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The 110-kDa polypeptide of spinach plastid DNA-dependent RNA polymerase: Single-subunit enzyme or catalytic core of multimeric enzyme complexes?

(chloroplasts/T7 RNA polymerase/*Escherichia coli* RNA polymerase)

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ABSTRACT **Highly purified RNA polymerase preparations from spinach chloroplasts contain seven major polypeptides of 150, 145, 110, 102, 80, 75, and 38 kDa. I find that RNA polymerase activity can be separated under defined conditions into three different fractions by heparin-Sepharose chromatography. Immunological analysis has shown that the first fraction contains RNA polymerase activity associated with all seven major polypeptides, and other studies have shown that some of these polypeptides (150, 145, 80, and 38 kDa) are associated with an RNA polymerase similar to the *Escherichia coli* enzyme. However, similar analyses of the remaining fractions show activity associated only with the 110-kDa polypeptide, suggesting the existence of a second kind of chloroplast RNA polymerase. Samples of this 110-kDa polypeptide purified by SDS/PAGE actively synthesize RNA in a reaction dependent on a supercoiled DNA template and the four ribonucleoside triphosphates. Hence, this polypeptide has all of the properties expected of a single-subunit RNA polymerase of the T7 bacteriophage type.**

Regulation of transcription in chloroplasts is still poorly understood. Originally, the existence of two different RNA polymerase activities was suggested by the finding that tRNA and mRNA genes in *Euglena* chloroplasts could be transcribed by a soluble enzymatic activity, whereas transcription of rRNA genes required a membrane-bound fraction (1). The activity of the soluble fraction was correlated with the expression of the *rpo* genes of the plastid genome, which are homologous to bacterial *rpo* genes that make up the bacterial RNA polymerase (2). However, neither of these fractions has been purified to homogeneity.

The most highly purified chloroplast RNA polymerase (cp RNA polymerase) preparations obtained from maize, spinach, and pea contain 7–14 polypeptides (3–6). In maize, some of these polypeptides (180, 120, 78, and 38 kDa) were shown to be products of the plastid *rpo* genes (5). In pea, some of these polypeptides (150, 54, and 51 kDa) have been confirmed as RNA polymerase subunits by photocrosslinking (7, 8). In preparations of spinach cp RNA polymerase, all of the major polypeptides were identified as components of active cp RNA polymerases through use of antibody-linked polymerase assays (ALPAs; ref. 3). However, these assays do not reveal whether these preparations contain only one active RNA polymerase or two.

In previous studies it has been shown that spinach leaf homogenates contain polypeptides that are immunologically related to $\beta\beta'$, α, and σ subunits of *Escherichia coli* RNA polymerase. The latter two polypeptides are separated from the $\beta\beta'$ homologs during PEI-cellulose chromatography. Specific initiation at the *rbcL* promoter requires both fractions, which supports the idea that this transcription is carried out by an RNA polymerase with a structure similar to the bacterial enzyme (4). However, cp RNA polymerase preparations lacking the α- and σ-like polypeptides are active in transcription. This led us to suggest the presence of a second type of RNA polymerase. In the present paper, I report studies that support the existence of a separate type of RNA polymerase in spinach chloroplasts, one that is active as a single polypeptide chain.

MATERIALS AND METHODS

Preparation of Spinach Chloroplast Extracts. Very young spinach plants (with leaves not larger than 5 cm) were harvested in the field. Intact and pure chloroplasts were isolated on Percoll gradients from 500 g of leaves as described (9). These preparations do not contain any detectable traces of mitochondrial contamination as shown by the absence of fumarase and phosphatidylethanolamine (10, 11). The enzyme was recovered from heparin-Sepharose by gradient elution with buffer A [50 mM Tris·HCl, pH 7.8/25% (vol/vol) glycerol/0.1% Triton X-100/0.1 mM EDTA] containing 100 mM to 1.5 M $(NH_4)_2SO_4$ followed by a step elution with 10 ml of 1.5 M $(NH_4)_2SO_4$ and 6 M urea in buffer A or by a two-step elution with buffer A containing 600 mM $(NH_4)_2SO_4$ followed by 1.5 M $(NH_4)_2SO_4$ and 6 M urea.

