The Virtual Embryo Project:
a computational framework for developmental toxicity

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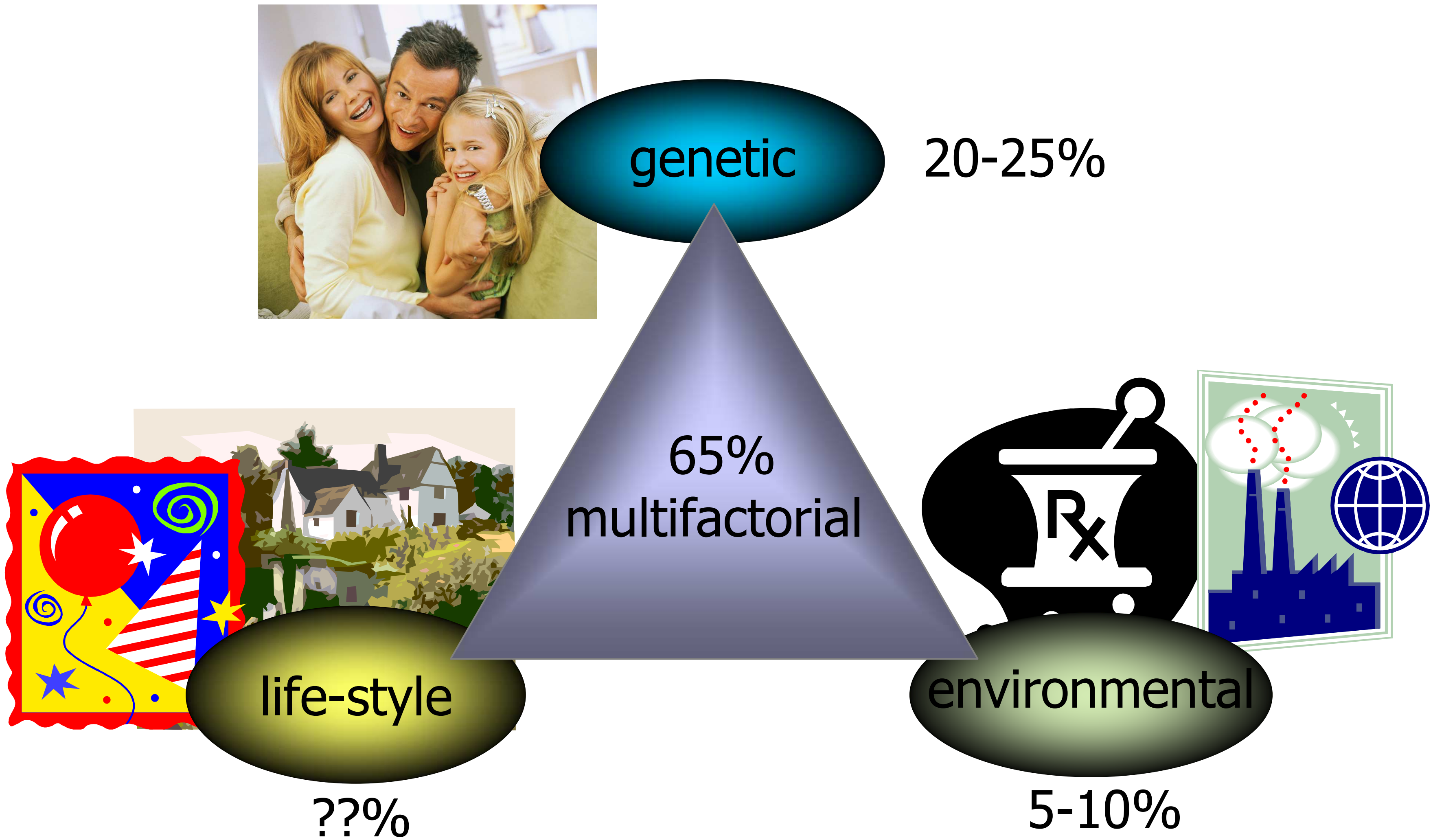
April 30, 2008

Outline

- scope of the problem
- new vision and strategy
- v-Embryo™
- developmental systems biology
- virtual tissues and simulators

Scope of the Problem:
developmental (prenatal) defects



Limitations of the current strategy

- developmental endpoints are likely sensitive to many chemicals a fetus may exposed to via the environment

- testing methods for developmental endpoints are expensive, time consuming, and use lots of animals

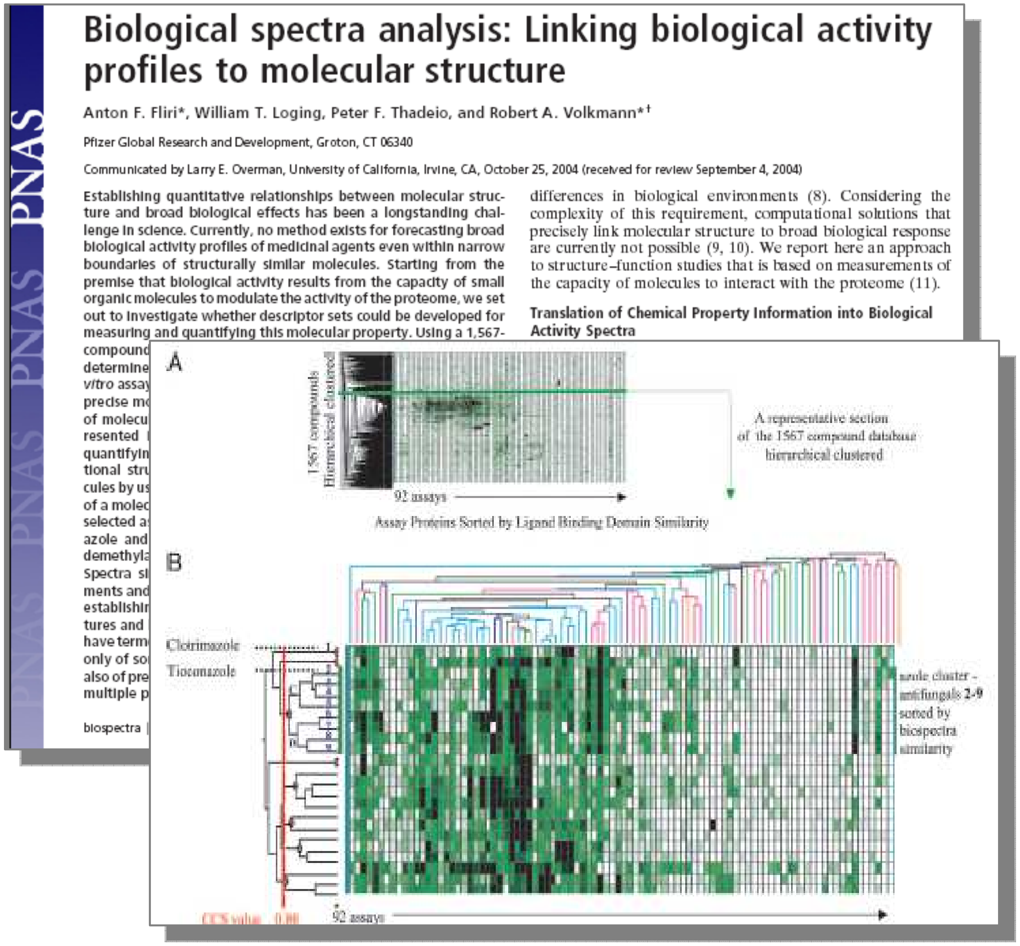
- not sensitive to developmental endpoints such as subtle malformations, functional defects or fetal programming

- nos. of chemicals requiring evaluation for developmental toxicity far exceeds what has and can be delivered

New strategy: systems biology

- genome-based technologies coupled with advances in robotics generates data comprehensively and efficiency
- use bioinformatics and computational biology to build systems-based models of detailed embryological events
- potential to significantly accelerate understanding of chemical mechanisms and toxicity pathways *in vitro*
- Holy Grail: revolutionize the way scientifically defensible predictions of toxicity *in vivo* can be based

high-throughput bioactivity profiling



PNAS PNAS PNAS PNAS PNAS

Biological spectra analysis: Linking biological activity profiles to molecular structure

Anton F. Fliri*, William T. Loging, Peter F. Thadeio, and Robert A. Volkmann*†

Pfizer Global Research and Development, Groton, CT 06340

Communicated by Larry E. Overman, University of California, Irvine, CA, October 25, 2004 (received for review September 4, 2004)

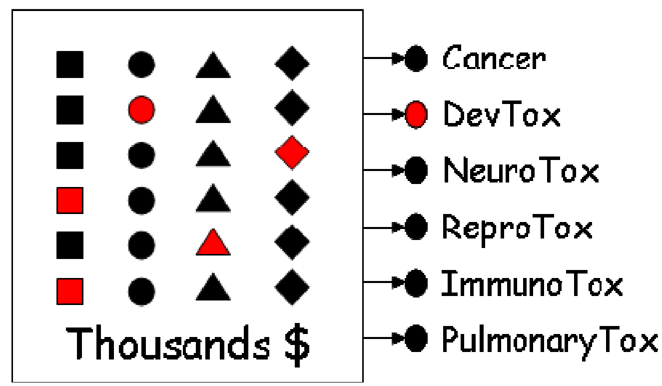
Establishing quantitative relationships between molecular structure and broad biological effects has been a longstanding challenge in science. Currently, no method exists for forecasting broad biological activity profiles of medicinal agents even within narrow boundaries of structurally similar molecules. Starting from the premise that biological activity results from the capacity of small organic molecules to modulate the activity of the proteome, we set out to investigate whether descriptor sets could be developed for measuring and quantifying this molecular property. Using a 1,567-compound ...

differences in biological environments (8). Considering the complexity of this requirement, computational solutions that precisely link molecular structure to broad biological response are currently not possible (9, 10). We report here an approach to structure–function studies that is based on measurements of the capacity of molecules to interact with the proteome (11).

