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A cDNA Clone for the 5′ Exon of Chloroplast ATP Synthase Subunit I Gene (*atpF*) from Broccoli (*Brassica oleracea* L. var. Italica) and Its Expression Pattern

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We isolated a cDNA clone, BLSC1, encoding 5′ exon of ATP synthase CF_0 subunit I from broccoli. BLSC1 is 285 nucleotides long which consists of a 5′ noncoding region of 34 nucleotides, a 5′ exon of 145 nucleotides and an intron of 106 nucleotides. The 5′ exon codes for 48 amino acids which reveals mostly hydrophobic. The amino acid sequence deduced from BLSC1 shares 83%, 83% and 91% identities with the genes coding for *atp*F from wheat, rice and spinach, respectively. Genomic Southern blot analysis for BLSC1 showed a typically strong signal for a gene located in the chloroplast genome. Northern blot analysis identified three major classes of transcripts showing strong positive signals in the leaves, but only trace amounts of the transcripts were identified in the other organs like stems, flower buds and roots.

Keywords: ATP synthase subunit I gene, *Brassica oleracea*, chloroplast gene, Southern hybridization, Northern hybridization

Chloroplast ATP synthase in higher plants is an energy-transducing multisubunit protein complex consisted of two functional parts, CF_1 and CF_0. CF_1 is an extrinsic membrane protein that catalyses the terminal step in photophosphorylation. CF_0 is an integral thylakoid membrane protein complex with the function of translocating protons across the thylakoid membranes (Hammes, 1983; Strotmann and Bickel-Sandkotter, 1984; Futai *et al.*, 1989; Junge, 1989). CF_1 is composed of five different subunits, denoted α, β, γ, δ and ε (*atp*A, *atp*B, *atp*C, *atp*D and *atp*E), with a subunit stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ (Moroney *et al.*, 1983). CF_0 contains four different subunits, I, II, III and IV (also called, *atp*F, *atp*G, *atp*H and *atp*I), with a stoichiometry of $I_1II_2III_{6\text{-}12}IV$ (Fromme, 1987; Grotjohann and Graber, 1990). The α, β and ε subunits of CF_1 and the I, III and IV subunits of CF_0 are encoded in the chloroplast genome and synthesized on the chloroplast ribosomes (Nelson *et al.*, 1980). In contrast, the γ and δ subunits of CF_1 and CF_0 subunit II are encoded in the nuclear genome and synthesized on the cytoplasmic ribosomes as a higher molecular weight precursor and imported into the chloroplasts (Herrmann *et al.*, 1983).

In higher plants, the gene for the subunit which is located far from the *atp*B-*atp*E cluster was initially isolated from tobacco (Deno *et al.*, 1983). The genes for the β and ε subunits were first sequenced from maize and spinach and are located upstream from *rbc*L on the opposite strand (Krebbers *et al.*, 1982). The genes for the IV, III and I subunits are clustered in this order just before *atp*A (Howe *et al.*, 1982; Bird *et al.*, 1985; Hennig and Herrmann, 1986). Unlike most other protein-encoding chloroplast genes from higher plants, the *atp*F genes contain a single intron (Bird *et al.*, 1985).

We isolated a cDNA clone from broccoli, designated BLSC1, showing high homologies to the previously reported ATP synthase CF_0 subunit I genes

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(*atp*F). The nucleotide sequence of BLSC1 was determined, and a putative open reading frame was deduced. The amino acid sequence deduced was compared with the previously reported genes for ATP synthase CF_0 subunit I. Expression pattern of *atp*F gene in the various organs of broccoli is discussed at the transcript level.

MATERIALS AND METHODS

Plant materials and bacteria

Brassica oleracea L. var. italica cv. Royal Green was grown in a green-house at 20± 2℃ under natural lighting. *Escherichia coli* strain HB101 was used for amplification and manupulation of the plasmids.

