

# Computational Embryology and Predictive Toxicology of Cleft Palate

Nisha S. Sipes<sup>1</sup>, M. Shane Hutson<sup>2</sup>, Nancy C. Baker<sup>1</sup>, Thomas B. Knudsen<sup>1</sup>

<sup>1</sup>US EPA, ORD, National Center for Computational Toxicology

<sup>2</sup>Depts of Physics & Astronomy and Biological Sciences, Vanderbilt University



**53<sup>rd</sup> Teratology Society Annual Meeting**

*June 25, 2013 Tucson, Arizona*

Platform Session 4: In Vitro, Computational, and Alternative Methods and Models for Screening Developmental Toxicants

## ***Disclosure***

**The authors of this research have no financial or other interests which pose a conflict of interest.**

**This research was funded by the United States Environmental Protection Agency, Office of Research and Development.**

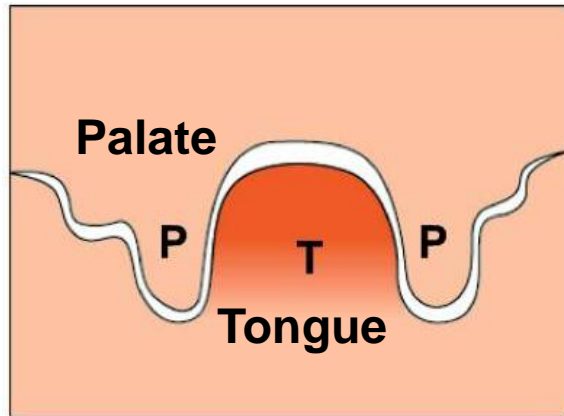
**Disclaimer: views are those of the presenter and do not necessarily reflect Agency policy nor imply endorsement of software used here.**

# What is Cleft Palate (CP)?

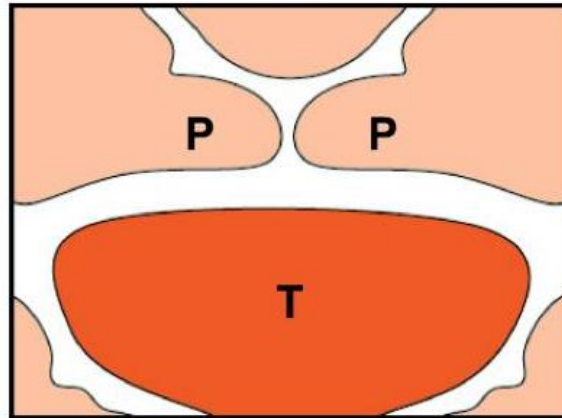
Disruption of secondary palate formation

