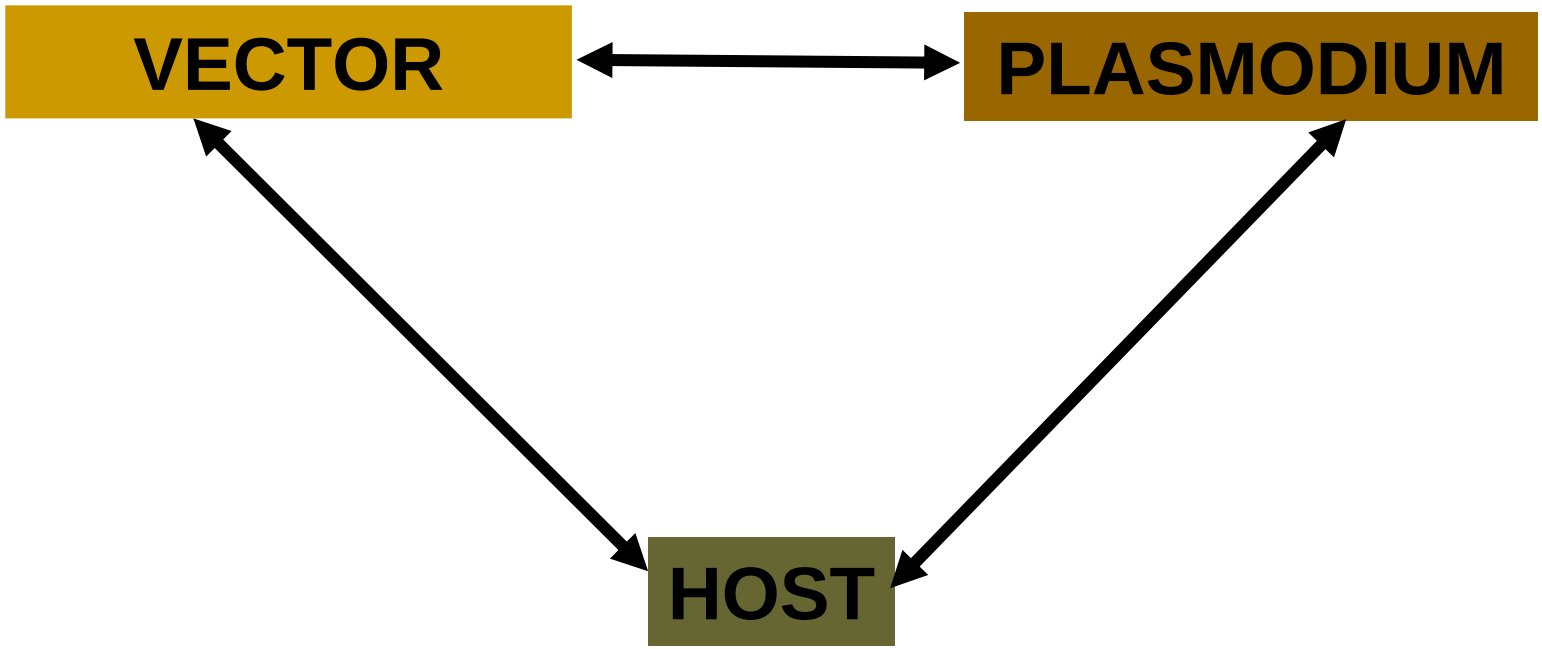
Genomic Analyses of Anopheles-Plasmodium Interactions

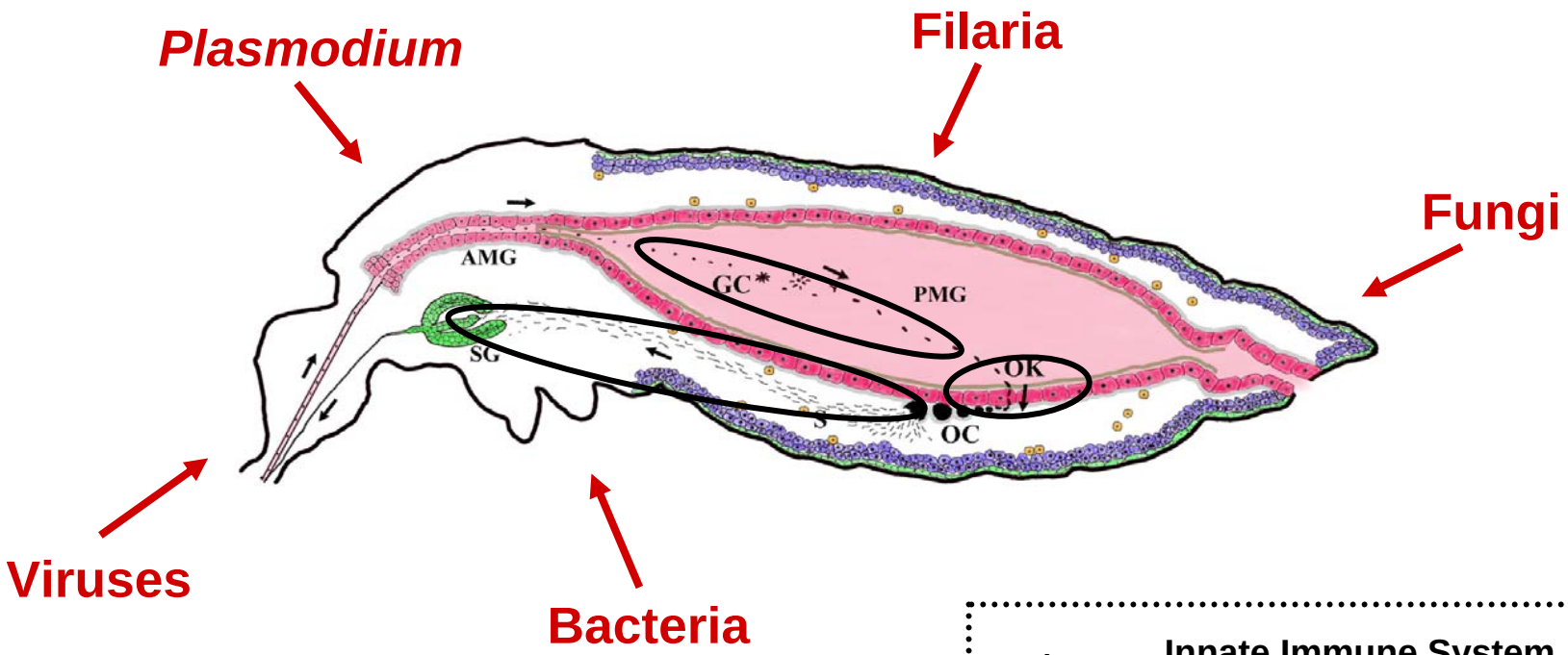
George Dimopolous, PhD

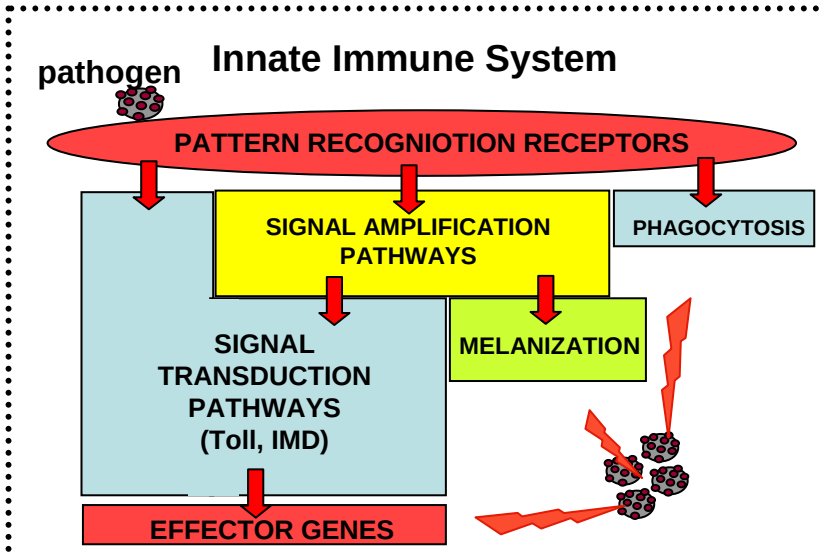
Disease Control



ERADICATING MALARIA

Mosquito: A Model For Innate Immunity

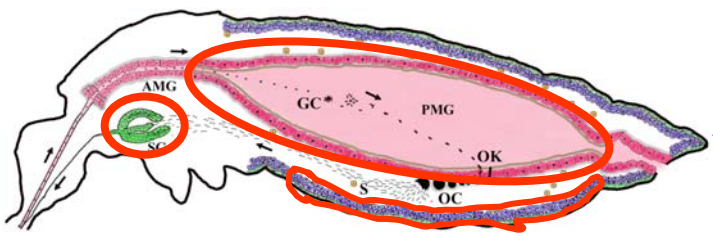




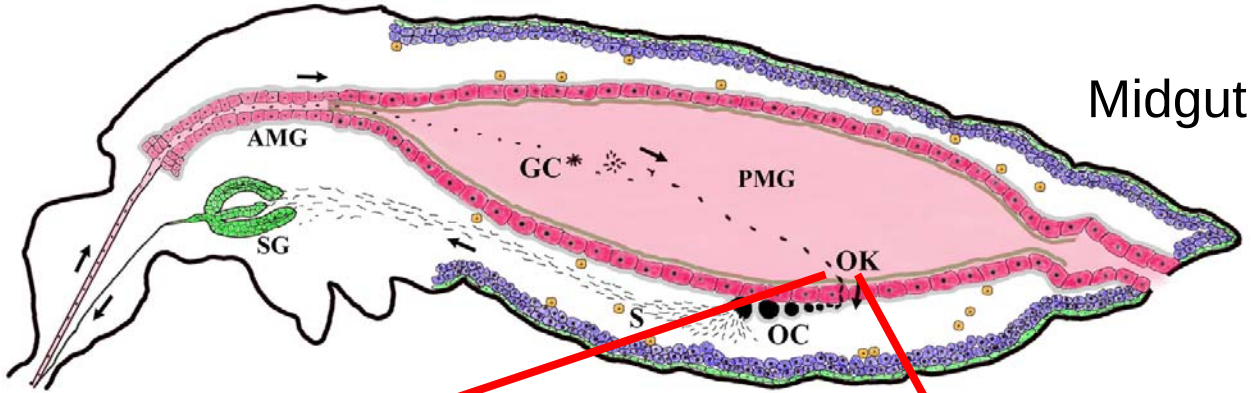
Strategies For Malaria Control

- 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
      - **EFFECTOR GENES**
        - Kill the parasite
          - Toxins
          - Mosquito immune system
        - Interfere with parasite – mosquito interactions
      - **PROMOTERS** (tissue & stage specific)
      - **GENETIC DRIVE SYSTEMS** (i.e. transposable elements, endosymbionts)



Anopheles Immune Defense Against *Plasmodium* Infection



Midgut invasion

Blood meal →

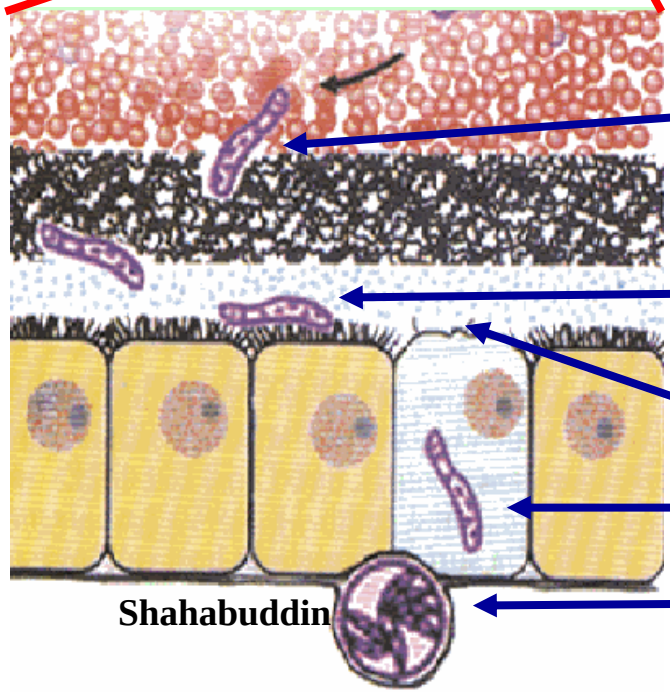
Peritrophic matrix →

Ectoperitrophic space →

Midgut epithelium →

Basal laminae →

Hemocoel →



Traversal of peritrophic matrix; parasite chitinase is activated by mosquito trypsin

