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Mini-review

Was photosynthetic RuBisCO recruited by acquisitive evolution from RuBisCO-like proteins involved in sulfur metabolism?

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Abstract

Genome analyses have revealed that the genomes of non-photosynthetic bacteria including *Bacillus subtilis* code for proteins similar to the large subunit of RuBisCO (called RuBisCO-like protein (RLP)). This raises a fundamental question as to their functional relationship to photosynthetic RuBisCO. Recently, we identified the RLP of *B. subtilis* as the 2,3-diketo-5-methylthiopentyl-1-phosphate enolase in the methionine salvage pathway. In this mini-review, we suggest functional and evolutionary links between *B. subtilis* RLP and photosynthetic RuBisCO. Furthermore, we propose that photosynthetic RuBisCOs evolved from RLPs similar to that found in *B. subtilis*.
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1. Introduction

Photosynthetic organisms produce ATP and NAD(P)H as chemical energy using sunlight, and fix CO_2 into an organic compound using this chemical energy in the Calvin cycle. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the key enzyme in this pathway. RuBisCO catalyzes the carboxylase reaction that fixes CO_2 in the substrate, ribulose-1,5-bisphosphate (RuBP) and produces two molecules of 3-phosphoglycerate [2,18]. The carboxylase reaction is the starting reaction in the Calvin cycle and fixed CO_2 is utilized as the carbon source for growth of photosynthetic organisms. Despite its obvious importance for creating biomass, RuBisCO has relatively inefficient kinetic properties. Two reasons account for this unexpected behavior. First, RuBisCO catalyzes a parasitic oxygenase reaction that

fixes O_2 into RuBP [2,18]. This oxygenase reaction antagonizes the carboxylase reaction in atmospheres in which O_2 is much more abundant than CO_2. The oxygenase reaction is the starting reaction of the so-called photorespiration process which releases CO_2 and wastes energy. Second, RuBisCO is a very slow catalyst whose turnover number is a few times per second, even when substrate is saturating [2,18]. As a consequence, the photosynthetic CO_2 assimilation rate is limited by RuBisCO in plants [20,36]. To overcome these disadvantages, plants are obliged to invest a large amount of nitrogen to synthesize RuBisCO, which accounts for about 50% of all proteins in leaves of plants [18]. RuBisCO is therefore the most abundant naturally occurring protein [7]. These disadvantages may reflect the constraints of RuBisCO molecular evolution when the Calvin cycle appeared.

Recently, genome analyses have revealed that non-photosynthetic organisms without the Calvin cycle, such as non-photosynthetic bacteria (including *Bacillus subtilis*) as well as Archaea possess genes with sequence similarity to the large subunit of RuBisCO [17]. A phylogenetic tree was proposed using the predicted amino acid sequences of the large subunit of RuBisCO and these homologues. In this tree the