Affects 1/700 live births worldwide

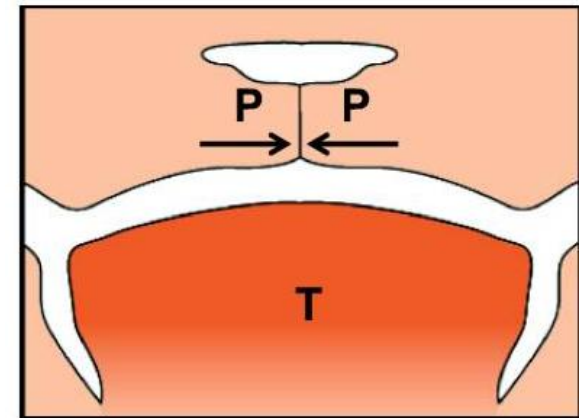
Mouse palatal development



**Growth**



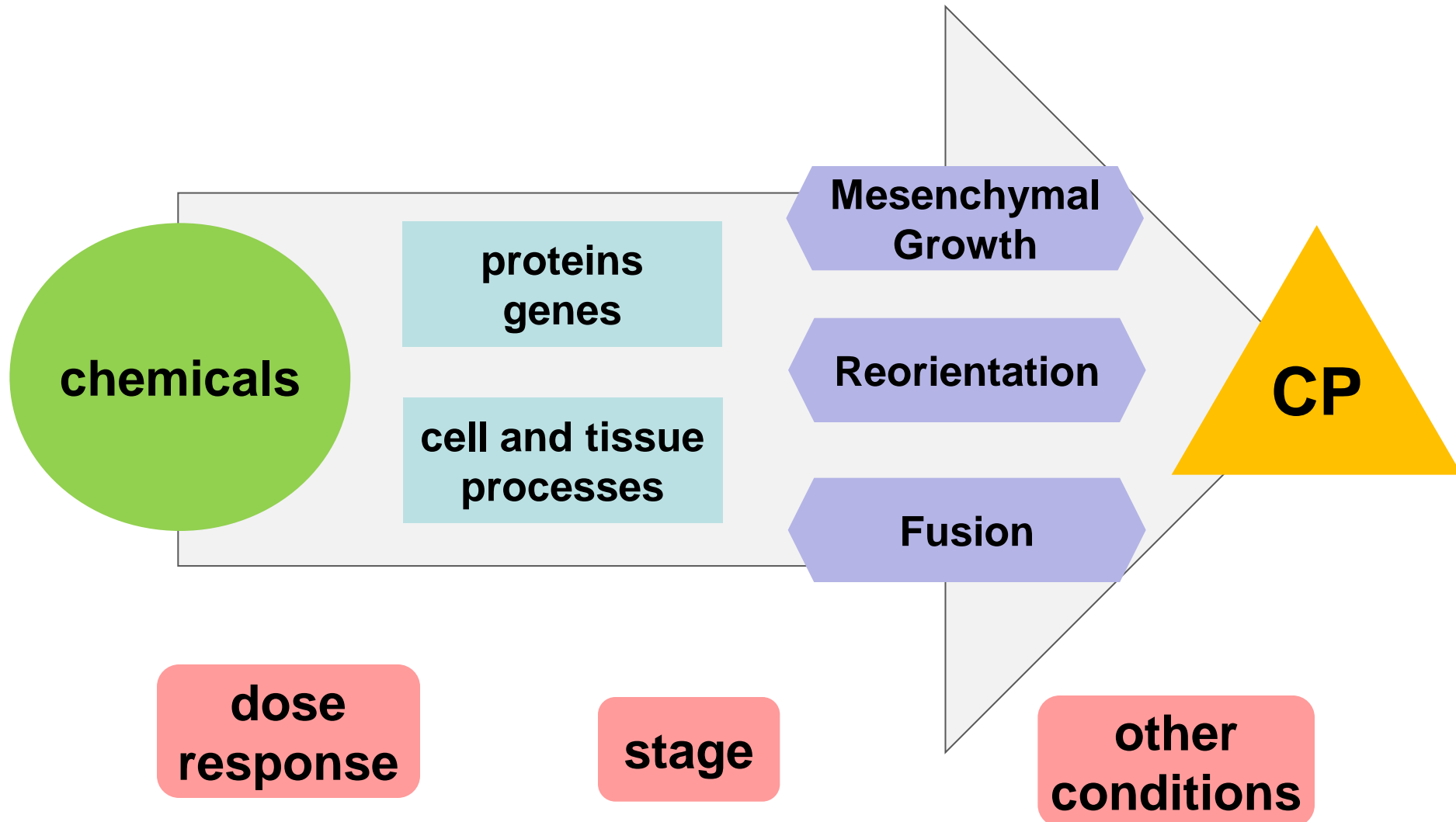
**Reorientation**



**Fusion**

# What Do We Want to Know?

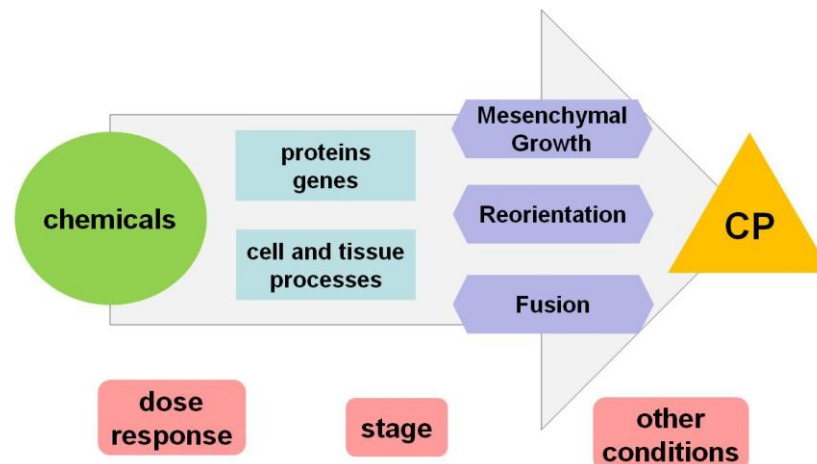
Environmental influences leading to CP



# A Systems Biology Framework

## Cleft Palate (CP)

1. Biological data from public literature (e-libraries)
2. High-throughput assay associations with CP
3. Adverse Outcome Pathway (AOP) elucidation
4. Multi-cellular/-scale computer simulation

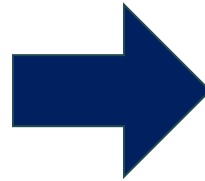


# 1. e-library Development

Literature Mining Using MeSH Annotations



*> 20 million total articles*



**effects/  
diseases**

**proteins/  
genes**

**chemicals**

**cell  
processes**

# 1. e-library Development

## Literature Mining Using MeSH Annotations

The screenshot shows a PubMed article page for PMID: 21254359. The article title is "Folate pathway and nonsyndromic cleft lip/palate". The abstract discusses the role of folate metabolism in cleft lip/palate. The MeSH terms are listed on the right side of the page, enclosed in a green box. The terms include: 5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase/genetics, Betaine-Homocysteine S-Methyltransferase/genetics, Carbon-Nitrogen Ligases/genetics, Cleft Lip/complications\*, Cleft Lip/ethnology, Cleft Lip/genetics, Cleft Palate/complications\*, Cleft Palate/ethnology, Cleft Palate/genetics, European Continental Ancestry Group/genetics, Folic Acid/metabolism\*, Genes/genetics\*, Genetic Predisposition to Disease\*, Hispanic Americans/genetics, Humans, Methylenetetrahydrofolate Reductase (NADPH2)/genetics, Nitric Oxide Synthase Type III/genetics, Polymorphism, Single Nucleotide/genetics\*, Reduced Folate Carrier Protein/genetics, and Thymidylate Synthase/genetics.

← → C H www.ncbi.nlm.nih.gov/pubmed/21254359

NCBI Resources How To

PubMed.gov  
US National Library of Medicine  
National Institutes of Health

Display Settings: Abstract

Birth Defects Res A Clin Mol Teratol. 2010;91(1):1-6.

**Folate pathway and nonsyndromic cleft lip/palate**

Blanton SH, Henry RR, Yuan Q, Mullik KA, et al. University of Miami Miller School of Medicine, Miami, FL.

**Abstract**

**BACKGROUND:** Nonsyndromic cleft lip/palate (NSCLP) is a common congenital anomaly. Folate supplementation with folic acid, a key component of the folate pathway, is known to reduce the risk of neural tube defects and may similarly reduce the risk of cleft lip/palate. However, the role of two common methylenetetrahydrofolate reductase (MTHFR) polymorphisms, C677T (rs1801133) and A1298C (rs1801131), in NSCLP is unclear, as few genes/SNPs have been associated with the folate pathway. We investigated whether these polymorphisms were associated with NSCLP in a large, multiethnic, case-control study.

**METHODS:** Fourteen folate metabolism genes were sequenced in 1,000 white and Hispanic NSCLP families.

**RESULTS:** Evidence for a risk association was found for the C677T polymorphism in the white group, whereas associations with MTHFR polymorphisms were not significant. The transmission of haplotypes and gene-environment interactions were also investigated.

**CONCLUSIONS:** These results suggest that folate metabolism may play a role in the etiology of NSCLP. Further studies are needed to provide support for other studies that have investigated the role of folate metabolism in NSCLP.

Copyright © 2010 Wiley-Liss, Inc.

PMID: 21254359 [PubMed - indexed for MEDLINE]

**Publication Types, MeSH Terms**

**LinkOut - more resources**

**MeSH Terms**

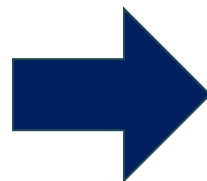
- 5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase/genetics
- Betaine-Homocysteine S-Methyltransferase/genetics
- Carbon-Nitrogen Ligases/genetics
- Cleft Lip/complications\*
- Cleft Lip/ethnology
- Cleft Lip/genetics
- Cleft Palate/complications\*
- Cleft Palate/ethnology
- Cleft Palate/genetics
- European Continental Ancestry Group/genetics
- Folic Acid/metabolism\*
- Genes/genetics\*
- Genetic Predisposition to Disease\*
- Hispanic Americans/genetics
- Humans
- Methylenetetrahydrofolate Reductase (NADPH2)/genetics
- Nitric Oxide Synthase Type III/genetics
- Polymorphism, Single Nucleotide/genetics\*
- Reduced Folate Carrier Protein/genetics
- Thymidylate Synthase/genetics

# 1. e-library Development

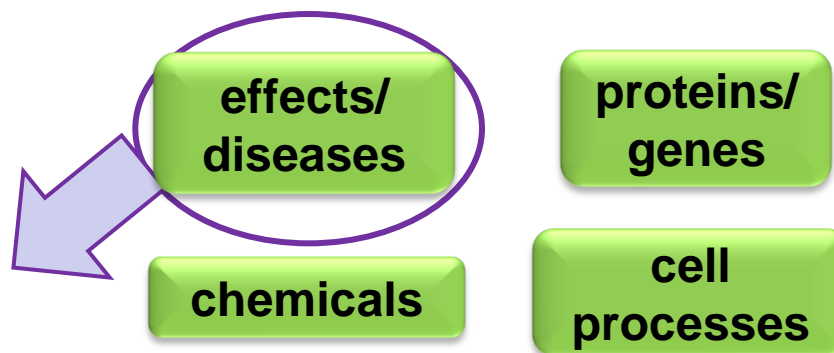
Literature Mining Using MeSH Annotations



