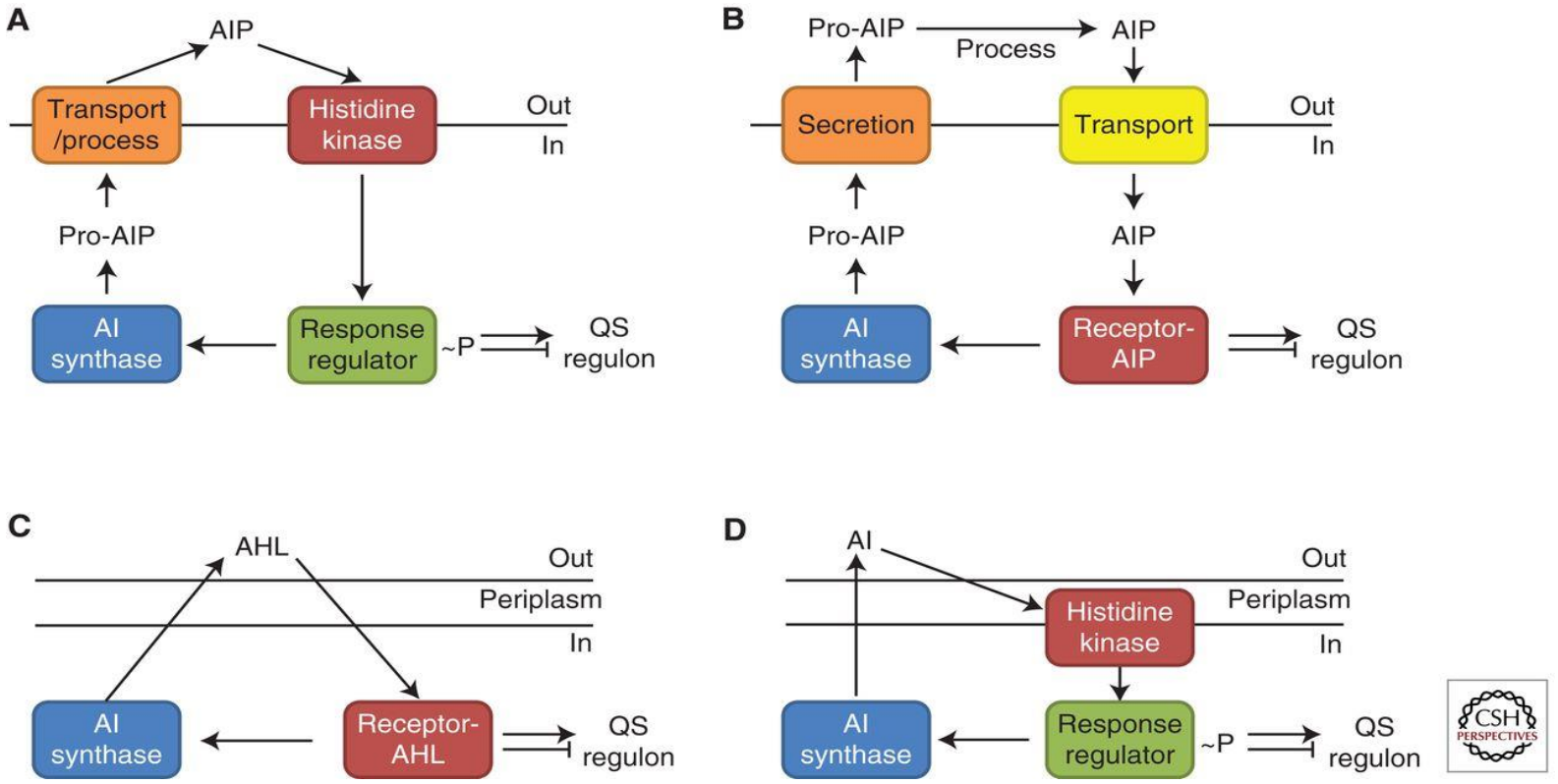
When a certain threshold level is reached, binding of an AIP to a receptor (membrane-bound sensor protein belonging to a **TCS**) Subsequently, the activated receptor binds and activated the cognate intracellular response regulator, which in turn will be activated. The activated response regulator influences the transcription of target genes.



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

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

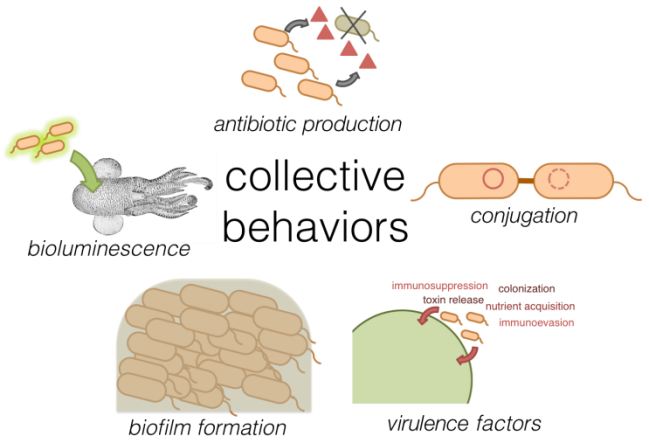
Autoinducing peptide (AIP) QS in Gram-positive bacteria by (*A*) two-component signaling, or (*B*) an AIP-binding transcription factor.
Small molecule QS in Gram-negative bacteria by (*C*) a LuxI/LuxR-type system, or(*D*) two-component signaling.

