Computational Modeling and Simulation of Developmental Toxicity

What can we learn from a virtual embryo?

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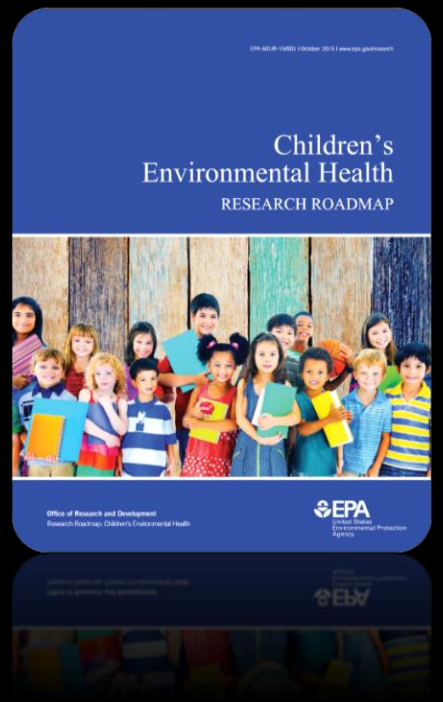
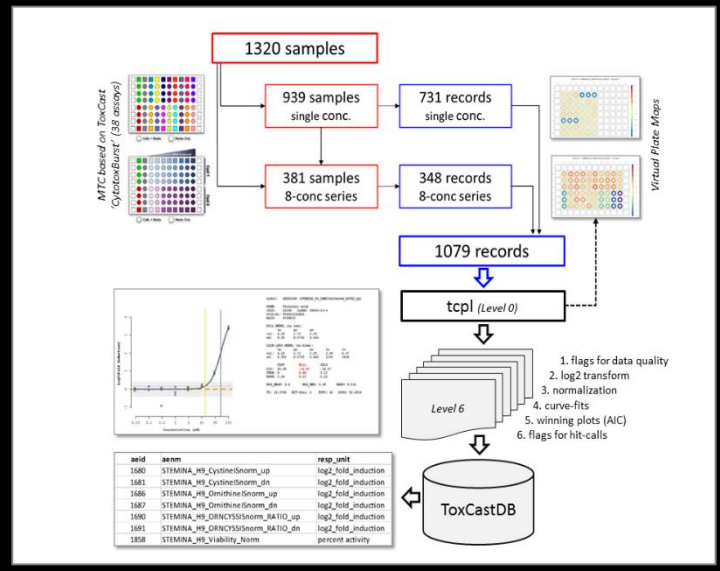
Developmental health and disease



- Unique vulnerability of fetuses, infants, and children has a critical role in setting health and environmental policy (Executive Order 13045).
- Frank R. Lautenberg Chemical Safety for the 21st Century Act of 2016 explicitly requires protection of children and pregnant women.
- EPA's Office of Children's Health Protection (OCHP) ensures that all EPA actions and programs address the unique vulnerabilities of children.
- Children's Environmental Health (CEH) is a cross-cutting research goal implemented in EPA/ORD's CEH research roadmap for 2016-2019.

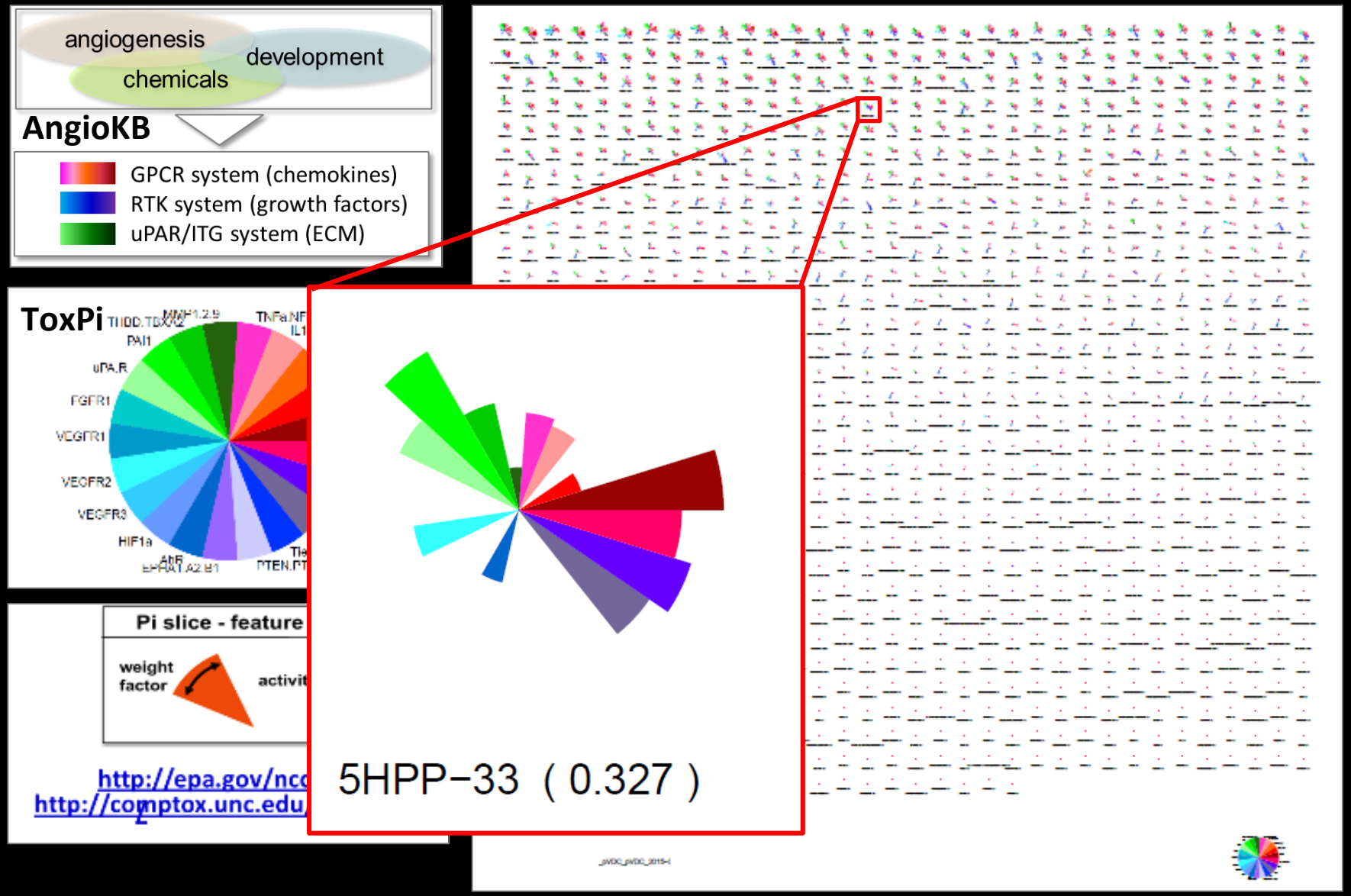
Predicting developmental toxicity

- Automated high-throughput assays are providing vast *in vitro* data streams for profiling the bioactivity of large chemical inventories in ToxCast/Tox21.
- One use of the high-throughput screening (HTS) data is to prioritize chemicals, based on their cellular and molecular bioactivity profiles, for potential developmental toxicity.
- 1066 ToxCast compounds were tested in a human stem cell-based assay (Stemina devTOX^{qP}).
- STM platform identified 190 positives with a 90% BA model (0.80 sensitivity, 1.00 specificity; n=33 reference compounds).

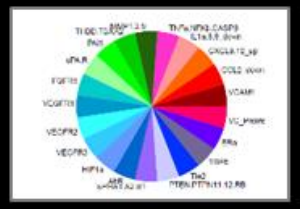


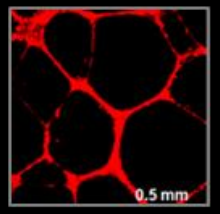
Source: Knudsen et al. (in preparation)

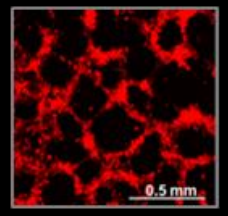
Angiogenesis: chemicals sorted by predicted potential to disrupt angiogenesis (pVDCs)

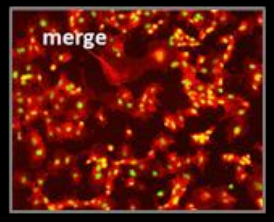


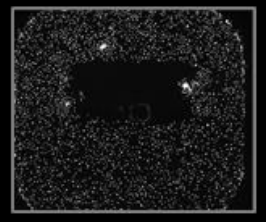
Validation: 38 pVDCs and non-pVDCs evaluated across 8 angiogenic platforms

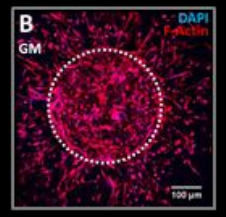


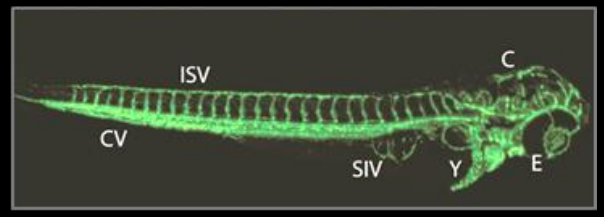


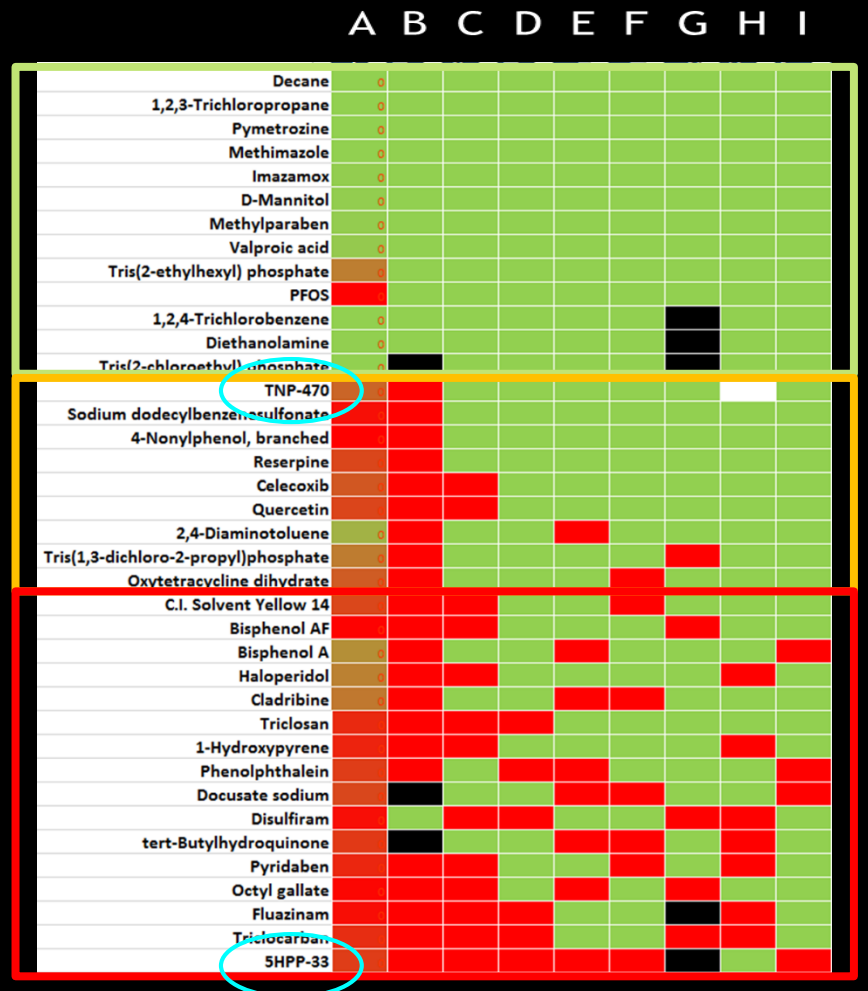






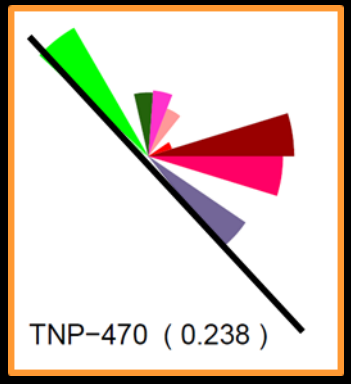


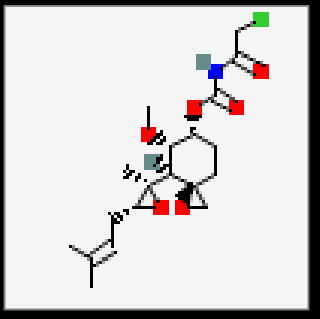


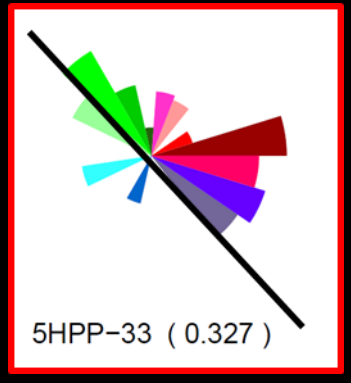


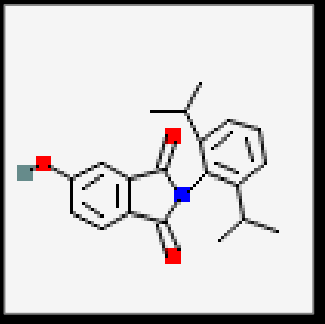
A ToxPi [1]
B FICAM tubulogenesis [2]
C Synthetic tubulogenesis [3]
D Matrigel tubulogenesis [3]
E nuCTNB [4]
F EC migration [4]
G angiogenic sprouting [5]
H TG-zebrafish [1]
I Vala tubulogenesis [2]

[1] Tal et al. Reprod Toxicol (submitted); [2] Knudsen et al., in prep; [3] Nguyen et al. Nature Bioengineering (submitted); [4] Belair et al. (2016) Acta Biomaterialia.

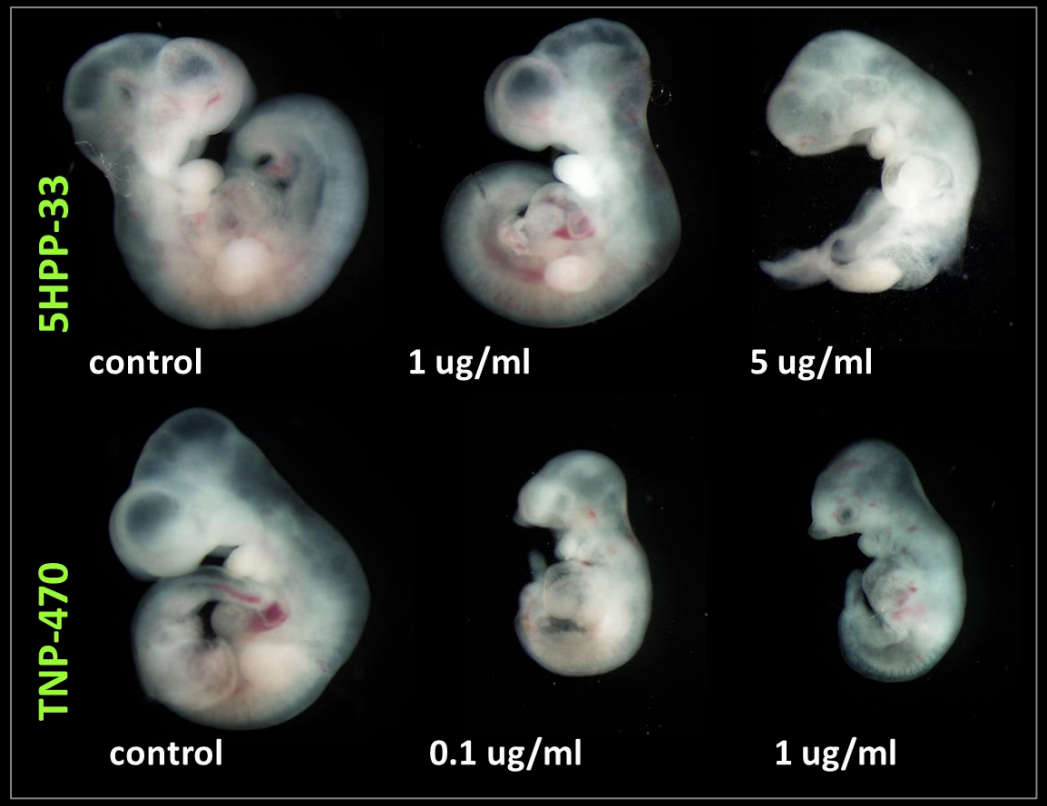


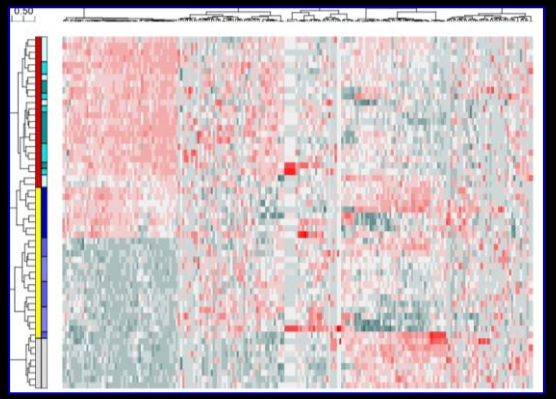


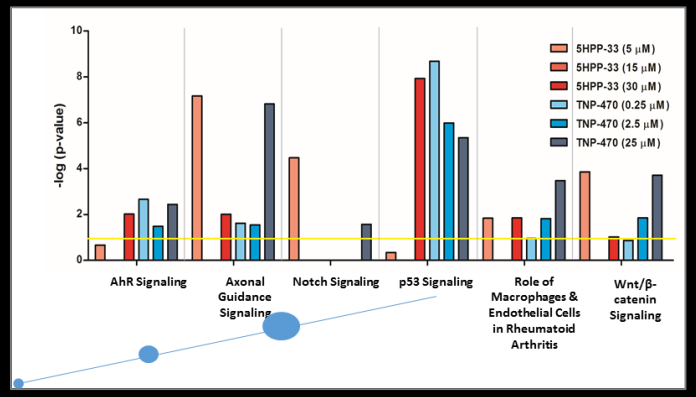




Rat WEC: GD10 embryos exposed for 48h







RNA-seq analysis: p53 was most significantly altered pathway in both cases (5HPP-33, TNP-470); alterations in Notch and Wnt expression unique to 5HPP-33.

