Comparative Genomics of DNA Recombination and Repair in Cyanobacteria: Biotechnological Implications

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Cyanobacteria are fascinating photosynthetic prokaryotes that are regarded as the ancestors of the plant chloroplast; the purveyors of oxygen and biomass for the food chain; and promising cell factories for an environmentally friendly production of chemicals. In colonizing most waters and soils of our planet, cyanobacteria are inevitably challenged by environmental stresses that generate DNA damages. Furthermore, many strains engineered for biotechnological purposes can use DNA recombination to stop synthesizing the biotechnological product. Hence, it is important to study DNA recombination and repair in cyanobacteria for both basic and applied research. This review reports what is known in a few widely studied model cyanobacteria and what can be inferred by mining the sequenced genomes of morphologically and physiologically diverse strains. We show that cyanobacteria possess many E. coli-like DNA recombination and repair genes, and possibly other genes not yet identified. E. coli-homolog genes are unevenly distributed in cyanobacteria, in agreement with their wide genome diversity. Many genes are extremely well conserved in cyanobacteria (mutMS, radA, recA, recFO, recG, recN, ruvABC, ssb, and uvrABCD), even in small genomes, suggesting that they encode the core DNA repair process. In addition to these core genes, the marine Prochlorococcus and Synechococcus strains harbor recBCD (DNA recombination), umuCD (mutational DNA replication), as well as the key SOS genes lexA (regulation of the SOS system) and sulA (postponing of cell division until completion of DNA reparation). Hence, these strains could possess an E. coli-type SOS system. In contrast, several cyanobacteria endowed with larger genomes lack typical SOS genes. For example, the two studied Gloeobacter strains lack alkB, lexA, and sulA; and Synechococcus PCC7942 has neither lexA nor recCD. Furthermore, the Synechocystis PCC6803 lexA product does not regulate DNA repair genes. Collectively, these findings indicate that not all cyanobacteria have an E. coli-type SOS system. Also interestingly, several cyanobacteria possess multiple copies of E. coli-like DNA repair genes, such as Acaryochloris marina MBIC11017 (2 alkB, 3 ogt, 7 recA, 3 recD, 2 ssb, 3 umuC, 4 umuD, and 8 xerC), Cyanothecce ATCC51142 (2 lexA and 4 ruvC), and Nostoc PCC7120 (2 ssb and 3 xerC).

Keywords: cyanobacteria, photoproduction, DNA recombination, DNA repair, genetic instability, insertion sequences, natural transformation, radiation resistance
INTRODUCTION

Cyanobacteria, the oldest and most diverse Gram-negative bacteria (Shih et al., 2013) are the only prokaryotes capable of oxygen-evolving photosynthesis (Hamilton et al., 2016). They are viewed as the ancestors of plant chloroplasts (Archibald, 2009), and as major producers of (i) the Earth’s atmospheric oxygen (Schopf, 2011) and (ii) the carbonates sedimentary deposits (Bosak et al., 2013; Benzerara et al., 2014).

Contemporary cyanobacteria produce a tremendous quantity of oxygen, and fix CO$_2$ (Jasson and Northen, 2010), NO$_3$ and N$_2$ (Zehr, 2011) into an enormous biomass that supports a large part of the food chain. N$_2$-fixing cyanobacteria can be used to fertilize soils (Singh et al., 2016), in place of industrial N-fertilizers whose production consumes large amounts of fossil fuels (Grizeau et al., 2015). In colonizing a wealth of wastewater ecosystems that contain high levels of nitrate and phosphate (Abed et al., 2014) and/or heavy metals, cyanobacteria could be used for wastewater treatment (Abed et al., 2014; Singh et al., 2016).

Cyanobacteria produce a wealth of natural products that can influence human health (antioxidants, vitamins, antibacterial, toxins (Williams, 2009; Dittmann et al., 2015; Kleigrew et al., 2016; Narainsamy et al., 2016). Hence, *Arthrospira* has served as a human food since time immemorial (Gao, 1998).

Cyanobacteria are also regarded as promising microbial factories for the production of chemicals from nature’s most plentiful resources: solar light, water, CO$_2$ (Lai and Lan, 2015; Savakis and Hellingwerf, 2015; Zhou et al., 2016). To reach this objective, it is necessary to (i) introduce and express in cyanobacteria the (heterologous) chemicals-producing genes they lack; (ii) redirect the photosynthetically-fixed carbon toward the production of the intended chemicals; (iii) increase the tolerance of the engineered cyanobacteria to the intended products and (iv) maintain, or increase, the genomic stability of the producer strains. These biotechnological works are mainly performed with the unicellular models *Synechocystis* sp. strain PCC6803, *Synechococcus* sp. strain PCC7942 (formerly *Anacystis nidulans* R2) and *Synechococcus* sp. strain PCC7002 (formerly *Agnellum quadruplicatum* PR6) that possess a small sequenced and manipulable genome (http://genome.microbedb.jp/cyanobase/). These cyanobacteria can take up and incorporate extracellular DNA into their chromosome to create insertion, deletion, or replacement mutations (Orkwiszewski and Kaney, 1974; Stevens and Porter, 1980; Grigorieva and Shestakov, 1982). They can also be manipulated with replicative shuttle vectors derived from (i) their endogenous plasmids (Kuhlemeier et al., 1981; Buzby et al., 1983; Chauvat et al., 1986), or (ii) the non-cyanobacterial plasmid RSF1010 (Mermet-Bouvier et al., 1993). Interestingly, this promiscuous plasmid replicates also in *Thermosynechococcus elongatus* (Mühlenhoff and Chauvat, 1996), *Prochlorococcus marinus* sp. strain MIT9313 (Tolonen et al., 2006), *Leptolyngbya* sp. strain BL0902 and *Nostoc punctiforme* sp. strain ATCC29133 (also registered as PCC73102) (Huang et al., 2010; Taton et al., 2014). Such RSF1010-derived plasmids proved useful tools for in vivo studies of (i) gene expression (Marraccini et al., 1993; Mermet-Bouvier and Chauvat, 1994; Mazouni et al., 1998; Fige et al., 2000; Mazouni et al., 2003; Huang et al., 2010; Dutheil et al., 2012); (ii) cell division (Mazouni et al., 2004; Marbouty et al., 2009), DNA repair (Domain et al., 2004); (iii) hydrogen production (Dutheil et al., 2012; Sakr et al., 2013; Ortega-Ramos et al., 2014); (iv) insertion sequence (Cassier-Chauvat et al., 1997); and (v) redox metabolism and responses to heavy metals (Poncelet et al., 1998; Marteyn et al., 2009, 2013).

Because of their photoautotrophic lifestyle, cyanobacteria are strongly challenged by DNA damages generated by solar UV rays and photosynthesis (for review see Cassier-Chauvat and Chauvat, 2015), likely explaining their resistance to radiations. Furthermore, many cyanobacteria engineered for biotechnological purposes appeared to be genetically unstable in using DNA recombination to inactivate/eliminate the newly introduced genes of industrial interest. Hence, a better understanding of DNA recombination and repair in cyanobacteria could help increasing their robustness and the genetic stability of the engineered strains. This would represent an important contribution toward the development of an economically viable photo-biotechnology. In this perspective, we used a comparative genomic approach (Table 1 and Supplemental Table 1), to show that cyanobacteria possess a large number of genes homolog to *Escherichia coli* DNA recombination and repair genes, including the key SOS players *lexA* and *sulA*. The presence/absence of these genes and information concerning their function and/or regulation indicate that some cyanobacteria may possess an *E. coli*-like SOS-type DNA repair system. These findings do not exclude the possible existence in cyanobacteria of other DNA repair genes, not yet identified.

