Safety Pharmacology Society, Washington DC
Symposium: “In silico Approaches to Safety Pharmacology”
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Computational Embryology – translational applications in modeling developmental toxicity

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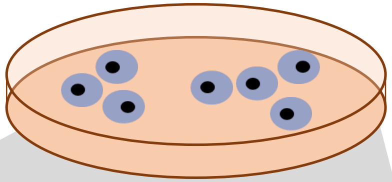
Problem statement

- Automated HTS assays enable rapid screening to help ‘decode the toxicological blueprint of active substances that interact with living systems’ [Sturla et al. 2014].
- Vast HTS data now in hand [https://comptox.epa.gov/dashboard], the need arises for novel *in silico* models that can advance efforts toward predictive toxicology.
- Reducing a complex biological system to simpler assays for chemical profiling disrupts the spatial and temporal dynamics that render a system complex in the first place.
- Toward predicting the potential for human toxicity with less reliance on vertebrate animal testing, we need *in silico* models that can rebuild this complexity.

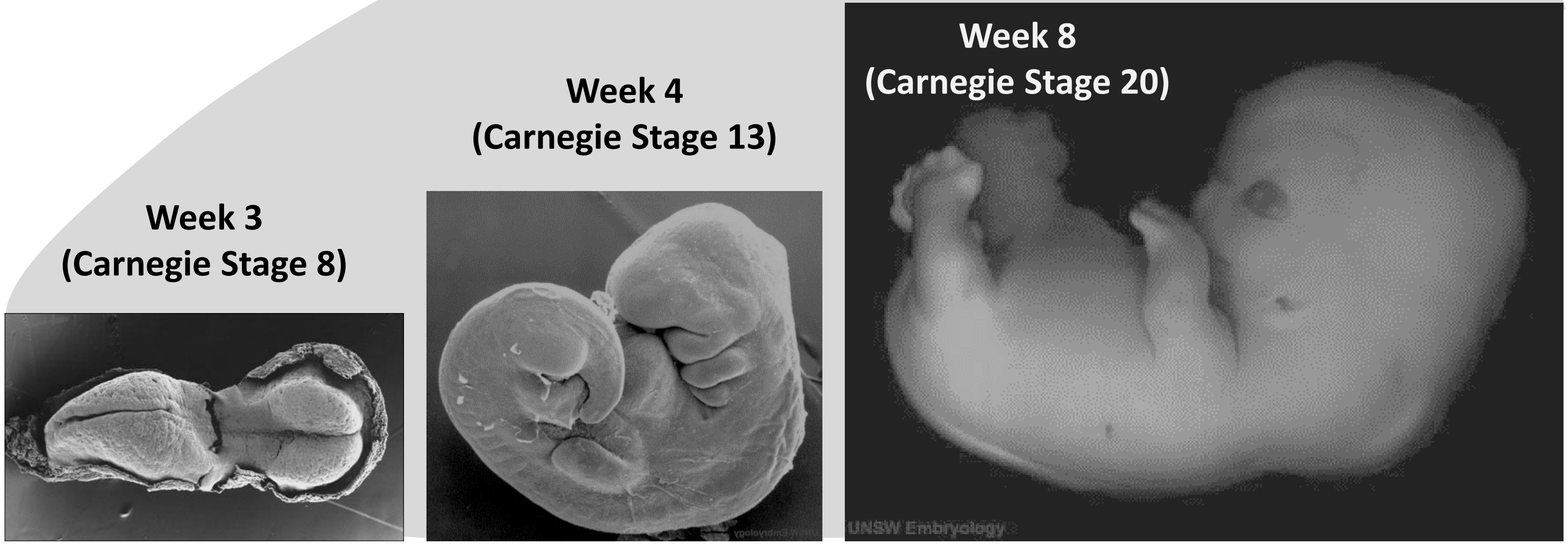
Developmental Toxicity



- Commonly evaluated by exposing pregnant rats and/or rabbits during gestation of major organ systems.

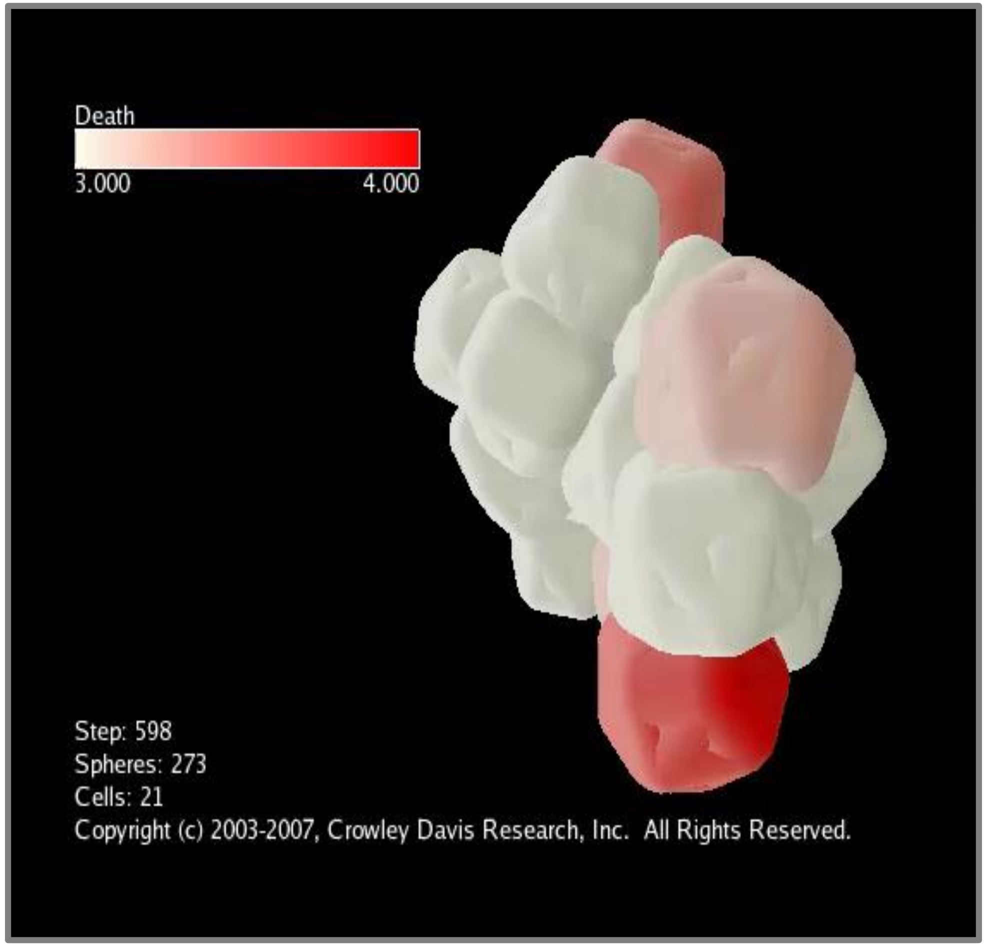
- Alternative (non-animal) methods must deal with the embryo and pregnancy as complex dynamical systems.

TIMELINE OF HUMAN EMBRYONIC DEVELOPMENT



SOURCE: https://embryology.med.unsw.edu.au/embryology

Anatomical homeostasis in a self-regulating 'Virtual Embryo'



SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Computational dynamics in a virtual embryo

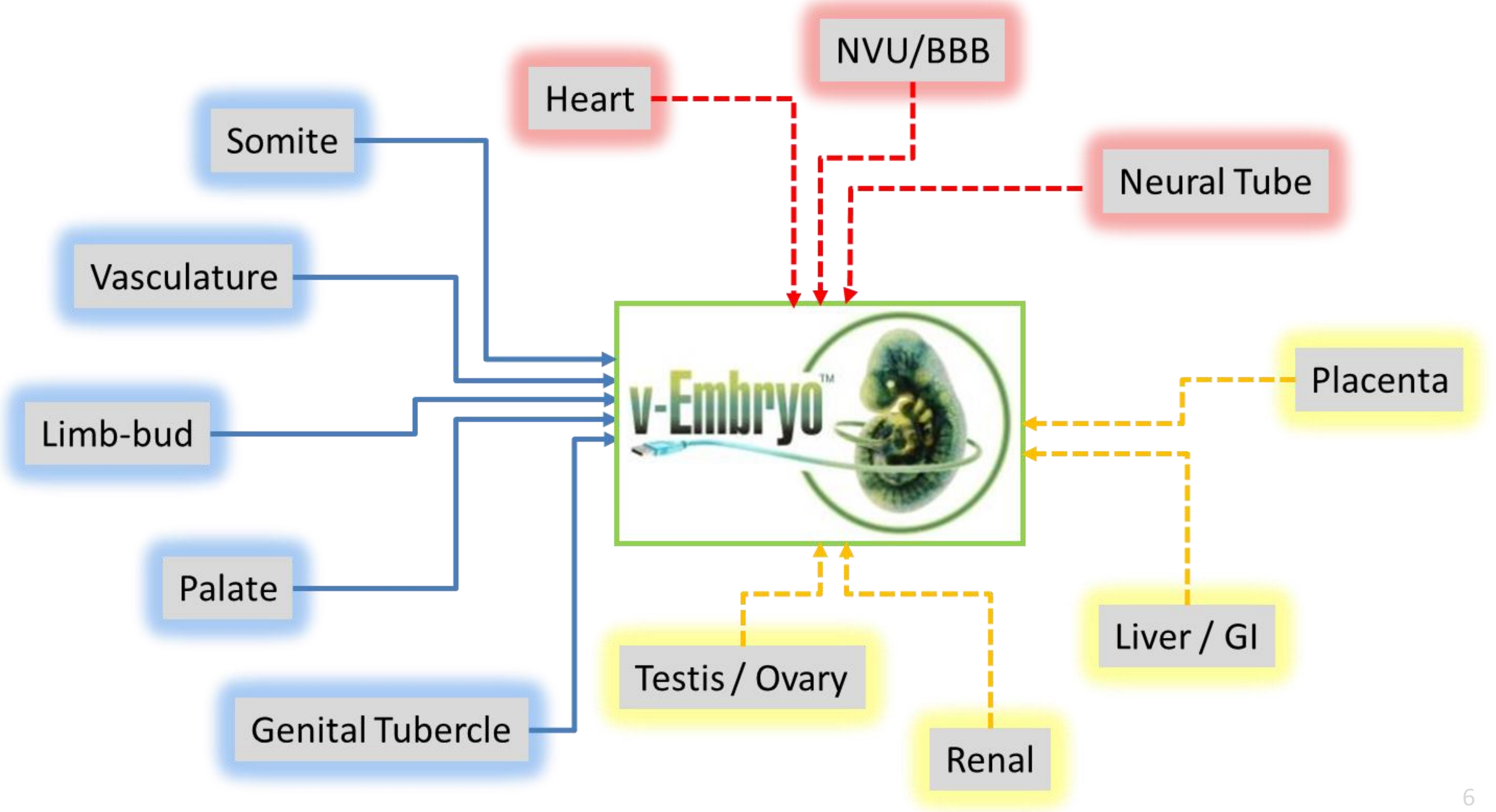
Hypothesis: cellular agent-based models (ABMs) can translate biomolecular lesion(s) into predictive models for developmental processes and toxicities.

Approach: build and test self-organizing morphogenetic fields *in silico* using an open-source modeling environment [www.compucell3d.org].

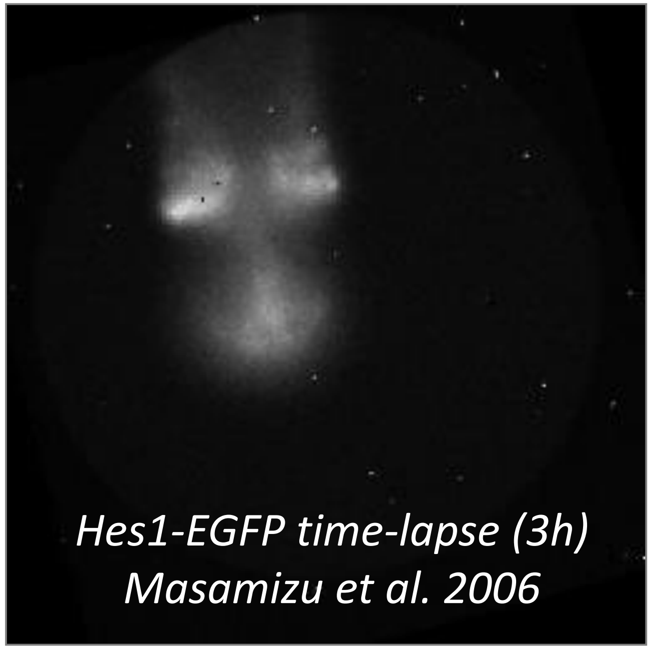
Input: A.I. cast into mathematically-defined cells (agents), synthetic gene circuits, and viscoelastic properties that can be perturbed with *in silico* or *in vitro* data.

