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Synthetic biology for CO_2 fixation

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Recycling of carbon dioxide (CO_2) into fuels and chemicals is a potential approach to reduce CO_2 emission and fossil-fuel consumption. Autotrophic microbes can utilize energy from light, hydrogen, or sulfur to assimilate atmospheric CO_2 into organic compounds at ambient temperature and pressure. This provides a feasible way for biological production of fuels and chemicals from CO_2 under normal conditions. Recently great progress has been made in this research area, and dozens of CO_2-derived fuels and chemicals have been reported to be synthesized by autotrophic microbes. This is accompanied by investigations into natural CO_2-fixation pathways and the rapid development of new technologies in synthetic biology. This review first summarizes the six natural CO_2-fixation pathways reported to date, followed by an overview of recent progress in the design and engineering of CO_2-fixation pathways as well as energy supply patterns using the concept and tools of synthetic biology. Finally, we will discuss future prospects in biological fixation of CO_2.

carbon dioxide fixation, synthetic biology, CO_2-fixation pathway, energy supply

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INTRODUCTION

Energy and the environment are two major issues that are closely related to human life. World energy consumption in 2011 reached 1.54×1,011 kW h, a 30% increase compared with an energy expenditure of 1.18×1,011 kW h in 2000 (Statistical review of world energy, 2013). Fossil fuels (coal, oil, and natural gas) contribute to over 75% of the world's energy consumption (International Energy Agency, 2014). The burning of fossil fuels has resulted in the massive release of carbon dioxide into the earth's atmosphere, which has generated worldwide concern regarding the associated greenhouse effect. It has been reported that worldwide CO_2 emission is increasing each year and reached 3.45×1,010 t in 2012 (Olivier et al., 2013). Therefore, the recycling of CO_2 wastes directly into fuels or chemicals is a potential approach to reduce carbon emission and to resolve the potential energy crisis.

Carbon atoms in CO_2 molecules are in their highest oxidation state, whereas those in common fuels and chemicals such as hydrocarbons, alcohols, and acids are in lower states. Energy input is thus required to synthesize fuels and chemicals from CO_2, which is one of the reasons why CO_2 is not extensively used in current chemical industries. However, autotrophic microbes can utilize light to fix atmospheric CO_2 through the well-known process of photosynthesis. Recently, much effort has been spent to take advantage of the abilities of autotrophic cyanobacteria and algae through metabolic engineering. The past five years have witnessed great success in this area. To date, dozens of fuels and chemicals including ethanol, butanol, lactic acid, acetone, isobutyraldehyde,

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isoprene, and oil can be synthesized from CO_2 by using engineered autotrophic microbes (Angermayr et al., 2012; Atsumi et al., 2009; Bentley and Melis, 2012; Dexter and Fu, 2009; Zhou, 2014; Lan and Liao, 2011; Lan and Liao, 2012; Zhou et al., 2012). Among them, CO_2-derived ethanol and lactic acid were produced at a level of grams per liter, demonstrating the potential of CO_2 for production of fuels and chemicals.

A deep understanding of natural CO_2-fixation pathways and rapid development of synthetic biology have provided us with new insights into this area of research. Great progress in new CO_2-fixation pathways and new energy supply patterns continue to emerge in recent years. To comprehensively introduce these new technological advances, we will first briefly introduce the six natural CO_2-fixation pathways. This will serve as a gateway to recent progresses in new CO_2-fixation pathways and energy supply patterns using synthetic biology (Chao et al., 2015). According to the Royal Academy of Engineering of UK, synthetic biology is defined as the design and engineering of biologically based parts, novel devices, and systems and the redesign of existing natural biological systems (The royal academy of engineering, 2009). Therefore, both design and engineering of synthetic CO_2-fixation pathways are included in this review.

NATURAL CO_2-FIXATION PATHWAYS

Six natural CO_2-fixation pathways have been reported to date (Figure 1), including the Calvin-Benson-Bassham cycle (hereafter, the Calvin cycle), the 3-hydroxypropionate cycle, the Wood-Ljungdahl pathway, the reductive tricarboxylic acid (TCA) cycle, the dicarboxylate/4-hydroxybutyrate cycle, and the 3-hydroxypropionate-4-hydroxybutyrate cycle. The Calvin cycle, the 3-hydroxypropionate cycle, and 3-hydroxypropionate-4-hydroxybutyrate cycle are aerobic, while the others pathways are anaerobic pathways because of the presence of certain oxygen-sensitive enzymes (Ducat and Silver, 2012).