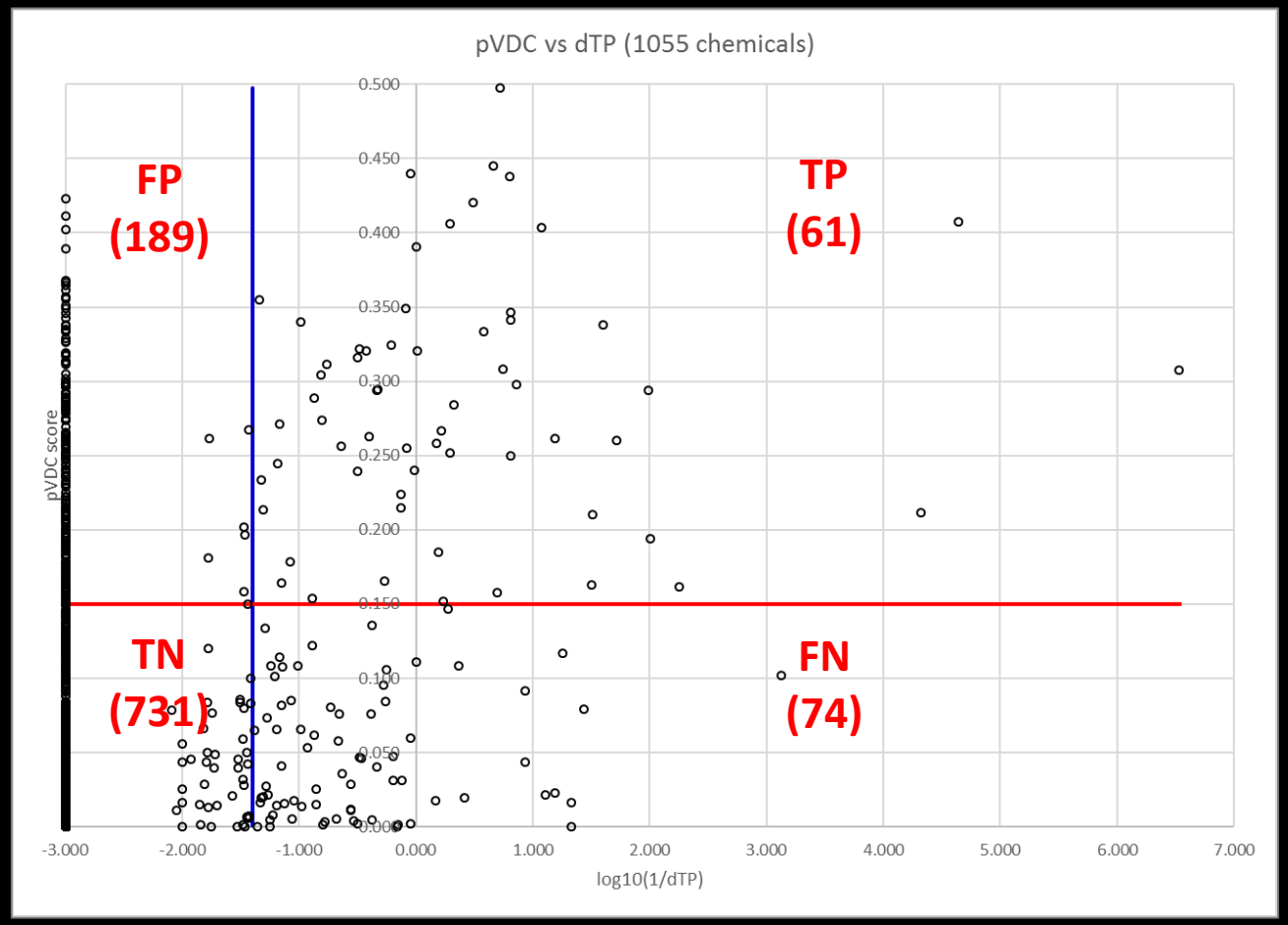
SOURCE: Franzosa et al. (in preparation)

5HPP-33: embryolethal ≥ 15 uM, AC50 = 21.2 uM; STM predicts human teratogenicity at ≥ 4.37 uM

TNP-470: dysmorphogenic ≥ 0.01 uM, AC50 = 0.038 uM; STM predicts human teratogenicity at ≥ 0.01 uM

SOURCE: Ellis-Hutchings et al. (submitted)

DevTox: how well does the pVDC score match-up with DevTox potential in a human system?



AOP-based **pVDC** score vs **DevTox** potential from the STM hES cell platform

Balanced Accuracy = 75.1%
(modeled on the 38-test set)

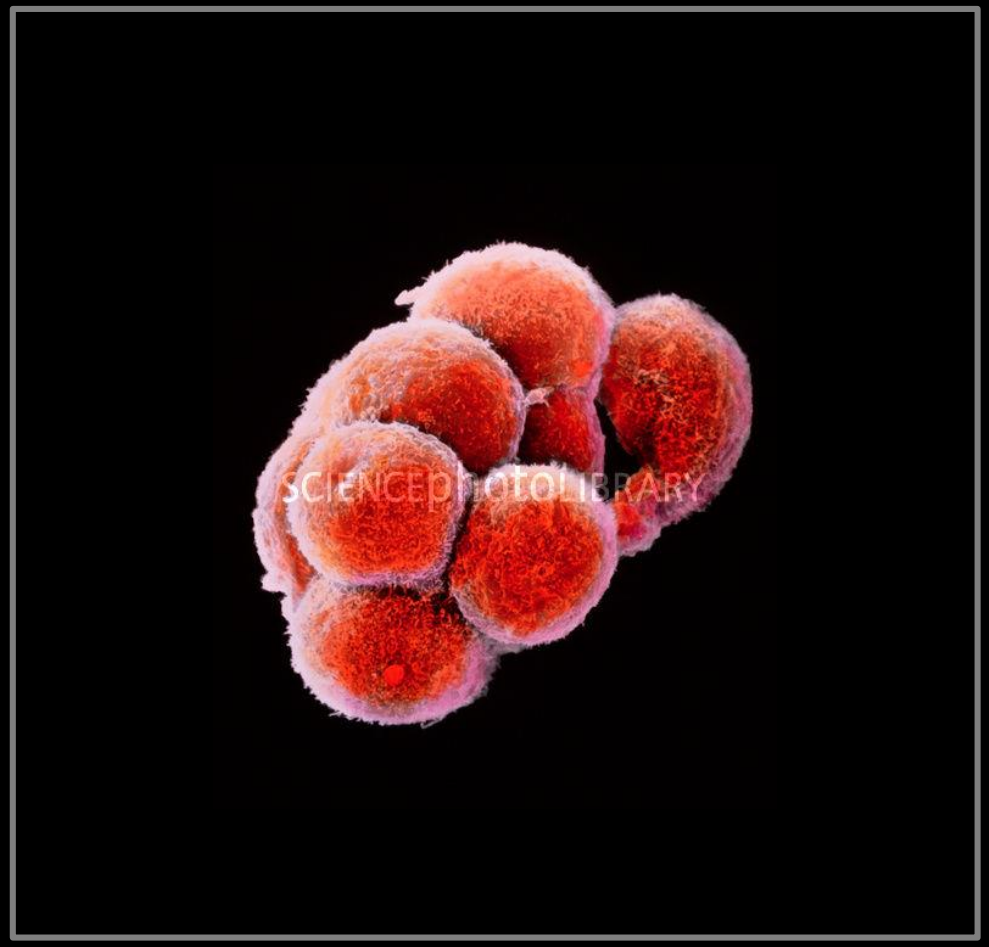
24.4% pVDC(+) also STM(+)
90.8% pVDC(-) also STM(-)

SOURCE: Knudsen et al., in preparation.

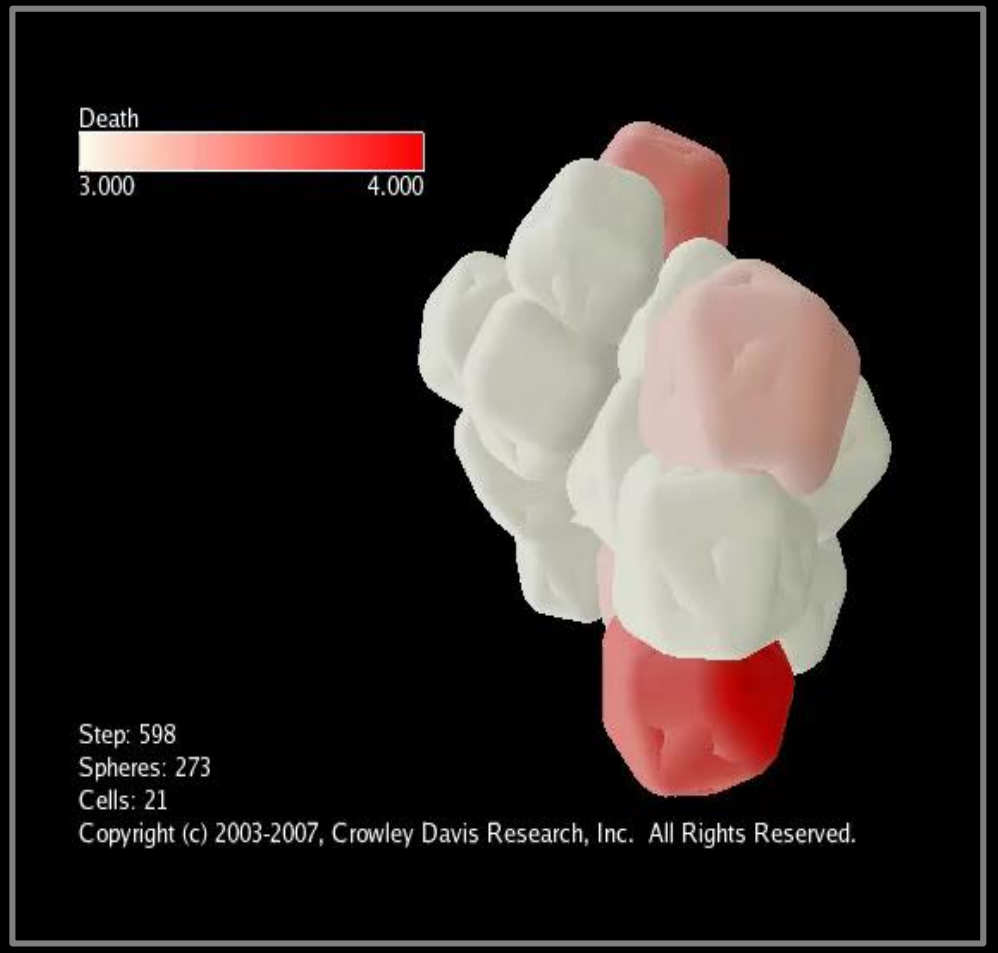
Virtual reconstruction of developmental toxicity

- The question of how tissues and organs are shaped during development is crucial for understanding (and predicting) human birth defects.
- While ToxCast HTS data may predict developmental toxicity with reasonable accuracy, mechanistic models are still necessary to capture the relevant biology.
- Subtle microscopic changes induced chemically may amplify to an adverse outcome but coarse changes may override lesion propagation in any complex adaptive system.
- Modeling system dynamics in a developing tissue is a multiscale problem that challenges our ability to predict toxicity from *in vitro* profiling data (ToxCast/Tox21).

Anatomical homeostasis in a self-regulating Virtual Embryo



Mouse Morula
SOURCE: Science Photo Library



SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Breathing life into a 'Virtual Embryo'

- **Hypothesis:** computer models that recapitulate a morphogenetic series of events can be used analytically (to understand) and theoretically (to predict) developmental toxicity.

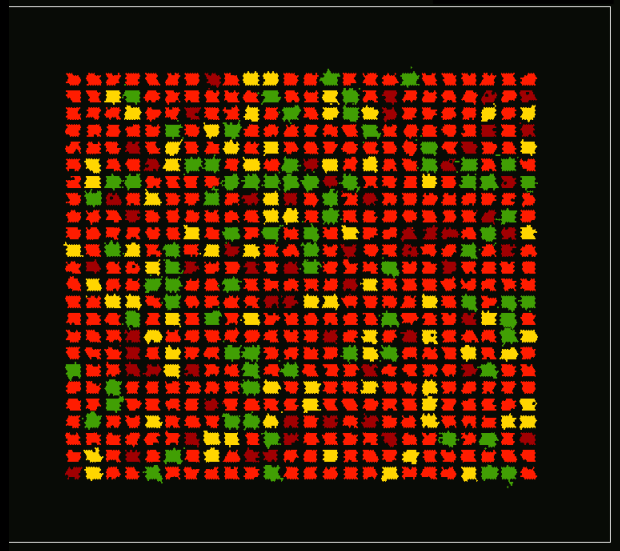
- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics from the bottom-up, cell-by-cell and interaction-by-interaction.

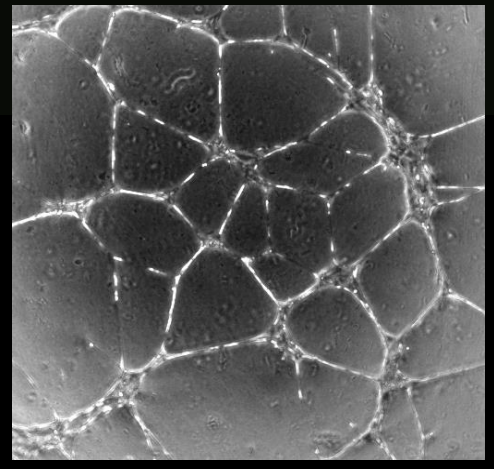
- **CompuCell3D:** open source modeling environment
  - engineered at Indiana University by James Glazier and colleagues;
  - steppables for distinct cell behaviors (growth, proliferation, apoptosis, differentiation, polarization, motility, ECM, signal secretion, …);
  - rules coded in Python for cell-autonomous 'agents' that interact in shared microenvironment and self-organize into emergent phenotypes.

