ESTIV 2022
*Models, biomarkers and assays for endocrine disruption
and developmental toxicity*

Computational systems models for human-predictive developmental toxicity

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Bringing the embryo into focus

Vast collections of bioactivity data from *in vitro* chemical profiling are now in hand (https://comptox.epa.gov/dashboard).

These complex datasets provide a new resource to examine key cellular and molecular determinants of developmental toxicity.

However, virtual reconstitution of a self-organizing system from unidimensional data (embryogeny) remains a challenge.

Of paramount importance:
- understanding how developmental cell fate and behavior is regulated,
- elucidating the systems-level dynamics of collective decision-making, and
- pinpointing how developmental perturbations are naturally buffered.

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:
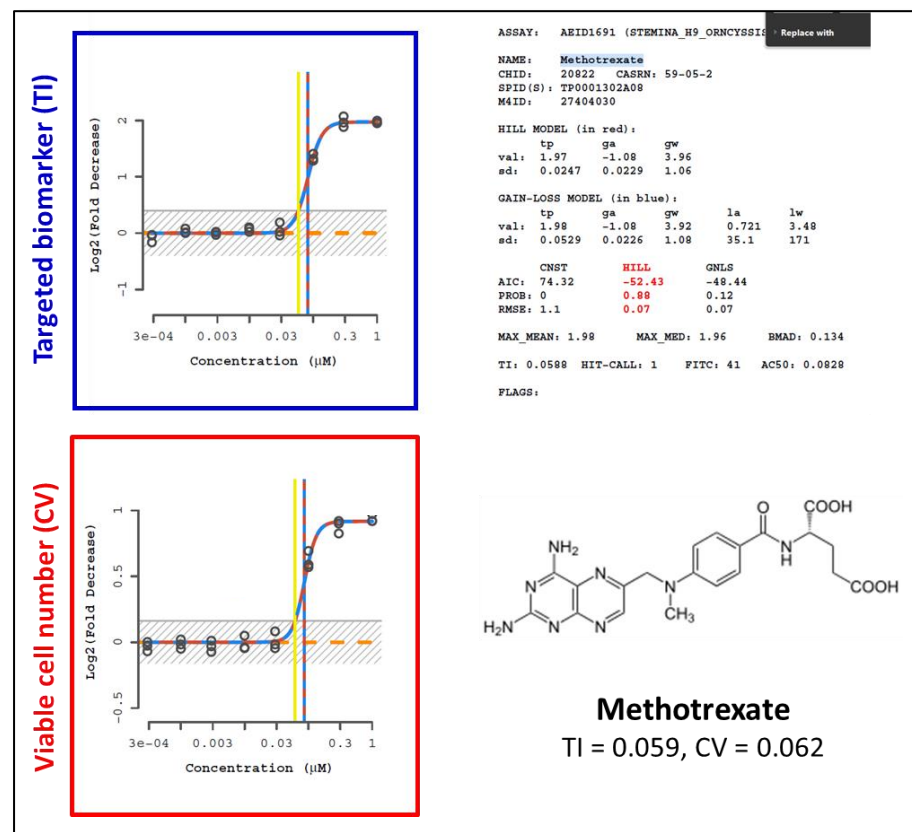

- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.

- **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).

ToxCast_STM: *devTOX^{qP} assay contracted from Stemina Biomarker Discovery*

- 1065 ToxCast Ph I/II chemicals at single-conc. or multi-conc.;

- Data tcpl-pipelined into ToxCast portfolio (now >1125 assays);

- Public data available in EPA's CompTox Chemicals Dashboard;

- 19.2% positivity rate in conc. based teratogenic potential.

https://comptox.epa.gov/dashboard

Methotrexate
TI = 0.059, CV = 0.062

Zurlinden et al. (2020) Toxicol Sci

Performance check

Balanced Accuracy 82% (0.65 sens, 1.00 spec) for 42 well-curated reference compounds.

Predictive sensitivity declined in concurrence with maternal toxicity and/or lower species concordance.

ATRA was most potent across 1065 compounds tested.

Scaling Criteria (ToxRefDB)
- BM-42 reference
- concordant, rat AND rabbit
- dLEL < mLEL, rat OR rabbit
- dLEL ≤ 200 mg/kg/day
- LEL for any study type

