The role of solute binding proteins in signal transduction

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Abstract
The solute binding proteins (SBPs) of prokaryotes are present in the extracytosolic space. Although their primary function is providing substrates to transporters, SBPs also stimulate different signaling proteins, including chemoreceptors, sensor kinases, diguanylate cyclases/phosphodiesterases and Ser/Thr kinases, thereby causing a wide range of responses. While relatively few such systems have been identified, several pieces of evidence suggest that SBP-mediated receptor activation is a widespread mechanism. (1) These systems have been identified in Gram-positive and Gram-negative bacteria and archaea. (2) There is a structural diversity in the receptor domains that bind SBPs. (3) SBPs belonging to thirteen different families interact with receptor ligand binding domains (LBDs). (4) For the two most abundant receptor LBD families, dCache and four-helix-bundle, there are different modes of interaction with SBPs. (5) SBP-stimulated receptors carry out many different functions. The advantage of SBP-mediated receptor stimulation is attributed to a strict control of SBP levels, which allows a precise adjustment of the system’s sensitivity. We have compiled information on the effect of ligands on the transcript/protein levels of their cognate SBPs. In 87% of the cases analysed, ligands altered SBP expression levels. The nature of the regulatory effect depended on the ligand family. Whereas inorganic ligands typically downregulate SBP expression, an upregulation was observed in response to most sugars and organic acids. A major unknown is the role that SBPs play in signaling and in receptor stimulation. This review attempts to summarize what is known and to present new information to narrow this gap in knowledge.

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Abbreviations: AI-2, autoinducer-2; CCR, carbon catabolite repression; LBD, ligand binding domain; Pi, inorganic phosphate; SBP, solute binding protein; TCS, two-component system.
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1. Introduction

Bacteria need to take up compounds from the extracytosolic space in order to survive. To this end, bacteria have evolved a variety of transmembrane transporters that permit the specific transport of a variety of compounds. Several transporter families, like ATP-binding cassette (ABC), tripartite ATP-independent periplasmic (TRAP), and tripartite tricarboxylate transporters (TTTs) employ solute binding proteins (SBPs) to capture the transport substrate in the extracytosolic space and to present it to the transmembrane receptor permeases [1]. These proteins thus play a central role in defining the substrate specificity of the transporter.

SBPs are found in all kingdoms of life [1] and form a superfamily composed of many families, as defined by different domain profiles in the Pfam [2] and InterPro [3] databases. Whereas SBPs in Gram-negative bacteria are present as diffusible proteins in the periplasm, in Gram-positive bacteria and archaea they are tethered to the external face of the cytoplasmic membrane [4]. Although SBPs vary largely in size, from 20 to 65 kDa, they share the same overall topology that consists of two lobes linked by a hinge region [5]. The transport substrate binds to the interface of both lobes, a process that frequently induces significant structural rearrangements [6,7].

Other major constituents of prokaryotic membranes are signal transduction receptors [8]. The function of these proteins is to sense extracytoplasmic signals and to initiate a cellular response leading to a more optimal adaptation to a given environmental condition. The most abundant signal transduction receptors are sensor kinases, chemoreceptors, diguanylate cyclases and phosphodiesterases, adenylyl cyclases, extracytosolic function sigma factors as well as Ser/Thr kinases and phosphatases [8]. Typically, these receptors are transmembrane proteins that contain an extracytoplasmic sensor or ligand binding domain (LBD) that is flanked by two transmembrane regions. Signal binding to the LBD creates a conformational change that is transmitted to the cytosolic part of the receptor to induce signaling cascades that lead to the final cellular response. Transmembrane signal transduction receptors mediate a variety of different responses. For example: (i) Sensor kinases form two component systems (TCSs) with response regulators that primarily regulate transcription [9]; (ii) Chemoreceptors form part of chemosensory pathways that primarily mediate chemotaxis but were also found to control second messenger levels or type IV pili-based motility [10]; (iii) Diguanylate cyclases/phosphodiesterases and adenylyl cyclases, respectively, control the levels of c-di-GMP and cAMP, second messengers that in turn regulate a variety of different cellular processes [11,12].

Transmembrane receptors employ many different LBD types for signal sensing [13,14] as exemplified by the 80 different LBD types identified in chemoreceptors [13]. Frequently, a given LBD type is shared by different receptor families [14,15], which is consistent with the notion that LBDs are modules that can be recombined with different signaling proteins. Although the function of many transmembrane signal transduction receptors has been identified by phenotypic and transcriptomic analyses of the corresponding bacterial mutants, the signals recognized by many of these receptors remain unknown [16,17]. The scarcity of information about the signals recognized by these receptors represents a major research need in the field because this knowledge is indispensable to fully understand the corresponding regulatory circuit [18]. Over the last years, significant progress in this area has been made by high-throughput ligand screening using individual LBDs [18,19]. However, in a number of cases screening did not identify any ligand, which is consistent with the notion that some LBDs may not bind signals directly. A possible explanation is that some LBDs interact with signal-loaded SBPs. There are a number of examples, reviewed in this article, in which signal transduction receptors are stimulated by an interaction with an SBP. Some of these SBPs thus carry out a double function, as they may be involved in transport and signal transduction. Since cellular SBP levels are frequently subject to tight control, it has been proposed that this mechanism offers the possibility of coordinating different, but physiologically related, processes such as transport and chemotaxis [20]. The study of SBP-activated chemoreceptors has shown that the overall responses are highly sensitive to SBP levels, thus permitting a better control of the sensitivity to specific ligands in response to their nutrient environment and to coordinate chemotaxis with ligand transport [21]. However, the cost for this capacity is the much narrower dynamic response range of SBP-stimulated receptors as compared to those that recognize signal molecules directly [21].

Here, we review the data available on SBP-stimulated signal transduction receptors and compile information on the effect of signal molecules on the expression of their cognate SBPs. SBP-stimulated receptors showed a wide phylogenetic spread, and these systems reveal a significant diversity in the structure of receptor LBDs as well as SBPs, suggesting that indirect sensing is a rather widespread mechanism. We propose that the relatively low number of characterized SBP-stimulated systems is the consequence of the technical complexity of identifying such systems rather than their low abundance.

2. Universality and diversity of SBP interactions with bacterial sensor proteins

We have compiled the information on bacterial receptors that are stimulated by the binding of SBPs in Table 1. There was a significant phylogenetic spread of the corresponding organisms including α-, β-, γ- and ε-Proteobacteria, Actinobacteria, Firmicutes as well as Archaea (Table 1). The genes encoding SBPs are often found in the vicinity of genes encoding the cognate transporters or signal transduction receptors (Tables 1 and 2). Remarkably, genes encoding SBPs that interact with chemoreceptors are primarily found in the vicinity of genes encoding transporters, whereas those for SBPs that stimulate sensor kinases are associated with signaling genes (Table 1). Receptors belong to four families, namely chemoreceptors, sensor kinases, GGDEF-EAL domain-containing diguanylate cyclases/phosphodiesterases, and Ser/Thr kinases (Fig. 1).

