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# *In vitro* expression of chloroplast genes in lysates of higher plant chloroplasts

(coupled transcription–translation/*Escherichia coli* lysate/*rbcL* gene/recombinant plasmid/transcript analysis)

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ABSTRACT **A DNA-dependent *in vitro*-coupled transcription–translation system has been prepared from lysates of isolated chloroplasts. These lysates are comparable to those of *Escherichia coli* in transcriptional and translational fidelity and efficiency in response to a given template DNA. When *Nicotiana tabacum* chloroplast DNA is used as template with chloroplast lysates (*N. tabacum* or spinach) or *E. coli* lysates, $NaDodSO_4$ gel analysis reveals similar polypeptide patterns that are distinct from the patterns obtained with *E. coli* DNA. Genes in recombinant plasmids containing chloroplast DNA are also expressed in these *in vitro* systems. DNA·RNA hybridization experiments show that transcripts are synthesized from most of the chloroplast genome. Newly synthesized large subunit of ribulosebisphosphate carboxylase/oxygenate and a transcript of the large subunit gene (*rbcL*) are observed in chloroplast lysates using as template chloroplast DNA or cloned fragments of tobacco chloroplast DNA that contain the large subunit gene. Results suggest that differential expression of chloroplast genes occurs *in vitro*. By using cloned chloroplast DNA templates in this homologous system, it is possible to identify and map structural genes for chloroplast proteins.**

There is considerable interest in examining regulation of expression of protein-coding genes present on chloroplast DNA. *In vitro* DNA-dependent coupled transcription–translation of chloroplast genes has been conducted using an *Escherichia coli* lysate (1–7). Linked systems, using *E. coli* polymerase in conjunction with rabbit reticulocyte and wheat germ extracts, have also been used to carry out transcription and translation of chloroplast genes in discrete steps. Other investigators have concentrated on translation of chloroplast mRNA *in vitro* in *E. coli*, rabbit reticulocyte, or wheat germ extracts (8–13). The DNA-dependent *E. coli* system has proven to be quite suitable for expression of well-characterized chloroplast genes (1–7, 14–16). Indeed, genes and protein-synthesizing systems of chloroplasts and *E. coli* share common features (17–19).

To date, studies using these heterologous systems have focused primarily on expression of chloroplast genes with prokaryotic structural features whose transcripts and encoded proteins are abundant *in vivo*. However, heterologous systems may differ from chloroplasts in regard to specific features of the transcription and translation machinery, as well as in regard to recognition of regulatory and processing signals present on chloroplast genes. For example, maize chloroplast RNA polymerase, in the presence of S factor, transcribes maize chloroplast DNA sequences in a supercoiled chimeric plasmid in preference to genes of the cloning vehicle (20). *E. coli* RNA polymerase does not respond to S factor and shows no preference for transcription of chloroplast DNA sequences (20). Furthermore, using the *E. coli* system, transcripts of spinach chloroplast DNA are synthesized that can encode proteins up to $M_r$ 50,000, but a significant proportion of low molecular weight polypeptides are synthesized *in vitro* (5, 21). This evidence suggests that translation of some chloroplast transcripts may be inaccurate (21) in the *E. coli* system. In addition, the *E. coli* system cannot be expected to process transcripts of intron-containing chloroplast genes that code for proteins (refs. 22–25; unpublished data) or to yield full-length polypeptides from these unprocessed transcripts.

In contrast to those of *E. coli*, chloroplast lysates must contain the macromolecular machinery including mRNA processing enzymes and other factors that effect the expression of chloroplast genes. Chloroplast-derived extracts can be expected to transcribe and translate chloroplast genes with high fidelity, resulting in gene products identical to those synthesized *in vivo*. We have developed and characterized a chloroplast lysate capable of *in vitro* transcription and translation of chloroplast genes (ref. 26; unpublished data). As an example of its general utility, this paper presents results of *in vitro* expression of the tobacco chloroplast ribulosebisphosphate carboxylase/oxygenase (RuBisCO) large subunit gene (*rbcL*) in chloroplast lysates.

## METHODS

**Plants, Bacterial Strains, and Plasmids.** *Nicotiana tabacum* cv "Turkish Samsun" was greenhouse grown. Spinach (from R. G. Jensen) was grown in controlled environment chambers. *E. coli* strain RR1 was from L. McReynolds, and *E. coli* strain Q13 was from the American Type Culture Collection. Plasmid pBR322 was amplified in *E. coli* strain RR1. Plasmid pNtSal6 contains the *Sal* 6 fragment (27) of *N. tabacum* chloroplast DNA inserted in pBR322 (27) such that *rbcL* gene on *Sal* 6 and the tetracycline-resistance gene (*tet*$^r$) of pBR322 are of opposite transcriptional polarity. Plasmid pZmBlB (28) was a gift of A. A. Gatenby. Antibody to RuBisCO holoenzyme was from R. G. Jensen.

