RESEARCH LETTER

Ploidy in cyanobacteria

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Abstract

A recently developed real-time PCR method for the determination of genome copy numbers was optimized for the application to cyanobacteria. Three species were chosen to represent a fresh water species, a salt water species, and two strains of a widely used laboratory species. *Synechococcus* PCC 7942 and *Synechococcus* WH7803 were found to contain 3–4 genome copies per cell and are thus oligoploid, confirming earlier publications. In contrast, *Synechocystis* PCC 6803 is highly polyploid. The motile wild-type strain contains 218 genome copies in exponential phase and 58 genome copies in linear and in stationary growth phase. The GT wild-type strain contains 142 genome copies in exponential phase and 42 genome copies in linear and stationary growth phase. These are the highest numbers found for any cyanobacterial species. Notably these values are much higher than the value of 12 genome copies published for the 'Kazusa' strain more than 20 years ago. The results reveal that for *Synechocystis* PCC 6803 strain differences exist and that the ploidy level is highly growth phase-regulated. A compilation of the ploidy levels of all investigated cyanobacterial species gives an overview of the genome copy number distribution and shows that monoploid, oligoploid, and polyploid cyanobacteria exist.

Introduction

Many eukaryotic species including ciliates, fish, flowering plants, and even some cell types of humans are polyploid, and advantages as well as disadvantages of polyploidy have been discussed in various reviews (e.g. Wendel, 2000; Osborn *et al.*, 2003; Comai, 2005; Thorpe *et al.*, 2007; Hegarty & Hiscock, 2008). In contrast, it is generally believed that prokaryotes typically contain a single copy of their chromosome. This is usually called 'haploidy', but as the term 'haploid' does not seem to make much sense in species without a diploid stage, the term 'monoploid' will be used throughout this contribution. The idea that prokaryotes are typically monoploid is a generalization from the results obtained with *Escherichia coli*, the best studied bacterium. *Escherichia coli* is monoploid when it is grown very slowly, e.g. with a doubling time of 16 h (Skarstad *et al.*, 1983). When the doubling time becomes shorter than the time to replicate and segregate the chromosome, *E. coli* starts a new round of replication before the previous round had been terminated, and thus the gene dosage of regions near the replication origin becomes higher than of regions near the terminus. This unequal gene dosage is called merodiploidy or merooligoploidy. Under optimal conditions, *E. coli* grows with a doubling time of 20 min and contains on average 6.8 origins and nearly two termini (Bremer & Dennis, 1996; Pecoraro *et al.*, 2011). The dependence of DNA content and growth rate shows that *E. coli* 'tries' to grow as monoploid as possible. Several other species of bacteria are truely monoploid, e.g. *Bacillus subtilis*, *Caulobacter crescentus*, and *Wolinella succinogenes* (Webb *et al.*, 1998; Pecoraro *et al.*, 2011).

However, several species of prokaryotes also have been described to be oligoploid or polyploid. A prominent example is *Deinococcus radiodurans*, which contains 5–8 genome copies (Hansen, 1978). It is long known that *D. radiodurans* can restore intact chromosomes from heavily fragmented chromosomes, which is not possible in monoploid species. Recently, it has been shown that this is a two-stage mechanism involving a high induction of DNA repair synthesis followed by recombination (Slade *et al.*, 2009). The efficient repair of a high number of double strand breaks (induced by irradiation or, more naturally, desiccation) is one evolutionary advantage of polyploidy for prokaryotes. Additional advantages include

gene redundancy, a low rate of spontaneous mutations, global regulation of gene expression, cell size increase, or the possibility of rapid cell divisions of pathogens after entering a host. Therefore, it might not be surprising that the number of known oligoploid and polyploid prokaryotic species outnumbers the monoploid species and it seems that monoploidy is not typical for prokaryotes, in contrast to the general belief. Polyploid, oligoploid, and monoploid species can co-exist within one group of prokaryotes, an example is the gamma-proteobacteria (Pecoraro *et al.*, 2011), whereas other groups like the euryarchaeota seem to be devoid of monoploid species (Hildenbrand *et al.*, 2011).

