Synthetic biology approaches for improving photosynthesis

Armin Kubis, Arren Bar-Even*

Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany

*Corresponding authors
Arren Bar-Even:
phone: +49 331 567-8910;
Email: bar-even@mpimp-golm.mpg.de

Keywords: carbon fixation, Rubisco, Calvin Cycle, photorespiration, carbon concentrating mechanisms, C4 metabolism

Highlight section

We discuss current efforts to boost plant carbon fixation using a synthetic biology approach, highlighting the engineering of Rubisco, optimizing the Calvin Cycle, introducing carbon concentrating mechanisms, and rewiring photorespiration.

Abstract

The phenomenal increase in agricultural yields that we have witnessed in the last century has slowed down as we approach the limits of selective breeding and optimization of cultivation techniques. To support the yield increase required to feed an ever growing population, we will have to identify new ways to boost the efficiency by which plants convert light into biomass. This challenge could be potentially tackled using state-of-the-art synthetic biology techniques to rewrite plant carbon fixation. In this review, we use recent studies to discuss and demonstrate different approaches for enhancing carbon fixation, including engineering Rubisco for higher activity, specificity, and activation; changing the expression level of enzymes within the Calvin Cycle to avoid kinetic bottlenecks; introducing carbon concentrating mechanisms such as inorganic carbon transporters, carboxysomes, and C4 metabolism; and rewiring photorespiration towards more energetically efficient routes or pathways that do not release CO_2. We conclude by noting the importance of prioritizing and combining different approaches towards continuous and sustainable increase of plant productivities.

Introduction

Selective breeding and optimization of cultivation techniques have historically driven increases in agricultural output. In the last century, these efforts have adopted a more scientific approach with the development of the Haber-Bosch process (Haber and Le Rossignol, 1909; Sutton *et al.*, 2008), and, later, the “green revolution” (Khush, 2001). Since 1961, global rice and wheat yields increased by 150% and 210%, respectively (FAO, 2018). However, we have recently started to witness stagnation in growth improvement of major crops such as rice in China (Peng *et al.*, 2009) or wheat in the USA (Ray *et al.*, 2012). This presents a major problem, as further yield increases are sorely needed to feed human population, especially considering global shift towards meat-dependent diets, use of arable lands to feed bio-refineries, deleterious effects of climate change, and continuous erosion of agricultural land (Godfray *et al.*, 2010; Tilman *et al.*, 2011).

Agricultural yield can be modeled as a product of three factors (Monteith and Moss, 1977; Fletcher *et al.*, 2011): (i) efficiency of intercepting light; (ii) efficiency of converting intercepted light into biomass; and (iii) the harvest index, i.e., the fraction of biomass that is captured in the harvested part. In the past, improved yields have largely been achieved by increasing the light capture efficiency and the harvest index; however, these two factors now appear to approach their practical limits (Long *et al.*, 2006). Therefore, the efficiency by which plants convert light to biomass has become the prime focus for further improvement (Long *et al.*, 2006). This efficiency is determined by two main processes, the light-dependent reactions, in which photoenergy is used for the generation of the cellular redox and energy carriers NADPH and ATP, and the light-independent reactions, which use these carriers to fix CO_2 and reduce it to organic carbon. The efficiency of both processes is unlikely to be improved by a classic selective breeding approach – as demonstrated by a recent study exploring 80 years of soybean breeding (Koester *et al.*, 2016) – but could be potentially increased by dedicated engineering (Zhu *et al.*, 2010). The focus of this review is the use of synthetic biology tools for boosting the efficiency and rate of carbon fixation. Rather than discuss the technical aspects of synthetic biology in plants – for which we refer the readers to other reviews (DePaoli *et al.*, 2014; Liu and Stewart, 2015; Boehm and Bock, 2018; Piatek *et al.*, 2018; Vazquez-Vilar *et al.*, 2018) – we emphasize conceptual strategies to boost carbon fixation. In particular, we discuss efforts aiming to improve carboxylation by Rubisco, optimize expression levels of enzymes within the Calvin Cycle, introduce carbon concentration mechanisms, and rewire photorespiration. We claim that multiple complementary strategies are paving the way towards substantial yield increases that are not feasible using conservative selective breeding techniques.

Engineering Rubisco

Rubisco, the key enzyme of the Calvin cycle, is probably the most abundant protein in the biosphere (Ellis, 1979; Raven, 2013), and is responsible for assimilating the vast majority of inorganic carbon (Raven, 2009). The enzyme catalyzes the condensation of ribulose 1,5-bisphosphate (RuBP) with CO_2 to give two molecules of glycerate 3-phosphate (G3P). Despite its key biochemical role, Rubisco is considerably slower than most enzymes in central metabolism (Bar-Even *et al.*, 2011). Moreover, Rubisco is not completely specific to CO_2 and also accepts O_2, leading to the formation of 2-phosphoglycolate (2PG) that needs to be reassimilated. In the C3 model plant *A. thaliana*, the carboxylation to oxygenation ratio was measured to be as low as 2.3:1 at high light conditions (Ma *et al.*, 2014). Suppressing oxygenation reactions by cultivating plants at elevated CO_2 concentrations has repeatedly shown to increase productivity. For example, a meta-analysis of 70 studies showed that rice yields increased by 23% when CO_2 concentrations were raised to 627 ppm (Ainsworth, 2008). These results indicate that engineering Rubisco for higher CO_2 specificity could substantially boost yield.

Approaches to improve Rubisco catalysis by random or side-directed mutagenesis have generally failed to yield substantial kinetic enhancements (Somerville and Ogren, 1982; Spreitzer *et al.*, 2005; Whitney *et al.*, 2011; Wilson *et al.*, 2016). Comparisons between Rubisco variants from a range of different organisms have revealed a trade-off between CO_2 specificity and carboxylation velocity (Tcherkez *et al.*, 2006; Savir *et al.*, 2010; Galmés *et al.*, 2014), although several recent studies challenge this finding (Young *et al.*, 2016; Cummins *et al.*, 2018). Considering this tradeoff, it actually seems that most Rubisco variants are well adapted to their intracellular environment. Still, as ambient CO_2 concentrations are changing at a rate faster than plants can adapt to, it was suggested that replacing plant Rubisco with another variant could boost carbon fixation by up to 25% (Zhu *et al.*, 2004; Orr *et al.*, 2016). Substituting one Rubisco variant with another is undoubtedly a challenging task, but was already demonstrated using homodimeric Rubisco from the α-proteobacterium *R. rubrum* (Whitney and Andrews, 2001) and, more recently, using a fast hexadecameric Rubisco from *S. elongates* (Lin *et al.*, 2014; Occhialini *et al.*, 2016). Coexpression of supporting chaperones, including the appropriate accumulation factors, can assist in producing an active Rubisco recombinantly, and can further facilitate efforts to enhance the kinetics of this key enzyme via mutagenesis (Aigner *et al.*, 2017).

