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# Genetic Regulation of *Corynebacterium glutamicum* Metabolism

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**Abstract** Physiological, biochemical and genetic studies of *Corynebacterium glutamicum*, a workhorse of white biotechnology used for amino acid production, led to a waste knowledge mainly about amino acid biosynthetic pathways and the central carbon metabolism of this bacterium. Spurred by the availability of the genome sequence and of genome-based experimental methods such as DNA microarray analysis, research on genetic regulation came into focus. Recent progress on mechanisms of genetic regulation of the carbon, nitrogen, sulfur and phosphorus metabolism in *C. glutamicum* will be discussed.

**Key words:** *Corynebacterium glutamicum*, genetic regulation, transcriptional regulators, carbon metabolism, nitrogen metabolism, sulfur metabolism, phosphorus metabolism, amino acid production

*C. glutamicum* was discovered as L-glutamate producing bacterium in 1957 and has since then become vital for the large-scale production of amino acids by fermentation processes. It is estimated that ~1,500,000 t of L-glutamate per year, ~550,000 t of L-lysine per year as well as smaller quantities of L-glutamine and branched-chain amino acids are produced by *C. glutamicum* strains [30, 56, 63]. Four decades of research on the physiology, biochemistry and genetics of *C. glutamicum*, and in particular on amino acid biosynthesis pathways and the central carbon metabolism, together with the establishment of industrial production processes, led to a profound knowledge about this Gram-positive bacterium (compiled in [17]), L-lysine as well as L-glutamate production strains are constantly improved by metabolic engineering (for review see [103]), but metabolic engineering also led to new products, e.g. L-serine [77], or enabled access to substrates which cannot be used by *C. glutamicum* wild type, e.g. xylose [38].

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The determination of the whole genome sequence of the *C. glutamicum* wild-type strain ATCC 13032 [33, 37] in combination with the development of genome-based technologies, such as DNA-microarray technology (for review see [101]) and proteomics (for review see [89]), marked an important breakthrough for fundamental and applied *C. glutamicum* research. Strain development benefited from partial genome re-sequencing as point mutations in genes known to be relevant for L-lysine production, e.g. in the pyruvate carboxylase gene *pyc* [78], could be identified [74]. Introduction of three point mutations to increase precursor supply ($pyc^{P458S}$), to increase flux into the L-lysine biosynthesis pathway ($lysC^{T311I}$) and to reduce flux to L-threonine ($hom^{V59A}$) into the genome of *C. glutamicum* wild type resulted in a strain which displayed a high L-lysine yield but which retained a high vitality and thus showed improved productivity [74]. As the supply of the cofactor NADPH is known to limit L-lysine production [59–62] additional mutations to increase flux in the pentose phosphate pathway ($gnd^{S361F}$, $zwf^{A243T}$) were introduced [21, 73]. Furthermore, introduction of a nonsense mutation in the malate:quinone oxidoreductase gene *mqo* improved L-lysine production presumably by reducing the tricarboxylic acid (TCA) cycle flux [34]. This approach, which has been named 'genome breeding' [74], successfully recapitulates known strain improvement strategies in the background of the *C. glutamicum* wild type leading to efficient L-lysine production while excluding the detrimental, but unavoidable mutations introduced by classical mutagenesis and screening or selection procedures. However, for further improvements of amino acid production and to fully exploit the biotechnological potential of *C. glutamicum* with respect to new products or metabolic capacities, an endeavor to integrate transcriptomics and proteomics with other 'omics' technologies into mathematical models of *C. glutamicum* followed by their iterative refinement has been initiated as discussed recently [103].

Genome-based approaches also advanced our understanding of fundamental aspects of *C. glutamicum*. Comparative

