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RESEARCH ARTICLE

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Transcription of succinate dehydrogenase subunit 4 (*sdh4*) gene in potato: detection of extensive RNA editing and co-transcription with cytochrome oxidase subunit III (*cox3*) gene

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Abstract The potato (*Solanum tuberosum* L.) mitochondrial *cox3*/*sdh4* gene cluster was previously identified by heterologous hybridization using a *Marchantia polymorpha sdh4* probe. In this work, this potato gene cluster was cloned, sequenced and its expression was evaluated. The gene sequence and gene locus organization were found to be similar to the corresponding gene cluster in other dicot species, where known. Northern hybridizations with potato and cauliflower (*Brassica oleraceae*) mtRNA and RT-PCR analyses using potato mtRNA indicated that *cox3* and *sdh4* are co-transcribed in both species, generating a complex transcription pattern, where several transcripts from 1.1 kb to 4.4 kb are found. The potato transcript from this cluster displayed 14 and 13 RNA-editing sites, in the *cox3* and *sdh4* genes respectively, which changed the codon identity to amino acids and created a *sdh4* partially edited stop codon. Forty-three cDNA clones were analyzed for editing process and revealed different partial-editing with no apparent sequential processing in the *sdh4* gene.

Keywords Mitochondrial DNA · Cytochrome oxidase subunit III · Succinate dehydrogenase subunit 4 · RNA editing · Potato

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Introduction

Analysis of the mitochondrial genome of different organisms has shown that they are greatly variable regarding gene organization, structure and expression. Interestingly, plant mitochondria possess the largest and most complex organellar genomes known. The sequencing of the angiosperm mitochondrial genome (Unseld et al. 1997; Kubo et al. 2000) has shown that they are further complicated by the presence of repeated sequences active in homologous recombination, which results in the simultaneous presence of molecules showing variable length and sequence organization. These repeated sequences allow intragenic recombinations leading to rearrangements (deletions, inversions, duplications), which might create pseudogenes and/or put genes together, which have been located in different genome regions during evolution.

The expression of plant mitochondrial genomes is achieved in a relatively complex way. Some of the characteristics of the mitochondrial transcription process which contribute to the transcription-complexity in plant mitochondria are the duplication of the transcribed region, the presence of multiple initiation and/or termination sites, the *cis*- and *trans*-splicing events involved in the processing of several genes, the RNA editing and the co-transcription of genes (for review, see Gray et al. 1992; Schuster and Brennicke 1994). Several plant mitochondrial genes are co-transcribed. Some of these polycistronic transcripts contain sequences coding for related products, such as ribosomal RNA (*rrn18*/*rrn5* in wheat; Falconet et al. 1984), ribosomal proteins (*rps13*/*rpl16* in maize; Hunt and Newton 1991) and ATPase subunits (*atpA*/*atp9* in maize; Covello and Gray 1991). However, co-transcribed genes involved in different metabolic pathways, such as *orf25*/*cox3* in rice (Liu et al. 1992) and *nad3*/*rps12* in wheat (Gualberto et al. 1988), are also found.

The coding capacity of the plant mitochondrial genome is approximately three times bigger than in other

organisms, although it encodes only a few percent of the genetic information needed for the biogenesis and correct function of the mitochondrial machinery. As an example, the *Arabidopsis thaliana* mitochondrial genome contains 57 genes (Unseld et al. 1997), coding for subunits of respiratory chain complexes, ribosomal proteins and at least 42 putative ORFs. However, although the coding capacity is similar in different angiosperm species, there is an intriguing diversity of gene complements, even among related species. It has been found that a given gene may be encoded in the mitochondrial DNA in one species but present in the nuclear genome of another (Brennicke et al. 1993). The gene coding for complex II subunit 4 (subunit of a mitochondrial membrane-bound complex) is an example of the genes showing diversity in genome location.

Gene coding for complex II subunits (succinate dehydrogenase) has been identified in bacterial and mitochondrial genomes from lower eukaryotes, such as algae and protists (Darlison and Guest 1984; Heizen et al. 1995; Leblanc et al. 1995; Burger et al. 1996). In other organisms, in general, complex II genes have been found in the nuclear genome (Morris et al. 1994; Au et al. 1995). However, in some plant species, such as *A. thaliana*, *Beta vulgaris*, *Lycopersicon esculentum*, *Marchantia polymorpha* and *Oenothera berteriana*, the gene coding for complex II subunit 4 (*sdh4* gene) was found in the mitochondrial genome (Oda et al. 1992; Giegé et al. 1998; Kubo et al. 2000; Adams et al. 2001).

This report describes the analysis of the *sdh4* gene from the potato mitochondrial genome, identified by heterologous hybridization with the homologous gene from *M. polymorpha*. The *sdh4* gene was found in a gene cluster that includes *cox3* and a partial *cox2* gene. The angiosperm conservation, sequence analysis, transcription and editing pattern of *cox3* and *sdh4* genes are described.

