United States Environmental Protection Agency

EPA's Virtual Embryo:
Modeling Developmental Toxicity

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Disclaimer: views are those of the presenter and do not necessarily reflect Agency policy nor imply endorsement of software used here

Biocomplexity Workshop, Indiana University
Quantitative Tissue Biology and Virtual Tissues
October 29, 2009

Overview

- scope of the problem
  - *assessing the toxicity of environmental chemicals*
- high-throughput screening (HTS)
  - *launching from the drug-discovery paradigm*
- computational (*in silico*) models
  - *molecular pathways and complex systems*
- virtual tissues
  - *building blocks for a virtual embryo*

Environmental chemicals

- need to understand health risks associated with 10K-30K chemicals in commerce and the environment
- current testing strategy is low throughput, costly, and relies on high-dose effects in animal studies
- only a minor fraction of chemicals of concern have data in sufficient depth or detail for risk assessment (IRIS <1K)
- scientific and practical needs for a different way to test chemicals and predict toxicities more efficiently

Vision of the new paradigm

NCCT (EPA), NCGC (NIH), NTP (NIEHS)

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REPORT IN BRIEF

July 2007

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.

Toxicity tests on laboratory animals are conducted to evaluate chemicals—including medicines, food additives, and industrial, consumer, and agricultural chemicals—for their potential to cause cancer, birth defects, and other adverse health effects. Information from toxicity testing serves as an important part of the basis for public health and regulatory decisions concerning toxic chemicals. Current test methods were developed incrementally over the past 50 to 60 years and are conducted using laboratory animals, such as rats and mice. Using the results of animal tests to predict human health effects involves a number of assumptions and extrapolations that remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about effects at lower doses or exposures. Test animals are typically observed for overt signs of adverse health effects, which provide little information about biological changes leading to such health effects. Often controversial uncertainty factors must be applied to account for differences between test animals and humans. Finally, use of animals in testing is expensive and time consuming, and it sometimes raises ethical issues.

Today, toxicological evaluation of chemicals is poised to take advantage of the on-going revolution in biology and biotechnology. This revolution is making it increasingly possible to study the effects of chemicals using cells, cellular components, and tissues—preferably of human origin—rather than whole animals. These powerful new approaches should help to address a number of challenges facing the

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POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

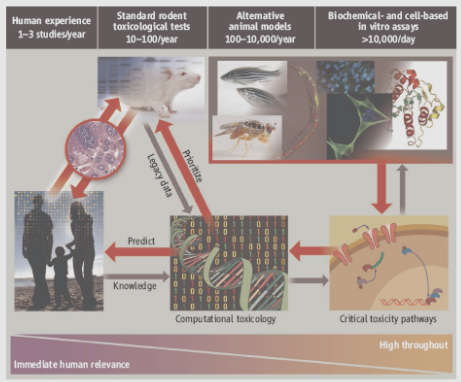
Francis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,



Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

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*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

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High Throughput Screening (HTS)

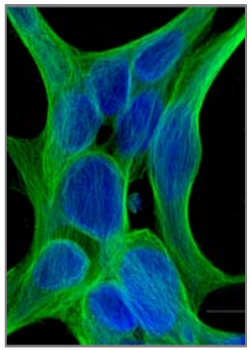
- uses automation to rapidly identify active compounds that modulate a particular biomolecular target or pathway
- activities are tested in an ‘assay’ using microtiter plates and a vehicle of DMSO
- assays are cell-based or cell-free (biochemical) systems and utilize recombinant technologies and human cells
- provides starting point for functional understanding of the particular biochemical process in drug development

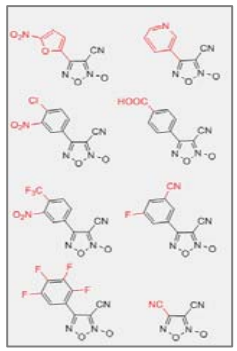
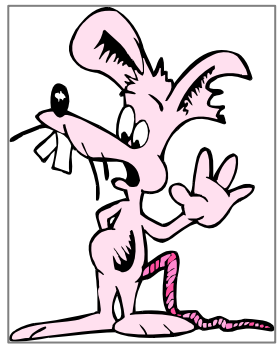
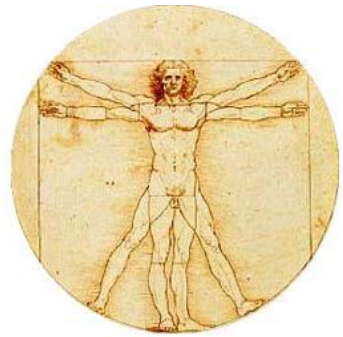
HTS predictive modeling

United States Environmental Protection Agency

drug development

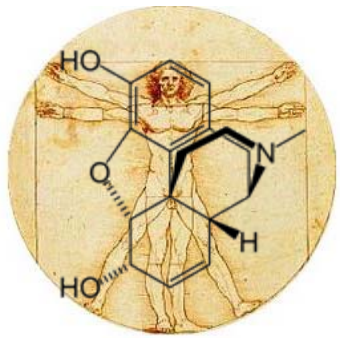
target identification → HTP *in vitro* screening → design actives → animal tests efficacy, safety → test in human patients

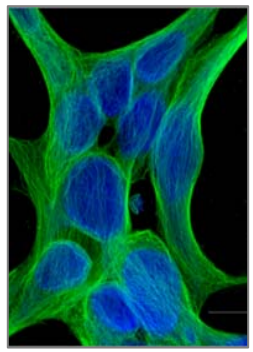


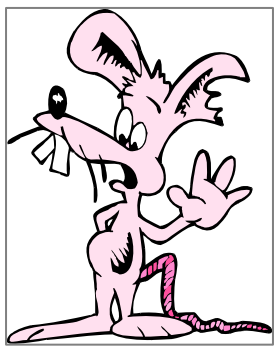




testing chemicals

unknown toxicity (or suspected) → HTP *in vitro* screening → target identification → predictive models → targeted tests (validation)





SOURCE: R Kavlock (2009), NCCT

Challenges and Opportunities

1. What fundamental targets emerge as we catalogue over a billion instances of *in vitro* perturbation?

2. What predictive signatures and pathways of toxicity will be unlocked from these HTS data?

3. How can we apply these data to different domains (e.g., predicting liver disease or developmental toxicity)?

4. Holy Grail: conquer the *in silico* reconstruction of tissues to evaluate biological plausibility of predictive signatures.

Developmental Toxicity

- chemical perturbation during formative stages of the reproductive cycle affecting:
  - embryo and fetal development (birth defects)
  - postnatal development (disabilities)
  - fertility and reproduction
  - general children's health

Prenatal development is a system within a system

Factors underlying this complexity

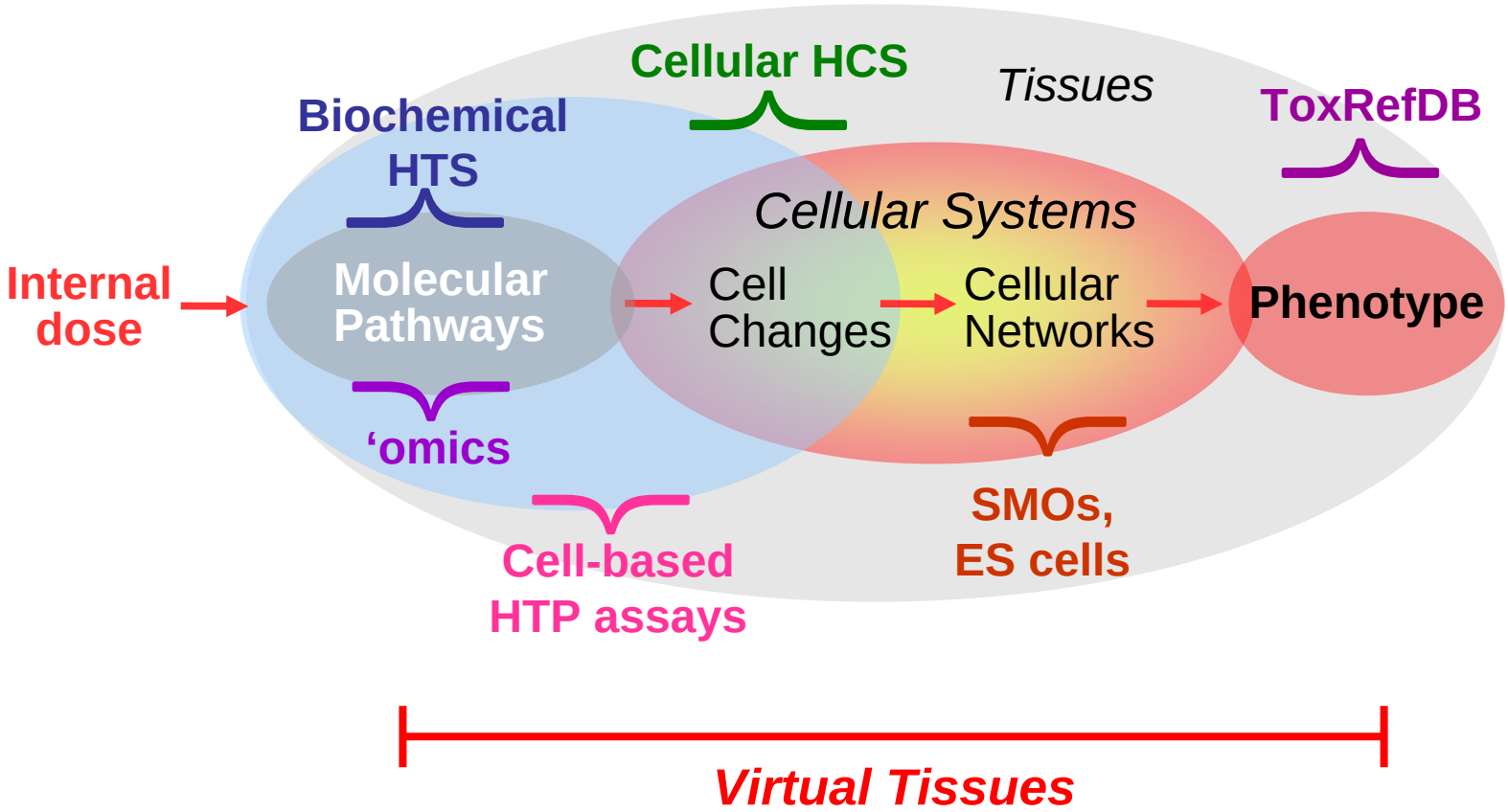
1. **TIMING:** morphogenesis and differentiation require precisely timed genetic signals and responses

