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New Insights into Molecular *Ehrlichia chaffeensis*-Host Interactions

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Abstract

Ehrlichia chaffeensis is an obligately intracellular bacterium that exhibits tropism for mononuclear phagocytes and survives by reprogramming the host cell. Here we review new information regarding the newly characterized effector molecules and the complex network of molecular host-pathogen interactions that the organism exploits enabling it to thrive and persist intracellularly.

Keywords

Ehrlichia; tandem repeat proteins; ankyrin proteins; effector; host-pathogen interaction

1. Introduction

Ehrlichia chaffeensis is an obligately intracellular Gram-negative bacterium and the etiologic agent of human monocytotropic ehrlichiosis (HME), an emerging life-threatening tick-borne zoonosis [1]. HME is a systemic disease resembling sepsis or toxic shock syndrome with symptoms that most commonly include fever, malaise, myalgia, and headache, and is frequently accompanied by hematologic abnormalities including leucopenia, thrombocytopenia, and anemia, and elevations in serum hepatic aminotransferases. Hospitalization is required in 40 to 60% of HME cases and approximately 3% of cases are fatal [1].

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E. chaffeensis exhibits tropism for mononuclear phagocytes, replicates within cytoplasmic vacuoles that have early endosomal characteristics, and survives by evading and/or suppressing the activation of innate and adaptive host defenses [2]. A primary strategy utilized by *Ehrlichia* to escape destruction is to interfere with immune activating signals produced by T cells rather than by inhibiting antigen presentation or T-cell activation [3]. Escape from phagocyte killing and intracellular persistence involves modulation of numerous host cell processes, including gene transcription, membrane trafficking, cell differentiation, activation and suppression of tyrosine and MAP kinase activity, downregulation of toll-like receptors and transcription factors, apoptosis, superoxide generation, lysosomal fusion, endosomal maturation, and transferrin receptor gene expression [4;5]. The inhibition of host MAP kinases by *E. chaffeensis* has been linked to the downregulation of transcription factors and corresponding target genes related to host defense [6]. After entry, *E. chaffeensis* blocks tyrosine phosphorylation of Janus kinase (Jak) and signal transducer and activator of transcription (Stat) signaling, inhibiting the anti-ehrlichial activity of IFN-γ [7]. Although many host cell processes are modulated by ehrlichiae, the effector proteins and host targets involved in the cellular reprogramming strategy to create a permissive host have been undefined. However, recent studies are providing new insight into the ehrlichial effector molecules responsible for modulating these important host-pathogen interactions.

Previous reviews have summarized the known cellular processes that are affected during *Ehrlichia* infection [2;5]. In this review, we focus on the newly defined *E. chaffeensis* effector proteins, 47-kDa tandem-repeat protein (TRP47) and 200-kDa ankyrin-repeat protein (Ank200) and their potential roles in pathobiology and disease pathogenesis. Molecular interactions between these effectors and specific host cell targets have been identified that are involved in controlling cellular processes such as gene transcription, cell signaling, cytoskeleton and vesicle trafficking, ATPase activity, apoptosis, and IFN-γ signaling, providing insights into the molecular mechanisms that *Ehrlichia* modulates to subvert host defenses and persist within mononuclear phagocytes.

2. Genome insights into *Ehrlichia* host-pathogen interactions

Complete genomes from three *Ehrlichia* species (*E. canis*, *E. chaffeensis*, and *E. ruminantium*) have been recently sequenced [8–10]. *Ehrlichia* have relatively small genome sizes (~1–1.5 Mb) with a high degree of genomic synteny, low G+C content (~30%) and one of the smallest genome coding ratios that is attributed to long non-coding regions and numerous long tandemly repeated sequences (TRs) [11]. The TRs appear to be actively created and deleted through a mechanism compatible with DNA slippage, but the mechanisms involved in this process are still unknown; however the generation of TRs by *Ehrlichia* appears to be a host adaptation mechanism [12]. TRs of different *Ehrlichia* species have no phylogenetic relationships and suggest that duplication occurred after diversification of the repeat-encoding DNA [12].