**Reagents.** [$^{35}$S]Methionine (800–1200 Ci/mmol; 1 Ci = 37 GBq), [$^{32}$P]UTP (600–1000 Ci/mmol), and 2,5-diphenyloxazole were from New England Nuclear. Restriction enzymes *Sal* I, *Xho* I, *Bam*HI, and *Eco*RI were from New England Biolabs. Percoll and protein A-Sepharose CL-4B were from Pharmacia. Miracloth was from Calbiochem–Behring. DNase I (bovine pancreas) and micrococcal endonuclease (*Staphylococcus aureus*) were from Sigma.

**Isolation of *E. coli* and Chloroplast DNA.** Cultures (200 ml) of *E. coli* RR1 were grown in Luria broth to late logarithmic phase, and cells were collected by centrifugation and washed with TE buffer (1 mM EDTA/10 mM Tris·HCl, pH 8.5).

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Abbreviations: RuBisCO, ribulosebisphosphate carboxylase/oxygenase; kbp, kilobase pair(s).

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Chromosomal DNA was isolated as described (29) with the following modifications: cells were resuspended in 50 ml of TES buffer (50 mM NaCl/1 mM EDTA/50 mM Tris·HCl, pH 8.0) with 2 mg of lysozyme per ml. After adding an equal volume of 2% $NaDodSO_4$ in TES buffer, the cell lysate was extracted three times with phenol saturated with TES. DNA from plasmids was isolated (29) from *E. coli* RR1 cells that were grown in Luria broth containing 50 μg of ampicillin per ml. DNA was purified on CsCl/ethidium bromide gradients. Chloroplast DNA was purified from *N. tabacum* leaves as described (30, 31), and absence of nuclear DNA contamination was evaluated from patterns of restriction enzyme digests after electrophoresis on 0.7% agarose gels (27).

**Preparation of *E. coli* Lysates for Transcription–Translation.** Cells of *E. coli* Q13 were grown at 37°C in 2-liter cultures of Luria broth. Cells were harvested at midlogarithmic phase and washed with buffer A (10 mM Tris acetate, pH 8.2/1 mM dithiothreitol/14 mM $Mg(OAc)_2$/60 mM KOAc). Lysates were prepared as described by Bottomley and Whitfeld (5).

**Isolation of Chloroplasts for Transcription–Translation.** *N. tabacum* leaves (500 g) were homogenized at 4°C in 2 liters of MCB (0.3 M mannitol/0.05 M Tris·HCl, pH 8.0/0.002 M EDTA/0.001 M 2-mercaptoethanol/1% bovine serum albumin) using a razor blade-equipped Waring blender (two 10-sec bursts at full speed). The brei was filtered through two layers each of cheesecloth and Miracloth. Chloroplasts were pelleted from the homogenate at 1250 × *g* for 10 min, resuspended in 20 ml of MCB, and layered on field-formed silicasol (Percoll) gradients (32) consisting of a mixture of 34 ml PPFB (5 g of polyethylene glycol 6000/1 g of Ficoll/1 g of bovine serum albumin brought to 100 ml with Percoll) and 64 ml of MCB. After centrifugation under the same conditions used to generate the gradients (32), intact chloroplasts were isolated and washed three times with 3 vol of MCB (26). No intact nuclei were observed by phase-contrast microscopic examination of these gradient-purified chloroplasts.

**Preparation of Chloroplast Lysates.** Gradient-purified chloroplasts resuspended in ½ vol of buffer A were lysed in a French press (7000 $lb/in^2$) or by three 10-sec pulses of a Branson sonifier cell disruptor W-350 (output setting 7) using a microprobe. Dithiothreitol (to 1 mM) was added and the thylakoid membranes were removed by centrifuging the lysate twice at 30,000 × *g* for 30 min and collecting the upper three-quarters of the supernatant (S-30) each time. The S-30 was incubated (5) to reduce endogenous activity or stored in liquid nitrogen for up to 6 months. If background activity was significant, the lysate was treated with micrococcal nuclease (33) and then dialyzed against buffer A for 2 hr at 4°C just prior to use in the transcription–translation reaction (26).

