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Review Article

Biological carbon fixation: From natural to synthetic

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ABSTRACT

The looming energy crisis and greenhouse effect are two of the greatest problems facing the sustainable development of humanity. Conversion of carbon dioxide (CO_2) into fuels and chemicals by organisms is a promising way to solve these problems. However, since the natural biological carbon fixation rate cannot meet the industrial demand, more efficient carbon fixation processes are urgently needed. With the rapid development of biotechnology and life sciences, more and more information about the natural carbon fixation processes have been revealed. The unrelenting efforts have been practiced for improving the carbon fixation efficiency by redesigning carbon fixation pathways and even introducing novel energy supply patterns. In this review, we summarized the recent achievements and discussed the future prospects on biological carbon fixation.

1. Introduction

The conversion of inorganic CO_2 into organic chemicals is the key step of the global carbon cycle. The organic carbon derived from CO_2 fixation is the major source of organic chemicals and energy for human consumption. Carbon atoms in CO_2 molecules are in their highest oxidation state, while those in common fuels and chemicals such as hydrocarbons, alcohols, and acids are in lower states. Energy input is thus required to synthesize organics from inorganic CO_2, which is one of the reasons why CO_2 is not extensively used in current chemical industries. Biological carbon fixation by autotrophs can utilize light or inorganic chemical energy to fix atmospheric CO_2 through evolved carbon fixation pathways. Moreover, the products of biological carbon fixation are diverse, comprising C2, C3, C4, and even much longer chain carbohydrates, which is more suitable as the biofuels and chemicals.

The total CO_2 fixation capacity of autotrophic organisms on earth reaches up to about 380 billion tons per year, but the specific carbon fixation efficiency is relatively low [1]. Taking cyanobacteria as an example, the carbon fixation rate is only 1 to 5 mg/L/h, which cannot meet the industrial demands with amount to 1 to 10 g/L/h [2]. During the past decade, researchers have engineered autotrophic microbes to produce a variety of biofuels and chemicals, such as ethanol, n-butanol, acetone, isobutyraldehyde, lactic acid, isoprene, 1,2-propanediol, methane, and biodiesel from CO_2 [2]. However, – limited by the efficiency of the natural carbon fixation, the productivity of these processes is still painfully low.

In addition to the low efficiency of the carbon fixation pathways, limited energy availability is also a bottleneck for carbon fixation. In nature, autotrophs can be classified into photoautotrophs and chemoautotrophs. Photoautotrophs use light as the energy source to fix carbon dioxide, while chemoautotrophs utilize reduced compounds such as molecular hydrogen, hydrogen sulfide and other inorganic compounds [3]. While photosynthesis is widespread in nature, its energy efficiency is generally no higher than 3% [4]. Although the natural carbon fixation processes cannot be utilized in industrial production at the moment, they provide a diversity array of carbon-fixing enzymes and corresponding pathways, and therefore a variety of possibilities for artificial engineering.

Recently, carbon fixation has been reconstructed to improve the carbon fixation rate and to increase the titer and productivity of biofuels and chemicals from CO_2. Based on synthetic biology, both carbon fixation pathways and energy supply modules can be redesigned. By freely combining different carbon fixation pathways and energy supply modules, new synthetic carbon fixation processes are expected to be developed, which will hopefully offer higher efficiencies than their natural counterparts. In this review, we summarized the recent advances in the research on biological carbon fixation from the perspective of synthetic biology, including the exploration of natural carbon fixation pathways, reconstruction and design of artificial carbon fixation pathways, engineering of energy supply patterns for carbon

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Table 1
Comparison of the six known natural carbon fixation pathways.

Pathways	Energy sources(s)	Input(s)	Output(s)	Discovered	Reference
Calvin cycle	Light	3 CO_2, 9 ATP, 6 NAD(P)H	Glyceraldehyde-3-phosphate	1948	Calvin & Benson, [5]
rTCA cycle	Light and Sulfur	2 CO_2, 2 ATP, 4 NAD(P)H	Acetyl-CoA	1966	Evans et al., [6]
WL pathway	Hydrogen	2 CO_2, 1 ATP, 4 NAD(P)H	Acetyl-CoA	1972	Schulman et al., [7]
3HP bicycle	Light	3 HCO_3^-, 5 ATP, 5 NAD(P)H	Pyruvate	1993	Strauss & Fuchs, [8]
Di-4HB cycle	Hydrogen and Sulfur	1 CO_2, 1 HCO_3^-, 3 ATP, 4 NAD(P)H	Acetyl-CoA	2007	Berg et al., [9]
3HP-4HB cycle	Hydrogen and Sulfur	2 HCO_3^-, 4 ATP, 4 NAD(P)H	Acetyl-CoA	2008	Huber et al., [10]

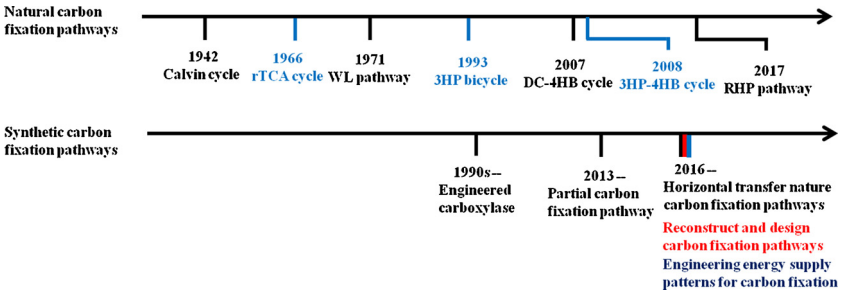

Fig. 1. The time axis of discovery of natural carbon fixation pathways and the development of synthetic carbon fixation pathways.

fixation, as well as challenges and future prospects of biological carbon fixation.