Carbon fixation via Rubisco can be potentially improved by means other than direct engineering of its catalytic parameters. The addition of a CO_2 molecule to an active site lysine, i.e., carbamylation, is a prerequisite for Rubisco activity (Lorimer and Miziorko, 1980), but can be hindered by the premature binding of RuBP or other sugar phosphates (Portis, 2002; Parry *et al.*, 2007). The

catalytic chaperone Rubisco activase (Rca) removes the sugar phosphate inhibitors from an inactive uncarbamylated enzyme or an inhibited carbamylated Rubisco (Portis, 2002). As the thermal instability of Rca was shown to constrain carbon fixation under moderate heat stress (Salvucci *et al.*, 2004), it has become an attractive target for engineering towards enhanced photosynthesis. For example, by increasing the thermostability of Rca in *A. thaliana*, improved photosynthesis and growth rate were demonstrated under a moderate heat stress (Kurek *et al.*, 2007; Kumar *et al.*, 2009). Similarly, overexpression of maize Rca in rice led to higher activation state of Rubisco in low light and faster response of photosynthesis when light intensities increased (Yamori *et al.*, 2012).

Optimizing expression of Calvin Cycle enzymes

G3P produced by Rubisco need to be metabolized by nine enzymes of the Calvin cycle to regenerate RuBP. This regeneration process, whose rate has to match that of Rubisco, is known to limit carbon fixation rate under certain conditions. Computational models have suggested that the natural distribution of enzymes within the Calvin Cycle is not optimal and could limit photosynthesis (Zhu *et al.*, 2007). Specifically, it was predicted that higher levels of sedoheptulose-1,7-bisphosphatase and fructose-1,6-bisphosphate aldolase, as well as enzymes linked to sink capacity, could support higher productivity.

Unsurprising, under elevated CO_2 concentrations, the rate of Rubisco becomes less limiting and carbon fixation is mostly constrained by RuBP regeneration. For example, studies of *N. tabacum* at 930 ppm CO_2 showed that reducing Rubisco levels by 30-50% did not inhibit growth (Masle *et al.*, 1993). Similar results were obtained in rice plants in which Rubisco levels were reduced by 65% at 1000 ppm CO_2. On the other hand, overexpression of sedoheptulose-1,7-bisphosphatase in *N. tabacum* at 585 ppm CO_2 resulted in higher carbon fixation rate (Rosenthal *et al.*, 2011). Similarly, at 700 ppm, increased levels of fructose-1,6-bisphosphate aldolase in *N. tabacum* led to increased biomass (Uematsu *et al.*, 2012).

Even at ambient CO_2 concentration, overexpression of limiting enzymes of the Calvin Cycle was shown to boost carbon fixation. In *N. tabacum*, overexpression of sedoheptulose 1,7-bisphosphatase (Lefebvre *et al.*, 2005) and fructose 1,6-bisphosphatase (Miyagawa *et al.*, 2001) increased photosynthetic rates and biomass. Similarly, the co-overexpression of sedoheptulose-1,7-bisphosphatase and fructose-1,6-phosphate aldolase enhanced photosynthesis and yield (Simkin *et al.*, 2015).

Establishing carbon concentrating mechanisms

To mitigate the problem of oxygenation, and further enable the use of faster (and less specific) Rubsico, multiple organisms have developed carbon concentrating mechanisms (CCMs) to concentrate CO_2 at the site of Rubisco. As C3 plants lack CCMs, it was proposed to introduce them to increase photosynthetic efficiency. Two main approaches are actively pursued: (A) introduction of biophysical CCMs from cyanobacteria and green algae (Long *et al.*, 2016; Rae *et al.*, 2017); and (B) introduction of C4 anatomy and metabolism (Hibberd *et al.*, 2008; Schuler *et al.*, 2016).

Biophysical CCM are found in cyanobacteria (Kupriyanova *et al.*, 2013) and in green algae like *C. reinhardtii* (Mackinder, 2018). In such CCM, bicarbonate is actively transported into the cytosol in which carbonic anhydrase is lacking. From there, bicarbonate is further transported into specialized compartments packed with Rubisco – carboxysomes in cyanobacteria and pyrenoids in green algae – where it is dehydrated to CO_2 by carbonic anhydrase. It is thought that both carboxysomes and pyrenoids present a diffusion barrier for CO_2 and O_2, keeping the former molecule in and the latter molecule out, and thus enhancing carboxylation and suppressing oxygenation (Mangan *et al.*, 2016).

Establishing biophysical CCM in plants is a challenging task that first requires the expression and correct localization of inorganic carbon transporters. It was suggested that the transporters themselves could increase carbon fixation rate albeit to a limited extent (McGrath 2014, Yin 2017). Indeed, overexpression of the putative-inorganic carbon transporter from cyanobacteria, ictB, in *A. thaliana*, tobacco, rice, and soybean was reported to increase photosynthetic rate and biomass (Lieman-Hurwitz *et al.*, 2003, 2005; Yang *et al.*, 2008; Simkin *et al.*, 2015; Hay *et al.*, 2017). In contrast, expression of other transporters from cyanobacteria or *C. reinhardtii* did not increase yield or improve growth, despite correct localization within the plant cells (Rolland *et al.*, 2016; Uehara *et al.*, 2016; Atkinson *et al.*, 2016). Optimizing transporter activity is therefore still an open challenge that needs to be resolved before commencing with the next step: assembly of Rubisco containing compartments. The establishment of these sophisticated structures would enable further increase in CO_2 concentration at the site of Rubisco and could therefore substantially enhance carbon fixation. Recently, simplified carboxysome structures were introduced into the chloroplasts of *N. tabacum* (Long *et al.*, 2018). Yet, these are expected to enhance photosynthesis only after combination with functional inorganic carbon transporters (McGrath and Long, 2014).

Engineering C4 metabolism

As an alternative to biophysical CCM, ongoing research is dedicated to introducing C4 metabolism into C3 plants (Schuler *et al.*, 2016). C4 metabolism utilizes the most efficient carbon fixation enzyme – PEP carboxylase – to temporarily capture inorganic carbon, which is then transported to the vicinity of Rubisco (Jenkins *et al.*, 1989). Specifically, PEP carboxylase in the mesophyll cells 'borrows' PEP and converts it to oxaloacetate, which is further metabolized to malate or aspartate. These C4 acids are transported to the bundle sheath cells and decarboxylated to release CO_2 next to RubsiCO, which is mainly localized in these cells. Pyruvate, the product of this decarboxylation, is then transported back to the mesophyll cells to regenerate PEP. Hence, the entire C4 cycle, which depends on a special anatomy termed "Kranz anatomy" (mesophyll cells surrounding bundle sheath cells), can be regarded as a sophisticated CO_2 pump that results in ~10 times higher concentration of inorganic carbon in the vicinity of RubsiCO (Jenkins *et al.*, 1989).

