Induction and Regulation of Chloroplast Replication in Mature Tobacco Leaf Tissue
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Induction and Regulation of Chlorophyll Maturation in Tobacco Leaf Tissue

ABSTRACT

Chlorophyll replication was induced in mature tobacco leaf cells by culturing leaf discs on a sterile medium composed of salts and nigerose. Chlorophyll replication was monitored by measuring the level of total chlorophyll following subtraction of the chlorophyll a content. Chlorophyll replication was observed to proceed most readily when the cell enlargement occurs in both light and dark. Chlorophyll replication in tobacco disc cultures proceeds most readily when the cell enlargement occurs in both light and dark. Chlorophyll replication is dependent on the replication of chloroplast DNA, and that the replication of chloroplast DNA occurs along with the replication of chromosomal DNA. The replication of chloroplast DNA is necessary for the induction of chlorophyll replication in tobacco leaf cells.

RESULTS

There is a positive correlation between the size of mature cells and the magnitude of chlorophyll replication in response to experimental conditions. The magnitude of chlorophyll replication is also variable. Replicate experiments, however, always show the same pattern, and there is a high degree of reproducibility between experiments. The magnitude of chlorophyll replication is dependent on the replication of chloroplast DNA, and that the replication of chloroplast DNA occurs along with the replication of chromosomal DNA. The replication of chloroplast DNA is necessary for the induction of chlorophyll replication in tobacco leaf cells.
chlorophyll content per gram fresh weight decreases drastically. The final chlorophyll content is again variable, but on the average there is no net loss of chlorophyll in kinetin-treated tissue. Material on S + S does lose chlorophyll during the experiment. Chloroplast size is not significantly changed by either medium.

The effect of kinetin concentration on replication is shown in Figure 2. The response is independent of concentration within the range used. The figure also shows that the kinetin effect can be duplicated with the cytokinin, benzyladenine.

Some chloroplast replication occurs when leaf tissue is cultured on a medium containing only salts and sucrose, and the effect of sucrose concentration on replication is shown in Figure 3. Sucrose seems to be somewhat limiting at 0.5 g/liter, but not at the concentration of 5 g/liter which is usually used in these experiments. Higher concentrations may even be slightly inhibitory and seem to inhibit chloroplast replication more than cell expansion, since the PN/CS ratio drops. Experiments with various sucrose concentrations in media containing kinetin showed no evidence for synergism, but in these experiments high sucrose concentrations were not inhibitory and the PN/CS ratio did not drop. The inhibitory effect of high concentrations of sucrose, and a general paucity of knowledge concerning the relationship between cell expansion and plastid replication, suggested the use of mannitol in the medium as an osmoticum. We thought that high osmotic tension in the medium might reduce cell expansion without directly influencing chloroplast replication, thus making it possible to separate the two events. Chloroplast replication, however, seems to be more sensitive to increasing mannitol concentra
tions than in cell expansion (Fig. 4), suggesting that mannitol has other, more specific effects on cell metabolism besides its action as an osmoticum.

Two other growth substances, IAA and GA were tested for their effects on chloroplast replication (Table I). They neither stimulate plastid replication nor cell expansion as much as kinetin.

Chloroplast replication, both with and without kinetin, is strictly light dependent (Fig. 5). The cells expand considerably in the dark, but the expansion on both types of media is less pronounced in the dark than in the light. Chloroplast numbers remain essentially at the initial value in the dark, so that PN/CS ratios drop drastically. The dark-induced inhibition

**Fig. 1.** Changes in chloroplast number per cell, cell size, and the ratio between them (PN/CS) over time in mature tobacco leaf discs cultured in the light on S + S + K medium. The points in the squares give the final values for the same parameters in tissue on S + S medium.

**Fig. 2.** Effect of different concentrations of kinetin (K) and benzyladenine (BA) on chloroplast number per cell in mature tobacco leaf discs cultured in the light for 7 days.

**Fig. 3.** Effect of different sucrose concentrations in a medium without kinetin on chloroplast number per cell and on the PN/CS ratio in mature tobacco leaf discs cultured in the light for 7 days.
can be released at any time. If leaf discs are kept in the dark for 7 days and then exposed to light, the number of chloroplasts increases (Fig. 6) just as in tissue that has not been pretreated in the dark. Cell size continues to increase, but at a slower rate than chloroplast number, and the PN/CS ratio rises.

The light dependency of chloroplast replication raises the question of the role of light in this phenomenon. The most obvious interpretation is photosynthesis, but these discs are provided with sucrose in the medium so gross energy requirements are met. It is conceivable, however, that photosynthesis could provide energy that is more readily available or provide some limiting substances for chloroplast division. The involvement of photosynthesis in replication was tested with DCMU. When 10 \( \mu M \) DCMU was added to a medium containing kinetin, chloroplast replication was almost completely inhibited, but cell expansion also tended to be inhibited. Experiments with lower concentrations showed that DCMU indeed inhibits both processes but that plastid replication is more sensitive (Fig. 7).

