GRC 2023

Cellular & Molecular Mechanisms of Toxicity

August 13-18, Andover NH

Session: "Modern Approaches in Developmental Toxicity Testing"

Computational (*in silico*) Cellular Dynamics In Developmental Toxicity

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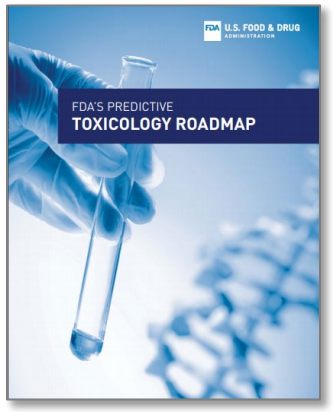
DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

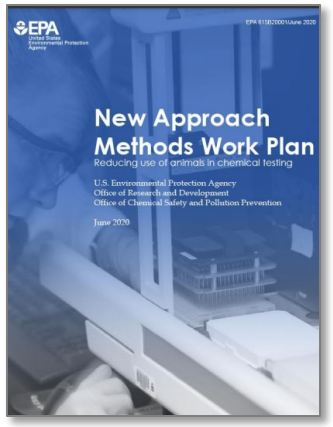
Disclosures

- **Disclaimer:** The views expressed are those of my own and do not reflect the views or policies of the USEPA.
- **Support:** EPA's Chemical Safety for Sustainability Research Program, Virtual and Complex Tissues Research Area.
- **Session:** ToxCast *in vitro* data will not be explained here; for details please refer to EPA's CompTox Chemicals Dashboard: **https://comptox.epa.gov/dashboard/**.
- **Conflicts of interest:** My role as Editor-in-Chief of Current Research in Toxicology (Elsevier) does not pose any conflicts of interest.

Regulatory drivers



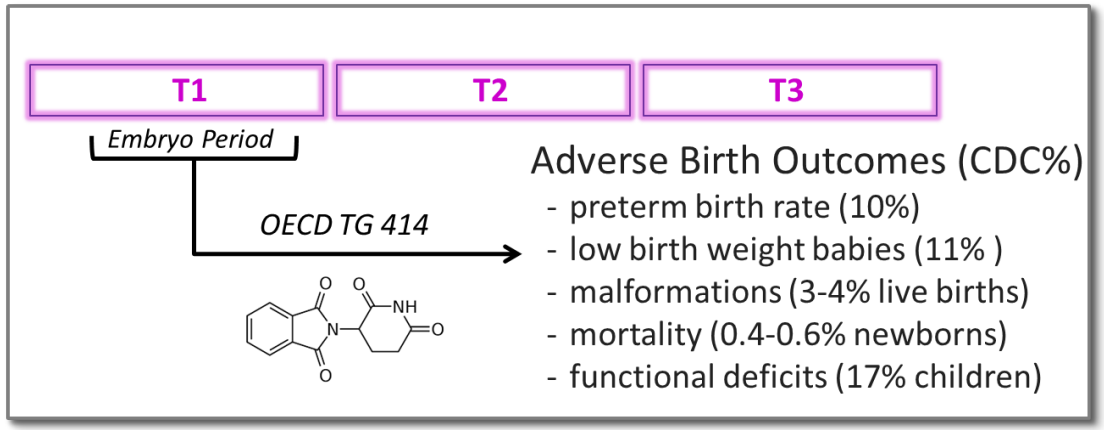
FDA's Predictive Toxicology Roadmap - created to identify the toxicology areas that could benefit from improved predictivity as well as promising new technologies that could potentially meet these needs and support animal 3Rs (Replacement, Reduction, and Refinement).
https://www.fda.gov



EPA's New Approach Methods Work Plan - created to prioritize agency efforts and resources toward activities that aim to reduce the use of animal testing while continuing to protect human health and the environment (NAMs).
https://www.epa.gov

Rundown

- NAMs that accurately predict human developmental toxicity are needed to supplant conventional testing in pregnant animal studies.
- Most *in vitro* assays lack the positional information, physical constraints, and regional organization of a mammalian system undergoing morphogenesis and development.
- This lecture will discuss practical and theoretical aspects of mechanistic modeling based on computational (*in silico*) dynamics for complex systems.



Take Home Message:
Computer models possessing emergent properties of a self-organizing dynamical system can render quantitative predictions of toxicity.

Pluripotent stem cell (PSC) models

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:

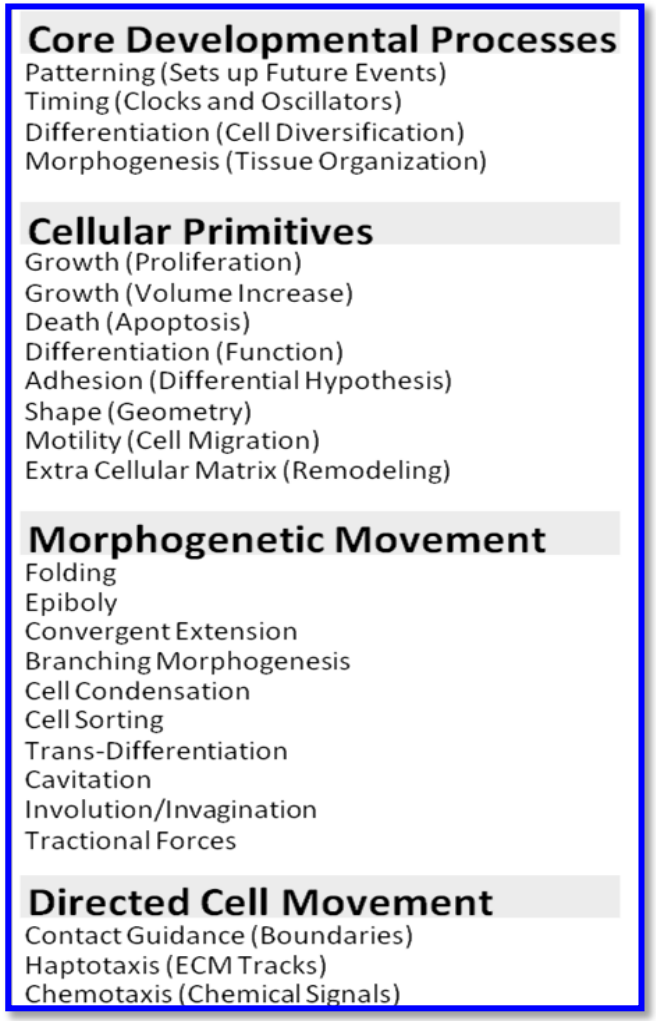
- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.
- **Pluripotency:** cells have the potential to form most of the different cell types comprising the embryo-fetus.
- **Autopoiesis:** capacity to self-organize into rudimentary tissues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

77-83% biomarker accuracy for PSC-based assays established from mouse [Panzica-Kelly et al. (2013) Toxicol Sci] or human [Palmer et al. (2013) Birth Def Res] cell lines.

Piersma et al. (2022) Curr Res Tox

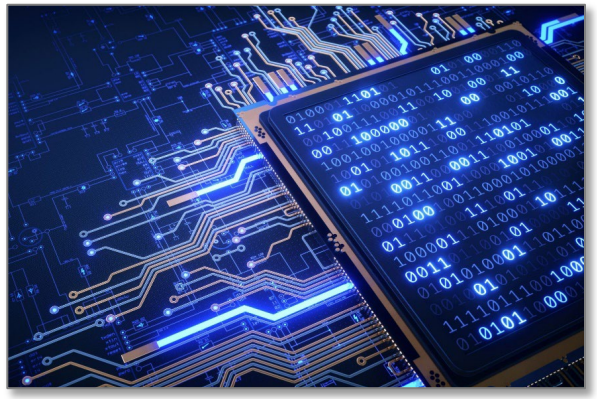
Morphogenesis is fundamentally complex

- PSC-based data generated on over 1065 industrial chemicals and failed pharma compounds in ToxCast.
  https://comptox.epa.gov/dashboard
- 432 ToxCast chemicals have DevTox studies in pregnant rats and/or rabbits, including 42 well-curated reference compounds.
- mPSC and hPSC assays registered a balanced accuracy (BAC) of 77-83% for the 42 reference DevTox compounds;
- however, BAC drops for studies with concurrent maternal toxicity, species discordance, missing biology or pathways.



Chandler et al. (2011) PLoS One; Zurlinden et al (2020) Tox Sci; Knudsen et al. (2020) Curr Opin Tox.

Computer Modeling and Simulation



© 2017. Published by The Company of Biologists Ltd | Development (2017) 144, 4214-4225 doi:10.1242/dev.151274

REVIEW

Computer modeling in developmental biology: growing today, essential tomorrow

James Sharpe[1,2,3,*,‡]

"Much as a microscope is a tool to help us see things that are too small for our own eyes, computer modeling is a tool that can help reveal the dynamics and predictions of an idea that is too complex for our own brains."
- Development, 2017

Mathematical models of physiological or chemical behavior in toxicological processes are often too complex to solve analytically.

