

Correlation between Polyribosome Level and the Ability to Induce Nitrate Reductase in Dark-grown Corn Seedlings¹

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ABSTRACT

Nitrate reductase can be induced in excised shoots of 3-day-old dark-grown *Zea mays* (var. WF9 × M14) seedlings in the absence of light. In contrast, leaves of 10-day-old dark-grown seedlings require a light treatment in order to induce enzymatic activity. Leaves of 10-day-old dark-grown seedlings contain a very low level of polyribosomes while 3-day-old shoots contain a very high level of polyribosomes. There is a gradual loss of polyribosomes from 3 to 10 days and a gradual loss of *in vitro* protein synthetic activity of the ribosome preparations. The loss of polyribosomes and decrease in their amino acid-incorporating activity correlate positively with the loss of ability to induce nitrate reductase activity as leaves of dark-grown corn seedlings age. These results corroborate and extend our previous results, in that light is not required for nitrate reductase induction *per se* in leaves of dark-grown seedlings but is required to reactivate the protein synthetic apparatus of older leaves.

Dark-grown seedlings are routinely used for enzyme induction studies in higher plants. In the case of nitrate reductase the effects of light (4, 9, 10) and substrate (1) on induction in dark-grown seedlings have been reported. While the role of substrate is well known, the effects of light have been questioned. Nitrate reductase activity was induced in 5-day-old dark-grown radish cotyledons in the absence of light (2). Other reports have shown a definite requirement for light for enzyme induction in 7- to 9-day-old seedlings (3, 9, 10). Jordan (4) reported a general decrease in nitrate reductase activity in barley leaves from dark-grown seedlings of increasing age even though nitrate was present in the nutrient medium. These results suggest that the ability to form an active nitrate reductase in dark-grown seedlings is lost with age.

In a previous report (9) we presented evidence consistent with the view that the requirement for light for the induction of nitrate reductase in 9-day-old corn leaves was for the development of an active protein-synthesizing apparatus as evidenced by polyribosome formation and not for enzyme induction *per se*. Polyribosomes increased from 27 to 42% of the ribosome population during the first 2 hr of light exposure. The ability to induce nitrate reductase under various conditions was positively correlated with the level of polyribosomes in the tissue. Other reports have also indicated that the level of poly-

ribosomes in 1-week-old dark-grown seedlings is quite low (7, 12). Thus although it is known that the percentage of polyribosomes in 7- to 9-day-old seedlings is low, little information is available on the state of the protein-synthesizing system during the initial days of seedling growth in darkness.

In this study we compared the ability to induce nitrate reductase in dark-grown seedlings (3 to 10 days old), in either light or darkness, with the level of polyribosomes and their *in vitro* protein synthetic activity. The results indicate that the loss of ability with age of dark-grown shoots or leaves to form an active nitrate reductase in darkness and the increasing lag period preceding enzyme formation in light are due to a loss of active polyribosomes as the seedlings age. The mechanism of the light activation of protein synthesis in the older leaves is under investigation.

MATERIALS AND METHODS

Plant Materials. *Zea mays* (var. WF9 × M14) was grown in vermiculite for 5, 7, or 10 days in a light-proof controlled environment growth chamber as previously described (9). Three-day-old seedlings were germinated in darkness in moist absorbant paper.

Enzyme Induction Studies. Three-day-old shoots were detached and used in their entirety (about 0.5 cm each) in induction studies. For 10-day-old seedlings the second leaves were harvested and trimmed to 10 cm. All induction treatments were carried out in 1500 ml of aerated solution in 4- × 6-inch plastic pans.

Nitrate Reductase Assay. Preparation of a cell-free extract and the assay for nitrate reductase activity were done as previously reported (10).

Preparation of Polyribosomes. Polyribosomes were prepared from 10-day-old leaves as previously described (9). For 3-, 5-, and 7-day-old seedlings the terminal 0.5-, 2-, and 3-cm shoot tips, respectively, were used without removing the coleoptile. All procedures were as reported above with the exception of the preparation of sucrose density gradients. In this study 0.8 ml of ribosome preparation, containing 18 A_{260} units, was layered on a 35-ml, 10 to 34% linear sucrose gradient and centrifuged at 81,500g for 105 min (Spinco SW 27, 1- × 3.5-inch tube).

