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*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:



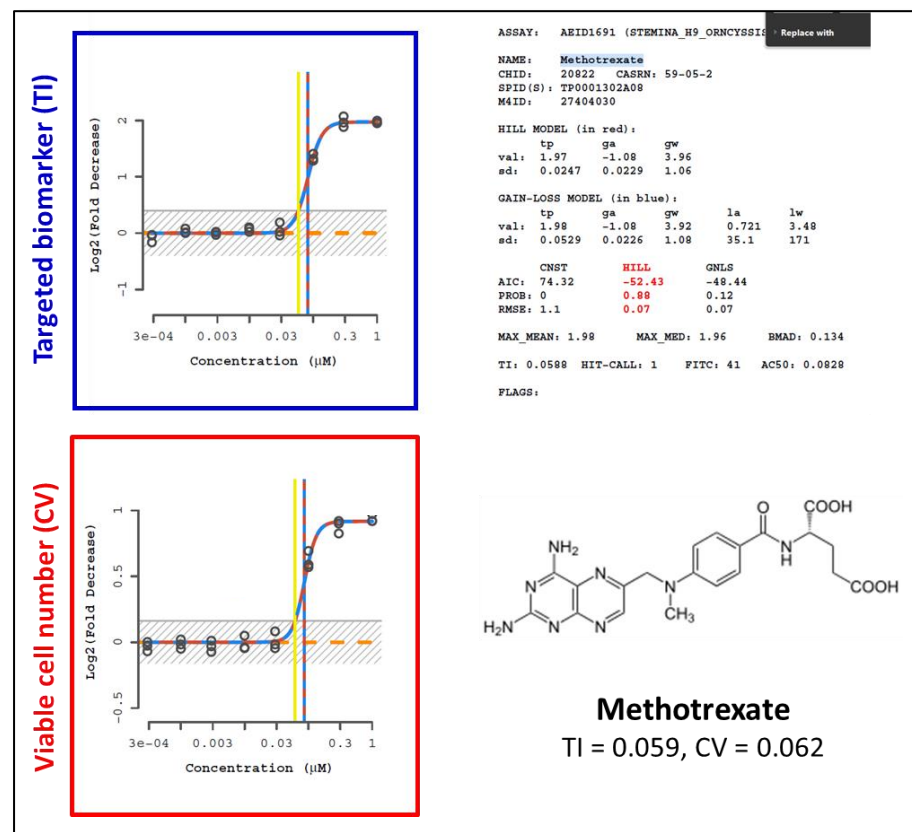
- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.
- **Pluripotency:** cells have the potential to form most of the different cell types comprising the embryo-fetus.
- **Autopoiesis:** capacity to self-organize into rudimentary tissues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



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Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity

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ABSTRACT

The Stemina devTOXquickPredict platform is a human pluripotent stem cell-based assay that predicts the developmental toxicity potential based on changes in cellular metabolism following chemical exposure (Palmer, J. A., Smith, A. M., Egnash, L. A., Conrad, K. R., West, P. R., Burnier, R. E., Donley, E. L. R., and Kirschner, P. R. (2013). Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. *Birth Defects Res. B Dev. Reprod. Toxicol.* 98, 363-363). Using this assay, we screened 1065 ToxCast phase I and II chemicals in single-concentration or concentration-response for the targeted biomarker (ratio of ornithine to cystine secreted or consumed from the media). The dataset from the Stemina (STM) assay is annotated in the ToxCast portfolio as STM. Major findings from the analysis of ToxCast STM dataset include (1) 19% of 1065 chemicals yielded a prediction of developmental toxicity, (2) assay performance reached 79%-82% accuracy with high specificity (> 84%) but modest sensitivity (< 67%) when compared with *in vivo* animal models of human prenatal developmental toxicity, (3) sensitivity improved as more stringent weights of evidence requirements were applied to the animal studies, and (4) statistical analysis of the most potent chemical hits on specific biochemical targets in ToxCast revealed positive and negative associations with the STM response, providing insights into the mechanistic underpinnings of the targeted endpoint and its biological domain. The results of this study will be useful to improving our ability to predict *in vivo* developmental toxicants based on *in vitro* data and *in silico* models.

Keywords: predictive toxicology; developmental toxicity; embryonic stem cells.

In 2007, the National Research Council published *Toxicity Testing in the 21st Century: A Vision and a Strategy* (National Research Council, 2007). This report addressed the potential for automated high-throughput screening (HTS) and high-content screening (HCS) assays and technologies to identify chemically induced biological activity in human cells and to develop predictive models of *in vivo* biological response that would replace or shift from traditional animal endpoint-based testing to human pathway-based risk assessment (Collins et al., 2008). Concurrent with the NRC 2007 report, the U.S. Environmental Protection

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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.

