Expression of Sarcomeric Mitochondrial Creatine Kinase in Antarctic Fishes

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Abstract

Creatine kinase (CK) plays an important role in the ATP energy cycle by acting as an "energy shuttle" between the mitochondrion and the site of ATP consumption. Sarcomeric mitochondrial creatine kinase (sMtCK) cDNA is found within the heart ventricle of both the icefish *Chaenocephalus aceratus* and the red-blooded Antarctic fish *Notothenia coriiceps*, but while *N. coriiceps* expresses CK as protein, separate studies within our lab have shown that CK protein is not expressed in *Chaenocephalus aceratus*. The goal of our research was to sequence sMtCK in two more fish: the icefish *Chionodraco rastrospinosus* and the red-blooded fish *Gobionotothen gibberifrons* and to determine whether sMtCK mRNA is expressed in icefish and red-blooded fish. We did this by performing PCR with degenerate and specific primers so as to amplify sMtCK, cloning the PCR products into *E. coli* and sequencing the clones. However, the PCR products could not be identified as sMtCK. We therefore concluded that sMtCK mRNA is not expressed in these two fish species. This conclusion is consistent with separate protein studies underway in our laboratory. Further studies are needed to determine whether these two fish species express ubiquitous MtCK, which is the other isoform of CK found in mitochondria.

Background

The sarcomeric isoform of creatine kinase (sMtCK) is an enzyme located between the inner and outer membranes of the mitochondrion; this location is thought to be important to its function in the energy production cycle of the cell (Schlattner, 2005). CK helps ensure that energy levels within cells remain stable. This is achieved by transferring the phosphate molecule from adenosine triphosphate (ATP) to creatine. The product, creatine phosphate, diffuses through the cytosol more efficiently than ATP, acting as an energy shuttle (Figure 1).

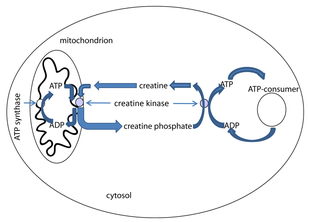

Figure 1. The role of creatine kinase and creatine as an energy shuttle between the mitochondria and the ATP consumer in the cytosol.

We hypothesized that sMtCK would be present within the cardiac cells of Antarctic fishes because of its role in energy regulation. Previous studies showed that sMtCK cDNA exists within the ventricle of Antarctic icefish *Chaenocephalus aceratus* (Winnard, 2003). However, preliminary data from our lab suggested that sMtCK protein is not expressed in the heart ventricle of this icefish, but is expressed in the closely-related red-blooded Antarctic fish *Notothenia coriiceps*. The aim of this study was to sequence sMtCK in the Antarctic icefish *Chionodraco rastrospinosus* and the red-blooded Antarctic fish *Gobionotothen gibberifrons* and then to measure the expression of sMtCK mRNA within the ventricle of icefishes and compare it to the level of CK mRNA in red-blooded Antarctic fishes.

Methods

- We performed PCR with a degenerate reverse primer and a gene-specific forward primer and cDNA from the heart ventricle of *C. rastrospinosus* and *G. gibberifrons* to amplify the sarcomeric mitochondrial creatine kinase (smtCK).

- We separated the PCR products using a 2% agarose gel and cut out bands having the appropriate number of base pairs (~750 bp) (Figure 2).

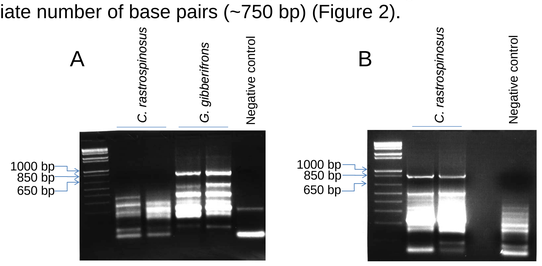

Figure 2. Results of gel electrophoresis with *G. gibberifrons* cDNA (A) and *C. rastrospinosus* cDNA (B). The length of the ladder fragments are indicated by the numbered arrows to the left of the image.

Methods (continued)

- We isolated the DNA from the cut-out bands using a QIAquick Gel Extraction Kit.

- We cloned the isolated DNA fragments through the use of TOPO TA Cloning kit by inserting the fragment into *E. coli*. We selected for *E. coli* with our DNA fragment by treating the LB plate with ampicillin and X-GAL (Figure 3).

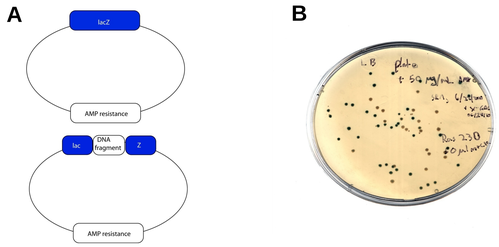

Figure 3. (A) Normal vectors express the lacZ gene. This allows *E. coli* to convert X-GAL into a product, which makes the colonies appear blue. However, the insertion of the DNA fragment into the vector disrupts lacZ gene expression, causing the colony to remain white. (B) Picture of an LB plate with white and blue *E. coli* colonies.

