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MINI-REVIEW

Botho Bowien · Bernhard Kusian

Genetics and control of CO_2 assimilation in the chemoautotroph *Ralstonia eutropha*

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Abstract The nutritional versatility of facultative autotrophs requires efficient overall control of their metabolism. Most of these organisms are Proteobacteria that assimilate CO_2 via the highly energy-demanding Calvin-Benson-Bassham reductive pentose-phosphate cycle. The enzymes of the cycle are encoded by *cbb* genes organized in *cbb* operons differing in size and composition, although conserved features are apparent. Transcription of the operons, which may form regulons, is strictly controlled, being induced during autotrophic but repressed to varying extents during heterotrophic growth of the bacteria. The chemoautotroph *Ralstonia eutropha* is one of the organisms studied extensively for the mechanisms involved in the expression of *cbb* gene systems. CbbR is a LysR-type transcriptional regulator and the key activator protein of *cbb* operons. The *cbbR* gene is typically located adjacent and in divergent orientation to its cognate operon. The activating function of CbbR seems to be modulated by metabolites signaling the nutritional state of the cell to the *cbb* system. Phosphoenolpyruvate is such a signal metabolite acting as a negative effector of *R. eutropha* CbbR, whereas NADPH has been proposed to be a coactivator of the protein in two other chemoautotrophs, *Xanthobacter flavus* and *Hydrogenophilus thermoluteolus*. There is evidence for the participation of additional regulators in *cbb* control. In the photoautotrophs *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, response regulator RegA of the global two-component signal transduction system RegBA serves this function. It is conceivable that specific variants of *cbb* control systems have evolved to ensure their optimal integration into regulatory networks operating in the diverse autotrophs characterized by different metabolic capabilities.

B. Bowien (✉) · B. Kusian
Institut für Mikrobiologie und Genetik,
Georg-August-Universität Göttingen,
Grisebachstrasse 8, 37077 Göttingen, Germany
e-mail: bbowien@gwdg.de,
Tel.: +49-551-393815, Fax: +49-551-399842

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Abbreviations *CBB* Calvin-Benson-Bassham · *LTTR* LysR-type transcriptional regulator · *PEP* Phosphoenolpyruvate · *RuBisCO* Ribulose-1,5-bisphosphate carboxylase/oxygenase

Introduction

The formation of organic matter in the biosphere depends upon the metabolic activity of the primary producers. These autotrophic organisms assimilate CO_2 as the major source of cell carbon. Oxygenic phototrophs such as the prokaryotic cyanobacteria and the eukaryotic algae and higher plants are quantitatively the dominating autotrophs with respect to global net carbon fixation. However, a great diversity of other prokaryotes, both phototrophs and chemotrophs, contributes to this fundamental biosynthetic process. The Calvin-Benson-Bassham (CBB) reductive pentose phosphate cycle is used as the metabolic pathway of CO_2 assimilation by the vast majority of these organisms. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the key enzyme of the cycle. Three additional pathways are known to operate exclusively in bacteria and archaea: (1) the reductive tricarboxylic acid cycle, (2) the 3-hydroxypropionate cycle, and (3) the reductive acetyl coenzyme A pathway. Whereas the cyclic routes occur in aerobic as well as anaerobic photo- and/or chemoautotrophs, the linear acetyl coenzyme A pathway appears to be restricted to anaerobic chemoautotrophs.

Proteobacterial aerobic chemoautotrophs and anaerobic phototrophs (purple bacteria) use the CBB cycle as the principal pathway of carbon assimilation (Gibson and Tabita 1996; Kusian and Bowien 1997; Shively et al. 1998). Numerous of these bacteria are facultative autotrophs characterized by a remarkable nutritional versatility. They are also able to grow heterotrophically on a great variety of organic substrates as carbon and energy sources or might

