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REVIEW PAPER

Engineering photosynthesis in plants and synthetic microorganisms

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Abstract

Photosynthetic organisms, such as cyanobacteria, algae, and plants, sustain life on earth by converting light energy, water, and CO_2 into chemical energy. However, due to global change and a growing human population, arable land is becoming scarce and resources, including water and fertilizers, are becoming exhausted. It will therefore be crucial to design innovative strategies for sustainable plant production to maintain the food and energy bases of human civilization. Several different strategies for engineering improved photosynthesis in crop plants and introducing novel photosynthetic capacity into microorganisms have been reviewed

Key words: C_4 photosynthesis; metabolic engineering; photorespiration; photosynthesis; synthetic biology; synthetic microorganisms.

Introduction

Photosynthesis provides the basis for life on earth by converting the energy contained in the photons of sunlight into chemical energy that is stored as reduced carbon in the form of carbohydrates, proteins, and fat. In addition, this process removes carbon dioxide from and releases oxygen to the atmosphere, thereby defining the atmosphere and driving the global carbon cycle. Over tens of thousands of years, the continuous growth of the human population on planet Earth was predominantly driven by the domestication and increasingly efficient production of crops, in particular, during the past two centuries. With rapidly decreasing fossil reserves of oil, coal, and natural gas, current plant productivity becomes increasingly important as source of energy but also as a commodity for industrial uses. The increasing draw on plant products for food and feed and the rising amount of plant biomass that is converted into fuels and industrial products, together with continued growth of the human population, threatens future growth. From 1961 to 2012, the human population more than doubled from approximately 3 billion to 7 billion people and a further increase to 9.3 billion is projected for the year 2050 (Source: FAOSTAT). During this time, the amount of arable land available for crop production has stayed almost flat, meaning that the arable land per capita is steadily decreasing (Fig. 1).

The data presented in Fig. 1 extrapolates the trends observed over the past 50 years into the future, until 2050. It becomes apparent from Fig.1 that the gap between the total cropland that is available and the area of cropland per capita is opening, which means that less and less arable land is going to be available for each person on the planet in the future. This trend is also illustrated in Fig. 2, showing that only the equivalent of 25% of the area of a soccer field will be available for crop production per capita in 2050.

Importantly, these scenarios do not account for cropland lost to erosion, salinity, urban growth, and other aggravating factors, such as changing patterns of meat consumption and thus are likely to represent a rather optimistic outlook.

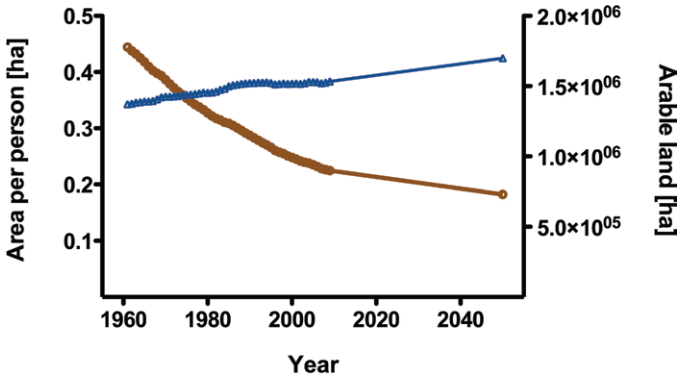

Fig. 1. Area of arable land per person (left axis, brown symbols) and total amount of arable land (right axis, blue symbols) from 1961 to 2010. Data points for the year 2050 have been extrapolated by linear regression from the trends between 1961 through 2010. Data taken from FAOSTAT (http://faostat.fao.org).

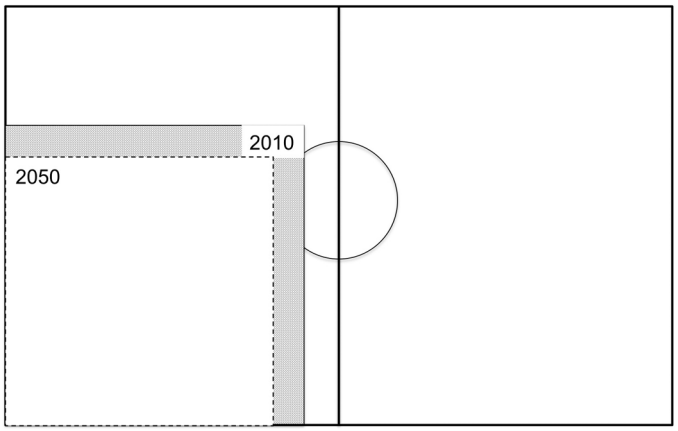

Fig. 2. Total area of arable land per capita in the years 2010 and 2050 in relation to a standard-sized soccer field (105 × 68 m; 7140 m^2).