DNA manipulations and preparation of a cDNA bank

Plasmid DNA in *E. coli* was extracted by alkaline lysis method (Sambrook *et al*., 1989). Restriction digestions, agarose gel electrophoresis, DNA fractionation from agarose gel and DNA ligation were performed by standard procedures (Sambrook *et al*., 1989; manufacturers' suggestions). Restriction and modifying enzymes were purchased from KOSCO (Korea) and Promega (U.S.A.). Extraction of total RNA from the young leaves of broccoli was done as described by Hong and Jeon (1987). Poly(A^+) RNAs were isolated from the total RNA using oligo(dT)-cellulose resin (Pharmacia, U.S.A.). cDNAs were synthesized by RiboClone cDNA Synthesis System using oligo $(dT)_{15}$ primer (Promega, U.S.A.) and put into pBR322 at *Eco*RV site. Labeling of DNA probes with [^{32}P]-dCTP for Southern hybridizations was carried out using Prime-a-Gene System (Promega).

Nucleotide sequencing and isolation of a gene for chloroplast ATP synthase subunit I

Clones in the cDNA bank were characterized by random sequencing. Overlapping subclones were generated by ExoIII digestion of the insert DNA to produce a set of unilaterally nested deletions. Nucleotide sequencing was performed by the dideoxy chain-termination method (Sanger *et al*., 1977) on double stranded DNA using Sequenase Version 2.0 (United States Biochemical Corporation, U.S.A.). Nucleotide sequences determined were analyzed by GenBank Internet System (U.S.A.) and PC gene software program (Univ. of Geneva, IntelliGenetics Inc. and Genefit SA, Swiss).

Southern blot analysis

Chromosomal DNA was isolated from the young leaves of broccoli and tobacco as described by Dellaporta *et al*. (1984). Extracted genomic DNA was digested with *Eco*RI and *Hin*dIII, electrophoresed on a 0.6% agarose gel and blotted onto Hybond-N membrane (Amersham, U.K.). The membrane was prehybridized and hybridized in 50% formamide and 5×SSPE at 37℃ and washed in 0.1×SSPE and 0.1% SDS at 37℃, and exposed to X-Ray film (Kodak, X-OMAT, U.S.A.) with two intensifying screens (DuPont, U.S.A.) at −70℃.

Northern blot analysis

Total RNA was prepared from flower buds, stems, leaves and roots of broccoli and subjected to northern hybridization. Northern hybridization was performed using ECL direct nucleic acid labeling and detection system (Amersham, U.K.). Ten g of total RNA was electrophoresed on an agarose gel containing 17.5% formaldehyde and blotted onto Hybond-N membrane. Prehybridization and hybrydization were performed in 0.5 M NaCl at 37℃. After hybridization, the membrane was washed in 6 M urea, 0.4% SDS and 0.5×SSPE for 20 min at 37℃ and washed again in 0.4% SDS and 0.5×SSPE for 20 min at 37℃ and 0.2×SSPE for 5 min at room temperature. The membrane was exposed to X-ray film for 1 h at room temperature. Size of the northern band was identified by comparing to the RNA ladder (Gibco/BRL, U.S.A.).

RESULTS AND DISCUSSION

By sequencing the clones in the cDNA library randomly, we isolated a putative clone, designated BLSC1, which shows high homologies to the previously reported chloroplast ATP synthase subunit I genes. BLSC1 is 285 nucleotides long which consists of a 5′ noncoding region of 34 nucleotides, a

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1                   TTTTTTTTATAGTTTAGCTAGAAGAGGAGATTAT

35     ATGAAAAATTTAACCGATTCTTTCGTTTACTTGGGTCACTGGCCA
       M  K  N  L  T  N  S  F  V  Y  L  G  H  W  P      15

80     TCCGCCGGGAGTTTCGGGTTTAATACCGATATTTTAGCAACAAAT
       S  A  G  S  F  G  F  N  T  D  I  L  A  T  N      30

125    CTAATAAATCTAAGTGTAGTCTTCGGTGTATTGATCTTTTTTGGA
       L  I  N  L  S  V  V  F  G  V  L  I  F  F  G      45

170    AAGGGAGTGTGTGTGAGTTGTTCATTTCAAGAATAGGCTGGATTC
       L  K  G  V  *                                    48

215    GTCCAGTGGCACTATAACTAGCAAAGAGGTCCTAATCCCGCGAAT

260    TACTTCTGAATACAAAATTCAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of BLSC1. "—" represents the ribosomal binding site and "★" indicates a presumtive initiation site of the intron sequence. The nucleotide sequence data reported will appear in the EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under the accession number U13703.

