TERATOLOGY v2.0

– building a path forward

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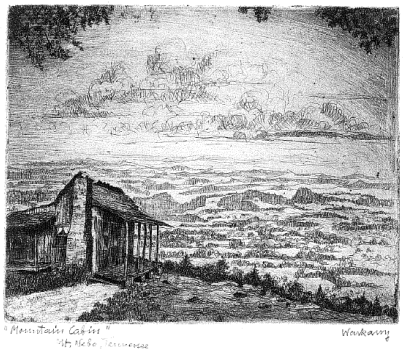

Joseph Warkany Lecture
Teratology Society - June 29, 2014
Bellevue, WA

Disclosure Slide

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No conflicts of interest to disclose.

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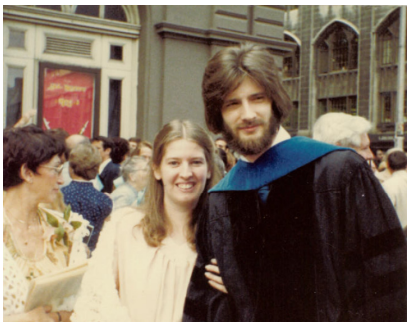

Thomas Jefferson University, Philadelphia, 1981

PhD training in Dave Kochhar's lab hammered on the importance of approaching teratogenesis as a multiscale problem: ***the integration of genetic-biochemical-mechanical factors over space and time is of fundamental concern.***

Having an incredibly supportive wife allowed my focus to remain on that problem for over 35 years -*Thanks, Cyn!*

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EPA's National Center for Computational Toxicology (2014)

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Pushing the Boundaries: potential 'Game-Changers'

One-liners solicited from ~35 Teratology Society members from different sectors, diverse expertise and age ranges; their responses in a nutshell (http://www.Wordle.net/):

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We can 'Push the Boundaries' by:

- "<advancing the> mechanistic understanding of gene-environment interactions." – *gd*
- "<identifying> a single major sensitivity gene for a given exposure <for> pre-conceptional genetic testing and counseling ..." - *rf*
- "<defining what is normal given the> virtually limitless combination of alleles and environments … in a global culture" – *cc*
- "<having> the tools and knowledge to understand the causes of most birth-defects rather than the minority that we do today." – *any*
- "<funding> new initiatives for understanding developmentally-mediated disorders" collaboratively." – ezf

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We can 'Push the Boundaries' by:

- "<applying> synthetic biology <to> the relationships between mechanistic effects and phenotypic consequences." – *sh*
- "<developing a pregnant> human-on-a-chip <platform> that incorporates microfluidics and is amenable to HTS." – *nk*
- "<when a> computer gives birth to a virtual infant." – *any*
- "modeling neurodevelopmental pathways in rodents, primates and humans, with extrapolation to C. elegans and zebrafish." – *emf*
- "<having> a unified dose response approach to cancer and non-cancer endpoints … raising the value of research in our field." – *any*

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We can 'Push the Boundaries' by:

- "… determining which species was correctly predicting human response for each different exposure …" – *rc*
- "… determining the molecular basis, or a refutation thereof, of a single unifying mechanism of teratogenesis". – *bb*
- "… <shifting> the emphasis toward a collaborative effort to find plausible predictive mechanistic models." – *ns*
- "<replacing> conventional descriptive methods with systems biology-based approaches …" - *ec*
- "<using> a systems level in silico model as the basis for a regulatory decision involving a developmental hazard." – *any*

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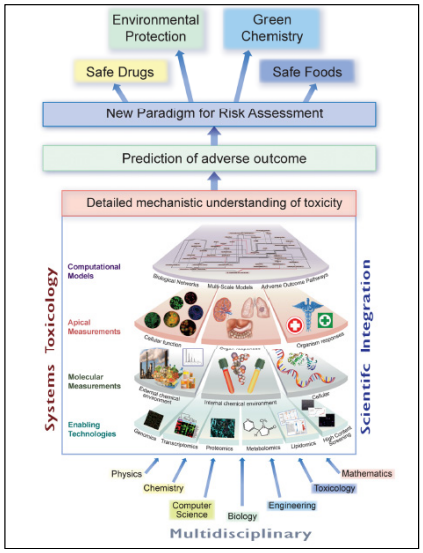
Systems Toxicology: *decoding the toxicological blueprint of active substances that interact with living systems*



Sturla et al. (2014) Chem Res Toxicol 27: 314-329

- detailed mechanistic, quantitative and dynamic understanding of toxicological processes;
- permitting ***prediction*** and accurate ***simulation*** of complex (emergent) adverse outcomes.