Angiogenesis

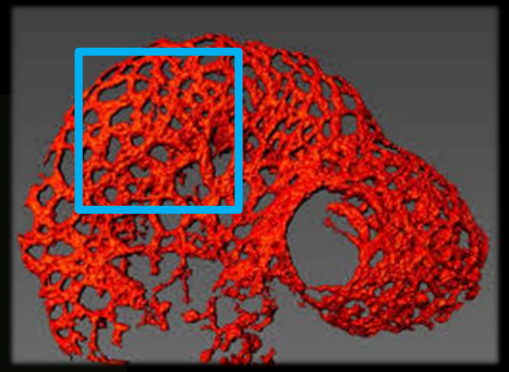
VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2

ABMS



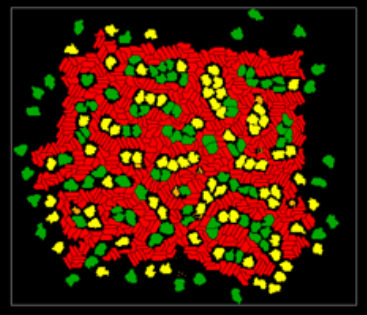


SOURCE: J Glazier, Indiana University

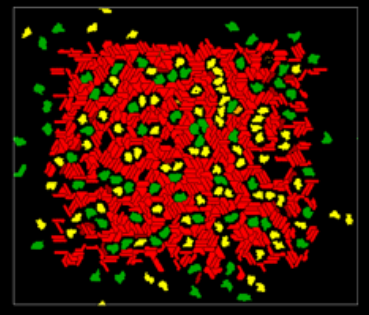


Exposure to 5HPP-33, a synthetic thalidomide analog

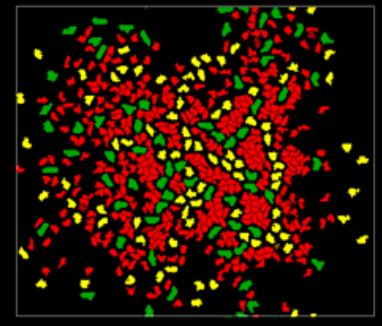
control



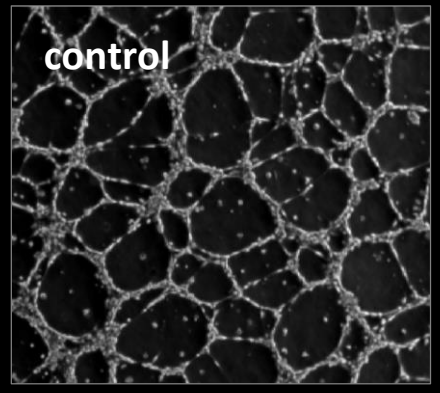
4 µM



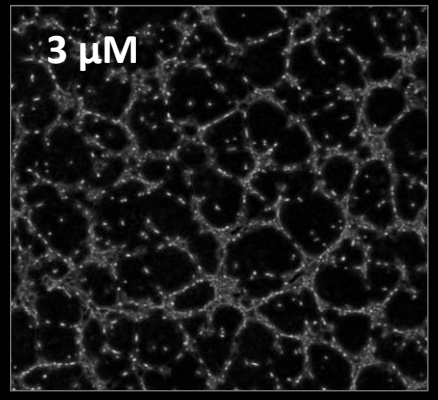
40 µM



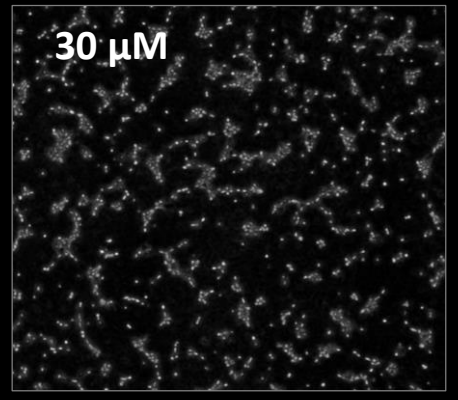
control



3 µM

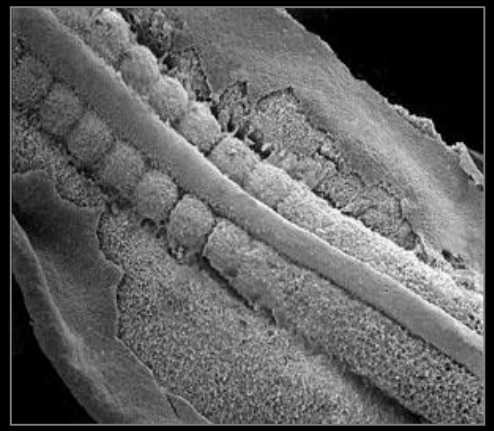


30 µM

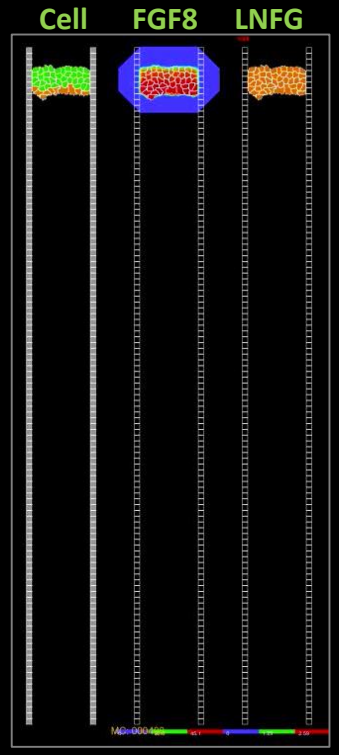


SOURCE: Kleinstreuer et al. 2013, PLoS Comp Biol

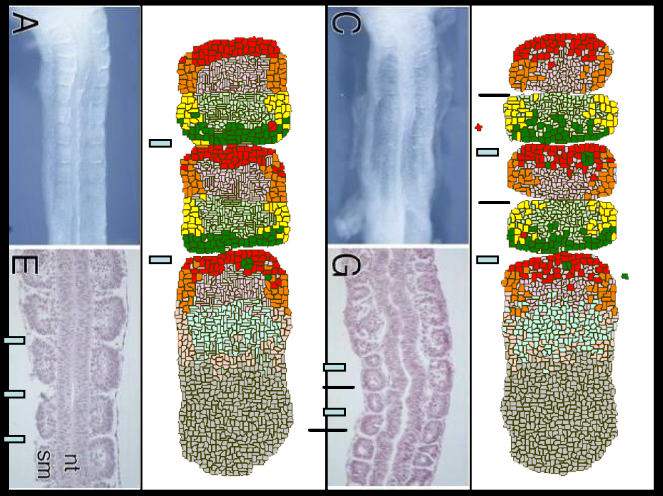
Somite formation



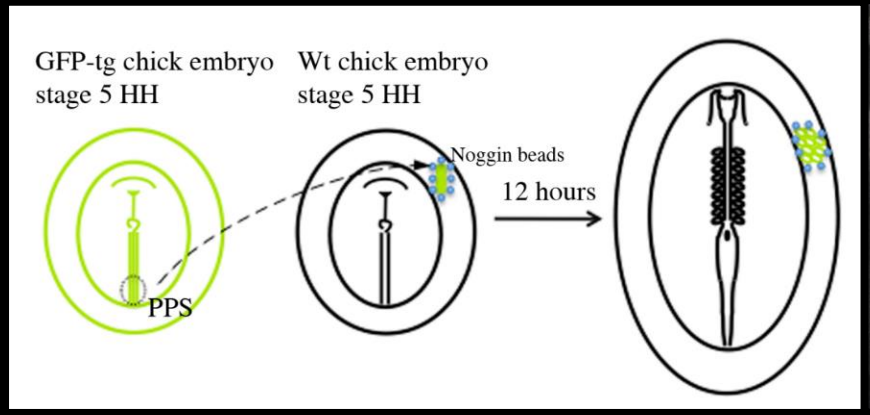
Hes1-EGFP time-lapse (3h)
Masamizu et al. 2006

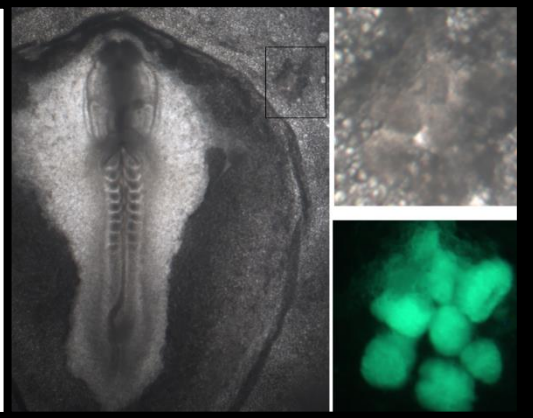


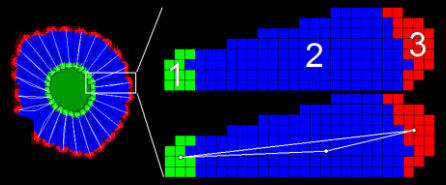
Clock and Wavefront model:
- *signal gradients along AP axis (eg, FGF8, RA)*
- *oscillating gene expression (eg, LFNG, Hes1)*
- *cell adhesion (eg, ND, ephrin system)*



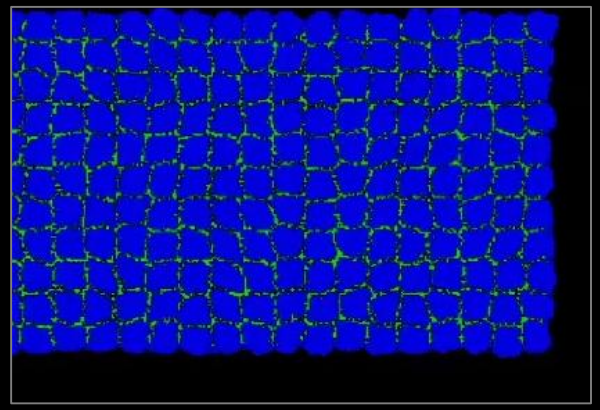
SOURCE: Hester et al. (2011) PLoS Comp Biol



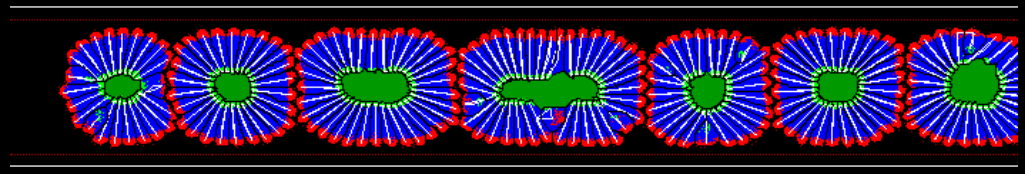




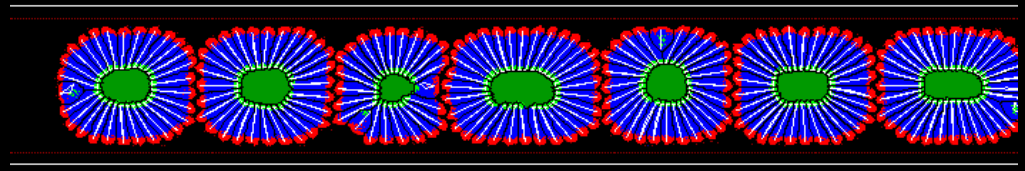
Epithelialization model:
- *clock genes do not oscillate*
- *somites form simultaneously*



Adding the Wavefront:
- *restores sequentiality*

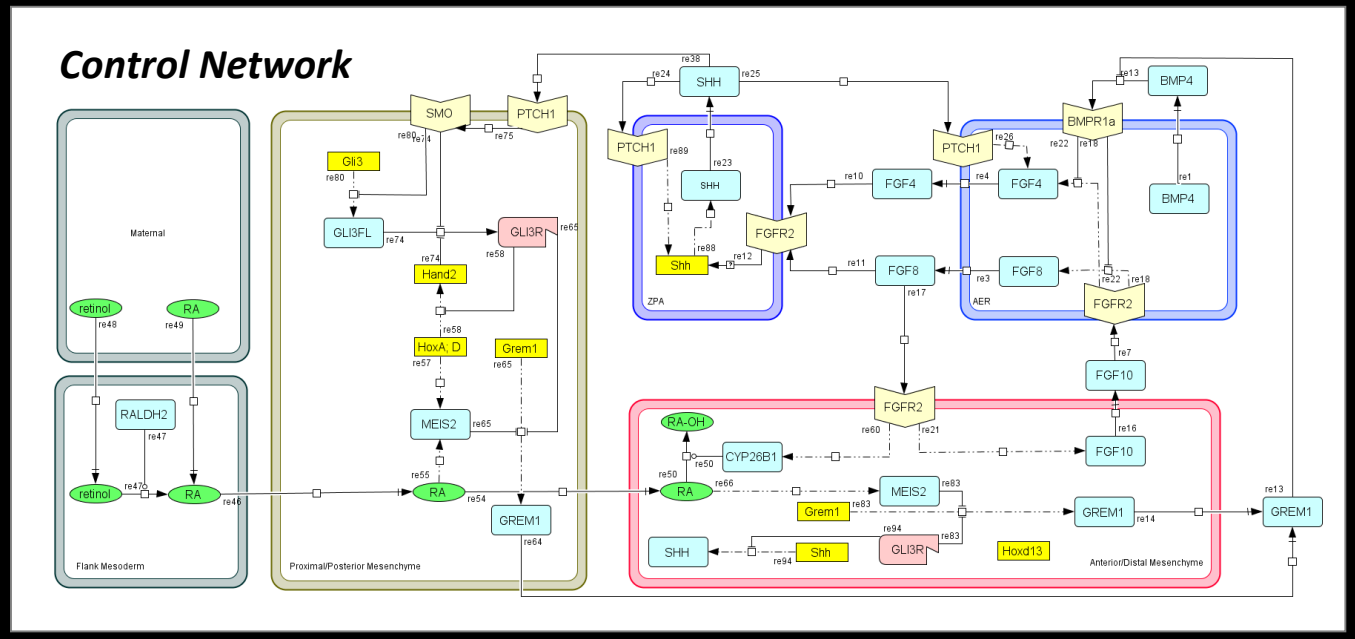


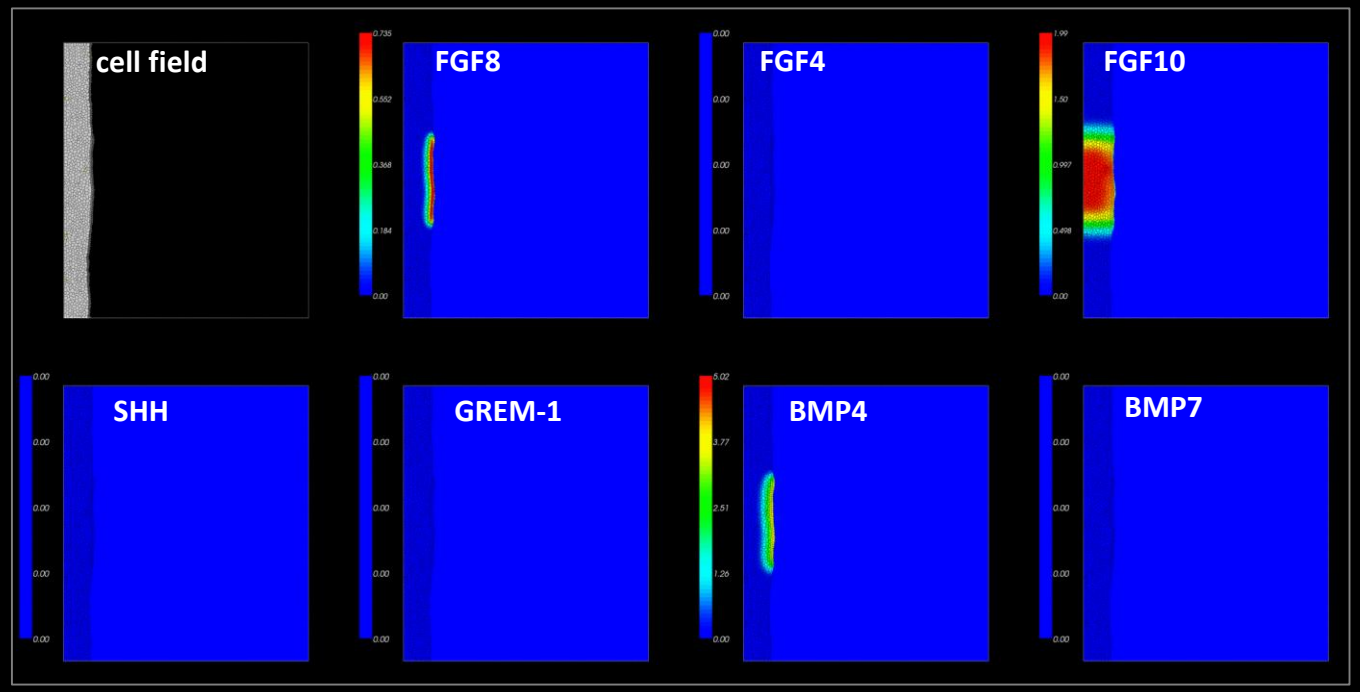
And adding the Clock:
- *improves regularity*

