SOT Risk Assessment Specialty Section (RASS)
Webinar April 12, 2023

Engineering Complex Systems for Predictive Toxicology in the Animal-free Zone

Modeling Cellular Dynamics *in silico*

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DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

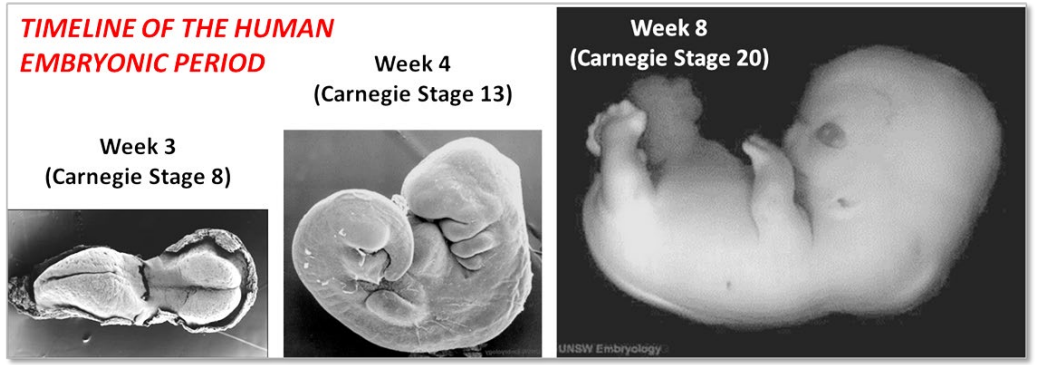
In a nutshell …

- New Approach Methods (NAMs) refers to 21st-C testing strategies that rely on *in vitro* data and *in silico* models to predict human toxicity with less reliance on animal testing.
- Complex NAMs that accurately predict the potential for human developmental toxicity are needed to succeed or supersede conventional testing in pregnant animals.
- Most *in vitro* assays lack the positional information, physical constraints, and regional organization of a multicellular system undergoing morphogenesis and development.
- Embryo-inspired computational (*in silico*) models with emergent, self-organizing capacity can simulate critical phase transitions during developmental processes and toxicities.
- Will a virtual tissue model of physical trajectory hold up to the mechanistic veracity needed to reliably predict toxicological outcome(s) in a complex system?

Developmental toxicity: *assessing chemical risks to the embryo*

"The first trimester is the most crucial to your baby's development. During this period, your baby's body structure and organ systems develop."

www.ucsfhealth.org

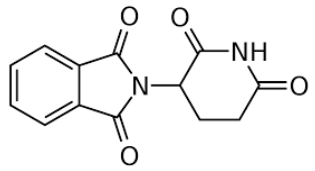


peak sensitivity (3rd – 8th wk)

Embryonic Period ***Fetal Period***

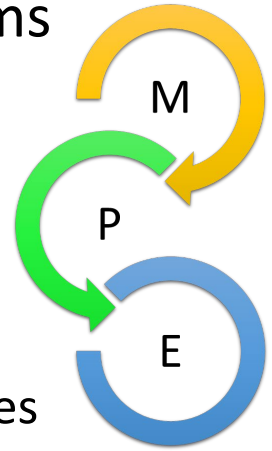
T1 **T2** **T3**

OECD TG 414
OPPTS 870.3700



Adverse Birth Outcomes
- preterm birth rate (10%)
- low birth weight babies (11%)
- malformations (3-4% live births)
- mortality (0.4-0.6% newborns)
- functional deficits (17% children)

Complex Systems
- gene networks
- multiscale
- autopoiesis
- canalization
- temporality
- state trajectories
- and more …



Pluripotent stem cell (PSC) assays

An active area of investigation and one of the most promising *in vitro* alternatives to pregnant animal testing for assessing developmental hazard potential; novel features:

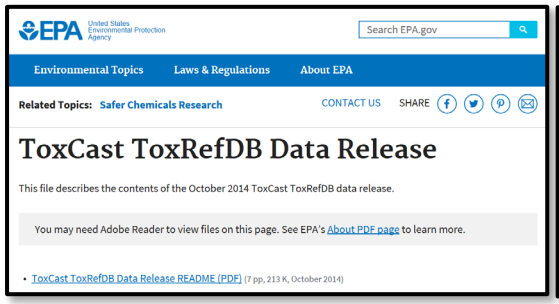
- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.
- **Pluripotency:** cells have the potential to form most of the different cell types comprising the embryo-fetus.
- **Autopoiesis:** capacity to self-organize into rudimentary tissues and more complex organoid structures.

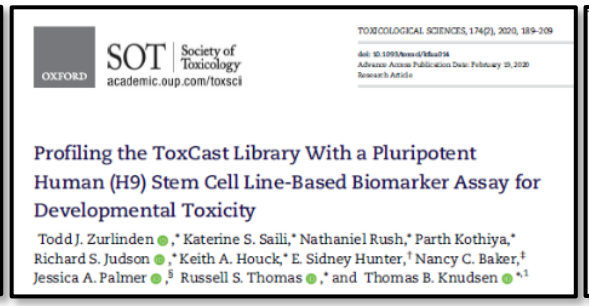
Established hPSC lines can recapitulate **some** of the biology driving embryogenesis during the period covered by guideline prenatal studies (e.g., OECD TG 414, OPPTS 870.3700).

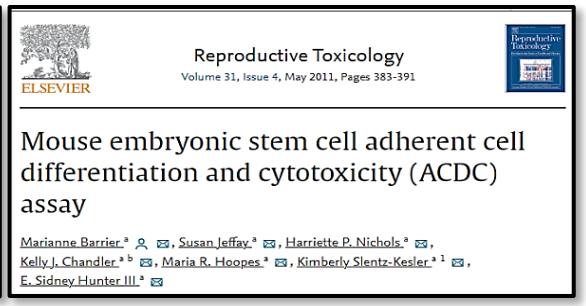
Translatability of PSC findings: *ToxCast PSC assay vs ToxRefDB fetal outcomes*

Weight of evidence for adverse fetal effects

WoE		hPSC	mESC
	n	*44*	*43*
	sens	0.654	0.208
	spec	1.000	1.000
	PPV	1.000	1.000
	NPV	0.667	0.441
	BAC	**0.833**	**0.721**
	n	*125*	*120*
	sens	0.444	0.195
	spec	0.800	0.797
	PPV	0.556	0.333
	NPV	0.719	0.656
	BAC	**0.637**	**0.495**
	n	*75*	*71*
	sens	0.417	0.250
	spec	0.843	0.894
	PPV	0.556	0.545
	NPV	0.754	0.700
	BAC	**0.655**	**0.623**
	n	*47*	*42*
	sens	0.345	0.208
	spec	0.778	0.944
	PPV	0.714	0.833
	NPV	0.424	0.472
	BAC	**0.569**	**0.653**
	n	*141*	*127*
	sens	0.260	0.258
	spec	0.757	0.882
	PPV	0.750	0.857
	NPV	0.267	0.303
	BAC	**0.508**	**0.580**







- Predictivity of hPSC and mPSC assays in ToxCast varies as a function of WoE for adverse fetal outcomes (n=432 chemicals with *in vivo* studies).
- Both platforms showed strong predictivity for well-curated developmental toxicants and non-toxicants, despite limited sensitivity (BAC 83.3% at its peak).
- Positive predictive value (PPV) is generally strong, meaning a positive PSC response is indicative of developmental hazard potential.
- Specificity is high although negative predictive value (NPV) drops when fetal effects are concurrent with maternal toxicity (BAC 50.8% at its worst).

https://comptox.epa.gov/dashboard

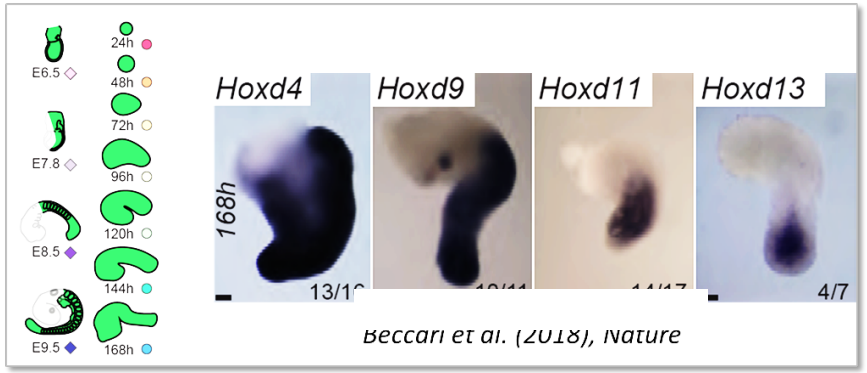
Bringing the embryo into focus

- Molecular biology and behavior of PSCs *in vitro* most closely resemble the naïve pluripotent state of the epiblast as the bilayered embryo enters gastrulation (primitive streak PS is a hallmark feature).
- Cultured PSCs can self-organize into rudimentary organs but lack positional information and physical constraints of an epiblast critical for '*decoding the genomic blueprint of the fetal body plan*'.

