Light regulation of specific mRNA species in *Lemna gibba* L. G-3

[polyadenylated mRNA/immunoprecipitation of translation products/precursor to small subunit of ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39)/protein synthesis]

ELAINE M. TOBIN

Biology Department and Molecular Biology Institute, University of California, Los Angeles, California 90024

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ABSTRACT Polyadenylated RNA was isolated from *Lemna gibba* L. G-3 and translated in a cell-free system from wheat germ. When plants were placed into complete darkness for 4 days, then returned to light for 18 hr, increased amounts of polyadenylated mRNA for at least two polypeptides were detected by *in vitro* translation over those amounts present in the dark. These two polypeptides have molecular weights of 32,000 and 20,000. The 20,000 dalton polypeptide was identified by immunoprecipitation as the precursor to the small subunit of the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase [3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39]. The polyadenylated mRNA that codes for the small subunit is not detectable by immunoprecipitation of translation products in the dark-treated tissue. Plants growing in the light actively synthesize both subunits of ribulose-1,5-bisphosphate carboxylase, but synthesis of these proteins was found to be greatly diminished in plants placed in darkness for 4 days. These results indicate that white light can dramatically affect the steady-state levels of specific polyadenylated mRNAs.

In previous work I have shown that white light induces qualitative changes in the mRNA complement of *Lemna gibba* which can be detected by translation of isolated mRNA and characterization of the polypeptide products by gel electrophoresis (1). There since have been a number of other reports of white light effects on mRNA in higher plants (2-5) and in a fungus (6). Giles *et al.* (3) presented evidence for an effect of light on the ability of mRNA to be translated in beans. In the cases of *Spirodela*, barley, peas, and *Fusarium*, the effect of light has been on the levels of mRNA (2,4-6).

In this report I will present evidence for the effect of white light on the mRNA of *Lemna* for a specific protein, the small subunit of ribulose-1,5-bisphosphate carboxylase [RuP₂; 3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39], as well as on the mRNA for another, as yet unidentified, protein. RuP₂ carboxylase is a soluble photosynthetic enzyme found in chloroplasts. This enzyme has a multimeric structure composed of two kinds of subunits. The large subunit is coded for by the chloroplast DNA (7) and synthesized in the chloroplasts (8). The small subunit is found as a part of the complete enzyme in the chloroplast, but it is coded for by the nuclear genome (9) and synthesized in the cytoplasm (10, 11). The small subunit has a molecular weight of 12,000-14,000 in higher plants. In *Chlamydomonas* it has been reported to be synthesized as a part of a larger molecular weight precursor polypeptide that is then cleaved to its final size (12). A 20,000-dalton polypeptide, immunoprecipitated by anti-small subunit serum, has also been reported in wheat (11) and in pea (4, 13). I have used a specific antibody against the small subunit of RuP₂ carboxylase from *Lemna* to show that such a large molecular weight precursor exists in *Lemna* and to identify the mRNA for this protein by immunoprecipitation of its translation product.

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MATERIALS AND METHODS

Growth of Plants. *Lemna gibba* L. G-3 were cultured aseptically in E medium (14) with 1% sucrose in a growth chamber under constant light at 27° ± 1°. *Nicotiana tabacum* L. 'Samsun' were grown in a greenhouse in Vermiculite.

Isolation of Poly(A) RNA. *Method 1.* Total cellular RNA was isolated by the phenol extraction procedure of Tobin and Klein (1). The cells were ground with buffer and extracted with phenol, and the nucleic acids were precipitated with ethanol. The RNA was further purified by extraction with 2-methoxyethanol and precipitation with cetyltrimethylammonium bromide.

Method 2. Polysomal RNA was isolated by the procedures of Jackson and Larkins (15) and Goldberg *et al.* (16) with some modifications. *Lemna* tissue was collected, rinsed with distilled water, and frozen in liquid nitrogen. The frozen tissue was ground to a powder with a mortar and pestle and then ground in a Waring Blendor for 20 sec with 2 vol of 0.5 M sucrose/0.4 M KCl/0.2 M Tris-HCl, pH 9.0/25 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)/35 mM MgCl₂/5 mM mercaptoethanol/0.07-0.1% diethylpyrocabonate. Such low levels of diethylpyrocabonate have been successfully used to recover undegraded RNA in *Drosophila* (17), and I have found that it greatly decreases RNA degradation in *Lemna*. After a low-speed centrifugation (1000 × g, 7 min), the supernatant was adjusted to 2% Triton X-100 and centrifuged at 16,000 × g for 20 min. This postmitochondrial supernatant was layered onto sucrose pads (1.8 M sucrose/40 mM Tris-HCl, pH 9.0/30 mM EGTA/5 mM mercaptoethanol) and the polysomes were pelleted by centrifugation at 59,000 rpm for 75 min in a Ti 60 rotor. The pellets were resuspended rapidly in dilute buffer (20 mM Tris-HCl, pH 8.5). After they were fully resuspended, the buffer was adjusted to 5 mM EGTA and 20 mM MgCl₂. The resuspended polysomes were digested with proteinase K (Beckman) at 50 μg/ml in 0.1% sodium dodecyl sulfate (NaDodSO₄) for 30-60 min and the RNA was precipitated with ethanol before being applied to the oligo(dT) columns.

