United States Environmental Protection Agency

Incorporating 'omics in the study of reproduction and development:

Virtual Tissue Models in Developmental Toxicity Research

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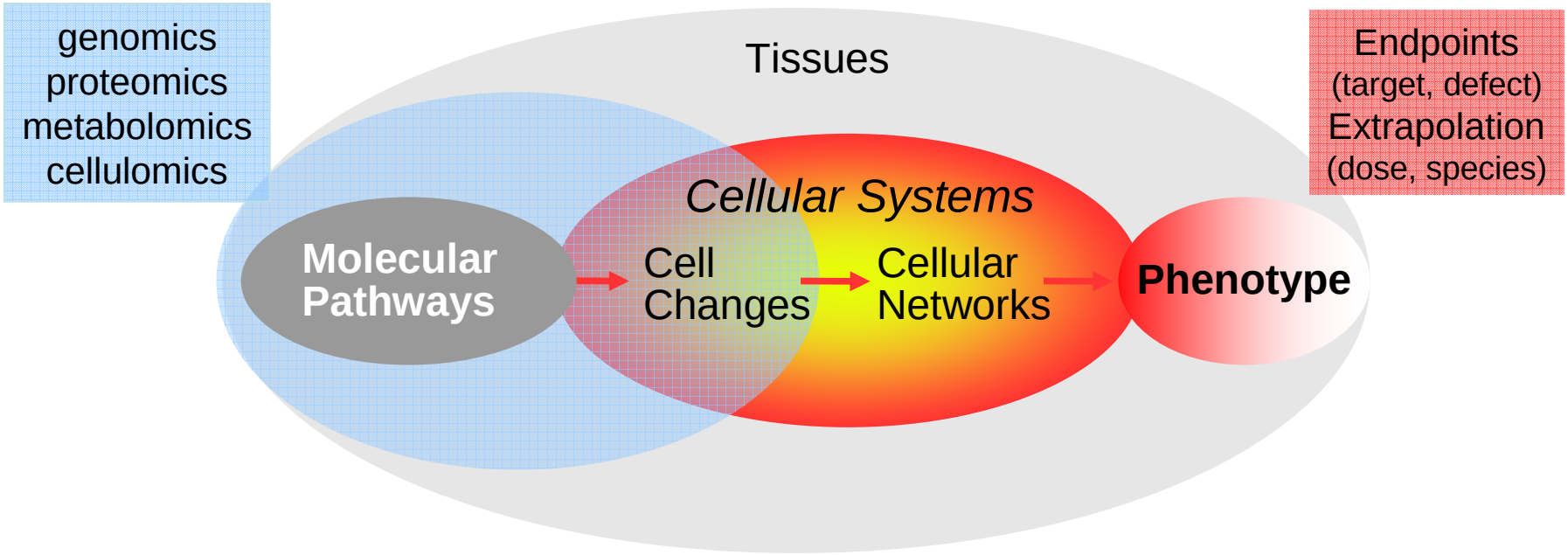
Office of Research and Development
National Center for Computational Toxicology

Toxicity: a *cross-scale* phenomenon



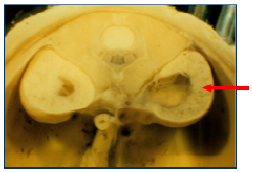
PARADIGM: cell changes are causally linked to perturbation of molecular pathways; tissue lesions are then propagated by dynamic cellular networks

CHALLENGE: computational models that integrate detailed information captured at different biological scales to predict key events leading to adverse outcomes

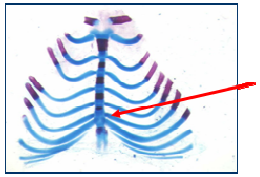
Profiling Developmental Activity

Toxicity Reference Database (ToxRefDB)

ToxRefDB holds source data captured from comprehensive *in vivo* studies
www.epa.gov/ncct/toxrefdb



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

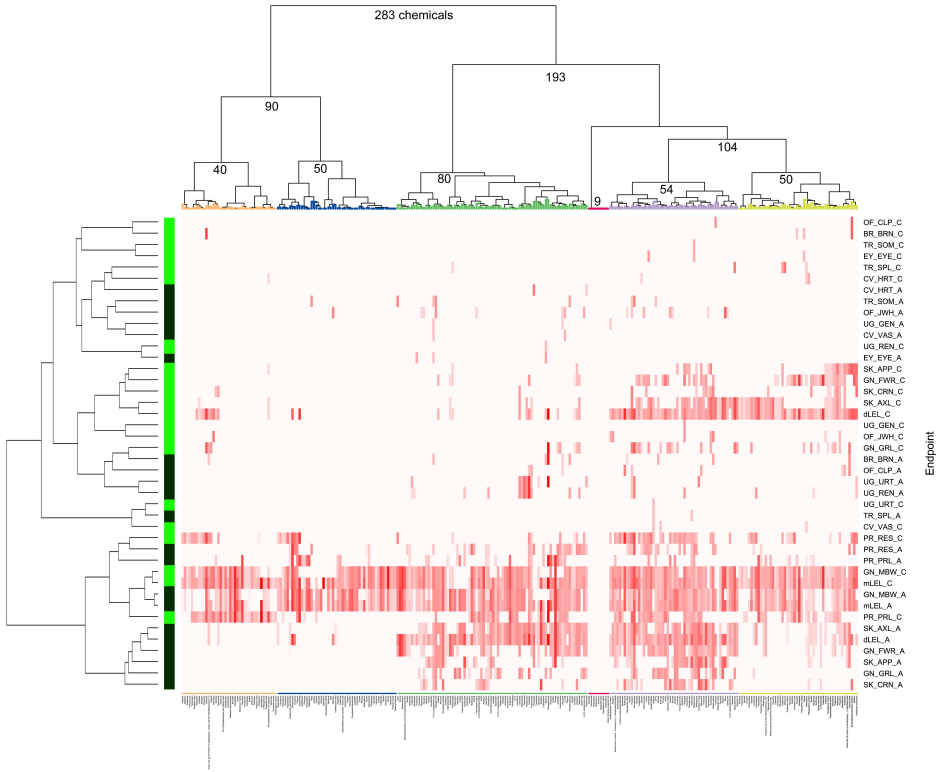


target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130

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

images from www.DevTox.org

387 chemicals, 988 annotated effects
www.DevTox.org, 751 prenatal studies



283 chemicals x 293 effects → 19 endpoint targets culled from rat and rabbit studies

SOURCE: Knudsen et al., submitted

Challenge: understanding the embryo as a complex dynamic system

- Input: ToxCast™ chemical library reveals a complex linkage between molecular target and fetal endpoint

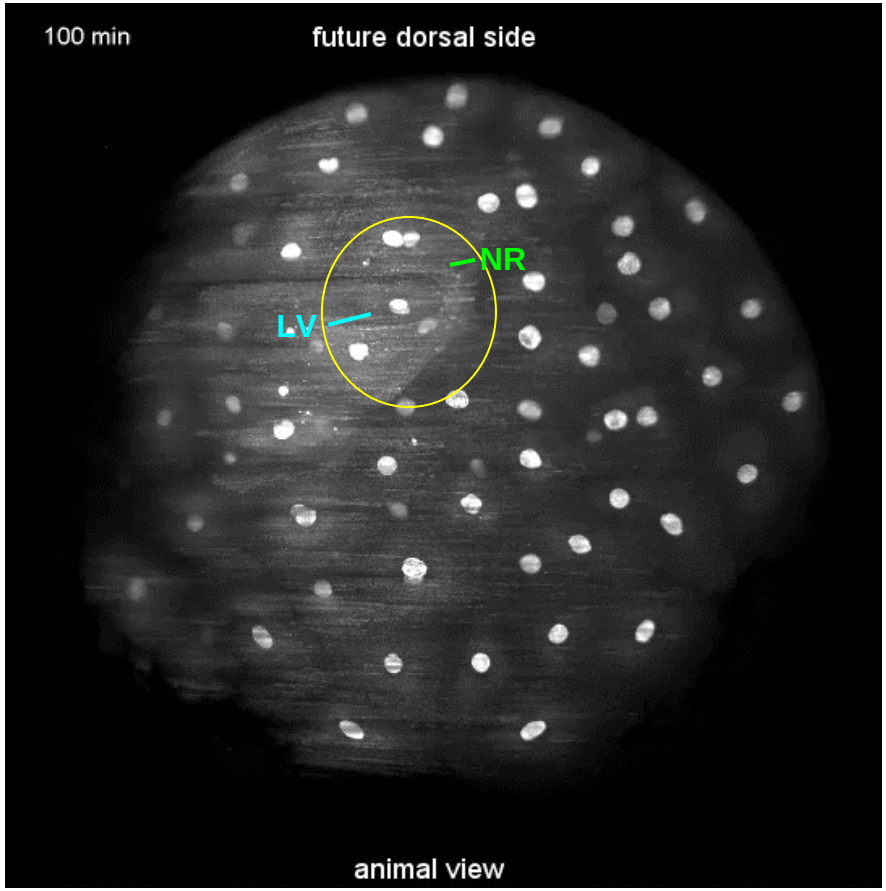
  1. which signaling networks and morphogenetic processes characterize sensitive systems at susceptible stages?

