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https://digitalcommons.odu.edu/biology_fac_pubs/259

Original Publication Citation

Sunyakumthorn, P., Petchampai, N., Kearney, M. T., Sonenshine, D. E., & Macaluso, K. R. (2012). Molecular characterization and tissue-specific gene expression of *Dermacentor variabilis* α-catenin in response to rickettsial infection. *Insect Molecular Biology, 21*(2), 197-204. doi:10.1111/j.1365-2583.2011.01126.x

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Insect Mol Biol. Author manuscript; available in PMC 2013 April 1.

Published in final edited form as:
Insect Mol Biol. 2012 April ; 21(2): 197–204. doi:10.1111/j.1365-2583.2011.01126.x.

Molecular characterization and tissue-specific gene expression of *Dermacentor variabilis* α-catenin in response to rickettsial infection

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Abstract

Alpha catenin is a cytoskeleton protein that acts as a regulator of actin rearrangement by forming an E-cadherin adhesion complex. In *Dermacentor variabilis*, a putative *α-catenin* (*Dvα-catenin*) was previously identified as differentially regulated in ovaries of ticks chronically infected with *Rickettsia montanensis*. To begin characterizing the role(s) of *Dvα-catenin* during rickettsial infection, the full-length Dvα-catenin cDNA was cloned and analyzed. Comparative sequence analysis demonstrates a 3069 basepair cDNA with a 2718 bp open reading frame with a sequence similar to *Ixodes scapularis* α-catenin. A portion of Dvα-catenin is homologous to the vinculin-conserved domain containing a putative actin binding region and β-catenin binding and dimerization regions. Quantitative RT-PCR analysis demonstrated that *Dvα-catenin* is predominantly expressed in tick ovaries and is responsive to tick feeding. The tissue-specific gene expression analysis of ticks exposed to *Rickettsia* demonstrates that *Dvα-catenin* expression was significantly downregulated at 12 hours post-exposure to *R. montanensis*, but not in *R. amblyommii*-exposed ovaries, compared to *Rickettsia*-unexposed ticks. Studying tick-derived molecules associated with rickettsial infection will provide a better understanding of the transmission dynamics of tick-borne rickettsial diseases.

Keywords

Dermacentor variabilis; *Rickettsia montanensis*; *R. amblyommii*; α-catenin

Introduction

Ticks are known as arthropod vectors for many pathogenic and nonpathogenic organisms of the genera *Anaplasma, Babesia, Borrelia, Ehrlichia* and *Rickettsia* (Sonenshine, 1993). In the United States, ticks are responsible for the transmission of more vector-borne diseases than any other group of arthropods, with recent reports of tick-borne rickettsioses, such as Rocky Mountain spotted fever (RMSF), human monocytic ehrlichiosis, and human granulocytic anaplasmosis increasing extensively over the last ten years (Dumler, 2010). Unique among the tick-borne rickettsial pathogens are the spotted fever group (SFG) *Rickettsia*, which in addition to potentially being transmitted horizontally via vertebrate

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hosts, are maintained by vertical transmission in tick populations. Despite sympatric distribution of multiple tick and SFG *Rickettsia* species, stable vertical transmission has likely contributed to the establishment of specific relationships between ticks and species of *Rickettsia*. The American dog tick, *Dermacentor variabilis*, is a vector and host for *Rickettsia rickettsii*, the causative agent of RMSF and *Rickettsia montanensis*, a *Rickettsia* considered nonpathogenic to humans (Mcdade & Newhouse, 1986). Despite the apparently transient infection of *D. variabilis* with other SFG *Rickettsia* (Williamson *et al.*, 2010), due to stable vertical transmission the most commonly encountered microbe is *R. montanensis* (Ammerman *et al.*, 2004; Smith *et al.*, 2010; Stromdahl *et al.*, 2010). The contributing molecular factors that facilitate rickettsial infection, including the host specificity are not well defined.