Engineering C4 photosynthesis in C3 plants has been outlined as a stepwise process (Schuler *et al.*, 2016) that includes alteration of plant tissue anatomy, establishment of bundle sheath morphology, as well as ensuring a cell-type specific enzyme expression. Although challenging, engineering a C3 plant to have C4 metabolism seems to be a feasible goal as it is known to have emerged independently at least 66 times in different phylogentic backgrounds (Sage *et al.*, 2012). Importantly, C3 plants already harbor the main enzymes of C4 metabolism, e.g., PEP carboxylase (Aubry *et al.*, 2011), and are known to shuttle carbon from the vasculature into the surrounding cells in a way similar to that of C4 plants (Hibberd and Quick, 2002; Brown *et al.*, 2010). This provides a solid basis to replicate the emergence of C4 metabolism by direct engineering.

Nevertheless, despite international efforts, a synthetic C4 plant has yet to be reported. Following Richard Feynman's famous quote "What I cannot create, I do not understand", it seems that incomplete understanding of C4 metabolism hampers its engineering. Specifically, the metabolic shuttling of intermediates between mesophyll and bundle sheath cells and the factors necessary to create Kranz anatomy are still not fully clear and need to be elucidated (Schuler *et al.*, 2016).

It might not be necessary to establish a complete C4 metabolism in order to improve carbon fixation. It was recently suggested that engineering a C3-C4 intermediate metabolism could enhance productivity (Schlüter and Weber, 2016). For example, in C3-C4 intermediate type I plants, photorespiratory glycine is transported from the mesophyll cells to the bundle sheath cells for decarboxylation. In the bundle sheath cells, the mitochondria are closely associated with the chloroplast, thereby enhancing re-assimilation of released CO_2 by nearby RubsiCO (Monson and Edwards, 1984; Rawsthorne *et al.*, 1988). Establishing this intermediary metabolism within C3

plants, besides being useful on its own to boost carbon fixation, would provide a milestone towards further engineering of complete C4 metabolism.

An interesting alternative engineering target is crassulacean acid metabolism (CAM). While C4 metabolism increases CO_2 concentration at the vicinity of Rubisco via spatial decoupling, CAM accomplishes the same goal via temporal decoupling. Specifically, inorganic carbon is temporarily fixed by the highly efficient PEP carboxylase during the night, when the stomata are open and CO_2 can freely enter the cell. Malate, the indirect product of the carboxylation, is stored within the vacuole. During the day, when the stomata are closed, malate is decarboxylated, releasing CO_2 and maintaining its high concentration for subsequent fixation by Rubisco and the Calvin Cycle. Besides increasing CO_2 concentration in the vicinity of Rubisco, CAM reduces water evaporation and increase water-use efficiency by 20-80% (Borland *et al.*, 2009), making CAM plants highly suitable for arid climates. Similarly to C4 metabolism, CAM has arisen multiple times in a taxonomically diverse range of plants, indicating that its necessary components exist in C3 plants which could potentially be engineered towards this unique carbon metabolism (DePaoli *et al.*, 2014). Furthermore, the existence of C3-CAM intermediate species and plants that switch between both metabolic modes further supports the potential of engineering C3 metabolism towards CAM (Borland *et al.*, 2011). Such engineering would require precise control of the activity key enzymes (e.g., PEP carboxylase, malic enzyme, and Rubisco), stomatal conductance, and intracellular transport (e.g., to and from the vacuole) (Borland *et al.*, 2014; DePaoli *et al.*, 2014; Yang *et al.*, 2015).

Rewiring photorespiration

2PG, the product of Rubisco's oxygenation activity, is recycled to the Calvin Cycle in a process termed photorespiration. This rather long pathway requires the shuttling of metabolites across multiple organelles and is considered inefficient as it dissipates energy by releasing ammonia and using oxygen as an electron acceptor. Moreover, photorespiration releases one CO_2 molecule in the recycling of two 2PG molecules and hence directly counteracts carbon fixation by the Calvin Cycle. The inefficiencies associated with the recycling of 2PG cannot be prevented by simply blocking photorespiration, as this pathway plays an essential role in plant metabolism (Somerville and Ogren, 1979) and reduction of its flux was shown to negatively affect photosynthesis (Servaites and Ogren, 1977; Wingler *et al.*, 1997; Heineke *et al.*, 2001). One explanation for this lies in the inhibitory effects exerted by several photorespiratory intermediates. For example, 2PG was shown to inhibit triosephosphate isomerase and sedoheptulose 1,7-bisphosphate phosphatase (Anderson, 1971; Flügel *et al.*, 2017), glyoxylate impairs Rubisco activation (Chastain and Ogren, 1989; Campbell and Ogren, 1990; Hausler *et al.*, 1996; Savir *et al.*, 2010), and glycine interferes with Mg^{2+} availability

(Eisenhut *et al.*, 2007). Based on these observations, it was suggested that increased photorespiratory flux could prevent the accumulation of inhibitory intermediates and enhance photosynthesis; indeed, this was demonstrated upon overexpression of components of the glycine cleavage system in *A. thaliana* (Timm *et al.*, 2012, 2015) and in *N. tabacum* (Lopez-Calcagno *et al.*, 2018).

While photorespiration cannot be avoided, it might be possible to replace the natural pathway with more efficient alternatives. The first bypass suggested in this regard was inspired by cyanobacterial photorespiration (Eisenhut, 2006), where glyoxylate is condensed and reduced to directly generate the key photorespiratory intermediate glycerate. This pathway was implemented in *A. thaliana* (Kebeish *et al.*, 2007) and later in *C. sativa* (Dalal *et al.*, 2015) using glycolate dehydrogenase, glyoxylate carboxyligase, and tartronic semialdehyde reductase from *Escherichia coli*. In both cases, this metabolic bypass, dissipating less energy and shifting CO_2 release from the mitochondria to the chloroplast, was shown to increase photosynthesis and biomass.