Analysis of RNA Polymerase Polypeptides by ALPAs. Proteins were precipitated with trichloroacetic acid at 0°C overnight, and polypeptides were separated by SDS/PAGE as described (3). Transfer to Immobilon-P membranes (Millipore) was performed at 400 mA in 25 mM Tris·HCl, pH 8.0/0.03% SDS under constant buffer circulation for 4 h and subsequently in 25 mM Tris·HCl at pH 8.0 for 1 h. cp RNA polymerase polypeptides were determined on the Immobilon filters by ALPAs (12). In the first binding reaction, antibodies raised against cp RNA polymerase purified from 4 kg of large leaves by subsequent chromatography on heparin-Sepharose, DEAE-cellulose, and PEI-cellulose (3) were bound to the polypeptides on the filter. In the second binding reaction, native chloroplast enzyme(s) corresponding to heparin-Sepharose fraction P1 was crosslinked to the denatured RNA polymerase polypeptides on the filters through the bound antibodies. Polypeptides that had bound active cp RNA polymerase were detected through *in vitro* transcription (for details see refs. 3 and 13). RNA polymerase polypeptides that are homologous to subunits of *E. coli* polymerase were determined by ALPAs using in the first binding reaction antibodies raised against *E. coli* holoenzyme and in the second binding reaction native *E. coli* polymerase (13).

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Abbreviations: cp RNA polymerase, chloroplast RNA polymerase; ALPA, antibody-linked polymerase assay.

Protein concentrations were measured using the method of Bradford (14).

Isolation and Renaturation of the 110-kDa Polypeptide. After precise localization, the 110-kDa protein or the β subunit of *E. coli* RNA polymerase was eluted from the Immobilon-P membrane with a solution of 1% Triton X-100 in 50 mM Tris·HCl at pH 9.0 (15). Proteins were precipitated with 4 vol of acetone and renatured as described for the σ subunit of *E. coli* polymerase (16). Briefly, the proteins were dissolved in 2.5 μl of 6 M guanidine hydrochloride in dilution buffer [50 mM Tris·HCl, pH 8.0/20% glycerol/bovine serum albumin (0.1 mg/ml)/150 mM NaCl/1 mM dithiothreitol/0.1 mM EDTA], kept for 15 min at room temperature, and then diluted stepwise 50-fold with dilution buffer over 3 h.

***In Vitro* Transcription.** For analysis of transcription products, *in vitro* transcription was performed at 30°C in 25-μl assays containing 44 mM Tris·HCl (pH 8.0), 80 μM dithiothreitol, 14 mM $MgCl_2$, 50 mM NaCl, 4 mM EDTA, 300 μM GTP, 300 μM ATP, 300 μM CTP, 5 μM UTP including 20 μCi (1 Ci = 37 GBq) of [^{32}P]UTP, 1 μg of DNA, and 2 μl of enzyme extract unless otherwise indicated. Reactions were stopped after 30 min by extraction with phenol/chloroform and precipitation with ethanol. In some cases, transcription products were treated with 2 units of pancreatic RNase or 40 units of RNase-free DNase at 30°C for 10 min before phenol/chloroform extraction. Transcription products were analyzed on 8% acrylamide/urea gels. For activity determination, *in vitro* transcription was performed in 100-μl assays containing 18 μM UTP including 5 μCi of [5,6-^{3}H]UTP. Incorporation of UMP into RNA was measured in a scintillation counter after precipitation of RNA on DEAE filter discs. Assays were carried out at 30°C for 30 min. One unit is the enzyme activity that incorporates 1 pmol of UMP into RNA per min. Incorporation is linear up to 30 min. Plasmid pUC18 containing the *rbcL* promoter region is described in ref. 4. Plasmid pTZ19-$P_{LS}T_a$ containing the T7 promoter, the spinach plastid *rbcL* gene promoter, and the *E. coli* threonine attenuator was kindly provided by E. M. Orozco and is described in detail (17).

