Recent developments in photorespiration research

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Abstract

Photorespiration is the light-dependent release of CO_2 initiated by Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) during oxygenic photosynthesis. It occurs during the biochemical reactions of the photorespiratory C_2 cycle, which is an ancillary metabolic process that allows photosynthesis to occur in oxygen-containing environments. Recent research has identified the genes for many plant photorespiratory enzymes, allowing precise functional analyses by reverse genetics. Similar studies with cyanobacteria disclosed the evolutionary origin of photorespiratory metabolism in these ancestors of plastids.

Introduction

Photorespiration, in contrast with day respiration and night respiration, is the light- and O_2-dependent release of CO_2 during oxygenic photosynthesis. It occurs since O_2 can substitute for CO_2 in the first reaction of photosynthetic CO_2 fixation, which is catalysed by Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase). In the active site, RubP (ribulose 1,5-bisphosphate) forms an enediolate intermediate, which reacts with CO_2 to form 3PGA (glycerate 3-phosphate). Owing to the chemical nature of the enediolate, O_2 can replace CO_2 as a substrate, forming 3PGA and 2PG (2-phosphoglycolate). In contrast with 3PGA, 2PG cannot directly enter the Calvin cycle, and its accumulation is toxic. Rubisco strongly favours CO_2 over O_2, but cellular CO_2 concentration is typically low and oxygen is produced in close vicinity to Rubisco. Moreover, in the atmosphere, the concentration of O_2 is two to three orders of magnitude higher than that of CO_2. Hence, 2PG is inevitably produced during oxygenic photosynthesis and the respective biosynthetic rates can be very high [1].

2PG enters the metabolism through conversion into 3PGA. This occurs in the photorespiratory C_2 cycle, which, regarding its central reactions, has been known for many years from careful biochemical studies in combination with the analysis of mutants isolated by classical approaches [2–4]. This pathway recovers three out of four 2PG carbon atoms and oxidizes one carbon atom to CO_2. The corresponding CO_2 loss is much higher than that from other respiratory processes. It amounts to approx. 20% of net photosynthesis under moderate conditions [5] and can be higher in warmer environments. Photorespiration is very low if plants are kept in artificially high CO_2 and is hence often erroneously perceived as a wasteful process without function. This view overlooks the fact that photorespiratory metabolism is an essential precondition of oxygenic photosynthesis. Nevertheless, further optimization of photorespiration (from the standpoint of agricultural productivity) appears to be possible, and corresponding attempts have delivered the first promising results (e.g. [6]).

Plant photorespiratory C_2 cycle

The plant photorespiratory C_2 cycle is complex and highly compartmentalized (Figure 1). Starting with 2PG synthesis by Rubisco, it requires eight more enzymatic reactions and needs several auxiliary enzymes that are distributed over four subcellular compartments: the chloroplast, the peroxisome, the mitochondrion and the cytosol.

The rates of RubP carboxylation and oxygenation are determined by the concentrations of CO_2, O_2 and RubP and by the kinetic properties of Rubisco. Consequently, there is much interest in identifying 'better' natural or mutant Rubisco variants and integrating them into plants. The latest strategies attempt to obtain Rubisco hypermorphs by directed evolution and genetic selection in engineered *Escherichia coli* and integrate them into plants [7]. This is not easy to achieve, because plant Rubisco comprises eight copies each of a plastome-encoded 55 kDa subunit and a nuclear-encoded 15 kDa subunit. Less complex forms exist in prokaryotes, but all Rubiscos are derived from primordial archaeal Rubisco-like proteins [8]. These ancestral enzymes evolved in an anaerobic environment. They have no Rubisco activity, but act as enolases in methionine-salvage pathways [9].

The 2PG originating from RubP oxygenation is hydrolysed by a plastidial PGP (2-phosphoglycolate phosphatase), PGP1. Plants also have a cytosolic PGP, but only deletion of the plastidial enzyme results in a 'photorespiratory phenotype', i.e. such mutants die in normal air and require elevated CO_2 for normal growth [10]. Glycolate moves out of the chloroplasts through a poorly characterized glycolate/glycerate antiporter and diffuses into the peroxisomes via porin-like channels [11].

