

The Gene Encoding Mitochondrial Succinate Dehydrogenase Subunit 4 Has Been Successfully Transferred to the Nuclear Genome in Pea, while Leaving an Original Sequence as a Pseudogene in the Mitochondrial Genome

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Abstract

A sequence homologous to a gene encoding succinate dehydrogenase subunit 4 (*sdh4*) was found in pea mitochondrial genome but non-functional. A functional *sdh4* gene was isolated from pea nuclear genome, suggesting gene transfer from the mitochondrion to the nucleus during evolution. As compared with mitochondrial *sdh4* genes, the pea nuclear *sdh4* gene has an extension in its N-terminal region, suggesting encoding a protein targeting signal to mitochondria. There are five introns and six exons in the pea nuclear *sdh4* gene. Sequence homology to mitochondrial *sdh4* is observed in the exon 6 but not in the other five exons, suggesting mitochondrial sequence was integrated downstream of the five exons. Comparison of nuclear *sdh4* genomic sequences from pea, *Arabidopsis* and rice suggests that the number and location of introns are different among the three. Therefore, *sdh4* genes may have evolved in the nuclei independently among plant species.

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Abbreviation

sdh4, succinate dehydrogenase subunit 4 gene.

According to the endosymbiont hypothesis, mitochondria are a resultant of endosymbiosis during evolution. It is known that over 90% of genetic information has already been transferred from the mitochondrion to the nucleus in mammals and insects, etc. In contrast, the genome size of plant mitochondria is much larger than those of animals and dozens of additional genetic information are found in plant mitochondrial genome by the complete nucleotide sequence analysis in liverwort, *Arabidopsis* and sugar beet (Oda *et al.*, 1992; Unseld *et al.*, 1997; Kubo *et al.*, 2000). This evidence may imply that plant mitochondrial genome is keeping rather ancient form as compared with animal mitochondrial genome, thus it will be a good model to know the evolutionary process of genetic information transfer. Gene transfer event

from the mitochondrion to the nucleus has been firstly found by Nugent and Palmer (1991) and examples of gene transfer events have been reported to date by several researchers including us (Kadowaki *et al.*, 1996; Kubo *et al.*, 1999 and references therein). In the present study, we report another case of gene transfer event in pea (*Pisum sativum*, L. var. Alaska).

The complex II of respiratory chain is involved in catalysis of the oxidation of succinate to fumarate and composed of four subunits, namely SDH1–SDH4 (see, Siedow, 1995 for a review). All of the subunits are encoded by nuclear genes in human and yeast, whereas genes for SDH3 and SDH4 (*sdh3* and *sdh4*) have been found in the mitochondrial genomes of some lower plants, a protist (Burger *et al.*, 1996) and several higher plants (Giegé *et al.*, 1998; Adams *et al.*, 2001). Recently, multiple losses and transfers to the nucleus of *sdh3* and *sdh4* genes have been reported in angiosperm (Adams *et al.*, 2001). The above results suggest that gene

transfer event of the *sdh4* gene from the mitochondrion to the nucleus is an on-going process in plants. Therefore, diversity of *sdh4*-gene content among plant species will give a clue to understand the process of gene transfer event.

In higher plants, *sdh4* genes have been found downstream of *cox3* gene in the mitochondrial genome of *Arabidopsis* and *Oenothera* by Giegé *et al.* (1998). They also suggested presence of the *sdh4*-like sequence in the mitochondrial genomes

