3304–3311 Nucleic Acids Research, 1994, Vol. 22, No. 16

Translation of *nad9* mRNAs in mitochondria from *Solanum tuberosum* is restricted to completely edited transcripts

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Received June 22, 1994; Revised and Accepted July 27, 1994 EMBL accession no. X79774

ABSTRACT

The pool of partially and completely edited mRNAs present in plant mitochondria could potentially be translated into a mixture of divergent proteins. This possibility was investigated for the *nad9* gene in potato by characterization of the mRNA population and the corresponding protein sequence. The deduced amino acid sequence of the *nad9* gene product has significant similarity to the nuclear-encoded 30 kDa subunit of the bovine and *Neurospora* NADH:ubiquinone oxidoreductase (complex I) and to the chloroplast *ndhJ* gene product. Immunoprecipitation of a 27 kDa *in-organello* ^{35}S labelled mitochondrial translation product with an antibody directed against the wheat *nad9* gene product demonstrates its functional expression in potato and wheat. Comparison of the *nad9* genomic DNA and cDNA sequences reveals seven codons to be changed by a C to U RNA-editing. Direct sequencing of RT-PCR products derived from cDNAs of different tissues of potato plants shows the presence of a significant portion of only partially edited *nad9* transcripts in the various tissues. Amino acid sequencing of internal peptides of the isolated 27 kDa protein from potato tubers demonstrates homogenous translation products of only completely edited *nad9* mRNAs even in the presence of partially edited mRNAs. This result suggests a pretranslational selection between edited and incompletely edited mRNAs in plant mitochondria.

INTRODUCTION

In higher plants the process of RNA-editing is known to selectively change individual cytidine to uridine ribonucleotides resulting in improved conserved homology of the encoded proteins (for review see 1). This change of individual mitochondrial codons appears to be a sometimes slow process, since individual cDNAs derived from the *cox2* and *nad3* transcripts for example exhibit various patterns of only partially edited mRNAs (2–4).

The translation of pools of partially edited transcripts by plant mitochondrial ribosomes would result in proteins with small differences in their amino acid sequences and could modulate mitochondrial functions. An altered mitochondrially encoded subunit of one of the respiratory chain complexes for example could affect the proton- and/or electron-transferring activity. In addition, specific kinetics of the RNA-editing process in individual plant organs and tissue-specific RNA-editing might have a regulatory role in mitochondrial gene expression.

Direct sequencing of two mitochondrially encoded proteins, the wheat ATP9 and the potato NAD7 polypetides, demonstrated that the amino acids specified by the edited codons of mtRNAs are indeed present in the isolated proteins (5,6). A correlation to partially edited sites in the respective mRNAs however was not possible in these instances. For the ATP9 protein all corresponding cDNA clones contain only the edited sequence. Statistical analysis of the degree of C to U transitions for the RNA-editing sites corresponding to the NH_2-terminally sequenced NAD7 protein found several *nad7* cDNAs to be only partially edited. In this case the presence of possible substoichiometric amounts of amino acid residues specified by the unedited codons could not be excluded due to the limited resolution of conventional NH_2-terminal amino acid sequencing. To investigate the presence of mixed protein products derived from partially edited mRNA populations with higher resolution and to decide whether unedited mRNAs can be translated, one of the protein subunits of complex I (NADH:ubiquinone oxidoreductase) has now been purified in sufficient amounts.

Subunits of complex I, the first electron coupling-site of the mitochondrial respiratory chain, are in almost all eukaryotes encoded by a conserved set of mitochondrial genes named *nad1* to *nad6* and *nad4L*. Higher plants with their large mitochondrial genomes (250–2500 kb) are thought to contain more protein-coding genes than the smaller organellar genomes of fungi and animals (7). Recently, two actively transcribed plant mtDNA regions in wheat and sugarbeet were found to encode open reading frames similar to the fungal and bovine nuclear-encoded 49 kDa and 30 kDa complex I subunits, respectively (8–10). These plant mitochondrial genes have been designated as *nad7* and *nad9*. The NAD7 and NAD9 proteins can be assigned to the group of 'intrinsic' complex I proteins, those 14 subunits which are in *E.coli* encoded by the *nuo* locus (11). It is suggested that only this minimal set of protein subunits assemble to form the prokaryotic enzyme active in proton-translocation and

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electron-transport functions (12). In the chloroplast genome a set of 11 open reading frames (*ndhA* to *ndhK*) have been identified by sequence homologies to code for analogous subunits of a potential enzyme involved in chlororespiration (13).

The composition of the plant mitochondrial complex I has been recently investigated (14–16). The assembly of at least 32 subunits suggests a complexity similar to that of the bovine and *Neurospora* enzymes. Some of the NH_2-terminal amino acid sequences of individual complex I subunits from plants show stretches of significant sequence conservation to known complex I proteins from bovine and/or *Neurospora* (14,15). The potato 27 kDa complex I subunit for example was thus proposed as the plant homologue of the bovine and *Neurospora* 30 kDa complex I proteins (15).

