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New isoforms of human mitochondrial transcription factor A detected in normal and tumoral cells

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ABSTRACT

Novel alternatively spliced variants of the human mitochondrial transcription factor A predicted by the computational tool ASPic were experimentally validated in different normal and tumoral human tissues by RT-PCR and DNA sequencing.
The comparison between the 5′UTR length and the distribution of the different transcripts showed that the transcripts with the shortest 5′UTR are present in all the investigated tissues, while the longest 5′UTR seems to be related to tissue-specificity. Studies about the localization and function of the most widely diffuse alternative isoform Tr6 were carried out.

1. Introduction

Alternative splicing (AS) is emerging as a major molecular mechanism capable to both extend the repertoire of functions produced by individual genes, through the expression of multiple transcripts encoding proteins with different biochemical and physical properties, and diversify the regulation of their expression through alternative 5′ and 3′UTRs (Graveley, 2001; Riva and Pesole, 2009). AS is an extremely economical means of increasing proteome diversity (Park and Graveley, 2007).

In general, humans appear to have higher levels of AS than other mammals, and vertebrates are thought to have higher AS frequency than invertebrates (Brett et al., 2002; Kim et al., 2007; Irimia et al., 2009). Recent analyses indicate that 92–94% human genes undergo AS and that frequently both AS and alternative cleavage and polyadenilation events vary among tissues (Wang et al., 2008). Thus, AS emerges as the rule, not the exception. mRNA and protein isoforms produced by alternative processing of primary RNA transcripts may differ in structure, function, localization or other properties (Black, 2003; Matlin et al., 2005). Recent studies have also shown that AS is important for determining developmental- and tissue-specific gene expression (Xie et al., 2002; Xu et al., 2002). Aberrant splicing forms are also associated with human diseases (Caceres and Kornblihtt, 2002). Tissue specific AS is usually regulated by a combination of tissue specific and ubiquitously expressed RNA binding factors that interact with *cis*-acting RNA elements to influence spliceosome assembly at nearby splice sites (Black, 2003; Matlin et al., 2005). Recently, it has been demonstrated that AS can also be regulated without the involvement of auxiliary splicing factors (Graveley, 2009).

It is now well established that splicing is frequently cotranscriptional and functionally coupled to transcription. Although in vitro splicing can occur irrespective of transcription, the process is less efficient than in vivo (Wetterberg et al., 2001; Bird et al., 2004) or in transcription-coupled in vitro systems (Das et al., 2006). The complexes involved in transcription and mRNA processing reactions usually share many of their components and in some cases can influence each other (Kornblihtt et al., 2004).

Over the last years, many important advances have been made in our understanding not only of the prevalence of AS and its regulation, but also of its biological and medical significance (Skotheim and Nees, 2007; Graveley, 2008).

In order to obtain a comprehensive overview of the splicing pattern of a given gene and of its implications, it is generally necessary to take into account both results obtained by computational approach and experimental results, since no individual method or algorithm is powerful and accurate enough to provide a complete picture of such a complex biological phenomenon. Our main interest was to investigate the tissues specificity of AS and the relationship between AS and cancer of the main mitochondrial transcription factor. So, we have searched alternative transcripts (ATs) of the *TFAM* gene, a nuclear

Abbreviations: mtDNA, mitochondrial DNA; TFAM, mitochondrial transcription factor A; COI, cytochrome c oxidase subunit I; LSP, light strand promoter; HSP, heavy strand promoter; CDS, coding sequence; HMG, high mobility group; UTR, untranslated region; AT, alternative transcript; AS, alternative splicing.

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gene encoding the mitochondrial transcription factor A, in different normal and tumoral human tissues, using both computational and experimental approach.

A short mRNA of *TFAM*, lacking exon 5, was found to be widely distributed in human tissues (Tominaga et al., 1993), representing 30% of the total *TFAM* transcript pool. The same type of splicing was found in rat, though at lower percentage (10%) (Mezzina et al., 2002). The absence of this exon does not modify the reading frame, permitting the translation of a 22 kDa protein lacking half the second HMG-box (Poulton et al., 1994). This *TFAM* alternative transcript is translated into a stable protein, although its stability is lower respect to the full length as we recently reported (Bruno et al., 2007).

2. Materials and methods

2.1. Bioinformatics

Novel *TFAM* variants were identified using ASPicDB (Castrignanò et al., 2008) (http://www.caspur.it/ASPicDB/index.php). This database was used to obtain sequence data of every transcript and protein isoform predicted, and also to identify intron/exon boundaries of the genomic sequence corresponding to each variant, essential for the design of isoform-specific primer sets.

2.2. Biological materials

High quality DNAse free total RNA from different normal or tumoral tissues was purchased from Ambion and Asterand.