However, it was shown that expression of only the first enzyme of the pathway, glycolate dehydrogenase, suffices to enhance photosynthesis. Supporting this, chloroplastic expression of glycolate dehydrogenase in *S. tuberosum* induced a 2.3-fold increase in tuber yield (Nölke *et al.*, 2014). This suggests that the benefits of glycerate-pathway might not stem from more efficient recycling of 2PG but rather from oxidation of glycolate to glyoxylate. Indeed, incubation with glyoxylate was shown to increase carbon fixation – potentially due to suppression of Rubisco oxygenation – in both tobacco leaf disks (Oliver and Zelitch, 1977) and soybean mesophyll cells (Oliver, 1980).

Another photorespiratory bypass involves the complete oxidation of 2PG to CO_2 via a catabolic pathway that consist of glycolate dehydrogenase, malate synthase, malic enzyme, and pyruvate dehydrogenase (Maier *et al.*, 2012). While the authors reported increased biomass and photosynthesis, it is still unclear which mechanism supports the beneficial effect of the pathway, as a theoretical model predicts a negative effect when 2PG is completely oxidized (Xin *et al.*, 2015).

Carbon-conserving photorespiration

As the main problem associated with photorespiration is (arguably) the release of CO_2, bypasses that do not lead to the loss of carbon could dramatically boost carbon fixation. Several synthetic carbon-conserving bypasses have been suggested. In the *de novo* 2PG salvage pathway (Ort *et al.*, 2015), 2PG was suggested to be reduced to 2-phosphoglycolaldehyde, which is subsequently condensed with dihydroxyacetone phosphate to give xylulose bisphosphate. This intermediate is

254 then dephosphorylated to xylulose 5-phosphate, a Calvin cycle metabolite. The main challenges of
255 this proposed bypass is the reversibility of most of its reactions (resulting in low driving force), the
256 low concentration of 2PG, and the inhibitory effect of xylulose bisphosphate (Yokota, 1991; Zhu and
257 Jensen, 1991; Parry *et al.*, 2007).

258 Recently, a systematic analysis identified multiple synthetic routes that can bypass photorespiration
259 without the release of CO_2. Several of these pathways involve the reduction of glycolate (the
260 concentration of which is considerably higher than that of 2PG) to glycolaldehyde, which then
261 undergoes an aldol condensation with a phosphosugar from the Calvin Cycle to generate a longer
262 chain phosphosugar that is reintegrated into Calvin Cycle (Bar-Even, 2018; Trudeau *et al.*, 2018). A
263 computational model indicated that these pathways can boost photosynthesis under all
264 physiologically relevant irradiation and intracellular CO_2 levels.

265 The operation of these carbon conserving bypass routes depends on the conversion of glycolate to
266 glycolaldehyde, but this activity is not supported by any known enzyme. To establish this activity two
267 enzymes were engineered (Trudeau *et al.*, 2018). First, acetyl-CoA synthetase from *E. coli* was
268 engineered to accept glycolate, thus generating glycolyl-CoA. Next, propionyl-CoA reductase from
269 *Rhodopseudomonas palustris* was engineered to accept glycolyl-CoA, reducing it to glycolaldehyde.
270 The cofactor specificity of this latter enzyme was switched, such that it could use NADPH – the
271 photosynthetic electron carrier – as an electron donor. The two engineered enzymes were
272 combined, in a test-tube, with fructose 6-phosphate aldolase (condensing glycolaldehyde with
273 glyceraldehyde 3-phosphate to generate arabinose 5-phosphate), arabinose 5-phosphate
274 isomerase, and phosphoribulokinase. Upon addition of glycolate and glyceraldehyde 3-phosphate,
275 NADPH and ATP were consumed and RuBP was found to accumulate (Trudeau *et al.*, 2018),
276 demonstrating the *in vitro* activity of an alternative photorespiration route that does not release CO_2.

277 It was further proposed to go beyond carbon conservation, and engineer a photorespiration bypass
278 that fixes CO_2 and thus directly support the activity of the Calvin Cycle. One such a carbon-positive
279 bypass was inspired by the 3-hydroxypropionate bicycle (Shih *et al.*, 2014). Here, glycolate is
280 oxidized to glyoxylate, which is then metabolized and further carboxylated to pyruvate. Towards the
281 implementation of this bypass, six non-native genes from *C. aurantiacus* were expressed in
282 cyanobacteria, but no distinct growth phenotype was evident.

283 In another study, glycolate was not recycled to the Calvin cycle but instead metabolized to acetyl-
284 CoA via the synthetic malyl-CoA-glycerate pathway (Yu *et al.*, 2018). This pathway can further be
285 used to generate acetyl-CoA from photosynthetic C3 sugars via an additional CO_2-fixing step,

thereby bypassing CO_2 release by pyruvate dehydrogenase. In cyanobacteria, the pathway facilitated a two-fold increase in bicarbonate assimilation.

Conclusions

The increasing number of studies demonstrating improved photosynthesis and growth by engineering different components of the light-dependent and independent reactions indicates that we are on the right path. Yet, many challenges are ahead of us. Beside the technical difficulties, which we did not discuss here and refer the reader to other reviews (DePaoli *et al.*, 2014; Liu and Stewart, 2015; Boehm and Bock, 2018; Piatek *et al.*, 2018; Vazquez-Vilar *et al.*, 2018), there is one key barrier that worth elaborating on, which is system complexity. Complex systems are notoriously difficult to engineer as the effect of even small changes can have substantial results that cannot be easily predicted. While mathematical models can help deal with such complexity, the lack of knowledge regarding many of involved components commonly hinders accurate prediction. Plant carbon metabolism provides an excellent example of a complex system, the response of which to changes is hard to foretell. Previous attempts to engineer carbon fixation demonstrate this vividly. Perhaps the best example is the engineering of photorespiration bypass routes as described above. While few bypasses were already shown to enhance photosynthesis, the cause of this effect is probably different than that originally suggested, as chloroplastic oxidation of glycolate suffices to support most of the beneficial effects. Unraveling this mystery would require deep understanding of the intricate interplay between all system components, a task which we have yet to fully achieve.

Moreover, while some engineering efforts show only minor benefits in isolation, the key for future improvements lies in the correct combination of multiple strategies. Indeed, first examples for beneficial cumulative effects have been reported (Simkin *et al.*, 2015). It is further clear that not all strategies can be implemented with similar ease. Overexpressing a Calvin Cycle enzyme, for example, is considerably easier than rerouting photorespiration via a synthetic pathway that does not release CO_2. It is therefore important to carefully choose targets for the near- and medium-future and progress in a way that ensures intermediate gains. For example, establishing a C3-C4 intermediate metabolism does not only provide a solid basis for further engineering of a complete C4 metabolism, but is expected to boost carbon fixation by itself. Once we gain the required proficiency in rewiring plant central metabolism, we can aim at even bigger targets, for example, replacing Rubisco with a set of enzymes, each responsible for a different catalytic step (Bar-Even, 2018), or replacing the Calvin Cycle with a synthetic carbon fixation pathway (Schwander *et al.*, 2016).

