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Photorespiration and the potential to improve photosynthesis

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The photorespiratory pathway, in short photorespiration, is an essential metabolite repair pathway that allows the photosynthetic CO_2 fixation of plants to occur in the presence of oxygen. It is necessary because oxygen is a competing substrate of the CO_2-fixing enzyme ribulose 1,5-bisphosphate carboxylase, forming 2-phosphoglycolate that negatively interferes with photosynthesis. Photorespiration very efficiently recycles 2-phosphoglycolate into 3-phosphoglycerate, which re-enters the Calvin–Benson cycle to drive sustainable photosynthesis. Photorespiration however requires extra energy and re-oxidises one quarter of the 2-phosphoglycolate carbon to CO_2, lowering potential maximum rates of photosynthesis in most plants including food and energy crops. This review discusses natural and artificial strategies to reduce the undesired impact of air oxygen on photosynthesis and in turn plant growth.

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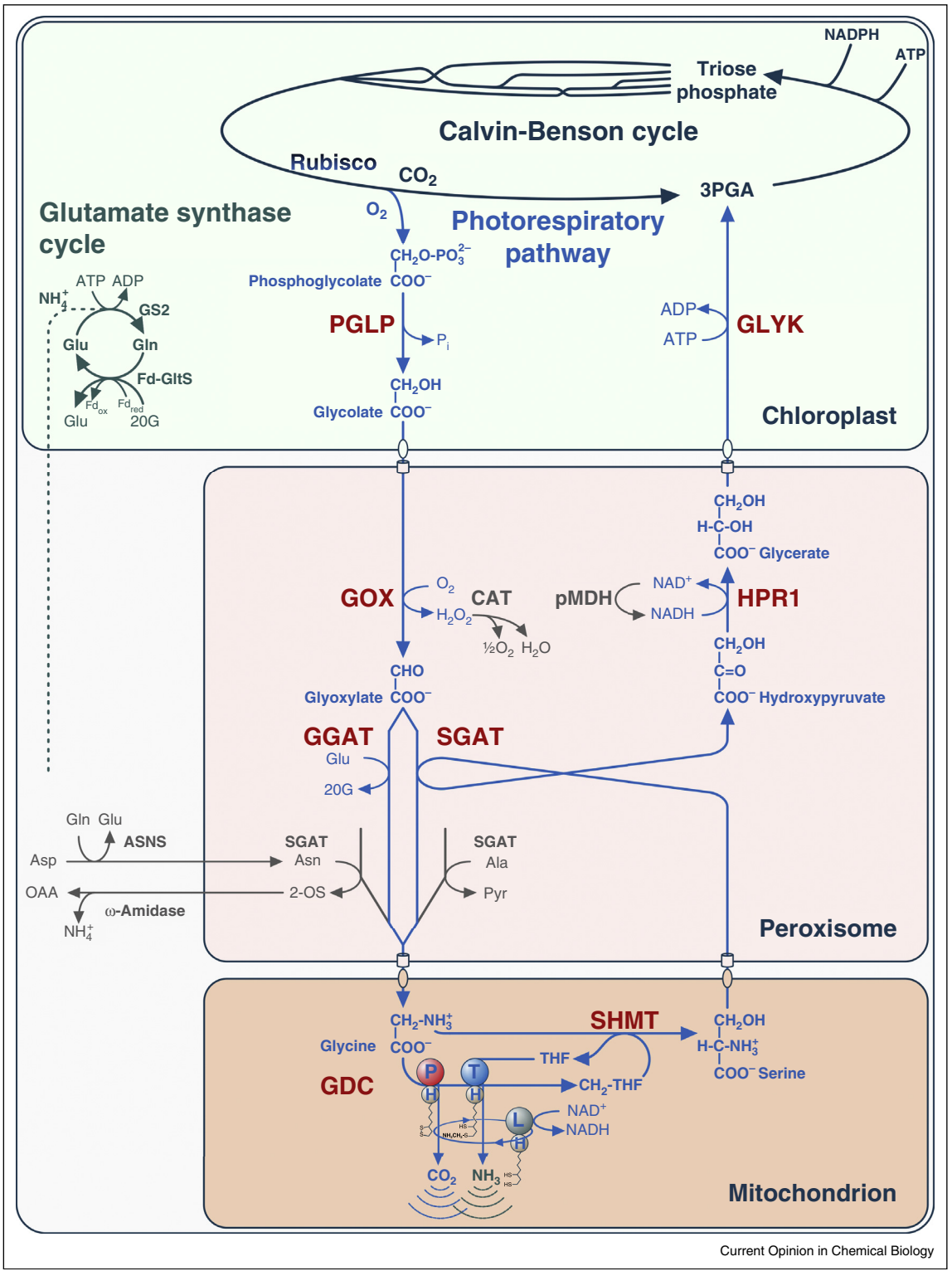
What is photorespiration?

