Engineering a Computable Epiblast for *in silico* Modeling of Developmental Toxicity

Kaitlyn N. Barham[1], Richard Spencer[2], Nancy Baker[3] and Thomas B. Knudsen[4]

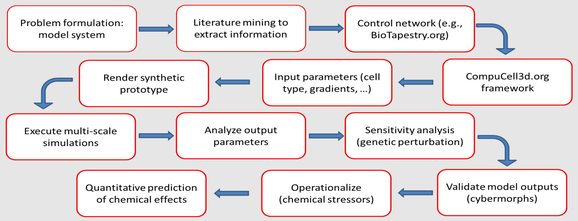
[1]Oak Ridge Associated Universities, [2]General Dynamics, [3]Pegasus and [4]Center for Computational Toxicology and Exposure, U.S. EPA, Research Triangle Park, NC

Barham.Kaitlyn@epa.gov

Introduction

- Virtual embryo computer models integrate biological systems with chemical specific information to make predictions about developmental toxicity.
- Here, we built and tested a dynamic epiblast stem cell agent-based model (ESABM) for mesoderm formation based on signaling networks patterning gastrulation.

Workflow



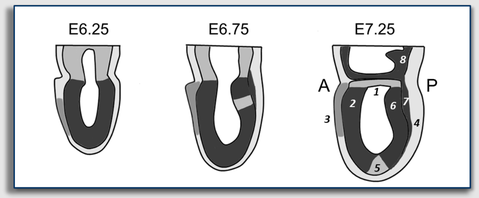
- Biological information from relevant literature on mouse or human stem cells was extracted and incorporated.
- ESABM [1] was built in CompuCell3D.org, encoding steppable modules called after each Monte Carlo Step (MCS, 7200 steps).
- The 3D simulation models the epiblast in mouse during embryonic days E6.25 – E7.25 (and human gestation days 14-17).
- Mesoderm formation was calibrated to *in toto* imaging of the mouse embryo at the single-cell level [2].
- ESABM enables self-organization of emergent phenotypes following targeted perturbations in the control system.
- Case examples tested chemical-bioactivity information from targeted ToxCast assays [https://comptox.epa.gov/dashboard/].
- For this, we mapped AC_{50} chemical bioactivity data from ToxCast to signal-dependent alterations in the ESABM control system.

REFERENCES

[1] Barham K, Spencer R, Baker NC and Knudsen TB (2024) Engineering a computable epiblast for in silico modeling of developmental toxicity *Reprod Toxicol* (submitted).

[2] McDole K, Guignard L, Amat F, Berger A, Malandain G, Royer LA, Turaga SC, Branson K and Keller PJ (2018) In toto imaging and reconstruction of post-implantation mouse development at the single-cell level *Cell* 175: 859-876.e33.

Key Signal-Responses in the Computer Model

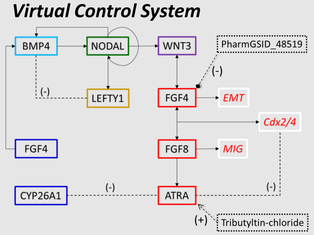


CELL TYPE SOURCE	SIGNAL	TARGET	EFFECT	CONSEQUENCE
ExE	BMP4	Epiblast	(+) *Nodal* expression	drives posterior fate
Epiblast	NODAL	ExE	(+) *Bmp4* expression	epiblast stabilization
Epiblast	NODAL	AVE	(+) *Lefty1*	pre-patterning
AVE	LEFTY1	Anterior epiblast	SMAD antagonism	posteriorization
Posterior epiblast	NODAL/BMP4	Primitive Node	*(+) Wnt3*	streak positioning
Primitive Node/Streak	FGF4	pre-ingression	facilitates EMT	(+) endomesoderm
Primitive Streak	FGF8	nascent mesoderm	facilitates migration	mesoderm dispersion
Primitive Streak	FGF4	CDX2/4	(+) HOX clock	HOX specification
Mesoderm	ATRA	CDX2/4	(-) HOX clock	HOX specification
TEST CASES (ToxCast)	**AC50**	**ASSAY READOUT**	**EXPOSURE**	**PREDICTED TOXICITY**
at-Retinoic acid (ATRA)	<10 nM	RARA → DR5	Maternal physiome	Expanded NODAL domain
Tributyltin-chloride	<10 nM	RXRA → DR5	Environmental	3'-HOX misallocation
PharmaGSID_48519	<10 nM	FGFR1 liganding	Kinase-directed inhibitor	5'-HOX misallocation

Section Profile (Anterior, Posterior)

1. Extraembryonic ectoderm (ExE)
2. Epiblast
3. Anterior Visceral Endoderm (AVE)
4. Hypoblast (embryonic)
5. Primitive Node
6. Primitive Streak (PS)
7. Embryonic mesoderm
8. Extraembryonic mesoderm