RESULTS AND DISCUSSION

Genomic Diversity of Cyanobacteria

In colonizing most waters (fresh, brackish and marine) and soils, where they face various challenges (Cassier-Chauvat and Chauvat, 2015), cyanobacteria have developed as widely diverse organisms (Narainsamy et al., 2013). Their genomes differ in size (from 1.44 to 12.07 Mb, ploidy (from two to more than 20 chromosome copies per cell) or GC content (30–60%), probably as a result from gains and losses of genes transferred by plasmids, insertion sequences (Alam et al., 1991; Cassier-Chauvat et al., 1997) and/or cyanophages (Hess, 2011; Shih et al., 2013). Most cyanobacteria possess a single circular chromosome, ranging from 1.44 Mb in size (the marine symbiotic strain UCYN-A) to 12.07 Mb (*Sytonema hofmanni* PCC7110) (Dagan et al., 2013). The well-studied strain *Synechocystis* PCC6803 has a 3.57 Mb chromosome, with a 48% GC content (http://genome.microbedb.jp/cyanobase/) and a copy number of 10–50 (Labarre et al., 1989; Griese et al., 2011). For the other models the values are 2.69 Mb, 55% and 2–5 for *Synechococcus* PCC7942 (Mann and Carr, 1974; Griese et al., 2011; Watanabe et al., 2015) and 3.00 Mb, 50%, and likely 2–5 for *Synechococcus* PCC7002 (Griese et al., 2011; Watanabe et al., 2015). *Synechocystis* PCC6803 also has seven plasmids, ranging from 2.3 Kb (Chauvat et al., 1986) to 119vKb...
Because of their photoautotrophic lifestyle, cyanobacteria are strongly challenged by solar UV rays and reactive oxygen species generated by photosynthesis (Cassier-Chauvat and Chauvat, 2015). Consequently, *Synechocystis PCC6803 and Synechococcus PCC7942* are found to be more resistant to UV than the (non-photosynthetic) bacterium *E. coli* where DNA repair is best known (Baharoglu and Mazel, 2014). *Synechocystis PCC6803* is also more resistant to gamma rays than *Synechococcus PCC7942* and *E. coli* in that order (the doses yielding 10% survival are 660, 230, and 130 Gy, respectively (Domian et al., 2004). Other cyanobacteria are even more radioresistant, almost as the champion bacterium *Deinococcus radiodurans* [100% survival at 5kGy (Moseley and Mattingly, 1971; Ito et al., 1983)]. These radiation-resistant cyanobacteria are *Chroococcidiopsis* [10% survival to 4–5 kGy of gamma rays (Billi et al., 2000), three *Anabaena* strains [they can grow at 5 kGy (Singh et al., 2010)] and *Arthrospira PCC8005* [it grows at 800 Gy (Badri et al., 2015)]. Thus, cyanobacteria might be used in the future for leaching (and/or sequestration) of radionuclides (Acharya and Apte, 2013).

### TABLE 1 | Reference of the genes from *Synechocystis* PCC6803 (all or slr), *E.coli* (eco) or *B.subtilis* (BSU) in the MBGD data base ([http://mbgd.genome.ad.jp/](http://mbgd.genome.ad.jp/)) used for searching their homologs in the studied cyanobacteria.

| Name     | Protein function                              | Gene id  |
|----------|-----------------------------------------------|----------|
| uvrA     | UvrA, excinuclease ABC subunit A              | slr1844  |
| uvrB     | UvrB, excinuclease ABC subunit B              | sll0459  |
| uvrC     | UvrC, excinuclease ABC subunit C              | sll0865  |
| uvrD     | UvrD, excinuclease ABC subunit C/helicaseII   | sll1143  |
| recA     | RecA, recombinase A                           | sll0569  |
| recBec   | RecB exonuclease V (RecBCD complex), beta subunit | eco:B2820 |
| recBcy   | Contains hhH domain and of nuclease of recB family | sll1686  |
| recC     | recC exonuclease V (RecBCD complex), gamma chain | eco:B2822 |
| recD     | recD exodeoxyribonuclease V, subunit alpha/ TraA family helicase | eco:B2819 |
| recF     | Recombination protein F RecF                  | sll1277  |
| recG     | ATP-dependent DNA helicase RecG              | sll0020  |
| recJec   | recJ ssDNA exonuclease, 5' -> 3'-specific     | eco:B2892 |
| recJcy   | single-stranded-DNA-specific exonuclease RecJ | sll1384  |
| recN     | DNA repair protein RecN                      | sll1520  |
| recO     | Recombination protein RecF                    | sll1426  |
| recO     | DNA gap repair protein                        | sll/eco:B2565 |
| ruvA     | Holiday junction DNA helicase RuvA            | sll0876  |
| ruvB     | Holiday junction DNA helicase RuvB            | sll0613  |
| ruvC     | Holiday junction resolvase RuvC              | sll0896  |
| mutH     | mutH methyl-directed mismatch repair protein  | eco:B2831 |
| mutL     | mutL DNA mismatch repair protein              | slr1199  |
| mutM     | Formamidoprimidine-DNA glycosylase            | slr1689  |
| mutS1    | DNA mismatch repair protein MutS              | sll1165  |
| mutS2    | recombination and DNA strand exchange inhibitor protein | sll1772 |
| mutT     | DNA mismatch repair protein Mutator Mut_like protein | sll134 |
| mutY1    | A/G specific adenin glycosylase yfhQ          | eco:B2961 |
| umuC     | umuC translesion error-prone DNA polymerase V subunit; | eco:B1184 |
| umuD     | SOS response UmuD protein                     | sll1523  |
| lexA     | lexA SOS function regulatory protein          | sll1626  |
| ssb      | ssb single-stranded DNA-binding protein       | sll0925  |
| dinB     | DNA polymerase IV                            | eco:B0231 |
| comA     | competence protein comEA, comA               | slr0197  |
| comE     | competence protein comEC, comEA comE         | sll1929  |
| comFA    | Competence protein ComF operon protein1       | BSU35470 |

(Continued)
Cyanobacteria can be Naturally Competent for Genetic Transformation Mediated by DNA Recombinations

The naturally transformable cyanobacteria *Synechococcus PCC7942, Synechococcus PCC7002*, and *Synechocystis PCC6803* can take up extracellular DNA and to recombine it into their own genome (Orkwisewski and Kaney, 1974; Stevens and Porter, 1980; Grigorieva and Shestakov, 1982). This capability served to create a wealth of insertions, deletions or replacement mutations (Lai and Lan, 2015; Savakis and Hellingwerf, 2015; Zhou et al., 2016).

Natural transformation is best studied in *Bacillus subtilis* and *Helicobacter pylori* (Dorer et al., 2011). DNA transported into the cytosol by the Com proteins (com for competence) is integrated into the recipient genome by the RecA, RecG, and RuvABC recombination proteins.

The com genes (Table 1) are widely distributed in cyanobacteria (Supplemental Table 1). *Synechocystis PCC6803, Synechococcus PCC7942*, and *Synechococcus PCC7002* harbor the comAEF genes (Supplemental Table 1). The *Synechocystis* PCC6803 genes *comA* and *comF* truly operate in transformation (Yoshihara et al., 2001), and *comF* is also involved in phototactic motility (Nakasugi et al., 2006). The role of *comE* could not be verified because the *comE*-depleted mutant dies rapidly (Yoshihara et al., 2001). By contrast, the *Prochlorococcus* cyanobacteria endowed with small genomes have no *comAEF* genes, excepted *P. marinus* MIT9303, and *P. marinus* MIT9313 that possess *comA, come*, and *ComF* (Supplemental Table 1). These strains also have the *recA, recG*, and *ruvABC* genes (Supplemental Table 1). We have verified in *Synechocystis* PCC6803 that *ruvB* operates in genetic transformation (Domain et al., 2004). These finding suggest that *P. marinus* MIT9313 may be transformable in appropriate conditions.

Recently, the CRISPR/Cas9 genome editing system, which enhances the recombination efficiency and accelerates the process for chromosome segregation, was used for efficient genome editing in cyanobacteria (Li et al., 2016; Wendt et al., 2016).

Cyanobacteria Genetically Engineered for Biotechnological Purposes can be Genetically Instable

Microbial organisms can genetically adapt themselves to their “laboratory” environment. This phenomenon explains the phenotypic differences observed between various sub-strains of the same organism cultivated in diverse laboratories. Hence, the four laboratory sub-strains of *Synechocystis* PCC6803 with different cell motility and/or ability to feed from glucose, harbor mutations, insertion or deletion, as compared to each others (Okamoto et al., 1999; Kanesaki et al., 2012; Trautmann et al., 2012).

Genetic instability can also be observed in strains genetically engineered for the synthesis of chemicals, where it can decrease the amplitude and/or durability of production. Genetic instability correlates with the toxicity of the products, and homologous recombination between repeated DNA motifs (Gellert and Nash, 1987; Holder et al., 2015), which are frequent in cyanobacteria (Elhai, 2015).

In the 61 articles reporting the genetic engineering of a model cyanobacterium for the synthesis of a biotechnological product, the level of production were analyzed only during short periods of times (usually not more than 30 days after the generation of the producer strains; Lai and Lan, 2015). Consequently, we know very little regarding genome (in)stability in engineered cyanobacteria growing under laboratory conditions. This genome (in)stability is an important issue in large industrial cultures that require many cell divisions of the engineered cyanobacteria. The longer the cultivation, the higher the probability of selecting spontaneous mutations decreasing the synthesis of the product to increase cell fitness.

A few studies reported the genetic instability of engineered cyanobacteria. We observed this phenomenon while attempting to use *Synechocystis PCC6803* for the production of a uniformly $^{13}$C-labeled mouse uron kinase (a serine protease). The uron kinase producing plasmid, which replicated stably in the *recA* mutatant of *E. coli*, invariably lost part of the uron kinase gene upon propagation in *Synechocystis* PCC6803 (Chauvat et al., 1988). Another *Synechocystis* PCC6803 strain harboring *Pseudomonas aeruginosa* genes cloned its chromosome (at the slr0168 neutral docking site) for lactic acid production, happened to rescue its growth by introducing a duplication (~160 bp) that generated premature stop codons into the *Pseudomonas* (NADPH/NADH) transhydrogenase gene (Angermayr et al., 2012).