genomics of the corynebacterial species *C. glutamicum*, *C. efficiens*, *C. diphtheria*, and *C. jeikeium* e.g. revealed which genes for DNA-binding transcriptional regulators occur in the genomes of all of these species and which are common to only a subset or an individual species [8]. Functional genomics analyses e.g. of all aminotransferase proteins inferred from the genome sequence of *Corynebacterium glutamicum* [58] or of all proteins of the L-methionine biosynthetic pathway [52, 88] have been performed to unravel their biochemical and/or physiological roles. Transcriptome analyses ([79, 101] added to the understanding of diverse aspects of *C. glutamicum* physiology, e.g. responses to altered availability of phosphorus, nitrogen, iron or magnesium [50, 75, 87, 92, 102], utilization of different carbon sources [23, 69, 95], glutamate and lysine production [23, 29, 48, 69, 80, 95] or serine catabolism [69]. Proteome analyses identified protein patterns specific for *C. glutamicum* cells responding to heat-shock [1], nitrogen limitation [90] valine addition [51] propionate utilization [13] or acetate utilization [23]. Phosphoproteome analysis revealed about 90 serine- or threonine-phosphorylated proteins [7] as likely substrates of the four serine/threonine protein kinases encoded in the *C. glutamicum* genome. Recently, the function of one of these protein kinases, PknG, was determined unraveling a fascinating mechanism of TCA cycle regulation [70]. Proteome comparisons of the wild type and a mutant lacking PknG, which was impaired in glutamine utilization, identified a 15-kDa protein named OdhI as a putative substrate of PknG. Purified PknG was shown to phosphorylate OdhI at threonine 14 and affinity chromatography showed that $OdhI^{T14A}$, a version of OdhI which cannot be phosphorylated at threonine 14, bound OdhA, the E1 subunit of ODH. Unphosphorylated OdhI inhibited ODH activity at nanomolar concentrations. Thus, this signaling cascade involving PknG and OdhI provides the molecular explanation for the reduced ODH activity that is essential for glutamate production by *C. glutamicum* [70].

Clearly, posttranscriptional control by attenuation [66], protein phosphorylation [70], protein-protein interaction [96], protein degradation [19, 20] as well as a plethora of allosteric control mechanisms of metabolic enzymes play important roles in *C. glutamicum* and allow adaptation of this bacterium to various environmental conditions. Transcriptional regulation, however, can be viewed as the most efficient regulatory process as the first step of gene expression, i.e. transcription, is controlled. The first transcriptional regulator characterized in *C. glutamicum* was LysG [5]. At elevated intracellular L-lysine, L-arginine, L-histidine or L-citrulline concentrations, LysG induces expression of *lysE* [5], which encodes an export system specific for L-lysine or L-arginine [100]. The physiological role of the divergently transcribed *lysE* and *lysG* genes is to ensure unimpaired growth at elevated L-lysine or L-arginine concentrations that arise during growth in the presence of peptides. Since then, a number of regulatory systems have been discovered. In the following, mechanisms of genetic regulation of the carbon, nitrogen, sulfur and phosphorus metabolism in *C. glutamicum* will be discussed.

## GENETIC REGULATION OF C-METABOLISM

*C. glutamicum* can utilize a variety of sugars and organic acids as sources of carbon and energy. Glucose is the preferred carbon source, albeit fructose and sucrose sustain as high growth rates as glucose and allow as efficient glutamate production [21]. However, lysine production on glucose media is more efficient than on fructose- or sucrose-containing media [21, 39]. Unlike *E. coli* and *B. subtilis* which show pronounced catabolite repression, *C. glutamicum* typically does not show diauxic growth or preferential carbon source utilization on mixtures of carbon sources, but co-utilizes the carbon sources present in the mixtures. Glucose has been shown to be cometabolised with acetate [104], propionate [13], fructose [16], lactate [95], pyruvate, serine [69], protocatechuate [65] and vanillate [65]. With the exception of L-glutamate [46], which only is utilized once glucose is exhausted, glucose does not repress uptake of less preferred carbon sources.

The characterization of acetate metabolism and its regulation played and still plays a very important role in furthering our understanding of *C. glutamicum* physiology and even served to validate 'omics' technologies for use with *C. glutamicum* [23, 28, 67, 101]. Growth of *C. glutamicum* on acetate as sole carbon source requires the acetate-activating enzymes acetate kinase and phosphotransacetylase [83], which convert acetate to acetyl-CoA, the glyoxylate cycle with its key enzymes isocitrate lyase and malate synthase [81, 82] fulfilling the anaplerotic demand and the gluconeogenic enzymes PEP carboxykinase [85] and fructose-1,6-bisphosphatase [86]. Interestingly, pyruvate kinase is essential for growth on acetate as repression of the malic enzyme gene *malE* precludes its role for the generation of pyruvate for biomasss formation [68]. The fact that during growth on acetate the specific enzyme activities of isocitrate lyase, malate synthase, acetate kinase and phosphotransacetylase are highly increased in comparison to growth on glucose [54, 81–83] and that *in vivo* carbon fluxes differed considerably between acetate- and glucose-grown *C. glutamicum* cells [104], initiated intensive studies of acetate-specific regulation [15, 22, 23, 28, 40, 41, 67, 105]. Meanwhile, three transcriptional regulators of acetate metabolism, RamA [15], RamB [22] and GlxR [41], and a transcriptional regulator of *acn* which encondes the TCA cycle enzyme aconitase [50] have been identified.

