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The sugar beet mitochondrial genome contains an ORF sharing sequence homology with the gene for the 30 kDa subunit of bovine mitochondrial complex I

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Abstract. From a sugar beet mitochondrial DNA library, we have isolated an open reading frame (ORF192) showing extensive homology to the gene for the 30 kDa subunit of the bovine mitochondrial complex I (NADH:ubiquinone reductase). The ORF192 was found to be actively transcribed to give an RNA of approximately 1.0 kb. We have designated this gene *nad9*. Transcripts from the *nad9* locus are edited by five C to U transitions, increasing similarity with the amino acid sequence of the corresponding bovine and *Neurospora crassa* polypeptides. Southern blot hybridization also indicates that *nad9* is present in the mitochondrial genomes of a variety of higher plant species.

Key words: Sugar beet – *nad9* – Mitochondrial gene – RNA editing

Higher plants have the largest and most complex mitochondrial genomes so far reported (reviewed in Newton 1988). The smallest plant mitochondrial DNA (mtDNA) known (208 kb, in *Brassica hirta;* Palmer and Herbon 1987) is already larger than the largest fungal mtDNA (176 kb, in *Agaricus bitorquis;* Hintz et al. 1985). Most animal mtDNAs comprise approximately 16 kb. One can thus pose the question whether the large size of plant mtDNAs reflects an increased coding capacity compared to animals and fungi. Makaroff and Palmer (1987) identified 24 abundant and distinct transcripts longer than 500 nucleotides in *Brassica campestris* mitochondria, and estimated that about 30% of the 218 kb mitochondrial genome is highly transcribed. It is also noteworthy that plant mtDNAs encode several genes which are not found in mitochondria of other groups of organisms; examples are the gene coding for the α subunit of ATPase (Braun and Levings III 1985), and genes for the ribosomal proteins S3 (Hunt and Newton 1991; Schuster and Brennicke 1991), S7 (Zhuo and Bonen 1993), S12 (Gualberto et al. 1988), S13 (Bland et al. 1986), S14 (Wahleithner and Wolstenholme 1988), S19 (Conklin and Hanson 1991), and L16 (Hunt and Newton 1991). We are interested in characterizing new coding sequences in sugar beet (*Beta vulgaris* L.) mtDNA. This communication presents evidence showing that a gene coding for a homologue of the 30 kDa subunit of bovine mitochondrial complex I (NADH dehydrogenase) is present in the sugar beet mitochondrial genome.

A mitochondrial genome library was constructed by cloning partially *Mbo*I-digested mtDNA from the sugar beet line TK81-0 (male-fertile cytoplasm) into the *Bam*HI site of the phage lambda DASH vector (Stratagene). In order to locate the actively transcribed mitochondrial genes, we screened our library with ^{32}P-labeled mitochondrial RNA (mtRNA) isolated from leaves of sugar beet plants. Among clones exhibiting positive hybridization signals and not related to previously identified genes was a clone designated #449. Hybridization of a total mtRNA probe to Southern blots of restriction digests of #449 demonstrated that significant hybridization was confined to the 4.8 kb *Eco*RI internal DNA fragment. This fragment was further subcloned into the plasmid vector pUC119. The sequence of the subclone was determined using the dideoxynucleotide chain termination method with Sequenase version 2.0 (United States Biochemicals).

Computer analysis of the sequence showed that the region examined here contained an uninterrupted open reading frame (ORF192) of significant size, with the potential to encode a 192 amino acid polypeptide (Fig. 1). A strong bias in favor of T or A in the third position of the codon (67.7% A+T vs. 33.3% G+C) is conspicuous, and is a feature of some other plant mitochondrial genes (Macfarlane et al. 1990). The deduced amino acid sequence of ORF192 was compared with protein sequences from the NBRF data base using GENETYX (SOFTWARE DEVELOPMENT) program. The putative sugar beet protein displays significant similarity with the 30