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

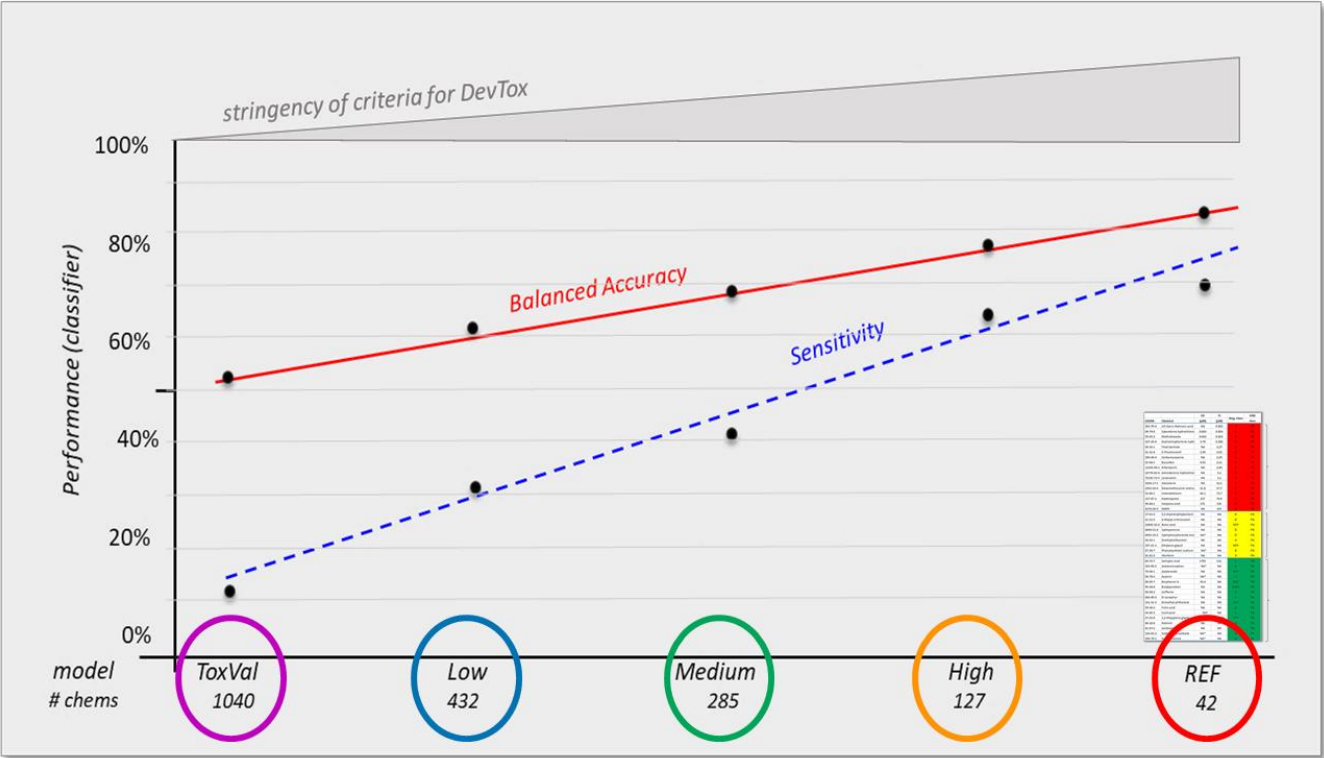
CAS#	Chemical	CV	(μM)		
302-79-4	all-trans-Retinoic acid	NA			
69-72-7	Salicylic acid	1795	513	C	TN
103-90-2	Acetaminophen	NA*	NA	B	TN
79-06-1	Acrylamide	NA	NA	NTP	TN
50-78-2	Aspirin	NA*	NA	C	TN
80-05-7	Bisphenol A	39.4	NA	NTP	TN
94-26-8	Butylparaben	NA	NA	GRAS	TN
58-08-2	Caffeine	NA	NA	B	TN
464-49-3	D-Camphor	NA	NA	C	TN
131-11-3	Dimethyl phthalate	NA	NA	NTP	TN
59-30-3	Folic acid	NA	NA	A	TN
54-85-3	Isoniazid	NA*	NA	C	TN
57-55-6	1,2-Propylene glycol	327552	246664	NTP	TN
68-26-8	Retinol	NA	NA	A	TN
81-07-2	Saccharin	NA	NA	A	TN
134-03-2	Sodium L-ascorbate	NA*	NA	A	TN
599-79-1	Sulfasalazine	NA*	NA	B	TN

ATRA was most potent across 1065 compounds tested.