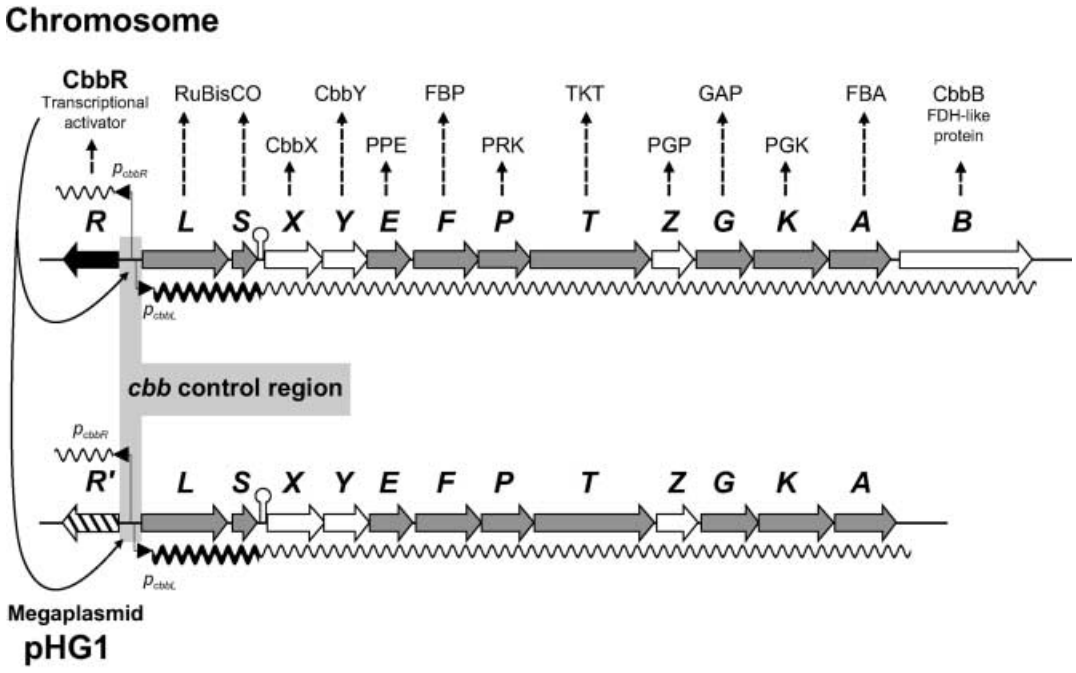


Fig. 1 Location and organization of the *cbb* regulon of the chemoautotroph *Ralstonia eutropha* H16 consisting of two highly homologous gene clusters. *Gray-shaded* genes encode CBB cycle enzymes. Abbreviations of gene products: *CbbR* LysR-type transcriptional activator binding to the operator of the *cbb* control region, *RuBisCO* ribulose-1,5-bisphosphate carboxylase/oxygenase made up of large (CbbL) and small (CbbS) subunits, *CbbX*, *CbbY* products of unknown function, *PPE* pentose-5-phosphate 3-epimerase, *FBP* fructose-1,6-/sedoheptulose-1,7-bisphosphatase, *PRK* phosphoribulokinase, *TKT* transketolase, *PGP* 2-phosphoglycolate phosphatase, *GAP* glyceraldehyde-3-phosphate dehydrogenase, *PGK* phosphoglycerate kinase, *FBA* fructose-1,6-/sedoheptulose-1,7-bisphosphate aldolase, *CbbB* formate dehydrogenase (FDH)-like protein. The *hatched cbbR (R´)* is the defective *cbbR* gene located on megaplasmid pHG1 of the strain. p_{cbbL} and p_{cbbR} designate the strong, inducible *cbb* operon and the weak, constitutive *cbbR* promoters, respectively. *Wavy lines* represent *cbb* transcripts, with different thicknesses indicating relative abundances. A hairpin structure (*symbol between S and X*) downstream of *cbbS* causes frequent premature transcription termination

even prefer mixotrophy at their habitats by utilizing inorganic and organic substrates simultaneously. Such flexibility implies the existence of regulatory networks in these organisms that enable efficient control of genes encoding relevant enzyme systems of carbon and energy metabolism because the energy demand for CO_2 assimilation is very high, particularly via the CBB cycle. In contrast, the obligate autotrophs typically living as chemo- or photolithotrophs rely on CO_2 assimilation, being unable to switch their metabolism between different modes of nutrition.

Autotrophic bacteria and archaea are generally not easily amenable to genetic analysis and manipulation. This is the main reason for the limited information available on the genetics of autotrophy in these organisms, although the increasing wealth of genomic data will improve the picture very soon. A growing number of Proteobacteria is being studied with respect to this feature, and some substantial progress has been made since the appearance of the last comprehensive reviews on the topic (Kusian and Bowien 1997; Shively et al. 1998). This mini-review will summarize some of the current knowledge about the organization and control of *cbb* gene systems with a special focus on the system present in the aerobic, facultatively chemoautotrophic β-proteobacterium *Ralstonia eutropha* H16.

The *cbb* operons

The organization of *cbb* genes in the various bacteria is diverse, but some features seem to be conserved. One or several *cbb* operons are present in the genomes of these organisms. *R. eutropha* H16 possesses the largest known *cbb* operons (Kusian and Bowien 1997; Shively et al. 1998). The chromosomal operon (cbb_c) of the strain has a length of about 15.2 kilobase pairs (kb) comprising 13 genes that encode eight of the ten CBB enzymes. A second, highly homologous operon (cbb_p) is located on the megaplasmid pHG1 and contains only 12 genes adding up to approximately 12.8 kb (Fig. 1). The transcription of each operon is directed by a single promoter, and there is no significant subpromoter activity within these unusually large transcriptional units. Nevertheless, strong differential expression of the promoter-proximal RuBisCO genes *cbbLS* and the remaining distal genes was observed and is primarily due to premature transcription termination directly downstream of *cbbS* (Schäferjohann et al. 1996).

Two tandemly arranged genes, *cbbX* and *cbbY*, that do not encode CBB enzymes are located adjacent to the *cbbLS* genes which encode a red-type form I (L_8S_8) RuBisCO (Watson and Tabita 1997) in *R. eutropha* (Fig. 1). Although the functions of their products are still unknown, CbbX is essential for autotrophic growth of the organism.

