Update section

Sequence

Isolation and nucleotide sequence of the potato mitochondrial gene for apocytochrome b

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The mitochondrial genome of *Solanum tuberosum* has not been studied in detail. Some physicochemical studies have been carried out [16] but to our knowledge no mitochondrial genes have been previously isolated. We have characterized the region of the potato mitochondrial (mt) genome containing the apocytochrome b gene (*cob*) and in this communication we report the gene sequence.

Mitochondrial DNA was isolated from potato tubers (S. tuberosum cv. Bintje) [8, 10]. A potato mitochondrial DNA library was constructed by cloning Sal I mitochondrial DNA fragments into pUC18. A 1.8 kb Eco RI fragment containing most of the cob gene from an alloplasmic line of wheat [11] was used as a probe to screen for the potato cob gene. Two Sal I clones (13 and 18 kb) were isolated and characterized by restriction mapping (Fig. 1A). The cob gene was localized on the restriction maps by Southern hybridization (result not shown).

The gene is present at least in two genomic

contexts differing in the 5' non-coding region (Fig. 1A). This suggests that, as reported for other plants [7, 9], homologous recombination between repeated sequences also occurs in the mitochondrial genome of potato.

Appropriate fragments from *cob* gene were subcloned into M13 bacteriophage derivatives and sequenced by the dideoxy termination method [12]. The sequence obtained is presented in Fig. 1B. The 1179 bp protein coding region shares extensive sequence identity at the nucleotide level with *cob* from broad bean (96.3%), *Oenothera* (97.0%), maize (95.2%), wheat (95.7%) and rice (95.3%) [17, 14, 3, 1, 11, 6]. As found in other plant mitochondrial genes, there is a strong bias toward the use of T (42.6%) in the third position of codons [3].

We also sequenced 371 bp of the 5' flanking region. Interestingly, the 5' upstream region shows a strong DNA sequence homology with the 5' flanking region of broad bean [17] (about 80% over 371 bp). On the other side homology

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDJB Nucleotide Sequence Databases under the accession number X58437 STCOB.