We here report analysis of the mitochondrial *nad9* gene coding for the 27 kDa protein subunit in *Solanum tuberosum* and its expression. A possible tissue-specific variation of the degree of mitochondrial RNA-editing in *nad9* transcripts was investigated in RT-PCR derived *nad9* cDNAs amplified from total RNAs of different potato plant tissues. A significant portion of the seven edited codons appears to be only partially edited in all of the different tissues. However, direct sequencing of internal peptides derived from proteolytic digests of the isolated NAD9 protein revealed exclusively the amino acid residue specified by the edited mRNA codon. This observation suggests that partially edited transcripts are not translated in plant mitochondria.

MATERIALS AND METHODS

Protein purification and analysis of mitochondrial translation products

Purification, NH_2-terminal amino acid sequencing of the potato 27 kDa protein, Western blotting and immunoreactions were performed as described (15). Analysis of *in-organello* ^{35}S labelled mitochondrial translation products and immunoprecipitation with an antibody directed against the wheat NAD9 protein (kind gift of J.-M. Grienenberger, Strasbourg) followed published protocols (17,6).

The 27 kDa protein was digested with Lys-C essentially under conditions described elsewhere (18). Lys-C peptides were separated by reverse-phase HPLC on a Vydac C_{18} column using a gradient of 0.1% TFA (trifluoro-acetic acid) in water vs. 0.1% TFA in acetonitrile solvent at 37°C. The purified peptides were sequenced on an ABI473A sequenator by automatic Edman degradation (15).

Analysis of potato genomic mtDNA and cDNA

Preparation of genomic mtDNA and the screening of a potato mitochondrial genomic library was performed as described (6). The degenerate oligonucleotide mixture 'nad9' (5′-ATGGAY-AAYCARTTYATHTTYAARTAYWSITGG-3′) used in Southern hybridizations was deduced from residues 1 to 11 of the NH_2-terminal amino acid sequence of the 27 kDa protein (15) and ^{32}P labelled by standard procedures.

Preparation of total RNA from potato green leaves, etiolated tuber shoots and tuber mitochondria was done according to ref. 19. RNA extracts were treated with 2 units of DNase I (RNase-free) for 15 min at 25°C and extracted once with phenol/chloroform. First-strand cDNA synthesis of DNase I treated RNA extracts with random hexamers was done using a commercial kit (Gibco BRL) according to the manufacturer's instructions. After RNase H treatment amplifications of genomic DNA and first-strand cDNAs were done with two *nad9* specific primers (nad9-3 5′-GTAAATCACTAGGTAGC-3′ and nad9-6 5′-CTTACGAAGGCAAATAGC-3′) for 30 cycles of 1 min at 94°C, 2 min at 50°C and 2 min at 72°C. In the first cycle denaturation at 94°C was extended to 4 min. Control experiments of amplifications from first-strand cDNAs were routinely done in parallel except that reverse transcriptase was replaced by DNase-free RNase.

Amplified *nad9* fragments were either cloned into pBluescript SK plasmids as described (6) or directly sequenced with an internal sequencing primer (nad9-fin 5′-TCAGTCTATTTCC-ATCAGCC-3′) according to the procedure described in ref. 20.

RESULTS

The 27 kDa subunit of the potato complex I is encoded by the mitochondrial *nad9* gene

The NH_2-terminal amino acid sequence of the 27 kDa complex I subunit from potato mitochondria showed a significant similarity to the N-termini of the nuclear-encoded bovine and *Neurospora* 30 kDa complex I subunit (15). The plant homologue of this subunit was found to be mitochondrially encoded in wheat and sugarbeet by the *nad9* gene (8,9). An oligonucleotide probe deduced from the potato 27 kDa sequence was used for Southern hybridizations to different restriction digests of total genomic and mitochondrial DNA from potato. Strong signals were detected in the digests of mtDNA (Fig. 1) whereas in the total cellular genomic DNA no additional hybridization signals could be detected (not shown), suggesting that a single locus in the mitochondrial genome of *Solanum tuberosum* codes for the 27 kDa protein. The same oligonucleotide probe was then used for

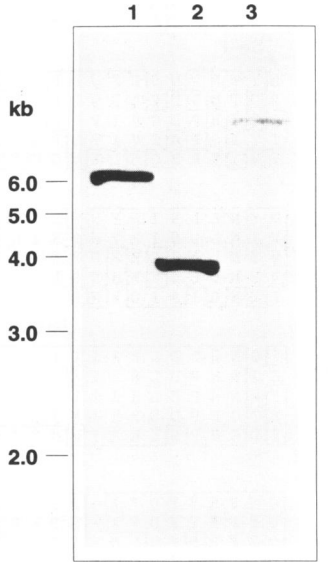

Figure 1. Southern blot analysis of potato genomic mtDNA probed with a degenerate oligonucleotide mixture derived from the NH_2-terminal sequence of the 27 kDa complex I subunit. Genomic mtDNA was digested with restriction enzymes, separated on an 1% agarose gel and hybridized after blotting with the ^{32}P-labelled oligonucleotide probe 'nad9'. Restriction digests in the different lanes are: lane 1, *Eco*RI, lane 2, *Bam*HI and lane 3, *Hin*dIII. Single hybridization signals in the mtDNA digests show *nad9* to be encoded by a unique genomic locus in potato.

screening a potato genomic mitochondrial library. A positive clone was isolated and shown to contain a 3.8 kb *Bam*HI genomic fragment with the complete coding region (Fig. 2). Sequencing of this genomic region identified an open reading frame of 573 base pairs (GenBank accession no. X79774). The deduced protein sequence shows more than 90% identity to the *nad9* open reading frame which has been recently identified in the wheat and sugarbeet mitochondrial genomes (8,9). According to the designation in these plants the potato mitochondrial gene was named *nad9*.

