MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Dec. 2006, p. 910–938 Vol. 70, No. 4
1092-2172/06/$08.00+0 doi:10.1128/MMBR.00020-06

Stimulus Perception in Bacterial Signal-Transducing Histidine Kinases

Thorsten Mascher,[1]* John D. Helmann,[2] and Gottfried Unden[3]*

Department of General Microbiology, Georg-August-University Göttingen, D-37077 Göttingen, Germany[1]; Department of Microbiology, Cornell University, Ithaca, New York 14853-8101[2]; and Institute of Microbiology and Wine Research, Johannes-Gutenberg-University Mainz, D-55099 Mainz, Germany[3]

INTRODUCTION

Life in the microbial world is characterized by continuous interactions between the bacterial cell and its environment. The ability of a bacterium to monitor environmental parameters, including osmotic activity and ionic strength, pH, temperature, and the concentrations of nutrients and harmful compounds, is a prerequisite for survival. For that purpose, bacteria have evolved surface-exposed signal transduction systems, typically comprised of transmembrane (TM) proteins that channel the input from sensory modules to intracellular responses. These TM signaling systems include the chemotaxis receptors, anti-σ:σ factor pairs, Ser/Thr protein kinases, and histidine protein kinases together with their cognate response regulators. This review focuses on the classical two-component systems (TCS) consisting of a usually membrane-bound sensor histidine protein kinase (HK) and a response regulator (RR), most often mediating differential gene expression (103, 108).

TCS allow adaptational responses to a huge variety of environmental stimuli, based on a simple modular system. Both proteins consist of (at least) two distinct domains. The HK harbors an N-terminal input domain that senses a specific

* Corresponding author. Mailing address for Thorsten Mascher: Georg-August-University, Dept. of General Microbiology, Grisebachstr. 8, D-37077 Göttingen, Germany. Phone: 49 (0) 551-3919862. Fax: 49 (0) 551-393808. E-mail: tmasche@gwdg.de. Mailing address for Gottfried Unden: Johannes-Gutenberg-University, Institute of Microbiology and Wine Research, Becherweg 15, D-55128 Mainz, Germany. Phone: 49 (0) 6131-39-23550. Fax: 49 (0) 6131-39-22695. E-mail: unden@uni-mainz.de.

stimulus, e.g., by binding or reacting with a signaling molecule or by interaction with a physical stimulus. The information is transduced through intramolecular conformational changes, resulting in the activation of the cytoplasmic transmitter domain (280). The transmitter, in turn, activates its cognate receiver, encoded by the N-terminal domain of the RR. The RR gives rise to the appropriate cellular response, which is mediated by the C-terminal effector (or output) domain of the RR through protein-protein interaction (e.g., chemotaxis) or protein-DNA interactions leading to differential gene expression.

The functional state of these two components is controlled by three phosphotransfer reactions: (i) the autophosphorylation of a conserved histidine in the transmitter domain of the sensor, (ii) the phosphotransfer to a conserved aspartate in the receiver domain of the RR (by the activity of the RR), and (iii) dephosphorylation of the RR to set the system back to the prestimulus state (202, 246). The phosphatase can be an intrinsic property (autophosphatase) of the RR or a phosphoprotein phosphatase activity of the kinase towards the regulator. However, external phosphatases are also common (129, 205, 206, 246). Some HKs show significant autophosphatase activity towards their own His-phosphate group. Additionally, some RRs also catalyze significant back transfer of the phosphate group to the corresponding His kinase (52).

With a few exceptions (i.e., *Mycoplasma* species), all bacterial genomes sequenced so far harbor multiple copies of genes encoding TCS. Typically, the structural genes for the HK and the cognate RR are organized in operons. In some bacteria, however, the two-component systems show an orphan organization at the gene level which impedes assigning sensor/regulator pairs and the identification of stimuli. This problem is prominent in *Myxococcus xanthus*, which contains a large number of TCS (146 HKs, including hybrid kinases), many of which are important for the control of complex differentiation programs such as fruiting body morphogenesis. More than 50% of the structural genes for the HKs are orphans and are separated by two or more genes from those of the next RR (238).

While some systems have been studied in great detail (most notably the paradigmatic systems EnvZ/OmpR, CheA/CheY, and NtrB/NtrC in *Escherichia coli*) (25, 109, 187) and transcriptome approaches have allowed initial genome-wide investigations on some TCS, many are still uncharacterized. Genome-wide sequence analyses to identify TCS have been performed for many bacteria, including *Bacillus subtilis* (59), *Escherichia coli* (178), *Pseudomonas aeruginosa* (223), *Corynebacterium glutamicum* (146), *Streptomcyes coelicolor* (107), and cyanobacteria (16). These analyses have been complemented, in some cases, by mutational and/or microarray approaches, as reported for *B. subtilis* (145), *Streptococcus pneumoniae* (153, 255), and *E. coli* (196, 286). It is anticipated that ongoing functional genomics approaches will rapidly advance our understanding of these large suites of sensory systems.

Sequence comparisons of TCS have been used to identify a number of conserved subfamilies. A structural classification of bacterial response regulators based on the diversity of output domains, domain architecture, and domain combinations was recently published (66). So far, comparisons of HK proteins have focused on their highly conserved intracellular catalytic (transmitter) domains (53, 87, 140). They show a homogenous composition of subdomains (or "boxes") and are generally cytoplasmically located (87, 280). A classification based on the H box of the kinase domain (containing the conserved site of autophosphorylation) was proposed by Fabret et al. (59) and has found widespread use. The most comprehensive and detailed sequence analysis, based on all six conserved boxes (the H, X, N, D, F, and G boxes) (see Fig. 2) in the transmitter domain (87), allows an even more precise subgrouping of HKs. A more recent classification (140), based on the sequence, organization, and predicted secondary structure of the H box, allowed the classification of archaeal HKs for the first time. These classification schemes are still evolving, as evidenced by the recent identification of a new subfamily of HKs, the HWE family (133). Although these analyses have focused specifically on the conserved features of the HK catalytic domain, they likely reflect the evolution of the TCS as a whole: in many cases specific subfamilies of HK are preferentially associated with specific subfamilies of RR proteins (87, 149). However, these classifications neglect functional aspects of the sensing and signal transduction process.

The principal biological function of TCS-mediated signal transduction manifests itself in the input (signal perception) and output (e.g., differential gene expression), rather than in the communication between its two components. Therefore, grouping HKs according to their input domains would reflect the biological role they play in the communication between a cell and its environment. While a number of novel conserved input domains were identified in HKs in recent years (5–7, 65, 67, 68, 186, 203, 288, 289), an overall classification based on the N-terminal sensor domains remains problematic, since these domains vary greatly in sequence, membrane topology, composition, and domain arrangement. All of these features have profound effects on sensing and signal transduction to the kinase domain. Therefore, this variability presumably reflects different principles in stimulus perception and processing, which are related to the topology and type of sensory domains but do not necessarily reflect the phylogenetic relationship of the sensor kinases. Consequently, a functional classification of HKs cannot be based on sequence alignments alone but rather requires the identification of domains and transmembrane helices and the prediction of the topological arrangement of these structures.

In order to arrange the large number of HKs according to functional aspects, the sensors are grouped here on the basis of their domain architecture, i.e., membrane topology, number of TM helices, and sequential arrangement of the sensory domain(s) within their N-terminal input domains. The available data clearly indicate that grouping HKs by these criteria is functionally related to the mechanism of sensing and signal transduction by the corresponding sensors but does not necessarily take into account phylogenetic aspects. Based on our analysis of the domain architecture and membrane topology of $\sim$ 4,500 sensor kinase sequences in the SMART database (229) (http://smart.embl-heidelberg.de/) and the published results on signal perception presented here, most (if not all) HKs fall into three major groups (Fig. 1).

The largest group, the periplasmic (or extracellular)-sensing HKs, includes proteins with an extracellular sensory domain which is framed by at least two TM helices (Fig. 1A). The kinase is localized in the cytoplasm (as for all other HKs). Thus, sensory and kinase domains are located in two different

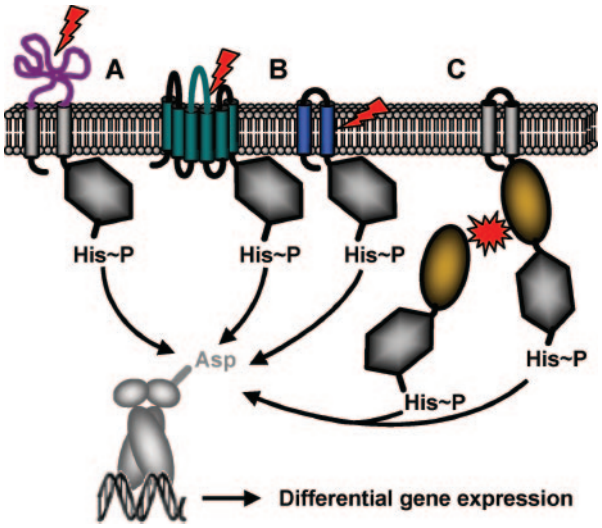

FIG. 1. Schematic representation of the three different mechanisms of stimulus perception. (A) Periplasmic-sensing HKs. (B) HKs with sensing mechanisms linked to the transmembrane regions (stimulus perception can occur either with the membrane-spanning helices alone or by combination of the transmembrane regions and short extracellular loops). (C) Cytoplasmic-sensing HKs (either soluble or membrane-anchored proteins). The stimulus is represented by a red arrow or red star. The parts of the proteins involved in stimulus perception are highlighted in color.

cellular compartments which are separated by a membrane, necessitating TM signal transduction. This type of membrane topology is typical for sensing solutes and nutrients.

The second group contains HKs with sensing mechanisms associated with the membrane-spanning helices. The unifying feature of this highly diverse group of sensor kinases is the presence of 2 to 20 transmembrane regions (TMR) implicated in signal perception. These TMR are connected by very short intra- or extracellular linkers; i.e., these sensors lack an obvious extracellular input domain (Fig. 1B). Therefore, the stimuli sensed either are membrane associated or occur directly within the membrane interface. Stimuli from within the membrane include the mechanical properties of the cell envelope (such as turgor or mechanical stress) or are derived from membrane-bound enzymes or other membrane-integral components. Other membrane-related stimuli include ion or electrochemical gradients, transport processes, and the presence of compounds that affect cell envelope integrity. Most quorum sensors from gram-positive bacteria also fall into this category. For the latter group, two of the TMR are connected by a short (20 to 50 amino acid residues) intra- or extracellular linker, which seems to be involved in stimulus perception. Signal transfer occurs from the membrane to the cytoplasmic kinase domain.

The third (and second-largest) group of sensor kinases, the cytoplasmic-sensing HKs, includes either membrane-anchored or soluble proteins with their input domains inside the cytoplasm (Fig. 1C). This class of sensor proteins detects the presence of cytoplasmic solutes or of proteins signaling the metabolic or developmental state of the cell or of the cell cycle. Other cytoplasmic TCS respond to diffusible or internal stimuli, such as O_2 or H_2, or stimuli transmitted by TM sensors.

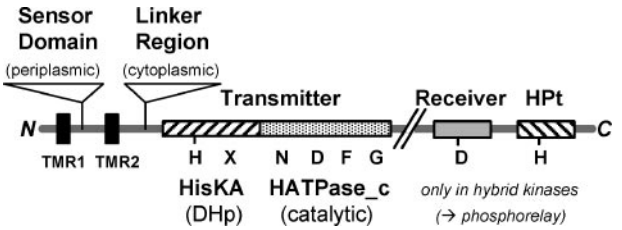

FIG. 2. Features, domains, and boxes of histidine protein kinases. The protein is symbolized by the gray line. The major domains are indicated by boxes, and their names are given above or below the line. Conserved boxes or amino acid residues are given below the line in one-letter code, according to the standard nomenclature (87, 280). The drawing is not to scale. See the text and Table 1 for details.

The subgroups within these three principal groups of HKs and their compositions of different sensory domains, as well as the stimulus perception mechanisms for well-characterized representatives from each group, will be the subject of the following sections of this review. It should be pointed out that all classes of sensors can contain, in addition to the principal features (Fig. 2), additional sensory or linker domains in various combinations. We also address the mechanism of intramolecular TM signal transduction and the occurrence and biological role of HKs that harbor more than one putative stimulus perception domain.

PERIPLASMIC-SENSING HISTIDINE KINASES

Periplasmic-sensing HKs represent the classical type and comprise the largest group of membrane-bound sensor kinases. As a definition and for reasons of simplicity, we use the term "periplasmic-sensing" HK throughout this review for all sensor kinases with a significantly large extracytoplasmic input domain, irrespective of their origin (i.e., HKs from both gram-negative and gram-positive bacteria). At present, databases contain about 2,500 members of this type. They consist of two regions: an N-terminal periplasmic sensing domain flanked by TM helices on either side (TMR1 and TMR2), followed by the C-terminal cytoplasmic transmitter domain (Fig. 2). The transmitter domain comprises a sequence with the conserved histidine residue for autophosphorylation (the H box) and ends with the highly conserved kinase (or catalytic) domain. The domain with the conserved His residue typically contains two α-helices (X box), which serve as a dimerization domain (DHp [dimerization and histidine phosphotransfer] or HisKA domain) (Table 1 and Fig. 2). The catalytic domain (HATPase) contains the conserved N, D, F, and G boxes with the respective highly conserved amino acid residues. This domain catalyzes autophosphorylation of the HKs. The RRs then catalyze their own phosphorylation, with the phosphoryl-HK as the phosphodonor (279, 280). In some cases, low-molecular-weight donors such as acetyl phosphate or, in vitro, also phosphoramidates can be used for RR phosphorylation (45, 158).

This prototypic domain organization can be varied by inclusion of "linker" regions, such as the HAMP or PAS domain (12, 276), between TMR2 and the transmitter domain or by additional phosphorylation domains downstream of the transmitter domain (Fig. 2). The linker domains vary considerably in size, extent, and type. The additional phosphorylation do-

TABLE 1. Names, term definitions, and features of conserved signaling and sensory domains

Domain[a]	Name origin	Localization	Size (aa)	Characteristics, conserved features, and/or remarks	Reference(s)
Signal transduction domains					
HAMP	Found in *H*K, *a*denylyl cyclase, *m*ethyl-carrier protein, *p*hosphatase	Cytoplasmic	~50	2 α-helices (coiled-coil structure); important linker domain for signal transduction from periplasmic input to cytoplasmic kinase domain; little sequence conservation	12, 276
HATPase_c	*H*istidine kinase-type *ATP*ase *c*atalytic domain	Cytoplasmic	~140	Catalytic domain of HK; phosphoryl transfer from ATP to HisKA domain; harbors the conserved N, D, F, and G boxes	87, 279, 280
HisKA/DHp	*His*tidine *k*inase *A* domain *d*imerization/*H*is *p*hosphotransfer	Cytoplasmic	~80	Dimerization and phosphoacceptor domain of HK; harbors the H box with the invariant histidine residue, the site of autophosphorylation in HK	87, 279, 280
HPt	*H*istidine *p*hospho*t*ransfer	Cytoplasmic	~100	Present at the N terminus in proteins which undergo autophosphorylation; contains an active histidine residue that mediates the phosphotransfer reactions	169
MA	*M*ethyl-*a*ccepting chemotaxis domain	Cytoplasmic	~260	Undergoes reversible methylation (at specific Glu residues) in response to attractants or repellants during bacterial chemotaxis; interacts with CheA-like HKs and CheW	27, 195, 222
REC	*Rec*eiver domain	Cytoplasmic	~100	N-terminal receiver domain of a response regulator; contains a phosphoacceptor site (an invariant aspartate residue) that is phosphorylated by HK proteins	200
Sensory input domains					
5TMR-LYT	*5 TM r*eceptor domain, *Lyt*S-like	Intramembrane	~90	Input domain with 5 TMR found in LytS-YhcK type HKs; little sequence conservation	5
7TMR-DISM	*7 TM r*eceptors with *d*iverse *i*ntracellular *s*ignaling *m*odules	Intramembrane	~200	Input domain with 7 TMR; little sequence conservation	5
CACHE	Found in Ca^{2+} channels and *che*motaxis receptors	Periplasmic	~80	Implicated in small-molecule binding	6
CHASE-CHASE6	*C*yclase/*H*is kinase-*a*ssociated *s*ensing *e*xtracellular	Periplasmic	150–300	Six individual groups of conserved putative periplasmic-sensing domains; predicted to sense diverse stimuli such as amino acids, peptides, cytokines, and turgor	7, 288
DISMED2	*DISM e*xtracellular *d*omain 2	Periplasmic	~130	Putative carbohydrate-binding domain at the N termini of most 7TMR-DISM sensor kinases; predicted to adopt an all-β-fold with a jellyroll topology	5
GAF	Found in c*G*MP phosphodiesterase, *a*denylyl cyclase, *F*hlA	Cytoplasmic	~150	One of the largest families of small-molecule binding units; PAS-like fold; predicted to bind cyclic nucleotides, such as cGMP/cAMP	13
KdpD	Domain found in *KdpD*-like HKs	Cytoplasmic	~210	Sensor domain of osmosensitive K^+ channel HKs; possibly the input domain responsible for sensing turgor pressure	267
MASE1	*M*embrane-*a*ssociated *se*nsor domain 1	Intramembrane	~280	8-TMR integral-membrane domain, with conserved residues (3 Pro, 3 Trp intrahelical); stimuli unknown; alternative name, 8TMR-UT	5, 186

Continued on following page

TABLE 1—*Continued*

Domain[a]	Name origin	Localization	Size (aa)	Characteristics, conserved features, and/or remarks	Reference(s)
MCP	*M*ethyl-accepting *c*hemotaxis *p*rotein	Transmembrane	500–600	TM sensor proteins consisting of a periplasmic input domain (CACHE, PAS, GAF, or TarH) and usually two cytoplasmic domains (HAMP and MA)	239, 244, 278
MHYT	Conserved *M*et, *H*is, Tyr, and *T*hr residues	Intramembrane	~190	6 TMR with short linkers; characteristic intrahelical MH(YT) motif in TMR2, TMR4, and TMR6; implicated in binding metals (e.g., copper)	67
PAS	Initially identified in the *P*ER, *A*RNT, and *S*IM proteins	Cytoplasmic	~110	Conserved and widely distributed redox sensor domain; binds heme, flavin, and adenine and senses light and oxygen (among others); often occurs duplicated and/or together with a C-terminal extension (PAC domain)	254, 289
PBPb	*P*eriplasmic solute-*b*inding *p*roteins, *b*acterial	Periplasmic	~220	Thought to directly bind substrate (amino acids or opines) close to the inner membrane; typical feature of BvgS-like HKs; often occurs duplicated	252
Phytochrome	Sensor of bacteriophytochromes	Cytoplasmic	~180	Photochromic photoreceptors that employ a bilin-type chromophore to act as a red/far-red-regulated reversible photoreceptor; bind linear tetrapyrroles	135, 265
Reg_prop	*Reg*ulatory *prop*eller	Periplasmic	~600	14 tandem repeats of 14 aa each form 2 7-bladed β-propellers	63
SS(S)F	*S*odium/solute *s*ymporter *f*amily	Intramembrane	~400	13 TMR; catalyze the uptake of a wide variety of solutes (including sugars, proline, and iodide) by sodium motive force	124, 125
TarH	*Tar-h*omologous domain	Periplasmic	~150	Ligand-binding domains of chemotaxis receptors (MCP); bind a huge variety of low-molecular-weight solutes such as amino acids, sugars (as attractants), and metal ions (as repellents)	172
USP	*U*niversal *s*tress *p*rotein family	Cytoplasmic	~130	Occur in KdpD-like HKs; conserved α/β-fold; function unclear (ATP binding?)	152, 236

[a] Names of domains derived from the SMART or Pfam database.

mains comprise receiver domains typical for RRs, with a conserved Asp residue for phosphorylation and an additional transmitter (histidine-containing phosphotransfer [HPt]) domain (Fig. 2). "Hybrid" kinases of this type constitute a phosphorelay, predominantly in gram-negative bacteria (285), whereas phosphorelay systems of gram-positive bacteria, such as the regulatory cascade of sporulation initiation in *B. subtilis* (58), normally consist of individual proteins mediating the stepwise phosphotransfer.

Sensor kinases sharing the prototypical architecture form a large and highly diverse group with regard to composition and function of the sensory or input domain (Fig. 3). The HKs are grouped primarily based on features of the periplasmic sensing domains, to reflect functional and sensory principles. The linker domain will be used in specific cases as an additional criterion for subgrouping, whereas transmitter domains will not be considered.

Despite a great diversity in sequence and stimulus specificity, the input domains of many sensors can be grouped by sequence alignment into a few families (Fig. 3 and Table 2). Most of the conserved domains (5–7, 67, 68, 186, 203, 288) are found also in other signaling and sensing systems. The functions of only a limited number of such sensing domains of HKs has been elucidated, in particular those of periplasmic (CitA, DcuS, and PhoQ) and cytoplasmic (FixL) PAS domains (40, 79, 85, 137, 144, 201, 219). Others are defined only by means of sequence conservation (such as the CHASE domains), and their functions and stimuli remain to be identified (65). Additionally, many HKs, including paradigmatic HKs such as EnvZ, do not contain conserved features in their periplasmic domains. Corresponding to the diversity of sensory domains, many different mechanisms of stimulus perception and processing can be anticipated, although these are mostly unknown. There are, however, a few well characterized examples that can serve as models.

Perhaps the simplest mechanism for signal detection by periplasmic-sensing HKs occurs by direct interaction between the sensor domain and a chemically defined small molecule, such as nitrate/nitrite for NarX or citrate for CitA (115, 242). For some of the periplasmic-sensing HKs, the binding site and structure of the

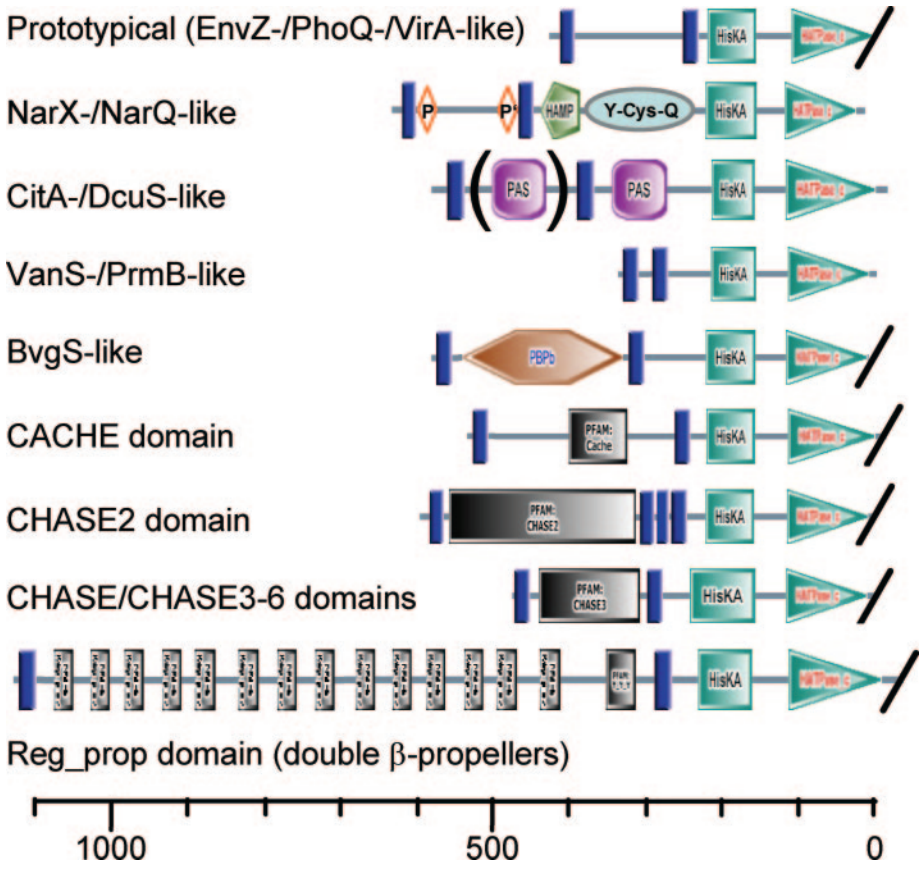

FIG. 3. Domain architecture of periplasmic-sensing histidine kinases. The figure is based on the graphical output of the SMART web interface at http://smart.embl-heidelberg.de (229), with modifications. The scale bar is in amino acids. Blue vertical bars represent putative transmembrane helices. Sizes and positions of conserved domains are indicated by the labeled symbols. Note that the transmitter domains are simplified, and as a default, only the HisKA and HATPase_c domains are shown. Additional cytoplasmic domains are possible and widespread but were ignored in all but obligatory cases (i.e., PAS domain for CitA-like HKs and HAMP domain for NarXQ-like HKs). A diagonal bar at the C terminus of the transmitter domain indicates the possible occurrence of hybrid kinases in that subgroup of sensor kinases. The periplasmic PAS domain of CitA/DcuS (in parentheses) is conserved by three-dimensional structure only and not by sequence. It is therefore not detectable by sequence analysis. VanS/PrmB-like proteins are described in the "Intramembrane-Sensing HKs: Cell Envelope Stress Sensors with Two TMR (LiaS/BceS-Like HKs)" section. See the text for details.

periplasmic binding domain has been determined (see below). In other cases, chemical stimuli are sensed indirectly through interaction with a periplasmic solute-binding protein, such as glucose by the sugar-binding protein ChvE for interaction with the *Agrobacterium tumefaciens* VirA sensor kinase (234). Alternatively, signaling may be triggered by mechanical, electrochemical, or concentration gradient stimuli, resulting in a conformational change of the input domain, as has been hypothesized for osmolarity or turgor sensors. There is also growing evidence that other periplasmic-sensing TCS use additional proteins, which transmit a primary signal to the HK, thereby complicating the identification of the primary stimulus. For most TCS the exact type of stimulus is not known. In the following descriptions, we will concentrate on characterizing general properties of the families, which will be exemplified by a few prominent members.