Therefore, we found it interesting to clarify the situation for another group of prokaryotes, the cyanobacteria. It has been described more than 30 years ago that *Anabaena cylindrica* and *Anabaena variabilis* are polyploid and contain 25 and 8–9 genome copies, respectively (Simon, 1977, 1980). In contrast, other species like *Synechococcus* WH8101 were shown to be monoploid (Armbrust *et al.*, 1989). Three species of cyanobacteria, representing a salt water species, a fresh water species, and a widely used laboratory species, were selected, and their genome copy numbers were quantified. A fast, sensitive, and precise real time PCR method was used that had originally been established for genome copy number quantification of haloarchaea (Breuert *et al.*, 2006), but has recently been applied successfuly to proteobacteria (Pecoraro *et al.*, 2011). In addition, a literature survey was performed and an overview of all cyanobacterial species with experimentally determined ploidy levels is given.

Materials and methods

Cyanobacterial strains, media and growth conditions

Two *Synechocystis* PCC 6803 wild-type strains were obtained from Annegret Wilde (University of Giessen, Germany). *Synechocystis* PCC6803 was isolated in 1968 by R. Kunisawa from a freshwater lake in California (Stanier *et al.*, 1971) and deposited at the Pasteur Culture Collection (PCC6803) and the American Type Culture Collection (ATCC 27184). Several variants arose and are currently under investigation. One strain will be called 'motile strain' (originally obtained from the lab of Sergey Shestakov, Moscow State University, in the cyanobacteria community known as 'Moscow strain'), the other will be called 'GT strain' (glusose tolerant; originally obtained from the lab of Martin Tichy, Trebon; in the cyanobacteria community known as 'Vermaas strain'). The genome sequence was determined from a third strain not used in this study. It was derived from the GT strain, and is known in the cyanobacteria community as the 'Kazusa strain' (Okamoto *et al.*, 1999).

Synechococcus elongatus PCC 7942 and *Synechococcus* sp. WH7803 were obtained from Wolfgang R. Hess (University of Freiburg, Germany). *Synechocystis* and *S. elongatus* were grown in BG11 medium (Rippka *et al.*, 1979). The marine *Synechococcus* sp. WH7803 was grown in artificial sea water (ASW; Waterbury & Wiley, 1988). All strains were grown at a temperature of 28 °C on a rotary shaker (120 r.p.m.) to prevent aggregation. They were grown phototrophically at a fluence rate of 10–60 photons m^{-2} s^{-1} (Osram daylight lamp LUMILUX de Lux L18W/954; Osram, Munich, Germany) with constant illumination. Growth was monitored spectroscopically at 750 nm.

Cell harvest, cell disruption and control of DNA integrity

Cultures for genome copy number determination were inoculated from precultures in the linear growth phase and grown to the respective optical densities (see text and tables). At the times of harvest, the cultures were checked microscopically to detect possible aggregation, which was not observed, and to determine cell densities using a Neubauer counting chamber. The cells of 40 mL culture were harvested by centrifugation (3200 ***g***, 30 min, room temperature). The supernatant was checked microscopically to verify that it was free of cells. The pellet was suspended in 2 mL distilled water. The cell density was determined microscopically using a Neubauer counting chamber. 0.5 mL of the cell suspension was mixed with either 0.75 g (*Synechocystis* PCC 6803) or 1 g (*S. elongatus* PCC 7942 and *Synechococcus* sp. WH7803) zirconia/silica beads (0.1 mm; Roth, Karlsruhe, Germany) in a 2 mL screw cup (Sarstedt, Nümbrecht, Germany). Cells were disrupted by shaking for 1.5 min (*Synechocystis* PCC 6803) or 2 min (*S. elongatus* PCC 7942 and *Synechococcus* sp. WH7803) in a Speedmill P12 (Analytik Jena, Jena, Germany). The cell density was determined again, and the values before and after cell disruption were used to calculate the efficiency of cell disruption. Cell debris was removed by centrifugation (15 000 ***g***, 20 min room temperature). 0.3 mL of the supernatant was used as cytoplasmic extract for further analysis. The integrity of genomic DNA was checked using analytical agarose electrophoresis. The extract was dialyzed against distilled water, and volumes prior and after dialysis were used to calculate the dilution.

