

Microphysiological Systems (MPS): Bridging Human and Animal Research
Session 3: Going from in vitro to in silico – data and developmental tools
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***Synthetic Microsystems, Computational Intelligence,
and Artificial Life***

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Developmental toxicity

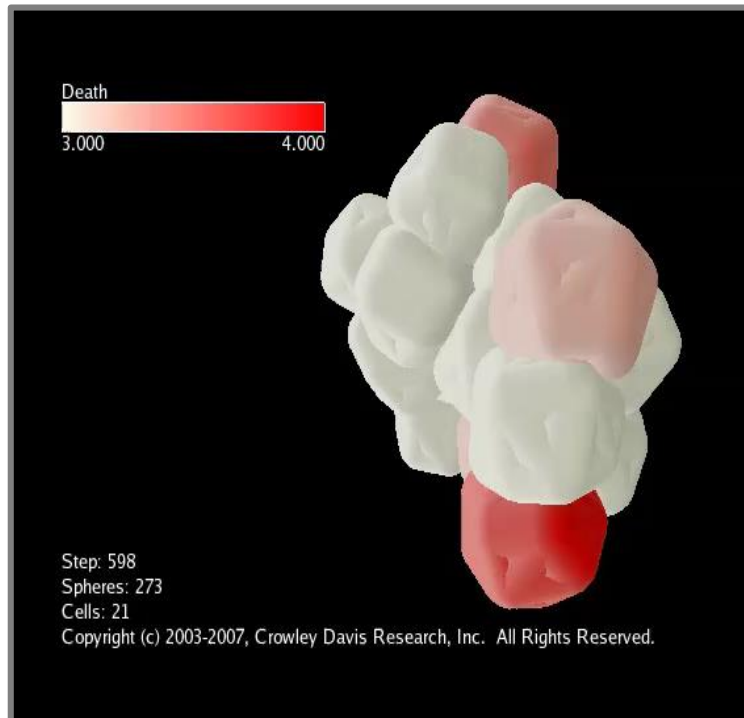
- Drug and chemical exposures during pregnancy can have profound and lifelong impacts on human health (developmental toxicity).
- Observation of fetal outcome in pregnant animal studies, most often in testing rats and/or rabbits, is the accepted means of developmental hazard identification.
- A guideline prenatal developmental toxicity study (e.g., OECD 414) is mechanistically complex, requires many animals, and potentially confounded by maternal effects.
- Query of prenatal developmental studies in EPA's ToxRefDB database found adverse fetal outcome for 53 of 283 (18.7%) chemicals tested in pregnant rats and rabbits.

Bridging animal-human studies

- Profiling 1065 ToxCast chemicals with a pluripotent stem cell (hPSC) assay showed a 19% positivity rate for teratogenic potential [Zurlinden et al. 2020].
- Closely matches the 18.7% positivity rate from concordant animal studies, but only a subset of the positives are detected by both *in vitro* and *in vivo* platforms.
- Discordance: (i) biology missed by the hPSC platform; (ii) concurrence of fetal outcomes with maternal toxicity; (iii) mesoscopic properties of complex systems.
- Motivation for synthetic microsystems, computational intelligence, and artificial life models of developmental processes and toxicities.

A more synoptic view ...

