Activation of 80S Maize Ribosomes by Red Light Treatment of Dark-grown Seedlings

R. L. TRAVIS, JOE L. KEY, AND CLEON W. ROSS
Department of Botany, University of Georgia, Athens, Georgia 30602

ABSTRACT

The in vitro protein synthetic activity of 80S ribosomes from leaves of dark-grown corn seedlings was enhanced (at low Mg2+ levels) by a 5-minute red light treatment applied 2 hours prior to tissue harvest. The effect was completely reversed by an immediate brief far red treatment, suggesting that ribosome activation is controlled by the phytochrome system. Experiments in which the interval between light treatment and tissue harvest was shortened indicate that the response was quite rapid. The initial increase in activity was detected within 30 minutes, followed by a rapid increase during the next 1.5 hours. No further increase occurred after 2 to 3 hours.

The change in ribosome activity relates, at least in part, to an increase in the level of peptidyl-tRNA associated with ribosomes. Removal of peptidyl-tRNA from light-treated ribosomes also completely reversed the red light effect. Activation of ribosomes by 2 to 3 hours continuous white light (as previously reported) differs from red light activation in that reversal of this response requires salt washing of the ribosomes in addition to removal of peptidyl-tRNA.

In earlier communications, we reported the transformation of 80S cytoplasmic monoribosomes to polyribosomes in several plant systems in response to various stimuli. In carrot disks (3, 6), polyribosome formation was enhanced by aging (tissue excised and incubated in water). Similar results were obtained with soybean basal hypocotyl ribosomes in response to auxin (9). In the carrot and soybean systems, the increase in polyribosome level was related to an increased activity of 80S monoribosomes. The increase in monoribosome activity was associated with an increase in the level of peptidyl-tRNA associated with the ribosomes. Removal of peptidyl-tRNA from the ribosomes completely reversed the activation response in each case.

Williams and Novelli (13) reported a light-enhanced increase in the level of polyribosomes in dark-grown maize seedlings with an associated increase in the level of in vitro leucine incorporation and poly(U)-directed phenylalanine incorporation. Red light was most effective in promoting ribosome activity. We also reported on the light activation of cytoplasmic monoribosomes in dark-grown corn leaves (11). In the corn leaf experiments, white light (approximately 1000 ft-c) was applied for 2 to 3 hr prior to ribosome preparation. This light response differs, however, from ribosome activation in the carrot and soybean systems in that removal of peptidyl-tRNA from the ribosomes did not completely reverse the light activation. Salt washing (0.5 M KCl) was required, in addition to stripping of peptidyl-tRNA, to effect complete reversal of the light response. In this communication, we have examined further the activation of cytoplasmic ribosomes by light. Emphasis is placed on light quality required to effect activation.

MATERIALS AND METHODS

Preparation of 80S monoribosomes, nitrogen treatments, and poly(U)-directed phenylalanine incorporation studies were done as previously described (9).

Growth of Plant Materials. Zea mays (var. WF9 × M14) was grown in vermiculite for 12 days in a light-proof controlled environment chamber. Fully expanded second leaves were used in all experiments. Twelve-day-old seedlings were used to minimize control ribosome activity. Although ribosomes lose activity rapidly if seedlings are maintained in darkness for 8 to 10 days a N2-gas treatment applied to the leaves immediately before ribosome preparation will further reduce activity (5, 11). By 12 days, activity has declined to a level where it is no longer affected by an anaerobic environment.

Light Treatments. Light treatments were applied in a controlled environment growth chamber. Except for the time course experiments, all plants were returned to darkness for 2 hr prior to ribosome preparation. Red light was supplied by two 250-w incandescent bulbs over a filter consisting of four layers of Dennison (Dennison Mfg. Co., Framingham, Mass.) red cellophane. Maximum transmission was at 675 nm. Far red light was obtained from the same light source utilizing a Westlake (Westlake Plastic Co., Lenni, Pa.) far red filter. Heat was filtered from the system by placing a glass pan containing 2 inches of water between the filter and light source. White light was supplied by a combination of 8 Sylvania fluorescent tubes and four 100-w incandescent bulbs. Blue and green light were supplied by a bank of eight Sylvania fluorescent tubes over filters of Dennison blue and green cellophane, respectively. Maximum transmission was at 430 nm for the blue filter and 550 nm for the green filter. Irradiance levels for the various light treatments are listed in Table II.

