Experimental and Applied Acarology (2005) 37: 215–229
DOI 10.1007/s10493-005-3261-8

The molecular and biological analysis of ixodid ticks histamine release factors

ALBERT MULENGA[1,2,*] and ABDU F. AZAD[1]
[1]*Department of Microbiology and Immunology, School of Medicine, University of Maryland Baltimore, Baltimore, MD 21201, USA;* [2]*Present address: Department of Entomology, College of Agriculture and Life Sciences, Texas A & M University, 2475 TAMU, College Station, TX 77843-2475, USA; *Author for correspondence (e-mail: a-mulenga@tamu.edu; phone: +1-979-458-4300)*

Received 1 July 2005; accepted in revised form 13 September 2005

Key words: Anti-tick vaccine development, Global expression, Histamine release factor, Ixodid ticks

Abstract. We previously described a *Dermacentor varibialis* (DV) cDNA that encodes a ubiquitously expressed and tick saliva-secreted functional histamine release factor (HRF) homolog. In this study gene specific primers based on DVHRF open reading frame nucleotide sequence were utilized to amplify three orthologs, from the wood tick, *D. andersoni* (DA), the black legged tick, the southern cattle tick, *Boophilus microplus* (BM) and the lone star tick, *Amblyomma americanum* (AA). At nucleotide level, sequence comparisons revealed 98 89 and 84% similarity to DVHRF for DAHRF, AAHRF and BMHRF, respectively, while predicted polypeptide comparisons revealed 98, 96 and 91% similarity for DAHRF, AAHRF and BMHRF respectively. Phylogenetically, the tick HRF clade, while distinct (100% bootstrap value), is closely related to other arthropods, but distantly related to vertebrate and protozoan clades. Consistent with sequence similarity analysis, a DVHRF-specific northern blotting probe hybridized a ~900 base pair (bp) mRNA band on all RNA blots. Likewise a mouse polyclonal antibody to *E. coli*-expressed recombinant (r) DVHRF, cross-reacted baculovirus-expressed non-fusion rAAHRF, rDAHRF, and rBMHRF. As revealed by northern blotting analysis of larvae and nymph RNA, DVHRF mRNA is expressed in both immature and mature ticks indicating that its transcription is not developmentally regulated. Unlike rHRF/TCTP proteins of other organisms, the calcium-binding function may not be conserved for tick HRF homologs as revealed by the $^{45}CaCl_2+$ overlay assay. Apparent global expression of DVHRF and its orthologs make this protein family an ideal target antigen for development of novel tick control strategies targeting multiple tick species.

Introduction

The histamine release factor (HRF)/translationally controlled tumor protein (TCTP) also known as p23 or p21 was originally detected in mouse ascites and erythroleukemia (Gachet et al. 1999). HRF/TCTP homologs have since been found in several organisms including, human (McDonald et al. 1995) plants (Ermolayev et al. 2003), yeast (Thaw et al. 2001), earthworms (Sturzenbaum et al. 1998), hemoparasites (*Plasmodium* spp. (McDonald et al. 2001), *Schistosoma mansoni* (Rao et al. 2002), *Wuchereria bancrofti* and *Brugia malayi* (Gnanasekar et al. 2002)), *Hydra vulgaris* (Yan et al. 2000), hematophagous (Mulenga et al. 2003a, b) and, non-hematophagous arthropods (Lee et al.

2004). Published functional studies describe HRF/TCTP homologs as growth-dependent, calcium-binding protein with capacity to induce secretion of histamine from basophils in an IgE-independent manor (Bommer and Thiele 2004). Other studies have demonstrated tubulin-binding function (Gachet et al. 1999) and induction of cytokine secretion from eosinophils (MacDonald et al., 2001). A recent molecular modeling study revealed that the *Schizosaccharomyces pombe* TCTP polypeptide was structurally closely related to the mammalian suppressor of Sec4 (Mss4/DSS4), a chaperone for GDP/GTP-free G proteins (Thaw et al. 2001). In related studies, HRF/TCTP was shown to interact with the translation elongation factor eEF1A and its guanine nucleotide exchange factor eEF1B-β (Cans et al. 2003). While the precise biological functions are unknown, ubiquitous expression, and structural conservation underscores the central role this protein is likely to play in the physiology of several organisms including ticks. From this stand point, and its association with allergy (Escura et al. 1998), up regulation in cancer proliferation (Vercouter et al. 2001; Stierum et al. 2003) and reduced expression in cancer remission Tuynder et al. 2002, 2004), HRF/TCTP is receiving tremendous research attention as a target for drug development against these conditions (Bommer and Thiele 2004).

We previously identified a *Dermancetor variabilis* (DV) HRF homolog that was differentially up regulated in *Rickettsia montana* infected ovaries (Mulenga et al. 2003a, b). We subsequently showed that both DVHRF mRNA and protein were ubiquitous, that the native protein was present in experimentally induced tick saliva and that its recombinant protein was able to induce histamine from basophils (Mulenga et al. 2003). Expression and apparent secretion of functional DVHRF into ixodid tick saliva during the feeding process is counter-intuitive in that ticks are known to counter the effects of histamine by secretion of histamine binding proteins (HBPs) into the feeding site to alleviate the impact of histamine (Chinery and Ayiteh-Smith 1977; Paessen et al. 1999, 2000; Bior et al. 2002; Sangamnatdej et al. 2002). Published studies (Koudstaal et al. 1978; Willadsen et al. 1979; Kemp and Bourne 1980; Paine et al. 1983) have demonstrated that histamine secreted into the feeding site not only affects the tick's capacity to attach onto host skin, but also affects its reproductive efficiency. Because of an apparent global expression exhibited by HRF/TCTP homologs, we wanted to investigate whether or not this also true for ixodid ticks. In this article we report on utilization of DVHRF ORF-specific primers, to clone orthologs from four other ixodid ticks, *D. andersoni, Boophilus microplus* and *Amblyomma americanum*. We provide evidence to show that the five tick HRF orthologs are not only conserved at sequence level but that their recombinant proteins are immuno-cross-reactive and that unlike homologs of most other organisms (Bommer and Thiel 2004), the calcium-binding function may not be conserved in ticks. We further show that, phylogenetically, while tick HRF clade is unique, it is closely related to other arthropod clades, hematophaous or not, but distantly related to vertebrate and protozoan.

