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Gene silencing of ribosomal protein P0 is lethal to the tick *Haemaphysalis longicornis*

Haiyan Gong [a], Min Liao [b], Jinlin Zhou [a], Tekeshi Hatta [a], Penglong Huang [a], Guohong Zhang [a], Hirotaka Kanuka [a], Yoshifumi Nishikawa [a], Xuenan Xuan [a], Kozo Fujisaki [a,b,*]

[a] *National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan*

[b] *Department of Veterinary Medicine, Kagoshima University, Korimoto 1-21-24, Kagoshima 890-0065, Japan*

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Abstract

Ribosomal protein P0 has been demonstrated to be a multifunctional protein in the large subunit of eukaryotic ribosome. In this study, a gene encoding ribosomal protein P0 termed HlP0 was isolated from a full-length salivary gland cDNA library previously constructed from the tick *Haemaphysalis longicornis*. The full-length cDNA of the HlP0 gene is 1141 bp, with an open reading frame (ORF) of 963 bp. The ORF of the HlP0 gene encodes a putative protein of 320 amino acid residues, with a predicted molecular mass of 35 kDa. Reverse transcription-polymerase chain reaction (RT-PCR) analysis indicated that the HlP0 gene transcript was expressed in all developmental stages and all tissues dissected from 4-day-fed adult ticks. Antibodies raised against recombinant HlP0 recognized a native protein with an expected molecular size of 35 kDa in all tested tissues. RNA interference of HlP0 gene was carried out by injecting ticks with PBS, green fluorescent protein (GFP) dsRNA, and HlP0 dsRNA. The results showed that ticks treated with HlP0 dsRNA obtained a strikingly lower body weight (2.63 ± 1.21 mg vs. 226.75 ± 74.80 mg in the PBS-injected group and 231.15 ± 51.32 mg in the GFP dsRNA-injected group, Student's *t*-test, $P < 0.01$), a lower engorgement rate (4% vs. 100% and 94.11%, respectively), and higher mortality (96% vs. 2.5% and 10.4%, respectively) after blood-sucking than the control groups. This suggests that ribosomal protein P0 is required for the blood ingestion and subsequent viability of *H. longicornis*. This is the first report of ribosomal protein P0 from ticks.
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Keywords: *Haemaphysalis longicornis*; Ribosomal protein P0; RNA interference

1. Introduction

Ticks are hematophagous arthropods that infest many host species. Once they attach to the host, their blood-sucking can cause irritation and infection of the skin, anemia, and paralysis (Vedanarayanan et al., 2004). More importantly, they transmit a variety of pathogens, such as viruses, bacteria, spirochetes, rickettsia, and protozoans to their hosts, which has been a major constraint to the improvement of the livestock industries. Moreover, 14 tick-borne diseases have been reported in international travelers, and the incidence of travel-associated tick-borne diseases is increasing as more people travel (Jensenius et al., 2006). To control ticks and tick-borne diseases, the frequent use of chemicals is not sustainable for environmental, medical, and

* Corresponding author at: Department of Veterinary Medicine, Kagoshima University, Korimoto 1-21-24, Kagoshima 890-0065, Japan. Tel.: +81 99 285 3569; fax: +81 99 285 3570.
E-mail address: tick@ms.kagoshima-u.ac.jp (K. Fujisaki).

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economic reasons. Vaccination is, thus, considered to be a promising alternative measure to control ticks. With respect to tick vaccine development, the mechanisms of tick activities, i.e., feeding, oviposition, and molting, need to be characterized. To date, a large array of studies has been carried out on the function of bioactive molecules from different tissues of ticks (Willadsen, 2004). Simultaneously, a series of proteins responsible for the secretion of proteins has been characterized (Karim et al., 2004, 2005). Nevertheless, proteins associated with protein synthesis in ticks remain unknown. It has been reported that the overall mass and protein in the salivary glands increased about 25-fold during tick feeding in female *Amblyomma americanum* (Shipley et al., 1993). New gene expression was observed in the salivary glands of female ticks after attachment, mating, and feeding (Oaks et al., 1991). This change in the protein expression level is directly related to the function of ribosomes.

