Application of synthetic biology in cyanobacteria and algae

Bo Wang1*, Jiangxin Wang1, Weiwen Zhang1 and Deirdre R. Meldrum1*

1 Center for Biosignatures Discovery Automation, The Biodesign Institute, Arizona State University, Tempe, AZ, USA
2 Biological Design Graduate Program, Arizona State University, Tempe, AZ, USA

Edited by: David Nielsen, Arizona State University, USA
Reviewed by: Shahe Aslani, University of California at Davis, USA
Christie A. M. Peeler, Colorado State University, USA

*Correspondence: Bo Wang and Deirdre R. Meldrum, Center for Biosignatures Discovery Automation, The Biodesign Institute, Arizona State University, 1001 South Millikin Avenue, Tempe, AZ 85287-6501, USA, e-mail: bo.wang.6@asu.edu; deirdre.meldrum@asu.edu

Current address: Weiwen Zhang, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, People’s Republic of China

INTRODUCTION

Cyanobacteria and algae are endowed with the complex photosynthesis systems (Mulkidjanian et al., 2006) which can absorb solar irradiation with a broad wave length and thereafter channel the absorbed energy to other forms of energy carriers such as chemicals (Chisti, 2007; Takahashi et al., 1998; van de Meenen et al., 2006) and electricity (Furukawa et al., 2006; Picciotta et al., 2010). Solar irradiation is a clean, abundant, and renewable energy resource and, if being properly and efficiently transferred, would be more than enough to power the entire human society (Rittmann, 2008). In addition, growing cyanobacteria and algae do not require arable land, which would eventually alleviate the increasing food prices due to the growing crop-based microalgae (Rittmann, 2008). In contrast, they can fix carbon dioxide (CO2), a type of greenhouse gas, during photosynthesis. Furthermore, cyanobacteria and algae grow faster than plants and bear relatively simple genetic background which is relatively easy to manipulate (Koksharova and Wolk, 2002).

As an emerging discipline that tackles biotechnology from a rational-design approach, synthetic biology aims to redesign existing biological systems or create artificial life (Benner, 2003; Endy, 2005; Mukherji and van Oudenaarden, 2009). In recent years, synthetic biology research has been focused on model species such as Escherichia coli and yeast, and has greatly boosted not only the in-depth understanding of the biological mechanisms in these cells, but also the capability and efficiency of these systems in the production of various useful products (Bennon, 2003; Lee and Lee, 2003; Martin et al., 2003; Martijn et al., 2003; Isaacs et al., 2004; Ro et al., 2006; Dwyer et al., 2007; Atsumi et al., 2008b; Imai et al., 2008; Keasling, 2008; Prather and Martin, 2008; Zhang et al., 2008, 2012; Bayer et al., 2009; Ma et al., 2009; Mukherji and van Oudenaarden, 2009; Steen et al., 2010; Yim et al., 2011). However, with over 40 cyanobacterial genome sequences1 and more than 60 algal genome sequences2 being completed and published, application of synthetic biology in cyanobacteria and algae has significantly lagged behind those in E. coli and yeast. Considering the aforementioned inherent merits of the photosynthetic microbes, we believe it would be of great scientific and application values to further develop synthetic biology tools and apply them in cyanobacteria and algae. We herein review the recent progresses and the challenges in developing and applying synthetic biology for cyanobacteria and algae.

TOOLS FOR SYNTHETIC BIOLOGY IN CYANOBACTERIA AND ALGAE

DEVELOPMENT OF “BIOTRICKS” FOR CYANOBACTERIA AND ALGAE

“BioBricks” stand for standardized DNA parts with common interface and can be assembled in living organisms. They are the basic interchangeable elements for regulating the genetic circuit3. Here we focus on the development of the most common BioBricks for cyanobacteria and algae (i.e., promoters, transcriptional terminators, ribosome binding sites, and other regulatory factors).
Promoters

Both native and foreign promoters have been evaluated in cyanobacteria, mostly using *Synechococcus elongatus* PCC 7942 (hereafter *Synechocystis* 6803) and *Synechocystis* sp. PCC 7942 (hereafter *Synechocystis* 7942) as model species (Table 1). The native promoters used are usually from genes essential to photosynthesis such as CO2 fixation (*Ptrc1O*, *Ptrc*, *Ptcp*, *Pcap*), photosystem I (*PisI*, *Pppx*,*Pppad*), and photosynthesis antenna protein phycocyanin (*PpCp*). A native nickel-inducible promoter, *PnrsB*, has also been successfully utilized to express phage lysis genes in *Synechocystis* 6803 (Liu and Curtiss, 2009). Besides the native promoters, a limited number of foreign promoters have also been characterized in cyanobacteria. The chimeric *Ptac/Pptrc* promoter, a strong promoter in *E. coli* used in *Synechocystis* 6803 showed that the strength of *Ptrc1O* (a version of the *Ptrc/Ptac* promoter) was more than fourfold higher than all versions of the promoter of native ribulose bisphosphate carboxylase/oxygenase (*RuBisCO*) large subunit, *PrbcL*, whereas the common *E. coli* promoters *Plac*, *Ppe*, and *Pct* exhibited very low or no detectable activities in the same system (Huang et al., 2010). Since currently very little is known about the performance of various native and foreign promoters in cyanobacteria, a systematic investigation on behaviors of various promoters in cyanobacteria would be important.

In algae, CaMV 35S and SV40 promoters from viruses have been used to express target genes (Bentley et al., 1990; Wang et al., 2010). However, the most effective promoters have been derived from highly expressed algal genes. For example, the widely used promoters for *Chlamydomonas* transformation have been derived from the 5′ untranslated region of the *Chlamydomonas reinhardtii* RuBisCO small subunit gene (*rbcS2*; Stevens et al., 1996), *Chlamydomonas* heat shock protein 70A gene (*hsp70A*; Schoda et al., 2000), marine diatom *fucoxanthin-chlorophyll a/c binding protein* gene *fbcP* (Apt et al., 1986; Miyagawa-Yamaguchi et al., 2011), *Dunaliella* duplicated carbonic anhydrase 1 (*DCA1*; Li et al., 2010; Liu et al., 2011), *Porphyra yezoensis* actin1 gene (*PyAct1*; Takahashi et al., 2010), and two *Nannochloropsis* unlinked violaxanthin/chlorophyll a-binding protein (VCP) genes, *VCP1* and *VCP2* (Kilian et al., 2011).

Table 1 | Selected promoters used in cyanobacteria.

| Promoters Sources | Gene(s) | Expression hosts | Reference |
|-------------------|---------|------------------|-----------|
| *Ptrc1O* | *Synechocystis* 6803, freel y lucerase, psb, adh, fer | *Synechocystis* 6803, *Takeshima* et al. (1994), Dang and Coleman 1999, *Liu* et al. (2011b), *Tan* et al. (2011) |
| *Ptrc* | *Synechocystis* 6803, accBCDA, accD, accA, ferB2 | *Synechocystis* 6803, *Takeshima* et al. (1994), *Liu* et al. (2011b) |
| *Ptac* | *Synechocystis* 7942 | *Synechocystis* 7942, *Takeshima* et al. (1994), *Takahama* et al. (2003), *Ducat* et al. (2011a) |
| *Pcap* | *Synechocystis* 6803, far, far1, far2, accBCDA | *Synechocystis* 6803, *Tan* et al. (2011) |
| *PisI* | *Synechocystis* 7942, eis, hydA, hydG, cvrbcLS | *Synechocystis* 7942, *Sakai* et al. (1997), *Takahama* et al. (2003), *Ducat* et al. (2011a) |
| *Pppx* | *Synechocystis* 6803, psb, adh, cvrbcL,5, iasp, taxA, farB1, farB2 | *Synechocystis* 6803, *Ikkii* et al. (2006), *Dexter* and *Fu* (2009), *Synechocystis* 7942, *Lindberg* et al. (2010), *Liu* et al. (2011b) |
| *Pppa* | *Synechocystis* 6803, luxAB | *Synechocystis* 6803, *Muraiamu* and *Hihara* (2006) |
| *Pppd* | *Synechocystis* 6803, luxAB | *Synechocystis* 6803, *Muraiamu* and *Hihara* (2007) |
| *Ppsb* | *Synechocystis* 6714, luxAB, accB, accC | *Synechocystis* 7942, *Imashimizu* et al. (2003) |
| *Pcat* | *Synechocystis* 6803, GPP | *Synechocystis* 6803, *Huang* et al. (2010) |
| *Pcap* | *Synechocystis* 6803, gal | *Synechocystis* 6803, *Liu* et al. (2011a) |
| *Pcap* | *Synechocystis* 6803, holB, endolin, auxiliary lysis enzyme | *Synechocystis* 6803, *Liu* and *Curtiss* (2009) |
| *Pff* | Coliphage T7, luxAB | *Alphanaba* sp. 7120, *Wilk* et al. (1993) |
| *Ppe* | *E. coli* atoF, adh, ter, Hbd, ctt, hydA, adi, cvrbcL, hydG | *Synechocystis* 7942, *Atsumi* et al. (2009), *Ducat* et al. (2011a), *Lan* and *Luo* (2011) |
| *PpCp* | *E. coli* pariE, atoF, acp, Hbd, ctt, cvrbcL, hydA, adi, cvrbcL, hydG | *Synechocystis* 7942, *Atsumi* et al. (2009), *Nakai* et al. (2009), *Atsumi* et al. (2009), *Huang* et al. (2010), *Niederholtmeyer* et al. (2010), *Lan* and *Luo* (2011) |
| *Ppe* | *E. coli* GPP | *Synechocystis* 6803, *Huang* et al. (2010) |
Transcriptional terminators
Placing a transcription terminator downstream of the introduced genes will prevent effects on the expression of genes adjacent to the insertion loci; meanwhile, placing a terminator upstream of the promoter of an introduced gene will also prevent any background transcription effect on the upstream genes (Adhya and Gottesman, 1982). So far only a few native and foreign terminators have been utilized in cyanobacteria, including the cyanobacterial RbICOCO terminator (Takeshima et al., 1994) and strong E. coli terminators such as rnl terminator (Geerts et al., 1995; Takashashi et al., 1999; Atsumi et al., 2000), bacteriophage T7 terminator (Lang and Haselkorn, 1991; Argotta et al., 2004), and rntT7T7E double terminator4 (Huang et al., 2010). Very little work has been conducted to characterize the termination efficiencies in cyanobacteria and algae.