Prototypical Periplasmic-Sensing Histidine Kinases

Prototypical periplasmic-sensing histidine kinases are composed of two TM helices with an intervening extracytoplasmic domain of 50 to 300 amino acids (aa), lacking large cytoplasmic linker regions. The sequences of the extracytoplasmic regions of most prototypical periplasmic-sensing HKs reveal no conserved or known sensing domains. Structural analysis of the PhoQ periplasmic domain revealed, however, a PAS domain, which was not recognized by sequence analysis (similar to the PAS domains of CitA and DcuS [40]). The other classes of periplasmic-sensing HKs are characterized by the presence of defined types of sensory domains in the periplasm and by the presence of extended linker domains. EnvZ, PhoQ, TorS, and VirA are important members of the prototypical HKs and will be discussed in more detail.

EnvZ, together with its cognate RR OmpR, plays a central role in the adaptation of *E. coli* to changes in extracellular osmolarity. EnvZ is one of the best-understood HKs, and studies of this protein have contributed enormously to our understanding of dimerization and phosphorylation reactions in membrane-bound HKs. The structures of the transmitter subdomains were solved by nuclear magnetic resonance (253, 257). The HisKA or DHp subdomain consists of two α helices that dimerize to form a four-helix bundle, which represents the

TABLE 2. Groups of bacterial histidine kinases according to the domain architectures of their sensing domains

Histidine kinase group	Transmembrane region (N terminal)		Cytoplasmic region (C terminal)		No. of kinases[e]	Bacterial group[f]	Reference(s)
	Architecture[a]	Length (aa)[b]	Architecture[c]	HK class[d]			
Periplasmic sensing					~2,500		
Prototypical sensors	TMR-(50–300 aa)-TMR	100–350	(HAMP)-HK-(REC-HPt)	NA[g]	<1,000	NA	18, 40, 139, 284
NarX/Q-like[h]	TMR-P-60 aa-P′-TMR	~180	HAMP-Y(Cys)Q-HK	HPK7	30	Proteobacteria	35, 242
CitA/DcuS-like	TMR-"PAS"-TMR	~200	PAS-HK	HPK5	20	Proteobacteria	144, 201, 219
VirA-like	TMR-200 aa-TMR	~300	HK-REC	HPK4	20	*Agrobacterium*	36, 234, 235
VanS-like	TMR-(25–30 aa)-TMR	~90	HK	HPK1a	10–20	Actinobacteria	104, 106
PrmB-like	TMR-(30–40 aa)-TMR	~90	HAMP-HK	HPK2a	10–20	Proteobacteria	281
PBPb sensors (BvgS-like)	TMR-PBPb$_{1–3}$-TMR	300–600	(PAS-PAC)-HK-(REC-HPt)	HPK1b	50	Proteobacteria	26, 148, 252
CACHE sensors	TMR-100-CACHE-50-TMR	~300	HK	NA[i]	3	NA[i]	6
CHASE-CHASE6 sensors	TMR-CHASE-TMR$_{1/3}$	~380	(HAMP)-(PAS-PAC)-HK	HPK1a/b	100[j]	NA[j]	7, 288
Reg-prop sensors	TMR-2βprop-(YYY)-TMR	~1,000	HK-REC-AraC-HTH	HPK1a	40	*Bacteroides*	63
TMR associated					~800		
LiaS-like	TMR-(5–25 aa)-TMR	~70	(HAMP)-HK	HPK7	20	*Firmicutes*	120, 151, 166, 167
BceS-like	TMR-(5–10 aa)-TMR	~60	HK	HPK3i	70	*Firmicutes*	166, 167, 194
DesK-like	4/5 TMR	~150	HK	HPK7	20	*Firmicutes*	3
RegB-like	3TMR-RB-3TMR	~200	HKcys	HPK3e	30	Proteobacteria	55
ComD/AgrC-like	6/7 TMR	~210	HK	HPK10	30	*Firmicutes*	98, 119, 160–162
ComP-like	8/10 TMR	300–350	HK	HPK7	10	*Firmicutes*	208
LuxN-like	8/10 TMR	~300	HK-REC	HPK4	10	*Vibrio*	62
PutP/CbrA-like (SSSF containing)	12–20 TMR	400–600	(PAS)-HK	HPK3d	30	Proteobacteria	124, 125
MHYT sensors	6 TMR	~300	HK-(REC-Hpt)	NA[i]	3	NA[i]	67
UhpB-like	MASE1	~300	HK	HPK7	20	Proteobacteria	130
MASE sensors	MASE1	250–400	(PAS-PAC)-HK-REC	HPK1b	10	Proteobacteria	5, 186
7TMR-DISM-like	TMR-(DISMED2)-7TMR	~200/400	HK-(REC)	HPK1b	30	Proteobacteria/ spirochetes	5
LytS-like	6TMR (TMR-5TMR-LYT)	~200	GAF-HK	HPK8	40	*Firmicutes*/ proteobacteria	5
Cytoplasmic sensing[k]					~1,600		65, 88
KdpD-like (membrane-anchored, N-terminal input)	KdpD-Usp-4TMR	~500	GAF-HK	HPK1a	100	*Firmicutes*/ proteobacteria	267
ArcB-like (membrane anchored, C-terminal input)	TMR-(10–20 aa)-TMR	~80	Leucine zipper-PAS-HK-REC-Hpt	HPK1b	20	Proteobacteria	163
FixL-like (membrane-anchored, C-terminal input)	2/3 TMR[m]	~100[m]	PAS-PAC-(PAS-PAC)-HK	HPK4	30	Proteobacteria	77, 79
CheA-like (soluble)			HPT-HK-CheW	HPK9	150	Proteobacteria	243, 244, 250, 278
Phytochrome sensors[l] (soluble)			PAS-GAF-phytochrome-HK	HPK3h	20		133, 135, 265

Continued on following page

TABLE 2—*Continued*

[a] Conserved domains were identified using the SMART tool (229). Sizes are averages and can vary greatly. TMR are putative. RB, RegB box; P/P′, boxes defined by Stewart (242); 2β-prop, two seven-bladed sensor-specific β-propellers, based on the presence of 14 Reg_prop domains (Pfam entry), arranged in tandem repeats. All other domains are as defined in the original publications or the SMART, Interpro, or Pfam database entries (see the text and references therein).
[b] Average size of the input domain, including all (flanking) transmembrane regions.
[c] Conserved domains were identified using the SMART tool (229). HK, histidine kinase (consisting of the SMART HisKA and HATPase_c domains); HKcys, histidine kinase domain bearing additional conserved (redox-active) cysteine residues; REC, receiver domain. All other domains are according to database entries or previous publications (see the text and references).
[d] Assignment is derived from multiple-sequence alignments of each group, based on the histidine kinase classification system of Grebe and Stock (87).
[e] Due to the dynamics in the availability of sequence information in the databases, the numbers given should be viewed as rough estimates, indicative only of the distribution and general importance of each group of sensor kinases.
[f] Predominant bacterial groups are given only if <70% of the corresponding sequences are derived from one phylum.
[g] NA, not applicable.
[h] According to Stewart (242), NarX- and NarQ-like sensors differ in the absence or presence of the cysteine-rich central domain, respectively.
[i] NA due to the small number of HKs containing these domains.
[j] CHASE to CHASE6 domain-containing sensors form six independent subgroups, belonging to different HK classes from different groups of organisms. Therefore, no details can be given for these kinases as a whole.
[k] Of the extremely diverse group of cytoplasmic-sensing HKs, only individual prominent examples are represented in this table. See the text for details. There are ~1,200 soluble kinases.
[l] There are 110 additional phytochrome sensors in plants.
[m] A number of FixL-like kinases are soluble proteins, lacking the TMR.

core of the transmitter domain (53, 257). The phospho-accepting His residue protrudes from the helices and is accessible to phosphorylation from the surface by the C-terminal catalytic HATPase subdomain. Thus, EnvZ is a functional dimer, with both the cytoplasmic transmitter and the periplasmic domains contributing to dimerization. The structures of the HisKA/DHp and HATPase domains gave important insights into the functions of the individual domains. Recently, the structure of the entire cytoplasmic transmitter, including the HAMP linker domain, of an HK was solved (165), which will help us to understand how the signal from TM helix 2 is received by the cytoplasmic domain and transferred to the kinase domain.

Despite the wealth of knowledge gained over the years on the function of this archetypical TCS, little is known about the mechanism of osmosensing by EnvZ. A close homolog, EnvZ of *Xenorhabdus nematophilus*, completely lacks a periplasmic domain [see "Intramembrane-Sensing HKs: Cell Envelope Stress Sensors with Two TMR (LiaS/BceS-Like HKs)"] but is still able to complement an *E. coli envZ* null mutant (251). In *E. coli* EnvZ, partial deletions of the periplasmic domain, or even a complete replacement with the periplasmic region of the nonhomologous PhoR of *B. subtilis*, did not significantly alter the process of osmosensing (156). These results call into question a direct and essential role of the periplasmic domain of *E. coli* EnvZ in osmosensing. Work on the yeast osmosensor Sln1 suggests that these kinases sense turgor as the key input stimulus. A systematic deletion/replacement analysis of the periplasmic domain of Sln1 suggests that only the integrity of the periplasmic domain as a whole is necessary for osmosensing, rather than specific amino acids sequence or regions (220). This work complements and supports the results obtained for *E. coli* EnvZ.

The PhoQ/PhoP TCS is important for the control of pathogenesis of *Salmonella* and other gram-negative bacteria. Multicellular organisms inhibit invading bacteria by use of cationic antimicrobial peptides, which contain a positive net charge and an amphipathic structure for interaction with negatively charged biological membranes (43). The bacteria acquire resistance to the antimicrobial peptides by modifying the cell surface, in particular lipopolysaccharide and lipid A, which are modified in antimicrobial peptide-resistant strains (174). The PhoQP TCS regulates modification of lipid A and other virulence factors, including those for antimicrobial peptide resistance (61, 91, 174, 217).

The sensor kinase PhoQ is activated at low concentrations of cations, such as Mg^{2+}, and by increasing concentrations of the antimicrobial peptides (i.e., during invasion of macrophages by the bacteria) but is repressed by high concentrations of divalent cations (70). Thus, these effectors have opposing effects on PhoQ function (Fig. 4). The crystal structure of the periplasmic PhoQ domain was determined in the Ca^{2+} bound state (40). The periplasmic domain belongs to the PAS domain family, despite an apparent lack of sequence similarity to PAS domain proteins. The structure matches that of CitA and DcuS (see below), but there is an insertion of two α-helices in the PAS fold. This insertion creates a flat and negatively charged surface on one side of the protein, which is derived from Glu and Asp residues. Unlike the PAS domains of DcuS and CitA, the PhoQ periplasmic domain contains no cavity or discrete binding pocket for ligand binding. It is proposed that binding of the antimicrobial peptides and Mg^{2+} occurs at the acidic surface at the membrane-proximal side of the protein, which is in close contact to the lipid surface of the cytoplasmic membrane (Fig. 4A). The acidic surface binds at least three Mg^{2+} ions, which are proposed to form cation bridges between the acidic region of PhoQ and the acidic membrane phospholipids (40) (Fig. 4A).

Ionic interactions tether the periplasmic domain of PhoQ to the membrane. In this state, the kinase domain of PhoQ is inactive (18, 40). The binding of antimicrobial peptides is suggested to displace the cations and to disrupt the interaction between PhoQ and the membrane. The antimicrobial peptides are suggested to function as a lever (Fig. 4B), lifting the periplasmic domain off the membrane. The structural distortion could be transmitted mechanically to the TM helices, with a resultant motion of the TM helices, resulting in the autophosphorylation of PhoQ. Thus, the stimuli of PhoQ (antimicrobial peptides and divalent cations) are not bound at a distinct binding pocket but function by resolving and forming interactions between PhoQ and the membrane surface (18).

Other prototypic periplasmic-sensing HKs, i.e., VirA, TorS,

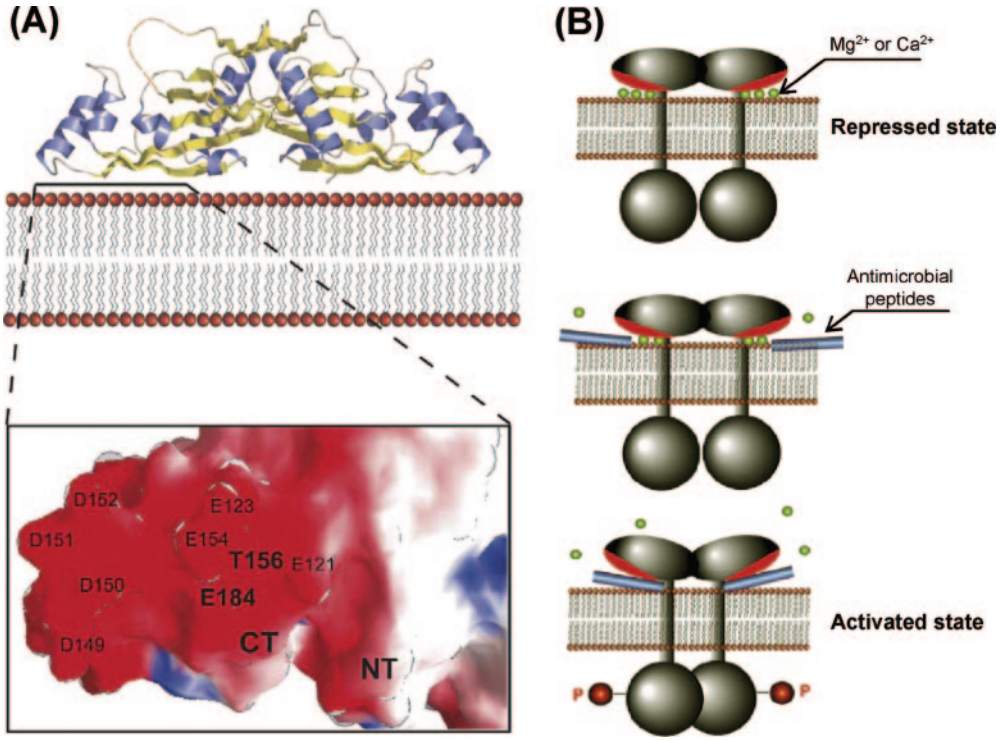

FIG. 4. Structure of the periplasmic input domain of PhoQ and model for the sensing mechanism of cations and antimicrobial peptides. (A) The crystal structure and charge profile of the surface facing the outer side of the cytoplasmic membrane are shown on the left. The residues important for coordinating the divalent metal ions are shown. The crystal structure of the dimeric PhoQ sensor domain (upper panel) forms a flat surface that comes in close contact to the membrane. The bottom part of this domain contains a highly negatively charged surface that participates in metal binding (lower panel, view from the membrane). Red represents negatively charged residues. NT, N terminus; CT, C terminus. (B) Working model for the competitive binding of Mg^{2+} and cationic antimicrobial peptides to PhoQ. Divalent cations, such as Ca^{2+} or Mg^{2+} (shown as green balls), bind to the acidic surface (red) and repress PhoQ activity by locking the PhoQ sensor domain in an inactive conformation (top panel). Cationic antimicrobial peptides interact with membrane phospholipids, thereby coming in close contact with the Ca^{2+} and Mg^{2+} binding sites of PhoQ. They compete with and displace divalent cations from PhoQ (middle panel). This provokes a conformational change of the input domain, which leads to autophosphorylation of the transmitter domain and thereby activation of PhoQ (lower panel). See the text for details. (Reprinted from reference 18 with permission from Elsevier.)

and BctE, sense stimuli by interaction of their periplasmic domains with other proteins in the same compartment. These proteins are often solute-binding proteins, which are part of binding-protein-dependent transport systems. Periplasmic solute-binding proteins are also used for sensing by methyl-accepting chemotaxis proteins (MCP) in bacterial chemotaxis (147, 252).

Expression of virulence (*vir*) genes in the gram-negative plant pathogen *Agrobacterium tumefaciens* is regulated by the VirAG TCS in response to acidic pH. The decrease in pH is caused by phenolic compounds (such as acetosyringone) that are released by wounded plant cells (reviewed in references 99 and 287). In addition, aldose monosaccharides (e.g., arabinose) exuded from wounded plant sites serve as strong enhancers of phenolic-induced HK activity (34, 235). The sugar is sensed by binding to the periplasmic sugar-binding protein ChvE, which in turn interacts with the 220-aa-long periplasmic input domain of VirA (36, 234). Direct sensing of phenolic compounds by VirA occurs in the cytoplasmic linker region between TMR2 and the HisKA domain, i.e., at a site different from that of ChvE interaction (20, 36, 49, 204, 277). Recently, it was established that the periplasmic domain is also involved in pH sensing (69).

The TorS/TorR two-component system of *E. coli* controls the expression of the *torCAD* operon, encoding the periplasmic trimethyl amine-*N*-oxide (TMAO) reductase (TorA), a TorA-specific chaperone (TorD), and the *c*-type cytochrome TorC (171). TorC is a membrane-bound protein and carries the catalytic domain containing pentaheme *c* on the periplasmic surface of the membrane. TorC interacts with the catalytic domain of TorA in the periplasm and forms a functional TorC-TorA respiratory complex of TMAO reductase (83). TorS detects TMAO presumably by its periplasmic region (123) and stimulates the expression of the *torCAD* operon. In addition, apoTorC lacking the heme C groups binds to the sensor TorS and negatively regulates kinase activity (i.e., when it is inactive and not able to form an active TorC-TorA respiratory complex). This direct protein-protein interaction involves the C-terminal part of TorC and the periplasmic domain of TorS (83).

Bordetella pertussis uses the BctDE TCS for controlling the expression of a citrate uptake system during growth on citrate (9). The citrate carrier is a tripartite tricarboxylate transporter, consisting of the BctAB membrane carrier proteins. BctC is an extracytoplasmic citrate-binding protein and represents the third component of the tripartite tricarboxylate transporter system. BctE is a prototypical HK with two TM helices and requires BctC for response to the citrate. Citrate-liganded

BctC interacts with the periplasmic sensing domain of BctE and controls the functional state of the sensor.

NarX/NarQ-Like Sensors of Environmental Nitrate and Nitrite

A second, well-characterized group of periplasmic-sensing HKs is represented by the NarX-NarQ-like sensors, which contain defined "boxes" or domains in the periplasm and extended linker domains between TMR2 and the transmitter domain (Fig. 3). In proteobacteria, anaerobic respiratory gene expression is controlled by one of two nitrate reductase (Nar) TCS, NarXL or NarQP, in response to environmental nitrate and nitrite. *E. coli* and *Salmonella enterica* contain both paralogs. The concentration of the two respiratory oxidants is sensed through ligand binding by the periplasmic domain (length, 115 aa) of the two corresponding HKs, NarX and NarQ. Multiple sequence alignments reveal two conserved stretches of 18 amino acid residues in length that flank each periplasmic side of the two TM helices (P and P′ boxes) (Fig. 3) (242). Alanine substitutions of highly conserved residues in the P box (but not in P′) strongly affected signal detection and were able to render NarX and NarQ in a constitutively active ("locked-on") or inactive ("locked-off") form (35, 242, 275). In addition to the conserved extracellular boxes, both types of Nar sensors have an extended cytoplasmic linker region. In the linker region, "locked-on" mutations that are dominant over "locked-off" mutations in the P box were identified (35, 131). Therefore, it was proposed that signal processing requires nitrate (or nitrite) detection in the periplasm by the P box, followed by signal transfer across the membrane and to the kinase, with the latter depending on transmission by the linker region. The linker region consists of a HAMP domain (12) and an unusual "Y-Cys-Q" module in front of the HisKA domain. Similarly to other HAMP linkers, the sequence immediately follows TMR2 and is predicted to form two short amphipathic α-helices, which are joined by an unstructured connector. Mutations in the HAMP linker disturb the function of NarX and NarQ, and it was concluded that the HAMP linker is required for proper signal transduction (10, 11). The central "Y-Cys-Q" module consists of three parts: (i) the Y box, a leucine-zipper like domain of 32 amino acids in length; (ii) the central cysteine cluster, which is present in NarX but missing in NarQ-like HKs; and (iii) the Q linker, which is reminiscent of glutamine-rich flexible interdomain linkers (Fig. 3) (242). While deletion and alanine replacement mutagenesis could establish a role of the Y box and Q linker in intramolecular signal transduction, no phenotype was observed in mutants lacking one or more of the conserved cysteine residues of the central region (242). The response regulator NarL is a member of the FixJ/LuxR family and is structurally and functionally well characterized (19). Taken together, the NarXQ-like HKs are distinct from most other periplasmic-sensing kinases due to conserved periplasmic and cytoplasmic domains involved in signal detection and transduction.