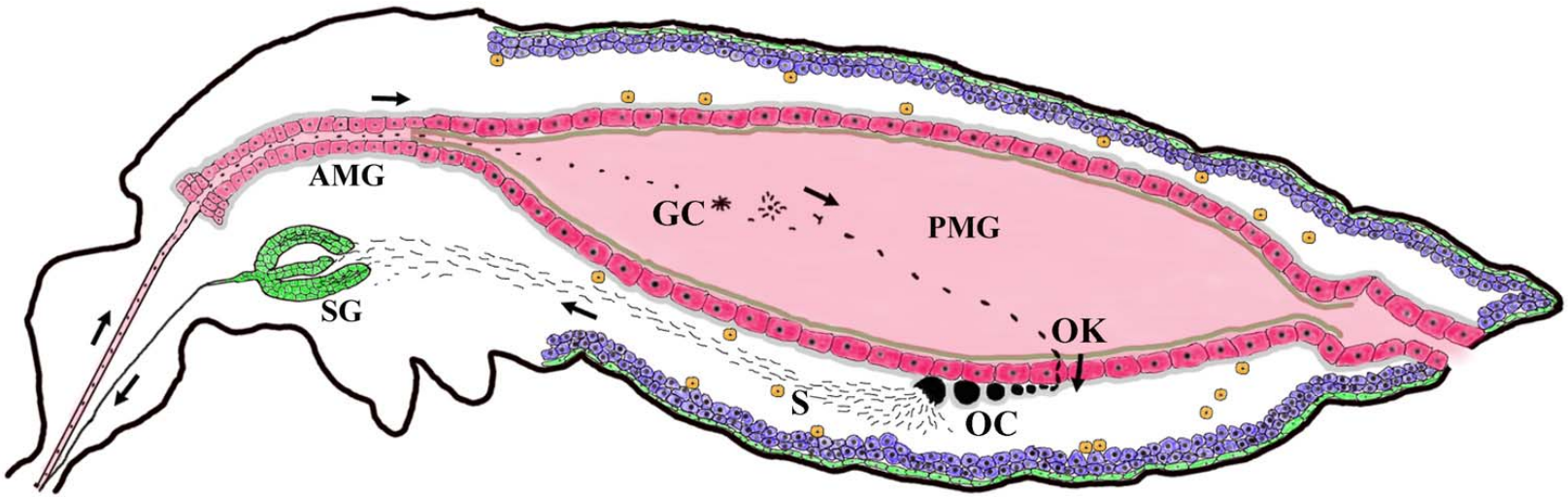
Attachment to microvilli carbohydrate.

Recognition of specific cell type

Invasion & traversal

Trigger for oocyst development

Anopheles Immune Defense Against *Plasmodium* Infection

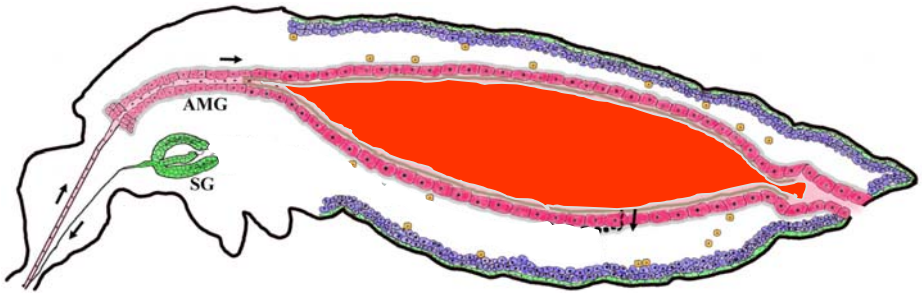


How do be assay the biological processes?

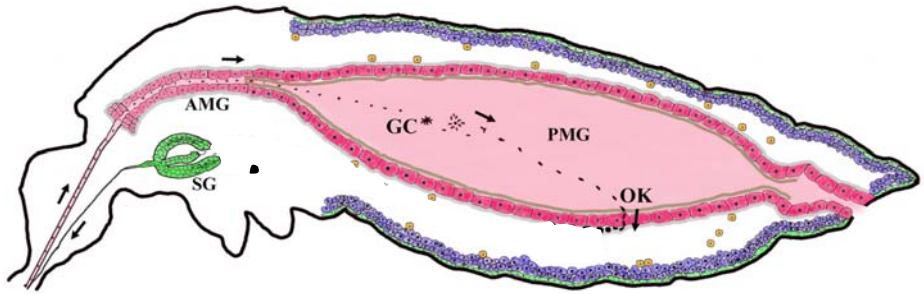
Control of physiological processes

transcription → transcript → translation → protein

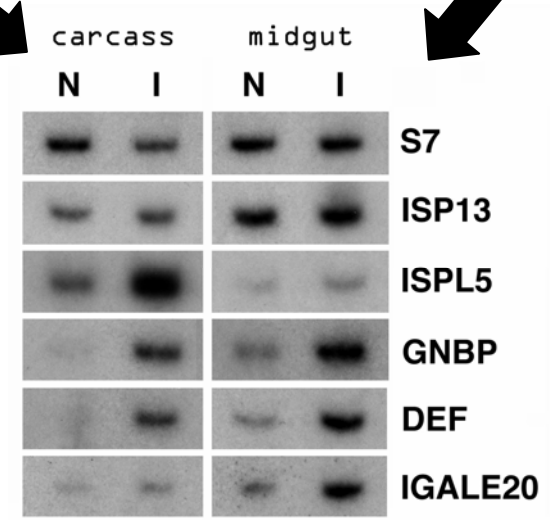
Transcription Analyses of Immune Responses to *Plasmodium*



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

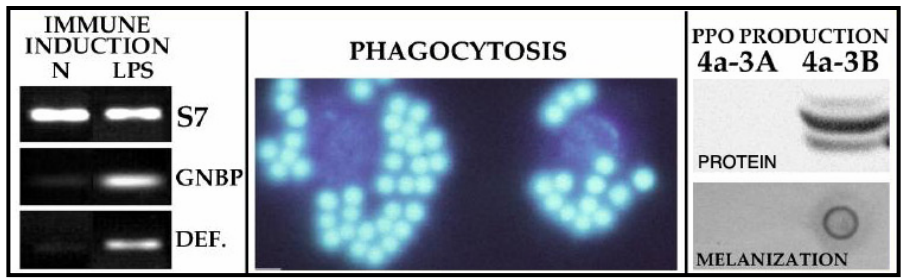
↓

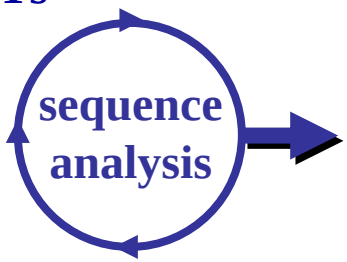
Sequencing 3840 random clones

↓

~6200 ESTs

cell lines

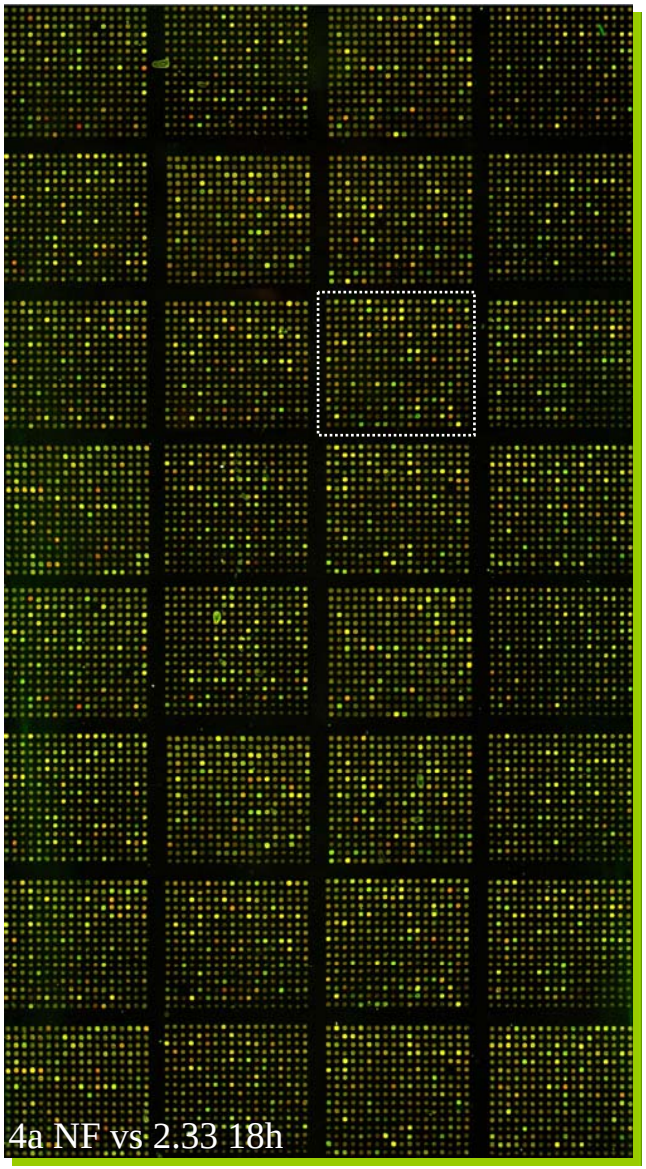




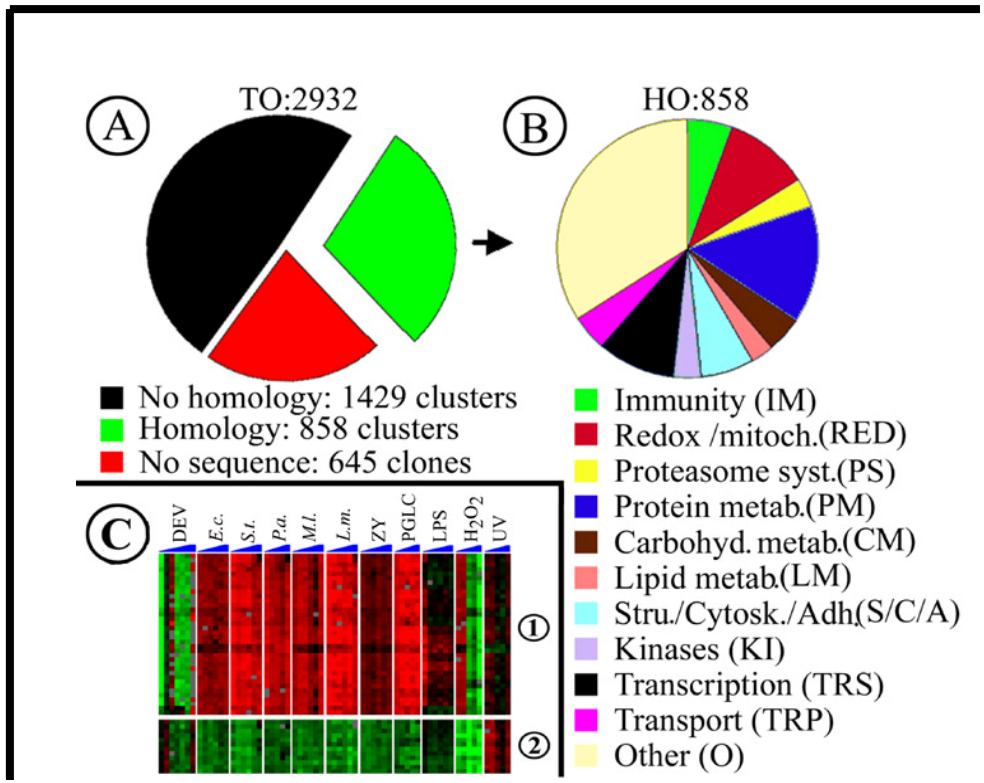
2287 unique genes
1118 genes with database hits
858 genes with assigned function
28 previously cloned *A.g.* genes
48 putative immunity genes