Ribosome binding sites
The ribosome binding sites (RBS) play a crucial role in initiating the translation of downstream target genes. Upon translation initiation, the 5'-terminal sequence of the 16S rRNA interacts with the core Shine–Dalgarno (SD) sequence of RBS by complementary pairing of the nucleic acids. For example, in cyanobacterium Synechocystis 6803, the 3'-terminal sequence of the 16S rRNA is AUUUAAGAGGA (Kaneko et al., 1996; Ma et al., 2002) and therefore the optimal complementary SD sequence should be AAAACCCGGGU (core SD sequence underlined). Heidorn et al. (2001) studied the efficiencies of different RBS in expressing GFP in Synechocystis 6803 and found that the RBS sequence UAGUGGAGG gave about twofold higher translation efficiency than a RBS sequence AUUAAAGAGGAG. The translation of downstream target genes will prevent effects on the expression of genes adjacent to the insertion loci; meanwhile, placing a terminator upstream of the promoter of an introduced gene will also prevent any background transcription effect on the upstream genes (Adhya and Gottesman, 1982). So far only a few native and foreign terminators have been utilized in cyanobacteria, including the cyanobacterial RbICOCO terminator (Takeshima et al., 1994) and strong E. coli terminators such as rnl terminator (Geerts et al., 1995; Takashashi et al., 1999; Atsumi et al., 2000), bacteriophage T7 terminator (Lang and Haselkorn, 1991; Argotta et al., 2004), and rntT7T7E double terminator4 (Huang et al., 2010). Very little work has been conducted to characterize the termination efficiencies in cyanobacteria and algae.

Endogenous enhancers
The transcription of an interest gene can be positively affected by placing in the gene cluster an enhancer, a short DNA fragment which interacts with certain proteins to enhance the transcription. In cyanobacteria, some light-responsive elements exhibit enhancer activities. For example, the 5'-untranslated regions of the psbAII and psbAIII genes of Synechococcus 7942 have been found as enhancers which can increase the expression of downstream genes by 4- to 11-fold when combined with an E. coli promoter (contI) in the Synechococcus 7942 host strain (Li and Golden, 1993). A recent study by Eichler-Stalberg et al. (2009) showed that inserting three introns from the native alga C. reinhardtii RBCS2 gene into the recombinant luciferase and erythrosopin resulted in up to fourfold increase of the expression levels. By fusing the recombinant luciferase with the endogenous Rubisco LSU protein, Muto et al. (2009) has achieved enhanced luciferase expression by 33-fold.

PLASMID VECTORS
Both integrative and replicative plasmids have been developed for cyanobacteria. In cyanobacteria, integrative plasmids are usually utilized as vectors to integrate foreign genes into the cyanobacterial genomes via homologous recombination (Golden et al., 1987; Eaton-Rye, 2004; Hedorn et al., 2011). Integrative plasmids usually cannot replicate themselves and would eventually be eliminated through cell division. Replicative plasmids are those which can replicate in host cyanobacteria and the replication properties can be descended to daughter cells. Replicative cyanobacterial plasmids can be classified into two types: those with replications of broad-host range plasmids (Mermet-Bouvier et al., 1993; Mermet-Bouvier and Chauvat, 1994; Ng et al., 2000; Huang et al., 2010) and those derived from endogenous cryptic plasmids (Reaston et al., 1982; Wolk et al., 1984; Lang and Haselkorn, 1991; Summers et al., 1995; Deng and Coleman, 1999; Argotta et al., 2004; Iwaki et al., 2006). Representative shuttle vectors for cyanobacteria are listed in Table 2. The copy numbers of the broad-host
Table 2 | Representative shuttle vectors for cyanobacteria.

| Cyanobacterial | Representative | Host cyanobacteria | Reference |
|----------------|---------------|--------------------|-----------|
| replicons | vectors | | |
| µD1 | pMB1 | Aramantha 7130, Aramantha 7118, Aramantha M-131, Nostoc 7524 | Watanabe et al. (1982), Wolk et al. (1984), Lang and Hasekóm (1991) |
| µC1 | pSU18, pSU18/202 | Nostoc sp. MAC 8069, Nostoc punctiforme | Lambert and Carr (1983), Summers et al. (1995), Aragüeta et al. (2004) |
| µH4 | pUC40 | Synechococcus 7942 | Kuhlemeyer et al. (1985), Iwaki et al. (2004) |
| µH4 | pCB4, pS11 | Synechococcus 7942 | Golden and Sherman (1983), Lunenburg and Coleman (1993), Deng and Coleman (1989) |
| pAQ1 | pAQ1E7 | Synechococcus 7002 | Bulby et al. (1994) |
| PBA1 | pARU19 | Synechococcus 6301 | Takashima et al. (1994) |
| RSF1010 | pARU10 | Synechocystis 6803, Synechocystis 6714, Synechococcus 7942, Synechococcus 6301, Anabaena 7120, Nostoc ATCC 29133 | Memelt-Bouvier et al. (1993), Memelt-Bouvier and Chauvat (1994), Ng et al. (2000), Huang et al. (2010) |

range RSF1010-derived plasmids have been reported as about 10 per chromosome in E. coli cells and 10–30 per cell in Synechocystis (Ng et al., 2000; Huang et al., 2010) which is slightly higher than the average copy number (approximately 10) of the Synechocystis chromosome (Eaton–Rye, 2004). Due to lack of an active partitioning mechanism, RSF1010-derived plasmids tend to be eliminated in cells and thus antibiotic selection pressure is required for the maintenance (Becker and Meyer, 1997; Meyer, 2009).

Plasmid vectors have been developed to transform algae (León-Bañares et al., 2004). Recombinant eukaryotic algal viruses as transformation vectors (Langridge et al., 1986) and Agrobacterium tumefaciens-mediated method (Kumar et al., 2011) were also successfully developed for both marine and freshwater algae (Wiang et al., 2010).

CODON USAGE

Since different organisms usually bear particular codon usage patterns, when a gene is cloned from one species and expressed in a second organism, some codons might become rare codons in the new host, leading to poor translation efficiency (Kane, 1995). Genes in cyanobacteria show a bias in use of synonymous codons (Ng et al., 2000; Huang et al., 2010) which is slightly higher than the average codon usage of a heterologous gene before it is transformed into an algal cell (Takahashi et al., 2010). The results showed that the codon-optimized IsPS showed remarkable improved expression, 10-fold higher than that of the native IsPS gene under control of the same promoter. The importance of codon optimization in algal genetic applications is also increasingly acknowledged. For instance, it has been shown that codon bias significantly affects the GFP expression in C. reinhardtii (Heitzer et al., 2007). As a result, in recent transgenic research, the codon-optimized luciferase gene was used in a green alga Gymnopus pectorale (Lerche and Hallmann, 2009) and the codon-modified β-glucuronidase gene was transformed in a red seaweed Porphyra yezoensis (Takahashi et al., 2010).