Zurlinden et al. (2020) Toxicol Sci

Knudsen et al. (2020) Curr Opin Toxicol
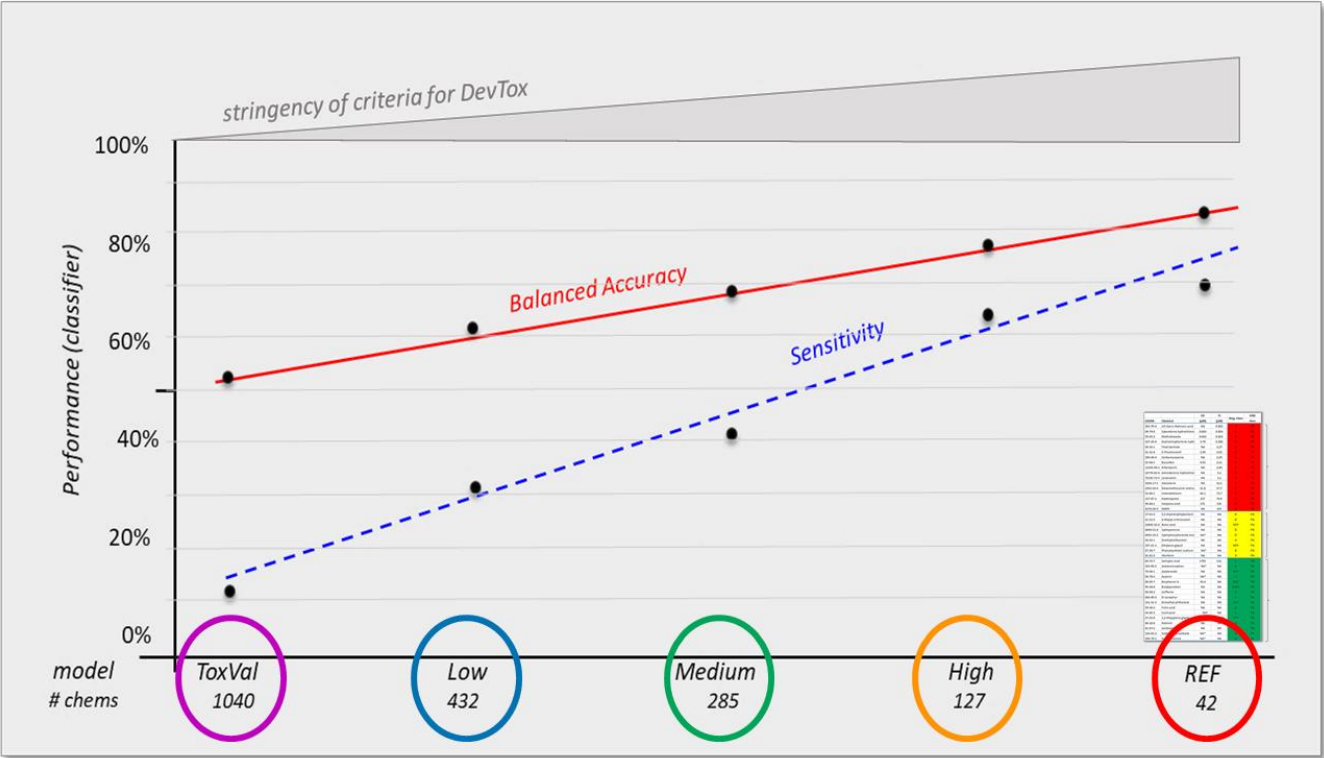
Translatability of hPSC findings to the intact embryo

Embryoid Body

Embryo (mouse)

Epiblast (human)

Kyoto collection
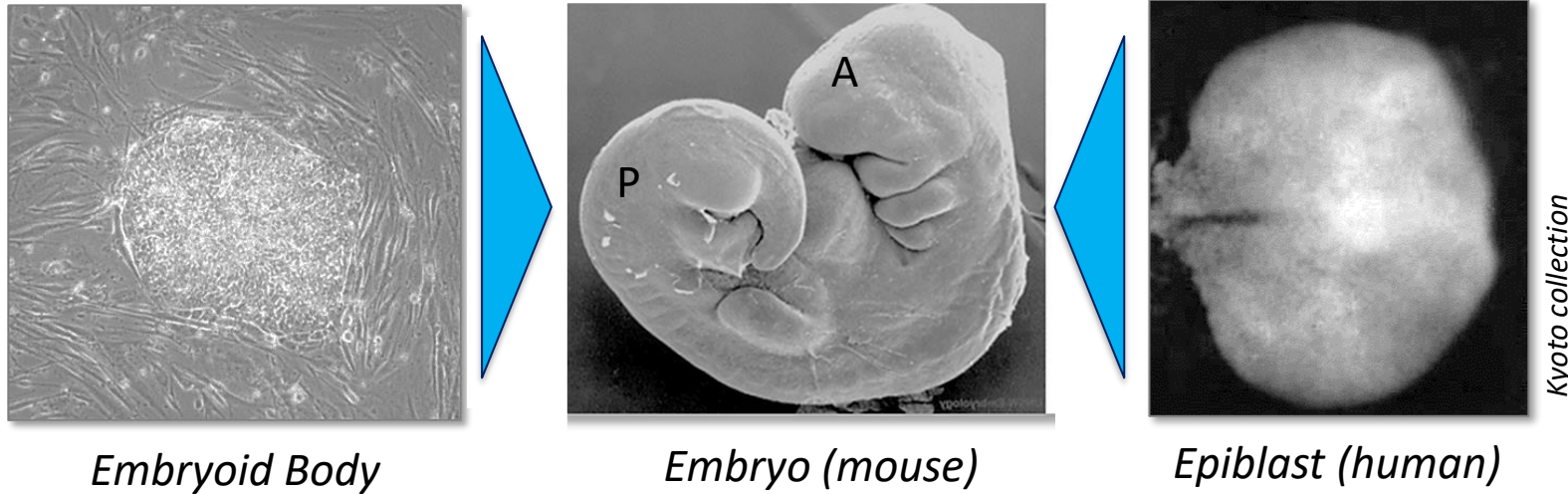
- The molecular biology and behavior of hPSCs in culture most closely resembles the 'epiblast' of an early embryo undergoing 'gastrulation'.

- Cultured hPSCs can self-organize into rudimentary tissues/organs but lack 'positional information' and physical constraints imposed through the multicellular epiblast;

- For example, the hallmark of gastrulation is primitive streak (PS) formation that establishes the anterior-posterior (AP) body axis and endo-mesodermal specification.

