Serotonin Signaling and Embryogenesis:
Physiological controls of Development

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Outline

• Introduction to meaning of organ asymmetry in embryos

• Basic facts about organ asymmetry with explanation

• Clinical significance of asymmetry

• How asymmetry is set-up
  • Physiological early mechanisms

• Summary
Knowing communication and patterning during development is important.

Answers critical questions of form and shape in developmental patterning of tissues and organs.

- Regenerative Medicine
Determining Left-Right asymmetry is a difficult problem!

Left-Right asymmetry is defined as a consistent difference across the midline axis (not developmental noise)

There are two problems to solve.

# Cells need to know their left side and right side.

# Cells need to know whether they are on the left side of the embryo/midline or the right side
Human Laterality
Brain, Heart and Viscera are consistently asymmetrical in all normal individual
Ways that body asymmetry can go wrong:

1. Situs solitus - normal situs - all organs on their correct side.

2. Situs inversus - complete mirror image reversal - all organs on the opposite side.
1) **Primary laterality syndromes** - *situs inversus totalis*, heterotaxia, isomerism affect more than 1 in 6,000 babies born to term. *Situs inversus totalis* involves no serious medical problems. Other laterality disturbances do.

2) Laterality defects accompany some other syndromes (holoprosencephaly, short-rib polydactily and renal-hepatic-pancreatic dysplasia syndromes).

3) Some syndromes have a unilateral presentation in tissues which normally have no asymmetric characteristics (e.g., cleft lip, and Holt-Oram syndrome, which results in left-sided upper limb malformations).

4) Reversed cerebral asymmetry is associated with breast cancer.

**Clinical implications of LR asymmetry:**


Asymmetry set up

Early events

Asymmetric gene cascade morphogenesis

Earliest coordination of LR asymmetry with DV and AP axes involving subcellular proteins (i.e. INV protein) possibly in only few cells (i.e. streak or Koller’s sickle in chick)

Processing of Vg-1 protein (Xenopus)

Nodal (lateral mesoderm of all species)

Other right-sided molecules?

Snail-related (chick)

activinβB

activinRLIα

Asymmetric morphogenesis

(modified after reviews by Yost, and Burdine & Schier)

ORGANOGENESIS
Understanding how Left-Right asymmetry is established

1. Organ Asymmetry – Only thing known until Mid-1990s
2. Amplification steps that set up organ asymmetry
3. Gradients of determinants (serotonin) that set up amplification steps
4. Bioelectrical signals that set up the gradients of determinants
5. Cytoskeletal chirality that sets up bioelectrical gradient

Development Time

Embryo
The last phase = organogenesis

Differential cues on L vs. R sides give rise to asymmetric organs via different migration, proliferation, and tensile forces
Amplification step: Asymmetric gene expression requires knowledge of L-R position with respect to the embryo;

Going from cells’ knowing which direction is L and R, to cells’ knowing which side of the midline L or R they are on

Asymmetric gene expression needs global position. L and R side must communicate at early stages

Cells communicate using Gap junctions to decide L-R identity

Neurons and synapses evolved by specializing these functions, first arising in somatic cells.
A unidirectional flow of small molecules through GJs causes a net L-R asymmetric gradient across midline. This asymmetric accumulation induces asymmetric gene expression – Amplification step.

Important questions:
1] How is a unidirectional flow maintained?
2] What is it that flows through Gap Junctions?

Developmental Bioelectricity:

slow, steady ion fluxes, electric fields, and **voltage gradients** endogenously generated and sensed by all cell types

(not the rapid action potentials in classical excitable cells nor effects of environmental electromagnetic exposure)

**Think this:**

| ![Image](image1.png) | ![Image](image2.png) |

| ![Image](image3.png) | ![Image](image4.png) |

**Not these.....**

**Not these:**
$V_{\text{mem}}$ functionally determines cell behavior in many morphogenetic contexts.


(after Binggeli and Weinstein, 1986)
Ion-pumps establish L-R voltage gradient across embryo


Serotonin: a neurotransmitter with many roles

5-Hydroxy Tryptamine – 5-HT (Serotonin)
Positively Charged

Total levels of maternal serotonin during embryogenesis
Maternal serotonin (5-HT) forms a voltage- and GJ-dependent rightward gradient in the 16-cell embryo


Serotonin triggers L-R asymmetric gene expression – Amplification steps

Ion pumps + GJs and unidirectional flow of 5-HT allows asymmetries generated level at single cell to be imposed on large multicellular fields in embryo.
A unified model of LR pattern formation

Fertilization and 1st cleavage allow LR computation to be made by a chiral component (cytoskeleton?)