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Figure 1 | Plant photorespiratory metabolism

Metabolic pathways constituting the core of the C_2 cycle, the photorespiratory nitrogen cycle and several associated reactions. AMT, ammonium transporter; OAA, oxaloacetate.

In the peroxisomes, the FMN-dependent GOX (glycolate oxidase) oxidizes glycolate to glyoxylate in two steps. First, FMN oxidizes glycolate and then the reduced FMN becomes re-oxidized by molecular O_2 in an H_2O_2-producing irreversible reaction. GOX is encoded by five redundant genes in *Arabidopsis*, which explains the lack of mutants with a photorespiratory phenotype. Notably, GOX-deficient maize requires high CO_2 conditions for survival, demonstrating the need of an intact photorespiratory metabolism not only in C_3 but also in C_4 plants [12]. The large amount of H_2O_2 produced during photorespiration is decomposed by the auxiliary enzyme CAT (catalase). Three *CAT* genes exist in *Arabidopsis*, where they form part of a general ROS (reactive oxygen species)-scavenging network, with *CAT2* being the most expressed gene in leaves. Functional deletion of *CAT2* causes severe effects in normal air, but not under non-photorespiratory conditions [13].

SGT (serine:glyoxylate aminotransferase) and GGT (glutamate:glyoxylate aminotransferase) work in parallel to convert glyoxylate into glycine. These two aminotransferases accept multiple substrate pairs, which causes some confusion in the naming of the enzymes and the coding genes. SGT preferably uses serine as the amino donor and is encoded by a single gene in *Arabidopsis* (*AGT1*) and in rice. Mutation of *AGT1* leads to a conditionally lethal high-CO_2-requiring phenotype [14]. GGT (re-)imports amino groups into the C_2 cycle from glutamate and can also use alanine, but not serine. Two very similar isoforms exist in *Arabidopsis* where GGT1 represents the major form in leaves [15,16].

Conversion of the two-carbon compound glycine into the three-carbon compound serine is a central function of the C_2 cycle and requires two mitochondrial enzymes, GDC (glycine decarboxylase) and SHMT (serine hydroxymethyltransferase). Both enzymes are highly susceptible to oxidation *in vivo* [17] and are likely targets for regulation by thioredoxin [18]. They are also essential components of general one-carbon metabolism (e.g. [19]).

GDC comprises four nuclear-encoded proteins. In the matrix of green-leaf mitochondria, they are all present at high concentrations and assemble to a fragile multienzyme

complex [20]. The smallest of these proteins, H-protein, interacts as a mobile substrate one after the other with P-, T- and L-protein. This happens through its lipoyl prosthetic group, and perturbation of mitochondrial lipoate biosynthesis hence impairs photorespiratory carbon flux [21]. First, P-protein extracts the aminomethylene moiety from glycine and ligates it to the lipoyl arm of H-protein to form S-aminomethylated H-protein. Next, T-protein transfers the lipoate-bound methylene group to THF (tetrahydrofolate) to produce CH_2-THF. In the course of these two reactions, the two lipoyl-sulfur atoms of H-protein become fully reduced and must be re-oxidized by L-protein. After completion of this last step, which requires NAD^+ and generates NADH, the GDC reaction cycle has been completed and S-oxidized H-protein is ready for re-use by P-protein. As the major result, the one-carbon compound CH_2-THF has been synthesized for subsequent use by SHMT. In addition, NADH and the waste products CO_2 and NH_3 were produced. Using malate as a vehicle, up to 50% of the generated reducing power goes to the cytosol. The remaining NADH is recycled within the mitochondria in a process that is largely uncoupled from ATP synthesis to rapidly provide NAD^+ for GDC [22]. Photorespiration hence affects the cellular $NADH/NAD^+$ balance and thus many other cellular processes including the tricarboxylic acid cycle [23] and nitrate assimilation [24,25].

SHMT completes the conversion of the two-carbon compound glycine into a three-carbon compound, serine. This occurs through the combination of CH_2-THF with a second molecule of glycine and, at the same time, regenerates THF for GDC. Other than GDC, SHMT activity is not restricted to the mitochondria. The reaction is fully reversible and specific isoforms decompose serine in other cellular compartments to provide CH_2-THF for biosynthetic reactions. Only one out of probably five SHMTs present in *Arabidopsis*, AtSHMT1, contributes significantly to photorespiratory metabolism [26]. Intriguingly, this enzyme is activated by Fd-GOGAT (ferredoxin-dependent glutamate synthase), which is normally responsible for ammonia assimilation in chloroplasts, but is also targeted to the mitochondria. Although the underlying mechanism remains speculative, and no effect was observed *in vitro*, activation occurs through physical interaction between the two proteins *in vivo* [27]. SHMT also produces considerable amounts of 5-formyl-THF. This compound strongly inhibits SHMT itself, and its recycling to THF is essential for the operation of the C_2 cycle [28].

