NRCPD-OUAVM Joint Research Report

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Project no: 2019-joint-10

1. Principal investigator

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Position: Professor

Affiliation: Faculty of Life and Environmental Sciences, University of Tsukuba, Japan

2. Project title:

Identification, Characterization and Functional Analysis of the Vitellogenin Receptor in the soft tick *Ornithodoros moubata*

3. Collaborating research group members at NRCPD

Name: Rika UMEMIYA-SHIRAFUJI

Position: Assistant Professor

4. Research period (in mm/dd/yyyy, and total number of years)

April 1, 2019 to March 31, 2020 1 year

5. Purposes and objectives

Our laboratory investigates the hormonal and nutritional regulation of egg protein synthesis in the soft tick *Ornithodoros moubata* in order to provide better understanding of reproductive in ticks for developing better strategies to control ticks. *O. moubata* is an excellent model species to investigate reproduction as regulated by feeding and mating. Presently, we focus on the incorporation of egg proteins into the oocytes by identifying and characterizing the vitellogenin receptor of *O. moubata* (*OmVgR*). During this study, we characterized the first *OmVgR* gene, we identified previously. *OmVgR* expression during the reproductive cycle of females was analyzed from the initiation of feeding until after the peak of egg laying by RT-PCR and Real-time PCR using whole ticks as well as tissues. In addition, *OmVgR* expression was also analyzed in ticks injected with Rapamycin an inhibitor of the TOR regulatory kinase to determine whether OmVgR expression is regulated by the nutrient signaling pathway.

6. Outline of research process

NCBI database was used for comparison of the *O. moubata VgR* (*OmVgR*) gene with *VgR* genes identified in other arthropods. The full sequence was then used to design 6 sets of primers to analyze the expression patterns of OmVgR in mated females by Real-time PCR. RNA was extracted and cDNA synthesized for tick samples at initiation of feeding, 2 hours, and 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20

days after engorgement. Presently, we are preparing samples from virgin females for the same times to compare with the mated female results. In addition, we assayed the expression of *OmVgR* in different tissues of mated engorged females. Tissues were pooled from several mated females 6 days after engorgement RNA extracted and cDNA synthesized for RT-PCR analysis. Finally, we analyzed *OmVgR* and *Vg* expression by Real-time PCR in both mated and virgin females injected with rapamycin, an inhibitor of the Target of Rapamycin (TOR) nutrient signaling kinase using RNA extracted from whole bodies of females after injection with rapamycin or the solvent (control).

7. Outline of research achievements

OmVgR shares the key motifs critical to proper functionality and shows the highest similarity to *VgRs* from hard ticks. Expression analysis revealed *OmVgR* is present at all times measured with the highest peaks appearing at 2 hours, 8 and 10 days after engorgement. Day 14 *OmVgR* expression coincides with the highest *OmVg* expression in these same samples. OmVg expression patterns were the same as seen in the previous study by Ogihara et al (2010). The above results show that *OmVgR* is expressed in mated engorged females from the initiation of feeding through the peak of egg laying. *OmVgR* expression occurs well before the expression of *OmVg* from day 4 and throughout the start of egg laying indicating *OmVgR* is present to function in incorporation of Vg in the oocytes.

Bands showed expression in the midgut, ovary and fat body but not the salivary glands. These three tissues function in Vg synthesis and egg development so expression of *OmVgR* indicates its importance in reproduction especially oocyte development in the ovary. However, expression in the midgut and fat body indicate it may also have other functions, studies need to be carried out to further elucidate these functions.

OmVg expression significantly decreased in both mated and virgin females when ticks were injected with rapamycin, but *OmVgR expression* didn't appear to be affected, except for an increase of *OmVgR* in rapamycin injected ticks on day 4. These results indicate that *OmVgR* is not directly regulated by the TOR nutrient pathway, but the increase in *OmVgR* expression in rapamycin treated mated females on day 4 remains to be explained.

8. Publication of research achievements

Studies are on going with plans to prepare and submit a paper on the identification and characterization of the first vitellogenin receptor gene from a soft tick, *Ornithodoros moubata*.

Plans to present the results at the 10th Tick and Tick-Borne Pathogen Conference were postponed until August 2021.

References

Horigane, M., T Shinoda, H. Honda and D. Taylor (2010) Characterization of a vitellogenin gene reveals two phase regulation of vitellogenesis by engorgement and mating in the soft tick *Ornithodoros moubata* (Acari: Argasidae). Insect Molecular Biology 19(4), 501-515.