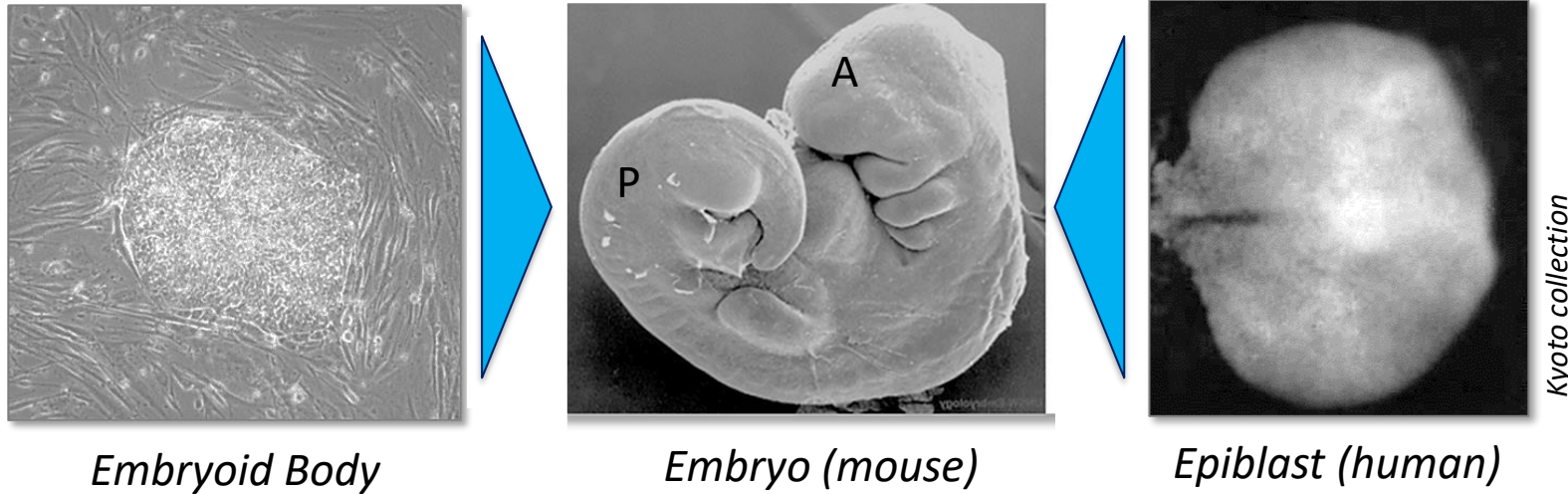
True Positive

False Negative

True Negative

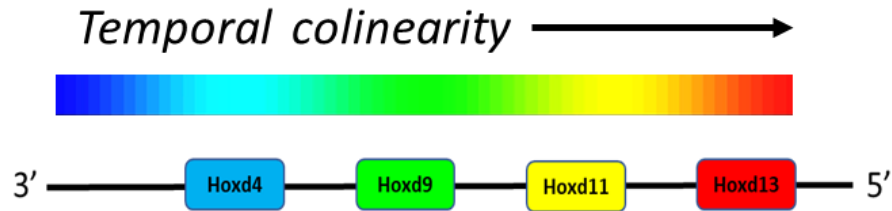


Translatability of hPSC findings to the intact embryo

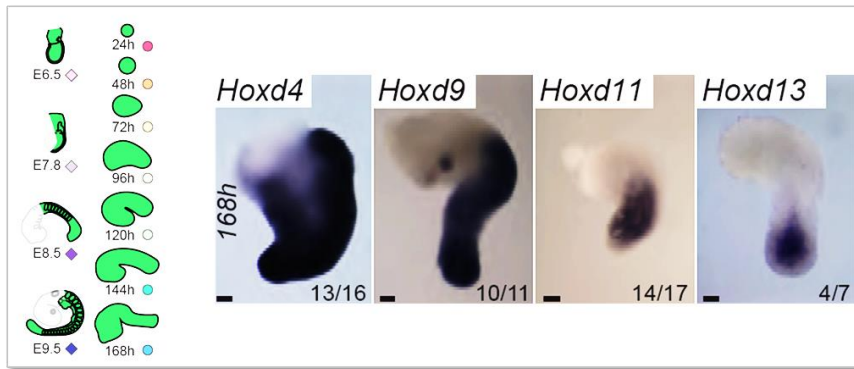


- The molecular biology and behavior of hPSCs in culture most closely resembles the 'epiblast' of an early embryo undergoing 'gastrulation'.
- Cultured hPSCs can self-organize into rudimentary tissues/organs but lack 'positional information' and physical constraints imposed through the multicellular 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