Real time PCR method for ploidy determination

To determine genome copy numbers, a real time PCR approach was applied (Breuert *et al.*, 2006; an overview is

given in Figure 1 of Pecoraro *et al.*, 2011). For each species, a fragment of about 1 kbp was amplified using standard PCRs with isolated genomic DNA as template. The primers are summarized in Table S1 (Supporting Information). The fragments were purified using preparative agarose gel electrophoresis and the AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The DNA mass concentrations were determined photometrically, and the concentrations of DNA molecules were calculated using the molecular weights computed with 'oligo calc' (www.basic.northwestern.edu/biotools). For each standard fragment, a dilution series was generated and used for real time PCR analysis in parallel with the dilution series of the respective cell extract. The 'analysis fragments' were 300–400 bp, and exact sizes and primers are summarized in Table S1. The real time PCR analyses were performed as previously described (Breuert *et al.*, 2006), but without glycerol addition. A negative control (no template control) was also included. By comparison of the C_t differences of the different dilutions, it was verified that the PCR was exponential at least up to the threshold DNA concentration used for the analysis (i.e. a 10-fold dilution corresponds to a C_t difference of about 3.32). The size of the analysis product and the absence of other products were verified using analytical agarose gel electrophoresis. A standard curve was generated and used to calculate the genome copy numbers present in the dilutions of the cell extract. Together with the known cell densities (see above), this number was used to calculate the genome copy number per cell. At least three independent experiments (biologic replicates) were performed for each species, and average values and standard deviations were calculated.

Spectroscopic method for ploidy determination

Dialyzed cytoplasmic extracts of *Synechocystis* PCC6803 (see above) were used to record spectra from 220 to 340 nm. The spectra had the typical shapes of nucleic acids spectra and E_{260}/E_{280} quotients typical for pure nucleic acids. The cell densities (see above) and the absorption at 260 nm were used to calculate the genome copy numbers per cell using the following parameters: absorption of one equals a DNA concentration of 50 μg mL^{-1}, the average molecular mass of one base pair is 660 g mol^{-1}, and the Avogadro number. The best value for the genome size is less clear, the chromosome size is 3.57 Mbp, and the genome size including plasmids is 3.96 Mbp. The plasmid copy number is unknown and e.g. in *Halobacterium salinarum,* two plasmids have a copy number of five, whereas the genome has a copy number of 25 (Breuert *et al.*, 2006). To take the unknown plasmid copy numbers into account, genome sizes of 3.96 Mbp (high plasmid copy number) and 3.65 Mbp (low plasmid copy number) were used to calculate the ploidy level of the chromosome.

It should be noted that in highly polyploid species, the absorbance of RNA is much lower than that of genomic DNA and can be neglected. A short calculation should demonstrate this point: *E. coli* cells growing with a doubling time of 100 min. contain about 7000 ribosomes per cell (Bremer & Dennis, 1996). If the same number is assumed for *Synechocystis* with a much longer doubling time, the cells would contain 3.2×10^7 nt ribosomal RNA, which makes up nearly 90% of cellular RNA. Fifty copies of a genome of 3.6 Mbp are equal to 3.6×10^8 nt. Therefore, under these conditions, DNA outnumbers RNA by more than a factor of 10.

Results and discussion

Optimization of the real time PCR method

The real time PCR method for the quantification of genome copy numbers had been established for haloarchaea (Breuert *et al.*, 2006), but, in the meantime, was also applied to methanogenic archaea and proteobacteria (Hildenbrand *et al.*, 2011; Pecoraro *et al.*, 2011). It has been validated against several independent methods, i.e. quantitative Southern blotting (Breuert *et al.*, 2006), DNA isolation, and spectroscopic quantification (Hildenbrand *et al.*, 2011), and the wealth of results published for *E. coli*, which was obtained by radioactive labeling and Fluorescence Activated Cell Sorter (FACS) analyses (Pecoraro *et al.*, 2011). In each case, the results of the real time PCR method were in excellent agreement with the respective independent method.