TRANSFORMATION OF CYANOBACTERIA AND ALGAE

Methods to introduce DNA into cyanobacteria include conjugation (Thiel and Wolk, 1987; Elhai and Wolk, 1988), electroporation (Zang et al., 2007), and natural transformation (Sheshakar and Khyen, 1976; Grigorieva and Shestakov, 1982; Kuhlemeyer and van Arkel, 1987). The methods have been well summarized in several recent reviews (Eaton–Rye, 2004; Koksharova and Wolk, 2002; Heidorn et al., 2011) and we suggest readers to refer these excellent reviews for details. Compared to cyanobacteria, transformation methods for algae are less developed and more complicated. Since the chloroplast and nucleus of alga C. reinhardtii were stably transformed more than two decades ago (Boynton et al., 1988; Debuchy et al., 1989; Fernández et al., 1989), different methods have been employed in algal transformation which include, but not limited to, particle bombardment, glass bead microinjection, electroporation and Agrobacterium tumefaciens-mediated transformation (León-Bañares et al., 2004; Coll, 2006; León and Fernández, 2007; Potvin and Zhang, 2010). Specifically, bombardment of target cells with DNA-coated metal particles turns to be an effective and highly reproducible method to transform algae. This method has been so far applied in the transformation of nuclear and chloroplast of many algal species such as C. reinhardtii, Volvox carteri, Chlorella sorokiniana, Chlorella ellipsoidea, Chlorella kessleri, Haematococcus pluvialis, Phaeodactylum tricornutum, and C. pectorale (Boynton and Gillham, 1993; Potvin and Zhang, 2010). In addition, agitatin of the cell wall-deficient algal cells with glass beads, polyethylene glycol (PEG) and foreign DNA has been used to transform algae such as C. reinhardtii, Dunaliella salina, and red alga Porphyra haitanensis (Kindle, 1990; Feng et al., 2009; Wang et al., 2010). Microinjection of the viral SV40 DNA or the chimeric construction pPYN2neo into the marine unicellular green alga Acetabularia mediterranea also resulted in a high yield and stable nuclear transformation (Neuhäusl et al., 1986);
nevertheless, it is hard to operate and the throughput of transformation is low. *Agrobacterium tumefaciens* has been used to mediate the transformation of *C. reinhardtii* (Kumar et al., 2004) and *H. pluvialis* (Kathiresan et al., 2009). Recently, it was discovered that the industrially relevant oil-producing alga *Nannochloropsis* sp. is haploid and can be transformed with high efficiency using high electric field electroporation. It has also been found that efficient stable transformation of this species via homologous recombination requires using linear DNA fragment rather than circular plasmid DNA (Kilian et al., 2011). However, the mechanism for the high homologous recombination efficiency is to be elucidated.

**APPLICATIONS OF MODIFIED CYANOBACTERIA AND ALGAE**

We focus here on recent progress in producing biofuels and other useful chemicals using genetically modified cyanobacteria and algae. For other applications, readers can refer to several other excellent reviews published recently (Radakovits et al., 2010; Qin et al., 2012). Other excellent reviews published recently (Radakovits et al., 2010; Qin et al., 2012). Efforts have been made from both process engineering and genetic engineering approaches to facilitate the lipid extraction (Liu and Curtiss, 2009; Liu et al., 2011a; Sheng et al., 2011). Specifically, Liu and colleagues have constructed inducible systems to conditionally express phage lysis genes and lipoysis enzyme genes in *Synechocystis* 6803 to trigger the cell lysis upon harvest and thus help lipid extraction from this species (Liu and Curtiss, 2009; Liu et al., 2011a). To produce secretable biofuels from a synthetic biology approach is another way to resolve above issues.

**BIOFUELS**

United States consumed 13.3 million barrels of petroleum per day for transportation purposes in 2009, accounting for 71% of all petroleum used (Energy Information Administration, 2010). Many alternatives to current liquid fuels have been proposed, including ethanol, 1-butanol, isobutanol, short-chain alcohols, short-chain alkanes, biodiesel (FAME, fatty acid methyl esters), fatty alcohols, alkenes, linear and cyclic isoprenoids (Lee et al., 2008; Connor and Atsumi, 2010). Current routes for biological production of fuels and chemicals are summarized in Figure 1. Traditionally people follow a two-step route to firstly collect plant biomass and then convert biomass to fuels by microbial fermentation (Stephanopoulos, 2007); whereas recently interest in microalgae is about two orders of magnitude higher and the cultivation land needed is around two orders of magnitude less (Chisti, 2007; Fernando et al., 2007; Liu and Curtiss, 2009). Efforts have been made from both process engineering and genetic engineering approaches to facilitate the lipid extraction (Liu and Curtiss, 2009; Liu et al., 2011a; Sheng et al., 2011). Specifically, Liu and colleagues have constructed inducible systems to conditionally express phage lysis genes and lipoysis enzyme genes in *Synechocystis* 6803 to trigger the cell lysis upon harvest and thus help lipid extraction from this species (Liu and Curtiss, 2009; Liu et al., 2011a). To produce secretable biofuels from a synthetic biology approach is another way to resolve above issues.

**Free fatty acids**

Enhanced production of free fatty acid (FFA) has already been achieved in *E. coli* through a series of genetic engineering (Davis et al., 2000; Lu et al., 2008). In a recent study, Liu et al. (2011b) engineered cyanobacterium *Synechocystis* strains to produce and secrete FFAs to up to 197 mg/L at a cell density of 1.0 × 10^9 cells/mL. The acetyl-CoA carboxylase (ACC) was overexpressed to drive the metabolic flux toward FFAs, while the fatty acid activation gene atsc (slr1609) was deleted to inactivate the FFAs degradation. Poly-β-hydroxybutyrate (PHB) synthesis genes (slr1993 and slr1994) and the phosphonatasecylase gene pha (slr2132) were deleted to block competitive pathways. Particularly, two genetic modifications turned to significantly increase the FFAs production and secretion: overexpression of thioesterase and weakening the polar peptidoglycan layer of the cell wall of *Synechocystis* 6803.

**Alkanes and alkenes**

Although it was known that some cyanobacteria can synthesize alkanes, the molecular mechanism had been mysterious until recently an alkane/alkene biosynthetic pathways were identified in cyanobacteria (Stein et al., 2010; Mendez-Perez et al., 2011). Stein et al. (2010) identified an alkane/alkene biosynthetic pathway that two successive biochemical reactions catalyzed by an acyl-ACP reductase and an aldehyde dehydrogenase, respectively, converts acyl-ACP (intermediates of fatty acid metabolism) to alkanes/alkenes. In order to increase the alkane production in cyanobacteria, heterologous expression of acyl-ACP reductase and aldehyde dehydrogenase genes (from *Synechococcus* 7942) has been achieved in *Synechocystis* 7002, which led to a total intracellular accumulation of n-alkane to up to 5% of the dry cell weight (Reppas and Ridley, 2010). In another research, Mendez-Perez et al. 2010; Qin et al., 2012). Efforts have been made from both process engineering and genetic engineering approaches to facilitate the lipid extraction (Liu and Curtiss, 2009; Liu et al., 2011a; Sheng et al., 2011). Specifically, Liu and colleagues have constructed inducible systems to conditionally express phage lysis genes and lipoysis enzyme genes in *Synechocystis* 6803 to trigger the cell lysis upon harvest and thus help lipid extraction from this species (Liu and Curtiss, 2009; Liu et al., 2011a). To produce secretable biofuels from a synthetic biology approach is another way to resolve above issues.

**Free fatty acids**

Enhanced production of free fatty acid (FFA) has already been achieved in *E. coli* through a series of genetic engineering (Davis et al., 2000; Lu et al., 2008). In a recent study, Liu et al. (2011b) engineered cyanobacterium *Synechocystis* strains to produce and secrete FFAs to up to 197 mg/L at a cell density of 1.0 × 10^9 cells/mL. The acetyl-CoA carboxylase (ACC) was overexpressed to drive the metabolic flux toward FFAs, while the fatty acid activation gene atsc (slr1609) was deleted to inactivate the FFAs degradation. Poly-β-hydroxybutyrate (PHB) synthesis genes (slr1993 and slr1994) and the phosphonatasecylase gene pha (slr2132) were deleted to block competitive pathways. Particularly, two genetic modifications turned to significantly increase the FFAs production and secretion: overexpression of thioesterase and weakening the polar peptidoglycan layer of the cell wall of *Synechocystis* 6803.

