

Possible role of an *Anopheles* transient receptor potential channel homologue in malaria parasite transmission

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Abstract

Midgut glycoproteins are potential receptors for pathogens such as arboviruses and malaria parasites that are transmitted by vector mosquitoes. An antiserum prepared against wheat germ agglutinin binding *Anopheles tessellatus* midgut proteins was used to screen an *An. gambiae* cDNA library. A clone homologous to the gene for a *Drosophila melanogaster* transient receptor potential-gamma cation channel protein was identified.

Introduction

Glycoproteins associated with the luminal surface of the mosquito midgut are potential receptors for mosquito transmitted pathogens such as malaria parasites [1-6] and arboviruses [7]. Antibodies against wheat germ agglutinin-binding midgut glycoproteins, ingested in an infective blood meal, block transmission of *Plasmodium vivax* and *P. falciparum* to the malaria vector *Anopheles tessellatus* [6]. To identify the relevant target midgut glycoproteins, a cDNA expression library of female *An. gambiae* abdomen was screened with the same antiserum against wheat germ agglutinin binding midgut glycoproteins.

Materials and Methods

Wheat germ agglutinin binding glycoproteins from sugar-fed female *An. tessellatus* midgut were purified and used to produce a rabbit antiserum as previously described [6]. The antiserum, pre-absorbed with *E. coli*, was used

to screen an *E. coli* lambda/bluescript (ZAP Express®/pBK-CMV; Stratagene, USA) cDNA library derived from female *An. gambiae* abdomen [8] according to standard procedures [9] modified by the manufacturer's instructions (Stratagene, USA). Cloned DNAs from antibody-reactive clones were excised and sequencing of these clones was performed from both the 5' and 3' ends. Nucleotide sequences were compared by BLAST search against the annotated *An. gambiae* Ensembl database (Wellcome Trust Sanger Institute, www.ensembl.org) and subsequently also with the *Drosophila melanogaster* genome.

Results

BLAST analysis of the 5'- and 3'-sequences of one unique clone obtained in the antibody screen, against the *An. gambiae* genome Ensembl database, showed that this was almost identical to a sequence in the contig CRA_x9P1GAV5CRW_68 present on the right arm of chromosome 3 of *An. gambiae* [Fig 1]. This *An. gambiae* sequence is homologous to the trp gamma gene product of *D. melanogaster*; (probability e-71 and 35% identity in amino acids; gbAAF53548.1(AE003652), which is a transient receptor potential cation channel protein, TRPgamma. The sequence of the predicted *Anopheles* protein showed several potential N-linked and O-linked glycosylation sites.

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Figure 1. Comparison of the DNA sequences of a part of the contig CRA_x9P1GAV5CRW_68 from the right arm of chromosome 3 of *An. gambiae* (Contig clone) and that of the antibody-reactive clone obtained using forward (clone E forw) and reverse (clone E rev) sequencing primers