Whereas knock-out of *cbbX* resulted in a loss of autotrophy, inactivation of *cbbY* did not cause a detectable alteration of the phenotype (B. Bowien, unpublished data). A similar organization with regard to *cbbX* and *cbbY* is found in the cbb_I gene cluster of the facultatively phototrophic α-proteobacterium *Rhodobacter sphaeroides* (Gibson and Tabita 1997). Interestingly, only photoautotrophic but not chemoautotrophic growth was impaired in a *cbbX*-deficient mutant of this bacterium. The *cbb* operons of the chemoautotrophs *Xanthobacter flavus* (Meijer et al. 1991), *Oligotropha carboxidovorans* (Santiago and Meyer 1997), and *Sinorhizobium meliloti* (Finan et al. 2001) also contain a *cbbX* gene, but not *cbbY*, located directly downstream of *cbbLS*. However, the association of *cbbX* with red-type form I RuBisCO genes is not restricted to bacteria. The plastid-encoded *rbcLS* (*cbbLS*) genes in red algae and cryptophytes are accompanied by a *cbbX* gene (Maier et al. 2000). The general linkage of *cbbX* to RuBisCO genes and the phenotype of the *R. eutropha cbbX* mutant suggest that the CbbX protein might be required for the synthesis of active forms of red-type form I RuBisCOs. CbbX contains a nucleotide-binding motif and was classified as member of a protein family including the *Bacillus* sporulation factor SpoVJ and some hypothetical mycobacterial proteins (Maier et al. 2000). The sporulation factor has been assigned to the AAA^+ class of chaperone-like ATPases associated with diverse cellular activities (Neuwald et al. 1999), and CbbX might therefore also be placed into this protein class.

It is of interest with regard to the potential function of CbbX that in some autotrophic bacteria a *cbbQ* instead of a *cbbX* gene is located downstream of *cbbLS* genes which encode green-type form I RuBisCOs (Yokoyama et al. 1995; Paoli et al. 1998; Baxter et al. 2002). In yet other bacteria, *cbbQ* was found to be associated with *cbbM* coding for form II (L_x) RuBisCOs (Shively et al. 1998; Hayashi et al. 1999). The *cbbQ* genes closely resemble the regulatory *nirQ* and *norQ* genes of denitrifying bacteria. The CbbQ protein hydrolyzes ATP and activates form I as well as form II RuBisCOs by inducing conformational changes of the enzymes (Hayashi et al. 1999; Hayashi and Igarashi 2002). NirQ from *Pseudomonas aeruginosa* was shown to activate the green-type form I RuBisCO of the chemoautotroph *Hydrogenophilus thermoluteolus* (Hayashi et al. 1998a). It is thus tempting to speculate that CbbX and CbbQ, although not being related, serve similar functions in activating red-type form I and green-type form I or form II RuBisCOs, respectively. Plant-type RuBisCO activases (Portis 1995), which are AAA^+ proteins, have not been detected in Proteobacteria. The *cbbLSQOYA* operon of *H. thermoluteolus* contains an unusual gene, *cbbO*. Like CbbQ, CbbO was reported to enhance the activity of *H. thermoluteolus* form I RuBisCO by a still unknown mechanism (Hayashi et al. 1997). A *cbbO* gene is also present downstream of *cbbQ* in the cbb_I gene cluster of the phototroph *Rhodobacter capsulatus* (Vichivanives et al. 2000) and was detected, although not linked to *cbbLSQ*, in the methanotroph *Methylococcus capsulatus* (Baxter et al. 2002).

Both *cbb* operons of *R. eutropha* H16 contain a *cbbZ* gene which encodes phosphoglycolate phosphatase (Fig. 1; Schäferjohann et al. 1993), the key enzyme of autotrophic glycolate metabolism in organisms operating the CBB cycle. The gene is also a cistron in the *cbbXYZ* operon within the cbb_I cluster of *Rba. sphaeroides* (Gibson and Tabita 1997) and in the cbb_{II} cluster of *Rba. capsulatus* (Vichivanives et al. 2000). The direct linkage of *cbbZ* with genes of CBB enzymes might be envisaged as the genetic manifestation of an interlocking between the CBB cycle and glycolate metabolism. In the presence of oxygen, 2-phosphoglycolate is formed by the action of the oxygenase function of RuBisCO and subsequently degraded to: (1) prevent accumulation of potentially toxic concentrations of the metabolite and (2) to salvage a part of its carbon that would otherwise be lost. The physiological significance of phosphoglycolate phosphatase in *R. eutropha* was studied by inactivating *cbbZ*. A *cbbZ* deletion mutant failed to grow organoautotrophically under air with formate as the energy source, but proliferated like the parent strain when cultivated at elevated CO_2 concentrations (>1%, v/v) suppressing the oxygenase activity of RuBisCO. Heterotrophic growth of the mutant was not affected (L. Grozdanov and B. Bowien, unpublished data). The high CO_2 requirement of the mutant during autotrophic growth is consistent with the anticipated metabolic role of phosphoglycolate phosphatase.

The 3′-terminal gene *cbbB* in the cbb_c operon of *R. eutropha* H16 encodes a formate-dehydrogenase-like protein. It is absent from the cbb_p operon of the strain (Fig. 1), thus the gene duplication event that presumably led to the formation of the two operons apparently did not include this gene. Until now, a *cbbB* gene has not been observed in any other of known *cbb* operons. The physiological significance of the CbbB protein in *R. eutropha* is not clear at present. Inactivation of *cbbB* did neither affect organoautotrophic growth on formate nor cause a conspicuous phenotype of the mutant (Bömmer et al. 1996).