**Alkanes and alkenes**

Although it was known that some cyanobacteria can synthesize alkanes, the molecular mechanism had been mysterious until recently an alkane/alkene biosynthetic pathways were identified in cyanobacteria (Stein et al., 2010; Mendez-Perez et al., 2011). Stein et al. (2010) identified an alkane/alkene biosynthetic pathway that two successive biochemical reactions catalyzed by an acyl-ACP reductase and an aldehyde dehydrogenase, respectively, converts acyl-ACP (intermediates of fatty acid metabolism) to alkanes/alkenes. In order to increase the alkane production in cyanobacteria, heterologous expression of acyl-ACP reductase and aldehyde dehydrogenase genes (from *Synechococcus* 7942) has been achieved in *Synechocystis* 7002, which led to a total intracellular accumulation of n-alkane to up to 5% of the dry cell weight (Reppas and Ridley, 2010). In another research, Mendez-Perez et al.
et al. (2011) identified the genes responsible for α-olefin biosynthesis in *Synechococcus* 7002. In addition, overexpression of the *arcB/CD* operon (which encodes ACC) in *Synechocystis* was also reported to enhance alkane/alkene production (Tan et al., 2011), consistent with the aforementioned results of FFAs production. Although it is believed there are certain alkane/alkene secretion pathways, the specific mechanisms are still under exploration (Radakovic et al., 2010).

**Ethanol**

Ethanol production via microbial fermentation has undergone a sharp increasing in the past decade for its utility as supplement in transportation fuel (Stephanopoulos, 2007; Energy Information Administration, 2010). In 1999, photosynthetic production of up to 230 mg/L ethanol has been reported using genetically engineered cyanobacterium *Synechococcus* 7942, in which an artificial operon of *pdc-adh* (genes originally from *Zymomonas mobilis*) was expressed under a *psaA* and *psaC* promoters via a shuttle vector pCB4 (Deng and Coleman, 1999). In a recent study, the *pdc-adh* expression cassette was integrated into the chromosome of *Synechocystis* 6803 at the *psaA2* locus. Driven by the light-inducible strong *psaA2* promoter, expression of *pdc-adh* resulted in ∼350 mg/L ethanol production by the engineered *Synechocystis* under high light (2000 μE/m²/s) conditions (Dexter and Fu, 2009). In algae, although many species have fermentative pathways to produce ethanol, the pathways are only functional under dark and anaerobic conditions (Hirayama et al., 1998). Algal ethanol is currently produced via heterotrophic fermentation of algal biomass using heterotrophs such as yeast and *E. coli* (Nguyen et al., 2009; Harun et al., 2010; Wargacki et al., 2012), which follows the two-step route (Figure 1). Direct photosynthetic production of ethanol by algae would be possible using a similar approach as being demonstrated in cyanobacteria by expressing foreign ethanol biosynthesis pathways, or by tuning the native regulatory pathways in algae.

**Isobutanol and 1-butanol**

Compared with ethanol, isobutanol and 1-butanol have much higher energy density. The energy density of butanol reaches 29.2 MJ/L, about 90% of that of gasoline, 32.5 MJ/L, and it is also less volatile and less corrosive than ethanol (Durre, 2007). Therefore, butanol is regarded as a better gasoline substitute. Recently, significant progress has been achieved for photosynthetic production of butanol. Liao and colleagues introduced an artificial isobutanol biosynthesis pathway into *Synechococcus* 7942 and the engineered strains were able to photosynthetically produce isobutyraldehyde and isobutanol at titers of 1100 and 7942, in which an artificial *accAB* operon was expressed. The 1-butanol production was increased by fourfold, up to ∼30 mg/L, under the same photosynthetic condition (Liao and Liao, 2012). Thus, the two-step route (<eqref{eq:1}>eqref{eq:1}</eqref{eq:1}> 7942, the 1-butanol was barely detectable (<eqref{eq:1}>eqref{eq:1}</eqref{eq:1}> 1 mg/L) in *C. reinhardtii* (Melis et al., 2000; Kruse et al., 2005; Ghirardi et al., 2007, 2009; Lee et al., 2010; Srirangan et al., 2011). Many cyanobacteria and algae naturally produce hydrogen as a secondary metabolite to balance the redox energetics. In order to fortify the hydrogen production, endeavor has been made to augment the electron flux, instead of the carbon flux, toward H₂ biosynthesis catalyzed by hydrogenases (2H⁺ + 2e⁻ → H₂). In alga *C. reinhardtii*, for instance, blocking the cyclic electron transfer around PSI turned to eliminate the possible electron competition for electron with hydrogenase;
As a result, the H2 evolution rate increased 5–13 times under a range of conditions (Kruze et al., 2005). Hydrogenase has been tethered to the PSI to obtain a much greater electron throughput and thus H2 evolution rate (Duza et al., 2006; Schwarze et al., 2010; Lubner et al., 2011). However, to date these experiments were all conducted in vitro and efforts need to be made from a synthetic biology approach to validate the concept in vivo. In another study, expression of an exogenous ferredoxin from Clostridium acetobutylicum in addition to the native ferredoxin could fortify the electron flow toward the hydrogenase HvdA via siphoning electrons from the fermentation of internal reducing equivalents (such as glycogen). As a result, the hydrogen production was enhanced by approximately twofold (Duca et al., 2011a) under light-dependent anoxic conditions. On the other hand, efforts (such as glycogen). As a result, the hydrogen production was enhanced by about 16-fold. Isoprene was eventually produced at a rate of ~50 mg/d dry cell/d under high light (~500 μE/m2/s) culture conditions. It is noteworthy that heterologous expression of IspE by replacing the pba2 gene did not affect photosynthesis significantly and depress the growth of the transformants (Lindberg et al., 2010), which was differed from the aforementioned ethylene-producing cyanobacteria (Sakai et al., 1997; Takahama et al., 2003).

**Acetone**

Acetone represents the simplest ketone which serves as a solvent and precursor for industrial chemicals (Vairava et al., 1996). Microbial production of acetone has been achieved in fermentation of Clostridium and recombinant E. coli using sugar as feedstocks (Bermejo et al., 1998). However, the maximal yield is merely 50% with the other half carbon being released as CO2 when hexose is the sole carbon source. Recently, through a combination of co-expression of the acetoacetate decarboxylase (aad) and coenzyme A transferase (cafAB) and deletion of the PHB polymerase (PhaEC) in Synechocystis 6803, 3–5 mg/L acetone has been produced under nitrogen and phosphate deprived, dark and anaerobic culture conditions. After deleting the phosphotransacetylase-encoding gene and the competitive acetate production was remarkably reduced and the acetone titer has been evidently increased to 36.0 mg/L in the culture (Zhou et al., 2012).

**Poly-β-hydroxybutyrates**

Cyanobacteria are the natural producers of PHB, a type of poly-hydroxyalkanoates (PHAs) that serves as biodegradable plastics (Hein et al., 1998; Taroncher-Oldenburg et al., 2008). However, the yield is very low and nutrient deprivation and acetate addition are usually necessary for accumulation of PHB (Wu et al., 2001). By introducing PHB biosynthesis genes from Ralstonia eutropha into Synechococcus 7942 coupled with nitrogen starvation and acetate supplementation, the PHB biosynthesis in the recombinant cyanobacteria has reached a maximum of 25.6% of the dry cell weight (Takahashi et al., 1998). Efforts in identifying gene disruptions which might contribute to increase of PHB accumulation were also made and several gene disruptions with positive effects were discovered (Yuo et al., 2009). Nevertheless, similar with other types of macromolecules PHB can not be secreted out of cells;
the required extraction process is energy-intensive and remains as one of the major hurdles for commercial applications (Chisti, 2007; Liu and Curtiss, 2009). As a result,3-hydroxybutyrate (3HB), the monomer of PHB and a building block molecule for other PHAs, has been successfully produced and secreted by genetically engineered E. coli (Lee and Lee, 2003; Liu et al., 2007; Teng et al., 2009). Hence, photosynthetic production of 3HB in cyanobacteria and algae might be a feasible approach to cope with the secretion problem.

**Lactic acid**

Lactic acid is another chemical that can serve as a building block for synthesizing biodegradable polyesters with valuable medical properties. It is also used as a preservative and acidulant in food industry, and can serve as an advanced nutrient for neuron cells (Wise et al., 2006). While conventional production of lactic acid relies on microbial fermentation of sugars (Wise et al., 2006), photosynthetic production of lactic acid using CO2 as carbon source has been recently demonstrated (Niederholtmeyer et al., 2010). Through heterologously expressing three genes, including ldhA, Bdp, and udhA, in cyanobacterium Synechococcus 7942, Niederholtmeyer et al. (2010) has accomplished production of lactic acid with a titer of ~56 mg/L under photosautotrophic culture condition. While LdhA catalyzes the conversion of pyruvate to lactate, expression of the lactate transporter gene Bdp turned to be essential for lactate secretion from the engineered Synechococcus strain (Niederholtmeyer et al., 2010). Repletion of NADH, a cofactor for LdhA, through expressing the NADPH/NADH transhydrogenase (encoded by udhA) greatly enhanced the lactate production but reduced the growth rate of Synechococcus (Niederholtmeyer et al., 2010).

