Plant Physiol. (1970) 46, 315–319

Nucleic Acid Metabolism during Greening and Unrolling of Barley Leaf Segments

Received for publication March 25, 1970

ROZANNE POULSON AND LEONARD BEEVERS
Department of Horticulture, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Barley (*Hordeum vulgare*) leaf segments unroll and green when illuminated. Illuminated segments also have an increased capacity for RNA synthesis. Part of this increased RNA synthesis may be attributed to an increased RNA polymerase activity. In addition, following illumination there is an increased formation of polysomes.

Analyses of RNA synthesized during illumination showed that the radioactive RNA which accumulated was predominantly associated with cytoplasmic ribosomal RNAs. It appears that the early phases of greening are achieved without chloroplast RNA synthesis. Following extended illumination there was evidence of chloroplast ribosomal RNA synthesis; however, this occurred after appreciable chlorophyll synthesis. Actinomycin D, which effectively inhibits RNA synthesis and leaf unrolling, restricts chlorophyll synthesis only during the later stages of illumination. 5-Fluorouracil inhibits the bulk of RNA synthesis but not greening, unrolling, or polysome formation. Studies with inhibitors of protein synthesis have demonstrated a requirement for protein synthesis concomitant with chlorophyll production and leaf unrolling.

Etiolated leaves enlarge and turn green following illumination. In grasses the increase in leaf size is due primarily to the unrolling of the leaf blade associated with an enlargement of the mesophyll cells (6). The greening process is characterized by the synthesis of chlorophyll and the development of mature chloroplasts (2, 13).

In addition to the normal enlargement and greening processes following illumination it has been shown (21) that there is a concomitant increase in the protein and nucleic acid content of the leaves. This increase in RNA and protein could arise as a result of increased cytoplasmic or chloroplast synthesis, or both. The relative importance of the two processes during the early phases of photoinduced leaf development has not been established. The observation that unrolling (1, 20) and greening (4, 12) can be prevented by inhibitors of protein and RNA synthesis suggest that the increase in RNA and protein levels must accompany unrolling and greening.

The present investigation attempts to determine the nature of the RNA synthesized following illumination, and an appraisal is made of the essentiality of RNA synthesis for unrolling and greening.

MATERIALS AND METHODS

Barley seedlings (*Hordeum vulgare*, L., var. Arivat) were grown in darkness at 23 C as previously described (20). The 7-mm segments were excised, starting 1 cm below the tip, from the primary leaves of 6.5-day-old seedings. Batches of 200 segments were incubated in 10 ml of 0.001 M sodium acetate buffer, pH 5.5, containing the appropriate test chemical and incubated at 25 C either in darkness or under 600 ft-c.

Synthesis of RNA was measured by the incorporation of ^{32}P-orthophosphate (neutralized with NaOH). In general 200 μc of carrier-free ^{32}P-orthophosphate were included during the terminal 6 hr of the incubation period. The segments were then washed in 0.1 M phosphate buffer, pH 7.0 and rinsed with deionized water. Total RNA was extracted by the sodium deoxycholate-phenol method as described by Click and Hackett (7), except that the pH of the extraction medium was adjusted to 8.0 and the concentration of bentonite was increased to 1%. The RNA pellet was dissolved in electrophoresis buffer, pH 7.8, containing 10% sucrose.

Total RNA was fractionated by polyacrylamide gel electrophoresis essentially according to the method of Loening and Ingle (14) except that 0.2% sodium lauryl sulfate was included in the buffer system. A constant current of 5 ma per gel was applied for 2 hr to remove free acrylamide, ammonium persulfate, and other impurities. The RNA sample (25 μg/25 μl) was then applied, and electrophoresis was continued for 2.3 hr. Absorption profiles were measured at 260 nm with a Gilford gel scanner attached to a Beckman DU spectrophotometer. The gels were frozen and cut into 0.5-mm slices with the Mickel gel slicer. Radioactivity of the gel slices was determined on a Nuclear-Chicago gas flow counter.