2. Exploring natural carbon fixation pathways

Six natural carbon fixation pathways have been discovered to date (Table 1, Fig. 1). The Calvin cycle was the first described CO_2 fixation pathway (1948), followed by the discovery of the reductive tricarboxylic acid cycle (rTCA cycle, 1966), the Wood-Ljungdahl pathway (WL pathway, 1972), the 3-hydroxypropionate bicycle (3HP bicycle, 1993), the dicarboxylate/4-hydroxybutyrate cycle (Di-4HB cycle, 2007), and the 3-hydroxypropionate-4-hydroxybutyrate cycle (3HP-4HB cycle, 2008) [5–10]. The Calvin cycle, the 3HP bicycle, and the 3HP-4HB cycle function well under aerobic conditions, while the rTCA cycle, the WL pathway, and the Di-4HB cycle contain certain oxygen-sensitive enzymes, and are therefore considered to only function anaerobically [11]. As described in Table 1, light, hydrogen, hydrogen sulfide and sulfur provide the energy needed by autotrophs to fix CO_2. The Calvin cycle is the most wide-spread carbon fixation pathway, and is the most thoroughly studied [12]. In fact, more than 90% of CO_2 in nature is fixed by plants, algae and microorganisms using this carbon fixation cycle. Unfortunately, the pathways other than the Calvin cycle are poorly understood, especially those from anaerobes. The anaerobic carbon fixation enzymes, CO dehydrogenate/acetyl-CoA synthase (from the WL pathway), 2-oxoglutarate synthase (from the rTCA cycle), and pyruvate synthase (from the Di-4HB cycle) should therefore be further characterized.

In addition to these six well-known natural carbon fixation pathways, the development of omics and systems biology enabled researchers to mine for new natural pathways. Very recently, Kono et al. reported a new carbon metabolic pathway named reductive hexulose-phosphate (RHP) pathway, which differs from the Calvin cycle in only a few steps. The enzymes catalyzing the pathway from fructose-6-phosphate (F6P) to ribulose-5-phosphate (Ru5P) are transketolase, sedoheptulose-1,7-bisphosphatase, and ribulose-5-phosphate 3-epimerase in the Calvin cycle. These enzymes are replaced by D-arabino-3-hexulose-6-phosphate synthase and 6-phospho-3-hexuloisomerase in the RHP pathway [13].This new pathway is present in methanogenic archaea. It consumes 1 mol of CO_2, 3 mol of ATP, and 2 mol of NADPH, to produces1 mol of formic acid. However, whether the RHP pathway allows autotrophy remains unknown.

3. Re-constructing and designing carbon fixation pathways

As the low carbon fixation efficiency of autotrophs is mainly limited by carboxylase, many studies focused on engineering those limiting enzymes. RuBisCO, the rate-limiting CO_2-fixing enzyme in the Calvin cycle, has long been the primary engineering target. Different strategies have been attempted in order improve the carbon fixation efficiency, including replacing the native RuBisCO with homologs from other sources, constructing a hybrid RuBisCO, and activity-directed selection for more efficient RuBisCO mutants (Table 2) [14–17]. In addition to engineering of RuBisCO, the carbon fixation efficiency of the Calvin cycle can be improved by overexpressing sedoheptulose-1, 7-bisphosphatase [18]. Additionally, enzyme engineering provides improved elements for constructing efficient carbon fixation pathways (Fig. 2).

Recently, using the key enzymes for carbon fixation, researchers also tried to introduce a carbon fixation ability into heterotrophic hosts, which enables the host to re-assimilate CO_2 at the expense of energy derived from external carbohydrates.The Calvin cycle is one of the most reconstructed pathways and its two key enzymes have been introduced into both *Escherichia coli* and *Saccharomyces cerevisiae* to enable CO_2 cycling and to increase the ethanol yield [19–22]. In another case,the introduction of a part of the 3-hydroxypropionate/4-hydroxybutyrate cycle in *Pyrococcus furiosus* enabled the production of 3-hydroxypropionicacid from CO_2 [23].

Due to the slow cell growth, inefficient protein expression and low chemical productivity of autotrophs, heterotrophs were considered more promising chassis cells for carbon fixation. Researchers therefore tried to reconstruct the complete carbon fixation pathways in the heterotrophs. In 2013, Mattozzi et al. divided the 3-hydroxypropionate cycle from *Chloroflexus aurantiacus* into four modules and expressed them separately in *Escherichia coli,* which was the first attempt to horizontally transfer a natural carbon fixation pathway [24]. In 2016, Antonovsky et al. evolved a fully functional Calvin cycle in *E. coli* [25]. Moreover, in 2018 there were two reports of reconstructing a WL pathway in *E. coli* to produce glycine and serine from CO_2 and formate [26,27].

Although the introduction of natural carbon fixation pathways into heterotrophic hosts may be more straight-forward, creating novel non-natural carbon fixation pathways can provide more possibilities. Bar-Even et al. computationally obtained a series of synthetic CO_2-fixation pathways that combined existing metabolic building blocks from various organisms, which provides more choices for constructing new