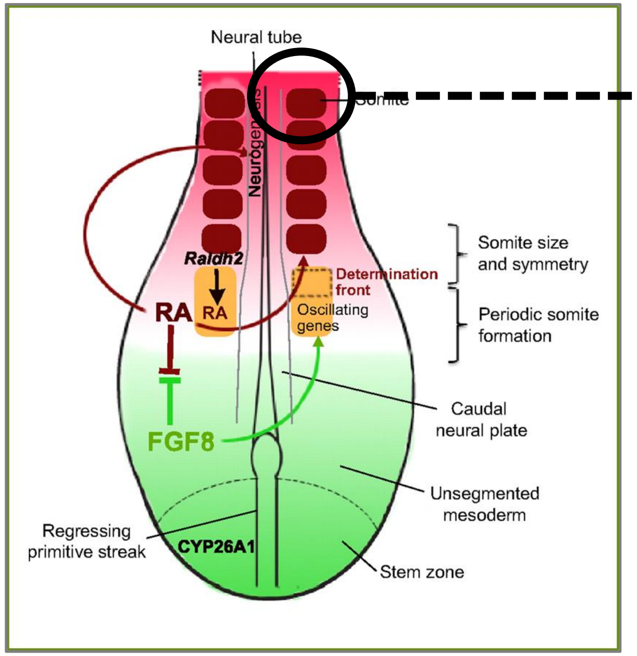
Emergence: simulation expresses individual cellular behaviors that collectively result in a morphogenetic series of events for the normal or perturbed system (cybermorphs).

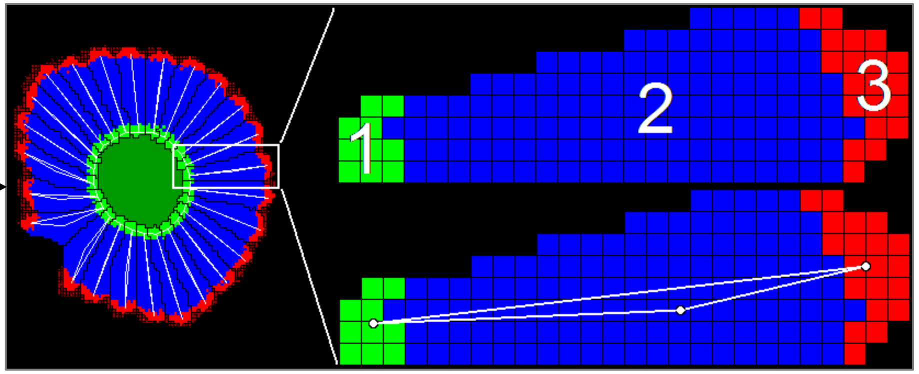
Output: probabilistic rendering of where, when and how a developmental defect might occur in response to defined lesions (genetic, environmental).


Heart
NVU/BBB
Neural Tube
Somite
Vasculature
Limb-bud
Palate
Genital Tubercle
v-Embryo™
Placenta
Liver / GI
Testis / Ovary
Renal

