Two-component signal transduction in *Corynebacterium glutamicum* and other corynebacteria: on the way towards stimuli and targets

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**Abstract** In bacteria, adaptation to changing environmental conditions is often mediated by two-component signal transduction systems. In the prototypical case, a specific stimulus is sensed by a membrane-bound histidine kinase and triggers autophosphorylation of a histidine residue. Subsequently, the phosphoryl group is transferred to an aspartate residue of the cognate response regulator, which then becomes active and mediates a specific response, usually by activating and/or repressing a set of target genes. In this review, we summarize the current knowledge on two-component signal transduction in *Corynebacterium glutamicum*. This Gram-positive soil bacterium is used for the large-scale biotechnological production of amino acids and can also be applied for the synthesis of a wide variety of other products, such as organic acids, biofuels, or proteins. Therefore, *C. glutamicum* has become an important model organism in industrial biotechnology and in systems biology. The type strain ATCC 13032 possesses 13 two-component systems and the role of five has been elucidated in recent years. They are involved in citrate utilization (CitAB), osmoregulation and cell wall homeostasis (MtrAB), adaptation to phosphate starvation (PhoSR), adaptation to copper stress (CopSR), and heme homeostasis (HrrSA). As *C. glutamicum* does not only face changing conditions in its natural environment, but also during cultivation in industrial bioreactors of up to 500 m³ volume, adaptability can also be crucial for good performance in biotechnological production processes. Detailed knowledge on two-component signal transduction and regulatory networks therefore will contribute to both the application and the systemic understanding of *C. glutamicum* and related species.

**Keywords** Histidine kinase · Response regulator · Sensors · Regulation · *C. diphtheriae*

**Introduction**

*Corynebacterium glutamicum* is a Gram-positive, facultative anaerobic, nonpathogenic soil bacterium which is used for the large-scale industrial production of the flavor enhancer L-glutamate (2.2 million tons in 2009) and the food additive L-lysine (1.5 million tons in 2011). Recent metabolic engineering studies have shown that *C. glutamicum* is also capable of producing a variety of other commercially interesting compounds, e.g. other L-amino acids (Wendisch et al. 2006a), D-amino acids (Stäbler et al. 2011), organic acids such as succinate (Okino et al. 2008; Litsanov et al. 2012a, b, c), diamines such as cadaverine (Mimitsuka et al. 2007) or putrescine (Schneider and Wendisch 2010), biofuels such as ethanol or isobutanol (Inui et al. 2004; Smith et al. 2010; Blombach et al. 2011), or proteins (Meissner et al. 2007). An overview of the product spectrum of *C. glutamicum* can be found in a recent review (Becker and Wittmann 2011). Due to its function as microbial cell factory, *C. glutamicum* has become a prominent model organism in industrial biotechnology and simultaneously for systems biology (Eggeling and Bott 2005; Wendisch et al. 2006b; Burkovski 2008). Another important aspect fostering research on *C. glutamicum* is its close phylogenetic
Protein Kinase subfamily HPK1, one to HPK5, membran

Basics of two-component signal transduction

Two-component systems (TCS) consist of a usually membrane-bound sensor kinase or histidine kinase (HK) and a response regulator (RR), which in most cases functions as transcriptional regulator. Both HKs and RRs are modular proteins (Fig. 1). Typical HKs are composed of a sensor domain, which is highly variable among different HKs, and a conserved cytoplasmic kinase core consisting of two distinct domains: a dimerization and histidine-phosphotransfer domain, designated HisKA domain in PFAM, and a C-terminal catalytic and ATP-binding (CA) domain, termed HATPase_c domain in the PFAM database (Punta et al. 2012). The HATPase domain binds ATP and catalyzes the transfer of the γ-phosphoryl group from ATP to the histidine residue, which is located within the HisKA domain. Several sequence motifs of the HATPase domain involved in ATP binding (G1, F, G2) are highly conserved. In many cases, additional domains such as HAMP domains are located between the N-terminal sensor domain and the C-terminal kinase core. Typical RRs are composed of a conserved N-terminal receiver domain (response_reg domain in PFAM), which contains the phosphorylatable aspartate residue, and a variable C-terminal effector or output domain. The HK responds to a certain stimulus by auto-phosphorylation of the conserved histidine residue in the HisKA domain and the phosphoryl group is subsequently transferred to the aspartate residue in the receiver domain of the RR in a reaction catalyzed by the RR. Phosphorylation activates (or in exceptional cases inhibits) the RR which then elicits a stimulus-specific response, usually the activation or repression of target genes (for reviews, see Stock et al. 1989, 2000; Bourret et al. 1991; Parkinson and Kofoid 1992; Mascher et al. 2006; Gao and Stock 2009). In this review, we summarize the experimental knowledge currently available for the TCS of the C. glutamicum type strain ATCC 13032 and we present an in silico analysis of TCS in Corynebacterium species for which complete genome sequences are available.

Two-component systems in C. glutamicum ATCC 13032

The first Corynebacterium genome that was completely sequenced and analyzed with respect to TCS was the one of C. glutamicum ATCC 1302 (Ikeda and Nakagawa 2003; Kalinowski et al. 2003). Genes for 13 HKs and 13 RRs were identified, all forming HK–RR or RR–HK pairs (Kocan et al. 2006). No orphan HKs or RRs were found as in many other bacteria like Escherichia coli and Bacillus subtilis (Mizuno 1997; Fabret et al. 1999). Bioinformatic analyses revealed that the HKs as well as the RRs of C. glutamicum can be classified into three different classes, as shown in Tables 1 and 2 which also list the locus tags given in the different genome annotations. According to the classification scheme of Grebe and Stock, seven HKs belong to Histidine Protein Kinase subfamily HPKγ, one to HPKγ, and the remaining five to HPKγ (Grebe and Stock 1999). Classification of the RRs according to their output domains
revealed a comparable distribution to the one of the sensor kinases: seven RRs belong to the OmpR family, one to the CitB family, and five to the LuxR family of RRs. Interestingly, all sensor kinases of HPK1 are paired with an OmpR-type response regulator, the HPK5-type sensor kinase is paired with the CitB-type RR, and all sensor kinases of HPK7 are paired with a LuxR-type RR (Fig. 1). All of the output domains of the *C. glutamicum* RRs contain DNA-binding motifs, suggesting that all of them function as transcriptional regulators (Kocan et al. 2006).