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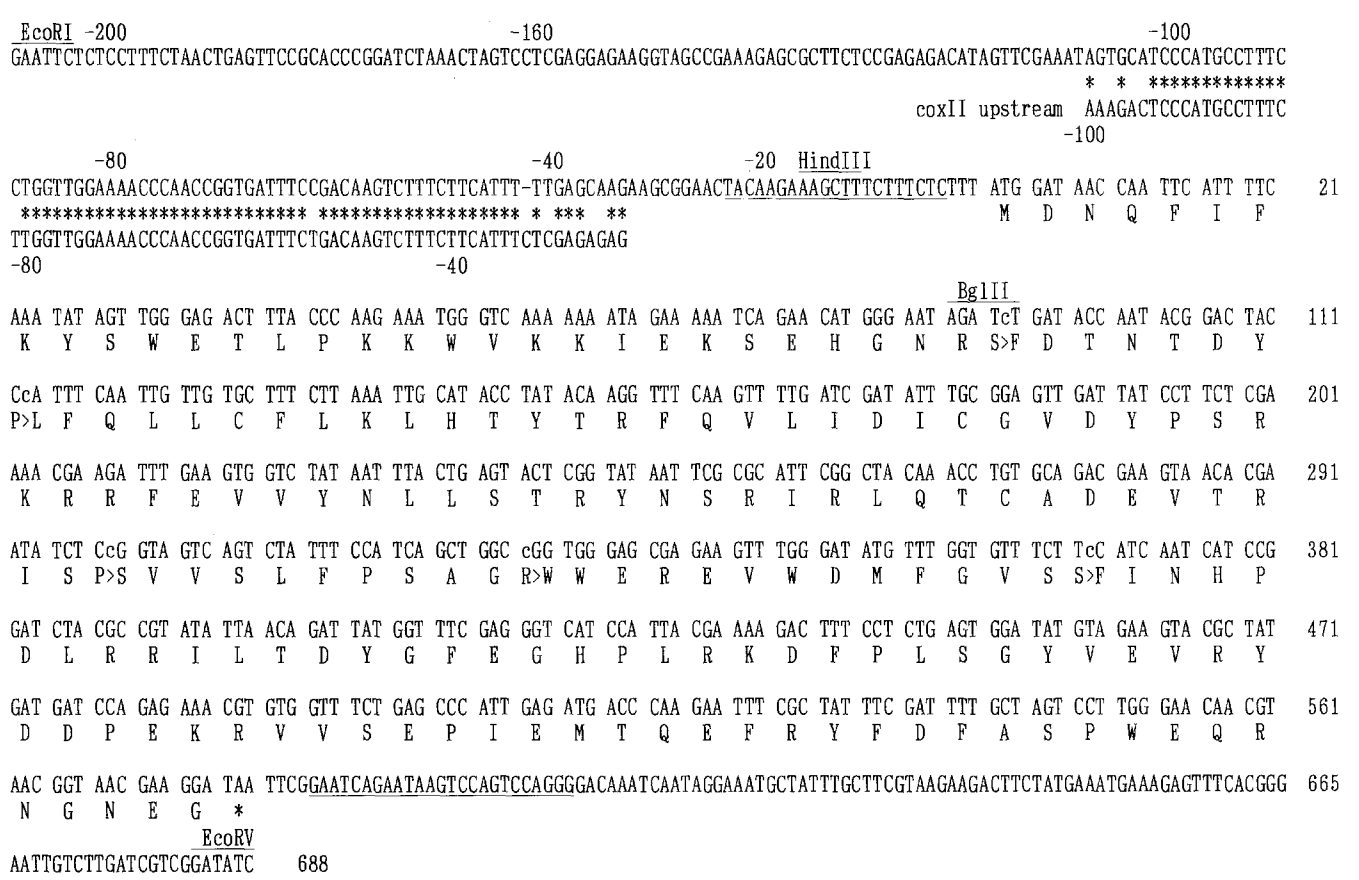