Aerobic CO_2-fixation pathways

The Calvin cycle (Figure 1A), as the most important CO_2-fixation pathway in nature from which all crop biomasses obtain their carbon, has attracted great attention from researchers (Stitt et al., 2010). It exists widely in plants, algae, cyanobacteria, and other organisms and is driven by light. This cycle was named after Melvin Ellis Calvin, who discovered it in the 1940s and won the Nobel Prize in Chemistry in 1961. One Calvin cycle converts three molecules of CO_2 to one molecule of glyceraldehyde 3-phosphate, with the consumption of nine ATP molecules and six nicotinamide adenine dinucleotide phosphate (NAD(P)H) molecules. It is the highest energy-consuming pathway among all six natural CO_2-fixation pathways. The CO_2-fixing enzyme, RuBisCO, is the rate-limiting enzyme in this cycle, with an average activity of 3.5 μmol min^{-1} mg^{-1} (Bar-Even et al., 2010). Moreover, O_2 in the air is a substrate of RuBisCO and competes with CO_2 for activity sites on the enzyme. Reaction with O_2 generates phosphoric glyoxylate, which releases CO_2 through subsequent photorespiration pathways.

The 3-hydroxypropionate cycle (Figure 1B) exists in photosynthetic green nonsulfur bacteria and is driven by light (Herter et al., 2001; Strauss and Fuchs, 1993). This cycle is the most complex, containing 16 enzymatic reaction steps that are catalyzed by 13 enzymes. In contrast to the Calvin cycle, which converts CO_2 to glyceraldehyde 3-phosphate, this cycle converts three molecules of HCO_3^- into one molecule of pyruvate, with the addition of five ATP and NAD(P)H molecules. There are two CO_2-fixing enzymes in this cycle: acetyl-CoA carboxylase and propionyl-CoA carboxylase.

Another archaeal aerobic CO_2-fixation pathway discovered in 2007 is the 3-hydroxypropionate-4-hydroxybutyrate cycle, which is driven by sulfur and hydrogen (Figure 1F) (Berg et al., 2007). This cycle synthesizes one molecule of acetyl coenzyme A from two molecules of HCO_3^-, four molecules of ATP, and four equal molecules of NAD(P)H. The two CO_2-fixing enzymes used are the same as those of the 3-hydroxypropionate cycle.

Anaerobic CO_2-fixation pathways

The Wood-Ljungdahl pathway (Figure 1C), which exists mainly in acetate-producing anaerobes, was identified in the 1970s by Harland G. Wood and Lars G. Ljungdahl (Ragsdale, 1997) and uses hydrogen as its energy source. It is the only non-cycle CO_2-fixation pathway, contains the fewest reaction steps, and consumes the least amount of energy. This pathway converts two molecules of CO_2 (or one molecule of CO_2 and one molecule of carbon monoxide) into one molecule of acetyl coenzyme A, using one ATP and four NAD(P)H molecules. It is therefore called the anaerobic acetyl coenzyme A pathway (Drake, 1994).

The reductive TCA cycle (Figure 1D) exists in photosynthetic green sulfur bacteria and anaerobic bacteria. This cycle generates one molecule of acetyl coenzyme A via two molecules of CO_2, with the consumption of two ATP and four NAD(P)H molecules (Evans et al., 1966; Kim et al., 1992). The two CO_2-fixing enzymes in this cycle are α-ketoglutarate synthase and isocitrate dehydrogenase. The enzyme α-ketoglutarate synthase is strictly anaerobic, with unknown activity. Isocitrate dehydrogenase has the highest activity amongst all CO_2-fixing enzymes listed in Table 1 (Berg, 2011).