In order to test for the essentiality of the *C. glutamicum* ATCC 13032 TCS, a deletion study was performed which revealed that all TCS genes except for *regX3* (= cgtr4, cg0484) could be deleted (Kocan et al. 2006). Thus, of the 13 TCS only the SenX3-RegX3 system appears to be essential for growth.

**The CitA-CitB system: control of citrate utilization**

The HK CitA and its cognate RR CitB of *C. glutamicum* belong to a family of TCS controlling the uptake and metabolism of citrate and dicarboxylates, the founding member being the citrate utilization (CitAB) TCS of *Klebsiella pneumoniae* (Bott et al. 1995). Citrate is a ubiquitous natural compound which can be utilized as a carbon and energy source by many bacterial species. Whereas anaerobic catabolism of citrate, which occurs for example in enteric bacteria...
(Bott 1997) and lactic acid bacteria (Bekal et al. 1998), requires a number of specific enzymes, in particular citrate lyase (Bott and Dimroth 1994), aerobic bacteria possessing a complete tricarboxylic acid cycle usually only require a citrate uptake system in order to be able to metabolize citrate.

*C. glutamicum* is able to grow aerobically in minimal medium with citrate as sole carbon and energy source (Polen et al. 2007; Brocker et al. 2009). When glucose is present in addition to citrate, both substrates are consumed simultaneously (Brocker et al. 2009), a feature typical for *C. glutamicum*. Global gene expression studies using DNA microarrays revealed that two putative citrate transport systems showed strongly increased expression in the presence of citrate, i.e. CitH (previously also named CitM or CitP) and TctABC (Polen et al. 2007). The former is a member of

| Histidine kinase | Locus tags | Class | Size (aa) | TMHs (aa position) | PFAM domains (aa position) | His ~ P site | Putative stimulus |
|-----------------|------------|-------|-----------|---------------------|-----------------------------|-------------|------------------|
| CitA            | Cg0089     | 5     | 551       | 27–47, 189–209      | HisKA not identif. HATPase_c 435–548 | 355         | Citrate          |
|                 | NCgl0067   |       |           |                     |                             |             |                  |
|                 | Cg0068     |       |           |                     |                             |             |                  |
| MtrB            | Cg0864     | 1     | 503       | 9–29, 175–195       | HAMP 172–241 HisKA 252–319 HATPase_c 365–475 | 266         | Unknown          |
|                 | NCgl0722   |       |           |                     |                             |             |                  |
|                 | Cg0755     |       |           |                     |                             |             |                  |
| PhoS (CgtS3)    | Cg2887     | 1     | 485       | 44–64, 184–204      | HAMP 185–255 HisKA 266–330 HATPase_c 373–484 | 276         | Phosphate limitation |
|                 | NCgl2517   |       |           |                     |                             |             |                  |
|                 | Cg2606     |       |           |                     |                             |             |                  |
| CopS            | Cg3284     | 1     | 399       | 16–36, 66–86        | HAMP 69–139 HisKA 143–207 HATPase_c 253–366 | 153         | Copper           |
|                 | NCgl2862   |       |           |                     |                             |             |                  |
|                 | Cg2964     |       |           |                     |                             |             |                  |
| HrrS (CgtS11)   | Cg3248     | 7     | 444       | 55–75, 90–110, 148–168 | HisKA_3 208–281 HATPase_c 316–413 | 217         | Heme             |
|                 | NCgl2835   |       |           |                     |                             |             |                  |
|                 | Cg2937     |       |           |                     |                             |             |                  |
| CgtS8 (ChrS)    | Cg2201     | 7     | 377       | 13–33, 36–56, 63–83, 105–125, 128–148 | HisKA_3 177–242 HATPase_c 279–371 | 186         | Heme             |
|                 | NCgl1935   |       |           |                     |                             |             |                  |
|                 | Cg2010     |       |           |                     |                             |             |                  |
| CgtS1           | Cg0331     | 1     | 489       | 14–34, 184–204      | HAMP 185–254 HisKA 258–322 HATPase_c 362–475 | 268         | Unknown          |
|                 | NCgl0269   |       |           |                     |                             |             |                  |
|                 | Cg0273     |       |           |                     |                             |             |                  |
| CgtS2           | Cg0997     | 1     | 479       | 31–51, 171–191      | HAMP 173–242 HisKA 246–318 HATPase_c 358–468 | 256         | Unknown          |
|                 | NCgl0840   |       |           |                     |                             |             |                  |
|                 | Cg0875     |       |           |                     |                             |             |                  |
| SenX3 (CgtS4)   | Cg0483     | 1     | 413       | 1–21                | HisKA 156–222 HATPase_c 267–378 | 166         | Unknown          |
|                 | NCgl0391   |       |           |                     |                             |             |                  |
|                 | Cg0403     |       |           |                     |                             |             |                  |
| CgtS5           | Cg2948     | 1     | 372       | 20–40, 71–91        | HAMP 73–143 HisKA 147–211 HATPase_c 257–370 | 157         | Unknown          |
|                 | NCgl2573   |       |           |                     |                             |             |                  |
|                 | Cg2663     |       |           |                     |                             |             |                  |
| CgtS6           | Cg3060     | 7     | 380       | 20–40, 57–77        | HisKA_3 170–229 HATPase_c 281–376 | 178         | Unknown          |
|                 | NCgl2667   |       |           |                     |                             |             |                  |
|                 | Cg2763     |       |           |                     |                             |             |                  |
| CgtS7           | Cg0707     | 7     | 423       | 79–99, 118–138, 142–162, 173–193, 199–219 | PspC 47–107 HisKA_3 241–309 HATPase_c 331–423 | 250         | Unknown          |
|                 | NCgl0585   |       |           |                     |                             |             |                  |
|                 | Cg0611     |       |           |                     |                             |             |                  |
| CgtS10          | Cg1083     | 7     | 419       | 48–68, 74–94, 109–129, 142–162, 165–185 | HisKA_3 230–297 HATPase_c 327–410 | 239         | Unknown          |
|                 | NCgl0911   |       |           |                     |                             |             |                  |
|                 | Cg0948     |       |           |                     |                             |             |                  |