Previous studies utilizing PCR-based transcriptional analyses in chronically SFG *Rickettsia*-infected ticks demonstrated differential expression of several tick-derived genes encoding ATPase, tubulin α-chain, glycine-rich protein, and α-catenin (Macaluso *et al.*, 2003; Mulenga *et al.*, 2003). Most molecules identified could be categorized as putative immune, stress response, or cytoskeletal associated molecules, such as α-catenin. In humans, α-catenin is able to bind various cytoskeleton proteins and regulates actin dynamics in the cells (Gates & Peifer, 2005; Drees *et al.*, 2005). This interaction is in part due to the molecule's two forms, one of which is a monomeric α-catenin that binds β-catenin, thereby forming an E-cadherin-dependent cell-cell adhesion complex linking to actin filaments. The other form is homodimeric α-catenin which binds actin filaments and inhibits the formation of the Arp2/3 and actin filament complex (Hartsock & Nelson, 2008). During host cell infection, many bacterial species utilize host α-catenin to mediate actin rearrangement in infected cells. For example, enterohemorrhagic and enteropathogenic *Escherichia coli* secrete a bacterial effector protein (EspB) into host cells that binds many host-derived proteins including α-catenin. EspB promotes α-catenin dimerization by competing with the Arp2/3 complex (Hamaguchi *et al.*, 2008; Kodama *et al.*, 2002). Likewise, during internalization of *Listeria monocytogenes* into epithelial cells, *Listeria* Internalin A binds to the E-cadherin-β-catenin complex on the host cell membrane, which is linked via α-catenin to actin filaments and recruits the cytoskeleton protein to the entry site (Sousa *et al.*, 2005). It has been demonstrated that in some species of SFG *Rickettsia*, actin polymerization is also required for bacterial invasion and motility during infection (Martinez & Cossart, 2004; Serio *et al.*, 2010). For example, *Rickettsia conorii* binds to host Ku70 and mediates actin polymerization via the Arp2/3 complex during internalization (Martinez & Cossart, 2004; Martinez *et al.*, 2005). Also, recent studies have identified a core set of actin-associated cytoskeletal proteins coupled with motility of *Rickettsia parkeri* in *Drosophila* cells (Serio *et al.*, 2010). In an *Ixodes scapularis* cell line (ISE6), *Rickettsia felis*, a flea-borne transitional group rickettsiosis agent interacts with tick cell surface histone H2B and depletion of histone H2B by RNAi and enzymatic treatment decreased rickettsial infection in the tick cells, suggesting a role of histone H2B in *R. felis* internalization into tick cells (Thepparit *et al.*, 2010). Despite progress in understanding rickettsial entry into host cells, the molecules and mechanisms of invasion of tick cells by SFG *Rickettsia* in ticks have not been identified.

Based on the previous identification of a partial transcript for putative host cytoskeletal components, the objectives of the current study were to characterize the α-catenin gene from *D. variabilis* and examine its association with rickettsial infection. Using functional bioassays, we tested the hypothesis that differential regulation of tick *α-catenin* during rickettsial infection is tissue-specific. Additionally, to begin to examine the basis of *Rickettsia*/tick pairing specificity, tick tissues were exposed to typical *Dermacentor*-associated *Rickettsia* (*R. montanensis*) or atypical non-*Dermacentor* associated *Rickettsia* (*Rickettsia amblyommii*) infection and expression of *Dvα-catenin* in response to each species was assessed. Understanding the tick-derived molecular response to rickettsial infection will

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provide insight into the specificity, transmission, and the ecology of tick-borne rickettsial infections.

Experimental procedures

Tick dissection

Rickettsia-free *D. variabilis* colonies were routinely maintained as described previously (Macaluso *et al.*, 2001). Unmated female ticks partially fed for 3 to 5 days were forcibly detached from host animals, washed twice in 70% ethanol, and rinsed with distilled water. Selected tick tissues (salivary glands, midgut, and ovary) were dissected out of the ticks, washed in sterile diethyl pyrocarbonate-treated water or fresh phosphate buffer saline (PBS, pH 7.4), and placed in RNA*later* (Ambion) until processed for nucleic acid extraction.

Nucleic acid extraction from tick tissues and cloning of Dvα-catenin full-length cDNA

As previously described (Mulenga *et al.*, 2004), ovaries from at least five *D. variabilis* were pooled, total RNA and subsequently mRNA were extracted using the NucleoSpin RNAII and NucleoTrap mRNA Mini kit (Clontech) according to the manufacturer's protocol. All RNA was stored at −80 °C until used. Cloning of full-length cDNA for Dvα-catenin was carried out using rapid amplification of cDNA ends (RACE) as previously described (Mulenga *et al.*, 2004). Briefly, 1 µg of mRNA extracted from ovaries was used to generate templates for 3' and 5' RACE using the SMART RACE cDNA synthesis kit (Clontech) according to the manufacturer's protocol. Following DNA sequencing of the putative *Dvα-catenin* fragment obtained by differential display PCR (Macaluso *et al.*, 2003), gene specific primers were designed to amplify the 5' [5'-GAAAGGTCGCTCAAAGCGGGC-3'] and 3' [5'-GCCTTCATCTTCCACACAACAATCGG-3'] ends. PCR products were routinely cloned into TOPO TA cloning vectors (Invitrogen) and sequenced (Macaluso *et al.*, 2003). MacVector software program (Accelrys) was used for DNA sequence analysis. Similarity comparisons to known proteins in the database were made by scanning DNA sequences against the GenBank database using tblastx.

Rickettsial culture

Two *Rickettsia* species, *R. montanensis* strain M5/6 and *R. amblyommii* strain Darkwater were routinely maintained and propagated in an African green monkey kidney cell line (Vero E6) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% FBS (Hyclone) in a humidified 5% CO_2 incubator at 34°C. For infection bioassays, when more than 80% of cells were infected, rickettsiae were semi-purified from *Rickettsia*-infected Vero E6 cells by detachment from the tissue culture flask, transferred to an Erlenmeyer flask containing sterile 3-mm borosilicate glass beads (Sigma), and vortexed at high speed for 5 min. The cell lysate was filtered through a 2-µm-pore size syringe filter (Millipore). The rickettsiae in the filtrate were collected by centrifugation at 16,000 × *g* at 4°C for 10 min. Rickettsial viability and enumeration were assessed as previously described by Sunyakumthorn *et al.* (2008). Briefly, purified rickettsiae were stained with a *Bac*Light viability stain kit (Invitrogen) and counted in a Petroff-Hausser bacteria counting chamber using a Leica microscope.