Several key genome features associated with host-pathogen interactions have been identified in *Ehrlichia*, including genes that encode tandem and ankyrin repeat containing proteins, actin polymerization proteins, a multigene family encoding outer membrane proteins, and a group of poly(G-C) tract (short sequence repeats) containing proteins [8]. In addition, mechanisms for the delivery of effector proteins have been identified, including many of the known type IV secretion system (T4SS) components [8–10]. The Sec-dependent and Sec-independent protein export pathways for secretion of protein across the inner membrane as well as a putative type I secretion system have also been identified. The *Ehrlichia* genomes also have genes that encode three response regulator two-component systems (TCS), a family of signal sensor, transduction, and response regulatory systems, composed of a pair

of a sensor histidine kinase and a response regulator, that allows bacteria to sense signals and respond to changes in their environment through specific gene activation or repression [9].

3. TRP and Ank proteins and the host immune response

E. chaffeensis and *E. canis* have a small subset of major immunoreactive proteins that react strongly with antibodies in sera from infected humans or dogs, and most of these proteins have recently been molecularly characterized [13]. Many of the molecularly characterized *E. chaffeensis* immunoreactive proteins contain tandem or ankyrin repeats [14–17]. Major continuous species-specific antibody epitope(s) have been mapped to the acidic serine-rich TRs of *E. chaffeensis*, TRP120, TRP47, and TRP32 [14–16], and in the TRs of *E. canis* orthologs TRP140, TRP36, and TRP19, respectively [14;16;18]. In addition, multiple species-specific antibody eptiopes have been mapped to acidic terminal domains of the Ank200s [17;19].

4. Outer membrane proteins and immune evasion

A unique superfamily of immunorective outer membrane proteins has been identified in the family *Anaplasmataceae*. *E. chaffeensis* has a paralogous family of 22 major outer membrane proteins (OMP-1/p28) arranged in single locus upstream from the *sec*A gene and downstream from a hypothetical transcriptional regulator gene [20]. Although recombination of the major outer membrane proteins of closely related *Anaplasma* spp. occurs to create antigenic diversity, there is no evidence that recombination of the *Ehrlichia* OMP-1 family occurs. Differential expression of the OMP-1 genes in ticks and animal hosts has been reported, suggesting that they play a role in host adaptation [21]. Expression of only one OMP-1 gene (OMP-1B) has been reported in ticks and tick cell lines that appear to involve a temperature sensitive regulation mechanism [21;22]. In contrast, all OMP-1 family members are expressed in mammalian hosts and cells and antibodies against all OMP-1 proteins have been detected in experimentally infected dogs [21;23]. Although the role of the proteins in antigenic variation and immune evasion is still uncertain, other characteristics have been identified for the OMP-1 proteins, including porin-like structural features, suggesting that they may facilitate nutrient acquisition [24].

5. TRP and Ank expression and secretion

E. chaffeensis exhibits two ultrastructural cell types, a small dense-cored (DC) form characterized by a dense nucleoid, and large replicating form, the reticulate cell (RC) that has uniformly dispersed nucleoid filaments. Ehrlichiae typically reside as microcolonies of bacteria within cytoplasmic vacuoles (morulae) derived from early endosomes [25]. The ultrastructural forms (DC and RC) can be distinguished phenotypically by the expression of two TRPs (TRP47 and TRP120) that are differentially expressed by DC ehrlichiae [14;26]. Immunoelectron microscopy has identified these TRPs, in addition to a non-differentially expressed TRP32, extracellularly associated with morular fibrillar matrix and the morula membrane, indicating that these proteins are secreted [14;15;26]. The expression of *E. chaffeensis* TRP120 and TRP47 by DC ehrlichiae suggests that one of their functions is related to attachment and entry, and some evidence of this function has been demonstrated with the TRP120 [26] as well as the TRP47 ortholog in *E. ruminantium* (Erum1110) [27]. Several effector TRPs have been reported to be secreted by the type III secretion system (T3SS), but the secretion mechanism utilized by ehrlichial TRPs is currently unknown. Recent study in our laboratory has indicated that *E. chaffeensis* TRP120, TRP47, TRP32, and Ank200 appear not to be secreted by VirB/ViD4 dependent Type IV secretion system (T4SS) (Wakeel and McBride, unpublished data). However, an *E. chaffeensis* Ank200 ortholog in *A. phagocytophilum*, AnkA, was recently reported to be secreted by a T4SS [28].

Although *E. chaffeensis* and *A. phagocytophilum* are closely related, they are different in many aspects such as tropism for different cell types and residence in different cytoplasmic compartments [29]. In addition, and *A. phagocytophilum* VirD4, the T4S substrate coupling protein, exhibits a higher identity with *Agrobacterium tumefaciens* VirD4 than *E. chaffeensis* [9;30].

