The Effect of Light and Inhibitors on Chloroplast and Cytoplasmic RNA Synthesis

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Abstract. Chloroplast RNA is synthesized in dark-grown radish cotyledons at about one-third the rate of that in the light. The synthesis, however, continues for longer in the dark and the percentage of chloroplast RNA can approach that in light-grown tissue. Light stimulates the synthesis and accumulation of both cytoplasmic and chloroplast RNA, but shows a 4-fold greater stimulation of the chloroplast RNA. Chloramphenicol, streptomycin and cycloheximide inhibit the synthesis of chloroplast RNA with little effect on cytoplasmic RNA. 5-Fluorouracil inhibits the synthesis of cytoplasmic RNA more than chloroplast RNA. Synthesis of the 0.56 × 10^6 mol wt chloroplast RNA is inhibited much less than the other ribosomal RNA components by actinomycin D.

One function of the chloroplast DNA may be to provide the template for the synthesis of chloroplast ribosomal RNA. Hybridization studies indicate that this is the case with Englera (14), but similar experiments with tobacco suggest that although the chloroplast DNA does contain cistrons for chloroplast ribosomal RNA, most of this RNA is coded for by the nuclear DNA (17). If chloroplast RNA is synthesized on the DNA in the chloroplast then the regulation of its synthesis should differ considerably from that of cytoplasmic RNA, which is synthesized in the nucleus. Studies with developing radish cotyledons have shown that synthesis of chloroplast and cytoplasmic RNAs can occur at different times. This, together with the similarity in the timing of chlorophyll and chloroplast RNA synthesis suggested that the latter may be light dependent (7). This paper compares the effect of light, and the effects of protein and RNA synthesis inhibitors on the synthesis of chloroplast and cytoplasmic ribosomal RNAs.

Materials and Methods

Radish seedlings (Raphanus sativus, L. var. Cherrybelle) were grown as previously described (8). Dark grown seedlings were maintained in complete darkness at 20° after the initial surface sterilization of the seed. Light grown seedlings received a daily 18 hr photoperiod of 1600 ft-c at 20°. The preparation and fractionation of total nucleic acid, and the measurement of RNA synthesis was as previously described (7, 11). When inhibitors were used the excised cotyledons were preincubated with the inhibitor for 1 hr before the addition of the radioactive precursor. Incubation in the presence of inhibitor and precursor was continued for 6 hr.

Results

Effect of Light. Total RNA was prepared at daily intervals from the cotyledons of dark and light grown seedlings. The cotyledons of the dark grown seedlings accumulate about 70% of the maximum RNA content of those grown in the light (fig 1). Transfer from the dark to light results in an increase in RNA accumulation, and a level comparable to the maximal light grown content of 120 μg RNA per pair of cotyledons is reached within 24 hr (fig 1). Fractionation of the total RNA by polyacrylamide gel electrophoresis shows that both light and dark grown tissue accumulates chloroplast RNA (1.1 M, 0.56 M, and 0.40 M), the difference being the amount of chloroplast RNA relative to cytoplasmic RNA (1.3 M and 0.70 M) (fig 2). Transfer from the dark to light results in an increased accumulation of the chloroplast RNA, giving a fractionation very similar to that of the light-grown material (fig 2). The amounts of the individual RNA components can be calculated from the gel fractionations (7), and figure 3 shows the amount of the chloroplast (1.1 M plus 0.56 M) and the cytoplasmic (1.3 M plus 0.70 M) RNA under the various light and dark treatments. The 1.1 M and 0.70 M RNAs were calculated assuming a breakdown of the 1.1 M RNA into 0.70 M plus 0.40 M pieces (7). After 4 days growth the accumulation of cytoplasmic RNA is only 12% higher in the light than in the dark, whereas light stimulates the accumulation of chloroplast RNA by 200% (fig 3). A similar effect is seen when the seedlings are

1 RNA components are referred to by their molecular weight in millions (7), i.e., 1.1 M RNA is RNA with molecular weight of 1.1 × 10^6.
Fig. 1. Effect of light on the accumulation of total nucleic acid during development of the radish cotyledon. Total nucleic acid content was determined at daily intervals during the growth of seedlings in the dark (●—●) and light (●—●). Seedlings were transferred from the dark to light after 3, 4, and 6 days (○―○), as indicated by the arrows.