For the former three families, the SBP binds to the LBD of the corresponding receptor protein. So far, there is only one characterized example of a SBP-stimulated Ser/Thr kinase, namely the tripartite GlnH/GlnX/PknG system [22]. GlnX is a transmembrane protein that has two 4HB LBDs at its periplasmic face. The binding of the GlnH SBP to GlnX generates a molecular stimulus that is transduced across the membrane to modulate the activity of the bound Ser/Thr kinase PknG [22]. The available data indicate that PknG phosphorylates primarily GarA, a regulatory protein that redirects metabolic fluxes towards the synthesis or degradation of glutamate by interacting with different enzymes involved in TCA cycle and glutamate metabolism [22–24]. More recent studies, however, have identified a significant number of physiological
| Signal transduction receptor Name | UniProt | Species | Phylogenetic category | LBD family (Pfam/InterPro) | Signal transduction receptor Name/Gene associated with | UniProt (size in kDa)* | Family Pfam/InterPro | Ligands (KD in μM) | PDB | Ref. |
|----------------------------------|---------|---------|----------------------|--------------------------|------------------------------------------------------|------------------------|----------------------|-------------------|------|------|
| Chemoreceptors                   |         |         |                      |                          |                                                      |                        |                      |                   |      |      |
| Tar                              | P76301  | E. coli | γ-Proteobacteria     | TarH (PF02203)           | MBP maltose binding protein/T Maltose binding protein/T  | P0AEX9 (43)            | SBP_bac_8 (PF13416) | D-maltose (1.5)    | 2LIG | [26,102] |
| Tap                              | P76300  | E. coli | γ-Proteobacteria     | TarH (PF02203)           | DppA dipeptide binding protein/T DppA dipeptide binding protein/T | P23847 (60)            | SBP_bac_5 (PF00496) | Various dipeptides, lower nM for Ala-Phe | 1DPE | [41,127] |
| Trg                              | P77448  | E. coli | γ-Proteobacteria     | TarH (PF02203)           | Galactose binding protein/T Galactose binding protein/T | P0AEE5 (35)            | Peripla_BP_4 (PF13407) | D-galactose (0.13) | 2GBP | 37-130 |
|                                  |         |         |                      |                          |                                                      |                        |                      |                   |      |      |
| Tsr                              | P02942  | E. coli | γ-Proteobacteria     | P76142 (37)              | Autoinducer-2                                         | PO2925 (31)            | Peripla_BP_4 (PF13407) | D-ribose (0.13)  | 2DR | [43,131] |
| TlpB                             | B5Z9N4  | H. pylori | α-Proteobacteria  | P54082 (38)              | OpuAC                                                | P02942 (31)            | Peripla_BP_4 (PF13407) | Spermidine (3.2) | 1POY | [45,46] |
|                                  |         |         |                      |                          |                                                      |                        |                      |                   |      |      |
| Sensor kinases                   |         |         |                      |                          |                                                      |                        |                      |                   |      |      |
| LuxQ                             | P54302  | Vibrio harvey | γ-Proteobacteria | LuxP (PF03908)                  | Al-2 (0.27)                                           | P54000 (41)            | Peripla_BP_4 (PF13407) | D-xylene (0.37) | 2HPI | [29,132] |
| LytS                             | A6LW08  | Clostridium beijerincki | Firmicutes | XyFU (PF03908)                  | Al-2 (0.27)                                           | A6LW07 (36)            | Peripla_BP_4 (PF13407) | D-xylene (0.37) | 2HPI | [29,132] |
| TorS                             | A0G8L85U58 | Vibrio parahaemolyticus | E. coli | TorS sensor (IPR037952)          | Triamcinolone-N-oxide (TMAO) (74) | Q87ID2 (37)           | Peripla_BP_1 (PF0532) | D-xylene (0.37) | 2HPI | [29,132] |
| TorS                             | P39453  | E. coli | γ-Proteobacteria     | TorS sensor (IPR037952)     | Trimethylamine N-oxide (TMAO) (74) | P38683 (38)           | Peripla_BP_1 (PF0532) | D-xylene (0.37) | 2HPI | [29,132] |
| HptS                             | Q2G1E0  | Staphylococcus aureus | Firmicutes | HptA (PF03908)                  | Glucose-6-phosphate (5), Galactose-6-phosphate (10) | X5DV1 (37)            | SBP_bac_11 (PF13531) | Glucose-6-phosphate (5), Galactose-6-phosphate (10) | 2HPI | [29,132] |
| VirA                             | P10799  | Agrobacterium tumefaciens | α-Proteobacteria | Not annotated (dCache-like) |                          | P54082 (38)            | Peripla_BP_4 (PF13407) | D-xylene (0.37) | 2HPI | [29,132] |
|                                  |         |         |                      |                          |                                                      |                        |                      |                   |      |      |
| AioS                             | U6A267  | Agrobacterium tumefaciens | α-Proteobacteria | Not annotated (dCache-like) |                          | G8XNW6 (34)            | Phosphonate-bd (PF12974) | As(III) (2.4) | 2HPI | [29,132] |
| BctE                             | A0A3J0PK6G6 | Bordetella pertussis | β-Proteobacteria | Not annotated (dCache-like) |                          | A8HV0 (35)             | TctC (PF03401) | Citrate | 2HPI | [29,132] |
| ChiS                             | Q5KUA1  | Vibrio harveyi | γ-Proteobacteria | Not annotated (dCache-like) |                          | Q9KUA3 (63)            | SBP_bac_5 (PF00496) | Glucose (1.4)   | 1ZTY | [58,138] |
| GtrS                             | PA3191  | Pseudomonas aeruginosa | γ-Proteobacteria | Not annotated (dCache-like) |                          | PA3190 (45)            | SBP_bac_1 (PF01547) | Glucose (1.4)   | 1ZTY | [58,138] |
Table 1 (continued)

| Signal transduction receptor | UniProt (size in kDa) | Family of LBDs | Phylogenetic category | LBD family (Pfam/InterPro) | Ligands (Km in μM) | Ref. |
|-----------------------------|-----------------------|----------------|----------------------|---------------------------|-------------------|-----|
| Degradase (Glu, Asp) | Q5RVL5 (31) | Saprospirales |- Proteobacteria | - | Norspermidine, Spermidine | [139,140] |
| Vibrio cholerae | Q85H50 (36) | Vibrio |- Proteobacteria | - | S-signal, ChvE | [139,140] |
| Serine/threonine kinase | P96254 (91) | GNB |- Proteobacteria | GlnH/SP | Asp (18) | [22–24] |
| Mycobacterium | P96255 (89) | MTB |- Proteobacteria | GlnH/SP | Glu (15) | [22–24] |
| Helicobacter pylori | P96257 (85) | H. pylori |- Proteobacteria | GlnH/SP | 6H1U, 6H20 | [22–24] |
| Tar chemoreceptor and the LytS sensor kinase | P96258 (81) | E. coli |- Proteobacteria | GlnH/SP | 6H2T | [22–24] |

a Genes associated with: T: transporter genes; SP: Signaling protein genes.
b Size including signal peptide.
c ChvE is also involved in chemotaxis; chvE mutants showed strongly reduced chemotaxis to D-galactose, D-glucose, L-arabinose, D-fucose, and D-xylose [141].

d The GlnH SBP binds to the transmembrane protein GlnX that interacts on the cytosolic side with the Ser/Thr kinase PknG.

e Genes associated with: T: transporter genes; SP: Signaling protein genes.
f The GlnH SBP binds to the transmembrane protein GlnX that interacts on the cytosolic side with the Ser/Thr kinase PknG.

HptS sensor kinases employ dCache-like domain structures. Bimodular LBDs of HptS sensor kinases consist of two HB modules (i.e., the dCache-like LBD of HptS) and one (i.e., the sCache-like LBD of HptS). The limited number of SBP-LBD co-crystal structures suggests that another layer of diversity resides in the mode by which SBPs interact with LBDs of similar structure. Based on extensive site-directed mutagenesis studies [33–35], the “mushroom” shaped model for the interaction of the maltose binding protein (MBP) with the Tar-LBD was established (Fig. 2A) [26].Alternatively, the XylFII SBP binds sideways to the LytS-LBD (Fig. 2B). Analogously, the LuxP SBP binds sideways to the LuxQ-LBD of the “LuxQ-periplasm” family that belongs to the dCache-LBD superfAMILY (Fig. 2D) [14]. In contrast, the binding mode of HptA to the dCache-like LBD of HptS is different since the SBP bridges two HptS-LBD monomers (Fig. 2E-F).

3. Chemoreceptors

By far the most thoroughly investigated chemotaxis system is that of Escherichia coli, and most fundamental aspects of chemotaxis have been discovered using this species [36]. It has four transmembrane chemoreceptors with a TarH type LBD in the periplasmic space as well as an aerotaxis receptor. There is direct evidence that three of these chemoreceptors, Tar, Trg and Tap, are activated by the binding of SBPs loaded with different sugars or dipeptides, mediating chemotaxis to these compounds (Table 1) [26,37–42]. More recent data also indicate that Trg is activated by the spermidine SBP PotD [43]. Interestingly, the Tar chemoreceptor is stimulated by the direct binding of aspartate as well as of the maltose binding protein, and both stimuli were found to be additive and independent [44]. In addition, there is also indirect evidence that the fourth E. coli chemoreceptor, Tsr, is also activated by direct binding of serine and interaction with an SBP [45,46]. E. coli showed strong attraction to the autoinducer-2 (AI-2) quorum sensing signal. As this response depends on Tsr as well as on the AI-2 binding SBP LsrB, it is very likely
Table 2
Regulation of the expression of SBPs at the transcriptional and protein levels by different ligand families and environmental cues.