Adapted from Dimopoulos et al. Proc Natl Acad Sci USA 2000;97:6619-6624

2001: A. gambiae Cell Line EST 4000 Array

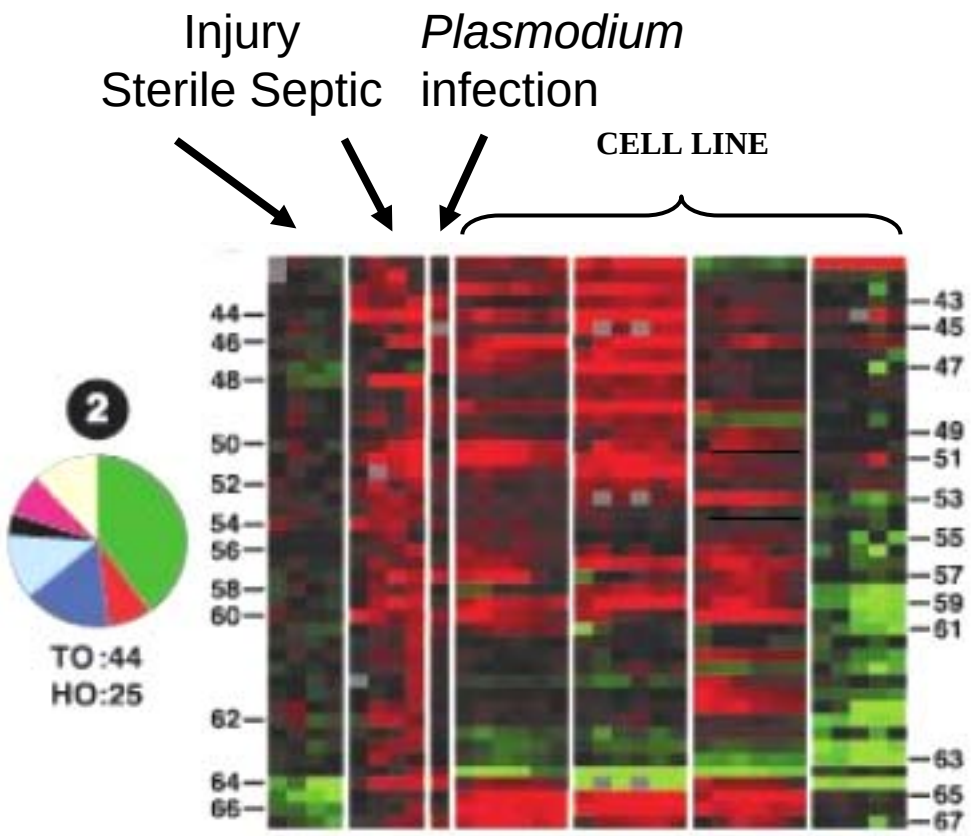


~2200 unique genes
~858 genes with assigned function

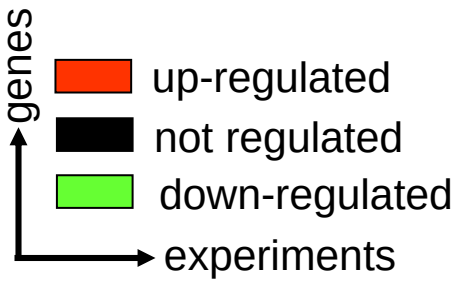


Dimopoulos *et al*., (2002) *Proc Natl Acad Sci U S A*. 99(13):8814-9.

Clustering of Cell Line and Mosquito Responses



Genes that are implicated in anti-*Plasmodium* defense.



Dimopoulos *et al*., (2002) *Proc Natl Acad Sci U S A*. 99(13):8814-9.

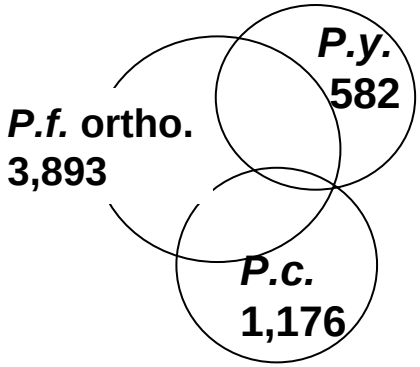
A. gambiae Genome Sequenced and Annotated in 2002

Size: ~260 mega bases

~13.000 predicted *Anopheles* proteins

GAMBER 22K: Anopheles gambiae – Plasmodium berghei microarray

P. berghei: ~5,6K genes

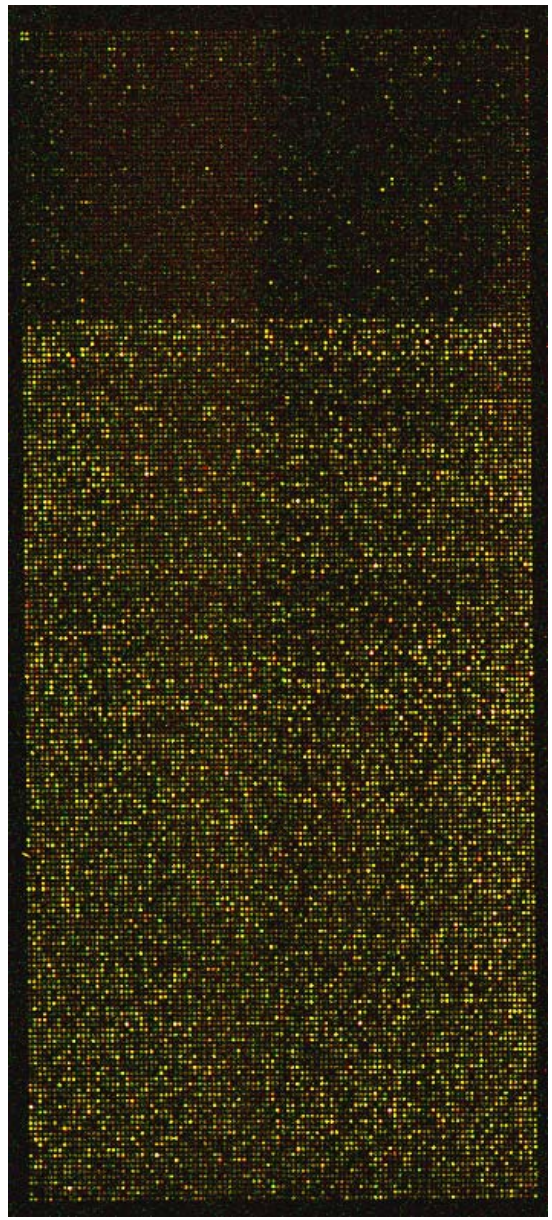


Collab .: N. Hall, TIGR

A. gambiae: ~ 13K genes

3' exon biased
exon specific oligos for 300 genes

Collab.: E. Monguin, Harvard



2004: Complete transcriptome

From Dong Y, et al. PLoS Pathog 2006;2(6):e52

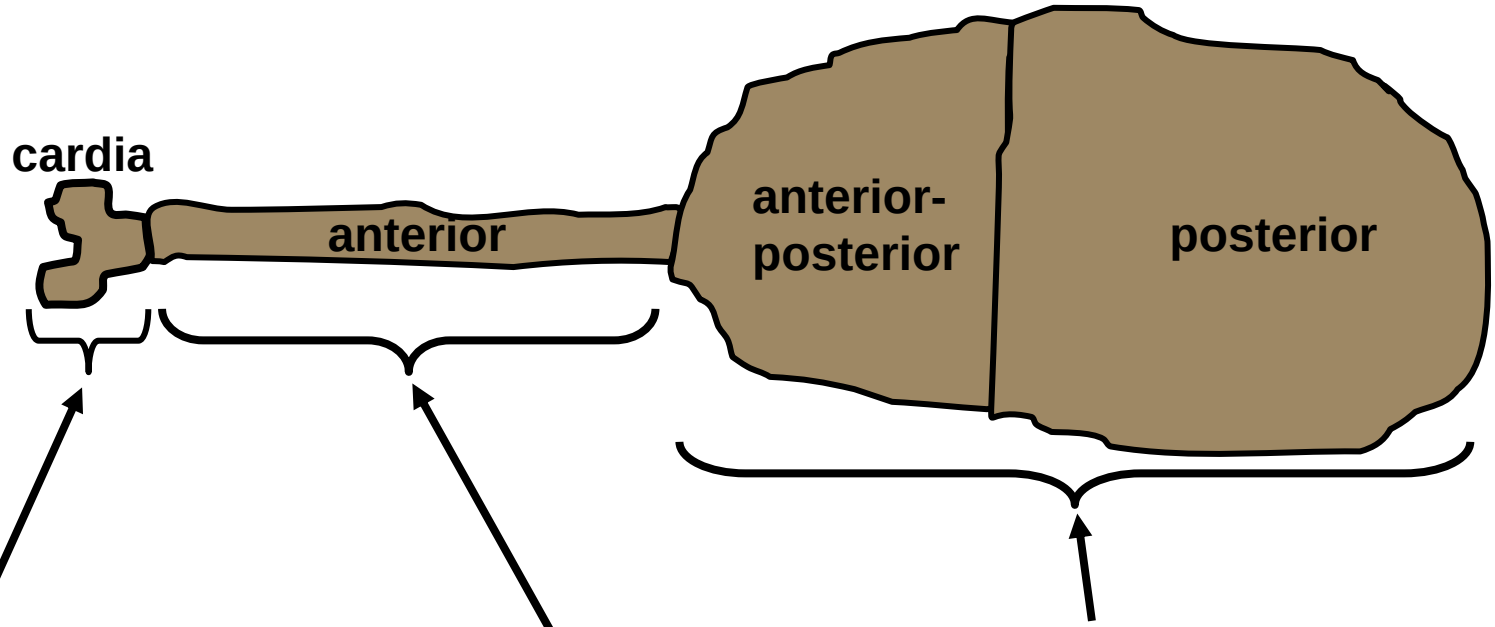
Gene Expression Profiling Can Assess and Identify:

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

Vector (Mosquito, Sandfly, Tsetse, etc…)
- Immune and other physiological responses to infection
- Resistance (refractoriness) & susceptibility genes
- Effector genes
- Tissue specific promoters
- Fitness signatures
- Insecticide resistance factors