***In Vitro* Transcription–Translation.** Components of the standard *in vitro* transcription–translation reaction mixture were 1–5 μg of DNA template, 8 μCi of [$^{35}$S]methionine, 20 μl of chloroplast lysate (60–70 μg of protein), 45 mM Tris acetate, pH 8.2, 60 mM KOAc, 11 mM $NH_4OAc$, 14 mM $Mg(OAc)_2$, 1% polyethylene glycol 6000, 2 mM ATP, 0.5 mM GTP, 0.5 mM CTP, 0.5 mM UTP, 0.5 mM cAMP, 10 mM phospho*enol*pyruvate, 0.8 μg of pyruvate kinase, 0.125 mg of *E. coli* tRNA per ml, 19 amino acids (0.2 mM), 0.14 mM pyridoxine HCl, 0.09 mM FAD, 0.09 mM NADP, 0.06 mM *p*-aminobenzoic acid, and 1.6 mM dithiothreitol. The 50-μl reaction mixture was incubated at 37°C for 45 min and then the reaction was stopped by the addition of 1.5 ml of cold acetone.

**Isolation and Analysis of *In Vitro* Transcripts.** *In vitro* transcription was in the same reaction mixture used for transcription–translation with the following exceptions: [$^{35}$S]-methionine was replaced with 0.2 mM methionine, [α-$^{32}$P]UTP (20 μCi per 50 μl of reaction mixture) was added, and UTP was deleted. Transcription proceeded for 45 min at 37°C. RNA was precipitated by the addition of NaOAc to 200 mM and 2 vol of ethanol. RNA was washed twice with 70% ethanol, dissolved in 50 μl of TE buffer and DNA was removed by adding 1 μg of DNase and incubating for 1 hr at 20°C. The solution was extracted with TE buffer-saturated phenol and the phenol was removed by ether extraction.

Two micrograms of *N. tabacum* chloroplast DNA was digested with *Sal* I or *Xho* I and the DNA was subsequently electrophoresed on a 0.65% agarose gel at room temperature in TBE buffer (0.089 M Tris·borate/0.089 M boric acid/0.0025 M EDTA, pH 8.0) at 50 V per 15-cm gel for 12 hr. DNA was transferred (34) to nitrocellulose paper (type BA85; Schleicher & Schuell) and hybridized with [$^{32}$P]RNA that had been labeled by *in vitro* transcription. Hybridization was performed as described (35), except KI was omitted. After hybridization, the filters were washed and air dried.

**Immunoprecipitation of the Large Subunit of RuBisCO from Lysates.** The transcription–translation mixtures were brought to 2% $NaDodSO_4$ and boiled for 3 min. The lysate was then diluted with 1 ml of Triton buffer (50 mM Tris·HCl, pH 7.8/0.15 M NaCl/2 mM EDTA/1% Triton X-100) and clarified at 20,000 × *g* for 10 min. Ten microliters of anti-RuBisCO holoenzyme serum was added to the supernatant. After incubation at 20°C for 1 hr, the antigen–antibody complex was bound to 5 mg (dry weight) of protein A-Sepharose by shaking gently for 1–2 hr. The Sepharose–protein complex was pelleted (6000 × *g*) and washed once with Triton buffer containing 1 M NaCl and three times with Triton buffer containing 0.15 M NaCl. The final Sepharose pellet was resuspended in 60 μl of gel electrophoresis sample buffer (36) and incubated at 60°C for 1 hr and then at 100°C for 3 min. After centrifugation at 6500 × *g*, the supernatant was subjected to $NaDodSO_4$ gel electrophoresis (36).

**Gel Analysis and Fluorography.** $^{35}$S-labeled proteins were resolved by slab gel electrophoresis (36). After electrophoresis, the gels were fixed for 1 hr in trichloroacetic acid/glacial acetic acid/methanol/water (1:1:3:5) and then placed in glacial acetic acid for 1 hr. The gel was then soaked for 90 min in 200 ml of 2,5-diphenyloxazole/acetic acid (15:85), washed in water for 1 hr, vacuum dried, and fluorographed with Kodak X-Omat film at −80°C (37).

