Evolution of Cellular Data Processing

Being a condition of survival, the ability to adapt to an ever-changing environment has accompanied life from the very first day. This means that even the most ancient and primitive cell must have been able to recognize and to rate relevant environmental signals. As explained in Chapter 1, this is done by a data-processing protein network that, particularly for unicellular organisms such as prokaryotes, fully resembles a nervous system, justifying the metaphor of a cellular "nanobrain." Investigation of prokaryotic signal transduction has shown that, in its basic biochemical mechanisms, such a primeval network does not differ fundamentally from signal processing networks in eukaryotes and the mammalian nervous system, thus nourishing the concept of a common evolutionary ancestry that had entered the stage in the beginning of life. A closer look at today's prokaryotes gives us some idea of how this might have occurred in the past. It immediately makes clear that the data-processing network must have evolved in parallel to the metabolic network.

3.1 Evolution of biological signal processing

Organisms are classified in three domains: bacteria and archaea (or archaebacteria), together constituting the prokaryotes, and eukaryotes. Eukaryotes differ from prokaryotes by a nuclear membrane and an intracellular compartmentalization in structures such as endoplasmic reticulum, Golgi apparatus, mitochondria, and chloroplasts. Genetic studies have revealed that eukaryotes are related more closely to archaea than to bacteria (Figure 3.1). This was a somewhat surprising discovery because archaea were formerly thought to be the primary

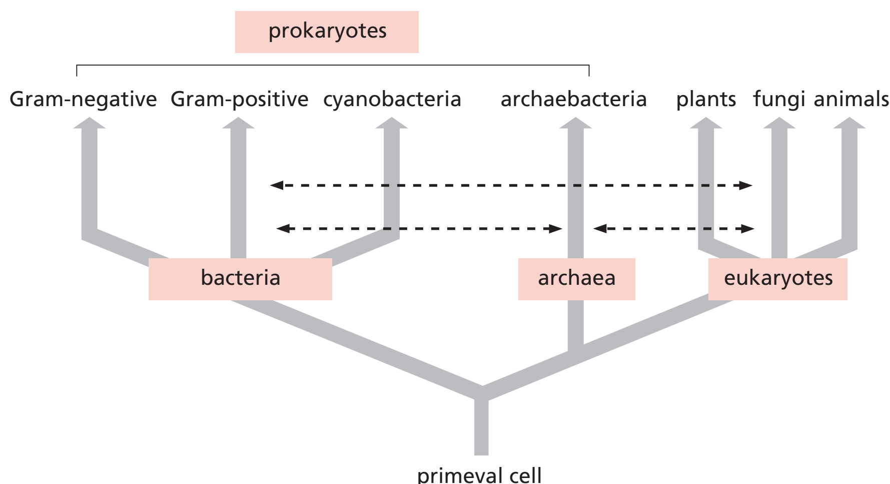


Figure 3.1 Phylogenetic three-domain tree Broken horizontal lines symbolize horizontal gene transfer. For other details see text.

organisms, in particular since they are settlers of extreme biotopes. The high degree of horizontal gene transfer between the three domains was another unexpected finding.

Once considered to be primitive, prokaryotes are now acknowledged as unexpectedly complex and highly organized forms of life. Their amazing ability to settle any possible ecological niche gives the reason for their overwhelming evolutionary success. Leaving aside adaptation by gene mutations, the cellular conditions of this ability are:

- rapid and reversible adaptation of gene expression and, thus, of the protein pattern (or "proteome") to the actual environmental conditions
- precise control of behavior such as motility, metabolism, and transport processes
- establishment of social interactions aimed at genetic recombination and division of labor in multicellular structures

To perform such adaptive responses, the cell must be a cognitive system. What are the primary environmental signals that a prokaryotic "nanobrain" has to decipher for the sake of survival? To exist in an unpredictable environment requires a permanent search for optimal conditions of life, or in other words, minimization of stress. It is conceivable, therefore, that the most elementary forms of signal processing are aimed at coping with stress situations such as starvation, disturbances of osmotic pressure, intoxication, dangerous radiation, and attack by predators. A cell unable to track down energy resources and blind to danger has no chance to survive. Thus, the processing of *environmental signals* stands at the very beginning (Figure 3.2). Social contacts or even the formation of multicellular colonies, including specialization of cells by differentiation aimed at a division of labor, may facilitate survival even more. Both require the evolution of an *intercellular* signaling system. It must be emphasized that, on the level of signal decoding, a cell does not make a distinction between environmental and intercellular signals: both are processed by the same network, provided the cell possesses the corresponding sensors or receptors.

A characteristic example of an ancient intracellular signaling reaction dealing with environmental stress is the **stringent response**. This is a primeval bacterial emergency reaction that saves energy. It leads to an almost complete stop of ribosome production and protein synthesis and, as a consequence, of cell proliferation. These are extremely expensive processes devouring almost 50% of the cellular energy. The stringent response is triggered by worsening life conditions, particularly starvation. Due to a lack of certain amino acids, uncharged tRNA accumulates at the ribosomes. As an immediate consequence, the ribosome-associated enzyme RelA, also known as stringent factor, becomes activated. RelA

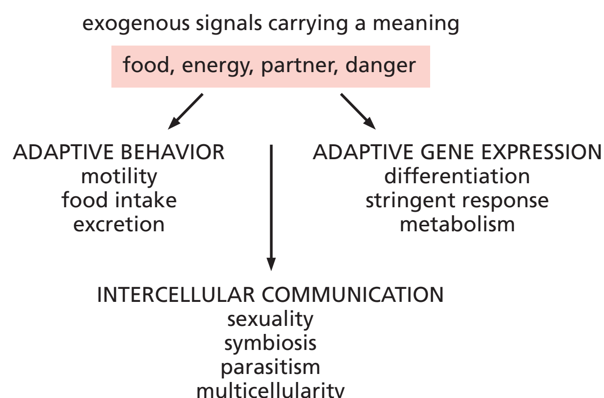


Figure 3.2 Evolution of biological signal processing All cells are able to adjust their behavior and pattern of gene expression to exogenous (environmental and intercellular) signals provided these carry a meaning such as food, energy, danger, and partner cells. The meaning is decoded by the cellular apparatus of data processing. The intercellular signaling systems of multicellular organisms have evolved from intercellular communication that prokaryotes require for sexual, symbiotic, and parasitic interactions as well as for the formation of multicellular collectives.

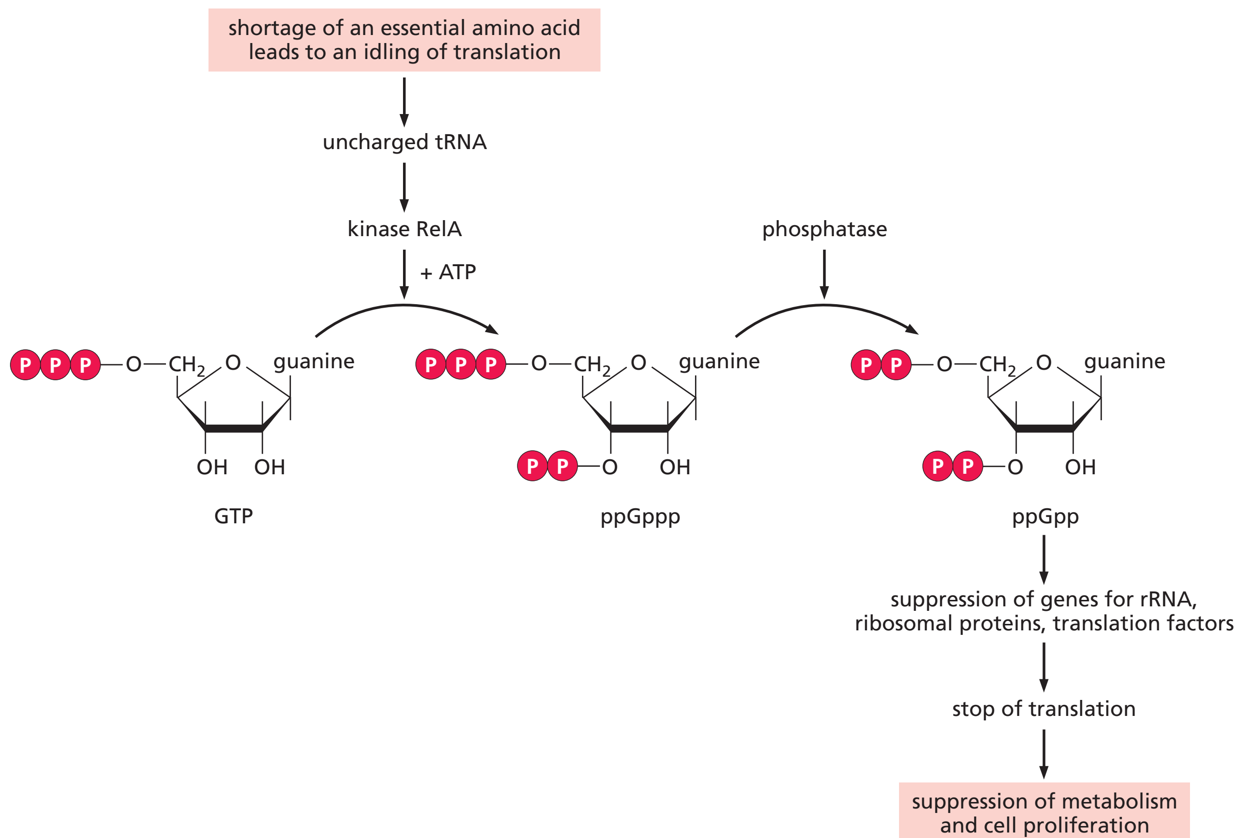
is a guanylate kinase that catalyzes the ATP-dependent pyrophosphorylation of GTP in position 3′, yielding ppGppp, which subsequently is dephosphorylated to guanosine 3′,5′-bis(diphosphate) (ppGpp) by a specific phosphatase (Figure 3.3). ppGpp has the function of an intracellular emergency signal or "**alarmone**," binding to the catalytic β-subunit of RNA polymerase and inhibiting, in particular, the *de novo* synthesis of ribosomal RNA, ribosomal proteins, translation factors, and tRNA. Cells containing a defective *relA* gene (*rel*axed mutants) are unable to adapt, literally growing to death in deficiency situations. Evolutionarily advanced species such as myxobacteria use the stringent response for a more sophisticated survival strategy, the formation of multicellular spore capsules (see Section 3.4.2).

The enzymatic apparatus of ppGpp synthesis, including RelA-homologous enzymes, has also been found in **plant chloroplasts**, which are thought to have evolved from bacteria. Like the bacterial system, the plant system is stimulated under stress situations, here in particular by wounding. Eukaryotes possess, in addition, other highly efficient means to turn down translation upon amino acid deficiency, operating by mechanisms that differ entirely from the stringent response (Sections 9.3 and 9.4).

Summary

By processing a wide variety of exogenous stimuli prokaryotes easily adapt to environmental conditions. The most elementary forms of signal processing are aimed at coping with emergency situations (stress). An example is provided by the stringent response, an adaptation of cellular protein synthesis to environmental stress. The response is based on the generation of intracellular messengers (alarmones such as ppGpp) that suppress RNA polymerase activity. Social interactions, cell differentiation and formation of multicellular structures depend on the evolution of *intercellular* signaling systems. Environmental and intercellular signals are processed by the same protein-DNA network.

Figure 3.3 Signaling of the stringent response in *E. coli* Stress situations such as a shortage of certain amino acids cause an idling of translation, resulting in activation of the ribosomal kinase RelA. The latter transfers a pyrophosphoryl group from ATP to the 3′-position of 5′-GTP. ppGppp thus generated becomes dephosphorylated enzymatically, yielding the "alarmone" ppGpp that acts as a transcriptional suppressor of genes encoding RNA and proteins required for translation. The result is an almost complete stop of protein synthesis. P, phosphate group.



3.2 Signal-controlled transport across cell membranes: the ancient way to communicate

The controlled uptake of food and the excretion of (toxic) metabolic end products stand at the very beginning of any communication between an organism and its environment. Since the lipid bilayer of the plasma membrane is impermeable for most substances, even the most primeval cells were forced to develop regulative transport mechanisms based on the function of special membrane proteins.

There are four types of transport processes:

(1) **Passive transport**, *along* a concentration gradient, is made possible by membrane channels or carrier proteins (uniporters) and therefore has been called "facilitated diffusion." Examples of uniporters are the glycerol transporter of *Escherichia coli* and the glucose transporters GluT1 and GluT4 in liver and muscle or fat tissue, respectively (Section 7.1.3).

(2) **Primary active transport**, *against* a concentration gradient, requires energy derived from enzymatic hydrolysis of ATP; that is, membrane-bound transport proteins have intrinsic ATPase activity or associate with ATPases. These transporters are discussed in more detail in Section 3.2.1.

(3) **Secondary active transport**, *against* a concentration gradient, derives the energy from an ion flow occurring along a concentration gradient and coupled with the transport process. Examples are the lactose transporter LacY driven by a proton gradient in *E. coli* and the Na^+-Ca^{2+} exchanger powered by a Na^+-gradient (Section 14.5.1).

(4) **Group translocation** consists of a targeted transport process coupled with an energy-delivering chemical modification of the molecule to be transported. In most cases this is achieved by phosphorylation (the translocation of a phosphate group) requiring the hydrolysis of an energy-rich phosphoric acid derivative such as ATP or phosphoenolpyruvate (PEP). This process is discussed in detail in Section 3.2.2.

Even a very simple prokaryote such as *Mycoplasma genitalium* (harboring only 470 genes) expresses one passive transporter, 13 ATP-powered transporters, and one ion-gradient-powered transporter as well as two group translocation systems. For *E. coli*—although with about 4300 genes it is by no means the most complex prokaryote (the soil bacterium *Sinorhizobium meliloti* has 6300 genes, as many as yeast)—the corresponding numbers are 7, 194, 74, and 22, respectively.

3.2.1 ATP-powered membrane transporters

The proteins of this group are ATPases catalyzing the *targeted* transmembrane transport of ions or larger molecules, frequently against a concentration gradient.

Ion pumps

The bacterial **F-type transporters** use ATP hydrolysis to generate a proton gradient across the cell membrane, driving numerous active transport processes. The reverse reaction results in ATP production. In fact, the ATP synthases of mitochondria and chloroplasts belong to the family of F-type transporters. The **P-type transporters** represent another family of ATP-driven ion pumps. Examples are provided by the Na^+/K^+-dependent ATPases (sodium pumps) and the Ca^{2+}-dependent ATPases (calcium pumps). **V-type transporters** found in the membrane of intracellular vacuoles are restricted to archaea and eukaryotes.

ABC transporters

The largest and most versatile subfamily of ATP-powered membrane transporters is the ABC transporters, named after the ATP-Binding Cassette, a particular ATP binding site in the ATPase domain. Characteristic structural features of this highly conserved cassette are the ATP-binding Walker A motif or P-loop and the Mg^{2+}-binding Walker B motif (ATP is bound as a Mg^{2+} complex). Both motifs are common to nucleoside-triphosphatases and kinases (see Section 2.4).

ABC transporters are modular proteins consisting of four domains: two transmembrane domains, frequently with six transmembrane helices each, and two cytoplasmic domains with one ATP binding site each (Figure 3.4). These domains may exist as separate subunits or may be integrated in one or two polypeptide chains. The transport channel is established by a circular arrangement of the transmembrane helices.

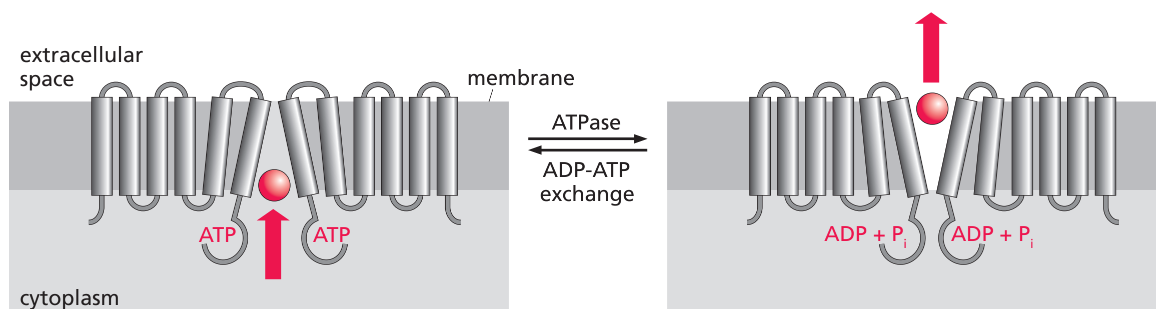
In *E. coli* the ABC transporters constitute a large gene family (74 genes). They transport a wide variety of substances, from simple ions, amino acids, and sugars to peptides and proteins, and each transporter is specific for the uptake or excretion of a distinct substance. The uptake of material is facilitated by selective binding proteins mostly found in the periplasm of Gram-negative bacteria. They function as a kind of receptor and interact with the ABC transporters.

One of the best-studied ABC transporters is the **MsbA** protein of *E. coli*. It is the only transporter that is absolutely essential for the bacterial cell. MsbA is a multidrug resistance protein, which exists in more than 30 homologs. These proteins are responsible for the excretion of lipophilic and toxic (foreign) substances such as drugs. An adaptive overexpression of MsbA is one of the mechanisms that renders bacteria resistant to antibiotics. Resistant bacteria are assumed to be the reason for approximately 60% of iatrogenic diseases.

The MsbA protein is composed of two polypeptide chains with six transmembrane helices and one ATP binding site each. It probably functions as a "flippase," trapping the substrate in a cage at the inner side of the membrane and releasing it to the outside upon a conformational change brought about by ATP hydrolysis (Figure 3.4).

The human homolog of MsbA is the multi-drug resistance protein **MDR1**, also called P-glycoprotein. Its adaptive overexpression in tumor cells is one of the mechanisms leading to the feared resistance against cytostatics during cancer chemotherapy. MDR1 is the prototype of a large family of eukaryotic ABC transporters with several functions. Another human ABC protein, the cystic fibrosis transmembrane conductance regulator or **CFTR**, is strictly speaking not a transporter but a hormone-controlled chloride channel regulating the function of mucous membranes (for more details see Section 14.7.2). A defective *cftr* gene is the cause of cystic fibrosis or mucoviscidosis, which is one of the most common human hereditary diseases. In both MDR1 and CFTR, the four domains of an ABC transporter share a single polypeptide chain.

Figure 3.4 Bacterial ABC transporter of type MsbA The protein is composed of two identical subunits with six transmembrane domains (cylinders) and one intracellular ATP-binding site each. ATP hydrolysis causes a conformational change, promoting the excretion of toxic substances (red ball) trapped in a cage. The transporter becomes reloaded upon ADP–ATP exchange.



3.2.2 Group translocation systems: primeval forms of data-processing networks combining food uptake with gene regulation

The best-known representatives of this family of membrane transporters are the PEP (phosphoenol pyruvate)-powered **phosphotransferase systems**, abbreviated as PTS. They combine membrane transport with the regulation of gene transcription.

Prokaryotes are capable of switching their genes on and off in a specific and signal-controlled mode in order to adapt to the actual conditions of the biotope. As far as the molecular mechanism is concerned, there is no fundamental difference between prokaryotic and eukaryotic gene transcription. In both cases, an initiation complex containing RNA polymerase and a series of regulatory proteins assembles at the gene at sites that have been marked by transcription factors. This is the key event and primary target of signaling (for details see Chapter 8). A characteristic feature of prokaryotes is that genes controlling an individual metabolic process (structural genes) are combined into functional units known as operons. Operons are transcribed as a whole into a polycistronic RNA and are under the control of both stimulatory and inhibitory gene sequences called promoters, enhancers, and silencers. These are the binding sites of transcription factors. Stimulatory transcription factors facilitate and inhibitory factors (repressors) inhibit the assembly of an active RNA polymerase complex. The major signaling events controlling transcription factor and repressor activity in both pro- and eukaryotes are noncovalent ligand binding and covalent protein phosphorylation. Moreover, repressor activity is frequently regulated by corresponding metabolic products acting via feedback loops or by special signaling reactions. The active form of a transcription factor is always a homo- or heterodimer, enabling a cooperative effect with an intrinsic noise filter (Section 1.3). Transcriptional control supports the idea formulated in Chapter 1 that signal processing is based on combinatorial processes. A cell contains many more genes than transcription factors. Only 5% of the 4290 genes of *E. coli* encode gene-regulatory proteins. How, then, is *specific* gene regulation achieved? The answer is that the majority of bacterial gene promoters are regulated by a set of different transcription factors rather than by a single regulatory protein. Because of this combinatorial principle, a wide variety of environmental stimuli may affect a large number of genes by use of only a rather limited number of regulatory proteins. The cooperation between activators and repressors renders these systems logical gates (see Section 1.5).

PTSs manage the uptake of a variety of sugars and sugar alcohols by substrate phosphorylation, using PEP as the phosphate donor and energy provider (Figure 3.5). Phosphorylation is the starting reaction of substrate metabolism such as glycolysis. The process is embedded in a network of interacting proteins that adjust behavior and genetic readout to the food supply. PTSs, showing the inseparable connection between metabolism and signal processing, indicate that even on a rather simple level the cellular response to an exogenous stimulus has to be treated as a systemic answer of the whole organism rather than as an isolated biochemical reaction.

PTSs are abundant in bacteria but have not yet been found in archaebacteria and eukaryotes. Even among bacteria they are differentially distributed. *E. coli* expresses 22 PTSs with different substrate specificities, while the 10 times smaller genome of *Mycoplasma genitalium* contains only two PTS genes (for the transport of glucose and fructose) and *Mycobacterium tuberculosis* has none.

PTSs are multiprotein complexes consisting of highly conserved and variable parts. A prototype is the glucose-transporting PTS^{Glc} of *E. coli.* Its conserved parts are the two cytoplasmic enzymes EI and HPr (Histidine-containing phosphocarrier Protein), while the variable part is the transport complex EII. EII is

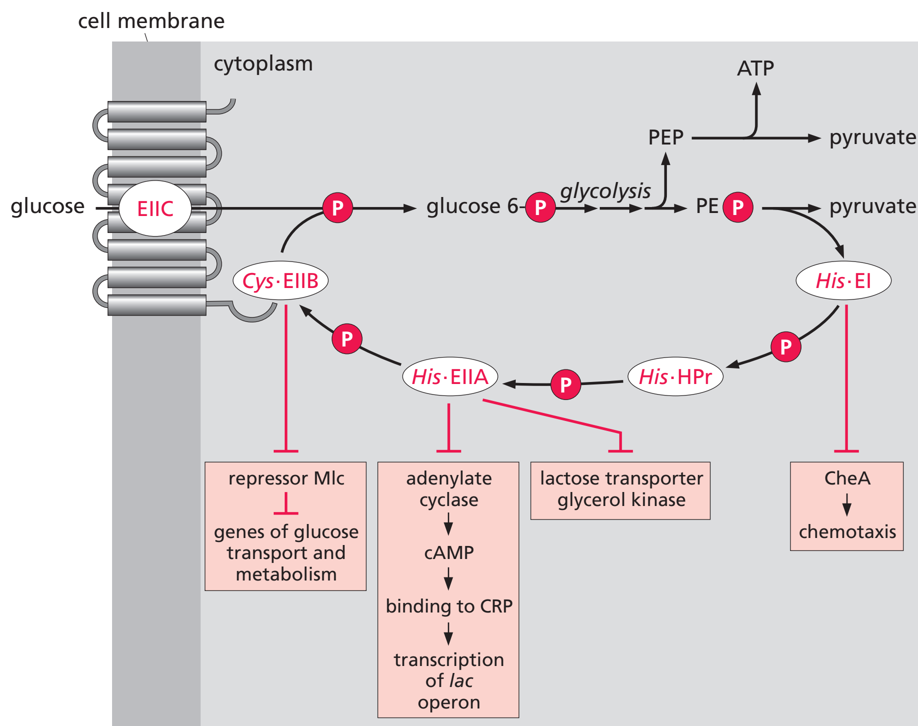


Figure 3.5 Phosphotransferase system (PTS) of *E. coli* The system couples the glycolytic pathway with glucose transport and regulatory reactions. Consisting of the cytoplasmic proteins EI, HPr, and EIIA and the membrane-bound channel complex EIIB/EIIC, it stepwise transfers a phosphate residue (P, red circle) from one of the two phosphoenolpyruvate (PEP) molecules derived from glucose 6-phosphate to glucose, which is taken up by the cell as glucose 6-phosphate. The second PEP molecule is used for ATP generation. When activated by glucose, the system changes cell motility and inhibits the uptake and metabolism of alternative nutrients (catabolite repression). The phosphorylated amino acids of the PTS proteins are depicted in italic type.

composed of three proteins: the membrane transporter EIIC and the associated proteins EIIA and EIIB (Figure 3.5). EI and HPr are separate cytoplasmic proteins, whereas the three EII subunits may either share one polypeptide chain (EIIABC; for instance, in the *N*-acetylglucosamine translocator of *E. coli*) or be partially separated (EIIA + EIIBC; for instance, in the glucose translocator of *E. coli*). EIIC contains eight transmembrane domains and as a dimer constitutes the transmembrane channel.

The homodimeric enzyme EI catalyzes the hydrolysis of PEP, transiently binding the phosphate residue at a histidine residue (His189 in *E. coli*); thus it is acting as a histidine autokinase. From there the residue migrates via HPr, EIIA, and EIIB to glucose, which as glucose 6-phosphate passes the membrane channel EIIC. In both HPr and EIIA the phosphate residue is also bound to His residues; however, in EIIB, which is the glucose phosphorylase proper, the phosphate residue interacts with a cysteine residue.