The locus tags are derived from the genome entries NC_006958, NC_003450, and BA000036, respectively. Classification was performed according to Grebe and Stock (1999). The transmembrane helices (TMHs, score above 1.2) were predicted by TopPred-II (Claros and von Heijne 1994). Domains and their position were calculated by PFAM (Punta et al. 2012). The phosphorylation sites (His ~ P) were predicted from sequence alignments.
the citrate-Mg\(^{2+}\)/H\(^{+}\)/citrate-Ca\(^{2+}\)/H\(^{+}\) symporter family (CitMHS), the latter belongs to the tripartite tricarboxylate transporter family (TTT). Expression of either \(\text{citH}\) or the \(\text{tctCBA}\) operon in \(E.\ coli\) enabled citrate utilization, confirming that both \(\text{CitH}\) and \(\text{TctABC}\) are functional citrate transporters (Brocker et al. 2009). Growth studies suggested that \(\text{CitH}\) is active with Ca\(^{2+}\) or Sr\(^{2+}\), but not with Mg\(^{2+}\), whereas \(\text{TctABC}\) is active with Mg\(^{2+}\) or Ca\(^{2+}\) but not with Sr\(^{2+}\). Evidence was obtained that 2 mM Ca\(^{2+}\) is sufficient to achieve maximal growth rates of \(C.\ glutamicum\) on citrate, whereas Mg\(^{2+}\) is required at 50-fold higher concentrations (Brocker et al. 2009). Either \(\text{CitH}\) alone or \(\text{TctABC}\) alone are sufficient for growth on citrate.

The genes encoding the CitAB TCS of \(C.\ glutamicum\) are located immediately upstream of \(\text{citH}\) and in opposite direction. The HK CitA (58.6 kDa) contains two putative transmembrane helices that border an extracytoplasmic domain extending from residues 48–188. The RR CitB (23.4 kDa) is composed of the receiver domain and a DNA-binding domain belonging to the CitB family. A \(C.\ glutamicum\) mutant

Table 2 Response regulators of \(C.\ glutamicum\) ATCC 13032

| Response regulator | Locus tags | Class | Size (aa) | PFAM domains (aa position) | Asp \(\sim\) P site | DNA-binding site | No. of target genes | Auto-regulation |
|--------------------|------------|-------|-----------|---------------------------|-----------------|----------------|-----------------|----------------|
| CitB               | Cg0090 NCgl0068 Cg0069 | CitB   | 218       | Response_reg 7–114 HTH_24 152–198 | D57     | n.d.         | 4               | no             |
| MtrA               | Cg0862 NCgl0721 Cg0754 | OmpR   | 226       | Response_reg 5–114 Trans_reg_C 146–222 | D53     | 8-bp tandem repeat | 25              | no             |
| PhoR (CgtR3)       | Cg2888 NCgl2518 Cg2607 | OmpR   | 235       | Response_reg 11–121 Trans_reg_C 156–230 | D59     | 8-bp tandem repeat | 17              | yes            |
| CopR               | Cg3285 NCgl2863 Cg2965 | OmpR   | 240       | Response_reg 15–124 Trans_reg_C 160–237 | D63     | 9-bp tandem repeat | 9               | yes            |
| HrrA (CgtR11)      | Cg3247 NCgl2834 Cg2935 | LuxR   | 212       | Response_reg 4–124 GerE 149–206 | D54     | n.d.         | 18              | n.d.           |
| CgtR8 (ChrA)       | Cg2200 NCgl1934 Cg2009 | LuxR   | 210       | Response_reg 4–122 GerE 147–204 | D54     | n.d.         | n.d.            | n.d.           |
| CgtR1              | Cg0330 NCgl0268 Cg0272 | OmpR   | 222       | Response_reg 4–114 Trans_reg_C 150–221 | D52     | n.d.         | n.d.            | n.d.           |
| CgtR2              | Cg0996 NCgl0839 Cg0874 | OmpR   | 232       | Response_reg 3–113 Trans_reg_C 152–227 | D51     | n.d.         | n.d.            | n.d.           |
| RegX3 (CgtR4)      | Cg0484 NCgl0392 Cg0404 | OmpR   | 232       | Response_reg 4–113 Trans_reg_C 154–230 | D52     | n.d.         | n.d.            | n.d.           |
| CgtR5              | Cg2947 NCgl2572 Cg2662 | OmpR   | 241       | Response_reg 16–125 Trans_reg_C 161–238 | D64     | n.d.         | n.d.            | n.d.           |
| CgtR6              | Cg3061 NCgl2668 Cg2764 | LuxR   | 206       | Response_reg 7–119 GerE 147–204 | D57     | n.d.         | n.d.            | n.d.           |
| CgtR7              | Cg0709 NCgl0586 Cg0612 | LuxR   | 230       | Response_reg 4–114 GerE 167–224 | D52     | n.d.         | n.d.            | n.d.           |
| CgtR10             | Cg1084 NCgl0912 Cg0949 | LuxR   | 203       | Response_reg 4–118 GerE 140–197 | D56     | n.d.         | n.d.            | n.d.           |

The locus tags are derived from the genome entries NC_006958, NC_003450, and BA000036, respectively. Classification was performed according to the output domains. The domains and their position were predicted by PFAM (Punta et al. 2012). The aspartate phosphorylation sites (Asp \(\sim\) P) were predicted from sequence alignments.
lacking the \(citAB\) genes was unable to grow with citrate as the sole carbon and energy source, but grew like wild type on glucose or pyruvate. The Cit\(^{-}\) phenotype could be abolished by transformation of the \(\Delta citAB\) mutant with a \(citAB\) expression plasmid, confirming that the CitAB TCS is required for citrate utilization (Brocker et al. 2009). DNA microarray and primer extension experiments revealed that the citrate-inducible expression of both \(citH\) and \(tctCAB\) is strictly dependent on the CitAB TCS. Furthermore, the purified RR CitB was shown to bind to the promoter regions of \(citH\) and \(tctCBA\) (Brocker et al. 2009). The exact DNA-binding motif has not yet been identified. According to these results, CitA serves as a sensor for extracellular citrate and triggers the phosphorylation of CitB, which then activates the expression of the citrate transport genes \(citH\) and \(tctCBA\) (Fig. 2).