6. Characteristics of TRP and Ank proteins

All of the characterized *E. chaffeensis* TRPs (TRP120, TRP47, and TRP32) and their orthologs exhibit similar biophysical characteristics including an acidic nature due to the predominance of acidic amino acid residues primarily in the TR region, abnormal electrophoretic mobilities, and a high frequency of polar amino acids, such as serine, particularly within TRs [14–16]. The comparison of TR amino acid usage has demonstrated that in spite of amino acid sequence variation of the TR among different ehrlichial species, conservation of amino acid usage is consistent. A total of 10 amino acids are used in all of the repeats, with a particularly high frequency of serine, threonine, alanine, proline, valine, aspartate, and glutamate [14–16]. Recent studies using mass spectrometry have determined that abnormal migration of *E. chaffeensis* TRPs during electrophoresis is attributed to the highly acidic TR regions and is not as result of large post translational modifications such as glycosylation, but rather the acidic nature of these repeat domains [15;16].

TRPs in pathogenic bacteria have been associated with host-pathogen interactions such as adhesion, actin nucleation and immune evasion. Examination of the *E. chaffeensis* TRPs has identified a relationship with several functional protein domains and motifs. The *E. chaffeensis* TRP47 contains seven 19-mer (ASVSEGDAVVNAVSQETPA) TRs that dominate the C-terminal region of the protein, and approximately half of the TRP47 is represented by the TR domain [31]. The TRP47 TR region exhibits homology with eukaryotic proteins including renin receptor/ATP6AP2/CAPER protein, DNA polymerase III subunits gamma and tau-conserved domain, and ribonuclease E suggesting similar functional characteristics [31]. Furthermore, new evidence indicates that the TRP47 is phosphorylated based on predicted tyrosine phosphorylation sites, mass spectrometry data consistent with addition of phosphate, and the fact that TRP47 can be immunoprecipitated with anti-phosphotyrosine antibodies (Wakeel and McBride, unpublished data). Other TRPs may be phosphorylated, such as TRP32, which has an unusually high frequency of tyrosine residues (20%) in the C-terminal tail [15].

Ehrlichia spp. are among only a few prokaryotes that are known to have ankryin repeat (Ank)-containing proteins. Ank is a ubiquitous eukaryote motif that mediates protein-protein interactions, and they are found in proteins that modulate many cellular functions such as transcriptional regulation, cell cycle, cytoskeleton organization, developmental regulation, signal transduction, toxicity, and the inflammatory response. The ankyrin structural repeat unit, Ank motif, is 33 amino acids long and contains two antiparallel helices and a beta-hairpin (helix-turn-helix). The Ank may occur in combinations with other types of domains and cooperatively fold into structures that mediate molecular recognition via protein-protein interactions. The most extensively studied Ank protein in *E. chaffeensis* is a 200 kDa protein (Ank200) that has central domain that has 19 Anks flanked by acidic (pI 4 to 5) C- and N-terminal domains that have a predominance of glutamate and aspartate residues [17]. In addition, like the TRPs, *E. canis* and *E. chaffeensis* Ank200s have a high proportion of polar amino acids, including serine and threonine [17;19].

7. Host cell gene expression during *E. chaffeensis* infection

E. chaffeensis significantly alters the transcriptional levels of approximately 5% of host genes within 24 hr of infection [4]. Genes that are modulated include those coding for

apoptosis inhibitors, regulation of cell cycle and differentiation, signal transduction, proinflammatory cytokines, biosynthetic and metabolic proteins, and membrane trafficking proteins. This transcriptional profile has provided new information on host cell processes targeted by *Ehrlichia* and revealed key themes in disease pathogenesis. Furthermore, the unique *Ehrlichia*-host interaction is illustrated by the fact that among intracellular bacteria only a relatively few host genes were found to be commonly induced during *E. chaffeensis* infection, while no genes were commonly repressed, suggesting that ehrlichial survival mechanisms have evolved distinctly from other intracellular pathogens [4].