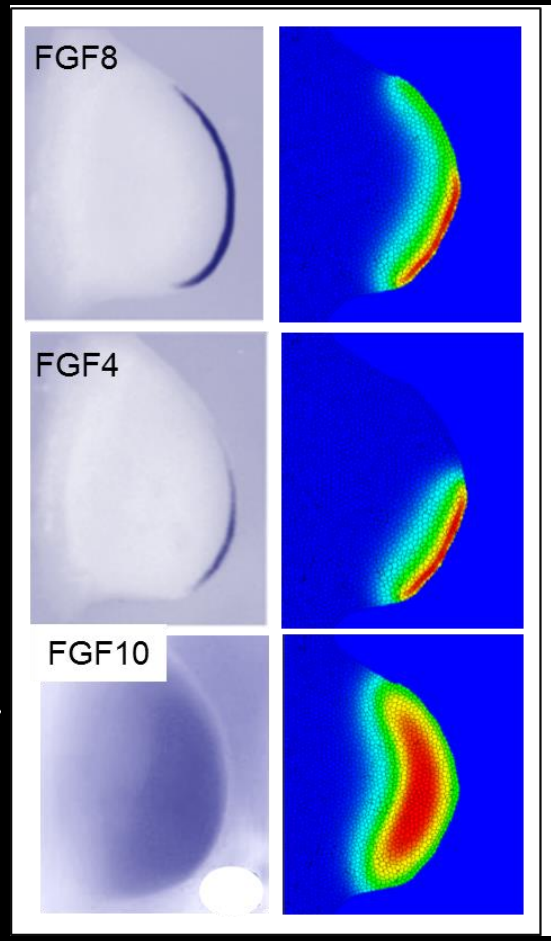


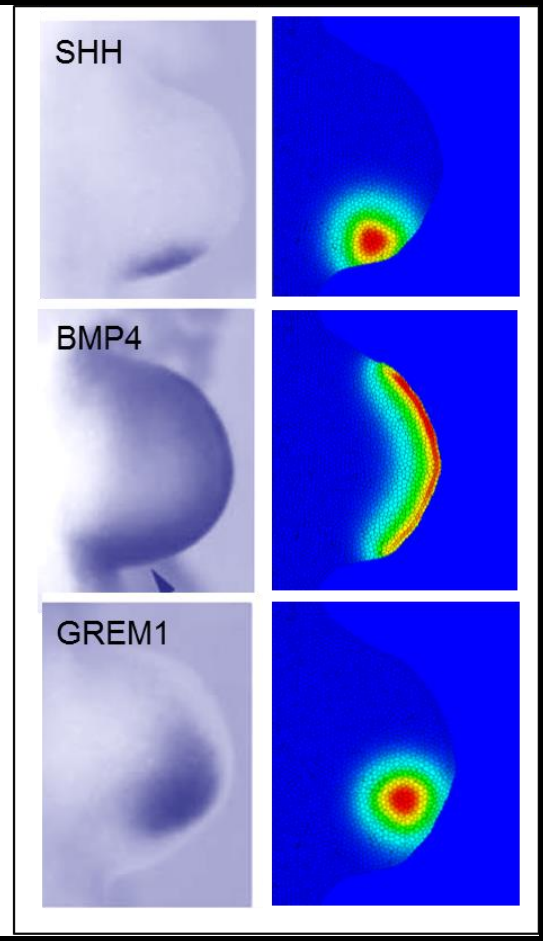
SOURCE: Dias et al. (2014) Science

Limb-bud outgrowth

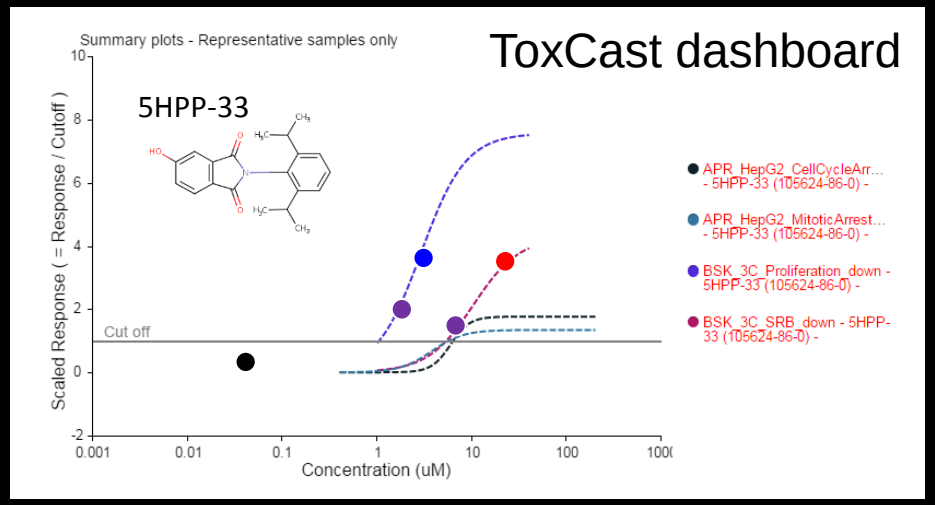


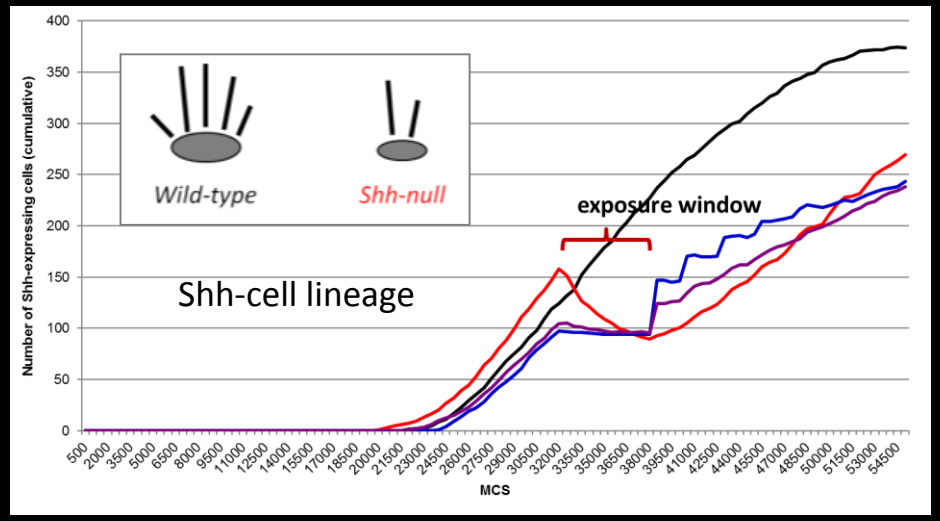


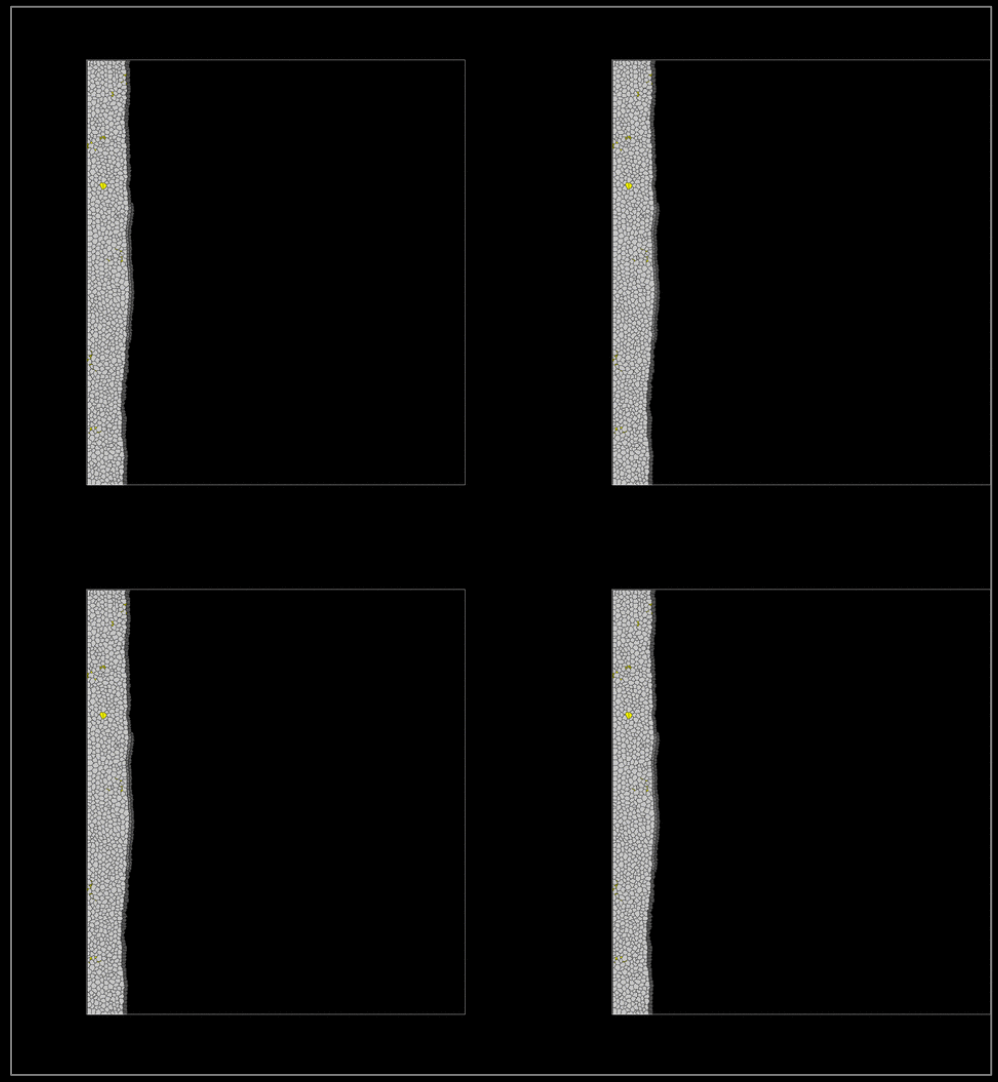




Teratogenesis *in silico*

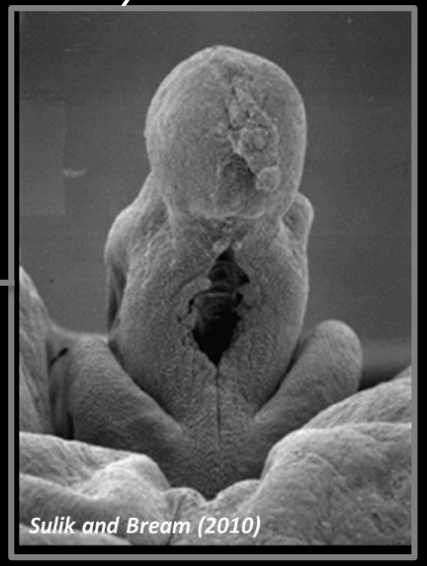




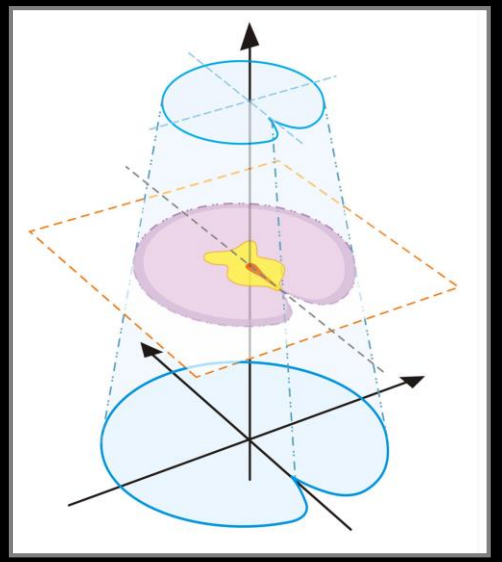


Genital Tubercle (GT) differentiation

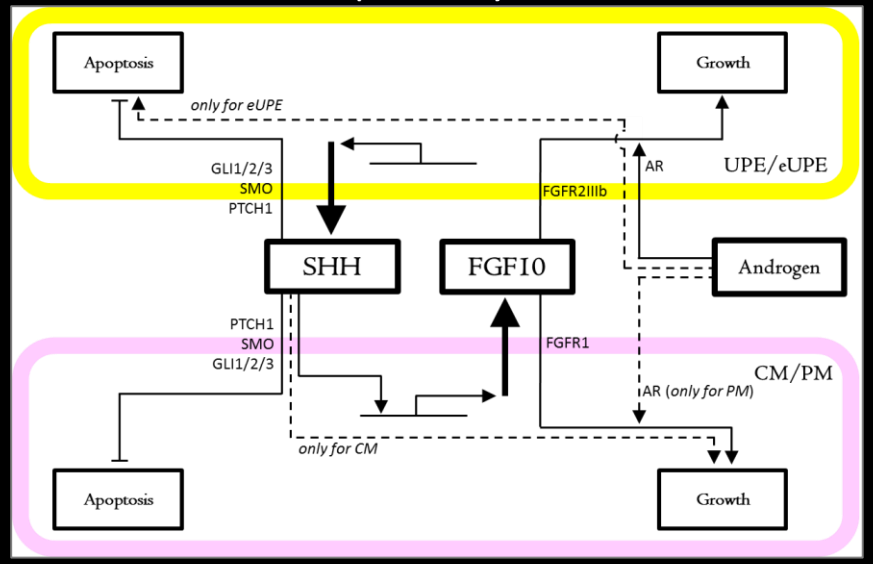
Embryonic GT



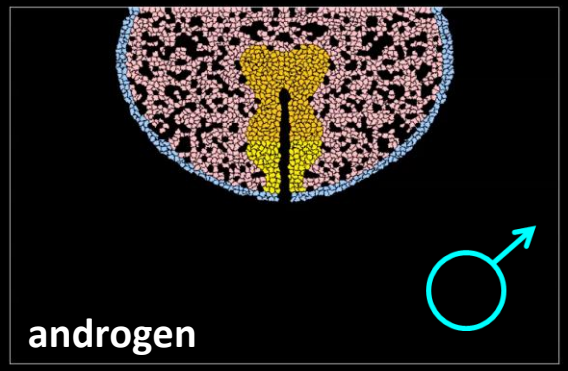
Abstracted GT

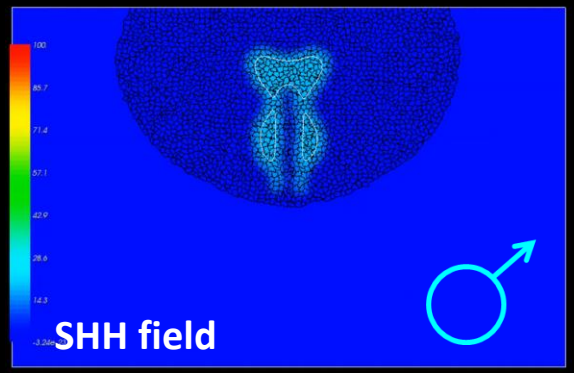


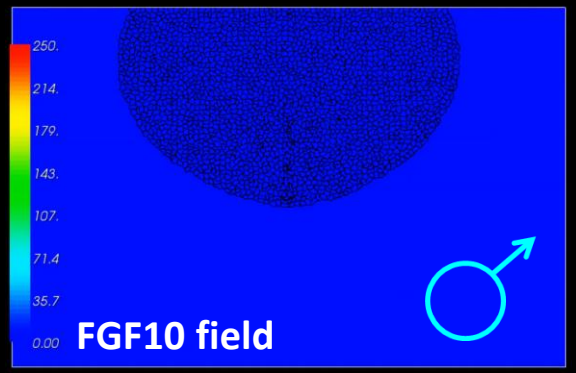
Control Network (mouse)

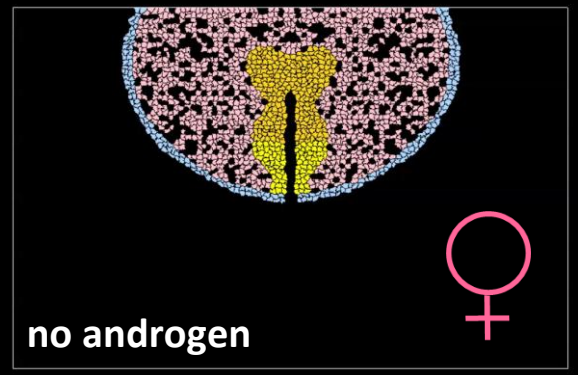


ABM simulation for sexual dimorphism (mouse GD13.5 – 17.5)



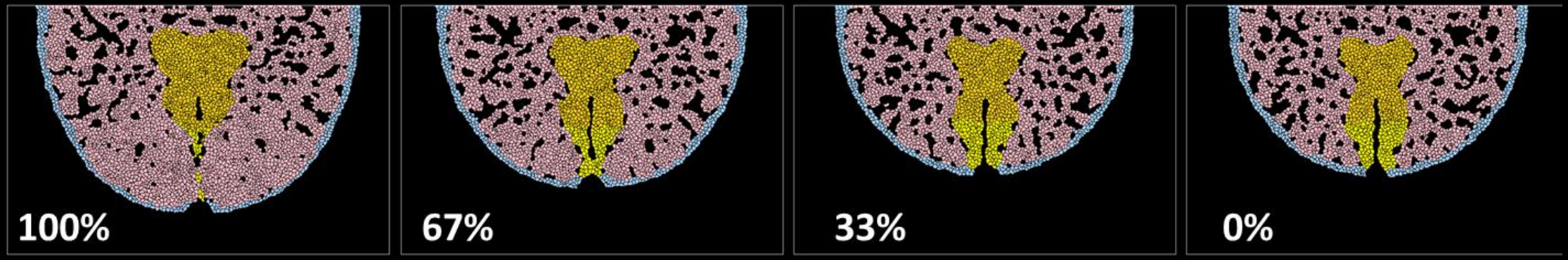




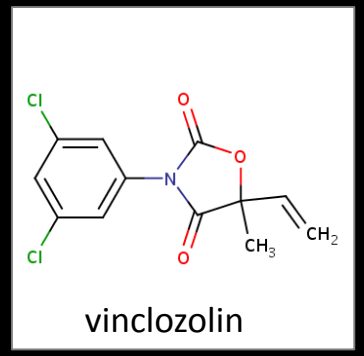


Urethral Closure: complex process disrupted in ‘hypospadias’

Driven by urethral endoderm (contact, fusion apoptosis) and androgen-dependent effects on preputial mesenchyme (proliferation, condensation, migration) via FGFR2-IIIb.

