Red-Far Red Reversible Effect on Polysome Formation in the Embryos of Pinus thunbergii Seeds

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ABSTRACT

Polysome formation in the embryos of Pinus thunbergii seeds was studied. Free ribosomes were dissociated to smaller subunits in a high salt buffer, but the complex ribosomes were not. The free ribosomes could be distinguished from monomer ribosomes derived from polysomes after RNase treatment. The monomer ribosomes present in the embryos of the dark-imbibed seeds were predominantly free ribosomes; very small quantities of polysomes could be detected in the embryos from dark-imbibed seeds. Such polysomes remained at a very low level during dark imbibition at least for a month. The level of polysomes increased 4 hours after a brief exposure to red light. The effect of red light on polysome formation was partially reversed when followed by far red light irradiation.

Monomer ribosomes can be distinguished from complex ribosomes carrying mRNA and peptidyl-tRNA in Hela cell (9), E. coli (1) and a higher plant (6), since the former are easily dissociated to two ribosomal subunits in a high salt buffer, but the latter are not. High resolution of polysomes in sucrose density gradient analysis is obtained by these techniques in combination with RNase hydrolysis of postmitochondrial supernatant.

It was reported previously that polysome formation in the embryos of pine seeds was detected within 24 hr after red light irradiation (8), but during the dark imbibition period no appreciable changes in ribosomal profiles were observed (3, 8).

The present report deals with (a) ribosomal profiles in the embryos of P. thunbergii seeds during dark imbibition of intact seeds; (b) polysome formation after red light irradiation; and (c) the possibility of phytochrome action on polysome formation.

MATERIALS AND METHODS

Seeds. Pinus thunbergii seed cones were collected at Kantoh Forest Tree Breeding Station in Ibaraki, Japan in November 1973. The cones were brought into a dark room and dried at 40°C to isolate the seeds. The seeds, unexposed to light, showed no germination (4, 8). These seeds were stored in the dark at 4°C until used in the experiments.

Lights. Red light (λ_max = 660 nm, 10^4 erg/cm^2-sec) and far red light (λ_max = 730 nm, 10^4 erg/cm^2-sec) were obtained from interference filters as described previously (4). To study the effects of red or far red light, the dark-imbibed seeds were irradiated and returned to the darkness. Thereafter, the embryos were isolated under the green safelight (8).

Preparation of Postmitochondrial Fraction. Fifteen embryos were homogenized in a Teflon homogenizer for about 30 sec in one of the following buffers: low salt buffer which contained 0.003 M MgCl_2, 0.024 M KCl, 0.02 M HEPES (pH 7.8), and 5% (w/v) sucrose (8); or high salt buffer which contained 0.05 M MgCl_2, 0.5 M NaCl, 0.02 M HEPES (pH 7.8), and 5% (w/v) sucrose. The homogenate was centrifuged at 13,000g for 15 min at 0°C, and the supernatant was named “postmitochondrial fraction.”

Preparation of Polysome Fraction. To study dissociation characteristics in high salt buffer of the monomer ribosomes derived from polysomes after RNase treatment, the following experiments were carried out. The postmitochondrial fraction (13,000g supernatant), in low salt buffer from 100 embryos from seeds germinating 7 days under the white fluorescent light, was layered on 2 ml of 60% (w/v) sucrose in low salt buffer, and was centrifuged at 200,000g (7) for 90 min (Hitachi 65P ultracentrifuge with an RP-65 rotor) to obtain ribosomal pellet. The pellet, resuspended in 1 ml of low salt buffer, was fractionated by a sucrose density gradient ranging 7.5 to 60% (w/v) in the same low salt buffer with a Hitachi RPS-40T rotor at 130,000g for 3 hr. Thereafter, the polysome fraction was collected by an ISCO density gradient fractionator. The collected polysome fraction was centrifuged at 225,000g for 3 hr with an RP-65 rotor again to obtain polysome pellet.

Sucrose Density Gradient Analysis. Immediately after preparation of the postmitochondrial fraction or polysome fraction, or after incubation with the pancreatic RNase (Miles) (1 ìg/ml) at 37°C for 5 min, the suspension was layered on 12 ml of sucrose density gradient ranging from 7.5 to 60% (w/v) in low salt buffer or high salt buffer. The gradients were centrifuged for 3 hr at 130,000g at 10°C with an RPS-40T rotor, then they were fractionated by an ISCO density gradient fractionator monitoring at 254 nm, with a 10 inch recorder connected.

Estimation of Polysome Levels. Relative ribosomal amounts were estimated by areas of corresponding absorbance peaks.

All experiments were repeated at least three times; similar results were obtained each time.