Together these phosphorylations are thermodynamically unstable, short-lived transition states of phosphotransferase reactions. [Note that the phosphoamide bond of phospho-His contains so much energy that the reaction ATP + protein ↔ ADP + protein-P is not shifted to the left only because the product (protein-P) becomes rapidly dephosphorylated by the subsequent reactions.] The more glucose available, the higher the rate of the cell's catabolic metabolism and the intracellular PEP level. Under such conditions the PTS machinery is running at top speed, phosphorylating glucose at a high rate and, thus, generating a rapid draining of phosphate. As a consequence, the steady-state concentrations of *phosphorylated* PTS proteins are low, whereas they rise upon glucose deficiency. The degree of phosphorylation of PTS components is used for a coupling of the transport process with accessory cellular functions. In fact, PTS represents the backbone of a simple but highly efficient signal-processing protein network enabling the cell to perfectly adapt to environmental conditions.

At a high glucose level, the now underphosphorylated EI inhibits the chemotaxis protein CheA, changing the motility pattern of the cell in such a way that it is able to approach the source of food (positive chemotaxis, see Section 3.3.4). Under

the same conditions, underphosphorylated EIIB inhibits the transcriptional repressor Mlc, thus ensuring continuously high activity of the genes encoding the enzymes of glucose transport and metabolism. Moreover, as long as sufficient glucose is available, underphosphorylated EIIA binds and inactivates the transporters for lactose and other sugars, preventing the costly uptake of less attractive nutrients for which the metabolic enzymes are not available, since the corresponding genes, such as the *lac* operon, are also repressed by EIIA. Only when the glucose supply is beginning to run low does this inhibition, called **catabolite repression**, become gradually canceled, enabling the cell to exploit alternative sources of food. The trigger for this genetic and metabolic changeover is the increase in PTS phosphorylation, by which a signal is released that changes the transcriptional pattern. In Gram-negative bacteria such as *E. coli*, this signal is **cyclic adenosine 3′,5′-monophosphate (cAMP).** It is generated from ATP when highly phosphorylated EIIA activates the membrane-bound enzyme adenylate cyclase (see Sidebar 3.1 and also Section 4.4.1 for a reaction scheme).

The function of cAMP is that of an intracellular signaling molecule or second messenger. To this end it binds to a cAMP receptor protein, CRP (also called Catabolite gene Activator Protein, CAP), and the complex thus formed interacts with the α-subunit of RNA polymerase, functioning as a co-activator for a series of catabolite-repressed gene complexes such as the *lac* operon (Figure 3.5).

Being an ancient control mechanism, cAMP signaling is by no means restricted to *E. coli*-like bacteria; many other prokaryotes express a wide variety of adenylate cyclase isoforms. This holds true even for most primitive species such as mycobacteria that have been found to produce cAMP in unusually large amounts. The genome of *Mycobacterium tuberculosis*, for instance, encodes no less than 17 nucleotide cyclases as well as several cAMP phosphodiesterases and other cAMP binding proteins, in particular putative transcription factors. Evidence is accumulating that highly pathogenic *Mycobacteria* (causing tuberculosis, leprosy, and other diseases) use cAMP signaling for proliferation and subversion of host defense mechanisms. Therefore, the corresponding proteins are considered to provide potential targets for novel therapeutic approaches, in particular against bacterial strains that have become resistant to conventional drug therapy.

The PTS mechanism depicted in Figure 3.5 applies also to Gram-positive bacteria. However, for catabolite repression they pursue another strategy, because

Sidebar 3.1 Prokaryotic adenylate cyclases Adenylate or adenylyl cyclases catalyze the formation of cAMP from ATP according to

$$\text{ATP} \rightarrow \text{cyclic } 3',5'\text{-AMP} + \text{pyrophosphate } (\text{PP}_i)$$

(the endergonic reaction is shifted to the right by the subsequent hydrolysis of pyrophosphate). Adenylate cyclases are ancient enzymes found in bacteria, archaea, and eukaryotes. Depending on the species, bacteria may express up to 40 different isoforms.

On the basis of primary sequence data, nucleotide cyclases are subdivided into six classes. Among these, three major families of prokaryotic adenylate cyclases are distinguished. Family 1 consists of adenylate cyclases of Gram-negative bacteria such as *E. coli* with a special function in catabolite repression. Family 2 contains adenylate cyclases of archaea and toxic adenylate cyclases of certain pathogenic bacteria, such as *Bacillus anthracis, Bordetella pertussis, Pseudomonas aeruginosa*, and *Bacillus bronchiseptica.* These enzymes are released together with other toxins into the host cell, causing a pathological overproduction of cAMP, for example, during anthrax and whooping cough. Family 3 consists of "eukaryotic" adenylate cyclases, some of which are found also in prokaryotes (for instance, in mycobacteria) but not in archaea (for details see Section 4.4.1).

Guanylate cyclases that transform GTP into cyclic 3′,5′-GMP seem to be entirely restricted to eukaryotes. Bacteria (but not archaea and eukaryotes) possess diguanylate cyclases that transform two GTP molecules into the second messenger cyclic di-GMP (see Section 3.4.2).

many of them—such as *Bacillus subtilis*—neither express a CRP-homologous protein nor produce cAMP. Instead, the catabolite-repressible genes are blocked by a transcriptional repressor that is removed upon glucose deficiency.

The key reaction is a phosphorylation of HPr at a serine (Ser) residue that competes with the EI-catalyzed His phosphorylation of HPr (which is still essential for the sugar uptake). The Ser phosphorylation requires ATP and is catalyzed by an HPr kinase/phosphatase. The latter is activated by a high intracellular level of ATP (and a low level of inorganic phosphate) as well as by intermediates of glycolysis; that is, under conditions of maximal glucose supply. In its structure and bifunctional activity (being both a kinase and a phosphatase), this primeval enzyme differs from all known protein kinases and phosphatases.

In contrast to the labile phospho-His bond, the ester bond of phospho-Ser is kinetically stable. Therefore, Ser-phosphorylated HPr is not a short-lived intermediate but can act as a transcriptional co-repressor by binding the so-called **catabolite control protein A (CcpA)**. This complex interacts with gene regulatory sequences named catabolite responsive elements, *cre*, and inhibits the transcription of catabolite-repressible operons (Figure 3.6). Upon glucose or ATP deficiency (or at a high level of intracellular inorganic phosphate), the kinase activity of the HPr kinase/phosphatase is turned down, whereas the phosphatase activity of the enzyme remains unchanged, now catalyzing the dephosphorylation of HPr. This leads to a dissociation of the HPr–CcpA complex and to an activation of the catabolite-repressed genes. Ser phosphorylation of HPr not only adjusts gene transcription to the food supply but also controls PTS by negative feedback, since the competing His phosphorylation, and thus the sugar uptake, is hindered.

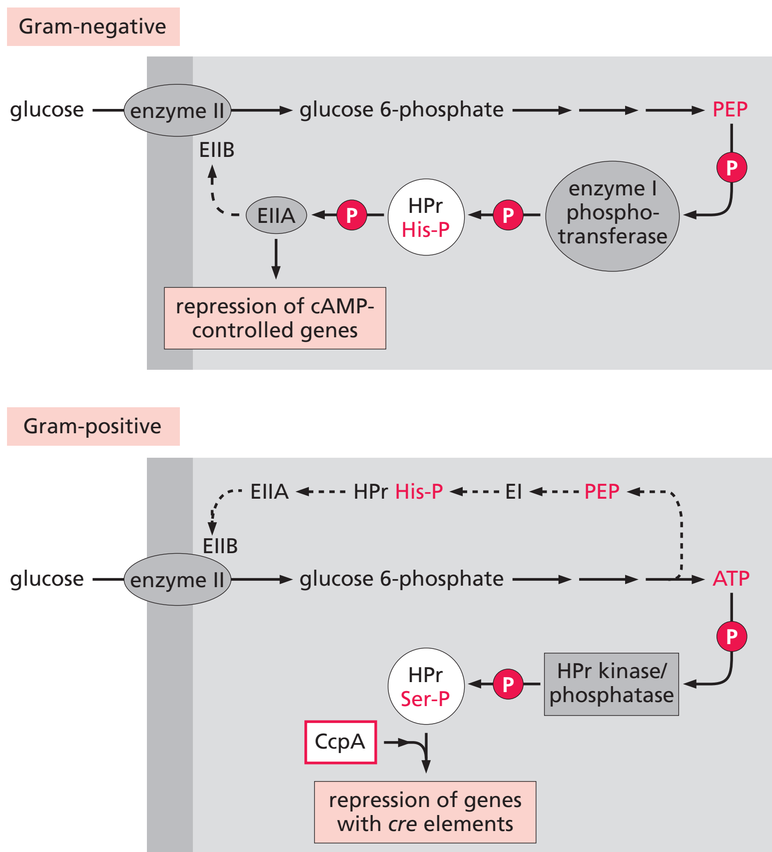


Figure 3.6 Catabolite repression in Gram-negative as compared with Gram-positive bacteria In Gram-negative species, the enzyme EIIA is underphosphorylated at high glucose concentration, inhibiting cAMP formation and thus the activation of cAMP-dependent (catabolite-repressed) genes. In Gram-positive bacteria, the PEP-dependent His phosphorylation competes with an ATP-dependent Ser phosphorylation of HPr. The Ser-phosphorylated HPr together with the protein CcpA inhibits transcription of catabolite-repressed genes.

Phosphotransferase system and evolution of signal transduction

PTS and catabolite repression are components of a data-processing protein network that regulates access to different nutrients in a highly economical manner. These components also cause the intake and metabolism of the particular carbon source to become adjusted at an optimal level that does not overtax the metabolic capacities of the cell. This enables bacteria to adapt perfectly to environmental conditions, albeit within their genetically programmed limits.

Of particular interest are the evolutionary aspects of the system. The network makes use of reversible protein phosphorylation, and the origin of this signaling reaction from short-lived enzymatic transition states becomes especially evident here. Moreover, with cAMP the prototype of a second messenger enters the stage for the first time. By transducing the effects of exogenous signals to the genome, this molecule has become an evolutionary model of success (though its function has changed from a cofactor of a gene-regulatory protein to an activator of protein kinases and ion channels that indirectly control transcription; see Sections 4.4.1 and 4.6.1). Further models of success are the two ways by which the activity of a transcription factor is controlled: either by ligand binding (cAMP–CRP) or by protein phosphorylation (Ser phosphorylation of HPr). These major principles of signaling to the genome have survived evolution up to today's most advanced organisms.

From one-component to multicomponent systems

The screening of domain libraries obtained by gene sequencing has shown that the majority of prokaryotic signal transducers combine just a single input with a single output domain, thus representing the simplest modular structure. Such proteins function as genuine binary switches. In order to distinguish them from the more complex two-component systems discussed below, these signaling devices are also called one-component systems. Most of them are transcriptional regulators with a DNA-binding and transactivating site as an output domain; some also have enzymatic functions. The input domains mostly recognize small intracellular molecules such as cAMP and metabolic products or are phosphorylated. The cAMP receptor protein CRP and the kinase substrate HPr provide examples of one-component systems.

One-component systems are probably the most ancient signaling proteins of all. The abundance of these simple transducers in prokaryotes indicates that, in the earlier days of evolution, linear signaling cascades with a rather moderate degree of cross talk may have predominated. One-component systems then seem to have evolved into more complex signal-transducing devices such as two-component systems (see Section 3.3) and multidomain proteins found in today's advanced bacteria and, in particular, in eukaryotes. Here a signaling protein with various input modules is able to communicate with numerous partner proteins, forming a multicomponent system and enabling intense cross talk between individual signaling cascades.

Summary

The controlled transport of material across cell membranes represents the most ancient form of communication between a cell and its environment. Transport against a concentration gradient must be powered by coupled ion flows or ATP hydrolysis. ATPase-coupled transporters that use ATP energy include the F, P, and V types, working as ion pumps, and ABC transporters (carrying an ATP-binding cassette) that are involved in food intake, control of electrolyte concentrations, and waste disposal, including the excretion of toxic substances. The phosphotransferase system (PTS) and the machinery of catabolite repression connect membrane transport with gene regulation. These components constitute a data-processing protein network that regulates access to individual nutrients in a highly efficient manner. This network enables the cell to adjust the uptake and metabolism of a particular carbon source to both the environmental situation

and its metabolic capacities. In evolution, PTS provides an early example of signaling protein phosphorylation, whose origin from short-lived enzymatic transition states is obvious. Moreover, with cAMP the prototype of an endogenous signal (second messenger) enters the stage together with two other successful models of evolution: control of transcription factor activity by either ligand binding (cAMP and cAMP receptor protein, CRP) or protein phosphorylation (His-containing phosphocarrier protein, HPr). CRP and HPr are prototypes of one-component systems, the most ancient and most abundant signal-transducing devices in prokaryotes.

3.3 Sensor-dependent signal processing: two-component systems

Up to a certain extent, membrane-bound transport systems are the cellular organs for food intake and waste excretion. In PTS, food intake is coupled with a simple but highly efficient network of signal processing, allowing an adaptation to the particular source of food. Nevertheless, and despite their selectivity, transport processes of this type are “blind” to the environment. PTS controls the motility of the cell by modulating the activity of the chemotaxis protein CheA, but it does not enable a behavior of prey-seeking, a tracking down of optimal biotopes, or even more important, an escape reaction in the case of danger. In fact, such blind survival programs are sufficient only for very simple prokaryotes living in a constant biotope, whereas more complex and free-living species require specific **sensor proteins** working independently of food intake and metabolism. These proteins enable the cell to adjust both its genetic readout and its behavior not only to the food supply but also to many other environmental influences.

Since most of the environmental factors do not pass or must not be allowed to penetrate the cell membrane, they cannot be recognized by the cytoplasmic one-component systems. As a consequence, sensor proteins had to be placed at the cell surface. This was done by an evolutionary trick: namely, to distribute input and output domains onto two different proteins, with one taking on the role of a membrane-bound sensor, and the other, of a cytoplasmic effector. In other words, a two-component system was made from a one-component system. In addition, an effective mechanism of signal transduction between both partners was designed.

Two-component systems dominate the communication between bacteria and environment. Their sensor proteins are receptors for environmental stimuli, the meaning of which can be decoded by the data-processing network of the cell, an ability that is essential for survival. The effector proteins of the sensors are called **response regulators**. They transmit the signal from the sensors to the metabolic and genetic apparatus of the cell. With a few exceptions, sensor genes and their cognate response regulator genes are organized in operons.

Between sensor and response regulator, the signal is transduced by protein phosphorylation, in particular by His phosphorylation, which plays a similar key role as for PTS (Figure 3.7). In fact, all sensor proteins are **His autokinases** or at least associated with His autokinases. When the sensors are integrated in the plasma membrane, such two-component systems represent a prototype of a transmembrane signaling device operating by post-translational protein modification. But two-component systems are also used for intracellular signaling. In this case the sensor domain of a membrane-bound His autokinase is facing the cytoplasmic side (such as in the case of the energy sensor Aer; see Figure 3.13), or the His autokinase even lacks transmembrane domains (for an example, see Figure 3.10). In fact, about a third of all His autokinases known seem to be cytoplasmic rather than membrane-bound enzymes. They are assumed to process endogenous signals such as metabolite concentrations, redox status, and acidity.

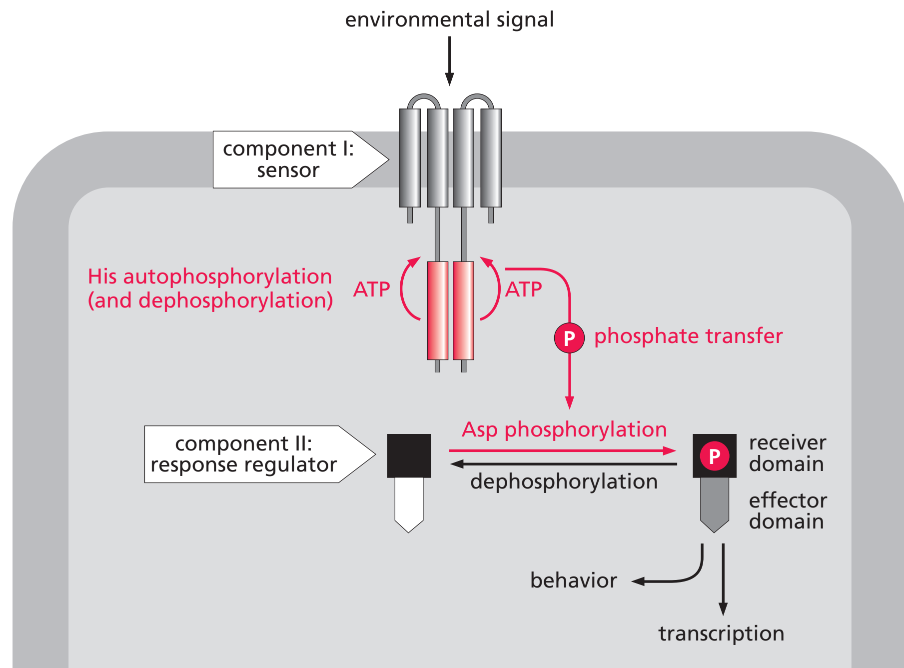


Figure 3.7 Prototype of a two-component system with a transmembrane sensor The scheme shows a dimeric sensor protein (component I) with four transmembrane domains and two cytoplasmic His autokinase domains (red) as well as the corresponding response regulator protein (component II). Upon activation, the sensor undergoes trans-autophosphorylation on a cytoplasmic His residue with ATP as a phosphate donor. The phosphate residue (P) is transferred to an Asp residue in the receiver (or input) domain of the response regulator. By the resulting long-range conformational change, the effector (or output) domain of the response regulator becomes activated. Some response regulators thus control the behavior, while others stimulate the genetic readout (aiming at metabolic adaptation) of the cell. From the receiver domain, the phosphate group is removed either by auto-dephosphorylation, by the sensor protein (exhibiting both His kinase and Asp phosphatase activities), or by separate phosphatases. Here, *activation* by phosphorylation is shown. However, depending on the system, dephosphorylation may also stimulate downstream signaling (with the phosphorylated response regulator being the inactive form). Sensors with two transmembrane domains per monomer are particularly abundant. However, there are also sensor His kinases with no transmembrane helices or up to 20 transmembrane helices per monomer (for examples see Figures 3.10 and 3.11).

However, little is known about their role in metabolic regulation. We are much better informed about the functions of membrane-bound His kinases operating as sensors for environmental stimuli. Here signal transduction occurs as shown in Figure 3.7.

When the sensor receives an environmental signal, it undergoes His autophosphorylation at its cytoplasmic domain with ATP as a phosphate donor. Since sensors are homodimers, the autophosphorylation occurs in *trans*: that is, the monomers phosphorylate each other. As in the PTS, the His-phosphorylated sensor protein represents a short-lived energy-rich transition state with a very high turnover rate (it is estimated that less than 1% of the His autokinases of a cell are phosphorylated at a given time) that serves as a phosphate donor for the phosphorylation of the response regulator. This occurs at a highly conserved aspartate (Asp) residue and changes the conformation and the activity of the response regulator. Since the response regulator catalyzes this phosphorylation itself, it might be defined as an Asp autokinase. However, apart from the fact that the term kinase is linked to ATP (or GTP) serving as a phosphate donor, the response regulator protein does not contain the typical protein kinase domains.

Though thermodynamically labile, the acyl–phosphate bond of phospho-Asp becomes stabilized by interactions within the polypeptide structure since its energy is used for the conformational change of the response regulator. Therefore, the dephosphorylation of response regulators requires enzymatic catalysis, contributed either by the response regulator itself, by the corresponding sensor His kinase (being a bifunctional kinase/phosphatase), or by a special response regulator phosphatase such as CheZ in bacterial chemotaxis (Section 3.3.4) or SpoOE in spore formation (Section 3.5.3). Depending on the type of response regulator, the lifespan of the phosphorylated state may vary between seconds and hours. Stimuli acting as input signals may control both the His autokinase activity and the response regulator phosphatase activity of the sensor.

For the cell, two-component systems provide extremely efficient means to adjust the genetic activity to the environment. To this end, in their N-terminal (mostly extracellular) domains the sensor His kinases are equipped with receptor

domains recognizing a huge variety of environmental stimuli. The structural variability of these receptor domains reflects the variability of the signals, whereas the cytoplasmic kinase domain is rather conserved.

Response regulators are signal-propagating proteins feeding the input signal into the data-processing protein–DNA network of the cell. Most of them are transcription factors exhibiting the characteristic architecture of a variable DNA-binding and transactivating domain combined with a conserved phospho-acceptor domain. Other response regulators may interact with proteins positioned downstream in a signaling cascade (see, for example, CheY in Figure 3.14), or the phospho-acceptor domains may be coupled with other signal-transducing domains, such as the catalytic centers of enzymes controlling the production of the intracellular messenger cyclic di-GMP (GGDEF and EAL; see Section 3.4.2) or post-translational protein modification by methylation (CheB; see Section 3.3.4). As far as their mode of function is concerned, response regulators seem to come close to digital (binary) switches (see Section 1.3). In the following sections, the mechanisms of action and cellular functions of two-component systems are explained and illustrated with selected examples.

3.3.1 To shrink or to burst: adaptation to osmotic pressure

For an organism living in an aqueous milieu, adaptation of cell volume and inside pressure to the osmolarity of the environment is a basic requirement of survival, since otherwise a cell would either burst or shrink and dry up. The osmoregulator of *E. coli* is the simplest of all two-component systems, demonstrating in an exemplary manner the characteristic features of these signal-transducing devices. It is composed of the osmosensor EnvZ (Env, envelope) and the response regulator OmpR (Omp, outer membrane protein), a transcription factor.The system controls the genes encoding the porins OmpF and OmpC (see Sidebar 3.2). These proteins constitute pores in the cell wall or outer membrane and regulate the transmembrane diffusion of hydrophilic and negatively charged molecules.

While the protein OmpF produces wide pores and is expressed at low osmotic pressure, OmpC forms narrow pores and is produced at high osmotic pressure, for instance, in the intestinal milieu, with its high concentration of dissolved substances of low molecular weight. Under such a condition of high osmolarity, the His autokinase activity of the sensor EnvZ is stimulated by a mechanism that is not yet fully understood. This results in an Asp phosphorylation of OmpR, which then induces transcription of the *ompC* gene while at the same time

Sidebar 3.2 Cell wall channels The family of **porin proteins** forms water-filled channels with a large diameter (1 nm) in the cell wall of Gram-negative bacteria as well as in the outer membrane of chloroplasts and mitochondria. In bacteria these channels serve the specific and unspecific exchange of material between environment and periplasm. The cell adjusts the expression of porins to the particular demands. For bacterial porins the exclusion limit of permeable molecules is at around 600 Da, with the transport following the concentration gradient passively. Though porins respond to changes of the membrane potential, the physiological role of this effect is still debated.

The pore of a porin consists of a β-barrel mostly composed of 16–22 anti-parallel β-sheets. A central bottle-neck formed by several loops connecting the β-sheets determines the substrate specificity of the channel. In the bacterial cell wall the porins are arranged as homotrimers. Parasitic bacteria may perforate the host cell membrane by means of porin-like toxins.

Another family of cell wall channels in Gram-negative bacteria is the **trans-periplasmic channel tunnels** of the type TolC. They have only one pore that again is formed by a β-barrel. Via a long tunnel domain they bridge the periplasm, directly interacting with transport proteins of the inner cell membrane. TolC channel tunnels are specialized for the export of proteins (for instance, bacterial toxins) and other molecules (for instance, substances toxic for the cell).

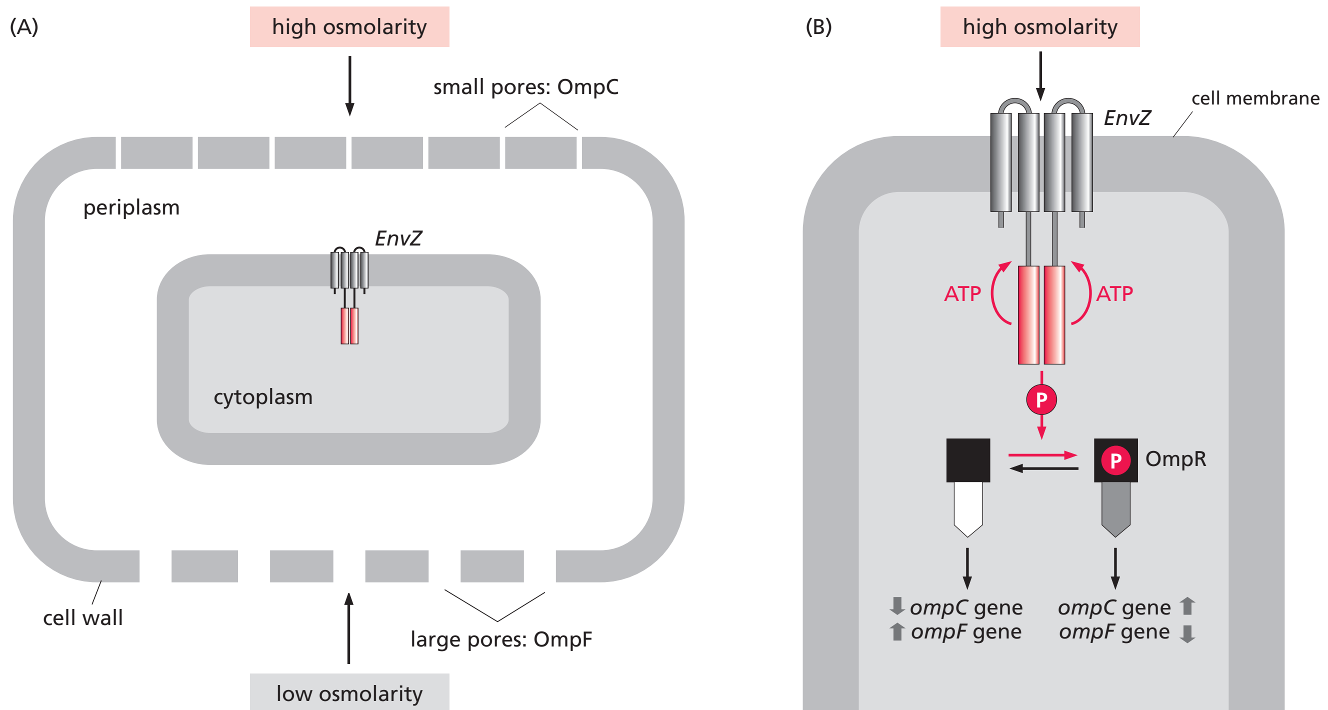


Figure 3.8 Two-component system of osmoregulation (*E. coli*) (A) Adaptation of pore size in the cell wall by osmolarity-controlled *de novo* synthesis of the pore proteins OmpC and OmpF. High osmolarity activates the sensor EnvZ in the cell membrane. (B) Control of *ompC/ompF* gene transcription by a two-component system consisting of the sensor His kinase EnvZ and the response regulator OmpR (P, phosphate). Dark gray vertical arrows symbolize inhibition or activation of gene transcription.

inhibiting *ompF* transcription (Figure 3.8). EnvZ is, thus, the prototype of a sensor responding to hyperosmotic stress. It is a protein of 450 amino acids exhibiting the characteristic architecture of a sensor His kinase, which always exists as a homodimer with one monomer phosphorylating the other one (trans-autophosphorylation, see Figure 3.9). Each monomer is anchored in the membrane by two N-terminal transmembrane helices flanking the signal recognition domain (this is the most abundant sensor type, but other forms with more than two transmembrane helices also occur; see Section 3.3.3).

