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# RECENT PROGRESS IN *BACILLUS SUBTILIS* TWO-COMPONENT REGULATION

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## 1. ABSTRACT

Two-component regulatory systems serve to control gene expression in response to environmental and physiological changes. They are widespread among a variety of organisms and most often found in prokaryotes. One of the gram-positive microorganisms *Bacillus subtilis* is a well-studied bacterium whose complete nucleotide sequence has been determined. Thus, it is now possible to study transcription of the whole genome with microarray analysis. In this review we summarize the recent progress in *B. subtilis* two-component regulatory systems by describing the known systems and those for which the function was recently assigned. Also included is an attempt to construct a partial transcriptional network involving several two-component systems. The studies described here are based on the data from traditional genetics and biochemistry, and from microarray analysis of 29 two-component systems.

## 2. INTRODUCTION

The two-component system in microorganisms is a ubiquitous signaling device, which is composed of a sensor kinase (SK) and a response regulator (RR) in the simplest style (1). In general, SK contains transmembrane segments that divide the protein into two parts, one containing an N-terminal sensory domain and the other a C-terminal catalytic domain. The catalytic part of SK phosphorylates its own histidine residue by responding to some environmental and metabolic signals. The phosphoryl group is then transferred to an aspartate residue within the conserved N-terminal receiver domain of the cognate RR. In most cases, RR carries a C-terminal helix-turn-helix motif, and upon phosphorylation it acts as a transcription factor to elicit appropriate adaptive responses. The system consisting of multiple His-Asp phosphorelay components is a more elaborated version of the two-component system. Crystallographic analysis of various SKs and RRs has provided the basis for deeper understanding of the molecular mechanism underlying the two-component signal transduction, although the number of SKs, RRs and their derivatives determined for their structure is limited. Several detailed reviews on these issues have been published (1- 3).

Availability of genome sequences of various microorganisms provides opportunities for a genome-wide comparison of various two-component systems among them. Bioinformatic analysis has revealed that a relatively

large number of the two-component system exist in free-living microorganisms, whereas the number of the signaling system is very limited for parasites. Indeed, *Escherichia coli* and *Bacillus subtilis*, free-living organisms belonging to the gram-negative and gram-positive clades, respectively, carry 30 SKs and 32 RRs (4, 5), and 37 SKs and 34 RRs (5, 6), respectively. In contrast, *Mycoplasma genitalium* and symbiotic *Buchnera* sp. have no such systems (5). In archea, distribution of this device is somewhat biased; *Methanococcus thermoautotrophilum* has 16 SKs and 8 RRs, while *M. jannaschii* (1.7 Mb) lacks such proteins, even though both species are classified into the same genus and are not parasites (5). In terms of structure, there are five hybrid SKs in *E. coli* that carry additional Hpt (Histidine phosphate transfer) or receiver domains (4), whereas no such hybrid structure is found in *B. subtilis* two-component signaling proteins. It is not known how and why such a difference has occurred in evolution.

Sequence determination of the whole *B. subtilis* genome (7) has made it possible to perform DNA microarray analysis on all the potential genes. In fact, target gene candidates for transcriptional regulators bearing a helix-turn-helix DNA binding motif have been obtained by this powerful tool (8-11). In this review, we focus on the recent progress in the study of *B. subtilis* two-component regulatory systems that carry a helix-turn-helix motif in the regulator with emphasis on the results of microarray analysis.

## 3. PROGRESS IN *B. SUBTILIS* TWO-COMPONENT REGULATORY SYSTEMS WITH KNOWN FUNCTION

The content of this section is confined to aspects of the known two-component regulatory systems of *B. subtilis*. For detailed description and information, readers should refer to the recently published reviews (12-16). In addition, the study on the phosphorelay system involving Spo0A is not included here for simplicity, since a large number of studies are reported in those reviews.