Acknowledgements

The authors thank Charlie Cotton for critical reading of the manuscript. This work was funded by the Max Planck Society and by the European Union's Horizon 2020 FET Programme under the grant agreement No 686330 (FutureAgriculture).

Key developments box

Assembly of Rubisco-containing carboxysomes in tobacco chloroplasts

Assembly of a simplified α-carboxysome in tobacco chloroplasts by replacing native Rubisco with large and small subunits of Rubisco from cyanobacteria and two key structural subunits. The introduction of carboxysomes to plant chloroplasts is a key step towards establishing a full biophysical carbon concentrating mechanism in higher plants (Long *et al.*, 2018).

Design and *in vitro* realization of carbon-conserving photorespiration

A systematic search and analysis of synthetic photorespiration bypass routes that do not release CO_2 reveals that these can enhance carbon fixation rate under all relevant physiological conditions. Two enzymes were engineered to jointly enable the reduction of glycolate to glycolaldehyde. The combination of these evolved enzymes with existing ones supported the *in vitro* recycling of glycolate to RuBP without the loss of CO_2, indicating the feasibility of carbon conserving photorespiration (Trudeau *et al.*, 2018).

The synthetic malyl-CoA-glycerate pathway supports photosynthesis

An *in vivo* demonstration of a synthetic pathway that can support photosynthesis in two ways. First, it can produce acetyl-CoA from C3 sugars without releasing CO_2. It can also assimilate photorespiratory glycolate without loss of carbon (Yu *et al.*, 2018).

Carbon fixation via a novel pathway *in vitro*

An *in vitro* reconstruction of a synthetic carbon fixing pathway, the CETCH cycle, based on highly efficient reductive carboxylation. The pathway, utilizing 17 enzymes that originate from 9 organisms, was optimized by a combination of enzyme engineering and metabolic proofreading (Schwander *et al.*, 2016).

Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field grown transgenic tobacco plants

Increased biomass upon overexpression of a limiting photorespiratory protein in tobacco grown in field conditions. This indicates that optimization of expression levels within native carbon fixation-related pathways could be harnessed to increase productivity, and that photorespiration could be improved even without the need for synthetic pathways (Lopez-Calcagno *et al.*, 2018).

The road to C_4 photosynthesis: evolution of a complex trait via intermediary states

A case for engineering C3-C4 intermediate metabolism as a way to increase photosynthetic efficiency and set the stage towards future realization of complete C4 metabolism. This study suggests that a detailed and mechanistic understanding of C3-C4 intermediates could provide valuable guidance for experimental designs aiming to boost carbon fixation (Schlüter and Weber, 2016).

Evolving Methanococcoides burtonii archaeal Rubisco for improved photosynthesis and plant growth

A demonstration of the use of directed laboratory evolution to improve the kinetic properties of Rubisco from an archaeal origin. The improved Rubisco variant was introduced to tobacco chloroplast and demonstrated to increase photosynthesis. Such protein engineering strategies could be used to address the kinetic limitations of key enzymes, thus supporting higher metabolic fluxes and boosting productivities (Wilson *et al.*, 2016).

References

Aigner H, Wilson RH, Bracher A, Calisse L, Bhat JY, Hartl FU, Hayer-Hartl M. 2017. Plant RuBisCo assembly in E. coli with five chloroplast chaperones including BSD2. Science **358**, 1272–1278.

Ainsworth EA. 2008. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biology **14**, 1642–1650.

Anderson LE. 1971. Chloroplast and cytoplasmic enzymes II. Pea leaf triose phosphate isomerases. Biochimica et Biophysica Acta - Enzymology **235**, 237–244.

Atkinson N, Feike D, Mackinder LCM, Meyer MT, Griffiths H, Jonikas MC, Smith AM, McCormick AJ. 2016. Introducing an algal carbon-concentrating mechanism into higher plants: location and incorporation of key components. Plant Biotechnology Journal **14**, 1302–1315.

Aubry S, Brown NJ, Hibberd JM. 2011. The role of proteins in C3 plants prior to their recruitment into the C4 pathway. Journal of Experimental Botany **62**, 3049–3059.

Bar-Even A. 2018. Daring metabolic designs for enhanced plant carbon fixation. Plant Science **273**, 71–83.

Bar-Even A, Noor E, Savir Y, Liebermeister W, Davidi D, Tawfik DS, Milo R. 2011. The moderately efficient enzyme: Evolutionary and physicochemical trends shaping enzyme parameters. Biochemistry **50**, 4402–4410.

Boehm CR, Bock R. 2018. Recent advances and current challenges in synthetic biology of the plastid genetic system and metabolism. Plant Physiology **3**, pp.00767.2018.

Borland AM, Barrera Zambrano VA, Ceusters J, Shorrock K. 2011. The photosynthetic plasticity of crassulacean acid metabolism: an evolutionary innovation for sustainable productivity in a changing world. New Phytologist **191**, 619–633.

Borland AM, Griffiths H, Hartwell J, Smith JAC. 2009. Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. Journal of Experimental Botany **60**, 2879–2896.

Borland AM, Hartwell J, Weston DJ, Schlauch KA, Tschaplinski TJ, Tuskan GA, Yang X,

Cushman JC. 2014. Engineering crassulacean acid metabolism to improve water-use efficiency. Trends in Plant Science **19**, 327–338.

Brown NJ, Palmer BG, Stanley S, *et al.* 2010. C $_4$ acid decarboxylases required for C $_4$ photosynthesis are active in the mid-vein of the C $_3$ species *Arabidopsis thaliana* , and are important in sugar and amino acid metabolism. The Plant Journal **61**, 122–133.

Campbell WJ, Ogren WL. 1990. Glyoxylate inhibition of ribulosebisphosphate carboxylase/oxygenase activation in intact, lysed, and reconstituted chloroplasts. Photosynthesis Research **23**, 257–268.

Chastain CJ, Ogren WL. 1989. Glyoxylate Inhibition of Ribulosebisphosphate Carboxylase/Oxygenase Activation State in vivo. Plant and Cell Physiology **30**, 937–944.

Cummins PL, Kannappan B, Gready JE. 2018. Directions for Optimization of Photosynthetic Carbon Fixation: RuBisCO's Efficiency May Not Be So Constrained After All. Frontiers in Plant Science **9**, 183.