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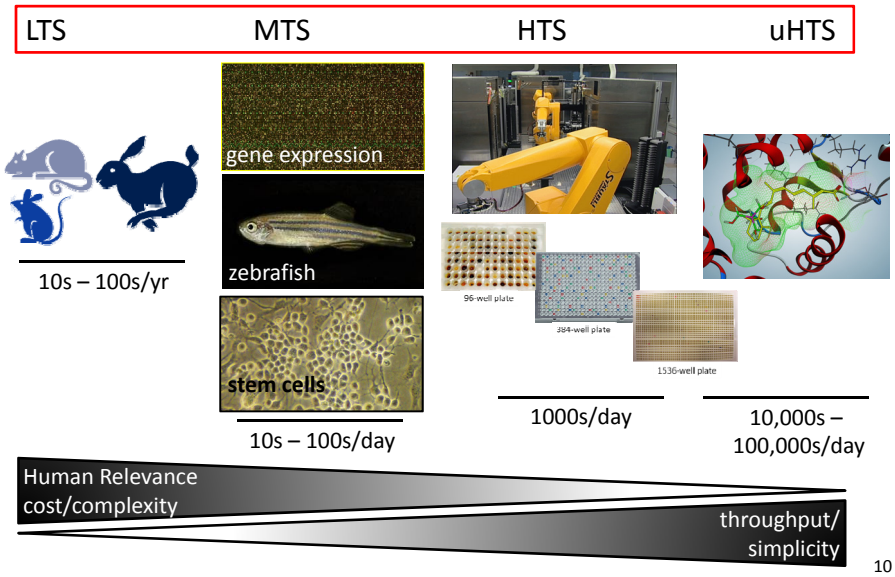
HTS/HCS platforms, advanced analytical tools, and computational models are transforming toxicology to a data-rich science.



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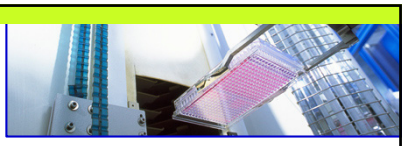
Chemical Testing under ToxCast and Tox21



- **ToxCast:** EPA research effort profiling >1060 chemicals across >800 *in vitro* assays (27M data points, ~1.7M conc. response curves). http://www.epa.gov/ncct/toxcast/
  - **Phase-I:** 310 data-rich chemicals (primarily pesticides) having over 30 years of traditional animal studies valued at $2B (completed 2011).
  - **Phase-II:** adds 767 chemicals (eg, industrial and consumer products, food additives, failed drugs) extend the broader chemical landscape (2014).
  - **Phase-IIIa:** adds 1001 compounds in a subset of ~100 assays (2014 -); E1K adds 880 chemicals in ~50 endocrine-related assays.
- **Tox21:** partnership of federal agencies.
  - 8193 chemicals in dozens of HTS assays (ongoing)
  - brings total number of chemicals to ~10,000

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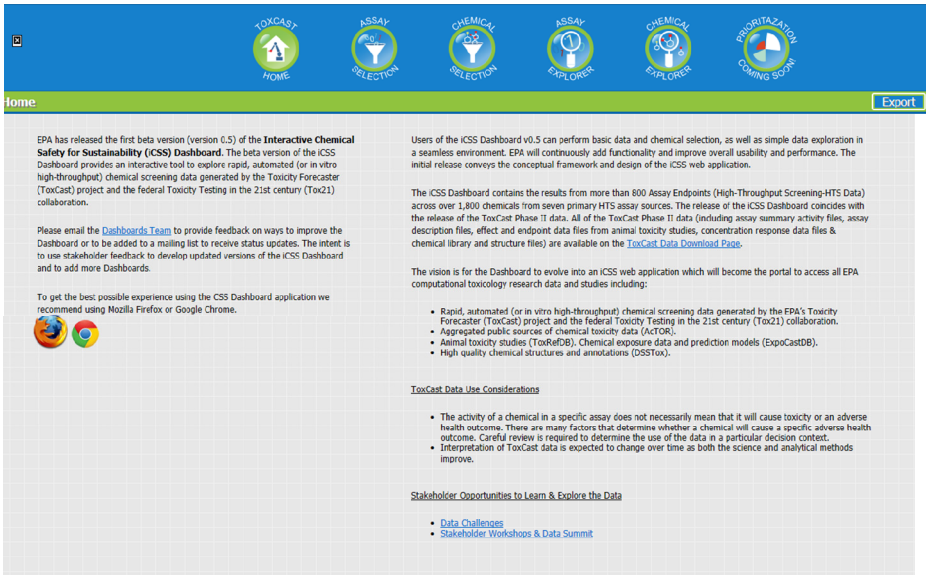
iCSS Dashboard: public delivery portal for ToxCast data



http://actor.epa.gov/dashboard/

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EPA's Children's Environmental Health (CEH) Research Roadmap *(see P40 by Sipes et al. in Tuesday's poster session)*

Overarching research goal: To provide the Agency and others with the information needed to incorporate consideration of early lifestage susceptibility and vulne

Priority

1. Know
   infor
2. Syste
   expo
3. Meth
   and t
4. Translational research to incorporate CEH into tools fit for purpose to inform community actions and decisions.