The isolation and fractionation of ribosomes from barley leaf sections was performed by a modification of the method described by Jachymcyzk and Cherry (10). Batches of 400 segments were frozen in liquid nitrogen and ground to a fine powder. The powder was homogenized in 0.25 M sucrose containing 0.02 M tris-HCl, pH 7.6; 0.01 M $MgCl_2$; 0.015 M KCl; 0.005 M β-mercaptoethanol; and 0.5% DOC.[1] Cycloheximide (3 mg/400 segments) was also included in the grinding mix. The homogenate was strained through Miracloth (Calbiochem), and the filtrate was centrifuged. The supernatant was layered over successive layers of 0.5 M and 1.6 M sucrose both containing 0.02 M tris-HCl, pH 7.6; 0.01 M $MgCl_2$; 0.015 M KCl; and 0.005 M β-mercaptoethanol (solution A), and centrifuged at 105,000g for 3 hr. The ribosomal pellet was resuspended in solution A. Polysomes were characterized by applying a 0.1-ml sample of the ribosome suspension onto a linear sucrose gradient (10–34%) containing solution A and centrifuged at 100,000g for 90 min. The distribution of ribosomes on the gradient was measured at 254 nm with an Isco density gradient fractionator.

Ribosome preparations similar to those used in the sucrose density gradient analysis but prepared in the absence of cycloheximide were used in studies of amino acid incorporation. The incubation medium was essentially that used by Mans and

[1] Abbreviations: DOC: deoxycholic acid; PEP: phosphoenolpyruvate.

Novelli (16, 17) and Williams and Novelli (24) and included the following in μmoles: tris-HCl, pH 7.6, 50; $MgCl_2$, 5; KCl, 8; β-mercaptoethanol, 2; ATP, 0.5; GTP, 0.15; PEP, 6.4; and 0.05 mg of crystalline pyruvate kinase; pea supernatant (1 mg of protein); barley leaf ribosomes (1 mg of protein); 0.5 μc of ^{14}C-leucine (315 μc/μmole) in a total volume of 0.5 ml. After incubation at 37 C for 30 min, trichloroacetic acid-insoluble material was collected and radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer (18).

RNA polymerase was extracted and assayed by a modification of the published procedure of Stout and Mans (22). Batches of 800 segments were frozen in liquid nitrogen and ground to a fine powder. The powder was homogenized in 10 ml of 0.05 M tris-HCl, pH 8.0, containing 0.25 M sucrose, 0.01 M $MgCl_2$, and 0.005 M β-mercaptoethanol. The homogenate was filtered through Miracloth, and the filtrate was centrifuged at 105,000*g* for 90 min. The supernatant was used as the crude enzyme preparation. Partial purification was achieved by adjusting the supernatant to 40% saturation by stepwise addition of $(NH_4)_2SO_4$. The solution was stirred and allowed to stand for 30 min at 2 C before the precipitate was collected by centrifugation. The precipitate was dissolved in 0.05 M tris-HCl, pH 8.0, containing 0.005 M β-mercaptoethanol and dialyzed for 4 hr.

The RNA polymerase assay mixture contained, in μmoles: tris-HCl, pH 8.0, 40; magnesium acetate, 20; β-mercaptoethanol, 40; NH_4Cl, 100; CTP, GTP, and ATP, 0.5 each; ^3H-UTP (200μc/μmole), 0.1; and 200 μg of calf thymus DNA; 0.4 ml of enzyme in a final volume of 0.8 ml. The mixture was incubated for 20 min at 35 C, and then 0.1-ml samples were removed and precipitated with 5 ml of ice-cold 10% trichloroacetic acid containing 1% sodium pyrophosphate. The precipitates were subsequently transferred to nitrocellulose membrane filters (Schleicher and Schuell, Bac-T-Flex B6) and washed with 50 ml of cold 5% trichloroacetic acid. The filters were dried and counted in a Packard Tri-Carb scintillation spectrometer.

Chlorophyll content was determined at 665 nm on 80% ethanol extracts. Protein was estimated by the method of Lowry *et al.* (15), with bovine serum albumin as a standard. Leaf width was measured with an overhead projector as previously described (20).

RESULTS

Effect of Illumination and Inhibitors. Illumination of etiolated barley leaf segments results in an increase in leaf width and an accumulation of chlorophyll (Fig. 1). These processes are influenced to varying extents by inhibitors of protein and RNA synthesis. A concentration of 5 mM chloramphenicol prevented chlorophyll accumulation in illuminated segments but did not affect unrolling. Cycloheximide prevented both leaf unrolling and chlorophyll production. It was observed that actinomycin D prevented unrolling of illuminated segments, but it did not restrict chlorophyll production during the early stages of illumination. 5-Fluorouracil treatment had no significant effect on either unrolling or greening.

