Two-Component Regulatory System

Two Component Regulatory Systems

A two-component regulatory system is a signal transduction system that uses the transfer of phosphoryl groups to control gene transcription and protein activity.

It has two major components: the first component is a sensor kinase (or histidine kinase); the second is a response regulator.

1. Sensor kinases usually posses two domains:

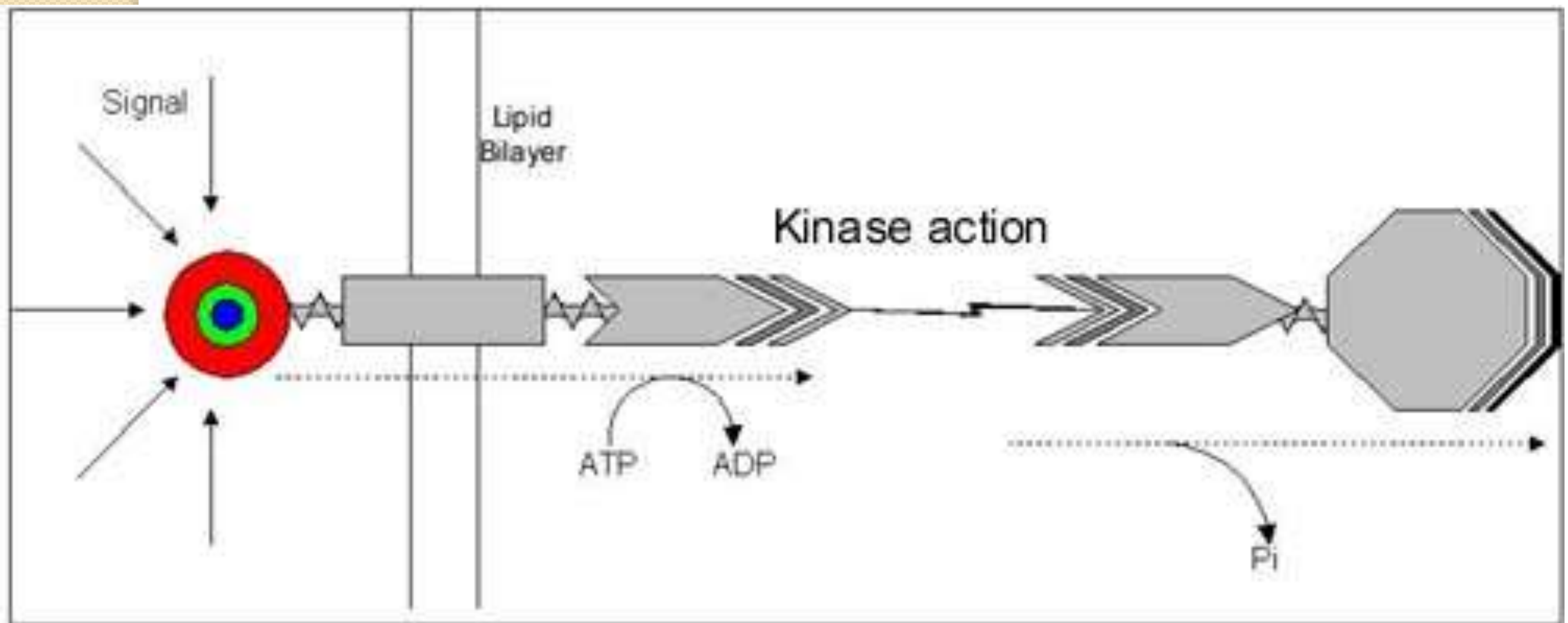
   1) Input domain(sensory domain): monitors environmental stimuli

      --Varies in length and amino acid sequences from one histidine kinase to another, conferring specificity for different stimuli.

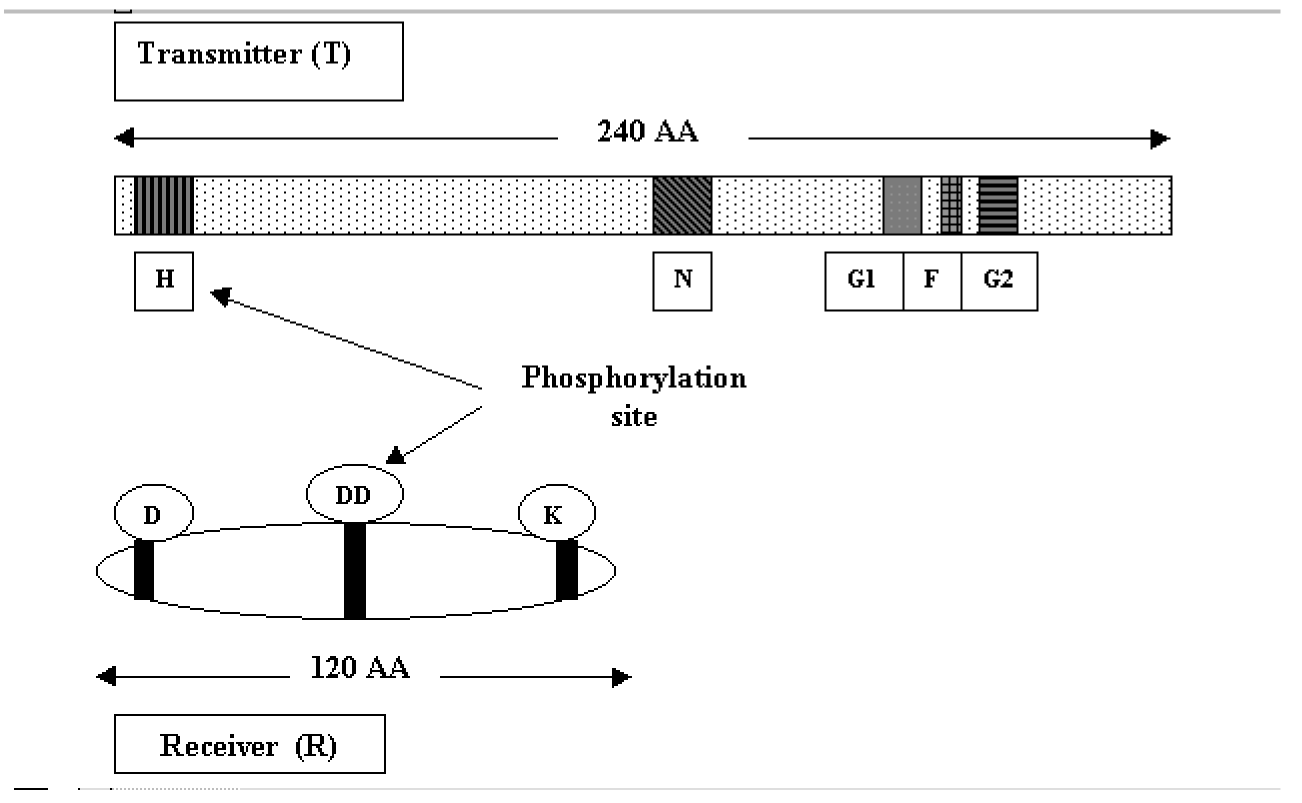
   2) Transmitter domain: auto-phosphorylates following stimulus detection

      --Shows high sequence conservation. It contains an invariant histidine residue that is phosphorylated in an ATP-dependent manner and short stretches of conserved amino acids, in particular two glycine-rich motifs involved ATP binding (the NG1FG2 motif).

2. Response regulator contains an amino-terminally located conserved receiver domain that is phosphorylated by the sensor kinase at a strictly conserved aspartate residue, leading to activation of the carboxy-terminal output domain.


Signal
Lipid
Bilayer
Kinase action
ATP
ADP
Pi

Sequence features and phosphorylation activities of communication modules



Two Component Regulatory System is widespread among living organisms

These signal transduction systems are found in all kingdoms of life, ranging from :

- Bacteria
- Archae
- Single-celled eukaryotic organisms
- Fungi
- Higher plants

Two Component Systems regulate diverse responses in many different organisms

nutrient acquisition

- ○nitrogen
- ○phosphorus
- ○carbon

•energy metabolism

- ○electron transport systems
- ○uptake and catabolic machinery

•virulence

- ○plasmid transfer
- ○degredative secretions
- ○toxin production
- ○adherence factors

•adaptation to physical or chemical aspects of the environment

- ○pH
- ○osmolarity
- ○light quality

•complex developmental pathways

- ○sporulation
- ○fruiting body development
- ○swarmer cell production

A single cell may have many two-component systems

- ***E. coli*** **has been found to have 45 gene products assigned to regulatory functions, with an additional 133 putative ones identified by analysis the complete genome sequence.**
- **Of these 178 proteins, 62 are likely to be part of two-component signal transduction pathways**
  - **26 histidine kinases (PSI-BLAST search of *E. coli* genome for signal transmitters)**
  - **36 response regulators (PSI-BLAST search of *E. coli* genome for signal receivers)**
  - **in 10 sub-families (families & functions)**

The BLAST search of *Pseudomonas aeruginosa* genome led to the identification of putative genes encoding 63 histidine kinases and 64 response regulators. Of these, 12 sensors and 18 regulators had been identified previously.

Two Component Regulatory Systems

EnvZ/OmpR - osmoregulation in *E. coli*
- **PhoR/PhoB - phosphate scavenging in *E. coli***
- **NtrB/NtrC - nitrogen assimilation in a variety of bacteria**
- **DctB/DctD - dicarboxylate transport in *Rhizobium leguminosarum***
- **VirA/VirG - virulence by *Agrobacterium tumefaciens***
- **KinA, KinB/SpoOF, SpoOA – sporulation in *Bacillus subtitlis***
- **CheA/Chey, CheB – chemotaxis in *E. coli***

Two-component regulatory pairs in bacteria

Organism	Histidine Kinase	Response Regulator	Adaptive Response
Agrobacterium tumifaciens	*VirA*	*VirG*	Virulence gene expression
Bacillus subtilis	*CheA*	*CheY & CheB*	Chemotaxis
Bacillus subtilis	*Com P*	*Com A*	Competence
Bacillus subtilis	*Degs*	*Deg U*	Competence, degradative enzyme production
Bacillus subtilis	*PhoR*	*PhoP*	Phosphate assimilation
Bacillus subtilis	*SpaK*	*SpaR*	Subtilin production
Bacillus subtilis	*SpoIIJ (KinA) & KinB*	*SpoOA & SpoOF*	Sporulation
Bordetella pertussis	*BvgS*	*BvgA*	Virulence gene expression
Enterococcus faecium	*Van S*	*VanR*	Synthesis of peptidoglycan precursors
Escherichia coli	*CheA*	*CheY & CheB*	Chemotaxis
Escherichia coli	*EnvZ*	*OmpR*	Osmoregulation
Escherichia coli	*NtrB*	*NtrC*	Nitrogen regulation
Escherichia coli	*PhoR*	*PhoB*	Phosphate assimilation
Lactococcus lactis	*NisK*	*NisR*	Nisin production
Staphylococcus aureus	*AgrB*	*AgrA*	Extracellular enzyme production
Streptococcus pneumoniae	*Com D*	*Com E*	Competence
Streptomyces lividans	*CutS*	*CutR*	Melanin production, copper metabolism
Vibrio fischeri	*LuxI*	*LuxR*	Bioluminescence

General Mechanism for the "Two-Component" Regulatory System

The input signal triggers a conformational change in the input domain of the sensor protein and consequently a conformational change in the transmitter domain of the sensor protein.