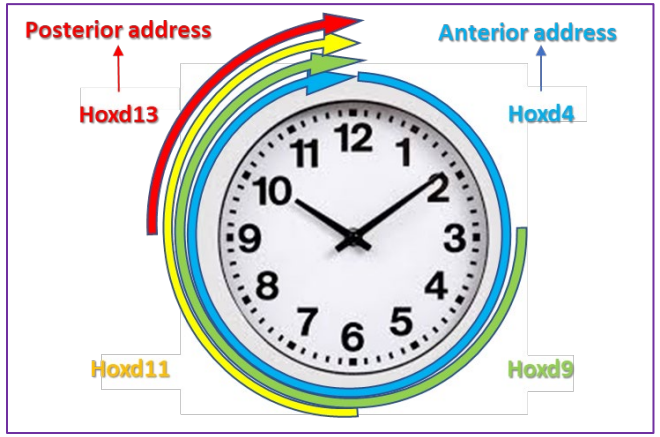
Gastruloid: *microsystem enabling self-organization of the body plan in vitro*

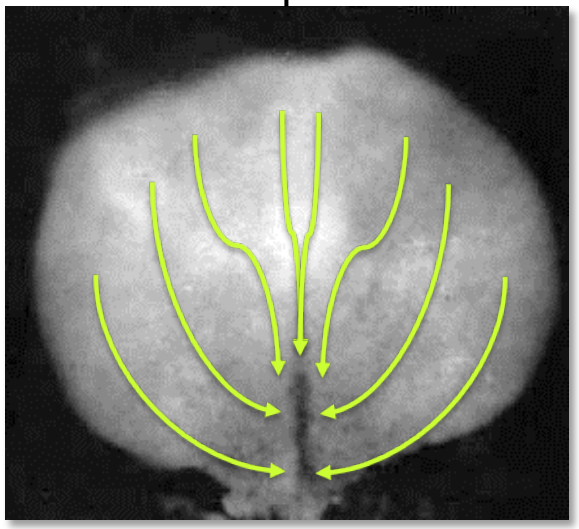
PSC-derived ‘gastruloid’ can establish anterior-posterior body axis *de novo*.

Engineering physical boundaries *in vitro*.



Beccari et al. (2018) Nature





Epiblast cells stream to PS and undergo EMT.

Temporal colinearity →

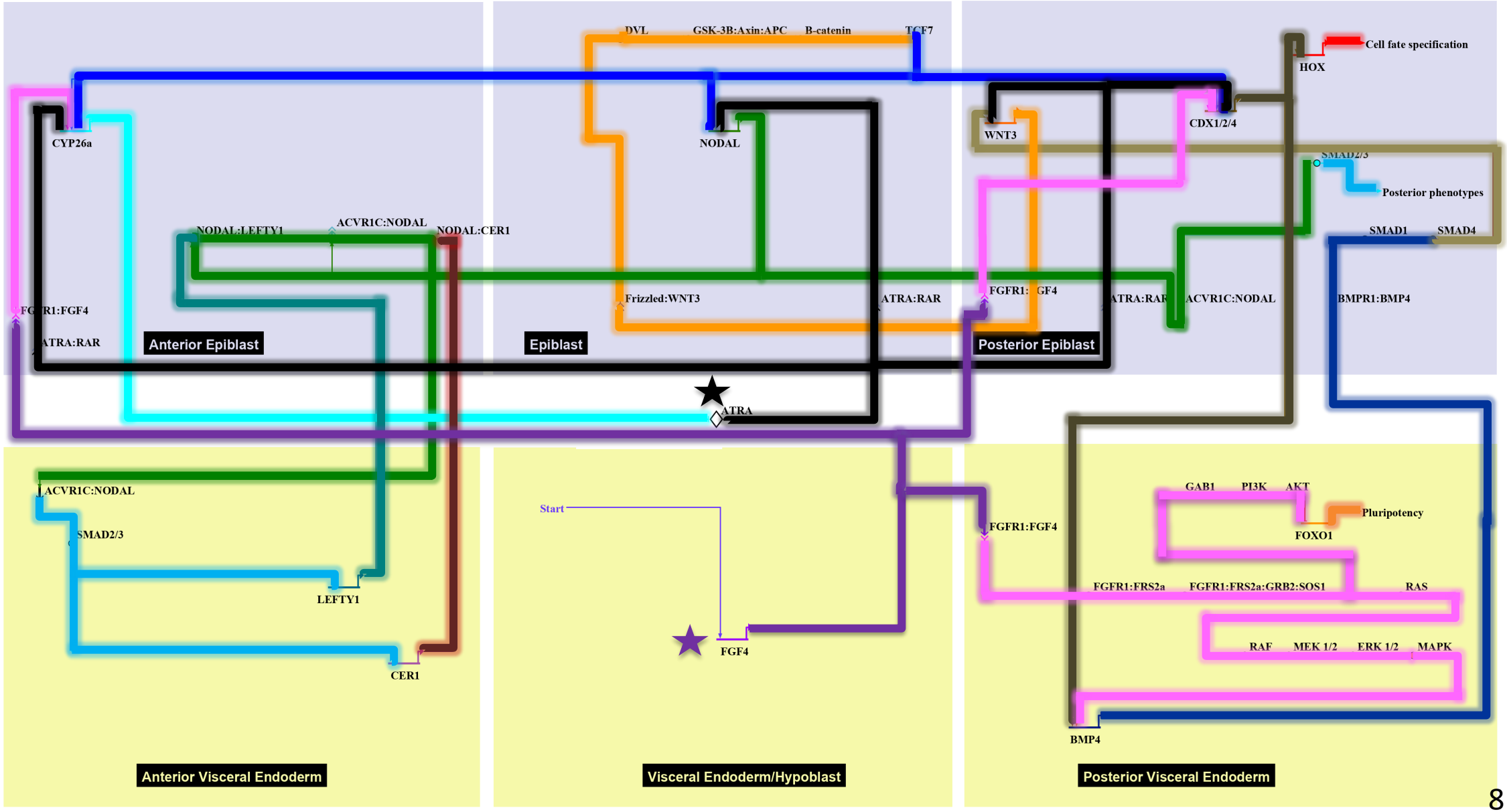
3′ — Hoxd4 — Hoxd9 — Hoxd11 — Hoxd13 — 5′

anterior → posterior specification (head-to-tail)

Think of it as a Hox-clock, where time is measured by progressive activation of homeobox (Hox) genes.

Time set as cells pass through PS; hence, position is key.

Flow of molecular regulatory information



Computational logic of a self-organizing system

Anatomical homeostasis in a self-regulating 'Virtual Embryo'

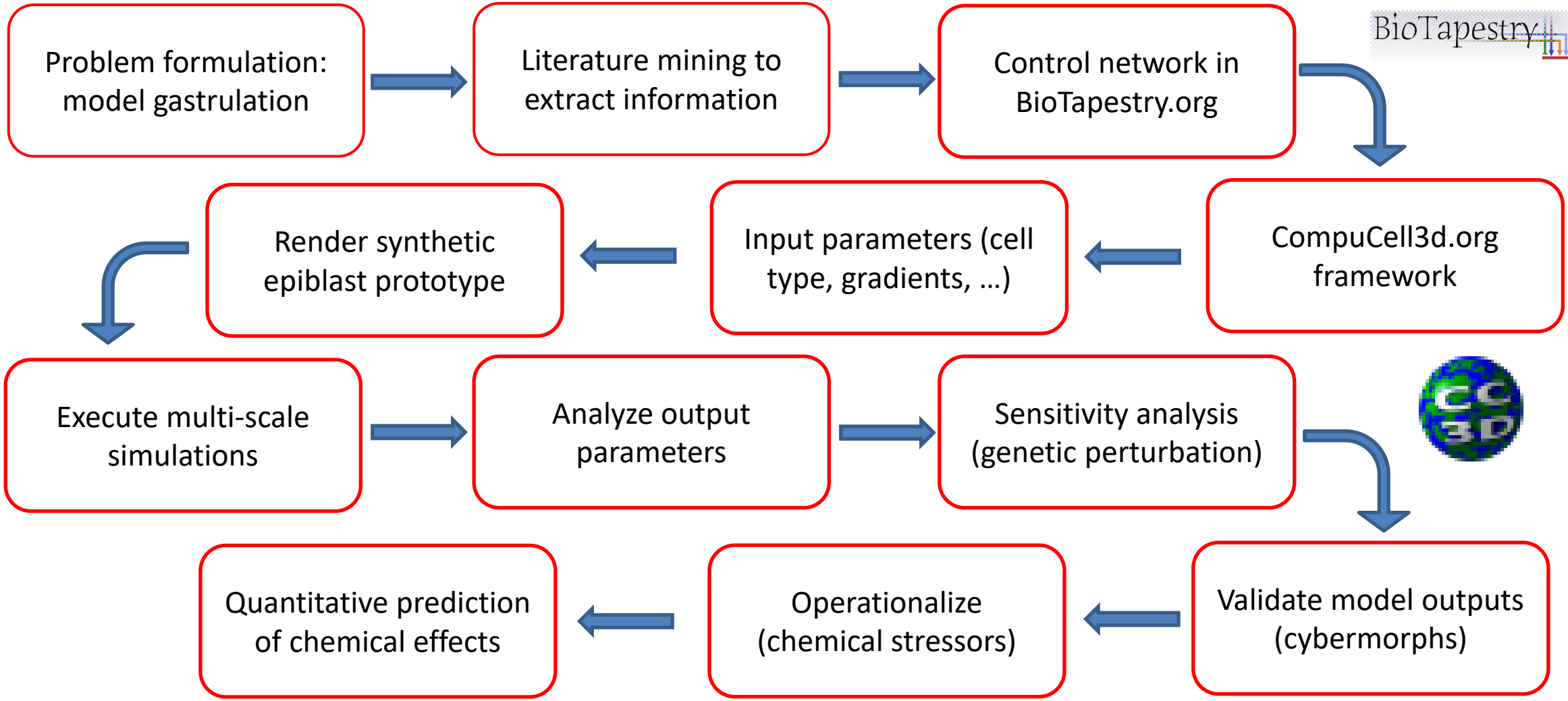
SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

- Embryo-inspired Agent-Based Model (SBM) of natural processes such as self-regulation and emergence.
- Computational intelligence (CI) evolves within the physical model by different phenotype-based protocols:
  - mathematical (phenotype-based algorithmic selection);
  - biological (fuzzy logic to fill in for incomplete information).
- Exploring mechanistic causation with artificial life through automation, synthetic control, and computer simulation.

