2013 Education Course: ***Principles of Teratology***
Teratology Society, Tuscon AZ

Teratogenic Mechanisms, Pathways and Processes

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Disclaimer:
the views are those of the presenter and do not necessarily reflect EPA policy.

Disclosure:
the presenter has no financial or other interests which pose a conflict of interest.

WHY UNDERSTANDING MECHANISMS IS IMPORTANT

- Most developmental defects have complex etiology following from interactions of gene-environment-lifestyle factors.
- Recognizing a teratogen is a very different problem than understanding its mechanism of action.
- Mechanistic information is essential to understanding how drugs and chemicals perturb development.
- Identifies important molecular initiating events for which rapid and cost efficient screens can be developed.
- Understanding mechanisms is needed for appropriate intervention and preventive public health strategies.

OUTLINE

Overview of mechanisms

Challenges of embryo-complexity

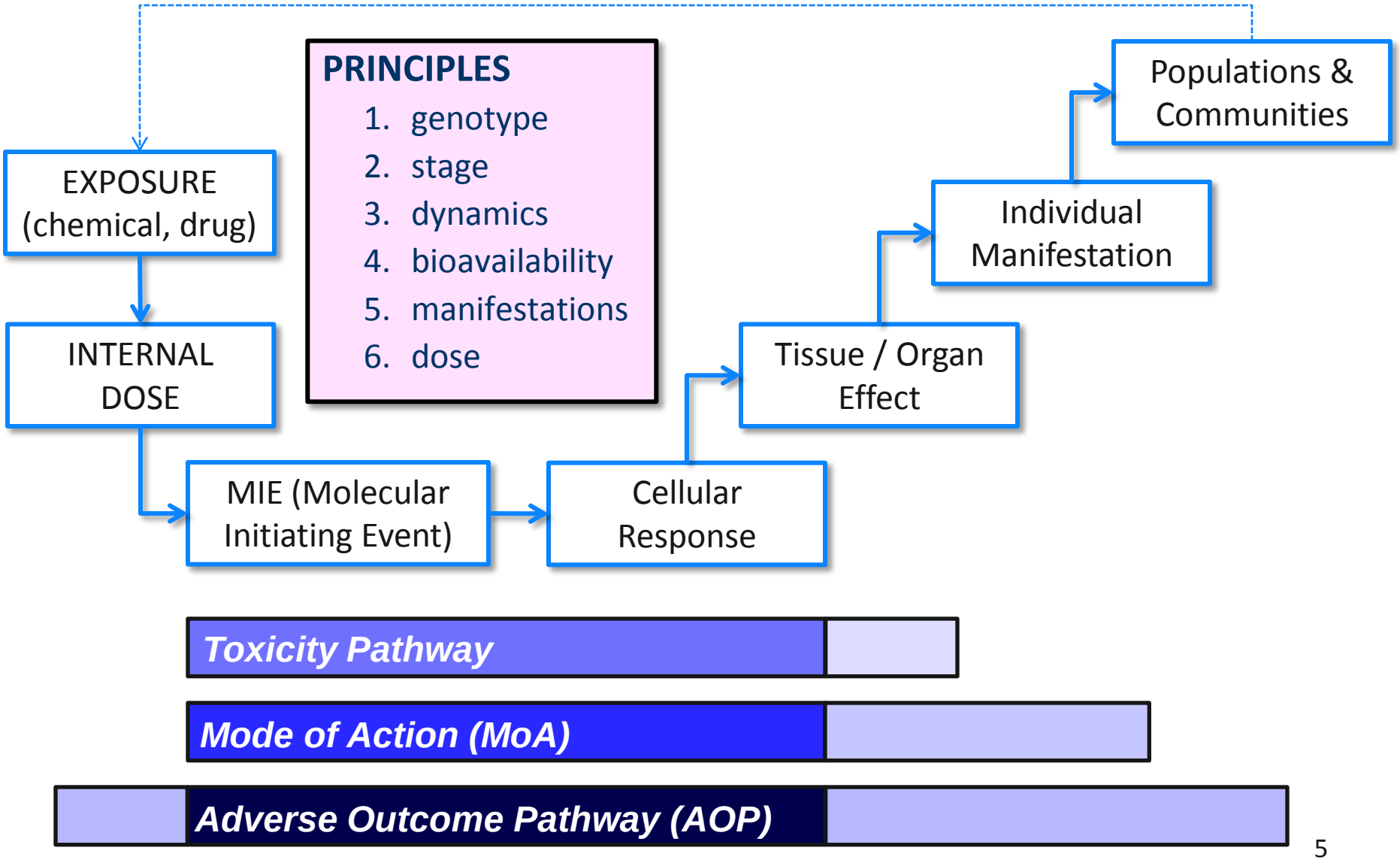
Signaling networks

Cell systems networks

WHAT DEFINES A TERATOGENIC MECHANISM?

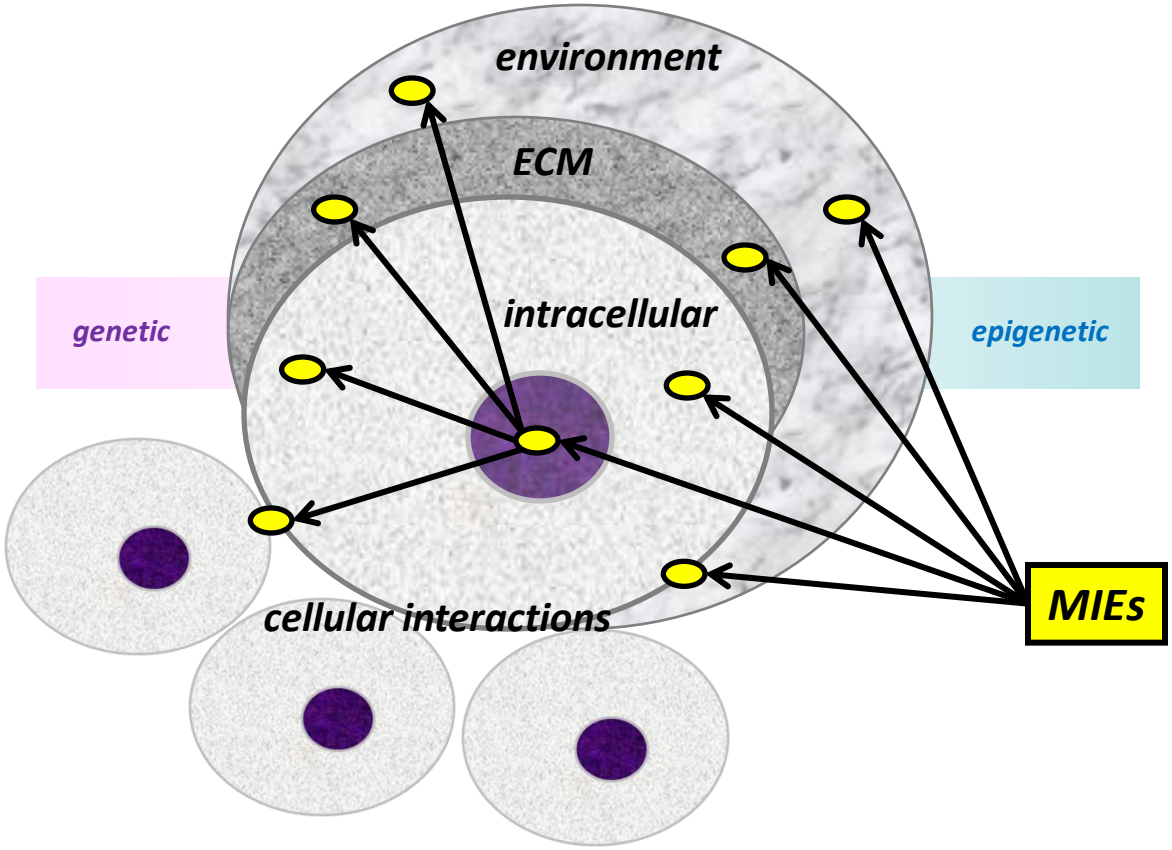
- The means by which a lesion is produced and propagated through a series of measurable events in development.
- Starts with exposure (eg, maternal) and ends with an adverse developmental outcome (eg, malformation).
- Implies detailed molecular knowledge of the initial point of chemical-biological interaction (initiating event).
- Considers downstream pathogenesis that can be linked to dynamic changes in cell fate and behavior.