Poly(A) RNA was isolated from the partially purified RNAs on oligo(dT) (T-3, Collaborative Research) columns. The RNA was dissolved in binding buffer (0.4 M NaCl/0.1% NaDodSO₄/10 mM Tris-HCl, pH 7.4) and applied to the column. After extensive washing with the binding buffer, the poly(A) RNA was eluted with 10 mM Tris-HCl, pH 7.4/0.05% NaDodSO₄ and precipitated with 2 vol of cold ethanol in the presence of 0.1 M NaOAc.

The RNA preparations isolated by the two methods were analyzed by sedimentation on sucrose gradients. The resuspended polysomes (method 2) were applied to step gradients (15:30:45:60%, 2:1:1:2, in 10 mM MgCl₂/10 mM KCl/40 mM

Abbreviations: RuP₂ carboxylase, ribulose-1,5-bisphosphate carboxylase; NaDodSO₄, sodium dodecyl sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

Tris-HCl, pH 8.5, left overnight at room temperature) and centrifuged for 60 min at 45,000 rpm in an SW 60 rotor. RNA (isolated by method 1 and by method 2) that did not bind to oligo(dT)-cellulose was applied to 5–20% sucrose gradients (in 0.1 M NaCl/1 mM EDTA/0.5% NaDodSO₄/10 mM Tris-HCl, pH 7.5) and centrifuged for 45 min at 59,000 rpm in an SW 60 rotor.

Translation Assay of mRNA. Poly(A) RNA was translated in a cell-free system from wheat germ (18), using [³⁵S]methionine to label the translation products. Usually, 1 µg of RNA was used in a 50-µl reaction mixture. The translated polypeptides were characterized on NaDodSO₄/polyacrylamide slab gels. The discontinuous gel system of O'Farrell (19) was used. The gels were either 10 or 12.5% acrylamide. Known molecular weight standards were used to calibrate each gel. After electrophoresis, the gel was stained with Coomassie blue and the radioactive translation products were located by fluorography (20).

Analysis of *In Vivo* Protein Synthesis. *In vivo* protein synthesis was assayed by adding 50–100 µCi of [³⁵S]methionine to 1 g of *Lemna* growing in 10 ml of E medium. The plants were harvested after 2 hr and the soluble and particulate proteins were extracted by the method of Cashmore (21). In this procedure, tissue is ground in a glass tissue grinder with 2 vol of extraction buffer (10 mM Tris glycine/1 mM phenylmethylsulfonyl fluoride/1% mercaptoethanol, pH 8.9). After a low-speed centrifugation to remove debris, the soluble and particulate components are separated by centrifugation at 27,500 × *g* for 30 min. The particulate proteins are solubilized by heating at 98° for 2 min in the presence of the extraction buffer and 1% NaDodSO₄. The proteins were characterized by electrophoresis on slab gels as described above, and the radioactivity was located by autoradiography. The bands of radioactivity on the autoradiograms represent the proteins being actively synthesized.