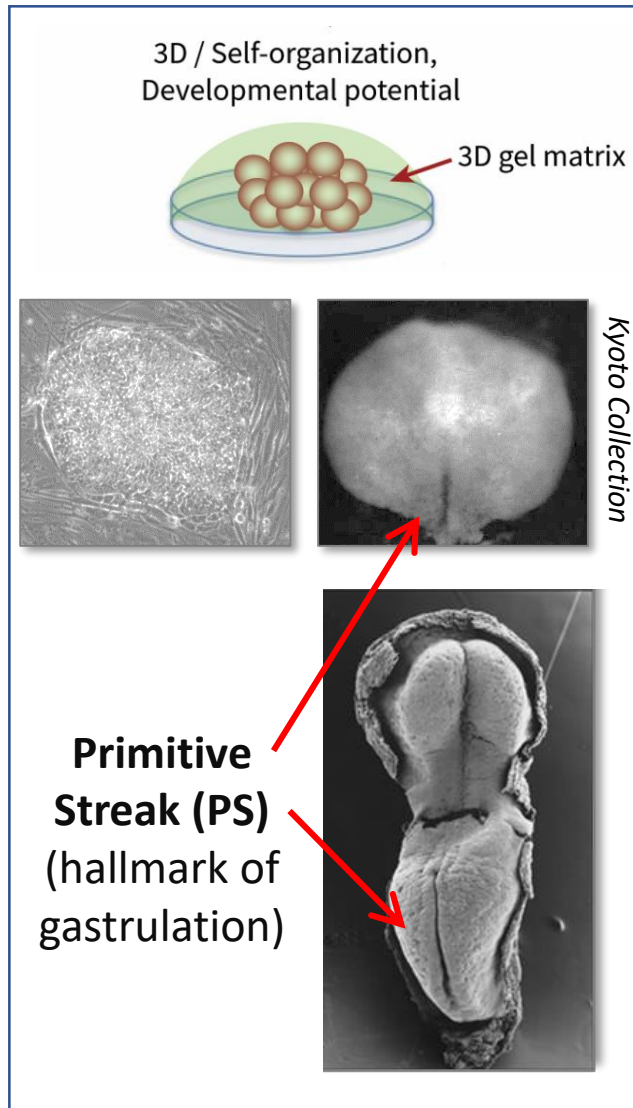
Anatomical homeostasis in a self-regulating 'Virtual Embryo'



- synthetic microsystems: human cell-based *in vitro* models aiming to recapitulate the microphysiology and integrated cellular behaviors of the physical system.
- computational intelligence: biological-inspired algorithms that use fuzzy logic to fill in for missing or incomplete information about the physical system.
- artificial life: bottom-up models of natural biological processes evolving through automation, synthetic control, and computer simulation.

*SOURCE: Andersen, Newman and Otter
(2006) Am. Assoc. Artif. Intel.*

Gastrulation: *decoding the genomic blueprint of the fetal body plan*



- hPSC cultures capture many aspects of development uniquely covered in guideline prenatal developmental toxicity studies.
- Unique properties of pluripotent stem cells: self-renewal, autopoiesis, and differentiate into most fetal cell lineages.
- Their molecular biology most closely resembles the epiblast of an early embryo (GD 5-7 mouse, week 2-3 human).
- Unlike the epiblast, an hPSC-derived 'embryoid body' is symmetrical and lacks 'positional information'.

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

Cellular dynamics in the epiblast

Key regulatory signals

STATE 1 - Naïve Pluripotency (self-renewal)

LIF/STAT3

ESC pluripotency signal

OCT4, SOX2, NANOG

pluripotency core triad

PI3K/AKT/MEK/ERK

signal transduction

STATE 2 - Primed Pluripotency (patterning)

FGF4

maintains *Bmp4* in ExE (GD 5.5)

BMP4

primes posterior cell fate

WNT3 *

pinpoints PS & propels pAVE

NODAL

induces primitive streak (GD 6.25)

LEFTY1/CER1

NODAL antagonists in AVE

STATE 3 – Determination (gastrulation)