Somite development

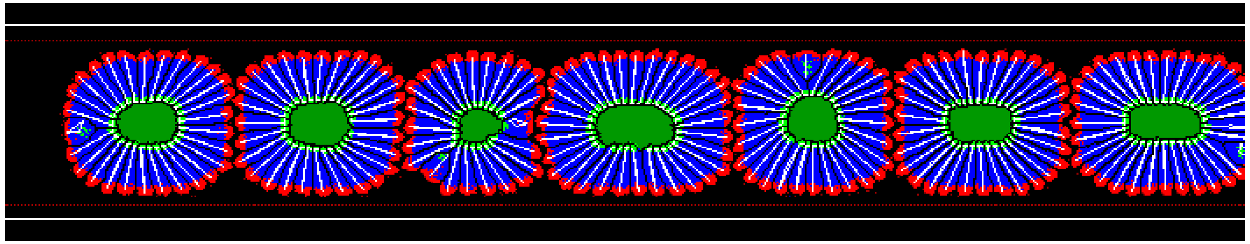


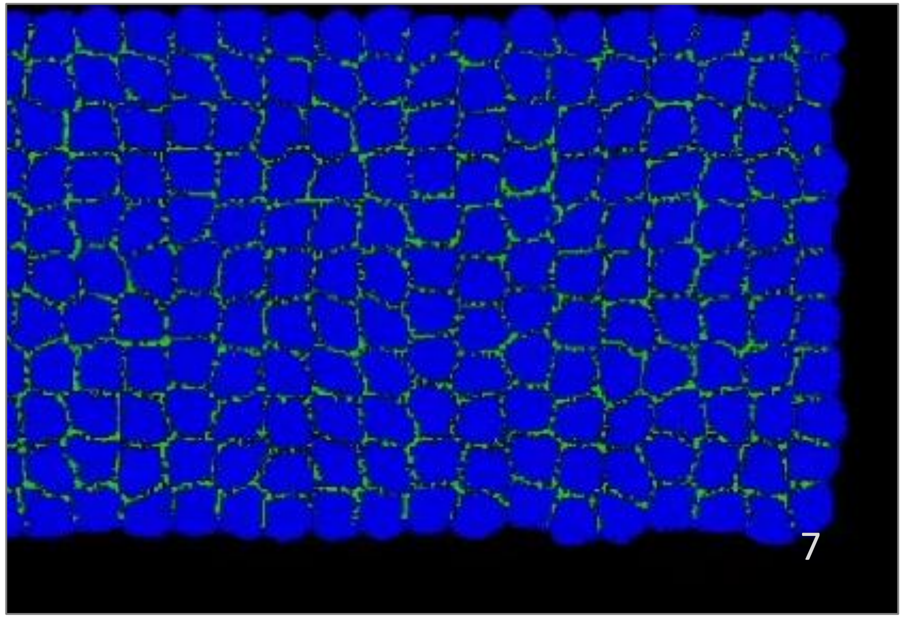




Differential cell adhesion

- *clock genes do not oscillate*
- *somites form simultaneously*

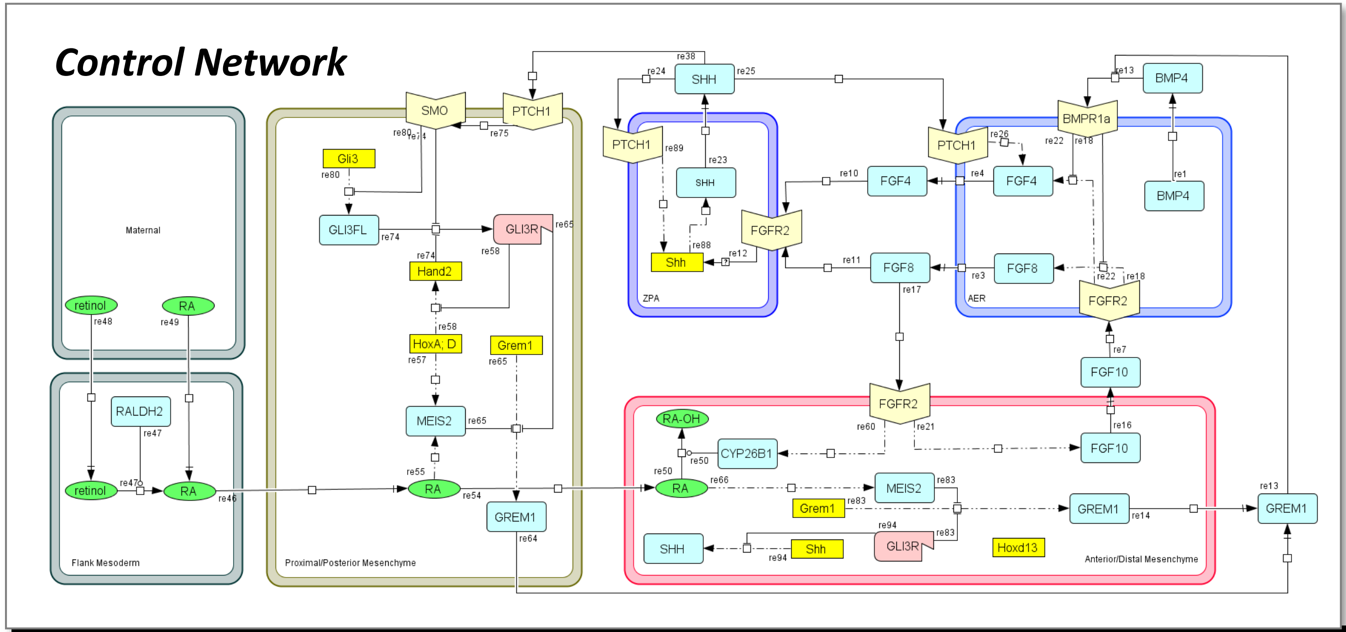


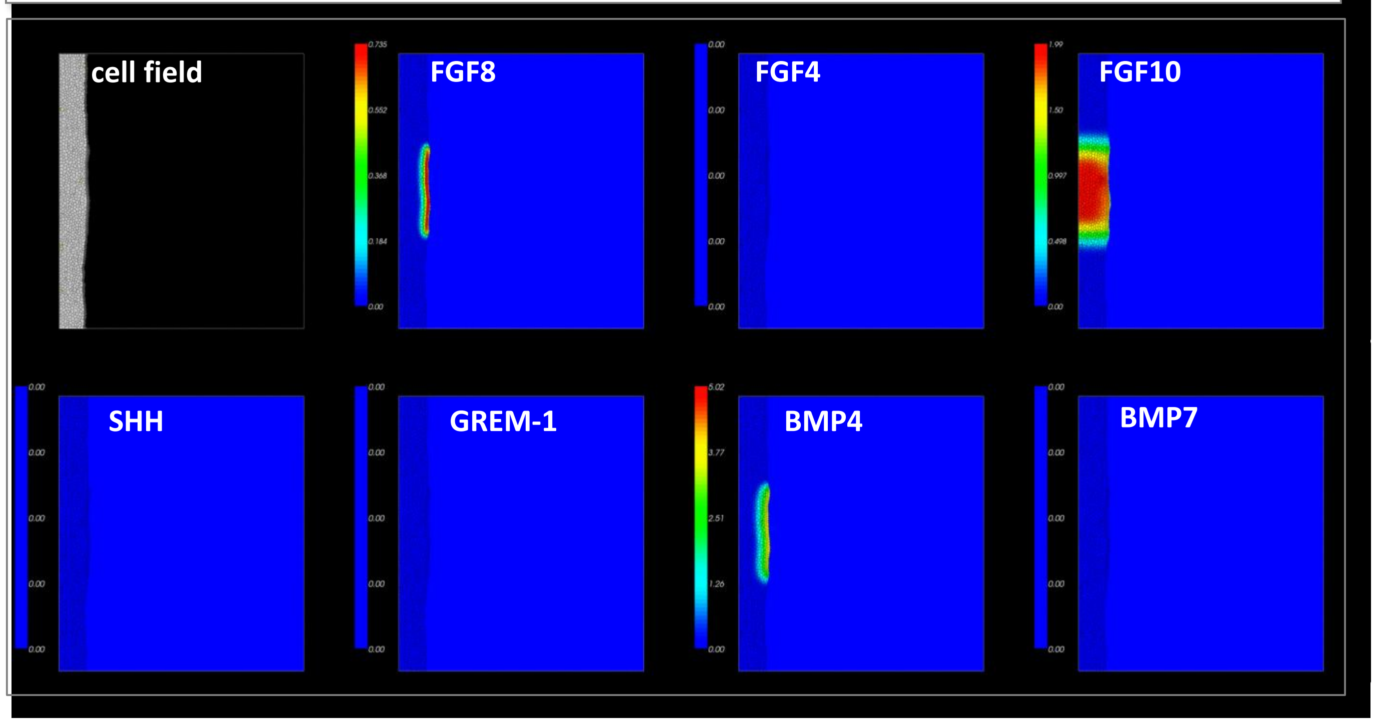


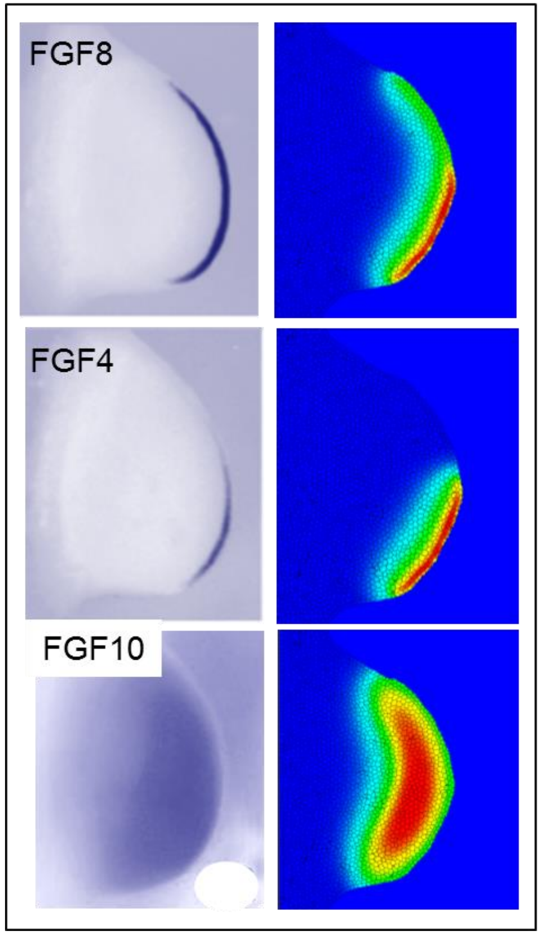
- *FGF8 wavefront restores sequentiality*
- *oscillatory clock improves regularity*