Research questions:

- By what biological *Adverse Outcome Pathways* do environmental contaminants contribute to important childhood health outcomes (adverse birth outcomes, obesity, cognitive disorders, asthma)?
- What are the systems-level influences of the chemical, natural and built environments on these health outcomes?
- How can we evaluate the cumulative risk of chemicals including the contribution of non-chemical stressors?

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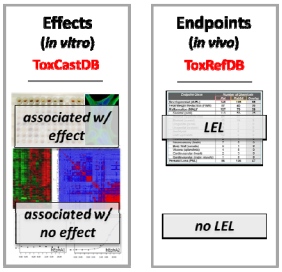
Predictive model: prenatal developmental toxicity (phase-I)

Feature	Description	Weight
RAR	Retinoic Acid receptor	0.58
GPCR	G-Protein-Coupled Receptors	0.55
TGFβ	Transforming Growth Factor β	0.38
MT	Microtubule organization	0.30
SENS_CYP	Cytochrome P450 (sensitive)	0.26
AP1	Activator protein 1	0.24
SLCO1B1	Organic anion transporter 1B1	0.11
CYP	CYPs (other)	0.06
HLA-DR	MHC complex	-0.38
PXR	Pregnane X receptor	-0.24
IL8	Interleukin 8	-0.23
PGE2	Prostaglandin E2 response	-0.18

Feature	Description	Weight
CCL2	Chemokine ligand 2 (MCP1)	1.15
IL	Interleukin (1a and 8)	0.39
CYP	Cytochrome P450	0.24
TGFβ	Transforming Growth Factor β	0.28
MESC	Mouse ES cells (J1)	0.13
SULT2A1	Sulfotransferase	-0.26
PGE2	Prostaglandin E2 response	-0.15

*Multivariate **Rat** Model*
71% balanced accuracy



*Multivariate **Rabbit** Model*
74% balanced accuracy

SOURCE: Sipes et al (2011) Toxicol Sci 124

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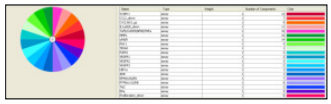
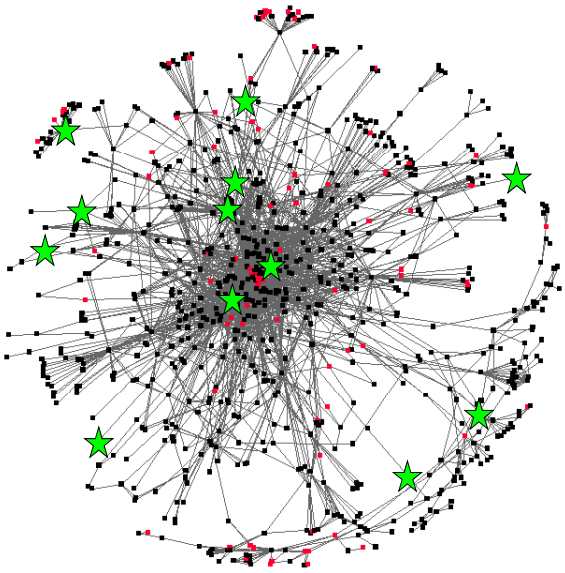
DevTox Model: features mapped to *GO* Biological Process

univariate DevTox features
multivariate DevTox features

★ *processes related to neovascularization (vasculogenesis and/or angiogenesis)*

Toxicity Prioritization Index (ToxPi) based on ToxCast for vascular disruption





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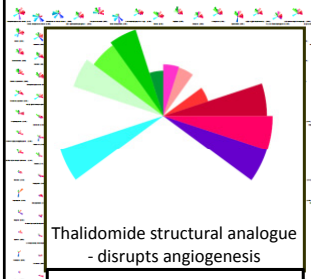
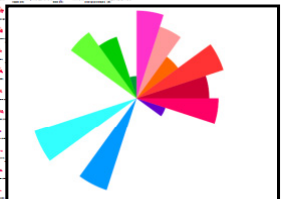
1060 Chemicals in ToxCast Ranked by pVDC score



Thalidomide structural analogue
- disrupts angiogenesis

5HPP-33 (0.683)



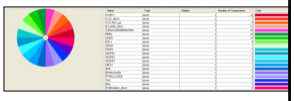
Mitocide/insecticide
- mitochondrial respiratory chain

Pyridaben (0.667)