Similarly, the *Synechococcus PCC7942* strain harboring the *Pseudomonas syringae* gene (*efe*) encoding the ethylene-forming enzyme (Fukuda et al., 1992; Sakai et al., 1997), managed to introduce short nucleotide insertions in *efe* to stop ethylene production and recover a healthy growth (Takahama et al., 2003). Another recombinant *Synechococcus PCC7942* strain could introduce a missense mutation in the *E. coli atoD* gene (acetoacetyl-CoA transferase) to decrease isopropanol production (Kusakabe et al., 2013).

In *Synechococcus PCC7002*, a recombinant strain managed to loose mannotol synthesis and recover healthy growth, in introducing a single-base deletion generating a stop codon in its *E. coli mannotol-1-phosphate dehydrogenase mtlD* gene (Jacobsen and Frigaard, 2014).

The *Synechocystis* PCC6803 and *Synechococcus PCC7002* recombinant strains producing the *Zymomonas mobilis* pyruvate decarboxylase enzyme (PDC) for ethanol production, could introduce mutations, insertions, deletions or mobile genetic elements (insertion sequences) into the *pdc* gene to stop ethanol production (Schulze et al., 2015).

Insertion sequences (ISs) are approximately 1 kbp long DNA segments found in the genome of most living organisms, where they can interrupt genes (Bennett, 2004). Generally, an IS comprises an inverted repeat DNA sequence flanking one or two genes encoding the mobilization protein (transposase), which drives the excision and reinsertion of IS in genomes.

Many cyanobacterial chromosomes and/or plasmids harbor a few or numerous copies of ISs, as the widely distributed IS families IS4, IS5, IS630 and IS200-605, which are regarded as ancestral (Lin et al., 2011). Though several *P. marinus* strains

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**Table 1**

| Gene | Description |
|------|-------------|
| *comA* | ComA protein |
| *comB* | ComB protein |
| *comC* | ComC protein |
| *comD* | ComD protein |
| *comE* | ComE protein |
| *comF* | ComF protein |
| *comG* | ComG protein |
| *comH* | ComH protein |
| *comI* | ComI protein |
| *comJ* | ComJ protein |
| *comK* | ComK protein |
| *comL* | ComL protein |
| *comM* | ComM protein |
| *comN* | ComN protein |
| *comO* | ComO protein |
| *comP* | ComP protein |
| *comQ* | ComQ protein |
| *comR* | ComR protein |
| *comS* | ComS protein |
| *comT* | ComT protein |
| *comU* | ComU protein |
| *comV* | ComV protein |
| *comW* | ComW protein |
| *comX* | ComX protein |
| *comY* | ComY protein |
| *comZ* | ComZ protein |
| *comAEF* | ComAEF operon |

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**Supplemental Table 1**

| Gene | Description |
|------|-------------|
| *recA* | RecA protein |
| *recB* | RecB protein |
| *recC* | RecC protein |
| *recD* | RecD protein |
| *recE* | RecE protein |
| *recF* | RecF protein |
| *recG* | RecG protein |
| *recH* | RecH protein |
| *recI* | RecI protein |
| *recJ* | RecJ protein |
| *recK* | RecK protein |
| *recL* | RecL protein |
| *recM* | RecM protein |
| *recN* | RecN protein |
| *recO* | RecO protein |
| *recP* | RecP protein |
| *recQ* | RecQ protein |
| *recR* | RecR protein |
| *recS* | RecS protein |
| *recT* | RecT protein |
| *recU* | RecU protein |
| *recV* | RecV protein |
| *recW* | RecW protein |
| *recX* | RecX protein |
| *recY* | RecY protein |
| *recZ* | RecZ protein |
| *ruvA* | RuvA protein |
| *ruvB* | RuvB protein |
| *ruvC* | RuvC protein |
| *ruvD* | RuvD protein |
| *ruvE* | RuvE protein |
| *ruvF* | RuvF protein |
| *ruvG* | RuvG protein |
| *ruvH* | RuvH protein |
| *ruvI* | RuvI protein |
| *ruvJ* | RuvJ protein |
| *ruvK* | RuvK protein |
| *ruvL* | RuvL protein |
| *ruvM* | RuvM protein |
| *ruvN* | RuvN protein |
| *ruvO* | RuvO protein |
| *ruvP* | RuvP protein |
| *ruvQ* | RuvQ protein |
| *ruvR* | RuvR protein |
| *ruvS* | RuvS protein |
| *ruvT* | RuvT protein |
| *ruvU* | RuvU protein |
| *ruvV* | RuvV protein |
| *ruvW* | RuvW protein |
| *ruvX* | RuvX protein |
| *ruvY* | RuvY protein |
| *ruvZ* | RuvZ protein |

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Many cyanobacterial chromosomes and/or plasmids harbor a few or numerous copies of ISs, as the widely distributed IS families IS4, IS5, IS630 and IS200-605, which are regarded as ancestral (Lin et al., 2011). Though several *P. marinus* strains
harboring a small genome have no IS, the frequencies of IS do not systematically increase with the genome size. Indeed, IS represent 10% of the 5.8 Mb genome of Microcystis aeruginosa NIES843, 1.5% of the 3.95 Mb genome of Synechocystis PCC6803, and 1% of the 7.2 Mb genome of Nostoc (Anabaena) PCC7120 (Lin et al., 2011). Consistent with the findings that transposable genes can be induced by stresses (Hernández-Prieto et al., 2016), several studies employing a positive selection procedure showed that ISs can be truly mobile in cyanobacteria. First, a recombinant Nostoc (Anabaena) PCC7120 strain harboring a plasmid encoding the B. subtilis SacB enzyme (levan sucrase), which kills cells incubated in the presence of sucrose, generated sucrose resistant mutants resulting from the disruption of the sacB gene by a mobile IS895 element (Alam et al., 1991).

Similarly, an IS5 element of Synechocystis PCC6803 was shown to be mobile in rescuing the growth of a conditionally lethal mutant by disrupting the repressor gene that normally blocks the transcription of an essential ferredoxin-encoding gene (Cassier-Chauvat et al., 1997; Poncelet et al., 1998). Other recently transposed IS4 elements were identified through Southern blotting and DNA sequencing analysis of three Synechocystis PCC6803 sub-strains (Okamoto et al., 1999).

In addition, the presence of multiple copies of an IS in a genome can promote homologous recombination, leading to genome rearrangements (inversions or deletions; Gellert and Nash, 1987) that can modify cell fitness. Moreover, ISs can be transferred between genomes by horizontal gene transfer mechanisms. Thus, ISs are an important force in genome evolution (Bennett, 2004).

So far very few studies attempted to decrease or eliminate the negative influence of IS on biotechnological production. In Corynebacterium glutamicum, the deletion of two major IS elements generated a cell chassis with an increased ability to stably produce recombinant proteins (Choi et al., 2015). A similar strategy could be tested in the genetically manipulable cyanobacteria Synechococcus PCC7942 and Synechococcus PCC7002 because they possess only one and ten transposase genes, respectively (http://genome.microbedb.jp/cyanobase/). In contrast, an IS-deletion strategy is not an appealing option for Synechocystis PCC6803 that possesses 128 transposase genes.

In E. coli, the stable propagation of recombinant DNA (usually cloned in plasmids) is achieved in strains where recA, the key DNA-recombination gene (Baharoglu and Mazel, 2014), has been inactivated to prevent unexpected DNA rearrangements. All cyanobacteria possess a recA gene (Acaroychoris marina MBIC11017 has 7 recA genes, Supplemental Table 1). The recA gene appeared to be indispensable to cell life in Synechococcus PCC7002 (Murphy et al., 1990), whereas it could be deleted from all chromosome copies in Synechocystis PCC6803 (Minda et al., 2005). The Synechocystis PCC6803 recA null mutant is bound to be of limited biotechnological interest because it is not only sensitive to UV-C, but also to standard fluence of white light required for cell growth. Furthermore, in being defective in DNA recombination a recA- mutant is not appropriate for genetic manipulation of the cyanobacterial chromosome (cloning of heterologous genes encoding the synthesis of biotechnological products and/or deletion of endogenous genes limiting the intended production).

