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Mitochondrion 5 (2005) 162–172

Mitochondrion

www.elsevier.com/locate/mito

Identification of three isoforms for mitochondrial adenine nucleotide translocator in the pufferfish *Takifugu rubripes*

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Received 13 August 2004; received in revised form 20 December 2004; accepted 26 January 2005

Abstract

Three adenine nucleotide translocator (ANT) genes were identified through in silico data mining of the *Fugu* genome database along with isolation of their corresponding cDNAs in vivo from the pufferfish (*Takifugu rubripes*). As a result of phylogenetic analysis, the ANT gene on scaffold_254 corresponded to mammalian ANT1, whereas both of those on scaffold_6 and scaffold_598 to mammalian ANT3. The ANT gene encoded by scaffold_6 was expressed ubiquitously in various tissues, whereas the ANT genes encoded by scaffold_254 and scaffold_598 were predominantly expressed in skeletal muscle and heart, respectively.

Keywords: Adenine nucleotide translocator; ADP/ATP carrier; Mitochondria; *Takifugu rubripes*; *Fugu* genome database

1. Introduction

Adenine nucleotide translocator (ANT) or ADP/ATP carrier, an integral component of the inner mitochondrial membrane, is the most abundant mitochondrial protein. It mediates exchange of ADP and ATP across the mitochondrial membrane, thereby linking ATP production in mitochondria to its functional utilization for energy requirements outside mitochondria (Klingenberg, 1981, 1989; Fiore et al., 1998). In mammals, three isoforms termed ANT1, ANT2, and ANT3 are known, and their distinct tissue distributions have been reported (Battini et al., 1987; Neckelmann et al., 1987; Houldsworth and Attardi, 1988; Cozens et al., 1989; Ku et al., 1990; Stepien et al., 1992; Yamazaki et al., 2002). ANT1 is predominantly expressed in heart and skeletal muscle of human, whereas ANT2 is expressed in kidney and liver. ANT3 is ubiquitously expressed but most remarkably in kidney (Stepien et al., 1992).

Abbreviations: ANT, adenine nucleotide translocator (ADP/ATP carrier); AUAP, abridged universal amplification primer; EST, expressed sequence tag; F_oF_1-ATPase, mitochondrial ATP synthase; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends.

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doi:10.1016/j.mito.2005.01.003

On the other hand, bovine (*Bos taurus*) ANT1 is expressed in heart, brain and kidney, but most remarkably in heart, whereas ANT2 is expressed in brain and kidney and ANT3 is ubiquitously expressed (Yamazaki et al., 2002). ANT1 is also predominantly expressed in heart and skeletal muscle of rat (*Rattus norvegicus*) and mouse (*Mus musculus*) (Shinohara et al., 1993; Ellison et al., 1996). Yamazaki et al. (2002) have shown the possibility for the absence of the ANT3 gene in rat. These tissue distributions of different ANT isoforms seem to reflect their different functions and associated tissue-specific energy metabolisms (Stepien et al., 1992), and therefore, it is interesting to examine expression profiles of ANT isoforms in various organisms.

In eurythermal temperate fish such as carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), temperature acclimation leads to an array of adaptational physiological changes to compensate for the effect of temperature variation on metabolic processes (Hazel and Prosser, 1974). Previously we showed, using two-dimensional electrophoresis, that mitochondrial ATP synthase (F_oF_1-ATPase) β-subunit of 55-kDa was increased following cold acclimation of carp (Kikuchi et al., 1999). Furthermore, the mRNA levels of F_oF_1-ATPase subunit encoded by the nuclear genes including β-subunit were nearly two-fold higher in carp acclimated to 10 °C than fish acclimated to 30 °C (Itoi et al., 2003). On the other hand, the transcripts of the subunits encoded by the mitochondrial genes in the 10 °C-acclimated carp were 6–7 times as much as those in the 30 °C-acclimated carp. In association with such differences, F_oF_1-ATPase activity measured at any temperatures in the range of 10–30 °C was nearly two-fold higher for the 10 °C- than 30 °C-acclimated fish. These quantitative and qualitative changes in carp F_oF_1-ATPase are thought to contribute to extra ATP production to compensate for the decline in metabolic rate at low temperatures. As described above, ANT is the most abundant protein in mitochondria and links ATP production to its consumption outside mitochondria. Bouchard and Guderley (2003) showed that rainbow trout changed mitochondrial respiration during temperature acclimation without changes in the amount of ANT, which constitutes a point of control of mitochondrial respiration (Groen et al., 1982). It has been suggested that the changes in mitochondrial respiration are due to the modification of fatty acid composition in mitochondrial membrane during temperature acclimation (Bouchard and Guderley, 2003). However, little information on fish ANT genes has been available except that a few expressed sequence tags (ESTs) are available for fish ANTs including ANT3 from zebrafish (*Danio rerio*) and ANT2 from goldfish. The goldfish ANT2 partial sequence has been recently published together with some expression data (Barreda et al., 2004).

The genomic sequence of the pufferfish (fugu) (*Takifugu rubripes*) has recently been sequenced to a draft level with approximately 95% coverage of the non-repetitive fraction of genome (http://fugu.hgmp.mrc.ac.uk; http://genome.jgi-psf.org/fugu/index.html) (Aparicio et al., 2002). Fugu has a haploid genome of about 365 Mb, which is 7.5 times smaller than the human genome, but is thought to retain a gene repertoire similar to that of human (Brenner et al., 1993). Thus, in general, the intergenic regions and introns in the fugu genome are relatively small in size and considerably less complex than their mammalian counterparts (Naito et al., 1998; Peixoto et al., 2000; Yu et al., 2001). Furthermore, the fugu genome shows a strong conservation of exon–intron boundaries with mammalian orthologues, and is therefore, an attractive model for analyzing genomic organization and promoter sequences (Naito et al., 1998; Peixoto et al., 2000; Yu et al., 2001).

In this study, we isolated three ANT genes from the *Fugu* genome database by in silico cloning using the nucleotide sequence of the human ANT1 gene as a probe. cDNA clones transcribed from the three ANT genes were also amplified by PCR using first strand cDNAs of fugu from heart and skeletal muscles as templates and their tissue distributions were determined by reverse transcription (RT)-PCR.

2. Materials and methods

2.1. Fish

Live specimens of fugu (*Takifugu rubripes*) (average body weight 50 g) were grown in laboratory aquariums at 20 °C. All fish were fed commercial pellets daily ad libitum and various tissues were collected after instant killing.