Volume 210, number 2, 153–156 FEB 04334 January 1987

Trans splicing in vivo: joining of transcripts from the 'divided' gene for ribosomal protein S12 in the chloroplasts of tobacco

Norihiro Zaita, Keita Torazawa, Kazuo Shinozaki and Masahiro Sugiura

Center for Gene Research, Nagoya University, Chikusa, Nagoya 464, Japan

Received 13 October 1986; revised version received 3 November 1986

The 'divided' gene for chloroplast ribosomal protein S12 consists of 3 exons and the first exon (5′-*rps*12) is located far from the second and third exons (3′-*rps*12) in the tobacco chloroplast genome. Northern blot hybridization revealed that both 5′-*rps*12 and 3′-*rps*12 are transcribed in the chloroplasts. Reverse transcription analysis indicated that *trans* splicing between the 5′-*rps*12 and 3′-*rps*12 transcripts occurs in vivo.

Chloroplast; *rps*12 gene; *trans* splicing; RNA sequence; (Tobacco)

1. INTRODUCTION

Chloroplast ribosomes in higher plants contain about 60 ribosomal proteins, one-third of which are known to be encoded by chloroplast DNA. The gene for chloroplast ribosomal protein S12 (*rps*12) was first identified in *Euglena* chloroplast DNA [1]. *Euglena rps*12 consists of 125 codons and has no intron. A portion of the tobacco *rps*12 was then found. This gene was split at least twice [2,3] and its two exons (from codon 39 to the last codon 123, 3′-*rps*12) were located in the large inverted repeats (IR_A and IR_B) of the chloroplast DNA [3]. Recently we have found the 5′-part of tobacco *rps*12 (from the initiation codon to codon 38, 5′-*rps*12) in the large single copy region of chloroplast DNA [4]. The 5′-*rps*12 was located 28 kbp downstream from 3′-*rps*12 in IR_B on the same strand, or 86 kbp downstream from 3′-*rps*12 in IR_A on the opposite strand. We have designated this gene structure as a 'divided' gene [5] and suggested a *trans* splicing pathway for the mRNA maturation [4]. Here we report the presence of an RNA species joined between the exon 1 and exon 2 sequences of tobacco *rps*12.

Correspondence address: M. Sugiura, Center for Gene Research, Nagoya University, Chikusa, Nagoya 464, Japan

2. MATERIALS AND METHODS

Total tobacco chloroplast RNA was prepared from young leaves as described [6] and treated with DNase I. Northern blot hybridization was carried out as in [7]. DNA probes were uniformly ^{32}P-labeled coding strands synthesized on recombinant M13 DNA templates [8]. Reverse transcription was performed as described [9,10] using reverse transcriptase from avian myeloblastosis virus. A 64-nucleotide *Sna*BI-*Hae*III fragment synthesized on the recombinant M13 DNA template and uniformly labeled with ^{32}P was used as a primer. The primer was annealed at 65°C for 30 min to 100 μg total tobacco chloroplast RNA in 30 μl of 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$ and 50 mM KCl. The base-specific reaction mixtures contained 5.5 μl DNA/RNA hybrid, 2 μl of 0.5 mM each of 4 dNTPs, 2 μl of either 1 mM ddNTPs, 1 μl reverse transcriptase (8 U), 1 μl of 100 mM DTT and 0.3 μl actinomycin D (2 mg/ml). Incubation was for 30 min at 42°C.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

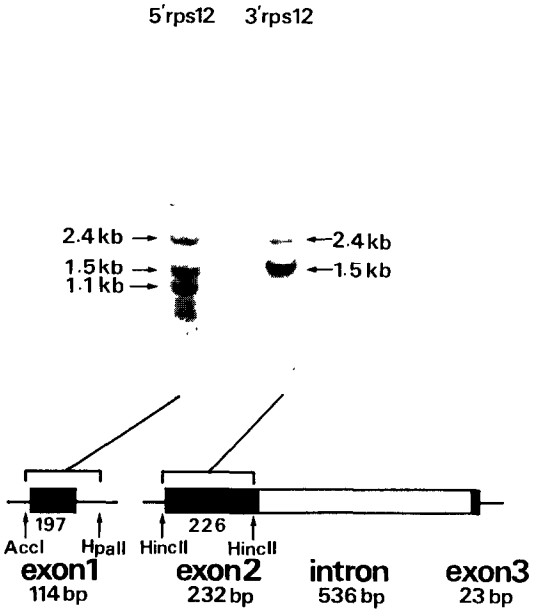
After hydrolysis of the RNA, the cDNA was analyzed by sequencing gel electrophoresis.

3. RESULTS AND DISCUSSION

Tobacco *rps*12 is divided into one copy of 5′-*rps*12 and two copies of 3′-*rps*12. The 5′-*rps*12 contains exon 1 of 38 codons and 3′-*rps*12 consists of exon 2 of 78 codons, a 536 bp intron and exon 3 of 7 codons as illustrated in fig.1.