## RESULTS

**DNA-Dependent Gene Expression in Chloroplast Lysates.** Chloroplast lysates active in transcription and translation of exogenous DNA were prepared by treating isolated chloroplasts from tobacco or spinach as though they were *E. coli* cells to be used for preparation of cell extracts capable of DNA-dependent gene expression (5). Since it is documented that *E. coli* extracts can express chloroplast genes (4, 5, 15), the quality of DNA-dependent gene expression in chloroplast lysates was compared with that obtained in *E. coli* extracts. Heterologous and homologous combinations of *E. coli* DNA and tobacco chloroplast DNA templates were used. For each template, similar polypeptide patterns (Fig. 1) were obtained in its homologous and heterologous milieu. Coupled transcription and translation was strictly template dependent, because there was undetectable background protein synthesis in the absence of exogenous DNA. The slight differences observed, with a given template, could be incomplete polypeptides from translation of unprocessed transcripts or could result from as yet uncharacterized differences in recognition of regulatory and/or processing signals for gene expression in chloroplasts and in *E. coli*.

**Transcription of Chloroplast Genes.** The observed DNA template dependency of polypeptide synthesis and the complexity of the polypeptide patterns observed implies that the chloroplast lysates had actively transcribed the template. To

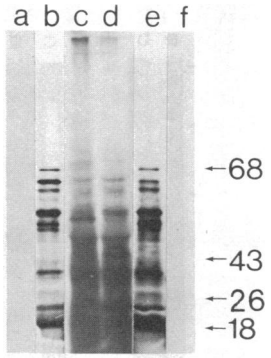


FIG. 1. DNA template-dependent polypeptide synthesis in extracts from *E. coli* (lanes a–c) and *N. tabacum* chloroplasts (lanes d–f). No DNA (lanes a and f), 3 μg of purified *E. coli* chromosomal DNA (lanes c and d), or 3 μg of chloroplast DNA (lanes b and e) was added to standard transcription–translation reactions. Prior to use, $CaCl_2$ to 2 mM and 8 units of micrococcal nuclease were added per 50 μl of lysate. After incubation for 15 min at 37°C, nuclease was inactivated by addition of EGTA to 2.5 mM and the lysates were dialyzed against buffer A. Preparation of samples for electrophoresis was as described in methods and by Bard *et al.* (26). A different exposure was used to photograph lanes c and d in order to reveal more detail. Positions of molecular weight markers ($\times 10^{-3}$) are indicated.

examine whether portions of the chloroplast genome were transcribed preferentially, transcription was done in the presence of [$\alpha$-$^{32}$P]UTP. The labeled transcripts were hybridized to filter-immobilized chloroplast DNA that had been digested with *Sal* I, *Bam*HI, and *Pvu* II restriction enzymes. Transcripts were representative of the entire chloroplast genome (Fig. 2) and transcription was rather uniform throughout the chloroplast genome.

Plasmid (pNtSal6), was used as template to assess whether protein-coding genes [*rbcL* (38), *atpB/E* (39, 40), and *petA* (41)] on the *Sal* 6 fragment (Fig. 3*A*) were transcribed. When [$^{32}$P]RNA transcripts were hybridized to *Xho* I digests of pNtSal6, *Xho* I fragment 7 [9.7 kilobase pairs (kbp)] was most intensely labeled (Fig. 3*C*, lane b). Other $^{32}$P-labeled signals were seen with *Xho* I fragments 2 (17.9 kbp) and 10 (5.4 kbp), which are within the *Sal* 6 fragment. Thus, *Sal* 6 transcripts were synthesized that hybridize to *Xho* I fragments (Fig. 3*B*) containing the *rbcL* (38), *atpB/E* (39, 40), and *petA* (41) genes (Fig. 3*A*). In another experiment, with

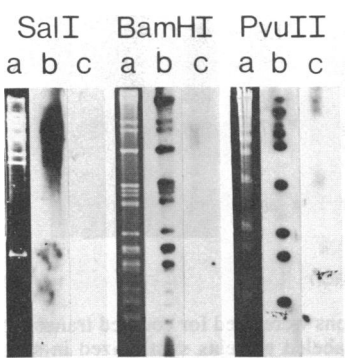


FIG. 2. Southern hybridization of [$^{32}$P]UTP-labeled RNA transcripts of tobacco chloroplast DNA in tobacco chloroplast lysates. Templates were total tobacco chloroplast DNA (lane b) or no DNA (lane c). Transcripts were hybridized to chloroplast DNA that had been digested with the indicated restriction enzymes, electrophoresed on a 0.7% agarose gel, and transferred to nitrocellulose paper. Lane a is a photograph of the agarose gel after staining with 1 μg of ethidium bromide per ml prior to transfer to nitrocellulose.

pNtSal6 as template, the [$^{32}$P]RNA transcripts were hybridized to an *Eco*RI/*Bam*HI double digest of pZmBIB (Fig. 3*B*), which contains the maize *rbcL* gene and part of the *atpB* gene (7, 43). Major transcripts hybridized to 2.0- and 0.45-kbp *rbcL* fragments, and minor transcripts were also observed that hybridize to the *atpB* gene and to the pBR322 vector (Fig. 3*C*, lane d). Similar results were observed for pNtSal6 transcripts in *E. coli* lysates (lane f); however, vector sequences appear to be more strongly transcribed, relative to the chloroplast sequences, than was observed in the chloroplast extracts (lane d).

