

# Anopheles fibrinogen immune lectins: a large conserved gene family fighting against *Plasmodium*

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## ABSTRACT

*Anopheles* mosquitoes are the major vectors of human malaria, but their ability to transmit malaria parasites are under large variations. The study of the molecular mechanisms that determine vectorial capacity is of the biggest interest, such as the immune genes implicated in anti-*Plasmodium* defences. The fibrinogen-domain immuno-lectin (FBN) family is evolutionary conserved immune gene family between mammals and invertebrates. The vertebrate ficolins are implicated in phagocytosis and complement activation, while the horseshoe crab and snail FBN genes have been implicated in bacteria binding, enhancement of antimicrobial activity and interaction with parasite (*Schistosoma*) components, respectively. The FBN proteins contain a pathogen-binding fibrinogen-like domain at their C-terminus and the N-terminal sequence is implicated in interaction with the N-terminus of other FBN proteins resulting in the formation of multimeric protein bundles with potential increased affinity and specificity to the pathogens. The *Drosophila* genome harbours only 13 FBN members while *Anopheles gambiae* has as many as 57 members with 39 of them well annotated. The reason for this remarkable expansion remains unknown, but has been speculated to be linked to hematophagy, the mosquito's microbial exposure or its interaction with *Plasmodium*. We have employed a comprehensive analysis which suggested them being implicated in functions other than anticoagulation. We demonstrate that about one third of 38 FBNs are induced by gram positive and negative bacteria as well fungus challenge in the mosquito, one fourth genes induced after *Plasmodium berghei* and *P. falciparum* challenge. Their function as antimicrobial and anti-*Plasmodium* components has been proved by RNAi mediated gene silencing assays. Of these genes the most interesting immunolectin, FBN9, is capable of specific binding to bacterial surface and it co-localizes with *P. berghei* ookinetes *in vivo*. It is implicated in phagocytosis and appears to form dimers upon binding to the bacteria. Further analyses are addressing the significance of FBN homo- or hetero- multimer formation in increasing the mosquito's pattern recognition receptor repertoire.

## Hypothesis

FBN immuno-lectins play essential roles in *A. gambiae* immune system and other processes. Through multimerization of FBNs mosquito generates increased number of pattern recognition receptor repertoires.

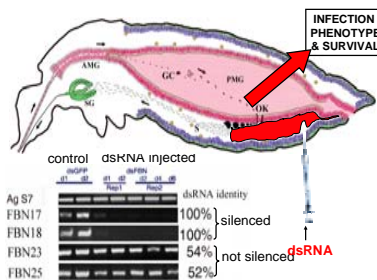
## Aims

- General molecular characterization of FBN genes.
- Investigate FBN genes implication in defense against microbes and malaria.

## Experimental approaches

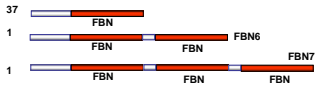
- Sequence and phylogenetic analysis
- Transcription analysis through semi-quantitative RT-PCR and Cluster program
- Investigate their roles in the anti-microbial and anti-malarial defense systems through Reverse genetic analysis (RNAi) and infection analysis
- Immuno-staining. Cellular co-localization of certain members of FBN after microbial and malarial parasites invasion.
- In vitro bacterial binding assays

## Reverse genetic analyses of gene function through RNAi mediated gene silencing



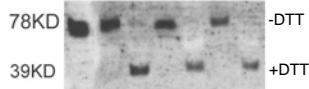
## Results

### Sequence features: Domain Organization



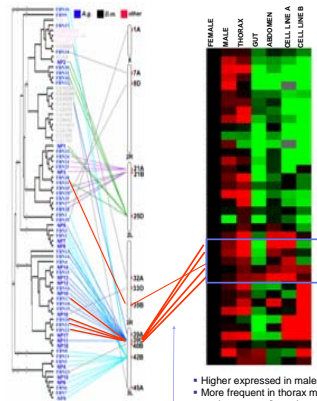
3 FBN immunolectins lack microorganism binding sites; 39 well annotated; 18 are not annotated  
PROMOTERS: 21 HAVE PREDICTED 5' UTR: 10 HAVE NF-kappa B regulatory elements