Herbicide/weed control
- acetohydroxyacid synthesis

Imazamox (0.02)

Toxicity Prioritization Index (ToxPi) for vascular disruption



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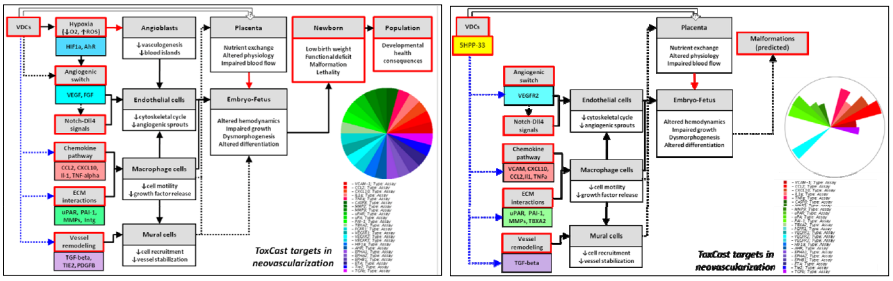
AOPs: putative vascular disrupting compounds (pVDCs) and their predicted impact on embryogenesis.

AOP framework model: known biology (MGI) and ToxCast HTS data

Activity predicted for 5HPP: anti-angiogenic Thalidomide analogue



SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res. C 93: 312-323
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996. doi:10.1371/journal.pcbi.1002996.

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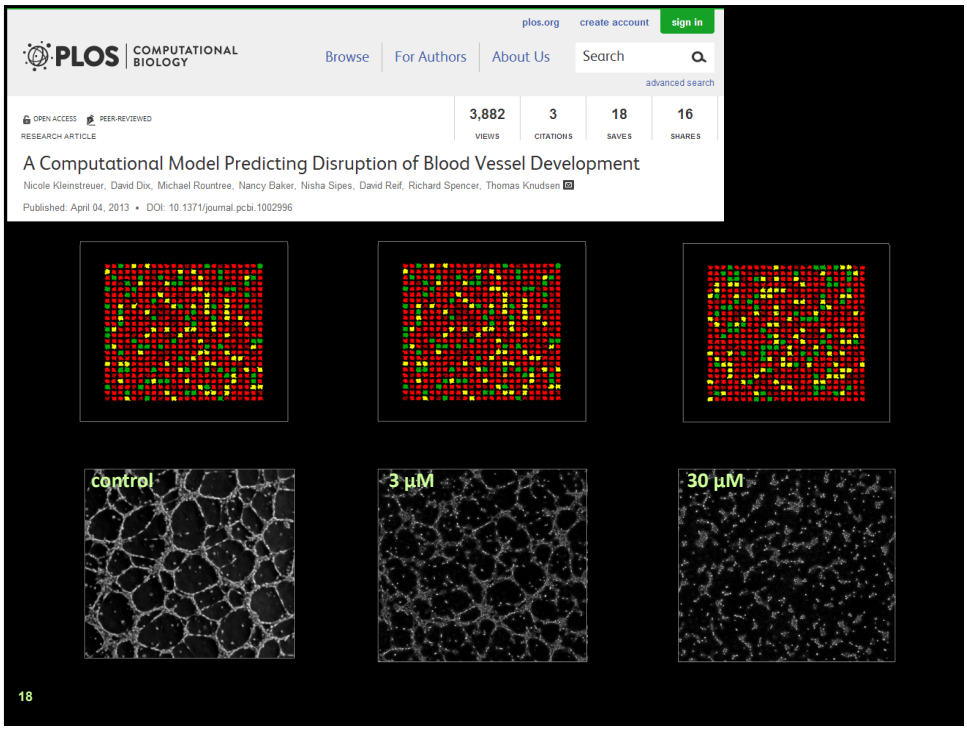

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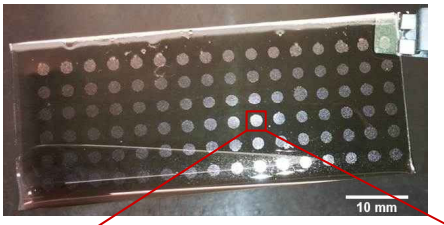
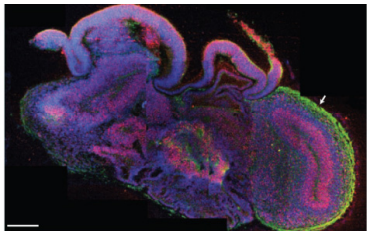
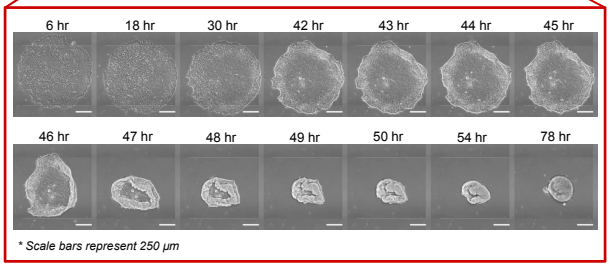
Minibrains and 3D Organotypic Culture Models

