ParA-like protein influences the distribution of multi-copy chromosomes in cyanobacterium Synechococcus elongatus PCC 7942

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Abstract

While many bacteria, such as Escherichia coli and Bacillus subtilis, harbour a single-copy chromosome, freshwater cyanobacteria have multiple copies of each chromosome per cell. Although it has been reported that multi-copy chromosomes are evenly distributed along the major axis of the cell in cyanobacterium Synechococcus elongatus PCC 7942, the distribution mechanism of these chromosomes remains unclear. In S. elongatus, the carboxysome, a metabolic microcompartment for carbon fixation that is distributed in a similar manner to the multi-copy chromosomes, is regulated by ParA-like protein (hereafter ParA). To elucidate the role of ParA in the distribution of multi-copy chromosomes, we constructed and analysed ParA disruptant and overexpressing strains of S. elongatus. Our fluorescence in situ hybridization assay revealed that the parA disruptants displayed an aberrant distribution of their multi-copy chromosomes. In the parA disruptant the multiple origin and terminus foci, corresponding to the intracellular position of each chromosomal region, were aggregated, which was compensated by the expression of exogenous ParA from other genomic loci. The parA disruptant is sensitive to UV-C compared to the WT strain. Additionally, giant cells appeared under ParA overexpression at the late stage of growth indicating that excess ParA indirectly inhibits cell division. Screening of the ParA-interacting proteins by yeast two-hybrid analysis revealed four candidates that are involved in DNA repair and cell membrane biogenesis. These results suggest that ParA is involved in the pleiotropic cellular functions with these proteins, while parA is dispensable for cell viability in S. elongatus.

INTRODUCTION

Cyanobacteria are prokaryotic micro-organisms; they manifest an oxygen-producing photosynthetic system similar to that of chloroplasts in higher plants. Several species of freshwater cyanobacteria harbour multiple genome copies per cell at all stages of their cell cycle [1–3]. This feature is in contrast to more traditionally studied bacteria, such as Escherichia coli and Bacillus subtilis, which typically contain only one or two complete chromosomal copies. The cyanobacterium Synechococcus elongatus PCC 7942 (hereafter referred to as S. elongatus), which has been used as a model organism for phototrophs, carries three plasmids. Our fluorescence in situ hybridization assay revealed that the parA disruptant the multiple origin and terminus foci are aggregated, which was compensated by the expression of exogenous ParA from other genomic loci. The parA disruptant is sensitive to UV-C compared to the WT strain. Additionally, giant cells appeared under ParA overexpression at the late stage of growth indicating that excess ParA indirectly inhibits cell division. Screening of the ParA-interacting proteins by yeast two-hybrid analysis revealed four candidates that are involved in DNA repair and cell membrane biogenesis. These results suggest that ParA is involved in the pleiotropic cellular functions with these proteins, while parA is dispensable for cell viability in S. elongatus.
Cyanobacterial cells contain carboxysomes, metabolic protein microcompartments for carbon fixation, which contain the majority of a cell’s ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) [20]. In the majority of cyanobacteria, carboxysomes are present in protein microcompartments for carbon fixation, which contain the ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) [20]. In S. elongatus, the carboxysome is similar in structure to that of the multi-copy chromosomes [21]. The mutation of chromosomal ParA and MreB, associating cytoskeletal proteins, specifically disrupts carboxysome ordering, indicating that ParA and MreB are involved in cellular compartmentalization in S. elongatus [18]. These observations leave open the possibility the carboxysomes interact with chromosomes and that ParA also participates in the direction of multi-copy chromosome localization. A similar study using FROS showed that deletion of parA does not affect the spacing of chromosomal DNA in S. elongatus [8]. Although FROS enables live-cell imaging, it requires more than 100 copies of tandem repeat sequences on each chromosome for labelling with fluorescent proteins, which could perturb DNA replication and subsequent cell proliferation processes [22]. In addition, in S. elongatus harbouring multi-copy chromosomes, the genetic construct used for FROS is unstable; cells stored for longer periods of time often lost tandem repeats [8]. Thus, it is necessary to examine chromosomal distribution in S. elongatus on simple genetic backgrounds.