Quantitative Reverse Transcription-PCR (qRT-PCR)

The PCR reaction reagents were mixed in 96-well plates containing 2 µl of cDNA, 2X iTaq SYBR Green Supermix with ROX (Bio-Rad), 100 mM each of forward and reverse primers in a total volume of 35 µl per reaction. The qRT-PCR primers used for catenin and actin were DvCat2555F (5'-CACCGATTGTTGTGTGGAAG-3') and DvCat2661R (5'-CTTTTTCTGTGAGCCCTTGC-3')] and [DvAct1424F (5'-

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CTTTGTTTTCCCGAGCAGAG-3') and DvAct1572R (5'-CCAGGGCAGTAGAAGACGAG-3')], respectively. A no-RT reaction (water was added instead of reverse transcriptase) was performed to verify the absence of genomic DNA. Ten microlitres of each reaction mixture were transferred into three wells of a 384-well plate and reacted in an ABI 7900HT unit (Applied Biosystems) at Louisiana State University, School of Veterinary Medicine using system software (SDS v2.3). Data for each sample was initially calculated as the percent difference in threshold cycle (C_T) value ($\Delta C_T = C_T$ *Actin* − C_T *α-catenin*).

Tissue-specific transcription of Dvα-catenin during bloodfeeding and rickettsial infection bioassay

To determine the specific expression of *Dvα-catenin* in tick tissues and response of *Dvα-catenin* expression during blood feeding, unfed (3 ticks) and 5-day fed female ticks (3 ticks) were dissected. Tick tissues (salivary glands, midgut, and ovary) were collected and stored in RLT buffer (QIAGEN) for RNA extraction. Total RNA was isolated from tick tissues using the RNeasy Mini kit (QIAGEN) according to the manufacturer's instruction. RNA was then treated with Dnase (Ambion) and purified using an RNA cleanup kit (Zymo Research). The synthesis of cDNA with random hexamers was carried out using 200 ng total RNA in 25 μl reaction volumes of an iScript reverse transcription kit (Bio-Rad).

In order to determine tissue-specific responses of ticks during rickettsial infection, backless ticks were generated according to a modified protocol of Bell (Bell, 1980) and used for rickettsial infection. In a laminar flow hood, forty-five unfed female *D. variabilis* ticks were cleaned with 70% ethanol for 2 min and 10% benzalkonium chloride solution for 5 min, and then rinsed with sterile water three times. The ticks were air-dried on sterile filter paper. Mouthparts and legs were excised to minimize contamination. Cleaned ticks were transversely cut along the perimeter of the alloscutum with a scalpel and the dorsal cuticle was taken off. The backless ticks were submerged in individual wells on a 96-well plate containing 200 μl of complete L15B medium and incubated at 34°C. After 24 h, the backless ticks were divided into three groups of 15 ticks per group. The unexposed group was incubated in L15B medium, while the second and third groups were exposed to *R. amblyommii* or *R. montanensis* (2.4×10^8 rickettsiae per tick per well), respectively. After 1 and 12 hours-post inoculation (hpi), the tick tissues (5 ticks per time time point) were dissected out, pooled, and kept in 100 μl RNA*Later* at −20°C. Total RNA (40 ng) was used for cDNA synthesis with an iScript reverse transcription kit (Bio-Rad). cDNA template (2 μl) was subjected to qPCR as described above.

Statistical analysis

Analysis of variance was conducted using the SAS statistical package (version 9.2) GLM procedure. The relative gene expression of α–catenin from unfed and 5 day fed tick tissues was examined for potential difference. For the backless tick bioassay, the relative gene expression was analyzed after rank transformation and a two-way interaction was examined (rickettsial infection and tick tissues) analysis. When overall significance was found, Tukey's honestly significant difference (HSD) post hoc test was performed to determine the pairwise difference of means of main effects. Pairwise *t*-tests of least-squares means were performed to determine any interaction effects of relative expression of *Dvα-catenin* between unfed and 5-day fed ticks, and unexposed and *Rickettsia*-exposed backless ticks. *P*-values of < 0.05 were considered significant.