Materials and methods

Isolation and analysis of mitochondrial DNA and RNA

Mitochondrial DNA (mtDNA) was isolated from etiolated seedlings of *Coix lacryma-jobi* L. cv Adlay, maize (*Zea mays*, cv AGF352), pea (*Pisum sativum*, cv Mikado), soybean (*Glycine max*, cv IAC-5), potato tubers (*Solanum tuberosum*, cv Binje) and cauliflower inflorescence (*Brassica oleracea*, from a local market).

Purified mitochondria were prepared as described by Vedel and Quetier (1974): mtDNA was obtained after mitochondrial lysis and CsCl–ethidium bromide centrifugation. mtRNA was prepared as described by Stern and Newton (1986), treated with DNAse I to remove the remaining DNA, then phenol extracted and precipitated. Standard procedures (Sambrook et al. 1989) were used for restriction enzyme digestions and agarose gel electrophoresis.

Southern and Northern hybridizations

Restriction-digested mtDNAs were transferred to Hybond-N filters (Amersham, UK) by standard procedures (Sambrook et al. 1989). DNA fragments used as probes were purified from gel-slices by electroelution and labeled by random hexamer priming. Heterologous hybridizations, using *M. polymorpha* mitochondrial *orf86a* as a probe against *Bam*HI-digested mtDNA from different angiosperm species, were performed under low stringency conditions (Siqueira et al. 2001). Homologous hybridizations and washes were performed under stringent conditions (Sambrook et al. 1989). Isolated mtRNA (5.0 µg/lane) was treated and then the samples were fractionated on 1.2% agarose gel and blotted onto Hybond-N. Probe labeling, washes and the full procedures are described by Siqueira et al. (2001).

cDNA synthesis and PCR amplification (RT-PCR)

cDNAs were synthesized using reverse DNaseI-treated mtRNA as templates, as described by Dias et al. 2000; Siqueira et al. 2001). The amplified products were purified by agarose gel electrophoresis before cloning. A control for the cDNA synthesis was performed by replacing reverse transcriptase with water in the reaction mixtures and verifying through PCR that there was no amplification product from that template.

cDNA cloning and sequencing

Standard procedures were used in the preparation, isolation and analysis of recombinant clones of *Escherichia coli* (Sambrook et al. 1989). DNA restriction fragments of interest were cloned into pBluescript vectors (Stratagene, USA) and the cDNAs were cloned into the pGEM-T vector system I (Promega, USA). Nucleotide sequencing was performed by the dye terminator-cycle method on an ABI Prism 310 sequencer (Applied Biosystems, USA). Both strands of the cDNA and genomic clones were sequenced. The sequence data were analyzed using the Lasergene system (DNA Star, USA).

PCR amplification of *orf86a* from *M. polymorpha* mtDNA

Aliquots of 10–20 ng of *M. polymorpha* total DNA were mixed with 2.5 mM $MgCl_2$ on a buffer, supplied by the manufacturer, containing 0.1 mM of each dNTP, 10 pmol of appropriate primers and 2.5 units of *Taq* DNA polymerase (Gibco BRL) in a final reaction volume of 100 µl. The amplification conditions were as described by Siqueira et al. (2001). The products of amplification were cloned into the pGEM-T vector and sequenced to confirm their identity before using them as probes in heterologous hybridizations.

Oligonucleotides

For PCR amplification of the *sdh4* gene from total DNA of *M. polymorpha*, the primers used were: marpo 86a 5′: 5′GAA ACC TTA GGG CAT TGG CTT CTT C3′ and marpo 86a 3′: 5′GAC TAT AAG GAG AGG GGA GAG ACG3′. For cDNA synthesis and RT-PCR amplification, the primers were: 1: 5′TCT ATC TAT TGG TGG GGA GGT3′, 2: 5′AAT ACC CCT ATT CTC CCT TCA T3′, 3: 5′ATG ATT GAA TCT CAG AGG CAT3′, 4: 5′AAG CCC GGT TCT CTT TGT CTT3′, 5: 5′CTA TTT GTC TCT ATC TAT TGG TG3′, 6: 5′AAT AGT GGA GGG TGC TTG ATA ATA3′, 7: 5′AGC TTT CCC ACT CCC TTT G3′, 8: 5′CGA GAG ACA AGA AAA CAT C3′, 9: 5′TAT GAG TTC GAT CCA TTA GGT TC3′, 10: 5′CAT CTG ACC AAG GAG CAT3′ and 11: 5′ACT GGC ATT TTG TAG ACG3′.