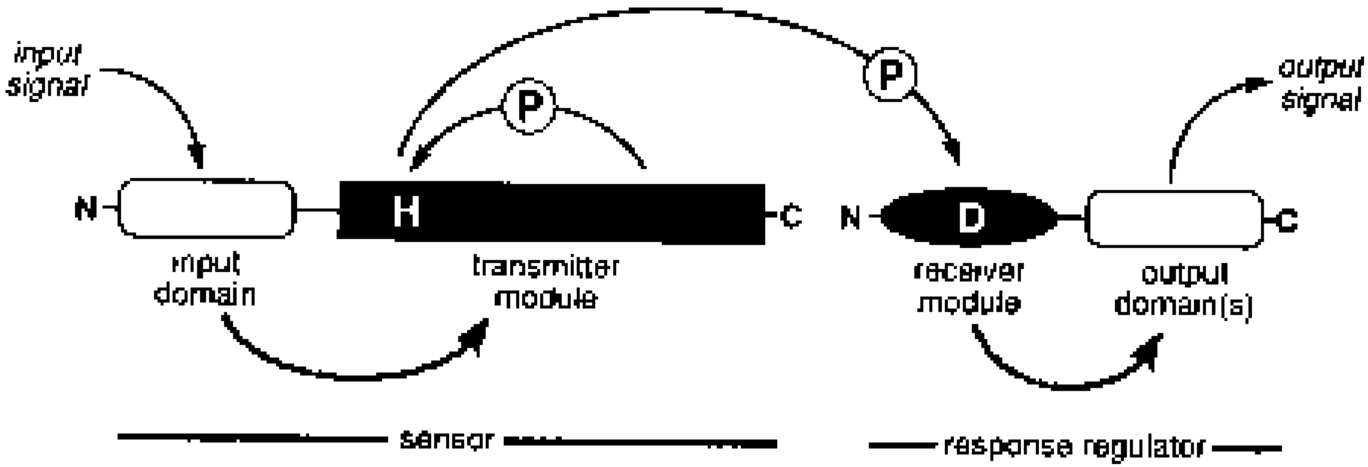
- **The conformational changes within the sensor protein stimulate the autophosphorylation of its transmitter domain; a phosphoryl group from ATP is transferred to the histidine residue.**

- **The unphosphorylated receiver domain of the response regulator protein associates with the transmitter domain, resulting in receiver phosphorylation.**

- **Receiver phosphorylation causes the output domain of the response regulator to undergo a conformational change and release an output signal.**

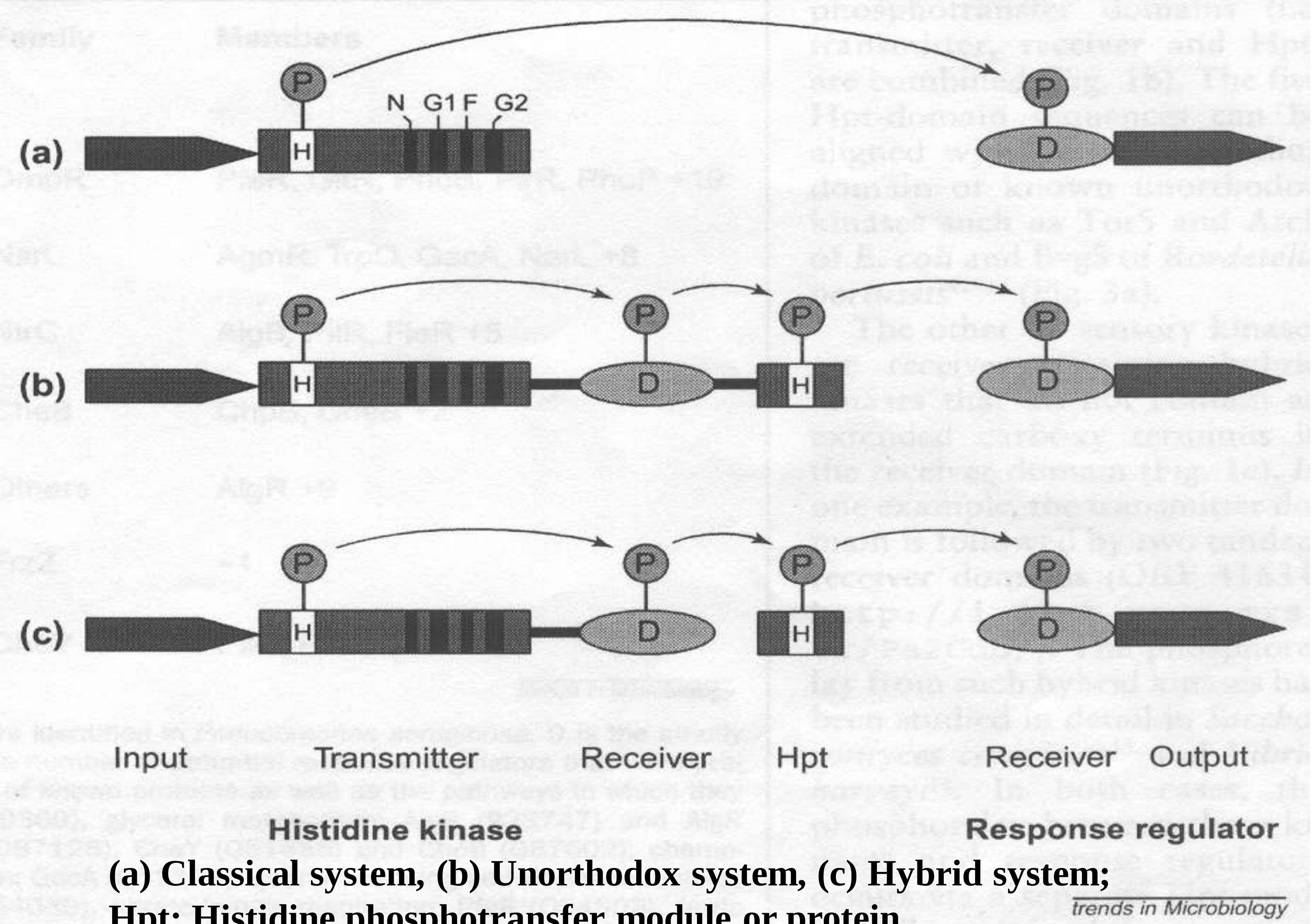
- **The output signal persists until receiver dephosphorylation occurs and interrupts the regulatory response.**

- **Receiver dephosphorylation is achieved by either:**
- **1) autophosphatase activity of the receiver domain**
- **2) transmitter-stimulated dephosphorylation**

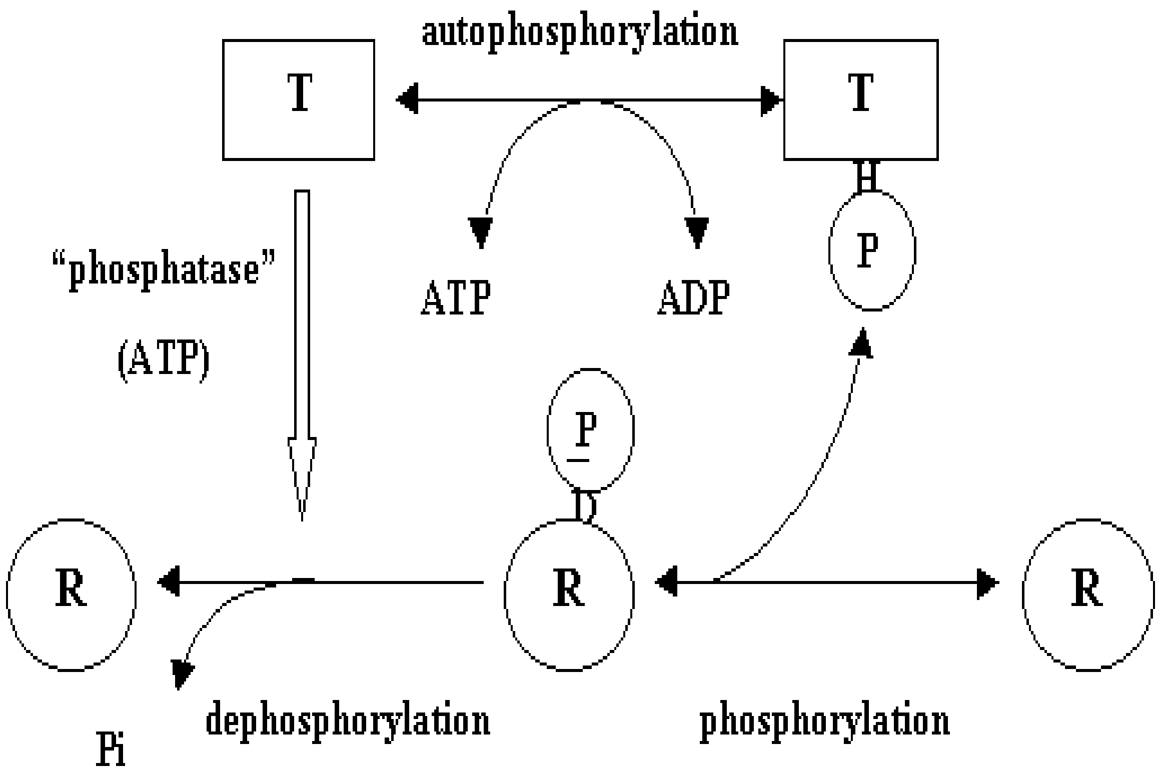


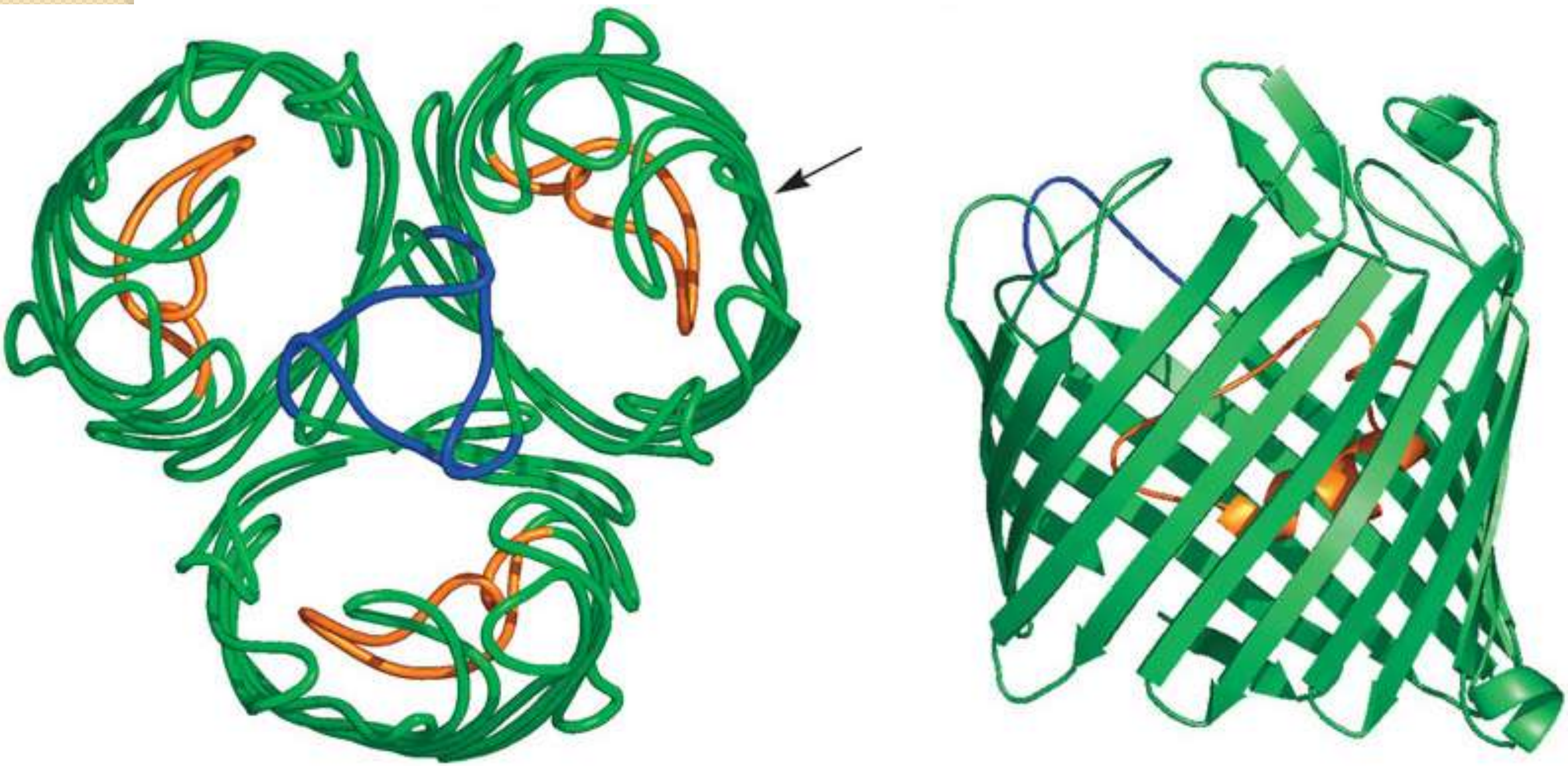
The "Two-Component" Regulatory System (Parkinson and Kofoid, 1992)

His-Asp phosphotransfer signaling between sensor kinase and response regulator



(a) Classical system, (b) Unorthodox system, (c) Hybrid system; Hpt: Histidine phosphotransfer module or protein


autophosphorylation
T
T
"phosphatase"
(ATP)
ATP
ADP
P
P
R
R
R
dephosphorylation
phosphorylation
Pi