RESULTS

Preparation of Three Different Chloroplast Fractions Containing RNA Polymerase Activities. A systematic analysis of chloroplast RNA polymerase activity using spinach leaves of field-grown plants of different ages has shown that activity is much higher in young, still-growing plants than in older, mature plants. Fig. 1 shows column chromatography of RNA polymerase fractions obtained from sucrose gradient-isolated chloroplasts starting with 4 kg of large leaves of mature plants as described (3). This elution profile is compared with that obtained from Percoll gradient-purified chloroplasts starting with 500 g of small leaves from very young plants. The total activity per gram of young leaves is much higher. In addition, the elution profile with the smaller amount of material gives two distinct peaks (designated P1 and P2), whereas in the older method only the first peak, P1, is present. The pooled fractions from young leaves are shown with bars at the bottom of the figure: P1 contained 1.7 mg of protein and had a specific activity of 44 units/mg. P2 contained 0.58 mg of protein and had a specific activity of 97 units/mg.

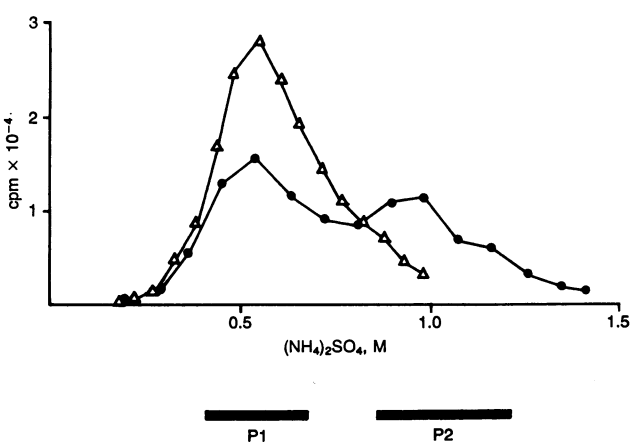

FIG. 1. Elution profiles of cp RNA polymerase activities from heparin-Sepharose. RNA synthesis of the different fractions was measured by incorporation of labeled UMP into RNA using nondenatured calf thymus DNA as template. △, Large leaves of mature plants; ●, small leaves of young plants.

Recently, we have demonstrated that a protein fraction containing σ-like polypeptides remains tightly bound to heparin-Sepharose in such fractionation and is eluted only with buffer containing 6 M urea and at least 1 M ammonium sulfate (4). After dialysis this fraction (P3) regains transcriptional activity. When P3 is isolated from 500 g of young leaves, one obtains about 0.2 mg of protein, with a specific activity of 60 units/mg after renaturation.

The polypeptide composition of RNA polymerase in fraction P1 from 500-g amounts of young leaves was found to be identical to that described previously, which has been characterized in some detail (3, 4). Hence, I focused my attention on the characterization of fractions P2 and P3.

Analysis of the Polypeptide Composition of RNA Polymerases in Fractions P2 and P3. Analysis of the polypeptide composition of fractions P2 and P3 by SDS/PAGE revealed a variety of polypeptides from 155 to 33 kDa (Fig. 2, lanes 1 and 5). We can determine which of these polypeptides are components of the RNA polymerase responsible for the activity of these fractions by using the ALPA technique (3, 13). In this technique, the polypeptides from each fraction are resolved by denaturing SDS/PAGE and transferred to an Immobilon-P membrane. The proteins fixed to the membrane are then treated with an excess of polyclonal antibodies raised to the purified cp RNA polymerase. These antibodies