An interesting way to limit genetic instability of engineered bacteria is to clone the product-synthesizing genes under the control of regulatable expression signals to afford a user-controlled synthesis of the potentially harmful product. Using such regulatory signals, one can grow the engineered strain up to a large biomass, before triggering the synthesis of the intended product, which, otherwise, could have impaired the fitness and/or the genetic stability of the producer. In cyanobacteria gene expression can be regulated by (i) light (psba2 promoter), (ii) the IPTG metabolite (lac promoter/repressor system), (iii) metals (cyanobacterial promoters coaT, ziaA, etc (Berla et al., 2013; Zhou et al., 2016), or (iv) the growth temperature [lambda phage pR promoter controlled by the cI857 temperature-sensitive repressor (Ferino and Chauvat, 1989; Mermet-Bouvier and Chauvat, 1994)]. As put forward by other workers (Berla et al., 2013) an ideal system should combine the following properties.

(a) “It should be inactive in absence of inducer”;
(b) “It should produce a predictable response to a given concentration of a regulator”;
(c) “The inducer should have no harmful effect on the host organism”;
(d) “The inducer should be cheap and stable under the growth conditions of the host”;
(e) “The inducible system should act orthogonally to the host cell’s transcriptional program (ideal transcriptional repressors should not bind to native promoters)”.

In our laboratory, we often used the temperature-controlled system that appeared to combine most of these advantages (Duthie et al., 2012; Marteyn et al., 2013; Ortega-Ramos et al., 2014) and references therein. This system tightly controls gene expression proportionally to growth temperatures i.e., absence of expression at temperature ≤30°C (the standard growth temperature of our favorite cyanobacterium Synechocystis PCC6803); intermediary expression at intermediate temperature 34–37°C; and strong expression at 39°C (where Synechocystis PCC6803 keep growing well). For instance, when this system was used to control the production of the heterologous enzymes chloramphenicol-acetyl-transferase and beta-galactosidase, which possess an easily quantified activity, the values were respectively ≤3 units (30°C); 700–1000 units (34–37°C) and 2000–4000 units (39°C) (Ferino and Chauvat, 1989; Mermet-Bouvier and Chauvat, 1994). Hence this system can be also used for basic research that requires the construction of conditionally-lethal mutants (Poncelet et al., 1998; Sakr et al., 2013).

**Distribution of Direct DNA-Damages Reversal Genes in Cyanobacteria**

From bacteria to higher eukaryotes, cells are continuously exposed to DNA damages generated by their own metabolism (Imlay, 2013) and/or exogenous sources (radiations, chemicals, etc). DNA lesions are repaired by conserved pathways that
have been extensively studied in *E. coli* (Baharoglu and Mazel, 2014). The simplest system, the direct damage reversal pathway, removes only the base-modifying agent in one single step (Resende et al., 2011) catalyzed by the AlkB demethylase, the Ogt alkyltransferase, and the Phr (photorepairs of pyrimidine) photolyase.

Using a comparative genomic approach, we found that the 76 cyanobacterial genome sequences in the MBGD data base (http://mbgd.genome.ad.jp/) possess many genes orthologous to *E. coli* DNA recombination and repair genes. The phr, alkB and ogt orthologs (Table 1) are distributed unevenly in cyanobacteria (Supplemental Table 1). The phr gene is present in almost all cyanobacteria including some, but not all, *P. marinus* strains endowed with a small genome (1.6–2.7 Mb). In agreement with the light fluence they receive in their oceanic biotopes (Biller et al., 2015), the high-light-adapted strains *P. marinus* MIT9515 and *P. marinus* MED4 possess phr, whereas the low-light-adapted strains *P. marinus* MIT9303 and *P. marinus* MIT9313 lack phr (Supplemental Table 1), and are light sensitive (Biller et al., 2015). The alkB and ogt genes are less frequent than phr. All three genes alkB, ogt, and phr are simultaneously present in several (twelve) studied cyanobacteria, such as *Nostoc (Anabaena)* PCC7120 (filamentous), and *Cyanothecae* PCC7425 (unicellular) where ogt is duplicated. The other (evolutionary distant) unicellular models *Synechocystis* PCC6803, *Synechococcus* PCC7942, and *Synechococcus* PCC7002 possess phr (Supplemental Table 1). *Synechocystis* PCC6803 has alkB but not ogt, *Synechococcus* PCC7942 has ogt (duplicated) but not alkB, and *Synechococcus* PCC7002 has neither alkB nor ogt. Interestingly, the symbiotic (marine) cyanobacterium UCYN-A has no phr, alkB, and ogt, in agreement with the fact that it possesses the smallest genome (1.44 Mb). The other symbiotic strain *Acaryochloris marina* MBIC11017 endowed with a larger genome (8.36 Mb) has two alkB, three ogt (including one on a plasmid) but no phr (Supplemental Table 1).

### Distribution of Nucleotide Excision DNA Repair Genes in Cyanobacteria

This pathway removes distortions of the double helix of DNA (pyrimidine dimers or DNA intra-strand cross-links), by excising a small group of bases (Baharoglu and Mazel, 2014). In *E. coli* the two-proteins complex UvrAB recognizes the DNA lesion; Uvrc generates a double incision on both sides of the lesion and the UvrD helicase removes the single-strand DNA carrying the lesion. The missing DNA is re-synthesized by the DNA polymerase I (Pol I), and subsequently sealed by a ligase.

All tested cyanobacterial genomes possess the *uvrABCD* single-copy genes (Supplemental Table 1), where *uvrA* and *uvrB* are not organized in operon (Supplemental Figure 1), unlike what occurs in *E. coli*. In some cyanobacterial genomes *uvrA*, *uvrB*, *uvrc*, and/or *uvrD* are clustered with another DNA repair gene, such as *phr* or *recN* (gene clusters a and c in Supplemental Table 1 and Supplemental Figure 1). In the radiation-resistant cyanobacterium *Arthrospira* PCC8005, *uvrBCD* were found to be upregulated by gamma rays (no information is provided for *uvrA*) (Badri et al., 2015).

### Distribution of Methyl-Directed DNA Mismatch Repair Genes in Cyanobacteria

This pathway corrects the mispaired DNA bases generated by replication errors (Putnam, 2016). In *E. coli*, MutS recognizes mispaired DNA bases and coordinates with MutH and MutL (nucleases), MutM, MutT and MutY (DNA glycosylases) and UvrD (helicase) to direct excision of the newly synthesized DNA strand (not yet methylated at GATC sites by the Dam methylase) up to the mismatch. The resulting gap is filled up by a DNA polymerase (likely PolIII) and a ligase (Putnam, 2016).

All tested cyanobacteria have *mutM* (Supplemental Table 1), which was shown in *Synechococcus* PCC7942 to operate in resistance to high light (Mühlenhoff, 2000). All cyanobacteria possess *mutS*, which occurs in two copies, excepted in *Crinalium epipsammum* PCC 9333 (Supplemental Table 1). By contrast, *mutH* is absent in all cyanobacteria. The genetic diversity of cyanobacteria is well illustrated with the presence/absence of *mutL*, *mutT*, and *mutY* (Supplemental Table 1), which lies in front of *recR* in a few cyanobacterial genomes (Table 1 and Supplemental Figure 1). Several *P. marinus* strains lack mutL, mutT, and mutY (Supplemental Table 1). In *Arthrospira* PCC8005 (radiation-resistant) *mutST* were upregulated by gamma rays (Badri et al., 2015).

The model strains *Synechocystis* PCC6803, *Synechococcus* PCC7942, *Synechococcus* PCC7002 and *Nostoc (Anabaena)* PCC7120 possess mutL, mutM, mutS (duplicated), mutT (excepted *Synechococcus* PCC7002), mutY (excepted *Synechococcus* PCC6803 and *Nostoc* PCC7120) (Supplemental Table 1). Thus, *Synechococcus* PCC7942 is best suited to study all these genes through deletion/over-expression in the otherwise same genetic context.

### Distribution of Recombinational DNA Repair Genes in Cyanobacteria

This pathway repairs double-stranded breaks and cross-links. In *E. coli*, single-strand DNA nicks are enlarged by the RecQ helicase and RecJ exonuclease, into gaps that are recognized by the proteins RecFOR. The double-strand DNA breaks (DSB) are recognized by the RecBCD proteins that form an exonuclease/helicase complex. Subsequently, the RecFOR/RecBCD complexes (and RecN) load RecA to initiate homologous recombination and DNA repair. RecA mediates synopsis, forming a Holliday junction. Replication fills gaps. RecG, Ssb (single-stranded DNA binding protein) and RuvAB mediate branch migration (stimulated by RadA), and RuvC resolves the junctions (Baharoglu and Mazel, 2014).

DNA recombination also involves the XerC-XerD complex. It converts dimers of the chromosome into monomers to permit their segregation during cell division, and it contributes to the segregational stability of plasmids (Resende et al., 2011; Buljubašić et al., 2013).

In many bacteria, such as *H. pylori* and *B. subtilis* the AddA and AddB proteins replace RecB and RecC, respectively (Dorer et al., 2011; Wigley, 2013).

All cyanobacteria contain *recA*, which occurs as seven copies in the large genome (8.36 Mb) of *A. marina* MBIC11017.
Four of these recA genes, possibly originating from gene duplication (Swingley et al., 2008), are located on four separate plasmids, while the other recA belong to the chromosome (Supplemental Table 1).