The NH_2-terminal sequence of the 27 kDa polypeptide as determined by direct protein sequencing corresponds to the N-terminus of the deduced NAD9 protein sequence and further confirms that this mitochondrial complex I subunit is encoded by the *nad9* gene. The deduced NAD9 protein contains 190 amino acid residues and has a calculated molecular mass of 22,656 Da. The high content of charged amino acids (31%) accounts for the apparent molecular mass of 27 kDa estimated by SDS-gel electrophoresis. It is noteworthy, that the NH_2-terminus of the isolated 27 kDa protein is accessible to Edman degradation suggesting that the first methionine residue has been post-translationally processed. Otherwise, the NH_2-terminal sequencing of the protein should have been blocked by the formylated initiator-methionine residue (6).

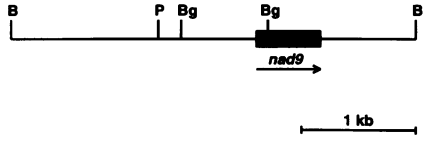

Figure 2. Organization and schematic map of the 3.8 kb mitochondrial genomic *nad9* clone. The coding region is shown as a black box, the direction of the reading frame is indicated by the arrow. Restriction sites are: B = *Bam*HI; Bg = *Bgl*II; P = *Pst*I. The nucleotide sequence of the *nad9* gene has been deposited in the Genbank under accession no. X79774.

Alignment of the potato NAD9 protein sequence to the related sequences of the nuclear-encoded 30 kDa complex I subunits from *Bos taurus* (21) and *Neurospora* (22) shows 51% and 46% sequence identity, respectively (Fig. 3). The deduced sequence of the *Marchantia* mitochondrial *orf212* (23) has 74% identity to the potato mitochondrial NAD9 sequence and identifies this ORF to potentially code for the NAD9 homologue of liverwort. Interestingly, the related bacterial complex I protein as deduced from the *E.coli nuoC* gene (Weidner *et al.*, 1993) shows a lower sequence similarity (34%) in comparison to the sequence identities between the 27 kDa potato mitochondrial protein and the nuclear-encoded bovine and *Neurospora* 30 kDa complex I proteins.