Translation of Chemical Property Information into Biological Activity Spectra

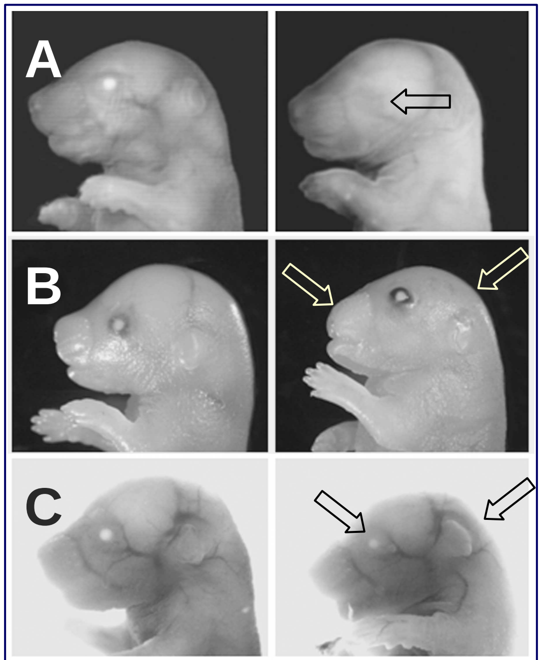
SOURCE: Fliri et al. (2005) PNAS 102: 261–266

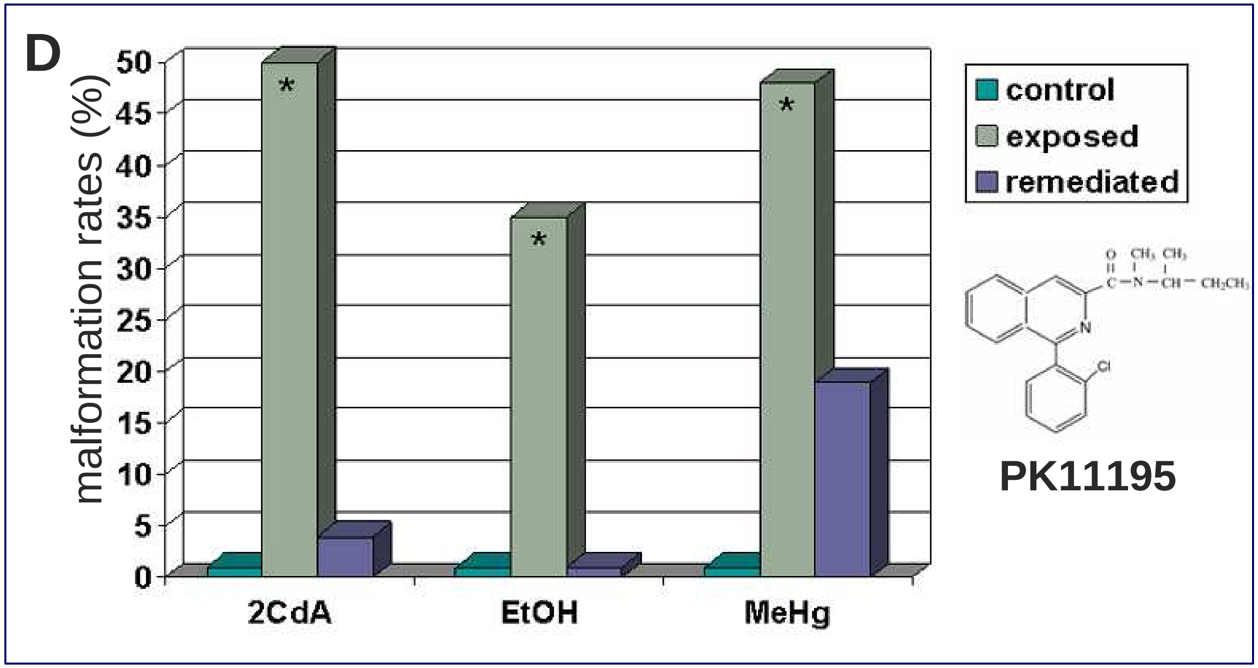
EPA's ToxCast™ Research Program: predictive signatures for thousands of environmental chemicals



SOURCE: David Dix, NCCT

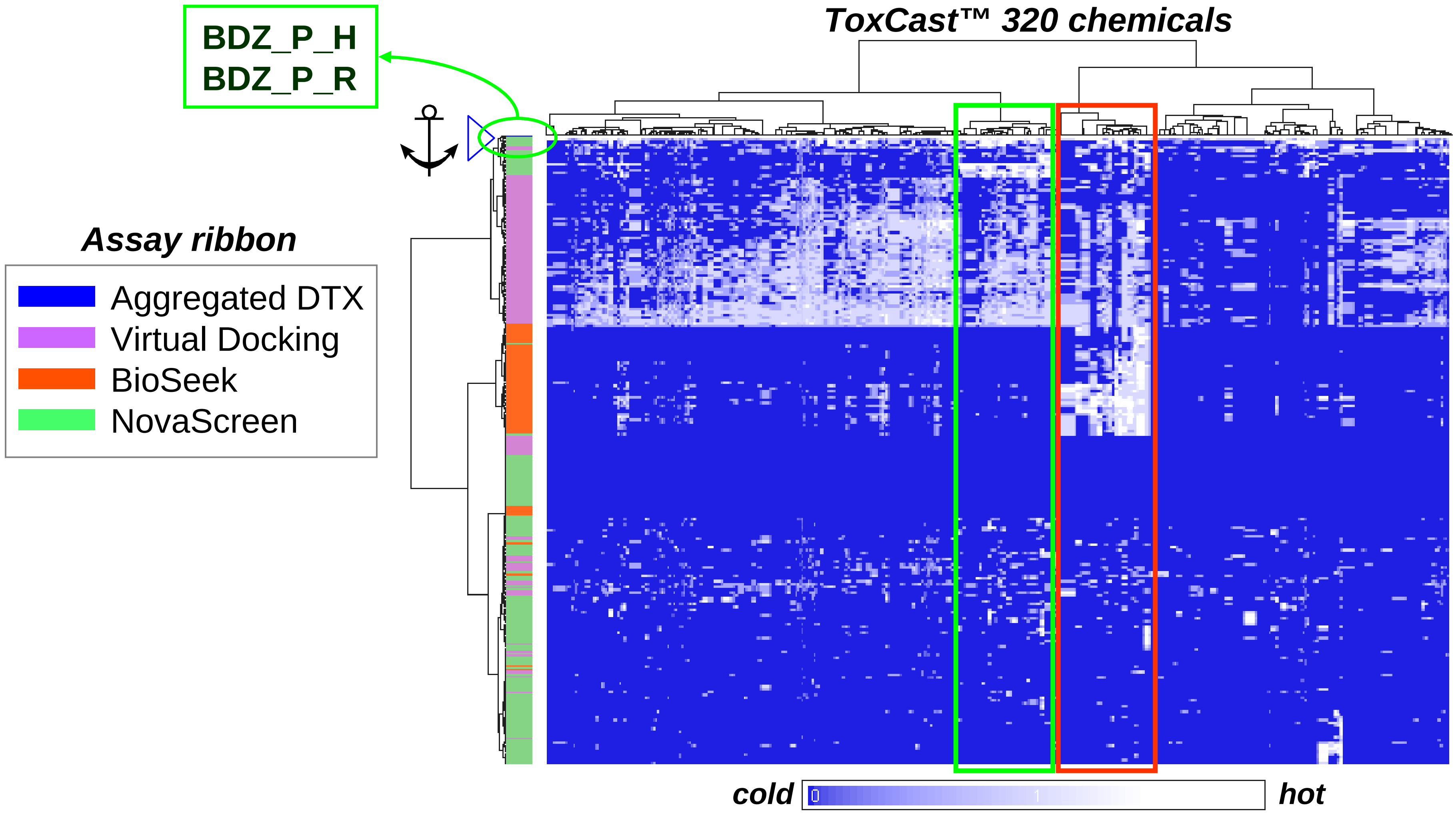
in vivo animal models



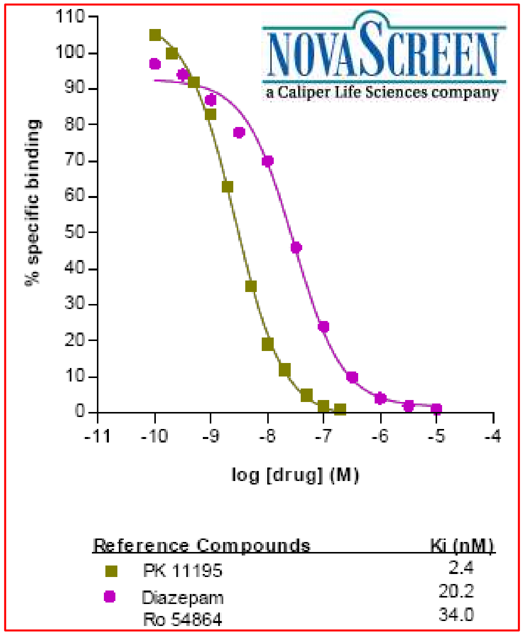


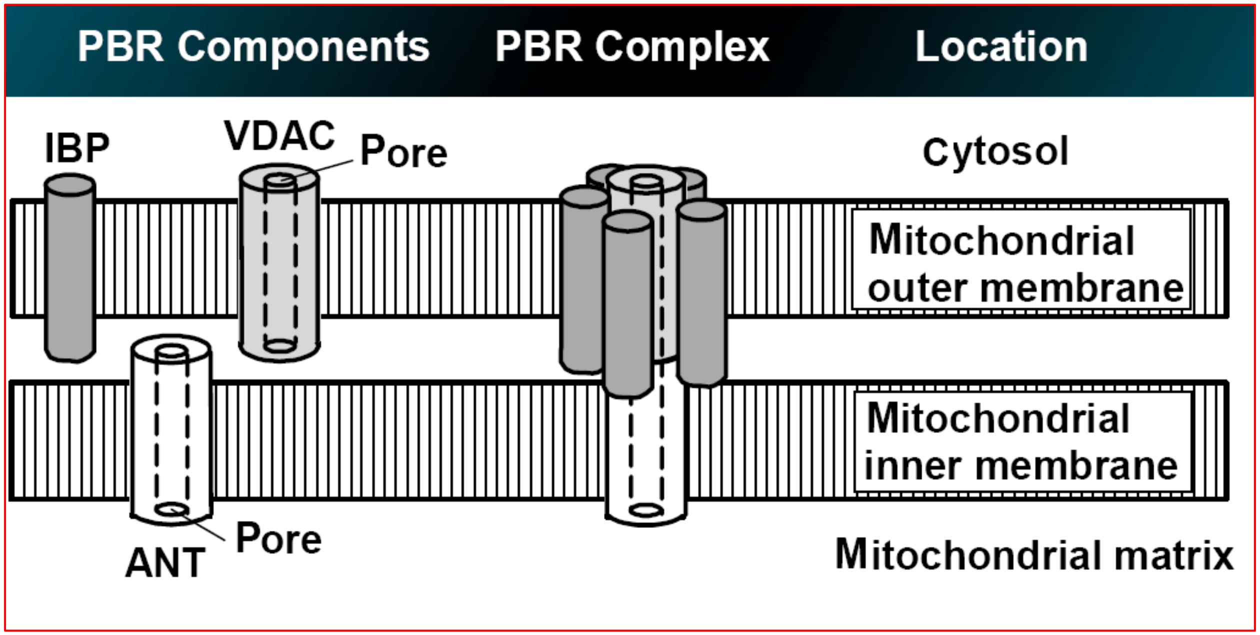
(A) Eye Reduction Defects (ERD) with 2-chloro-2'-deoxyadenosine (**2CdA**) on day 8
(B) Fetal Alcohol Syndrome (FAS) with ethanol (**EtOH**) exposed on gestation day 8
(C) Fetal Minamata Disease (FMD) with methylmercury (**MeHg**) exposed on day 9
(D) Remediation: co-exposed to mitochondrial *BDZ_P* ligand (**PK11195**) on day 8 or 9

bioactivity profile: ~280 chemicals from ~1300 prenatal studies (ToxRefDB_3700) clustered by ~380 assays and anchored to aggregate developmental toxicity



BDZP: peripheral-type benzodiazepine receptor (new name TSPO for mitochondrial 18K TranSPOrter)