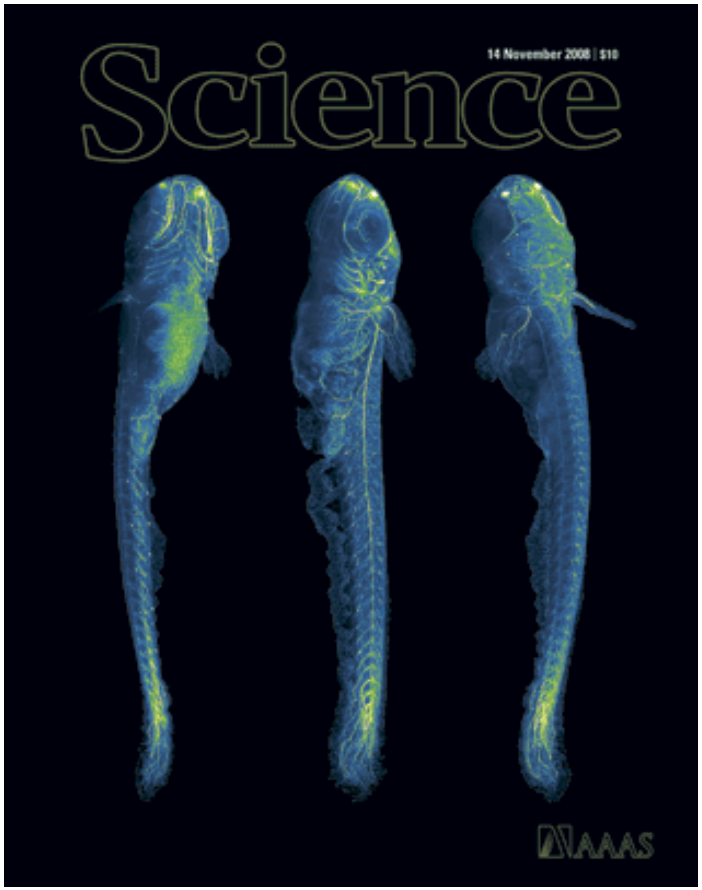
  2. what computational tools can we use to navigate the complexity of state changes in a dynamical system?

  3. can computational models be built to predict dysmorphogenesis across chemical class, dose, species, stage, and genetic makeup?

- Output: modular reconstruction of a developing tissue using computer models that execute rules in morphogenesis

Digital embryo: ZFET morphogenesis

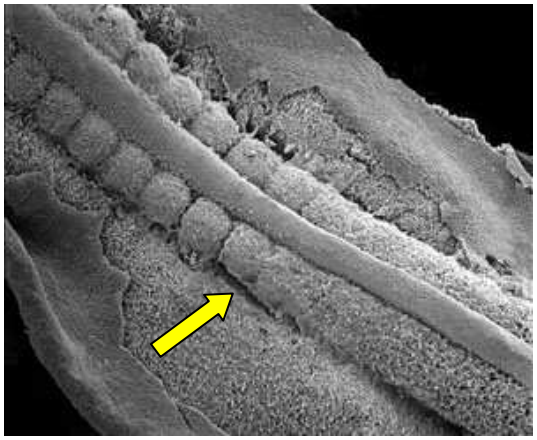


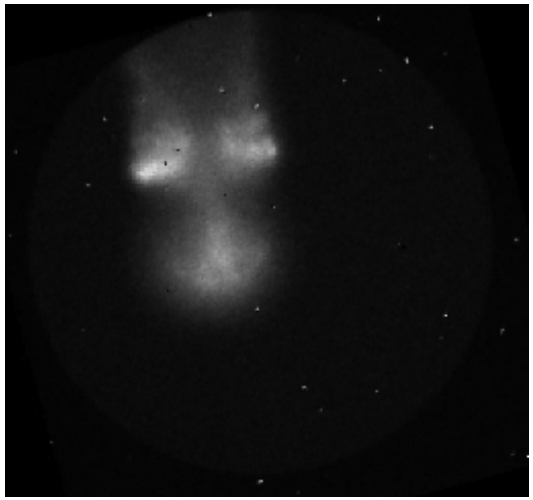


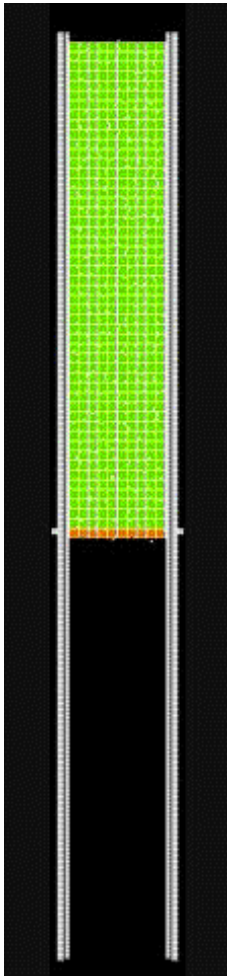
Reconstruction of zebrafish early embryonic development tracking H2B-EGFP by DSLM at 90s intervals over 18h

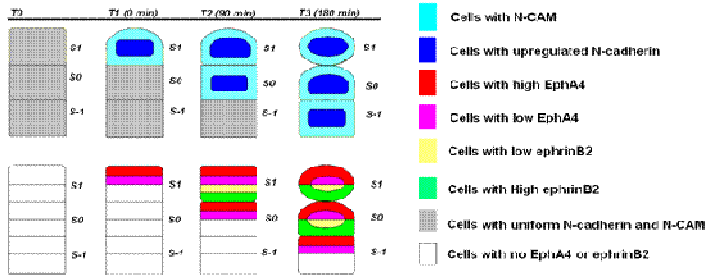
Source: Keller et al. (2008) Science 322: 1065-1069

Cell-based models (CC3D):
vertebrate segmentation clock

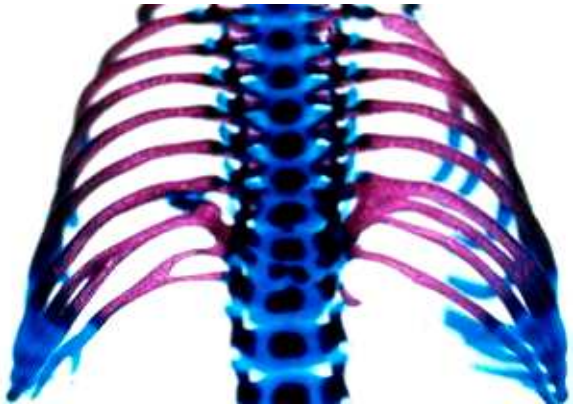








In silico model, CompuCell3D software
SOURCE: Jim Glazier, Indiana University



Prenatal exposure, boric acid
SOURCE: John Rogers, RTD/EPA

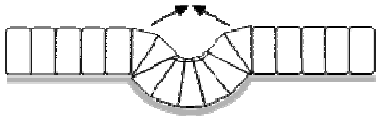
Hes1-EGFP time-lapse (3h) clock-wavefront
SOURCE: Masamizu et al. (2006) PNAS USA 103:1313-18

Morphogenesis toolbox

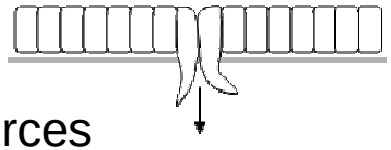
Core developmental processes
- patterning (sets up future events)
- timing (clocks and oscillators)
- differentiation (cell diversification)
- morphogenesis (tissue organization)

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

Morphogenetic movement
- folding
- epiboly



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



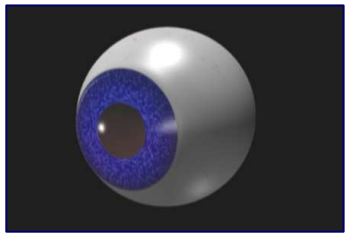
- tractional forces

Directed cell movement
- contact guidance (boundaries)
- haptotaxis (ECM tracks)
- chemotaxis (chemical signals)