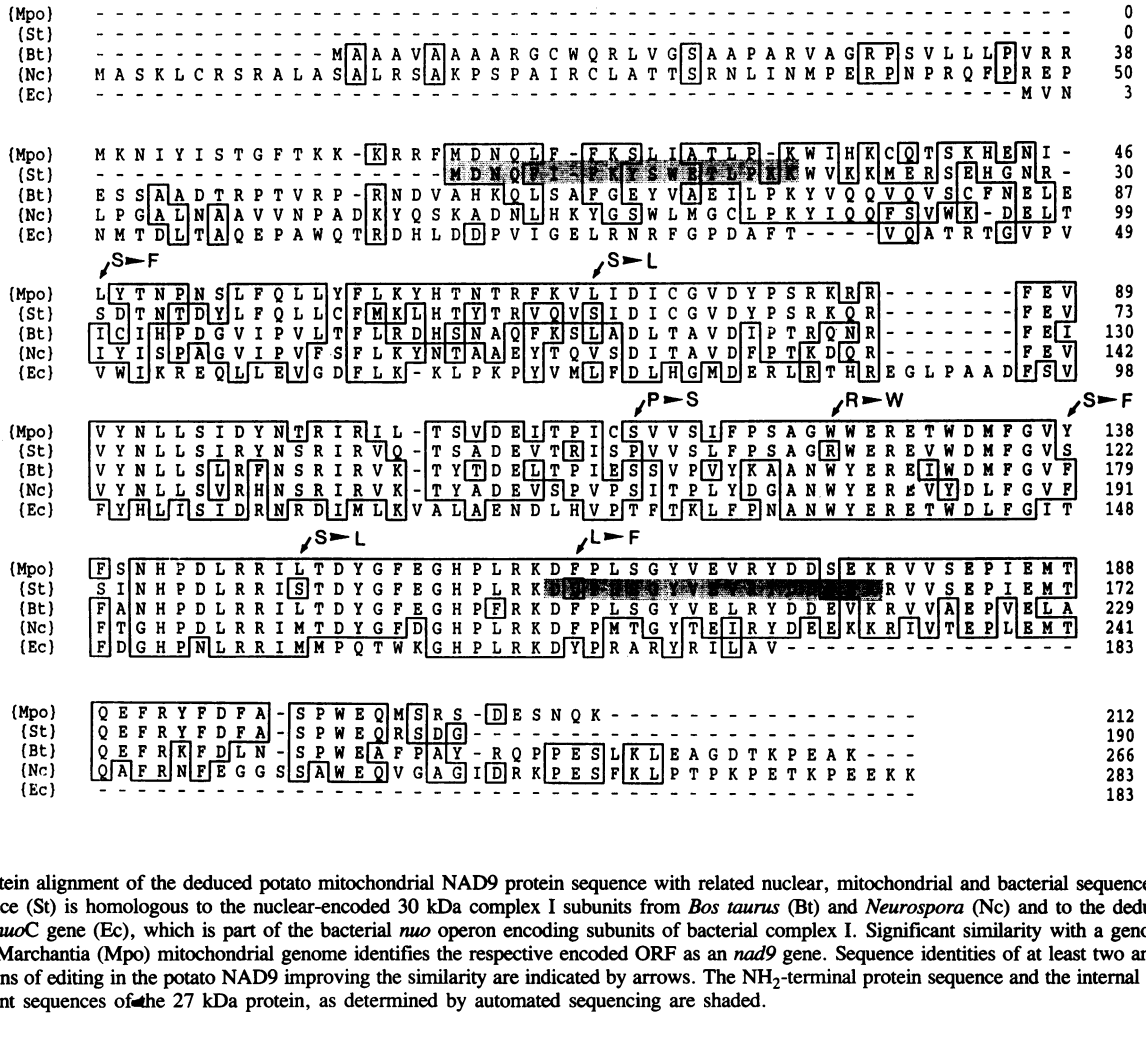

Figure 3. Protein alignment of the deduced potato mitochondrial NAD9 protein sequence with related nuclear, mitochondrial and bacterial sequences. The potato NAD9 sequence (St) is homologous to the nuclear-encoded 30 kDa complex I subunits from *Bos taurus* (Bt) and *Neurospora* (Nc) and to the deduced sequence of the *E.coli nuoC* gene (Ec), which is part of the bacterial *nuo* operon encoding subunits of bacterial complex I. Significant similarity with a genomic sequence region of the Marchantia (Mpo) mitochondrial genome identifies the respective encoded ORF as an *nad9* gene. Sequence identities of at least two amino acids are boxed. Positions of editing in the potato NAD9 improving the similarity are indicated by arrows. The NH_2-terminal protein sequence and the internal Asp_{146}/Lys_{162} Lys-C fragment sequences of the 27 kDa protein, as determined by automated sequencing are shaded.

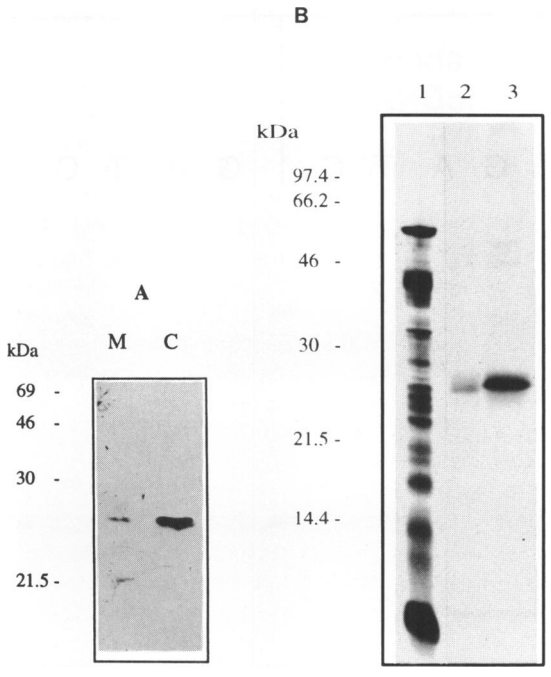

Figure 4. Western blot analysis and immunoprecipitation of the potato 27 kDa protein. **(A)** Total potato mitochondrial membrane proteins (lane M) and a purified complex I preparation (lane C) were fractionated on 15% (w/v) SDS-PAGE. After electrophoresis the proteins were blotted onto nylon membranes and probed with an antibody raised against the expression product of the mitochondrial *nad9* gene of wheat (8). **(B)** Mitochondria extracted from potato tubers and wheat seedlings were used for *in-organello* translation and the radiolabelled proteins were incubated with the *nad9*-antiserum. Immunoprecipitates from wheat (lane 2) and potato (lane 3) were loaded on a 15% SDS-PAGE and autoradiographed after electrophoresis. For comparison, an autoradiograph of total *in-organello* translation products from potato is shown in lane 1.

The mitochondrial *nad9* gene codes for a 27 kDa protein in potato and wheat mitochondria

A polyclonal antibody directed against the wheat NAD9 expression product specifically recognizes a 27 kDa protein in a Western blot of total potato mitochondrial membrane extracts (Fig. 4a). The intensity of the immuno-reacting protein band is markedly enriched in the preparation of the potato mitochondrial complex I confirming this subunit to be part of the NADH-dehydrogenase.

In order to test for functional expression of the investigated potato mitochondrial *nad9* gene, the wheat NAD9 antibody was used for immunoprecipitation experiments. The mitochondrial translation products were labelled with ^{35}S methionine by *in-organello* protein synthesis in mitochondria isolated from potato tubers and then incubated with the antibody for subsequent precipitation of antibody-antigen complexes. As control the analogous experiment was done with wheat ^{35}S labelled *in-organello* translation products. Autoradiographs of SDS-PAGE separated immunoprecipitates of the wheat and potato mitochondrial translation products detect only a single radiolabelled band with an apparent size of 27 kDa (Fig. 4b). This result suggests the mitochondrial *nad9* gene to code for the 27 kDa of the mitochondrial complex I at least in potato and in wheat confirming its functional expression.