Purification of the Small Subunit of RuP₂ Carboxylase. In order to prepare antibodies to the small subunit of RuP₂ carboxylase, the protein was purified in a two-step procedure involving sucrose gradients and polyacrylamide gel electrophoresis. *Lemna* plants were ground either with sand in a mortar or in a Waring Blender. Grinding buffer was either: A, 0.15 M NaOAc/25 mM Tris-HCl, pH 7.8; or B, 0.2 M NaCl/0.1 M Tris-HCl, pH 8.5 with 20% Dowex. The buffer-to-tissue ratio was 1.5:1. After the supernatant was clarified by centrifugation (10 min, 35,000 × *g*), it was concentrated 12- to 15-fold in an Amicon ultrafiltration apparatus using a PM30 membrane. The concentrated supernatant was applied to sucrose gradients (5–30% in 15 mM MgOAc/25 mM Tris-HCl, pH 7.8) and centrifuged in an SW 27 rotor at 26,000 rpm for 15–21 hr. Under these conditions, the large RuP₂ carboxylase enzyme complex (*M_r* > 500,000) moves into the gradient ahead of the bulk of other proteins (22). The partially purified enzyme fractions were pooled and precipitated with ammonium sulfate (40% saturation), and the subunits were dissociated and dissolved in electrophoresis sample buffer [62.5 mM Tris-HCl, pH 6.8/2.25% NaDodSO₄/5% mercaptoethanol/15% (vol/vol) glycerol/0.0025% bromophenol blue]. The final purification step was by electrophoresis on a preparative slab gel 3 mm thick (23). The O'Farrell discontinuous gel system was used (see above) and the 12.5% acrylamide gel was prepared without wells so that up to 1 ml of sample could be applied evenly across the top. After electrophoresis, the gel was stained briefly (1–2 min) with 0.25% Coomassie blue and destained for 15–30 min in distilled water to locate the small subunit band. A longitudinal strip was excised and more thoroughly stained to locate possible impurities. The portion of the gel containing the small subunit was excised, lyophilized, and ground to a powder. Protein was

eluted from the powder by soaking in water overnight, and the protein concentration was determined by the method of Lowry *et al.* (24).

Preparation of Antibodies. Antibodies to the purified small subunit were obtained from rabbits after injection of the powdered gel strip mixed with saline and Freund's complete adjuvant (Calbiochem) (23). Three injections were made: 2 mg subcutaneously, 0.4 mg 1 week later, and a final injection of 0.4 mg in the footpads at 14 weeks. Sera were tested by double-diffusion assays on Ouchterlony plates. The highest titer was found 4 weeks after the final injection.

Immunoprecipitation. The rabbit antiserum was used to identify the small subunit precursor in the translation products by the method of Higgins *et al.* (25), which utilizes goat anti-rabbit serum to precipitate the rabbit antibody-antigen complex. Translations were carried out in 450-µl reaction mixtures. A 200-µl portion was used for the immunoprecipitation, another 200 µl served as a control with nonimmune serum, and the remainder was applied to the gel to characterize the total translation products. In addition, controls were included in which the endogenous translation products (no added mRNA) were tested with immune serum. The precipitation procedure involved the addition of 10 µl of serum to 200 µl of translation products in 1 ml of phosphate-buffered saline with 2% Triton X-100 and 1% deoxycholate. The mixture was incubated for 30 min at 37°, 100 µl of goat anti-rabbit serum was added, and the incubation was continued for another 60 min. The precipitate was pelleted through 1 M sucrose with the appropriate unlabeled amino acid (10 mM) and washed several times. The immunoprecipitate was dissolved in sample buffer (see above) with the addition of 6 M urea and characterized by electrophoresis and fluorography in parallel with the total translation products.

RESULTS

RNA Isolation. A sedimentation profile of resuspended polysomes isolated by method 2 from light-grown plants shows that relatively large polysomes could be obtained (Fig. 1). The modal size ranged from 6 to 8 in most preparations. In order

FIG. 1. Sedimentation profile of resuspended polysomes.

to check for degradation, the non-poly(A) RNA isolated by the two different methods was characterized on sucrose gradients. There was no apparent degradation of rRNAs when RNA was isolated from polysomes (method 2), but some degradation was detected when the RNA was isolated by the phenol extraction procedure from the total cellular RNA (method 1).

Characterization of Poly(A) RNA by Translation *In Vitro*. Products of translations of poly(A) RNA isolated from two different plant species and by the two RNA isolation methods are compared in Fig. 2. The fluorogram of the translation products shows numerous discrete polypeptides over a large molecular weight range. Differences in translation products were evident between *Lemna* (lanes 2 and 3) and tobacco (lane 1). For example, tobacco had a relatively intense band at about 20,000 daltons that was less intense in the *Lemna* samples, whereas *Lemna* had relatively more intense bands than tobacco just above and below this region. Other differences between the two species occurred around 30,000 and 42,000 daltons. On the other hand, the translation products of the two samples of *Lemna* poly(A) RNA were not detectably different whether the poly(A) RNA was isolated from total cellular RNA (lane 3) or from the polysomes (lane 2). Because some degradation occurred during the longer procedure (method 2) of isolation of total RNA, the bands in lane 3 were less conspicuous in the higher molecular weight region of the gel, but longer exposure of the film has shown that lane 3 contains all the bands that are visible in lane 2.