To give a short overview, genomic DNA was used as a template in a conventional PCR reaction to amplify a fragment of about 1 kbp. A dilution series of this fragment was prepared and used for real time PCR analysis. A fragment of about 300 bp, internal to the standard fragment, was amplified. The results were used to generate a standard curve. To determine the genome copy number, cells were lysed and a dilution series of the resulting cell extract was analyzed using real time PCR in parallel to the standards. The results allowed calculating the number of genome copies in the cell extract and, in combination with the cell density of the culture, the ploidy level.

The following points have to be optimized for every new species under investigation and were optimized for the three species of cyanobacteria used in this study: (1) the cell density has to be quantified with a very low variance, (2) it has to be verified that culture growth is highly reproducible, (3) the method of cell disruption has to be about 100% effective yet leaving the genomic

DNA intact, and (4) the real time PCR has to be truly exponential.

For cyanobacteria, the method for cell disruption turned out to be the most critical point. Several standard methods (sonification, enzymatic murein digestion, 'normal shaking' with glass beads) could not be used, either because the efficiency of cell lysis was too low or because damage of the genomic DNA was too high. Shaking the cells in a Speedmill with 0.1 mm glass beads led to satisfactory results, lysis efficiency was higher than 90%, and the genomic DNA was only slightly damaged (fragment sizes from 4 kbp to >20 kbp, data not shown). The amount of beads and shaking time were optimized for every species. To exemplify the results, Fig. S1 (Supporting Information) shows one typical example of a real time PCR analysis (Fig. S1a), a standard curve (Fig. S1b), a melting point analysis, and an analytical agarose gel of the analysis fragments (Fig. S1c, d). At least three independent cultures were analyzed (and each culture was analyzed at least in triplicates), and average values and standard deviations (SD) were calculated.

Synechococcus elongatus PCC 7942 and *Synechococcus* WH7803 are oligoploid

Synechococcus elongatus PCC 7942 grew with a doubling time of 24 h. An average growth curve of three cultures is shown in Fig. S2. The results of genome quantification of three independent cultures are summarized in Table 1. At an $OD_{750\,nm}$ of 0.6, *S. elongatus* contained about four genome copies per cell and thus the species is oligoploid. This is termed 'exponential phase', although growth of the cultures was not truly exponential, but the $OD_{750\,nm}$ of 0.6 was prior to the onset of the linear growth phase (compare Fig. S2). This value is in accordance with the previously published value of 3–5 genome copies that was obtained using FACS analysis (Mori *et al.*, 1996). Stationary phase cells also contained 3–4 genomes per cell. Therefore, in *S. elongatus*, the ploidy level is not growth phase-regulated, in contrast to many other species.

The results of genome quantification for *Synechococcus* WH7803 are also summarized in Table 1. This species also contained between three and four genome copies at an $OD_{750\,nm}$ of 0.6 and during stationary phase, and is thus oligoploid. Again, this is in accordance with an earlier study that applied FACS analysis for genome copy number determination and found 2–4 copies per cell (Binder & Chisholm, 1990). Taken together, the freshwater as well as the salt water species were found to be oligoploid, irrespective of the applied method for quantification (based either on one specific site of the genome (this study) or the average DNA content), growth in continuous light (this study) or growth in light–dark cycles (Mori *et al.*, 1996), and the growth phase.