By scoring expression of a transcriptional fusion of the promoter of the malate syntase gene *aceB* in clones of a *C. glutamicum* genomic library [41], overexpression of *glxR*

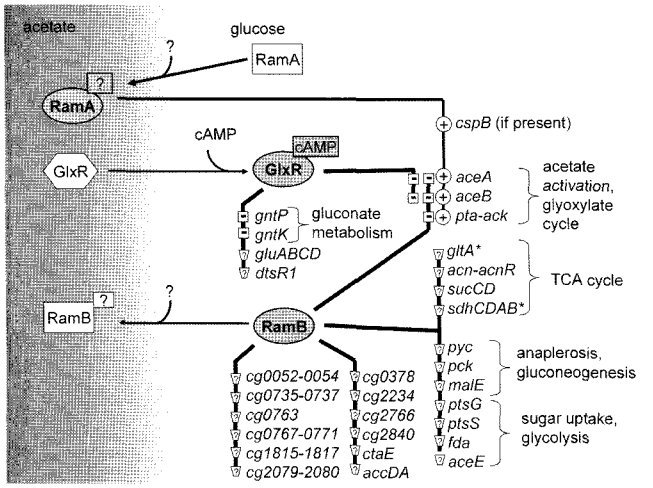


**Fig. 1.** Model of the regulation of carbon metabolism in *C. glutamicum* by RamA, RamB and GlxR.
Transcriptional repression is indicated by minus signs in squares, activation by plus signs in circles and question marks indicate possible negative or positive regulation. For details see text.

was found to repress *aceB* transcription during growth on acetate [40]. GlxR was shown to occur as a dimer and to bind to the *aceB* promoter in a cAMP-dependent manner *in vitro* (Fig. 1). As the intracellular cAMP concentration was about fourfold higher during growth on glucose as compared to growth on acetate, it was proposed that cAMP-GlxR represses *aceB* transcription during growth on glucose while *aceB* transcription is derepressed during growth on acetate [40]. Recently, it was suggested that GlxR serves a wider regulatory role of carbon metabolism (Fig. 1) as transcription of the gluconate utilization genes *gntP* and *gntK*, which encode gluconate permease and gluconate kinase, appears to be repressed by cAMP-GlxR [55]. Thus, the presumed target genes of GlxR including *aceB*, *gntP*, and *gntK* are subject to repression by glucose and as demonstrated for *gntP* and *gntK* also by fructose or sucrose [55]. Interestingly, repression of *gluABCD* by cAMP-GlxR might be the molecular cause for the glucose-glutamate diauxie [46] as bioinformatic analyses suggest repression of the *gluABCD* operon as well as of *aceA*, *dtsR1*, *cspB*, and *acn-acnR* by GlxR [2, 55]. Because repression by GlxR during growth on glucose was not reverted by gluconate [55], it is currently unknown whether glucose and gluconate are utilized in a sequential manner or simultaneously. Future studies will elucidate the complete GlxR regulon and determine intracellular cAMP concentrations under a variety of growth conditions.

RamA is a LuxR-type regulator identified as a protein binding to the *pta-ack* and *aceA/aceB* promoter regions [15]. RamA was shown to be essential for growth on acetate as sole carbon source. Deletion of *ramA* resulted in low specific activities of acetate kinase, phosphotransacetylase, isocitrate lyase and malate synthase when grown on glucose/acetate mixtures [15]. Similarly, expression of transcriptional fusions of the *pta-ack* and *aceA/aceB* promoter regions was very low under these conditions. Thus, RamA activates transcription of these genes (Fig. 1). Purified RamA specifically binds to a tandem A/C/TG$_{4-6}$T/C sequence present in the *pta-ack* and *aceA/aceB* promoter regions as demonstrated by EMSA studies using intact or mutated promoter DNA fragments [15]. The effector of RamA activity is not yet known, although acetate, acetyl-phosphate, CoA, 2-oxoglutarate, NAD, NADH, cAMP and cGMP were demonstrated not to interfere with RamA binding to its target DNA [15]. It was noted, however, that lower RamA concentrations were needed for binding to the *aceA/aceB* promoter DNA when ATP or ADP were present and higher RamA concentrations when acetyl-CoA was present [15]. Acetyl-CoA or a derivative thereof has previously been implicated in transcriptional regulation of *aceA*, *aceB*, and *pta-ack* [105].

