

A novel creatine kinase cDNA whose transcript shows enhanced testicular expression

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A cDNA (TCK1) encoding a creatine kinase (CK) subunit has been isolated from a cDNA library prepared from rainbow trout testis poly(A) + RNA. The predicted amino acid sequence for this CK subunit showed a novel amino-terminal coding region. Northern blot analyses demonstrated preferential expression of this CK subunit in testis relative to other CK containing organs. Southern blot analysis suggested a single copy number for this CK gene.

ATP levels in vertebrate cells are largely regulated by creatine kinase (EC 2.7.3.2). At least three dimeric isoforms BB, BM and MM, and a related mitochondrial form exist in mammals [1]. Distinct patterns of tissue-specific CK isotype expression have been demonstrated [2]. The vertebrate testis often contain protein variants [3] and generate large amounts of ATP, hence, it is a good candidate organ for a CK variant.

We have prepared independent trout testis cDNA libraries in either lambda gt11 [4] or Bluescript (Stratagene) plasmids from mid-stage testis RNA and have screened them for cDNAs showing preferential or unique expression in the testis. A cDNA, TCK1 (1312 bp), has been isolated encoding a creatine kinase subunit of 383 amino acids with a predicted molecular weight of 43 010.

The complete nucleic acid sequence aligned with the predicted amino acid sequence is depicted in Fig. 1. A homology comparison [5] of the DNA sequence to the EMBL and Genbank nucleic acid sequence databases (releases 19.0 and 60.0, respectively) and of the predicted amino acid sequence to the NBRF-protein sequence database (release 21.0) showed, respectively, an optimum sequence homology of 78.5% to the rabbit brain CK DNA sequence (AC No.: RABCKB) and 85.6% to the chicken brain CK amino acid sequence (AC No.: A24793). The predicted sequence of the fourteen

amino-terminal residues was unlike any CK sequence previously reported. The possibility of a cloning or sequencing artefact for this region can be ruled out by the following observations. First, other reading frames in this region cannot generate the usual consensus CK sequence. Second, additional 5'-end cDNA CK clones isolated from an independently constructed cDNA library showed the identical coding sequence. As TCK1 was isolated from a spermatogenic testis, an organ rich in mitochondria, the possibility exists that this cDNA represents a mitochondrial CK clone. However, TCK1 has only limited homology to any of the known mitochondrial CK sequences [6,7] and is more homologous to B- and M-CK subunit sequences. Although it has been reported that there is exceptional homology of the 3'-untranslated region of B-CK cDNAs [8], comparison of this region of TCK1 to all other reported CK sequences showed no significant homologies.

The novel sequence characteristics of TCK1 raise the possibility that its transcript shows testis-specific or testis-enhanced expression. This possibility is addressed by the Northern blot analysis depicted in Fig. 2. When the entire TCK1 fragment was used as a probe (Fig. 2A), CK transcripts were detected in testis, brain, small intestine, heart, and at very low levels, in spleen and kidney. Variations in the CK transcript sizes detected may reflect different sizes of CK subunit transcripts or may be the result of differences in post-transcriptional modifications such as polyadenylation. No CK transcripts were detected in the gill or liver RNA preparations although prolonged autoradiograph exposures were not performed. When the 3'-untranslated region of TCK1 was used as a probe to a replicate Northern blot (Fig. 2B), however, CK transcripts were detected almost exclusively in the testis. Brain CK transcripts were

The sequence data in this paper have been submitted to the EMBL/Genbank Datalibraries under the accession number X53859.

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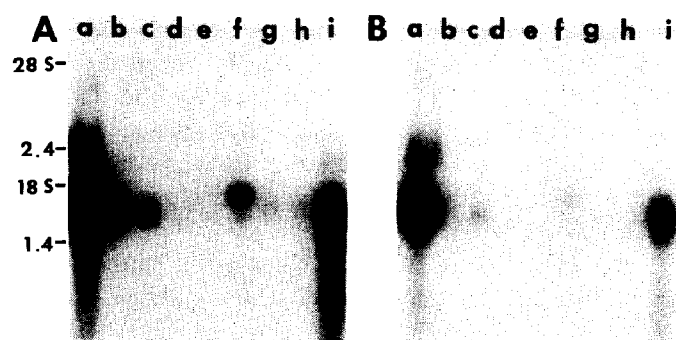


Fig. 2. Northern blot analysis of total RNA isolated from a variety of trout organs and probed with the complete TCK1 clone (panel A) or its 3'-untranslated region (panel B). Replicate blots were made from a 1.5% agarose-formaldehyde gel by standard methods [13]. Lanes a-h contained 10 μ g of total RNA isolated from testis, brain, small intestine, gill, liver, heart, spleen and kidney. Lane i contained 5 μ g of testis RNA. The blot depicted in panel A was hybridized under standard conditions [13] using the entire TCK1 cDNA radiolabelled to high specific activity by random priming [12]. The replicate blot depicted in Panel B was hybridized with the 132 bp *Mae*III/*Eco*RI 3'-untranslated region fragment of TCK1. The positions of RNA molecular weight markers (Bethesda Research Laboratories) and of ribosomal RNAs are indicated to the left of panel A. Only that portion of the autoradiograph showing hybridization is depicted.

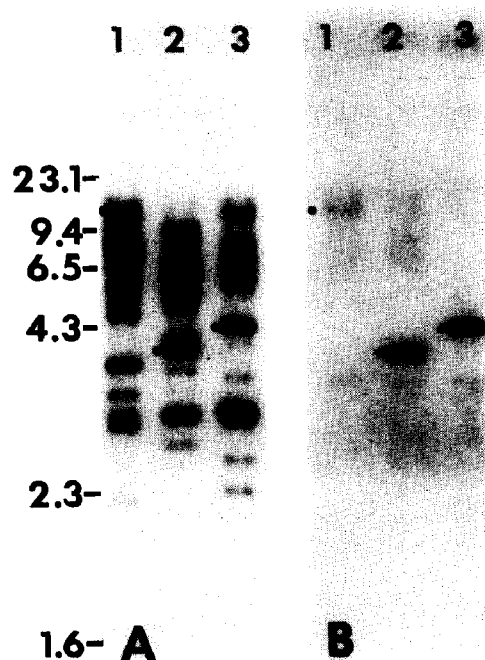


Fig. 3. Southern blot analysis of trout genomic DNA probed with the complete TCK1 clone (panel A) or its 3'-untranslated region (panel B). Replicate blots were made from a 0.8% agarose-TBE gel by standard methods [13]. Radioactive probe generation and hybridization conditions were as described in the legend to Fig. 1. Lanes 1-3 contained 10 μ g of DNA digested with the restriction enzymes *Eco*RI, *Hind*III, and *Xba*I, respectively, under conditions recommended by the enzymes' manufacturers. The positions of DNA molecular weight markers are indicated to the left of panel A. The hybridizing bands common to both probes are indicated by the dots (●). Only that portion of the autoradiograph showing hybridization is depicted.

statement is only speculative and this region of TCK1 may turn out to be in common with other trout B- and M-CK cDNA clones. The answers to these questions will require the isolation of other CK cDNA clones from the trout and from the testis of other species.

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