The question how citrate is sensed by CitA has not yet been answered experimentally for the protein of \(C.\) glutamicum, but for the HKs CitA from \(K.\) pneumoniae and \(E.\) coli. For \(K.\) pneumoniae it was shown that the isolated periplasmic domain CitAP binds citrate, presumably the dianionic form H-citrate\(^{2-}\), in a 1:1 stoichiometry with a \(K_d\) of 5 \(\mu\)M at pH 7 (Kaspar et al. 1999). In the case of \(E.\) coli CitA, the periplasmic domain bound citrate with a \(K_d\) of about 0.3 \(\mu\)M at pH 7 (Kaspar and Bott 2002). The crystal structure of \(K.\) pneumoniae CitAP in complex with citrate was the first one solved for a periplasmic domain of a HK and revealed a PAS-fold, a versatile ligand-binding structural motif. The groups responsible for citrate binding were identified as Thr-58, Arg-66, His-69, Ser-101, Leu-102, Lys-109, Ser124, and Arg-107. Four of these (R66, H69, R107, and K109) had been identified before as important for citrate binding by showing that their exchange to alanine increased the \(K_d\) 38- to \(\geq\)300-fold (Gerharz et al. 2003). In a subsequent study, structures of \(K.\) pneumoniae CitAP in the citrate-free and citrate-bound states were solved and their comparison showed that ligand binding causes a considerable contraction of the sensor domain (Sevvana et al. 2008). This contraction may represent the molecular switch that activates transmembrane signaling in the receptor, causing a piston-like movement of the second transmembrane helix towards the periplasm. In \(C.\) glutamicum CitA, all of the citrate-binding residues of \(K.\) pneumoniae CitA except Thr-58 and Ser-101 are conserved, suggesting that also the corynebacterial CitA protein directly senses the presence of citrate via its extracytoplasmic domain.

**The MtrB-MtrA system: osmoregulation and control of cell wall metabolism**

The MtrAB TCS, which was the first one studied in \(C.\) glutamicum, is highly conserved in sequence and genomic
organization in actinobacteria (Hoskisson and Hutchings 2006) and the RR MtrA was shown to be essential in M. tuberculosis (Zahrt and Deretic 2000). The RR MtrA of C. glutamicum is a 24.9 kDa protein with an OmpR-type DNA-binding output domain (Fig. 1). In contrast to M. tuberculosis, deletion of the mtrA gene alone and together with mtrB was possible in C. glutamicum. ΔmtrAB mutant cells exhibited a pleiotropic phenotype. The cells were elongated, segmented, and some showed irregular septum formation. In addition, they were more sensitive to penicillin and vancomycin, inhibitors of transpeptidases in cell wall synthesis, but more resistant to ethambutol, which interferes with the synthesis of the arabinogalactan moiety of the cell wall in Corynebacterineae (Belanger et al. 1996). These facts implied that the MtrAB TCS is somehow involved in cell wall homeostasis (Möker et al. 2004).

DNA microarray analysis comparing the ΔmtrA or the ΔmtrAB mutant with the wild type combined with different types of MtrA-DNA-interaction studies (ChIP-chip analyses, DNA affinity chromatography, and electrophoretic mobility shift assays) revealed 22 MtrA target genes/operons, some being transcriptionally activated and some being transcriptionally repressed by MtrA (Fig. 3; Brocker and Bott 2006; Brocker et al. 2011). These contrary functions of activation and repression correlate with the position of the MtrA binding site in the promoter region of the corresponding target gene(s): it is located in the vicinity of the −10 region when MtrA acts as a repressor, whereas it is located upstream of the −35 region, when MtrA acts as an activator. In vitro phosphorylation of MtrA by phosphorymidate caused dimerization of the response regulator and enhanced its DNA-binding affinity, indicating that MtrA is activated by phosphorylation. The DNA-binding site of MtrA as determined by experimental work and bioinformatics was found to be a loosely conserved 8-bp direct repeat separated by a 3-bp linker (consensus sequence (A/G)TAACATtn(A/G)TAACAT), whose length is important for MtrA binding (Brocker et al. 2011). Direct repeats are often found as binding sites of OmpR-type regulators (Gupta et al. 2006; Hickey et al. 2011 and references therein). Based on structural studies with PhoB of E. coli it was proposed that upon phosphorylation OmpR-type RRs get activated by a mechanism in which the receiver domains form a twofold symmetric dimer while the DNA-binding domains bind to DNA with tandem symmetry (Bachhawat et al. 2005). The results obtained for MtrA of C. glutamicum are in accordance with such a mechanism.

Most of the MtrA target genes code for proteins of unknown function. Among the characterized target genes, betP and proP code for uptake carriers for the compatible solutes glycine betaine and proline or ectoine, respectively, which are involved in the response to hyperosmotic stress (Peter et al. 1998; Morbach and Krämer 2005). The genes mepA, mepB, and nlpC code for putative cell wall peptidases, csbD for a homolog of a protein belonging to the σB-dependent general stress regulon in B. subtilis (Pragai and Harwood 2002), and rpf2 for a resuscitation promoting factor (Hartmann et al. 2004). The phenotype of the ΔmtrAB mutant together with the function of the proteins encoded by the MtrA target genes indicated that the MtrAB system is involved in cell wall metabolism and the osmo-stress response. This conclusion is in agreement with experimental results on MtrA proteins of mycobacteria, which show high sequence identity to MtrA of C. glutamicum (e.g. 74 % to MtrA of M. tuberculosis [MtrA_Mt]). In M. smegmatis, downregulation of mtrA expression using an antisense mRNA technique resulted in elongated cells and an increased sensitivity to the antituberculosis drugs isoniazid and streptomycin (Li et al. 2010). In mycobacteria, several MtrA target genes have been described, such as the promoter regions of dnaA and fprB coding for the initiator protein of DNA replication and the major secreted immunodominant antigen Ag85B, respectively, and the chromosomal origin of replication orfC (Fol et al. 2006; Li et al. 2010; Rajagopalan et al. 2010). MtrA_Mt is constitutively expressed throughout growth in human macrophages (Haydel and Clark-Curtiss 2004) and MtrA can be detected in sera from TB patients (Singh et al. 2001) indicating that the MtrAB TCS is involved in pathogenesis in M. tuberculosis.