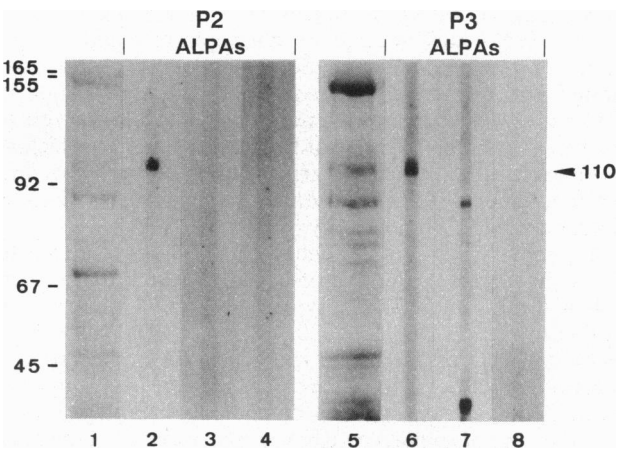

FIG. 2. Analysis of the polypeptide composition and characterization of RNA polymerase polypeptides of P2 and P3 by ALPAs. P2 and P3 proteins were separated by SDS/PAGE and transferred to Immobilon-P membranes. Proteins were localized by staining with amido black (lanes 1 and 5). The part of the membrane charged with P2 or P3 proteins was cut into three equal strips. After destaining with 45% methanol, the membrane strips were saturated with bovine serum albumin and analyzed as follows: lanes 2 and 6, ALPAs using antibodies raised against purified cp RNA polymerase in the first binding reaction and heparin-Sepharose-purified cp RNA polymerase corresponding to P1 obtained from 4 kg of large leaves in the second binding reaction; lanes 3 and 7, ALPAs using antibodies raised against the *E. coli* holoenzyme in the first binding reaction and *E. coli* RNA polymerase holoenzyme in the second binding reaction; lanes 4 and 8, control experiments as indicated in the text.

bind to the polypeptides on the membrane, but since each antibody molecule has two binding sites, many bound antibodies retain free antibody binding sites. These are directed solely against the specific polypeptide to which they are attached on the membrane. Partially purified, native cp RNA polymerase is now added and will attach specifically to those antibodies that are fixed to denatured RNA polymerase polypeptides (subunits and/or transcription factors) on the membrane. Finally, the location of polypeptides that have bound native cp RNA polymerase is determined by incubation of the membrane with a DNA template and labeled nucleoside triphosphates to detect polymerase activity.

The use of this technique to analyze the polypeptides of our earlier preparations of purified spinach cp RNA polymerase implicated a large number of polypeptides from 155 kDa down to 33 kDa as RNA polymerase components, including polypeptides shown to crossreact with the *E. coli* RNA polymerase subunits (3). However, to my surprise, ALPA analyses of fractions P2 and P3 show only a single active component, which coincides with the 110-kDa polypeptide (Fig. 2, lanes 2 and 6). Hence, the activity of these fractions appears to be unrelated to the *E. coli*-like cp RNA polymerase activity.

Fractions P2 and P3 were also screened for polypeptides homologous to *E. coli*-like RNA polymerase subunits by ALPA analyses using *E. coli* RNA polymerase in the second binding step (Fig. 2, lanes 3 and 7). P2 contains no *E. coli*-related polypeptides, whereas P3 contains the previously studied σ-like polypeptides of 33 and 90 kDa (4). A variety of control experiments were done to rule out eventual artifacts of the ALPA assays. They include (*i*) performing the transcription reaction without template DNA to exclude the possibility that the ALPA-reactive polypeptides result from binding of labeled nucleotides to the active center of native polymerase, (*ii*) incubation of the filter with the labeled nucleotide alone to show that it is not bound directly to one of the polypeptides on the filter, (*iii*) incubation of the filter with labeled DNA to exclude binding of the native enzyme through the DNA template, and (*iv*) transcription assay of the polymerase with [γ-^{32}P]ATP to rule out the possibility that the signal results from end-labeling of the DNA. Lanes 4 and 8 are representative of all of these controls. In earlier studies it was shown that preimmune serum does not give any ALPA-reactive band with cp or *E. coli* RNA polymerase (3).

