United States
Environmental Protection
Agency

Enhancing the Quality of Predictions for
Developmental Toxicity Based on Alternative Methods
EUROTOX2016, Seville - Sept 5, 2016

Computational Modeling and Simulation of Developmental Toxicity

v-Embryo™

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Anatomical homeostasis in a self-regulating multicellular system

SOURCE: Tim Otter, – with permission

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

v-Embryo™

Can a computer model of the developing embryo translate cellular disruptions into a prediction of dysmorphogenesis?

and if so …

How might such models be used with high-performance computing ***analytically*** (to understand) and ***theoretically*** (to predict) adverse developmental outcomes for different exposure scenarios?

e.g., chemicals, non-chemical stressors, drugs, mixtures, lifestages, …

Multicellular Agent-Based Models (ABMs)

- Computer models that recapitulate multiple signaling networks and coordinated cell behaviors.

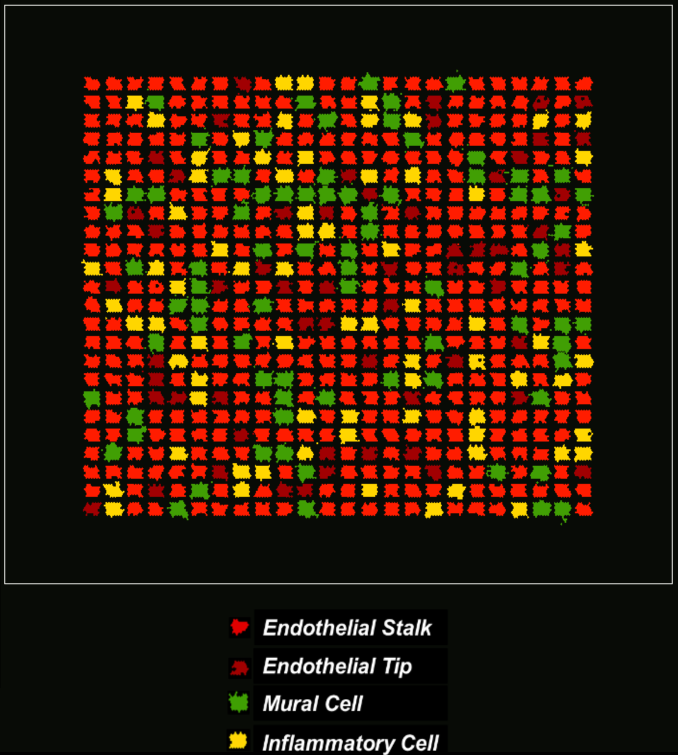
- Running ABMs with real (*in vitro*) or synthetic (*in silico*) data is a heuristic to predict emergent responses following perturbation.

- Comparing simulated outcomes with reference experiments tells how well the ABM performs.

- Can use them to translate screening-level data from chemical-biology into predictive toxicology of a developmental hazard.

Angiogenesis

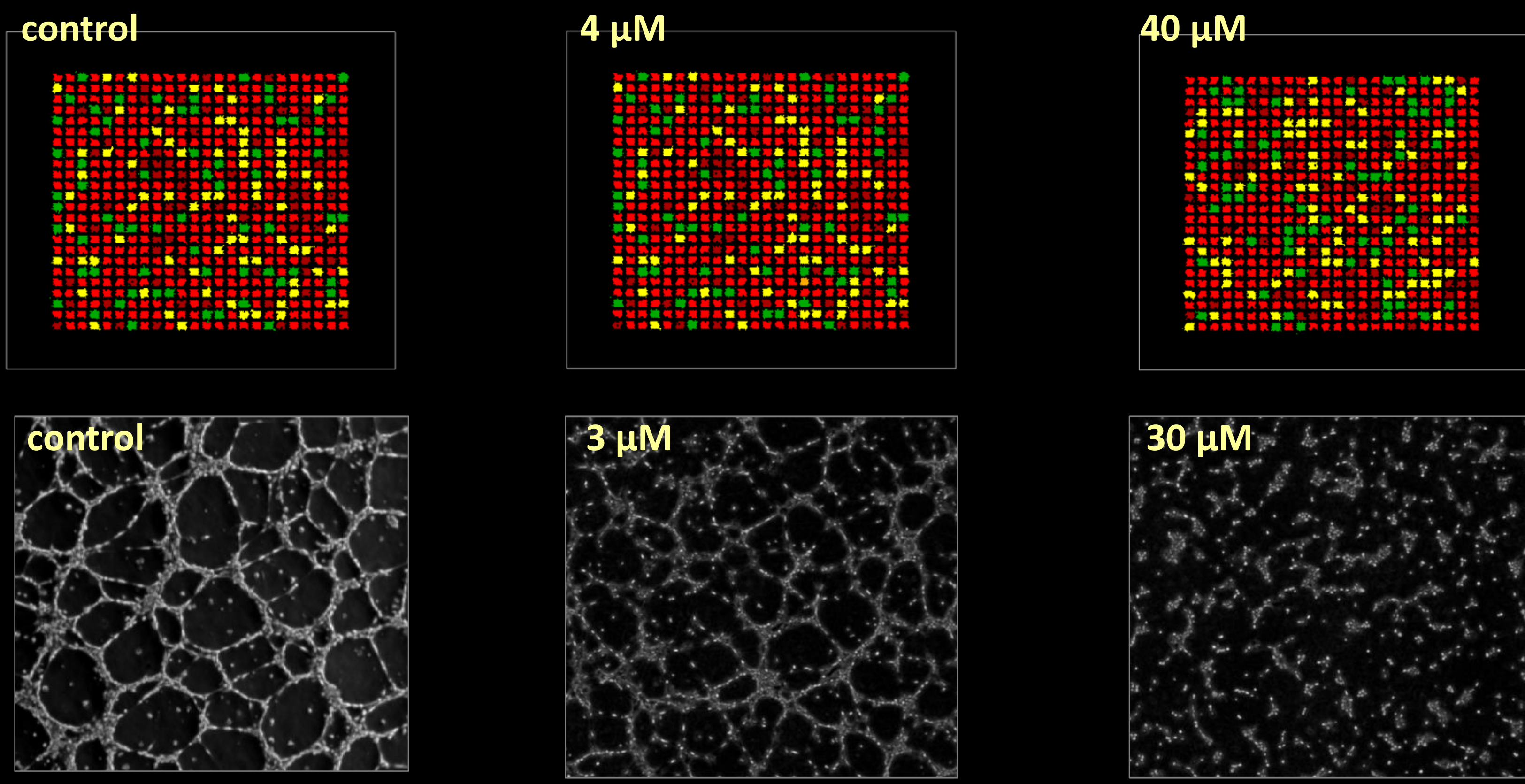
VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2



- individual rules assigned to low-level ‘agents’ (cells)
- agents interact in a shared environment *
- executing the biology leads to emergent features
- models run differently each time (stochastic)
- each run reveals one possible solution

* *CompuCell3D.org is an open-access environment for cell-oriented modeling developed at Indiana University by J Glazier and colleagues*

5HPP-33 concentration response predicted *in silico from* ToxCast and demonstrated *in vitro* with a human endothelial cell assay