Northern blot hybridization was carried out to determine whether 5′-*rps*12 and 3′-*rps*12 are expressed in the chloroplasts. Total tobacco chloroplast RNA was electrophoresed in 1.0% agarose gels and transferred to nylon membrane sheets (Biodyne A). The RNA blot was hybridized with the 197-nucleotide *Acc*I-*Hpa*II fragment containing 5′-*rps*12 and the 226-nucleotide *Hin*cII fragment containing exon 2 of 3′-*rps*12. The 5′-*rps*12 probe hybridized to 3 major RNA bands of 2.4, 1.5 and 1.1 kb and several minor bands. The 3′-*rps*12 probe hybridized to 2 main bands of 2.4 and 1.5 kb and several faint bands (fig.1). These results indicate that both *rps*12s are expressed in the chloroplasts. The major 1.5 and 2.4 kb RNAs are common RNA species hybridized with both probes.

Reverse transcription analysis was then performed to detect a possible splice junction of the exon 1 and exon 2 sequences. The 64-nucleotide *Sna*BI-*Hae*III coding fragment (34–98 nucleotides downstream from the 5′-end of exon 2) was used as a primer and total tobacco chloroplast RNA as template. The sequence ladders shown in fig.2 clearly indicate the presence of an RNA species joined between the exon 1 and exon 2 sequences. No sequence corresponding to the 18 nucleotides shown in fig.2 could be found in the entire 155844 bp tobacco chloroplast DNA [5]. The sequence ladders diverge little after the junction site (fig.2), indicating that the major 1.5 and 2.4 kb RNAs are spliced mRNAs or spliced intermediates. The faint ladders after the junction site fit the 5′-flanking DNA sequence of exon 2 (see fig.3), suggesting the presence of a small amount of the precursor mRNA from 3′-*rps*12. Based on the location, 5′-*rps*12 and 3′-*rps*12 cannot be transcribed as a single transcript. We therefore conclude that the two separate transcripts from 5′-*rps*12 and 3′-*rps*12 are spliced in *trans* in the chloroplasts.



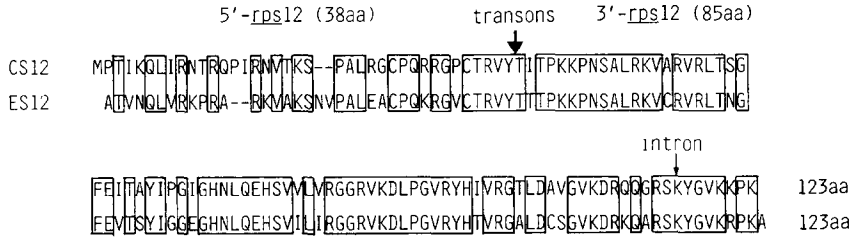


Fig.1. Detection of transcripts for CS12. Autoradiographs of RNA blots of total tobacco chloroplast RNA hybridized with the ^{32}P-labeled 5′-*rps*12 and 3′-*rps*12 probes. Size markers are calf liver 28 S and 18 S rRNAs, *E. coli* 23 S and 16 S rRNAs, tobacco chloroplast 5 S rRNA and *E. coli* tRNA-Glu. The lower part indicates the structure of 5′-*rps*12 and 3′-*rps*12, the location of DNA probes used and the predicted tobacco CS12 sequence compared with that of the *E. coli* S12 (ES12) (74% homology).

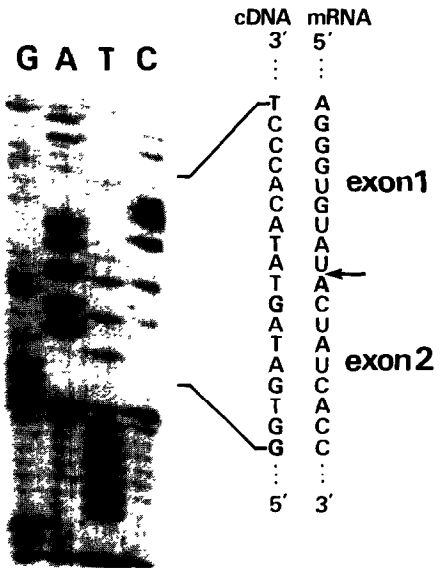


Fig.2. Determination of the splice junction. cDNA synthesis was carried out in the presence of ddNTP using a 64-nucleotide *Sna*BI-*Hae*III coding fragment as a primer and total tobacco chloroplast RNA as template. The arrow indicates the splice junction.

It has been reported that an antiserum against *E. coli* ribosomal protein S12 cross-reacts with a 30 S ribosomal protein from spinach chloroplasts and that its M_r is similar to that of *E. coli* S12 [11]. Therefore, the *trans* spliced products are most likely to function as mRNA for a tobacco S12 protein (CS12). In vitro *trans* splicing has been observed between two separate mRNA precursors prepared in vitro by SP6 RNA polymerase transcription [12,13]. The tobacco *rps*12 is, to our knowledge, the first example of genes requiring *trans* splicing.