В

GCAAGATGATCAGTCCGAGAGTGCT6GAGAGAGAGAGAGAGAGGGTAAAAACCTCTCTTATTCGGTCACCGAGAAGTCGGACGACTCTTCAGTAACCCAGGGTGATCCGACCCCTTCGACG -135 CTITTITCGCTGTATACCCCCTCCATCTTCGGA6GTGGAAGAAGGGTACTCACATTTTAATACATAGTAGGGCCCCAGAACGCTAAAAGGT6GGGGAACAAGAGTTGTCACGATAGAA - 36 1 Met Thr Ile Arg Asn Gin Arg Leu Ser Leu Leu Lys Gin Pro 11e Ser Ser Thr Leu Asn Gin His Leu Ile Asp Tyr AAGAGAAAAAAAAAAA ATG ACT ATA AGG AAC CAA CGG CTC TCT CTT CAT AAA CAA CCT ATA TEC TCC ACA CTT AAT CAG CAT TIG ATA GAT TAT 78 50 Pro Thr Pro Ser Asn Leu Ser Tyr Trp Trp Gly Phe Gly Ser Leu Ala Gly Ile Cys Leu Val Ile Gin Ile Val Thr Gly Val Phe Leu CCA ACC CCG AGC AAT CTT AGT TAT TEG TEG EGE TIC GET TCE TTA ECE EGT ATT TET TTA ETC ATT CAG ATA ETE ACT EGC ETT TIT TTA 168 Ala Met His Tyr Thr Pro His Val Asp Leu Ala Phe Asn Ser Val Glu His Ile Met Arg Asp Val Glu Gly Gly Trp Leu Leu Arg Tyr GCT ATG CAT TAC ACA CCT CAT GTG GAT CTA GCT TTC AAC AGC GTA GAA CAC ATT ATG AGA GAT GTT GAA GGG GGC TGG TTG CTC CGT TAT 258 100 Met His Ala Asn Gly Ala Ser Met Phe Phe Ile Val Val His Leu His Ile Phe Arg Gly Leu Tyr His Ala Ser Tyr Ser Ser Pro Arg ATG CAT GCT AAT GGG GCA AGT ATG TTT TTC ATT GTG GTT CAC CTG CAT ATT TTT CGT GGT CTA TAT CAT GCC AGT TAT AGC AGT CCT AGG 348 ٨ Glu Phe Val Arg Cys Leu Gly Val Val Ile Phe Leu Leu Met Ile Val Thr Ala Phe Ile Gly Tyr Val Leu Pro Trp Gly Gln Met Ser GAA TIT GTT CGG TGT CTC GGA GTT GTA ATC TTC CTA TTA ATG ATT GTG ACA GCT TTT ATA GGA TAT GTC CTA CCT IGG GGT CAG ATG AGC 438 ۸ 150 Phe Trp Gly Ala Thr Val Ile Thr Ser Leu Ala Ser Ala Ile Pro Val Val Gly Asp Thr Ile Val Thr Trp Leu Trp Gly Gly Phe Ser TIT TEG GEA GCT ACA GTA ATC ACA AGC TTA GCT AGC GCC ATA CCT GTA GTA GEA GAT ACC ATA GTE ACT TEG CTT TEG GET GEG TIC TCC 528 200 Val Asp Asn Ala Thr Leu Asn Arg Phe Phe Ser Leu His His Leu Leu Pro Phe Ile Leu Val Gly Ala Ser Leu Leu His Leu Ala Ala GTG GAC AAT GCC ACC TTA AAT CGT TTT TTT AGT CTT CAT CAT TTA CTC CCC TTT ATT TTA GTA GGC GCC AGT CTT CTT CAT CTG GCC GCA 618 ٨ Leu His GIn Tyr Gly Ser Asn Asn Pro Leu Gly Val His Ser Glu Met Asp Lys Ile Ala Ser Tyr Pro Tyr Phe Tyr Val Lys Asp Leu TTG CAT CAA TAT GGA TCC AAT AAT CCA TTG GGT GTA CAT TCA GAG ATG GAT AAA ATT GCT TCT TAC CCT TAT TIT TAT GTA AAG GAT CTT 708 250 Val Gly Trp Val Ala Phe Ala Ile Phe Phe Ser Ile Trp Ile Phe Tyr Ala Pro Asn Val Leu Gly His Pro Asp Asn Tyr Ile Pro Ala GTA GGT TGG GTA GCT TTT GCT ATC TTT TCC ATT TGG ATT TTT TAT GCT CCT AAT GTT TTG GGG CAT CCC GAC AAT TAT ATA CCT GCT 798 Asn Pro Met Ser Thr Pro Pro His Ile Val Pro Glu Trp Tyr Phe Leu Pro Ile His Ala Ile Leu Arg Ser Ile Pro Asp Lys Val Gly AAT CCG ATG TCC ACC CCG CCT CAT ATT GTG CCA GAA TGG TAT TTC CTA CCG ATC CAT GCC ATT CTT CGT AGT ATA CCT GAC AAA GTG GGA 888 300 Gly Val Ala Ala Ile Ala Pro Val Phe Ile Cys Leu Leu Ala Leu Pro Phe Phe Lys Ser Met Tyr Val Arg Ser Ser Phe Arg Pro GET GTA SEC GCA ATA GEA CCA GTT TIT ATA TET CTE TTE SECT TTA CCC TIT TIT AAA AGT ATE TAT GTA CET AGT TCA AGT TTT CEC CCE 978 350 Ile His Gin Gly Ile Phe Trp Leu Leu Ala Asp Cys Leu Leu Leu Gly Trp Ile Gly Cys Gin Pro Val Giu Ala Pro Phe Val Thr ATT CAC CAA 66A ATA TTT T66 TT6 CTT TT6 6C6 6AT T6C TTA CTA CTA 66T T66 ATC 66A T6T CAA CCT 6T6 6A6 6CA CCC TTT 6TT ACT 1068 Ile Gly Gln Ile Ser Pro Leu Val Phe Phe Leu Phe Phe Ala Ile Thr Pro Ile Leu Gly Arg Val Gly Arg Gly Ile Pro Asn Ser Tyr ATT GEA CAA ATT TCT CCT TTA GTT TTC TTC TTG TTC TTT GCC ATA ACG CCC ATT CTG GGA CGA GTT GGA AGA GGA ATT CCT AAT TCT TAC 1158 ٨ 393 Thr Asp Glu Thr Asp His Thr ACE GAT GAG ACT GAT CAC ACC TGA TTAGTGAGAAATTCTTACACCAATCATTTACGAGTGGGTAATACACCCAAGAATTTACAAGCGGAATGAAGG 1260

Fig. 1. A. Restriction map of Sal I fragments carrying the cob gene from Solanum tuberosum cv. Bintje. The filled bar represents the cob coding region. Direction of transcription is shown by an arrow. A, Ava I; B, Bam HI; Cl, Cla I; E, Eco RI; H, Hind III; P, Pst I; S, Sal I; Sm, Sma I; X, Xho I; Xb, Xba I (all Eco RI and Ava I sites are not indicated). B. Nucleotide sequence of the

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extends only a limited distance, approximately 50 bp, into the purine-rich flanking region of *cob* genes from *Oenothera*, maize, wheat and rice [14, 3, 1, 11, 6].

