One Ring To Bind Them All: Is Heme Biosynthesis A Factor In *Wolbachia*-Filarial Nematode Endosymbiosis?

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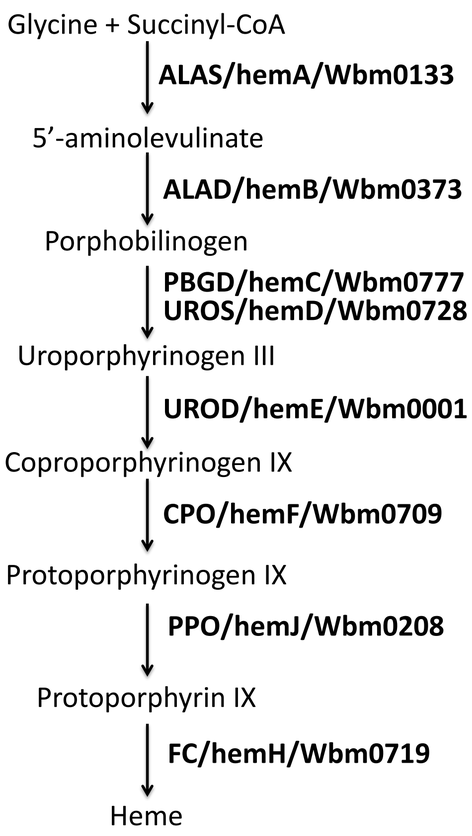
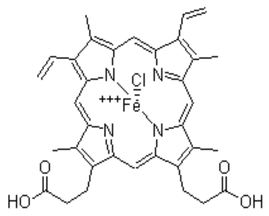
Introduction:

Transmitted by insect vectors, human nematode-based filariasis causes debilitating diseases affecting nearly 150 million people with 1.2 billion individuals at risk in 80 countries. Genomic sequencing has revealed that many human filarial nematodes, such as *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, (causative agents of lymphatic filariasis (LF)) and *Onchocerca volvulus* (causative agent of onchocerciasis (river blindness)) contain the obligate endosymbiont, *Wolbachia*. Genome sequencing of *B. malayi (Bm)* and its *Wolbachia* (*wBm)* identified a number of metabolites implicated in the host-endosymbiont interaction, one of which was heme, a co-factor in a number of enzymes and essential to many biological processes. Although the *Bm* genome encodes a functional ferrochelatase gene (the final step in the heme biosynthetic pathway and a product of lateral gene transfer), like other nematodes, it is incapable of synthesizing heme. However, the *wBm* genome contains a functional heme synthesis pathway, leading to the hypothesis that *wBm* may supply *Bm* with heme.

Our laboratory is exploiting the use of biochemistry, cytology and Next-Generation sequencing to further investigate patterns of *Bm* and *wBm* heme trafficking.

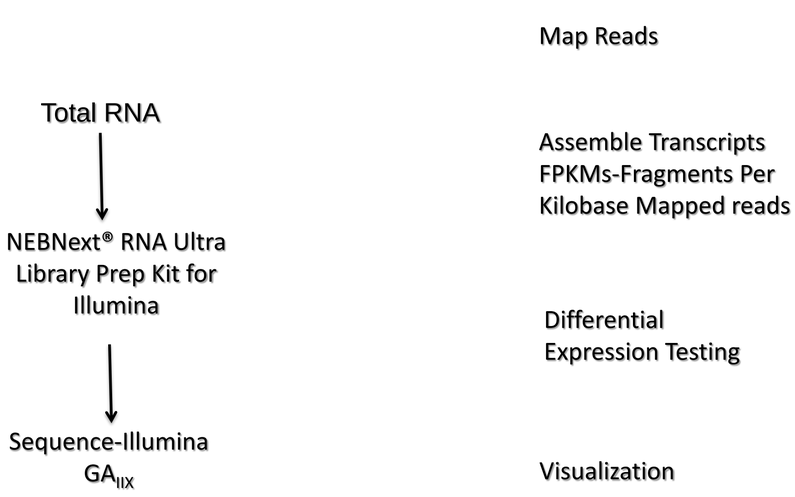




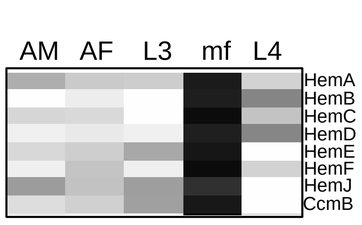
Heme Biosynthetic Pathway of *Wolbachia (wBm)*. Heme is synthesized through a well-defined evolutionarily conserved pathway. The eukaryotic/prokaryotic/*Wolbachia* gene names for the enzymes involved in each step of the pathway are given in bold.

ALAS, 5-aminolevulinate synthase; ALAD, 5-aminolevulinate dehydratase (also known as PBGS, porphobilinogen synthase,); PBGD, porphobilinogen deaminase; UROS, uroporphyrinogen III synthase; UROD, uroporphyrinogen III decarboxylase; CPO, coproporphyrinogen IX oxidase; PPO, protoporphyrinogen IX oxidase; FC, ferrochelatase.