In the cytoplasmic part of all sensor kinase dimers, a bundle of four helices constitutes a dimerization domain containing the His residues to be phosphorylated (so-called H-box) and the binding sites for the response regulator. The catalytic domain of the His kinase is localized in the C-terminal domain and oriented in such a way that trans-autophosphorylation of the subunits becomes possible (Figure 3.9). The dimerization domain of EnvZ also harbors the active center of an Asp-specific protein phosphatase that catalyzes the dephosphorylation of the response regulator, thus terminating signal transduction.

As EnvZ represents a typical sensor kinase, OmpR, a 27 kDa protein, is the prototype of a response regulator (and of a signal-transducing protein in general), consisting of an N-terminal regulatory or receiver domain and a C-terminal effector or transmitter domain. The conserved receiver domain contains the characteristic structural elements of a response regulator including the essential Asp residue. Its trans-autophosphorylation with His-phosphorylated EnvZ as a phosphate donor is again facilitated by a homodimerization of OmpR and induces a conformational change enabling the dimer to interact with the regulatory sequences of the *ompC* and *ompF* genes.

Like EnvZ, OmpR also exhibits Asp-specific protein phosphatase activity: it is able not only to activate but also to inactivate itself, thus, together with EnvZ, controlling the lifespan of the active state. So, the response regulator has the qualities of a switching element with a built-in timer. Such devices play an important role in the transformation of analog into digital signals and vice versa.

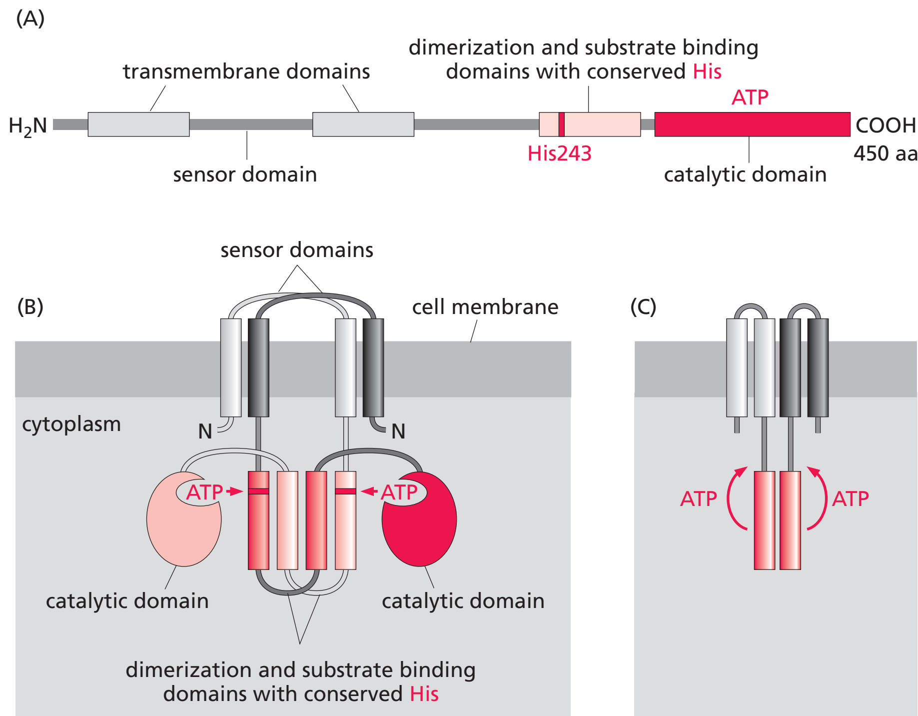


Figure 3.9 Sensor His kinase EnvZ of *E. coli* (A) Simplified domain structure of an EnvZ monomer with the strategic His residue 243 undergoing trans-autophosphorylation and the catalytic domain binding ATP. (B) Membrane anchoring and schematic folding geometry of an EnvZ homodimer. (C) Simplified scheme as used in other figures. The arrows symbolize His autophosphorylation.

3.3.2 Nitrogen fixation and hydrogen utilization

Enzymatic reduction of molecular nitrogen to ammonia represents a fundamental biochemical process essential for life on earth. It is managed by certain bacteria and archaea such as the rhizobia living as symbionts in leguminosae. Requiring the hydrolysis of 12 ATP per one N_2, the reduction of nitrogen ranks among the most expensive (that is, energy-consuming) biochemical reactions and is therefore under strict control:

$$N_2 + 6H^+ + 6e^- + 12ATP + 12H_2O \rightarrow 2NH_3 + 12ADP + 12P_i$$

The nitrogenases catalyzing the process are mostly molybdenum- (or vanadium-) containing iron–sulfur proteins with a complex quaternary structure. They become expressed only when nutritious nitrogen sources, such as amino acids and ammonia salts, are not available.

Nitrogenase expression is controlled by a two-component system consisting of the sensor NtrB and the response regulator NtrC (Ntr = nitrogen). NtrB is a prototype of a *cytoplasmic* sensor His kinase lacking transmembrane domains (other examples of sensor His autokinases without transmembrane domains, such as HoxJ, KinA, and CheA, are described later). The protein monitors the intracellular state of nitrogen metabolism. In the presence of good nitrogen sources, NtrB is blocked by an inhibitor protein in order to avoid an unnecessary and costly nitrogen reduction. With a deficiency of good nitrogen sources, NtrB phosphorylates the response regulator, which in turn stimulates the transcription of a gene encoding another transcription factor, NifA (Nif = nitrogen fixation). As a master key, NifA controls the whole collection of genes required for nitrogen fixation including that of nitrogenase (Figure 3.10).

Nitrogenases are extremely oxygen-sensitive enzymes. Nevertheless, the cell needs oxygen for ATP generation, which is then used as energy supply for nitrogen reduction. To adapt to an optimal oxygen concentration (micro-aerobic conditions), therefore, rhizobia possess an additional two-component system consisting of the sensor His kinase FixL and the response regulator FixJ [Fix =

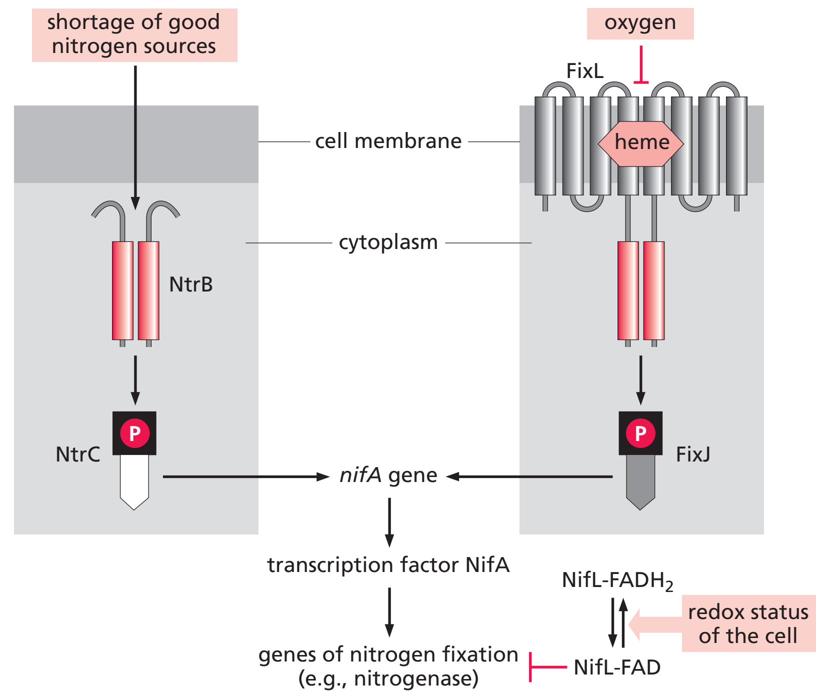


Figure 3.10 Signaling pathways for the regulation of nitrogen fixation The figure shows the cytoplasmic NtrB/NtrC two-component system inducing transcription of genes of nitrogen fixation (left), the membrane-bound FixL/FixJ two-component system controlling the adaptation to oxygen pressure (right), and the cytoplasmic NifL system expressed by some species to adjust gene transcription to the cellular redox state (bottom right). P, phosphate. For further details see text.

Sidebar 3.3 PAS and GAF domains As sensory domains recognizing a wide variety of input signals, these ubiquitous protein interaction motifs play a major role in both pro- and eukaryotic signal processing. Among the prokaryotic signal transducers, various His autokinases, adenylate cyclases, phosphodiesterases, ATPases, and diguanylate cyclases (Section 3.4.2) contain such domains functioning as regulatory elements.

PAS domains consisting of 100–300 amino acids are widely distributed among pro- and eukaryotes. The name PAS refers to three proteins where these domains were discovered originally:

- Period protein of *Drosophila*, involved, like homologous vertebrate proteins, in the regulation of genes controlling diurnal rhythms
- ARNT protein (Aryl hydrocarbon Receptor Nuclear Translocator) of vertebrates; among others, it controls (together with the aryl hydrocarbon receptor) genes encoding detoxifying enzymes (Section 8.4.3)
- Sim protein, a developmental regulator of *Drosophila*

In eukaryotes, PAS domains mainly establish homo- and heterotypical interactions between proteins. They are found in many signal-transducing proteins including protein kinases, receptors, and ion channels, as well as in phytochromes and other photoreceptor proteins of plants. The considerably smaller PAS domains of prokaryotes are distantly related to the eukaryotic domains. They are found mainly in signaling proteins such as the oxygen sensor FixL, the hydrogen sensor HoxJ, the sporulation-controlling sensor KinA, the aerotaxis-controlling sensor Aer, and the light sensor PYP.

GAF domains are named after the three different proteins in which they were first found: cGMP-phosphodiesterase, Adenylate cyclase (of the cyanobacterium *Anabaena*), and FhlA, a transcription factor of *E. coli.* They rank among the most ancient, widespread, and abundant small-molecule binding protein motifs and are found in organisms ranging from cyanobacteria to humans. Known ligands are the cyclic nucleotides cAMP and cGMP (that is not found in prokaryotes), while other ligands have yet to be identified.

GAF domains are distinct from cyclic nucleotide binding domains found in the bacterial transcription factor CRP and in eukaryotic effector proteins such as cAMP- and cGMP-activated protein kinases, cyclic nucleotide gated ion channels, and the Rap exchange factor EPAC (see Section 4.6.1). The regulatory domains of eukaryotic cGMP-controlled phosphodiesterases (Section 4.4.2) are GAF domains. The *Anabaena* adenylate cyclase is superactivated by positive feedback through an interaction of cAMP with GAF domains.

(nitrogen) fixation]. FixL represents a sensor with four transmembrane domains. It harbors a structurally unusual heme molecule bound by a PAS domain (see Sidebar 3.3). This heme serves as the oxygen sensor: at low oxygen concentrations, the system is active and the phosphorylated response regulator induces the transcription of the *nifA* gene, whereas at higher oxygen concentrations, oxygen occupies the Fe^{2+} of heme, thus inhibiting the His kinase activity of FixL (Figure 3.10). By this means an uneconomical synthesis of nitrogenase is avoided under conditions where the enzyme is running the risk of being inactivated by oxygen.

Another protective mechanism has been found, in particular in *Klebsiella* species. Here the operon encoding NifA contains an additional gene encoding the regulator protein NifL. By means of an associated flavin adenine dinucleotide (FAD), NifL monitors the redox state of the cell: in the presence of oxygen, the cofactor is oxidized to FAD and NifL represses transcription of the genes of nitrogen fixation (Figure 3.10). The reduced form NifL-$FADH_2$, indicating low oxygen pressure, does not exhibit this repressive effect. In contrast to the heme of FixL, here the redox cofactor is not a component of a sensor His kinase.

Hydrogen utilization

Many prokaryotes are able to cleave hydrogen, which accumulates as a byproduct of the nitrogenase reaction or is produced by anaerobic fermentation, into two protons and two electrons, resembling the oxidation of H_2 to water. This ancient reaction, which originally may have emerged in an oxygen-free primeval atmosphere, is catalyzed by hydrogenases and used as an energy supply in a wide variety of bacteria and archaea where the expression of hydrogenases is controlled by a two-component system. The sensor domain of the corresponding His kinase (for instance, the cytoplasmic HoxJ in *Ralstonia eutropha*, with Hox standing for H-oxidation) is via a PAS domain associated with a regulatory hydrogenase (HoxBC), an iron–nickel–sulfur protein acting as the H_2 sensor. The system's response regulator (here HoxA) represses transcription of the hydrogenase genes when phosphorylated. Hydrogen inhibits the His kinase of the sensor, thus promoting the accumulation of nonphosphorylated response regulator that induces gene transcription. This example demonstrates that the phosphorylation of a response regulator may be both a positive and a negative signal, a situation analogous to osmoregulation in yeast (see Section 3.5.1) and chemotaxis of *E. coli* (see Section 3.3.4).

3.3.3 Phosphorelays: devices for fine-tuning and cross talk

A rather baroque type of two-component system, transducing the signal along cascades of several interconnected phosphorylations, is represented by phosphorelays. In such relays each sensor His kinase delivers the phosphate residue to an Asp residue of a separate receiver domain, from where it migrates again to a His residue of a so-called HPt (His-containing Phosphotransfer) domain and finally to an Asp residue of a response regulator. HPt domains, which exhibit bundles of four helices, superficially resemble dimerization domains of sensor His kinases (Figure 3.9). The domains of a phosphorelay are either separate proteins or subdomains of one polypeptide chain. Like the PTS cascade, phosphorelays provide additional locations for cross talk with the signal-processing protein network, as well as probably more efficient noise suppression.

The phosphorelays most thoroughly studied are those controlling the sporulation of bacteria of the species *Bacillus* and *Clostridium*. Sporulation is triggered by environmental stress signals such as starvation. These signals are transduced by several sensor His kinases (for instance, the five membrane-bound or cytoplasmic sensor kinases KinA–E of *Bacillus subtilis*) to a highly conserved phosphorelay controlling the transcription of sporulation genes (Figure 3.11). The variety of stress signals is reflected by a corresponding variability of the sensors' extracellular receiver domains.

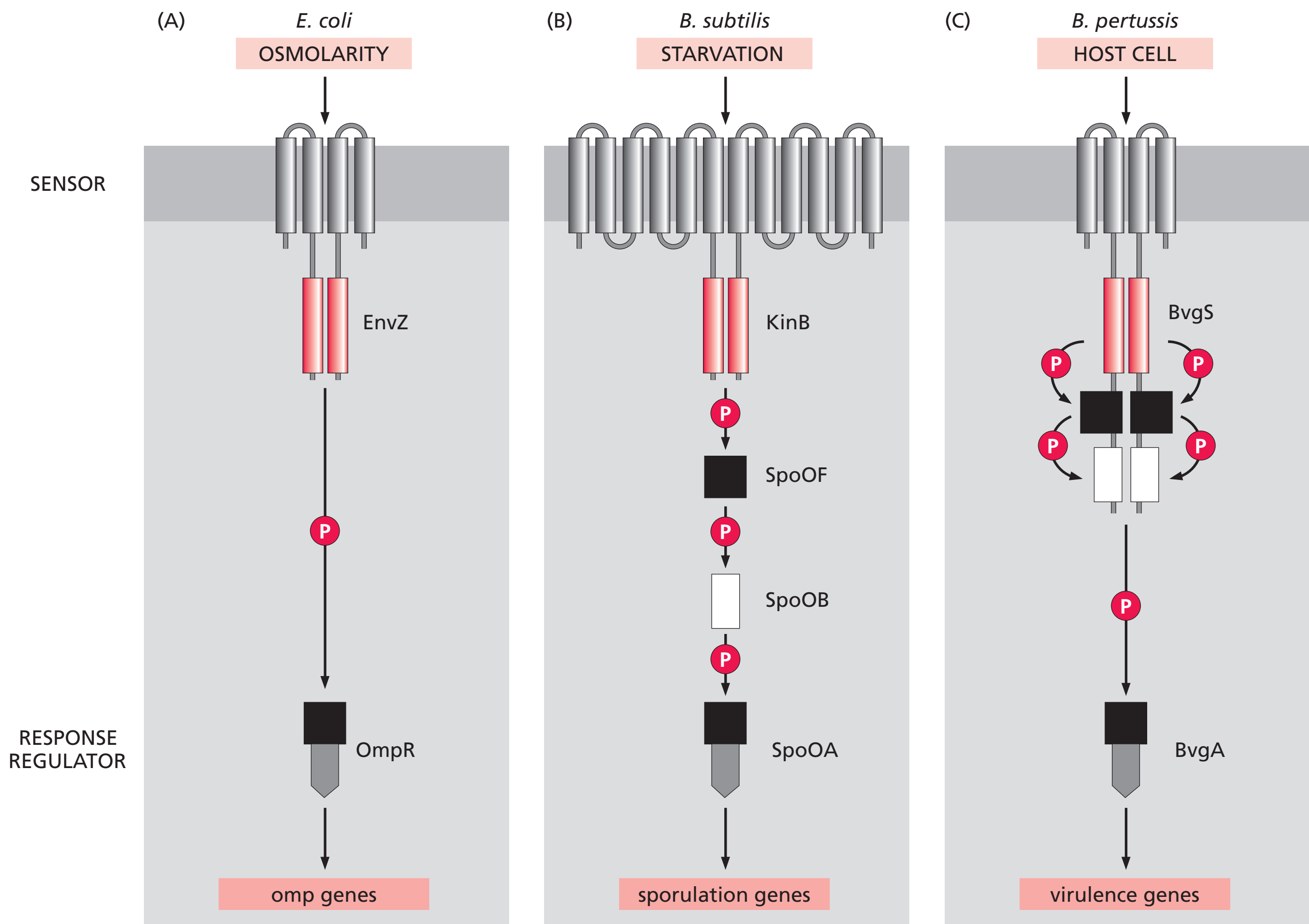


Figure 3.11 Selected two-component systems of bacteria (A) Simple system of osmolarity control, shown for comparison (see also Figure 3.8). (B, C) More complex phosphorelay systems. Red cylinder, His kinase domain; black square, receiver domain (site of Asp phosphorylation) of a response regulator; gray wedge-shaped symbol, effector domain of a response regulator; white rectangle, HPt domain.

KinB, exhibiting 12 transmembrane domains per dimer, is a prototype of a group of sensors that functionally resemble the G-protein-coupled receptors of eukaryotes (Chapter 5); like those, they bind their ligands in the cavity formed by the gobletlike structure of multiple transmembrane helices. Such sensors may contain up to 40 transmembrane domains per dimer. Examples include the quorum sensors monitoring population signals in bacterial colonies (see Section 3.4.1). Another well-known phosphorelay regulates the transcription of the virulence genes of *Bordetella pertussis*. Here all components of the relay including the sensor are found on a single polypeptide chain.

3.3.4 The "nanobrain" of prokaryotes: learning by doing

A brain interprets sensory impressions and calculates a behavioral pattern aiming at adaptation. The data-processing protein network of bacteria does the same, albeit at a lower scale. The major type of prokaryotic behavior consists of controlled changes of motility. Thanks to the inspiring observations made by the German botanist Wilhelm Pfeffer at the end of the 19th century, it became clear that bacteria respond to beneficial and hazardous stimuli by seeking and flight movements, respectively. Such targeted movements are called taxis, and depending on the environmental stimulus, one distinguishes between chemotaxis, phototaxis, thermotaxis, osmotaxis, magnetotaxis, galvanotaxis (electricity), and thigmotaxis (touch). Representing a classical example of quasi-intelligent behavior, chemotaxis of *E. coli* ranks among the best-investigated physiological reactions of prokaryotes and is considered to be at present the most thoroughly studied mechanism in all biological data processing.

Chemotactic behavior

Prokaryotes qualified for taxis move by means of long flagella performing rotating or oscillating motions. In the absence of a stimulus, the cell executes a random walk, which in the case of *E. coli* consists of forward movements (swimming phases) that become interrupted after about 1 s each by a tumbling phase lasting for about 0.1 s. During each tumbling phase, the cell randomly orients into a new direction for swimming. This motility pattern results in an aimless three-dimensional zigzag course. For swimming the flagella are rotating counterclockwise, bundling to some kind of a propeller, whereas for tumbling the rotation is switched into a clockwise mode resulting in a disintegration of the flagellar bundle (in other species, the mode of rotation can be the other way around). Possessing only a forward and a neutral gear, the flagellar motor of *E. coli* is rather simple. Other species such as the soil bacterium *Sinorhizobium meliloti* are able to activate their flagella separately and stepwise, thus changing their speed, as well as to move in curves and to switch into a reverse gear.

The flagellar apparatus of bacteria is a complex device composed of many different proteins. Being an "electric motor," it obtains its energy from ion gradients across membranes. The rotation is brought about by sequential, possibly electrostatic interactions between rotor and stator proteins that are arranged in a circular pattern (Figure 3.12). The molecular mechanism for changing the direction of rotation is not yet fully understood.

When a bacterial cell picks up a stimulus, it changes the random walk into a targeted seeking or escape movement depending on whether the stimulus is an attractant (such as food) or a repellent (such as a poison). To this end, the swimming phases become prolonged when oriented correctly or shortened when oriented in the wrong direction. The cell is thus able to measure the concentration gradient of a substance and promptly adjust its behavior to the result obtained. A bacterium is too small to monitor a spatial concentration gradient, for example, between front and back. Instead, prokaryotes recognize *temporal* differences of concentration. To do this the cell must remember where it was before. In other words, targeted movements require learning and memory. Therefore, the data-processing protein apparatus of tactic behavior not only controls the flagellar machine but also must be able to learn and to adapt in a very short time, enabling the bacterial cell to make its decisions in a fraction of a second. As one may expect, bacterial chemotaxis can also be misused, for instance, by myxobacteria and myxamoebeae, which produce attractive signals to lure *E. coli,* their favored prey.

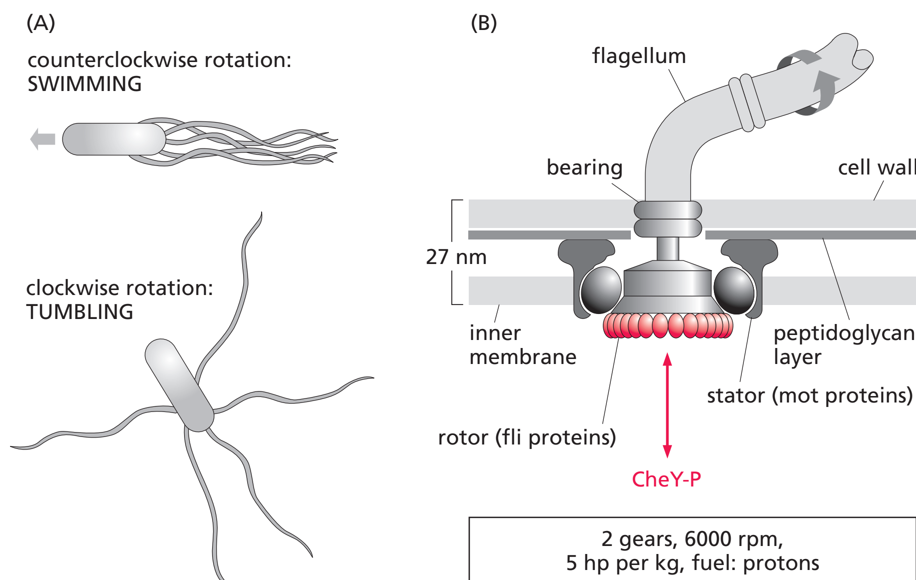


Figure 3.12 Motility apparatus of *E. coli* (A) Two modes of movement. Depending on the direction of rotation, the 5–7 helical flagella either form a bundle, working like a propeller, or fall apart. (B) Schematic representation of the flagellar motor and its interaction with phospho-CheY (CheY-P).