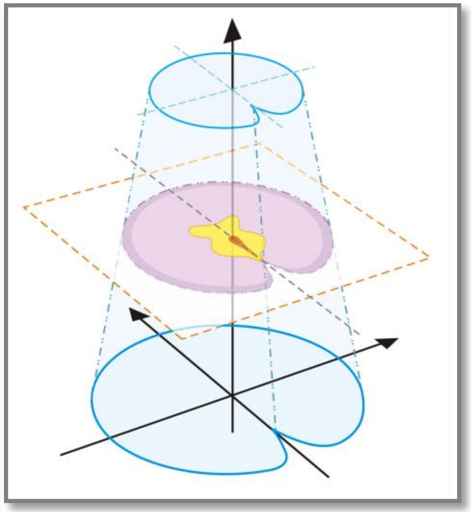


SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol

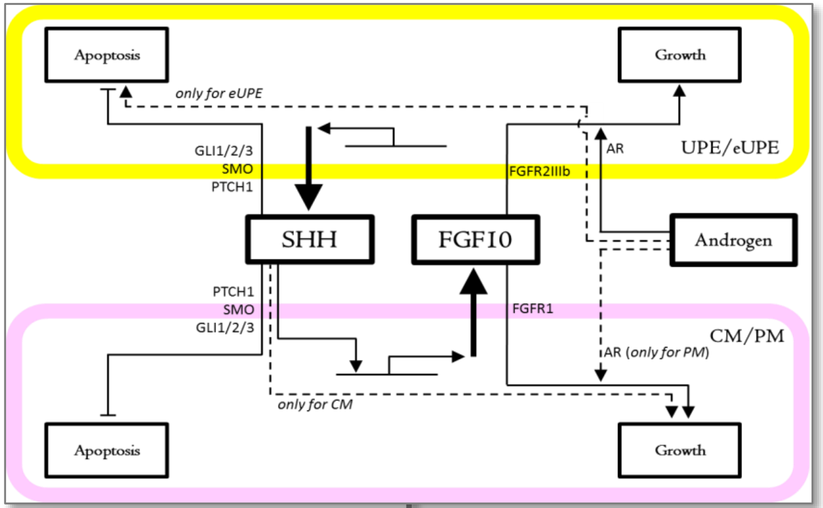
Modeling Genital Tubercle Development

Embryonic GT

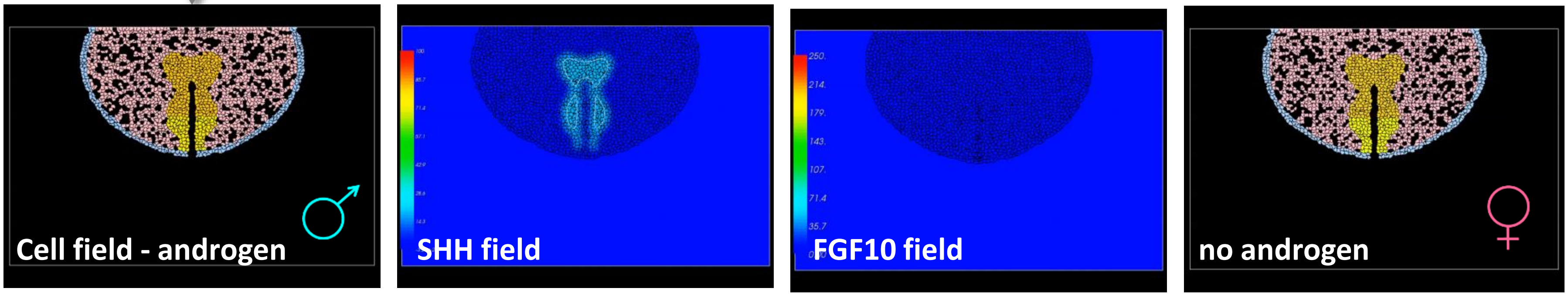
Abstracted GT



Control Network (mouse)



ABM simulation for sexual dimorphism (MCS 4000 = GD13.5 – 17.5)

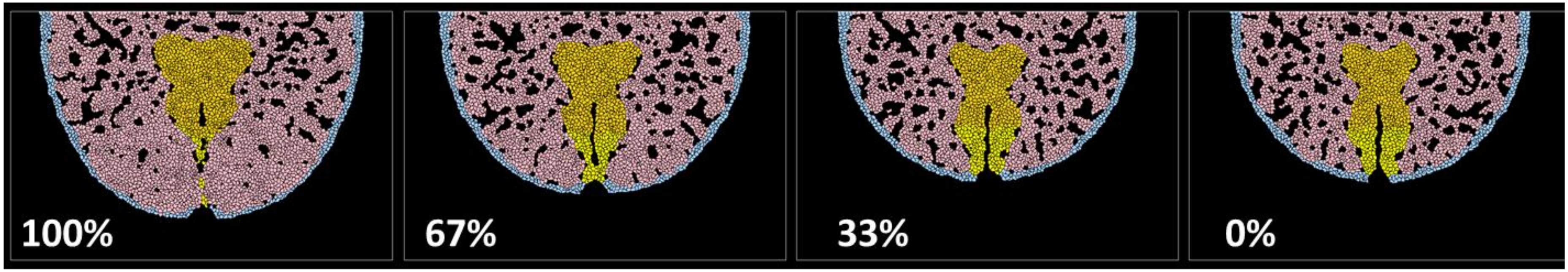


- sexually indifferent at MCS 0 (GD13.5)
- androgen production by fetal testis introduced at MCS 2000 (GD15.5)
- sexual dimorphism evaluated at MCS 4000 (GD17.5)

Urethral Closure: complex process disrupted in 'hypospadias'

- Driven by urethral endoderm (contact, fusion apoptosis) and preputial mesenchyme (proliferation, condensation, migration).

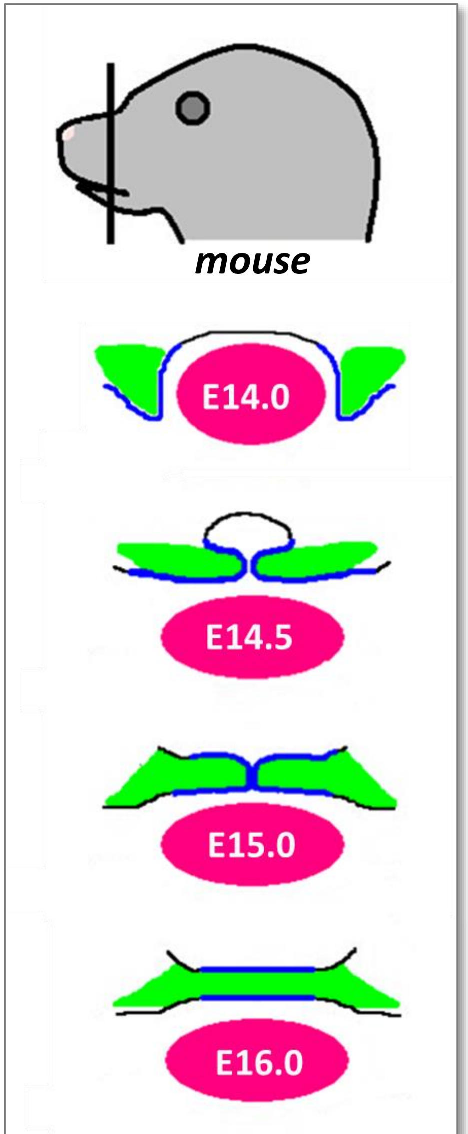
- Disruption of SHH, FGF10, or AR signaling leads to urethral closure defects (e.g., hypospadias).



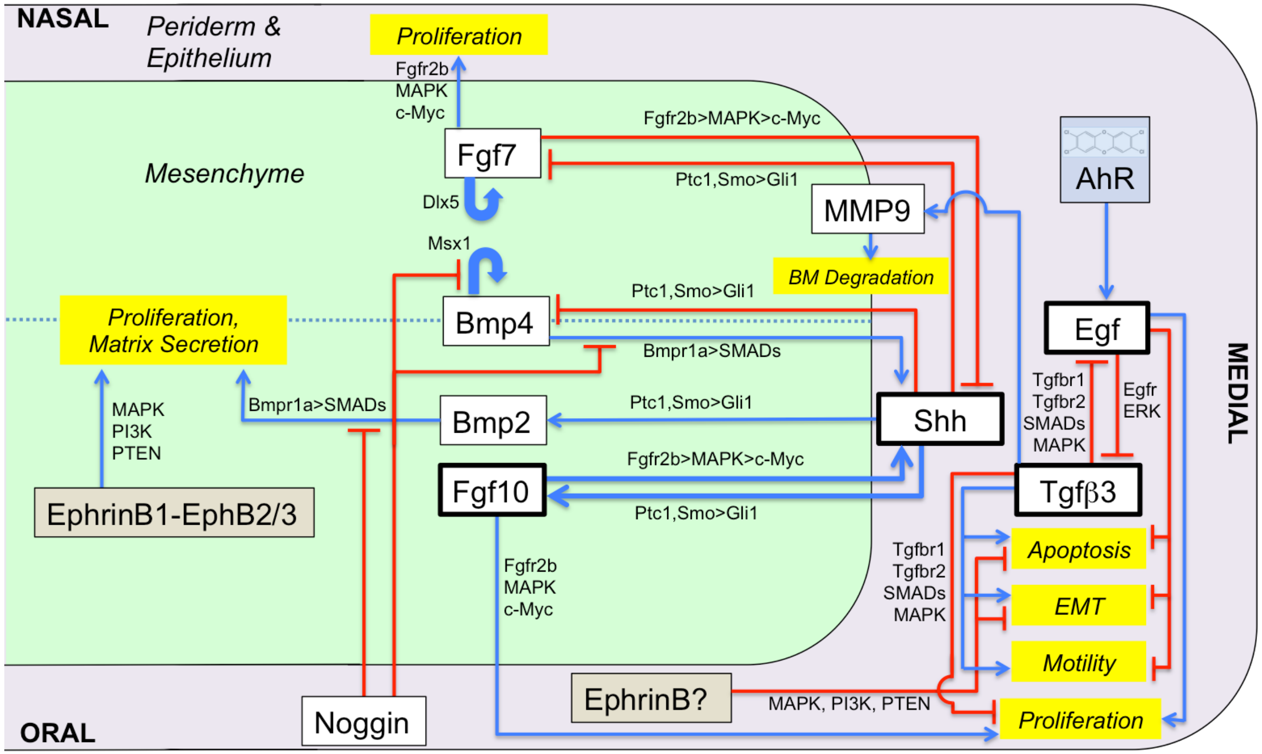
Androgenization *(n = 10 sims)*	***Phenotype (MCS 4000)*** Septation	Fusion	Conden.	Closure Index
100%	6/10	8/10	10/10	0.80
67%	2/10	5/10	10/10	0.57
33%	0/10	4/10	0/10	0.13
0%	0/10	2/10	0/10	0.07