Amino Acid Incorporation Studies. Amino acid incorporation was studied using modifications of the methods of Mans and Novelli (5, 6) and Williams and Novelli (11) as previously published (9). Soluble factors for all assays were prepared from 48-hr dark-germinated corn shoots using the method of Williams and Novelli (11).

Light Treatments. High intensity white light pretreatments and light for enzyme induction studies were applied in a controlled environment growth chamber. Light intensity was 1000 ft-c and temperature was maintained at 24 C.

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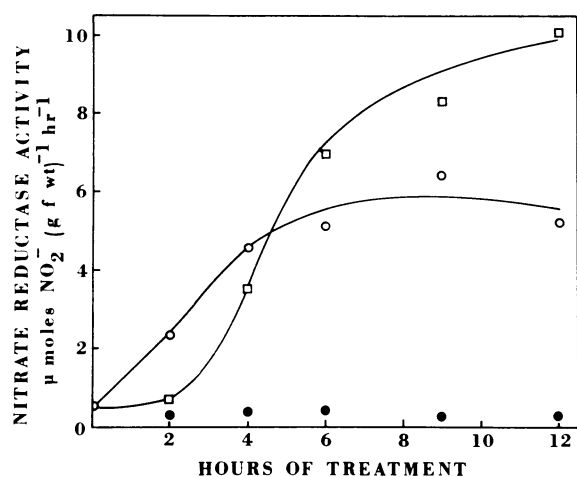


FIG. 1. Activity of nitrate reductase in dark-grown corn seedlings incubated in 50 mM KNO_3 in light. \circ : 3-day-old excised shoots; \square : 10-day-old detached leaves; \bullet : 3-day-old excised shoots or 10-day-old detached leaves incubated without nitrate. Light was supplied at 1000 ft-c.

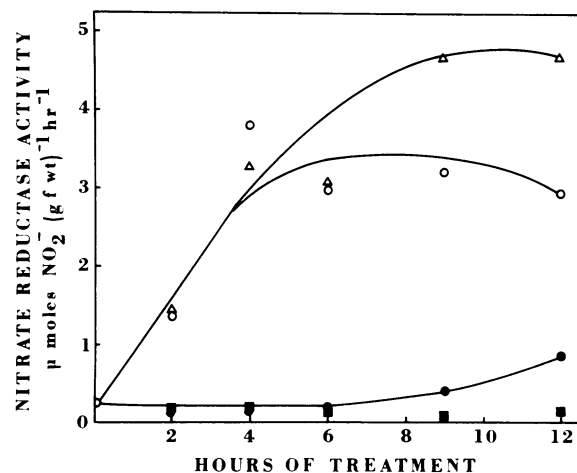


FIG. 2. Activity of nitrate reductase in dark-grown corn seedlings incubated in 50 mM KNO_3 in darkness. \circ : 3-day-old excised shoots minus glucose; \triangle : 3-day-old excised shoots plus 50 mM glucose; \bullet : 10-day-old detached leaves minus glucose; \blacksquare : 10-day-old detached leaves plus glucose.

RESULTS AND DISCUSSION

Induction of nitrate reductase in light was compared in excised 3-day-old dark-grown shoots and leaves of 10-day-old dark-grown seedlings (Fig. 1). In the young shoots enzymatic activity increased with an initial burst during the first 4 to 6 hr in light and then remained constant throughout the remainder of the treatment period. Induction in 10-day-old leaves occurred only after a 2- to 4-hr lag period. However, activity continued to increase throughout the treatment period. These results suggest that the young shoots maintained the ability to form an active nitrate reductase rapidly when placed in light in the presence of nitrate, while older leaves had lost this ability and hence exhibited a significant lag period before enzymatic activity increased. When either age tissue was placed in light in the absence of nitrate no significant increase in nitrate reductase activity occurred.