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Results

Full-length Dvα-catenin cDNA and sequence analysis

Gene specific primers designed from a *Dvα-catenin* fragment obtained by differential display PCR (Macaluso *et al.*, 2003) were used to clone the full-length α-catenin cDNA. After sequence analysis (blastx), the 3069 bp full-length cDNA was designated *Dvα-catenin* (Genbank accession number HM755938). A putative 2718 bp ORF encodes an expected 905 amino acid protein with a calculated molecular weight of 96 kDa. The deduced amino acid sequence is shown in Figure 1. A multiple sequence alignment of Dvα-catenin amino acids showed the highest similarity to tick and insect α-catenin with 94.7% identity to *Ixodes scapularis* α-catenin (Genbank accession number XP002413819), 87.7% to *Pediculus humanus corporis* α-catenin (Genbank accession number XP002429770) and *Aedes aegypti* mosquito α-catenin (Genbank accession number XP001657216), and 85.6% to *Drosophila melanogaster* α-catenin (Genbank accession number NP524219), compared to *Homo sapiens* α-catenin (82.7% similarity, Genbank accession number NP004380) (Figure 1).

Conserved domains were identified using NCBI Conserved Domain Search Service (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=cdd). The Dvα-catenin amino acid sequence is homologous to a vinculin conserved domain at amino acid positions 19–865 and contains a putative F-actin binding region at the C-terminus as well as β-catenin binding and α-catenin dimerization regions at the N-terminus (Figure 2).

mRNA expression of Dvα-catenin in tick tissues and response to feeding

To determine the mRNA expression profile of *Dvα-catenin* in different tick tissues and its responses to blood feeding, total RNA samples from different tick tissues (salivary glands, midgut, and ovary) of unfed and 5-day fed ticks were subjected to qRT-PCR assay. Results show that expression of *Dvα-catenin* is significantly greater in tick ovary, compared to salivary gland and midgut tissues in unfed ticks. Feeding leads to a decline in the expression of this gene in all tissues, with the change in the ovary being significant (Figure 3) suggesting a response to blood feeding.

Tissue-specific gene expression of Dvα-catenin in response to rickettsial infection

In order to assess the differential expression of *Dvα-catenin* in response to rickettsial infection in tick tissues and *Rickettsia* species-specific manner, an *ex vivo* study of tick tissues (backless tick bioassay) was performed. The tick dorsal integument was removed and backless ticks were exposed to equal amounts of either *R. montanensis* or *R. amblyommii*. After 1 and 12 hpi, total RNA of salivary glands, midguts, and ovaries from unexposed and *Rickettsia*-exposed ticks were subjected to qRT-PCR assay. Similar to the comparison between unfed and fed ticks, *Dvα-catenin* expression was greatest in tick ovary; no difference associated with rickettsial infection was identified at 1 hpi. A similar pattern of expression was observed at 12 hpi; however, a significant decrease of *Dvα-catenin* expression was observed in the *R. montanensis*-exposed ovary, compared to unexposed ticks (Figure 4).

Discussion

The present study describes the molecular and biological characterization of *Dvα-catenin* from the American dog tick, *D. variabilis*. Multiple alignments demonstrate that α-catenin is molecularly conserved among species of ticks and other arthropods, as well as in humans. Consistent with other organisms, the characteristics of the deduced amino acid sequence shows homology with vinculin protein, containing putative α-catenin dimerization, β-catenin

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binding domain, and the actin binding domain (Pokutta *et al.*, 2008). The conserved nature of the molecule suggests homologous function in cell structure within ticks.

In addition to molecular characterization, this study sought to assess the activity of *Dvα-catenin* in ticks. Female ixodid adult ticks are known to feed for extended periods of time (1 to 2 weeks), and dynamic changes in tick gene activity are associated with tick feeding (Aljamali *et al.*, 2009; Chalaire *et al.*, 2011; Mulenga & Khumthong, 2010a; Mulenga & Khumthong, 2010b). Most tick genes responsive to blood feeding are upregulated in salivary glands during tick feeding and related to manipulation of blood flow, blood digestion, and host immune responses, (Aljamali *et al.*, 2009). *Dvα-catenin* is constitutively expressed in the tick tissues assessed including salivary glands, midgut, and ovary, with significantly greater expression in the ovary, compared to other tissues. During the slow phase of feeding, expression is significantly downregulated. After 5-days of feeding, expression of *Dvα-catenin* was still greatest in the ovary, although significantly downregulated compared to unfed ticks. Downregulation of *Dvα-catenin* expression in individual salivary glands and midguts was not significant. While it appears *Dvα-catenin* is responsive to feeding, the reason for this observed regulation is unknown.