Leung et al. (2016) ***Reproductive Toxicology***

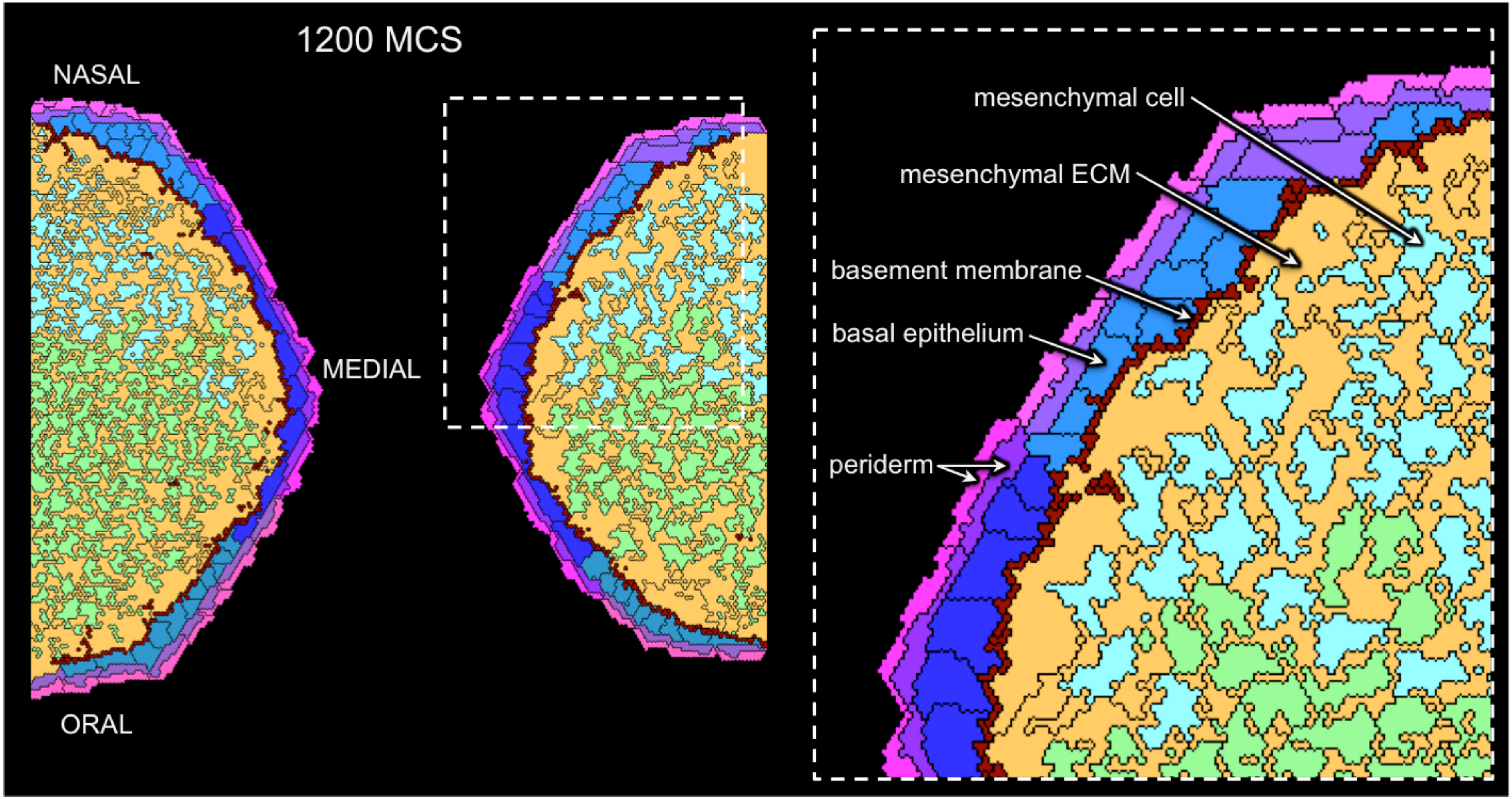
Modeling Palatal Development



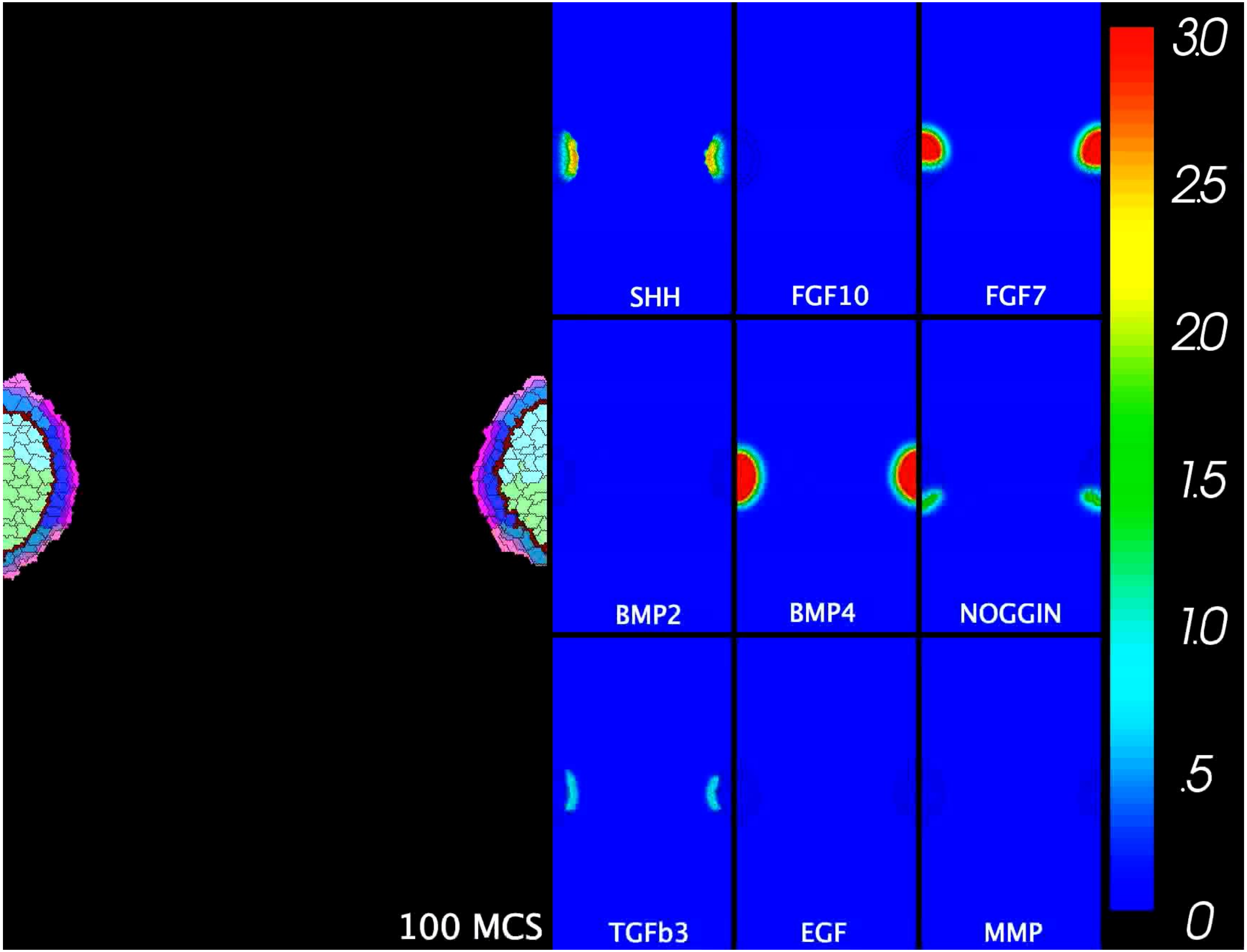
- E12.5 initial outgrowth of palatal shelves
- E13.5 expansion alongside the tongue
- E14.5 elevate, meet, and adhere at medial edge
- E15.5 fusion complete, mesenchymal confluence
- E16.5 osteogenic differentiation