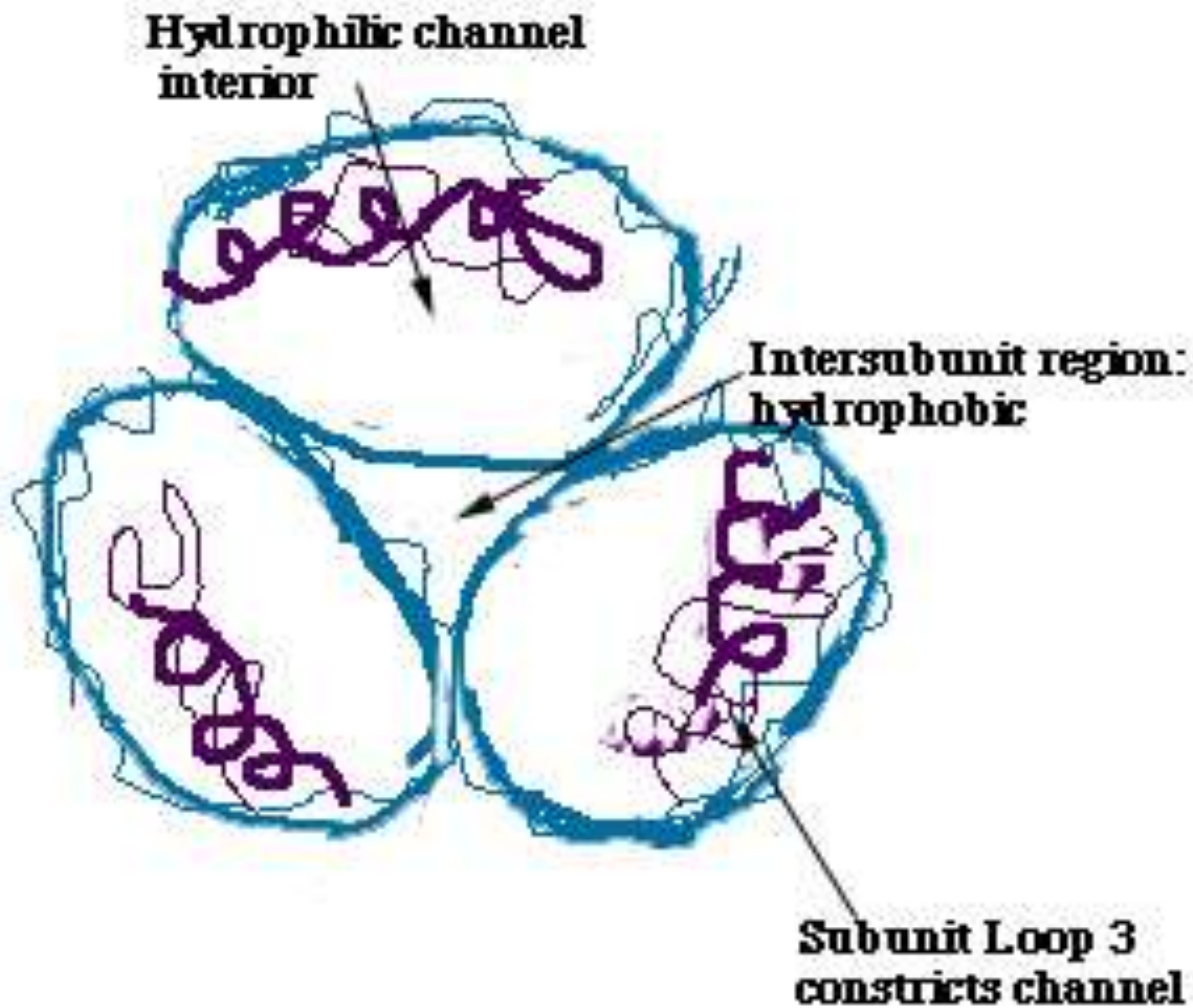


(a) Porin trimer (b) OmpF side view

Hiroshi Nikaido, *MMBR* 67(4):593-656 ASM/2003, Fig 2/p. 598

Porin Proteins

Fig. B1



Hydrophilic channel interior
Intersubunit region: hydrophobic
Subunit Loop 3 constricts channel

Table 1

Porin	Channel size (23)	Molecular Weight	Expression at low osmolarity	Expression at high osmolarity
OmpF	0.58 nm radius	38,306 Da	high	repressed, very low
OmpC	0.54 nm radius	37,083 Da	very low	high

Fig. B2

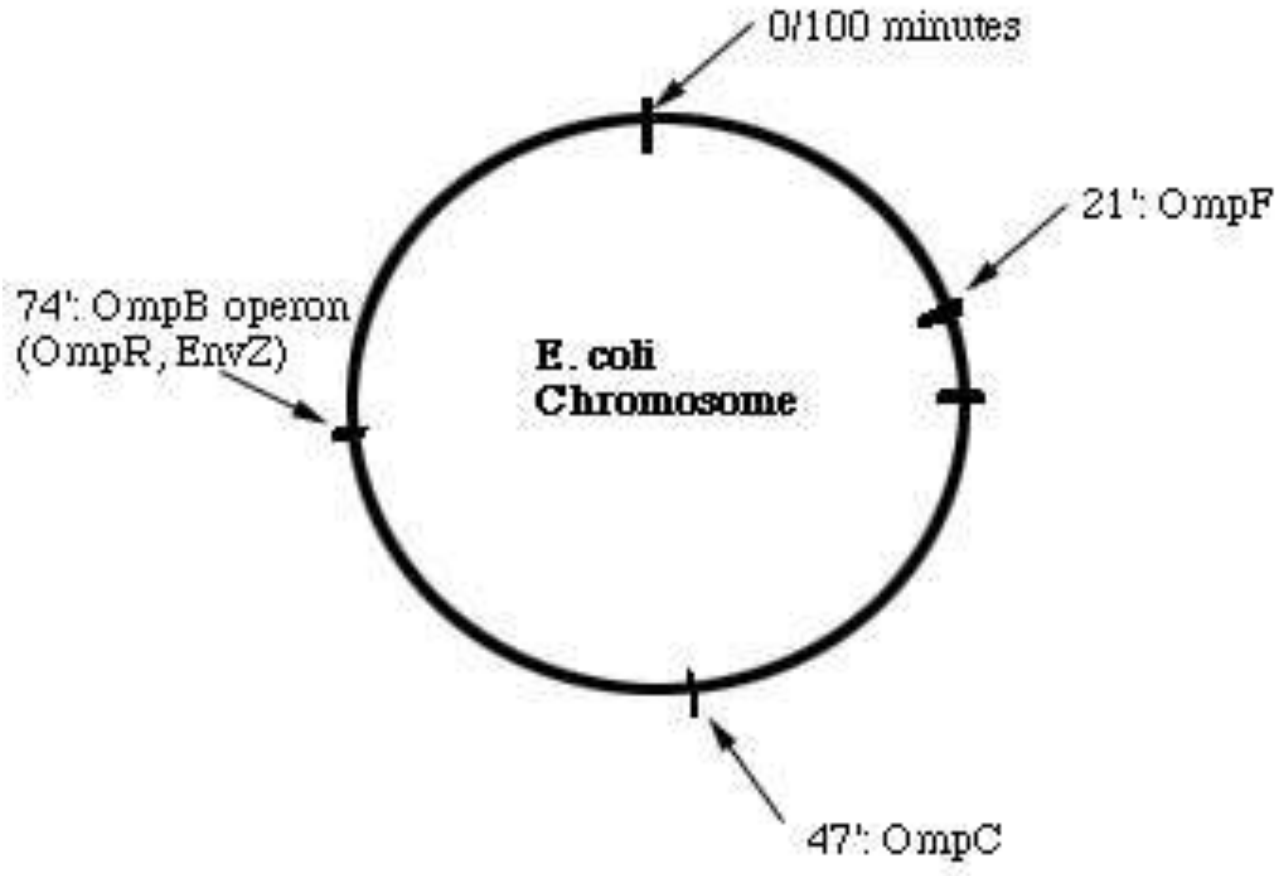
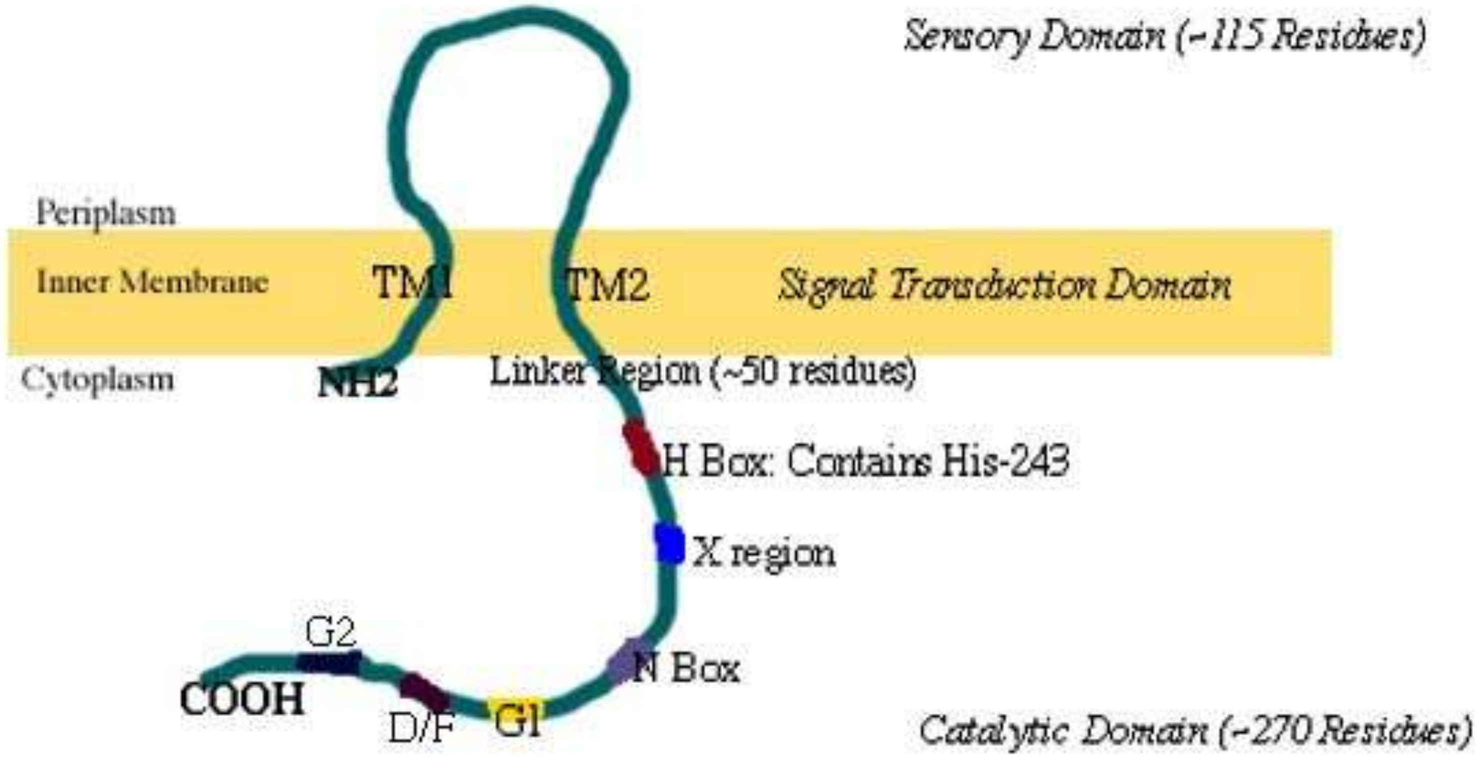
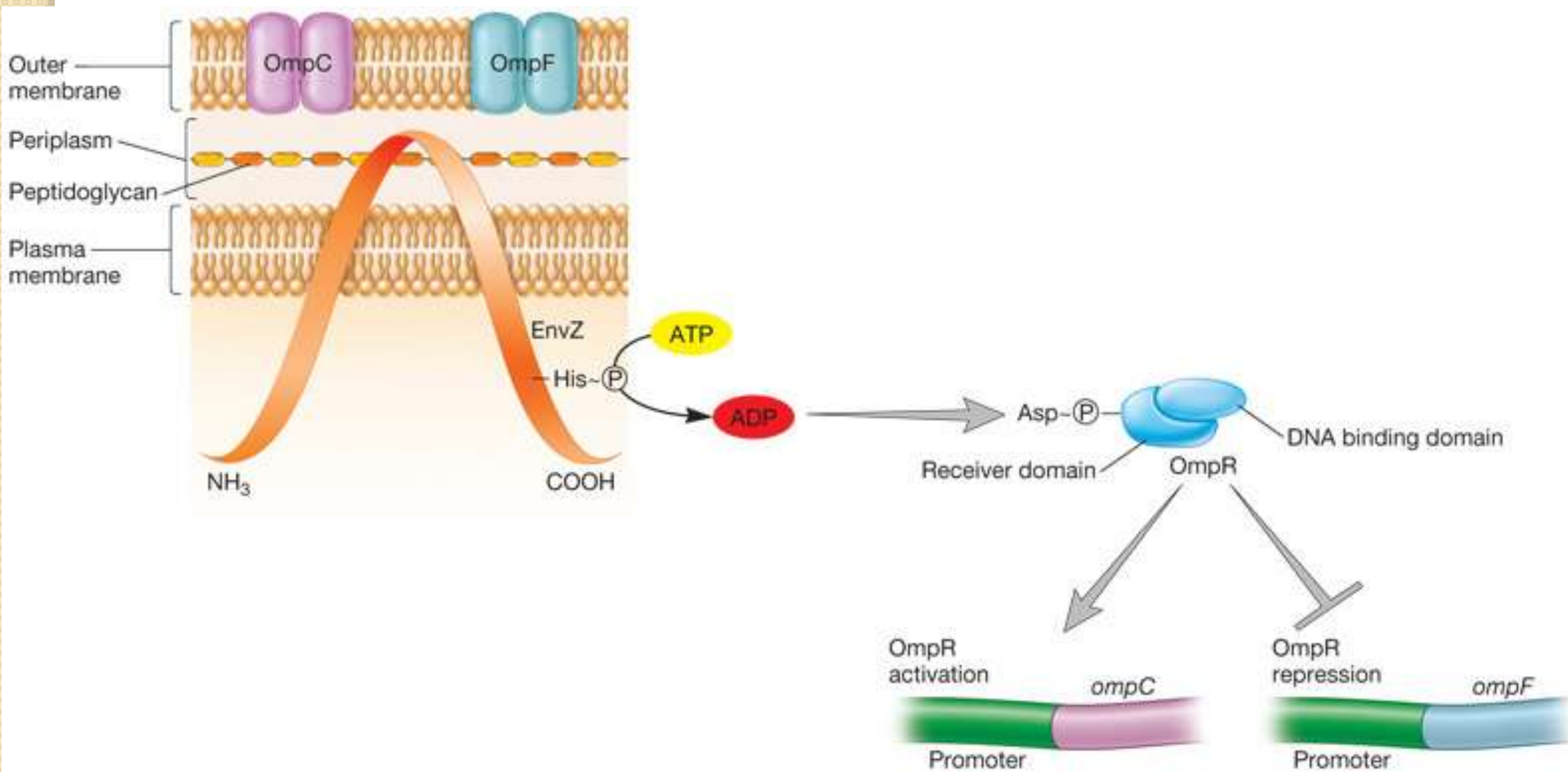