Irrespective of the decreasing amount of cropland that is available per capita, due to the Green Revolution, the production of food calories per day and person has steadily increased by approximately 1.3-fold from 2200 kcal d^{-1} $capita^{-1}$ to 2794 kcal d^{-1} $capita^{-1}$ between 1961 and 2007 (Fig. 3). This is a major achievement, given that the human population has grown 2.2-fold during this time period. However, this success came at a cost – the 2.2-fold increase in food calories available per capita that was achieved over the past 50 years is associated with a 7.3-fold increase in the amount of nitrogenous fertilizer applied (Fig. 3) and a more than 3-fold higher input of phosphate (Data: FAOSTAT). That is, the input required to achieve higher yields is outpacing the output in crop productivity, which clearly is unsustainable in the mid- to long term. In particular, for phosphate, this trend is of special concern since the stocks of this mineable resource are limited and will eventually be depleted (Elser and Bennett, 2011).

Taken together, these data and trends clearly highlight the need for producing more plant products with a lower input of

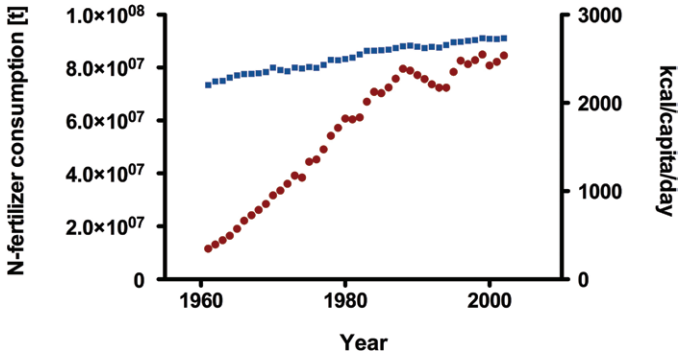

Fig. 3. Nitrogenous fertilizer consumption (left axis, red symbols) and kilocalories produced as crop plants (right axis, blue symbols) between 1961 and 2002.

resources. Traditional plant breeding has managed to keep up with growing demands to date. This did, however, require a massive increase in the input of resources, such as mineral nutrients and water. For the future, new paradigms are needed for the improvement of crop plants that afford higher efficiency. In addition, strategies should be developed that reduce the draw on crop products as an energy source or commodity for industrial purposes. This might become possible by engineering the capacity to perform photosynthesis into organisms that currently do not have this trait, such as yeasts or biotechnologically interesting bacteria. Several different avenues in engineering photosynthesis, from improving crop plants to the engineering of novel microorganisms, are reviewed here.

Engineering photosynthesis in crop plants

Central to many strategies to improve crop yield potential is addressing the limitation by RubisCO, a rate-limiting enzyme in plant photosynthesis (Yokota and Shigeoka, 2008). Engineering RubisCO with the objective of producing higher plant rbcL and rbcS genes that code for improved forms of the enzyme has been a primary objective for enhancing photosynthetic efficiency (Parry *et al*., 2003, 2007; Whitney *et al*., 2011*a*). Despite recent progress with engineering hybrid RubisCO enzymes on the basis of C_4-RubisCOs (Ishikawa *et al*., 2011; Whitney *et al*., 2011*b*), to date most of the efforts resulted in limited success because the incorporation of engineered genes into chloroplasts DNA is still a challenge and especially for chloroplast transformation in major crop plants (Maliga and Bock, 2011).

Bypasses to photorespiration

In parallel to these attempts aimed at reducing the oxygenase activity of RubisCO, approaches aimed at redirecting photorespiratory fluxes by installing synthetic bypasses to circumvent mitochondrial CO_2 release in C_3 plants were recently put forward by three different groups (Kebeish *et al*., 2007; Carvalho *et al*., 2011; Maier *et al*., 2012) (Fig. 4).

Although photorespiration is important in all oxygenic organisms because it efficiently removes phosphoglycolate, a potent inhibitor of photosynthesis, in higher plants it may result in a

loss of at least 25% (with enhanced losses at high temperatures and low stomata aperture) of the carbon fixed (Leegood *et al*., 1995). Moreover, during photorespiration, considerable amounts of organically-bound nitrogen are released as ammonia, which must be re-assimilated in a process that consumes a substantial amount of energy (Leegood *et al*., 1995). Thus, a reduction in the rate of photorespiration represents, in principle, a major contribution towards an increased biomass production in C_3-plants.