Results and discussion

Organization of the *sdh4* locus in potato mtDNA

In a previous study of transcribed sequences in the angiosperm mitochondrial genome homologous to *M. polymorpha* mitochondrial sequences, the *sdh4* locus

in a 1.8-kb *Pvu*II potato mtDNA fragment (Siqueira et al. 2001) was identified. The *sdh4* gene was identified using the *M. polymorpha* mitochondrial *sdh4* gene as a probe in heterologous hybridizations against a filter containing angiosperm mtDNA (cauliflower, *Coix*, maize, pea, potato, soybean). The results showed a very strong hybridization signal in potato and cauliflower mtDNA and weaker intensities of the observed bands in the other species (data not shown). Cloning and sequencing of the 1.8-kb *Pvu*II *sdh4* homologous potato mtDNA fragment indicated a 363-bp fragment showing 98% similarity with the *sdh4* sequence from *A. thaliana* (*orf95*; Giegé et al. 1998) and the presence of the *cox3* gene sequence immediately upstream from the *sdh4* gene. Similarly, in *Arabidopsis* and *Oenothera*, the *sdh4* sequence in potato overlaps 3′ of the end of the *cox3* gene and shows two methionine codons, which could be used to initiate translation. The second ATG is located 23 codons downstream from the initial ATG and shares the TG nucleotides with the TGA stop codon of the *cox3* gene. Since the sequence of the *cox3* gene was interrupted by one of the terminal *Pvu*II sites of the fragment, two *cox3* angiosperm consensus sequence-based primers (primers 3, 6) were used to amplify the missing portion of the *cox3* gene from potato mtDNA. The amplification product was cloned, sequenced and its sequence was added to the 5′ terminus of the 1.8-kb *Pvu*II fragment. Thirty-three base pairs downstream from the stop codon of the *sdh4* gene, 25 codons similar to the final portion of the *cox2* gene were found (GenBank accession number AF280607).

Conservation of the *cox3*/*sdh4* gene locus organization in different angiosperm species

The heterologous hybridization experiments performed using gene *sdh4* from *M. polymorpha* mtDNA as a probe, identified a homologous sequence in the mitochondrial genome of cauliflower, pea, potato and soybean (dicot species), but not in *Coix* and maize (monocot species; data not shown). In order to verify the organization of the *cox3*/*sdh4* locus found in potato, heterologous hybridizations were performed, employing the wheat *cox3* gene and potato *sdh4* gene (not including the *cox3*-overlapping region of the *sdh4* gene) as probes.

Results showed that the *M. polymorpha sdh4* and potato *sdh4* probes identified bands with identical length in dicot species, suggesting that they are the same fragments. The weak intensity signal obtained in pea and soybean hybridization employing a potato *sdh4* probe might be explained by a lesser similarity to the potato *sdh4* sequence. Effectively, the homologous soybean sequence present in the sequence database showed only the initial 58 nucleotides homologous to the potato *sdh4* gene, indicating the lack of most of this gene. *Coix* and maize mtDNA showed no homologous fragment for either probe. In this way, cauliflower, pea and soybean mitochondrial genomes have conserved the *sdh4* sequence or, at least, a part of this sequence. Hybridizations with the wheat *cox3* gene as a probe revealed the same initially identified fragments by *cox3* and/or *sdh4* probes in the cauliflower, pea and soybean genomes (data not shown).

The *cox3* gene sequences from other plants published or found in the databases were evaluated in order to identify whether the *sdh4* gene was conserved downstream of this gene. The comparison indicated that there are sequences homologous to *sdh4* gene downstream of the *cox3* gene in most of those species. Even though the similarity to the mitochondrial *sdh4* sequence among different species remained high (96–98%), the functionality and length of this gene sequence conserved in the mitochondrial genome during evolution was different for each species. Hence, if the *sdh4* gene present in potato mitochondrial genome is considered a model for comparison with the others, the conservation of the *sdh4* gene might be classified in three categories.

1. Conserved coding sequence: in *Oenothera* (Giegé et al. 1998) and potato, the *sdh4* sequence has apparently been conserved in almost its totality, showing at least 100 amino acids homologous among themselves.
2. Coding sequence interrupted by stop codons: *A. thaliana* (Giegé et al. 1998), sugar beet (Kubo et al. 2000) and sunflower (*Helianthus annuus*, Quagliariello et al. 1990) have conserved the *sdh4* sequence (sequences showing 95% similarity with potato *sdh4* gene), but mutations in the coding sequence produce stop codons interrupting the ORF in these species (Fig. 1).
3. Coding sequence interrupted by deletions: in broad bean (*Vicia faba*, Macfarlane et al. 1990), soybean (Grabau and Gengenbach 1989) and monocots, such as *Aegilops columnaris* (U46766), maize (McCarty et al. 1988), rice (*Oriza sativa*, Kaleikau et al. 1990) and wheat (*Triticum aestivum*, Gualberto et al. 1990a), the major portion of the *sdh4* gene sequence has been lost. A small sequence corresponding to approximately to 80 and 58 nucleotides of the *sdh4* gene was conserved in bwad bean and soybean respectively, while sequences containing from 47 to 53 nucleotides were conserved in monocot species.