RNA Synthesis in Illuminated Segments. Polyacrylamide gel electrophoresis of RNA extracted from barley leaf segments showed that six distinct fractions were present (Fig. 2). These fractions coincide with the 1.3 M, 1.1 M, 0.7 M, 0.56 M, 0.4 M, and soluble components described by Loening and Ingle (14). The 1.1 M, 0.56 M, and 0.4 M components are considered to be exclusively chloroplast in nature. It is apparent that these chloroplast ribosomal RNAs are equally prominent in preparations from both etiolated and illuminated segments. Thus there is no absolute light requirement for the synthesis of chloroplast rRNA in the barley leaf.

Investigations of the incorporation of radioactivity from ^{32}P-orthophosphate into RNA components indicated that very little radioactivity was incorporated into leaf segments maintained in the dark for 6 hr. In the comparable illuminated segments more isotope was incorporated into RNA, and radioactivity accumulated in the regions of cytoplasmic rRNA (1.3 and 0.7 M) and the soluble RNA. There was no detectable accumulation of radioactivity into the chloroplast rRNAs (1.1, 0.56, and 0.4 M).

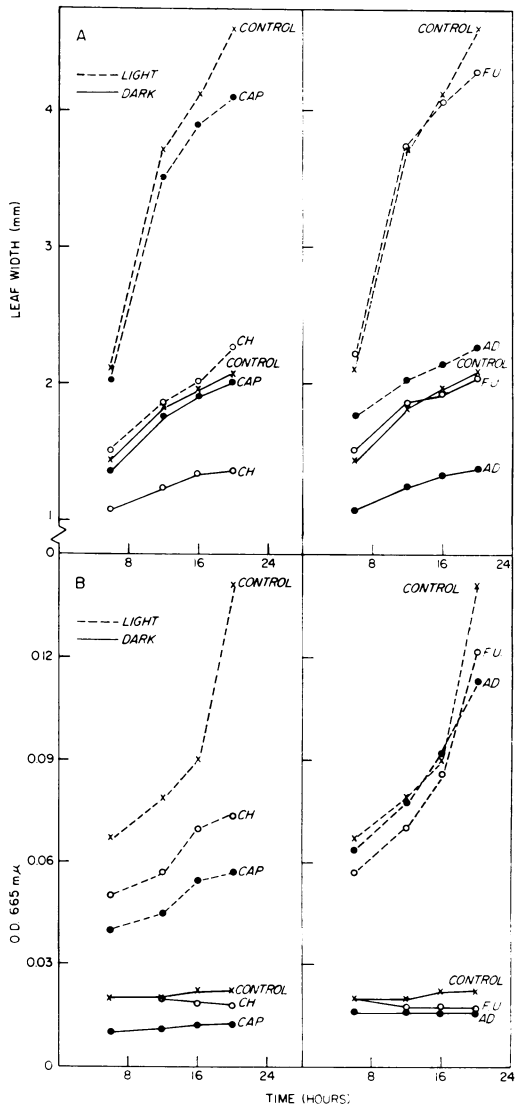

FIG. 1. Effect of 5 mM chloramphenicol (CAP), 40 μM cycloheximide (CH), 5 mM 5-fluorouracil (FU), and 40 μM actinomycin D (AD) on leaf unrolling (A); chlorophyll development (B) of barley segments incubated in either the light or dark for 20 hr.

When ^{32}P-orthophosphate was included during the terminal 6 hr of a 16-hr illumination period, the radioactivity accumulated in the cytoplasmic ribosomal and soluble RNA regions. At this time, however, there was incorporation of ^{32}P into the chloroplast rRNAs. Segments incubated in the dark for 16 hr did not incorporate isotope into the chloroplast rRNA regions.

We have confirmed that the radioactive RNA accumulated during the early phases of illumination was predominantly cytoplasmic and not chloroplast in nature by gel electrophoresis of ^{32}P-labeled RNA extracted from illuminated barley leaf segments in the presence of ^{14}C-labeled 23 S RNA prepared from *Escherichia coli*.