Carvalho *et al*. (2011) proposed bypassing the photorespiratory nitrogen cycle by introducing *E. coli* glyoxylate catabolic reactions into *Nicotiana tabacum* to metabolize glyoxylate to hydroxypyruvate directly in peroxisomes (Fig. 4). It was expected that this pathway would compete with the aminotransferases that convert glyoxylate to glycine, thus avoiding ammonia production in the photorespiratory nitrogen cycle. Unfortunately, although the transgenic plants produced contained the transgenes, the product of one of the transgenes was not detectable in any of the transgenic lines. Thus, the functionality and efficacy of this proposed pathway still remains to be proven.

The introduction of two alternative intrachloroplastic glycolate oxidation pathways proved to be beneficial, strongly suggesting that it is possible to improve the growth of *A. thaliana* by diverting part of the glycolate formed by the oxygenase activity of RubisCO away from the photorespiratory pathway. In one approach the *E. coli* gycolate catabolic pathway was introduced into *A. thaliana* chloroplasts (Kebeish *et al*., 2007) (Fig. 4). An increase in biomass and in the apparent rate of CO_2 assimilation were observed in plants producing glyoxylate by

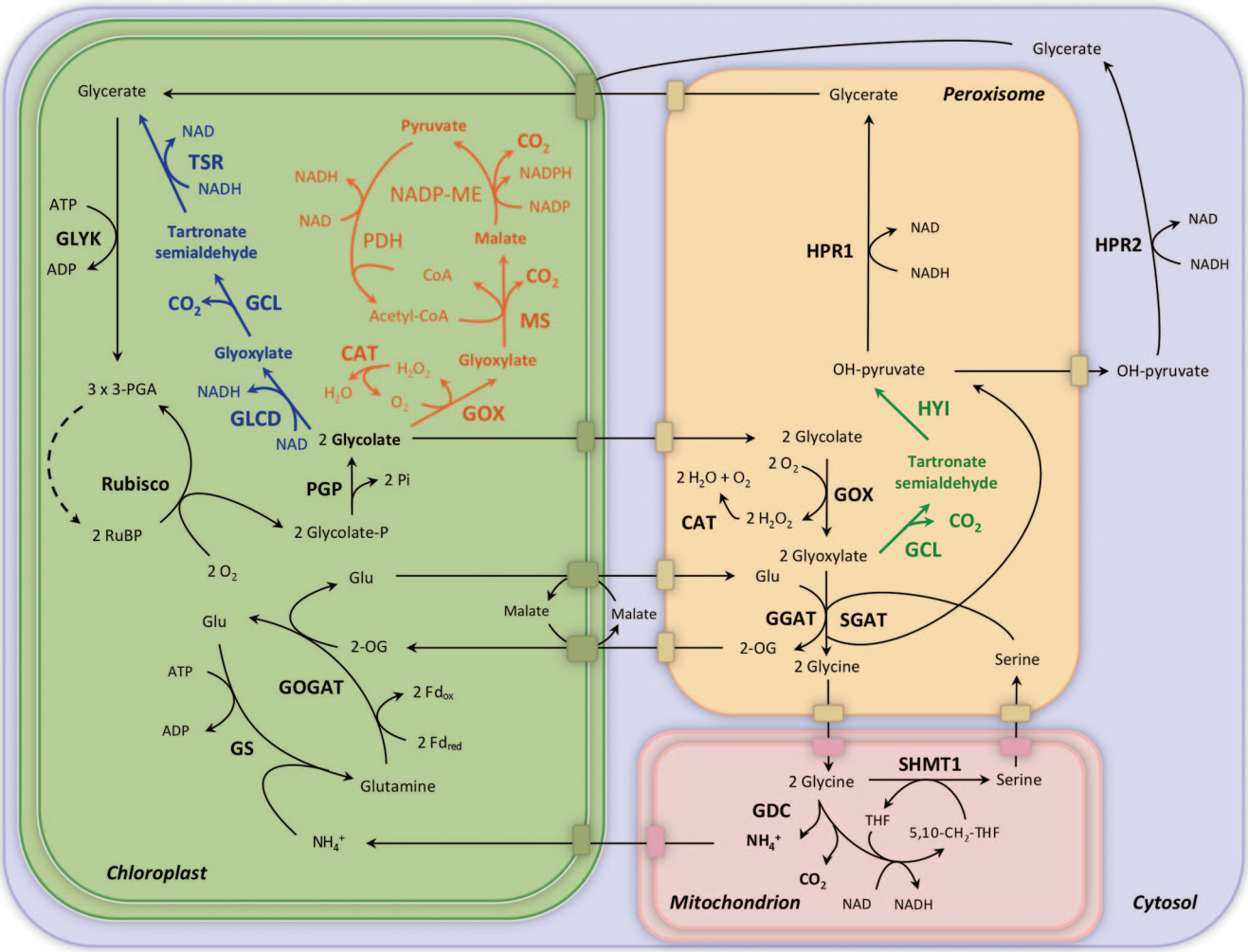

Fig. 4. Synthetic bypasses to circumvent mitochondrial CO_2 release during photorespiration. The photorespiratory pathway (black) is short-circuited in the chloroplasts by the bacterial glycolate pathway (blue) or by a complete glycolate oxidation pathway (red) or, alternatively, in the peroxisomes by glyoxylate catabolic reactions to produce hydroxypyruvate. Enzymes over-expressed for the full functioning of these pathways are highlighted in bold case. Rubisco, ribulose-1,5-*bis*phosphate carboxylase/oxygenase; PGP, phosphoglycolate phosphatase; GOX, glycolate oxidase; CAT, catalase; GGAT, glutamate-glyoxylate aminotransferase; GDC, glycine decarboxylase; SHMT, serine hydroxymethyl transferase; SGAT, serine-glyoxylate aminotransferase; HPR, hydroxypyruvate reductase; GLYK, glycerate kinase; GS, glutamine synthetase; GOGAT, glutamine-oxoglutarate aminotransferase; GLCD, glycolate dehydrogenase; GCL, gyoxylate carboligase; HYI, hydroxypyruvate isomerase; TSR, tartronate semialdehyde reductase; MS, malate synthase; NADP-ME, NADP-malic enzyme; PDH, pyruvate dehydrogenase. RuBP, ribulose-1,5-*bis*phosphate; THF, tetrahydrofolate; 5,10-CH_2-THF, 5,10-methylene-THF. (Modified from Maurino and Peterhansel, © 2010 and reproduced with permission from Elsevier.)