Besides activating transcription of genes of acetate metabolism RamA might serve additional functions. Recently, it has been shown that RamA activates transcription of the surface-layer protein gene *cspB* in *C. glutamicum* ATCC 14067 (Fig. 1) [26]. The best studied *C. glutamicum* wild-type strain, ATCC 13032, lacks an S-layer present in closely related *C. glutamicum* strains such as ATCC 14067 [11, 12, 27]. The fact that S-layer protein synthesis was shown to depend on the carbon source with lactate addition showing the most pronounced stimulatory effect [94] and a role for RamA in activiation of *cspB* [27] establishes a link between carbon metabolism and S-layer formation. RamA likely plays further roles also in the *C. glutamicum* wild-type strain ATCC 13032, since a deletion mutant lacking RamA showed a reduced biomass yield on glucose [15]. Future work will establish the RamA regulon, its effector and its role in regulating glucose metabolism.

Regulator of acetate metabolism B, RamB, represses transcription of the *pta-ack* operon and the *aceA* and *aceB* genes (Fig. 1) [22]. Deletion and mutation analysis of the promoter regions of these genes allowed identifying highly conserved 13-bp motifs (AA/GAACTTTGCAAA) as cis-regulatory elements for acetate-dependent expression. RamB was purified by DNA affinity chromatography as a protein specifically binding to the promoter region of the *pta-ack* operon [22]. Purified RamB protein was shown to bind specifically to both the *pta-ack* and the *aceA/aceB* promoter regions. A deletion mutant lacking RamB exhibited high-level specific activities of acetate kinase, phosphotransacetylase, isocitrate lyase, and malate synthase, regardless of the carbon source, while *C. glutamicum* wild type showed high-level specific activities only in the presence of acetate. Similarly, expression analysis of transcriptional fusions of the *pta-ack* and the *aceA/aceB* promoter regions revealed that this deregulation takes place at the level of transcription [22]. Thus, RamB represses transcription of *pta-ack*, *aceA*, and

*aceB* in the absence of acetate, while RamA activates transcription of these genes in the presence of acetate.

The acetate stimulon comprises more genes than the known target genes of RamB (*aceA*, *aceB*, *pta-ack*). A bioinformatic analysis of the genome sequence revealed that variants of the *cis*-regulatory motif of RamB identified upstream of *aceA*, *aceB*, and *pta-ack* also occur in the promoter regions of 28 other genes (Fig. 1), 11 of which belong to the acetate stimulon. Two or even three motifs were located in front of the genes encoding pyruvate carboxylase, PEP carboxykinase, citrate synthase, subunit E1 of the pyruvate dehydrogenase complex, and PTA-AK. Further work will have to address whether all of these 28 genes belong to the RamB regulon, whether RamB always functions as a repressor and what metabolite modulates its function.

The flux through the TCA cycle is about fourfold higher during growth on acetate than on glucose [104], but the specific activities of citrate synthase and isocitrate dehydrogenase are relatively constant on various carbon sources [18]. The fact that the specific aconitase activities are 2.5–4 fold higher on propionate, citrate, or acetate than on glucose [50], suggested that aconitase is a major control point of tricarboxylic acid cycle activity in *C. glutamicum*. A gene encoding a TetR-type transcriptional regulator was found downstream of the aconitase gene *acn* [50]. This gene was named *acnR* as AcnR could be shown to repress *acn* expression. AcnR functions as a homodimeric repressor by binding to the *acn* promoter in the region from 11 to 28 relative to the transcription start, thus presumably preventing binding of RNA polymerase [50]. This AcnR binding motif and the organization of its gene into an operon together with *acn* is conserved in corynebacteria and mycobacteria [50]. While the activity of TetR-type repressors are typically modulated by low-molecular weight effectors, the nature of the effector of AcnR remains unknown to date although a number of central metabolites were tested for their ability to prevent AcnR from binding to the *acn* promoter [50].