**Sugars**

Fresh water cyanobacteria accumulate solutes such as glucosylglycerol and sucrose when they are exposed to salt stress (Hagenmann, 2011). By knocking out the app gene (which contributes to the biosynthesis of glucosylglycerol) from the Synechocystis 6803 genome, Miao et al. (2003) achieved sucrose accumulation of up to 44 mg/L(Dow) after 0.9 M salt shock for 96 h. In another study, overexpression of invA, gll, and galU genes in Synechococcus 7942 resulted in up to 45 mg/L total hexose production (including glucose and fructose) in the culture supplemented with 200 mM NaCl (Niederholtmeyer et al., 2010). While InvA catalyzes the conversion of sucrose to glucose and fructose, expression of the glucose or fructose transporter GLF (encoded by gll gene) was essential for glucose or fructose secretion. Additional expression of GalU enhanced the biosynthesis of intracellular precursors and thus further increased the hexose sugar production by over 30% in the culture (Niederholtmeyer et al., 2010).

**CHALLENGES AND OPPORTUNITIES OF SYNTHETIC BIOLOGY IN CYANOBACTERIA AND ALGAE**

Despite of promising progresses, there are challenges ahead for synthetic biology to reach its full power in modifying cyanobacteria and algae for biotechnological applications. Here we briefly discuss the challenges and possible strategies.

**IMPROVING TOOLS FOR GENETIC MANIPULATION**

**Effective “BioBricks”**

Although a few “BioBricks” have been characterized in cyanobacteria, the limit number of gene expression elements would not fulfill the need of synthetic biology in cyanobacteria. After an initial gene expression, a fine-tuning of the gene expression is usually the next step in order to further optimize the properties of the genetically engineered strains, which requires a good number of “BioBricks.” Currently most of the “BioBricks” were collected from E. coli, but the E. coli “BioBricks” might behave differently in cyanobacteria. For example, tightly regulated IPTG-inducible lacI/Ptac gene expression system does not work as well in cyanobacteria as it does in E. coli (Huang et al., 2010). Thus, systematic collection and characterization of “BioBricks” in cyanobacteria is necessary. Additionally, in contrast to various commercialized E. coli and yeast strains that have been genetically modified to serve as chassis for different purposes, there are few such cyanobacterial or algal species available nowadays. To design and construct a series of chassis strains is thus an urgent task. Moreover, to our knowledge, there has been no study of the performance of a given BioBrick in different cyanobacterial species. We assume that a defined BioBrick might behave differently across cyanobacterial species and the efficiency of the BioBrick need to be characterized for each cyanobacterial species.

**Improved transformation efficiency**

Standardized transformation vectors/protocols have been established for model cyanobacteria, such as Synechococcus and Synechocystis, although the transformation efficiency still needs further improvement (Eaton-Rye, 2004; Heidorn et al., 2011). However, the transformation methods for model filamentous cyanobacteria, such as Anabaena and Spirulina, are still under development (Ducat et al., 2011b), and so far no genetic engineering has been conducted in the marine N2-fixing cyanobacterium Trichodesmium despite significant interest on its ability of peaking the fixation of CO2 and N2 simultaneously during the day time (Chen et al., 1998; Ierman-Frank et al., 2001). In vivo restriction activities have been demonstrated as an important barrier for introducing foreign DNA into cyanobacterial cells (Elhai et al., 1997; Koksharova and Wolk, 2002). Hence, it would be helpful to construct methylation-defect cyanobacterium host strains or to establish in vitro systems that can methylate the foreign DNA before transformation. Additionally, since the bacteriophage λ recombination system has greatly improved the E. coli transformation efficiency (Yu et al., 2000), we propose that high-efficiency homologous recombination in cyanobacterial cells might be achievable by developing a proper cyanophage recombination system.

In order to improve transformation efficiency of other algal species, endeavors could be made to uncover the mechanism behind the recently discovered truth that highly efficient homologous recombination occurs after electroporation of the industrially relevant oil-producing alga Nannochloropsis sp. (Kilian et al., 2011). Recently, through an approach of ex vivo assembly of the chloroplast genome before bombarding it into the green alga C. reinhardtii, O’Neill et al. (2012) demonstrated that simultaneous and multi-loci genetic modifications of the chloroplast of the green
alga C. reinhardtii could occur after one single round of transformation, providing an alternative method to improve the efficiency of multiple-gene transfer.

**IMPROVING PHOTOSYNTHESIS EFFICIENCY**

Although the solar energy conversion efficiencies of algae and cyanobacteria are 2–3 fold higher than those of crop plants, the efficiencies are still low with yields around 5–2% during the growing season and around 3% in bioreactors on an annual basis (Blankenship et al., 2011). A recent study on in silico modeling of the reconstructed photosynthetic process revealed that the regulation of the photosynthesis activity is quite complex and a high degree of cooperativity of nine alternative electron flow pathways is responsible for optimized photosynthesis performance in Synechocystis 6803 (Nogales et al., 2012).

**Light harvesting**

The photosynthetic microorganisms in nature have been selected by the abilities to reproduce but not by the ability to produce a maximal amount of biomass or specific products. In order to thrive in the wild environment, cyanobacteria and algae have maximized their expression of pigments and antennas to compete with the competitors for sunlight. However, when monoculture was employed to produce high-density of biomass or maximal titers of specific products in photo-bioreactors, excessive photon capture by the cells in the surface layer can block the light availability to the cells underneath (Melis, 2009). To address this issue, studies have been conducted on minimizing the size of the photosystem antenna complex through various strategies, such as by expressing truncated light-harvesting antenna complex (LHC) mutants (Blankenship et al., 2011; Ort and Melis, 2011; Work et al., 2012), by down-regulating the expression of LHC through RNA interference (RNAi) and expression of LHC translation repressor in both cyanobacteria and algae (Mussungu et al., 2007; Work et al., 2012).

For example, the photosynthetic activity (measured by oxygen evolution) was about threefold higher in the alga strain StnSLR3 (with LHC being down-regulated via RNAi) than in the parent strain Stn3 (without RNAi) after 100 min of high-light treatment; the cell growth rate also increased under high-light conditions after the LHC was down-regulated via RNAi (Mussungu et al., 2007). Another bold proposal was to increase the photosynthesis efficiency by extending the light absorption range of the photosystems in cyanobacteria and algae (Blankenship et al., 2011).

As the chlorophyll, carotenoids, and other accessory pigments in cyanobacteria and algae capture only visible region of the spectrum of solar radiation (400 to 700 nm), about 50% of the incident solar energy is dissipated and wasted during photosynthesis. Moreover, since the two photosystems compete for light with the same wavelengths, the overall efficiency is significantly reduced. Thus, it was proposed that one of the two photosystems be engineered to extend the absorption maxima to ~1100 nm, approximately doubling the solar photon capture, by heterologously expressing bacteriochlorophyll B (Blankenship et al., 2011).

**CO₂ fixation**

RuthisCO is an essential enzyme in photosynthetic carbon fixation in Calvin–Benson–Bassham (CBB) cycle, catalyzing the combination of ribulose-1,5-bisphosphate with CO₂. However, the reaction is slow. In addition, RuthisCO can also take O₂ as substrate in addition to CO₂ which further lower the carbon fixation efficiency. A recent study has revealed that despite slow catalytic turnover and confused CO₂/O₂ substrate specificity, RuthisCOs might have been nearly perfectly optimized (Tcherkez et al., 2006).

In nature, cyanobacteria and some algae have evolved certain CO₂-concentrating mechanisms (CCMs) to increase the CO₂ fixation efficiencies. In cyanobacteria, RuthisCOs are sequestered together with carbonic anhydrase in carboxysomes, polyhedral microcompartments (MCPs) with proteinaceous shells. Anhydrase catalyzes the conversion of HCO₃⁻ to CO₂ which is trapped by MCPs for RuthisCOs. Because CCMs can result in much higher CO₂ concentration, and thus higher CO₂/O₂ ratio, around the RuthisCOs, the carbon fixation efficiency is greatly increased (Espie and Kimber, 2011). It has been found that Synechococcus 7942 cells with more carboxysomes exhibited higher CO₂ fixation rates (Savage et al., 2010). Heterologous expression of Synechococcus 6301 rbcLS (that encodes RuthisCO) in Synechococcus 7942 also led to more efficient CO₂ fixation and higher yield of isobutyraldehyde in the genetically modified isobutyraldehyde-producing strain (Atsumi et al., 2009). Besides, overexpression of bicarbonate transporters has also been proposed to improve the photosynthesis efficiency (Price et al., 2011). Alternatively, RuthisCO-independent carbon fixation pathways have been proposed. A recent work using in silico modeling of the recombination of existing metabolic building blocks showed that some of the proposed carbon fixation cycles have overall higher kinetic rates (Bar-Even et al., 2010).