| SBP                          | Gene/ Gene associated with | SBP family/Pfam | Species                  | SBP ligands               | Experimental conditions                                      | Fold change | Ref. |
|-----------------------------|---------------------------|------------------|--------------------------|---------------------------|-------------------------------------------------------------|-------------|------|
| AatJ                        | PA1342/T<sup>TH</sup>     | SBP_bac_3/PF00497 | Pseudomonas aeruginosa   | L-Glu                     | 5 mM L-Glu vs 5 mM L-Arg                                     | 2.6<sup>b</sup> | [142]|
| HsdB                       | spa_1337/T<sup>TH</sup>   | SBP_bac_5/PF00496 | Streptococcus pneumoniae | Oligopeptides             | 10 mM Arg vs 0.05 mM Arg                                     | 2.2<sup>b</sup> | [143]|
| ApbA                       | spa_0109/T<sup>TH</sup>   | SBP_bac_3/PF00497 | S. pneumoniae            | Arg                       | 10 mM Arg vs 0.05 mM Arg                                     | 10.1<sup>b</sup> | [143]|
| ArtI                       | artI                      | SBP_bac_3/PF00497 | E. coli                  | Arg, ornithine            | 0.6 mM Arg vs no Arg                                         | 3.4<sup>a</sup> | [121]|
| ArtJ                       | artJ                      | SBP_bac_3/PF00497 | E. coli                  | Arg                       | 5.7 mM Arg vs no Arg                                         | No change   | [144]|
| atu2422                    | atu2422/T<sup>TH</sup>    | Peripla_BP_6/PF13458 | Agrobacterium fabrum    | GABA, L-Pro, L-Ala, L-Val | 1 mM GABA vs no GABA                                         | 4.7<sup>b</sup> | [146]|
| atu4243                    | atu4243/T<sup>TH</sup>    | SBP_bac_8/PF13416 | A. fabrum                | GABA                      | 1 mM GABA vs no GABA                                         | No change   | [147]|
| DppA                       | dppA                      | SBP_bac_5/PF00496 | E. coli                  | Dipeptides                | Reduced DppA levels in the presence of casamino acids       | Reduced dppA transcript levels in the presence of casamino acids | [148]|
| GltI                       | gltI/T<sup>TH</sup>       | SBP_bac_3/PF00497 | E. coli                  | Glu, Asp                  | LB medium vs human urine                                      | -4.1<sup>b</sup> | [146]|
| HisJ                       | hisJ/T<sup>TH</sup>       | SBP_bac_3/PF00497 | E. coli                  | His, Arg                  | LB medium vs human urine                                      | -3.4<sup>a</sup> | [146]|
| LAGL                        | argT/T<sup>TH</sup>       | SBP_bac_3/PF00497 | E. coli                  | Lys, Arg, ornithine       | 0.6 mM Arg vs no Arg                                         | No change   | [121]|
|LivJ                        | CjJ81176_1038/T<sup>TH</sup> | Peripla_BP_6/PF13458 | Campylobacter jejuni    | Leu, Ile, Val             | 4 g/l glucose vs 10 mg/l glucose                             | -2.5<sup>b</sup> | [108]|
|LivK                        | livK/T<sup>TH</sup>       | Peripla_BP_6/PF13458 | E. coli                  | Leu                       | 4 g/l glucose vs <10 mg/l glucose, fed-batch conditions<sup>8</sup> | -8.3<sup>a</sup> | [109]|
|MetQ                        | metQ/T<sup>TH</sup>       | Lipoprotein_9/PF03180 | E. coli                  | Leu                       | CDM medium (0.7 mM L-Leu, 0.2 mM L-Ile, 0.9 mM L-Val) vs CDM-LIV medium (no L-Leu, L-Ile, L-Val) | No change   | [149]|
|MppA                        | mppA<sup>TH</sup>         | SBP_bac_5/PF00496 | E. coli                  | Murein peptide L-alamyl-gamma-D-glutamyl-meso-diaminopimelate | Reduced MppA levels in the presence of casamino acids         | Reduced MppA levels in the presence of casamino acids<sup>7</sup> | [108]|
|OppA                        | oppA/T<sup>TH</sup>       | SBP_bac_5/PF00496 | E. coli                  | Two and five amino acids-long peptides | 4 g/l glucose vs <10 mg/l glucose, fed-batch conditions<sup>8</sup> | -2.2<sup>a</sup> | [108]|
|PEB1a                       | CjJ9221c/T<sup>TH</sup>   | SBP_bac_3/PF00497 | C. jejuni                | Asp, Glu                  | 7.5% (v/v) O<sub>2</sub> vs 1.9% (v/v) O<sub>2</sub><sup>8</sup> | 5.6<sup>b</sup>/13.3<sup>a</sup> | [152]|
|AioX                        | aioX<sup>HP</sup>         | Phosphonate-bd/PF12974 | Agrobacterium tumefaciens | As(III)                   | 100 µM As(III) vs no As(III)                                | 3.3<sup>a</sup> | [98]|

<sup>a</sup>Ref. 141; <sup>b</sup>Ref. 142; <sup>c</sup>Ref. 143; <sup>d</sup>Ref. 144; <sup>e</sup>Ref. 145; <sup>f</sup>Ref. 146; <sup>g</sup>Ref. 147; <sup>h</sup>Ref. 148; <sup>i</sup>Ref. 149; <sup>j</sup>Ref. 150; <sup>k</sup>Ref. 151; <sup>l</sup>Ref. 152.
Table 2 (continued)

| SBP       | Gene/ Gene associated with | SBP family/Pfam | Species                      | SBP ligands         | Experimental conditions                                                                 | Fold change |
|-----------|---------------------------|------------------|------------------------------|---------------------|----------------------------------------------------------------------------------------|-------------|
| CeuE      | ceuE/T^                  | Peripla_BP_2/PF01497 | C. jejuni                   | Fe(III)-siderophore complexes | Iron replete (40 μM FeSO₄) vs iron-chelated, iron replete (40 μM Fe₂(SO₄)₃) vs iron-chelated | −3.0^a      |
| CeuE      | HP_1561/T^               | Peripla_BP_2/PF01497 | Helicobacter pylori         | Ni-(L-His)_2        | 0.5 mM Ni(II) vs no Ni(II) Non-chelated iron (high iron) vs iron-chelated (low iron)^b | −6.5^b      |
| FatB      | VV2_0842/-               | Peripla_BP_2/PF01497 | Vibrio vulnificus           | Ferric vulnibactin  | Non-chelated iron (high iron) vs iron-chelated (low iron)^b | −12.9^b     |
| FbpA      | NGO0217/T^               | SBP_bac_1/PF01547  | Neisseria gonorrhoeae       | Fe(III), Ga(III)    | Iron replete (100 mM Fe(NO₃)₃) vs iron-chelated                                        | −6.1^c      |
| FeC       | fecB/T^                  | Peripla_BP_2/PF01497 | E. coli                     | Fe(III)-citrate     | High iron citrate (1 mM citrate, 100 μM FeSO₄) vs low iron citrate (1 mM citrate, chelated iron) | −3.5^c      |
| FeC       | fecB/T^                  | Peripla_BP_2/PF01497 | E. coli                     | Fe(III)-citrate     | High iron citrate (1 mM citrate, 100 μM FeSO₄) vs low iron citrate (1 mM citrate, chelated iron) | −3.5^c      |
| FepB      | fepB/T^                  | Peripla_BP_2/PF01497 | E. coli                     | Ferric-enterobactin complexes | Iron replete (100 mM FeSO₄) vs iron-chelated                                        | −10.6^d     |
| VcFhuD    | VCO395_A2582/T^          | Peripla_BP_2/PF01497 | Vibrio cholerae             | Hydroxamate and catecholate type xenosiderophores | LB vs bovine milk (most iron is chelated or bound to proteins) | −8.3^e      |
| HbpaA     | hbpA/-                   | SBP_bac_5/PF00496  | Haemophilus influenzae      | Reduced and oxidized glutathione, heme, hemin | 10 μg/ml heme vs heme-deficient medium | 6.2^e       |
| Ihbp      | HD1816/T^                | ZnuA/PF01297      | Haemophilus ducreyi         | Heme                | 100 μg/ml heme vs 15 μg/ml heme | −3.9^f      |
| CphmuT    | hmuT/T^                  | Peripla_BP_2/PF01497 | Corynebacterium pseudotuberculosis | Heme              | Non-chelated (high iron) vs iron-chelated (low iron) | −1.9^g      |
| CgHmuT    | hmuT/T^                  | Peripla_BP_2/PF01497 | Corynebacterium glutamicum | Heme                | 36 μM FeSO₄ vs 1 μM FeSO₄ | −8.8^h      |
| YphmuT    | hmuT/T^                  | Peripla_BP_2/PF01497 | Yersinia pestis             | Heme                | 40 μM FeCl₃ vs iron-chelated (low iron) | −6.8^h      |
| HtxB      | htxB/T^                  | No data           | Pseudomonas stutzeri        | Hypophosphate, phosphate | 2 mM Pi vs 0.1 mM Pi | −10.6^i     |
| IdiA      | Tery_3377/-              | No data           | Trichodesmium erythraeum   | Fe(II)              | 2 mM Pi vs 0.1 mM phosphate 2 mM Pi vs 0.1 mM phosphate | −13.2^j      |
| MntC      | mntC/T^                  | ZnuA/PF01297      | N. gonorrhoeae              | Mn(II), Zn(II)      | Mn(II) excess vs Mn(II)-chelated increased MntC levels in the presence of iron chelator | −17.5^k     |
| ModA      | modA/T^                  | No data           | E. coli                     | Molybdate, chromate, perrenenate | 100 μM Mo(II) vs no Mo(II) | −50^l       |
| NikA      | nikA/T^                  | SBP_bac_5/PF00496  | E. coli                     | Ni(II)              | 250 μM Ni(II) vs no Ni(II) 1 μM Ni(II) vs no Ni(II) | −5.33^m      |
| NikZ      | cj1584cT^                | SBP_bac_5/PF00496  | C. jejuni                   | Ni(II)              | 10 mM nitrate vs no nitrate^e | −3.6^n       |
| PhnA      | phnA/T^                  | No data           | E. coli                     | Phosphonate, 2-aminophosphonate | 2 mM Pi vs 0.2 mM Pi | −3466^o     |
| PstB      | ptaS/T^                  | PBP_like_2/PF12849 | P. aeruginosa               | Pi                  | 1 mM Pi vs 0.2 mM Pi | −223^p       |
| PstS1     | ptaS1/T^                 | PBP_like_2/PF12849 | Mycobacterium tuberculosis | Pi                  | 3.6 mM Pi vs Pi starvation | −2.24^q      |
| PstS3     | ptaS3/T^                 | PBP_like_2/PF12849 | M. tuberculosis             | Pi                  | 3.6 mM Pi vs Pi starvation | −6.5^r       |
| PtxB      | ptxB/T^                  | No data           | P. stutzeri                 | Pi, hypophosphate, | 2 mM Pi vs 0.1 mM Pi | −15^s       |