over-expression of the first enzyme of this pathway and those plants producing glycerate by expression of the whole pathway. Moreover, a similar decreased post-illumination CO_2 burst in all transgenic lines indicated that reduced photorespiratory fluxes were achieved with this approach (Kebeish *et al*., 2007). In a second approach, enhanced CO_2 fixation and growth improvement were obtained in *A. thaliana* plants expressing an alternative chloroplastic glycolate catabolic cycle in which glycolate is completely oxidized within the chloroplast to two molecules of CO_2 (Maier *et al*., 2012) (Fig. 4). Because both of the engineered pathways are localized to chloroplasts, CO_2 released as a product of the pathway in close proximity to RubisCO can directly be refixed. It is worth mentioning here that, using both approaches, no significant deviation of the CO_2 compensation point from the wild-type value was observed. In addition, these synthetic pathways have the advantage compared with photorespiration that no ammonia is released in any of its enzymatic steps. Thus, less reassimilation of ammonia would be required, resulting in a potential improvement in the plant nitrogen use efficiency. The energy costs of the introduced pathways are lower than 'normal' photorespiration (Maurino and Peterhansel, 2010; Maier *et al*., 2012). Interestingly, the plants produced by Klebeish *et al*. (2007) and Maier *et al*. (2012) showed enhanced biomass only when grown in short days and presented flatter and thinner leaves, whereas no apparent differences to the wild type were observed in long days. Although the precise causes of this change in morphology is a matter for future investigations, it was suggested that it might result from signalling through altered metabolic status (Maier *et al*., 2012). A challenge for the future is to develop optimized versions of these basic approaches to introduce the glycolate catabolic cycles into plants with agronomical importance (http://www.3to4.org/). It also remains to be tested as to whether reducing the flux through photorespiration by enzymatic bypasses would adversely affect nitrate assimilation due to reduced export of reducing equivalents from the chloroplast to the cytoplasm (Bloom *et al*., 2010).

