

*Incorporating 'omics in the study of reproduction and development:*

# Virtual Tissue Models in Developmental Toxicity Research

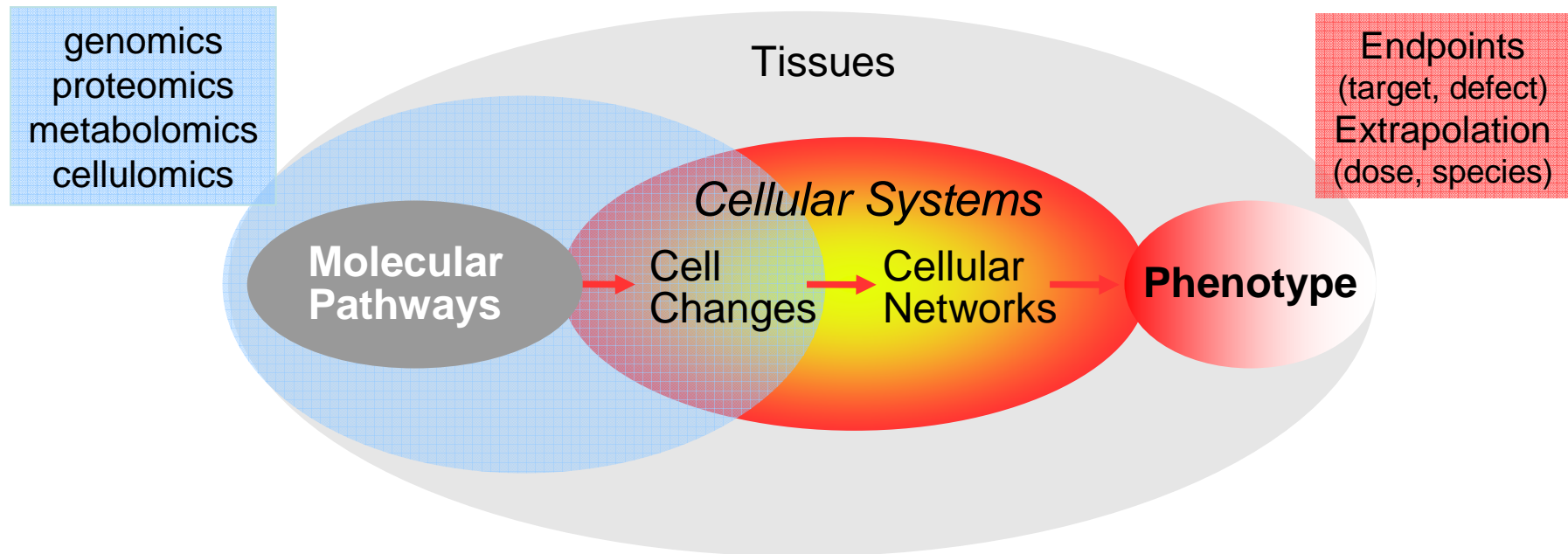
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National Center for Computational Toxicology & \*Lockheed Martin**

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY



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

## 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)

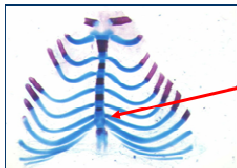
ToxRefDB holds source data captured from comprehensive *in vivo* studies

[www.epa.gov/ncct/toxrefdb](http://www.epa.gov/ncct/toxrefdb)

images from [www.DevTox.org](http://www.DevTox.org)



**target:** kidney  
**description:** absent renal papilla  
**code:** UG\_REN\_3.1060.5013

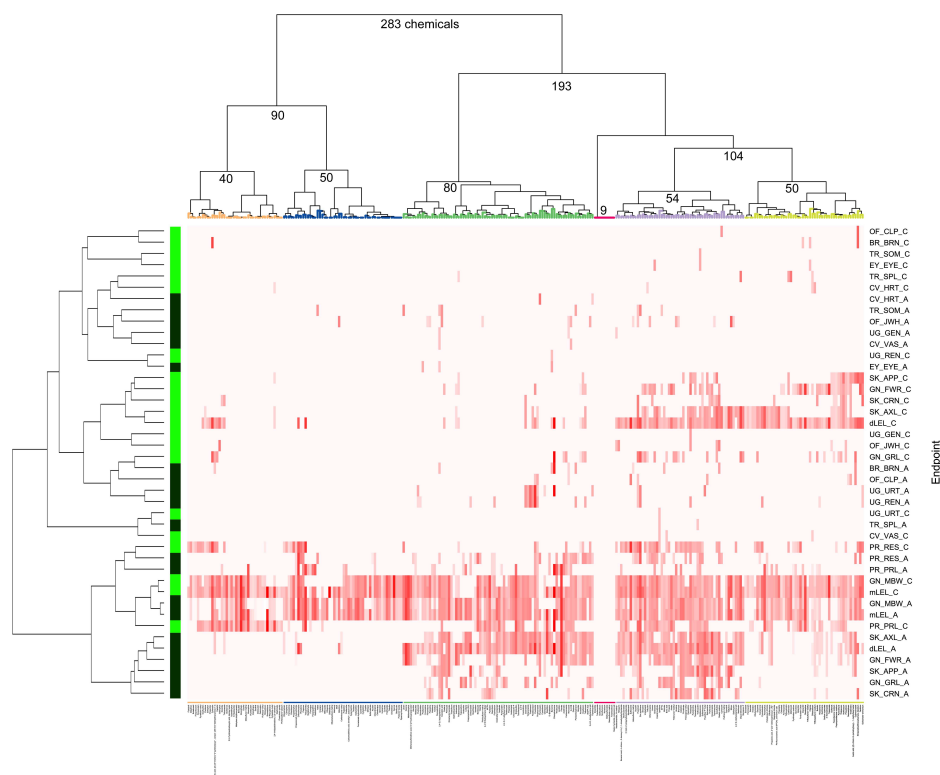


**target:** sternebra  
**description:** incomplete ossification  
**code:** SK\_AXL\_2.1099.5130



**target:** hindpaw  
**description:** polydactyly (digit I)  
**code:** SK\_APP\_2.1051.5234

387 chemicals, 988 annotated effects  
[www.DevTox.org](http://www.DevTox.org), 751 prenatal studies

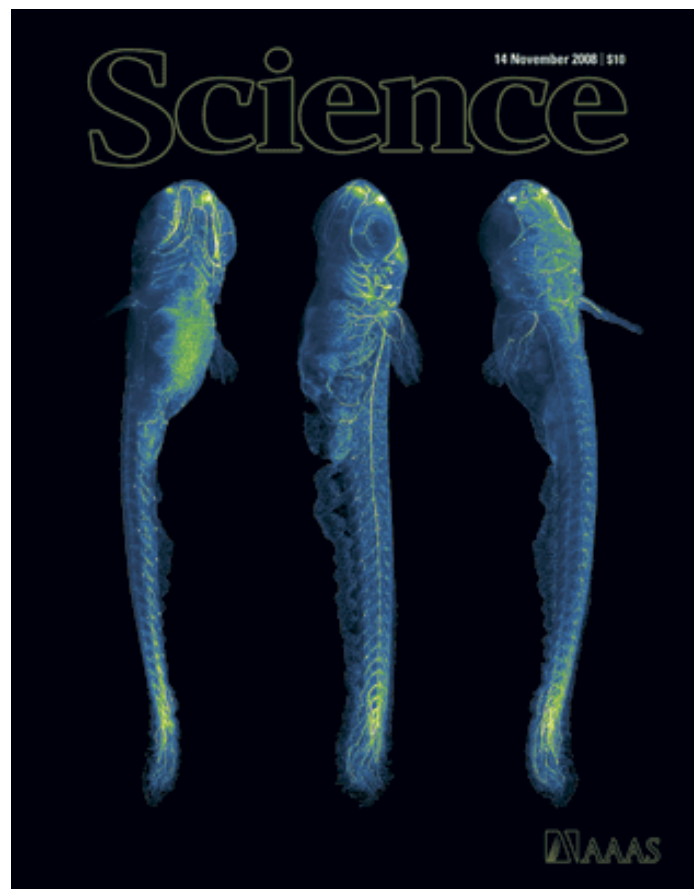
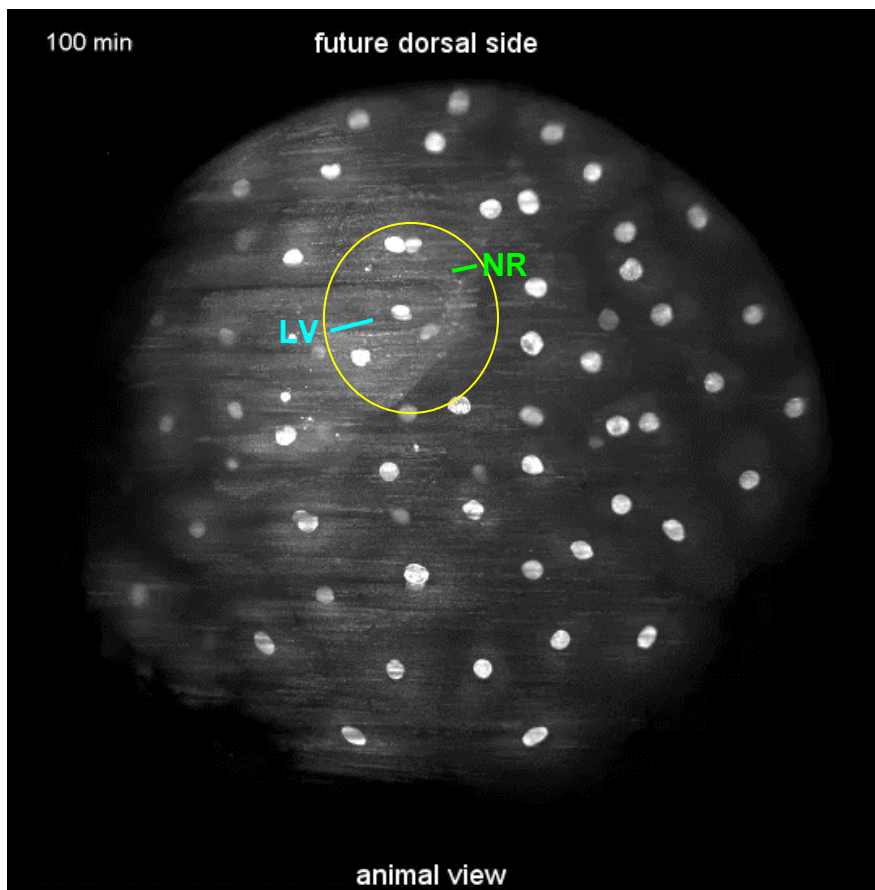