Like recA, rada and recG are present in all cyanobacteria, and radA is duplicated in *Cyanothece* PCC7425, *M. aeruginosa NIES-843* and UCYNA (Supplemental Table 1). It is the only duplicated gene in the very small UCYNA genome (1.44 Mb).

Many cyanobacteria have two copies of recJ and recQ genes. They are noted as recJec or recJy, or recQec or recQcy (rec for *E. coli*, cy for cyanobacteria), according to their high (recIec, recQec) or low (recJy, recQcy) sequence similarity with their *E. coli* counterparts (Table 1 and Supplemental Table 1). This is true for *Synechococcus* PCC7002 and *Nostoc* PCC7120, where these duplicated genes can be studied and compared through deletion/over-expression. In *Arthrospira* PCC8005 (radiation-resistant), recGIQ were found to be upregulated by gamma rays (Badri et al., 2015). In contrast a few cyanobacteria has neither recJ nor recQ, as *P. marinus* MIT9315 (Supplemental Table 1). Also interestingly, the low-light-adapted *P. marinus* MIT9313 and *P. marinus* MIT9303 possess the recQ genes (and ogt and the competence genes comE and comFC), which are not present in other *Prochlorococcus* (Supplemental Table 1). In addition, both *P. marinus* MIT9313 and *P. marinus* MIT9303 lack the phr gene, which occurs in other *Prochlorococcus* (Supplemental Table 1), in agreement with their light-sensitivity (Billier et al., 2015). Collectively, these findings support the proposal that *P. marinus* MIT9303 and *P. marinus* MIT9313 belong to the same clade, which diverged early from the other *Prochlorococcus* clades (Sun and Blanchard, 2014; Billier et al., 2015).

Almost all cyanobacteria have the single-copy genes recF, recO and recR, excepted *Cyanobacterium aponinum* PCC10605, *C. epipsammum* PCC9333 and *Cylindrospermum stagnale* PCC7417 which lack recR (Supplemental Table 1)

The recBCD genes are less conserved in cyanobacteria. For instance, the strain UCYN-A that possesses recFOR has no recBCD genes (Supplemental Table 1). Most *P. marinus* strains and several marine *Synechococcus* strains possess recBCD. Most of these strains possess two recB copies, noted recBec (good similarity with *E. coli* recB) or recBcy (cy for cyanobacteria, low similarity with *E. coli* recB). In these strains, recBec belongs to the same genomic region than recC and recD (cluster f in Supplemental Table 1 and Supplemental Figure 1). In a few other cyanobacteria recD is duplicated (*Microcoleus* PCC7113) or triplicated (*A. marina* MBIC11017 and *N. punctiforme* PCC73102), irrespectively of the presence /absence or recBec and recBcy (Supplemental Table 1). The well-studied model cyanobacteria lack recB, recC, or recD. Both *Synechocystis* PCC6803 and *Nostoc* (Anaabaena) PCC7120 lack recBec and recC, while both *Synechococcus* strains PCC7942 and *Synechococcus* PCC7002 lack recCD.

The recN gene is present in all cyanobacteria to the noticeable exception of *Chamaesiphon minutus* PCC6605. Interestingly the RecN protein was absent in mature heterocysts of *Anabaena* PCC7120, the differentiated nitrogen-fixing cells that have lost the ability to divide (Hu et al., 2015).

In some cyanobacteria a few rec genes are clustered together (*recBCD* see cluster f in Supplemental Table 1 and Supplemental Figure 1), or with other DNA repair genes, including *umuA* (cluster a) or *mutY* (cluster n; Supplemental Table 1 and Supplemental Figure 1).

All cyanobacteria have a ssb gene, which is repeated in a few strains. For instance, ssb is duplicated in *Nostoc* (Anaabaena) PCC7120 and *A. marina* MBIC11017, while it is triplicated in *Chroococcidiopsis thermals* PCC7203 and quadruplicated in *Cyanothece* PCC7822 (Supplemental Table 1). In these cyanobacteria (excepted *Nostoc* (Anaabaena) PCC7120) one ssb copy is propagated on a plasmid. One of the two *Nostoc* PCC7120 ssb genes, (alr0088, but not alr7579) was shown to be involved in the tolerance to UV and mitomycin C which causes formation of DNA adducts (Kirti et al., 2013).

The ruvABC genes are present in all cyanobacteria, to the noticeable exception of *G. kilaueensis* JS1 which lacks *ruvC* (Supplemental Table 1). The *ruvA* and *ruvB* genes are not adjacent unlike their operonic *E. coli* counterparts. Furthermore, *ruvA* is duplicated in *Trichodesmium erythraeum* ISM101, while *ruvC* is quadruplicated in *Cyanothece* ATCC51142 and quadruplicated in *Cyanothece* PCC7822 (Supplemental Table 1). In *Synechocystis* PCC6803 *ruvB* was shown to be dispensable to cell growth in standard laboratory conditions, and to operate in the resistance to UV and H2O2 (Domain et al., 2004).

Unlike recAN and *ruvABC*, xerC is a rare gene in cyanobacteria (Supplemental Table 1). It occurs in a single copy in a few strains, as UCYN-A, *Synechococcus* PCC7942, *Synechococcus* PCC7002 and *Synechocystis* PCC6803, or in several copies in *Cyanothece* PCC7424, *Cyanothece* PCC7822 (two copies), *Nostoc* (Anaabaena) PCC7120 (three copies), *A. marina* MBIC11017 (eight copies).

In bacteria, homologous recombination preferentially initiates at highly repeated, oligomeric DNA sequences designated as Chi (crossover hotspot instigator) sites. In *E. coli*, the Chi site used by RecBCD is 8 bases (GCTGTTGG), whereas in *B. subtilis* Chi used by AddAB is just 5 bases (AGCGG) (Wigley, 2013). Similarly, the GGATCGC sequence is overrepresented in many cyanobacteria where one or more methylases recognize some portion of the sequence (Elhai, 2015). In *Synechocystis* PCC6803 the repeated sequence HIP1 (Highly Iterated Palindrome) is associated to a CGATCG-specific methylase (M.Ssp6803I) that is required for rapid growth (Elhai, 2015).

**Distribution of Mutagenic DNA Repair Genes in Cyanobacteria**

The above-mentioned repair systems usually remove the initial DNA lesions and provide the genetic material back to its original state. When facing many DNA injuries cells start synthesizing several proteins (endonucleases, polymerases and ligases) to accelerate DNA repair, even though there may be some incorporated errors. In this case, the replicative DNA polymerase PolIII, which cannot replicate damaged DNA, is replaced by other polymerases PolIV (encoded by *dinB*) and PolV (encoded by *umaCD*), which replicate damaged DNA in a mutagenic manner (Baharoglu and Mazel, 2014).
The *umuCD* genes (Table 1) are unevenly distributed in cyanobacteria (Supplemental Table 1). Many strains have no *umuCD*, like UCYN-A (small genome) and *Synechococcus PCC7002*. Others possess *umuCD*, such as *Nostoc* (Anabaena) PCC7120, *Synechococcus PCC7942*, *Synechocystis PCC6803*, and the *Prochlorococcus* strains. A few strains harbor a duplication of *umuC* (*Synechococcus PCC6312*) and/or *umuD* (*Cyanobium gracile PCC6307* and *Synechococcus PCC6312*). *A. marina MBIC11017* possesses three *umuC* and four *umuD* (Swingley et al., 2008). In some cyanobacteria *umuDC* are clustered together, nearby *ruvA* (cluster z in Supplemental Table 1 and Supplemental Figure 1).

The gene *dinB* (Table 1) is present in a very few cyanobacteria, such as *A. marina MBIC11017*, *Anabaena PCC7120* and *G. kilaeuaensis JS1* (Supplemental Table 1).

Distribution of the Key *E. coli*-Type SOS Genes LexA and SuluA in Cyanobacteria

In many bacteria, the so-called “SOS” regulatory system is the main transcriptional circuit that detects DNA damages and regulates the repair systems according to cells needs (Baharoglu and Mazel, 2014). The SOS response is activated when RecA binds single-stranded DNA and generates a nucleofilament triggering the auto-proteolysis of the LexA regulator. In *E. coli*, LexA normally represses about 40 SOS genes (recABC, *ruvABC*, *etc.*) by binding to its cognate LexA-box sequence on their promoters (5′-tactgtatatatacAGta-3′; the upper cases indicate the conserved nucleotides), thereby precluding their transcription (Baharoglu and Mazel, 2014). One of the SOS-controlled gene codes for the key SuluA protein that delays cell division until DNA damages are repaired.