*> 20 million total articles*



**Cleft Lip / Palate**



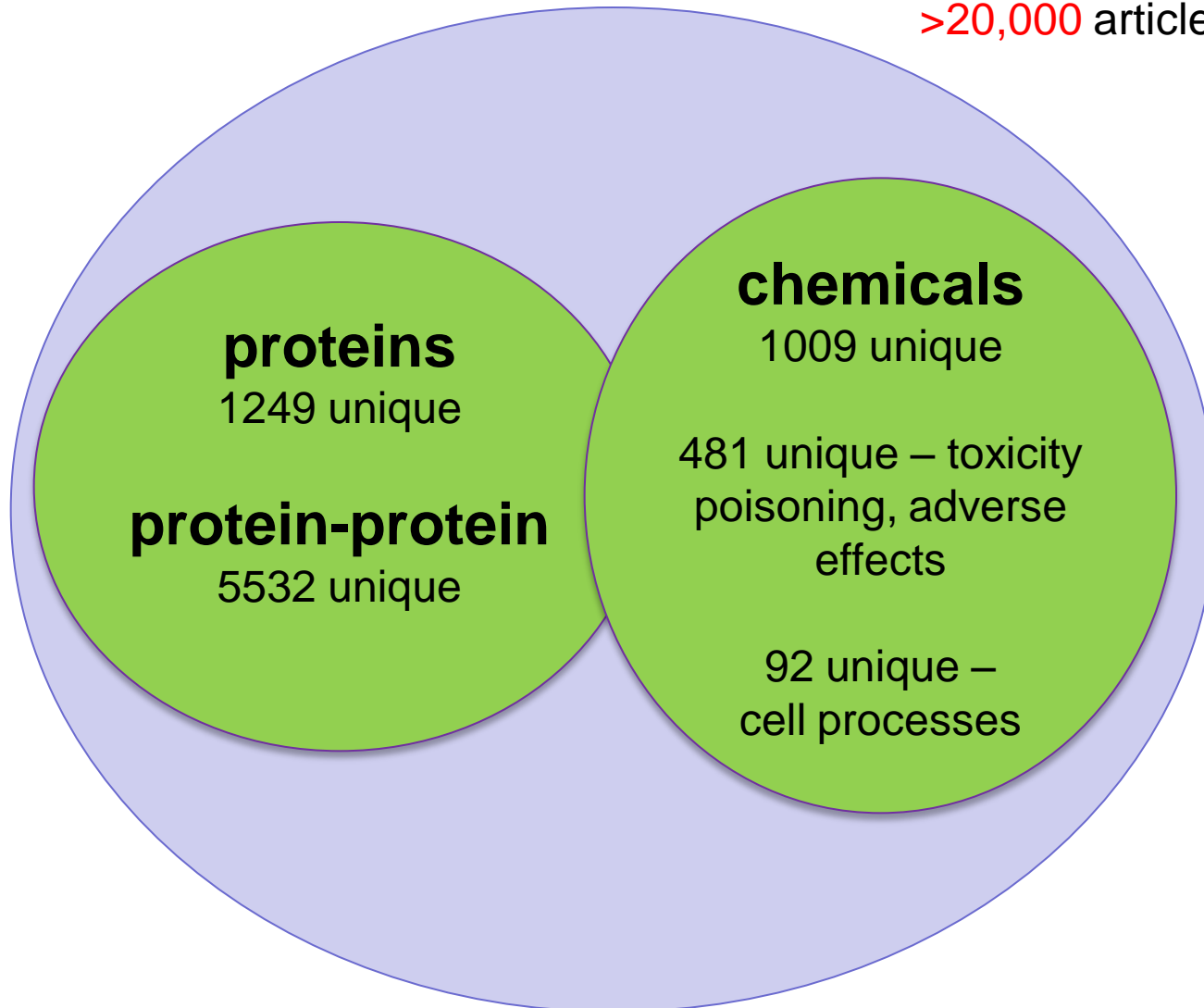
*via computer languages and database software*



# 1. e-library Development

## Cleft Lip/Palate Articles

>20,000 articles



# 1. e-library Development

## CLP Chemicals and Cell Processes

	A	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
1	Cleft Palate Chemicals and Cell Processes																															
2	Article counts for co-occurring MeSH terms annotated with drug effects																															
3	Chemicals	G0 Phase	G1 Phase	G2 Phase	S Phase	Cell Death	Apoptosis	Anoikis	Autophagy	Cell Dedifferentiation	Cell Differentiation	Adipogenesis	Gametogenesis	Oogenesis	Spermatogenesis	Sperm Maturation	Hematopoiesis	Erythropoiesis	Hematopoiesis, Extramed	Leukopoiesis	Lymphopoiesis	Myelopoiesis	Neurogenesis	Cell Fusion	Cell Growth Processes	Cell Enlargement	Cell Proliferation	Cell Division	Cell Movement	Cell Aggregation	Cell Migration Inhibition	Chemotaxis
22	Betamethasone	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
23	bis(tri-n-butyltin)oxide	0	0	0	0	0	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	
24	Bromodeoxyuridine	1	1	1	3	10	10	0	0	0	14	0	0	0	1	0	0	0	0	0	0	0	1	0	0	17	28	4	0	0	0	
25	butylbenzyl phthalate	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	
26	Cacodylic Acid	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	6	0	0	0	0	
27	Caffeine	0	0	7	2	1	9	0	0	0	5	0	0	0	2	0	0	0	0	0	0	0	0	1	0	2	13	1	1	0	0	
28	Carbaryl	0	0	0	0	1	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	
29	Carmustine	0	0	2	0	2	3	0	0	0	1	0	0	0	0	0	4	1	0	0	0	0	0	0	0	1	15	1	0	0	0	
30	Chlorpyrifos	0	0	0	0	6	10	0	0	0	14	1	1	2	2	0	0	0	0	0	0	0	4	0	0	4	4	1	0	0	0	
31	Corticosterone	0	0	0	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	
32	Cortisone	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
33	cyclopamine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

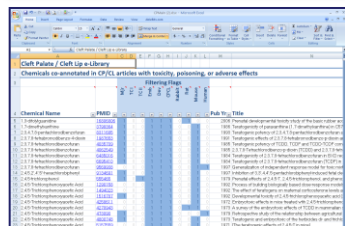
# 1. e-library Development

## CLP Chemicals and Cell Processes

	A	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
1	Cleft Palate Chemicals - AOP Mat																															
2	Article	A	B	C	D	F	G	H																								
	1	Cleft Palate Chemicals - AOP Mat		<<-- Back																												
	2	Cell Processes - detail view																														
	3			Filtering Flags																												
	4	ChemName	Process	Tox	Palate	PMID	PubYr	Title																								
	1018	Caffeine	G2 Phase	1	0	<a href="#">1944378</a>	1991	Effects of caffeine and cycloheximide during G																								
	1019	Caffeine	S Phase	1	0	<a href="#">8613687</a>	1996	UV-B-induced cell cycle perturbations, micronu																								
	1020	Caffeine	S Phase	1	0	<a href="#">7506363</a>	1994	A case of caffeine-mediated cancellation of mi																								
	1021	Caffeine	Cell Death	1	0	<a href="#">8512808</a>	1993	Caffeine potentiates the lethality of tumour ne																								
3	Chem	1022	Caffeine	Apoptosis	1	0	<a href="#">15039113</a>	2004	1,2-bis(2-aminophenoxy)ethane-N,N,N'-tetra																							
22	Beta	1023	Caffeine	Apoptosis	1	0	<a href="#">10769661</a>	2000	Sensitization and caffeine potentiation of cispl																							
23	bis(t	1024	Caffeine	Apoptosis	1	0	<a href="#">12378022</a>	2002	Caffeine induces apoptosis in human neurobla																							
24	Bron	1025	Caffeine	Apoptosis	1	0	<a href="#">9458292</a>	1998	Enhancement of CDDP cytotoxicity by caffeine																							
25	buty	1026	Caffeine	Apoptosis	1	0	<a href="#">12395097</a>	2002	Caffeine-induced neuronal death in neonatal r																							
26	Caco	1027	Caffeine	Apoptosis	1	0	<a href="#">12884404</a>	2003	Apoptosis induced by different doses of caffei																							
27	Caffe	1028	Caffeine	Apoptosis	1	0	<a href="#">8299722</a>	1994	Enhancement of CDDP cytotoxicity by caffeine																							
28	Carb	1029	Caffeine	Apoptosis	1	0	<a href="#">16709440</a>	2007	The enigmatic effects of caffeine in cell cycle a																							
29	Carn	1030	Caffeine	Apoptosis	1	0	<a href="#">8512808</a>	1993	Caffeine potentiates the lethality of tumour ne																							
30	Chlo	1031	Caffeine	Cell Differentiation	1	0	<a href="#">22470550</a>	2012	Exploring the caffeine-induced teratogenicity o																							
31	Cort	1032	Caffeine	Cell Differentiation	1	0	<a href="#">7948410</a>	1994	In vitro study of teratogenic effects of caffeine																							
32	Cortiso	1033	Caffeine	Cell Differentiation	0	0	<a href="#">2885939</a>	1987	Potentiating effect of caffeine on embryotoxic																							