The key activator CbbR

CbbR has been identified as an essential regulatory protein activating the transcription of *cbb* operons in many autotrophic proteobacteria. The presence of (a) *cbbR* gene(s) is a general feature in these organisms (Kusian and Bowien 1997; Shively et al. 1998). A *cbbR* gene is typically located immediately upstream of and in divergent orientation to the *cbb* operon being under control of the activator gene. In *R. eutropha* H16 both *cbb* operons are preceded by *cbbR* (Fig. 1), although the copy located on plasmid pHG1 is defective due to several short deletions. The chromosomally encoded CbbR activates both operons (Windhövel and Bowien 1991). In organisms possessing more than one *cbb* operon: (1) only one *cbbR* copy might be present, (2) each operon might be associated with a *cbbR* gene, (3) in exceptional cases, like the obligate chemoautotroph *Hydrogenovibrio marinus* MH-110 (Hayashi et al. 1998b), *cbbR* might be lacking or not located in the vicinity of the operons.

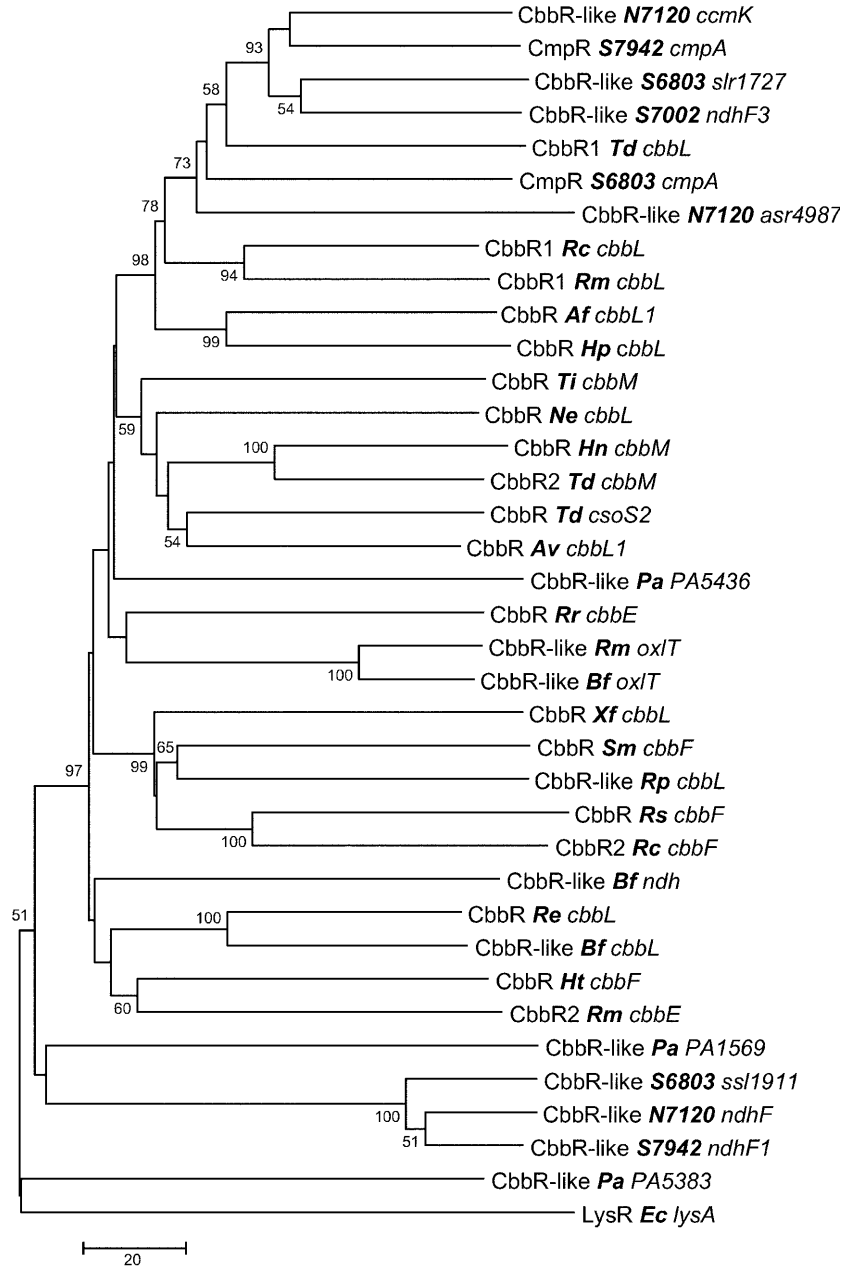


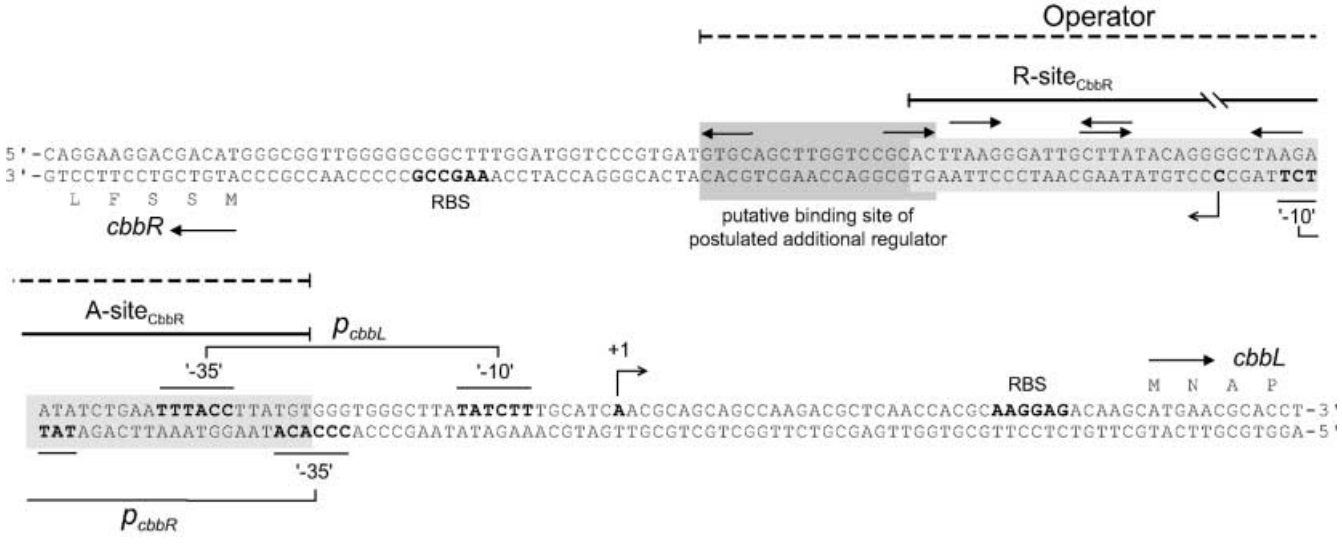
Fig. 2 Phylogenetic tree of CbbR and CbbR-like proteins. The amino acid sequences of proteins with significant similarities to known CbbRs were aligned employing the program ClustalX version 1.81 (Thompson et al. 1997). The tree was generated by the neighbor-joining method based on a protein distance matrix by means of the MEGA2 program (Kumar et al. 2001). The branch lengths correspond to the number of substitutions per sequence, with the reference bar representing 20 substitutions. Bootstrap values higher than 50% are indicated. Proteins whose genes are not linked to known *cbb* genes or operons were designated as CbbR-like. LysR was included to differentiate between CbbR and other members of the LysR family. The abbreviations indicate the organismal origin of the regulatory gene together with a neighboring gene which is not necessarily the regulatory