The *lexA* gene (Table 1) is unevenly distributed in cyanobacteria. It is absent in both *Arthospira PCC8005* (Badri et al., 2015) and NIES39, and in several strains of the genus *Gloeobacter*, *Oscillatoria* and *Synechococcus* (including *Synechococcus PCC7942*, Supplemental Table 1), similarly to what found in other bacteria as *H. pylori* (Dorer et al., 2011) and *Streptococcus pneumoniae* (Baharoglu and Mazel, 2014). By contrast, *lexA* is present in the other tested cyanobacteria (it is duplicated in *Cyanothece ATCC51142*). The marine cyanobacteria of the genus *Prochlorococcus* and *Synechococcus* share a very similar *lexA* (clade C), while other strains possess a slightly different *lexA* (clade B), such as *A. marina MBIC11017*, and both *Nostoc PCC7120* and *Synechocystis PCC6803* (Li et al., 2010). Interestingly, the *Synechocystis PCC6803* *lexA* gene appeared to regulate carbon assimilation (Domain et al., 2004) and cell motility (Kizawa et al., 2016), but not DNA recombination and repair (Domain et al., 2004). Furthermore, the *Nostoc PCC7120* *LexA* protein has a RecA-independent autoproteolytic cleavage (Kumar et al., 2015).

The *suluA* homolog is present in almost all cyanobacteria, to the noticeable exception of *Gloeobacter violaceus PCC7421*, *G. kilaeuaensis JS1*, *Anabaena sp. 90* and UCYN-A (Supplemental Table 1). In *Synechocystis PCC6803*, *suluA* appeared to be indispensable to cell life and division (Raynaud et al., 2004).

The DNA Repair Genes Present in all Cyanobacteria Likely Encode the Core Process

Many genes are present in the 76 studied cyanobacteria (*mutM*, *radA*, *recA*, *recFO*, *recG*, *recN*, *ruvABC*, *ssb*, and *uvrABCD*) (Table 1 and Supplemental Table 1), including the marine strain UCYN-A that possesses the smallest genome (1.44 Mb), and numerous marine strains *Prochlorococcus* and *Synechococcus* also endowed with a small genome (1.65–Mb). Similarly, *mutS*, *recN*, and *ruvC* are present in almost all cyanobacteria (Supplemental Table 1), namely *Thermosynechococcus NK55a* (absence of *mutS*), *Cyanothece PCC51142* (absence of *recN*) and (*G. kilaeuaensis JS1* absence of *ruvC*). Consequently, we propose that the genes *mutMS*, *radA*, *recA*, *recFO*, *recG*, *recN*, *ruvABC*, *ssb*, and *uvrABCD* encode the core DNA repair system of cyanobacteria.

A few other genes are also very well conserved (Supplemental Table 1), such as *recR* (absent in *C. stagnale PCC7417*, *Cyanobacterium aponinum PCC10605* and *C. epipsammum PCC9333*), *phr* (absent in *A. marina MBIC11017*, and four *Prochlorococcus* strains: SS120, MIT9211, MIT9303 and MIT9313), and *sulA* (absent in UCYN-A, *Anabaena* sp. 90, and the two *Gloeobacter* strains *G. violaceus PCC7421* and *G. kilaeuaensis JS1*).

By contrast, *mutH* is absent in all cyanobacteria (Supplemental Table 1) while *dinB* occurs in only five cyanobacteria (*G. kilaeuaensis JS1*, *Nostoc* (Anabaena) PCC7120, *N. punctiforme PCC73102*, Rivularia PCC7116, and *A. marina MBIC11017*), and *recC* occurs mostly in the marine *Prochlorococcus* and *Synechococcus* strains.

*Acaryochloris marina MBIC11017* Possesses the Largest Panel of DNA Repair Genes Some of which Occurring in Multiple Copies in the Chromosome and/or Plasmids

The cyanobacteria *A. marina* are unique in that they use chlorophyll d to absorb far-red light for photosynthesis. *A. marina MBIC11017* possesses a large genome (836 Mb) comprising a circular chromosome (6.5 Mb) and nine plasmids (2.13–374 Kb, (Swingley et al., 2008)). Consistent with its large genome size, *A. marina MBIC11017* possesses almost all DNA repair genes observed in cyanobacteria, to the noticeable exception of *recC*. In addition to the core genes (*mutMS*, *radA*, *recA*, *recFO*, *recG*, *recN*, *ruvABC*, *ssb*, and *uvrABCD*) *A. marina MBIC11017* has the following genes *alkB*, *dinB* (rare in cyanobacteria), *lexA*, *mutLTY*, *phr*, *ogt*, *mutLTY*, *recJQR*, *sulA*, *ssb*, *umuCD*, and *xerC* (Supplemental Table 1). Several of these genes occur in multiple copies (some located on plasmids): *alkB* (two copies), *mutS* (two copies), *ogt* (three copies), *recA* (seven copies, four of them located on four distinct plasmids), *recD* (three copies, two of them propagated on plasmid), *recJ* (two copies), *recQ* (two copies), *ssb* (two copies), *umuC* (three copies including two plasmid copies), *umuD* (four copies including...
two plasmid copies), and xerC (eight copies, including six on plasmids).

The role of the DNA repair genes of *A. marina* MBIC11017 cannot be studied in this host because it has no genetic system yet. However, these genes can be studied in the genetic models *Synechocystis* PCC6803, *Synechococcus* PCC7942, *Synechococcus* PCC7002 or *Nostoc* (*Anabaena*) PCC7120, and their future DNA repair mutants. Hence, it would be interesting to study (and compare) the capability of each of the seven *A. marina* MBIC11017 recA genes to complement the detrimental absence of the endogenous recA gene of *Synechococcus* PCC7002 (Murphy et al., 1990). If so, the responses of the resulting mutants to DNA damaging agents could be further studied and compared to those of the *Synechococcus* PCC7002 wild-type strain.

**Together, the Evolutionary-Distant Genetic Models *Synechocystis* PCC6803, *Synechococcus* PCC7942, *Synechococcus* PCC7002 and *Nostoc* (*Anabaena*) PCC7120 Possess almost all DNA Repair Genes**

The cyanobacterial core DNA repair genes (*mutMS, radA, recA, recFO, recG, recN, ruvABC, ssb, and uvrABCD*) can be investigated in any genetic models *Synechocystis* PCC6803, *Synechococcus* PCC7942, *Synechococcus* PCC7002 and/or *Nostoc* (*Anabaena*) PCC7120, through deletion and/or over-expression, and phenotypic analysis of the resulting mutants (resistance to DNA damaging agents, etc).

Besides the core DNA repair genes, *Synechocystis* PCC6803, the best-studied model, can be used to investigate alkB, lexA, mutL, mutS (a second copy), mutT, phr, recBcy, recD, recQcy, sulA, umuC (two copies), and umuD (Supplemental Table 1). The genes missing in *Synechocystis* PCC6803 (*dinB, ogt, mutY, recBec, recC, recJcy, recjec, and recQec*) can be studied in the other models (Supplemental Table 1): *Synechococcus* PCC7942 (*mutY, recBec, and the two copies of ogt and rec*)), *Synechococcus* PCC7002 (*mutY, recBec, the two copies of recJ and recQec*) and *Nostoc* PCC7120 (*dinB, ogt, the two copies of recI, and recQec*). By contrast, recC in occurring only in the marine cyanobacteria *Synechococcus* and *Prochlorococcus*, with no genetics, cannot be studied in its truly natural genetic context. Nevertheless, recC can be investigated in any model cyanobacteria mentioned above.

So far only the *ruvB* and *lexA* genes of *Synechocystis* PCC6803 have been studied in vivo. While *ruvB* was found to operate in DNA-recombination, *lexA* appeared to regulate carbon assimilation (Domain et al., 2004) and cell motility (Kizawa et al., 2016) but not DNA repair (Domain et al., 2004).

**The *E. coli*-Like SOS Model for DNA Repair is Possibly Valid for the Marine *Prochlorococcus* and *Synechococcus* Cyanobacteria, but not for *Gloeobacter*, *Synechocystis* PCC6803, and *Synechococcus* PCC7942**

In addition to the core DNA repair genes (*mutMS, radA, recA, recFO, recG, recN, ruvABC, ssb, and uvrABCD*) the small genomes (1.6–2.7 Mb) of the marine cyanobacteria *Prochlorococcus* and *Synechococcus* possess several genes frequently absent in larger cyanobacterial genomes (*recBCD* and *umuCD*; Supplemental Table 1). *Prochlorococcus* and *Synechococcus* also have homologs of *lexA* and *sulA*, which encode the key *E. coli* SOS proteins LexA (regulation of the SOS system) and SulA (postponing of cell division until completion of DNA reparation) (Baharoglu and Mazel, 2014). Furthermore, recA and uvrA are induced by UV in *Prochlorococcus* and *Synechococcus* (no information is provided for the other genes), as occurs in *E. coli* (Mella-Flores et al., 2012). The distribution of DNA repair genes in *Prochlorococcus* and *Synechococcus* marine strains suggest that they may possess an *E.coli*-like SOS system. This hypothesis is consistent with the fact that the mutation rate of *Prochlorococcus* is similar to that of *E. coli* (Billier et al., 2015).