Hybridizations employing the *sdh4* gene as a probe against total and mitochondrial DNA from maize and sugar cane have indicated that there is probably no copy in the mtDNA of this gene (data not shown). The *Magnolia grandiflora cox3* 3′ region (Perrota et al. 1996) does not extend far enough to allow any conclusion with regards to the presence of a complete *sdh4* gene. The available sequence indicates that its last 50 nucleotides (downstream from the *cox3* gene) are homologous to the *sdh4* gene. This great variation in the *sdh4* gene conservation among different species is unexpected, when compared with the high conservation of other respiratory chain genes. However, the magnitude of variation observed for gene *sdh4* seems to be similar to the variation of ribosomal protein genes in angiosperm mitochondrial

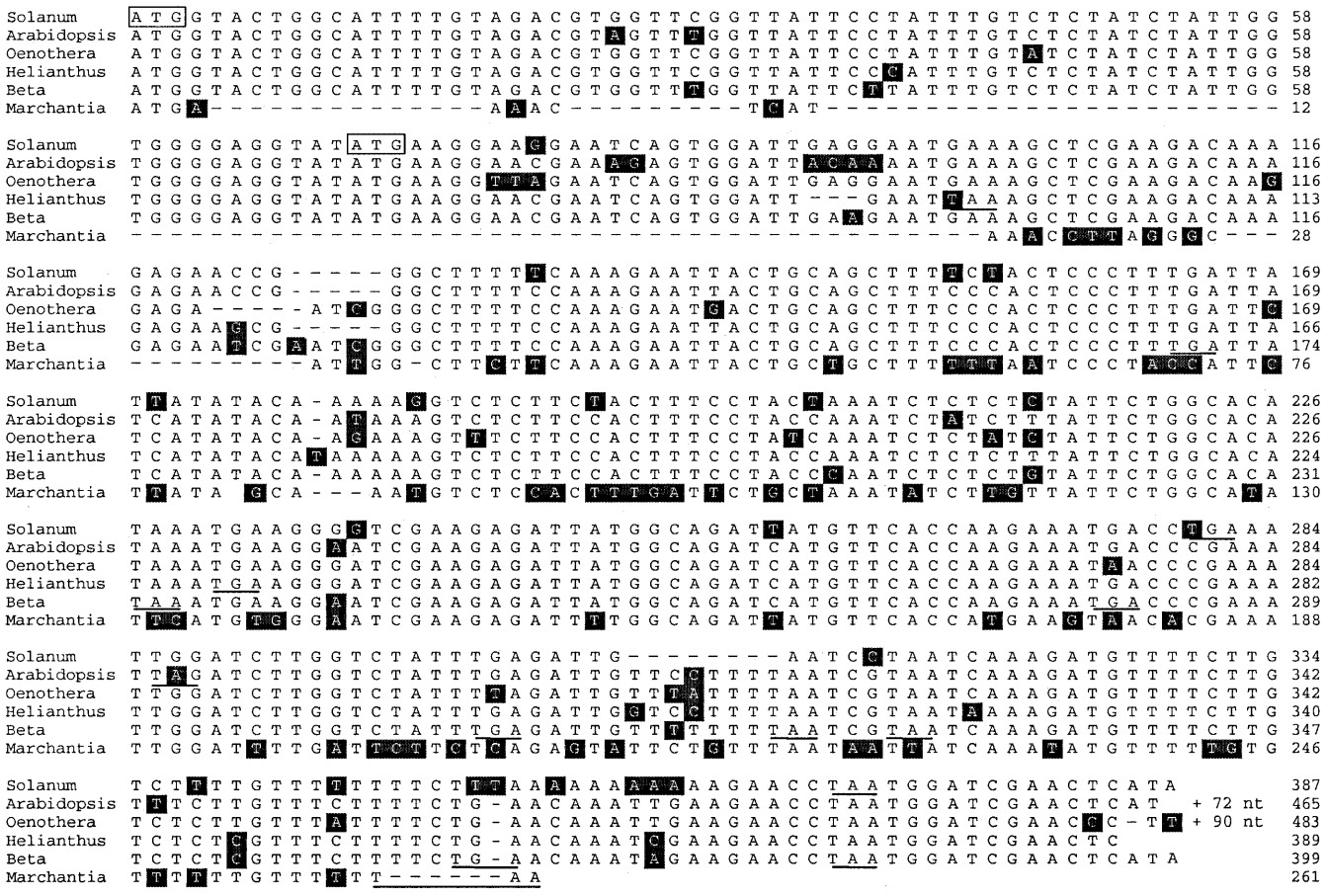

Fig. 1. Nucleotide sequence comparison, showing a high similarity between the *sdh4* gene sequences from potato (cDNA; *Solanum*) and *Arabidopsis thaliana*, *Oenothera* (Giegé et al. 1998), sunflower (*Helianthus*; Quagliariello et al. 1990), sugar beet (*Beta*; Kubo et al. 2000) and *Marchantia polymorpha* (Oda et al. 1992). Stop codons interrupting reading frames are *underlined*. The two putative *sdh4* ATG genes are *boxed*. The *black squares* indicate nucleotides which differ between the sequences