2. **SENSITIVITY:** metabolic and regulatory pathways are prone to genetic errors and environmental disruptions

3. **MULTIPLICITY:** simple lesions propagated to complex phenotypes & complex lesions → simple phenotypes

4. **MATERNAL:** impact of maternal exposure biology and physiology during prenatal and lactational stages

ToxCast™ Bioactivity Profiling



ToxRefDB

Toxicity Reference Database

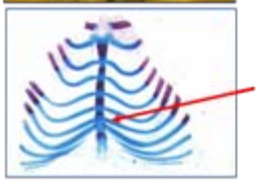
- **source data:** 2073 guideline studies for 480 chemicals, mostly pesticides (>$2B worth)
- **prenatal studies:** 751, mostly rat and rabbit, testing 387 chemicals (283 chemicals tested in both species)
- **annotation:** 988 terms for maternal and fetal effects based on enhanced *DevTox.org* lexicon
- **endpoints:** lowest effect levels for maternal (mLEL) and developmental (dLEL) parameters and cLELs

www.epa.gov/ncct/toxrefdb/

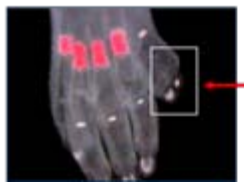
Profiling developmental toxicity

in vivo endpoints
(target, description)

target: kidney
description: absent renal papilla
code: UG_REN_3.1060.5013

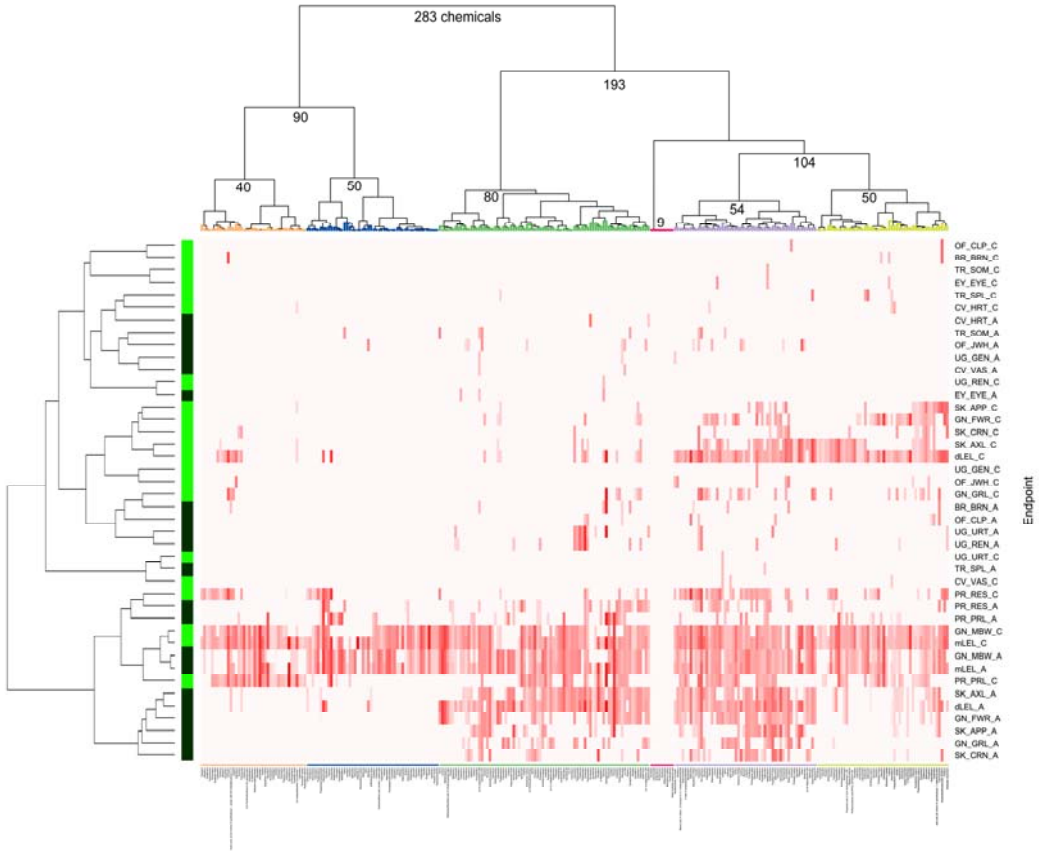


target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130



target: hindpaw
description: polydactyly (digit I)
code: SK_APP_2.1051.5234

images from www.DevTox.org



ToxRefDB 387 chemicals, 751 prenatal studies, 988 effects annotated

283 chemicals x 293 effects → 19 target systems from rat (■) and rabbit (■) studies

