Engineering a Computable Epiblast for *in silico* Gastrulation and Predictive Modeling of Developmental Toxicity with *in vitro* Data from the ToxCast Stem Cell Assay

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Background

- The U.S. EPA is working to develop New Approach Methods (NAMs) to study chemical effects on prenatal development as an alternative to animal testing. One area of interest is virtual tissue modeling in which developmental events are recapitulated *in silico*.
- A human pluripotent stem cell (hPSC) assay on over 1,000 chemicals has been utilized to generate the ToxCast portfolio, which can predict developmental toxicity with ~80& balanced accuracy [1].
- The developmental potential of hPSCs is closely reflected by the mammalian 'epiblast' due to its differentiation into the primary germ layers (endoderm, mesoderm, ectoderm) during gastrulation.
- An *in silico* epiblast encompassing cellular dynamics during gastrulation provides a platform for predictive toxicology through dynamical modeling and simulation of developmental phenotypes.

Gastrulation

- This vital process establishes the fundamental body plan through transition from a bilayer symmetrical disc to a trilayered structure with bilateral, dorso-ventral, and antero-posterior axes.
- Gastrulation occurs during embryonic days E5.5-E7.5 in mouse and the 3rd week of human gestation, and has recently been recapitulated from hPSCs in a bioengineered microsystem [2].
- At E5.5, FGF triggers a signaling cascade that primes the epiblast for BMP4 to polarize the body axis. A hallmark of the process is formation of the 'primitive streak' in the posterior midline.
- Epiblast cells migrate through the primitive streak and differentiate into mesodermal fates destined for different regions depending on timing and position setting the Hox clock [3].
- An agent-based model of the epiblast was constructed in the CompuCell3D.org modeling environment to operationalize the cell-cell regulatory signals established from the literature.
- Our virtual epiblast quantitatively self-organizes a primitive streak and subsequent mesodermal populations destined for different regions of the embryo.

References

[1] Zurlinden TJ, Saili KS, Rush N, Kothiya P, Judson RS, Houck KA, Hunter ES, Baker NC, Palmer JA, Thomas RS, Knudsen TB. Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity. *Toxicol Sci*. 2020 Apr 1;174(2):189-209. doi: 10.1093/toxsci/kfaa014. PMID: 32073639; PMCID: PMC8527599.

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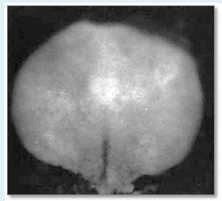
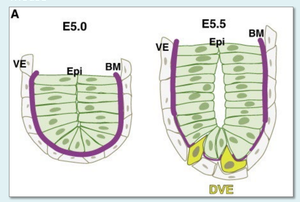
[3] Bardot ES, Hadjantonakis AK. Mouse gastrulation: Coordination of tissue patterning, specification and diversification of cell fate. Mech Dev. 2020 Sep;163:103617. doi: 10.1016/j.mod.2020.103617. Epub 2020 May 27. PMID: 32473204; PMCID: PMC7534585.

Human

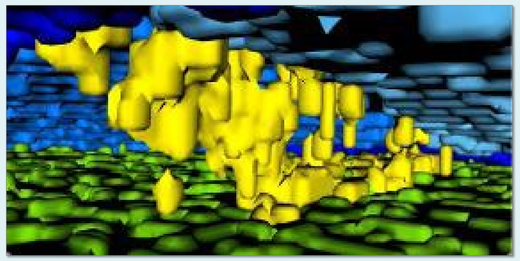


Epiblast from the Kyoto Collection

Mouse

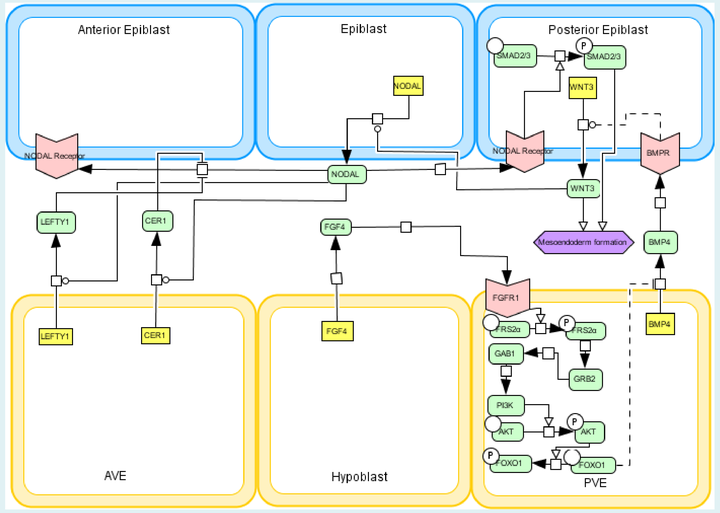


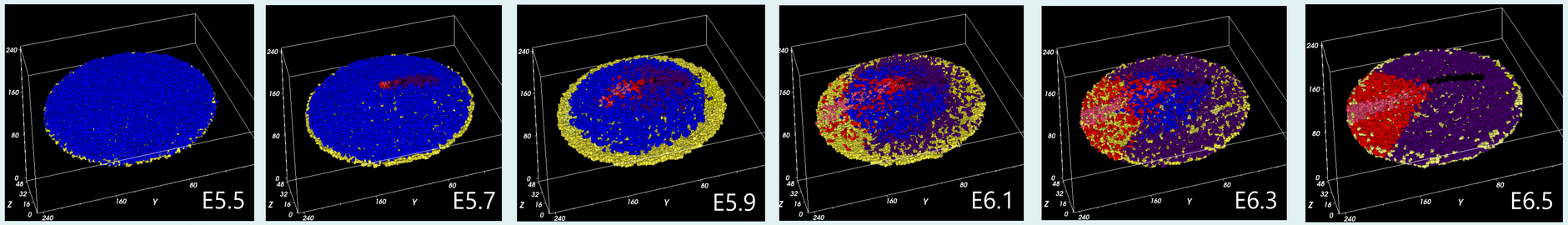
Matsuo and Hiramatsu (2017) Mech Devel



The key regulatory network at play during patterning was determined from a review of the current body of literature on mouse and human gastrulation. A signaling cascade begins with the release of FGF4 from the hypoblast and results in anterior-posterior patterning due to the limited action of NODAL on only the posterior cells of the epiblast.

FGF4 stimulates the secretion of BMP4 from the posterior visceral endoderm (PVE). BMP4 stimulates release of WNT3 from the posterior epiblast, which causes NODAL secretion from the epiblast. The presence of NODAL causes the anterior visceral endoderm (AVE) to locally secrete LEFTY1 and CER1, which both prevent NODAL from binding to anterior epiblast cells. The interdependencies of these key proteins is integral to predicting the altered phenotypes caused by embryonic toxicological exposure. These proteins have been encoded into our model to observe altered phenotypes based on chemical perturbations of these pathways.





Color	Name
	Medium
	Epiblast
	VisceralEndoderm

	Chordamesoderm
	ParaxialMesoderm
	LateralPlateMesoderm

	Organizer
	PrimitiveStreak

We engineered a fully computable model of the mouse epiblast from E5.5 through E6.5 that demonstrates the formation of the primitive streak and subsequent epithelial-mesenchymal transition of epiblast cells, which differentiate and self-organize into separate mesodermal domains. ToxCast chemical and bioactivity data is currently being utilized in case studies to screen embryonic phenotypic perturbations invoked by environmental stressors.

Model Video

DISCLAIMER: This poster does not represent EPA Policy.