Synchocystis PCC 6803 is highly polyploid

First, the motile *Synechocystic* PCC 6803 wild-type strain was analyzed. An average growth curve of three independent cultures is shown in Fig. S3. The results of genome copy number determination are summarized in Table 2. The doubling time at the cell harvest in linear growth phase ($OD_{750\,nm}$ = 0.6) was around 20 h. *Synechocystis* PCC 6803 turned out to be highly polyploid, and it contained nearly 60 genomes per cell, both in linear and in stationary growth phase. As this value is very high and in fact higher than any value published until now for any cyanobacterial species, the genome copy number in stationary phase cells was also determined using an independent method, namely spectroscopic determination of the DNA concentration. The average values of 57.9 (if the plasmid copy number would be low) and 53.3 (if the plasmid copy number would be high) genomes per cell were in excellent agreement with the real time PCR result, and thus underscored that *Synechocystis* PCC 6803 is highly polyploid.

An earlier study had also shown that this species is polyploid, but the reported value of 12 genome copies per cell for the 'Kazusa' wild-type of *Synechocystis* PCC 6803 (Labarre *et al.*, 1989) is much lower than the value

Table 1. Genome copy numbers of *Synechococcus elongatus* PCC 7942 and *Synechococcus* sp. WH 7803

		Exponential phase		Stationary phase	
Culture	$OD_{750\,nm}$	Genome copies	Average ± SD	Genome copies	Average ± SD
S. elongatus PCC 7942					
1	0.6	4.2		3.1	
2	0.6	3.6	4.0 ± 0.3	3.5	4.0 ± 1.3
3	0.6	4.2		5.5	
Synechococcus sp. WH 7803					
1	0.6	2.6			
2	0.6	4.3	3.6 ± 0.9		
3	0.6	3.8			

Table 2. Genome copy numbers of two *Synechocystis* PCC 6803 strains

	Exponential phase			Linear phase			Stationary phase	
No.	$OD_{750\,nm}$	Genome copies	Average ± SD	$OD_{750\,nm}$	Genome copies	Average ± SD	Genome copies	Average ± SD
Motile wild-type strain								
1	0.1	210.3		0.6	66.1		52.9	
2	0.1	218.3	218.0 ± 7.6	0.6	46.7	58.0 ± 10.1	61.0	57.6 ± 4.2
3	0.1	225.5		0.6	61.2		58.9	
GT wild-type strain								
1	0.1	144.5		0.6	41.1		42.1	
2	0.1	136.1	142.2 ± 5.3	0.6	46.3	47.2 ± 6.6	45.2	43.3 ± 2.8
3	0.1	146.0		0.6	54.1		39.7	

determined in this study. The reason for the discrepancy is not obvious, as in the previous study also the lysis efficiency was quantified, genome size was underestimated by only 32%, and the colorimetric assay for DNA quantification probably cannot be that wrong. The same medium was used, and a similar doubling time of 15–20 h was reported. Therefore, it might be that both reports are correct and that the ploidy level of various strains of the species *Synechocystis* PCC 6803 are different.

To test this hypothesis, another wild-type strain of *Synechocystis* PCC 6803 was used, i.e. the so-called GT wild-type. This strain was chosen because genotypes segregate much faster in the GT strain than in the motile strain in the course of chromosomal mutant construction (Annegret Wilde, personal communication). Average growth curves of three independent cultures are shown in Fig. S4, and again, cells in linear growth phase and in stationary phase were analyzed. The results are also shown in Table 2. The GT wild-type was also highly polyploid; however, the genome copy number was with 42 genome copies nearly 30% lower than that of the motile wild-type, verifying that different strains of PCC 6803 vary in their ploidy level.

Notably, the 12 genome copies reported for the 'Kazusa' strain (Labarre *et al.*, 1989) are much lower compared with the 42 and 58 genome copies of the two other wild-type strains analyzed in this study. Three explanations appear possible: (1) the 'Kazusa' strain highly deviates from the other two strains, (2) the genome copy number changed during the last 20 years of cultivation in the laboratory and today the ploidy level of the 'Kazusa' strain is higher than in 1989, (3) strains cultivated for long times under identical names in different laboratories accumulated different mutations, including mutations that affect the ploidy level, and thus 'identical' strains have different ploidy levels in different laboratories.