Three-day-old dark-grown shoots and leaves of 10-day-old dark-grown seedlings were given nitrate in darkness to determine whether they maintained or lost the ability to form an ac-

tive nitrate reductase independent of light (Fig. 2). Enzymatic activity increased rapidly in the 3-day-old shoots without a significant lag period and then leveled off after 4 to 6 hr. The final level of activity was approximately 70% of that attained in light. The addition of glucose to the induction medium increased enzymatic activity in the shoots to nearly the same level that was induced in the light. This indicates that a lack of energy in the detached shoots limited the development of nitrate reductase activity in darkness relative to that which occurred in light. In control leaves of 10-day-old dark-grown seedlings enzymatic activity did not increase in darkness. When glucose was supplied, activity increased up to about 1 unit during the treatment period.

In a previous report (9) we suggested that the requirement for light for the development of nitrate reductase in corn leaves was related to the formation and maintenance of active polyribosomes. Figure 3 suggests that the results obtained in this study relate to a loss of polyribosomes during the 10-day dark period. Three-day-old dark-grown shoots contained a high level of polyribosomes (Fig. 3A), correlating with the ability to form an active nitrate reductase without a significant lag when nitrate was made available. By 10 days the polyribosomes decreased to a very low level relative to the level of monoribosomes (Fig. 3D). The percentage of monoribosomes and ribosomal subunits increased with age concomitantly with the decrease in polyribosomes. Ribosomes prepared from 5- and 7-day-old shoots (Fig. 3, B and C) were included to show the progressive loss of polyribosomes from 3 to 10 days.

It has been reported that polyribosomes increase significantly in leaves of older dark-grown seedlings during the first few hours in light (7, 9, 12) and that the newly formed polyribosomes have a much higher protein synthetic activity per unit of polyribosome than the pre-existing dark polyribosome population (9, 12). These reports suggest that the lag period before nitrate reductase induction is a result of a time requirement for the formation of active polyribosomes. Once a significant level of polyribosomes was present (after about 2 hr), induction occurred at a rate similar to that of 3-day-old shoots. The absence of a significant lag period in 3-day-old shoots is apparently related to the presence of a relatively high level of active polyribosomes.

^{14}C -Leucine incorporation studies were carried out to determine that the ribosome preparations were capable of incor-

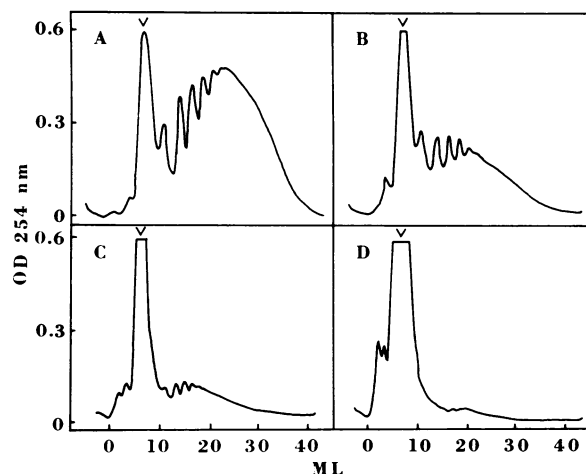


FIG. 3. Ribosome profiles from dark-grown corn seedlings. A: 3-day-old shoots; B: 5-day-old shoots; C: 7-day-old shoots; D: 10-day-old second leaves. Related assays of nitrate reductase for A and D are shown in Figures 1 and 2. Arrows denote monoribosome peaks.

porating amino acids into protein. Ribosomes prepared from 3-day-old shoots attained the highest level of incorporation over the 30-min assay period (Fig. 4). As tissue age increased, the ability of the ribosomes to incorporate ^{14}C -leucine into protein decreased, correlating with the decrease in the percentage of polyribosomes in the preparation. The level of endogenous incorporation by the ribosome preparations in the absence of supernatant factors was quite low and did not increase during the assay period.