Citrate and C_4-Dicarboxylate Sensor Proteins CitA and DcuS

CitA/DcuS-like HKs respond to the environmental concentrations of citrate (CitA) or C_4-dicarboxylates and citrate (DcuS) and, together with their cognate RRs CitB/DcuR, regulate genes encoding carriers and enzymes for the degradation of externally supplied citrate or C_4-dicarboxylates, in particular under anaerobic growth conditions. Metabolism of endogenously produced citrate or C_4-dicarboxylates is not regulated by CitA/DcuS. In binding studies, the periplasmic domains of CitA of *Klebsiella pneumoniae* and *E. coli* function as high-affinity citrate receptors with K_D values in the micromolar range (136). The molecular interactions between the sensor domain and its ligand have been elucidated through the cocrystal structure of the periplasmic domain of CitA of *K. pneumoniae* and mutagenesis (Fig. 5) (75, 137, 219). The structure of the corresponding domain of the C_4-dicarboxylate sensor DcuS of *E. coli* was determined in solution by nuclear magnetic resonance (Fig. 5) (201). The extracellular input domains of both sensors adopt a PAS-like fold with a core structure consisting of four or five β-strands, which is flanked by three N-terminal α-helices and one C-terminal α-helix. There is only one small α-helix located within the β-strands (201). The nearest structural neighbor is the PAS domain of the photoactive yellow protein from *Halorhodospira halophila* (28), which has a similar tertiary structure but shows no obvious sequence similarity. The β-strand structure

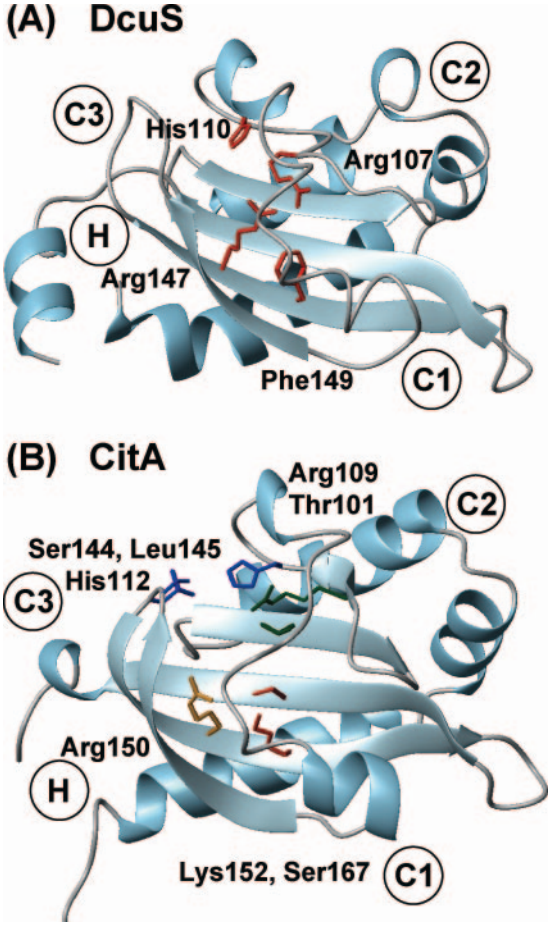

FIG. 5. Structures of the periplasmic sensing domains of DcuS (A) and CitA (B). The structures for the periplasmic domains of CitA and DcuS are derived from http://www.rcsb.org. The residues required for C_4-dicarboxylate sensing by DcuS (144, 201) and direct binding of citrate to CitA (75, 219) are shown. The corresponding sites are highlighted in the structure.

forms the bottom of the citrate/C_4-dicarboxylate binding pocket, which is framed by the α-helices. Citrate is bound at three carboxylate-binding sites, C1 to C3 (Fig. 5). Each of the carboxylic groups is liganded by one basic amino acid residue (Lys, Arg, or His) and at least one further residue with a hydroxyl side chain (Ser and Thr). The residues form extensive hydrogen bonds to the carboxylic groups. The hydroxyl group of citrate is bound at the hydroxyl site by an Arg residue through hydrogen bonding. Replacement of most of the residues by mutation inactivates citrate binding and the function of CitA. The tight interaction and binding of citrate are reflected by high-affinity binding of H-citrate^{2-} to the isolated periplasmic domain (75, 137).

The C_4-dicarboxylate binding site of DcuS is known at lower resolution but shows remarkable similarity to that of CitA (144, 201). In contrast to CitA, DcuS has a broad substrate specificity and accepts all C_4-dicarboxylates; the dicarboxylic groups have to be present with a distance of 3.1 to 3.8 Å for efficient activation of DcuS. Small polar (i.e., hydroxyl, amino, or thiol) side groups at position C2 or C3 of the C_4-dicarboxylates, such as in malate, aspartate, or thiosuccinate, have no negative effect. The carboxylate groups are essential but can be replaced by a nitro group, such as in nitropropionate. Residues Arg_{107}, His_{110}, Arg_{147}, and Phe_{149} are essential for stimulation by the C_4-dicarboxylates (144) and are homologous to the basic residues at C1 to C3 and the hydroxyl (H) site of CitA. This suggests that for binding of the dicarboxylates, similar sites as for binding of the C_6-tricarboxylate citrate are required.

DcuS/CitA homologs are found in many different bacteria, mostly proteobacteria but also gram-positive bacteria (115). The DcuS homolog in *B. subtilis* (DctS or YdbF) represents an interesting variation (15). DctS has an extracytoplasmic domain (predicted to be 140 aa), which is similar in size to the domain of *E. coli* DcuS (135 aa). DctS, however, requires a periplasmic C_4-dicarboxylate-binding protein for sensing C_4-dicarboxylates (15), although the essential residues for C_4-dicarboxylate binding by *E. coli* DcuS (Arg_{107}, His_{110}, Phe_{120}, Arg_{147}, and Phe_{149}) are conserved (144).

As described above, the structures of subdomains have been determined for various membrane-bound HKs (CitA, DcuS, EnvZ, and PhoQ). However, for none of these sensors are high-resolution structures of all domains known, let alone the structure of the intact full-length protein. Therefore, detailed models for the mechanism of signal transduction in periplasmic-sensing HKs are lacking. Nevertheless, comparisons of ligand-bound and free periplasmic sensory domains enabled researchers to develop first ideas on the mode of ligand sensing and signal transduction across the membrane.

Proposed mode of signal perception and transduction by DcuS/CitA. Structural studies suggest that ligand binding may trigger conformational changes that are propagated into the TM helices, thereby presumably affecting kinase dimerization and/or activity. The N- and C-terminal ends of the periplasmic domains of DcuS and CitA consist of long α-helices, which protrude to the surface of the globular structure at the membrane-proximal side and presumably are directly continued in TM helices 1 and 2. In the CitA structure, determined with bound ligand (citrate), the N- and C-terminal helices have a nearly parallel arrangement. In contrast, the corresponding helices in DcuS have an inclined arrangement with respect to each other. This arrangement presumably does not reflect the situation in the intact protein and could be caused by the lack of the following TM helices 1 and 2 in the domain structure or by the lack of bound ligand. Conformational movements in the periplasmic domain upon binding of the stimulus may therefore be propagated into the TM helices, providing a mechanism for signal transduction into and across the membrane. As described above, the mechanism of signal transmission could be similar to the mode suggested for PhoQ, but the initial event is different. For CitA/DcuS, binding of the ligand is proposed to cause a conformational change in the periplasmic domain which is transmitted to the transmembrane helices. In contrast, PhoQ binding of the antimicrobial peptide is thought to lift the periplasmic domain from the membrane surface by displacing the Mg^{2+} ions. The movement could then be transmitted in a lever-like mechanism to the TM helices. Conformational movements in the TM helices could involve a piston stroke mechanism, as suggested for signal transfer in the MCP Tar (197). However, signal transfer by other mechanisms such as torsional movement, including rotation of the TM helices against each other, as suggested earlier for MCP (39), cannot be excluded.

Sensor Kinases with PBPb Sensing Domains

Periplasmic-sensing HKs with periplasmic binding-protein (PBPb) sensing domains are exemplified by BvgS and EvgS of *Bordetella* spp. and *E. coli*, respectively (21, 41, 148). The sensors harbor periplasmic domains reminiscent of high-affinity periplasmic solute-binding proteins that mediate substrate binding in ABC transporters. The PBPb domains (up to three can occur side by side in the periplasmic domain of a single HK of this type) are located between TMR1 and TMR2 (Fig. 3). On the basis of sequence similarities, the PBPb domains can be grouped in eight families that correlate with the nature of the substrate bound. PBPb domain family (or cluster) 3 is homologous to periplasmic binding proteins specific for polar amino acids and opines (252). Some HKs of this group show a complex architecture of their transmitter domain, including a PAS domain in the linker region and a receiver and HPt domain at the C-terminal end of the kinase. Other (albeit so-far-uncharacterized) members contain only the "standard" HisKA/HATPase_c domains in their cytoplasmic transmitter regions.

The model TCS of this class is BvgAS of *Bordetella pertussis* (148), which controls expression of virulence genes and biofilm development in this human-pathogenic bacterium, the etiological agent of whooping cough (148, 175). BvgS responds to environmental stimuli such as temperature, magnesium sulfate, or nicotinic acid, but the actual stimulus has not yet been chemically defined (148). The stimuli are thought to be perceived by the PBPb domains. The cytoplasmic PAS domains of BvgS and EvgS are sensitive to water-soluble quinone analogs in vitro (26). It has been suggested that BvgS and EvgS respond in this way to the redox state of the ubiquinone from the aerobic respiratory chain (30), reminiscent of the O_2-sensing HK ArcB of *E. coli* (174) (see below). BvgS-homologous systems have been described for *Bordetella* species and other pathogenic bacteria, all of which regulate complex behavioral shifts to a pathogenic life style: EvgAS controls the expression of a drug efflux pump in *E. coli* (138); AstRS controls swarming motility, phase variation, and stationary-phase adaptation in

Photorhabdus luminescens (46); and CblSRT regulates the expression of cable pili in *Burkholderia cenocepacia* (256).

Novel Conserved Periplasmic Sensing Domains: CACHE, CHASE, and Reg_prop

Recently, sequence analyses led to the identification of several new types of sensory domains found in TCS, MCP, and signal transducing adenylate cyclases (Table 1) (65). The CACHE domain (Ca^{2+} channels, *che*motaxis receptors) was the first extracytoplasmic sensing domain in this group that was identified by multiple-sequence alignments of sensor proteins (6). Initial experimental data indicate that the CACHE domain is involved in small-molecule binding. The CACHE domains of McpB and McpC of *B. subtilis* are important for sensing amino acids and carbohydrates, respectively (71, 94). So far, only a few HKs with CACHE domains are found in the databases. The CACHE domain of the sensor kinase DctB is crucial for sensing dicarboxylates in *Rhizobium leguminosarum* (218).

Multiple-sequence alignments of extracellular regions from membrane-bound signaling proteins led to the identification of six different conserved extracellular sensing domains, termed CHASE to CHASE6 (for *c*yclase/*h*istidine kinase-*a*ssociated *s*ensing *e*xtracellular) (7, 288). As their name indicates, these domains (length, 150 to 300 aa) are found in the N-terminal region of HKs, adenylate cyclases, and predicted diguanylate cyclases/phosphodiesterases. Additionally, CHASE2 domains occur in serine/threonine kinases, and CHASE3 domains occur in MCP. CHASE domains are flanked by predicted TMR and are often, but not necessarily, found in association with other conserved cytoplasmic signaling domains, such as PAS, GAF, or HAMP (Table 1). CHASE2, however, is always followed by three TM helices, but never a HAMP domain (Fig. 3). About 100 CHASE domain-containing HKs can be found in the databases. They are predominantly encoded in the genomes of cyanobacteria but can also be found in proteobacteria, gram-positive bacteria, and even in some archaea (7, 182, 288). So far, only one bacterial CHASE domain-containing HK has been analyzed with regard to its biological function: VsrA, a CHASE3 domain-containing HK, is required for the expression of virulence factors in *Pseudomonas solanacearum* (227). CHASE3-containing HKs can also be found in the genome sequences of other pathogenic bacteria, such as *Legionella pneumophila* and *Bacillus anthracis* (288). Analysis of the CyaA adenylate cyclase from *Myxococcus xanthus* indicates that the CHASE2 domain might function in osmosensing (288).

A novel type of periplasmic-sensing HK has been identified in the human gut symbiont *Bacteroides thetaiotaomicron*. The periplasmic input domain has a length of more than 1,000 amino acids and harbors 14 tandem repeats of a Reg_prop domain. Sequence homology indicates that these repeats are likely to form two seven-bladed β-propellers (63, 213). The role of these structures for sensing (either directly or indirectly through protein-protein interaction) remains to be determined. These kinases are found in large numbers in this bacterium. It has been suggested that the Reg_prop HKs are crucial (together with a plethora of extracytoplasmic-function σ factors) for the regulation of the organism's large repertoire of genes for the metabolism of complex polysaccharides in response to their availability in the gut environment (283). Similar HKs with Reg_prop domain containing input regions are so far only found in *Xanthomonas campestris*.

General Roles of Extracytoplasmic Sensor Domains in Stimulus Perception

As shown in this section, there is a remarkable flexibility and variation in the type of sensory domains of periplasmic-sensing HKs. In many cases, the role of the periplasmic domain in signal sensing is unproven, controversial, or even questionable, such as (and most notably) in the case of *E. coli* EnvZ (see above). Another prominent example is the phosphate sensor PhoR of *B. subtilis*. By domain architecture, this protein has all features of a classical periplasmic-sensing HK: the N terminus of this sensor kinase contains two TM helices flanking an extracytoplasmic domain of 120 aa. However, strains expressing PhoR derivatives in which the extracytoplasmic domain between the two TM domains, or even the complete N terminus up to the cytoplasmic side of TM helix 2, are deleted (PhoR is expressed as a soluble, cytoplasmic protein in the latter case) respond almost normally to PhoR-mediated phosphate limitation (233). The authors of this and another study on the homologous PhoR sensor kinase from *E. coli* could demonstrate that an extended cytoplasmic linker region, termed the C_2 region, between TM helix 2 and the kinase domain is necessary and sufficient for sensing phosphate limitation (228, 233). Therefore, PhoR has to be regarded as a membrane-anchored, cytoplasmic-sensing HK rather than a periplasmic-sensing HK. These findings raise the possibility that the extracytoplasmic domains of some sensor proteins classified as periplasmic-sensing HKs are unnecessary for stimulus perception (102). Alternatively, some periplasmic-sensing HKs are mixed periplasmic/cytoplasmic-sensing HK that detect additional, so-far-unidentified stimuli.

Remarkably, different types of sensing domains have been adopted for the same or similar stimuli. This can be exemplified by the sensors for C_4-dicarboxylates: the C_4-dicarboxylate is perceived by a PAS-like domain in DcuS from *E. coli*, a four-helix bundle domain in Tar (aspartate) MCP of *E. coli*, and the CACHE domain in the succinate sensing DctB of *Rhizobium leguminosarum* (6, 114, 218, 219). Osmosensing is mediated by an even larger diversity of sensor proteins that even belong to completely different families: *E. coli* EnvZ is a prototypical periplasmic-sensing HK, while its functional homolog from *Xenorhabdus nematophilus* is a small intramembrane-sensing HK (see below). The osmosensors KdpD from *E. coli* and MtrB from *Corynebacterium glutamicum* are also HKs but are completely different from EnvZ (128, 179) (see below). In addition, the carriers BetP from *C. glutamicum* and ProU from *E. coli* and other organisms are also able to sense osmolarity (216, 226).

The recent identification of new conserved periplasmic domains by sequence analyses indicates that additional, so-far-uncharacterized periplasmic sensing domains might exist, which further extend the number and types of domains (and presumably stimulus perception mechanisms) used by periplasmic-sensing HKs. Thus, our understanding of signal perception and transmission by periplasmic-sensing HKs is still at the very beginning.

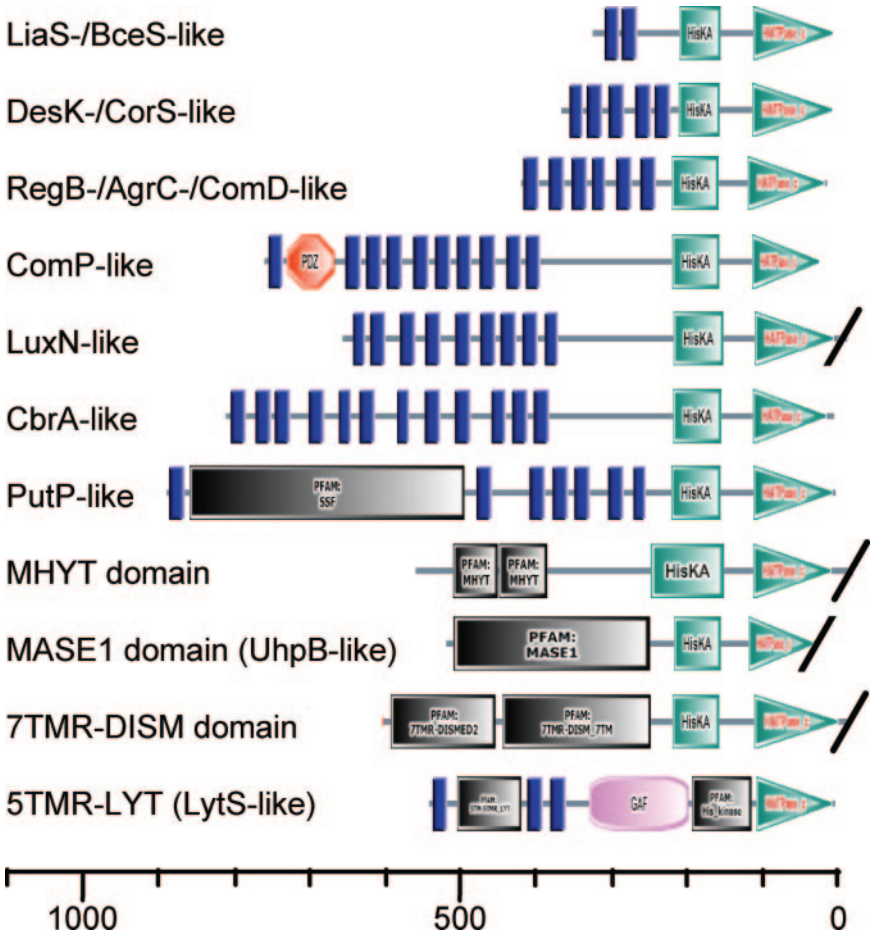

FIG. 6. Domain architecture of histidine kinases with sensing mechanisms linked to the transmembrane regions. The figure is based on the graphical output of the SMART web interface at http://smart.embl-heidelberg.de (229), with modifications. The scale bar is in amino acids. Blue vertical bars represent putative transmembrane helices. Sizes and positions of conserved domains are indicated by the labeled symbols. Note that the transmitter domains are simplified, and as a default, only HisKA and HATPase_c are shown. See the text for details.

HISTIDINE KINASES WITH SENSING MECHANISMS LINKED TO THE TRANSMEMBRANE REGIONS

In this highly diverse group of sensor kinases, the TM helices appear to play a central role in stimulus perception. The N-terminal input domains of all HKs listed in this section consist primarily of putative TMR, most of which have only short or no significant (extra)cytoplasmic linkers between these TMR (Fig. 6). Their grouping is based on their function and the number of TM helices but most likely also reflects their phylogenetic relationship, as indicated by the sequence conservation of their respective transmitter regions (Table 2). Six subgroups can be differentiated: (i) small sensor kinases with 2 TMR lacking any significant extracytoplasmic linker (LiaS/BceS-like intramembrane-sensing HKs), which are involved in sensing cell envelope stress or mediating ABC transporter-coupled detoxification processes in gram-positive bacteria; (ii) DesK-like thermosensors with 4 or 5 TMR that respond to membrane fluidity; (iii) RegB-like global sensor kinases with 6 TMR; (iv) quorum-sensing HKs with 6 to 10 TMR, again occurring primarily in gram-positive bacteria (AgrC/ComD-, ComP-, and LuxN-like HKs); (v) HKs with 12 to 20 TMR which show homology to transport proteins (CbrA- and PutP-like HKs); and (vi) HKs with "unknown conserved" input domains of 6 to 8 TMR (MHYT-, MASE1-, 7TMR-DISM-, and 5TMR-LYT-containing HKs). Proteins harboring these domains have been identified by bioinformatic analyses and are grouped according to their input domains.

Intramembrane-Sensing HKs: Cell Envelope Stress Sensors with Two TMR (LiaS/BceS-Like HKs)

The term intramembrane-sensing histidine kinase (IMHK) was originally coined for sensor kinases of the cell envelope stress stimulon of *B. subtilis* (167). The HKs of the three TCS involved—LiaS, BceS, and YvcQ—share striking similarities in their domain organization that led to the initial definition: small sensor kinases of less than 400 aa in total length with a short sensing domain consisting of two deduced TM helices with a extracytoplasmic linker of less than 25 aa between them. It was proposed that these kinases sense their stimuli at or within the membrane interface, hence their name (167). Two major groups can be distinguished, based on genomic context and sequence conservation: (i) LiaS-like sensors as part of three-component systems and (ii) BceS-like kinases that are

linked, by function and genomic context, to ABC transporters (166). Both groups occur almost exclusively in gram-positive bacteria with a low G+C content (*Firmicutes*).

LiaS-like HKs. So far, only two members of the LiaS-like subgroup of IMHKs have been described in detail: the eponymous sensor LiaS from *B. subtilis* and VraS from *Staphylococcus aureus*. The kinases of both TCS sense the presence of cell-wall-active antibiotics. LiaS is induced by antibiotics (including bacitracin, vancomycin, ramoplanin, and cationic antimicrobial peptides) that interfere with the lipid II cycle in cell wall biosynthesis (168, 210). The VraS sensor kinase responds to an even broader spectrum of cell wall antibiotics, such as glycopeptides, β-lactams, bacitracin, and D-cycloserine (151). Two additional LiaRS homologs also respond to the presence of cell wall antibiotics: TCS03 of *Streptococcus pneumoniae* and YvqEC of *Bacillus licheniformis* are activated by the presence of sublethal concentrations of vancomycin and bacitracin, respectively (92, 271), indicative of a general role of all LiaS-homologous IMHKs in the detection of cell envelope stress. Recently, it was found that LiaF, a membrane protein, whose gene is topologically linked to *liaSR* in the genomes of all species harboring LiaS homologs, is crucial for the LiaS-mediated sensing mechanism: in a *liaF* deletion mutant, the LiaRS system is constitutively "on," thereby no longer necessitating a stimulus for full activity (120). Therefore, LiaF, together with LiaRS, constitutes a cell envelope stress-sensing three-component system. The corresponding HKs are phylogenetically conserved and belong to the HPK7 subfamily (166).

BceS: a two-component system–ABC transporter connection. The largest distinct group of IMHKs is characterized by the location of their structural genes adjacent to (usually upstream of) those encoding ABC transporters. About 70 proteins belong to this phylogenetically conserved subgroup (HPK3i) (Table 2), and almost all are from gram-positive bacteria with a low G+C content (166). The genes encoding the TCS and the ABC transporter are expressed as independent transcriptional units and together form specific and efficient detoxification units: It is thought that the HK senses the presence of sublethal concentrations of harmful compounds (such as the cell wall antibiotic bacitracin) and activates its cognate RR, which in turn strongly induces the expression of the adjacent ABC transporter, which subsequently facilitates removal. Such a topological and functional link of the TCS and ABC transporter operons was observed earlier in the *Bacillus*/*Clostridium* group of low-G+C gram-positive bacteria and is well documented for *B. subtilis* (121, 122, 166, 167, 185, 194).

Miscellaneous IMHKs. About 50 additional, phylogenetically unrelated proteins from *Firmicutes*, *Actinobacteria*, and *Proteobacteria* share the overall domain architecture of IMHKs. So far, only a few examples have been described in any detail. The GtcRS is located adjacent to the *grsAB* operon of *Bacillus brevis*, which encodes enzymes of the biosynthesis of the peptide antibiotic gramicidin S (260). The SaeRS TCS is part of a complex regulatory network that controls the expression of virulence determinants in *S. aureus* (81, 82). So far, only one IMHK from gram-negative bacteria has been investigated: EnvZ of *Xenorhabdus nematophilus* is homologous to the "classical" osmosensor EnvZ of *E. coli* in its cytoplasmic C-terminal domain, but it lacks an extracytoplasmic domain. While the periplasmic domains have diverged extensively, EnvZ from *X. nematophilus* was still able to complement a Δ*envZ* mutant of *E. coli* to sense changes in environmental osmolarity and properly regulate the phosphorylation levels of the cognate RR OmpR of *E. coli* (251).

IMHK-like periplasmic-sensing HKs: VanS/PrmB-like proteins. Two phylogenetically unrelated groups of cell envelope stress-sensing HKs show striking similarities to IMHKs with regard to their overall size and domain organization: (i) small VanS-like HKs of the VanB type, the sensors of vancomycin resistance in gram-positive bacteria, and (ii) PmrB/BasS-like HKs mediating resistance to cationic antimicrobial peptides in *E. coli* and *Salmonella* spp. (89, 90, 282). However, in both cases experimental evidence points towards a role of the short extracytoplasmic linker between the two TMR in signal perception. VanS-like HKs interact with vancomycin through their short extracellular sensing domain (25 to 30 aa), presumably by binding the drug directly (104, 106). The same is true for PmrB/BasS-like HKs, with a periplasmic linker of 30 to 35 amino acids between the TMR. It has been demonstrated that PmrB senses ferric iron through two conserved ExxE motifs in this short extracytoplasmic sensor domain (281). Therefore, both VanS- and PrmB-like proteins are periplasmic-sensing HKs (Fig. 3).