Figure 1

| | | | | | |
|--------------|-------|--|--------------|-------|--|
| Contig clone | 18750 | gttgatgaagggcttcaatataagccaccgcccggcaccggacccggc | Contig clone | 19449 | cgggacgggaaggagcacgcaccactatcacttcatttcggcgcgt |
| clone E rev | 14 | -----gcccggcaccggacccgggccc | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 18800 | agcatttgaccccgtgctggagtattgctgcagctgccggaccattcg | Contig clone | 19499 | ggcagcactgaagcgaagcggaaaaagtctccaacgcccaactcca |
| clone E rev | 34 | agcatttgaccccgtgctggagtattgctgcagctgccggaccattcg | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 18850 | ccgcacgagctgttgcggcgctgttggcccgggttgacaccgaagaa | Contig clone | 19549 | gcccgtgctggagccaccgtgcgagctgccctggtaacgaacgcgat |
| clone E rev | 84 | ccgcacgagctgttgcggcgctgttggcccgggttgacaccgaagaa | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 18900 | ggcactgcaccaggtgaatccgatgcatcagttatgctgcagctgcaga | Contig clone | 19599 | actacgacgggtaatacaagcagtaaggtacgttggactttgcagctag |
| clone E rev | 134 | ggcactgcaccaggtgaatccgatgcatcagttatgctgcagctgcaga | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 18950 | gctcgttcggggagaacaacaaccgatccatcgctggcaaaagctggca | Contig clone | 19649 | ccgagtagcgggacattgatttagggagctttagatattggttgttt |
| clone E rev | 184 | gctcgttcggggagaacaacaaccgatccatcgctggcaaaagctggca | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19000 | ccgaagttaactccaaaagctccaggatcgacacaagaagcgggg | Contig clone | 19699 | gctggcagtgccacgtacctaacacaggggtggcattccaattgattact |
| clone E rev | 234 | ccgaagttaactccaaaagctccaggatcgacacaagaagcgggg | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19050 | tccgttgaattatcaaaaacctcaaaaaaggcaccatttaacgatgct | Contig clone | 19749 | ttcattgccattaccacagtgatgtgacgcactctgcgggggtgtcat |
| clone E rev | 282 | ----- | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19100 | ttctcctccgagggaacgcttattgaagcggccaaacatggcagcgtgt | Contig clone | 19799 | tcgctgttgattacagcagctaccacattacgataattcattcaaacga |
| clone E rev | 282 | -----ggaacgcttattgaagcggccaaacatggcagcgtgt | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19150 | cgaagttatcgggcctcccgatcgggaagactgggtgtgcaaccacgggt | Contig clone | 19849 | atcaagctctatagtgcaatgagcaatatctcttgggtggcgacactg |
| clone E rev | 319 | cgaagttatcgggcctcccgatcgggaagactgggtgtgcaaccacgggt | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19200 | ccgggtcggggcgaatcgccagccacagcaatagcccggcgtcggatga | Contig clone | 19899 | acacgcttctcaactccgaggtactgaacgtgcttccacgctac |
| clone E rev | 369 | ccgggtcggggcgaatcgccagccacagcaatagcccggcgtcggatga | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19250 | agccatcacggagtcaccgtccgattcgaaccgagcctggatcgcccgg | Contig clone | 19949 | cgacgcggcgcaagcgcgaaccggaagcaccggcagcgtacggccg |
| clone E rev | 419 | agccatcacggagtcaccgtccgattcgaaccgagcctggatcgcccgg | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19300 | acgtcgagcagtgctccgatccacacaagcatcacaatcagcgcaccgg | Contig clone | 19999 | gcccggcacgagcgaacgcaaaagccagcagcactcagcagcagcgt |
| clone E rev | 469 | acgtcgagcagtgctccgatccacacaagcatcacaatcagcgcaccgg | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19350 | acggaccggggg-caccaagatcggcggcagcagcgtggaagtgcgtga | Contig clone | 20049 | cgaaggtcggcaccgttaacacctccaccagggagagagcgtcgtac |
| clone E rev | 519 | acggaccggggg-caccaagatcggcggcagcagcgtggaagtgcgtga | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| Contig clone | 19399 | cgacgacgacagcagcagcgggacggccacggcgaacggcaagaacggg | Contig clone | 20099 | agcagccggcgagcgggtgcccggccatcagctggccgtgcacaagcac |
| clone E rev | 569 | cgacgacgacagcagcagcgggacggccacggcgaacggc----- | clone E rev | 606 | ----- |
| clone E forw | 946 | ----- | clone E forw | 946 | ----- |
| | | | Contig clone | 20149 | cagaagatcccgcgaggaaacagctccaacgaacctgtctacgctt |
| | | | clone E rev | 606 | -----cggaagaa----- |
| | | | clone E forw | 946 | ----- |
| | | | Contig clone | 20199 | gcaggagcagcgtgatagcagtgatggtggtggtgaggcgtcag |
| | | | clone E rev | 615 | ----- |
| | | | clone E forw | 946 | ----- |