SOURCE: Knudsen et al. (2009) Repro Tox 28: 209-19

ToxCast™ bioactivity profiling

United States Environmental Protection Agency

Biochemical HTS assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

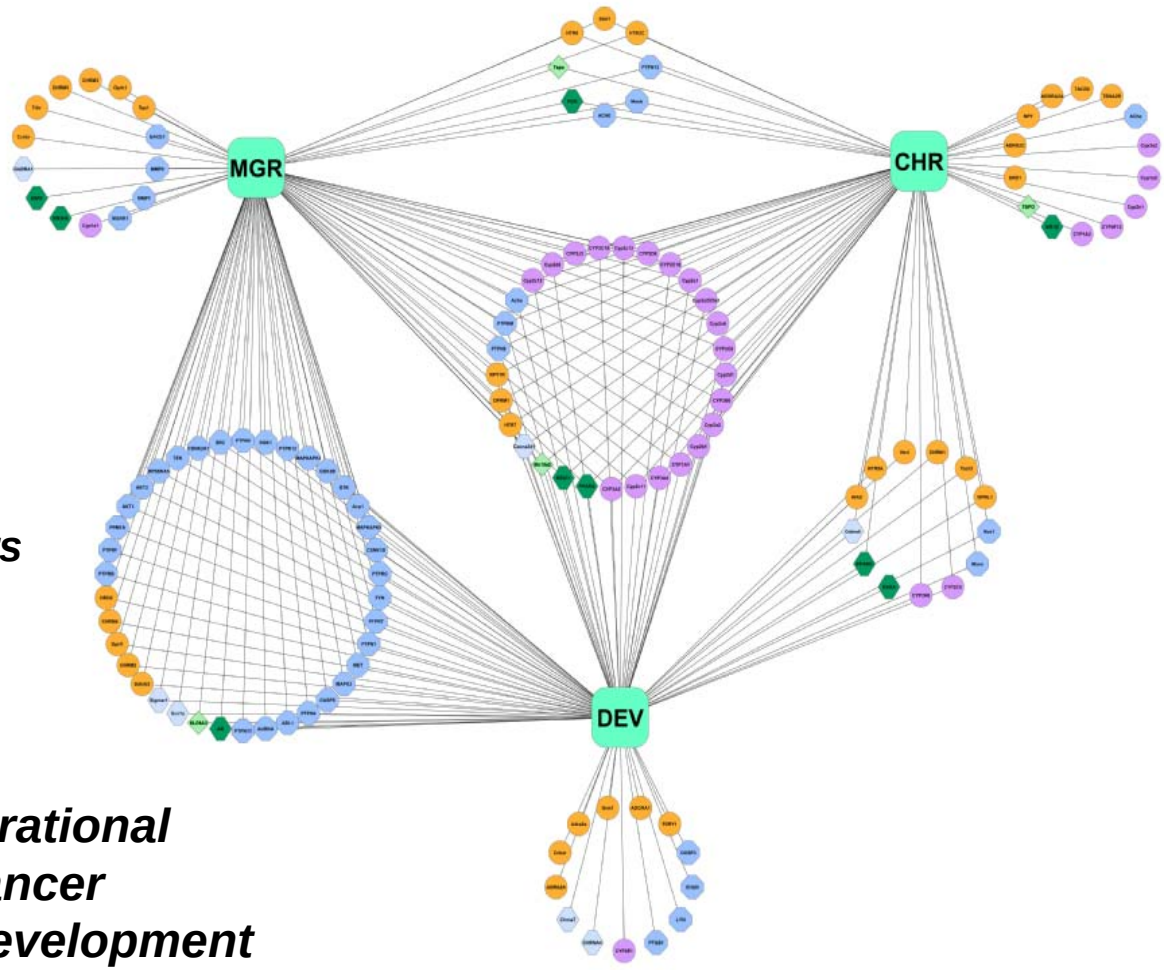
309 chemicals x 467 endpoints

Cell-based assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

Biochemical HTS screen

309 chemicals x 239 biochemical assays



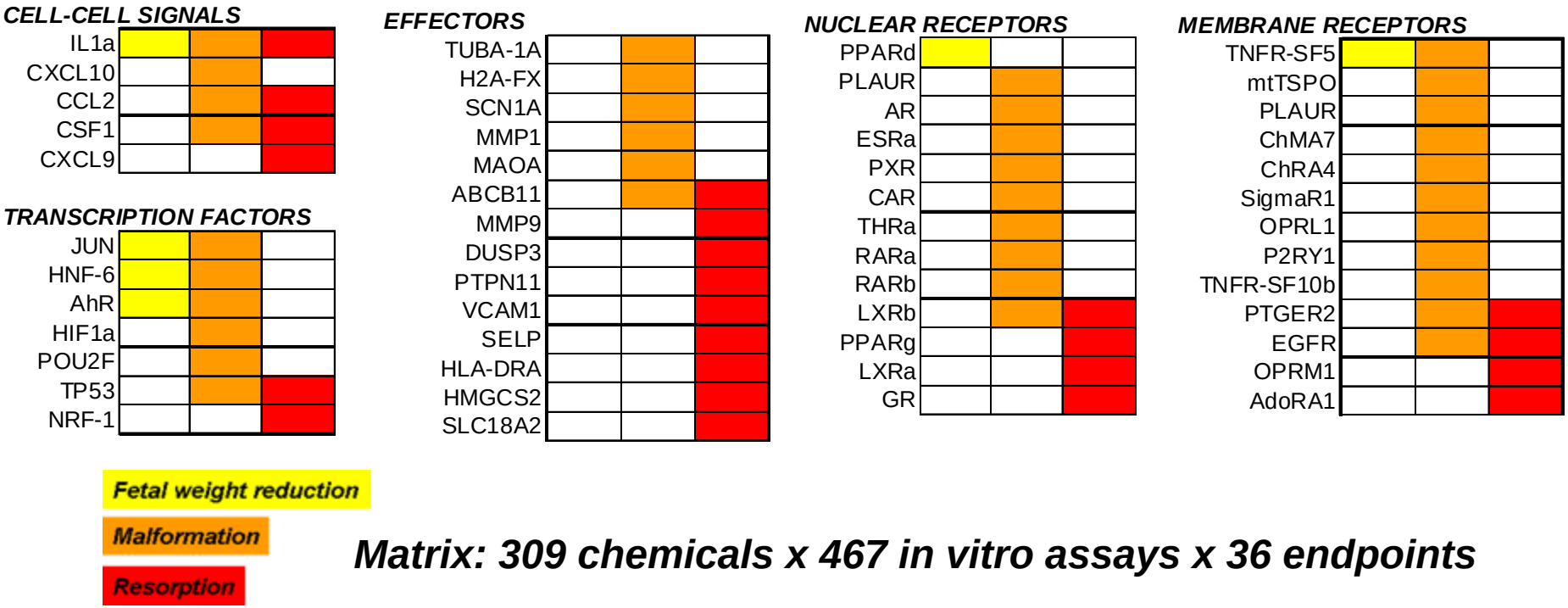
- ***CYP450***
- ***effector enzymes***
- ***GPCRs***
- ***nuclear receptors***
- ***channels, transporters***

- ***ENDPOINTS***
  - ***MGR: multigenerational***
  - ***CHR: chronic/cancer***
  - ***DEV: prenatal development***

SOURCE: Knudsen et al. (submitted)

Targets of Developmental Toxicity

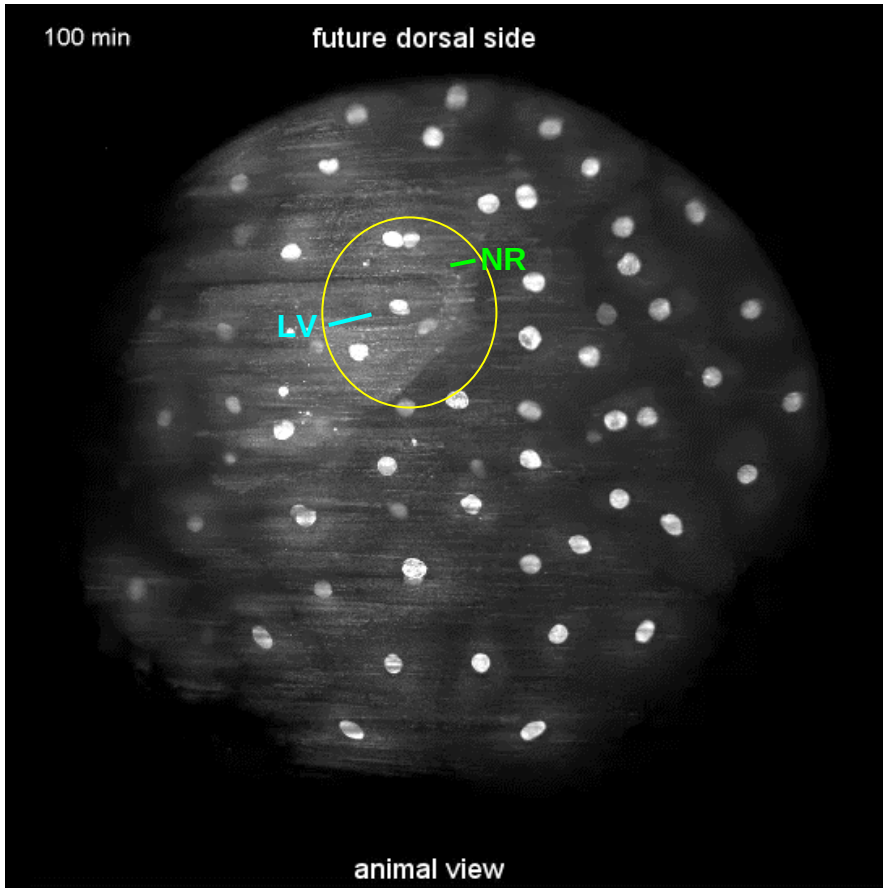
predicted from ToxCast™by machine learning

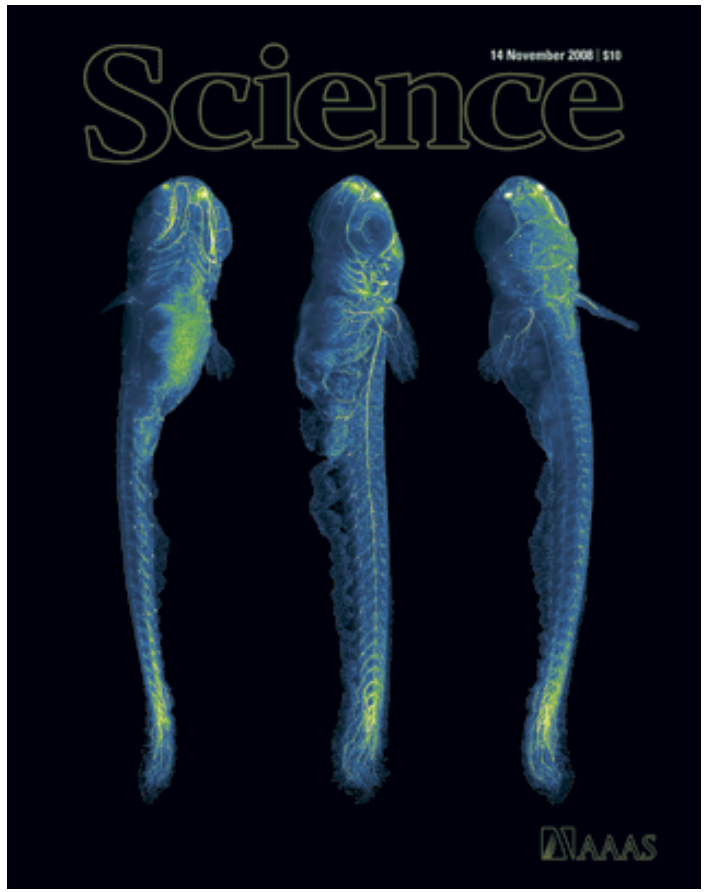


Matrix: 309 chemicals x 467 in vitro assays x 36 endpoints

Environmental chemicals that hit these in vitro assay targets also produced developmental effects in prenatal studies