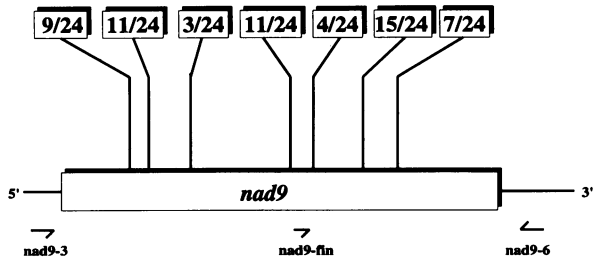

Figure 5. Statistical analysis of the degree of editing at inidividual sites. The nucleotide sequences of 24 RT-PCR derived *nad9* cDNA clones were determined between the two primers (nad9-3 and nad-6) spanning the complete *nad9* coding region. The number of edited versus total nucleotides found at the individual editing sites are given in the boxes. The primer nad9-fin was used for direct sequencing of RT-PCR amplification products.

The *nad9* transcripts are edited

Two oligonucleotide primers flanking the *nad9* coding region were used for PCR with randomly primed first-strand cDNAs of total RNA from potato tuber mitochondria as templates. The expected products of ~790 base pairs were cloned into pBluescript plasmids and sequenced. Comparison of the genomic DNA and PCR derived cDNA sequences showed seven codons to be changed by C to U RNA-editing. All seven editing sites change the affected triplet to code for another amino acid improving the similarity between the plant mitochondrial NAD9 protein and the nuclear-encoded 30 kDa mammalian and fungal proteins (Fig. 3).

A total of 24 *nad9* cDNA clones were sequenced to examine the degree of editing. The majority of the analysed clones were incompletely edited and various patterns of partial editing along the cDNAs were observed (Fig. 5).

Editing of *nad9* transcripts in different potato tissues

To determine whether the reduced level of completely edited *nad9* transcripts in tubers merely reflects the expected slower physiological functions of dormant storage organs like potato tubers, editing levels were compared in other tissues. Total RNA extracts from tuber mitochondria, etiolated shoots and green leaves were then used for randomly primed first-strand cDNA synthesis and subsequenctly amplified with the primer pair nad9-3 and nad9-6 (see Fig. 5). The RT-PCR products obtained from the different potato tissues were then used for direct sequencing reactions. A PCR product amplified from total genomic DNA was sequenced in parallel. The internal sequencing primer used in these reactions allowed the determination of the nucleotides covering the 3′ part of the *nad9* coding region where three RNA-editing sites are located (Fig. 5).

The sequencing gels showed at these sites the presence of both T and C nucleotides in the amplified cDNAs from all three different potato tissues (Fig. 6). The respective T versus C reaction intensities display variations between the three different editing sites. Furthermore, variant degrees of edited versus unedited nucleotides can be detected between potato tubers cDNAs and shoots and leaves cDNAs (Fig. 6). The degrees of partial editing in tuber RT-PCR products as detected here by direct sequencing generally correspond to the results obtained from sequencing of individual *nad9* cDNA clones from the same tissue (Fig. 5) and suggest the presence of uncompletely edited and therefore variant *nad9* transcripts.