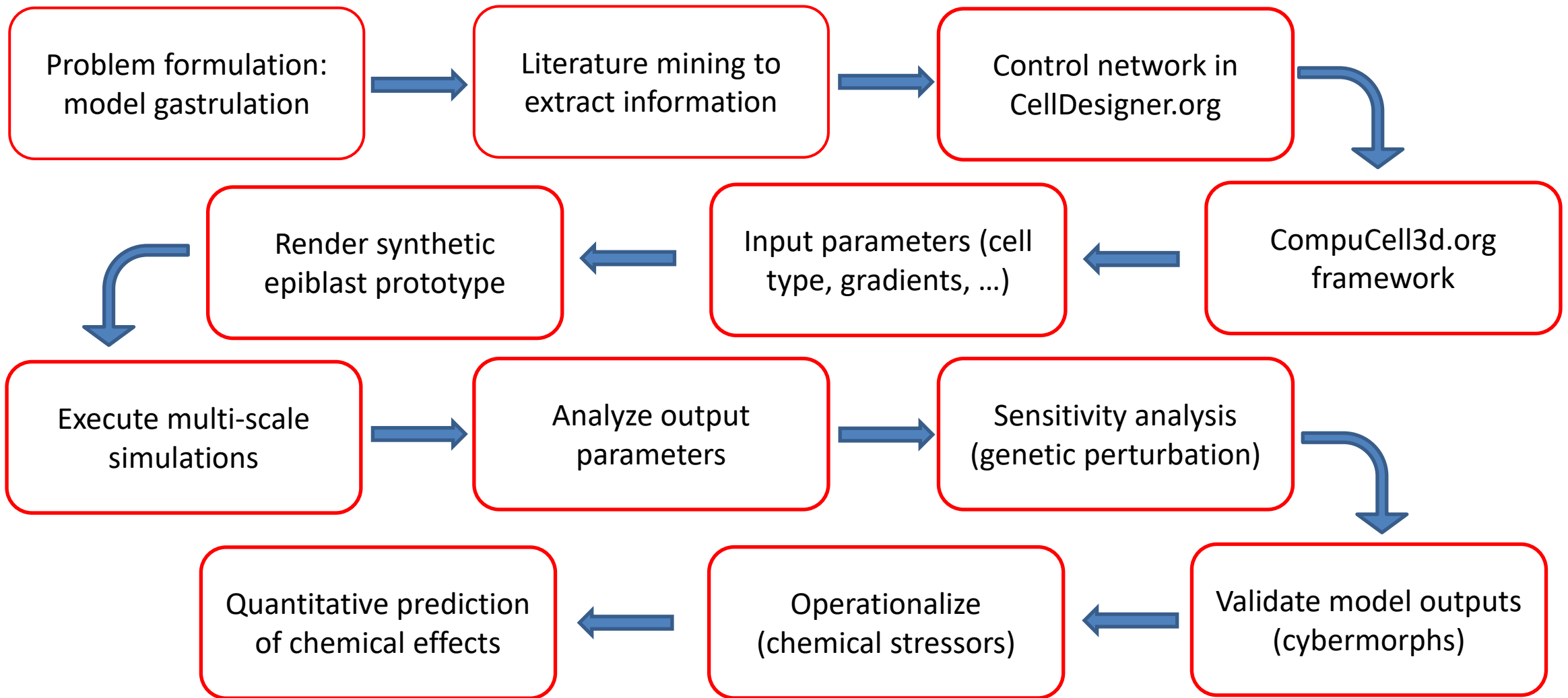
- mESC-derived ‘gastruloid’ self-organizes *Hox* gene expression profiles in a quasi-normal pattern;
- spatio-temporal colinearity reflects AP polarity of the embryo *in vivo* (but still no PS);
- epiblast cell migration through the PS coincides with the regional specification of mesoderm;
- this process 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

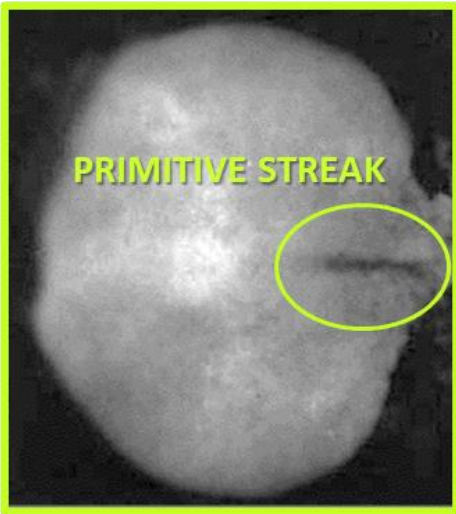
Cellular Agent-Based Model (ABM): *‘smart model’ to explore toxicodynamics.*

- 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](#)).

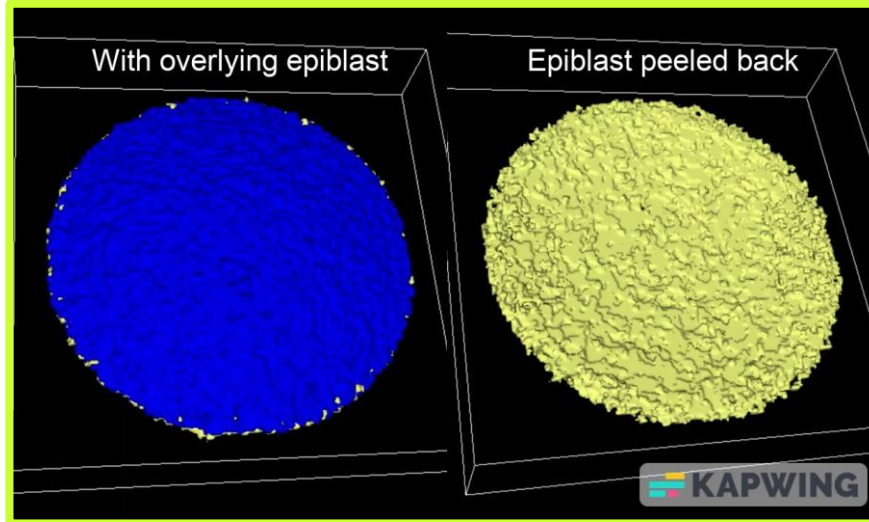
ESABM: *workflow for computational reconstruction of cell dynamics in the epiblast*