Experimental procedures

Ticks and RNA extraction

Partially fed adult *D. variabilis* and *A. mericanum* ticks as well as unfed adult, nymph and larvae *D. variabilis* were provided by our collaborator, Dr. Sonenshine at Old Dominion University. Immortalized *D. andersoni* (DAE100) and *B. microplus* (BME26) cell lines were kindly provided by Dr. Timothy Kurtti at the University of Minnesota. Total RNA extraction was done using the Trizol reagent according to instructions by the manufacturer (Invitrogen, Carlsbad, CA). Whole ticks were first rinsed in 70% ethanol, air dried, pulverized in liquid nitrogen, and immediately transferred to the Trizol reagent. Following a quick rinse in DPEC treated-water, tick cells collected from culture flasks were homogenized directly in Trizol and processed for RNA extraction.

Cloning of full-length DVHRF ortholog cDNA and sequence analysis

Following instructions by the manufacturer of the kit (Invitrogen), DVHRF-ORF specific primers (sense (ATGCTGATTTTTAAGGATAAGATC) and antisense (CATTTTTTCTTCATCCAGACCATG) were used in single step RT-PCR to clone HRF ORFs of *D. andesoni* (DA), *A. americanum* (AA) and *B. microplus* (BM) according to instructions by the manufacturer. Following sequencing, a gene specific primer was designed and used in 5′ rapid amplification of cDNA ends (RACE) to clone the 5′ end cDNA fragment according to instructions by the manufacturer (Clontech, Palo Alto, CA). Following sequencing of the 5′ end fragment, a sense primer based on the extreme 5′ end was used in 3′ RACE to clone full-length cDNA. Templates for 3′ and 5′ RACE were synthesized from 5 μg of total RNA extracted from DA, AA, IS and BM using the SMART RACE cDNA synthesis kit according to the manufacturer's protocol (ClonTech). PCR products were routinely cloned in TOPO TA cloning vectors (Invitrogen). The plasmid template for sequencing was spin column purified using the SV miniprep kit (Promega, Madison, WI) and nucleotide sequences were determined using the universal M13 forward or reverse primers, as well as gene specific primers when necessary. Samples were sequenced by the dye terminator method on a 373 automated fluorescence sequencing system (Applied Biosystems, Foster City, CA). DNA sequence analysis utilized the MacVector software program (Accelrys, San Diego, CA). For similarity comparisons to known proteins on the database, DNA sequences were scanned against the GenBank database using BlastX.

Phylogenetic tree analysis

The phylogeny tree was constructed from the dataset of 39 HRF/TCTP polypeptide sequences downloaded from GenBank and five tick HRF homologs (Table 1) using the neighbor joining method provided in the MacVector DNA analysis software package. Specifications were set for bootstrap values at 500 replications, gaps proportionately distributed and correction for distance set to Poisson distribution. The tree was rooted from an unrelated outlier, a known calcium binding *Mus musculus* tropomyosin protein (Table 1).

Northern blotting analysis

To investigate whether a DVHRF-specific probe will hybridize to its orthologs, total RNA extracted from DV, DA, AA and BM were subjected to northern blotting analysis. Similarly, to investigate whether transcription of DVHRF was developmentally regulated, total RNA of immature ticks (larvae and nymph) were also subjected to northern blotting analysis. Samples (3–7.5 μg total RNA per lane) treatment, electrophoresis and transfer to Hybond N + membranes were done as described in Northern-Max protocol (Ambion, Austin, TX). Following transfer, processing of the RNA blots was done as previously described (Mulenga et al. 2003b). The membranes were washed to a final stringency of 0.1× SSC plus 0.1 % SDS at 65 °C twice for 15 min each and subsequently exposed to an X-ray film for 40 min at −80 °C.

Expression of recombinant (r) DVHRF and its orthologs

Baculovirus expression

Insect cell expression of rDVHRF and its orthologs (DA, AA and BM) was done according to instructions provided in the user manual of Bac to Bac baculovirus expression system (Invitrogen). Utilizing DVHRF ORF primers (above) with added *Spe*I/*Xho*I restriction enzymes for unidirectional cloning, coding regions were amplified and sub-cloned into the pFASTBAC donor plasmid (invitrogen) that was digested with appropriate enzymes. The pFASTBAC-tick HRF constructs were used to transform DH10Bac competent cells to allow for site directed transposition of the expression cassette into the expression bacmid. Recombinant bacmids were transfected into sf9 cells (Invitrogen) using Cellfectin according to instructions by the manufacturer (Invitrogen) to produce the primary baculovirus stock. Following amplification, viral stocks were used for protein expression. Expression was confirmed by tricine SDS-PAGE with Coomassie blue staining and western blotting analysis as described below.

Table 1. HRF/TCTP polypeptides used to construct the phylogeny tree.