### 3.1. DegS-DegU

The DegS-degU two-component system was identified as a regulatory system controlling the synthesis of extracelluar degradative enzymes (17). It has been demonstrated that the phosphorylated form of DegU stimulates degradative enzyme synthesis at the transcription level. Since DegS does not contain transmembrane amino acid sequences, it is likely to be a cytosolic protein and senses some intracellular signal to induce degradative enzyme synthesis. DegU is also involved in competence development, but it is the unphosphorylated form of DegU that stimulates this process (17). DegU is required for the synthesis of ComK, the competence transcription factor that is necessary for competence development (18, 19). It has been shown that DegU is necessary for *comK* transcription because it primes the productive interaction of ComK with its own promoter, thus triggering the *comK* autoregulatory circuit that results in a rapid increase in ComK concentration (20). Thus, DegU is unusual in that it is active in two forms, and the cells' decision to produce degradative enzymes or to develop competence is determined by the phosphorylation state of DegU. Since DegU regulates different targets in two forms, it is called a molecular switch (17). The observation that unphosphorylated DegU is functional in activating competence development may be related to the following two cases. In alginate biosynthesis in *Pseudomonas aeruginosa*, only the unphosphorylated but not the phosphorylated forms of the RRs, AlgB and AlgR, activate the target gene (21). In *Azotobacter vinelandii*, phosphorylation of NifL at the His site known to be the phosphorylation site of the SKs is not necessary for the activation of the cognate RR NifA, suggesting that unphosphorylated NifA has a functional activity (22).

The DegS-DegU system is involved in salt stress response. Expression of the genes for exocellular alkaline protease, *aprE*, and wall associated protein, *wap*, is downregulated by the presence of high salt, whereas that of levansucrase gene, *sacB*, is upregulated (23, 24). The effects of salt on *wap* and *sacB* expression are not seen in *degS* or *degU* null mutants, indicating that the phosphorylated DegU is involved in salt stress response .

Stabilization of phosphorylated DegU inhibits transcription of *sigD* encoding an alternative sigma factor, SigD (25, 26). It has also been suggested that phosphorylated DegU inhibits the activity of SigD (26). This sigma factor directs transcription of the class three motility and chemotaxis genes (the *sigD* regulon genes) (27). We have observed in microarray analysis that overproduction of DegU in a *degS*-deleted strain resulted in repression of most of the genes in the *sigD* regulon without a decrease in *sigD* transcription (10), in agreement with the previous observations.

In addition to DegS-DegU, there are several factors that exert positive effects on *aprE* expression (17, 28). These include DegR, DegQ, ProB, SenS and TenA. The positive effects of the first four factors on *aprE* expression depend on the presence of the functional DegS-DegU. In the case of DegR, the positive effect was shown to be due to stabilization of phosphorylated DegU in the cell (29). Among the five factors, disruption of *degR* and *degQ* results in significant reduction in *aprE-lacZ* expression, while *tenA* and *senS* disruption moderately affects the fusion expression. On the other hand, *aprE-lacZ* expression is delayed by *senS* deletion, although whether this effect is via DegS-DegU system remains to be studied (28).

Microarray analysis revealed many genes under DegU regulation whose function is known or unknown (10), in addition to the already known target genes such as those involved in protease synthesis (*aprE*, *nprE* and *ispA*). The known genes include those for ATP synthase, siderophore synthesis, polyketide synthase, flagellar synthesis etc. It is interesting to note that *epr*, an extracellular serine protease gene, is negatively regulated by DegU unlike the regulation of the other protease genes. This may suggest a role of Epr in a cellular process other

than degradative enzyme synthesis. That the number of genes regulated by DegU is large indicates that the DegS-DegU system is a global one involved in various aspects of cell function.