The species *Synechocystis* PCC 6803 was isolated from freshwater in California more than 40 years ago (Stanier *et al.*, 1971). Several mutations are known that occurred during its further 'evolution in the laboratory'. The sequenced 'Kazusa' strain contains insertion elements at four places of the genome that were devoid of an insertion element in the original isolate (Okamoto *et al.*, 1999). In addition, the sequenced 'Kazusa' strain contains a frameshift mutation in the gene encoding a protein kinase that is not present in other strains (Kamei *et al.*, 2001). It will be interesting to unravel how different strains differ in their ploidy level. An in-depth analysis including several samples of each of the three wild-type strains obtained from different laboratories around the world will be needed to clarify the situation. In any case, all *Synechocystis* PCC 6803 strains analyzed until now are polyploid, and we could show that the ploidy levels of different strains vary. For experiments that are sensitive to the ploidy level, this should be taken into account and the ploidy level of the strain under investigation should be quantified.

Anonymous reviewers of the first version of this article pointed out that we only analyzed the linear and the stationary growth phase, and that an analysis of exponentially growing cells would also be desirable. Therefore, again three independent cultures of both strains were grown and were harvested during exponential growth at an $OD_{750\,nm}$ of 0.1. The results are included in Table 3. Surprisingly, it turned out that the GT wild-type contained 142 genome copies per cells and the motile wild-type contained 218 genome copies per cell, much higher values than in linear and stationary growth phase. Several bacterial and archaeal species exhibit growth phase-dependent regulation of their ploidy levels and have higher genome copy numbers in exponential phase than in stationary phase (Hildenbrand *et al.*, 2011; Pecoraro *et al.*, 2011). *Synechocystis* PCC 6803 adds to this list, but is extraordinary in that the genome copy number is already down-regulated in linear growth phase.

The genome copy numbers of 218 and 142 in exponentially growing cells of the two *Synechocystis* strains are considerably higher than the 120 genome copies per cell that have been reported for *Buchnera*, a symbiotic bacterium with a reduced genome size (Komaki & Ishik-

Table 3. Overview of cyanobacterial species with experimentally determined ploidy levels and selected parameters

Species	Growth temperature (°C)	Doubling time (h)	Genome size (Mbp)	Average genome copy No.	Ploidy	References
Anabaena cylindrica	30	18.5	–	25	Polyploid	Simon (1977)
Anabaena variabilis	30	–	7.1	5–8	Oligoploid	Simon (1980)
10 *Microcystis* strains	20	stat.ph.	–	1–10	Oligoploid	Kurmayer & Kutzenberger (2003)
Anabaena sp. PCC 7120	28	–	7.2	8.2	Oligoploid	Hu *et al.* (2007)
Prochlorococcus	–	–	1.7	–	Monoploid	Vaulot *et al.* (1995)
Synechococcus elongatus PCC 7942	28	24	2.8	3.9/3.3*	Oligoploid	This study
S. elongatus PCC 7942	30	11, LDC	2.8	3–5	Oligoploid	Mori *et al.* (1996)
Synechococcus sp. PCC 6301	38	5 to >50	2.7	2–6 to >1–2†	Oligoploid	Binder & Chisholm (1990)
Synechococcus sp. WH 7803	28	–	2.4	3.6	Oligoploid	This study
Synechococcus sp. WH 7803	25	–	2.4	2–4	Oligoploid	Binder & Chisholm (1995)
Synechococcus sp. WH 7805	25	15	2.6‡	1	Monoploid	Binder & Chisholm (1995)
Synechococcus sp. WH 8101	25	17	3.2‡	1	Monoploid	Armbrust *et al.* (1989)
Synechococcus sp. WH8103	25	22	2.7‡	1–2	Monoploid	Binder & Chisholm (1995)
Synechocystis sp. PCC 6803 (motile)	28	20	3.6	218/58/58*	Polyploid	This study
Synechocystis sp. PCC 6803 (GT)	28	20	3.6	142/47/43*	Polyploid	This study
Synechocystis sp. PCC 6803 ('Kazusa')	30	15–20	3.6	12	Polyploid	Labarre *et al.* (1989)

stat.ph., stationary phase; LDC, light–dark cycles.

*Exponential phase/linear phase/stationary phase.