Microarray analysis during infection has shown that *E. chaffeensis* appears to manipulate genes related to three primary areas of the host response. First, *E. chaffeensis* represses the transcription of cytokines involved in the early innate immune response and cell-mediated immune response to intracellular microbes, including host cell cytokines that modulate innate and adaptive immunity to intracellular bacteria such as IL-12, IL-15, and IL-18, which are repressed. These cytokines play fundamental roles in stimulating NK cells and T helper 1 cells to produce gamma interferon (IFN-γ), which then activates macrophages to kill phagocytized bacteria. IL-12 and IL-15 also activate NK cells and cytotoxic T lymphocytes to kill cells infected with intracellular bacteria. Thus, repression of IL-12, IL-15, and IL-18 suggests that modulating these cytokines is critical to the survival of *E. chaffeensis*.

Second, *E. chaffeensis* up-regulates NF-κB and apoptosis inhibitors, which may enhance host cell survival. Apoptosis is an innate mechanism of host defense used to prevent proliferation of internalized bacteria. *E. chaffeensis* infection induce apoptosis inhibitors such as IER3 (immediately early response 3), BirC3 (baculoviral IAP repeat-containing protein 3), and BCL2, but inhibits apoptosis inducers such as BIK (BCL2-interacting killer) and BNIP3L (BCL2/adenovirus E1B 19-kDa interacting protein 3-like) during the early stage of infection, thus impairing host cell apoptosis and maintaining a prolonged growth opportunity for ehrlichiae.

Third, *E. chaffeensis* inhibits the transcription of genes involved in membrane trafficking. *E. chaffeensis* lives in an early endosome and inhibits the maturation of the endosome to evade destruction by lysosomal enzymes [29]. *E. chaffeensis* represses the production of Rab5, SNAP23, and STX16 (syntaxin 16) during infection, most dramatically during the first hour of infection. *E. chaffeensis* induces the production of vimentin, a reservoir for SNAP23 [32]. Thus, *E. chaffeensis* appears to modulate phagosome-lysosome fusion by apregulating expression of Rab5 and SNAPs in the macrophage. A current model of vesicle fusion is explained by the SNARE hypothesis which proposes that the docking and fusion of vesicles with the plasma membrane are mediated by the specific interaction of vesicle proteins (v-SNARE and SNAR receptor) with the target plasma membrane protein (t-SNARE). Among the proteins implicated are syntaxins, which have at least 16 members (synaptosome-associated proteins; SNAPs). These proteins form a complex that juxtaposes the two membranes to be fused, and this interaction is regulated by Rab5, a small GTPase of the Rab family. Depletion of Rab5 inhibits the fusion of the phagosome containing *Listeria monocytogenes* with lysosomes [33].

8. *E. chaffeensis* TRP47 network of host interactions

Progress in understanding the specific interactions of TRP47 with the host has been advanced by a recent study demonstrating interactions between *E. chaffeensis* TRP47 and multiple host proteins including polycomb group ring finger 5 (PCGF5), Src protein tyrosine kinase FYN (FYN), protein tyrosine phosphatase non-receptor type 2 (PTPN2), adenylate cyclase-associated protein 1 (CAP1), and immunoglobulin lambda-like polypeptide 1

(IGLL1) with distinct cellular functions associated with signaling, transcriptional regulation, vesicle trafficking, and cellular proliferation and differentiation [31] (Figure 1). Furthermore, the potential importance of the TRP47 in pathobiology is supported by the recent studies demonstrating that the TRP47 gene is the most highly expressed gene in the *E. chaffeensis* transcriptome (Kuriakose and McBride, unpublished data). Although, the relevance of these ehrlichiae-host molecular interactions in the context of ehrlichial pathobiology remains to be determined, the host targets identified suggest that TRP47 is a multifunctional effector that plays an important role in establishing bacterial infection and promoting intracellular survival.

8.1 Host cell signaling and TRP47

Tyrosine phosphorylation of host and/or bacterial proteins has been implicated in signaling pathways triggering the entry of many intracellular pathogens. Tyrosine kinases are known to be involved in ehrlichial entry; however, the specific kinases involved have not been determined [34]. The association of TRP47 with tyrosine kinase FYN and the intracellular DC form of *E. chaffeensis* suggests that it may be recruited by TRP47 to facilitate the entry process. FYN specifically phosphorylates caveolin-1 and is required for coxsackievirus internalization and infection via caveolin-associated vesicles to polarized epithelial cells [35]. FYN was not observed associated with *E. chaffeensis* RCs demonstrating a selective association with DC ehrlichiae that express TRP47. *E. chaffeensis* TRP47 is strikingly similar to *Chlamydia trachomatis* serovars L2, Tarp, which is an immunoreactive, secreted (T3SS), highly acidic (pI 4) protein that migrates abnormally on SDS-PAGE, contains six near-identical tandem repeats, and is tyrosine phosphorylated by the Abl kinase at the site of entry and associated with recruitment of actin [36].

