Final Progress Report September 30 1999

Texas Imported Fire Ant Research and Management Project

Title of project: Characterization of fire ant G protein-coupled receptors (GPCRs)

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Major accomplishments to date (Sep. 1/97- September 30/99):

We have cloned several fire ant cDNAs from worker ants from a monogyne colony using cDNA that we synthesized.

1. We have fully sequenced a proton channel (16kDa) subunit from the Vacuolar ATPase, a ubiquitous pump that energizes intracellular compartments and apical epithelia in insects. This is a recent finding, not previously reported. We recently performed a northern blot of whole workers from a polygyne colony and obtained transcript sizes of 10, 5 and 2 kb. The expected size of the transcript was about 2 kb as this is the size present in other insects such as budworm, *Heliothis virescens* and the hornworm, Manduca sexta. The significance of these other two transcripts is unknown but it is possible that there is more than one gene for this protein, or that the mRNA is spliced in more than one site. It would be interesting to determine if the bigger transcripts encode more than one open reading frame (multiple proteins).
2. We have cloned partially a G protein-coupled receptor, a serotonin receptor. This cDNA may encode a receptor involved in regulating key functions in the fire ant.
3. Most importantly we have discovered a cDNA fragment from an orphan transporter in the fire ant, most similar to the glucose transporter of vertebrates. There is only one known insect sugar transporter, this has recently (1999) been cloned from Drosophila melanogaster. However this fruit fly transporter belongs to a different group than the putative sugar transporter that we have identified. The transporter we have partially sequenced is similar to the nervous system type of transporters in vertebrates. This opens up a new line of research and to the discovery of critical proteins in the fire ant that could be selectively disrupted for control: The only source of energy of the nervous system is glucose. This has also implications for other hymenopterans, such as other pest ants or the honeybee.
4. We have partially sequenced a ribosomal protein and a fragment of myosin heavy chain.
5. A student partially supported by this project graduated with a Ph.D. in Toxicology.

Goals achieved:

We made a variety of discoveries as indicated above. With respect to the original goals of the proposal we succeeded in using the strategy of cloning by sequence similarity to obtain a G protein-coupled receptor cDNA, from a putative a serotonin receptor. This research has potential for commercial developments. A Ph. D. student supported under this research graduated in May 1999.

Relevance to the Texas Imported Fire Ant Research and Management Project:

The research and discoveries under this project address a fundamental aspect of fire ant basic science; the gene discovery needed to target vulnerable systems in the fire ant. This research can lead to the development of various insecticides, repellents, antifeedants, etc. It has the potential to also interfere with the aggressive behavior of fire ants through the manipulation of the serotoninergic system.

Publications submitted/published; presentations/posters presented at national technical meetings/ conferences:

1. This research is described in detail in a Ph. D. dissertation by my former student, Sam K. Frazier, submitted in May 1999. The title of the dissertation is:

Three aspects of intercellular communication in insects: biosynthesis of Manduca sexta adipokinetic hormone and cloning of G protein-coupled receptors and other membrane proteins from the red imported fire ant, Solenopsis invicta *Buren.*

2. A poster of this research was presented by Sam Frazier during the Toxicology Graduate Student Forum, College of Veterinary Medicine, August 26, 1998. TAMU.

Signature: Date:

Patricia Pietrantonio September 30 1999

Appendix to final report 97/99

Northern blot of fire ant (*S. invicta* Buren) 16 kDa V-ATPase subunit c (30 June99-September 99)

In order to determine the number of transcript(s) and transcript size(s) encoding the V-ATPase subunit c, a northern blot was performed.

Methods

Extraction of total RNA

1) Polygyne fire ant workers (3.5 g) of were taken from storage at -80°C and placed in liquid N_2, then ground by hand with a mortar and pestle.
2) Powder from the ground ants was taken from the mortar and placed immediately into 35 ml TRIzol™ Reagent (Gibco BRL Gaithersburg, MD) and incubated 5 minutes at RT.
3) The mixture was centrifuged for 10 min at 10,397 g, 4°C; pellet and lipid layer were discarded.
4) To the supernatant, 7 ml of chloroform was added and mixed by shaking vigorously by hand for 15 sec. The mixture was then incubated for 3 minutes at RT.
5) The solution was centrifuged for 15 min at 10,397 g, 4°C; the aqueous phase was retained and the organic phase discarded.
6) 18-ml isopropanol were then mixed with the aqueous phase; RNA was allowed to precipitate overnight.
7) RNA solution was centrifuged at 10,397 g, 4°C for 10 minutes; supernatant was removed and discarded.
8) RNA pellet was allowed to air dry for 15 minutes, then was dissolved in 2 ml DEPC-treated H_2O and stored at -20°C.

Poly-A+ selection

Fire ant mRNA was isolated by performing poly A^+ selection on a 1.5-ml aliquot of total RNA. Oligotex mRNA purification kit (Qiagen Inc., Valencia, CA) and its accompanying batch protocol was used to purify poly A^+ mRNA.