In that regard, the 25-residue cutoff value for the periplasmic linker might indeed be a critical threshold to differentiate "true" IMHKs from related but periplasmic-sensing HKs involved in cell envelope stress response (166).

DesK-Like Thermosensors with Four to Six TMR

The cytoplasmic membrane is the primary selective barrier between a bacterial cell and its environment. Its function critically depends on the physical state of the lipid bilayer, which in turn is strongly influenced by the temperature. Low temperature leads to a phase transition from a disordered liquid-crystalline phase to a more rigid and ordered gel-like phase. In order to maintain a functional membrane under such conditions, cells can lower the melting point of their membranes either by incorporation of de novo-synthesized branched-chain fatty acids or by desaturation of fatty acid moieties in the existing membrane. The mechanism of temperature-dependent adaptation of membrane fluidity and its regulation have been studied intensively in the two paradigmatic species *E. coli* and *B. subtilis* and have been reviewed recently (164).

In *B. subtilis*, regulation of membrane fluidity is mediated by the cold shock-inducible expression of the *des* gene, encoding a fatty acid desaturase (2, 4). Its expression is controlled by the DesRK TCS. At higher growth temperatures (membrane in the liquid state), the bifunctional sensor kinase DesK is predominantly in the phosphatase state, thereby repressing *des* expression. At lower temperatures, DesK switches to a dominant kinase activity, thereby activating its cognate RR DesR, which results in a strong induction of *des* expression (3). It has been suggested that DesK senses these temperature changes through its hydrophobic N-terminal region, which consists of four or five TM regions (105). Experimental evidence points towards membrane fluidity as the stimulus of DesK-mediated *des* expression, which requires the presence of the N-terminal TM region of the protein (42). However, the mechanism of

temperature sensing by this N-terminal input domain has not yet been addressed experimentally.

CorS of *Pseudomonas syringae* is another thermosensor that is not homologous to DesK. It resembles DesK with regard to the N-terminal hydrophobic input domain, consisting of six TM regions, with only very short or no periplasmic linkers in between (DesK-like HK; four to five TM regions) (Fig. 6). In contrast to the case for DesK, which is conserved in the *Firmicutes* group of gram-positive bacteria, no CorS homologs can be found in the databases. CorS regulates the thermoresponsive production of the phytotoxin coronatine, a polyketide that is synthesized predominantly at lower temperatures (31). LacZ/PhoA fusions confirmed the presence of six TMR, and deletion analysis indicated an important role of the N-terminal TM helix for thermosensing (237). However, as for DesK, it remains to be investigated whether membrane fluidity (a physical property of the membrane) or its chemical composition serves as a trigger for CorS activation.

RegB/PrrB-Like Redox-Responding Global Sensor Kinases with Six TMR

RegBA and PrrBA were originally identified as TCS involved in the anaerobic induction of the photosystem in *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* 2.4.1, respectively (56, 181). RegBA and PrrBA are global regulatory systems that are highly conserved in photosynthetic and nonphotosynthetic alpha- and gammaproteobacteria and regulate energy-generating and -utilizing processes such as photosynthesis, carbon fixation, hydrogen oxidation, denitrification, aerobic and anaerobic respiration, electron transport, and aerotaxis in response to redox energy or catabolic [H] supply of the cell (55). RegB and PrrB are bifunctional proteins, exerting both kinase and phosphatase activities. While the kinase activity is regulated by the redox state of the electron transfer chain, the phosphatase activity is assumed to be constitutive (215). The input domains of RegB/PrrB consist of six TM helices (38, 198), which are required for stimulus perception (215). A mutation in the cytoplasmic loop between TM helix 2 and TM helix 3 causes constitutive (i.e., oxygen-insensitive) kinase activity in vivo (56). The aerobic respiratory chain has been shown to supply the signal by interaction of the cytochrome cbb_3 oxidase with PrrB/RegB (55, 191–193). In a defined in vitro system, cytochrome oxidase cbb_3 stimulated the phosphatase/kinase activity ratio of PrrB in the presence of O_2, resulting in a decrease of PrrA-phosphate and thus of the active state of PrrA (193). Sensing and optimal kinase activity required the TM sensing domain of PrrB.

For optimal signal transfer and sensing by RegB/PrrB, the gene products of the *senC*/*prrC* genes, which are cotranscribed with *regA*, are required. SenC/PrrC are predicted membrane-spanning copper-binding proteins, and their inactivation results in an oxygen-insensitive phenotype (57, 170). SenC is suggested to modulate RegB activity or to form a signaling link between cytochrome cbb_3 oxidase and RegB (192, 249). RegB contains a redox-sensitive cysteine residue in the cytoplasmic domain which becomes oxidized in the presence of oxygen, resulting in the formation of an intermolecular disulfide and an inactive RegB tetramer (248, 249). The oxidation of the cysteine residue by oxygen might occur via cytochrome cbb_3 oxidase or directly.

In *R. sphaeroides*, the expression of photosynthesis genes is regulated by light in addition to oxygen. The regulation by light is initiated by the photosynthetic reaction center and transmitted by the electron transport chain and the PrrBA TCS. In addition, there is a blue-light photoreceptor, AppA, which interferes with the PrrBA-dependent regulation (95). Finally, it has been suggested that RegB in *R. capsulatus* may respond to additional stimuli, such as the flow of intermediates through the bacteriochlorophyll biosynthetic pathway (1).

In summary, RegB is a well-characterized redox sensor, which senses the redox state of the aerobic electron transport chain by interaction with cytochrome oxidase, in contrast to other O_2 sensors, which interact with molecular O_2 (such as FixL) or with the respiratory quinones (i.e., ArcB [see below]). Still, many mechanistic details, such as the role of the TM helices in signal perception and in the interaction between PrrB/RegB and the oxidase, have to be elucidated.

Peptide Quorum Sensors with 6 to 10 TMR (AgrC/ComD-, ComP-, and LuxN-Like HKs)

AgrC/ComD- and ComP-like HKs are sensors of peptide-dependent quorum-sensing systems, a widespread mechanism of cell density-responsive regulation in gram-positive bacteria. ComP-like HKs differ in the architecture of the input domain from those of the AgrC/ComD group (Fig. 6): ComP-like HKs tend to have 8 to 10 TM regions (based on computer predictions), and some include a larger (about 50-aa) extracellular loop between TM helix 1 and TM helix 2 that is important for signal detection, while AgrC/ComD-like HKs seem to have six TM regions and lack the extracellular loop region. The two groups are also phylogenetically distinct: ComP-like HKs belong to the HPK7 subfamily, whereas AgrC/ComD-like HK exclusively comprise the very unique HPK10 subfamily, which has already been recognized as being restricted to peptide quorum-sensing HKs (98, 280). Within the latter group, AgrC- and ComD-like kinases can be differentiated by the chemical nature of their stimuli: AgrC-like HKs sense cyclic thiolactone-containing autoinducing peptides (AIPs), while ComD-like sensors bind unmodified GG leader-type peptides (98, 160). The precursors of these signaling peptides harbor a characteristic double-glycine leader that is removed concomitant with its export (184). A number of excellent reviews on this topic have been published (48, 51, 141, 142, 160, 232, 247). Therefore, we will concentrate our description on stimulus detection only. The systems share a common mechanism for intraspecies communication. The signaling peptides are expressed at a constant low level as inactive cytoplasmic precursors. They are actively secreted, usually after posttranslational modifications. Examples include cleavage of a leader peptide (*S. pneumoniae* CSP), introduction of lanthionine bridges (lantibiotics such as subtilin or nisin), or isoprenylation (*B. subtilis* ComX). Peptide processing and secretion are often coupled processes. The active signaling peptides accumulate in the environment, and when they reach a certain threshold concentration, their presence is detected by the corresponding HK. These proteins, in turn, activate their cognate RR, resulting ultimately in a differential expression (usually upregulation) of the target genes,

including the regulatory system, the signaling peptide, and its export/modification/sensing system (positive feedback loop). The concentration of the stimulating molecule is therefore a measure of the cell density of organisms having the same pherotype (i.e., those that are able to excrete and sense the same peptide species). Activation of the corresponding TCS leads to a sudden burst in peptide release, thereby ensuring that the cells of a culture become activated at the same time to achieve synchronization. Examples include the development of competence for genetic transformation in the genera *Bacillus* (ComX-ComAP) and *Streptococcus* (ComC-ComDE), transition to the virulence state in *Staphylococcus* (AgrBCD), and bacteriocin production in various *Firmicutes* bacteria (48, 51, 141, 142, 160, 232, 247).

AgrC is the sensor kinase of one of the four known TCS regulating staphylococcal virulence. AgrC mediates quorum sensing and is activated upon binding of the self-encoded AIP. The interaction between the signaling peptide and the sensing domain of *S. aureus* AgrC, the sole receptor of AIPs in staphylococci (116), has been studied in great detail. Localization, membrane topology, and enzymatic kinase function demonstrate that AgrC is a membrane-bound HK with six TM helices (157). Extensive studies on AgrC architecture and AIP/AgrC interaction resulted in the model of a two-step process of stimulus perception. First, the AIP interacts with the sensor domain by entering a hydrophobic pocket formed by the TM helices in a non-sequence-specific manner. In a second step, sequence-specific hydrophilic interactions between the AIP and amino acid residues in the last extracellular loop of the AgrC sensing domain leads to activation of the kinase and autophosphorylation (160). The sequences of AIPs (*agrD* genes) and of the corresponding *agrB* and *agrC* genes, which encode proteins for the export and the TCS, are highly variable. They comprise a functional unit that defines the pherotype of an individual strain (50). A genetic and pherotypic polymorphism of all peptide-specific parts of the signal perception mechanism has also been described for the competence quorum-sensing systems in *S. pneumoniae* and other streptococci (97, 274) and *B. subtilis* (258, 259). The AgrC sensor kinases detect only peptides of their own pherotype, and this specificity is based on the TM-sensing domain of the kinase alone: engineered fusion kinases composed of heterologous N-terminal input and C-terminal transmitter domains responded only to the peptide that corresponded to the pherotype of the sensor domain (118, 161). The membrane topology and mechanism of stimulus perception of two additional quorum-sensing HKs of this class, PlnB and ComP, have been investigated to some extent.

PlnB is a sensor kinase regulating transcription of the *pln* bacteriocin biosynthesis locus of *Lactobacillus plantarum*. It responds to the extracellular presence of the inducing peptide pheromone IP-C11 and activates the two cognate RRs PlnC and PlnD, which regulate the transcription of the bacteriocin operons *plnEFI* and *plnJKLR* as well as the regulatory *plnABCD* operon itself (47). A membrane topology with six TM helices is predicted for PlnB, but experiments suggest the presence of seven TM helices with a large N-terminal extracytoplasmic loop of 24 amino acids, as has also been predicted for the competence sensors ComD from *S. pneumoniae* (98, 118). The peptide pheromone interacts with the membrane-integral N-terminal input domain (118), and three amino acid residues (Asp54, Ser58, and Leu61) in the extracellular loop between TM helix 2 and TM helix 3 that are important for peptide/sensor interaction have been identified (119). The corresponding residues are also critical for ComD activity, indicating a common mechanism of peptide sensing, which could be similar to that described for AgrC (119). Thus, peptide quorum sensing apparently occurs by a common mechanism involving nonspecific hydrophobic interaction at the TMR, as well as specific interactions of the inducing peptide with polar residues in the extracytoplasmic loop of the sensor domain (situated between TM helices 2 and 3 for PlnB and ComD and between TM helices 3 and 4 for AgrC).

The ComPA TCS is required for the development of competence for genetic transformation, a process embedded in the complex developmental program during the adaptation of *B. subtilis* to nutrient limitations (183). ComP senses the presence of the ComX pheromone, a modified decapeptide (8), and in turn activates its cognate RR ComA. This ultimately leads to the activation of the competence transcription factor ComK, which controls the expression of genes encoding the DNA uptake and recombination machinery (37, 93). Membrane topology studies based on PhoA/LacZ fusion studies suggest that the input domain of ComP consists of eight TM helices. Two periplasmic linkers in the first four TM regions are important for binding of the ComX pheromone (208). A domain-based sequence analysis predicts the presence of 10 TM helices for three ComP homologs, with only one large extracellular loop remaining (equivalent to the first extracellular loop [Fig. 6]). This large loop contains a PDZ domain (domain present in *P*SD-95, *D*lg, and *Z*O-1/2) with a length of about 70 amino acids. PDZ domains occur primarily in higher eukaryotes but have also been identified in bacterial proteins such as the cell envelope stress protease HtrA (214). They are involved in binding of (poly)peptides, indicative of a role in ComX binding. Therefore, the domain architecture of some ComP proteins, such as the one analyzed by Piazza et al. (208), closely resembles that of LuxN-like kinases (Fig. 6). The role of the PDZ domain in peptide sensing in the ComP-like sensors has not been addressed so far.

Interestingly, LuxN/CqsS-like quorum sensors from gram-negative bacteria, which respond to homoserine lactone, show a very similar domain architecture: the input domains of both kinases consist of eight or nine putative TM regions (Fig. 6). While the corresponding quorum-sensing systems have been investigated intensively with respect to signal transduction and gene regulation (see references 51, 64, and 232 for recent reviews), nothing is known about the mechanism of signal perception by the input domains of these sensor kinases. Therefore, it can only be speculated, by comparison to the gram-positive quorum sensors, that it might also involve direct binding of the autoinducers, possibly through the concerted action of TM regions and specific residues in the loop regions in between.

CbrA- and PutP-Like Proteins with More than 10 TMR: Sensor Kinases with Fused Secondary Carrier Domains

Databases contain about 10 HKs with N-terminal input domains of 12 to 20 TM helices. One such TCS, CbrAB of

Pseudomonas aeruginosa, controls the expression of several catabolic pathways in response to a stimulus reflecting the intracellular carbon/nitrogen ratio. Mutants defective in the corresponding genes are severely impaired in the use of mannitol, glucose, pyruvate, and citrate as sole carbon sources and in the use of several amino acids (arginine, histidine, and proline) and polyamines as sole carbon and nitrogen sources (189). The input domains of CbrA and its homologs show a significant degree of sequence conservation in the TM regions and the connecting loops. The sensing domains are homologous to Na^+/solute symporters (SSSF domain [Table 1]) such as *E. coli* PutP or PanF (96, 124). This class of transporters uses the free energy stored in electrochemical Na^+ gradients for the uptake of solutes, including proline (PutP) and pantothenate (PanF) (125). While the membrane topology (126, 212, 272) and biochemistry of the carriers has been studied in great detail (reviewed in reference 125), no data are available on the corresponding sensor kinases. It is tempting to speculate that these HKs sense the presence of solutes during uptake. If this is true, these kinases would represent a unique type of "transmembrane-sensing" HKs that detect their stimulus during transport. This type of sensing is reminiscent of glucose sensors of *Saccharomyces cerevisiae*, which are homologous to secondary glucose carriers that have lost transport function (199).

Novel Conserved Putative TMR-Associated Sensing Domains with Six to Eight TMR: MHYT, MASE1, 7TMR-DISM, and 5TMR-LYT

A number of putative membrane-integral sensing domains have been identified in recent years by comparative genomics, none of which have been further addressed experimentally with regard to the mechanism of stimulus perception (Fig. 6).

The MHYT domain was the first conserved integral membrane sensor domain identified (67) and is present predominantly in diguanylate cyclases but also in a number of so-far-uncharacterized HKs. It consists of six TM helices and was named for its characteristic and highly conserved MH(Y) residues in the center of TM helices 2, 4, and 6. The membrane topology indicates that these residues might coordinate a copper ion, suggesting a role in O_2, CO, or NO sensing (67). However, no functional characterization of an MHYT domain has been published so far.

Comparative genomic analysis identified two conserved putative *m*embrane-*a*ssociated *se*nsor domains, MASE1 and MASE2 (186). MASE1 (but not MASE2) domains were found in sensor kinases (in addition to chemoreceptors and guanylate cyclases). They consist of six putative TM helices that harbor several conserved Trp residues. Some, but not all, MASE1-containing HKs harbor additional C-terminal cytoplasmic-sensing domains, such as PAS or GAF (see below and Table 1). Neither sequence nor putative topology allows speculations on the nature of the stimuli sensed. No data are available on signal perception for MASE-containing HKs. So far, only one MASE1-containing HK has been genetically analyzed. UhpABC of *E. coli* regulates the expression of a glucose-6-phosphate (glucose-6-P) transporter, UhpT, in response to the extracellular presence of the inducer (glucose-6-P) that is recognized by UhpC, a TM protein that interacts with UhpB, the cognate sensor kinase. The sensor domain of UhpB consists of eight TMR, including one large extracellular loop connecting TMR7 and TMR8 (111). The interaction between UhpC/glucose-6-P and UhpB is thought to cause a conformational change, resulting in the autophosphorylation of UhpB and the activation of the UhpA RR (130). The cooperativity of glucose-6-P, UhpC, and UhpB has been investigated in detail (263), but the mechanism of signal perception by UhpB and the role of the MASE1 domain have not been addressed. The MASE1 domain has also been described as a conserved TM domain in signal perception by another group and termed the 8TMR-UT domain (5).

Serpentine or seven-transmembrane helix receptors (7TMR) are widespread in eukaryotic signal transduction (209). These input domains can occur individually or in combination with additional extracytoplasmic globular domains that specifically interact with a ligand. It has recently been shown through comprehensive sequence profiling analysis and comparative genomics that serpentine receptors are also quite common in bacteria (5). These signaling domains, termed 7TMR-DISM (*7 TM r*eceptors with *d*iverse *i*ntracellular *s*ignaling *m*odules) (Table 1), occur in combination with transmitter domains of bacterial HKs but also with signal-transducing domains from other sensory proteins, such as diguanylate cyclases, phosphatases, DNA-binding domains, and other noncatalytic domains involved in sensing and signal transduction. Many of the 7TMR-containing HKs also harbor an N-terminal extracellular putative ligand-binding domain (DISMED [*DISM e*xtracellular *d*omain]) (Table 1) (5). So far, only one of these HKs has been functionally investigated: RetS-dependent signal transduction is important for reciprocally regulating acute infection and chronic persistence in *Pseudomonas aeruginosa* and is required for the expression of the type III secretion system and other virulence factors and for the repression of genes involved in formation of the exopolysaccharide components of the biofilm matrix (86). A recent mutational study demonstrates that the periplasmic DISMED domain is expendable for HK function. In contrast, deletion of six of the seven TMR of the 7TMR-DISM domain results in a truncated protein that, while still being anchored to the cytoplasmic membrane, is no longer able to complement a *retS* mutant (154), underscoring the importance of the TMR for signal perception in this group of HKs. However, it is not yet clear whether these proteins receive their stimulus from the outside (periplasm) or from within the membrane.

Another conserved input domain, the 5TMR-LYT domain, has been proposed for LytS- and YhcK-like HKs (5) but so far has not been experimentally addressed.

Models for Stimulus Perception Associated with TMR

HKs with putative input domains consisting primarily of TMR represent the phylogenetically most diverse subgroup of sensor kinases and also the least well understood with regard to the mechanism of stimulus perception. They show a large variance in the overall protein size. A common feature is the presence of at least six TM helices (except for of LiaS/BceS-like HKs [see below]) that do not show a significant degree of sequence conservation and, with few exceptions, lack larger cytoplasmic- or periplasmic-sensing portions between them (Fig. 6). This seems to indicate a

proposed sensing mechanism that takes place within or is directly related to the membrane interface, although experimental support for this conjecture is still sparse. It was suggested for gram-positive quorum-sensing HKs that the signaling peptide first interacts with the sensor rather nonspecifically within a hydrophobic environment provided by the TM helices (160), i.e., within the membrane interface. While structural information is lacking, it could be envisioned that the hydrophobic pocket is formed by the 12 TM helices of a HK dimer. Is this model of stimulus perception also applicable for the other HKs with putative TMR-associated sensing mechanisms? It would explain the comparably large number of TM helices characteristic for most of them (Fig. 6). Following this hypothesis, the TM regions would form a hydrophobic compartment buried inside the cytoplasmic membrane for interaction with a lipophilic compound that functions as a stimulus. This stimulus could be linked to or even buried within the cytoplasmic membrane or could come from the environment. Alternatively, the TM helices could function as "antennas" that probe a certain chemical or physical parameter of the cytoplasmic membrane itself. Such a mode of stimulus perception has been suggested for the thermosensors (42, 105).

The only exceptions to the rule seem to be the small cell envelope stress-sensing LiaS/BceS-like HKs for which the term intramembrane sensing was originally coined (167). These proteins harbor only two TM helices with almost no spacing between them (less than 10 aa for most). Still, they might fit the mold, since they are functionally associated with multiple TM-spanning proteins that are (LiaS-like HKs) or might be (BceS-like HKs) involved in the mechanism of signal perception.

For the LiaS protein, it was recently demonstrated that a third protein, LiaF, is absolutely required for LiaS function and might be involved in the mechanism of stimulus perception (120). This protein harbors four TM helices (S. Jordan and T. Mascher, unpublished result). Assuming that both LiaS and LiaF function as dimers, the overall LiaFS sensory complex would consist of 10 to 12 TM helices.

BceS-like HKs are functionally coupled to ABC transporters. The ABC transporter functions primarily as a detoxification pump that is strongly induced only in the presence of its substrate. However, a basal level of expression comparable to the corresponding TCS was observed, at least in case of the bacitracin efflux pump BceAB, even in the absence of bacitracin (194). It is tempting to speculate that this basal expression of the ABC transporter, which by itself must be able to recognize its substrate, is necessary for the formation of a sensory complex between the ABC transporter and the cognate HK in case of BceS-like proteins, a situation somewhat reminiscent of the CbrA/PutP-like HKs with fused secondary carrier domains (see above). While such a mechanism for stimulus perception would be fascinating, it is purely speculative at the moment and needs to be addressed experimentally.

CYTOPLASMIC-SENSING HISTIDINE KINASES

The input domains of cytoplasmic-sensing HKs reside within the cytoplasm. Consequently, their stimuli are derived from the cytoplasm or from the cytoplasmic side of the membrane. Sensor proteins of this type can be (i) membrane integral with N- or C-terminal cytoplasmic sensing domains, (ii) cytoplasmic proteins associated with membrane-integral proteins, or (iii) of permanent cytoplasmic location. Most of the membrane-bound cytoplasmic-sensing HKs can be identified due to the presence of conserved cytoplasmic input domains (such as PAS or GAF [Table 1]). However, membrane-integral, cytoplasmic-sensing HKs (the first subgroup mentioned above) lacking clearly identifiable sensing domains are hard to identify and might be indistinguishable from sensors of the other two major groups described above until the precise region(s) involved in signal detection can be defined.

Membrane-bound cytoplasmic-sensing HKs typically contain two to four TM helices without significant periplasmic loops (Fig. 7). The sequence gives no indication of stimulus perception by periplasmic or membrane-integral domains. The cytoplasmic sensory domains can be located N or C terminal of the TM helices (Fig. 7).

Cytoplasmic-sensing HKs show a very high degree of diversity in their domain architecture, especially the largest subgroup of soluble sensor kinases (Table 2). Therefore, they cannot be classified by means of their domain architecture (with the noteworthy exception of CheA-like HKs). These proteins usually harbor one to several copies of PAS domains, often including a C-terminal extension (PAC), sometimes combined with other domains, such as GAF (Table 1; Fig. 7). Only a very limited number of these proteins have been investigated to date. Therefore, only some well-characterized examples of cytoplasmic-sensing HKs will be described in the following sections.