target. *Af cbbL1*, *Acidithiobacillus ferrooxidans* (data base accession number BAA01917); *Av cbbL1*, *Allochromatium vinosum* (AAA23327); *Bf cbbL*, *Burkholderia fungorum* (http://www.jgi.doe.gov); *Bf ndh*, *B. fungorum* (http://www.jgi.doe.gov); *Bf oxlT*, *B. fungorum* (http://www.jgi.doe.gov); *Ec lysA*, *Escherichia coli* (AAB40486); *Hn cbbM*, *Halothiobacillus neapolitanus* (AAD02441); *Hp cbbL*, *Hydrogenophaga pseudoflava* (AAD10333); *Ht cbbF*, *Hydrogenophilus thermoluteolus* (BAA95688); *Ne cbbL*, *Nitrosomonas europaea* (http://www.jgi.doe.gov); *N7120 ccmK*, *Nostoc* sp. PCC 7120 (NP_484905); *N7120 asr4987*, *Nostoc* sp. PCC 7120 (NP_489026); *N7120 ndhF*, *Nostoc* sp. PCC 7120 (NP_487994); *Pa PA1569*, *Pseudomonas aeruginosa* (AGG04959); *Pa PA5383*, *P. aeruginosa* (AAG08767); *Pa PA5436*, *P. aeruginosa* (AAG08822); *Re cbbL*, *Ralstonia eutropha* (AAA21982); *Rm cbbE*, *Ralstonia metallidurans* (http://www.jgi.doe.gov); *Rm cbbL*, *R. metallidurans* (http://www.jgi.doe.gov); *Rm oxlT*, *R. metallidurans* (http://www.jgi.doe.gov); *Rc cbbF*, *Rhodobacter capsulatus* (AAC32304); *Rc cbbL*, *Rba. capsulatus* (AAC32308); *Rs cbbF*, *Rhodobacter sphaeroides* (P52690); *Rp cbbL*, *Rhodopseudomonas palustris* (http://www.jgi.doe.gov); *Rr cbbE*, *Rhodospirillum rubrum* (AAB27779); *S6803 cmpA*, *Synechocystis* sp. PCC 6803 (NP_442731); *S6803 slr1727*, *Synechocystis* sp. PCC 6803 (NP_441244); *S6803 ssl1911*, *Synechocystis* sp. PCC 6803 (NP_440469); *Sm cbbF*, *Sinorhizobium meliloti* (NP_436736); *S7002 ndhF3*, *Synechococcus* PCC7002 (AAB62183); *S7942 cmpA*, *Synechococcus* sp. PCC7942 (BAB18722); *S7942 ndhF1*, *Synechococcus* sp. PCC7942 (AAG59995); *Td cbbM*, *Thiobacillus denitrificans* (AF046931); *Td cbbL*, *T. denitrificans* (AAD02439); *Td csoS2*, *T. denitrificans* (AAG60036); *Ti cbbM*, *Thiomonas intermedia* (*'Thiobacillus intermedius'*) (AAC24963); *Xf cbbL*, *Xanthobacter flavus* (CAA80406)