Another TRP47 interacting protein, PTPN2, is a protein tyrosine phosphatase (PTP) also known as T cell PTP (TC-PTP) that catalyzes the dephosphorylation of phosphotyrosine peptides and regulates phosphotyrosine levels in signal transduction pathways. PTPs are known to regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation [37;38]. Multiple substrates of PTPN2 include CSF-1R, EGFR, PDGFR, IR, p52Shc, Jak1, Jak3, Stat1, Stat3, Stat5a/b, and Stat6 [38]. The *in vivo* and *in vitro* analyses indicate that PTPN2 could control cytokine signaling events by its negative action on the Jak/Stat pathway. The loss of PTPN2 results in Stat5 hyper-activation, increased production of gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin (IL)-12, and inducible nitric oxide synthase (iNOS), increased tyrosine phosphorylation, recruitment of a Grb2/Gab2/Shp2 complex to the CSF-1 receptor, and enhanced activation of ERK, and may affect transcription factor PU.1 signaling.

PTPN2 is ubiquitously expressed with particularly high expression in hematopoietic tissues. It is remarkable, not only by the fact that it appears to broadly influence hematopoietic cell development, but recent findings also demonstrate a role in several human diseases from autoimmune disease to cancer [37]. The Jak/Stat pathway is inhibited by monocytotropic *E. chaffeensis* [7], and supports the possibility that TRP47 may not only be involved in the inhibition of IFN-γ-induced tyrosine phosphorylation of Stat1, Jak1, and Jak2 by interacting with PTPN2, but also in the regulation of cellular development.

8.2 Vesicle trafficking, apoptosis and TRP47

Membrane trafficking is a cellular process that has been identified as an *Ehrlichia* target by multiple studies. A specific interaction between TRP47 and the multifunctional protein CAP1 has been defined that appears to occur at the morula membrane interface, where CAP1 localizes with the DC morulae adjacent to the morula boundaries (membrane) [31]. The distribution pattern of CAP1 was remarkably different in *E. chaffeensis*-infected cells as

compared to uninfected cells, where it is primarily associated with the plasma membrane, indicating that distribution of this protein is altered as a result of *E. chaffeensis* infection [31]. CAPs were originally identified in yeast as a component of the adenylyl cyclase complex, and yeast cells deficient in CAPs are defective in cytoskeleton organization. Although CAPs do not regulate cAMP in animal cells, their role in regulation of actin remodeling in response to cellular signals is widely conserved. CAP1 is a highly conserved monomeric actin binding protein that contains actin (C-terminal), adenylyl cyclase and cofilin (N-terminal), and Src homology 3 (SH3) and profilin (central region) binding domains and plays an active role in actin turnover [39]. Genetic studies in yeast have implicated CAP1 in vesicle trafficking and endocytosis. In mammalian cells, CAP1 is associated with SH3 domain-dependent mAbp1-dynamin complex involved in receptor-mediated endocytosis [40]. Many intracellular bacteria including *Listeria, Rickettsia, Burkholderia, Shigella* and *Mycobacterium* species, subvert cellular actin dynamics to facilitate their movement within the host cytosol and to infect neighboring cells while evading host immune surveillance and promoting their intracellular survival [41]. Thus, in an effort to survive in the intracellular niche *Ehrlichia* may manipulate the mononuclear phagocyte cytoskeleton components such as actin by modulating CAP1.

Ehrlichiae, like chlamydiae, inhibit apoptosis early in infection, but while chlamydiae induce cell death at the end of the infection cycle, ehrlichial exit mechanisms remain undefined [42;43]. Interestingly, CAP1 has also been implicated in promoting apoptosis by functioning as an actin shuttle to mitochondria. Similar to cofilin, BAD, and BAX, CAP1 rapidly translocates to mitochondria independent of caspase activation where it promotes apoptosis [44]. Associations between ehrlichial morulae and mitochrondria have been consistently observed [25]. Thus, the TRP47 and CAP1 interaction may serve a dual function by facilitating endocytosis and vesicle trafficking, and promoting apoptosis in the late stages of infection.