ACTIVIN A

mesoendoderm formation

FGF8

Hox clock

ATRA

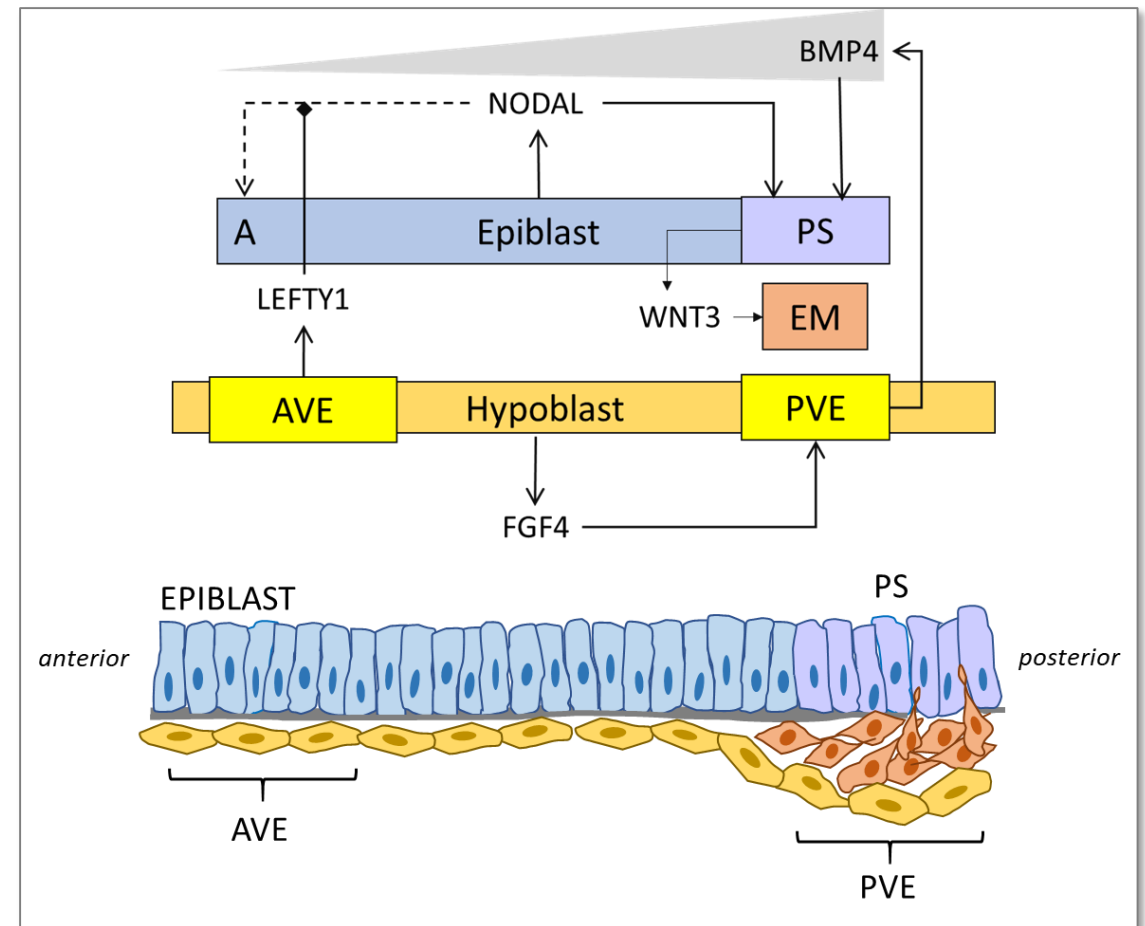
mutual antagonism with Fgf8

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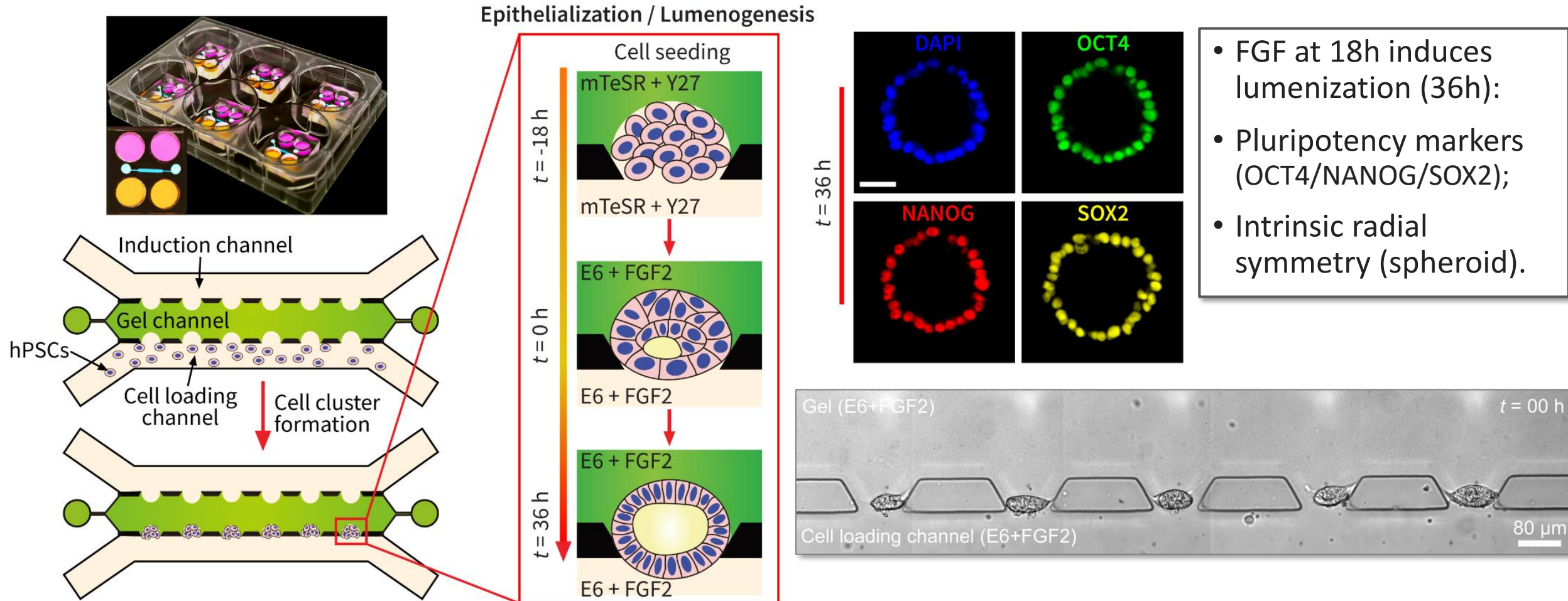
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SOURCE: Tam et al. (2006) Curr Opin Gen Dev