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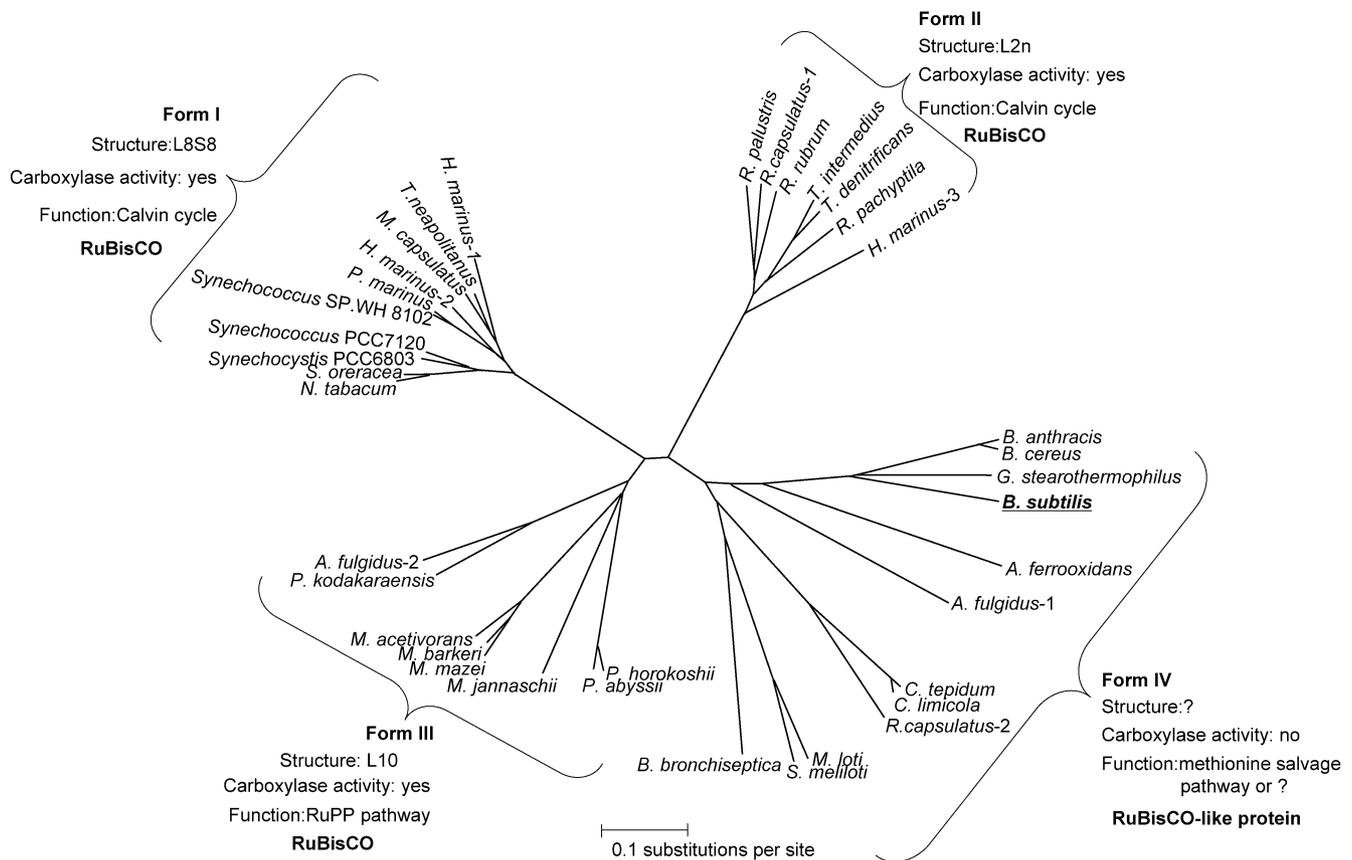
Fig. 1. Phylogenetic tree constructed from the deduced amino acid sequences of the large subunits of RuBisCO (forms I and II) and RLPs (forms III and IV) from various organisms. The multiple sequence alignment and the tree were produced with CLUSTALW and TREE VIEW Ver. 1.5.3. Full names of species are as follows: *S. oleracea, Spinacia oleracea; N. tabacum, Nicotiana tabacum; P. marinus, Prochlorococcus marinus; H. marinus, Hydrogenovibrio marinus; M. capsulatus, Methylococcus capsulatus; T. neapolitanus, Thiobacillus neapolitanus; R. capsulatus, Rhodobacter capsulatus; R. capsulatus, Rhodobacter capsulatus; R. rubrum, Rhodospirillum rubrum; T. intermedius, Thiobacillus intermedius; T. denitrificans, Thiobacillus denitrificans; R. pachyptila* symbiont, *Riftia pachyptila* endosymbiont bacterium; *M. jannaschii, Methanococcus jannaschii; P. horikoshii, Pyrococcus horikoshii; P. kodakaraensis, Pyrococcus kodakaraensis; M. acetivorans, Methanosarcina acetivorans; M. barkeri, Methanosarcina barkeri, M. mazei, Methanosarcina mazei; P. abyssii, Pyrococcus abyssii; A. fulgidus, Archaeoglobus fulgidus; B. anthracis, Bacillus anthracis; B. cereus, Bacillus cereus; B. subtilis, Bacillus subtilis; G. stearothermophillus, Geobacillus stearothermophillus; A. vinosum, Allochromatium vinosum; C. limicola, Chlorobium limicola; C. tepidum, Chlorobium tepidum; M. loti, Mesorhizobium loti; S. meliloti, Sinorhizobium meliloti* and *B. bronchiseptica, Bordetella bronchiseptica. H. marinus, R. capsulatus* and *A. fulgidus* possess a few genes for the large subunit of RuBisCO and/or RLP. L and S refer to large and small subunits, respectively.

proteins are classified into four forms (Fig. 1) [17]. Form I consists of eight large and eight small subunits of 50–55 and 12–18 kDa, respectively. It is distributed widely among photosynthetic organisms such as higher plants, eukaryotic algae, cyanobacteria and photosynthetic and chemoautotrophic proteobacteria [35]. Form II is composed only of the large subunits. It is found in some photosynthetic proteobacteria, chemoautotrophic bacteria and marine eukaryotic dinoflagellates [35]. Form III is composed of only large subunits. It is found in Archaea. Each one of these three forms possesses all the amino acid residues known to participate in catalysis by RuBisCO and catalyzes both carboxylation and oxygenation of RuBP [8,9,35]. The existence of form III with the catalyzing ability of RuBisCO has been an enigma, because Archaea lack the gene for phosphoribulokinase which catalyzes the formation of RuBP from ribulose-5-phosphate (Ru5P) [37]. Recently, Finn and Tabita reported that RuBP was not synthesized from Ru5P but from 5-phospho-D-ribose-1-pyrophosphate (RuPP) in

