Developmental Computation with Embryonic Stem Cells

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Introduction

Objective: Engineer a generalizable computational agent-based model (ABM) that quantitatively simulates embryonic stem (ES) cell behaviors *in silico*.

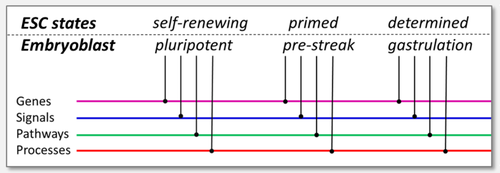
Rationale: Human ES cell lines (hESCs) are a promising *in vitro* platform for development hazard prediction due to their capacity for self-renewal, broad differentiation potential, and self-organization [1].

Hypothesis: An *in silico* cellular dynamics model with sufficient morphological programming logic of the human epiblast provides a deep-learning platform to translate *in vitro* profiling data from ToxCast [2] into a quantitative simulation of *in vivo* dysmorphogenesis.

Background

ToxCast_STM: a metabolic biomarker assay of teratogenicity in pluripotent hESC cells (H9 line) [3] registered hits on 202 of 1065 ToxCast chemicals [4]. Performance-based models accurately classified two-thirds of those with robust *in vivo* developmental toxicity outcomes. Recursive enrichment models inferred PI3K-FOXO signaling as a major determinant of domain sensitivity [4] and predicted several pathway domains missed by the assay [5].

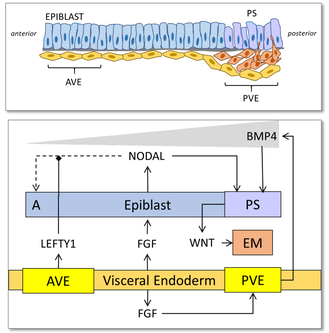
Pluripotency: cultured ESCs progress from a ground state (naïve pluripotency) to a primed state upon LIF withdrawal setting the stage for lineage determination. RNA profiling shows these cells naturally resemble the early epiblast stage of embryogenesis [6]. Cultured hESCs exposed to BMP4 may self-organize into radially symmetrical patterns of germ layers when geometrically confined [7]; however, synthetic hESC microsystems with localized BMP4 delivery break the symmetry and display reproducible axial patterning [8].



Information from the literature used to guide construction of ES-ABM.

References:
[1] Martello, Smith (2014) Ann Rev Cell Dev Biol 30 https://doi.org/10.1146/annurev-cellbio-100913-013116
[2] Thomas et al. (2019) Toxciol Sci 169 https://doi.org/10.1093/toxsci/kfz058
[3] Palmer et al. (2013) Birth Defects Res 98 https://doi.org/10.1002/bdrb.21078
[4] Zurlinden et al. (2020) Toxicol Sci (in press) https://doi.org/10.1093/toxsci/kfaa014
[5] Zurlinden et al. (2020) SOT Abstract 2049, Poster #383 (manuscript in preparation)
[6] Cheng et al. (2019) Cell Reports 26 https://doi.org/10.1016/j.celrep.2019.02.031
[7] Simunovic et al. (2019) Nat Cell Biol https://doi.org/10.1038/s41556-019-0349-7
[8] Manfrin et al. (2019) Nat Meth 26 https://doi.org/10.1038/s41592-019-0455-2
[9] Yu, Cui (2016) Development 143 https://doi.org/10.1242/dev.137075

Conceptual systems model (epiblast)

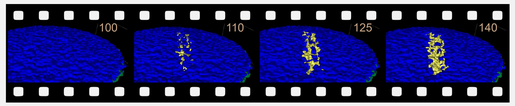


Signaling network: complex interactions between the epiblast and extraembryonic cells set up the major body plan:

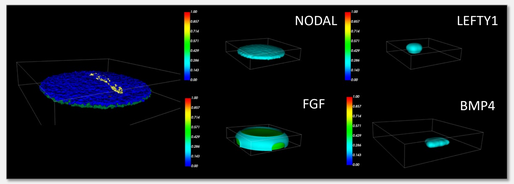
- gastrulation establishes the midline (anterior-posterior) body axis and primary germ layers that will form the embryo proper;
- hallmark is the primitive streak (PS), a transient structure that extends by circular cell movements driven by FGF and WNT;
- positioned by NODAL commuted from the epiblast and BMP4 from posterior visceral endoderm (PVE) inducing Wnt3a expression;
- anterior visceral endoderm (AVE) is a major organizing center via signals that antagonize NODAL and BMP4 .