| | | | | | |
|---|---------------------|--|---|---------------------|---|
| Contig clone clone E rev clone E forw | 20249 615 946 | taggggcaggtggagcgtgtgacacgtccggcgaccggctcagcactggt ----- ----- | Contig clone clone E rev clone E forw | 21099 615 846 | gctaccaacaacggagactcatatgacgcaccctggagacaggggggg ----- gttaccacaacacggagactcatatgacgcacccttgagacaggggggg |
| Contig clone clone E rev clone E forw | 20299 615 946 | cagtcgttggccacgtccacgtctacgtcgtgccgaagattccggcgct ----- ----- | Contig clone clone E rev clone E forw | 21149 615 796 | -----tcattctctttaaaccgatagctctgtaagggacgggtctgatg ----- ggggggtcattctctttaaaccgatagctctgtaagggacgggtgtgatg |
| Contig clone clone E rev clone E forw | 20349 615 946 | aacgccactacggggcatttacctccagggtggttataggggagac ----- ----- | Contig clone clone E rev clone E forw | 21193 615 746 | caagcgcgcacacctaaactacacgatacatcttaggcacgataaga ----- caaccgcgcacacctaaactaaacacgatacaccttaggcacgataaga |
| Contig clone clone E rev clone E forw | 20399 615 946 | ggtagcgcgcaaggggtgcgactattgtatgtaaatgggtgctggcg ----- ----- | Contig clone clone E rev clone E forw | 21243 615 696 | aagacatactacaaccgctccgaatccaataaagttagttaagcaga ----- aagacatactacaaccgctccgaatccaataaagttagttaaacaga |
| Contig clone clone E rev clone E forw | 20449 615 946 | ctcaaggcgttgagcgcacgtctaacggaacaatgagtgtaaaactgct ----- ----- | Contig clone clone E rev clone E forw | 21293 615 646 | tagattacttatcagcataaaagagtaaaaccagttgattagtagggtgc ----- tagattacttatcagcataaaagagtaaaaccagttgattagtagggtgc |
| Contig clone clone E rev clone E forw | 20499 615 946 | aaaagcatcaatttaaacgtggtggcgaagaagtaaggcgaatggtta ----- ----- | Contig clone clone E rev clone E forw | 21343 615 596 | cgaggagtgtataaggagtgacggggacacgcaaatcgaggatcgcaac ----- cgaggagtgtataaggagtgacggggacacgcaaatcgaggatcgcaac |
| Contig clone clone E rev clone E forw | 20549 615 946 | aagacgcaagacgagttgaaatgacatctcggcaactactctgcagatgg ----- ----- | Contig clone clone E rev clone E forw | 21393 615 546 | agtgttgaacgctaacacacatcagacatccgagtaattgaaatagcaa ----- agtgttgaacgctaacacacatcagacatccgagtaattgaaatagcaa |
| Contig clone clone E rev clone E forw | 20599 615 946 | ctggagcaacgcgttcgtgtgatggtgtaagaaaatcctctgttcg ----- ----- | Contig clone clone E rev clone E forw | 21443 615 496 | aaactaacacaagaagtaaaaggaggccttataatgtaacatgtaaa ----- aacataacacaagaagtaaaaggaggccttataatgtaacatgtaaa |
| Contig clone clone E rev clone E forw | 20649 615 946 | aggagagagataggagtttaggcctaaaaaggaaccgtaaccggctcg ----- ----- | Contig clone clone E rev clone E forw | 21493 615 446 | catgatgtgatgtgtatcagtaacaaaagagaacagaacaaacca ----- catgatgtgatgtgtatcagtaacaaaagagaacagaacaaacca |
| Contig clone clone E rev clone E forw | 20699 615 946 | taacagagcccgatcctgaccgaatgatcccatcctcggacggtgtcgt ----- -----catcattg----- | Contig clone clone E rev clone E forw | 21543 615 396 | cgaaccactataatgataacgtacgtctctcgttcaaggacgatta ----- cgaaccactataatgataatgtacgtctctcgttcaaggacgatta |
| Contig clone clone E rev clone E forw | 20749 615 938 | gctgtcgtctatattccatacagcgtcaaccttggggcagcagctgta ----- ----- | Contig clone clone E rev clone E forw | 21593 615 346 | gacagtaacaaaaacaacaaacaaagcattgaattgggacgcgatcat ----- gacagtaacaaaaacaacaaacaaagcattgaattgggacgcgatcat |
| Contig clone clone E rev clone E forw | 20799 615 938 | gatcatggacacgatcgggagcgggaaatggagtgcaacagtgacagtt ----- ----- | Contig clone clone E rev clone E forw | 21643 615 296 | tagagcagatgtagatggaacttcgatcaaacgtaaaaaaa-ctagta ----- tagagcagatgtagatggaacttcgatcaaacgtaaaaaaaactagta |
| Contig clone clone E rev clone E forw | 20849 615 938 | gaatgttggtgtagctgtaaggcatatagatgtataacgataccaat ----- ----- | Contig clone clone E rev clone E forw | 21692 615 246 | agcaacattaacaatttattagtcgtaattgggagcagaaaaccatgt ----- agcaacattaacaatttattagtcgtaattgggagcagaaaaccatgt |
| Contig clone clone E rev clone E forw | 20899 615 938 | acaatacaaaaaggaccaggtctcttggagggtgctcggctcggcgca ----- ----- | Contig clone clone E rev clone E forw | 21742 615 196 | tcgcgtagacgtaagacaagataactatcgtagtgagagaacgatag ----- tcgcgtagacactaagacaagataactatcgtagtgagagaactatag |
| Contig clone clone E rev clone E forw | 20949 615 938 | gcaccaacaatcccgtggtgccgctggaggagtagctgttaaacatc ----- ----- | Contig clone clone E rev clone E forw | 21792 615 146 | agagaaaaaggagagaaagagagaaggtggttgaaacgaataaaagat ----- agagagaaaaaggagagaaagagagaaggtggttgaaacgaataaaagat |
| Contig clone clone E rev clone E forw | 20999 615 938 | attgttaagctattggaactctgacaggaacaaccaagaggacagcgcg ----- -----taagctatt-gaactctgacag-acaaccaagaggacagcgcg | Contig clone clone E rev clone E forw | 21842 615 96 | aaaattataaaagggttagctcgtcgtgctaaaagcaactagtagctaa ----- aaaattataaaagggttagctcgtcgtgctaaaagcaactagtagctaa |
| Contig clone clone E rev clone E forw | 21049 615 896 | gctagtgtagctgtaagaccgctccttcccataaccgttgatgtgtt ----- gctagtgtagctgtaagaccgctccttcccataaccgttgatgtgtt | Contig clone clone E rev clone E forw | 21892 615 46 | acacgacagatttagtagtgaagaaatgaacaaaaactataaatta ----- acacgatagatttagtagtgaagaa----- |