Membrane-Anchored HKs with N-Terminal Cytoplasmic Sensing Domains

Membrane-anchored HKs with N-terminal cytoplasmic sensing domains form a relatively small subgroup of about 100 members within the cytoplasmic-sensing HKs. The osmosensor KdpD represents the prototype of this subgroup (reviewed in reference 128). KdpD controls the fast adaptive response of *E. coli* to changes in turgor or osmolality. Upon osmotic upshift or K^+ limitation, *E. coli* induces the high-affinity K^+ uptake ATPase KdpFABC as a first response. The induction is effected by the KdpDE TCS. The HK KdpD consists of a large N-terminal domain (about 400 aa), four TM helices, and a C-terminal transmitter domain (Fig. 7). Sensing of osmolality occurs indirectly by measuring the intracellular parameters K^+, ATP concentration, and ionic strength, which respond to osmolality changes (132). KdpD shows a complex structure of functional domains. It consists of an N-terminal cytoplasmic input domain, which contains a Walker A and B motif, forming an ATP-binding domain, a Usp domain (152, 236), the four TMR, and an Arg cluster located C terminal of TM helix 4 (127, 129). High osmolality and K^+ limitation within the cells stimulate kinase activity of KdpD and autophosphorylation. Stimulation involves direct interaction of the N- and the C-terminal cytoplasmic domains. In addition, osmolality or related parameters also control binding of the N-terminal domain to the membrane surface. At low osmolality (or high intracellular K^+), KdpD is converted to a phosphatase. Deletion of the TM helices demonstrated that they are not essential for sensing K^+ but may be important for correct positioning of the N- and C-terminal cytoplasmic domains (100, 101). Mutants harboring point mutations in TM helix 1 were mainly affected in

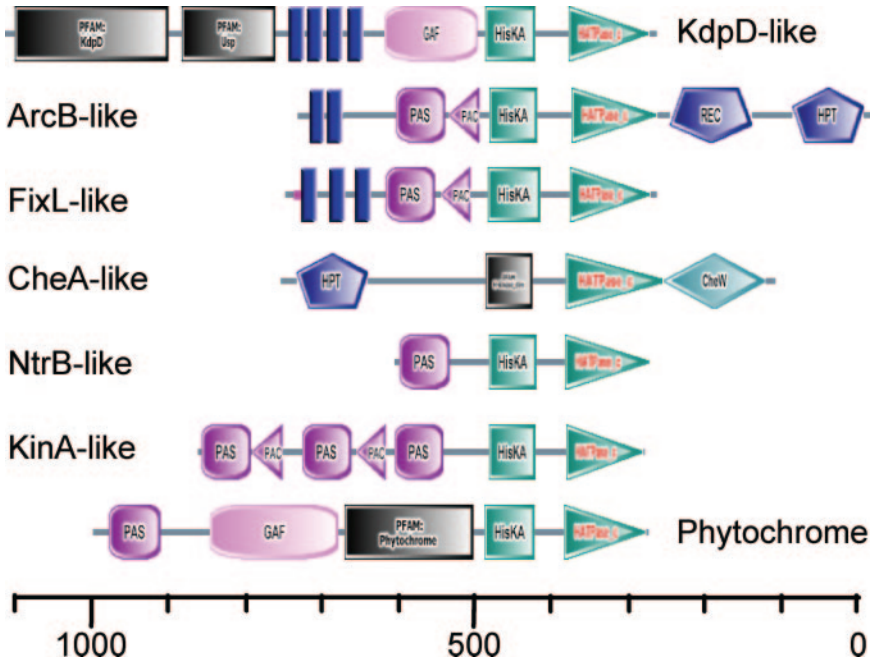

FIG. 7. Domain architecture of some examples of cytoplasmic-sensing histidine kinases. The figure is based on the graphical output of the SMART web interface at http://smart.embl-heidelberg.de (229), with modifications. The scale bar is in amino acids. Blue vertical bars represent putative transmembrane helices. Sizes and positions of conserved domains are indicated by the labeled symbols. See the text for details.

sensing osmolality, but not K^+, suggesting that sensing of both parameters can be dissected and that osmosensing depends mainly on membrane-integral amino acid residues (240). More recently, an in-depth mutational study clearly demonstrated that the K^+ sensor is located in the cytoplasmic C-terminal domain (224). Taken together, these findings indicate that KdpD seems to be a cytoplasmic-sensing HK (K^+) that also has a TMR-associated sensing mechanism (osmolality).

Membrane-Anchored HKs with C-Terminal Cytoplasmic Sensing Domains

Membrane-anchored HKs with C-terminal cytoplasmic sensing domains include some well-characterized examples of sensory HKs. The O_2 sensor FixL from *Bradyrhizobium japonicum* or *Rhizobium meliloti*, together with its corresponding response regulator FixJ, regulates the transcription of N_2 fixation genes in root-nodulating bacteria (76). FixL contains four TM helices, which are followed by a cytoplasmic PAS (~150 aa) and the C-terminal transmitter domain (~240 aa) (Fig. 7). The PAS domain is formed by two crossed monomers with an α-helix at the contact site. Each of the PAS monomers contains a ferrous heme molecule. Under high O_2 tension, O_2 is bound as the sixth ligand, resulting in kinase inactivation. The kinase is in the active state in deoxy-FixL, causing autophosphorylation of FixL, phosphorylation of FixJ, and transcription of N_2 fixation genes under O_2-limiting conditions (78, 80, 84). In constructs lacking the membrane anchor, the cytoplasmic part of FixL, comprising the PAS and kinase domains (FixL*), retains the ability to bind O_2, and the kinase activity responds to O_2 binding (76, 180). These findings suggest that the membrane anchor is not essential for O_2 sensing. The crystal structures of *B. japonicum* FixL in the ferric (Fe^{3+}), ligand-free, and CN^--bound forms were solved (CN^- mimicking bound O_2). The structures show that O_2 sensing is based on structural changes triggered by heme association/dissociation of the O_2 (84). High-resolution crystal structures of FixL from *R. meliloti* and *B. japonicum*, together with spectroscopic data, suggest that binding of O_2 to the distal heme (sixth ligand) site affects the kinase domain (84, 176, 177). The sixth site is covered by a hydrophobic triad of amino acids (including Ile209 and Ile210 in *R. meliloti*), which limits access of O_2 to the site. Binding of O_2 (as Fe-O-O) displaces the hydrophobic amino acid residues, in particular Ile209. The related structural changes initiate signal transduction from the heme via the input domain to the kinase domain. Thus, the initial events of signal perception and the beginning of signal transfer in FixL are well characterized. The necessity for a membrane association of FixL is not obvious. O_2 gains rapid access to the cytoplasm by diffusion even under low oxygen tension and active respiration. Intracellular O_2 tension in *E. coli* and other bacteria appears to be similar to the extracellular O_2 tension and can be used as a measure for sensing extracellular O_2 tension (14, 24, 262). Therefore, the reason for the localization of direct O_2 sensors at the membrane is not clear.

The ArcBA TCS of *E. coli* is important for regulation of facultative anaerobic metabolism in response to O_2 availability. Under anaerobic conditions, ArcB is in the active state and phosphorylates the RR ArcA. ArcA~P represses genes of aerobic metabolism and activates genes involved in fermentative metabolism (112, 159). ArcB is an unorthodox sensor kinase with two N-terminal TM helices (aa 22 to 77) and a short periplasmic loop of 16 aa. It is a hybrid kinase with three cytoplasmic domains (Fig. 7) (catalytic domain with conserved

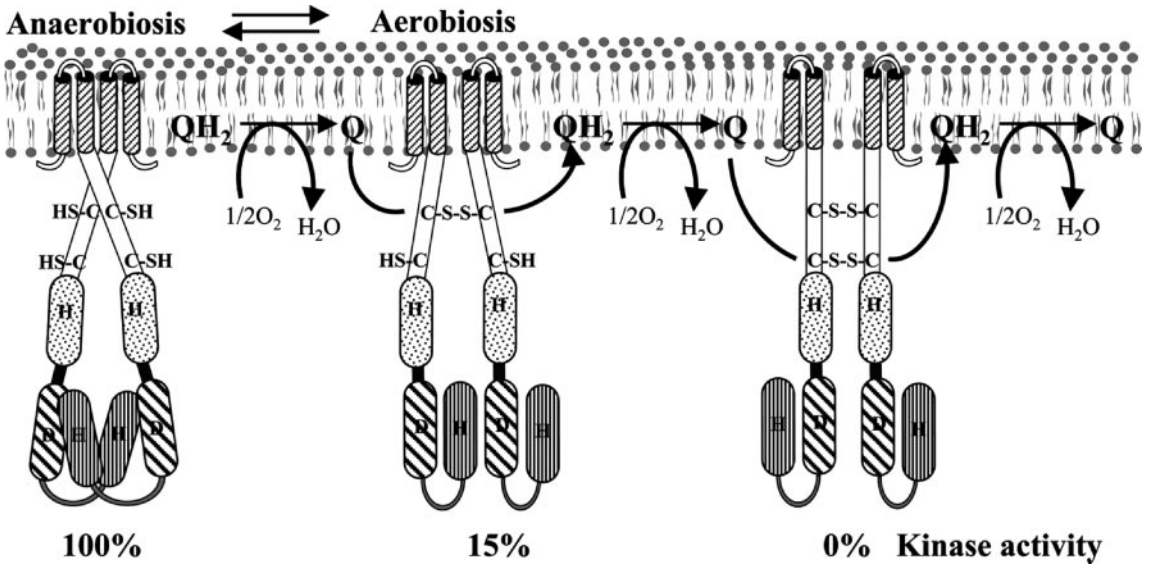

FIG. 8. Model for the redox regulation of the ArcB sensor kinase of *E. coli* through inactivation by oxygen. Upon a shift from anaerobic to aerobic growth conditions, the respiratory quinone (ubiquinone [Q]) becomes oxidized by oxygen via the respiratory chain. The oxidized quinones oxidize Cys180 of ArcB, resulting in an intermolecular Cys180/Cys180 disulfide bridge of the ArcB dimer. The resulting conformational changes lead to a reduced kinase activity of ArcB. Under fully oxic conditions, when the quinones are maximally oxidized by the respiratory chain, the quinones oxidize Cys241 of the ArcB linker domain. The additional conformational changes completely silence ArcB kinase activity. (Reprinted from reference 163 with permission of the publisher. Copyright 2004 National Academy of Sciences, U.S.A.).

H292, receiver domain with conserved D575, and C-terminal HPt domain with conserved His717) (110, 113). The linker between TM helix 2 and the transmitter/catalytic domain includes a leucine zipper (aa 77 to 115) and a PAS domain (aa 115 to 263) (73). Anoxic conditions cause autophosphorylation and intramolecular phosphoryl transfer of ArcB (H292→D576→H717), resulting in the phosphorylation of ArcA. Under nonstimulating conditions, ArcB functions as an ArcA~P phosphatase (73). ArcB senses the absence of O_2 indirectly by an intermediate of the aerobic respiratory chain at the membrane. Oxidized quinones, present under oxic conditions, silence the kinase activity of ArcB (74). A recent model (163) suggests that the quinones oxidize two Cys thiol residues in the PAS domain of ArcB, Cys180 and Cys241, in two steps. In the first step, the Cys_{180} residues of the ArcB dimer are oxidized, yielding an intermolecular Cys180-Cys180 disulfide. A second oxidation by quinones forms the Cys241-Cys241 intermolecular disulfide. The completely reduced dimer has full kinase activity, and the fully oxidized ArcB has lost kinase activity completely (Fig. 8). The disulfides are thought to cause a domain rearrangement and to disturb the interaction of the kinase domains.

The structure of the PAS domains, as well as its localization and that of the Cys residues relative to the membrane, is not known. Therefore, it is not clear how the membrane-integral quinones can oxidize the cytoplasmic Cys180 and Cys241. In a similar case, it has been shown that reduced quinones reduce periplasmic Cys residues of the membrane-bound protein disulfide reductase DsbB (17), and the quinone site of the NADH:ubiquinone oxidoreductase seems to be distant from the membrane, too (290). Presumably, the membrane-integral quinones are able to react at sites outside the membrane, when hydrophobic parts of the protein enable emerging of the reactive head group from the membrane. Thus, it appears that ArcB is a membrane-bound sensor kinase with a cytoplasmic sensor domain that controls kinase activity. The regulatory agent (oxidized quinones) is derived from the membrane. Therefore, membrane association is essential for ArcB function, representing a principal difference compared to FixL function.

Soluble HKs Associated with Membrane-Integral Sensory Proteins

There is a considerable number of soluble HK sensors, which are permanently or transiently associated with membrane-bound sensor proteins that function as the primary stimulus input site. The prototype of this type of sensory HK is CheA, which regulates chemotaxis in proteobacteria (25). The complex formed between the soluble HK CheA and MCP such as Tar has been studied in great detail and reviewed extensively in recent years (25, 29, 173, 239, 243, 244, 250, 266, 270). Therefore, it will be mentioned only briefly here, and readers are referred to the papers cited above for in-depth information. CheA, together with its cognate RR CheY, forms a soluble TCS controlling the flagellar motors of enteric bacteria. The CheAY TCS is conserved in many other bacteria and archaea (250). Upon CheA-mediated phosphorylation, CheY diffuses to the flagellar motor. Thereby, CheY-P controls motor and flagellar rotation and swimming of the bacteria (245, 270). The activity of the HK is regulated by the sensory MCP, which forms a receptor-CheA-CheW ternary complex. As described above, the MCP senses the extracellular stimuli either directly by the periplasmic binding site (e.g., Asp for Tar) or a binding protein (e.g., the maltose-binding protein for Tar) (22, 23, 60). Formation of the complex and stable signal transduc-

tion require stabilization of the complex by the coupling protein CheW (72, 188, 230). The signaling domain of the MCP in the complex consists of two helices (α7 and α8) and a loop between the helices, which are important for formation of the ternary complex and kinase regulation. Overall, soluble CheA-like HKs perceive their stimuli by interaction with the primary sensor such as the MCP.

Soluble, Cytoplasmic-Sensing HKs

Soluble HKs sense cytoplasmic stimuli, including (i) signals from cellular metabolism (e.g., NtrBC), (ii) the developmental or DNA replication state of the cell (e.g., KinA), or (iii) environmental stimuli that have direct access to the cell by diffusion or transmission (e.g., H_2 for HoxJA or light for phytochrome photoreceptors). Some prominent examples will be discussed to demonstrate the variety in function and composition of the cytoplasmic sensor HK.

NtrB is a paradigmatic soluble HK. The NtrBC TCS regulates the expression of genes important for the assimilation of ammonia and for the utilization of alternative N compounds (221). NtrB is a bifunctional HK, either catalyzing the autophosphorylation of NtrB (autokinase activity) and thereby serving as the phosphodonor for NtrC or promoting the rapid dephosphorylation of NtrC-P (phosphatase activity) (117, 273). This switch in activity is modulated by the PII protein, which senses the N supply of the cell. Under conditions of N excess, PII is present in the deuridylylated form. It then interacts with NtrB and converts it to a phosphatase. Under N limitation, PII is found in the uridylylated form (PII-UMP). PII-UMP does not interact with NtrB, which then functions, in the unbiased state, as an autokinase.

NtrB consists of C-terminal transmitter (aa 189 to 350) and N-terminal sensor (aa 1 to 120) domains, separated by an intervening H domain which carries the conserved His residue. The N-terminal sensor domain seems to be essential for stabilization of NtrB in the phosphatase conformation, whereas the H domain represents the site for PII interaction and signal input for switching from autokinase to phosphatase after binding of PII (132, 150, 211). The PAS-like sensor domain could be used for sensing additional unknown signals.

KinA is a key HK for the input of intracellular stimuli into the phosphorelay controlling onset of sporulation of *B. subtilis* (183, 207). KinA contains three PAS domains of uncertain function. The N-terminal PAS domain is important for spore formation and is a ATP-binding domain (241). It has been suggested that KinA senses the energy state of the cell in this way. In addition, KinA is regulated by binding of the inhibitor proteins Sda and KipI, which inhibit autophosphorylation of KinA (32, 225, 269).

In *Ralstonia eutropha*, the expression of the genes for hydrogenase and accessory genes requires activation by a soluble cytoplasmic-sensing TCS, the HoxJA proteins. Recognition of H_2 is mediated by a signal transduction/sensory complex formed between the kinase HoxJ and a sensory [Ni-Fe]-hydrogenase, HoxBC, which functions as the H_2 sensor (143, 155, 231). In the absence of H_2, HoxJ has a kinase activity, resulting in phosphorylation and inactivation of the cognate RR HoxA. This inactivation of an RR by phosphorylation stands in contrast to the case for most RRs, which usually become activated upon phosphorylation. In the presence of H_2, the sensory hydrogenase HoxBC becomes reduced by H_2 and inhibits the autophosphorylation of HoxJ by direct interaction and complex formation at the input domain, resulting in a dephosphorylated RR and transcriptional activation of the hydrogenase genes. Therefore, the function of the HoxJA TCS depends on signal input by the sensor hydrogenase HoxBC. This close association is reflected by the genomic organization of the corresponding genes (*hoxABCJ* are jointly transcribed), the formation of the sensory HoxBC-HoxJ complex, and at the functional level. A homologous system, HupUV-HupTR, has been described for *Rhodobacter capsulatus* (54).

Bacteriophytochromes were recognized as a new family of HKs by recent phylogenetic analysis of HK proteins (134, 135, 264, 265). These soluble proteins contain a phytochrome sensor domain, previously thought to be restricted to plants, where it is involved in light perception. The bacterial (BphPS) and cyanobacterial (Cphs) phytochromes all contain a GAF domain and often an additional N-terminal PAS domain (Fig. 8). By combining phylogenetic and biochemical studies, a role of all three conserved domains in photoreception was established. The phytochrome domain binds a bilin-type linear tetrapyrrol as a chromophore (44, 265). The GAF domain houses the bilin lyase activity, and a conserved Cys residue in the PAS domain is also important for chromophore binding (135). Several members of the family show autokinase activity in vitro, demonstrating their role in a phosphorelay. The physiological role of the (cyano-)bacterial phytochrome HK is not known.

Soluble Cytoplasmic-Sensing HKs: the "Missing Link" between Two-Component and One-Component Signal Transduction?

It should be pointed out that only about one-fifth of all HKs (fewer than 1,000) are soluble, cytoplasmic-sensing proteins. This seems to be a surprisingly small number, given the plethora of potential signals occurring within a bacterial cell. One convincing explanation can be found in the recent recognition of the importance and abundance of so-called "one-component systems" that are predominately used in bacteria for sensing of cytoplasmic signals (261). Their use represents a simplified design of signal transduction in case of singular cytoplasmic signals by directly fusing the input domain to an output domain, thereby omitting the unnecessary and energy-consuming phosphotransfer step of TCS. Sensor and output domains of one-component systems are evolutionary related to those of TCS. For example, RocR, a transcriptional regulator controlling arginine utilization in *B. subtilis* (33), closely resembles the NtrBC TCS (described above) with regard to its N-terminal PAS input and C-terminal helix-turn-helix DNA-binding domain, but it lacks the TCS-typical transmitter and receiver domains necessary for phosphotransfer (261). The specific role of one-component systems in cytoplasmic sensing and signal transduction processes is underscored by their cellular localization: while one-component systems hardly exist for TM signal transduction, they outnumber TCS in case of cytoplasmic signal perception by more than an order of magnitude (261).

COMBINATION OF SENSING DOMAINS

A large number of sensor kinases (~1,000) harbor more than one (putative) input domain, but the functional roles of these combined sensory domains are unclear so far. In cytoplasmic-sensing HK proteins, a single input domain seems to be the exception rather than the rule. Very often, PAS domains are duplicated (or even multiplied) in one single sensor kinase: the kinase KinA of *B. subtilis* harbors an array of three copies of PAS domains in its N-terminal input domain (241, 268), and about 100 cytoplasmic-sensing HKs harbor PAS together with GAF domains. The soluble phytochrome sensors also harbor additional GAF and sometimes PAS domains.

A combination of periplasmic and cytoplasmic sensing domains is also quite common and is even a characteristic feature of some subgroups listed among the periplasmic-sensing HKs (Table 2). Almost all BvgS-like sensors harbor a cytoplasmic PAS domain in addition to the periplasmic PBPb domain. Even the DcuS/CitA-like kinases, archetypes for periplasmic-sensing proteins, have an additional cytoplasmic PAS domain with uncertain roles. The same is true for the so-far-uncharacterized CHASE-type sensors.

The regular occurrence of sensor kinases with more than one sensory domain raises the question of the biological gain from such a protein architecture. The most obvious explanation seems to be the possibility of integrating and/or amplifying different input signals to maximize sensitivity. This concept is best exemplified by the signal integration and amplification mechanism of CheA in the chemotactic response, where the information of different chemoreceptors of various specificity, clustered in thousands in a tightly arranged array at the cell poles, is integrated through a relatively small number of CheA kinase proteins, thereby maximizing sensitivity and overall gain (29, 30, 239, 244).

A special form of this additive effect is exemplified by the "enhancer" stimulation of VirA: the cytoplasmic input domain of VirA is activated by phenolic compounds from the host plant cell (99, 287). This stimulation is strongly enhanced by the presence of aldose monosaccharides in the medium, which are also released by wounded plant cells (34, 235) and bound by a periplasmic sugar-binding protein, ChvE (36, 234). While phenolic compounds represent the primary signal for VirA autophosphorylation, activation of the periplasmic input domain has a strong additive enhancer effect on VirA activity.

It can be envisioned that the effects of combining sensory domains could be not only additive ("and" switch) but also inhibitory/mutually exclusive ("not" switch). Thereby, stimulatory information would be processed only in the absence of another signal that, if present, prevents autophosphorylation. Alternatively, different input stimuli that represent the same "type" of information for the cell may be sensed through a single HK with multiple input domains ("either-or" switch), thereby saving the cell the costs of expressing more than one protein for that purpose.

CONCLUSIONS AND OUTLOOK

Twenty years have passed since two-component systems were first recognized as a fundamental principle in bacterial signal perception and transduction (190). Still, our knowledge on the mechanisms of stimulus perception by the corresponding HK proteins is sparse and restricted to numerous vague indications, with only a few well-characterized examples. Here, we have summarized evidence from both the published work and a comparative genomics analysis, indicating that HK domain architecture correlates with the type of stimulus sensed. Therefore, grouping of HK proteins according to the domain architecture of the N-terminal input domain reflects functional aspects of stimulus perception and represents a useful functional classification tool.

Moreover, the data shown in Table 2 demonstrate a good correlation between sensing-domain architecture and sequence conservation in the transmitter domain, as well as a defined phylogenetic distribution for most groups of the corresponding sensor kinases, which is indicative for a phylogenetic relationship (i.e., a common evolutionary history), at least for the HKs addressed herein. While a certain domain architecture of the input domain is normally linked to a specific transmitter domain (conserved by sequence and therefore reflecting a phylogenetic relationship), the converse is not always true. ComP-like kinases can be clearly identified through the domain architecture of their input domain, and all corresponding proteins belong to the HPK7 subfamily (87) and are derived from *Firmicutes* bacteria, which is indicative of a common phylogenetic background. However, the members of the HPK7 subfamily, while being clearly related based on sequence conservation, come from a variety of functional groups and phyla. They also use different mechanisms of stimulus perception and are involved in responding to completely unrelated stimuli, such as nitrate (NarX/Q-like sensors), cell envelope stress (LiaS-like sensors), membrane fluidity (DesK-like sensors), and signaling peptides (ComP-like sensors). Only rarely is one type of input domain linked exclusively to a specific transmitter domain. For example, ComD/AgrC-like peptide quorum sensors from *Firmicutes* bacteria belong to HPK10, a subfamily of HKs that is also restricted to these HK proteins (Table 2), as has been observed previously (98, 280).