### 3.2. ComP-ComA

This system serves as a quorum-sensing device to regulate expression of the genes for competence development (14, 15). For *B. subtilis* cells to become competent, sufficient concentration of the ComX pheromon, a 10-amino-acid peptide, is required. The pheromon is excised from the cytoplasmic 55-amino-acid precursor and modified at the tryptophan residue probably by an isoprenoid residue in a ComQ-dependent manner (30). The secreted ComX pheromon interacts with the second extracellular loop of ComP, resulting in autophosphorylation of ComP (14, 31). The ComA RR receives phosphate from the phosphorylated ComP and activates the genes involved in competence development. The *comPA* genes lie immediately downstream of the *comQX* genes. Among several *Bacillus* species, the amino acid sequences of ComQ, ComX and a part of the N-terminal, extracellular region of ComP show species-specific diversity, which results in the species-specific activation of the ComA regulon (32, 33). Another extracellular factor has been identified as CSF, which is believed to cause stimulation of ComA phosphorylation probably through inhibition of an aspartyl-phosphate phosphatase, RapC, whose target may be phosphorylated ComA (14). Transcription of *rapC* is stimulated by phosphorylated ComA, indicating an autoregulatory loop between ComA and RapC (34). On the other hand, CSF accumulated at high concentrations in stationary culture is thought to inhibit the ComP kinase activity (14). Phosphorylated ComA activates the *srfA* operon encoding the enzymes involved in surfactin synthesis (14, 15). The *srfAB* region encodes another small protein required for competence development, ComS. Moreover, it was suggested that ClpX might be required for ComA-dependent transcription of *srfA*, although the exact mechanism is unknown (35).

Microarray analysis revealed ComA targets such as the genes for antibiotic (surfactin) synthesis and aspartyl-protein specific phosphatases, RapA and RapF, in addition to many unknown genes (10, 36). Apparently the ComP-ComA system is involved in regulation of genes other than those involved in competence.

### 3.3. PhoR-PhoP

A DNA microarray analysis on the PhoP regulon revealed *glnQ*, *yjdB* and *yycP* as the newly identified genes (10). A proteome analysis has also been carried out on phosphate-starved cells (37), and the results show that there are several genes whose expression is low-phosphate inducible but independent of PhoRP. In an attempt to identify the sensing process of low-phosphate concentration by PhoR, an extensive deletion analysis of the N-terminal region of PhoR was performed (38). Surprisingly, deletions of not only the periplasmic loop but also the transmembrane and intracellular linker regions did not abolish the low-phosphate inducible synthesis of alkaline phosphatase, indicating that the kinase domain of PhoR is sufficient for the signal sensing of phosphate concentrations. It should be noted that the periplasmic region of the sensor kinases, for instance NarX and NarQ, is implicated in the signal-accepting process (39, 40).

### 3.4. ResE-ResD

ResE-ResD regulates genes required for both anaerobic and aerobic respiration including *narGHIJ* (respiratory nitrate reductase), *nasDEF* (assimilatory nitrite reductase), *hmp* (flavohemoglobin), *ctaABC* (cytochrome caa3 oxidase), *qoxABCD* (cytochrome aa3 quinol oxidase) and so forth (41). Extensive analysis of the interaction of ResD with the upstream regions of these genes has been carried out, for example, on *ctaA*, *fnr* (transcriptional regulator), *hmp* and *nasD* (42, 43). Interestingly, the homologous system of *S. aureus*, SrrA-SrrB, regulates expression of virulence genes by responding to environmental oxygen levels (44). DNA microarray analysis was carried out for ResE-ResD, and several ResD-controlled genes have been identified (9).

## 4. ELUCIDATION OF ROLES OF TWO-COMPONENT SYSTEMS ENCODED BY GENES PREVIOUSLY GROUPED AS 'Y-GENES'

Recently, several two-component systems that were grouped in so-called y-gene products (function not yet identified) have been characterized. These include the YycG-YycF, DesK-DesR, DctS-DctR, CssS-CssR and CitS-CitT systems. We will describe functional roles or limited characterization of these two-component factors.

### 4.1. YycF-YycG

The *yycG-yycF* operon encodes a two-component system essential for cell viability (45, 46). The basis of the essential nature of this system is not well understood, although the recent finding that YycG controls one of promoters of the *ftsAZ* operon necessary for cell division (46) may give a hint to its essential nature. YycG-YycF is conserved among several eubacteria including *S. aureus* (47). In *S. aureus*, high concentrations of sucrose and NaCl in medium partially suppress the conditional lethal phenotype of *yycF*. Further, this conditional mutant is hypersensitive to macrolide and lincosamide antibiotics even in the presence of the *ermB* gene that specifies resistance to these antibiotics, suggesting that YycF-YycG might be involved in cell permeability control through modulation of the cell wall or cell membrane composition (47).