283 chemicals x 293 effects → 19 endpoint targets  
culled from rat and rabbit studies

## **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

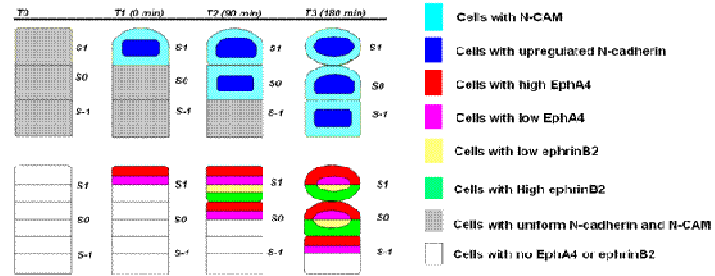
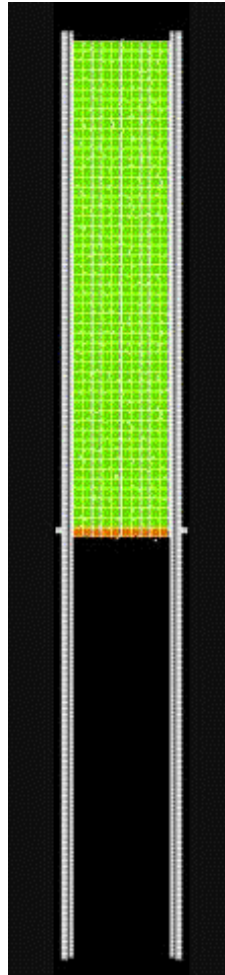
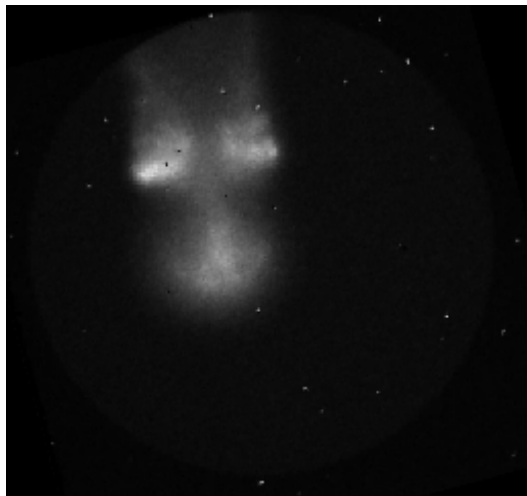
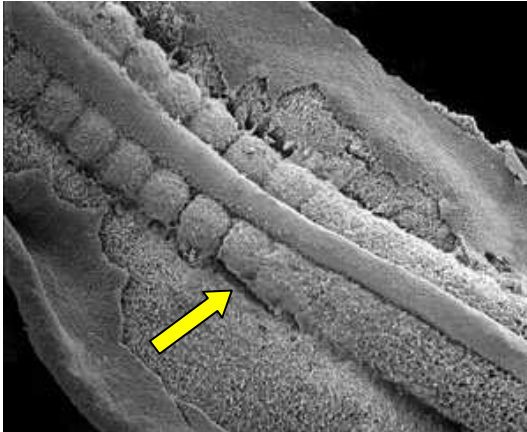
# Digital embryo: ZFET morphogenesis



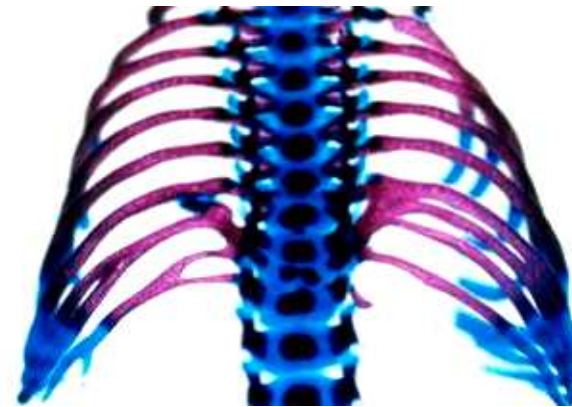
Reconstruction of zebrafish early embryonic development  
tracking H2B-EGFP by DSLM at 90s intervals over 18h



# 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

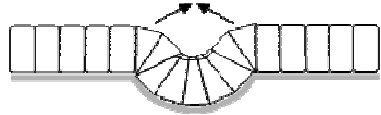
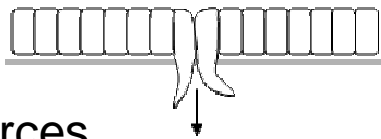
## Core developmental processes

- 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)