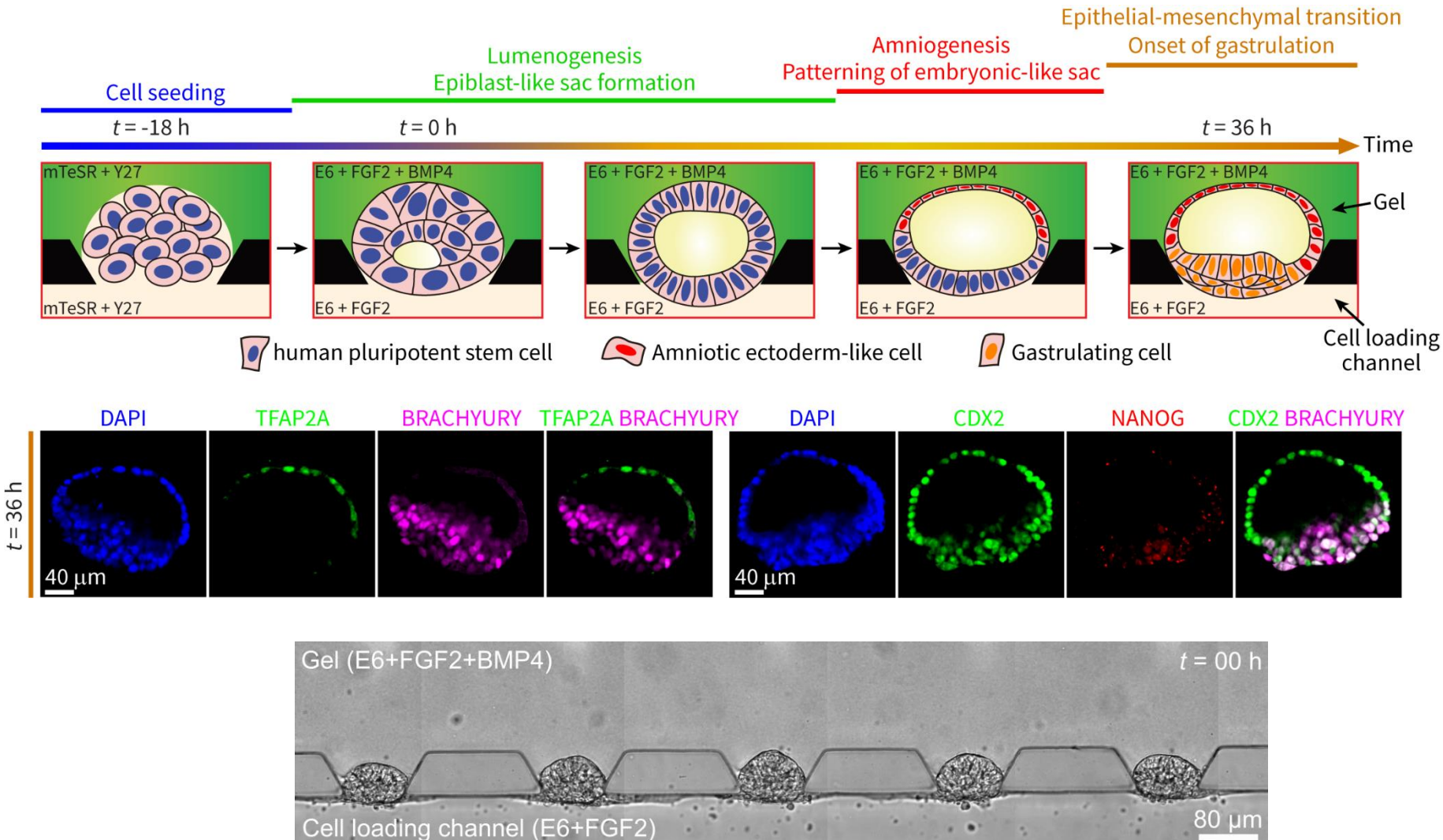
Morphological programming logic



Synthetic epiblast: *geometrically confined hPSCs seeded in MPS device*



Breaking the symmetry with BMP4



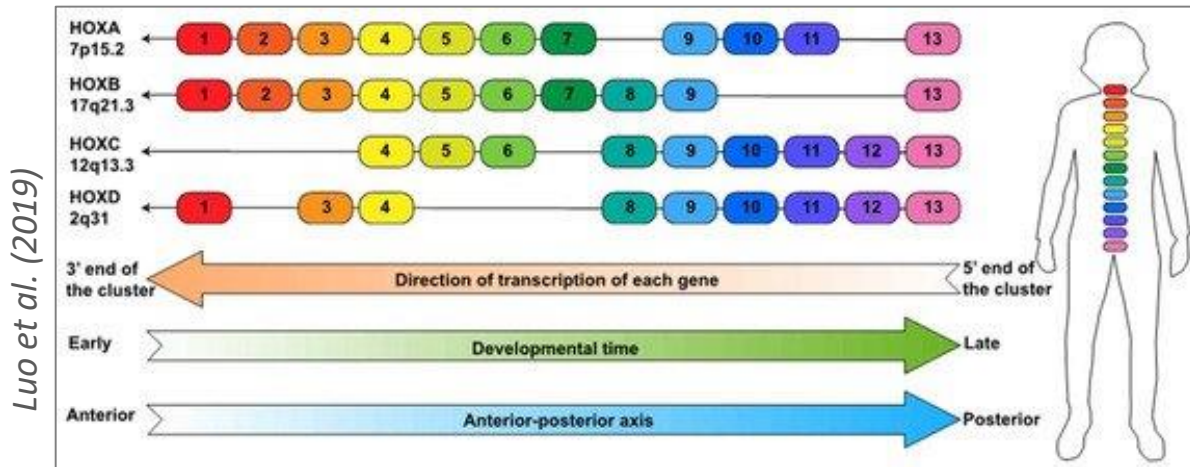
- BMP4 gradient breaks intrinsic symmetry.
- BMP4 primes posterior cell fate.
- Distinct axial domains emerge.
- Pluripotency advances to a determined state.
- But a bona fide primitive streak has not formed.

Bottom-up synthetic and computational models

- MPS reveals the intrinsic capacity of hPSCs to render cell patterns that can be organized with microfluidics.
- FGF2 and BMP4 is a start, but still other extrinsic signals needed to position a PS (NODAL, LEFTY1, WNT3).
- Agent-Based Models (ABMs): nature-inspired agents (cells) and rules (behaviors) set in motion as a self-organizing system [CompuCell3d.org modeling environment].
- ‘Smart models’: computational intelligence with fuzzy logic to simulate properties governing cell fate and behavior where rules are inexact or knowledge incomplete.
- Dynamic translation of genetic errors or biomolecular lesions translated into a probabilistic rendering of adverse developmental outcome (‘cybermorphs’).