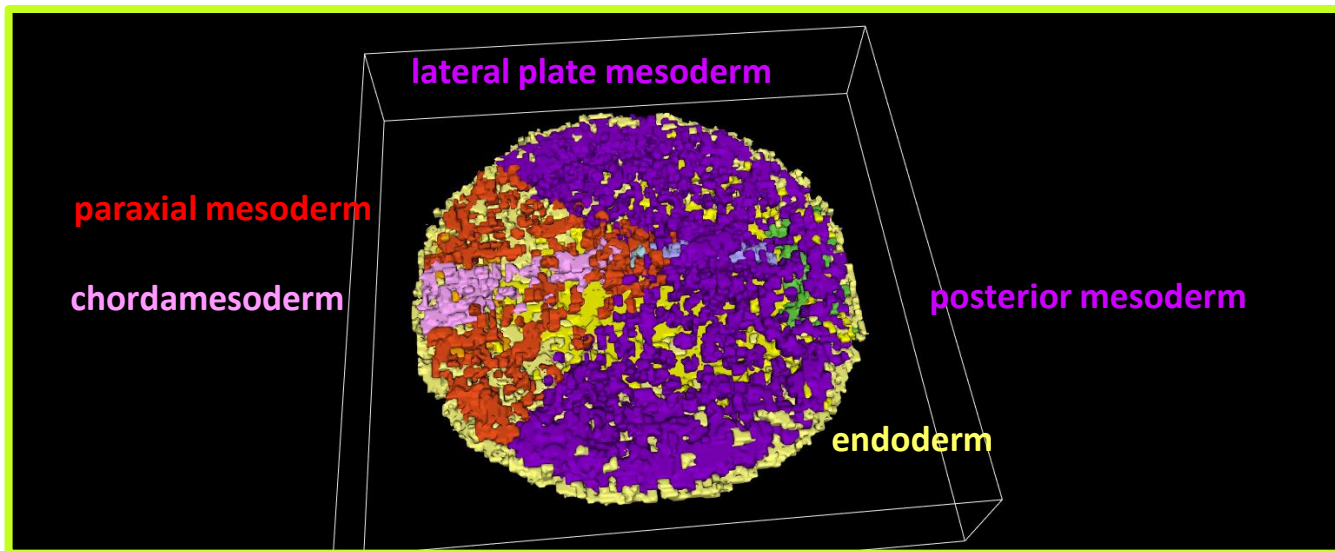
Quasi-normal gastrulation: *simulated in a virtual environment*



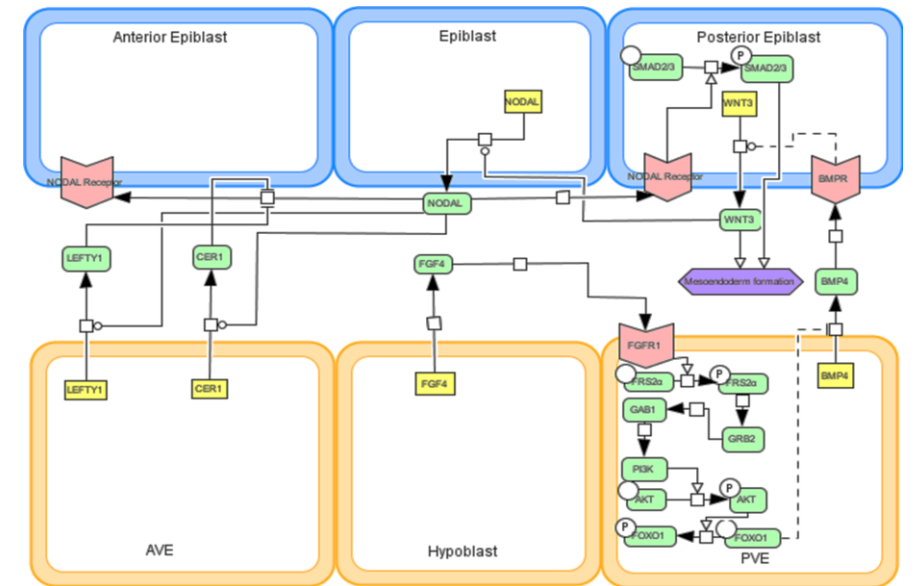
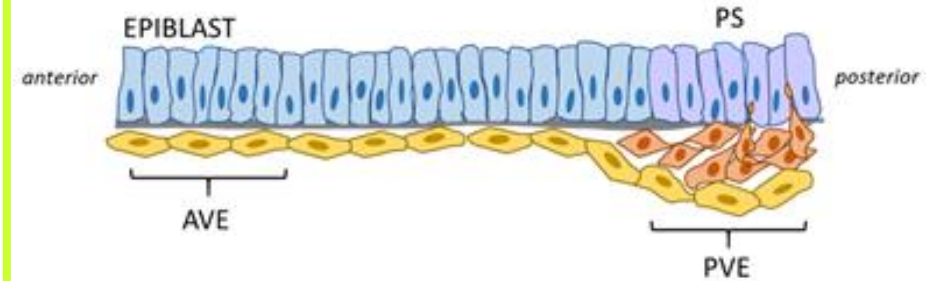
Epiblast (in vivo)
3rd week gestation