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

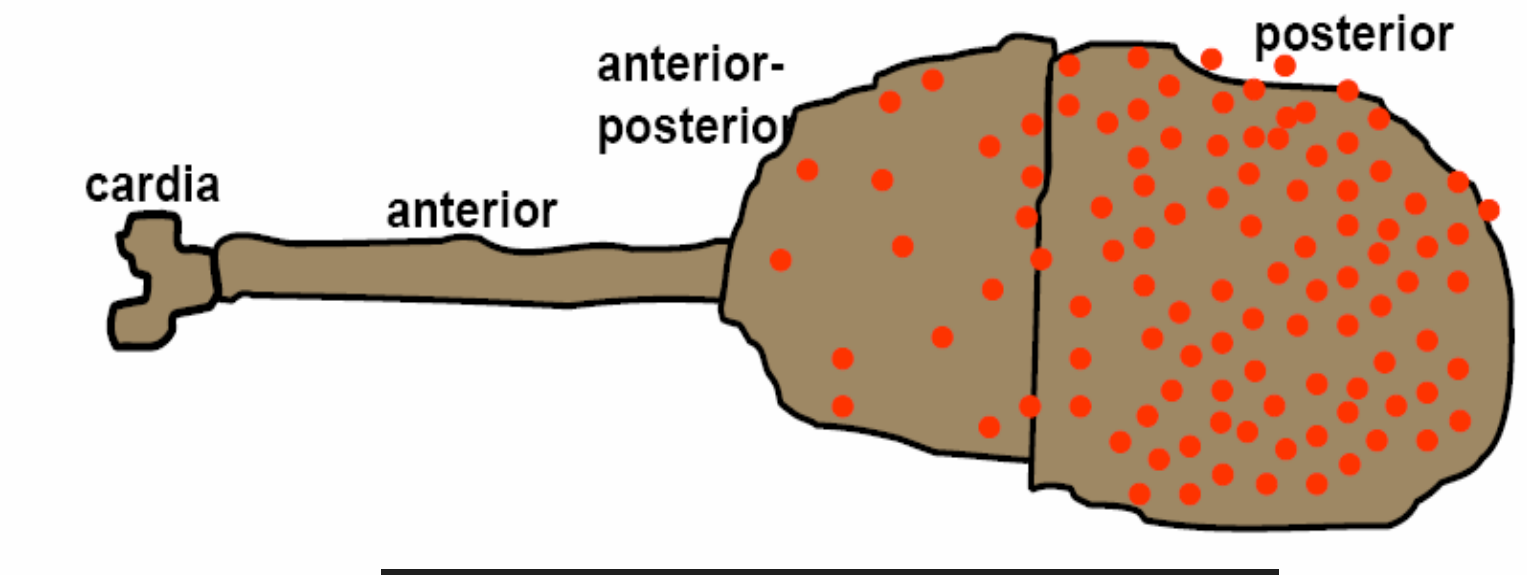


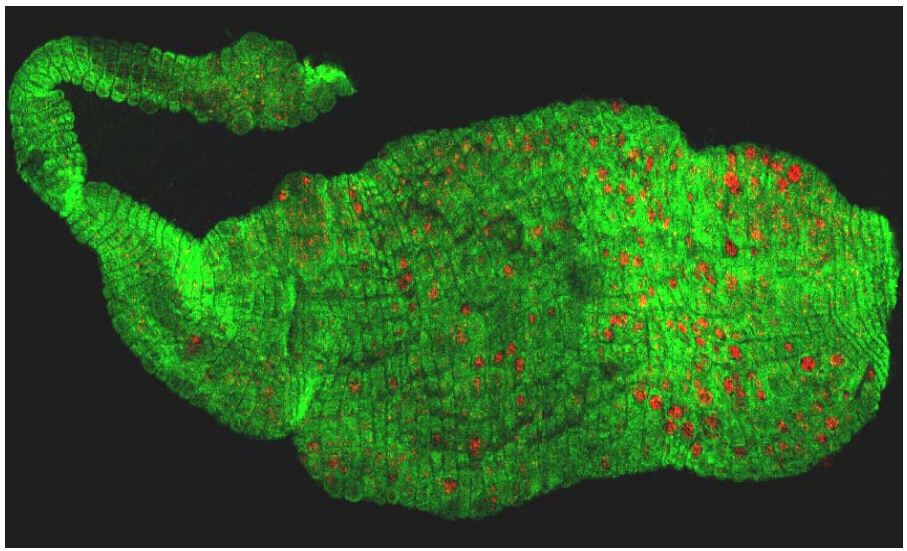
-***Cardia***
secretion of type II Pm
expression of defensin
secretory cells

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

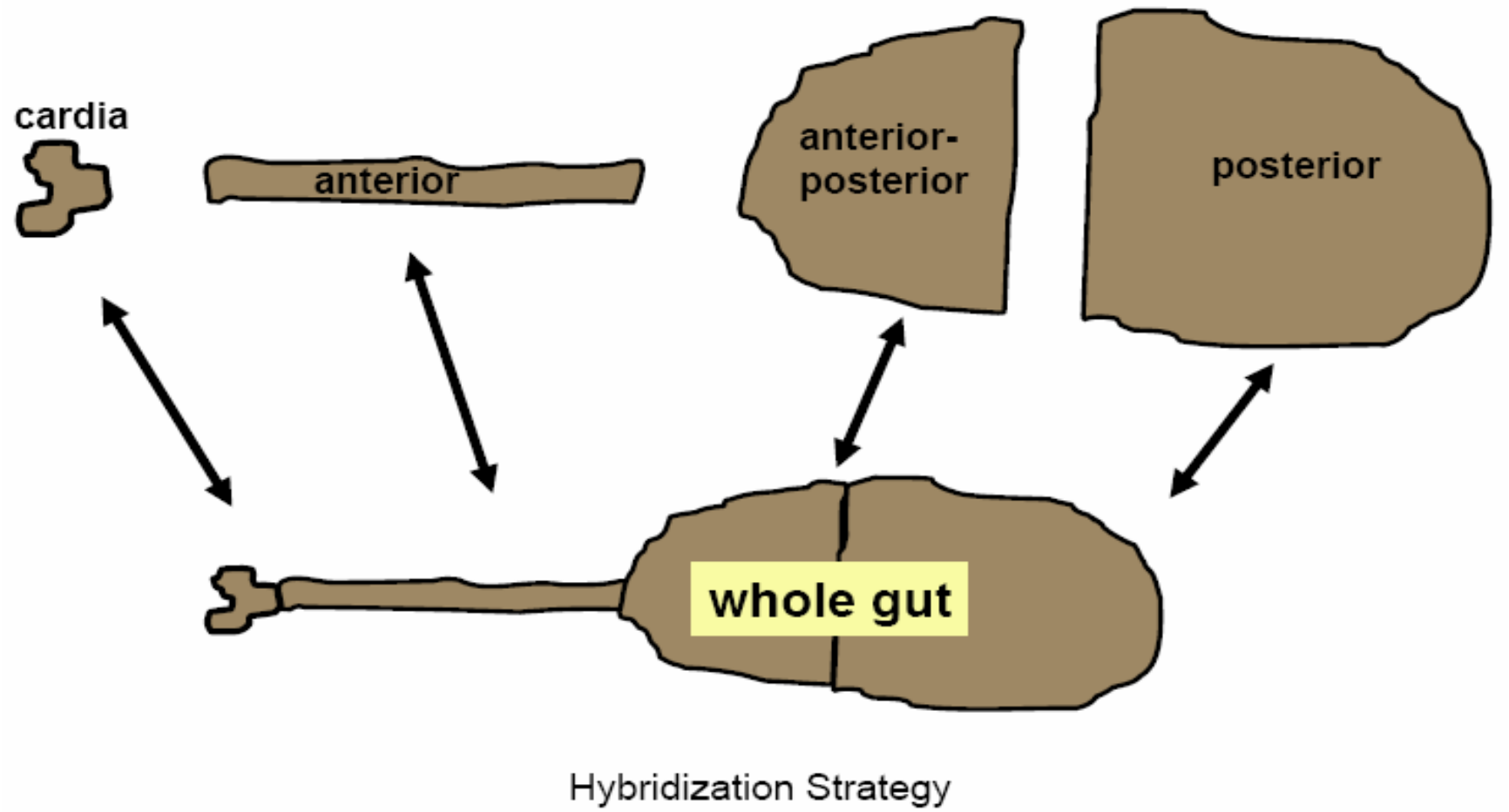
- Posterior midgut
synthesis/secretion of proteolytic enzymes
absorption of blood digestion products
distension (25um → 2.5um high)
stretching of basal lamina
different cell types