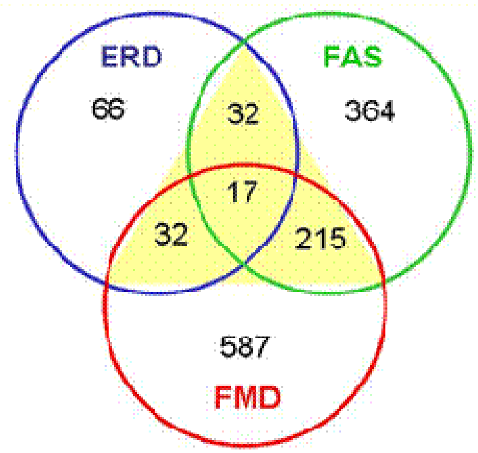




Putative functions:

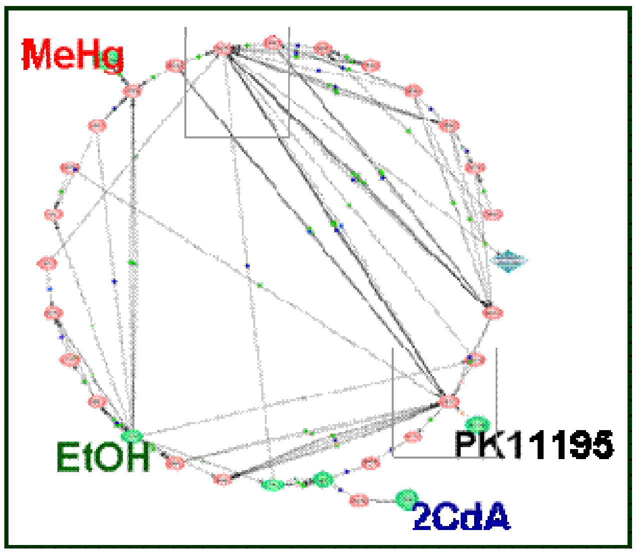
steroidogenesis (StAR)
oxygen sensing (NRF1)
apoptosis (BCL2)
genomic signals (p53)

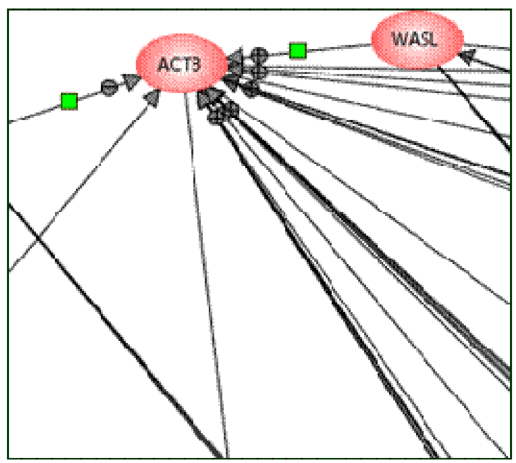
core gene network in early embryos

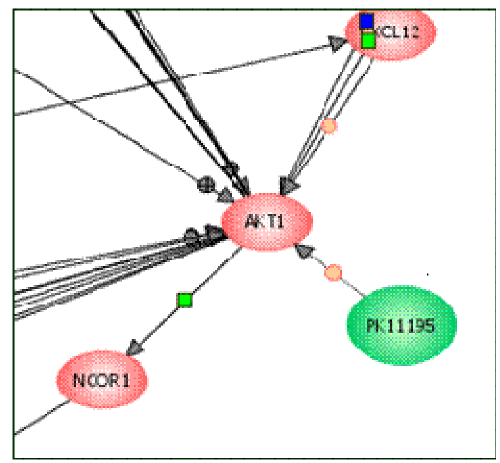


296 genes with altered mRNA expression
In the embryonic forebrain at 3.0h post-exposure

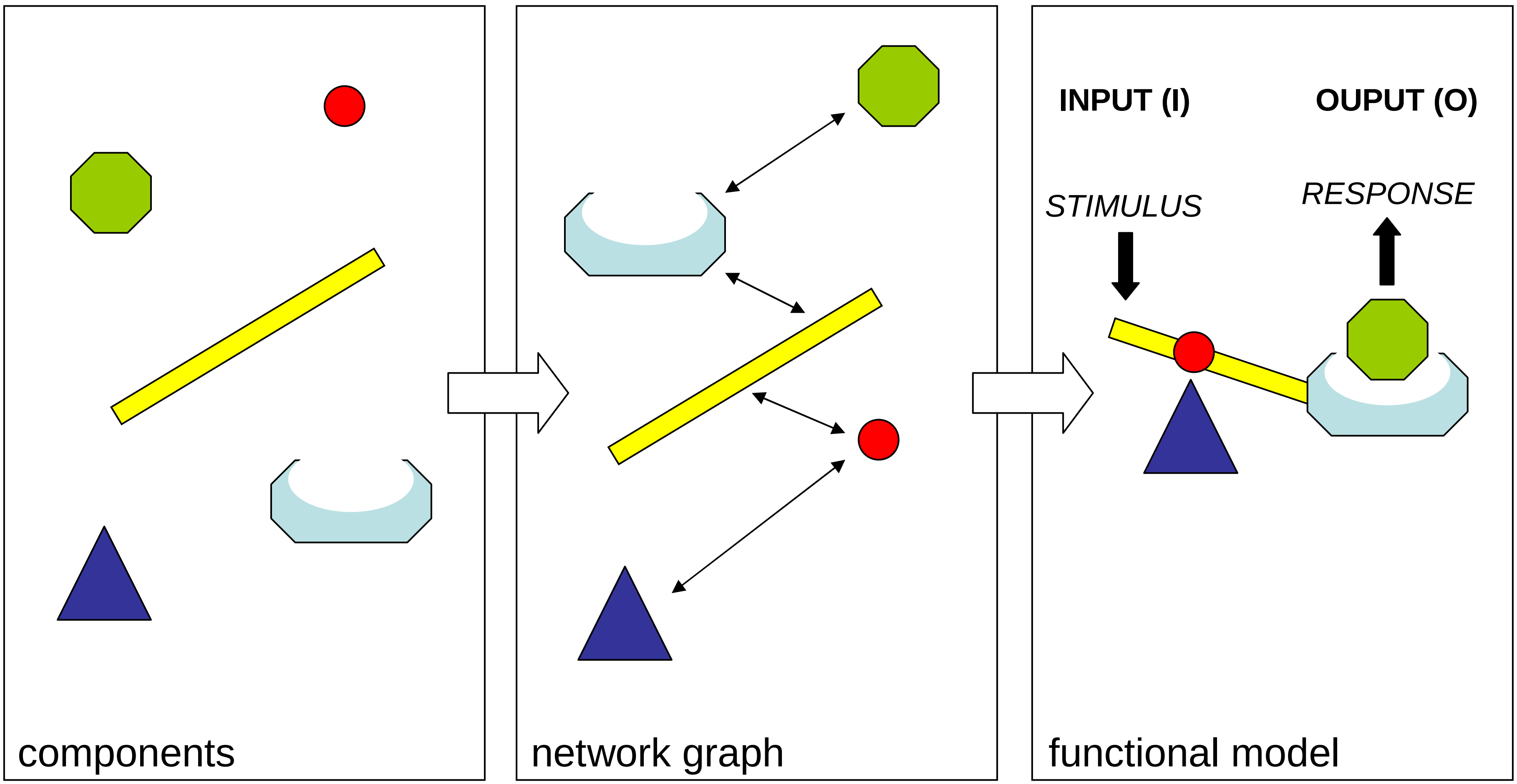
KEGG PATHWAY	*LIST*	*P value*
RIBOSOME	55	2.52E-06
FOCAL ADHESION	41	0.010225
CALCIUM SIGNALING PATHWAY	36	0.006316
INSULIN SIGNALING PATHWAY	27	0.034363
PHOSPHATIDYLINOSITOL SIGNALING SYSTEM	23	0.005534
GAP JUNCTION	20	0.037796
LONG-TERM DEPRESSION	18	0.016329
ADHERENS JUNCTION	17	0.045554
GLYCOLYSIS / GLUCONEOGENESIS	16	0.011854
LONG-TERM POTENTIATION	15	0.029993
PROTEASOME	12	0.003279
TYPE II DIABETES MELLITUS	12	0.028197







computational systems models:
why mathematical modeling is urgently needed



Based on MW Covert (2006) Integrated regulatory and metabolic models. In: Computational Systems Biology, edited by A Kriete and R Eils, Elsevier Academic Press (page 194)

in the new vision …

… our ability to create mathematical models describing the function of biological networks will become just as important as traditional lab skills and thinking - D Butler (2001) Nature 409, 758-760

"Molecular biology took Humpty Dumpty apart … mathematical modeling is required to put him back together again …"