The amino acid sequence deduced for potato apocytochrome b is also shown in Fig. 1B. We have not considered possible RNA editing events [2, 4, 5] (the universal genetic code has been used). The deduced sequence corresponds to an hydrophobic polypeptide 393 amino acids long, with a predicted molecular weight of 43420 Da and with overall homologies of 93.4-97.5% with amino acid sequences deduced for other plant cob genes [1, 3, 6, 11, 14, 17]. The potato apocytochrome b contains the four conserved histidine residues (88, 102, 189 and 203) implied to bind the two electron-carrying protohaem prostethic groups of cytochrome b [3, 13], and conforms to a proposed model in which the protein is folded into nine hydrophobic transmembrane domains [13, 18]. Predicted amino acid sequences of the carboxy terminal portion of plant apocytochromes b show variability [11]: the potato polypeptide is identical in that region to the Oenothera polypeptide.

Candidate positions for RNA editing are shown by carets in Fig. 1B. These editing events lead to radical amino acid substitutions in *Oenothera* and/or wheat, thus improving the degree of conservation between the predicted plant protein sequences and homologous polypeptide sequences from other organisms [4, 15].

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References

- Boer PH, McIntosh JE, Gray MW, Bonen L: The wheat mitochondrial gene for apocytochrome b: absence of a prokaryotic ribosome binding site. Nucl Acids Res 13: 2281–2292 (1985).
- Covello PS, Gray MW: RNA editing in plant mitochondria. Nature 341: 662–666 (1989).
- Dawson AJ, Jones VP, Leaver CJ: The apocytochrome b gene in maize mitochondria does not contain introns and is preceded by a potential ribosome binding site. EMBO J 3: 2107-2113 (1984).
- Gualberto JM, Lamattina L, Bonnard G, Weil JH, Grienenberger JM: RNA editing in wheat mitochondria results in the conservation of protein sequences. Nature 341: 660–662 (1989).
- 5. Hiesel R, Wissinger B, Schuster W, Brennicke A: RNA editing in plant mitochondria. Science 246: 1632–1634 (1989).
- Kaleikau EK, André CP, Doshi B, Walbot V: Sequence of the rice mitochondrial gene for apocytochrome b. Nucl Acids Res 18: 372 (1990).
- Levings III CS, Brown GG: Molecular biology of plant mitochondria (Review). Cell 56: 171–179 (1989).
- Neuburger M, Journet EP, Bligny R, Carde JP, Douce R: Purification of plant mitochondria by isopycnic centrifugation in density gradients of percoll. Arch Biochem Biophys 217: 312–323 (1982).
- Newton KJ: Plant mitochondrial genomes: organization, expression and variation. Ann Rev Plant Physiol Plant Mol Biol 39: 503-532 (1988).
- Ricard B, Echeverría M, Cristophe L, Litvak S: DNA synthesis in isolated mitochondria and mitochondrial extracts from wheat embryos. Plant Mol Biol 2: 167–175 (1983).

Solanum tuberosum cv. Bintje mitochondrial cob gene and its derived protein sequence. The purine-rich stretch preceding the initiation codon is overlined: this region shows high homology with analogous regions in all known plant cob genes. Termination codon is boxed. C to U conversions detected in *Oenothera* and/or wheat cob mRNA and leading to radical amino acid substitutions are shown by carets (positions where nucleotide is already a T in potato are not included). Some restriction sites are underlined.

- Saalaoui E, Litvak S, Araya A: The apocytochrome b from an alloplasmic line of wheat (*T. aestivum* cytoplasm *T. timopheevi*) exists in two differently expressed forms. Plant Sci 66: 237-246 (1990).
- Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467 (1977).
- 13. Saraste M: Location of haem-binding sites in the mitochondrial cytochrome b. FEBS Lett 166: 367-372 (1984).
- Schuster W, Brennicke A: TGA-termination codon in the apocytochrome b gene from *Oenothera* mitochondria. Curr Genet 9: 157–163 (1985).
- 15. Schuster W, Hiesel R, Wissinger B, Brennicke A: RNA editing in the cytochrome b locus of the higher plant

Oenothera berteriana includes a U to C transition. Mol Cell Biol 10: 2428–2431 (1990).

- Vedel F, Quetier F: Physico-chemical characterization of mitochondrial DNA from potato tubers. Biochim Biophys Acta 340: 374–387 (1974).
- Wahleithner JA, Wolstenholme DR: Ribosomal protein S14 genes in broad bean mitochondrial DNA. Nucl Acids Res 16: 6897–6913 (1988).
- Widger WR, Cramer WA, Herrmann RG, Trebst A: Sequence homology and structural similarity between cytochrome b of mitochondrial complex III and the chloroplast b₆-f complex: Position of the cytochrome b hemes in the membrane. Proc Natl Acad Sci USA 81: 674–678 (1984).