Cellular Agent-Based Model (ABM)

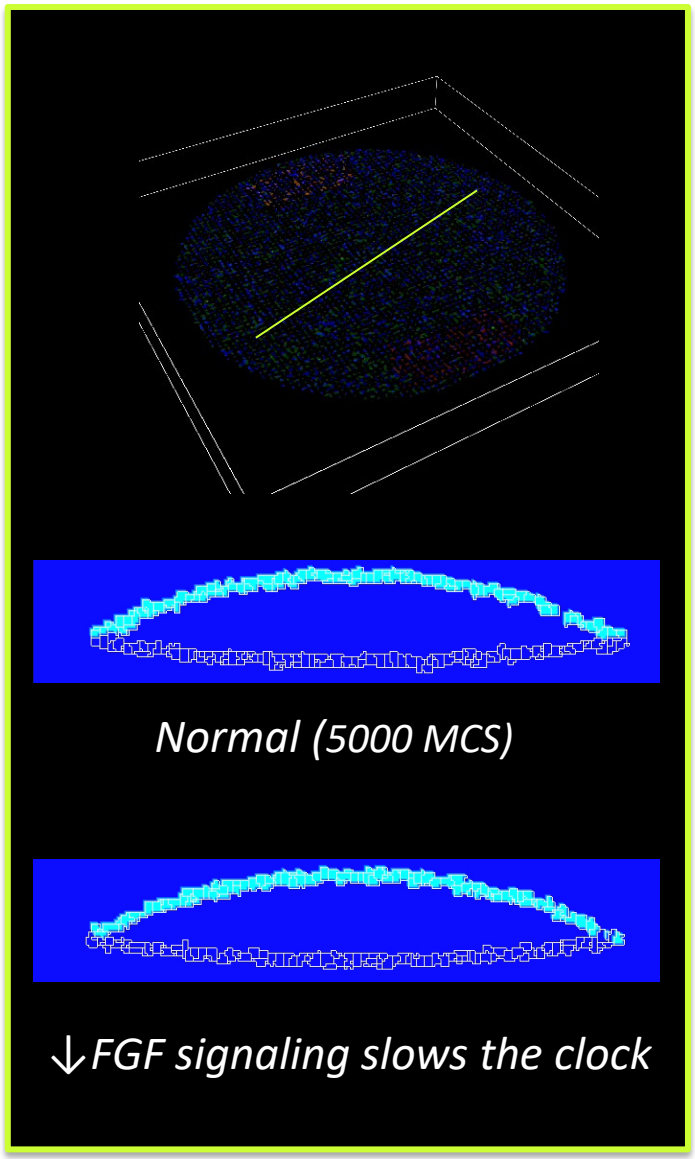
- Nature-inspired *agents* (cells) and *rules* (behaviors) set into motion as a self-organizing virtual system, using an open-source modeling environment (**CompuCell3d.org**).
- Soft-computing uses 'fuzzy logic' to simulate forces or properties governing cell activity where rules are inexact or knowledge incomplete (**computational intelligence**).
- Change course in response to a particular situation or stimulus, such as genetic errors or biomolecular lesions fed into the model from real world data (**sensitivity analysis**).
- Probabilistic rendering of where, when and how a particular condition might lead to an adverse developmental outcome (**cybermorphs**).
- End-game: run countless perturbation scenarios and/or uncover critical phenomenon explaining an altered phenotype (**perturbation matrices**).

Workflow: *computational ABM reconstructing stem cell dynamics*



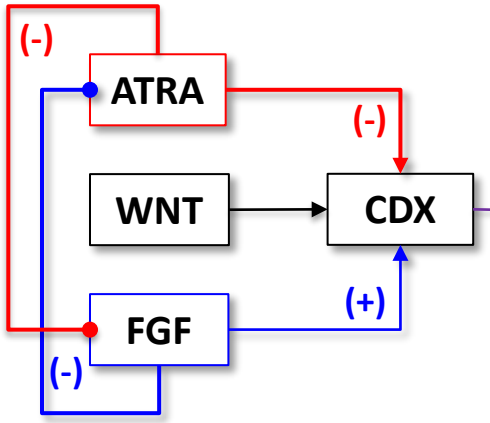
Kaitlyn Barham and Richard Spencer, manuscript in preparation

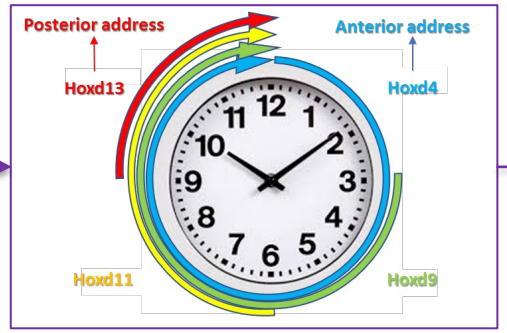
Gastruloid *in silico*: *reconstructing the morphological programming logic in silico*



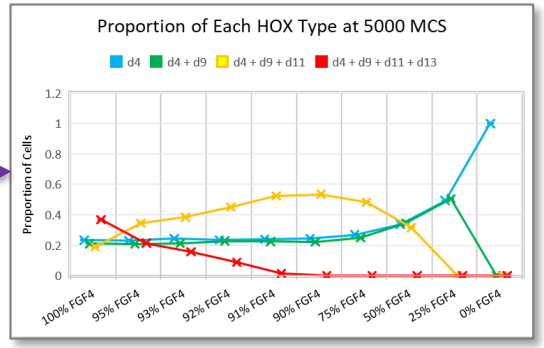
K Barham, R Spencer (work in progress)

- ESABM generates axial (chordomesoderm), paraxial (somitic), lateral (limbs, external genitalia), and posterior (caudal, blood) mesoderm.





