Programming Microphysiological Systems for Children's Health Protection

Thomas B. Knudsen, PhD
Developmental Systems Biologist
US EPA, National Center for Computational Toxicology
Chemical Safety for Sustainability Research Program
Virtual Tissue Models (VTM) project
knudsen.thomas@epa.gov
ORCID 0000-0002-5036-596x

Society for Experimental Biology and Medicine
Symposium: Progress Toward Adoption of Microphysiological Systems in Biology and Medicine
Chicago, April 24, 2017

DISCLAIMER: The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the US EPA

Drivers for Innovation

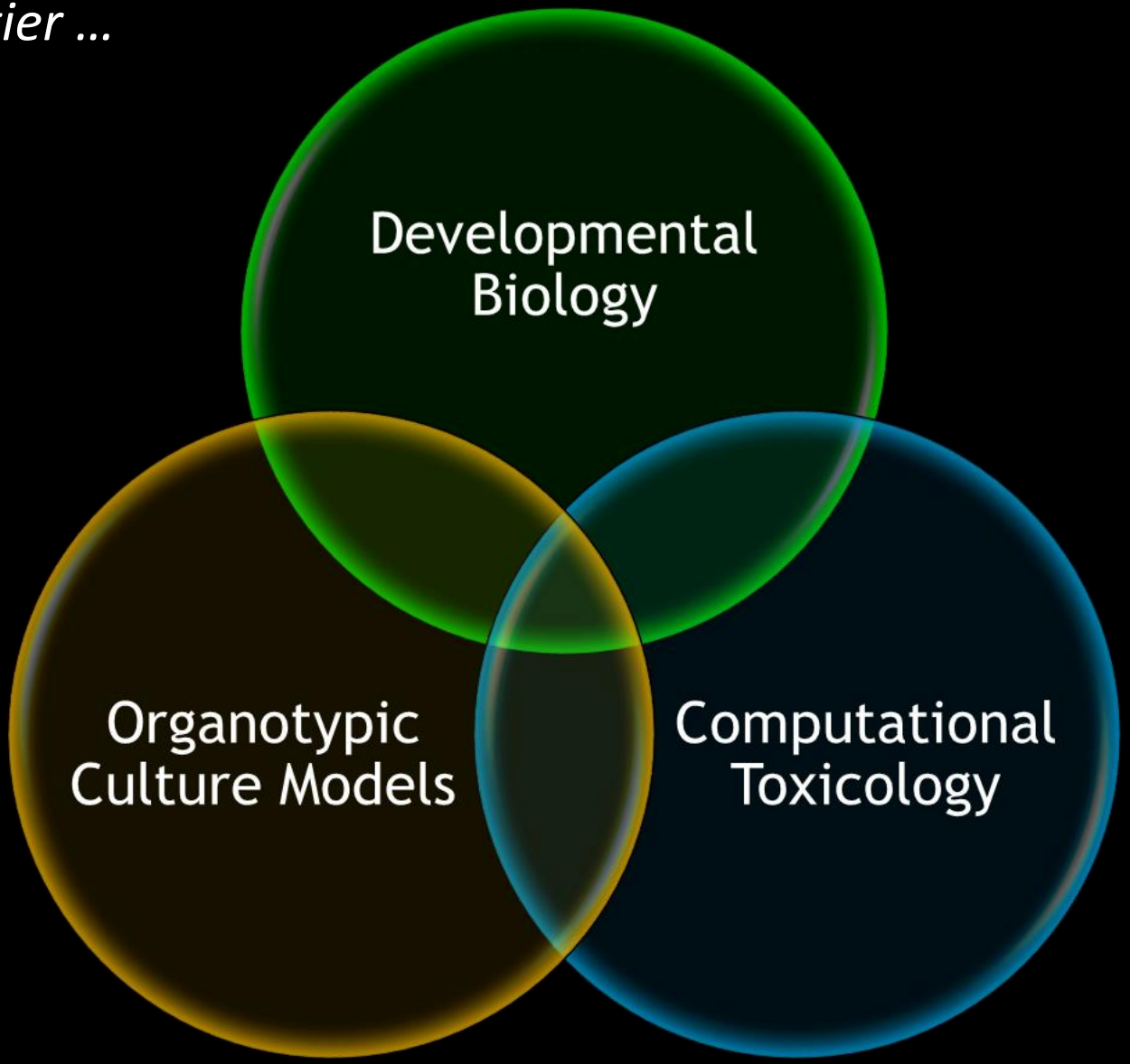
- Chemical regulation challenged by >85,000 chemicals listed on EPA's inventory under TSCA (Toxic Substances Control Act.
  - current animal-based methods do not scale to the problem

- Automated high-throughput screening (HTS) assays now providing vast *in vitro* data streams for predictive toxicology.
  *- **computational models needed to translate HTS data into human physiology***

- TSCA reform (2016) explicitly requires consideration of impacts to pregnant women and children as susceptible populations.
  *- **complexity of human development poses a critical challenge for translation***

http://www.ncats.nih.gov/

Predictive Toxicology: *the final frontier …*

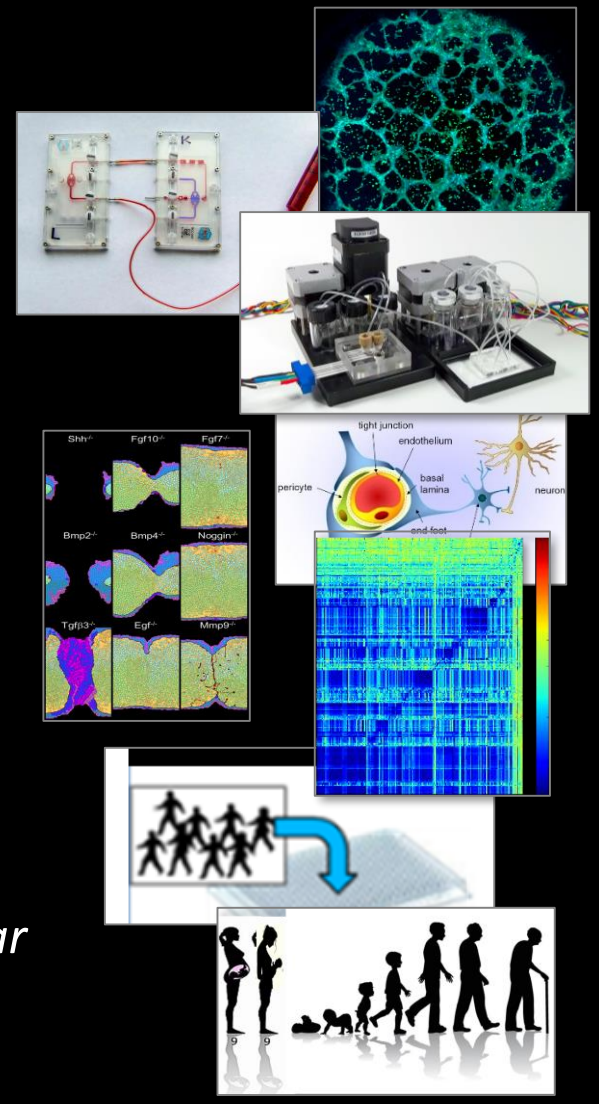


Hypothesis-based testing: engineered human micro-tissues, miniorganoids, and microphysiological systems can support children's health protection research and development:

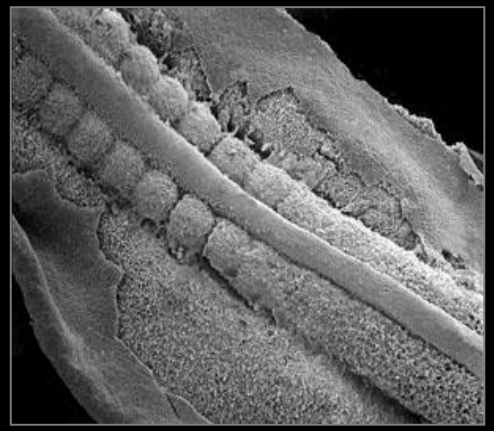
- **organotypic culture models (OCMs) -** *novel tools to predict developmental toxicity in a human system at a more physiological level than possible with conventional embryonic stem cell culture models;*

- **adverse outcome pathways (AOPs) -** *many human birth defects are mechanistically-linked to critical processes in the embryo such as tissue fusion, epithelial-mesenchymal transition, vascularization, biomechanical shaping, …*

- **virtual tissue models (VTMs) -** *provide empirical data for reconstructing cellular dynamics in computer models of the embryo that can be used to simulate adaptive and adverse responses in a dose-time series relationship.*



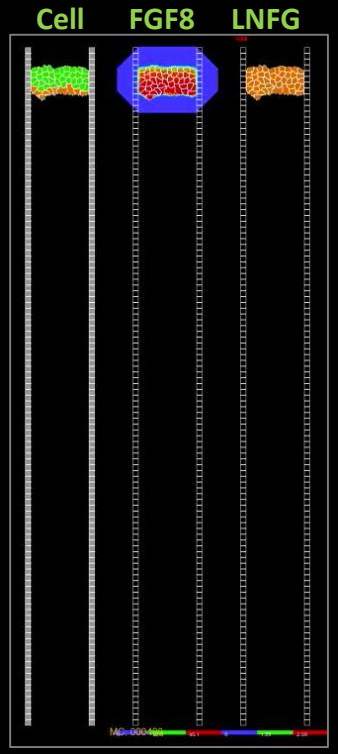
Somite formation



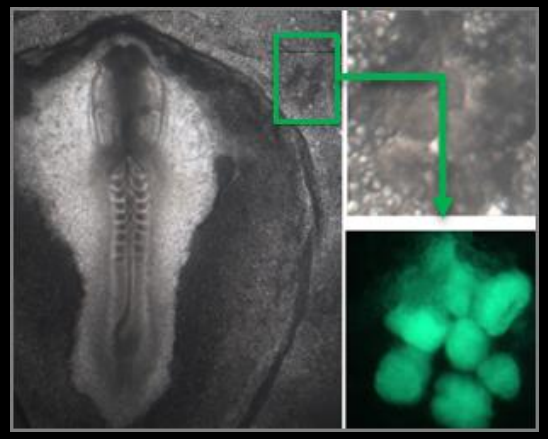
Hes1-EGFP time-lapse (3h)
Masamizu et al. 2006