PRINCIPLES ARE EMBEDDED ACROSS SCALES



After: Ankley et al. (2010) Environ Toxicol Chem 29: 730-741

MOLECULAR INITIATING EVENT (at the Site of Action)



- In general, teratogenesis is initiated by chemical-biological interactions at a molecular level.
- Initial interaction may be covalent binding to proteins / DNA (i.e. reactive chemistry) ...
- … or non-covalent interactions (receptors, enzymes) in which potency drives toxicity.
- MIEs represent a primary event anchoring the AOP to a cascade of pathogenesis.

After: Saxén (1976) J Embryol exp Morph 36: 1 -12

KNOWN TERATOGENIC MECHANISMS

- Large number of teratogens and adverse developmental outcomes makes it difficult to pinpoint unifying mechanisms.

- 6 principal teratogenic mechanisms based on associations of major birth defects with medications used by women of reproductive age:

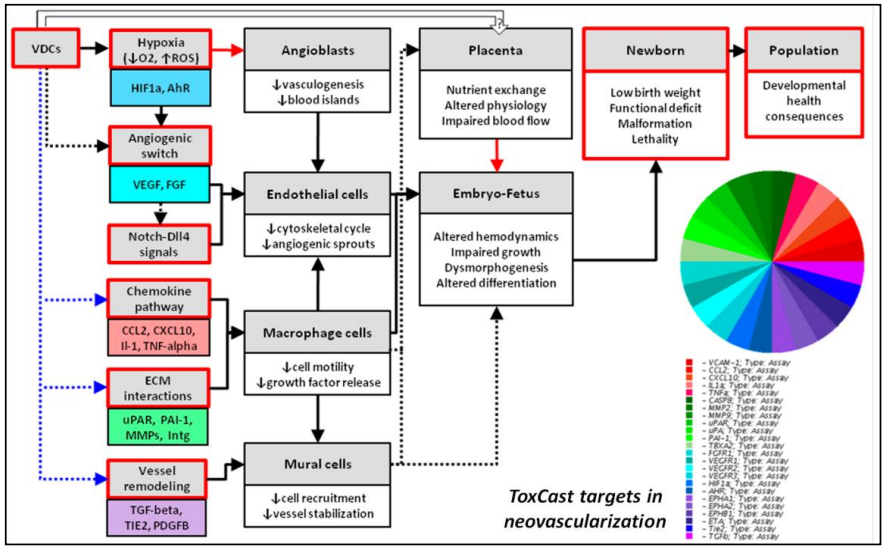
1. Folate antagonism
2. Neural crest cell disruption
3. Endocrine disruption
4. Oxidative stress
5. Vascular disruption
6. Specific receptor- or enzyme-mediated teratogenesis