8.3 Immunoglobulin lambda-like protein 1 and TRP47

IGLL1 is also interacts with TRP47 and shows colocalization on the surface of morulae. IGLL1 gene encodes one of the surrogate light chain subunits and is a member of the immunoglobulin gene superfamily and does not undergo rearrangement [45]. The preB cell receptor is composed of a membrane-bound Ig Mu heavy chain in association with a heterodimeric surrogate light chain (IGLL1) and is found on the surface of proB and preB cells [45]. The preB cell receptor is involved in transduction of signals for cellular proliferation, differentiation from the proB cell to the preB cell stage, allelic exclusion at the Ig heavy chain gene locus, and promotion of Ig light chain gene rearrangements [45]. Thus, the significance of the interaction between TRP47 and IGLL1 might involve signaling and development, but suggests a novel role for IGLL1 in the macrophage and one that will require further study to understand.

8.4 Gene silencing complex and TRP47

The TRP47 interacting partner, PCGF5 has been associated with DNA-dependent regulation of transcription, metal ion binding, and protein-protein interactions. It has a specialized Zn-finger domain consisting of 40 to 60 residues that binds two atoms of zinc, is defined by the 'cross-brace' motif involved in protein-protein interactions [46;47]. PCGF5 is related to the polycomb group proteins (transcriptional repressors) Bmi-1/PCGF4 and Mel-18/PCGF2 that play important role in the regulation of Hox gene expression, X-chromosome inactivation, tumorigenesis, and self-renewal, maintenance of pluripotency of stem cells, and stimulation of E3 ubiquitin ligase activity. Thus, it appears that TRP47-expressing DC ehrlichiae may recruit PCGF5 in an effort to modulate host cell gene expression to favor survival. Altered gene expression in *E. chaffeensis*-infected cells has been reported, but the mechanisms

involved are largely unknown [4]. However, a recent study [48] has demonstrated that *E. chaffeensis* Ank200 targets host cell genes related to apoptosis, ATPase activity, and transcription and may modulate transcription.

9. *Ehrlichia chaffeensis* Ank200

The *E. chaffeensis* Ank 200 is a large immunoreactive protein that contains 19 ankyrin repeats in a centralized domain flanked by acidic terminal domains [17;48]. *E. chaffeensis* Ank200 is a nuclear translocated protein that lacks a classic nuclear localization signal (NLS); however, its specific association with host cell DNA motifs suggests that it plays an important direct role in modulating host cell gene transcription. Nuclear effector proteins have been reported recently in several intracellular human bacterial pathogens including *Ehrlichia, Anaplasma, Shigella* and *Yersinia*. It is well documented that *E. chaffeensis* modulates host cell gene transcription, and this can occur through multiple pathways and host-pathogen interactions. However, directly targeting genes in the host cell nucleus identifies an interesting new and relatively unexplored mechanism that pathogens, including *Ehrlichia*, utilize to modulate host gene transcription.

9.1 Ank 200 interaction with host *Alu* elements

E. chaffeensis Ank200 is translocated to the nuclei of *Ehrlichia*-infected mononuclear phagocytes where it interacts with an adenine-rich motif in promoter and intronic *Alu* elements, [48]. *Alu* elements are short interspersed mobile DNA elements distributed in a nonrandom manner that comprise approximately 5–10% of the human genome and are thought to be involved in transcriptional regulation as a carrier of cis regulatory elements [49;50]. *Alu* elements have known transcription factor binding sites including all MEF2 family members, HNF1.03, OC.2, BARX2 and PAX4 [51]. The association of Ank200 with *Alu* elements suggests that Ank200 could affect gene transcription globally through *Alu*-mediated transcriptional control mechanisms. The global analysis of binding sites of Ank200 demonstrated that this protein binds to multiple regions distributed on nearly every chromosome via direct DNA interaction or with other DNA-binding proteins.

9.2 Ank200 targets apoptosis, ATPase and transcriptional regulatory genes

Chromatin immunoprecipitation (ChIP) and microarrays (ChIP-chip) analysis has identified a subset of Ank200 target genes that have been classified into three gene ontology (GO) databases (biological processes, molecular function and cell structure). These included genes associated with transcriptional regulation (DNA and RNA), apoptosis, ATPase activity, and structural associations with the nucleus [48]. Interestingly, the most targeted genes are associated with cellular processes that are known to be modulated by *E. chaffeensis*, including apoptosis, ATPase activity, and regulation of gene transcription. In addition, Ank200 also appears to bind genes associated with transcription, and there is evidence that a large number of genes associated with transcription are modulated during *E. chaffeensis* infection [4]. ATPase activity is present in ehrlichial inclusions, and genes associated with ATPase activity also appear to be targets of *E. chaffeensis* Ank200 [48].