## 2. High throughput links to CP

*in vivo data*



*e-libraries* (25)

+

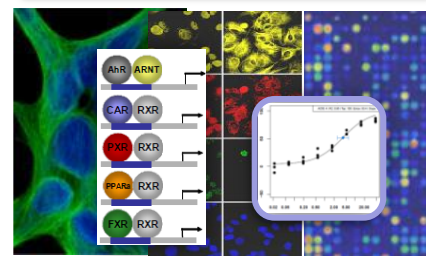


*ToxRefDB* (31)

**CP  
teratogens**

**non-CP  
teratogens**

*in vitro testing*



*ToxCastDB*

**> 700 assays**  
**Concentration response**

## 2. High throughput links to CP

Assay Name	Description
APR_CellCycleArrest_72hr	cell-cycle arrest
BSK_CASM3C_Proliferation_down	decreased proliferation
BSK_CASM3C_SAA_up	serum amyloid
ATG_ERE_CIS	estrogen receptor
ATG_GR_TRANS	glucocorticoid receptor
ATG_RARa_TRANS	retinoic acid receptor
ATG_RARb_TRANS	retinoic acid receptor
ATG_RARg_TRANS	retinoic acid receptor
ATG_RXRa_TRANS	retinoid x receptor
ATG_RXRb_TRANS	retinoid x receptor
NVS_ADME_hCYP19A1	cytochrome p450
NVS_ENZ_hCASP5	caspase 5
NVS_ENZ_hElastase	elastase
NVS_ENZ_hFGFR1	fibroblast growth factor
NVS_ENZ_hPTPN11	phosphatase
NVS_ENZ_oCOX1	cyclooxygenase
NVS_GPCR_bH1	histamine GPCR
NVS_GPCR_gH2	histamine GPCR
NVS_GPCR_gLTD4	leukotriene GPCR
NVS_GPCR_hAdoRA1	adenosine GPCR
NVS_GPCR_hAdoRA2a	adenosine GPCR
NVS_GPCR_rAdra1_NonSelective	adrenergic GPCR
NVS_GPCR_rAdra1A	adrenergic GPCR
NVS_GPCR_rAdra1B	adrenergic GPCR
NVS_GPCR_rAdra2_NonSelective	adrenergic GPCR
NVS_GPCR_rSST	somatostatin GPCR
NVS_OR_gSIGMA_NonSelective	sigma receptor
NVS_IC_rCaBTZCHL	ion channel
NVS_IC_rNaCh_site2	ion channel

½ in e-libraries

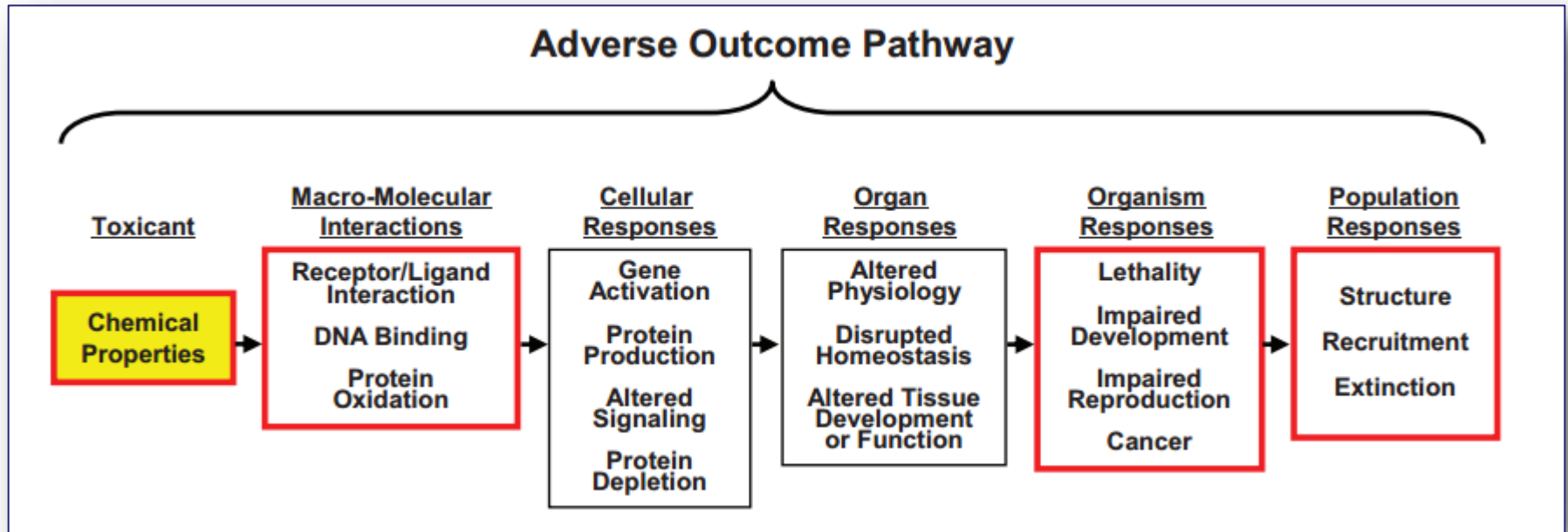
**Statistical  
associations**



**Quantitative  
chemical-assay  
concentration  
response**

# 3. Adverse Outcome Pathway (AOP)

conceptual and practical tool to capture multiscale descriptions of a toxicological process