(continued on next page)
| SBP | Gene| Gene associated with | SBP family/Pfam | Species | SBP ligands | Experimental conditions | Fold change | Ref. |
|-----|-----|----------------------|-----------------|---------|-------------|-------------------------|-------------|-----|
| Sbp | XAC1017/T^a | No data | Peripla_BP_2/PF01497 | Xanthomonas citri | Sulfate | 2 mM Pi vs 0.1 mM phosphate | –20^a | [173] |
| VatD | V2_1012/T^a | Peripla_BP_2/PF01497 | V. vulnificus | Ferric aerobactin, ferric vulnibactin | | 2 mM Pi vs 0.1 mM hypophosphate | –17^a | [156] |
| ViiP | viiP/T^a | Peripla_BP_2/PF01497 | V. cholerae | Ferric vibriobactin | | 2 mM sulfate vs 1 mM sulfate/C0 | –1.9^a | [174] |
| ZnuA | znuA/T^a | ZnuA/PF01297 | E. coli | Zn(II), Co(II), Cu(II), Cu(I), Cd(II) | | 10 mM ZnSO_4 vs 3 mM ZnSO_4/C0 | 20e | [175] |
| Organic acids | adpC (RPA4515)/SP^a | TctC/PF03401 | Rhodopseudomonas palustris | Adipate, 2-oxoadipate, trans-trans-muconate, pimelate, suberate, azelate | 1 mM adipate vs no adipate | 5.8d | [103] |
| BctC | hp3867/T^a & SP^a | TctC/PF03401 | Bordetella pertussis | Citrate | 10 mM citrate vs no citrate | 16.9 | [89] |
| CouP | RPA1789/T^a | Peripla_BP_6/PF13458 | R. palustris | p-coumarate, ferulate, caffeate, cinnamate | 3 mM p-coumarate vs 3 mM benzoate | 5.7d/4.3g | [177] |
| MatC | RPA494/T^a | TctC/PF03401 | R. palustris | L- and D-malate, succinate, fumarate, L- and D-Met | 10 mM succinate vs 3 mM p-coumarate | No changeb | [177] |
| SiaP | siaP/T^a | DctP/PF03480 | H. influenzae | Sialic acid, N-acetylneuraminic acid, N-glycolyneuraminic acid | 10 mM sialic acid vs 0.1 mM sialic acid | 3.3d | [178] |
| TarP | RPA1782/T^a | DctP/PF03480 | R. palustris | p-coumarate, ferulate, caffeate, cinnamate | 3 mM p-coumarate vs 10 mM succinate | 2.3d/1.2e | [177] |
| TauA | tauA/T^a | OpuAC/PF04069 | E. coli | Taurine, N-(2-acetamido)-2-aminoethanesulfonic acid, 2-(N-morpholino)ethanesulfonate | 250 mM taurine vs 250 mM sulfate | 143d | [88] |
| Polyamines and quaternary ammonium compounds | betS | BCCT/PF02028 | Sinorhizobium (Ensifer) meliloti | Glycine betaine, proline betaine | 1 mM glycine betaine vs no glycine betaine | No changec | [179] |
| ChoX | choX/T^a | OpuAC/PF04069 | S. meliloti | Choline, acetylcholine | 7 mM choline vs no choline | No changec | [180] |
| NspS | VC0704/SP^a | SBP_bac_8/PF13416 | V. cholerae | Spermidine, norspermidine, spermine | Increased vs low c-di-GMP levels | 2.9^b | [181] |
| OpuAC | opuAC/T^a | OpuAC/PF04069 | Bacillus subtilis | Glycine betaine, proline betaine, arsenobetaine, dimethylglycine | 1 mM glycine betaine vs no glycine betaine | –2.2^b | [124] |

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| SBP | Gene| Gene associated with | SBP family/Pfam | Species | SBP ligands | Experimental conditions | Fold change | Ref. |
|-----|-----|----------------------|-----------------|---------|-------------|------------------------|------------|-----|
| PotD | potD | SBP_bac_8/PF13416 | E. coli | Spermidine, putrescine | 0.5 M NaCl vs no NaCl\(^{11}\) Increased transcription under osmotic stress\(^{11}\) | –2\(^{11}\) | [182] |
| ProX | proX | OpuAC/PF04069 | E. coli | Glycine betaine, proline betaine | 0.7 M sorbitol vs no sorbitol\(^{12}\) | 10.0\(^{12}\) | [184] |
| SpuD | spuD | SBP_bac_8/PF13416 | P. aeruginosa | Putrescine | 20 mM putrescine vs no putrescine | 8.4\(^{14}\) | [185] |
| SpuE | spuE | SBP_bac_8/PF13416 | P. aeruginosa | Spermidine | 20 mM spermidine vs no spermidine | 14\(^{14}\) | [185] |
| Mono-, oligo- and polysaccharides | | | | | | | |
| AguE | TM_0432 | SBP_bac_1/PF01547 | Thermotoga maritima | \(\alpha\)-1,4-digalacturonate | 10 mM pectin vs 10 mM D-ribose | 24\(^{15}\) | [186] |
| AlgQ2 | TM_0642 | SBP_bac_1/PF01547 | Sphingomonas sp. A1 | Alginate oligosaccharides | 0.5% (w/v) alginate vs 0.5% (w/v) glucose | 27.8\(^{16}\) | [94] |
| AraF | araF | Peripla_BP_1/PF00532 | E. coli | L-Arabinose, D-fucose | 10 mM cellobiose vs 10 mM D-ribose | 5\(^{17}\) | [186] |
| BglE | TM_0031 | SBP_bac_5/PF00496 | T. maritima | Cellobiose and laminaribiose | 0.6 mM GlcNAc vs no GlcNAc | No change\(^{18}\) | [102] |
| CBP | VC_0620 | SBP_bac_5/PF00496 | V. cholerae | Chitin oligosaccharides ([GlcNAc]_n) | 0.6 mM GlcNAc vs no GlcNAc | No change\(^{19}\) | [102] |
| ChvE | chvE | Peripla_BP_4/PF13407 | A. tumefaciens | Galactose, glucuronic acid, galacturonic acid, arabinose and glucose | D-fucose (3\(^{\circ}\) or 5\(^{\circ}\) mM) vs no D-fucose | 6\(^{20}\)/129\(^{20}\) | [188,189] |
| | | | | | D-galactose (3\(^{\circ}\) or 5\(^{\circ}\) mM) vs no D-galactose | 6\(^{20}\)/increased ChvE levels\(^{20}\) |
| | | | | | D-glucose (3\(^{\circ}\) or 5\(^{\circ}\) mM) vs no D-glucose | 6\(^{20}\)/increased ChvE levels\(^{20}\) |
| | | | | | 5 mM glucuronic acid vs no glucuronic acid | No changes\(^{21}\)/increased ChvE levels\(^{21}\) |
| GltB | PA3190 | SBP_bac_1/PF01547 | P. aeruginosa | Glucose | 10 mM glucose vs no glucose | 64.5\(^{22}\) | [71] |
| MalE | mae | SBP_bac_1/PF01547 | E. coli | Maltose, maltotriose, maltotetrose | 1 g/l glucose vs 0.1 g/l glucose\(^{23}\) | –67.4\(^{23}\) | [109] |
| MalE | lmo2125 | SBP_bac_8/PF13416 | Listeria monocytogenes | Maltose | 25 mM maltose vs no maltose | 58 M maltose vs no maltose | 12.0/24.0\(^{24}\) | [190] |
| | | | | | 0.2% (w/v) arabinose vs no arabinose | –12.0/24.0\(^{24}\) | |
| | | | | | | | | |