genomes. It has been suggested that factor(s) such as nuclear transfers, functional substitution by another protein or the protein being dispensable in certain plants (Palmer et al. 2000) could explain this ribosomal protein gene variation among different species. Additional analysis must be carried out to unveil this unexpected variation of *sdh4* gene conservation. Nonetheless, considering the importance of the SDH4 protein in the respiratory chain and the *cox2* gene (Nugent and Palmer 1991) as an example of variation for a mitochondrial gene location, the most likely explanation for loss (deletion and/or silencing) of the *sdh4* gene from the mitochondrion is its transfer to the nuclear genome. Palmer et al. (2000) and Adams et al. (2001) suggested that the *sdh4* gene in the angiosperm mitochondrial genome was subjected to several separate losses.

Southern hybridization employing the *sdh4* gene (without the *cox3* overlapping region) as a probe against total DNA from cauliflower and potato indicated that there are no nuclear copies of this gene in either species (data not shown). Thus, the mitochondrial *sdh4* gene must be functional, producing the correct SDH4 protein for the normal activity of the cauliflower and potato mitochondria. Downstream from the partial *cox2* sequence (placed downstream from the *sdh4* gene in potato) there are 32 nucleotides which are highly (93–100% similarity) conserved in other mitochondrial genomes. This sequence is found in *A. thaliana* (Unseld et al. 1997), *Citrillus lanatus* (AF288042) and sugar beet (Kubo et al 2000) and seems to be involved in mitochondrial genome rearrangement.

In conclusion, a high conservation of the *cox3*/*sdh4* gene organization was found among the species evaluated. In monocots, the complete *sdh4* gene sequence is no longer present in the mitochondrial genome. Nonetheless, some initial codons of the *sdh4* gene are placed after the *cox3* stop codon, indicating that it was the original *sdh4* mitochondrial genomic position. This result suggests that the *sdh4* gene has been transferred to the nuclear genome in several species, during the plant species evolution. Sequence analysis or hybridization experiments have suggested that the functional *sdh4* gene is probably in the nuclear genome in *A. thaliana* (Giegé et al. 1998), broad bean (Macfarlane et al 1990), soybean (Grabau and Gengenbach 1989), sugar beet (Kubo et al. 2000), sunflower (Quagliariello et al. 1990) and monocot species; but it is not there in cauliflower, *Oenothera* and potato (Giegé et al. 1998). This finding

agrees with the angiosperm *sdh4* conservation analysis of Adams et al. (2001), which indicated several different events when *sdh4* was lost from the mitochondrial genome during the plant species evolution.

Transcription and RNA-editing analysis of the *cox3*/*sdh4* locus

Northern blot hybridization was carried out with total mtRNA from potato and cauliflower, using potato *cox3* and *sdh4* genes as probes (Fig. 2). The *cox3* probe revealed multiple transcripts in both species, ranging over 1.1–4.4 kb. Among these, the potato 4.4 kb transcript and the cauliflower 2.4 kb and 2.0 kb transcripts were also identified by the *sdh4* probe, strongly suggesting co-transcription of these two genes (Fig. 2).

To characterize the extent and sites of RNA editing in *cox3*/*sdh4* transcripts, cDNA covering the coding region was obtained by RT-PCR using a primer set, as indicated in Fig. 3. The 43 cDNAs revealed 14 C-to-U RNA editing events in *cox3* and 13 in the *sdh4* coding region. These 27 C-to-U editing sites resulted in 11 and 7 codon modifications in the *cox3* and *sdh4* genes, respectively, corresponding to 4.2% of the COX3 and 5.7% of the SDH4 amino acid sequences. Considering the length of both gene sequences, it is worth noting that *sdh4* has twice as many edited sites as *cox3*. However, 50% of the editing events in the *sdh4* gene are silent mutations. A comparison of the *cox3* editing positions in *Solanum* and other angiosperms (Table 1) showed that ten sites have already been found in one of the four species analyzed. Thus, it appears that these positions have a common phylogenetic origin. Nine out of the ten common potato editing sites are found in *Magnolia* (Perrota et al. 1996) and wheat (Gualberto et al. 1990b), six in *A. thaliana* (Giegé and Brennicke 1999) and four in *Olea europaea* (Perrota et al. 1997). The fact that, comparatively, there are more editing sites in common between potato and wheat (Gualberto et al. 1990b), which is a monocot species, than among the other three dicot species, is intriguing. Editing at sequence positions 231, 267, 494 and 503 has been found only in potato. Two of the four species-specific editing sites (sequence positions 231, 267) are silent. The first silent editing site present in *Solanum* is only found in *Magnolia* (Perrota et al. 1996). The silent editing sites in *Arabidopsis* (Giegé and Brennicke 1999), *Olea* (Perrota et al. 1997) and wheat (Gualberto et al. 1990b) are also species-specific, thus illustrating that editing at silent sites is variable between different plant species. The same situation is observed for the *sdh4* silent editing sites in *Solanum* and *A. thaliana* (Giegé et al. 1998; Table 2).

