DNA Contents of Cell Nuclei in Two Cyanidiophyceae:
Cyanidioschyzon merolae and Cyanidium caldarium Forma A

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DNA contents of cell nuclei in the unicellular red algae Cyanidioschyzon merolae and Cyanidium caldarium Forma A were estimated to be approximately 8 Mbp and 18 Mbp, respectively, using video-intensified microscope photon-counting system (VIMPCS) after staining with 4′-6-diamidino-2-phenylindole (DAPI) (Suzuki et al. 1992). In contrast, Malezka (1993) and Takahashi et al. (1993) estimated that cell nuclei in C. merolae contain approximately 11.7 Mbp and 12.2 Mbp, respectively, using pulsed-field gel electrophoresis (PFGE). This discrepancy appears to be due to the low AT contents in C. merolae and C. caldarium Forma A (Suzuki et al. 1992) and preferential binding by DAPI to AT-rich DNA (Kapuscinski and Szer 1979). In this study, we again measured DNA contents of cell nuclei in the two algae using VIMPCS after staining with DAPI, Schiff’s reagent, and propidium iodide (PI). Schiff’s reagent binds to purine residues (Lesser 1953) and the stainability of PI is affected by DNA topology (Prosperi et al. 1994). The DNA contents of cell nuclei in C. merolae and C. caldarium Forma A were calculated based on the GC content of these algal DNAs (Suzuki et al. 1992), and on the values obtained by VIMPCS following DAPI, Feulgen, and PI staining.

Materials and methods

Cyanidioschyzon merolae 10D and C. caldarium Forma A were grown in Allen’s medium as previously described (Nagashima et al. 1981). Cells were collected by centrifugation at 4000 g for 5 min before staining.

Saccharomyces cerevisiae, which has a genome size of 13.56 Mbp, was generously provided by Bio-Rad Laboratory (strain YNN 295) and used as a standard material. Cells were collected by centrifugation at 3000 g for 5 min before staining.

DAPI staining: C. merolae, C. caldarium Forma A and S. cerevisiae cells were fixed in 0.5% glutaraldehyde in TAN buffer [20 mM Tris-HCl, pH 7.7, 0.5 mM EDTA, 1.2 mM spermidine, 7 mM 2-mercaptoethanol, and 0.4 mM phenylmethylsulfonyl fluoride] and stained with 0.4 µg/ml DAPI.

Feulgen Staining: Cells were fixed in 75% ethanol and 25% acetic acid, spun down and incubated in 1 N HCl at 60°C for 7 min. The cells were spun down again and stained with standard Schiff’s reagent (Feulgen and Rossenbeck 1924).

Propidium iodide staining: Staining was performed according to the method described by Suzuki et al (1986). Cells were fixed in 70% ethanol for more than 30 min. Cells were then washed twice in autoclaved distilled water, and suspended in 10 mM Tris-HCl, pH 8.0, with 15 mM NaCl. The cells were spun down and treated with 1 mg/ml RNase A in the same buffer at
37°C for 2 hr or more. Before use, RNase was boiled for 5 min to avoid DNase activity. The cells were spun down again and suspended in NS buffer [0.25 M sucrose, 1 mM EDTA, 7 mM 2-mercaptoethanol, 0.8 mM PMSF, 1 mM magnesium chloride, 0.1 mM calcium chloride, 0.1 mM zinc sulfate and 20 mM Tris-HCl, pH 7.6]. The cells were then stained with 10 μg/ml PI and 1 mg/ml RNase in NS buffer.

After staining, a cover slip was placed over the sample, which was then squashed. Samples were irradiated by ultraviolet (UV) light for DAPI and green light for Schiff’s reagent and PI, and then examined by an epifluorescence microscope (BHS-RFK; Olympus, Tokyo, Japan) equipped with phase-contrast optics. Photographs were taken using Neopan 400 film (Fuji Photo Film Co., Tokyo, Japan). The fluorescence intensities from cell nuclei were determined using VIMPCS (Hamamatsu Photonics Ltd., Hamamatsu, Japan).

