

Foraging gene in black-legged tick, *Ixodes scapularis*:
preliminary sequence information

Kevin Dougherty

Department of Biological Sciences

Fordham University, 441 E. Fordham Rd.

Bronx, NY 10458

Abstract

The foraging (*for*) gene encodes a cGMP-dependent protein kinase (PKG) and has been linked to variation in feeding phenotypes of the honey bee, the fruit fly, and the nematode. The objective of this study was to examine the *for* gene in black-legged tick, *Ixodes scapularis*. Using primers designed from an alignment of known mRNA transcripts, tick DNA was isolated and sequenced.

Introduction

Despite variation in selective pressures, both the sequence and function of many genes are often conserved across metazoa (Fitzpatrick and Sokolowski 2004). It is assumed that these highly conserved genes encode proteins with a vital role (e.g. metabolic function, development, etc) in the biology of all animals. Researchers have taken this candidate gene approach to examine the genetic underpinnings of behavioral plasticity (Fitzpatrick et al. 2005). For instance, the foraging (*for*) gene encodes cGMP-dependent protein kinase (PKG) and potentially has a conserved role in food-related behaviors. Variation in foraging has been linked to the cGMP signal transduction

pathway in the fruit fly, *Drosophila melanogaster* (Osborne et al. 1997, Kalderon and Rubin 1989), the honey bee, *Apis mellifera* (Ben-Shahar et al. 2002), and the nematode, *Caenorhabditis elegans* (Fujiwara et al. 2002). To further our understanding of when the link between the *for* gene and behavior evolved, behavioral genetic analyses need to be undertaken with non-model genetic organisms (Fitzpatrick and Sokolowski 2004).

Despite the medical and agricultural significance of many arthropods, there are still large gaps in our understanding of their biology. Ticks vector numerous etiological agents, yet relatively little is known about the tick genome (Hill and Wikel 2005). Black-legged ticks, *Ixodes scapularis*, are involved in the transmission of Lyme Disease (*Borrelia burgdorferi*), human granulocytic ehrlichiosis (*Anaplasma Phagocytophilum*), and babesiosis (*Babesia microti*). Sequence information on the tick ortholog of the foraging gene could represent a novel target for tick-borne disease control.

The objectives of this study were to expand the scope of comparative eukaryotic analyses, examine the *for* gene in ticks, and contribute to the *Ixodes scapularis* genome project. Specific primers designed from an alignment of four orthologs of the *for* gene were used in a PCR reaction with tick DNA. I sequenced the PCR product and compared it with the mRNA transcripts used in the alignment.

Materials and Methods

Total DNA Extraction

Total genomic DNA was extracted from a pool of adult ticks using the DNeasy Tissue Kit (Qiagen, Germantown, MD). The extraction followed the manufacturer's protocol for isolation of DNA from insects with a few slight modifications. Ten ticks were placed in a 1.5-ml microcentrifuge tube, frozen in liquid nitrogen, and crushed using

a pestle. The pulverized ticks were incubated overnight in 180 μ L of buffer ATL and 20 μ L of proteinase K at 55°C. The tube was vortexed occasionally during the incubation. The only other deviation from the manufacturer's protocol was the final step. DNA was eluted from the spin column twice with 50 μ L of warm deionized water (70°C). DNA from *D. melongaster* was isolated in a similar fashion to serve as a positive control for the primers.

Primer Design and PCR

I designed primers from an alignment of PKG mRNA transcripts from four insect species; *Drosophila melanogaster* (NM_058140), *Bombyx mori* (AF465600), *Anopheles gambiae* (XM_319605), and *Apis mellifera* (AF469010). The primers (forAF1 5' CAGATGAAGAAGGCCAG 3' and forBR1 5' CATCAGCATGTACAGGTACT 3') recognized a highly conserved region of the mRNA transcript and were designed to amplify a 140 bp product of genomic DNA.

Each 25 μ l polymerase chain reaction contained 2-200 ng template DNA, 2.5mM dNTPs, 50 mM MgCl₂, 5 pmol of each primer, and 0.175 μ l Taq DNA polymerase (5 U/ μ l) in 10x PCR buffer. Reaction cycling conditions were as follows: 94°C (5 min); 50 cycles of 94°C (30s), 56°C (30s), 72°C(30s); then 72°C for 7 min. PCR products were stained with ethidium bromide and visualized under UV light.