The archaeal anaerobic CO_2-fixation pathway—the dicarboxylate/4-hydroxybutyrate cycle (Figure 1E)—was discovered in 2008. This cycle uses sulfur and hydrogen as energy sources (Huber et al., 2008). One molecule each of CO_2 and

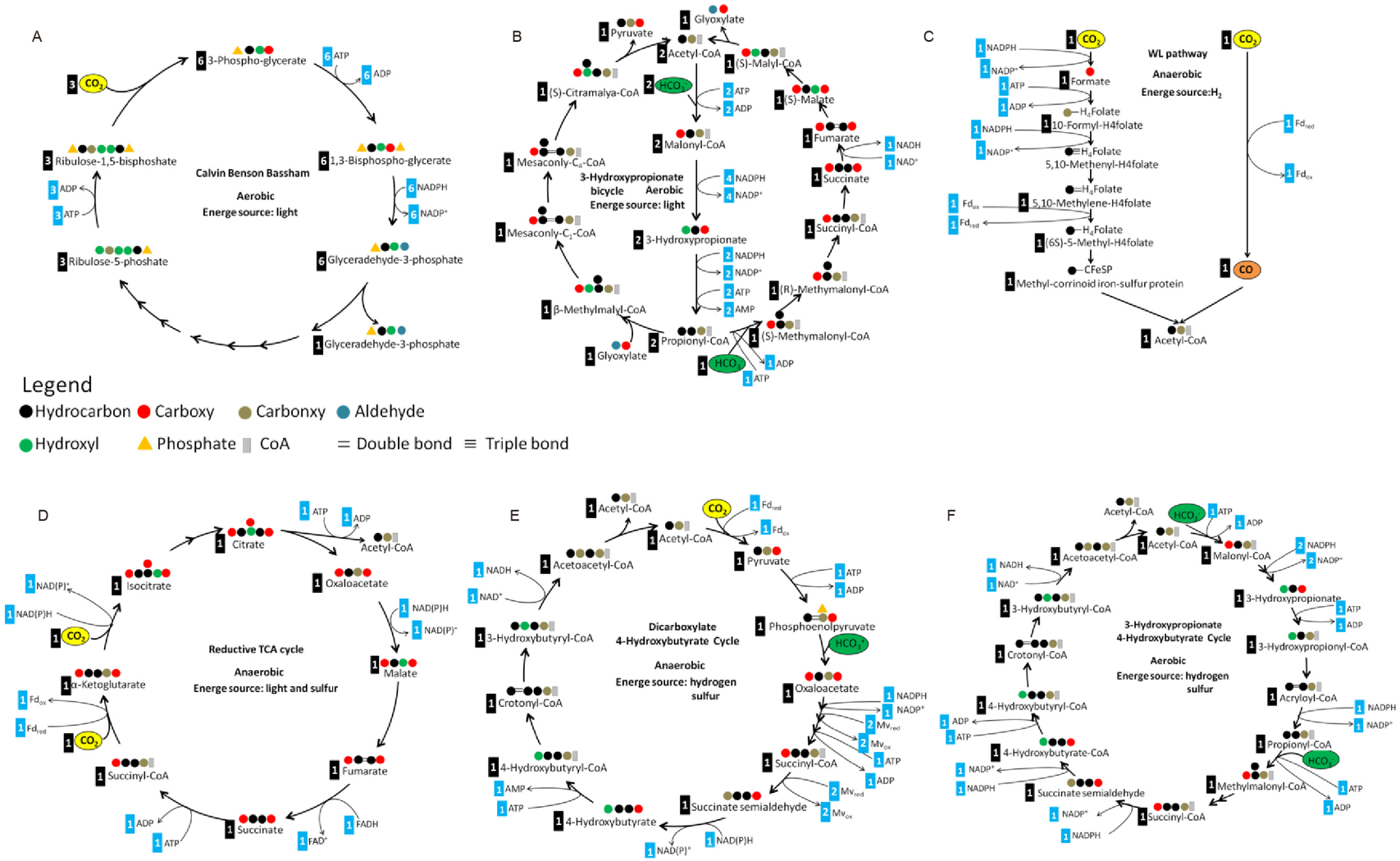

Figure 1 Six natural CO_2-fixation pathways. A, Calvin cycle; B, 3-hydroxypropionate cycle; C, Wood-Ljungdahl pathway; D, reductive TCA cycle; E, dicarboxylate/4-hydroxybutyrate cycle; F, 3-hydroxypropionate/4-hydroxybutyrate cycle.