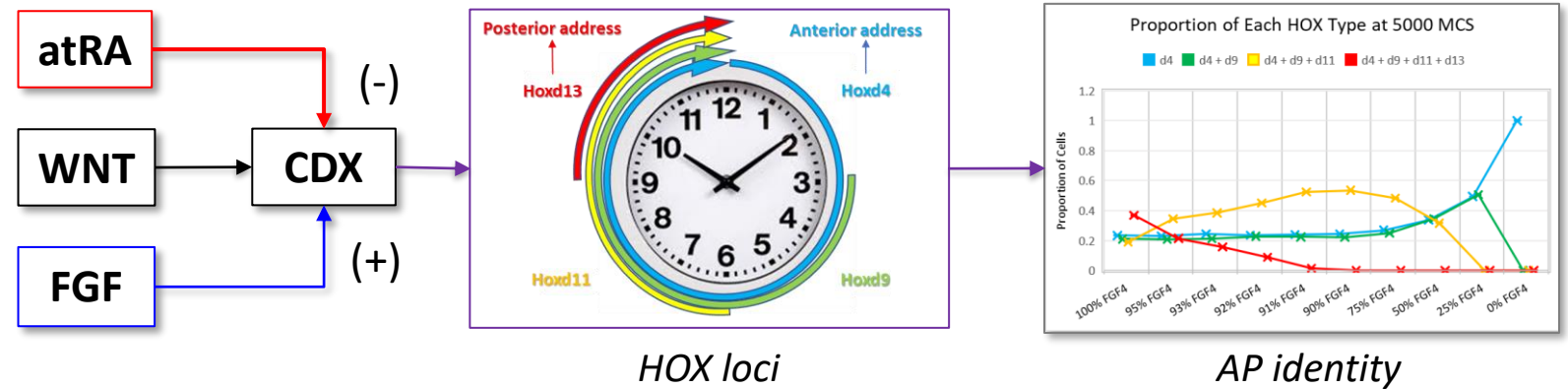
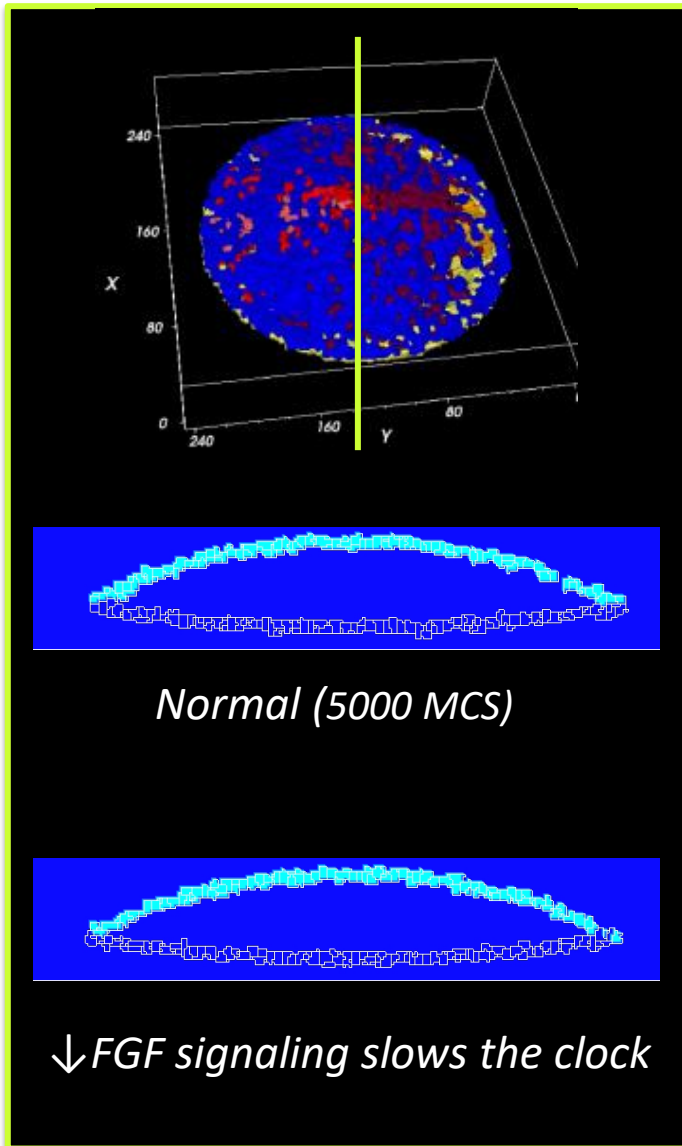
Epiblast (in silico)
(mouse E5.5 – E6.5)



