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Comparative Analysis of the Action of Cytokinin and Light on the Formation of Ribulosebisphosphate Carboxylase and Plastid Biogenesis

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Abstract. The role of cytokinin in plastid biogenesis was investigated in etiolated rye leaves (*Secale cereale* L.) and compared with the effect of white light. Cytokinin deficiency of the leaves was induced by early excision of the seedling roots and reversed by the application of kinetin. The cytokinin supply had a much greater influence on plastid biogenesis than on leaf growth in general. The activities of several chloroplastic enzymes were increased 200%–400% after kinetin treatment of cytokinin-depleted leaves. The activity of ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) and the amount of fraction-I protein even showed a sevenfold increase. In cytokinin-depleted leaves the development of ribulose-1,5-bisphosphate carboxylase and NADP-glyceraldehyde-phosphate dehydrogenase was specifically and markedly inhibited by actinomycin D. The inhibition was partially or even completely overcome after treatment with kinetin. However, under all conditions, RNA synthesis of the leaves was only partially inhibited by actinomycin D. According to immunologic studies, all dark-grown leaves, in addition to the complete enzyme, contained an excess of free small subunit of ribulose-1,5-bisphosphate carboxylase that was absent in mature light-grown leaves. The most striking accumulation of free small subunit protein occurred in cytokinin-depleted dark-grown leaves, indicating a deficiency of the plastidic synthesis of the large subunit. The capacity as well as the activity of plastidic protein synthesis was preferentially increased by cytokinin and light. Cytokinin increased the amount of plastidic ribosomes per leaf and relative to the amount of cytoplasmic ribosomes. While the percentage of cytoplasmic ribosomes bound as polyribosomes was little affected by the cytokinin supply, the proportion of plastidic polyribosomes was increased from 11% to 18% after kinetin treatment of cytokinin-depleted leaves. In the light, the proportion of plastidic polyribosomes reached 39% of the total plastidic ribosomes.

Key words: Chloroplast biogenesis – Cytokinins – Enzyme development – Photoregulation – Ribulose-1,5-bisphosphate carboxylase – *Secale*.

Introduction

In various systems cytokinins exert a specific control over chloroplast differentiation. In some strains of tobacco callus, kinetin was required for differentiation and greening of the chloroplasts (Stetler and Laetsch, 1965; Seyer et al., 1975), and in cultured tobacco leaf discs, kinetin stimulated chloroplast replication (Laetsch and Boasson, 1972). Concomitantly with the stimulation of chloroplast differentiation, cytokinins increased the synthesis of chlorophyll and of chlorophyll precursors in cucumber cotyledons (Fletcher and McCullagh, 1971; Fletcher et al., 1973; Wôzny and Szweykowska, 1975). Also in barley leaves (Averina and Shlyk, 1972) and lupin cotyledons (Mlodzianowsky and Gezela, 1974) chlorophyll formation was promoted by kinetin. In developing rye leaves, we have described striking and specific cytokinin-dependent increases in the activities of chloroplast-specific enzymes, such as RuBP carboxylase and NADP-GAP dehydrogenase (Feierabend, 1969; Feierabend, 1970b). Similar observations have been made in cucumber cotyledons by Harvey et al. (1974). Even in mature bean leaves kinetin applications increased the activities of photosynthetic enzymes (Treharne et al., 1970). While the latter phenomenon was ascribed to some enzyme activation in mature leaves,

Abbreviations: RuBP carboxylase = ribulose-1,5-bisphosphate carboxylase; NADP-GAP dehydrogenase = NADP-dependent glyceraldehyde-3-phosphate dehydrogenase

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inhibitor experiments suggested that in developing leaves cytokinins controlled the amount of enzyme protein formed, at least for the RuBP carboxylase (Feierabend, 1970b; Harvey et al., 1974). This was further confirmed during the present studies by comparing the amounts of fraction-I protein.