MtrB (54.7 kDa), the cognate HK of MtrA, contains two putative transmembrane helices which border an extracytoplasmatic domain of ~151 aa. To search for the stimulus sensed by MtrB, Strep-tagged MtrB was purified, reconstituted into proteoliposomes and its activities, autophosphorylation and phosphoryl group transfer to MtrA, were compared in the absence and presence of different stimuli (Möker et al. 2007a). Potassium ions were shown to stimulate MtrB activity, but this effect was also observed for DcuS of E. coli, a sensor kinase involved in recognition of C₄ dicarboxylates. Therefore, K⁺ seems to have a general stimulation effect on HKs rather than being the stimulus of MtrB to sense hyperosmotic stress (Möker et al. 2007a). In subsequent studies, membrane shrinkage was excluded as being the specific stimulus for MtrB. Various compounds such as amino acids, sugars, and polyethylene glycols were shown to activate MtrB, presumably not by binding to a specific binding site, but by changing the hydration state of MtrB. As this activation was independent of the periplasmic domain of ~151 aa. To search for the stimulus sensed by MtrB, Strep-tagged MtrB was purified, reconstituted into proteoliposomes and its activities, autophosphorylation and phosphoryl group transfer to MtrA, were compared in the absence and presence of different stimuli (Möker et al. 2007a). Potassium ions were shown to stimulate MtrB activity, but this effect was also observed for DcuS of E. coli, a sensor kinase involved in recognition of C₄ dicarboxylates. Therefore, K⁺ seems to have a general stimulation effect on HKs rather than being the stimulus of MtrB to sense hyperosmotic stress (Möker et al. 2007a). In subsequent studies, membrane shrinkage was excluded as being the specific stimulus for MtrB. Various compounds such as amino acids, sugars, and polyethylene glycols were shown to activate MtrB, presumably not by binding to a specific binding site, but by changing the hydration state of MtrB. As this activation was independent of the periplasmic loop and the HAMP domain (Fig. 1), the kinase domain was proposed to sense hypertonicity (Möker et al. 2007b).

Immediately downstream of mtrA–mtrB, the gene lpqB is located which encodes a lipoprotein of unknown function. In M. tuberculosis, it has been shown that the LpqB protein interacts with the extracellular domain of MtrB, thereby influencing MtrA phosphorylation and expression of the MtrA target gene dnaA (Nguyen et al. 2010). Hence, MtrAB...
**Fig. 3** Regulon of the MtrAB two-component system of *C. glutamicum* showing its involvement in osmeregulation and cell wall metabolism. Genes shown in red are repressed and genes shown in green are activated by MtrA. Target proteins with known function are indicated. For genes highlighted in yellow, a binding of the response regulator MtrA upstream of these genes was observed, but the mRNA level of the genes was not altered in mutants lacking mtrAB or mtrA together with LpqB seem to form a three-component system, as already suggested previously (Hoskisson and Hutchings 2006).

The PhoS-PhoR system: coping with phosphate starvation

Phosphorus is one of the macromolecules of all cells and makes up 1.5–2.1 % of the cell dry weight of *C. glutamicum* (Liebl 2005). Inorganic phosphate (P_i) is the preferred phosphorus source of *C. glutamicum* and half-maximal growth rates are obtained at a P_i concentration of about 0.1 mM (Monod constant; Ishige et al. 2003). Besides P_i, various other inorganic and organic phosphates can serve as phosphorus sources for *C. glutamicum* (Wendisch and Bott 2005; Wendisch and Bott 2008). When P_i is abundant, inducible (psi) genes, increases (Ishige et al. 2003). From the kinetics of the transcriptional response, the following strategy to cope with Pi limitation was deduced. The first activation of expression of the group of genes, called phosphate starvation response (psr) genes, was the only two-component genes whose expression was rapidly (within 10 min) and transiently induced after a shift from P_i excess to P_i starvation (Ishige et al. 2003). Both results suggested that the PhoRS TCS was involved in the adaptation to P_i limitation. Transcriptome comparisons and primer extension studies of the ΔphoRS mutant and the wild type demonstrated that none of the psi genes except pstSCAB was induced in the mutant within 60 min after a shift from P_i excess to P_i limitation. Activation of the pstSCAB genes was weaker in the ΔphoRS mutant than in the wild type (Kocan et al. 2006).

The HK PhoS (52.4 kDa) contains two transmembrane helices delimiting an extracytoplasmic domain of about 120 amino acids and in the cytoplasm a HAMP domain followed by the HisKA and HATPase domains. The RR PhoR (26.4 kDa) is composed of an N-terminal receiver domain and a C-terminal output domain of the OmpR family (Fig. 1). In vitro studies revealed that the cytoplasmic part of PhoS showed constitutive autokinase activity and allowed rapid phosphorylation of PhoR. PhoR ~ P bound with different affinity to eight promoters of psi genes/operons, i.e. pstSCAB, phoRS, phoC, ushA, ugpAEBC, nucH, phoH1, and glpQ1 (Fig. 4). In addition, PhoR ~ P bound to the promoter of the porin gene porB, whose expression was reduced in the ΔphoRS mutant, and to the promoter of the pitA gene encoding a low-affinity secondary phosphate transporter (Schaaf and Bott 2007). Expression of pitA was reduced after a shift to P_i limitation and PhoR ~ P might act as a repressor of pitA. The affinity of unphosphorylated PhoR was about fivefold lower than that of PhoR ~ P, indicating that the latter is the active form of the protein (Schaaf and Bott 2007). The PhoR binding sites in the pstSCAB promoter and in the phoRS promoter were defined as 19-bp motifs composed of a loosely conserved 8-bp tandem repeat separated by a 3-bp linker, whose length is important for binding. The highest affinity was found for an artificial motif containing two perfect 8-bp tandem repeats: CCTGTTGAatatCCTGTTAA (Schaaf and Bott 2007). Again, this type of binding motif is in accord with that proposed for OmpR-type regulators (Bachhawat et al. 2005). The position of the binding motifs in the two promoters suggested different mechanisms of interactions with the RNA polymerase (Schaaf and Bott 2007).

The positive autoregulation of the phoRS genes, which were supported by reporter gene fusions, could be...
Fig. 4  Role of the PhoRS two-component system of *C. glutamicum* in phosphate starvation. Genes shown in green are activated by PhoR, the *pitA* gene shown in red is repressed by PhoR. The function of some target proteins is indicated.
responsible for the successive expression of the psi genes. Whereas uninduced levels of PhoR ~ P could be sufficient to induce the high-affinity pstSCAB and phoRS promoters, elevated levels of PhoR ~ P might be required for induction of the lower affinity target promoters. Consequently, the PhoRS system may function as a rheostat rather than a simple switch.

