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Organization and regulation of *cbb* CO_2 assimilation genes in autotrophic bacteria

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Abstract

The Calvin-Benson-Bassham cycle constitutes the principal route of CO_2 assimilation in aerobic chemoautotrophic and in anaerobic phototrophic purple bacteria. Most of the enzymes of the cycle are found to be encoded by *cbb* genes. Despite some conservation of the internal gene arrangement *cbb* gene clusters of the various organisms differ in size and operon organization. The *cbb* operons of facultative autotrophs are more strictly regulated than those of obligate autotrophs. The major control is exerted by the *cbbR* gene, which codes for a transcriptional activator of the LysR family. This gene is typically located immediately upstream of and in divergent orientation to the regulated *cbb* operon, forming a control region for both transcriptional units. Recent studies suggest that additional protein factors are involved in the regulation. Although the metabolic signal(s) received by the regulatory components of the operons is (are) still unknown, the redox state of the cell is believed to play a key role. It is proposed that the control of *cbb* operon expression is integrated into a regulatory network.

Keywords: Autotrophy; Calvin cycle; *cbb* gene; CO_2 fixation; Regulation

Contents

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1. Introduction

Primary production of organic carbon in the biosphere is based on the assimilation of CO_2 by autotrophic organisms. The oxygenic photosynthesizers like higher plants, algae and cyanobacteria contribute the overwhelming portion to global net CO_2 fixation [1]. Anoxygenic photosynthetic bacteria such as the purple and green bacteria are secondary producers together with chemolithotrophic bacteria and chemolithotrophic archaea. During evolution a number of different mechanisms of CO_2 assimilation developed in the various groups of autotrophs. Three of the known carbon reduction pathways are cyclic reaction sequences: the reductive pentose phosphate cycle (Calvin-Benson-Bassham cycle; Fig. 1) [2], the reductive tricarboxylic acid cycle [3,4] and the 3-hydroxypropionate cycle [5,6]. To date the non-cyclic reductive acetyl-CoA pathway [7] has not been detected in any phototroph.

Whereas the Calvin cycle is found in both eu- and prokaryotic autotrophs, the other routes appear to be confined to bacteria and/or archaea, although evidence for the occurrence of the reductive tricarboxylic acid cycle in the green alga *Chlamydomonas reinhardtii* has been reported [8]. Among the prokaryotes the Calvin cycle is the principal pathway in cyanobacteria and the majority of the proteobacteria. With

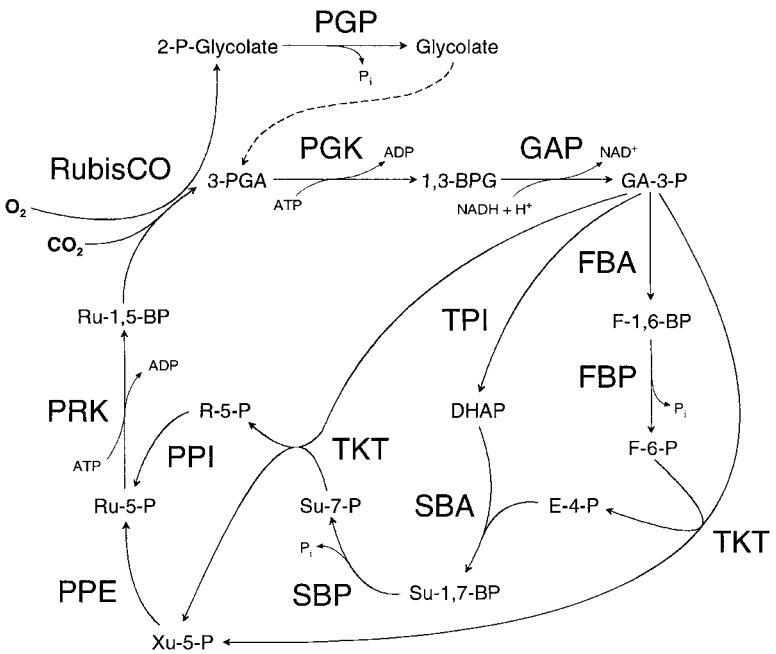

Fig. 1. Reactions and enzymes of the Calvin cycle including the initial reaction of the photo-chemorespiratory pathway of glycolate oxidation. The following abbreviations are used for (i) metabolites: 1,3-BPG, 1,3-bisphosphoglycerate; DHAP, dihydroxyacetone phosphate; E-4-P, erythrose-4-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; GA-3-P, glyceraldehyde-3-phosphate; 3-PGA, 3-phosphoglycerate; R-5-P, ribose-5-phosphate; RuBP, ribulose-1,5-bisphosphate; Ru-5-P, ribulose-5-phosphate; Su-1,7-BP, sedoheptulose-1,7-bisphosphate; Su-7-P, sedoheptulose-7-phosphate; Xu-5-P, xylulose-5-phosphate; (ii) enzymes: FBA, fructose-1,6-bisphosphate aldolase; FBP, fructose-1,6-bisphosphatase; GAP, glyceraldehyde-3-phosphate dehydrogenase; PGK, 3-phosphoglycerate kinase; PGP, phosphoglycolate phosphatase; PPE, pentose-5-phosphate 3-epimerase; PPI, pentose-5-phosphate isomerase; PRK, phosphoribulokinase; RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SBA, sedoheptulose-1,7-bisphosphate aldolase, SBP, sedoheptulose-1,7-bisphosphatase, TKT, transketolase; TPI, triosephosphate isomerase.

the remarkable exception of some halobacteria [9,10], the key enzymes of the cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) and phosphoribulokinase (PRK), were not detected in archaea. However, the halobacteria have not yet been shown to be capable of autotrophic growth. Surprisingly, the autotrophic, methanogenic archaeon *Methanococcus jannaschii* using the reductive acetyl-CoA pathway of CO_2 assimilation contains a gene, whose derived product resembles the large subunit of RubisCO [11]. RubisCO and PRK have also been found in the obligately methylotrophic bacterium *Methylococcus capsulatus* [12]. A PRK-like gene obviously not related to autotrophic growth has recently been identified in the heterotrophic enterobacterium *Escherichia coli* [13]. Fixation and reduction of CO_2 via the Calvin cycle requires considerably more metabolic energy than through the alternative routes [6,14].

According to their general metabolic capabilities, two major groups of autotrophic organisms are distinguished among the photo- and chemotrophs, the obligate and facultative autotrophs. The photo- and chemolithotrophs are characterized by obligate autotrophy, i.e., reliance on CO_2 as main source of cell carbon. Facultative autotrophs exhibit greater nutritional flexibility enabling the organisms to grow on organic substrates as alternative carbon and energy sources [15]. Some aerobic, chemotrophic bacteria, e.g., the hydrogen-oxidizing bacteria, and the phototrophic purple non-sulfur bacteria are typical representatives of these versatile organisms. They may also grow mixotrophically by utilizing inorganic and organic carbon and/or energy substrates simultaneously.

Efficient control of the corresponding pathways on both the activity and the synthesis level of the enzyme systems is essential for facultative autotrophs to cope with varying nutritional regimes. Carbon and energy metabolism are undoubtedly linked by a complex regulatory network which awaits elucidation. The major methodological advances in molecular genetics during the past decade allowed to initiate genetic investigations that are required to provide insight into the principles controlling autotrophic metabolism. Such studies have been performed with several different bacteria using the Calvin cycle for CO_2 assimilation. In this article the current knowledge of Calvin cycle genetics and regulation in chemo- and phototrophic bacteria will be summarized. However, the cyanobacteria will largely be excluded from the discussion, since an excellent recent review is available on this aspect of the topic [16].

2. Organization and genomic location of *cbb* genes

Except for the reactions catalyzed by RubisCO, PRK and sedoheptulose-1,7-bisphosphatase the remaining 10 reactions of the Calvin cycle (see Fig. 1) are also involved in the central carbon metabolism (glycolysis/gluconeogenesis, pentose biosynthesis) of heterotrophs and facultative autotrophs. Thus, in order to avoid ambiguity in gene designations, a uniform nomenclature was proposed for genes of bacterial enzymes that exclusively operate in the cycle [17]. The *cbb* genes encode enzymes and products related or presumed to be related to functions of the cycle, including genes with a regulatory function in the expression of *cbb* transcriptional units (Fig. 2). These new designations will be used throughout this article, but not applied to cyanobacterial genes.