In terms of the essential two-component system, such systems are also reported in other bacteria. One example is the CtrX-CtrA pair that regulates the cell cycle of *Caulobacter crescentus* (48). CtrA is widely conserved in alpha-proteobacteria, and proved to be essential for cell growth in symbiotic bacterium, *Sinorhizobium meliloti* (49). In *Helicobacter pylori*, there are three RRs that are essential for growth (50).

### 4.2. DesK-DesR

DesK-DesR (formerly YocF-YocG) has been demonstrated to activate the *des* (formerly YocE) gene

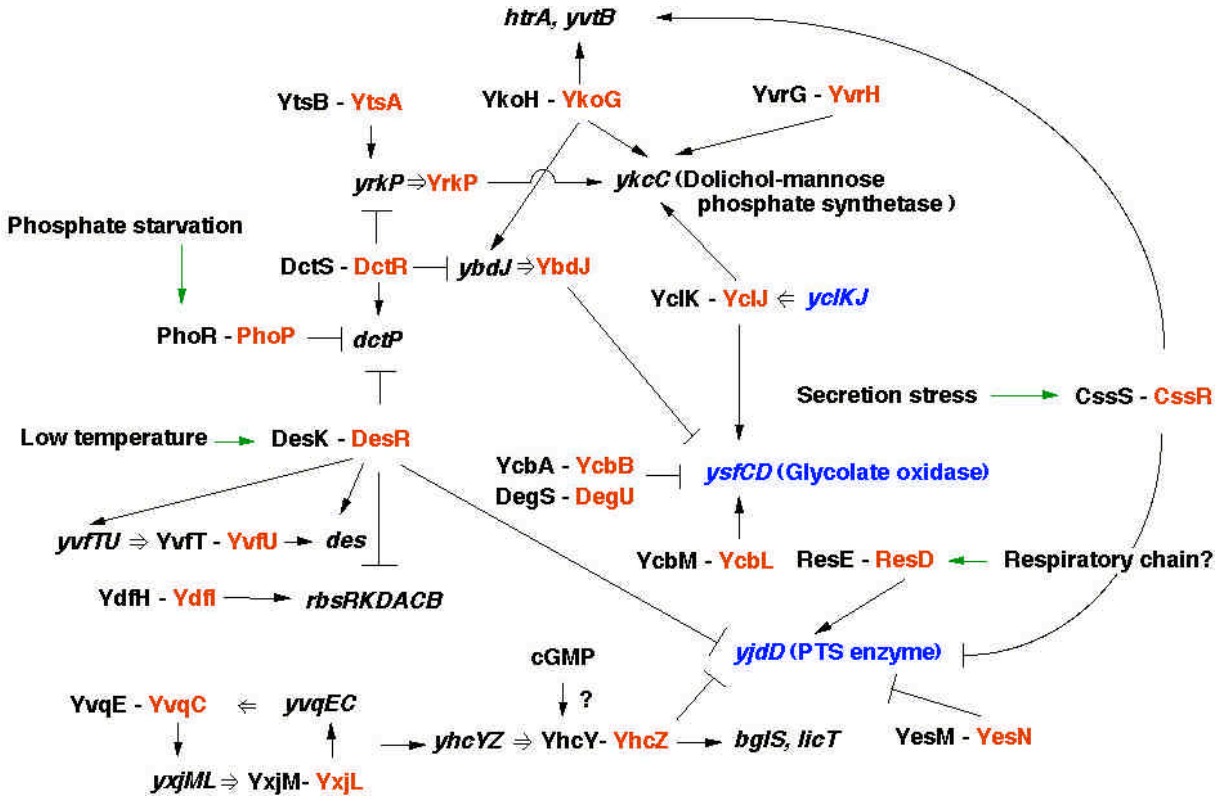


**Figure 1**. Transcription network involving two-component systems. Black single-lined arrows and T-formed lines show positive and negative transcription regulation, respectively. Green arrows indicate environmental signal inputs. Double-lined arrows depict the synthesis of the gene products from the two-component regulatory genes. The red and blue letters represent RRs and the genes induced under anaerobic conditions, respectively. For simplicity not all the target genes for each two-component system are shown, nor the interaction between ResE-ResD and PhoR-PhoP (13).