To elucidate the role of ParA in the distribution of multi-copy chromosomes in S. elongatus, we established fluorescence in situ hybridization (FISH) microscopy and analysed the disruptant of ParA in S. elongatus. The parA disruptant showed aberrant chromosome distribution and a UV-C-sensitive phenotype, which were compensated by the expression of exogenous ParA from a genomic neutral site. Additionally, overexpression of parA showed severe inhibition of cell division at late growth stage, suggesting that a large excess of ParA indirectly perturb the regulation of cell division. Screening of the ParA-interacting proteins by yeast two-hybrid analysis revealed four candidates that are involved in DNA repair and cell membrane biogenesis. Our data provide new knowledge, not only on a function of ParA, but also for a distribution mechanism of multi-copy chromosomes in S. elongatus.

**METHODS**

**Culture conditions**

S. elongatus strain mutants were derived from laboratory WT and contained an approximately 50 kb deletion (711 254 to 759 931) in the genome [6]. The ΔparA strains were also established in other laboratory strains carrying a complete genome, which were obtained from Professor Kondo’s laboratory at Nagoya University (NU) and a commercial vendor (LT; Life Technologies, Gaithersburg, MD, USA). As the standard condition, the WT and its derivatives were grown phototrophically at 30°C under continuous illumination (40 μE m⁻² s⁻¹) in BG-11 medium with 2% bubbling CO₂. When necessary, kanamycin, chloramphenicol, and spectinomycin were added to the media at final concentrations of 10, 7.5 and 40 μg ml⁻¹, respectively.

**Construction of the disruptants**

The parA disruptant was constructed as follows. The 0.6 kb upstream (primers PA-1 and PA-2, Table S1) and down-stream flanking regions (primers PA-5 and PA-6) of parA and the kanamycin-resistance gene cassette (primers PA-3 and PA-4) were amplified by PCR using S. elongatus chromosomal DNA and pUC4K (Pharmacia) as templates. Next, the fragments were recombined by recombinant PCR using primers PA-1 and PA-6, and the resulting fragment was used to transform the WT strain into a kanamycin-resistant strain. The gene construct is shown in Fig. S2. Disruption of the target genes was confirmed by PCR (Fig. S3a, b). Following the same procedure we obtained the mreB disruptants; the primer sets were mreB: MB-1–6 (Table S1).

**Construction of the strain expressing ParA at a neutral site**

To express haemagglutinin (HA)-tagged parA in an S. elongatus chromosomal neutral site (Synpcc7942_2498 gene locus), the parA fragment of S. elongatus was amplified by PCR from genomic DNA using the primer sets PA-Bam-F and PA-Sac-R (Table S1). After digestion with restriction enzymes, the fragment was cloned into pNSHA [6] digested with BamHI/Sall in order to fuse the HA-tag with the N-terminal portion of parA. The resulting plasmid was used for transformation of the WT and ΔparA strains to spectinomycin-resistant strains. These strains, named NSparA and ΔparA-NSparA (Fig. S2), were used to analyse the overexpression (Fig. 5) or complementation of ParA (Fig. 2).

**Construction of the strain expressing HA-tagged ParA at an intact chromosomal site**

We also constructed another strain expressing C-terminal HA-tagged ParA from an intact chromosomal site of parA (Fig. S2). This was amplified by PCR from genomic DNA using the primer sets PA-Eco-F and PA-Sac-R (Table S1), and the fragment was cloned into pNSHA digested with EcoRI/Sall (pNSparA1HA). The C-terminal HA-tagged parA (primers PA-Eco-F and KM-HA-R, Table S1), its downstream flanking regions (primers PA-5 and PA-6) and the kanamycin-resistance gene cassette (primers HA-Km-F and PA-4) were amplified by PCR using pNSparA1HA, pUC4K and S. elongatus chromosomal DNA, respectively, as templates. The fragments were recombined by PCR using primers PA-Eco-F and PA-6, and the resulting fragment was used to transform the WT strain into a kanamycin-resistant
strains. PCR and Sanger sequencing showed that parA tagged with HA and the kanamycin-resistant gene were correctly introduced into the intact chromosomal site.