2.1. Chemotrophs

The information on number and organization of *cbb* genes in the different organisms examined so far varies considerably. Among the chemoautotrophs the facultative hydrogen- and formate-oxidizing bacterium *Ralstonia eutropha* H16 (formerly *Alcaligenes eutrophus* H16 [18]) is characterized by a very peculiar clustering of *cbb* genes on two different replicons of its genome, the chromosome and the megaplasmid pHG1 [19,20]. The self-transmissible plasmid comprises about 450 kb and also carries the genes of the hydrogen-oxidizing system of the organism [21] adjacent to its *cbb* genes. Both clusters have an identical arrangement of *cbb* genes which encode all Calvin cycle enzymes with the exception of triosephosphate isomerase and ribose-5-phosphate isomerase (Fig. 2). They are strongly homologous with an average nucleotide identity of about 93% for the regions extending from the regulatory gene *cbbR* to *cbbA* [22–28]. As expected, the preservation of the se-

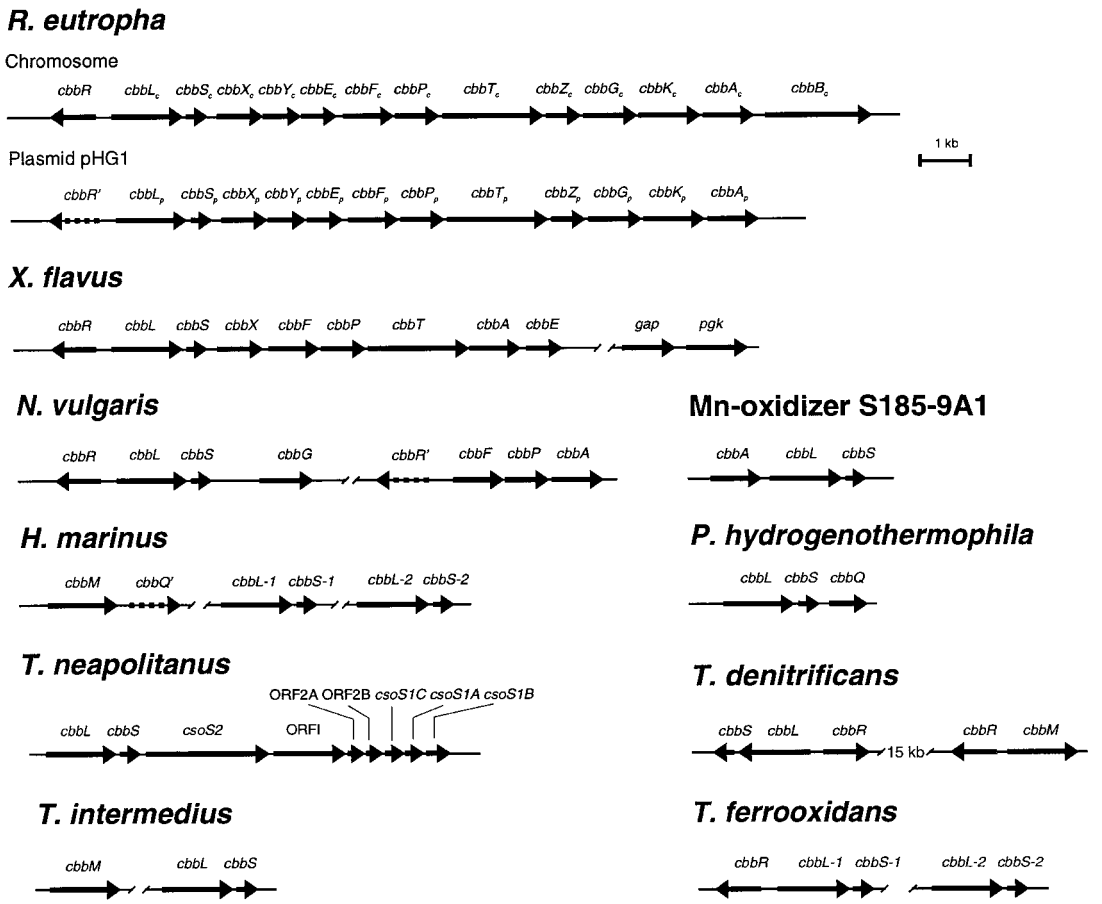

Fig. 2. Organization of *cbb* genes in chemotrophic bacteria. Designations of genes and their products: *cbbR*, CbbR (LysR-type transcriptional activator); *cbbLS*, large and small subunit of form I RubisCO, respectively; *cbbM*, form II RubisCO; *cbbE*, PPE; *cbbF*, FBP/SBP; *cbbP*, PRK; *cbbT*, TKT; *cbbZ*, PGP; *cbbG* and *gap*, GAP; *cbbK* and *pgk*, PGK; *cbbA*, FBA/SBA; *cbbB*, formate dehydrogenase-like gene of unknown function. The functions of *cbbX*, *cbbQ* and *cbbY* gene products are also unknown, but possibly related to the Calvin cycle. *csoS1*(*C,A,B*) and *csoS2* encode carboxysomal proteins. Indices c and p refer to chromosomal and plasmid locations, respectively, of the *cbb* genes in *R. eutropha* H16. Non-functional genes are indicated by broken arrows. The length of the reference bar corresponds to 1 kb.

quence is higher in the open reading frames than in the intergenic stretches. Such very high degree of homology suggests that the two clusters presumably originate from a gene duplication event. However, the chromosomal cluster contains at least one additional gene, $cbbB_c$, located immediately downstream of $cbbA_c$ and encoding a formate dehydrogenase-like protein of unknown function. A mutant of *R. eutropha* H16 deficient in $cbbB_c$ did not exhibit a phenotype discernibly different from that of the wild-type strain [29]. The thirteen and twelve genes of the chromosomal ($cbbL_c$ through $cbbB_c$) and plasmid-borne cluster ($cbbL_p$ through $cbbA_p$), respectively, have the same relative orientation (Fig. 2) and form single transcriptional units reaching sizes of about 15.2 kb (cbb_c operon) and 12.8 kb (cbb_p operon) [28–30]. The divergently oriented regulatory *cbbR* gene, which was first discovered in this organism [31], forms a monocistronic operon within the chromosomal cluster. Although its counterpart *cbbR'*, located on pHG1 (Fig. 2), is functionally deficient due to a 28-bp deletion near the 5′ terminus [23], it is also actively transcribed [26].

Apart from $cbbB_c$ two other genes of the *cbb* op-

eron, *cbbX* and *cbbY* located immediately downstream of the RubisCO genes *cbbLS*, could so far not be assigned specific functions [24]. Individual or simultaneous inactivation of these genes neither abolished the expression of the remaining genes of the operon nor did it affect the ability of such mutants to grow autotrophically (J. Schäferjohann and B. Bowien, unpublished results). It is thus concluded that the gene products CbbX and CbbY are either not required for autotrophy and the synthesis/function of Calvin cycle enzymes or their functions might be replaced by other proteins in the mutants.

The location of *cbbZ* encoding phosphoglycolate phosphatase [25] within the *cbb* operon is of particular interest in view of the physiological role of this enzyme during autotrophic growth of the organism. Phosphoglycolate is produced by the oxygenase activity of RubisCO at relatively low CO_2 and high O_2 concentrations as they may occur in the natural habitat of *R. eutropha*. The metabolite must be dephosphorylated to glycolate (i) to avoid accumulation of the compound, which is a potential inhibitor of triosephosphate isomerase [32], and (ii) to oxidatively recycle the formed glycolate into the Calvin cycle via the D-glycerate pathway. A similar carbon-salvaging glycolate metabolism operates during photorespiration in plants [33]. The metabolic interlocking of autotrophic carbon reduction and oxidation routes is manifested on the genetic level in *R. eutropha*, although the pHG1-borne $cbbZ_p$ of strain H16 is apparently not expressed. Interestingly, the products of *cbbY* and *cbbZ* exhibit significant similarity [25]. A functional phosphoglycolate phosphatase gene has also been detected in *E. coli* [34].