The mechanism by which the PhoRS TCS senses phosphate limitation is still an open issue. Experiments in our laboratory with purified PhoS reconstituted into proteoliposomes argued against the idea that the environmental Pᵢ concentration is directly sensed by PhoS. For the PhoR-PhoB TCS of E. coli, which performs a similar function in the phosphate starvation response as PhoRS in C. glutamicum, a model was proposed in which the phosphate ABC transporter PstSCAB is responsible for sensing the periplasmic Pᵢ concentration and transfers this information to the sensor kinase PhoR, whereby also the PhoU protein plays a role (Hsieh and Wanner 2010). As a PhoU homolog is also present in C. glutamicum (encoded by cg2842), a similar mechanism as suggested for E. coli might be involved in Pᵢ sensing by C. glutamicum.

The CopS-CopR system: handling copper stress

Due to its ability to change between Cu(II) and Cu(I), copper serves as a redox cofactor for many enzymes, such as cytochrome c oxidases (Ridge et al. 2008). However, free copper ions can trigger the formation of reactive oxygen species and lead to sulphydryl depletion. Therefore, high copper concentrations are toxic for cells. Most organisms have the ability to adapt to elevated copper concentrations by preventing the accumulation of free intracellular copper ions. Suitable strategies are for example the induction of copper exporters, of copper chaperons, or of multicopper oxidases to get rid of toxic intracellular copper levels, to sequester free copper ions, or to oxidize Cu(I) to the less toxic Cu(II), respectively (Osman and Cavet 2008).

Except for two putative multicopper oxidases, the only copper-dependent enzyme currently known in C. glutamicum is cytochrome aa₃ oxidase, which forms a supercomplex with the cytochrome bc₁ complex (Niebisch and Bott 2001, 2003; Bott and Niebisch 2003). As this supercomplex is critical for aerobic respiration and oxidative phosphorylation, copper is also required by C. glutamicum. On the other hand, copper concentrations of ≥50 μM inhibited growth of C. glutamicum (Schelder et al. 2011). DNA microarray studies revealed that the genes encoding the HK CopS (cg3284) and the RR CopR (cg3285) as well as the up- and downstream genes (cg3286-cg3289 and cg3283-cg3281) showed strongly increased expression when cells were cultivated in the presence of 21 μM copper rather than at the routinely used 1 μM, indicating that this gene region is important for the adaptation to copper stress (Schelder et al. 2011).

The relevance of the CopSR TCS for copper homeostasis was confirmed by the finding that a ΔcopSR mutant showed an increased susceptibility to copper ions, but not to nickel, manganese, zinc, silver, cobalt, lead, or cadmium ions. This phenotype was reversed by plasmid-borne copRS expression in the ΔcopSR mutant (Schelder et al. 2011). The HK CopS (43.0 kDa) presumably contains two transmembrane helices bordering a small extracytoplasmic region of about 30 amino acids, a HAMP domain and the characteristic HisKA and HATPase domains (Table 1 and Fig. 1). The RR CopR (26.7 kDa) is composed of a receiver domain and a DNA-binding domain of the OmpR family (Table 2 and Fig. 1). DNA microarray studies revealed no differences in gene expression between the ΔcopSR mutant and the wild type when the strains were cultivated in glucose minimal medium with 1 μM copper, whereas 43 genes displayed a more than threefold altered mRNA level when the strains were grown in the presence of 21 μM copper. In particular, the genes cg3286-cg3289, which are located upstream of copS in reverse orientation showed 50- to 100-fold lower expression in the ΔcopSR mutant, whereas expression of the three genes downstream of copS, cg3283-cg3281 was reduced only by a factor of about two. Binding studies with purified CopR uncovered a single binding site located in the intergenic region between copR and cg3286, which represents a 9-bp tandem repeat separated by 2-bp (TGAAGATT'TnnT-GAAGATT'T). Phosphorylation by acetyl phosphate was shown to enhance the binding affinity of CopR to its DNA target about sixfold. Reporter gene assays indicated that CopR activates both the cg3286 and the copR promoter. According to these data, the CopSR system is activated by elevated copper levels and phosphorylated CopR activates expression of the cg3286-cg3287-cg3288-cg3289 genes and of the copR-copS-cg3283-cg3282-cg3281 genes (Fig. 5).

Some proteins encoded by these CopR target genes can obviously be linked to copper homeostasis: CopB (encoded by cg3281) is a putative copper export ATPase which exports copper out of the cytoplasm into the extracytoplasmic space, where a putative multicopper oxidase (encoded by cg3287) can possibly oxidase Cu(I) to the less toxic and less membrane-permeable Cu(II). The function of the putative secreted copper-binding protein Cg3282 in copper homeostasis could be sequestration of excess copper ions or delivery of copper ions from CopB to the multicopper oxidase. The function of the other encoded proteins is still unclear.

Although there is clear evidence that CopS senses elevated copper concentrations, the mechanism of sensing has not yet been clarified. In principle, either CopS alone could be responsible for detection, or one or more additional...
proteins could be involved, such as a copper-binding protein or a copper transporter. For the copper-responsive HK CinS from *Pseudomonas putida*, a putative copper binding site has been identified which includes two histidine residues in the periplasmic loop (Quaranta et al. 2009). The periplasmic loop of CopS (LFHDHMLMTGREDPSLELFHAEQAYRDAN) also contains three histidine residues as well as two methionine residues, which might also be involved in direct copper binding as shown for other copper-binding proteins (Davis and O’Halloran 2008).

### The HrrS-HrrA system: control of heme homeostasis

Heme is a cofactor of various enzymes, in particular complexes of the respiratory chain, but can also serve as a source of iron. In *C. glutamicum*, prominent heme-containing enzymes are succinate dehydrogenase, also called succinate: menaquinone oxidoreductase (Kurokawa and Sakamoto 2005), the cytochrome *bc*$_1$-aa$_3$ supercomplex (Niebisch and Bott 2001, 2003; Sone et al. 2001), cytochrome *bd* oxidase (Kusumoto et al. 2000), respiratory nitrate reductase (Bott and Niebisch 2003), or catalase. Thus, both aerobic and anaerobic respiration of *C. glutamicum* are strictly dependent on heme (Bott and Niebisch 2003; Nishimura et al. 2007; Takeno et al. 2007) as well as the detoxification of reactive oxygen species generated by respiration. Heme biosynthesis in *C. glutamicum* occurs via the CS pathway that uses glutamate as the substrate for the synthesis of δ-aminolevulinic acid (Bott and Niebisch 2003).