As an important component of the translation of protein, the ribosome is universal to all organisms. Ribosomes in mammals consist of four RNA species and a vast number of ribosomal proteins (RPs). Among the RPs in eukaryotic cells, acidic phosphoproteins P0, P1, and P2 form a complex, presumably as the pentameric form $(P1)_2/(P2)_2$/P0 (Shimizu et al., 2002). P0 protein herein is a multifunctional protein. It contributes RNA-binding domain to the binding of $(P1)_2/(P2)_2$/P0 complex to 28S rRNA (Uchiumi and Kominami, 1992), and is associated with the interaction of the complex with the elongation factor, eEF2 (Justice et al., 1999). Moreover, the dephosphorylated P0 detaches from the ribosome and is transported to the nucleus, where it showed apurinic/apyrimidinic endonuclease (APE) activities that are involved in DNA repair (Sanchez-Madrid et al., 1981; Yacoub et al., 1996). In order to describe the function and significance of ribosomal protein P0, the disruption of protein P0 was previously performed using P-element insertion (Frolov and Birchler, 1998) and C-terminal truncation (Griaznova and Traut, 2000; Hagiya et al., 2005). In addition to the above methods, interference with the gene by double-stranded RNA (dsRNA) transfection has been employed in the study of P0 in mosquito cells (Jayachandran and Fallon, 2003).

RNA interference (RNAi), the sequence-specific degradation of mRNA mediated by homologous dsRNA, has become a valuable tool for determining the biological role of genes. To date, RNAi of genes has been performed in several kinds of arthropods, including mosquito (Attardo et al., 2003; Boisson et al., 2006), fruit flies (Boutros et al., 2004), silkworm (Ohnishi et al., 2006), and ticks (Hatta et al., 2007; Zhou et al., 2006).

In this study, we isolated a ribosomal protein P0 from the adult tick *Haemaphysalis longicornis* and investigated its transcription profiles in different developmental stages and tissues. Moreover, through RNAi of ribosomal protein P0 gene, we determined that normal expression of P0 is essential for the feeding and survival of ticks. The down-regulation of P0 led to the failure of blood-sucking and subsequent death of ticks.

2. Materials and methods

2.1. Ticks

The Okayama strain of hard tick *H. longicornis* (Fujisaki, 1978) was infested on the ears of Japanese white rabbits (SPF, Japan Laboratory Animals, Tokyo, Japan). To obtain desired tissues from partially fed ticks, unfed adult ticks were allowed to feed for 4 days on rabbits and then manually detached. The recovered ticks were immediately subjected to dissection under a microscope, and tissues were collected in a cold PBS buffer. The collected tissues were stored at −80 °C until use.

2.2. Sequencing and analysis of the gene

A full-length salivary gland cDNA library was constructed using the vector-capping method as described previously (Kato et al., 2005), and a total of 10,000 recombinant transformants from the library were randomly selected and partially sequenced to form the database of expressed sequence tags (Harnnoi et al., 2007). From the database, two sequences containing a ribosomal P0 encoding insert were selected and fully sequenced using an automated sequencer (ABI prism 310 Genetic Analyzer, Applied Biosystems) (Boldbaatar et al., 2006) by three primers, T7 forward, P0 gene-specific (HlP0-con, listed in Table 1), and T3 reverse primers. The obtained full-length cDNA sequence of ribosomal P0 was then analyzed using a basic local alignment search tool (BLAST) (NCBI: http://www.ncbi.nlm.nih.gov).

2.3. In vitro expression of recombinant HlP0 and preparation of the anti-rHlP0/TrX serum

The HlP0 gene was PCR amplified using primers HlP0-E-F and HlP0-E-R (Table 1), and non-directionally cloned into the *Bam*H I site of the expression vectors pET32a and pGEX-4T-3 (Amersham Pharmacia

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