AN INCREASE IN RNA POLYMERASE ACTIVITY AFTER ILLUMINATION OF DARK-GROWN MAIZE SEEDLINGS

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Received September 25, 1967

Williams and Novelli (1964) demonstrated that illuminating dark grown seedlings of Zea mays enhanced the amino acid incorporating activity of ribosomes isolated from them. Mans (1966) inferred that the increase in amino acid incorporating activity resulted from an increase in the level of messenger RNA associated with the ribosomes isolated from the illuminated seedlings. Stout and Mans (1967) have isolated an RNA polymerase (Nucleoside-triphosphate: RNA nucleotidyl transferase, EC 2.7.7.6) from corn seedlings and have characterized its product as DNA-like RNA. Our experiments were designed to establish a "light effect" on the amino acid incorporating system isolated from maize seedlings and to assay the preparations from the same seedlings for DNA-dependent RNA polymerase activity. The results indicate a direct correlation between an increase in amino acid incorporating activity and RNA polymerase activity after illumination of dark grown seedlings.

Kernels of Zea mays (WF9 X Bear 38) were germinated in the dark on two trays lined with filter paper. After five days, at 23C, one tray of etiolated seedlings was exposed to incandescent or fluorescent light (approximately 3.9×10^4 Lux) for 1.5 hr and then incubated for an additional 2 hr in the

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* Public Health Service Postdoctoral Fellowship (5-F2-CA-28, 208-02) from National Cancer Institute. Present address is Biology Department, Virginia Polytechnic Institute, Blacksburg, Virginia 24061.

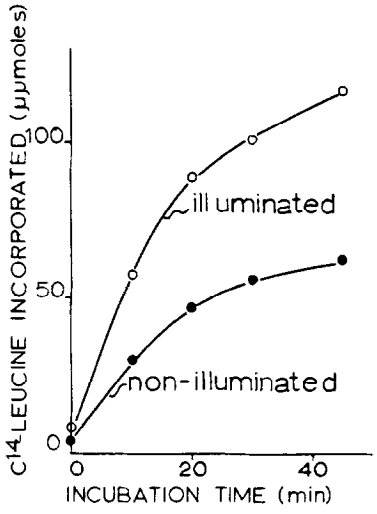

Figure 1. L-leucine-^{14}C incorporation into hot, trichloroacetic acid, precipitated product from illuminated and non-illuminated seedlings. The cell-free reaction mixture contained, in a final volume of 0.5 ml: 0.05 mg crystalline pyruvic kinase, 0.39 mg 2 day-old maize, high speed, supernatant protein, 1.0 mg ribosomal protein, 50 μmoles Tris-Cl (pH 8.0 @ 30 C), 5μmoles ATP-K, 0.15 μmoles GTP-K, 5 μmoles $MgCl_2$, 3.7 μmoles potassium phosphoenol pyruvate, and 0.075 μmoles L-leucine-^{14}C (specific activity 25 mc/mmole). The reaction mixture was incubated at 30 C and aliquots were removed to filter paper discs at the indicated times and assayed for radioactivity as described by Mans and Novelli (1961). One μμmole of L-leucine-^{14}C incorporated is equal to 33.3 counts/min.

dark. The subsequent incubation in the dark is necessary for significant stimulation of amino acid incorporation. Shoots were harvested (in total darkness) from both trays of seedlings and dropped into liquid nitrogen. A fine powder was prepared from each batch of frozen shoots with a motor-driven mortar and pestle. Ribosomes were prepared from half of each powder with a ground glass homogenizer and isolated by the procedure of Wettstein et al. (1963). The ribosomes were assayed for amino acid incorporating activity in the presence and absence of a high speed supernatant component prepared from two-day-old etiolated seedlings according to the methods of Mans and Novelli (1964). RNA polymerase was isolated from the remaining half of each powder using a French pressure cell as described by Mans (1966). Activity was mea-

sured in crude homogenates, high speed supernatant fractions and ammonium sulfate precipitated fractions prepared from them as described by Stout and Mans (1967). Calf thymus DNA was used as added template in the enzyme assay.