Digital embryo: image-based reconstruction of a zebrafish embryo

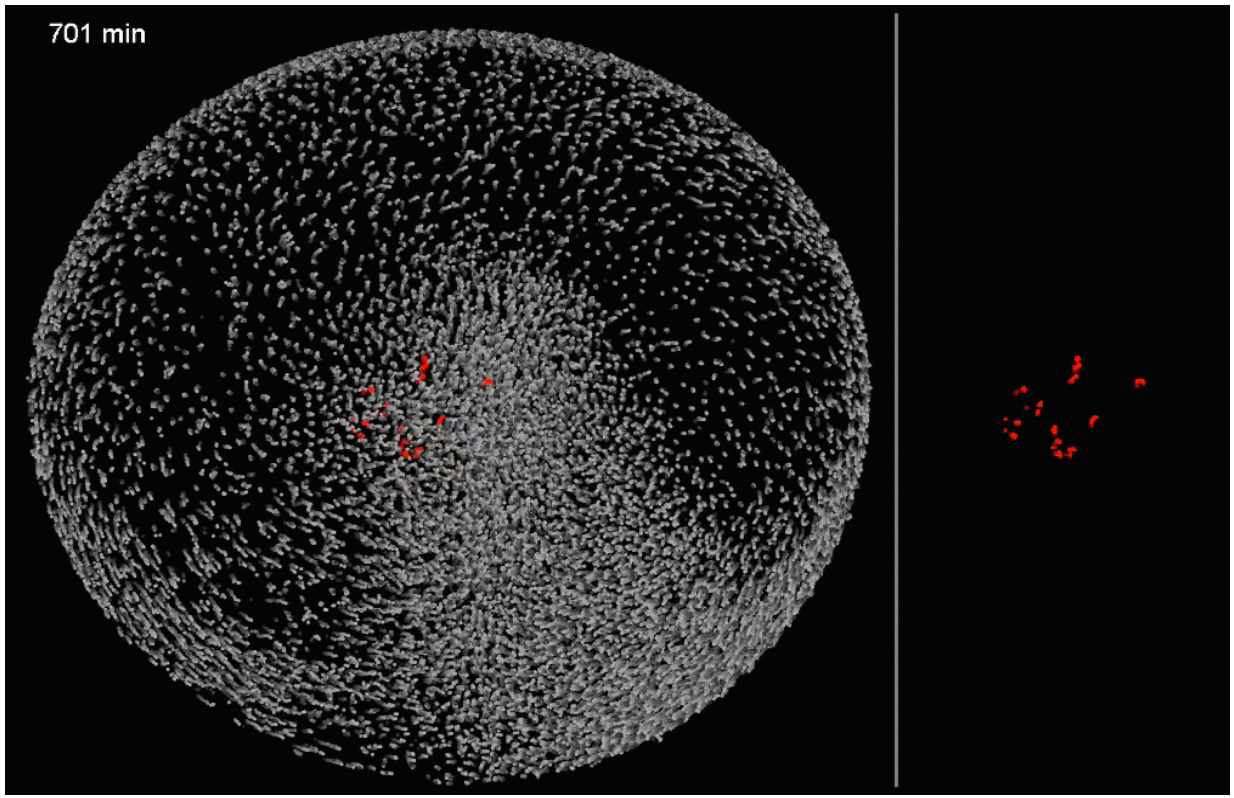




early embryonic development tracked with H2B-EGFP
by DSLM at 90s intervals over 18h

Source: Keller et al. (2008) Science 322: 1065-69

Reverse engineering morphogenesis



optic vesicle development in early zebrafish embryos

Source: Keller et al. (2008) Science 322: 1065 – 1069

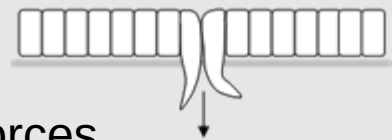
Morphogenetic processes

Core developmental processes
- patterning (sets up future events)
- timing (clocks and oscillators)
- differentiation (cell diversification)
- morphogenesis (tissue organization)

Cellular primitives
- growth (proliferation)
- death (apoptosis)
- differentiation (function)
- adhesion (DAH)
- shape (geometry)
- motility (cell migration)
- ECM (remodeling)

Morphogenetic movements
- folding
- epiboly

- convergent extension
- branching morphogenesis
- cell condensation
- cell sorting
- trans-differentiation
- cavitation
- involution



- tractional forces

After: Bard (2005) J Anat 206: 1 - 16

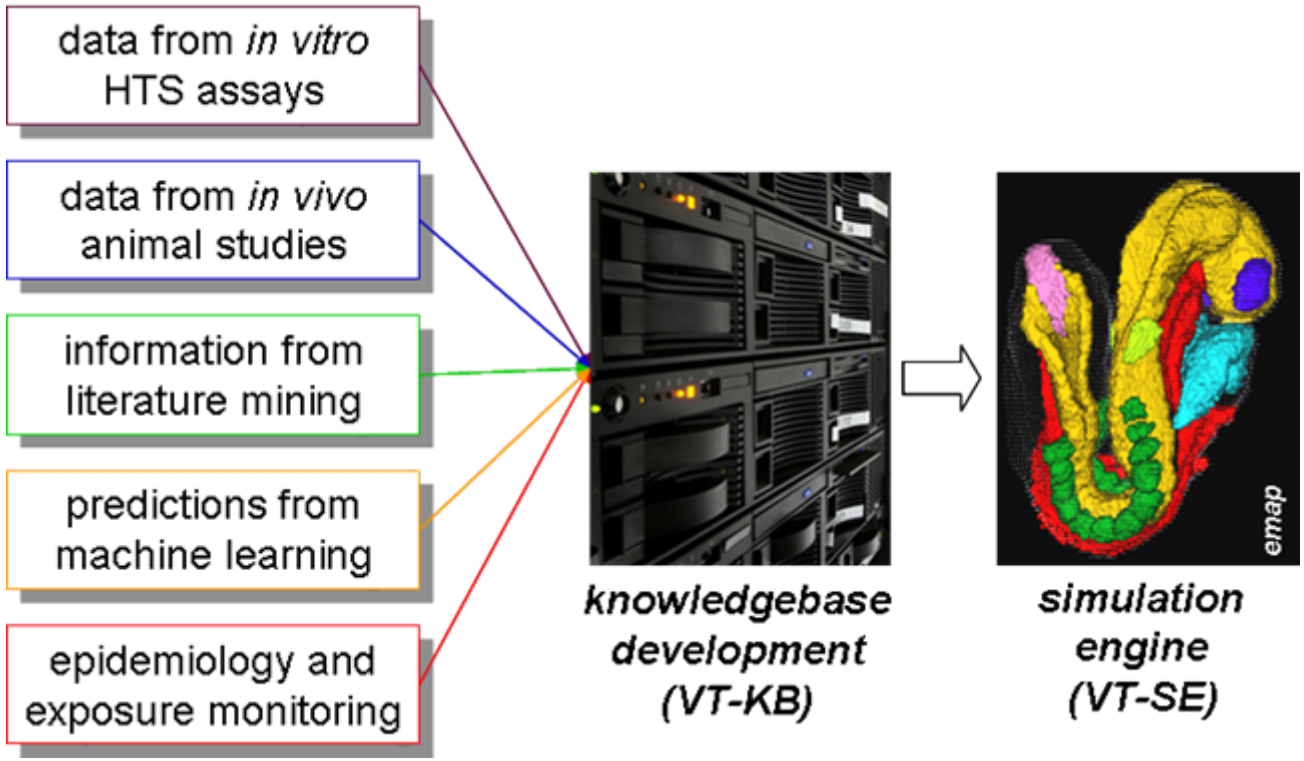
VT: Virtues for Systems Modeling

- incorporate knowledge of embryology and cell signaling pathways relevant to developmental processes
- enable the emergence of higher-order of organization using cells as autonomous agents
- implement predictive signatures from *in vitro* profiles (ToxCast) and analyze developmental trajectories
- determine biological plausibility of the predictors based on *in vivo* profiles (ToxRefDB)