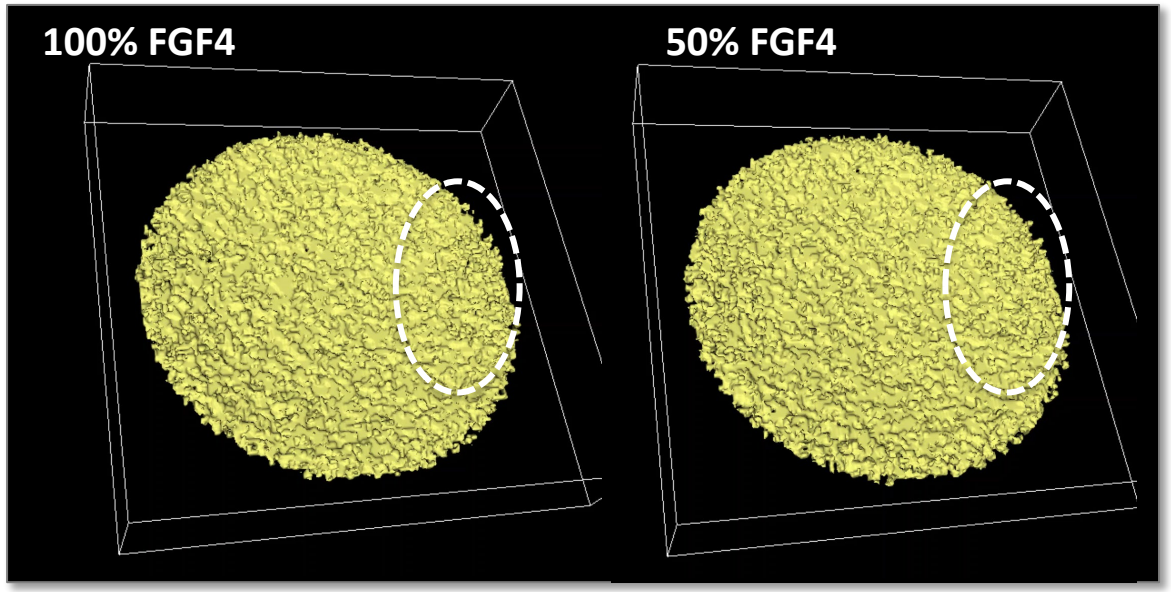
Hox clock

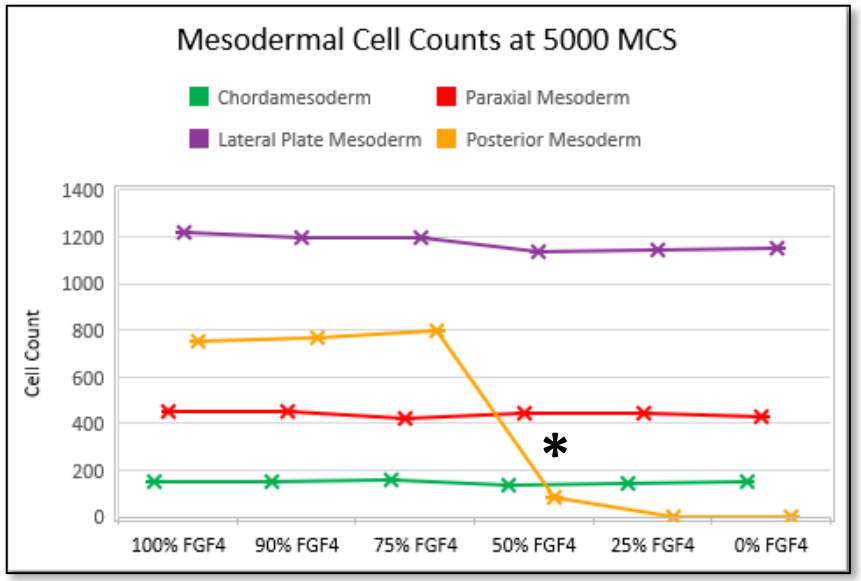


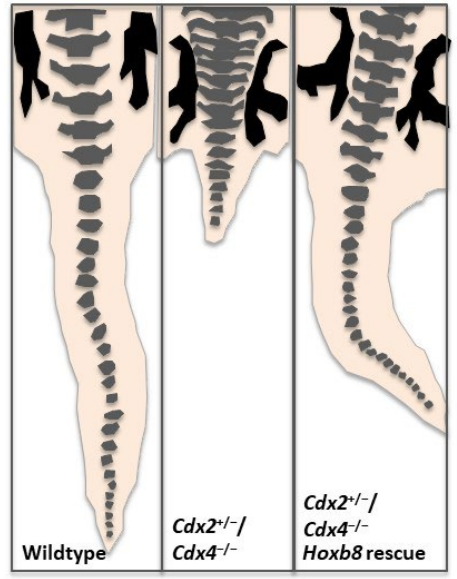
regional specification

- Rate of the Hox clock is controlled by CDX genes that regulate AP identity based on local signaling (ATRA, WNT, FGF).
- ESABM can '*recode the genomic blueprint of the fetal body plan*' for evaluating chemical effects on regional specification of mesoderm.

Hacking the model: *FGF4 cybermorphs*



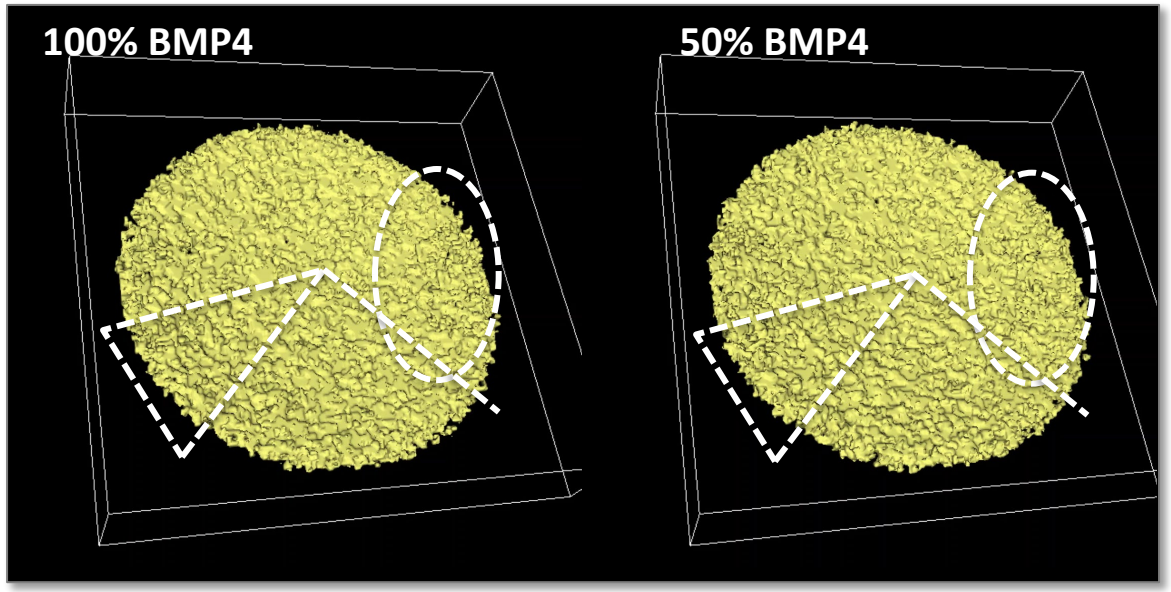


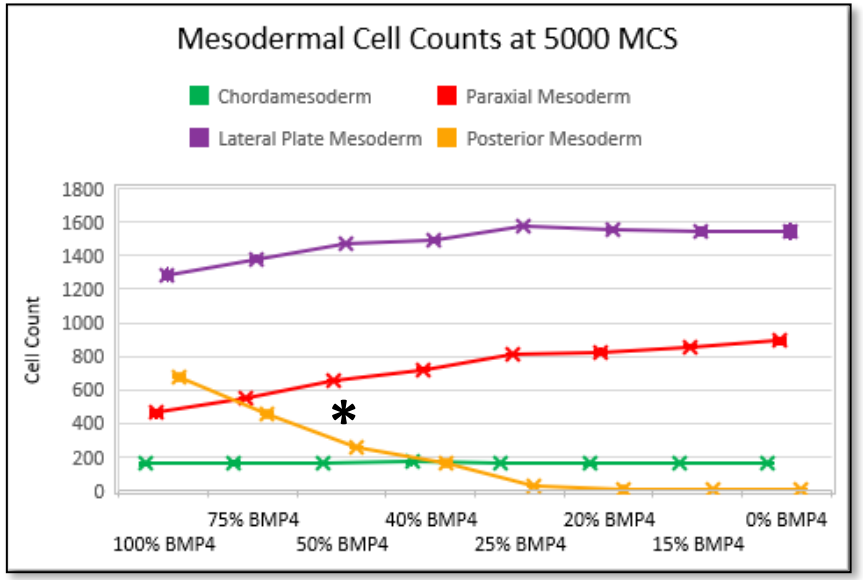


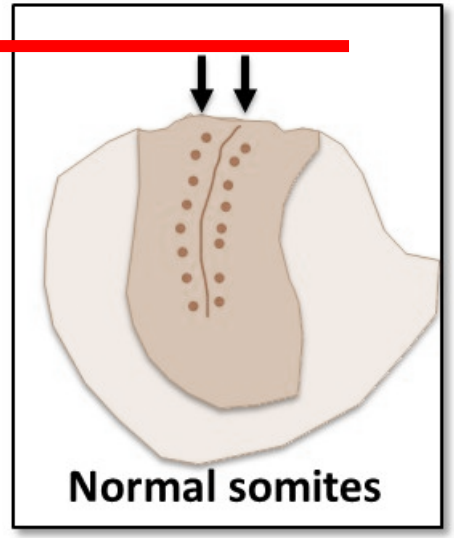
after Young et al. (2009) Dev Cell

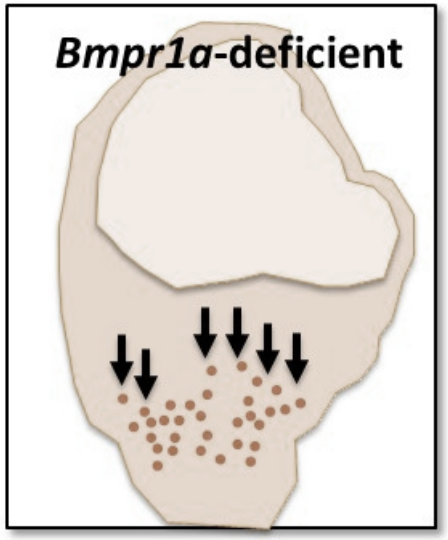
- FGF4 is a positive determinant of CDX-dependent regulation of the HOX clock;
- progressive activation of CDX specifies more posterior mesodermal cell fates;
- FGF4 knockdown in the model had a critical effect on posterior mesoderm formation (*);
- 50% FGF4-cybermorph recapitulates functional inactivation of *Cdx2/4* in mice.