Figure 1 shows the increase in amino acid incorporating activity of ribosomes isolated from illuminated and non-illuminated maize seedlings. These data are comparable in initial rate and final specific activity to those of Williams and Novelli (1964) and, therefore, represent the "light effect" which they described.

There is an increase in the level of DNA-dependent RNA polymerase activity in all of the fractions isolated from illuminated seedlings. The increased activity can be measured as incorporation of ^{14}C-AMP, as shown in Table I, or as incorporation of ^{32}P-CMP. Incubation mixtures of crude homogenates contained endogenous corn DNA in addition to added calf thymus DNA. The data reported represent the increment of RNA synthesized in response to the calf thymus DNA. The other fractions were essentially devoid of diphenylamine reacting material. Both the crude homogenates and the 200,000 g supernatant fractions incorporated AMP in the absence of added DNA. However, the level of this incorporation was the same for preparations from illuminated and non-illuminated seedlings. A comparison of the data in experiment I with those of experiment II indicates that the greater the "light effect" on amino acid incorporation, the greater the increase in RNA polymerase activity in all the fractions assayed. The difference in level of response of the seedlings to illumination in the two experiments may reflect a difference in the quality (incandescent and fluorescent) of the light used to illuminate the dark grown seedlings. The supernatant fraction resulting from a 20 min, 20,000 g centrifugation of a homogenate prepared with a glass homogenizer contained RNA polymerase activity. Therefore, it is not essential to utilize the French pressure cell for isolation of the soluble RNA polymerase from corn seedlings as reported by Mans and Novelli (1964).

We have drawn several conclusions from these results. Illumination of

Table I

EFFECT OF ILLUMINATION ON ETIOLATED SEEDLINGS

Assay	Non-illuminated	Illuminated	Increase[4]
	μμmoles	μμmoles	%
	Experiment I (Incandescent)		
Amino Acid Incorporation[1]	66	81	23
RNA Polymerase[2]			
Crude Homogenate	63	74	17
200,000 g supernatant fraction	83	107	29
$(NH_4)_2SO_4$ precipitate	178	235	32
20,000 g supernatant fraction[3]	45	63	40
	Experiment II (Fluorescent)		
Amino Acid Incorporation[1]	53	83	57
RNA Polymerase[2]			
Crude Homogenate	186	248	33
200,000 g supernatant fraction	211	323	53
20,000 g supernatant fraction[3]	65	97	49

[1] ^{14}C-leucine incorporated per mg ribosomal protein in 30 min at 30 C as described in figure 1.

[2] DNA dependent ^{14}C-AMP incorporated per mg protein per 10 min at 30 C.

[3] 20,000 g supernatant fraction was obtained prior to addition of potassuim deoxycholate in the preparation of ribosomes.

[4] Calculated as: $\frac{\text{illuminated} - \text{non-illuminated}}{\text{non-illuminated}}$ (100)

dark grown seedlings with a short exposure to white light enhances the level of RNA polymerase detected in cell-free preparations 3.5 hr after illumination. Other experiments suggest that the enhancement is an increased level of activity of enzyme protein already present and not an increased amount of enzyme. The "light effect" is not manifest as a change in the template activity of the

endogenous DNA of the maize seedlings. The increased polymerase activity in the illuminated seedlings was found in the presence of calf thymus DNA as the sole source of template DNA (the 200,000 g supernatant fraction and ammonium sulfate precipitates). Highly polymerized DNA isolated from etiolated corn seedlings also satisfies the template requirement for polymerase isolated from both illuminated and non-illuminated seedlings. These experiments do not preclude an interaction between a regulator component and any template DNA, altered by illumination of dark grown seedlings.

This work was supported by the Atomic Energy Commission; Report Number NYO-3536-7: and is Scientific Article No. A1364 Contribution No. 3948 of the Maryland Agricultural Experiment Station (Department of Botany).

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