Virtual Embryo

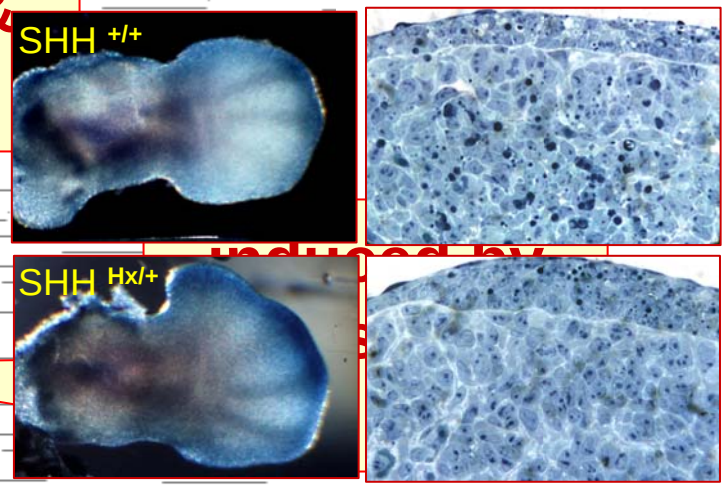
- Cell-based simulations
- Morphogenesis Manager

CompuCell3D
MorphMan



Limb Morphogenesis

cell growth & death

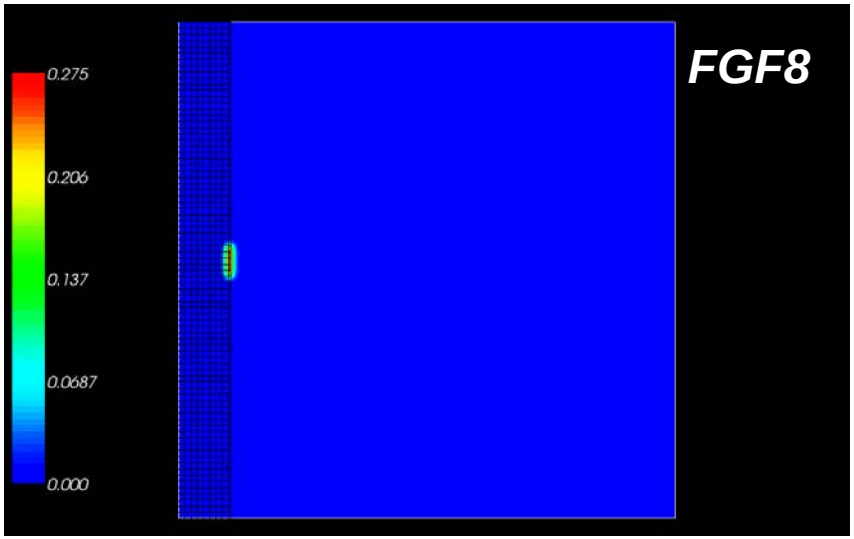


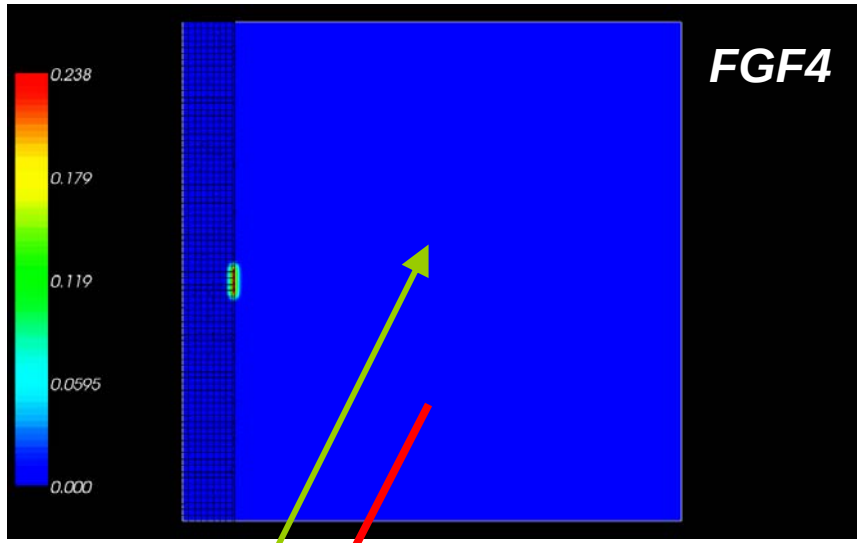
GOAL: disruptions in cell signaling

Polarized limb outgrowth

Modeled in www.***CompuCell3d***.org environment
N Poplawski (chick limb) → M Rountree (mouse limb)