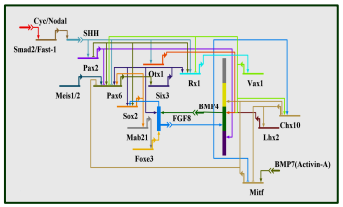
Source: Bard (2005) J Anat 206: 1 - 16

EPA's Virtual Embryo

1. abstraction: represent the morphogenetic blueprint of the target system

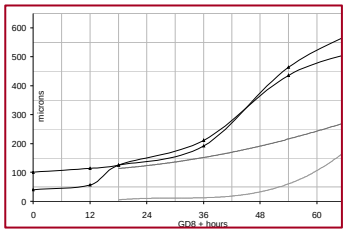


2. system wiring: build signaling networks for core developmental processes



3. cell-based rules: computer program (CC3D) implementing cellular primitives

4. computational model: execute developmental trajectories *in silico*

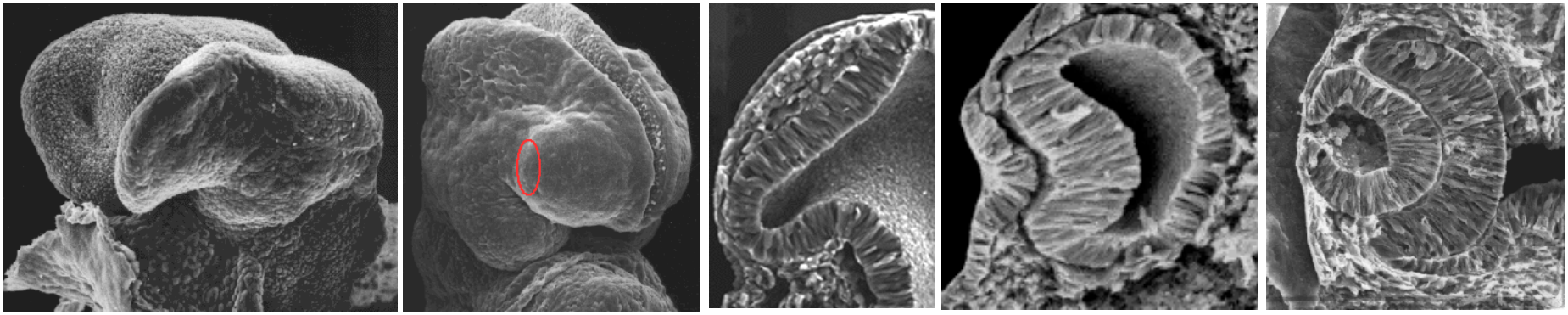


Early eye development

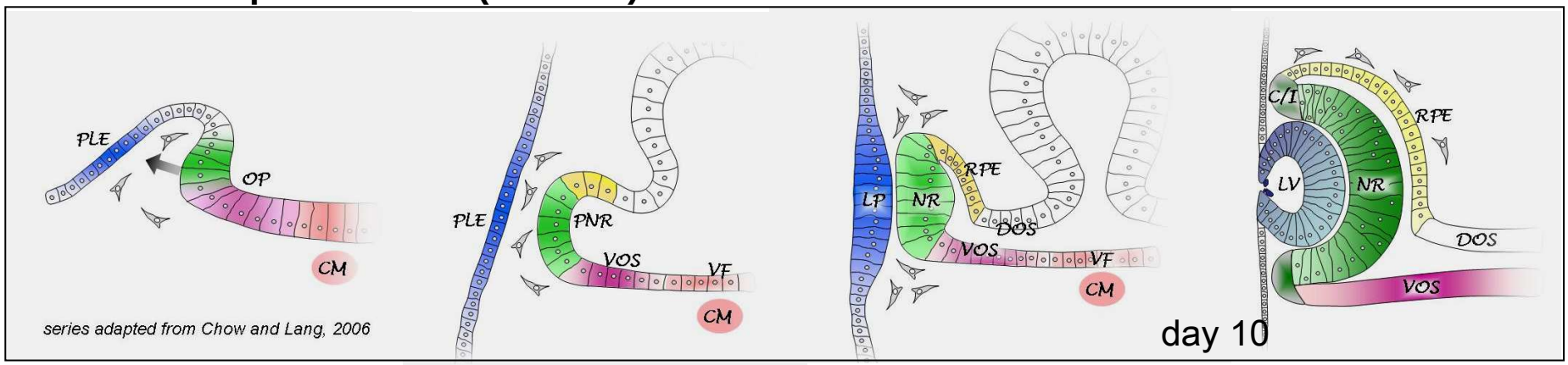
~days 8-11 mouse = ~20-37 days human gestation

1

SEM series *K Sulik*, http://www.med.unc.edu/embryo_images/unit-eye/eye_htms/eyetoc.htm

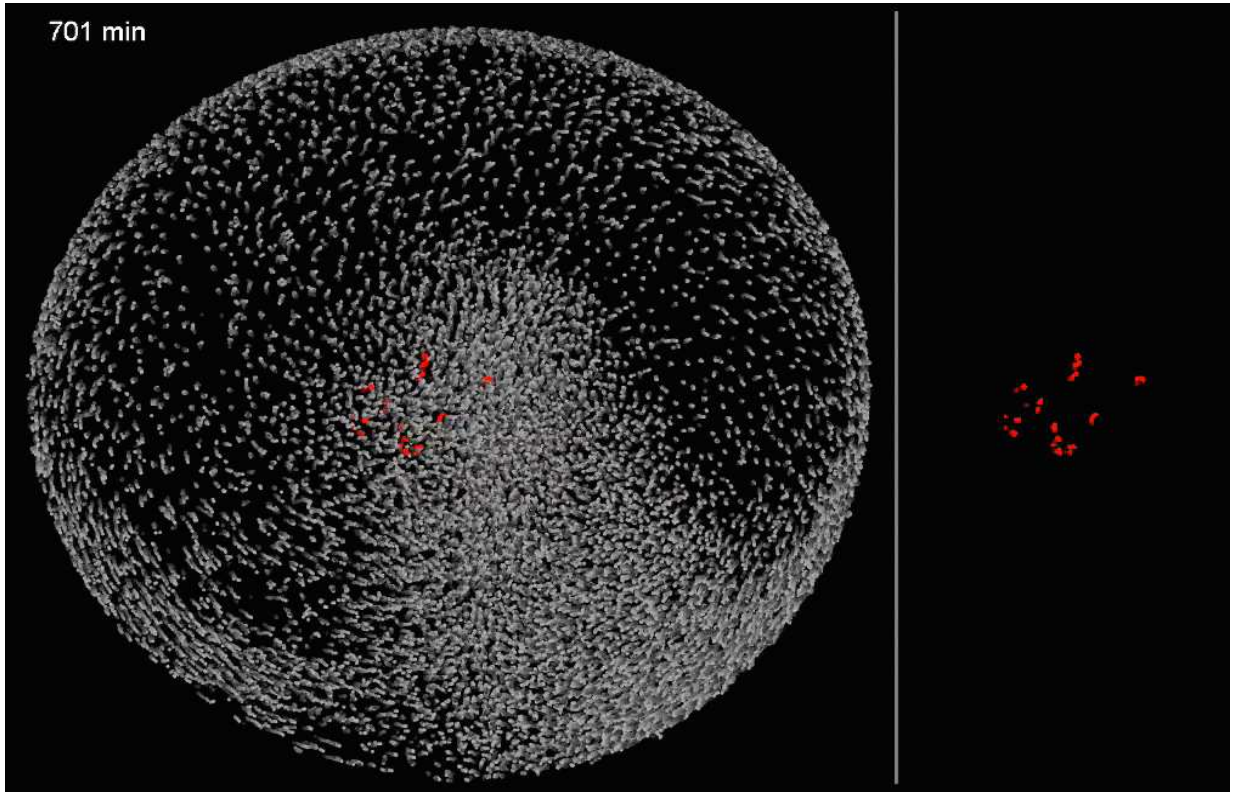


Anatomical representation (TS12-18)



A real morphogenetic blueprint

1

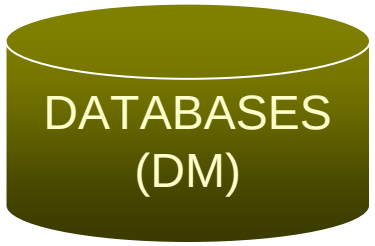


reverse-engineering the cellular dynamics of optic vesicle formation in the zebrafish embryo using DSLM

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

Building a signaling network

2



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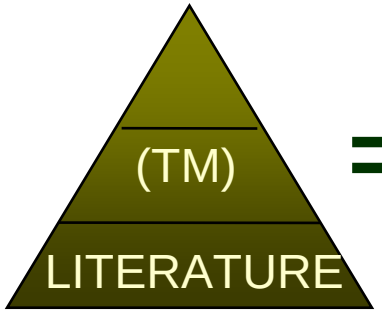


=

- summarization
- classification
- clustering
- ontologies
- concepts
- correlations
- causality

- structured
- computable
- easier to curate
- QA/QC
- semantics/metadata
- accurate but laborious

