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研究課題名（和文）Molecular studies on nutrient regulation of reproduction and immune responses in a tick

研究課題名（英文）Molecular studies on nutrient regulation of reproduction and immune responses in a tick

研究代表者
Ｔａｙｌｏｒ　ＤｅＭａｒ（Taylor, DeMar）

筑波大学・生命環境系・教授

研究者番号：５０２６１７７２

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研究成果の概要（和文）：マダニは、動物と人間の両方にとって、ウイルス、細菌、原虫などの多くの病気の病原体の重要な媒介物です。この研究の焦点は、Ornithodoros moubataの繁殖と免疫を調節するメカニズムをよりよく理解することでした。ダニは卵を生産するために血粉を必要とし、卵タンパク質生産、卵黄形成の調節は栄養素とホルモンによって調節されます。マダニの細胞プロセスの調節におけるTORの重要性を研究している研究者はほとんどいません。この助成金で行われた実験は、TORの抑制剤の注射が、血リンパ中の卵タンパク質のレベルを減少させ、そして卵子の産卵に導く卵巣における卵発生の欠如をもたらすことを明らかにしました。

研究成果の学術的意義や社会的意義
This research provides a clearer understanding of factors regulating reproduction and immune responses in a tick. This knowledge can lead to the development of better methods for controlling ticks by targeting different factors in the regulatory pathways and possibly prevent disease transmission.

研究成果の概要（英文）：Ticks are important vectors of many viral, bacterial, protozoan and other disease pathogens to both animals and humans. The focus of this research was to better understand the mechanisms regulating the reproduction and immunity in a model tick Ornithodoros moubata. Ticks require a blood meal to produce eggs and the regulation of egg protein production, vitellogenesis, is regulated by nutrients and hormones. The Target of Rapamycin (TOR) kinase has been shown to be the key regulatory factor for these mechanisms. Injection of rapamycin, an inhibitor of TOR, decreased the levels of egg proteins in the hemolymph and resulted in lack of egg development in the ovaries leading to the laying of no eggs. Results showed TOR expression peaks occur before the Vg gene peak indicating TOR may be important in regulating Vg expression. We also identified and characterized the relish gene in the immune responses to test the role of nutrients in the regulation of both the Toll and Imd pathways.

研究分野：Tick Physiology (Reproduction & Nutrition)

キーワード：tick　blood feeding　nutrient signalling　reproduction　immunity　Target of Rapamycin

１．研究開始当初の背景

Ticks are obligate hematophagous arthropods that infest all classes of terrestrial vertebrates and transmit a wide variety of viruses, spirochaetes, proteobacteria, protozoa, fungi and filarial nematodes (Sonenshine 1991). Ticks play key roles in the maintenance and transmission of new and reemerging diseases to domestic and animals as well as humans. Diseases directly affect the food security and health safety of numerous countries, for example 99% of cattle are infected by anaplamosis in South Africa (Moyo and Masika, 2009). Good methods for the control of ticks and the disease organisms they transmit are lacking. Studies on the environmental factors affecting vector competence are common, but few studies have investigated the physiology of the tick vector despite its importance in factors determining vectorial capacity. It is essential to understand the physiological processes, particularly mechanisms regulating growth, development, reproduction and immune responses, to understand disease vector competence. Ticks require a blood meal for growth, development and reproduction. To date we have focused on two main areas of tick physiology, 1) regulation of immune responses (Nakajima et al., 2001; 2002; 2003; 2005) and 2) regulation of reproduction (Ogihara et al., 2007; Horigane et al., 2007; 2008; 2010) using the soft tick *Ornithodoros moubata* as a model species. This soft tick is an excellent model species because it feeds within 2 hrs allowing us to synchronize physiological regulatory signals to determine their importance in the regulation of egg protein synthesis, immune responses and other physiological mechanisms. In addition, we have developed a system to assay the survival of transmissible and non-transmissible microorganisms in this tick species (Takano et al., 2013). These previous studies will allow us to determine the effects of nutrition on hormonal and gene regulatory systems for reproduction in *O. moubata*. The studies outlined below have set the stage for us to carry out this investigation. Our studies on the regulation of reproduction show the physiological regulation of reproduction can be easily separated into two phases, a nutritionally regulated phase and a hormonally regulated phase (Horigane et al. 2010). We have elucidated the ecdysteroid titers throughout the reproductive cycle (Ogihara 2007), identified the EcR nuclear receptor (Horigane et al., 2007) and RXR nuclear receptor (Horigane et al. 2008) as well as the vitellogenin gene (Horigane et al., 2010) in this species. These results clearly show ecdysteroids are essential for the regulation of tick vitellogenesis and set the stage for further studies to elucidate the role of nutritional factors in the regulation of successful reproduction.

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２．研究の目的

Ticks are important vectors of many viral, bacterial, protozoan and other disease pathogens to both animals and humans. The focus of this research was to better understand the mechanisms regulating the reproduction and immunity in a model tick *Ornithodoros moubata*. Ticks require a blood meal to produce eggs and the regulation of egg protein production, vitellogenesis, is regulated by nutrients and hormones. Previous studies have elucidated the mechanisms of hormonal regulation for the production of mature eggs. The funding from this grant has allowed us to make progress in understanding the importance of nutrients or the blood meal in the regulation of reproduction. In addition, ticks have an immune response to protect

themselves from harmful pathogens, but are still able to transmit numerous pathogens to the animal or human hosts they feed on. Our previous research has shown that *O. moubata* produces antimicrobial peptides to prevent attack of themselves from harmful pathogens. We also showed that the production of these antimicrobial peptides is regulated by the TOLL Immune Pathway that is commonly seen in other animals, particularly insects. The funding from this grant has also allowed us to further understand the regulation of the immune responses and their importance in the transmission of diseases by ticks.