The presence of dual *cbb* gene clusters is not a special feature of *R. eutropha* H16. Reiteration of *cbb* genes on chromosomes and megaplasmids has been demonstrated for several strains of the species with the surprising exception of the type strain [35–38]. The latter harbors pHG2 lacking at least $cbbLS_p$ and the PRK gene $cbbP_p$, if not the complete *cbb* gene cluster. It does not show the heterotrophic derepression of RubisCO and PRK that seems to be correlated with a megaplasmid location of the corresponding genes (see Section 3.1). Plasmid pHG21-a of the related strain *Alcaligenes hydrogenophilus* M50 (or 1978) also contains a second set of *cbb* genes [38,39]. Since the megaplasmids from all of these strains carry the genetic information for the hydrogen-oxidizing system, they confer the lithoautotrophic trait upon the organisms. The heavy-metal-resistant strain CH34 of *R. eutropha* does not contain a pHG-type plasmid [40] and consequently has only chromosomal *cbb* genes, which are completely absent in the non-autotrophic strain JMP134 [38].

Genes encoding the large subunit of RubisCO (*cbbL*) and PRK (*cbbP*) were detected in several aerobic, carbon monoxide-oxidizing bacteria. These genes are duplicated in *Oligotropha carboxydovorans* OM5 with one copy located on the chromosome and the second on plasmid pHCG3 [41], which is reminiscent of the situation in *R. eutropha*. Also, RubisCO genes are reportedly located on the chromosome as well as on plasmid pPB13 of the nitrite-oxidizing nitrifier *Nitrobacter hamburgensis* X_{14} [42]. The hydrogen oxidizer *Acidovorax facilis* K carries the *cbb* genes exclusively on plasmid pHG22-a, whereas strain J of the same species only has chromosomal *cbb* genes [43]. An exclusive plasmid location of these genes has also been demonstrated in the sole Gram-positive bacterium studied so far in this respect, the hydrogen oxidizer *Rhodococcus opacus*. Strains MR11 and MR22 of the organism carry the genes on the linear plasmids pHG201 and pHG205, respectively [44]. In contrast, the symbiotic N_2-fixing bacterium *Bradyrhizobium japonicum* that is able to grow chemoautotrophically as a free-living organism has chromosomal *cbb* genes [45].

A cluster of *cbb* genes has been located on the chromosome of *Xanthobacter flavus* H4-14 (Fig. 2), which is able to use methanol in addition to formate or hydrogen as energy source for autotrophic growth [46]. This region comprises at least seven genes of Calvin cycle enzymes that are organized in a basically similar order as the corresponding genes in *R. eutropha* H16 [47,48]. Also, a *cbbX* gene is positioned downstream of the RubisCO genes, emphasizing a possible role of *cbbX* in the autotrophic carbon metabolism of these organisms. An additional resemblance concerns the relative position and orientation of the *cbbR* gene. However, two other genes, *gap* and *pgk*, encoding GAP and PGK, respectively, that also operate in the cycle, are part of a separate operon not physically linked to the *cbb* gene cluster [49].

Two unlinked clusters of *cbb* genes were discovered in the nitrite oxidizer *Nitrobacter vulgaris* T3

[50]. Their organization bears only partial similarity to the clusters of *R. eutropha* and *X. flavus*, although the typical arrangement within the gene groups *cbbR-cbbLS* and *cbbFP* is also conserved in *N. vulgaris* (Fig. 2). However, due to only partial sequence information it is premature to postulate a complete *cbbR* upstream of *cbbLS*. We also noticed a significant resemblance to *cbbR* genes when analyzing the sequence upstream of *cbbF* in divergent orientation relative to the *cbbFPA* cluster. The similarity of potential polypeptide products to CbbR proteins is, however, distributed over all three reading frames, suggesting the possible presence of a non-functional *cbbR* copy in this position.

Considerably less information is available on *cbb* genes of other chemoautotrophs. It mostly relates to the regions of RubisCO genes. In the thermophilic hydrogen oxidizer *Pseudomonas hydrogenothermophila* TH-1 a novel gene of unknown function, *cbbQ* (Fig. 2), which is similar to *nirQ* of the denitrification gene cluster from *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*, was detected immediately downstream of *cbbLS* [51]. The obligately autotrophic hydrogen and sulfur oxidizer *Hydrogenovibrio marinus* is the only organism known so far to possess three sets of RubisCO genes (Fig. 2), two sets of *cbbLS* encoding the conventional plant-type form I (L_8S_8) RubisCO [52,53] and one additional *cbbM* for the structurally simple form II (L_{2-6}) enzyme [54,55]. Downstream of *cbbM* we detected a region of high similarity to *cbbQ* of *P. hydrogenothermophila*. The region comprises the whole gene, but the similarity of the deduced polypeptide products is spread in several sections over three reading frames, indicating the presence of a non-functional *cbbQ* gene.

The obligately autotrophic iron and sulfur oxidizer *Thiobacillus ferrooxidans* Fe1 contains two identical sets of *cbbLS* genes (Fig. 2) with only *cbbLS-1* preceded by the regulatory *cbbR* gene. Both copies of the RubisCO operon are probably active [56,57]. The occurrence of two sets of RubisCO genes has also been observed with other strains of *T. ferrooxidans* [58,59]. A form I RubisCO gene cluster and a form II gene, separated by about 17 kb, were detected in the facultatively anaerobic sulfur oxidizer *Thiobacillus denitrificans* [60], and each set is preceded by a *cbbR* gene (J.M. Shively, personal communication). Form I and form II genes are also present in *Thiobacillus intermedius* (Fig. 2) [61]. In *Thiobacillus neapolitanus* the *cbbLS* genes are clustered with downstream carboxysome genes [62]. The manganese-oxidizing bacterium SI85-9A1, which may be unable to grow autotrophically, contains a gene cluster consisting of *cbbA* and *cbbLS* [63], resembling the arrangement of the corresponding genes in the *cbb* form I operon of the phototroph *Rhodobacter sphaeroides* (see Section 2.2).

2.2. Phototrophs

The *cbb* genes of the purple non-sulfur bacterium *R. sphaeroides* have been extensively studied [64]. This facultatively photosynthetic organism harbors two chromosomes (I and II) [65], both of which carry a *cbb* gene cluster of different organization (Fig. 3). On chromosome I, one pentacistronic operon encoding Calvin cycle enzymes including the form I RubisCO is followed by the *cbbXYZ* operon [66]. The gene arrangement in this cluster is quite different from those found in the *cbb* regions of *R. eutropha* and *X. flavus*. However, a divergently oriented regulatory gene *cbbR* [67] is also located immediately upstream of the cbb_I operon. The presence of *cbbX*, *Y* and *Z* represents the second reported case, besides *R. eutropha*, for the occurrence of all three genes within a *cbb* cluster. A defect in *cbbX* impaired the photoautotrophic growth of *R. sphaeroides*, but the function of this gene is apparently not essential for chemoautotrophic growth of the organism [66]. The cyanobacterium *Synechococcus* sp. PCC7942 also contains a *cbbZ*-like gene, which is, however, not linked to other *cbb* sequences [68]. The cbb_{II} operon is located on chromosome II and comprises the $cbbM_{II}$ gene coding for a form II RubisCO (L_4 or L_6; Fig. 3). Although this setup results in a partial duplication of Calvin cycle genes as in a number of chemoautotrophs, the genetics of the *cbb* system in *R. sphaeroides* differs significantly from that of the organisms already mentioned. A *cbbR* gene seems to be absent from the cbb_{II} cluster, but instead four open reading frames (ORFV, ORFU1, ORFU2 and ORFW) that may be involved in the regulation of the cbb_I-cbb_{II} regulon were localized directly upstream of $cbbF_{II}$ [69].