– Schnell et al. (2007) Am Sci 95:134

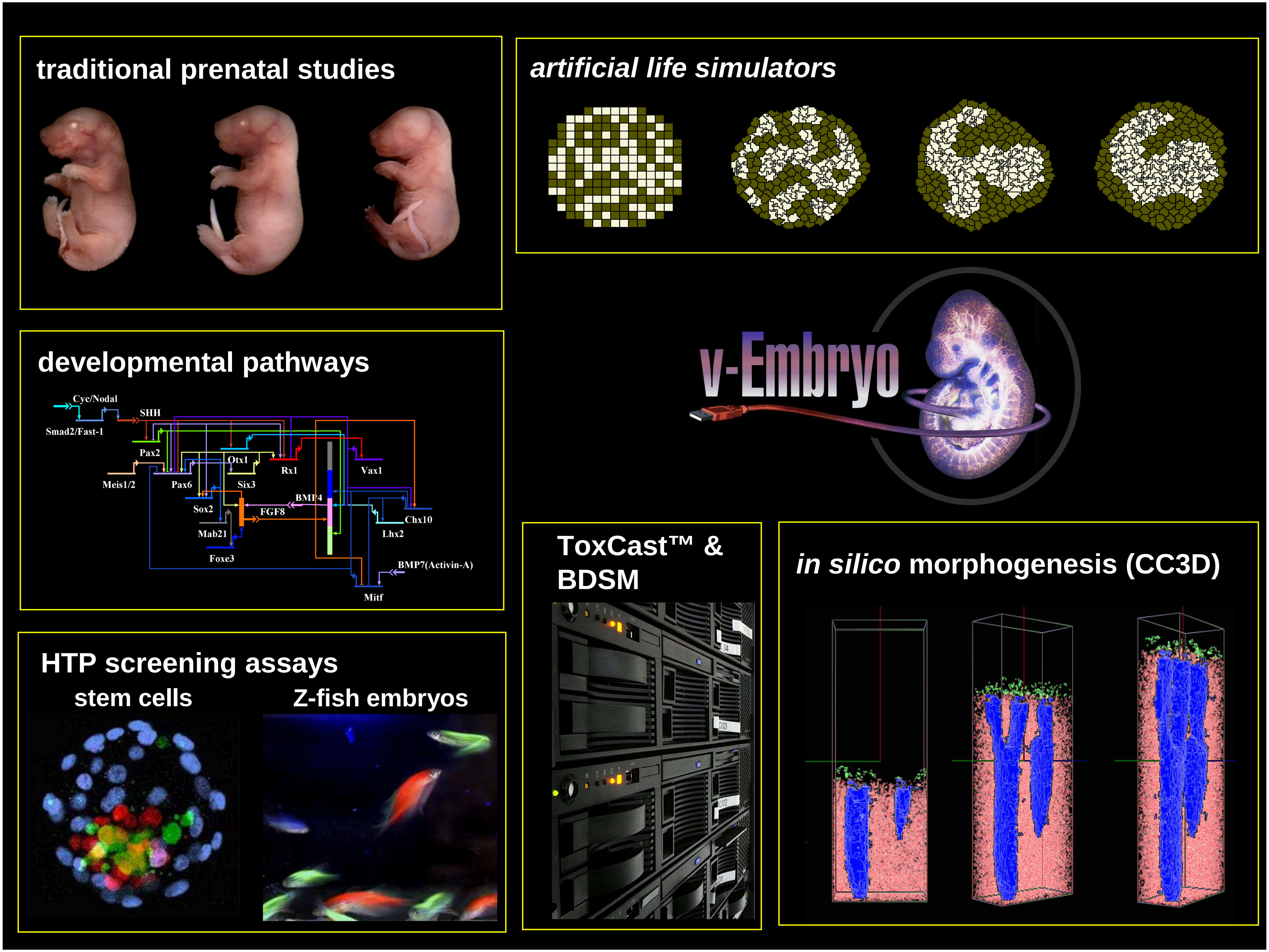
THE VIRTUAL EMBRYO

SOURCE: Matt Shipman, Risk Policy Report, Vol 15, No.2 - January 8 2008

- far-reaching new program to develop a sophisticated computer model of a human embryo …

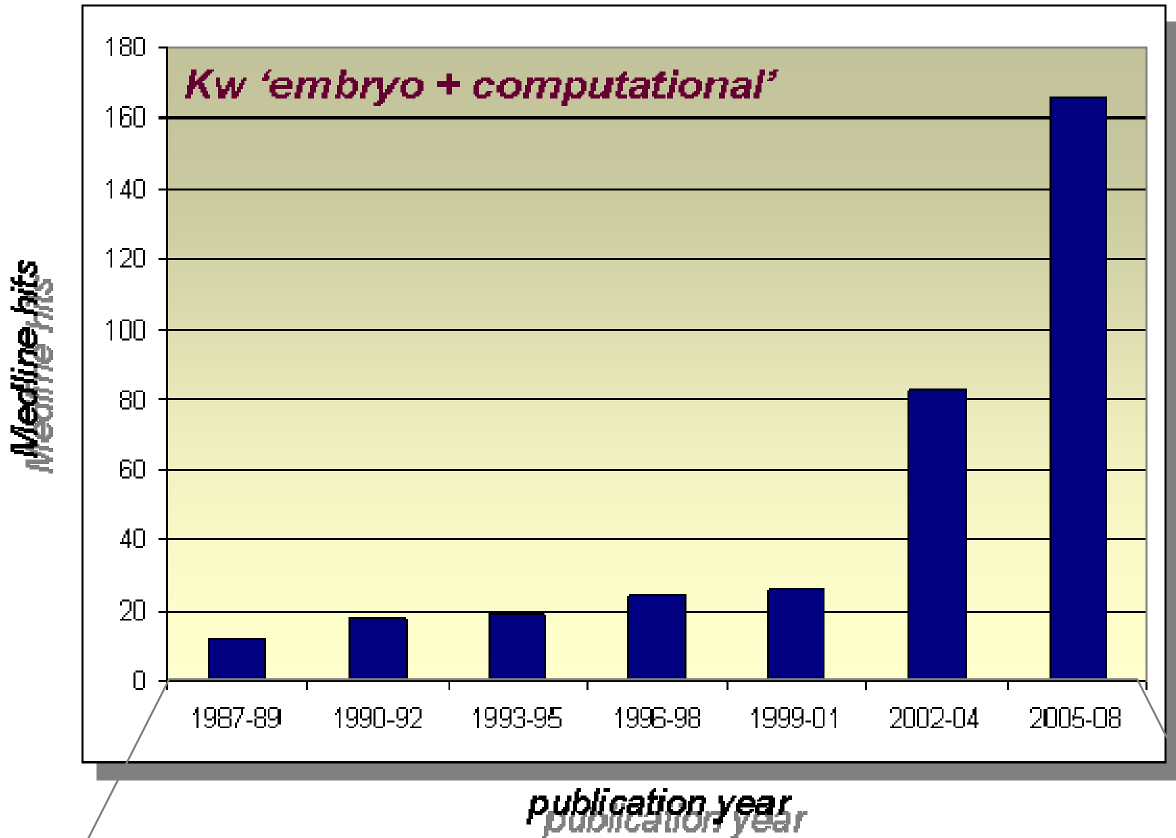
- help regulators better understand the developmental risks posed by chemicals and other environmental stressors

- help the agency better identify sensitive subpopulations and, ultimately, allow researchers to rapidly predict a chemical's potential developmental toxicity


traditional prenatal studies
artificial life simulators
developmental pathways
Cyc/Nodal
SHH
Smad2/Fast-1
Pax2
Otx1
Rx1
Vax1
Meis1/2
Pax6
Six3
BMP4
Sox2
FGF8
Chx10
Mab21
Lhx2
Foxe3
BMP7(Activin-A)
Mitf
v-Embryo
ToxCast™ &
BDSM
in silico morphogenesis (CC3D)
HTP screening assays
stem cells
Z-fish embryos

- S. Kubrick, 1968 “Starchild”

computational embryology:
impact of the human genome project



descriptive biology	◁	cell biology biochemistry	molecular biology	genomics bioinformatics	systems biology	▷ *virtual biology*

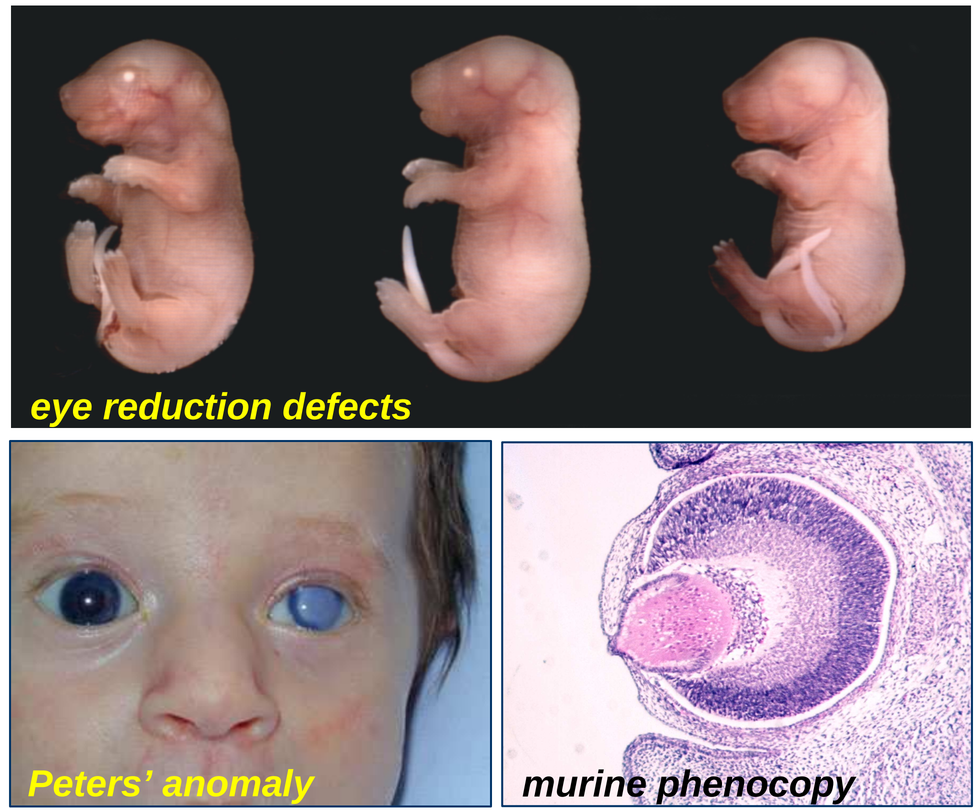
Developmental Systems Biology

- virtual tissues and artificial life simulators as models to study morphogenesis and predict defects *in silico*
- addresses how mechanisms at one scale (cellular) interact to produce higher level (tissue) phenomena
- just as molecular networks determine local cell behavior, discrete cellular networks drive morphogenesis
- myriad of agents that can disrupt morphogenesis requires understanding systems at a network-level

research prototype:
vertebrate eye development

Attributes:

- good literature base
- human relevance
- reciprocal tissue interactions
- morphogenetic cell death
- range of ocular phenotypes
- often non-syndromic
- genetic susceptibility
- environmental sensitivity
- developmental ontology
- self-regulating gene networks
- conservation of cell signaling
- mitochondrial dependency
- cellular interaction networks
- simple/complex

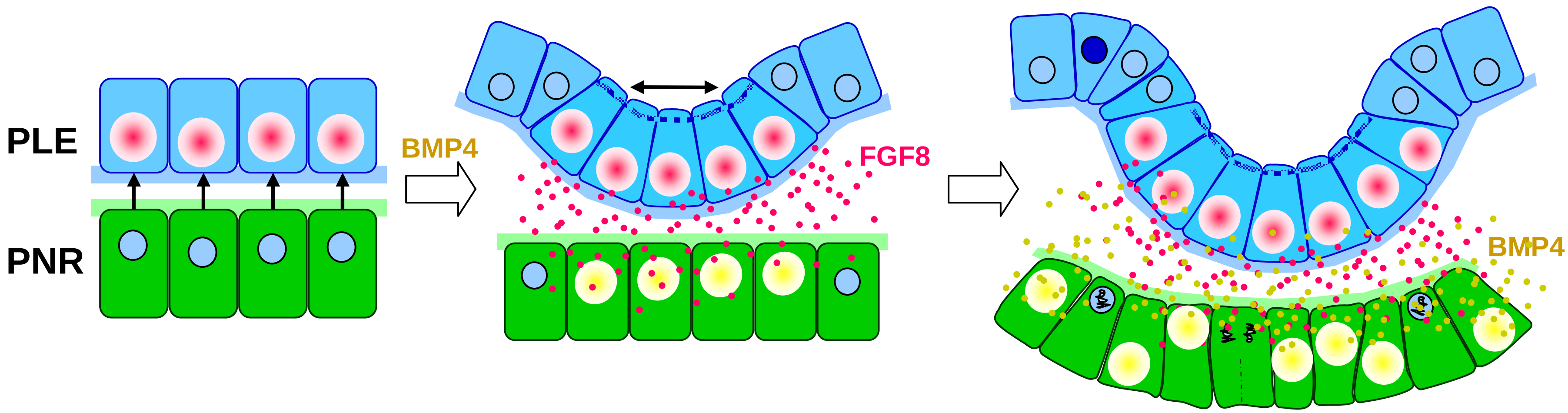


SOURCE: Wubah et al. (2001) Teratology 64: 154-169
Charlap et al. (2003) Birth Def Res A 67: 108-115

