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INFLUENCE OF A CYTOKININ AND ITS RECEPTOR ON THE SYNTHESIS OF PROTEIN IN ISOLATED COTTON PLANT CHLOROPLASTS AND ANALYSIS OF THE PROTEIN—PEPTIDE PRODUCTS OF THIS INTERACTION

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The kinetics of transcription and translation and the influence upon it of the cytokinin-receptor complex have been investigated in isolated cottonplant chloroplasts. It has been established that this complex changes the qualitative and quantitative spectrum of the water-soluble chloroplast proteins. It is postulated that the proteins synthesized de novo *belong to the proteins of the ribulosebiphosphate—carboxylase complex.*

The interrelationship and the diversity of the physicochemical processes lying at the basis of the hormonal regulation of the vital activity of the plant organism are due, on the one hand, to the structure of specific phytohormone receptors and, on the other hand, to their participation in processes of recognition and discrimination at the membrane and genetic levels. In particular, a series of plant proteins has been discovered that possesses receptor properties, and it has been shown that their characteristic function is their capacity, in complexes with the corresponding phytohormones, for activating the synthesis of RNA *in vitro* and *in vivo*. However, the question of the activation products at the level of the genome has remained little-studied and, consequently, questions are still open that are connected with the selectivity and polyfunctionality of the phytohormones, their interaction with membranes, and other aspects of this problem.

By using model systems with isolated chromatin from cottonplant chloroplasts it has been possible to show that a hormone-receptor complex not only activates RNA polymerase but also induces the synthesis of protein [1].

It has been shown that, on the interaction of a cytokinin hormone—receptor complex with chromatin, three new, in comparison with a control, polypeptides are formed having M 14, 18, and 20 kDa. However, in view of the nonribosomal nature of the synthesis of these polypeptides, they apparently cannot be assigned to the final products of the hormone-receptor interaction. It is most likely that they are intermediate producers of the type of signal polypeptides responsible, for example, for the expression of definite genes.

In a search for specific proteins determining the physiological response of the cell to a hormonal signal, we have turned our attention to the capacity of cytokinins for regulating the synthesis of the key element of photosynthesis — ribulose-biphosphate carboxylase (RBPC), which, consequently, may play the role of one of the key products in the expression of genes stimulated by cytokinins [2].

Since RBPC is mainly localized in the chloroplasts (about 50% of the total protein content) the latter form the most convenient material for studying the biosynthesis of this enzyme. In view of this, we isolated chloroplasts from the leaves of two-month cotton plants and, in them, investigated the influence of benzylaminopurine (BAP) and its complex with the receptor protein (cytokinin-binding protein — CBP) on the processes of transcription and translation. As can be seen from Figs. 1 and 2, the kinetics of transcription and translation have an intermediate nature between the eukaryotic and prokaryotic types of synthesis of RNA and protein. This agrees well with the endosymbiotic hypothesis of the origin of chloroplasts in the course

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TABLE 1. Influence of BAP, CBP, and the BAP–CBP Complex on the Synthesis of Protein and of RNA in a System with Isolated Chloroplasts

Preparations	Incorporation of ^{33}P-UTP, pulses/min	Incorporation, %	Incorporation of ^{35}S-methionine, pulses/min	Incorporation, %
Control (isolated chloroplasts (CPs))	22429	100	303257	100
CPs + BAP	33501	147.8	322392	106.3
CPs + CBP	23045	104.3	321924	106.2
CLs + BAP + CBP	38852	173.3	383150	127.9

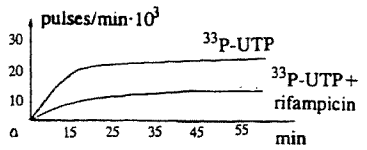

Fig. 1

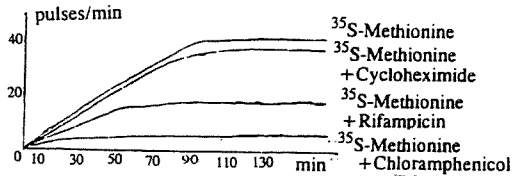

Fig. 2

Fig. 1. Kinetics of the synthesis of RNA in cotton plant chloroplasts.

Fig. 2. Kinetics of the synthesis of proteins in cotton plant chloroplasts.

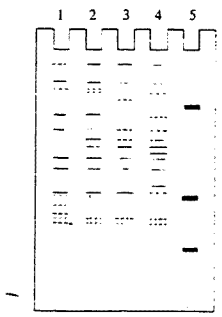

Fig. 3. Electophoretogram of the water-soluble chloroplast proteins: *1*) control; *2*) BAP; *3*) CBP; *4*) BAP + CBP; *5*) mixture of standards.

of evolution. The inhibition of the process by chloramphenicol and its insensitivity to cycloheximide showed the participation of the 70S ribosomes in protein synthesis and the absence of 80S ribosomes from the system.

The addition of BAP, CBP, and their complex to the system with isolated chloroplasts substantially stimulated the incorporation of ^{33}P-UTP into the RNA and of ^{35}S-methionine into the proteins. As can be seen from Table 1, the hormone-receptor complex increased the degree of their incorporation by 73 and 27%, respectively.

Electrophoretic analysis of the fraction of water-soluble chloroplast proteins (Fig. 3) showed that, in response to the actions of the hormone, the receptor, and their complex, the synthesis was induced of five polypeptides with *M* 18 to 60 kDa, absent from the control. In addition to the appearance of new protein bands on an electrophoretogram, an increase in the amount of the proteins also present in the control was observed. As shown by radiometric analysis (Table 2), the activity of the RBPC increased simultaneously.

EXPERIMENTAL

The chloroplasts were isolated from the leaves of two-month cotton plants of the Andizhan variety by a procedure described previously [3]. RNA polymerase activity was determined as in [4]. For the analysis of transcription, the chloroplasts

TABLE 2. RBPC Activity in Cotton Plant Chloroplasts

Preparations	RBPC activity, pulses/min/mg of protein	%
Control — isolated chloroplasts (CPs))	1500 ± 20	100
CPs + BAP	2450 ± 15	163.3
CPs + CBP	1900 ± 20	126.7
CPs + BAP + CBP	2800 ± 20	186.7

were suspended in a small volume of cooled buffer (300 mM sorbitol, 0.5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1 mM Na_2HPO_4, 2 mM EDTA, 35 mM Tris-HCl, pH 7.8). To 0.5 ml of the chloroplast suspension was added 50 μl of ^{35}S-methionine (product of Radiopreparat, Tashkent, molar activity 11PBq/mole) with a total activity of 1 mBq, and the mixture was incubated with irradiation by sunlight for predetermined times at 25°C. The reaction was stopped by the addition of 1 ml of cooled acetone.

Isolation of the Total Labeled Proteins. The proteins that had deposited were centrifuged off, washed free from unbound radioactivity with acetone, and, after the elimination of the latter, dialyzed against water and lyophilized.

The analysis of the translation products was made by electrophoresis in PAAG in the presence of sodium dodecyl sulphate by Laemmli's method [5].

The RBPC activity was determined radiometrically from the incorporation of $^{14}CO_2$ from $NaHCO_3$ into the acid-stable reaction products, as in [6].

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