Northern blot

Poly-A+ selected RNA was precipitated and resuspended in RNA sample loading buffer (Sigma). Following denaturation, the RNA was loaded in a gel (1% agarose, 0.66M formaldehyde, 1X MOPS) and run at 60 V for 2 ½ hours. Following electrophoresis, the gel was briefly soaked in DEPC water to remove formaldehyde, then in 20x SSC for 45 min. RNA in the gel was then transferred to a Schleicher & Schull Nytran membrane using a conventional upwards transfer protocol. After the membrane was allowed to dry, a DNA positive control was spotted onto an unused portion of the membrane.

Labeling of probe/Hybridization with ^{32}P

1) ULTRAhyb solution in heated in hybe oven set to 68°C; heating blocks set to 98°C and 37°C.
2) Template diluted to 2.5 ng/µl. Template used was gel-purified insert from the V-ATPase PCR product.
3) Once ULTRAhyb had dissolved, 10-20 ml were added to a bottle and used to pre-hybridize the membrane at 42°C for approximately 30 minutes.

Decaprime labelling:

1) Thawed ^{32}P-dCTP.
2) G50 column placed in collection tube inside 15-ml conical; spun 2 minutes to remove buffer, then column placed in new collection tube inside a new conical.
3) In Eppendorf tube, 10 µl of template DNA (25 ng), 2.5 µl 10x Decamer solution, and 1.5 µl water were mixed (total volume 14 µl).
4) DNA solution was heated to 98°C for 7 minutes, then quick frozen in liquid N_2.
5) DNA solution partially thawed then placed on ice.
6) Added 5 µl Reaction buffer (-dCTP), 5 µl α 32P-dCTP, and 1 µl of Klenow to the mix.
7) Incubated at 37°C for 10 minutes.
8) Added 1µl of 0.5 M EDTA to stop the reaction.
9) Added solution to G50 column and spun 4 minutes to collect probe.
10) Collected probe and checked 2 µl in scintillation counter to determine specific activity.
11) Probe denatured by adding 1/3 volume 2N NaOH, then added to hybridization tube with membrane.
12) Hybridized overnight (16 h) at 42°C.
13) Total specific activity of the radioactive probe was estimated at $3x10^7$ cpm.

Washing/detection

1) Membrane washed 2X 5 minutes in 2X SSC, 0.1% SDS at 42°C.
2) Membrane washed 2X 15 minutes in 0.1X SSC, 0.1% SDS at 50°C.
4) Blot allowed to air dry on a piece of Whatman 3M paper.
5) Exposures to X-ray film done at -80°C.

Materials:

α-^{32}P dCTP. NEN Life Science Products, Inc. Boston, MA.
ULTRAhyb™. Ambion, Inc. Austin, TX.
Ambion DECAprime II™ Random Priming DNA Labeling kit. Ambion, Inc. Austin, TX.Kit contains: Klenow, 10x decamer solution, 5x reaction buffer minus dATP, 5x reaction buffer minus dCTP, 0.5 M EDTA, water, and control DNA.

Select® G-50 spin columns with STE buffer. 5 Prime→3 Prime, Inc, Boulder, CO.

Results

Autoradiography revealed strong positive bands at sizes of approximately 10 kb, 5 kb, and 2 kb (Figures 1 and 2). Transcripts of 10 and 5 kb were not expected for such a small protein. An additional northern blot experiment using excess (2 M) formaldehyde in the agarose gel was run to insure that all RNA is completely denatured and resolve the sizes of the positive bands. The same results were obtained.

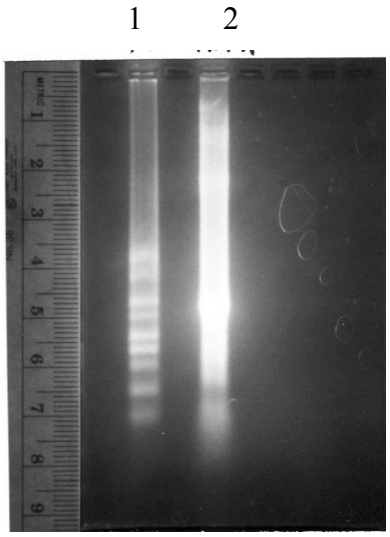

Fig. 1. Agarose gel, fire ant mRNA (Lane 2). Lane 1. Marker= Sigma RNA markers R7020.

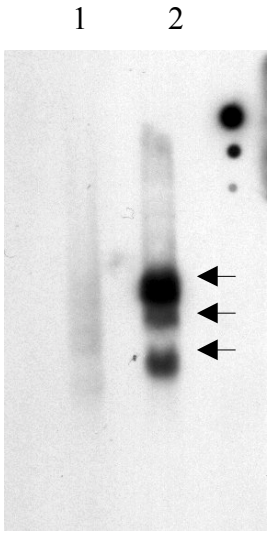

Fig. 2. Autoradiograph of northern blot from gel in Fig. 1. Arrows from top to bottom at 10-, 5- and 2-kb. Lanes 1 and 2 as in Fig.1.

Discussion

The detection of transcripts of 10- and 5-kb was unexpected while the detection of the 2kb transcript was expected since this transcript size has been reported for this gene in lepidopteran larvae (Pietrantonio and Gill, 1995). The 10-kb transcript appears to be more abundant according to the signal detected in the northern blot, followed by the 2kb and lastly the 5kb transcripts.