Table 1 Summary of the six natural CO_2-fixation pathwaysa)

	Organisms	Energy	Species	Reaction numbers	Total reaction equations	ATP/CO_2 (mol/mol)	NAD(P)Ha /CO_2 (mol/mol)	CO_2-fixing enzymes	Specific activity μmol min^{-1} mg^{-1} (CO_2/HCO_3^-)	Reference
A	Plant Algae Cyanobac-teria	Light	Maize *Scenedesmus* sp. *Synechocystis* sp.	13	$3CO_2$+9ATP+ 6NAD(P)H→ GA-3P+ 9ADP+6$NAD(P)^+$+8Pi	3	2	RuBisCO (EC: 2.1.1.127)	3.5	(Bar-Even et al., 2010; Calvin, 1949; Calvin and Massini, 1952)
B	Green nonsulfur bacteria	Light	*Chloroflexus aurantiacus*	16	$3HCO_3^-$+5ATP+ 5NAD(P)H→Pyruvate+ 3ADP+2AMP+3Pi+ 2PPi+5$NAD(P)^+$	1.67	1.67	Acetyl-CoA carboxylase (EC: 6.4.1.2) Propionyl-CoA carboxylase (EC: 6.4.1.3)	18 30	(Bar-Even et al., 2010; Herter et al., 2001; STRAUSS and FUCHS, 1993)
C	Anaerobic bacteria	Hydro-gen	*Clostridium ljungdahlii*	8	2CO_2+ATP+2NAD(P)H+ 2Fd_{red}+CoASH→AcCoA+ ADP+Pi+2$NADP^+$+2Fd_{ox}	0.5	2	Formate dehydrogenase (EC: 1.2.1.2) CO dehydro-genate/Acetyl-CoA synthase (EC: 2.3.1.169)	2.34 0.46	(Drake, 1994; Ragsdale, 1997)
D	Green sulfur bacteria	Light Sulfur	*Chlorobiumthio-sulfatophilum*	9	2CO_2+2ATP+2NAD(P)H+ FADH+Fd_{red}+CoASH→ AcCoA+2ADP+2Pi+ 2$NAD(P)^+$+FAD^++Fd_{ox}	1	2	2-Oxoglutarate synthase (EC: 1.2.7.3) Isocitrate dehydrogenase (EC: 1.1.1.87)	– 53	(Bar-Even et al., 2010; Evans et al., 1966; Kim et al., 1992)
E	Archaea	Hydro-gen Sulfur	*Ignicoccus hospitalis*	14	CO_2+HCO_3^-+3ATP+ NAD(P)H+Fd_{red}+4MV_{red}+ CoASH→AcCoA+2ADP+ AMP+2Pi+2PPi+$NAD(P)^+$ +Fd_{ox}+ 4MV_{ox}	1.5	2	Pyruvate synthase (EC: 1.2.7.1) Phospho-enolpyruvate car-boxylase (EC: 4.1.1.31)	– 35	(Bar-Even et al., 2010; Huber et al., 2008)
F	Archaea	Hydro-gen Sulfur	*Metallosphaera sedula*	16	2HCO_3^-+4ATP+ 4NAD(P)H+CoASH→ AcCoA+3ADP+3Pi+ AMP+PPi+4$NADP^+$	2	2	Acetyl-CoA carboxylase (EC: 6.4.1.2) Propionyl-CoA carboxylase (EC: 6.4.1.3)	18 30	(Bar-Even et al., 2010; Berg et al., 2007)

a) 1 Fd_{red}=1 NAD(P)H; 1 FADH=1 NAD(P)H; 2 MV_{re}=1 NAD(P)H

HCO_3^- are used to synthesize one molecule of acetyl coenzyme A, consuming three ATP and four NAD(P)H molecules. The CO_2-fixing enzymes in this cycle are pyruvate synthase and phosphoenolpyruvate carboxylase. Pyruvate synthase is another strictly anaerobic enzyme with unknown activity. It is reported that the KM of phosphoenolpyruvate carboxylase to HCO_3^- is the smallest amongst all carboxylases listed in Table 1 (Oleary, 1982), demonstrating its high affinity for HCO_3^-. Notably, the doubling time of autotrophic archaea Ignicoccus hospitalis, which utilizes this CO_2-fixation pathway, is only 1 h under optimal growth conditions (Jahn et al., 2007). This may be partly contributed by the strong affinity of phosphoenolpyruvate carboxylase.