Isolation of the 110-kDa Polypeptide and *in Vitro* Transcription. The results obtained by ALPAs show that fractions P2 and P3 are transcriptionally active even though none (P2) or only some (P3) of the *E. coli*-like RNA polymerase polypeptides are present. Further, the RNA polymerase activity is correlated with only the 110-kDa polypeptide. A simple explanation for these results would be that the 110-kDa polypeptide is a second RNA polymerase, distinct from the *E. coli*-like enzyme. To test this directly, polypeptides from fractions P2 and P3 were separated by SDS/PAGE and transferred to Immobilon-P, and the location of the 110-kDa polypeptide was determined by antibody reactivity (Fig. 3, lanes 1 and 2). For this experiment, antibodies were monitored by peroxidase, which gives many more bands than by ALPA assays. The membrane region containing the 110-kDa polypeptide (lane 2) was excised. The protein was eluted, precipitated, and renatured as described in *Materials and Methods*. The total quantity of the eluted protein cannot easily be determined because the buffer contains bovine serum albumin (100 μg/ml), but the activity obtained is reproducible for preparations carried out with the same amount of protein (200 μg).

As a template for transcription, I used three different DNAs. A pUC18 plasmid with a 550-bp insert containing the promoter region for the chloroplast *rbcL* gene (4) was used to detect general transcriptional activity, to determine the

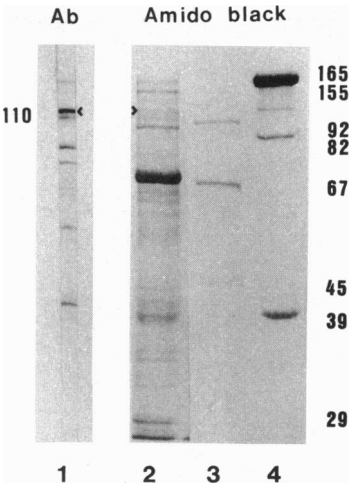

FIG. 3. Polypeptide pattern of fraction P2/P3 of heparin-Sepharose-purified cp RNA polymerase. Two hundred-microgram aliquots of protein were separated by SDS/PAGE and transferred to Immobilon-P, and polypeptides were stained with amido black (lane 2). RNA polymerase polypeptides were revealed on one of the aliquots by incubation with antibodies raised against purified cp RNA polymerase (lane 1; Ab). Molecular size markers correspond to protein test mixture 4 from Serva (29, 45, 67, and 92.5 kDa; lane 3) and subunits of the *E. coli* RNA polymerase (165, 155, 82, and 39 kDa; lane 4). The 110-kDa polypeptide used for renaturation is marked with >.

length of produced transcripts in the absence of a strong terminator, and to test the effect of supercoiling. A dependence of template activity on supercoiling has been reported for partially purified cp RNA polymerase activities (18, 19). The determination of the size of the transcripts is important to rule out the possibility that the 110-kDa polypeptide represents a DNA primase, because it has been shown that DNA primase of pea chloroplasts sediments as a 115- to 120-kDa polypeptide on glycerol gradients (20). Transcription activity with this template was measured by gel electrophoresis (Fig. 4); relatively long transcripts are seen over the range of 1000–3000 nt (lane 7). The production of such long transcripts rules out the possibility that the 110-kDa polypeptide represents a DNA primase. The transcription activity was dependent on all four nucleoside triphosphates (lanes 1 and 2) and supercoiled DNA template (lane 6). The products are sensitive to ribonuclease (lane 3). To confirm that a sole subunit of a multimeric enzyme like the *E. coli* RNA polymerase cannot give such a type of transcriptional activity, I also isolated and renatured the β subunit of the *E. coli* enzyme (lane 5). I have not attempted to characterize the transcription initiation site on this template.