Fig. 2. Fractionation of nucleic acid prepared from the cotyledons of light and dark grown radishes. Total nucleic acid was fractionated by gel electrophoresis for 3 hr. A) Dry seed, B) 4 days growth in the dark, C) 4 days growth in the light and D) 3 days growth in the light following 4 days in the dark. The RNA components are referred to as their molecular weight in millions.

Fig. 3. Effect of light on the accumulation of cytoplasmic and chloroplast RNA. Fractionations of total nucleic acid, such as are shown in figure 2, were quantitated after making corrections for breakdown of the 1.1 M RNA (7). The amounts of chloroplast RNA (1.1 M plus 0.56 M) and cytoplasmic RNA (1.3 M plus 0.70 M) were calculated during growth in the dark (●—●), light (●—●) and after transfer from dark to light (○―○) as indicated by the arrow.

RNA is a kinetic rather than an absolute effect. The rate of accumulation of the chloroplast RNA is 5 μg/day in the dark compared with 15 μg/day in the light. However, the accumulation continues for a longer period in the dark, and levels comparable to the light-grown content are finally reached (fig 3). The differential effect of light on cytoplasmic and chloroplast RNA is also seen when the synthesis of the different RNAs is determined in excised cotyledons by the incorporation of radioactive precursors.

### Table 1. Effect of Light on the Synthesis of RNA

<table>
<thead>
<tr>
<th></th>
<th>Total RNA</th>
<th>10^2 cts per min per 25 μg total RNA</th>
<th>Chloroplast RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>32P orthophosphate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>67.0</td>
<td>43.9</td>
<td>14.2</td>
</tr>
<tr>
<td>Light</td>
<td>88.5</td>
<td>52.0</td>
<td>24.8</td>
</tr>
<tr>
<td>% Stimulation by light</td>
<td>32</td>
<td>19</td>
<td>75</td>
</tr>
<tr>
<td><strong>14C bicarbonate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>2.94</td>
<td>2.12</td>
<td>0.49</td>
</tr>
<tr>
<td>Light</td>
<td>6.30</td>
<td>3.74</td>
<td>1.86</td>
</tr>
<tr>
<td>% Stimulation by light</td>
<td>114</td>
<td>76</td>
<td>280</td>
</tr>
</tbody>
</table>
Actinomycin D
Averages
5-Fluouracil
triazole
0.51
Cycloheximide
Streptomycin
100
1001
-22
control
the
calculate
2
synthesis
of
radioactivity
RNA
Inhibition
effect,
and
5-fluouracil
RNA
more
inhibited
these
3
inhibition
68
selective inhibition
at
I
not
small
to
\text{torv}
effect
of
RNA
is
suitable
stimulation
chloroplast
(280 \%)
and
cytoplasmic
(76 \%)
RNA
corporation
(table I).

Effect of Inhibitors. Low concentrations of chloramphenicol almost completely prevent the synthesis of chloroplast RNA while having no inhibitory effect on cytoplasmic RNA synthesis; in fact a small stimulation of cytoplasmic RNA synthesis is consistently observed (fig 4, table II). Streptomycin shows a similar selective inhibition, although not as clear cut as chloramphenicol. Cycloheximide at 1 \(\mu\)g/ml inhibits the synthesis of both chloroplast and cytoplasmic RNA, but at 0.5 \(\mu\)g/ml a clear selective inhibition of chloroplast RNA synthesis is seen, 68 \% and 85 \% inhibition compared to 6 \% inhibition of cytoplasmic RNA synthesis. With these 3 protein synthesis inhibitors the synthesis of the smaller ribosomal-RNA (0.70 and 0.56 M) is inhibited more than the synthesis of the larger ribosomal RNA (1.3 M and 1.1 M).

Neither of the 2 RNA synthesis inhibitors, 5-fluouracil and actinomycin D, show any striking differential effect, but the synthesis of cytoplasmic 1.3 M RNA is consistently inhibited more than that of the chloroplast RNA by 5-fluouracil (table II). Inhibition of 0.70 M RNA was not determined since the radioactivity in this region was broader and not coincident with the 265 m\(\mu\) optical density peak after 5-fluouracil treatment (fig 4). Although actinomycin D inhibited the synthesis of all the RNA components, synthesis of the chloroplast 0.56 M RNA was consistently the least inhibited. With these 2 inhibitors of RNA synthesis it is noticeable that the synthesis of the larger ribosomal component is inhibited more than that of the smaller.