The manipulation of host cytoskeletal molecules by microorganisms is not unprecedented. In vertebrate host cells, many species of bacteria including *Listeria, Shigella, Rickettsia, Burkholderia*, and *Mycobacterium* are able to modulate rearrangement of actin cytoskeleton in order to invade host cells (Dramsi & Cossart, 1998; Gouin *et al.*, 2004; Hamaguchi *et al.*, 2008; Sousa *et al.*, 2005). For SFG *Rickettsia*, multiple species utilize host cytoskeletal components (actin-based) for movement in the host cells (Gouin *et al.*, 2004; Heinzen *et al.*, 1999; Heinzen, 2003). Dvα-catenin is one of several tick-derived molecules, which were identified as differentially expressed in *R. montanensis*-infected tick ovary. Specifically, transcript analysis suggested that expression was greater in tick ovary constitutively infected with *R. montanensis*, compared to uninfected tick ovary (Macaluso *et al.*, 2003). The association between *Dvα-catenin* and *Rickettsia* infection in the ovary is not clear. During tick feeding after attachment and ingestion of host blood, oocytes begin to further develop in tick ovary (Sonenshine, 1993). Based on the metabolic coupling of tick and rickettsiae, including the feeding responsive reactivation of rickettsiae in tick tissues (Hayes & Burgdorfer, 1982), it is possible that SFG *Rickettsia* utilize α-catenin to modulate actin rearrangement in order to invade neighboring host cells during this period, resulting in an increase in *Dvα-catenin* expression. The bioassay developed in the current study was able to capture the influence of rickettsial infection on host cell *Dvα-catenin* expressionat the early stages of infection. In contrast to the constitutively-infected cohort of ticks examined previously (Macaluso *et al.*, 2003), this study demonstrated that acute infection of *R. montanensis*, which is a typical *Rickettsia* for *D. variabilis*, downregulated *Dvα-catenin* expression in the ovary at 12 hpi. The differences in tick experimental models, including chronic versus acute infection and fed versus unfed ticks, between the previous study (Macaluso *et al.*, 2003) and the current study make comparison difficult. However, we speculate that in chronically infected ticks, *R. montanensis* is reactivated in response to blood feeding; resulting in upregulated *Dvα-catenin* expression required for invasion and movement inside the host cells. It has been demonstrated recently that the surface cell antigen 4 (Sca4) of *R. prowazekii* binds to and activates vinculin which, in turn, binds to host cytoskeleton (Park *et al.*, 2011). Interestingly, *Dvα-catenin* also contains a vinculin-conserved domain which may also bind to rickettsial Sca4 and alter host cytoskeleton. Whether or not the rickettsiae are manipulating the host expression of *α–catenin*, or if we measured the host response to insult, cannot be determined from the experiments in this study. Understanding the utilization of host molecules during chronic infection may be important for identifying the mechanisms for stable vertical transmission.

The current study examined acute infection and demonstrated that after 12 hpi *Dvα-catenin* expression is downregulated. We suggest this is a tick response to control the level of rickettsial infection in the ovary by downregulation of the *Dvα-catenin*, thus preventing utilization of the cytoskeletal components necessary for cell invasion. Differential regulation of other *D. variabilis*-derived molecules, including a Kunitz protease inhibitor, has been described during *R. montanensis* challenge in tick midgut (Ceraul *et al.*, 2011). If as described for other bacteria, and α-catenin is a component of cell entry, *Rickettsia* may downregulate *α-catenin* expression upon initial infection of the ovary in order to inhibit infection and transovarial transmission of second rickettsial species. This competitive interaction is also known as the interference phenomenon, and has been observed in SFG *Rickettsia* infections of ticks (Burgdorfer *et al.*, 1981; Macaluso *et al.*, 2002). Many studies have demonstrated a specific receptor/ligand complex facilitating rickettsial internalization in vertebrate cells (Cardwell & Martinez, 2009; Chan *et al.*, 2009; Martinez *et al.*, 2005; Riley *et al.*, 2010). A role for α-catenin in receptor/ligand binding in tick ovary during rickettsial infection is not known and further characterization is needed in order to understand the molecular interaction between *Rickettsia* and tissues central for sustained vertical transmission.

Transmission of SFG *Rickettsia* among ticks is complex as the tick serves as both the vector and reservoir. However, not all rickettsial species are horizontally transmitted by ticks and vertical transmission occurs with specificity as demonstrated transovarial transmission is limited to a few pairings (Macaluso *et al.*, 2002). Despite the overlapping distribution of several tick and rickettsial species, combined field and laboratory studies suggest that the biological association between ticks and rickettsial species is specific. In order to study these relationships and examine the tick tissue-specific response, a modified tick tissue culture of backless ticks (Bell, 1980) was used to identify the alteration of *Dvα-catenin* expression only during *R. montanensis* infection; suggesting that the tick response was specific to certain *Rickettsia* species. Although not a substitute for the natural route of infection, the backless tick bioassay was chosen for several reasons. First, the assay allowed for delivery of known numbers of rickettsiae with equal opportunity to expose, or infect, all organs. Several studies have examined the tick response to microbial challenge using body cavity inoculation or oral challenge via capillary feeding (Baldridge *et al.*, 2007; Ceraul *et al.*, 2011; Kocan *et al.*, 2008; Mulenga & Khumthong, 2010a). While this will allow for assessment of infection in the gut, the dissemination of rickettsiae after exposure is not clear. Because this study also intended to examine *Dvα-catenin* in the ovary during acute infection, whole organ exposure was desired. Through normalization of dissected ticks over 24 hours, and direct comparison to uninfected controls, the backless tick model is a functional bioassay to capture organ-specific responses.