Gel purification, Ligation and Transformation

Amplified sequences were separated on a 1% agarose gel, excised from the gel, and purified using Rapid Gel Extraction System (Marligen Biosciences Inc.). PCR products were eluted in 28 μ l of warm dH₂O (70°C). I then ligated the PCR products into

pGEMT vectors (Promega, Madison, WI). The vectors were transformed into JM109 cells and incubated overnight in media containing luria broth, ampicillin, Xgal and IPTG. DNA was purified from inoculated colonies using the Rapid Plasmid Purification Kit (Marligen Biosciences Inc.).

DNA Sequencing

Nucleotide inserts were sequenced using AmpliCycle Sequencing Kit (Perkin Elmer), a modified Sanger dideoxy sequencing method. Each 8 µl sequencing reaction contained 2 µl of either ddGTP, ddATP, ddTTP, or ddCTP termination mixtures and 6 µl of a master mixture (10X buffer, 50 fmol template DNA, α33-ATP, SP6 primer). Each reaction was covered with 12 µl mineral oil and placed in an Applied Biosystems Thermal Cycler. Reaction cycling conditions were as follows: 94°C (3 min); 35 cycles of 94°C (30s), 58°C (30s), 72°C (1 min). PCR products were run on a denaturing polyacrylamide gel. The nucleotide sequence was visualized using autoradiography and aligned with known sequences using MacVector 6.5.3.

Results

Total genomic DNA was extracted from a pool of ten adult ticks. Specific primers (forAF1 5' CAGATGAAGAAGGCCAG 3' and forBR1 5' CATCAGCATGTACAG-GTACT 3') were designed and used to isolate and sequence a small fragment (180bp) of the PKG gene in ticks (Figure 1). Unfortunately, I was unable to obtain quality sequence information before the end of funding.

Tick PCR Products



Figure 1. Amplified sequences (180 bp) were separated on an agarose gel. Faint bands were combined, purified using the Rapid Gel Extraction System (Marligen Biosciences Inc.), and cloned using pGEMT vector (Promega).

Discussion

Tick gene data are a valuable resource for studying tick biology, host-tick-bacteria relationships, and evolutionary biology (Hill and Wikel 2005). Despite potential problems with gene prediction among distantly related species, I was able to amplify tick DNA using specific primers. During future efforts, I will utilize RACE methods to isolate and sequence cDNA using degenerative primers. Once sequence information is obtained, I will examine the expression of *Isfor* (*I. scapularis* ortholog of the *for* gene) in relation to tick questing behavior (e.g. larvae versus adult questing phenotypes).

Acknowledgements

I am eternally grateful to Dr. Rubin for the opportunity to pursue my interests in his laboratory. I would also like to thank the brave teaching assistants, Jinsong Qiu and Lisa Sarran, for their tireless efforts. Special thanks to Jinsong for his incredible patience.

References

- Ben-Shahar, Y., A. Robinson, M.B. Sokowski, and G.E. Robinson. 2002. Influence of gene action across different time scales on behavior. *Science* 296: 741-744
- Fitzpatrick, M.J. and M.B. Sokolowski. 2004. In search of food: the evolutionary link between cGMP-dependent protein kinase (PKG) and behavior. *Integr. Comp. Biol.* 44: 28-36.
- Fitzpatrick, M.J., Y. Ben-Shahar, H.M. Smid, L.E.M. Vet, G.E. Robinson, and M.B. Sokolowski. 2005. Candidate genes for behavioral ecology. *Trends in Ecology and Evolution* 20: 96-104.
- Fujiwara, M., P. Sengupta, S.L. McIntire. 2002. Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4cGMP-dependent protein kinase. *Neuron* 36:1091-1102.
- Hill, C.A. and S.K. Wikel. 2005. The *Ixodes scapularis* Genome Project: an opportunity for advancing tick research. *Trends in Parasitology* 21: 151-153.
- Kalderon, D. and G.M. Rubin. 1989. cGMP-dependent protein kinase genes in *Drosophila*. *Journal of Biological Chemistry* 264:10738-10748.
- Osborne K.A., A. Robinson, E. Burgess, S. Butland, R.A. Shaw, A. Coulhard, H.S. Pereira, R.J. Greenspan, and M.B. Sokowski. 1997. Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* 277: 834-836