Dalal J, Lopez H, Vasani NB, *et al.* 2015. A photorespiratory bypass increases plant growth and seed yield in biofuel crop Camelina sativa. Biotechnology for Biofuels **8**, 1–22.

DePaoli HC, Borland AM, Tuskan GA, Cushman JC, Yang X. 2014. Synthetic biology as it relates to CAM photosynthesis: challenges and opportunities. Journal of Experimental Botany **65**, 3381–3393.

Eisenhut M. 2006. The Plant-Like C2 Glycolate Cycle and the Bacterial-Like Glycerate Pathway Cooperate in Phosphoglycolate Metabolism in Cyanobacteria. PLANT PHYSIOLOGY **142**, 333–342.

Eisenhut M, Bauwe H, Hagemann M. 2007. Glycine accumulation is toxic for the cyanobacterium *Synechocystis* sp. strain PCC 6803, but can be compensated by supplementation with magnesium ions. FEMS Microbiology Letters **277**, 232–237.

Ellis RJ. 1979. The most abundant protein in the world. Trends in Biochemical Sciences **4**, 241–244.

FAO 'Food and Agriculture Organization of the United Nations'. 2018. FAOSTAT Database.

Fletcher AL, Brown HE, Johnstone PR, de Ruiter JM, Zyskowski RF. 2011. Making sense of yield trade-offs in a crop sequence: A New Zealand case study. Field Crops Research **124**, 149–156.

Flügel F, Timm S, Arrivault S, Florian A, Stitt M, Fernie AR, Bauwe H. 2017. The Photorespiratory Metabolite 2-Phosphoglycolate Regulates Photosynthesis and Starch Accumulation in Arabidopsis. The Plant cell **29**, 2537–2551.

Galmés J, Kapralov M V, Andralojc PJ, Conesa MÀ, Keys AJ, Parry MAJ, Flexas J. 2014. Expanding knowledge of the Rubisco kinetics variability in plant species: Environmental and evolutionary trends. Plant, Cell and Environment **37**, 1989–2001.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. 2010. Food Security: The Challenge of Feeding 9 Billion People. Science **327**, 812–818.

Haber F, Le Rossignol R. 1909. Production of ammonia. US1202995A, filed August 13, 1909, and issued October 31, 1916

Hausler RE, Bailey KJ, Lea PJ, Leegood RC. 1996. Control of photosynthesis in barley mutants with reduced activities of glutamine synthetase and glutamate synthase III . Aspects of glyoxylate metabolism and effects of glyoxylate on the activation. Planta **44**, 388–396.

Hay WT, Bihmidine S, Mutlu N, Hoang K Le, Awada T, Weeks DP, Clemente TE, Long SP. 2017. Enhancing soybean photosynthetic CO2 assimilation using a cyanobacterial membrane protein, ictB. Journal of Plant Physiology **212**, 58–68.

Heineke D, Bykova N, Gardeström P, Bauwe H. 2001. Metabolic response of potato plants to an antisense reduction of the P-protein of glycine decarboxylase. Planta **212**, 880–887.

Hibberd JM, Quick WP. 2002. Characteristics of C4 photosynthesis in stems and petioles of C3 flowering plants. Nature **415**, 451–454.

Hibberd JM, Sheehy JE, Langdale JA. 2008. Using C4 photosynthesis to increase the yield of rice—rationale and feasibility. Current Opinion in Plant Biology **11**, 228–231.

Jenkins CLD, Furbank RT, Hatch MD. 1989. Mechanism of C4 Photosynthesis: A Model Describing the Inorganic Carbon Pool in Bundle Sheath Cells. PLANT PHYSIOLOGY **91**, 1372–

1381.

Kebeish R, Niessen M, Thiruveedhi K, Bari R, Hirsch H-J, Rosenkranz R, Stabler N, Schonfeld B, Kreuzaler F, Peterhansel C. 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in Arabidopsis thaliana. Nature Biotechnology **25**, 593–599.

Khush GS. 2001. Green revolution: the way forward. Nature Reviews Genetics **2**, 815–822.

Koester RP, Nohl BM, Diers BW, Ainsworth EA. 2016. Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars. Plant, Cell & Environment **39**, 1058–1067.

Kumar A, Li C, Portis AR. 2009. Arabidopsis thaliana expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynthesis Research **100**, 143–153.

Kupriyanova E V, Sinetova MA, Cho SM, Park Y Il, Los DA, Pronina NA. 2013. CO2-concentrating mechanism in cyanobacterial photosynthesis: Organization, physiological role, and evolutionary origin. Photosynthesis Research **117**, 133–146.

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G. 2007. Enhanced Thermostability of Arabidopsis Rubisco Activase Improves Photosynthesis and Growth Rates under Moderate Heat Stress. The Plant Cell **19**, 3230–3241.

Lefebvre S, Lawson T, Zakhleniuk O V, Lloyd JC, Raines CA, Fryer M. 2005. Increased Sedoheptulose-1,7-Bisphosphatase Activity in Transgenic Tobacco Plants Stimulates Photosynthesis and Growth from an Early Stage in Development. PLANT PHYSIOLOGY **138**, 451–460.

Lieman-Hurwitz J, Asipov L, Rachmilevitch S, Marcus Y, Kaplan A. 2005. Expression of cyanobacterial ictB in higher plants enhanced photosynthesis and growth. Plant Responses to Air Pollution and Global Change. Tokyo: Springer Japan, 133–139.

Lieman-Hurwitz J, Rachmilevitch S, Mittler R, Marcus Y, Kaplan A. 2003. Enhanced photosynthesis and growth of transgenic plants that express *ictB* , a gene involved in HCO_3^- accumulation in cyanobacteria. Plant Biotechnology Journal **1**, 43–50.

Lin MT, Occhialini A, Andralojc PJ, Parry MAJ, Hanson MR. 2014. A faster Rubisco with

potential to increase photosynthesis in crops. Nature **513**, 547–550.

Liu W, Stewart CN. 2015. Plant synthetic biology. Trends in Plant Science **20**, 309–317.

Long BM, Hee WY, Sharwood RE, *et al.* 2018. Carboxysome encapsulation of the CO2-fixing enzyme Rubisco in tobacco chloroplasts. Nature Communications **9**, 3570.

Long BM, Rae BD, Rolland V, Förster B, Price GD. 2016. Cyanobacterial CO2-concentrating mechanism components: function and prospects for plant metabolic engineering. Current Opinion in Plant Biology **31**, 1–8.

Long SP, Zhu XG, Naidu SL, Ort DR. 2006. Can improvement in photosynthesis increase crop yields? Plant Cell Environ **29**, 315–330.