**Probe preparation for FISH**

Three DNA fragments encompassing the sequences surrounding the oriC (primers Ori1-F and Ori1-R, Ori2-F and Ori2-R, and Ori3-F and Ori3-R) and three fragments around the putative terC region (primers Ter1-F and Ter1-R, Ter2-F and Ter2-R, and Ter3-F and Ter3-R) were amplified by PCR and used to make probe templates. Fluorescence-12-dUTP (Roche Diagnostics) was used as a labelling substrate and was incorporated into the template DNA fragments using a random primed DNA labelling kit (Roche). After removal of any remaining non-incorporated substrate by ethanol precipitation, the labelled probe DNA (oriC region: total 13 694 bp; terC region: total 13 566 bp) was dissolved in hybridization solution (50% formamide, 2×SSC, and 100 µg ml⁻¹ salmon sperm DNA). The probe DNA in the hybridization mixture was sonicated for 3 min using a Covaris S-2 sonicator (Covaris, Woburn, MA, USA). Prior to hybridization, it was denatured by heating to 100 °C for 10 min and then used to perform FISH.

**FISH**

*S. elongatus* and its derivatives were incubated on BG-11 plates for 1 week and diluted to OD

\[\text{OD}_{570} = 0.1\]

with fresh BG-11 liquid medium. In the parA complemented strain, ΔparA-NS1AparA, 1 µM IPTG was added to the medium for the expression of exogenous ParA. In the case of the spot test, exponentially growing cells (OD

\[\text{OD}_{750} = 0.5\]

) were diluted and spotted on BG-11 plates and then irradiated with or without UV-C (254 nm) using UV lamps (UVP, Upland, CA, USA) at dose rates of 150 and 300 J m⁻². In order to evaluate the survival rate against UV-C irradiation, the colony formation ratio was compared. A total of 20 ml of exponential culture was transferred to a plastic dish and irradiated with UV-C at dose rates of 200 and 400 J m⁻². Cells were harvested and spread on solid medium before and after the UV irradiation. The plates were then kept overnight in the dark at 30 °C to prevent photoreactivation, unless mentioned otherwise. After the dark incubation, plates were transferred to light conditions for 4 days.

**Yeast two-hybrid screening and the specificity test**

Yeast two-hybrid analysis for the library screening was carried out according to the method described previously, with minor modifications [23]. Full-length (primers PA-Eco-F and PA-Sal-R, Table S1), the N-terminal (primers PA-Eco-F and PA-143-Bam-R) or the C-terminal (primers PA-144-Eco-F and PA-Sal-R) region of parA was PCR-amplified and cloned into pGBT, a GAL4 DNA-binding domain fusion vector [24]. The plasmids were transformed into yeast P159-4Aa derivatives with haploid strains, using TRP1 as the selective marker, and used for the library screening. Positive protein interactions between the bait and prey were detected by the ability of the cells to grow on SC-LWH plates [synthetic complete (SC) plates lacking Leu, Trp, and His] supplemented with 1 mM 3-aminotriazole. The pGAD derivatives were extracted from the positive colonies and the insert fragments were sequenced. The specificity test was carried out as described previously [23]. Bait–prey pairs for the specificity test were spotted onto the selection plates and incubated for 5 days.

**RESULTS**

**Localization of multi-copy chromosomes in S. elongatus cells**

To study the dispositional organization and distribution mechanism of multi-copy chromosomes in *S. elongatus*, we performed FISH analysis, which allows direct examination of chromosomal distribution with a simple genetic background. Exponentially growing cells were fixed and stained using a fluorescein-labelled DNA probe, covering the 13 kb region around the ori, and then observed by fluorescence microscopy. We observed the green fluorescent foci in the *S. elongatus* cells to be distributed along the major axis of the cell (Fig. 1, WT). The number of ori-foci was correlated with cell size (Fig. 1b, WT) and DNA content profile (Fig. 1c, d, WT). A similar pattern was observed when we used the ter probe, which covers the region opposite to ori in the *S. elongatus* chromosome (Fig. S4, WT). The number of ter-foci was comparable to that of ori-foci (Figs 1c and...
S4c), indicating that only a small number of the replication origins in the multi-copy chromosomes were undergoing DNA replication even in the exponential phase, as we reported previously [5, 6]. This is in contrast to E. coli and B. subtilis that contain one or two chromosomes.