(continued on next page)
| SBP | Gene/ Gene associated with* | SBP family/Pfam | Species | SBP ligands | Experimental conditions | Fold change | Ref. |
|-----|-----------------------------|------------------|---------|-------------|--------------------------|------------|-----|
| MalE1 | TM_1204/T* | SBP_bac_8/PF13416 | T. maritima | maltotriose, maltotriose, β-(1-4)-mannanotetraose | 5 g/l trehalose vs 5 g/l glucose | Increased expression in trehalose | [192] |
| MalE2 | TM_1839/T* | SBP_bac_8/PF13416 | T. maritima | maltose, maltotriose, trehalose | 5 g/l lactose vs 5 g/l glucose | Increased expression in lactose | [192] |
| MalE2 | TM_1839/T* | SBP_bac_8/PF13416 | T. maritima | maltose, maltotriose, trehalose | 5 g/l maltose vs 5 g/l glucose | Increased expression in maltose | [193] |
| MglB (or GBP) | mglB/T* | Peripla_BP_4/PF13407 | E. coli | D-Galactose, D-glucose | 0.01-10 mM galactose vs no galactose | Strong induction of mglB transcription | [92] |
| MglB (or GBP) | mglB/T* | Peripla_BP_4/PF13407 | T. maritima | Mannose, mannobiose, lactose | 4 g/l glucose vs <10 mg/l glucose | 1.5 | [108] |
| MnBP3 | TM_1223/T* | SBP_bac_5/PF00496 | T. maritima | Mannose, mannobiose, cellobiose, laminaribiose, xylobiose, mannosapentaose, cellopentaose, xylopentaose, laminaripentaose, mannohexaose | 0.25% (w/v) mannose vs 0.25% (w/v) arabinose | Up-regulated | [195] |
| MnBP6 | TM_1226/T* | SBP_bac_5/PF00496 | T. maritima | Mannose, mannosapentaose, cellobiose, laminaribiose, xylobiose, mannosapentaose, cellopentaose, xylopentaose, laminaripentaose, mannohexaose | 0.25% (w/v) mannose vs 0.25% (w/v) arabinose | Up-regulated | [195] |
| RBP | TM_0958/T* | Peripla_BP_4/PF13407 | T. maritima | D-ribose | 10 mM D-ribose vs 10 mM L-arabinose | 22 | [186] |
| RbsB | rbB/T* | Peripla_BP_4/PF13407 | E. coli | D-ribose | 10 mM D-ribose vs 10 mM L-trehalose | 42 | [93] |
| ThuE | thuE/T* | SBP_bac_1/PF01547 | S. meliloti | Trehalose, maltose | 4 g/l glucose vs <10 mg/l glucose | 4.4 | [108] |
| XloE | TM_0071/T* | SBP_bac_5/PF00496 | T. maritima | Xylobiose, xylotriose | 0.25% (w/v) mannose vs 0.25% (w/v) arabinose | Up-regulated | [186] |
| YtfQ | ytfQ/T* | Peripla_BP_4/PF13407 | E. coli | Galactofuranose, arabino, galactose, talose, allose, ribose | 1 g/l glucose excess vs 0.1 g/l glucose | No change | [109] |
| Cofactors and terminal electron acceptors | | | | | | | |
| BtuF | synpcc7002_o0635/T* | Peripla_BP_2/PF01497 | Synechococcus sp. PCC 7002 | Cyano-cobalamin, cobalamin | 4 mg/L cobalamin vs no cobalamin | 18.1 | [197] |
| TorT | torT/SP* | Peripla_BP_1/PF00532 | E. coli | Trimethylamine N-oxide (TMN) | Anaerobic vs aerobic growth | 2.5 | [97] |
| Opines and quorum sensing molecules | | | | | | | |
| SBP Gene | Gene associated with | SBP family/Fam | Species                  | SBP ligands                                                                 | Experimental conditions                                                                 | Fold change | Ref.  |
|----------|----------------------|-----------------|--------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-------------|-------|
| AccA     | accA/T^a             | SBP_bac_5/PF00496 | *A. fabrum*              | Agrocinopine D, agrocinopine-3'-O-benzoate, agrocin 84, agrocinopine A, D-glucose-2-phosphate, L-arabinose-2-isopropylphosphate, L-arabinose-2-phosphate | 20 µM agrocinopines vs no agrocinopines (under phosphate limiting conditions; 0.1 mM Pi) | 4.5^e       | [198] |
| LuxP     | luxP/SP^a            | Peripla_BP_4/PF13407 | *Vibrio harveyi*        | AI-2                                                                         | 1 µM AI-2 vs no AI-2                                                                    | No change^e | [199] |
| LsrB     | lsrB/T^a             | Peripla_BP_4/PF13407 | *E. coli*                | AI-2                                                                         | 100 µM AI-2 vs no AI-2                                                                   | 10.5^b      | [200] |
| LsrB     | SM_b21016/T^a        | Peripla_BP_4/PF13407 | *S. meliloti*            | Al-2                                                                         | 80 µM Al-2 vs no Al-2                                                                    | 13.0^c      | [201] |
| NocT     | atu0207/T^a          | SBP_bac_3/PF00497 | *A. tumefaciens*         | Nopaline, pyronopaline, octopine                                            | Nopaline/pyronopaline mix (1 mM) vs no nopaline/pyronopaline                           | 54.5/68.1^d | [202] |

^a Genes associated with: T: transporter genes; SP: Signaling protein genes.

^b Microarray data.

^c Western blot.

^d Quantitative real-time PCR.

^e Reporter gene expression.

^f Northern hybridizations.

^g Mass spectrometry proteome analysis.

^h Protein expression profiling (2D protein gels followed by mass spectrometry/peptide mass fingerprinting).

^i Exponentially modified protein abundance index [emPAI].

^j RNA-sequencing.

^k SDS-PAGE gel analysis.

^l Induction was only observed in a nanAsiaB double mutant that can neither catabolize nor activate sialic acid.

^m Primer extension analyses.

^n Lowest and highest fold-change values measured at the different pH values ranging from 4.75 to 5.75.

^o In vitro transcription assays.

^p Genomic SELEX screenings.

^q Regulation mediated by non-cognate ligands or by environmental cues associated with SBP function.
that these two proteins interact in the periplasm [45,46]. The notion that all four chemoreceptors of the model organism most-studied for chemotaxis are activated by SBP binding gives further support to the notion that this type of sensing is widespread.

Apart from the E. coli, there are relatively few characterized chemoreceptor – SBP interactions. A well-characterized example is Pseudomonas aeruginosa chemotaxis to inorganic phosphate (Pi) [47,48], which is a central signal molecule that controls bacterial virulence [49]. Pi chemotaxis is due to the action of two chemoreceptors, CtpH and CtpL, that respond to high and low Pi concentrations, respectively [47,48]. The concerted action of both receptors thus provides an expansion of the response range. The two receptors employ different sensing mechanisms: whereas CtpH recognizes Pi directly via its TarH type LBD, CtpL contains an HBM domain, GGDEF: diguanylate cyclase; EAL: phosphodiesterase. In some cases binding of ligand-free SBP to receptor protein has been observed.

The functions and regulons of sensor kinases, diguanylate cyclases/phosphodiesterases and Ser/Thr kinases

The functions and regulons of sensor kinases, diguanylate cyclases/phosphodiesterases and Ser/Thr kinases that are stimulated by SBP binding are listed in Table 3. The SBP-stimulated systems carry out a number of different functions, including transport, respiration, compound catabolism or virulence, suggesting that there is no apparent restriction to the function of SBP-stimulated systems. These data also support the idea that these regulons are associated with specific and well-defined functions. As indicated above, the only characterized SBP-stimulated Ser/Thr kinase is PknG, which phosphorylates several target proteins that are involved in very different cellular processes [25]. Several studies of SBP-activated sensor kinases have provided insight into the sensing and transmembrane signaling mechanism that is summarized below.

4. Sensor kinases, diguanylate cyclases/phosphodiesterases and Ser/Thr kinases

The functions and regulons of sensor kinases, diguanylate cyclases/phosphodiesterases and Ser/Thr kinases that are stimulated by SBP binding are listed in Table 3. The SBP-stimulated systems carry out a number of different functions, including transport, respiration, compound catabolism or virulence, suggesting that there is no apparent restriction to the function of SBP-stimulated systems. These data also support the idea that these regulons are associated with specific and well-defined functions. As indicated above, the only characterized SBP-stimulated Ser/Thr kinase is PknG, which phosphorylates several target proteins that are involved in very different cellular processes [25]. Several studies of SBP-activated sensor kinases have provided insight into the sensing and transmembrane signaling mechanism that is summarized below.

4.1. The sensing mechanism

SBP-stimulated systems are composed of three molecules that mutually interact. The thermodynamics of this interaction have been established for the TorS-LBD/TorT/TMAO system [28] that is illustrated in Fig. 3.

Two apoTorT molecules were found to bind to the TorS-LBD dimer with high affinity (K_d=1.79 μM). ApoTorT bound its signal molecule TMAO (trimethylamine N-oxide) with a relatively modest affinity of 74 μM. However, TMAO recognition by the TorS-LBD/TorT complex occurred with negative cooperativity, with the two respective K_d values being 1.36 and 121 μM. This result indicates that TMAO binds preferentially to the SBP when it is associated to the sensor 

![Fig. 1. Schematic of the different membrane proteins that bind solute binding proteins (SBPs) and their corresponding primary functions. LBD: Ligand binding domain, GGDEF: diguanylate cyclase; EAL: phosphodiesterase. In some cases binding of ligand-free SBP to receptor protein has been observed.](image-url)
kinase as compared to the free form. Analytical ultracentrifugation studies also showed that TMAO binding to apoTorT increases its affinity for TorS-LBD, a finding that was also reflected in decreased atomic mobility at the TorS-LBD/TorT interface. In a similar way, the CBP SBP interacts with the non-canonical DNA binding sensor kinase ChiS to repress its activity, and the binding of chitin oligosaccharides by the CBP/ChiS complex activates ChiS without triggering the dissociation of the complex [58]. Of note is that these findings do not agree with a previous publication that suggested a ligand-induced dissociation of CBP from ChiS [59]. However, the sensing mechanisms established for these systems may not be generally applicable to all SBPs. For example, the working model of the MbaA-NspS system proposes that the binding of spermidine to the SBP NspS induces its dissociation from the MbaA sensor domain [60].

4.2. The mechanism of signal transduction: multiple events leading to a lateral displacement of transmembrane helices

The currently available data suggest that there may be multiple mechanisms by which signal-loaded SBPs control sensor autokinase...
eral displacement of the sensor kinase transmembrane regions also causes a latency transition observed in the LuxQ-LuxP system [28]. In this asymmetry transition that is the opposite of the symmetry-to-asymmetry transition reported for the TorS system [28]. The authors reported the structures of the two complexes were almost identical, indicating that isopropanol binding had the same effect as TMAO binding in the crystallization buffer) in the TorT ligand-binding site [28].

In the absence of the AI-2 signal, LuxP and LuxQ-LBD form hetero-dimers [29]. Upon signal binding, two LuxP/LuxQ-LBD pairs interact to form an asymmetric hetero-tetramer. In this asymmetric tetramer, both LuxQ-LuxP pairs are related by a rotation of 140 degrees, which represents a significant asymmetry. As a consequence of this asymmetry, the transmembrane helices are displaced laterally, which may be the molecular stimulus triggering changes in autokinase activity [29].

In the absence of the AI-2 signal, LuxP and LuxQ-LBD form hetero-dimers [29]. Upon signal binding, two LuxP/LuxQ-LBD pairs interact to form an asymmetric hetero-tetramer. In this asymmetric tetramer, both LuxQ-LuxP pairs are related by a rotation of 140 degrees, which represents a significant asymmetry. As a consequence of this asymmetry, the transmembrane helices are displaced laterally, which may be the molecular stimulus triggering changes in autokinase activity [29]. The mechanism of the LuxQ-LuxP system thus involves the signal-induced introduction of protein asymmetry and the formation of the hetero-tetramer complex. Analogously, the XylFII-LytS system, the authors propose that the formation of this signal-induced complex formation also causes a lateral displacement of the sensor kinase transmembrane regions [27].