of other dicotyledonous plants. In addition, partial nucleotide sequences of *sdh4*-homologues have also been reported in the mitochondrial genomes of several higher plants (Adams *et al.*, 2001). In order to know its gene organization in pea, DNA gel blot analysis was conducted by using the *Arabidopsis* mitochondrial *sdh4* sequence as a probe. Mitochondrial and nuclear DNAs were isolated from young seedlings of pea as described in Umbeck and Gengenbach (1983) and Rivin *et al.* (1982), respectively, and used for hybridization analysis. A signal was detected in pea mitochondrial DNA (Fig. 1, left panel), suggesting presence of an *sdh4*-homologous sequence in pea mitochondrial genome. The *sdh4*-homologous sequence was isolated from a pea mitochondrial DNA library with the *Arabidopsis* *sdh4* probe and the nucleotide sequence was determined. Nucleotide sequence alignment shows that the pea mitochondrial sequence shows 87% sequence homology to the *Arabidopsis* *sdh4* sequence but lacks a C-terminal portion of evolutionarily conserved region (Figs. 2 and 4). It is unlikely that the open reading frame of the pea *sdh4* gene is divided by some other machinery, such as a group II intron or *trans*-splicing because there was no such structure in the downstream region. These results strongly suggest that pea mitochondrial *sdh4* sequence can not encode functional SDH4. The process of disruption of the original *sdh4* gene in pea seems to be different form that of the *Arabidopsis* *sdh4* whose open reading frame is interrupted by an internal stop codon (Giegé *et al.*, 1998).

To examine the gene transfer event, DNA gel blot analysis of pea nuclear DNA was conducted. A signal was detected in the nuclear DNA with each of two restriction enzymes (Fig. 1, right panel). This signal appears to be derived from nuclear DNA, not from mitochondrial DNA, because the

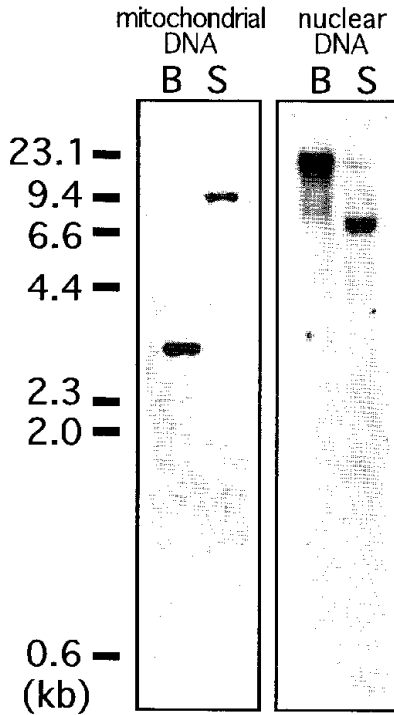


Fig. 1 DNA gel blot analysis of *sdh4* gene. Mitochondrial (2 μ g) and nuclear DNAs (5 μ g) of pea were digested with *Bam*HI (B) and *Sac*I (S), respectively, electrophoresed through a 0.7% agarose gel and blotted onto a nylon membrane. An *Arabidopsis* mitochondrial *sdh4* sequence (Giegé *et al.*, 1998) was used as a probe. Molecular weight standards are shown at left.



Fig. 2 Nucleotide sequence alignment of *sdh4* genes from pea (this study) and *Arabidopsis* (Giegé *et al.*, 1998) mitochondrial genomes. Identical nucleotides are indicated by asterisks. Putative initiation codons in the original *sdh4* genes are enclosed. Pea mitochondrial *sdh4* sequence has been deposited in the EMBL/GenBank/DBJ databases under the Accession no. AB070890.

size of each signal in the nuclear DNA is different from that in the mitochondrial DNA. It is likely that the *sdh4*-homologous sequence is present as a single copy in pea nuclear genome by the DNA gel blot analysis. Perhaps, an additional copy showing low homology to *sdh4* might be present because a weak signal (15 kb) was also detected in the nuclear DNA (Fig. 1, lane S in right panel). This result suggests presence of another *sdh4*-homologous sequence in pea nucleus. In order to isolate the nuclear *sdh4* sequence, a pea nuclear cDNA library was constructed from poly(A)⁺ RNA and screened by using the *Arabidopsis sdh4* probe. Nucleotide sequence of the largest cDNA clone is shown in Fig. 3B. It

contains an open reading frame capable of encoding 223 amino acids and the predicted amino acid sequence shows significant homology to known SDH4 peptides (Fig. 4). Thus we concluded that this sequence encodes mitochondrial SDH4. As compared with mitochondrial-encoded *sdh4* genes, the pea nuclear *sdh4* gene has an extension of 130 amino acids in its N-terminal region (Fig. 4). This extension is likely to include a targeting signal for protein import into mitochondria because it has typical amino acid composition of mitochondrial targeting signals: many hydroxylated and positively charged amino acids; and less negatively charged amino acids. In addition, this region has the