***Develop predicted target-to-outcome pathways of CP teratogens***

**CP teratogens**

**Molecular**  
Target pathways

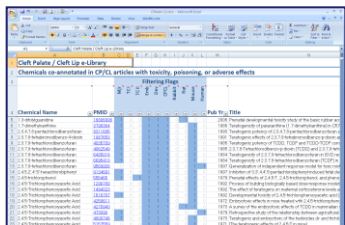
# 3. Cleft Palate AOP Framework

**Cellular**  
Cell-level behaviors

**Tissue**  
Palatal shelf

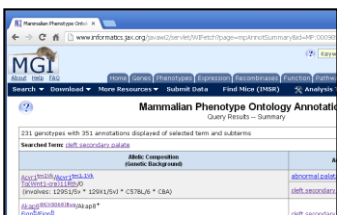
**Organ**  
Apposition

**Outcome**  
Structural  
malformation



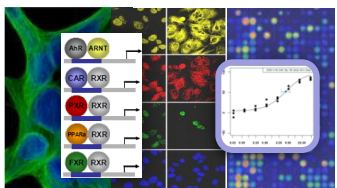
Chemical Name	CAS	PubMed Title
1,2-Dichloroethane	107-06-2	128777
1,3-Dichlorobenzene	95-13-8	128778
1,4-Dichlorobenzene	95-50-1	128779
1,5-Dichlorobenzene	95-50-1	128780
1,6-Dichlorobenzene	95-50-1	128781
1,7-Dichlorobenzene	95-50-1	128782
1,8-Dichlorobenzene	95-50-1	128783
1,9-Dichlorobenzene	95-50-1	128784
1,10-Dichlorobenzene	95-50-1	128785
1,11-Dichlorobenzene	95-50-1	128786
1,12-Dichlorobenzene	95-50-1	128787
1,13-Dichlorobenzene	95-50-1	128788
1,14-Dichlorobenzene	95-50-1	128789
1,15-Dichlorobenzene	95-50-1	128790
1,16-Dichlorobenzene	95-50-1	128791
1,17-Dichlorobenzene	95-50-1	128792
1,18-Dichlorobenzene	95-50-1	128793
1,19-Dichlorobenzene	95-50-1	128794
1,20-Dichlorobenzene	95-50-1	128795

**1. e-libraries**

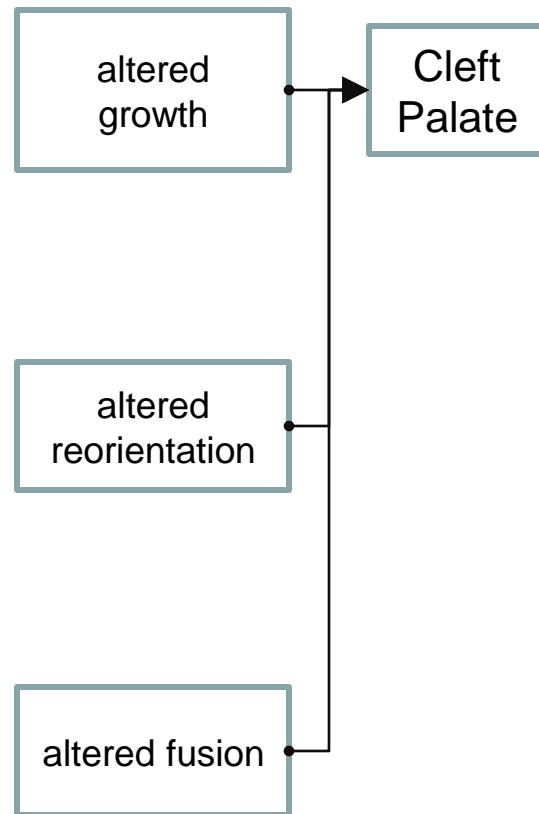


Gene	Phenotype
Alx1	abnormal palate
Alx2	abnormal palate
Alx3	abnormal palate
Alx4	abnormal palate
Alx5	abnormal palate
Alx6	abnormal palate
Alx7	abnormal palate
Alx8	abnormal palate
Alx9	abnormal palate
Alx10	abnormal palate
Alx11	abnormal palate
Alx12	abnormal palate
Alx13	abnormal palate
Alx14	abnormal palate
Alx15	abnormal palate
Alx16	abnormal palate
Alx17	abnormal palate
Alx18	abnormal palate
Alx19	abnormal palate
Alx20	abnormal palate
Alx21	abnormal palate
Alx22	abnormal palate
Alx23	abnormal palate
Alx24	abnormal palate
Alx25	abnormal palate
Alx26	abnormal palate
Alx27	abnormal palate
Alx28	abnormal palate
Alx29	abnormal palate
Alx30	abnormal palate
Alx31	abnormal palate
Alx32	abnormal palate
Alx33	abnormal palate
Alx34	abnormal palate
Alx35	abnormal palate
Alx36	abnormal palate
Alx37	abnormal palate
Alx38	abnormal palate
Alx39	abnormal palate
Alx40	abnormal palate
Alx41	abnormal palate
Alx42	abnormal palate
Alx43	abnormal palate
Alx44	abnormal palate
Alx45	abnormal palate
Alx46	abnormal palate
Alx47	abnormal palate
Alx48	abnormal palate
Alx49	abnormal palate
Alx50	abnormal palate
Alx51	abnormal palate
Alx52	abnormal palate
Alx53	abnormal palate
Alx54	abnormal palate
Alx55	abnormal palate
Alx56	abnormal palate
Alx57	abnormal palate
Alx58	abnormal palate
Alx59	abnormal palate
Alx60	abnormal palate
Alx61	abnormal palate
Alx62	abnormal palate
Alx63	abnormal palate
Alx64	abnormal palate
Alx65	abnormal palate
Alx66	abnormal palate
Alx67	abnormal palate
Alx68	abnormal palate
Alx69	abnormal palate
Alx70	abnormal palate
Alx71	abnormal palate
Alx72	abnormal palate
Alx73	abnormal palate
Alx74	abnormal palate
Alx75	abnormal palate
Alx76	abnormal palate
Alx77	abnormal palate
Alx78	abnormal palate
Alx79	abnormal palate
Alx80	abnormal palate
Alx81	abnormal palate
Alx82	abnormal palate
Alx83	abnormal palate
Alx84	abnormal palate
Alx85	abnormal palate
Alx86	abnormal palate
Alx87	abnormal palate
Alx88	abnormal palate
Alx89	abnormal palate
Alx90	abnormal palate
Alx91	abnormal palate
Alx92	abnormal palate
Alx93	abnormal palate
Alx94	abnormal palate
Alx95	abnormal palate
Alx96	abnormal palate
Alx97	abnormal palate
Alx98	abnormal palate
Alx99	abnormal palate
Alx100	abnormal palate