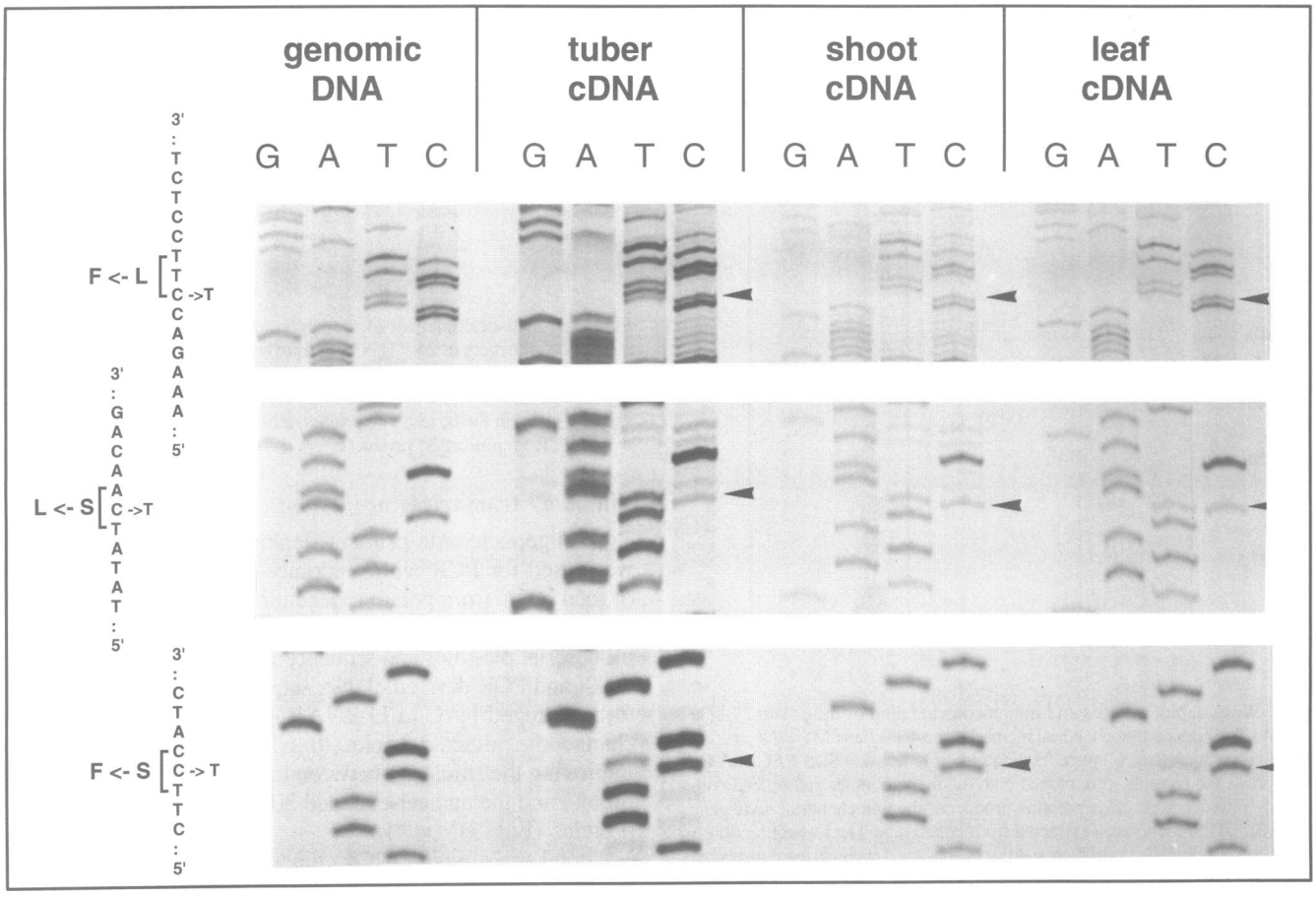

Figure 6. Analysis of different tissues for RNA-editing. Potato genomic mtDNA and DNase I-treated total RNAs isolated from tuber mitochondria, tuber shoots and green leaves were used for randomly primed first-strand cDNA synthesis. For amplification of *nad9* cDNA sequences the two primers nad9-3 and nad9-6 were used. The PCR products obtained after 30 cycles were purified on 1.5% (w/v) agarose gels and subjected to direct sequencing. The internal primer (nad9-fin, see Fig. 5) used in the reactions yields the *nad9* coding sequence covering the last three editing sites. In the left margin the sequence around the individual editing site, the respective edited nucleotide and the resulting amino acid change are indicated. Arrowheads mark editing sites where both, C and T nucleotides could be detected in the autoradiographs of the sequencing gels indicative for the presence of both edited and unedited mRNAs. For comparison, sequencing reactions were performed with amplified tuber genomic DNA. Small relative differences in the degrees of editing are apparent at the individual editing positions between the different tissues (see text).

The NAD9 protein contains only amino acids specified by edited *nad9* transcripts

The presence of a significant portion of partially edited *nad9* transcripts raises the question whether the pool of slightly variant mRNAs is translated into polypeptides having small differences in their amino acid sequences. In order to unequivocally test the presence of different NAD9 polypeptides, direct amino acid sequencing of relevant peptides of the 27 kDa complex I subunit was performed. The 27 kDa protein was first digested with endoproteinase Lys-C and the fragments obtained were separated by reverse-phase HPLC (Fig. 7). Several of the proteolytic peptides containing amino acids specified by either the un-edited or edited codons were found to be not suitable for N-terminal sequence analysis. In these peptides the corresponding un-edited triplet codes for a serine, an amino acid residue showing only weak signals in the HPLC profiles of the PTH-amino acids and which thus makes the detection of small substoichiometric amounts difficult. In addition, interpretation problems can arise by the progressively higher backgrounds resulting from incomplete Edman degradation in succeeding N-terminal sequencing cycles. To unequivocally determine whether variant NAD9 polypetides exist, the peptides spanning the region from Asp_{146} to Lys_{162} (see Fig. 3) were selected for direct protein sequencing. Within the corresponding coding region only about one third of the *nad9* cDNAs amplified from tuber RNAs showed a C to U transition at editing position seven (Fig. 5 and Fig. 6). The Asp_{146}/Lys_{162} peptide is expected to contain a phenylalanine encoded by the edited *nad9* mRNAs or a leucine encoded by the unedited mRNAs. In the HPLC profiles obtained from the sequencing steps of this peptide a clear peak for exclusively phenylalanine could be detected (Fig. 8). NAD9 peptides having a leucine residue at this position should be identifiable by a small, but nevertheless clearly detectable peak for this amino acid. Peaks for the unedited and edited peptides could possibly be separated by a mobility shift in the reverse-phase HPLC introduced by the small difference in the hydrophobicity of the peptide containing a leucine residue. However, sequencing of all surrounding peaks showed to contain either no peptide moiety or to represent Lys-C peptides of another NAD9 protein region (data not shown).

DISCUSSION

The plant mitochondrially encoded NAD9 protein is a subunit of the peripheral part of complex I

Including the *nad9* gene a total of nine subunits of the NADH-dehydrogenase (complex I) has by now been shown to be expressed by the mitochondrial genetic system of higher plants. From biochemical and structural studies on the plant mitochondrial complex I at least 32 subunits have been suggested to assemble into the functional enzyme (14–16). Most of the subunits of complex I are suggested to be accessory proteins, since the 'minimal' bacterial and probably evolutionary progenitor 'core' enzymes in *E.coli*, *Rhodobacter* and *Paracoccus denitrificans* comprise only 14 subunits (11, 24, 25).