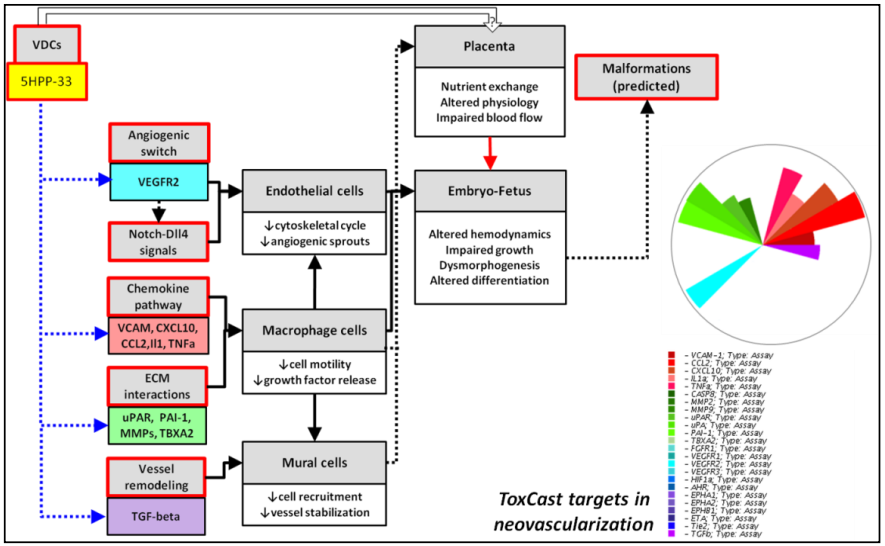
After: van Gelder et al. (2010) Hum Rep Update 16: 378-394.

EMBRYONIC VASCULAR DISRUPTION

AOP framework model:
known biology and ToxCast HTS data

AOP predicted from 5HPP:
anti-angiogenic Thalidomide analogue





SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res. C 93: 312-323.

SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996. doi:10.1371/journal.pcbi.1002996.

CASE FOR THALIDOMIDE EMBRYOPATHY

CRBN
cereblon (proteasome)

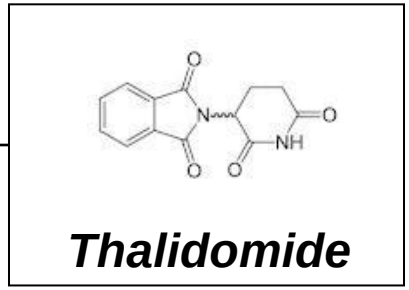
cell-cell signaling
molecular gradients (FGF8)

cellular behaviors
growth and apoptosis

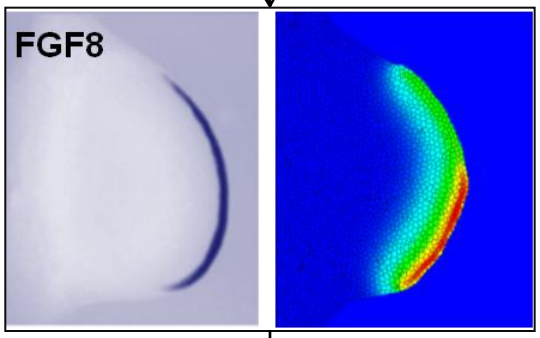
embryonic vasculature
vascular disruption

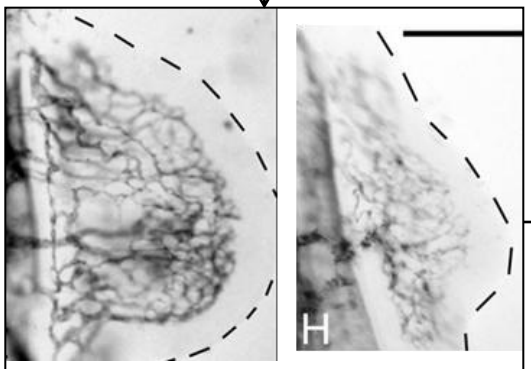
early limb-buds
dysmorphogenesis

birth defects
limb malformations



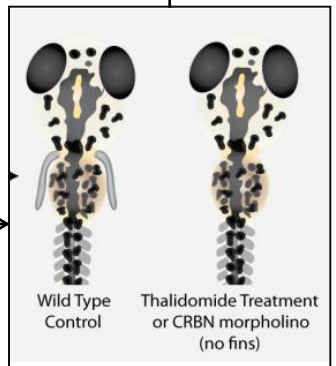
Thalidomide





Therapontos et al. 2009, PNAS 106

Short or missing limbs in humans, monkeys, rabbits, zebrafish (but not rodents)



Taylor et al. 2010, Cell Com.Signal.