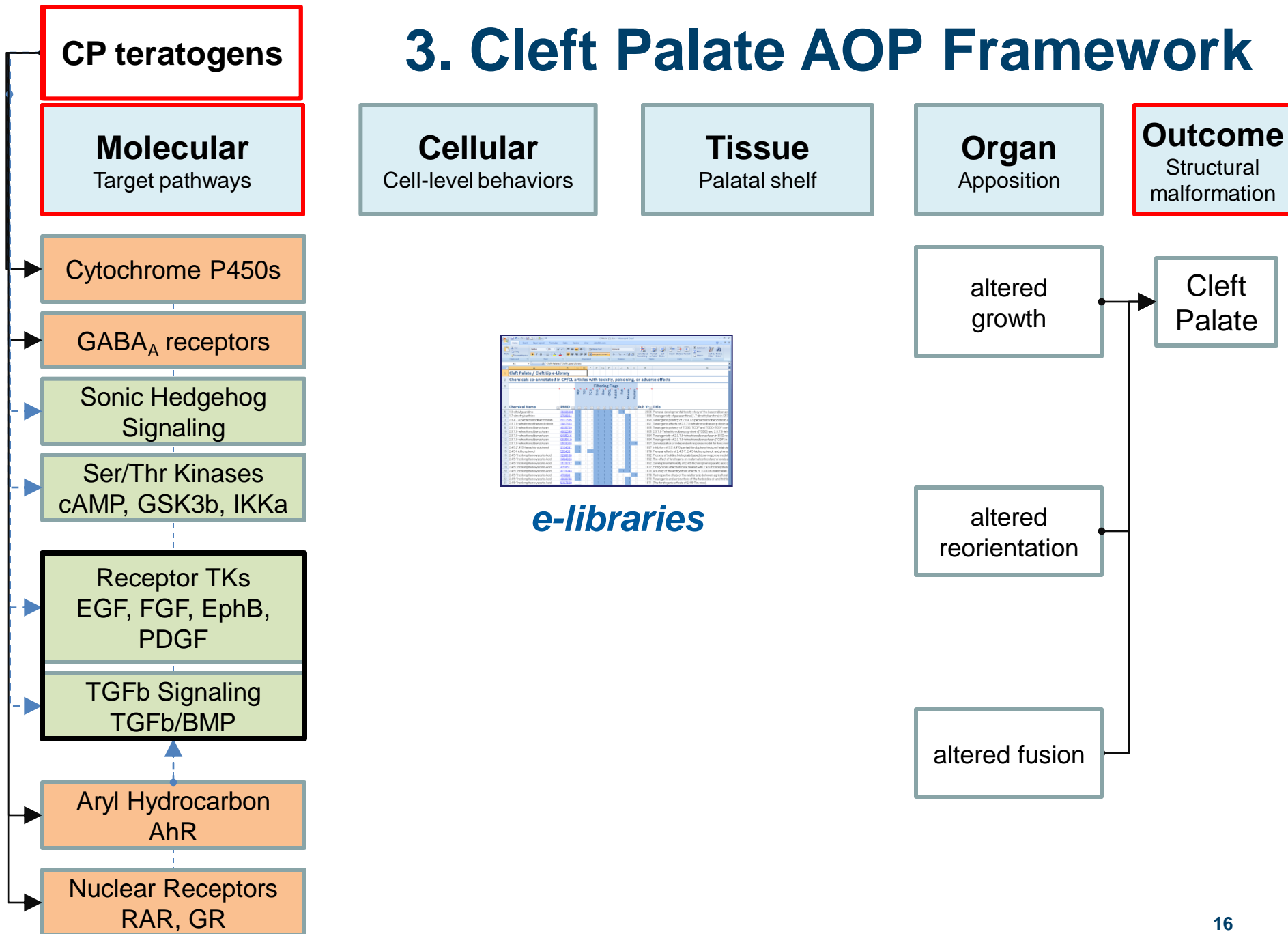
**2.informatics.**  
**jax.org**



**3. ToxCast**

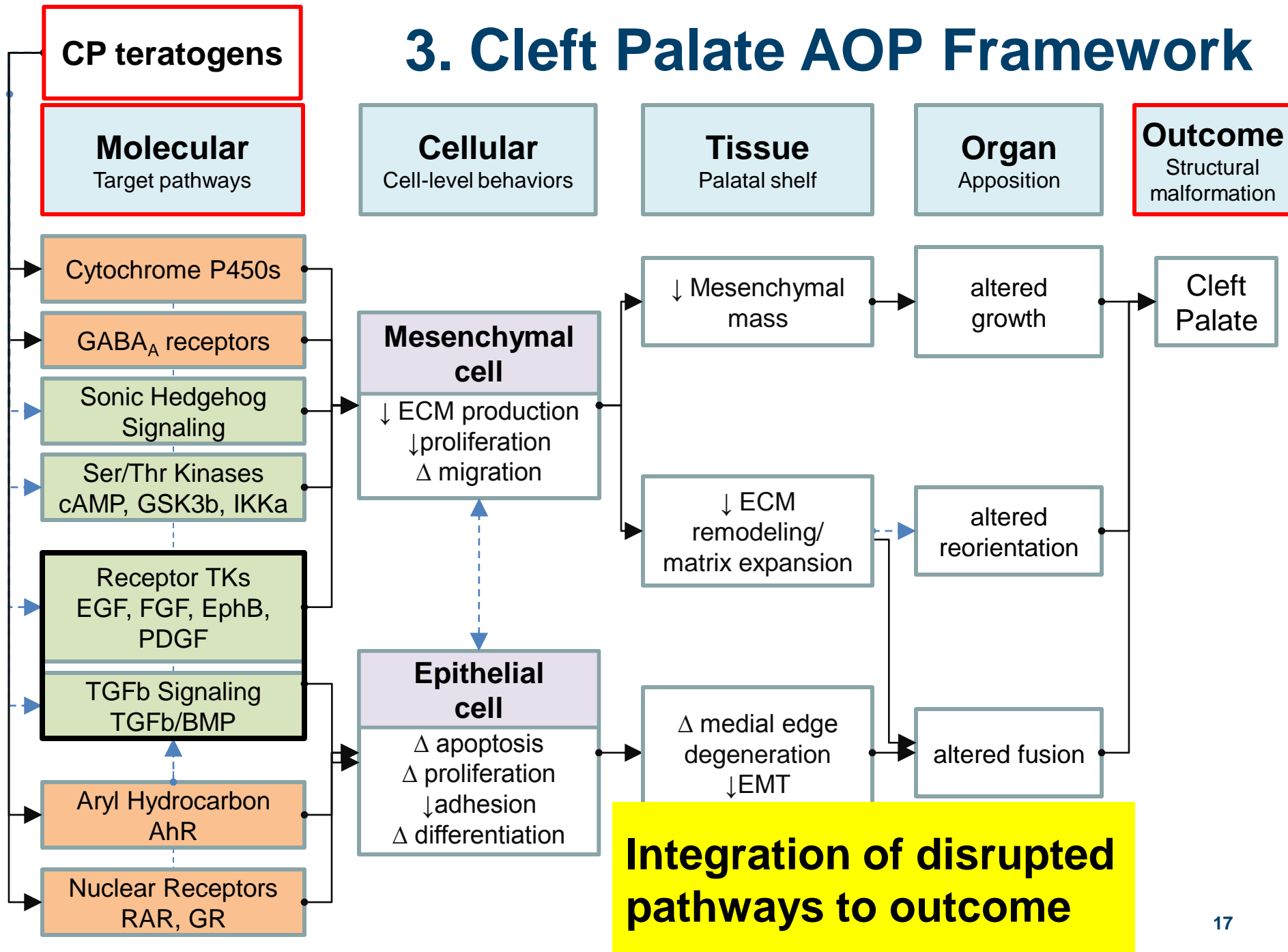


### 3. Cleft Palate AOP Framework





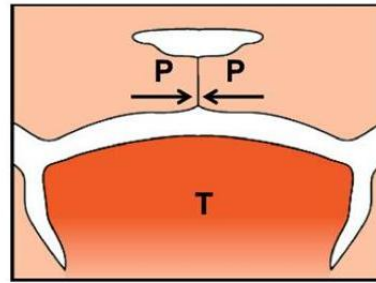
# 3. Cleft Palate AOP Framework



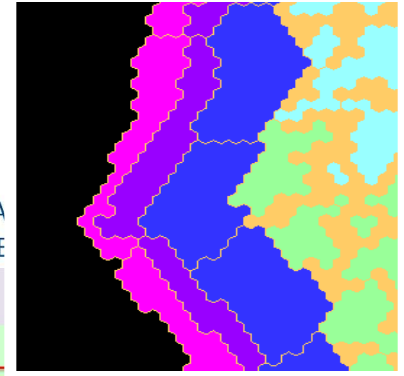
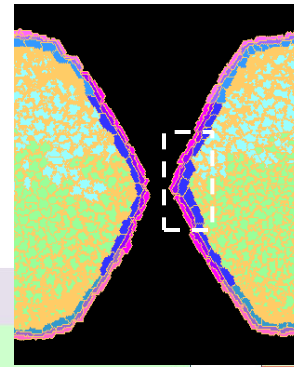
# 4. Multicellular Computer Simulation