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A. MKNLTNSFVYLGHWPSAGSFGFNTDILATNLINLSVVFGVLIEFGKGV 48
B. ***V*H***F*A**********************T**V***---**** 45
C. ****T******A*********L************T**V***---**** 45
D. ***V*D***F***********F***************L***---**** 45
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Fig. 2. Alignment of amino acid sequences for the 5′ exons of *atp*F gene in broccoli (A), wheat (B), rice (C) and spinach (D). "*" indicates the identical amino acid sequences; "---" was added to maximize alignment.

5′ exon of 145 nucleotides and an intron of 106 nucleotides (Fig. 1). Nucleotide sequence of BLSC1 shows "GAGGAG", the underlined nucleotide sequence, which is the remarkably conserved ribosomal binding site in plant chloroplast genome. BLSC 1 also contains a putative intron sequence starting as "GU" like other *atp*F genes reported from plants.

The 5′ exon codes for 48 amino acids and reveals a mostly hydrophobic domain. The region of 26th-48th amino acid residues is likely a transmembrane helix domain (Kyte and Doolittle, 1982). The amino acid sequence for BLSC1 shares 83%, 83% and 91% amino acid identities with the 5′ exon of *atp*F genes from wheat (Birds *et al.*, 1985), rice (Hiratsuka *et al.*, 1989) and spinach (Hennig and Herrmann, 1986), respectively (Fig. 2). The 5′ noncoding region and the intron region show 85% nucleotide sequence identity to the spinach *atp*F gene (Henning and Herrmann, 1986).

When the total DNAs of broccoli and tobacco plants were digested with *Eco*RI and *Hin*dIII and then hybridized with ^{32}P-labeled BLSC1, the Southern band patterns for broccoli and tobacco were very alike. Single strong Southern bands were observed at about 4.5 kb for *Eco*RI and 1.6 kb for *Hin*dIII in both plants (Fig. 3). The strength, i.e., at least 100 times stronger than a typical Southern signal observed for a gene coded in the nuclear genome, and the size of the Southern bands observed in broccoli indicates that *atp*F gene is also localized in the chloroplast genome in broccoli like in tobacco.

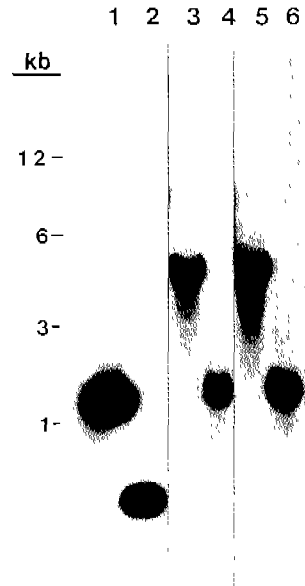

Fig. 3. Southern blot analysis of broccoli and tobacco DNAs with ^{32}P-labeled BLSC1. Broccoli (3 and 4) and tobacco (5 and 6) DNAs were digested with *Eco*RI (3 and 5) and *Hin*dIII (4 and 6). 1, BRL's 1 kb DNA ladder; 2, *Bam*HI/*Eco*RI digest of pBLSC1.