Fig. 1. Nucleotide and derived amino acid sequences of the *nad9* (ORF192) gene in sugar beet mitochondria. The sequence has been deposited under accession number D16539 in the EMBL data base. Numbering of nucleotides is from the predicted translation start of *nad9*. Cytosines altered by RNA editing are indicated by *lowercase* letters. Oligonucleotide primers used for RT-PCR are *underlined*. Restriction sites are indicated for *Bgl*II, *Eco*RI, *Eco*RV and *Hin*dIII. Sequence alignment of the *nad9* gene with sugar beet *coxII* 5′ flanking region (Senda et al. 1991) is also shown

kDa subunit polypeptide of complex I from bovine mitochondria (57% homology) and the corresponding 31 kDa protein from *Neurospora crassa* (51%), both of which are encoded by the nuclear genome (Pilkington et al. 1991; Videira et al. 1990) (Fig. 2). As shown in Fig. 2, the sugar beet protein is 57 or 67 amino acids shorter at its amino-terminus than the corresponding bovine and *N. crassa* sequences, respectively. Our comparison indicated that the best match between this protein and sequences registered in the data base is with the product of ORF212 from the liverwort mitochondrial genome (Oda et al. 1992) (Fig. 2). Similarities were also detected with the deduced protein sequences encoded by the tobacco chloroplast gene *ndhJ* (Shinozaki et al. 1986) and the liverwort chloroplast gene *ndh9* (Ohyama et al. 1986).

There have been several reports of mitochondrial genes coding for seven subunits of NADH dehydrogenase in higher plants: *nadl* (Chapdelaine and Bonen 1991; Wissinger et al. 1991; Conklin et al. 1991), *nad2* (Binder et al. 1992), *nad3* (Gualberto et al. 1988), *nad4* (Lamattina and Grienenberger, 1991), *nad4L* (Brandt et al. 1992), *nad5* (Knoop et al. 1991; Pereira de Souza et al. 1991) and *nad6* (Haouazine et al. 1992; Nugent and Palmer 1993). *Trypanosoma brucei* mtDNA also contains additional genes for other subunits (7 and 8) of NADH dehydrogenase (Koslowsky et al. 1990; Souza et al. 1992). Hence, we have designated the ORF192 *nad9*.

An examination of the sequences upstream of the *nad9* locus revealed an interesting homology with the sugar beet *coxII* 5′ flanking sequence. As seen in Fig. 1, the homology includes a region of 61 nucleotides extending from position −43 upstream from the ORF192 initiation codon to position −103. This corresponds to positions −35 to −95 relative to the *coxII* start codon (Senda et al. 1991).

The expression of *nad9* was investigated in Northern blot experiments. A *Bgl*II-*Eco*RV fragment, which contains most of coding region and 3′ flanking sequence (see Fig. 1), was used to probe a Northern blot of total mtRNA from sugar beet leaves. The probe hybridized to a single transcript of about 1.0 kb (data not shown), indicating that the *nad9* is transcribed in mitochondria.

RNA editing has been found in almost all of the protein coding genes in higher plant mitochondria analyzed to date (reviewed in Gray et al. 1992; Wissinger et al. 1992). To determine whether this is the case for sugar beet *nad9*, the complete region of the gene was amplified by RT-PCR. DNase-treated mtRNA was subjected to reverse transcription using the primer 5′-CCCTGGACTGGACTTATTCTGATTC-3′, which