It is still open how many of the genes coding for subunits of complex I are located within the plant mitochondrial DNA or have already been transferred to the nucleus during evolution of plants. The bovine and *Neurospora* 30 kDa complex I subunits which are the homologues of the plant NAD9 protein are nuclear-encoded in these organisms. During evolution of mammals and fungi the corresponding *nad9* gene has most likely been transferred to the nucleus whereas in plants this gene has been retained in the organellar genome. The low sequence similarity found between the mitochondrial sequence and the sequence deduced from the homologous *E.coli* gene confirms that the former endosymbionts originated not from this phylogenetic branch of bacteria but rather from the α-purple subdivision (26).

The bovine 30 kDa subunit has been localised in the peripheral part of complex I (13). Studies on the *Neurospora* enzyme have shown that the peripheral part in this fungus is assembled exclusively by nuclear-encoded proteins independently from the membrane part (27). The homologous plant protein NAD9, and as recently shown also the NAD7 protein (6,10), are the first examples for mitochondrially encoded components of complex I in plants which are not located in the hydrophobic membrane part. Therefore, in plants the peripheral part of complex I is assembled by both nuclear-encoded subunits and at least the two mitochondrially encoded subunits NAD7 and NAD9. Whether this has further implications on a plant-specific assembly pathway of complex I and whether its functional activities are influenced by the obviously differently coordinated expression of nuclear and mitochondrial genes remains to be investigated.

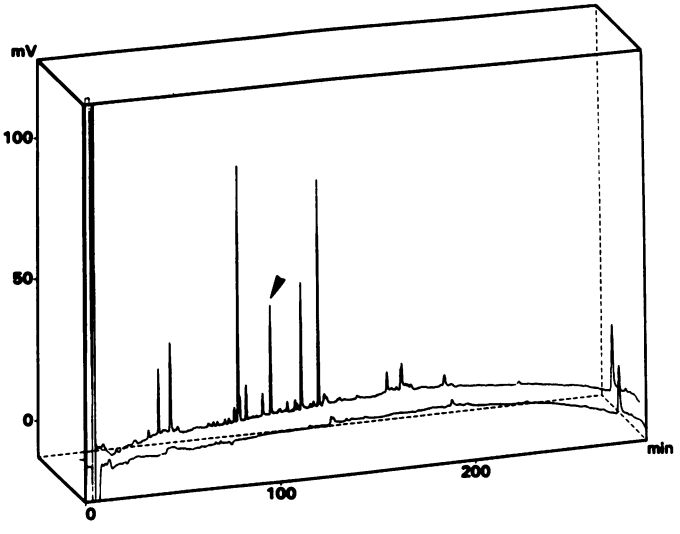

Figure 7. HPLC-profile of the 27 kDa complex I subunit from potato mitochondria after enzymatic fragmentation. The 27 kDa protein band was excised from a preparative complex I protein blot and incubated with endoproteinase Lys-C. The generated peptides were separated by reverse-phase HPLC and elution of the peptides was monitored at 220 nm. The line in front represents the background baseline. The Asp_{146}/Lys_{162} fragment is indicated by the arrowhead.

RNA-editing of the potato *nad9* mRNAs in different tissues

In the different plant tissues altered requirements for mitochondrial respiratory activities could be modulated by the