Gastrulating embryo: *quasi-normal self-organizing in vitro*
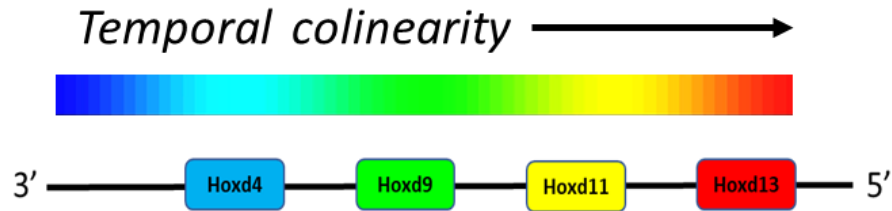
engineered microsystem: gastruloid
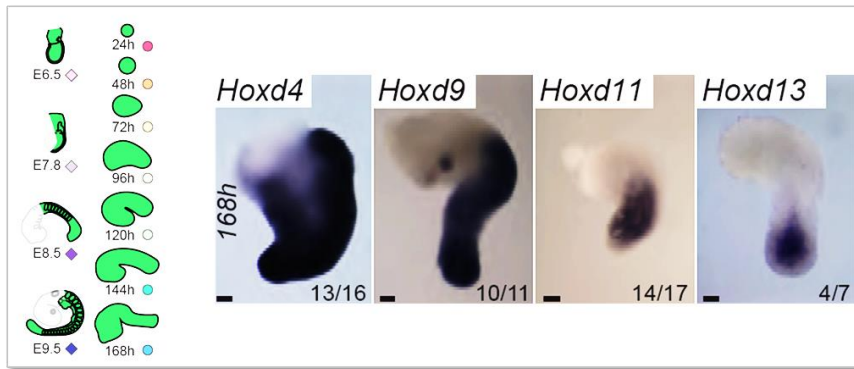
Beccari et al. (2018) Nature

- mESC-derived 'gastruloid' self-organizes *Hox* gene expression profiles in a quasi-normal pattern;

- spatio-temporal colinearity reflects AP polarity of the embryo *in vivo* (but still no PS);

- epiblast cell migration through the PS coincides with the regional specification of mesoderm;

- this process is critical in '*decoding the genomic blueprint of the fetal body plan*' and sensitive to perturbation (eg, retinoids).

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

Cellular Agent-Based Model (ABM): *'smart model' to explore toxicodynamics.*

- 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**).

- Can change course in response to a particular situation or stimulus, such as genetic errors or biomolecular lesions fed into the dynamic 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**).