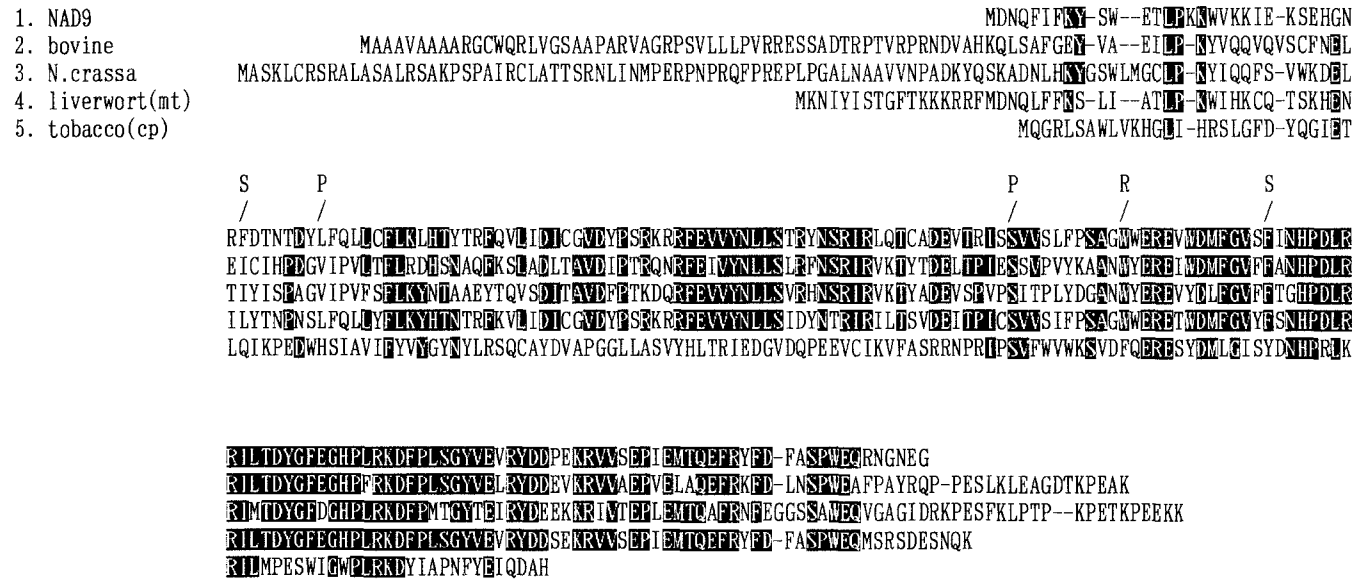

Fig. 2. Alignment of the predicted NAD9 protein sequence of sugar beet mitochondria (1) with the corresponding nuclear-encoded sequences from the bovine (2) and *N. crassa* (3) genomes, and with protein sequences encoded in liverwort mitochondrial ORF212 (4) and tobacco chloroplast *ndhJ* (5). Amino acids encoded by the genomic sequence at the sites of RNA editing are given above the sequence alignment. Gaps were introduced into the sequences in order to maximize the alignment. Amino acid residues common to at least three of the sequences are highlighted in *black*

is complementary to the 3′ region of the termination codon of *nad9* (Fig. 1). The resulting cDNA was amplified by PCR using the primer 5′-TACAAG AAAGCTTTCTTTCTC-3′, which is identical to the sequence upstream of the *nad9* initiation codon (Fig. 1). Amplified fragments were then cloned into pUC119. Sequencing of four independent cDNA clones revealed five C to U changes (Fig. 1). All of these modifications occur in the first or second position of the codon, leading to alterations in amino acid specification. Note also that the editing events improve the match with the bovine and *N. crassa* sequences (Fig. 2).

Southern analysis with the *Bgl*II-*Eco*RV probe (see Fig. 1) revealed hybridization to single fragments of *Eco*RI- and *Hind*III-digested sugar beet mtDNA, 4.8 and 1.4 kb, respectively, in length (data not shown). This indicates that the *nad9* sequences only occur in this arrangement in the sugar beet mitochondrial genome. We next wished to ascertain whether the *nad9* is present in other plant mitochondrial genomes, because mitochondrial protein gene content is known to be generally well conserved among a wide range of plant species. The *Bgl*II-*Eco*RV probe was used to hybridize with mtDNAs from rice (cv. Kita-ake), soybean (cv. Harosoy), and common bean (cv. Kotsubu-ryokuto no. 1), and total cellular DNAs from apple (cv. Sekai-ichi), and beech. Positive hybridization signals were detected for all plant species examined (data not shown). We think that the hybridization profiles in apple and beech represent mtDNA for the following reasons: first, the intensity of hybridization appears too great for a single-copy nuclear gene (data not shown); second, computer analysis failed to reveal the presence of sequences homologous to chloroplast DNAs of tobacco (Shinozaki et al. 1986), rice (Hiratsuka et al. 1989) and liverwort (Ohyama et al. 1986) within the probe used. Our results thus demonstrate that the *nad9* sequence is conserved among mitochondrial genomes from a variety of higher plants.

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