**Coupled Transcription and Translation of *rbcL* Gene.** Since the *rbcL* gene was transcribed from chloroplast DNA templates, we endeavored to discover whether this gene was also expressed *in vitro* with fidelity at the level of translation. Newly synthesized large subunit RuBisCO was immunoprecipitable from tobacco and spinach chloroplast lysates by using total tobacco chloroplast DNA as template (Fig. 4 *A* and *B*). In the absence of added template, or with *E. coli* DNA template, no immunoprecipitable polypeptide was detected. Chloroplast lysates synthesized large subunit of identical apparent molecular weight to that found *in vivo*, because the electrophoretic mobility of both proteins was identical (Fig. 4*B*). Plasmid pNtSal6, which contains the *rbcL* gene, also directed the synthesis of immunoprecipitable large subunit of RuBisCO (Fig. 4 *A* and *B*). Thus, the *rbcL* gene was actively transcribed in our chloroplast lysates (Fig. 3), and immunoprecipitable large subunit must have resulted from translation of full length *in vitro*-synthesized transcripts.

Supercoiled plasmid preparations were excellent templates for *rbcL* gene expression in these chloroplast lysates. Fig. 4*C* shows that large subunit polypeptide was a major translation product of coupled transcription–translation in *N. tabacum* chloroplast lysates and was readily detected without resort to immunoprecipitation. No large subunit was detected if the large subunit gene had been cleaved with *Eco*RI and *Bam*HI prior to the transcription–translation reaction (Fig. 4*C*). Other prominent translation products of $M_r$ 13,000, 57,000, and 38,000 (Fig. 4*C*) may correspond to cytochrome *f* and to $\beta$ and $\varepsilon$ subunits of chloroplast coupling factor 1, respectively (40, 41).

## DISCUSSION

We have developed a DNA-dependent homologous *in vitro* system capable of expressing chloroplast genes from total chloroplast DNA and cloned fragments of chloroplast DNA. This system also transcribes and translates *E. coli* DNA, and presumably other prokaryotic DNA. The chloroplast and *E. coli* systems are of comparable efficiency, because similar levels of transcription and translation activities are observed using the same amount of template in lysates from each source that had been adjusted to standard protein concentrations (3–3.5 mg/ml).

Since the chloroplast lysate is a homologous system for expressing chloroplast genes, it was expected that proteins actively synthesized *in vivo* would, likewise, be actively synthesized *in vitro*. This is demonstrated for the large subunit of RuBisCO, which is one of the most intensely labeled polypeptides synthesized *in vitro*. In addition to the *rbcL*-coded protein, the four other major polypeptides synthesized with pNtSal6 as template (Fig. 4*C*) probably correspond to products of the *atpB*, *atpE*, and *petA* genes (38, 40, 41) as well as the plasmid-coded $\beta$-lactamase. Thus, polypeptide patterns observed by using chloroplast DNA templates indicate that the same set of chloroplast genes are actively transcribed and translated *in vivo* and *in vitro*.

*In vitro* transcripts were examined for evidence that might suggest differential recognition of certain promoters or tran-