Influence of Inhibitors on RNA Synthesis. Incubation of illuminated segments of chloramphenicol, cycloheximide, actinomycin D, or 5-fluorouracil for 10 hr before a 6-hr exposure to ^{32}P-ortho-

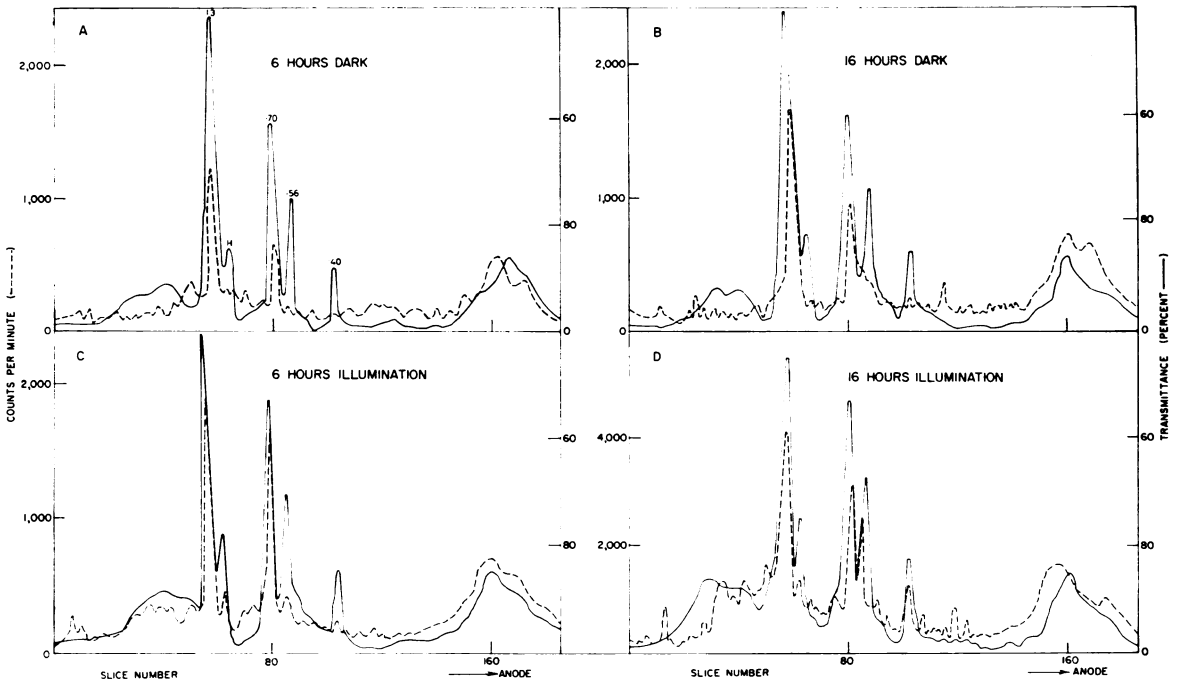

FIG. 2. Polyacrylamide gel electrophoretic patterns of nucleic acids from barley leaf segments incubated with ^{32}P-orthophosphate during the terminal 6 hr of each treatment. A: 6-hr incubation in the dark; B: 16-hr incubation in the dark; C: 6-hr incubation in the light; D: 16-hr incubation in the light.

phosphate reduced the capacity of the leaf segments to incorporate radioactivity into RNA (Fig. 3). At the concentrations used there was no evidence of specific effects of the inhibitors on the synthesis of any of the RNA components. Thus from these data it is not possible to ascribe the differential effects of the inhibitors on unrolling and greening to their specific effects on RNA synthesis.

RNA Polymerase. Assays of the DNA-dependent soluble RNA polymerase activity were performed on extracts from illuminated and nonilluminated barley leaf segments. It was found (Table I) that in the crude preparation RNA polymerase activity was low and enzyme activity did not increase appreciably during a 16-hr illumination period. Enzyme preparations from illuminated segments had consistently higher polymerase activity than preparations from nonilluminated segments. The differences in activity were more apparent in partially purified extracts. The activity of the partially purified preparations from segments illuminated for 12 and 16 hr was greater than that from segments receiving only 6 hr of illumination. Thus it appears that part of the increase in RNA content which occurs during illumination could be ascribed to an increase in RNA polymerase. When enzyme activity was expressed on a specific activity basis, it was observed that segments illuminated for 6 hr had a 34% greater polymerase activity than controls, whereas when results are expressed on a per segment basis, the activity of the illuminated segments is only 20% above the controls. These data suggest that light stimulates the preferential synthesis or activation of soluble RNA polymerase protein.