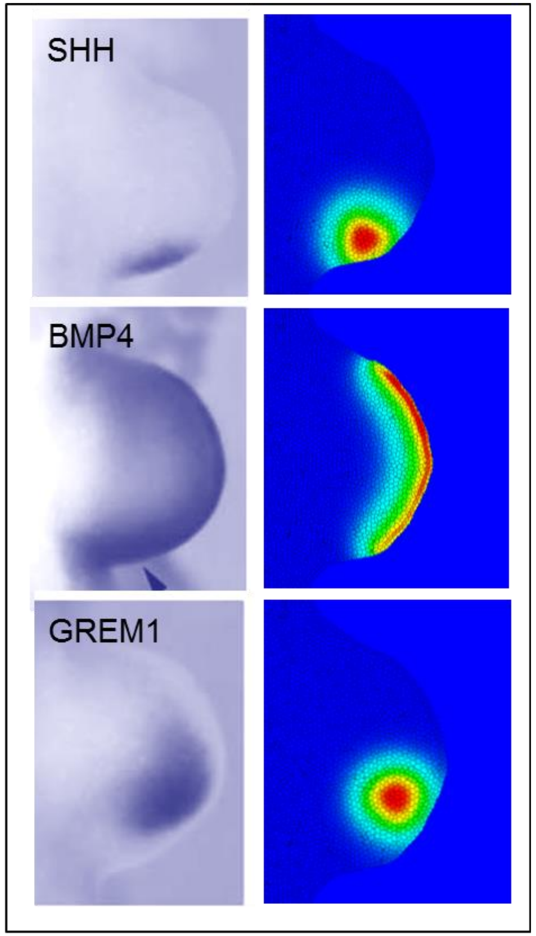
SOURCE: Dias et al. (2014) Science

Limb-bud outgrowth



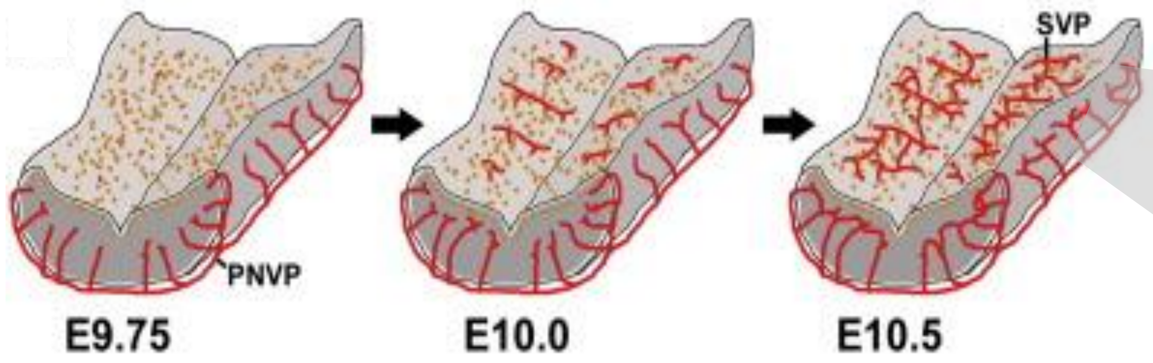


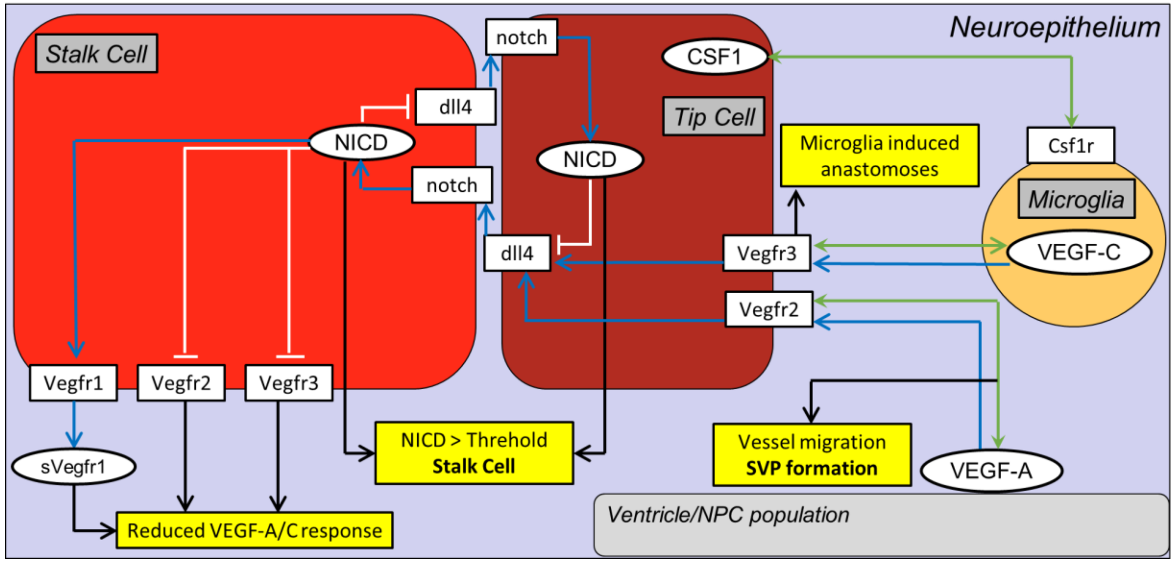


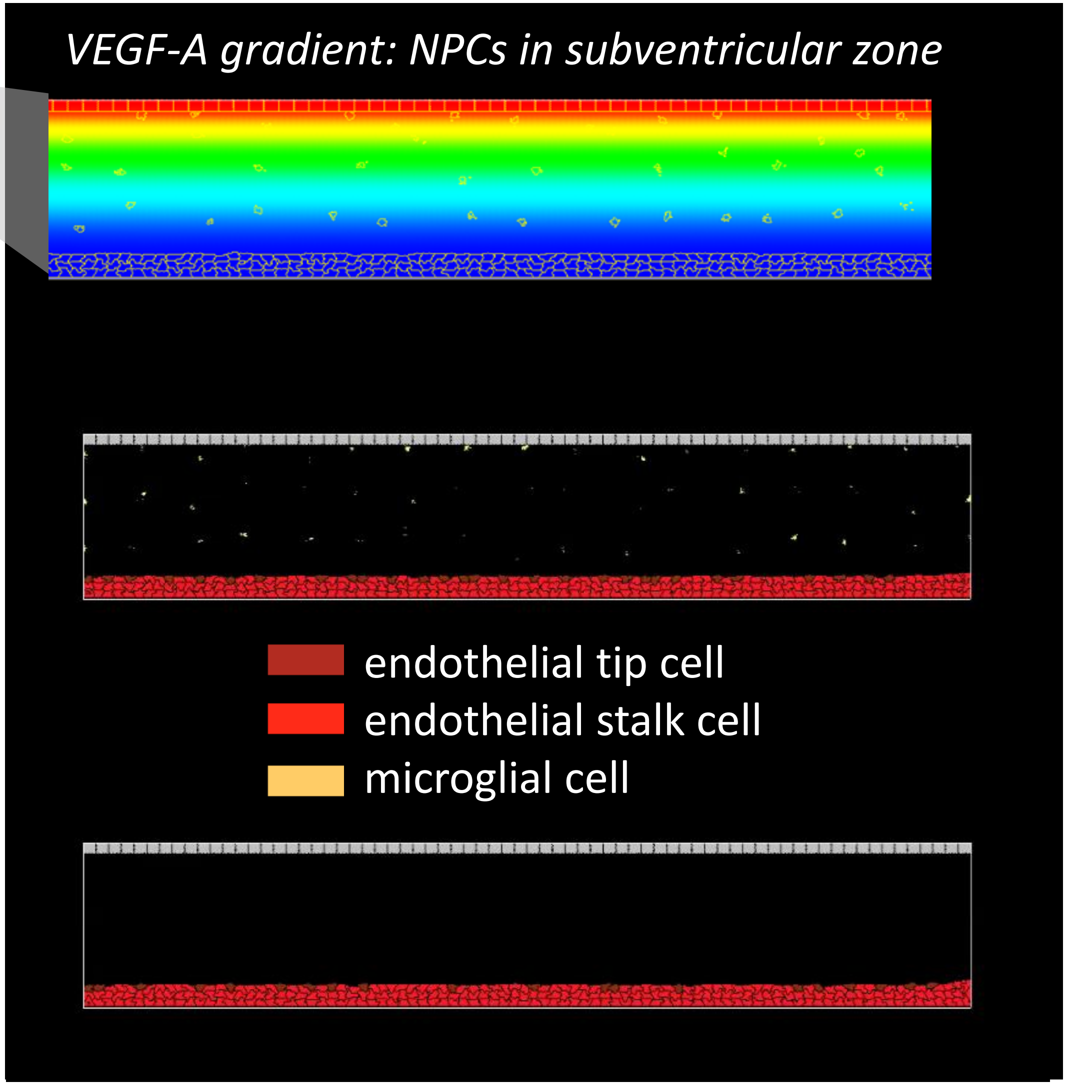


Brain angiogenesis

Tata et al. (2015) *Mechanism Devel*

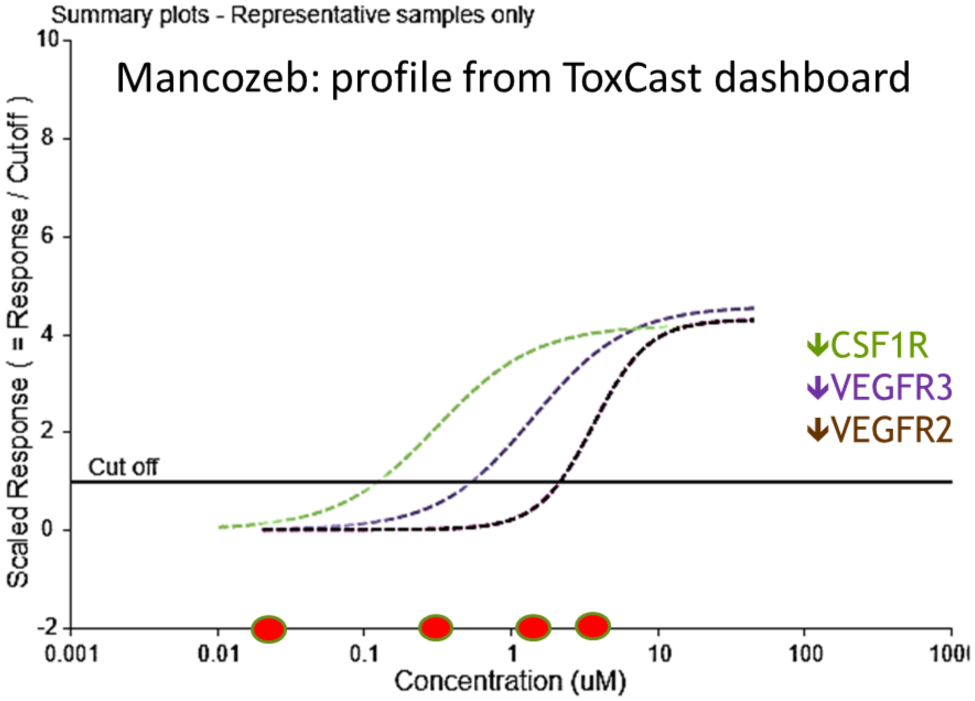


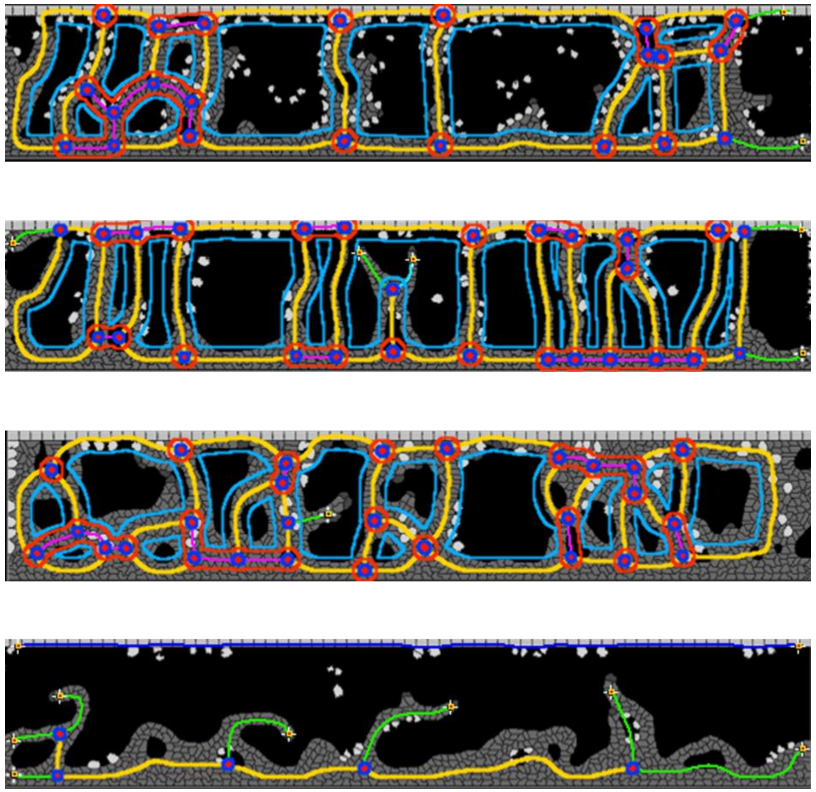




SOURCE: Zurlinden, Kate Saili (2018) – NCCT, unpublished

Executing a simulated dose-response

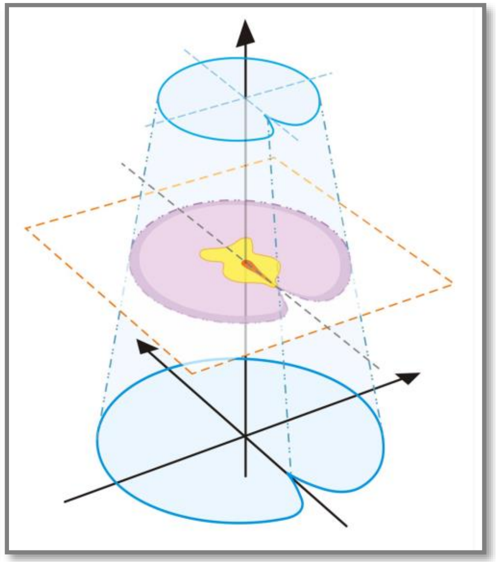


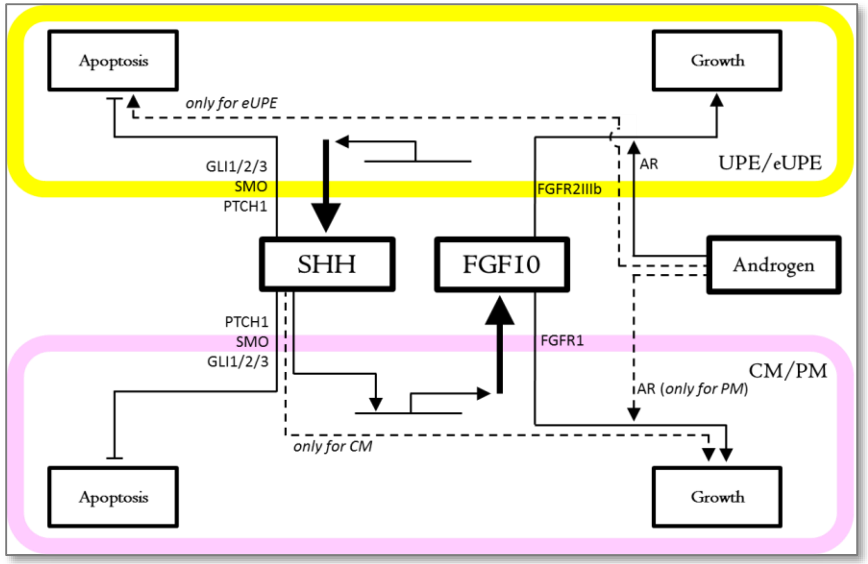


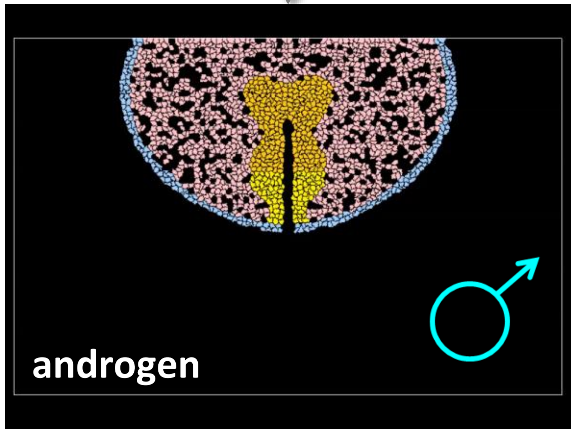
Critical concentration of Mancozeb on brain angiogenesiis:

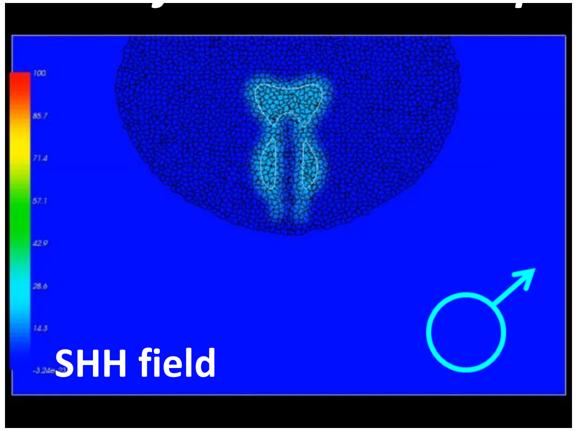
- predicted from *in silico* model ~0.5 µM (Zurlinden, NCCT)
- observed in 3D organotypic culture model of the hNVU ~0.3 µM (Daly, UWisc)

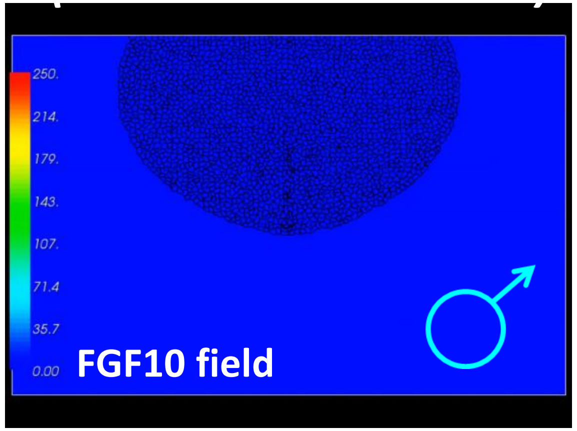
Sexual dimorphism: *genital tubercle development*