This leads to asymmetric localization of ion channels and pumps

Differential ion exchange with the outside world leads to a LR voltage gradient

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This leads to asymmetric localization of ion channels and pumps

Differential ion exchange with the outside world leads to a LR voltage gradient

DV-patterning pathways (Wnts, etc.) set up a large-scale GJC region surrounding a zone of isolation. Small molecule determinants are randomly distributed.

The existing voltage difference electrophoreses charged small molecule determinants in a preferred direction, subject to gap junctional selectivity.

Asymmetric gene expression driven by serotonin signaling

Early asymmetric markers initiate cascade of asymmetric signaling molecules which in turn guide the chiral morphogenesis of the viscera

Instructive Shape Information Resides in Bioelectrical Networks among all Cell Types

+ other layers (mechanical forces, etc.)
Bioelectric Prepattern Determines Transcriptional Domains during craniofacial morphogenesis

Dany Adams

Physiological Prepattern underlying (controlling) gene expression:

Pax6, Frizzled, Wnts, BMPs

Genetic Prepattern underlying (controlling) subsequent morphology

Transmembrane voltage reporter dye during craniofacial patterning:

O’Donnell et al., 2006

Endogenous $V_{\text{mem}}$ distribution

Change $V_{\text{mem}}$ pattern using ANY channel

Altered gene expression

Altered craniofacial anatomy

Frog embryo’s “Electric Face” as prepattern
Bioelectric signals also sculpt planarian and human faces

Control RNAi

H,K-ATPase RNAi

normal head

head with wrong proportions

Control Intact

normal head bioelectric pattern

Mutated Kir2.1 channel causes Andersen-Tawil Syndrome in human babies

Showing low set ears, micrognathia and retrognathia
Reinforcing bioelectrical signals rescues active notch-induced brain defects
Bioelectric signals can triggering regeneration of complex structures

Outgrowth with distal patterning induced (and still growing)

Hind-leg amputation

The regenerated leg has both sensation and mobility:
Implications

Serotonin and ion channels have important functions in development

1) Careful of drugs - SSRIs, anti-epileptics, anti-arrythmics during pregnancy

2) Opportunity for electroceuticals --- To fix defects
Thank you to:

Principal Investigator: Michael Levin

Post-docs:
Gary McDowell – Establishment of left-right asymmetry
Célia H-R, and Justin Guay - apoptosis, sodium flux, tail and appendage regeneration
Junji Morokuma - gap junctions and planarian stem cells
Douglas Blackiston - melanocytes and K⁺ channels
Vaibhav Pai - ion flux and eye/brain induction, dynamics of bioelectrical networks
Daniel Lobo - symbolic modeling of regeneration
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Douglas Blackiston - melanocytes and K⁺ channels
Vaibhav Pai - ion flux and eye/brain induction, dynamics of bioelectrical networks
Daniel Lobo - symbolic modeling of regeneration

Students:
Fallon Durant – V_{mem} and planaria regeneration
Brook Chernet – V_{mem} and oncogene-mediated tumor formation
Maria Lobikin - V_{mem} as a regulator of metastasis

Technical assistance:
Rakela Lubonja - lab manager
Erin Switzer - animal husbandry
Joan Lemire, Jean-Francois Pare - molecular biology

Collaborators:
Dany Adams - V-ATPase in asymmetry, regeneration, craniofacial patterning
Christopher Martyniuk – microarray and gene analysis
David Kaplan - human MSC differentiation and biodome sleeves
Douglas Brash - cancer
Paul C. W. Davies - top-down causation models
John Y. Lin - optogenetics control of V_{mem}

Model systems: tadpoles, worms, zebrafish, and chick embryos

Reagents:
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Thomas Knopfel, M. Montero-Lomeli, M. Lu - ion transporter constructs

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Inspiration: Lionel Jaffe, a pioneer of bioelectricity
time for questions
Cytoskeletal direction orients asymmetric placement of ion pumps.


