Summer 2009

Functional characterization of SCP1 in Procambarus clarkii using RNA interference

Robert Carpenter

Functional characterization of SCP1 in *Procambarus clarkii* using RNA interference

Robert O. Carpenter '10, Christopher M. Gillen, Department of Biology, Kenyon College, Gambier, OH

Abstract

SCP1 is an invertebrate EF-hand calcium binding protein that is hypothesized to play an important role in muscle relaxation. SCP1 is highly expressed in axial abdominal muscle (tail), and is especially abundant in fast-twitch muscle fibers. We have found three variants of the *Procambarus clarkii* sarcoplasmic calcium binding protein (pcSCP1a, pcSCP1b, pcSCP1c). In this study, we use RNA interference (RNAi) to reduce the expression of all three variants and explore the function of pcSCP1. We used relative quantification real-time PCR to evaluate the expression of pcSCP1 in control and dsRNA injected crayfish, using variant-specific primers, primers from the non-variable region, and 18s ribosomal RNA as an endogenous control. We injected 559 bp dsRNA to reduce pcSCP1 expression, resulting in an average 55% reduction of expression in comparison to controls. In individual crayfish, the amount of pcSCP1 reduction varied from no reduction to 10-fold reduction compared to controls. Upon visual inspection, crayfish injected with dsRNA were found to be lethargic when compared to controls. The use of RNAi helped us visualize the functional effects of decreased pcSCP1 expression, and provides a great tool for further investigation into proteins that are believed to assist in the muscle relaxation system.

Introduction

Sarcoplasmic Calcium Binding Protein (SCP) is hypothesizedto play an important/significant role in muscle relaxation of fast-twitch muscle fibers, similar to the function of vertebrate parvalbumin [1,2].

The freshwater crayfish, *Procambarus clarkii*, has been used to study the expression of SCP and other genes that play a role in regulating calcium transport. The unique molting cycle of P. clarkii transports an extensive amount/quantity of calcium and consequently provides an excellent model to study genes involved in regulating calcium transport.

SCP has been shown to have the highest expression in the fast-twitch portions of the posterial axial muscle (tail) of crayfish. In order to understand the function of the calcium binding protein, we used RNA interference to knock down the expression of the SCP gene. Injecting double-stranded RNA blocks the expression of the SCP gene and allows one to see the effect its absence on the crayfish. **Therefore, our aim was to probe the function of SCP by associating the expression of the gene with a change in movement or behavior.**

References

[1] Lexie White, Suzanne Rohrback, Christopher Gillen, unpublished data.
[2] Cox, J.A., Stein, E.A. (1981) Characterization of a New Sarcoplasmic Calcium-Binding Protein with Magnesium-Induced Cooperativity in the Binding of Calcium. Biochemistry 20: 5430-5436.
[3] BLOCK-iT RNAi TOPO Transcription Kit, For TOPO-mediated generation of templates and production of double stranded RNA (dsRNA) for use in RNA interference (RNAi) analysis. (2005) Invitrogen.

Acknowledgements

I would like to thank Professor Chris Gillen for his advice and assistance throughout the project. I would also like to thank Aaron Yeoh for his assistance in the lab. This was a Kenyon College Summer Science Scholars research opportunity that was funded by the National Science Foundation.

Materials and Methods

Freshwater crayfish were obtained from Carolina Biological Supply and acclimated in water tanks at room temperature (23ºC) for one week. Crayfish were paired based on weight and pairs were injected with either crayfish saline (7.4pH) or dsRNA in crayfish saline (500ng/µL). Crayfish were sacrificed after 48hours and their tail tissues were harvested and frozen at -80ºC. Total RNA was isolated using the RNA STAT-60 reagent (Tel Test "B"), and DNA was removed with Turbo DNA-free kit (Ambion). Total RNA was reverse transcribed into cDNA using random hexamers with reverse transcriptase (Applied Biosystems). The amount of cDNA was quantified using Real-Time PCR and comparing dCT values between the control crayfish and dsRNA injected crayfish.

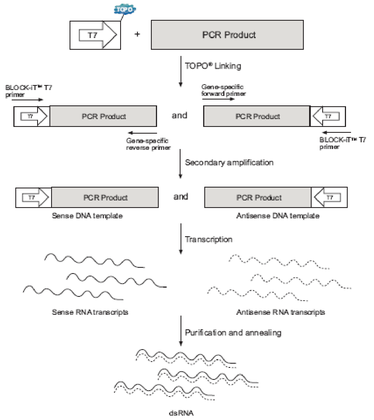

Image from Block-iT RNAi TOPO Transcription Kit Manual (Invitrogen), [3].

Figure 1. *Synthesis of dsRNA.* Oligonucleotide primers were designed to amplify a 559 bp region of the crayfish's SCP gene. The amplified regions were linked to T7 promoter sequences (Invitrogen) and sense and antisense sequences were amplified using the target primers. Sense and antisense templates from secondary amplification were used for RNA transcription. Equal concentrations of ssRNA transcripts were combined to form dsRNA.