For example, by coupling the phosphoenolpyruvate carboxylase and the core of the natural C4 carbon fixation cycle, the overall CO₂ fixation rate was predicted as 2–3 folds higher than that of the CBB cycle which employs RuthisCO (Bar-Even et al., 2010).

**OVERCOMING THE OXIDATIVE STRESS**

Since cyanobacteria and algae are oxygenic microorganisms, the abundant oxygen evolved by splitting water during photosynthesis process becomes an issue for expressing oxygen-sensitive enzymes. For example, either [NiFe] or [FeFe] hydrogenase required for biological production of H₂ has low oxygen-tolerance (Lee et al., 2010), and the nitrogenases which fix N₂ into NH₄⁺ are also extremely oxygen-sensitive (Fay, 1992). From a broader prospect, this oxygen sensitivity issue could be crucial for successful expression of a large number of pathways from anaerobic microorganisms in oxygenic cyanobacteria and algae. To address the issue, efforts have been made to obtain oxygen-resistant enzymes from nature or through mutagenesis. For example, hydrogenases with better oxygen-tolerance have been found from Desulfovibrio fructovorans by a single V74M mutation (Dementius et al., 2009). Alternatively, temporal segregation of oxygenic photosynthesis and hydrogen biosynthesis would be another option. In nature, many cyanobacterial species have evolved the mechanism to photosynthetically fix CO₂ during day time while fix N₂ by the oxygen-sensitive nitrogenases during night (Fay, 1992). Thereby, the solar energy can
be firstly fixed into carbohydrates, such as starch, during oxy-
genic photosynthesis and then be utilized to power the oxygen-sensitive reactions during dark anoxic conditions. In addition, spatial seg-
regation could be used. Hydrogenosomes can be localized to certain advantageous space, such as being expressed in heterocysts, to
avoid the oxidative stress (Ray, 1992). Recent studies on bacte-
rial MCPs assembling might have provided another opportunity to spatially segregate incompatible oxygenic and oxygen-sensitive processes (Fan et al., 2010; Heinhorst and Cannon, 2010; Bonacci et al., 2012). Moreover, Mehler reaction can be used to overcome the oxidative stress (Meher, 1951; Asada, 2006). Mehler reaction has been evolved to overcome the intracellular oxidative stress by scavenging reactive oxygen species in cyanobacteria and chloro-
plasts (Kana, 1993; Asada, 2006). For instance, during N2-fixation period Mehler reaction consumes ~75% of gross O2 production and therefore maintains the O2 concentration at a low level (Kana, 1993; Milligan et al., 2007). However, Mehler reaction consumes reductant significantly (Asada, 2006); thus, in the future it will be
of great interest and of vital importance to maintain the activity locally around the oxygen-sensitive enzymes rather than in the
entire cytoplasmic environment.

SYSTEMATIC APPROACHES

Functional genomics, i.e., transcriptomics, proteomics, and metabolomics, would greatly promote the development of synthetic biology in cyanobacteria and algae. Albeit the genomes of some many species of cyanobacteria and algae have been sequenced2-3, a large portion of the sequenced genomes have not yet been annotated and the regulatory networks are still very poorly understood. The study of cyanobacterial and algal transcriptomes, proteomes, and metabolomes would allow for identification of new genes, pathways and regulatory networks which are essential to expand the size and diversity of the pool of genetic tools for synthetic biology. For example, recent transcriptomics studies on Synecocystis 6803 has enhanced the understanding of the transcriptional regulation in this photosyn-
thetic microorganism which revealed that approximate two-thirds of the transcriptional start sites give rise to asRNAs and noncod-
ing RNAs (ncRNAs), indicating that asRNAs and ncRNAs play an important role in cyanobacterial genetic regulation (Mitschke et al., 2011). We expect that omics may be the key to collect information about the interactions and regulations to develop a sustainable green chemistry industry.

Metabolic modeling

Although most synthetic biology research in cyanobacteria and algae focus on local pathway optimization, comprehensive syn-
thetic biology summons optimization of the genetic network and metabolic flux at the systems level. Genome-scale metabolic mod-
eling allows theoretically evaluating the impact of genetic and environmental perturbations on the biomass yield and metabolic flux distribution and allows predicting the optimal metabolic flux profile to maximize the value of a given objective function (Shastri and Morgan, 2009; Knoop et al., 2010; Yoshikawa et al., 2011). The in silico modeling may thus provide a systematic approach to design an optimal metabolic network to maximize the production of the interest biofuel or chemical. Such genome-scale metabolic network models have been constructed for cyanobacteria and algae, and have been utilized to predict new targets to improve product yields and new pathways (Shastri and Morgan, 2005; Knoop et al., 2010; Dal’Molin et al., 2011; Yoshikawa et al., 2011). However, the reconstruction of the global metabolic networks is still in the infancy stage and the simulation results rely signifi-
cantly on the included pathways. For instance, with ambiguities in metabolic networks in Synechocystis 6803, the estimated metabolic fluxes could be significantly different from the experimental results (Yoshikawa et al., 2011). In order to refine the quality of the recon-
structed metabolic networks and thus the simulation of metabolic flux, it is inevitable to couple with experimental characterization of the metabolic networks in cyanobacteria and algae (Yoshikawa et al., 2011). As an example, by firstly investigating the in vivo activities of the purified relevant enzyme products (heterologously expressed in E. coli) and subsequently verifying their in vivo activi-
ties in the native host Synechococcus 7002, Zhang and Bryant (2011) reported that two enzymes could functionally compensate for the missing 2-oxoglutarate dehydrogenase in the TCA cycle. Further database searches indicated that homologs of these two enzymes occur in all cyanobacteria but Prochlorococcus and marine Synechococcus, which overturned the previously widely accepted assumption that cyanobacteria possess an incomplete TCA cycle (Meeke, 2011; Zhang and Bryant, 2011). Such discoveries would be of utter importance to reconstruct qualified in silico models for simulating the metabolic flux in the future.

CONCLUSION

Owing to the relatively simple genetic contents and the ability to capture solar energy, fix CO2, grow fast and directly syn-
thesize specific products, cyanobacteria and algae have become excellent candidates for building autotrophic cell factories to pro-
duce renewable surrogate fuels and chemicals. With a large pool of genome sequences and improved genetic tools being available, application of synthetic biology in these photosynthetic microorgan-
isms are highly desirable. In recent years, exciting results have been achieved not only in understanding of the fundamen-
tal molecular mechanisms but also in producing various interest products, such as biofuels and chemicals, utilizing cyanobacte-
ria and algae as the production platforms. Nevertheless, synthetic biology in cyanobacteria and algae is still in its infancy and syn-
thetic biologists are facing great challenges and opportunities in addressing various issues, such as improving the tools for genetic manipulation, enhancing light harvesting, increasing CO2 fix-
ation efficiency, and overcoming of the intracellular oxidative stress. Systematic approaches, such as functional genomics and metabolic modeling, may also diversify the genetic tools and help the metabolic network design. It is doubtful that synthetic biol-
ogy would be indispensable for the future success in applying cyanobacteria and algae for various biotechnological purposes.

ACKNOWLEDGMENTS

This work was supported by the NEPTUNE fund granted to Deirdre R. Meldrum at ASU. Weiwen Zhang is currently funded by a grant from National Basic Research Program of China (National “973” program, project No. 2011CBA00803).
REFERENCES

Adhikary, S., and Gottman, M. (1982). Promoter overcrash: transcription through a promoter may inhibit its activity. Cell 29, 979-994.

Apt, K. E., Krude-Panich, P. G., and Grosomann, A. R. (1998). Stable nuclear transformation of the diatom Phaeodactylum tricornutum. Mol. Gen. Genet. 252, 572-579.

Atsumi, S., Higashide, W., and Liao, J. C. (2006). Production and scavenging of reactive oxygen species in cyanobacterial and their functions. Plant Physiol. 141, 391-398.

Atsumi, S., Cann, A. F., Connor, M. R., Becker, E., and Meyer, R. (1997). Acquisition of nuclear transformation of the cyanobacterium Escherichia coli for 1-butanol production. Metab. Eng. 10, 305-315.

Atsumi, S., Hanai, T., and Liao, J. C. (2006). Non-destructive pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 437, 36-39.

Atsumi, S., Higashide, W., and Liao, J. C. (2009). Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat. Biotechnol. 27, 1177-1180.

Bar-Even, A., Nuss, E., Lewis, N. E., and Milo, R. (2010). Design and analysis of synthetic carbon fixation pathways. Proc. Natl. Acad. Sci. USA 107, 6363-6368.

Bay, T. S., Wolmark, D. M., Temo, K., Misry, K. A., Santi, D. V., and Voigt, C. A. (2009). Synthesis of methyl halides from biomass using engineered Escherichia coli. BMC Genomics 13, 605-615.