Photorespiration, in contrast to the light-independent processes of mitochondrial respiration, is the light-dependent consumption of O_2 and coupled release of CO_2 that occurs simultaneously with photosynthetic CO_2 uptake and O_2 release in all plants, algae and cyanobacteria. Rates of plant photorespiration can be very high, especially under conditions of high temperature and water shortage. Globally, the process re-liberates an estimated 29 Gt of freshly assimilated carbon per year into the atmosphere [1,2]. On the molecular level, the term photorespiration also connotes the photorespiratory pathway as an integral component of the photosynthetic-photorespiratory supercycle [3,4]. Photorespiration starts when the CO_2 fixation enzyme ribulose 1,5-bisphosphate carboxylase (RuBP carboxylase/oxygenase; Rubisco) of the Calvin–Benson cycle (CB cycle) fixes O_2 instead of CO_2 [5]. Oxygenation of RuBP forms 3-phosphoglycerate (3PGA) and 2-phosphoglycolate (2PG), whereas carboxylation of RuBP forms 2 mol 3PGA. In C_3 plants, every third to fourth molecule of RuBP is oxygenated rather than carboxylated at the present day air CO_2/O_2 ratio (0.04% CO_2/20.95% O_2) [1,6]. Accordingly, large amounts of 2PG are produced during the day. Photorespiration recycles two molecules of 2PG into one molecule of 3PGA; thus, only 25% of organic carbon is lost as CO_2 whereas 75% is salvaged and used to synthesize RuBP, refilling the CB cycle.

The photorespiratory pathway involves more than 20 different enzymes and (mostly unidentified) transporters that are distributed over at least three compartments in plant cells, the chloroplast, the peroxisome, and the mitochondrion (Figure 1). Rubisco generates 2PG in the chloroplast. 2PG phosphatase (PGLP) dephosphorylates 2PG into glycolate, which is exported from the chloroplast into the cytosol by the recently discovered glycolate/glycerate antiporter [7••] and then diffuses into the peroxisome. In the peroxisome, glycolate oxidase (GOX) catalyses the O_2-dependent irreversible oxidation of glycolate to glyoxylate giving rise to H_2O_2, which is quickly detoxified by catalase (CAT). Still in the peroxisome, glyoxylate becomes transaminated to glycine by the parallel action of glutamate:-glyoxylate aminotransferase (GGAT) and serine:glyoxylate aminotransferase (SGAT). The required glutamate is imported from the chloroplast by exchange against malate via dicarboxylate antiporters. Glycine then moves into the mitochondrion where the glycine decarboxylase multienzyme system (GDC) and serine hydroxymethyltransferase (SHMT) convert two molecules of glycine to one molecule of serine, NH_3 and CO_2. In the oxidative decarboxylation step, GDC reduces NAD^+ and to NADH. Serine is exported from the mitochondrion back to the peroxisome to return its amino group to glyoxylate in the SGAT reaction, producing hydroxypyruvate (HP). Next, another peroxisomal enzyme, HP reductase (HPR1), reduces HP to glycerate. The necessary NADH is produced from malate oxidation by peroxisomal malate dehydrogenase. The glycerate returns into the chloroplast to become phosphorylated by glycerate 3-kinase (GLYK) to finally yield 3PGA. This CB cycle intermediate is used to regenerate the Rubisco acceptor molecule RuBP. Several more enzymes are essential for the entire photorespiratory metabolism for example to re-assimilate the photorespiratory NH_3 in the photorespiratory nitrogen