Cancer human lung cells, H1299 line, whose characteristics are specified on the www.lgcpromochem-atcc.com., were grown in DMEM (Dulbecco's modified Eagle's medium, Eurobio, Courtaboeuf, France) supplemented with 10% fetal bovine serum at37°C in a humidified incubator containing 5% CO_2.

Chinese hamster ovary cells (CHO) were grown in Ham's F-12 nutrient mixture supplemented with 10% fetal bovine serum.

2.3. RNA extraction

Total RNA from H1299 cells was obtained by using the Qiagen RNeasy® Mini Kit (including the RNase Free Dnase set) as described by the manufacturer. RNA concentration was determined by spectrophotometric measurements at 260, 280, and 230 nm using NanoDrop® ND 1000 spectrophotometer (ThermoFisher Scientific Inc., MI., Italy).

2.4. cDNA synthesis

cDNA was synthesized from 1 μg of total RNA using SuperScript® III Reverse Transcriptase as described by the manufacturer (Invitrogen).

2.5. PCR and cloning of partial cDNA of TFAM alternative transcripts

Specific oligonucleotide primer pairs for AT were designed against predicted sequences by ASPicDB and constructed using Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and primer3 (http://frodo.wi.mit.edu/) software. The primers sequences are reported in Table 1. Amplification products were purified using Nucleo Spin® extract II (Macherey Nagel), legated into the pGEM T easy vector (Promega), and transformed into high efficiency Escherichia coli JM109 Competent Cells (Promega). Positive colonies were selected and plasmids were purified using the QIAprep® Spin Miniprep Kit (QIAGEN). All procedures were carried out according to the manufacturer's instructions. Purified plasmids were sent to the Primm Sequence Service (Primm srl, Italy) for sequencing.

The 5′RACE was carried out using the 5´RACE System for Rapid Amplification of cDNA Ends, Version 2.0 (Invitrogen). Sequence of the primers used is reported in Table 1.

Table 1
Alternative transcripts validation by RT-PCR.

ISOFORMS	PCR product LENGTH (bp)	PRIMERS	LOCALIZATION	SEQUENCE
TFAM.Ref	**754**	ForBamKoz	Exon 1	acggatccgagcgatggcgtttctccgaagcat
		RevExpEco	Exon 7	aagaattcttaacactcctcagcacca
TFAM3	**230**	Tfam-3 for	Between exons 1 and 2[a]	Ccaaagaaacctcatgctaaag
		Tfam3-Rev	Between exons 2[a] and 7	ttaacactcctcagccaccat
TFAM.tr2	**449**	For5′UTRlong	5′UTR long	tgcgccaattccgccccg
		Rev Tr2	Exon 3[a]	attaacaattccctgagccag
TFAM.tr3	**685**	AsTr4For2-3	Between exons 2[a] and 3	gtccaaagaaacctatgcaa
		AsRev3′UTR	3′UTR	tgctggcagaagtccatgag
TFAM.tr4	**645**	AsTr4For2-3	Between exons 2[a] and 3	gtccaaagaaacctatgcaa
		Poly A	Between UTR and poly A	
TFAM.tr5	**816**	For5′UTRlong	5′UTR long	tgcgccaattccgccccg
	344	Rev 6-7	Between exons 6 and 7	tgaatatataattccttttcagagt
		Nested ASfor2-3	Between exons 2 and 3	aagctcagaacccagatgcaa
		Rev6-5	Between exons 6 and 5	Agcttttcctgcggtgaatca
TFAM.tr6	**658**	ForBamKoz	Exon 1	accgatccgagcgatggcgtttctccgaagcat
		RevExpEco	Exon 7	aagaattcttaacactcctcagcacca
TFAM.tr7	**831**	For5′UTRlong	5′UTR long	tgcgccaattccgccccg
	195	Rev Tr7	Exon 6[a]	ttgagactaacaaccgtaatact
		Seminested For 4-5	Between exons 4 and 5	aaaaaagagttaacactgcttg
		Rev Tr7	Exon 6[a]	ttgagactaacaaccgtaatact
TFAM.tr7Δ5	**735**	For5′UTRlong	5′UTR long	tgcgccaattccgccccg
	100	Rev Tr7	Exon 6[a]	ttgagactaacaaccgtaatact
		Seminested For 4–6	Between exons 4 and 6	aaaaaaagaaaagctgaagactgt
		Rev Tr7	Exon 6[a]	ttgagactaacaaccgtaatact
TFAM.tr8	**1561**	For Tr8	Exon 1[a]	ctcagggaattgttaataaagt
		RevTr8Tr3Tr6	3′UTR	acacaattcctagctcacagg
TFAM.tr9	**574**	For5′UTRlong	5′UTR long	tgcgccaattccgccccg
		Rev Tr9	Exon 2[a]	tcctgcagtctctgaatgctt
TFAM.tr10		nd	nd	nd

[a] Exon number is referred to the specified transcript.