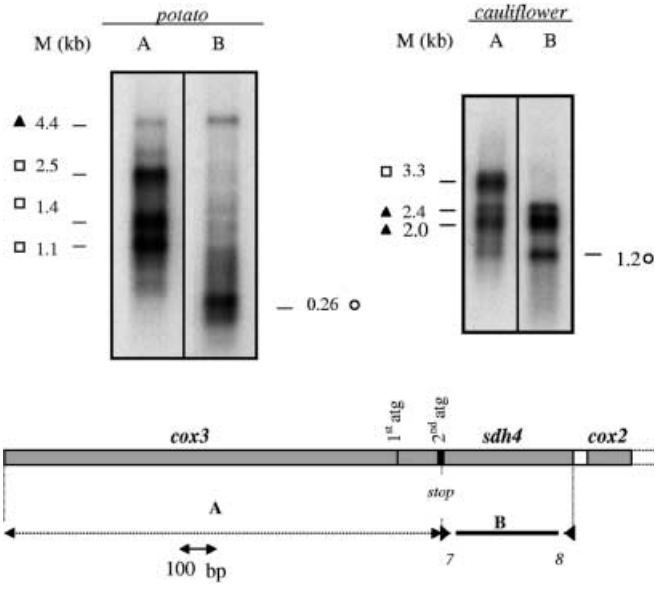

Fig. 2. RNA hybridizations of the *cox3* and *sdh4* transcripts in potato and cauliflower. Total mtRNAs (5 μg) were denatured and applied to each lane of a 1.2% agarose/formaldehyde gel, transferred to a Nylon membrane and hybridized with 32P-labeled fragments. The probes used were *cox3* (*A*) and *sdh4* (*B*); and both are indicated by *horizontal bars* in the *lower figure*. *M* An RNA ladder (9.5–0.24 kb) was used to estimate molecular size. *Solid triangles* indicate transcripts identified by both probes, *empty squares* identify *cox3* transcripts and *empty circles* indicate specific *sdh4* transcripts

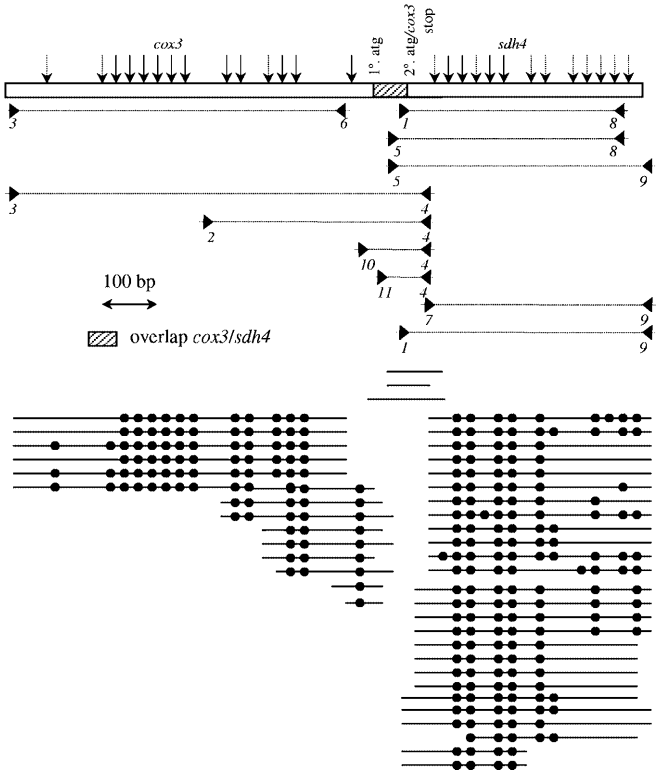

Fig. 3. *cox3*- and *sdh4*-editing sites found in 43 sequenced potato cDNA clones. The cDNA amplification strategy is indicated by *arrows* and *dotted lines* below the *horizontal bar*. *Numbers below the arrows* identify primer numbers. The *dotted arrows* above the *horizontal bar* indicate the partial-editing sites and the *continuous arrows* represent non-partial-editing sites. *Horizontal bars with solid circles* indicate sequenced cDNA clones with edited sites

Table 1. Number, sequence position and codon affected by RNA-editing in the *cox3* gene of potato, *Arabidopsis thaliana* (Giegé and Brennicke 1999), *Marchantia grandiflora* (Perrota et al. 1996), *Oenothera europaea* (Perrota et al. 1997) and wheat (Gualberto et al. 1990b). Codons *in bold* represent partial-editing sites. – Editing site absent, *C* editing site (C-to-U editing), *NS* number of non-silent editing sites, *S* number of silent editing sites, *sil* silent editing site, *Total* total number of editing sites by species