9.3 Ank200 modulation genes associated with pathobiology

Analysis of Ank200 gene targets identified a number of genes that have been linked to pathogenesis and immune evasion. Specific Ank200 target genes of potential importance that are significantly upregulated or silenced during infection are TNF-α, Jak2 and CD48. Although TNF-α expression is not induced early in infection (<48 hr) [52;53], TNF-α expression is upregulated approximately 30-fold by day 5 post infection [48]. Several studies have demonstrated that overproduction or high serum concentration of TNF-α on day 7 post infection is closely associated with the fatality in severe HME [54;55]. This study

provided evidence that *E. chaffeensis* Ank200 may contribute to the induction of TNF-α by binding directly with promoter and upregulating gene transcription.

It is known that *E. chaffeensis* enters host monocytes by clathrin-independent, receptor-mediated endocytosis [29;56]. However, the detailed mechanism of entry is still unclear, and the identity of the receptor remains undefined. In recent studies, the entry and establishment of infection of *E. chaffeensis* has been associated with caveolae and unidentified host GPI-anchored proteins [57]. CD48, a caveolae-associated GPI-anchored protein, recognized as a receptor for bacterial uptake is strongly upregulated during *E. chaffeensis* infection and is a Ank200 target gene. This situation suggests that *E. chaffeensis* may modulate gene transcription of cellular receptors associated with entry [48].

One of the primary mechanisms by which *E. chaffeensis* survives in the host cell appears to be the ability to block macrophage responsiveness to IFN-γ. *E. chaffeensis* blocks IFN-γ induced tyrosine phosphorylation of Jak and Stat by raising PKA activity in THP-1 cells [7]. Furthermore, Jak2 transcription appears to be silenced during *E. chaffeensis* infection and Jak/Stat genes are also Ank200 targets, suggesting that *E. chaffeensis* uses multiple strategies, including directly modulating genes associated with the Jak/Stat pathway [4]. It is not clear if Ank200 modulates the expression of these genes, but the fact that these genes are associated with ehrlichial pathobiology and pathogenesis and are Ank200 targets suggests that *Ehrlichia* modulates these innate immune response effectors by multiple mechanisms.

10. Conclusions

Ehrlichia TRPs and Ank proteins appear to be key effectors in novel host-pathogen interactions associated with important host cell processes. Recent studies have provided new insight into the complex host-pathogen interactions that require further exploration and definition of the molecular strategies and specific mechanisms involved in evading host defenses and modulation of host cell processes by *Ehrlichia*. New information on *E. chaffeensis* TRP47 interactions with PCGF5, FYN, PTPN2, CAP1, and IGLL1 demonstrates the multifunctional nature of ehrlichial effector proteins and the complexity of host interactions that may ultimately regulate gene transcription, vesicle trafficking, and cell signaling pathways. Modulation of host cell transcription by *Ehrlichia* and the mechanisms involved have been advanced significantly by the identification of a nuclear effector, Ank200, and provide insight into the mechanisms that the pathogen uses to directly modulate host gene transcription. Functional studies on *Ehrlichia* effectors will advance our understanding of the complex network of interactions between obligatory intracellular pathogens and their hosts. Such studies will likely reveal more complex interactions that are unique to these organisms as well as provide new insights into the cell biology and the complex relationship between the pathogen and the host. Future studies focusing on these bacterial effector mechanisms may not only increase our understanding *E. chaffeensis* pathobiology, but also provide us clues to rationally design preventive and therapeutic compounds that can control HME and other intracellular pathogens.