Poly(A) RNA was also isolated by the two methods from plants put into darkness for 4 days, and these translation products were compared. As found in the light-grown tissue, the mRNAs obtained by the two extraction procedures gave essentially identical translation products *in vitro* (data not shown).

Identification of One of the Translation Products of Poly(A) RNA. The small subunit of RuP₂ carboxylase was purified in order to obtain antibodies to this protein. The initial purification step on sucrose gradients selects for very large molecular weight protein and thus no low molecular weight contaminants were found near the small subunit after electrophoresis on the polyacrylamide gels (see Fig. 5). Antibodies

obtained in rabbits were used to identify this protein among the translation products of poly(A) RNA. The results of such an immunoprecipitation from RNA obtained from light-grown plants are shown in Fig. 3. The principle species precipitated had a molecular weight of 20,000. The small subunit of the RuP₂ carboxylase has a molecular weight of 12,000 in *Lemna* (see Fig. 5); therefore, the precipitated translation product represents the precursor to the small subunit.

Effect of Light on the mRNA Population. Plants were grown in constant light and then placed in total darkness for 4 days. After harvest of a portion of the plants in the dark, the remaining plants were illuminated for up to 20 hr before harvest. Poly(A) RNA was isolated from both sets of plants and translated in the *in vitro* wheat germ system. In a second experiment the translation products from both the light- and dark-treated plants were immunoprecipitated with antiserum to the small subunit of RuP₂ carboxylase. The fluorogram of the translation products and the immunoprecipitates is shown in Fig. 4. The light treatment resulted in a dramatic increase of at least two mRNA species, which code for proteins of molecular weights of 20,000 and 32,000. There was no detectable immunoprecipitate from translation products of RNA extracted from plants that had been in the dark for 4 days (lane D-I). The antiserum to the small subunit of RuP₂ carboxylase did precipitate the 20,000 dalton band that appears in response to light (lane L-I). Therefore, the white light treatment clearly increases the level of poly(A) RNA coding for the small subunit of RuP₂ carboxylase.

***In Vivo* Protein Synthesis.** *In vivo* protein synthesis was assayed by incorporation of [³⁵S]methionine in plants growing in constant light or in darkness for 4 days (Fig. 5). The total proteins contained in the plant tissue are shown by the stained gel (A), and the newly synthesized proteins are shown on the autoradiogram of the gel (B). The plants took up and incorporated similar amounts of [³⁵S]methionine in a 1-hr labeling period in the light or in the dark. The radioactive bands in the light experiment are darker than those in the dark experiment because of a longer exposure to the x-ray film. The proteins were divided into soluble and particulate fractions before

FIG. 2. Fluorogram of translation products of poly(A) mRNA isolated from: light-grown plants. Electrophoresis on a 12.5% polyacrylamide gel, RNA isolated from: lane 1, tobacco polysomes; lane 2, *Lemna* polysomes; lane 3, *Lemna* total RNA.

FIG. 3. Immunoprecipitation of the precursor to the small subunit of RuP₂ carboxylase. Fluorogram of translation products and immunoprecipitate. Poly(A) mRNA was isolated from polysomes of light-grown plants; electrophoresis was on a 12.5% polyacrylamide gel. Lanes: 1, control immunoprecipitate of total translation products (with nonimmune serum); 2, endogenous translation products (no added mRNA); 3, total translation products; 4, immunoprecipitate of total translation products.

FIG. 4. Light effects on mRNA levels. Fluorogram of translation products and immunoprecipitates. Plants were placed in darkness for 4 days and then returned to white light. (Left) Poly(A) mRNA was isolated from total RNA. Electrophoresis was on a 10% polyacrylamide gel. Lanes: D, RNA extracted at the end of the dark period; L, RNA extracted 20 hr after return of the plants to light. (Right) Poly(A) mRNA was isolated from polysomes and electrophoresis was on a 12.5% polyacrylamide gel. Lanes: D, RNA extracted at the end of the dark period; L, RNA extracted 18 hr after return of the plants to light; D-I, immunoprecipitate of sample D; L-I, immunoprecipitate of sample L; C, control immunoprecipitate of sample L with nonimmune serum.

electrophoresis. The pattern of protein synthesis in the light appears to be reflected in the pattern of the total proteins. The two subunits of RuP₂ carboxylase were found in the soluble fraction at 54,000 and 12,000 daltons. Fig. 5C shows the positions of the two subunits on the gel from a partially purified preparation of the enzyme. When plants had been in darkness for 4 days, the two subunits were still present in the tissue.