Figure 1



The photorespiratory pathway and its interconnection with photosynthetic Calvin–Benson cycle and NH_3 assimilation in higher plants. (2OG, 2-oxoglutarate; 2-OS, 2-oxosuccinamate; 3PGA, 3-phosphoglycerate; Ala, alanine; Asn, asparagine; ASNS, asparagine synthetase; Asp, aspartate; CAT, catalase; FD-GltS, ferredoxin-dependent glutamate synthase; GDC, glycine decarboxylase complex; GGAT, glutamate:glyoxylate aminotransferase; Gln, glutamine; Glu, glutamate; GLYK, glycerate 3-kinase; GOX, glycolate oxidase; GS2, glutamine synthetase; HPR1, hydroxypyruvate reductase; OAA, oxaloacetate; PGLP, phosphoglycolate phosphatase; Pyr, pyruvate; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; SGAT, serine:glyoxylate aminotransferase; SHMT, serine hydroxymethyltransferase.)

cycle [8] or to remove inhibitory 5-formyl tetrahydrofolate produced in a side-reaction of SHMT [9•].

Photorespiration is an energy-demanding process that formally requires a total of 3.25 mol ATP and 2 mol NADPH per one oxygenation of RuBP, which is about one-third of the total energetic costs of CO_2 fixation in air [10]. Whereas the photorespiratory core pathway (net reaction: 2 mol $2PG + O_2 \rightarrow 3PGA + CO_2 + NH_3$; see Figure 1) consumes 1 mol of ATP for the phosphorylation of glycerate to 3PGA via GLYK (0.5 mol ATP per oxygenation event), the reassimilation of the released ammonia into glutamine and the conversion of 3PGA into RuBP via the CB cycle is highly energy-demanding.

Why is there photorespiration?

The high energy demand of photorespiration and particularly the inherent loss of freshly assimilated CO_2 raise the question why this process exists? The answer is relatively simple: the photorespiratory pathway in essence renders the CB cycle insensitive towards oxygen. All oxygenic phototrophs rely on the CB cycle with Rubisco as the key carboxylating enzyme. The chemistry of the Rubisco-catalysed reaction dictates that competitive oxygenation occurs whenever O_2 is present. The essential role of photorespiration for enabling oxygenic photosynthesis is clearly supported by many mutant studies, which showed that knocking out genes encoding photorespiratory enzymes results in the so-called 'photorespiratory phenotype', that is such mutants cannot grow in ambient air but can be rescued in air with a 20-fold to 30-fold higher CO_2 concentration than normal, where RuBP oxygenation becomes inhibited [11,12]. Non-viability in normal air is due to a combination of at least two effects: RuBP deprivation of the CB cycle and inhibition by 2PG of key enzymes such as chloroplastic triosephosphate-isomerase and phosphofructokinase [3]. Additionally, glyoxylate affects CB cycle operation by the inhibition of Rubisco activase [13], and glycine accumulation to some extent segregates magnesium from cellular metabolism, which is the reason for the slow growth of glycine-accumulating cyanobacterial mutants [14]. Photorespiration prevents or at least minimizes these harmful processes, and this comes at a price.

The photorespiratory pathway is an ancient process and varies among organisms

It was initially thought that photorespiration evolved in response to the low CO_2 and high O_2 concentrations prevailing when streptophytes (comprising charophytes, bryophytes, and vascular plants) colonized land and higher plants developed [15]. It is now considered most likely that the basics of photorespiratory metabolism co-evolved together with oxygenic photosynthesis in cyanobacteria [12]. The present view is essentially based on two lines of evidence. First, plant-like photorespiratory metabolism was demonstrated in cyanobacteria [16••], green algae [17] and more recently in red algae [18•]. Second, the reconstructed phylogenies of photorespiratory enzymes reflect their ancient origins in different groups of prokaryotes that served as eukaryotic host cell or as endosymbionts for the origin of mitochondria and plastids [19].