In the present investigation we tried to further investigate the mechanism of the specific influence of cytokinin on the development of chloroplastic enzymes in young rye leaves. Two problems were of main interest. (1) The molecuar site of cytokinin action: Does the development of chloroplast-specific enzymes require transcription or might it be explained by some translational control ? (2) The cellular site of the cytokinin action: An overwhelming number of chloroplastic proteins and even the small subunit of the RuBP carboxylase are synthesized on cytoplasmic ribosomes and seem to be coded by nuclear DNA (Feierabend and Schrader-Reichhardt, 1976; Feierabend and Wildner, 1978; for reviews see Ellis et al., 1973; Ellis, 1976). Therefore, the question arose as to whether the selective influence of cytokinin on the development of chloroplast-specific enzymes was mediated by some primary effect on the nuclear-cytoplasmic or by an exclusive influence on the chloroplastic biosynthetic system, and how are the activities of the two systems coordinated? To estimate changes in the capacities of the two systems of protein synthesis, the amounts of 80 S and 70 S ribosomes were compared under different cytokinin level, and the respective polyribosome patterns were used as indicators of their actual activities. The degree of interdependence of the 80 S and 70 S translation systems was tested by following the degree of synchronization in the formation of the subunits for RuBP carboxylase.

Materials and Methods

Plant Material and Growing Conditions

Plant material and growing conditions were described by de Boer and Feierabend (1978). Continuous far-red light was generated as described previously (Feierabend, 1969).

Extraction and Separation of Nucleic Acids

Nucleic acids were extracted as described by Schäfers and Feierabend (1976). High-molecular-weight nucleic acids were separated by electrophoresis on 2.5% polyacrylamide gels according to Loening (1967), as described by Schäfers and Feierabend (1976).

Isolation of Ribosomes and Polyribosomes

a) Monoribosomes. Monoribosomes were isolated after dissociation of the polyribosomes by incubating the leaves under N_2, as described by Feierabend and Schrader-Reichhardt (1976).

b) Polyribosomes. For the estimation of the plastidic polyribosomes, leaves were frozen in liquid nitrogen, finely powdered, homogenized in grinding medium, and total polyribosomes were separated on sucrose gradients as described for the preparation of cytoplasmic polyribosomes (de Boer and Feierabend, 1978), except that the gradient buffer contained 20 mM $MgCl_2$. During fractionation of the gradients the fractions containing either the polyribosomes, or the monoribosomes and ribosomal subunits were collected separately. The ribosomal particles were sedimented by a 2.5-h centrifugation at 170,000 *g*. The resulting sediments were dissolved in a small volume of 36 mM Tris-phosphate, pH 7.6, containing 1 mM EDTA and 10 mM $MgCl_2$. Sodium dodecylsulfate was added to give 2% (wt/vol) final concentration. Samples were analyzed for their rRNA composition by polyacrylamide gel electrophoresis as described above.

Preparation of Cell-Free Extracts

For each extraction, the first leaves of ten seedlings were washed and ground at 2° with a pestle in a prechilled mortar with 0.05 M Tris-HCl buffer, pH 7.5, containing 4 mM dithioerythritol. The homogenates were adjusted to a final volume of 10 ml, filtered through a sintered-glass funnel (Schott & Gen., No. 3D1), and centrifuged for 5 min at 120 *g*. The supernatants were used for the assay of enzyme activities. For the estimation of fraction-I protein (electrophoresis, precipitation), leaf extracts were prepared as described above and centrifuged for 10 min at 1,000 *g* and subsequently for 45 min at 160,000 *g*.

Electrophoresis of Fraction-I Protein

Proteins of leaf extracts were separated by electrophoresis on polyacrylamide gels. The gel system No. 1 of Maurer (1971) was used, except that the acrylamide concentration was 5%. Electrophoresis was performed in the analytical cell (3 mm thickness) of a DESAGA flat-bed electrophoresis apparatus, model No. 146 300, connected to an Ortec 4100 Pulsed Power Supply operating at 350 V, 1 µF and 300 pulses/s. Gels were stained with fast-green according to Bertolini et al. (1976). The protein band of the fraction-I protein was identified by comparison with the electrophoretic mobility of purified rye RuBP carboxylase (Feierabend and Wildner, 1978). The gels were scanned at 630 nm with the linear transport device of the Gilford model 240 recording spectrophotometer. For quantitative comparisons the peak areas for the fraction-I protein were cut from the paper of the recorded absorbance profiles and compared by weighing.