0/100 minutes
21': OmpF
74': OmpB operon
(OmpR, EnvZ)
E. coli
Chromosome
47': OmpC

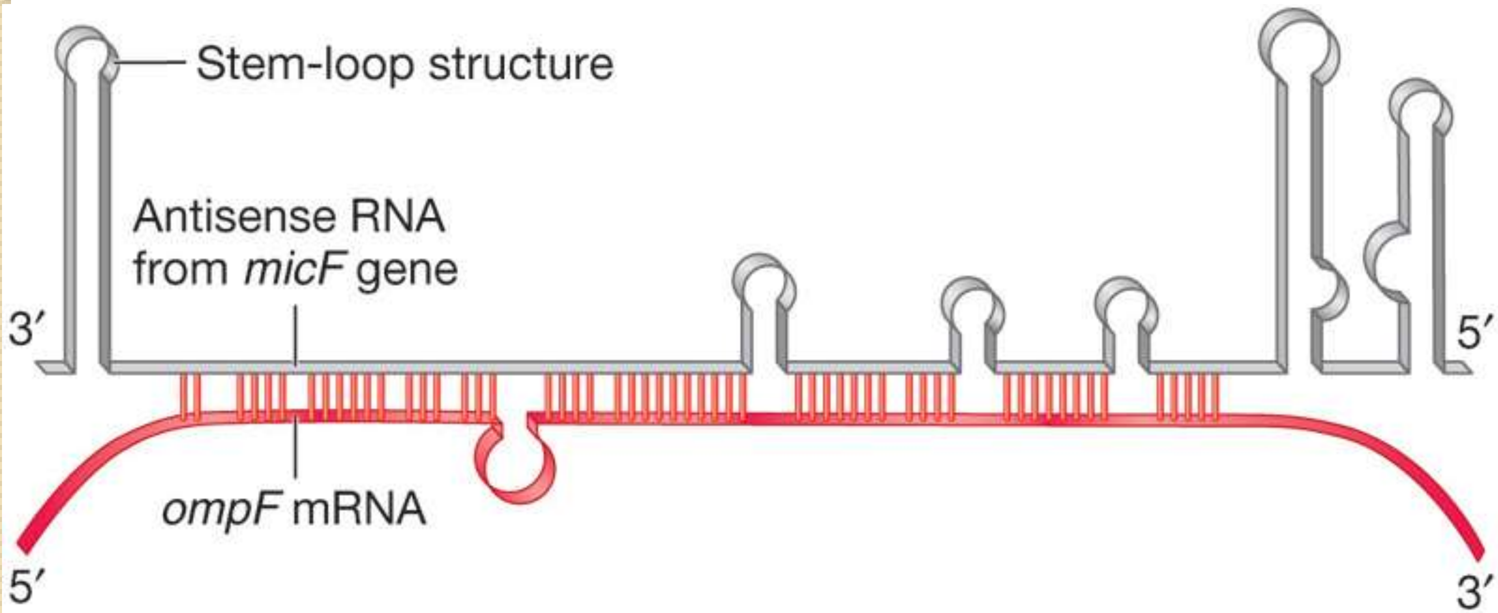
Fig. B3



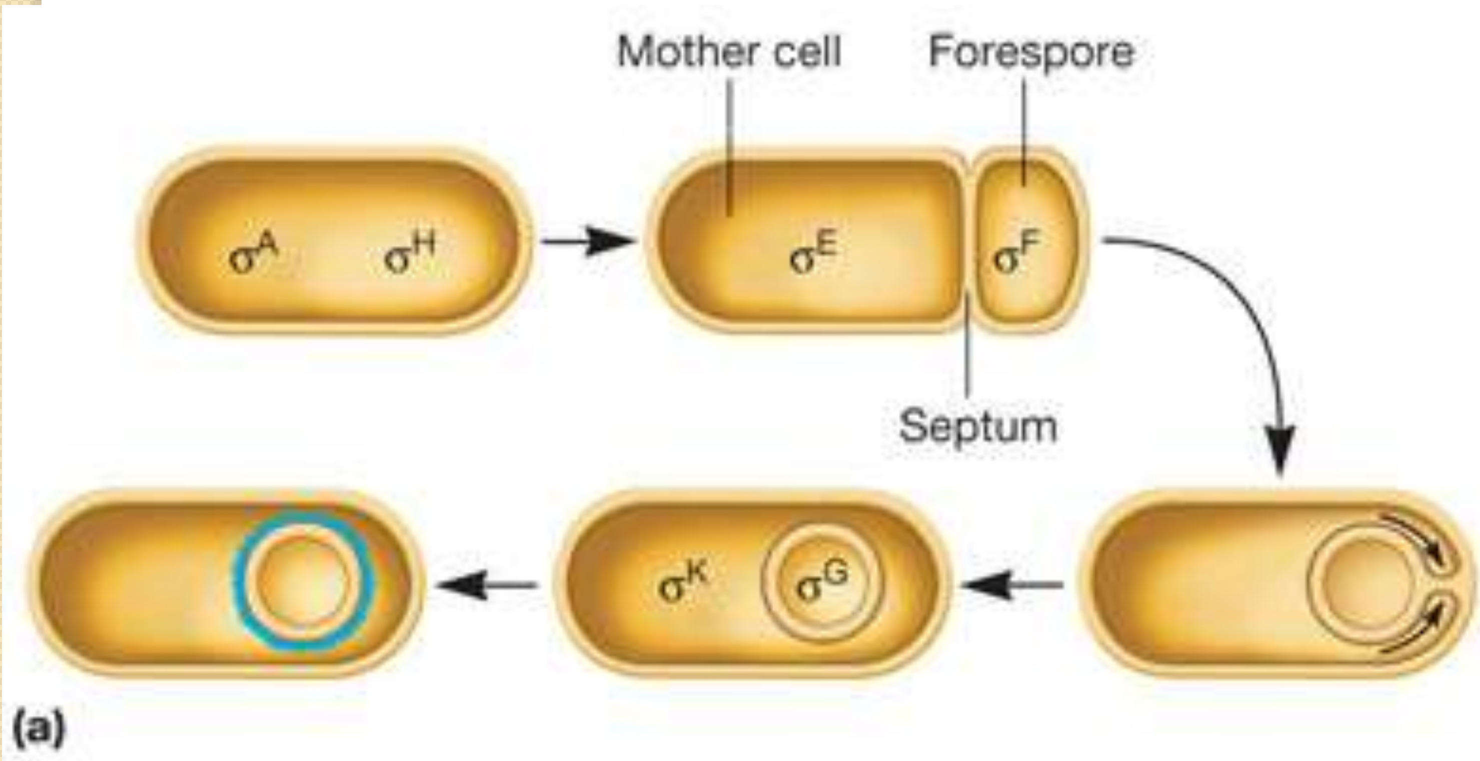
Adapted from Hsing, et.al. (15)



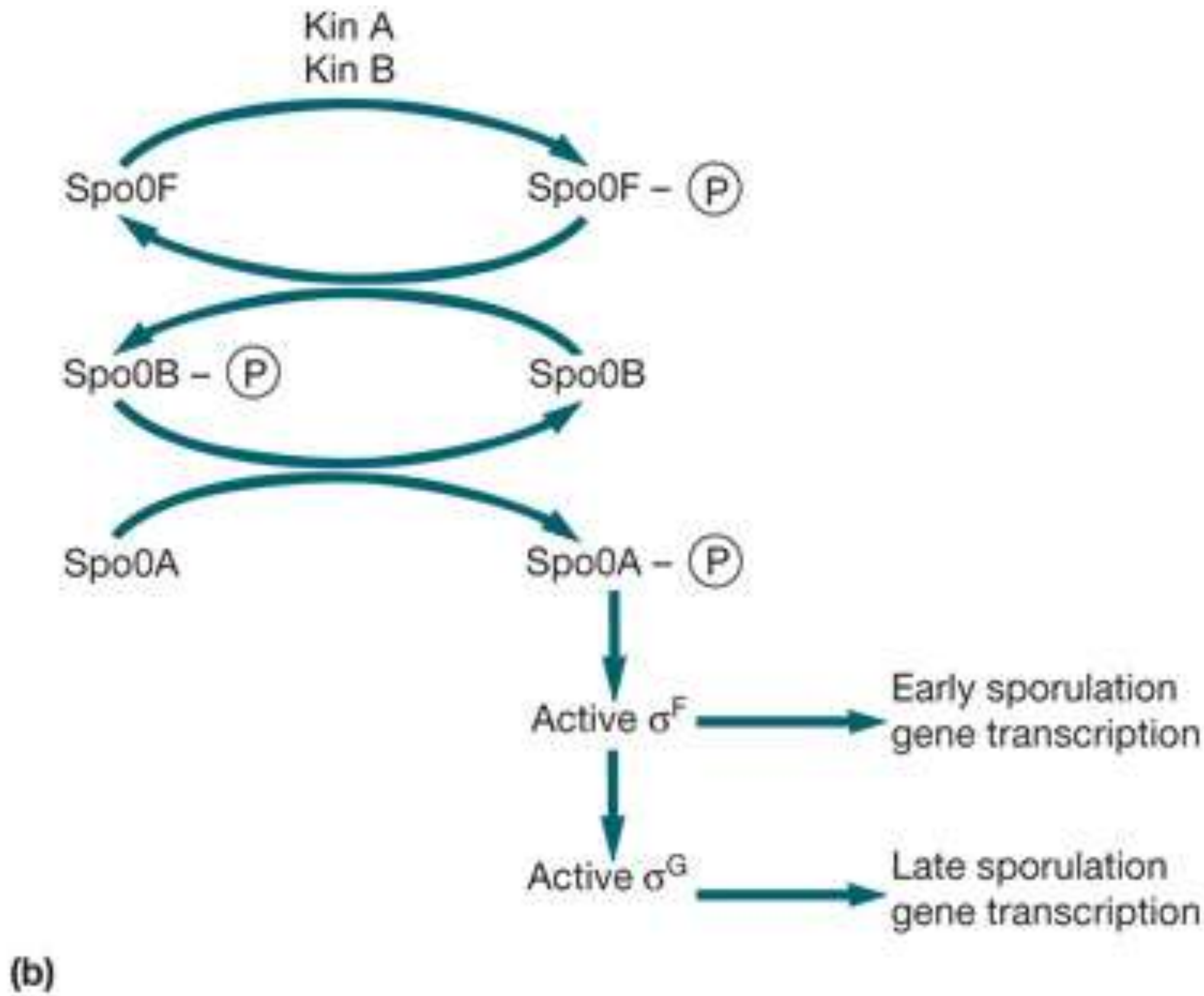
Two Component Signal Transduction System and the Regulation of Porin Proteins