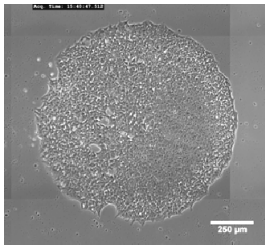
SOURCE: W Murphy, U Wisconsin

Lancaster et al.(2013) Nature 501: 373-379









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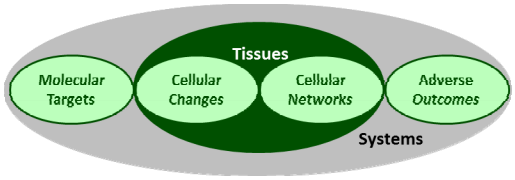
Hallmarks of Transformation



- ***Embryogenesis*** is a multicellular process – we need to visualize, analyze and model the dynamic nature of cellular interactions.
- so is ***Teratogenesis*** - even a few cell-cell interactions, disrupted at a critical time in development, can have an impact children's health.
- major future ***Challenge*** - integrate the dynamics of these processes at different spatial scales during normal and abnormal development.
- a ***Predictive*** understanding depends on a global strategy to interact 'big-data' with 'principles of teratology' at a systems level.

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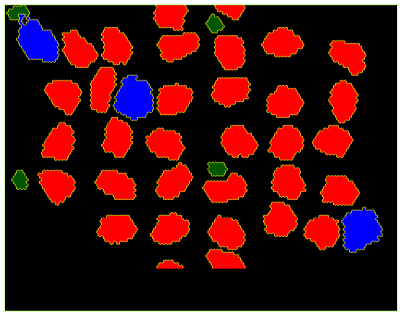
Agent-Based Model (ABMs)

Biological rules assigned to low-level 'agents' that then interact in a shared environment to display high-order (emergent) features.

In vitro

In silico



SOFTWARE: www.CompuCell3D.org
James Glazier and colleagues, Indiana U

- *macrophage*
- *RBC*
- *bug*

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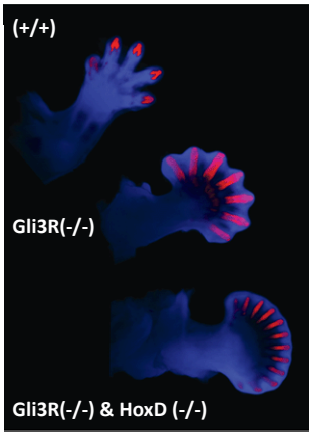
FINS *to* LIMBS (… and back)

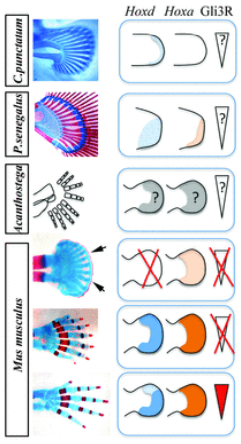
Evolution and Development of the pentadactylous autopod

HoxD (fin rays) → Gli3R (pentadactyly) → Gli3R (polydactyly) → HoxD ('fin' rays)

Boot et al. (2008) Nat Met 5: 609



Vogel (2012) Science 338: 1406
(with permission from J Sharpe)



Sheth et al. (2012) Science 338: 1476

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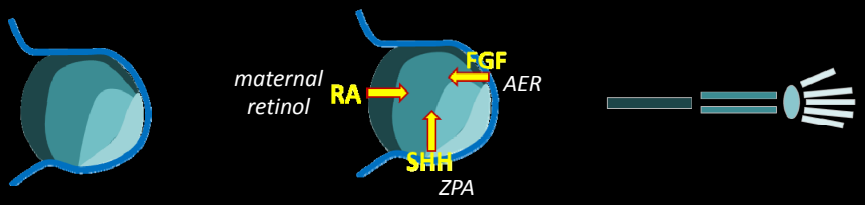
Morphogenesis: *limb development*

PRE-PATTERNING *(specification)* → **SIGNALING** *(organization)* → **INDUCING** *(differentiation)*