Amino Acid Incorporation Studies. Five A560 units of ribosomes were incubated for 20 min at 37 C with 1 µc of 8H-amino acid mixture (reconstituted protein hydrolysate). Magnesium concentration was 10 mM. Other constituents of the reaction mixture were as previously described (11) except that poly(U) was omitted. The reaction mixture was then layered over a linear 10 to 34% sucrose gradient and centrifuged at 39,000
rpm (Spinco SW 41 rotor) for 180 min at 3 C. The distribution of ribosomes in the gradient was monitored with a continuous recording ISCO Model D density gradient fractionator. Tendon fractions were collected. The samples were precipitated with an equal volume of 10% trichloroacetic acid and, collected on Whatman GFA glass fibre disks, washed with 5% trichloroacetic acid and dried. Radioactivity was determined in a Packard liquid scintillation spectrometer.

**Formation of H-Pep tidylpuromycin.** Peptidylpuromycin formation was studied by the method of Evins (2) with the following modifications. Five A_{260} units monoribosomes were incubated for 30 min at 37 C in a 1-ml reaction mixture (11) containing 5 μg of H-puromycin (3000 mc/mmole) and 10 μg of carrier puromycin. The reaction was terminated by the addition of 5 ml of 10% trichloroacetic acid, filtered on a Whatman GFA glass fibre disk, washed with 50 ml of 5% trichloroacetic acid, dried, and counted in a Packard liquid scintillation spectrometer.

**RESULTS AND DISCUSSION**

The effect of a brief red light treatment on the activity of 80S cytoplasmic ribosomes is shown in Table I. At a low Mg^{2+} concentration (10 mm) ribosomes from red light-treated leaves were approximately twice as active as at poly(U)-directed phenylalanine incorporation as ribosomes from dark-control leaves. As the Mg^{2+} concentration was increased to 12.5 mm, the level of enhancement decreased. Repeated experiments have shown that little variability occurs in the relative enhancement by red light of ribosome activity at low Mg^{2+} levels (10 mm). While actual levels of activity will vary between experiments, ribosomes from red light-treated tissue are consistently twice as active as ribosomes from dark-control leaves. Conversely, at high Mg^{2+} levels (30 mm), phenylalanine incorporation will vary, often by as much as 25% within and between experiments. This type of variability is evident in the data presented in Table I. Repeated experiments, however, indicate that levels of incorporation at 30 mm Mg^{2+} are similar for this set of treatments. Table I shows further that a N_{2} gas pretreatment applied immediately before ribosome preparation reversed the effect of red light. A similar pretreatment applied to dark-control leaves had no effect on ribosome activity. An 8-min far red treatment immediately following red light reversed the red light effect. As the interval between red light application and far red treatment was increased the level of far red reversibility rapidly declined (Fig. 1).

**Table 1. Poly(U)-directed \(^{14}\)C-Phenylalanine Incorporation by 80S Ribosomes from Leaves of Dark-grown Corn Seedlings**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mg^{2+} Conc. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>rpm/Asc unit ribosome:30 min</td>
</tr>
<tr>
<td>Dark control</td>
<td>4260</td>
</tr>
<tr>
<td>Dark + nitrogen(^1)</td>
<td>4240</td>
</tr>
<tr>
<td>Red (5 min)</td>
<td>8510</td>
</tr>
<tr>
<td>Red (5 min) + far red (8 min)</td>
<td>4110</td>
</tr>
<tr>
<td>Red (5 min) + nitrogen(^1)</td>
<td>4385</td>
</tr>
</tbody>
</table>

\(^1\) Two hr after treatment leaves were excised and submerged in water for 1 hr with N_{2} gas bubbled through the water. These leaves were then illuminated by red light for 30 min.

![FIG. 1. Influence of increasing time interval between red and far red light treatments on the reversibility of red light-enhanced monoribosome activation. Twelve-day-old dark-grown seedlings were given a 5-min red light treatment followed by either an immediate 8-min far red treatment (zero time control) or a similar far red treatment applied 30 min to 2 hr later. Except during exposure periods, all seedlings remained in darkness. Monoribosomes were prepared from second leaves.](Diagram)

Similar Mg^{2+} responses have been reported for ribosome preparations from fungal (8), mammalian (1), and other higher plant systems (6, 9, 11). In all cases, the enhanced activity at low Mg^{2+} levels of ribosomes from treated relative to control tissue was related to an increase in the level of peptidyl-tRNA associated with ribosomes from the treated tissue. Van der Zeijst et al. (12) recently described two types of poly(U)-directed phenylalanine incorporation by yeast ribosomes. At low Mg^{2+} levels phenylalanine was incorporated only into pre-existing polypeptides. This type of incorporation is mediated by the interaction between ribosomes carrying endogenous peptidyl-tRNA and poly(U). At high Mg^{2+} levels true poly(U)-dependent oligophenylalanine synthesis occurred.