Immunoprecipitation of Fraction-I Protein

A monospecific rabbit antiserum to RuBP carboxylase was used, which has been described in detail by Feierabend and Wildner (1978). For a quantitative estimation of fraction-I protein, 0.2 ml of a cell-free leaf extract were mixed with 0.065 ml of antiserum, which was sufficient for a complete precipitation of the RuBP carboxylase from all treatments tested. After mixing at room temperature the precipitations were kept overnight at 4°. After centrifugation the precipitates were washed 4 times with 2% (wt/vol) NaCl. The final precipitate was dissolved in 0.05 *N* NaOH and the protein content was determined with the Lowry procedure (Gerhard and Beevers, 1968).

Immunoelectrophoresis

Immunoelectrophoresis in agarose gels was performed according to standard techniques (Ouchterlony, 1970) with the Camag equipment (see Feierabend and Wildner, 1978). For immunoelectrophoresis leaf extracts were prepared with 50 mM tricine-KOH buffer, pH 8.0, containing 10 mM $MgSO_4$, 4 mM dithioerythritol and 1.5% (vol/vol) Triton X-100. Per 20 6-day-old leaves, 5 ml of the grinding medium were used for kinetin-treated and light-grown leaves, 2.5 ml for leaves of intact dark-grown seedlings, and 1 ml for leaves of derooted dark-grown seedlings. The homogenates were centrifuged for 30 min at 48,000 *g* and 4 µl of the supernatants were applied per well for immunoelectrophoresis.

Enzyme Assays

Enzyme activities were determined spectrophotometrically at 25°. Fructose-1,6-bisphosphatase (EC 3.1.3.11), NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13), NADP-dependent malate dehydrogenase (EC 1.1.1.82), phosphoribolokinase (EC 2.7.1.19), transketolase (EC 2.2.1.1), and ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) were assayed as described previously (Feierabend and Schrader-Reichhardt, 1976).

Carotenoids

Total carotenoids were estimated from 80% acetone extracts according to Metzner et al. (1965).

Determination of Plastid Sizes

Sizes of plastids were determined in sections of fixed leaf tissues, as described previously (Feierabend, 1970a).

All experiments were performed 3–8 times. Figure 4 shows the results of representative experiments. All other data are averages of the measurements. Standard deviations are indicated.

Results

Development of Chloroplastic Enzymes

The influence of cytokinin on the development of chloroplastic enzymes was investigated in the leaves of dark-grown rye seedlings depleted of their endogenous cytokinin supply by early excision (on day 1 of germination) of the seedling roots (Feierabend, 1969; de Boer and Feierabend, 1978). Since both kinetin and light promote the development of chloroplastic enzymes, leaves of intact and untreated light-grown seedlings were investigated for comparison.

The cytokinin-induced changes of chloroplastic enzyme activities (Table 1) were much greater than those of leaf growth in general that were described in the preceding publication (de Boer and Feierabend, 1978). Such a specific influence of cytokinin has already been described for RuBP carboxylase and for NADP-GAP dehydrogenase (Feierabend 1969, 1970b) and was now also observed for several other chloroplast-specific enzymes and for the carotenoid contents. The activities of all plastid enzymes were considerably more affected by the excision of the seedling roots than was general leaf growth. Subsequent kinetin treatments resulted in 2–4-fold, for RuBP carboxylase even in 7-fold increases (Table 1). Parameters of general leaf growth were at maximum increased by 100% under such conditions (de Boer and Feierabend, 1978). Quantitative determinations of the fraction-I protein contents by electrophoresis and by precipitation with specific antibodies documented that the changes in RuBP carboxylase activity represented corresponding changes in the amounts of the enzyme protein (Fig. 1). After kinetin treatment some but not all of the plastid enzymes reached activities in darkness that were approximately as high as those in the control leaves in light (Table 1).