Over geologic times, a number of adaptations occurred leading to variations in the photorespiratory metabolism. Most cyanobacteria metabolize 2PG not only by the plant-like photorespiratory cycle, but can also convert 2 mol glyoxylate into glycerate using the bacterial glycerate pathway with tartronate-semialdehyde as intermediate [16••]. This pathway also releases one CO_2 per two molecules of 2PG but does not require transamination of glyoxylate and hence does not release ammonia, which makes it more energy efficient. Moreover, some cyanobacterial strains have the potential to completely oxidize glyoxylate into CO_2 [16••]. Cyanobacteria as well as chlorophytes evolved glycolate dehydrogenase-(GDH)-based photorespiration, which is not producing the by-product H_2O_2 and gains NAD(P)H, in contrast to GOX-based photorespiration in other eukaryotic alga [18•,20] and plants.

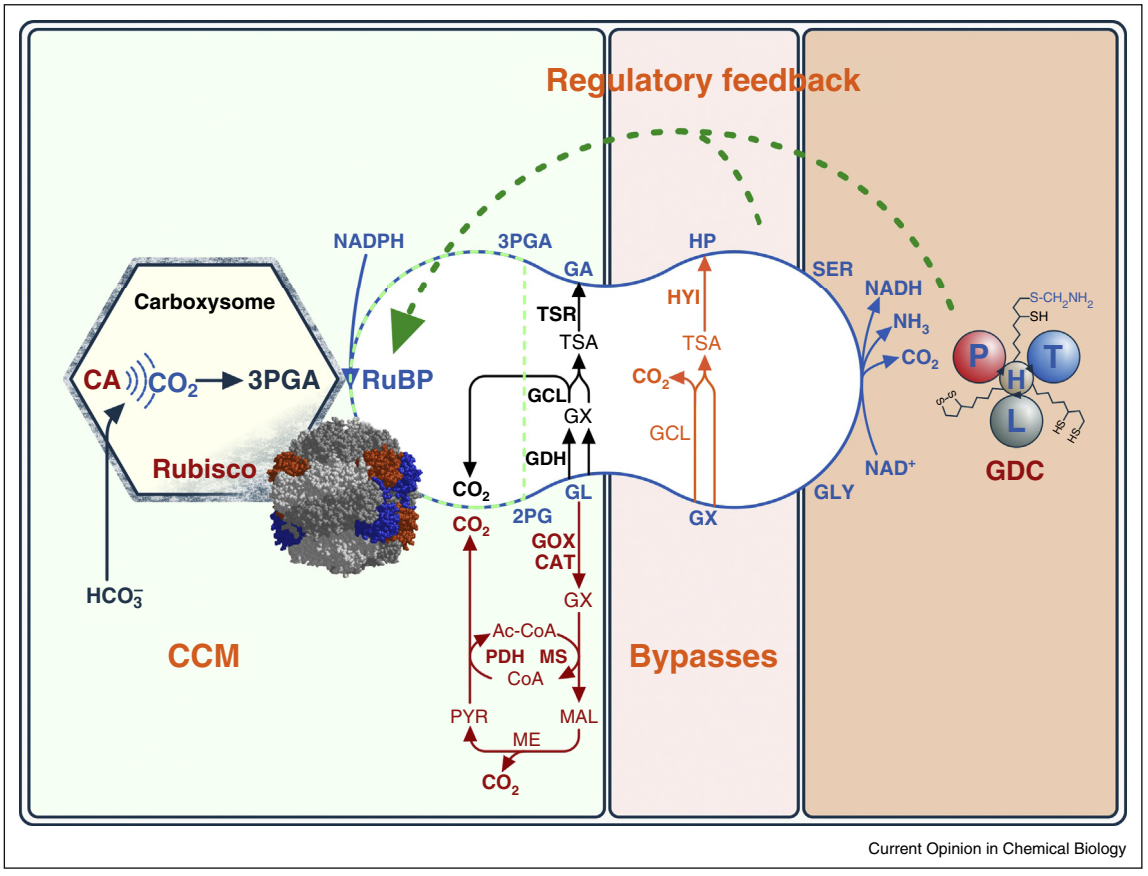
Photorespiration is a major metabolic pathway and a target for crop improvement