Under anaerobic conditions initiation of peptide synthesis in plant tissue is prevented while read-out and release of nascent protein continues, leading to the production of free or run-off ribosomes (4). This effect is manifested by a total loss of polyribosomes from the ribosome population leaving only monoribosomes and ribosomal subunits. Thus, the N_{2} gas treatment effectively strips ribosomes of peptidyl-tRNA. The results presented in Table I suggest that red light-mediated ribosome activation is also related to an increased level of peptidyl-tRNA associated with the ribosomes. When peptidyl-tRNA was removed from treated ribosomes, the effect was reversed. This observation was further extended by comparing the rates of incorporation of a mixture of H-amino acids into nascent polypeptide (Fig. 2). Monoribosomes from red light-treated leaves incorporated twice as much radioactivity into nascent protein as did control ribosomes. Again the response was completely reversed by an immediate far red treatment. Actual levels of peptidyl-tRNA can be measured by incubating ribosomes with H-puromycin. Ribosomes containing peptidyl-tRNA bind puromycin, a peptide bond is formed between puromycin and the nascent peptide, and the peptide is subsequently released as peptidylpuromycin. Each ribosome can form only a single molecule of peptidylpuromycin. Ribosomes
peptidyl-puromycin as ribosomes from dark-control leaves (Table II). Far red light applied immediately after the red light treatment reversed the red light effect.

Red light-activated ribosomes differ from ribosomes isolated from leaves given 2 to 3 hr continuous light prior to extraction (11). As noted in the introduction, ribosomes from leaves treated for 2 to 3 hr with continuous light require a salt washing treatment in addition to removal of peptidyl-tRNA to completely reverse the light enhancement of amino acid incorporation. Further, a 2 to 3 hr light treatment caused a decrease in the Mg\(^{2+}\) level required for optimal phenylalanine incorporation, whereas a brief red or white light treatment did not affect the Mg\(^{2+}\) optima (activity levels are similar for ribosome preparations for 20 to 30 mM Mg\(^{2+}\), data not included). The reason for the differential response of the ribosomes to a brief light treatment versus 2 to 3 hr continuous light is not fully understood. The ease of reversal of the response by either a far red light or N\(_2\)-gas pretreatment suggests, however, that an early initial response at the level of the ribosome is controlled by phytochrome.

The effectiveness of red light in activating the ribosomes and the subsequent reversal by a brief far red light treatment suggests that ribosome activation may be under the control of the phytochrome system. To further test this hypothesis, the effectiveness of green and blue light in activating ribosomes was tested (Table III). As in the previous experiment, the seedlings were returned to darkness for 2 hr after light treatment and before preparation of ribosomes. Green light increased only slightly (9%) phenylalanine incorporation by the ribosomes. Blue light produced a significant (27%) although less pronounced enhancement than did red light. A 1-min red light treatment produced only one-third the activation obtained with a 5-min exposure. Five minutes of white light applied 2 hr prior to ribosome preparation was as effective as 5 min red light in ribosome activation.

The time course of ribosome activation by red light is presented in Figure 3. The seedlings were given a 5 min red light treatment then returned to darkness for various time intervals up to 3 hr prior to ribosome preparation. There was a rapid increase in ribosome activity without a significant lag (i.e. within 30 min after light treatment). Ribosome activity continued to increase rapidly up to 2 hr after light treatment. After 2 hr no further increase in activity occurred. In subsequent experiments, the time course was continued up to 12 hr with no further increase in ribosome activity.

