STIMULATION OF AN IN VITRO AMINO ACID INCORPORATING SYSTEM BY ILLUMINATION OF DARK-GROWN PLANTS[1]

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A number of morphological and biochemical changes occur in the leaves of most higher plants after dark-grown shoots are exposed to light. One characteristic effect is a relatively rapid and extensive increase in total leaf protein (De Deken-Grenson, 1954; Mego and Jagendorf, 1961). This change is accompanied by the development of a number of enzymatic activities not found in the etiolated leaf (Marcus, 1960; Tolbert and Gailey, 1955; and Keister, Jagendorf, and San Pietro, 1962). Because this effect of light represents a unique and presumably specific regulation of protein synthesis, we have attempted to study the phenomenon further in vitro. This preliminary report is concerned with the effect of light on dark-grown seedlings as reflected by the amino acid incorporating activity of subsequently prepared ribosomes.

METHODS

The procedures developed previously in this laboratory (Mans and Novelli, 1964) for growth of maize seedlings and preparation of the amino acid incorporating system were modified as described below. Maize seeds were germinated on moist filter paper in the dark at 23 to 24° C. On the fifth day, the seedlings were illuminated for an hour with a 300-watt incandescent spot lamp at a distance of 90 cm, using a dilute $CuSO_4$ solution as a heat filter. After exposure to light, the

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seedlings were returned to the dark for various intervals (usually 2 hours). The portion of the shoots above the coleoptilar node was harvested and the cells were disrupted under liquid nitrogen in a motor-driven mortar and pestle. The ribosomes were prepared according to a modified Wettstein procedure (Wettstein et al., 1963). After rinsing the surface, the resultant pellet was carefully suspended at a concentration of 5 to 10 mg protein per ml in a medium of 0.01 M Tris-Cl (pH 7.6 at 0° C), 0.005 M $MgCl_2$, and 0.015 M KCl.

The crude supernatant fraction was prepared from the shoots of dark-grown seedlings and represents material not sedimented at 150,000 X g for 90 minutes. Maize supernatant, prepared in this manner, was used in all experiments described below. Omission of either ribosomes or supernatant from the reaction mixture described in Table 1 results in negligible activity.

Soybean and bean seeds were germinated in moist vermiculite in the dark at 28° C. The small primary leaves were harvested after light treatment on the sixth day of growth. Ribosomes were prepared as indicated above.

Amino acid incorporation into protein was assayed on filter paper discs as described by Mans and Novelli (1961). Protein was determined by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

The specific activities of ribosomal preparations from dark-grown plants are compared with those from similar plants after light treatment in Table 1. These data illustrate the magnitude of the light response and the limits of variability normally encountered in our preparations. Ribosomes from younger maize seedlings are stimulated similarly (about 60 to 70%), although preparations from shoots of 2-day old seedlings exhibit little or no response if the shoot length averages less than 0.5 to 0.7 cm. Our observation that light also affects the activity of subsequently prepared ribosomes from bean and soybean leaves leads us to suggest that the undeveloped leaf tissue may be the locus of this light response in maize.

The kinetics of a typical assay in which maize ribosomes were used are shown in Fig. 1. These data show that the rate of amino acid incorporation, as well as the extent of total incorporation, is greater per unit of ribosomal protein in preparations from light-treated plants. Estimates of the RNA content of these preparations, based on absorbancy measurements at 260 mμ, indicate that the RNA:protein ratio is unaf-

Table 1

Effect of Light Treatment on C^{14}-Leucine Incorporation by Ribosomes Subsequently Prepared from Dark-Grown Plants

Particle Source	cpm Incorporated/mg Protein		Stimulation
	Untreated	Treated	%
Maize Shoot	2,185	3,710	70
	1,877	3,193	70
	2,359	4,196	78
	1,893	2,882	52
Bean Leaf	2,992	7,780	160
	2,742	8,220	199
Soybean Leaf	4,470	12,130	172

The data are expressed as cpm of C^{14}-Leucine incorporated into the acid-insoluble product per mg ribosomal protein. The reaction mixture contained, in a final volume of 0.5 ml: 0.05 mg pyruvic kinase, crude maize supernatant (0.5 to 1.0 mg protein), 0.1 ml ribosomes (0.5 to 1.0 mg protein), and the following in μmoles: Tris buffer, pH 7.6, 50; ATP, 0.5; GTP, 0.15; $MgCl_2$, 5.0; phosphoenol pyruvate, 6.4; KCl, 8.0; C^{14}-Leucine (spec. act. 49.2), 0.004. The reaction was initiated by adding ribosomes and was assayed after 30 minutes at 37° C.

