ABSTRACT

The levels of chloroplast DNA in a cultured phototrophically grown soybean (Glycine max [L.] Merr. & Corsoy) cell line were determined by molecular hybridization. The cells were also grown photomixotrophically and heterotrophically in suspension cultures and the level of plastid DNA was found to be constant at approximately 26% of the total cellular DNA in all three growth modes. By comparison, total cellular DNA extracted from plants of the same variety used as the explant source for the cultured cells contained 12.3 to 18.9% (leaves and seeds) and 6.1 to 8.9% (roots) plastid DNA.

Plastid DNA contains genes whose products, in conjunction with proteins from the cytoplasm, are required for photosynthesis in higher plants (4). The levels of plastid DNA observed in plant cells can vary according to age, developmental stage, and tissue type of the cell in question (15). The number of plastome copies available as transcriptional templates presumably influences, to some extent, the ability of the cell to grow photosynthetically. For instance, the level of chloroplast DNA in cultured tobacco cells was observed to be 2.5- to 2.8-fold higher in suspension cultures grown autotrophically or mixotrophically in the light than in those grown heterotrophically in the dark (2). The elevation of chloroplast DNA levels in photosynthetically active cells suggests a correlation between plastome copy number and the cellular need for reduced carbon and energy.

Recently, Horn et al. (7) described a cell line (SB) of soybean that is capable of photoautotrophic growth. This cell line contains more Chl and fixes CO₂ at a faster rate than any photomixotrophically cultured cell line thus far studied (7). Furthermore, SB cells that are grown heterotrophically in the dark are unpigmented but when placed in the light are able to green rapidly (5, 7), thereby providing an excellent model system for studying the effects of light on the synthesis of chloroplast components and plastid development.

The objective of this study was to measure the percentage of plastid DNA in SB cell suspensions grown photoautotrophically, photomixotrophically, or heterotrophically. The levels of plastid DNA in various tissues of soybean plants were also examined for comparison with the chloroplast DNA content found in the suspension cells.

MATERIALS AND METHODS

Plants and Tissue Culture. Seeds of Glycine max (L.) Merr. & Corsoy and the SB cell line were obtained from M. Horn and J. Widholm, Department of Agronomy, University of Illinois, Urbana. The seeds were germinated in the dark on sterile Murashige and Skoog agar (12) containing 1% sucrose and the resulting plants were maintained in either dark or light for 15 d until used as a source of DNA. Photoautotrophic cells were grown on KT (7) media devoid of a carbon source, in air containing 5.0% CO₂ and under 10,000 lux of white light. Photomixotrophic cells were grown on KT media containing 3% sucrose under 10,000 lux of white light. Heterotrophic cells were grown on KT media containing 3% sucrose in the dark. The suspension cultures in all three growth modes were maintained with constant shaking and were initiated from photoautotrophically growing callus.

DNA Isolation. DNA was extracted and purified from plant material by a modification of the Murray and Thompson procedure (13) as described previously (6). Recombinant plasmids containing fragments of soybean chloroplast DNA were isolated as described earlier (6). Some of the recombinant plasmids were a kind gift from D. T. N. Pillay, University of Windsor, Canada.

Determination of Nuclear DNA Content. Nuclei were isolated from SB suspension cultures on silica sol gradients as described by Willmitzer and Wagner (18). The concentration of purified nuclei was measured by light microscopy and the DNA concentration determined by the fluorometric method of Vytasek (19).

Radiolabeling of DNA. SB suspension cultures were radiolabeled in vivo by adding 8 μCi/ml of [³H]-methylthymidine (40 Ci/mmol) to each culture every 12 h for a total of 72 h. This length of time (approximately two cell divisions) was chosen to ensure uniform labeling of all the DNA species. For radiolabeling in vitro, DNA isolated from either plant tissue, seed, or suspension cultures was labeled with ³²P by T4 polynucleotide kinase (11) or by random primer extension as previously detailed (6).

All radiochemicals were obtained from Amersham Corporation, Arlington Heights, IL.

Quantitative Molecular Hybridization. The quantitative hybridization procedure used here has been previously described in detail (2, 3, 6). Briefly, 5 μg of cloned soybean chloroplast DNA was covalently bound to nylon membranes (GENESCREEN PLUS, from New England Nuclear) and radiolabeled total cellular DNA from either plant tissue, seed, or suspension culture was hybridized to the bound cloned chloroplast DNA. Hybridization was conducted at 65°C for 60 h in a solution containing 0.5 M NaHPO₄ (pH 7.2), 1% SDS, and 1 mM EDTA. Each hybridization reaction contained an internal control probe consisting of cloned chloroplast DNA 100% homologous to that bound on the membrane. After hybridization, the membranes were washed

1 SB refers to the specific soybean cell line obtained from J. Widholm and described in Ref. (7).
and dried and the amount of radioactivity bound was determined by liquid scintillation counting. The level of plastid DNA is expressed as the percentage of input cellular DNA hybridized to the membrane bound cloned chloroplast DNA after correction for the efficiency of hybridization as determined by the amount of internal control probe bound (3, 6).

RESULTS AND DISCUSSION

Plastid DNA levels were found to remain roughly constant in suspension cultures of SB cells regardless of their mode of growth (Table 1). Cells whose DNA was labeled in vivo by continuous growth on low concentrations (~0.2 mm) of [3H]thymidine contained 22.5 to 25.8% plastid DNA whether grown in the light or in the dark. Radiolabeling with this concentration of thymidine was not observed to inhibit growth of the cells and resulted in specific activities of DNA ranging from 1.4 to 2.7 x 10^6 cpm/μg of DNA.

