GENE NOTE

Ubiquitous expression of a gene encoding for uncoupling protein isolated from the thermogenic inflorescence of the dead horse arum Helicodiceros muscivorus

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1 The nucleotide sequence data reported was deposited in DDBJ under the accession number AB088762.
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Abstract

Uncoupling proteins (UCPs) are a family of mitochondrial inner membrane proteins that have been implicated in heat production in mammalian cells. The inflorescences of several members of the arum lily family (Araceae) have also been shown to produce heat during flowering, but the involvement of UCP-mediated heat production in plants is not known. In this work a gene has been isolated termed HmUCPa that encodes for a putative uncoupling protein from Helicodiceros muscivorus, a highly thermogenic arum lily. RT-PCR analysis revealed that the expression of HmUCPa was ubiquitously found, both in thermogenic male florets and appendix, and the non-thermogenic female florets, spathe and club-shaped organs of the spadix. These results suggest that HmUCPa is not primarily involved in organ-specific heat production in H. muscivorus.

Key words: Arum lily, heat production, Helicodiceros muscivorus, mitochondria, uncoupling protein (UCP).

Thermogenesis occurs in several species of the arum lily family (Araceae), cycads (Cycadaceae), water lilies (Nymphaeaceae), palms (Cyclanthaceae), custard apples (Annonaceae), lotus (Nelumbonaceae), and other primitive seed plants (reviewed by Seymour and Schulze-Motel, 1997). To date, heat production in these plants has been believed to be associated with a large increase in the activity of the cyanide-insensitive pathway, a non-phosphorylating electron transport route regulated by the alternative oxidase, which is unique to plant mitochondria (Vanlerbergh and McIntosh, 1997).

In mammals, on the contrary, a mitochondrial protein called uncoupling protein (UCP) has been shown to play an important role in heat production (Bouillaud et al., 2001). UCPs are found in the inner membrane of the mitochondria and allow a transmembrane H+ flux, which uncouples respiration from ATP synthesis, permitting the dissipation of chemical energy as heat. In thermogenic plants, however, the molecular mechanism underlying UCP-mediated heat production remains to be determined.

Recently, it has been discovered that the dead horse arum Helicodiceros muscivorus, which originally lives on a few islands in the Mediterranean, shows strong thermogenicity in the appendix and male florets (RS Seymour et al., unpublished data). To elucidate the possible involvement of UCP-related heat production in H. muscivorus, screening for cDNA clones encoding for the putative uncoupling protein was undertaken.

Degenerate primers (Zf1, 5'-CCYTYGAYACIGCIAAR-3'; Zr1, 5'-ACWTTCCAISYICCIAWIC-3') were deduced from the regions conserved among the UCP family (Ito, 1999) and a major PCR product of approximately 0.6 kb was amplified on cDNA prepared from total RNA of thermogenic male florets. The amplified cDNA fragment showed a high sequence homology to SfUCP, a gene encoding for plant UCP isolated from the thermogenic spadix of skunk cabbage (Ito, 1999). A full-length cDNA fragment of H. muscivorus, termed HmUCPa, was further obtained by 5'- and 3'-RACE (SMART™ RACE cDNA amplification kit, Clontech). HmUCPa is 1178 nucleotides in length excluding the poly-A tail, and encodes a putative protein of 304 amino acids. The deduced protein sequence is 87.8% identical to that of the SfUCP derived from skunk cabbage (Ito, 1999) and possesses three typical mitochondrial carrier signature domains, six membrane-spanning domains, and one nucleotide binding domain that are characteristic of the known UCP family. The HmUCPa protein shows 81.3%, 80.2%, 76.3%, 73.9%, 72.8%, 72.5%, and 71.7% identity to potato StUCP (Laloi et al., 1997), AtPUMP (Maia et al., 1998), OsUCP2 (Watanabe and Hirai, 2002), AtUCP2 (Watanabe et al., 1999), WhUCP1b (Murayama and Handa, 2000), WhUCP1a (Murayama and Handa, 2000), and OsUCP1 (Watanabe and Hirai, 2002), respectively, and around 47% identity to the human UCP isofoms.