Hacking the model: *BMP4 cybermorphs*





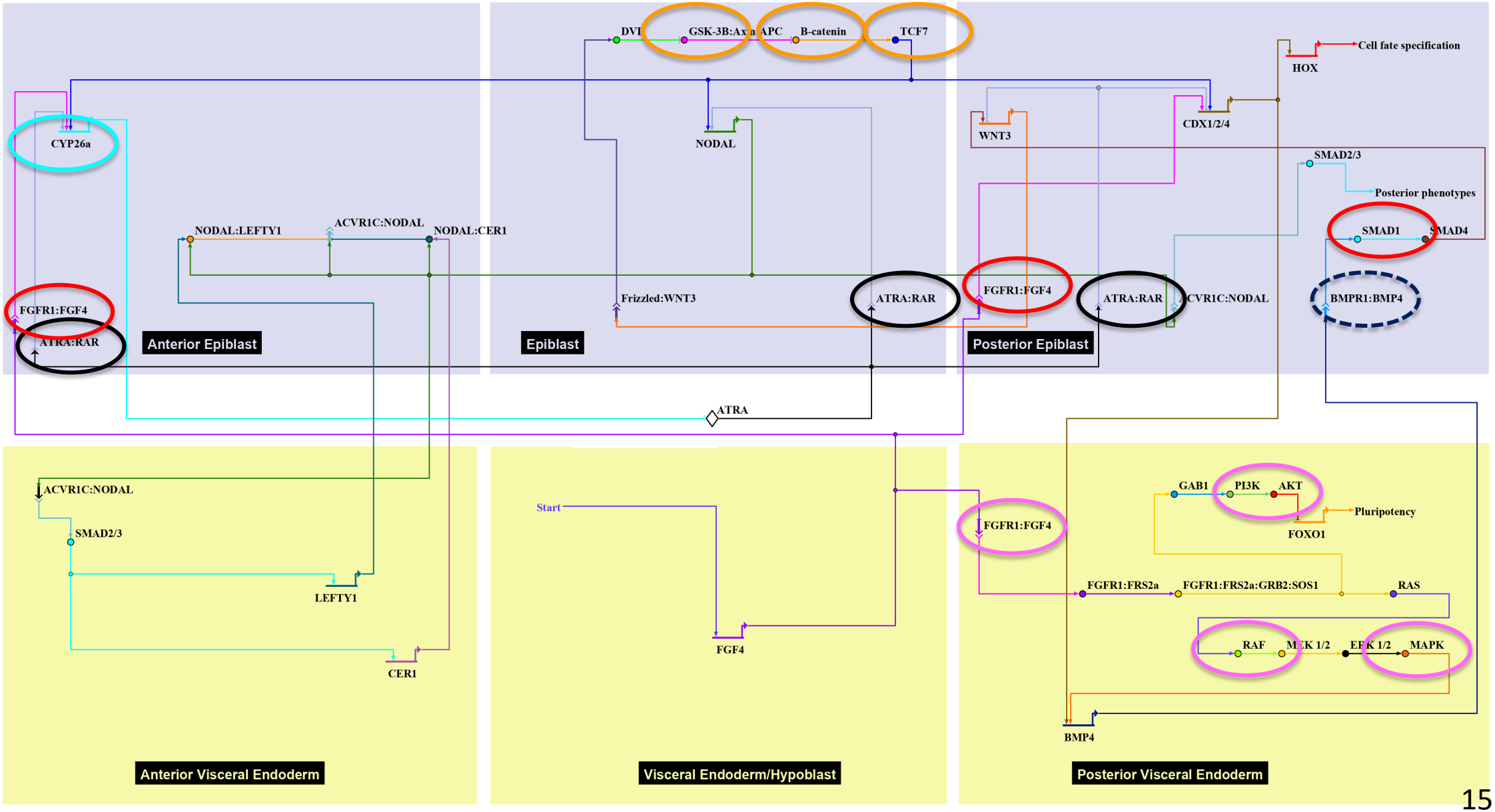




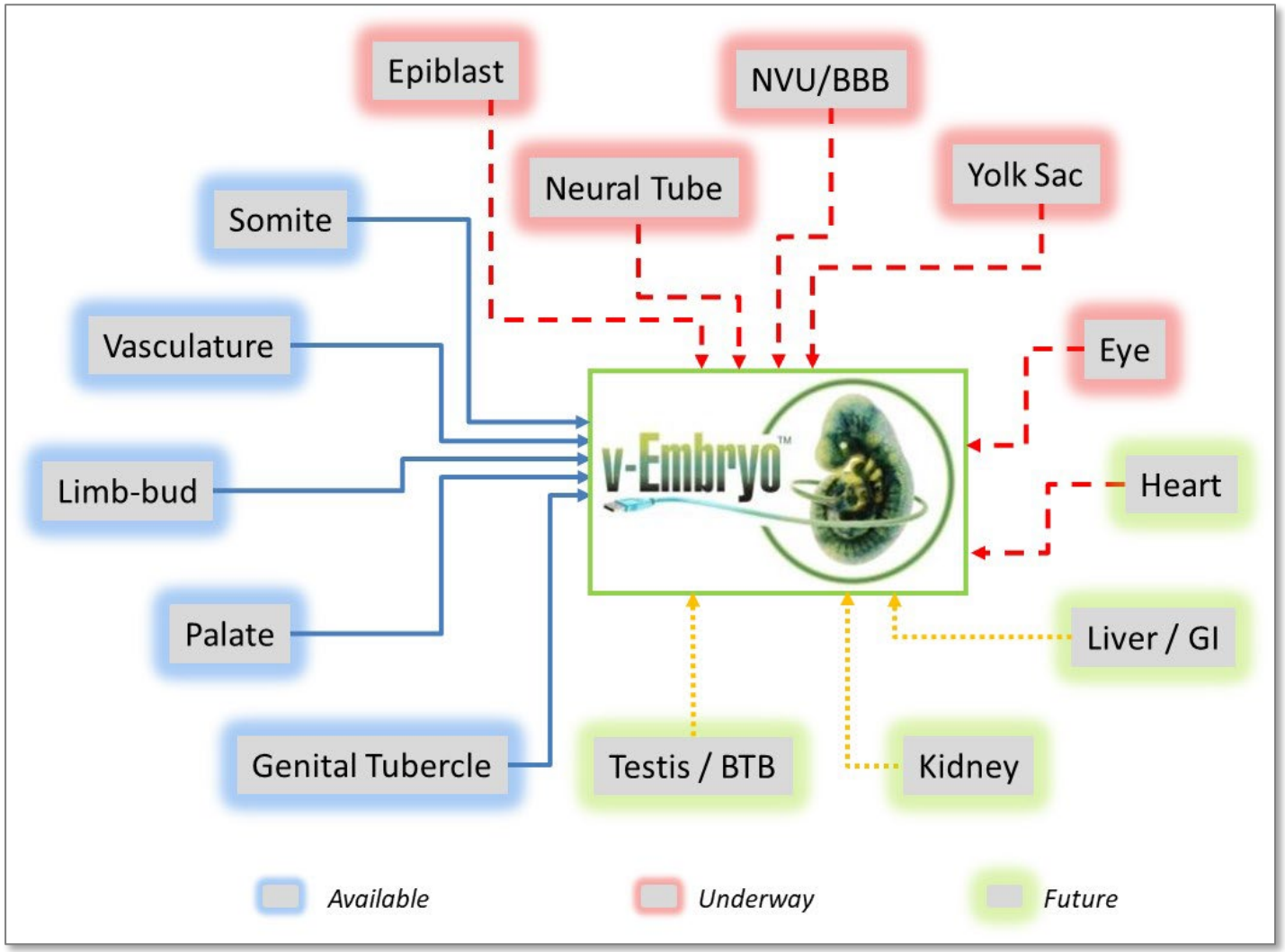
- BMP4 is maintained by FGF4 and primes posterior fate of the mesoderm;
- BMP4 in the epiblast regulates recruitment of prospective paraxial mesoderm;
- Conditional *Bmpr1a*-knockdown anteriorizes mesoderm, expanding the paraxial field;
- 50% BMP4-cybermorph recapitulates functional deficit in *Bmpr1a*-deficient mice.

after Miura et al. (2006), Development

ToxCast/Tox21 bioactivity nodes

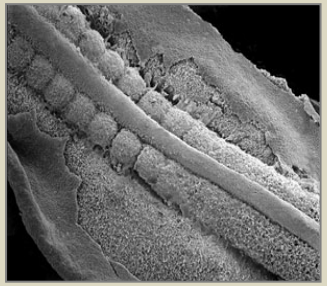


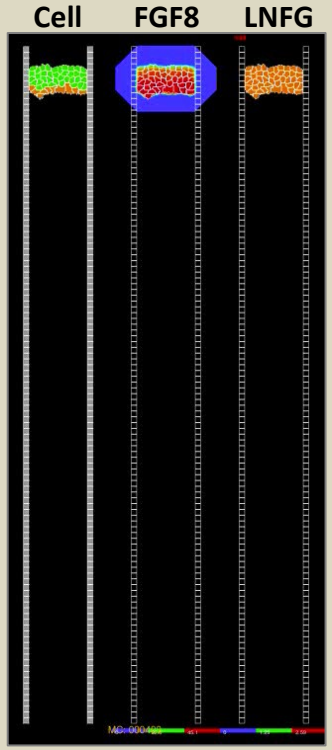
Toward a Virtual Embryo



- Cellular ABMs built to biological specification.
- Uniquely simulate complex morphogenetic processes.
- Amenable to mechanistic titration with NAMs data in space & time.
- Proof-of-concept shown for several birth defects.