Key event: lens vesicle ←→ optic vesicle

cell-based processes driving the natural system

PATTERNING	**MORPHOGENESIS**	**SELECTIVE GROWTH**	**CELL DIFFERENTIATION**
cell interaction	**cytoskeletal remodeling**	**proliferation and apoptosis**	**crystallins, Photorec. genes**



cell-based processes driving the formal system

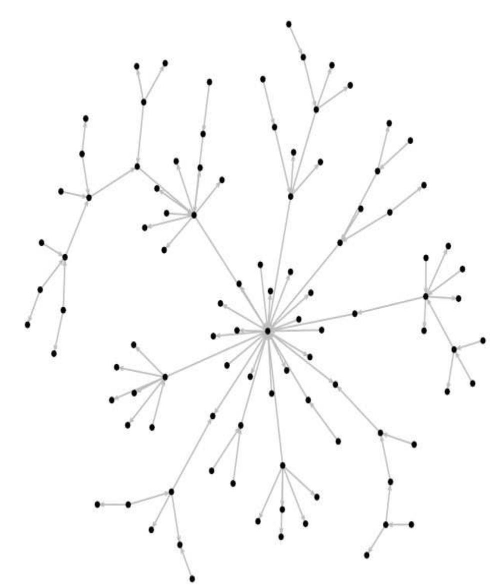
NETWORK LOGIC	**CELLULAR AUTOMATA**	**BIOCHEMICAL FIELDS**	**STATE TRANSITIONS**
information flow	**cellular Potts model**	**Reaction-Diffusion eq.**	**cell type & state module**

gene regulatory networks (GRNs)

INPUT SIGNAL → **PROCESSOR** → **OUTPUT RESPONSE**

Developmental Signals

Wnt, TGFβ, Shh, RTK, Notch-Delta, NF-kB, PCD, nuclear hormone receptors, RPTPs, receptor GC, cytokines, NO, GPCRs, integrins, CADs, gap junction, ligand-gated cation channels, UPR, p53

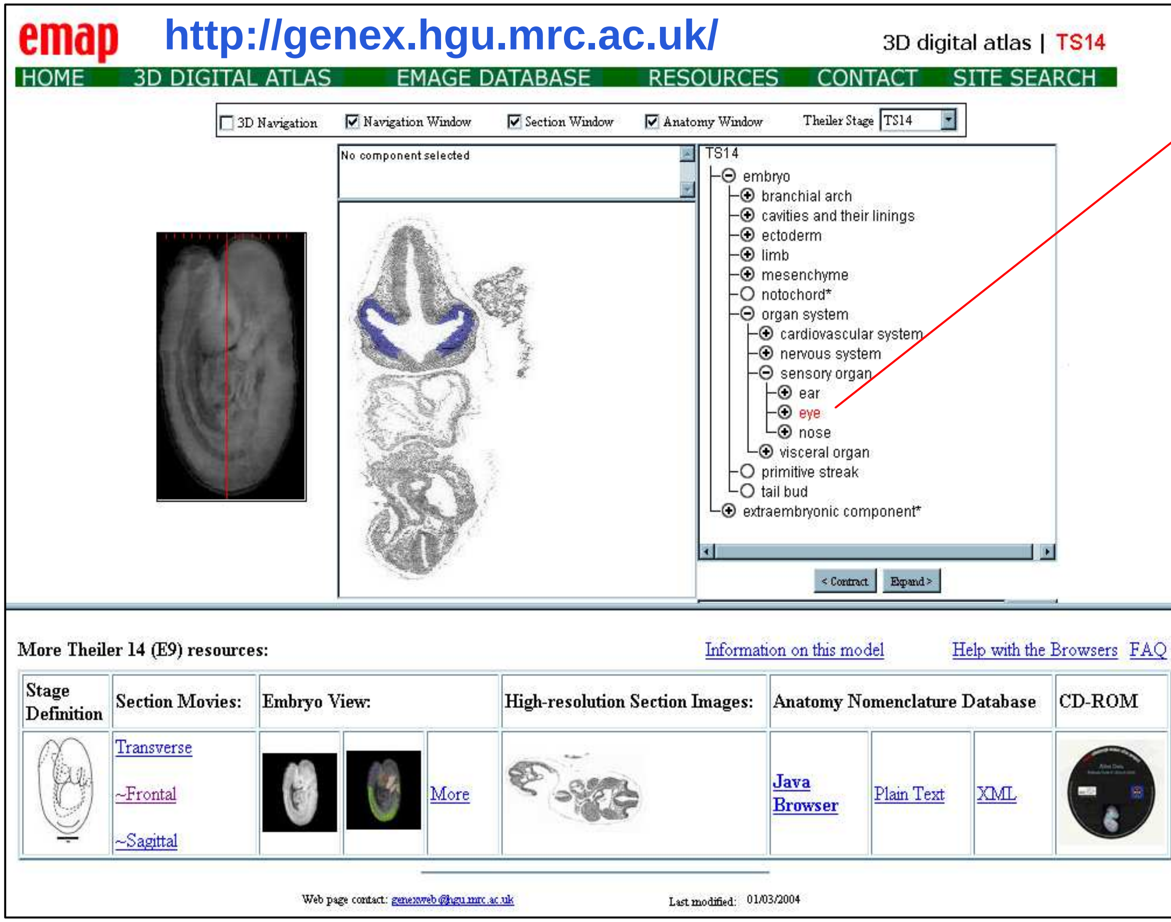


Morphoregulatory Responses

patterning
proliferation
apoptosis
differentiation
adhesion
motility
shape
ECM remodeling

building developmental GRNs:

