The Role of the Circadian Clock in Fat Body Transcriptomics and Metabolomics
Circadian Rhythms

Input Pathways

Circadian Clock

Output Pathways

Inputs

Outputs
Molecular Clock

Nucleus

Cytoplasm

E-box

per

tim

PER

TIM

PER

TIM

CYCLE

CLOCK
Central and Peripheral Clocks

Metabolism

Brain

Fat Tissue

Liver

Fat Body

Brain
Major Questions

1. How do central and peripheral clocks regulate metabolism?

2. What is the role of the fat body clock in metabolism?
Experimental Design
Targeting Brain and Fat Body Clocks

Brain
clk856-GAL4

Fat Body
to-GAL4
VSlsp-GAL4
Selectively Targeting Fat Body Clocks: to vs lsp

- to- and lsp-GAL4 both selectively target the fat body.
- lsp-GAL4 is more selective.
CRISPR-Cas9 Gene Editing

Nucleus

Cytoplasm

E-box

per

tim

CRISPR
Effectively Disrupting Fat Body Clocks: to vs lsp

CRISPR eliminates clocks most effectively when paired with to-GAL4.
Summary: Targeting and Disrupting Fat Body Clocks

<table>
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<th>lsp-GAL4</th>
<th>to-GAL4</th>
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<tbody>
<tr>
<td>Targeting</td>
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<td>✓</td>
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<td>Clock Disruption</td>
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*to-GAL4 minimally disrupts clocks outside of the fat body.

Conclusion: We will use to-GAL4 to drive CRISPR and eliminate PER expression in the fat body.
Experimental Design
Major Questions

1. How do central and peripheral clocks regulate metabolism?

2. What is the role of the fat body clock in metabolism?
Experimental Groups

1. Intact Clocks

2. KO Brain Clock

3. KO Brain Clock

4. KO Fat Body Clock

*With Induced Feeding
Activity rhythms are under the control of central brain clocks, not fat body clocks.
Sample Collection
• Every 2 hours for 48 hours

Data Acquisition
• Mass Spectrometry

Data Analysis
• Identify metabolites

Results: If certain metabolite levels change after clock elimination, their associated metabolic processes are circadian regulated.
Challenges

- Number of flies
- Bacterial infection in fly stocks (Summer – Fall, 2022)
  - Required antibiotic treatment in food
- Troubleshooting protocols

**Metabolomics Sample Size**

\[
\begin{align*}
24 \text{ collections} & \div 1 \text{ fly group} = 600 \text{ flies} \div 1 \text{ fly group} \\
4 \text{ fly groups} & \times 600 \text{ flies} \div 1 \text{ fly group} = 2,400 \text{ flies} \\
2 \text{ replicates} & \times 2,400 \text{ flies} \div 1 \text{ replicate} = 4,800 \text{ flies total}
\end{align*}
\]
Conclusions

• to-GAL4 selectively targets and effectively eliminates fat body clock function
• Our metabolomics collection protocol is optimized and scheduled for early summer

Future Directions

• **Metabolomics**: Determine what **metabolites** are impacted by loss of the circadian clock
• **Transcriptomics**:  
  • Optimize collection protocol  
  • Determine what **genes** are impacted by loss of the circadian clock
Questions?