Discussion

During the growth of etiolated tissue proplastids develop to a size comparable with the mature chloroplast but have a very characteristic internal structure.

### Table II. Inhibition of Cytoplasmic and Chloroplast RNA Synthesis

<table>
<thead>
<tr>
<th>Inhibitor ((\mu)g/ml)</th>
<th>1.3 M</th>
<th>0.70 M</th>
<th>1.1 M</th>
<th>0.56 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol 100 (^1)</td>
<td>-22</td>
<td>6</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>300</td>
<td>-8</td>
<td>-1</td>
<td>91</td>
<td>100</td>
</tr>
<tr>
<td>1000</td>
<td>62</td>
<td>71</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin 100</td>
<td>5</td>
<td>7</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>500</td>
<td>10</td>
<td>16</td>
<td>66</td>
<td>83</td>
</tr>
<tr>
<td>Cycloheximide 0.5 (^2)</td>
<td>6</td>
<td>6</td>
<td>68</td>
<td>85</td>
</tr>
<tr>
<td>1.0 (^2)</td>
<td>53</td>
<td>69</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>3-Amino-1,2,4 triazole 2</td>
<td>-10</td>
<td>-1</td>
<td>-1</td>
<td>-21</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>-3</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>5-Fluouracil 50</td>
<td>70</td>
<td>...</td>
<td>57</td>
<td>34</td>
</tr>
<tr>
<td>100</td>
<td>80</td>
<td>...</td>
<td>67</td>
<td>49</td>
</tr>
<tr>
<td>162 (^3)</td>
<td>85</td>
<td>...</td>
<td>62</td>
<td>54</td>
</tr>
<tr>
<td>Actinomycin D 10 (^4)</td>
<td>79</td>
<td>68</td>
<td>92</td>
<td>53</td>
</tr>
</tbody>
</table>

\(^1\) Averages from 2 separate experiments.

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![Fig. 4. Inhibition of synthesis of RNA components. Cotyledons from 3 day radish seedlings were excised and incubated in the absence (A) and presence of 100 \(\mu\)g/ml chloramphenicol B, 0.5 \(\mu\)g/ml cycloheximide C) and 162 \(\mu\)g/ml 5-fluouracil D) for 1 hr. \(^{32}\)P orthophosphate was then added, and the incorporation during the following 6 hr was used as a measure of RNA synthesis. Total RNA was prepared and fractionated by gel electrophoresis for 3.5 hr.](https://www.plantphysiol.org)
the prolamellar body (4). Electron micrographs of such proplasts in etiolated maize (9) and in the radish (unpublished data) show many ribosome-like particles. Furthermore, 70s ribosomes, the same size as those found in the mature chloroplast, have been isolated from proplasts of dark-grown beans (3). Fractionation of the RNA of dark-grown radish cotyledons shows the presence of RNA components (1.1 M and 0.56 M) characteristic of the chloroplast (figs 2 and 3). Thus microscopy, ribosome preparations and RNA analysis all agree that chloroplast ribosomes are found in etiolated tissue.

Further development of the proplast, such as the synthesis of chlorophyll and the organization of lamellae into grana, requires light. Light appears to have little effect on the content of chloroplast ribosomes, since Jacobsen et al. (9) observed a rather lower concentration of ribosome-like particles in the mature chloroplast than in the proplast from etiolated maize, and Boardman (3) found similar ratios of 70s and 80s ribosomes from both dark and light-grown bean leaves. However, the more detailed studies presented here show a definite light effect. Comparison of light and dark-grown tissue of the same age shows that the accumulation of chloroplast RNA is stimulated by light. Growth in the light for 4 days results in the accumulation of 28 µg of chloroplast RNA per pair of cotyledons compared to 8 µg in the dark control. The accumulation is stimulated by 90% within 24 hr on transferring tissue from the dark to light (fig 3). The light requirement for chloroplast RNA accumulation is not absolute, since a slow accumulation occurs in the dark. The rate of accumulation is about one-third of that in the light, but it continues for a much longer time. The accumulation of cytoplasmic RNA, however, is very similar in light and dark, reaching a maximum content around day 4 and then decreasing. Consequently tissue grown in the dark for longer periods contains a relatively high proportion of chloroplast RNA, comparable to that of light grown tissue.