ESABM: *workflow for computational reconstruction of cell dynamics in the epiblast*
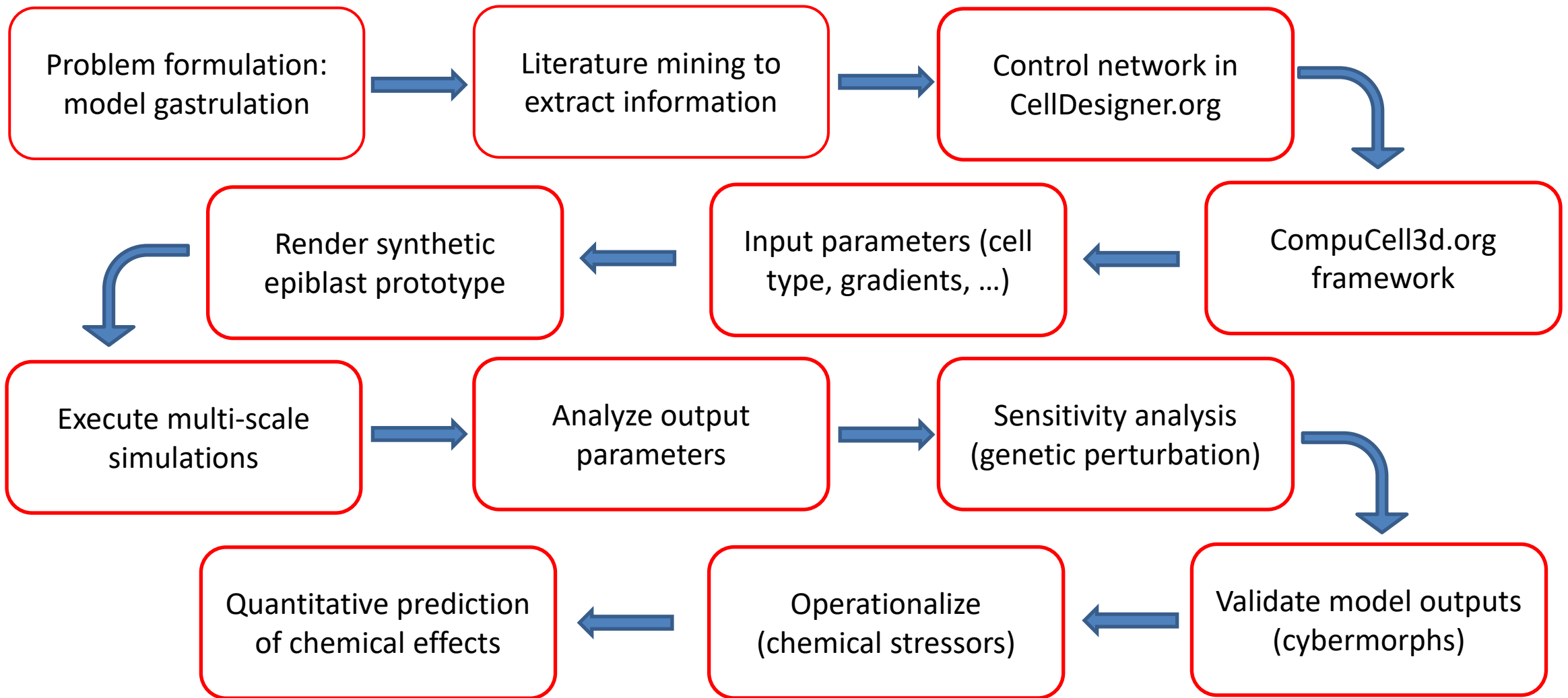
Quasi-normal gastrulation: *simulated in a virtual environment*
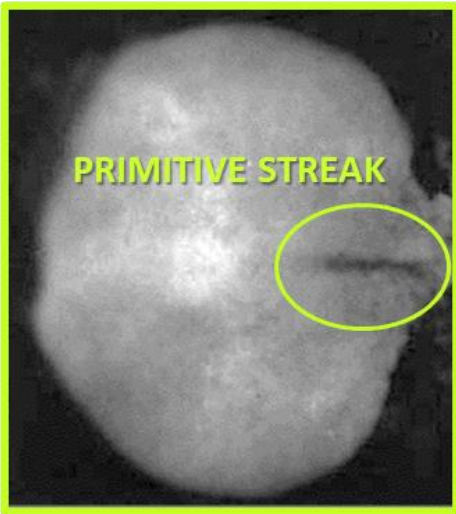
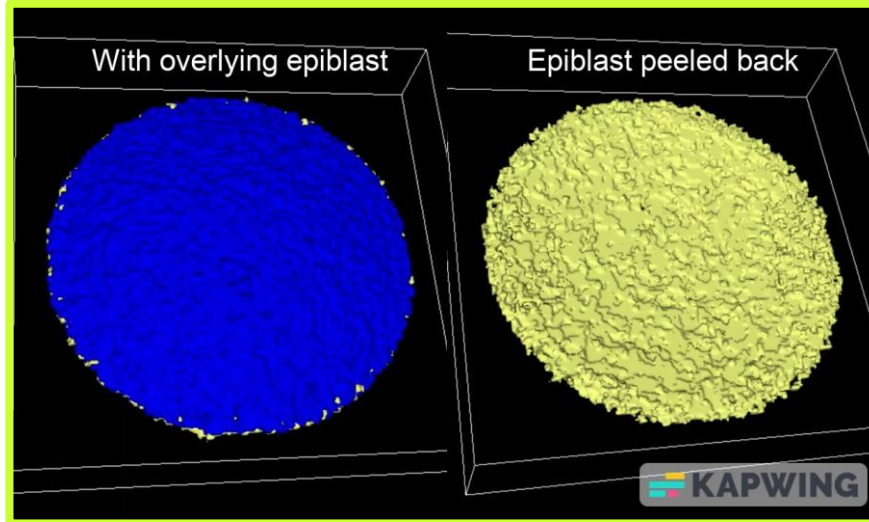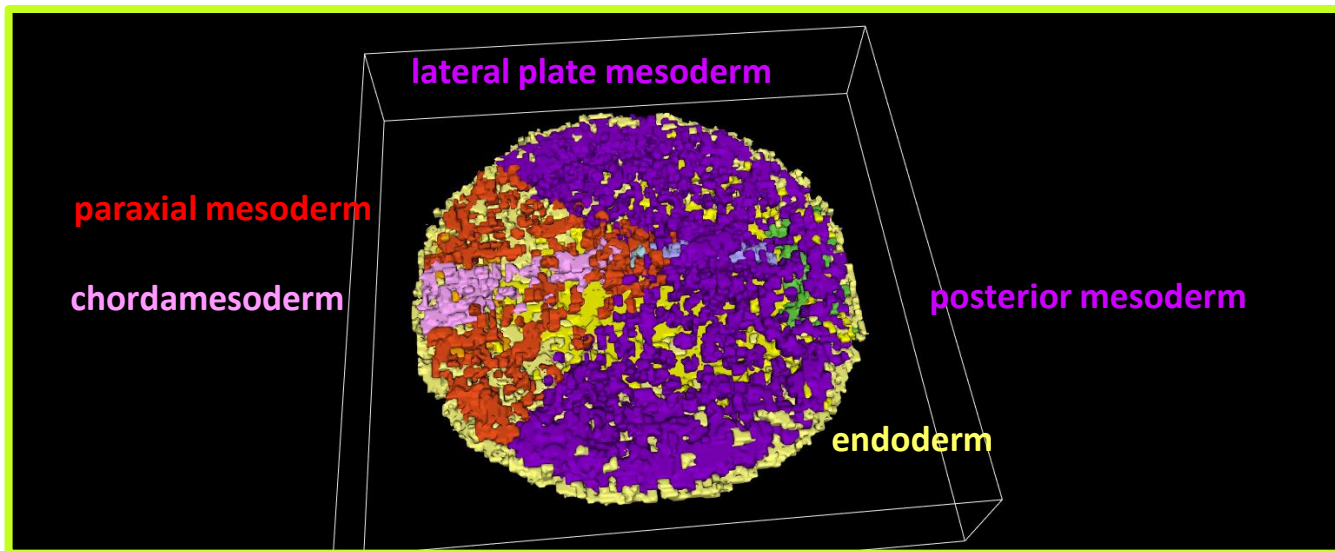Epiblast (in vivo)
3rd week gestation