the methanogenic Archaeon *Methanoccocus jannaschii* [10] and this form III should function as RuBisCO in the RuPP pathway. Therefore, we should classify the Archaean RuBisCO homologue in form III as RuBisCO. Interestingly, the X-ray structure of the Archaea form III RuBisCO of *Pyrococcus kodakaraensis* has been solved and it was reported that this RLP was a decamer composed only of large subunits, with a pentagonal ring-like structure [21]. Form IV was found in non-photosynthetic bacteria including *B. subtilis*, other photosynthetic bacteria without the Calvin cycle and Archaea. Form IV probably participates in a metabolic pathway other than the Calvin cycle, because form IV catalyzes neither carboxylase nor oxygenase reactions using RuBP [3]. Therefore, RuBisCO homologues in form IV are called RuBisCO-like protein (RLP) [3]. A phylogenetic kinship between RLP and RuBisCO is very interesting because some of the organisms possessing genes for RLP are expected to have emerged before evolutionary completion of the Calvin cycle [14,15].
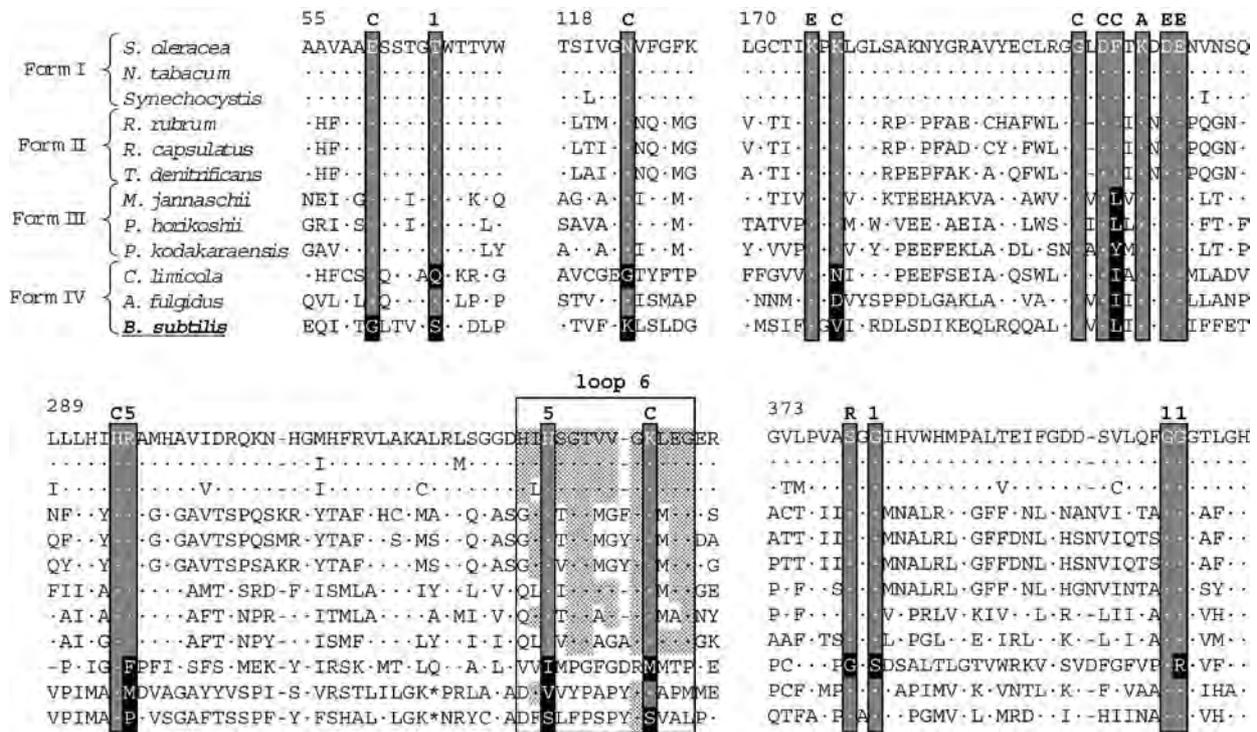
Fig. 2. Partial multiple sequence alignment of the large subunits of RuBisCO (forms I, II and III) and RLPs (form IV) (adapted from Hanson and Tabita [17]). Only sequences around the residues involved in the RuBisCO reaction are aligned. Active-site residues are white letters and are highlighted in gray. C above the alignment indicates residues involved in catalysis; in particular, E shows residues for enolization of RuBP. 1 and 5 above the alignment indicate residues involved in binding of the phosphate groups on C1 and C5 of RuBP, respectively. The residue showing R binds to RuBP but not to phosphate. White letters in black boxes indicate active-site residues that have been replaced by other amino acids. Conserved residues in loop 6 are highlighted in light gray. The alignment is numbered according to the sequence of the large subunit of RuBisCO from spinach.

Recently, we demonstrated that *B. subtilis* RLP catalyzed the 2,3-diketo-5-methylthiopentyl-1-phosphate enolase reaction as the fourth step in the methionine salvage pathway, a part of sulfur metabolism [3]. This was the first report on the biochemical function of RLP. In this mini-review, we further explore possible functional and evolutional links between RLP and photosynthetic RuBisCO.

2. *B. subtilis* RLP in the phylogenetic tree of photosynthetic RuBisCO and RLPs

RLPs of non-photosynthetic Bacteria, photosynthetic Bacteria and one Archaeon are classified in form IV in a phylogenetic tree produced using genome-predicted amino acid sequences (Fig. 1). *B. subtilis* RLP belongs to form IV along with counterparts in the same genus *Bacillus*, *Bacillus cereus* and *Bacillus anthracis*, the green sulfur Bacteria *Chlorobium tepidum* and *Chlorobium limicola*, the sulfate-reducing Archaeon *Archaeoglobus fulgidus* and the root nodule Bacteria *Mesorhizobium loti* and *Sinorhizobium meliloti* (Fig. 1). In contrast, Archaean RuBisCO are mostly form III (Fig. 1). Although RLPs are found in non-photosynthetic Bacteria, photosynthetic Bacteria and one Archaeon, we do not have a straightforward physiological or environmental connection accounting for the presence of

RLP in these microorganisms. The predicted amino acid sequence of *B. subtilis* RLP shows about 23% identity to form I and II RuBisCO, and shows about 30% identity to form III RuBisCO. In the clade of form IV, *B. subtilis* RLP shows 34 and 25% identity to the RLPs of *A. fuldidus* and *C. limicola*, respectively.

