

# The mosquito hyper-variable pattern recognition receptor, AgDscam, in the anti-*Plasmodium* defense



Yuemei Dong and George Dimopoulos

W. Harry Feinstone Department of Molecular Microbiology and Immunology,  
Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe Street,  
Baltimore, MD 21205-2179, USA.

## ABSTRACT

Mosquitoes transmit a broad range of human parasitic and viral diseases, within which malaria is the most devastating insect-borne disease. Though *Anopheles* mosquitoes are the major vectors of human malaria, their ability to transmit malaria parasites are under large variations. The dynamic immune interaction between the vector host and the malaria pathogen determines the success of *Plasmodium* development and continuation of the subsequent disease transmission cycle. The study of the molecular mechanisms that determine the recognition of the pathogens are of the biggest interest in the mosquito innate immune system which may provide clues for developing future strategies to control mosquito-borne diseases. In a previous study, our data from splice form gene expression profile, RNAi mediated gene silencing, phagocytosis assays and *in vitro* bacterial binding assays establish AgDscam is a hyper-variable pattern recognition receptor of the mosquito's innate immune system. By alternative splicing it can generate pattern recognition receptor repertoires in the mosquito innate immune system which can effectively recognize a variety of pathogens despite the lack of a highly sophisticated adaptive immune surveillance system. Here we show that AgDscam can co-localize with both rodent and human malaria parasites in the mosquito midgut epithelium. *In vitro* ookinete binding assay again suggested AgDscam can bind to the parasites. siRNA mediated depletion of AgDscam with siRNAs pool specific to exon 4.1, 4.8, 4.11, 6.11, 10.27 from mosquitoes resulted in increased permissiveness to *P. falciparum* infection with 65% increase of oocyst numbers, while single siRNA gene silencing of either one of these splice forms resulted in no significant change of infection level. The qRT-PCR and microarray hybridization assays based on a CombiMatrix platform, with probes more specific to the different 101 exons, suggested that the regulators of signaling transduction pathways (Rel1, Rel2, Cactus, Caspar) are involved in the regulation of alternative splicing of AgDscam. The gene silencing of Rel1 and Rel2 resulted in a decreased colocalization of AgDscam with *P. falciparum* parasites in the *Anopheles* midgut epithelium. Further studies are undergoing to identify the splice forms which have the most significance in defense against malaria parasites.

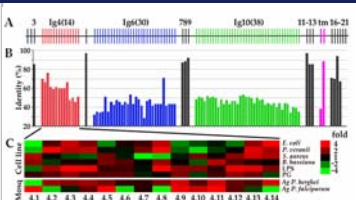
## OBJECTIVES

- Investigate the interaction between AgDscam and *Plasmodium* parasites
- Investigate the significance of alternative splicing in defense against malaria parasites
- Investigate the regulation of immune signaling pathways (IMD, Toll) in the expression of different AgDscam alternative splicing isoforms

## EXPERIMENTAL APPROACHES

- Investigate the roles of isoforms in the anti-malarial defense systems through Small interference Reverse genetic analysis (siRNAi) and infection analysis
- Expression analysis by CombiMatrix microarray hybridization
- Immuno-staining: *In vivo* and *In vitro* co-localization of DSCAM with *P. falciparum* ookinetes

## BACKGROUNDS



A. AgDscam gene organization is displayed with the 101 exons. The variable Ig domain exon cassettes are displayed as Ig4, Ig6 and Ig10. The transmembrane domain exons (T M) are displayed in pink color and non-spliced exons are displayed in black. C. Differences in AgDscam exon 4 transcript representations between different challenge. Expression has been determined with real-time quantitative PCR analyses and expression ratios have been log2 transformed. (Dong et al., 2006. PLOS Biol. 4: 1137-1146.)

## RESULTS

### AgDscam kills *P. falciparum* in a splice-form specific manner

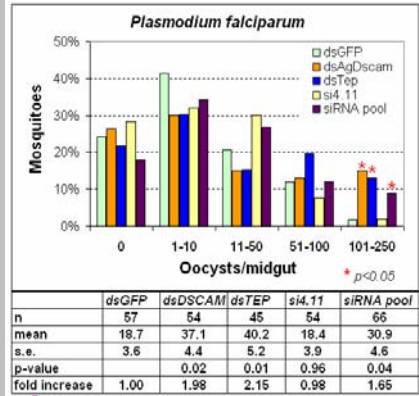


Figure 1: dsRNA and siRNA mediated depletion of AgDscam from adult female mosquitoes resulted in increased permissiveness to *P. falciparum* infection, as indicated by a 98% increase in oocysts numbers. siRNAs pool silencing with siRNAs specific to exon 4.1, 4.8, 4.11, 6.11, 10.21, 10.27 can achieve similar result with 65% increasing of the oocysts number; while single siRNA silencing of 4.11 (or other exons including in the pool, as a prove of principal only siRNA from exon 4.11 is included here) can not achieve significant effect in terms of *P. falciparum* infection. The figure presents the frequency distributions of oocysts pooled from 3 independent assays where mean indicates mean intensity of infection (oocysts number) plus/minus standard error and n indicates the total number of mosquitoes in each experiment. Infection levels in AgDscam silenced mosquitoes were comparable to the positive control *Tep1* dsRNA treated.