Epiblast (in silico)
(mouse E5.5 – E6.5)

Morphological Programming Logic
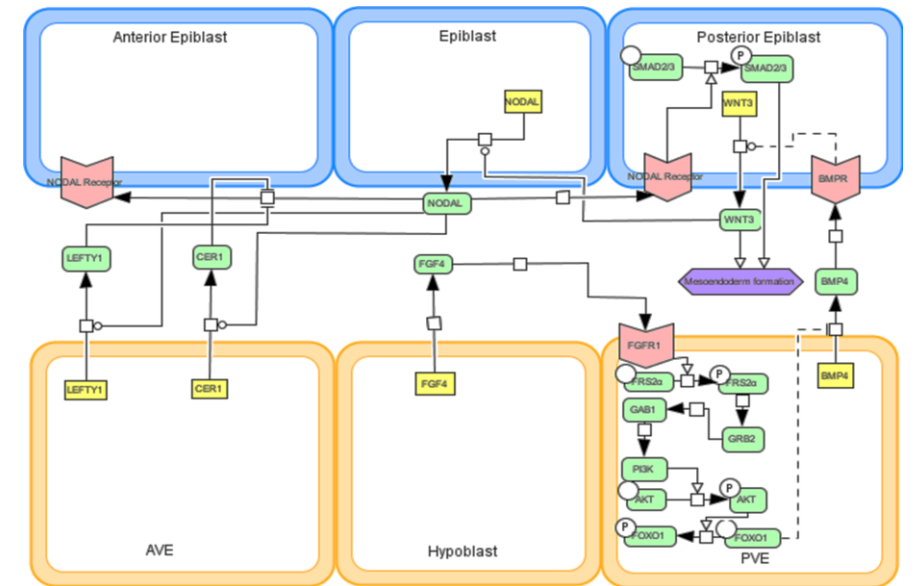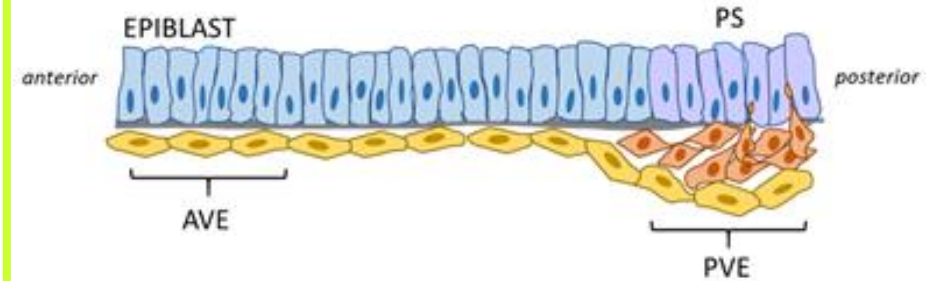
Patterning: *computable specification of regional mesoderm*

Normal (5000 MCS)

↓FGF signaling slows the clock

atRA → (-) → CDX

WNT → CDX

FGF → (+) → CDX

CDX →

HOX loci
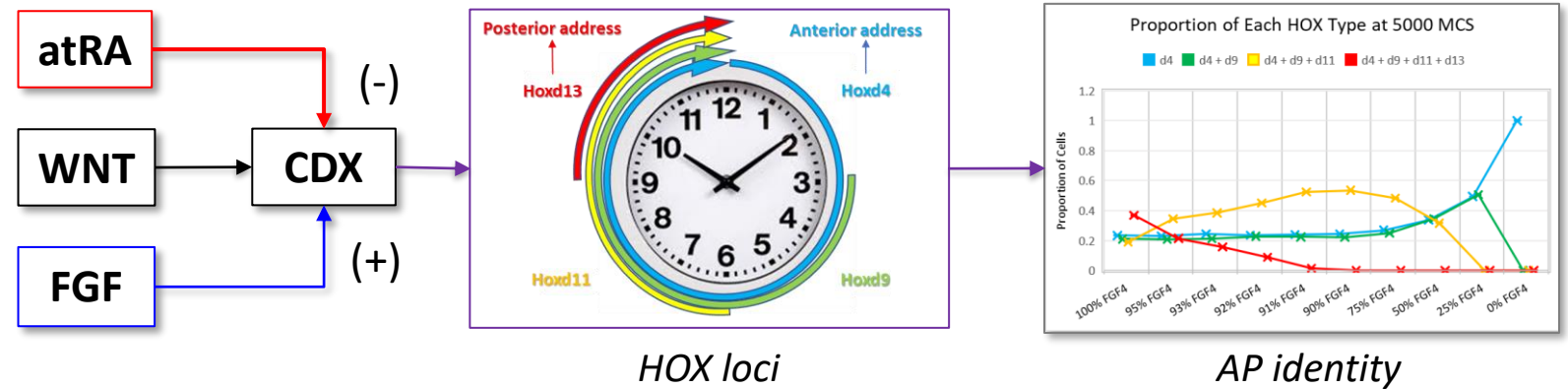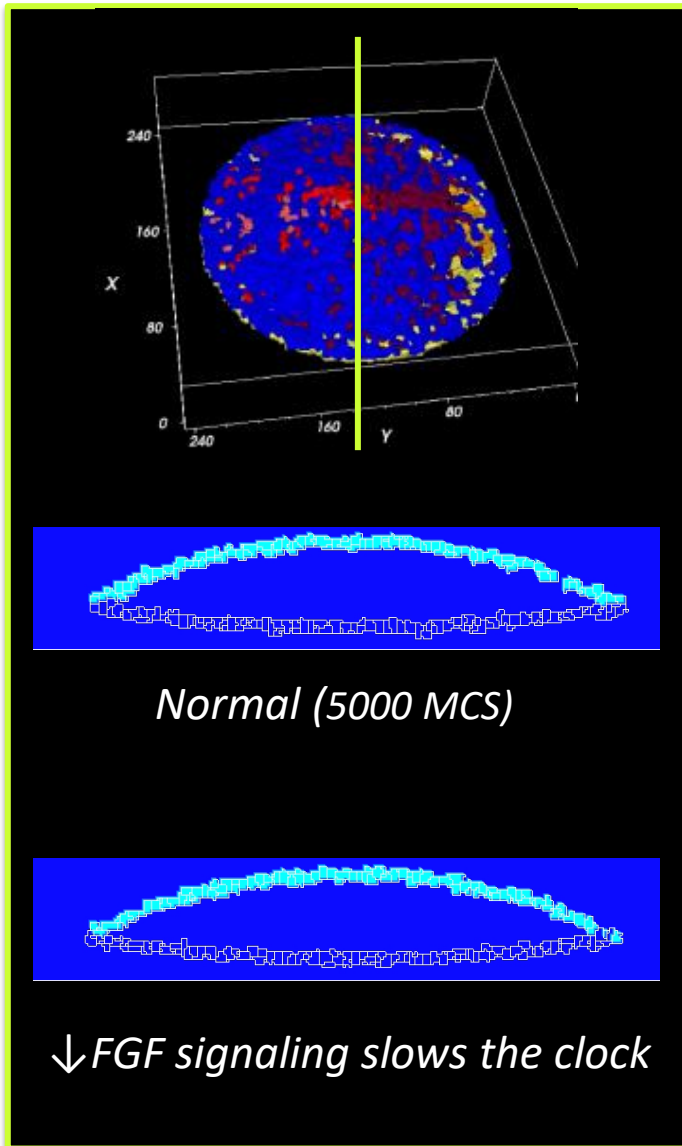
AP identity