Although *Rhodobacter capsulatus* is closely related to *R. sphaeroides*, it possesses only a single chromo-

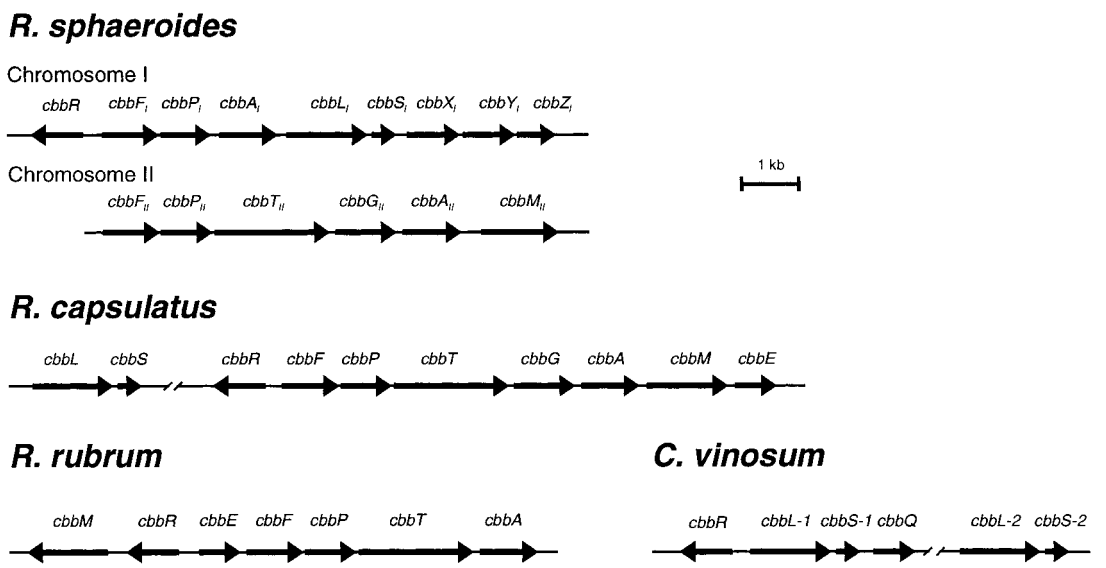

Fig. 3. Organization of *cbb* genes in phototrophic bacteria.The designations of genes and their products are the same as those used in Fig. 2. Indices I and II refer to locations of the *cbb* genes on chromosomes I and II, respectively, of *R. sphaeroides*. Length of reference bar: 1 kb.

some [70], which carries two unlinked *cbb* gene clusters (Fig. 3). The smaller cluster contains the form I RubisCO *cbbLS* genes, whereas the larger one including the form II RubisCO *cbbM* gene closely resembles the cbb_{II} operon of *R. sphaeroides* [71]. However, the form II cluster of *R. capsulatus* comprises an additional *cbbE* gene [72]. A single *cbb* gene cluster has been located in another purple non-sulfur bacterium, *Rhodospirillum rubrum* [73,74]. The cluster is unusually organized in that the *cbbR* gene is flanked by two *cbb* operons, downstream by the form II RubisCO *cbbM* gene and upstream by *cbbEFPTA* (Fig. 3). *R. rubrum* produces only a form II RubisCO (L_2), and the presence of *cbbLS* genes in this organism was not reported. A different situation prevails in the purple sulfur bacterium *Chromatium vinosum*, in which two unlinked sets of form I RubisCO have been characterized [75,76]. The functional *cbbLS-1* operon is located adjacent to a *cbbR* gene and also comprises *cbbQ*, whereas the potential *cbbLS-2* operon (Fig. 3) is apparently inactive [77].

2.3. Summarizing aspects

Comparison of *cbb* gene organization in the chemo- and anaerobic photoautotrophic bacteria studied so far reveals several features. (i) Although number of and overall arrangement of genes within the clusters are species-specific, in a few cases even strain-specific, there is a considerable conservation of the gene order within some blocks such as *cbbLSX* or *cbbLSQ* and *cbbFPT*. (ii) Reiteration or duplication of *cbb* genes, regardless of their transcriptional activity, are not exceptional. (iii) Plasmid-borne *cbb* genes are rather widespread. Surprisingly, however, no plasmid location of these genes was found to date in phototrophs. (iv) *cbbR* is a general regulatory (activator) gene linked to the *cbb* operons and forms a monocistronic operon within the *cbb* gene clusters. It occurs not only in (all) facultative, but also in some of the obligate autotrophs, a surprising feature since the latter (*T. ferrooxidans*, *T. denitrificans*) are characterized by a lack of nutritional versatility, making constitutive transcriptional activity of their *cbb* operons a necessity. In some bacteria the gene might be present in more than one functional copy, and special attention in this respect should be directed to the organisms with multiple *cbb* gene clusters. An interesting observation is the presence of two genes in the cyanobacterium *Synechocystis* sp. PCC6803, that exhibit significant similarity to *cbbR* [78]. Both genes

are not physically linked to known *cbb* genes of the organism. Whether or not these genes are involved in the regulation of cyanobacterial CO_2 assimilation remains to be elucidated.

The physiological function of *cbbX* is still unknown. However, the distribution of the gene or its homologs extends to non-chlorophytic algae. Like in bacteria the gene is located directly downstream of the RubisCO genes in the chloroplast genomes of the red algae *Porphyra purpurea* [79] and *Antithamnion* sp. [80], although it apparently is a rudimentary copy in the latter case [24]. Moreover, the presence of *cbbX* in these algae is accompanied by a high homology of their RubisCO genes to those of the autotrophic proteobacteria *R. eutropha*, *X. flavus* and *R. sphaeroides* [81,82], which were shown to possess *cbbX*. In the chloroplast of the diatom *Odontella sinensis* a homolog of *cbbX* is located about 12.4 kb upstream of the RubisCO genes [83].

Operation of the Calvin cycle in facultative autotrophs at least requires the additional expression of the RubisCO and PRK genes upon shift from heterotrophic to autotrophic growth. The reactions catalyzed by the remaining enzymes of the cycle do also function in the general carbon metabolism under all growth conditions and might be provided by enzymes encoded by independently expressed genes. However, in *R. eutropha* [29,30,84], *X. flavus* [47,48], *R. sphaeroides* [85–87] and *R. capsulatus* [71] *cbbLS*/*cbbM* and *cbbP* are organized in large operons together with other special genes of cycle enzymes. Such setup is best suited to ensure coordinate expression of these genes. The location of RubisCO and PRK genes in separate transcriptional units as in *N. vulgaris* [50] and *R. rubrum* [74] requires the coordinate expression to be indirectly accomplished by regulatory means. The tandem arrangement of the *cbbLS* genes is a very strongly conserved feature, and the genes often form a dicistronic operon, which is even found (designated as *rbcLS*) in cyanobacteria [88], the cyanelle of the biflagellate protist *Cyanophora paradoxa* [89] and in the chloroplast genomes of some non-chlorophytic algae [79,80,83,90].

A consequence of strictly regulated *cbb* genes in facultative autotrophs (see Section 3) is a requirement of additional genes that encode the corresponding isoenzymes involved in the central carbon metabolism during heterotrophic growth of these organisms. In fact, preliminary physical evidence for the presence of *fbp* (FBP) and *gap* (GAP) genes in *R. eutropha* have been obtained [27,91]. Two FBP/SBP isoenzymes were isolated from autotrophic cells of *R. opacus* MR11. The enzyme showing nearly equivalent FBP and SBP activities is only synthesized in autotrophic cells, whereas the other enzyme with a high FBP/SBP activity ratio is constitutively produced and thus operates in heterotrophically growing cells of this bacterium [92]. There is preliminary evidence that *R. opacus* MR11 also forms FBA/SBA isoenzymes, a constitutive class II enzyme and a class I enzyme additionally produced in autotrophic cells [38]. Likewise, *X. flavus* uses two FBP/SBP isoenzymes of comparable substrate specificities (CbbF and Fbp, respectively) for the different modes of growth [93] and employs a class II FBA/SBA (CbbA) in the Calvin cycle, but a class I enzyme (Fba) in heterotrophic carbon metabolism [48]. A second transketolase gene, *tkt*, was detected immediately upstream of *gap-pgk* (see Section 2.1) in *X. flavus* [49]. *R. capsulatus* also possesses an extra transketolase gene, *tktA*, which is not linked to the *cbb* gene clusters [94].