The size of the plastids of cytokinin-depleted leaves was significantly smaller than in control or kinetin-treated leaves (Table 2). There was no major difference in the size of the plastids from leaves of dark-grown control plants, kinetin-treated leaves, or seedlings that had received continuous far-red light (Table 2). Following exposure to far-red light, activities of plastid enzymes were strongly increased without

Table 1. Activities of several chloroplastic enzymes and carotenoid contents in the first leaves of 7-day-old rye seedlings grown in light (a) or at different cytokinin levels in the dark: (b) control, (c) roots excised after day 1 of germination, (d) roots excised on day 1, kinetin applied on day 2 of germination. Enzyme activities are nmoles substrate reacted per min per first leaf. Carotenoids are µg per leaf

	(a) Light	(b) Dark, control	(c) Dark, −roots	(d) Dark, +kinetin
RuBP carboxylase	84 ± 3	34 ± 6	10 ± 3	65 ± 4
Fructosebisphosphatase	97 ± 5	18 ± 3	5 ± 1	22 ± 3
Phosphoribulokinase	1,236 ±220	186 ±10	100 ±11	349 ±27
Transketolase	110 ± 16	49 ± 8	20 ± 2	70 ±12
NADP-Malate dehydrogenase	49 ± 4	15 ± 4	5 ± 0.3	24 ± 3
Carotenoids	17.8± 1.2	2.6± 0.2	1.2± 0.2	3.8± 0.6

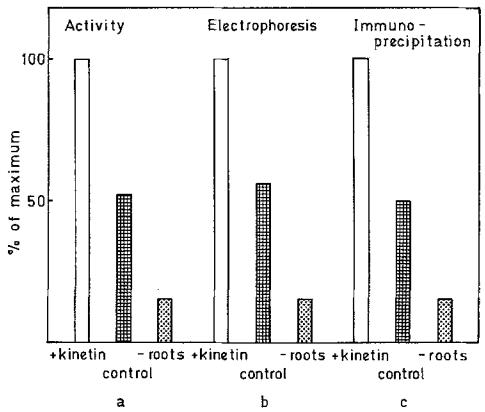

Fig. 1a–c. Comparison of the activities of RuBP carboxylase **a** with the relative amounts of fraction-I protein determined after electrophoresis of leaf extracts **b** or by precipitation with a specific antiserum to RuBP carboxylase **c** in the leaves of 7-day-old dark-grown rye seedlings. Roots were excised at day 1 of germination (−roots). Kinetin was applied to derooted seedlings on day 2 of germination (+kinetin). Units are per cent of the measurements in kinetin-treated leaves

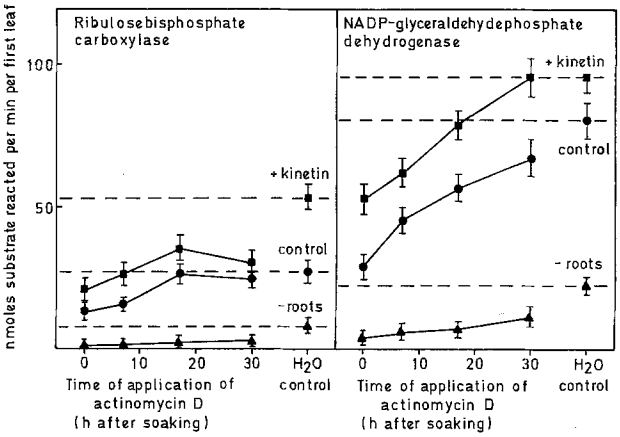

Fig. 2. Effect of actinomycin D at various application times on the activities of RuBP carboxylase and of NADP-GAP dehydrogenase in the first leaves of 7-day-old rye seedlings grown in the dark at different levels of cytokinin. ● control seedlings, ▲ seedling roots excised after day 1 of germination, ■ roots excised at day 1, kinetin applied on day 2 of germination