Taken together, these observations argue in favor of a combined functional/phylogenetic classification of sensor kinases based on input domain architecture, phylogenetic heritage, genomic context, and sequence conservation, rather than by virtue of the conserved features of the transmitter domain alone. Unfortunately, such a functional HK classification, as described in this review, is at the moment far from being comprehensive: the defined subgroups of HKs summarized in Table 2 so far only account for about 20% of all sensor kinases in the databases. While most (if not all) kinases can be linked to one of the three major groups (or a combination of them), the number of variations and individual exceptions within each group is sometimes very large, especially within the periplasmic-sensing HKs. It appears that the input domains of HKs were shuffled and duplicated and that new input and/or transmitter domains were added during evolution. This includes the combination of input domains (see above) or the formation of hybrid-type sensor kinases, harboring additional receiver domains at the C terminus for integrating more complex signal transduction pathways (285).

Some of the input domains for which experimental data on the mechanism of signal perception are available have only their respective domain architecture in common and do not

share a significant degree of sequence similarity (i.e., the peptide quorum sensors). On the other hand, there is hardly any functional information on the sensing mechanism of HKs with conserved input domains that were identified by comparative genomics. Additionally, the sensory domains of those kinases best understood with regard to the mechanism of stimulus perception (i.e., DcuS/CitA-like, PhoQ-like, or AgrC/ComD-like HKs) do not represent large subgroups of sensor kinases.

The input domains of many prototypical periplasmic-sensing HKs show a high degree of sequence diversity. As long as a further subgrouping based on both domain architecture and sequence conservation of the input domain is not feasible, these proteins must be regarded as individuals. Without experimental evidence on most of these sensory kinases, this observation can be interpreted in two ways. (i) The high degree of sequence diversity can be interpreted as a protein-specific evolution to facilitate the perception of unique stimuli (or, alternatively, a unique mechanism of stimulus perception). (ii) It might also be that a number of N-terminal domains in these sensor kinases are evolutionary remnants without a functional role in signal perception, as has been suggested previously (102). This interpretation is especially tempting for those HK proteins that harbor additional (i.e., cytoplasmic) putative input domains. However, the success of the first comprehensive sequence analyses on signal perception domains (5–7, 65, 67, 68, 186, 203, 288, 289) raises the hope that additional signal perception domains will be identified by applying more sophisticated bioinformatic search algorithms hand-in-hand with "classical" approaches of molecular biology. Determination of additional input domain structures might be a key in identifying common features of signal perception, especially for the periplasmic-sensing HKs.

Clearly, our present knowledge on the mechanism of signal perception by bacterial HKs is rudimentary, at best, compared to the overwhelming diversity of signaling domains. It seems that we have barely begun to understand the complexity of sensor kinase-mediated signal perception that, based on a simple and exchangeable modular design, allows bacteria to cope with life in an ever-changing environment. However, the available data together with comprehensive analyses of the sequence and domain architecture of more than 4,000 HKs in the databases enabled us to group HKs according to their input domains and sensing mechanisms. We hope that this overview will stimulate and intensify research on the modes of signal perception of different groups of HKs, in order to finally understand and view sensor kinases for what they really are: biochemical senses of the bacterial cell.

ACKNOWLEDGMENTS

We thank Michael Bott and Tarek Msadek for critical reading of the manuscript and helpful suggestions and comments.

Work in the authors' labs was supported by the Deutsche Forschungsgemeinschaft and grants from the Fonds der Chemischen Industrie (to T.M. and G.U.) and by NIH grant GM047446 (to J.D.H.).

ADDENDUM IN PROOF

Since the manuscript was accepted for publication, two new structures of bacterial signaling domains were published. Work by M. Hulko et al. (Cell **126:**929–940, 2006) shows that the HAMP domain (connecting the periplasmic sensor domains or TM helices with the cytoplasmic transmitter domain in many HKs) forms a four-helix structure in the homodimer. Rotation of the helices is suggested to transduce the signal to the transmitter domain. The structure of the periplasmic sensory complex $LuxPQ_P$ with and without bound autoinducer-2 (a furanosyl borate diester that acts as a quorum-sensing signal in the bioluminescent marine bacterium *Vibrio harveyi*) revealed that its binding causes major conformational changes within the LuxPQ periplasmic region. It is suggested that these changes affect the positioning of the transmembrane helices of the sensor kinase LuxQ, thereby transducing the signal to the cytoplasmic transmitter domain, which ultimately inhibits its kinase activity (M. B. Neiditch et al., Cell **126:**1095–1108, 2006).

REFERENCES

1. **Abada, E. M., A. Balzer, A. Jager, and G. Klug.** 2002. Bacteriochlorophyll-dependent expression of genes for pigment-binding proteins in *Rhodobacter capsulatus* involves the RegB/RegA two-component system. Mol. Genet. Genomics **267:**202–209.
2. **Aguilar, P. S., J. E. Cronan, Jr., and D. de Mendoza.** 1998. A *Bacillus subtilis* gene induced by cold shock encodes a membrane phospholipid desaturase. J. Bacteriol. **180:**2194–2200.
3. **Aguilar, P. S., A. M. Hernandez-Arriaga, L. E. Cybulski, A. C. Erazo, and D. de Mendoza.** 2001. Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*. EMBO J. **20:**1681–1691.
4. **Aguilar, P. S., P. Lopez, and D. de Mendoza.** 1999. Transcriptional control of the low-temperature-inducible *des* gene, encoding the Δ5 desaturase of *Bacillus subtilis*. J. Bacteriol. **181:**7028–7033.
5. **Anantharaman, V., and L. Aravind.** 2003. Application of comparative genomics in the identification and analysis of novel families of membrane-associated receptors in bacteria. BMC Genomics **4:**34.
6. **Anantharaman, V., and L. Aravind.** 2000. Cache—a signaling domain common to animal Ca(2+)-channel subunits and a class of prokaryotic chemotaxis receptors. Trends Biochem. Sci. **25:**535–537.
7. **Anantharaman, V., and L. Aravind.** 2001. The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacterial receptors. Trends Biochem. Sci. **26:**579–582.
8. **Ansaldi, M., D. Marolt, T. Stebe, I. Mandic-Mulec, and D. Dubnau.** 2002. Specific activation of the *Bacillus* quorum-sensing systems by isoprenylated pheromone variants. Mol. Microbiol. **44:**1561–1573.
9. **Antoine, R., I. Huvent, K. Chemlal, I. Deray, D. Raze, C. Locht, and F. Jacob-Dubuisson.** 2005. The periplasmic binding protein of a tripartite tricarboxylate transporter is involved in signal transduction. J. Mol. Biol. **351:**799–809.
10. **Appleman, J. A., L. L. Chen, and V. Stewart.** 2003. Probing conservation of HAMP linker structure and signal transduction mechanism through analysis of hybrid sensor kinases. J. Bacteriol. **185:**4872–4882.
11. **Appleman, J. A., and V. Stewart.** 2003. Mutational analysis of a conserved signal-transducing element: the HAMP linker of the *Escherichia coli* nitrate sensor NarX. J. Bacteriol. **185:**89–97.
12. **Aravind, L., and C. P. Ponting.** 1999. The cytoplasmic helical linker domain of receptor histidine kinase and methyl-accepting proteins is common to many prokaryotic signalling proteins. FEMS Microbiol. Lett. **176:**111–116.
13. **Aravind, L., and C. P. Ponting.** 1997. The GAF domain: an evolutionary link between diverse phototransducing proteins. Trends Biochem. Sci. **22:**458–459.
14. **Arras, T., J. Schirawski, and G. Unden.** 1998. Availability of O_2 as a substrate in the cytoplasm of bacteria under aerobic and microaerobic conditions. J. Bacteriol. **180:**2133–2136.
15. **Asai, K., S. H. Baik, Y. Kasahara, S. Moriya, and N. Ogasawara.** 2000. Regulation of the transport system for C_4-dicarboxylic acids in *Bacillus subtilis*. Microbiology **146:**263–271.
16. **Ashby, M. K., and J. Houmard.** 2006. Cyanobacterial two-component proteins: structure, diversity, distribution, and evolution. Microbiol. Mol. Biol. Rev. **70:**472–509.
17. **Bader, M., W. Muse, D. P. Ballou, C. Gassner, and J. C. Bardwell.** 1999. Oxidative protein folding is driven by the electron transport system. Cell **98:**217–227.
18. **Bader, M. W., S. Sanowar, M. E. Daley, A. R. Schneider, U. Cho, W. Xu, R. E. Klevit, H. Le Moual, and S. I. Miller.** 2005. Recognition of antimicrobial peptides by a bacterial sensor kinase. Cell **122:**461–472.
19. **Baikalov, I., I. Schroder, M. Kaczor-Grzeskowiak, K. Grzeskowiak, R. P. Gunsalus, and R. E. Dickerson.** 1996. Structure of the *Escherichia coli* response regulator NarL. Biochemistry **35:**11053–11061.
20. **Banta, L. M., R. D. Joerger, V. R. Howitz, A. M. Campbell, and A. N. Binns.** 1994. Glu-255 outside the predicted ChvE binding site in VirA is crucial for sugar enhancement of acetosyringone perception by *Agrobacterium tumefaciens*. J. Bacteriol. **176:**3242–3249.
21. **Bantscheff, M., A. L. Perraud, A. Bock, K. Rippe, V. Weiss, M. Glocker, and R. Gross.** 2000. Structure-function relationships in the Bvg and Evg two-component phosphorelay systems. Int. J. Med. Microbiol. **290:**317–323.
22. **Bass, R. B., M. D. Coleman, and J. J. Falke.** 1999. Signaling domain of the

aspartate receptor is a helical hairpin with a localized kinase docking surface: cysteine and disulfide scanning studies. Biochemistry **38:**9317–9327.
23. **Bass, R. B., and J. J. Falke.** 1999. The aspartate receptor cytoplasmic domain: in situ chemical analysis of structure, mechanism and dynamics. Structure Fold Des. **7:**829–840.
24. **Becker, S., G. Holighaus, T. Gabrielczyk, and G. Unden.** 1996. O_2 as the regulatory signal for FNR-dependent gene regulation in *Escherichia coli*. J. Bacteriol. **178:**4515–4521.
25. **Bilwes, A. M., S.-Y. Park, C. M. Quezada, M. I. Simon, and B. R. Crane.** 2003. Structure and function of CheA, the histidine kinase central to bacterial chemotaxis, p. 48–73. *In* M. Inouye and R. Dutta (ed.), Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
26. **Bock, A., and R. Gross.** 2002. The unorthodox histidine kinases BvgS and EvgS are responsive to the oxidation status of a quinone electron carrier. Eur. J. Biochem. **269:**3479–3484.
27. **Bollinger, J., C. Park, S. Harayama, and G. L. Hazelbauer.** 1984. Structure of the Trg protein: homologies with and differences from other sensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81:**3287–3291.
28. **Borgstahl, G. E., D. R. Williams, and E. D. Getzoff.** 1995. 1.4 Å structure of photoactive yellow protein, a cytosolic photoreceptor: unusual fold, active site, and chromophore. Biochemistry **34:**6278–6287.
29. **Bray, D.** 2002. Bacterial chemotaxis and the question of gain. Proc. Natl. Acad. Sci. USA **99:**7–9.
30. **Bray, D., M. D. Levin, and C. J. Morton-Firth.** 1998. Receptor clustering as a cellular mechanism to control sensitivity. Nature **393:**85–88.
31. **Budde, I. P., B. H. Rohde, C. L. Bender, and M. S. Ullrich.** 1998. Growth phase and temperature influence promoter activity, transcript abundance, and protein stability during biosynthesis of the *Pseudomonas syringae* phytotoxin coronatine. J. Bacteriol. **180:**1360–1367.
32. **Burkholder, W. F., I. Kurtser, and A. D. Grossman.** 2001. Replication initiation proteins regulate a developmental checkpoint in *Bacillus subtilis*. Cell **104:**269–279.
33. **Calogero, S., R. Gardan, P. Glaser, J. Schweizer, G. Rapoport, and M. Debarbouille.** 1994. RocR, a novel regulatory protein controlling arginine utilization in *Bacillus subtilis*, belongs to the NtrC/NifA family of transcriptional activators. J. Bacteriol. **176:**1234–1241.
34. **Cangelosi, G. A., R. G. Ankenbauer, and E. W. Nester.** 1990. Sugars induce the *Agrobacterium* virulence genes through a periplasmic binding protein and a transmembrane signal protein. Proc. Natl. Acad. Sci. USA **87:**6708–6712.
35. **Cavicchioli, R., R. C. Chiang, L. V. Kalman, and R. P. Gunsalus.** 1996. Role of the periplasmic domain of the *Escherichia coli* NarX sensor-transmitter protein in nitrate-dependent signal transduction and gene regulation. Mol. Microbiol. **21:**901–911.
36. **Chang, C. H., and S. C. Winans.** 1992. Functional roles assigned to the periplasmic, linker, and receiver domains of the *Agrobacterium tumefaciens* VirA protein. J. Bacteriol. **174:**7033–7039.
37. **Chen, I., and D. Dubnau.** 2004. DNA uptake during bacterial transformation. Nat. Rev. Microbiol. **2:**241–249.
38. **Chen, W., A. Jager, and G. Klug.** 2000. Correction of the DNA sequence of the *regB* gene of *Rhodobacter capsulatus* with implications for the membrane topology of the sensor kinase RegB. J. Bacteriol. **182:**818–820.
39. **Chi, Y. I., H. Yokota, and S. H. Kim.** 1997. Apo structure of the ligand-binding domain of aspartate receptor from *Escherichia coli* and its comparison with ligand-bound or pseudoligand-bound structures. FEBS Lett. **414:**327–332.
40. **Cho, U. S., M. W. Bader, M. F. Amaya, M. E. Daley, R. E. Klevit, S. I. Miller, and W. Xu.** 2006. Metal bridges between the PhoQ sensor domain and the membrane regulate transmembrane signaling. J. Mol. Biol. **356:**1193–1206.
41. **Cotter, P. A., and A. M. Jones.** 2003. Phosphorelay control of virulence gene expression in *Bordetella*. Trends Microbiol. **11:**367–373.
42. **Cybulski, L. E., D. Albanesi, M. C. Mansilla, S. Altabe, P. S. Aguilar, and D. de Mendoza.** 2002. Mechanism of membrane fluidity optimization: isothermal control of the *Bacillus subtilis* acyl-lipid desaturase. Mol. Microbiol. **45:**1379–1388.
43. **Dathe, M., and T. Wieprecht.** 1999. Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. Biochim. Biophys. Acta **1462:**71–87.
44. **Davis, S. J., A. V. Vener, and R. D. Vierstra.** 1999. Bacteriophytochromes: phytochrome-like photoreceptors from nonphotosynthetic eubacteria. Science **286:**2517–2520.
45. **Deretic, V., J. H. Leveau, C. D. Mohr, and N. S. Hibler.** 1992. In vitro phosphorylation of AlgR, a regulator of mucoidy in *Pseudomonas aeruginosa*, by a histidine protein kinase and effects of small phospho-donor molecules. Mol. Microbiol. **6:**2761–2767.
46. **Derzelle, S., S. Ngo, E. Turlin, E. Duchaud, A. Namane, F. Kunst, A. Danchin, P. Bertin, and J.-F. Charles.** 2004. AstR-AstS, a new two-component signal transduction system, mediates swarming, adaptation to stationary phase and phenotypic variation in Photorhabdus luminescens. Microbiology **150:**897–910.
47. **Diep, D. B., R. Myhre, O. Johnsborg, A. Aakra, and I. F. Nes.** 2003. Inducible bacteriocin production in *Lactobacillus* is regulated by differential expression of the *pln* operons and by two antagonizing response regulators, the activity of which is enhanced upon phosphorylation. Mol. Microbiol. **47:**483–494.
48. **Dirix, G., P. Monsieurs, B. Dombrecht, R. Daniels, K. Marchal, J. Vanderleyden, and J. Michiels.** 2004. Peptide signal molecules and bacteriocins in Gram-negative bacteria: a genome-wide in silico screening for peptides containing a double-glycine leader sequence and their cognate transporters. Peptides **25:**1425–1440.
49. **Doty, S., M. Yu, J. Lundin, J. Heath, and E. Nester.** 1996. Mutational analysis of the input domain of the VirA protein of *Agrobacterium tumefaciens*. J. Bacteriol. **178:**961–970.
50. **Dufour, P., S. Jarraud, F. Vandenesch, T. Greenland, R. P. Novick, M. Bes, J. Etienne, and G. Lina.** 2002. High genetic variability of the *agr* locus in *Staphylococcus* species. J. Bacteriol. **184:**1180–1186.
51. **Dunny, G. M., and S. C. Winans.** 1999. Cell-cell signaling in bacteria. ASM Press, Washington, D.C.
52. **Dutta, R., and M. Inouye.** 1996. Reverse phosphotransfer from OmpR to EnvZ in a kinase−/phosphatase+ mutant of EnvZ (EnvZ.N347D), a bifunctional signal transducer of *Escherichia coli*. J. Biol. Chem. **271:**1424–1429.
53. **Dutta, R., L. Qin, and M. Inouye.** 1999. Histidine kinases: diversity of domain organization. Mol. Microbiol. **34:**633–640.
54. **Elsen, S., O. Duche, and A. Colbeau.** 2003. Interaction between the H_2 sensor HupUV and the histidine kinase HupT controls HupSL hydrogenase synthesis in *Rhodobacter capsulatus*. J. Bacteriol. **185:**7111–7119.
55. **Elsen, S., L. R. Swem, D. L. Swem, and C. E. Bauer.** 2004. RegB/RegA, a highly conserved redox-responding global two-component regulatory system. Microbiol. Mol. Biol. Rev. **68:**263–279.
56. **Eraso, J., and S. Kaplan.** 1995. Oxygen-insensitive synthesis of the photosynthetic membranes of *Rhodobacter sphaeroides*: a mutant histidine kinase. J. Bacteriol. **177:**2695–2706.
57. **Eraso, J. M., and S. Kaplan.** 2000. From redox flow to gene regulation: role of the PrrC protein of *Rhodobacter sphaeroides* 2.4.1. Biochemistry **39:**2052–2062.
58. **Errington, J.** 2003. Regulation of endospore formation in *Bacillus subtilis*. Nat. Rev. Microbiol. **1:**117–126.
59. **Fabret, C., V. A. Feher, and J. A. Hoch.** 1999. Two-component signal transduction in *Bacillus subtilis*: how one organism sees its world. J. Bacteriol. **181:**1975–1983.
60. **Falke, J. J., and G. L. Hazelbauer.** 2001. Transmembrane signaling in bacterial chemoreceptors. Trends Biochem. Sci. **26:**257–265.
61. **Fields, P. I., E. A. Groisman, and F. Heffron.** 1989. A *Salmonella* locus that controls resistance to microbicidal proteins from phagocytic cells. Science **243:**1059–1062.
62. **Freeman, J. A., B. N. Lilley, and B. L. Bassler.** 2000. A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. Mol. Microbiol. **35:**139–149.
63. **Fülöp, V., and D. T. Jones.** 1999. Beta propellers: structural rigidity and functional diversity. Curr. Opin. Struct. Biol. **9:**715–721.
64. **Fuqua, C., M. R. Parsek, and E. P. Greenberg.** 2001. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu. Rev. Genet. **35:**439–468.
65. **Galperin, M. Y.** 2004. Bacterial signal transduction network in a genomic perspective. Environ. Microbiol. **6:**552–567.
66. **Galperin, M. Y.** 2006. Structural classification of bacterial response regulators: diversity of output domains and domain combinations. J. Bacteriol. **188:**4169–4182.
67. **Galperin, M. Y., T. A. Gaidenko, A. Y. Mulkidjanian, M. Nakano, and C. W. Price.** 2001. MHYT, a new integral membrane sensor domain. FEMS Microbiol. Lett. **205:**17–23.
68. **Galperin, M. Y., A. N. Nikolskaya, and E. V. Koonin.** 2001. Novel domains of the prokaryotic two-component signal transduction systems. FEMS Microbiol. Lett. **203:**11–21.
69. **Gao, R., and D. G. Lynn.** 2005. Environmental pH sensing: resolving the VirA/VirG two-component system inputs for *Agrobacterium* pathogenesis. J. Bacteriol. **187:**2182–2189.
70. **Garcia Vescovi, E., F. C. Soncini, and E. A. Groisman.** 1996. Mg^{2+} as an extracellular signal: environmental regulation of *Salmonella* virulence. Cell **84:**165–174.
71. **Garrity, L. F., S. L. Schiel, R. Merrill, J. Reizer, M. H. Saier, Jr., and G. W. Ordal.** 1998. Unique regulation of carbohydrate chemotaxis in *Bacillus subtilis* by the phosphoenolpyruvate-dependent phosphotransferase system and the methyl-accepting chemotaxis protein McpC. J. Bacteriol. **180:**4475–4480.
72. **Gegner, J. A., D. R. Graham, A. F. Roth, and F. W. Dahlquist.** 1992. Assembly of an MCP receptor, CheW, and kinase CheA complex in the bacterial chemotaxis signal transduction pathway. Cell **70:**975–982.
73. **Georgellis, D., O. Kwon, P. De Wulf, and E. C. Lin.** 1998. Signal decay through a reverse phosphorelay in the Arc two-component signal transduction system. J. Biol. Chem. **273:**32864–32869.
74. **Georgellis, D., O. Kwon, and E. C. C. Lin.** 2001. Quinones as the redox