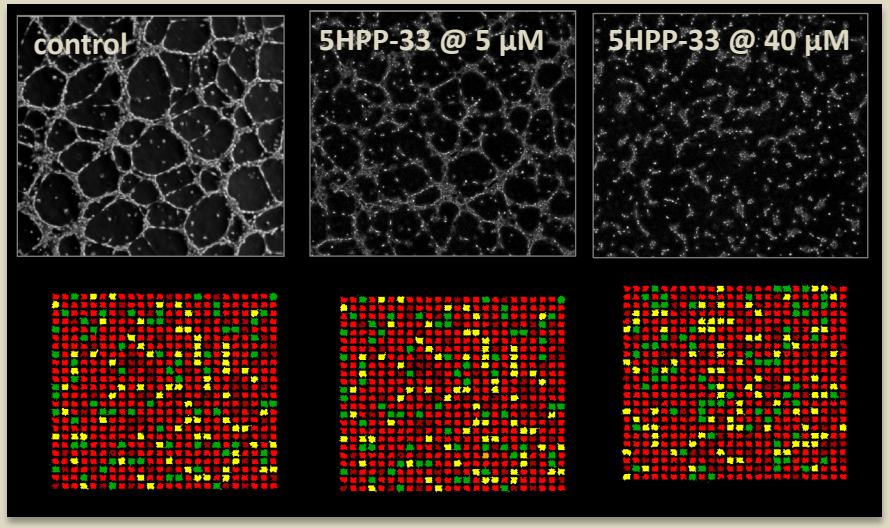
Somites



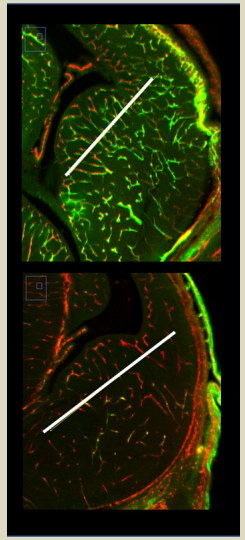


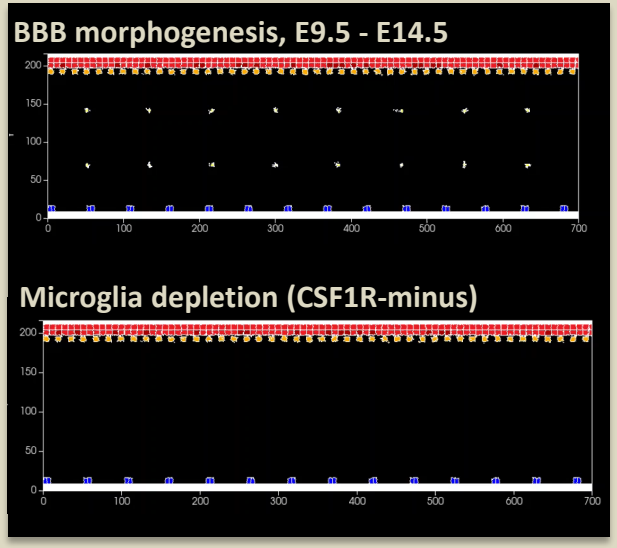
Hester et al. (2011) PLoS Comp Biol

Vasculature



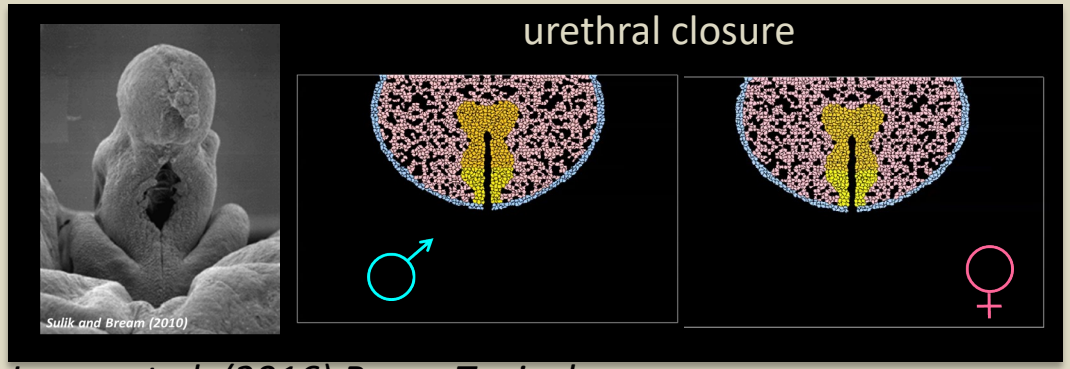
Kleinstreuer et al. (2013) PLoS Comp Biol



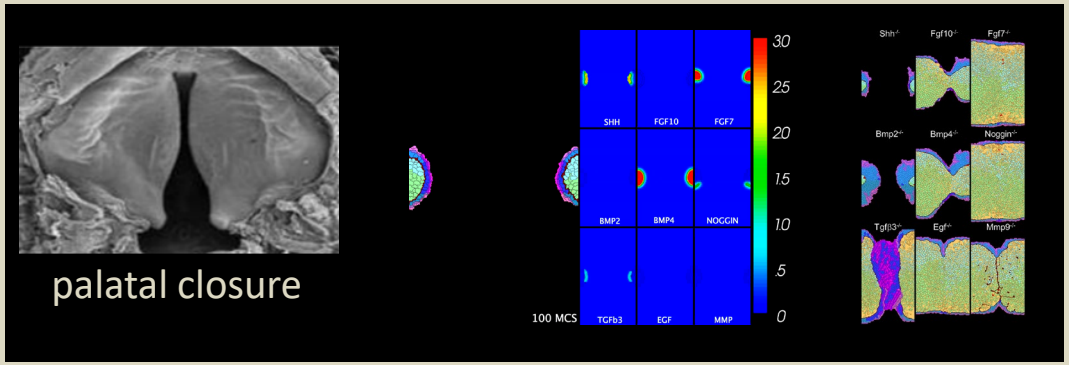


Naphade et al. (2023), submitted

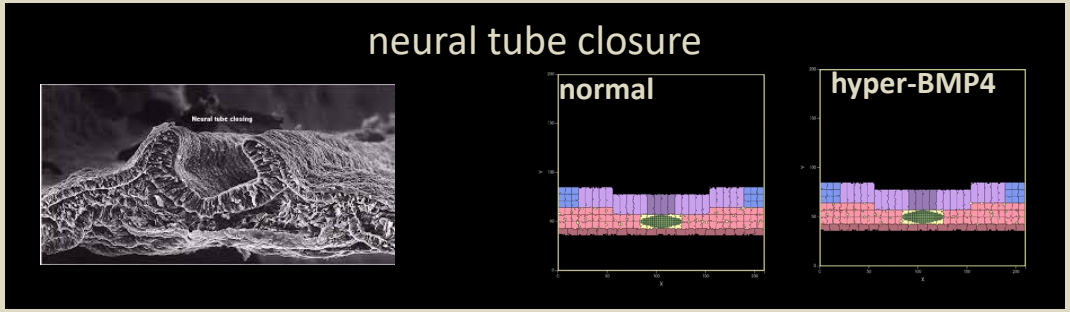
Morphogenetic Fusion



Leung et al. (2016) Repro Toxicol

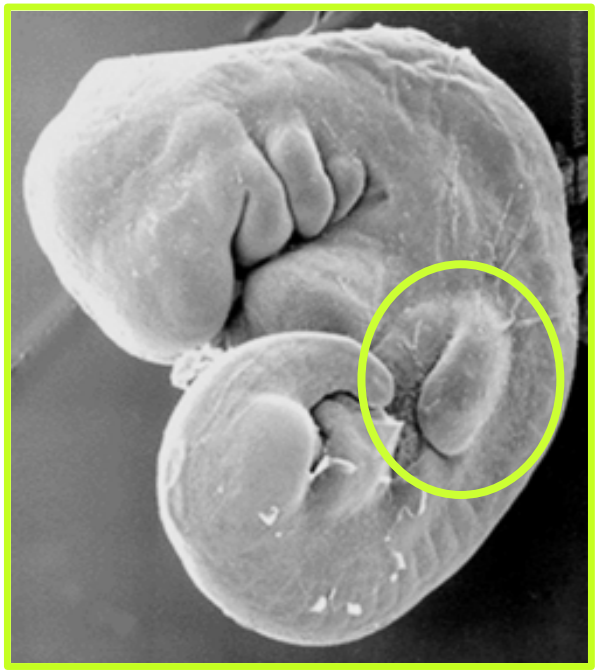


Hutson et al. (2017) Chem Res Toxicol



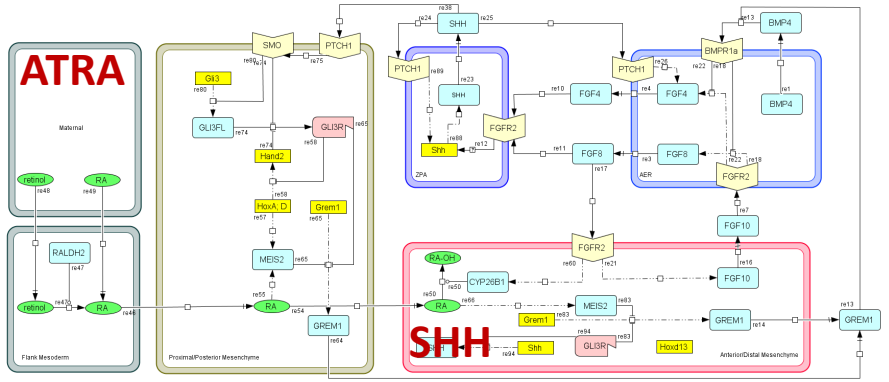
Berkhout et al. (2023), work in progress