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*

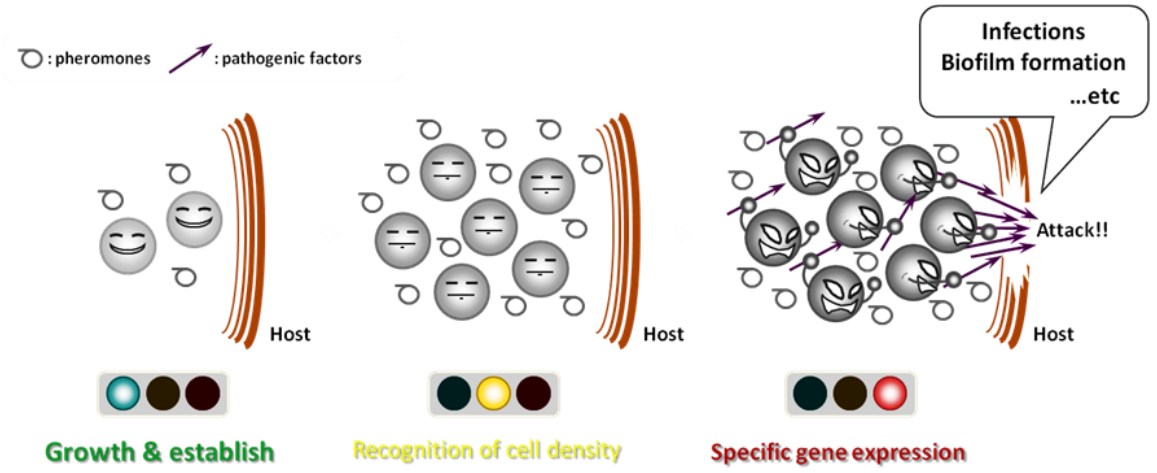
- **Sporulation** QS upregulates spore-forming genes in *Bacillus subtilis and other Gram+ bacteria*

- **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

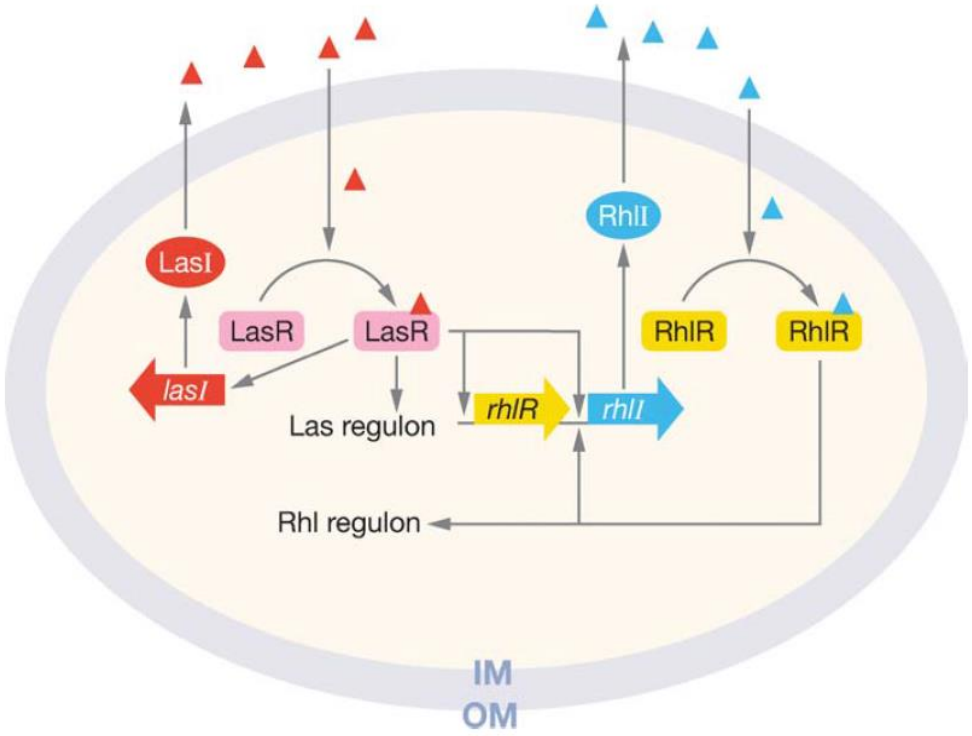
- Activation of virulence genes at low population density would result in the generation of host defence responses, thus providing the host an early lead over the invading bacteria.
- In order to guarantee their survival, certain pathogens evade the synthesis of virulence factors until their number makes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