To determine whether transcription with the 110-kDa polypeptide was selective and could use specific transcription initiation and termination sites, I used a supercoiled plasmid template with two different kinds of promoters, each reading toward a strong terminator. In this plasmid (pTZ19-$P_{LS}T_a$; ref. 17) the terminator is the strong, *rho*-independent *E. coli thr* attenuator, which efficiently terminates transcription by either the *E. coli* or the bacteriophage T7 RNA polymerase (17). The two promoters are the bacteriophage T7 promoter and the chloroplast *rbcL* gene promoter; the latter is efficiently used by *E. coli* RNA polymerase. Transcription from the T7 promoter gives a 365-nt RNA, whereas transcription from the *E. coli*-like *rbcL* promoter gives a 227-nt RNA; each of these is terminated by the *thr* attenuator. In addition there are also some longer transcripts resulting from readthrough of the attenuator (17). This expected pattern of transcripts is found in control experiments using purified T3 and T7 RNA

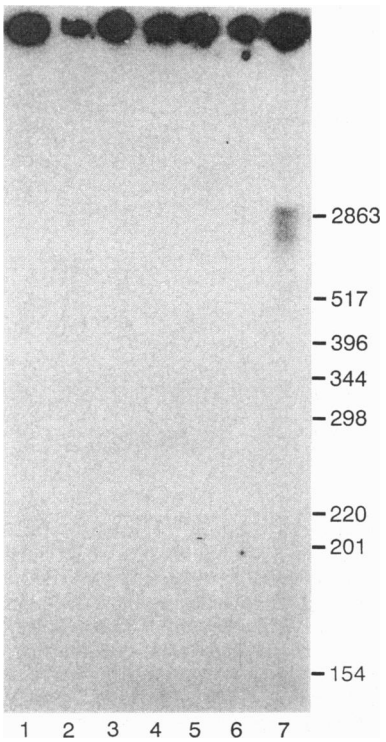

FIG. 4. Transcription of supercoiled and linear DNA. Transcription was performed as described using pUC18 containing the *rbcL* promoter region as template. The template in its supercoiled form was transcribed by 2 μl of the 110-kDa enzyme. In addition to 20 μCi of [^{32}P]UTP (5 μM), the reaction mixture contained only ATP and CTP (lane 1), ATP and GTP (lane 2), or ATP, GTP, and CTP (lanes 7 and 3). In lane 3, transcription products were subsequently digested with pancreatic RNase. Further control reactions were performed without DNA (lane 4) and with 2 μl of the renatured β subunit of *E. coli* RNA polymerase (lane 5). In lane 6, the template was transcribed in its linearized form by the 110-kDa enzyme. The migration of molecular size markers is indicated at the right.

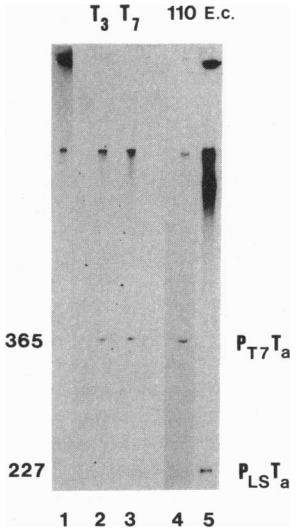

FIG. 5. Transcription of supercoiled DNA containing two different promoters and one strong terminator. The template represents the plasmid pTZ19-$P_{LS}T_a$ containing the T7 promoter (P_{T7}), the spinach plastid *rbcL* gene promoter (P_{LS}), and the *E. coli* threonine attenuator (T_a). Lane 1 shows the end-labeled, linearized plasmid. Transcription of 1 μg of DNA was performed by using 2 μl of the 110-kDa enzyme (lane 4). Transcription of 400 ng of DNA was performed with 2 units of T3 (lane 2), 1 unit of T7 (lane 3), or 1 unit of *E. coli* RNA polymerase (lane 5). Control reactions, as outlined in Fig. 4, lanes 1–4, gave the same results as in Fig. 4 and are not shown.

polymerases (Fig. 5, lanes 2 and 3) and purified *E. coli* RNA polymerase (lane 5).