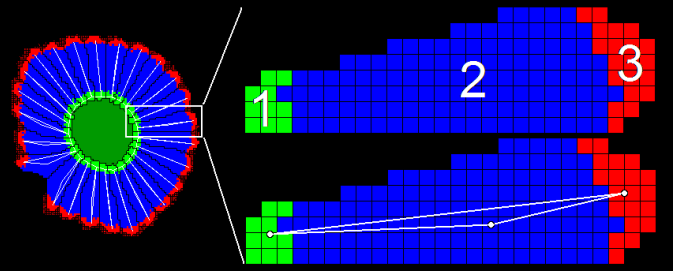
Clock and Wavefront Simulation

- *oscillating gene expression (eg, Hes1, LNFG)*
- *signal gradients along AP axis (eg, FGF8, RA)*
- *differential cell adhesion (eg, ND, ephrin system)*



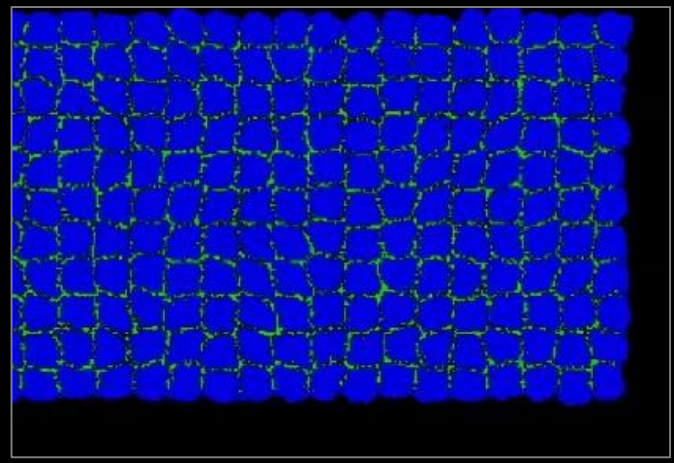
SOURCE: Hester et al. (2011) PLoS Comp Biol



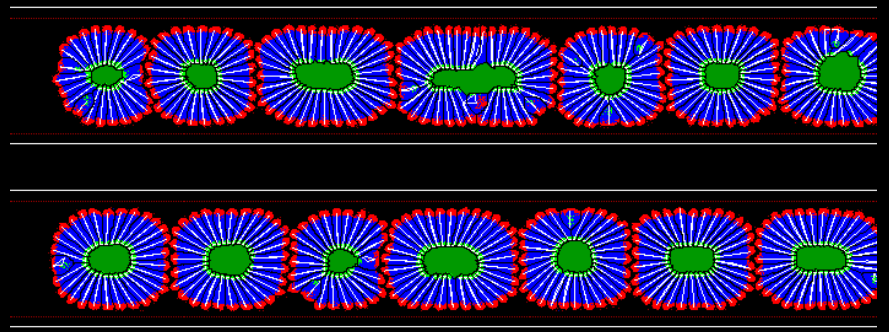


Epithelialization Model

- *clock genes do not oscillate*
- *somites form simultaneously*

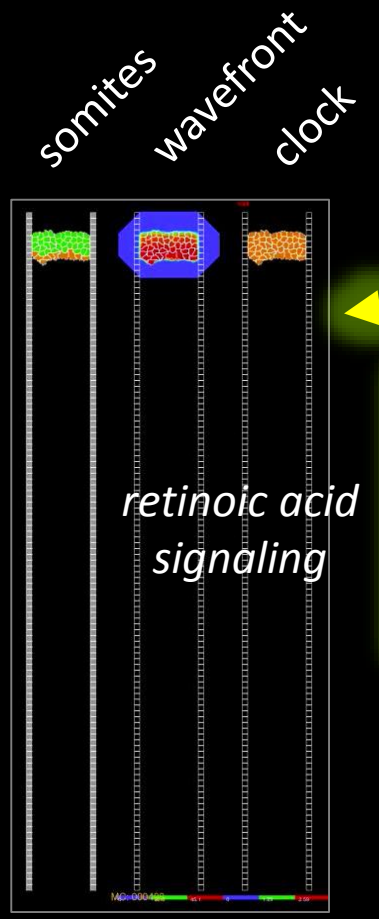


- *Adding the wavefront restores sequentiality*
- *Adding the clock improves regularity*

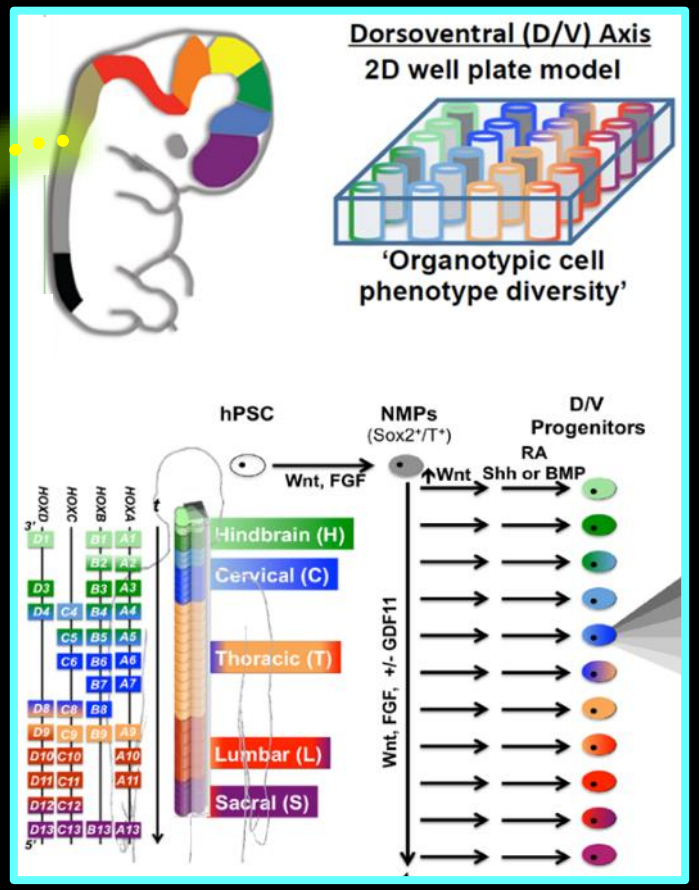


SOURCE: Dias et al. (2014) Science

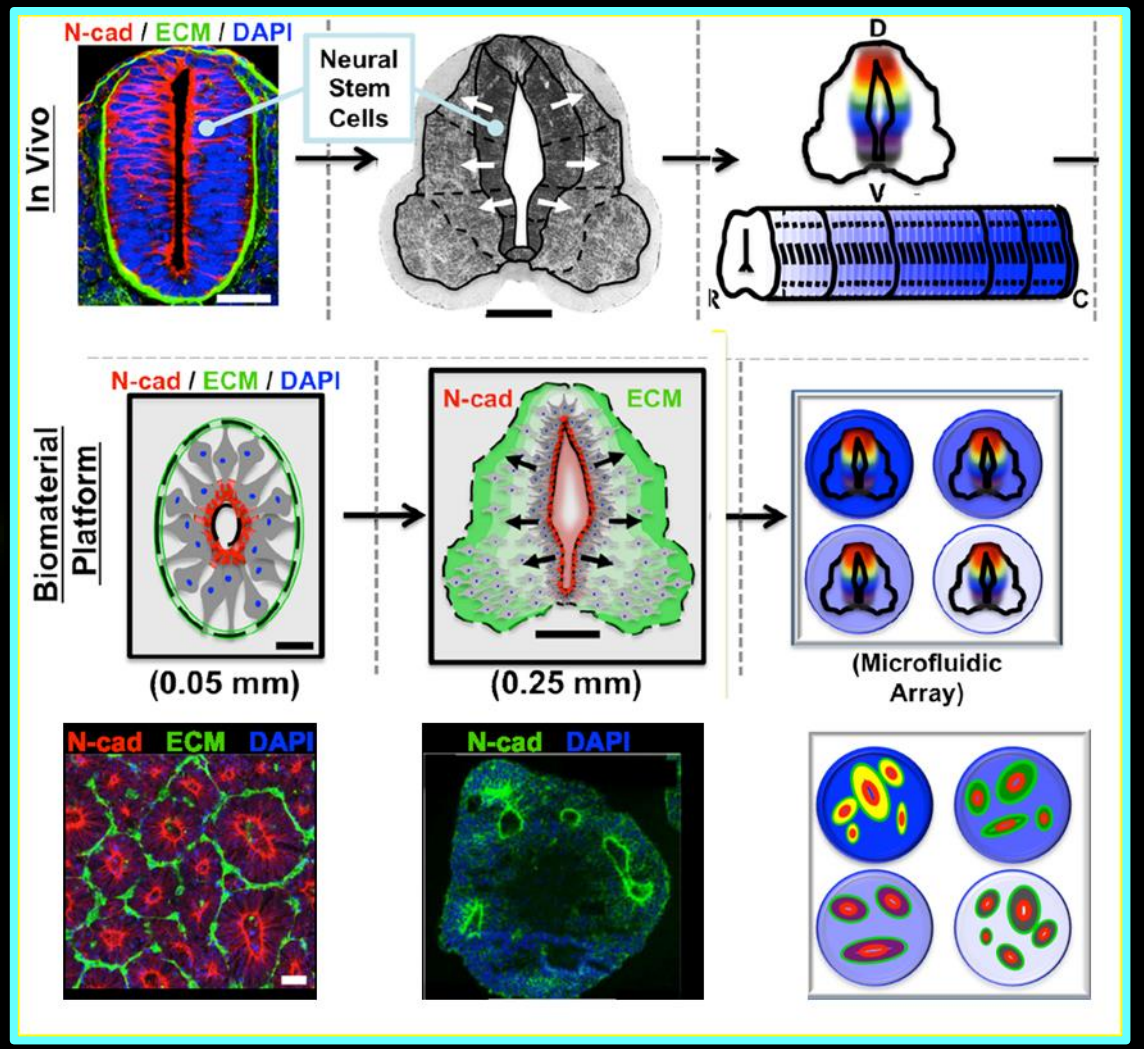
Micropatterning: *regionally-diverse stem cell arrays for the human neural tube*



Glazier's somite model (*in silico*)