encoding the delta5-lipid desaturase upon temperature downshift (51; Figure 1). This enzyme introduces a double bond at the delta5-posision of the acyl-chains of membrane phospholipid, resulting in a change of the physical state of cell membrane. The alteration of membrane lipid composition leads to adaptation to cold temperatures. The DesR protein has been shown to bind to the regulatory region of the *des* gene, in which a cis-acting sequence for DesR, TCAT-N9-ATGA, was identified. Unsaturated fatty acids, the products of the delta5-lipid desaturase (Des), may serve as negative signaling molecules for DesK-DesR, because addition of these molecules inhibits the *des* transcription. Thus, there is a regulatory loop involving DesK-DesR and Des in terms of the cold shock response. Taken together, DesK appears to sense a change of membrane fluidity.

### 4.3. DctS-DctR

DctS-DctR (formerly YdbF-YdbG) is involved in transport of the C4-dicarboxilic acids, fumarate and succinate, mediated through the DctP permiase (YdbH, 52). The *dctSRP* genes form an operon. DctR regulates *dctP* expression (Figure 1), and the cis-element on the regulatory region of *dctP* has been determined. Addition of fumarate and succinate induces the expression of *dctP*, suggesting that these C4-dicarboxilic acids might be ligands for DctS. Interestingly, a putative ligand-binding protein, DctB, does not seem to be involved in incorporation of C4-dicarboxilic acids, but is required for efficient induction of *dctP*. The DctBSRP system does not take part in malate transport unlike the *E. coli* counterpart involving the DcuS-DcuR system (53).

The expression of *dctP* is negatively regulated by PhoP and DesR as shown by microarray analysis (11). Thus, this might indicate that the transport of C4-dicarboxilic acids and adaptation of the cells to phosphate starvation or low temperature are mutually exclusive.

### 4.4. CssS-CssR

It has been shown that the CssS-CssR (formerly YvqB-YvqA) system regulates the gene encoding membrane-associated protease HtrA, which is thought to degrade misfolded and aggregated proteins (54; Figure 1). Thus, this system is required for quality control of exported proteins at the cell surface as in the case of the CpxA-CpxR system in *E. coli* (55, 56).

Our DNA microarray analysis revealed that CssR also positively regulates expression of *yvtB* (Figure 1), one of the two paralogues of *htrA* in *B. subtilis* (11). The expression of *htrA* and *yvtB* is also positively regulated by YkoG, the RR of the YkoH-YkoG system, suggesting involvement of this system in degradation of misfolded

**Table 1.** Genes for transcription factors regulated by RRs

| RR | Positive Regulation | Negative Regulation |
|---|---|---|
| DesR | | *rbsR* |
| YbdK | | *ykoM* |
| YcbB | | *bkdR* |
| DctR | | *paiA, sigZ, kipR, adaA* |
| YdfI | *ydgJ, rsbR* | |
| YufM | *comK, ydfL* | |
| YvcP | *ytzE, yoqD, yonN* | |

proteins. The two systems, CssS-CssR and YkoH-YkoG, are closely related in both the transmembrane domain organization of the SKs and the amino acid sequences of the SKs and RRs (6).

### 4.5. CitS-CitT

The CitS-CitT system regulates *citM*, the gene for a citrate transporter, and CitT binds to the regulatory region of *citM* (57, 58). The extracellular domain of CitS may sense citrate, as does its homologue in *Klebsiella pneumoniae* (59).

### 4.6. YclJ-YclK

The genes coding for the YclK-YclJ two-component system is induced in an anaerobic environment (9). Among the YclJ-regulated genes (11) identified by microarray analysis, are *ysfCD*, *ykuO* and *dhbAB* whose expression is induced in anaerobic growth (9).