**DISCUSSION**

Chloroplasts in palisade cells of mature tobacco leaves cover most of the available wall space, and they often appear to squeeze each other out of shape. This suggests that cell enlargement might be a precondition for chloroplast replication. Granick (3) also suggested that the number of chloroplasts per cell may be related to the available cell surface, and recently Honda et al. (4) showed a strong correlation between cell size and number of chloroplasts.

Our data indicate that cell enlargement in the light and in

![Graph](image)

**Fig. 4.** Effect of mannitol added to \( S + S + K \) medium on chloroplast number per cell and on cell size in mature tobacco leaf discs in the light for 7 days.

<table>
<thead>
<tr>
<th>Treatment in the Light</th>
<th>Percentage of Initial Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroplast No./cell</td>
</tr>
<tr>
<td>( S + S )</td>
<td>148</td>
</tr>
<tr>
<td>( S + S + )k (0.5 mg/liter)</td>
<td>279</td>
</tr>
<tr>
<td>( S + S + )IAA (0.5 mg/liter)</td>
<td>197</td>
</tr>
<tr>
<td>( S + S + )GA (0.5 mg/liter)</td>
<td>172</td>
</tr>
</tbody>
</table>

**Table 1.** Effect of Various Growth Substances on Chloroplast Replication and Cell Expansion in Mature Tobacco Leaf Discs Cultured on Sterile Media for 1 Week in the Light

![Graph](image)

**Fig. 5.** Changes in chloroplast number per cell, cell size, and PN/CS ratio in mature tobacco leaf discs cultured for 7 days in light and dark on both \( S + S \) and \( S + S + K \) media.

![Graph](image)

**Fig. 6.** Release of dark inhibition of chloroplast replication in mature tobacco leaf discs. Discs were kept in the dark for 7 days on medium with or without kinetin and then exposed to light for 7 days on the same medium.

The presence of certain minimal nutrients does induce chloroplast replication and that specific factors are not required for this process. Under these conditions, the cells in the leaf discs expand to two or three times their original size, and the number of chloroplasts increases. The curves for the processes are very similar to those for cell expansion and mitochondrial replication obtained by Juniper and Clowes (7). The addition of kinetin to the medium greatly enhances chloroplast replica-
Fig. 7. Comparison of chloroplast number per cell, cell size, and PN/CS ratio in discs cultured in the light both with and without DCMU.

It is suggested that photosynthesis is involved, although it is disturbing that DCMU also inhibits cell expansion. In some cases there is more inhibition with DCMU than occurs in darkness. If photosynthesis is required for chloroplast replication, this may also explain the observation that high concentrations of sucrose and mannitol inhibit chloroplast replication more than cell expansion (Figs. 3, 4). It has been shown (6) that even at relatively low soil moisture tension photosynthesis may be inhibited. Osmotic stress would have the same effect. It is known, however, that high sugar concentrations have a bleaching effect on chloroplasts (15). High sucrose concentrations inhibit chlorophyll synthesis in etiolated tobacco leaf discs exposed to light (8), and the higher concentrations of sucrose and mannitol caused a considerably greater loss of chlorophyll from the tissue in the present study. Although the mechanism of this sugar effect is not understood, it is likely that anything which has an adverse effect on chlorophyll metabolism will influence chloroplast behavior as well.

If chlorophyll is the pigment involved in the light effect, then etioplasts react differently than chloroplasts. Etioplasts in tissue exposed to light begin to divide immediately, whereas there is no detectable amount of chlorophyll, and consequently no photosynthesis, until between 16 and 24 hr after exposure to light (1). Recent experiments have shown this to be a low energy response, so it is possible that the light-dependent response in mature leaves is a phytochrome-mediated phenomenon. It is expected that work in progress will solve this problem.

The light effect also complicates the relationship between cell size and chloroplast replication, because cell enlargement occurs in the dark while replication does not (Fig. 5). The kinetin stimulation of cell enlargement in the dark is almost eliminated, and cell enlargement on both types of media is less than in the light. This experiment demonstrates that cell enlargement does not always induce chloroplast replication. It remains true, however, that replication does not occur in the absence of cell enlargement.

When the cells are exposed to light after they have doubled in size in the dark, the stimulus for chloroplast replication is not a gradual increase in available space, but the sudden removal of a block in a situation where space is not limiting. This relationship between cell enlargement and chloroplast replication suggests it might be possible to induce synchronous replication of chloroplasts, and the precise regulation would be a definite advantage in experiments concerned with chloroplast autonomy.

LITERATURE CITED