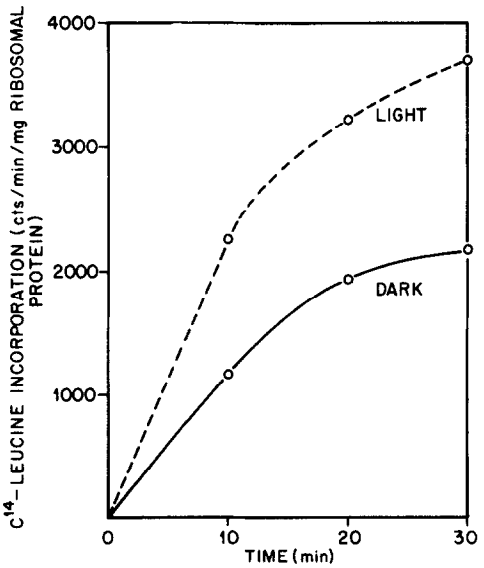

Fig. 1. Kinetics of light-stimulated C^{14}-leucine incorporation. Data are expressed as cpm incorporated into the acid-insoluble product per mg ribosomal protein. Assay conditions are described in Table 1.

fected by the light treatment. Furthermore, because the same supernatant material was used in all comparative experiments, possible changes in the amino acid level of the crude supernatant from light-treated plants (De Deken-Grenson, 1954) are not responsible for the stimulation observed. Thus the light effect described in the present investigation is an intrinsic feature of the ribosomal preparation and appears to reflect an enhanced capacity of these particles for protein synthesis.

Fig. 2 illustrates the development of the light effect in maize shoots as a function of time after initiation of the light treatment. Under these experimental conditions, no significant stimulation is observed in less than 2 hours, and the full extent of the light effect is reached within 3 hours. Similarly, a lag after light treatment is reported for in vivo studies with etiolated leaves on the development of enzymatic activities associated with photosynthetic CO_2 fixation (Tolbert and Gailey, 1955), the capacity for sustained chlorophyll synthesis (Virgin, 1958) and the ability to degrade starch (Klein, Price, and Mitrakos, 1963).

The results described in this report demonstrate that, under these conditions, ribosomes isolated from light-treated plants show a markedly enhanced capability for C^{14}-leucine incorporation into protein. This finding, together with the observa-

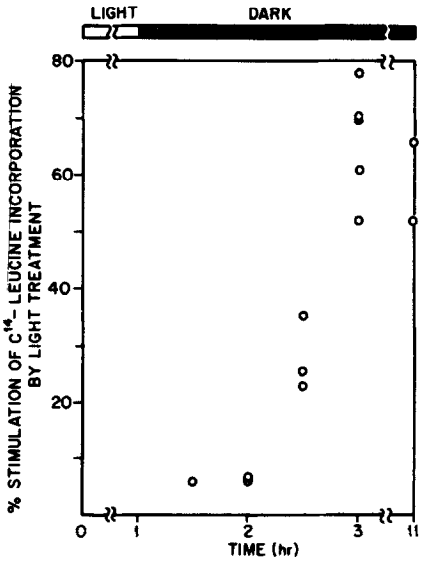

Fig. 2. Development of the light-stimulated incorporation of leucine by ribosome fractions prepared at various times after treatment. Data are expressed as percent increase in activity over controls. Assay conditions are described in Table 1.

tions that this stimulation is not restricted to corn shoots and that a distinct lag period is required for development of the phenomenon, are consistent with previous in vivo studies cited above which demonstrate the light-induced synthesis of leaf protein in intact plants.

Further studies regarding the nature of this light effect are in progress.

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