Plasmodium Infection of the Midgut



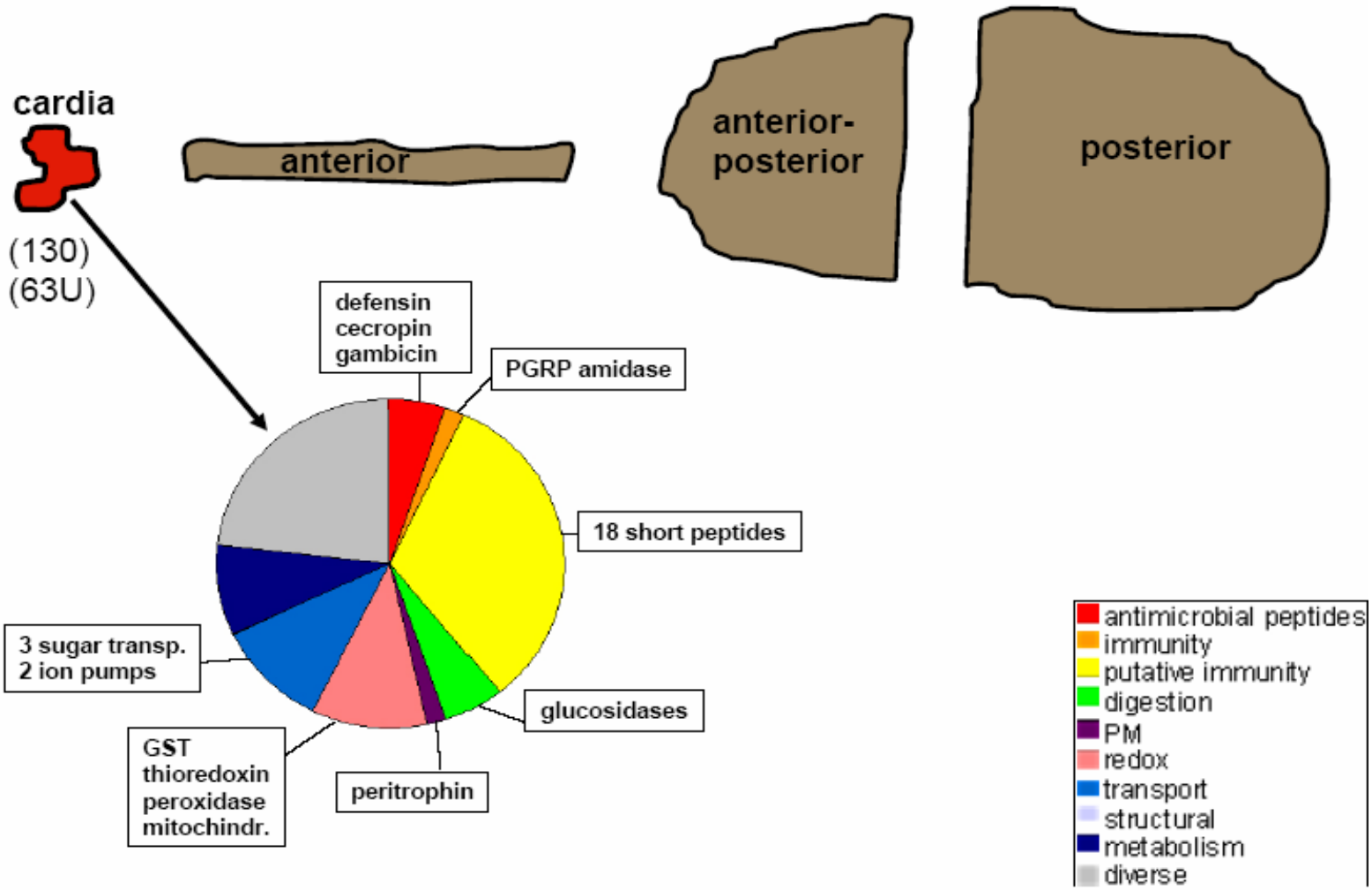


Transcriptomic Dissection of the Midgut

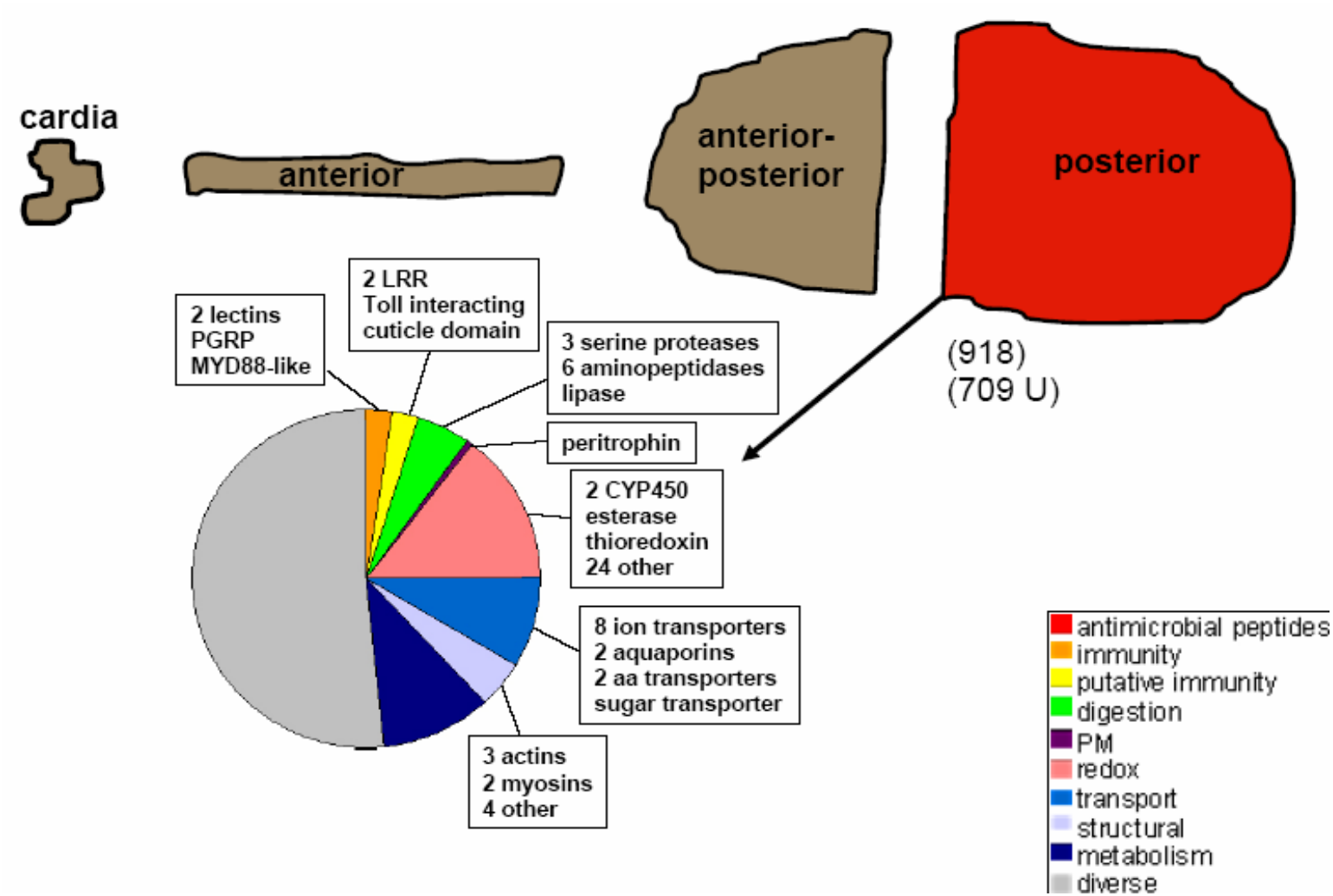


Hybridization Strategy

Transcriptomic Dissection of the Midgut



Transcriptomic Dissection of the Midgut



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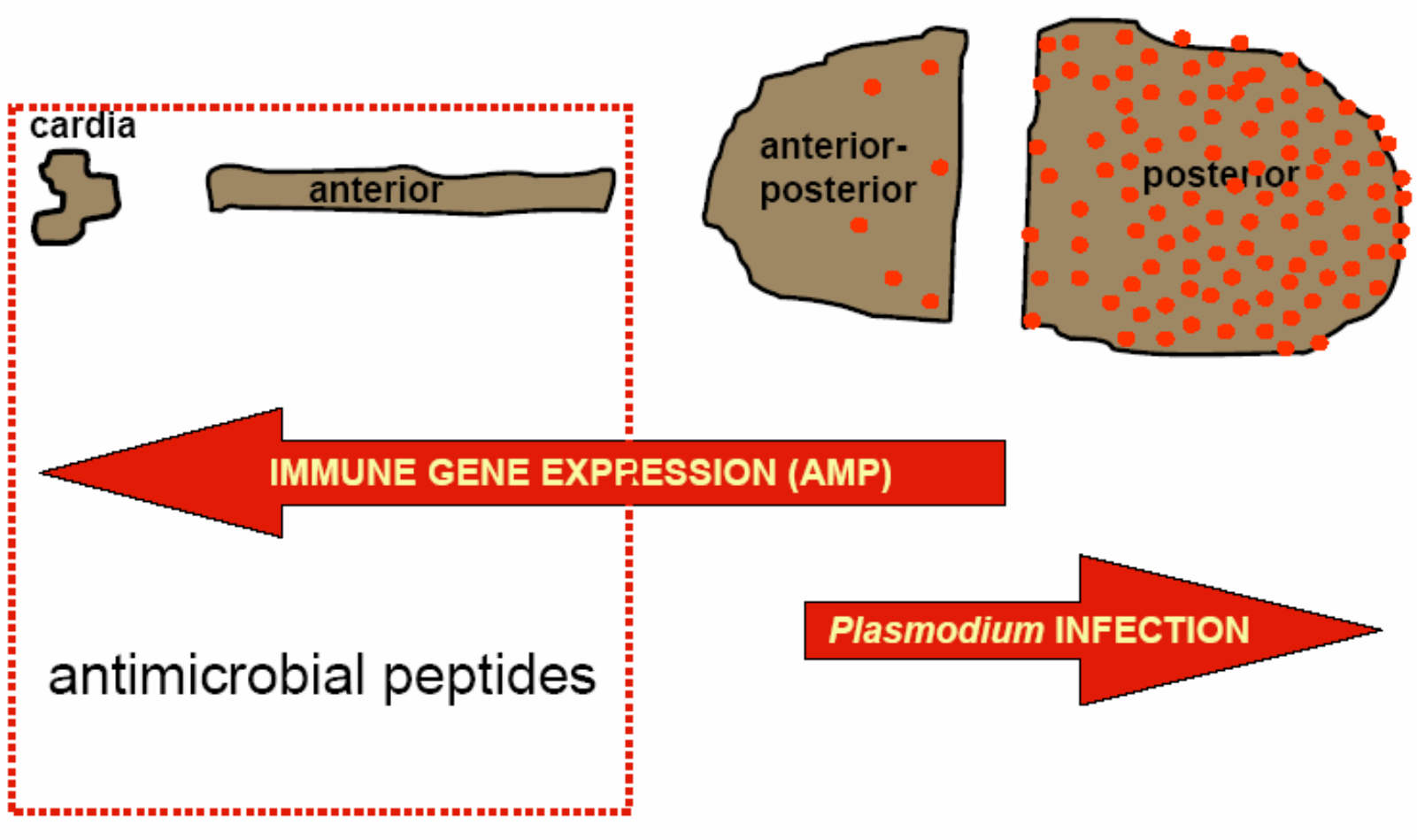
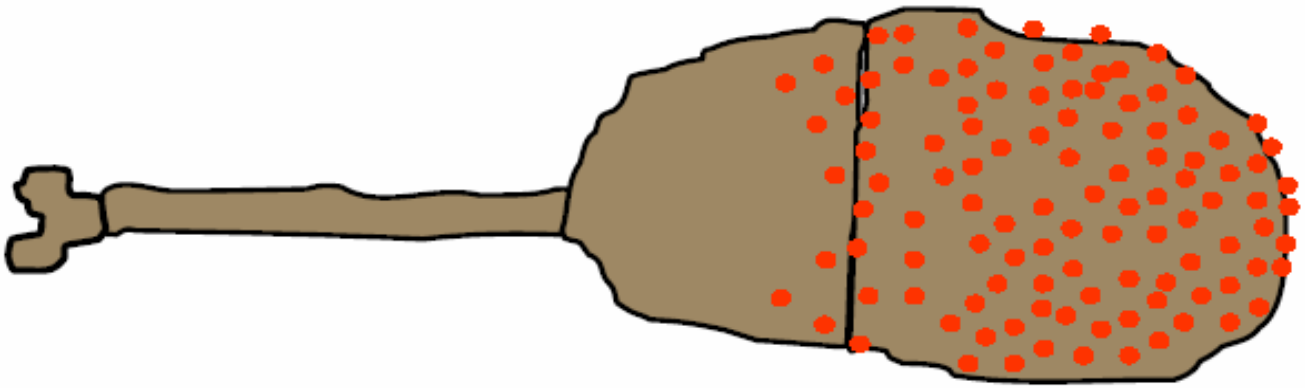
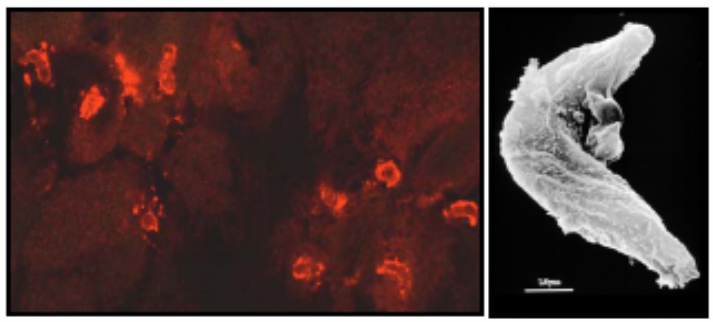
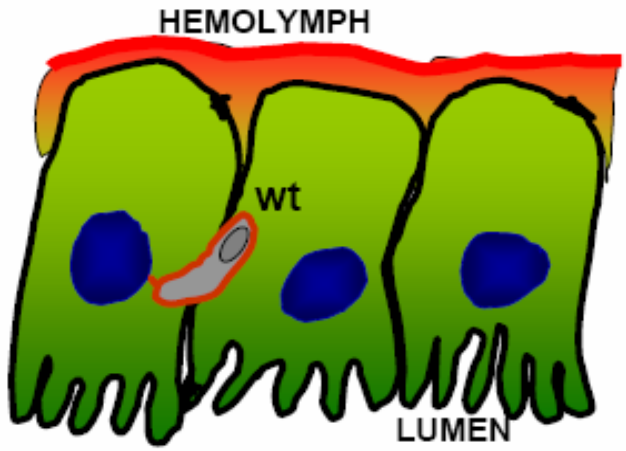