We note that *ysfCD* expression is regulated by five RRs, i.e., positively by YcbL and YclJ, and negatively by YbdJ, DegU and YcbB (11; Figure 1). YsfC and YsfD show high similarities in amino acid sequence to *E. coli* glycolate oxidase subnuits, the *glcD* and *glcEF* gene products, respectively (60). The *glcEF* locus was originally reported to consist of two genes, but resequencing by the *E. coli* genome project revealed that they constitute a single gene. Glycolate oxidase catalyzes the generation of glyoxylate, which is a starting compound for the synthesis of 3-phosphoglycerate in the glycerate pathway as well as malate in the glyoxylate cycle. The fact that five two-component systems participate in the regulation of *ysfCD* might suggest an importance of glycolate oxidase in *B. subtilis* metabolism.

### 4.7. LytS-LytT

This system resembles *S. aureus* LytS-LytR, which controls the rate of autolysis probably through murein hydrolase activity (61). *S. aureus* LytR regulates *lrgA* and *lrgB* encoding murein hydrolase exporter and murein hydrolase, respectively (62). The expression of the *B. subtilis* counterparts of those genes, *ysbA* and *ysbB*, was also increased in our microarray analysis. These results, however, have to be taken with caution. In our experimental system, the RR gene, *lytT*, was placed downstream of an IPTG-inducible P*spac* promoter on a multicopy plasmid, and the SK gene, *lytS*, in the host cell was disrupted by insertion of a plasmid containing an IPTG-inducible promoter (10). Thus, upon induction with IPTG for overproduction of the RR, the expression of the genes downstream of the disrupted SK gene is also induced unless a transcriptional terminator is present. Since *ysbAB* are located downstream of the *lytS* sensor gene, the results on *ysbAB* expression are not conclusive. Another *B. subtilis* homologue of *lrgA* is *ywbH* that is negatively regulated by LytT (11). The biological meaning of this adverse phenomenon is unclear.

## 5. TRANSCRIPTIONAL NETWORK WITH LINKAGES FORMED AMONG TWO-COMPONENT SYSTEMS

We have attempted to take a general view on the regulatory cascade of RRs bearing a helix-turn-helix motif from the results of microarray analyses, although a more complete one will be obtained when more information on regulatory factors becomes available. A partial network is depicted in Figure 1. For reasons of clarity, we did not include Spo0A in our analysis because of its roles in many cellular processes, although a microarray analysis of the Spo0A regulon has been reported (8). It should be emphasized that both the primary and secondary target genes regulated by the RRs are included in the Figure, i.e., the genes under direct regulation of the RRs as well as those under indirect regulation via some transcriptional regulator(s) that are under the regulation of the RRs. Also in Table 1, we show known and putative transcription factors that were found to be regulated by RRs (11), which will provide the basis for expanding the transcriptional network.

The comprehensive DNA microarray analysis of two-component systems in *B. subtilis* revealed several direct linkages between RRs, in which a RR affects expression of the gene encoding another RR. If the regulatory connections between RRs are identified, it is expected that a global transcriptional network that is inclusive of many two-component systems will eventually be uncovered.

The first linkage includes the known system, DesK-DesR (Figure 1). The DNA microarray analysis uncovered target genes other than the *des* gene, i.e., *yvfTU* encoding a two-component system whose function is unknown (11). This YvfT-YvfU two-component system regulates a few genes including *des* and *yvfR* encoding a putative ABC transporter (63). Thus, it is likely that, through the YvfT and DesK sensors, various signals including a low temperature shift may induce expression of the target genes, such as *des*, although the biological significance of this redundant regulation is unclear. DesR and another RR, YdfI, regulate negatively and positively the *rbsRKDACB* operon involved in ribose transport (64), respectively. Interestingly, the RRs DesR, YdfI and YvfT show high similarities in their amino acid sequences. Moreover, according to Fabret and others (6), their cognate SKs, DesK, YdfH and YvfT are classified into Group II in which the members bear four transmembrane domains at the N-terminal region. The interaction among multiple two-component systems is reminiscent of the previous observations that *phoPR* expression requires ResD-binding to the *phoPR* promoter region and that PhoP and ResD have extensive similarities in their amino acid sequences (13). In *Salmonella* an interaction between the PhoP-PhoQ