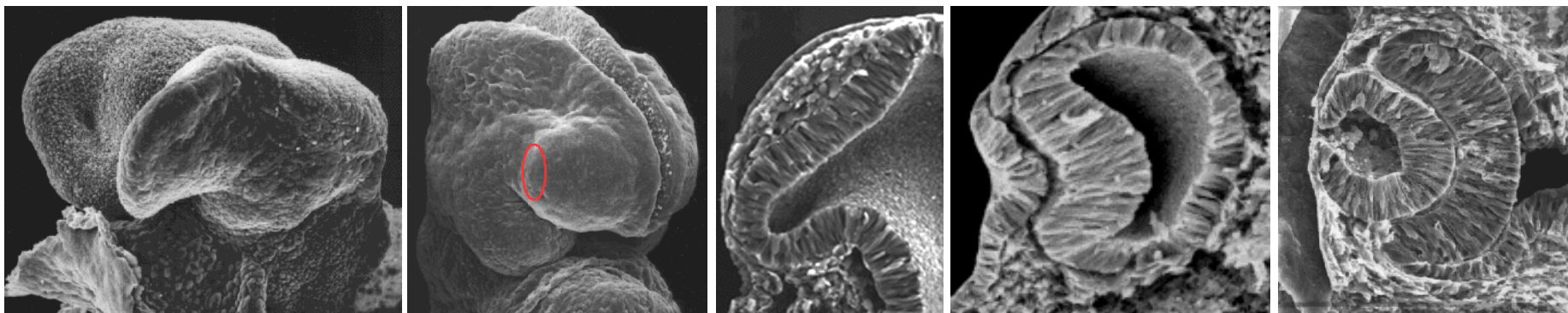




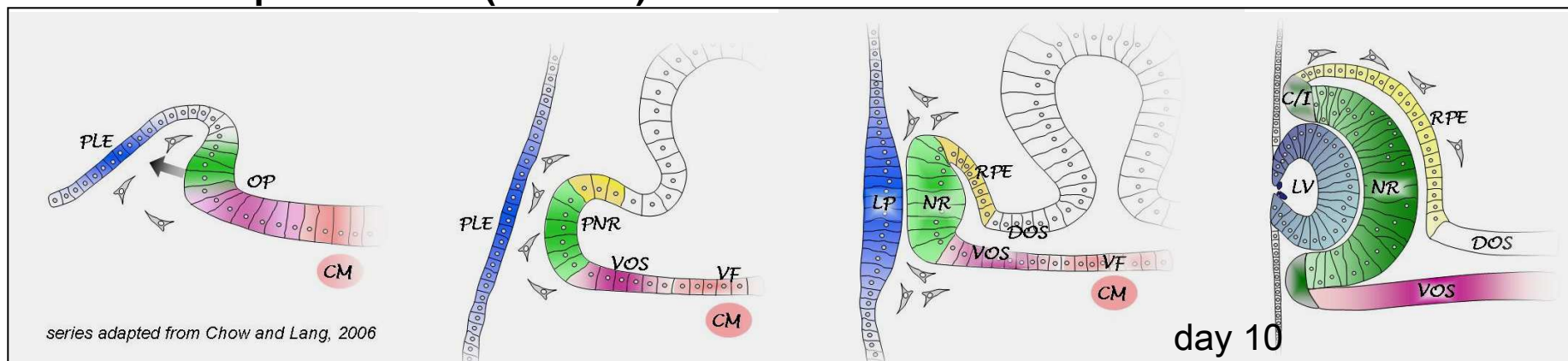
# Early eye development

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

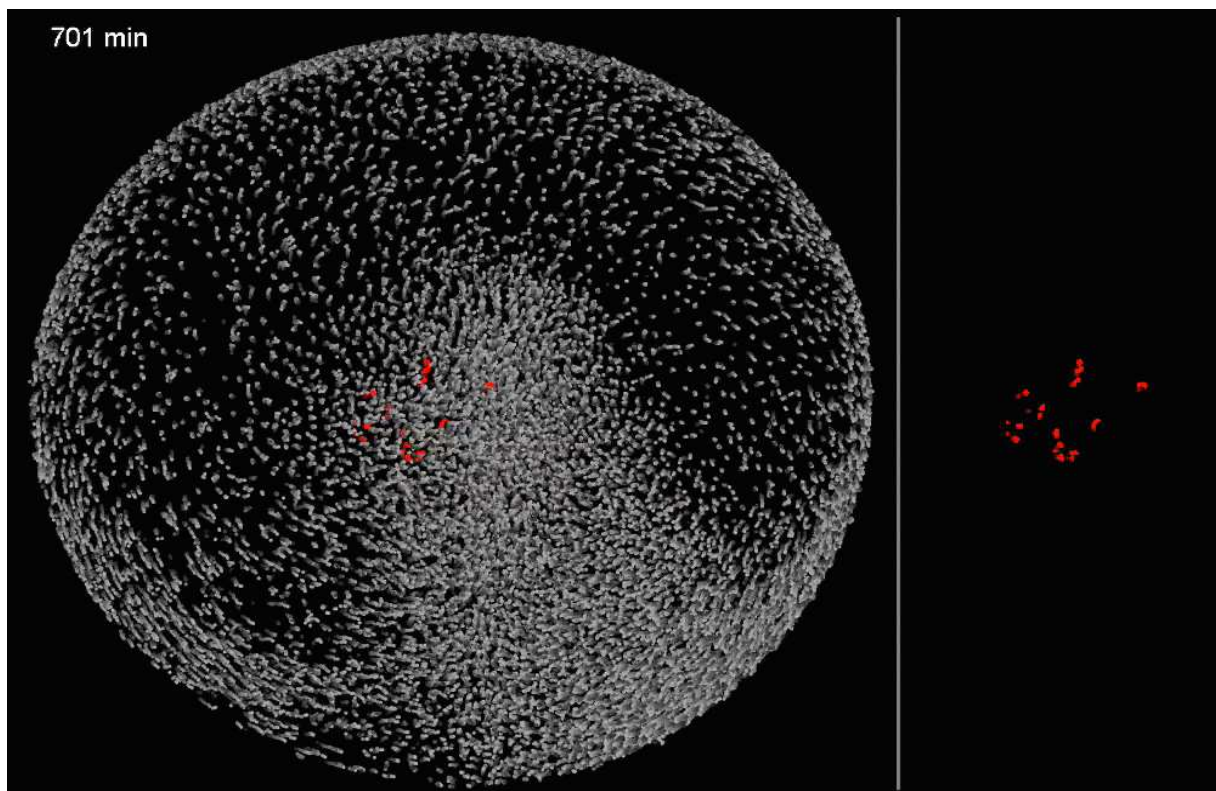
SEM series *K Sulik*, [http://www.med.unc.edu/embryo\\_images/unit-eye/eye.htm](http://www.med.unc.edu/embryo_images/unit-eye/eye.htm) <https://eyetoc.htm>



## Anatomical representation (TS12-18)

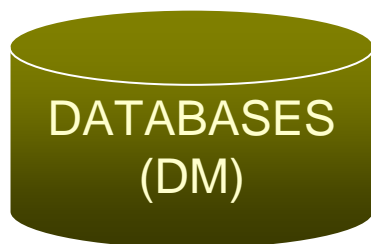


# A real morphogenetic blueprint

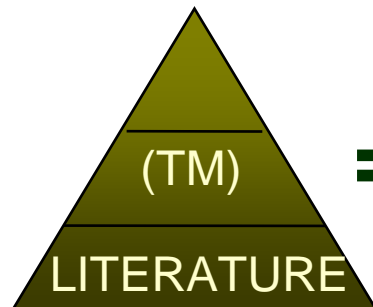


reverse-engineering the cellular dynamics of optic vesicle formation in the zebrafish embryo using DSLM

# Building a signaling network




+




=

- 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

# Gene expression databases:

EMAGE: Edinburgh Mouse Atlas Gene Expression

MPO: Jax Mouse Phenotype Ontology Browser

**emap** <http://genex.hgu.mrc.ac.uk/> 3D digital atlas | TS14

HOME 3D DIGITAL ATLAS EMAGE DATABASE RESOURCES CONTACT SITE SEARCH

3D Navigation  Navigation Window  Section Window  Anatomy Window Theiler Stage TS14

No component selected


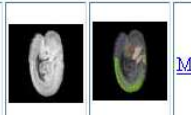

TS14

- embryo
  - branchial arch
  - cavities and their linings
  - ectoderm
  - limb
  - mesenchyme
  - notochord\*
  - organ system
    - cardiovascular system
    - nervous system
  - sensory organ
    - ear
    - eye
    - nose
  - visceral organ
  - primitive streak
  - tail bud
- extraembryonic component\*

< Contract Expand >

More Theiler 14 (E9) resources:

[Information on this model](#) [Help with the Browsers](#) [FAQ](#)

Stage Definition	Section Movies:	Embryo View:	High-resolution Section Images:	Anatomy Nomenclature Database	CD-ROM
	Transverse			<a href="#">Java Browser</a>	
	~Frontal				
	~Sagittal				

Web page contact: [genexweb@hgu.mrc.ac.uk](mailto:genexweb@hgu.mrc.ac.uk) Last modified: 01/03/2004

63 genes (TS12-18)