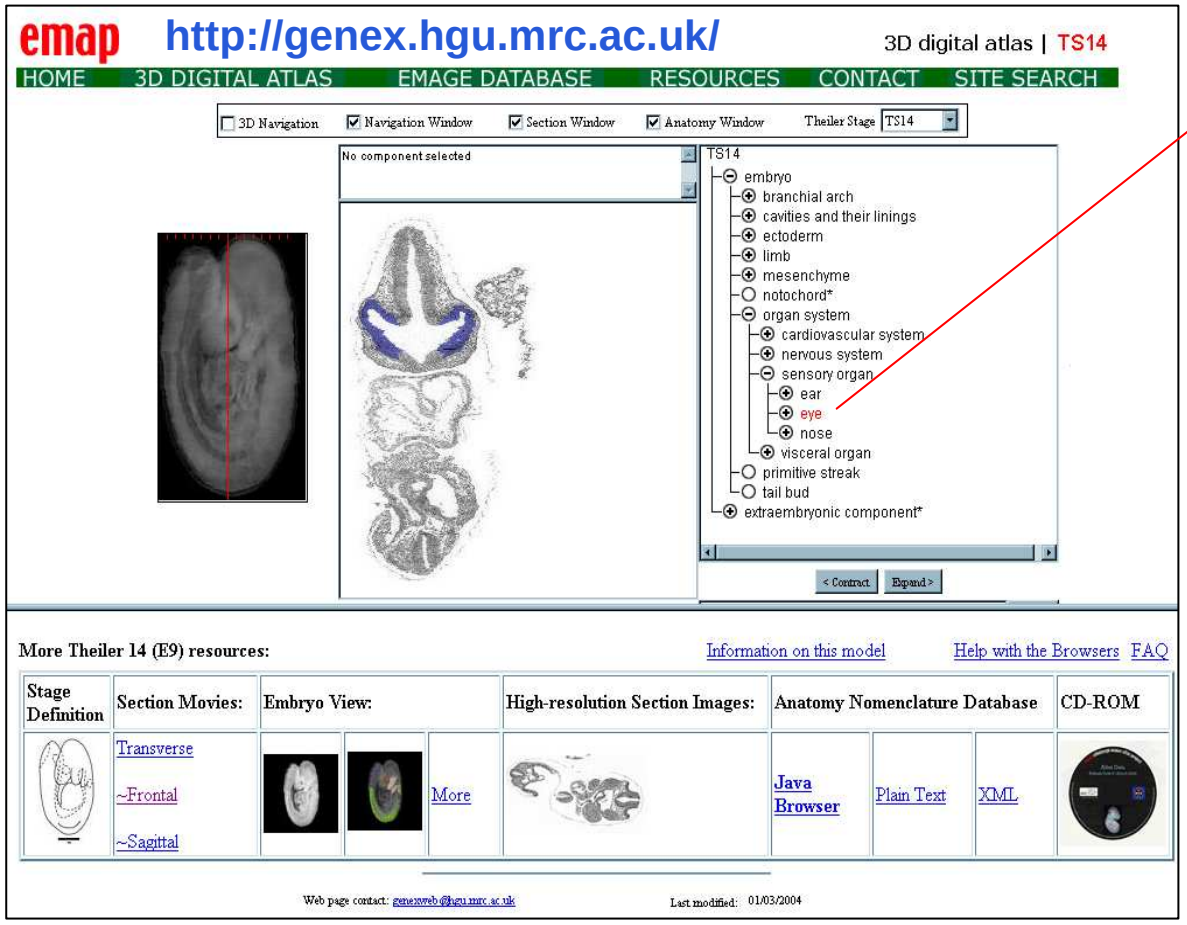
- unstructured
- algorithms needed to put in computable form
- difficult to manage and control
- noisy (filtering becomes essential)
- ambiguous - ***cricket the game or cricket the bug?***
- coarse but fast

SOURCE: Amar Singh, LHM

Gene expression databases:

EMAGE: Edinburgh Mouse Atlas Gene Expression
MPO: Jax Mouse Phenotype Ontology Browser

2

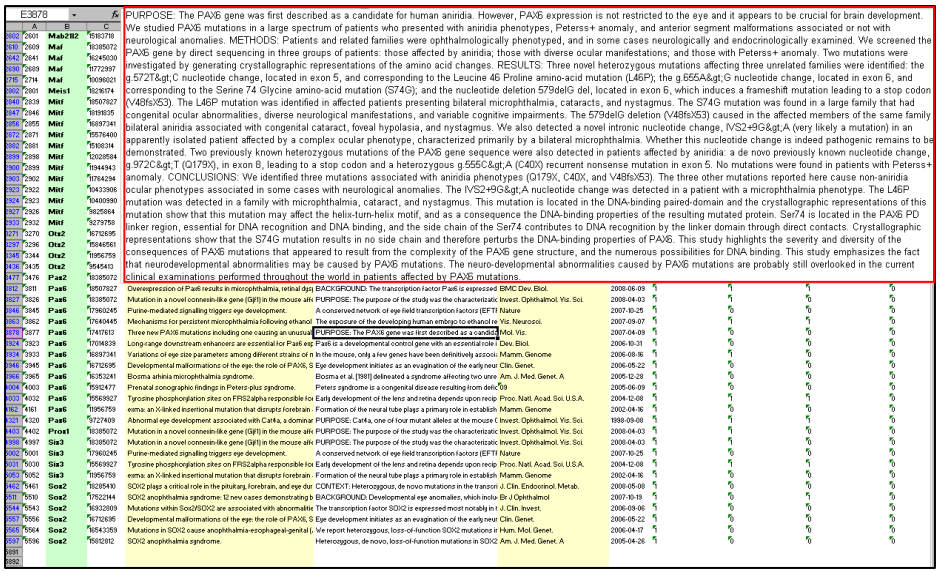


63 genes (TS12-18)