†Longer doubling times correspond to lower genome copy numbers.

‡Calculated from relative fluorescence and genome size of WH7803.

awa, 1999). To our knowledge, a higher value has been reported only for *Epulopiscium* sp. that contains tens of thousands of genome copies (Mendell *et al.*, 2008). However, *Epulopiscium* sp. is a giant bacterium exhibiting cell lengths in excess of 600 μm. Therefore, *Synechocystis* PCC 6803 has the highest ploidy level of any 'normal' sized prokaryote. However, it is unclear whether such high ploidy levels also exist in natural habitats, or whether this is an artifact of decades of cultivation in the laboratory. *Synechocystis* PCC 6803 was isolated 40 years ago and has been cultivated in the laboratory since then (Stanier *et al.*, 1971). Therefore, an in-depth analysis (see above) should also include fresh isolates of *Synechocystis* PCC6803 as well as samples from different culture collections that had been kept frozen since their submission.

Ploidy in cyanobacteria

A literature search was performed to identify (hopefully) all cyanobacterial species with experimentally determined ploidy levels. Table 3 summarizes the results together with selected features. Three species are polyploid and contain at least 10 genome copies. They belong to different genera and grow either as single cells or as filaments. More than ten species are oligoploid and contain between three and nine genome copies. Again, among them are unicellular and filamentous species of several genera. Four species are monoploid, and thus monoploidy is not the rule, but an exception in cyanobacteria. The ploidy level is highly variable in cyanobacteria similar to the proteobacteria (Pecoraro *et al.*, 2011). One genus can harbor monoploid and oligoploid species (*Synechococcus*) or oligoploid and polyploid species (*Anabaena*). There is no obvious correlation between the number of genome copies and any of the listed features, i.e. genome size, growth temperature, and doubling time. Various evolutionary advantages of oligo- and polyploidy for prokaryotes exist. As has been extensively studied with *D. radiodurans*, one of the advantages is resistance against double strand breaks that can be induced by X-ray irradiation (an artificial situation) and desiccation (regularly occurring in natural habitats). In fact, it could be shown that the resistance of polyploid *Synechocystis* PCC 6803 against X-ray irradiation is much higher than that of the oligoploid *Synechococcus* PCC 7942 (Domain *et al.*, 2004). Another advantage is that the cells can live without a stringent control of equal chromosome segregation,

and for *Synechocystis* PCC 6803 and *Nostoc* sp. PCC 7120, it has indeed been shown that the amount of DNA in the two newborn daughter cells after cell division is not always identical, but can vary (Hu *et al.*, 2007; Schneider *et al.*, 2007). An additional advantage is gene redundancy, which opens the possibility that under unfavorable conditions, mutations are induced in some genome copies, whereas the wildtype information is retained in others. It has indeed been shown that heterozygous cells of *S. elongatus* PCC 7942 and of *Synechocystis* PCC 6803 can be selected, at least under laboratory conditions (Labarre *et al.*, 1989; Spence *et al.*, 2004; Takahama *et al.*, 2004; Nodop *et al.*, 2008). Heterozygous strains have also been selected of two halophilic and methanogenic archaea, *Haloferax volcanii* and *Methanococcus maripaludis*. In both cases, it was shown that in the absence of selection gene conversion leads to the equalization of genomes and reappearance of homozygous cells (Hildenbrand *et al.*, 2011; Lange *et al.*, 2011). By analogy, we predict that gene conversion also operates in oligo- and polyploid species of cyanobacteria. The higher efficiency of gene replacement with linear DNA compared with circular DNA in *Synechocystis* PCC 6803 indicates that this is really the case (Labarre *et al.*, 1989).

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Selected results to exemplify the real time PCR method for genome copy number quantification.
Fig. S2. Average growth curve of *Synechococcus* PCC 7942.
Fig. S3. Average growth curve of motile *Synechocystis* PCC 6803.
Fig. S4. Average growth curve of the GT strain of *Synechocystis* PCC 6803.
Table S1. Oligonucleotides and PCR fragments.

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