The CbbR proteins belong to the family of bacterial LysR-type transcriptional regulators (LTTR; Schell 1993) and seem to form a subfamily because of their relatively high homology. LTTRs are characterized by three functional domains: an amino-terminal domain containing a DNA-binding helix-turn-helix structural motif, a central domain presumably involved in effector recognition, and a carboxy-terminal domain probably required for response and multimerization. The occurrence of *cbbR*(-like) genes is not confined to the Proteobacteria, but extends to cyanobacteria, the green plastids of glaucocystophytes as well as the red plastids of red algae, heterokont algae and cryptomonads (Maier et al. 2000). A phylogenetic analysis disclosed the existence of several subgroups among CbbR homologs. Surprisingly, these proteins are also present in nonautotrophic bacteria such as *P. aeruginosa* and *Burkholderia fungorum*, and some organisms contain multiple paralogs (Fig. 2). Closer inspection of the genome sequence data available for *B. fungorum* LB400 (http://www.jgi.doe.gov) revealed that the strain carries a *cbb* gene cluster (*cbbLSXFPTAE* not linked to *cbbR*), suggesting a potential autotrophic capability. Cyanobacterial CbbR homologs do not control the transcription of *cbb* genes but participate in the adaptation of *Synechocystis* and *Synechococcus* to inorganic carbon starvation (low CO_2/HCO_3^-) and osmotic stress (Figge et al. 2001; Omata et al. 2001). The data suggest that CbbR(-like) proteins are not only involved in the regulation of *cbb* and other operons related to CO_2 metabolism but might play a more global regulatory role.

The *cbb* control region of *R. eutropha*

LTTRs typically bind to the control regions of their target operons at a partially symmetric sequence element which contains the T-N_{11}-A motif centered about 65 bp upstream of the transcription start site (Schell 1993). The intergenic segments between *cbbR*/*cbbR´* and $cbbL_c$/$cbbL_p$ of *R. eutropha* H16 are 167 bp long and contain the complete control region of the pertaining *cbb* operons. Thirteen nucleotide positions differ between the chromosomal and the plasmid-borne control regions. Within these regions the CbbR-binding site occupies positions –75 to –24 relative to the transcription start site and overlaps the –35 box of the σ^{70}-dependent *cbb* promoter p_{cbbL} (Kusian and Bowien 1995; Fig. 3). DNase I footprinting performed in the presence of bound CbbR revealed a hypersensitive site at positions –47 and –48, indicating DNA bending caused by the activator. The CbbR-binding site almost completely covers the divergently oriented σ^{70}-type *cbbR* promoter p_{cbbR}, suggesting negative autoregulation of *cbbR* transcription. Two subsites of the operator were recognized, both of which are occupied by a presumably dimeric CbbR. The p_{cbbL}-distal subsite is believed to be the recognition (R-) site, the proximal one the activation (A-) site. Even slight alterations in the spacing of the subsites by introducing 1- or 2-bp insertions abolished the activation of p_{cbbL}. An absolutely correct relative alignment of the subsites is obviously required for productive binding of CbbR to allow activation of transcription by proper interaction with RNA polymerase (Kusian and Bowien 1995). Specific binding to their target *cbb* operators has been demonstrated with CbbR from *Acidithiobacillus ferrooxidans* (Kusano and Sugawara 1993), *X. flavus* (van Keulen et al. 1998), *Rba. sphaeroides* (Dubbs et al. 2000), and *H. thermoluteolus* (Terazono et al. 2001). Apart from CbbR, specific binding of two additional proteins to the *cbb* control

Fig. 3 Nucleotide sequence of the chromosomal intergenic region between the *cbbR* and *cbbL* genes of *R. eutropha* H16. The segment contains the *cbb* control elements comprising the operator, the *cbb* operon promoter p_{cbbL}, and the *cbbR* promoter p_{cbbR}. Activator CbbR binds to the R- and A-sites (*light shading*) within the operator, whereas the *dark-shaded* area upstream of the R-site represents the potential binding site of a second regulatory protein. The *arrows* above the sequence indicate symmetrical motifs (GTGC-N_{10}-GCAC, TAAG-N_6-CTTA, CTTA-N_9-TAAG). *Bent arrows* are positioned at the transcription start points (+1) of the genes. The NH_2-terminal amino acid sequences of the gene products CbbR and CbbL are also given. *RBS* Ribosome-binding site



region of *R. eutropha* H16 has been observed. Both proteins, however, were found not to be involved in *cbb* control (T. Jeffke, C. Höfle, B. Kusian, and B. Bowien, unpublished data). The DNA region immediately upstream of the $CbbR_{R\text{-}site}$, comprising at least 18 bp and containing a symmetrical motif (GTGC-N_{10}-GCAC; Fig. 3), is required for full activation of p_{cbbL}. It is hypothesized to be the binding site of an additional *cbb* regulatory protein that is different from the two proteins. This site together with the CbbR-binding site would make up the *cbb* operator of *R. eutropha* (Fig. 3).