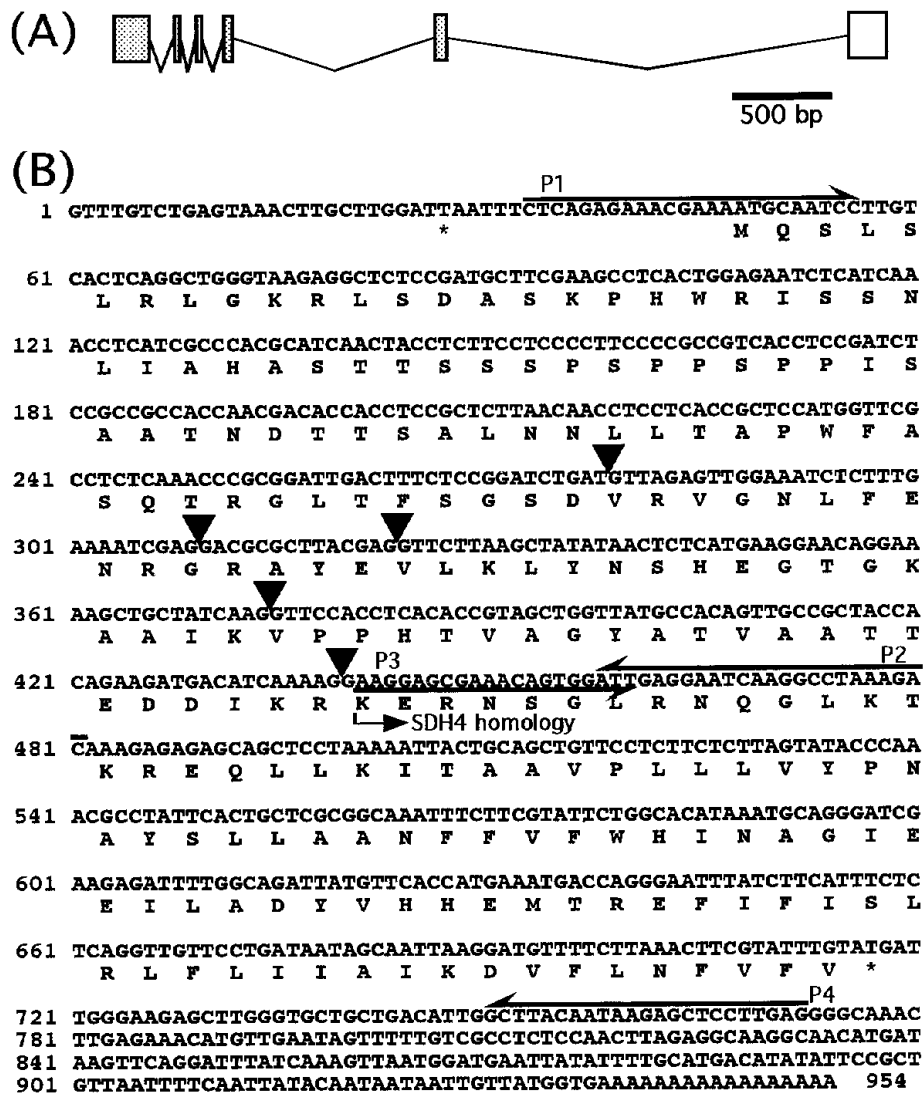


Fig. 3 (A) Schematic representation of pea nuclear *sdh4* gene. Open and dotted boxes represent mitochondrial SDH4-coding region and an N-terminal extension, respectively. Introns are shown by bent lines. (B) cDNA and deduced amino acid sequences of pea nuclear *sdh4* gene. Translational stop codons are represented by asterisks. Intron sites are indicated by filled triangles. The locations of primers used for PCR analysis to determine the genomic sequence are given by horizontal arrows. A region homologous to mitochondrial SDH4 protein is shown by a bent arrow. The cDNA and genomic sequences of pea nuclear *sdh4* have been deposited in the EMBL/GenBank/DBJ databases under the Accession nos. AB070891 and AB070892, respectively.

