Interactions between D/V and A/P patterning revealed in a 3D atlas of blastoderm gene expression

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3D gene expression imaging

- \textit{In situ}–hybridization
  - Anti-sense RNA-probes, Tyramide Signal Amplification
  - Sytox labeled DNA
- 2-photon fluorescent confocal microscopy
1. Segment individual nuclear volumes based on Sytox intensity

2. Record 3D location and expression levels associated with each nucleus.

<table>
<thead>
<tr>
<th>id, x, y, z, Vn, Vc, Sytox, Cy3_n,...</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 102.36, 142.14, 112.00, 207.96, 605.36, 52.18, 23.55,...</td>
</tr>
<tr>
<td>2, 264.63, 172.01, 79.36, 281.73, 599.90, 82.12, 31.67,...</td>
</tr>
<tr>
<td>3, 225.91, 174.99, 88.65, 185.79, 418.35, 85.32, 35.63,...</td>
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<td>...</td>
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</tbody>
</table>
D/V variations in the A/P patterning system

1. A/P gene expression levels vary around D/V circumference and change over time

2. Nuclear density varies in both A/P and D/V

3. Changing morphology interacts with spatial patterns of gene expression
d/v patterning of ftz expression levels

Intensity of ftz stripes (averaged over 143 embryos)

azimuthal angle

dorsal ventral dorsal dorsal ventral dorsal
d/v expression levels of ftz, eve, prd, slp1 stripes

stripe #
1  2  3  4  5  6  7

ftz (143 embryos)

eve (183 embryos)

prd (17 embryos)

slp1 (21 embryos)
Relative expression of *hb* and *eve* vary along the d/v coordinate.
Movement of anterior *ftz* boundaries

<table>
<thead>
<tr>
<th>Stage</th>
<th>Percentage</th>
<th>Number of Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>4-8%</td>
<td>44 embryos</td>
</tr>
<tr>
<td>Mid</td>
<td>26-50%</td>
<td>42 embryos</td>
</tr>
<tr>
<td>Late</td>
<td>76-100%</td>
<td>57 embryos</td>
</tr>
</tbody>
</table>
Movement of *Kr* expression pattern boundaries

- **Early:** 4-8% (11 embryos)
- **Mid:** 26-50% (10 embryos)
- **Late:** 76-100% (15 embryos)
D/V variations in the A/P patterning system

1. A/P gene expression levels vary around D/V circumference and change over time

2. Nuclear density varies in both A/P and D/V

3. Changing morphology interacts with spatial patterns of gene expression
0% membrane invagination... ...100% membrane invagination
Pre-gastrulation nuclear movements
dorsal view
Density change verified with live imaging

- **fixed embryos**
  - early stage 5
  - anterior
  - posterior

- **live embryos**
  - late stage 5
  - anterior
  - posterior

- dorsal
- ventral
Pattern Dynamics

Nuclear movement

Changing expression
D/V variations in the A/P patterning system

1. A/P gene expression levels vary around D/V circumference and change over time

2. Nuclear density varies in both A/P and D/V

3. Changing morphology interacts with spatial patterns of gene expression
$gd7$ mutant (dorsalized)

wildtype

toll$^{10B}$ mutant (ventralized)
ftz stripe locations in $gd^7$ (dorsalized)
ftz stripe locations in *toll^{10B}* (ventralized)
Conclusions

• A large dataset of quantitative 3D expression patterns and morphology

• A/P gene expression levels vary around D/V circumference and change over time

• Morphology is dynamic even prior to gastrulation and affects the patterns expression we observe
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http://bdtnp.lbl.gov