Although light stimulates the synthesis and accumulation of both cytoplasmic and chloroplast RNA, the stimulation of chloroplast RNA is about 4-fold greater than that of the cytoplasm. This 4-fold selective effect of light on chloroplast RNA is shown both by the increased accumulation of RNA on transfer from dark to light (90% chloroplast versus 22% cytoplasmic), fig 3) and by the stimulation of incorporation into RNA of (32P) phosphate (75% versus 19%, table I) and (14C) carbon dioxide (280% versus 76%, table I).

Protein synthesis by the 70s ribosomes of bacteria differs in certain respects from that of 80s ribosomes of higher organisms. Of particular interest is their different sensitivity to certain antimetabolites, such as chloramphenicol and cycloheximide. Protein synthesis by 70s ribosomes is more sensitive to chloramphenicol than is 80s directed protein synthesis (15). Since the chloroplast contains 70s ribosomes and 80s ribosomes are present in the cytoplasm, similar selective effects may be expected between chloroplast and cytoplasmic protein synthesis. Spencer (16) has shown that in vitro protein synthesis by chloroplast ribosomes is sensitive to chloramphenicol, presumably due to the high binding affinity of 70s ribosomes for this antibiotic (1). Chloramphenicol inhibits the synthesis of nitrite reductase, which is located in the chloroplast, much more than the cytoplasmic synthesis of nitrate reductase (12, 13). Similarly the synthesis of other chloroplast proteins, ribulose-1,5 diP carboxyylase, NADP-glyceraldehyde-3-P dehydrogenase and Fraction I protein are inhibited by chloramphenicol but not by cycloheximide (15). Inhibition of protein synthesis, however, invariably results in the inhibition of RNA synthesis (6), so that these selective inhibitors of protein synthesis might be expected to show a similar selective inhibition of RNA synthesis. Chloramphenicol does indeed selectively inhibit the synthesis of chloroplast ribosomal RNA with no effect on cytoplasmic RNA synthesis (table II). Similar selective inhibition is shown by streptomycin. Such results suggest at least that the proteins of the chloroplast ribosome are synthesized within the chloroplast on 70s ribosomes.

Cycloheximide on the other hand, which inhibits protein synthesis by 80s but not by 70s ribosomes (5), should not interfere with chloroplast protein synthesis. Cycloheximide inhibits the synthesis of nitrate reductase more than nitrite reductase, and the inhibition of non-chloroplast proteins such as isocitrate lyase (D. Graham and R. M. Smillie, personal communication) while not inhibiting the chloroplast proteins ribulose-1,5 diP carboxyylase, NADP-glyceraldehyde-3-P dehydrogenase and Fraction I protein (15). In contrast to experiments with animal cells (5), in plant tissues cycloheximide markedly inhibits the accumulation of radioactive precursors into RNA as well as into protein (6, 10). At concentrations of 1 to 2 µg/ml total RNA synthesis is severely impaired (6, 10) (table II), but at lower concentrations there is a large inhibition (68% and 85%) of accumulation of chloroplast RNA with only a small effect (6% and 6%) on cytoplasmic RNA. This suggests that some 80s protein synthesis, extremely sensitive to cycloheximide, is necessary for the accumulation of chloroplast ribosomal RNA in addition to the 70s protein synthesis as indicated by the chloramphenicol inhibition. Such results suggest that the nucleus exercises some control over chloroplast development.

The herbicide, 3-amino-1,2,4 triazole, which inhibits the formation of normal chloroplasts and causes the complete loss of chloroplast ribosomes in light grown wheat (2), has no effect on the synthesis of chloroplast RNA under the conditions of these experiments (table II).

Synthesis of 1.3 M cytoplasmic RNA is inhibited more than that of chloroplast RNA by 5-fluorouracil, and both 5-fluorouracil and actidin inhibit
the synthesis of the smaller ribosomal RNA much less than the larger component. In particular the small chloroplast component (0.56 m) is relatively insensitive to actinomycin inhibition. This greater sensitivity of larger ribosomal RNA to RNA synthesis inhibitors contrasts with the greater sensitivity of the smaller ribosomal RNAs towards protein synthesis inhibitors.

The large selective effect of light on chloroplast RNA synthesis, and the varying degrees of differential inhibition of the synthesis of chloroplast and cytoplasmic RNA illustrate that compartmentalization of RNA synthesis occurs within the plant cell, which suggests that chloroplast RNA is made somewhere other than the nucleus—presumably in the chloroplast.

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Literature Cited