Serine donates its amino group to glyoxylate, and, in the penultimate step of the C_2 cycle, the resulting hydroxypyruvate becomes reduced to glycerate by the peroxisomal NADH-dependent HPR (hydroxypyruvate reductase) 1. NADH cannot permeate the peroxisomal membrane and must be provided by pMDH (peroxisomal malate dehydrogenase), which oxidizes malate imported from the chloroplasts and the mitochondria via the cytosol. In contrast with the deleterious effect of blocks in other C_2 cycle reactions, the deletion of neither HPR1 nor pMDH strongly impairs plant growth [29,30]. This is because excessive hydroxypyruvate can move to the cytosol, where

it is reduced by the auxiliary enzyme NADPH-dependent HPR2. Hydroxypyruvate flux through the cytosol is small in moderate environments and is probably defined by the rate of NADH supply to HPR1. It is speculated that the co-operation of peroxisomal and cytosolic reactions allows more flexibility in the short-term adaptation of photorespiratory metabolism to changing environmental conditions [29].

In the chloroplasts, GLYK (glycerate 3-kinase) completes the C_2 cycle by returning three out of four 2PG carbon atoms back to the Calvin cycle in the form of 3PGA. GLYK is the only 3PGA-producing glycerate kinase. Most bacteria and all animals use glycerate kinases that produce glycerate 2-phosphate [31,32].

The C_2 cycle requires glutamate to transaminate glyoxylate and it generates ammonia during CH_2-THF synthesis from glycine. These processes and the refixation of photorespiratory ammonia together form the photorespiratory nitrogen cycle [2,33]. Refixation of ammonia occurs by two chloroplast-localized enzymes, GS2 (glutamine synthetase 2) and Fd-GOGAT. GS2 combines glutamate and ammonia to form glutamine. Fd-GOGAT then conveys the glutamine amido group to 2OG (2-oxoglutarate) to provide fresh glutamate for both transamination and ammonia refixation. Interestingly, *Arabidopsis* GS2 is dual-targeted to both chloroplasts and mitochondria, indicating that some photorespiratory ammonia (and CO_2) could be fixed directly within the mitochondria, but the physiological significance of such a process remains to be shown [34,35]. Entry of 2OG into the chloroplasts and the exit of glutamate are accomplished by two separate translocators, DiT1 for 2OG and DiT2 for glutamate, which operate in a coupled manner since they both counter-exchange malate [11,36]. Apart from these two translocators and the porine-like peroxisomal channels, little is known about transport proteins involved in photorespiration.

Origin of photorespiration

Photorespiration, as a consequence of the catalytic mechanism of CO_2 fixation by Rubisco, reflects the origin of oxygenic photosynthesis 3.5 billion years ago in the anaerobic environment of the Pre-Cambrian ocean [37,38]. Approx. 1.2 billion years ago, this capability was transferred from cyanobacteria via endosymbiosis to eukaryotes leading to the evolution of algae and plants [39].

Approx. 500 million years ago, when land plants appeared on Earth, O_2 levels had increased from probably ∼2% at the time of the endosymbiotic event to approximately the levels we have today, but CO_2 was still 15-fold higher than in our present atmosphere. Massive photosynthetic activity in the Carboniferous period (360–300 million years ago) then resulted in an intermittent fall in CO_2 levels and a rise in O_2 levels to more than 30% [40,41]. Probably at about this time in Earth history, i.e. long after the primary endosymbiotic event leading to plastids, cyanobacteria evolved highly efficient CCMs (CO_2-concentrating mechanisms) to rapidly capture inorganic carbon, present even at a very low concentration [42]. CCMs comprise high-affinity CO_2- and