Potato			*A. thaliana*			*M. grandiflora*			*O. europaea*			Wheat		
Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid
CT*C*	69	L→sil	–	–	–	CT*C*	69	L→sil	–	–	–	–	–	–
–	–	–	*C*CA	112	P→S	*C*CA	112	P→S	–	–	–	–	–	–
GT*C*	231	V→sil	–	–	–	–	–	–	–	–	–	–	–	–
C*C*T	245	P→L	C*C*T	245	P→L	C*C*T	245	P→L	C*C*T	245	P→L	C*C*T	245	P→L
T*C*T	257	S→F	T*C*T	257	S→F	T*C*T	257	S→F	–	–	–	T*C*T	257	S→F
–	–	–	–	–	–	C*C*G	263	P→L	–	–	–	–	–	–
TT*C*	267	F→sil	–	–	–	–	–	–	–	–	–	–	–	–
*C*TT	289	L→F	–	–	–	*C*TT	289	L→F	–	–	–	*C*TT	289	L→F
–	–	–	–	–	–	*C*TT	298	L→F	–	–	–	–	–	–
–	–	–	–	–	–	*C*GG	304	R→W	*C*GG	304	R→W	–	–	–
T*C*T	311	S→F	T*C*T	311	S→F	T*C*T	311	S→F	T*C*T	311	S→F	T*C*T	311	S→F
T*C*T	314	S→F	T*C*T	314	S→F	T*C*T	314	S→F	T*C*T	314	S→F	T*C*T	314	S→F
–	–	–	–	–	–	*C*GG	388	R→W	–	–	–	–	–	–
C*C*T	413	P→L	C*C*T	413	P→L	C*C*T	413	P→L	–	–	–	C*C*T	413	P→L
–	–	–	–	–	–	C*C*C	419	P→L	C*C*C	419	P→L	–	–	–
C*C*T	422	P→L	C*C*T	422	P→L	C*C*T	422	P→L	–	–	–	C*C*T	422	P→L
GCT	494	A→V	–	–	–	–	–	–	–	–	–	–	–	–
G*C*T	503	A→V	–	–	–	–	–	–	–	–	–	–	–	–
T*C*A	512	S→L	–	–	–	T*C*A	512	S→L	–	–	–	T*C*A	512	S→L
–	–	–	–	–	–	T*C*C	527	S→F	–	–	–	T*C*C	527	S→L
–	–	–	–	–	–	–	–	–	–	–	–	AC*T*[a]	531	T→sil
T*C*C	566	S→F	–	–	–	T*C*C	566	S→F	T*CC*	566/567	S→F/–	T*C*C	566	S→F
–	–	–	–	–	–	T*C*C	602	S→F	–	–	–	–	–	–
–	–	–	TT*C*	603	F→sil	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	T*C*A	605	S→L	–	–	–	–	–	–
–	–	–	–	–	–	T*C*G	653	S→L	T*C*G	653	S→L	–	–	–
–	–	–	–	–	–	*C*GG	754	R→W	*C*GG	754	R→W	*C*GG	754	R→W
–	–	–	–	–	–	C*C*A	764	P→L	C*C*A	764	P→L	C*C*A	764	P→L
Total = 14, S = 3, NS = 11			Total = 8, S = 1, NS = 7			Total = 22, S = 1, NS = 21			Total = 10, S = 1, NS = 9			Total = 13, S = 1, NS = 12		

[a] U-to-C editing

Editing in the potato *sdh4* gene is extensive, involving 13 C-to-U nucleotide transitions. Seven editing events alter the significance of the codon; and three of these events also occur in *A. thaliana* (Giegé et al. 1998). Six out of the seven codon alterations improve the similarity between the potato and *Marchantia* (Oda et al. 1992) SDH4 protein. The editing site at sequence position 280 (Fig. 1), which originates a stop codon, is noteworthy. This editing occurs two amino acids before the stop codon present in the *A. thaliana sdh4* mitochondrial sequence (Giegé et al. 1998), although it does not occur in tomato (Adams et al. 2001), the phylogenetically nearest species to potato. There are five other editing sites identified after the edited stop codon in potato. The silent editing events correspond to almost 50% of the editing in the *Solanum sdh4* gene. This fact indicates that silent editing is remarkably important in the editing process of this gene. Seven editing sites present in the potato *sdh4* gene are common with sites found in *A. thaliana* (Giegé et al. 1998), *Podophyllum* (Adams et al. 2001) and tomato (Table 2). As expected, all editing sites existing in the tomato *sdh4* gene (Adams et al. 2001) are present in potato; and *Euphorbia* (Adams et al. 2001) does not share any *sdh4*-editing site with potato (Table 2).