Sup *E. coli* *P. veronii* *B. subtilis*



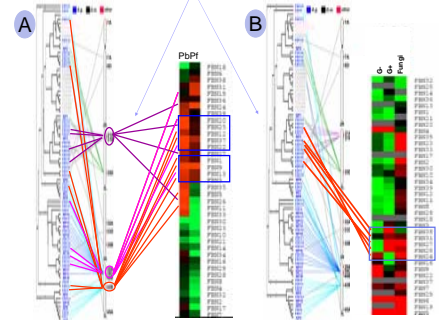
In vitro bacteria binding assay shows that FBN9 can bind to both gram negative and gram positive bacteria and form dimers when binding to bacteria

### Tissue specific FBNs gene expression profile

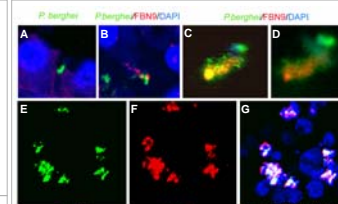


- Higher expressed in males
- More frequent in thorax may suggest they are hemocytes/hemolymph specific
- Complexity of expression pattern suggested diverse functions

### Strong correlation between expression, phylogeny and chromosomal location



A: FBN Regulation in *Plasmodium berghei* (Pb) and *P. falciparum* (Pf)  
B: in G<sup>+</sup>, G<sup>-</sup> and fungi challenged mosquitoes



### FBN9 colocalizes with bacteria, plasmodium

A-D: FBN9 (red) colocalizes with *P. berghei* (GFP parasites, green); A, negative control with only serum staining; B-C, with FBN9 antibody staining; E-G: FBN9 (red) colocalizes with *E. coli* (FITC label, green); E: FITC *E. coli* only; F: FBN9 staining; G: superimpose of *E. coli*, FBN9 and DAPI staining

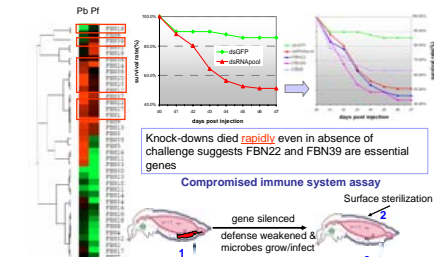
## Summary

- Complexity of expression pattern suggested diverse functions
- Strong correlation between transcription, phylogeny and chromosomal location
- Antimicrobial and anti-malaria function
- Complement and synergistic functions in immunity
- FBN9, FBN22 and FBN39 play very essential roles in the immune system
- FBN9 colocalizes with bacteria, *plasmodium*, and it forms dimers when binding to the bacteria.

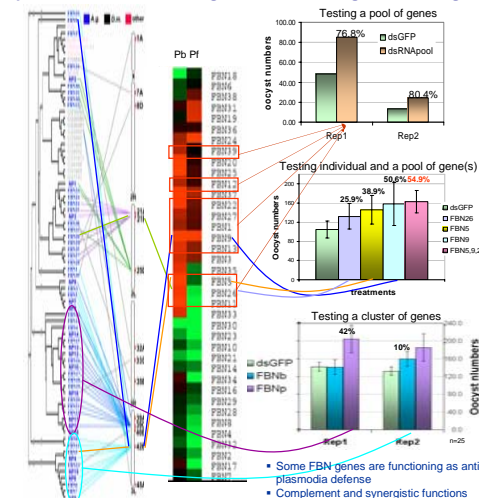
## Acknowledgements

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### Depletion of FBNs results in proliferation of opportunistic bacteria



### analyses of genes function for implication in anti-plasmodial defense through RNAi mediated gene silencing



Bac1: *Shigella flexneri*  
Bac2: *Asaia bogorensis*  
BacX: *Pseudomonas* sp.