3. Regulation of *cbb* operons

Since assimilation of CO_2 via the Calvin cycle is a highly energy-demanding process, autotrophs have evolved various sophisticated mechanisms to control the activity of this pathway. Facultative autotrophs in particular must have the means to properly adjust the differing carbon fluxes under autotrophic and heterotrophic growth conditions. Although the enzymes of the cycle are subject to metabolic regulation of their activities [38,95], the basic control is exerted at the gene level, i.e., by modulating transcription. The transcriptional activity of the *cbb* genes is usually reflected by the activity profiles of their enzyme products, assuming that there is a general correlation between enzyme activity and enzyme content. RubisCO and in some cases also PRK activities are therefore routinely taken to represent expression of *cbb* operons.

3.1. Chemotrophs

Complete or partial repression of Calvin cycle enzymes during heterotrophic growth is a characteristic property of facultative chemoautotrophs, which is in accordance with the physiological needs of these organisms [38]. Regarding this regulatory aspect, *R. eutropha* is the most thoroughly studied chemoautotroph. The two *cbb* operons of strain H16 are highly expressed in litho- (H_2 as energy source) as well as organoautotrophically (formate) grown cells. The expression level increases even further upon CO_2 limitation imposed during lithoautotrophic growth [31,96]. In contrast, heterotrophic growth on most organic acids is characterized by virtually complete repression of the operons. A few substrates including fructose and citrate cause partial derepression up to about one third of the normal autotrophic expression level [97]. This 'gratuitous' activation of the operons, termed heterotrophic derepression, is apparently dependent on the presence of a plasmid-borne copy of the *cbb* operon [30,37]. An interesting feature of substrates causing heterotrophic derepression is the induction of the operons up to autotrophic levels during mixotrophic growth in the presence of H_2 or formate [98]. This induction does not depend on the plasmid-borne operon. The severe repression caused by other substrates is, however, not relieved. Except for the heterotrophic derepression, the observed expression of the *cbb* operons of *R. eutropha* under different growth conditions is compatible with a control mainly determined by the redox or energy state of the cells.

Transcript analyses and studies with operon fusions confirmed that the observed regulatory pattern in *R. eutropha* is the consequence of variations in the activity of the *cbb* operon promoter [20,26]. There is a pronounced differential expression of the 5′-terminal RubisCO genes and the residual genes of the *cbb* operons that results in an about 5-fold molar abundance of RubisCO over PRK [99]. The differential expression is due to frequent premature transcription termination immediately downstream of the *cbbLS* genes, resulting in high *cbbLS* transcript levels, while those of the other genes located further downstream in the operon are much less abundant [20,84]. A primary transcript encompassing the whole operon was not detected. The transcripts related to the remaining genes were found to cover different parts of the operon and to vary in size (R. Bednarski and B. Bowien, unpublished results). These findings might indicate that posttranscriptional processing and/or segmental degradation of the primary transcript is additionally involved in differential expression of the *cbb* operon genes of *R. eutropha*.

A regulatory pattern basically similar to that of *R. eutropha* was found for the single *cbb* operon in *X. flavus*. High expression prevails in litho- (H_2) or organoautotrophically (methanol, formate) grown cells, but heterotrophic growth on all substrates tested was not accompanied by any derepression of the operon. On the other hand, mixotrophic growth with various organic substrates in the presence of H_2, methanol or formate induced the key enzymes of the Calvin cycle [100,101], again suggesting that the redox or energy state of the cell may be sensed to produce the regulatory signal required for the expression of the *cbb* operon. The single *gap-pgk* operon of *X. flavus* is constitutively expressed to provide the organisms with GAP and PGK during both heterotrophic and autotrophic growth. However, it is additionally induced in autotrophically growing cells, indicating coordinate control of the *cbb* and *gap-pgk* operons. The coordination is achieved by action of the transcriptional activator CbbR [49,102]. In agreement with corresponding observations in *R. eutropha*, the *cbb* operon of *X. flavus* is subject to conspicuous differential gene expression. The mRNA of the 5′-terminal *cbbLSX* genes was found to be about six times as abundant as the *cbbFP* partial transcript. These findings have been interpreted as the result of processing of the primary large transcript into products of different stability [47].

Formation of RubisCO and PRK is also repressed in the facultative organoautotroph *Pseudomonas oxalaticus*, which is able to utilize formate for autotrophic growth, when cultured on acetate or fructose [103,104]. Interestingly, like in *R. eutropha* H16, fructose may cause only partial repression [105]. Heterotrophic derepression of RubisCO and PRK was reported for *R. opacus* MR11 during growth on pyruvate, but not on fructose [106]. Complete repression of RubisCO in acetate- or glycolate-grown cells was observed in *Paracoccus denitrificans*, another facultative autotroph capable of using H_2, methanol, methylamine or formate as energy sources [107,108],

as well as in *Acidovorax facilis* grown on various organic substrates [109]. The N_2-fixing symbionts *B. japonicum* and *Sinorhizobium meliloti* induce/derepress RubisCO only during autotrophic growth with H_2 or formate as free-living organisms [110,111].

The synthesis of RubisCO even appears to be regulated in the obligate autotrophic sulfur oxidizer *T. neapolitanus*. Similar to *R. eutropha*, CO_2 limitation leads to a significant increase of the enzyme [112]. Enhanced formation of special RubisCO-containing polyhedral inclusion bodies, the carboxysomes, accompanies the induction [113]. Carboxysomes are assumed to promote the efficiency of CO_2 fixation under low concentrations of inorganic carbon [114]. Carboxysomal genes are located directly downstream of the *cbbLS* genes in this organisms [62] (see Fig. 2). However, in the obligate autotroph *Thiomicrospira pelophila* CO_2 limitation does not stimulate RubisCO synthesis [112], whereas it does so in the facultative autotroph *Paracoccus versutus* [115]. The facultative autotroph *T. intermedius* harbors carboxysomes and exhibits high RubisCO activity during autotrophic ($S_2O_3^{2-}$ as energy source) and mixotrophic growth ($S_2O_3^{2-}$ plus glutamate), but heterotrophically grown cells lack both RubisCO and carboxysomes [116]. In another facultative autotroph, *Thiobacillus novellus*, which does not form carboxysomes, RubisCO synthesis also seems to be completely repressed in heterotrophic cells [117]. Among the nitrifiers only the facultative autotroph *Nitrobacter agilis* was reported to show repression of RubisCO in the presence of acetate [118].

3.2. Phototrophs

The regulation of synthesis of Calvin cycle enzymes in response to various inorganic and organic compounds used as electron donors and/or carbon sources is phenotypically similar in the phototrophic purple bacteria and in chemoautotrophs. This might be expected since a number of photosynthetic bacteria, particularly non-sulfur purple bacteria, are also able to grow aerobically in the dark as chemotrophs [95,119,120]. In the phototrophs the Calvin cycle is not only the major route of carbon assimilation under photoautotrophic conditions with H_2 or reduced sulfur compounds as electron sources, but must also operate when CO_2 has to serve as electron sink during photoheterotrophic growth to dissipate excess reducing equivalents generated by the oxidation of organic substrates [121,122]. Highly reduced compounds, such as propionate or butyrate, increase the requirement for compensatory CO_2 reduction relative to the utilization of the more oxidized malate or succinate. Accordingly, repression in *R. sphaeroides* [123], *R. rubrum* [124], *Rhodobacter blasticus* [125] and *Rhodopseudomonas acidophila* [126] during growth on the latter substrates reduces the level of RubisCO by at least one order of magnitude compared to growth on the more reduced compounds. Like in chemoautotrophs (see Section 3.1), limited supply of CO_2 causes a strong derepression of RubisCO in photoautotrophic cells of *R. rubrum* [124,127,128] and *R. sphaeroides* [123]. On the other hand, high CO_2 concentrations (5%) in the gas atmosphere repress the synthesis of both RubisCO and PRK in *R. sphaeroides* during photoheterotrophic growth on malate to a level substantially below that observed at low concentrations (0.05%) [129]. Exposure of phototrophically grown *R. sphaeroides* to aerobiosis or aerobic chemoheterotrophic growth of the organism completely represses RubisCO synthesis [130], suggesting the involvement of oxygen or the redox state of the cell in the regulation [131,132]. The need to maintain the redox balance thus plays an essential role in the control of CO_2 fixation during phototrophic growth of non-sulfur purple bacteria. This principle of regulation may also prevail in the sulfur purple bacterium *Chromatium vinosum*, which shows partial repression of RubisCO in cells growing photoheterotrophically with pyruvate. Again, CO_2 deprivation during photoautotrophic cultivation ($S_2O_3^{2-}$ as electron donor) of the organism leads to significant derepression of the enzyme beyond its normal autotrophic level [133].