## GENETIC REGULATION OF N-METABOLISM

Inorganic ammonium is the preferred nitrogen source for growth of *C. glutamicum*, but also a number of organic nitrogen sources, e.g. L-glutamate, L-glutamine, L-alanine, L-serine, L-threonine, L-asparagine, peptides, urea or creatinine can be utilized [10]. Membrane-potential-driven transport of (methyl)ammonium is mediated by AmtA, AmtB and presumably by a third system [64]. L-glutamate may be taken up by the ATP-driven GluABCD system [49] or by the membrane-potential-driven GltS system [98]. Urea is taken up by ATP-driven transport [3], while creatinine uptake is mediated by the permease CrnT [6].

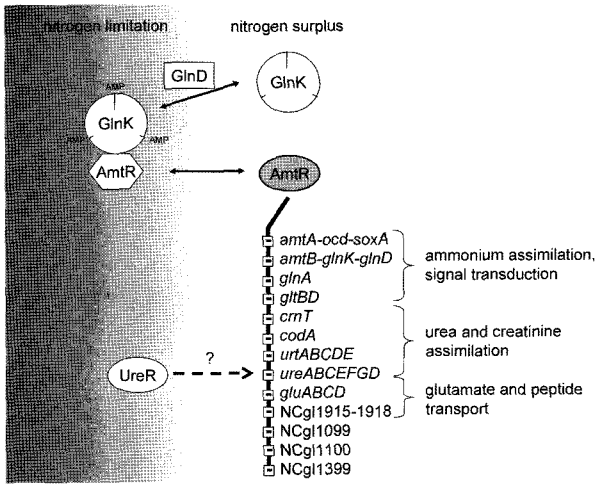


**Fig. 2.** Model of the regulation of nitrogen metabolism in *C. glutamicum* by AmtR, UreR, GlnD and GlnK.
Symbols are as indicated in Fig. 1. For details see text.

The two main pathways of ammonium assimilation in *C. glutamicum* are catalyzed by glutamate dehydrogenase (GDH) and glutamine synthetase (GS)-glutamine 2-oxoglutarate-aminotransferase (GOGAT) [97]. All nitrogen sources are ultimately converted to L-glutamine and L-glutamate directly, e.g. amino acids by transaminases [58], or indirectly via ammonium, e.g. after cleavage by urease [71] or creatinase [6]. *In-vivo* $^{15}N$ NMR analysis revealed that in *C. glutamicum* 28% of ammonium is assimilated via the GS reaction in glutamine, 72% via the GDH reaction in glutamate, while other reactions, e.g. that of alanine dehydrogenase, did not contribute to ammonium assimilation [97].

Nitrogen regulation by the master regulator AmtR and the signal transduction proteins GlnK and GlnD (Fig. 2) has been reviewed recently [91]. In short, when ammonium becomes scarce, GlnK, the only $P_{II}$-type protein in *C. glutamicum*, is adenylylated by the GlnD protein [72]. Adenylated GlnK protein is cytosolic, while unmodified GlnK is, at least in part, sequestered to the cytoplasmic membrane by binding to the ammonium transporter AmtB (but not to AmtA) [96]. Membrane-sequestration prevents proteolysis of GlnK by the proteases FtsH, ClpCP and ClpXP [96]. In its adenylated form GlnK binds to the TetR-type transcriptional repressor AmtR and prevents binding of AmtR to its target genes. AmtR, the master regulator of nitrogen regulation in *C. glutamicum* [36], represses transcription of at least 33 genes [4] encoding enzymes for ammonium assimilation, signal transduction as well as transport and utilization of organic nitrogen sources.

The AmtR binding site, 5'-tttCTATN$_6$AtAGat/aA-3', is an imperfect palindromic sequence [4] with highly conserved and less conserved bases (capital and small letters, respectively). Binding of AmtR to binding sites identified in the *C. glutamicum* genome by bioinformatics upstream

of 12 transcription units (and two divergently oriented genes) was verified experimentally [4]. AmtR binding sites are located -8 to -370 bp upstream of the translational start codon. As the transcriptional start sites have not been determined, it remains to be seen whether AmtR binding precludes binding of RNA polymerase to the -10 and/or -35 promoter regions of the target genes/operons or whether repression occurs by another mechanism. AmtR control of the *vanABK* operon was shown to be indirect [65], but the respective transcriptional regulator has not yet been identified. The operon *vanABK* encodes vanillate demethylase, an enzyme required for the utilization of vanillate or ferulate as carbon source, and a putative uptake system for protocatechuate, the product of the vanillate demethylase reaction [65]. Protocatechuate is a constituent of *C. glutamicum* minimal media such as CgXII medium and facilitates iron acquisition [57]. However, *vanABK* was not identified as part of the iron stimulon [50] and it neither belongs to the regulon of RipA, the regulator of iron proteins A [107], nor to the regulon of DtxR, the master regulator of iron-dependent gene expression in *C. glutamicum* [9, 106].