Transcriptomics: Using NextGen sequencing technology to examine RNA profiling of *Wolbachia*



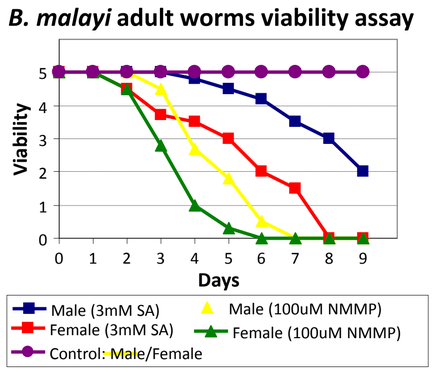
Differential expression: *Wolbachia* heme *biosynthesis* genes and genes related to heme binding and utilization are up-regulated, especially in microfilaria.



Heme synthesis and transport appear to be linked to heme binding/utilization in a stage-specific manner.

B. malayi ex vivo viability assays with heme pathway inhibitors – succinyl acetone & N-methyl mesoporphyrin show *Wolbachia* heme biosynthesis is required for worm survival

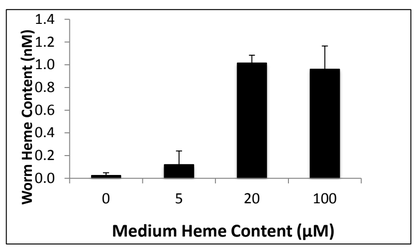
***B. malayi* adult worms viability assay**



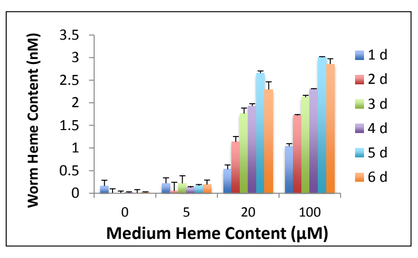
-Both adult worms & microfilaria are killed by heme pathway inhibitors

(NMMP—FC inhibitor)
(SA- ALAD inhibitor)

This suggests *Wolbachia* heme biosynthesis is critical for worm survival. However, heme can also be taken up from the media.

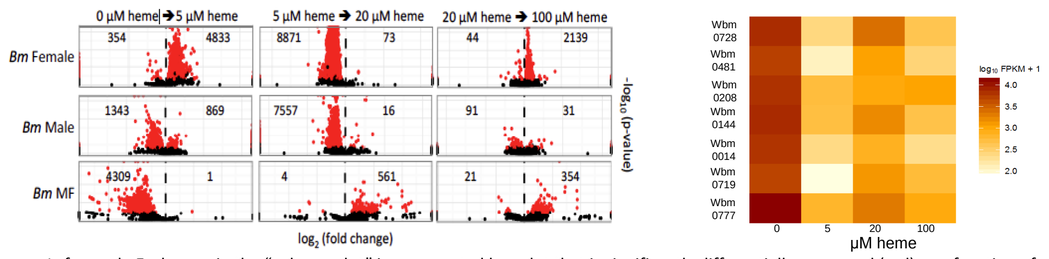


Total worm heme content increases with increasing medium heme concentration



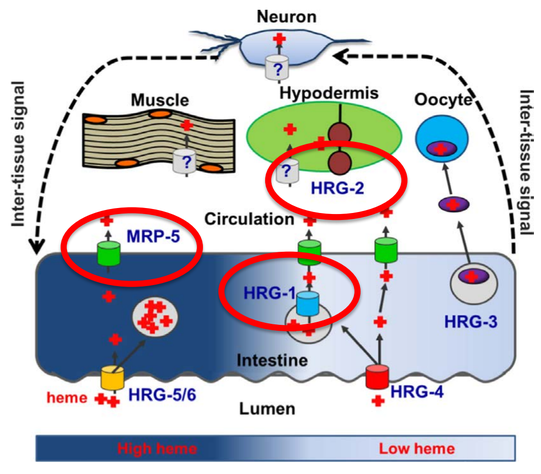
Total worm heme content increases over time

Addition of heme to the media induces "up" and "down" regulation of genes (especially in microfilaria). Interestingly, heme addition down-regulates most *Wolbachia* heme biosynthesis genes.



Left panel: Each gene in the "volcano plot" is represented by a dot that is significantly differentially expressed (red) as a function of increasing the heme concentration. Right panel: *Wolbachia* biosynthesis genes are down-regulated as a function of increasing heme.

The Search for "Heme Response Genes" (HRGs)



- **HRG-1**
  - **high affinity transporter**
  - **CeHRG-1/BmHRG-1**
    - **39% identity/60% similar**
- **MRP-5**
  - **heme exporter**
  - **CeMRP-5/BmMRP-5**
    - **48% identity/66% similar**
- **HRG-2**
  - **heme transport into hypodermis**
  - **CeHRG-2/BmHRG-2**
    - **31% identity/51% similar**

Heme homeostasis-*B. malayi* similar, but not identical to, *C. elegans*, a free-living nematode

Summary:

--A conundrum exists for understanding the role of heme biosynthesis. *Wolbachia* genomics, biochemistry and inhibition studies suggest the pathway is essential for worm survival and expression studies suggest heme regulates *Wolbachia* heme biosynthesis genes.

--Yet *Brugia* has the functional genes for heme uptake and distribution. In *B. malayi* and the related nematode *D. immitis,* expression studies indicate heme biosynthesis genes are up-regulated in male and female tissues along with Sec and Type IV secretion system components. Genes encoding heme transport and distribution from the extracellular environment are present and functional.

--We hypothesize that *Wolbachia* helps supply heme for fertility (oogenesis) and perhaps for other requirements for additional heme. One example may be for worm survival in the insect vector host, where exogenous heme may be negligible.

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