**Characterization of parA and mreB in the distribution of multi-copy chromosomes**

To investigate the distribution mechanism of multi-copy chromosomes in *S. elongatus*, we focused on a chromosomal parA gene (Synpcc7942_1833), which encodes a homologue of orphan ParA in *S. elongatus*. Consistent with previous observations [8, 18], we were able to isolate the complete disruptant of chromosomal parA (Fig. S3a). While the growth of the ΔparA strain was similar to that of the WT strain (Fig. S3c, d), few remarkable ori-foci signals were observed in the ΔparA strain (Fig. 1a, ΔparA). We analysed the correlation between the number of ori-foci and DNA content in the ΔparA strain. The number of ori-foci in the ΔparA cells was significantly lower (one–two ori-foci per cell) than that of the WT strain (Fig. 1c). Nevertheless, the amount of DNA in the ΔparA strain was similar to that of the WT strain (Fig. 1d). The distribution of the ter-foci was similar to that of the ori-foci in the ΔparA strain (Fig. S4, ΔparA). These results indicate that the ori and ter regions in the ΔparA strain are aggregated in the cell.

We additionally analysed the positioning of the ori-foci in an mreB knockdown mutant (mreb^KD^) that has a reduced copy number of mreb genes in the multi-copy chromosomes (Fig. S3b). Sphere-shaped cells were observed in the culture of the mreb^KD^ strain, as reported in a previous study [18]. The ori-foci in the spherical mreb^KD^ cells were disorganized, suggesting that MreB is also involved in chromosomal positioning in *S. elongatus*.

**Complementation of parA in the ΔparA background**

To elucidate the effect of parA disruption, we tested the effects of complementation with parA. The HA-tagged

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**Fig. 1.** Distribution of the ori regions in *S. elongatus* WT, ΔparA and mreb^KD^ strains. (a) Cells grown on BG-11 plates for 1 week were cultured for 24 h under light conditions. Fixed cells were examined by FISH using ori probes that covered roughly 13 kb of each region. Merged images of FISH foci (FF) and intrinsic chlorophyll fluorescence (Chl) or DAPI-stained cells (DS) are shown. Scale bar: 2 µm. (b–d) Comparison between the number of ori-foci and DNA content in WT and disruptant cells. (b) Numbers of FISH foci derived from the ori probe by cell length. (c) Profiles of ori-foci. Ratios of the cell number containing each focus among the total 300 cells observed were shown. (d) DNA content profiles of the WT and mutant strains. Each disruptant culture was analysed by flow cytometry.
parA, placed under the control of the IPTG-inducible promoter, was introduced into a chromosomal neutral site in the WT and ΔparA strains. The resulting strains were named NS\textsubscript{HA}parA and ΔparA-NS\textsubscript{HA}parA, respectively (Fig. S2). In addition, we also generated a strain carrying a C-terminally HA-tagged parA at the native parA locus, named ParA\textsubscript{HA} (Fig. S2). Western blot analysis of the ParA\textsubscript{HA} strain showed an endogenous expression level of ParA. The level of ParA was transiently increased 1 day after the culture and decreased 3 days after the culture (Fig. 2a). When 1 µM IPTG was added to the ΔparA-NS\textsubscript{HA}parA culture, the expression level of exogenous ParA was similar to that of the ParA\textsubscript{HA} strain, although the ParA expression level was significantly higher in the presence of 10 µM IPTG (Fig. 2a). Next, we analysed the distribution of the ori-foci in ΔparA-NS\textsubscript{HA}parA cells in the culture containing 1 µM IPTG, and observed that the number of ori-foci was restored to a level similar to that of the WT (Fig. 2b–d). This suggests that the aggregation of multi-copy chromosomes is caused by the dysfunction of ParA.

**ParA-dependent chromosomal positioning in several laboratory strains**

It has been reported that parental strains with different genetic backgrounds occasionally cause a variation in experimental results [25, 26]. Actually, our laboratory strain lacks approximately 50 kb (711 254 to 759 931) in the genome [6], and we thus analysed the effects of parA disruption on various genetic backgrounds. ΔparA strains were established in other laboratory strains that were obtained from Professor Kondo’s laboratory at Nagoya University (NU) and a commercial vendor (LT; Life Technologies, Gaithersburg, MD, USA). Both ΔparA strains (NU- and LT-ΔparA) showed a distribution of ori-foci similar to that of our ΔparA strain (Fig. 3). A similar FISH pattern of ter-foci was also observed between our ΔparA and the NU-ΔparA strains (Fig. S4, NU-ΔparA). These observations suggest that ParA plays a
critical role in chromosomal positioning regardless of the genetic background.