As heme is an iron-containing porphyrin, heme metabolism is intimately connected with iron metabolism. Similar to copper, iron is both essential as protein cofactor but also dangerous as ferrous iron catalyzes the formation of reactive oxygen species. Therefore, organisms have evolved sophisticated strategies to ensure sufficient iron supply, but to avoid high, toxic intracellular iron concentrations (Andrews et al. 2003). In *C. glutamicum*, DtxR has been identified as the master regulator of iron homeostasis (Brune et al. 2006; Wennerhold and Bott 2006; Frunzke and Bott 2008). Among the target genes repressed by DtxR under iron excess were several ones related to heme metabolism, i.e. a putative operon predicted to encode a secreted heme transport-associated protein (*htaA*, cg0466) and an ABC transporter for heme uptake (*hmaTUY*, cg0467-cg0468-cg0469), a putative operon predicted to encode to further secreted heme transport-associated proteins (*htaB-htaC*, cg0470-cg0471), another putative secreted heme transport-
associated protein (htaD, cg3156), and the hmuO gene (cg2445) encoding heme oxygenase (Wennerhold and Bott 2006). The presence of genes related to heme import and degradation which are derepressed under iron limitation suggested that the non-pathogenic C. glutamicum can use heme as an iron source. In fact, non-toxic concentrations of hemin (2.5 μM) as sole iron source allowed comparable growth rates and cell yields as the same concentration of FeSO₄ (Frunzke et al. 2011). Mutants lacking either the hmu operon (htaA-hmuTUV) or the htaBC operon showed a slight, but significant growth defect when hemin was supplied as iron source, whereas growth of a ΔhmuO mutant lacking heme oxygenase was strongly impaired. These phenotypes support the involvement of the corresponding proteins in heme uptake and degradation. Transcriptome studies revealed that all of the aforementioned genes related to heme metabolism and a few additional ones showed increased expression levels when heme was used as sole iron source (Frunzke et al. 2011).

The set of target genes repressed by DtxR under iron-sufficient conditions in C. glutamicum also includes genes for transcriptional regulators, in particular those for the AraC-type regulator RipA (Wennerhold et al. 2005) and for the RR CgtR11. The gene cgtrR11 (hrrA) is located downstream of cgtsR11 (hrrS) encoding the cognate HK, which however is not repressed by DtxR (Wennerhold and Bott 2006). Due to the high sequence identity of the CgtSR11 system of C. glutamicum to the HrrSA TCS of C. diphtheriae (Bibb et al. 2007), it was renamed accordingly. The C. diphtheriae HrrSA system was shown to be involved in the heme-dependent activation of hmuO and repression of hemA, encoding the heme biosynthesis enzyme glutamyl-tRNA reductase (Bibb et al. 2007). A ΔhrrA mutant of C. glutamicum showed a strong growth defect on agar plates containing hemin as sole iron source, suggesting that also the C. glutamicum HrrSA system plays a role in heme metabolism. Transcriptome comparisons of the ΔhrrA mutant and the wild type and in vitro studies with purified HrrS and HrrA protein led to the identification of six promoter regions to which HrrA binds and to the definition of the HrrA regulon (Frunzke et al. 2011). The DNA binding motif of HrrA, which is composed of a receiver domain and a LuxR-type DNA binding domain (Fig. 1), has not yet been determined.

Like many other RR, HrrA functions both as an activator and as a repressor. The genes activated by HrrA – P code for heme oxygenase (hmuO), for subunit III of cytochrome aa₃ oxidase and the three subunits of the cytochrome bc₁ complex (ctaE-qcrCAB operon), and for subunit I of cytochrome aa₃ oxidase (ctaD). The genes repressed by HtrA – P code for 11 proteins involved in heme biosynthesis and cytochrome c maturation (hemE-hemY-hemL-cg0519-ccsX-ccdA-resB-resC; hemA-hemC; hemH). Thus, when heme is available, HrrSA stimulates heme degradation and the synthesis of the heme-containing cytochrome bc₁-aa₃ supercomplex and at the same time reduces heme biosynthesis. It thus plays a key role in heme homeostasis, together with the master regulator DtxR (Fig. 6).

The HrrSA TCS is assumed to be activated by heme, but biochemical studies confirming this assumption are not yet available. The HK HrrS (Fig. 1) is predicted to contain three transmembrane helices that border two extracytoplasmic regions of about 54 and 38 amino acid residues and the conserved HisKA and HATPase domains (Kocan et al. 2006). Heme recognition could occur either at the periplasmic side or within the cytoplasmic membrane.

### The CgtSR8-CgtR8 system: a second TCS possibly involved in heme homeostasis

Besides HrrSA, a second TCS of C. glutamicum, CgtSR8, might also be involved in the regulation of heme homeostasis. The CgtSR8 system shows high sequence identity to the ChrSA system of C. diphtheriae, which activates hmuO expression and represses hemA expression in a heme-dependent manner (Schmitt 1999; Bibb et al. 2005, 2007). More recently, also expression of the hrtBA genes of C. diphtheriae, which code for an ABC transporter conferring resistance to heme toxicity, was reported to be activated by ChrSA in this bacterium (Bibb and Schmitt 2010). Genes homologous to hrtBA (cg2202, cg2204) are also present in the C. glutamicum genome immediately upstream of cgtSR8 in opposite orientation and expression of both genes was activated by heme (Frunzke et al. 2011). Furthermore, the RR HrrA was shown to bind to the cgtS8-hrtB intergenic region and expression of cgtSR8 and hrtBA was increased two- and ten-fold, respectively, in the ΔhrrA mutant during growth with heme as sole iron source. These data suggest that CgtSR8 might have a similar function as ChrSA in C. diphtheriae and that HrrSA and CgtSR8 of C. glutamicum, which show sequence similarity to each other, are interrelated (Frunzke et al. 2011).

For ChrS of C. diphtheriae, experimental evidence was provided that it functions as heme sensor. The autophosphorylation of purified ChrS reconstituted into proteoliposomes was shown to be stimulated by 1 μM hemin, but not by other metalloporphyrins and iron. In addition, UV-spectra supported a direct interaction between ChrS and hemin (Ito et al. 2009). The N-terminal region of ChrS (and CgtS8) is predicted to contain five transmembrane helices and the deduced topology was supported by PhoA and LacZ fusions (Bibb and Schmitt 2010). Distinct amino acid substitutions (R34A, Y61F, R70A, D75N, F114N) in the N-terminal region of ChrS were found to inhibit or prevent heme-dependent
activation of the hrtB promoter or to cause heme-independent, constitutive promoter activation (H21L). These data support a role of the N-terminal ChrS region in heme sensing and signal transfer to the kinase domain (Bibb and Schmitt 2010).