The essential 19 amino acid residues in RuBisCO required for carboxylase and oxygenase reactions have been revealed by analysis of point mutation and X-ray structures [17]. In *B. subtilis* RLP, 8 of these amino acid residues are substituted by different amino acids (Fig. 2). In RLP of *C. limicola* and *A. fuldidus* of the same clade, 10 and 4 amino acid residues are substituted, respectively. In contrast, only one amino acid residue is substituted in Archaea form III RuBisCO (Fig. 2). Form III also conserves a loop domain called loop 6 which is essential for catalysis [1,18], but amino acid residues in loop 6 of form IV are substituted by other amino acid groups (Fig. 2). This makes it easy to explain why form III can function as RuBisCO but RLP cannot.

3. The function of *B. subtilis* RLP in the methionine salvage pathway

The *mtnW* gene for the RLP of *B. subtilis* is the first gene in the *mtnWXBD* operon, which is close to the *mtnKA*
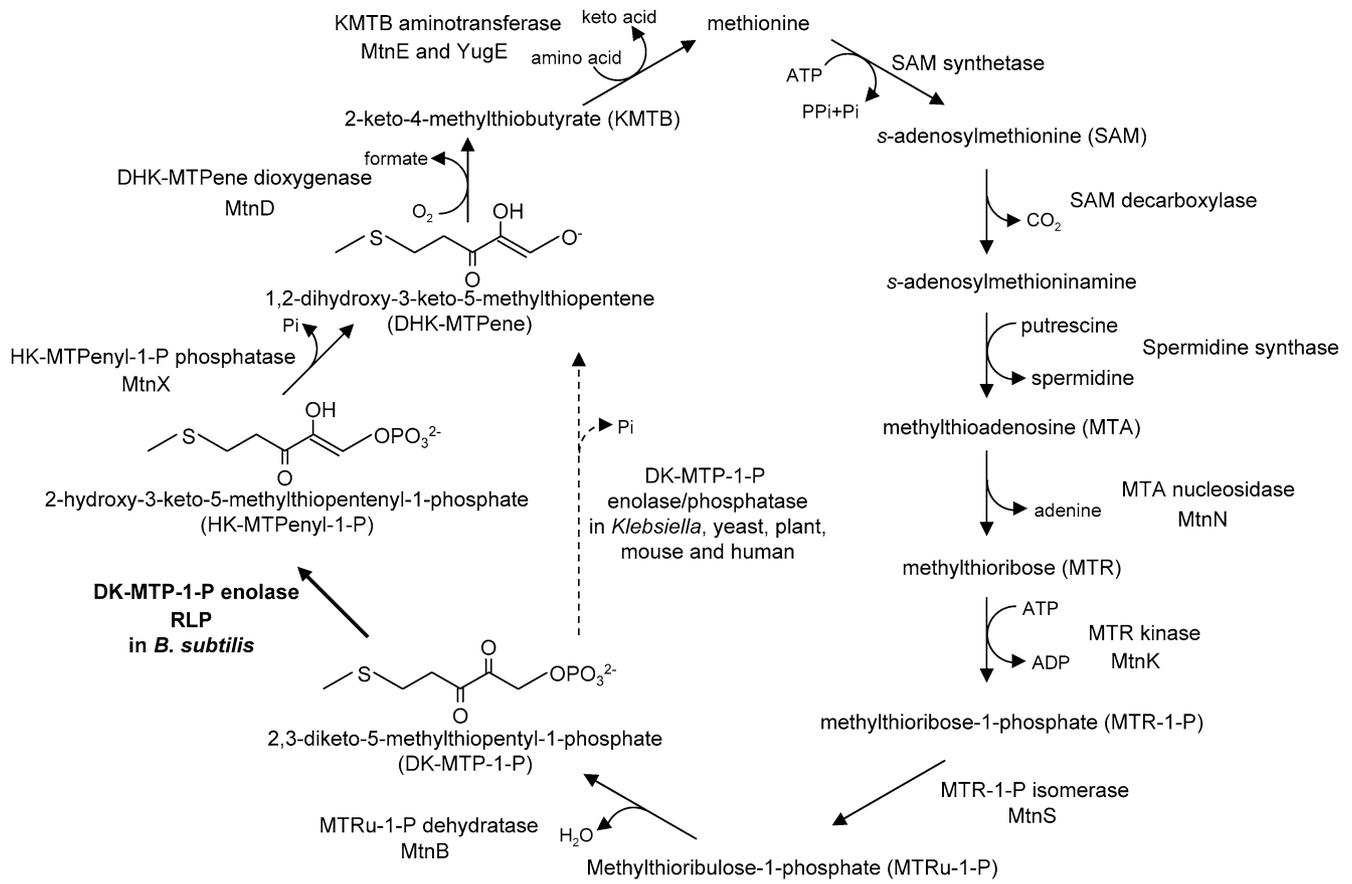
Fig. 3. The methionine salvage pathway in *B. subtilis*. The reaction step catalyzed by the enolase/phosphatase in *Klebsiella* and eukaryotic organisms is shown by a broken line.