DESIGNING AND ENGINEERING CO_2-FIXATION PATHWAYS BY SYNTHETIC BIOLOGY

Research progress in this area is summarized in Table 2, which can be divided into three parts: (i) computer-aided design of new CO_2-fixation pathways and relocation of natural CO_2-fixation pathways; (ii) engineering CO_2-fixation pathways by increasing the CO_2 supply; and (iii) engineering CO_2-fixation pathways by enhancing activities of CO_2-fixing enzymes.

Design and relocation of CO_2-fixation pathway

Designing an efficient CO_2-fixation pathway is the ultimate aim of synthetic biology, but is still faced with great challenges at the current stage. There is only one reported work on this technology: in 2010, Bar-Even et al. computationally obtained a series of synthetic CO_2-fixation pathways that combined existing metabolic building blocks from various organisms, based on the properties of approximately 5,000 natural enzymes (Bar-Even et al., 2010). The kinetics, energetics, and topologies of both synthetic and natural pathways were compared. One synthetic pathway, which employed the most effective CO_2-fixing enzyme, phosphoenolpyruvate carboxylase, was based on the C4 cycle and was predicted to be two to three times faster than the Calvin cycle. However, construction of such a cycle was still restricted by uncertainties in the expression, activity, stability, and regulation of all enzymes in this pathway.

Recently, relocation of natural CO_2-fixation pathways has

Table 2 Recent progress in designing and engineering CO_2-fixation pathways by synthetic biology

	Results	Year	Reference
Design and relocation of CO_2-fixation pathway	Designed alternative synthetic CO_2-fixation pathways by computer	2010	(Bar-Even et al., 2010)
	Divided the 3-hydroxypropionate cycle from *Chloroflexus aurantiacus* into four sub-pathways and expressed them separately in *Escherichia coli*	2013	(Mattozzi et al., 2013)
	Produced 3-hydroxypropionate from CO_2 by *Pyrococcus furiosus* introduced with partial 3-hydroxypropionate/4-hydroxybutyrate cycle from *Metallosphaera sedula*	2013	(Keller et al., 2013)
	Recycled CO_2 in an engineered *E. coli* with introduction of partial cyanobacterial Calvin cycle	2013	(Zhuang and Li, 2013)
	Developed a relative quantification approach to calculate the CO_2-fixation efficiency in *E. coli* with partial cyanobacterial Calvin cycle	–	(Gong et al., 2015)
	Increased ethanol yield in *Saccharomyces cerevisiae* with partial cyanobacterial Calvin cycle	2013	(Guadalupe-Medina et al., 2013)
Engineering the CO_2-fixation pathway by increasing the CO_2 supply	Reconstructed cyanobacterial carboxysome in *E. coli*	2012	(Bonacci et al., 2012)
	Improved CO_2-fixation efficiency of an CO_2-fixing *E. coli* by introduction of carbonic anhydride	–	(Gong et al., 2015)
	Introduced a bypass photorespiration pathway into the *E. coli* glycolate metabolic pathway to release CO_2 into the chloroplast	2007	(Kebeish et al., 2007)
Engineering the CO_2-fixation pathway by improving the CO_2-fixing enzyme	Developed an activity-directed selection method for RuBisCO and increased the specific carboxylation activity of RuBisCO in *Synechococcus* sp. PCC 7002 by 85%	2014	(Cai et al., 2014)
	Increased thermotolerance of RuBisCO activase from *Arabidopsis thaliana* to improve the stability of RuBisCO	2009	(Kumar et al., 2009)
	Replaced the tobacco RuBisCO with cyanobacteria RuBisCO and observed significantly increased growth rate of tobacco under high concentration of CO_2	2014	(Lin et al., 2014)
	Constructed a hybrid RuBisCO from different RuBisCO large and small subunits and studied its enzymatic properties	–	(Genkov et al., 2010; Ishikawa et al., 2011)
	Reported that over-expressing the sedoheptulose-1-7 bisphosphatase improves photosynthetic carbon gain and yield	2011	(Rosenthal et al., 2011)