CHALLENGES OF EMBRYO-COMPLEXITY

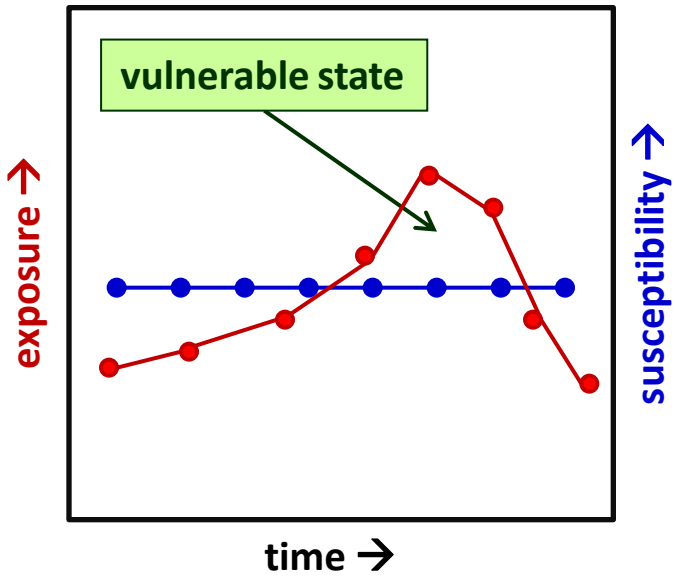
Several levels of complexity in pregnancy and development contribute to an incomplete understanding of teratogenic mechanisms:

- embryo is a biological complex system
- propagation of events across scales
- MIEs come and go as development advances
- windows of vulnerability open and close at different stages
- individual biological variability in mother and conceptus

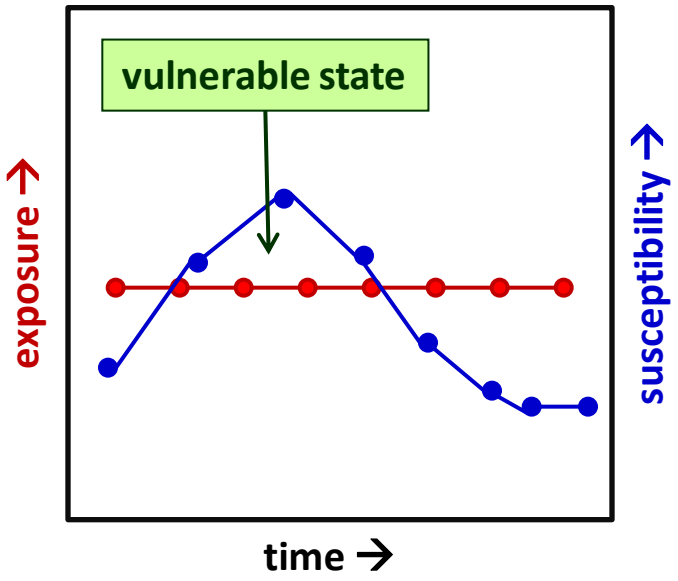
EMBRYOGENESIS & PREGNANCY

- Susceptibility reflects the dynamic interplay between ***developmental program*** and ***maternal environment***