Cellular Agent-Based Models (ABMs): *a theoretical approach.*

Anatomical homeostasis in a self-regulating 'Virtual Embryo'

Shared by Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

- Inspired by natural morphogenetic processes such as multicellular dynamics, emergence, and self-regulation.

- Physical models evolve in 'artificial life' simulators utilizing different phenotype-based protocols:

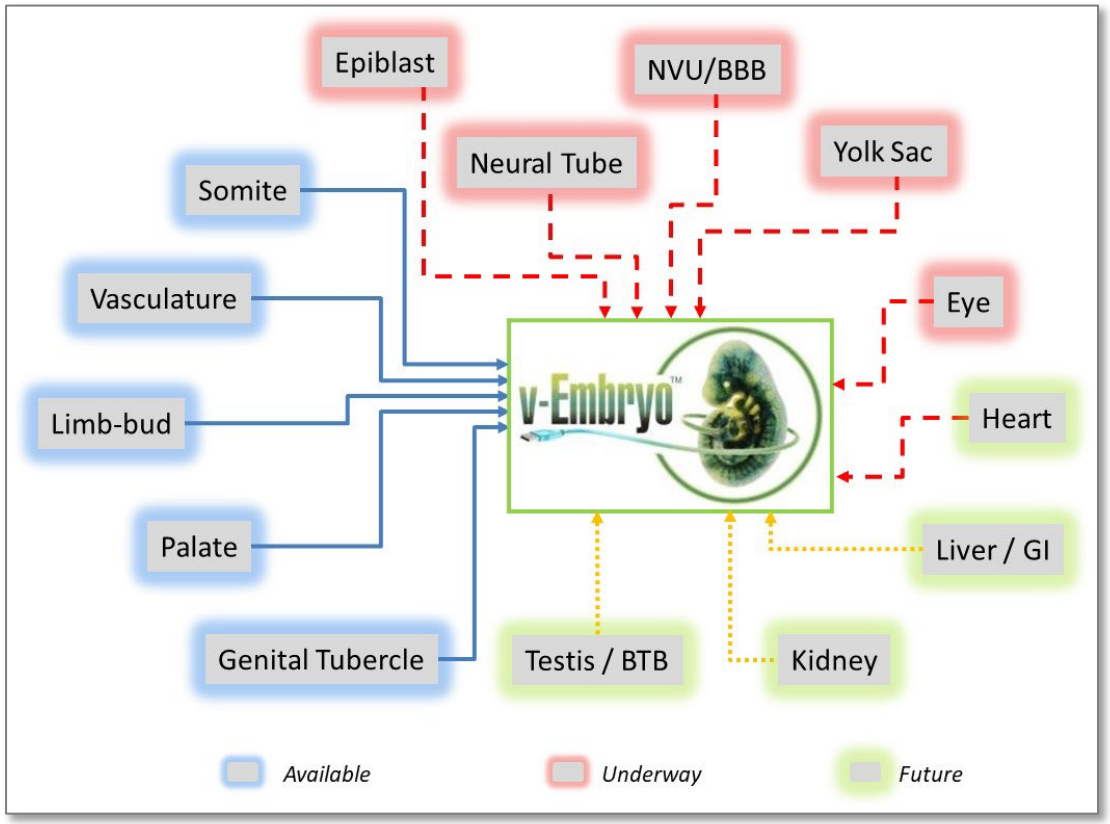
  - mathematical (AI-based algorithmic selection);
  - biological (fuzzy logic to fill in missing information).

Translational goals:
Isolating critical phenomena in developing tissues following genetic dysfunction and/or chemical injury.

Unique Qualities: *what cell ABMs bring to mechanistic prediction in toxicology*

- 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 (**cybermorphs**).
- Running countless perturbation scenarios return a probabilistic rendering of how a particular condition might lead to an adverse phenotype (**perturbation matrices**).

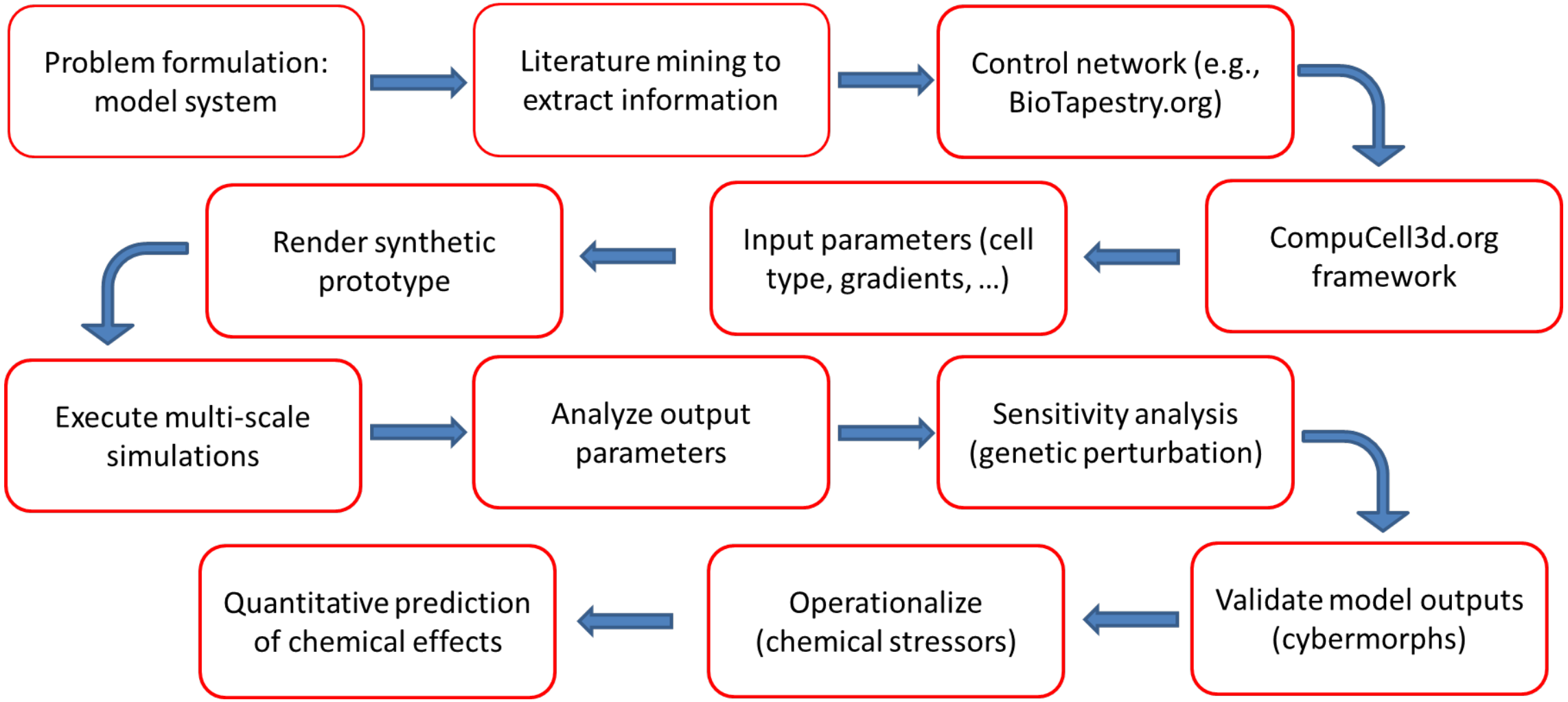
Blue Sky Vision: *toward a fully computable virtual (in silico) embryo*



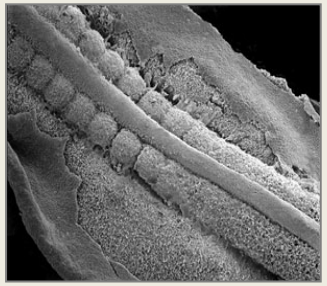
- Computer simulation can extend data-driven models for mechanistic prediction.
- Modular systems can be anchored to known phenotypes (mouse, zebrafish, human).
- Models transcend empty prediction by integrating toxicology with embryology.

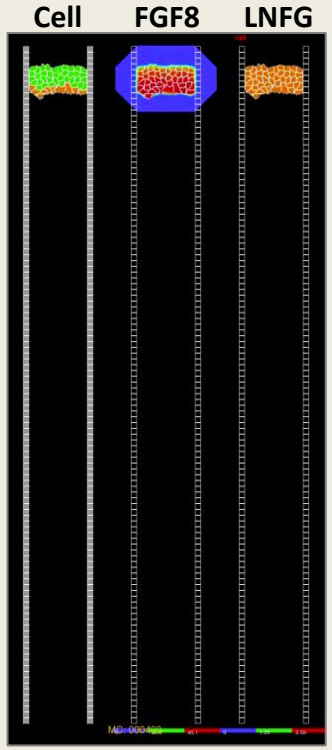
Two challenges for regulatory acceptance:
- filling critical information gaps (input);
- establishing scientific confidence (output).