received much attention, as engineering natural CO_2-fixing autotrophic microbes is usually difficult. In 2013, Mattozzi et al. divided the 16 steps of the 3-hydroxypropionate cycle from *Chloroflexus aurantiacus* into four sub-pathways and expressed each sub-pathway in *Escherichia coli* (Mattozzi et al., 2013). Each sub-pathway was found to be functional, which provided a basis for the potential synthesis of CO_2-fixing *E. coli*. In the same year, Keller et al. expressed a part of the 3-hydroxypropionate/4-hydroxybutyrate cycle from the archaea *Metallosphaera sedula* (optimum growth temperature of 73°C) in another archaea *Pyrococcus furiosus* (optimum growth temperature of 100°C) (Keller et al., 2013). This engineered strain can synthesize a valuable industrial chemical, 3-hydroxypropionic acid, from CO_2, using hydrogen as the energy source.

The above two studies successfully introduced a natural CO_2-fixation pathway into another host but failed to direct the carbon flux from the CO_2-fixation pathway into the host's central metabolic network. In order to conjugate the introduced CO_2-fixation pathway with the central metabolic network so that the fixed carbon can be efficiently utilized by the host for cell growth, two intermediates in the Calvin cycle, ribulose 5-phosphate (Ru5P) and 3-phosphoglycerate (3PGA), were selected as nodes to connect the host's central pentose phosphate pathway with the glycolysis pathway. By constructing a CO_2-fixing bypass in the central metabolic pathways, CO_2 recycling and increased ethanol yield were observed in *E. coli* (Zhuang and Li, 2013) and *Saccharomyces cerevisiae* (Guadalupe-Medina et al., 2013), respectively. However, these studies were unable to determine the amount of CO_2 that had been fixed by the central metabolic pathways. Recently, we have developed a relative quantification method to calculate the ratio of carbon flux from the CO_2-fixation pathway and the central metabolic pathway by LC/MS/MS detection of ^{13}C and unlabeled metabolites (Gong et al., 2015).

After reconstructing the carbon fixation pathway, researchers must consider methods to further improve the efficiency of carbon fixation. There are two methods for this improvement. The first is to increase the concentration of inorganic carbon substrates. The second is to enhance the metabolic flux. Current research is mainly focused on reconstructing enzymes in the carbon fixation pathways.

Engineering of CO_2-fixation pathways via increase in CO_2 supply

Cyanobacteria and C4 plants employ the carbon-concentrating mechanism (CCM) to increase intracellular inorganic carbon concentrations. CCM is accomplished by the organelle carboxysome in cyanobacteria. Bicarbonate is transported into the carboxysome, converted to CO_2 by carbonic anhydrase, and catalyzed by the encapsulated RuBisCO therein. The protein shell of carboxysome is positively charged and thus acts as a barrier to prevent loss of CO_2 and facilitates build-up of CO_2 around RuBisCO. It has been reported that CO_2 concentration in the carboxysome is approximate a 1,000-fold higher than that of the outside (Badger and Price, 2003). In C4 plants, atmospheric CO_2 is first captured by the highly active phosphate pyruvate carboxylase in the mesophyll cells to produce 4-carbon organic acids such as malate and oxaloacetate. These 4-carbon organic acids are transported into the bundle sheath cells to release CO_2 by actions of decarboxylases and then converted to energy-rich molecules such as glucose by RuBisCO therein. The CO_2 concentration is approximate 10-fold higher in the bundle sheath cells compared to outside the cells, as these cells can prevent the diffusion of CO_2.

Much work has been done to simulate CCMs. In 2012, Bonacci et al. introduced shell proteins of cyanobacterial carboxysome into *E. coli* and observed the assembly of icosahedral complexes in *E. coli* (Bonacci et al., 2012). This was the first evidence to suggest the possibility that reconstruction of the CCM in a heterologous host can induce heterotrophic CO_2-fixation. However, the function of this synthetic carboxysome in the heterologous host was not reported. We recently introduced cyanobacterial carbonic anhydrase, a key enzyme in the cyanobacterial CCM, into *E. coli*. Improved CO_2-fixation efficiency was found in the engineered CO_2-fixing *E. coli*, demonstrating that the CCM can also be transplanted into heterotrophic microbes (Gong et al., 2015). Ideas on introducing the CCM from cyanobacteria or C4 plants into C3 crops to improve the photosynthetic efficiency of the latter (Covshoff and Hibberd, 2012; Price et al., 2011; Price et al., 2013) have been reported, but much research is still needed on the topic.