MODEL A
constant biology, varied environment

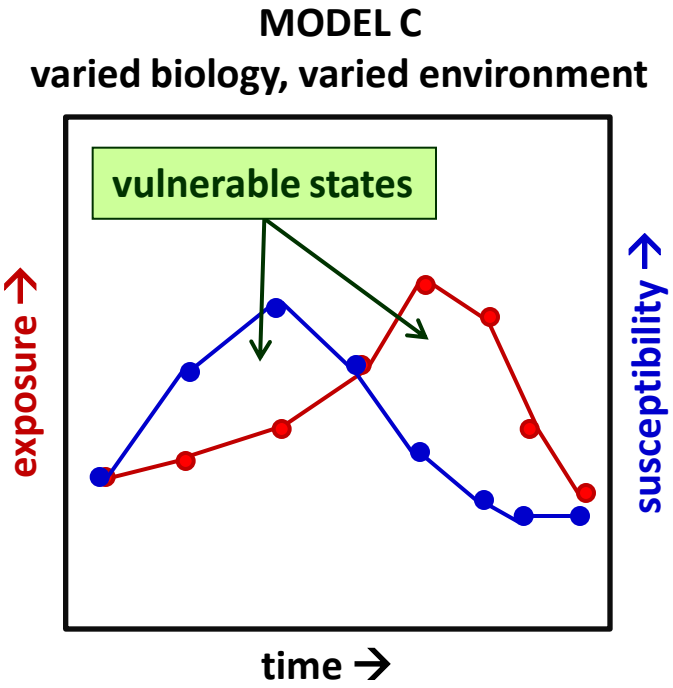


MODEL B
varied biology, constant environment



EMBRYOGENESIS & PREGNANCY

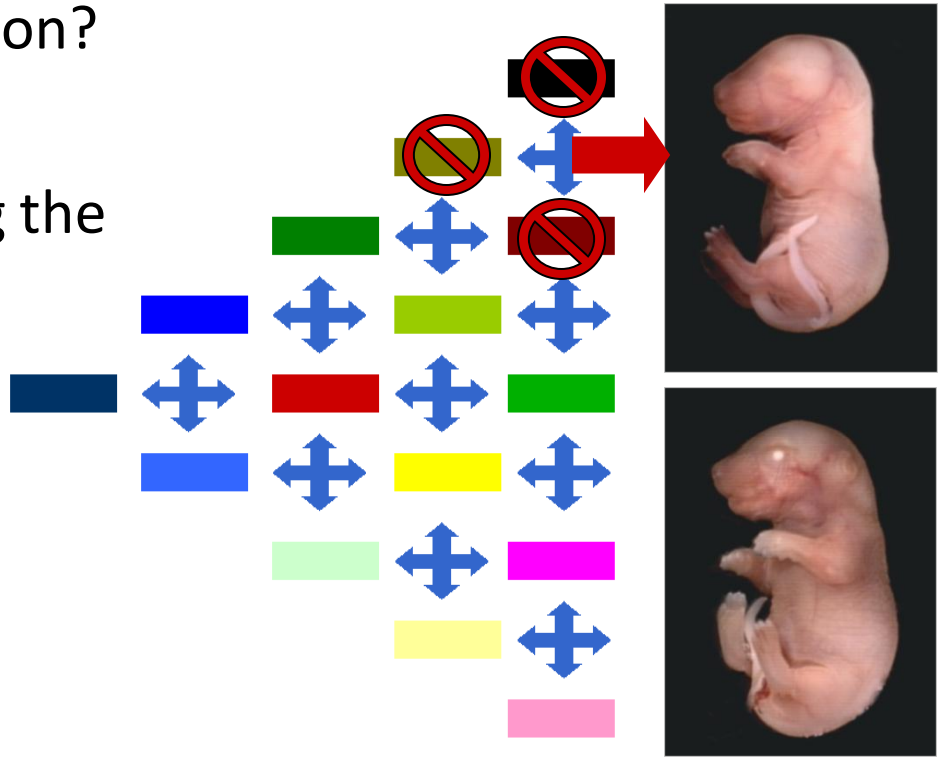
- Susceptibility reflects the dynamic interplay between ***developmental program*** and ***maternal environment***