operon [32]. These operons have S-box riboswitches that regulate the expression of the genes involved in sulfur metabolism in *B. subtilis* [27]. In these operons, MtnD is highly homologous to the 1,2-dihydroxy-3-keto-5-methyl-thiopentene dioxygenase and MtnK was identified as the methylthioribose kinase [34] which suggested that both operons were functioning in the methionine salvage pathway (Fig. 3) [13,28,31]. Therefore, we predicted that *B. subtilis* RLP would catalyze a reaction step somewhere in this pathway [3]. Some bacteria [13,33], yeasts [25], plants [22], rats [39] and humans [12] utilize the methionine salvage pathway. In this pathway, organic sulfur is salvaged from methylthioribose (MTR), which is derived from the methylthioadenosine (MTA) that is a byproduct of the synthesis of spermidine [13,31]. In the pathway that has been proposed in *Klebsiella pneumoniae*, MTR is phosphorylated to MTR-1-phosphate (MTR-1-P) by a kinase, and then MTR-1-P is isomerized by an isomerase to methylthioribulose-1-phosphate (MTRu-1-P) [11]. MTRu-1-P then undergoes a dehydration reaction that is catalyzed by a dehydratase and yields 2,3-diketo-5-methyl-thiopentyl-1-phosphate (DK-MTP-1-P) [11]. DK-MTP-1-P is converted to 1,2-dihydroxy-3-keto-5-methylthiopentene (DHK-MTPene) via the intermediate, 2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate (HK-MTPenyl-1-P), by

a bifunctional enolase/phosphatase [4,29]. Finally, DHK-MTPene is converted to formate and 2-keto-4-methyl-thiobutyrate (KMTB) by a dioxygenase [38,39] and KMTB is transaminated to methionine [5]. This pathway regenerates reduced sulfur and metabolically links it to polyamine biosynthesis, but details of the physiological roles of this pathway remain obscure. Each step of this pathway has been predicted in *Klebsiella* sp. by analysis of metabolic intermediates but two enzymes, MTR-1-P isomerase and MTRu-1-P dehydratase, were unknown. Moreover, each reaction step in this pathway was uncharacterized in *B. subtilis*, except for that catalyzed by MTA nucleosidase, MTR kinase and KMTB aminotransferase encoded by *mtnN* [30,32], *mtnK* [34] and *mtnE* [5], respectively.

In order to identify the reaction catalyzed by RLP, we analyzed the functions of proteins encoded in the *mtnWXBD* and *mtnKS* operons using recombinant proteins purified from *Escherichia coli*. We used ^{1}H NMR and UV–vis spectroscopy measurements to do this [3]. In summary of these experiments, we identified *B. subtilis* RLP as the DK-MTP-1-P enolase which catalyzes enolization from DK-MTP-1-P to HK-MTPenyl-1-P and the other four enzymes in the methionine salvage pathway were identified: MtnX, MtnB, MtnD and MtnS are HK-MTPenyl-1-P phosphatase, MTRu-

1-P dehydratase, DHK-MTPene dioxygenase, MTR-1-P isomerase, respectively (Fig. 3) [3].

The function of form IV RLP is still unclear, except in *B. subtilis*. However, *B. anthracis* and *B. cereus* possess operons homologous to *mtnWXBD* (ban-3242-3245 for *B. anthracis* in: AnthraList: http://bioinfo.hku.hk/GenoList/index.pl?database=anthralist and *ykrW* to *Z* for *B. cereus* in CERELIST: http://bioinfo.hku.hk/GenoList/index.pl?database=cerelist) and *mtnKS* (ban-3239-3240 for *B. anthracis* in AnthraList and *ykrTS* for *B. cereus* in CERELIST) in *B. subtilis*. The ordering of each gene, including the RLP gene in the operons, is also the same as that of *B. subtilis*. This fact suggests strongly that RLP functions as a DK-MTP-1-P enolase in the methionine salvage pathway in these *Bacillus* species. It was reported that form IV RLP of *C. limicola* is involved in the response to oxidative stress and also in sulfur metabolism [17]. The fact that *C. limicola* RLP is involved in sulfur metabolism and oxidative stress is very interesting, because *C. limicola* RLP may catalyze the same reaction as that of *B. subtilis* RLP in the methionine salvage pathway. In addition, this RLP may be involved in response to oxidative stress through methionine salvage pathway-linked polyamine synthesis, because polyamines function as antioxidants [6].