**Decreased viability of the parA disruptant against UV-C treatments**

The functional significance of parA deficiency was investigated by assessing cell growth in parA disruptants, because loss of ParA function has a disadvantageous effect on survival in environmental conditions. Although we assessed growth and viability at high temperature and in high light stress conditions, significant differences were not observed (data not shown). However, after UV-C irradiation treatment, cell viability was notably lower in the ΔparA strain compared with WT and parA-complement cells (Fig. 4). These results suggest that the aggregation of ori and ter regions and/or abnormal distribution of carboxysomes in parA disruptants reduced their tolerance to UV-C stress. In contrast, no significant differences in viability after UV-C irradiation were observed in the mreB KD strain.

**Effect of ParA overexpression**

To explore other roles of ParA, we analysed the effect of ParA overexpression in *S. elongatus*. Western blot analysis of the NSHAparA strain using anti-HA antiserum showed a marked increase in the HA-tagged ParA protein level after induction with 1 mM IPTG (Fig. 5a). While there was no effect on cell mass (Fig. 5b), the increment of cell number was clearly inhibited 4 days after the addition of IPTG (Fig. 5c). Flow cytometry revealed that cell size and DNA content were significantly increased in the presence of IPTG (Fig. 5d–f). Spot tests on each day showed that the number of viable cells was decreased in the presence of IPTG (Fig. S5). These results indicate that excess ParA protein inhibits cell division during the late growth phase in *S. elongatus*.

**ParA-interacting proteins**

To reveal the functions of ParA further, we explored the ParA-interacting proteins by yeast two-hybrid screening using an *S. elongatus* genomic library [23]. Using the C-terminal domain of ParA (ParA-C, 144–252 aa) as bait for the screening, we succeeded in fishing many independent clones whereas no prey clones were obtained with the full-length or N-terminal domain as bait. After sequencing more than 70 positive clones, we carried out specificity tests (Fig. S6) and identified reliable candidates by obtaining...
multiple fragments, encoding different regions of the same proteins. These were Synpcc7942_1761, Synpcc7942_2009, Synpcc7942_2131 and Synpcc7942_2563 (ORF1761, ORF2009, ORF2131 and ORF2563 respectively), all encoding hypothetical proteins. The fragments of each protein obtained from the library are schematically represented

Fig. 3. Effects of parA disruption under different genetic backgrounds. As parental strains, S. elongatus obtained from Nagoya University (NU) or a commercial vendor (LT; Life Technologies) were used. (a) Distribution of ori-foci in ΔparA strains. Merged images of ori-foci and intrinsic chlorophyll fluorescence (left) or DAPI-stained cells (right) are shown. Scale bar: 2 µm. (b) Numbers of ori-foci by cell length. (c) Profiles of ori-foci. Ratios of the cell number containing each focus among the entire 300 cells observed were shown.

Fig. 4. Viability test after UV-C irradiation. (a) Cells grown on BG-11 plates for 1 week were cultured for 24 h under light conditions. After adjustment to OD750=0.5, each culture was diluted, spotted onto BG-11 plates and irradiated with UV-C (254 nm) at a dose rate of 150 or 300 J m⁻². The plates were then incubated overnight in the dark at 30 °C to prevent photoreactivation. (b) Quantification of cell viability after the UV-C irradiation. The cultures, incubated for 24 h, were transferred to a plastic dish and irradiated with UV-C at dose rates of 200 and 400 J m⁻². Cells were harvested and spread on a solid medium. Open circle: WT, closed circle: ΔparA strain, open square: ΔparA-NSHAparA strain.
along with the full-length proteins (Fig. 6). The two fragments of ORF1761 contain the same region (189–389 aa) within the TolC domain (accession no. COG1538), conserved among the outer membrane efflux protein family [27]. ORF2131 fragments were sorted into three groups derived from the C-terminal region (458–634 aa) that contains the MltE domain (COG0741) exhibiting lytic murein transglycosylase [28]. The regions of the ORF2009 and ORF2563 fragments were also overlapped (ORF2009, 127–507 aa; ORF2563, 119–448 aa). These proteins share the same SbcC domain (COG0419) belonging to the SMC_N family [29]. Likewise, a fragment of SMC protein (Synpcc7942_2045) was also obtained from the library screening (Fig. S6). The ORF2009, ORF2563 and SMC share the same domain, although the aa sequences of these proteins are particularly divergent, indicating that it is the structure of the SMC_N domain that is necessary for the interaction with the C-terminal region of ParA. These ParA-interacting proteins might be associated with ParA in the various cellular processes: subcellular distribution of chromosome and carboxysome, cell division and UV-stress response.