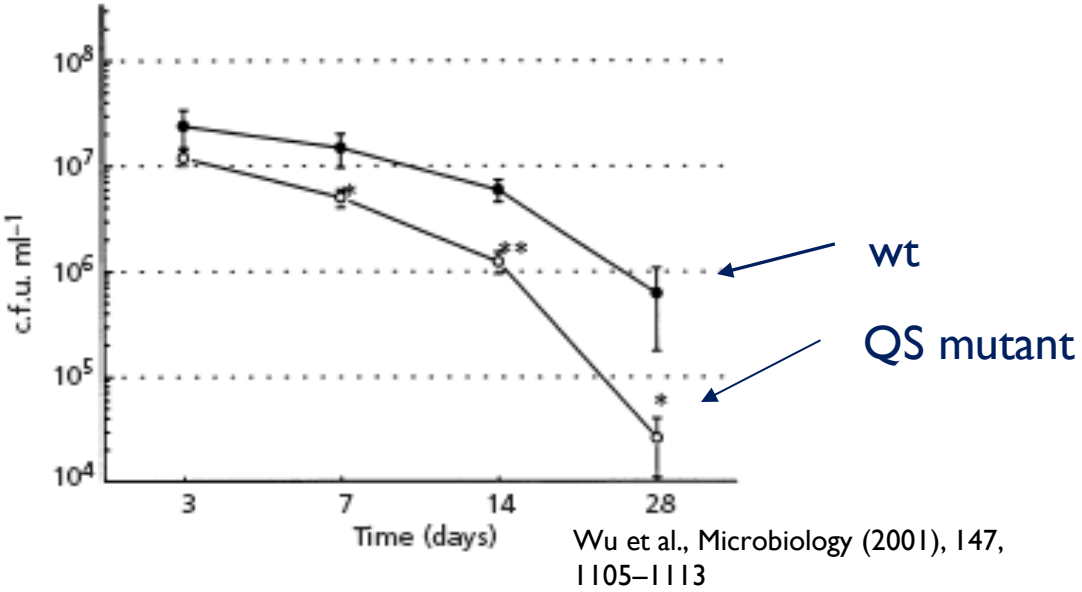
A hierarchical circuity network indeed produces **a temporally ordered sequence of gene expression** (multiple virulence traits including exoproteases and exotoxins production, siderophores, several secondary metabolites and biofilm formation) that may be critical for the ordering of early and late events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



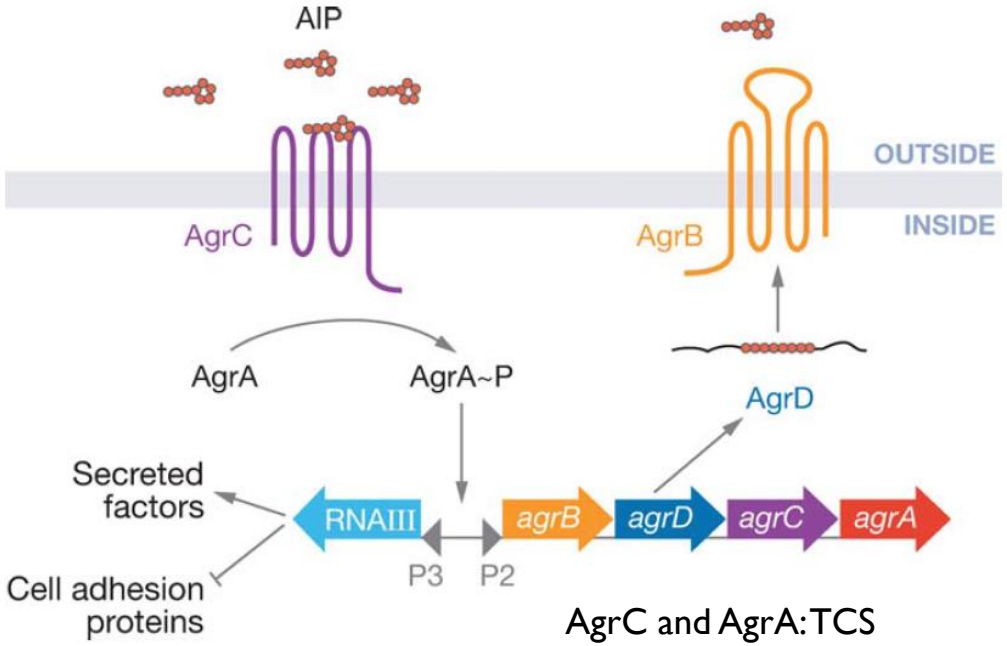
Wu et al., Microbiology (2001), 147, 1105–1113

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