4. Functional link between the *B. subtilis* RLP and photosynthetic RuBisCO

By comparing the reaction of *B. subtilis* RLP to that of photosynthetic RuBisCO, we were able to make interesting conjectures. *B. subtilis* RLP functions in sulfur metabolism, which is very distinct from photosynthetic carbon metabolism. However, when a detailed investigation of the catalytic reaction is undertaken, RLP has common enzymatic features with photosynthetic RuBisCO. The structure of DK-MTP-1-P as a substrate of *B. subtilis* RLP is very similar to that of RuBP [3], except that in DK-MTP-1-P, the OH-group and proton on C3 of RuBP are replaced by a carbonyl group (Fig. 4). Also, in DK-MTP-1-P, the OH-group on C4 and the phosphate group on C5 of RuBP are replaced by a proton and a methylthio group, respectively (Fig. 4). The reaction catalyzed by RuBisCO consists of three sequential, partial reactions: enolization of C2–C3 of RuBP, carboxylation or oxygenation on attack by CO_2 or O_2 on C2 of the enediol, and hydrolysis [2,18]. The first catalyzing step, enolization, is the same catalyzing reaction as that of RLP (Fig. 4). Thus, RLP catalyzes the same reaction as RuBisCO for a substrate whose structure is similar to that of RuBP. As mentioned above, *B. subtilis* RLP has conserved 11 amino acid residues among the 19 amino acid residues essential for catalysis by RuBisCO (Fig. 2). Among these 11 amino acid residues, *B. subtilis* RLP retains amino acid residues for binding the phosphate group on C1 of RuBP but, as expected, not for the phosphate group on C5 or for loop 6 (Fig. 2) [3]. Substitutions of amino acid
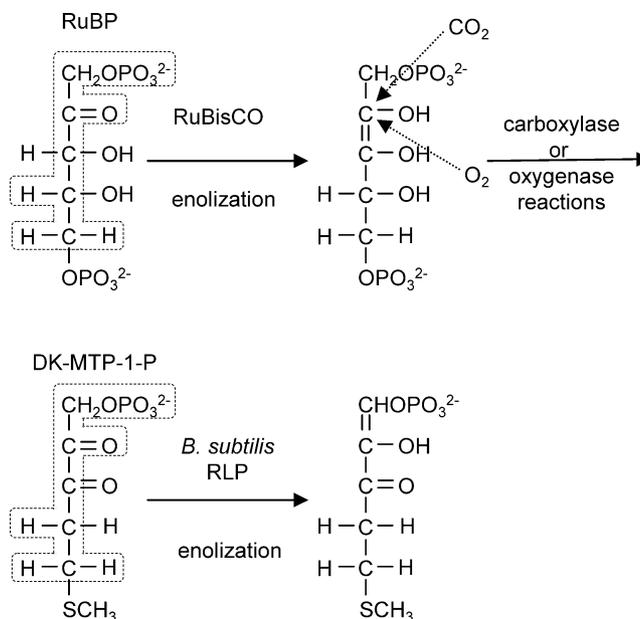

Fig. 4. Comparison of catalytic reactions with *B. subtilis* RLP and photosynthetic RuBisCO.

residues for binding the phosphate group on C5 of RuBP could be attributed to the methylthio group on C5 of DK-MTP-1-P. It has been established that deletion of loop 6 from RuBisCO prevents carboxylation and oxygenation reactions, but retains the ability to catalyze the enolization of RuBP [23]. K175, D203 and E204 residues are also essential amino acid residues for enolization of RuBP, and mutation of these residues leads to a lack of enolization activity [16,19]. *B. subtilis* RLP conserves all these amino acid residues (Fig. 2). This observation supports the hypothesis that the RLP-catalyzed enolization of DK-MTP-1-P does not require the amino acid residues for binding the phosphate group on C5 of RuBP nor loop 6. RuBisCO has to be activated by CO_2 and Mg^{2+} at the K201 residue forming a carbamate to catalyze both carboxylase and oxygenase reactions [2,18]. RLP also conserves this K201 residue (Fig. 2). In our experiments, *B. subtilis* RLP showed DK-MTP-1-P enolase activity even when we did not perform any activating treatment in a reaction mixture including high CO_2 concentration [3]. It is unclear whether this lysine residue, or carbamylation of this residue, is important for RLP activity. However, this residue could be essential for activity of RLP because it is conserved in all RLPs and photosynthetic RuBisCO (Fig. 2).

We predicted that photosynthetic RuBisCO might have the ability to catalyze the DK-MTP-1-P enolase reaction because there were some common features between *B. subtilis* RLP and photosynthetic RuBisCO. Wild-type *B. subtilis* can grow in a culture medium including MTA [3,30], a metabolite in the methionine salvage pathway, as the sole source of sulfur, but the RLP-deficient mutant cannot [3]. The gene for RuBisCO from photosynthetic bacteria, *Rhodospirillum rubrum*, rescued the RLP-deficient mutant when they were

grown on MTA medium [3]. However, recombinant Ru-BisCO of *R. rubrum* catalyzed the DK-MTP-1-P enolase reaction at a much slower rate than RLP from *B. subtilis* [3]. Thus photosynthetic RuBisCO catalyzes the same reaction as that catalyzed by RLP or DK-MTR-1-P enolase from *B. subtilis*. It is interesting that *B. subtilis* RLP cannot catalyze carboxylase and oxygenase reaction catalyzed by photosynthetic RuBisCO, while photosynthetic RuBisCO retains DK-MTP-1-P enolase activity catalyzed by *B. subtilis* RLP. From the standpoint of sharing a common catalytic reaction between *B. subtilis* RLP and RuBisCO, it is possible that *B. subtilis* RLP and photosynthetic RuBisCO evolved from a common ancestral protein.