HRF/TCTP polypeptide source	GeneBank accession no.
Mus musculus tropomyosin[a]	X64831
Hydra vulgaris	U76187
Trypanosoma brucei	AAX80036
Entomoeba hystolytica	EAL51230
Cryptosporidium parvum	EAK89103
Theileria annulata	CA173774
Plasmodium knowlesi	P84152
P. falciparum	NP_703454
P. chabaudi	CAA84320
P. yoeli	EAA16837
P. berghei	CAA96616
Dictyostelium discoideum	XP_639361
Zea mays	AAN40686
Arabiptosis thaliana	AAM66134
Pisum sativum	AAB19090
Medicago sativa	X98618
Galus galus	NM_205398
Ratus norveticus	NP_446319
Orytagolus caniculus	AJ277093
Pan troglodytes	XM_509662
Bos taurus	NP_001014410
Mus musculus	NM_009429
Human	CAH72035
Xenopus tropicalis	BC080968
X. laevis	BC043811
Schistosoma mansoni	AF358139
S. japonicum	AAB42079
Schizosacharomyces pombe	CAA93806
Candida albicans	EAK99010
Panaeus monodon	AA061938
Anopheles gambiae	EAA08161
Aedes albopictus	AAV90736
Apis mellifera	XP_623154
Bombyx mori	BAC99978
Drosophila pseudoobscura	EAL29084
D. yakuba	AAR09822
D. melanogaster	AAF54603
Boophilus microplus	DQ009479
Amblyomma americanum	DQ009481
Dermacentor andersoni	DQ009480
D. variabiis	AAL75585
Chaenorhabitis elegans	CAB02099
Brugia malayi	AAC47622
Wuchereria bancrofti	AAK71499

[a]Outlier for rooting the tree.

E. coli *expression*

For *E. coli* expression, construction of expression plasmids of DAHRF, AAHRF and BMHRF and induction of recombinant protein expression was done as previously described (Mulenga et al. 2003b). The pET 32 *E. coli* expression system produces His-tag fused recombinant proteins to allow for affinity purification on metal element charged columns. Briefly, DVHRF ORF primers with added *Eco*RV/*Sac*II restriction enzyme sites for unidirectional cloning were used to amplify the coding regions. Following transformation of BL21 (DE3) pLysS host cells, expression was induced by addition of isopropyl-*β*-D-thiogalactopyranoside (IPTG) to final concentration of 0.9 mM/ml of culture and incubation continued for 6 h at 37 °C. Recombinant proteins were purified by affinity chromatography under native conditions as according to instructions in the pET system manual (Novagen, Madison, WI).

SDS-PAGE and western blot analyses

Utilizing standard protocols, *E. coli* and insect cell expression samples were, respectively, subjected to glycine and tricine SDS-PAGE using 4–20 or 10–20% pre-cast poly-acrylamide gels (Invitrogen). Following electrophoresis, gels were either stained with Coomassie blue or processed for western blotting analysis. Following transfer, membranes were probed with polyclonal antibodies to *E. coli*-expressed rDVHRF and naïve mouse serum according to instructions provided in the Western breeze chemiluminescent substrate kit (Invitrogen). Positive signals were captured by autoradiography.

Calcium-binding assay

Calcium-binding of *E. coli*-expressed rDVHRF, rDAHRF and rAAHRF was evaluated using a $^{45}CaCl_2+$ overlay assay as described elsewhere (Arcuri et al. 2004). Approximately 15 μg of affinity purified, *E. coli*-expressed rDVHRF and its orthologs were resolved on a 10–20% gradient poly-acrylamide gel and transblotted onto nitrocellulose membranes (Invitrogen) according to standard protocols. Bovine serum albumin and crude mouse heart muscle protein extracts were used for negative and positive controls respectively. Following transfer, the membranes were briefly washed in buffer (60 mM KCl, 5 mM $MgCl_2$, 10 mM imidazole–HCl (pH 6.8). The washed membranes were incubated in the same buffer containing 10 μCi/ml of $^{45}CaCl_2+$ (GE healthcare, Waukesha, WI) for 20 min. The membranes were subsequently subjected to two 5 min washes in 30% ethanol and air dried for 10–30 min. The air dried membranes were exposed to X-ray films at room temperature for 5–30 min and protein associated radioactivity determined by autoradiography.

Results

Cloning, sequence and RNA analyses

While attempting to identify consensus domains from a multi-sequence analysis, we observed that HRF/TCP homologs of the same genus showed very high sequence similarity approaching 100% around the 5′ and 3′ ends of the ORF (results not shown). We wanted to investigate whether this observation was also consistent for ixodid ticks. Here we report on utilization of DVHRF ORF-specific PCR primers (Mulenga et al. 2003b) to amplify four orthologs, from *A. americanum* (AA), *D. andersoni* (DA) and *B. microplus* (BM) (Figure 1). Internal gene-specific primers from highly conserved regions of the DVHRF ORF (see Figure 1) were used in 5′ and 3′ RACE to clone full length cDNAs of AAHRF (GeneBank accession no. DQ009481), BMHRF (DQ009479) and DAHRF (DQ009480). Pair wise alignments of coding regions revealed 98, 89 and 84% similarity to DVHRF for DAHRF, AAHRF and BMHRF, respectively, while predicted polypeptides similarity to DVHRF protein were 98% for DAHRF, 96% for AAHRF and 91% for BMHRF (Figure 1 and Table 2). Similar to the DVHRF (Mulenga et al. 2003), predicted polypeptides of its orthologs do not have leader sequences and are predicted to be cytoplasmic with 70% reliability as revealed by the psort (site) analysis. Consistent with DNA analysis data, a DVHRF-specific northern