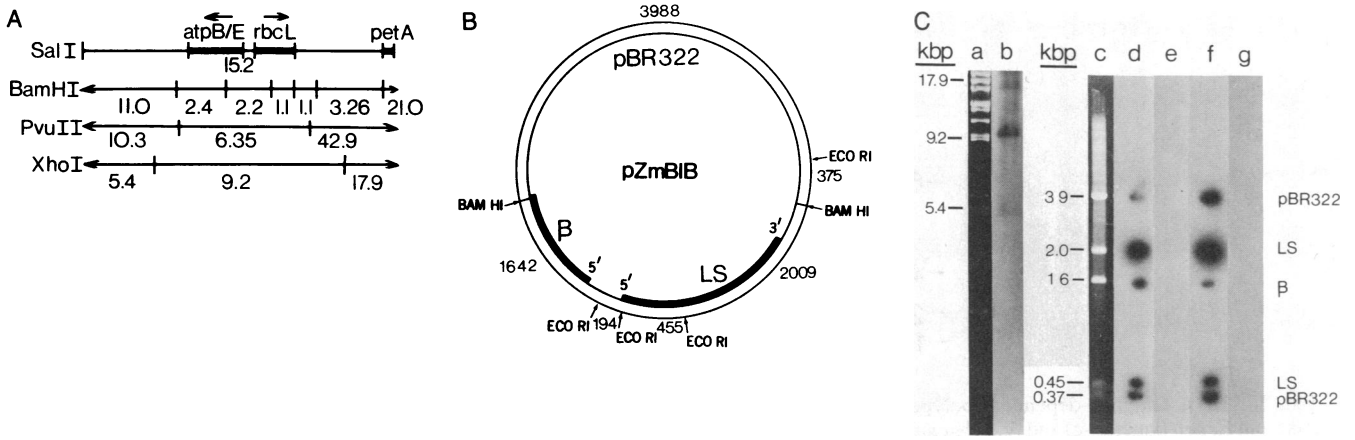


FIG. 3. Transcription of tobacco *rbcL* gene in chloroplast lysates. (*A*) Map of *Sal* 6 fragment of tobacco chloroplast DNA indicating restriction sites (28, 42) and known genes in the fragment (38–41). *Xho* I, *Pvu* II, and *Sal* I sites are according to ref. 42. Arrows on the map for each enzyme indicate fragments that extend beyond the borders of *Sal* 6. Direction of transcription is indicated where known. (*B*) Map of *Eco*RI and *Bam*HI sites of pZmBIB. Location of *rbcL* (43) and *atpB/E* (7) genes and transcription polarities are indicated. (*C*) [$^{32}$P]UTP labeled RNA obtained from transcription, without added template (lanes e and g), or with pNtSal6 (lanes b, d, and f) in an *N. tabacum* (lanes b, d, and e) and *E. coli* (lanes f and g) lysate. This labeled RNA was hybridized (lane b) to total chloroplast DNA that had been digested with *Xho* I, electrophoresed on a 0.7% agarose gel, and transferred to nitrocellulose paper. Lane a is a photograph of the *Xho* I digest after staining the gel with ethidium bromide. The labeled RNA was also hybridized (lanes d–g) to plasmid pZmBIB DNA that had been digested with *Bam*HI and *Eco*RI, electrophoresed on a 0.7% agarose gel, and transferred to nitrocellulose paper (lane c). Lane c is a photograph of the ethidium bromide-stained gel of pZmBIB digested with *Bam*HI and *Eco*RI. LS, large subunit.

scriptional regulation of chloroplast gene expression. With linear total chloroplast DNA as template, Southern hybridization analysis (Fig. 2) indicated a uniform distribution of sequences representing the whole chloroplast genome. The relative hybridization of transcripts to DNA fragments containing the *rbcL* gene was not significantly different from that of other fragments. However, analysis of pNtSal6 transcripts (Fig. 3*C*, lane b) suggested at least 2- to 3-fold greater transcription, on a molar basis, of sequences hybridizing to the 9.2-kbp *Xho* I fragment (containing the *rbcL*, *atpB*, and *atpE* genes), relative to both the 17.9-kbp *Xho* I fragment (containing the *petA* gene) and the 5.4-kbp *Xho* I fragment (gene content unknown).

The appearance of uniform transcription of total chloroplast DNA could result from averaging of the hybridization signals from different sized transcripts of several genes on each restriction fragment. Since the chloroplast DNA template was linear, while the plasmids used were closed-circular supercoiled molecules, the tertiary structure of the template may affect promoter recognition and the differential transcription of specific chloroplast genes. Our observations are in agreement with results of transcriptional studies of open circular and supercoiled plasmids containing cloned maize chloroplast genes (28).