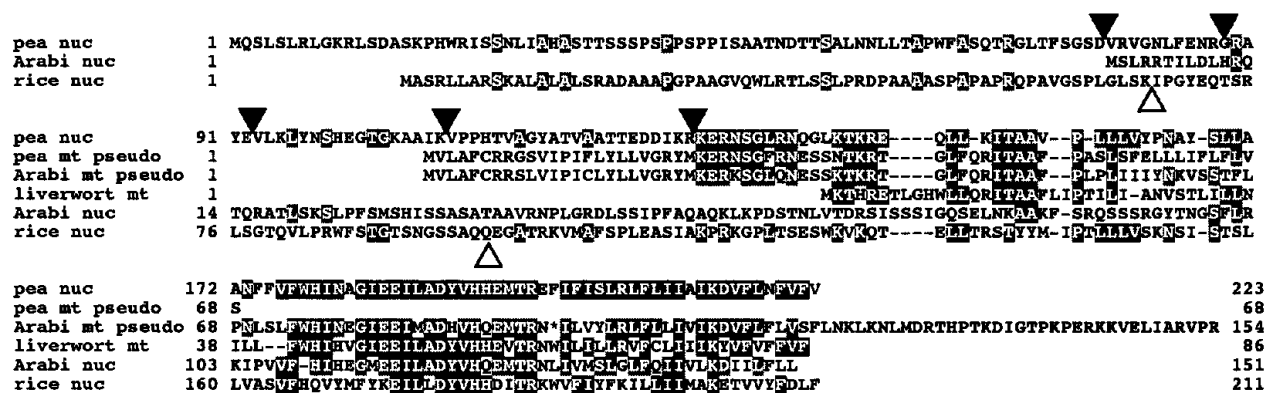


Fig. 4 Amino acid sequence alignment of SDH4. Deduced amino acid sequence of pea nuclear *sdh4* gene (this study) is aligned with that encoded in pea mitochondrion (this study), *Arabidopsis* mitochondrion and nucleus (Giegé *et al.*, 1998; Adams *et al.*, 2001), rice nucleus (DDBJ Accession no. AP003266) and liverwort mitochondrion (Oda *et al.*, 1992). Amino acid residues identical to pea nuclear SDH4 are indicated by black boxes. Intron sites in pea and rice nuclear *sdh4* genes are indicated by filled and open triangles, respectively. *Arabidopsis* nuclear *sdh4* gene has no intron sequence.

potential of forming an amphiphilic α -helix structure which suggested to be important for protein import into mitochondria (data not shown). The origin of the N-terminal extension is not clear because database search analyses and plaque hybridization experiments could not find out other known genes.

In order to examine the history of pea genomic *sdh4* gene in evolution, PCR was conducted with primers P1–P4 (locations of primers are shown in Fig. 3B) and pea nuclear DNA as a template. With primer pair P3–P4, products of the same size with the cDNA sequence were amplified. In contrast, larger products were obtained with primer pairs P1–P2 and P1–P4. Nucleotide sequence analysis of the amplified products shows presence of five introns and six exons in the pea nuclear *sdh4* gene (Fig. 3). The intron sequences vary, in length, from 100 nt to 2080 nt (the genomic sequence is not shown here but available in the EMBL/GenBank/DDBJ databases under the Accession no. AB070892). There was no intron in the mitochondrial SDH4-homologous region. Sequence homology to mitochondrial *sdh4* is observed in the exon 6 but not in the former five exons, suggesting mitochondrial sequence was integrated downstream of the five exons. Very recently, nuclear *sdh4* genes have been reported in several angiosperms (Adams *et al.*, 2001). Of these, the *Arabidopsis* and rice *sdh4* genes have found to contain no and two introns, respectively, which is contrast to the pea *sdh4*, showing diversity of genomic organization among plant species. Moreover, the N-terminal extensions are totally different among the three plant species (Fig. 4). The above results suggest that *sdh4* genes have evolved independently among plant species after the transfer

events or gene transfer event happened after split of the plants.

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