S.No.	Gene 1	Gene 2	Search 1	Search 2	PMID	Web link	Title	Author	Read	Relevant	New Inform
1	cxcr	shh	fetal or embryo	mouse or mic	12283802	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12283802</a>	A limb-specific mouse	Trujillo, V. et al.	Y	N	
2	cxcr	shh	fetal or embryo	mouse or mic	10853897	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10853897</a>	Direct action of the notochord	Mahler, F., Akbar	Y	N	
3	cxcr	shh	fetal or embryo	mouse or mic	10894227	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10894227</a>	Two distinct cell popul	Gentile, J., et al.	Y	N	
4	cxcr	shh	fetal or embryo	mouse or mic	10462011	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10462011</a>	Expression of acyl and	Shihb, U., et al.	Y	N	
5	cxcr	shh	fetal or embryo	mouse or mic	10742416	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10742416</a>	Complex expression of	Hadamirin, C.	Y	N	
6	shh	shh	fetal or embryo	mouse or mic	11731484	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11731484</a>	Shh, the MDV5 and	Sun, Z., Hoek	Y	N	
7	shh	shh	fetal or embryo	mouse or mic	10801036	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10801036</a>	Vax1, a novel homeo	Hakone, M., et al.	Y	Y	
8	shh	shh	fetal or embryo	mouse or mic	10955509	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10955509</a>	Sonic hedgehog is not	Schwartz, H., et al.	Y	N	
9	shh	shh	fetal or embryo	mouse or mic	10103070	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10103070</a>	Shh regulates the fore	Reuter, E., et al.	Y	N	
10	Pax2	pax2	fetal or embryo	mouse or mic	17470285	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17470285</a>	Rybo, a polycomb com	Petry, M., Warty	Y	Y	
11	Pax2	pax2	fetal or embryo	mouse or mic	17168116	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17168116</a>	Lmx1b, a essential fo	Guo, C., Qiu, H.	Y	N	
12	Pax2	pax2	fetal or embryo	mouse or mic	10934993	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10934993</a>	A role for GHRH in ear	Wu, S., Page, Y.	Y	Y	
13	Pax2	pax2	fetal or embryo	mouse or mic	14713874	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/14713874</a>	Pax2, a lipoprotein	Chen, G., et al.	Y	Y	
14	Pax2	pax2	fetal or embryo	mouse or mic	12970747	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12970747</a>	Paired-Box genes an	Murakami, A., et al.	Y	N	
15	Pax2	pax2	fetal or embryo	mouse or mic	12758174	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12758174</a>	Retinal pigmented ep	Burns, N., Miy, Y.	Y	Y	
16	Pax2	pax2	fetal or embryo	mouse or mic	11807880	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11807880</a>	Shc1, a vertebrate ho	Hess, T., et al.	Y	Y	
17	Pax2	pax2	fetal or embryo	mouse or mic	11458094	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11458094</a>	Shc1 regulates the for	Reuter, E., et al.	Y	N	
18	Pax2	pax2	fetal or embryo	mouse or mic	11008333	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11008333</a>	Spatial specification of	Schwartz, M., et al.	Y	N	
19	Pax2	pax2	fetal or embryo	mouse or mic	10801036	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10801036</a>	Vax1, a novel homeo	Hakone, M., et al.	Y	Y	
20	Pax2	pax2	fetal or embryo	mouse or mic	10054661	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10054661</a>	Etropic bone morphog	Gallien, J., et al.	Y	N	
21	Pax2	pax2	fetal or embryo	mouse or mic	10811669	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10811669</a>	Ceroidin, ocular lev	Hornes, S., et al.	Y	Y	
22	Pax2	pax2	fetal or embryo	mouse or mic	10955509	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10955509</a>	Sonic hedgehog is not	Schwartz, H., et al.	Y	N	
23	Pax2	pax2	fetal or embryo	mouse or mic	10297866	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10297866</a>	Pax genes and organo	Dahl, E., Kozak, Y.	Y	N	
24	Pax2	pax2	fetal or embryo	mouse or mic	11251545	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11251545</a>	Roles of Pax genes in	Stavova, A., et al.	Y	N	
25	pax2	pax2	fetal or embryo	mouse or mic	11243024	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11243024</a>	The homeobox gene in	Kaneko, A., et al.	Y	Y	
26	pax2	pax2	fetal or embryo	mouse or mic	17821048	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17821048</a>	PAX6 and SOX2 dep	mouse, M., Kan, Y.	Y	Y	
27	pax2	pax2	fetal or embryo	mouse or mic	17470285	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17470285</a>	Rybo, a polycomb com	Petry, M., Warty, Y.	Y	Y	
28	pax2	pax2	fetal or embryo	mouse or mic	17064077	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17064077</a>	Shc1 activation of Pax2	Lu, W., Lapan, Y.	Y	Y	
29	pax2	pax2	fetal or embryo	mouse or mic	10921066	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10921066</a>	Understand the bas	Uchikawa, M., et al.	Y	Y	
30	pax2	pax2	fetal or embryo	mouse or mic	10855260	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10855260</a>	The involvement of m	Reuter, E., et al.	Y	Y	
31	pax2	pax2	fetal or embryo	mouse or mic	11852703	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11852703</a>	Requirement of mesod	Mokkova, N., et al.	Y	N	
32	pax2	pax2	fetal or embryo	mouse or mic	10581674	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10581674</a>	Interplay of Pax2 and	Scardifil, L., et al.	Y	Y	
33	pax2	pax2	fetal or embryo	mouse or mic	12711853	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12711853</a>	Pax2 autoregulation in	Avra, S., Nish, Y.	Y	Y	
34	pax2	pax2	fetal or embryo	mouse or mic	12842482	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12842482</a>	Cell-autonomous inhi	Yamada, R., et al.	Y	Y	
35	pax2	pax2	fetal or embryo	mouse or mic	11083887	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11083887</a>	Pax2 activity in the kn	Ashley, P., et al.	Y	N	
36	pax2	pax2	fetal or embryo	mouse or mic	10811892	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10811892</a>	BMX4 is essential fo	Paruta, V., Ho, Y.	Y	Y	
37	pax2	pax2	fetal or embryo	mouse or mic	10810865	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10810865</a>	Development of Sp2, 2	Kamada, Y., et al.	Y	Y	
38	pax2	pax2	fetal or embryo	mouse or mic	17470285	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17470285</a>	Rybo, a polycomb com	Petry, M., Warty, Y.	Y	Y	
39	pax2	pax2	fetal or embryo	mouse or mic	17064077	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17064077</a>	Shc1 activation of Pax2	Lu, W., Lapan, Y.	Y	Y	
40	pax2	pax2	fetal or embryo	mouse or mic	10791010	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10791010</a>	Identification of agene	Lu, S., et al.	Y	Y	
41	pax2	pax2	fetal or embryo	mouse or mic	10683827	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10683827</a>	Transcriptional regul	Grise, N., et al.	Y	Y	
42	pax2	pax2	fetal or embryo	mouse or mic	10368004	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10368004</a>	Raii2 expression in	Mic, F., Miki, Y.	Y	Y	
43	pax2	pax2	fetal or embryo	mouse or mic	12842482	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12842482</a>	Cell-autonomous inhi	Yamada, R., et al.	Y	Y	
44	pax2	pax2	fetal or embryo	mouse or mic	11967299	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11967299</a>	Shc1 promotes the for	Reuter, E., et al.	Y	Y	
45	pax2	pax2	fetal or embryo	mouse or mic	11458094	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11458094</a>	Shc1 regulates the fore	Reuter, E., et al.	Y	Y	
46	pax2	pax2	fetal or embryo	mouse or mic	10502109	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10502109</a>	Mouse Dlx3, a homeo	Davis, B., Shih, Y.	Y	Y	
47	pax2	pax2	fetal or embryo	mouse or mic	10097211	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10097211</a>	Shc1 overexpression in	Lu, F., Wu, Y.	Y	Y	
48	pax2	pax2	fetal or embryo	mouse or mic	10611515	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10611515</a>	Shc1, a mitosis homeo	Lu, F., et al.	Y	Y	
49	pax2	pax2	fetal or embryo	mouse or mic	10811892	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10811892</a>	Development of Sp2, 2	Kamada, Y., et al.	Y	Y	
50	vax1	chx10	fetal or embryo	mouse or mic	17470285	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/17470285</a>	Rybo, a polycomb com	Petry, M., Warty, Y.	Y	Y	
51	chx10	chx10	fetal or embryo	mouse or mic	11385160	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11385160</a>	Requirement for Mib2	Yamada, R., et al.	Y	N	
52	chx10	chx10	fetal or embryo	mouse or mic	10420417	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10420417</a>	Loss of retinal progen	Colla, B., Hoek, N.	Y	Y	
53	chx10	chx10	fetal or embryo	mouse or mic	10459106	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10459106</a>	Shc1, a mitosis homeo	Lu, F., et al.	Y	Y	
54	chx10	chx10	fetal or embryo	mouse or mic	11023863	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/11023863</a>	Extracellular mesench	Fukushima, S., et al.	Y	Y	
55	chx10	chx10	fetal or embryo	mouse or mic	10803182	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/10803182</a>	Signaling and transcr	Nguyen, M., et al.	Y	Y	
56	Pax6	Mis	fetal or embryo	mouse or mic	12183884	<a href="#">http://www.ncbi.nlm.nih.gov/pubmed/12183884</a>	Mis homeoprotein de	Zhang, X., Fan, Y.	Y	Y	