Lopez-Calcagno PE, Fisk S, Brown KL, Bull SE, South PF, Raines CA. 2018. Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field grown transgenic tobacco plants. Plant Biotechnology Journal.

Lorimer GH, Miziorko HM. 1980. Carbamate formation on the .epsilon.-amino group of a lysyl residue as the basis for the activation of ribulosebisphosphate carboxylase by carbon dioxide and magnesium(2+). Biochemistry **19**, 5321–5328.

Ma F, Jazmin LJ, Young JD, Allen DK. 2014. Isotopically nonstationary 13C flux analysis of changes in Arabidopsis thaliana leaf metabolism due to high light acclimation. Proceedings of the National Academy of Sciences of the United States of America **111**, 16967–72.

Mackinder LCM. 2018. The *Chlamydomonas* CO_2 -concentrating mechanism and its potential for engineering photosynthesis in plants. New Phytologist **217**, 54–61.

Maier A, Fahnenstich H, von Caemmerer S, Engqvist MK, Weber AP, Flugge UI, Maurino VG. 2012. Transgenic Introduction of a Glycolate Oxidative Cycle into A. thaliana Chloroplasts Leads to Growth Improvement. Front Plant Sci **3**, 38.

Mangan NM, Flamholz A, Hood RD, Milo R, Savage DF. 2016. pH determines the energetic efficiency of the cyanobacterial CO 2 concentrating mechanism. Proceedings of the National Academy of Sciences **113**, E5354–E5362.

Masle J, Hudson GS, Badger MR. 1993. Effects of Ambient CO2 Concentration on Growth and

Nitrogen Use in Tobacco (Nicotiana tabacum) Plants Transformed with an Antisense Gene to the Small Subunit of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase. Plant physiology **103**, 1075–1088.

McGrath JM, Long SP. 2014. Can the cyanobacterial carbon-concentrating mechanism increase photosynthesis in crop species? A theoretical analysis. Plant physiology **164**, 2247–61.

Miyagawa Y, Tamoi M, Shigeoka S. 2001. Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. Nature Biotechnology **19**, 965–969.

Monson RK, Edwards GE. 1984. C 3 - C 4 Intermediate Photosynthesis in Plants. BioScience **34**, 563–574.

Monteith JL, Moss CJ. 1977. Climate and the Efficiency of Crop Production in Britain [and Discussion]. Philosophical Transactions of the Royal Society B: Biological Sciences **281**, 277–294.

Nölke G, Houdelet M, Kreuzaler F, Peterhänsel C, Schillberg S. 2014. The expression of a recombinant glycolate dehydrogenase polyprotein in potato (Solanum tuberosum) plastids strongly enhances photosynthesis and tuber yield. Plant Biotechnology Journal **12**, 734–742.

Occhialini A, Lin MT, Andralojc PJ, Hanson MR, Parry MAJ. 2016. Transgenic tobacco plants with improved cyanobacterial Rubisco expression but no extra assembly factors grow at near wild-type rates if provided with elevated CO2. Plant Journal **85**, 148–160.

Oliver DJ. 1980. The effect of glyoxylate on photosynthesis and photorespiration by isolated soybean mesophyll cells. Plant physiology **65**, 888–92.

Oliver DJ, Zelitch I. 1977. Increasing photosynthesis by inhibiting photorespiration with glyoxylate. Science (New York, N.Y.) **196**, 1450–1.

Orr DJ, Alcântara A, Kapralov M V, Andralojc PJ, Carmo-Silva E, Parry MAJ. 2016. Surveying Rubisco Diversity and Temperature Response to Improve Crop Photosynthetic Efficiency. Plant physiology **172**, 707–717.

Ort DR, Merchant SS, Alric J, *et al.* 2015. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proceedings of the National Academy of Sciences of the United States of America **112**, 8529–36.

Parry MAJ, Keys AJ, Madgwick PJ, Carmo-Silva AE, Andralojc PJ. 2007. Rubisco regulation: a role for inhibitors. Journal of Experimental Botany **59**, 1569–1580.

Peng S, Tang Q, Zou Y. 2009. Current Status and Challenges of Rice Production in China. Plant Production Science **12**, 3–8.

Piatek AA, Lenaghan SC, Neal Stewart C. 2018. Advanced editing of the nuclear and plastid genomes in plants. Plant Science **273**, 42–49.

Portis AR. 2002. Rubisco activase - Rubisco's catalytic chaperone. Photosynthesis research **75**, 11–27.

Rae BD, Long BM, Förster B, Nguyen ND, Velanis CN, Atkinson N, Hee WY, Mukherjee B, Price GD, McCormick AJ. 2017. Progress and challenges of engineering a biophysical CO2-concentrating mechanism into higher plants. Journal of Experimental Botany **68**, 3717–3737.

Raven J. 2009. Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments. Aquatic Microbial Ecology **56**, 177–192.

Raven JA. 2013. Rubisco: still the most abundant protein of Earth? New Phytologist **198**, 1–3.

Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW. 1988. Distribution of photorespiratory enzymes between bundle-sheath and mesophyll cells in leaves of the C3?C4 intermediate species Moricandia arvensis (L.) DC. Planta **176**, 527–532.

Ray DK, Ramankutty N, Mueller ND, West PC, Foley JA. 2012. Recent patterns of crop yield growth and stagnation. Nature Communications **3**, 1293.

Rolland V, Badger MR, Price GD. 2016. Redirecting the Cyanobacterial Bicarbonate Transporters BicA and SbtA to the Chloroplast Envelope: Soluble and Membrane Cargos Need Different Chloroplast Targeting Signals in Plants. Frontiers in Plant Science **7**, 1–19.

Rosenthal DM, Locke AM, Khozaei M, Raines CA, Long SP, Ort DR. 2011. Over-expressing the C3 photosynthesis cycle enzyme Sedoheptulose-1-7 Bisphosphatase improves photosynthetic carbon gain and yield under fully open air CO2 fumigation (FACE). BMC Plant Biology **11**, 123.

Sage RF, Sage TL, Kocacinar F. 2012. Photorespiration and the Evolution of C 4 Photosynthesis. Annual Review of Plant Biology **63**, 19–47.

Salvucci ME, Crafts-Brandner SJ, Salvucci ME. 2004. Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. Plant physiology **134**, 1460–70.

Savir Y, Noor E, Milo R, Tlusty T. 2010. Cross-species analysis traces adaptation of Rubisco toward optimality in a low-dimensional landscape. Proceedings of the National Academy of Sciences **107**, 3475–3480.

Schlüter U, Weber APM. 2016. The Road to C_4 Photosynthesis: Evolution of a Complex Trait via Intermediary States. Plant and Cell Physiology **57**, 881–889.