Responses to *Plasmodium* Invasion of the Midgut





R.E. Sinden



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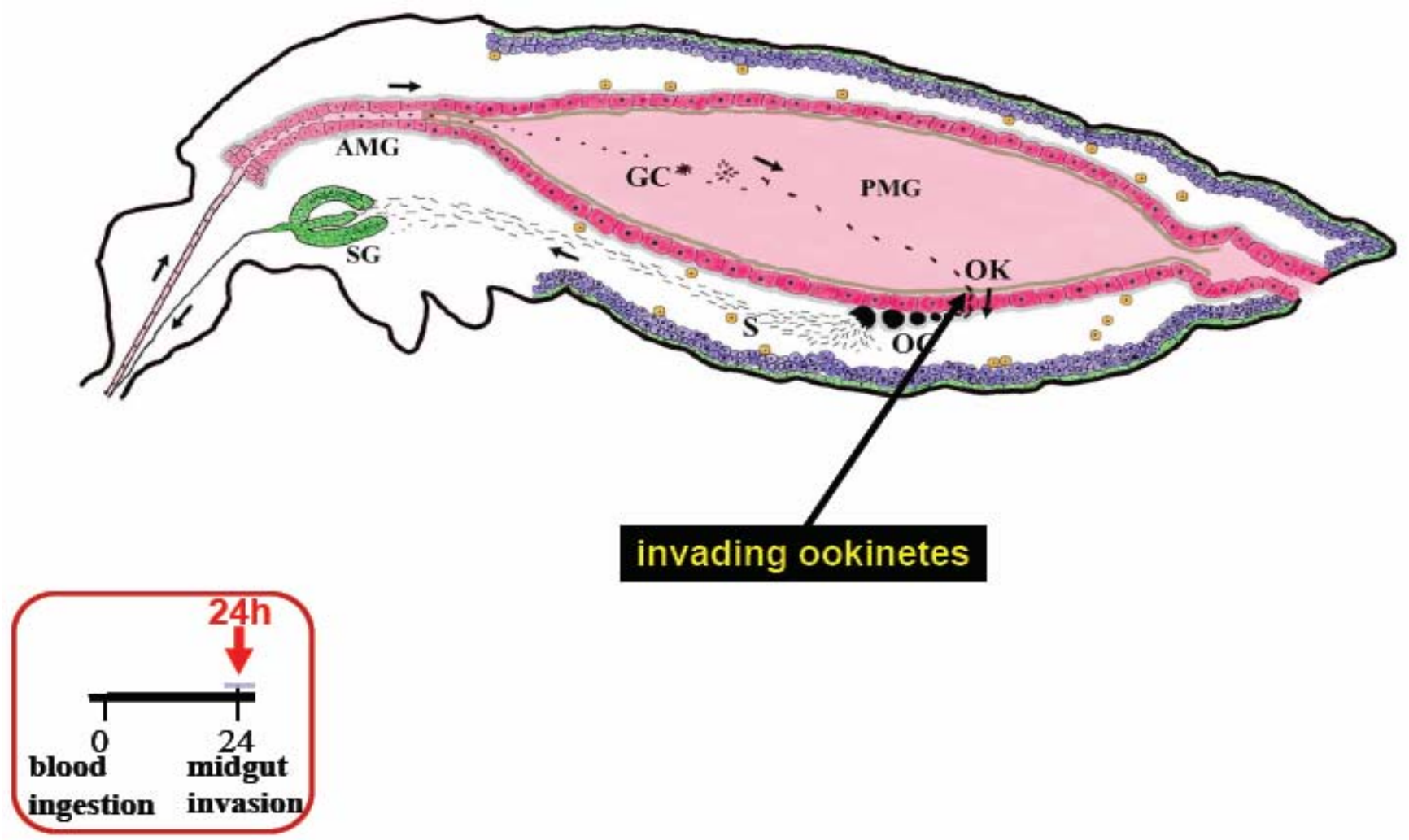
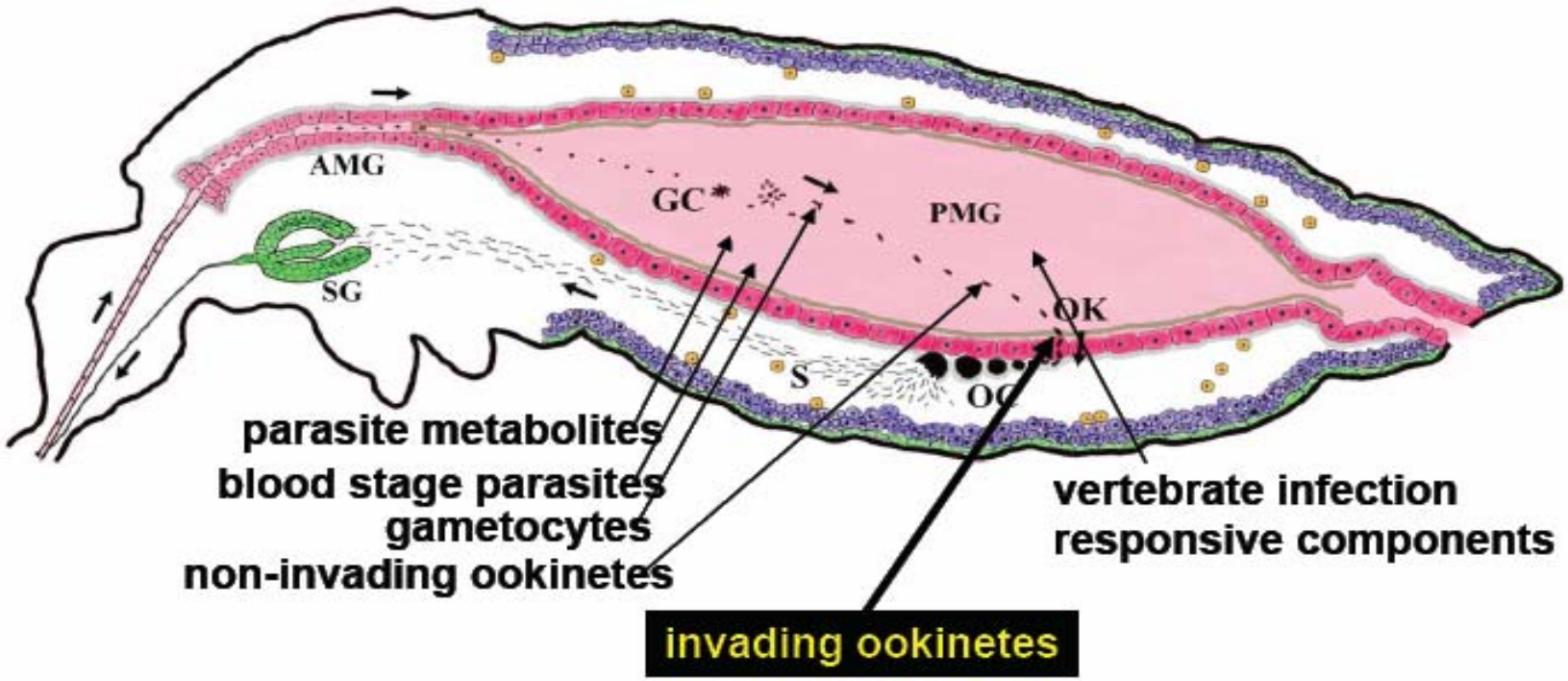
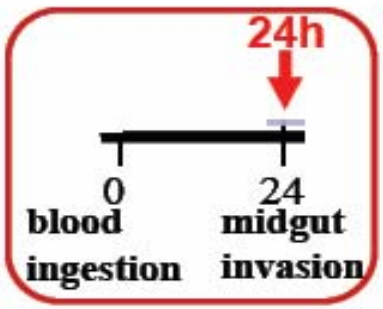