cbb regulation in *R. eutropha*

As a facultative autotroph *R. eutropha* H16 controls expression of its two *cbb* operons very strictly to restrict wasting of energy by operating the CBB cycle when CO_2 assimilation is not needed. Both operons are simultaneously transcribed, thus forming a regulon under major control of the transcriptional activator CbbR (Windhövel and Bowien 1991; Kusian et al. 1995). Lithoautotrophic growth with hydrogen or organoautotrophic growth with formate as energy sources leads to high induction/derepression of the operons that is enhanced by CO_2 limitation, whereas strong repression prevails during heterotrophic growth on most organic substrates such as pyruvate. Inactivation of the *cbbR* gene results in an autotrophically negative phenotype due to total loss of transcription from p_{cbbL}. The weakly constitutive transcription of *cbbR* is only slightly increased in induced cells. Mutational modifications in p_{cbbL} were used to study the regulation of p_{cbbL} in more detail. The closer the –35 and/or –10 boxes matched those of the σ^{70} consensus promoter of *E. coli*, the higher was the basal activity of the modified p_{cbbL}. The partially constitutive mutant promoters retained the activation under inducing conditions, even in a CbbR-free background. These findings were interpreted as indirect evidence for the participation of (an)other regulator(s), besides CbbR, in the transcriptional control of the *R. eutropha cbb* operon promoter (Jeffke et al. 1999).

In *Rba. sphaeroides* the two-component global regulatory system PrrB-PrrA is directly involved in the positive regulation of the cbb_I and cbb_{II} operons (Qian and Tabita 1996). The response regulator PrrA was shown to bind at two promoter-proximal sites located immediately upstream of the CbbR-binding region and at two additional promoter-distal sites of the cbb_I operon (Dubbs et al. 2000). RegA (homolog of PrrA) also activates of cbb_I and cbb_{II} operons of *Rba. capsulatus* (Vichivanives et al. 2000). Since the PrrBA/RegBA signal transduction system was originally found to be implicated in the regulation of photosynthesis genes (Eraso and Kaplan 1994) and genes of the nitrogen metabolism (Joshi and Tabita 1996) of *Rba. sphaeroides* and *Rba. capsulatus*, *cbb* transcription control is apparently interlocked with the gene regulation of other major metabolic segments in these phototrophs. The two-component systems RegSR and ActSR in the symbiotic N_2-fixing proteobacteria *Bradyrhizobium japonicum* and *S. meliloti*, respectively, both being facultative chemoautotrophs, are homologous to PrrBA and RegBA. There is preliminary evidence that RegR and ActR might also function as activators, in addition to CbbR, of the *cbb* operons of these organisms (Emmerich et al. 2000; Fenner et al. 2000).

A central question concerns the signals acting in the control of *cbb* operons in the various autotrophic bacteria. These signals are thought to be generated intracellularly in the carbon and/or energy metabolism rather than to originate directly from the external environment (Kusian and Bowien 1997; Shively et al. 1998). The search for (a) *cbb* signal metabolite(s) in *R. eutropha* disclosed phosphoenolpyruvate (PEP) as a negative effector of CbbR, demonstrated by in vitro transcription analyses employing the purified activator, DNA-dependent RNA polymerase and p_{cbbL}-carrying templates (Grzeszik et al. 2000). CbbR-activated transcription from partially constitutive p_{cbbL} (see above) was inhibited by PEP at concentrations within the physiological range (0.2–2.0 mM). None of many metabolites including oxidized and reduced pyridine dinucleotides (NAD[P]/H), tested separately or in combinations, exerted significant effects on the transcription activity. Thus, CbbR from *R. eutropha* is a sensor of the intracellular PEP concentration. PEP increases the affinity of the activator to its operator target site, resulting in a diminished activating potential of CbbR. The metabolite does, however, not phosphorylate the activator (C. Höfle, B. Kusian, and B. Bowien, unpublished data). Since PEP is an indirect product of the CBB cycle, this feedback-type control responds to the carbon state of the cell. The wild-type p_{cbbL} remained silent in the in vitro transcription system presumably due to a missing additional protein required for promoter activation.

In contrast, CbbR of *X. flavus* has been described as a NADPH sensor (van Keulen et al. 1998). NADPH was shown to enhance the binding of CbbR to its cognate *cbb* operator and to relax CbbR-induced DNA bending. The determined K_{dNADPH} of 75 μM corresponds well to the intracellular concentration of NADPH observed in *X. flavus* (van Keulen et al. 2000). The findings suggest that the expression of the *cbb* and *gap-pgk* operons in *X. flavus* is primarily controlled by the energetic state of the cell. Further evidence will be required to verify this conclusion. CbbR from *H. thermoluteolus* also interacts specifically with NADPH, resulting in altered binding of the activator to the *cbb* operator (Terazono et al. 2001). So far, *cbb* signal metabolites other than PEP and NADPH have not been identified in autotrophs. It is well conceivable that the various autotrophs possess distinct CbbR proteins interacting with different ligands.