Sl.No.	Gene 1	Gene 2	Search 1	Search 2	PMID	Web link	Title	Authors	Read	Relevant	New Informati
1	cyc	shh	fetal or embryo	mouse or mic	12783802	http://www.nc	A temperature-sensitive	Tian J., Yam	Y	Y	N
2	cyc	shh	fetal or embryo	mouse or mic	10952887	http://www.nc	Direct action of the noc	Müller F., Albe	Y	Y	
3	cyc	shh	fetal or embryo	mouse or mic	10694427	http://www.nc	Two distinct cell popul	Odenthal J., V	Y	N	N
4	cyc	shh	fetal or embryo	mouse or mic	8946241	http://www.nc	Expression of axial and	Strähle U., Bl	Y	N	N
5	cyc	shh	fetal or embryo	mouse or mic	8674416	http://www.nc	Complex expression of	Heuptmann G	Y	N	N
6	sfh	pax2	fetal or embryo	mouse or mic	11731484	http://www.nc	Vhnf1, the MODY5 and	Sun Z., Hopki	Y	N	N
7	sfh	pax2	fetal or embryo	mouse or mic	10603036	http://www.nc	Vax1, a novel homeobo	Hallonet M., H	Y	Y	Y
8	sfh	pax2	fetal or embryo	mouse or mic	9655820	http://www.nc	Sonic hedgehog is not	Schauerte H.,	Y	N	N
9	sfh	otx1	fetal or embryo	mouse or mic	15105370	http://www.nc	Otx2 regulates the extr	Puelles E., An	Y	N	N
10	Pax2	pax6	fetal or embryo	mouse or mic	17470285	http://www.nc	Rybp, a polycomb com	Pirity M., Wan	Y	YN (develop	Y
11	Pax2	pax6	fetal or embryo	mouse or mic	11716916	http://www.nc	Lmx1b is essential for	Guo C., Qiu H	Y	N	N
12	Pax2	pax6	fetal or embryo	mouse or mic	16934893	http://www.nc	A role for GnRH in eat	Wu S., Page	Y	Y	Y
13	Pax2	pax6	fetal or embryo	mouse or mic	14713874	http://www.nc	Prep1.1 has essential	Deflorian G.,	Y	D?	Y
14	Pax2	pax6	fetal or embryo	mouse or mic	12970747	http://www.nc	Paired-like homeobox	Muratovska A	Y	N	N
15	Pax2	pax6	fetal or embryo	mouse or mic	12758174	http://www.nc	Retinal pigmented epit	Baumer N., M	Y	Y	Y
16	Pax2	pax6	fetal or embryo	mouse or mic	11804780	http://www.nc	Dach1, a vertebrate ho	Heanue T., Da	Y	YN (Day13	N
17	Pax2	pax6	fetal or embryo	mouse or mic	11468894	http://www.nc	Six3 promotes the forn	Lagutin O. V.,	Y	Y	N
18	Pax2	pax6	fetal or embryo	mouse or mic	11003833	http://www.nc	Spatial specification of	Schwarz M., D	Y	Y	N
19	Pax2	pax6	fetal or embryo	mouse or mic	10603036	http://www.nc	Vax1, a novel homeobo	Hallonet M., H	Y	Y	Y
20	Pax2	pax6	fetal or embryo	mouse or mic	10051661	http://www.nc	Ectopic bone morphog	Golden J., Bri	Y	N	N
21	Pax2	pax6	fetal or embryo	mouse or mic	9831649	http://www.nc	Zebrafish contains two	Nornes S., Ch	Y	Y	Y
22	Pax2	pax6	fetal or embryo	mouse or mic	9655820	http://www.nc	Sonic hedgehog is not	Schauerte H.,	Y	N	N
23	Pax2	pax6	fetal or embryo	mouse or mic	9297966	http://www.nc	Pax genes and organog	Dahl E., Kose	Y	Y	N
24	Pax2	pax6	fetal or embryo	mouse or mic	8126546	http://www.nc	Roles of Pax-genes in	Stoykova A., G	Y	N	N
25	pax6	lx1	fetal or embryo	mouse or mic	12142224	http://www.nc	The homeobox gene m	Kawahara A.,	Y	Y	Y (Dld not und
26	pax6	sox2	fetal or embryo	mouse or mic	17820048	http://www.nc	PAX6 and SOX2-depe	Inoue M., Kan	Y		
27	pax6	sox2	fetal or embryo	mouse or mic	17470285	http://www.nc	Rybp, a polycomb com	Pirity M., Wan	Y	YN	Y
28	pax6	sox2	fetal or embryo	mouse or mic	17060077	http://www.nc	Six3 activation of Pax6	Liu W., Lagut	Y	Y	Y
29	pax6	sox2	fetal or embryo	mouse or mic	15924696	http://www.nc	Understanding lens di	Uchikawa M.,	Y		
30	pax6	sox2	fetal or embryo	mouse or mic	15855760	http://www.nc	The involvement of ne	Reza H., Yasu	Y	Y	
31	pax6	sox2	fetal or embryo	mouse or mic	15652703	http://www.nc	Requirement of mesod	Molotkova N.,	Y	N	N
32	pax6	sox2	fetal or embryo	mouse or mic	15659874	http://www.nc	Interplay of Pax6 and S	Kondoh H., Uc	Y	YN*	YN*
33	pax6	sox2	fetal or embryo	mouse or mic	12710953	http://www.nc	Pax6 autoregulation in	Aota S., Nakaz	Y	Y	Y
34	pax6	sox2	fetal or embryo	mouse or mic	12642482	http://www.nc	Cell-autonomous involv	Yamada R., M	Y	N*	Y*
35	pax6	sox2	fetal or embryo	mouse or mic	11069887	http://www.nc	Pax6 activity in the lens	Ashery-Padan	Y	N*	N
36	pax6	sox2	fetal or embryo	mouse or mic	9851982	http://www.nc	BMP4 is essential for l	Furuta Y., Hog	Y	Y*	Y*
37	pax6	sox2	fetal or embryo	mouse or mic	9809885	http://www.nc	Involvement of Sox1, 2	Kamachi Y., Uc	Y	Y	N
38	pax6	six3	fetal or embryo	mouse or mic	17470285	http://www.nc	Rybp, a polycomb com	Pirity M., Wan	Y	YN (develop	Y
39	pax6	six3	fetal or embryo	mouse or mic	17060077	http://www.nc	Six3 activation of Pax6	Liu W., Lagut	Y	Y	Y
40	pax6	six3	fetal or embryo	mouse or mic	16765103	http://www.nc	Identification of genes	Lord-Grignon	Y	Y*	Y*
41	pax6	six3	fetal or embryo	mouse or mic	15569927	http://www.nc	Tyrosine phosphorylati	Gotoh N., Ito	Y		
42	pax6	six3	fetal or embryo	mouse or mic	15366004	http://www.nc	Rald2 expression in o	Mic F., Molotk	Y		
43	pax6	six3	fetal or embryo	mouse or mic	12642482	http://www.nc	Cell-autonomous involv	Yamada R., M	Y	N	N
44	pax6	six3	fetal or embryo	mouse or mic	11956759	http://www.nc	eyes- an X-linked inse	Cunningham	Y	N	N
45	pax6	six3	fetal or embryo	mouse or mic	11468894	http://www.nc	Six3 promotes the forn	Lagutin O. V.,	Y	Y	N
46	pax6	six3	fetal or embryo	mouse or mic	10502109	http://www.nc	Mouse Dach, a homolo	Davis R., She	Y	Y*	YN*
47	pax6	six3	fetal or embryo	mouse or mic	10090721	http://www.nc	Six3 overexpression in	Loosli F., Win	Y	Y	Y
48	pax6	six3	fetal or embryo	mouse or mic	9651515	http://www.nc	Six3, a medaka homol	Loosli F., Kös	Y	Y	Y
49	sox2	foxe3	fetal or embryo	mouse or mic	12642482	http://www.nc	Cell-autonomous involv	Yamada R., M	Y	N	N
50	vax1	chx10	fetal or embryo	mouse or mic	17470285	http://www.nc	Rybp, a polycomb com	Pirity M., Wan	Y	YN (develop	Y
51	chx10	lhx2	fetal or embryo	mouse or mic	15385160	http://www.nc	Requirement for Mab2	Yamada R., M	Y	N	N
52	chx10	mif	fetal or embryo	mouse or mic	16420417	http://www.nc	Loss of retinal progeni	Coles B., Hor	Y	N	N
53	chx10	mif	fetal or embryo	mouse or mic	15459106	http://www.nc	Transdifferentiation of	Rowan S., Chi	Y	N	N
54	chx10	mif	fetal or embryo	mouse or mic	11023863	http://www.nc	Extraocular mesenchy	Fuhrmann S.,	Y	Y	Y
55	chx10	mif	fetal or embryo	mouse or mic	10903182	http://www.nc	Signaling and transcrip	Nguyen M., A	Y	Y	Y
56	Pax6	Meis	fetal or embryo	mouse or mic	12183364	http://www.nc	Meis homeoproteins di	Zhang X., Frie	Y	Y	Y

Literature-mining: establishing relevant gene-gene and gene-phenotype associations

2



TM PubMed returned 5,889 records for these genes in development across 4 species (rat, mouse, zebrafish, human)

Computing relevance scores for association of gene-malformation in the eye based on co-occurrence of DevTox terms in the abstracts

EMAGE	DevTox	Pa	Pb	Pab	Sscore	Rscore
Wnt2b	Microphthalmia	38	365	1	3.065753	0.486537
Vax1	Ocular coloboma	11	120	3	96.64091	1.985161
Tbx3	Microphthalmia	47	365	1	2.478694	0.394223
Tbx2	Microphthalmia	55	365	1	2.118157	0.325958
Sox2	Anophthalmia	221	193	3	2.990786	0.475785
Sox2	Ocular coloboma	221	120	1	1.603394	0.20504
Sox2	Small lens	221	103	1	1.868031	0.271384
Six6	Anophthalmia	23	193	2	19.15837	1.282359
Six6	Microphthalmia	23	365	1	5.065158	0.704593
Six6	Retina fold	23	88	1	21.00889	1.322403
Six3	Anophthalmia	94	193	4	9.375372	0.971989
Six3	Microphthalmia	94	365	5	6.196736	0.792163
Six3	Ocular coloboma	94	120	1	3.769681	0.576305
Six3	Open eye	94	153	1	2.956612	0.470794
Six3	Retina fold	94	88	1	5.140474	0.711003
Six3	Small lens	94	103	1	4.391861	0.642649
Si	Small lens	444	103	1	0.929808	-0.03161
Rax	Anophthalmia	15	193	1	14.68808	1.166965
Rax	Ocular coloboma	15	120	1	23.62333	1.373341
Rax	Retina fold	15	88	1	32.21364	1.50804
Pax6	Anophthalmia	587	193	2	0.750669	-0.12455
Pax6	Microphthalmia	587	365	11	2.183109	0.339075
Pax6	Ocular coloboma	587	120	5	3.018313	0.479764
Pax6	Open eye	587	153	2	0.946922	-0.02369
Pax6	Small lens	587	103	10	7.032963	0.847138
Pax2	Aphakia	335	29	1	4.376943	0.641171
Pax2	Microphthalmia	335	365	1	0.347757	-0.45872
Pax2	Ocular coloboma	335	120	16	16.92418	1.228508
Otx2	Anophthalmia	239	193	1	0.921846	-0.03534
Otx2	Microphthalmia	239	365	2	0.974884	-0.01105
Otx2	Open eye	239	153	1	1.162852	0.065524
Msx2	Microphthalmia	200	365	1	0.582493	-0.23471
Mitf	Microphthalmia	100	365	42	48.92942	1.68957
Mitf	Retina fold	100	88	1	4.832045	0.684131
Meis1	Microphthalmia	42	365	1	2.773777	0.443072
Bmp4	Anophthalmia	518	193	1	0.425331	-0.37127
Bmp4	Microphthalmia	518	365	2	0.449802	-0.34698