Chloroplasts may arise from the development of proplastids or from the redifferentiation of etioplasts, amyloplasts or chromoplasts depending on the organs. Expression patterns of BLSC1 gene in various organs were examined. Northern blot analysis of total RNAs extracted from 3 mm flower buds, stems, leaves and roots of broccoli for BLSC1 gene also showed typical patterns for chloroplast genes. Three strong bands at about 4.6 kb, 3.4 kb and 1.7 kb were observed from leaves. Two bands were apparent at about 3.4 kb and 1.7 kb for flower buds and stems, and at about 3.4 kb and 2.5 kb for roots. Intensity of the northern bands was much stronger for leaves compared to that in other organs (Fig. 4). Multiple northern bands indicate that *atp*F gene is transcribed as a part of polycistronic transcript. Differences in the largest size of the northern bands observed from

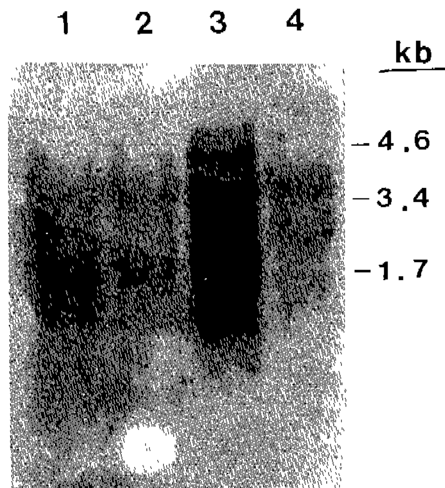

Fig. 4. Northern hybridization pattern of BLSC1 transcripts in broccoli. Total RNAs isolated from flower buds (1), stems (2), leaves (3) and roots (4) were northern hybridized with ^{32}P-labeled BLSC1.

each organ could indicate differences in the rate of processing for the primary transcript or the point of transcriptional termination. The size difference for the smaller transcripts among organs, i.e., 2.5 kb for roots and 1.7 kb for other organs, probably reflects difference in the processing.

Transcription of ATPase genes in tobacco and spinach showed polycistronic transcription of the gene. The primary transcript was 3.0 kb in tobacco (Shinozaki *et al.*, 1986) and 3.2 kb in spinach (Green and Hollingsworth, 1992). Green and Hollingsworth (1994) also investigated tissue-specific expression pattern of the gene and identified identical transcript sizes for the gene in various organs, such as root, stem, leaf and flower, although the level of transcript showed big differences. Genomic Southern data shown here clearly indicates that chloroplast genome, at least around the *atp*F gene, is well conserved between the different plant species. On the other hand, the northern data shown here indicates differences in the transcription of ATP synthase genes among different plant species. Size difference for the largest transcript of ATP synthase gene suggests different polycistronic transcription unit among the plant species. Size difference of the transcripts for the various organs in broccoli suggests that a different splicing of the primary transcript depends on the organ. The difference observed should be related to the level of ATP synthase in the plastids. Control of chloroplast gene expression is heavily influenced posttranscriptionally (Gruissem, 1989), and the data presented in this paper represents an example of diversity in the splicing of the primary transcript in chloroplast genes which are thought to be well conserved in plant kingdom.

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브로꼴리 葉綠體 ATP Synthase Subunit I 遺傳子의 5′ Exon 部位 cDNA 클론 및 이의 발현 양상

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적 요

브로꼴리로부터 엽록체 ATP synthase subunit I 유전자의 5′ exon 부위에 상응하는 cDNA가 클론되어 BLSC1으로 명명되었다. BLSC1 클론은 285개의 염기를 가지며, 이들은 34개의 염기로 된 5′ noncoding 부위, 145개의 염기로 된 5′ exon, 그리고 106개의 염기로 된 intron 부위를 포함한다. 5′ exon 부위는 대부분 소수성 성질을 갖는 48개의 아미노산을 암호화하고 있다. BLSC1 클론으로부터 연역된 폴리펩티드의 아미노산 서열은 밀, 벼 그리고 시금치의 폴리펩티드와 각각 83%, 83% 그리고 91% 상동성을 갖는다. 또한, Southern 분석은 BLSC1 유전자가 엽록체 유전자상에 존재함을 알려준다. Northern 분석에 의해 BLSC1 유전자가 인지하는 3종류의 전사체가 잎, 줄기, 뿌리 그리고 꽃눈에서 발현되며, 잎에서 가장 많은 양으로 발현됨을 확인하였다.

주요어 : ATP synthase 구조단위 I 유전자, *Brassica oleracea*, 엽록체 유전자, Southern 분석, northern 분석

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