Responses to *Plasmodium* Invasion of the Midgut





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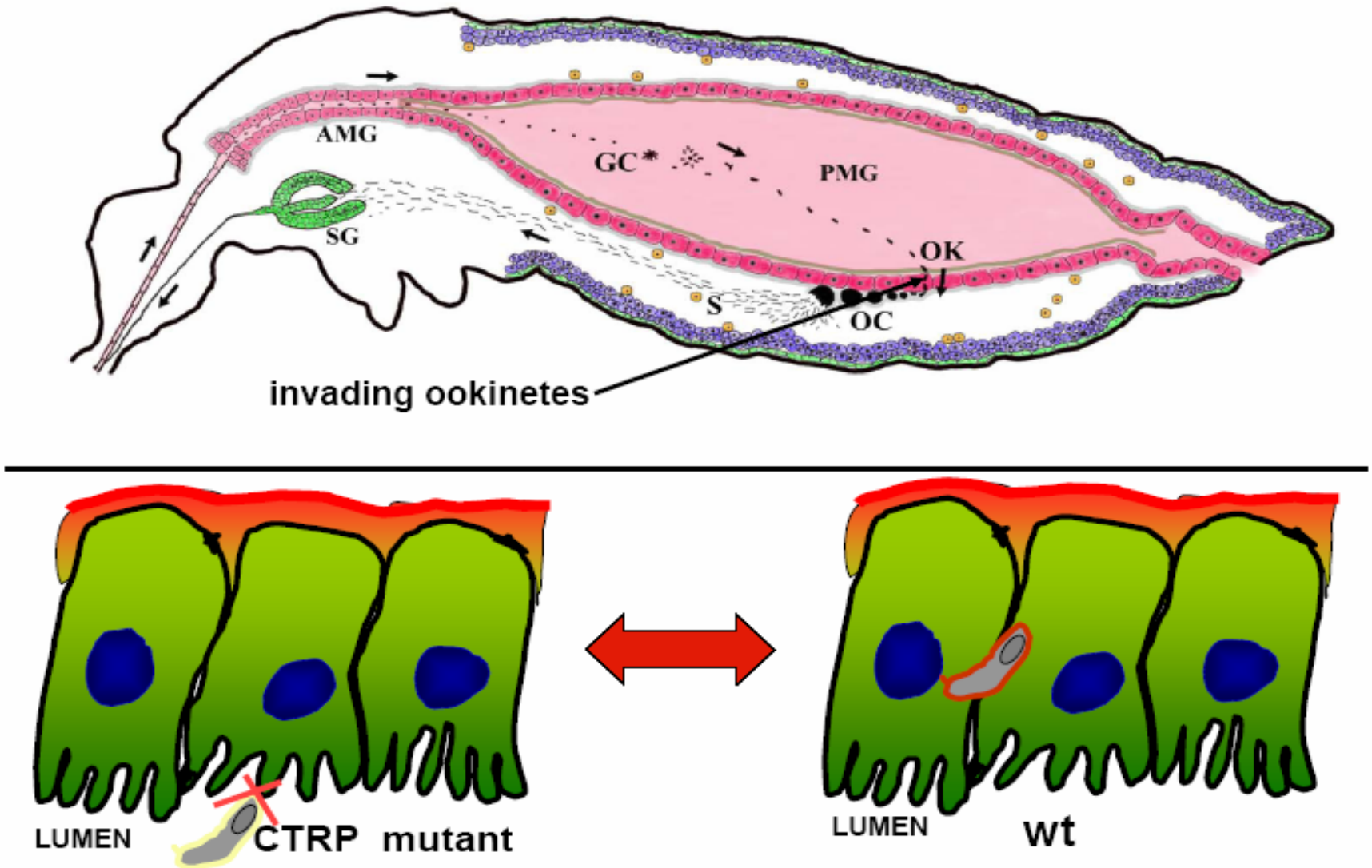
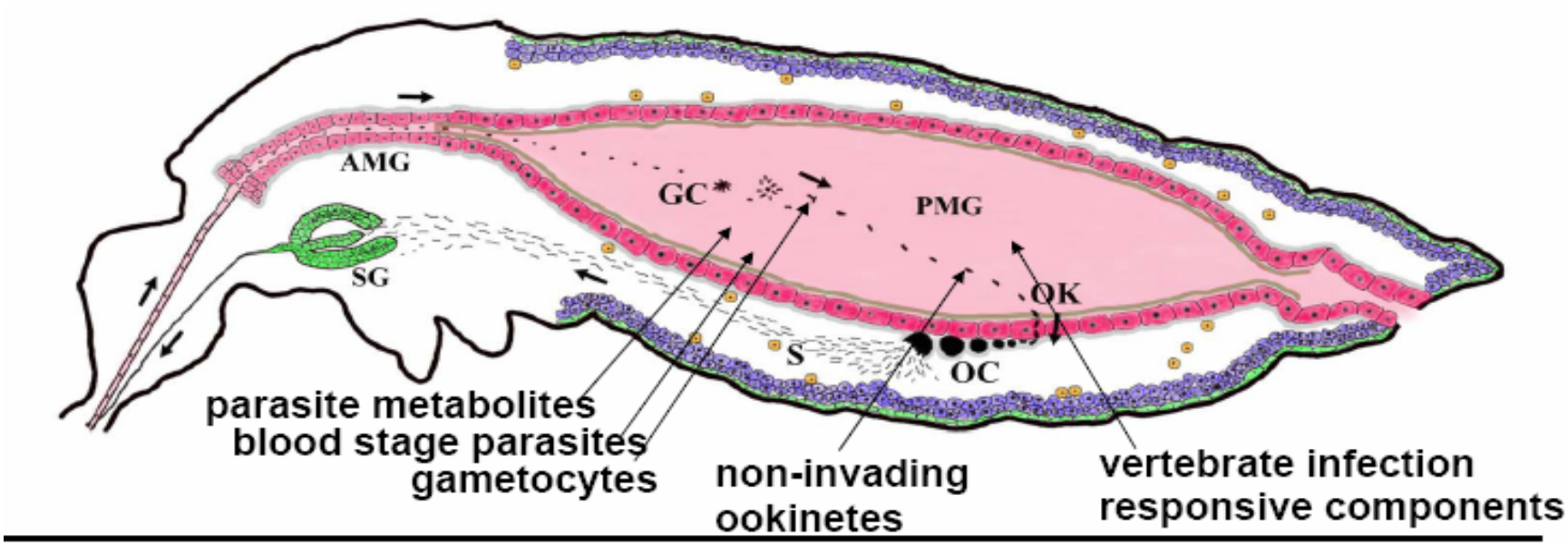
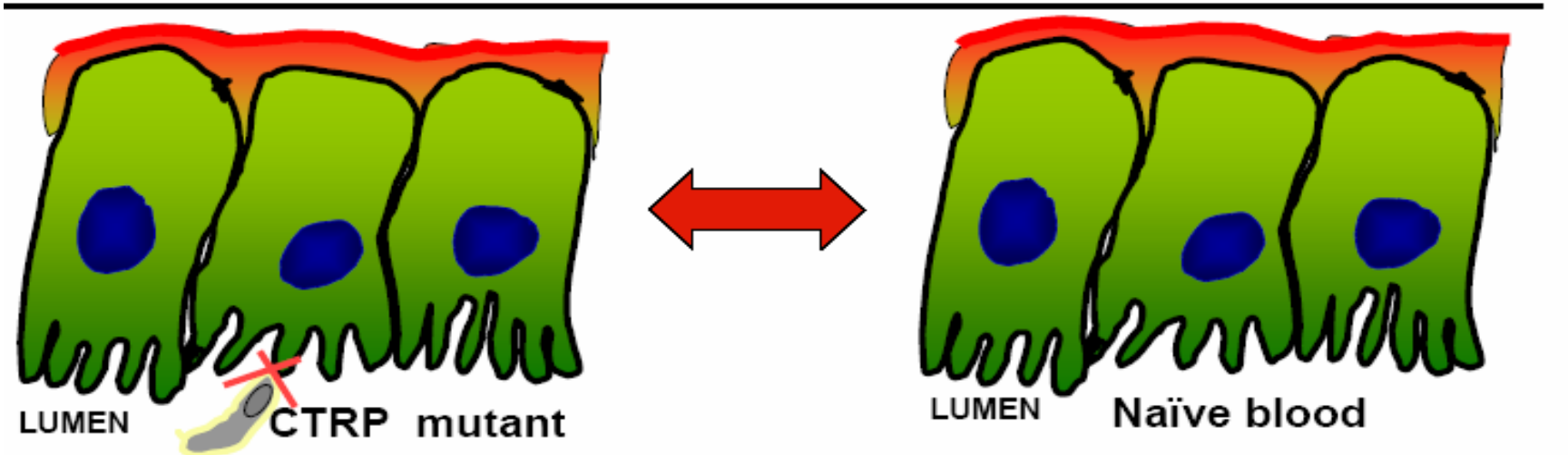
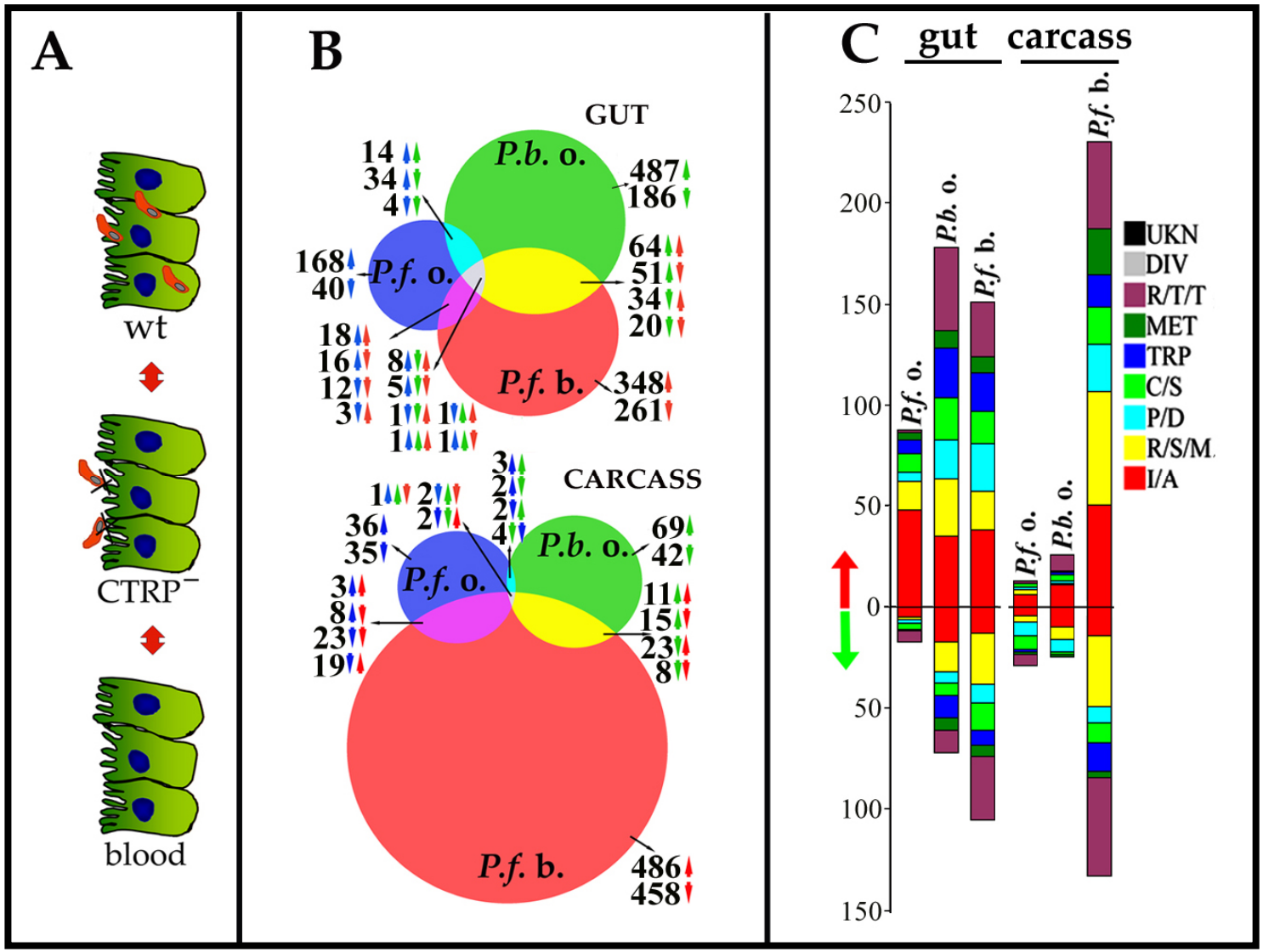


Responses to *Plasmodium* Invasion of the Midgut





Responses to *Plasmodium* Invasion of the Midgut

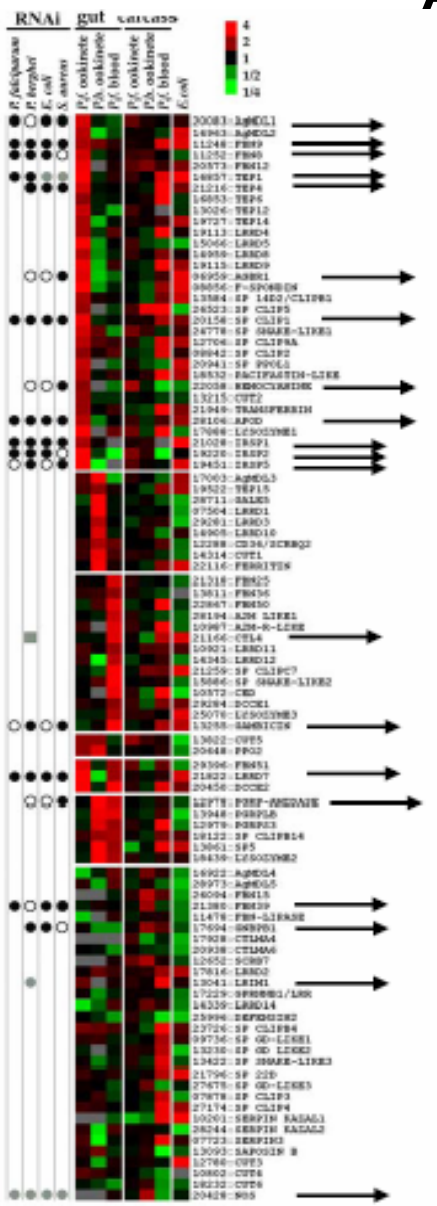


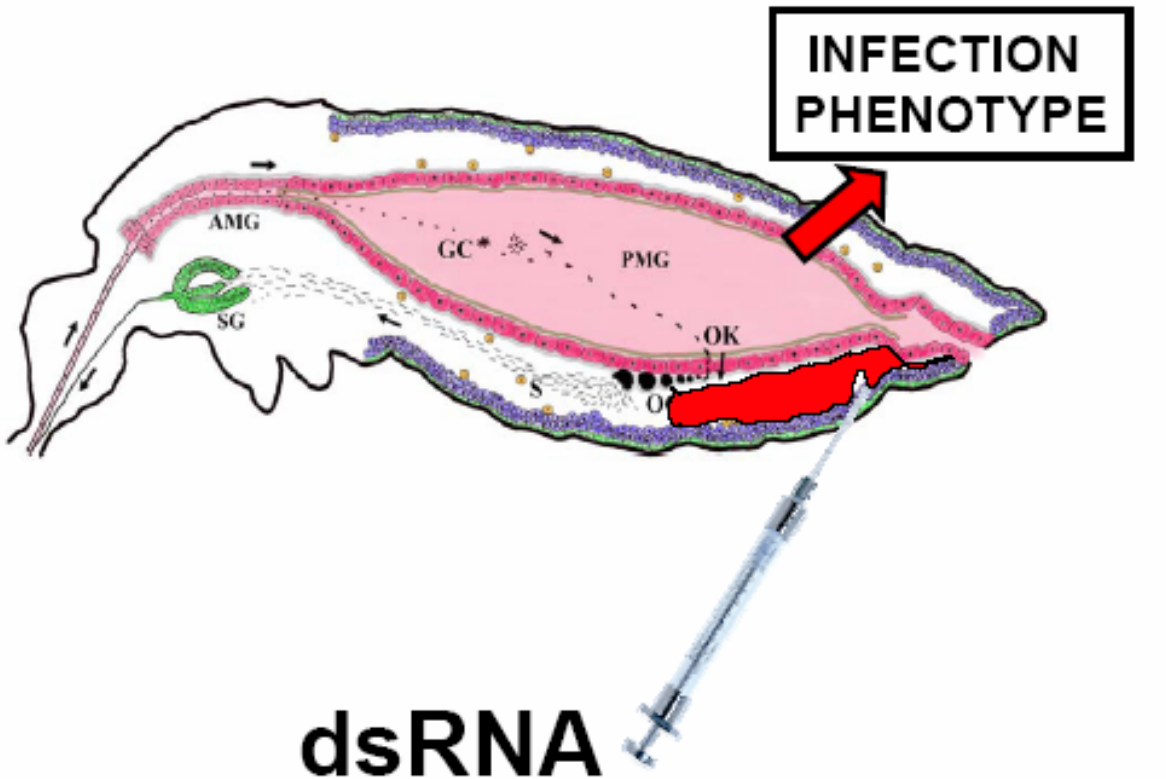
From Dong Y, et al. PLoS Pathog 2006;2(6):e52