Table 2. Influence of cytokinin and light on the size of plastids in mesophyll cells of the first leaves of 5-, or 7-day-old etiolated rye seedlings grown in complete darkness (a–c) or in continuous far-red light (d). (b)-roots: seedling roots were excised on day 1 of germination, (c) +kinetin: derooted seedlings were treated with kinetin on day 2 of germination

Age	(a) Control	(b) −Roots	(c) +Kinetin	(d) Far-red
5 days	3.3	2.0	3.2	3.0 µm length
	2.2	1.4	2.2	2.2 µm breadth
7 days	2.8	2.3	3.4	3.0 µm length
	2.0	1.6	2.4	2.3 µm breadth

greening (Feierabend, 1969). In addition to their size, plastids may, however, differ in number.

Effect of Actinomycin D

In order to examine whether transcription was needed, the cytokinin-induced increases of the activities of RuBP carboxylase and of NADP-GAP dehydrogenase were investigated in the presence of actinomycin D. The effect of actinomycin D depended on the time of its application and on the cytokinin level of the leaves. In cytokinin-deficient leaves RuBP carboxylase was virtually absent in the presence of the inhibitor, and the increase of NADP-GAP dehydrogenase activity was inhibited by 50%–80%, even when the actinomycin D was added as late as 30 h after soaking (Fig. 2). In intact control seedlings and in kinetin-treated derooted seedlings, NADP-GAP dehydrogenase reached, also in the presence of actinomycin D, much higher activities than in cytokinin-deficient leaves. When the seedlings were treated with actinomycin D at 30 h and kinetin was applied at 48 h of germination, the activity of NADP-GAP dehydrogenase was as high as in the absence of the inhibitor and 8.3 times higher than in the absence of kinetin. RuBP carboxylase behaved similarly (Fig. 2). In intact seedlings an inhibition was observed only when actinomycin D was applied earlier than 17 h after imbibition. Kinetin treatment restored high levels of enzyme activity in cytokinin-depleted leaves (10–20-fold increases) in the presence of actinomycin D. However, in contrast to the results with NADP-GAP dehydrogenase, RuBP carboxylase of kinetin-treated leaves reached maximally 50–60% of the activity measured in the absence of the inhibitor.

Size and Activity of the Translation Systems

To compare the capacities of the cytoplasmic and the plastidic systems of protein synthesis, the amounts of 80 S and 70 S ribosomes were determined by estimating their specific high-molecular-weight rRNA species after polyacrylamide gel electrophoresis of the nucleic acids isolated from the leaves. Under all experimental conditions, the ratio of the cytoplasmic 25 S to 18 S rRNA was approximately 2.0, as expected. The plastidic rRNA, however, had a ratio of 23 S to 16 S of between 1.4 and 1.7 for light-grown and

derooted seedlings, and a lower ratio of 1.2–1.3 for dark-grown kinetin-treated and untreated control seedlings (de Boer, 1976). According to Ingle (1968) such low 23 S to 16 S ratios indicate an instability of the 23 S rRNA. Therefore, in order not to underestimate the plastidic rRNA contents, measurements of the 16 S rRNA alone were used for following the behavior of the plastidic rRNA (Fig. 3).