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

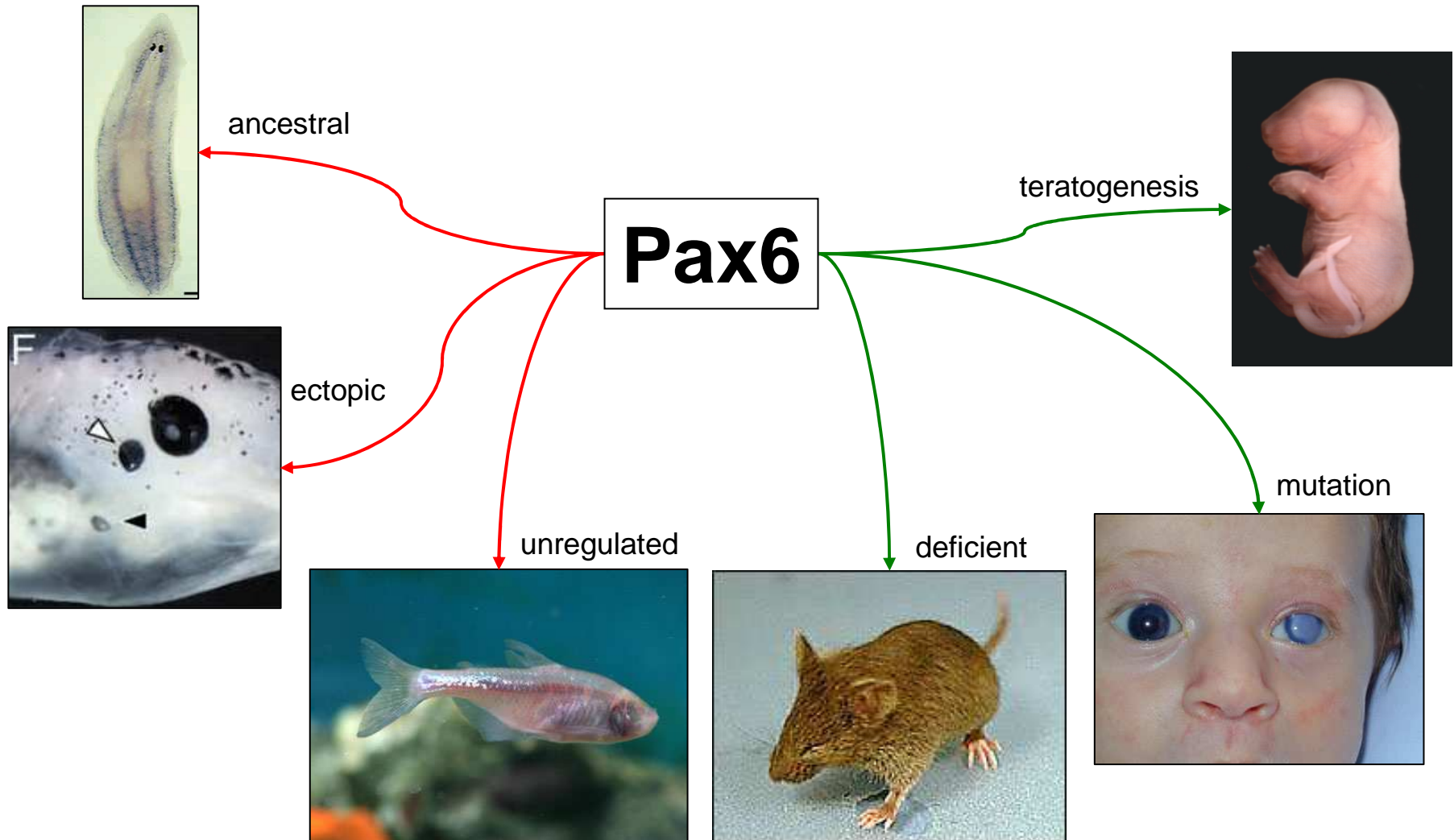
**PURPOSE:** The *PAX6* gene was first described as a candidate for human aniridia. However, *PAX6* expression is not restricted to the eye and it appears to be crucial for brain development. We studied *PAX6* mutations in a large spectrum of patients who presented with aniridia phenotypes, Petersen+ anomaly, and anterior segment malformations associated or not with neurological anomalies. **METHODS:** Patients and related families were ophthalmologically phenotyped, and in some cases neurologically and endocrinologically examined. We screened the *PAX6* gene by direct sequencing in three groups of patients: those affected by aniridia, those with Petersen+ anomaly, and those with Petersen+ anomaly. Two mutations were investigated by generating crystallographic representations of the amino acid changes. **RESULTS:** Three novel heterozygous mutations affecting three unrelated families were identified: the g.5721>g.C nucleotide change, located in exon 5, and corresponding to the Leucine 46 Proline amino-acid mutation (L46P); the g.655A>g.G nucleotide change, located in exon 6, and corresponding to the Serine 74 Glycine amino-acid mutation (S74G); and the nucleotide deletion 579delG del, located in exon 6, which induces a frameshift mutation leading to a stop codon (V486S/S3). The L46P mutation was identified in affected patients presenting bilateral microphthalmia, cataracts, and nystagmus. The S74G mutation was found in a large family that had congenital ocular abnormalities, diverse neurological manifestations, and variable cognitive impairments. The 579delG deletion (V486S/S3) caused in the affected members of the same family bilateral aniridia associated with congenital cataract, foveal hypoplasia, and nystagmus. We also detected a novel intronic nucleotide change, IVS2+9G>g.A (very likely a mutation in an apparently isolated patient affected by a complex ocular phenotype, characterized primarily by a bilateral microphthalmia. Whether this nucleotide change is indeed pathogenic remains to be demonstrated. Two previously known heterozygous mutations of the *PAX6* gene sequence were also detected in patients affected by aniridia: a de novo previously known nucleotide change, g.572C>g.T (G178R), in exon 6, leading to a stop codon and a heterozygous g.555>g.T.A (C43D) recurrent nonsense mutation in exon 5. No mutations were found in patients with Petersen+ anomaly. **CONCLUSIONS:** We identified three mutations associated with aniridia phenotypes (G178Y, C43D, and V486S/S3). The three other mutations reported here cause non-aniridia ocular phenotypes associated in some cases with neurological anomalies. The IVS2+9G>g.A nucleotide change was detected in a patient with a microphthalmia phenotype. The L46P mutation was detected in a family with microphthalmia, cataract, and nystagmus. This mutation is located in the DNA-binding paired-domain and the crystallographic representations of this mutation show that this mutation may affect the helix-turn-helix and as a consequence the DNA-binding properties of the resulting mutated protein. Ser74 is located in the PAX6 PD-linker region, essential for DNA recognition and DNA binding, and the side chain of the Ser74 contributes to DNA recognition by the linker domain through direct contacts. Crystallographic representations show that the S74G mutation results in no side chain and therefore perturbs the DNA-binding properties of *PAX6*. This study highlights the severity and diversity of the consequences of *PAX6* mutations that appeared to result from the complexity of the *PAX6* gene structure, and the numerous possibilities for DNA binding. This study emphasizes the fact that neurodevelopmental abnormalities may be caused by *PAX6* mutations. The neurodevelopmental abnormalities caused by *PAX6* mutations are probably still overlooked in the current clinical examinations performed throughout the world in patients affected by *PAX6* mutations.

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

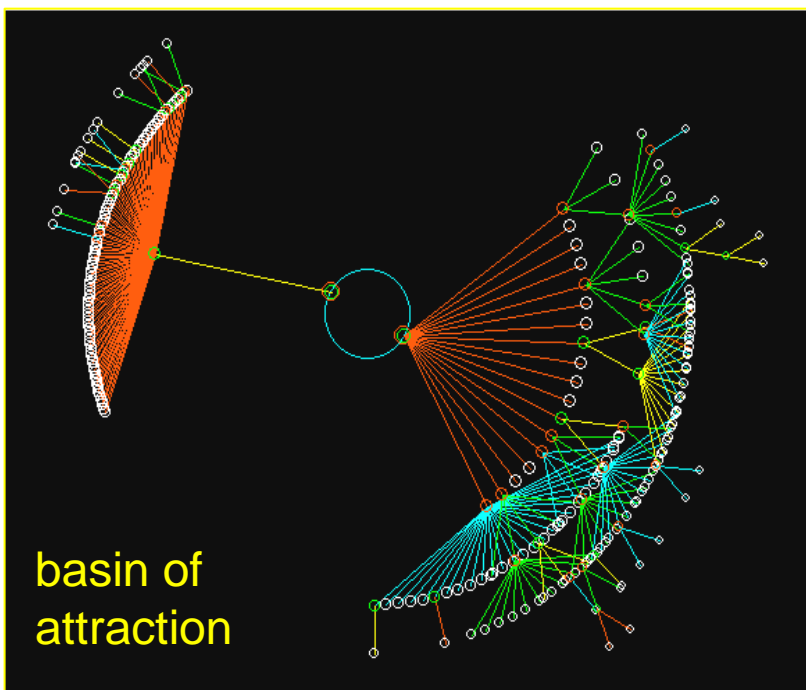
Computing relevance scores for association of gene-malformation in the eye based on co-occurrence of DevTox terms in the abstracts