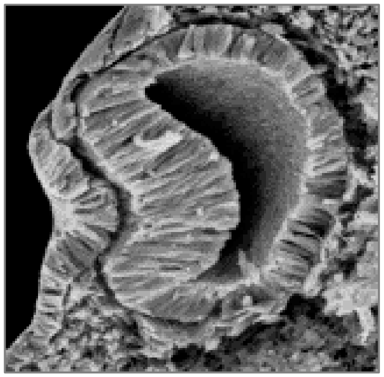
capturing data from EMAGE database

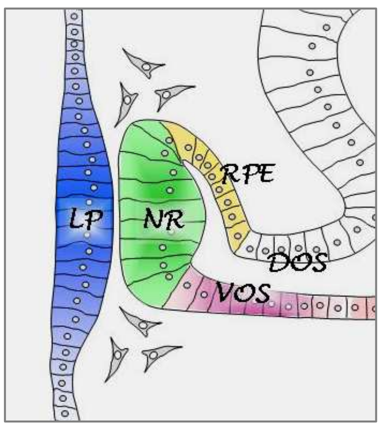


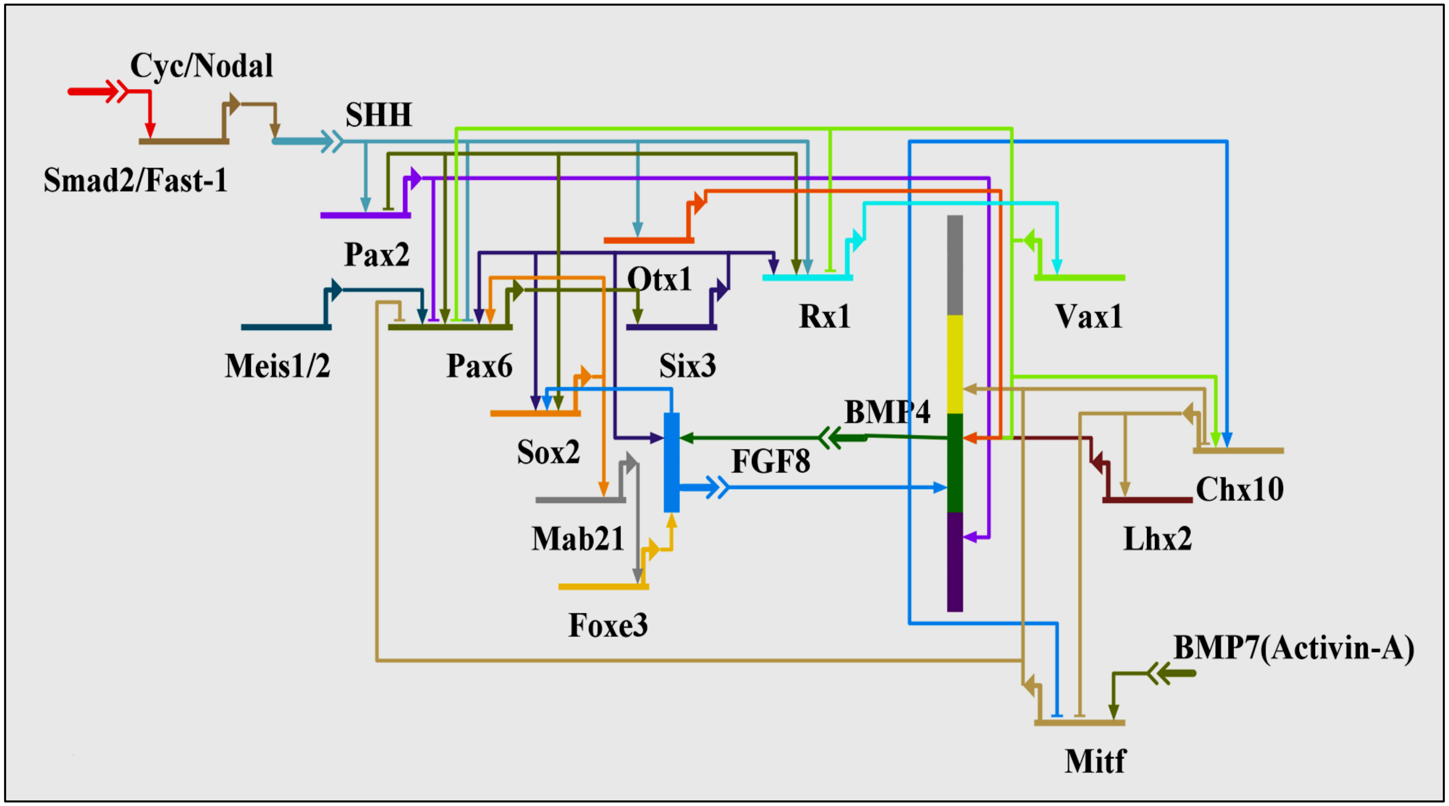
63 genes (TS12-18)
captures 2164 PMIDs

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 mice	12783802	http://www.ncbi	A temperature-sensitiv	Tian J., Yam C	Y	Y	N
2	cyc	shh	fetal or embryo	mouse or mice	10952887	http://www.ncbi	Direct action of the nod	Müller F., Albe	Y	Y	Y
3	cyc	shh	fetal or embryo	mouse or mice	10694427	http://www.ncbi	Two distinct cell popula	Odenthal J., v	Y	N	N
4	cyc	shh	fetal or embryo	mouse or mice	8946241	http://www.ncbi	Expression of axial and	Strähle U., Bla	Y	N	N
5	cyc	shh	fetal or embryo	mouse or mice	8674416	http://www.ncbi	Complex expression of	Hauptmann G	Y	N	N
6	shh	pax2	fetal or embryo	mouse or mice	11731484	http://www.ncbi	vhnf1, the MODY5 and	Sun Z., Hopki	Y	N	N
7	shh	pax2	fetal or embryo	mouse or mice	10601036	http://www.ncbi	Vax1, a novel homeobo	Hallonet M., H	Y	Y	Y
8	shh	pax2	fetal or embryo	mouse or mice	9655820	http://www.ncbi	Sonic hedgehog is not	Schauerte H.,	Y	N	N
9	shh	otx1	fetal or embryo	mouse or mice	15105370	http://www.ncbi	Otx2 regulates the exte	Puelles E., An	Y	N	N
10	Pax2	pax6	fetal or embryo	mouse or mice	17470285	http://www.ncbi	Rybp, a polycomb com	Pirity M., Wan	Y	YN (develop	Y
11	Pax2	pax6	fetal or embryo	mouse or mice	17166916	http://www.ncbi	Lmx1b is essential for	Guo C., Qiu H	Y	N	N
12	Pax2	pax6	fetal or embryo	mouse or mice	16934393	http://www.ncbi	A role for GnRH in earl	Wu S., Page L	Y	Y	Y
13	Pax2	pax6	fetal or embryo	mouse or mice	14711874	http://www.ncbi	Prep1.1 has essential g	Deflorian G., T	Y	D?	Y
14	Pax2	pax6	fetal or embryo	mouse or mice	12970747	http://www.ncbi	Paired-Box genes are f	Muratovska A.	Y	N	N
15	Pax2	pax6	fetal or embryo	mouse or mice	12756174	http://www.ncbi	Retinal pigmented epith	Bäumer N., M	Y	Y	Y
16	Pax2	pax6	fetal or embryo	mouse or mice	11804780	http://www.ncbi	Dach1, a vertebrate ho	Heanue T., Da	Y	YN (Day13 (	N
17	Pax2	pax6	fetal or embryo	mouse or mice	11458394	http://www.ncbi	Six3 promotes the form	Lagutin O., Zh	Y	Y	N
18	Pax2	pax6	fetal or embryo	mouse or mice	11003833	http://www.ncbi	Spatial specification of	Schwarz M., C	Y	Y	N
19	Pax2	pax6	fetal or embryo	mouse or mice	10601036	http://www.ncbi	Vax1, a novel homeobo	Hallonet M., H	Y	Y	Y
20	Pax2	pax6	fetal or embryo	mouse or mice	10051661	http://www.ncbi	Ectopic bone morphog	Golden J., Bra	Y	N	N
21	Pax2	pax6	fetal or embryo	mouse or mice	9831649	http://www.ncbi	Zebrafish contains two	Nornes S., Cla	Y	Y	Y
22	Pax2	pax6	fetal or embryo	mouse or mice	9655820	http://www.ncbi	Sonic hedgehog is not	Schauerte H.,	Y	N	N
23	Pax2	pax6	fetal or embryo	mouse or mice	9297966	http://www.ncbi	Pax genes and organog	Dahl E., Kosel	Y	Y	N
24	Pax2	pax6	fetal or embryo	mouse or mice	8126546	http://www.ncbi	Roles of Pax-genes in	Stoykova A., G	Y	N	N
25	pax6	rx1	fetal or embryo	mouse or mice	12142024	http://www.ncbi	The homeobox gene m	Kawahara A.,	Y	Y	Y (Did not und
26	pax6	sox2	fetal or embryo	mouse or mice	17825048	http://www.ncbi	PAX6 and SOX2-depe	Inoue M., Kam	Y		
27	pax6	sox2	fetal or embryo	mouse or mice	17470285	http://www.ncbi	Rybp, a polycomb com	Pirity M., Wan	Y	YN	Y
28	pax6	sox2	fetal or embryo	mouse or mice	17066077	http://www.ncbi	Six3 activation of Pax6	Liu W., Laguti	Y	Y	Y
29	pax6	sox2	fetal or embryo	mouse or mice	15926496	http://www.ncbi	[Understanding lens dif	Uchikawa M.,	Y		
30	pax6	sox2	fetal or embryo	mouse or mice	15855760	http://www.ncbi	The involvement of neu	Reza H., Yasu	Y	Y	Y
31	pax6	sox2	fetal or embryo	mouse or mice	15652703	http://www.ncbi	Requirement of mesod	Molotkova N.,	Y	N	N
32	pax6	sox2	fetal or embryo	mouse or mice	15558474	http://www.ncbi	Interplay of Pax6 and S	Kondoh H., U	Y	YN*	YN*
33	pax6	sox2	fetal or embryo	mouse or mice	12710953	http://www.ncbi	Pax6 autoregulation me	Aota S., Nakaj	Y	Y	Y
34	pax6	sox2	fetal or embryo	mouse or mice	12642482	http://www.ncbi	Cell-autonomous involv	Yamada R., M	Y	N*	Y*
35	pax6	sox2	fetal or embryo	mouse or mice	11069887	http://www.ncbi	Pax6 activity in the lens	Ashery-Padan	Y	N*	N
36	pax6	sox2	fetal or embryo	mouse or mice	9851982	http://www.ncbi	BMP4 is essential for l	Furuta Y., Hog	Y	Y*	Y*
37	pax6	sox2	fetal or embryo	mouse or mice	9609835	http://www.ncbi	Involvement of Sox1, 2	Kamachi Y., U	Y	Y	N
38	pax6	six3	fetal or embryo	mouse or mice	17470285	http://www.ncbi	Rybp, a polycomb com	Pirity M., Wan	Y	YN (develop	Y
39	pax6	six3	fetal or embryo	mouse or mice	17066077	http://www.ncbi	Six3 activation of Pax6	Liu W., Laguti	Y	Y	Y
40	pax6	six3	fetal or embryo	mouse or mice	16765103	http://www.ncbi	Identification of genes	Lord-Grignon	Y	Y*	Y*
41	pax6	six3	fetal or embryo	mouse or mice	15569927	http://www.ncbi	Tyrosine phosphorylati	Gotoh N., Ito M	Y		
42	pax6	six3	fetal or embryo	mouse or mice	15366004	http://www.ncbi	Raldh2 expression in o	Mic F., Molotk	Y		
43	pax6	six3	fetal or embryo	mouse or mice	12642482	http://www.ncbi	Cell-autonomous involv	Yamada R., M	Y	N	N
44	pax6	six3	fetal or embryo	mouse or mice	11956759	http://www.ncbi	exma: an X-linked inse	Cunningham D	Y	N	N
45	pax6	six3	fetal or embryo	mouse or mice	11458394	http://www.ncbi	Six3 promotes the form	Lagutin O., Zh	Y	Y	N
46	pax6	six3	fetal or embryo	mouse or mice	10502109	http://www.ncbi	Mouse Dach, a homolo	Davis R., She	Y	Y*	YN*
47	pax6	six3	fetal or embryo	mouse or mice	10090721	http://www.ncbi	Six3 overexpression ini	Loosli F., Win	Y	Y	Y
48	pax6	six3	fetal or embryo	mouse or mice	9651515	http://www.ncbi	Six3, a medaka homolo	Loosli F., Köst	Y	Y	Y
49	sox2	foxe3	fetal or embryo	mouse or mice	12642482	http://www.ncbi	Cell-autonomous involv	Yamada R., M	Y	N	N
50	vax1	chx10	fetal or embryo	mouse or mice	17470285	http://www.ncbi	Rybp, a polycomb com	Pirity M., Wan	Y	YN (develop	Y
51	chx10	lhx2	fetal or embryo	mouse or mice	15385160	http://www.ncbi	Requirement for Mab21	Yamada R., M	Y	N	N
52	chx10	mitf	fetal or embryo	mouse or mice	16420417	http://www.ncbi	Loss of retinal progenit	Coles B., Hors	Y	N	N
53	chx10	mitf	fetal or embryo	mouse or mice	15459106	http://www.ncbi	Transdifferentiation of	Rowan S., Ch	Y	N	N
54	chx10	mitf	fetal or embryo	mouse or mice	11023863	http://www.ncbi	Extraocular mesenchym	Fuhrmann S.,	Y	Y	Y
55	chx10	mitf	fetal or embryo	mouse or mice	10903182	http://www.ncbi	Signaling and transcrip	Nguyen M., A	Y	Y	Y
56	Pax6	Meis	fetal or embryo	mouse or mice	12183364	http://www.ncbi	Meis homeoproteins di	Zhang X, Frie	Y	Y	Y

GRN for early eye development: built by semi-automated database + literature mining