Cell Signaling



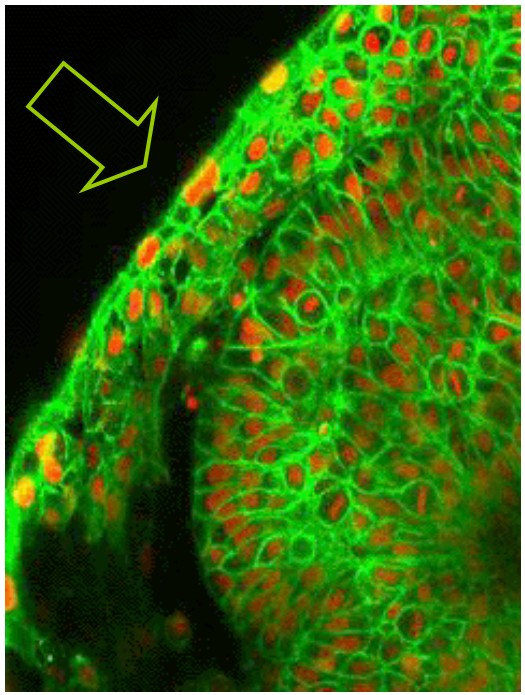


Patterning of limb outgrowth

FGF4 supports SHH niche

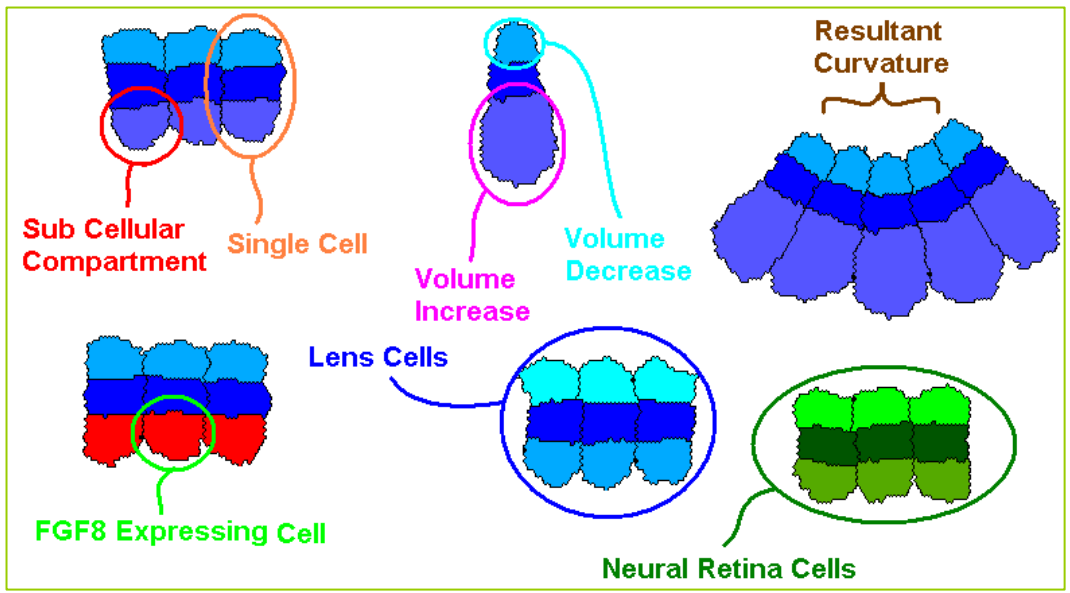
SHH organizes the paw

Example: process of lens induction & invagination

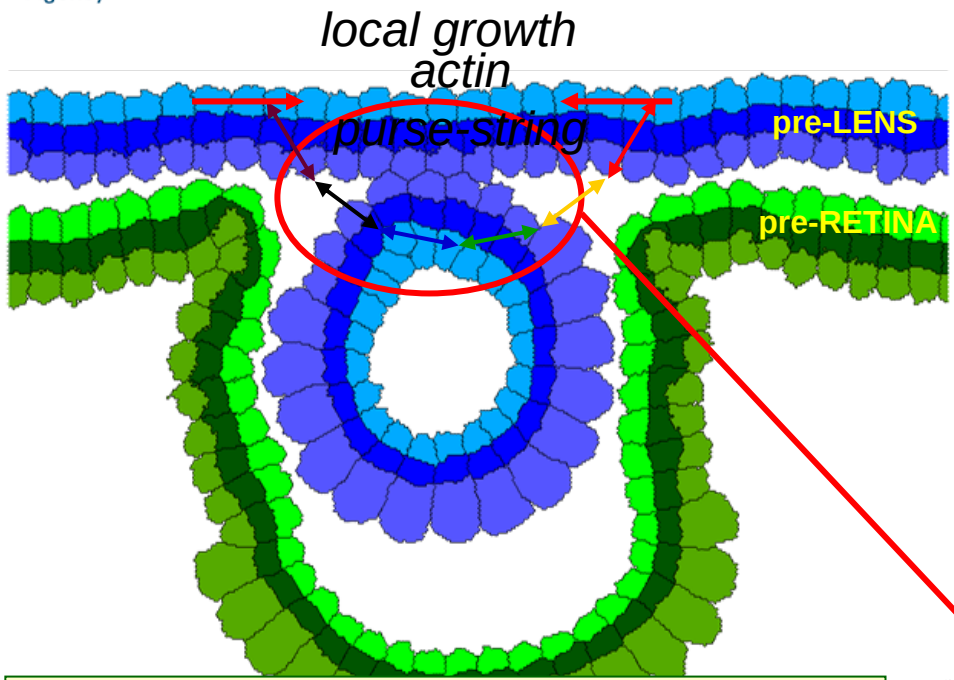


Zebrafish embryo eye
SOURCE: CB Chien lab (2009)
http://chien.neuro.utah.edu/

Driven by changes in cell growth, shape and adhesion



Lens Vesicle Formation



modeled with CompuCell3d
www.compucell3d.org

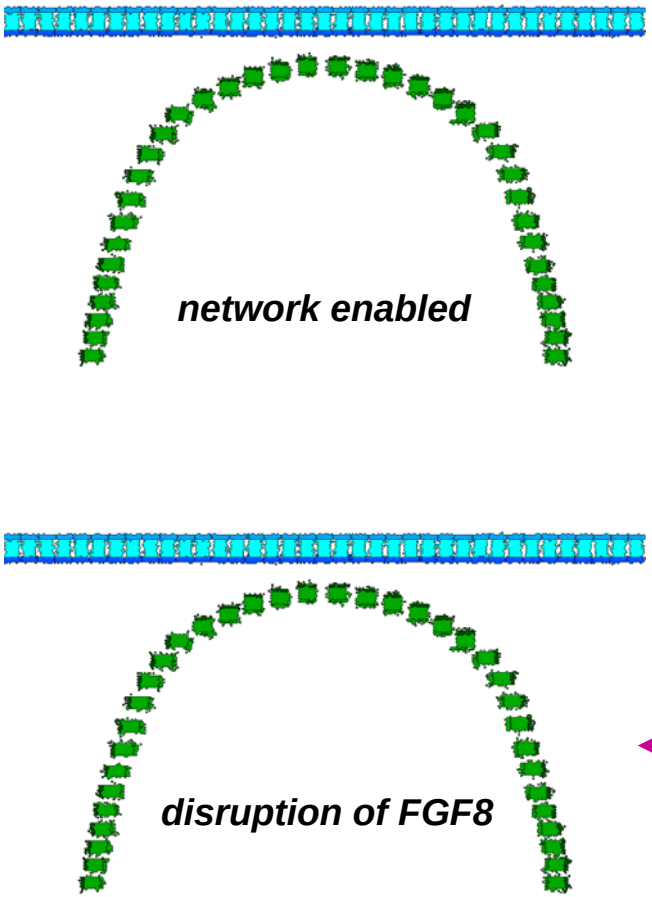
mediated by cell shape changes

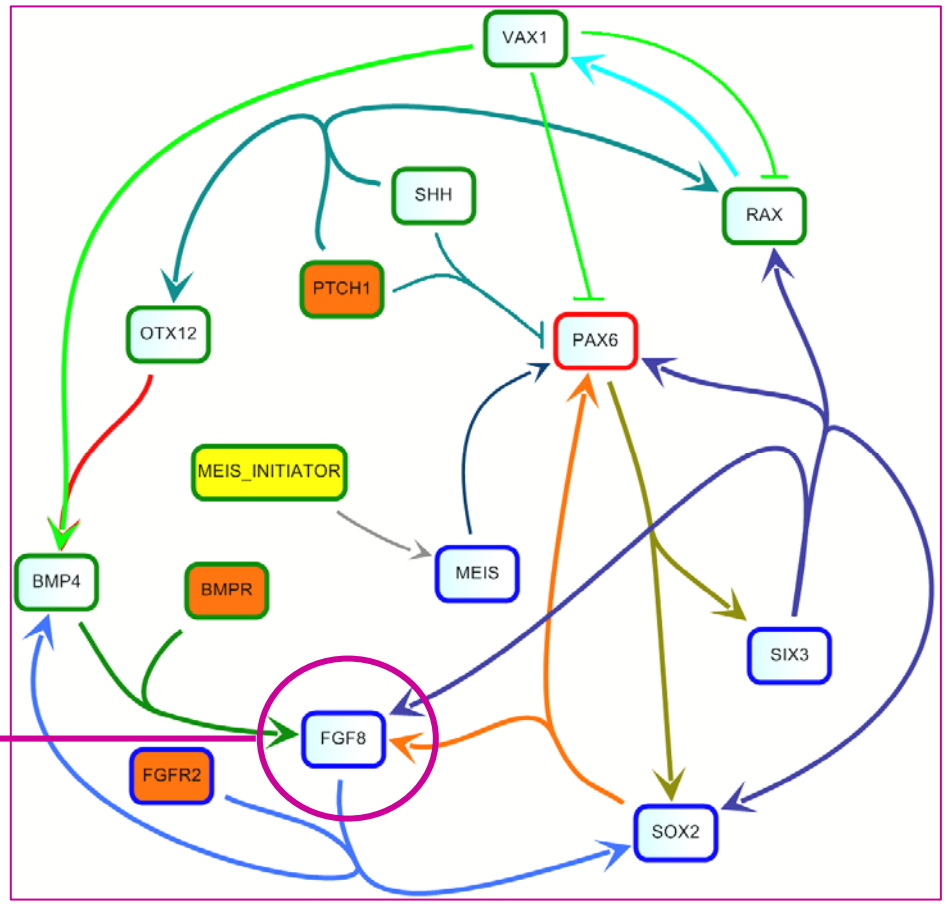
lens placode invaginates

edges will anneal

lens vesicle detaches

Coupling to Gene Regulatory Network





***sine oculis* network (lens invagination)**

ToxCast™ predictors

How do we introduce predictors?

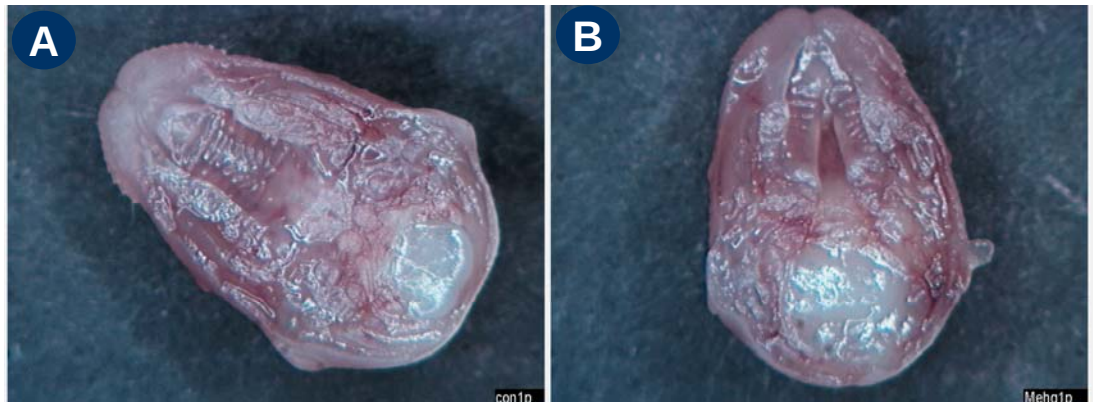
EXAMPLE:

cleft palate (B)

chemicals = 12

assays = 37

relative risk (avg) = 6.24



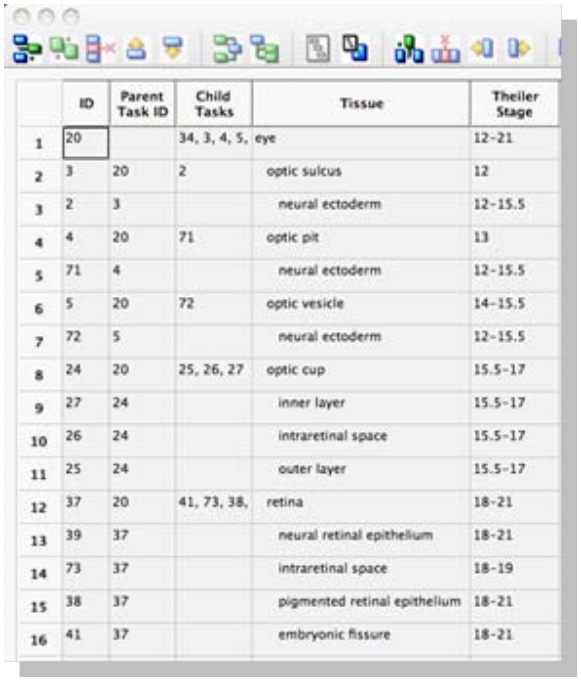
Assay True Positives

TP = 10 (WNT-CTNB, RAR, RXRa, TGFB and AHR)
TP = 9 (IGF-1, PDGF, WNT, EGF)
TP = 8 (chemokine production, epithelial-mesenchymal transformation, p53, hypoxia)
TP = 7 (type-II TGFbeta receptor, SMAD protein nuclear translocation)
TP = 5 (VEGF signaling, AP-1)
TP = 4 (regulation of TGFB2 production, regulation of cyclic AMP)
TP = 3 (hGR, glucocorticoid receptor activity, OPRM1, mu opioid receptor activity)