Regulation of Translation by Antisense RNA

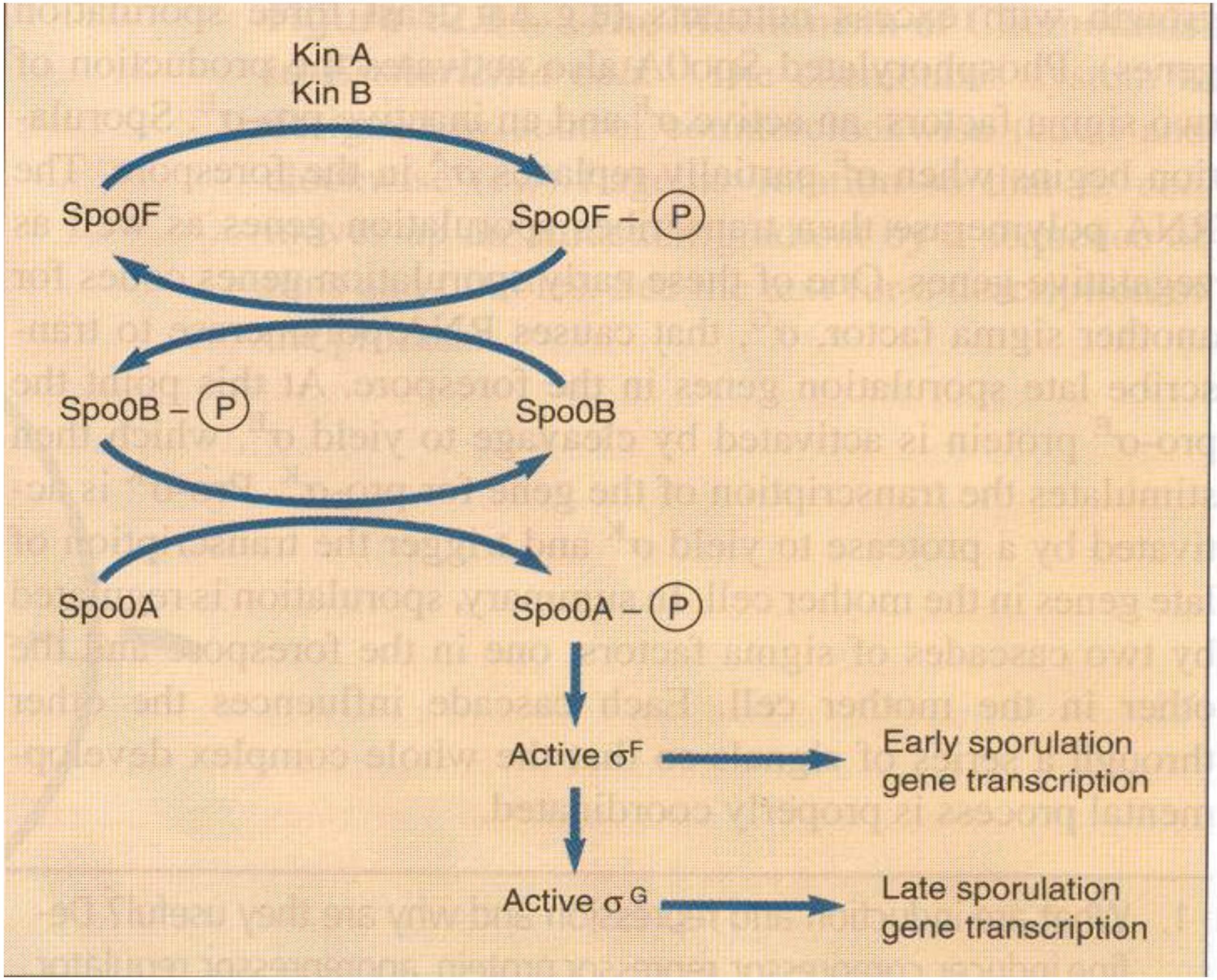


The initiation of sporulation is governed in part by the activities of two spatially separated sigma factors

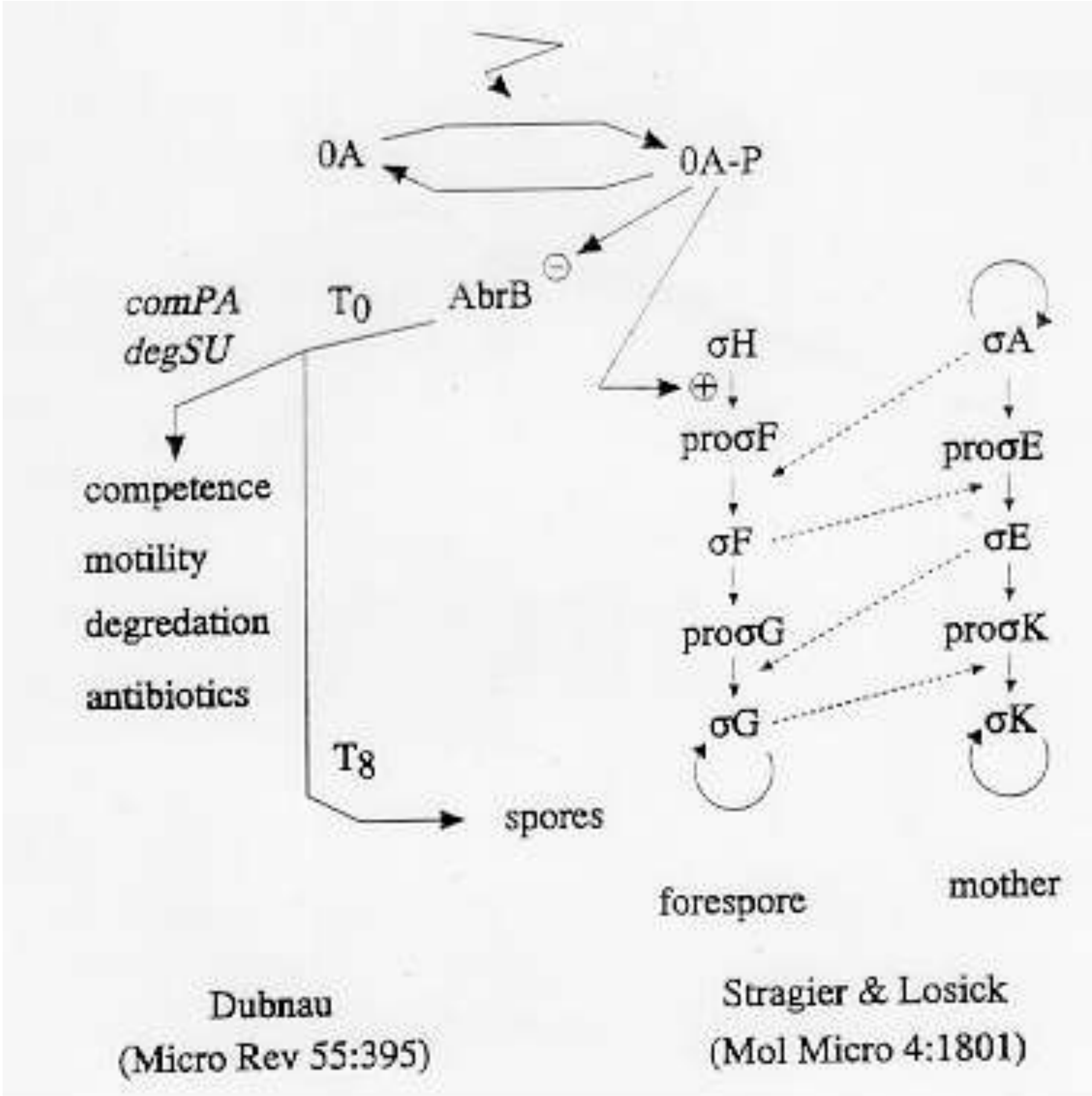


The activation of sigma F is accomplished through a phosphorelay system

Sporulation control in *Bacillus subtilis*



Phosporylated SpoOA also negatively controls transcription of *abrB*, which encodesa repressor of *spoOH*. The *spoOH* gene encodes yet another sigma factor needed during speculation. This negative control of *abrB* actually appears to cause much of the early gene expression during speculation initiation.



0A
0A-P
AbrB
comPA
degSU
T0
competence
motility
degredation
antibiotics
T8
spores
σH
proσF
σF
proσG
σG
σA
proσE
σE
proσK
σK
forespore
mother
Dubnau
(Micro Rev 55:395)
Stragier & Losick
(Mol Micro 4:1801)

Chemotaxis

Microorganisms are able to sense chemicals in their environment and either move toward them or away from them

Run

1. A forward swimming motion
2. Flagellum rotates in a counterclockwise direction (ccw)

Tumble

1. A tumbling motion (to readjust the moving direction of cell)
2. Flagellum rotates clockwise (cw)

Chemotaxis is a unique two component regulatory system (phosphorelay system)

Chemoreceptor：methyl-accepting clemotaxis proteins (MCPs)

