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SCIENCE DIRECT

Gene 345 (2005) 173–182

GENE
SECTION
FUNCTIONAL GENOMICS

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Cellular expression and alternative splicing of *SLC25A23*, a member of the mitochondrial Ca^{2+}-dependent solute carrier gene family

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Received 2 July 2004; received in revised form 27 September 2004; accepted 17 November 2004
Available online 7 January 2005
Received by M. D'Urso

Abstract

The transport of metabolites across the inner mitochondrial membrane is mediated by a large superfamily of mitochondrial solute carrier (MSC) proteins. A novel human member of the MSC gene family named *SLC25A23*, with homologs in mammalian and non-mammalian species has been recently identified together with two close paralogs, *SLC25A24* and *SLC25A25*. These genes encode the human isoforms of the ATP-Mg/Pi carrier described in whole mitochondria. We report here the cellular expression and alternative splicing of *SLC25A23*. The gene encodes a 468 amino acids polypeptide, named SCaMC-3, with a bipartite structure typical of calcium-binding mitochondrial solute carrier (CaMSC) proteins. The amino-terminal portion harbors three canonical EF-hand calcium-binding domains while the carboxyl-terminal portion of SCaMC-3 has the characteristic features of the MSC superfamily. Northern blot analysis reveals the presence of the transcript in brain, heart, skeletal muscle, liver and small intestine. The *SLC25A23* gene undergoes alternative splicing suggesting a modular nature of the encoded product. Three out of four putative protein isoforms lack a significant portion of the third mitochondrial carrier signature. The most common SCaMC-3 isoform shows a mitochondrial subcellular localization when transfected in HeLa cells and is able to bind calcium by Ca^{2+}-dependent mobility shift assays. We believe that our study will contribute to a better knowledge of this family of mitochondrial carriers.

Keywords: Mitochondria; Solute carrier; Metabolite transport; EF-hands; Calcium-binding

1. Introduction

The mitochondrial solute carrier (MSC) proteins are one of the three major classes of proteins localized in the mitochondrial inner membrane together with the electron transport chain complex and the ATP synthase complex. MSCs transport compounds through the inner mitochondrial membrane to link the biochemical pathways in the cytosol with those in the mitochondrial matrix. The human genome likely encodes 48 different mitochondrial carriers (Kunji, 2004) that constitute a superfamily of related proteins characterized by the presence of three repeated regions, each about 100 amino acids long whose hydrophobic profile is indicative of two transmembrane helices joined by an

Abbreviations: MSC, mitochondrial solute carrier; *SLC25A23*, solute carrier family 25 member 23; cDNA, DNA complementary to RNA; bp, base pair(s); EF-hand, calcium elongation factor-binding loop; CaMSC, calcium-binding mitochondrial solute carrier; SCaMC-3, Small Calcium-binding Mitochondrial Carrier 3; EST, expressed sequence tag; PAC, P1 plasmid-derived artificial chromosomes; BAC, bacterial artificial chromosome; PCR, polymerase chain reaction; EDTA, Ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; EGTA, Ethylene glycol-bis(2-amino-ethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PAGE, polyacrylamide-gel electrophoresis; nt, nucleotide(s); kDa, kilodalton(s); aa, amino acid(s); chr, chromosome(s).

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doi:10.1016/j.gene.2004.11.028

extensive hydrophilic region. The carriers also have a signature motif, which contains the P-X-[D/E]-X-X-[R/K] sequence and is conserved in all members of the family and in all three repeats (Prosite PS50920) (Kuan and Saier, 1993; Kobayashi et al., 1999). MSCs are encoded by nuclear genes and have to be imported into the mitochondrial membranes. They lack an N-terminal targeting sequence and follow a unique import pathway involving the interaction with specialized import components in the outer membrane, the intermembrane space, and the inner membrane (Truscott and Pfanner, 1999). MSCs probably function as homodimers, each monomer being folded in the membrane into six transmembrane segments (Palmieri, 2004).