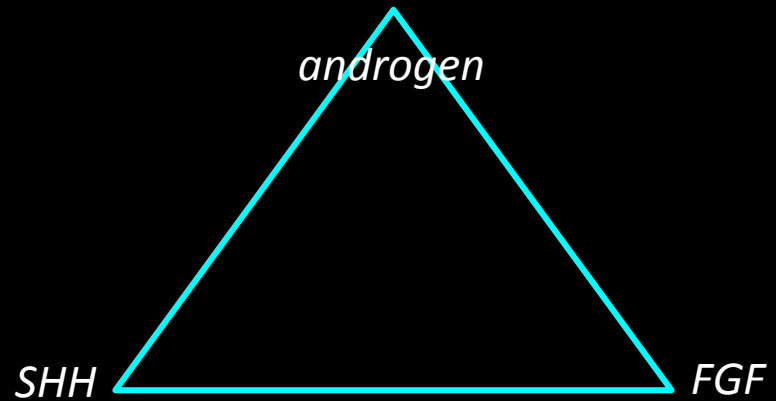


Leung et al. (2016) ***Reproductive Toxicology***

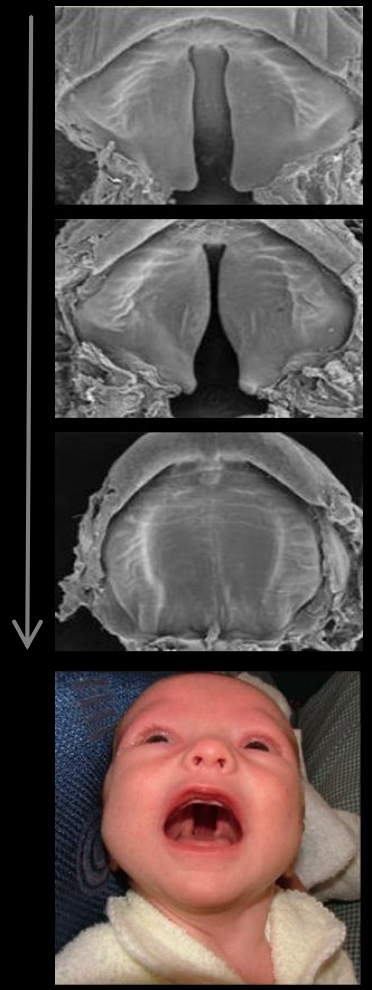


vinclozolin

Androgenization	
(n = 10 sims)	Closure Index
100%	0.80
67%	0.57
33%	0.13
0%	0.07

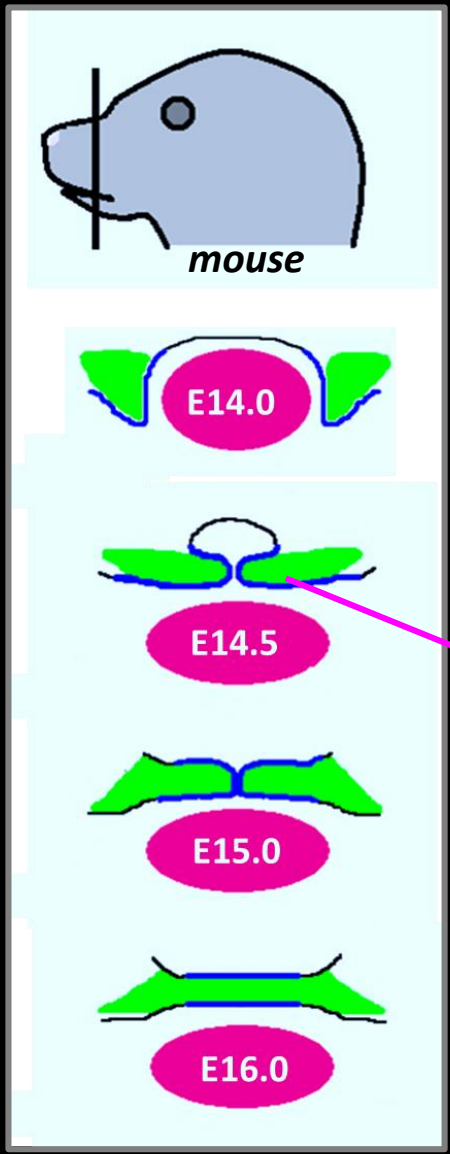


Cleft Palate

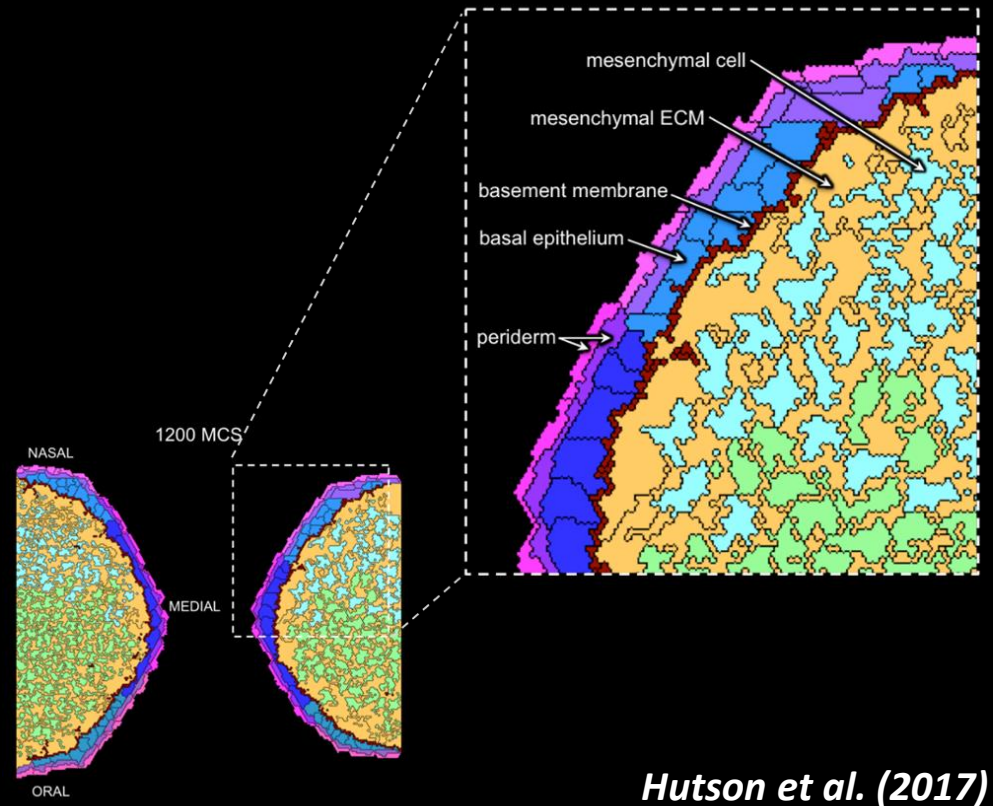


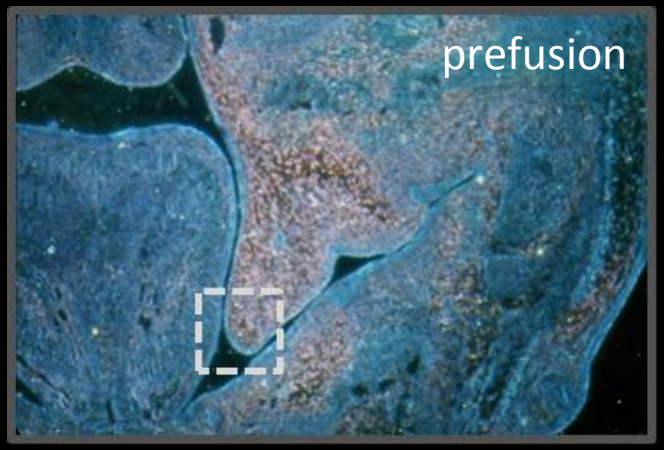
- Most prevalent craniofacial birth defect (annually ~7000 newborns in the USA and ~200,000 worldwide).
- Etiology is multifactorial: interaction of genetic, environmental, and lifestyle factors.
- Vulnerable period encompasses outgrowth of right-left palatal process in the oral cavity of the 1st trimester embryo.
- Local disruption of epithelial-mesenchymal interaction impairs outgrowth and fusion of the palatal processes.

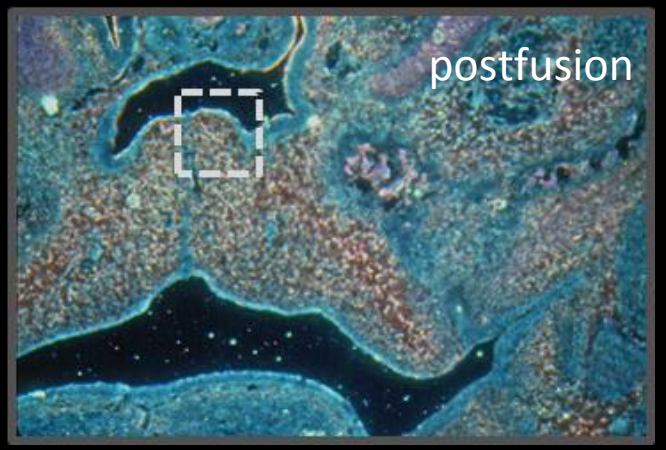
Modeling Palatal Development



- E12.5 initial outgrowth of palatal shelves
- E13.5 expansion alongside the tongue
- E14.5 elevate, meet, and adhere at medial edge
- E15.5 fusion complete, mesenchymal confluence
- E16.5 osteogenic differentiation

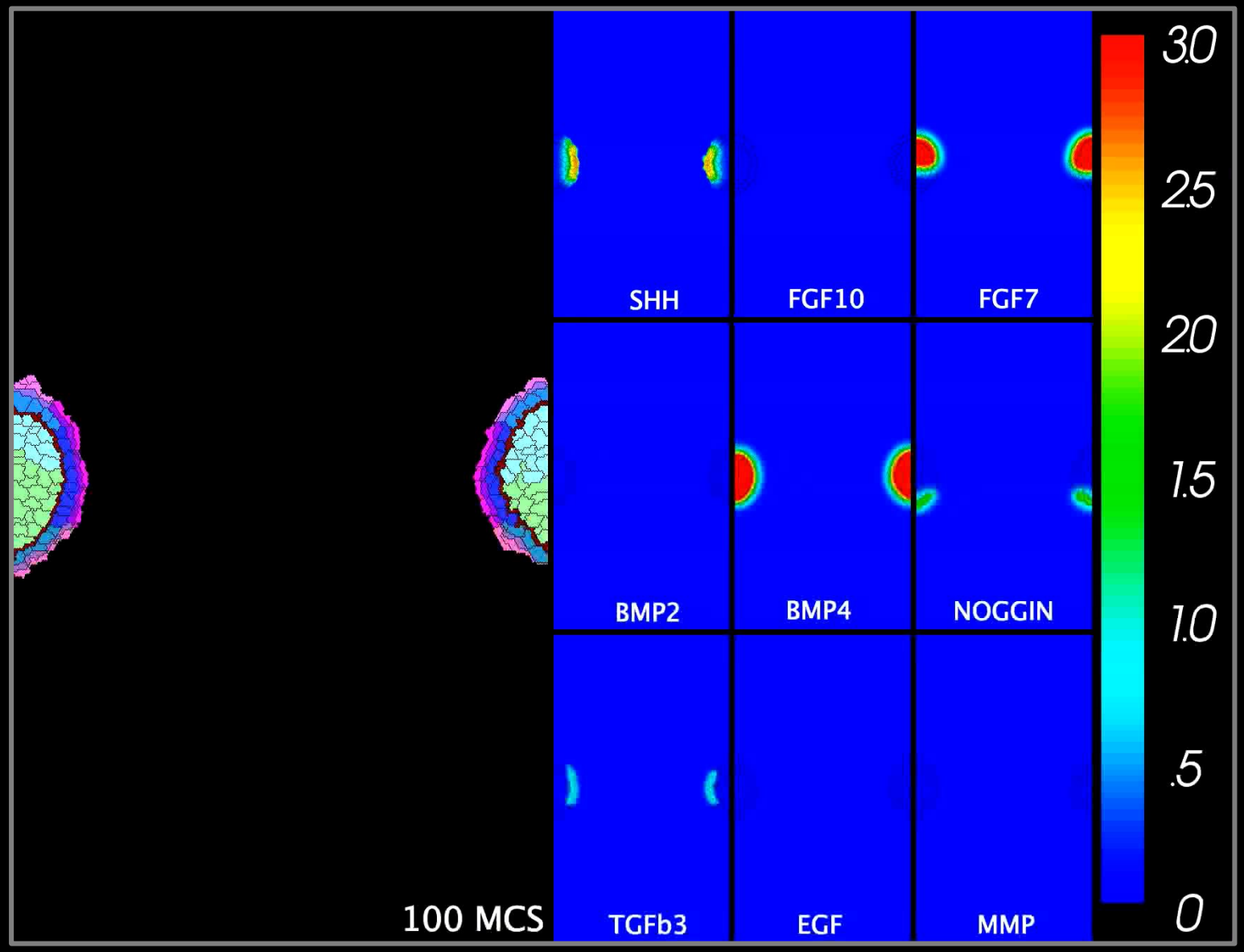






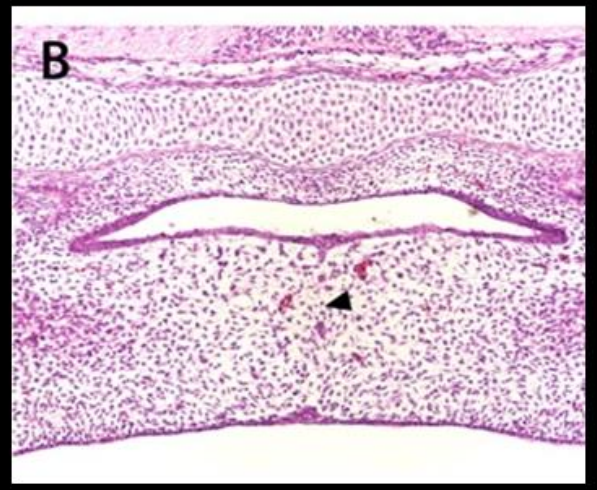
Hutson et al. (2017) (Chem Res Toxicol, in revision)