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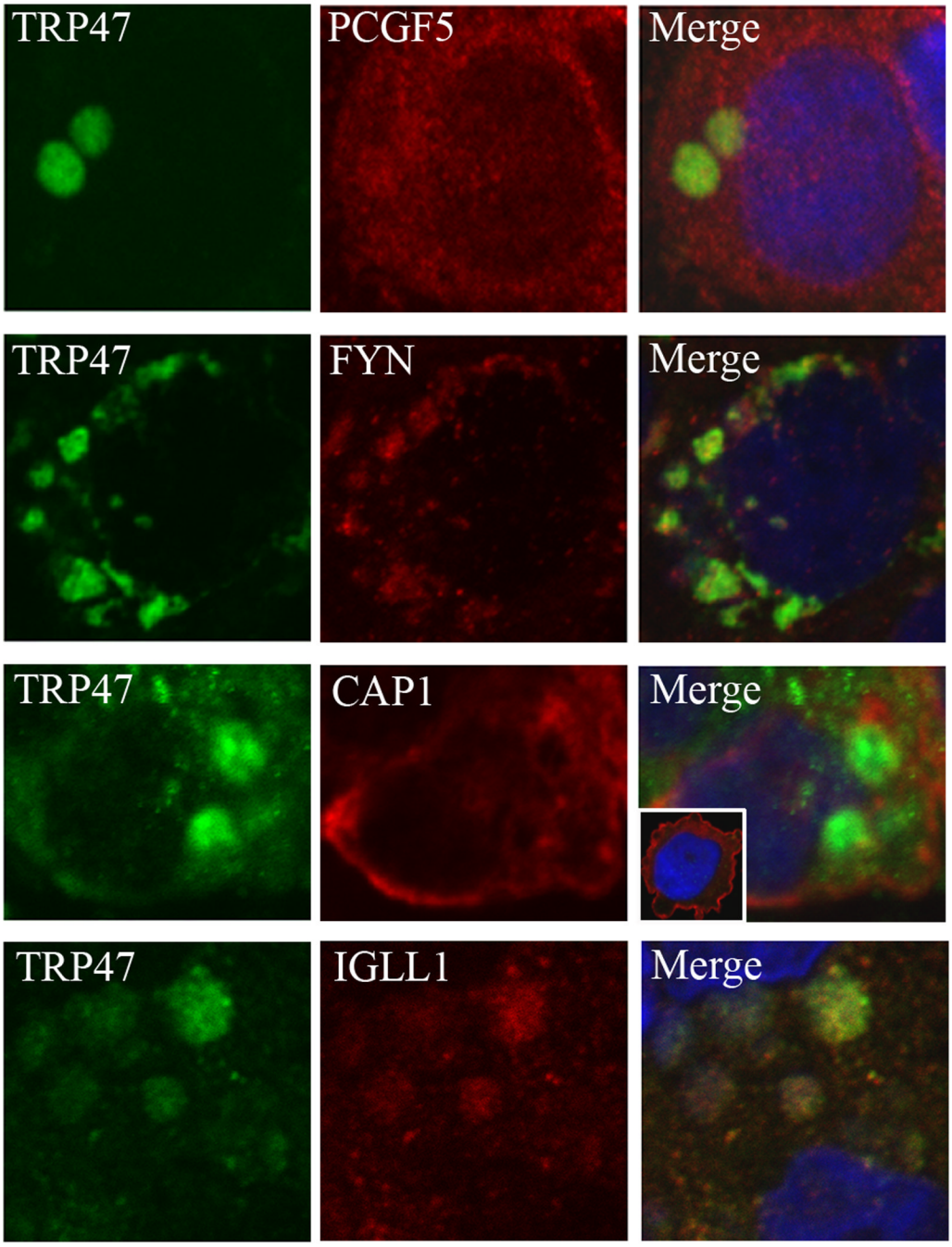

Fig. 1.

Colocalization of *E. chaffeensis* TRP47 with PCGF5, FYN, CAP1, and IGLL1 in *E. chaffeensis*-infected THP-1 cells. THP-1 cells were infected with *E. chaffeensis* and 3 days postinfection were dually labeled and examined by confocal microscopy. The panels on the left were labeled with TRP47 (green), middle panels labeled either with PCGF5, FYN, CAP1, or IGLL1 (red) and the panels on the right are merged images. PCGF5, FYN, CAP1, and IGLL1 colocalize with *E. chaffeensis* TRP47-labeled morulae (right panels, merged images). In the inset (TRP1 + CAP1 merged panel), a normal uninfected THP-1 cell reveals that CAP1 is mainly associated with plasma membrane, while in the *E. chaffeensis*-infected

THP-1 cell, CAP1 is distributed in cytoplasm and associated with *E. chaffeensis*-containing morulae.