Processing of chemotactic signals

Tactic signals are processed by a highly conserved two-component system that in its construction differs somewhat from the systems discussed above. The major difference is that sensor and His kinase are separate proteins and the His kinase interacts with two different response regulators that affect behavior rather than gene transcription by controlling the flagellar motor and the cellular memory. The separation of sensor protein and His kinase is highly economical since it allows a single signal-processing apparatus to be stimulated by a wide variety of environmental signals, thus enabling an integrated response of the cell.

Apart from the lack of intrinsic His kinase activity, the sensors of tactic signals resemble conventional sensor His kinases in that they exist as dimers with mostly two pairs of transmembrane helices with a variable signal recognition site at the outside (for Gram-negative bacteria, the periplasmic side) and a conserved cytoplasmic domain. These chemoreceptors are known as **methyl-accepting chemotaxis proteins (MCPs)** since they become methylated at several carboxyl groups in the course of a chemotactic response. The degree of change in methylation that occurs during signal processing and subsequently results in an inhibition of receptor activity resembles a bacterial short-term memory store. This feature is discussed in more detail later in the chapter.

E. coli expresses five different MCPs responding to certain sugars, amino acids, dipeptides, and oxygen as well as to changes in pH and temperature. To be recognized by an MCP, sugar molecules have to bind first to specific receptor proteins in the periplasm (Figure 3.13). The oxygen-sensing MCP called Aer binds the redox cofactor FAD at its cytoplasmic side via PAS domains. FAD is coupled to an electron transport chain and signals, by its redox state, either a surplus or a shortage of oxygen. Aer enables a more general **energy taxis** such as seeking for energy-rich food, the metabolism of which activates the cellular electron transport chains. By this means the cell may find a biotope with an optimal ratio between oxygen and oxidizable metabolic substrates. Other examples of energy-tactic sensors are the Ser receptor Tsr (Figure 3.13) and the stress sensor ArcB of *E. coli*. Tsr does not contain a redox cofactor but probably responds by a conformational change to pH fluctuations generated by redox reactions. ArcB contains a reactive dithiol group in a PAS domain, which under aerobic conditions becomes oxidized to an intramolecular disulfide group (Section 3.5.6).

While Aer represents an indirect oxygen sensor, other species such as *B. subtilis* and haloarchaea express direct oxygen sensors coupled with a heme moiety. All

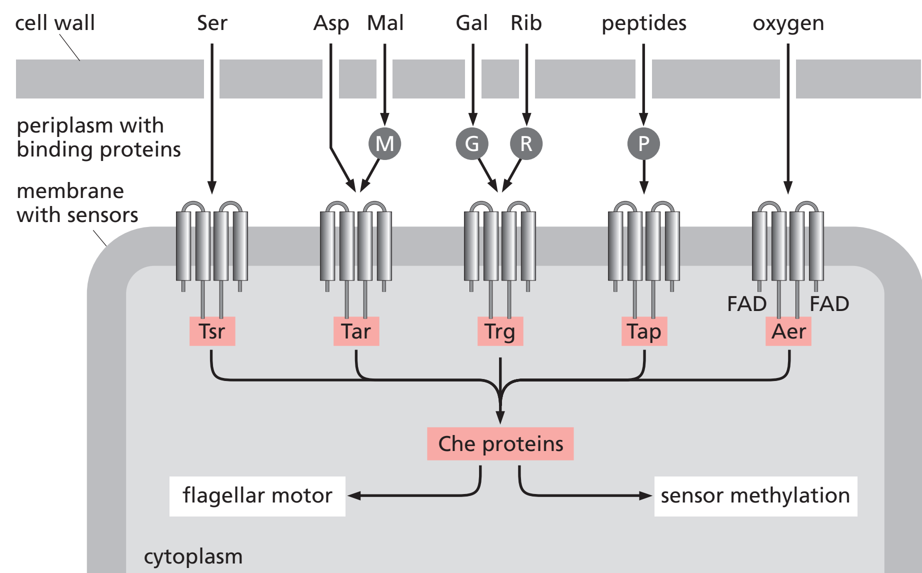


Figure 3.13 Chemotaxis sensors of *E. coli* Depicted are the five membrane-integrated sensor proteins Tsr, Tar, Trg, Tap, and Aer, together with their preferred ligands. M, G, R, and P are periplasmic binding proteins for various sugars and peptides. The Che proteins, including His autokinases and response regulators, are the common downstream effectors of the sensors, controlling both rotation of the flagellar motor and, in a negative feedback loop, methylation of the sensor proteins. Ser, serine; Asp, aspartate; Gal, galactose; Mal, maltose; Rib, ribose; FAD, flavin adenine dinucleotide.

in all, the sensor array of *E. coli* appears to be rather meager, since there are bacteria that may express up to 10 times more different sensor proteins.

The apparatus of tactic data processing is highly conserved (Figure 3.14). It consists of the so-called chemotaxis proteins, two of which are response regulators:

- CheA, a cytoplasmic His autokinase
- CheW, an adaptor protein linking the sensor protein with CheA
- CheY, the response regulator controlling the flagellar motor
- CheB, the response regulator controlling adaptation, which is a demethylase for the sensor protein
- CheZ, an Asp-specific protein phosphatase for signal termination, supporting the weak autophosphatase activity of CheY
- CheR, a methyltransferase catalyzing methylation of the sensor protein by *S*-adenosylmethionine

It should be noted that the term "chemotaxis protein" is somewhat misleading, because the system processes not only chemotactic but all kinds of tactic signals. In fact, separation of the sensor domain from the His autokinase domain CheA has the advantage that a large amount of sensors can be coupled via the adaptor CheW with the same signal-processing network. We shall encounter this principle of domain separation again when discussing eukaryotic signal transduction.

Some models have been developed to explain the mechanism of signal transduction from the sensor to the His kinase CheA. They postulate either a rotation or a shift of the sensor's subunits. There is, for instance, experimental evidence indicating that a tiny pistonlike movement (of 0.16 nm) of the outer transmembrane segments of the sensor is sufficient to inhibit the His kinase activity of CheA (Figure 3.15). During such a minimal shift, the interactions between the side chains of neighboring helices would be preserved, providing the sensor with the properties of an elastic spring that automatically returns into the relaxed position upon termination of the signal. However, how a tolerable signal-to-noise ratio is maintained under such conditions remains an open question. Apart from

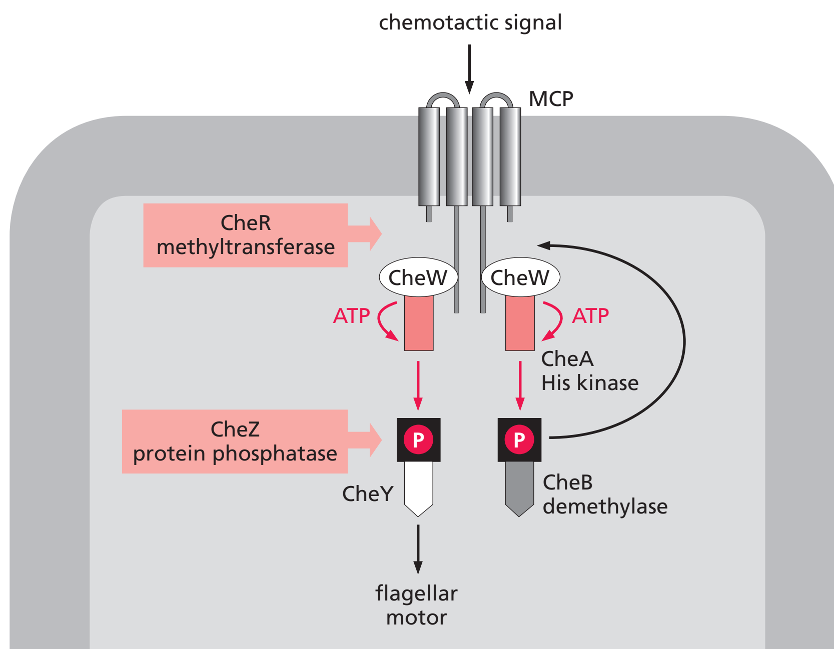


Figure 3.14 Chemotactic two-component system of *E. coli* The autophosphorylated His kinase CheA (red), coupled by the adaptor protein CheW to the membrane-bound sensor (MCP), is the phosphate (P) donor for Asp phosphorylation of the response regulators CheY (controlling the flagellar motor) and CheB (demethylating the sensor). Opponents are the CheY-specific protein phosphatase CheZ and the methyltransferase CheR. Both are constitutively active. The phosphorylated CheY switches the flagellar motor towards clockwise rotation. Red arrows indicate phosphotransfer reactions.

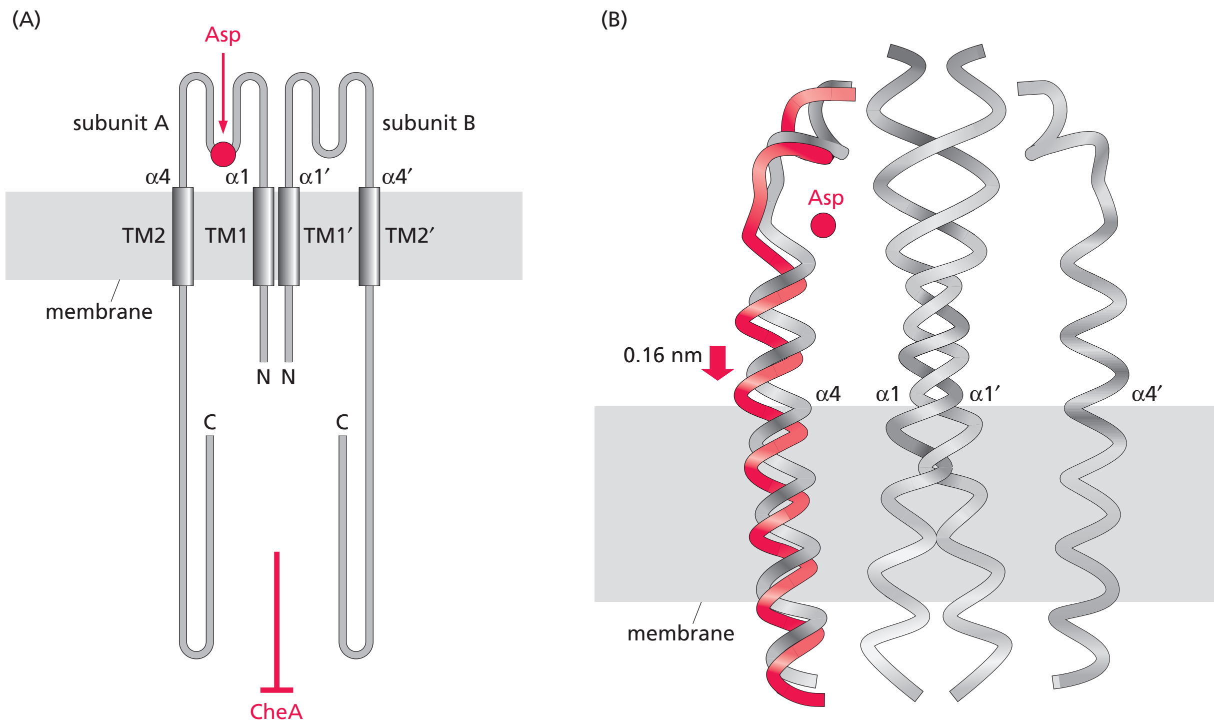


Figure 3.15 Model of transmembrane signaling by a chemotaxis sensor (*E. coli*) (A) Schematic representation of the aspartate sensor homodimer inhibiting the His kinase CheA upon binding of aspartate. (B) Vertical shift (red arrow) of the polypeptide chain α4 of the transmembrane domain TM2 induced by aspartate binding. (B, adapted from J.J. Falke et al., *Annu. Rev. Cell Dev. Biol.* 13, 457–512, 1997.)

this, it should be kept in mind that CheA is controlled not only by (chemo)taxis sensors but also by PTS (Figure 3.5).

The structure of His kinase CheA differs clearly from that of sensor His kinases such as EnvZ (Figures 3.16 and 3.17). Though CheA also exists as a dimer, the His phosphorylation occurs in N-terminal HPt domains rather than in the helix bundle of the dimerization domains. Moreover, each CheA monomer contains a regulatory domain for interaction with the adaptor protein CheW and the sensor as well as a substrate-binding domain for interaction with the response regulators.

Intelligent behavior by adaptation

In *E. coli* the activity of the His kinase CheA and thus the degree of phosphorylation of the Che proteins is high in the *absence* of attractants (or in the *presence* of repellents). In other words, the interaction of an MCP sensor with nutrients inhibits the Che signaling system. Due to the Asp phosphorylation, the response regulator CheY loses contact with CheA, instead interacting as an allosteric regulator with the switching protein FliM of the flagellar motor. This event causes the motor to rotate clockwise, into neutral gear, resulting in a tumbling of the cell. Conversely, the binding of an attractant inhibits CheA. As a consequence, dephosphorylation of CheY by CheZ gains the upper hand, interrupting the interaction between CheY and FliM and switching the motor into counterclockwise rotation, into forward gear. [Note that in other species (such as *B. subtilis*), attractants may *stimulate* and repellents *inhibit* CheA activity, providing an instructive example for the ambiguity of signaling.]

In parallel, the degree of sensor methylation becomes changed: while the methyltransferase CheR is permanently active, the demethylase CheB is stimulated by Asp phosphorylation. As a result the degree of methylation becomes diminished in neutral gear, during the tumbling phase, and increases upon switching into forward gear, during the swimming phase (Figure 3.18).

The stepwise methylation of several (four or five) methyl groups gradually desensitizes the sensor, probably because the piston movement of the transmembrane

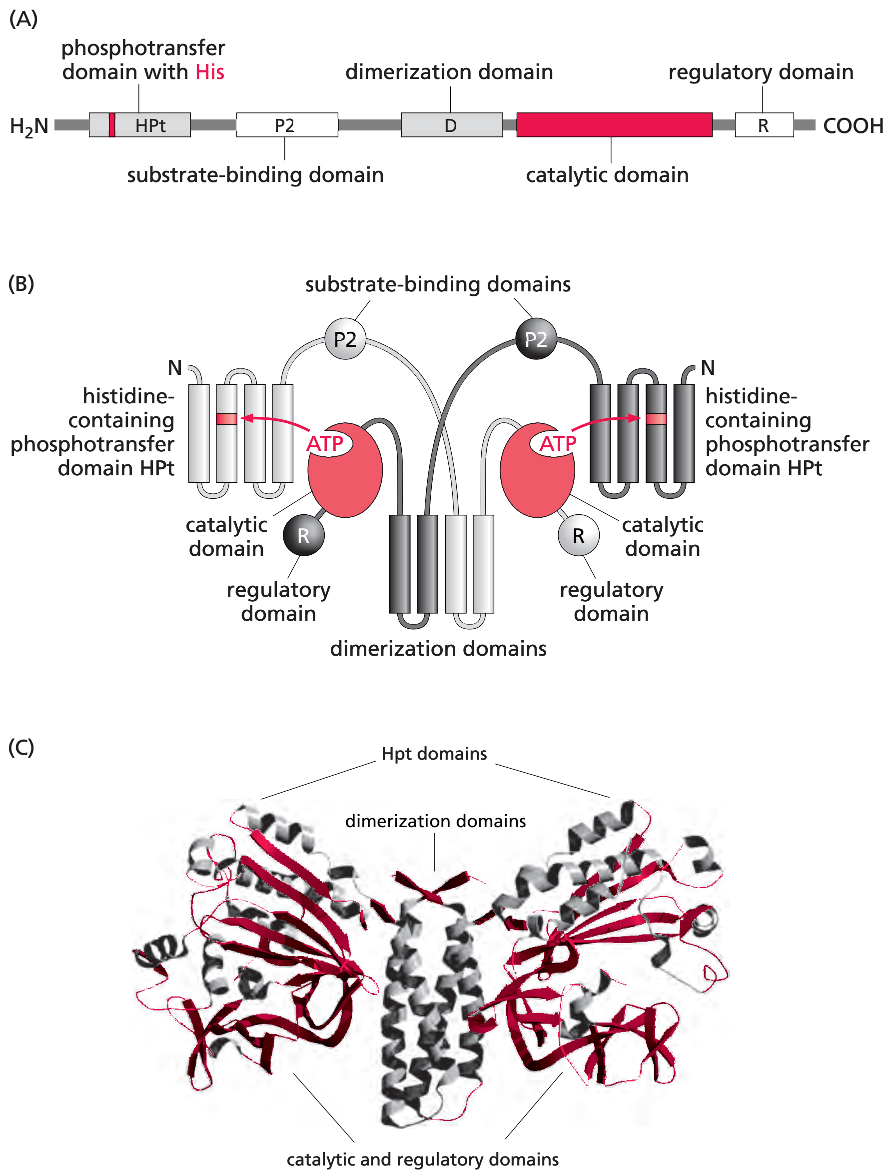


Figure 3.16 Histidine kinase CheA
(A) Domain structure of CheA.
(B) Schematic folding geometry of the homodimer. Autophosphorylated His residues are shown as red boxes. Catalytic domains with ATP-binding site are shaded red. (C) Molecular model of the CheA dimer based on X-ray crystallography.

segments becomes more and more blocked. This leads to reactivation of the His kinase CheA and thus to termination of the swimming phase. The system is switched back to position 0 and can now respond to the next higher attractant concentration, again with maximal sensitivity. In other words, the system has become adapted.

Our sensory organs are subject to similar adaptation, albeit following an entirely different mechanism. Such adaptive processes guarantee that the sensory apparatus remains fully sensitive over a wide range of signal intensity (at least 5 orders of magnitude). Thus, a targeted movement of bacteria is possible: without adaptation, the cell would become maximally excited at a minimal signal intensity, inevitably letting it go over the top.

Mainly due to the high phosphatase activity of CheZ, the degree of CheY phosphorylation changes within milliseconds, whereas sensor methylation is maintained for seconds. This difference is due to both the continuous activity of the methyltransferase CheR and the relatively slow auto-dephosphorylation of the phosphorylated demethylase CheB, which is *not* accelerated by CheZ. Due to this sluggishness, the sensor methylation plays the role of a molecular short-term

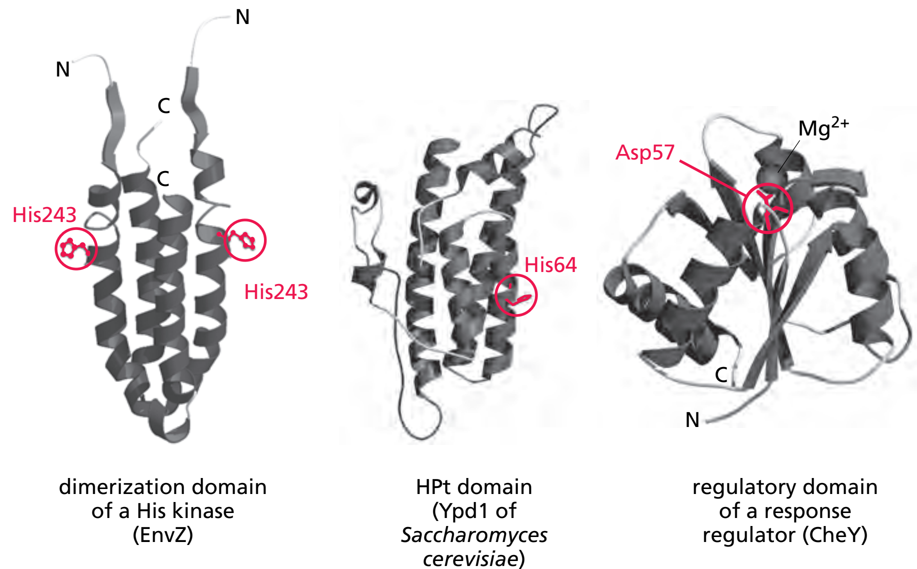


Figure 3.17 Active centers of some two-component proteins (Adapted from A.H. West and A.M. Stock, *Trends Biochem. Sci.* 26, 369–376, 2001.)

memory: the degree of methylation is high when the cell was in an area of high attractant concentration *immediately before*, and vice versa. It is easily understood that adaptation of the data-processing apparatus and learning or memory are two sides of the same coin; for example, I am eager to learn as long as my curiosity has not become satisfied or as long as I have not become adapted!

The prokaryotic "nanobrain"

Sensors and chemotaxis proteins are integrated into stable complexes (Figure 3.19). This fixed wiring guarantees a particularly rapid signal processing as well as a selective adaptation to individual stimuli. In the cell membrane, many thousands of such complexes are combined into large formations localized predominantly in the anterior pole of the cell. This sensor field may be formed by hexagonal lattices consisting of six triads of sensor dimers, each with associated CheW and CheA proteins (Figure 3.20). Such lattices are assumed to exhibit a high degree of cooperativity. This means that allosteric changes of conformation induced by the ligands in a few sensors spread across a larger distance. Such an arrangement is a potent signal amplifier and allows the transformation of digital input signals into analogous output signals. The result is a gradual behavior of the cell paired with high sensitivity. In principle, this situation resembles sensory signal processing in higher organisms. The (chemo)taxis system has been described, therefore, as a prokaryotic "nanobrain." Indeed, the phosphorylation and methylation pattern of the taxis proteins, or the excitation pattern of the

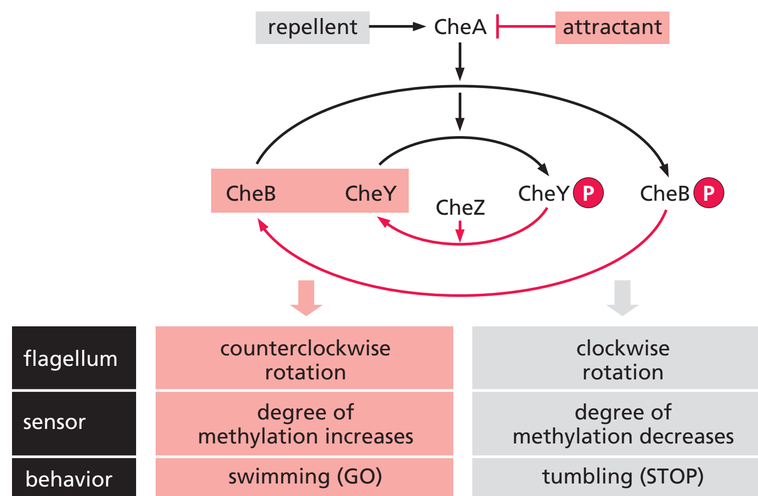


Figure 3.18 Chemotactic responses of *E. coli* Attractants inhibit and repellents stimulate the CheA-catalyzed phosphorylation of response regulators CheB and CheY, resulting in the opposite responses shown in the boxes. In the absence of CheA activity (lack of an attractant or contact with a repellent), CheB undergoes slow auto-dephosphorylation, whereas CheY is dephosphorylated rapidly by the phosphatase CheZ. As a consequence, the methylated state is more persistent than the phosphorylated state, providing a short-term memory store.

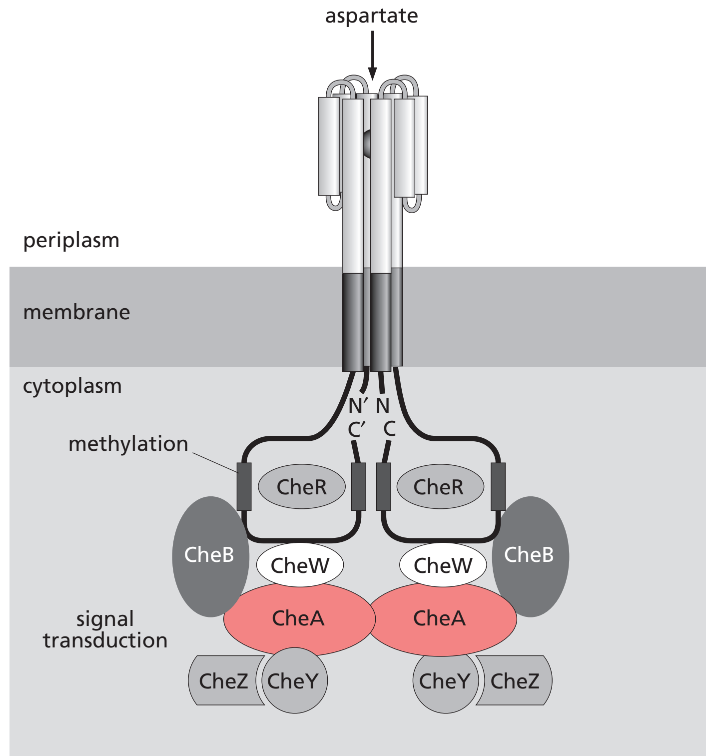


Figure 3.19 Chemotactic signaling complex of *E. coli* Schematic representation of the aspartate receptor Tar and the associated Che proteins. Helical domains of Tar are symbolized by cylinders. (Adapted with modifications from D.F. Blair, *Annu. Rev. Microbiol.* 49, 489–522, 1995.)