EMAGI	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



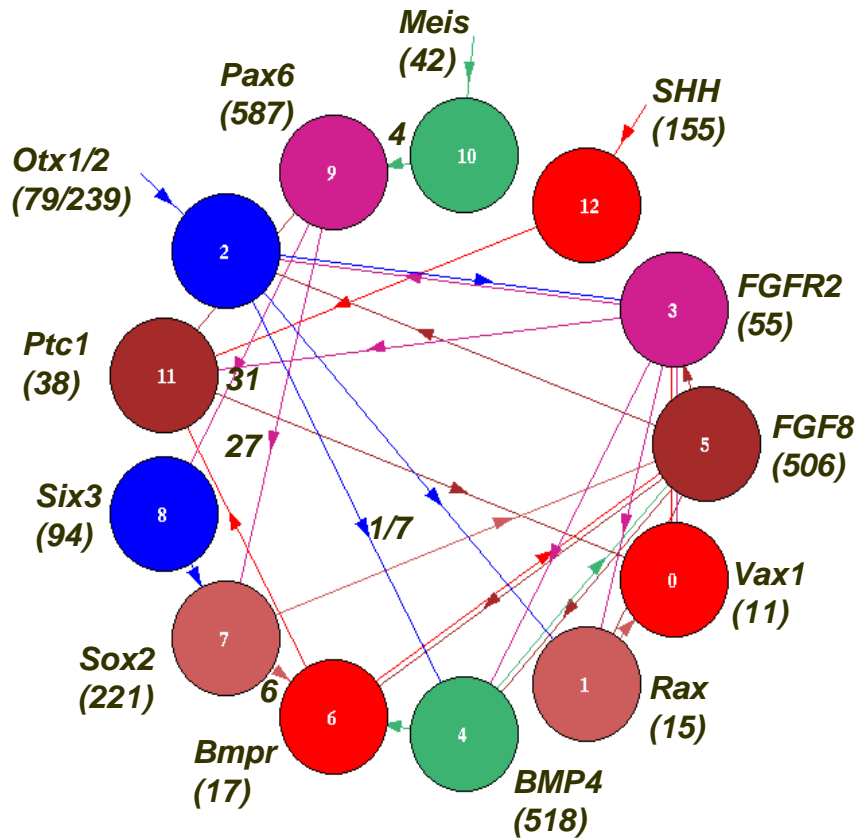




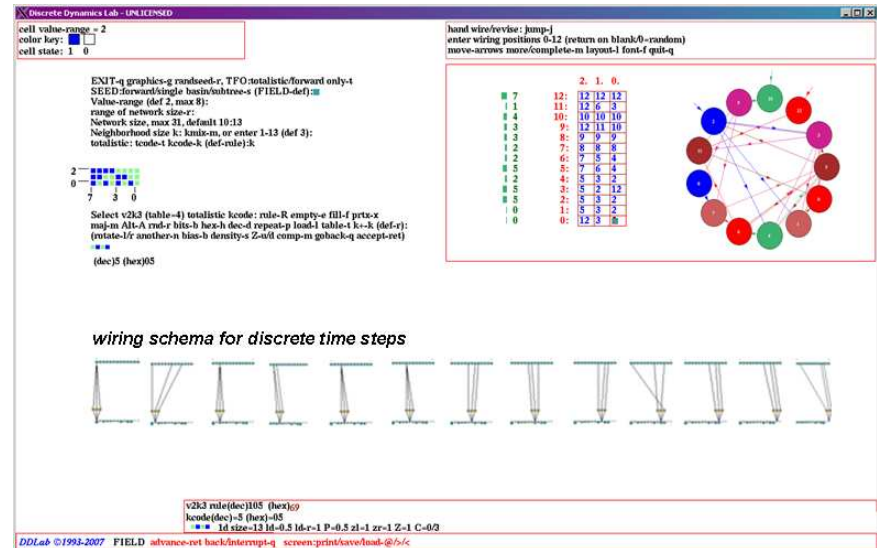
## DDNs: 'state machines'

- 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



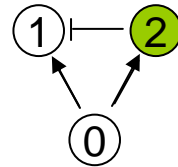
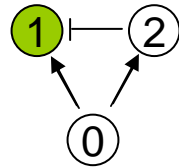
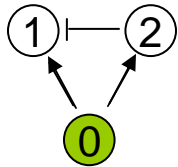
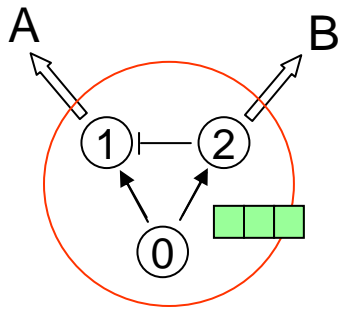
7 transcription factors  
3 receptor systems  
3 signal ligands



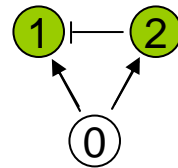
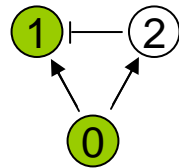
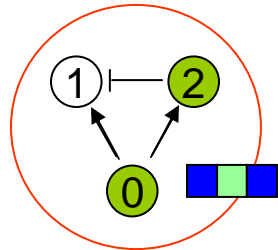
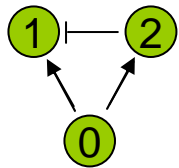
Software: A Wuensche, <http://www.ddlab.com/>

network size (n) = 13 nodes  
network connectivity (k) = 3  
Boolean states (2<sup>n</sup>) = 8192

# Simple RBN state dynamics

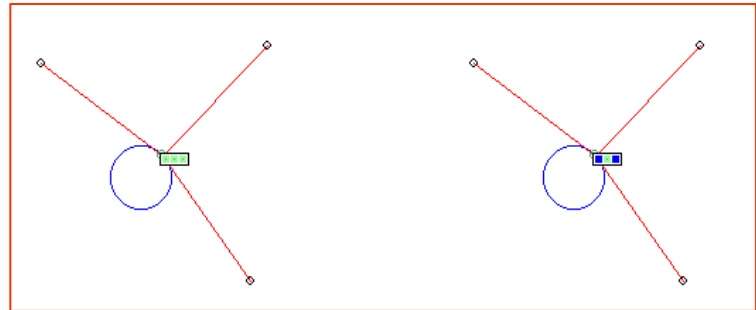


topology  
n=3  
k=2  
2<sup>3</sup> states

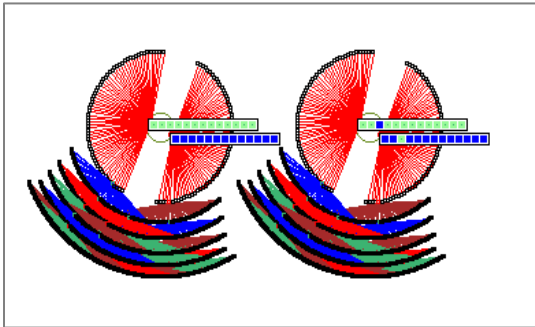


rules  
0 → 1  
0 → 2  
2 ⊣ 1  
2 > 0

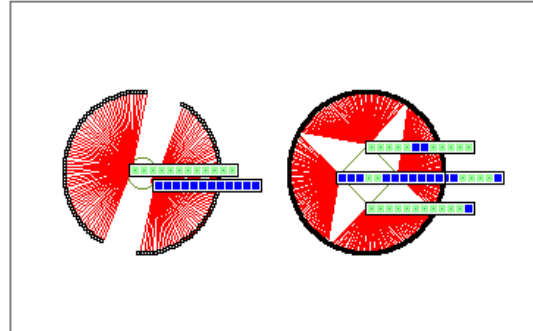
stable patterns  
(attractors)



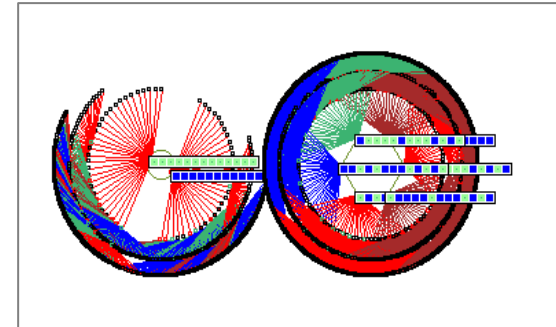
# State attractor field of some more complex networks