Workflow


Problem formulation: model system
Literature mining to extract information
Control network (e.g., BioTapestry.org)
CompuCell3d.org framework
Input parameters (cell type, gradients, …)
Render synthetic prototype
Execute multi-scale simulations
Analyze output parameters
Sensitivity analysis (genetic perturbation)
Validate model outputs (cybermorphs)
Operationalize (chemical stressors)
Quantitative prediction of chemical effects

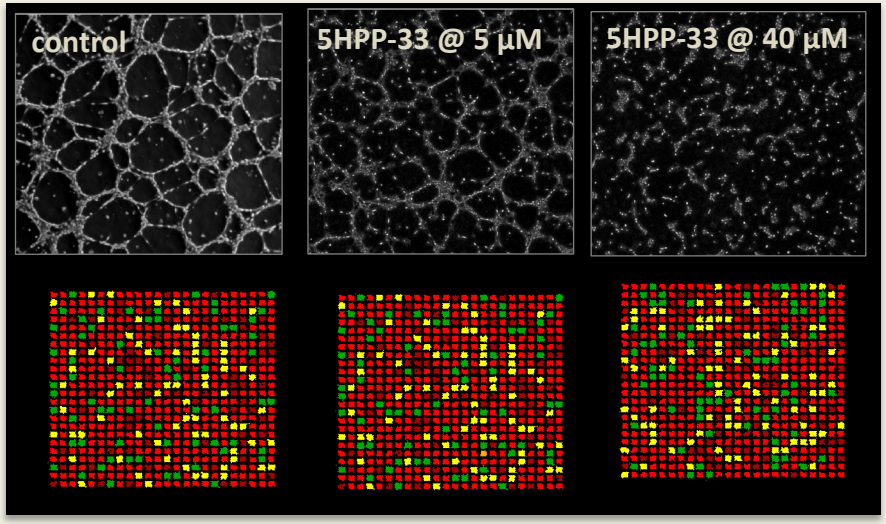
Somites



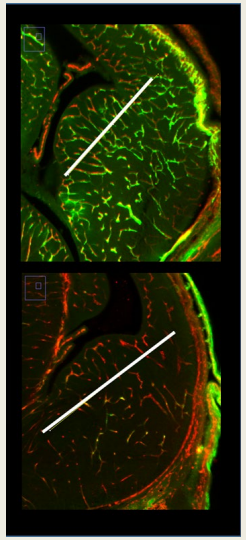


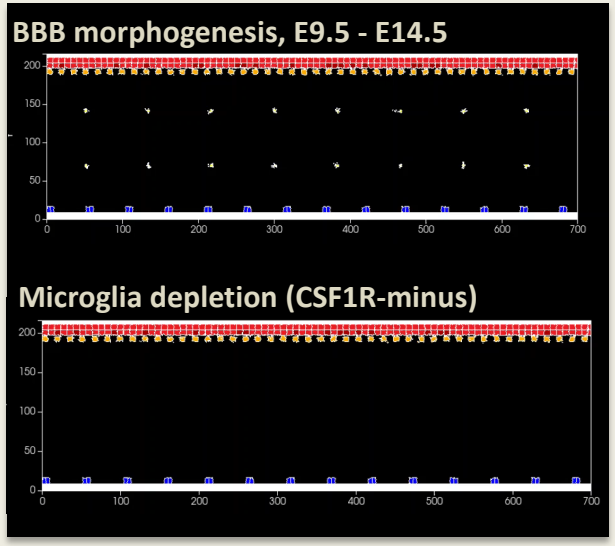
Hester et al. (2011) PLoS Comp Biol

Vasculature



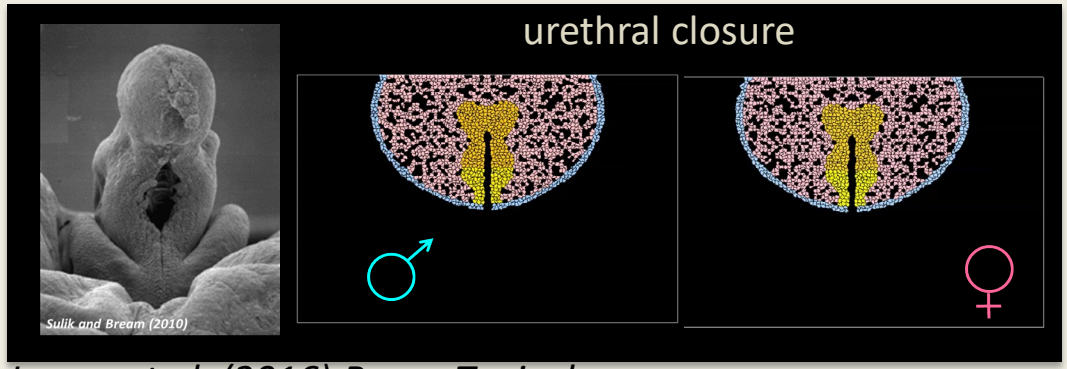
Kleinstreuer et al. (2013) PLoS Comp Biol



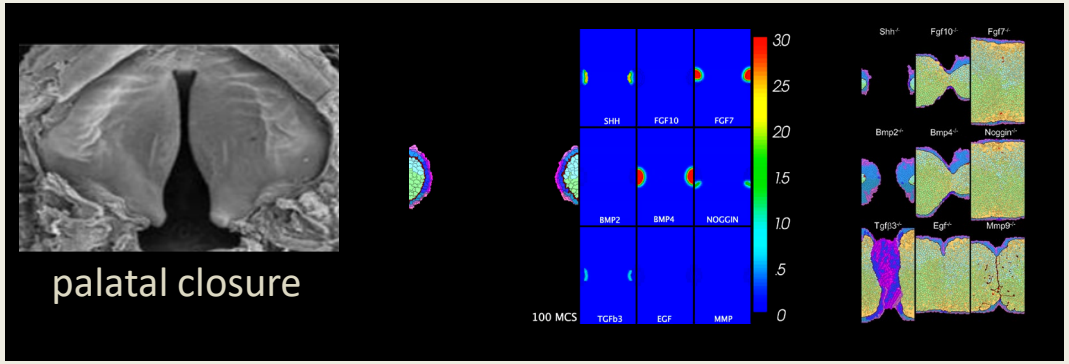


Naphade et al. (2023), Curr Opin Toxicol

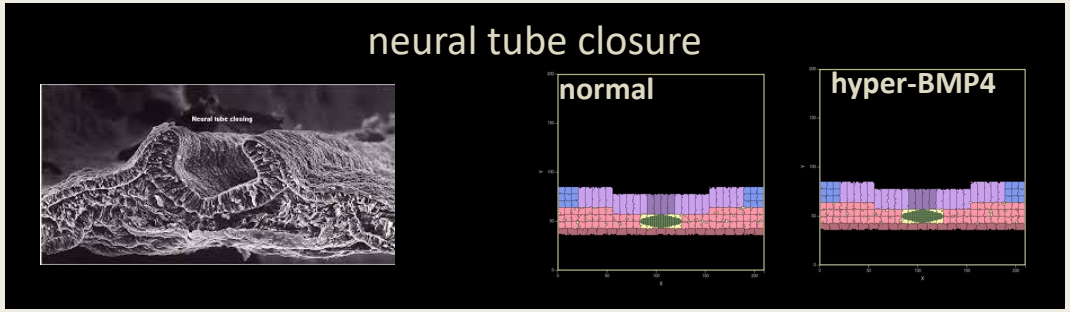
Morphogenetic Fusion



Leung et al. (2016) Repro Toxicol



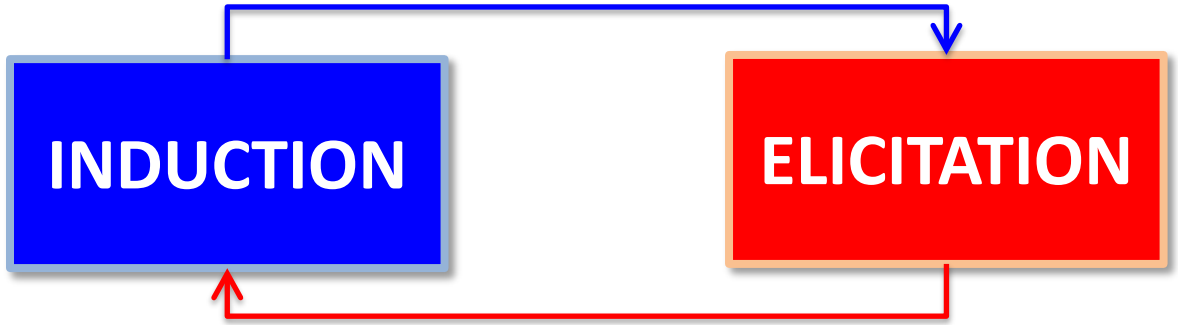
Hutson et al. (2017) Chem Res Toxicol



Berkhout et al. (2023), work in progress

Strategy: *reconstructing early effects on signal kinematics and toxicodynamics*

Forward engineering - *suppose we know a molecular effect (eg, ToxCast lesion), how far can an ABM take us to hypothesizing an apical outcome?*