Morphogenesis Manager

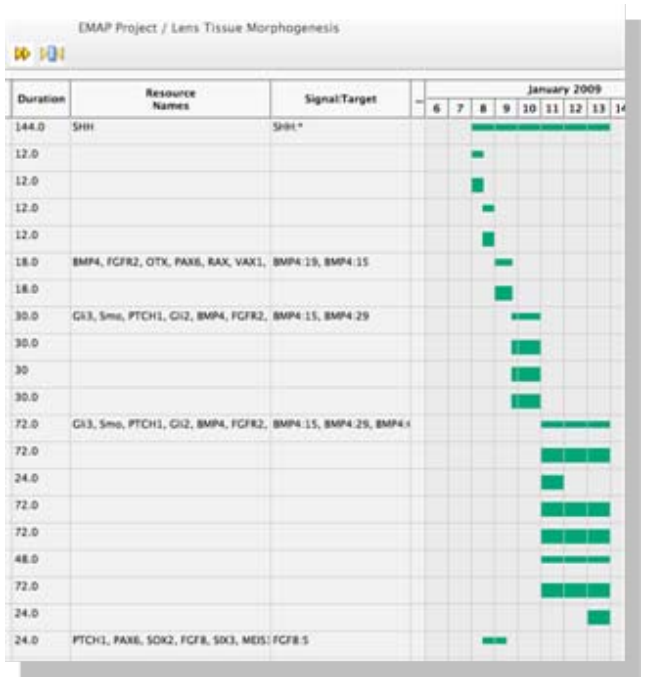
MorphMan: open-source control center for reading & organizing multiple inputs from knowledgebase → CC3D

GanttPV Tissue Project

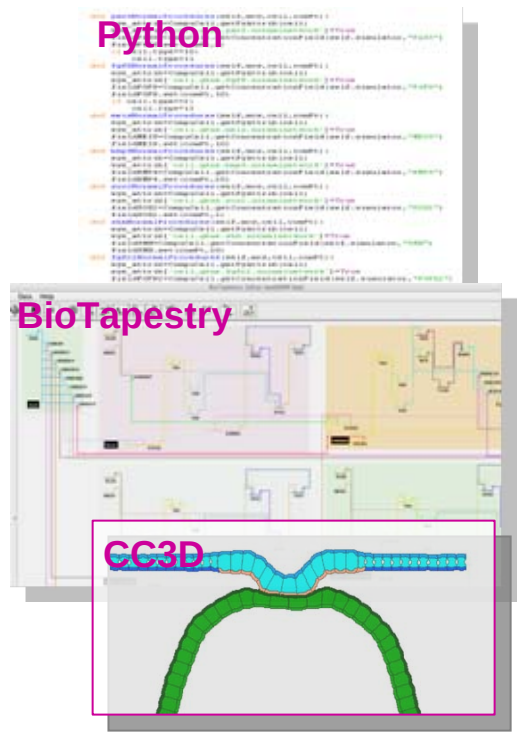


	ID	Parent Task ID	Child Tasks	Tissue	Theiler Stage
1	20		34, 3, 4, 5,	eye	12-21
2	3	20	2	optic sulcus	12
3	2	3		neural ectoderm	12-15.5
4	4	20	71	optic pit	13
5	71	4		neural ectoderm	12-15.5
6	5	20	72	optic vesicle	14-15.5
7	72	5		neural ectoderm	12-15.5
8	24	20	25, 26, 27	optic cup	15.5-17
9	27	24		inner layer	15.5-17
10	26	24		intraretinal space	15.5-17
11	25	24		outer layer	15.5-17
12	37	20	41, 73, 38,	retina	18-21
13	39	37		neural retinal epithelium	18-21
14	73	37		intraretinal space	18-19
15	38	37		pigmented retinal epithelium	18-21
16	41	37		embryonic fissure	18-21

Gantt chart project timeline

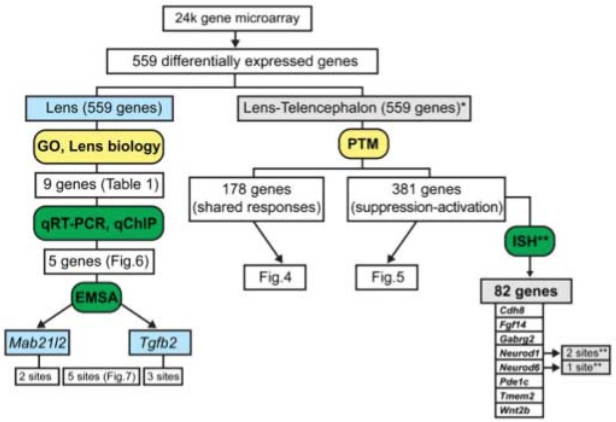


Outputs

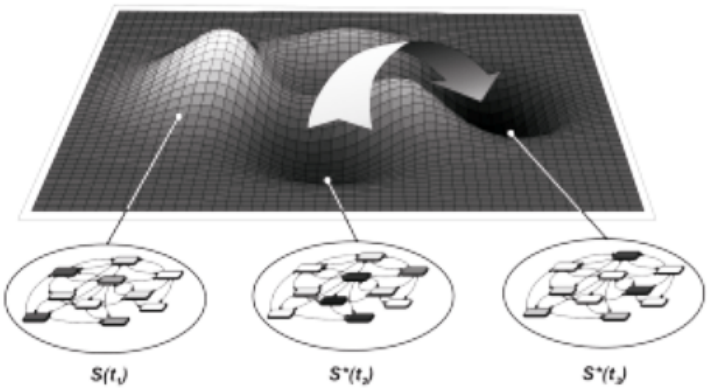


Entering cellular process data

Gene Networks



Network States



Gantt Project Resources

EMAP Project / Lens Molecule Resources

	Symbol	Name	Classification	Activated By	Repressed By	Signal Ligand	Notes
1	SHH	sonic hedgehog	signal ligand				
2	PTCH1	patched homolog	receptor			SHH	
3	Smo	smoothened homolog	signal transducer	PTCH1	PTCH1		
4	Gli2	GLI-Kruppel family member	transcription factor	Smo			
5	Gli3	GLI-Kruppel family member	transscription factor	Smo			
6	BMP4	bone morphogenetic protein	signal ligand	OTX, VAX1			anophthalmia-microphthalmia (AM)
7	BMPR1	bone morphogenetic protein re	receptor			BMP4	
8	Msx2	homeobox, msh-like 2	transcription factor				regulated by BMP
9	FGF8	fibroblast growth facto	signal ligand				
10	FGFR2	fibroblast growth factor recepto	receptor			FGF8	
11	MEIS1	Meis homeobox	transcription factor				
12	OTX	orthodenticle homolog	transcription factor	FGFR2, Gli2	Gli3		microphthalmia, retinal dystrophy
13	PAX6	paired box gene 6	transcription factor	SOX2, Gli2, MEIS1	Gli3		heterozygous human aniridia and Pete
14	PAX2	paired box gene 2	transcription factor				PAX6 spatial mutually exclusive, poste
15	RAX	retina and anterior neural fold h	transcription factor	Gli2	Gli3		
16	Chx10	Vsx2, visual system homeobox	transcriptions factor				
17	SIX3	sine oculis-related homeobox	transcription factor	PAX6			
18	SOX2	SRY-box containing gene	transcription factor	BMPR1, SIX3			BMP4 can replace
19	VAX1	ventral anterior homeobox cont	transcription factor	RAX			ventral gradient bias with Tbx5
20	Tbx5	T-box 5	transcription factor				dorsal gradient bias vith VAX1
21	Mab21l1	mab-21-like 1	eye development	PAX6			regulated by PAX6
22	Tgfb2	transforming growth factor, bet	cell soma	PAX6			regulated by PAX6