Sensorkinase：CheA

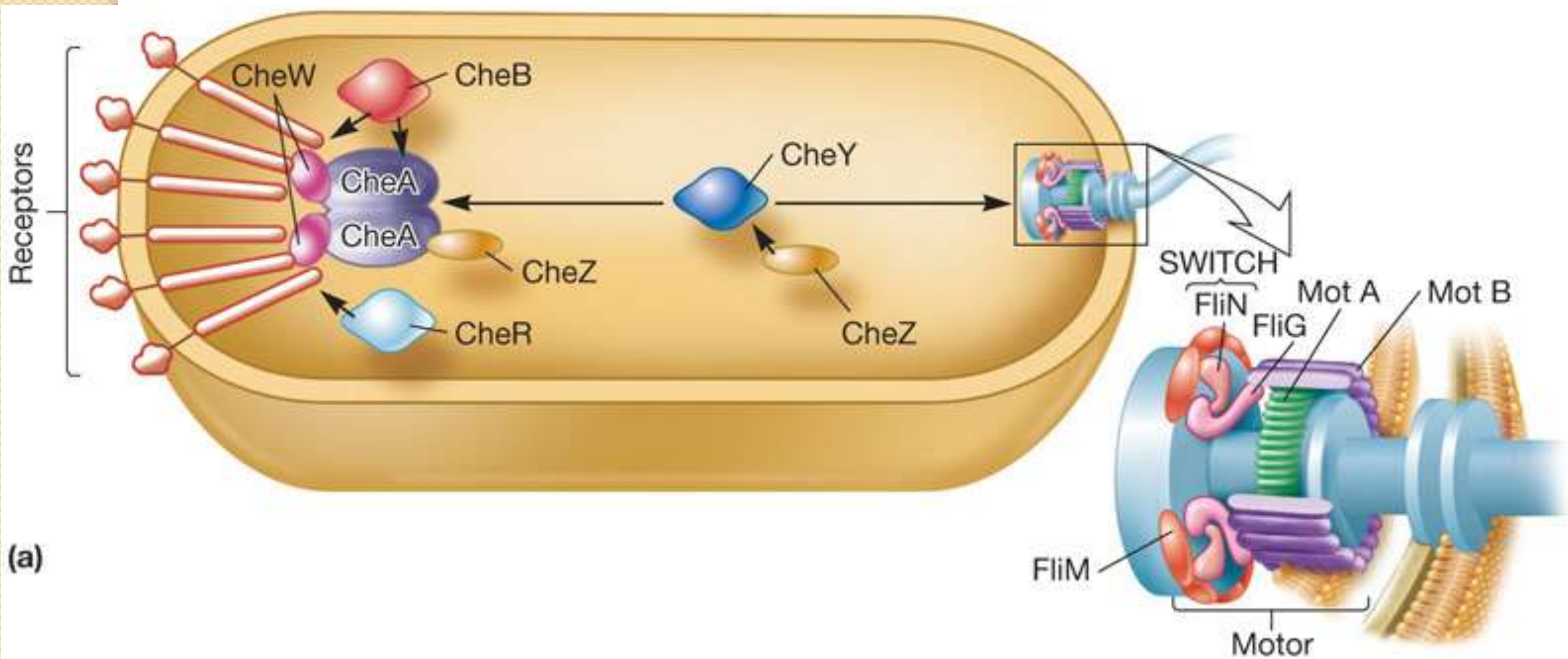
Response regulator：CheY

Chew

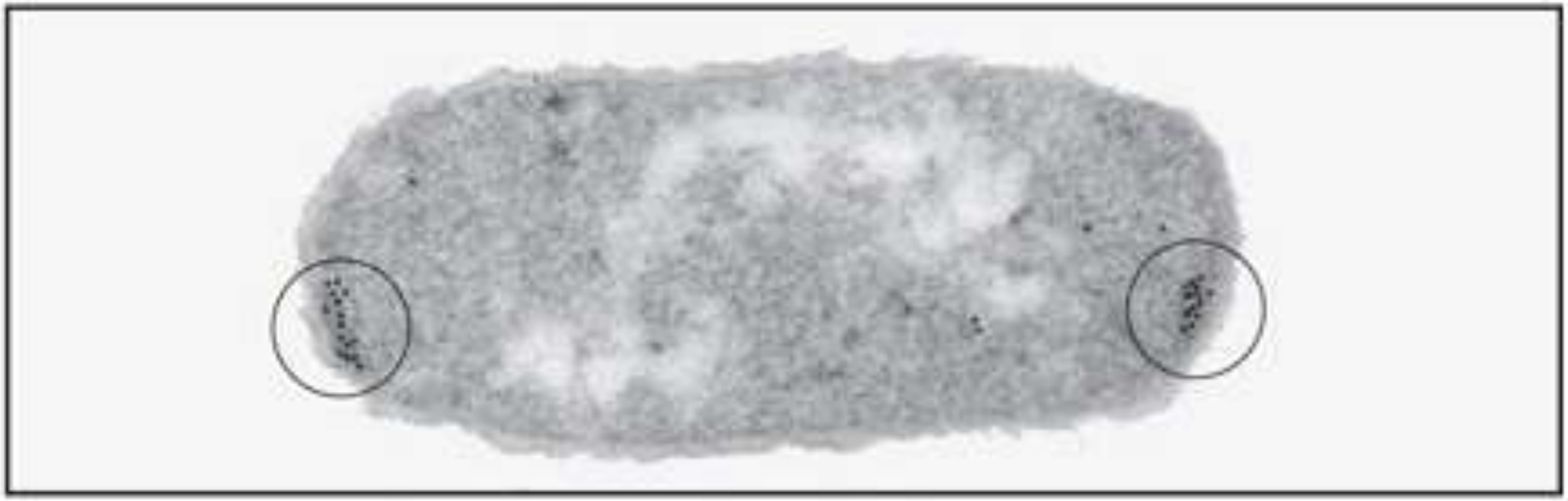
CheR：MCP-specific methyl transferase

CheB：MCP-specific methylesterase

CheZ：phosphatase

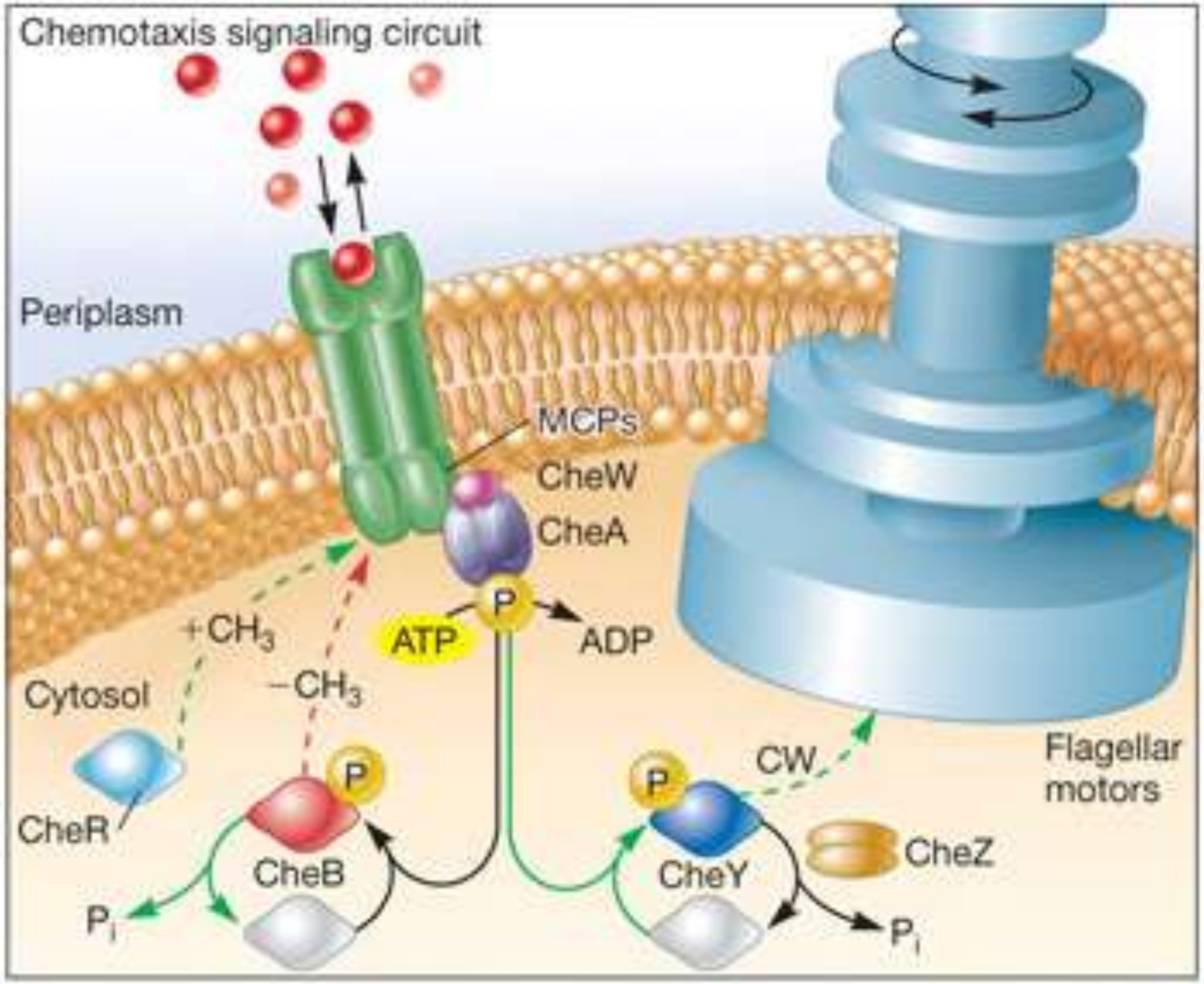


The methyl-accepting chemotaxis protein (MCPs) form clusters associated with the CheA and CheW proteins.



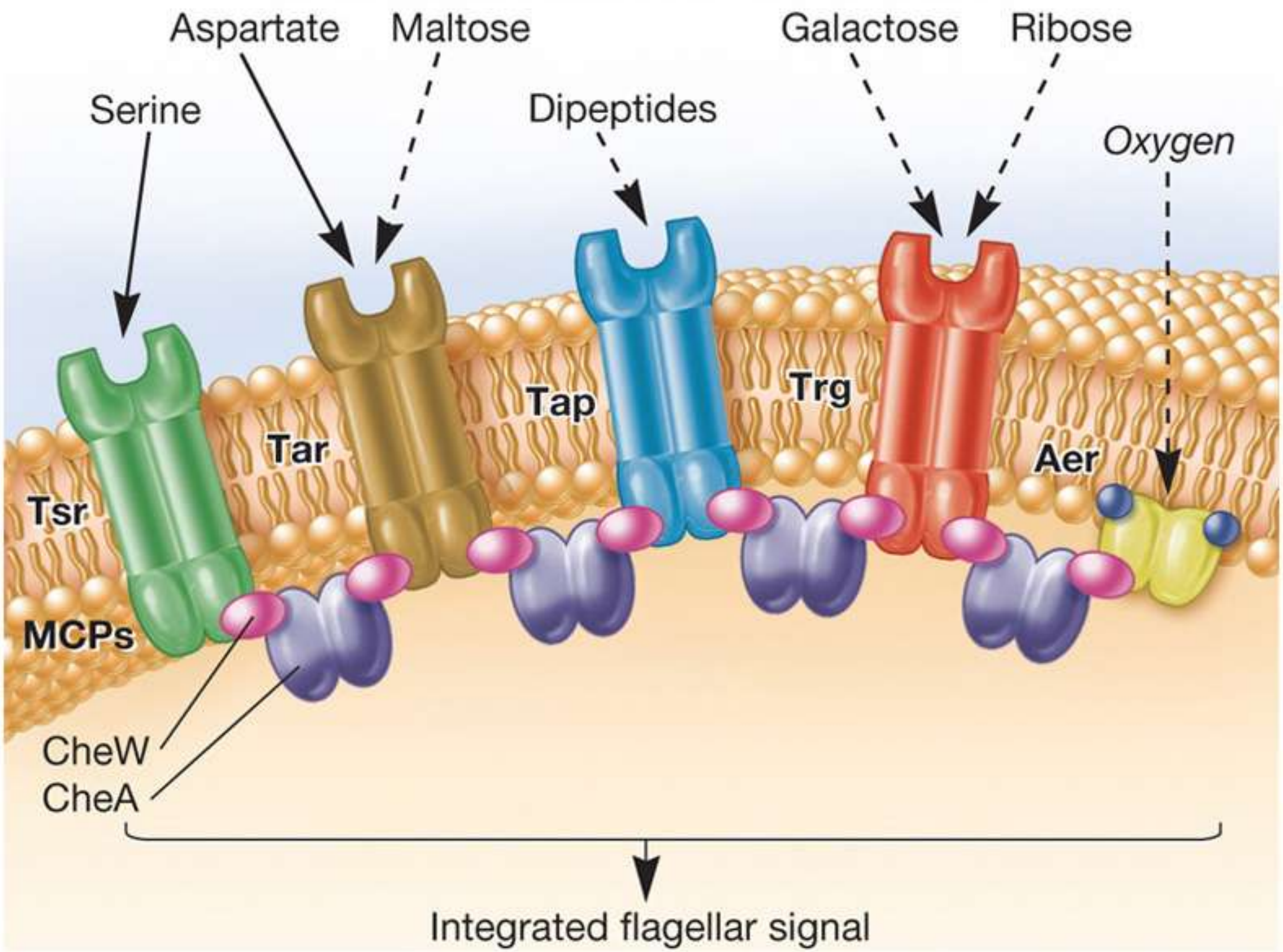
(b)

MCPs, CheW, CheA complexes form large clusters of receptors at either end of the cell

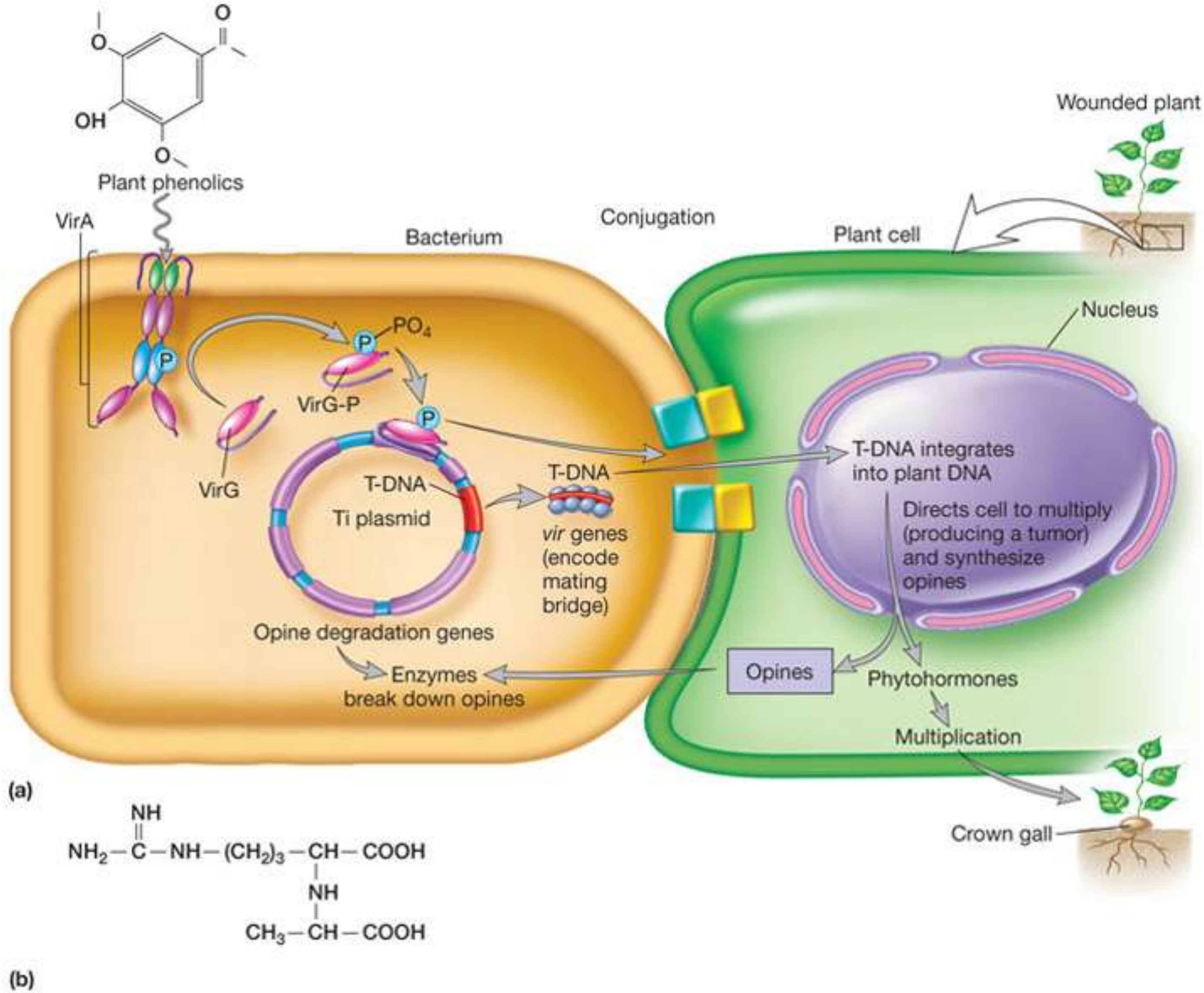


(c)

The chemotactic signaling pathways of *E. coli*.