In *R. sphaeroides* the *cbb* gene regulation is complicated by the presence of the two different *cbb* operons, cbb_I and cbb_{II}, which are independently controlled. Whereas form I RubisCO predominates in photoautotrophic cells, the synthesis of the form II enzyme is relatively favored during photoheterotrophic growth [123,134]. The presence of dimethyl sulfoxide as an alternative electron acceptor for CO_2

partially represses the synthesis of form II RubisCO [122]. This observation is in accordance with the notion that the form II is more important than the form I enzyme for keeping the redox poise in the cells [121,129]. RubisCO deletion mutants, which are incapable of photoautotrophic growth with H_2, rely on the presence of dimethyl sulfoxide for photoheterotrophic growth [121]. They do, however, grow photoautotrophically with reduced sulfur compounds ($S_2O_3^{2-}$ or S^{2-}), indicating a conditional operation of (an) alternative CO_2 assimilation pathway(s) in *R. sphaeroides* and *R. rubrum* [135]. Individual inactivation of the RubisCO genes in *R. sphaeroides* revealed that a functional lack of one *cbb* operon affects the expression of the other in a partially compensatory manner, suggesting a complex control exerted by a regulatory network [86,134]. In fact, an oxygen-sensory two-component signal transduction system, RegA (PrrA)-RegB (PrrB), has been discovered in *R. sphaeroides* [136–138] and *R. capsulatus* [139,140] that is part of a regulatory cascade controlling the synthesis of the photosystem [141]. The system appears to have a more global significance in the non-sulfur purple bacteria as it is also involved in the control of N_2 fixation and CO_2 assimilation [132,142]. It is required for the positive regulation of both *cbb* operons as well as for expression of the genetic information encoding the putative alternative CO_2 assimilation pathway(s) in *R. sphaeroides* [142].

Transcription of the *cbb* operons in *R. sphaeroides* yields large transcripts, which are apparently relatively unstable. Much smaller segments representing different genes or gene groups of the operons are far more abundant in the cells [87,129,131]. In analogy to the situation prevailing in the chemotrophs *R. eutropha* and *X. flavus* (see Section 3.1), posttranscriptional processing of the primary transcript is likely to play a role in the generation of differential *cbb* mRNA abundance. Stable RubisCO-specific transcripts were also observed in *R. rubrum* [128] and *C. vinosum* [133].

4. Function of activator CbbR

The *cbbR* gene, which was detected in most of the organisms mentioned above (see Figs. 2 and 3), encodes an activator protein assigned to the LysR family of transcriptional regulators [143,144]. Mutations in *cbbR* of *R. eutropha* [23] and *X. flavus* [145] inactivated expression of the *cbb* operons in these organisms. A corresponding mutant of *R. sphaeroides* showed that a functional *cbbR* is absolutely required for expression of the form I *cbb* operon while that of the form II operon is only partially dependent on the product of this gene [67]. It has been speculated that the *cbbR* gene product of *C. vinosum* may function as both activator and repressor of its cognate *cbbLS* operon, depending on the availability of the effector molecule [77]. The *cbbR* genes known so far are located in divergent orientation immediately upstream of (one of) the regulated *cbb* operons. The spacing between *cbbR* and these operons varies from 91 bp in *R. sphaeroides* [67] to 243 bp in *N. vulgaris* [50]. The resulting intercistronic region is presumed to contain the promoters of the *cbb* operon and of *cbbR* as well as the binding site of CbbR (operator) and is thus referred to as *cbb* control region.

The purified native CbbR from *R. eutropha* is a dimeric protein, although a tetramer has been observed at low temperature (4°C) [146]. A determination of the stoichiometry of CbbR binding will be required to ascertain the quaternary structure of the binding protein species. Like all other LysR-type regulators, the CbbR protein is characterized by an NH_2-terminal DNA-binding domain containing a helix-turn-helix structural motif. These regulators bind different, low-molecular-mass effectors, which act as coregulators by inducing conformational changes in the proteins. The central domain of the various regulators is thought to be involved in effector binding and to be structurally less conserved than the DNA-binding domain [144]. In contrast to this general pattern, the central domains of the CbbR proteins are remarkably conserved, and it is, therefore, tempting to speculate that these proteins interact with similar, if not the same, effectors (signal metabolites) in the different organisms. Effectors of CbbR have not been recognized so far.

Usually LysR-type regulators bind to promoter regions at a partially symmetric sequence containing the T-N_{11}-A motif typically centered about 65 bp upstream of the transcription start site of the regulated operon [147]. Binding of CbbR to the *cbb* control region has been demonstrated in vitro for

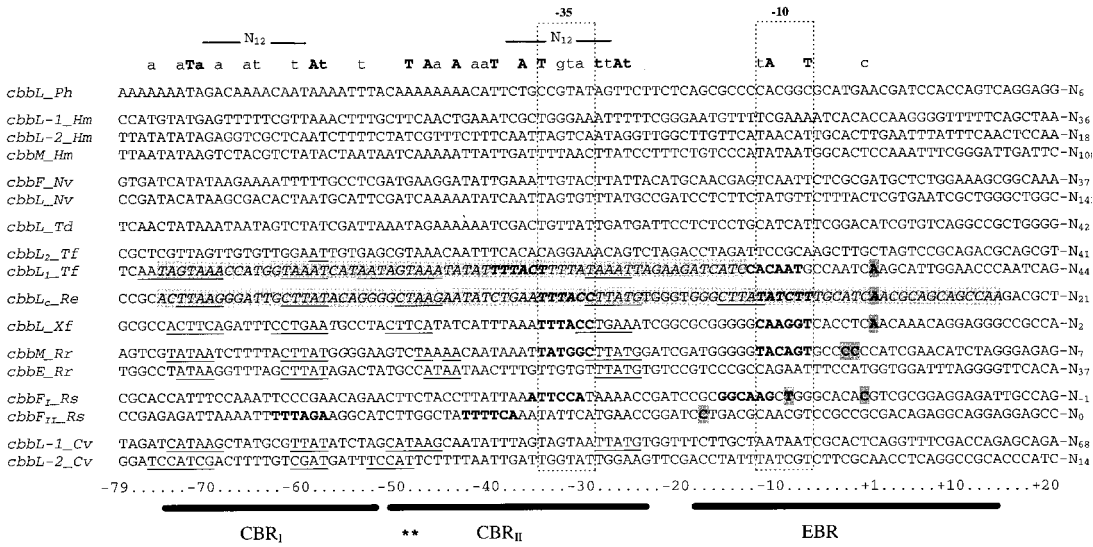

Fig. 4. Multiple alignment of identified and presumed control regions of *cbb* operons from various autotrophic bacteria. Sequences were aligned using the program MACAW, version 2.05 [148], and are labelled by the 5′-terminal gene in the respective operon together with the initials of the bacterial name (see Figs. 2 and 3). The subscripts of N at the right ends indicate the number of intervening bases in front of the 5′ end of the operon. The numbering of bases refers to the positions of the verified transcriptional start sites (+1) of the *T. ferrooxidans*, *R. eutropha* and *X. flavus* operons. Verified start sites and corresponding −10/−35 regions of σ^{70}-type promoters are printed in bold typeface, start sites are additionally highlighted by shading. The CbbR-binding regions (operator) of *T. ferrooxidans* and *R. eutropha* are printed in italic typeface and are also shaded. Subsites CBR_I, CBR_{II} and EBR of the *cbb* control region of *R. eutropha* (see text) are indicated by the bold lines below the aligned sequences. Sequence motifs forming (incomplete) direct or inverted repeats are underlined. Asterisks mark a proposed center of CbbR-induced DNA bending in the *R. eutropha* operator. Dashed boxes surround −10/−35 regions of putative promoters as derived from the alignment. Conserved bases are shown above the alignment: n, base frequency 50–59%; **n**, frequency 60–69%; N, frequency 70% or higher in the respective position. Putative CbbR-binding motifs ($T\text{-}N_{12}\text{-}A$) located within the most conserved region are also indicated. Abbreviations of bacteria names: Cv, *C. vinosum*; Hm, *H. marinus*; Nv, *N. vulgaris*; Ph, *P. hydrogenothermophila*; Re, *R. eutropha*; Rr, *R. rubrum*; Rs, *R. sphaeroides*; Td, *T. denitrificans*; Tf, *T. ferrooxidans*; Xf, *X. flavus*.