Becker, E., Buehler, C., Franzmaier, I. M., and Steuer, R. (2012). The diversity of cyanobacterial metabolism: genome analysis of multiple photosynthetic microorganisms. BMC Genomics 13, 601-619.

Becker, E., and Meyer, R. (1997). Acquisition of nuclear transformation of the cyanobacterium Escherichia coli for 1-butanol production. Metab. Eng. 10, 305-315.

Benson, S. F. (2005). Act natural. Nature 431, 118.

Berman-Frank, I., Lundgren, P., Chen, Y. B., Kupper, H., Kelker, Z., Benjam, B., and Falkowshi, P. (2001). Segregation of nitrogen fixation and photosynthetic marine in the cyanobacterium Trichodesmium. Science 294, 1334-1337.

Benner, S. L., Wolfe, N. E., and Papoutsakis, T. E. (1999). Expression of Chlorovinylacetate oxidizing ACCase genes in E. coli for acetone production and adequate detoxification. Appl. Environ. Microbiol. 65, 2803-2809.

Blankenship, R. E., Tiede, D. M., Bivins, B., and Jay, E. (1994). Determination of the optimal aligned spacing between the translation initiation codon of mRNAs. Eukaryot. Cell 1, 252, 572-579.

Boynton, J. E., Gillham, N. W., Harries, E. H., Hosler, J. P., Johnson, T. M., Shark, K. B., and Sanford, J. E. (1991). Improvement of efficiency: a quantitative analysis. Proc. Natl. Acad. Sci. USA 87, 7666-7672.

Bubhaly, R., Purton, S., and Rickard, J. C. (2010). The argininosuccinate lyase gene of Chlamydomonas reinhardtii: an important tool for nuclear transformation and for correlating the genetic and molecular maps of the Arabidopsis. J. Biotechnol. 148, 12-20.

Dokshina, Y., Es, L. E., Shoh, C. R., Cho, K. M., and Liao, J. C. (2011). Enhancing carbon dough length of 1-butanol pathway for human symthesis from glucose by engineered Escherichia coli. J. Am. Chem. Soc. 133, 11599-11610.

Dorn, S., Marian, J., Miller, E. N., and Gonzalez, R. (1980). Evolution of higher plants and alga. cyanobacteria. Plant Physiol. 65, 1180-1185.

Durre, F. (2007). Algal cells: an attractive biofuel. Biotechnol. J. 2, 1523-1534.

Dyer, D. J., Kohankar, M. A., Haro, B., and Collins, J. S. (2007). Glycerol inhibitors induce an oxidative stress damage cellular death pathway in Escherichia coli. J. Mol. Biol. 37, 939-944.

Elahi, I., Vaplak, A., Mars, P., Souza, P. B., and Wolk, C. F. (1997). Reduction of conjugal transfer efficiency by three restriction activities of Arabidopsis sp. strain PCC 7120. J. Bacteriol. 179, 1998-2005.

Elahi, I., and Wolk, C. F. (1998). Conjugative transfer of DNA by the male gametophyte of Arabidopsis thaliana. Methods Mol. Biol. 167, 477-744.

Franklin, R. E. (2010). Foundations for understanding and development of cyanobacteria. Nature 468, 449-453.

Fu, X., Chang, S., Lin, Y., Rebe, C. M., Crowley, C. S., Jefferson, R. E., Yama, T. O., and Bobik, T. A. (2010). Short N-terminal sequences package proteins into bacterial microcompartments. Proc. Natl. Acad. Sci. USA 107, 7509-7514.

Fry, P. (1992). Orange solutions of nitrogen fixation in cyanobacteria. Microbiol. Rev. 56, 340-373.

Fung, S., Sui, L., Lu, H., and Lu, P. (2009). Improvement of efficiency of genetic engineering of Donela salina by glass beads method. Mil. Biol. J. Rep. 36, 1435-1439.
Gerostemas, G., and Shinohara, S. (1982). Transformation in the cytochrome system of cyanobacteria \( \text{sp.} \). FEBS Lett. 153, 305.

Heidorn, T., Camsund, D., Huang, H. L., and Kruse, O. (2010). Design and characterization of a synthetic gene expression system. \( \text{J. Mol. Biol.} \) 395, 203–218.

Kane, J. F., and Lindblad, P. (2011). Synthetic biology. \( \text{Mol. Syst. Biol.} \) 7, 515.

Koike, K., and Wolke, C. (2002). Genetic tools for cyanobacteria. \( \text{Appl. Microbiol. Biotechnol.} \) 56, 123–137.

Koumoundourou, C., Stavrakakis, I., and Drivas, M. (2009). Improved photosynthetic hydrogen production in microalgae. \( \text{J. Biol. Chem.} \) 284, 4919–4925.

Kühn, C. J., Thomas, A., van der Ende, A., van Loon, L. C. W., and van der Meer, K. A. (1998). Host-vector systems for gene cloning in cyanobacteria. \( \text{Adv. Biochem. Eng. Biotechnol.} \) 58, 123–137.

Kühn, C. J., van der Ende, A., van Loon, L. C. W., and van der Meer, K. A. (1998). Host-vector systems for gene cloning in cyanobacteria. \( \text{Microbiotechnology, Ecotoxicology and Bioremediation September 2012| Volume 3| Article 344 | "fmicb-03-00344" — 2012/11/4 — 14:56 — page 12 — #12}
Wang et al. Synthetic biology of cyanobacteria and algae

León, R., and Fernández, E. (2007). 

Langridge, P., Brown, J. W. S., Pintor-Lan, E. I., and Liao, J. C. (2012). A TP

Trends Biotechnol.

carbonic anhydrase gene 1 from

Stable nuclear transformation of
green cell-factories.

D., Galván, A., and Fernández, E.

achievements and problems.

Nuclear transformation of eukary-

toacid production of enantiomerically

photosynthetic isoprene production

lated leader regions of cyanobacterial

J. Bacteriol.

G. (1986). Expression of zein genes

and Keasling J. D. (2008). Metabolic

B. E. (2010). Biological hydrogen pro-

BMC Biotechnol.

McNeely, K., Xu, Y., Bennette, N.,

Mascarelli, A. L. (2009). A dupli-

Plant Physiol.

Lubner, C. E., Applegate, A. M., Knörzer,

Liu, X., and Curtiss, R. III. (2009).

Lu, X. (2010). A perspective: Photosyn-

synthetic biology to fuels.

Acetabularia mediterranea

−

616, 1–11.

353–354.

28, 262–271.

83x1122]Wang et al. Synthetic biology of cyanobacteria and algae

León, R., and Fernández, E. (2007).

Langridge, P., Brown, J. W. S., Pintor-Lan, E. I., and Liao, J. C. (2012). A TP

Trends Biotechnol.

carbonic anhydrase gene 1 from

Stable nuclear transformation of
green cell-factories.

D., Galván, A., and Fernández, E.

achievements and problems.

Nuclear transformation of eukary-

toacid production of enantiomerically

photosynthetic isoprene production

lated leader regions of cyanobacterial

J. Bacteriol.

G. (1986). Expression of zein genes

and Keasling J. D. (2008). Metabolic

B. E. (2010). Biological hydrogen pro-

BMC Biotechnol.

McNeely, K., Xu, Y., Bennette, N.,

Mascarelli, A. L. (2009). A dupli-

Plant Physiol.

Lubner, C. E., Applegate, A. M., Knörzer,

Liu, X., and Curtiss, R. III. (2009).

Lu, X. (2010). A perspective: Photosyn-

synthetic biology to fuels.

Acetabularia mediterranea

−

616, 1–11.

353–354.