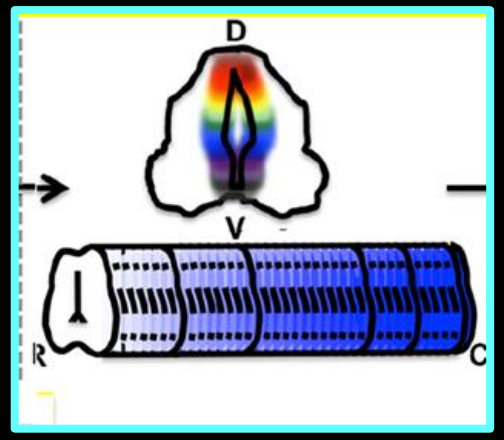


Ashton's neural tube model (*in vitro*)

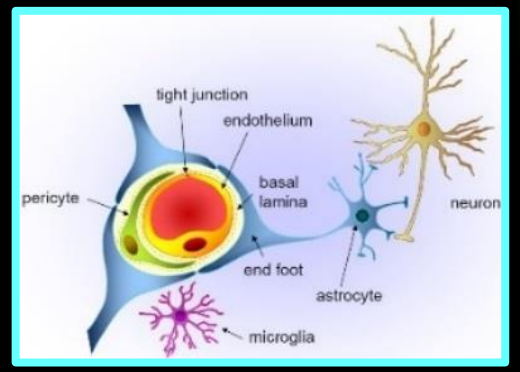


SOURCE: Randolph Ashton, U Wisconsin H-MAPS Center [Lippman et al. 2015; Knight and Ashton 2015]

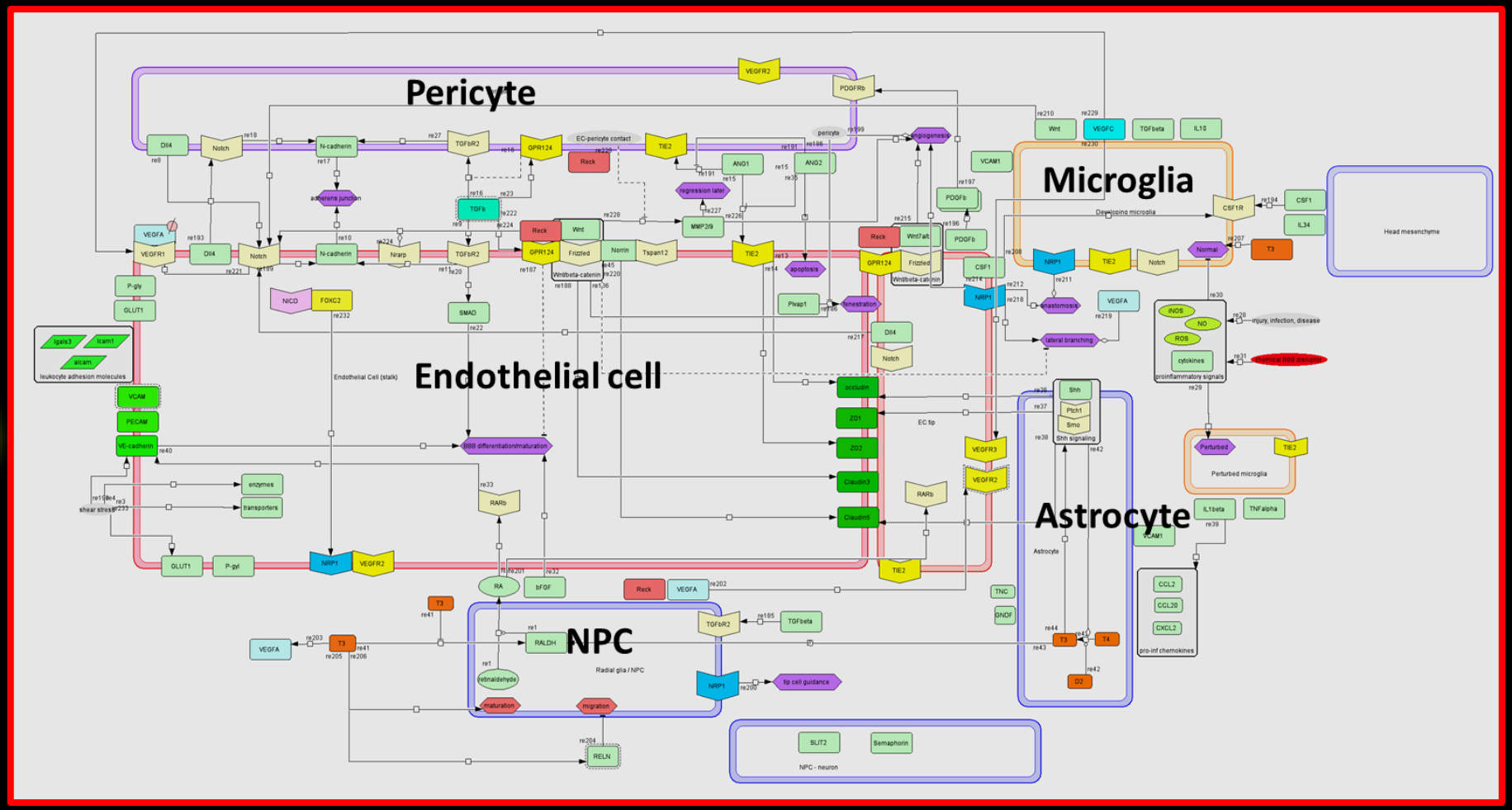
Vascularization of the Neural tube



Ashton's neural tube model (*in vitro*)



Neurovascular OCM (W Murphy – U Wisc)

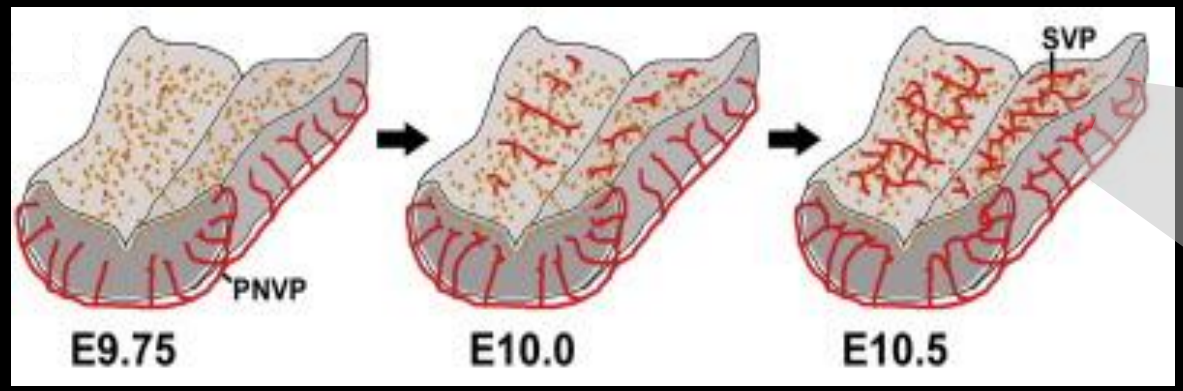


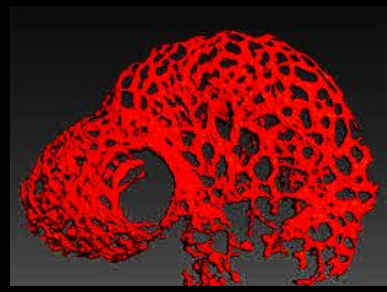
Saili's control network for blood-brain barrier development

Saili et al. (2017) manuscript under internal review

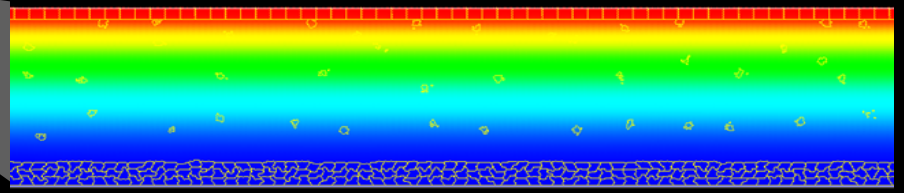
Modeling Brain Angiogenesis

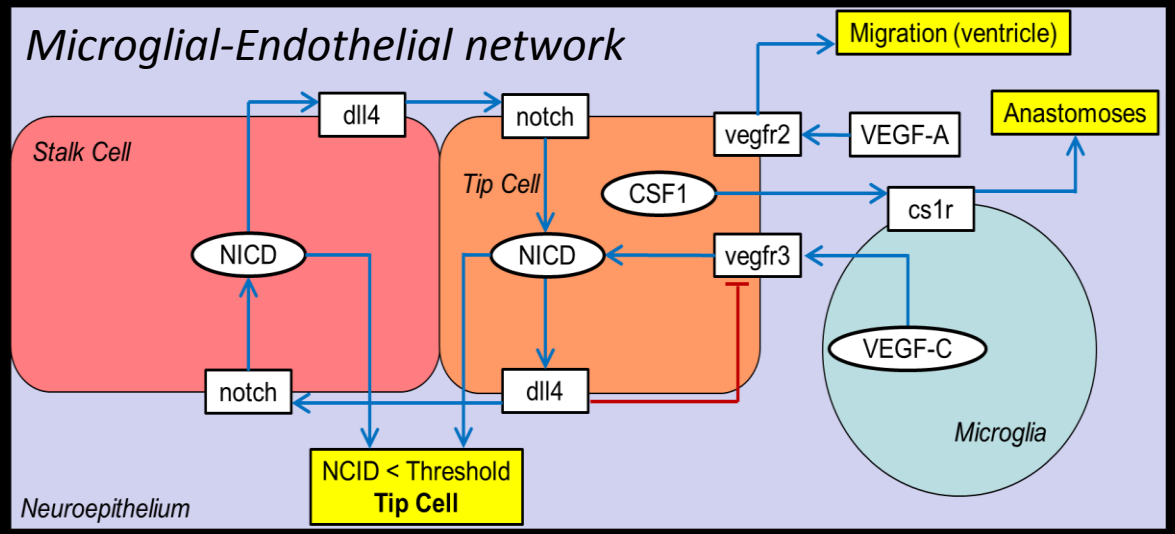
Tata et al. (2015) *Mechansim Devel*

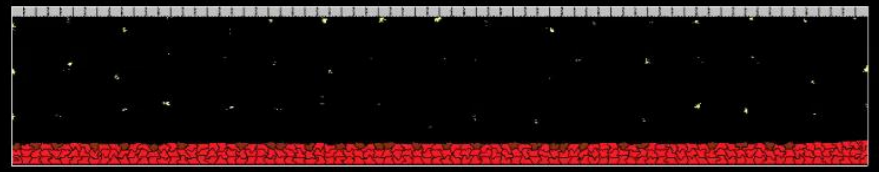




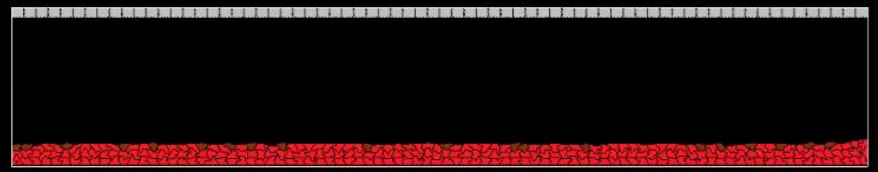
VEGF-A gradient: NPCs in subventricular zone