**DISCUSSION**

In this study, we revealed the pleiotropic roles of ParA, which is involved in the distribution of multi-copy chromosomes, cell division and UV-stress response, besides the carboxysome localization. We established a FISH technique in *S. elongatus* that enabled direct examination of chromosomal distribution and observed the multi-copy chromosomes to be equally spaced along the major axis of the cell (Fig. 1a). The ΔparA strain showed aberrant chromosomal distribution in not only our laboratory strain (Fig. 1, ΔparA), but also other strains (Fig. 3). In the ΔparA strain, the multi-copy chromosomes were aggregated as one or two ori/ter-foci (Figs 1 and S4).

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**Fig. 5.** Effects of parA overexpression. The ParA inducible strain grown on BG-11 plates for 1 week was cultivated in liquid BG-11 with or without 1 mM IPTG (final conc.) and harvested at the indicated days after transfer to BG-11 liquid medium. (a) Expression levels of HA-tagged ParA and DnaK2. Samples containing 10 µg of total protein were analysed by Western blot, using anti-HA and anti-DnaK2 antibodies. (b–f) Effects of ParA overexpression. Growth curve (b) and cell number (c) under cultivation with (open circle) or without (closed circle) or without 1 mM IPTG (closed circle). (d–f) Cells cultivated with (+) or without (−) 1 mM IPTG for 7 days were harvested at each time point. Fixed cells were stained with SYTOX Green and subjected to analysis. DNA content profiles (d) and relative cell size (e) were analysed using flow cytometry. (f) Cell morphology 7 days after cultivation was examined by fluorescence microscopy. Bright-field images (bright) and SYTOX Green-stained images are included. Scale bar: 10 µm.
ΔparA), which were clearly compensated by the expression of exogenous ParA from a neutral site (Fig. 2). Therefore, we concluded that ParA protein is involved in the distribution of multi-copy chromosomes. In *S. elongatus* cells, the ParA protein localizes from one pole to the opposite pole in an oscillatory manner and is involved in the positioning of the carboxysome [18]. It has been reported that Soj, a homologue of chromosomal ParA in *B. subtilis*, binds non-specifically to DNA through positive charges located on one face of the protein [30, 31]. These observations imply that the positioning of chromosomes is determined through the oscillation of ParA. Our comprehensive study of the protein–protein interactions using yeast two-hybrid screening discloses the specific interaction between ParA and SMC_N family proteins: ORF2009, ORF2563 and SMC. SMC_N family proteins regulate the compaction and segregation of chromosomes [32], and hence may contribute, along with ParA, to chromosomal distribution in *S. elongatus*. In contrast to our observations, Jain *et al.* [8] reported that parA deletion did not affect chromosome partitioning, when analysed by FROS. We assume that differences in experimental and culturing conditions are responsible for the disparity of the phenotypes seen with the ΔparA strain. In FROS, live cells are grown on an agarose pad and examined by fluorescence microscopy [8], while we examined the cells under liquid culture conditions, where the growth is faster than that on the plate.