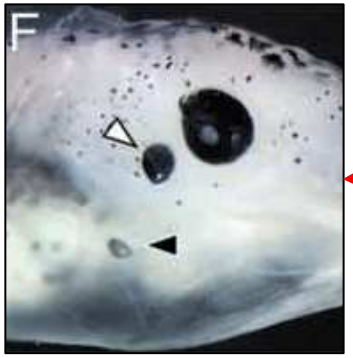
Pax6: master selector of the eye

2

ancestral

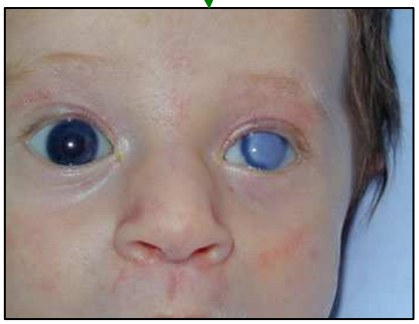
Pax6

teratogenesis



ectopic

mutation

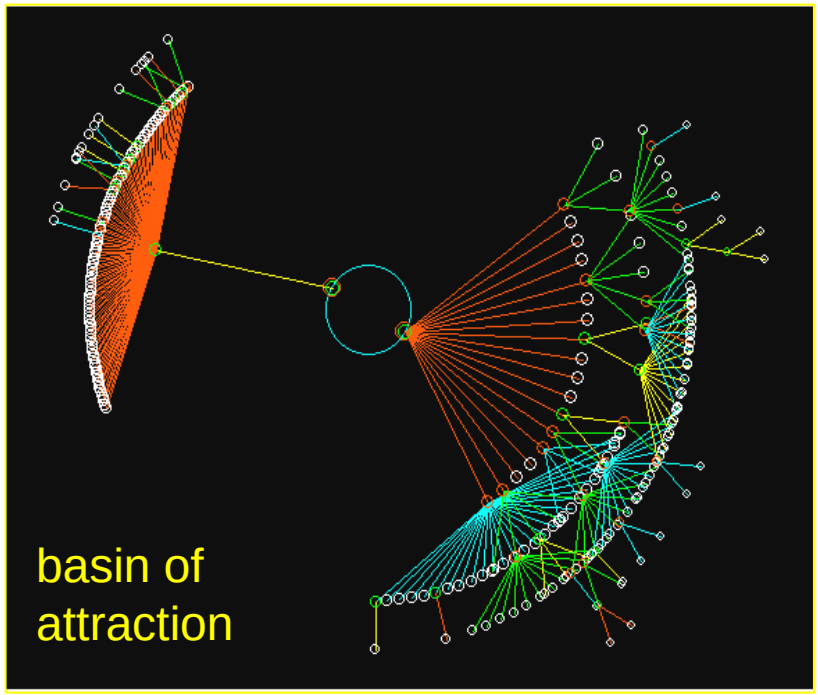


unregulated

deficient

Discrete Dynamical Network (DDN)

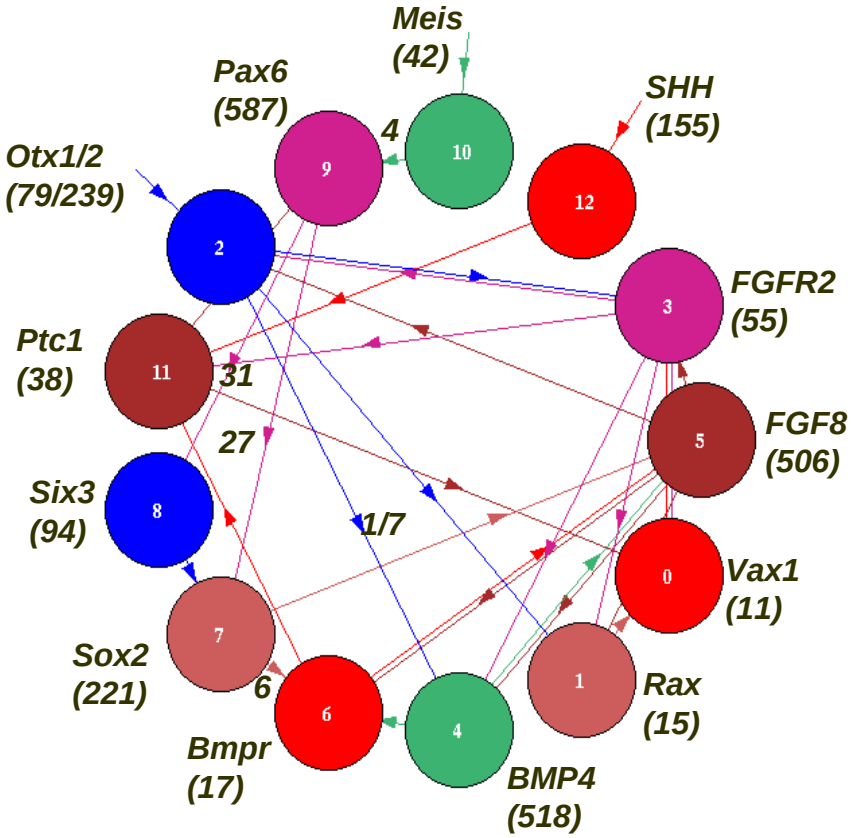
2

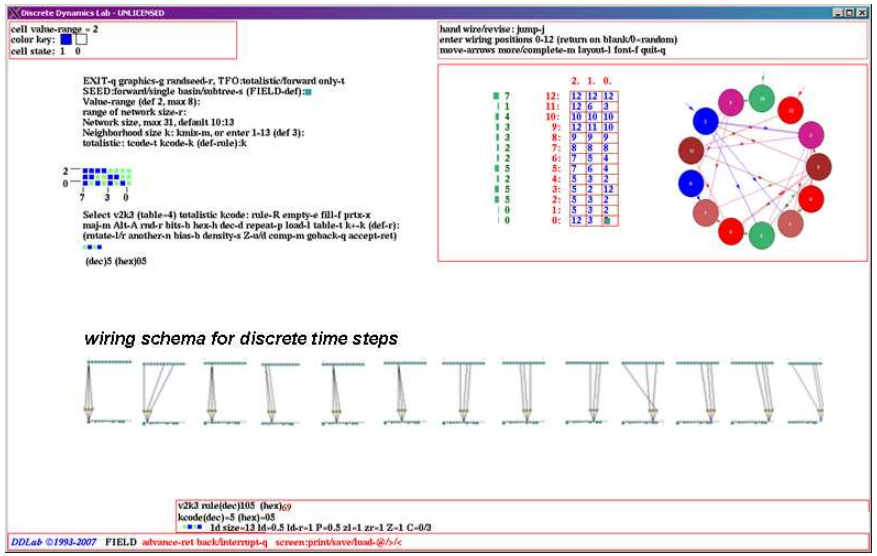


DDNs: ‘state machines’

- analysis of state trajectories
- follow effects of chemical perturbation
- run network forward to find attractor states
- run backwards to disclose historical paths

System wiring diagram



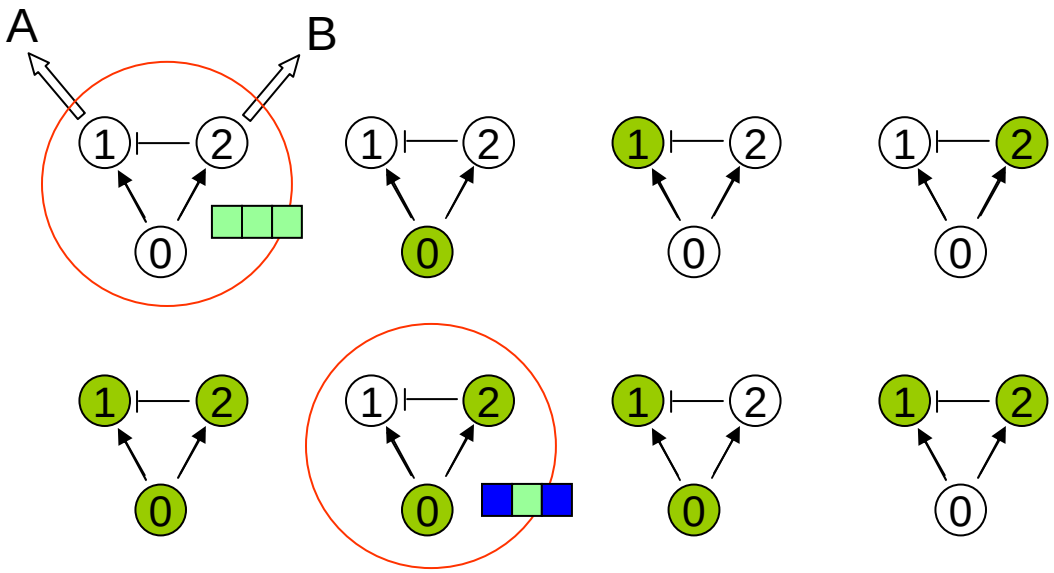


Software: A Wuensche, http://www.ddlab.com/

7 transcription factors
3 receptor systems
3 signal ligands

network size (n) = 13 nodes
network connectivity (k) = 3
Boolean states (2^n) = 8192

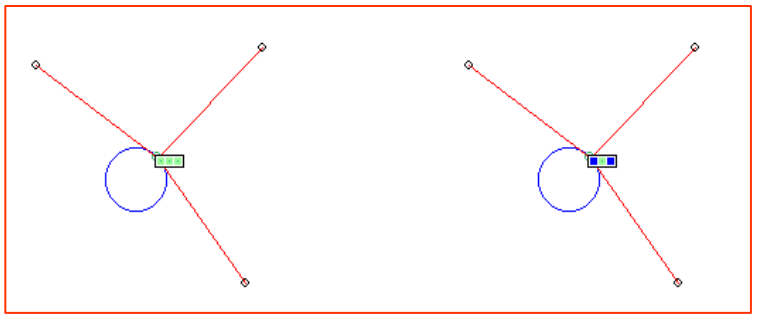
Simple RBN state dynamics



topology
n=3
k=2
2^3 states

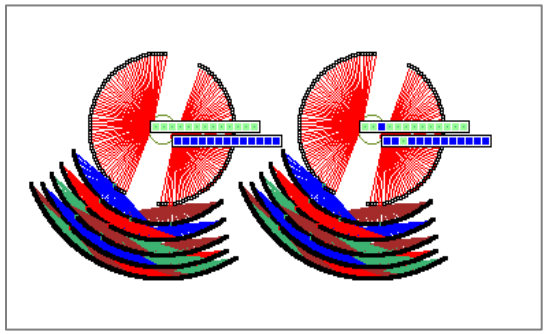
rules
0→1
0→2
2 ┤ 1
2 > 0

stable patterns (attractors)