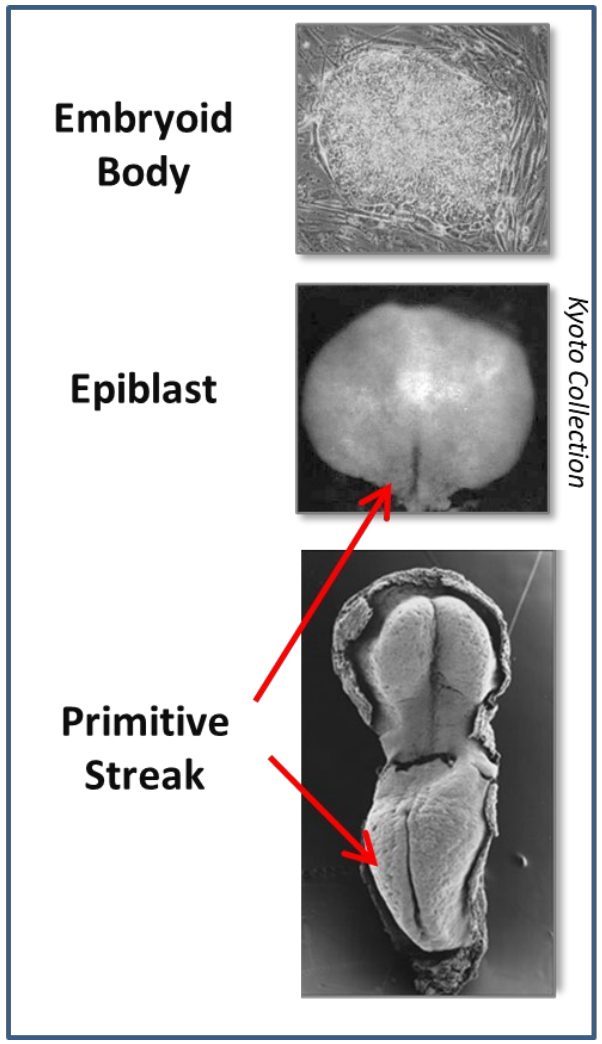


Reverse-engineering - *suppose we know an apical outcome (eg, DevTox), how far can an ABM take us to inferring a molecular mechanism?*

Hypothesis:

Morphological programming logic of the embryo provides a platform for computational intelligence that can translate *in vitro* bioactivity into a mechanistic *in silico* model.

Gastrulation: *remarkable example of a self-organizing system*

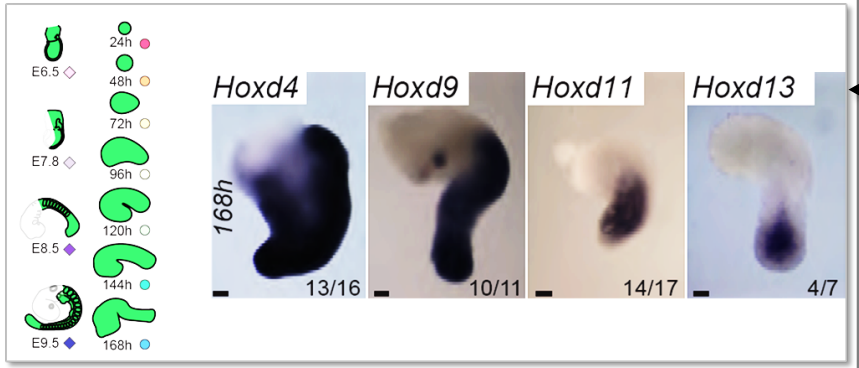


- The molecular biology and behavior of hPSCs in culture most closely resembles the **epiblast** of an early embryo during gastrulation.
- Although cultured hPSCs can form most cell types in the fetus they lack **positional information** of an intact epiblast.
- An anatomical hallmark in most *Vertebrates* is the **primitive streak** through which a genomic body plan unfolds.
- Cell migration through the primitive streak is essential for spatial organization, regional specification, and lineage determination.

"It is not birth, marriage, or death, but gastrulation which is truly the most important time in your life." - Lewis Wolpert

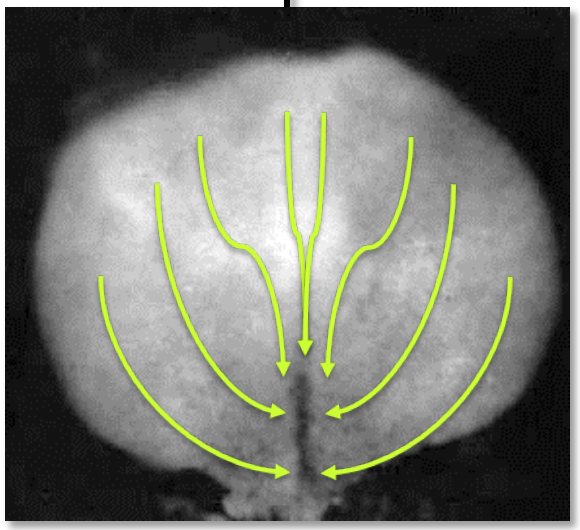
Autopoiesis: *temporal colinearity of homeobox (Hox) genes that pattern the embryo*

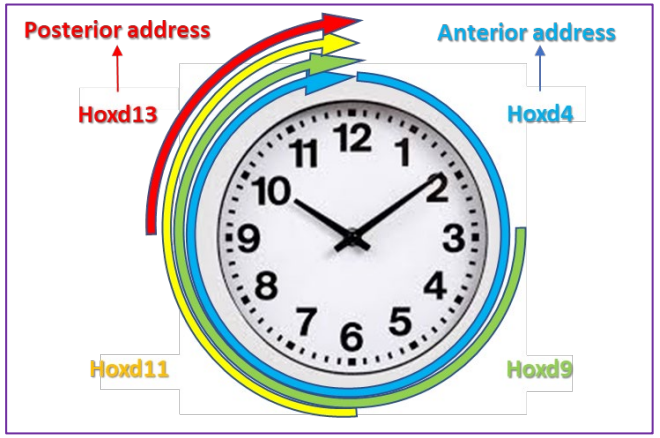
Rudimentary gastruloid self-organizes the anterior-posterior body axis.



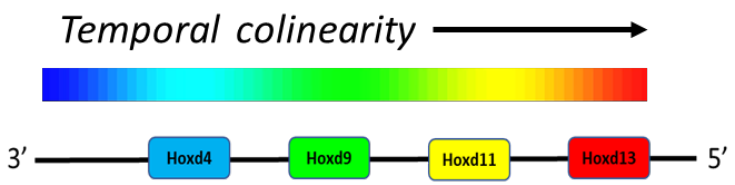
Beccari et al. (2018) Nature

Critical mass of PSCs aggregated into an *in vitro* microsystem.





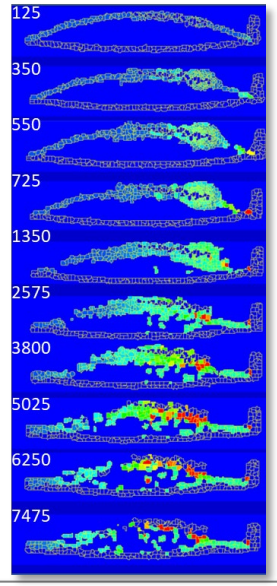
Think of it as a Hox-clock, where time is measured by nested activation of *Hox* genes in succession.

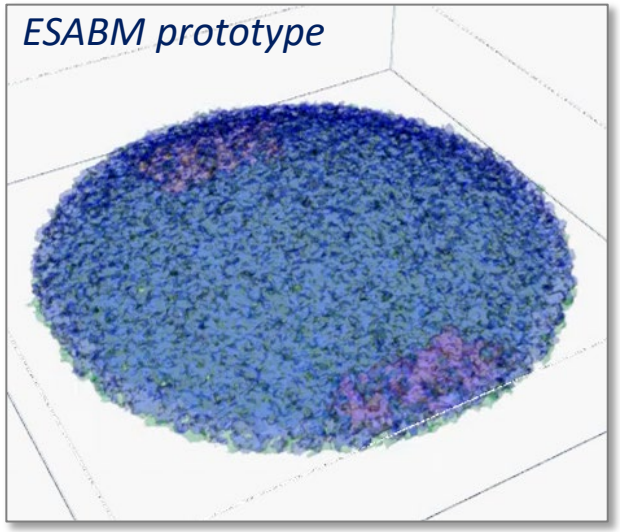


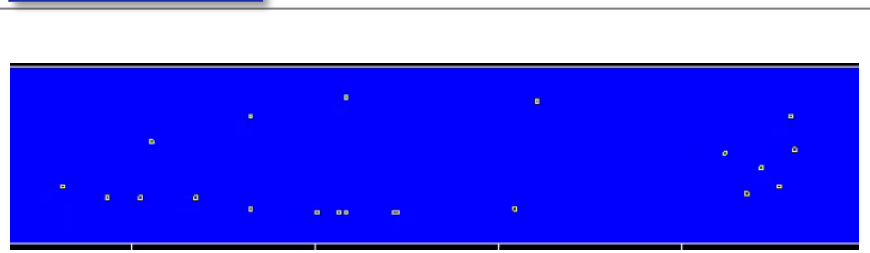
anterior → posterior specification (head-to-tail)

Time is set at the PS: cell position is a key parameter in clonal transmission of fate.

