Differential mechanical coupling between cardioblasts guides heart morphogenesis

Timothy Saunders
Cell matching is essential in development


https://www.youtube.com/watch?v=A9zLKmt2nHo
Drosophila cardiogenesis as simple matching system

Mismatched hearts have beating defects
Cell matching is an active process
Quantifying cell matching

Mis-Matched Contact Length

\[ \frac{\text{Mis-Matched Contact Length}}{\text{Total Contact Length}} = \text{Mismatch} \]
Quantifying cell matching
Cell matching robust at boundary of different cell types
Cell matching imprecise without different cell types
Filopodia are selectively binding to distinct cell types

Hand>moesin-GFP
Svp heart cells have weaker filopodia binding

Svp\textgreater\text{moesin-GFP}

So what is the underlying mechanism?
Screen focusing on known neural cell matching molecules

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>shg</td>
<td>DE-cadherin</td>
</tr>
<tr>
<td>CadN</td>
<td>DN-cadherin</td>
</tr>
<tr>
<td>Nrt</td>
<td>Neurotactin</td>
</tr>
<tr>
<td>Nrg</td>
<td>Neuroglian</td>
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<tr>
<td>Con</td>
<td>Connectin</td>
</tr>
<tr>
<td>Fas1</td>
<td>Fascilin I</td>
</tr>
<tr>
<td>Fas2</td>
<td>Fascilin II</td>
</tr>
<tr>
<td>Fas3</td>
<td>Fascilin III</td>
</tr>
<tr>
<td>Ten-m</td>
<td>Tenascin major</td>
</tr>
<tr>
<td>Ten-a</td>
<td>Tenascin accessory</td>
</tr>
<tr>
<td>Dscam 1</td>
<td>Down syndrome cell adhesion molecule 1</td>
</tr>
<tr>
<td>Pvf3</td>
<td>PDGF- and VEGF-related factor 3</td>
</tr>
</tbody>
</table>
Fas3 is involved in neuronal cell matching

No observed phenotype in null mutant

Chiba et al. Nature 1995
Altering Fas3 expression effects filopodia contact time

Svp>UAS-moesin-GFP

Fas3 up-regulation

Svp> UAS-moesin-GFP
UAS-Fas3
How does the Fas3 expression pattern affect cell matching?
Ten-m is expressed in a complementary fashion to Fas3
Fas3 / Ten-m double mutant has severe matching defects
**Wild-type Embryos**

- Tin-positive CBs initiate contacts

**Embryos with reduced Fas3 expression in the heart**

- Svp-positive CBs initiate contacts

**Partly-matched Heart**

- Weak Adhesion Unstable and Seperated Filopodia Contacts (caused by low expression of both Fas3 and Ten-m) (mediated by Ten-m when losing Fas3 competition)

**Well-matched Heart**

- Strong Adhesion Stable and Extented Filopodia Contacts (mediated by strong Fas3 expression)

- Weak Adhesion Unstable and Seperated Filopodia Contacts (potentially caused by binding competition)

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**Legend**

- Tin-positive CB
- Svp-positive CB
- Filopodia
- Fas3
- Ten-m

**Zhang et al. Developmental Cell (2018)**
Is differential adhesion sufficient to ensure precise cell matching?

\[ E_a = K (L_{cell} - L_{0,a})^2 - (\epsilon_{aa} \cdot x + \epsilon_{ab} \cdot y) \]
\[ E_b = K (L_{cell} - L_{0,b})^2 - (\epsilon_{bb} \cdot x + \epsilon_{ab} \cdot y) \]

Tlili et al. Biorxiv 2019 (653535)
Cell matching consistent with an equilibrium energy approach

\[ E_a = K(L_{cell} - L_{0,a})^2 - (\epsilon_{aa} \cdot x + \epsilon_{ab} \cdot y) \]
\[ E_b = K(L_{cell} - L_{0,b})^2 - (\epsilon_{bb} \cdot x + \epsilon_{ab} \cdot y) \]