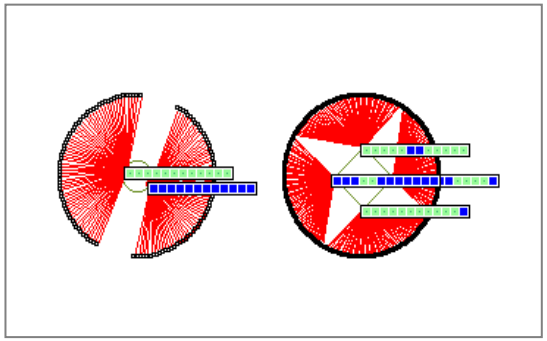


Attractor field (n=3, k=2)
modeled with DDLab v2k2 rule(dec)14

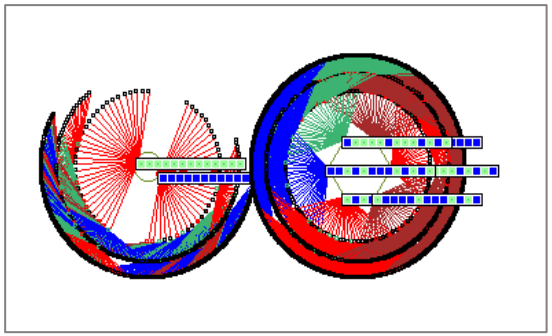
State attractor field of some more complex networks



ordered dynamics
on or ***off*** configurations
(**STATE A**, **STATE B**)



chaotic dynamics
loss of Pax6 function

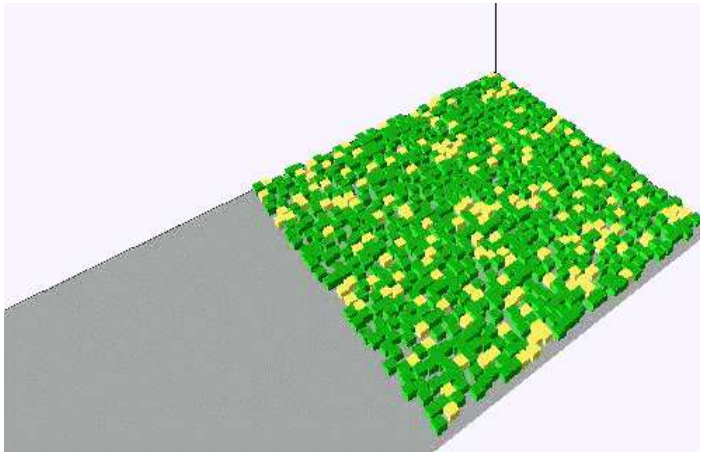


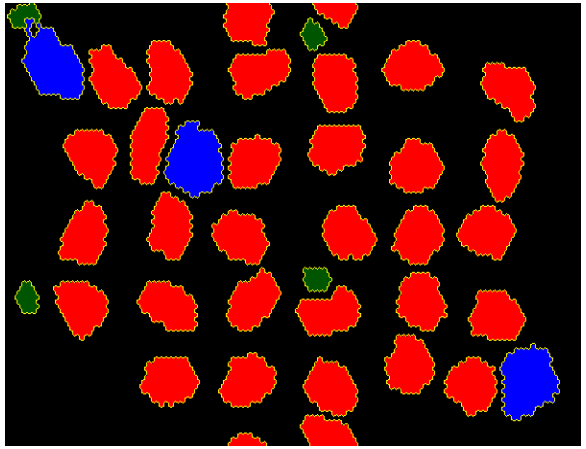
random dynamics
random assortment

network size (n) = 13 nodes
network connectivity (k) = 3
Boolean states (2^n) = 8192

Peter's Anomaly

Cell-based models:
CompuCell 3D (CC3D)



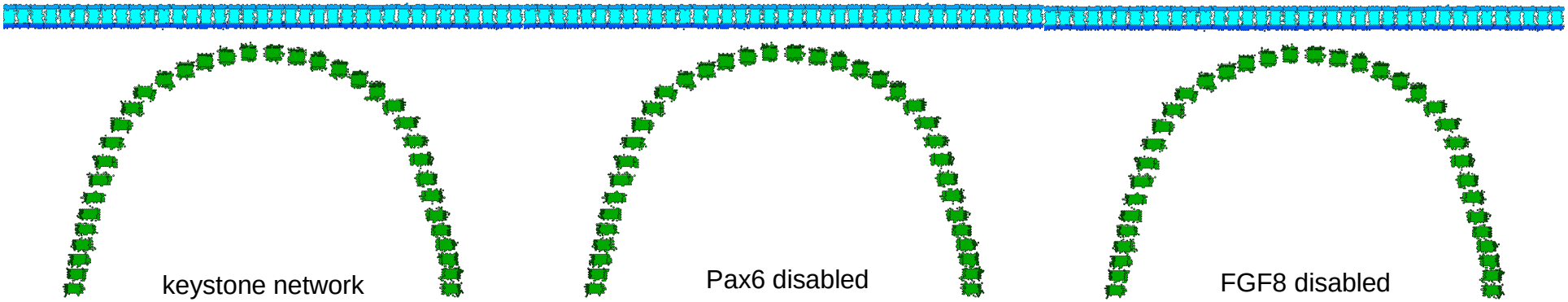


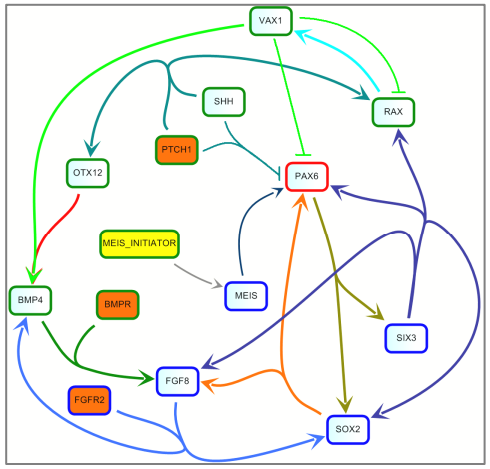
Source: Glazier, Marrakesh (2008)

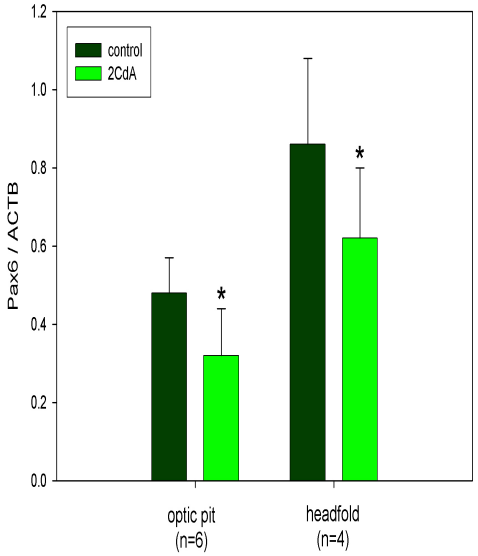
Source: M Rountree, NCCT (2009)

Predictive arrays: disrupting specific nodes in the network code for lens induction

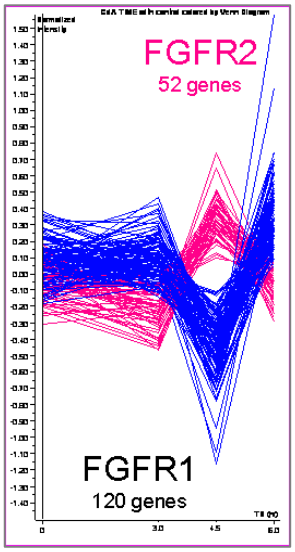
4





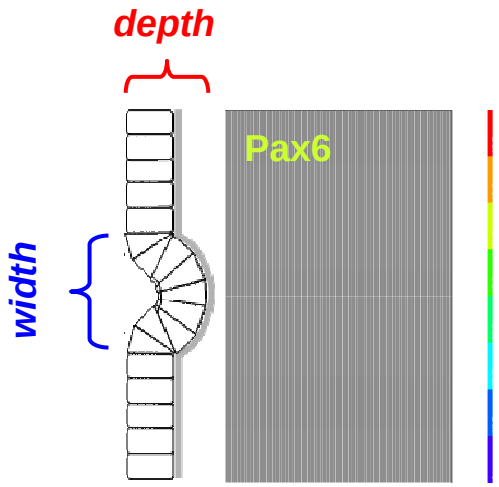


Pax6 expression (3h)

