

A tobacco chloroplast DNA sequence possibly coding for a polypeptide similar to *E. coli* RNA polymerase β -subunit

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DNA sequencing has revealed a long open reading frame (ORF) in the large single-copy region of tobacco chloroplast DNA. This ORF consists of 1070 codons and its deduced amino acid sequence shows about 39% homology to that of the β -subunit of *E. coli* RNA polymerase. This finding raises a possibility that some of the chloroplast RNA polymerase subunits are coded for by the chloroplast genome.

(Tobacco) Chloroplast DNA sequence RNA polymerase *rpoB* gene

1. INTRODUCTION

Chloroplasts in higher plants contain their own DNA and RNA polymerase. Chloroplast RNA polymerase in higher plants has been suggested to be nuclear encoded [1–4]. However, sequences hybridized with the β -subunit gene (*rpoB*) of *Escherichia coli* RNA polymerase have been found in *Chlamydomonas* chloroplast DNA as well as nuclear DNA [5]. We have sequenced one of the tobacco chloroplast DNA regions which hybridized with the *E. coli rpoB* probe and found an open reading frame whose amino acid sequence resembles that of the *E. coli* β -subunit. This finding raises the interesting possibility that chloroplasts in higher plants are an additional site of synthesis of their RNA polymerase subunits.

2. MATERIALS AND METHODS

Transducing phage λ rif^d18 was kindly provided by Dr A. Ishihama and its 6.0% *EcoRI* fragment was used as the *rpoB* probe. Southern blot hybridization was carried out as described [6]. The clone bank of tobacco (*Nicotiana tabacum* var. Bright Yellow 4) chloroplast DNA and recombi-

nant plasmid pTB7 are described in [7]. DNA sequence was determined as described [8] and analyzed using the GENETYX program (Software Development Co., Tokyo).

3. RESULTS AND DISCUSSION

Using the clone bank of tobacco chloroplast DNA [7], we searched for the chloroplast DNA regions homologous to the *E. coli rpoB* by hybridization with the probe. Recombinant plasmid pTB7 which contains consecutive 3.0, 3.2, 1.1 and 3.6 kbp *Bam*HI fragments (Ba13, Ba12a, Ba25 and Ba10b, respectively, [7]) was one of the several clones which gave a weak signal. We sequenced portions of the cloned *Bam*HI fragments in pTB7 and found a 3210 open reading frame on the same strand as the gene for H⁺-ATPase α -subunit (*atpA*) (figs 1 and 2).

The predicted polypeptide is 1070 amino acid residues long and 272 residues shorter than the *E. coli* β -subunit (1342 residues) [10]. When gaps are introduced, the amino acid sequence deduced from ORF₁₀₇₀ has about 39% homology with that of the *E. coli* β -subunit (fig.3). This homology is comparable to that (23–68%) of ribosomal proteins [6].

Recently Lerbs et al. [4] have analyzed in vitro

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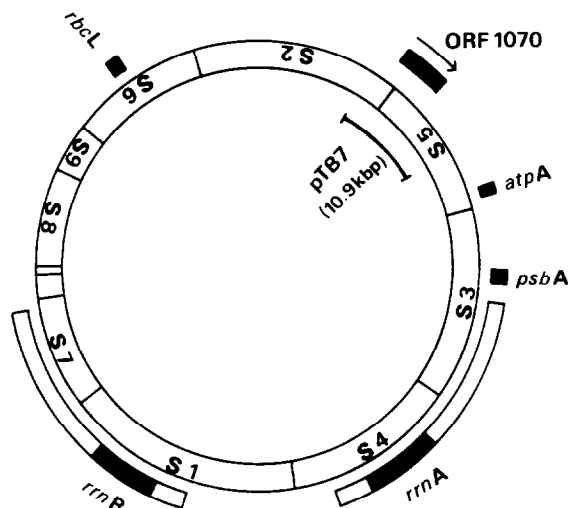


Fig.1. Position of the cloned fragment in pTB7 and of ORF₁₀₇₀ on the *Sal*I map of tobacco chloroplast DNA. The arrow indicates the direction of possible transcription.

translated products of both chloroplast RNA and poly(A)⁺ nuclear RNA in spinach and suggested that all chloroplast RNA polymerase polypeptides are coded for by the nuclear genome [4]. From their study, ORF₁₀₇₀ could be a pseudogene. Another possibility is that chloroplasts have two RNA polymerase species, one coded for by nuclear genomes and the other by chloroplast genomes which is expressed in restricted conditions, e.g. in etiochloroplasts. Two types of RNA polymerase activity have been isolated from *Euglena* chloroplasts [9].