Jordan (4) reported that a 10-min high intensity white light pretreatment 6 hr before placing dark-grown, 7-day-old barley leaves in light removed the lag period preceding enzyme induction. Similar results were obtained in this study with 10-day-old dark-grown corn leaves (Fig. 5). Although the light pretreatment did not completely eliminate the lag period before induction, it was shortened significantly. This slight lag is indicative of the time requirement for the leaves to take up nitrate and possibly to form template or messenger RNA for directing the synthesis of the enzyme. Ribosome profiles from similarly treated leaves (Fig. 6) indicate that the short light pretreatment enhanced polyribosome formation to a level equal to that of leaves after a 2-hr light treatment (approximately 42% polyribosomes). Williams and Novelli (11) reported similar increases in bean leaf polyribosomes after a 30-min red light treatment followed by 2.5 hr of darkness. This level of polyribosomes, as an indicator of the protein synthetic ability of the tissue, is apparently sufficient to allow nitrate reductase synthesis. Thus, when pretreated leaves were placed in light with nitrate available, enzymatic activity increased without a significant lag period (Fig. 5).

It appears that dark-grown corn seedlings contain a rapidly degenerating protein-synthesizing system. Young shoots (3 days old) maintain the ability to form an active nitrate reductase rapidly with or without light. Older leaves (10 days old) possess a much degraded protein-synthesizing system as evidenced by a low level of polyribosomes and require a light treatment to re-establish the polyribosome level (and thus an active protein synthetic apparatus) before enzymatic activity can be induced. The reinitiation of polyribosome formation and the associated induction of nitrate reductase does not require continuous light energy; however, continuous light over the 12-hr treatment period is required for maximal polyribosome formation and maximal increases in nitrate reductase.

The effect of light in "activating" the protein synthetic ap-

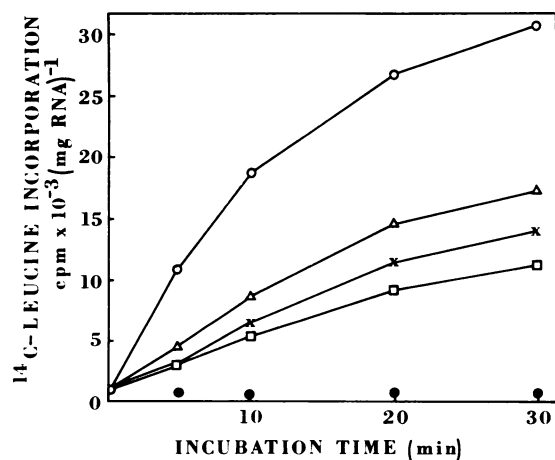


FIG. 4. ^{14}C -Leucine incorporation by ribosomes isolated from dark-grown corn seedlings. \circ : 3-day-old shoots; Δ : 5-day-old shoots; \times : 7-day-old shoots; \square : 10-day-old second leaves; \bullet : 3-, 5-, or 7-day-old shoots or 10-day-old leaves minus supernatant factors.

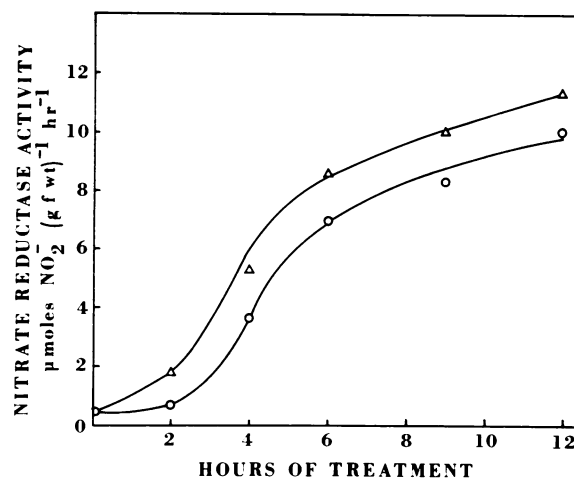


FIG. 5. Effect of high-intensity light pretreatment given 6 hr before beginning of 12-hr illumination treatment on the light-induced development of nitrate reductase in second leaves of 10-day-old dark-grown seedlings. Leaves were incubated in 50 mM KNO_3 . \circ : control; Δ : pretreated. Pretreatment was for 10 min at 1000 ft-c.