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



chaotic dynamics  
*loss of Pax6 function*



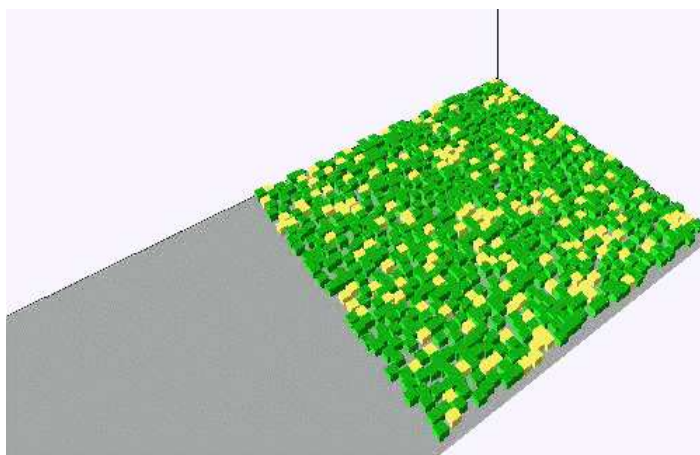
random dynamics  
*random assortment*

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

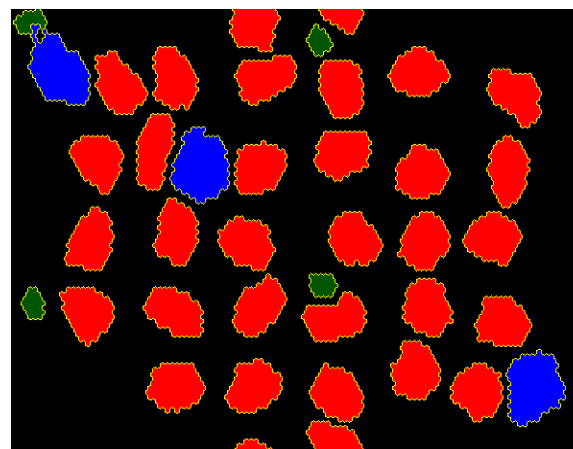


*Peter's Anomaly*

# Cell-based models: CompuCell 3D (CC3D)

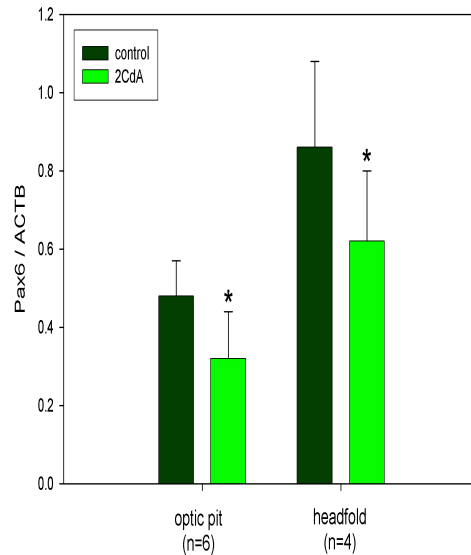
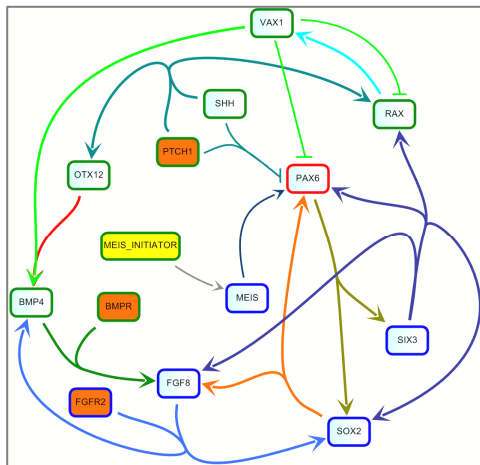
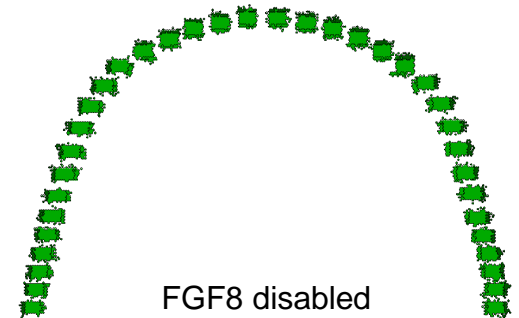
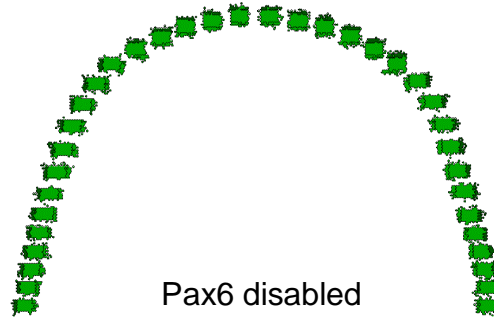
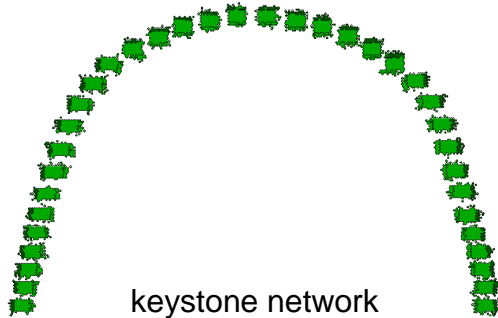


*Source: Glazier, Marrakesh (2008)*

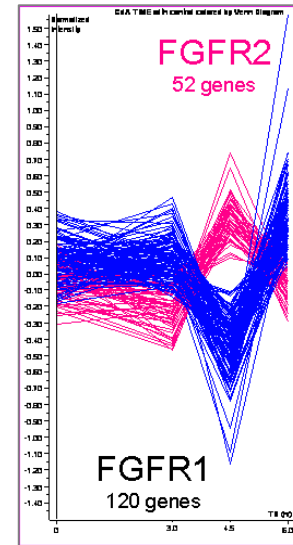


*Source: M Rountree, NCCT (2009)*

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



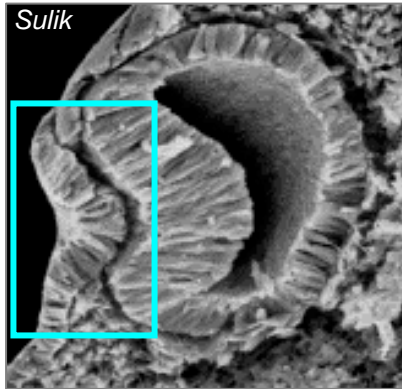
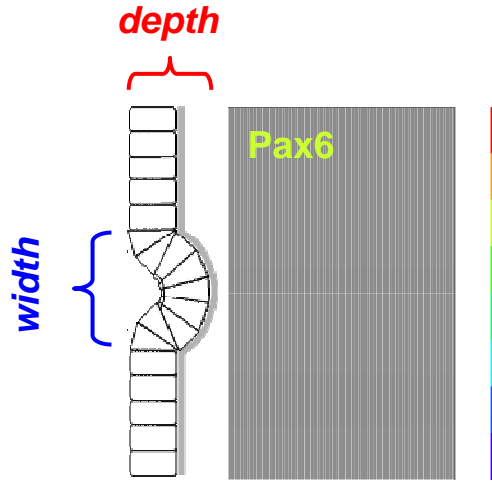
Pax6 expression (3h)



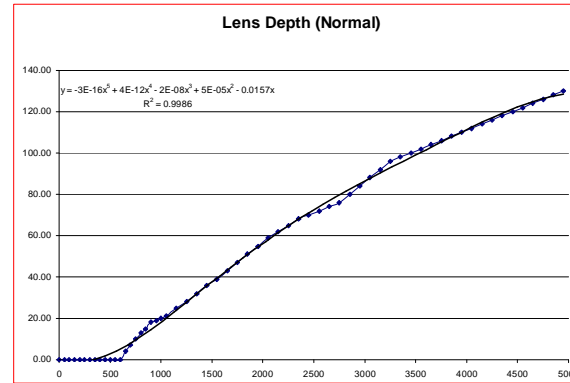
gene profiles  
(0 - 6h)



