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

1.5-Fold Stage
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
Wild type on $fbn-1$ RNAi in $fbn-1$ (ns67)

PIN on $fbn-1$ RNAi in $fbn-1$ (ns67)
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
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

Few L1s → Not enhancing

Observe plates → Hatch 1 day → Synchronize (remove adult worms)

Many L1s → Microscopy → >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 domans, EGF-like domains, RGD domains and eLRRon domains
aECM enhancers RNAi (*lin-35; sym-4* background)
Anterior morphogenesis & resistance to biomechanical forces

- **MEC-8**
- **FBN-1**
- **SYM-3**
- **SYM-4**
- **RAB-11**

aECM components

Trafficking target(s)
aECM Enhancers RNAi (\textit{fbn-1} background)

![Bar chart showing the percent PIN for different treatments.](chart.png)

- **Control**
- **Glycosylated, Oscillating**
- **Glycosylated, Oscillating, Secreted**
- **Oscillating, Secreted**
- **Other**

**Treatments:**
- Vector
- fbn-1 RNAi
- K09F6.3
- C01G6.3 RNAi
- clec-88
- ugt-25
- Iron-15
Future Directions

- Finish screening enhancers with \textit{fbn-1}
- Test more enhancers
- Look for expression in epidermis
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