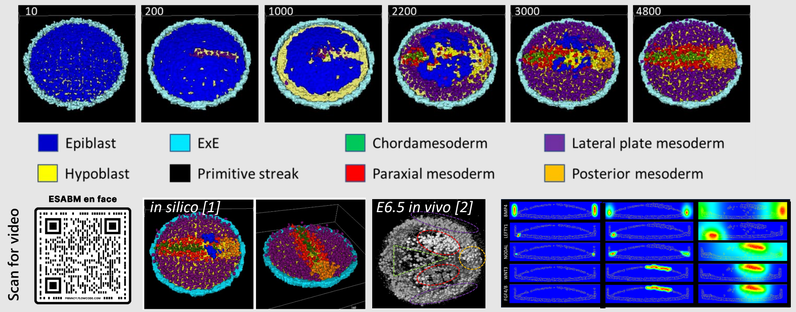
Virtual Control System



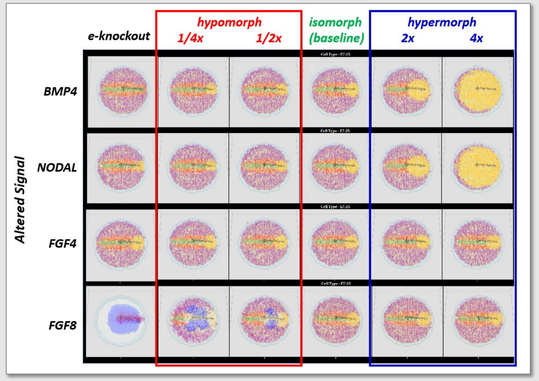
- Virtual development is triggered by BMP4 release from the ExE and NODAL from the epiblast.
- NODAL stabilizes BMP4, amplifies itself, and induces LEFTY1 (a BMP4 antagonist) in the AVE.
- The NODAL-BMP4-WNT3 axis drives PS formation through regulation of FGF signaling.
- FGF8 from nascent mesoderm drives cell dispersion between epiblast and hypoblast layers.
- FGF4 paces the autonomous Homeobox (HOX) clock running in the epiblast via CDX2/4.
- all-trans retinoic acid (ATRA) must be removed for proper FGF and NODAL signaling (<E7.5).

Simulated Mesodermal Fate Map

Embryonic disc: wave of epiblast stem cell progression *en face.* Nascent mesodermal cells pass through the PS and are colored by prospective fate. The epiblast (*blue*) is fully consumed in this simulation (steps number time frames). Self-emergent *in silico* fate [1] compares to E6.5 mouse reconstructed by *en toto* imaging [2]. AP signal domains shown in mid-longitudinal sections.



Fate Maps Predicted from *in silico* Perturbation



Mesodermal population density (predicted):

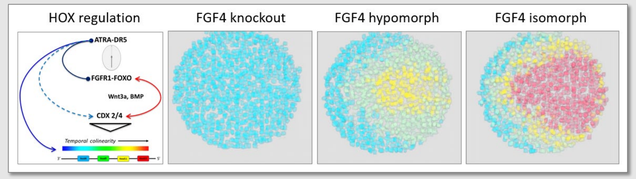
- Regional fate is determined by cell position and timing as it traverses the PS:
  - chordamesoderm (green)
  - paraxial mesoderm (red)
  - lateral plate mesoderm (magenta)
  - posterior mesoderm (yellow).
- Population densities following simulated perturbation of control signals compared to baseline simulations (isomorphs):
  - e-knockout (complete loss)
  - hypomorphs (2- or 4-fold reduction)
  - hypermorph (2- or 4-fold increase).
- Anterior-Posterior patterning is driven by BMP4-WNT3 and NODAL-LEFTY1 signals.

CELL FIELD ARRAY

Autonomous Homeobox specification (HOX clock):

- Mesodermal specification is determined by nested activation of *Hox* genes assigned in colinear fashion (3' → 5') and fixed once a cell passes through the PS. The pattern is visualized below for *Hoxd4/d9/d11/d13* activation.
- Rate of the HOX clock in ESABM is paced by the WNT3 → FGF → ATRA → CDX axis. Chemical interference with FGF-signaling (e.g., phamaGSID_48519) or precocious activation of ATRA-signaling (e.g., tributyltin-chloride) are predicted to misregulate the HOX clock, causing mesoderm to carry the wrong *Hox* address to their regions.

FGF4 HOX ACTIVATION



Conclusions

- Virtual embryo is a novel approach to visualize cellular trajectories, map toxicodynamics, and predict adverse phenotypes in ways difficult to accomplish *in vivo*.
- ESABM platform enables *in silico* integration of molecular effects data with known embryology to model chemical effects data on epiblast patterning and gastrulation.
- Temporality at present covers early gastrulation events but can be scaled to ensemble models at other stage for mechanistic prediction of developmental toxicity.