Arthropods and microbes are known to have intimate relationships and many associations between arthropods and their endosymbionts are beneficial (Werren *et al.*, 2008). For example, a beneficial effect of the agent of human granulocytic anaplasmosis (HGA), *Anaplasma phagocytophilum*, in *Ixodes scapularis* was suggested via regulation of tick-derived molecules (Neelakanta *et al.*, 2010). For the SFG *Rickettsia*, it is likely that if there is decreased fitness associated with infection by some species of *Rickettsia* (Niebylski *et al.*, 1999; Burgdorfer & Brinton, 1975), and if harboring a non-lethal species is actually beneficial to the tick host (Burgdorfer *et al.*, 1981), then a specific tick/*Rickettsia* relationship would develop. The intricate mechanisms that facilitate this infection are unknown. The current study provides a pathway to identifying the molecules associated with rickettsial infection of the tick vector and a bioassay to assess the tissue-specific nature of these relationships. Ultimately, studying specific interactions between tick vectors and *Rickettsia* will lead to a better appreciation of ecology and epidemiology of tick-borne rickettsial diseases.

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Acknowledgments

We thank Christopher Paddock for *R. amblyommii* (Darkwater strain), Also, Chutima Thepparit, Supanee Hirunkanokpun, Britton Grasperge, and Mark Guillotte for their technical assistance. We also thank Jacqueline Macaluso for helpful comments. This research was supported by the National Institutes of Health (AI077784). This work was also part of P. Sunyakumthorn's doctoral dissertation.

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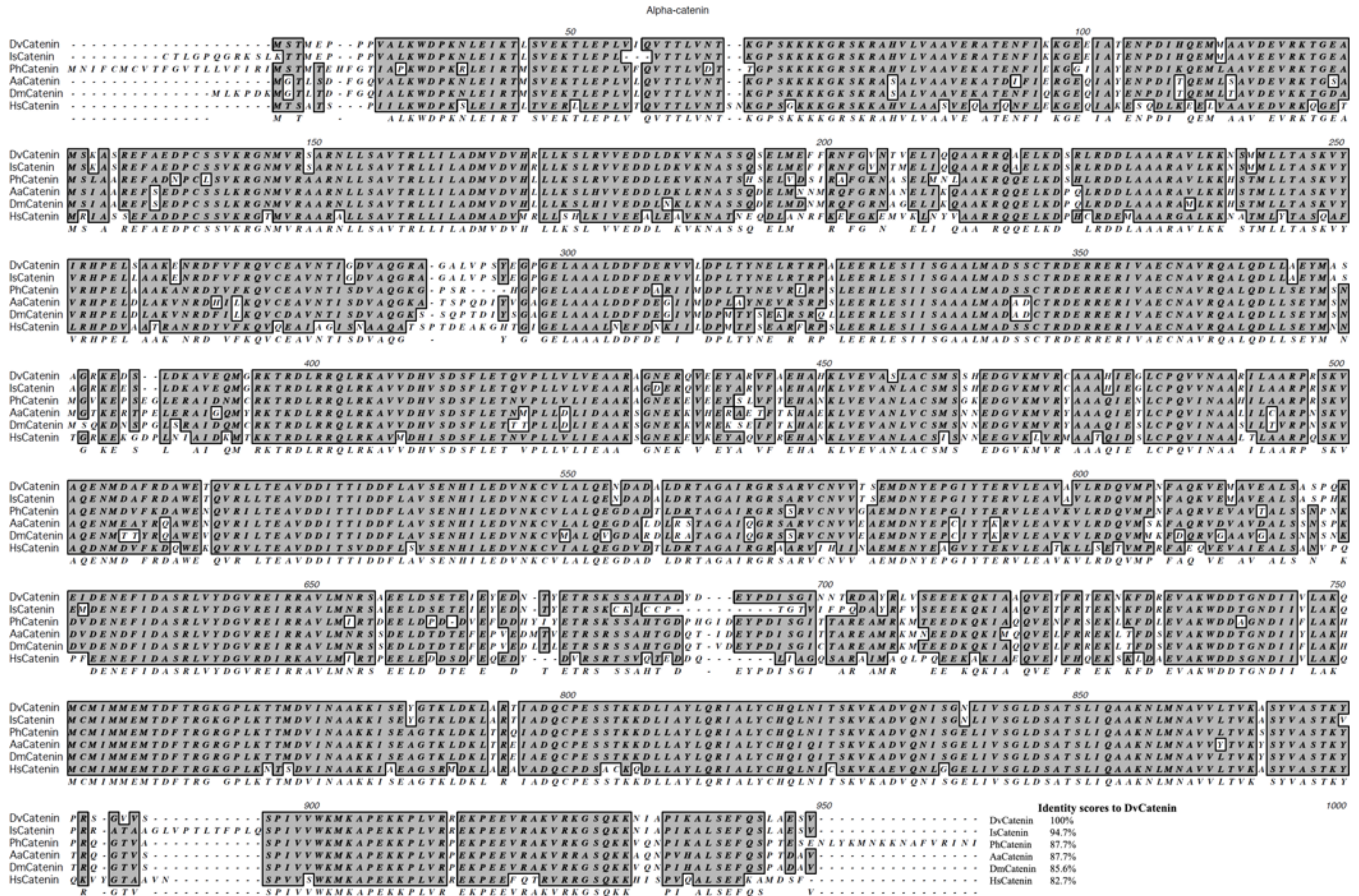