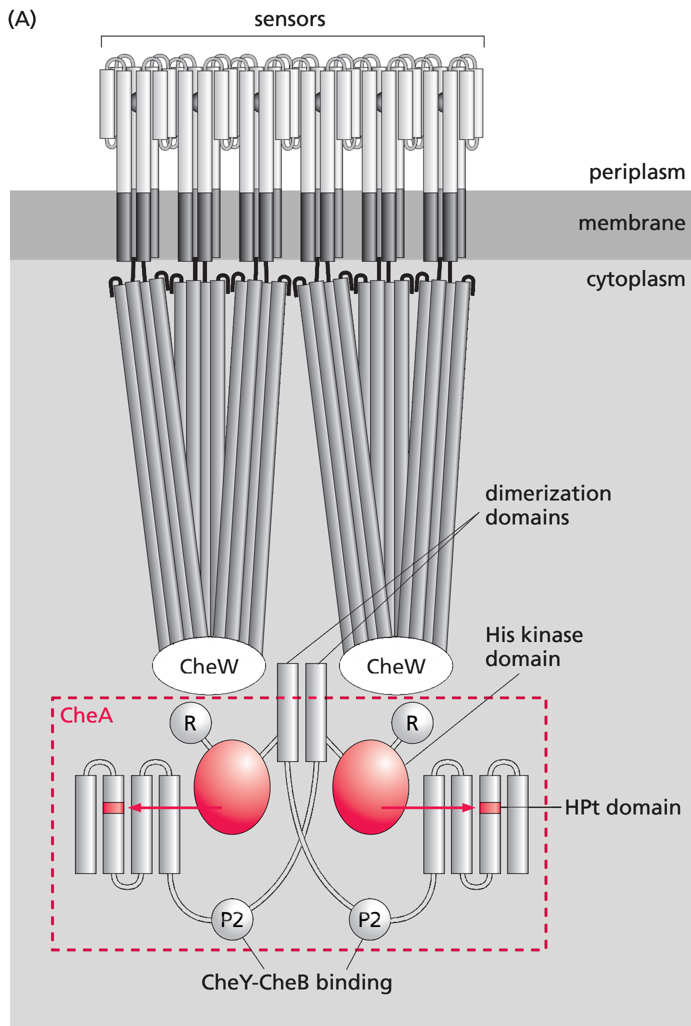
"nanoneurons", somehow portrays the actual environmental situation just as the excitation pattern of brain neurons does. It should be kept in mind, however, that the taxis system itself is embedded into a larger data-processing protein network, and thus it represents only a part of the bacterial "nanobrain."

Finally, it should be noted that although signal processing operates at the level of the single cell, chemotactic behavior is a systemic phenomenon involving a large number of cells that resemble a school of fishes. As we shall see in Section 3.4.1, the cells communicate by means of so-called quorum signals. These are hormone-like metabolites that also interact with sensors coupled to the chemotaxis system of signal transduction. Thus, in a particularly clear manner, bacterial chemotaxis illustrates a major challenge of today's research: to understand the function of a data-processing network and the emergence of new properties from complex systems, one has to proceed from studying single molecules or single cells to an investigation of large populations of interacting partners. That is, one must go beyond the reductionist approach (see Chapter 17).

Bacterial IQ

A closer look at the prokaryotic cell, with its clear conditions, strongly supports the metaphor introduced in Chapter 1 of the data-processing protein network as the "brain of the cell." Indeed, bacterial signal transduction widely fulfills basic requirements of a neural network, such as performing logical operations by parallel data processing using nontrivial algorithms that are able to adjust. Are bacteria really intelligent?

A partial affirmative answer to this question is given in the definition of the chemotactic response as quasi-intelligent behavior. But what is intelligence? Primarily it may be understood as an organism's potential to deal with environmental (and endogenous) challenges in a rational and flexible way. On the molecular level, the number of signaling proteins relative to the size of the genome may be taken as a rough measure of intelligence. This cellular IQ seems to be a matter of lifestyle: the higher the environmental challenges, the higher the IQ value. Analysis of a large number of prokaryotic genes has shown a clear



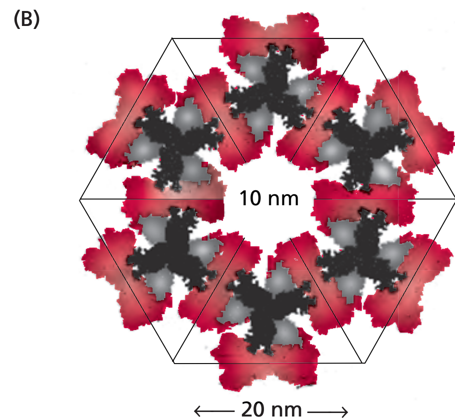


Figure 3.20 Architecture of a sensor–CheA complex in the inner membrane of *E. coli* (A) CheA dimer interacting, via its regulatory domains (Figure 3.15), with two CheW adaptors. On average, three sensor molecules are bound to each CheW molecule via their extended (hairy) cytoplasmic domains. Several thousand such complexes constitute the bacterial nanobrain. (B) Model of a lattice of chemotactic receptor complexes, shown looking onto the plasma membrane (receptors are in black, CheW adaptor proteins are in gray, and CheA His kinases are in red). It is assumed that the sensory field of the cell membrane is formed by a large number of such hexagonal structures. (A, adapted with modifications from J. Stock and M. Levit, *Curr. Biol.* 10, R11–R14, 2000. B, adapted with modifications from T.S. Shimizu et al., *Nat. Cell Biol.* 2, 792, 2000.)

tendency for high IQ values in free-living microbes that encounter an ever-changing environment, whereas the price of living in luxury and idleness is stultification. Thus, the majority of symbiotic and parasitic microbes get by on an absolute minimum of signal transducers, frequently being devoid of two-component systems including that of chemotaxis. On this scale *E. coli*, the pet of microbiologists, turns out to be rather stupid. For instance, in comparison with free-living Gram-negative bacteria, which sometimes express more than 50 different chemotactic MCPs, the five MCPs of *E. coli* look rather poor, and the same holds true for other signal-transducing proteins (for example, 30 *E. coli* His autokinases versus up to 200 types in other species). This shows the limited value of studies relying just on one or a few “dumb” species.

It must be emphasized that our definition of intelligence is somewhat narrow since it does not include what is known as associative memory. This wonderful property enables us to extract common parameters from countless details that help us to recognize and categorize objects, subjects, and systems as related even when they are not identical. The absence of associative memory is a serious problem of today’s computer technology, as demonstrated by daily experience. Whether prokaryotes in particular, or single cells in general, possess an equivalent of associative memory remains to be shown.

3.3.5 Phototaxis and the “invention” of rhodopsin

Phototaxis is defined as a targeted movement directed by light. It has been studied in more detail for **halobacteria**. These are a subclass of archaea inhabiting

extreme biotopes, that are, for instance, high in salinity, such as the Dead Sea (salt concentration 4.3 M as compared to 0.6 M in ocean water). Halobacteria are able to utilize sunlight as an energy supply for ATP production. Light, particularly at short wavelengths, is also a deadly hazard. To deal with this problem, halobacteria have developed phototactic systems that recognize short-wavelength light as a repellent and long-wavelength light as an attractant. This enables the cells to actively find optimal environmental conditions.*

The molecular system decoding light signals resembles that of chemotaxis, though the sensors are, of course, quite different. Halobacteria express two types of light sensors or halobacterial transducers (Htrs): one (HtrI) for long-wavelength light and the other (HtrII) for short-wavelength light. Both resemble chemotaxis sensors as they interact with CheA and the other chemotaxis proteins upon illumination. However, being unable to respond to light on their own, the Htr proteins form complexes with two light-sensitive proteins, the sensory rhodopsins SRI and SRII. When the SRI/HtrI complex becomes stimulated, for instance by orange light, the cell responds with seeking behavior, whereas an escape reaction is induced by activation of the SRII/HtrII complex, for instance by blue light. In other words, prokaryotes equipped with such a signal-processing system are able to “see” colors.

Rhodopsins are proteins with seven transmembrane domains arranged in a goblet-shaped pattern. At the outside, this structure has bound retinal (vitamin A aldehyde) as an imide (Schiff base) at the ε-amino group of a lysine residue. In the absence of light, the retinal is locked in the *all-trans* configuration and the nitrogen of the imide bond is protonated, carrying a positive charge. Upon illumination, retinal isomerizes into the 13-*cis* form, resulting in an allosteric rearrangement of the transducer protein Htr that causes an activation of the phototactic system.

In addition to the phototactic sensor rhodopsins (SR type), other microbial rhodopsins are known. These participate in the transformation of light into metabolic energy. In fact, these bacterio- and halorhodopsins (BR and HR) play the role of light-powered ion pumps serving the generation of ATP and the control of the cellular pH value. Upon illumination, BR produces a proton gradient across the cell membrane by transferring, through a conformational rearrangement, the proton bound to the imide nitrogen from the inside to the outside of the cell. By powering a membrane-bound ATP synthase, the proton gradient then transforms light energy into chemical energy (Figures 3.21 and 3.22). HR uses the positive charge of the protonated Schiff base to bind chloride ions electrostatically. Here the proton does not dissociate from the illuminated protein but, again due to a light-dependent conformational rearrangement, the N–H dipole is folded down, releasing the chloride ion into the interior of the cell. This transport process regulates intracellular acidity.

Halobacteria perfectly adjust to environmental conditions. When there is ample food and oxygen (that is, the oxygen signal is received by heme-containing aerotaxis sensor HtrVIII and transduced to the Che complex), only light protection by the HtrII/SRII system is needed. Consistently, the energy-supplying HRs and BRs become expressed only upon oxygen shortage in the water, as caused, for instance, by an increase of the salt concentration due to evaporation.

Evolution of rhodopsins

Rhodopsins are universal light sensors that are classified in two families: microbial rhodopsins (type 1) and rhodopsins of the animal eye (type 2). Whether both

*Simpler systems of light protection are found in many prokaryotes. **Photoactive yellow protein** (**PYP**) has been investigated in more detail since in biophysics it provides a favored model for studies of the effects of light on protein conformation. PYP is a cytoplasmic protein containing *p*-hydroxycinnamic acid as a light-sensitive pigment. Short-wavelength light, via a molecular rearrangement of the pigment, induces a conformational change that evokes an escape reflex.

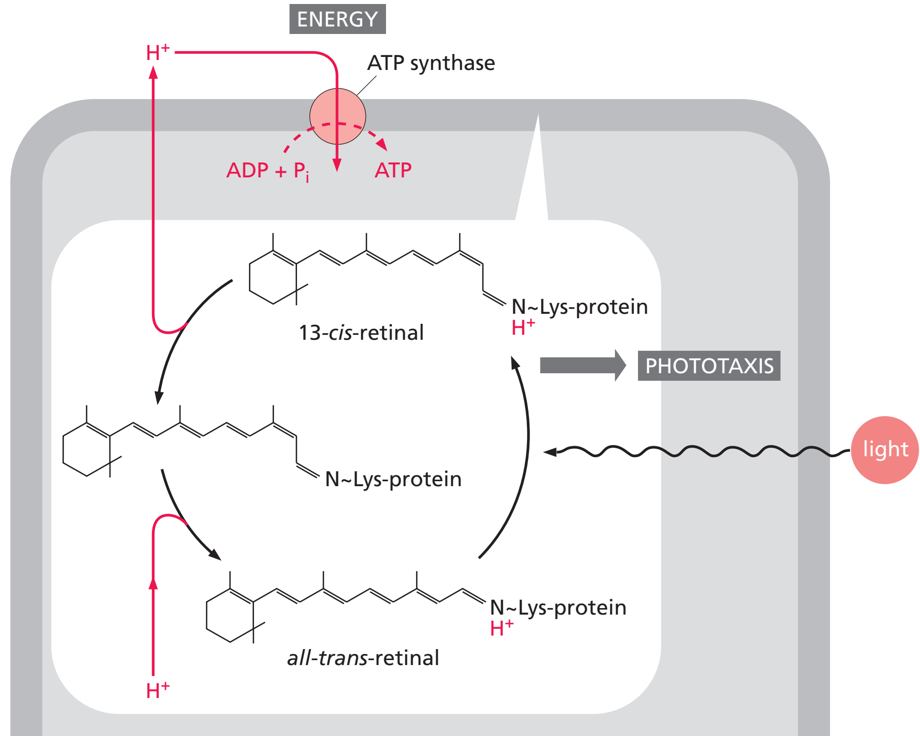


Figure 3.21 Reaction cycle of archaebacterial rhodopsin In the inset the reactions occurring in the cell membrane are shown: light-dependent *cis–trans* isomerization of retinal bound as a Schiff base at a lysine residue of rhodopsin. As a consequence, the conformation of the membrane-bound rhodopsin is changed in such a way that a proton bound to the Schiff base is released into the extracellular space. The reflux of the protons following an electrical charge gradient powers a membrane-bound ATP synthase. In halorhodopsin, the protons are not released but facilitate the influx of chloride ions.

types developed independently or are the result of lateral gene transfer is still a matter of debate. Microbial type 1 rhodopsins are by no means restricted to haloarchaea but have been found in a wide variety of prokaryotes as well as in unicellular eukaryotes, green algae, and fungi. Thus, the type 1 rhodopsin CSR (Chlamydomonas Sensory Rhodopsin) is found in the light-sensitive organelles of *Chlamydomonas* algae (Figure 3.23).

The molecular architecture of rhodopsin, with its seven transmembrane helices and an extracellular ligand binding site, is one of the most successful standard models of evolution. It serves as a receptor for retinal and light as well as for a wide variety of other signaling molecules, though the receptors of higher eukaryotes are probably not descendants of the microbial rhodopsins (see Section 5.1).

Light sensors of cyanobacteria

Other prokaryotes using light as an energy supply are the cyanobacteria or blue-green algae. They are considered to be the evolutionary "inventors" of

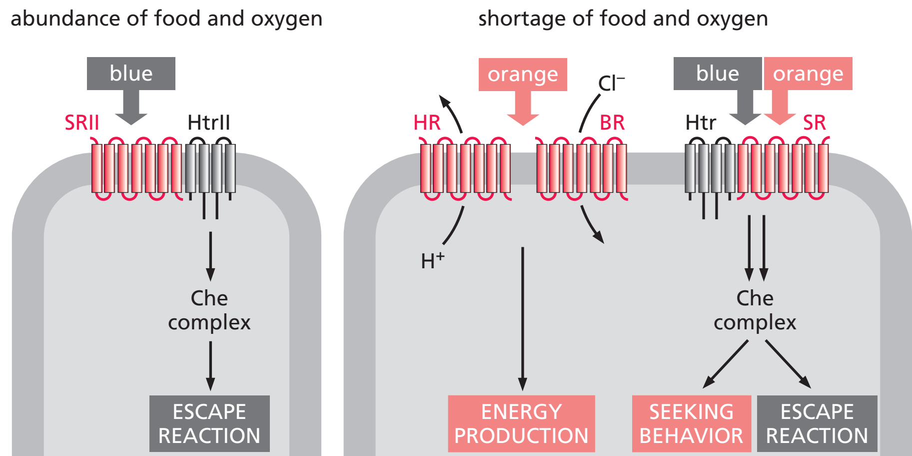


Figure 3.22 Phototaxis of haloarchaea Different rhodopsins (red) are shown integrated into the plasma membrane and serving energy-delivering ion transport (center) and controlling, as complexes with corresponding sensors, phototactic movements. Note that in the situation depicted by the right figure seeking behaviour is triggered by the HtrI/SRI complex, whereas an escape reaction is induced by the HtrII/SRII complex. For further details see text.

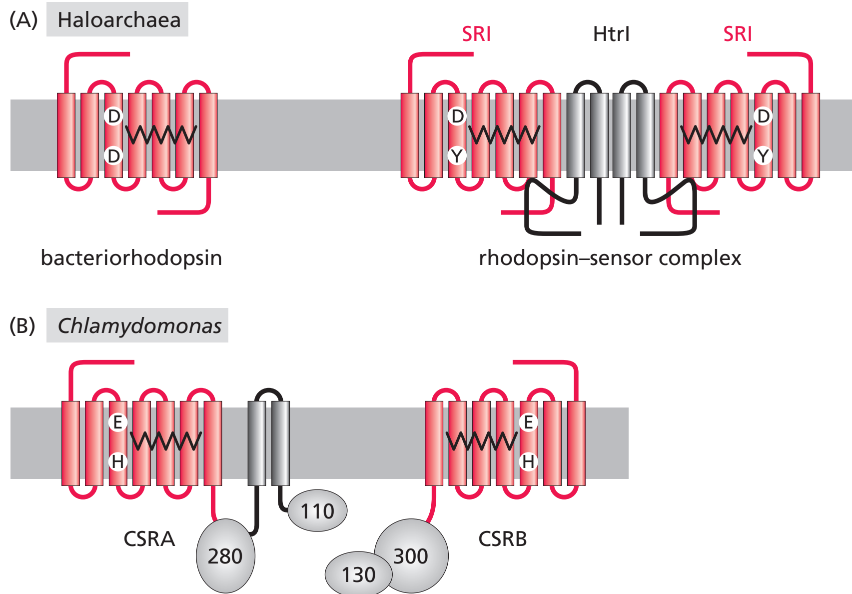


Figure 3.23 Rhodopsins of the microbial type (A) Two haloarchaeal rhodopsins are depicted: ion-transporting bacteriorhodopsin (left) and the rhodopsin–sensor complex of phototactic signal transduction (right). Retinal is symbolized by the zigzag line. The rhodopsin–sensor complex differs from the ion-transporting bacteriorhodopsin in that a negatively charged aspartate residue (D) in the third transmembrane helix, essential for ion translocation, is replaced by a neutral tyrosine residue (Y). Each sensor homodimer (HtrI) is shown to interact via its cytoplasmic domains with two rhodopsin molecules. Upon illumination, the transmembrane helices of the rhodopsins are shifted and interact with the transmembrane helices of the sensor, thus inducing signal transduction along the Che pathway. (B) Two sensoric rhodopsins (CSRA and CSRB) found in the light-sensitive structures of the unicellular eukaryote *Chlamydomonas.* They provide examples of light receptors that are not coupled to the chemotaxis complex. Instead, in both molecules rhodopsin is covalently bound to large proteins (characterized by the molecular weights given in kDa) that evoke a light-dependent influx of Ca^{2+} ions by functioning as transmembrane ion channels or by interacting with separate channel proteins. The Ca^{2+} current regulates the phototactic motility of the cell.

chlorophyll-dependent photosynthesis, being the ancestors of plant chloroplasts. In cyanobacteria, phototaxis is controlled by the phycochromes phycoerythrin and phycocyanin rather than by rhodopsins. Moreover, by means of these light-sensitive proteins, the cell is able to utilize light with wavelengths for photosynthesis to which chlorophyll is relatively insensitive.

The light-sensitive pigments of phycochromes are two tetrapyrrole derivatives, phycoerythrobilin (for short-wavelength light) and phycocyanobilin (for long-wavelength light), which are structurally related to bile pigments. Light induces an isomerization of the pigment molecules, acting as an input signal for the phototaxis system (Figure 3.24). In addition, as in rhodopsin, a proton is released and fed into the photosynthetic complex. Apart from the Che apparatus, another two-component system is used by the cell to adjust the expression of

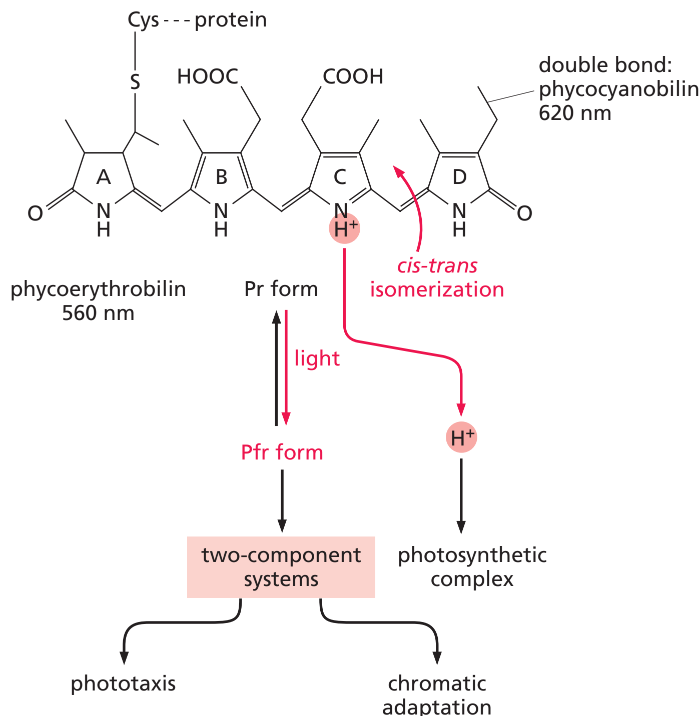


Figure 3.24 Light-sensitive reaction of cyanobacteria Light-dependent isomerization of the tetrapyrrole pigments in the phycochrome proteins is shown. The light-activated Pfr form activates two-component systems of phototaxis and chromatic adaptation. Absorption maxima of the pigments are given.

phycochromes to the light conditions, a response called chromatic adaptation. The phycochromes are the evolutionary ancestors of the phytochromes serving light adaptation in plants (Section 3.5.1).

Bacterial signal transduction: more elaborate than previously assumed

The sequencing of almost 200 prokaryotic genomes has provided a wealth of data showing that prokaryotic signal processing is a theme with many variations. Thus, depending on the species, putative sensor domains of transmembrane proteins are fused with His autokinases, adenylate cyclases, serine/threonine (Ser/Thr)-specific protein kinases, protein phosphatases, diguanylate cyclases (a novel type of second-messenger generating enzymes; see Sidebar 3.4), and DNA-binding domains. These interactions indicate the existence of transmembrane signaling mechanisms that go beyond the conventional two-component system, albeit in most cases the extracellular input signals as well as the downstream effector proteins of such receptorlike proteins are not known. Moreover, such enzymatically active domains are integrated in various cytoplasmic proteins, which by the presence of typical protein and DNA interaction domains are characterized as potential transducers in an intracellular signaling network. These data, which are derived from genomic screening, need to be validated by biochemical studies. Nevertheless, they show that the prokaryotic cell has mastered the task of constructing new protein species by recombining a limited set of modules in a virtuoso manner, thus laying the foundations for most of the signaling processes found in mammalian cells.

Summary

Two-component systems provide the standard model of receptor-coupled signal processing in prokaryotes. The sensor expresses His autokinase activity or couples with His autokinase. The effector protein, the response regulator, usually is a transcription factor. About two-thirds of all sensors are transmembrane proteins processing environmental stimuli; the rest are found in the cytoplasm processing intracellular signals. Upon activation by a signal, the sensor undergoes ATP-dependent His autophosphorylation, immediately transmitting the phosphate residue to a regulatory Asp residue of the response regulator. Phosphatase activities render the signaling process reversible. Adaptation of *E. coli*-like bacteria to osmotic pressure provides a simple example of the function of a two-component system. Depending on the phosphorylation status, the corresponding response regulator induces transcription of genes encoding either small or large cell wall pores. Genes encoding the enzymatic machinery of bacterial nitrogen fixation are under the control of several two-component systems that adjust transcription to both the availability of nitrogen sources and the toxic potential of oxygen. Oxygen-sensing systems are coupled with heme or redox cofactors. Expression of the enzymes used for the primeval energy-supplying oxidation of hydrogen is controlled by two-component systems containing specific H_2-sensor proteins. Phosphorelays resemble two-component systems but consist of more than two partners. Such phosphorylation cascades offer additional possibilities for control, network formation (cross talk), and signal fine-tuning. By means of a universal two-component system consisting of individual sensors and a common apparatus of signal transduction (Che complex), prokaryotes are able to adjust their movement pattern to a wide variety of chemical and physical stimuli acting as either attractants or repellents. A characteristic feature of this system is that various sensor proteins couple noncovalently (via adaptor proteins) with a separate His kinase, and the response regulators are proteins that control the motility and adaptive behavior of the cell. Adaptation enabling targeted, quasi-intelligent movement is due to sensor desensitization through reversible protein methylation. The phosphorylation and methylation patterns represent a short-term memory store of the cell. In light-sensitive halobacteria the phototactic sensors of two-component systems are coupled with rhodopsin proteins containing *all-trans*-retinal as a light-sensitive pigment.

Upon illumination, the retinal is isomerized to the 13-*cis* form and the chemotaxis system is activated, regulating the movement pattern and the adaptive behavior of the cell. Other microbial rhodopsin types function as ion pumps and use light as an energy source. They are also found in unicellular eukaryotes and fungi. In cyanobacteria, the light-sensitive components coupling with corresponding two-component systems are tetrapyrrole pigments bound by phycochrome proteins, the precursors of plant phytochromes.

3.4 From vagabonds to societies: "bacterial hormones"

If hormones are understood as mediators of *inter*cellular communication in metazoans, a term such as "bacterial hormones" appears to be nonsensical at first glance. However, most prokaryotes are by no means loners but are capable of amazing social performances, thereby acting like multicellular organisms. This process begins with the formation of characteristically shaped colonies and culminates in complex, differentiated structures that consist of millions of cells. Examples are provided by the chainlike colonies of certain cyanobacteria, the predatory spheres of myxococci, the fruiting bodies of myxobacteria, the hunting packs of *Proteus mirabilis*, and the so-called biofilms. Frequently, such structures are aimed at the division of labor through cell differentiation.

3.4.1 Quorum sensing and auto-inducers

For aggregation and differentiation, cells communicate by signals. Among such **social signals**, the so-called quorum sensing has been investigated in detail. Quorum sensing monitors the population density of a collection of cells to find out whether or not it makes sense to start an expensive genetic program—such as sporulation or biofilm formation—which requires a larger group of cells to become effective. The principle of quorum sensing is simple: since cells continuously release the social signal, its concentration is a direct function of cell density, and the target genes become activated above a distinct threshold concentration.

Pioneering experiments were performed on *Vibrio fischeri*. This Gram-negative symbiont inhabits the light-producing organs of certain fishes and cephalopods, causing bioluminescence. In return for this the host protects the settlers from enemies and provides food. For the host, light production makes sense only when the intensity of bioluminescence exceeds a certain value. However, the prokaryote produces light only at a sufficiently high population density (at least 10^{10} cells/mL, as compared with a density of a few hundred cells per milliliter in sea water). Therefore, bioluminescence is triggered by a population-dependent signal that is released by the cells. This auto-inducer stimulates neighboring cells to transcribe the *lux* genes that encode bioluminescence enzymes such as luciferases (more information on such enzymes is found in Section 5.2) and enzymes catalyzing the production of the auto-inducer.