In other experiments, DNA was isolated from cells grown photoautotrophically, photomixotrophically, or heterotrophically and subsequently radiolabeled in vitro by either T4 polynucleotide kinase or by random primed extension with the Kleivow fragment of E. coli DNA polymerase I. Hybridization of these cellular DNAs to cloned chloroplast DNA revealed a plastid DNA content of 23.5 to 30.5% (Table 1). The range of values observed in all three growth modes and labeling methods was 22.5 to 30.5% plastid DNA with an average value of 26%. This level is nearly 2-fold higher than the level (14% of total cellular DNA) of chloroplast DNA found in photoautotrophically grown tobacco (Nicotiana tabacum) cells (2). Furthermore, tobacco suspension cultures exhibit a light dependent regulation of chloroplast DNA synthesis since plastome levels were 2.5- to 2.8-fold higher in light grown cells than in dark grown cells (2). By contrast, the consistently high plastome DNA levels reported here for soybean cells grown in either light or dark suggest that the SB cell line does not regulate chloroplast DNA synthesis in response to light. The fact that SB cells synthesize the same amount of plastid DNA in either light or dark is not due to a general lack of light response by the cells since unpigmented heterotrophically grown SB cells rapidly accumulate Chl when placed in the light (7). Moreover, Erdös et al. (5) have shown that ribulose biphosphate carboxylase activity is stimulated 7-fold and that two polypeptide components of the light harvesting complex appear during “greening” of heterotrophically grown SB cells.

Table 1. Chloroplast DNA Content in Suspension Cultures of Soybean

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>In vitro labeled</th>
<th>In vivo labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Seed</td>
<td>18.4</td>
<td>15.1</td>
</tr>
<tr>
<td>Light grown leaves</td>
<td>18.9</td>
<td>15.0</td>
</tr>
<tr>
<td>Dark grown leaves</td>
<td>17.8</td>
<td>12.3</td>
</tr>
<tr>
<td>Light grown roots</td>
<td>8.9*</td>
<td></td>
</tr>
<tr>
<td>Dark grown roots</td>
<td>8.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Autotrophic cells</td>
<td>26.5</td>
<td>30.5</td>
</tr>
<tr>
<td>Mixotrophic cells</td>
<td>26.6</td>
<td>30.2</td>
</tr>
<tr>
<td>Heterotrophic cells</td>
<td>25.0</td>
<td>23.5</td>
</tr>
</tbody>
</table>

* Radiolabeled with [α-32P]dCTP by random primer extension.  
  b 5' end labeled with [γ-32P]ATP by T4 polynucleotide kinase.  
  c Radiolabeled by growth for 72 h in media containing [3H]thymidine.  
  d Average of two determinations.

To determine the levels of plastid DNA in intact plants of the soybean variety (Corsoy) used to initiate the SB line, total cellular DNA from either etiolated or light grown 15 d old plants was hybridized to membrane bound cloned soybean chloroplast DNA. No significant differences in plastid DNA levels were observed between plants grown for 15 d in the dark or light (Table 1). However, leaves from both dark and light grown plants contained approximately two times the plastid DNA found in roots of the same plants (15.0–18.9% for leaves compared to 6.1–8.9% for roots). DNA isolated from soybean seeds contained 15.1 to 18.4% plastid DNA, which is essentially the same as that value observed in leaves (Table 1). These data are in agreement with numerous literature reports showing leaves to contain more plastid DNA than roots (1, 14–17). However, previous reports concerning plastome levels in etiolated and light grown plants have indicated that light grown shoots contain 3 to 4 times more plastid DNA than etiolated shoots of similar age (8, 10). Apparently, the leaves of the plant used as the explant source for the SB line are able to synthesize plastid DNA at the same rate in the presence or absence of light for at least 15 d past germination. This may be analogous to the situation in pea leaves, where the levels of chloroplast DNA were the same whether etiolated or grown in the light for 1 d (9).

The data presented here demonstrate that cultured SB cells contain a considerably higher percentage of plastid DNA than the plant from which they were derived (Table 1). The nuclear DNA content of SB cultures was measured to be 11.1 pg of DNA per nucleus. Since the haploid soybean genome consists of 1.95 pg of DNA (10), it seems unlikely that a reduction of nuclear DNA occurred during establishment of the cell line and, therefore, it is reasonable to assume that the increased percentage of plastid DNA observed in SB cells is due to an increase in the number of plastomes per cell (4,000–12,500 copies per cell in intact plants compared to 15,000–20,000 copies per cell in SB suspension culture).

Horn et al. (7) have previously reported the high photosynthetic efficiency of the SB cell line when grown photoautotrophically and Erdös et al. (5) have described a greening system utilizing SB cells that underscores the importance of the SB cell line as a model system for studying the molecular events involved in chloroplast development. The results reported here further characterize the cell line by establishing the levels of plastid DNA under three different growth modes and demonstrates that SB cells constitutively maintain an abnormally high level of plastid DNA regardless of the quantity of light or reduced carbon available to them.

LITERATURE CITED

6. HEINHORST S, G CANNON, A WEISSBACH 1985 Plastid and nuclear DNA synthesis are not coupled in suspension cells of Nicotiana tabacum. Plant Mol Biol 4: 3–12
8. INAMINE G, B NASH, H WEISSBACH, N BROTM 1985 Light regulation of the

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synthesis of the large subunit of ribulose-1,5-bisphosphate carboxylase in pea: Evidence for transcriptional control. Proc Natl Acad Sci USA. In press