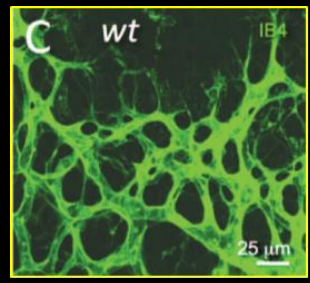


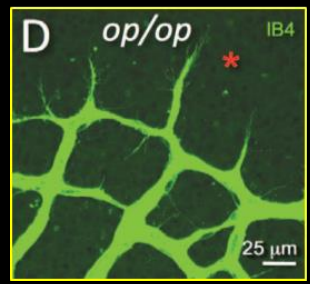




endothelial tip cell
endothelial stalk cell
microglial cell



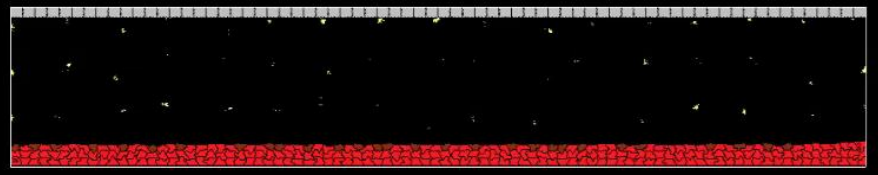




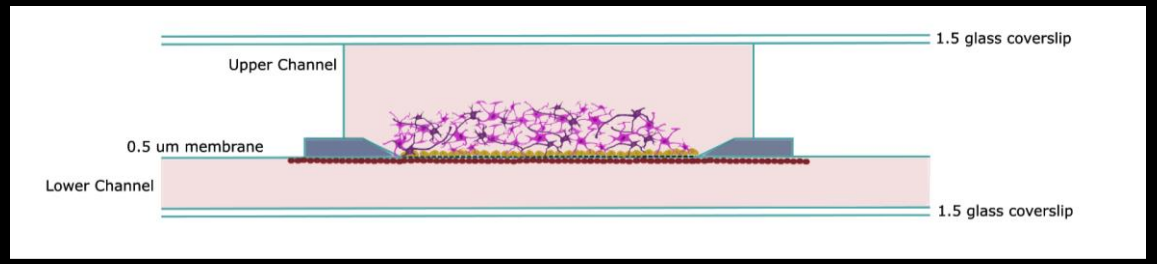
Rymo et al. (2011) PLoS one

SOURCE: T Zurlinden – NCCT (2017)

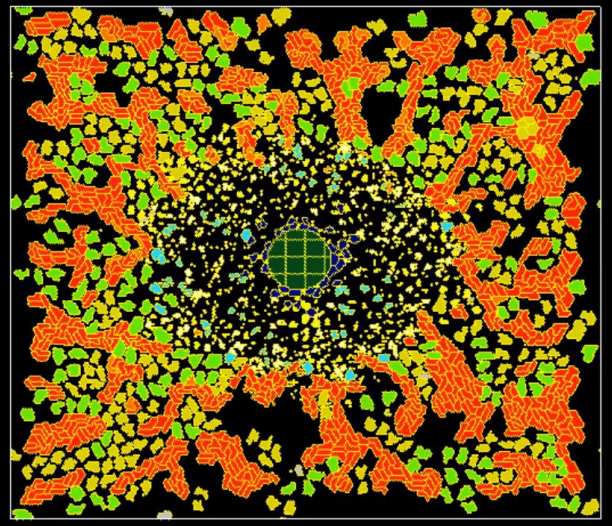
Modeling the fetal Neurovascular unit (NVU)



Zurlinden's brain vascularization model (*in silico*)

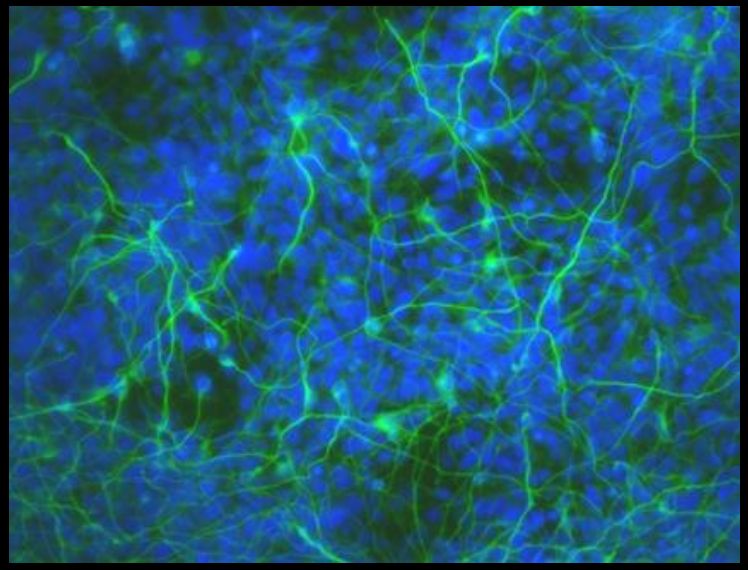


Ibidi hNVU device
(A Schwab / S Hunter – NHEERL)



endothelial stalk cell
endothelial tip cell
macrophage
mural cell

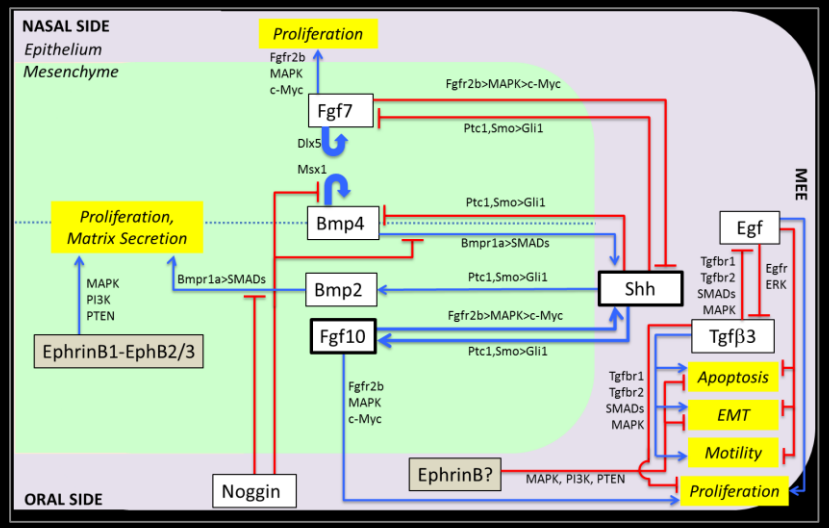
NPC
microglia
pericyte
astrocyte



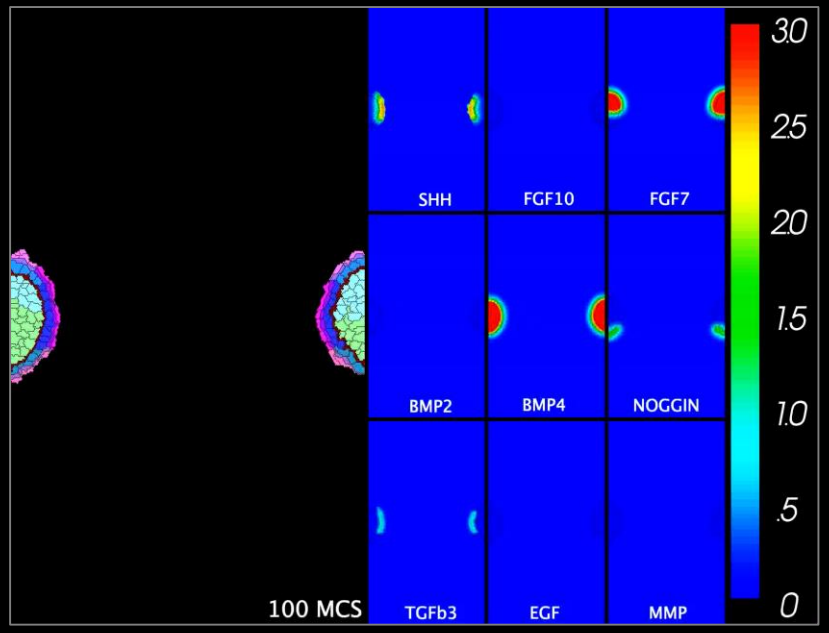
computational NVU (cNVU)