Designing a new CCM is an alternative strategy to reconstructing CCMs from cyanobacteria. In 2007, Kebeish et al. developed a new approach to increase CO_2 concentration in plant chloroplasts (Kebeish et al., 2007). They introduced three genes of the *E. coli* glycolate catabolic pathway into *Arabidopsis thaliana* chloroplasts. This new pathway replaced plant photorespiration, which occurred in the peroxisomes and the mitochondria. Therefore, CO_2 that should have been released into the cytoplasm through photorespiration was released into the chloroplasts. As a result, CO_2 concentrations in chloroplasts were increased for carbon fixation by RuBisCO. This design provided a new alternative photorespiration pathway that can improve photosynthesis and possibly increase crop yield.

Engineering the CO_2-fixation pathway by enhancing CO_2-fixing enzymes

RuBisCO, the rate-limiting CO_2-fixing enzyme in the Calvin cycle, has long been the primary engineering target, since CO_2-fixation efficiency was believed to be associated with the crop production. RuBisCO is a bifunctional enzyme with

both carboxylation activity towards CO_2 and oxygenation activity toward O_2. Therefore, manipulation of RuBisCO activity involves enhancement of its extremely slow carboxylation activity and reduction of its oxygenation activity. However, engineering of RuBisCO has made little progress in the past ten years, as it has a complex hexadecamer structure, but lacks sufficient structure-function relationships. Recent engineering by directed evolution was successful in improving its heterologous expression in *E. coli*, but still failed to improve its carboxylation activity and selectivity (Whitney et al., 2011). This year, we developed an activity-directed selection system for RuBisCO and successfully improved the specific carboxylation activity of RuBisCO from *Synechococcus* sp. PCC 7002 by 85% (Cai et al., 2014). Mutant analyses revealed that all mutations occurred in the small subunit, emphasizing the long-term overlooked contribution of the small-subunit to its catalytic activity.

Another engineering target is RuBisCO activase, which is required by some RuBisCO for activation prior to every catalytic cycle. Kumar et al. found that improving the thermal stability of RuBisCO activase increased stability of RuBisCO within a certain temperature range (Kumar et al., 2009).

To further enhance the CO_2-fixation efficiency of crop plants as a means to increase crop production, chimeric RuBisCOs from various sources have been reported. Lin et al. knocked-out the large subunit of RuBisCO in tobacco and inserted genes for large and small RuBisCO subunits from *Synechococcus elongatus* PCC7942. The transgenic tobacco was able to grow at high CO_2 concentrations. This work was the first step to implement the carbon concentrationg mechanisms from cyanobacterial to tobacco, with the potential of increasing its photosynthetic efficiency (Lin et al., 2014). Genkov et al. replaced the small subunit of *Chlamydomonas* RuBisCO with that of plants (e.g., spinach, Arabidopsis, sunflower). Compared with the *Chlamydomonas* RuBisCO, the engineered RuBisCO hybrids demonstrated high selectivity, albeit at a decreased catalytic efficiency (Genkov et al., 2010). Aside from engineering of RuBisCO, increasing Calvin cycle intermediates also improved efficiencies of carbon fixation. For example, Rosenthal et al. over-expressed sedoheptulose-1,7-bisphosphatase in tobacco to increase the reproduction rate of ribulose-1,5-bisphosphate (RuBP). This consequently increased efficiency of CO_2 fixation and growth of tobacco (Rosenthal et al., 2011).

DESIGNING ENERGY SUPPLY PATTERNS FOR CO_2-FIXATION

Energy input is required for CO_2 fixation. Autotrophic microbes naturally employ light, sulfur, and hydrogen as their energy source, while energy for heterotrophic CO_2 fixation comes mainly from metabolism of sugar. Recently, new energy supply patterns have been attempted for CO_2 fixation in both autotrophic and heterotrophic microbes, the results of which have paved an exciting starting point in this field.