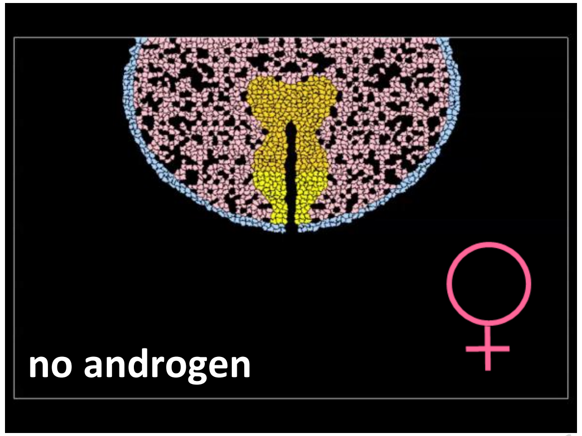




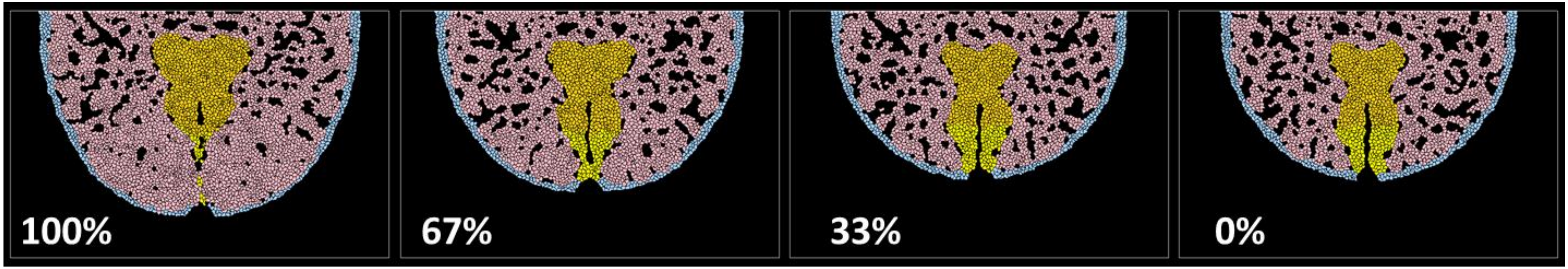


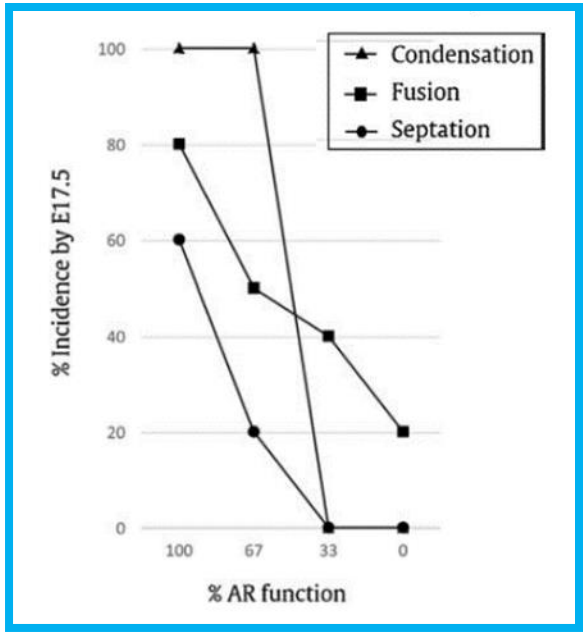






Androgen virulization: *closure rates @4000 MCS* $\int androgen\ supply$

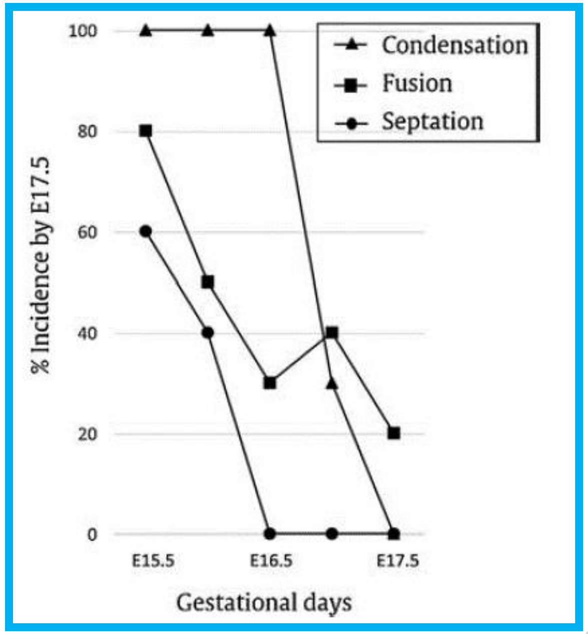




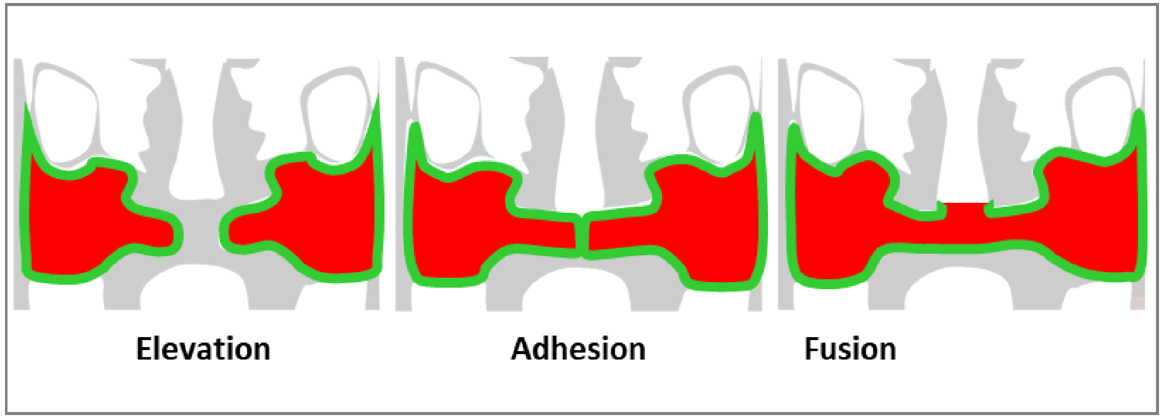
Closure indices (simulated, n=10)