Discussion

Characterising midgut molecules involved in pathogen-mosquito interactions may help in developing new methods of controlling diseases transmitted by mosquitoes, e.g. by impairing vector competence [10] as achieved experimentally for *An. stephensi* through transgenesis [11]. The midgut proteins interacting with malaria parasites are likely to be located in the posterior midgut which is where the blood meal is digested and parasite development and invasion occurs. The midgut-specific genes from *An. gambiae* characterised so far include those encoding for structural and digestive proteins [12,13], immune response genes of *An. gambiae* that are activated during midgut invasion by ookinetes [8] as well as genes for defensive proteins such as cecropin [14] and defensin [15] that are expressed in the anterior midgut. A cell surface mucin specifically expressed in the midgut of *An. gambiae* has been characterised by screening a cDNA library of *An. gambiae* midgut with an antiserum against female *An. gambiae* peritrophic membrane proteins [13].

TRP cation channel protein in *Drosophila* has been detected in photoreceptors and nerve cell axons [16]. A human analogue has been reported in hepatocytes [17]. More recently a TRP cation channel protein has been identified as having a role in calcium signalling and fluid transport in the *Drosophila* Malpighian tubule epithelium [18]. The related *Anopheles* protein detected here could be a membrane glycoprotein cation transporter present on the luminal surface of the midgut cells, although this remains to be confirmed in expression studies. Implication of the gene in the interaction between mosquito and pathogen can be addressed by generating specific antibody against this gene and testing for invasion inhibition or interference RNA (RNAi) mediated gene silencing and subsequent *Plasmodium* infection or in vitro interaction assays between recombinant protein and *Plasmodium*. Since ingestion of the antiserum to midgut glycoproteins reduces infectivity of malaria parasites to *An. tessellatus* [6], proteins such as the TRP cation channel, and the cell surface mucin [8] warrant further investigation as potential targets for transmission blocking vaccines and other methods for interfering with malaria transmission.