By contrast, several findings indicate that the *E.coli*-like SOS model for DNA repair is not valid for all cyanobacteria. The strongest evidence is that two cyanobacteria *G. violaceus* PCC7421 and *G. kilaueensis* JS1 have none of the two key SOS genes *lexA* and *sulA*, and they also lack *alkB*, *recBC* and *xerC* (Supplemental Table 1). Similarly, *Synechococcus* PCC7942 (and its sister strain PCC6301) has no *lexA*, *alkB*, *dinB*, and *recCD*, while *Anabaena* sp. 90 lacks *sulA*, *dinB*, *ogt*, *recBCD*, and *umuCD*. *Synechocystis* PCC6803 possesses *lexA*, but it does not regulate DNA repair genes; it controls carbon assimilation (Domain et al., 2004) and cell motility (Kizawa et al., 2016). Furthermore, the *Synechocystis* PCC6803 *lexA* and *recA* genes are not induced by UV-C as occur in *E. coli*, actually they are downregulated by UV-C (Domain et al., 2004) (*lexA* is also negatively regulated by UV-B (Huang et al., 2002)). In addition, the *Synechocystis* PCC6803 *lexA* and *recA* promoters have neither *E. coli*-like nor *B. subtilis*-like SOS boxes (Domain et al., 2004). Similarly, no SOS box was found in the promoter region of the *Synechococcus* PCC7002 *recA* gene (Murphy et al., 1990). Furthermore, the *lexA* gene of *Anabaena* PCC7120 was neither induced by UV-B nor mitomycin C. In addition, the *Synechocystis* PCC6803 LexA protein has a RecA-independent autoproteolytic cleavage (Kumar et al., 2015).

In *Synechococcus* PCC7942, the Weigle-reactivation of irradiated phage (As-1) was neither induced by mitomycin-C nor nalidixic acid, unlike what was found in *E.coli* (Lanham and Houghton, 1988).

**CONCLUSION**

From bacteria to higher eukaryotes, cells are equipped with various conserved systems to repair DNA damages generated by their own metabolism (Imlay, 2013) or exogenous sources (solar UV, gamma radiations, chemicals, etc.). Inevitably, some DNA lesions are not correctly repaired leading to mutations that can influence cell fitness (Baharoglu and Mazel, 2014).

For historical reasons, DNA recombination and repair in prokaryotes have been mostly studied in the (non-photosynthetic) bacterium *E. coli* (Baharoglu and Mazel, 2014). Unlike *E.coli*, cyanobacteria are continuously exposed to DNA damages generated by solar UV rays and their own
photosynthetic metabolism (Cassier-Chauvat and Chauvat, 2015). As a likely consequence, all tested cyanobacteria were found to be more radiation resistant than E. coli. It is also important to study DNA recombination and repair in cyanobacteria for biotechnological purposes, since many recombinant strains appeared to be genetically unstable. They somehow managed to inactivate the (newly-introduced) heterologous genes of industrial interest. Thus, a better understanding of DNA recombination and repair in cyanobacteria may lead to increasing the genetic stability of biotechnologically important strains, an important industrial goal.

Using a comparative genomic approach, we found that cyanobacteria possess many genes orthologous to E. coli DNA recombination and repair genes, notwithstanding the possibility that cyanobacteria have other, as yet unidentified, such genes. These E. coli-like genes are unevenly distributed in cyanobacteria, in agreement with their wide genome diversity, in a way consistent with the size of their genomes, i.e., large genomes tend to possess more DNA repair genes than small genomes. Most of these E. coli-like genes are scattered throughout cyanobacterial genomes, suggesting that there is a mechanism for their coordinate regulation or that they are mostly expressed constitutively. Many DNA repair genes (mutMS, radA, recA, recFO, recG, recN, ruvABC, ssb, and uvrABCD) are extremely well conserved in cyanobacteria, including in the Prochlorococcus and Synechococcus marine strains which possess very small genomes (1.44–2.7 Mb). Consequently, we propose that these genes encode the core DNA repair system of cyanobacteria. These marine Prochlorococcus and Synechococcus cyanobacteria also have the genes recBCD (DNA recombination), umuC (mutational DNA replication), and the key SOS genes lexA (regulation of the SOS system) and sulA (postponing of cell division until completion of DNA repairation). These findings suggest that the marine Prochlorococcus and Synechococcus cyanobacteria may possess an E. coli-type SOS system.

In contrast, other cyanobacteria endowed with larger genomes lack some of the SOS key genes (lexA, sulA, recBCD, or umuC). For instance, G. violaceus PCC7421 and G. kiluaeaensis JS1 lack lexA, recBC, and sulA (they also lack alkB and xerC). Synechococcus PCC7942 has neither lexA nor recBCD. Furthermore, the lexA gene of Synechocystis PCC6803 is not involved in the regulation of DNA repair genes (Domain et al., 2004). Collectively, these findings suggest that the E.coli-like SOS model for DNA repair is likely not valid for all cyanobacteria. The cyanobacterium A. marina MBIC11017 possesses the most complete, and complex, set of DNA repair genes: alkB (two copies), dinB (rare in cyanobacteria), lexA, mutL, mutM, mutS (two copies), mutT, mutY, ogt (three copies), phr, radA, recA (seven copies, four of them located on plasmids), recD (three copies, including two plasmidic copies), recF, recG, recJ (two copies), recN, recO, recQ (two copies), recR, ruvABC, ssb (two copies), sulA, umuC (three copies including two plasmid copies), umuD (four copies including two plasmid copies), uvrABCD and xerC (eight copies, including six on plasmids). However, A. marina MBIC11017 has not all DNA repair genes, since it lacks recC. All cyanovascular DNA repair genes naturally present (or not) in the few (evolutionary distant) genetic models Synechocystis PCC6803, Synechococcus PCC7002, Synechococcus PCC7942 and Nostoc (Anabaena) PCC7120, can be studied through deletion and/or over-expression, and analysis of the corresponding mutants (e.g., resistance to DNA damaging agents). Such works would be most welcome since little is known about DNA recombination and repair in cyanobacteria. So far, only the recA, ruvB, and lexA genes have been studied in vivo. The recA gene appeared to be indispensable in Synechococcus PCC7002 (Murphy et al., 1990), and dispensable in Synechocystis PCC6803 (Minda et al., 2005). The Synechocystis PCC6803 recA-null mutant was sensitive to UV-C and white light. The Synechocystis PCC6803 ruvB gene was found to operate in DNA-recombination, while lexA appeared to regulate carbon assimilation (Domain et al., 2004) and cell motility (Kizawa et al., 2016), but not DNA repair (Domain et al., 2004). We hope that this review will stimulate future studies of DNA recombination and repair in cyanobacteria so as to answer the following questions, among others. Do cyanobacteria possess DNA recombination and repair genes with no counterpart in a non-photosynthetic and radiation-sensitive bacterium such as E. coli? What is the specificity/redundancy of the various copies of the repeated genes of cyanobacteria (for example of the seven recA genes of A. marina MBIC11017)? What are the molecular mechanisms responsible for the high radiation-resistance of some cyanobacteria (for instance Chroococcidiopsis). How to improve the genetic stability of cyanobacterial strains engineered for biotechnological puposes?

E. coli DNA Recombination and Repair in Cyanobacteria

AUTHOR CONTRIBUTION
CC and FC conceived the study. CC, TV, and FC carried out the literature search and analyzed the data. CC, TV, and FC wrote the paper.

ACKNOWLEDGMENTS
TV was a recipient of PhD thesis fellowship from the CEA-Saclay France.

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2016.01809/full#supplementary-material

Supplemental Table 1 | Distribution of DNA repair genes in cyanobacteria. The presence (indicated by the number of copies) or absence (0) of the gene is indicated along with the letters referring to the conserved gene clusters depicted in Supplemental Figure 1.