Figure 1. Multiple sequence comparison of α-catenin amino acid sequences

The Dvα-catenin deduced amino acid sequence was aligned with *Ixodes scapularis* α-catenin (IsCatenin, accession No. XP002413819), *Pediculus humanus corporis* α-catenin (PhCatenin, accession No. XP002429770) *Aedes aegypti* α-catenin (AaCatenin, accession No. XP001657216), *Drosophila melanogaster* α-catenin (DmCatenin, accession No. NP524219), and *Homo sapiens* α-catenin (HsCatenin, accession No. NP004380). Alignment was performed using MacVector software. Shaded gray indicates conserved amino acid residues. The identity scores to Dvα-catenin were derived from pairwise alignment using ClustalW 1.83 software.

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Figure 2. Putative protein binding sites of Dvα-catenin

Numbers correspond to amino acids of the protein sequence. Shaded gray region is vinculin conserved domain containing β-catenin, α-catenin, and F-actin binding regions.

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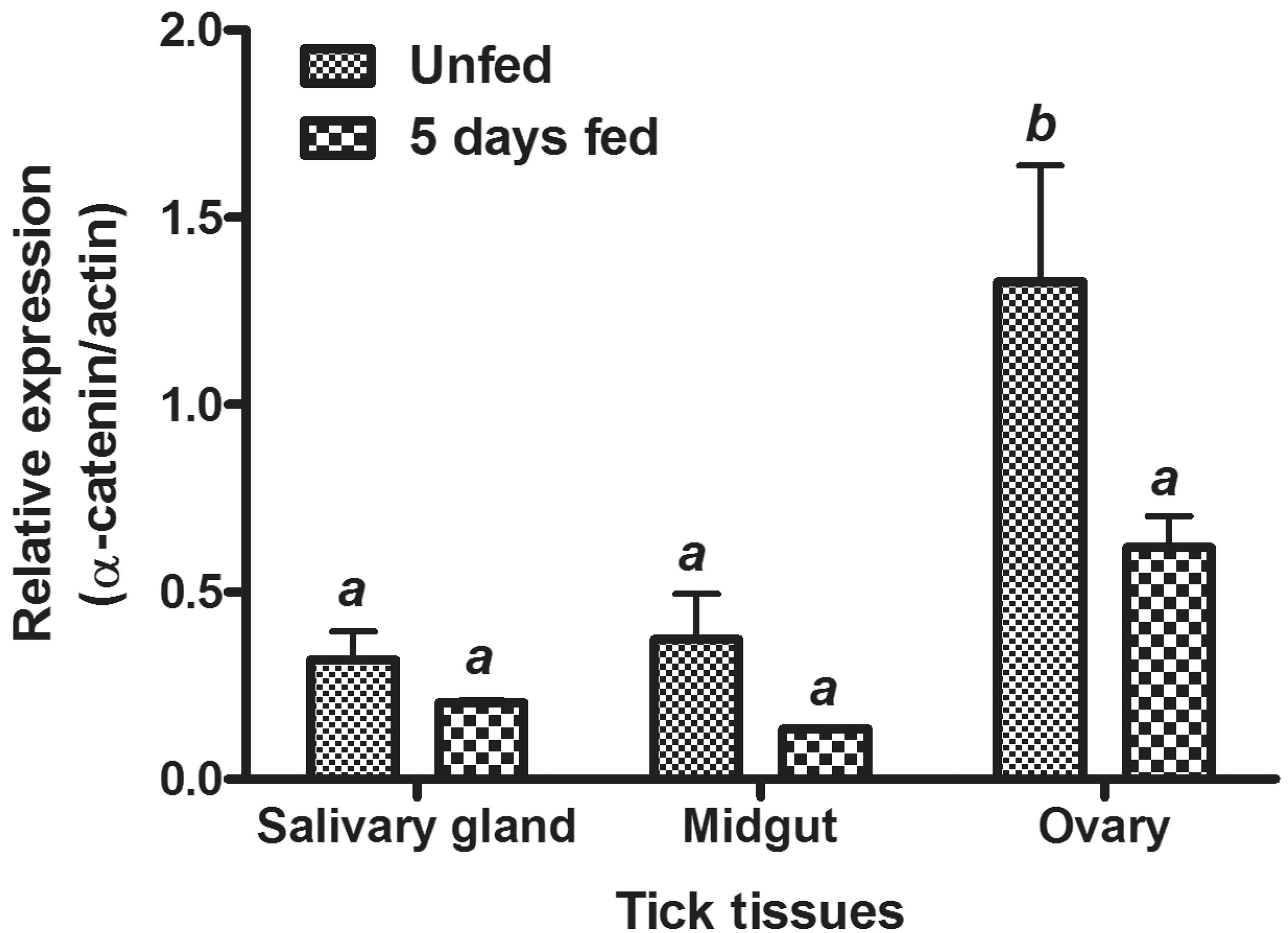

Figure 3. Tissue-specific expression of Dvα-catenin mRNA expression in unfed and 5-days fed *D. variabilis*

Total RNA was extracted from tick tissues (salivary glands, midguts, and ovaries) and expression of *Dvα-catenin* was determined by qRT-PCR assay. Transcription level of *Dvα-catenin* was normalized to tick *actin*. Data shown are mean relative expression. Error bar represents standard error of means (SEM). The bars with same letter are not significantly different ($P \leq 0.05$).