R. eutropha [23,146], *T. ferrooxidans* [57] and *X. flavus* [145]. In *T. ferrooxidans* CbbR binds within a segment encompassing positions −76 to −14 relative to the transcriptional start of the *cbb* operon (Fig. 4). The corresponding segment in *R. eutropha* is very similarly located between positions −76 and −27 and was designated as central binding region (CBR). At high concentrations of CbbR it even extended to position +13, forming an extended binding region (EBR). Promoter activities and binding abilities associated with subfragments of the control region indicated the presence of two independent CbbR-binding subsites (CBR_I and CBR_{II}) in *R. eutropha* that are occupied in a cooperative manner [146]. A differential occupation of the two sites was not observed, although operators of several other systems controlled by LysR-type proteins do exhibit differential occupation of subsites in response to the concentration of the regulator and/or effector [144]. Interestingly, the binding of CbbR in *X. flavus* might correspond to the latter type [145].

The relative locations of the CBR in *R. eutropha* and of the corresponding region in *T. ferrooxidans* agree very well with those found for other regulatory systems of the LysR type [144]. An overlap of the operator with the −35 region of the regulated promoter is presumed to enable contacts between the regulatory protein and the α-subunits of RNA polymerase that are essential in the activation process [149]. Insertions of one or two bp introduced upstream of the −35 region between CBR_I and CBR_{II} of *R. eutropha* inactivated the *cbb* operon promoter, probably by altering the phasing and positions of the subsites relative to each other and to

the promoter. While CbbR binding itself was not influenced by the insertions, the essential activator-polymerase contacts were possibly disturbed. A hypersensitive site (Fig. 4) observed in a DNase I footprint suggested that binding of CbbR introduces a DNA bend within the CBR [146]. DNA bending within transcriptional control regions including those dependent on LysR-type regulators appears to be of functional importance in many systems [150]. Studies on the binding of CbbR from phototrophic bacteria were not reported.

4.1. Regulation of cbbR

The narrow spacing and divergent orientation of *cbbR* genes and their cognate *cbb* operons place the promoters of both transcriptional units as well as the operator in the intercistronic *cbb* control region (see Section 4). Such compact arrangement of transcriptional signal sequences has important regulatory implications for the systems, especially for their *cbbR* promoters. In *R. eutropha* H16 *cbbR* has been shown to be transcribed as a 1.4-kb mRNA at low level from two distinct, σ^{70}-type promoters that are active under different growth conditions. Promoter P_{Ra}, which is functional in autotrophic cells, partially overlaps the *cbb* operon promoter, P_L, and is completely contained within the CBR. A second promoter, P_{Rp}, used during heterotrophic growth, was detected further upstream within the region transcribed from P_L [26,146]. This might be the reason for its inactivation in autotrophic cells. Although interference of CbbR binding to the operator with transcription from both *cbbR* promoters would not be surprising, the observed promoter activity in autotrophic cells is even significantly higher than in heterotrophic cells. Thus, activation of P_L simultaneously stimulates P_{Ra}. As removal of the CBR_I operator subsite strongly derepresses the promoter activity, the low-level transcription of *cbbR* appears to result from negative autoregulation of the gene [146]. This mode of control as a consequence of an overlapping operator-promoter arrangement is typical of LysR-type transcriptional regulators [144]. However, since complete deletion of the *cbbR* gene did not relieve the repression of P_R activity in *R. eutropha* (N.-H. Gropp and B. Bowien, unpublished results), it is tempting to postulate the existence of a second protein, which also binds to the *cbb* control region and acts in concert with CbbR to regulate the operon. Data on the regulation of *cbbR* genes in other autotrophs were not reported yet, but the conserved arrangement of *cbbR* genes and their cognate *cbb* operons suggest a mode of regulation similar to that observed in *R. eutropha*.

4.2. The cbb control region

Since CbbR-directed activation appears to be a common principle in the regulation of *cbb* operons, it is reasonable to assume a conservation of operator and promoter structures with respect to both their sequences and relative positions within the *cbb* control regions in different organisms. In search for such conserved structures the available upstream sequences of *cbb* operons from chemo- and photoautotrophs were subjected to an alignment analysis, which also included *P. hydrogenophila* and *H. marinus*, although the presence of a *cbbR* gene has not yet been demonstrated in these organisms.

The comparison revealed that the control regions from *R. eutropha* [26], *T. ferrooxidans* (*cbbLS-1* operon) [57] and *X. flavus* [47] exhibit the highest resemblance among the analyzed sequences (Fig. 4). Similarities are particularly evident between positions −30 and −50 and positions −60 and −73 relative to the known transcriptional start sites of the corresponding *cbb* operons as well as around the start sites themselves. However, the assigned or presumed −10 regions of the σ^{70}-type promoters are less conserved than the strikingly similar −35 regions. Site-directed mutagenesis has confirmed the locations of the −10/−35 regions of the *cbb* operon promoter from *R. eutropha* (C. Kaiser, N.-H. Gropp and B. Bowien, unpublished results). The upstream regions of both operons from *R. rubrum* [74] fit well into the overall alignment with a remarkably high similarity between the two sequences. The transcriptional start site of the *cbbM* monocistronic operon, as determined for photolithoautotrophically grown cells [128], is located 2 or 3 bp closer to the proposed −10 region than those in the other promoters. A significant resemblance between the proposed two *cbb* control regions of *R. sphaeroides* is also apparent. In contrast to the form I region, for which the localized transcriptional start site and the presumed

promoter conform well to the alignment [151], the positions of these sequence elements are found further upstream in the form II region [69]. Both regions from *R. sphaeroides* show a relatively low overall similarity to the other sequences (Fig. 4).

Transcriptional start sites for the *cbb* operons of the other organisms remain to be determined. Nevertheless, the alignment reveals potential CbbR-binding and promoter sites of conspicuous though somewhat varying similarity upstream of position −25 in the different sequences (Fig. 4). The presence of two subsites for CbbR binding within the CBR of *R. eutropha* allows a hypothetical assignment of functions to the conserved sequence motifs. While the partial inverted repeats around positions −72 and −60 may represent one binding site corresponding to CBR_I, the second site, equivalent to CBR_{II}, may be formed by the inverted repeats around positions −40 and −27. Mutations within the latter site of *T. ferrooxidans* decreased CbbR binding to the control region [57], supporting this assumption. Interestingly, one segment of the partial inverted repeat is also present in the EBR of *R. eutropha* that is occupied in vitro only at high CbbR-operator ratios. This structural element could explain binding of CbbR in the EBR, but its function in vivo is unclear.

Although T-N_{11}-A motifs thought to be characteristic of operators recognized by LysR-type regulators [147] are found in some of the proposed *cbb* control regions [69,74], a highly conserved sequence segment located around position −65 is absent. Instead, the alignment revealed a very similar motif, T-N_{12}-A, present in both potential CbbR-binding sites (Fig. 4). The downstream part in both of these motifs contains a short conserved sequence, CTTATG in *R. eutropha*, as incomplete direct repeats centered at positions −60 and −28. In some cases the conserved bases at position −60 also form an incomplete inverted repeat with those around position −72. Like in other gene systems controlled by LysR-type regulators [144], an A-rich segment separates the two binding sites that contains the suggested center of CbbR-induced bending of the *cbb* control region from *R. eutropha* [146].

A certain degree of conservation within the various *cbb* control regions can be deduced from the sequence alignment data, but evidence for the functional significance of presumed operator-promoter elements must await experimental analysis in most cases. One source of uncertainty in the reliable alignment of the sequences originates from the relatively high A+T content of these regions [23,57,77]. Another difficulty in interpreting the data arises from observations that the dependency of *cbb* gene activation by CbbR varies with different systems and may be influenced by (an) additional protein factor(s) [69,132,142].