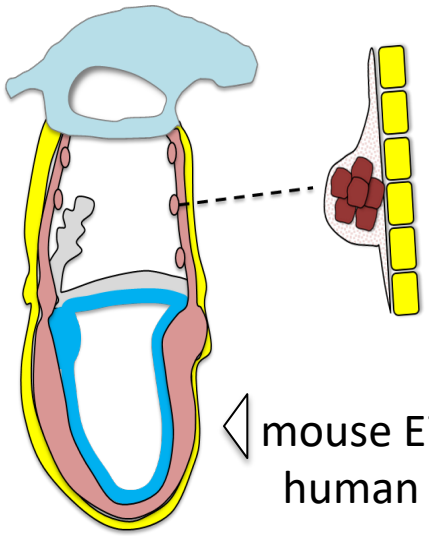
Example: *encoding the genomic blueprint of the embryonic axis during gastrulation*



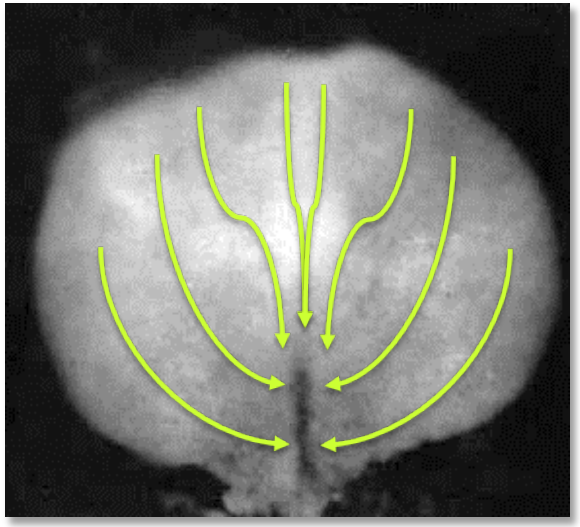


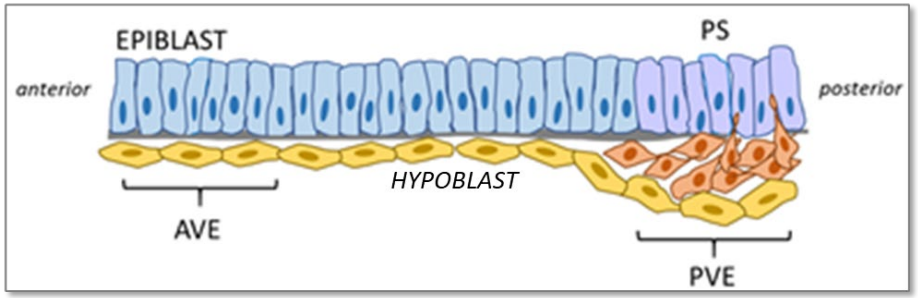


Cell fields and colinear *Hox* gene activation can be modeled in compucell3d.org.



mouse E7.5
human 3rd week

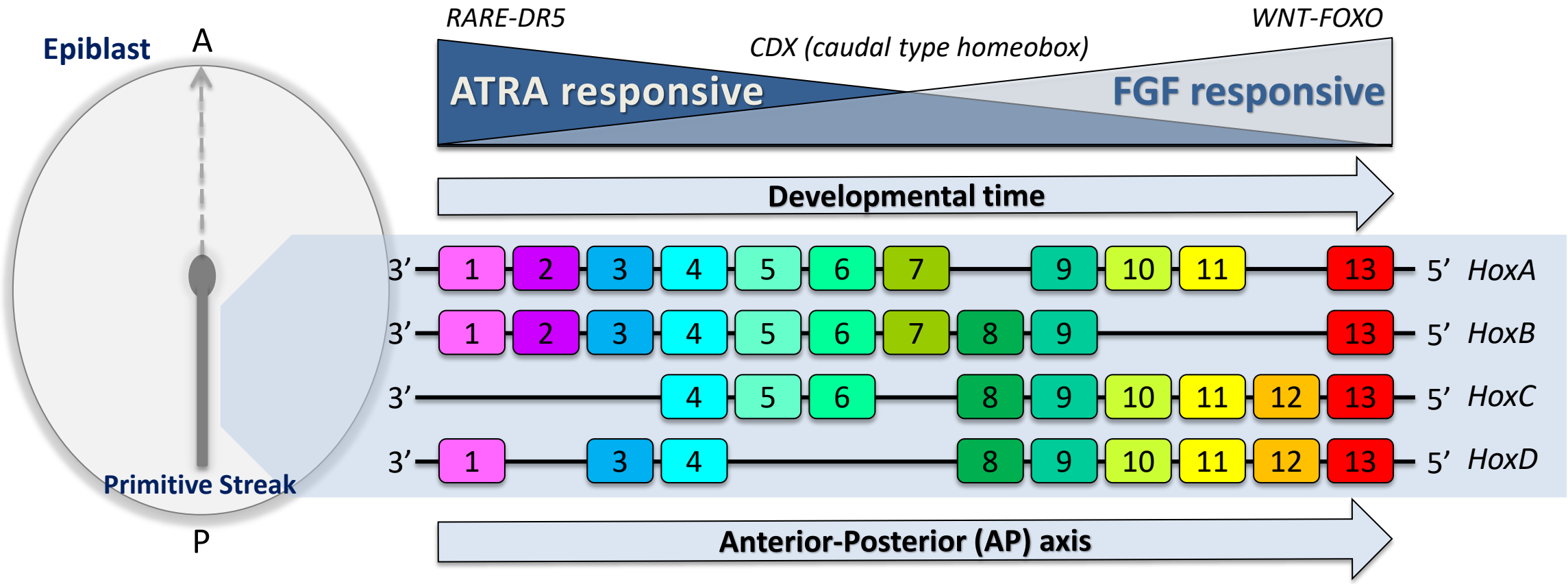




PSCs in the epiblast stream to the Primitive Streak (PS) where they undergo EMT → mesoderm.

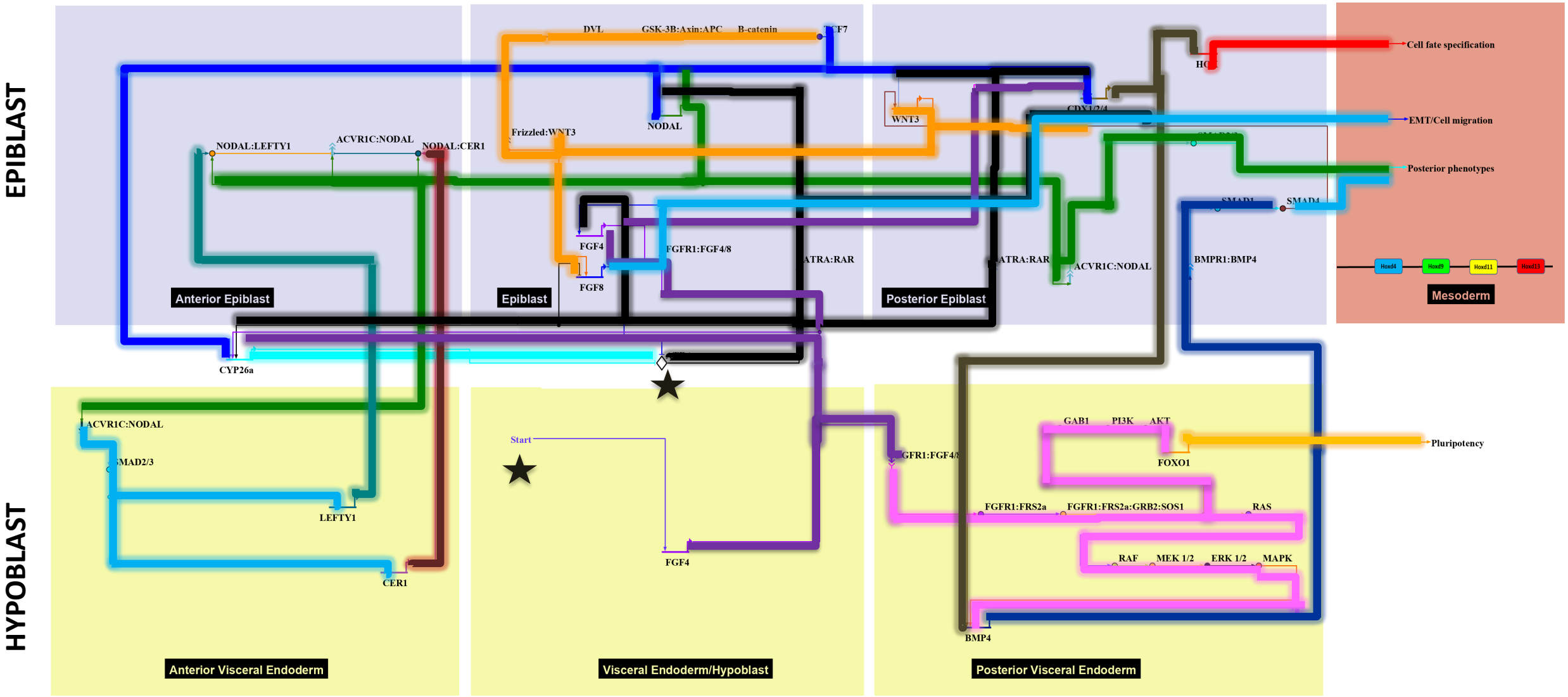
Richard Spencer, EMVL; Kaitlyn Barham, ORAU