- As each epiblast stem cell passes through the PS, its AP molecular identity is locked in time sync'd to an autonomous 'HOX clock'.

- Timing is dependent on a cell's position in the epiblast, which determines how long it takes the cell to reach the PS.

- 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 AP identity of mesoderm.

Hacking the model: *FGF4 cybermorphs*
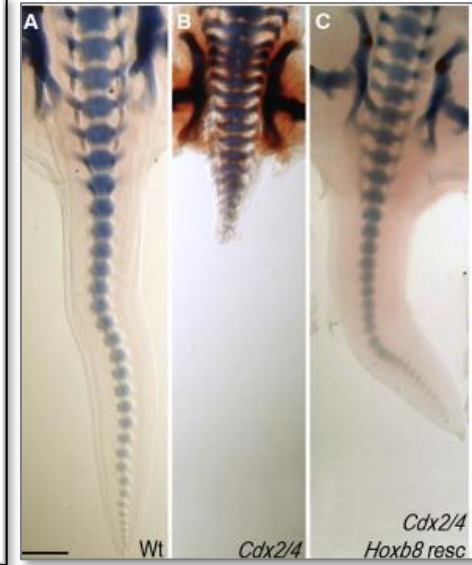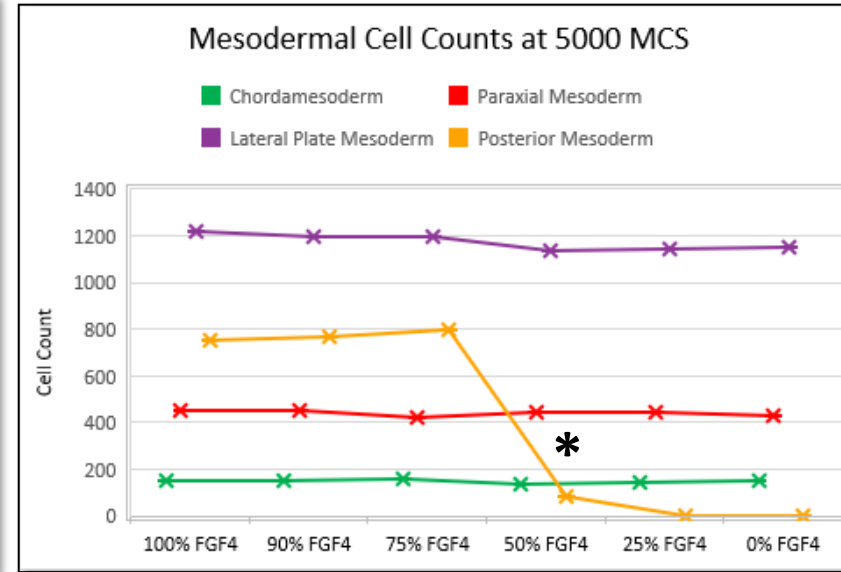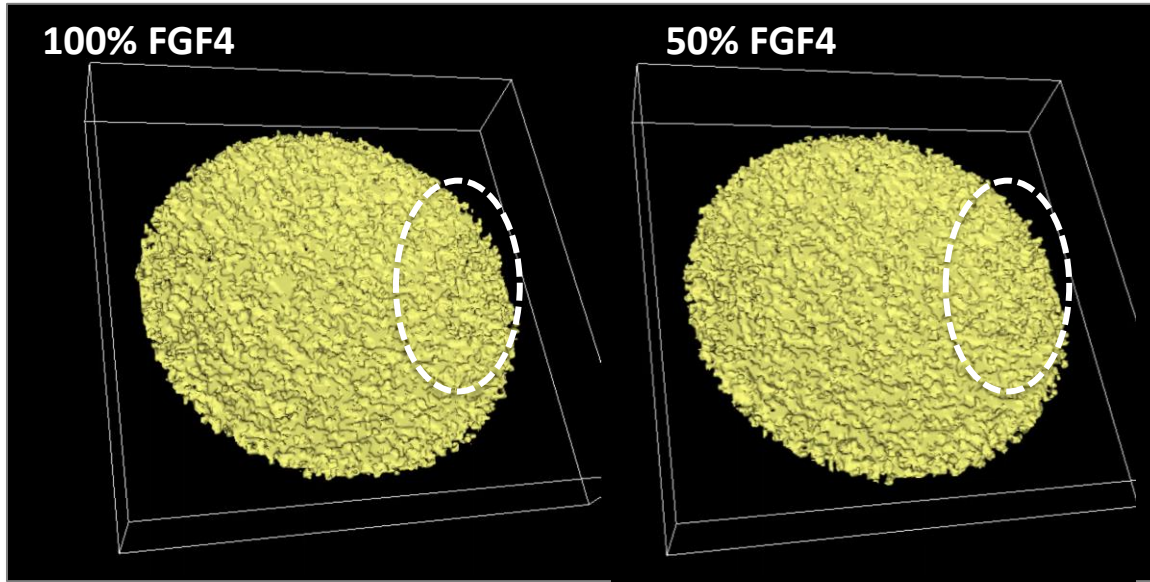
Young et al. (2009) Dev Cell