In addition to its utility in studying expression of already characterized chloroplast genes, the chloroplast lysate sys-

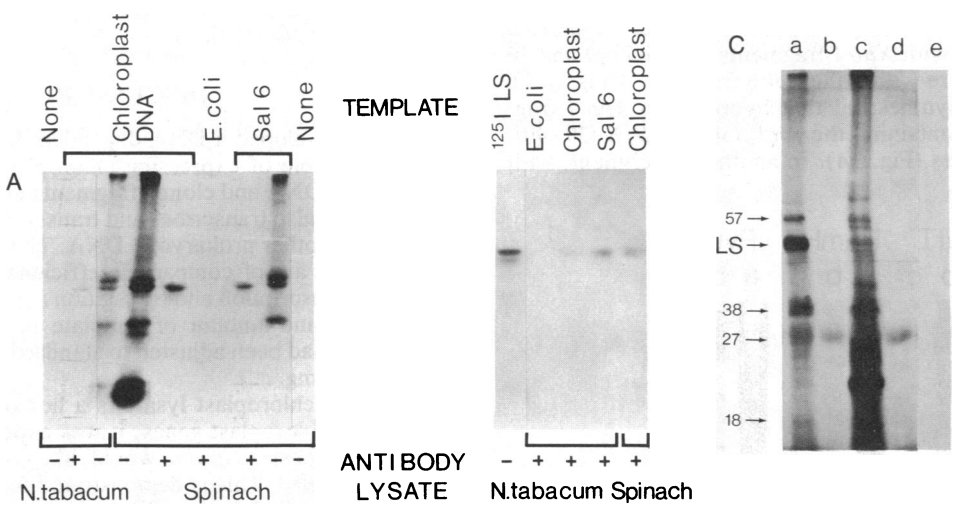


FIG. 4. DNA-dependent *rbcL* gene expression in chloroplast lysates. Standard conditions were used for coupled transcription–translation and for immunoprecipitation of the large subunit of RuBisCO. (*A*) Fluorograph of $^{35}$S-labeled proteins synthesized in spinach or tobacco chloroplast lysates. Proteins were products of coupled transcription–translation of tobacco chloroplast DNA template, *E. coli* DNA, or the pNtSal6 plasmid. The proteins were either complexed with fraction I antibody that had been conjugated to protein A-Sepharose, precipitated, and then electrophoresed on a $NaDodSO_4$/polyacrylamide gel or directly precipitated and subjected to electrophoresis. (*B*). Fluorograph of $^{35}$S-labeled proteins synthesized in spinach or tobacco chloroplast lysates. Proteins were products of coupled transcription–translation of tobacco chloroplast DNA template, *E. coli* DNA, or the pNtSal6 plasmid. The proteins were complexed with fraction I antibody that had been conjugated to protein A-Sepharose, precipitated, and then electrophoresed on a $NaDodSO_4$/polyacrylamide gel. Lane a contains purified large subunit (LS) that was isolated from tobacco and labeled with $^{125}$I. (*C*) Fluorograph of [$^{35}$S]methionine-labeled proteins synthesized in a *N. tabacum* lysate. Templates were pNtSal6 DNA (closed circular) (lane a), pNtSal6 DNA digested with *Eco*RI/*Bam*HI (lane b), total *N. tabacum* chloroplast DNA (linear fragment) (lane c), pBR322 DNA (lane d), none (lane e). LS indicates large subunit of RuBisCO.

tem should facilitate detection and identification of as yet unknown chloroplast structural genes and the proteins they encode. *In vitro* expression of intron-containing chloroplast genes will also be an important application of this system. Studies of transcriptional and translational regulation of specific chloroplast genes are now possible *in vitro* in a homologous system. Detection and identification of specific regulatory molecules should result from appropriate studies using this system. These considerations, the results presented here, and the ability to reconstitute full activity after lyophilization (ref. 26; unpublished data) suggest that these crude chloroplast extracts will become tools of choice for *in vitro* analysis of chloroplast gene expression and regulation.