Conclusions

Organization and transcriptional control of *cbb* genes in autotrophic bacteria show both common characteristics and differences. Conserved features concern the occurrence of *cbb* operons, which comprise at least the RuBisCO

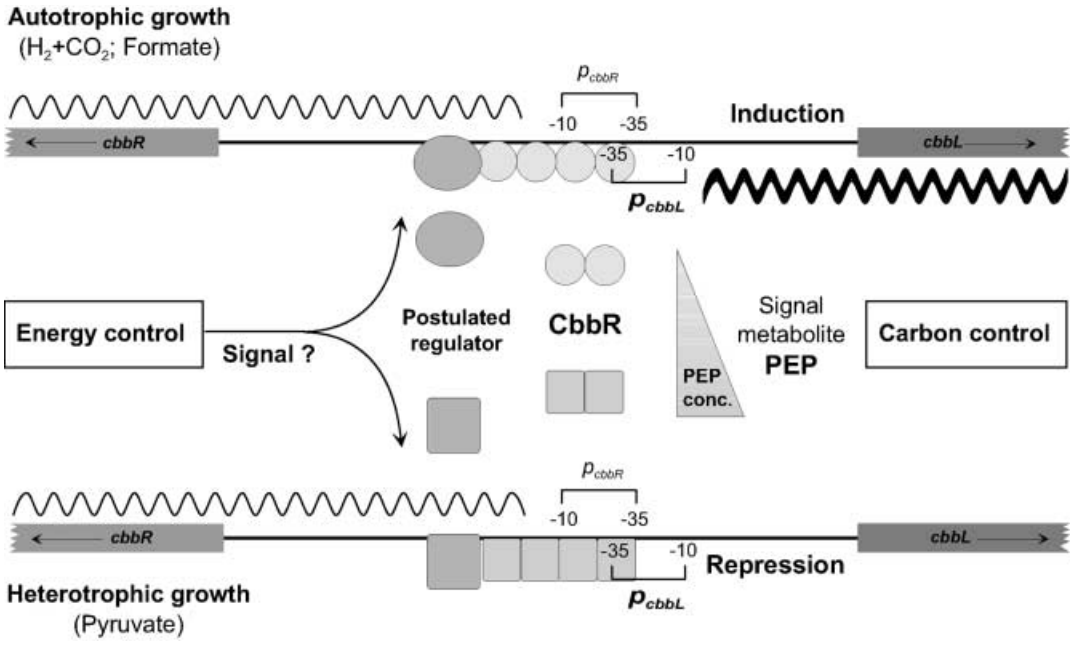


Fig. 4 Model for the transcriptional control of the *cbb* operons in *R. eutropha* H16. Autotrophic growth causes induction, heterotrophic growth results in repression of p_{cbbL}. The higher intracellular concentration of the negative effector PEP in heterotrophic cells leads to inhibition of the activating function of CbbR (carbon control of the system). A postulated but still unknown second regulator, which possibly senses the energetic state of the cell, is required for activation of p_{cbbL} (energy control). *Differently shaped symbols* for CbbR and the postulated regulator indicate presumed different conformations of the proteins. The *wavy lines* represent the *cbb* transcripts with different thicknesses indicating relative abundances

genes *cbbLS* or *cbbM*. Larger *cbb* operons often contain typical blocks of genes like *cbbLSX*, *cbbLSQ*, *cbbMQ* and *cbbFPT*, suggesting that these individual gene groups were originally recruited as such and might have common evolutionary backgrounds. But apart from these unifying aspects, overall sizes and gene arrangements of *cbb* operons vary considerably among organisms (Kusian and Bowien 1997; Shively et al. 1998). The activator gene *cbbR* is usually located in the direct neighborhood of its cognate *cbb* operon, in this way clustering structural and regulatory genetic information necessary for operation of the CBB cycle. In future studies of *cbb* control systems several aspects of CbbR function should be considered: (1) DNA target specificity, (2) mechanism of promoter activation, (3) interaction with effectors, (4) interaction with other proteins, (5) cross-talk between CbbR paralogs of an organism, (6) interspecies specificity of CbbR orthologs, and (7) role of CbbR in integrating *cbb* control into superimposed regulatory networks.

The *cbb* regulon of *R. eutropha* H16, consisting of two large, duplicated *cbb* operons located on separate replicons, is being extensively analyzed. Coordinate expression of the operons requires CbbR encoded by the single, chromosomally located *cbbR* gene. However, the observed regulatory properties of the system cannot be explained conclusively unless at least one additional regulator protein, possibly an activator, is invoked that contributes to the fine-tuning of p_{cbbL} activity. This protein might serve as a sensor of the energetic state of the cell, perhaps signaling the availability of reduced pyridine dinucleotides. In *X. flavus* and *H. thermoluteolus* CbbR was proposed to be an NADPH sensor, but such function is unlikely for the protein of *R. eutropha*. Instead the *R. eutropha* activator is a PEP sensor, the activity of which is modulated by the negative effector. Identification of PEP as a signal metabolite, indicating the cellular carbon state, is in agreement with earlier physiological studies that suggested PEP to be a negative and a reduced metabolite (possibly NAD[P]H) to be a positive effector of RuBisCO formation in the organism (Im and Friedrich 1983). A tight linkage between the carbon and energy states in the *cbb* control in *R. eutropha* – and probably in other autotrophs as well – appears to be likely. The collective evidence permits a model for *cbb* control in *R. eutropha* that accounts for the current observations (Fig. 4). A crucial aspect in validating the model will be the identification of the postulated additional regulator and the potential signal metabolite supposed to be sensed by the protein. This regulator must not necessarily be PrrA/RegA – as in *Rba. sphaeroides* and *Rba. capsulatus* – or a homolog of it. The integration of *cbb* gene control into overall cellular regulation is apparently different in the various autotrophs, depending on their metabolic capabilities which evolved during adaptation to diverse habitats. Specific variants of the basically CbbR-mediated *cbb* control exist in individual (groups of) organisms that are characterized by different metabolic signals and additional regulators.

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