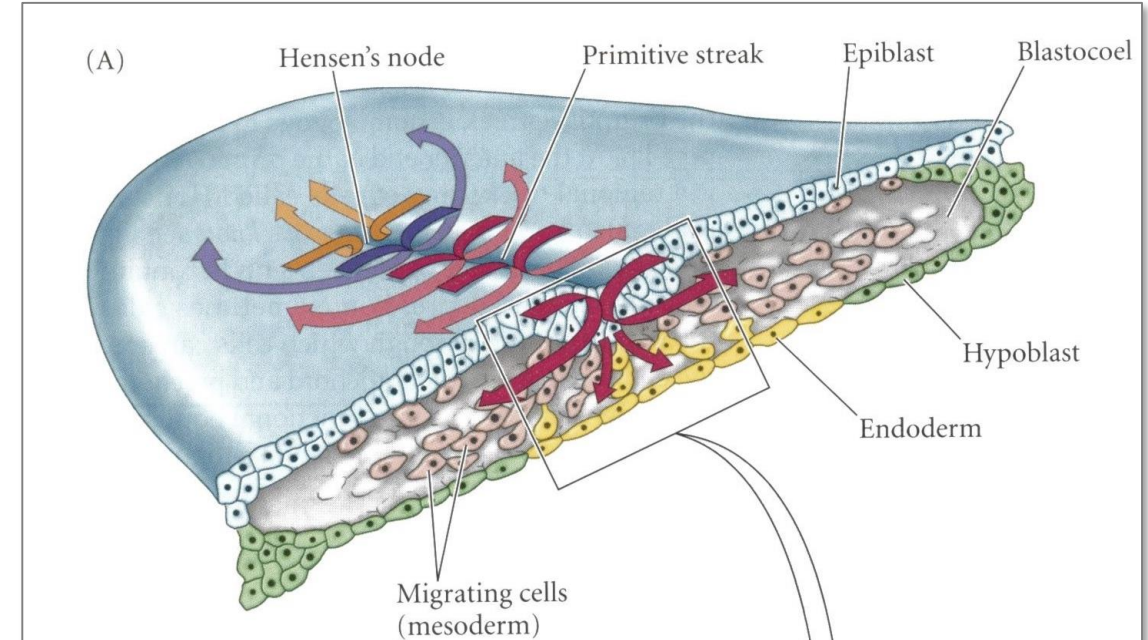
Positional Information and mesoderm formation