Morphological Programming Logic

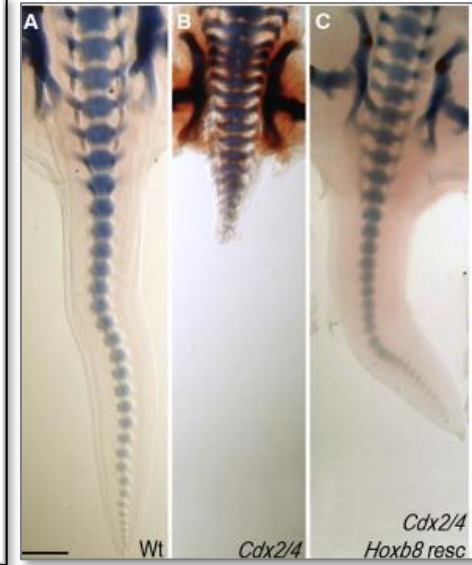
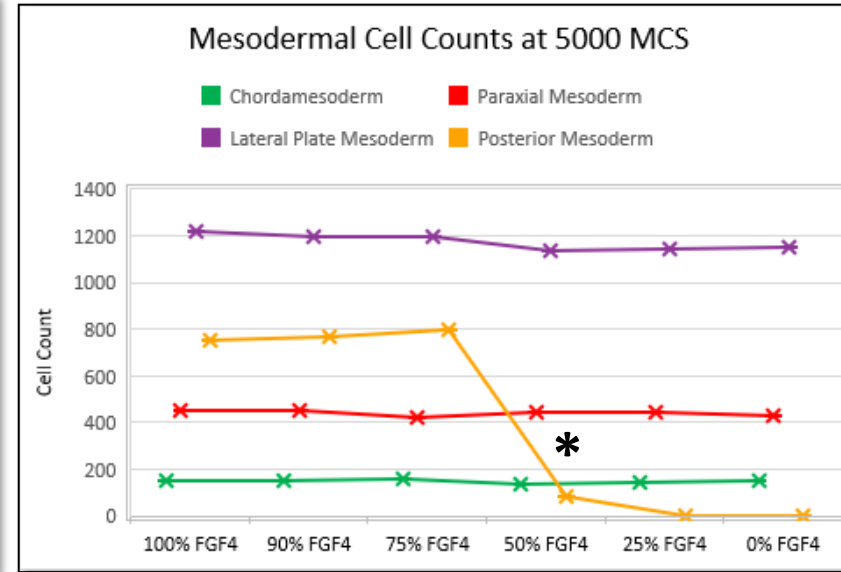
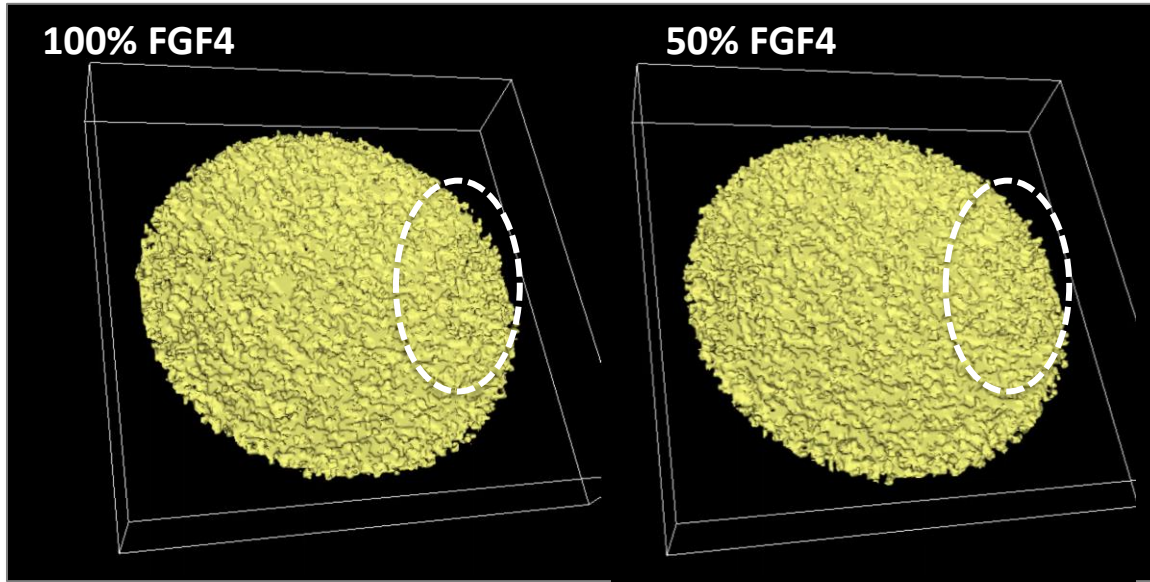


Patterning: *computable specification of regional mesoderm*



- 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.