Modeling Palatal Development

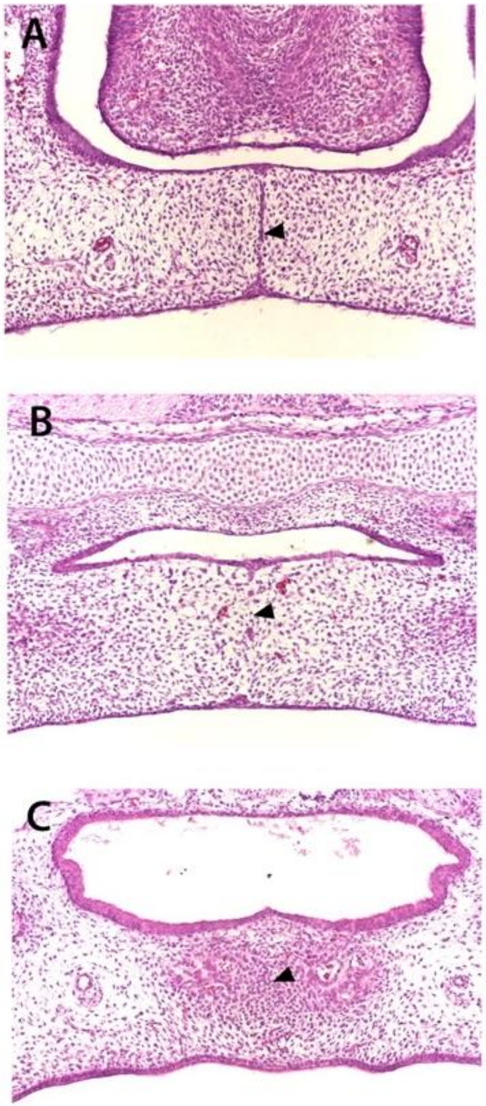


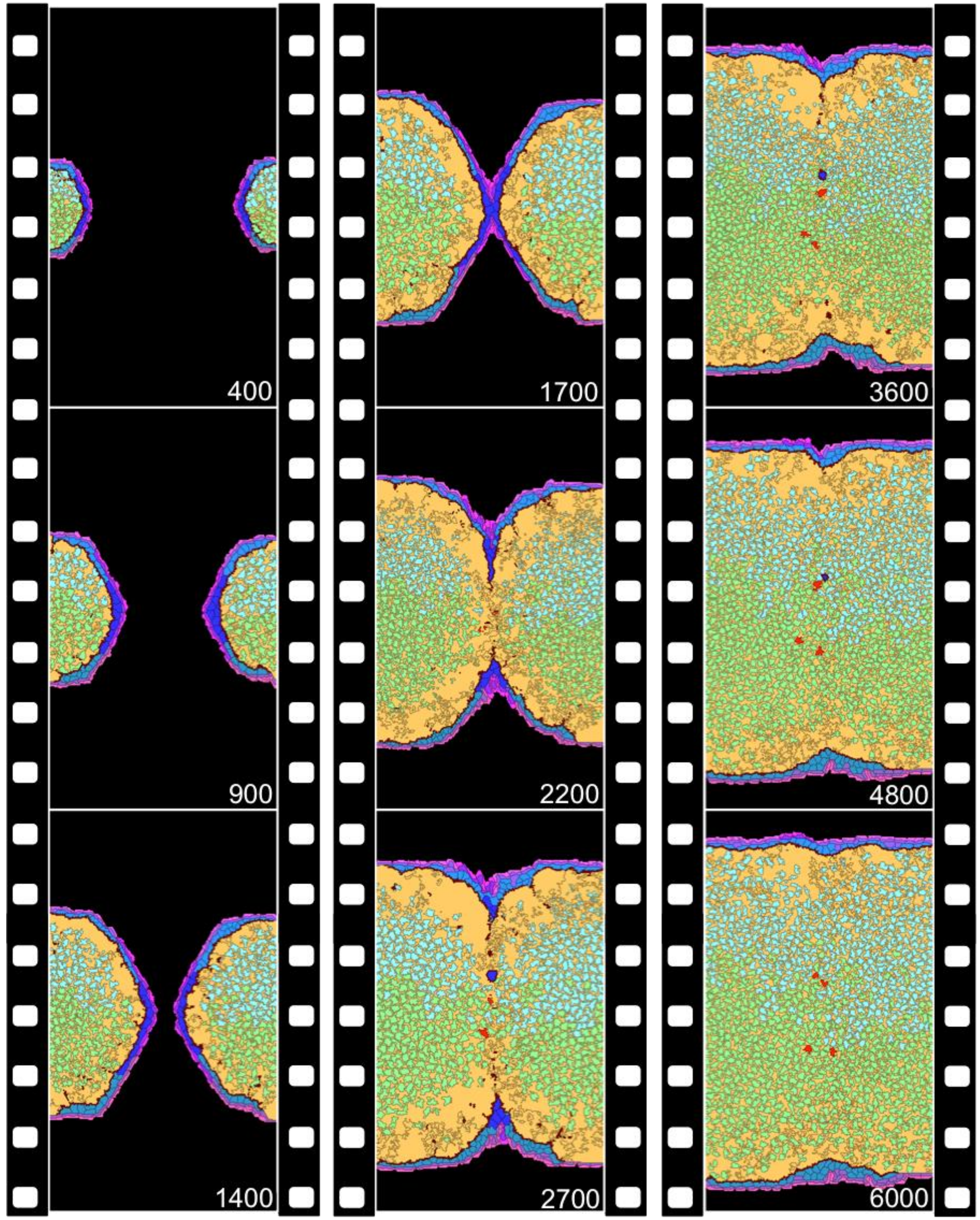
Hutson et al. (2016) manuscript under review