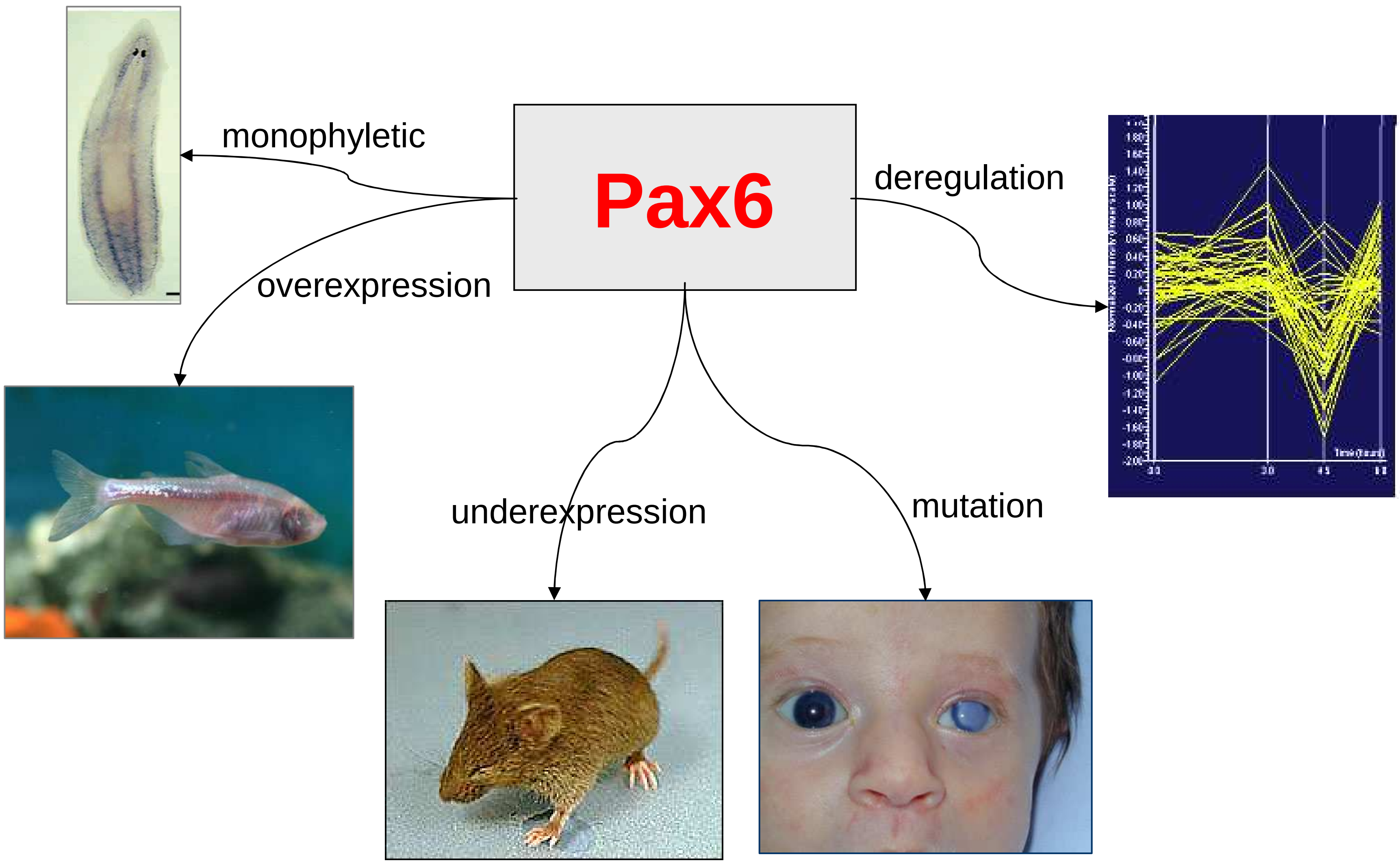




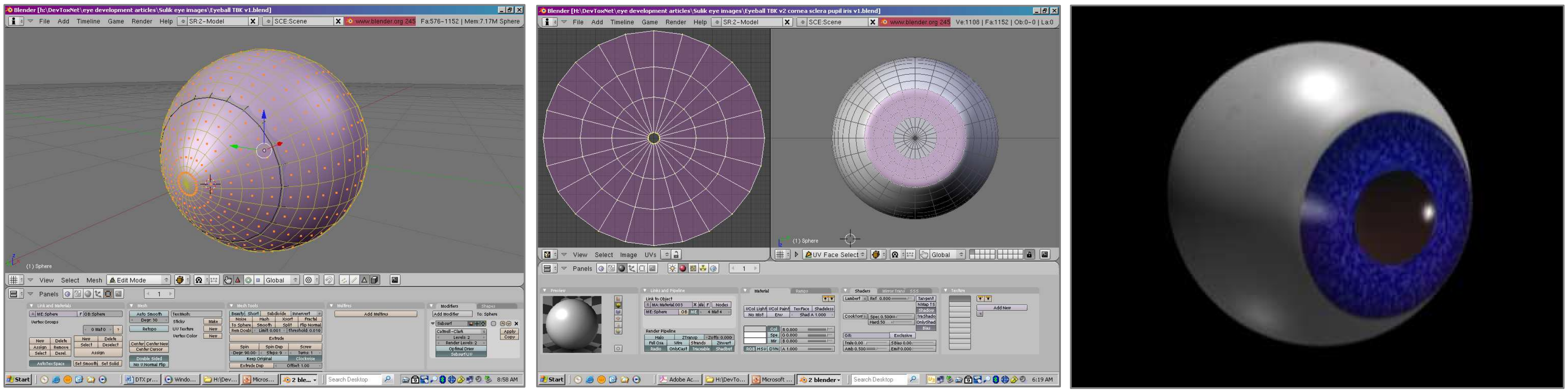


SOURCE: Knudsen and Singh (2008), manuscript in preparation

consequences of perturbing GRNs

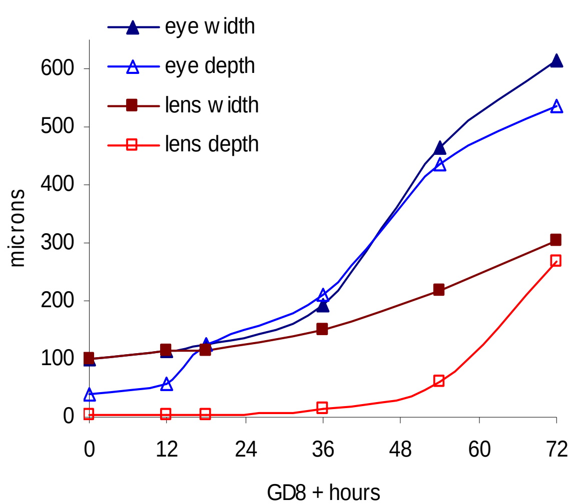


simulation: surface representation



Model of the eye constructed with Blender 2.5 software, **Blender**foundation, http://www.blender.org

MORPHOMETRY: on-line atlases and SEM image banks of mouse eye development



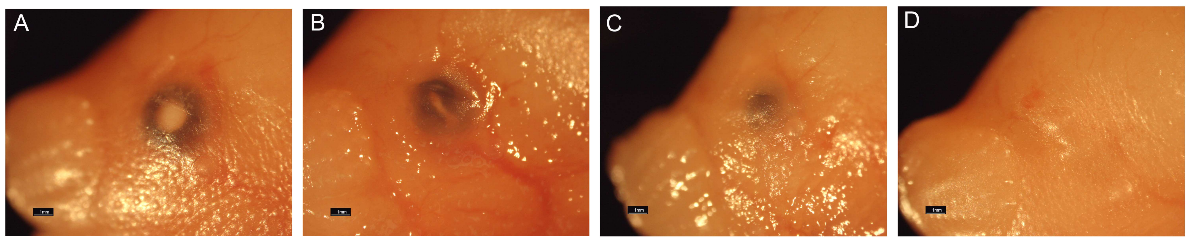
$$y = a * b^{x}$$

Parameter	Coefficient	Standard error	P	R, adj R sqr
eye width				
variable a	67.8043	18.4709	0.0089	0.98150301
variable b	1.0279	0.0034	< 0.0001	0.95433389
eye depth				
variable a	77.3446	22.6413	0.0269	0.96849494
variable b	1.0282	0.0048	< 0.0001	0.92247805
lens width				
variable a	67.7712	5.0620	< 0.0001	0.99358307
variable b	1.0172	0.0010	< 0.0001	0.98425755
lens depth				
variable a	0.7208	0.1232	0.0043	0.99939671
variable b	1.0857	0.026	< 0.0001	0.99924413

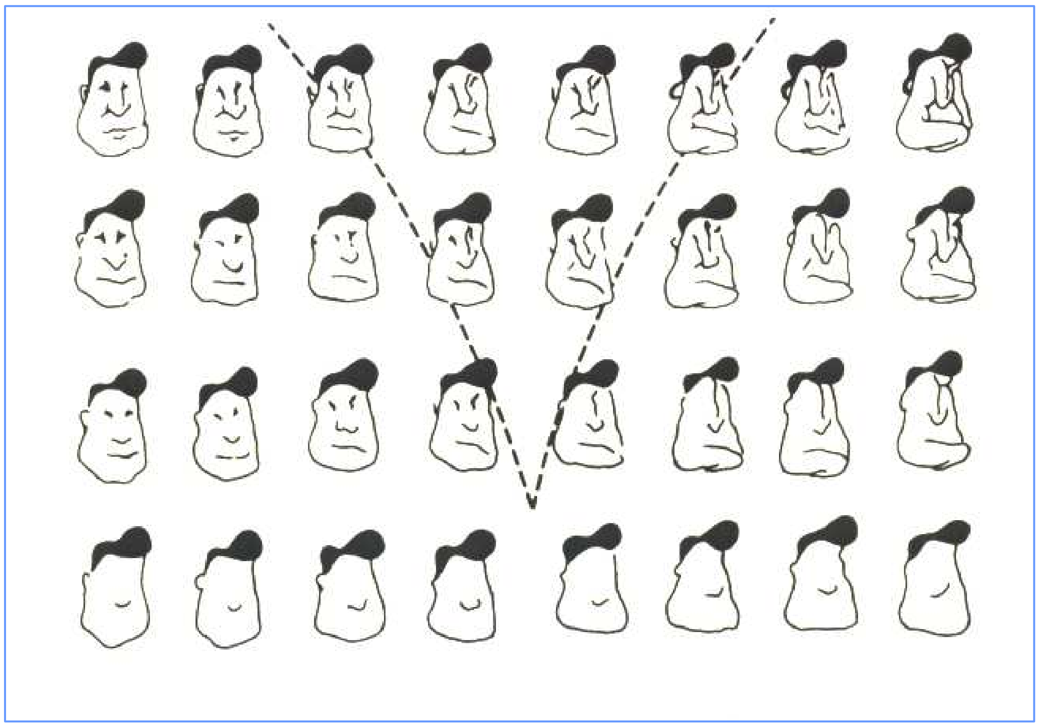
challenge: modeling catastrophe *in silico*