Members of this family are involved in the transport of several heterogeneous substrates such as ADP/ATP, phosphate, citrate, fumarate/succinate, carnitine, ornithine, aspartate/glutamate, etc. (Palmieri, 2004). A subfamily of MSCs that are calcium-binding mitochondrial solute carriers (CaMSCs) was recently identified in mammalian and non-mammalian species. CaMSCs are characterized by the presence of additional domains named EF-hands, calcium-binding motifs present in different calcium-binding proteins. One of these CaMSCs, Aralar, is specifically expressed in excitable tissues and localizes to mitochondria (del Arco and Satrustegui, 1998). Subsequently, another protein called Aralar2 (78% identical to Aralar) was identified, showing the same subcellular localization of Aralar, but with a different expression pattern, being confined to liver and to non-excitable tissues (del Arco et al., 2000). Aralar2 is identical to Citrin, the product of the gene mutated in type-II citrullinaemia (Kobayashi et al., 1999). Aralar and Citrin correspond to two isoforms of the mitochondrial aspartate/glutamate carrier. Weber et al. (1997) isolated from rabbit intestine another member of this subfamily named Efinal, which was reported to localize mainly to peroxisomes with a minor fraction detectable in mitochondria. The fact that both organelles use solute carriers of the same superfamily underlines the close relationship between mitochondria and peroxisomes. The presence of calcium-binding domains corresponding to the EF-hands in some of these proteins opens up the possibility that these polypeptides might be involved in the handling of calcium or in calcium regulated metabolite transport in these subcellular compartments. More recently, a human subfamily of three Small Calcium-binding Mitochondrial Carriers named SCaMC or APC (ATP-Mg/Pi carriers) has been described (del Arco and Satrustegui, 2004; Fiermonte et al., 2004): the *SLC25A24* gene is the human ortholog of the rabbit Efinal protein (Weber et al., 1997), *SLC25A25* is the human ortholog of the rat MCSC protein (Mashima et al., 2003), while *SLC25A23* represents a completely novel gene. These genes encode the human isoforms of the ATP-Mg/Pi carrier described in the past in whole mitochondria (Aprille, 1993). Their main function is probably to catalyze the net uptake or efflux of adenine nucleotides into or from the mitochondria.

In the present work we describe the studies we have performed on the cellular expression and alternative splicing of *SLC25A23* gene.

2. Materials and methods

2.1. Isolation of human SLC23A25

We have initially determined the full-insert sequence of the IMAGE cDNA clone 1565949 (1430 bp, GenBank accession no. AJ512835). A bioinformatic analysis of the EST database revealed that the vast majority of IMAGE clones deriving from the same transcriptional unit have a common 3′ sequence that differs from that of cDNA clone 1565949 (data not shown). This finding indicates that clone 1565949 probably represents a rare transcript isoform of this gene. To isolate the most common isoform, we devised a RT-PCR-based strategy using primers derived from both clone 1565949 sequence and 3′ ESTs in dbEST (data not shown). This effort, together with the characterization of four additional IMAGE cDNA clones corresponding to the *SLC25A23* transcript (188405, 1238507, 1527325 and 2408186), allowed us to assemble the complete sequence of the cDNA (3425 nt, GenBank accession no. AY750170). More recently a putative full-length cDNA clone MGC2615 (IMAGE cDNA clone 3356718) has been retrieved from the Mammalian Gene Collection and characterized (GenBank accession no. BC001656). The 3′ sequence of clone MGC2615 also differs from that of the most common transcript isoform.

IMAGE cDNA clones have been obtained from the UK Human Genome Mapping Project (HGMP) Resource Centre. Automated sequencing (using an Applied Biosystem ABI 3100 fluorescent sequencer) was performed using vector and gene specific oligonucleotide primers. Sequence assembly and editing was performed using both the AutoAssembler version 1.4 (Perkin Elmer-Applied Biosystem) and DNA Strider 1.2 (Marck, 1988) software programs.

2.2. Bioinformatic analysis

dbEST searches were performed as previously described (Banfi et al., 1998). Human PAC or BAC genomic sequences were analyzed using the NIX software at the HGMP (Borsani et al., 1998).

Multiple sequence alignment was performed using the ClustalW algorithm (Thompson et al., 1994). *SLC25A23* nucleotide and amino acid sequences were compared to the non-redundant sequence databases present at the NCBI (National Center for Biotechnology Information) using BLAST version 2.0 (Altschul et al., 1997).