Supplemental Figure 1 | Conserved genomic organization around the DNA repair genes in cyanobacterial genomes. Genes are represented by boxes pointing in the direction of their transcription. DNA repair genes are colored in red. Genes encoding hypothetical proteins are indicated as “ho.”
Li, H., Shen, C. R., Huang, C. H., Sung, L. Y., Wu, M. Y., and Hu, Y. C. (2016). CRISPR-Cas9 for the genome engineering of cyanobacteria and succinate production. Metab. Eng. 38, 293–302. doi: 10.1016/j.mib.2016.09.006
Li, S., Xu, M. L., and Su, Z. C. (2010). Computational analysis of LexA regulons in Cyanobacteria. Bmc Genet. 11:257. doi: 10.1186/1471-2164-11-257
Lin, S., Haas, S., Zemot, T., Xiao, P., Vingron, M., and Li, R. (2011). Genome-wide comparison of cyanobacterial transposable elements, potential genetic diversity indicators. Gene 473, 139–149. doi: 10.1016/j.gene.2011.10.011
Mann, N., and Carr, N. G. (1974). Control of macromolecular-composition and cell division in blue-green-alga Anacystis-Nidulans. J. Gen. Microbiol. 83, 399–405. doi: 10.1002/00221287-83-2-399
Marpouty, M., Saguez, C., Cassier-Chauvat, C., and Chauvat, F. (2009). ZipN, an FtsA-like orchestrator of divisome assembly in the model cyanobacterium Synechocystis PCC6803. Mol. Microbiol. 74, 409–420. doi: 10.1111/j.1365-2958.2009.06873.x
Marraccini, P., Bulleau, S., Cassier-Chauvat, C., Mermet-Bouvier, P., and Chauvat, F. (1993). A conjugative plasmid vector for promoter analysis in several cyanobacteria of the genera Synechococcus and Synechocystis. Plant Mol. Biol. 23, 905–909. doi: 10.1007/BF00021546
Marteyn, S., Lu, J., and Marois, C. (2015). A novel, autoinducible bioluminescent reporter for Bioluminescence imaging in a cyanobacterium. Front. Microbiol. 6, 608. doi: 10.3389/fmicb.2015.00608
Marteyn, B., Sakr, S., Farci, S., Bedhomme, M., Chardonnet, S., Decottignies, P., et al. (2013). The Synechocystis PCC6803 MerA-like enzyme operates in the reduction of both mercury and uranium under the control of the Glutaredoxin 1 Enzyme. J. Bacteriol. 195, 4138–4145. doi: 10.1128/JB.00272-13
Mazouni, K., Bulleau, S., Cassier-Chauvat, C., and Chauvat, F. (1998). Promoter element spacing controls basal expression and light inducibility of the cyanobacterial secA gene. Mol. Microbiol. 30, 1113–1122. doi: 10.1046/j.1365-2958.1998.01145.x
Mazouni, K., Domain, F., Cassier-Chauvat, C., and Chauvat, F. (2004). Molecular analysis of the key cytokinetic components of cyanobacteria: PisZ, ZipN and MinCDE. Mol. Microbiol. 52, 1145–1158. doi: 10.1111/j.1365-2958.2004.04042.x
Mazouni, K., Domain, F., Chauvat, F., and Cassier-Chauvat, C. (2003). Expression and regulation of the crucial plant-like ferredoxin of cyanobacteria. Mol. Microbiol. 49, 1019–1029. doi: 10.1046/j.1365-2958.2003.03609.x
Mella-Flores, D., Six, C., Ratin, M., Partensky, F., Le Corguille, G., et al. (2012). Prochlorococcus and Synechococcus have Evolved Different Adaptive Mechanisms to Cope with Light and UV Stress. Front. Microbiol. 3:285. doi: 10.3389/fmicb.2012.00285
Mermet-Bouvier, P., Cassier-Chauvat, C., Marraccini, P., and Chauvat, F. (1993). Transfer and replication of Rd1010-derived plasmids in several cyanobacteria of the general Synechocysts and Synechococcus. Curr. Microbiol. 27, 323–327. doi: 10.1007/BF01568955
Mermet-Bouvier, P., and Chauvat, F. (1994). A conditional expression vector for the cyanobacteria Synechocystis sp. strains PCC6803 and PCCC6714 or Synechococcus sp. strains PCC7942 and PCC6301. Curr. Microbiol. 28, 145–148. doi: 10.1007/BF01571055
Minda, R., Ramachandani, J., Joshi, V. P., and Bhattacharjee, S. K. (2005). A homologous recA mutant of Synechocystis PCC6803: construction strategy and characteristics eliciting a novel RecA independent UVC resistance in dark. Mol. Genet. Genomics 274, 616–624. doi: 10.1007/s00438-005-0054-z
Moseley, B. E. B., and Mattingly, A. (1971). Repair of irradiated transforming deoxyribonucleic acid in wild type and a radiation-sensitive mutant of Micrococcus Radiodurans. J. Bacteriol. 105, 976–+. Mühlenhoff, U. (2000). The FAPY-DNA glycosylase (Fpg) is required for survival of the cyanobacterium Synechococcus elongatus under high light irradiance. FEMS Microbiol. Lett. 187, 127–132. doi: 10.1111/j.1574-6941.2000.tb12859.x
Mühlenhoff, U., and Chauvat, F. (1996). Gene transfer and manipulation in the thermophilic cyanobacterium Synechococcus elongatus. Mol. Gen. Genet. 252, 43–100. doi: 10.1007/BF02172309
Murphy, R. C., Gasparich, G. E., Bryant, D. A., and Porter, R. D. (1990). Nucleotide-sequence and further characterization of the Synechocystis Strain PCC 7002 recA gene - complementation of a cyanobacterial RecA mutation by the Escherichia coli recA gene. J. Bacteriol. 172, 967–976.
Nakasugi, K., Svenson, C. J., and Neilan, B. A. (2006). The competence gene, comf, from Synechocystis sp strain PCC 6803 is involved in natural transformation, phototactic motility and piliation. Microbiology 152, 3623–3631. doi: 10.1099/mic.0.29189-9

Narainsamy, K., Farci, S., Braun, E., Junot, C., Cassier-Chauvat, C., and Chauvat, F. (2016). Oxidative-stress detoxification and signalling in cyanobacteria: the crucial glutathione synthesis pathway supports the production of ergothioneine and ophthalmate. Mol. Microbiol. 100, 15–24. doi: 10.1111/mmi.13296

Narainsamy, K., Marteyn, B., Sakr, S., Cassier-Chauvat, C., and Chauvat, F. (2013). “Genomics of the pleiotropic glutathione system in cyanobacteria,” in Genomics of Cyanobacteria, eds F. Chauvat and C. Cassier-Chauvat (Amsterdam: Academic Press, Elsevier), 157–188.

Okamoto, S., Ikeuchi, M., and Ohmori, M. (1999). Experimental analysis of Prochlorococcus strain MIT9313: green fluorescent protein expression from an RSF1010 plasmid and Tn5 transposition. Appl. Environ. Microbiol. 72, 7607–7617. doi: 10.1128/AEM.02034-06

Trautmann, D., Voss, B., Wilde, A., Al-Babili, S., and Hess, W. R. (2012). Microevolution in cyanobacteria: re-sequencing a motile strain of Synechocystis sp. PCC 6803. DNA Res. 19, 435–448. doi: 10.1093/dnares/dss024

Watanabe, S., Ohbayashi, R., Kanesaki, Y., Saito, N., Chibazakura, T., Soga, T., et al. (2013). Intensive DNA Replication and Metabolism during the Lag Phase in Cyanobacteria. PLoS ONE 9:e89372. doi: 10.1371/journal.pone.0089372

Wendt, K. E., Ungerer, J., Cobb, R. E., Zhao, H., and Pakrasi, H. B. (2016). CRISPR/Cas9 mediated targeted mutagenesis of the fast growing cyanobacterium Synechococcus elongatus PCC 7942. Mol. Microbiol. 100, 15–24. doi: 10.1111/mmi.13296

Williams, P. G. (2009). Panning for chemical gold: marine bacteria as a source of new therapeutics. Trends Biotechnol. 27, 45–52. doi: 10.1016/j.tibtech.2008.10.003

Yoshihara, S., Geng, X. X., Okamoto, S., Yura, K., Murata, T., Go, M., et al. (2009). Ecological genomics of marine picocyanobacteria. Microbiol Mol. Biol. Rev. 73, 249–+. doi: 10.1128/MMBR.00035-08

Chopf, J. W. (2011). The paleobiological record of photosynthesis. Photosyn. Res. 107, 87–101. doi: 10.1007/s11120-010-9577-1

Shih, P. M., Wu, D. Y., Latifi, A., Axen, S. D., Fewer, D. P., Talla, E., et al. (2013). Improving the coverage of the cyanobacterial phytoplankton using diversity-driven genome sequencing. Proc. Natl. Acad. Sci. U.S.A. 110, 1053–1058. doi: 10.1073/pnas.1217071110

Singh, J. S., Kumar, A., Rai, A. N., and Singh, D. P. (2016). Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front. Microbiol. 7:529. doi: 10.3389/fmicb.2016.00529

Stevens, S. E., and Porter, R. D. (1980). Transformation in Agmenellum quadruplicatum. Proc. Natl. Acad. Sci. U.S.A. 77, 6052–6056. doi: 10.1073/pnas.77.10.6052

Sun, Z., and Blanchard, J. L. (2014). Strong genome-wide selection early in the evolution of Prochlorococcus resulted in a reduced genome through the loss of a large number of small effect genes. PLoS ONE 9:e88837. doi: 10.1371/journal.pone.0088837

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.