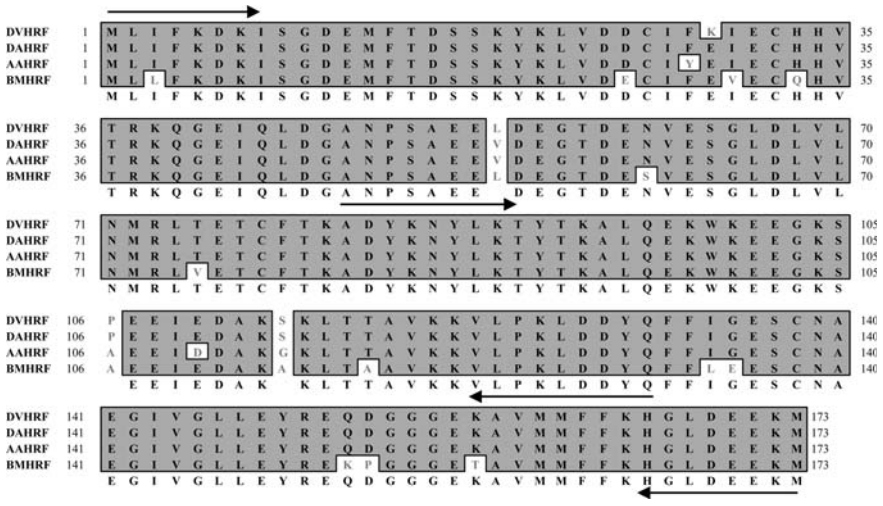


Figure 1. Multi-sequence alignment of the *D. variabilis* HRF homolog (DVHRF) predicted polypeptide (Mulenga et al. 2003) and its orthologs from *D. andersoni* (DA), *B. microplus* (BM) and *A. americanum* (AA). Similarities are boxed and shaded gray, differences are open and not shaded. Conserved (CONS) are listed at the bottom of the alignment. Numbers indicate amino acid position. Underlined regions indicate PCR primer regions. Arrow heads indicate sense (→) and anti-sense (←) primers.

Table 2. % Similarity comparisons to DVHRF open reading frame.

	Nucleotide	Amino acid
DAHRF	98	98
AAHRF	89	96
BMHRF	84	91

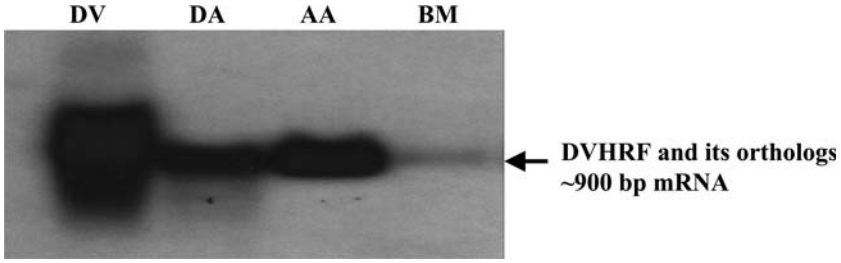


Figure 2. Northern blotting analysis of total RNA extracted from *D. variabilis* (DV), *D. andersoni* (DA), *A. americanum* (AA) and *B. microplus* (BM). An α^{32}P-dATP-labeled DVHRF-specific probe was used to probe the membrane and the positive signal captured by autoradiography.

blotting probe cross-hybridized a ~900 base pair mRNA band on DA, AA and BM RNA blots (Figure 2).

Phylogenetic analysis

Using the neighbor joining method, we successfully constructed a phylogenetic tree rooted from an unrelated outlier, a *Mus musculus* tropomyosin which is a known calcium-binding protein (Figure 3). From the *M. musculus* tropomyosin outlier, five groups (A–E) and lone *Hydra vulgaris*, branched off. Branches B and C are not supported by bt values indicating a distant relationship among members within these branches. On the other hand, branches A, D and E are supported by bt values of 86, 100 and 87%, respectively, indicating high similarity among clades represented in these branches. Within the A-branch, the tick, mosquito and hemo-nematode clades are strongly supported by 100% bt values indicating that clades within the A branch while closely related, are distinct. It is also important to note that, clades of hematophagous arthropods (Hpv), vertebrates hosts (Vh) and vector borne hemoparasites (Vbhp) belong to distinct groups supported by high bootstrap values.

Protein expression and western blotting analysis

SDS-PAGE and Coomassie blue staining analyses of sequentially harvested spent medium and cells revealed that rDVHRF and its orthologs expressed

as soluble proteins and were secreted into culture medium (results not shown). In agreement with sequence and northern blotting analyses data (Figures 1 and 2, Table 1), a mouse polyclonal antibody to *E. coli*-expressed rDVHRF (Mulenga et al. 2003b) reacted to comparable intensity on protein blots of rDVHRF and its orthologs (Figure 4). This result demonstrated that polypeptides of DVHRF and its orthologs are cross-immuno reactive.