- 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*
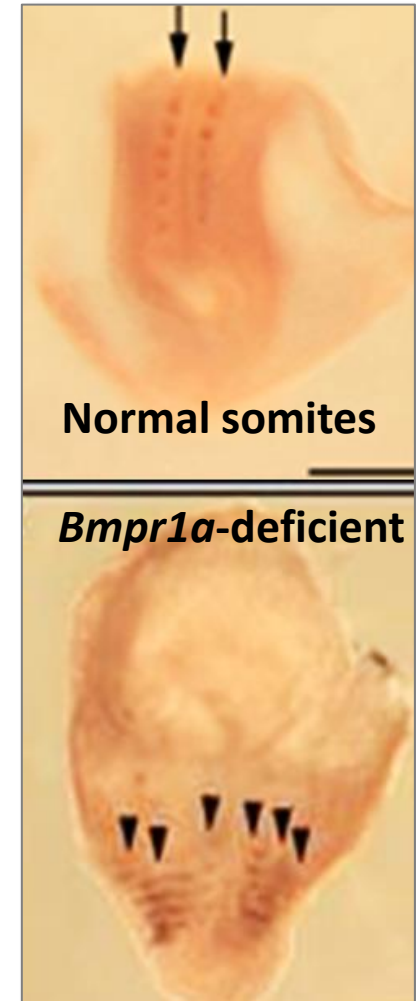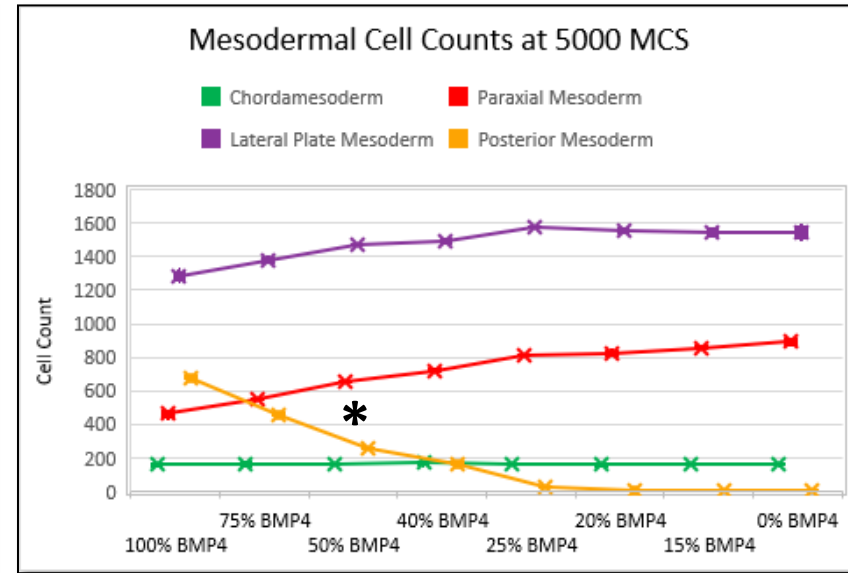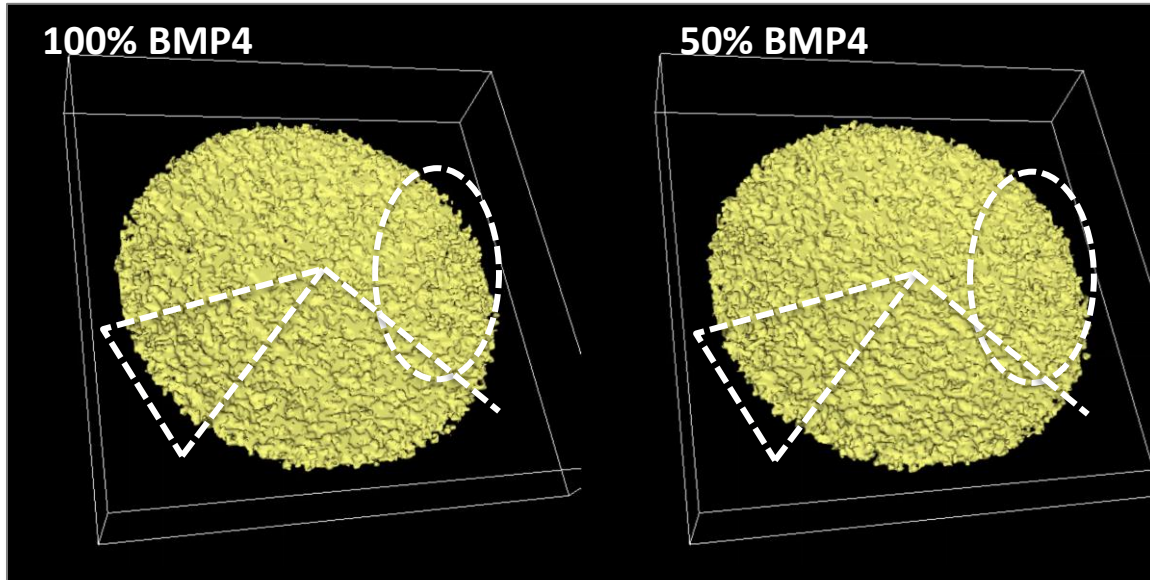
- BMP4 is maintained by FGF4 and primes posterior fate of the mesoderm;