C_3 to C_4: increasing photosynthetic efficiency and decreasing the input of water and N-fertilizer through implementing a C_4 engine in C_3 plants

Plants using the C_4 pathway of photosynthesis possess a better nitrogen investment and water- and radiation-use efficiencies in warm conditions compared with C_3 plants (Snaydon, 1991; Ghannoum *et al*., 2011). CO_2 concentrations of 500 µM can be reached in bundle sheath chloroplasts of C_4 species, resulting in a saturated net CO_2 fixation and much decreased rates of photorespiration in ambient air. During the last 10–15 years various attempts were conducted to transfer C_4 traits in C_3 plants to improve their photosynthetic performance. But positive effects on photosynthesis by expressing single C_4 enzymes or combinations of them into C_3 species have not been reported to date (Ishimaru *et al*., 1997; Häusler *et al*., 2001, Ku *et al*., 2001; Fahnenstich *et al*., 2007). Attempts to create a single-cell C_4 mini-cycle in transgenic rice plants demonstrated that insertion of the C_4 cycle basic enzymatic machinery without adding the rest of the C_4 engine, such as the cellular compartmentalization and the transport systems, is not enough enhance C_3 crop photosynthesis (Miyao *et al*., 2011).

At present, new attempts towards transferring C_4 properties to C_3 crops are being made in an effort to achieve a major increase in global rice production and a posterior transfer into other crops such as wheat and rape (http://C4rice.irri.org; http://www.3to4.org/). The aim of the metabolic C_4 engineering approach is to generate a two-celled NADP-ME C_4 cycle in rice by expressing the classical enzymes of the pathway in the appropriate cell types. Engineering the C_4 CO_2-concentrating system into C_3 plants represents one of the most ambitious undertakings. While developing a more efficient and higher yielding form of rice might require many years to achieve, the efforts towards this end will also lead to a much better understanding of the function of C_4 photosynthesis. This comprises knowledge about the establishment and regulation of C_4 (Kranz) anatomy, the genetic control of vein patterning, and C_4-type chloroplasts development (Bräutigam *et al*., 2011; Kajala *et al*., 2011; http://www.3to4.org/). Specifically, understanding the molecular biology and biochemistry of the major C_4 enzymes will be crucial for successfully introducing functional proteins into the metabolic context of C_3 plants and the discovery of the key genes controlling the expression of C_4 photosynthesis can dramatically accelerate the success of this challenging project. Furthermore, this knowledge might also provide new ways to improve the efficiency of existing C_4 crops (e.g. to resist environmental stresses better).

Paradigms for entirely novel CO_2 assimilation pathways in plants

The rate of carbon fixation can become a growth-limiting factor in modern agriculture and therefore increasing the carbon fixation rate using alternative pathways could be a promising avenue for improving photosynthesis. Using the entire repertoire of approximately 5000 metabolic enzymes known to occur in nature, Bar-Even *et al*. (2010) computationally explored and identified alternative carbon fixation pathways that combine existing metabolic building blocks from various organisms. Some of the possible synthetic pathways would have improved overall kinetic rates over their natural counterparts (Bar-Even *et al*., 2010, 2011). For example, a cycle employing the most efficient carboxylating enzyme, phospho*enol*pyruvate carboxylase, and the core enzymes of the C_4 cycle, is predicted to be two to three times faster than the Calvin–Benson cycle (Fig. 5). The feasibility of introduction of entirely *in silico* predicted CO_2 assimilation pathways into plants although exciting, faces many uncertainties, such as (i) adjusting expression and activity of the enzymes to the required levels, as they may be influenced *in vivo* by stability, regulation, and localization and (ii) the integration into the endogenous metabolic network.

Other improvements in carbon fixation could be achieved by installing complete carbon-concentrating mechanisms into plant chloroplasts (Ducat and Silver, 2012). This synthetic biology effort is an attractive possibility as Bonacci *et al*. (2012) demonstrated that functional carboxysomes could be obtained in *E. coli*.

Optimizing antenna size

As the absorptive capacity of light-gathering antenna can exceed metabolic capacity, plants and photosynthetic microbes could also