Auto-inducers are not restricted to luminous bacteria but have been found in many other species. As far as Gram-negative bacteria are concerned, auto-inducers are almost exclusively **acyl-homoserine lactones** (Figure 3.26), differing from each other by the acyl residue and exhibiting species-specific effects. This provides quorum sensing with a high degree of privacy. Acyl-homoserine lactones are lipophilic enough to penetrate cellular membranes with increasing cell density, thereby "soaking" the whole population.* The corresponding cellular receptors are transcription factors that by convention are called LuxR in reference to the bioluminescence system, although they are by no means involved

*Free diffusion requiring a minimum of water solubility is only one way to distribute social signals within a cell population. Highly hydrophobic molecules have been shown to be packed in vesicles that are pinched off from the outer membrane of certain Gram-negative bacteria to become fused with the membrane of a neighboring cell.

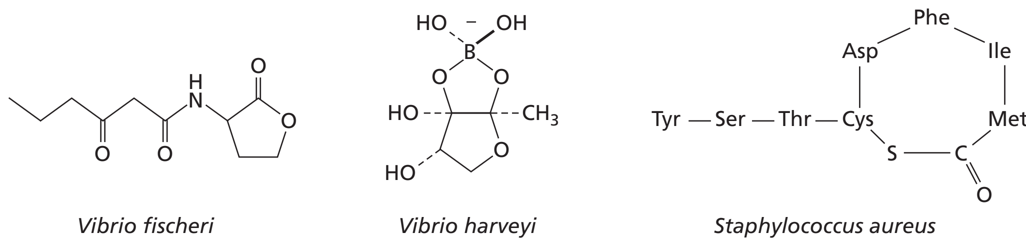


Figure 3.26 Some auto-inducers of quorum sensing Shown are an acyl-homoserine derivative (left), a furanosylborate diester (middle), and a cyclic octapeptide (right) from different microorganisms.

only in light production. They may control many other population-dependent functions including the formation of spores, colonies, and biofilms as well as of virulence factors (these are illness-causing toxins and proteins used by pathogenic microorganisms for the invasion of host cells). Upon binding of a specific auto-inducer, the LuxR factors dimerize and either induce or repress the transcription of individual operons (Figure 3.27). This mechanism of action closely resembles that of steroid hormones, which in higher eukaryotes interact with so-called nuclear receptors (Section 8.3).

Gram-positive bacteria do not produce acyl-homoserine lactones. Instead their auto-inducers are short, partially **modified peptides** released from larger precursor proteins (an example, the cyclic octapeptide of *Staphylococcus aureus,* is shown in Figure 3.26). In some species this signaling system is completed by another one using **furanosylborate diesters** as auto-inducers (Figure 3.26). This provides an interesting and rare example of the utilization of boron in a biological system. Evidently the arsenal of "bacterial hormones" is much more elaborate than hitherto assumed, since novel factors have been found that differ chemically from modified peptides, acyl-homoserine lactones, and furanosylborate diesters. They include derivatives of oligosaccharides, dihydroxypentadienones, butyrolactones, quinolones, and others, with many of them exhibiting quite exotic structures.

In contrast to acyl-homoserine factors the auto-inducers of Gram-positive species are unable to penetrate cellular membranes. Instead, their effects are mediated by highly specific two-component systems controlling the transcription of corresponding genes (Figure 3.27). The analogy to peptide hormones of higher eukaryotes that interact with protein kinase-coupled receptors is striking. Thus, auto-inducers control the activity of transcription factors either directly by binding or indirectly by phosphorylation. These two mechanisms have been preserved in the course of evolution and still represent the major signaling routes leading to gene regulation (Section 8.3.1).

Prokaryotes, but also algae and flowering plants, produce inhibitors of quorum sensing, thus outcompeting and keeping in check populations of competitor, parasitic, and symbiotic bacteria. There is some evidence that human cells also use anti-quorum strategies to fight bacterial infections. For instance, cells from bronchial epithelium have been shown to inactivate the auto-inducer controlling the virulence genes in *Pseudomonas aeruginosa*. It is clear that the investigation of such defense mechanisms and the potential clinical application of synthetic inhibitors offer highly attractive possibilities in medicine.

3.4.2 Biofilms and fruiting bodies: benefits of multicellularity

Population-dependent signals control not only parasitism and symbiosis but also the aggregation of bacteria into multicellular, differentiated structures such as biofilms and fruiting bodies. Biofilms are large bacterial aggregates growing on surfaces. In these complex structures the cells are embedded like tissue cells into an extracellular polysaccharide matrix that is produced by the cell collective. Common examples are tooth plaques, the slimy coat of stream pebbles, mats of bacteria lining water pipes, and the bacterial colonies in the ruminant stomach. The discovery of biofilms has revolutionized the widely held idea of prokaryotes

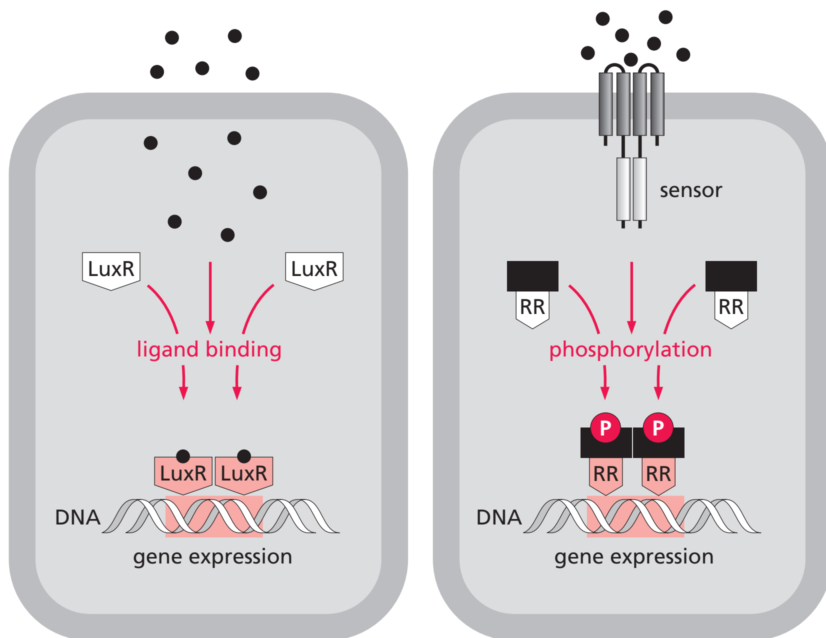


Figure 3.27 Mechanisms of action of auto-inducers: two major pathways along which extracellular signals control gene transcription Depending on the chemical structure, auto-inducers (black dots) activate transcription factors either by direct interaction (left, Gram-negative bacteria) or via a receptor-controlled phosphorylation cascade (right, two-component system of Gram-positive bacteria). LuxR, transcription factors first studied in the bioluminescence system; RR, response regulator; P, phosphate. For other details see text.

Sidebar 3.4 Cyclic di-GMP, a novel second messenger of bacteria Recently an endogenous signaling molecule has been identified that promotes production of the extracellular matrix of biofilms. Cyclic diguanosine monophosphate (c-di-GMP) was originally discovered as an activator of cellulose synthase in certain bacterial species. The number of putative functions has expanded considerably, including, in addition to biofilm formation, intercellular communication in myxobacteria, development of flagella, pathogen–host interactions, phage resistance, and photosynthesis in cyanobacteria.

C-di-GMP is produced from GTP by diguanylate cyclases and inactivated by cognate phosphodiesterases (Figure 3.25). These enzymes are characterized by specific sequence motifs such as GGDEF for the cyclases and EAL for the phosphodiesterases. Such motifs are found in a wide variety of bacterial proteins, including response regulators of two-component systems. The cyclases and phosphodiesterases become activated by external signals. This puts c-di-GMP in the rank of a genuine second messenger, albeit most of its effector molecules are still not known. Bacteria with a "high IQ" may express more than 60 different diguanylate cyclase isoforms, indicating a key role of these enzymes in prokaryotic signal processing. Some of these enzymes are parts of integral transmembrane proteins carrying an extracellular sensor domain, the ligands of which are still unknown.

Considering the high resistance of biofilms to conventional antibacterial drugs and the restriction of c-di-GMP to bacteria, the enzymes controlling the metabolism and effects of c-di-GMP are emerging as attractive targets for novel therapeutic strategies. Cyclic 3′,5′-GMP, a typical second messenger of eukaryotic cells, has not been found in prokaryotes.

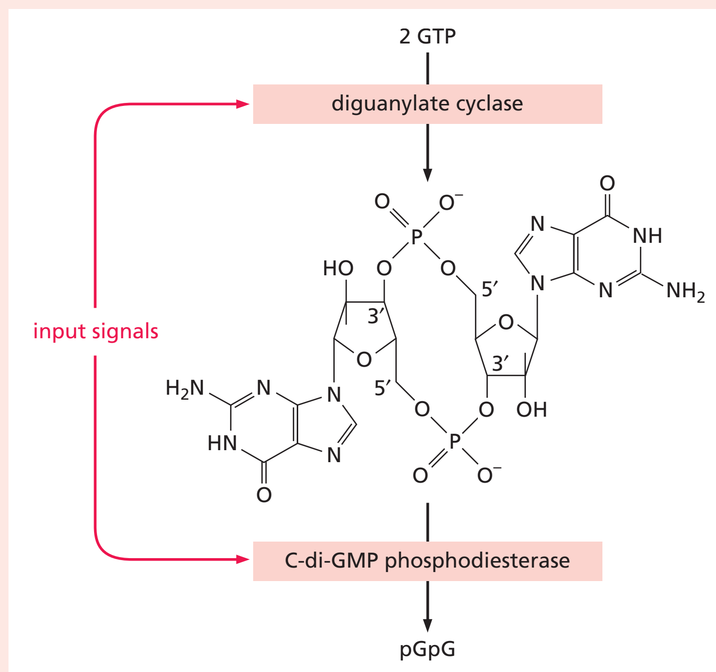


Figure 3.25 Signal-controlled biosynthesis and inactivation of cyclic di-GMP

as “primitive” unicellular organisms. Indeed, rather than single cells living as vagabonds, biofilms are the predominant form of prokaryotic life. As indicated by fossils, the history of such multicellular structures reaches back to the early days of evolution.

Biofilms are highly organized three-dimensional structures with fluid channels running through a spongelike body. The microbes inhabiting a biofilm may belong to one or to several different species. The individual cell types are organized in colonies that communicate with each other and divide labor, for instance, breaking-up of food, protection from stress factors, and parasexual exchange of genetic material. The degree of cell differentiation, expressed as a pattern of genetic activity, depends on the position in the biofilm.

Like metazoans, such highly organized forms of prokaryotic life can exist only when there is a permanent exchange of intercellular signals. Here the autoinducers of quorum sensing play a key role. Because of their resistance to antibiotics and disinfectants, biofilms are a major cause of acute bacterial intoxications and chronic infections. The investigation of the signaling mechanisms is, therefore, of practical value.

One of the most complex physiological processes in the prokaryotic world is the sophisticated survival strategy of **myxobacteria.** In emergency situations they develop fruiting bodies, consisting of thousands of cells that have become differentiated to spores. This type of cell differentiation is triggered by the ancient prokaryotic stress reaction of the stringent response, resulting in an almost complete stop of cell proliferation (Section 3.1). In addition, stressed myxobacteria release proteases into the extracellular space that hydrolyze extracellular proteins. The amino acids thus produced serve both as food and as quorum signals (called A-signals) that are processed by two-component systems, enabling the cell to monitor whether enough cells are available to generate a fruiting body. When this is the case, the cells emit aggregation signals of still-unknown structure, inducing chemotactic responses. The cell that happens to be the strongest transmitter develops into an aggregation focus, gathering together some 100,000 other cells. The aggregate then differentiates into a fruiting body. The differentiation is controlled by a morphogenetic signal protein (C-signal) exposed at the cell surface, interacting with sensors at the surface of neighboring cells. This juxtacrine signal transduction strongly resembles events regulating the embryonic development of vertebrates (see, for example, Sections 7.1.4, 7.3, and 16.1). By this means, *Myxococcus xanthus* produces fruiting bodies and also differentiates alternatively into another multicellular structure, the so-called predatory sphere, serving the collective catching of prey.

In their mobility mode, myxobacteria differ from less complex species such as *E. coli* in that they have no flagella but execute sliding movements resembling that of amoebae. They possess two genetically fixed programs of mobility, one for single cells and the other for groups of cells sliding like slugs on a slimy secretion. Both programs are probably coordinated by a signaling cascade that formerly had been found only in animals. In this cascade, a transmembrane tyrosine kinase (MasK of *M. xanthus*) interacts with a GTPase (MglA) that, among all prokaryotic G-proteins, is most similar to the small G-proteins of the eukaryotic Ras family. Although a role of MasK as a signal receptor has not been firmly established, these findings strongly indicate a fluent transition between prokaryotic and eukaryotic mechanisms of signal processing.

Myxobacteria developed approximately 2 billion years ago, representing the most ancient multicellular organisms. Independently, multicellularity was also “invented” by eukaryotic myxamoebae or slime molds. The modes of motility, aggregation, and differentiation, most thoroughly studied for *Dictyostelium discoideum*, are quite similar to those of myxobacteria, though they employ other mechanisms. This provides a fascinating example of evolutionary convergence (Figure 3.28).

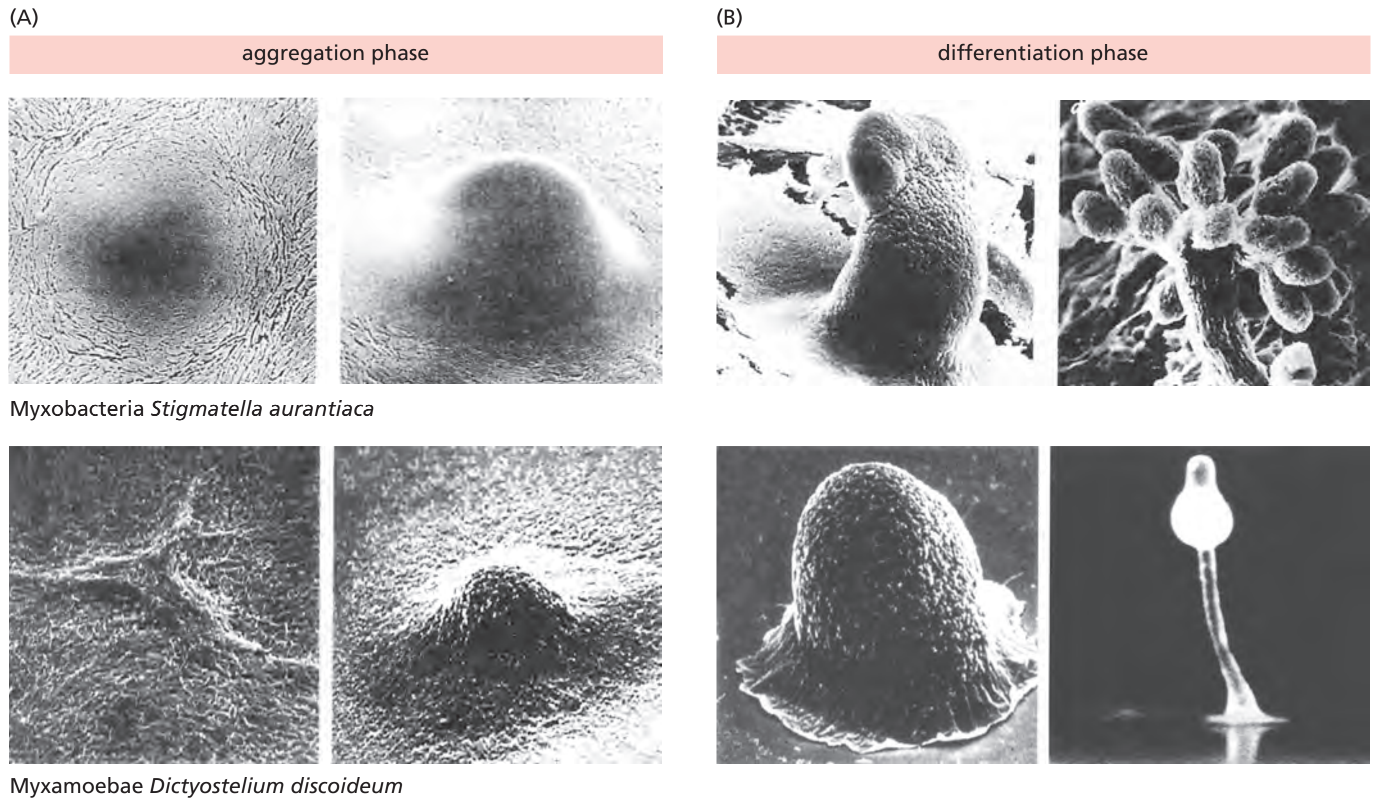


Figure 3.28 Aggregation and differentiation of myxobacteria as compared with myxamoebae In both cases, apparently amorphous cellular aggregates (A) develop into fruiting bodies (B). (Myxobacteria, adapted from J.A. Shapiro, *Sci. Am.*, 256, 82–89, June 1988. Myxamoebae, adapted from B. Alberts et al., Molecular Biology of the Cell. New York and London: Garland Publishing, 1983.)

There is a blurred dividing line between the auto-inducers of quorum sensing and bacterial **pheromones.** These cell-derived attractants are small peptides controlling various aspects of social life. In *Enterococcus faecalis*, for instance, they induce the exposure of surface proteins for "sexual" contacts (serving the exchange of genetic material). This function resembles that of plant gamones or animal sexual hormones. In other species pheromones control the uptake of free DNA or the invasion of tissues by pathogenic bacteria. The effects of the prokaryotic pheromones known thus far are mediated by two-component systems.

Summary

The formation and function of bacterial colonies and complex multicellular structures (such as biofilms and fruiting bodies) are controlled by intercellular signals including factors that monitor the population density (quorum sensing). In Gram-negative species, such hormone-like auto-inducers are acyl-homoserine lactones able to penetrate the cell membrane and to interact directly with transcription factors. The auto-inducers of Gram-positive species are modified peptides, furanosylborate diesters, and other compounds. Unable to pass the membrane, they instead interact with sensors of corresponding two-component systems that transduce the signal through Asp phosphorylation of transcription factors, or response regulators. Thus, intercellular signaling by "hormones" and the major principles of transcriptional regulation—either ligand binding or phosphorylation of transcription factors—are of prokaryotic origin.

3.5 From bacteria to humans: evolution of signaling mechanisms

Since cells cannot survive without communicating with the environment, the mechanisms of cellular signal processing must have developed in parallel with the mechanisms of food intake, energy metabolism, and reproduction. Indeed, there are fluid transitions between metabolic and signaling reactions, and even

the most primitive prokaryotes possess a complex arsenal of signal-processing proteins, while more advanced species communicate via hormone-like factors. A comparison of different species clearly indicates that practically all eukaryotic mechanisms of signal transduction must have emerged from prokaryotic precursors. Examples of these precursors, such as membrane transporters, second messengers, protein kinase-coupled and rhodopsin-like receptors, and the pathways of transcription factor control, have been described above. In the following sections, the discussion of evolutionary aspects is expanded to include standard biochemical devices of signal transduction such as kinases, phosphatases, GTPases, proteases, redox enzymes, and ion channels.

3.5.1 "Prokaryotic" protein phosphorylation in eukaryotes

Phosphorylation is a key reaction of energy metabolism, and at the same time is the most versatile and perhaps most ancient mechanism for post-translational control of protein function. Most probably, protein phosphorylation was applied by the precursors of archaea and bacteria. In the course of evolution, several large families of protein kinases and phosphatases have emerged (see Section 2.6 for an overview).

The His-specific autokinases of prokaryotic two-component systems represent a transition between the phosphotransferases of energy metabolism and signal-processing protein kinases. In the course of prokaryotic evolution, they have become models of success: 1.5% of the genome of *E. coli* includes 30 genes encoding His kinases and 32 genes encoding response regulators. *Nostoc punctiformis*, *Myxococcus xanthus*, and cyanobacteria express more than 150 different two-component systems. Only very simple bacteria such as mycoplasma can survive without two-component systems. They probably have lost the corresponding genes due to their special life conditions (see remarks on the "bacterial IQ" in Section 3.3.4).

Histidine kinases and two-component systems are also expressed by archaebacteria, slime molds, fungi, and plants. It is assumed that they had been taken over from bacteria by horizontal gene transfer. Apart from the mitochondrial genome, no such genes have been found in animals. All eukaryotic two-component systems are constructed as phosphorelays, thus providing more possibilities for interactions and signaling cross talk.

Only assumptions can be made concerning the reason why, on the way to eukaryotes, bacterial His phosphorylation has fallen into oblivion, being replaced by Ser/Thr/Tyr phosphorylation. Because the phosphoric ester bonds are much more stable than the phospho-His and phospho-Asp bonds, they certainly provide a better memory effect, more precise regulation, and a superior signal-to-noise ratio, albeit at the price of speed.

Yeast

The bakers' yeast *Saccharomyces cerevisiae* has only one His kinase gene and three response regulator genes encoding a system for **osmolarity control** (Figure 3.29). A striking feature of this system is its connection with a "modern" Mitogen-Activated Protein (MAP) kinase module (Section 2.6.5), which has not yet been found in prokaryotes. In fact, yeast cells express several MAP kinase modules with different functions (see Section 11.1). By phosphorylating the corresponding transcription factors, one of these modules stimulates genes encoding proteins that protect the cell from osmotic stress, for instance, enzymes of glycerol production. Under normal conditions this module is inhibited by the phosphorylated response regulator SsK1. Hypertonic stress blocks the associated sensor His kinase Sln1, resulting in dephosphorylation of the response regulator and, in turn, activation of the MAP kinase module. Via the interconnected HPt protein Ypd1, the His kinase Sln1 can phosphorylate a second response regulator, Skn7, which does not control a MAP kinase module but functions as a transcription

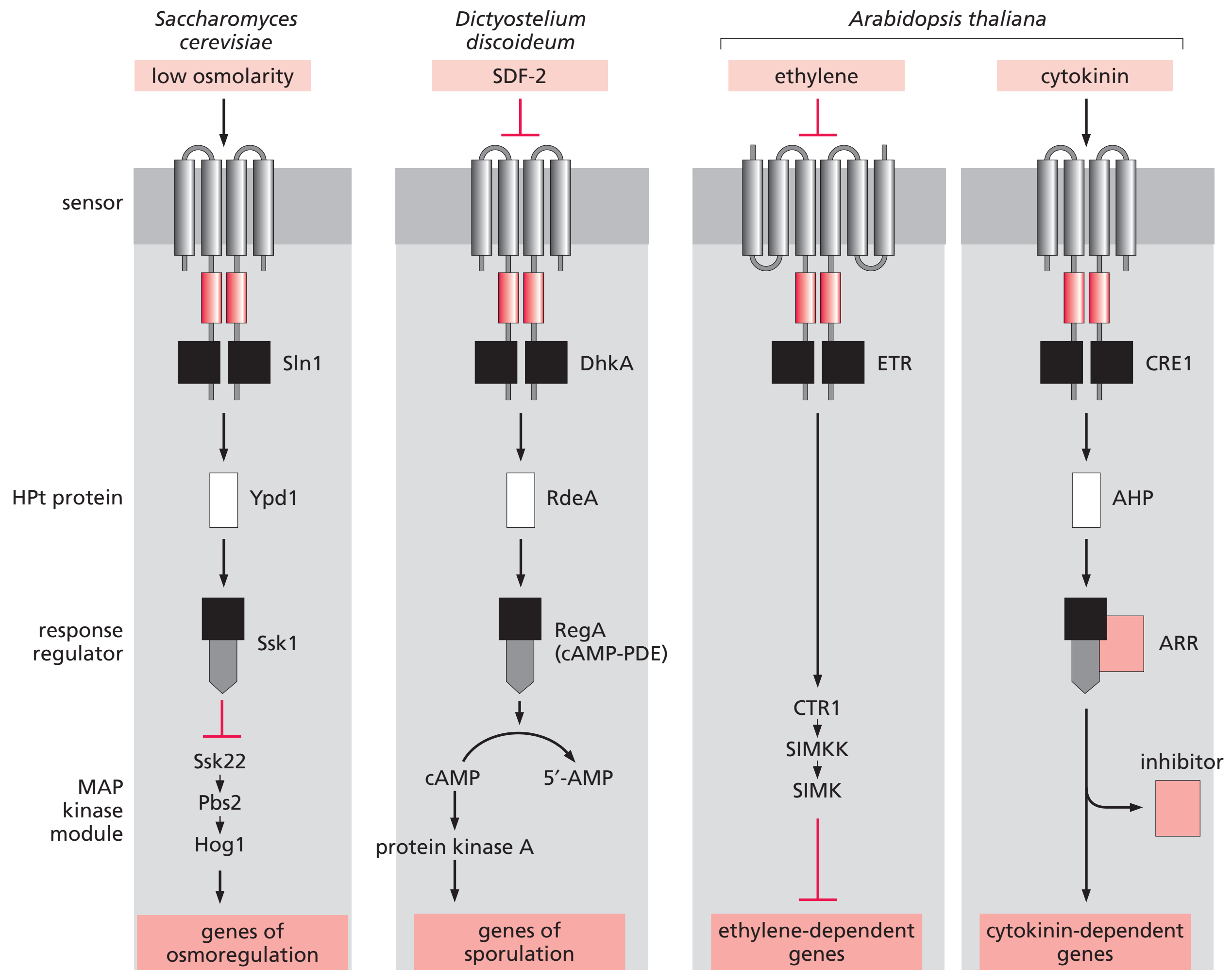


Figure 3.29 "Two-component" phosphorelays of eukaryotes Red cylinder, His autokinase domain; black square, Asp-containing receiver domain; white rectangle, HPt domain; gray wedge-shaped symbol, effector domain of response regulator. For details see text.

factor of additional stress genes. An analogous system has been found in the fission yeast *Schizosaccharomyces pombe*.