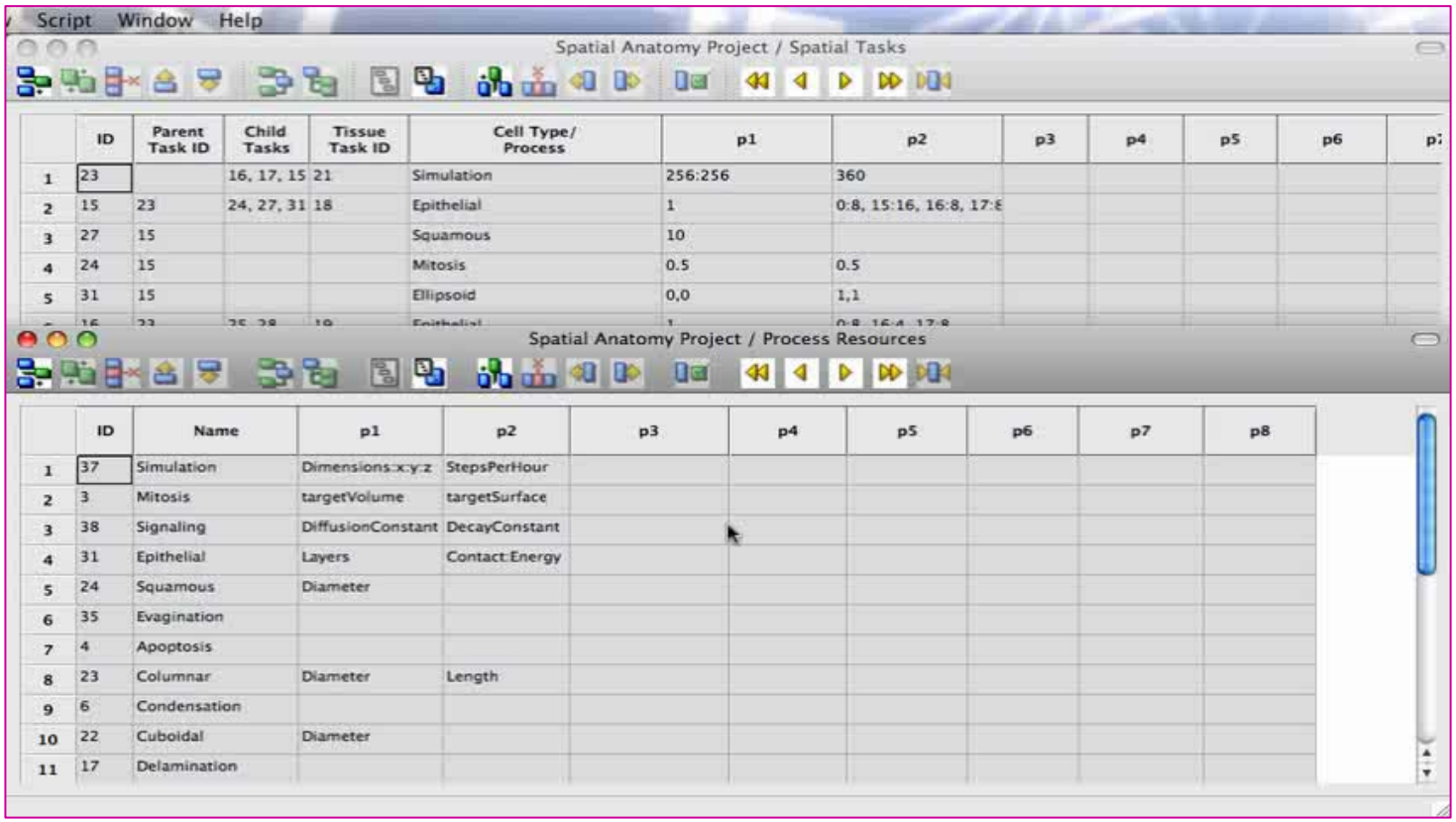
Can add more columns
(e.g., chemically-perturbed system)

Wolf LV, Yang Y, Wang J, Xie Q, Braunger B, et al. (2009) Identification of Pax6-Dependent Gene Regulatory Networks in the Mouse Lens. PLoS ONE 4(1): e4159.

Huang, S, Ingber, DE. (2006) A Non-Genetic Basis for Cancer Progression and Metastasis: Self-Organizing Attractors in Cell Regulatory Networks. *Breast Disease* 26: 27–54

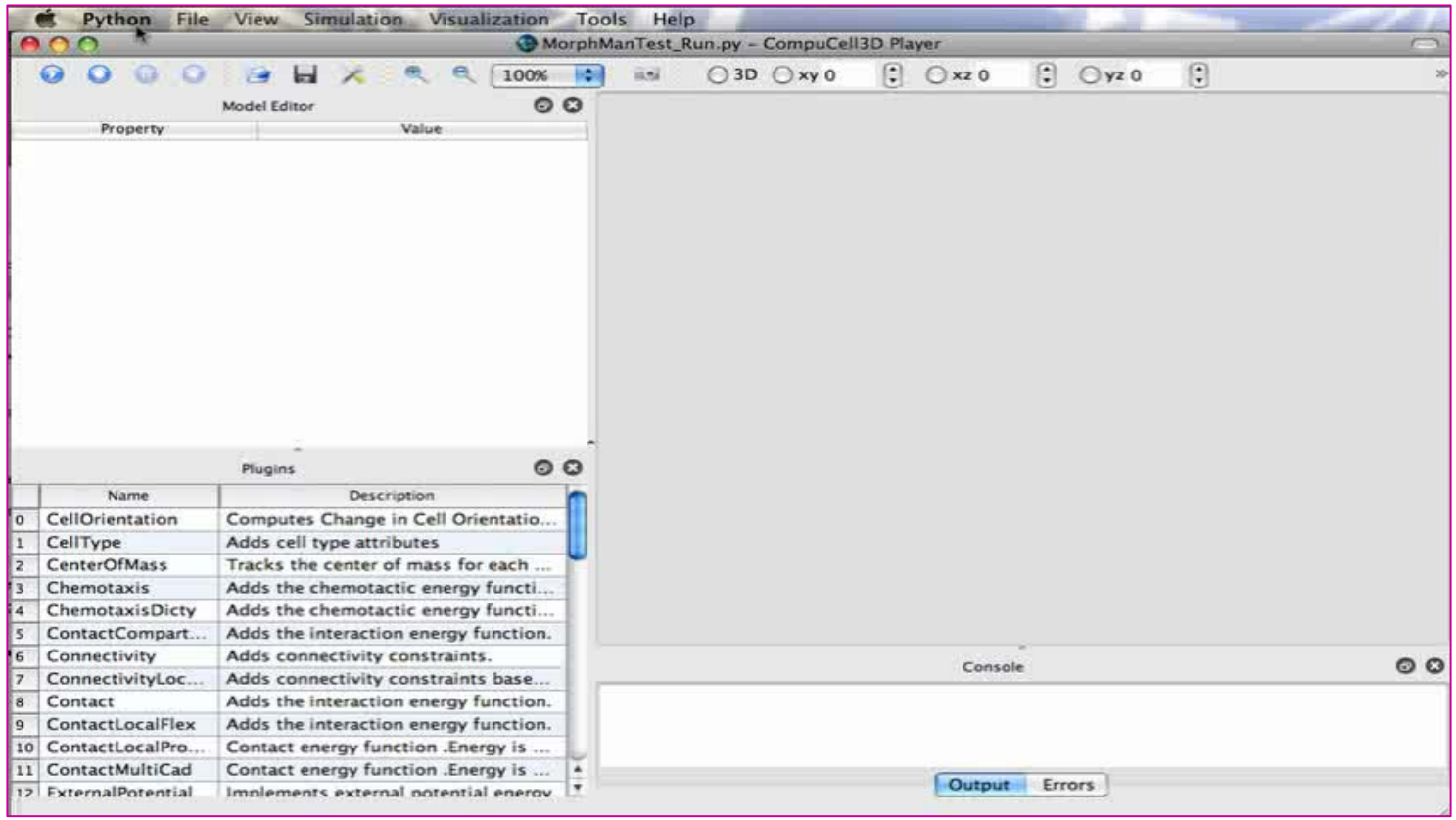
Output example

sample Gantt spatial setup exported to CompuCell3D …



Output example

. . . then Gantt exports are imported into CompuCell3D and run



Self-regulating cellular systems

"…it's alive…"

Anatomical homeostasis in a 'virtual embryo' modeled with Endogenics software

SOURCE: R Newman and T Otter Crowley Davis Research, Inc. (http://www.cdres.com/)

Our Research Network

Virtual Embryo (NCCT)
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Holly Mortensen

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