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Engineering carbon fixation with artificial protein organelles

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Based on projections for global population growth, current techniques for improving agricultural yields will not be able to address future demands for major food crops. Improving photosynthetic efficiency by engineering carbon fixation has been identified as one of the most important approaches for increasing agricultural output. Recent studies indicate that introducing cyanobacterial-like carbon concentrating mechanisms (CCMs) into plant chloroplasts represents a promising strategy for enhancing plant photosynthesis. Here, we give a general outline for transferring CCMs to plants. The proposed trajectory includes introducing bicarbonate transporters and CO_2-fixing organelles into plant chloroplasts as well as minimizing stromal carbonic anhydrase (CA) activity. We focus on different approaches for constructing compartments that co-localize the CO_2-fixing enzyme D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and CA, aimed at increasing RuBisCO turnover and decreasing wasteful photorespiration. We consider strategies based on cyanobacterial carboxysomes and on other protein-based compartments, specifically encapsulin nanocompartments. Finally, recent advances in expressing catalytic and structural carboxysomal components in plants will be highlighted.

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Introduction

The past achievements of the Green Revolution are today challenged by several developments. In recent decades, population growth has outpaced improvements in agricultural productivity with conservative projections estimating a world population of 9.7 billion in 2050 and 11.2 billion in 2100 (United Nations, World Population Prospects, the 2015 Revision). Continuously rising CO_2-levels in the atmosphere leading to accelerated anthropogenic climate change will result in increased flooding, loss of agricultural land and droughts [1]. In addition, a growing demand for biofuels and biomass production will lead to increased competition for arable land. Thus, there is an urgent need to improve agricultural productivity beyond yield potentials achievable by traditional methods [2]. In plants, photosynthetic efficiency in terms of light energy converted to biomass is only *ca.* 1% and has been identified as one of the most promising targets for improving agricultural productivity [3].

An important limitation of photosynthetic carbon acquisition is the enzyme D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Because of RuBisCO's low turnover number (between 1 and 10 s^{-1}, http://brenda-enzymes.org) and its poor selectivity between CO_2 and O_2, RuBisCO-catalyzed CO_2-fixation is one of the main factors impeding efficient flux through the Calvin–Benson–Bassham (CBB) cycle. Under ambient conditions, RuBisCO fixes up to two molecules of O_2 for every five CO_2-fixation cycles [4]. This RuBisCO side reaction is referred to as photorespiration and the resulting toxic product (glycolate-2-phosphate) needs to be recycled via the investment of additional energy and reducing equivalents [5]. It is estimated that 30% of photosynthetic output is lost via photorespiration [6]. The inability of RuBisCO to efficiently discriminate between CO_2 and O_2 is a result of its evolutionary history. Although, the true roots of the carboxylation activity of RuBisCO are currently contested [7,8], it is clear that the evolutionary origin of RuBisCO traces back to a time when atmospheric O_2 levels were minimal [9,10]. Thus, ancient RuBisCO was selected for its ability to carboxylate ribulose-1,5-bisphosphate, but not for minimizing photorespiration [11–13].

Different photosynthetic organisms have evolved a variety of systems to address the inherent limitations of RuBisCO. C3 plants, including important food crops like rice, wheat, barley and soybean, overcome RuBisCO's catalytic inefficiency by accumulating large amounts of enzyme with RuBisCO representing up to 50% of soluble protein [14]. C4 plants like corn, sugarcane and grasses as well as CAM (crassulacean-acid metabolism) plants have evolved carbon-concentrating mechanisms (CCMs) which increase the effective molarity of CO_2 near RuBisCO resulting in enhanced CO_2-fixation [15,16].

Algae on the other hand have evolved CCMs consisting of active uptake systems for inorganic carbon, leading to highly increased intracellular concentrations of bicarbonate. Many algae also contain pyrenoids, shell-less protein aggregates that co-localize RuBisCO and CA among other protein components [17]. Cyanobacteria use a multicomponent CCM that consists of specific CO_2 and bicarbonate uptake systems and a proteinaceous organelle, the carboxysome, which encapsulates RuBisCO and carbonic anhydrase (CA) [18–20]. Co-localization of RuBisCO and CO_2-generating CA saturates RuBisCO's active site with CO_2, thus strongly reducing wasteful photorespiration (Figure 1a).