Results

Figures 1a–c are fluorescent microphotographs showing cell nuclei and organelle nuclei in C. merolae after staining with DAPI, Schiff’s reagent and PI, respectively. After staining with DAPI, nuclei in C. merolae emitted weaker blue-white fluorescence than those in S. cerevisiae (Fig. 1a). However, after staining with Schiff’s reagent, nuclei in C. merolae emitted as much red light as those in S. cerevisiae (Fig. 1b). In addition, after staining with PI, nuclei in C. merolae emitted stronger red light than those in S. cerevisiae (Fig. 1c). A similar trend was seen with C. caldarium Forma A. The fluorescence intensity of C. caldarium Forma A relative to that of S. cerevisiae increased after staining with DAPI, Schiff’s reagent, and PI, in that order (Figs. 1d–f). Schiff’s reagent binds to purine residues, and DAPI binds to AT. Therefore, the present results suggest that the GC content of cell-nuclear DNA in S. cerevisiae is lower than those in C. merolae and C. caldarium Forma A, and that the fluorescence intensities of cell nuclei after staining with DAPI depend on the GC contents of cell nuclei. Therefore, it is difficult to estimate the DNA content of cell nuclei in C. merolae and C. caldarium Forma A by comparison to the DNA content of cell nuclei in S. cerevisiae after DAPI staining. The results of PI staining, which is affected by DNA topology, indicated the difference of DNA structure among C. merolae, C. caldarium Forma A and S. cerevisiae.

To confirm these results, we estimated the fluorescence intensities of cell nuclei in C. merolae and C. caldarium Forma A using VIMPCS (Table 1). The fluorescence intensities of cell nuclei in C. merolae and C. caldarium Forma A after staining with DAPI, Schiff’s reagent and PI were approximately 0.55, 0.99 and 1.75 times, and 1.05, 2.54 and 5.07 times as much as those in S. cerevisiae, respectively.

The $\rho_{csl}$ values of cell-nuclear DNA in C. merolae and C. caldarium RK-1 were 1.716 and 1.715, respectively (Suzuki et al. 1992). Since the $\rho_{csl}$ value of C. caldarium Forma A has not yet been determined, we used the $\rho_{csl}$ value of C. caldarium RK-1, which is very similar to C. caldarium Forma A. The relation between $\rho_{csl}$ and GC content is;

$$[G+C]\% = 1020.6 (\rho_{csl} - 1.6606)$$ (Delay 1970).

Therefore, the GC contents of C. merolae and C. caldarium RK-1 are believed to be 56.5% and 55.6%, respectively. The GC content of S. cerevisiae is 36.2% (Chargaff 1950). Based on these data and the present results, we determined that the DNA contents of the cell nucleus in C. merolae and C. caldarium Forma A after staining with DAPI, Schiff’s reagent, and PI were approximately 11 Mbp, 13 Mbp and 24 Mbp, and 21 Mbp, 35 Mbp and 69 Mbp, respectively (Table 2). These results indicate that the DNA content per cell nucleus in C. merolae is approximately half that in C. caldarium Forma A. Since the value calculated from the results
Fig. 1. Fluorescent photomicrographs showing cell nuclei and organelle nuclei in *C. merolae* (a–c), and *C. caldarium* Forma A (d–f) after staining with DAPI (a and d), Schiff's reagent (b and c) and PI (c and f). The fluorescence intensities of their cell nuclei were compared to those of *S. cerevisiae*. Large arrows, cell nuclei in *C. merolae* or *C. caldarium* Forma A; arrow heads, their organelle nuclei; small arrows, cell nuclei in *S. cerevisiae*. In order of increasing intensities relative to *S. cerevisiae*, photomicrographs are those of DAPI (top; a and d), Feulgen staining (middle; b and e) and PI (bottom; c and f). Scale bar represents 1 μm.

of Feulgen staining was close to that calculated from PFGE resolution patterns (Fig. 2) by Takahashi et al. (14.2 Mbp, in press), we concluded that the ploidy of *C. merolae* cells is haploid and the DNA content of the cell nucleus in *C. merolae* is approximately 13 Mbp. The DNA
Table 1. The fluorescence intensities of cell nuclei in *C. merolae* and *C. caldarium Forma A* after staining with DAPI, Schiff's reagent (Feulgen staining) or propidium iodide (PI). The values represent the intensity relative to that of *S. cerevisiae*.