## GENETIC REGULATION OF S-METABOLISM

*C. glutamicum* can utilize inorganic sulfate and thiosulfate, but also aliphatic sulfonates and sulfonate esters as sources of sulfur for growth [52]. Assimilatory reduction of inorganic sulfate as well as degradation of sulfonates and sulfonate esters leads to sulfite and ultimately to sulfide. The predominant sulfur-containing molecules within the *C. glutamicum* cell are the amino acids L-cysteine and L-methionine. L-cysteine biosynthesis proceeds from L-serine via O-acetyl serine (CysE, [25]) and sulfur is incorporated either from sulfide by *O*-acetylserine sulfhydrolases A and B (CysK, CysM) or from thiosulfate by *O*-acetylserine sulfhydrolases B (CysM). In the initial catalytic step of L-methionine biosynthesis homoserine is converted to *O*-acetyl homoserine (MetX, [76]). Two alternative pathways, direct sulfhydrolation by *O*-acetylhomoserine sulfhydrylase (MetY; [32] and transsulfuration by cystathionine-γ-synthase (MetB; [31]) and cystathionine β-lyase (AecD; [31, 42]) operate in *C. glutamicum* [32, 53] and yield homocysteine. Homocysteine is also generated when cystathionine-γ-synthase (MetB) produces homolanthionine from homocysteine and *O*-acetyl homoserine as a side reaction [47] followed by cleavage of homolanthionine by cystathionine-β-lyase (MetC) to oxobutanoate, a precursor of isoleucine biosynthesis. Homocysteine is converted to L-methionine in a reaction requiring methyl-tetrahydrofolate, which originates from L-serine in the reactions of serine hydroxymethyltransferase (GlyA, [93]) and $N^{5,10}$-methylene tetrahydrofolate reductase (MetF), by vitamin B12-independepent methionine synthase (MetE) or by vitamin B12-dependent methionine synthase (MetH). The general methyl donor *S*-adenosylmethionine is derived of L-methionine by *S*-adenosylmethionine synthase (MetK; [24]).

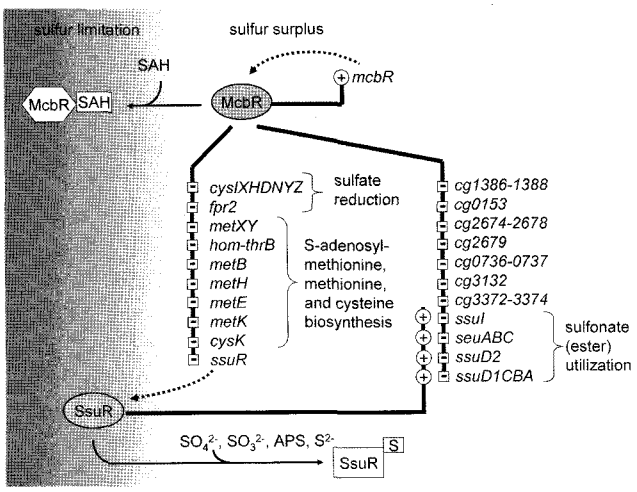


**Fig. 3.** Model of the regulation of sulfur metabolism in *C. glutamicum* by McbR and SsuR.
Symbols are as indicated in Fig. 1. For details see text.