Embryonic disc: *encoding the genomic blueprint of the embryonic axis in silico*



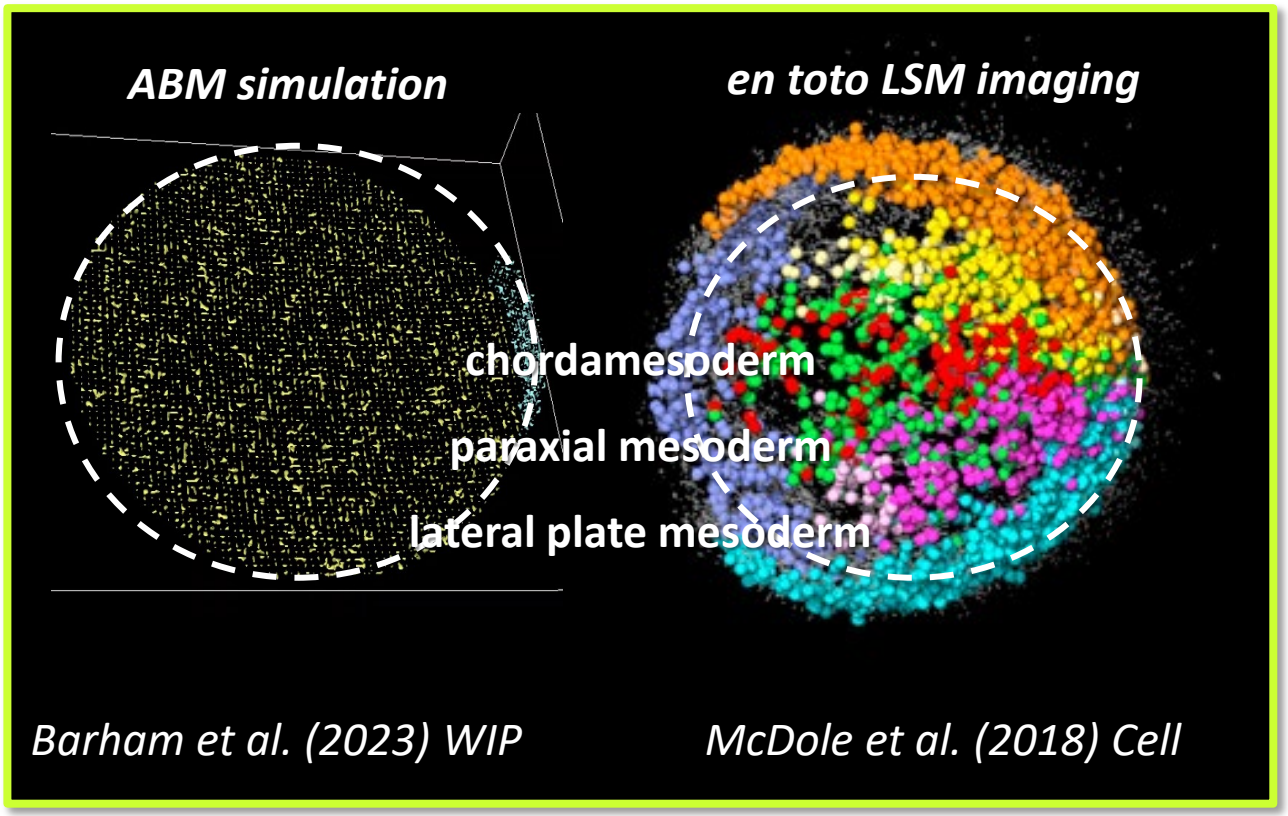
Homeobox (*Hox*) genes are downstream effectors of *all-trans* retinoic acid (ATRA) and Fibroblast Growth Factor (FGF) signaling that paces their colinear expression.

ATRA and FGF Signaling: *flow of molecular regulatory information*



Barham et al. (manuscript in preparation)

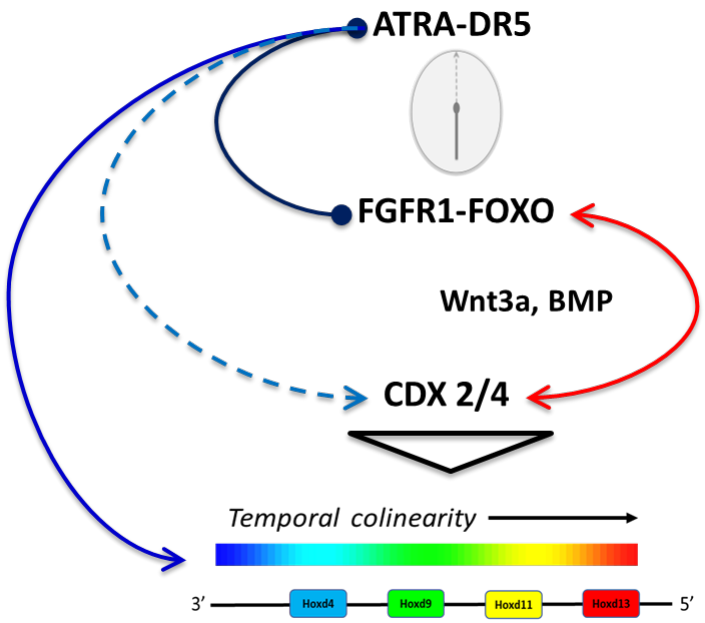
Epiblast dynamics: *putting morphological programming logic into motion*



Quoting McDole: *"… there is little mixing of the different mesodermal layers once cells exit the primitive streak. This suggests that when and where a cell exits the streak is of critical importance to determining its final fate, …"*

Barham et al. (manuscript in preparation)