Transcription of this template with the purified 110-kDa polypeptide gives a surprising result. The predominant transcript is an RNA with a mobility corresponding to that of the 365-nt T7 RNA polymerase transcript (Fig. 5, lane 4). There is no detectable product in the 227-nt region. The tentative conclusions from this experiment are that the 110-kDa activity can initiate transcription at or near the T7 promoter and responds efficiently to the *thr* attenuator termination signal.

DISCUSSION

Chromatography of RNA polymerase preparations from purified spinach chloroplasts obtained from very young leaves results in separation of three transcriptionally active fractions that differ in their RNA polymerase polypeptide compositions. This separation was found when the original purification procedure (3) was modified by using spinach leaves of very young plants and a smaller amount of leaf material. The first chromatographic peak (P1) corresponds in polypeptide composition to that obtained in the original procedure. The second peak (P2) contains only the non-*E. coli*-like 110-kDa polypeptide. A third fraction (P3) recovered from heparin-Sepharose by elution under denaturing conditions also contains the 110-kDa polypeptide and regains transcriptional activity during dialysis. In P2 and P3, the 110-kDa polypeptide could be shown to be a component of an active RNA polymerase activity by using the immunochemical ALPA assays, which couple active cp RNA polymerase to separated polypeptides on a membrane. Thus the question arises whether this 110-kDa polypeptide represents a common subunit of enzyme complexes found in the three fractions or is itself a single-subunit RNA polymerase.

Results demonstrate that the 110-kDa RNA polymerase polypeptide, which is not immunologically related to any of the *E. coli* RNA polymerase polypeptides, has all of the properties expected of a single-subunit DNA-dependent RNA polymerase core enzyme: it produces RNA in a DNA-dependent manner and only in the presence of all four nucleoside triphosphates. *In vitro*, the polypeptide may have the properties of a holoenzyme because it appears to recognize the T7 bacteriophage promoter. However, I have not yet succeeded in purifying the 110-kDa enzyme to homogeneity using conventional methods such as chromatography on heparin-Sepharose, DEAE-cellulose, or PEI-cellulose. Further purification, starting with P2 or P3, has always resulted in complete loss of enzymatic activity.

The transcription initiation of the renatured 110-kDa polypeptide at or near the T7 promoter is rather surprising. I have not yet mapped the start site exactly by primer extension or nuclease S1 mapping. T7 and T3 RNA polymerases are highly specific for their respective promoters. However, T7 RNA polymerase can synthesize RNA at a low rate starting from a T3 promoter and vice versa (21). If I compare these phage enzymes with the 110-kDa plastid enzyme, I find three surprising similarities: (*i*) They have about the same molecular mass. (*ii*) Under 30- to 60-min assay conditions, much more RNA is produced when the template is in a supercoiled form rather than in a linear form (under the same conditions T7 RNA polymerase produces ≈400-fold more RNA with supercoiled than with linear DNA; see ref. 17). (*iii*) They may use the same sequences for initiation of transcription. These similarities between the 110-kDa plastid enzyme and the phage polymerases suggest the possibility of structural homologies between the two kinds of polymerase as well. In this regard, it may be significant that the single-subunit RNA polymerase from mitochondria is structurally homologous to the phage enzymes (22).