Morphing NAMs data across levels of biological organization

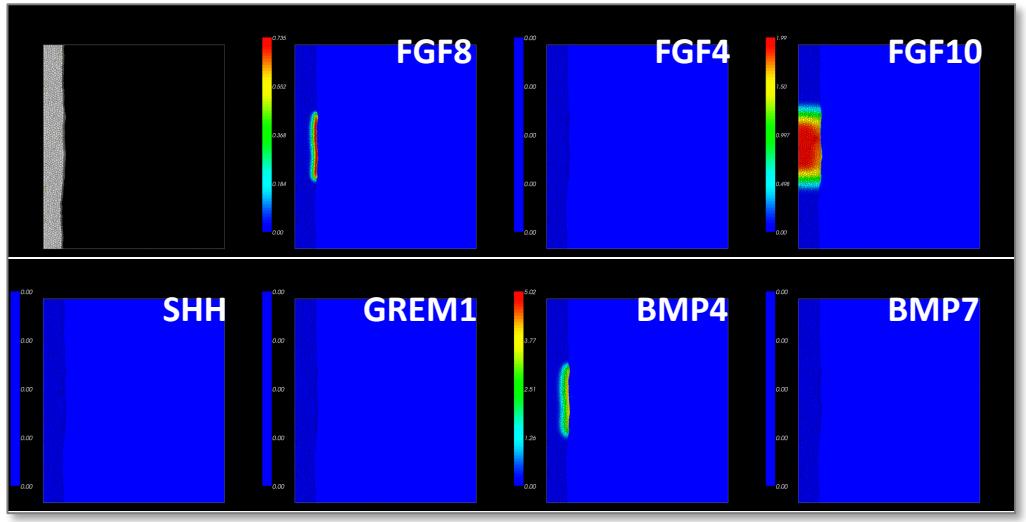


Early limb development
(~4-weeks gestation)

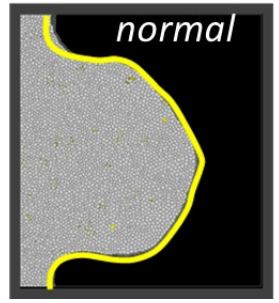
Control system: biological network

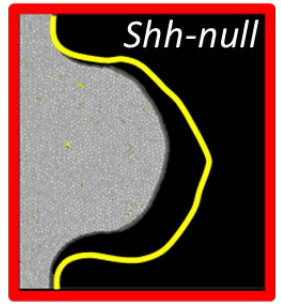


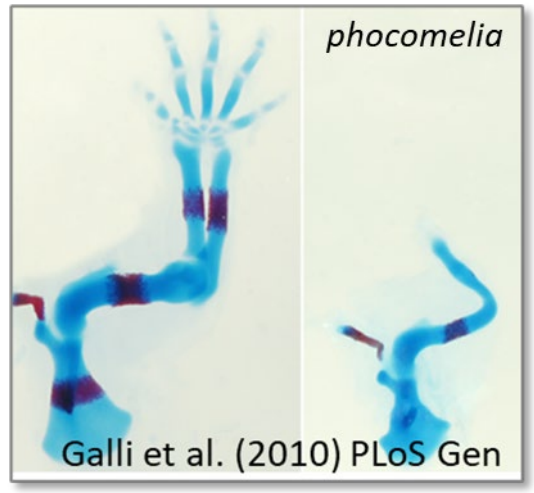
Cell agent-based model (compucell3d.org)



Hacking the model (cybermorphs)





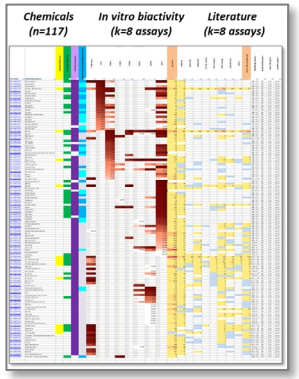


Morphing NAMs data across levels of biological organization

Bioactivity (ToxCast/Tox21)

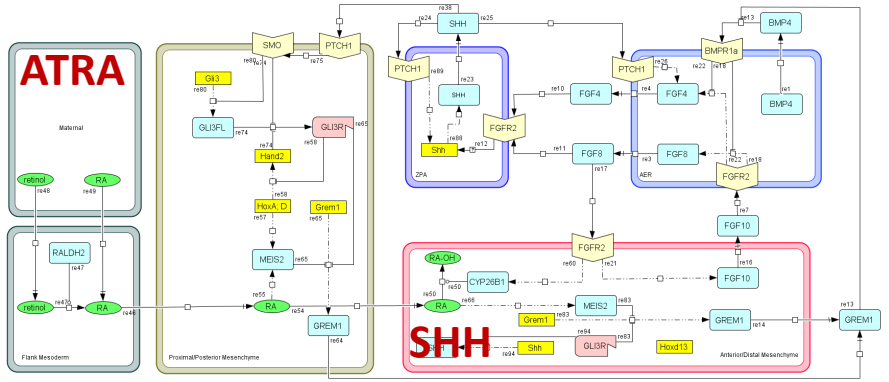
- Data on over 1400 HTS assays
- Literature mining (AbstractSifter)
- Machine learning (Classifiers)

117 chemicals active on MIEs in the ATRA pathway and invoke skeletal embryopathies.

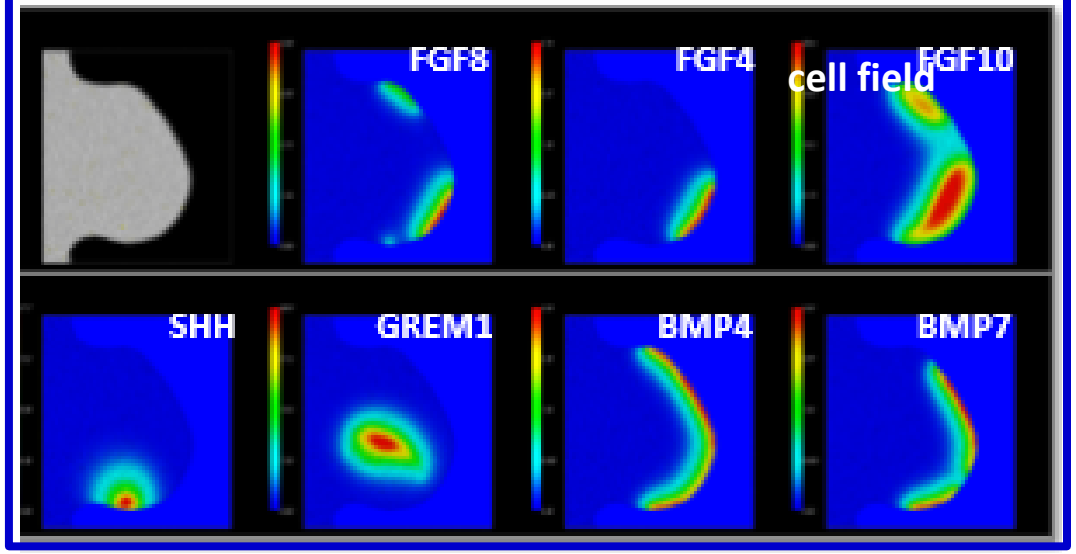


Simulating an ATRA overload *in silico:* 'cybermorph' foreshadows distal deficiencies.

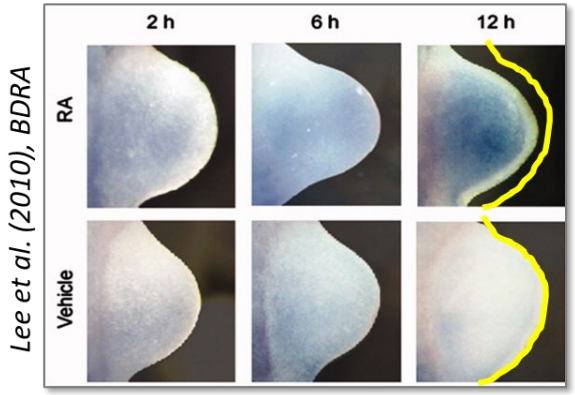
Control system: biological network

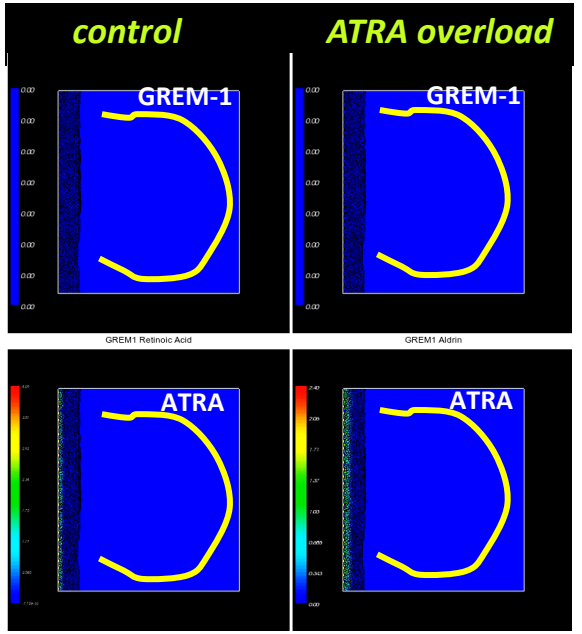


Cell agent-based model (compucell3d.org)



ATRA overload





Toward a Virtual Cornea:

An Agent-Based Model to Study Interactions between the Cells and Layers of the Cornea under Homeostasis and following Chemical Exposure