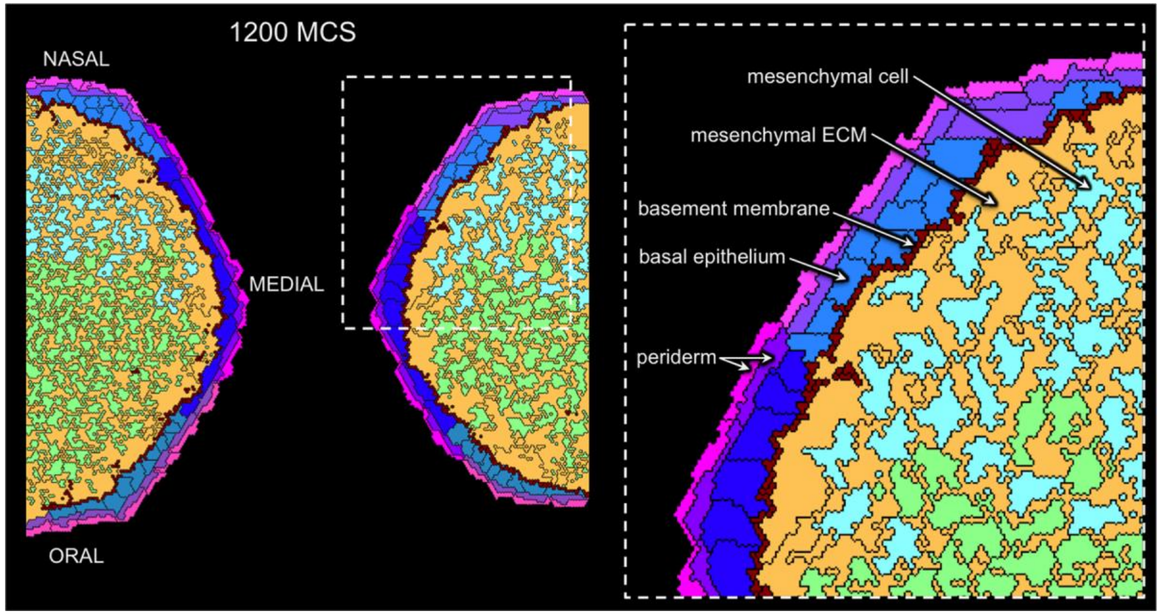
LEFT: androgen insufficiency

RIGHT: delayed virulization

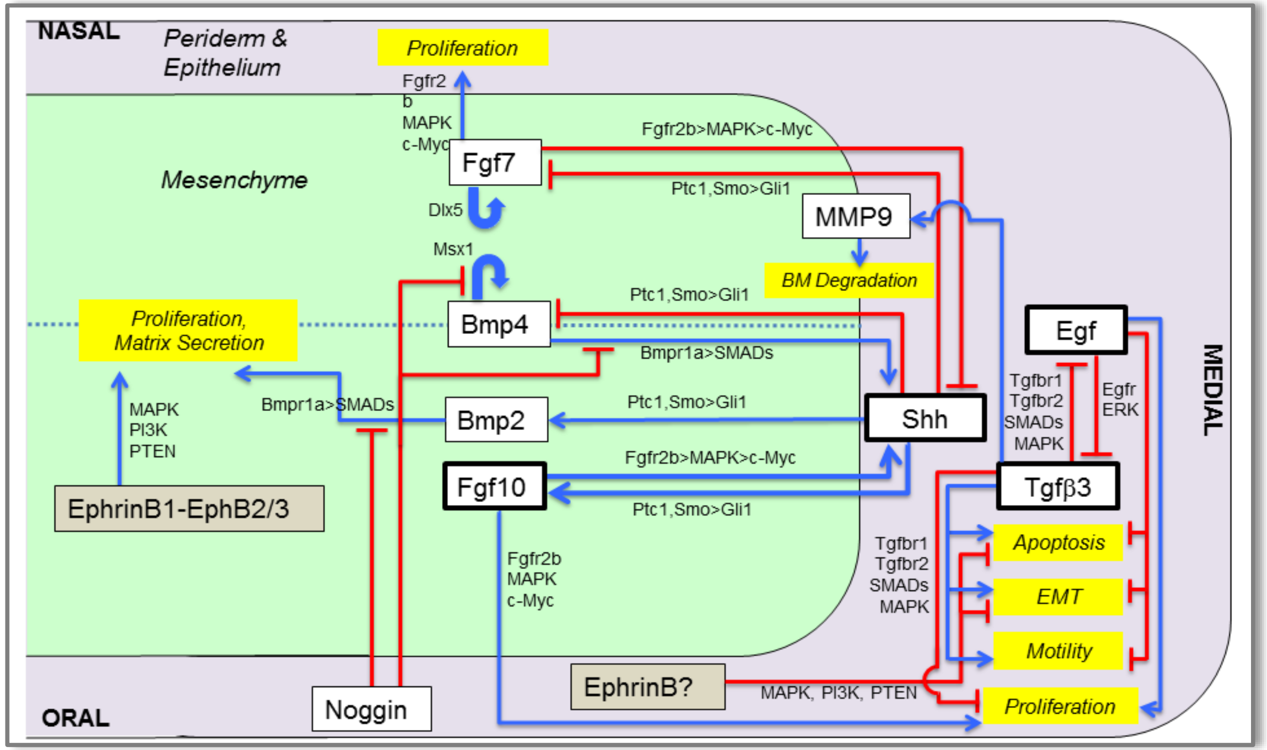


Palate morphogenesis: *structurally simple, genetically complex*



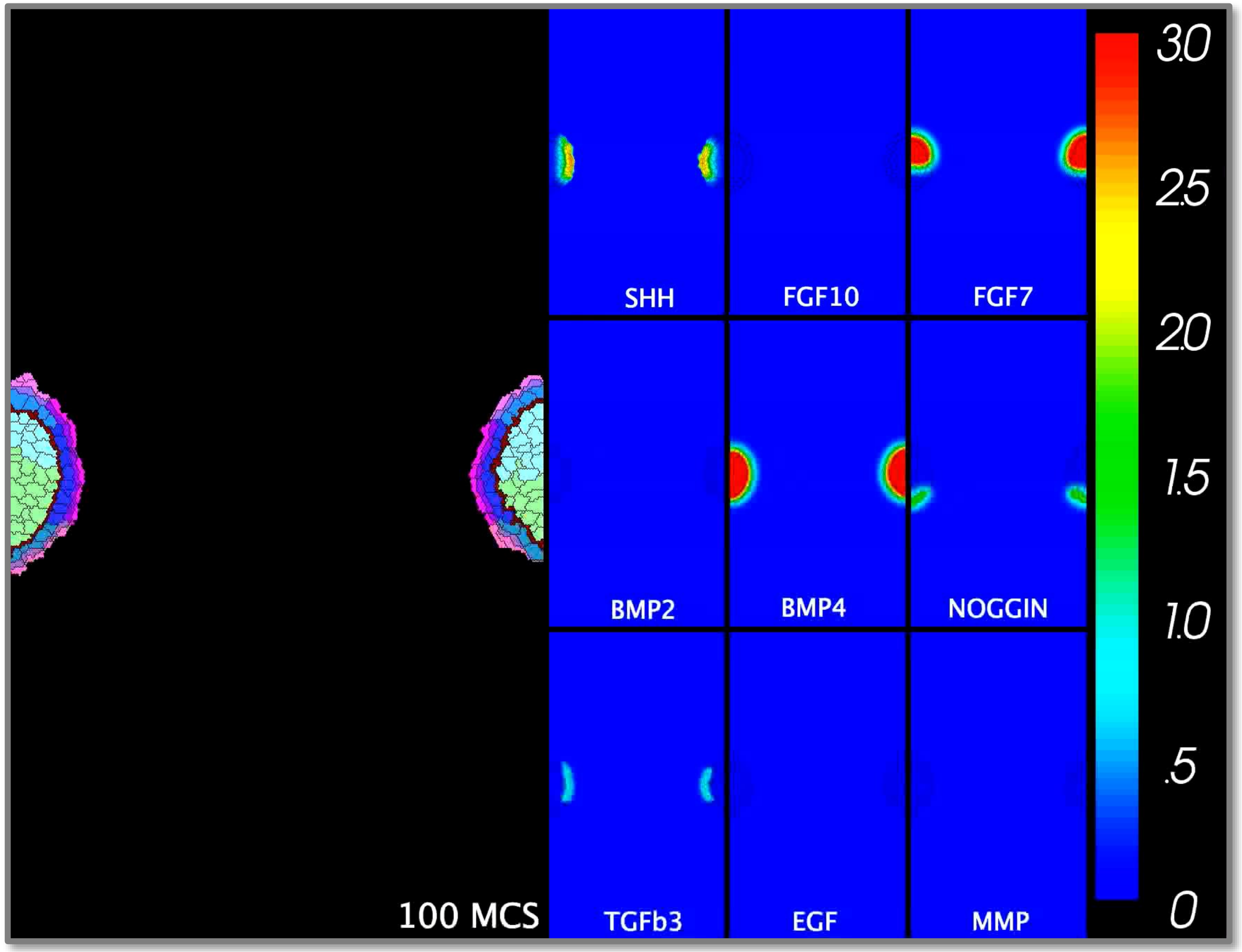


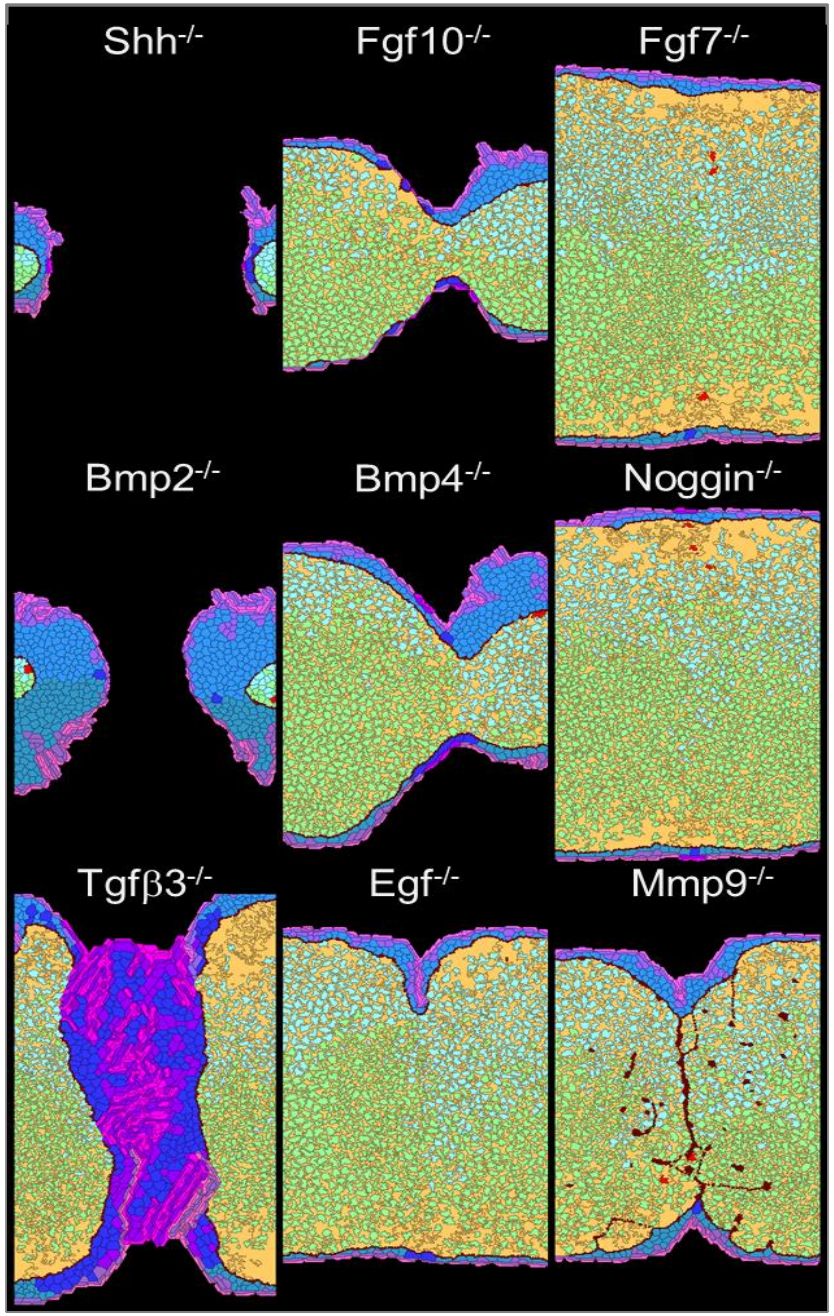
- A.I. = synthetic cell signaling networks



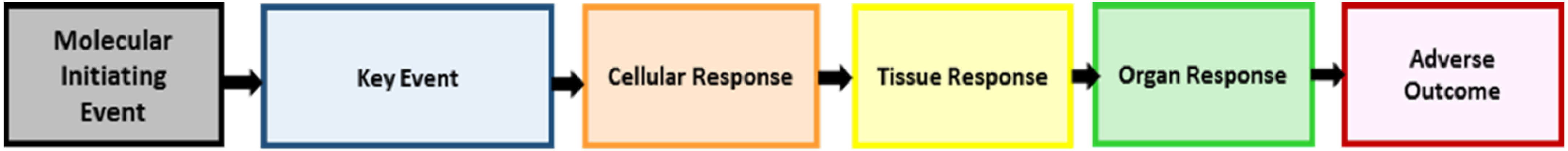
SOURCE: Hutson et al. (2017) Chem Res Toxicol

Hacking the control network





Cleft palate: *multiple mechanisms inferred from ToxCast*



ToxCast Chemicals

summarized by ToxCast gene score and chemotype for machine-learning

Animal studies

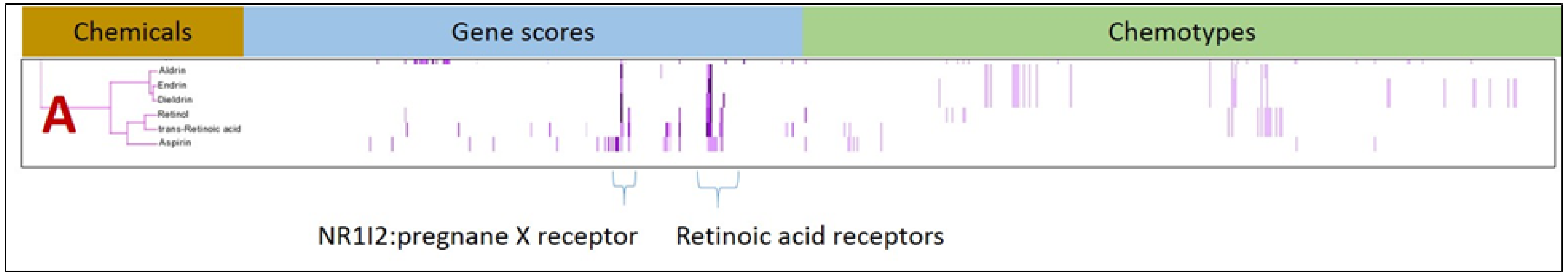
63 of 500 chemicals associated with cleft palate in ToxRefDB or biomedical literature

AOP clusters

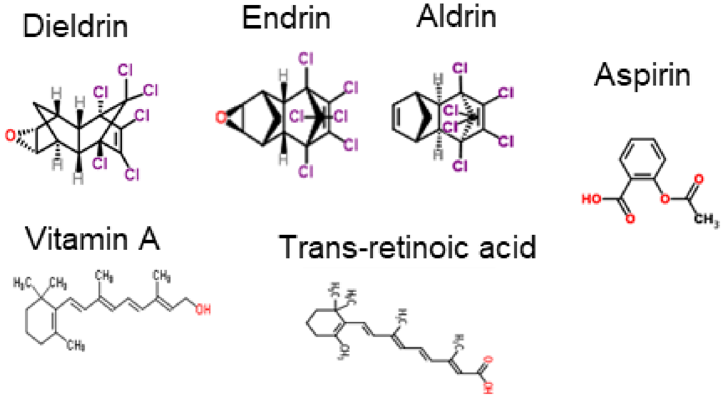
6 mechanistic pathways inferred from integration of HTS data with chemical structure.

Baker et al. (manuscript in clearance)

i)

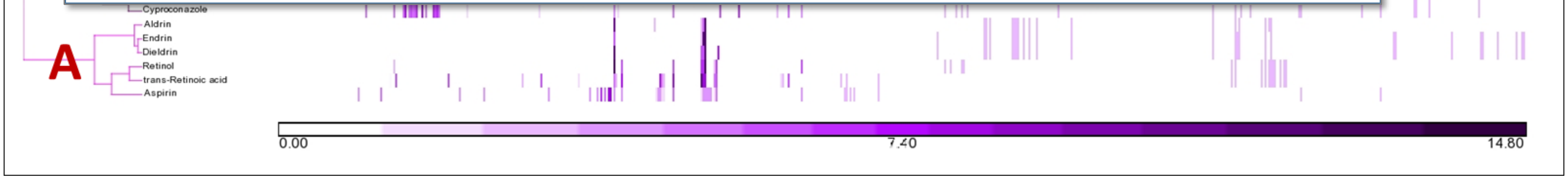


ii)

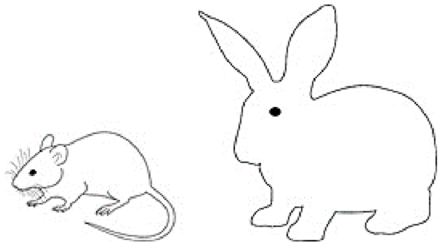


iii)

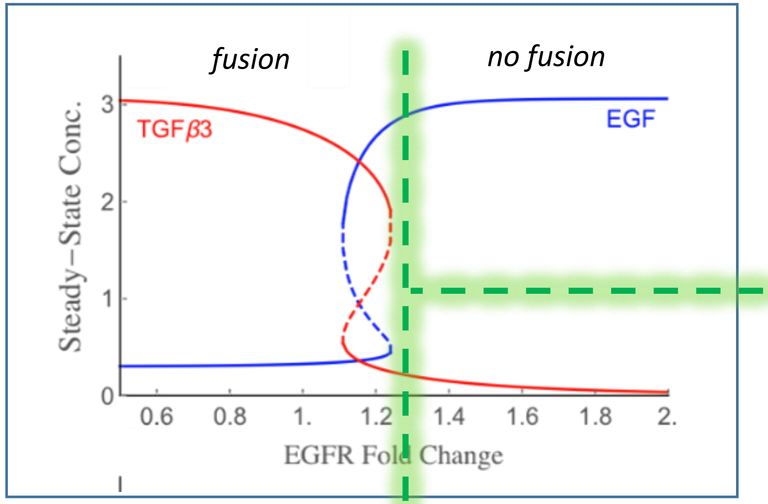
Chemical	Cleft palate? 0/1	Gene Score RARG
Retinol	1	13.5
Dieldrin	1	12.0
Endrin	1	11.9
Aldrin	1	11.6
Bromuconazole	0	10.8
Tributyltin chloride	1	8.6
trans-Retinoic acid	1	8.2
Rotenone	0	7.7
Fenpyroximate (Z,E)	0	7.6
Chlorothalonil	0	6.7
Triflumizole	0	6.5
SSR126768	0	6.4
Tebufenpyrad	0	6.3
... > 50 more chemicals		



Mathematical model: *EGF/TGF β3 bistable switch*

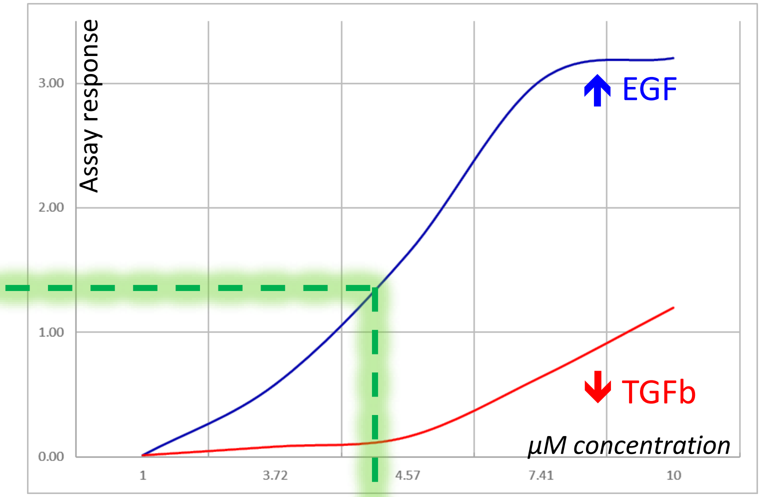


INPUT: switch dynamics



tipping point predicted by computational dynamics (hysteresis switch)

Captan in ToxCast



OUTPUT: tipping point mapped to concentration response (4 µM)