RESULTS AND DISCUSSION

Ribosomes of embryos isolated from dark-imbibed seeds appeared to be a homogeneous preparation of monomer ribosomes in low salt buffer, as shown in Figure 1A (8). However, in high salt buffer, ribosomes of the same embryos were dissociated into ribosomal subunits (Fig. 1B). When the sample was treated with...
pancreatic RNase (1 μg/ml) at 37 C for 5 min in high salt buffer, a little monomer ribosome peak was detected (Fig. 1C). Separation of the larger subunit peak (Fig. 1D) was much sharper after the incubation than without incubation (Fig. 1B). The incubation at 37 C alone in high salt buffer did not produce the monomer ribosomes (Fig. 1D). Since the monomer ribosome was detected only after RNase treatment, the monomer ribosomes in Figure 1C were confirmed to be those of polysomal origin. The same sort of experiments was done on light-treated seed embryos, and the results were the same except for changes in quantities (not shown).

The responses of polysomal fraction to high salt buffer were further characterized (Fig. 2). The purified polysomes in high salt buffer showed a profile as in Figure 2A. When the polysome fraction was incubated with the pancreatic RNase at 37 C, monomer ribosomes were produced (Fig. 2B). In contrast to free monomer ribosomes, those induced after RNase treatment did not dissociate into ribosomal subunits, even in high salt buffer (Fig. 2B). The results in Figures 1 and 2 demonstrate that by using high salt buffer and pancreatic RNase treatment a good operational criterion for separating free and complex ribosomes with a high resolution is possible in this system. Very small quantities of polysomes could be determined after conversion of polysomes to monomer ribosomes with RNase in high salt buffer.

During dark imbibition of Pinus thunbergii seeds, polysomes were scarcely detected in the previous experiments using low salt buffer (8). The more sensitive method mentioned above demonstrated that during dark imbibition of seeds a very small quantity of polysomes was present (Fig. 3D). The level of polysomes in the embryos of dark-imibed seeds was maintained at a ground level during prolonged dark imbibition for 5 days to 35 days (Fig. 4). Although Fountain and Bewley (2) indicated that protein synthesis increased during the imbibition phase in dormant lettuce seeds, the present experiments demonstrated that a constant polysome level in pine embryos in the dark imbibition was maintained at least for a month.

Whether polysomes are present in embryos from dry seeds and during the first several hours of imbibition still remains to be studied, inasmuch as ribosomes prepared from such embryos were not dissociated into ribosomal subunits in high salt buffer. This
suggested that monomer ribosomes in such embryos (5, 8) are in a specific state. Our preliminary experiments indicate that the monomer ribosomes were dissociated into subunits only after incubation at 37°C in high salt buffer.

After the irradiation of red light at 5 or 30 days of dark imbibition, the polysome level showed an increase with a time lag of about 4 hr (Fig. 4). The time lag for polysome formation after red light irradiation is apparently much smaller in the high salt buffer experiments than in the previous low salt buffer experiments (8), when examined by our sensitive methods to detect polysome level. The time length of dark imbibition before red light irradiation did not affect the length of the lag period before the increase of polysomes (Fig. 4).

In P. thunbergii seeds isolated from cones in the dark, a promoting effect of red light on germination was not reversed by an equal quantity of far red light (4). By increasing the quantity of far red light, the red light effect was reversed (unpublished results).

With these red-far red reversible seeds, effects of red and far red light on polysome formation were determined. The results indicate that the new method using high salt buffer plus pancreatic RNase is sensitive enough to detect differences in levels of polysomes after various light treatments (Fig. 5). The effect of red light on polysome level was partially reversed by subsequent far red light irradiation (Table I). Therefore, such polysome formation appears to be mediated by phytochrome action.

The new sensitive method described in this report can be used in other systems where low levels of polysomes are suspected.
POLYSOMAL LEVELS IN PINE SEED GERMINATION

Table I. Red-Far Red Reversible Effects on Polysome Level in *Pinus thunbergii* Seed Embryos

After light treatment at 7 days of dark-imbibition, the seeds were returned to the dark. Ribosomal profiles in high salt buffer 24 hr after light treatment were analyzed and polysome levels were estimated. Polysome levels after light treatment are averages of three replications. R: red light (λ_{max} = 660 nm, 10^4 erg/cm^2·sec); FR: far red light (λ_{max} = 730 nm, 10^4 erg/cm^2·sec).

<table>
<thead>
<tr>
<th>Light Treatments</th>
<th>Polysome Levels 24 hr after Light Treatment</th>
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<tbody>
<tr>
<td>10 min R</td>
<td>100</td>
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<tr>
<td>10 min R + 30 min FR</td>
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<td>30 min FR</td>
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LITERATURE CITED