The ratio of both the cytoplasmic and the plastidic rRNA to DNA was lowest in light-grown leaves and considerably higher in all dark-grown leaves, thus confirming the results obtained for the total rRNA (de Boer and Feierabend, 1978, Fig. 5). Between days 5 and 7 the ratio of rRNA per DNA decreased, except for the plastidic rRNA of kinetin-treated leaves (Fig. 3). The content of cytoplasmic rRNA per DNA was considerably higher in cytokinin-depleted than in kinetin-treated dark-grown leaves. The content of plastidic rRNA per DNA was, however, higher in kinetin-treated than in cytokinin-depleted leaves. The ratio of plastidic to cytoplasmic rRNA increased with age. In addition, it was much higher (about twice as high) for the leaves of intact control seedlings and for kinetin-treated leaves than for cytokinin-depleted leaves (Fig. 3). Thus cytokinins seem to promote selectively the formation of plastidic ribosomes. Similar results were also obtained by comparisons of isolated ribosomes, whereas the differences observed were not as marked. However, ribosome yields have been shown to change under different growth conditions (Gordon et al., 1975).

Since the content of polyribosomes seems to be correlated with the rate of protein synthesis (Short et al., 1974; Smith, 1976), it was used as an indicator for the activity of the cytoplasmic and plastidic translation systems. In order to estimate the polyribosome proportion of the plastids, first total polyribosomes were prepared, which included 80 S as well as 70 S polyribosomes. The contents of plastidic ribosomes in the polyribosome and in the monoribosome plus subunit fractions were then determined from the amounts of the plastid-specific 16 S rRNA after electrophoretic separation. The percentage of the plastidic ribosomes bound as polyribosomes was much higher in light, as compared to all dark-grown leaves. It was lowest in the cytokinin-depleted leaves and clearly increased after application of kinetin (Table 3). The cytoplasmic polyribosome proportion was similarly determined from the total polyribosome separations by comparison of the cytoplasmic rRNA in the polyribosome and in the monoribosome plus subunit fractions (Table 3). Except that all percentages were somewhat lower, the results in general confirm the data obtained from separations of 80 S polyribosomes alone (de Boer and Feierabend, 1978; Figs. 6 and 7), i.e., that the proportion of cytoplasmic polyribosomes was little affected by the cytokinin supply but clearly increased by light.

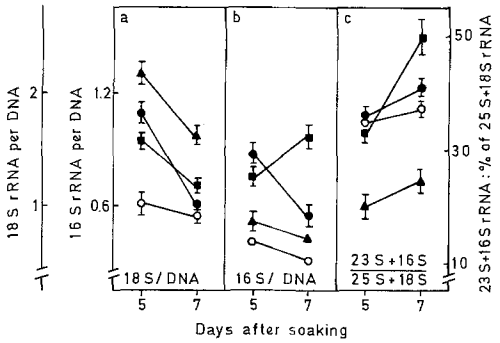

Fig. 3a–c. Changes of the relative amounts of cytoplasmic 18 S **a** and of plastidic 16 S **b** rRNA and of the ratio of plastidic to cytoplasmic rRNA **c** in the first leaves of rye seedlings grown in light (○) or at different cytokinin supplies in the dark: ● control seedlings, ▲ seedling roots excised after day 1 of germination, ■ roots excised on day 1, kinetin applied on day 2 of germination

Table 3. Influence of cytokinin and white light on the plastidic and cytoplasmic polyribosome proportions in the first leaves of 7-day-old rye seedlings. Percentages of the 16 S rRNA and of the 18 S rRNA of total ribosomes found in the polyribosome fraction. Seedling roots were excised on day 1 of germination (−roots). Kinetin was applied to derooted seedlings on day 2 of germination (+kinetin)

Conditions	Percentage in polyribosomes of	
	16 S rRNA (%)	18 S rRNA (%)
−Roots	10.9 ± 0.7	29.0 ± 2.1
+Kinetin	17.6 ± 1.5	31.8 ± 2.4
Control, dark	12.0 ± 2.1	29.0 ± 2.1
Control, light	38.9 ± 1.6	49.1 ± 0.9

Coordination of the Formation of the Subunits of RuBP Carboxylase

Previous work with specific antibodies (Feierabend and Wildner, 1978) showed that at young stages of normal leaf development and in plastid ribosome-deficient leaves the small subunit of RuBP carboxylase was formed either in excess to, or even in the complete absence of, the large subunit of this enzyme. After immunoelectrophoresis, a precipitation arc of free small subunit was found and identified, which was different from the precipitation arc of the complete RuBP carboxylase (Feierabend and Wildner, 1978). In all dark-grown leaves such a free small subunit protein occurred in addition to the complete