SHH
FGF10
FGF7
BMP2
BMP4
NOGGIN
TGFb3
EGF
MMP
100 MCS
3.0
2.5
2.0
1.5
1.0
.5
0

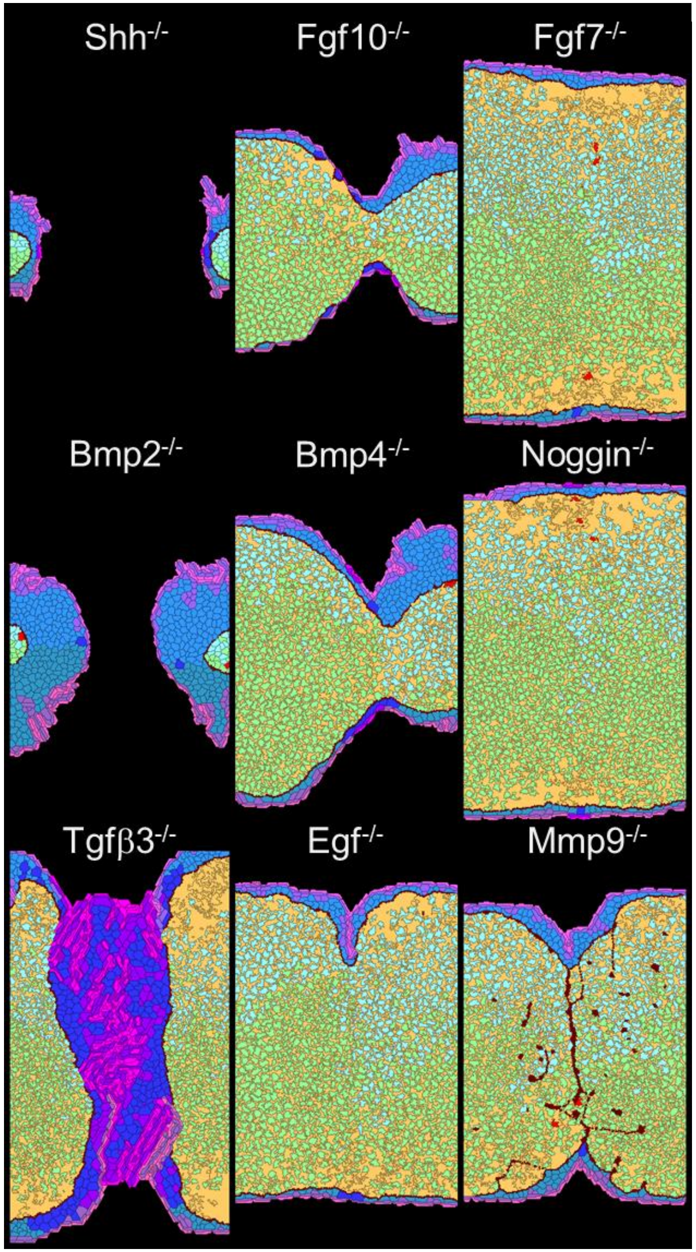
ABM for Fusion

Jin and Ding (2006) Development





Hacking the Control Network: *in silico* knockouts



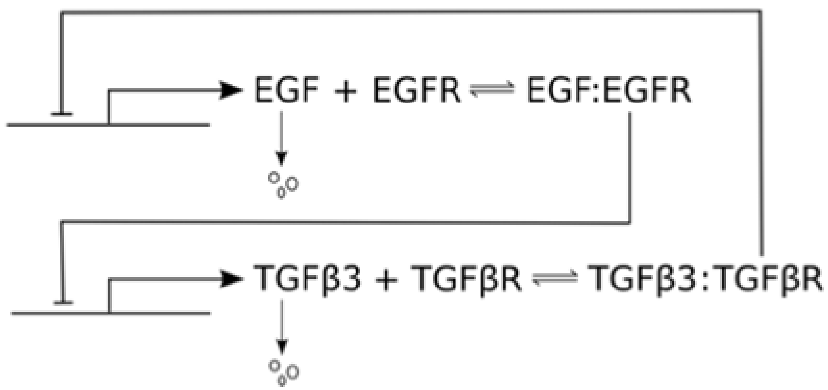
Outgrowth to MEE contact (MCS 200-2000)

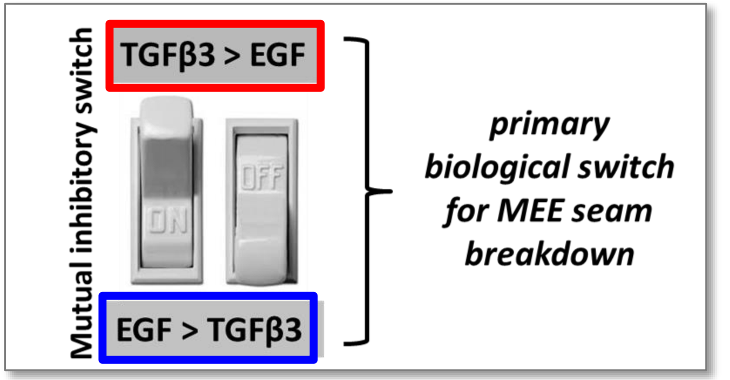
- SHH emanating from MEE is the primary driver of mesenchymal proliferation and ECM production.
- FGF10, BMP2, BMP4 are main effectors in the mesenchyme and feedback onto the epithelium.
- FGF7, Noggin are negative effectors in the mesenchyme, and feedback onto epithelium.

MES breakdown (MCS 2000-3000)

- TGFβ3 triggers MEE cells to programmed cell death (apoptosis), epithelial-mesenchymal transition (EMT), or migration (retraction).
- EGF has the opposite effect, maintaining MEE proliferation and survival.

TGF-EGF switch as a molecular target

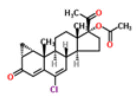




- MEE expression of TGFβ3 peaks just before adhesion, whereas EGFR expression drops (e.g., switch is flipped).
- Several teratogens ↑EGFR expression, induce MEE proliferation, and disrupt fusion (e.g., switch not flipped): Retinoic acid, Hydrocortisone, TCDD [Abbott 2010].

ToxCast profiling of 63 cleft palate teratogens

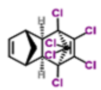
Cyproterone acetate



Triamcinolone

Chemical	Cleft palate	Gene score NR3C1 (glucocorticoid receptor)
Cyproterone acetate	1	4.74