Figure 2 | Cyanobacterial photorespiratory metabolism

Cyanobacterial 2PG metabolism comprises overlapping plant-like (grey) and bacterial pathways [46]. Plant GOX-like enzymes and GLYK are present only in filamentous nitrogen-fixing cyanobacteria. In other cyanobacteria, GLCDHs produce glyoxylate, and 3PGA is formed by the co-operation of 2PGA-forming glycerate kinases (GK) with phosphoglyceromutase (PGM). Cyanobacteria can circumvent the glycine-into-serine conversion by directly converting glyoxylate into hydroxypyruvate by using glyoxylate carboligase (GCL) and TSR. Some species, including *Synechocystis*, are able to completely decompose glyoxylate to CO_2 via oxalate decarboxylase (ODC) and formate dehydrogenase (FDH).
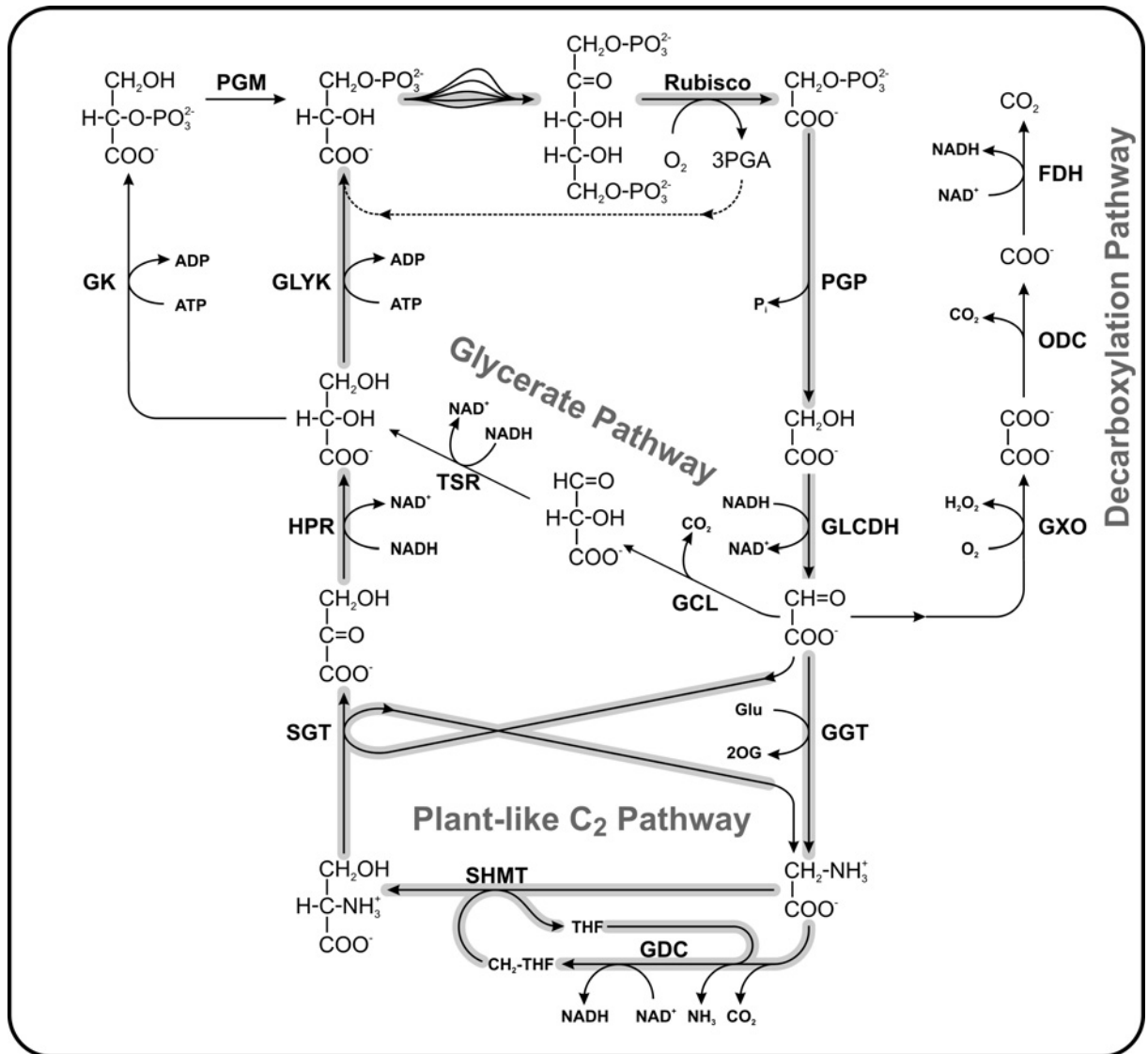
bicarbonate-uptake systems in combination with a sequestration of Rubisco in carboxysomes, in which a very high CO_2 concentration favours carboxylation and inhibits RubP oxygenation. It was hence long thought that 2PG metabolism is incomplete in cyanobacteria and that corresponding carbon fluxes are negligible.