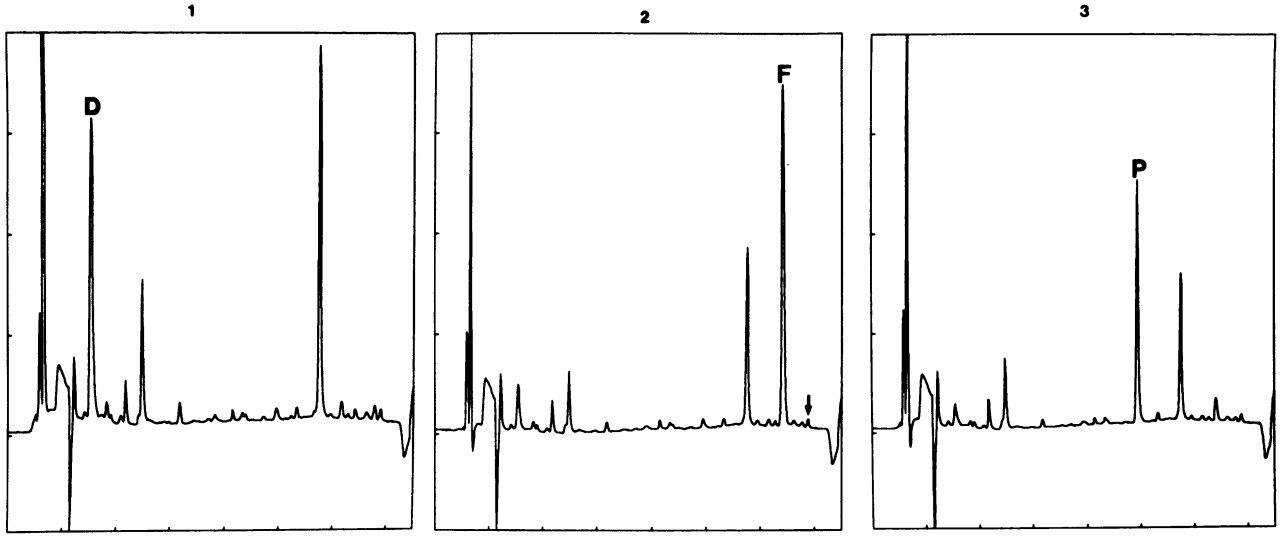

Figure 8. Determination of the amino acid sequence of the Asp_{146}/Lys_{162} fragment of the 27 kDa protein. The HPLC elution profiles of the phenylthiohydantoin (PTH) amino acids of the first three cycles are shown. The horizontal and vertical axes indicate the time and absorbance of the elution profiles, respectively. Identified PTH-amino acid residues in the individual sequencing cycles are indicated in the one-letter code. In the second cycle only a peak of phenylalanine can be detected. The arrow indicates the position where leucine elutes in the amino acid standard profile.

expression of the mitochondrial *nad* genes, possibly post-transcriptionally at the level of RNA-editing. In trypanosomes a developmental regulation of expression has been observed for the degree of editing of the mitochondrially encoded ND7 and ND8 transcripts in the two life-cycle forms (28, 29). Editing occurs preferentially in the bloodstream form.

We have investigated the tissue-specific pattern of differential editing in photosynthetic, germinating or storage potato tissues. Considerable amounts of partially edited *nad9* transcripts are present in leaves, shoots and tubers. The small tissue-specific variation at individual editing sites observed between cDNAs from potato leaves and tubers might have slight indirect and post-transcriptional effects on the expression of the *nad9* gene. Slower rates of RNA-editing could have the consequences that these mitochondrial transcripts are more affected by endo- or exonucleolytic degradation so that the translation rates of some genes are reduced. Mitochondrial respiratory activities providing energy are essential in non-photosynthetic tissues. It would be interesting to see whether the rates of RNA-editing are light-regulated and differ between tissues exposed to darkness or to illumination. A study on the pattern of chloroplast RNA-editing in different tissues and organs of spinach showed, however, that in etiolated and illuminated leaves no differences exist in the editing patterns of the photosynthetic genes *pbsF* and *pbsL* (30).

Partially edited *nad9* mRNAs are not realized into polymorphic proteins

The mRNA populations of several plant mitochondrial genes are found to be a mixed pool of completely and partially edited transcripts (2–4, 6). Translation of completely as well as partially edited transcripts would affect the predicted amino acid sequences of plant mitochondrial proteins and would result in a mixture of slightly divergent proteins. The utilization of different mRNAs in translation would allow divergent polypeptides to be generated from a single gene analogous to gene families in eukaryotic nuclear genomes. To investigate this possibility we determined the amino acid sequence of a part of the potato mitochondrially encoded NAD9 protein where a significant portion of *nad9* mRNAs is not edited. The high resolution sequence analysis of the internal Lys-C peptides of the NAD9 protein shows that only polypeptides derived from completely edited transcripts are present in the plant mitochondrial complex I.

This result raises the question whether partially edited transcripts are excluded from translation. If translation of low amounts of partially edited *nad9* transcripts is allowed, these products might not be detectable due to their instability resulting from the lack of integration into the functional enzyme. Evidence against this possibilty is given by transgenic experiments with tobacco plants where the protein products derived from unedited *atp9* sequences appear to be stable in plant mitochondria (31). The presence of the 'unedited', abnormal ATP9 protein, however, is phenotypically associated with mitochondrial dysfunctions and induction of male-sterility. Effects of differential editing have been also reported for a CMS phenotype in rice, where the mRNAs of an altered mitochondrial *atp6* gene are not edited like the normal *atp6* transcripts and the production of an aberrant ATP6 protein causes sterility (32). Our results show that NAD9 proteins derived from partially edited transcripts are not present in the functional mitochondrial complex I. Even in light of these observations, it will be rather difficult to rule out the possibility that variant NAD9 polypeptides not integrated into the enzyme are post-translationally degraded by rapid proteolysis.

Circumstantial evidence clearly supports the view that selection occurs at the mRNA level and determines the accessibility of only completely edited mRNAs for translation. In the polysomal RNA fraction only a minor population of partially edited transcripts have been found (4). From the results presented in this report, which show that no protein moiety corresponding to partially edited transcripts is present, we propose that translation and RNA-editing are partitioned in plant mitochondria. A specific and mechanistically unknown selection process allows only edited mRNAs to enter translation. It has been postulated that unprocessed transcripts are captured in the 'editosomal' complex and are not released for protein synthesis before the mRNAs are completely edited (4). In yeast it has been shown that activation factors are required for translation of several mitochondrial transcripts (33). Although such factors still await to be characterized in higher plant mitochondria, they could possibly play a role in the selection process determining only completely edited mRNAs for translation.

ACKNOWLEDGEMENTS

We greatfully acknowledge the support of Dr C.J.Leaver and the expert assistance of A. Liddell in performing the immunoprecipitations. We are grateful to L.Gäbler for his help in cloning the *nad9* gene and to Tse-I Lin for the isolation of potato complex I. We wish to thank Dr J.-M.Grienenberger, Strasbourg, for the kind gift of NAD9 antibodies. This study was supported by grants from the Deutsche Forschungsgemeinschaft, from the Bundesministerium für Forschung und Technologie and the Anglo-German Foundation of the Deutscher Akademischer Austauschdienst and the British Council.

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