Polysome Levels. Absorbancy profiles of ribosomal preparations prepared from barley leaf segments and subjected to sucrose density gradient centrifugation are shown in Figure 4. Characteristically, there is a monosome peak at the top of the gradient with increasing polysomic associations toward the bottom of the gradient. In etiolated leaf segments 45% of the ribosomes occur as polysomes; exposing the segments to 1 hr of light increases this percentage to 73%. A 6-hr illumination period only increases the population of ribosomes occurring as polysomes to 75%.

The population of polysomes was reduced by actinomycin D (Table II), as might be expected if actinomycin D blocks the synthesis of mRNA. 5-Fluorouracil had no effect on the polysome level of barley leaf tissue, indicating that, while the bulk of RNA synthesis is inhibited, mRNA synthesis is not (11). Cycloheximide-treated tissue showed a small decrease in the percentage of ribosomes occurring as polysomes.

The four minor peaks to the right of the monosome peak probably represent subunits of the 80 S and 70 S ribosomes. These subunits are present in both illuminated and nonilluminated segments and may constitute a pool from which 80 S and 70 S ribosomes are assembled during periods of active protein synthesis.

The polysomic nature of the material to the left of the monosome peak has been verified by incubation with RNase. Also, preparations similar to those used in the sucrose density gradient analysis, but prepared in the absence of cycloheximide, were active in incorporating ^{14}C-leucine into trichloroacetic acid precipitable material. The conditions for amino acid incorporation by the ribosome preparations are presented in Table III. Ribosomes prepared from illuminated leaf segments showed a greater capacity for ^{14}C-leucine incorporation into trichloroacetic acid insoluble material in comparison to nonilluminated segments. During the initial 6 hr of illumination the capacity of the ribosomes to support amino acid incorporation increased 116%. Thereafter, however, increasing the duration of illumination did not elicit an additional increase in leucine-incorporating capacity.

DISCUSSION

The visible photomorphogenic changes which occur following illumination of etiolated leaves, expansion, and greening are accompanied by an increased capacity for RNA synthesis (3, 13). These observations have raised several questions. First, what kind of RNA is synthesized; and secondly, which RNA fractions are necessary for the photomorphogenic changes?