- **Cell-level model**

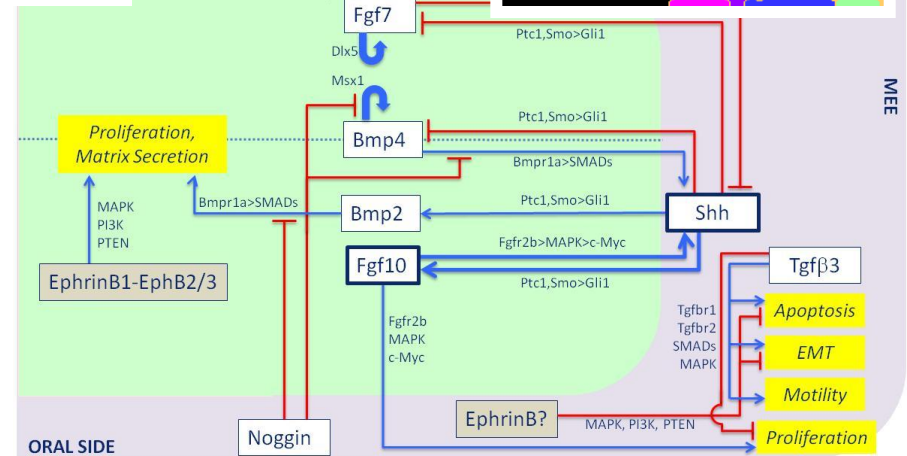
- Simulation is driven by biological networks and rules