FIG. 5. *In vivo* protein synthesis. (Light) Plants were labeled in the light for 2 hr with [³⁵S]methionine. The soluble and particulate proteins were subjected to electrophoresis on a 12.5% polyacrylamide gel. (Dark) At the end of 4 days of darkness plants were labeled for 2 hr with [³⁵S]methionine. The soluble and particulate proteins were subjected to electrophoresis on a 12.5% polyacrylamide gel. (A) Total protein, stained with Coomassie blue. (B) Autoradiogram of [³⁵S]-methionine-labeled proteins. (C) Partially purified RuP₂ carboxylase, stained with Coomassie blue. The sample was applied to the gel after sucrose gradient centrifugation and ammonium sulfate precipitation. S, soluble proteins; P, particulate proteins.

FIG. 6. Lack of small subunit precursor poly(A) mRNA in dark-treated plants. Fluorogram of the translation products and immunoprecipitate on a 12.5% polyacrylamide gel. RNA was extracted from plants after 4 days of darkness. Lanes: 1, endogenous translation products from wheat germ extract; 2, immunoprecipitate of lane 1; 3, RNA extracted from total RNA; 4, immunoprecipitate of lane 3; 5, immunoprecipitate of lane 6; 6, RNA extracted from polysomes.

However, there was apparently little or no synthesis of these two subunits in the dark, although a number of other proteins still were being synthesized. The pattern of protein synthesis 18 hr after return of plants to light was the same as in plants grown in constant light (unpublished data). These results are consistent with the apparent lack of mRNA for the small subunit of RuP₂ carboxylase in dark-treated plants.

Absence of Small Subunit mRNA in Darkness. Because a question arises as to whether the mRNA for the small subunit of RuP₂ carboxylase might be present but not translated after 4 days of darkness, I attempted to detect this mRNA by translation in the *in vitro* wheat germ system followed by immunoprecipitation with the antiserum to the small subunit. Poly(A) RNA isolated from dark-treated plants by both isolation methods was used. The fluorogram was overexposed to increase the possibility of detecting any immunoprecipitate band in the region of 20,000 daltons. Lane 1 of Fig. 6 shows that the wheat germ system has endogenous mRNA that is translated into a few radioactive components, but none of these was precipitated by antibody to the small subunit of RuP₂ carboxylase. The sample in lane 3 was translated from poly(A) RNA isolated from the total RNA and that in lane 6, from poly(A) RNA isolated from polysomes. Lanes 4 and 5 show respective immunoprecipitates from those samples. No mRNA for the small subunit of RuP₂ carboxylase was detectable by these methods whether the RNA was isolated from polysomes or from total RNA. Therefore, little or no poly(A) mRNA for this protein is present in these cells.

DISCUSSION

The presence of mRNA for the small subunit of RuP₂ carboxylase among poly(A) RNAs from *Lemna* was established by immunoprecipitation of its translation product with antiserum to the pure protein. In *Lemna*, as in other species (4, 11-13), the initial translation product has a larger molecular weight than the small subunit of the enzyme and therefore must represent a precursor that is cleaved to its final size after translation. The additional amino acid sequence is of substantial size in

Lemna. This precursor has not yet been demonstrated *in vivo*. Dobberstein *et al.* (12) have speculated that the additional amino acid sequence of the precursor functions in some way to transport the small subunit across the chloroplast envelope.

The poly(A) mRNA for the small subunit was not detectable by immunoprecipitation of *in vitro* translation products in tissue that had been in the dark for 4 days. This result could explain the lack of detectable synthesis of the small subunit *in vivo* in dark-treated plants.

The light treatment had a dramatic effect on the levels of at least two poly(A) mRNAs. One of these mRNAs was identified as the mRNA for the small subunit of RuP₂ carboxylase. The other has not yet been identified, but it seems reasonable to postulate that it might code for the protein moiety of the chlorophyll *a-b* protein. This protein is the major thylakoid membrane protein and its amount is known to be modulated by light (26). In addition, Apel and Kloppstech (2) have recently shown that light affects the level of mRNA coding for this protein in barley.

Light treatment might affect the amount of mRNA for these proteins through changed rates of synthesis, of processing of the initial transcript in the nucleus, or of degradation. Alternatively, light might somehow affect the attachment of these mRNAs onto polysomes. Therefore, comparisons were made of translation products of poly(A) RNA that was isolated either from polysomes (method 2) or from total cellular RNA (method 1). In the latter case, one would expect to obtain nonpolysomal RNA, including RNA that might have been present as ribonucleoprotein, that might code for polypeptides not represented in the mRNA on the polysomes.