We also revealed that the ΔparA strain is sensitive to UV-C treatment. It has been reported that *S. elongatus* and *Synechocystis* sp. PCC 6803 were more resistant to UV-C rays than *E. coli* [33]. Since DNA is a primary target of UV radiation, it is reasonable that polyploid cyanobacteria exhibit higher UV-stress tolerance than *E. coli*, which harbour single-copy chromosomes per cell. Our observations described in this work imply that the compartmentalization of multi-copy chromosomes is also important for UV-stress tolerance. It has been reported that UV irradiation induces repair and resynthesis of damaged DNA and proteins in cyanobacterial cells [34]. The appropriate distribution of multi-copy chromosomes (and carboxysomes) may be necessary for the recovery process. The ORF2009 and ORF2563 proteins, which interact with ParA, contain the SbcC domain. It has been reported that SbcC is involved in a wide variety of DNA repair and maintenance functions, including homologous recombination and non-homologous end joining [29]. Thus, these proteins may contribute to UV-stress tolerance, along with ParA.
Besides the SbcC domain proteins, our yeast two-hybrid analysis revealed specific interactions of ParA with ORF1761 and ORF2131, containing a TolC and MltE domain, respectively. In *E. coli*, the function of each homologue, TolC (homologue of ORF1761) and Slt70 (homologue of ORF2131) has been revealed. TolC is an outer membrane porin, which is involved in the efflux of several hydrophobic and amphiphatic molecules [27], and Slt70, involved in the quality control of peptidoglycan, localizes to the cell envelope where it binds to the outer surface of the murein sacculus [28]. In *S. elongatus*, however, there is yet no information about the function and subcellular localization of ORF1761 and ORF2131. Although we cannot rule out the possibility that these interactions are non-specific, our *in silico* analysis using the PSORT algorithm (http://psort1.hgc.jp/form.html) suggested that both ORF1761 and ORF2131 localize on the inner membrane as well as ParA (data not shown). This observation indicates that ParA may interact with ORF1761 and ORF2131 around the inner membrane. Further studies on these interactions are underway to determine the function of ParA.

We observed that excess ParA protein causes a deleterious effect on the growth of *S. elongatus*. In the presence of excess ParA, cell doubling and viability were significantly reduced (Figs 5c and S5), while cell mass was not affected (Fig. 5b). In addition, both DNA content and cell size were significantly increased (Fig. 5d–f). These effects began to emerge at a late stage in the growth phase, indicating that excess ParA indirectly inhibits cell division. ParA shares homology with MinD (Fig. S1), which plays a role in regulating cell septum position in bacteria, including *S. elongatus* [8, 35, 36]. In *B. subtilis*, MinD overexpression leads to the emergence of filamentous cells [37], while the phenotype of ParA overexpression in *S. elongatus* caused cellular hypertrophy, which is distinct to the filamentous phenotype. These observations indicate that excess ParA inhibits cell division via a different mechanism from that of MinD in *S. elongatus*. We have previously reported that the addition of nalidixic acid, an inhibitor of DNA gyrase, also leads to the emergence of giant cells [5], suggesting that excess ParA affects DNA topology and/or replication.

We also observed disorder of the chromosomal distribution in the *mreB*<sup>KD</sup> strain (Fig. 1). MreB has an important role in organizing cell wall biosynthesis, thus, aberrant chromosomal distribution of MreB seems to be a secondary consequence of the perturbed shape and cell wall biosynthesis. In rod-shaped bacteria such as *E. coli* and *V. cholerae*, the malfunction of MreB leads to the emergence of anucleate cells [38–40]. In *S. elongatus*, although the ParA and MreB proteins participate in the distribution of multi-copy chromosomes, the ∆parA and *mreB*<sup>KD</sup> strains did not show any anucleate cells (Table S2). A similar observation has been
reported for the AmreB strain in *Anabaena* sp. PCC 7120 [41]. These findings imply that polyplid cyanobacteria have an active mechanism to segregate chromosomes, even with aberrant positioning in a ΔparA background, yet how aggregated chromosomes segregate in a ΔparA strain is largely unknown. In *Synechocystis* 6803, which does not possess *mreB* and *parA*, multiple chromosomal copies segregate randomly into two daughter cells during cell division [42]. To elucidate the chromosomal segregation mechanisms in polyplid cyanobacteria, further studies on the segregation machinery and its regulators in various cyanobacterial species is necessary.

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**Conflicts of interest**

All authors have approved the manuscript and agree with submission to Microbiology. There are no conflicts of interest to declare.

**References**


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