Spatially-dynamic ABMS for palate development

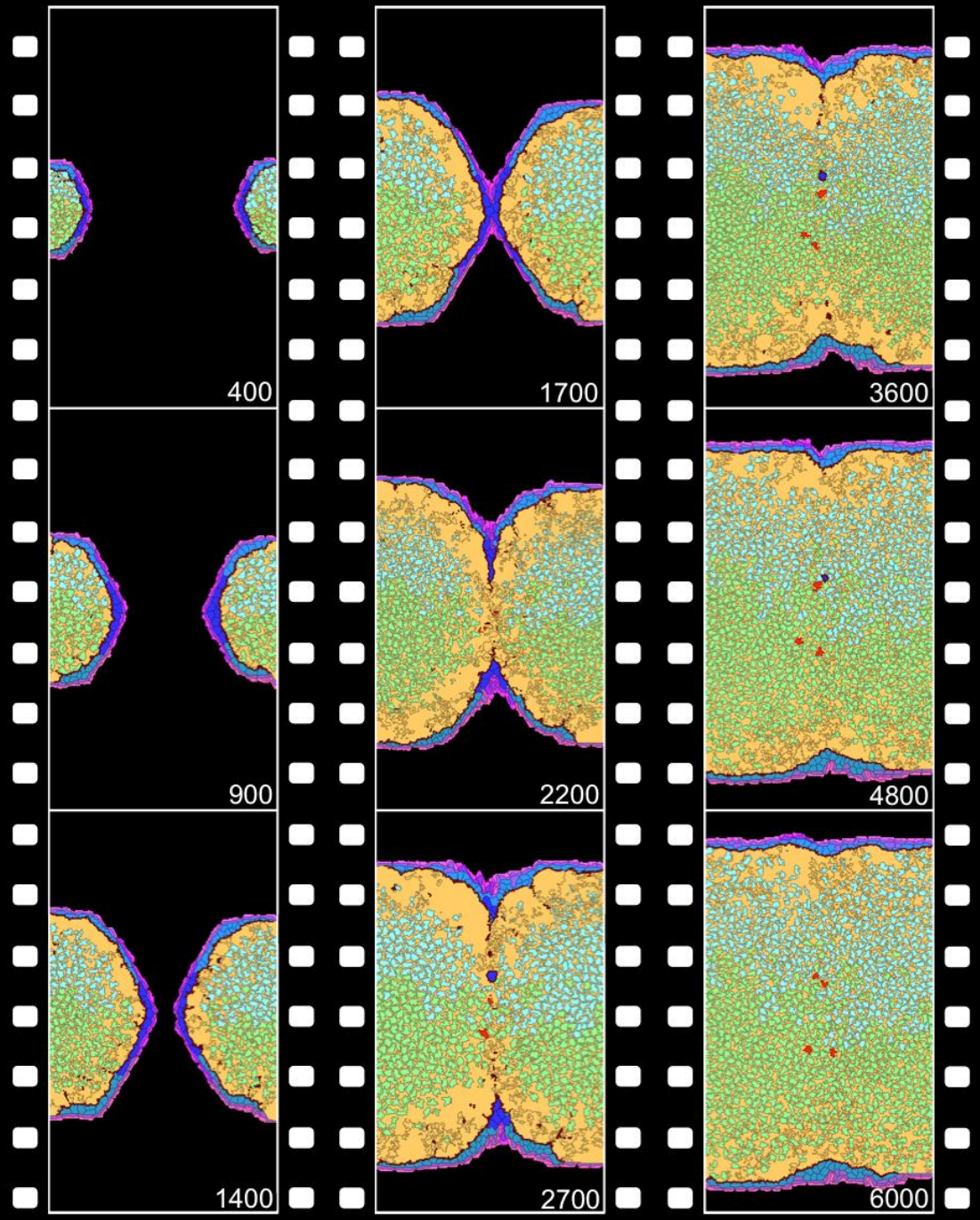


Morphogenetic fusion

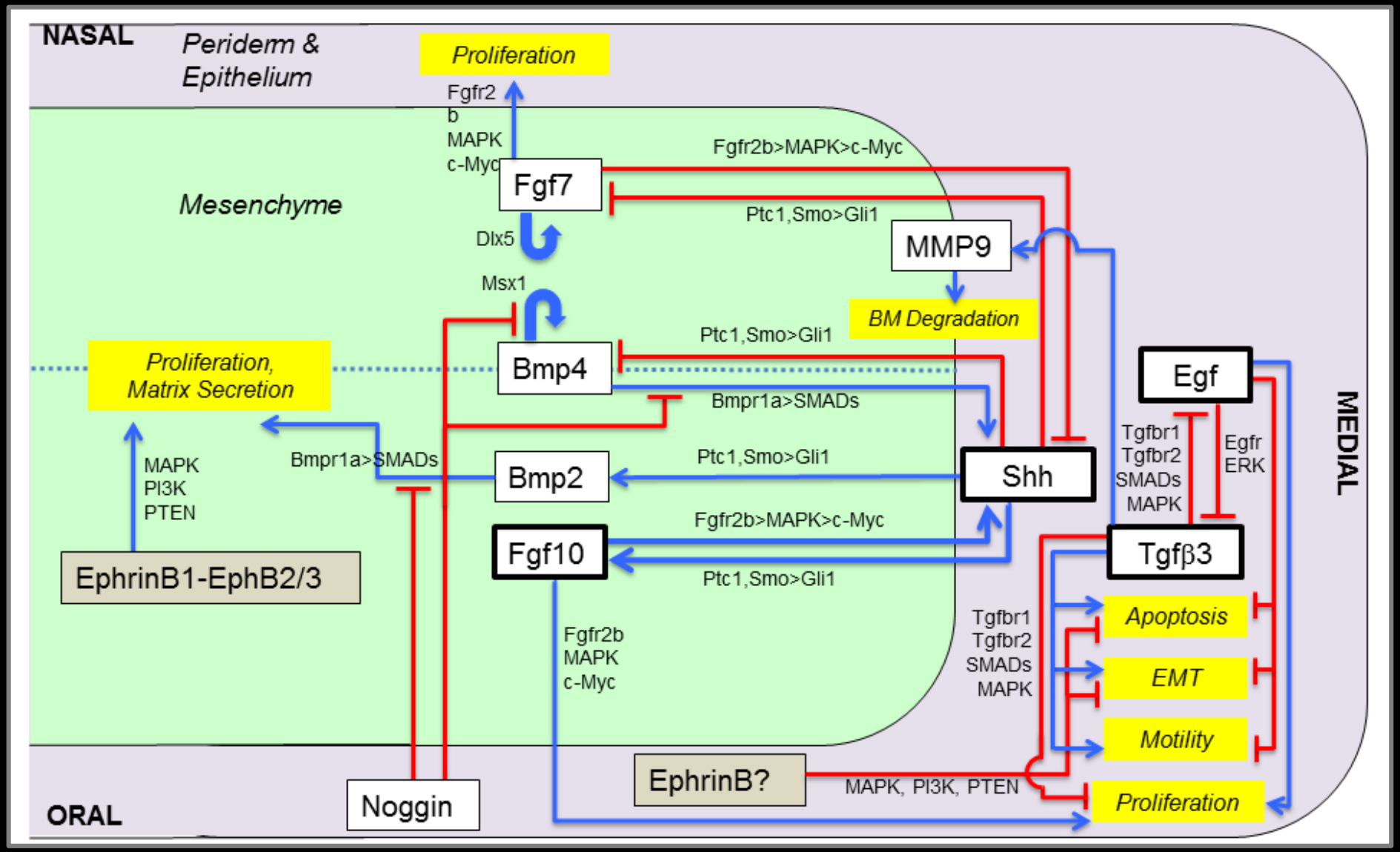
Jin and Ding (2006) Development



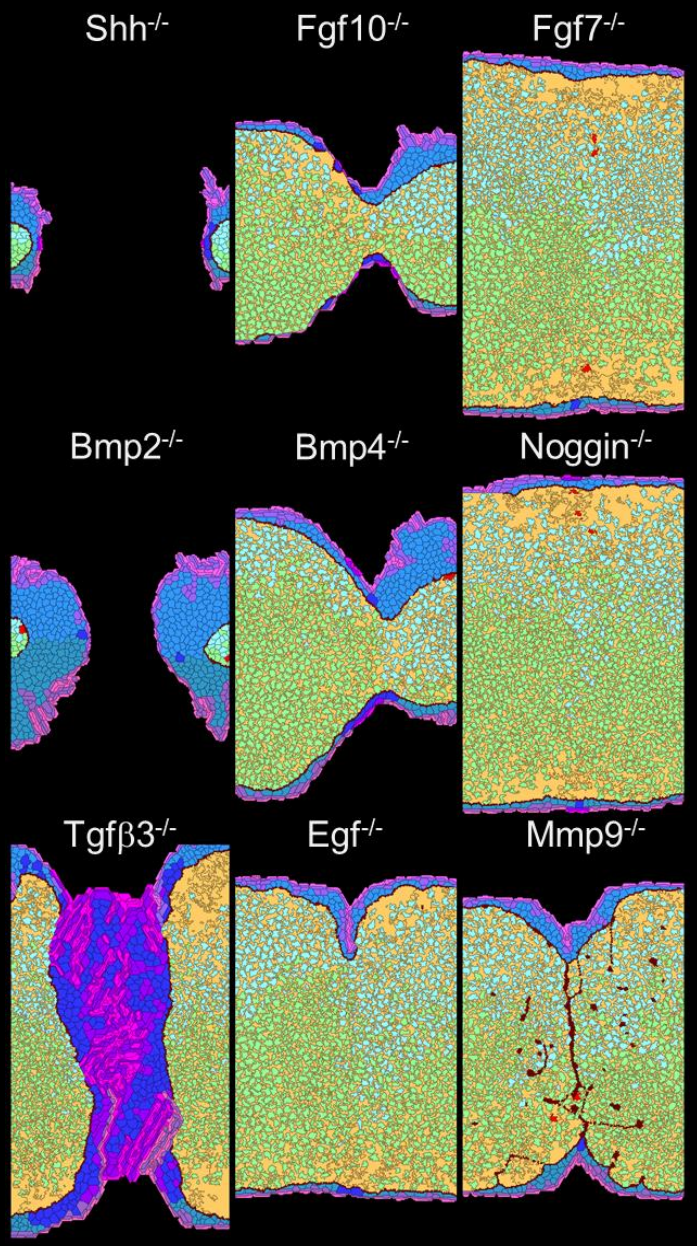
MES breakdown is programmed genetically to coincide with MEE apposition



Control network



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



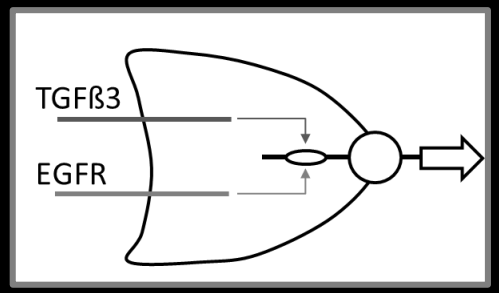
Signals driving outgrowth to apposition and MEE contact (MCS 200-2000)

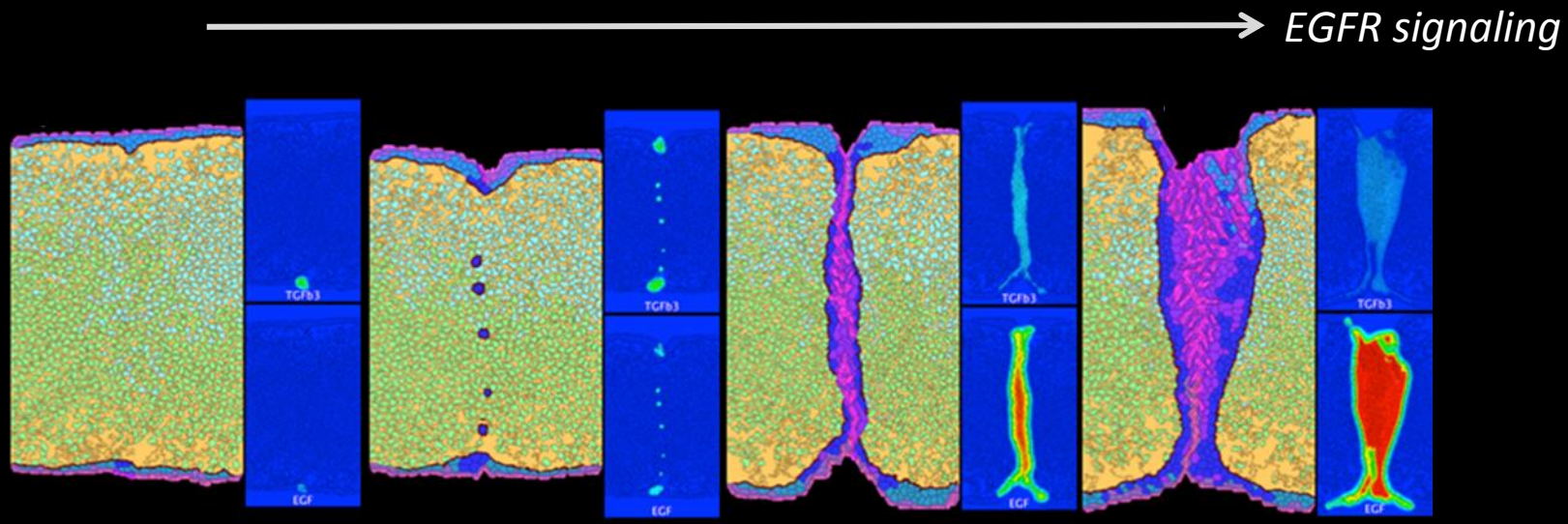
- SHH from the MEE drives mesenchymal proliferation and ECM production via FGFs/BMPs.
- Positive and negative feedback loops modulate epithelial-mesenchymal signaling cell-by-cell and interaction-by-interaction.

Signals driving MES breakdown (MCS 2000-3000)

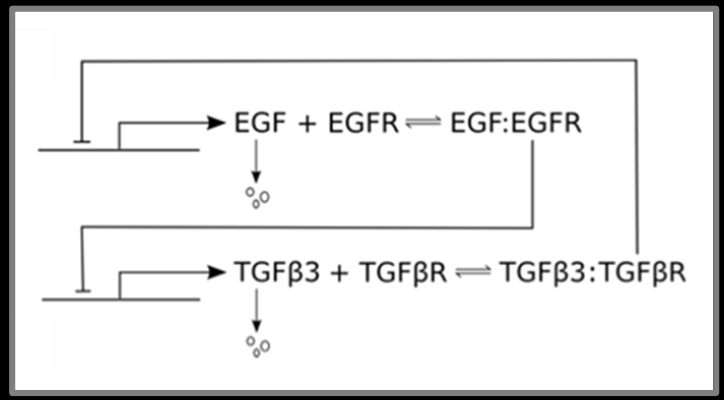
- TGFβ3 triggers MEE cells to undergo apoptosis (PCD), epithelial-mesenchymal transition (EMT), and migration (retraction).
- EGF has the opposite effect, maintaining MEE cell growth, proliferation, and survival.

Messin' with the switch: system fragility and fault tolerance



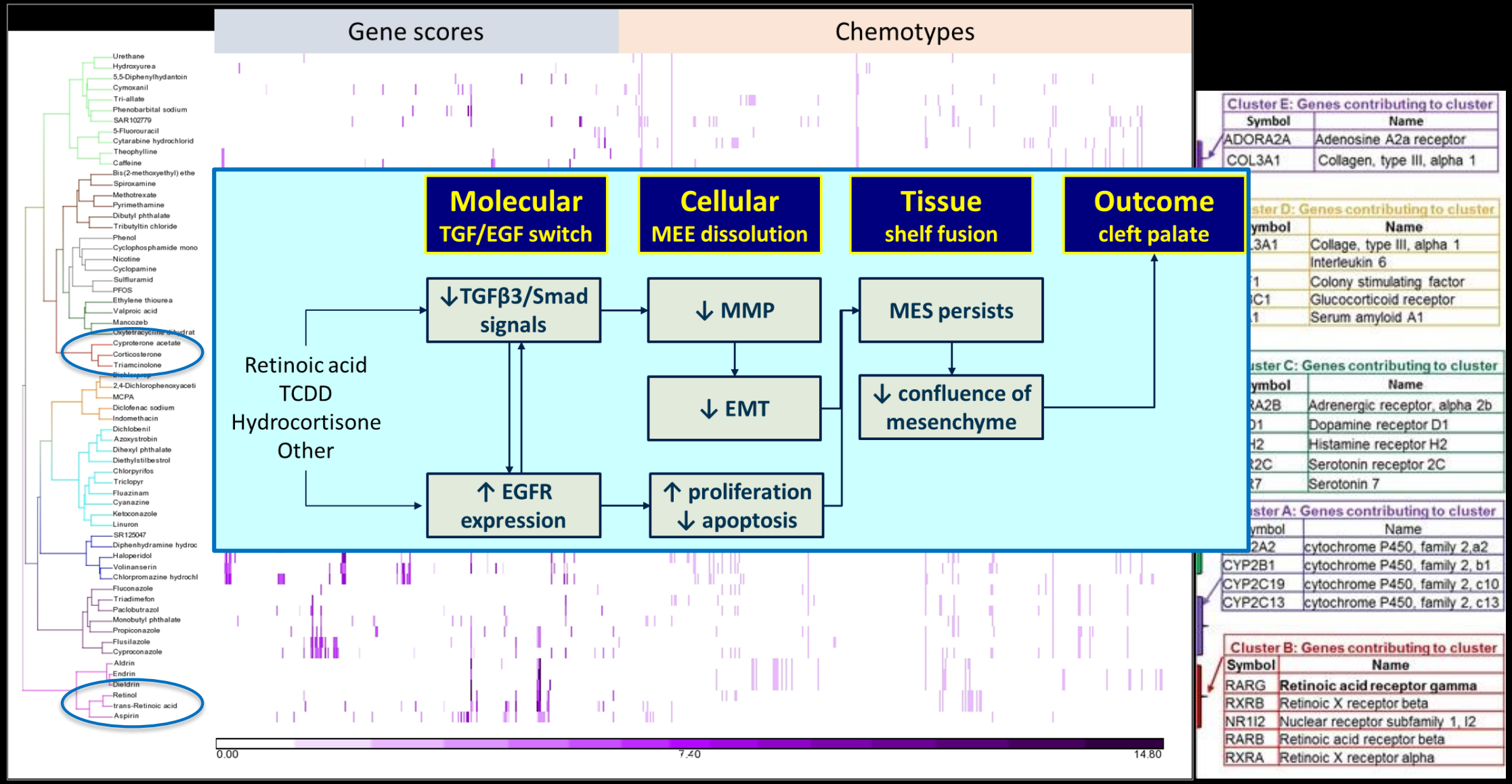


- A mutual inhibitory gene regulatory circuit exhibits switch-like behavior in the MEE.
- EGFR expression normally wanes several hours prior to MEE apposition to flip the switch to the TGFβ3 state.
- Several cleft palate teratogens are known to maintain EGFR expression (Retinoic acid, Hydrocortisone, TCDD) [Abbott 2010]).