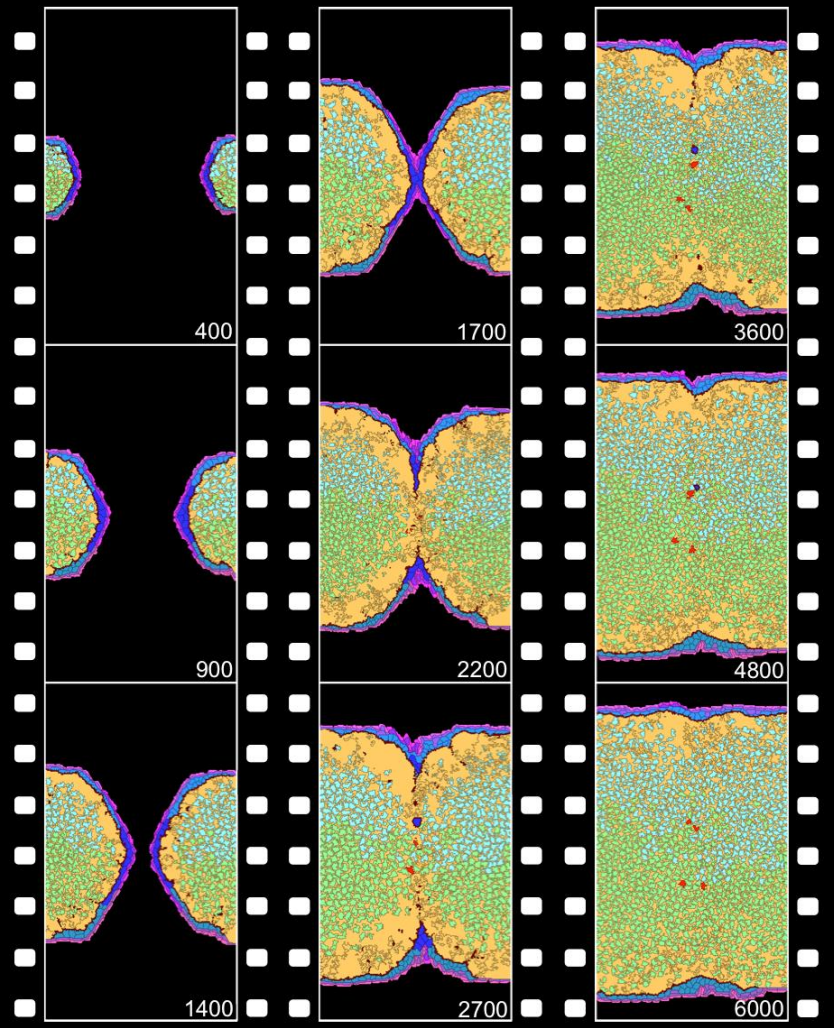
Morphogenetic fusion: *palate development (in vivo)*

Control network

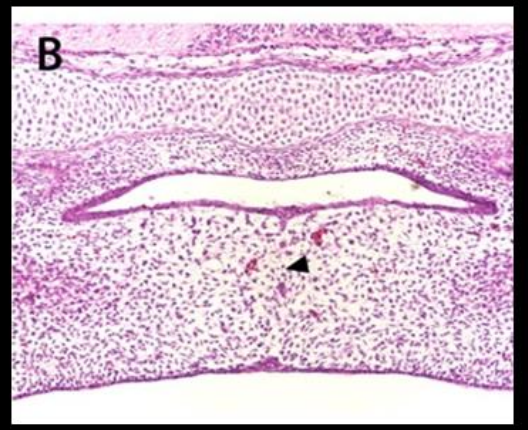


Anterior simulation





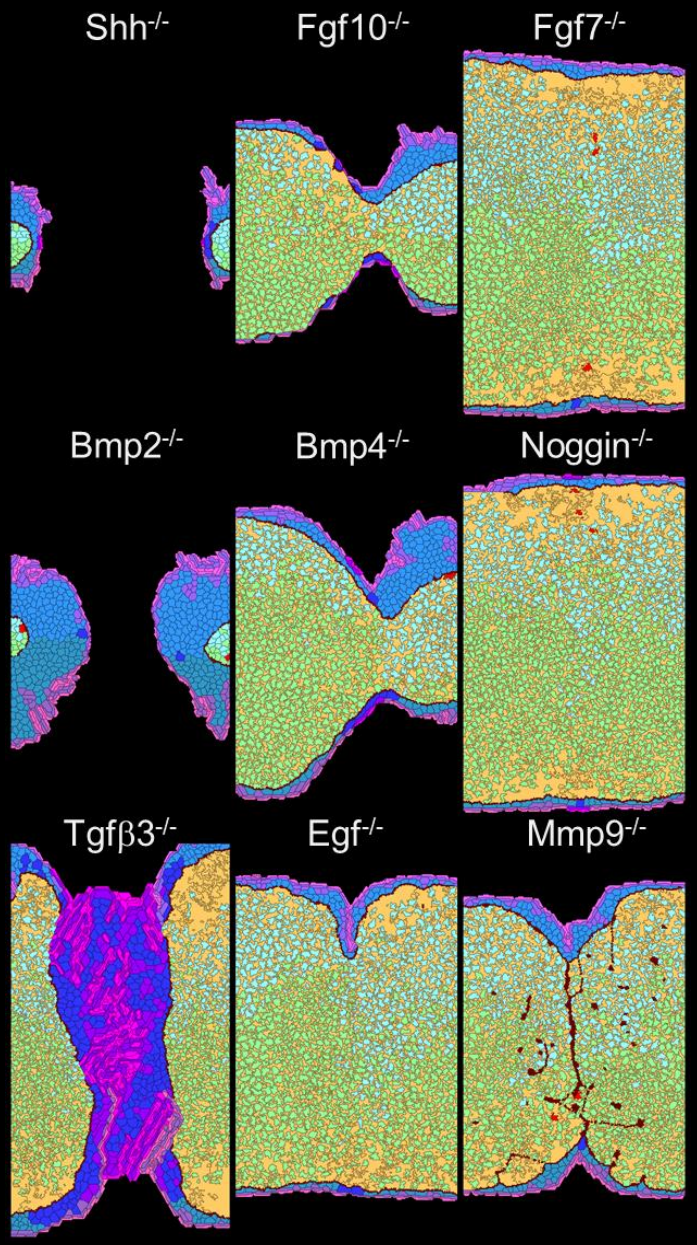
Jin and Ding (2006) Development



MEE breakdown is programmed genetically to coincide with apposition

SOURCE: Hutson et al. (2017) Chem Res Toxicol

Hacking the Control Network: *in silico* knockouts → 'Cybermorphs'

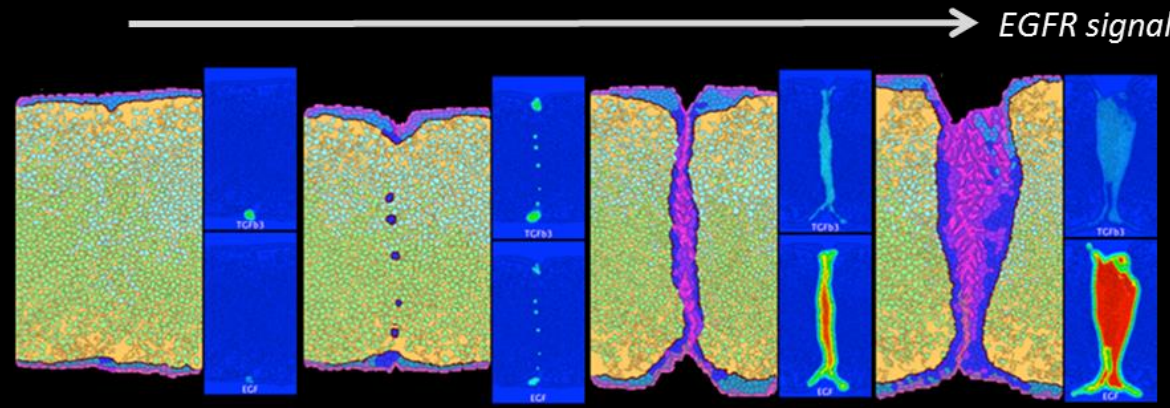


SHH signaling drives outgrowth *(MCS 200-2000)*

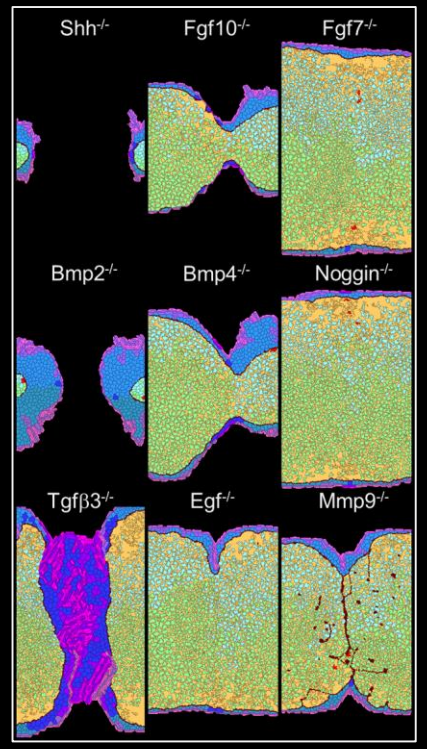
- SHH::FGF and SHH::BMP stimulate mesenchymal cell proliferation and ECM production

TGF-beta signaling drives fusion *(MCS 2000-3000)*

- TGFβ3::EGF signaling switches epithelial cell fate from survival (high EGFR) to regression (low EGFR).