Dictyostelium discoideum

Containing 20 His kinase and phosphorelay genes, respectively, this slime mold is presently known to be the eukaryote equipped with the most two-component systems. Among these systems, however, only one is understood in more detail in that it has been found to be involved in the coordination of cell differentiation by controlling the production of the signal molecule cyclic AMP. For *Dictyostelium*, cAMP has two functions: it is a chemotactic attractant for the aggregation of single cells to a multicellular structure and it is a morphogenetic signal inducing the differentiation of spore capsules. These effects of cAMP are mediated by G-protein-coupled receptors (see Chapter 5). The action of cAMP depends on its concentration: at low levels it is an attractant, at high levels a morphogen. The cAMP level is controlled by two enzymes, an adenylate cyclase catalyzing biosynthesis from ATP and a phosphodiesterase catalyzing hydrolysis to inactive 5′-AMP. cAMP phosphodiesterase (cAMP-PDE) is a C-terminal subdomain of a response regulator, RegA, and becomes activated by Asp phosphorylation of RegA. This phosphorylation is stimulated by two sensor His kinases, DhkA and DhkB (*Dictyostelium* histidine kinases), that are inhibited upon binding of their ligands; Slime mold Differentiation Factor SDF-2; and discadenin, a cytokinin derivative. As a result, the interconnected HPt protein RdeA and the response regulator RegA become dephosphorylated, the phosphodiesterase activity is suppressed, and the level of cAMP increases (Figure 3.29). Another sensor His kinase of *Dictyostelium* is probably involved in the adaptation to osmolarity.

Plants

Plants express a limited number of two-component systems. For instance, 11 His kinase genes and 16 response regulator genes have been found in the genome of *Arabidopsis thaliana* (as compared with more than a thousand genes encoding "eukaryotic" protein kinases). Some of these systems mediate the response to osmotic stress and to the phytohormones cytokinin and ethylene as well as light adaptation by phytochromes.

In structure and function, the **osmosensor Athk1** (*Arabidopsis thaliana* histidine kinase 1) resembles the corresponding sensor Sln1 of yeast. In both cases the His kinase activity is depressed by hypertonic stress. Athk1 is found predominantly in root cells.

The gaseous plant hormone **ethylene** controls processes of senescence such as fruit ripening and the fall of leaves. *Arabidopsis* expresses at least five different ethylene receptors, which are tissue-specifically distributed. These are sensor His kinases that inhibit the expression of ethylene-inducible genes when activated. Ethylene blocks the His kinase activity. Like the osmosensor system of yeast, the signaling cascade includes a MAP kinase module with the Ser/Thr kinase CTR1 acting as a MAP3 kinase (Figure 3.29). As functional antagonists of ethylene, **cytokinins** promote cell division and retard senescence. In *Arabidopsis*, two sensor His kinases have been identified as cytokinin receptors. Upon phosphorylation, the corresponding response regulators become released from a complex with an inhibitor protein (Figure 3.29).

Phytochromes emerged from the phycochromes of cyanobacteria (see Section 3.3.5). They are used by plants to adapt growth, flowering, germination, and periodic movements to the light situation. The light signals are processed by a "degenerate" two-component system with a His kinase-related Ser/Thr kinase coupled to the sensor. By means of this system, the transcription of genes encoding proteins of light adaptation is controlled. "Degenerate" His kinases have also been found in prokaryotes such as *B. subtilis*, indicating that the change of amino acid specificity is not a result of eukaryotic evolution.

Other light sensors of plants include the phytochrome-related cryptochromes, a group of flavoproteins with unknown functions, and the phototropins. The latter, which are flavoproteins with an additional Ser/Thr kinase activity, are involved in the control of light-directed movements or phototropisms. A response regulator-like protein also plays a role in the regulation of day–night rhythms (photoperiodicity) of *Arabidopsis.*

Animals

The genomes of animals sequenced thus far do not contain His kinase and response regulator genes. An exception is mitochondria, the matrix of which contains two Ser/Thr kinases exhibiting some sequence homology with His kinases. These enzymes are subunits of the large dehydrogenase complexes metabolizing pyruvate and other α-keto acids. The dehydrogenase activity is suppressed by phosphorylation. This reaction is stimulated by protein deficiency, indicating that it provides a protective mechanism against a counterproductive degradation of amino acids.

The occurrence of degenerate His kinases in mitochondria is explained by the evolution of these organelles from endosymbiotic prokaryotes. It should be noted that in contrast to the labile His phosphorylation, the Ser/Thr phosphorylations catalyzed by degenerate His kinases are stable post-translational modifications.

Since conventional two-component systems are not found in animals, they provide interesting targets for novel antibacterial drugs, which are under development.

3.5.2 "Eukaryotic" protein phosphorylation in prokaryotes

At the transition from prokaryotes to eukaryotes, the "prokaryotic" His kinases were widely replaced by Ser/Thr and Tyr kinases. Nevertheless, the adjective "eukaryotic" commonly used for these enzymes is somewhat misleading, since they are by no means restricted to eukaryotes but are found, together with the corresponding protein phosphatases, in the majority of prokaryotes as well. In fact, in 29 out of 35 prokaryotic genomes sequenced, corresponding genes have been identified (for His kinases this ratio is 26:35). Even mycoplasma lacking His kinases express "eukaryotic" Ser/Thr kinases. Obviously these enzymes have a long evolutionary history, probably going back to a primeval form that emerged prior to the separation of bacteria, archaea, and eukaryotes. In contrast, His kinases seem to be genuine bacterial "inventions" taken over by other species through horizontal gene transfer. Such a concept does not contradict the fact that some microbes are devoid of either bacterial His kinases or "eukaryotic" kinases and phosphatases. As a rule, such species are highly adapted pathogenic organisms, such as mycoplasma and rickettsia, as well as inhabitants of isolated biotopes with constant environmental conditions. It is assumed that the corresponding genes devoted to the processing of environmental stimuli turned out to be unnecessary and were eliminated.

The functions of "eukaryotic" protein phosphorylation in prokaryotes are understood only in a few cases. Examples are the kinase Pkn1 of *Myxococcus xanthus*, which is involved in sporulation, and the kinase AfsK, which phosphorylates the transcription factor AfsR and thus regulates the biosynthesis of antibiotics in *Streptomyces coelicolor.* In eukaryotes, with very few exceptions, Ser/Thr phosphorylation and Tyr phosphorylation are catalyzed by different protein kinases. However, in most of the prokaryotic kinases, this specification is not found (thus they are frequently called STY kinases). Nevertheless, Tyr phosphorylation seems to be a rare event in microbes, found only in a very few species such as streptomycetes and myxobacteria. Moreover, *specific* Tyr kinases have been detected as yet only in myxobacteria but in no other prokaryotes. Such enzymes, in particular the receptor-coupled forms, are more or less restricted to animals. Their equivalents in plants (and some prokaryotes) are the "receptorlike" Ser/Thr kinases, of which animals express only a few types (see Sections 2.6.3 and 6.1).

Kinases unrelated to both His kinases and eukaryotic kinases are called atypical. Examples of atypical kinases in prokaryotes are provided by the bifunctional protein kinases/phosphatases (see Section 3.5.3). Atypical kinases of eukaryotes include the myosin heavy-chain kinases of *D. discoideum* and the related elongation factor 2 kinase (eEF2K, see Section 9.3.4) as well as the kinase BCR (known as a fusion partner of the Abl Tyr kinase of chronic myeloid leukemia, see Section 7.1.1) and several protein kinases related to phosphatidylinositol 3-kinase (Section 12.9.1). In Figure 3.30 an attempt is made to schematically derive the evolutionary history of current protein kinase families from their present distribution.

3.5.3 Evolution of protein phosphatases

Among the protein phosphatases, the Ser/Thr-specific enzymes of the PPP family (Section 2.6.4) seem to be phylogenetically most ancient. Like Ser/Thr kinases, they probably were introduced by the common primeval precursor of pro- and eukaryotes. In contrast, the bacterial enzymes of the PPM family (Section 2.6.4) are thought to have been acquired from eukaryotes by horizontal gene transfer since they have not yet been found in archaea. A similar assumption is made concerning Tyr-specific phosphatases, identified in a few, mostly pathogenic, bacterial species where they play a role as virulence factors (see Sidebar 3.5). No genes of the dual-specific Cdc25 phosphatases regulating the cell cycle have yet been found in prokaryotes.

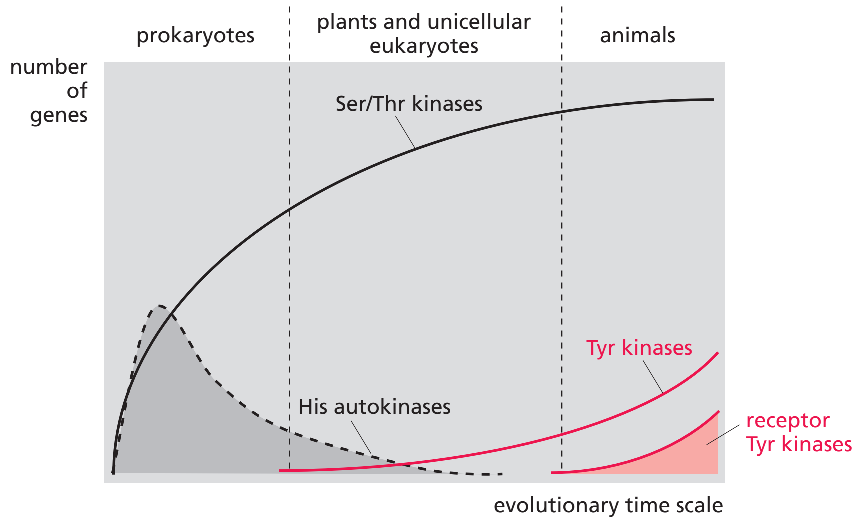


Figure 3.30 Tentative scheme illustrating the evolution of different protein kinase families This schematic sketch (which is not to scale) is based on the distribution of kinase families in today's organisms.

The phospho-His and phospho-Asp residues generated by two-component systems are hydrolyzed either nonenzymatically or by the intrinsic phosphatase activity of the bifunctional His kinases and response regulators. There are, in addition, Asp-specific phosphatases such as CheZ (chemotaxis), SpoOE (sporulation of *B. subtilis*), RapA and B (sporulation), and the His-specific phosphatase SixA (adaptation of *E. coli* to oxygen deficiency). These enzymes are monospecific: they have only one substrate protein each and are considered to be the result of highly specialized development. CheZ dephosphorylates exclusively the response regulator CheY, and SixA affects only the HPt domains of the sensor ArcB, the cytoplasmic part of which is a typical phosphorelay consisting of a His kinase, an Asp kinase, and an HPt domain.

Other extreme specialists are the bifunctional bacterial Ser/Thr kinases/phosphatases, represented by the isocitrate dehydrogenase kinase/phosphatase (AceK) of *E. coli* and the HPr-kinase/phosphatase of *B. subtilis*. AceK is of historical interest since it was the first protein kinase found in prokaryotes. The enzyme inhibits isocitrate dehydrogenase by phosphorylating a strategic Ser residue in the catalytic center, thus inhibiting a starter reaction of the citrate cycle and shifting the isocitrate metabolism to the glyoxylate pathway:

$$NADP^+ + \text{isocitrate} \rightarrow NADPH + \alpha\text{-ketoglutarate} + H^+ + CO_2$$

In the kinase, the same catalytic center is responsible for both phosphorylation and dephosphorylation. Dephosphorylation occurs through a re-transfer of the protein-bound phosphate residue to ADP. The subsequent hydrolysis of the ATP thus formed renders the reaction exergonic. In other words, the kinase/

Sidebar 3.5 Phosphotyrosine phosphatases as virulence factors: God's scourge of the Middle Ages Some bacteria produce Tyr-specific protein phosphatases of the conventional subfamily (see Section 2.6.4). They are assumed to have acquired this ability from eukaryotes by horizontal gene transfer. The bacterial phosphatases are extremely potent virulence factors. The enzyme YoPH of plague bacteria (*Yersinia*), together with some additional toxins, is thought to be responsible for the depopulation of large areas of Europe and Asia in the Middle Ages. YoPH is injected by the bacteria into the host cell and destroys focal adhesions—the contacts to other cells and to the extracellular matrix—thus inducing cell death by apoptosis. A comparably destructive effect, in this case on the cytoskeleton, is exhibited by the Tyr-specific phosphatases STP of *Salmonella typhimurium* and MPtpB of *Mycobacterium tuberculosis*. Other examples of the fatal consequences of a disturbance by bacteria of cellular data processing are provided by the cholera, pertussis, and diphtheria toxins attacking G-proteins and by the pathogenic adenylate cyclases of anthrax. They will be discussed in more detail in Section 4.3.3.

phosphatase is also an ATPase. Such a mechanism appears to be unique and is not a precedent in evolution. It also holds true for HPr kinase/phosphatase which is, however, devoid of ATPase activity. Obviously both enzymes represent evolutionary impasses.

3.5.4 Evolution of regulatory GTPases

In addition to protein kinases and phosphatases, GTPases constitute a particularly important family of signal-transducing proteins. There are two major types of GTPases: the FtsZ/tubulin family and the G-protein family. Both seem to have evolutionary histories comparable to that of protein kinases, since the corresponding genes have been found in all prokaryotes studied so far.

The prokaryotic **FtsZ proteins** derive their name from a group of *E. coli* proteins involved in cell division. In their three-dimensional architecture and their functions they closely resemble the eukaryotic β-tubulins, though very little sequence homology exists. FtsZ proteins control the directed polymerization of cytoplasmic filaments or protofilaments in prokaryotes, whereas β-tubulins regulate the formation of microtubules in eukaryotic cells. Protofilaments are components of the Z-ring that separates the daughter cells during mitosis, while microtubules constitute the mitotic spindle as well as cilia and flagella. As expected, FtsZ-related proteins have also been found in mitochondria and chloroplasts.

The **G-proteins** are not related to FtsZ proteins and β-tubulins. As biochemical switches they regulate a wide variety of cellular processes, causing spatial and temporal coordination and irreversibility (Section 2.4). First the translation of mRNA has to be mentioned, which is controlled by the GTPase subfamily of initiation and elongation factors. The prokaryotic translation factors are summarized in Table 3.1 (for details see Section 9.1). This mechanism is highly conservative and common to all forms of life. Equally abundant are G-proteins such as the signal recognition particles and their receptors, which manage the transport of newly synthesized polypeptide chains across membranes (see Section 9.2). They have prokaryotic counterparts such as the G-proteins Ffh and FtsY of *E. coli.*

Additional prokaryotic G-proteins seem to be involved in ribosome formation, namely the Era (*E.coli* ras-like) proteins, facilitating the maturation of ribosomal RNA; the EngA proteins and the ribosome small subunit-dependent GTPases RsgA promoting the assembly of large and small ribosomal subunits, respectively; and the ribosome-associated Obg proteins, which may function as stress sensors to measure the fall of the cellular GTP level during a stringent response.

Table 3.1 Prokaryotic translation factors

Factor	Function
IF1	dissociation of the ribosome, binding of initiator tRNA
IF2	GTPase, formation of the 70S initiation complex
IF3	ATPase, binding and scanning of mRNA
EF-Tu	GTPase, binding of aminoacyl tRNA at the A-site
EF-Ts	GDP–GTP exchange factor of EF-Tu
EF-G	GTPase, translocation of peptidyl-tRNA
RF1	release of the completed polypeptide
RF2	release of the completed polypeptide
RF3	GTPase, termination

Homologous GTPases playing a role in ribosome biogenesis are found also in other organisms. Gene mutations have shown that most of the prokaryotic G-proteins are essential.

The search for receptor-coupled heterotrimeric G-proteins in prokaryotes has been in vain. This supports the conclusion that prokaryotic transmembrane signaling is strongly dominated by the two-component mechanism. Counterparts of small G-proteins of the Ras, Rho, Rab, Ran, and Arf types are also widely absent from prokaryotes, perhaps with the exception of the Ras-related GTPase MglA of myxobacteria (mentioned previously) and an Arf-related protein involved in the formation of magnetosomes in magnetic bacteria.

Taken together, the prokaryotic G-protein mechanism appears to be simple when compared with the elaborate G-protein networks in eukaryotic cells. In fact, as explained in Chapter 10, the majority of eukaryotic G-proteins regulate the traffic between intracellular compartments, which are absent from prokaryotes.

3.5.5 Sensors of oxidative stress

Life under an oxygen atmosphere is a risky venture. Organisms may profit from aerobic conditions that enable highly efficient energy production. However, they are also endangered by the aggressive chemical properties of oxygen and its metabolic products (Section 2.2). To deal with oxidative stress, aerobic bacteria have developed simple but efficient protective mechanisms by which they recognize dangerous reactive oxygen species (ROS) and initiate countermeasures. In this situation, the mechanistic principle of signal processing operates, in which certain signaling proteins become post-translationally modified by oxidation of iron-sulfide groups or cysteinyl–SH groups.

To make this clear, let us examine two ROS-controlled transcription factors of *E. coli*: the superoxide anion radical sensor SoxR and the H_2O_2 sensor OxyR. **SoxR** contains as a redox cofactor a [Fe_2-S_2] iron sulfide entity complexed by cysteinyl residues. This so-called iron sulfur cluster (which is found in many redox enzymes) typically undergoes single electron transitions when reacting with free radicals such as superoxide anion radicals. As a result the conformation of SoxR is changed facilitating the binding to DNA. In turn, genes that encode enzymes degrading the highly toxic superoxide anion radical are activated. The prototype of such enzymes is superoxide dismutase catalyzing the reaction $2O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2$. The activation of SoxR is reversible because the factor is reduced by NADH/NADPH. The transcription factor **OxyR** takes care of the elimination of hydrogen peroxide that is generated along this and other metabolic pathways. OxyR represents the second family of ROS sensors that contain as a redox center oxidable cysteinyl residues instead of an iron sulfur cluster. Such proteins are less reactive with free radicals but are especially suited to react with peroxides that oxidize the thiol groups of Cys to sulfenic acid and disulfide groups (Section 2.2). As for SoxR this modification is reversible becoming reduced by NADH/NADPH via glutathione as an intermediate reductant. In the oxidized state, OxyR induces the activity of genes controlling peroxide metabolism. Another example of a bacterial redox sensor containing a reactive dithiol configuration is the His kinase ArcB. It will be discussed in more detail in Section 3.5.6.

Saccharomyces cerevisiae possesses a similar protective system; however, the sensor and transcription factors are separated. The yeast peroxide sensor is **Orp1**, a protein that in contrast to OxyR is not a transcription factor but exhibits the properties of a peroxidase, which is an enzyme that catalyzes the oxidation of substrates by hydrogen peroxide. In the course of this reaction, two cysteine residues of Orp1 become oxidized, forming a disulfide bridge or sulfenic acid groups that immediately become reduced again by another SH protein, **Yap1**. Yap1 is a transcription factor controlling several genes taking care of antioxidant

production. It is inactivated by thioredoxin-catalyzed reduction. An adaptor protein, Ybp1, brings the sensor Orp1 in close contact with Yap1.

As compared with the bacterial OxyR, the yeast system impressively demonstrates the evolutionary change from a simple peroxide sensor to a H_2O_2-sensitive complex of signal processing consisting of a sensor, an adaptor, and an effector. As discussed in more detail in Section 2.2, in eukaryotes, ROS indeed play a double role as both stress factors that have to be eliminated and intracellular signaling molecules. An example for ROS signaling in higher eukaryotes is found in Section 7.1.

3.5.6 Signal-controlled proteolysis

In eukaryotes, controlled degradation as well as proteolytic activation of signal-transducing proteins is a major regulatory mechanism. As a rule, proteins are marked for degradation by ubiquitylation to become hydrolyzed subsequently in proteasomes. This principle finds its precursor in prokaryotes. An example is the activation of the **alternative sigma factor σ^S** of *E. coli* (see Sidebar 3.6). This factor becomes up-regulated only in stress situations such as starvation, high temperature, or osmotic crisis, and directs the RNA polymerase to genes specialized for stress protection. Under stress-free conditions, σ^S is kept at a low level by permanent proteolytic degradation. To be recognized by the protease ClpXP (a bacterial counterpart of the proteasome), the σ factor needs to be labeled by binding the recognition factor **RssB** (note the striking analogy to ubiquitylation). RssB, a protein, is active only when phosphorylated, thus providing an input terminal for stress signals that are mediated by a two-component system with the His kinase **ArcB** as a sensor and RssB as a response regulator. ArcB is an energy sensor that, under aerobic conditions, exists in an oxidized disulfide form. Upon energy shortage indicated by oxygen deficiency, ArcB is reduced to the dithiol form and its His kinase activity is suppressed. As a consequence, the σ factor becomes resistant to proteolytic degradation. Such a mechanism, which instead of inducing *de novo* synthesis only alters the steady-state concentration of a regulatory protein, has the advantage of responding very rapidly to changes in the environment. This principle is expensive but obviously essential for coping with stress situations.

The release of active signaling proteins from membrane-bound precursors by intramembrane proteolysis (Chapter 13) has also been introduced by prokaryotes. An example is the **alternative sigma factor σ^K** directing RNA polymerase to

Sidebar 3.6 Sigma factors The RNA polymerase of *E. coli* consists of a core and a regulatory subunit called σ-factor. The core is a heterotetrameric protein composed of two α-subunits, one β-subunit, and one β′-subunit. It is able to bind to DNA and to catalyze RNA biosynthesis but is unable to recognize the promoter sequences of individual genes. Such selectivity is conferred by the σ-factor. *E. coli* possesses seven different σ-subunits exhibiting different promoter specificities. Everyday needs are satisfied by the σ^{70}-subunit, a protein of 63 amino acids. The additional six σ-factors become activated during stress situations and control the transcription of “emergency genes” such as those encoding heat-shock proteins (Section 2.5.1).

σ^{70} has two binding sites for DNA, with the C-terminal site recognizing double-stranded regions and the N-terminal site recognizing single-stranded regions. The corresponding interaction sequences of DNA are a TTGACA hexamer at –35 bp (35 base pairs upstream of the transcription start) and a TATAAT hexamer, the **TATA-box,** localized in a double-stranded area as well as at –10 bp, where the double strand becomes unwound into single strands. This unwinding, also called melting, is a prerequisite for strong binding between DNA and the RNA polymerase complex. σ^{70} interacts with these two sites only as a complex with the core enzyme due to an auto-inhibitory conformation of the σ-factor where the N-terminal sequence blocks the binding site for the DNA sequence at –35 bp. This inactive form becomes unfolded to the active conformation upon binding to the core enzyme.

sporulation genes. σ^K becomes released from a precursor protein when *B. subtilis* receives environmental signals inducing sporulation. This signaling reaction is a perfect counterpart of the animal notch system (see Section 13.1.1).

3.5.7 Prokaryotic ion channels

Ion channels are absolutely essential for metabolism, equalization of osmotic pressure, and regulation of intracellular acidity. In parallel they were employed for data processing from the very beginning of life. Clearly the evolutionary history of ion channels dates back as far as that of protein kinases and GTPases. Only the simplest prokaryotes, such as some mycoplasma, seem to be devoid of the corresponding genes, leaving open the question whether they had been lost by adaptive processes. Research on prokaryotic ion channels has made an important and significant contribution to our understanding of these signal-propagating devices, in particular since several channel proteins could be crystallized and studied by X-ray analysis. The overall result emerging from these studies is that all basic structures and mechanisms of action of ion channels were introduced by prokaryotes.

A common structural feature of ion channels is a series of transmembrane helices (at least four) forming a central pore that, through its width and electrostatic charge, determines the channel's ion selectivity (Section 2.10.3). Prokaryotic ion channels that have been investigated in great detail include the mechanosensitive channel MscL, several potassium and sodium channels, and the chloride channel ClC. Each of these channels is a prototype of a large and evolutionary extremely successful family of channel proteins. Representatives of prokaryotic cation channels are shown in Figure 3.31.

Mechanosensitive channels

These channels are abundant in both prokaryotes and eukaryotes. Their major role is that of an excessive pressure valve protecting cells from disruption by hypo-osmotic shock. Gating of the channels is triggered by the horizontal tension of the plasma membrane due to a swelling of the cell. This tension causes an irislike twisting of the channel's transmembrane domains, leading to a widening of the central pore (Figure 3.32). Such a response is particularly essential for free-living organisms that are permanently endangered by a hypo-osmotic situation, for instance, rain.

Lacking a ligand-binding site as well as an ion selectivity filter and a voltage sensor, **MscL** (L stands for large conductance) is one of the simplest ion channels. It represents the primeval form of the so-called S5–S6 channels (see Figure 3.31 and Section 2.10.3) and might be a precursor of the ligand-controlled ion channels of higher eukaryotes. In prokaryotes, however, no ligand-controlled channels except the glutamate-dependent potassium channel GluR0 (see below) have been found.