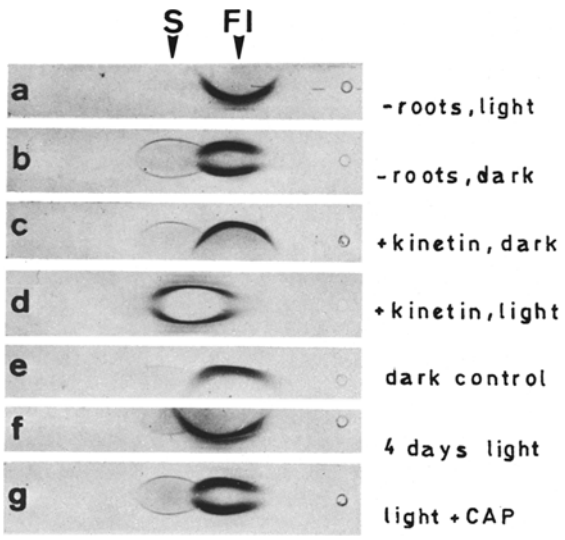

Fig. 4a–g. Immunoelectrophoresis of extracts from rye leaves grown under different cytokinin levels and light conditions. Leaf extracts in the antigen wells were from 6-day-old derooted light **a**, or dark-grown **b**, kinetin-treated dark- **c**, or light-grown **d**, or untreated dark-grown control seedlings **e**. **f** Leaf extract of normal 4-day-old light-grown seedlings **g** Leaf extracts from 4-day-old light-grown seedlings treated with D-threo-chloramphenicol (2 mg/ml) on day 3. The different leaf extracts were adjusted to similar concentrations of RuBP carboxylase (see Methods). The troughs contained antiserum to RuBP carboxylase. *FI* Precipitation arc of fraction-I protein; *S* precipitation arc of small subunit

RuBP carboxylase (Fig. 4). Although accurate quantitative estimations were not possible, it is evident from Figure 4, that among etiolated leaves the accumulation of free small subunit, relative to the amount of the complete enzyme, was strongest in the cytokinin-deficient leaves and was least striking in the untreated control leaves. In all mature light-grown leaves, however, whether cytokinin-depleted or kinetin-treated, free small subunit was no longer present. Only the very young stages of light-grown leaves (3-day-old) contained an excess of small subunit protein, which disappeared with age (Fig. 4; see also Feierabend and Wildner, 1978). In light-grown leaves a heavy accumulation of free small subunit, similar to that observed in dark-grown cytokinin-deficient leaves was, however, induced and preserved when the chloroplastic protein synthesis was inhibited with chloramphenicol (Fig. 4g).

