Recursive and Combinatorial Signaling in C. elegans Development

**Germ Layers and Animal Evolution**

- **Ecdysoza** (e.g., nematodes, flies)
- **Lophotrochozoa**
  - **Diploblasts**
  - **Sponges**
  - **Choanoflagellates**
- **Deuterostomes**

**Germ Layers in a Vertebrate**

- **Ectoderm**: skin, nervous system
- **Mesoderm**: skeleton, muscle, etc.
- **Endoderm**: gut, lungs, etc.

Credit: W. Smith

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Quantum cosmology

*indeterminate*

Nematode development

*determinate*

*Determinacy of C. elegans development*

Sir John Sulston
Major cell types made by founder cells

Gene regulatory cascade for mesendoderm
Recursive and Combinatorial Signaling in C. elegans development
Recursive and Combinatorial Signaling in C. elegans development

**formation of the C. elegans endoderm**

- Specification
- Gastrulation
- Cell division
- Differentiation

**MED-1/2 GATAs specify mesendoderm**

- SKN-1
- Maternal
- Zygotic

**med(-)**

- "C"
- (Mesectoderm) "C"
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Mesendoderm development

END-1/3 GATAs specify endoderm

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END-1/3 specify endoderm

SKN-1
MED-1,2
END-1,3

E fate

wild type  ubiquitous

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ELT-2/7 GATAs control gut differentiation

- SKN-1 (maternal)
- MED-1,2
- END-1,3
- ELT-2,7 (differentiation)
- gut structural proteins, enzymes

properties of endoderm regulatory cascade

- zygote
- EMS
- MS
- E

- SKN-1
- MED-1,2
- END-1,3
- ELT-2,7 (E fate)

specification

differentiation
properties of endoderm regulatory cascade

Features
1. Redundancy (GATA)
2. Cell division
3. Dedicated functions

MED-1 defines a novel GATA class

consensus

<table>
<thead>
<tr>
<th>GATA factor</th>
<th>H G A T A R</th>
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<tbody>
<tr>
<td>MED-1</td>
<td>A A G T A T A C</td>
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</tbody>
</table>
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### fourteen putative MED targets

<table>
<thead>
<tr>
<th>gene</th>
<th>product</th>
<th>MED sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>F58E10.2</td>
<td>end-1</td>
<td>** **</td>
</tr>
<tr>
<td>F58E10.5</td>
<td>end-3</td>
<td>** ** **</td>
</tr>
<tr>
<td>F35H8.7</td>
<td>wee-1.1</td>
<td>** **</td>
</tr>
<tr>
<td><em>ceh-20/F31E3.2</em></td>
<td>homeobox</td>
<td>** **</td>
</tr>
<tr>
<td>F58G4.4</td>
<td>LAG-2-like</td>
<td>** **</td>
</tr>
<tr>
<td>C32E12.5</td>
<td>Sox family (HMG)</td>
<td>** **</td>
</tr>
<tr>
<td>ZK849.2</td>
<td>RCC1</td>
<td>** **</td>
</tr>
<tr>
<td>T07D1.2</td>
<td>unknown</td>
<td>** **</td>
</tr>
<tr>
<td>ZK177.10, ZK177.1</td>
<td>T-box/unknown</td>
<td>** ** **</td>
</tr>
<tr>
<td>T11A5.5</td>
<td>oxygen transport</td>
<td>** **</td>
</tr>
<tr>
<td>C17C3.7, C17C3.10</td>
<td>bHLH (2)</td>
<td>** **</td>
</tr>
<tr>
<td>B0303.8, 9</td>
<td>unknown</td>
<td>** ** **</td>
</tr>
</tbody>
</table>

### correlation with embryonic transcriptome

5/12 detected

Baugh et al., 2003
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sox-1::GFP in E, MS descendants

Asymmetric cell division

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P2 induces E fate

EMS

MS fate

E fate

P2 induces E fate

EMS

MEDs

POPs

MEDs

ENDs

MS fate

E fate

Wnt

MAPK

POPs

POPs
Models for POP-1 repression

**MS**

**MED displacement**

**MED inhibition**

*in vivo* detection of protein-DNA interactions

**fusion protein**

GFP

transcription factor

**target array**
MED-1 binds end-3 promoter

specification of E and MS

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recursive POP-1 asymmetry

Lin et al. (1998) Cell 92, 229–239

dynamics of POP-1

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**coalescence of POP-1**

**identification of PLP-1**

- LG VR
- end-1/LG VR
- end-3
- Lef-1 site
- PLP-1 = pur alpha
- POP-1

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asymmetry of nuclear PLP-1

POP-1 and PLP-1 in asymmetry

A ↔ POP-1 → P

POP-1

PLP-1 (initial)
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1. P$_2$ induces E fate
   - Wnt
   - MAPK

2. MAP kinase pathway and PLP-1
   - mom-4(-)
   - lit-1(-)
   - mom-4(-);lit-1(-)

MOM-4 -> LIT-1 -> Nuclear PLP-1
**RNAi screen**

dsRNA → “instant gene knockout”

feed bacteria expressing dsRNA

gene-wide screen (~19,000 genes)

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**RNAi library screen**

worms

mutant progeny (+2 days)

E. coli expressing dsRNA
Reduced end expression results in excess gut cells

Wildtype

Normal end-1,3

E

Weak end-1/3, skn-1, med-1/2, plp-1

Reduced end-1,3

20 cells

22~32 cells

Pattern of gut cells in C. elegans
### RNAi screen summary

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Lethal</th>
<th>Viable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (248)</td>
<td>%</td>
</tr>
<tr>
<td>I slight excess</td>
<td>13 5%</td>
<td>7 0.3%</td>
</tr>
<tr>
<td>II large excess</td>
<td>9 3%</td>
<td>0 0%</td>
</tr>
<tr>
<td>III subnormal</td>
<td>0 0%</td>
<td>4 0.2%</td>
</tr>
<tr>
<td>IV faint express.</td>
<td>1 0.4%</td>
<td>37 1.7%</td>
</tr>
<tr>
<td>V no expression</td>
<td>12 5%</td>
<td>0 0%</td>
</tr>
<tr>
<td>VI abn. pattern</td>
<td>37 15%</td>
<td>8 0.4%</td>
</tr>
</tbody>
</table>

#### Research Group

![Image of the research group]
Recursive and Combinatorial Signaling in C. elegans development
Recursive and Combinatorial Signaling in *C. elegans* development

**RNAi screen/gut number**

Leukena Cheam  
Isabella Mengarelli

James Vang

Not pictured: Cricket Wood

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