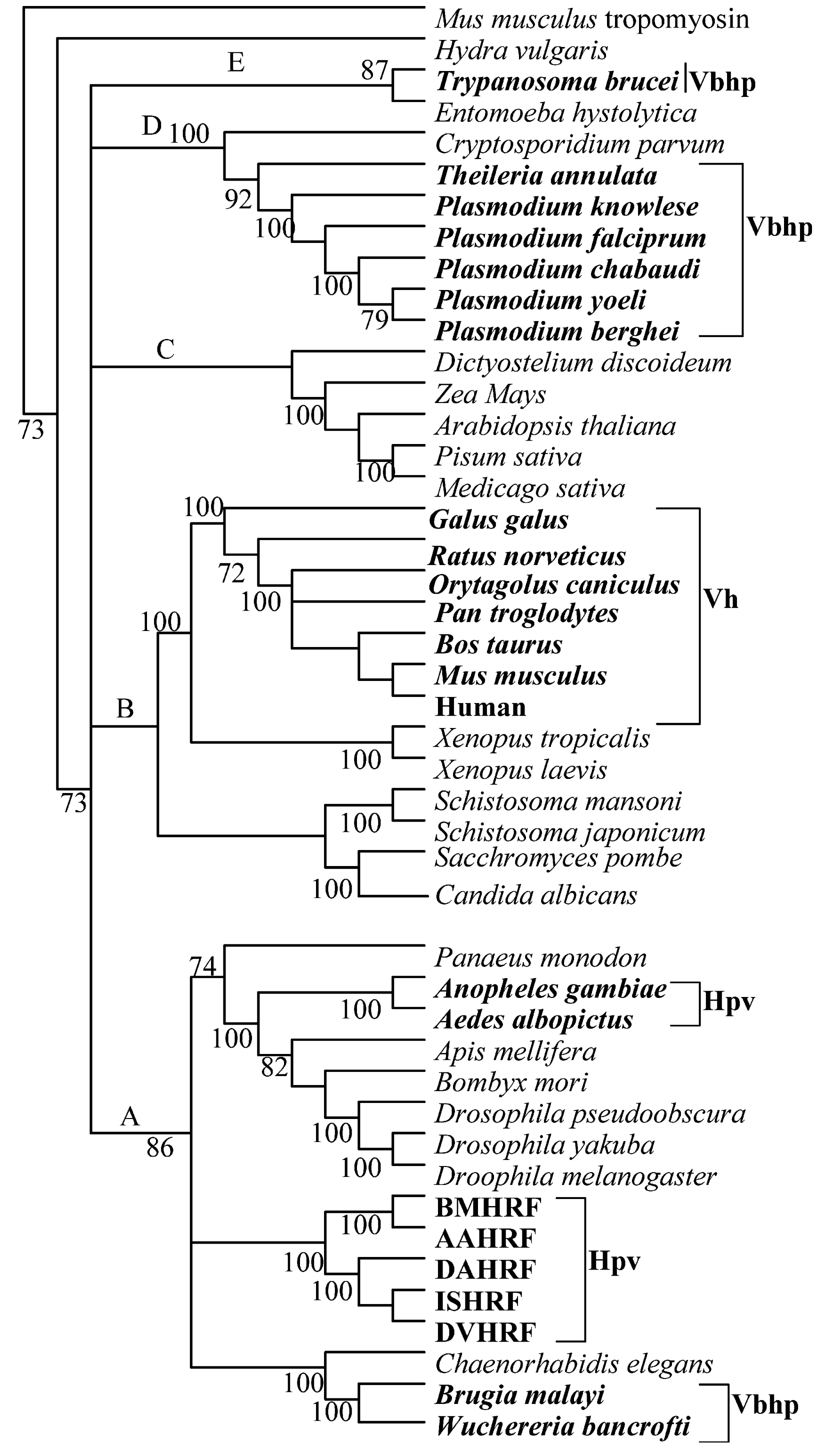
Due to low expression levels observed with the insect cell expression system, baculovirus-expressed rDVHRF and its orthologs were not purified. To generate sufficient amounts of proteins for down stream assays, DVHRF orthologs were expressed in *E. coli* as described (Mulenga et al. 2003b). Except for BMHRF which expressed as insoluble fractions and were removed from the assay, AAHRF and DAHRF expressed as soluble fractions and were successfully affinity purified as provided in the pET expression system user manual (Novagen) (results not shown).

Calcium-binding assay

Most studied HRF/TCTP orthologs do have a calcium binding and induction of secretion of histamine functions (Bommer and Thiele 2004). In the previous study, we showed that rDVHRF was able to induce secretion of histamine from basophils (Mulenga et al. 2003b). In this study we wanted to investigate whether the calcium-binding function was also conserved in ixodid tick HRF homologs. As revealed by the $^{45}CaCl_2$ overlay assay, no calcium-binding activity was detectable for *E. coli*-expressed rDVHRF, rDAHRF and rAAHRF above background control (results not shown). Two diffuse bands at 20 and 45 kDa were detected on the mouse heart muscle positive control blot, probably representing myosin light chain and tropomyosin. Our conclusion should be considered cautiously due to the possibility of not having correct folding of *E. coli*-expressed tick proteins.

Transcription of DVHRF is not developmentally regulated

We previously showed that neither translation nor transcription of DVHRF were dependent upon the uptake of a blood meal by ticks (Mulenga et al 2003b). In this study we tested whether or not transcription of DVHRF mRNA transcription was developmental stage specific. As shown in Figure 5 the DVHRF mRNA was transcribed through out the tick's developmental cycle suggesting that its transcription was not developmentally regulated. Since both DAHRF and BMHRF were amplified from total RNA that was prepared from immortalized embryonic cell lines, we assume that tick HRF is also expressed in tick eggs although this was not done.