For the following reasons, it is very unlikely that the 110-kDa polypeptide results from mitochondrial contamination of the plastid preparation: (*i*) It is a relatively abundant polypeptide in the polymerase preparation. In enzyme preparations purified from P1, the 110-kDa polypeptide is present in the same amounts as the *E. coli*-like polymerase polypeptides (3). It is rather unlikely that a mitochondrial contamination copurifies over three different columns not only in the same way but also in the same abundancy as the chloroplast enzyme. (*ii*) The 110-kDa polypeptide reacts with antibodies prepared against purified cp RNA polymerase from P1, and it reacts strongly in the ALPA assays in which native chloroplast enzyme corresponding to P1 is coupled in the second binding reaction. (*iii*) The renatured 110-kDa polypeptide of fraction P1 gives the same result in transcription assays as that of P2/P3 (data not shown). (*iv*) Plastid preparations do not contain any traces of soluble mitochondrial enzymes (absence of fumarase activity) and mitochondrial membranes (absence of phosphatidylethanolamine).

The *in vivo* initiation sites of the 110-kDa plastid enzyme are entirely speculative. I would like to present four hypotheses that may serve for further characterization of this protein: (*i*) Transcription initiation *in vivo* has also been demonstrated in several cases at sequences lacking an *E. coli*-like promoter sequence (23–25). It was speculated that these transcripts could result either from transcription by a polymerase other than an *E. coli*-like RNA polymerase or from regulation of transcription by usage of specific initiation factors. These genes could be targets for the 110-kDa enzyme. (*ii*) On the other hand, the T7 promoter and mitochondrial promoters serve to prime DNA replication (26–29). Since termination of transcription in plastids is still a matter of question (17, 30), transcription could start near the origin of replication and proceed without termination, where tRNAs and hairpin structures serve as signals for subsequent processing as in mitochondria (31). (*iii*) Transcriptional activities of lysed chloroplasts can be separated into soluble activities and transcriptionally active chromosomes (TAC; refs. 1, 32, and 33). High-salt purified TAC activities have been analyzed from chloroplasts of *Euglena gracilis* (34) and spinach (35, 36). The transcriptionally active chromosome of *Euglena* chloroplasts contains three major polypeptides of 116–118, 83–88, and 24–26 kDa (34). Immunological analysis of the polypeptides of the spinach high-salt TAC shows that it contains only the 110-kDa RNA polymerase polypeptide (35). Hence, the 110-kDa polypeptide analyzed here could represent the solubilized form of the TAC activity. (*iv*) In this paper, it is shown that I can separate the 110-kDa polypeptide from the *E. coli*-like polymerase polypeptides. However, I cannot yet separate the *E. coli*-like polypeptides from the 110-kDa polypeptide. Therefore, I cannot exclude the possibility that the 110-kDa polypeptide is also a functional polypeptide of the multisubunit enzyme that contains the *E. coli*-like polypeptides.

As the 110-kDa polypeptide reacts in *in vitro* studies like a core enzyme and is not immunologically homologous to any of the subunits of *E. coli* RNA polymerase, I conclude that it is not the product of the chloroplast *rpoB* gene as previously suggested (37). The existence of an RNA polymerase that is not the protein product of the plastid *E. coli*-like *rpo* genes could explain the recently observed phenomenon that a plastid genome that does not contain *rpo* genes is nevertheless transcribed (38, 39). Evidence for the existence of a functioning non-chloroplast-encoded RNA polymerase also comes from analysis of gene expression of ribosome-deficient plastids, where a chloroplast-encoded *E. coli*-like enzyme cannot be made up (40). It is interesting to note that the plastid *rpo* genes are preferentially expressed under this condition.

The fact that the 110-kDa enzyme (P2) is only well separable from the *E. coli*-like enzyme if RNA polymerase is prepared from very young plants indicates that the abundance of the two types of RNA polymerases changes during plant development. It is tempting to speculate that the 110-kDa enzyme preferentially transcribes the plastid genome during early stages of chloroplast development until the *E. coli*-like plastid-encoded subunits are made up.

I am grateful to Dr. E. M. Orozco for kindly providing the transcription plasmid pTZ19-$P_{LS}T_a$. I thank Dr. J. Joyard for measuring fumarase activity and phosphatidylethanolamine content of purified chloroplast preparations and Prof. Dr. M. J. Chamberlin for helpful discussions during the preparation of the manuscript.

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