Increasing the Copy Number of \( rbcL \) Genes in \textit{Chlamydomonas reinhardtii} Chloroplast Genome Affects neither Cell Proliferation nor Distribution of Pyrenoid Antigens to Rubisco Holoenzyme Antibody

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Summary A higher copy number of the \( rbcL \) gene in \textit{Chlamydomonas reinhardtii} genome upregulates synthesis of Rubisco gene products (Uchida \textit{et al.} 2005). To determine the influence of \( rbcL \) gene number increase on the viability and morphology of the cell, we examined the growth speed, size of pyrenoid and density of antigen to Rubisco holoenzyme antibody in the three-\( rbcL \) (3L) transformant. There were no differences detected in these phenotypes between the 3L transformant and the control transformant.

Key words \textit{Chlamydomonas reinhardtii}, Chloroplast gene transformation, \( rbcL \), Pyrenoid.

Materials and methods

Culture of \textit{Chlamydomonas} transformants

The three-\( rbcL \) (3L) \textit{Chlamydomonas} transformant and the control transformant have been re-
ported elsewhere (Uchida et al. 2005). Cells were cultured in liquid HSM medium (Sueoka 1960) in Erlenmeyer flasks. The flasks were supplied with air bubbles, or were shaken continuously at a rotation of 100 rpm, under 16 : 8 h light/dark photoregime at 25°C. Cold white fluorescent light (model FLR40S, Matsushita) was provided at a photon flux of 30 $\mu$E m$^{-2}$ s$^{-1}$.

Density of cells was estimated from optical density at 750 nm (Sager and Granick 1953).

**Measurement of pyrenoid diameter**

Cell suspension aliquots of each transformant were withdrawn, dropped onto compartments isolated by silicone grease on a single glass slide, covered with a coverslip, and observed under a Nomarski differential interference-contrast microscope (model BX60, Olympus) equipped with an eyepiece micrometer. The diameter of the pyrenoids in 100 cells from each transformant was measured.

**Immunoelectron microscopy**

Cells were fixed with 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 mM cacodylate buffer pH 7.2, dehydrated, embedded in LR white (Polyscience) that contained 0.5% 2-ethoxy-2-phenylacetophenone using a UV illuminator (model TUV, Dohan EM), and thin-sectioned using a microtome (model Ultracut-E, Reichert). The sections were loaded on nickel grids, blocked with 5% skim milk in PBS for 1 h, washed in 0.5% Tween 20 in PBS, treated with primary antibody against Rubisco holoenzyme (Uchida et al. 2005) that had been diluted 1 : 1200 in PBS for 1 h, washed three times with 0.5% Tween 20 in PBS, treated with secondary antibody conjugated with 10-nm gold particles (British Bio Cell) that had been diluted 1 : 100 in PBS, washed three times

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pyrenoid diameter$^a$ (mean$^b$±SD)</th>
<th>Gold particle number per $\mu$m$^2$ (mean$^c$±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-rbcL</td>
<td>2.0±0.26</td>
<td>442±89.0</td>
</tr>
<tr>
<td>Control</td>
<td>2.0±0.37</td>
<td>422±58.0</td>
</tr>
</tbody>
</table>

$^a$ Diameter determined by Nomarski differential interference-contrast microscopy.

$^b$ Mean values of one hundred counts.

$^c$ Mean values of the immunogold numbers counted on 5 cell sections.

$^d$ Cytoplasm region was adopted as background.
with 0.5% Tween 20 in PBS, and then with distilled water. The section was stained with uranyl acetate and viewed with an electron microscope (model H-7100, Hitachi). No signals were detected on the pyrenoid after preimmune serum treatment.

**Results and discussion**

To examine the effect of \( rbcL \) gene increase on cell proliferation, we checked the growth rate of three-\( rbcL \) (3L). Fig. 1 shows the growth curves of 3L and the control. In logarithmic and stationary phases, no obvious growth difference between the 3L and the control was observed.

We examined the size, fine structure and distribution of antigen to anti-Rubisco holoenzyme antibody in the 3L pyrenoid. The pyrenoid diameter in 3L was the same as that in the control (Fig. 2C). The electron microscopic image of pyrenoid in 3L after cryofixation and staining with uranyl acetate and lead citrate was similar to that of the control (data not shown). Analysis of 3L by immuno-electron microscopy, revealed the Rubisco antigen distributed evenly on the entire pyrenoid region of electron dense region (Fig. 2A) and the anti-Rubisco antibody labeling density in the pyrenoid was 442 particles/\( \mu \)m\(^2\) (Fig. 2C). These results were consistent and almost equal to those in the control (Figs. 2B, C). LSU and SSU accumulation are almost the same in 3L as in the control, while synthesis of LSU and SSU is markedly more upregulated in 3L than in the control (Uchida et al. 2005). The data presented in this report might correspond to the absence of differences in the Rubisco subunit accumulation between the 3L and the control. Previous reports indicate that LSU is synthesized and pooled in excess in association with chaperonin before being assembled with equal moles of SSU to form Rubisco holoenzyme (Roy 1989, Gutteridge and Gatenby 1995). The LSU pool before this assembly might be outside the pyrenoid.

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**References**