Schuler ML, Mantegazza O, Weber APM. 2016. Engineering C_4 photosynthesis into C_3 chassis in the synthetic biology age. The Plant Journal **87**, 51–65.

Schwander T, Von Borzyskowski LS, Burgener S, Cortina NS, Erb TJ. 2016. A synthetic pathway for the fixation of carbon dioxide in vitro. Science **354**, 900–904.

Servaites JC, Ogren WL. 1977. Chemical inhibition of the glycolate pathway in soybean leaf cells. Plant physiology **60**, 461–6.

Shih PM, Zarzycki J, Niyogi KK, Kerfeld CA. 2014. Introduction of a synthetic CO2-fixing photorespiratory bypass into a cyanobacterium. Journal of Biological Chemistry **289**, 9493–9500.

Simkin AJ, McAusland L, Headland LR, Lawson T, Raines CA. 2015. Multigene manipulation of photosynthetic carbon assimilation increases CO2 fixation and biomass yield in tobacco. Journal of Experimental Botany **66**, 4075–4090.

Somerville CR, Ogren WL. 1979. A phosphoglycolate phosphatase-deficient mutant of Arabidopsis [9]. Nature **280**, 833–836.

Somerville CR, Ogren WL. 1982. Genetic modification of photorespiration. Trends in Biochemical Sciences **7**, 171–174.

Spreitzer RJ, Peddi SR, Satagopan S. 2005. Phylogenetic engineering at an interface between large and small subunits imparts land-plant kinetic properties to algal Rubisco. Proceedings of the National Academy of Sciences of the United States of America **102**, 17225–30.

Sutton MA, Simpson D, Levy PE, Smith RI, Reis S, van Oijen M, de Vries W. 2008.

Uncertainties in the relationship between atmospheric nitrogen deposition and forest carbon sequestration. Global Change Biology **14**, 2057–2063.

Tcherkez GGB, Farquhar GD, Andrews TJ. 2006. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proceedings of the National Academy of Sciences **103**, 7246–7251.

Tilman D, Balzer C, Hill J, Befort BL. 2011. Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci U S A **108**, 20260–20264.

Timm S, Florian A, Arrivault S, Stitt M, Fernie AR, Bauwe H. 2012. Glycine decarboxylase controls photosynthesis and plant growth. FEBS Letters **586**, 3692–3697.

Timm S, Wittmiß M, Gamlien S, Ewald R, Florian A, Frank M, Wirtz M, Hell R, Fernie AR, Bauwe H. 2015. Mitochondrial Dihydrolipoyl Dehydrogenase Activity Shapes Photosynthesis and Photorespiration of *Arabidopsis thaliana*. The Plant Cell **27**, 1968–1984.

Trudeau DL, Edlich-Muth C, Zarzycki J, *et al.* 2018. Design and in vitro realization of carbon-conserving photorespiration. Proceedings of the National Academy of Sciences **115**, E11455–E11464.

Uehara S, Adachi F, Ito-Inaba Y, Inaba T. 2016. Specific and Efficient Targeting of Cyanobacterial Bicarbonate Transporters to the Inner Envelope Membrane of Chloroplasts in Arabidopsis. Frontiers in Plant Science **7**, 1–8.

Uematsu K, Suzuki N, Iwamae T, Inui M, Yukawa H. 2012. Increased fructose 1,6-bisphosphate aldolase in plastids enhances growth and photosynthesis of tobacco plants. Journal of Experimental Botany **63**, 3001–3009.

Vazquez-Vilar M, Orzaez D, Patron N. 2018. DNA assembly standards: Setting the low-level programming code for plant biotechnology. Plant Science **273**, 33–41.

Whitney SM, Andrews TJ. 2001. Plastome-encoded bacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) supports photosynthesis and growth in tobacco. Proceedings of the National Academy of Sciences **98**, 14738–14743.

Whitney SM, Sharwood RE, Orr D, White SJ, Alonso H, Galmés J. 2011. Isoleucine 309 acts as a C4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco)

carboxylation rate in Flaveria. Proceedings of the National Academy of Sciences of the United States of America **108**, 14688–93.

Wilson RH, Alonso H, Whitney SM. 2016. Evolving Methanococcoides burtonii archaeal Rubisco for improved photosynthesis and plant growth. Scientific Reports **6**, 22284.

Wingler A, Lea PJ, Leegood RC. 1997. Control of photosynthesis in barley plants with reduced activities of glycine decarboxylase. Planta **202**, 171–178.

Xin C-P, Tholen D, Devloo V, Zhu X-G. 2015. The benefits of photorespiratory bypasses: how can they work? Plant physiology **167**, 574–85.

Yamori W, Masumoto C, Fukayama H, Makino A. 2012. Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. The Plant Journal **71**, 871–880.

Yang S-M, Chang C-Y, Yanagisawa M, Park I, Tseng T-H, Ku MSB. 2008. Transgenic Rice Expressing Cyanobacterial Bicarbonate Transporter Exhibited Enhanced Photosynthesis, Growth and Grain Yield. Photosynthesis. Energy from the Sun. Dordrecht: Springer Netherlands, 1243–1246.

Yang X, Cushman JC, Borland AM, *et al.* 2015. A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter, drier world. New Phytologist **207**, 491–504.

Yokota A. 1991. Carboxylation and Detoxification of Xylulose Bisphosphate by Spinach Ribulose Bisphosphate Carboxylase/Oxygenase. Plant and Cell Physiology **32**, 755–762.

Young JN, Heureux AMC, Sharwood RE, Rickaby REM, Morel FMM, Whitney SM, Raines C. 2016. Large variation in the Rubisco kinetics of diatoms reveals diversity among their carbon-concentrating mechanisms. Journal of Experimental Botany **67**, 3445–3456.

Yu H, Li X, Duchoud F, Chuang DS, Liao JC. 2018. Augmenting the Calvin–Benson–Bassham cycle by a synthetic malyl-CoA-glycerate carbon fixation pathway. Nature Communications **9**, 2008.

Zhu G, Jensen RG. 1991. Xylulose 1,5-Bisphosphate Synthesized by Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase during Catalysis Binds to Decarbamylated Enzyme. Plant physiology **97**, 1348–53.

Zhu X-G, Long SP, Ort DR. 2010. Improving Photosynthetic Efficiency for Greater Yield. Annual Review of Plant Biology **61**, 235–261.

Zhu XG, Portis AR, Long SP. 2004. Would transformation of C3crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. Plant, Cell and Environment **27**, 155–165.

Zhu X-G, de Sturler E, Long SP. 2007. Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant physiology **145**, 513–26.