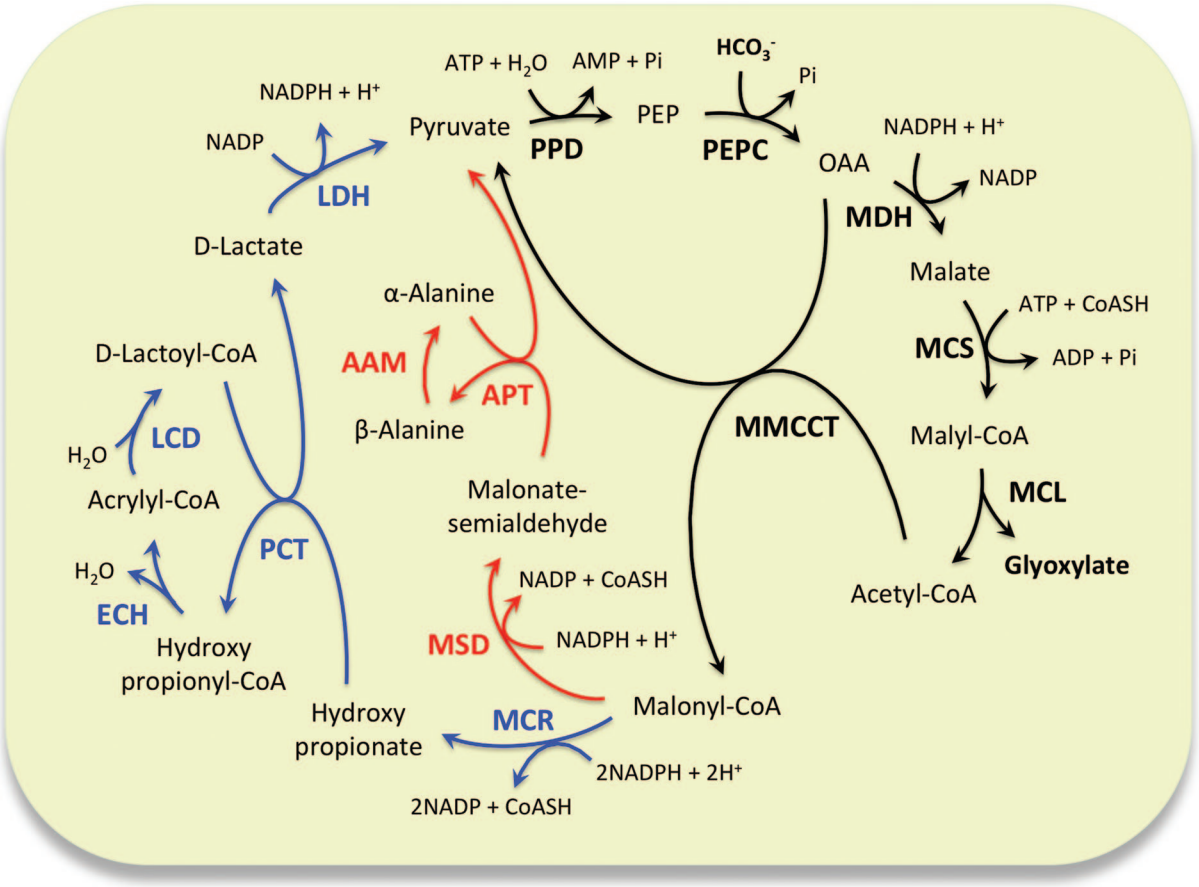

Fig. 5. Proposed CO_2 assimilation pathway employing phospho*enol*pyruvate carboxylase, and the core enzymes of the C_4 cycle. (Modified from Bar-Even *et al*., 2010, and reproduced by kind permission © 2010 of the National Academy of Sciences, USA.) PPD, pyruvate water/phosphate dikinase; PEPC, PEP carboxylase; MDH, malate dehydrogenase; MCS, malyl-CoA synthetase; MCL, malyl-CoA lyase; MMCCT, methylmalonyl-CoA carboxytransferase; MSD, malonate-semialdehyde dehydrogenase; APT, β-alanine-pyruvate transaminase; AAM, alanine 2,3-aminomutase; MCR, malonyl-CoA reductase; PCT, propionate CoA transferase; ECH, enoyl-CoA hydratase; LCD, lactoyl-CoA dehydratase; and LDH, lactate dehydrogenase.

benefit from a reduced need to engage photoinhibitory pathways under high light intensity (Ducat and Silver, 2012). Greater light penetration beyond the top layers mitigates efficiency losses associated with light saturation. In this regard, a canopy structure with vertically oriented upper leaves can enhance light distribution within the canopy, thus improving the efficiency of photosynthetic C fixation (Ort *et al*., 2010). Down-regulation of chlorophyll synthesis in *Chlamydomonas* rendered cultures of increased productivity at higher irradiances because they can transmit more light and are less sensitive to photoinhibition (Beckmann *et al*., 2009). Thus, optimizing antenna size could be a strategy to maximize photosynthetic efficiency of crop monocultures (Anten, 2005).