The Methyl-Accepting Chemotaxis Proteins of *E.coli.*



Functions of Genes Carried on the *Agrobacterium* Ti Plasmid

The GacS/GacA Two-Component System

- **The GacS/GacA two component system is present in a wide variety of Gram-negative bacteria and has been studied mainly in enteric bacteria and fluorescent pseudomonads**
- **This system controls the production of secondary metabolites and extra-cellular enzymes involved in pathogenicity to plants and animals, biocontrol of soilborne plant diseases, ecological fitness, or tolerance to stress.**
- **Usually the GacS/GacA system positively controls the expression of target genes, but negative control exerted directly or indirectly by this system has also been reported.**
- **In *Pseudomonads fluorescences* CHA0, an antagonist of root-pathogenic fungi, the GacS/GacA system tightly controls the expression of antifungal secondary metabolites (e.g. *hcnA* for hydrogen cyanide synthase) and exoenzymes (e.g. *aprA* for major exoprotease) at a posttranscriptional level, involving the RNA-binding protein and global negative regulator of secondary metabolism RsmA.**
- **RpoS (sigma-S or sigma-38) controls a large number of genes that are expressed during postexponential growth and under various stress condition in *E. coli* and other bacteria. In *Erwinia carotovora*, RpoS positively control s *rsmA* expression.**
- **A region surrounding the *hcnA* ribosome-binding site, about 11 nucleotides in length, is instrumental for GacA control. Exactly the same region is also involved in the repression by RsmA, suggesting the RsmA may be a downstream element in the GacS/GacA regulatory cascade.**

The GacS/GacA Two-Component System (continued)

- **In *P. flourescences* CHA0, Rsm Z, a regulatory RNA of 127 nucleotides, can complex the RsmA protein and allow the translation of target mRNA to proceed.**
- **Expression of *rsmZ* depended on GacA, increased with increasing population density (could be the consequence of some type of quorum sensing), and was stimulated by the addition of a solvent-extractable extracellular signal produced by strain CHA0 at the end of exponential growth. This signal appeared to be unrelated to *N*-acyl-homoserine lactones.**
- **Overexpression of RsmZ effectively suppressed the negative effect of *gacS* and *gacA* mutations on target genes, i,e., *hcnA* and *aprA*.**
- **Mutational inactivation of *rsmZ* resulted in reduced expression of these target genes in the presence of added signal. Overexpression of *rsmA* had a similar, but stronger negative effect.**
- **These results support a model in which GacA upregulates the expression of regulatory RNAs, such as RsmZ of strain CHA0, in response to a bacterial signal. By a titration effect, RsmZ may then alleviate the repressing activity of RsmA on the expression of target mRNAs.**

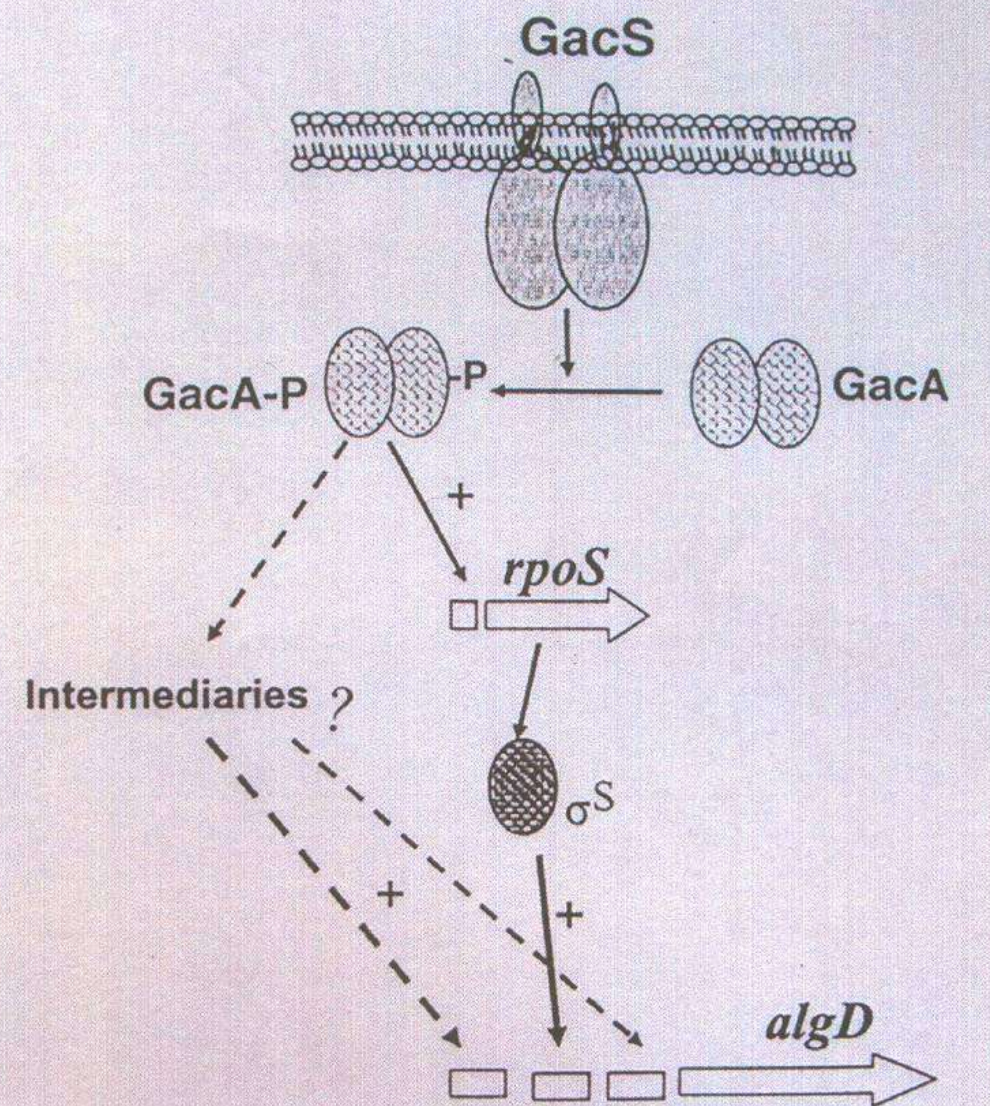


FIG. 5. Model for regulation of *algD* expression by the global regulators GacA and σ^S.

Quorum sensing

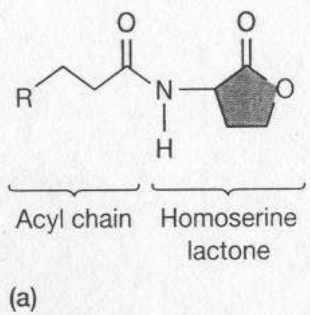
Quorum Sensing (Autoinduction)

The process in which bacteria monitor their own population density by sensing the levels of signal molecules (sometimes called autoinducers) that are released by the microorganisms. When this signal molecules reach a threshold concentration, quorum-dependent genes are expressed.

It could be that autoinducer is a way to determine the extent of diffusion and mixing in a cell's immediate environment. When there is too much diffusion and mixing , it would not make sense to release molecules such as proteases, siderophore, antibiotics, toxins, and virulence factors.

Quorum sensing has been found among both gram-negative and gram-positive bacteria.

The most common signal molecules in gram-negative bacteria are acyl homoserine lactones (AHLs). These are small molecules composed of a 4- to 14-carbon acyl chain attached by an amide bond to homoserine lactone.



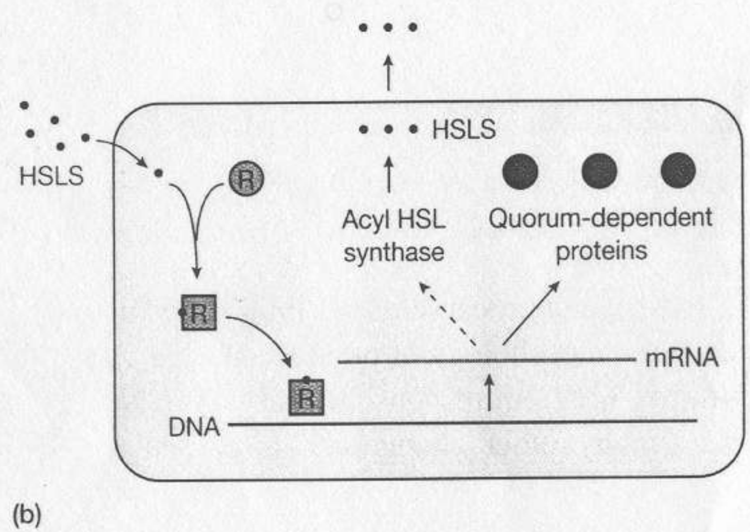
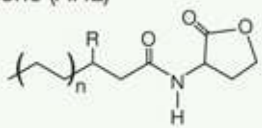
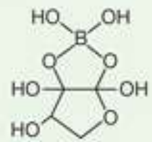
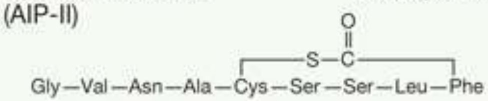
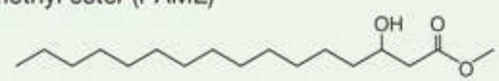
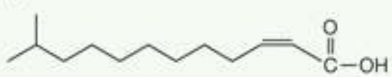
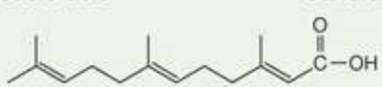
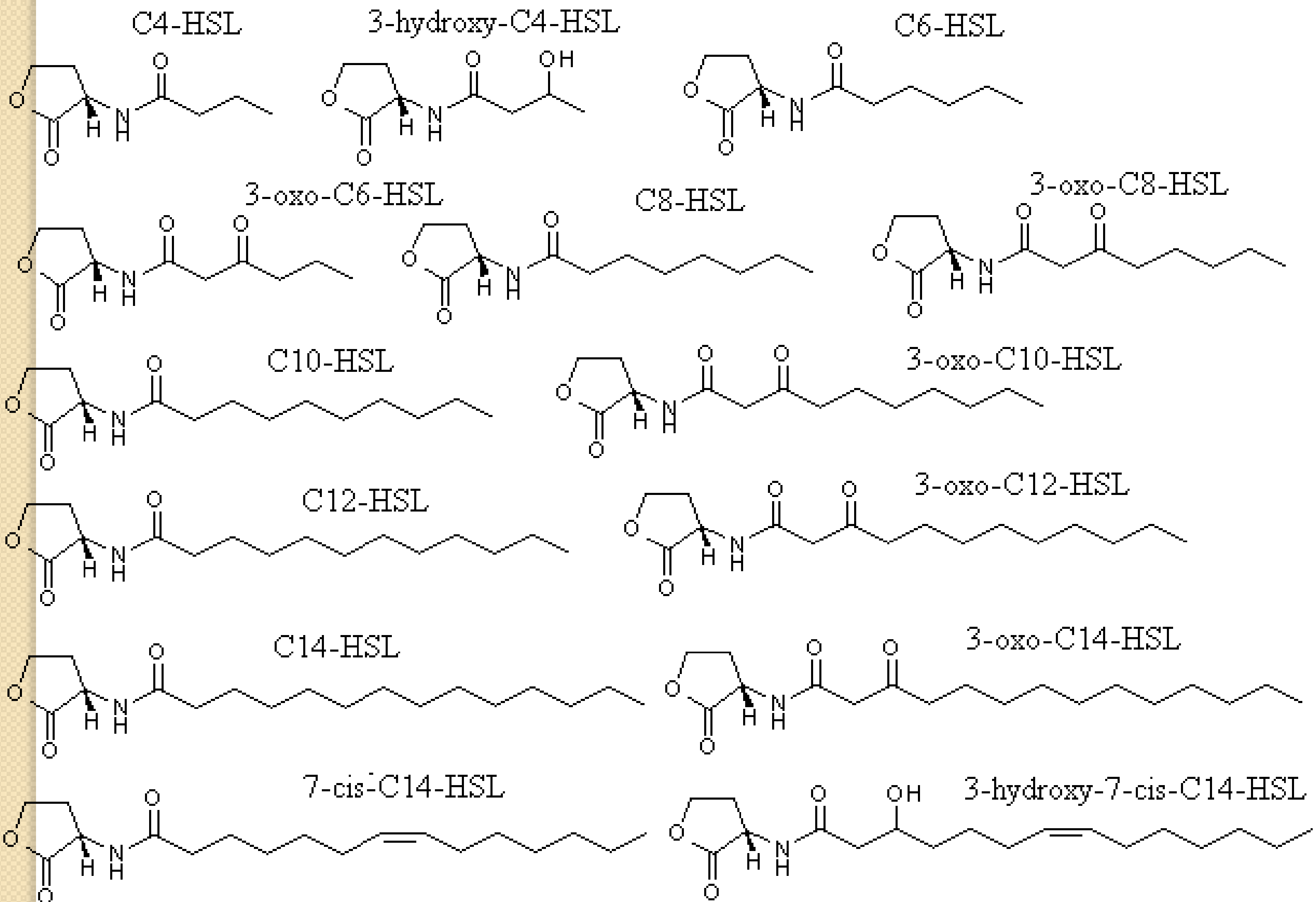
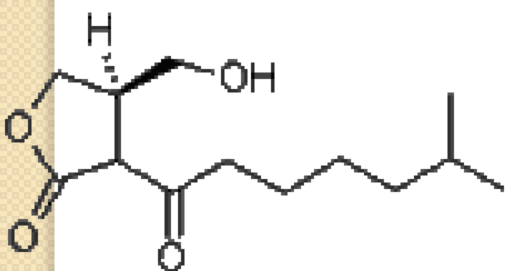


Figure 6.20 Quorum Sensing in Gram-Negative Bacteria. (**a**) A generalized structure for acyl homoserine lactone, the best-known quorum sensing signal or autoinducer. (**b**) A schematic diagram giving an overview of the way in which quorum sensing functions in many gram-negative bacteria. The receptor protein that acts as an inducer is labeled R. The dashed lines indicate that acyl HSL synthase is not always made in response to the autoinducer. See text for more details.