<table>
<thead>
<tr>
<th></th>
<th>DAPI</th>
<th>Feulgen</th>
<th>PI</th>
</tr>
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<tbody>
<tr>
<td><em>C. merolae</em></td>
<td>0.55±0.11</td>
<td>0.99±0.18</td>
<td>1.75±0.50</td>
</tr>
<tr>
<td><em>C. caldarium</em></td>
<td>1.05±0.16</td>
<td>2.56±0.63</td>
<td>5.07±1.49</td>
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Table 2. Estimated DNA contents of cell nuclei in *C. merolae* and *C. caldarium Forma A* calculated by their fluorescence intensities and GC contents. Cell nuclei were stained with DAPI, Schiff's reagent (Feulgen staining), or propidium iodide (PI).

<table>
<thead>
<tr>
<th></th>
<th>DAPI</th>
<th>Feulgen</th>
<th>PI</th>
<th>GC contents</th>
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<tbody>
<tr>
<td><em>C. merolae</em></td>
<td>11 Mbp</td>
<td>13 Mbp</td>
<td>24 Mbp</td>
<td>56.5%*</td>
</tr>
<tr>
<td><em>C. caldarium</em></td>
<td>21 Mbp</td>
<td>35 Mbp</td>
<td>69 Mbp</td>
<td>55.6%**</td>
</tr>
</tbody>
</table>

* calculated from ρ_{oct} (Suzuki et al. 1992). ** calculated from ρ_{oct} (Ohta et al. 1992).

The content of the cell nucleus in *C. caldarium Forma A* is believed to be approximately 35 Mbp, which was also calculated from the results of Feulgen staining. Based on this result, Feulgen staining seems to be most reliable of the three reagents for estimating genome size.

Discussion

Coleman and Maguire (1995) estimated the genome size of Ochromonas plastid DNA by staining with DAPI and ethidium bromide, using T4 particles as a reference. DAPI and ethidium bromide gave similar estimated values. The GC contents of Ochromonas plastid DNA and T4 particles are 31% (Charles 1977) and 34.4% (Schmidt and Hearst 1969), respectively. Therefore, the reagent may not to be considered when there is little difference in the GC contents. However, if there is a large difference in GC contents, such as among the Cyanidiochlorophyceae *C. merolae* and *C. caldarium* and *S. cerevisiae*, the binding preferences of the reagents may affect the estimation. Suzuki *et al.* (1992) estimated the genome size of *C. merolae* to be 8 Mbp after staining with DAPI using VIMPCS. The estimated genome size was
approximately 14 Mbp using PFGE resolution patterns (Takahashi et al. in press). This discrepancy was attributed to a disregard for the GC contents and the binding preferences of DAPI. In this study, the genome sizes of C. merolae and C. caldarium Forma A were estimated to be approximately 13 Mbp and 35 Mbp, respectively, which are similar to the values estimated from Feulgen staining. The results that the estimated genome size using PFGE was similar to that after staining with Schiff's reagent indicated that the ploidy of C. merolae is haploid. DAPI, which shows a logarithmic relation of AT contents (Dexhelet et al. 1989), is unsuitable for estimating genome sizes. PI, which is base-unspecific, but depends on DNA topology, showed different results from those of Feulgen. Therefore, GC contents of nuclear DNAs and the binding preferences of staining reagents should be considered when estimating genome sizes.

Summary

The DNA contents in the cell nuclei of the Cyanidiophyceae C. merolae and C. caldarium Forma A were estimated using VIMPCS after staining with DAPI, Schiff's reagent or PI. For C. merolae, the obtained data were compared with those of PFGE. The results indicated that the genome sizes of C. merolae and C. caldarium Forma A were approximately 13 Mbp and 35 Mbp, respectively. Since the values obtained by PFGE were very close to those estimated from Feulgen staining, Schiff's reagent, which binds to purine residues and is not affected by GC content, is considered to be the most reliable of these three reagents for estimating DNA contents.

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