63 cleft palate (animal) teratogens in ToxCast

Baker et al. (in preparation)



Cluster E: Genes contributing to cluster

Symbol	Name
ADORA2A	Adenosine A2a receptor
COL3A1	Collagen, type III, alpha 1

ster D: Genes contributing to cluster

ymbol	Name
L3A1	Collage, type III, alpha 1
	Interleukin 6
1	Colony stimulating factor
C1	Glucocorticoid receptor
1	Serum amyloid A1

uster C: Genes contributing to cluster

ymbol	Name
RA2B	Adrenergic receptor, alpha 2b
D1	Dopamine receptor D1
H2	Histamine receptor H2
R2C	Serotonin receptor 2C
R7	Serotonin 7

ster A: Genes contributing to cluster

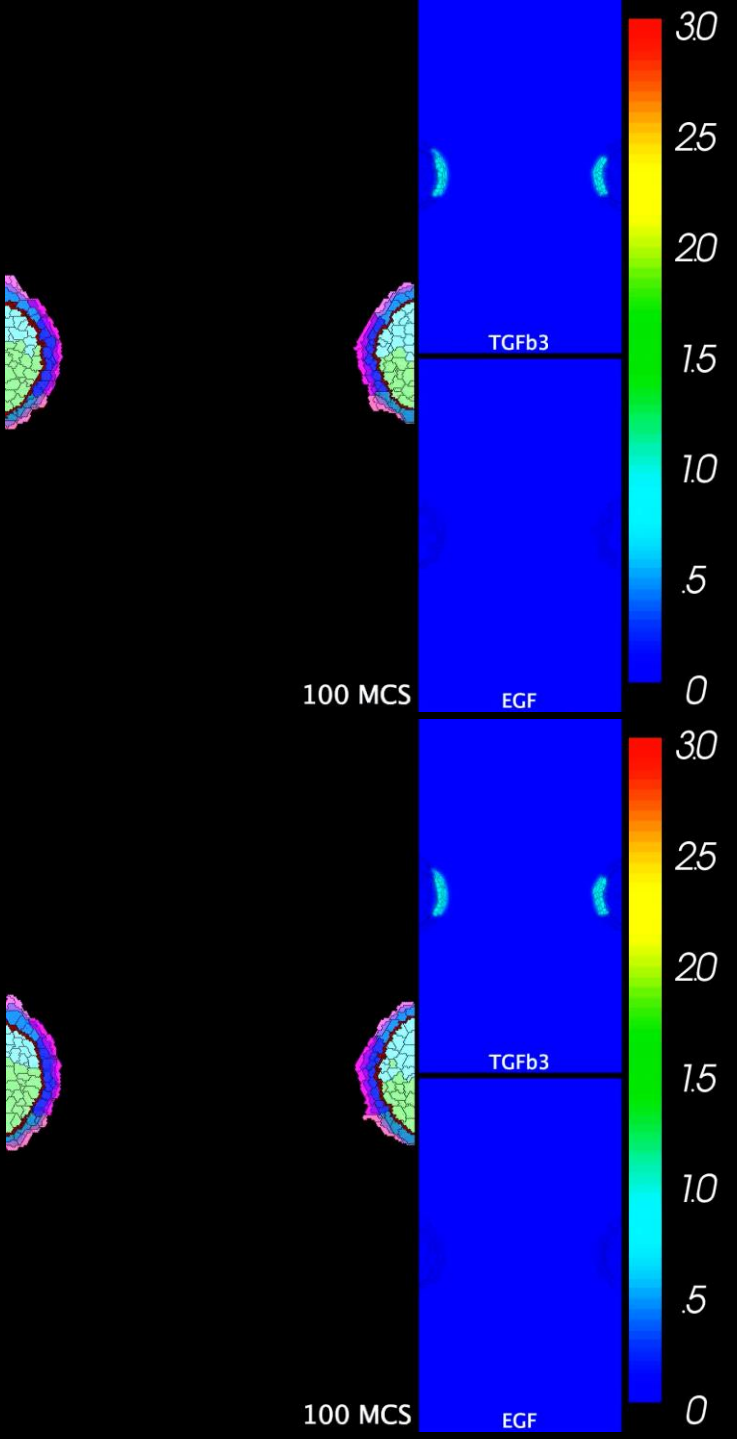
ymbol	Name
2A2	cytochrome P450, family 2,a2
CYP2B1	cytochrome P450, family 2, b1
CYP2C19	cytochrome P450, family 2, c10
CYP2C13	cytochrome P450, family 2, c13

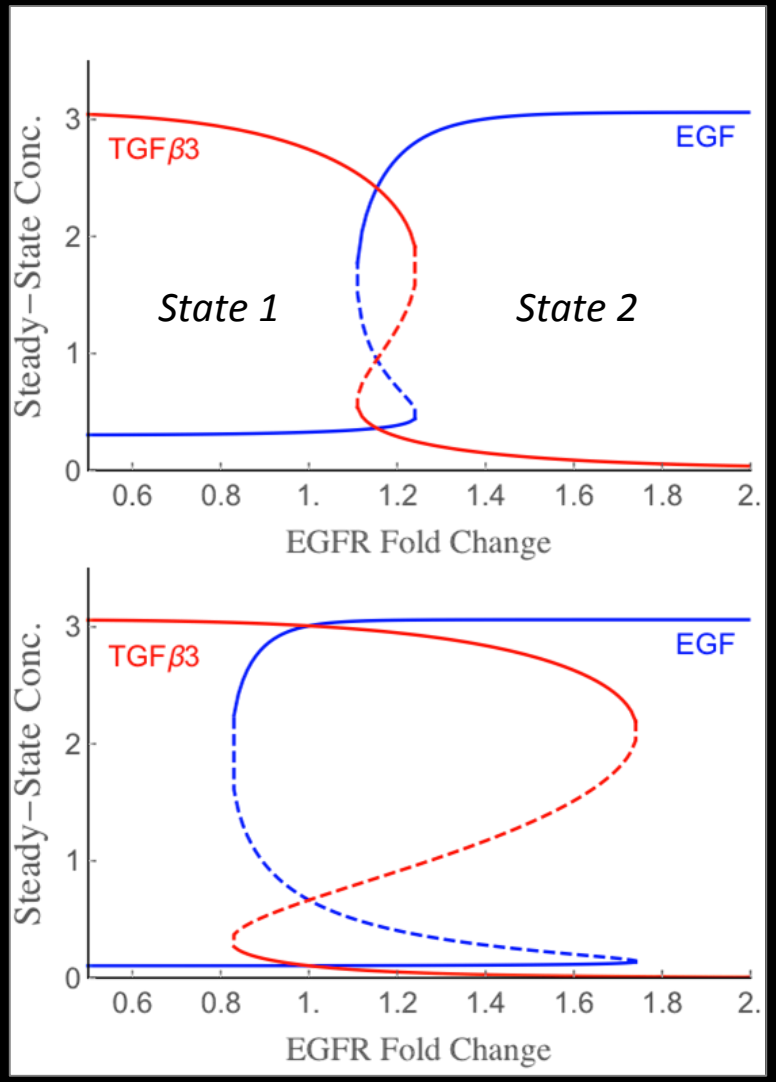
Cluster B: Genes contributing to cluster

Symbol	Name
RARG	**Retinoic acid receptor gamma**
RXRB	Retinoic X receptor beta
NR1I2	Nuclear receptor subfamily 1, I2
RARB	Retinoic acid receptor beta
RXRA	Retinoic X receptor alpha

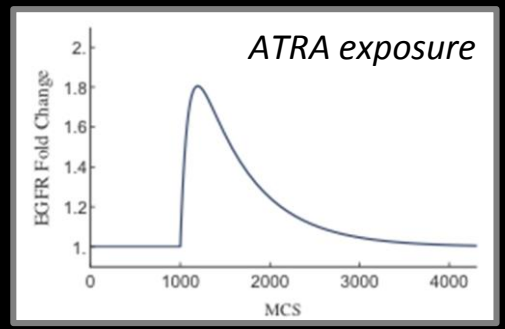
Dissecting circuit dynamics *in silico*:

two scenarios for differential teratogenicity





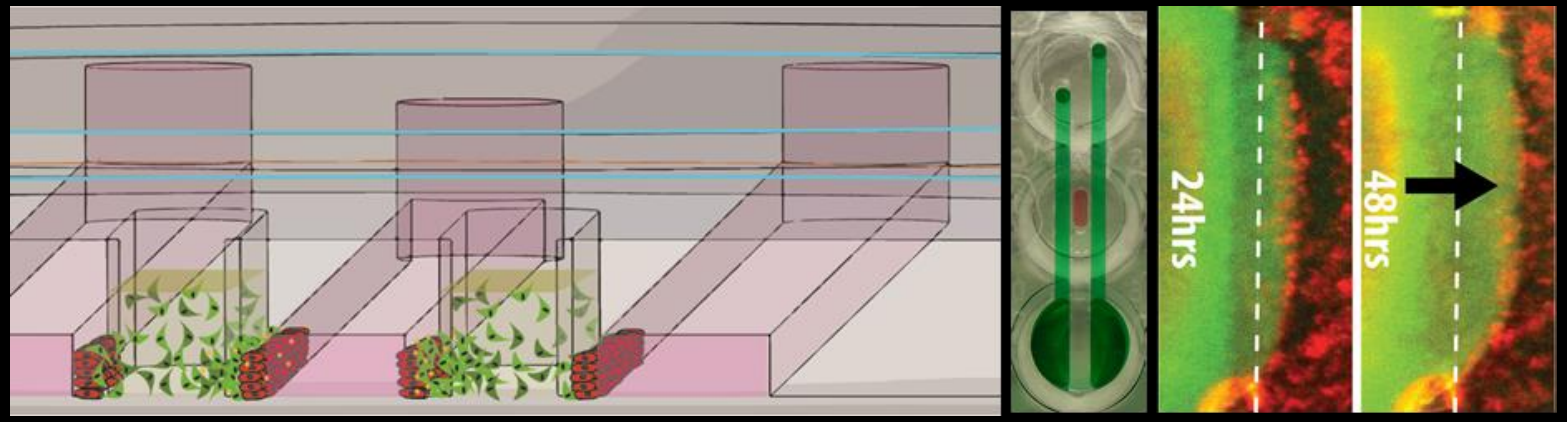
tipping point >1.8x (n=24)
(reversible)



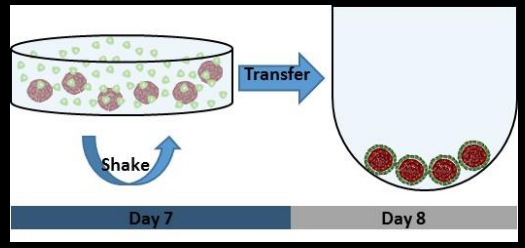
tipping point ~1.5x (n=16)
(not reversible)

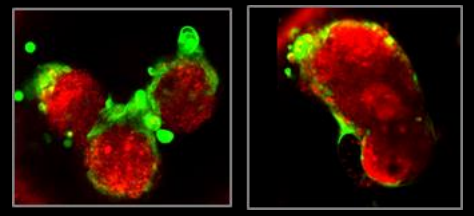
Microscale systems: engineered 3D OCMs that use human cells for predictive toxicology

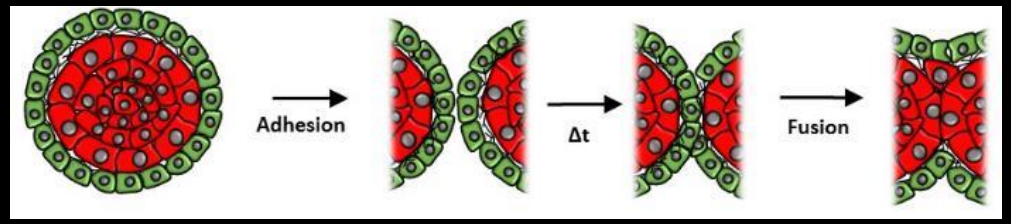
Beebe lab – U Wisconsin, HMAPS [Johnson et al.] – with permission

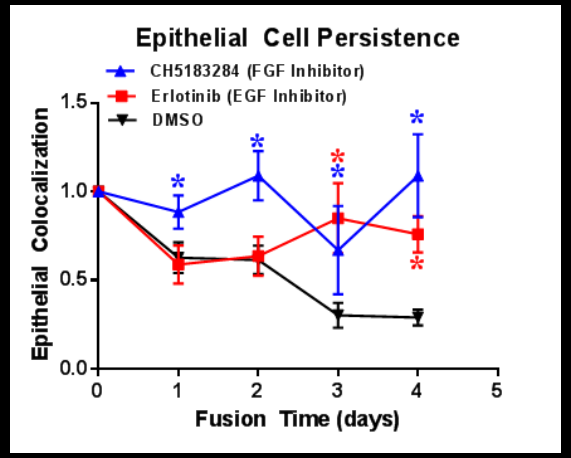


Abbott lab - NHEERL/TAD [Belair et al.] – with permission









VTM WORKFLOW

bioactivity profiles

⇩

biological circuits

⇩

cellular dynamics

⇩

ABMS 'cybermorphs'

⇩

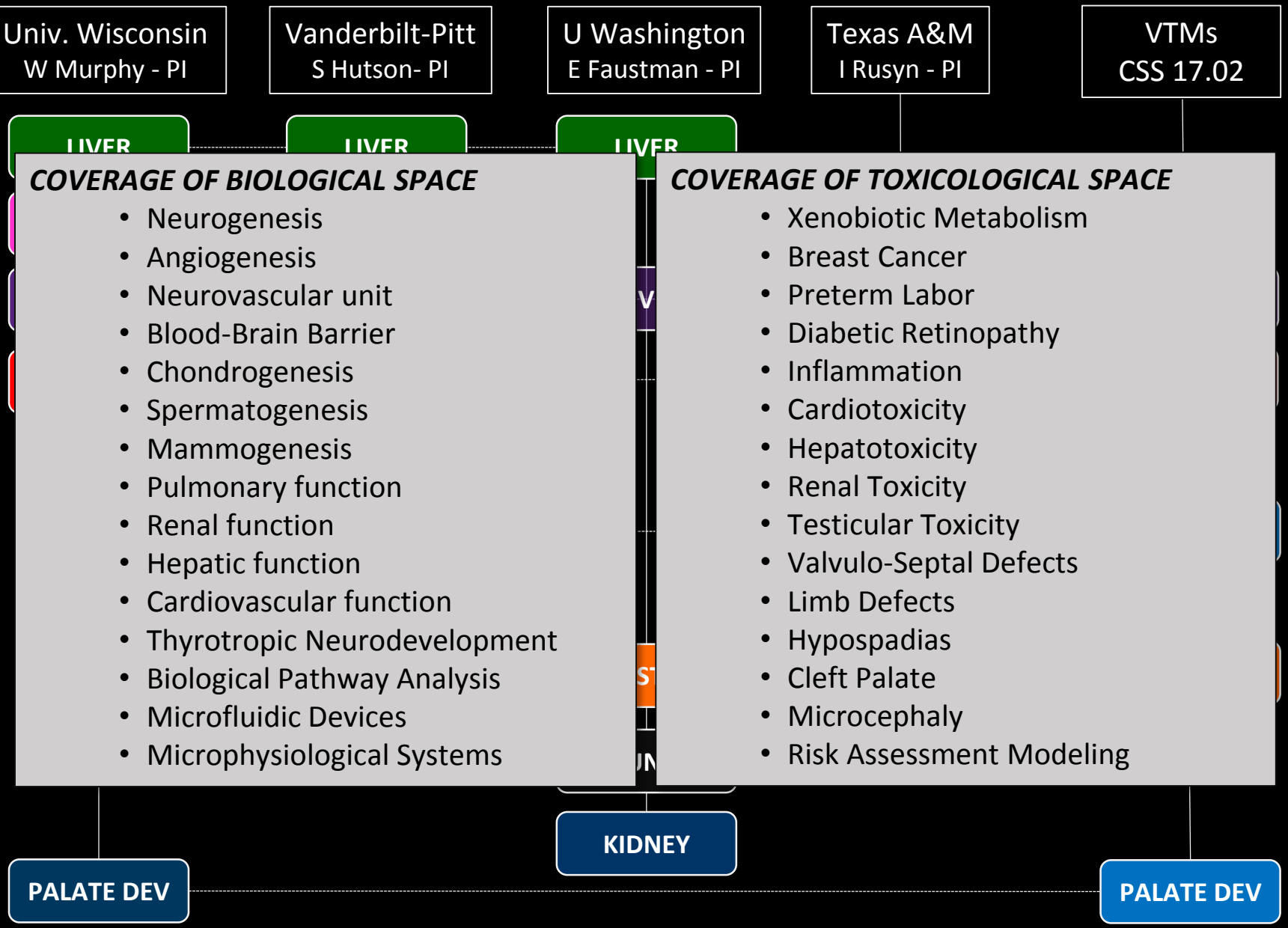
organotypic cultures

⇩

functional analysis

Integration with OCM-PT STAR Centers

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program Grant #



Computational and Organotypic Modeling of Microcephaly

Knudsen TB[1], Baker NC[2], Faustman EM[3], Murphy WL[4] and Daly W[4].