In the crystallographic study of the TorS-TorT system [28], the authors used a covalently linked TorS-LBD dimer that makes it impossible to observe individual SBP-LBD complexes as observed in the two systems just discussed. The authors reported the structures of the apoTorS/TorQ-LBD complex as well as of complex structures that contained the TMAO signal or isopropanol (present in the crystallization buffer) in the TorQ ligand-binding site [28]. The structures of the two complexes were almost identical, indicating that isopropanol binding had the same effect as TMAO binding. In these structures, the TorS-LBD dimer formed a complex with two TorT monomers. In the signal-free structure, this hetero-complex was asymmetric and characterized by two distinct TMAO-binding sites. The thermodynamic studies mentioned above show that TMAO binds both sites with negative cooperativity (Fig. 3). The symmetry of the hetero-complex is achieved when TMAO binds to both sites, and the authors suggest that asymmetry must be reinforced by TMAO binding to the first, high-affinity site. Therefore, signal binding in this system causes an asymmetry-to-symmetry transition that is the opposite of the symmetry-to-asymmetry transition observed in the LuxQ-LuxP system [28].

A crystallographic study of the HptA/HptS-LBD complex has been reported recently [30]. The HptS-LBD belongs to the family of dCache domains that are composed of two α/β modules, generally referred to as membrane distal and membrane proximal mod-

Table 3
The functions of SBP-stimulated signal transduction receptors.

| Receptor | SBP | Function of system | Regulon/Comment | Ref. |
|----------|-----|-------------------|-----------------|-----|
| Chemoreceptors | | | | |
| Tar | MBP | Chemoattraction to maltose | | [26] |
| Tag | DppA | Chemoattraction to dipeptides | | [41] |
| Trg | GBP | Chemoattraction to galactose | | [38,4243] |
| Rbp | GBP | Chemoattraction to ribose | | |
| PotD | Chemoattraction from spermidine | | |
| Tsr | LsvB | Chemoattraction to Al-2 | | [45,46] |
| T+ | | | | |
| TiplB | AibA | Chemoattraction from Al-2 | | [56] |
| CptL | PsIS | Chemoattraction to low Pi concentrations | | [48] |
| BasT | BasB | Chemoattraction to amino acids | | [53] |
| CosT | CosB | Chemoattraction to quaternary amines | | [53] |
| Sensor kinases | | | | |
| LuxQ | LuxP | Quorum sensing | Five small regulatory RNAs (Qyr1-5) that target the master quorum-sensing regulator LuxR | [203] |
| LytS | XylFII | D-xylose transport | xylFGH operon encoding an D-xylose ABC transporter | [134] |
| TorS | TorT | Respiration on TMAO | torCAD operon encoding the trimethylamine oxide (TMAO) reductase system | [204] |
| HptS | HptA | Glucose-6-phosphate transport | hprt encoding the hexose phosphate transporter | [137] |
| VirA | ChvE | Virulence | vir genes and the repABC operon whose expression leads to the insertion of A. tumefaciens T-DNA into TmaI plasmid into host cells, causing tumor formation | [205] |
| AioS | AioX | Control of As(III) oxidation | aioBA genes encoding an As(III) oxidase | [206] |
| BctE | BctC | Citrate transport | bctCBA operon encoding a trisaccharide transporter | [89] |
| ChiS | CBP | Degradation of chitin | 50 genes, most of which encode proteins involved in chitin catabolism | [59] |
| GtrS | GltB | Glucose | gltBFGK-oprB operon encoding glucose transporter | [71] |
| Diguanylate cyclase/c-di-GMP phosphodiesterases (GGEDEF/EAL) | | | | |
| MbaA | NspS | Control of biofilm formation | Changes in c-di-GMP levels that alter expression of vps genes (among others), encoding proteins for biofilm polysaccharide synthesis | [207] |
| ScrC | ScrB | Motility and capsular polysaccharide production | Changes in c-di-GMP levels that alter expression of laf (lateral flagellum) and cps (capsular polysaccharide) genes, resulting in altered swarming motility and colony morphology | [208] |
| Serine/threonine kinase | | | | |
| GlnX/PknG | | Control of glutamate levels by regulating the activities of enzymes involved in TCA cycle and glutamate metabolism; evidence for additional PknG phosphorylation targets | PknG phosphorylates the regulatory protein GarA. Recent mass spectrometry approaches identified novel candidate PknG substrates that have roles in metabolism, cell wall synthesis and protein processing, translation and folding | [22,24,25,209] |

* The transmembrane protein GlnX interacts with the periplasmic SBP GlnH and with the Ser/Thr kinase PknG in the cytosol.
In the apo form, a hetero-tetramer was observed in which apo HptA SBP interacted with the membrane distal modules of both HptS-LBD monomers (Fig. 2E). However, binding of the signal molecule glucose-6-phosphate (G-6-P) caused large structural rearrangements. The result of these rearrangements was that HptA linked the membrane-distal module of one HptS-LBD monomer with the membrane-proximal module of the other (Fig. 2F). These large structural rearrangements caused a tilting of HptS-LBD, leading to a lateral displacement of both transmembrane regions in a manner reminiscent of the other systems studied [30].

Cache domains are the most abundant extracytosolic bacterial sensor domains [13,14,62] and the majority of Cache domains are present in their dCache configuration. A large number of dCache domains have been studied, and, in almost all cases, ligands bind to the membrane-distal module [63–66]. That circumstance raised the question as to the function of the membrane-proximal module. The X-ray study of HptS-LBD in a complex with HptA described above may provide an answer to this question. It was shown that signal-bound HptA links two HptS-LBDs by establishing contacts with the membrane-distal and membrane-proximal modules [30]. Other than the finding that lactate binds to the membrane-proximal module of the TlpC chemoreceptor of H. pylori [67], this is the first demonstration of a role for the membrane-proximal module of dCache domains in the signaling process.

Further studies will show to what degree other dCache domains operate by the same mechanism.

The signal transduction mechanism for systems that sense signals directly is still subject to debate [61]. Ligand binding at LBDs has been shown to induce a number of different reorientations, such as helical rotation, piston shifts or helix scissoring movements. Frequently, these changes occur concomitantly, and several reorientations of the receptor LBD, like scissoring movements, are compatible with lateral displacements of the transmembrane regions [68–70]. Further research will be required to determine to what degree lateral displacements of transmembrane regions cause the activation, whether direct or indirect, of ligand-triggered signal-transduction receptors.

5. GltB: A SBP that interacts with a sensor kinase and chemoreceptors

Another layer of complexity of SBP-mediated regulation is added by the observation that the glucose-sensing SBP GltB interacts with the periplasmic domain of the GtrS sensor kinase as well as with the LBD of the PctA and McpN chemoreceptors [71]. The GtrS-LBD had been previously shown to bind two glucose derivatives, 2-ketogluconate and 6-phosphogluconate, and to regulate the expression of genes involved in glucose metabolism [72]. Xu et al. show that the binding of glucose-loaded GltB is essential for the GtrS-mediated control of genes involved in glucose transport [71]. Chemoreceptors PctA and McpN were previously found to bind amino acids and nitrate, respectively, and to mediate chemotaxis to these compounds [73–75]. Although the authors of this study demonstrate the binding of GltB to the LBDs of PctA and McpN, they did not explore whether this resulted in chemotaxis. GtrS, PctA and McpN each possesses a different type of LBD. The LBD of GtrS is not recognized by any domain profile, whereas PctA and McpN possess a dCache and PilJ type LBD, respectively [64,75]. The PctA chemoreceptor is a prime example of the complexity of bacterial signal transduction. Apart from binding amino acids [64] and GltB [71], PctA also mediates chemotaxis to AI-2 [76] and histamine [63]. It has been shown that the PctA-LBD binds AI-2 directly with high affinity [76], whereas it has been

Fig. 3. The mechanism of TorT and TMAO recognition by the ligand-binding domain of TorS. Because sensor kinases form stable dimers in the membrane, experiments were conducted using a dimeric TorS-LBD in which both monomers are covalently linked by a 25 amino acid flexible linker. The thermodynamic parameters derived from analytical ultracentrifugation and isothermal titration calorimetry experiments are indicated. Data were taken from [28].
proposed that PctA mediates histamine chemotaxis through binding an unidentified SBP [63].

6. Additional studies suggesting SBP-mediated stimulation of signal transduction receptors

There are a number of other studies that provide indirect evidence for activation of signaling receptors by SBP binding. One example is the mechanism for chemotaxis of Spingomonas sp. strain A1 to the polysaccharide pectin. The SBP SPH1118 binds pectin, and a knockout mutation in the corresponding gene abrogates pectin chemotaxis [77], suggesting an indirect activation mechanism. However, the molecular mechanism by which a polysaccharide interacts with an SBP remains to be established. Furthermore, the Tlpl chemoreceptor of Campylobacter jejuni mediates chemotaxis to aspartate [78]. However, the 3D structure of the LBD revealed that the binding pocket is too small to accommodate this ligand and the individual Tlpl-LBD failed to bind aspartate. The authors thus suggest that Tlpl is activated by SBP binding [78]. Another example is that of the McpC chemoreceptor of Bacillus subtilis, which supports chemotaxis to 17 amino acids. However, binding studies revealed that only 11 of them bound directly to the purified McpC-LBD [79]. To investigate a potential involvement of SBPs, the authors conducted pull-down experiments with immobilized McpC-LBD. This study detected interactions of McpC with the amino acid-sensing SBPs ArtP, MetQ and YckB. The authors thus conclude that McpC employs two different mechanisms based on direct and indirect sensing [79]. Furthermore, chemotaxis of P. aeruginosa towards histamine is based on the concerted action of three chemoreceptors: TlpQ, PctA and PctC [63]. Whereas TlpQ-LBD bound histamine with nanomolar affinity, no direct binding was detected for the PctA and PctC chemoreceptors, which are known to bind different amino acids directly [64]. These data suggest that the histamine signal mediated by PctA and PctC signaling depends on their interaction with one or more histamine-binding SBPs. The Tlp1, McpC, PctA and PctC chemoreceptors all possess dCache-type LBDs. This observation, in combination with the available structural and functional data (Fig. 2, Table 1), suggests that SBP-mediated receptor stimulation may be a property of many dCache domains.