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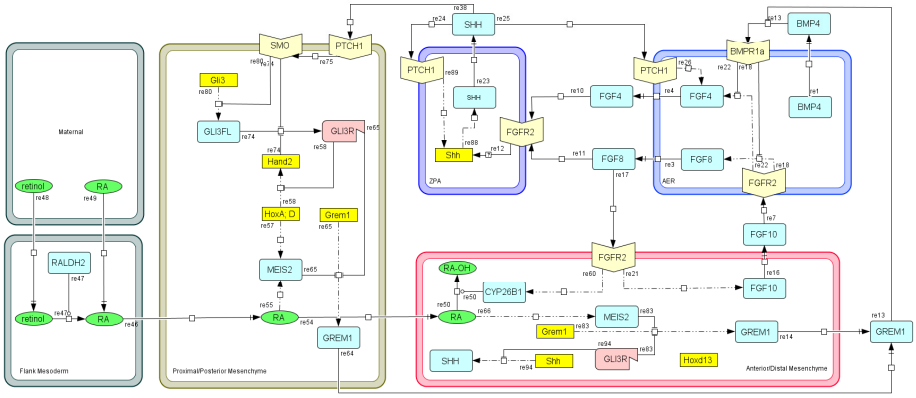
Limb outgrowth: *control network*



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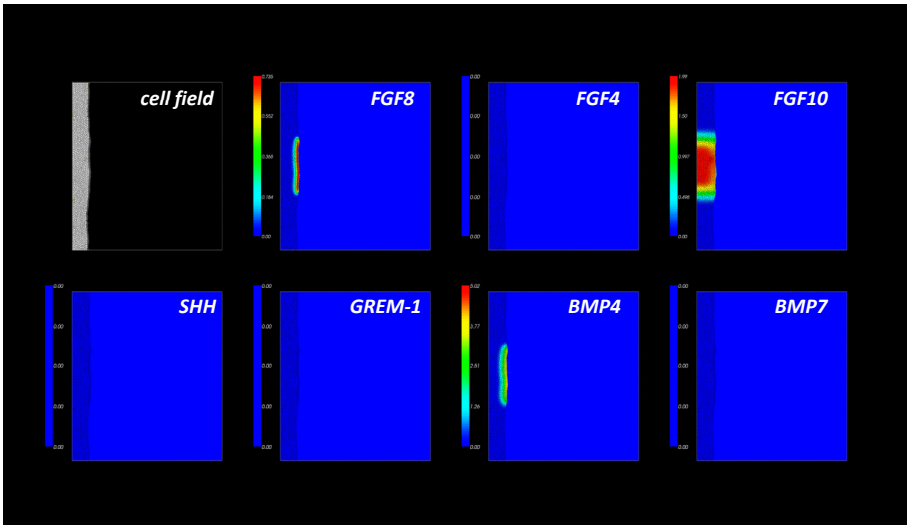
Agent-Based Model: *CompuCell3D* simulation of key spatial temporal gradients patterning limb-bud outgrowth



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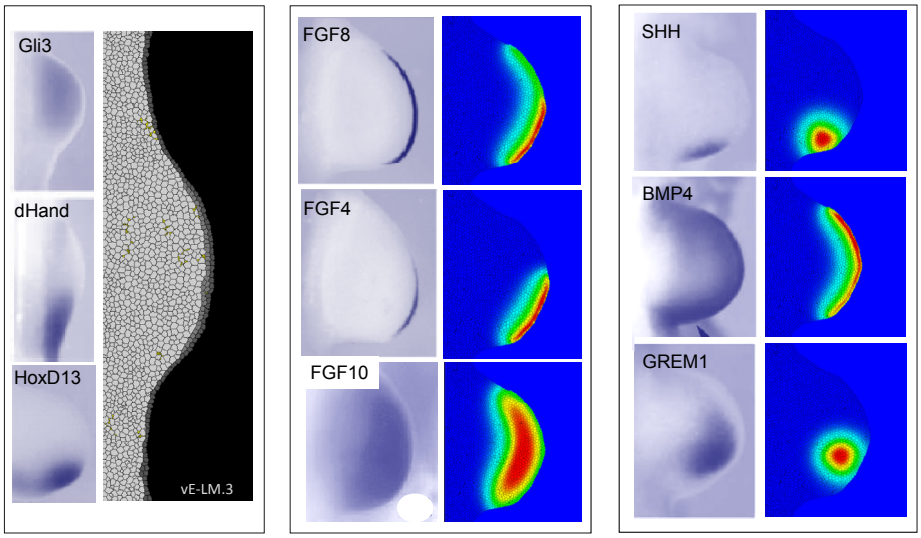
SOURCE: Ahir, Knudsen et al. (NCCT)