ATRA and FGF Signaling: *flow of molecular regulatory information*

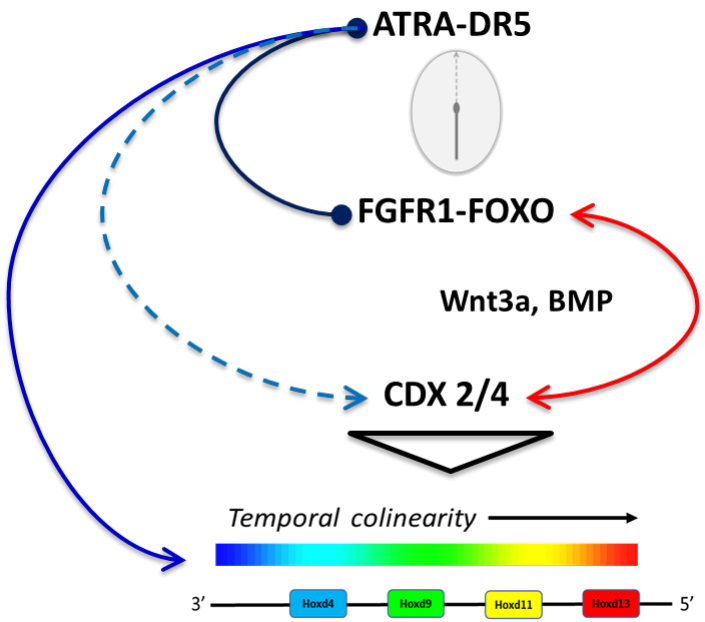


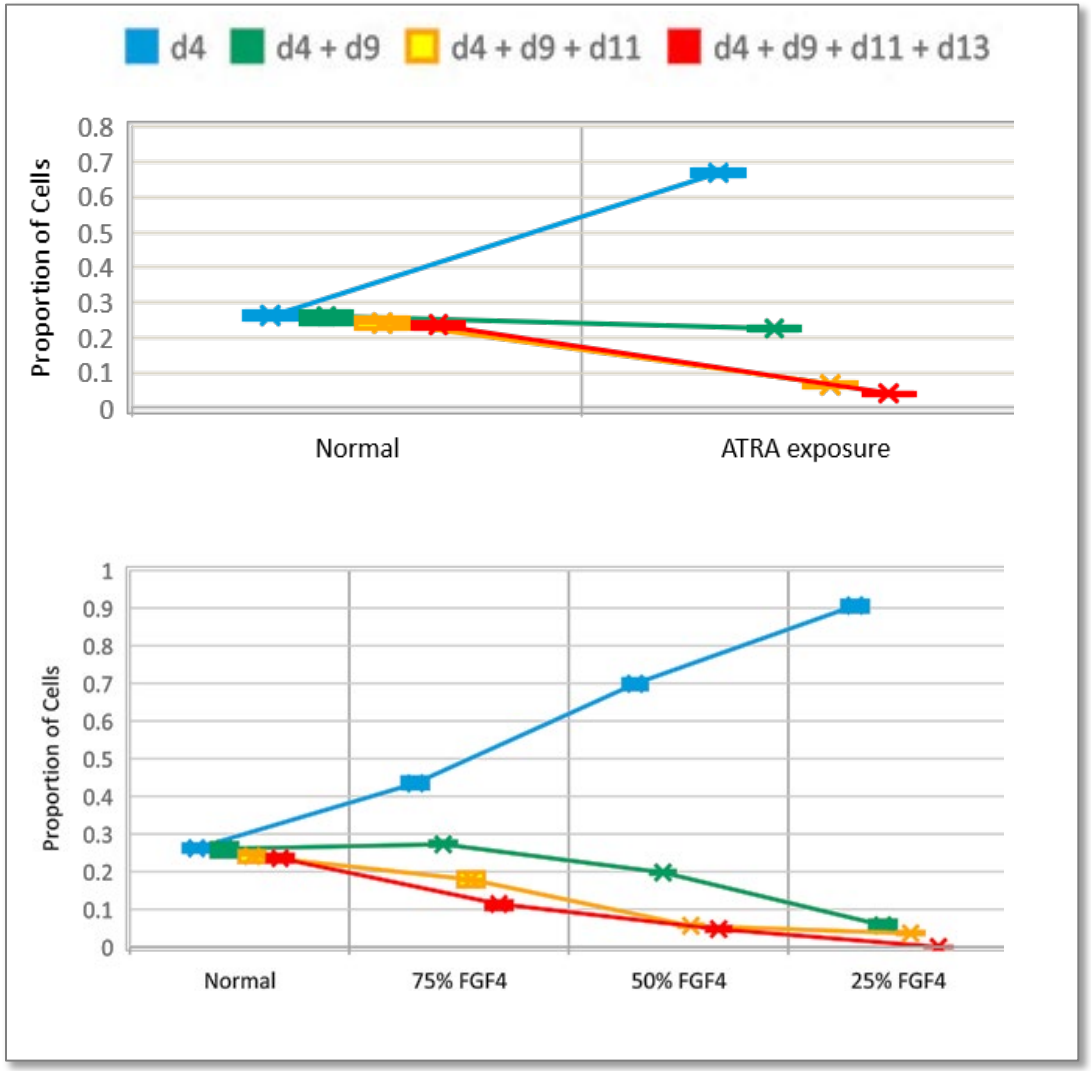
- Autonomous Hox clock is set as mesoderm internalizes at a pace regulated by ATRA-FGF-CDX-HOX signaling network.
- ATRA accelerates 3'-Hox gene activation → anterior *Hox* gene assignment of nascent mesoderm.
- FGFR1 accelerates 5'-Hox gene activation → posterior *Hox* gene assignment of nascent mesoderm.

Model Predictions:

Excessive ATRA or insufficient FGF signaling anteriorizes nested *Hox* code … mesodermal cells establish position and may carry the wrong *Hox* address into organogenesis.

ATRA and FGF Signaling: *flow of molecular regulatory information*

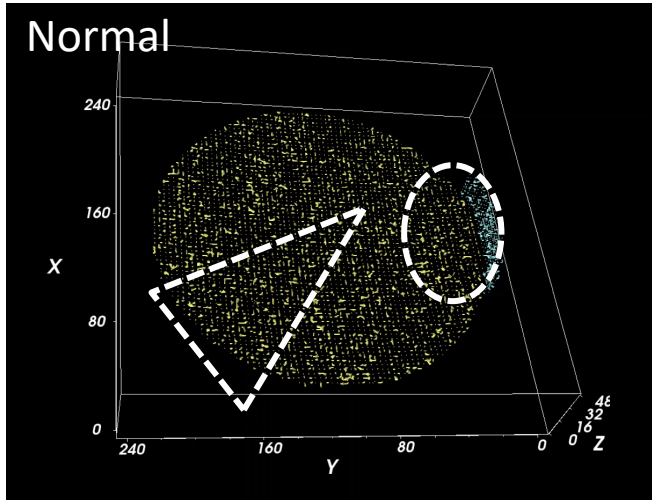


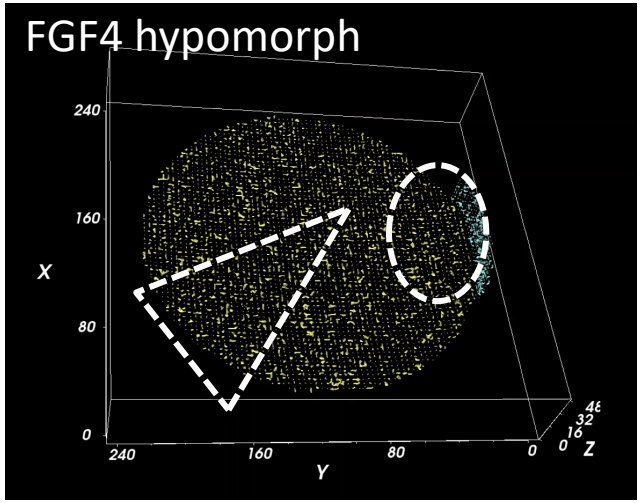


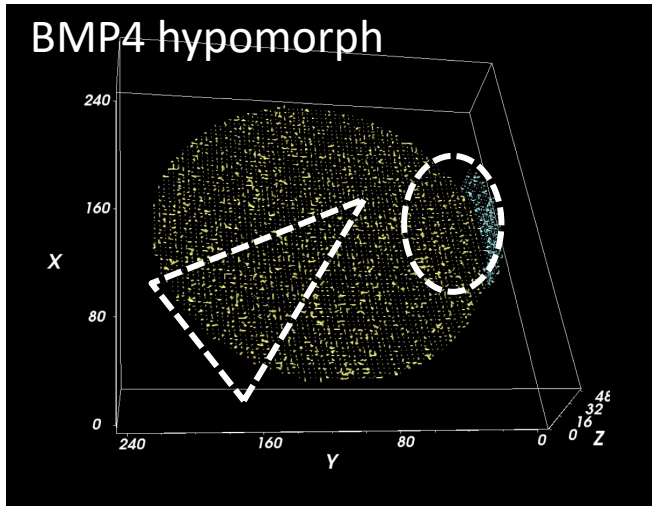
Model output:
Both conditions anteriorize the nested *Hox* address of nascent mesoderm

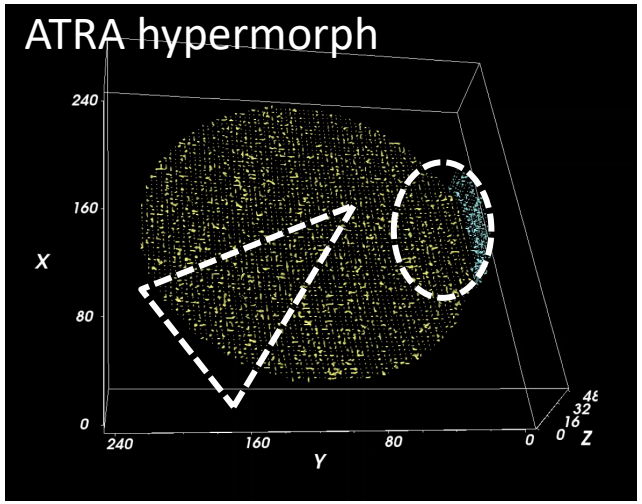
Barham et al. (manuscript in preparation)