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References

1. Ramasamy MS, Kulasekera R, Wanniarachchi IC, Srikrishnaraj KA, Ramasamy R. 1997. Interactions of human malaria parasites, *Plasmodium vivax*, *P. falciparum* with the midgut of *Anopheles* mosquitoes. *Med Vet Entomol.* 11: 290-296.
2. Beier JC. 1998. Malaria parasite development in mosquitoes. *Annu Rev Entomol.* 43: 519-543.
3. Ramasamy MS, Ramasamy R. 1990. Effects of anti-mosquito antibodies on the infectivity of the rodent malaria parasite *Plasmodium berghei* to *Anopheles farauti*. *Med Vet Entomol.* 4: 161-166.
4. Billingsley PF, Sinden RE. 1997. Determinants of malaria-mosquito specificity. *Parasitol Today* 13: 297-301.
5. Zieler H, Nawrocki JP, Shahabuddin M. 1999. *Plasmodium gallinaceum* ookinetes adhere specifically to the midgut epithelium of *Aedes aegypti* by interaction with a carbohydrate ligand. *J. Exp Biol.* 202: 485-495.
6. Ramasamy R, Wanniarachchi IC, Srikrishnaraj KA, Ramasamy MS. 1997b. Mosquito midgut glycoproteins and recognition sites for malaria parasites. *Biochim Biophys Acta* 1361: 114-122.
7. Ramasamy MS, Sands M, Kay BH, Fanning I, Lawrence GW, Ramasamy R. 1990. Anti-mosquito antibodies reduce the susceptibility of *Aedes aegypti* to arbovirus infection. *Med Vet Entomol.* 4: 49-55.
8. Dimopoulos G, Seeley D, Wolf A, Kafatos FC. 1998. Malaria infection of the mosquito *Anopheles gambiae* activates immune-responsive genes during critical transition stages of the parasite life cycle. *EMBO J.* 17: 6115-6123.

9. Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning – a laboratory manual. 2nd edition Cold spring Harbour Laboratory Press, New York.
10. Beaty BJ. 2000. Genetic manipulation of vectors: a potential novel approach for control of vector-borne diseases. *Proc Natl Acad Sci USA* 97: 10295-10297.
11. Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M. 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* 417: 452-455.
12. Muller HM, Catteruccia F, Vizioli J, della Torre A, Crisanti A. 1995. Constitutive and blood meal-induced trypsin genes in *Anopheles gambiae*. *Exp Parasitol*. 81: 371-385.
13. Shen Z, Dimopoulos G, Kafatos FC, Jacobs-Lorena M. 1999. A cell surface mucin specifically expressed in the midgut of the malaria mosquito *Anopheles gambiae*. *Proc Natl Acad Sci USA* 96: 5610-5615.
14. Vizioli J, Bulet B, Charlet M, Lowenberger C, Blass C, Muller HM, Dimopoulos G, Hoffmann J, Kafatos FC, Richman A. 2000. Cloning and analysis of a cecropin gene from the malaria vector mosquito, *Anopheles gambiae*. *Insect Mol Biol*. 9: 75-84.
15. Dimopoulos G, Richman A, Muller HM, Kafatos FC. 1997. Molecular immune responses of the mosquito *Anopheles gambiae* to bacteria and malarai parasites. *Proc Natl Acad Sci USA* 94: 11508-11513.
16. Xu XZ, Chien F, Butler A, Salkoff L, Montell C. 2000. TRPgamma, a *Drosophila* TRP-related subunit, forms a regulated cation channel with TRPL. *Neuron* 26: 647-657.
17. Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD. 2000. TRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nature Cell Biol*. 2: 695-702
18. MacPherson MR, Pollock VP, Kean L, Southall TD, Giannakou ME, Broderick KE, Dow JAT, Hardie RC, Davies SA. 2005. Transient receptor potential-like channels are essential for calcium signaling and fluid transport in a *Drosophila* epithelium. *Genetics* 169: 1541-1552.