Fusion



- **Gene, protein, pathway incorporation**
- **AOP visualization**



**TIME**

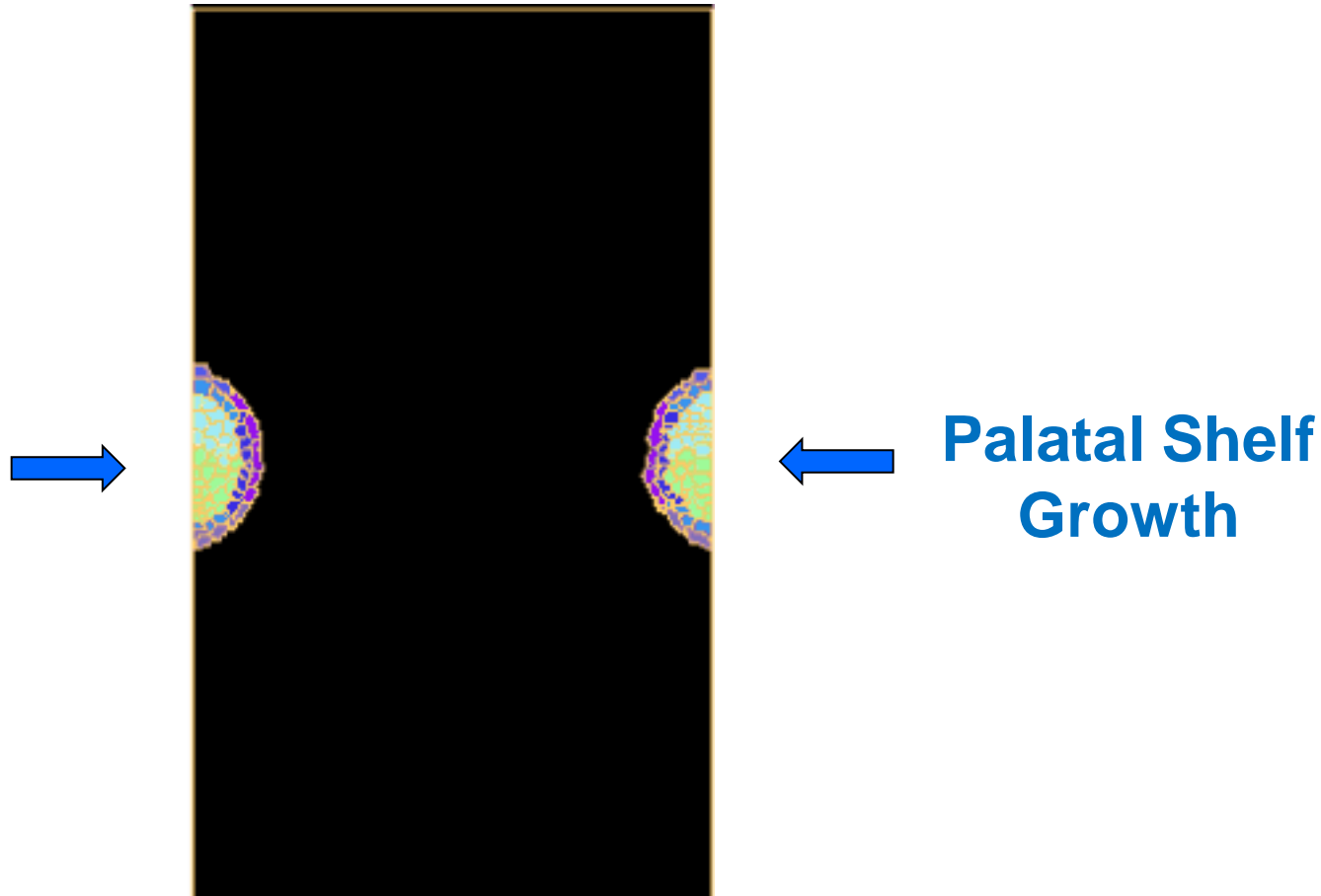
**DOSE  
RESPONSE**

**STAGE  
SPECIFICITY**

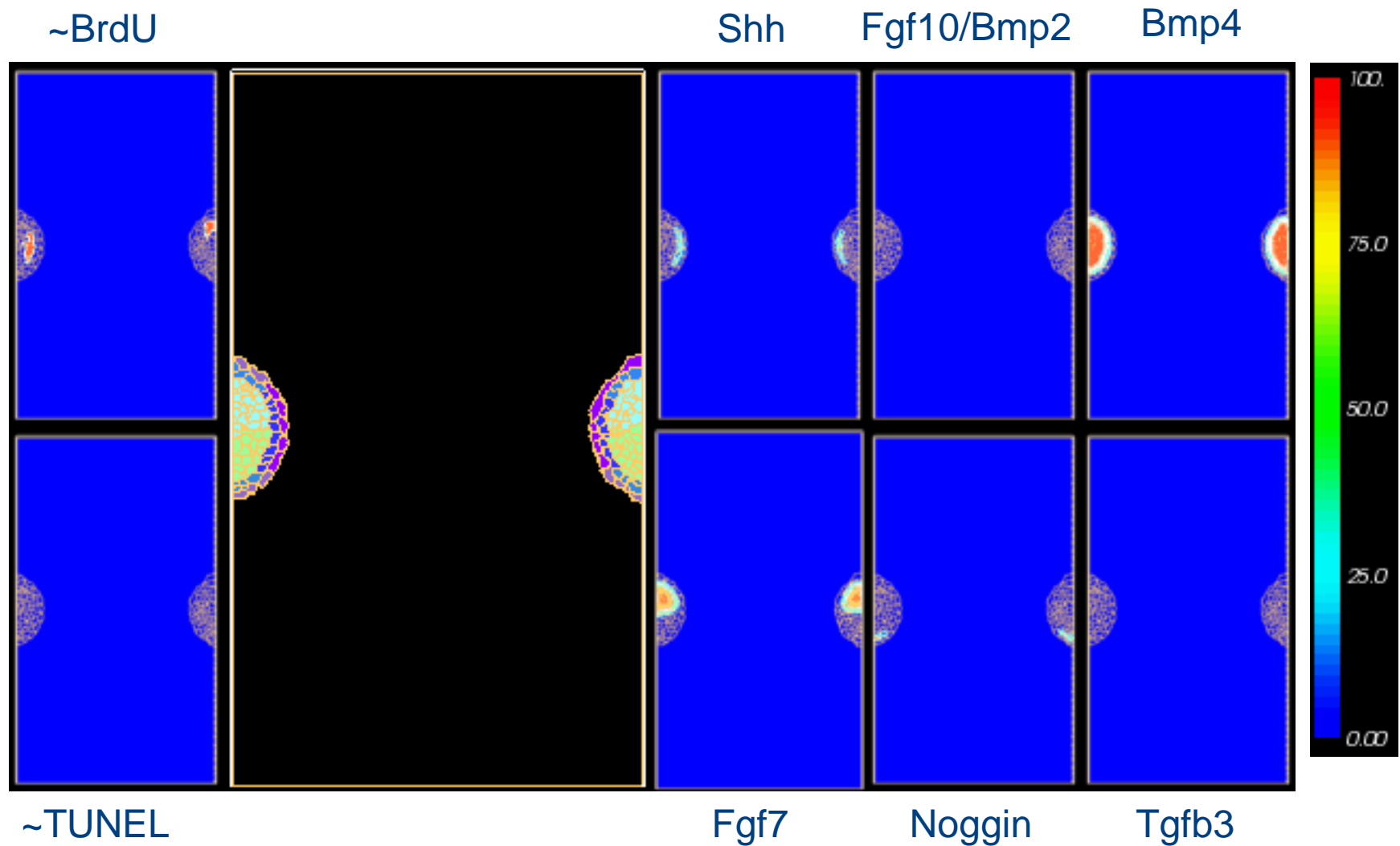
**PREDICTIONS**

# 4. Multicellular Computer Simulation

## Normal Palatal Fusion



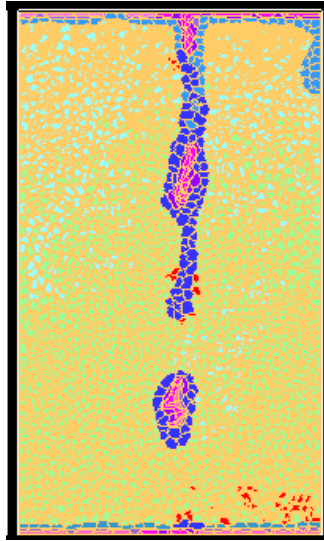
# 4. Multicellular Computer Simulation



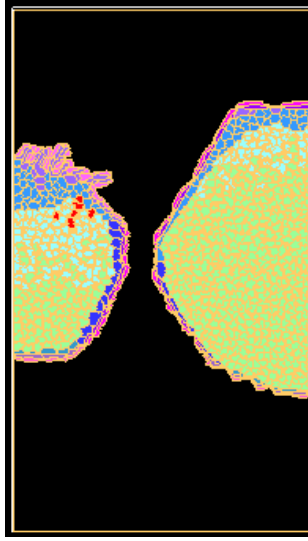
# 4. Multicellular Computer Simulation

*in silico*  
knockouts

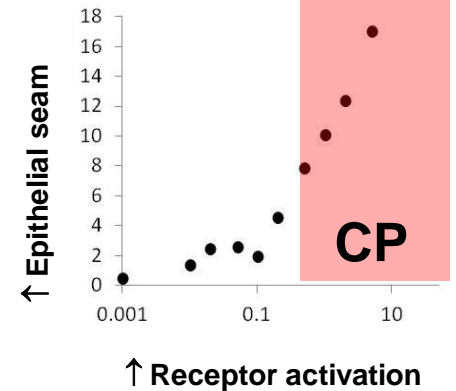
Tgfb3<sup>-/-</sup>



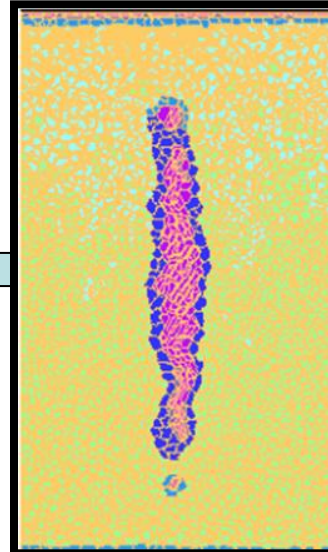
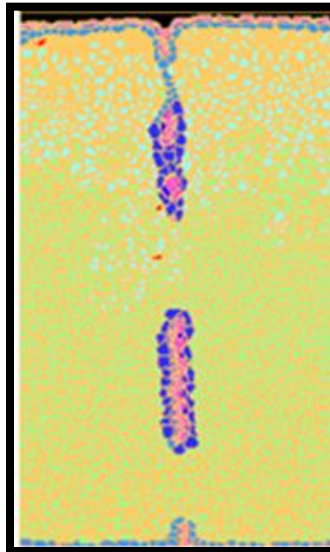
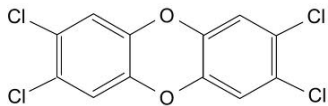
Fgf10<sup>-/-</sup>



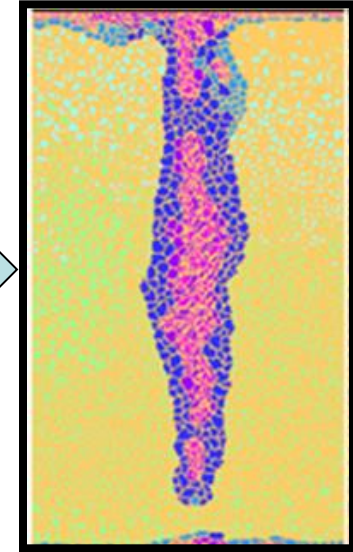
Chemical exposure



*in silico*  
toxicant  
exposure



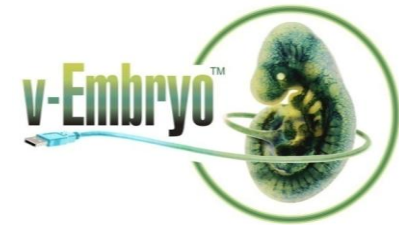
highest dose



# Summary

- ❖ Toxicity in the embryo is an expression of complex and interwoven events that follow from cellular perturbation
- ❖ Systems biology framework can be used to integrate information
  - ❖ e-libraries *publically available articles*
  - ❖ ToxCast assays *dose response*
  - ❖ AOPs *fill in sequence of events*
  - ❖ *in silico* computational models *predictive capability + time & dose*
- ❖ Systems-level models that recapitulate *in vivo* biology can be used to integrate information for making predictions about potential chemical hazards

# Thank you!



<http://www.epa.gov/ncct/>

## Virtual Embryo (NCCT)

Thomas Knudsen

Nancy Baker (LM)

Shane Hutson (Vanderbilt)

Maxwell Leung

Richard Spencer

## Virtual Embryo (NHEERL)

Barbara Abbott

Kelly Chandler (NCCT)

Sid Hunter

Stephanie Padilla

Tamara Tal

## ToxCast (NCCT)



THE  
TERATOLOGY  
SOCIETY  
BIRTH DEFECTS RESEARCH • EDUCATION • PREVENTION  
EST. 1960

## Postdoctoral Fellow Travel Award