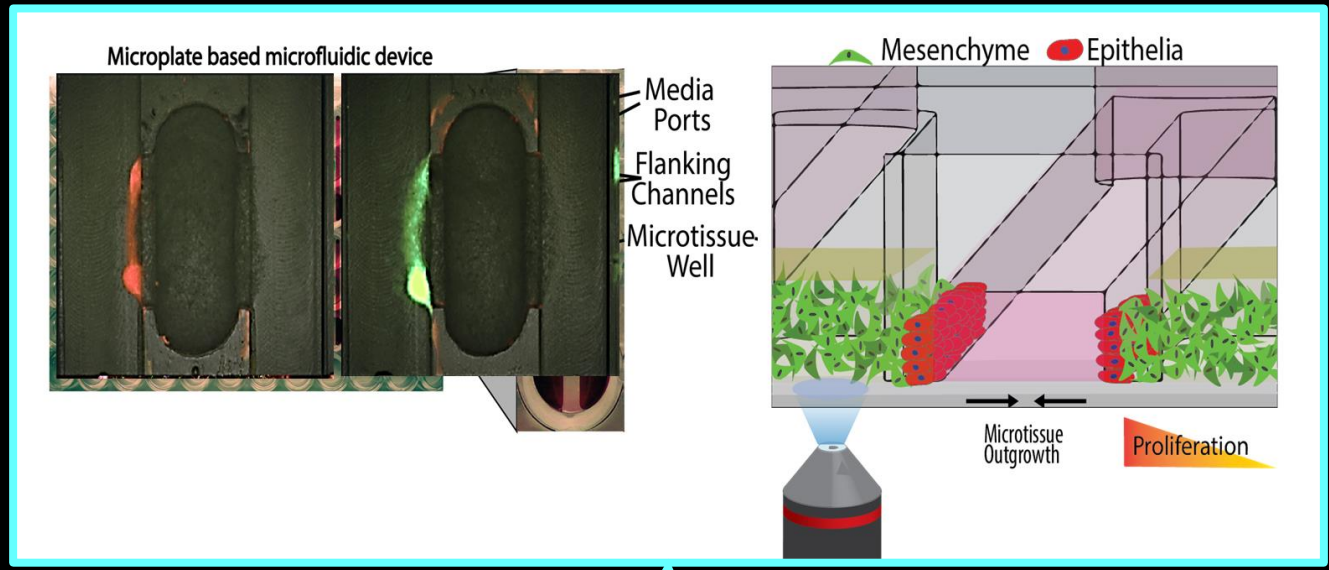


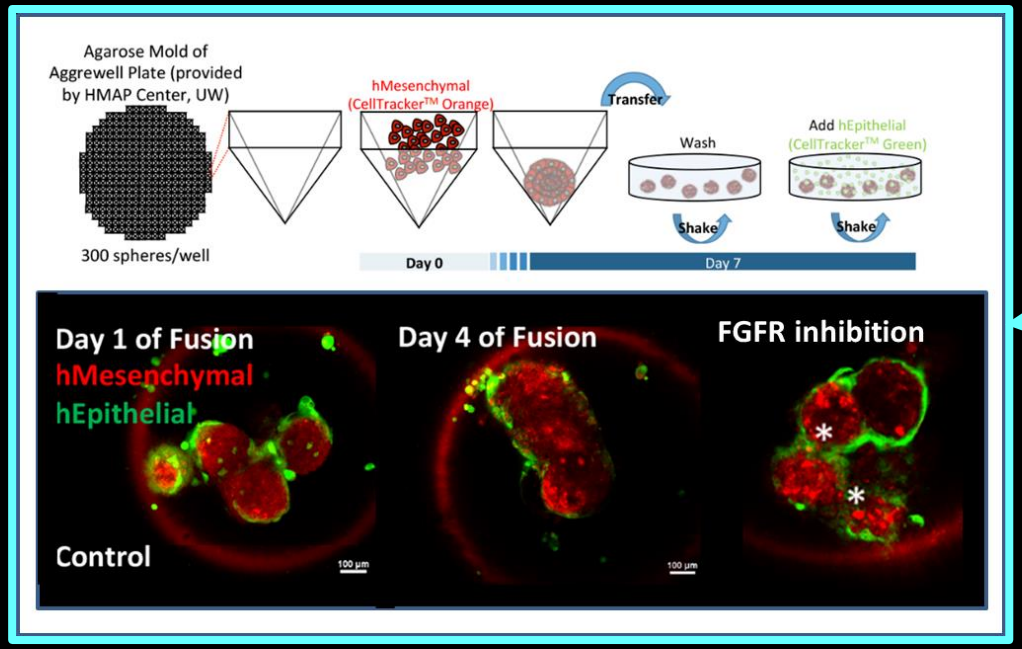
Cleft Palate: *recapitulating palatogenesis and pathogenesis in vitro*



Hutson's fusion model (*in silico*)

SHH-driven outgrowth (B Johnson / D Beebe – U Wisc)

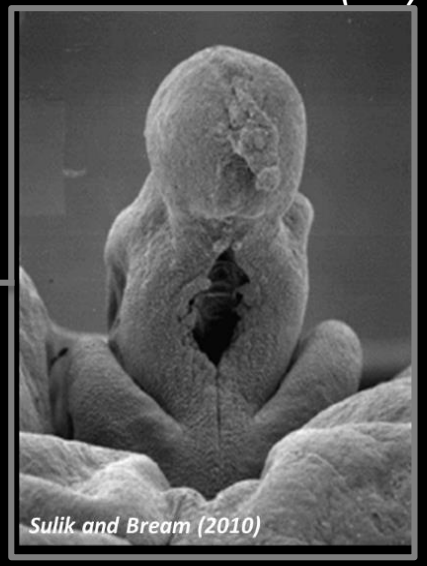


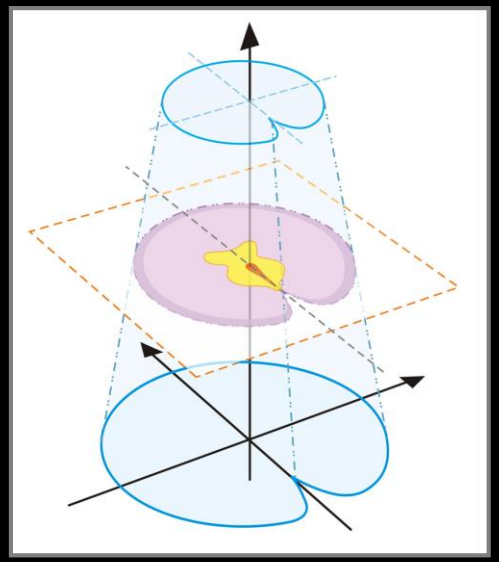


Epithelial fusion (D Belair / B Abbott – NHEERL)

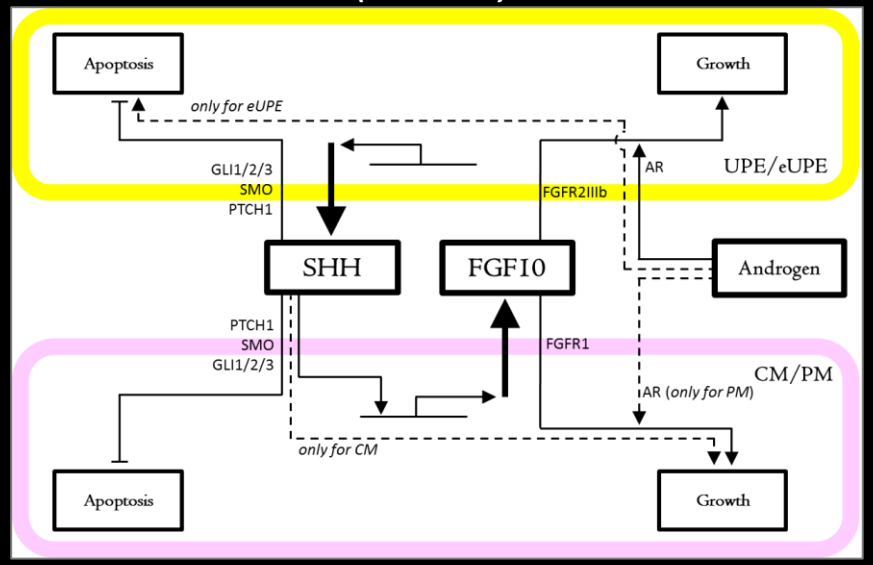
Genital Differentiation

Genital tubercle (GT)

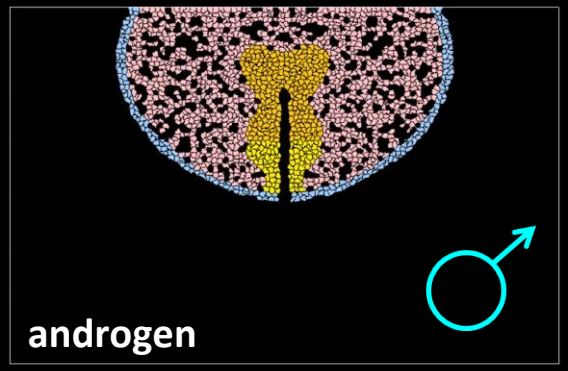


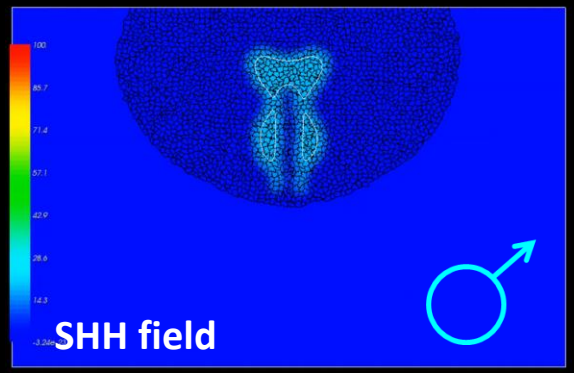


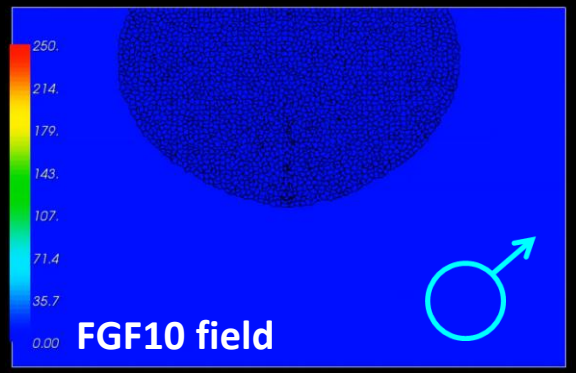
Control Network (mouse)

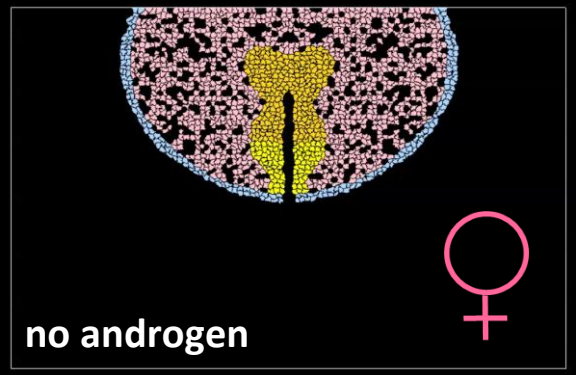


ABM simulation for sexual dimorphism (mouse GD13.5 – 17.5)