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





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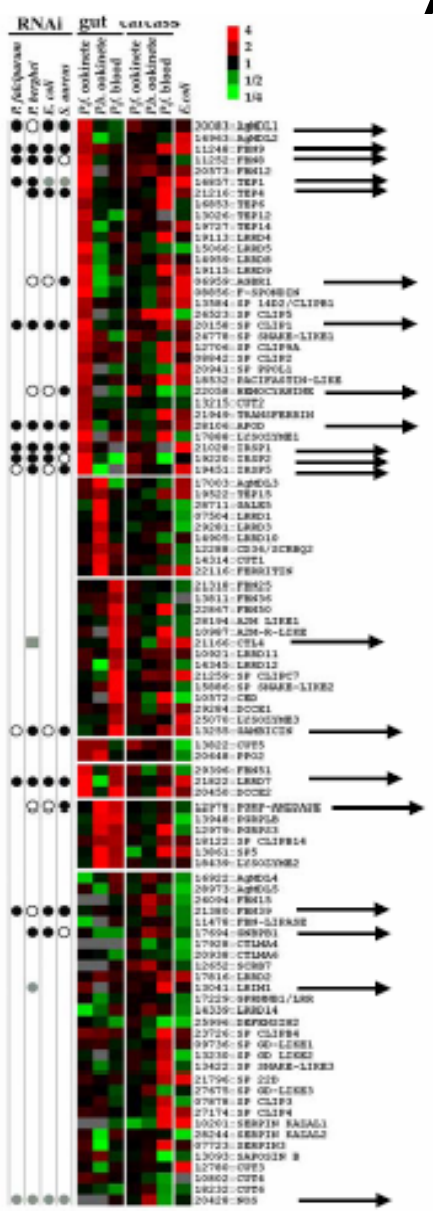
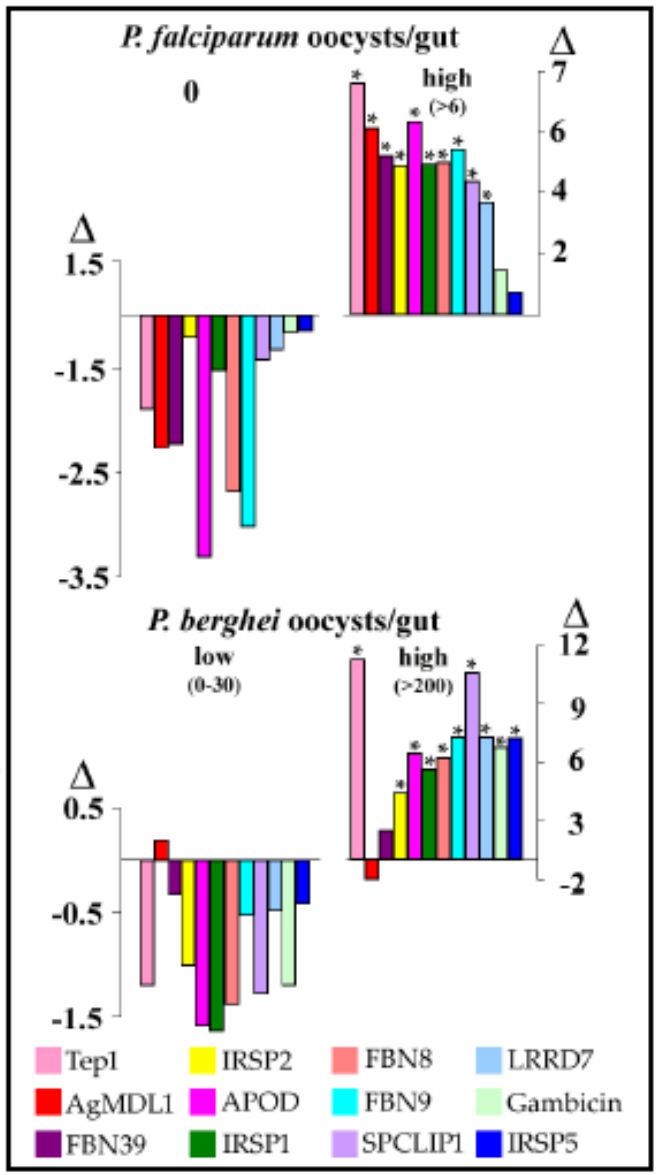
Reverse Genetic Analyses of Anti-*Plasmodium* Function





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.**

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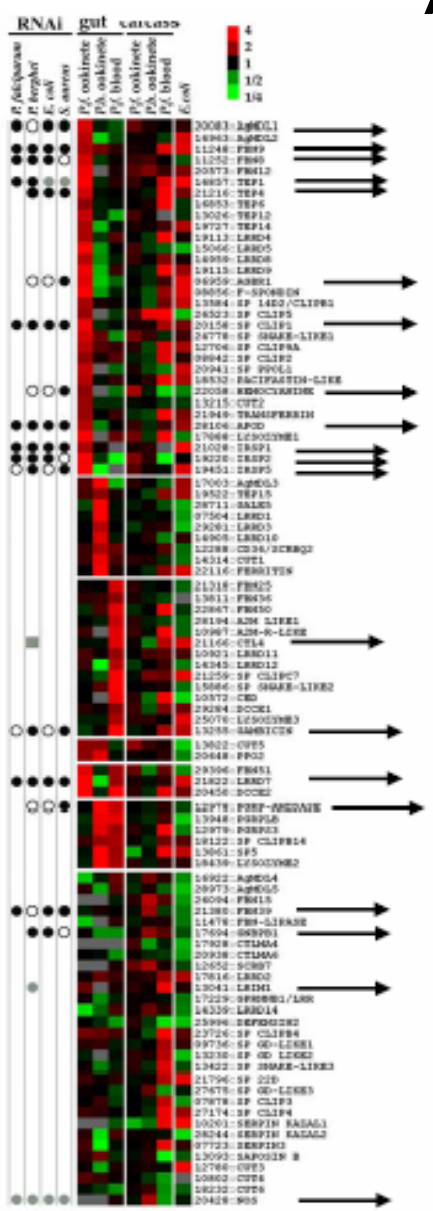
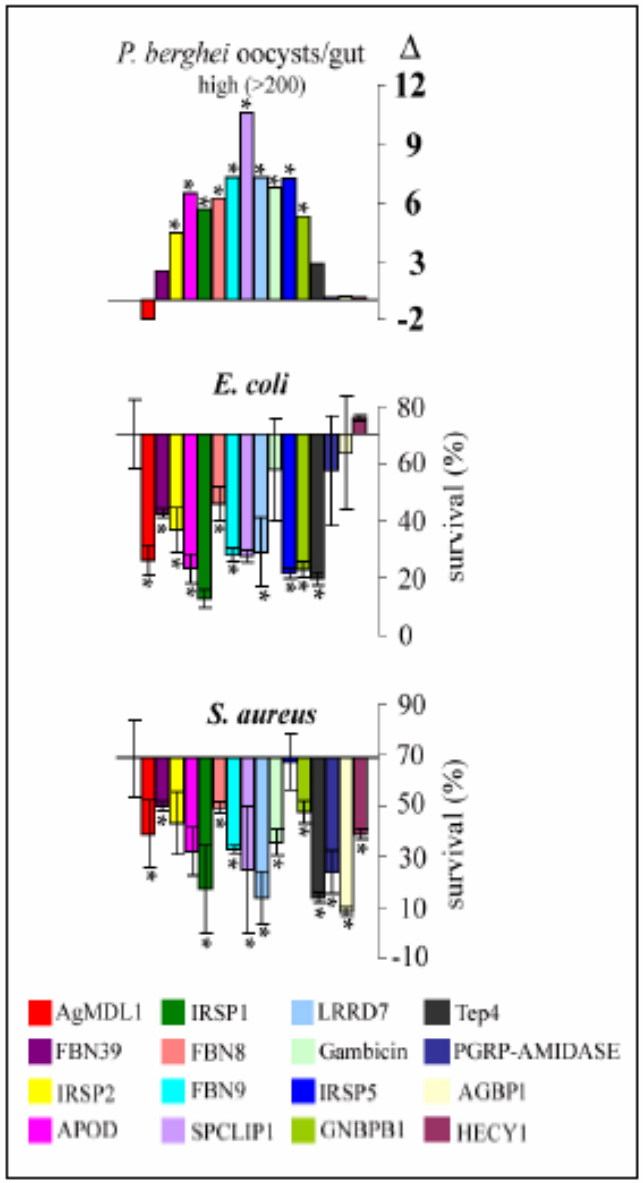
Reverse Genetic Analyses of Anti-*Plasmodium* Function





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.*

From Dong Y, et al. PLoS Pathog 2006;2(6):e52

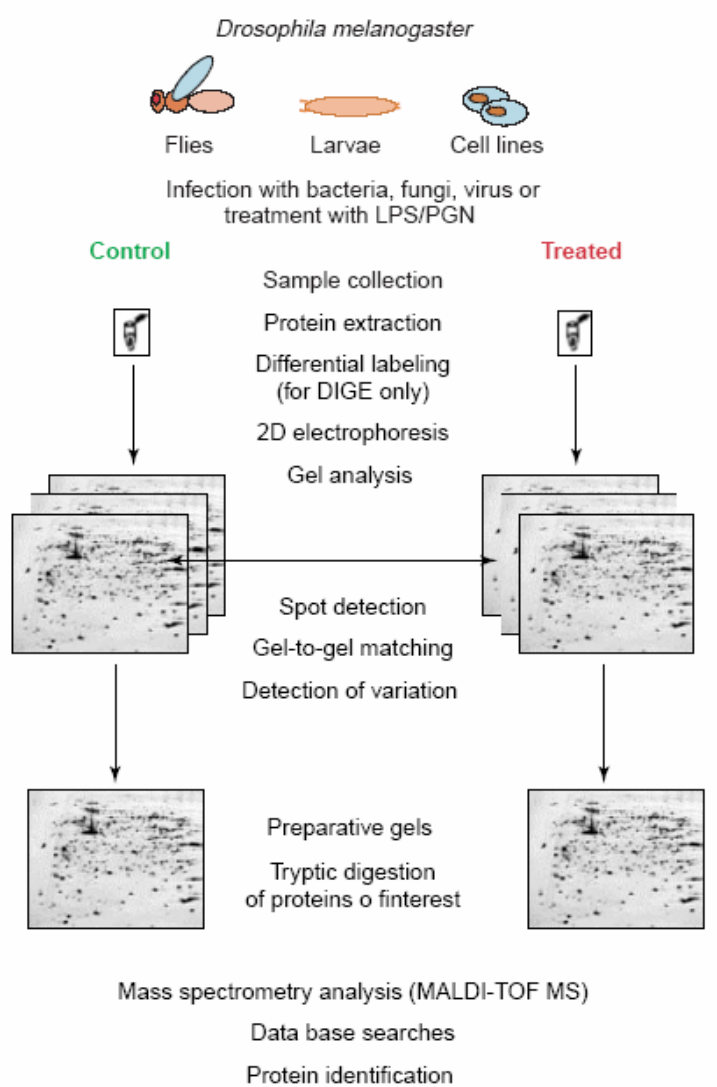
Immune Defense Against *Plasmodium* in the Midgut

- 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



transcript

↓

protein

↓

function

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low resolution

Engstrom (2004) *Trends in Biotechnology* Vol.22 No.11