Mus musculus tropomyosin
Hydra vulgaris
Trypanosoma brucei
Vbhp
Entomoeba hystolytica
Cryptosporidium parvum
Theileria annulata
Plasmodium knowlese
Plasmodium falciprum
Plasmodium chabaudi
Plasmodium yoeli
Plasmodium berghei
Vbhp
Dictyostelium discoideum
Zea Mays
Arabidopsis thaliana
Pisum sativa
Medicago sativa
Galus galus
Ratus norveticus
Orytagolus caniculus
Pan troglodytes
Bos taurus
Mus musculus
Human
Vh
Xenopus tropicalis
Xenopus laevis
Schistosoma mansoni
Schistosoma japonicum
Sacchromyces pombe
Candida albicans
Panaeus monodon
Anopheles gambiae
Aedes albopictus
Hpv
Apis mellifera
Bombyx mori
Drosophila pseudoobscura
Drosophila yakuba
Droophila melanogaster
BMHRF
AAHRF
DAHRF
ISHRF
DVHRF
Hpv
Chaenorhabidis elegans
Brugia malayi
Wuchereria bancrofti
Vbhp
E
D
C
B
A
87
100
92
100
100
79
100
100
73
100
72
100
100
100
73
100
100
74
100
100
82
86
100
100
100
100
100
100
100

Figure 3. Phylogenetic tree analyses: GenBank downloads (Table 1) of HRF/TCTP, DVHRF (Mulenga et al. 2003) and its orthologs predicted polypeptides were used in the phylogeny tree construction using the neighbor joining method. Main branches from *Hydra vulgaris* are marked with A–E (bold). The bootstrap values (a measure of reliability) are indicated at the node of each clade. Glades for hematophaous vector arthropods (**Hpv**), vector borne hemoparasites (**Vbhp**) and vertebrate hosts (**Vh**) are bolded.

Discussion

We previously reported that the American dog tick, *D. variabilis* encoded a functional histamine release factor homolog (HRF) that was apparently secreted into tick saliva during feeding (Mulenga et al. 2003b). In the present study we describe cloning and biological characterization that orthologs of DVHRF are conserved in at least four ixodid tick species tested here. In humans, HRF homologs have been reported to be involved in a wide range of important physiological functions. While properties of human HRF homologs may not be true for ticks, apparent secretion into tick saliva during feeding and conservation of DVHRF and its orthologs in ixodid ticks make this protein family an attractive candidate for development of vaccines targeting multiple tick species.

Whether or not the diversity among HRF/TCTP homologs as revealed by phylogenetic analysis is an indicator of how the function of this protein family may have diverged through evolution is unknown at present. From published studies (Bommer and Thiele 2004), key properties for the HRF/TCTP protein family, induction of secretion of histamine from basophils and calcium binding are apparently conserved in both parasites and their vertebrate host. In the context of arthropod–host–pathogen interactions, expression of HRF/TCTP homologs by hematophagous vector arthropods, vector-borne hemoparasites and, the vertebrate hosts is very intriguing. The question is, what impact if any does pathogen expressed HRF/TCTP protein, for instance have on their invertebrate and vertebrate hosts. All parasitic versions of HRF/TCTP proteins appear to be secreted into the vertebrate host organism. We showed that native DVHRF was present in experimentally induced tick saliva (Mulenga et al. 2003b) suggesting that it was injected into the host during tick feeding. In related studies, *S. mansoni* (Rao et al. 2002), *W. bancrofti* and *B. malayi* (Gnanasekar et al. 2001) HRF/TCTP proteins are secreted into the host and are thought to cause an inflammatory infiltration of eosinophils through chemotaxis. McDonald et al. (2001) have shown by western blotting analysis, that *P. falciparum* TCTP is secreted into the host and its recombinant protein is capable of inducing histamine secretion from human basophils and IL-8 from eosinophils, although at reduced efficiency than the human version. Interestingly *P. yoeli* TCTP was shown to bind the anti-malarial drug, artemisinin and increased TCTP expression correlated with increased resistance against this

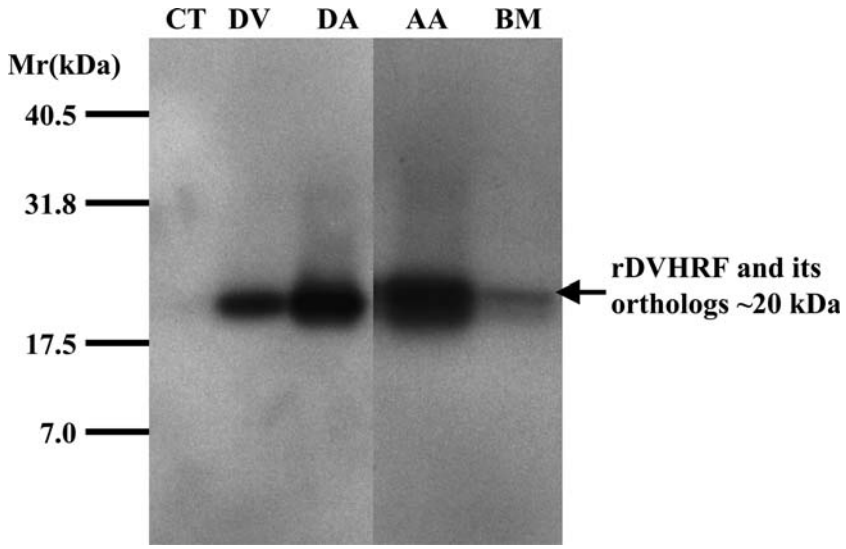


Figure 4. Western blotting analyses: A mouse polyclonal antibody to *E. coli*-expressed rDVHRF was used to probe protein blots of baculovirus-expressed rDVHRF (DV), rDAHRF (DA), rAAHRF (AA) and rBMHRF (BM). CT (negative control) was a culture supernatant from cells transfected with an empty expression bacmid. Please note that the negative control naïve mouse serum blot is not shown.

drug (Walker et al. 2000). With reference to interactions with the vertebrate host, it will be interesting to compare and contrast the functions the HRF/TCTP protein family in hematophagous vector arthropods and vector borne hemoparasites.

