Genomic Analyses of Anopheles-Plasmodium Interactions

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Disease Control

VECTOR  \rightarrow  HOST  \rightarrow  PLASMODIUM

ERADICATING MALARIA
Mosquito: A Model For Innate Immunity

**Signal Transduction Pathways** (Toll, IMD)

**Signal Amplification Pathways**

**Phagocytosis**

**Melanization**

**Effector Genes**

**Pattern Recognition Receptors**

- **Plasmodium**
- **Filaria**
- **Viruses**
- **Bacteria**
- **Fungi**

Innate Immune System

Pathogen
Strategies For Malaria Control

- Kill, or avoid contact with the vector mosquito
  - insecticides, repellents, bednets, genetic control

- Kill the parasite in
  - human
    - Drugs
    - Vaccines
  - mosquito
    - Transmission blocking vaccines
    - Genetic modification of mosquito

- Toxins
  - Interfere with parasite – mosquito interactions
    - Mosquito immune system

- EFFECTOR GENES
- PROMOTERS
  - (tissue & stage specific)

- GENETIC DRIVE SYSTEMS
  - (i.e. transposable elements, endosymbionts)
Anopheles Immune Defense Against Plasmodium Infection

- Blood meal
- Peritrophic matrix
- Ectoperitrophic space
- Midgut epithelium
- Basal laminae
- Hemocoel

- Midgut invasion
- Traversal of peritrophic matrix; parasite chitinase is activated by mosquito trypsin
- Attachment to microvilli carbohydrate
- Recognition of specific cell type
- Invasion & traversal
- Trigger for oocyst development

Shahabuddin
Anopheles Immune Defense Against Plasmodium Infection

How do we assay the biological processes?

Control of physiological processes

transcription → transcript → translation → protein
Transcription Analyses of Immune Responses to *Plasmodium*

naïve blood

*Plasmodium* infected blood

Quantitative RT-PCR of immune marker genes

Adapted from Dimopoulos et al., EMBO J 1998;17:6115-6123.
Anopheles Gambiae Gene Discovery Project (Year 2000)

Normalized cDNA libraries

 Sequencing 3840 random clones

 ~6200 ESTs

2287 unique genes
1118 genes with database hits
858 genes with assigned function
28 previously cloned A.g. genes
48 putative immunity genes

Adapted from Dimopoulos et al. Proc Natl Acad Sci USA 2000;97:6619-6624
2001: A. gambiae Cell Line EST 4000 Array

~2200 unique genes
~858 genes with assigned function

Clustering of Cell Line and Mosquito Responses

Injury
Sterile Septic

Plasmodium infection

CELL LINE

Genes that are implicated in anti-Plasmodium defense.

A. gambiae Genome Sequenced and Annotated in 2002

Size: ~260 mega bases

~13,000 predicted Anopheles proteins
GAMBER 22K: Anopheles gambiae – Plasmodium berghei microarray

\[ P. \text{berghei:} \sim 5,6K \text{ genes} \]

\[ P.f. \text{ortho.} \\
3,893 \]

\[ P.y. \text{ } 582 \]

\[ P.c. \text{ } 1,176 \]

Collab.: N. Hall, TIGR

\[ A. \text{gambiae:} \sim 13K \text{ genes} \]

3’ exon biased
exon specific oligos for 300 genes

Collab.: E. Monguin, Harvard

Gene Expression Profiling Can Assess and Identify:

**Host** (Human mouse, rat, etc..)
- Immune & other physiological responses to infection
- Resistance & susceptibility genes

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<tr>
<th><strong>Vector</strong> (Mosquito, Sandfly, Tsetse, etc…)</th>
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<tr>
<td>• Immune and other physiological responses to infection</td>
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<tr>
<td>• Resistance (refractoriness) &amp; susceptibility genes</td>
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<tr>
<td>• Effector genes</td>
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<tr>
<td>• Tissue specific promoters</td>
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<td>• Fitness signatures</td>
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<td>• Insecticide resistance factors</td>
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**Pathogen** (Plasmodium, Leishmania, viruses, bacteria, etc)
- Virulence factors
- Infection stage specific markers
- Drug and vaccine target genes
- Other disease control target genes
Functional Compartmentalization of the Midgut

- **Cardia**
  - secretion of type II Pm
  - expression of defensin secretory cells

- **Anterior midgut**
  - sugar absorption region
  - no enzymatic activity
  - no change in morphology
  - cells with less microvilli

- **Posterior midgut**
  - synthesis/secretion of proteolytic enzymes
  - absorption of blood digestion products
  - distension (25μm → 2.5μm high)
  - stretching of basal lamina
  - different cell types
Plasmodium Infection of the Midgut
Transcriptomic Dissection of the Midgut
Transcriptomic Dissection of the Midgut
Transcriptomic Dissection of the Midgut

- Cardia
- Anterior
- Anterior-posterior
- Posterior

- 2 lectins
- PGRP MYD88-like
- 2 LRR Toll interacting cuticle domain
- 3 serine proteases
- 6 aminopeptidases
- Lipase
- Peritrophin
- 2 CYP450 esterase thiorredoxin
- 24 other
- 8 ion transporters
- 2 aquaporins
- 2 aa transporters
- Sugar transporter
- 3 actins
- 2 myosins
- 4 other

Legend:
- Antimicrobial peptides
- Immunity
- Putative immunity
- Digestion
- PM
- Redox
- Transport
- Structural
- Metabolism
- Diverse
Transcriptomic Dissection of the Midgut

antimicrobial peptides

Immune Gene Expression (AMP)

Plasmodium Infection
Responses to *Plasmodium* Invasion of the Midgut
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Responses to *Plasmodium* Invasion of the Midgut

- **Conclusions**
  - *P. berghei* ookinete invasion results in a broader response, comprising more genes. A likely result of the higher infection level.
  
  - Invasion by *P. falciparum* induces a larger number of immune genes, that are qualitatively different.
  
  - Malaria infected blood triggers a broader physiological response including immune responses. Are these important for anti-*Plasmodium* defense?
Reverse Genetic Analyses of Anti-Plasmodium Function

Reverse Genetic Analyses of Anti-Plasmodium Function

8 genes have effect on both
2 genes are specific for *P. f.*
2 genes are specific for *P. b.*

*Anopheles* anti-Plasmodium defense is mostly universal, with some parasite species specific activities/mechanisms.

Reverse Genetic Analyses of Anti-Plasmodium Function

All genes with effects on Plasmodium development influence survival upon bacteria challenge. 4 genes with effect on survival upon bacteria challenge have no effect on Plasmodium development.

Anopheles is using components of its antimicrobial defense system to combat Plasmodium.

Immune Defense Against *Plasmodium* in the Midgut

• Conclusions
  – *Anopheles* anti-*Plasmodium* defense is mostly universal, with some parasite species specific activities/mechanisms.
  – *Anopheles* is using components of its antimicrobial defense system to combat *Plasmodium*.
Anopheles - Plasmodium Interactions

• Don’t forget the Pathogen
• Parallel transcription analysis of *Anopheles* and *Plasmodium* can identify expression signatures of interacting processes.
• See: Xu *et al.* 2005 Molecular & Biochemical Parasitology
Proteome Analyses of Insect Immune Responses