As outlined before, the repair of the consequences of RuBP oxygenation occurs very efficiently, salvaging three out of four glycolate carbons for photosynthesis [12], but nevertheless comes at the cost of losing some freshly assimilated CO_2 and extra energy that is required for the recycling of 2PG into RuBP and particularly of photorespiratory NH_3 into glutamate nitrogen. This is why photorespiration has been a key target of crop improvement for decades and the respective approaches gained fresh momentum in recent years [21,22•]. Present-day strategies focus on the improvement of Rubisco properties [23] and the establishment of CO_2-concentrating mechanisms (CCMs) into C_3 plants [24,25,26] to reduce 2PG production, the optimisation of the photorespiratory pathway to achieve 2PG recycling for example without NH_3 release [27], the exploitation of regulatory feedback from the photorespiratory pathway to the CB cycle to enhance gross photosynthesis [3], and finally the generation of artificially designed CO_2-assimilation pathways [28•] (Figure 2).

Reducing photorespiratory activity by alternative Rubisco variants and CO_2-concentrating mechanisms

Photorespiration is initiated by the low CO_2 specificity of Rubisco, which also catalyses the oxygenase reaction leading to the necessity of 2PG recycling. Concerning the 'improvement' of Rubisco, for example by directed evolution [29], significant progress was made in recent years though additional effort will be necessary to produce transgenic plants with improved photosynthesis

Figure 2



Schematic display of strategies aiming to improve plant growth by manipulating photorespiration. Strategies to improve Rubisco properties and carboxylation efficiency by generating CCMs such as cyanobacterial carboxysomes in C_3 plant chloroplasts are displayed at the left side. Artificial bypasses (explained in the text) to decrease photorespiratory NH_3 release and/or to increase CO_2 concentrations in the chloroplasts are shown in the central part. The GDC scheme at the right side represents recent findings that increased GDC activity leading to enhanced photorespiratory flux improved photosynthesis and plant growth most likely due to regulatory feedback on Calvin–Benson cycle activity. (2PG, 2-phosphoglycolate; 3PGA, 3-phosphoglycerate; Ac-CoA, acetyl-CoA; CA, carbonic anhydrase; CAT, catalase; GA, glycerate; GDC, glycine decarboxylase complex; GCL, glyoxylate carboligase; GDH, glycolate dehydrogenase; GL, glycolate; GLY, glycine; GOX, glycolate oxidase; GX, glyoxylate; HP, hydroxypyruvate; HYI, hydroxypyruvate isomerase; MAL, malate; ME, malate enzyme; MS, malate synthase; PDH, pyruvate dehydrogenase; PYR, pyruvate; RuBP, ribulose 1,5-bisphosphate; SER, serine; TSA, tartronate-semialdehyde; TSR, tartronate-semialdehyde reductase.)

[30,31••]. It shall be noted that some authors argue Rubisco may be nearly perfectly optimised for the natural habitats of the respective species [32•,33•].

A different approach aims at exploiting several naturally occurring CCMs by which C_4 plants, algae and cyanobacteria increase the CO_2 concentration near Rubisco thereby increasing carboxylation and suppressing oxygenation [34]. C_4 plants perform C_3 photosynthesis that is supported by a specific CCM, the so-called C_4 cycle. C_4 photosynthesis has independently evolved in more than 65 plant lineages including highly productive crops such as sugar cane and maize [35], which also involved the early re-localization of photorespiration into bundle sheath to enhance there the local CO_2 concentration [36•,37]. The complex 'C_4 syndrome' of morphological and biochemical features requires concerted operation of many genes, and it is easily understandable that artificial C_3 to C_4 conversion of crops such as rice will be a long-term task [26,38]. Recently, it is also discussed how to establish a cyanobacterial CCM in C_3 plants (see Figure 2). This CCM comprises several bicarbonate and CO_2 uptake systems to accumulate bicarbonate inside the cells, which then diffuses into a proteinaceous micro-compartment containing Rubisco, the carboxysome, where carbonic anhydrase converts the bicarbonate to CO_2 [39••]. The feasibility of introducing carboxysomes into other organisms has been tested in *Escherichia coli* [40]. Tobacco plants expressing cyanobacterial Rubisco and the carboxysome internal structural protein CcmM produced aggregates of these two proteins in the chloroplast, which resembled an early carboxysome