### Rel1 and Rel2 regulate the AgDscam alternative splicing (CombiMatrix microarray hybridization with probes specific to 101 exons)

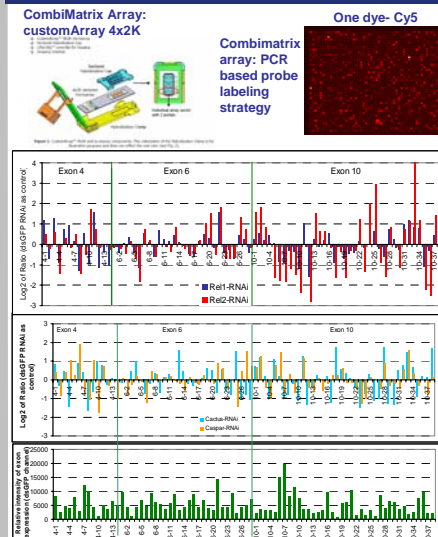


Figure 3. AgDscam isoform expression in the Rel1, Rel2, Cactus and Caspar depletion *A. gambiae* Sua5B cell lines. The signal ratios of gene silenced samples (Rel1-RNAi, Rel2-RNAi, Cactus-RNAi, Caspar-RNAi) versus dsGFP treated cells controls were first averaged and log2 transformed. Within the upper panel, log2 ratio is plotted against individual exons, with the name of each exon marked on the x-axis. Within the lower panel, the relative intensity of expression from each exon is plotted which shows the amount of each splice form present in the dsGFP treated control samples.

## CONCLUSIONS

- AgDscam involves in defense against *Plasmodium* parasites, and it associates with *Plasmodium* ookinetes
- The regulation of immune signaling pathways (IMD, Toll) plays roles in AgDscam alternative splicing

### *In vitro* colocalization of AgDscam with *P. falciparum* ookinetes

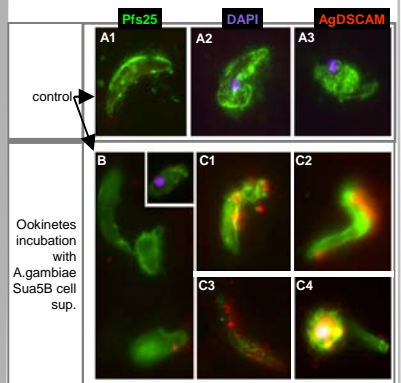


Figure 4. *In vitro* colocalization of AgDscam with *P. falciparum* ookinetes. A1: Ookinete is stained with Pfs25 antibody (green) alone; A2-A3: Ookinetes are stained with Pfs25 and DAPI (blue); B-C: ookinetes were first incubated with *A. gambiae* Sua5B cell supernatant which has AgDscam protein, then the ookinetes were stained with Pfs25, DAPI, and with AgDscam preimmune only (B), or with AgDscam antibody (C1-C4). The ookinetes were prepared from mosquitoes midgut homogenization about 24 to 28 hours after *P. falciparum* gametocytes bloodmeal.

### Rel2 and Caspar regulates the colocalization of AgDscam with *P. falciparum* parasites in the mosquito midgut epithelium

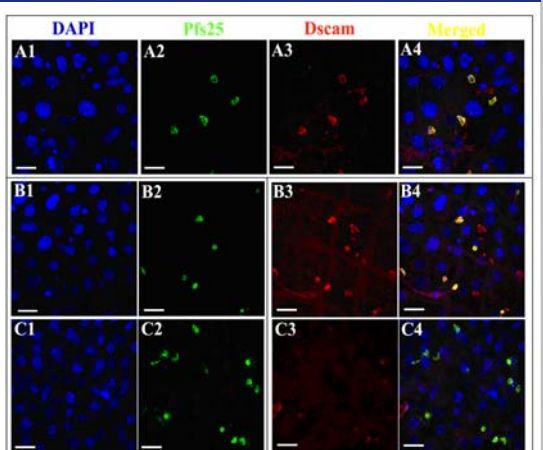


Figure 4. Colocalization of AgDscam and Parasites in the Mosquito Midgut. Double staining of midgut tissues of dsGFP treated control (A1-A4), dsCaspar treated (B1-B4) and dsRel2 treated (C1-C4) mosquitoes 24 hpi with the *P. falciparum* stained with Pfs25 antibody. Scale bars are equal to 10 μm. AgDscam colocalizes stronger with parasites on the Caspar depletion midgut epithelium, and most of the AgDscam-labeled ookinetes have less staining of Pfs25. In the Rel2 gene silenced mosquitoes, there is less staining of AgDscam with *P. falciparum* parasites.

## FUTURE DIRECTIONS

- Identify the splice forms which have the most significance in defense against malaria parasites
- Further study to investigate the regulation of immune signaling pathways (IMD, Toll) in AgDscam alternative splicing

## ACKNOWLEDGEMENTS

- Johns Hopkins mosquito and parasitology core facilities
- supported by WHO/TRD, The Ellison Medical Foundation, Johns Hopkins School of Public Health and Johns Hopkins Malaria Research Institute, NIH