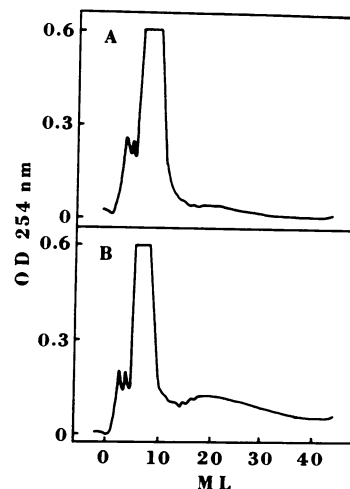


FIG. 6. Effect of high-intensity light pretreatment given 6 hr before harvest on polyribosome level in second leaves of 10-day-old dark-grown seedlings. A: Control; B: pretreated. Pretreatment was for 10 min at 1000 ft-c.

paratus which was monitored in these studies by following polyribosome levels and their *in vitro* amino acid-incorporating activity is unknown. The results of Williams and Novelli (12), Stout *et al.* (8), and Poulson and Beevers (7) are suggestive of a role of light at the level of enhancement of RNA synthesis. Thus if the 10-day-old leaves cannot synthesize the required RNA in response to nitrate, an active nitrate reductase would not be formed even though the tissue was able to synthesize some protein on pre-existing polyribosomes which presumably would not contain mRNA for nitrate reductase synthesis. Alternatively, 10-day-old dark-grown leaves might be able to synthesize the necessary RNA in response to nitrate, but this newly synthesized RNA might not be incorporated into the initiation complex, resulting in a failure of nitrate reductase induction. With this possibility one envisages some type of translational control, possibly at the level of formation of the initiation complex. The results of Williams and Novelli (12) and of Travis *et al.* (9), which show a higher amino acid-incorporating activity per unit of polyribosome in ribosomes prepared

from leaves of light-treated relative to dark-grown seedlings, are suggestive of a role of light in modifying some "translational" control system. These possible roles of light in activating protein synthesis are under study.

LITERATURE CITED

1. BEEVERS, L. AND R. H. HAGEMAN. 1969. Nitrate reduction in higher plants. *Annu. Rev. Plant Physiol.* 20: 495-522.
2. BEEVERS, L., L. E. SCHRADER, D. FLESHER, AND R. H. HAGEMAN. 1965. The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings. *Plant Physiol.* 40: 691-698.
3. CHEN, T. M. AND S. K. RIES. 1969. Effect of light and temperature on nitrate uptake and nitrate reductase activity in rye and oat seedlings. *Can. J. Bot.* 47: 341-343.
4. JORDAN, W. R. 1968. Control of nitrate assimilation by light regulated synthesis and degradation of nitrate and nitrite reductases in *Hordeum vulgare* L. Ph.D. thesis, University of California, Davis.
5. 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.
6. MANS, R. J. AND G. D. NOVELLI. 1964. Stabilization of the maize seedling amino acid incorporation system. *Biochim. Biophys. Acta* 80: 127-136.
7. POULSON, R. AND L. BEEVERS. 1970. Nucleic acid metabolism during greening and unrolling of barley leaf segments. *Plant Physiol.* 46: 315-319.
8. 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.
9. TRAVIS, R. L., R. C. HUFFAKER, AND J. L. KEY. 1970. Light-induced development of polyribosomes and the induction of nitrate reductase in corn leaves. *Plant Physiol.* 46: 800-805.
10. TRAVIS, R. L., W. R. JORDAN, AND R. C. HUFFAKER. 1970. Light and nitrate requirements for the induction of nitrate reductase activity in *Hordeum vulgare*. *Physiol. Plant.* 23: 678-685.
11. WILLIAMS, G. R. 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.
12. WILLIAMS, G. R. AND G. D. NOVELLI. 1968. Ribosome changes following illumination of dark-grown plants. *Biochim. Biophys. Acta* 155: 183-192.