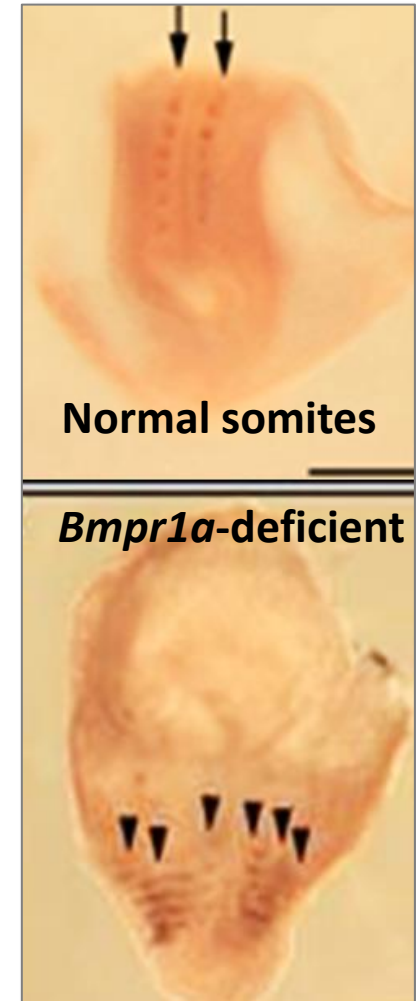
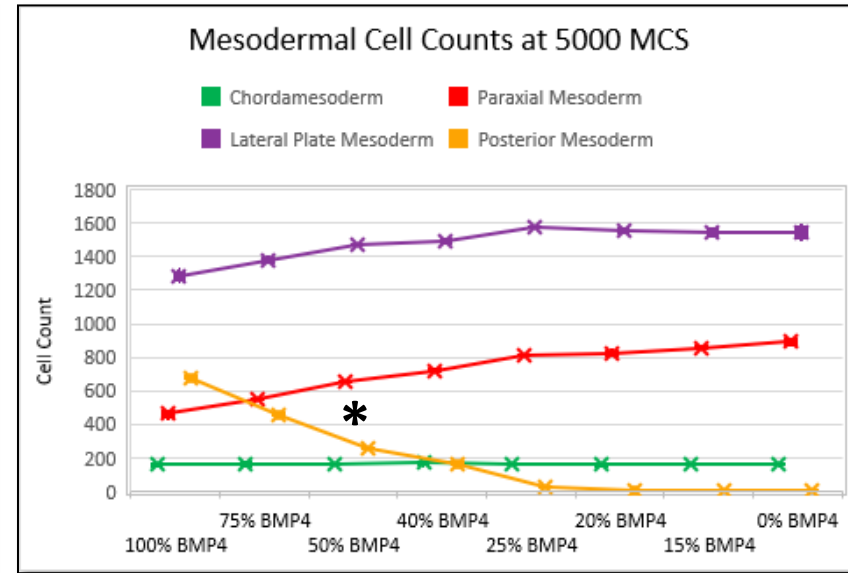
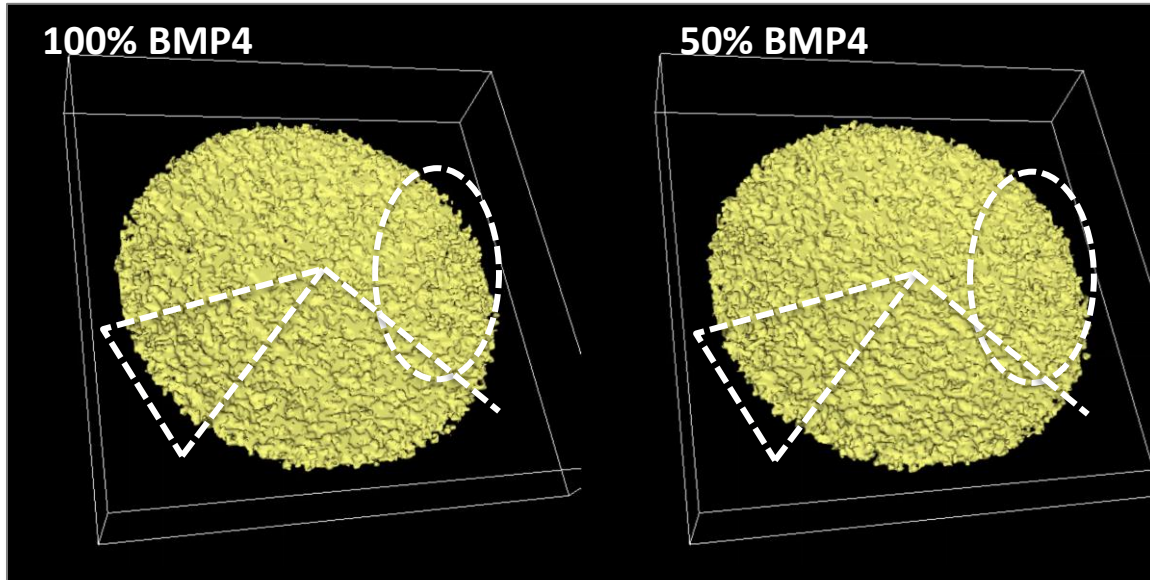
Hacking the model: *FGF4* cybermorphs



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

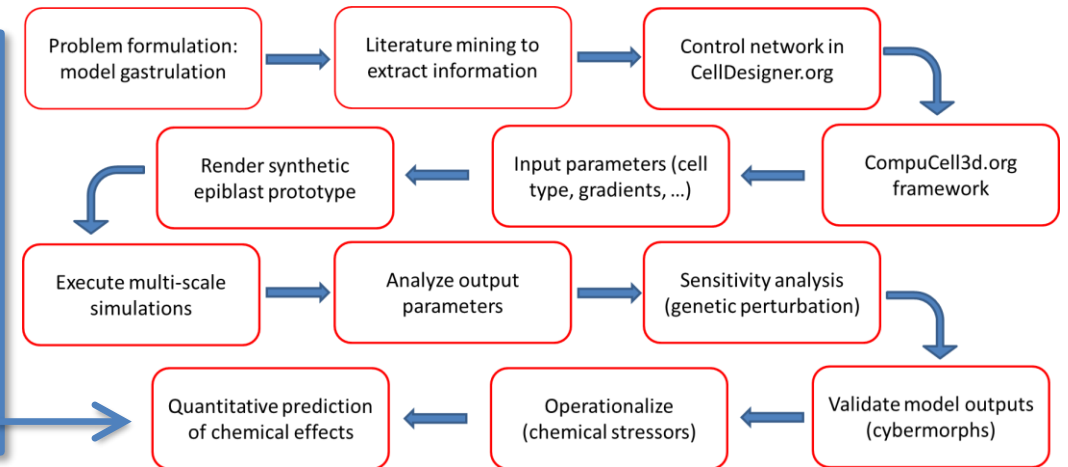


Miura et al. (2006) *Devel*

- 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*-deficient mice.

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



- 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.



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<http://www2.epa.gov/sites/production/files/2015->