It was only recently that new genetic data allowed the identification of a number of genes that are involved in cyanobacterial 2PG metabolism, for example genes for TSS (tartronic semialdehyde synthase) and TSR (tartronic semialdehyde reductase), two components of the bacterial glycer-

ate pathway converting glyoxylate into glycerate. In addition, a number of genes were identified that encode proteins with high similarities to enzymes of the plant C_2 cycle [43].

Most of the functional analyses have been performed with *Synechocystis* sp. PCC 6803. This unicellular freshwater cyanobacterium is genetically transformable by exogenous DNA, can be grown heterotrophically, and hence represents a popular model organism for reverse genetics experiments. Systematic deletion of all genes that could be involved in 2PG metabolism disclosed three overlapping pathways (Figure 2).

Blockade of one of these pathways, which is very much reminiscent of the plant C_2 cycle, by inactivating GDC only slightly decreased growth in the photorespiratory condition of normal air [44]. Blockade of the glycerate pathway by inactivation of TSR also produced only minor effects. The combination of both blocks in a double-mutant distinctly reduced the performance, but was still not lethal [45]. Only the additional inactivation of oxalate decarboxylase, which is part of a third metabolic route in some cyanobacteria including *Synechocystis*, finally resulted in a triple-mutant that could survive only in high CO_2, but not in normal air [46]. At least some of the observed detrimental effects were due to suboptimal concentrations of available Mg^{2+}, caused by the formation of co-ordination complexes with accumulating glycine [47]. This three-branch model of 2PG metabolism was supported further by simultaneous inactivation of two GLCDHs (glycolate dehydrogenases) present in *Synechocystis*. This blockade, which is located upstream of the branching into the three separate routes, led to a massive accumulation of glycolate, and the mutant very much resembled the triple-mutant mentioned above: it was unable to grow in normal air, but grew healthily in a CO_2-enriched environment.

Cyanobacteria are a very diverse group of organisms, and it is not surprising that variations also exist in photorespiratory metabolism. Such differences exist among the cyanobacteria and between cyanobacteria and plants. For example, none of the known cyanobacterial genomes harbours a plant-like PGP. On the other hand, the genomes of the marine cyanobacterium *Prochlorococcus* SS120 and of the nitrogen-fixing *Anabaena* PCC 7120 harbour genes for plant-type GOX, whereas the conversion of glycolate into glyoxylate in *Synechocystis* is rather mediated by GLCDH. Similarly, a plant-type GLYK is present in *Prochlorococcus* and in *Anabaena*, but not in *Synechocystis*, which has a bacterial-type glycerate kinase [32] and hence requires phosphoglyceromutase to produce 3PGA and complete the pathway.

These analyses show that cyanobacteria, the earliest organisms to perform oxygenic photosynthesis, produce significant amounts of photorespiratory 2PG. As a valuable resource, 2PG is not just disposed into the environment in the form of glycolate as was believed formerly. Apparently, the production of 2PG as well as its recovery and recycling into 3PGA have been essential features of oxygenic photosynthesis, and many enzyme components of the metabolic pathways responsible have been endosymbiotically conveyed to plants [46].

Future challenges

Application of reverse genetics and the availability of many new tools allowed the identification of individual components of the photorespiratory C_2 cycle at the gene level and analysing cross-talk to other metabolic pathways. A much higher complexity than imagined of photorespiratory metabolism and its integration in whole-cell metabolism became apparent. Future research will shed more light on the evolutionary processes leading to plant photorespiration, analyse the passage of photorespiratory metabolites through organellar membranes, and find out whether and how photorespiration is regulated in the context of whole-cell metabolism and changing environments.

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