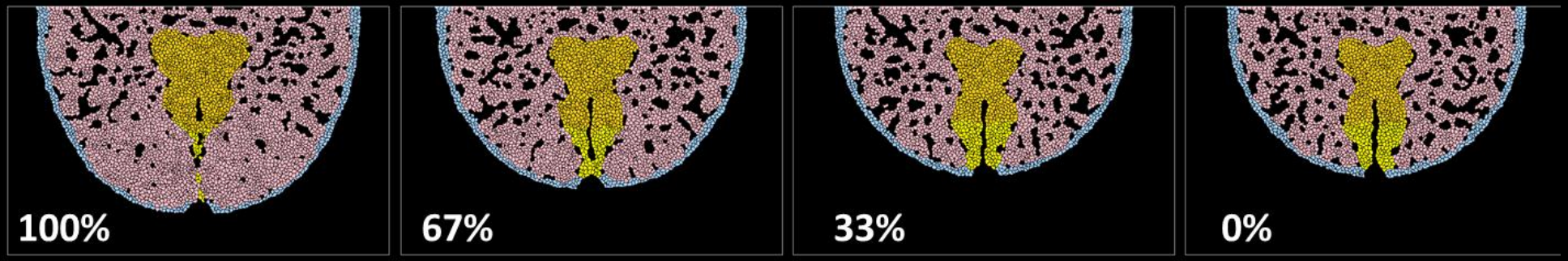




SOURCE: Leung et al. (2016) Reprod Tox

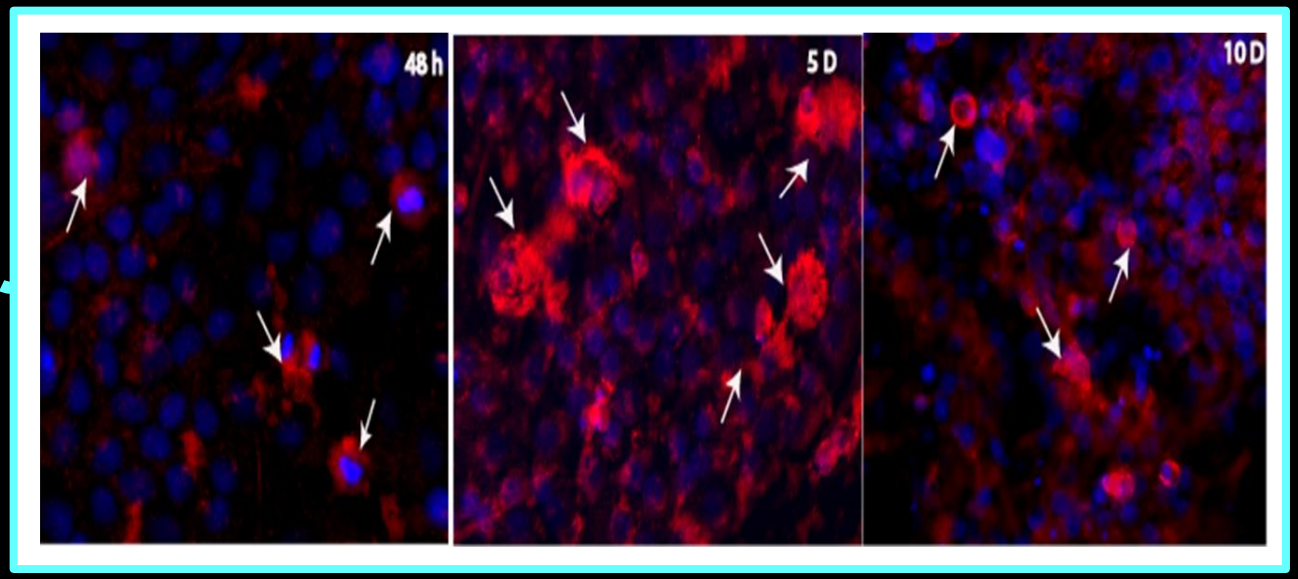
Hypospadias: *recapitulating urethrogenesis and pathogenesis in vitro*

Predicted impact of fetal testosterone deficiency on genital tubercle differentiation



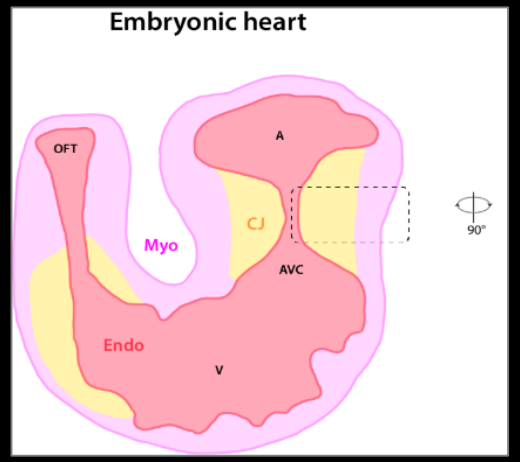
Leung's fusion model
Reprod Toxicol (2016)

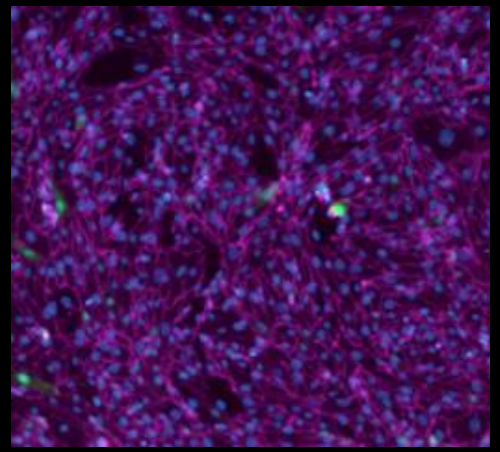
Faustman's OCM for testicular development
SOURCE: Harris et al. (2016) Toxicol In Vitro

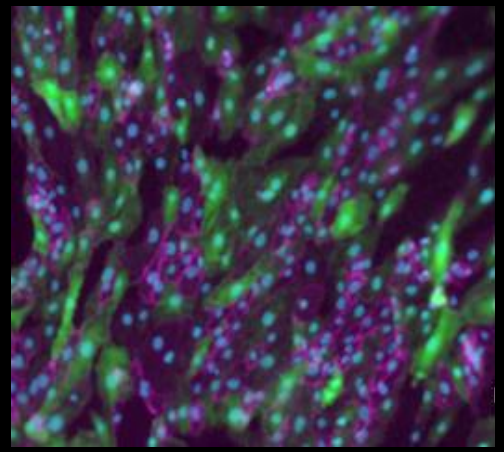


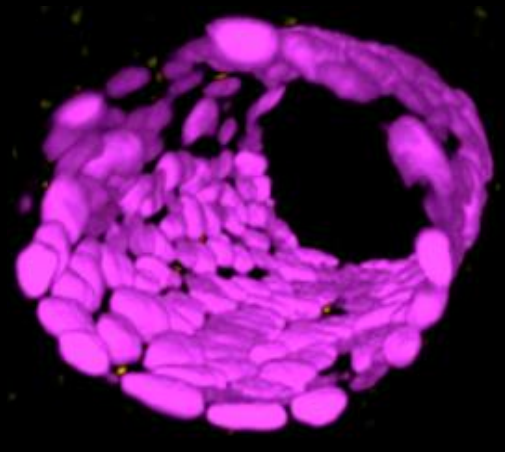
Epithelial-Mesenchymal Transition:

delay or disruption underlies some congenital malformations (e.g., valvulo-septal heart defects)