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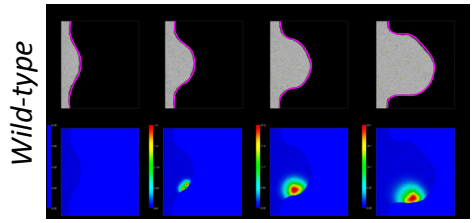
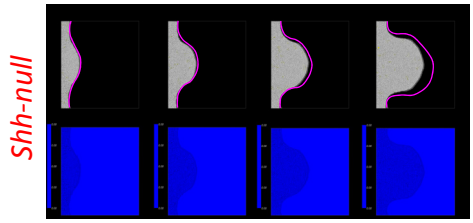
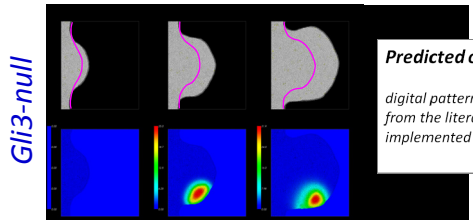
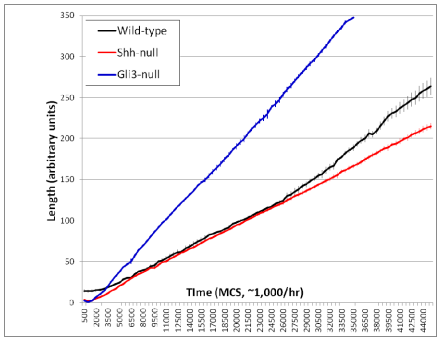
ISH (mouse literature) vs ABM







Rate of outgrowth *(n=5 simulations)*



Predicted outcomes

digital patterns inferred from the literature; not yet implemented in the model

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HYPOTHESIS

a computer model that executes the spatial and temporal dynamics of biological networks in the embryo can be used predictively to simulate developmental toxicity.

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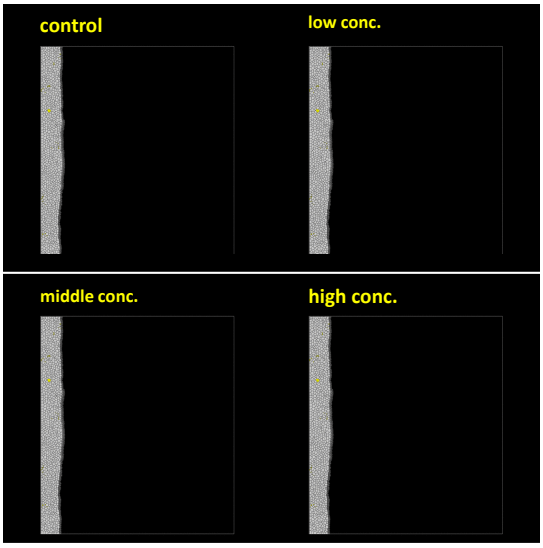
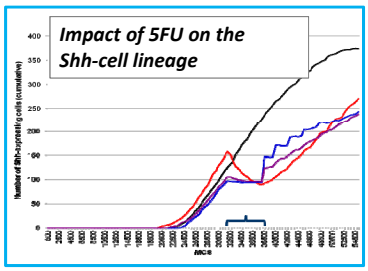
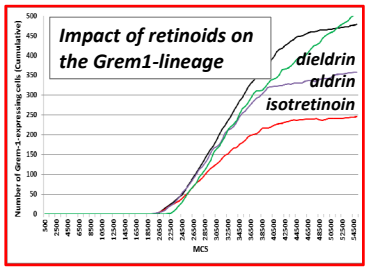
Translating *in vitro* data into simulation







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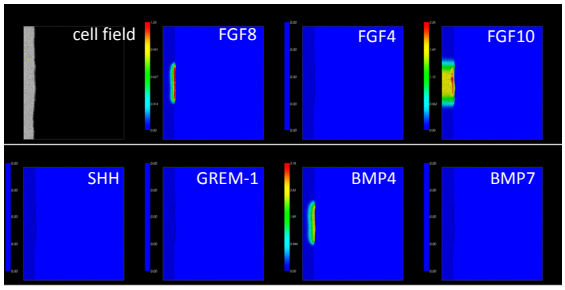
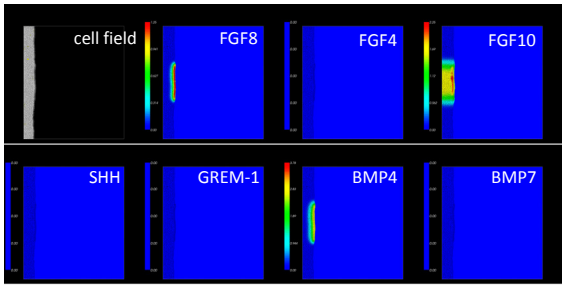
Failability: running the models backward to reveal the earliest signs of failure (e.g., onset of Shh expression)



Run #3
Onset of Shh expression is slightly ahead in this run, leading to an posterior bias in the FGF10 domain.