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L Y K F P K I E D T D Q E I E F Q L F V E T Y Q L V E P L I K E R
AGATGCTGTGTATGAATCACTCACATATTCTTCTGAATTATATGTATCCGCGGGATTAATTTGGAAAAACAGTAGGGATATGCAAGAACAACAATTTT 300
D A V Y E S L T Y S S E L Y V S A G L I W K N S R D M Q E Q T I F
ATCGGAACATTCTCTAATGAATTCCTGGGAACCTTCTATAGTCAATGGAATATATAGAATTGTGATCAATCAATATTGCAAGTCCCGGTATTTATT 400
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R S E L D H N G I S V Y T G T I I S D W G R S E L E I D R K A R
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I W A R C V S R K Q K I S I L V L S S A M G L N L R E I L E N V C Y
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P E I F L S F L S D K E R K K I G S K E N A I L E F Y Q Q F A C V G
GTGGCGATCCGGTATTTTCTGAATCCTTATGTAAGGAATTACAAAAGAAATTTCTTCAACAAAGATGTGAATTAGGAAGGATTTGGTGCAGGAAATATGAA 800
G D P V F S E S L C K E L Q K K F F Q Q R C E L G R I G R R N M N
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R R L N L D I P Q N N T F L L P R D I L A A A D H L I G L K F G M
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G A L D D M N H L K N K R I R S V A D L L Q D Q F G L A L V R L E N
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G Q I L A D G A A T V G E L A L H G K N V L V A Y M P W E G Y N S
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I L Q E M L T Y K S D H I R A R Q E V L G T T I I G G T I P N P E D
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A P E S F R L L V R E L R S L A L E L N H F L V S E K N F Q I N R
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K E A

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Fig.2. DNA sequence of ORF₁₀₇₀. The RNA-like strand (strand B) is presented. The deduced amino acid sequence is shown below the DNA sequence.

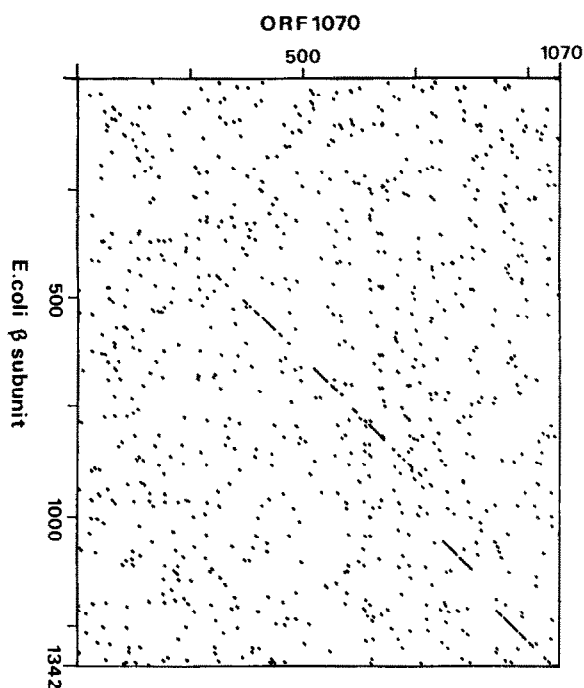


Fig.3. Dot matrix analysis between the deduced amino acid sequences of ORF₁₀₇₀ and *E. coli* β -subunit. The matrix program used for analysis scores a dot for 3 out of 4 identities.

ACKNOWLEDGEMENT

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