5. A hypothesis on the molecular evolution of photosynthetic RuBisCO from the RLP of *B. subtilis*

The reaction steps from DK-MTP-1-P to DHK-MTPene are catalyzed by one bi-functional enzyme, DK-MTP-1-P enolase/phosphatase, in *Klebsiella* species [4,29], but by two separate enzymes DK-MTP-1-P enolase (RLP) and HK-MTPene-1-P phosphatase in *B. subtilis*, as described above (Fig. 3) [3]. Genomes of some Gram-negative bacteria, yeast, *Arabidopsis*, *Drosophila*, mice and humans, possess homologous genes for the enzymes DK-MTP-1-P enolase/phosphatase proposed to function in the pathway of *Klebsiella* sp. The amino acid identities of the orthologues of DK-MTP-1-P enolase/phosphatase in these organisms with those found in *Klebsiella* sp. are 40% for cyanobacteria (Cyanobase/WH8102: SYNW1964), 37% for yeast (PIR: S30843), 42% for *Arabidopsis* (GB: At5g53850), 43% for *Drosophila* (FlyBase: Fbgn0037305), 39% for mice (Mouse Genome Informatics: CGI 1915120) and 40% for humans (GB: AF113125). The gene for the orthologue of RLP is not found in yeast, mice or humans, but the orthologue for RuBisCO in photosynthetic organisms is. Accordingly, DK-MTP-1-P enolase/phosphatase probably functions in the methionine salvage pathway of these organisms. Amino acid sequences of enolase/phosphatase are not at all homologous to those of *B. subtilis* RLP. Therefore, when we consider the evolution of the methionine salvage pathway, there are two pathways, an enolase form and an enolase/phosphatase form, where DK-MTP-1-P enolase or DK-MTP-1-P enolase/phosphatase functions, respectively [32]. When this pathway first appeared, ancient organisms would have utilized either the enolase form or the enolase/phosphatase form of this pathway.

Gupta and his colleagues proposed a linear bacterial phylogeny by analyzing conserved inserts and deletions in highly conserved catalytic domain of various proteins, and according to their findings Gram-positive low G + C Bacteria including *B. subtilis* may represent the direct progeny of the most ancient bacteria [14,15]. Moreover, their hypothesis proposed that Gram-positive low G + C Bacteria emerged earlier than Archaea, Gram-negative Bacteria,

cyanobacteria or photosynthetic Bacteria [14]. This hypothesis would be compatible with the enolase form of the methionine salvage pathway in *B. subtilis* being relatively primitive, and *B. subtilis* RLP would have functioned in this primitive pathway, because organisms that emerged later than *B. subtilis*, in their evolutionary hypothesis, possess the genes for enolase/phosphatase. Furthermore, if *B. subtilis* had not gained the gene for RLP by lateral gene transfer from photosynthetic organisms, RLP might be an ancestor of photosynthetic RuBisCO. In a standard phylogenetic tree of the large subunits of RuBisCO, the RLP from *B. subtilis* is not included on any branch that includes RuBisCO, nor on branches that include form III RuBisCO with RuBP-carboxylation activity (Fig. 1). The codon usage and the G + C content of the gene for RLP are typical of this organism. The literature [26] suggests that genes such as the gene for RLP were probably not derived by lateral transfer of a gene for a RuBP-carboxylating enzyme from another unrelated organism, for example in this case, an archaean or photosynthetic bacterium. Together with Gupta's hypothesis, these observations suggest that an ancestral RLP that evolved into that of *B. subtilis* might be an ancestor of photosynthetic RuBisCO, and that archaean RuBisCO and photosynthetic RuBisCO with activities of carboxylation and oxygenation might have evolved from that particular RLP, rather than from another common ancestral protein.

Within this frame of thought, we propose that the common ancestor of photosynthetic RuBisCO has to be found in the RLPs involved in the methionine salvage pathway (Fig. 5). It is conceivable that one of the duplicate RLPs might have lost its job in this pathway while allowing RuBisCO to occur. Later on, or in a particular environment, an enolase/phosphatase might in parallel have begun to function in this step instead of RLP after recruitment of an enolase/phosphatase. However, RLP would not have vanished in evolution and might have evolved into archaean RuBisCO and photosynthetic RuBisCO by acquisition of amino acid residues necessary for binding a phosphate group on C5 of RuBP, loop 6 and others. In addition, the Calvin cycle may have been completed by emergence of photosynthetic RuBisCO which evolved from *B. subtilis* RLP; consequently, chemoautotrophic and photosynthetic bacteria utilizing the Calvin cycle for CO_2 fixing might have emerged, and then plants could prosper in the earth. In this way, the existence of RLP may be a demonstration of the success story of how protein functioning in a pathway such as the methionine salvage pathway became the key enzyme in photosynthesis and the most abundant protein on earth. RLP of *B. subtilis* might be a "living fossil" of photosynthetic RuBisCO's ancestor.