Captan in ToxRefDB

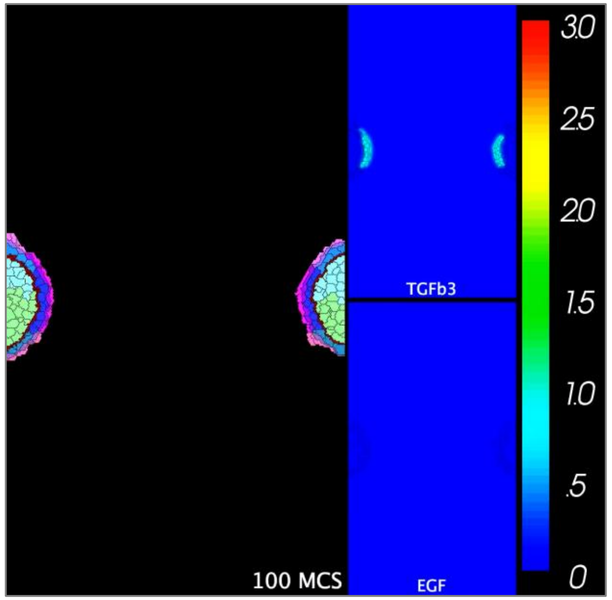
NOAEL = 10 mg/kg/day

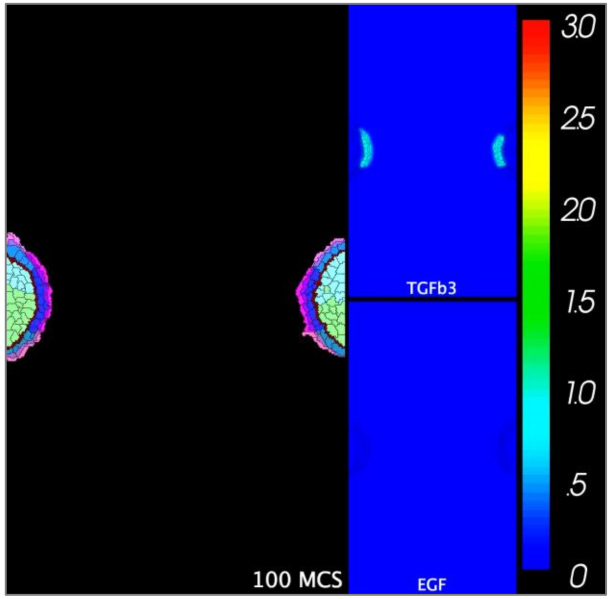
LOAEL = 30 mg/kg/day

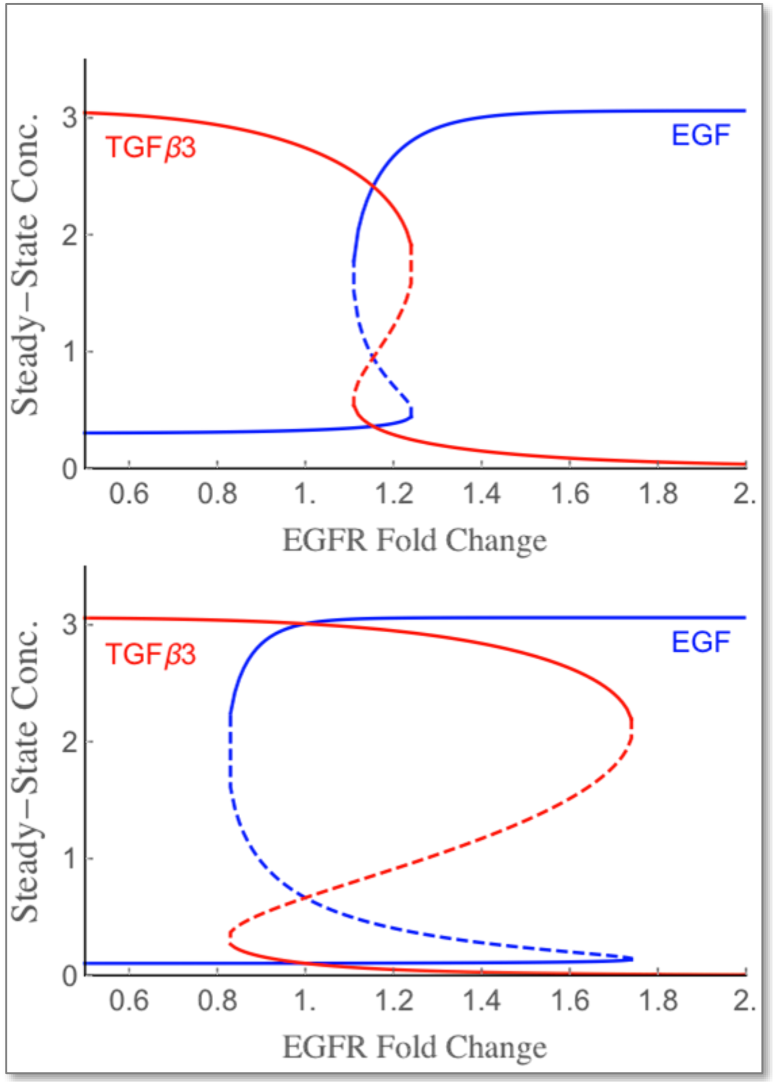
human HTTK model

2.39 mg/kg/day would achieve a steady state of 4 µM in fetal plasma

Messin' with the switch: *two scenarios for bistable dynamics*



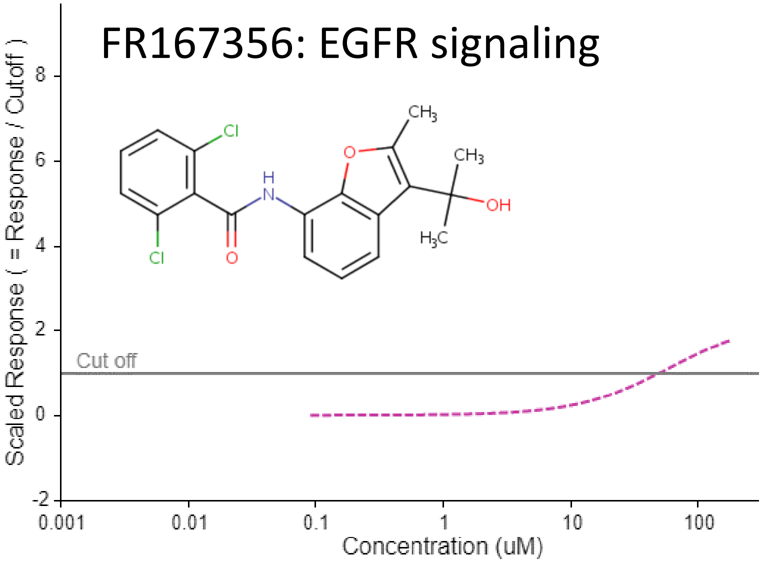




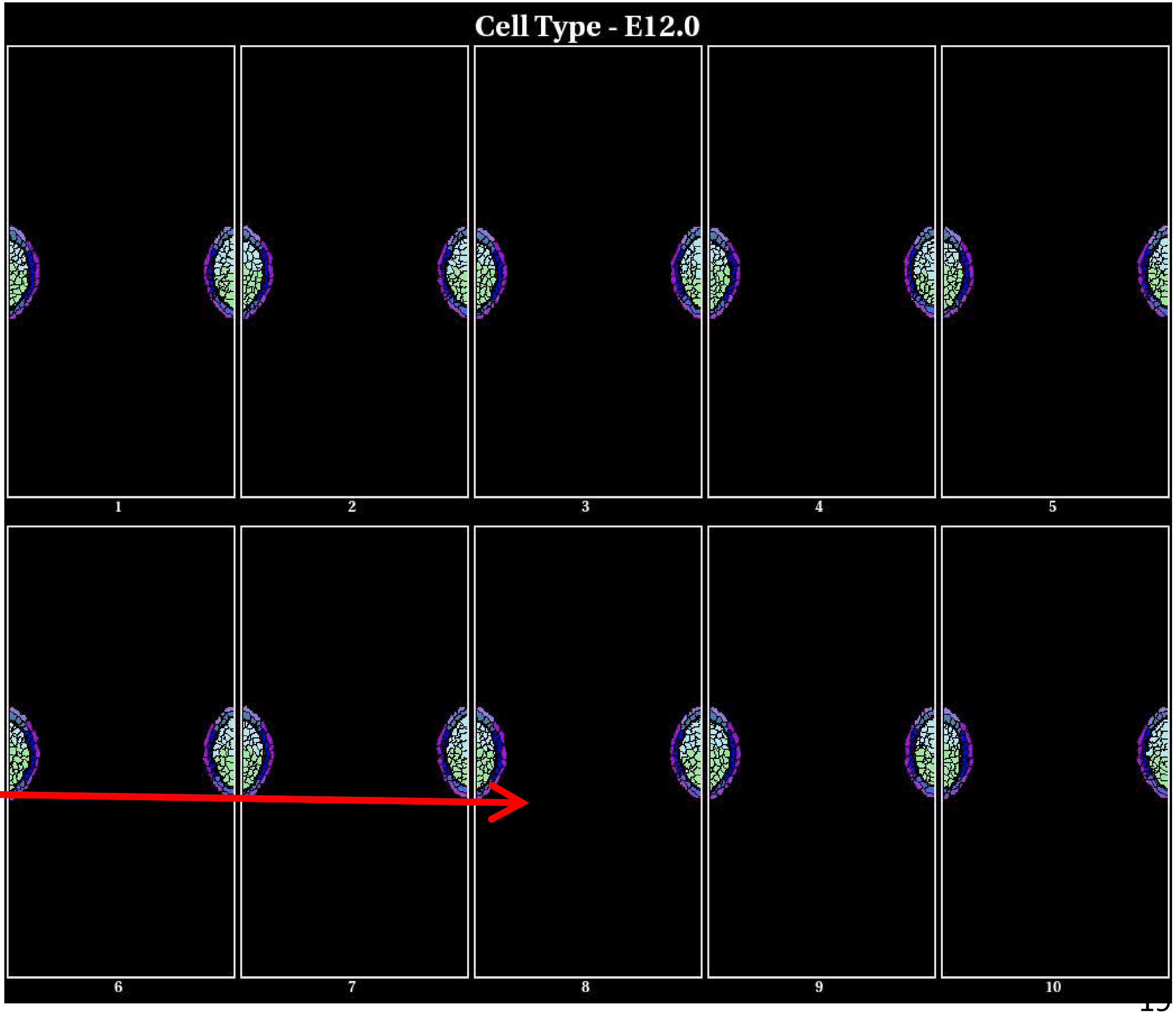
Narrow hysteresis:
less resilient but reversible