28, 262–271.
sequence database status for the year 2000. Nucleic Acids Res. 28, 2921.
Nishidate, G., Nishida, U., G., de Groot, E. J., and Schneider, H. G. (1986). High yield and stable transformation of the unicellular green alga Acetabularia by microinjection of 5′-SDF DNA and p5′Zeo. EMBO J. 5, 1437–1444.
Ng, W. O., Zolada, R., Wang, Y., Tay, J. S., and Palacios, H. R. (2000). Flick, the major photosynthetic factor in the cyanobacteria Synechocystis sp. strain PCC 6803 for a chlorophyll-a-specific DNA phasmid. Arch Microbiol. 173, 412–416.
Nguyen, M. T., Che, S. P., Le, J., Lee, J. H., and Sim, S. J. (2009). Hydrothermal acid pretreatment of Chlamydomonas reinhardtii biomass for ethanol production. J. Microbiol. Biotechnol. 19, 161–166.
Niethammer-Meyer, H., Wohlfarth, B. T., Savage, D. F., Silver, P. A., and Way, J. C. (2010). Engineering cyanobacteria to synthesize and export hydrophobic products. Appl. Environ. Microbiol. 76, 3462–3466.
Niogret, S., Gaudinmond, S., Knight, R. M., Palsson, B. O., and Thiele, I. (2012). Detailing the optimal design of multiple genes. Biotechnol. Adv. doi: 10.1016/j.biotechadv.2012.05.004. (Published ahead of print).
Noble, M. A., Kurjan, J., O’Neill, B. M., Mikkelson, K. L., Gutierrez-Pisciotta, J. M., Zou, Y., and Baskakov, I. (2012). Synthetic ribosome binding sites to control protein expression. Nat. Biotechnol. 27, 946–950.
Sadegh, D. F., Alonso, B., Chen, A. H., and Silver, P. A. (2010). Spatially ordered dynamics of the bacterial carbon fixation machinery. Science 327, 1209–1211.
Safarova, M., Blatkov, D., and Bork, C. F. (2008). The HSPA7A promoter as a tool for the improved expression of transgene in Chlamydomonas. Plant J. 52, 121–131.
Sáenz, A., Kocak, M. J., Rognes, M., and Lenz, O. (2010). Requirements for construction of a functional hybrid complex of photosystem I and (Nfi)-hydrogenase. Appl. Environ. Microbiol. 76, 2641–2651.
Sharkey, T. D., Wilesby, A. E., and Donohue, A. R. (2008). Isoprene emission from plants why and how. Annu. Rev. 101, 5–14.
Shresta, A. A., and Morgan, J. A. (2005). Flux balance analysis of photosynthetic metabolism. Biochim. Biophys. Acta 21, 1617–1626.
Sheng, J., Vannelli, B. R., and Rittman, B. E. (2011). Evaluation of methods to extract and quantify lipids from Synechocystis PCC 6803. Biomass Bioenerg. 35, 1697–1701.
Shimokawa, T., and Kihara, N. T. (1979). Evidence for genetic transformation in Blue-green algae Anacystis nidulans. Mol. Gen. Genet. 177, 937–939.
Shimokawa, T., and Kihara, N. T. (1979). Evidence for genetic transformation in Blue-green algae Anacystis nidulans. Mol. Gen. Genet. 177, 937–939.
Sinclair, A., and Feinberg, A. M. (2008). Opportunities for reprograming bioenergy using microorganisms. Biotechnol. Bioeng. 103, 205–212.
Sierra, D. A., Talcott, E. L., and Meeks, J. C. (1995). Genetic evidence of a major role for cyanobacteria in nitrogen fixation and dark growth of the cyanobacterium Nostoc PCC 2953. J. Bacteriol. 177, 6366–6374.
Takahama, K., Matsushita, M., Naga- hama, K., and Ogura, T. (2003). Construction and analysis of a recombinant cyanobacterium expressing a dimethylallyl diphosphate synthase in nitrogen fixation and dark growth of the cyanobacterium Nostoc PCC 2953. J. Bacteriol. 175, 6366–6374.
Takahashi, H., Miyake, M., Tokiwa, Y., and Iwasa, Y. (1998). Improved accumulation of poly-3-hydroxybutyrate by a recombinant cyanobacterium. Biotechnol. Lett. 20, 183–190.
Takahashi, M., Uy, S. A., Naga, K., and Mikami, K. (2010). Isolation and regeneration of transiently transformed protoplasts from gusasepholic blight of the marine red alga Porphyra yezoensis. Electron J. Biotechnol. 15, 2.
Takishima, Y., Suguna, M., and Hagi- vara, H. (1996). A novel expression vector for the cyanobacterium, Synechocystis PCC 6801. Gene 169, 181–188.
Takao, Y., Tan, L., Gao, Q., Wang, W., Qi, F., and Lu, A. (2011). Photosynthesis-driven carbon fixation in Chlamydomonas reinhardtii. Metab. Eng. 15, 169–176.
Takara-Oldenburg, G., Nishida, K., and Stephanopoulos, G. (2000). Identification and analysis of the polyhydroxyalkanoate-specific beta-ketothiolase and acetoacetyl CoA reductase genes in the cyanobacterium Synechocystis sp. strain PCC 6803. Appl. Environ. Microbiol. 66, 4400–4408.
Takahashi, M., Uy, S. A., Naga, K., and Mikami, K. (2010). Isolation and regeneration of transiently transformed protoplasts from gusasepholic blight of the marine red alga Porphyra yezoensis. Electron J. Biotechnol. 15, 2.
Takishima, Y., Suguna, M., and Hagi- vara, H. (1996). A novel expression vector for the cyanobacterium, Synechocystis PCC 6801. Gene 169, 181–188.
Takahashi, H., Miyake, M., Tokiwa, Y., and Iwasa, Y. (1998). Improved accumulation of poly-3-hydroxybutyrate by a recombinant cyanobacterium. Biotechnol. Lett. 20, 183–190.
Takahashi, M., Uy, S. A., Naga, K., and Mikami, K. (2010). Isolation and regeneration of transiently transformed protoplasts from gusasepholic blight of the marine red alga Porphyra yezoensis. Electron J. Biotechnol. 15, 2.
Takishima, Y., Suguna, M., and Hagi- vara, H. (1996). A novel expression vector for the cyanobacterium, Synechocystis PCC 6801. Gene 169, 181–188.
Takahashi, H., Miyake, M., Tokiwa, Y., and Iwasa, Y. (1998). Improved accumulation of poly-3-hydroxybutyrate by a recombinant cyanobacterium. Biotechnol. Lett. 20, 183–190.
Wang et al. Synthetic biology of cyanobacteria and algae

Tyson, K. E., Jin, Y. S., Espinosa, F. A., and Stephanopoulos, G. (2009). Identification of gene disruptions for increased poly-3-hydroxybutyrate accumulation in Synechocystis PCC 6803. Biotechnol. Prog. 25, 1235–1243.

van de Monte, A. M., Hofmann-Marrtitz, M. F., Vermaas, W. F., and Beisson, B. W. (2006). The three-dimensional structure of the cyanobacterial Synechocystis sp. PCC 6803. Arch. Microbiol. 184, 259–278.

Wang, J., Jiang, P., Cui, Y., Guan, X., and Qin, S. (2010). Gene transfer into conchospores of Porphyra hexaspora (Bangiales, Rhodophyta) by glass bead agglomeration. Physiologia 49, 353–360.

Wang, Z., T. Ulrichs, N., Jin, S., W. Fischenschmidt, S., and Goodenough, U. (2009). Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless Chlorella vulgaris. Jaffrey Cell 6, 1858–1908.

Wanggacki, A. J., Erkan, E., Wu, M., N., Rajagopalan, D. D., Santos, C. N., Kim, P., Cooper, S. R., Raimer, R. M., Hermann, A., Zipur, A. B., Lakhmanyanamani, A., Kashyapra, Y., Bakers, D., and Yoshikawa, Y. (2009). An engineered microbial platform for direct biofuel production from brown macroalgae. Science 325, 308–313.

Wu, Y., Kim, J. N., and Ryu, H. W. (2006). Botanical production of lactic acid and its recent applications. Jour Zoolaich. Jour. 44, 163–172.

Wolff, C. P., Elhai, J., Katzir, T., and Hol-land, D. (1991). Amplification of a transcriptional pattern formed during development of Anabaena Mut. Microbiol. 7, 441–445.

Work, V., D’Adamo, S., Radakovits, R., Linkersen, K. E., and Porse, V. M. (2012). Improving photosynthesis and metabolic networks for the competitive production of phototroph-derived biofuels. Curr. Opin. Biotechnol. 23, 290–297.

Wu, Y., Wu, Q., and Shen, Z. Y. (2011). Accumulation of poly-beta-hydroxybutrate in cyanobacterium Synechocystis PCC6803. Biotechnol. Biofuels 7, 441–452.

Yang, S. F. and Hoffman, N. E. (1984). Mechanism for hydrogenation of acetone to isopropanol and of carbon oxides to methanol over copper-containing oxide catalysts. J. Mol. Catal. A Chem. 11, 455–468.

Zang, X., Liu, B., Liu, S., Arunakur, R., Jinkerson, R. E., and Pose- schen, D., Zhao, F., Bao, Q., and Wu, J. (2012). Carbon usage patterns and adaptive evolution of marine unicellular cyanobacteria Synechococcus and Prochlorococcus. Mol. Phylogenet. Evol. 62, 206–215.

Zaravan, T., M., Pyun, L., Makarova, D. V., and Kruger, T. A. (1996). Mechanisms for hydrogenation of acetone to isopropanol and of carbon oxides to methanol over copper-containing oxide catalysts. J. Mol. Catal. A Chem. 11, 455–468.

Zhang, K., Sawaya, M. R., Eisenberg, D. N., and Ma, Y. (2012). Designing and creating a modularized synthetic pathway in cyanobacterium Synechocystis enables production of acetone from carbon dioxide. Microb. Eng. 14, 398–409.