Discussion

In young etiolated rye leaves depleted of their endogenous cytokinin supply by early excision of the seedling roots (Feierabend, 1969), two sets of cytokinin effects were distinguished: (1) Some general stimulation of the overall leaf growth (de Boer and Feierabend, 1978), and (2) a much greater, specific influence on the development of chloroplastic enzymes. The development of chloroplastic enzymes required a greater cytokinin supply than leaf growth in general. While the amounts of several general growth parameters were at maximum doubled after kinetin treatment of cytokinin-depleted leaves (de Boer and Feierabend, 1978) the activities of chloroplastic enzymes were increased 3- to 7-fold. The increased RuBP carboxylase activity was accompanied by a corresponding increase in the amount of enzyme protein. This supports the conclusion drawn from previous inhibitor experiments, i.e., that the cytokinin-induced increase in RuBP carboxylase activity resulted from a de novo synthesis of the enzyme (Feierabend, 1970b; Harvey et al., 1974). In the present work actinomycin D was applied in order to examine whether the cytokinin-induced increase of the chloroplastic enzyme activities and particularly of the RuBP carboxylase was also dependent on transcription. The results were, however, equivocal. On the one hand the development of the newly appearing chloroplastic enzymes RuBP carboxylase and NADP-GAP dehydrogenase was specifically and markedly inhibited by actinomycin D (in contrast to other enzymes present from the beginning; see de Boer and Feierabend, 1978) in cytokinin-depleted leaves. After addition of kinetin, inhibition of the development of RuBP carboxylase and of NADP-GAP dehydrogenase was largely or even completely overcome. Such results would routinely be interpreted as indicating that the cytokinin-induced rise in RuBP carboxylase activity was partially dependent on transcription and that the increase in NADP-GAP dehydrogenase activity was relatively independent of transcription. On the other hand, RNA synthesis of the leaves seemed to be only partially inhibited after treatments with actinomycin D (de Boer and Feierabend, 1978). Therefore, from the available information it cannot be decided whether the inhibition of the two chloroplastic enzymes in cytokinin-depleted leaves really resulted from a selective action of the inhibitor on the transcription of some minor but specific mRNA fractions, or was due to some nonspecific or even toxic effects.

From the fact that cytokinin preferentially enhanced the accumulation of chloroplastic enzymes, it cannot be concluded that its action was mainly restricted to biosynthetic events within the plastids themselves, because most of the chloroplastic enzymes tested, such as NADP-GAP dehydrogenase, phosphoribulokinase, NADP-malate dehydrogenase etc., are synthesized on cytoplasmic ribosomes (Feierabend and Schrader-Reichhardt, 1976). Cytokinins seem to enhance the realization of an integral specific differ-

entiation pattern within the leaf cell that includes chloroplast differentiation and the synthesis of chloroplast constituents, wherever their site of synthesis, but additionally also concerns other accompanying processes of the developing leaves, such as the biogenesis of the peroxisomes (de Boer and Feierabend, 1974).

The cytokinin requirement of plastidic protein synthesis, however, seemed to be greater than that of the cytoplasmic synthesis of chloroplast-specific proteins. RuBP carboxylase, which needs plastidic protein synthesis for the formation of its large subunit, showed a much greater response to changes of the cytokinin level than do chloroplastic enzymes of cytoplasmic origin. Even the formation of the small subunit of the RuBP carboxylase seemed to be less sensitive to a cytokinin deficiency than did the formation of the complete enzyme. The marked accumulation of an excess of free small subunit in cytokinin-depleted and in all dark-grown leaves documented the fact that the formation of the large and not that of the small subunit was limiting the synthesis of the complete enzyme under these conditions. According to our results cytokinin selectively promotes the plastidic protein synthesis, which produces the large subunit of RuBP carboxylase as one of its major products (Ellis et al., 1973; Ellis, 1976) in two ways: (1) through an enlargement of the capacity of the plastidic translational machinery within the leaf cells, as indicated by the relative increase of the plastidic ribosome content, and (2) through a stimulation of the activity of the existing translational system, as indicated by the increase of the ratio of plastidic polyribosomes to monoribosomes.

A similar predominant accumulation of plastidic rRNA was observed in cucumber cotyledons under the influence of cytokinin (Mikulovich et al., 1977) and was mediated in peas and white mustard via phytochrome (Scott et al., 1971; Thien and Schopfer, 1975), which is also known to enhance chloroplast biogenesis. In bean leaves, light and phytochrome, additionally, also increased the proportion of plastidic polyribosomes, (Smith, 1976; Klein and Pine, 1977). In our experiments with rye leaves light, too, induced a striking increase in the proportion of plastidic polyribosomes, which was much greater than that observed after kinetin treatment in the dark. This may explain the fact that in all dark-grown rye leaves, even with a high cytokinin supply, an excess of free small subunit of the RuBP carboxylase was present (Fig. 4). Light was obviously needed in order for the rate of the plastidic synthesis of the large subunit to become equal to that of the small subunit in the cytoplasm so that no surplus of the latter was left.

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