Run #4
Onset of Shh expression is slightly behind in this run, leading to an anterior bias in the FGF10 domain.

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The Multiscale Problem

- Small disturbances at the cellular-molecular level might cascade into big effects as the system evolves to higher scales;
- (or large disturbances might be buffered prior to any observable outcome).

da Vinci's sketch of turbulent water flow captures this complexity in even this simple phenomenon;
SOURCE: Heng (2014) Am Scientist 102: 174-177

- Uncertainty on the microscopic scale (e.g., how disruption in one cell impacts the behavior of others) hinders our ability to predict the outcome at a macroscopic scale.

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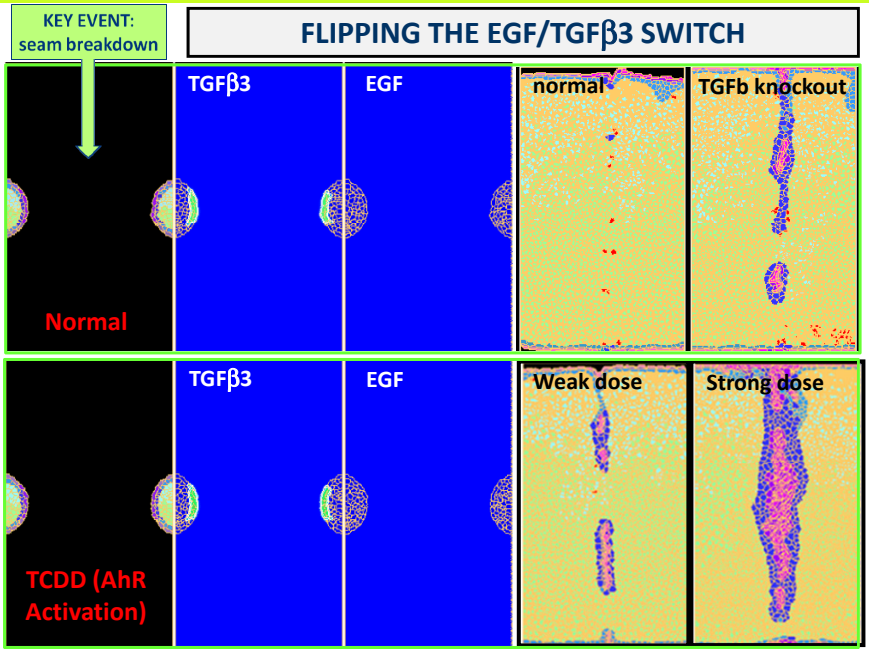

SOURCE: Hutson et al. (manuscript in preparation).

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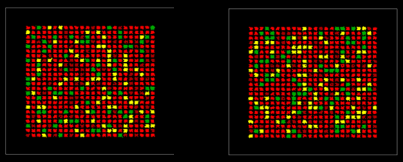
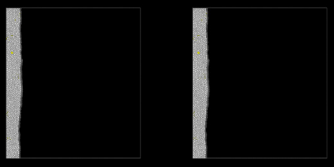
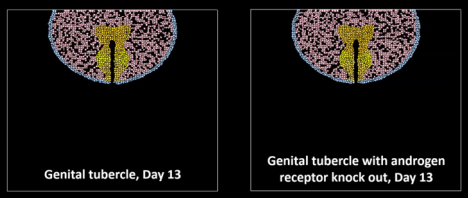
Blood vessel development disrupted by Thalidomide



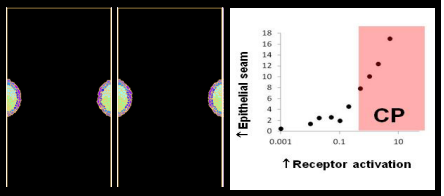
Limb-bud outgrowth disrupted by 5FU-induced mesenchymal loss



Genital tubercle outgrowth disrupted by AR-knockout



Palatal fusion disrupted by TCDD-induced AhR hyperactivation



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Benefits and challenges of computational model for predicting developmental toxicity

- reconstructing spatial dimension and function (systems response)
- predicting impacts of cellular changes on dynamics (trajectories)
- quantifying the 'un-measureable' (lesion propagation)
- parameter sweeps to isolate key elements (sensitivity analysis)
- high-throughput hypothesis testing (mechanistic understanding)
- pinpointing nascent events underlying 'emergent' biology
- surrogate for missing data or information (knowledge gaps)
- probing pathway interactions (convergence, cumulative)
- simulating different exposure scenarios (ADME)
- not a living entity (can only code rules as we understand them)
- finding sweet-spot to enable, but not over-specify performance
- how complex do these systems model need to be (reality check)
- extending them for lifestage considerations / life-course model

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VIRTUAL EMBRYO TEAM

www.epa.gov/ncct/v-Embryo/

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