A phylogenetic tree constructed from the UCP protein sequence alignments confirmed that they are distributed into two major groups, mammalian and plant UCPs (Fig. 1). On the branch of plant UCPs, Helicodiceros UCP protein was grouped with thermogenic plant species. These results indicate that HmUCPa encodes a novel isofom of plant UCPs that are derived from thermogenic plant species.

To determine the involvement of HmUCPa gene in heat production of H. muscivorus, RT-PCR analyses with various RNAs were performed (Fig. 2). Because heat production occurs only in the appendix and the male florets in H. muscivorus (RS Seymour et al., unpublished data), one would expect that the mRNA expression of HmUCPa is restricted to these organs. However, the
UCP1, 2, and 3 (Bouillaud et al., 1985; Boss et al., 1997; Fleury et al., 1997), StUCP (Laloi et al., 1997), OsUCP1 and OsUCP2 (Watanabe et al., 1997), AtPUMP (Maia et al., 1985; Boss et al., 1999), AtUCP1 and AtUCP2 (Watanabe and Hirai, 2002), WhUCPa and WhUCPb (Murayama and Handa, 2000), and HmUCPa. The numbers above and below the branches indicate the distance from the common ancestor.

Fig. 1. Phylogenetic relationship among plant and human UCPs. The tree was drawn by the UPGMA method using amino acid sequences of human UCP1, 2, and 3 (Bouillaud et al., 1985; Boss et al., 1997; Fleury et al., 1997), StUCP (Laloi et al., 1997), AtPUMP (Maia et al., 1998), AtUCP2 (Watanabe et al., 1999), OsUCP1 and OsUCP2 (Watanabe and Hirai, 2002), WhUCPa and WhUCPb (Murayama and Handa, 2000), and HmUCPa. The numbers above and below the branches indicate the distance from the common ancestor.

Fig. 2. Expression analysis of HmUCPa. Primer sets for HmUCPa (Sp1: 5’-GGAAAGGTATAGTACCAGGCTTGCATC-3’, Sp2: 5’-GGAACCA-ATACAAACCGCAAC-3’) were expected to amplify a 476 bp fragment. PCR reactions with the cDNA (RT+) and total RNA without reverse transcriptase reaction (RT-) were carried out with 19 amplification cycles as described previously (Ito et al., 1999). Amplified products were analysed by electrophoresis and visualized by hybridization with a full-length HmUCPa cDNA as a probe (Ito et al., 1999). Ethidium bromide-stained gels, showing undegraded rRNA are also included at the bottom of the panel.

expression of the mRNA was detected in all examined organs, including the non-thermogenic female florets, spathe and club-shaped organs of the spadix. Control experiments without reverse-transcriptase reaction confirmed that there was no DNA contamination in each RNA (Fig. 2). Thus, it is postulated that the mRNA accumulation from the HmUCPa gene is independent of the organ-specific heat production in H. muscivorus.

UCPs identified from non-thermogenic plants such as potato and rice also possess all the typical features reported for known mammalian UCPs (Laloi et al., 1997; Watanabe and Hirai, 2002). The results also confirmed that the existence of the UCP gene itself might not necessarily imply its participation in the heat production in other thermogenic plant species, although further expression analysis of its gene product is necessary. Alternatively, another UCP isoform such as SFUCPb, a Sylcomarces gene product which lacks the fifth transmembrane domain (Ito, 1999), or an alternative oxidase, which is unique to plant mitochondria, might contribute to the organ-specific heat production in H. muscivorus. Moreover, ubiquitous expression of HmUCPa mRNA also suggests participation of its product in more general cellular metabolism. Indeed, it has recently been shown that UCPs may contribute to the reduction of reactive oxygen species in mammalian mitochondria (Echtay et al., 2002). It remains essential therefore to establish role(s) of UCPs derived from thermogenic and non-thermogenic plant species.

Acknowledgements

This work was supported by the Program for Promotion of Basic Activities for Innovative Biosciences (Japan) and the Australian Research Council.

References


