Short Communication

The Site of Synthesis of Two Chloroplast Cytochromes in Chlamydomonas reinhardi

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The analysis of steps of genetic transcription and translation specifying certain of the chloroplast components of the unicellular green alga, Chlamydomonas reinhardi, has been the subject of several publications from this laboratory (1, 4, 7-10, 13, 16, 17). The cytochromes are of particular interest in this regard, for the chloroplasts of C. reinhardi contain at least three—cytochrome 553, cytochrome 559, and cytochrome 563 (5, 6, 12)—and there is some evidence to suggest (6, and B. Epel, unpublished) that there may be more than one cytochrome 559. The synthesis of cytochromes 553 and 563 has been shown to depend on translational steps that occur on chloroplast ribosomes, for their synthesis in synchronous cultures is prevented by antibiotics that inhibit translation on chloroplast ribosomes (1). An earlier paper from this laboratory reported (13) that the level of these two cytochromes was not affected in cells of a mutant strain of C. reinhardi in which the chloroplasts contain only 5 to 10% of the number of chloroplast ribosomes found in wild-type cells (9). The discrepancy between the results obtained with synchronous cultures of wild-type cells and chloroplast ribosome-deficient mutant strain, ac-20, has prompted the reinvestigation of the cytochrome content of the mutant strain, particularly in view of the fact that the determination of the cytochromes reported in the earlier work was based on light-induced absorbance changes in chloroplast fragments, a less sensitive method of determination than one based on measurements of the chemically reduced-minus-oxidized difference spectra of solubilized cytochromes 553 and 563.

In this paper we show that both cytochrome 553 and 563 are deficient in mixotrophically grown cells of ac-20 in which there is a drastic reduction in the number of chloroplast ribosomes. We also show that the cytochrome content increased when cells of the mutant strain are placed under phototrophic growth conditions where the level of chloroplast ribosomes increases to at least 25% of the level characteristic of the wild-type strain (9). Thus, the results are in accord with the observation (1) that chloroplast ribosomes play an essential role in the synthesis of cytochromes 553 and 563.

MATERIALS AND METHODS

Organisms and Culture Conditions. The wild-type strain 137c of C. reinhardi and the mutant strain ac-20 derived from it were used in the experiments described here. Liquid cultures of these strains were maintained at 25°C and under 4000 lux from daylight fluorescent lamps. The culture medium was either a minimal salts medium (15) or the same minimal medium supplemented with 0.2% (w/v) sodium acetate. Cells grown in the light in minimal medium are referred to here as phototrophic cells. Cells grown in the light in acetate-supplemented medium are referred to as mixotrophic cells.

Transfer Experiments. Following the transfer of mixotrophic cells of ac-20 to minimal medium there is an increase in the number of chloroplast ribosomes (9). Shortly after this increase has occurred there is a recovery of photosynthetic capacity (13, 17). For the transfer experiment described in this paper a culture of mixotrophic cells was harvested, washed in minimal medium, and then resuspended in minimal medium and placed in the light. Samples of cells were withdrawn at 0, 8, and 13 hr after the transfer of the culture to the light, and the cells were analyzed for their cytochrome and chlorophyll content. By the 8th hr in the light the number of chloroplast ribosomes has recovered the level characteristic of phototrophic cells (9).

Cytochrome Determinations. Acetone powders of cells were prepared as described by Armstrong et al. (1), and the cytochromes 553 and 563 were extracted from them by the procedure of De Petrocellis et al. (5). The amounts of the cytochromes were determined from their reduced-minus-oxidized difference spectra as recorded with a Cary model 14 spectrophotometer. Cytochrome 553 was reduced by adding 4 μmoles of ascorbate, pH 6.2, and was oxidized by the addition of 4 μmoles of hypochlorite (5). The amount of cytochrome 553 was calculated from the difference molar extinction coefficient (εM553 nm - εM559 nm) of 2.5 × 10³ (3).

Cytochrome 563 is reducible by dithionite but not by ascorbate (11). For the determination of cytochrome 563, 4 μmoles of ascorbate, pH 6.2, were added to one cuvette and 4 μmoles of ascorbate plus a few grains of dithionite were added to the other. The amount of cytochrome 563 was calculated from the difference molar extinction coefficient (εM563 nm - εM563 nm) of 2.0 × 10³ (3).

Chlorophyll and Cell Numbers. The chlorophyll content of cells was determined by a modification (2) of the method of MacKinney (14). Cell numbers were obtained with the aid of a hemacytometer.

RESULTS AND DISCUSSION

The cytochrome and chlorophyll content from a typical experiment of mixotrophically grown cells of the wild-type strain are given in Table I. Phototrophically grown cells have similar

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Table I. Chlorophyll and Cytochrome 553 and 563 Content of Cells of Wild-Type and ac-20 C. reinhardtii

<table>
<thead>
<tr>
<th>Strain and Culture Conditions</th>
<th>Chlorophyll Content</th>
<th>Cytochrome Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m4molecules/10^6 cells</td>
<td>Cyt. 553</td>
</tr>
<tr>
<td>Wild-type (mixotrophic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.7</td>
<td>0.095</td>
<td>0.238</td>
</tr>
<tr>
<td>ac-20 (mixotrophic)</td>
<td>14.1</td>
<td>0.029</td>
</tr>
<tr>
<td>ac-20 (phototrophic)</td>
<td>14.5</td>
<td>0.039</td>
</tr>
</tbody>
</table>

amounts of the two cytochromes, but they have about one-third less chlorophyll (8, 17). Cell size is also less by about one-third. The amount of chlorophyll 553 reported here is in good agreement with that reported earlier for C. reinhardtii by De Petrocellis et al. (5). However, the amount of cytochrome 563 reported by De Petrocellis et al. (5) is less than that reported here, perhaps because our extractions were carried out in the cold. The amount of cytochrome 563 we report here is similar to that obtained by Boardman and Anderson (3), who also did their extraction in the cold.

As the data in Table I show, both mixotrophically and phototrophically grown cells of ac-20 are, in comparison to wild-type cells, deficient in chlorophyll, cytochrome 553, and cytochrome 563. Whereas the chlorophyll deficiency is the same under the two growth conditions, the cytochrome deficiencies are more extreme in the mixotrophically grown cells. Thus, the most marked decrease in cytochromes 553 and 563 is seen in cells in which there is a drastic reduction in the number of chloroplast ribosomes. This relationship is in accord with the observation that translational steps on chloroplast ribosomes are required for the synthesis of cytochromes 553 and 563 (1).

The results of transfer experiments further substantiate the relationship between chloroplast ribosomes and the two cytochromes, for the recovery of the cytochromes occurs under conditions that are known to result in the recovery of chloroplast ribosomes (9). As the data summarized in Table II show, there is no change in the chlorophyll content of the cells following the transfer, but their content of cytochromes 553 and 563 is seen to increase by 20 to 30% by the 13th hr of the transfer experiment.

In summary, the results given here and those reported earlier from experiments with synchronous cultures (1) support the contention that chloroplast ribosomes are involved in the synthesis of cytochromes 553 and 563.

LITERATURE CITED