In an earlier communication, we reported that light enhanced the development of polyribosomes in dark-grown corn leaves (10). Although there was a significant increase in polyribosome level during the initial 2-hr light treatment, the major increase occurred after 2 hr in light. Williams and Novelli (13) reported a 2-hr lag between light treatment and any measurable increase in the ability of ribosomes from corn seedlings to incorporate \(^{14}\)C-leucine into acid-insoluble protein. These results reflect the time requirement for the development of

---

**Fig. 2.** Effect of red and far red light on the ability of cytoplasmic monoribosomes to incorporate \(^3\)H-amino acids into nascent protein. A: Monoribosomes from second leaves of 12-day-old dark-grown corn seedlings; B: monoribosomes from similar leaves harvested 2 hr after a 5-min red light treatment; C: monoribosomes from similar leaves to which an 8-min far red treatment was applied immediately following exposure to red light. Monoribosome peak is denoted by 80.

**Table II.** Effect of Light Treatment on \(^3\)H-Peptidylpuromycin Formation by 80S Ribosomes from Dark-grown Corn Leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^3)H-Peptidylpuromycin (pmoles/mg RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>71</td>
</tr>
<tr>
<td>Red (5 min)</td>
<td>125</td>
</tr>
<tr>
<td>Red (5 min) + far red (8 min)</td>
<td>76</td>
</tr>
</tbody>
</table>

---

**Table III.** Effect of Light Quality on \(^{14}\)C-Phenylalanine Incorporation by Ribosomes from Leaves of Dark-grown Corn Seedlings

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>Irradiance ((\mu)E cm(^{-2}))</th>
<th>Enhancement (%) increase over dark control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green (5 min)</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>Blue (5 min)</td>
<td>120</td>
<td>27</td>
</tr>
<tr>
<td>Red (1 min)</td>
<td>300</td>
<td>36</td>
</tr>
<tr>
<td>Red (5 min)</td>
<td>300</td>
<td>93</td>
</tr>
<tr>
<td>White (5 min)</td>
<td>300</td>
<td>116</td>
</tr>
</tbody>
</table>

---

**Fig. 3.** Effect of red light on the in vitro protein synthetic activity of cytoplasmic monoribosomes. Corn seedlings were grown for 12 days in darkness, given 5 min red light and returned to darkness for various time intervals before ribosomes were prepared from second leaves. One \(A_{164}\) unit of monoribosomes was incubated with 150 \(\mu\)g poly(U) and 0.5 \(\mu\)g of \(^{14}\)C-phenylalanine for 30 min at 37 C.
polyribosomes prior to leucine incorporation. Recently, Pine and Klein (7) reported that a 10-min red light treatment induced cytoplasmic polyribosome formation in etiolated bean leaves. Our results indicate that during the initial lag period, prior to the appearance of polyribosomes, the monoribosomes are being "activated" or altered by light. That polyribosomes probably begin to accumulate even before 2 hr is indicated by the early increase in ribosome activity (30 min) and the significant increase in corn leaf polyribosomes by 2 hr as previously reported (10).

CONCLUSIONS

The in vitro protein synthetic activity of cytoplasmic monoribosomes at low Mg\textsuperscript{2+} levels was enhanced by red light. Since the response was completely reversed by a brief far red light treatment, this type of ribosome activation is apparently under the control of the phytochrome system. The absence of a response to green light and only a slight response to blue light further supports this conclusion.

The change in ribosome activity relates, at least in part, to an increase in the level of peptidyl-tRNA associated with the ribosomes. In this regard the response of corn ribosomes to red light is similar to other types of ribosome activation that we have observed (e.g. carrot ribosome activation in response to aging and soybean hypocotyl ribosome activation in response to auxin). However, the nature of the initial change at the level of the ribosome is yet to be elucidated. The increased level of peptidyl-tRNA associated with ribosomes in response to the treatment, either in this system or the other higher plant systems we have studied, is probably a secondary response. Acrylamide gel electrophoresis profiles of carrot ribosomal proteins indicated that at least two additional protein components are associated with ribosomes from aged tissue relative to ribosomes from fresh tissue (6). Similar studies are in progress to determine whether red light activation of corn ribosomes is associated with a change in the ribosomal protein complement. Any change in ribosome-associated proteins which increased the ability of the ribosome to participate in protein synthesis would effectively increase the level of peptidyl-tRNA associated with the ribosomes.

Although RNA synthesis, presumably mRNA, is essential for the monoribosome to polyribosome transformation (3, 9), the changes in ribosome activity noted here, and in other systems (1, 6, 8, 9, 11), were independent of, and in addition to, possible changes in mRNA synthesis or availability. This can be concluded since ribosome activation was measured in an in vitro assay system in the presence of an excess of synthetic mRNA.

LITERATURE CITED