Bilateral gynandromorphs suggest midline is linked to first cleavage
Ion channel/pump oncogenes

Ion channels are implicated by genetic screens

channelopathies
(huge under-estimate due to compensation/redundancy)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Morphogenetic role or LOF phenotype</th>
<th>Species</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>TMEM16A chloride channel</td>
<td>Tracheal morphogenesis</td>
<td>Mouse</td>
<td>(Rock et al., 2008)</td>
</tr>
<tr>
<td>Kir7.1 potassium channel</td>
<td>Melanosomes development</td>
<td>Zebrafish</td>
<td>(Iwashita et al., 2006)</td>
</tr>
<tr>
<td>Cx41.8 gap junction</td>
<td>Pigmentation pattern</td>
<td>Zebrafish</td>
<td>(Watanabe et al., 2006)</td>
</tr>
<tr>
<td>Cx45 gap junction</td>
<td>Cardiac defects (cushion patterning)</td>
<td>Mouse</td>
<td>(Kumai et al., 2000; Nishii et al., 2001)</td>
</tr>
<tr>
<td>Cx43 gap junction</td>
<td>Fin regeneration</td>
<td>Zebrafish</td>
<td>(Hoptak-Solga et al., 2008)</td>
</tr>
<tr>
<td>Cx43 gap junction</td>
<td>Oculodentodigital dysplasia (ODDD), heart defects (outflow tract and conotruncal), left-right asymmetry</td>
<td>Human, mouse</td>
<td>(Britz-Cunningham et al., 1995; Debeer et al., 2005; Ewart et al., 1997; Pizzuti et al., 2004; Reaume et al., 1995)</td>
</tr>
<tr>
<td>Kir2.1 potassium channel</td>
<td>Wing patterning</td>
<td>Drosophila</td>
<td>(Dahal et al., 2012)</td>
</tr>
<tr>
<td>Cx43 gap junction</td>
<td>Fin size and pattern regulation</td>
<td>Zebrafish</td>
<td>(Davy et al., 2006; Lovine et al., 2005; Sims et al., 2009)</td>
</tr>
<tr>
<td>Cx43 gap junction</td>
<td>Osteoblast differentiation in bone patterning</td>
<td>Mouse</td>
<td>(Civitelli, 2008)</td>
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<tr>
<td>Kir2.1 potassium channel</td>
<td>Craniofacial morphogenesis (Andersen-Tawil syndrome) and limb patterning</td>
<td>Mouse</td>
<td>(Bendahhou et al., 2003; Dahal et al., 2012)</td>
</tr>
<tr>
<td>CFTR chloride channel</td>
<td>Bilateral absence of vas deferens</td>
<td>Human</td>
<td>(Uzun et al., 2005; Wilschanski et al., 2006)</td>
</tr>
<tr>
<td>Girk2 potassium channel</td>
<td>Cerebellar development</td>
<td>Mouse</td>
<td>(Hatten et al., 1986; Patil et al., 1996; Rakic and Sidman, 1973a, b)</td>
</tr>
<tr>
<td>GABA-A receptor (chloride channel)</td>
<td>Craniofacial patterning (Angelman Syndrome, cleft palate) and limb defects</td>
<td>Mouse</td>
<td>(Culiat et al., 1995; Homanics et al., 1997; Miller and Becker, 1975; Wee and Zimmerman, 1985)</td>
</tr>
<tr>
<td>KCNH2 K⁺ channel</td>
<td>Cardiac patterning</td>
<td>Mouse</td>
<td>(Teng et al., 2008)</td>
</tr>
<tr>
<td>NHE2 Na⁺/H⁺ exchanger</td>
<td>Epithelial patterning</td>
<td>Drosophila</td>
<td>(Simons et al., 2009)</td>
</tr>
<tr>
<td>V-ATPase proton pump</td>
<td>Wing hair patterning</td>
<td>Drosophila</td>
<td>(Hermle et al., 2010)</td>
</tr>
<tr>
<td>KCNQ1 potassium channel</td>
<td>Abnormalities of rectum, pancreas, and stomach</td>
<td>Mouse</td>
<td>(Than et al., 2013)</td>
</tr>
<tr>
<td>KCNQ1 potassium channel</td>
<td>Inner ear defects - Jervell and Lange-Nielsen syndrome</td>
<td>Human, mouse</td>
<td>(Casimiro et al., 2004; Chouabe et al., 1997; Rivas and Francis, 2005)</td>
</tr>
<tr>
<td>Kir6.2 potassium channel</td>
<td>Craniofacial defects</td>
<td>Human</td>
<td>(Glynn et al., 2004)</td>
</tr>
<tr>
<td>NaV channels</td>
<td>Spina bifida, heart, CNS, and neck defects</td>
<td>Human</td>
<td>(Fonager et al., 2000)</td>
</tr>
<tr>
<td>NaV 1.5, Na⁺/K⁺-ATPase</td>
<td>Cardiac morphogenesis</td>
<td>Zebrafish</td>
<td>(Chopra et al., 2010; Shu et al., 2003)</td>
</tr>
<tr>
<td>Innexin gap junctions</td>
<td>Foregut development, cuticle (epithelial) patterning</td>
<td>Drosophila</td>
<td>(Bauer et al., 2002; Bauer et al., 2004)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ion Translocator Protein</th>
<th>Species</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>NaV1.5 sodium channel</td>
<td>Human</td>
<td>(House et al., 2010b; Onkal and Djamgoz, 2009)</td>
</tr>
<tr>
<td>EAG-1 potassium channel</td>
<td>Human</td>
<td>(Pardo et al., 1999)</td>
</tr>
<tr>
<td>KCNK9 potassium channel</td>
<td>Mouse</td>
<td>(Pei et al., 2003)</td>
</tr>
<tr>
<td>Ductin (proton V-ATPase component)</td>
<td>Mouse</td>
<td>(Saito et al., 1998)</td>
</tr>
<tr>
<td>SLC5A8 sodium/butyrate transporter</td>
<td>Human</td>
<td>(Gupta et al., 2006)</td>
</tr>
<tr>
<td>KCNE2 potassium channel</td>
<td>Mouse</td>
<td>(Roepke et al., 2010)</td>
</tr>
<tr>
<td>KCNQ1 potassium channel</td>
<td>Human, mouse</td>
<td>(Lee et al., 1997; Than et al., 2013; Weksberg et al., 2001)</td>
</tr>
<tr>
<td>SCN5A voltage-gated sodium channel</td>
<td>Human</td>
<td>(House et al., 2010a)</td>
</tr>
<tr>
<td>Metabotropic glutamate receptor</td>
<td>Mouse, Human</td>
<td>(Martin et al., 2012; Song et al., 2012; Speyer et al., 2012)</td>
</tr>
</tbody>
</table>
If cilia initiate asymmetry de novo, and are a widely-relevant, global LR symmetry compass, how good is the fit to actual data?