While MscL channels occur predominantly in bacteria, a second family of mechanosensitive channels is distributed more widely. These are the structurally

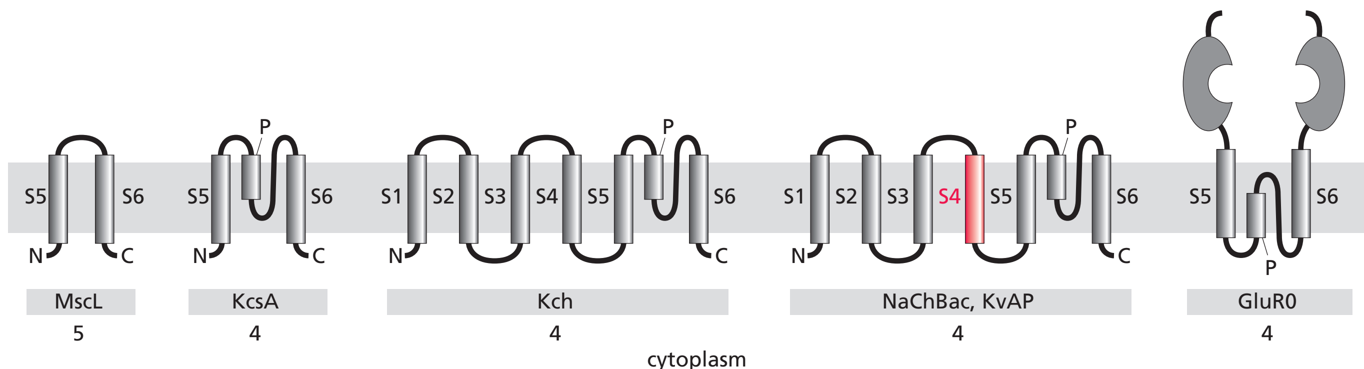


Figure 3.31 Subunits of prokaryotic ion channels of the S5–S6 family: precursors of eukaryotic channels Mechanosensitive channel MscL is the prototype of the following eukaryotic ion channels: ATP-receptor cation channel P2X (Section 16.6); epithelial sodium channel ENaC (Section 14.3); and several neuropeptide receptor ion channels of invertebrates. Potassium channel KcsA resembles the inwardly rectifying potassium channel K_{ir} of eukaryotes. In other eukaryotic K^+ channels, two KcsA-like subunits (TWIK) or one KcsA-like subunit and one Kch-like subunit (TOK) share one polypeptide chain each (Section 14.4). Potassium channel Kch resembles the cyclic nucleotide-gated ion channels of higher eukaryotes (Section 14.5.7). Voltage-dependent sodium and potassium channels NaChBac and KvAP may be precursors of voltage-dependent Na^+-, K^+-, and Ca^{2+}-channels of eukaryotes (see Sections 14.2, 14.4.1, and 14.5.2). Glutamate-dependent potassium channel GluR0 is probably the precursor of the ionotropic glutamate receptor cation channels of vertebrates (Section 16.4). Gray cylinders, transmembrane domains and P-loops (P); red cylinder, transmembrane helix with voltage sensor; dark gray domains, extracellular Glu-binding site of GluR0. Numbering S1–S6 of the transmembrane domains refers to the voltage-dependent K^+ channel of eukaryotes. The number of subunits constituting a channel is shown beneath the name.

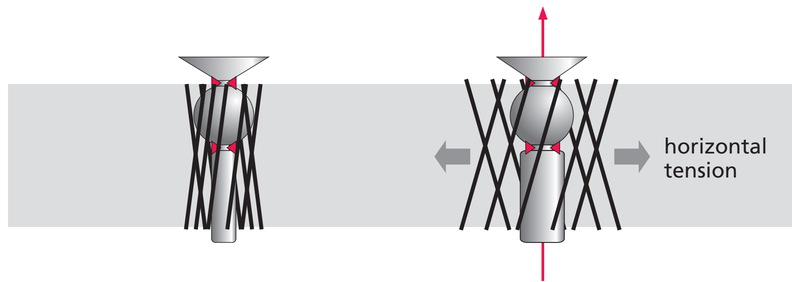


Figure 3.32 Model of the mechanosensitive channel MscL The channel pore is depicted in a three-dimensional schematic manner (bottleneck structures are shown in red). Transmembrane helices are represented by black lines. The channel is gated by a horizontal tension of the membrane caused by the swelling of the cell in a hypo-osmotic environment.

highly variable **MscS channels** (S stands for small conductance). The corresponding channel of *E. coli* consists of seven symmetrically arranged subunits with three transmembrane helices each and becomes gated by both mechanical stress and membrane depolarization, thus representing a primeval form of voltage-dependent ion channels.

Potassium and sodium channels

A prokaryotic prototype of these channels is **KcsA** (K^+channel of streptomyces A) isolated from *Streptomyces lividans* (homologous channels of *E. coli* are KefB and KefC). X-ray analysis has revealed a subunit structure that is characterized by two transmembrane helices and a P-loop rendering the channel selective for K^+ ions (the problem of channel selectivity is discussed in more detail in Section 2.10.3). Each channel consists of four subunits representing the primeval form of all ion-selective potassium channels of the S5–S6 family (Figure 3.31). The KcsA channel opens at low pH, probably due to an interaction of protons with the extracellular domains. As in the case of the MscL channel, this gating is assumed to be caused by an irislike twisting of the transmembrane helices (Figure 3.33).

The structure of KcsA resembles that of inwardly rectifying potassium channels of vertebrates (Section 14.4.2). A special species of this channel type is the potassium channel **MthK** of *Methanobacterium thermoautotrophicum (*Figure 3.33). It contains an extended cytoplasmic C-terminal domain that binds Ca^{2+} ions resulting in channel gating. X-ray crystallography has provided a deep insight into channel structure and the gating process (Figure 3.34). Functionally MthK resembles the mammalian Ca^{2+}-gated potassium channels BK and SK that, however, have a more complex structure (Section 14.4.1).

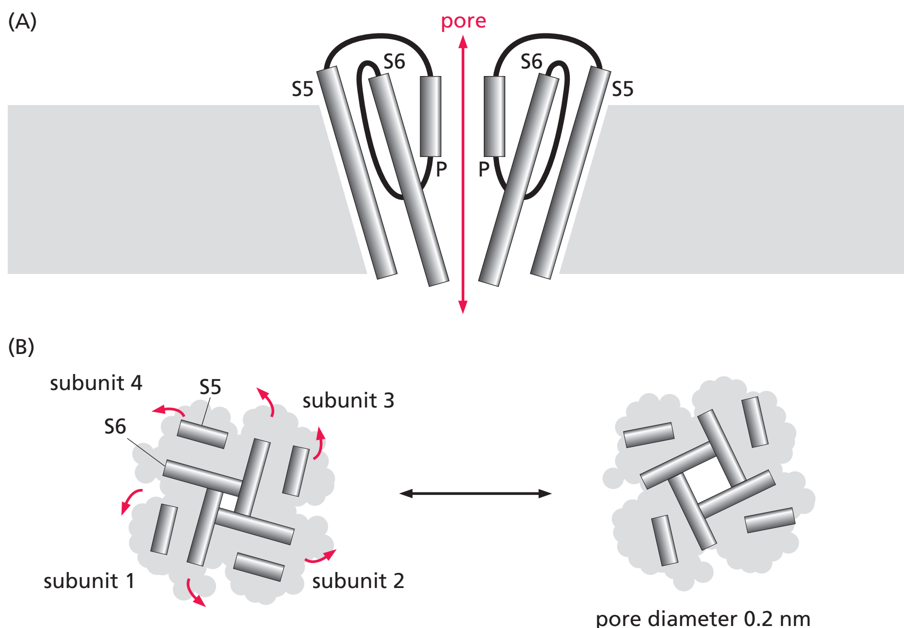


Figure 3.33 Schematic representation of architecture and gating of the KcsA channel (A) Arrangement of transmembrane helices S5 and S6 as well as the P-loop (P) in the plasma membrane (cross section showing two of the four subunits of the channel). (B) Gating of the central pore by an irislike twisting of the transmembrane helices.

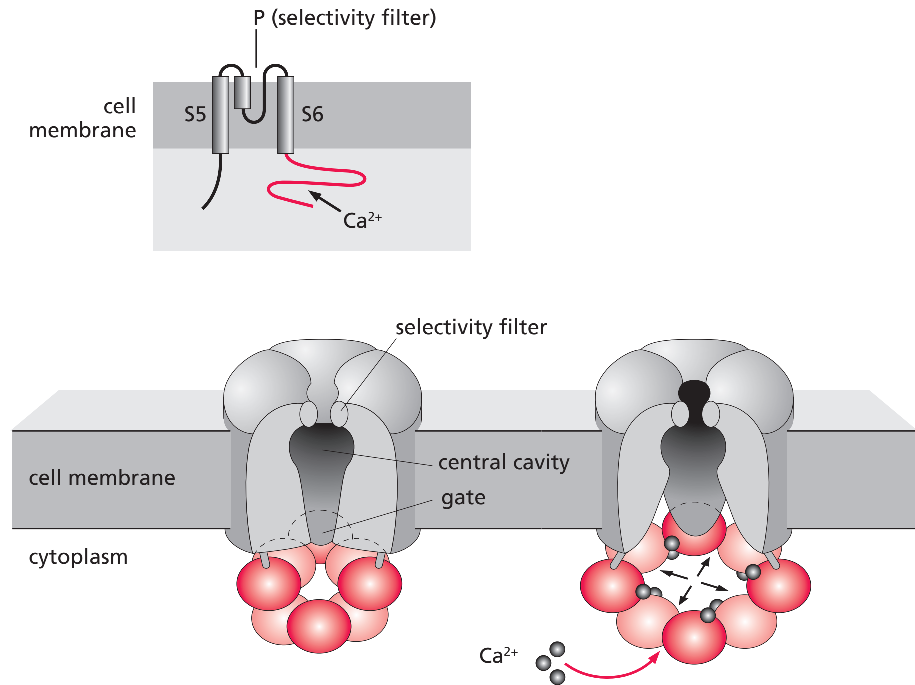


Figure 3.34 Gating of the Ca^{2+}-dependent K^{+} channel MthK The insert shows the schematic structure of one of the four channel subunits with the transmembrane segments S5 and S6 and the P-loop (P) forming the selectivity filter. The extended C-terminal domain (red) contains Ca^{2+} binding sites. Below a space-filling model of the channel based on X-ray crystallography is shown in a closed (left) and in an open form (right). Channel gating is due to a conformational change of the ring of C-terminal domains (red) that is induced by Ca^{2+} binding. (Modified from M. Schumacher & J.P. Adelman, *Nature* 417, 501–502, 2002.)

Voltage-dependent cation channels of vertebrates are typically composed of four subunits with six transmembrane domains and a P-loop each whereby transmembrane segment S4 acts as a voltage sensor (Section 14.1). An analogous structure has been found for prokaryotic cation channels, such as the potassium channel **Kch** of *E. coli*. While Kch does not significantly respond to voltage, other bacterial channels do: the **voltage-dependent Na^{+}-channel** of *Bacillus halodurans* (Figure 3.31) and the archaeal potassium channel **KvAP** of *Aeropyrum pernix*. Certainly, these channels are evolutionary precusors of vertebrate voltage-dependent ion channels. Their four subunits resemble those of Kch; however, transmembrane segment S4 together with a part of S3 acts as a voltage sensor (see Figure 14.3 in Section 14.1). The elucidation of the KvAP structure has revolutionized our concepts of ion channel gating (see Section 14.1). While the function of the potassium channel is not yet understood, the sodium channel has been proposed to be a part of the flagellar motor, which in this species is supplied by energy derived from a Na^{+} gradient across the cell membrane. It is possible that these channels also participate in regulation of the very negative ion potential (ranging between –100 and –150 mV) of bacterial cells. Cooperating with mechanosensitive channels they may, in addition, protect the cell from osmotic stress. The subunit and domain architecture of voltage-dependent ion channels is also found in cyclic nucleotide-gated channels that play a key role in vertebrate sensory signal processing (Chapter 15). The ancestor of these channels might be represented by the cyclic nucleotide-gated potassium channel **MloK1** of *Mezorhizobium loti*, a plant symbiont.

A **glutamate-dependent potassium channel GluR0**, which has been found in some bacterial species, has evolved from the fusion of a KscA-like channel protein with a periplasmic binding protein for glutamate. GluR0 is considered to represent the evolutionary precursor of the ionotropic Glu receptors of higher eukaryotes (Section 16.4).

Chloride channels

Like the S5–S6 channels, the chloride or anion channels also originate from prokaryotic precursors, though not all prokaryotes have the corresponding genes. X-ray analysis of the channel protein **EcClC** of *E. coli* has revealed an unexpectedly complex structure. This channel is composed of two identical

subunits with 18 strongly twisted helices each, 17 of them penetrating the cell membrane partially or completely. In contrast to cation channels, each subunit of EcClC forms a pore; that is, the channel has two pores (Figure 3.35). This structure seems to be prototypical for all chloride channels found as yet (Section 14.7). The function of the prokaryotic chloride channels is unclear. They may participate in regulation of the cell volume. Because of the small volume of a bacterial cell (10^{-12} mL on average), the gating of ion channels must be precisely terminated; otherwise, intolerable changes of the ion concentration and osmotic pressure would occur. How this control is achieved is still an open question.

Water channels

A large and ubiquitous family of membrane channels is specialized for the transport of water and small polyalcohols, which on their own cannot penetrate the lipid bilayer. These channels are called **aquaporins** (Aqp, only for water) and **glyceroaquaporins** (Glp, for water and for polyalcohols such as glycerol). Both types are widely distributed among prokaryotes and participate in osmoregulation and volume control, respectively. Human cells express 10 different aquaporins. The channels AqpZ and GlpF of *E. coli* have been studied in detail. Their structure seems to be representative for all (glycero)aquaporins. Each channel is composed of four subunits, each of which contains four transmembrane helices and two P-loops and forms one pore; that is, the complete channel has four pores (Figure 3.34). Bottlenecks and charged amino acid residues guarantee the selectivity of the pores. Hydrated ions are too large to pass through the pore, and the charge of the pore is insufficient for stripping the ion from water molecules. Therefore, water channels are impassable for ions and other charged molecules.

Summary

While Ser/Thr-specific protein kinases have been found in all species studied thus far, His autokinase-based two-component systems are restricted to prokaryotes and, to a smaller extent, slime molds, fungi, and plants. In animals, only two kinases that structurally resemble prokaryotic His autokinases (but phosphorylating Ser and Thr residues) have been identified in mitochondria. With one exception (myxobacteria), Tyr-specific protein kinases seem to be restricted to animals. Receptor-coupled Tyr kinases are particularly late inventions of evolution. Protein phosphatases are phylogenetically as ancient as protein kinases. His autokinases are bifunctional, exhibiting both kinase and phosphatase activity. In addition, monofunctional His-, Asp-, and Ser/Thr-specific phosphatases have been found in prokaryotes. Some bacterial Tyr phosphatases acquired from animals by horizontal gene transfer play a fatal role as virulence factors. The most ancient G-proteins are translation factors and proteins that catalyze the transmembrane transport of newly synthesized

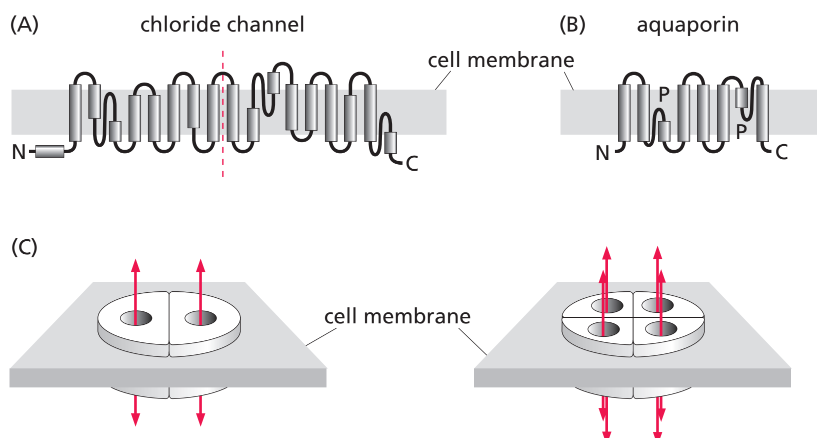


Figure 3.35 Membrane topology of chloride and water channels (A) Schematic representation of a chloride channel subunit showing 18 helices (gray cylinders). Note the internal symmetry caused by an anti-parallel repeat of the first nine helices (see also Section 14.7 for more details). (B) Subunit of an aquaporin water channel showing six transmembrane helices and two P-loops. (C) Channels composed of two and four identical subunits, respectively, exhibiting either two or four pores.

polypeptide chains. Most prokaryotes have been found to express neither small G-proteins of the Ras superfamily nor receptor-coupled trimeric G-proteins. Redox signaling through reversible oxidation of SH groups in proteins is an ancient principle of regulation. In prokaryotes it may have developed from mechanisms that protected the organism from oxidative stress. Mechanisms resembling both the ubiquitin-controlled degradation of signaling proteins and the proteolytic release of active factors from protein precursors are also found in prokaryotes. In bacteria such signaling events play an important role in stress situations. All ion and water channels of eukaryotes can be traced back to prokaryotic precursors. In prokaryotes such channels are involved in cell volume control, regulation of the osmotic pressure, and maintenance of the membrane potential. The role of voltage-sensitive bacterial ion channels is not yet fully understood.

Further reading

Alexandre G & Zhulin IB (2001) More than one way to sense chemicals. *J. Bacteriol.* 183, 4681–4686.

Anantharaman V, Iyer LM & Aravind L (2007) Comparative genomics of protists: new insights into the evolution of eukaryotic signal transduction and gene regulation. *Annu. Rev. Microbiol.* 61, 453–475.

Anishkin A & Kung C (2005) Microbial mechanosensation. *Curr. Opin. Neurobiol.* 15, 397–405.

Baker MD, Wolanin PM & Stock JB (2006) Systems biology of bacterial chemotaxis. *Curr. Opin. Microbiol.* 9, 187–192.

Bart H, Aktories K, Popoff MR et al. (2004) Binary bacterial toxins: biochemistry, biology, and applications of common *Clostridium* and *Bacillus* proteins. *Microbiol. Mol. Biol. Rev.* 68, 373–402.

Bassler BL & Losick R (2006) Bacterially speaking. *Cell* 125, 237–246.

Beier D & Gross R (2006) Regulation of bacterial virulence by two-component systems. *Curr. Opin. Microbiol.* 9, 143–152.

Booth IR, Edwards MD & Miller S (2003) Bacterial ion channels. *Biochemistry* 42, 10045–10053.

Braeken K, Moris M, Daniels R et al. (2006) New horizons for (p)ppGpp in bacterial and plant physiology. *Trends Microbiol.* 14, 45–54.

Bren A & Eisenbach M (2000) How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation. *J. Bacteriol.* 182, 6865–6873.

Brückner R & Titgemeyer F (2002) Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. *FEMS Microbiol. Lett.* 209, 141–148.

Caldon CE, Voong P & March PE (2001) Evolution of a molecular switch: universal bacterial GTPases regulate ribosome function. *Mol. Microbiol.* 41, 289–297.

Camilli A & Bassler BL (2006) Bacterial small-molecule signaling pathways. *Science* 311, 1113–1116.

Chang G & Roth CB (2001) Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science* 293, 1793–1800.

Chow B & McCourt P (2007) Plant hormone receptors: perception is everything. *Genes Dev.* 20, 1998–2008.

Davidson AL & Chen J (2004) ATP-binding cassette transporters in bacteria. *Annu. Rev. Biochem.* 73, 241–268.

D'Autreaux B & Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.* 8, 813–824.

Deutscher J, Francke C & Postma W (2006) How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. *Microbiol. Mol. Biol. Rev.* 70, 939–1031.

Fuqua C & Greenberg EP (2002) Listening in on bacteria: acyl-homoserine lactone signaling. *Nat. Rev.* 3, 685–692.

Galperin MJ (2005) A census of membrane-bound and intracellular signal transduction proteins in bacteria: Bacterial IQ, extroverts and introverts. *BMC Microbiol.* 5, 35–54.

Galperin MJ (2004) Bacterial signal transduction network in a genomic perspective. *Environ. Microbiol.* 6, 552–567.

Gao R, Mack TR & Stock AM (2007) Bacterial response regulators: versatile regulatory strategies from common domains. *Trends Biochem. Sci.* 32, 225–234.

Gonzalez JE & Keshavan ND (2006) Messing with bacterial quorum sensing. *Microbiol. Mol. Biol. Rev.* 70, 859–875.

Gottesman S (2003) Proteolysis in bacterial regulatory circuits. *Annu. Rev. Cell Dev. Biol.* 19, 565–587.

Heelingwerf KJ (2005) Bacterial observations: a rudimentary form of intelligence? *Trends Microbiol.* 13, 152–158.

Henke JM & Bassler BL (2004) Bacterial social engagements. *Trends Cell Biol.* 14, 648–656.

Hoff WD, Jung KH & Spudich Jl (1997) Molecular mechanism of photosignaling by archaeal sensory rhodopsins. *Annu. Rev. Biomol. Struct.* 26, 223–258.

Jenal U & Malone J (2006) Mechanism of cyclic-di-GMP signaling in bacteria. *Annu. Rev. Genet.* 40, 385–407.

Kaiser D (2004) Signaling in myxobacteria. *Annu. Rev. Microbiol.* 58, 75–98.

Kenelly PJ (2003) Archaeal protein kinases and protein phos-

phatases: insights from genomics and biochemistry. *Biochem. J.* 370, 373–389.

Kenelly PJ (2002) Protein kinases and protein phosphatases in prokaryotes: a genomic perspective. *FEMS Microbiol. Lett.* 206, 1–8.

Koretke KK, Lupas AN, Warren PV et al. (2000) Evolution of two-component signal transduction. *Mol. Biol. Evol.* 17, 1956–1970.

Laursen BS, Soerensen HP, Mortensen KK et al. (2005) Initiation of protein synthesis in bacteria. *Microbiol. Mol. Biol. Rev.* 69, 101–123.

Lloyd G, Landini P & Busby S (2001) Activation and repression of transcription initiation in bacteria. *Essays Biochem.* 37, 17–31.

Mascher T, Helmann JD & Unden G (2006) Stimulus perception coupling in bacterial signal-transducing histidine kinases. *Microbiol. Mol. Biol. Rev.* 70, 910–938.

Mason P & Kay R (2000) Eukaryotic signal transduction via histidine-aspartate phosphorelay. *J. Cell Sci.* 113, 3141–3150.

Miller MB & Bassler BL (2001) Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55, 156–199.

Paulsen IT, Nguyen L, Sliwinski MK et al. (2000) Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. *J. Mol. Biol.* 301, 75–100.

Ryan RR, Fouhy Y, Lucey JF et al. (2006) Cyclic di-GMP signaling in bacteria: recent advances and new puzzles. *J. Bacteriol.* 188, 8327–8334.

Shapiro JA (1988) Bacteria as multicellular organisms. *Scientific Am.* 256, 82–89.

Shenoy AR & Visweswariah SS (2006) New messages from old messengers: cAMP and mycobacteria. *Trends Microbiol.* 14, 543–550.

Sherratt DJ (2003) Bacterial chromosome dynamics. *Science* 301, 780–785.

Shimkets LJ (1999) Intercellular signaling during fruiting-body development of *Myxococcus xanthus*. *Annu. Rev. Microbiol.* 53, 525–549.

Siebold C, Flükiger K, Beutler R et al. (2001) Carbohydrate transporters of the bacterial phosphoenolpyruvate: sugar phosphotransferase system (PTS). *FEBS Lett.* 504, 104–111.

Spudich JL (2006) The multitalented microbial sensory rhodopsins. *Trends Microbiol.* 14, 480–487.

Stock AM, Robinson VL & Goudreau PN (2000) Two-component signal transduction. *Annu. Rev. Biochem.* 68, 183–215.

Stoodley P, Sauer K, Davies DG et al. (2002) Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* 56, 187–209.

Szurmant H & Ordal GW (2004) Diversity of chemotaxis mechanisms among the bacteria and the archaea. *Microbiol. Mol. Biol. Rev.* 68, 301–319.

Tamayo R, Pratt JT & Camilli A (2007) Roles of cyclic diguanylate in the regulation of bacterial pathogenesis. *Annu. Rev. Microbiol.* 61, 131–148.

Thomasson B, Link J, Stassinopoulos AG et al. (2002) MglA, a small GTPase, interacts with a tyrosine kinase to control type IV pili-mediated mobility and development of *Myxococcus xanthus*. *Mol. Microbiol.* 46, 1399–1413.

Toledano MB, Delaunay A, Monceau L et al. (2004) Microbial H_2O_2 sensors as archetypical redox signaling modules. *Trends Biochem. Sci.* 29, 351–357.

Ulrich LE, Koonin EV & Zhulin IB (2005) One-component systems dominate signal transduction in prokaryotes. *Trends Microbiol.* 13, 52–56.

Ulrich LE & Zhulin IB (2007) MiST: a microbial signal transduction database. *Nucleic Acids Res.* 35, D386–D390.

Urrao T, Yamaguchi-Shinozaki K & Shinozaki K (2000) Two-component systems in plant signal transduction. *Trends Plant Sci.* 5, 67–74.

Warner JB & Lolkema JS (2003) CcpA-dependent carbon catabolite repression in bacteria. *Microbiol. Mol. Biol. Rev.* 67, 475–490.

West AH & Stock AM (2001) Histidine kinases and response regulator proteins in two-component signaling systems. *Trends Biochem. Sci.* 26, 369–376.

Wilson DN & Nierhaus KH (2007) The weird and wonderful world of bacterial ribosome regulation. *Crit. Rev. Biochem. Mol. Biol.* 42, 187–219.

Wolanin PM, Thomason PA & Stock JB (2002) Histidine protein kinases: key signal transducers outside of the animal kingdom. *Genome Biol.* 3, review S3013.