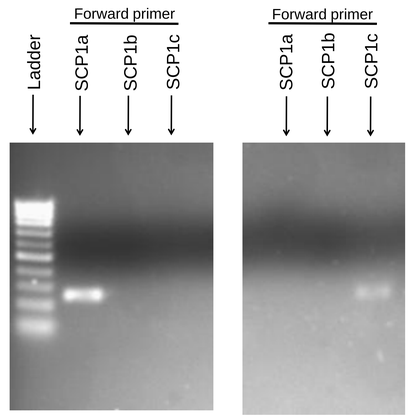

Figure 2. Gel electrophoresis of PCR reactions showing primer specificity. SCP1a and SCP1c forward primers only amplified products from plasmid templates corresponding to their respective variant. The same reverse primer was used in all reactions.

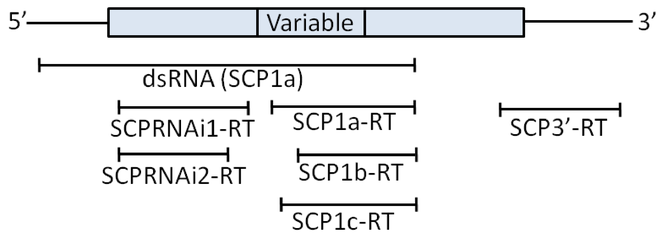

Figure 3. Map of SCP cDNA including important primers used. The section highlighted in light blue is the open reading frame. The variable region is also labeled. dsRNA was created using the SCP1a DNA variant template.

Future Work

- Characterize the expression of other genes that play a role in calcium transport (SERCA, PMCA, CaM) in response to a decrease in SCP expression.
- Exercise the crayfish after injection to quantify the sluggishness realized by the crayfish due to RNA interference.
- Design dsRNA to selectively interfere with each splice variant, so we can visualize potential functional difference between the splice variants.

Results

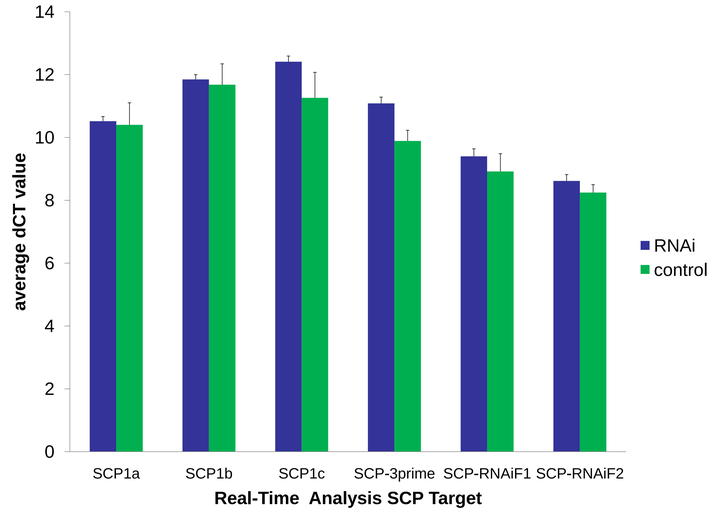

Figure 4. Effect of injected dsRNA on expression of tail SCP in *P. clarkii* using Real-Time PCR analysis. dCT is the difference between 18s and SCP in the number of cycles needed to amplify cDNA to a threshold value. A higher dCT value is indicative of lower SCP expression. These data are from a single batch of crayfish (n=3 for each treatment). Error bars are standard deviation.

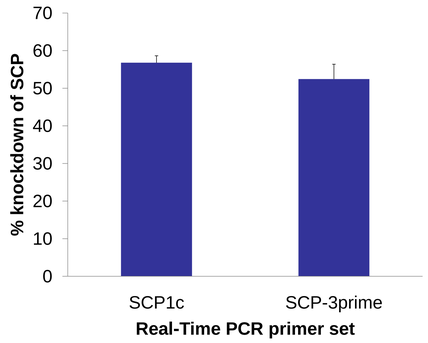

Figure 5. The percent change in SCP expression between control and dsRNA injected crayfish. Data are average of two different trials. Error bars are standard deviation. (n=7 control, n=6 dsRNA)

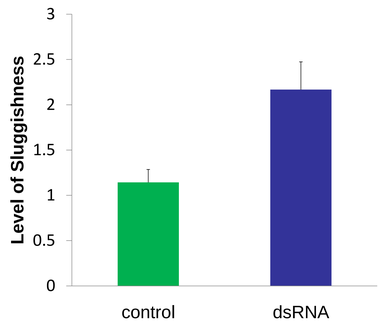

Figure 6. Comparison between control and dsRNA crayfish on their level of sluggishness 48 hours after injection. Level of sluggishness is from 1-3 with 1 meaning no change in movement, 2 signifying some decrease in movement, and 3 as substantial sluggishness or no movement. (n=7 control, n=6 dsRNA)

Conclusions

- **Real-Time PCR showed that dsRNA injected crayfish had decreased expression of SCP in comparison to controls. We were able to consistently decrease expression of pcSCP1 by ~50%, leading to a significant change between dsRNA injected individuals and control crayfish.**
- **Visual assessment of the crayfish after injection revealed that crayfish injected with double-stranded RNA were consistently more sluggish than control crayfish.**
- **Our data support a role for SCP in relaxation of fast-twitch muscle fibers.**