Cell migration through the primitive streak



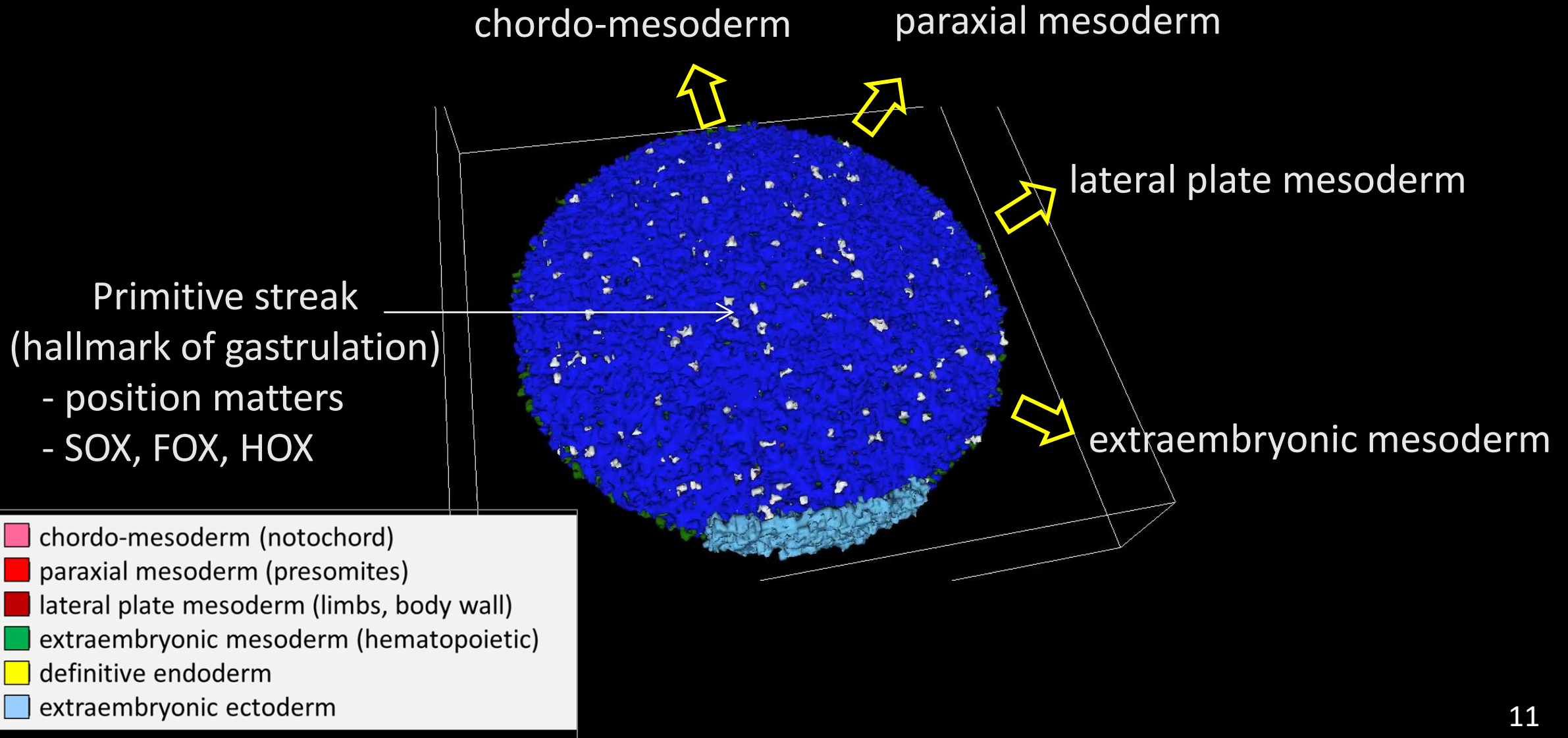
- HOX pattern 'primed' in the epiblast by FGF8-*CDX1* signaling (cell autonomous HOX clock).
- HOX pattern is 'determined' as epiblast cells pass through the primitive streak.

Gilbert, Developmental Biology



- Migration routes of cells passing through the PS to establish endomesodermal populations.
- A→P fate of a cell is based on epiblast position, which determines when and where it ingresses.