In Bacillus subtilis, the SBP-encoding gene ydbE is located next to genes encoding the sensor kinase–response regulator pair YdbFG. YdbE shows homology to SBPs that sense C4-dicarboxylates, and the YdbFG TCS regulates the utilization of fumarate and succinate [80]. Inactivation of either ydbF or ydbE abolished this regulatory activity, and it was suggested that YdbE plays a sensory role by stimulating the YdbFG TCS [80]. Furthermore, the genes encoding homologs of the A1–2 SBP LsrB are typically found next to the A1–2 transporter. Interestingly, in Treponema primitia and Treponema azotonutricium lsrB genes are located next to genes encoding sensor kinase/response regulator pairs, suggesting a participation in TCS signaling rather than transport [81].

7. Regulation of SBP levels

What are the advantages of SBP-mediated receptor stimulation as compared to systems that recognize signals directly? The overall responses of SBP-activated chemoreceptors have been shown to be highly sensitive to the SBP levels, permitting more-precise control of the sensitivity to specific ligands in response to their nutrient environment [21]. Although regulation of the expression of SBPs has been reviewed several times [82–86], the role of ligands on the expression of their cognate SBPs has not been analysed on a global scale. To assess the effect of different ligand families on SBPs levels, we have surveyed the results of studies on 88 SBPs from 27 different phylogenetically diverse bacterial species with different lifestyles, including plant, animal and human pathogens, beneficial plant-associated bacteria, as well as non-pathogenic marine and soil bacteria. These SBPs bind a wide range of compounds, including amino acids, peptides, organic acids, sugars, polyamines, quaternary amines, cofactors, opines, quorum-sensing molecules or inorganic nutrients. The effects of these ligands on the expression of their cognate SBPs are compiled in Table 2, and the resulting patterns are illustrated in Fig. 4.

A major conclusion of this study is that 87 % of the ligands modulated the expression of their cognate SBPs (Table 2). The magnitude of regulation stretches from a 3460-fold downregulation of phnD expression in the presence of 2 mM as compared to 0.2 mM Pi [87] to a 143-fold upregulation in the expression of tauA in the presence of taurine [88]. Although the regulatory proteins that control the expression of several of the SBPs have been identified [89–98], the corresponding molecular mechanisms of the regulation are not the focus of this review.

7.1. Sugars and organic acids generally upregulate the expression of cognate SBPs

Sugars and organic acids are often preferred carbon sources for bacteria [99–101], and SBPs have been identified for mono-, oligo- and polysaccharides, tricarboxylic acid cycle intermediates, sugar acids and aromatic acids (Table 2). The analysis of the expression of 27 sugar and organic acid SBPs from 11 different bacterial genera revealed a preferential upregulation in the presence of their cognate ligands (Table 2). Notable examples were the genes encoding CBP and TauA, whose expression was upregulated more than 100-fold in the presence of chitin oligosaccharides [102] and taurine [88], respectively. In some cases, different regulatory effects were observed at different ligand concentrations. For example, the transcript levels of adpC encoding an aliphatic dicarboxyylate SBP were increased in the presence of micromolar concentrations of its dicarboxyylate ligands but reduced in the presence of millimolar concentrations of these dicarboxyylate SBPs [103]. As shown in Table 1, the SBPs BctC, ChvE and CBP stimulate the sensor kinases BctE [89,104], VirA [105–107] and ChiS [59,102], respectively, and their cognate ligands upregulate the expression of BctC, ChvE and CBP at the levels of both transcription and protein synthesis (Table 2).

In contrast to the general tendency that sugars enhance SBP expression, the presence of an excess of glucose reduced the expression of the sugar-binding, amino acid-binding and peptide-binding SBPs LAO/ArgT, MalE, MppA, MglB, OppA, RbsB and YtfQ either at the transcript or protein level (Table 2). This effect was mainly due to a global regulatory mechanism, termed carbon catabolite repression (CCR), that modulates up to 10% of all genes in a given bacterium [99–101]. The mechanism implies that in the presence of preferred carbon sources, such as glucose in Escherichia coli and Bacillus subtilis, the expression of genes involved in the catabolism and transport of non-preferred carbon sources is repressed. As shown in Table 2, CCR plays an important role in the regulation of multiple SBPs in E. coli and Salmonella typhimurium. A mechanistic reason for the CCR-mediated SBP repression may be related to the fact that E. coli preferentially uses the phosphoenolpyruvate–carbohydrate phosphotransferase system for glucose transport (independent of SBPs) when growing on millimolar concentrations of sugars and switches to high affinity transport systems dependent on SBPs under glucose-limiting conditions [82,108,109]. Thus, the synthesis of a number of ABC transporter SBPs is upregulated at low glucose concentrations (Table 2).

7.2. Downregulation of SBPs by inorganic ligands

Phosphorous and metal ions like iron, nickel, manganese and zinc are essential elements for many biological processes. They also
play important roles in bacterial signaling by modulating gene expression [110–114] and chemotactic processes [48,115,116]. The analysis of the expression of 28 phosphorous and metal ion SBPs from 16 different bacterial species revealed that these elements strongly repress the expression of their cognate SBPs (Table 2). Phosphorous and metal ions have a limited bioavailability due to their low abundance or insolubility. SBPs for these compounds are generally characterized by a very high affinity for their cognate ligands, permitting responses at very low concentrations. Representative examples are the binding of molybdate and Pi to their cognate SBPs ModA and PstS, respectively, that occurs with a much higher affinity (K_a ~ 10 nM) than in other compound families [48,117]. In addition, the intracellular concentration of these elements needs to be finely balanced to avoid cellular toxicity. The repression of SBPs in response to elevated concentrations of metal ions and inorganic nutrients is a mechanism to maintain homeostatic intracellular levels [85,86,111,113,114]. As shown in Table 1, the SBP AioX binds arsenite and subsequently stimulates the TCS AioSR [118,119]. In contrast to the effect of most inorganic compounds on SBP expression, arsenite induced aioX expression (Table 2), which may be due to the fact that AioSR induces the expression of the genes involved in arsenite oxidation (Table 3), a process that reduces the toxicity of the ligand.

7.3. Dissimilar effects of amino acids, polyamines and quaternary ammonium compounds on cognate SBPs expression

Contrary to what was observed for sugars and organic acid SBPs, the expression of SBPs specific for amino acids and peptides in E. coli was generally downregulated at both the gene expression and protein levels in the presence of their cognate ligands (Table 2). An interesting case is that of the four E. coli SBPs ArtJ, ArtI, HisJ and LAO/ArgT that bind arginine (Table 2) [120]. These SBPs are encoded in two different clusters, artJ-hisJQMP and artIPIQM-artJ, which also encode the ABC-type transporters HisQMP and ArtQPM, respectively [9]. The expression of the genes encoding ArtJ, ArtI and HisJ, but not ArgT, was downregulated in the presence of arginine in a regulatory process mediated by the transcriptional repressor ArgR [91,121]. Whereas a strong downregulation was observed for artJ, only a weak repression was noted for hisJ and artI (Table 2).

Current data suggest that this differential regulation is associated with the different physiological functions of ArtJ, ArtI, HisJ and LAO/ArgT. Thus, the arginine-specific ArtJ as well as the arginine-binding and ornithine-binding SBP ArtI were suggested to be mainly involved in the uptake of amino acids for biosynthetic processes [91,120]. In contrast, the expression of argT is activated by the nitrogen regulatory protein NtrC in response to nitrogen limitation [122], and ArgT may be primarily implicated in the transport of arginine under conditions of nitrogen starvation. HisJ binds histidine with a 10-fold higher affinity than arginine [91] and its weak repression by arginine (Table 2) may ensure efficient histidine uptake when arginine is abundant. The primary role of amino acid-binding and peptide-binding SBPs is the uptake of nutrients for growth, but the dipeptide-binding SBP DppA of E. coli is involved in both chemotaxis and transport (Tables 1 and 3) and its expression was downregulated at both the transcriptional and protein synthesis levels in the presence of casamino acids (Table 2).

The expression profiles of polyamine-binding and quaternary ammonium compound-binding SBPs in the presence of their cognate ligands was dissimilar, with these SBPs showing either upregulation, downregulation or unaltered expression (Table 2). Several of these SBPs bind the osmoprotectants glycine betaine and proline betaine, and their expression was induced in E. coli and B. subtilis under conditions of osmotic stress to permit a rapid adjustment of the intracellular osmotic strength (Table 2) [123,124]. In accordance with the regulation of the expression of SBPs by environmental cues that are associated with the SBP function, the expression of torT encoding a SBP that binds the terminal electron acceptor TMAO was upregulated under anaerobic conditions [97]. In addition, second messengers were also found to modulate the expression of SBPs. As indicated above, the SBP NspS of V. cholerae forms a signaling system with the transmembrane GGDDEF-EAL protein MbaA to control biofilm formation in response to polyamines [125]. Increased c-di-GMP intracellular levels were shown to upregulate the expression of nspS (Table 2).