signal for the Arc two-component system of bacteria. Science **292:**2314–2316.
75. **Gerharz, T., S. Reinelt, S. Kaspar, L. Scapozza, and M. Bott.** 2003. Identification of basic amino acid residues important for citrate binding by the periplasmic receptor domain of the sensor kinase CitA. Biochemistry **42:**5917–5924.
76. **Gilles-Gonzalez, M. A., G. S. Ditta, and D. R. Helinski.** 1991. A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. Nature **350:**170–172.
77. **Gilles-Gonzalez, M. A., and G. Gonzalez.** 2005. Heme-based sensors: defining characteristics, recent developments, and regulatory hypotheses. J. Inorg. Biochem. **99:**1–22.
78. **Gilles-Gonzalez, M. A., and G. Gonzalez.** 1993. Regulation of the kinase activity of heme protein FixL from the two-component system FixL/FixJ of *Rhizobium meliloti*. J. Biol. Chem. **268:**16293–16297.
79. **Gilles-Gonzalez, M.-A., and G. Gonzalez.** 2004. Signal transduction by heme-containing PAS-domain proteins. J. Appl. Physiol. **96:**774–783.
80. **Gilles-Gonzalez, M. A., G. Gonzalez, M. F. Perutz, L. Kiger, M. C. Marden, and C. Poyart.** 1994. Heme-based sensors, exemplified by the kinase FixL, are a new class of heme protein with distinctive ligand binding and autoxidation. Biochemistry **33:**8067–8073.
81. **Giraudo, A. T., A. Calzolari, A. A. Cataldi, C. Bogni, and R. Nagel.** 1999. The *sae* locus of *Staphylococcus aureus* encodes a two-component regulatory system. FEMS Microbiol. Lett. **177:**15–22.
82. **Giraudo, A. T., A. L. Cheung, and R. Nagel.** 1997. The *sae* locus of *Staphylococcus aureus* controls exoprotein synthesis at the transcriptional level. Arch. Microbiol. **168:**53–58.
83. **Gon, S., C. Jourlin-Castelli, L. Theraulaz, and V. Mejean.** 2001. An unsuspected autoregulatory pathway involving apocytochrome TorC and sensor TorS in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **98:**11615–11620.
84. **Gong, W., B. Hao, and M. K. Chan.** 2000. New mechanistic insights from structural studies of the oxygen-sensing domain of *Bradyrhizobium japonicum* FixL. Biochemistry **39:**3955–3962.
85. **Gong, W., B. Hao, S. S. Mansy, G. Gonzalez, M. A. Gilles-Gonzalez, and M. K. Chan.** 1998. Structure of a biological oxygen sensor: a new mechanism for heme-driven signal transduction. Proc. Natl. Acad. Sci. USA **95:**15177–15182.
86. **Goodman, A. L., B. Kulasekara, A. Rietsch, D. Boyd, R. S. Smith, and S. Lory.** 2004. A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. Dev. Cell **7:**745–754.
87. **Grebe, T. W., and J. B. Stock.** 1999. The histidine protein kinase superfamily. Adv. Microb. Physiol. **41:**139–227.
88. **Green, J., and M. S. Paget.** 2004. Bacterial redox sensors. Nat. Rev. Microbiol. **2:**954–966.
89. **Gunn, J. S., K. B. Lim, J. Krueger, K. Kim, L. Guo, M. Hackett, and S. I. Miller.** 1998. PmrA-PmrB-regulated genes necessary for 4-aminoarabinose lipid A modification and polymyxin resistance. Mol. Microbiol. **27:**1171–1182.
90. **Gunn, J. S., S. S. Ryan, J. C. Van Velkinburgh, R. K. Ernst, and S. I. Miller.** 2000. Genetic and functional analysis of a PmrA-PmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar Typhimurium. Infect. Immun. **68:**6139–6146.
91. **Guo, L., K. B. Lim, J. S. Gunn, B. Bainbridge, R. P. Darveau, M. Hackett, and S. I. Miller.** 1997. Regulation of lipid A modifications by *Salmonella typhimurium* virulence genes *phoP-phoQ*. Science **276:**250–253.
92. **Haas, W., D. Kaushal, J. Sublett, C. Obert, and E. I. Tuomanen.** 2005. Vancomycin stress response in a sensitive and a tolerant strain of *Streptococcus pneumoniae*. J. Bacteriol. **187:**8205–8210.
93. **Hamoen, L. W., G. Venema, and O. P. Kuipers.** 2003. Controlling competence in *Bacillus subtilis*: shared use of regulators. Microbiology **149:**9–17.
94. **Hanlon, D., and G. Ordal.** 1994. Cloning and characterization of genes encoding methyl-accepting chemotaxis proteins in *Bacillus subtilis*. J. Biol. Chem. **269:**14038–14046.
95. **Happ, H. N., S. Braatsch, V. Broschek, L. Osterloh, and G. Klug.** 2005. Light-dependent regulation of photosynthesis genes in *Rhodobacter sphaeroides* 2.4.1 is coordinately controlled by photosynthetic electron transport via the PrrBA two-component system and the photoreceptor AppA. Mol. Microbiol. **58:**903–914.
96. **Häse, C. C., N. D. Fedorova, M. Y. Galperin, and P. A. Dibrov.** 2001. Sodium ion cycle in bacterial pathogens: evidence from cross-genome comparisons. Microbiol. Mol. Biol. Rev. **65:**353–370.
97. **Håvarstein, L., R. Hakenbeck, and P. Gaustad.** 1997. Natural competence in the genus *Streptococcus*: evidence that streptococci can change pherotype by interspecies recombinational exchanges. J. Bacteriol. **179:**6589–6594.
98. **Håvarstein, L. S.** 2003. Intercellular communication in Gram-positive bacteria depends on peptide pheromones and their histidine kinase receptors, p. 341–363. *In* M. Inouye and R. Dutta (ed.), Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
99. **Heath, J. D., T. C. Charles, and E. W. Nester.** 1995. Ti plasmid and chromosomally encoded two-component systems important in plant cell transformation by *Agrobacterium* species, p. 367–386. *In* J. A. Hoch and T. J. Silhavy (ed.), Two-component signal transduction. ASM Press, Washington, D.C.
100. **Heermann, R., K. Altendorf, and K. Jung.** 2003. The N-terminal input domain of the sensor kinase KdpD of *Escherichia coli* stabilizes the interaction between the cognate response regulator KdpE and the corresponding DNA-binding site. J. Biol. Chem. **278:**51277–51284.
101. **Heermann, R., A. Fohrmann, K. Altendorf, and K. Jung.** 2003. The transmembrane domains of the sensor kinase KdpD of *Escherichia coli* are not essential for sensing K^+ limitation. Mol. Microbiol. **47:**839–848.
102. **Hoch, J. A.** 2000. Two-component and phosphorelay signal transduction. Curr. Opin. Microbiol. **3:**165–170.
103. **Hoch, J. A., and T. J. Silhavy (ed.).** 1995. Two-component signal transduction. ASM Press, Washington, D.C.
104. **Hong, H. J., M. I. Hutchings, J. M. Neu, G. D. Wright, M. S. Paget, and M. J. Buttner.** 2004. Characterization of an inducible vancomycin resistance system in *Streptomyces coelicolor* reveals a novel gene (*vanK*) required for drug resistance. Mol. Microbiol. **52:**1107–1121.
105. **Hunger, K., C. L. Beckering, and M. A. Marahiel.** 2004. Genetic evidence for the temperature-sensing ability of the membrane domain of the *Bacillus subtilis* histidine kinase DesK. FEMS Microbiol. Lett. **230:**41–46.
106. **Hutchings, M. I., H. J. Hong, and M. J. Buttner.** 2006. The vancomycin resistance VanRS two-component signal transduction system of *Streptomyces coelicolor*. Mol. Microbiol. **59:**923–935.
107. **Hutchings, M. I., P. A. Hoskisson, G. Chandra, and M. J. Buttner.** 2004. Sensing and responding to diverse extracellular signals? Analysis of the sensor kinases and response regulators of *Streptomyces coelicolor* A3(2). Microbiology **150:**2795–2806.
108. **Inouye, M., and R. Dutta (ed.).** 2003. Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
109. **Inouye, M., R. Dutta, and Y. Zhu.** 2003. Regulation of porins in *Escherichia coli* by the osmosensing histidine kinase/phosphatase EnvZ, p. 27–47. *In* M. Inouye and R. Dutta (ed.), Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
110. **Ishige, K., S. Nagasawa, S. Tokishita, and T. Mizuno.** 1994. A novel device of bacterial signal transducers. EMBO J. **13:**5195–5202.
111. **Island, M. D., and R. J. Kadner.** 1993. Interplay between the membrane-associated UhpB and UhpC regulatory proteins. J. Bacteriol. **175:**5028–5034.
112. **Iuchi, S., and E. C. Lin.** 1988. *arcA* (dye), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. Proc. Natl. Acad. Sci. USA **85:**1888–1892.
113. **Iuchi, S., Z. Matsuda, T. Fujiwara, and E. C. Lin.** 1990. The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the *arc* modulon. Mol. Microbiol. **4:**715–727.
114. **Iwama, T., Y. Ito, H. Aoki, H. Sakamoto, S. Yamagata, K. Kawai, and I. Kawagishi.** 2006. Differential recognition of citrate and a metal-citrate complex by the bacterial chemoreceptor Tcp. J. Biol. Chem. **281:**17727–17735.
115. **Janausch, I. G., E. Zientz, Q. H. Tran, A. Kroger, and G. Unden.** 2002. C_4-dicarboxylate carriers and sensors in bacteria. Biochim. Biophys. Acta **1553:**39–56.
116. **Ji, G., R. Beavis, and R. Novick.** 1995. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc. Natl. Acad. Sci. USA **92:**12055–12059.
117. **Jiang, P., A. A. Pioszak, M. R. Atkinson, J. A. Peliska, and A. J. Ninfa.** 2003. New insights into the mechanism of the kinase and phosphatase activities of *Escherichia coli* NRII (NtrB) and their regulation by the PII protein, p. 143–164. *In* M. Inouye and R. Dutta (ed.), Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
118. **Johnsborg, O., D. B. Diep, and I. F. Nes.** 2003. Structural analysis of the peptide pheromone receptor PlnB, a histidine protein kinase from *Lactobacillus plantarum*. J. Bacteriol. **185:**6913–6920.
119. **Johnsborg, O., L. H. Godager, and I. F. Nes.** 2004. Identification of a region involved in the pheromone receptor function of the histidine kinase PlnB. Arch. Microbiol. **182:**450–457.
120. **Jordan, S., A. Junker, J. D. Helmann, and T. Mascher.** 2006. Regulation of LiaRS-dependent gene expression in *Bacillus subtilis*: identification of inhibitor proteins, regulator bindings sites and target genes of a conserved cell envelope stress-sensing two-component system. J. Bacteriol. **188:**5153–5166.
121. **Joseph, P., G. Fichant, Y. Quentin, and F. Denizot.** 2002. Regulatory relationship of two-component and ABC transport systems and clustering of their genes in the *Bacillus*/*Clostridium* group, suggest a functional link between them. J. Mol. Microbiol. Biotechnol. **4:**503–513.
122. **Joseph, P., A. Guiseppi, A. Sorokin, and F. Denizot.** 2004. Characterization of the *Bacillus subtilis* YxdJ response regulator as the inducer of expression for the cognate ABC transporter YxdLM. Microbiology **150:**2609–2617.
123. **Jourlin, C., A. Bengrine, M. Chippaux, and V. Mejean.** 1996. An unorthodox sensor protein (TorS) mediates the induction of the *tor* structural genes in response to trimethylamine N-oxide in *Escherichia coli*. Mol. Microbiol. **20:**1297–1306.

124. **Jung, H.** 2002. The sodium/substrate symporter family: structural and functional features. FEBS Lett. **529:**73–77.
125. **Jung, H.** 2001. Towards the molecular mechanism of Na(+)/solute symport in prokaryotes. Biochim. Biophys. Acta **1505:**131–143.
126. **Jung, H., R. Rubenhagen, S. Tebbe, K. Leifker, N. Tholema, M. Quick, and R. Schmid.** 1998. Topology of the Na+/proline transporter of *Escherichia coli*. J. Biol. Chem. **273:**26400–26407.
127. **Jung, K., and K. Altendorf.** 1998. Individual substitutions of clustered arginine residues of the sensor kinase KdpD of *Escherichia coli* modulate the ratio of kinase to phosphatase activity. J. Biol. Chem. **273:**26415–26420.
128. **Jung, K., and K. Altendorf.** 2002. Towards an understanding of the molecular mechanisms of stimulus perception and signal transduction by the KdpD/KdpE system of *Escherichia coli*. J. Mol. Microbiol. Biotechnol. **4:**223–228.
129. **Jung, K., and K. Altendorf.** 1998. Truncation of amino acids 12–128 causes deregulation of the phosphatase activity of the sensor kinase KdpD of *Escherichia coli*. J. Biol. Chem. **273:**17406–17410.
130. **Kadner, R. J.** 1995. Expression of the Uhp sugar-phosphate transport system of *Escherichia coli*, p. 263–274. *In* J. A. Hoch and T. J. Silhavy (ed.), Two-component signal transduction. ASM Press, Washington, D.C.
131. **Kalman, L. V., and R. P. Gunsalus.** 1990. Nitrate- and molybdenum-independent signal transduction mutations in *narX* that alter regulation of anaerobic respiratory genes in *Escherichia coli*. J. Bacteriol. **172:**7049–7056.
132. **Kamberov, E. S., M. R. Atkinson, and A. J. Ninfa.** 1995. The *Escherichia coli* PII signal transduction protein is activated upon binding 2-ketoglutarate and ATP. J. Biol. Chem. **270:**17797–17807.
133. **Karniol, B., and R. D. Vierstra.** 2004. The HWE histidine kinases, a new family of bacterial two-component sensor kinases with potentially diverse roles in environmental signaling. J. Bacteriol. **186:**445–453.
134. **Karniol, B., and R. D. Vierstra.** 2003. The pair of bacteriophytochromes from *Agrobacterium tumefaciens* are histidine kinases with opposing photobiological properties. Proc. Natl. Acad. Sci. USA **100:**2807–2812.
135. **Karniol, B., J. R. Wagner, J. M. Walker, and R. D. Vierstra.** 2005. Phylogenetic analysis of the phytochrome superfamily reveals distinct microbial subfamilies of photoreceptors. Biochem. J. **392:**103–116.
136. **Kaspar, S., and M. Bott.** 2002. The sensor kinase CitA (DpiB) of *Escherichia coli* functions as a high-affinity citrate receptor. Arch. Microbiol. **177:**313–321.
137. **Kaspar, S., R. Perozzo, S. Reinelt, M. Meyer, K. Pfister, L. Scapozza, and M. Bott.** 1999. The periplasmic domain of the histidine autokinase CitA functions as a highly specific citrate receptor. Mol. Microbiol. **33:**858–872.
138. **Kato, A., H. Ohnishi, K. Yamamoto, E. Furuta, H. Tanabe, and R. Utsumi.** 2000. Transcription of *emrKY* is regulated by the EvgA-EvgS two-component system in *Escherichia coli* K-12. Biosci. Biotechnol. Biochem. **64:**1203–1209.
139. **Khorchid, A., M. Inouye, and M. Ikura.** 2005. Structural characterization of *Escherichia coli* sensor histidine kinase EnvZ: the periplasmic C-terminal core domain is critical for homodimerization. Biochem. J. **385:**255–264.
140. **Kim, D., and S. Forst.** 2001. Genomic analysis of the histidine kinase family in bacteria and archaea. Microbiology **147:**1197–1212.
141. **Kleerebezem, M., and L. E. Quadri.** 2001. Peptide pheromone-dependent regulation of antimicrobial peptide production in Gram-positive bacteria: a case of multicellular behavior. Peptides **22:**1579–1596.
142. **Kleerebezem, M., L. E. Quadri, O. P. Kuipers, and W. M. de Vos.** 1997. Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol. Microbiol. **24:**895–904.
143. **Kleihues, L., O. Lenz, M. Bernhard, T. Buhrke, and B. Friedrich.** 2000. The H_2 sensor of *Ralstonia eutropha* is a member of the subclass of regulatory [NiFe] hydrogenases. J. Bacteriol. **182:**2716–2724.
144. **Kneuper, H., I. G. Janausch, V. Vijayan, M. Zweckstetter, V. Bock, C. Griesinger, and G. Unden.** 2005. The nature of the stimulus and of the fumarate binding site of the fumarate sensor DcuS of *Escherichia coli*. J. Biol. Chem. **280:**20596–20603.
145. **Kobayashi, K., M. Ogura, H. Yamaguchi, K. Yoshida, N. Ogasawara, T. Tanaka, and Y. Fujita.** 2001. Comprehensive DNA microarray analysis of *Bacillus subtilis* two-component regulatory systems. J. Bacteriol. **183:**7365–7370.
146. **Kočan, M., S. Schaffer, T. Ishige, U. Sorger-Herrmann, V. F. Wendisch, and M. Bott.** 2006. Two-component systems of *Corynebacterium glutamicum*: deletion analysis and involvement of the PhoS-PhoR system in the phosphate starvation response. J. Bacteriol. **188:**724–732.
147. **Kondoh, H., C. B. Ball, and J. Adler.** 1979. Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **76:**260–264.
148. **König, J., A. Bock, A. L. Perraud, T. M. Fuchs, D. Beier, and R. Gross.** 2002. Regulatory factors of *Bordetella pertussis* affecting virulence gene expression. J. Mol. Microbiol. Biotechnol. **4:**197–203.
149. **Koretke, K. K., A. N. Lupas, P. V. Warren, M. Rosenberg, and J. R. Brown.** 2000. Evolution of two-component signal transduction. Mol. Biol. Evol. **17:**1956–1970.
150. **Kramer, G., and V. Weiss.** 1999. Functional dissection of the transmitter module of the histidine kinase NtrB in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **96:**604–609.
151. **Kuroda, M., H. Kuroda, T. Oshima, F. Takeuchi, H. Mori, and K. Hiramatsu.** 2003. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. Mol. Microbiol. **49:**807–821.
152. **Kvint, K., L. Nachin, A. Diez, and T. Nystrom.** 2003. The bacterial universal stress protein: function and regulation. Curr. Opin. Microbiol. **6:**140–145.
153. **Lange, R., C. Wagner, A. de Saizieu, N. Flint, J. Molnos, M. Stieger, P. Caspers, M. Kamber, W. Keck, and K. E. Amrein.** 1999. Domain organization and molecular characterization of 13 two-component systems identified by genome sequencing of *Streptococcus pneumoniae*. Gene **237:**223–234.
154. **Laskowski, M. A., and B. I. Kazmierczak.** 2006. Mutational analysis of RetS, an unusual sensor kinase-response regulator hybrid required for *Pseudomonas aeruginosa* virulence. Infect. Immun. **74:**4462–4473.
155. **Lenz, O., and B. Friedrich.** 1998. A novel multicomponent regulatory system mediates H_2 sensing in *Alcaligenes eutrophus*. Proc. Natl. Acad. Sci. USA **95:**12474–12479.
156. **Leonardo, M. R., and S. Forst.** 1996. Re-examination of the role of the periplasmic domain of EnvZ in sensing of osmolarity signals in *Escherichia coli*. Mol. Microbiol. **22:**405–413.
157. **Lina, G., S. Jarraud, G. Ji, T. Greenland, A. Pedraza, J. Etienne, R. P. Novick, and F. Vandenesch.** 1998. Transmembrane topology and histidine protein kinase activity of AgrC, the *agr* signal receptor in *Staphylococcus aureus*. Mol. Microbiol. **28:**655–662.
158. **Lukat, G. S., W. R. McCleary, A. M. Stock, and J. B. Stock.** 1992. Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors. Proc. Natl. Acad. Sci. USA **89:**718–722.
159. **Lynch, A. S., and E. C. Lin.** 1996. Responses to molecular oxygen, p. 1526–1538. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
160. **Lyon, G. J., and R. P. Novick.** 2004. Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. Peptides **25:**1389–1403.
161. **Lyon, G. J., J. S. Wright, A. Christopoulos, R. P. Novick, and T. W. Muir.** 2002. Reversible and specific extracellular antagonism of receptor-histidine kinase signaling. J. Biol. Chem. **277:**6247–6253.
162. **Lyon, G. J., J. S. Wright, T. W. Muir, and R. P. Novick.** 2002. Key determinants of receptor activation in the *agr* autoinducing peptides of *Staphylococcus aureus*. Biochemistry **41:**10095–10104.
163. **Malpica, R., B. Franco, C. Rodriguez, O. Kwon, and D. Georgellis.** 2004. Identification of a quinone-sensitive redox switch in the ArcB sensor kinase. Proc. Natl. Acad. Sci. USA **101:**13318–13323.
164. **Mansilla, M. C., L. E. Cybulski, D. Albanesi, and D. de Mendoza.** 2004. Control of membrane lipid fluidity by molecular thermosensors. J. Bacteriol. **186:**6681–6688.
165. **Marina, A., C. D. Waldburger, and W. A. Hendrickson.** 2005. Structure of the entire cytoplasmic portion of a sensor histidine-kinase protein. EMBO J. **24:**4247–4259.
166. **Mascher, T.** 2006. Intramembrane-sensing histidine kinases: a new family of cell envelope stress sensors in Firmicutes bacteria. FEMS Microbiol. Lett. **264:**133–144.
167. **Mascher, T., N. G. Margulis, T. Wang, R. W. Ye, and J. D. Helmann.** 2003. Cell wall stress responses in *Bacillus subtilis*: the regulatory network of the bacitracin stimulon. Mol. Microbiol. **50:**1591–1604.
168. **Mascher, T., S. L. Zimmer, T. A. Smith, and J. D. Helmann.** 2004. Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. Antimicrob. Agents Chemother. **48:**2888–2896.
169. **Matsushika, A., and T. Mizuno.** 1998. The structure and function of the histidine-containing phosphotransfer (HPt) signaling domain of the *Escherichia coli* ArcB sensor. J. Biochem. (Tokyo) **124:**440–445.
170. **McEwan, A. G., A. Lewin, S. L. Davy, R. Boetzel, A. Leech, D. Walker, T. Wood, and G. R. Moore.** 2002. PrrC from *Rhodobacter sphaeroides*, a homologue of eukaryotic Sco proteins, is a copper-binding protein and may have a thiol-disulfide oxidoreductase activity. FEBS Lett. **518:**10–16.
171. **Mejean, V., C. Iobbi-Nivol, M. Lepelletier, G. Giordano, M. Chippaux, and M. C. Pascal.** 1994. TMAO anaerobic respiration in *Escherichia coli*: involvement of the *tor* operon. Mol. Microbiol. **11:**1169–1179.
172. **Milburn, M. V., G. G. Prive, D. L. Milligan, W. G. Scott, J. Yeh, J. Jancarik, D. E. Koshland, Jr., and S. H. Kim.** 1991. Three-dimensional structures of the ligand-binding domain of the bacterial aspartate receptor with and without a ligand. Science **254:**1342–1347.
173. **Miller, A. F., and J. J. Falke.** 2004. Chemotaxis receptors and signaling. Adv. Protein Chem. **68:**393–444.
174. **Miller, S. I., R. K. Ernst, and M. W. Bader.** 2005. LPS, TLR4 and infectious disease diversity. Nat. Rev. Microbiol. **3:**36–46.
175. **Mishra, M., G. Parise, K. D. Jackson, D. J. Wozniak, and R. Deora.** 2005. The BvgAS signal transduction system regulates biofilm development in *Bordetella*. J. Bacteriol. **187:**1474–1484.
176. **Miyatake, H., M. Mukai, S.-i. Adachi, H. Nakamura, K. Tamura, T. Iizuka, Y. Shiro, R. W. Strange, and S. S. Hasnain.** 1999. Iron coordination struc-