6. Perspective

RLPs, archaean RuBisCO and photosynthetic RuBisCO are valuable effective model proteins for the general study of the molecular evolution of enzymes. *B. subtilis* RLP is
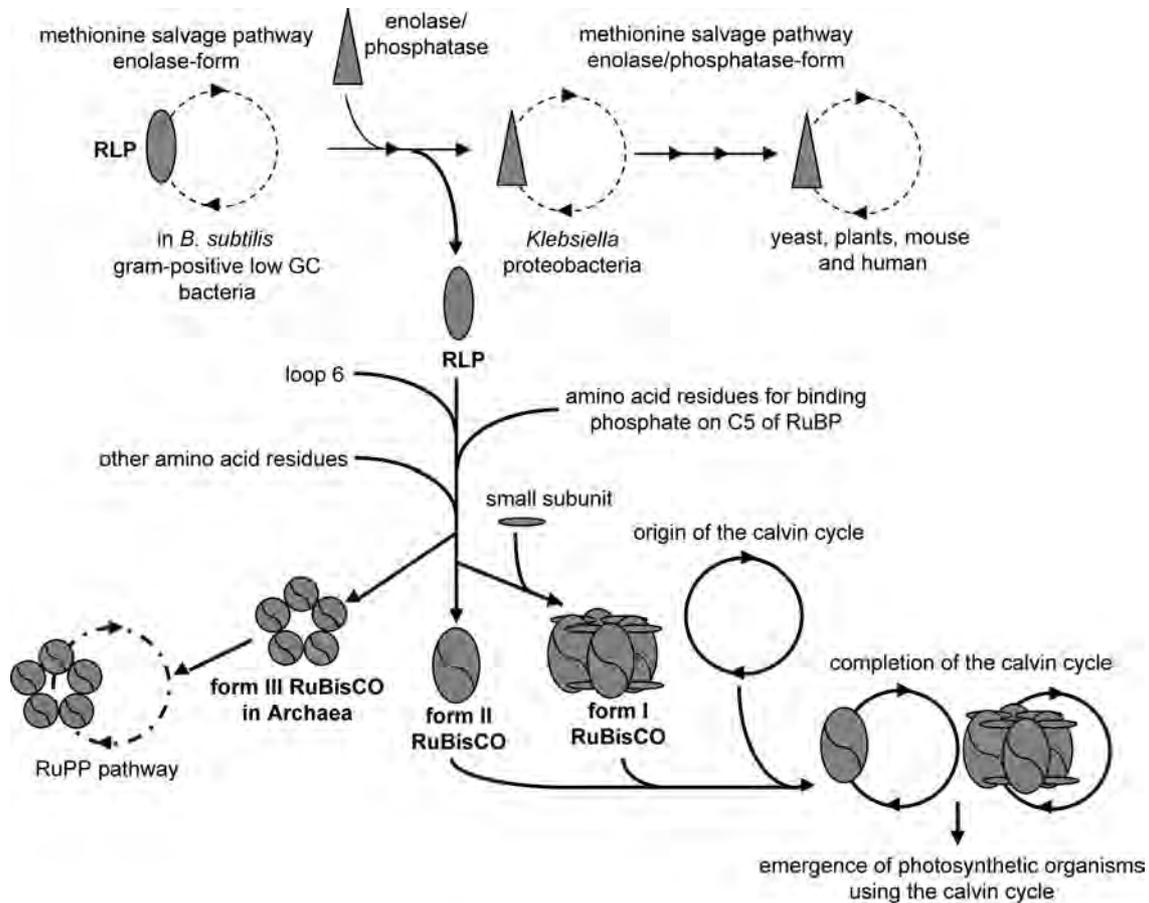
Fig. 5. A hypothesis of the molecular evolution of photosynthetic RuBisCO from *B. subtilis* RLP.

the sole RLP whose function has been identified biochemically [3]. However, our results with the RLP of *B. subtilis* suggest that RLPs of other Bacteria and Archaea might also catalyze, at least in vitro, the same reaction as *B. subtilis* RLP or a partial reaction of RuBisCO in a metabolic pathway. Identification of the function of other RLPs should provide more information about the molecular evolution of photosynthetic RuBisCO from RLPs.

For the generation of plants performing highly efficient photosynthesis, many researchers throughout the world have been trying to create, by mutational methods, a "super RuBisCO" which has a higher turnover of carboxylation and higher specificity for CO_2 than extant photosynthetic RuBisCO [24]. However, this vision has not yet been achieved.

If RLP is RuBisCO's ancestor, the inefficient kinetic properties of photosynthetic RuBisCO may be hidden in evolutionary history from RLP to RuBisCO. Therefore, we might have access to their causes by inducing artificial evolution of RLP into photosynthetic RuBisCO using mutational methods in vitro. If we could create a mutant of RLP that can catalyze either the carboxylase or oxygenase reaction using artificial evolution, amino acid residues responsible for the specificity for CO_2 or O_2 may be revealed. RLP could be used as the starting material for creating a "super RuBisCO", rather than extant photosynthetic RuBisCO that

may be less amenable to catalytic improvement. We may succeed in creating a "super RuBisCO" leading to another type of molecular evolution distinct from that which photosynthetic RuBisCO followed in nature.

Acknowledgements

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