8. Conclusions and research needs

The studies reviewed here strongly suggest that SBP-mediated activation of transmembrane receptors is a general and widespread
phenomenon. Researchers working in the field are thus encouraged to design experimental strategies, using either genetic or protein biochemistry approaches, to identify further systems. The overall final objective of these research efforts, which will lead to a larger number of characterized systems, is to get a more comprehensive understanding of which signaling systems are stimulated by direct binding and which by SBP-based mechanisms. Accumulating information will also enable us to see more clearly the physiological constraints that have led to the evolution of receptor activation by direct and indirect binding. Central questions still to be answered are:

1. SBPs and receptor LBDs form superfamilies composed of many individual families. Do members of all or only some families participate in indirect receptor activation? Is it possible to identify sequence features specific for SBP-LBD interactions?

2. Whereas signal-loaded SBPs stimulate receptors, much less information is available on the role of the SBP apo forms. What are the affinities of apo- and holo-SBPs for their respective targets? What is the effect of apo-SBP binding on receptor activity?

3. Several receptors bind signals directly as well as in complexes with SBPs. How frequent are such systems and what is the flexibility in the response afforded by these dual input mechanisms for receptor activation?

4. What is the concentration of SBPs in the periplasmic space?

5. What factors determine whether the expression of an SBP is regulated by its cognate ligand?

6. What is the evolutionary history of the receptor proteins that are activated by both direct and indirect ligand binding?

The answers to these questions will not only increase our fundamental knowledge about signal transduction mechanisms but also may offer alternative strategies to fight pathogenic bacteria by interfering with their ability to sense, respond and adapt to their environment.

CRediT authorship contribution statement

Miguel A. Matilla: Conceptualization, Data curation, Funding acquisition, Writing – original draft, Writing – review & editing. Álvaro Ortega: Conceptualization, Data curation, Writing – review & editing. Tino Krell: Conceptualization, Data curation, Funding acquisition, Writing – original draft, Writing – review & editing.

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References

[1] Scheepers GH, Lycklama a Nijeholt JA, Poolman B. An updated structural classification of substrate-binding proteins. FEBS Lett 2016;590(23):4393–401.
[2] El-Gebali S, Mistry J, Bateman A, Eddy, S.R., Luciani, A, et al. 2019. The Pfam protein families database in 2019. Nucleic Acids Res. 47, D427-D432.
[3] Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., et al., 2019. Several receptors bind signals directly as well as in complexes with SBPs. How frequent are such systems and what is the flexibility in the response afforded by these dual input mechanisms for receptor activation?

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References

[1] Scheepers GH, Lycklama a Nijeholt JA, Poolman B. An updated structural classification of substrate-binding proteins. FEBS Lett 2016;590(23):4393–401.
[2] El-Gebali S, Mistry J, Bateman A, Eddy, S.R., Luciani, A, et al. 2019. The Pfam protein families database in 2019. Nucleic Acids Res. 47, D427-D432.
[3] Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., et al., 2019. InterPro in 2019: improving coverage, classification and access to protein sequence annotations. Nucleic Acids Res. 47, D351-D360.
Kokoeva MV, Storch KF, Klein C, Oesterhelt D. A novel mode of sensory
Laganenka L, Colin R, Sourjik V. Chemotaxis towards autoinducer 2 mediates
Richarme G. Interaction of the maltose-binding protein with membrane
Daddaoua A, Molina-Santiago C, la Torre Jd, Krell T, Ramos J-L. GtrS and GltR
Gardina PJ, Bormans AF, Hawkins MA, Meeker JW, Manson MD. Maltose-
Metcalf WW, Wanner BL. Construction of new beta-glucuronidase cassettes
Aksamit RR, Koshland DE. Identification of the ribose binding protein as the
Björkman AJ, Binnie RA, Zhang H, Cole LB, Hermodson MA, Mowbray SL. A
Machuca MA, Johnson KS, Liu YC, Steer DL, Ottemann KM, Roujeinikova A. The
Glekas GD, Mulhern BJ, Kroc A, Duelfer KA, Lei V, Rao CV, et al. The
Rader BA, Wreden C, Hicks KG, Sweeney EG, Ottemann KM, Guillemin K. R
Zaborin A, Romanowski K, Gerdes S, Holbrook C, Lepine F, Long J, et al. Red
Rodríguez-Arce I, Al-Jubair T, Euba B, Fernández-Calvet A, Gil-Campillo C, Rowe JL, Starnes GL, Chivers PT. Complex transcriptional control links Chivers PT, Sauer RT. Regulation of high affinity nickel uptake in bacteria. Ni2 Weber A, Ko White AK, Metcalf WW. The htx and ptx operons of Angerer A, Braun V. Iron regulates transcription of the Momma K, Mishima Y, Hashimoto W, Mikami B, Murata K. Direct evidence Patzer SI, Hantke K. The ZnuABC high-affinity zinc uptake system and its Miyamoto K, Kosakai K, Ikebayashi S, Tsuchiya T, Yamamoto S, Tsujibo H. Rech S, Deppenmeier U, Gunsalus RP. Regulation of the molybdate transport Alonzo S, Heyde M, Laloi P, Portalier R. Analysis of the effect exerted by M.A. Matilla, Á. Ortega and T. Krell Computational and Structural Biotechnology Journal 19 (2021) 1786–1806.

[163] Ibraim IC, Parise MTD, Parise D, Sfeir MZT, de Paula Castro TL, Wattam AR, Rogers MB, Sexton JA, DeCastro GJ, Calderwood SB. Identification of an Howlett RM, Hughes BM, Hitchcock A, Kelly DJ. Hydrogenase activity in the Blum, S. E., Goldstone, R. J., Connolly, J. P. R., Reperant-Ferter, M., Germon, P., Rech S, Deppenmeier U, Gunsalus RP. Regulation of the molybdate transport Alonzo S, Heyde M, Laloi P, Portalier R. Analysis of the effect exerted by M.A. Matilla, Á. Ortega and T. Krell Computational and Structural Biotechnology Journal 19 (2021) 1786–1806.

[163] Ibraim IC, Parise MTD, Parise D, Sfeir MZT, de Paula Castro TL, Wattam AR, Rogers MB, Sexton JA, DeCastro GJ, Calderwood SB. Identification of an Howlett RM, Hughes BM, Hitchcock A, Kelly DJ. Hydrogenase activity in the Blum, S. E., Goldstone, R. J., Connolly, J. P. R., Reperant-Ferter, M., Germon, P., Rech S, Deppenmeier U, Gunsalus RP. Regulation of the molybdate transport Alonzo S, Heyde M, Laloi P, Portalier R. Analysis of the effect exerted by M.A. Matilla, Á. Ortega and T. Krell Computational and Structural Biotechnology Journal 19 (2021) 1786–1806.
Bansal T, Jesudhasan P, Pillai S, Wood TK, Jayaraman A. Temporal regulation of enterohemorrhagic Escherichia coli virulence mediated by autoinducer-2. Appl Microbiol Biotechnol. 2008;78(5):811–9.

Pereira, C. S., McAuley, J. R., Taga, M. E., Xavier, K. B. & Miller, S. T. (2008). Sinorhizobium meliloti, a bacterium lacking the autoinducer-2 (AI-2) synthase, responds to AI-2 supplied by other bacteria. Mol Microbiol. 70, 1223-1235.

Lang J, Vigouroux A, Planamente S, El Sahili A, Blin P, Aumont-Nicaise M, et al. Agrobacterium uses a unique ligand-binding mode for trapping opines and acquiring a competitive advantage in the niche construction on plant host. PLoS Pathog. 2014;10(10):e1004444. https://doi.org/10.1371/journal.ppat.1004444.

Tu KC, Bassler BL. Multiple small RNAs act additively to integrate sensory information and control quorum sensing in Vibrio harveyi. Genes Dev. 2007;21(2):221–33.

Méjean V, Iobbe-Nivol C, Lepelletier M, Giordano G, Chippaux M, Pascal M-C. TMAO anaerobic respiration in Escherichia coli: involvement of the tor operon. Mol Microbiol. 1994;11(6):1169–79.

Brencic A, Winans SC. Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. Microbiol Mol Biol Rev. 2005;69(1):155–94.

Kashyap DR, Rotero LM, Franck WL, Hassett DJ, McDermott TR. Complex regulation of arsenite oxidation in Agrobacterium tumefaciens. J Bacteriol. 2006;188(3):1081–8.

Karatan E, Duncan TR, Watnick PI. NspS, a predicted polyamine sensor, mediates activation of Vibrio cholerae biofilm formation by norspermidine. J Bacteriol. 2005;187(21):7434–43.

Boles BR, McCarter LL. Vibrio parahaemolyticus scrABC, a novel operon affecting swarming and capsular polysaccharide regulation. J Bacteriol. 2002;184(21):5946–54.

Cowley, S., Ko, M., Pick, N., Chow, R., Downing, K. J., Gordhan, B. G., Betts, J. C., Mizrahi, V., Smith, D. A., Stokes, R. W. & Av-Gay, Y. (2004). The Mycobacterium tuberculosis protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth in vivo. Mol Microbiol. 52, 1691-1702.