NHR-49/PAARα and HLH-30/TFEB cooperate for C. elegans host defense via a flavin-containing monooxygenase

Khursheed A. Wani
Laboratory of Javier Irazoqui
Department of Microbiology and Physiological Systems
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*C. elegans* natural habitat poses a challenge to survive

Natural habitats and substrates of *C. elegans*  

(Hinrich Schulenburg, and Marie-Anne Félix; 2017)
C. elegans lack conventional innate immune response pathways

- It is not clear whether C. elegans sole TLR, TOL-1, functions in innate immunity.
- C. elegans genome does not encode NFkB.
- Unlike mammals, C. elegans lack cell-mediated immunity.
**C. elegans** show transcriptional response to pathogens

- Anti-microbial peptides
- Lysozymes
- Lectins

(D. coniospora, M. nematophilum)

(Picture modified from Kim D, 2008)

(Work carried out in Fred Asubel, Jonathan Hodgkin, Jonathan Ewbank, Dennis Kim, Alejandro Aballay, Emily Troemel, Read Pukkila-Worley, & Javier Irazoqui labs)
C. elegans show a pathogen-specific transcriptional response

(Irazoqui JE et al; 2010)
S. aureus destroys C. elegans intestinal epithelial cells

(Irazoqui et al, 2010)
C. elegans fight infection by the induction of host defense genes

(ilys-3/snb-1)

E. coli S. aureus

(clec-60/snb-1)

E. coli S. aureus

(Irazoqui JE et al; 2010)
S. aureus infection poses a nutritional challenge

How much of the host response is due to infection as opposed to the nutritional challenge?
Can we separate metabolic stress from infection?

Wani et al.; under revision
Identification of infection-specific gene signature

Wani et al; under revision
FMO-2 is a highly-induced infection-specific gene

- **fmo-2** encodes flavin-containing monooxygenase
- Detoxification of xenobiotic substances
- In Arabidopsis, FMO1 functions in host defense

**Table:**

| E. coli | S. aureus | Starvation |
|--------|-----------|------------|
| H02F09.3 | ech-9 | Y65B4BR.1 |
| C50F7.5 | srr-6 | Y47H9C.1 |
| K08C7.4 | irg-5 | C33A12.19 |
| C33A12.19 | clec-52 | pals-39 |
| mpk-2 | \( \log_2 \text{FoldChange}=7.278 \) | |

*Wani et al; under revision*
FMO-2 is a pathogen-specific host defense gene

FMO-2 mRNA levels (relative to E. coli)

- E. coli
- S. aureus
- P. aeruginosa

Survival over time after transfer to S. aureus

Wild type vs. fmo-2(-)****

Wani et al; under revision
FAD and NADPH motifs are evolutionarily conserved.

**FAD binding motif**

- **GXGXXG**

**NADPH binding motif**

- **GXGXX(G/A)**

**G10A**

**G191A G193A**

**G191A G193A**
FMO-2 catalytic activity is required for host defense

| Survival (%) | Time after transfer to S. aureus (h) |
|--------------|-------------------------------------|
| Wild type    | 100                                 |
| fmo-2(FAD mutant) | 90*****                            |
| fmo-2(NADPH mutant) | 80*****                            |
| fmo-2(FAD+NADPH mutant) | 70*****                            |

Wani et al; under revision
\textit{fmo-2} induction is required for host defense
HLH-30/TFEB is important for host response to infection

**hlh-30::hlh-30::gfp**

- Non-pathogenic *E. coli*
- *S. aureus*

~80% HLH-30-dependent genes

(Visvikis O et al; 2014)
**fmo-2 induction is independent of HLH-30/TFEB**

![Plot](image)

**Wild type**
- UP in Starvation
- UP in Infection

**hlh-30(-)**
- UP in Infection

**UP in Infection**
- 99 genes
- **fmo-2**, **fip-1**
- **fmo-3**, **nlp-34**

**Wild type**
- 6 genes
- **clec-60**, **clec-70**, **lys-4**, **mpk-2**, **sma-5**, **kreg-1**

**hlh-30(-)**
- **clec-52**, **C33A12.19**, **C54F6.12**, **K08C7.4**, **Y47H9C.1**

Wani et al; under revision
HLH-30/TFEB is partially required for *fmo-2* induction during infection

![Graph showing *fmo-2* mRNA induction](image)

- **Wild type**: Red bars
- **hlh-30(-)**: Black bars
- **hlh-30 (-); Philh-30; fmo-2 ns**: Green bars

**Gene expression levels** (relative to Wild type + E. coli):
- 0.1
- 1
- 100
- 1,000
- 10,000

*E. coli* and *S. aureus* images show wild type and *hlh-30(-)* conditions.

Wani et al; under revision
What else does regulate *fmo-2* induction during infection?
NHR-49/PPARα is essential for *fmo-2* induction during infection

Wild type

*nhr-49(-)*

**E. coli**

**S. aureus**

*Pfmo-2::gfp*

(Wild type)

(E. coli)

(S. aureus)

Nonpathogenic *E. coli*

*S. aureus*

(E. coli)

(S. aureus)

*Pfmo-2::gfp*

(Wild type)

(*nhr-49(-)*; *Pnhr-49::nhr-49* (Rescue))

Wani et al; under revision
NHR-49/ PPARα is necessary and sufficient for host survival

Wani et al; under revision
HLH-30 and NHR-49 regulate host defense via fmo-2 induction
Summary

Wani et al; under revision
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fmo-2 mutants are hypersensitive to infection

Wani et al; under revision
NHR-49 gain-of-function causes *fmo-2* overexpression

**Graph:**
- Y-axis: Relative expression of *fmo-2* (fmo-2/snb-1)
- X-axis: Wild type, nhr-49(gf1)
- Data points: Wild type (gray), nhr-49(gf1) (red)
- Legend: Non-pathogenic *E. coli* (gray), S. aureus (red)

**Images:**
- *E. coli* and S. aureus under Wild type and nhr-49(gf2) conditions
- Pfmo-2::gfp fluorescence in *E. coli* and S. aureus

**Text:**
- NHR-49 gain-of-function causes *fmo-2* overexpression
- Pfmo-2::gfp fluorescence
- Relative expression comparison: Wild type vs. nhr-49(gf1)
- **Statistical significance:**
  - Wild type: S. aureus
  - nhr-49(gf1): S. aureus

**Note:**
- **Non-pathogenic E. coli**
- **S. aureus**
- **Pfmo-2::gfp** fluorescence
- **Wild type**
- **nhr-49(gf2)**
NHR-49 functions cell non-autonomously for host defense and fmo-2 induction

Intestinal rescue
- Non-pathogenic E. coli
- S. aureus
- Wild type
- nhr-49(-)
- nhr-49(-); Pgly-19::nhr-49****

Muscle rescue
- fmo-2

Neuronal rescue
- Non-pathogenic E. coli
- S. aureus
- Wild type
- nhr-49(-)
- nhr-49(-); Prgef-1::nhr-49****

Epidermis rescue
- fmo-2

Relative expression (fmo-2/snb-1)
- Wild type
- nhr-49(-)
- nhr-49(-); Pgly-19::nhr-49

Non-pathogenic
- E. coli
- S. aureus

fmo-2
- **
- ***
- **
Loss of NHR-49 is stronger than loss of HLH-30

Wani et al; under revision
HLH-30 functions downstream/parallel to NHR-49 for host defense and *fmo-2* induction
Does HLH-30 function downstream/parallel to NHR-49?
HLH-30 functions downstream/parallel to NHR-49 for host defense and fmo-2 induction

Wani et al; under revision