[1]USEPA, National Center for Computational Toxicology; and [2]Lockheed-Martin, Research Triangle Park NC

[3]University of Washington, UW-PT Center, Seattle WA; and [4]University of Wisconsin, HMAPs Center, Madison WI

Microcephaly and Maternal Zika Infection

- Cluster of birth defects in N Brazil linked to mosquito-borne Zika virus by the Brazilian Health Ministry (November) and validated by CDC (December) [1].

Science (2016)

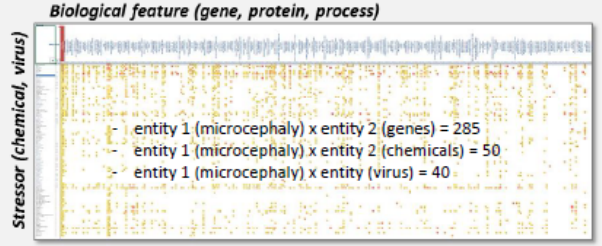
N Engl J Med (2016)

"On the basis of this review, we conclude that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies."

- Reduction in brain volume, ventricular dilations, brain calcifications, retinal defects, and placental insufficiency are all part of the congenital Zika story.
- A broader scientific need exists for Adverse Outcome Pathway (AOP) models of microcephaly because it has many possible causes [2]:
  - intrauterine infections (e.g., Rubella, CMV, ZikV)
  - inborn errors of metabolism (e.g., urea cycle, mitochondriopathies)
  - maternal smoking, drug and alcohol abuse
  - environmental chemicals (e.g., methylmercury)
  - genetic factors (autosomal recessive traits; microdeletions, duplications)
  - prenatal malnutrition, socioeconomic factors, ...
- OBJECTIVE: capture information on 'microcephaly' into an AOP framework.

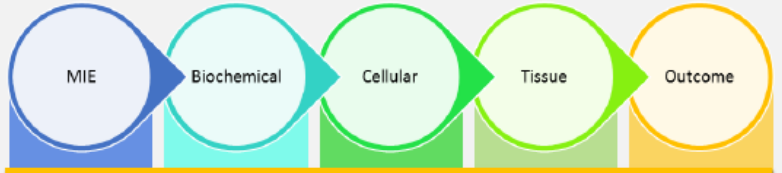
Microcephaly Information Retrieval

- MGI Mammalian Phenotype Browser: 'microcephaly' (MP:0000433) returns 85 gene associations including candidate genes for microcephaly in humans.
- ToxRefDB returns ~75 chemicals invoking dilated ventricles/hydrocephaly (39), and/or reduced brain size/cellular mass (40).
- *MicrocephalyConnections* tool sweeps literature (PubMed) to produce a multidimensional database of MeSH co-annotations (350,651 records).



- ToxCast high-throughput screening (HTS) data for bioactivity profiling (https://actor.epa.gov/dashboard/), in progress.

Adverse Outcome Pathway (AOP)



KEY EVENT 4: *reduced neurogenic capacity in the ventricular zone of the brain, leading to cortical thinning and reduced brain size at the 2nd trimester.*

- Human brain size is determined by the number of neurogenic cells available to form neocortex and is a function of the precursor pool size of neuroprogenitor cells (NPCs) [3].
- NPCs self-replicate in the ventricular zone of the brain during 1st trimester; this growth period is followed by differentiation to form neocortex by the 2nd trimester [4].

KEY EVENT 3: *altered neuroprogenitor growth kinetics, leading to hypoplasia of the neurogenic niche in the 1st trimester.*

- Zika virions infect hNPCs (but not hES cells or neurons) *in vitro*, and the resulting consequences on cell growth/apoptosis has a demonstrable effect on hNPC-derived neurosphere size [5,6,7].
- Chemical injury (alcohol, methylmercury) during rodent neurodevelopment alters NPC pool sizes via adverse effects on the growth kinetics (cell growth/migration/apoptosis) [8].

KEY EVENT 2: *misorientation of hNPC mitotic division, leading to premature loss of neuroprogenitors from the proliferative cycle.*

- hNPCs divide symmetrically before switching to asymmetrical (neurogenic) divisions; premature switching (or apoptosis) results in loss of NPCs from the proliferative cycle [3,4].
- Alignment of the mitotic spindle determines the polarity of mitotic divisions to self-replicate hNPCs (equal division) or spinoff a daughter cell that enters the neurogenic lineage (unequal division) [9].

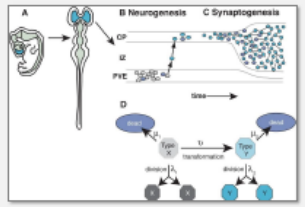
KEY EVENT 1: *dysregulation of the centrosome cycle, leading to misalignment of the centrioles and the microtubule organizing center of the cell.*

- Many candidate genes for human 'primary microcephaly' function in the structural organization and regulation of the centrosome, containing two centrioles at right angles to each other [10].
- Transcriptomic analysis showed hNPCs express five genes for human primary microcephaly (MCPH1, ASPM, CENJ, STIL, CDSRAP2) as an indication of an centrosomal cycle for further investigation of this hypothesis.

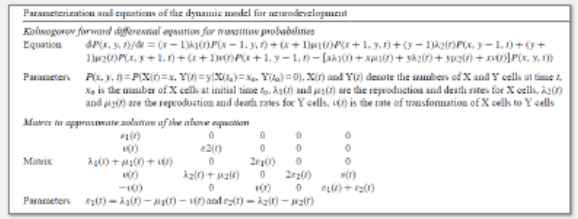
MOLECULAR INITIATING EVENT: *ToxCast bioactivity profiles are a resource for building predictive signature(s) for microcephaly.*

- 30 ToxRefDB chemicals that invoke decreases in brain developmental parameters had bioactivity profiles mapping to one or more targets in a ToxCast assay (top gene scores = p53, NRF2, PXR, AhR, HSF1, VDR, ...).
- Bioactivity profiles are being used to build predictive signatures for microcephaly that can be used in modeling the system, both *in silico* (computational) and *in vitro* (human brain mimics).

Computational Model of Neurodevelopment

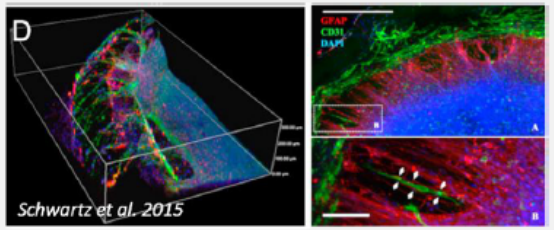


LEFT: model for the neurogenic switch to assess criticality of NPC loss on neurodevelopment [8]. BELOW: parameters to simulate NPC dynamics mathematically following chemical (or viral) exposure [9]. This model can be applied to simulate Key Events 3-4 in the AOP.



Human Brain Mimics

Dynamics of hNPC growth, migration, and apoptosis for the computational model can be assessed in miniorganoids developed from hNPCs + iPSC-derived endothelial and microglia [11]. Studies are planned to develop human brain mimics from microcephalic patient-derived iPSCs and to provide evidence for Key Events 1-3 in the AOP for chemicals and Zika.



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[5] Tang et al. (2016) Cell Stem Cell 18
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[9] Faustman et al. (2005) Envi Toxicol Pharmacol 19
[10] Barbelanne and Tsang (2014) Biomed Res International
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DISCLAIMER: The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

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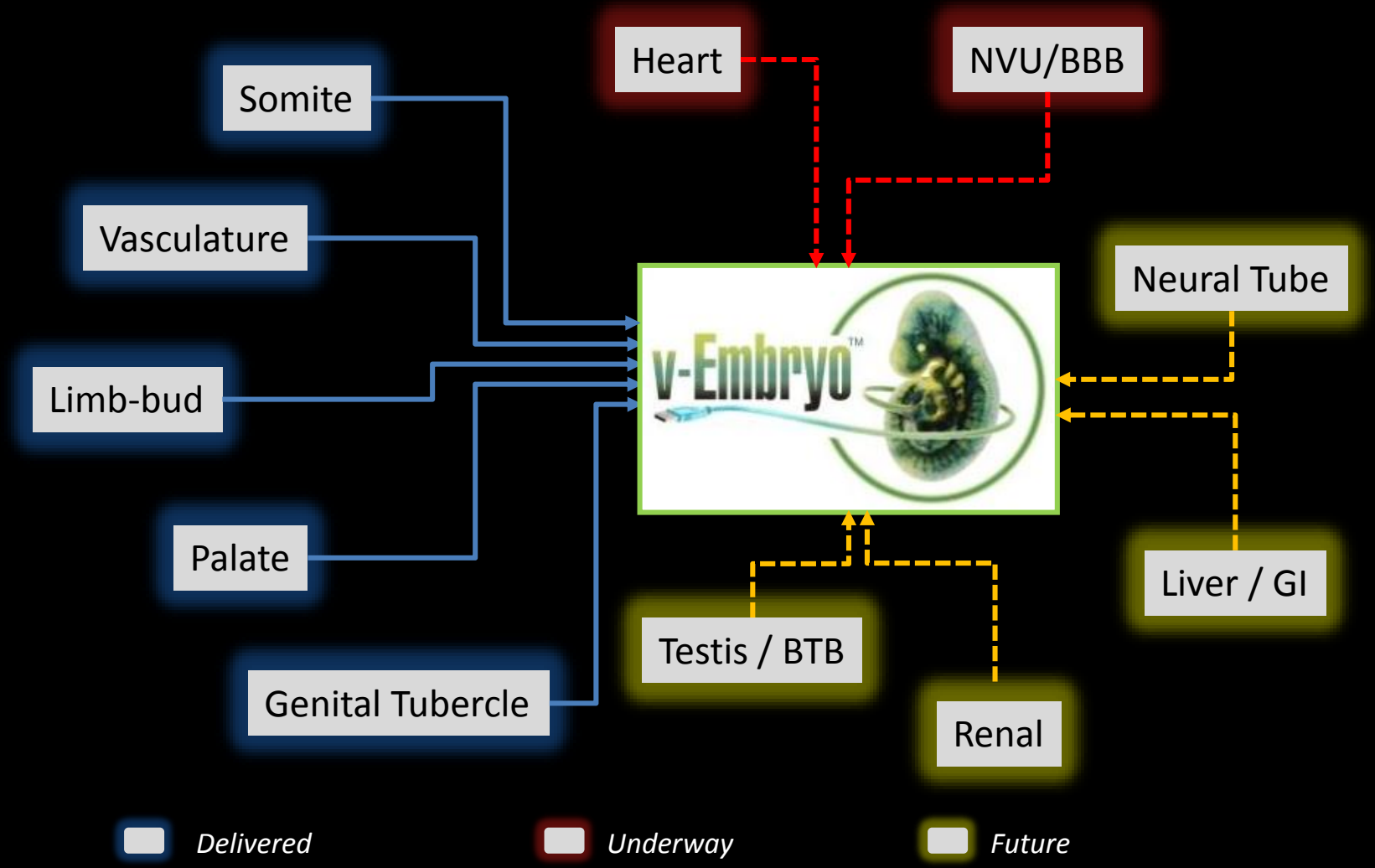
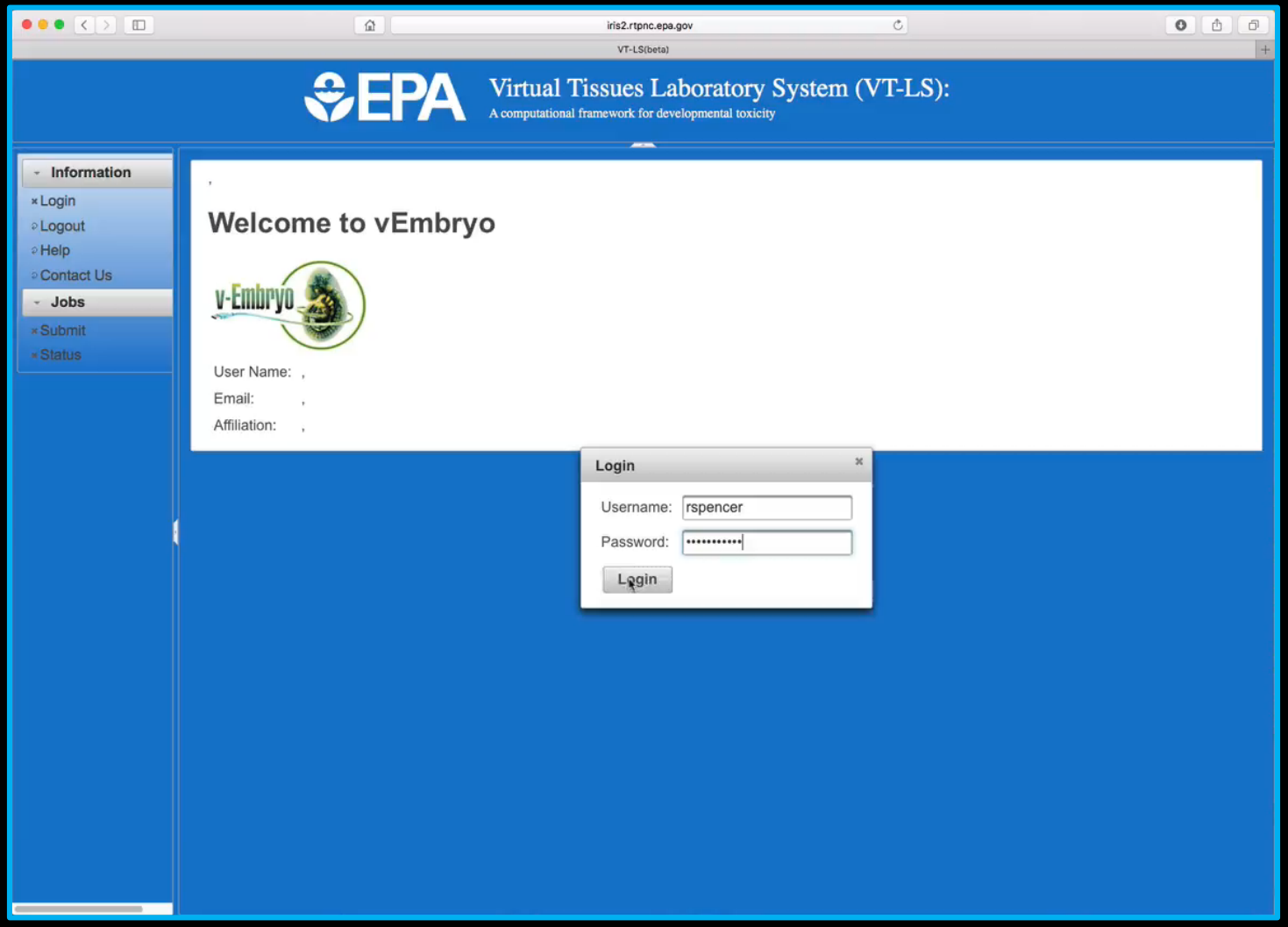
Toward a 'Virtual Embryo'



- *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 (revision).*
- *Leung et al. (2016) Reprod Toxicol.*
- *Zurlinden/Saili et al. (FY17 product).*
- *Hunter et al. (FY18 product).*
- *Your name here.*

Virtual Tissue Laboratory System (VTLS)



EMVL: Spencer, Balabin, Cathy, Transue, Howard

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- Bhavesh Ahir – NCCT (now U Illinois – Chicago)
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- Nisha Sipes – NCCT (now NTP)
- Richard Spencer – Leidos / EMVL
- Nancy Baker – Leidos / NCCT
- Ed Carney† – Dow Chemical Company
- Rob Ellis-Hutchings – Dow Chemical Company
- Tuula Heinonen – U Tampere / FICAM
- Jessica Palmer – Stemina Biomarker Discovery
- Parth Kothiya – NCCT (now Indiana U)
- James Glazier – Indiana U (TIVS)
- Shane Hutson – Vanderbilt U (VPROMPT)
- Kate Saili – NCCT
- Todd Zurlinden – NCCT
- BeiBei Cai – Vala Sciences
- Dan Rines – Vala Sciences
- Jill Franzosa – NCCT (now CSS)
- Brian Johnson – U Wisconsin (HMAPS)
- Eric Nguyen – U Wisconsin (HMAPS)
- William Murphy – U Wisconsin (HMAPS)
- William Daly – U Wisconsin (HMAPS)
- Tamara Tal – NHEERL/ISTD
- David Belair – NHEERL/ISTD
- Barbara Abbott – NHEERL/ISTD
- Imran Shah - NCCT
- Alex Tseutaki – HS intern

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