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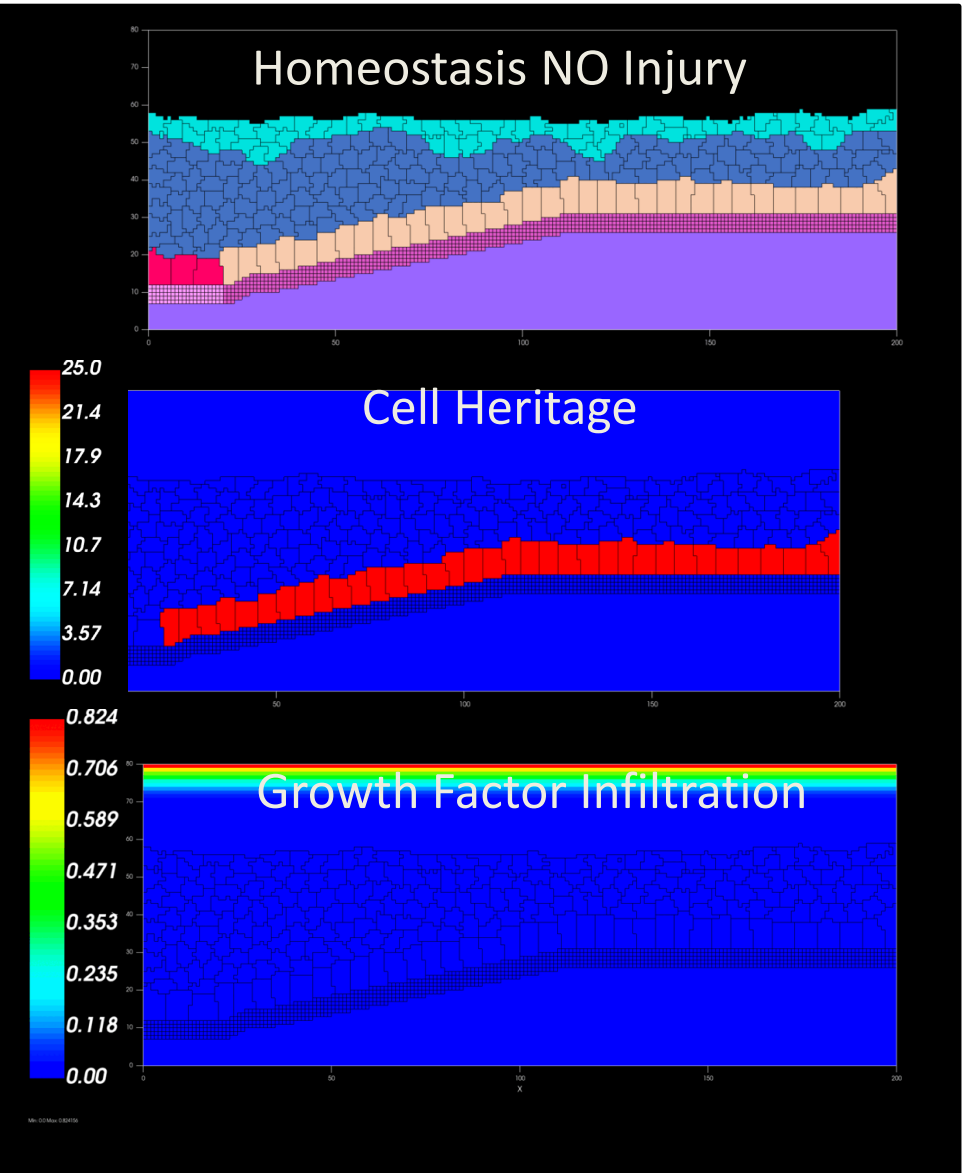

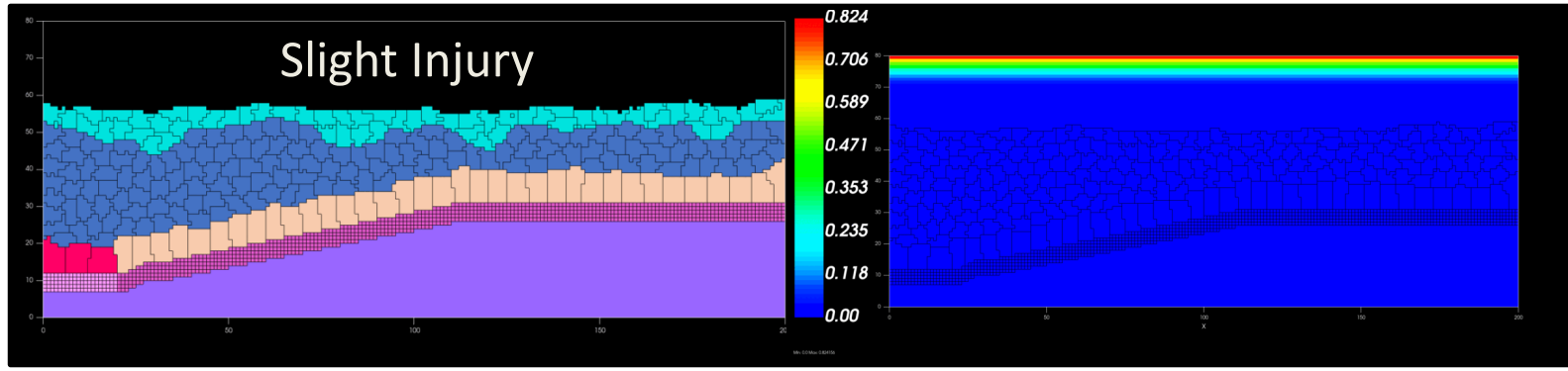

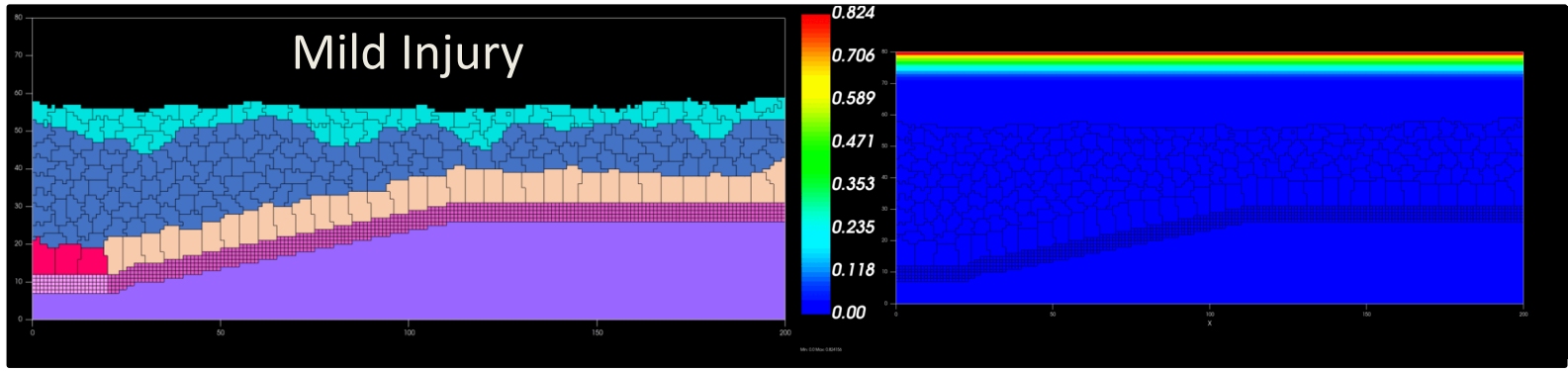

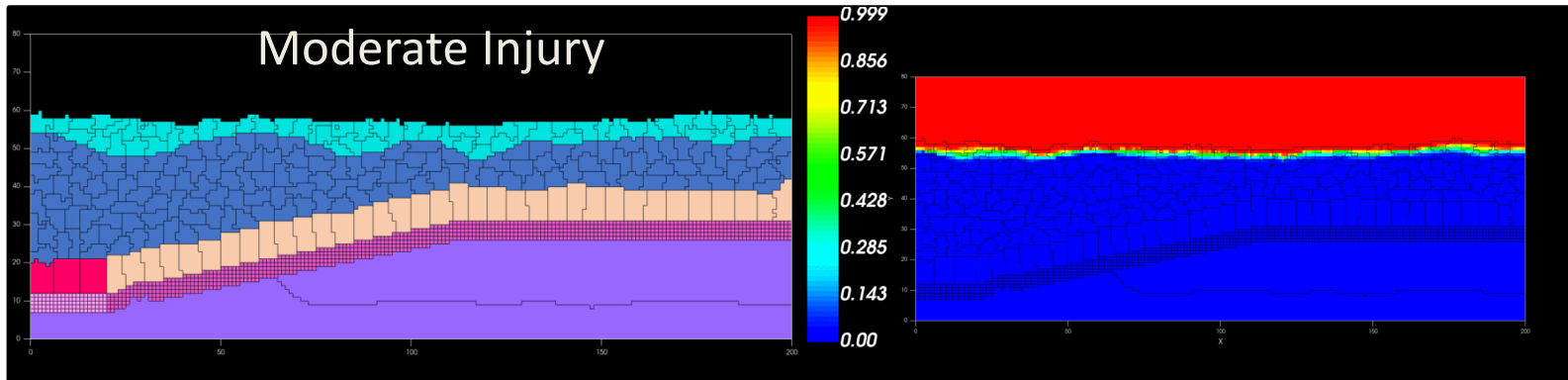

Summary

- *In silico* reconstitution of a self-organizing embryo from unidimensional data (eg, embryogeny) remains a challenge.
- Virtual tissue models are a novel approach to: (i) visualize cellular trajectories; (ii) map toxicodynamics; and (iii) predict adverse phenotype (cybermorphs).
- A fully computable virtual embryo (synbryo) may be a distant goal, but modular systems that bring toxicodynamics to life can pinpoint critical phenomena.
- Such models would allow a user to simulate limitless ‘what-if’ scenarios quantitatively, similar to computer models used for engineering complex physical systems.

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