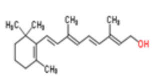
Aldrin



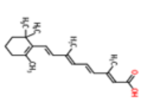
Endrin

Dieldrin

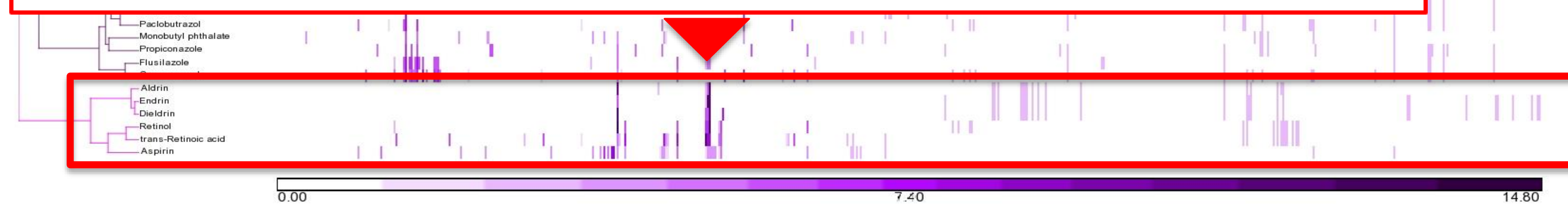
Retinol



Trans-retinoic acid

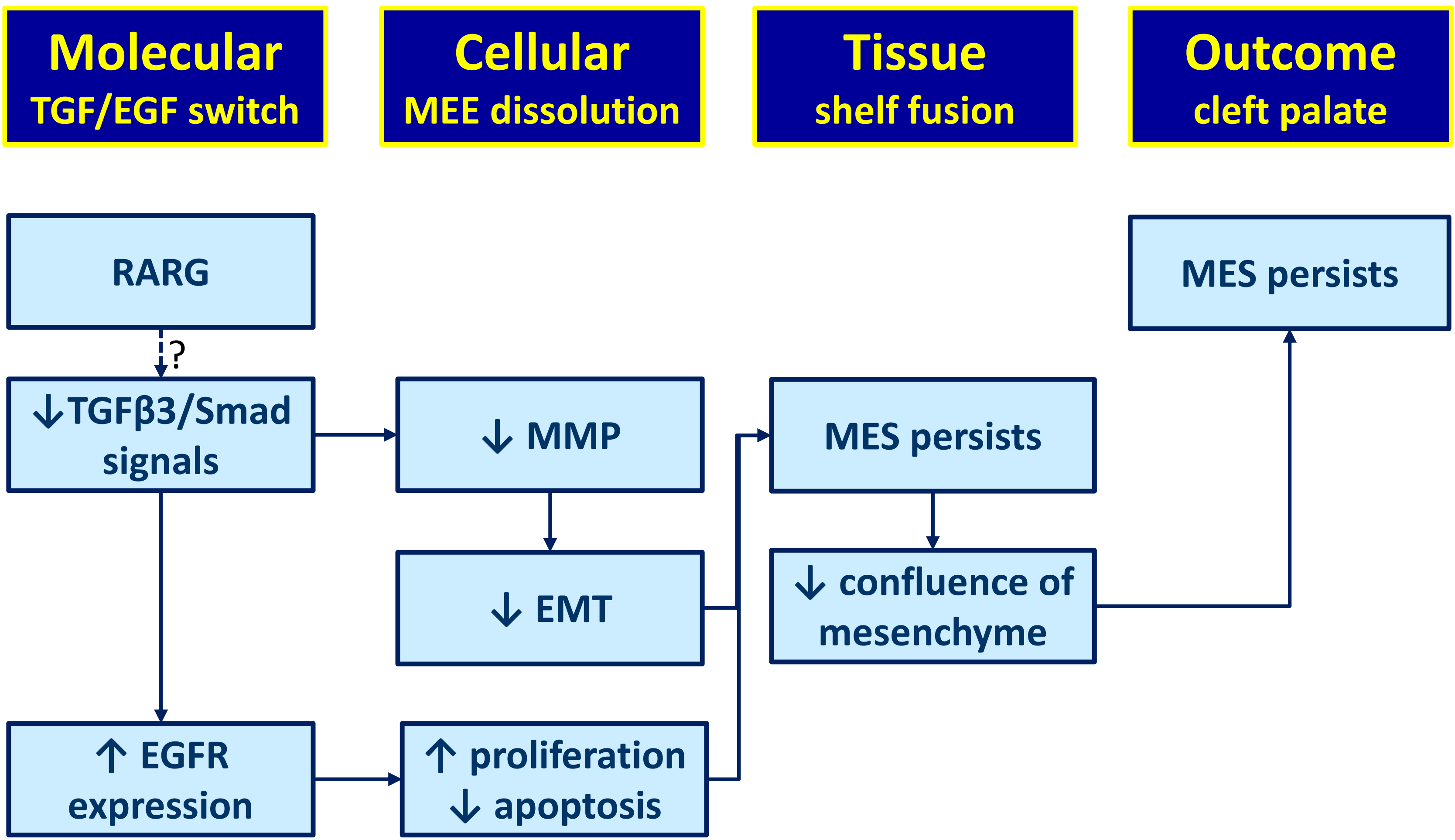


Chemical	Cleft palate	Gene score RARG (retinoic acid receptor gamma)
Aldrin	1	11.6
Endrin	1	11.9
Dieldrin	1	12.0
Retinol	1	13.5
Trans-retinoic acid	1	8.2
Tributyltin chloride	1	8.6
Bromuconazole	0	10.8

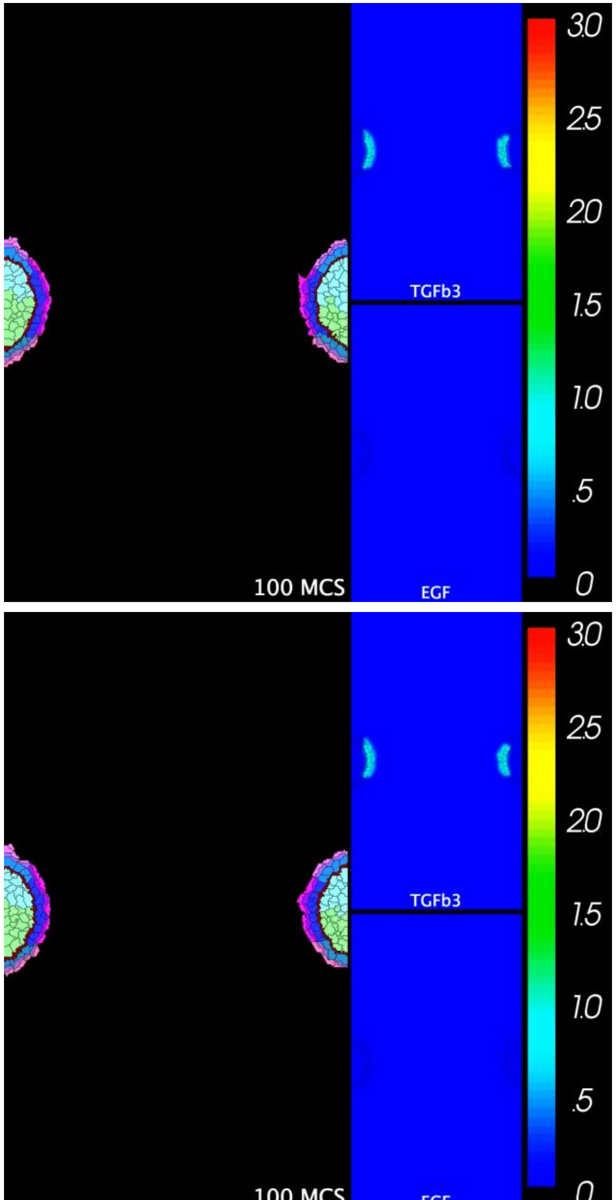


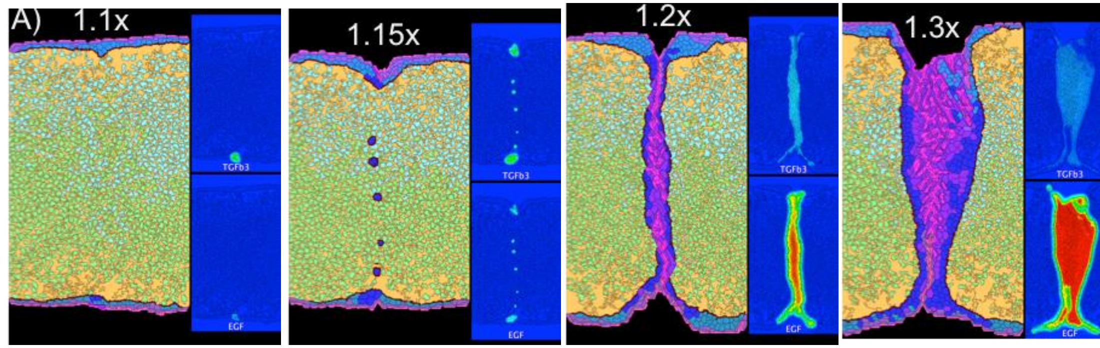
Baker et al. (2016) manuscript in preparation

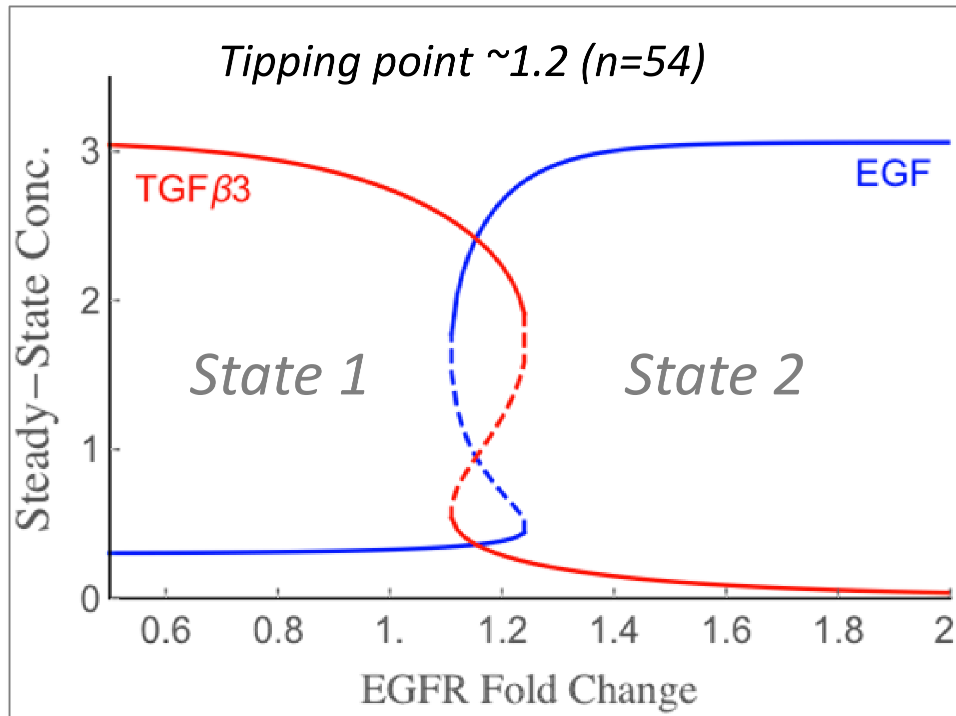
Putative AOP for Retinoic acid *(one of several!)*