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1. Erion, J. L., Tarnowski, J., Weissbach, H. & Brot, N. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3459–3463.
2. Zurawski, B., Bottomley, W. & Whitfeld, P. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6260–6264.
3. Howe, C. J., Bowman, C. M., Dyer, T. A. & Gray, J. C. (1982) *Mol. Gen. Genet.* **186,** 525–530.
4. Whitfeld, P. R. & Bottomley, W. (1980) *Biochem. Int.* **1,** 172–178.
5. Bottomley, W. & Whitfeld, P. R. (1979) *Eur. J. Biochem.* **43,** 31–39.
6. Willey, D. L., Huttly, A. K., Phillips, A. L. & Gray, J. C. (1983) *Mol. Gen. Genet.* **189,** 85–89.
7. Krebbers, E. T., Larrinua, I. M., McIntosh, L. & Bogorad, L. (1982) *Nucleic Acids Res.* **10,** 4985–5002.
8. Cuming, A. C. & Bennett, J. (1981) *Eur. J. Biochem.* **118,** 71–80.
9. Driesel, A. J., Speirs, J. & Bohnert, H. J. (1980) *Biochim. Biophys. Acta* **610,** 297–310.
10. Westhoff, P., Nelson, N., Bunemann, H. & Herrmann, R. G. (1981) *Curr. Genet.* **4,** 109–120.
11. Langridge, P. (1981) *FEBS Lett.* **123,** 85–89.
12. Reisfeld, P., Mattoo, A. K. & Edelman, M. (1982) *J. Biochem.* **124,** 125–129.
13. Chua, N. H. & Schmidt, G. W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6110–6114.
14. Alt, J., Westhoff, P., Sears, B. B., Nelson, N., Hurt, E., Hauska, B. & Herrmann, R. G. (1983) *Embo J.* **2,** 979–986.
15. Westhoff, P., Alt, J., Nelson, N., Bottomley, W., Bunemann, H. & Herrmann, R. G. (1983) *Plant Mol. Biol.* **2,** 95–107.
16. Erion, J. L., Tarnowski, J., Peacock, S., Caldwell, P., Redfield, B., Brot, N. & Weissbach, H. (1983) *Plant Mol. Biol.* **2,** 279–290.
17. Bohnert, H. J., Crouse, E. J. & Schmitt, J. M. (1982) in *Encyclopedia of Plant Physiology*, eds. Parthier, B. & Boulter, D. (Springer, Berlin), pp. 475–526.
18. Bottomley, W. & Bohnert, H. J. (1982) in *Encyclopedia of Plant Physiology*, eds. Parthier, B. & Boulter, P. (Springer, Berlin), pp. 533–596.
19. Whitfeld, P. R. & Bottomley, W. (1983) *Annu. Rev. Plant Physiol.* **34,** 279–310.
20. Jolly, S. O. & Bogorad, L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 822–826.
21. Whitfeld, P. R., Spencer, D. & Bottomley, W. (1973) in *Biochemistry of Gene Expression in Higher Organisms*, eds. Pollok, J. & Lee, W. J. (Australian and New Zealand Book, Sydney), pp. 504–522.
22. Steigler, G. L., Matthews, H. M., Bingham, S. E. & Hallick, R. B. (1982) *Nucleic Acids Res.* **10,** 3427–3444.
23. Hollingsworth, M. J., Johanningmeier, V., Karabin, G. D., Steigler, G. L. & Hallick, R. B. (1984) *Nucleic Acids Res.* **12,** 2001–2017.
24. Zurawski, G., Bottomley, W. & Whitfeld, P. R. (1984) *Nucleic Acids Res.* **16,** 6547–6558.
25. Montandon, P. E. & Stutz, E. (1983) *Nucleic Acids Res.* **11,** 5877–5892.
26. Bard, J. D. J., Bourque, D. P. & Zaitlin, D. (1985) *Methods Enzymol.* **118,** in press.
27. Hildebrand, M., Jurgenson, J. E., Ramage, R. T. & Bourque, D. P. (1985) *Plasmid*, in press.
28. Gatenby, A. A., Castleton, J. A. & Saul, M. W. (1981) *Nature (London)* **291,** 117–121.
29. Davis, R. W., Bottstein, D. & Roth, J. R. (1980) *A Manual for Genetic Engineering* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 116–118.
30. Kolodner, R. D. & Tewari, K. K. (1975) *Biochim. Biophys. Acta* **402,** 372–390.
31. Jurgenson, J. E. (1980) Dissertation (University of Arizona, Tucson), pp. 38–39.
32. Schmitt, J. M., Benhke, H.-D. & Herrmann, R. G. (1974) *Exp. Cell Res.* **85,** 63–72.
33. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
34. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–577.
35. Whitfeld, P. R., Herrmann, R. G. & Bottomley, W. (1978) *Nucleic Acids Res.* **5,** 1741–1751.
36. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
37. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
38. Shinozaki, K. & Sugiura, M. (1982) *Gene* **20,** 91–102.
39. Shinozaki, K., Deno, H., Kato, A. & Sugiura, M. (1983) *Gene* **24,** 147–155.
40. Fluhr, R., Fromm, H. & Edelman, M. (1983) *Gene* **25,** 271–280.
41. Lin, C. M. & Kung, S. D. (1984) *Theor. Appl. Genet.* **68,** 213–218.
42. Seyer, P., Kowallik, K. V. & Herrmann, R. G. (1981) *Curr. Genet.* **3,** 189–204.
43. McIntosh, L., Poulsen, C. & Bogorad, L. (1980) *Nature (London)* **288,** 556–560.