Parallel genetic pathways contribute to epidermal structure and resistance of biomechanical force during *C. elegans* development

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Sensory Depression in Wild-type

• Wild-type embryos form a small indentation at the anterior end at 1.5-fold stage

• Transient structure
  • Usually disappears at 3-fold stage

• Occurs as pharynx elongates during early embryogenesis
  • Biomechanical force
Pharynx Ingressed (PIN) Phenotype

• Posterior displacement of pharynx and buccal capsule
• In mec-8; sym-3/4 mutants the sensory depression deepened at 3-fold stage forming keyhole structure
• Inability to withstand biomechanical pulling force
• Results in larvae that are unable to feed
• Larval lethality
Adult (1110-1150 μm) (capable of egg laying)

Young adult (900-940 μm)

L4/adult molt

L4 (820-650 μm)

L3/L4 molt

Dauer (400 μm)

L3 (490-510 μm)

L2/L3 molt

L2 (360-380 μm)

L1/L2 molt

L1 (250 μm)

Hatching

crowding

starvation

high temp

Predauer (L2d)

L1/L2d molt

up to 4 months

ex utero development (24 h)

1.5-fold

2-fold

3-fold

Comma

13 hr

8 hr

10 hr

8 hr

Gastrula (approximately 30-cell)

in utero development (150 min)

First cleavage (40 min)

eggs laid at

8 hr

IntroFig6
Extracellular Matrix

• Provides structure and protection
• Improper ECM leads to
  • Developmental disorders
  • Birth defects
  • Connective tissue disorders
• Studied in basement membranes, little is known about apical ECM (aECM)
Mec-8

- MEC-8 is highly conserved in vertebrates
- Regulates the alternative splicing of a conserved basement membrane protein
- RNAi of *fbn-1* strongly enhances Pin in sym-3, sym-4 and mec-8 mutants
Anterior morphogenesis & resistance to biomechanical forces

MEC-8 → FBN-1

SYM-3 → SYM-4
FBN-1

• Critical embryonic sheath component that prevents defects of the epidermis by actinomyosin contractions
• Shares homology to human fibrillins
  • Mutation in human FBN1 lead to Marfan Syndrome
  • Mutations in human FBN2 lead to Beals Syndrome
  • Mutations in human FBN3 linked to Waill-Marchesani Syndrome
Sym-3/4

- Both SYM-3/4 are highly conserved in vertebrates
- SYM-3
  - Associated with membrane tethering
  - Regulate endocytosis
- SYM-4
  - Controls endocytic recycling
  - Exocytosis
Methods

Grow RNAi in LB-AMP-TET media → 16 hours at 37°C → Spot plates 200 μL culture/plate → Dry 1 day → Pick worms 4-5 L4s/plate → Lay 1-2 days → Synchronize (remove adult worms)

Few L1s → Not enhancing

Observe plates → Hatch 1 day → Microscopy

Many L1s → >20% PIN for lin-35; sym-4

>40% PIN for fbn-1 → Enhancing
aECM Components

- Screen to look for likely aECM components using genes from three data sets:
  - N-glycosylated
  - Oscillating
  - Secreted
- Proteins with ZP domains, EGF-like domains, RGD domains and eLRRon domains
aECM enhancers RNAi (\textit{lin-35}; \textit{sym-4} background)
Anterior morphogenesis & resistance to biomechanical forces

- MEC-8
- FBN-1
- aECM components
- RAB-11
- SYM-3
- SYM-4
- Trafficking target(s)
aECM Enhancers RNAi (*fbn-1* background)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent PIN</th>
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<tbody>
<tr>
<td>Vector</td>
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<tr>
<td>fbn-1</td>
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<tr>
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<tr>
<td>C01G6.3</td>
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<td>Glycosylated, Oscillating, Secreted</td>
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<tr>
<td>Oscillating, Secreted</td>
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<tr>
<td>Other</td>
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Legend:
- Control
- Glycosylated, Oscillating
- Glycosylated, Oscillating, Secreted
- Oscillating, Secreted
- Other
Future Directions

• Finish screening enhancers with *fbn-1*
• Test more enhancers
• Look for expression in epidermis
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