We propose to designate the 3′-flanking sequence of exon 1 as 'transon' 1 and the 5′-flanking sequence of exon 2 as 'transon' 2. The sequence GTGCGAC at the 5′-end of transon 1 and the sequence GTCAACTTTTCC at the 3′-end of transon 2 fit the conserved boundary sequences of most chloroplast introns [14]. As shown in fig.3, a long complementary structure (about 70 bp) can be constructed between transon 1 and transon 2. This structure, if any, may be necessary for or at least enhance *trans* splicing as has been reported in *trans* splicing in vitro [12,13].

A divided *rps*12 structure has also been found in liverwort chloroplast DNA [15], rice, sugar beet and broad bean chloroplast DNAs (unpublished). This suggests that *trans* splicing is one of the general processing pathways in chloroplasts of a variety of plants.

ACKNOWLEDGEMENT

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture (Japan).

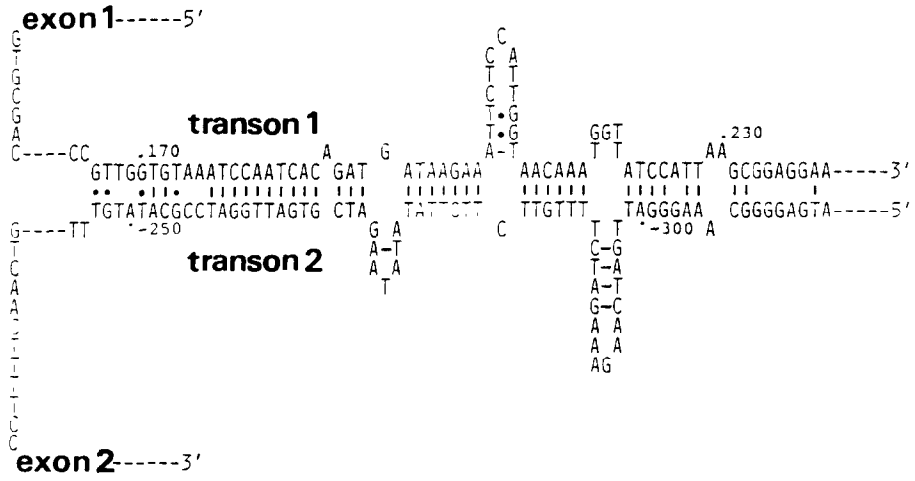


Fig.3. Possible complementary structure between transon 1 and transon 2. Sequences flanking exons fit the conserved boundary sequences of most chloroplast introns. Numbers show nucleotides from splice junctions.

REFERENCES

[1] Montandon, P.-E. and Stutz, E. (1984) Nucleic Acids Res. 12, 2851–2859.
[2] Hallick, R.B., Gingrich, J.C., Johanningmeier, U. and Passavant, C.W. (1985) in: Molecular Form and Function of the Plant Genome (Van Vloten-Doting, L. et al. eds) NATO ASI Series, vol.83, pp.211–220, Plenum, New York.
[3] Fromm, H., Edelman, M., Koller, B., Goloubinoff, P. and Galun, E. (1986) Nucleic Acids Res. 14, 883–898.
[4] Torazawa, K., Hayashida, N., Obokata, J., Shinozaki, K. and Sugiura, M. (1986) Nucleic Acids Res. 14, 3143.
[5] Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. and Sugiura, M. (1986) EMBO J. 5, 2043–2049.
[6] Shinozaki, K. and Sugiura, M. (1982) Gene 20, 91–102.
[7] Ohme, M., Kamogashira, T., Shinozaki, K. and Sugiura, M. (1985) Nucleic Acids Res. 13, 1045–1056.
[8] Messing, J. (1983) Methods Enzymol. 101, 20–78.
[9] Levy, S., Sures, I. and Kedes, L.H. (1979) Nature 279, 737–739.
[10] Leer, R.J., Van Raamsdonk-Duin, M.M.C., Mager, W.H. and Planta, R.J. (1984) FEBS Lett. 175, 371–376.
[11] Bartsch, M. (1985) J. Biol. Chem. 260, 237–241.
[12] Solnick, D. (1985) Cell 42, 157–164.
[13] Konarska, M.M., Padgett, R.A. and Sharp, P.A. (1985) Cell 42, 165–171.
[14] Shinozaki, K., Deno, H., Sugita, M., Kuramitsu, S. and Sugiura, M. (1986) Mol. Gen. Genet. 202, 1–5.
[15] Fukuzawa, H., Kohchi, T., Shirai, H., Ohyama, K., Umesono, K., Inokuchi, H. and Ozeki, H. (1986) FEBS Lett. 198, 11–15.