PROPAGATION OF EVENTS ACROSS SCALES

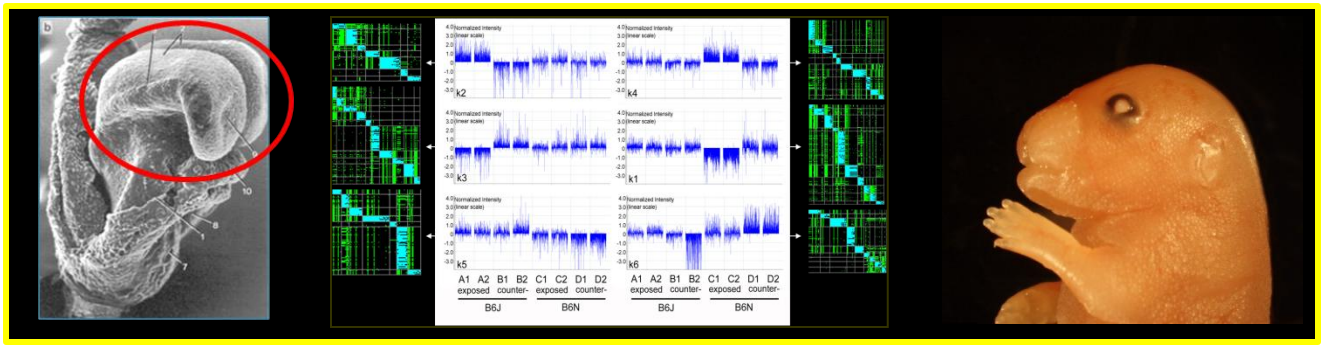
- How do disruptions at the molecular level propagate to higher levels of biological organization?

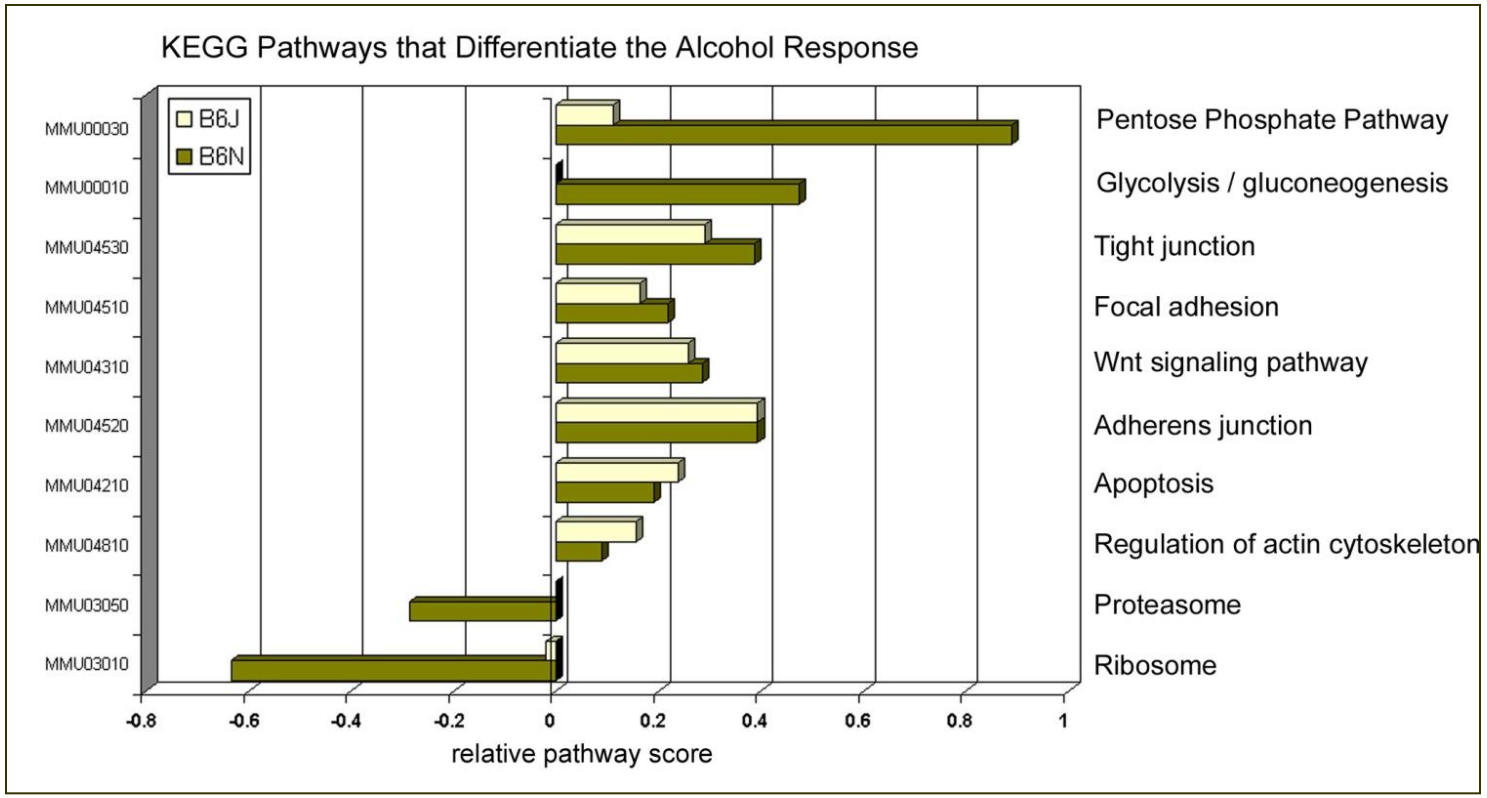
- Important issue is identifying the intermediate *key events*:
  - *necessary:* must occur to continue the chain of events;
  - *sufficient:* if it occurs the adverse effect will emerge.