- BMP4 in the epiblast regulates recruitment of prospective paraxial mesoderm;

- Conditional *Bmpr1a*-knockdown anteriorizes mesoderm, expanding the paraxial field;

- 25% BMP4-cybermorph recapitulates functional deficit *Bmpr1a*-deficienct mice.

Miura et al. (2006) Devel

Next steps

AC50s (μM)	Signal	FGF4	BMP4	WNT
	Target	FGFR1	SMAD1	GSK3b
ToxCast chemical (CASRN)	179465-71-5	0.041	NA	NA
	686756-87-6	0.005	0.082	8.669
	8018-01-7	1.156	NA	0.641
	12427-38-2	25.140	NA	0.272
	9006-42-2	20.682	NA	9.576

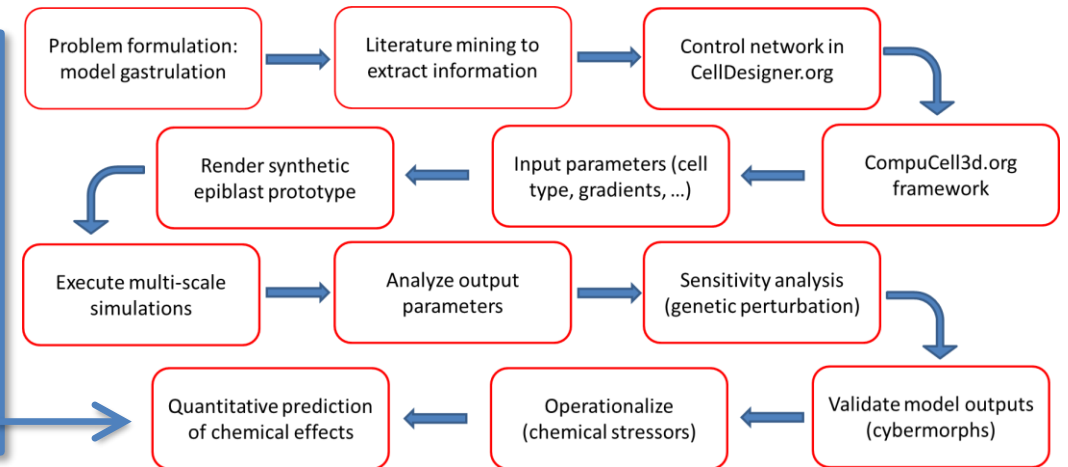Flowchart: Problem formulation: model gastrulation → Literature mining to extract information → Control network in CellDesigner.org → CompuCell3d.org framework → Input parameters (cell type, gradients, …) → Render synthetic epiblast prototype → Execute multi-scale simulations → Analyze output parameters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

- Translate data from ToxCast *in vitro* bioactivity profiles into dynamic simulations for computational rendering of critical phenomenon (*in silico* toxicodynamics).

- Extends the predictivity of data from *in vitro* stem cell culture into a computational model that propagates biomolecular lesions into emergent tissue-level phenotypes (cybermorphs).

- A fully computable synthetic embryo ('synbryo') may be a distant goal, but modular *in silico* systems can bring spatial biology of a critical process to life.

Acknowledgements

Center for Computational Toxicology and Exposure

Nancy Baker (Leidos)

Kaitlyn Barham (ORAU, Univ North Carolina)

Sid Hunter (BCTD)

Todd Zurlinden (Postdoct, now CEPHEA)

… and many on the Virtual and Complex Tissue Models Project (VCTM, CSS 405)

Environmental Modeling and Visualization Lab (EMVL)

Richard Spencer (General Dynamics)

Indiana University

James Glazier

TJ Seto