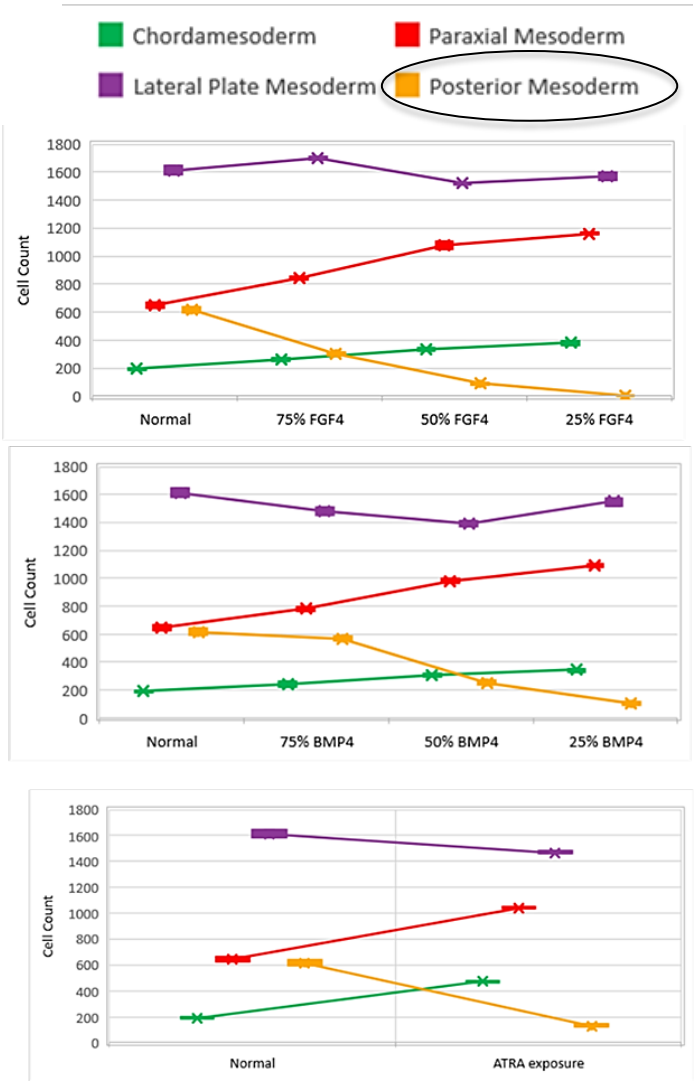
Hacking the model: *cybermorphs elicited from synthetic disruption*





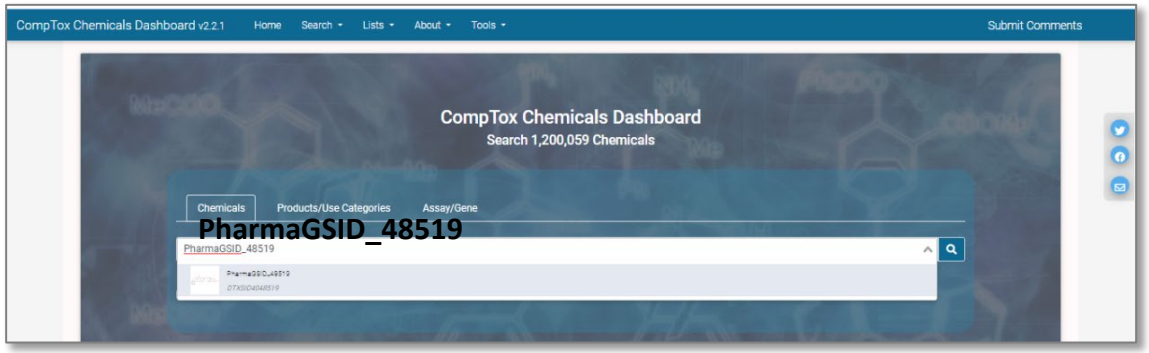


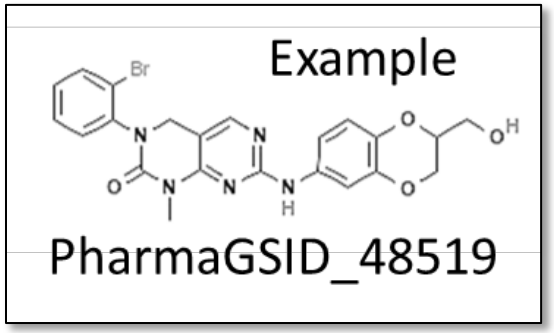




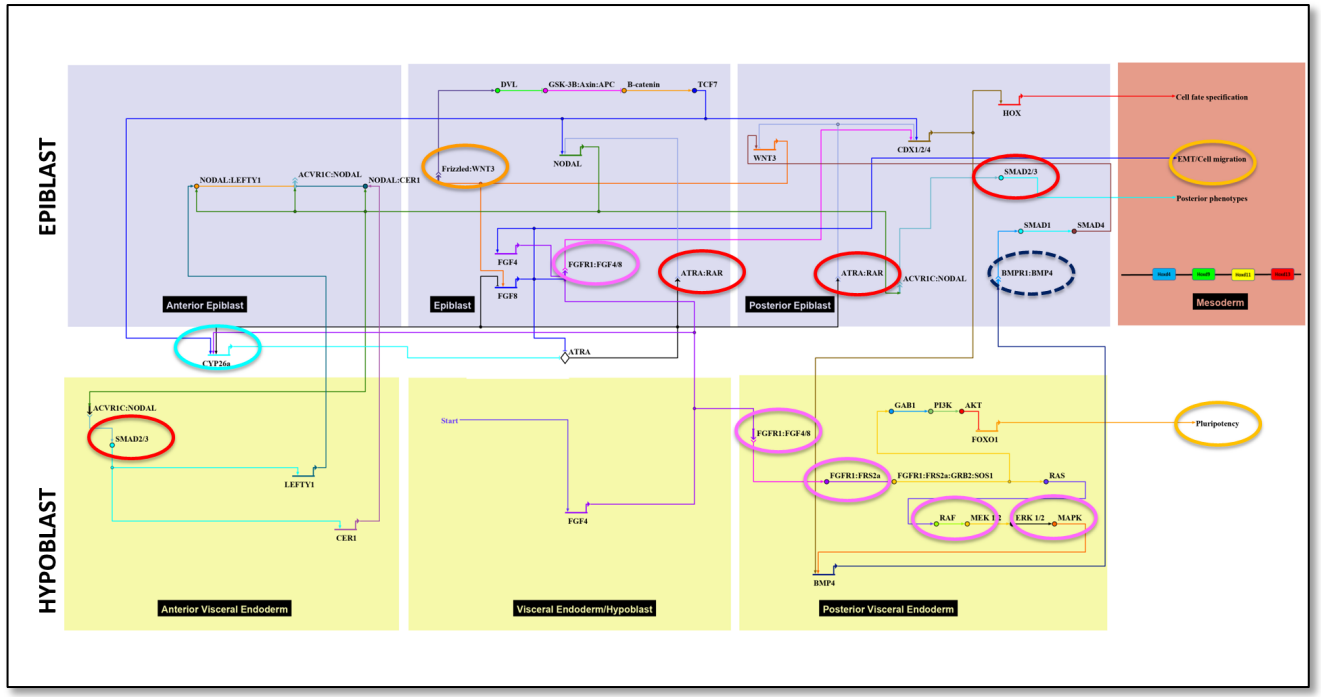
Barham et al. (manuscript in preparation)

Mapping the response: *direct assays with data hit-calls in ToxCast/Tox21*





Platform	Assay Target	µM Hit-call	Feature
NovaScreen	hFGFR1_dn	0.01	FGF signaling
NovaScreen	hSRC_dn	0.01	FGF signaling
NovaScreen	hRAF_dn	0.01	FGF signaling
Httr	EMT	0.36	MCF7 gene signature
Tox21	SBE_dn	0.57	SMAD binding
AttaGene	DR5_up	1.22	ATRA signaling
Stemina DevTox^{qP}	ORN/CYSS	1.22	hPSC DevTox threshold
BioSeek	TFGb1_dn	10.0	BMP signaling
Tox21	Gli3_dn	11.3	SHH antagonist
Tox21	Wnt_dn	15.0	WNT antagonist
Tox21	SMAD3_dn	15.4	SMAD3 phosphorylation
Httk	Fetal plasma	0.84 mg/ml	Plasma steady state



https://comptox.epa.gov/dashboard

Computational Biology

- Cell ABMs are a novel approach to: (i) visualize cellular trajectories; (ii) map toxicodynamics; and (iii) predict adverse phenotype (cybermorphs).

- *In silico* reconstitution of a self-organizing embryo from *in vitro* data (eg, embryogeny) remains a challenge, and these are theoretical models (artificial life).

- 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.

Thank you!

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Challenge Questions

Translational: What could a comprehensive suite of human-relevant synthetic (*in silico*) models bring to toxicity testing in the animal-free zone?

Investigational: How complex must intelligent cellular systems models be for accurate phenotypic translation?

Operational: What best practices are best suited for NAMs implementation, circa 2025? How far can artificial life go towards replacing animal testing, circa 2035?

Communication: given proof-of-concept with small working prototypes, what factors would make stakeholders more (or less) comfortable using these types of computer models in place of a whole organism?