Sulfur metabolism is transcriptionally regulated by the regulatory proteins McbR and SsuR (Fig. 3). McbR acts as the master regulator of almost all genes of sulfur metabolism [84] including its own gene and the regulator genes *ssuR*. McbR of *C. glutamicum* was shown to directly control expression of 45 genes in 22 operons that share a conserved motif in their promoter regions, the inverted repeat 5'-TAGAC-$N_6$-GTCTA-3', and code for proteins involved in all aspects of transport and metabolism of the macroelement sulfur, i.e. methionine and cysteine biosynthesis, sulfate reduction, uptake and utilization of sulfur-containing compounds [84]. Band-shift experiments revealed that *S*-adenosylhomocysteine, a product of *S*-adenosylmethionine-dependent transmethylation reactions, prevented the binding of McbR to the conserved sequence motif. This regulatory link between sulfur and $C_1$ metabolism is also known in *E. coli*, but in this bacterium the biosynthetic endproduct *S*-adenosylmethionine functions as a co-repressor of the methionine apo-repressor MetJ from *E. coli* [14]. In contrast, *S*-adenosylhomocysteine functions as an inducer in *C. glutamicum* and causes derepression of the McbR regulon [84]. A sulfur salvage pathway was proposed to exist because *cg0860* encodes a putative enzyme hydrolyzing *S*-adenosylhomocysteine to yield homocysteine, a direct precursor of L-methionine and *S*-adenosylmethionine. Although *cg0860* is not regulated by McbR, the accumulation of *S*-adenosylhomocysteine might be a good indicator of insufficient supply of L-methionine or *S*-adenosylmethionine and thus the *S*-adenosylhomocysteine concentration might reflect the general sulfur status of the *C. glutamicum* cell.

In the absence of sulfate, sulphonate sulfur utilization regulator (SsuR) [44] of *C. glutamicum* activates expression of 9 genes in 4 operons (*ssuI*, *seuABC*, *ssuD2*, and *ssuD1CBA*) coding for the enzymes involved in sulfonate and sulfonate ester utilization [45]. The SsuR binding site comprises a T-rich, a GC- and an A-rich domain with a length of six, nine and six nucleotides, respectively. SsuR binding sites could only be identified in the promoter regions of *ssuI*, *seuABC*, *ssuD2*, and *ssuD1CBA*, where they are located upstream of the −35 promoter regions. Binding of SsuR to its binding sites *in vitro* was prevented by sulfate as well as by the metabolites of assimilatory sulfate reduction adenosine 5'-phosphosulphate, sulfite and sulfide [44]. All genes belonging to the SsuR regulon and *ssuR* itself, which is not autoregulated, are part of the McbR regulon [84]. This hierarchical regulatory organization ensures that genes required for sulfonate and sulfonate ester utilization are only expressed under conditions of a low sulfur status, i.e. when *S*-adenosylhomocysteine accumulates, and when in addition supply of reduced sulfur by assimilatory sulfate reduction is insufficient. Thus, *S*-adenosylhomocysteine inactivates McbR under conditions of a limiting *S*-adenosylmethionine supply and expression of the McbR regulon genes including the SsuR regulon genes and *ssuR* is derepressed. Maximal expression of the SsuR regulon genes is ensured by activation by SsuR when low concentrations of sulfate, adenosine 5'-phosphosulphate, sulfite and sulfide indicate insufficient supply of reduced sulfur by assimilatory sulfate reduction. Currently, it is not known whether *C. glutamicum* can detect the presence of sulfonates or sulfonate esters and whether this would be of relevance for regulation of their metabolism.

Transcription of the genes of assimilatory sulfate reduction, of cysteine biosynthesis and for degradation of organosulfur sources is activated in *E. coli* by the transcriptional regulator CysB in the presence of the co-activator *N*-acetyl-serine, an isomer of *O*-acetyl-serine [99]. The *C. glutamicum* genome encodes a protein sharing 25% identical amino acids with *E. coli* CysB [52]. However, currently it is not known whether CysB plays a role in sulfur-dependent transcriptional regulation in *C. glutamicum*. Additional regulators might play a role in regulation of the sulfate reduction operon *cysIXHDNYZ* in *C. glutamicum*. DtxR, the central regulator of iron metabolism in *C. glutamicum* [9, 106], may be involved in regulation of *cysIXHDNYZ*. Some genes of the *cysIXHDNYZ* operon and the divergently oriented *fpr2*, which is not essential for sulfate reduction, showed increased expression in the absence of DtxR in one study [9], but not in another [106]. While binding of purified DtxR to the *fpr2* promoter DNA was shown *in vitro*, the physiological significance of regulation of *fpr2* and presumably also of *cysIXHDNYZ* by DtxR requires further studies.