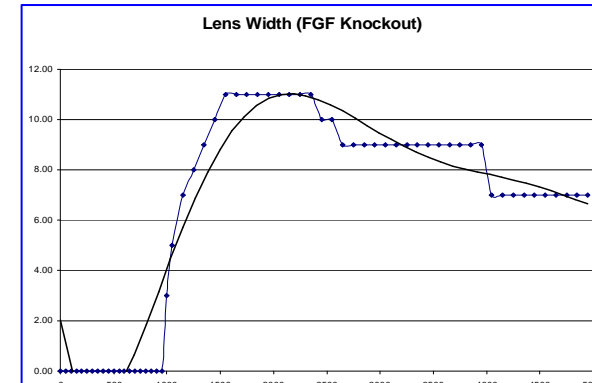
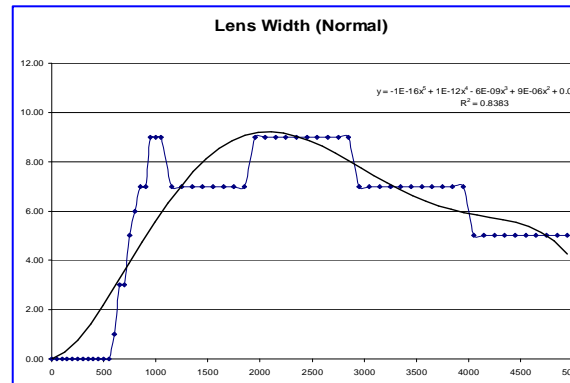
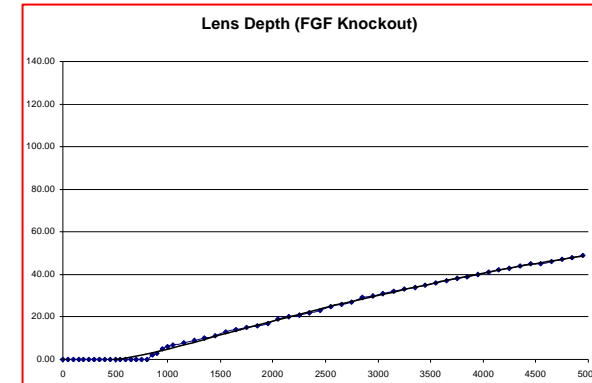
# Developmental trajectory of lens: 5000 MC-steps in CC3D (24h in mouse)



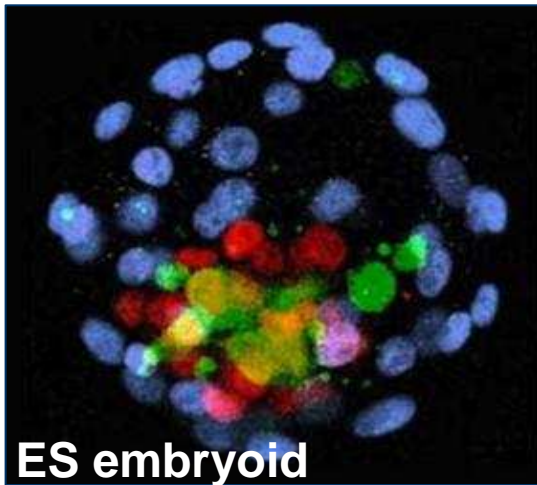
network enabled



FGF8 disabled



# Emergence: self-repair



**ES embryoid**

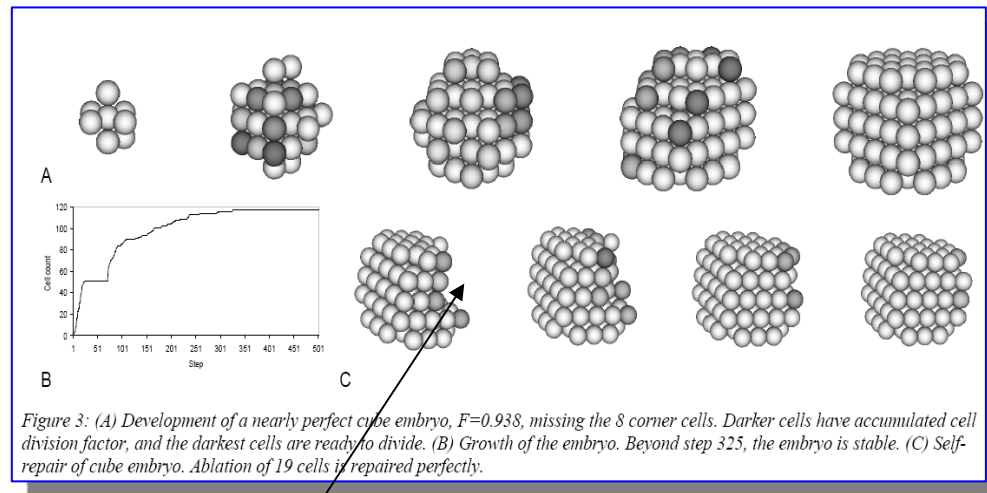
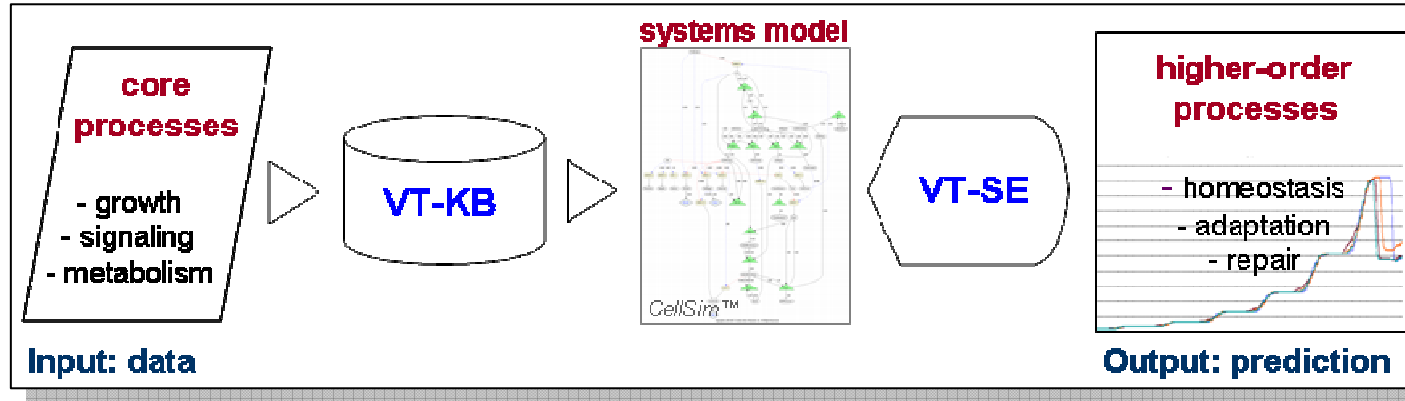


Figure 3: (A) Development of a nearly perfect cube embryo,  $F=0.938$ , missing the 8 corner cells. Darker cells have accumulated cell division factor, and the darkest cells are ready to divide. (B) Growth of the embryo. Beyond step 325, the embryo is stable. (C) Self-repair of cube embryo. Ablation of 19 cells is repaired perfectly.

deleted cells

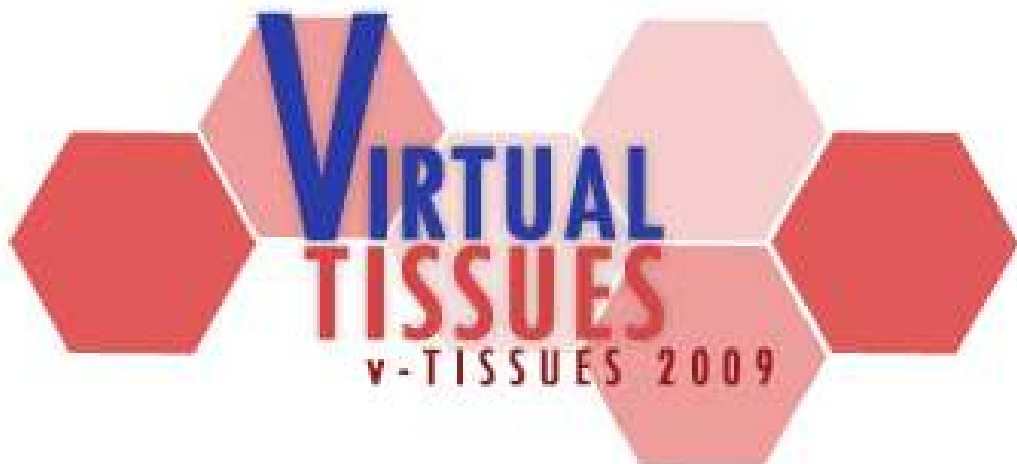
**SOURCE: CellSim™, R Newman, 2006, Crowley Davis Research, Inc. (<http://www.cdres.com/>)**

# 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-Tissues 2009**

First International Workshop on Virtual Tissues

Hosted by National Center for Computational Toxicology

April 21-22 EPA Campus, RTP, NC

Please register to participate

[www.epa.gov/ncct/virtual\\_tissues](http://www.epa.gov/ncct/virtual_tissues)



- ❖ **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

Michael Rountree

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/>***