ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Expression of uncoupling protein and alternative oxidase depends on lipid or carbohydrate substrates in thermogenic plants

by

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Biol. Lett. (doi:10.1098/rsbl.2005.0338)

Supporting Materials

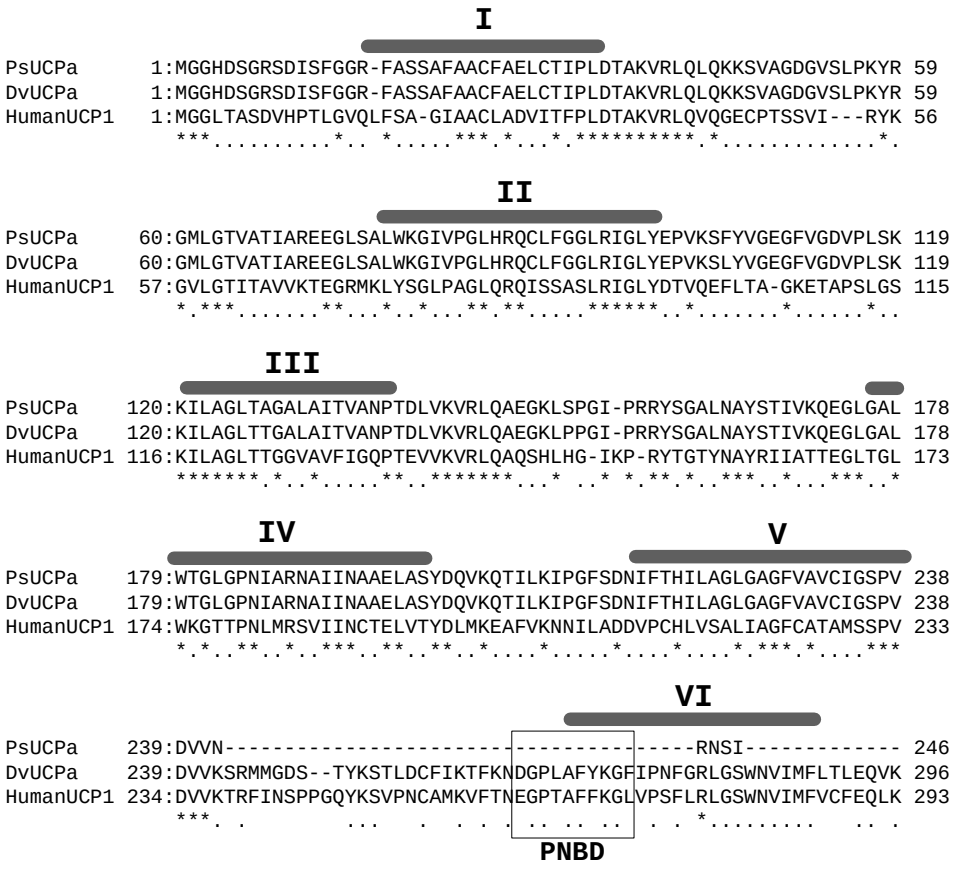
PCR cloning and expression analysis

To obtain partial cDNA fragments for *UCP* genes, degenerate primers (Zf1, 5'-CCI YTI GAY ACI GCI AAR-3'; Zr1, 5'-ACW TTC CAI SYI CCI AEI C-3') were used for PCR amplifications. Full-length cDNAs for *Philodendron selloum* and *Dracunculus vulgaris*, isolated by 5'- and 3'-RACE (SMART^{TM} RACE cDNA amplification kit, Clontech Laboratories Inc., Palo Alto, CA), were termed *PsUCPa* (DNA Data Bank of Japan accession no. AB189674) and *DvUCPa* (AB189672), respectively.

To obtain partial cDNA fragments for *AOX* genes, primers were designed based on the cDNA sequence for the *SgAOX*. Oligonucleotide primers used were Sgaox_F0 (5'-AAA GAG GAC GGC TCC GAG TGG C-3') and Sgaox_R1 (5'-ATC AGT GGT ACC CGA GCG GCG C-3'). Full-length cDNAs for *Philodendron selloum* and *Dracunculus vulgaris*, isolated by a 5'- and 3'-RACE were termed *PsAOX* (AB190213) and *DvAOX* (AB189673), respectively. Sequence data were assembled and analyzed using the GENETYX-WIN software system, version 5.2.3 (Software Development Inc., Tokyo, Japan).

Expression analyses of isolated cDNAs in various tissues in *P. selloum* and *D. vulgaris* were performed by RT-PCR according to the previous report (Ito *et al*. 2003).

Briefly, primer sets for *PsUCPa* (Sp1: 5'- TCT TCT GCA ACT CTC CCG ATT-3', Sp2: AGC AGT GGT ATC AAC GCA GAG T-3'), *DvUCPa* (Sp1: 5'- ATA CCA AGA CGT TAT TCG GGT GCA T-3', Sp2: 5'- CAC CCG AAT AAC GTC TTG GTA TGC C-3'), *PsAOX* (Sp1: 5'- CCG GGC GAT GAT GCT GGA GA-3', Sp2: 5'- CCC GCT GTC GAT GTC CTT GA -3'), and *DvAOX* (Sp1: 5'- CCA ACT TGC ATG CCT GCA GGT C-3', Sp2: 5'- TAT TCG AAT TCG AGC TCG GTA C-3') were used for PCR reactions with 19 amplification cycles, and the amplified products were electrophoresed and transferred to a Hybond N^+ membrane (Amersham Biosciences Inc., Piscataway, NJ), hybridized with a specific cDNA probe for each cDNA, and visualized by the AlkPhos Direct labelling and Detection System (Amersham Biosciences Inc. Piscataway, NJ) following the manufacturer's instructions.



Supplementary Figure 1. Alignment of the deduced amino acid sequences of the putative PsUCPa and DvUCPa proteins with human UCP1. The sequences are shown in single letter codes. Aristerisks indicate perfect matches and dots represent conservative changes within all sequences. The gaps introduced to optimize the sequence alignment are represented by dashes. Six predicted transmembrane regions (I-VI) are indicated by horizontal shadow lines. The potential purine-nucleotide binding domain (PNBD) is boxed.