5. Conclusions and perspectives

Although assimilation of CO_2 is the main function of the Calvin cycle during autotrophic growth of both chemo- and phototrophic bacteria, this capacity also makes the cycle an important electron sink during photoheterotrophic growth of phototrophs [122]. Under such conditions in the absence of electron acceptors other than CO_2, these organisms have no alternative means to dissipate excess reducing power generated by the oxidation of organic substrates. Chemotrophs, on the other hand, obligately depend on respiration for energy generation during autotrophic growth. Only under certain nutritional conditions may these bacteria require the electron-dissipating function of the cycle. The cycle thus has a broader physiological significance in phototrophs. The presence of RubisCO and/or PRK in non-autotrophic bacteria and archaea, which have not been shown yet to assimilate CO_2 via the Calvin cycle, is perhaps related to this special metabolic role of the cycle.

Recent studies on the molecular biology of CO_2 assimilation have substantially widened the knowledge about organization and regulation of *cbb* genes in chemo- and phototrophic bacteria. The genes were found to be clustered in many of the organisms, forming operons of varying size and internal organization. However, some organizational patterns are conserved, raising questions about a possible common origin of *cbb* operons/genes and their recruitment from the existing gene pool of a cell. Horizontal transfer may have played a major role in spreading *cbb* genes among different bacteria. The ever growing sources of sequence data will enable more comprehensive phylogenetic analyses with *cbb* genes and corresponding genes related to the central heterotrophic carbon metabolism to be performed in

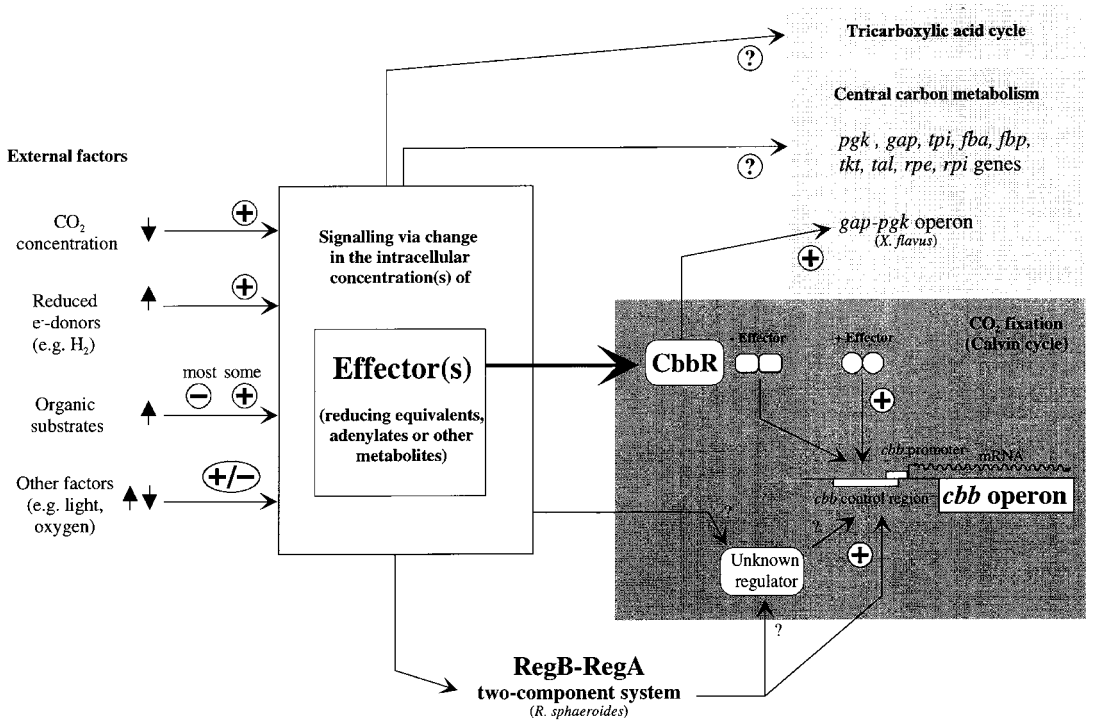

Fig. 5. Hypothetical regulatory network controlling the expression of *cbb* genes and genes of metabolic pathways related to the Calvin cycle in autotrophic bacteria. Pathways of signal transduction are shown by arrows with questions marks indicating uncertain relationships. Encircled signs describe the effect of an signal on the regulated system. The small arrows behind the external factors symbolize relative changes in concentration of these factors to cause signalling. Genes and their products: *fba*, FBA; *fbp*, FBP; *gap*, GAP; *pgk*, PGK; *rpe*, PPE; *rpi*, PPI; *tal*, transaldolase; *tkt*, TKT; *tpi*, TPI.

pursuit of these questions. Isoenzymes operating in either the Calvin cycle or the heterotrophic carbon metabolism are possibly part of the usual enzyme inventory of facultative autotrophs. Whether isoenzymes also exist in this metabolic segment of obligate autotrophs is an open matter. Future research on the genetics of the cycle should include Gram-positive bacteria, since so far only Gram-negative proteobacteria have been studied in this respect.

Two common regulatory features are noticed for *cbb* operons in chemo- and phototrophic bacteria, the availability of CO_2 and reducing power to the cells. Limited supply of CO_2 appears to generally cause derepression/induction of the operons, even in obligate autotrophs. In the presence of sufficient electron donor, lack of CO_2 will presumably lead to a relative surplus of reducing equivalents (NADH/NADPH) and energy (ATP) and to a drop in the concentration of central carbon metabolites. Based on this rationale, the redox state and/or the energy charge of the cells, as integrative parameters, are the most plausible sources of (a) metabolic signal(s) transduced to the *cbb* genes within a regulatory network (Fig. 5).

The nature of these signals as well as their transduction pathways are unknown. A direct action of external factors as inducers of *cbb* gene expression seems unlikely. The occurrence of heterotrophic derepression of *cbb* operons in some facultative chemoautotrophs is not easily reconciled with the redox/energy state of the cells as potential controlling parameters. However, an alteration in intracellular concentration of a metabolite may produce the required signal. Since the Calvin cycle is a biosynthetic pathway end-product repression would be a possible mechanism to explain the regulation on a physiological basis. Phosphoenolpyruvate and acetyl-coenzyme A, which are indirect end-products of the cycle,

have been proposed as potential signal metabolites in *R. eutropha* [98] and *P. oxalaticus* [152], respectively, but evidence for these proposals is lacking. The different responses in *cbb* gene regulation with respect to organic growth substrates suggest the presence of integrative signalling pathways and/or more than one regulator involved in the control of *cbb* operons in the various bacteria. Regardless of the type of signal, the information is eventually communicated to the CbbR activator protein that undoubtedly is an immediate regulator of *cbb* operons (Fig. 5).

Additional regulators are assumed to function either indirectly or directly in concert with CbbR to affect activation/repression of cbb operons. The action of CbbR (and possibly such regulators) may not be limited to *cbb* operons sensu stricto as indicated by the involvement of the activator in the regulation of the *gap-pgk* operon in *X. flavus* [49]. It is conceivable that the control of other genes of central carbon metabolism is also integrated into the presumed regulatory network (Fig. 5). The RegB-RegA two-component regulatory system, identified in *R. capsulatus* and *R. sphaeroides*, is part of the network in the latter [142]. Functionally equivalent systems are perhaps present in other photo- and chemoautotrophs. In order to avoid futile respiratory loss of fixed carbon, transition from heterotrophic to autotrophic growth in facultative autotrophs is expected to be accompanied by a reduction of the oxidative capacity of the tricarboxylic acid cycle. Future studies should thus address a potential inclusion of genes related to this cycle in the control of autotrophic carbon metabolism. There probably is a number of still unknown regulatory and enzymic functions, which are related to CO_2 assimilation. The product of the *aut* gene from *R. eutropha* that is essential for autotrophic growth of the organism [153] is merely an indication of the potential diversity of components integrated into the autotrophic system.

Our current knowledge suggests the existence of both common principles and differences in *cbb* gene regulation between chemo- and photoautotrophs. Moreover, differences in regulatory details are likely to be present within these groups of organisms. Continuing research efforts will be necessary to elucidate signals, signal transducers and other components participating in the intriguing genetic control of CO_2 assimilation in autotrophic bacteria.

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