**Table 2.** Genes for ABC transporters (ATP-binding protein) regulated by RRs

| RR | Positive Regulation | Negative Regulation |
|---|---|---|
| PhoP | *glnQ, pstB1, pstB2* | |
| ResD | *cydC* | |
| DesR | | *rbsA* |
| YccH | *natA* | |
| YclJ | *yclH* | |
| YdfI | *rbsA* | *cydC* |
| YfiK | | *appF, yqiZ* |
| YhcZ | *yknU* | |
| YkoG | *yclH* | |
| YtsA | *yvcR* | |
| YufM | | *ythP, cydC* |
| YvcP | *ytsC, yxdL* | |
| YvfU | *yvfR* | |
| YvrH | *ycbN, ykfD, sunT, ptsB2, yxdL* | |

and PmrA-PmrB systems has been reported, i.e., PhoQ activates *pmrAB* through a small protein, PmrD, during growth in low-phosphate medium (65).

The second linkage consists of YtsB-YtsA, DctS-DctR, YkoH-YkoG, *yrkP* and *ybdJ* (Figure 1). YtsA and DctR regulate expression of the *yrkPQ* operon by exerting, respectively, positive and negative control. On the other hand, YkoG and DctR exert positive and negative effects on *ybdJ* expression, respectively. Thus, the two RRs, YrkP and YbdJ, are located downstream from two two-component systems. One of the target genes of YrkP is *ykcC* that probably encodes dolichol phosphate mannose synthetase (11). This enzyme is believed to be involved in glycosylation of proteins, based on the comparison with the eukaryotic counterpart, although protein glycosylation is rare and its role is unclear in prokaryotes (66). Recently, it was reported that glycosylation of a cell envelope protein is involved in phage infection in *Streptomyces coelicolor* (67). Therefore, the observation that the four RRs, YclJ, YkoG, YrkP and YvrH regulate *ykcC* expression might suggest that they are involved in some extracellular processes through the regulation of protein glycosylation. We have observed direct binding of His-tagged YrkP to the upstream region of the *ykcBC* operon (unpublished data).

The final linkage detected is different from those described above in that two RRs form a closed regulatory circuit and regulate a third RR. YvqC activates expression of the *yxjL* gene, whose gene product then activates *yvqC* expression (Figure 1). Both YvqC and YxjL activate the expression of the *yhcZ* gene encoding another RR. These interactions suggest that various signals are integrated at the three SKs, YvqE, YxjM and YhcY and activate expression of the genes under YhcZ regulation. These RRs show the closest similarity to DegU in the amino acid sequences among the *B. subtilis* RRs (6).

Of the YhcZ-regulated genes, two with known function were detected, i.e., *bglS*(*licS*) and *licT* encoding extracellular beta-glucanase and the antiterminator for *bglS*, respectively (68, 69). YhcZ inhibits expression of *yjdD*, which encodes a PTS enzyme homologue. We note that *yjdD* expression is regulated by 5 regulators, i.e., positively by ResD (9) and negatively by DesR, YhcZ, YesN and CssR (11). This might suggest an important role of YjdD in the cell. It is interesting to note that YvqE and YxjM, the SKs located upstream of YhcZ (Figure 1), carry transmembrane and extracytoplasmic regions, while YhcY lacks such a region (6). Thus, these signal transduction systems might integrate both extra- and intracellular information, leading to expression or repression of the downstream genes.

## 6. PUTATIVE FUNCTIONS OF 'y-GENE' TWO-COMPONENT SYSTEMS

On the basis of clustering of the functions of presumptive target genes regulated by RRs, possible roles of several two-component systems could be deduced. For instance, YfiK appears to repress the expression of biosynthesis genes for certain amino acids such as arginine, threonine and leucine-isoleucine-valine (11). Thus, YfiJ-YfiK could play a role in the metabolic control of amino acid biosynthesis.

YdfI also seems to participate in amino acid biosynthesis, as the *proHJ* genes encoding redundant enzymes for the first and third steps of proline biosynthesis, respectively (70), were identified as the targets. Proline is known as a major endogeneously produced osmoprotectant in *B. subtilis* (71). Therefore, it may be reasonable that *proHJ* expression is induced under high salt conditions (70). It is possible that YdfH-YdfI might be involved in this salt-induction process.