３．研究の方法

The focus of the experiments for the project was on identification and characterization of the TOR genes by extraction of mRNA from fed female ticks in order to analyze the importance of nutrients in the vector competence of *Ornithodoros moubata*. Standard molecular techniques to synthesize the cDNA and obtain partial fragments of the two receptors based on primers designed from high homology regions of these receptor genes identified from a hard tick (Umemiya-Shirafuji et al. 2012) and other arthropod species. RT-PCR was used to confirm the quality of the samples and whether the primers were properly designed. Ticks were kept in an incubator at 30℃ and 70±2% humidity. Real-time PCR was used to determine expression patterns of the TOR and vitellogenin (Vg) genes in females several days before and up to 20 days after feeding. Identification and characterization of the expression patterns of the relish gene was also similarly carried out to allow for comparisons with the relish expression patterns.

Synthesis and injection of double stranded RNA to determine the effects of TOR silencing on expression of the Vg, REL, Relish genes was carried out, but the. dsRNA silencing did not successfully knockdown these genes. The dsRNA experiments are now being repeated. Ticks injected with rapamycin, an inhibitor of TOR, were also similarly analyzed for up to 30 days after feeding to confirm the expression of Vg and production of eggs to confirm whether TOR expression is important in the regulation of tick reproduction and immunity.

４．研究成果

Nutrient regulation of egg production

In most animals, nutrient uptake is communicated to the cell through upregulation of the Nutrient Signaling Pathway, which regulates most cellular processes including protein synthesis. The Target of Rapamycin (TOR) kinase has been shown to be the key regulatory factor for these mechanisms. Few researchers have studied the importance of TOR in the regulation of cellular processes in ticks. Experiments carried out during the first year of this grant revealed that the injection of rapamycin, an inhibitor of TOR, decreases the levels of egg proteins in the hemolymph and results in lack of egg development in the ovaries leading to the laying of no eggs until the rapamycin is metabolized and the TOR kinase can resume its regulatory function. Subsequent molecular studies revealed TOR inhibition by rapamycin injection greatly reduced expression of the egg protein gene showing the nutrient signaling pathway is essential for reproduction in ticks. Subsequently, a putative TOR sequence was identified. Focus of the research for the second year of the grant was to confirm the sequence of the TOR gene fragment and analyze the expression of the TOR gene at different times after engorgement and different tissues of mated female ticks. Results showed TOR expression peaks occur before the Vg gene peak indicating TOR may be important in regulating Vg expression. Expression of the TOR and Vg gene were analyzed the RT-PCR in the midgut, ovary and fat body on day 6 after engorgement, day of the Vg expression peak, showing the TOR gene is expressed in 3 tissues, but Vg is expressed only in the midgut and fat body. The final year experiments focused on trying to confirm the full sequence of the TOR gene and develop the RNAi assays for the analysis of the effects of TOR knockdown on the expression of the egg proteins and the hormone receptors that were shown previously to regulate reproduction in ticks. However, problems with the construction of effective double stranded RNA for the knockdown of the TOR gene prevented the completion of these experiments. They are still in progress and expected to be completed this year.

Nutrient Signaling and Immune Responses

Ticks also have an immune system that helps protect them for pathogens that will harm them. Despite this they are still able to transmit various pathogens. Goal of this part of the research plan was to determine the role of nutrition in the tick immune system. Before we could test the role of TOR in the regulation of the immune responses, it was necessary to identify and characterize the importance of the relish gene in the immune responses, so we could test both the Toll and Imd pathways. During the first two years of the grant we were able to identify the relish gene and characterize its time and tissues of expression. However, similar to the reproductive gene studies, we were unable to construct an effective double stranded RNA to confirm the functions of relish and the role of TOR knockdown on the expression of the immune genes. This work is also still in progress and similarly will likely be completed by the end of this fiscal year.

The above results have given us a much clearer understanding of the factors regulating the expression of genes related to reproduction and immune responses in the soft tick *Ornithodoros moubata*. This basic knowledge of these processes can be used in the development of better methods for controlling ticks by targeting different factors in the regulatory pathways of these mechanisms. In order to do this, further experiments are needed to identify the other factors not yet elucidated in the pathways as well as experiments to design and test these targets. The results here and previously can also help understand these mechanisms in other animals and the evolutionary development of the pathways, because ticks are considered to be a more primitive organism. The development of more effective control mechanisms based on the knowledge of tick reproduction and immunity can lead to reduced populations of ticks and possibly methods to prevent the transmission of diseases by ticks.

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〔産業財産権〕
○出願状況（計 0 件）

○取得状況（計 0 件）

〔その他〕
ホームページ等

６．研究組織

(1)研究分担者
研究分担者氏名：なし

ローマ字氏名：

所属研究機関名：

部局名：

職名：
研究者番号（8 桁）：

(2)研究協力者

研究協力者氏名：轡田圭又
ローマ字氏名：Keisuke Kutsuwada

研究協力者氏名：中武章子
ローマ字氏名：Shoko Nakatake

研究協力者氏名：Cornelia Wagner