Conservation of the C. glutamicum two-component systems in other species of corynebacteria

Except for the TCS of C. glutamicum ATCC 13032 described above and the HrrSA and ChrSA systems of C.
### Table 3 Two-component signal transduction systems in *Corynebacterium* species

| Two-component system | Presence in the indicated *Corynebacterium* strains<sup>a</sup> |
|----------------------|---------------------------------------------------------------|
|                      | Cgl<sup>b</sup> | CglR<sup>b</sup> | Cau<sup>b</sup> | Cdi<sup>b</sup> | Cef<sup>b</sup> | Cje<sup>b</sup> | Ckr<sup>b</sup> | Cpt<sup>b</sup> | Cur<sup>b</sup> | Cvb<sup>b</sup> | Cac1 | Cac2 | Cam | Cbo | Cge | Cgc1 | Cgc2 | Cli | Cma1 | Cma2 | Cpg | Cre | Cst | Ctu |
| CitAB                | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| MtrBA                | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| PhoSR                | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CopSR                | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| HrrSA                | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR8               | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR1               | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR2               | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR4               | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR5               | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR6               | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR7               | +                  | +<sup>d</sup> | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| CgtSR10              | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| cgR_2292             | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| cgR_2299             | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |
| cgR_0540/0541        | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  | +                  |

+, Genes encoding the sensor kinase and the response regulator are present

<sup>a</sup>Cgl, *Corynebacterium glutamicum* ATCC 13032 (Ikeda and Nakagawa 2003; Kalinowski et al. 2003); CglR, *Corynebacterium glutamicum* R (Yukawa et al. 2007); Cau, *Corynebacterium aurimucosum* ATCC 700975 (Trost et al. 2010a); Cdi, *Corynebacterium diphtheriae* NCTC-13129 (Cerdeno-Tarraga et al. 2003); Cef, *Corynebacterium efficiens* YS-314 (Nishio et al. 2003); Cje, *Corynebacterium jeikeium* K411 (Tauch et al. 2005); Ckr, *Corynebacterium kroppenstedtii* DSM 44385 (Tauch et al. 2008a); Cpt, *Corynebacterium pseudotuberculosis* FRC41 (Trost et al. 2010b); Cur, *Corynebacterium urealyticum* DSM 7109 (Tauch et al. 2008b); Cvl, *Corynebacterium ulcerans* BR-AD22 (Trost et al. 2011); Cva, *Corynebacterium variabile* DSM 44702 (Schröder et al. 2011); Cac1, *Corynebacterium accolens* ATCC 49725; Cac2, *Corynebacterium accolens* ATCC 49726; Cam, *Corynebacterium amycolatum* SK46; Cho, *Corynebacterium bovis* DSM 20582; Cge, *Corynebacterium genitale* ATCC 33030; Cgc1, *Corynebacterium glucuronolyticum* ATCC 51866; Cgc2, *Corynebacterium glucuronolyticum* ATCC 51867; Cli, *Corynebacterium lipophiloflavum* DSM 44291; Cma1, *Corynebacterium matruchotii* ATCC 14266; Cma2, *Corynebacterium matruchoti* ATCC 33806; Cpg, *Corynebacterium pseudogenitalium* ATCC 33035; Cre, *Corynebacterium resistens* DSM 45100; Cst, *Corynebacterium striatum* ATCC 6940; Ctu, *Corynebacterium tuberculostearicum* SK141

<sup>b</sup>Completed and published genomes

<sup>c</sup>Only present in Cac2

<sup>d</sup>Only the gene encoding the response regulator is present (cgR 0730)
diphtheriae, no other TCS of corynebacteria have been experimentally studied to our knowledge. However, a variety of genome sequences of Corynebacterium species were determined in recent years, which are of interest because of their pathogenicity, their role in cheese ripening, or in amino acid production. We performed an in silico analysis of these genomes to determine the conservation of the C. glutamicum TCS. The results of this analysis are summarized in Table 3 and Table S1, which lists the GI numbers of the orthologous proteins.

In C. glutamicum strain R, genes for 13 sensor kinases and 14 response regulators are found (Yukawa et al. 2007). Homologs of CgtSR6 and of CgtS7 are absent, while two TCS not present in strain ATCC 13032 are found in strain R (cgR_2292, cgR_2299, cgR_0540, cgR_0541). Thus, even in strains of the same species the presence of TCS can vary.

Considering their conservation in Corynebacterium strains with known genome sequence, the TCS can be divided into three groups. Group A contains three highly conserved TCS, namely MtrAB, PhoSR and CgtSR4 (SenX3/RegX3). They are present in all (MtrAB) or all except for one (PhoRS) or two (SenX3/RegX3) species analyzed here. It can be assumed that these TCS play important roles in the physiology of corynebacteria, which is supported in the case of MtrAB and PhoRS by the large regulons and in the case of CgtR4 by its essentiality in C. glutamicum ATCC 13032. Group B involves four TCS that are present in 12–18 of the 22 species analyzed here: CopSR, HrrSA, CgtSR2, and CgtSR7. The function of the latter two is not yet known. Finally group C contains nine TCS that are found in one to nine species. Of these, only the function of the CitAB and the ChrSA (CgtSR8) system is currently known.

**Concluding remarks**

The work summarized above illustrates that significant progress was achieved in understanding the role of TCS in C. glutamicum since the genome sequence was published in 2003 and allowed the in silico identification of these signal transduction systems. Cellular functions were elucidated for five of the 13 TCS present in the type strain ATCC 13032 by using the following approaches: (1) comparison with TCS of known functions from other bacteria; (2) inspection of the genomic environment of the TCS genes; (3) search for phenotypes of deletion mutants lacking a particular TCS; and (4) identification of the target genes of the RR. The five characterized systems include CitAB (citrate uptake), MtrAB (osmoregulation and cell wall homoeostasis), PhoSR (phosphate limitation), CopSR (copper stress), and HrrSA (heme homoeostasis). The stimuli sensed by the corresponding HK are predicted to be citrate (CitA), phosphate limitation (PhoS), copper ions (CopS), and heme (HrrS); however, direct biochemical evidence for these predictions is not yet available. Further studies along the lines outlined above should allow to elucidate the roles of all 13 TCS of C. glutamicum. The resulting knowledge will contribute to a systemic understanding of this species and can be used for optimization of strains or process conditions used for industrial purposes.

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