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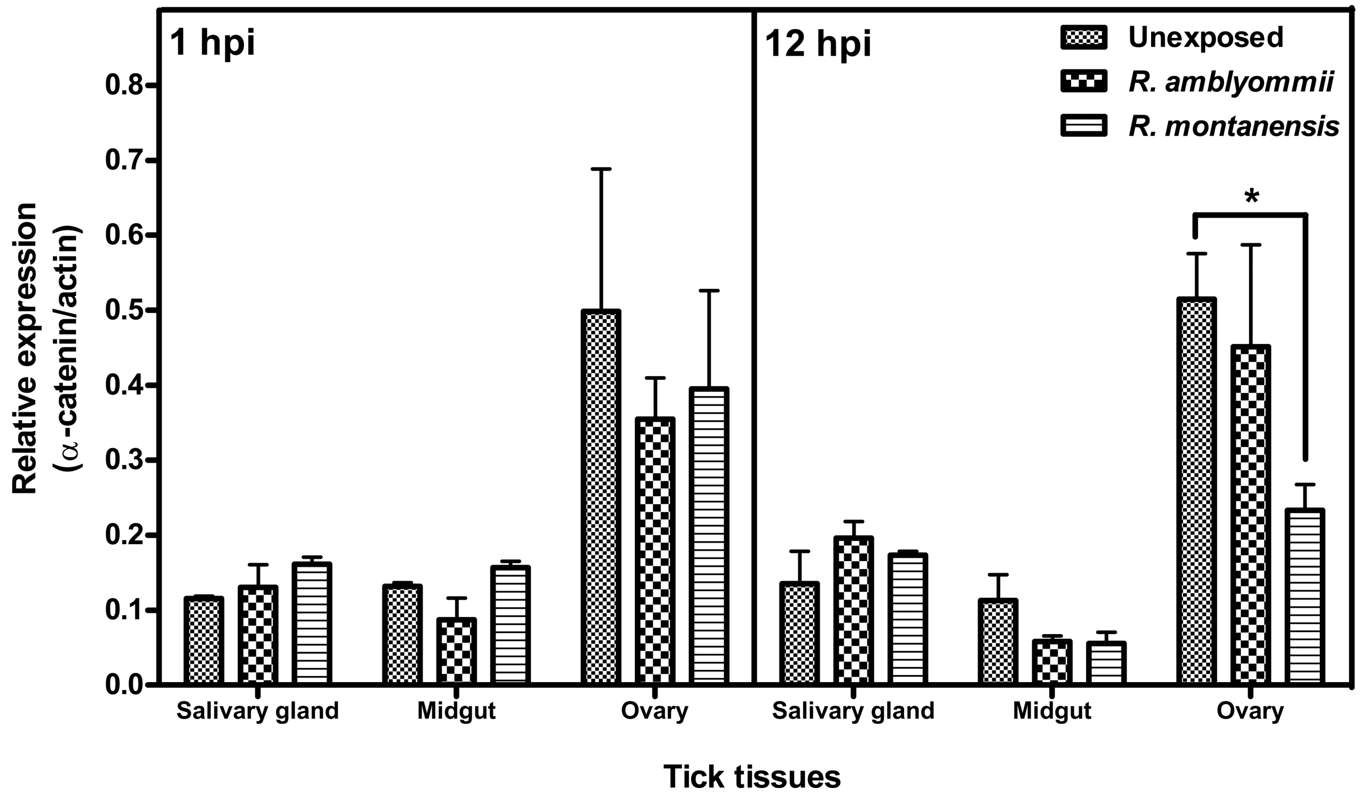

Figure 4. Dvα-catenin mRNA expression in backless ticks during *R. montanensis* and *R. amblyommii* exposure

Unfed *D. variabilis* female ticks were transversely cut along the perimeter of alloscutum, and the dorsal cuticle was taken off. Then, backless ticks were exposed to *R. montanensis* or *R. amblyommii* and incubated at 34°C. After 1 and 12 hpi, tick tissues were dissected and total RNA was extracted. Total RNA from each tissue was analyzed for expression of *Dvα-catenin* by qRT-PCR assay. Transcription level of *Dvα-catenin* was normalized to tick *actin*. Data shown are mean relative expression from two experiments. Error bar represents standard error of means (SEM). Asterisk indicates significant difference ($P \leq 0.05$).