The results show that the same translation products are obtained whether the poly(A) RNA is isolated from polysomes or from total cellular RNA. These results are obtained whether the plants were grown in the light or dark. The only difference seen is that the largest molecular weight translation products are less predominant when the RNA is isolated by the longer procedure from the total cellular RNA (method 1). Because there is evidence for some degradation of rRNA when method 1 is used, it seems reasonable to suppose that the longer mRNAs are relatively more likely to be degraded in this procedure than when the RNA is isolated from the polysomes. In any case, there does not seem to be a class of poly(A) mRNA that is present in the cells but not on polysomes. Thus, there is no indication of a selective recruitment of the mRNA population into polysomes induced by light. The simplest explanation consistent with the data is that light results in an increase in the level of two specific poly(A) mRNAs, one of which codes for the small subunit of RuP₂ carboxylase. The possibility that light treatment leads to polyadenylation of previously existing messenger or messenger precursor has not been excluded.

The photoreceptor for this response has not been identified, although unpublished work in this laboratory indicates that the low-energy phytochrome response is not involved in the increase in the carboxylase small subunit.

Note Added in Proof. Further experiments with another immunoprecipitation method using *Staphylococcus aureus* to adsorb the antigen-antibody complex (27) have enabled me to detect an extremely low level of small subunit precursor among the translation products of poly(A) RNA isolated from tissue after 4 days of darkness.

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1. Tobin, E. M. & Klein, A. O. (1975) *Plant Physiol.* **56**, 88-92.
2. Apel, K. & Kloppstech, K. (1978) *Eur. J. Biochem.* **85**, 581-588.
3. Giles, A. B., Grierson, D. & Smith, H. (1977) *Planta* **136**, 31-36.
4. Highfield, P. E. & Ellis, R. J. (1978) *Nature (London)* **271**, 420-424.
5. Rosner, A., Jakob, K. M., Gressel, J. & Sagher, D. (1975) *Biochem. Biophys. Res. Commun.* **67**, 383-391.
6. Schrott, E. L. & Rau, W. (1977) *Planta* **136**, 45-48.
7. Chan, P.-H. & Wildman, S. G. (1972) *Biochim. Biophys. Acta* **277**, 677-680.
8. Blair, G. E. & Ellis, R. J. (1973) *Biochim. Biophys. Acta* **31**, 223-234.
9. Kawashima, N. & Wildman, S. G. (1972) *Biochim. Biophys. Acta* **262**, 42-49.
10. Gray, J. C. & Keckwick, R. G. O. (1974) *Eur. J. Biochem.* **44**, 491-500.
11. Roy, H., Patterson, R. & Jagendorf, A. T. (1976) *Arch. Biochem. Biophys.* **172**, 64-73.
12. Dobberstein, B., Blobel, G. & Chua, N.-H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1082-1085.
13. Cashmore, A. R., Broadhurst, M. K. & Gray, R. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 655-659.
14. Cleland, C. F. & Briggs, W. R. (1969) *Plant Physiol.* **44**, 503-507.
15. Jackson, A. O. & Larkins, B. A. (1976) *Plant Physiol.* **57**, 5-10.
16. Goldberg, R. B., Hoschek, G., Kamalay, J. C. & Timberlake, W. E. (1978) *Cell* **14**, 123-131.
17. Lengyel, J. A. & Penman, S. (1977) *Dev. Biol.* **57**, 243-253.
18. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2330-2334.
19. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007-4021.
20. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83-88.
21. Cashmore, A. R. (1976) *J. Biol. Chem.* **251**, 2848-2853.
22. Goldthwaite, J. J. & Bogorad, L. (1971) *Anal. Biochem.* **41**, 57-66.
23. Tjian, R., Stinchcomb, D. & Losick, R. (1974) *J. Biol. Chem.* **250**, 8824-8828.
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, J. R. (1951) *J. Biol. Chem.* **193**, 265-275.
25. Higgins, T. J. V., Zwar, J. A. & Jacobsen, J. V. (1976) *Nature (London)* **260**, 166-169.
26. Thorner, J. P. & Alberte, R. S. (1976) in *The Enzymes of Biological Membranes*, ed. Martonosi, A. (Plenum, New York), Vol. 3.
27. Kessler, S. J. (1975) *J. Immunol.* **115**, 1617-1624.