Following short periods of illumination, radioactivity accumu-

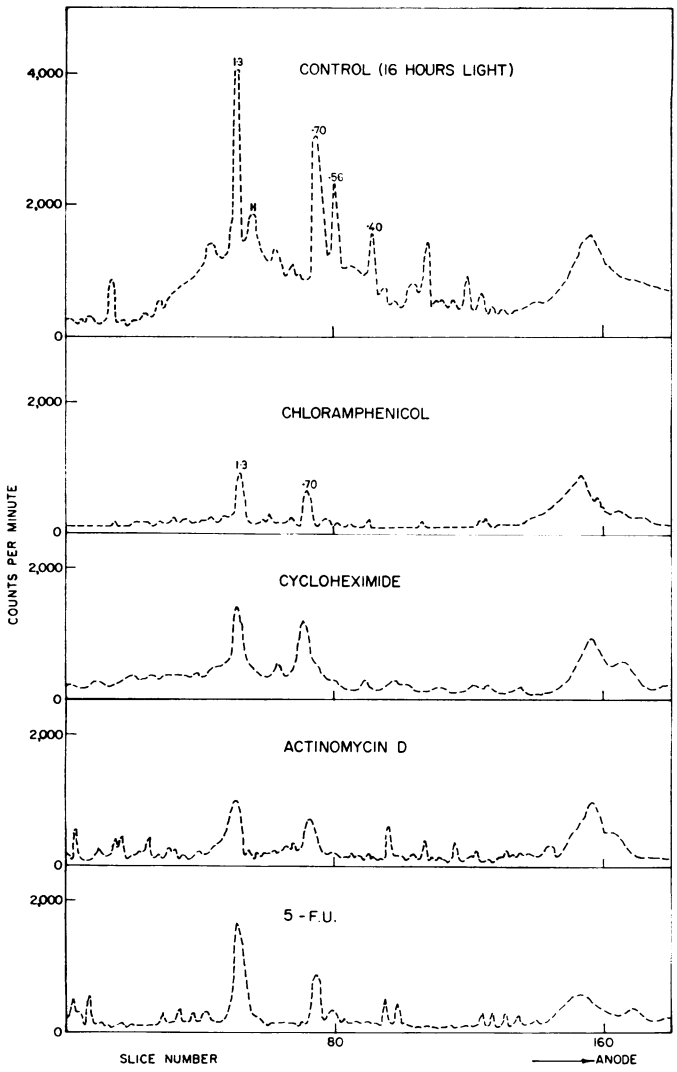

FIG. 3. Effect of 5 mM chloramphenicol, 40 μM cycloheximide, 40 μM actinomycin D, and 5 mM 5-fluorouracil on incorporation of radioactivity from ^{32}P-orthophosphate into RNA components of illuminated barley leaf segments.

Table I. *RNA Polymerase in Etiolated and Illuminated Barley Leaf Segments*

Treatment	Nonilluminated		Illuminated	
	μμmoles ^3H-UMP incorporated/ reaction	μμmoles ^3H-UMP incorporated/ mg protein	μμmoles ^3H-UMP incorporated/ reaction	μμmoles ^3H-UMP incorporated/ mg protein
hr				
105,000*g* fraction				
6	32	33	39	41
12	33	34	41	44
16	34	34	43	48
$(NH_4)_2SO_4$ fraction				
6	85	128	99	161
12	87	131	108	195
16	87	134	110	207

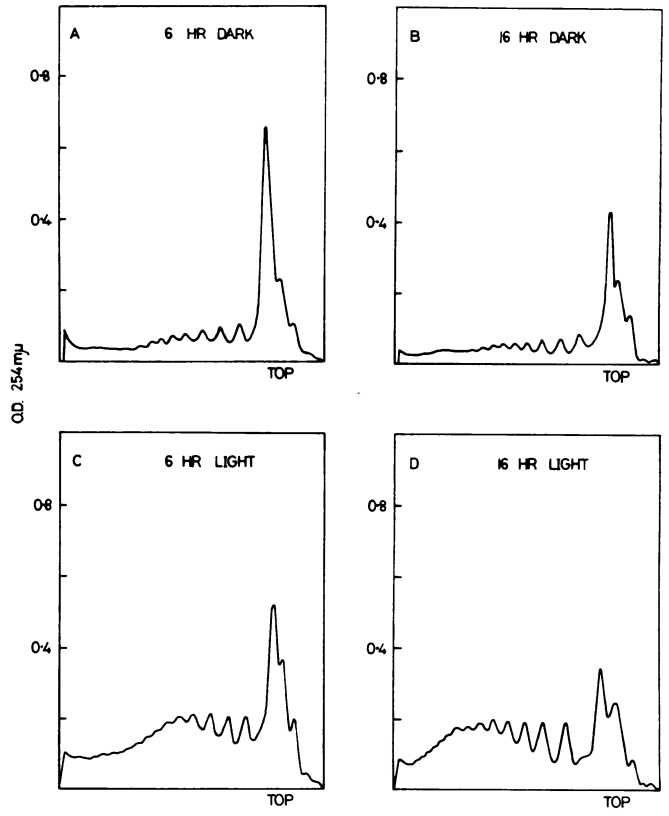

FIG. 4. Sucrose density gradient profiles of ribosome preparations from barley leaf segments. A: 6-hr dark; B: 16-hr dark; C: 6-hr light: D: 16-hr light.

lated predominantly in the cytoplasmic rRNA fractions. Thus, during the early phases of chlorophyll production, there is no requirement for the specific synthesis of chloroplast rRNA. This observation agrees with the concept of Boardman (2). The incorporation of radioactive phosphate into chloroplast rRNA during the later stages of illumination indicates that there is a period of chloroplast rRNA synthesis following illumination. However, this occurs after the segments have synthesized appreciable amounts of chlorophyll. In contrast, Bogorad (3, 5) observed that illumination resulted in a rapid preferential synthesis of plastid rRNA. These conflicting observations may be due to different plant tissue or tissue age. In this respect Ingle (9) demonstrated that in greening radish cotyledons the period of active chloroplast rRNA synthesis was short, whereas cytoplasmic rRNA was continuously synthesized.