Broad hysteresis:
more resilient but irreversible

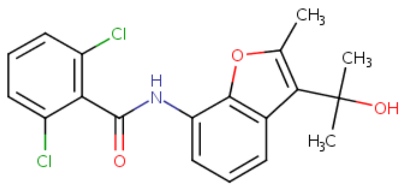
Simulated dose-response

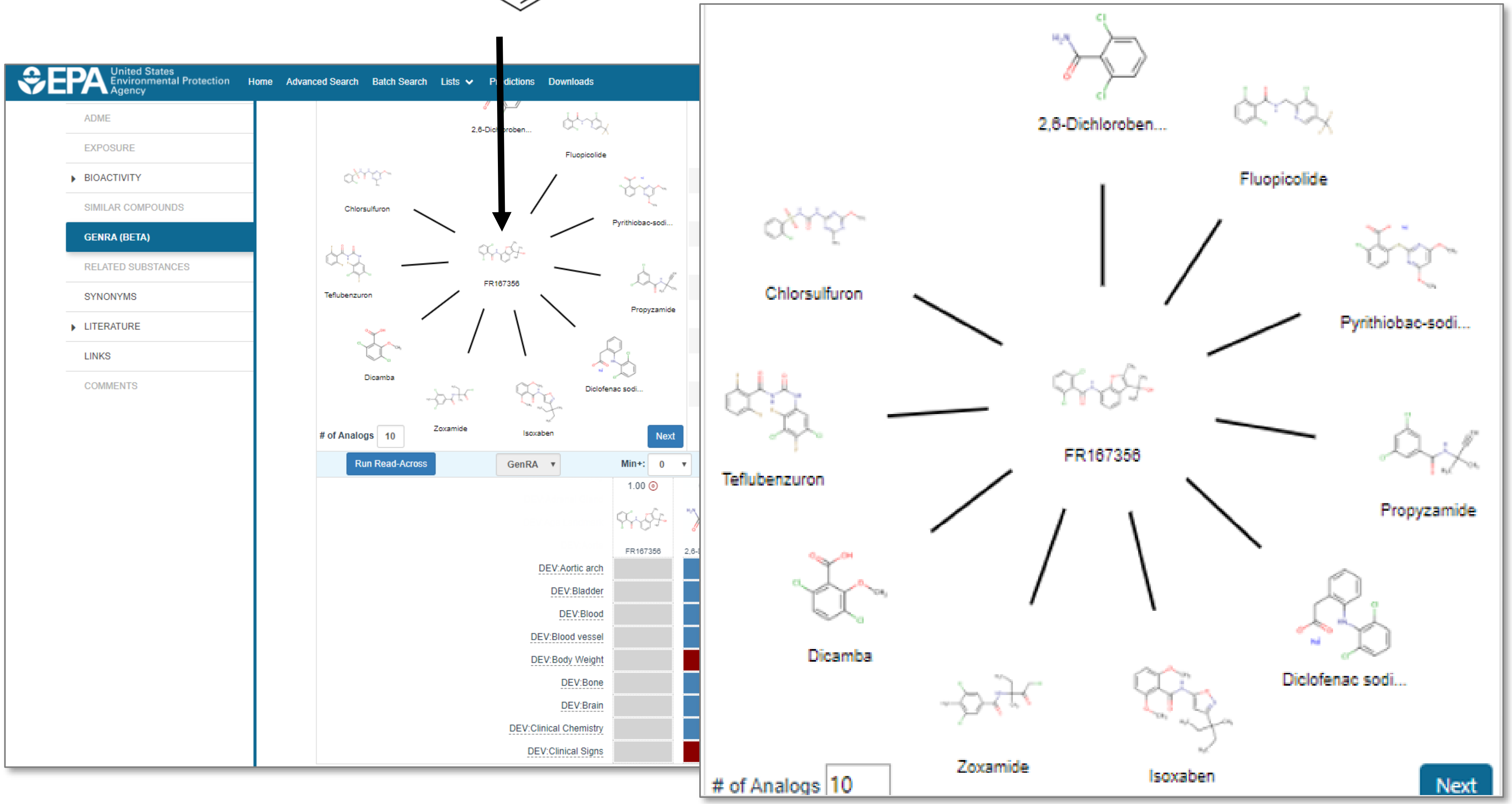


Tipping point predicted
in topological context

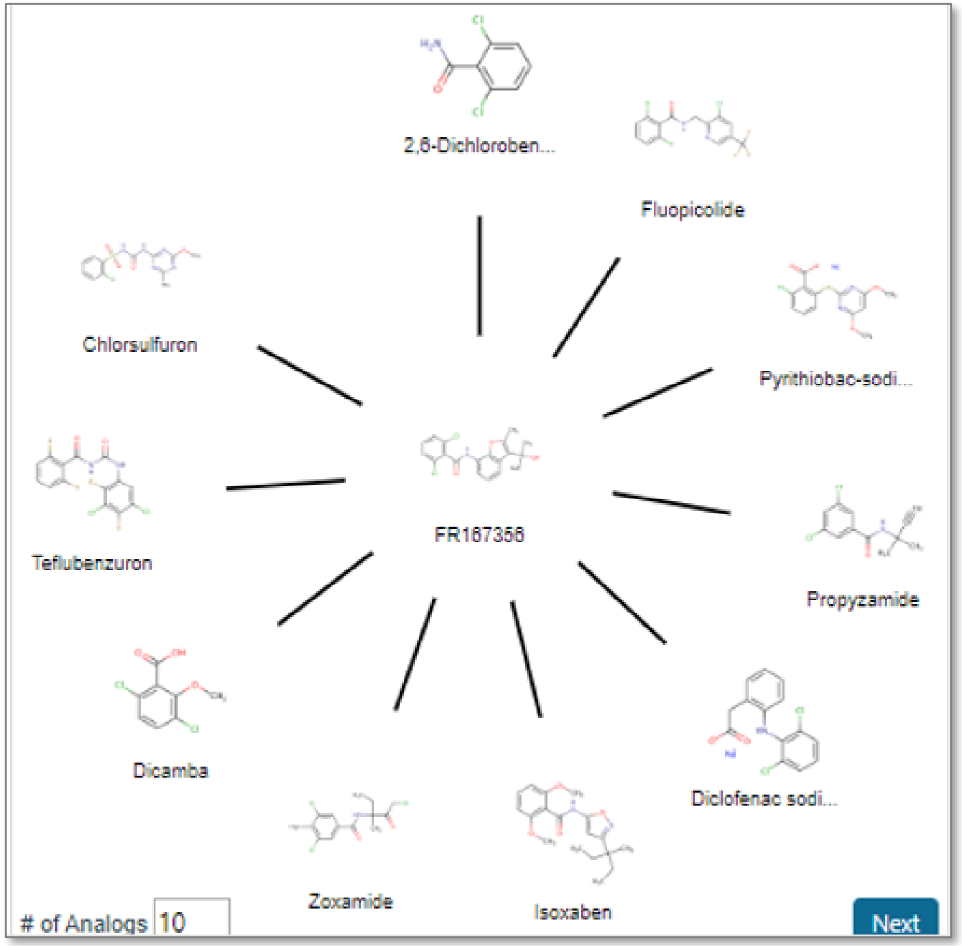


Read across: CompTox chemicals dashboard





- **FR 167356** developed as a selective inhibitor of osteoclast vacuolar H+-ATPase (V-ATPase)
- V-ATPase functions in a critical bone formation pathway and lytic bone disease (osteoporosis)
- No DevTox information available in open literature
- Chemotype neighbors Indomethacin and Diclofenac are NSAIDs that disrupt murine palatal fusion *in vitro*

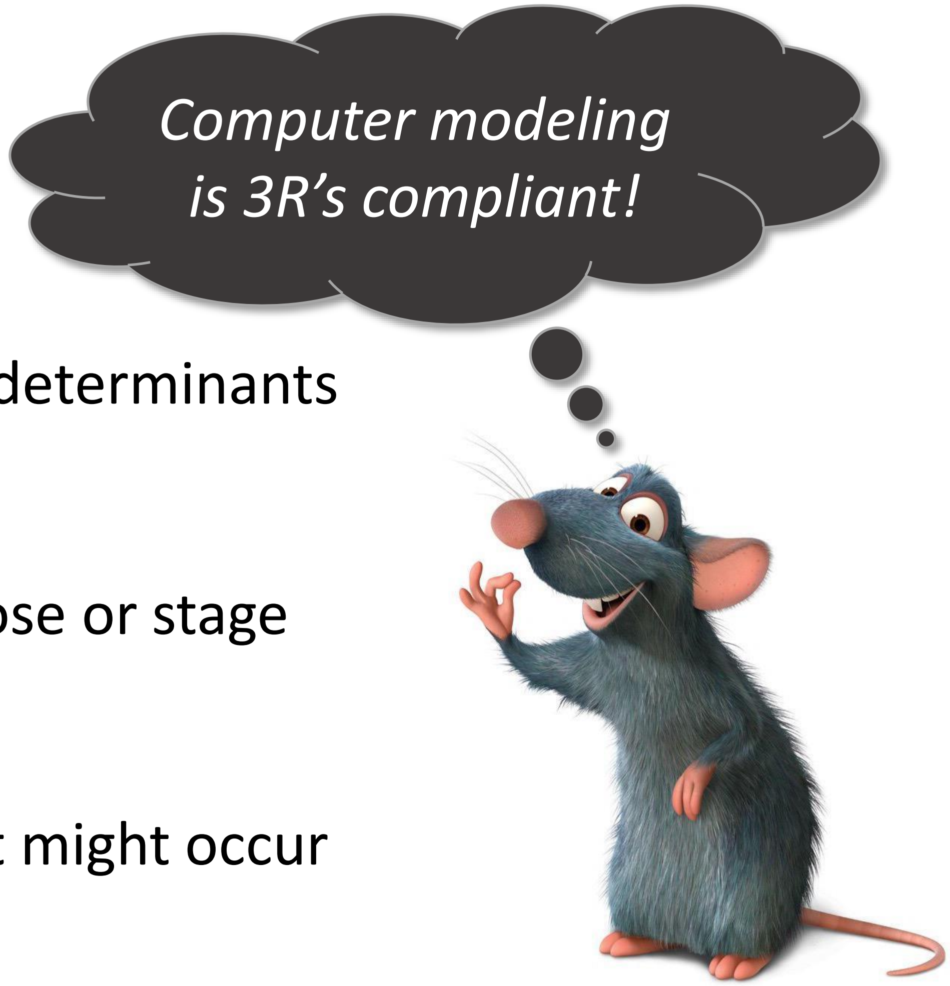


1	Abstract Sifter	Landscape View					
2			Update Article Counts / View / hide queries / Heat Map by column / Heat Map by row				
3			5684	(reproduction AND (toxicity OR abnormal OR adverse effects))	nital, Hereditary, and Neonatal Diseases and Abnormalities[	palate OR palate/drug effects) not surgery Not operati	bone AND development
4	DSSTOX link to Dashboard	Preferred Name	Chemical / entity query	ReproTox	DevTox	CP	bone
5		Indomethacin	Indomethacin	482	468	7	174
6	DTXSID3037208	Diclofenac sodium	15307-79-6 OR Diclofenac sodium OR Diclofenac	143	37	1	18
7	DTXSID4024018	Dicamba	1918-00-9 OR Dicamba	9	1	0	0
8	DTXSID1048174	FR167356	174185-16-1 OR FR167356 OR FR 167356 OR "FR 167356"[nm]	0	0	0	4
9	DTXSID7022170	2,6-Dichlorobenzami	2008-58-4 OR 2,6-Dichlorobenzamide	0	0	0	0
10	DTXSID7034624	Fluopicolide	239110-15-7 OR Fluopicolide	0	0	0	0
11	DTXSID8032673	Pyrithiobac-sodium	123343-16-8 OR Pyrithiobac-sodium OR pyrithiobac sodium	0	0	0	0
12	DTXSID2020420	Propyzamide	23950-58-5 OR Propyzamide OR pronamide	1	0	0	0
13	DTXSID8024159	Isoxaben	82558-50-7 OR Isoxaben	1	0	0	0
14	DTXSID9032581	Zoxamide	156052-68-5 OR Zoxamide	0	0	0	0
15	DTXSID6042440	Teflubenzuron	83121-18-0 OR Teflubenzuron	6	0	0	0
16	DTXSID7023980	Chlorsulfuron	64902-72-3 OR Chlorsulfuron	0	0	0	0
17	DTXSID4032619	Halofenozide	112226-61-6 OR Halofenozide OR N-4-chlorobenzoyl-N'-benzoyl-N'-tert-I	5	0	0	0
18	DTXSID0034774	Noviflumuron	121451-02-3 OR Noviflumuron	0	0	0	0
19	DTXSID7052862	Cumyluron	99485-76-4 OR Cumyluron	0	0	0	0
20	DTXSID4047672	Flubendiamide	272451-65-7 OR Flubendiamide	5	0	0	0
21	DTXSID3032628	Methoxyfenozide	161050-58-4 OR Methoxyfenozide	11	0	0	0
22							

SOURCE: Nancy Baker, NCCT

Computer modeling and simulation:

- reconstruct basic modules of embryogenesis *in silico* cell-by-cell and interaction-by-interaction;
- execute tissue simulations that advance through critical determinants of phenotype;
- simulate *in vitro* data under various *in vivo* scenarios - dose or stage response, critical pathways, non-chemical stressors, …;
- probabilistic rendering of where, when and how a defect might occur under different exposure scenarios.



Virtual Tissue Models: Predicting How Chemicals Impact Human Development

Special Thanks

Barbara Abbott – NHEERL / TAD
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James Glazier – Indiana University
Sid Hunter – NHEERL / ISTD
Brian Johnson – U Wisconsin / HMAPS STAR
Nicole Kleinstreuer – NCCT (now NTP / NICEATM)
William Murphy – U Wisconsin / HMAPS STAR
Kate Saili – NCCT
Richard Spencer – Leidos / EMVL
Todd Zurlinden – NCCT