assembly complex [31••]. First attempts to express cyanobacterial bicarbonate transporter in plant cells and their successful targeting to the chloroplast envelope have also been published [41,42].

Bypassing sections of the photorespiratory pathway

Several strategies were reported which aim at short-circuiting sections of the photorespiratory pathway in plants to optimise the recycling of 2PG [27]. The first report concerned the introduction of the bacterial glycerate pathway (see Figure 2), which converts glyoxylate into glycerate, bypassing the formation of glycine and its conversion into serine, which is accompanied by the release of NH_3 [43•]. To this end, three subunits of glycolate dehydrogenase (GDH) as well as tartronate-semialdehyde (TSA) synthase and TSA reductase from *E. coli* were fused to chloroplastic import sequences and co-expressed in Arabidopsis. The resulting transgenic plants showed reduced photorespiration and increased biomass yield under short day conditions. Interestingly, improved growth was unexpectedly also observed in plants overexpressing GDH alone. This raises the question of whether or not the glycerate pathway was functional in these transgenic plants. In a follow-up study, this group reported that overexpression of an artificially generated polyprotein comprising all three *E. coli* GDH subunits also enhanced photosynthesis and tuber yield in potato [44]. These authors speculated that the resulting glyoxylate was completely oxidized to CO_2 within the chloroplast by pyruvate dehydrogenase (PDH) [45], boosting CO_2 fixation by Rubisco. A similar strategy was used with the biofuel crop *Camelina sativa*, where overexpressing the complete or partial glycerate pathway in chloroplasts also resulted in improved growth and higher seed yields [46].

Full oxidation of glycolate to CO_2 at the site of its origin was attempted by overexpressing GOX, malate synthase (MS) and CAT in Arabidopsis chloroplasts [47]. This strategy aimed to increase the chloroplastic CO_2 concentration and to reduce the overall flux through the cycle saving energy for NH_3 reassimilation (see Figure 2). However, considering that the operation of this bypass would release even more CO_2 from glycolate, it is surprising that improved photosynthetic performance and better growth were observed. The natural decarboxylation pathway from cyanobacteria [16••] represents another possibility to achieve complete decarboxylation of photorespiratory glycolate in the chloroplast, which has not been tested yet.

The bacterial glycerate pathway has also been expressed in peroxisomes of tobacco, where the substrate glyoxylate of this pathway naturally occurs [48]. The peroxisomal expression of glyoxylate carboligase (GCL) and HP isomerase (HYI) (see Figure 2), however, did not have beneficial effects on photosynthesis and growth, in contrast to the chloroplastic expression of the glycerate pathway discussed above [43•]. Similar observations were made when the proteins of the cyanobacterial glycerate pathway were over-expressed in Arabidopsis after fusion with peroxisomal import sequences [own unpublished observations].

Notably, although higher photosynthetic rates and yields were demonstrated in most of these studies, not one of them provided qualitative or quantitative evidence for the claim that the anticipated bypass is indeed functional *in planta*, for example by genetic experiments, flux analysis or the demonstration that less ammonia is released by photorespiration in the transgenic plants. Without such evidence it is well possible and perhaps even likely that the observed positive effects on photosynthesis and growth are due to intervention into the regulatory interplay between the photorespiratory pathway and CO_2 fixation as discussed below, which however does not make the above reports less interesting. As glycine is the major substrate for oxidative phosphorylation in limiting CO_2 [49] it is also difficult to predict whether and to what extent bypassing the mitochondrial part of photorespiration would affect ATP synthesis and in turn sucrose synthesis in the cytosol.