## GENETIC REGULATION OF P-METABOLISM

*C. glutamicum* can utilize inorganic phosphate and organophosphates, but not phosphonates, as sole sources of phosphorus for growth [102]. The growth rate of *C. glutamicum* with inorganic phosphate as source of phosphorus is half-maximal at a concentration of about 0.1 mM [35]. *C. glutamicum* responds to phosphate starvation by changing expression of a number of genes involved in phosphorous metabolism [35, 102]. Using whole-genome *C. glutamicum* DNA microarrays [101] the phosphate starvation stimulon and the kinetics of the phosphate starvation response of *C. glutamicum* could be determined [35]. Among the genes of the phosphate starvation stimulon the *pstSCAB* operon coding for an ABC-type phosphate uptake system is induced first when phosphate becomes scarce. With a short delay the *ugpABCE* operon encoding an ABC-type *sn*-glycerol 3-phosphate transport system and the gene for glycerophosphoryldiester phosphodiesterase (*glpQ1*) are induced to allow uptake of *sn*-glycerol 3-phosphate as alternative source of phosphorus. Subsequently, the genes *ushA* (5'-nucleotidase, [87], *nucH* (extracellular nuclease), *phoH1* (ATPase of unknown function) and *cg0689* are induced. Finally, a number of further genes mainly coding for proteins of unknown function are induced. Thus, the strategy of *C. glutamicum* to cope with phosphate limitation is to induce 1) a high-affinity system for phosphate uptake, 2) a system for uptake and utilization of the organophosphate *sn*-glycerol 3-phosphate and 3) systems allowing to access inorganic phosphate present in non-transportable organophosphates.

The genes of one of the 13 two-component regulatory systems of *C. glutamicum* showed transiently increased mRNA levels 10 min after shifting to medium with a limiting phosphate concentration [35]. A mutant lacking this two-component regulatory system was specifically impaired in its ability to grow under phosphate limitation as revealed in an exhaustive functional genomics analysis of two-component regulatory systems of *C. glutamicum* [43]. Therefore, the genes lacking in this strain were named *phoS* (encoding the sensor kinase) and *phoR* (encoding the response regulator). Comparing the transcriptomes of *C. glutamicum* wild type and of the deletion mutant Δ*phoRS* it could be shown that phosphate starvation induction of all genes of the phosphate starvation stimulon, with the exception of the *pstSCAB* operon, within 1 h after a shift from phosphate excess to phosphate limitation requires the PhoS-PhoR two-component regulatory system (Fig. 4) [43]. The timed gradual induction of the genes of the phosphate starvation stimulon suggests that the number and/or sequence of the PhoR binding sites differ between genes/operons showing fast and slow induction profiles. The partial induction of the *pstSCAB* operon in a mutant lacking PhoS-PhoR [43] suggests that (an) additional regulator(s) is/are involved in control of *pstSCAB* expression.

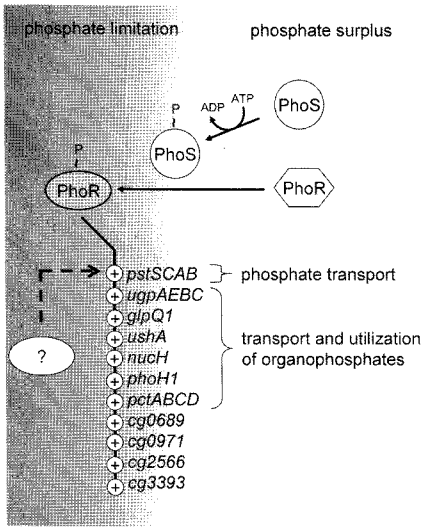


**Fig. 4.** Model of the regulation of phosphorus metabolism in *C. glutamicum* by the two-component regulatory system PhoS and PhoR.
Symbols are as indicated in Fig. 1. For details see text.

## OUTLOOK

In the last three years 'omics' technologies have boosted our knowledge on metabolic regulation in *C. glutamicum*. Until recently some thought that *C. glutamicum* is a rather simple bacterium devoid of efficient regulatory mechanisms (few isozymes, little allosteric control, hardly any transcriptional regulation) and that this might even explain why it lends itself to be used as an ideal producer of amino acids. It is clear now that *C. glutamicum* has many multi-facetted regulatory mechanisms which we have just begun to unravel and which often are novel and without precedence in the model bacteria *E. coli* and *B. subtilis*. In future, we will have to study genetic regulation in *C. glutamicum* in its full complexity, find out parallels to the taxonomically related pathogenic mycobacteria and apply our knowledge on genetic regulation to metabolic engineering.

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