Engineering photosynthesis into microorganisms and/or photosynthetic microorganisms

Recent technological advances and synthetic biology are being implemented to design and engineer complex regulatory and metabolic networks in microorganisms (Johnson and Schmidt-Dannert, 2008; Ducat *et al*., 2011, 2012; Jensen *et al*. 2012). This would eventually allow the generation of designer bacteria with new useful properties, such as improved capture of solar energy to be used as energy sources to drive the natural or engineered production of high-value feedstocks and fuels (Johnson and Schmidt-Dannert, 2008). The implementation of this technology also relies on the use of both protein structure-guided and *in vitro* evolution strategies to alter and optimize the catalytic function of enzymes in the assembled pathways.

Moreover, the use of photosynthetic microorganisms, such as cyanobacteria, could reduce hte cost of carbohydrate feedstock during most bio-industrial processes, especially if chemical synthesis could be coupled with the use of CO_2 as a carbon source (Ducat *et al*., 2011). On the other hand, light availability, its capture efficiency, and its conversion to chemical energy constrain the upper yield of photosynthesis-driven metabolism. In this way, genetic approaches to construct cyanobacterial strains with improved photosynthetic efficiencies would improve product yield from several metabolic pathways.

Light-driven biosynthesis in bacteria

Progress towards the introduction of light-driven proton pumping or anoxygenic photosynthesis into non-photosynthetic bacteria is being made, which might allow for biosynthesis using

high-potential electron donors such as mixed acids from the waste-stream of a previous fermentation (Johnson and Schmidt-Dannert, 2008). It is suggested that either an increase in the transmembrane proton potential or the reduction of quinones involved in the respiratory electron-transport chain would be responsible to integrate light-driven processes with bacterial metabolism (Johnson and Schmidt-Dannert, 2008). Successful attempts in this area are the recovery of *E. coli* strains (i) able to pump protons when archeal or bacterial rhodopsins were functionally expressed (Kamo *et al*., 2006; Gourdon *et al*., 2008) and (ii) to assemble a complete light-driven photosystem when a complete cluster of genes encoding an entire proteorhodopsin-based photosystem was expressed (Martinez *et al*., 2007). Other studies explored the generation of transmembrane potential through light-driven mechanisms to boost normal *E. coli* metabolism. Some examples are enhanced synthesis of ATP (Racker and Stoeckenius, 1974), active transport of small solutes (Yerushalmi *et al*., 1995), and flagellar motion (Walter *et al*., 2007). With regard to the engineering of anoxygenic photosynthesis, although many proteins and cofactors (e.g. pigment–protein complexes, assembly factors, chaperones) should be co-expressed for its whole integration, only results on the expression of individual components, such as light-driven proton pumps to generate additional metabolic energy were reported (Johnson and Schmidt-Dannert, 2008).

Oxygenic photosynthesis could also drive biosynthesis in an engineered organism using light, CO_2, and water. Until now the full Calvin cycle has been expressed in *E. coli* (Parikh *et al*., 2006). Here again, the expression of all components required to re-create non-cyclic oxygenic photosynthesis could be a long-term prospect.

Sucrose production in cyanobacteria

Photosynthetic productivity can be enhanced by the optimization of the source/sink balance to minimize photosynthetic inefficiencies, for example, by rerouting carbon flux in photosynthetic microorganisms. Recently, Ducat *et al*. (2012) engineered *Synechococcus elongatus* to a sucrose-exporting cyanobacterium by expressing a sucrose:proton symporter and thus expanding the photosynthetic sink. This genetically modified strain can export sucrose to concentrations greater than 10 mM. This was accompanied by increased biomass, enhanced photosystem II activity, carbon fixation, and chlorophyll content most probably due to the relaxation of photoinhibition. This approach might even be more promising if sugar efflux transporters, such as the recently discovered SWEETS (Chen *et al*., 2010), were used.