Improving gross photosynthesis by increasing photorespiratory enzymatic capacity

Regulatory feedback from the photorespiratory pathway to the CB cycle was long presumed as numerous experiments had consistently shown that affecting photorespiratory carbon flow impairs photosynthetic CO_2 fixation [50]. In the reverse direction, it was also observed that overexpression of some photorespiratory enzymes, speeding up flux through the photorespiratory cycle, improves photosynthesis and plant growth. For example, overexpression of two individual GDC proteins in Arabidopsis, the so-called H-protein [51••] and dihydrolipoamide dehydrogenase (L-protein subunit) [52•], lowered the CO_2 compensation points in combination with higher net-CO_2 uptake rates and better growth. Similar results were reported for rice overexpressing mitochondrial SHMT [53]. Cause(s) and effect(s) are not yet known at the molecular level, but it appears that the capacity of the mitochondrial reactions could control overall photorespiratory flux and that photorespiratory activity could regulate the activity of the CB cycle (see Figure 2). For example, shifting Arabidopsis plants from high CO_2 into ambient air typically results in the massive accumulation of glycine [54], which also supports the notion that mitochondrial glycine-to-serine conversion limits the overall photorespiratory flux.

In addition to regulating photosynthesis, photorespiration has also major impact on several other fundamental processes of plant metabolism. There are clear hints that the reassimilation of NH_3 released during photorespiration supports nitrate assimilation by C_3 plants [55]. Recently,

it has been also demonstrated that photorespiratory Pi-cycling confers growth advantage to woody plants even when grown in low-O_2 environments [56]. Thus, in addition to strategies that aim at decreasing photorespiration, targeted modulation of the enzymatic capacity of critical steps in the photorespiratory pathway may be another promising way by which photosynthesis and associated metabolic pathways can be optimised to achieve crop improvement.

Implementation of newly designed CO_2 fixation pathways into plants

The CB cycle including Rubisco with the later additions of the photorespiratory pathway and in some organisms CCMs evolved over several billion years in response to changing environments. Now, photosynthetic metabolism is presumably perfectly adapted to the conditions dictated by the results of the evolution of the particular organism and by the present atmosphere. These constraints imply that it will be difficult to establish highly efficient crops that do not require the photorespiratory pathway. Synthetic biology may possibly open the way to implement artificial carbon fixation pathways [21,28•] and 2PG salvage routes. Such hypothetical pathways have been computed using approximately 5000 known metabolic enzymes and compared on the basis of their kinetic and energetic properties with the result that some of them could be distinctly superior to the CB cycle [57]. A notable advance into this direction was the recent introduction of the 3-hydroxypropionate cycle, which is used for CO_2 fixation by the phototrophic bacterium *Chloroflexus aurantiacus* [58], into the cyanobacterium *Synechococcus elongatus*, where it could function as additional CO_2 fixation pathway and as potential photorespiratory bypass [59•]. At present, functionality of the introduced pathway in *S. elongatus* was demonstrated, but improved growth or related phenotypic alterations were not observed maybe due to the operation of an efficient CCM present in this cyanobacterium. Recently, some of these newly emerging strategies have been extensively reviewed [28•].

Conclusions

The photorespiratory pathway allows the CB cycle to operate in the presence of oxygen and thus is a key constituent of plant metabolism. Having crop yields and molecular breeding in mind, sensible approaches to improve photosynthesis will not aim at eradicating photorespiration but rather attempt to maximise net carbon gain. This goal can be achieved by establishing CCMs in C_3 crops, maybe in combination with a Rubisco that has a better carboxylation-to-oxygenation ratio. The exploitation of regulatory interactions between the individual parts of the photosynthetic-photorespiratory metabolic network to increase photosynthesis or streamlining the photorespiratory pathway by the introduction of artificial routes for the conversion of glycolate to glycerate are two more presently pursued strategies. Finally, synthetic biology approaches could allow introducing artificial glycolate-utilizing pathways that improve net photosynthetic carbon gain. The combined application of these approaches will open many avenues to improve the plant carbon assimilation with the final aim to improve crop yield.

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