Our long term objective is to utilize DVHRF or any of its orthologs to immunize against multiple tick species. Conservation of antigenic epitopes is

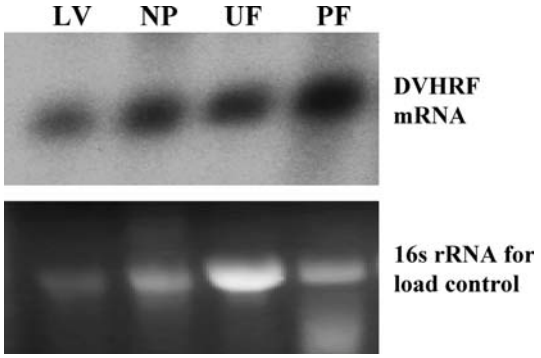


Figure 5. Transcription analysis of DVHRF mRNA in immature *D. variabilis* ticks: Total RNA blots of unfed larvae (LV) and Nymphs (NP) as well as unfed (UF) and partially fed (PF) adult ticks were probed with a α^{32}P-dATP-labeled DVHRF probe. Positive signals were captured by autoradiography.

a key property for candidate anti-tick vaccine antigens targeting multiple tick species. Thus cross-immunoreactivity among tick HRF homolog polypeptides as revealed by our western blotting analysis data is encouraging. The implication of this finding is that, an immune response against one tick homolog will be effective against all members of the genera. The mouse polyclonal antibody used in this study was produced against thioredoxin-fused rDVHRF protein which carried a His-tag (Mulenga et al. 2003b). A possibility existed that the immunogenecity exhibited by *E. coli*-expressed rDVHRF could have been due to antigenic epitopes on the thioredoxin and/or His-tag portions of the protein. However, the fact that rDVHRF and its orthologs used in this assay were expressed from a vector which did not produce a His-tag-fused protein rules out this possibility, but rather show that the observed immunological cross-reactivity was due to conserved tick HRF antigenic epitopes.

Findings presented in this study that transcription of DVHRF mRNA is not developmentally regulated and the fact that neither translation nor transcription is dependent on blood meal feeding (Mulenga et al. 2003) may suggest that DVHRF and its orthologs may play no direct role in facilitation of blood meal uptake by ticks. Our assertion here could well be supported by the fact that tick HRF homologs are closely related to non-blood feeders as revealed by phylogenetic analysis. As a candidate antigen for development of anti-tick vaccines, apparent lack of a role in facilitation of blood meal feeding by ticks is not an attractive property. We are however encouraged by the apparent wide involvement of HRF homologs in several physiological functions (Bommer and Thiele 2004) which if immunologically blocked could incapacitate the tick's ability as a vector and pest. It is also interesting to note that immunizing against a molecule like DVHRF will be effective against all developmental stages of the tick which can be very attractive when dealing with a single-host tick such as *B. microplus*. We are currently focused on studies to elucidate the physiological roles of DVHRF and its orthologs in tick physiology and at the tick–host interface.

Acknowledgements

We thank Drs. Sonenshine and Kurti for providing the ticks and tick cells used in this study. This work was supported in part by funds from NIAID/NIH.

References

Arcuri F., Papa S., Carducci A., Romagnoli R., Liberatori S., Riparbelli G.M., Sanchez J.C., Tosi P. and del Vecchio M.T. 2004. Translationally controlled tumor protein (TCTP) in the human prostate and prostate cancer cells: expression, distribution, and calcium binding activity. Prostate 60: 130–140.

Bior A.D., Essenberg R.C. and Sauer J.R. 2002. Comparison of differentially expressed genes in the salivary glands of male ticks, *Amblyomma americanum* and *Dermancetor andersoni*. Insect Biochem. Mol. Biol. 32: 645–655.

Bommer U. and Thiele B. 2004. The translationally controlled tumor protein (TCTP). Int. J. Biochem. Cell. Biol. 36: 379–385.

Cans C., Passet B.J., Shalak V., Portebois V.N., Crible V., Amzallag N., Allanic D., Tufino R., Argentin M., Moras D., Fiucci G., Goud B., Mirande M., Amson R. and Telerman A. 2003. Translationally controlled tumor protein acts as a guanine nucleotide dissociation inhibitor on the translation elongation factor eEF1A. Proc. Natl. Acad. Sci. USA 100: 13892–13897.

Chinery W.A. and Ayiteh-Smith E. 1977. Histamine blocking agent in the salivary gland homogenate of the tick *Rhipicephalus sanguineus*. Nature 265: 366–367.

Ermolayev V., Weschke W. and Manteuffel R. 2003. Comparison of Al-induced gene expression in sensitive and tolerant soybean cultivars. J. Exp. Botany 54: 2745–2756.

Escura R.B., Shroeder J.T. and MacDonald S.M. 1998. HrHRF: function and regulation. Drug News Perspect 11: 223–229.

Gachet Y., Tournier S., Lee M., Lazaris-Karatzas A., Poulton T. and Bommer U.A. 1999. The growth-related, translationally controlled protein P23 has properties of a tubulin binding protein and associates transiently with microtubules during the cell cycle. J. Cell Sci. 112: 1257–1271.