Equilibrate and measure matching

Input cell sizes

Equilibrium energy approach

Mismatch

Svp cells

Tin cells

Cell size (\(\mu m\))

0 5 10 15 20

Svp cells

Tin cells

Cell number

0 20 40 60 80 100
Cell matching consistent with an equilibrium energy approach

\[ E_a = K(L_{cell} - L_{0,a})^2 - (\epsilon_{aa} \cdot x + \epsilon_{ab} \cdot y) \]
\[ E_b = K(L_{cell} - L_{0,b})^2 - (\epsilon_{bb} \cdot x + \epsilon_{ab} \cdot y) \]

Behaviour depends on

\[ \gamma = \frac{\epsilon_{TT} - \epsilon_{ST} + (\epsilon_{SS} - \epsilon_{ST})}{K} \]

Potts model

\[ \epsilon_{TT}/K \]

\[ \epsilon_{SS}/K \]
Mismatch in wild-type and deformed hearts

Cell alignment best at boundaries between cell types

Alignment consistent with observed cases of cardioblast number variation

Tlili et al. Biorxiv 2019 (653535)
Coupling between waves of Myosin-II and filopodia adhesion regulates cell matching
Known waves of Myosin-II in cardioblasts during matching

**Drosophila**

Zipper: Myosin II heavy chain  
Squash: Myosin II light chain

What is the function of this wave?

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Vogler et al., JCB 2014
Myosin-II waves have period ~ 4 minutes

Zipper::GFP  Hand>Moesin::mCherry

Interval Time : 260 ± 45 s
Counterbalance of filopodia adhesion and Myosin II

Weak adhesion
- Broken contacts
- Retracted filopodia

Strong adhesion
- Contacts stabilise
- Drives cells matching

Actin-Dependent Stabilisation

Myosin-II Dependent Contraction
Filopodia retraction correlates with Myosin II appearance at cell leading edge.
Reduction in Myosin-II results in reduced cell matching
Strong force between filopodia in wildtype hearts

Hand>Moe::GFP

with Y. Toyama
Strong force between filopodia in wildtype hearts

Hand>Moe::GFP
Force between filopodia reduced in Zip knockdown

Hand>Moe::GFP; Zip-RNAi
Force between filopodia reduced in Zip knockdown

Hand>Moe::GFP; Zip-RNAi
Force between filopodia reduced in Zip knockdown

Instantaneous Recoil Velocity

\[ \text{RV}_{\text{Filopodia}} = 0.15 \pm 0.04 \mu \text{m/s} \]

\[ \text{RV}_{\text{Zip-RNAi}} = 0.10 \pm 0.07 \mu \text{m/s} \]

\[ \text{RV}_{\text{Gap}} = 0.02 \pm 0.03 \mu \text{m/s} \]
Myosin II over-activation also reduces cell matching

Suggests levels of Myosin are optimised to enable precise cell matching
Mechanical testing of cellular interactions

Weak adhesion
- Broken contacts
- Retracted filopodia

Strong adhesion
- *Some* contacts stabilise
- Drives precise cells matching

Extra strong adhesion
- Contacts *too* stable
- Unable to correct for mistakes in matching

Cdc42

Myosin-II waves

Filopodia adhesions

Cdc42

Cell Mismatch

Reduced Myosin-II activity

Wildtype

Myosin-II over-activation

Effective Adhesion Force
Acknowledgements

Current lab
Jason Lai
Tricia Loo
Mario Mendieta
Sunandan Dhar
Shaobo Zhang
Prabhat Tiwari
Vaishali Yadav
Veena Venugopal

Former lab members
Chris Amourda
Jeronica Chong
Anqi Huang
Veena Venugopal

Collaborators
David Garfield (Humboldt)
Martin Loose (Austria)
Enrique Martin-Blanco (Spain)
Jose Munoz (Spain)
Ivo Telley (Portugal)
Yusuke Toyama (MBI)
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