New energy supply for CO_2-fixation in autotrophic microbes

To date, electricity is the sole new energy that autotrophic microbes can utilize. In 2012, Li et al. reported that an engineered *Ralstonia eutropha* H16 could utilize electricity for CO_2 fixation to produce higher alcohols such as 3-methyl-1-butanol and isobutanol (Li et al., 2012). Another example used the concept of a reverse microbial fuel cell to transform electricity to energy forms that can be used by microbes. Electricity was first used to reduce nitrite to ammonia, the latter of which can be used as an energy source for cell growth and CO_2 fixation in the chemoautotroph *Nitrosomonas europaea* (Khunjar et al., 2012).

New energy supplies for CO_2-fixation in heterotrophic microbes

Engineering of heterotrophs to utilize electricity or light as the sole energy resource has been reported. For electricity utilization, it has been reported that electrical current can be directly applied to a gram-positive bacterium to produce methane from CO_2 by electromethanogenesis (Cheng et al., 2009). In 2010, Nevin et al. used a graphite electrode to provide electrons for the acetogenic heterotroph *Sporomusa ovata*, which was grown in biofilm form on the cathode surface (Nevin et al., 2010). The electrons were then used by the bacterial strain to reduce CO_2 to produce acetate and a small amount of 2-oxobutyrate.

There are two strategies in utilization of light. Direct light utilization includes reconstruction of natural photosystems in heterotrophic microbes. Compared with photosystems I and II, the proteorhodopsin photosystem is a relatively simple one. In 2007, Martinez et al. expressed six genes from the proteorhodopsin photosystem of marine picoplankton into *E. coli* and successfully enabled photophosphorylation in *E. coli* exposed to light (Martinez et al., 2007). Indirect utilization of light first converts light to electricity *in vitro* and then provides electricity for microbes to convert CO_2 into organic compounds (Yu, 2012). This strategy was inspired by the development of photovoltaic technology and by the fact that some microbes are already capable of utilizing electricity. Other energy resources (e.g., heat, mechanical, and nuclear energy), currently unavailable for microbes, can also be transformed into electricity *in vitro* to be used by microbes.

CONCLUDING REMARKS

Conversion of CO_2 to fuels and chemicals is an area of great interest, as it provides potential solutions to both environmental and energy issues. Homogeneous and heterogeneous catalytic hydrogenation and photocatalysis are capable of con-

verting CO_2 into energy through chemical reactions. However, CO_2 is not used extensively as a raw material in the current industry, possibly because large amount of input energy is required to reduce the CO_2 molecules. Billions of years of natural evolution have created a biological route for CO_2 fixation. Although great success has been made in microbial production of fuels and chemicals from CO_2 during the past five years, achieving this on an industrial scale is still not feasible. Inefficiencies in CO_2 fixation mainly lie in inefficient natural pathways and the energy supply.

Six natural carbon-fixation pathways have been reported to date. Because the Calvin cycle is the primary pathway in plants, algae, and cyanobacteria and the pathway enzymes can be easily expressed heterologously, most engineering efforts have been directed towards the Calvin cycle. However, the past ten years of research have made little progress. Aside from difficulties in engineering RuBisCO, the energy requirements of the Calvin cycle are the highest amongst the six pathways. Therefore, this pathway may not be the best choice for CO_2 fixation. We believe that with new developments in synthetic biology, such as the computer-aided design of new synthetic CO_2-fixation pathways, there will be an increase in manipulation of other CO_2-fixation pathways.

Concerning the energy supply issue, solar energy is the cheapest resource. However, the reconstruction of complex biological photosynthesis systems is very difficult. Based on preliminary results on usage of electric energy by microbes and the development of photovoltaic technology, multiple energy resources may be used for CO_2 fixation.

We have already seen that with the powerful concepts and tools of synthetic biology, researchers are able to design and engineer new CO_2-fixing elements, pathways, and energy supply systems. We believe that further progress will continue to be made in this field of research. In the near future, synthetic modules or microbes may be used in the industry to produce fuels and chemicals from CO_2.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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