small changes in nonlinear system → sudden shifts in behavior

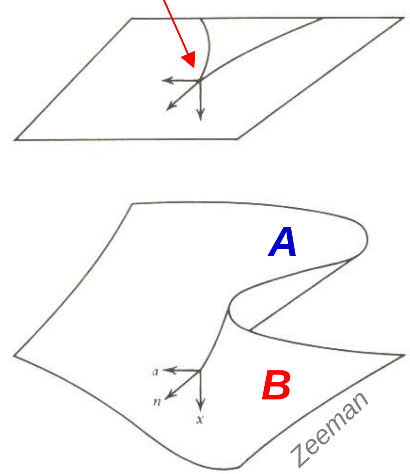
STATE A ←------→ **STATE B**

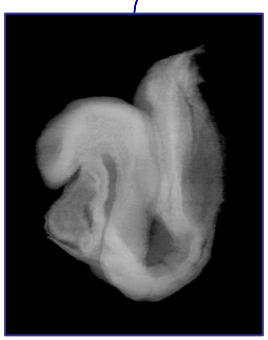


Gestation →



critical point

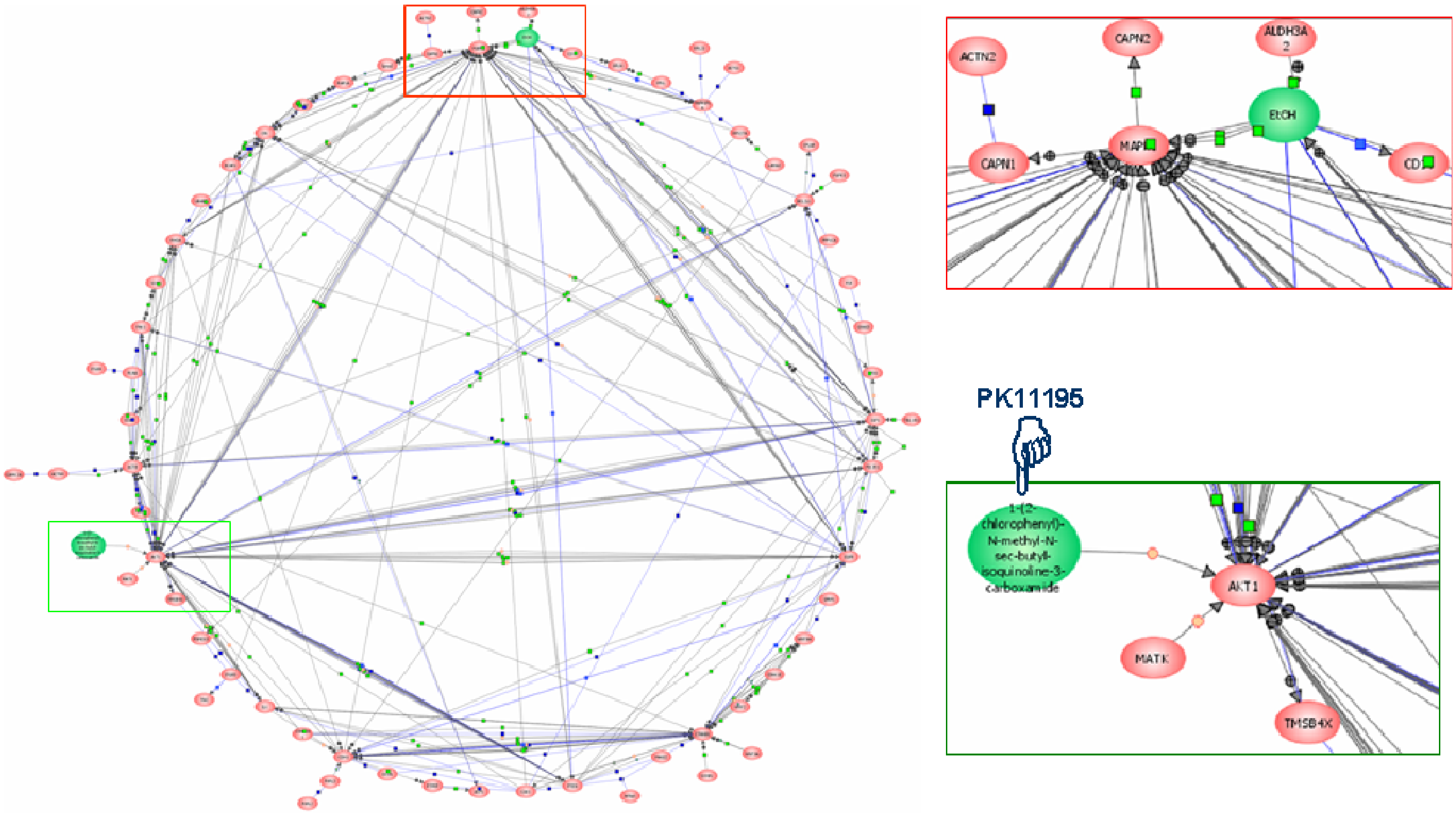




exposure

SOURCE: Saunders, 1980, Cambridge University Press NY

FAS network: genes affected in the rudimentary forebrain during maternal alcohol exposure



SOURCE: Green ML, Singh AV, Zhang Y, Nemeth KA, Sulik KK and Knudsen TB (2007) Reprogramming of genetic networks during initiation of the fetal alcohol syndrome. Devel Dynam 236: 613-631

Functional models: differential cell adhesion in mouse embryos modeled *in silico*

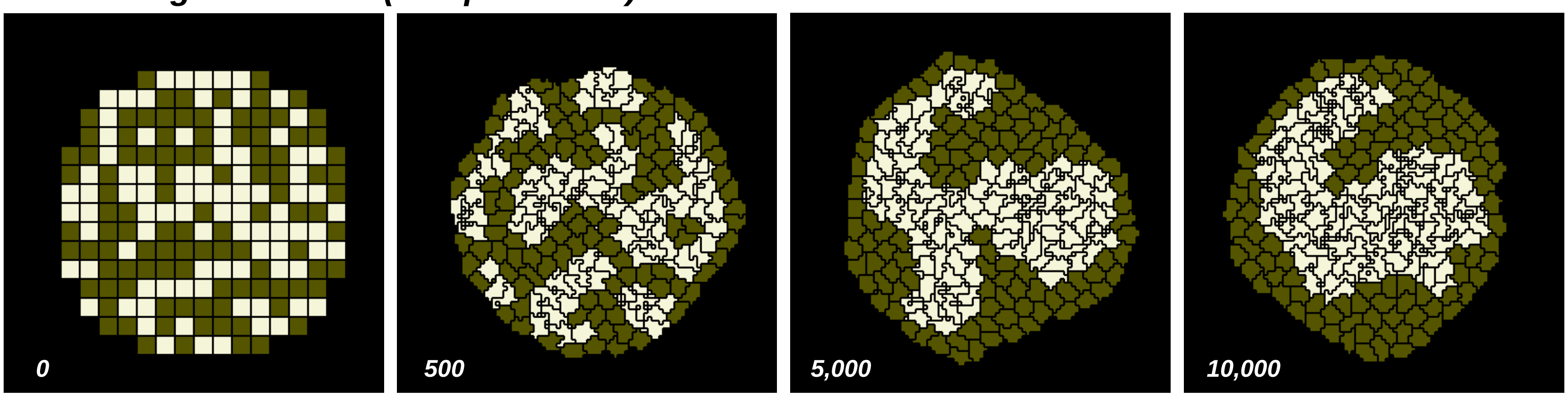
Cell autonomous sorting (biological)

receptor-mediated cell adhesion genes

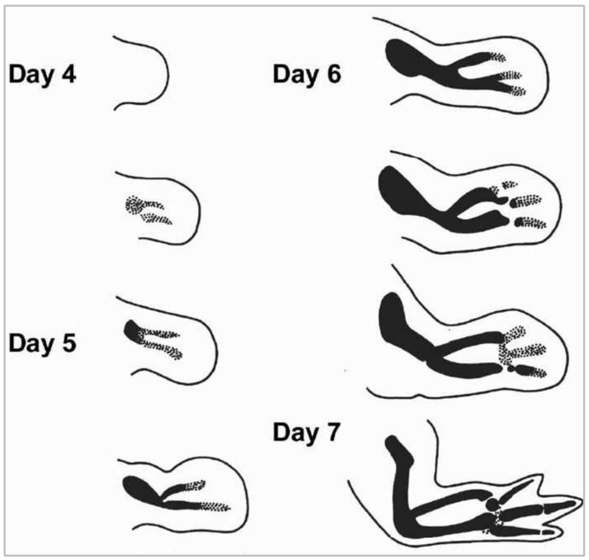
Gene	B6J	B6N	*B6J-B6N differential*
Procollagen 1 alpha 2	↑	↑	+ 0.12
Procollagen 6 alpha 2	↑	--	+ 0.74
Integrin alpha 3	↑	↓	+ 1.09
Integrin beta 3	↓	--	- 0.42
Caveolin-1	↑	--	+ 0.75
L1 cell adhesion molecule	--	↓	+ 0.20
Focal adhesion kinase (PTK2)	--	↓	+ 1.28
Ephrin b1	↑	↓	+ 1.38
Ephrin b2	--	↓	+ 1.61
Ephrin b3	↑	--	+ 0.88
Ephrin a5	↑	↓↓	+ 2.24
EPH receptor A2	--	↓	+ 0.68
EPH receptor A4	--	↓	+ 1.34
IGF-1	↑	--	+ 0.35
IGF-1 receptor	--	↓	+ 0.70

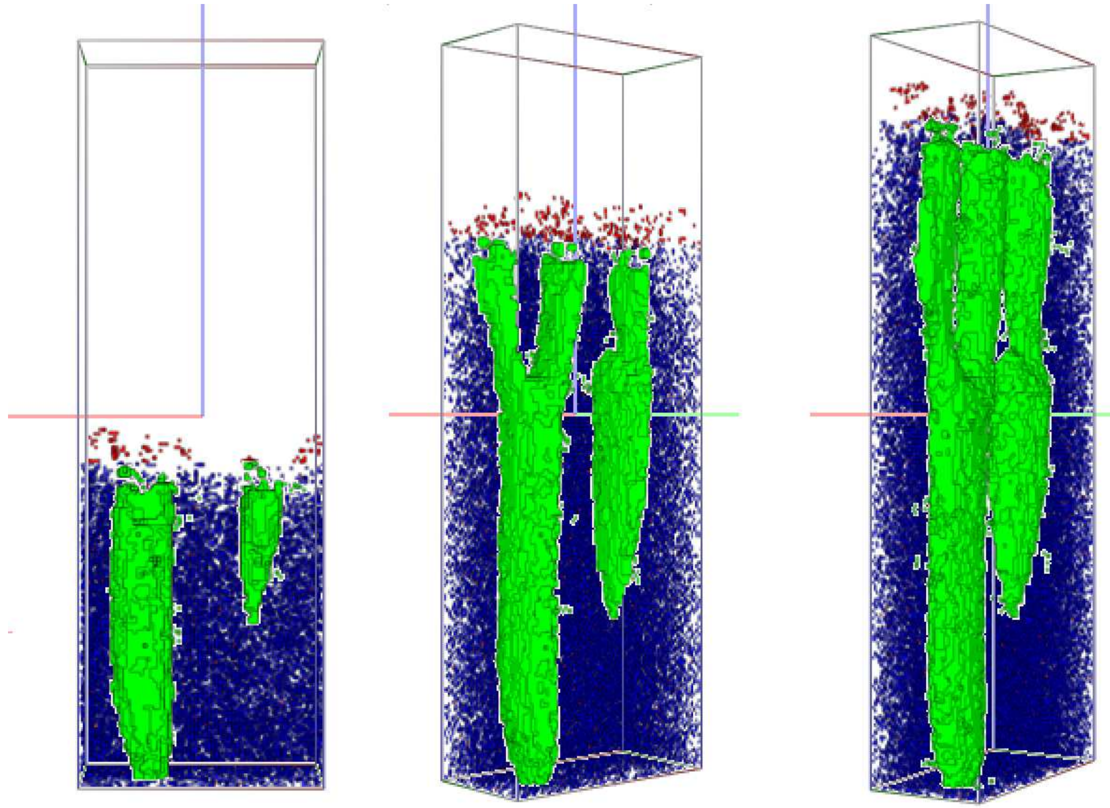
Subset of significantly altered genes in focal adhesion linked by functional classification and expression profile; up-/down regulation at threshold of 0.75-fold change. The differential is given in log(2) units (eg, 1 = two-fold change).

Cell sorting automaton (computational)



toolbox for *in silico* morphogenesis:
agent-based model of chick limb development





CompuCell3D Rendering at steps 1750, 2750, 3250

SOURCE: Cickovski et al. (2005) IEEE/ACM Trans Comput Biol Bioinfor 2: 1-15

Complicated for sure but inevitably …

- computational models will be needed to unravel biological complexity
- virtual tissues and artificial life simulators will become more common research techniques
- *in silico* experiments can test the complex factors that influence morphogenesis and teratogenesis
- *e*-science will need to close the gap between virtual biology and the real world

Acknowledgements

University of Louisville
Caleb Bastian
Maia Green
Denis Kinane
Ken Knudsen
Kim Nemeth
Nafeesa Owens
Bruno Ruest
Amar Singh
Reetu Singh

NIEHS National Institute of Environmental Health Sciences

NIAAA National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health

NCCT/EPA
Jerry Blancato
David Dix
Rocky Goldsmith
Keith Houck
Richard Judson
Bob Kavlock
Matt Martin
Imran Shah
Amar Singh (LHM)

Sid Hunter (NHEERL)
Stephanie Padilla (NHEERL)
Richard Spencer (EMVL)

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