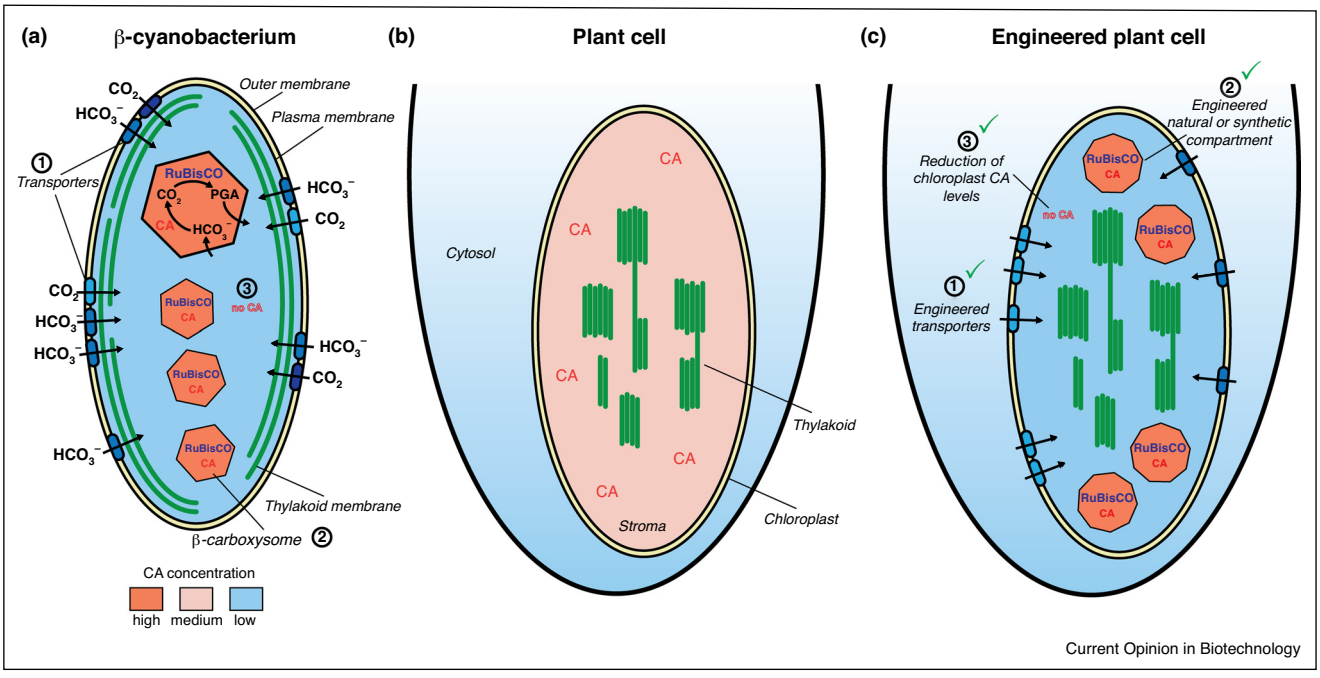
To address the need for improved photosynthetic CO_2-fixation of agriculturally important plants, a number of strategies have been suggested. They include improving the catalytic properties of RuBisCO [21,22•], engineering accessory proteins like RuBisCO activase [2] and engineering other enzymatic components of the CBB cycle to improve carbon flux [5]. Alternative approaches are aimed at engineering synthetic photorespiration bypasses [23,24] and at constructing synthetic CO_2-fixation pathways utilizing alternative carbon-fixing enzymes [25–27]. Mining the natural genetic variation of phototrophic organisms to identify superior enzymes and pathways has also been suggested [19]. Finally, improving the working conditions of RuBisCO by installing non-native CCMs has been identified as a promising strategy to improve plant photosynthetic efficiency [5,18,20].

In this review we will focus on current efforts aimed at transferring cyanobacterial CCMs to C3 plants. The prospects of engineering artificial minimal protein organelles and the challenges of transplanting them to C3 chloroplasts will be discussed.

Engineering carbon-concentrating mechanisms in plants

Compared with plant RuBisCO, cyanobacteria have evolved faster enzymes that are more sensitive to O_2. What makes cyanobacteria nonetheless thrive in high oxygen environments is their sophisticated CCM. By using active bicarbonate transporters and CO_2 uptake systems, high concentrations of inorganic carbon are accumulated within the cyanobacterial cytosol (>20 mM) leading to an up to 1000-fold accumulation ratio [29]. In addition, RuBisCO and CA are encapsulated inside carboxysome microcompartments leading to a high local concentration of CO_2 around the RuBisCO active site. The selectively permeable protein shell of the carboxysome minimizes internal O_2 concentrations thus

Figure 1



Introducing a cyanobacterial-like CCM to plant chloroplasts.
(a) The β-cyanobacterial CCM consists of dedicated transporters (1), a CO_2-fixing organelle called the carboxysome (encapsulating RuBisCO and CA) (2) and CA, specifically localized to carboxysomes and absent from the cytosol (3). **(b)** Simplified plant cell and chloroplast. **(c)** To introduce an optimized CCM to plant chloroplasts, all three components need to be transferred resulting in an engineered plant cell with increased carbon fixing capabilities. Importantly, co-localization of RuBisCO and CA inside a protein organelle does not have to be based on carboxysomes but can be achieved using other, more engineerable, compartments like encapsulins.