tures of oxygen sensor FixL characterized by Fe K-edge extended X-ray absorption fine structure and resonance Raman spectroscopy. J. Biol. Chem. **274:**23176–23184.
177. **Miyatake, H., M. Mukai, S. Y. Park, S. Adachi, K. Tamura, H. Nakamura, K. Nakamura, T. Tsuchiya, T. Iizuka, and Y. Shiro.** 2000. Sensory mechanism of oxygen sensor FixL from *Rhizobium meliloti*: crystallographic, mutagenesis and resonance Raman spectroscopic studies. J. Mol. Biol. **301:** 415–431.
178. **Mizuno, T.** 1997. Compilation of all genes encoding two-component phosphotransfer signal transducers in the genome of *Escherichia coli*. DNA Res. **4:**161–168.
179. **Möker, N., M. Brocker, S. Schaffer, R. Krämer, S. Morbach, and M. Bott.** 2004. Deletion of the genes encoding the MtrA-MtrB two-component system of *Corynebacterium glutamicum* has a strong influence on cell morphology, antibiotics susceptibility and expression of genes involved in osmoprotection. Mol. Microbiol. **54:**420–438.
180. **Monson, E. K., M. Weinstein, G. S. Ditta, and D. R. Helinski.** 1992. The FixL protein of *Rhizobium meliloti* can be separated into a heme-binding oxygen-sensing domain and a functional C-terminal kinase domain. Proc. Natl. Acad. Sci. USA **89:**4280–4284.
181. **Mosley, C. S., J. Y. Suzuki, and C. E. Bauer.** 1994. Identification and molecular genetic characterization of a sensor kinase responsible for coordinately regulating light harvesting and reaction center gene expression in response to anaerobiosis. J. Bacteriol. **176:**7566–7573.
182. **Mougel, C., and I. B. Zhulin.** 2001. CHASE: an extracellular sensing domain common to transmembrane receptors from prokaryotes, lower eukaryotes and plants. Trends Biochem. Sci. **26:**582–584.
183. **Msadek, T.** 1999. When the going gets tough: survival strategies and environmental signaling networks in *Bacillus subtilis*. Trends Microbiol. **7:**201–207.
184. **Nes, I. F., D. B. Diep, L. S. Havarstein, M. B. Brurberg, V. Eijsink, and H. Holo.** 1996. Biosynthesis of bacteriocins in lactic acid bacteria. Antonie Leeuwenhoek **70:**113–128.
185. **Neumüller, A. M., D. Konz, and M. A. Marahiel.** 2001. The two-component regulatory system BacRS is associated with bacitracin 'self-resistance' of *Bacillus licheniformis* ATCC 10716. Eur. J. Biochem. **268:**3180–3189.
186. **Nikolskaya, A. N., A. Y. Mulkidjanian, I. B. Beech, and M. Y. Galperin.** 2003. MASE1 and MASE2: two novel integral membrane sensory domains. J. Mol. Microbiol. Biotechnol. **5:**11–16.
187. **Ninfa, A. J., P. Jiang, M. R. Atkinson, and J. A. Peliska.** 2000. Integration of antagonistic signals in the regulation of nitrogen assimilation in *Escherichia coli*. Curr. Top. Cell Regul. **36:**31–75.
188. **Ninfa, E. G., A. Stock, S. Mowbray, and J. Stock.** 1991. Reconstitution of the bacterial chemotaxis signal transduction system from purified components. J. Biol. Chem. **266:**9764–9770.
189. **Nishijyo, T., D. Haas, and Y. Itoh.** 2001. The CbrA-CbrB two-component regulatory system controls the utilization of multiple carbon and nitrogen sources in *Pseudomonas aeruginosa*. Mol. Microbiol. **40:**917–931.
190. **Nixon, B. T., C. W. Ronson, and F. M. Ausubel.** 1986. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. Proc. Natl. Acad. Sci. USA **83:**7850–7854.
191. **O'Gara, J. P., J. M. Eraso, and S. Kaplan.** 1998. A redox-responsive pathway for aerobic regulation of photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1. J. Bacteriol. **180:**4044–4050.
192. **Oh, J.-I., and S. Kaplan.** 2002. Oxygen adaptation. The role of the CcoQ subunit of the *cbb3* cytochrome c oxidase of *Rhodobacter sphaeroides* 2.4.1. J. Biol. Chem. **277:**16220–16228.
193. **Oh, J. I., I. J. Ko, and S. Kaplan.** 2004. Reconstitution of the *Rhodobacter sphaeroides* cbb3-PrrBA signal transduction pathway in vitro. Biochemistry **43:**7915–7923.
194. **Ohki, R., Giyanto, K. Tateno, W. Masuyama, S. Moriya, K. Kobayashi, and N. Ogasawara.** 2003. The BceRS two-component regulatory system induces expression of the bacitracin transporter, BceAB, in *Bacillus subtilis*. Mol. Microbiol. **49:**1135–1144.
195. **Okumura, H., S.-I. Nishiyama, A. Sasaki, M. Homma, and I. Kawagishi.** 1998. Chemotactic adaptation is altered by changes in the carboxy-terminal sequence conserved among the major methyl-accepting chemoreceptors. J. Bacteriol. **180:**1862–1868.
196. **Oshima, T., H. Aiba, Y. Masuda, S. Kanaya, M. Sugiura, B. L. Wanner, H. Mori, and T. Mizuno.** 2002. Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. Mol. Microbiol. **46:**281–291.
197. **Ottemann, K. M., W. Xiao, Y. K. Shin, and D. E. Koshland, Jr.**1999. A piston model for transmembrane signaling of the aspartate receptor. Science **285:**1751–1754.
198. **Ouchane, S., and S. Kaplan.** 1999. Topological analysis of the membrane-localized redox-responsive sensor kinase PrrB from *Rhodobacter sphaeroides* 2.4.1. J. Biol. Chem. **274:**17290–17296.
199. **Özcan, S., J. Dover, and M. Johnston.** 1998. Glucose sensing and signaling by two glucose receptors in the yeast *Saccharomyces cerevisiae*. EMBO J. **17:**2566–2573.
200. **Pao, G. M., and M. H. Saier, Jr.** 1995. Response regulators of bacterial signal transduction systems: selective domain shuffling during evolution. J. Mol. Evol. **40:**136–154.
201. **Pappalardo, L., I. G. Janausch, V. Vijayan, E. Zientz, J. Junker, W. Peti, M. Zweckstetter, G. Unden, and C. Griesinger.** 2003. The NMR structure of the sensory domain of the membranous two-component fumarate sensor (histidine protein kinase) DcuS of *Escherichia coli*. J. Biol. Chem. **278:** 39185–39188.
202. **Parkinson, J. S.** 1993. Signal transduction schemes of bacteria. Cell **73:**857–871.
203. **Pas, J., M. von Grotthuss, L. S. Wyrwicz, L. Rychlewski, and J. Barciszewski.** 2004. Structure prediction, evolution and ligand interaction of CHASE domain. FEBS Lett. **576:**287–290.
204. **Peng, W.-T., Y.-W. Lee, and E. W. Nester.** 1998. The phenolic recognition profiles of the *Agrobacterium tumefaciens* VirA protein are broadened by a high level of the sugar binding protein ChvE. J. Bacteriol. **180:**5632–5638.
205. **Perego, M.** 1998. Kinase-phosphatase competition regulates *Bacillus subtilis* development. Trends Microbiol. **6:**366–370.
206. **Perego, M., C. Hanstein, K. M. Welsh, T. Djavakhishvili, P. Glaser, and J. A. Hoch.** 1994. Multiple protein-aspartate phosphatases provide a mechanism for the integration of diverse signals in the control of development in *B. subtilis*. Cell **79:**1047–1055.
207. **Phillips, Z. E., and M. A. Strauch.** 2002. *Bacillus subtilis* sporulation and stationary phase gene expression. Cell Mol. Life Sci. **59:**392–402.
208. **Piazza, F., P. Tortosa, and D. Dubnau.** 1999. Mutational analysis and membrane topology of ComP, a quorum-sensing histidine kinase of *Bacillus subtilis* controlling competence development. J. Bacteriol. **181:**4540–4548.
209. **Pierce, K. L., R. T. Premont, and R. J. Lefkowitz.** 2002. Seven-transmembrane receptors. Nat. Rev. Mol. Cell Biol. **3:**639–650.
210. **Pietiäinen, M., M. Gardemeister, M. Mecklin, S. Leskela, M. Sarvas, and V. P. Kontinen.** 2005. Cationic antimicrobial peptides elicit a complex stress response in *Bacillus subtilis* that involves ECF-type sigma factors and two-component signal transduction systems. Microbiology **151:**1577–1592.
211. **Pioszak, A. A., P. Jiang, and A. J. Ninfa.** 2000. The *Escherichia coli* PII signal transduction protein regulates the activities of the two-component system transmitter protein NRII by direct interaction with the kinase domain of the transmitter module. Biochemistry **39:**13450–13461.
212. **Pirch, T., S. Landmeier, and H. Jung.** 2003. Transmembrane domain II of the Na+/proline transporter PutP of *Escherichia coli* forms part of a conformationally flexible, cytoplasmic exposed aqueous cavity within the membrane. J. Biol. Chem. **278:**42942–42949.
213. **Pons, T., R. Gomez, G. Chinea, and A. Valencia.** 2003. Beta-propellers: associated functions and their role in human diseases. Curr. Med. Chem. **10:**505–524.
214. **Ponting, C. P.** 1997. Evidence for PDZ domains in bacteria, yeast, and plants. Protein Sci. **6:**464–468.
215. **Potter, C. A., A. Ward, C. Laguri, M. P. Williamson, P. J. Henderson, and M. K. Phillips-Jones.** 2002. Expression, purification and characterisation of full-length histidine protein kinase RegB from *Rhodobacter sphaeroides*. J. Mol. Biol. **320:**201–213.
216. **Racher, K. I., R. T. Voegele, E. V. Marshall, D. E. Culham, J. M. Wood, H. Jung, M. Bacon, M. T. Cairns, S. M. Ferguson, W. J. Liang, P. J. Henderson, G. White, and F. R. Hallett.** 1999. Purification and reconstitution of an osmosensor: transporter ProP of *Escherichia coli* senses and responds to osmotic shifts. Biochemistry **38:**1676–1684.
217. **Rebeil, R., R. K. Ernst, B. B. Gowen, S. I. Miller, and B. J. Hinnebusch.** 2004. Variation in lipid A structure in the pathogenic yersiniae. Mol. Microbiol. **52:**1363–1373.
218. **Reid, C. J., and P. S. Poole.** 1998. Roles of DctA and DctB in signal detection by the dicarboxylic acid transport system of *Rhizobium leguminosarum*. J. Bacteriol. **180:**2660–2669.
219. **Reinelt, S., E. Hofmann, T. Gerharz, M. Bott, and D. R. Madden.** 2003. The structure of the periplasmic ligand-binding domain of the sensor kinase CitA reveals the first extracellular PAS domain. J. Biol. Chem. **278:**39189–39196.
220. **Reiser, V., D. C. Raitt, and H. Saito.** 2003. Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. J. Cell Biol. **161:**1035–1040.
221. **Reitzer, L. J.** 1996. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, L-alanine, and D-alanine, p. 391–407. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
222. **Rice, M. S., and F. W. Dahlquist.** 1991. Sites of deamidation and methylation in Tsr, a bacterial chemotaxis sensory transducer. J. Biol. Chem. **266:**9746–9753.
223. **Rodrigue, A., Y. Quentin, A. Lazdunski, V. Mejean, and M. Foglino.** 2000. Two-component systems in *Pseudomonas aeruginosa*: why so many? Trends Microbiol. **8:**498–504.
224. **Rothenbücher, M. C., S. J. Facey, D. Kiefer, M. Kossmann, and A. Kuhn.** 2006. The cytoplasmic C-terminal domain of the *Escherichia coli* KdpD protein functions as a K^+ sensor. J. Bacteriol. **188:**1950–1958.
225. **Rowland, S. L., W. F. Burkholder, K. A. Cunningham, M. W. Maciejewski,**

A. D. Grossman, and G. F. King. 2004. Structure and mechanism of action of Sda, an inhibitor of the histidine kinases that regulate initiation of sporulation in *Bacillus subtilis*. Mol. Cell **13:**689–701.
226. **Rübenhagen, R., H. Rönsch, H. Jung, R. Krämer, and S. Morbach.** 2000. Osmosensor and osmoregulator properties of the betaine carrier BetP from *Corynebacterium glutamicum* in proteoliposomes. J. Biol. Chem. **275:**735–741.
227. **Schell, M. A., T. P. Denny, and J. Huang.** 1994. VsrA, a second two-component sensor regulating virulence genes of *Pseudomonas solanacearum*. Mol. Microbiol. **11:**489–500.
228. **Scholten, M., and J. Tommassen.** 1993. Topology of the PhoR protein of *Escherichia coli* and functional analysis of internal deletion mutants. Mol. Microbiol. **8:**269–275.
229. **Schultz, J., F. Milpetz, P. Bork, and C. P. Ponting.** 1998. SMART, a simple modular architecture research tool: identification of signaling domains. Proc. Natl. Acad. Sci. USA **95:**5857–5864.
230. **Schuster, S. C., R. V. Swanson, L. A. Alex, R. B. Bourret, and M. I. Simon.** 1993. Assembly and function of a quaternary signal transduction complex monitored by surface plasmon resonance. Nature **365:**343–347.
231. **Schwartz, E., U. Gerischer, and B. Friedrich.** 1998. Transcriptional regulation of *Alcaligenes eutrophus* hydrogenase genes. J. Bacteriol. **180:**3197–3204.
232. **Shapiro, J. A.** 1998. Thinking about bacterial populations as multicellular organisms. Annu. Rev. Microbiol. **52:**81–104.
233. **Shi, L., and F. M. Hulett.** 1999. The cytoplasmic kinase domain of PhoR is sufficient for the low phosphate-inducible expression of Pho regulon genes in *Bacillus subtilis*. Mol. Microbiol. **31:**211–222.
234. **Shimoda, N., A. Toyoda-Yamamoto, S. Aoki, and Y. Machida.** 1993. Genetic evidence for an interaction between the VirA sensor protein and the ChvE sugar-binding protein of *Agrobacterium*. J. Biol. Chem. **268:**26552–26558.
235. **Shimoda, N., A. Toyoda-Yamamoto, J. Nagamine, S. Usami, M. Katayama, Y. Sakagami, and Y. Machida.** 1990. Control of expression of *Agrobacterium* vir genes by synergistic actions of phenolic signal molecules and monosaccharides. Proc. Natl. Acad. Sci. USA **87:**6684–6688.
236. **Siegele, D. A.** 2005. Universal stress proteins in *Escherichia coli*. J. Bacteriol. **187:**6253–6254.
237. **Smirnova, A. V., and M. S. Ullrich.** 2004. Topological and deletion analysis of CorS, a *Pseudomonas syringae* sensor kinase. Microbiology **150:**2715–2726.
238. **Søgaard-Andersen, L.** 2005. Personal communication.
239. **Sourjik, V.** 2004. Receptor clustering and signal processing in *E. coli* chemotaxis. Trends Microbiol. **12:**569–576.
240. **Stallkamp, I., K. Altendorf, and K. Jung.** 2002. Amino acid replacements in transmembrane domain 1 influence osmosensing but not K^+ sensing by the sensor kinase KdpD of *Escherichia coli*. Arch. Microbiol. **178:**525–530.
241. **Stephenson, K., and J. A. Hoch.** 2001. PAS-A domain of phosphorelay sensor kinase A: a catalytic ATP-binding domain involved in the initiation of development in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **98:**15251–15256.
242. **Stewart, V.** 2003. Nitrate- and nitrite-responsive sensors NarX and NarQ of proteobacteria. Biochem. Soc. Trans. **31:**1–10.
243. **Stock, J. B., and M. Levit.** 2000. Signal transduction: hair brains in bacterial chemotaxis. Curr. Biol. **10:**R11–R14.
244. **Stock, J. B., M. N. Levit, and P. M. Wolanin.** 2002. Information processing in bacterial chemotaxis. Sci. STKE, PE25.
245. **Stock, J. B., and M. G. Surette.** 1996. Chemotaxis, p. 1103–1129. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
246. **Stock, J. B., M. G. Surette, M. Levit, and P. Park.** 1995. Two-component signal transduction systems: structure-function relationships and mechanisms of catalysis, p. 25–52. *In* J. A. Hoch and T. J. Silhavy (ed.), Two-component signal transduction. ASM Press, Washington, D.C.
247. **Sturme, M. H., M. Kleerebezem, J. Nakayama, A. D. Akkermans, E. E. Vaugha, and W. M. de Vos.** 2002. Cell to cell communication by autoinducing peptides in gram-positive bacteria. Antonie Leeuwenhoek **81:**233–243.
248. **Swem, L. R., S. Elsen, T. H. Bird, D. L. Swem, H. G. Koch, H. Myllykallio, F. Daldal, and C. E. Bauer.** 2001. The RegB/RegA two-component regulatory system controls synthesis of photosynthesis and respiratory electron transfer components in *Rhodobacter capsulatus*. J. Mol. Biol. **309:**121–138.
249. **Swem, L. R., B. J. Kraft, D. L. Swem, A. T. Setterdahl, S. Masuda, D. B. Knaff, J. M. Zaleski, and C. E. Bauer.** 2003. Signal transduction by the global regulator RegB is mediated by a redox-active cysteine. EMBO J. **22:**4699–4708.
250. **Szurmant, H., and G. W. Ordal.** 2004. Diversity in chemotaxis mechanisms among the bacteria and archaea. Microbiol. Mol. Biol. Rev. **68:**301–319.
251. **Tabatabai, N., and S. Forst.** 1995. Molecular analysis of the two-component genes, *ompR* and *envZ*, in the symbiotic bacterium *Xenorhabdus nematophilus*. Mol. Microbiol. **17:**643–652.
252. **Tam, R., and M. H. Saier, Jr.** 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol. Rev. **57:**320–346.
253. **Tanaka, T., S. K. Saha, C. Tomomori, R. Ishima, D. Liu, K. I. Tong, H. Park, R. Dutta, L. Qin, M. B. Swindells, T. Yamazaki, A. M. Ono, M. Kainosho, M. Inouye, and M. Ikura.** 1998. NMR structure of the histidine kinase domain of the *E. coli* osmosensor EnvZ. Nature **396:**88–92.
254. **Taylor, B. L., and I. B. Zhulin.** 1999. PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol. Mol. Biol. Rev. **63:**479–506.
255. **Throup, J. P., K. K. Koretke, A. P. Bryant, K. A. Ingraham, A. F. Chalker, Y. Ge, A. Marra, N. G. Wallis, J. R. Brown, D. J. Holmes, M. Rosenberg, and M. K. Burnham.** 2000. A genomic analysis of two-component signal transduction in *Streptococcus pneumoniae*. Mol. Microbiol. **35:**566–576.
256. **Tomich, M., and C. D. Mohr.** 2004. Genetic characterization of a multicomponent signal transduction system controlling the expression of cable pili in *Burkholderia cenocepacia*. J. Bacteriol. **186:**3826–3836.
257. **Tomomori, C., T. Tanaka, R. Dutta, H. Park, S. K. Saha, Y. Zhu, R. Ishima, D. Liu, K. I. Tong, H. Kurokawa, H. Qian, M. Inouye, and M. Ikura.** 1999. Solution structure of the homodimeric core domain of *Escherichia coli* histidine kinase EnvZ. Nat. Struct. Biol. **6:**729–734.
258. **Tortosa, P., L. Logsdon, B. Kraigher, Y. Itoh, I. Mandic-Mulec, and D. Dubnau.** 2001. Specificity and genetic polymorphism of the *Bacillus* competence quorum-sensing system. J. Bacteriol. **183:**451–460.
259. **Tran, L.-S. P., T. Nagai, and Y. Itoh.** 2000. Divergent structure of the ComQXPA quorum-sensing components: molecular basis of strain-specific communication mechanism in *Bacillus subtilis*. Mol. Microbiol. **37:**1159–1171.
260. **Turgay, K., and M. A. Marahiel.** 1995. The *gtcRS* operon coding for two-component system regulatory proteins is located adjacent to the *grs* operon of *Bacillus brevis*. DNA Seq. **5:**283–290.
261. **Ulrich, L. E., E. V. Koonin, and I. B. Zhulin.** 2005. One-component systems dominate signal transduction in prokaryotes. Trends Microbiol. **13:**52–56.
262. **Unden, G., and J. Schirawski.** 1997. The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signals and reactions. Mol. Microbiol. **25:**205–210.
263. **Verhamme, D. T., P. W. Postma, W. Crielaard, and K. J. Hellingwerf.** 2002. Cooperativity in signal transfer through the Uhp system of *Escherichia coli*. J. Bacteriol. **184:**4205–4210.
264. **Vierstra, R. D.** 2003. Cyanophytochromes, bacteriophytochromes, and plant phytochromes: light-regulated kinases related to bacterial two-component regulators, p. 273–295. *In* M. Inouye and R. Dutta (ed.), Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
265. **Vierstra, R. D., and S. J. Davis.** 2000. Bacteriophytochromes: new tools for understanding phytochrome signal transduction. Semin. Cell Dev. Biol. **11:**511–521.
266. **Wadhams, G. H., and J. P. Armitage.** 2004. Making sense of it all: bacterial chemotaxis. Nat. Rev. Mol. Cell Biol. **5:**1024–1037.
267. **Walderhaug, M. O., J. W. Polarek, P. Voelkner, J. M. Daniel, J. E. Hesse, K. Altendorf, and W. Epstein.** 1992. KdpD and KdpE, proteins that control expression of the *kdpABC* operon, are members of the two-component sensor-effector class of regulators. J. Bacteriol. **174:**2152–2159.
268. **Wang, L., C. Fabret, K. Kanamaru, K. Stephenson, V. Dartois, M. Perego, and J. A. Hoch.** 2001. Dissection of the functional and structural domains of phosphorelay histidine kinase A of *Bacillus subtilis*. J. Bacteriol. **183:**2795–2802.
269. **Wang, L., R. Grau, M. Perego, and J. A. Hoch.** 1997. A novel histidine kinase inhibitor regulating development in *Bacillus subtilis*. Genes Dev. **11:**2569–2579.
270. **Webre, D. J., P. M. Wolanin, and J. B. Stock.** 2003. Bacterial chemotaxis. Curr. Biol. **13:**R47–R49.
271. **Wecke, T., B. Veith, A. Ehrenreich, and T. Mascher.** 2006. Cell envelope stress response in *Bacillus licheniformis*: integrating comparative genomics, transcriptional profiling, and regulon mining to decipher a complex regulatory network. J. Bacteriol. **188:**7500–7511.
272. **Wegener, C., S. Tebbe, H. J. Steinhoff, and H. Jung.** 2000. Spin labeling analysis of structure and dynamics of the Na(+)/proline transporter of *Escherichia coli*. Biochemistry **39:**4831–4837.
273. **Weiss, V., G. Kramer, T. Dünnebier, and A. Flotho.** 2002. Mechanism of regulation of the bifunctional histidine kinase NtrB in *Escherichia coli*. J. Mol. Microbiol. Biotechnol. **4:**229–233.
274. **Whatmore, A. M., V. A. Barcus, and C. G. Dowson.** 1999. Genetic diversity of the streptococcal competence (*com*) gene locus. J. Bacteriol. **181:**3144–3154.
275. **Williams, S. B., and V. Stewart.** 1997. Discrimination between structurally related ligands nitrate and nitrite controls autokinase activity of the NarX transmembrane signal transducer of Escherichia coli K-12. Mol. Microbiol. **26:**911–925.
276. **Williams, S. B., and V. Stewart.** 1999. Functional similarities among two-component sensors and methyl-accepting chemotaxis proteins suggest a role for linker region amphipathic helices in transmembrane signal transduction. Mol. Microbiol. **33:**1093–1102.
277. **Wise, A. A., L. Voinov, and A. N. Binns.** 2005. Intersubunit complementation of sugar signal transduction in VirA heterodimers and posttransla-

tional regulation of VirA activity in *Agrobacterium tumefaciens*. J. Bacteriol. **187:**213–223.

278. **Wolanin, P. M., and J. B. Stock.** 2004. Bacterial chemosensing: cooperative molecular logic. Curr. Biol. **14:**R486–R487.
279. **Wolanin, P. M., and J. B. Stock.** 2003. Transmembrane signaling and the regulation of histidine kinase activity, p. 73–122. *In* M. Inouye and R. Dutta (ed.), Histidine kinases in signal transduction. Academic Press, San Diego, Calif.
280. **Wolanin, P. M., P. A. Thomason, and J. B. Stock.** 2002. Histidine protein kinases: key signal transducers outside the animal kingdom. Genome Biol. **3:**REVIEWS301.
281. **Wösten, M. M., L. F. Kox, S. Chamnongpol, F. C. Soncini, and E. A. Groisman.** 2000. A signal transduction system that responds to extracellular iron. Cell **103:**113–125.
282. **Wösten, M. M. S. M., and E. A. Groisman.** 1999. Molecular characterization of the PmrA regulon. J. Biol. Chem. **274:**27185–27190.
283. **Xu, J., H. C. Chiang, M. K. Bjursell, and J. I. Gordon.** 2004. Message from a human gut symbiont: sensitivity is a prerequisite for sharing. Trends Microbiol. **12:**21–28.
284. **Yaku, H., and T. Mizuno.** 1997. The membrane-located osmosensory kinase, EnvZ, that contains a leucine zipper-like motif functions as a dimer in *Escherichia coli*. FEBS Lett. **417:**409–413.
285. **Zhang, W., and L. Shi.** 2005. Distribution and evolution of multiple-step phosphorelay in prokaryotes: lateral domain recruitment involved in the formation of hybrid-type histidine kinases. Microbiology **151:**2159–2173.
286. **Zhou, L., X.-H. Lei, B. R. Bochner, and B. L. Wanner.** 2003. Phenotype microarray analysis of *Escherichia coli* K-12 mutants with deletions of all two-component systems. J. Bacteriol. **185:**4956–4972.
287. **Zhu, J., P. M. Oger, B. Schrammeijer, P. J. J. Hooykaas, S. K. Farrand, and S. C. Winans.** 2000. The bases of crown gall tumorigenesis. J. Bacteriol. **182:**3885–3895.
288. **Zhulin, I. B., A. N. Nikolskaya, and M. Y. Galperin.** 2003. Common extracellular sensory domains in transmembrane receptors for diverse signal transduction pathways in bacteria and archaea. J. Bacteriol. **185:**285–294.
289. **Zhulin, I. B., B. L. Taylor, and R. Dixon.** 1997. PAS domain S-boxes in archaea, bacteria and sensors for oxygen and redox. Trends Biochem. Sci. **22:**331–333.
290. **Zickermann, V., M. Bostina, C. Hunte, T. Ruiz, M. Radermacher, and U. Brandt.** 2003. Functional implications from an unexpected position of the 49-kDa subunit of NADH:ubiquinone oxidoreductase. J. Biol. Chem. **278:**29072–29078.