Partial editing in potato *cox3*/*sdh4* gene cluster

Sequence analysis of 43 cDNA clones revealed partially edited clones for both genes. The number of partially edited sites and the extent of editing differed between the *cox3* and *sdh4* genes. Three and eight partially edited sites, respectively, were found in *cox3* and *sdh4* genes; and, in both genes, 50% of them are silent. This finding suggests that *cox3*-editing sites are edited before *sdh4*-editing sites. Considering the number and importance of these partial-editing events in the alteration of the coding sequence, one may suggest that they might represent a transition state of either losing or gaining new editing sites.

Considering the eight partial-editing sites in *sdh4*, four are silent alterations, three change the codon specificity and one originates a stop codon. It is important to note that, in the gene sequence after the editing site originating at the stop codon (sequence position 211, Table 2), all preceding edits are partially and differentially processed at a low frequency. Thus, the editing pattern for this gene portion is totally different from the 5′ gene region, suggesting that, in the *sdh4* gene, there is a polarity in the editing process

Table 2. Number, sequence position and codon affected by RNA-editing in the *sdh4* gene of potato, *A. thaliana* (Giegé et al. 1998), tomato (Adams et al. 2001), *Podophyllum* (Adams et al. 2001) and *Euphorbia* (Adams et al. 2001). Codons *in bold* represent partial editing sites. – Editing site absent, *C* editing site (C-to-U editing), *NS* number of non-silent editing sites, *S* number of silent editing sites, *sil* silent editing site, *Total* total number of editing sites by species

Potato			*A. thaliana*			Tomato			*Podophyllum*			*Euphorbia*		
Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid	Affected codon	Base number	Changed amino acid
–	–	–	–	–	–	–	–	–	AT*C*	15	I→sil	–	–	–
–	–	–	A*C*C	53	T→I	–	–	–	–	–	–	–	–	–
TT*C*	63	F→sil	–	–	–	–	–	–	–	–	–	–	–	–
TT*C*	84	F→sil	–	–	–	TT*C*	84	F→sil	TT*C*	84	F→sil	–	–	–
–	–	–	–	–	–	–	–	–	*CC*A	85/86	P→S/ S→L	–	–	–
C*C*A	86	P→L	C*C*A	86	P→L	C*C*A	86	P→L	–	–	–	–	–	–
AT*C*	102	I→sil	–	–	–	–	–	–	AT*C*	102	I→sil	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	A*C*A	104	T→I
TC*C*	123	S→sil	–	–	–	TC*C*	123	S→sil	–	–	–	–	–	–
C*C*A	134	P→L	C*C*A	134	P→L	C*C*A	134	P→L	–	–	–	–	–	–
–	–	–	AT*C*	171	I→sil	–	–	–	–	–	–	–	–	–
***C*AT**	190	H→Y	*C*AT	190	H→Y	*C*AT	190	H→Y	–	–	–	–	–	–
***C*GA**	211	R→[a]	–	–	–	–	–	–	–	–	–	–	–	–
TT*C*	243	F→sil	–	–	–	–	–	–	–	–	–	–	–	–
***C*TT**	244	L→F	–	–	–	–	–	–	–	–	–	–	–	–
***C*TC**	277/279	L→F/sil	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	CT*C*	279	L→sil	–	–	–	–	–	–
T*C*T	284	S→F	–	–	–	T*C*T	284	S→F	–	–	–	–	–	–
Total = 13, S = 6, NS = 7			Total = 5, S = 1, NS = 4			Total = 7, S = 3, NS = 4			Total = 5, S = 3, NS = 2			Total = 1, S = 0, NS = 1		

[a] Stop codon generated after editing

(Fig. 3). The edit resulting in a stop codon was found to be processed in only 30% of the sequenced cDNA clones. Four partial *sdh4* editing sites were found processed only once in 26 sequenced cDNA. In spite of the low processing frequency of these sites, they seem to be real editing sites and not enzyme or sequencing errors, as no other base change was found in the 43 analyzed cDNAs. In order to reduce the possibility of reproducing an eventual reverse transcriptase and/or polymerase error in most clones, the cDNA clones were obtained from different cDNA synthesis reactions and amplifications. Furthermore, the possibility of obtaining four enzyme/sequencing errors in C-to-U transitions for the same gene is extremely low. The causes for this differential editing remain unclear and it is suggested that incompletely edited pre-RNAs reflect intermediates in the editing process (Mulligan et al. 1999). Analyzed as a whole, the *sdh4* gene partial-editing results suggest that there might be translation of incompletely edited mtRNA. This appears to be possible for the *sdh4* gene translation, especially due to the presence of a partially edited stop codon.

Taken together, the editing results for the potato *sdh4* gene and Southern and Northern hybridization results using the *sdh4* sequence as a probe against total and mtDNA and mtRNA from cauliflower and potato suggest that the *sdh4* gene is probably functional in the mitochondria of both these species.

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