- We picked two white colonies from each LB plate and allowed them to grow overnight in LB media. We isolated the vectors by using a QIAprep Spin Miniprep Kit.

- We then prepared the samples for sequencing using a Big Dye Terminator Cycle Sequencing Kit. The Big Dye reactants amplify the DNA but randomly terminates the extension, generating fragments of different sizes.

- The products of the Big Dye reaction were then filtered through sephadex-filled spin columns so as to removed the unused reactants from the Big Dye reaction.

- We then sent the samples to the Core Lab at the University of Alaska Fairbanks for sequencing.

Results

- After receiving the sequences from the Core Lab, we analyzed them and used Genbank to search for similar sequences. In theory, if our sequence closely matched with the sMtCK sequence from another organism, it would tell us that sMtCK existed within *C. rastrospinosus* and *G. gibberifrons*.

- Although our sequences aligned with other known sequences, there was insufficient coverage (< 24%) to identify our sequences as sMtCK (Figure 4).

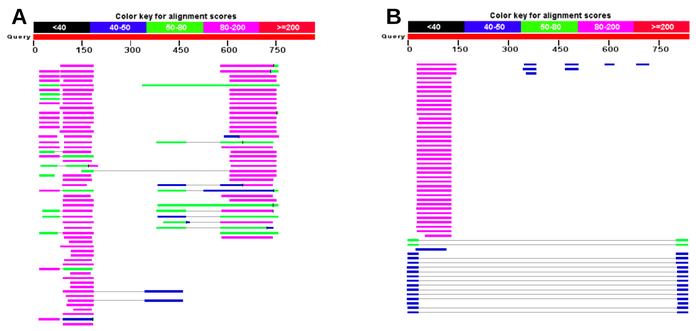

Figure 4. Blast results from GenBank for *C. rastrospinosus* (A) and *G. gibberifrons* (B). Under optimum conditions, there will be red bars, as this means that there is a close match, whereas white regions within the sequence show no match at all.

- These results indicate that there is mostly likely no cDNA for sMtCK within the heart ventricle of *C. rastrospinosus* or *G. gibberifrons*, and therefore no expression of sMtCK mRNA.

- Our findings are consistent with studies in our lab which compare sMtCK protein expression between icefish and red-blooded fish. These protein studies showed that no sMtCK protein was expressed in mitochondria isolated from the heart ventricle of *C. rastrospinosus* or *G. gibberifrons*.

Results (continued)

- Although the sequences from *C. rastrospinosus* and *G. gibberifrons* did not match any known sequence, a previous study by Winnard and colleagues (2003) and a study in our lab have already sequenced sMtCK in *C. aceratus* and *N. coriiceps*.

- We used this information to create primers to measure the cDNA levels of sMtCK in the icefish *C. aceratus* as compared to the red-blooded fish *N. coriiceps* (Figure 5).

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N. coriiceps F ctggccggacgctactacggcctgcaagacctgacagacaaggagcagcagcagctcatt
N. coriiceps R ctggccggacgctactacggcctgcaagacctgacagacaaggagcagcagcagctcatt
C. aceratus    ctggccggacgctactacggcctgcgagacctgacagacaaggagcagcagcagctcatt
               ************************* **********************************

N. coriiceps F gacgaccacttcctgtttgacaagcctatctctcctctgttgacatgtgcctttatggcc
N. coriiceps R gacgaccacttcctgtttgacaagcctatctctcctctgttgacatgtgcctttatggcc
C. aceratus    gacgaccacttcctgtttgacaagcctgtctctcctctgttgacatgtgcctttatggcc
               ************************* **********************************

N. coriiceps F agagactggcctgacgccaggggcatctggcacaataatgaaaagacctttctaatctgg
N. coriiceps R agagactggcctgacgccaggggcatctggcacaataatgaaaagacctttctaatctgg
C. aceratus    agagactggcctgacgccagaggcatctggcacaacaatgagaagaccttcctgatctgg
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Figure 5. The alignment of parts of sMtCK sequence from *N. coriiceps* and *C. aceratus*. The blue line shows a conserved splice site, the blue letters indicate the site to which the reverse primer binds, the red letters indicate the site to which the forward primer binds, and the stars show bases that match between the two species.

Future direction

- Due to time constraints, we were unable to measure the actual expression of sMtCK in the heart ventricles of *C. aceratus* and *N. coriiceps.*

- These studies only focused on one form of mitochondrial creatine kinase. Besides the sarcomeric form of CK, there is also an ubiquitous form of CK in mitochondria. It is possible that *C. rastrospinosus* and *G. gibberifrons* express the ubiquitous form but not the sarcomeric form of CK. It is also possible that for some reason, the primers failed to amplify the sMtCK; perhaps sMtCK exists at such a low level that we could not detect it.

- To determine whether or not *C. rastrospinosus* and *G. gibberifrons* express the ubiquitous MtCK (uMtCK), similar experiments to this one could be conducted. These experiments could use PCR with degenerate primers that bind to uMtCK instead of sMtCK.

- If uMtCK was found and sequenced in the heart ventricle of *C. rastrospinosus* and *G. gibberifrons*, quantitative real-time PCR could be performed to measure the expression of uMtCK in the heart ventricle of these fishes.

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