The increased incorporation of radioactivity into RNA following illumination may be partly due to the observed increase in RNA polymerase level. A similar increase in soluble polymerase activity following illumination has been reported by Stout, Parenti, and Mans (23). The failure to detect an increased RNA polymerase activity in crude preparations with increasing illumination time may be due to the fact that purification removes a ribonuclease present which could cause an underestimation of RNA polymerase activity. The rapid increase in soluble RNA polymerase activity and the increased accumulation of radioactive phosphate into the cytoplasmic rRNA following illumination suggest that the soluble RNA polymerase functions in the synthesis of cytoplasmic RNA. Bogorad (3) has also reported a light-stimulated increase of RNA polymerase activity in maize leaves. However, in these studies the increased activity appeared to be confined to a particulate polymerase associated with the plastid fraction. In assays of RNA polymerase activity associated with organelles or chromatin, there is some ambiguity as to whether changes in polymerase activity represent changes in enzyme level or an altered DNA template availability. In the present investiga-

Table II. *Polysome Content of Barley Leaf Segments*

Ribosomes were isolated from barley leaf segments at 6 and 16 hr after initiation of the treatments. The percentage of polysomes was determined from the area under the monosome and polysome tracings of the gradient profiles.

Treatment	Polysomes	
	6 hr	16 hr
	%	%
Nonilluminated	45	48
Illuminated	75	76
Illuminated in presence of		
Actinomycin D, 40 μM	65	52
5-Fluorouracil, 5 mM	71	73
Cycloheximide, 40 μM	63	62
Chloramphenicol, 5 mM	...	72

Table III. *Leucine Incorporation into Protein by Ribosomes from Barley Leaf Segments*

Treatment	Nonillum-inated	Illum-inated
	cpm	
1 hr	1403[1]	1637
6 hr	1337	2901
16 hr	1616	3048
+RNase	...	128
−ATP	...	2448
−GTP	...	1148
−ATP, GTP, PEP + pyruvate kinase	...	106
−Supernatant	...	101
−Ribosomes	...	96
+19 L-amino acids	...	2754
Complete system		3048

[1] Results are expressed as cpm incorporated into trichloroacetic acid-precipitable material with the assay conditions described in "Materials and Methods."

tion the use of a soluble enzyme and a standardized DNA level precluded an altered template.

The light-stimulated formation of polysomes is similar to that reported by Williams and Novelli (24, 25). Ribosomal preparations from illuminated leaf segments have a greater capacity for amino acid incorporation than preparations from nonilluminated segments.

The inhibition of both greening and leaf unrolling by cycloheximide indicated that protein synthesis must accompany the photomorphogenic changes. The prevention of greening but not leaf unrolling by chloramphenicol is consistent with the concept that leaf unrolling is achieved by protein synthesis involving cytoplasmic ribosomes whereas chlorophyll synthesis requires the functioning of chloroplast ribosomes. However, the observed inhibition of greening by cycloheximide does not agree with this concept. The conflicting data can be reconciled if chlorophyll synthesis involves the participation of both cytoplasmic and chloroplast ribosomes whereas the unrolling process requires only the cytoplasmic component. As indicated by Kirk and Allen (12), it cannot be determined whether inhibition of protein synthesis prevents chlorophyll production by inhibiting the synthesis of essential enzymes involved in chlorophyll synthesis or whether the inhibitors impede the formation of structural proteins required for the formation of membranes with which the chlorophyll becomes associated.

The inhibition of unrolling but not greening by actinomycin D indicates a differential requirement for RNA synthesis in the two processes. Furthermore, the failure of 5-fluorouracil to inhibit unrolling indicates that the initial photoinduced unrolling is dependent upon the synthesis of a restricted complement of RNA.

Insofar as it has been shown that the synthesis of D-RNA (11) is insensitive to inhibition by 5-fluorouracil (11), and since ribosomal preparations from 5-fluorouracil-treated segments contained polysomes whereas preparations from actinomycin D-treated segments had a preponderance of monosomes, it is possible that the sole requirement for RNA synthesis during early photomorphogenesis is the production of mRNA. The synthesis of this component facilitates photoinduced unrolling; greening can occur in the absence of detectable RNA synthesis.