Engineering synthetic plastid-containing (micro-) organisms: synthetic endosymbioses

Symbiotic relationships between different organisms have been found in virtually every ecosystem where they fulfil ecologically important roles. Endosymbiosis, with one symbiotic partner (the endosymbiont) living intracellularly within the second partner (the host), is the most intimate form of symbiosis. Host partners in these relationships are typically eukaryotes whereas the endosymbionts are either pro- or eukaryotes (Nowack and Melkonian, 2010). Endosymbiotic relationships range from nitrogen fixation by *Rhizobia* in plant root nodules to the symbiosis of photosynthetic organisms and heterotrophic hosts. The latter relationships comprise endosymbioses of cyanobacteria and algae with a large number of unicellular protists (Nowack and Melkonian, 2010), with fungi (lichens), with several metazoans, for example, species of the phyla *Mollusca* (giant clams), *Porifera* (sponges), *Cnidaria* (corals), and even vertebrates, such as the spotted salamander, leading to 'photosynthetic animals' (Kerney *et al*., 2011; Rumpho *et al*., 2011). In almost all of these cases the endosymbiosis with animals is temporary. That is, the transmission of photosynthetic symbionts is horizontal and each generation will have to acquire its photosynthetic partners anew from the environment (Johnson, 2011; Rumpho *et al*., 2011). Some of the underlying reasons are that (i) the endosymbionts are not transmitted to the germline, thereby preventing the development of a permanent photosynthetic animal; (ii) the lack of integration of the cell cycles of host and endosymbiont cells; and (iii) the absence of protein targeting systems permitting the host to deliver essential proteins to the endosymbiont.

To overcome these hurdles to the establishment of stable, vertically inheritable synthetic endosymbioses, it would be desirable to recapitulate the process of plastid endosymbiosis in the laboratory by employing a synthetic experimental evolutionary biology approach (Weber and Osteryoung, 2010). Clearly, this is a major challenge that is far from being solved. However, the concepts of synthetic biology could be used to bridge the gap between evolutionary theory and functional biology by engineering a novel organism from existing and newly designed parts. Symbiotic relationships have recently begun to be exploited in synthetic biological networks of increasing complexity (Agapakis *et al*., 2011). While most of these studies are aimed at engineering synthetic dual-organism systems of free-living microorganisms for biotechnology (Waks and Silver, 2009), several were designed to analyse the process of the establishment of symbiosis itself (Harcombe, 2010; Hosoda *et al*., 2011). In several of these studies, auxotrophs of different bacteria or yeast, which are able to complement the metabolic restraints of the respective partners were grown together, thereby demonstrating the successful establishment of synthetic obligate symbioses (Harcombe, 2010; Wintermute and Silver, 2010; Hosoda *et al*, 2011). Approaches to establish non-photosynthetic and photosynthetic endosymbioses can be traced back to more than 40 years ago when it was attempted to introduce isolated chloroplasts into mouse fibroblast cells (Nass, 1969). More recent attempts include the introduction of heterotrophic and phototrophic bacteria or isolated chloroplasts into different host cells, for example, spinach chloroplasts into *Neurospora crassa* cells (Vasil and Giles, 1975), the cyanobacteria *Anabaena variabilis* and *Gloeocapsa* into tobacco and maize cells (Burgoon and Bottino, 1976; Meeks *et al*., 1978), and genetically engineered *Bacillus subtilis* cells into a human cell line (Bielecki *et al*., 1990). In many of these studies the bacterial cells and even chloroplast were found to be stable for hours or even days within heterotrophic eukaryotic cells. However, no division of chloroplasts or bacteria within

their host cells has been reported and no stable endosymbiotic relationship was achieved.

While it is unlikely that engineering of synthetic, plastid-containing organisms will lead to systems that match the photosynthetic efficiency of palisade parenchyma cells, it has significant potential in biotechnological applications, such as the production of biofuels and high-value biomaterials. The advantage of such synthetic plastid-bearing organisms is inherent to their design principles: A genetically modifiable chassis, such as yeast cells, would be engineered to become the plastid recipient through (multiple) gene additions and knockouts. The plastid recipient (synthetic endosymbiont host) can then be engineered to exploit the organic carbon backbones generated by photosynthesis through the plastid. That is, instead of engineering the capacity to conduct photosynthesis into a microorganism by the stepwise addition of genes, as outlined in the chapters above, the capacity to conduct photosynthesis is acquired wholesale, through the integration of a functional plastid. Of course, this would require engineering of the synthetic host's genome to harbour the genes required for plastid maintenance. This latter point might feasibly be yeast, given that the first partially synthetic chromosomes have already been engineered and were shown to function when introduced into yeast cells (Dymond *et al*., 2011). In addition to conferring the ability to conduct photosynthesis to its host, the introduced synthetic plastid also affords its host with an additional reaction compartment with distinctive biosynthetic capacities, such as the ability to synthesize fatty acids or terpenes, which increases the metabolic and biotechnological versatility of the synthetic dual-organism system.

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