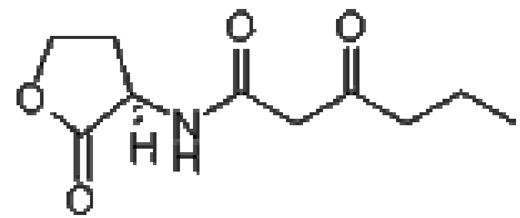
Signal and Structure	Representative Organism	Function Regulated
N-acyl homoserine lactone (AHL) 	*Vibrio fischeri* *Agrobacterium tumefaciens* *Erwinia carotovora* *Pseudomonas aeruginosa* *Burkholderia cepacia*	Bioluminescence Plasmid transfer Virulence and antibiotic production Virulence and biofilm formation Virulence
Furanosylborate (AI-2) 	*Vibrio harveyi*	Virulence
Cyclic thiolactone (AIP-II) 	*Staphylococcus aureus*	Virulence
Hydroxy-palmitic acid methyl ester (PAME) 	*Ralstonia solanacearum*	Virulence
Methyl dodecenoic acid (DSF) 	*Xanthomonas campestris*	Virulence
Farnesoic acid 	*Candida albicans*	Dimorphic transition and virulence

Representative Cell-Cell Communication Molecules

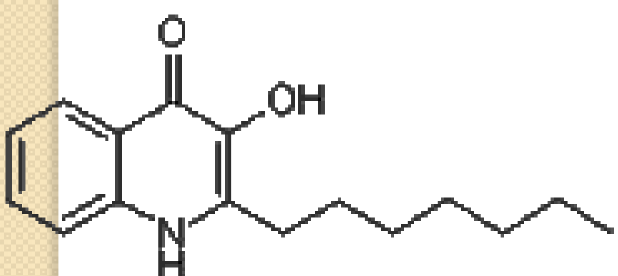

C4-HSL
3-hydroxy-C4-HSL
C6-HSL
3-oxo-C6-HSL
C8-HSL
3-oxo-C8-HSL
C10-HSL
3-oxo-C10-HSL
C12-HSL
3-oxo-C12-HSL
C14-HSL
3-oxo-C14-HSL
7-cis-C14-HSL
3-hydroxy-7-cis-C14-HSL



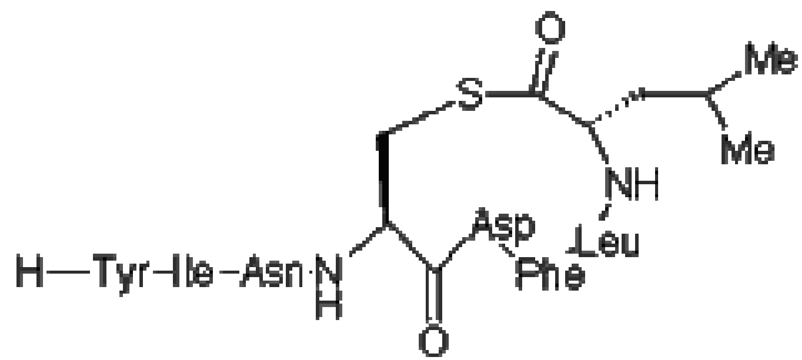
γ–butryolactone from *Streptomyces griseus*



3-oxo-C6-HSL from *Vibrio fischeri*



2-heptyl-3-hydroxy-4-quinolone
from *Pseudomonas aeruginosa*



cyclic thiolactone from type III
Staphylococcus aureus

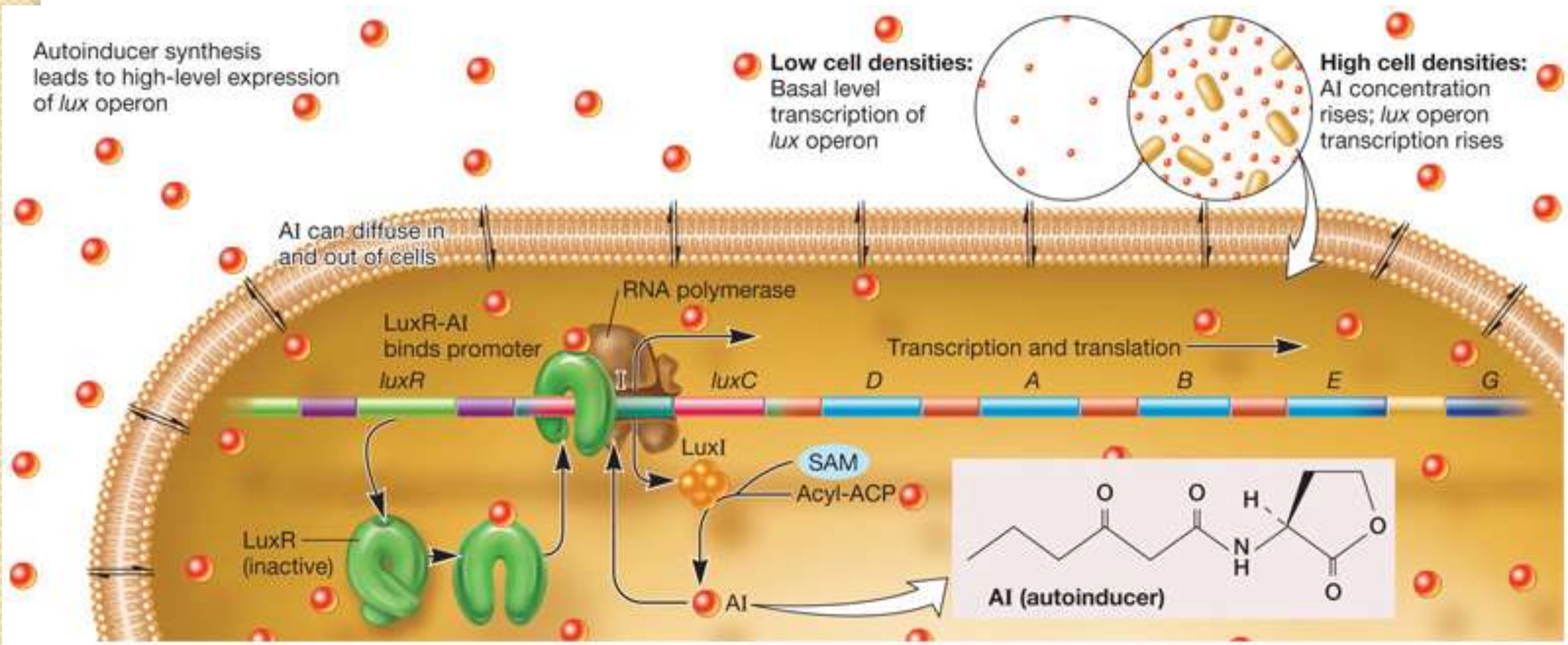
Vibrio fischeri

Signal (3-oxo-6-HSL , an N-acyl homoserine lactone or AHL) is synthesized by the protein LuxI and sensed by the protein LuxR.

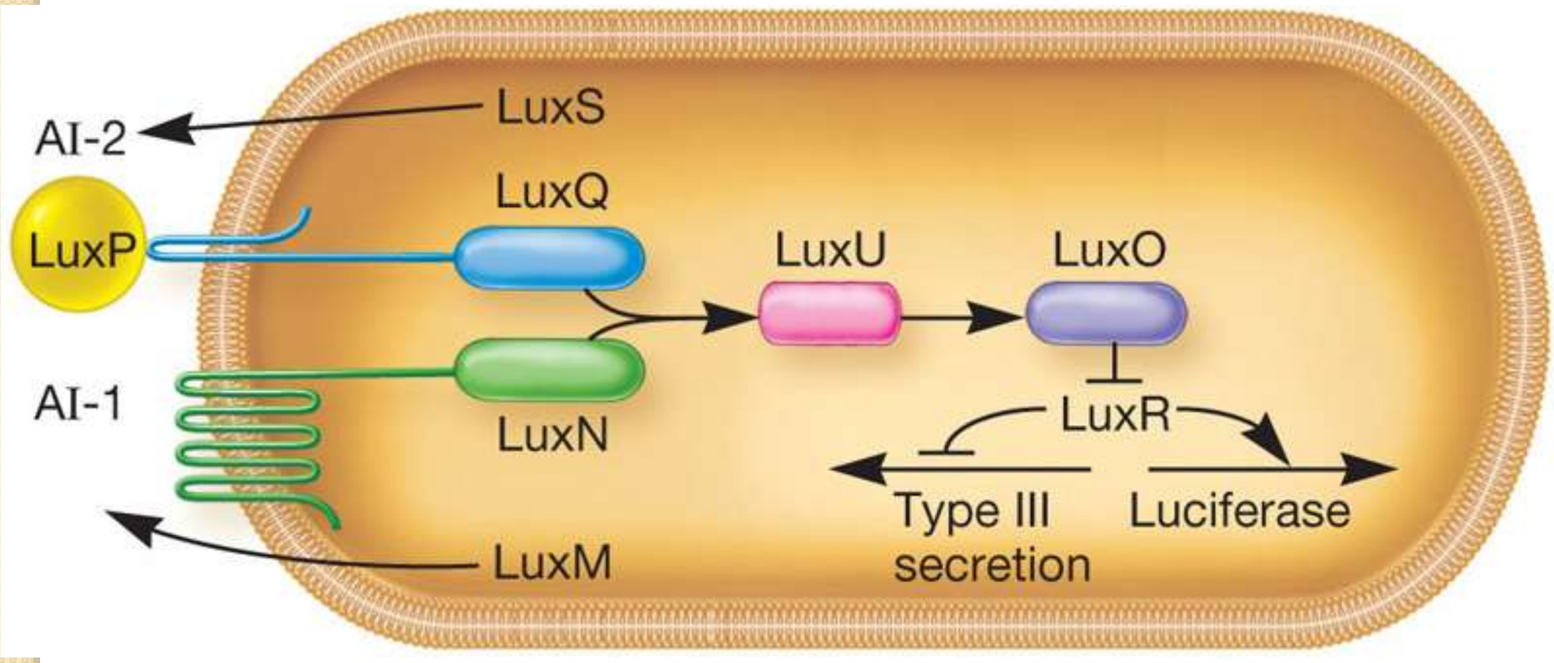
V. harveyi

It has two quorum sensing systems

1. AHL signal (3-hydroxy-C4-HSL) ,which is generated by the protein LuxM, is received by the LuxN protein.
2. AI-2, generated via Lux S is received by the Lux P and Lux Q proteins.
   Lux N contain both sensor Kinase and response regulator domains of two component systems.



Quorum Sensing in *V. fischeri*



Quorum sensing in *V.harveyi.*

Gram positive bacteria have been shown to communicate using a number of different QS signals

1. Many employ post-translationally modified peptides created from larger precursors.
   1) These peptides are usualy secreted by ATP-binding cassette (ABC) transporters.
   2) Some interact with membrane bound sensor Kinases that transduce a signal across the membrane.
   3) Others are transported into the cell by oligopeptide permeases, where they then interact with intracellular receptors.

   Examples:
   1) Virulence in *Staphylococcus aureus*.
   2) Competence for DNA-uptake in *Bacillus subtilis* and *Streptococcus pneumoniae*.
   3) Sporulation in *B. subtilis.*
   4) Conjugal plasmid transfer in *Enterococcus faecalis*.
   5) Bacteriocin production in lactic acid bacteria.

2. Butyrolactone

   This is used by several *Streptomyces* species to control production of antibiotics and antibiotic resistance.

3. AI-2 signal molecule synthesized by Lux S protein, but no homologues

   of the receptors for AI-2 have been identified.