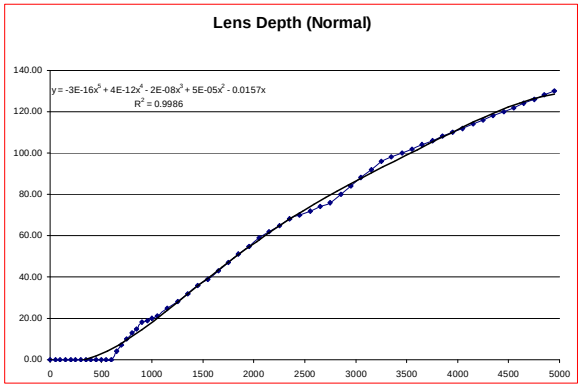


gene profiles
(0 - 6h)

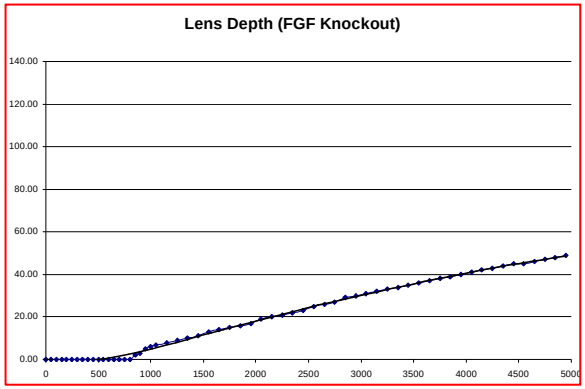
Developmental trajectory of lens:
5000 MC-steps in CC3D (24h in mouse)

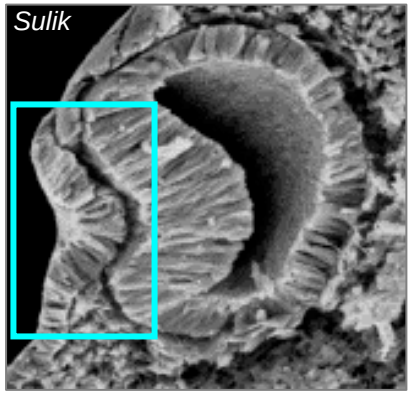


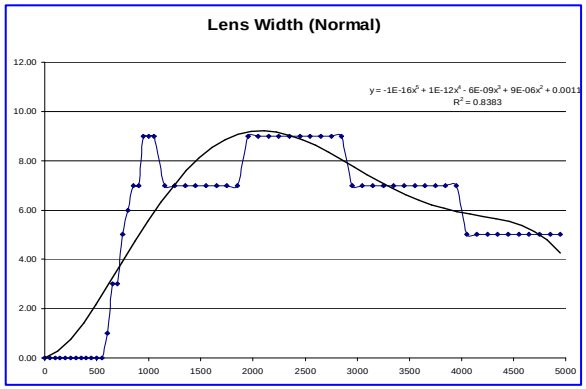
network enabled

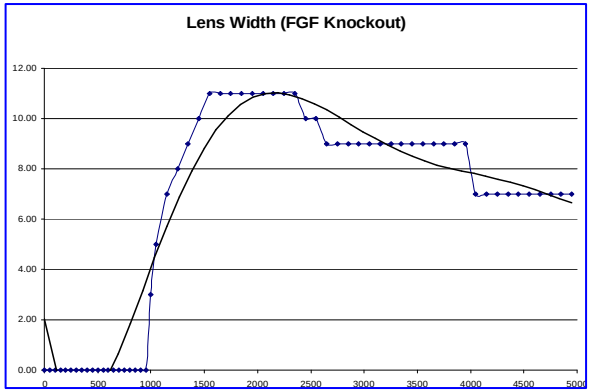


FGF8 disabled

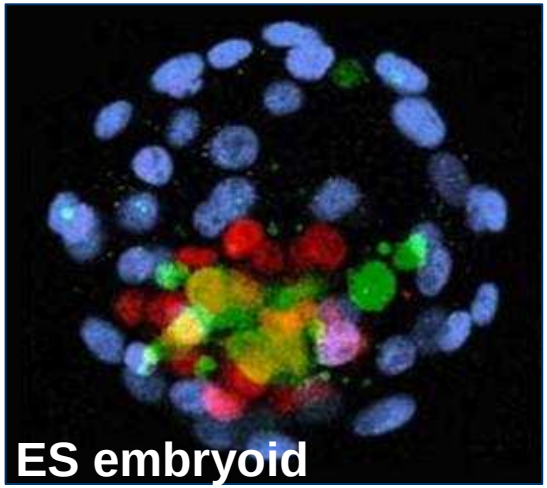


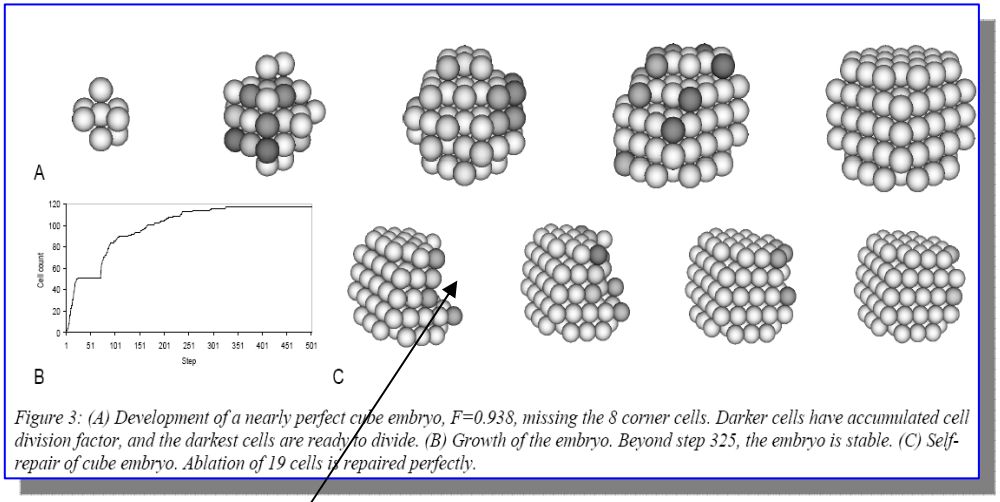






Emergence: self-repair

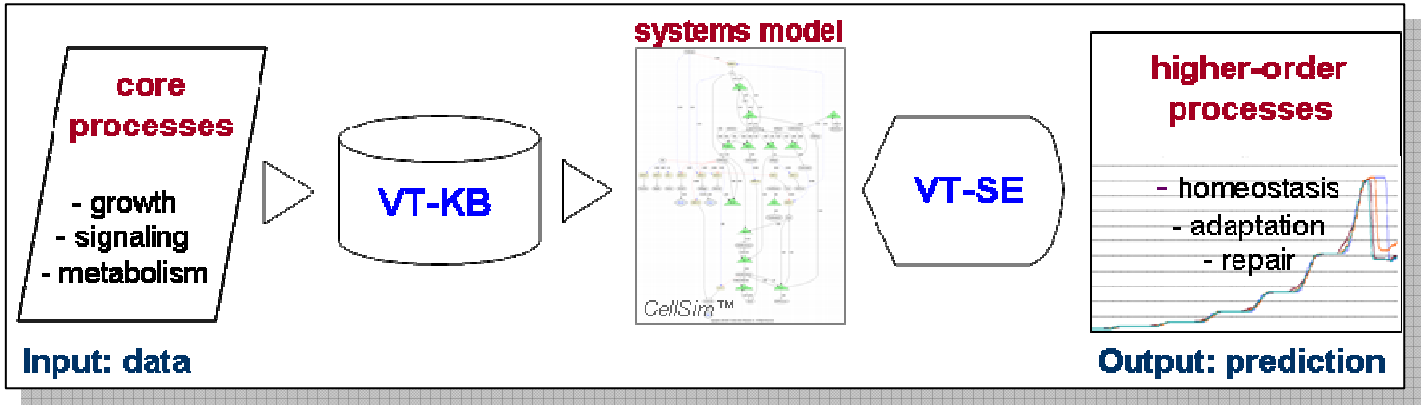




deleted cells

SOURCE: CellSim™, R Newman, 2006, Crowley Davis Research, Inc. (http://www.cdres.com/)

Can explore conditions that would be too difficult on a real embryo …



- HTP hypothesis testing (sweeps)
- self-regulating dynamic models
- responses to stimuli / injury / mutation
- discover key parameters in the system

v-Embryo™

United States Environmental Protection Agency

v-Tissues 2009

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Summary

- **Motivation:** scientific needs to understand mechanisms of toxicity and predict developmental defects from complex datasets
- **Research goal:** simulate as embryonic tissues react to perturbation across chemical class, system, stage, genetic makeup, dose and time
- **Data needs:** detailed knowledge of molecular embryology, cell signaling pathways, and cellular phenotypes
- **Output models:** modular reconstruction of a developing embryo from cell-based models of morphogenesis and differentiation

Acknowledgements

Virtual Embryo
Amar Singh
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Richard Spencer

Virtual Tissues
Imran Shah
Rory Conolly

ToxCast™
Keith Houck
David Dix
Richard Judson
Bob Kavlock

EPA's Virtual Embryo Project is seeking fellows, partners, & research collaborators: visit http://www.epa.gov/ncct/v-Embryo/