Gnanasekar M., Rao K.V.N., Chen L., Narayanan R.B., Geetha M., Scott A.L., Ramaswamy K. and Kaliraji P. 2002. Molecular characterization of a calcium binding translationally controlled tumor protein homologue from the filarial parasites *Brugia malayi* and *Wuchereria bancrofti*. Mol. Biochem. Parasitol. 121: 107–118.

Kemp D.H. and Bourne A. 1980. *Boophilus microplus*: the effect of histamine on the attachment of cattle-larvae, studies *in vivo* and *in vitro*. J. Parasitol. 80: 487–496.

Koudstaal D., Kemp D.H. and Kerr J.D. 1978. *Boophilus microplus*: rejection of larvae from British breed cattle. Parasitology 76: 379–386.

Lee M.J., Kusakabe T., Kawaguchi Y., Miyagawa Y., Takahashi M., Mon H., Nho S. and Koga K. 2004. Molecular cloning and characterization of the translationally controlled tumor protein gene in *Bombyx mori*. Comp. Biochem. Physiol. Part B 139: 35–43.

McDonald S.M., Bhisutthibhan J., Shapiro T.A., Rogerson S.J., Taylor T.E., Tembo M., Langdon J.M. and Meshnick S.R. 2001. Immune mimicry in mamalaria: *Plasmodium falciparum* secretes a functional histamine-releasing factor homologue *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. USA 98: 10829–10832.

McDonald S.M., Rafnar T., Langdon J. and Lichtenstein L.M. 1995. Molecular identification of an Ige-dependent histamine-releasing factor. Science 269: 688–690.

Mulenga A., Macaluso K.R. and Azad A.F. 2003a. Dynamics of *Rickettsia*-tick interactions: identification and characterization of differentially expressed mRNAs in uninfected and infected. Insect Mol. Biol. 12: 185–193.

Mulenga A., Macaluso K.R., Simser J.A. and Azad A.F. 2003b. The American dog tick encodes a functional histamine release factor homolog. Insect Biochem. Mol. Biol. 33: 911–919.

Paessen G.C., Adams P.L., Harlos K., Nuttal P.A. and Stuart D.I. 1999. Tick histamine binding proteins: isolation, cloning, and three-dimensional structure. Mol. Cell 3: 661–671.

Paesen G.C., Adams P.L., Nuttal P.A. and Stuart D.L. 2000. Tick histamine-binding proteins: lipocalins with a second binding cavity. Biochim. Biophys. Acta 1482: 92–101.

Paine S.H., Kemp D.H. and Allen J.R. 1983. *In vitro* feeding of *Dermacentor andersoni* (stiles): effects of histamine and other mediators. Parasitology 86: 419–428.

Rao K.V.N., Chen L., Gnanasekar M. and Ramaswamy K. 2002. Cloning and characterization of a calcium binding, histamine-releasing protein from *Schitosoma mansoni*. J. Biol. Chem. 277: 31207–31213.

Sangamnatdej S., Paessen G.C., Slovak M. and Nuttall P.A. 2002. A high affinity serotonin- and histamine-binding lipocalin from tick saliva. Insect Mol. Biol. 11: 79–86.

Stierum R., Gaspari M., Dommels Y., Ouatas T., Pluk H., Jespersen S., Vogels J., Verheckx K., Groten J. and van Ommen B. 2003. Proteome analysis reveals protein associated with proliferation and differentiation of colorectal cancer cell line Caco-2. Biochem. Biophys. 1650: 73–91.

Sturzenbaum S.R., Kille P. and Morgan A.J. 1998. Identification of heavy metal induced changes in the expression patterns of the translationally controlled tumor protein (TCTP) in the earthworm *Lumbicus rubellus*. Biochem. Biophys. Acta 1398: 294–304.

Thaw P., Baxter N.J., Hounslow A.M., Clive P., Waltho J.P. and Graven J. 2001. Structure of TCTP reveals unexpected relationship with guanine nucleotide free chaperones. Nat. Struct. Biol. 8: 701–704.

Tuynder M., Fiucci G., Prieur S., Lespagnol A., Geant A., Beaucourt S., Duflaut D., Besse S., Susini L., Cavarelli J., Moras D. and Amson R. 2004. Translationally controlled tumor protein is a target of tumor reversion. Proc. Natl. Acad. Sci. USA 101: 15364–15369.

Tuynder M., Susini L., Prieur S., Besse S., Fiucci G., Amson R. and Telerman A. 2002. Biological models and genes of tumor reversion: cellular reprogramming through tpt1/TCTP and SIAH-1. Proc. Natl. Acad. Sci. USA 99: 14976–14981.

Vercouuter-Eduart A.S., Czeszak X., Crepin M., Lemoine J., Boilly B., Le Bourhis X., Peyrat J.P. and Hondermarck H. 2001. Proteomic detection of changes in protein synthesis induced by fibroblast growth factor-2 in MCF-7 human breast cancer cells. Exp. Cell Res. 262: 59–68.

Walker D.J., Pitsch J.L., Peng M.M., Robinson B.L., Peters W., Bhisutthibhan J. and Meshnick S.R. 2000. Mechanisms of artemisinin resistance in the rodent malaria pathogen, *Plasmodium yoeli*. Antimicrob. Agents Chemother. 44: 344–347.

Willadsen P., Wood G.M. and Riding G.A. 1979. The relation between skin histamine concentration, histamine sensitivity, and the resistance of cattle to the tick, *Boophilus microplus*. Z. Parasitenkd 59: 87–93.

Yan L., Fei K., Bridge D. and Sarras M.P.Jr 2000. A cnidarian homologue of translationally controlled tumor protein (P23/TCTP). Dev. Genes Evol. 210: 507–511.