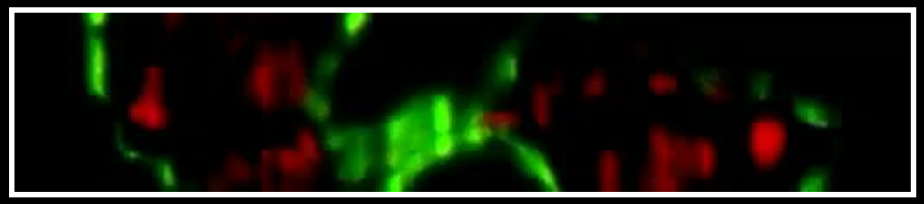






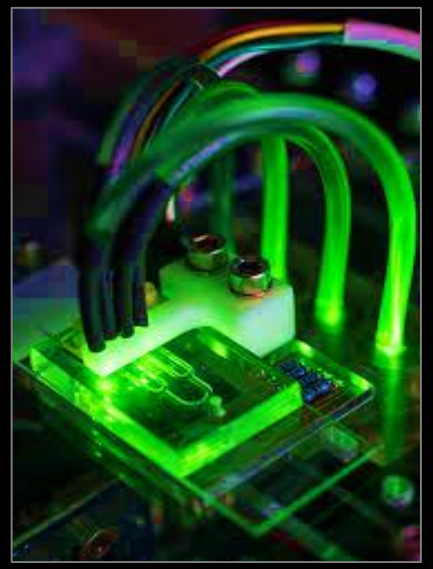


SOURCE: K Grode / S Hunter - NHEERL

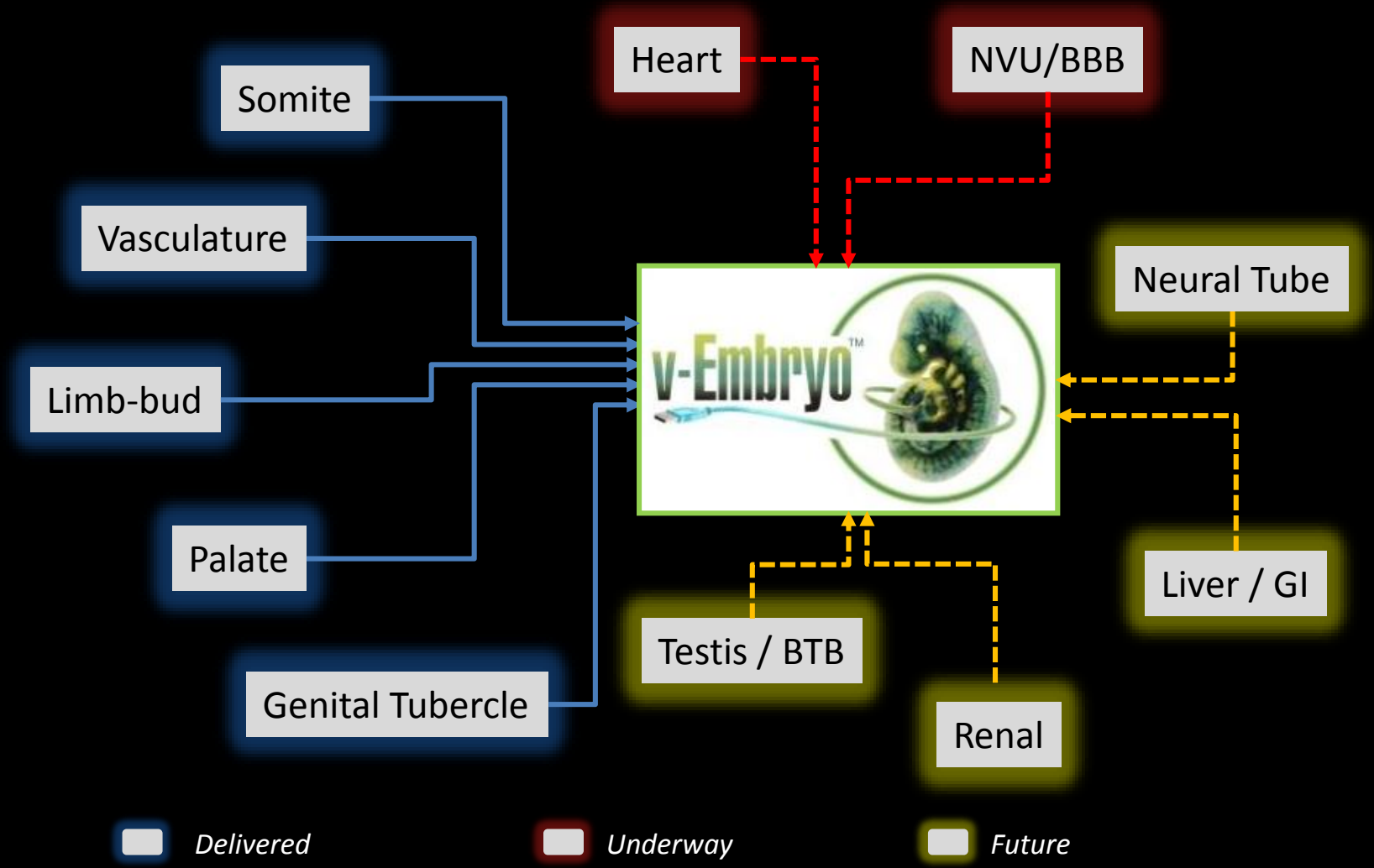


Zfish embryo heart at 72 hpf
SOURCE: Scherz et al. (2008) Development

… but endocardial EMT does not occur in a static environment: need to "go with the flow" (K Grode / D Belair – NHEERL)



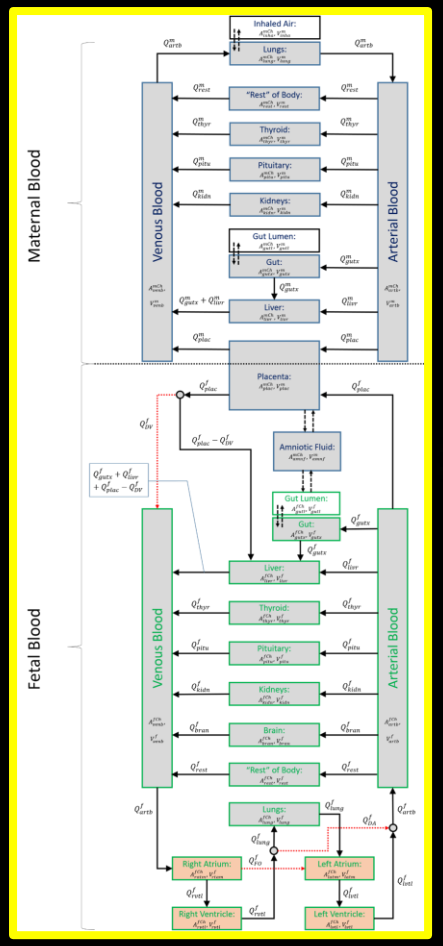
Grand Challenge:
a predictive 'virtual embryo'



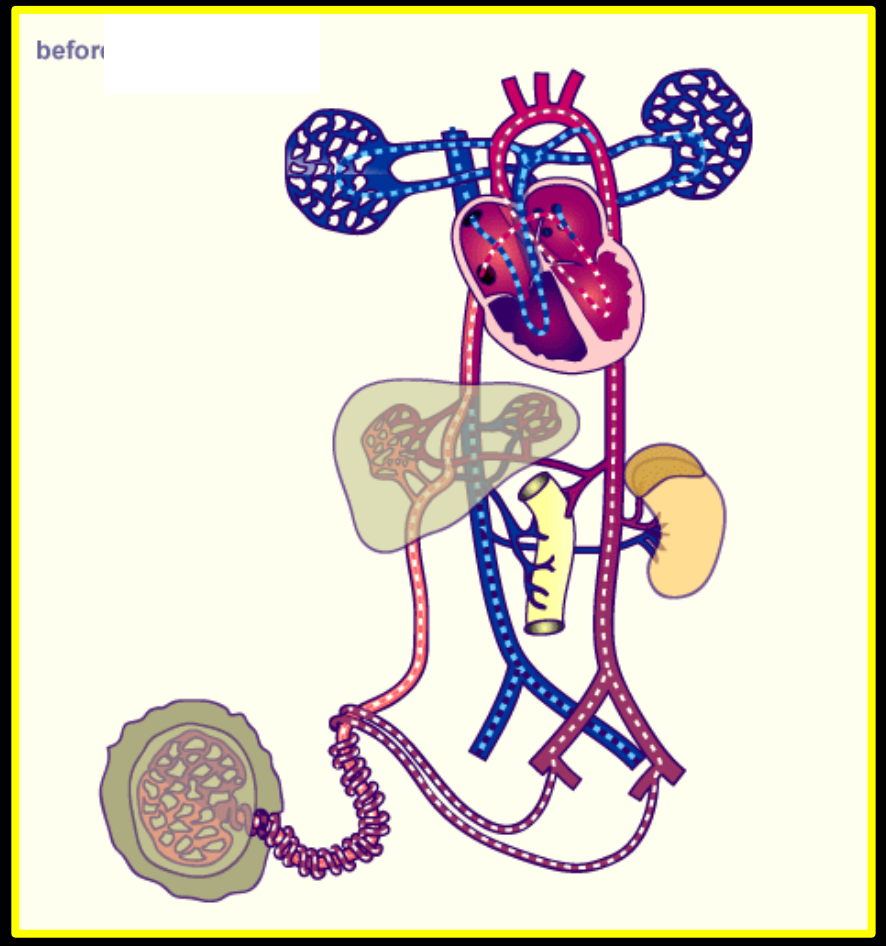
- *Hester et al. (2011) PLoS Comp Bio; Dias et al (2014) Science*
- *Kleinstreuer et al. (2013) PLoS Comp Bio.*
- *Ahir et al. (MS in preparation).*
- *Hutson et al. (2017) Chem Res Toxicol.*
- *Leung et al. (2016) Reprod Toxicol.*
- *Zurlinden et al. (FY17 product).*
- *Hunter et al. (FY18 product).*
- *Your name here.*

Fetal circulation and Microfluidic circuits:

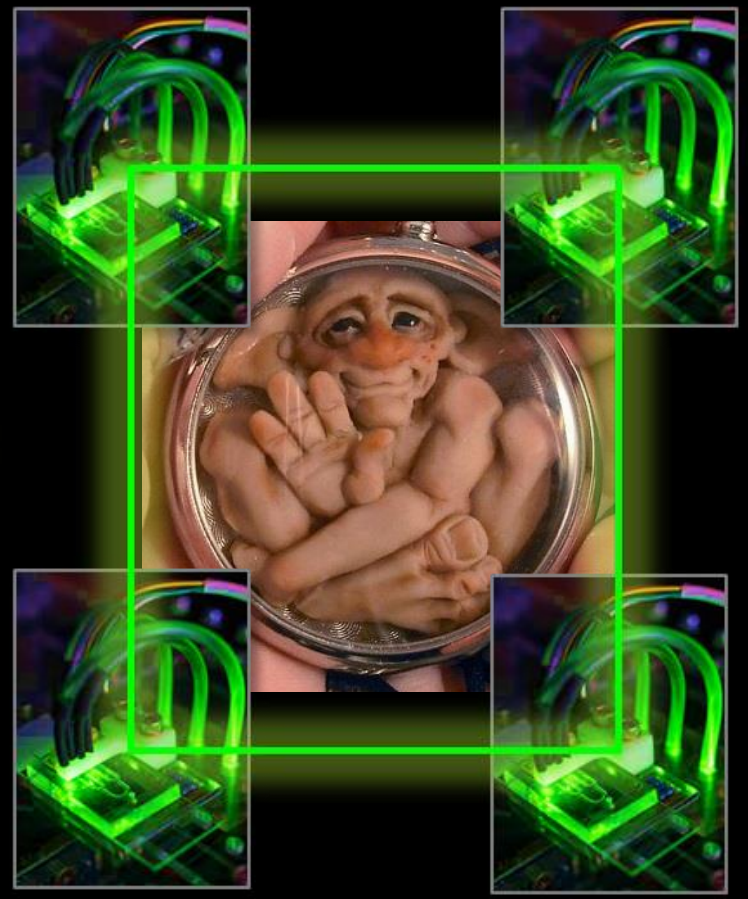
delay or disruption underlies some congenital malformations (e.g., valvulo-septal heart defects)



Fetal PBPK model
Kapraun et al. (NCCT)



Fetal Circulatory System
(A Kreislaufvor)



Testing the Homunculus

Virtual Reconstruction of Developmental Toxicity

Computer models (eg, virtual embryo) in parallel with morphoregulatory platforms (eg, OCMs) and kinetic models can work together seamlessly to support children's health research on causal mechanisms for:

- structural malformations
- neurodevelopmental impairment
- cardiovascular defects
- low birth weight?
- preterm labor?
- other (childhood asthma, obesity, metabolic syndrome)?

SOURCE: Knudsen, Klieforth and Slikker (2017) Exp Biol Med (submitted)

SOT Contemporary Concepts in Toxicology Conference

Predictive Toxicology and Preventive Medicine for Healthy Children

November 14–16, 2018 | Washington, DC Area

Special Thanks

- Barbara Klieforth – EPA / NCER
- Max Leung – NCCT (now CalEPA)
- Kate Saili – NCCT
- Todd Zurlinden – NCCT
- Nancy Baker – Leidos / NCCT
- Richard Spencer – ARA / EMVL
- James Glazier – Indiana U
- Sid Hunter – NHEERL / ISTD
- Kyle Grode – NHEERL/ISTD
- Andrew Schwab – NHEERL/ISTD
- Barbara Abbott – NHEERL/TAD
- David Belair – NHEERL/TAD
- John Wikswo – Vanderbilt U
- Shane Hutson – Vanderbilt U
- Bill Murphy – U Wisconsin
- Brian Johnson – U Wisconsin

EPA STAR OCM-PT Centers

- Shane Hutson – Vanderbilt U (VPROMPT)
- Bill Murphy – U Wisconsin (HMAPS)
- Elaine Faustman – U Washington (UW-PTC)
- Ivan Rusyn – Texas A&M U (CT-AOP)

http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf