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Remodeling of tick cytoskeleton in response to infection with Anaplasma phagocytophilum

Alejandro Cabezas-Cruz\textsuperscript{1,2}, Pilar Alberdi\textsuperscript{3}, James J. Valdes\textsuperscript{1,4}, Margarita Villar\textsuperscript{3}, Jose de la Fuente\textsuperscript{3,5}

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1. ABSTRACT

The obligate intracellular pathogen Anaplasma phagocytophilum infects vertebrate and tick hosts. In this study, a genome-wide search for cytoskeleton components was performed in the tick vector, Ixodes scapularis. The available transcriptomics and proteomics data was then used to characterize the mRNA and protein levels of I. scapularis cytoskeleton components in response to A. phagocytophilum infection. The results showed that cytoskeleton components described in other model organisms were present in the I. scapularis genome. One type of intermediate filaments (lamin), a family of septins that was recently implicated in the cellular response to intracellular pathogens, and several members of motor proteins (kinesins and dyneins) that could be implicated in the cytoplasmic movements of A. phagocytophilum were found. The results showed that levels of tubulin, actin, septin, actin-related proteins and motor proteins were affected by A. phagocytophilum, probably to facilitate infection in I. scapularis. Functional studies demonstrated a role for selected cytoskeleton components in pathogen infection. These results provided a more comprehensive view of the cytoskeletal components involved in the response to A. phagocytophilum infection in ticks.

2. INTRODUCTION

Ticks are blood feeding arthropod ectoparasites that transmit pathogens causing diseases in humans and animals worldwide (1). Among these pathogens, Anaplasma phagocytophilum (Rickettsiales: Anaplasmataceae) is an obligate
intrinsic bacterial proteins transmitted by Ixodes spp. in the United States, Europe, Africa, and Asia (1–3). Diseases caused by A. phagocytophilum include human granulocytic anaplasmosis (HGA), equine and canine granulocytic anaplasmosis, and tick-borne fever (TBF) of ruminants (1–3).

A. phagocytophilum infects vertebrate granulocytes, and tick midgut, hemocytes, and salivary glands (2–4). The development of A. phagocytophilum is complex and coordinated with the tick feeding cycle. Infection and colonization of ticks occurs within midgut cells and then subsequently in other tissues including the salivary glands from where transmission occurs during feeding (3). To establish infection, A. phagocytophilum induce complex changes in tick and vertebrate host cells mediated by different mechanisms (4). These mechanisms appear to be common to ticks and vertebrate hosts, and include but are not limited to remodeling of the cytoskeleton, inhibition of cell apoptosis, manipulation of the immune response, and modification of cell epigenetics and metabolism (4).

During infection, A. phagocytophilum induce cytoskeleton remodeling through actin reorganization or spectrin differential regulation in both vertebrate host and ticks (5, 6). These results suggested that A. phagocytophilum induce cytoskeleton remodeling for infection and multiplication in tick cells. However, the mechanisms used by A. phagocytophilum for remodeling of tick cytoskeleton have not been fully characterized.

To better characterize the mechanisms used by A. phagocytophilum to remodel cytoskeleton during infection of tick cells, the dynamics of the cytoskeleton was characterized in the tick vector, I. scapularis in response to pathogen infection. First, the composition of tick cytoskeleton was annotated using the recently published genome of I. scapularis (7). Then, previously published transcriptomics and proteomics data (8, 9) was used to characterize the mRNA and protein levels of cytoskeleton components in response to A. phagocytophilum infection of I. scapularis nymphs, female midguts and salivary glands, and ISE6 cultured cells were obtained from previously published results (8, 9) and deposited at the Dryad repository database, NCBI’s Gene Expression Omnibus database and ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD002181 and doi: 10.6.019/PXD002181. The identified genes encoding for cytoskeleton components were searched against the transcriptomics and proteomics data to characterize their mRNA and protein levels in response to A. phagocytophilum infection.

3. MATERIALS AND METHODS

3.1. Annotation of the cytoskeleton components in the I. scapularis genome

The I. scapularis genome (7) was searched with the speciﬁc names of genes encoding for cytoskeleton components. When records were not obtained using speciﬁc enzyme names, then the I. scapularis genome was searched with the Blastp tool from the Basic Local Alignment Search Tool (BLAST) using the human orthologs as ‘query’ (10, 11). The sequences with the lowest E-value were selected. The conserved domains of identiﬁed protein sequences were classiﬁed using the protein families database Pfam (12). The I. scapularis orthologs found in the tick genome were double-checked by searching the Homo sapiens genome database using as queries the tick homologs identiﬁed in the previous step.

3.2. Characterization of the I. scapularis mRNA and protein levels in response to A. phagocytophilum infection

The quantitative transcriptomics and proteomics data for uninfected and A. phagocytophilum-infected I. scapularis nymphs, female midguts and salivary glands, and ISE6 cultured cells were obtained from previously published results (8, 9) and deposited at the Dryad repository database, NCBI’s Gene Expression Omnibus database and ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD002181 and doi: 10.6.019/PXD002181. The identiﬁed genes encoding for cytoskeleton components were searched against the transcriptomics and proteomics data to characterize their mRNA and protein levels in response to A. phagocytophilum infection.

3.3. Phylogenetic analysis of I. scapularis septins

To genetically characterize the I. scapularis septins, a phylogenetic analysis was conducted using sequences from mammals (H. sapiens and Mus musculus), insects (Anopheles gambiae and Drosophila melanogaster) and chelicerates (Limulus polyphemus, Stegodyphus mimosarum and Metaseiulus occidentalis) (Figure 1). The sequences were aligned with the Multiple Alignment using Fast Fourier Transform program (MAFFT, version 7) configured for the highest accuracy (13, 14). Non-aligned regions were removed with Gblocks (version 0.9.1b) (15). The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model implemented in Molecular Evolutionary Genetics Analysis (MEGA, version 6) software (16, 17). Reliability of internal branches was assessed using the bootstrap replicates (1000 bootstrap replicates). Graphical representation and editing of the phylogenetic tree was performed with MEGA6.

3.4. Immunoﬂuorescence assay in I. scapularis midguts and salivary glands

Female ticks fed on A. phagocytophilum-infected and uninfected sheep and shed with 3% paraformaldehyde in 0.2% sodium cacodylate buffer were embedded in parafﬁn and used to prepare sections.
Anaplasma remodel tick cytoskeleton

Figure 1. Classification of I. scapularis septins. A maximum likelihood phylogenetic tree was built using the amino acid sequences of the septins identified in I. scapularis and their orthologs (names of the orthologs and accession numbers are shown in parentheses): I. scapularis (SEPT Group 2B, EEC07992; SEPT1, EEC12890; SEPT3, EEC02884; SEPT4/5, EEC07178; SEPT7A, EEC06789 and SEPT7B, EEC07929); H. sapiens (SEPT1, NP_443070; SEPT2, AAH14455; SEPT3, AAQ00517; SEPT4, AAH18056; SEPT5, AAH25261; SEPT6, AAH76547; SEPT7, AAH63640; SEPT8, NP_001092281; SEPT9, AAH21952; SEPT10, AAH21449; SEPT11, AAH36165; SEPT12, AAH36191 and SEPT14, NP_097249); M. musculus (SEPT1, NP_059489; SEPT2, NP_055021; SEPT3, XP_006521054; SEPT4, XP_006532651; SEPT5, AAH45333; SEPT6, NP_064326; SEPT7, NP_003898; SEPT8, XP_011247135; SEPT9, XP_0065333807; SEPT10, XP_011241558; SEPT11, NP_001009818; SEPT12, XP_006522641 and SEPT14, Q9DA77); A. gambiae (SEPT1, XP_312292; SEPT2, XP_309077; SEPT3, XP_311306 and PNUT, XP_309277); D. melanogaster (SEPT1, NP_523430; SEPT2, NP_523417; SEPT4, NP_728003; SEPT5, NP_651961 and PNUT, P40797); L. polyphemus (SEPT1, XP_0137860505; SEPT2, XP_013779075; SEPT3, XP_013794450; SEPT5, XP_013777903 and SEPT7, XP_013775106); S. mimosarum (SEPT1, KFM64660; SEPT2, KFM59146; SEPT4, KFM75885 and SEPT7, KFM73665) and M. occidentalis (SEPT Group 2B, XP_003748008; SEPT, XP_003748009; SEPT7 and XP_003743448; SEPT9, XP_003744831). Bootstrap values (1000 bootstrap replicates) for internal branches are shown.
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Table 1. Sequences of siRNAs and oligonucleotide primers for real-time RT-PCR of tick transcripts

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...needle, centrifuged at 200 x g for 5 min to remove cells debris and intact cells and the volume adjusted to 12 ml with L-15B300 culture medium then 500 ul was added per well or they were mock infected by adding 500 ul/ well of culture medium alone. Cells were incubated for an additional 72 h, collected, and processed for DNA and RNA extraction. Total RNA was used to analyze tropomyosin gene knockdown by real-time RT-PCR with respect to the Rs86 control. DNA was used to quantify the A. phagocytophilum levels by msp4 PCR as described previously (6). A. phagocytophilum DNA levels were compared between treated and control cells by Student’s t-test with unequal variance between treatment and control siRNA treated cells (P=0.05; N=6 biological replicates).

3.6. Analysis of tick cell mRNA levels by real-time RT-PCR

Total RNA was extracted from uninfected and A. phagocytophilum-infected ISE6 cells using TriReagent (Sigma, St. Louis, MO, USA) following the manufacturer’s recommendations. Real-time RT-PCR was performed on RNA samples with gene-specific primers (Table 1) at 55°C using the iScript One-Step RT-PCR Kit with SYBR Green and the iQ5 thermal cycler (Bio-Rad, Hercules, CA, USA) following the manufacturer’s recommendations. A dissociation curve was run at the end of the reaction to ensure that only one amplicon was formed and that the amplicons denatured consistently in the same temperature range for every sample (21). The mRNA levels were normalized against ribosomal protein S4 (rps4; 22) using the genNorm method (ddCT method as implemented by Bio-Rad iQ5 Standard Edition, Version 2.0.) (23). The results were compared by Student’s t-test with unequal variance between test and control siRNA treated cells (P=0.05; N=6 biological replicates).

4. RESULTS

4.1. Cytoskeleton components identified in other organisms are present in the I. scapularis genome

The eukaryotic cytoskeleton is composed of three major protein superfamilies, actin, tubulin, and...
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intermediate filaments (IFs) (24). However, recently septins have been regarded as the fourth component of the cytoskeleton (25). Actin and tubulin form dynamic actin filaments and microtubules, respectively. Around 50 different types of specialized proteins that form more static filaments compose the filamentous structures of the IFs (26). The proteins forming the IFs have been grouped into six groups (Types I-VI) based on similarities between their amino acid sequences. These groups include acidic and neutral keratins (Types I and II, respectively), vimentin, desmin, glial fibrillary acidic protein and peripherin (Type III), neurofilament (NF) proteins NF-L, NF-M and NF-H and α-internexin (Type IV), nuclear laminas (Type V), and nestin (Type VI) (26). Most types of IFs are cytoplasmic, but laminas are localized in the cell nucleus (26). Septins are GTP-binding proteins that form hetero-oligomeric complexes and higher-order structures, which play important roles as scaffolds for protein recruitment as well as diffusion barriers for subcellular compartmentalization, notably during host-microorganism interactions (25). Septins have been found in both unicellular and multicellular organisms (27).

In addition to Types I-VI IF basic components, there are other proteins that regulate or interact with them and give full functionality to the cytoskeleton. Among these proteins are the tropomodulins (Tmods), leiomodins (Lmods), tropomyosins, profilins, formins, and actin-depolymerizing factors (ADF) (24). The Tmod family includes four Tmods and three Lmods in humans. Tmods cap the pointed ends of actin filaments in actin cytoskeleton structures in a developmentally regulated and tissue-specific manner (28). Tropomyosins are actin-binding proteins that constitute core components of the microfilament and regulate actin dynamics (29). Profilins are ubiquitous actin monomer binding proteins that regulate microfilament polymerization (30). Formins are a family of proteins that govern different aspects of cellular physiology by remodeling actin and microtubules (31). Finally, ADFs depolymerize actin filaments and are modulators of actin filament dynamics and other cellular processes, from signal transduction to the cytonuclear trafficking of actin (32).

The major cytoskeleton components found in other organisms were identified in the I. scapularis genome (Table 2). In agreement with previous reports that arthropods do not have most cytoplasmic IFs (33), we did not identify IFs Types I, II, III, IV and VI in the genome of I. scapularis. However, two nuclear laminas, members of Type V IFs were identified (Table 2). Additionally, 6 septins, 7 actins and 16 tubulins, including 7 α-tubulins, 6 β-tubulins, 1 γ-tubulin, 1 ε-tubulin, and 1 δ-tubulin were also identified (Table 2). Of the 6 families of actin-related proteins analyzed (ADF, Tmod, Lmod, Tm, prof and formin), only Lmod was not identified in the genome of I. scapularis (Table 1). Comparatively, the molecular components identified in the I. scapularis cytoskeleton are found in other organisms such as H. sapiens, D. melanogaster and Saccharomyces cerevisiae (Table 3). However, more tubulins were found in I. scapularis when compared to D. melanogaster and S. cerevisiae (Table 3). For motor proteins, 10 kinesins and 20 dyneins were identified in the I. scapularis genome (Table 4).

4.2. Classification of the I. scapularis septins

Because of the importance of septins in the molecular interactions between hosts and microorganisms (25), the I. scapularis septins were characterized in more detail using a phylogenetic analysis (Figure 1). The number of septins varies between different organisms. For example, humans have 13 septins (SEPT1-SEPT12 and SEPT14); S. cerevisiae have 7 septins (SceCdc3, SceCdc10, SceCdc11, SceCdc12, SceShs1, SceSpr28, SceSpr3), and D. melanogaster have 5 septins (DmePrut, DmeSep1, DmeSep2, DmeSep4, and DmeSep5) (25, 27, 34) (Table 3). Based on the phylogenetic analysis, animal septins were classified into groups 1A, 1B and 2B (27). Six septins were identified in I. scapularis (Table 2 and 3), and were represented in the three groups of animal septins (Figure 1). Using amino acid sequences, four I. scapularis septins were classified unambiguously as SEPT1, SEPT3, and two copies of SEPT7 (A and B) (Figure 1). The clustering of the other two I. scapularis septins did not allow for a definitive classification. One septin clustered as SEPT4/5, and the other clustered within Group 2B, but without a clear phylogenetic relationship to any particular septin family member (Figure 1). Another classification grouped human septins into four groups named as SEPT2 (including SEPT1, SEPT2, SEPT4 and SEPT5), SEPT3 (including SEPT3, SEPT9 and SEPT12), SEPT6 (including SEPT6, SEPT8, SEPT10, SEPT11, and SEPT14), and SEPT7 (including SEPT7 only) (25). Based on this classification, I. scapularis septins were represented only in groups SEPT2, SEPT3 and SEPT7.

4.3. The mRNA and protein levels of I. scapularis cytoskeleton components vary in response to A. phagocytophilum infection in a tissue-specific manner

The finding of the genes encoding major eukaryotic cytoskeleton components in I. scapularis suggested the existence of an evolutionarily conserved cytoskeleton in ticks that have not been fully characterized. The tick response to A. phagocytophilum infection is largely regulated at the transcriptional level (8, 9, 35). Furthermore, the existence of histones and histone modifying
Table 2. Annotation of cytoskeleton components in the I. scapularis genome

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Anaplasma remodel tick cytoskeleton

The results showed mRNA/protein-specific and tissue-specific changes in the cytoskeleton components in infected and uninfected samples (Figure 2). At the protein level, many proteins were not identified by mass spectrometry, but for the identified proteins, the results showed a low (23%) correlation between differential regulation at the mRNA and protein levels (Figure 2). The cytoskeleton components with similar regulation at the mRNA and protein levels included α-tubulin (N=2), β-tubulin (N=4), actin (N=1), septin (N=1), prolin (N=1), lamin (N=1), tropomyosin (N=1), kinesin (N=3), and dynein (N=2) (Figure 2). The α- and β-tubulins showed a variable profile with tissue-specific signatures in response to pathogen infection (Figure 2). Actin regulation at the mRNA and protein levels was similar in nymphs only, and their levels were significantly lower in infected ticks when compared to uninfected controls (Figure 2). The mRNA and protein levels for Sept1 significantly increased in response to infection in tick midguts only (Figure 2). In contrast, the levels of all kinesins and dyneins that showed a similar differential regulation at the mRNA and protein levels significantly increased in response to infection in both tick midguts and salivary glands (Figure 2). However, the mRNA and protein levels for two kinesins were significantly lower in infected ISE6 cells when compared to uninfected controls (Figure 2). Regarding actin-related proteins, prolin mRNA and protein levels significantly decreased in response to infection in both tick midguts and salivary glands, while lamin and tropomyosin levels significantly increased in midguts in response to infection (Figure 2). As in previous experiments (6), these results supported cytoskeleton remodeling with tissue-specific changes during A. phagocytophilum infection in I. scapularis.

4.4. Functional studies support a role for cytoskeleton remodeling during A. phagocytophilum infection of tick cells

The actin-related protein tropomyosin was selected for functional studies due to its role in the signalling environment to regulate the cytoskeleton (29). Furthermore, tropomyosin protein levels increased in tick midguts and ISE6 cells, and decreased in salivary glands in response to A. phagocytophilum.

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</tr>
<tr>
<td>S. cerevisiae</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
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</tr>
</tbody>
</table>

Data on the number of all proteins, except septins, in H. sapiens, D. melanogaster and S. cerevisiae were collected from Gunning et al. (2015). Data on the number of septins were collected from Mostowy and Cossart (2012) for H. sapiens and S. cerevisiae, and from Adam et al. (2000) for D. melanogaster. Abbreviations: ADF, actin-depolymerizing factor; Tmod, tropomodulin; Lmod, leiomodin; Tm, tropomyosin.

Table 3. Number of cytoskeleton components in I. scapularis and other species
infection (Figure 2). The antibody against tropomyosin used for immunoFluorescence recognized the protein in muscle, midguts and salivary glands of I. scapularis fed female ticks (Figure 3A). The results of the immunoFluorescence assay in I. scapularis female ticks validated the proteomics results by showing higher and lower protein levels in tick midguts and salivary glands, respectively in infected ticks when compared to uninfected controls (Figure 3B). A 60% tropomyosin silencing was obtained after gene knockdown in tick ISE6 cells when compared to Rs86 control siRNA-treated cells. The A. phagocytophilum DNA levels decreased in tropomyosin siRNA-treated cells when compared to controls (Figure 3C), supporting an effect of tropomyosin knockdown on reducing pathogen infection. Taken together, these results supported a role for cytoskeleton remodeling during A. phagocytophilum infection of tick cells.

5. DISCUSSION

Anaplasma phagocytophilum are obligate intracellular pathogens that infect and multiply in both vertebrate and invertebrate tick hosts. These bacteria have one of the smallest genomes (1.47 Mb), and represent a paradigm for reductive evolution (36–38).
Remarkably, A. phagocytophilum have a broad host range, and several tick species can act as vectors for these bacteria (3, 39, 40). Therefore, the development of host-specific strategies for infection would have represented a high fitness cost. Instead, recent findings on the molecular characterization of tick-host-pathogen interactions revealed that A. phagocytophilum uses common strategies for infection of ticks and vertebrate hosts (4). Among these common strategies is the remodeling of the cytoskeleton (4).

In general, it is widely accepted that intracellular bacteria manipulate the actin cytoskeleton to assist pathogen infection, replication and dissemination (41–43). However, little is known about the function of tick cytoskeleton components during intracellular bacterial infection (43). Here, we found that I. scapularis ticks have the same cytoskeleton components described in other organisms such as H. sapiens, D. melanogaster and S. cerevisiae. In human neutrophils, A. phagocytophilum infection induces cytoskeleton rearrangement through the regulator of the actomyosin cytoskeleton, ROCK1 (44). Recently, a global proteomics analysis revealed that A. phagocytophilum infection changes the protein representation profile of cytoskeleton components in the human promyelocytic leukemia cell line, HL60 (45). In particular, keratin, Arp2/3 protein complex, α-actinin-4, galecin-1, plastin-2 were significantly under-represented, while kinesin-like protein 2 and cofilin were significantly over-represented in HL60 in response to infection (45).

Several components of the I. scapularis cytoskeleton such as tubulin, actin, actin-related proteins and motor proteins were differentially expressed/represented in response to A. phagocytophilum infection. The role of kinesins and dyneins during the intracellular movement of Anaplasma spp. within host cells remains to be established, but our results suggested that in addition to cytoskeleton rearrangement, motor proteins may play an important role during infection by A. phagocytophilum (Figure 4). The rickettsia Orientia tsutsugamushi, the causative agent of scrub typhus that also infects vertebrate and invertebrate hosts, uses dynein to propel themselves from the cell periphery to the microtubules organizing center (MTOC) (46). Furthermore, Chlamydia trachomatis inclusion membrane protein CT850 interacts with dynein to promote appropriate positioning of the inclusion at the MTOC (47).

In agreement with our results, Macaluso et al. (50) reported that α-tubulin was differentially expressed in response to Rickettsia montanensis infection of Dermacentor variabilis ticks. They found that the α-tubulin gene was down-regulated in tick midguts, and up-regulated in salivary glands in response to rickettsial infection (50). However, herein we found that the expression of the α-tubulin genes identified in I. scapularis did not change significantly in the salivary glands of infected ticks when compared to uninfected controls. The differences in α-tubulin regulation in salivary glands between R. montanensis and A. phagocytophilum infected ticks may reflect...
pathogen-specific and/or tick-specific responses. However, in agreement with the results of Macaluso et al. (50), two of the α-tubulin genes identified in I. scapularis were down-regulated, while all α-tubulin proteins were under-represented in midguts in response to infection.

In this study, seven septins were identified in the genome of I. scapularis. Septins are recently discovered and important components of eukaryotic cytoskeleton (25). The bacteria Listeria monocytogenes and Shigella flexneri exploit the host actin cytoskeleton for their own motility and they are attached to long actin tails in the cytosol of the host (51). Septins form a cage around these bacteria-related actin tails, surround bacterial bodies and were suggested to constitute a cellular defense mechanism against pathogens (52, 53). In the case of Shigella flexneri (25), septin cages limit bacterial motility and dissemination. The response of tick septins to A. phagocytophilum infection was complex. Most septins were down-regulated and under-represented at the mRNA and protein levels, respectively, probably reflecting tick response to A. phagocytophilum infection. The response of tick septins to A. phagocytophilum infection was complex. Most septins were down-regulated and under-represented at the mRNA and protein levels, respectively, probably reflecting tick response to A. phagocytophilum infection. However, I. scapularis SEPT1 and SEPT4/5 proteins were over-represented in salivary glands and midguts, respectively, probably reflecting tick response to A. phagocytophilum infection (Figure 4). In agreement with these results, different septins seem to have distinct roles during bacterial invasion processes (25).

The decrease in A. phagocytophilum infection levels in tick ISE6 cells after tropomyosin gene knockdown, suggested that the pathogen remodels tick cytoskeleton, at least in part, by increasing the levels of these actin-related proteins in tick midguts (Figure 4). In tick salivary glands, the decrease in protein representation in response to infection may be associated with tick response to limit pathogen infection. The mechanism by which A. phagocytophilum manipulate protein levels in tick cells is unknown, but may include modification of host cell epigenetics by bacterial regulators secreted through the T4 secretion system (T4SS) and other mechanisms (35).

Figure 3. Role of tropomyosin in A. phagocytophilum infection of I. scapularis. (A) Representative image of a fed I. scapularis female tick showing tropomyosin recognition in muscle, midgut (G) and salivary gland (SG) tissues stained with rat anti-tropomyosin monoclonal antibodies (green, FITC) and DAPI (blue). (B) Representative images of immunofluorescence analysis of uninfected and A. phagocytophilum-infected adult female I. scapularis midguts (G) and salivary glands (SG). Tick tissues were stained with preimmune control serum or rat anti-tropomyosin monoclonal antibodies (green, FITC) or DAPI (blue) and superimposed (FITC+DAPI). Bars, 20 μm. Red arrows illustrate the positive staining for tropomyosin in tick G and SG. (C) A. phagocytophilum DNA levels were determined in infected ISE6 tick cells treated with tropomyosin siRNAs or control Rs86 SiRNA, shown as average ± S.D., and compared between groups by Student's t-test with unequal variance (P=0.0.3; N=6 biological replicates).
similar to those obtained before for other genes and proteins in response to A. phagocytophilum infection, showing tissue-specific differences in the response to pathogen infection (8). The transcriptomics and proteomics data of I. scapularis nymphs and female midguts and salivary glands in response to A. phagocytophilum infection has been validated in several experiments (8, 9, 35). As previously discussed, these results suggested that differences between mRNA and protein levels could be due to delay between mRNA and protein accumulation which requires sampling at different time points and/or the role for post-transcriptional and post-translational modifications in tick tissue-specific response to A. phagocytophilum infection (8, 9, 35). In fact, some components of the cytoskeleton are regulated at the transcriptional (55) and translational (56) levels, while others are affected by post-translational modifications (57).

In summary, here we showed that A. phagocytophilum remodel tick cytoskeleton for infection (Figure 4). The results showed that tubulin, actin, septin, actin-related proteins and motor proteins were affected by A. phagocytophilum infection in I. scapularis, and suggested mechanisms by which remodeling of the cytoskeleton assists pathogen infection and dissemination (Figure 4). The present work provides a more comprehensive view of the cytoskeletal components involved in infection and host response to A. phagocytophilum infection in ticks. These results provided additional evidences to support that A. phagocytophilum uses similar strategies to infect vertebrate hosts and ticks, including the remodeling of the cytoskeleton.

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Anaplasma remodel tick cytoskeleton


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