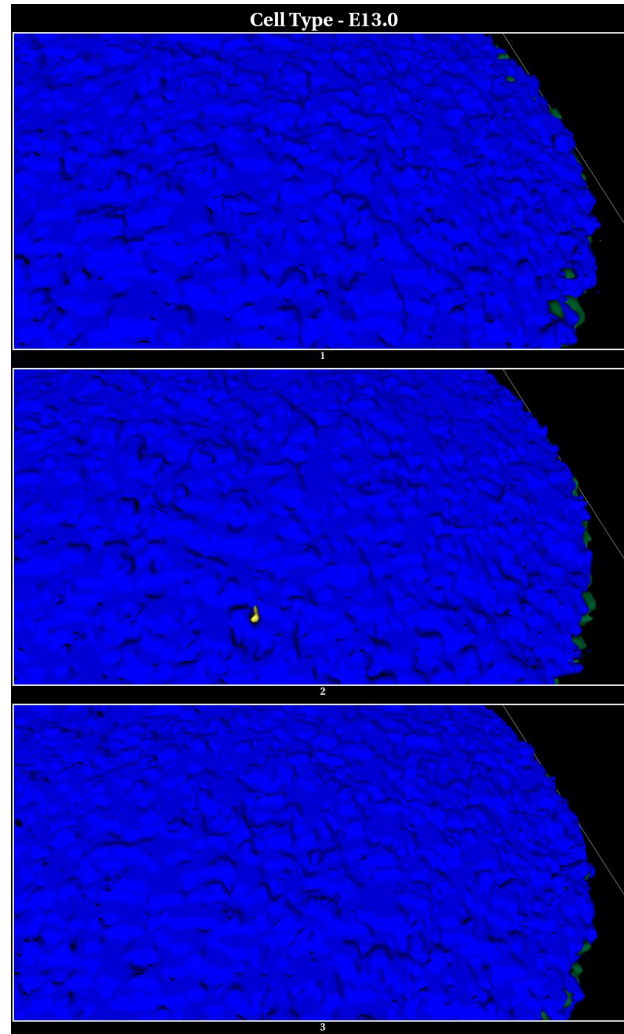
Spatial dynamics of mesodermal birth



Synoptic manifold for developmental computation with ToxCast data

Chemical effects
data from *in vitro*
profiling

- FGF signaling has a critical role in PS formation (FGF \rightarrow BMP4 \rightarrow WNT); the number of WNT-positive cells (yellow) is a proxy measure.
- Metrics for individual cells and signals can be computed over time, to track the impact of various perturbations introduced from *in vitro* chemical profiling data ('cybermorphs').



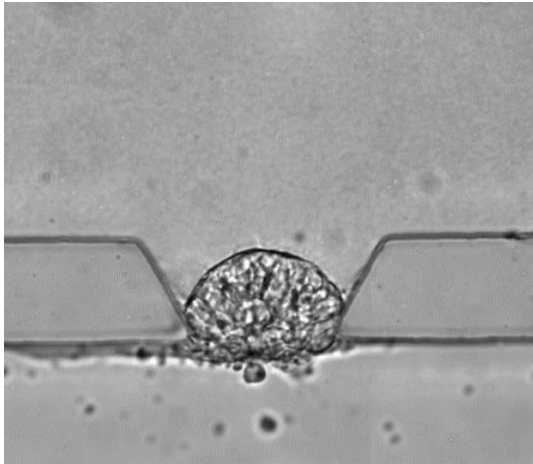
Control
(405 cells ingress)

75% reduction of FGF
(260 cells ingress)

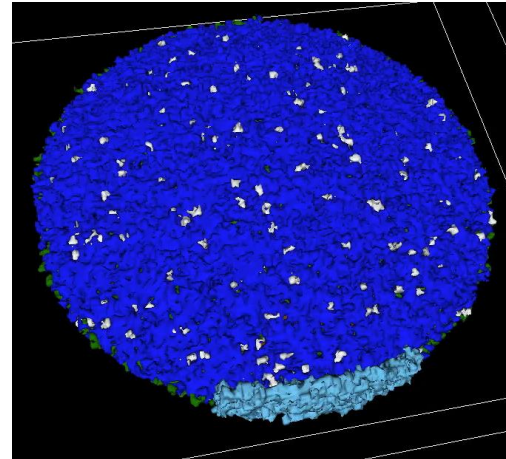
90% reduction of FGF
(207 cells ingress)

Quasi-gastrulation: *recoding the genomic blueprint of the fetal body plan?*

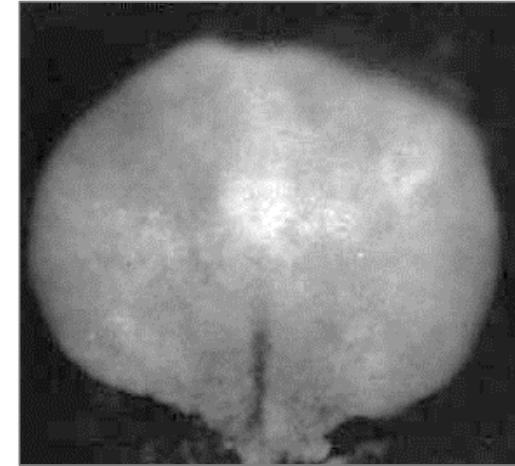
In vitro



In silico



In vivo



- A synoptic view can translate theories into testable hypotheses, sensitivity analysis, generate predictions, and design new experiments.
- *In silico* toxicodynamics will help address uncertainties in translatability of hPSC chemical effects data to the intact embryo:
 - put in motion *in vitro* profiling data
 - add positional information to hPSC platforms
 - infer regional specification for data-driven models
 - quantitatively simulate what chemical exposures would do in an automated system
 - provide inferences on developmental effects in a 3Rs-compliant manner.

Acknowledgements



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

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