Expression of the genes for ABC transporters of sodium ion, *natA* and *natB*, is induced by ethanol stress (72). Because their expression is regulated by YccH (11), the YccG-YccH system might be involved in $Na^+$ ion export. One could speculate that the system helps the cell to exclude cytoplasmic $Na^+$ by activating *natAB* gene expression when the integrity of membrane as a permeability barrier is damaged by agents such as alcohols. We have observed specific binding of His-tagged YccH to the regulatory region of the *natAB* operon (unpublished data).

## 7. RELATIONSHIP BETWEEN TWO-COMPONENT SYSTEMS AND TRANSPORTERS

It appears from the microarray analysis that two-component regulatory systems are associated with ABC transporters. In fact as shown in Table 2, we found 24 genes including 7 redundant ones that are under the regulation of 14 RRs (10, 11). In addition it is possible that the genes for the 3 putative ABC transporters, *ytsCD*, *yvcRS* and *yxdLM* are under regulation of the two-component systems YtsAB, YvcPQ and YxdJK, respectively. However, since the former genes are located immediately downstream of the latter genes, a definitive answer could not be obtained because of the limitation of our microarray method as described above. The association between the two-component systems and ABC transporters may suggest a role in ion or solute balance.

**Table 3.** Domains detected in *B. subtilis* SKs

| Domain | Function | SKs |
|---|---|---|
| Cache | Small ligand binding | YufL, DctS |
| PAS | FAD, heme, and cinnamic acid binding | ResE, PhoR, DctS, KinA, YufL, CitS, YycG |
| GAF | cGMP binding | LytS, YhcY |

The data are derived from URL (http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/micr.html)

## 8. DOMAIN ORGANIZATION OF SKs IN *B. SUBTILIS*

Accumulation of sequence information on more than 50 complete microbial genomes allows examination of domain architecture of SKs and RRs by comparison of a large number of two-component systems with each other. Sequence analysis of *B. subtilis* two-component systems has revealed conserved domains in SKs such as Cache, PAS and GAF but not in RRs that had been unknown previously (5; Table 3). There are seven SKs bearing the PAS domain in *B. subtilis* as shown in Table 3. KinA, a component of phosphorelay involved in the initiation of sporulation, has three PAS domains, and their roles in dimerization and enzymatic activity have been characterized (73). However, the function of the PAS domains found in other SKs remains uncharacterized.

There are no experimental data on the roles of the GAF (cGMP binding) and Cache (small ligand binding) domains of *B. subtilis* SKs. YhcY contains a putative GAF domain. This might be involved in sensing the intracellular concentration of GTP through binding of cGMP, because expression of *bglS*, which is under YhcY regulation, is affected by the intracellular GTP pool (74).

The Cache domain was initially detected in the extracellular region of a chemosensary receptor, MCP, as a small ligand-binding domain. A Cache domain was identified in DctS (5). It might be possible that the domain is involved in binding of C4-dicarboxilic acids.

## 9. PERSPECTIVE

Since two-component regulatory systems are not usually essential for growth, it is likely that their role is to provide the cell with an advantage to cope with the changing environments. *B. subtilis* inhabits the soil where temperature, nutrients, humidity etc. are rarely optimal for growth. Therefore, it is possible that it has developed a wide variety of adaptive devices including two-component regulatory systems, and together with transcriptional regulators they play important roles in many aspects of environmental stresses. To know more about *B. subtilis*, it is important to establish a transcriptional network in which transcription regulators and two-component systems are included, in addition to the studies on signal transduction of individual two-component systems. Microarray analysis is a suitable technique for such purposes. Following the completion of the microarray analysis on the two-component regulatory systems that affect transcription, studies on transcriptional regulators including those which carry a helix-turn-helix motif in the molecule are now in progress. For instance, we and others have analyzed the genes regulated by ComK, a master regulator of competence development, by microarray analysis (75, 76). Many genes were identified as members of the ComK regulon including *smf*, the disruption of which resulted in a decrease in transformation efficiency. The other group has reported the results of a microarray analysis on ScoC (77). All of these results demonstrate that the DNA microarray analysis is a powerful tool for analyzing global gene regulation.

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