<table>
<thead>
<tr>
<th>Experimental question:</th>
<th>cilia model predicts:</th>
<th>cytoplasmic model predicts:</th>
<th>chromatid segregation model predicts:</th>
<th>experimental result:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Should mutation of kinesin, dynemin, MTOC, and PCP proteins randomize LR?</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should viscosity changes at the node randomize LR?</td>
<td>YES</td>
<td>no, unless cilia amplify</td>
<td>no, unless cilia amplify</td>
<td>YES</td>
</tr>
<tr>
<td>Should chick embryos have asymmetric gene expression before node forms?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should frog embryos establish asymmetric gradients long before cilia form?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should disruption of cytoskeleton or of physiological asymmetries only during the first couple of cleavages randomize LR?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should chick node receive LR information from lateral tissue?</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Should embryos (human &amp; newt) randomize if split at the 2 cell stage?</td>
<td>NO</td>
<td>YES</td>
<td>yes</td>
<td>YES(^1)</td>
</tr>
<tr>
<td>Should the brains of primary ciliary dyskinesia patients have normal laterality?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should any mutants exist with abnormal ciliary flow but normal LR asymmetry?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should animals and plants with no cilia and no node be able to establish asymmetry using some of the same molecules as ciliated vertebrates?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Should organizers induced past the first few cleavages be randomized?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin of asymmetry:</th>
<th>Centriole + PCP</th>
<th>Centriole + PCP</th>
<th>Mitotic apparatus + PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification by:</td>
<td>Cilia-driven fluid flow in node during gastrulation</td>
<td>Intracellular localization of bioelectric machinery at cleavage stages</td>
<td>PCP-aligned differential chromatid segregation</td>
</tr>
</tbody>
</table>

So,

Many of the mouse genetic studies do not distinguish among the two models. Cilia definitely appear to do something, perhaps a parallel, later pathway?
New Concepts, Tools
(next steps for us)

• Target morphology encoding in bioelectrical networks as a true memory (incorporating ideas from cognitive science)

• Spontaneous symmetry-breaking in bioelectrical networks (modeling of self-organizing voltage patterns in tissues for guided self-assembly in synthetic biology)

• Optogenetics for non-excitable cells - cracking the bioelectric code by reading/writing electrical patterns in vivo

• Biomedical applications
  • cancer diagnosis, suppression
  • wearable bioreactors for rat limb regeneration

• AI tools for a new Bioinformatics of Shape