Dynamical systems model (quasi-gastrulation)

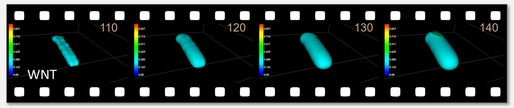
ES-ABM: small working prototype of the human bilaminar disc embryo implemented in compucell3d.org modeling environment; agent-based model has approximately 10K interacting cells.



Cell field: epiblast viewed from above (top) during PS formation; yellow cells mark the prospective ingression axis in response to WNT signaling; pseudotime in the frame.



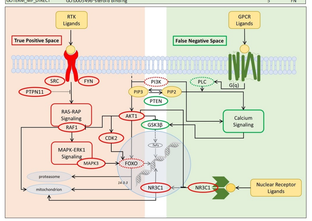
Signal fields: NODAL (epiblast) is antagonized by LEFTY1 (AVE); FGF (visceral endoderm) induces BMP4 (PVE); NODAL + BMP4 position WNT (PS).



WNT marker: WNT is an indicator of the PS, here represented as a quantitative tracing of endomesodermal (EM) ingression.

Imputing domains of applicability

Annotation System	Keystone Pathway / Process	# MIEs	Class
GOTERM_BP_DIRECT	GO:0051404#regulation of phosphatidylinositol 3-kinase signaling	8	TP
KEGG_PATHWAY	hsa04068:FoxO signaling pathway	8	TP
KEGG_PATHWAY	hsa04510:Focal adhesion	13	TP
GOTERM_BP_DIRECT	GO:0007220~phospholipase C-activating G-protein coupled receptor signaling pathway	10	FN
INTERPRO	IPR001723:Steroid hormone receptor	7	FN
GOTERM_MF_DIRECT	GO:0005499~vitamin D binding	5	FN

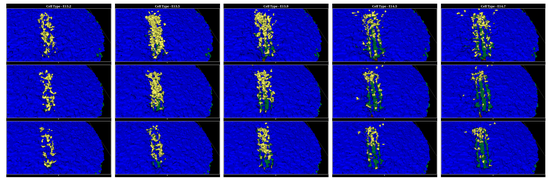


Biological domain: From the ToxCast_STM assay, we correlated the hESC predictive biomarker to potential MIEs (molecular initiating events) within the domain of applicability for developmental toxicity [4]. This is consistent with the importance of AKT-PI3K signaling in regulating pluripotency states [9].

- TP, true positive (red zone)
- FN, false negative (green zone)

Given FGF signals has a crucial effect in preserving hESC properties [9], we simulated cell ingression as a surrogate for FGF → BMP4 → WNT.

WNT-dependent PS ingression: quasi-gastrulation cybermorphed for different degrees of cell ingression (yellow cells) over the same pseudotime period.



WNT at 100% strength (405 cells ingress)

WNT at 25% strength (260 cells ingress)

WNT at 10% strength (207 cells ingress)

SUMMARY

A multicellular agent-based model (ES-ABM) was engineered to recapitulate cellular dynamics of the human epiblast during gastrulation. The small working prototype (preliminary model):

- sets up the two major organizing centers that position the anterior-posterior body axis (AVE, NODE) in the epiblast and enables cellular ingression through the primitive streak.
- provides a surrogate of WNT-dependent germ layer formation and will eventually host a relevant signaling network (FGF, NODAL, BMP4, WNT).
- enables a virtual (*in silico*) approach to integrate HTS *in vitro* HTS data (e.g., ToxCast/Tox21) with the logic of cell-cell signaling networks for predictive toxicology.

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