LITERATURE CITED

1. Beevers, L., J. A. Pearson, B. Loveys, and P. F. Wareing. 1970. Phytochrome and hormonal control of expansion and greening of etiolated wheat leaves. Planta 90: 286–294.
2. Boardman, N. K. 1967. Chloroplast structure and development. *In*: A. San Pietro, E. A. Greer, and T. J. Army, eds., Harvesting the Sun. Academic Press, New York. pp. 211–230.
3. Bogorad, L. 1967. Chloroplast structure and development. *In*: A. San Pietro, F. A. Greer, and T. J. Army, eds., Harvesting the Sun. Academic Press, New York. pp. 191–210.
4. Bogorad, L. and A. B. Jacobson. 1964. Inhibition of greening of etiolated leaves by actinomycin D. Biochem. Biophys. Res. Commun. 14: 113–117.
5. Bogorad, L. and A. B. Jacobson. 1965. RNA metabolism in chloroplasts. Fed. Proc. 24: 664.
6. Burström, H. 1942. Über Entfeltung und Einrollen eines mesophilen Grasblattes. Bot. Not. 7: 351–362.
7. Click, R. E. and D. P. Hackett. 1966. The isolation of ribonucleic acid from plant, bacterial or animal cells. Biochim. Biophys. Acta 129: 74–84.
8. Ingle, J. 1968. The synthesis and stability of chloroplast ribosomal RNAs. Plant Physiol. 43: 1448–1454.
9. Ingle, J. 1968. The effect of light and inhibitors on chloroplast and cytoplasmic RNA synthesis. Plant Physiol. 43: 1850–1854.
10. Jachymcyzk, W. J. and J. H. Cherry. 1968. Studies on messenger RNA from peanut plants: in vitro polyribosome formation and protein synthesis. Biochim. Biophys. Acta 157: 368–377.
11. Key, J. L. 1966. Effects of purine and pyrimidine analogues on growth and RNA metabolism in the soybean hypocotyl—The selective action of 5-fluorouracil. Plant Physiol. 41: 1257–1264.
12. Kirk, J. T. O. and R. L. Allen. 1965. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. Biochem. Biophys. Res. Commun. 21: 523–530.
13. Kirk, J. T. O. and R. A. E. Tilney-Bassett. 1967. The Plastids. W. H. Freeman and Co., London.
14. Loening, U. E. and J. Ingle. 1967. Diversity of RNA components in green plant tissues. Nature 215: 363–367.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1961. Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
16. Mans, R. J. and G. D. Novelli. 1961. Measurement of the incorporation of radioactive amino acids into protein by a filter paper disk method. Arch. Biochem. Biophys. 94: 48–53.
17. Mans, R. J. and G. D. Novelli. 1964. Stabilization of the maize seedling incorporating system. Biochim. Biophys. Acta 80: 127–136.
18. Marcus, A., J. Feeley, and T. Volcani. 1966. Protein synthesis in imbibed seeds. III. Kinetics of amino acid incorporation, ribosome activation, and polysome formation. Plant Physiol. 41: 1167–1172.
19. Pogo, B. G. T. and A. O. Pogo. 1964. DNA dependence of plastid differentiation inhibition by actinomycin D. J. Cell. Biol. 22: 296–301.
20. Poulson, R. and L. Beevers. 1970. Effects of light and growth regulators on leaf unrolling in barley. Plant Physiol. In press.
21. Rhodes, M. J. C. and E. W. Yemm. 1963. The development of chloroplasts and the synthesis of proteins in leaves. Nature 200: 1077–1080.
22. Stout, E. R. and R. J. Mans. 1968. Partial purification and properties of RNA polymerase from maize. Biochim. Biophys. Acta 134: 327–336.
23. Stout, E. R., R. Parenti, and R. J. Mans. 1967. An increase in RNA polymerase activity after illumination of dark-grown maize seedlings. Biochem. Biophys. Res. Commun. 29: 322–326.
24. Williams, G. and G. D. Novelli. 1964. Stimulation of an in vitro amino acid incorporating system by illumination of dark-grown plants. Biochem. Biophys. Res. Commun. 17: 23–27.
25. Williams, G. R. and G. D. Novelli. 1968. Ribosome changes following illumination of dark-grown plants. Biochim. Biophys. Acta 155: 183–195.