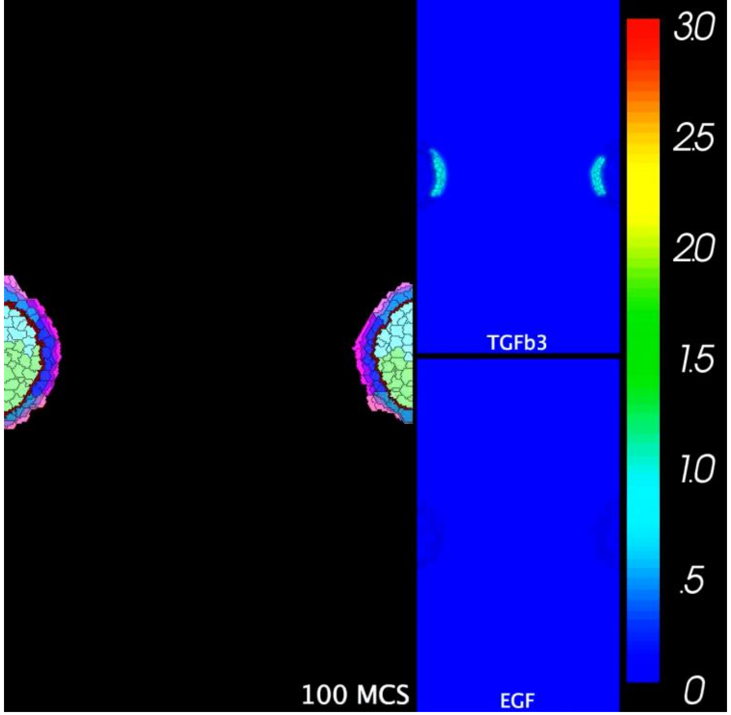
TGF-EGF circuit dynamics

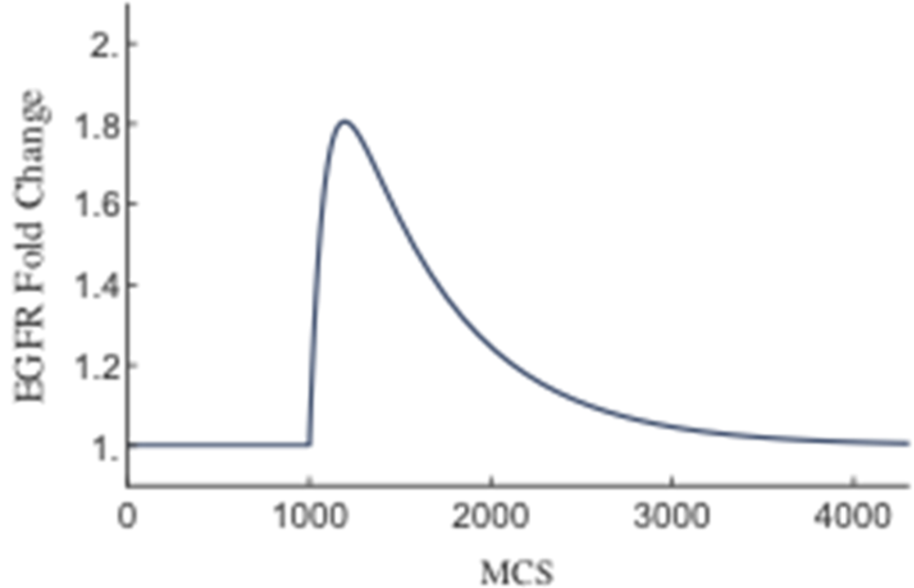


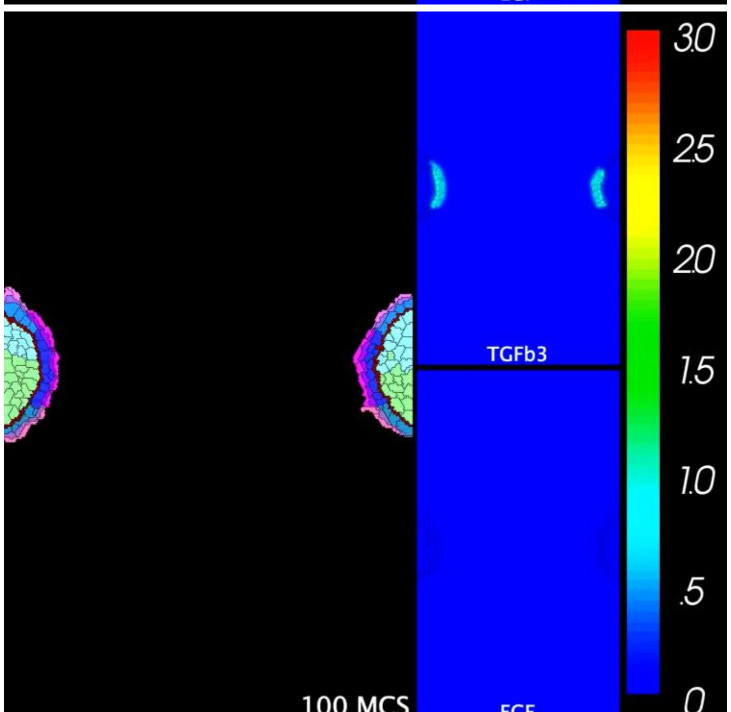


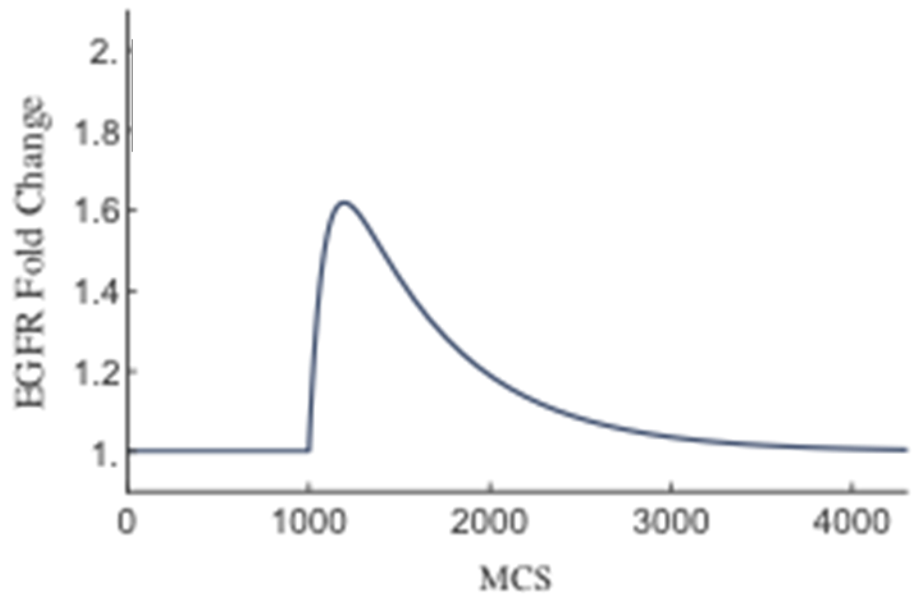


Acute Exposure

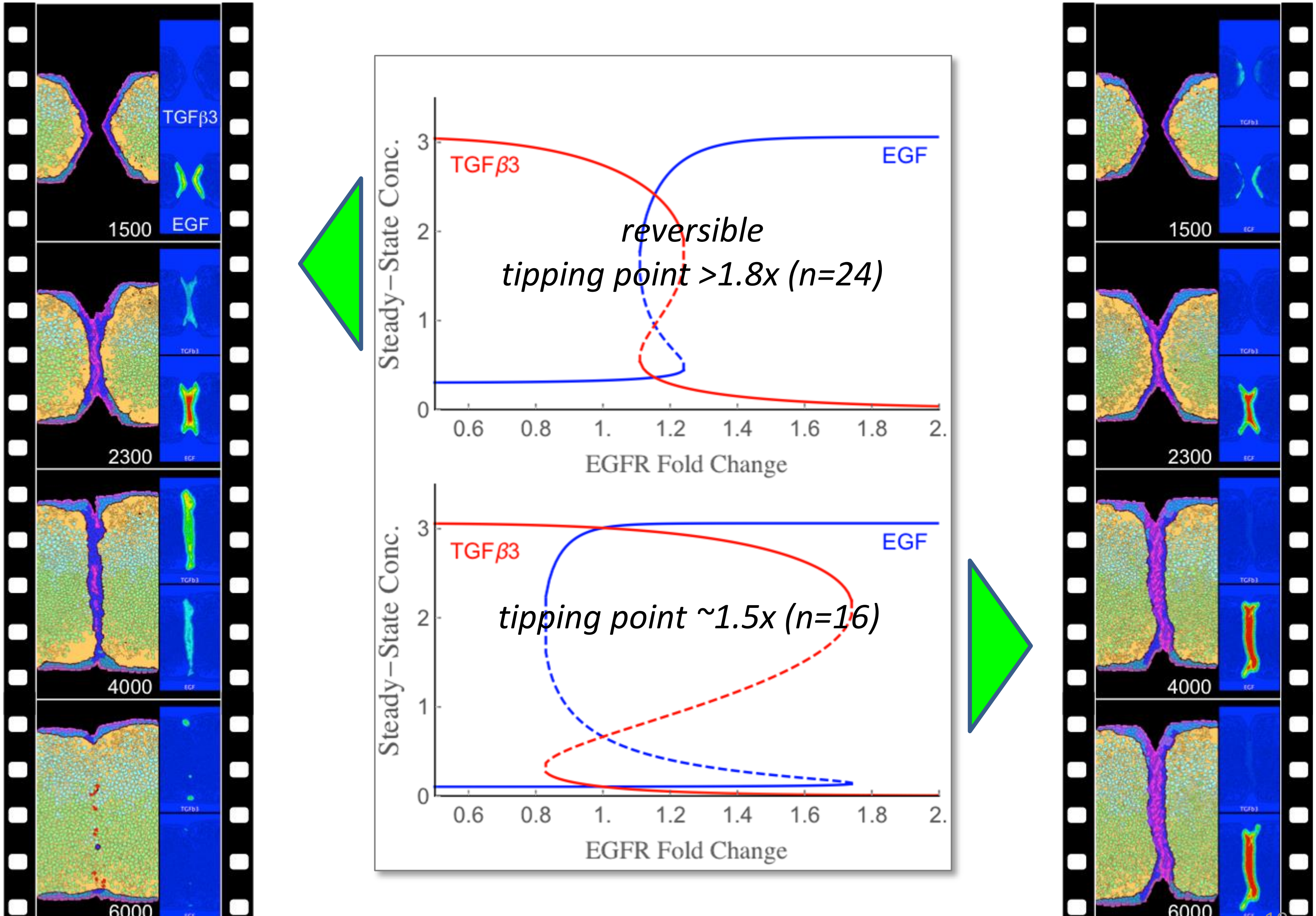






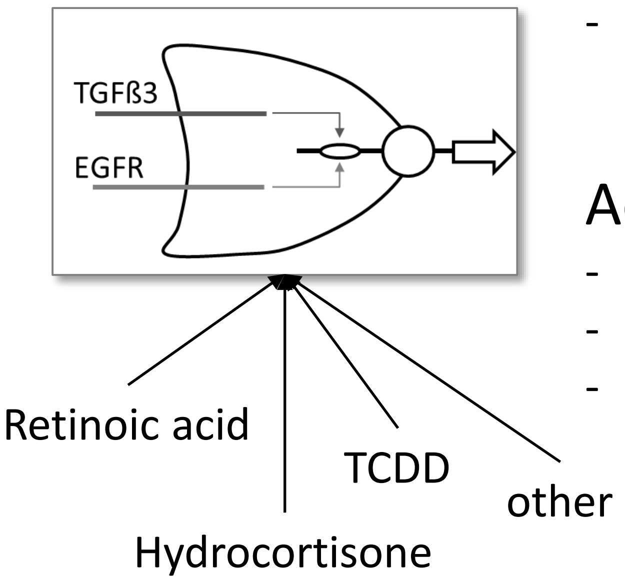


Impact of the bifurcation zone (acute exposure)



TGF-EGF switch (predicted impact)

Molecular
TGF/EGF switch

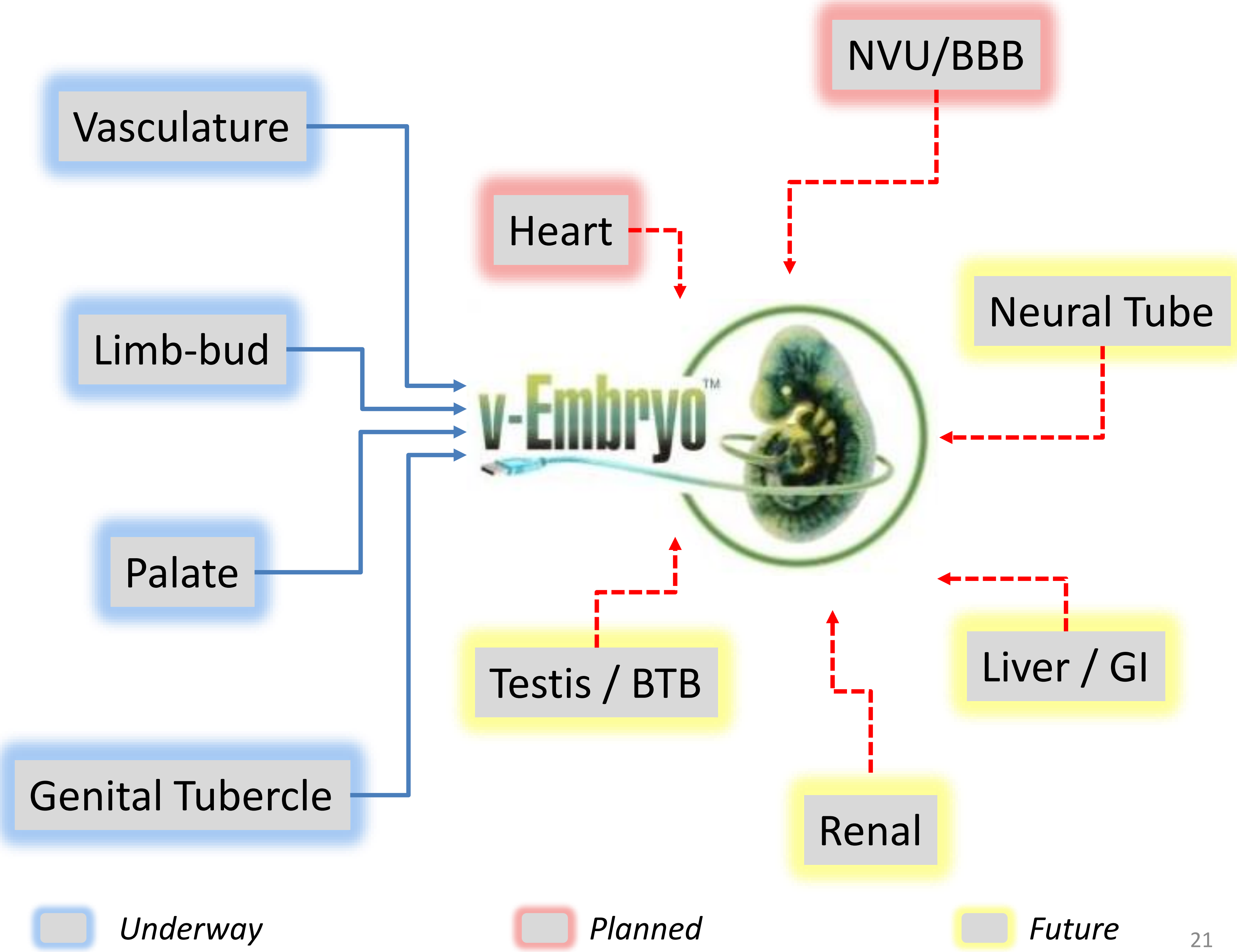


Chronic exposure scenario

- low hysteresis system tips at ~1.2x EGFR (n=54)
- high hysteresis system tips at ~1.2x EGFR (n=32)
- width of bifurcation zone does not seem to matter

Acute exposure scenario

- low hysteresis system tips at >1.8x EGFR (n=24)
- high hysteresis system tips at ~1.5x EGFR (n=16)
- more canalization with a narrow bifurcation zone



Vasculature
Limb-bud
Palate
Genital Tubercle
Heart
NVU/BBB
v-Embryo™
Neural Tube
Testis / BTB
Renal
Liver / GI
Underway
Planned
Future

Special Thanks

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- W Daly – U Wisconsin – STAR
- W Muphy – U Wisconsin / STAR
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- D Belair – NHEERL / TAD
- Sid Hunter – NHEERL / ISTD
- Max Leung – NCCT (now U Pittsburgh)
- Jill Franzosa – NCCT (ORISE)
- Nicole Kleinstreuer – NCCT (now NIEHS/NTP)
- Nisha Sipes – NCCT (now NIEHS/NTP)
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- Nancy Baker – Leidos / NCCT
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- B Cai – Vala Sciences
- D Rines – Vala Sciences
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- J Glazier – Indiana U / STAR
- Shane Hutson – Vanderbilt U / STAR
- K Saili – NCCT
- T Zurlinden - NCCT

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Virtual Tissue Models: Predicting How Chemicals Impact Human Development

http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf

National Center for Computational Toxicology