- Key events of an AOP are *in vivo*, leading to adverse effects in whole organisms; however, HTS/HCS approaches may be used to provide support and data to evaluate an AOP.

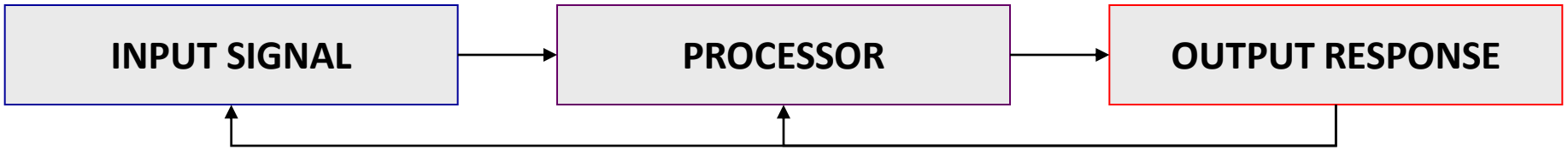
GENOMICS REVEALS RESPONSIVE PROCESSES





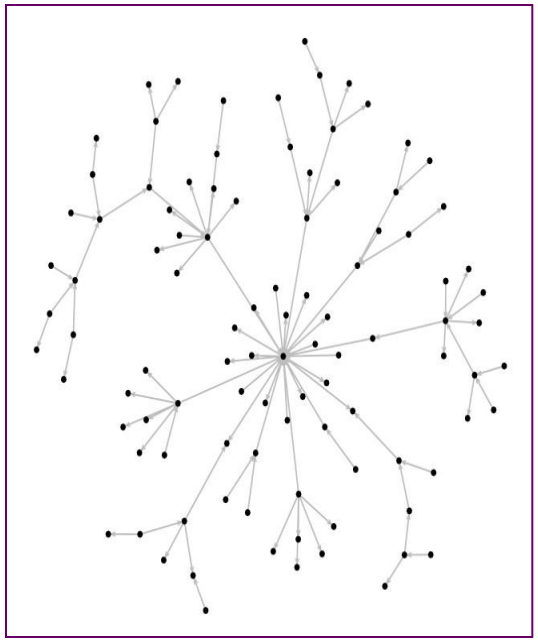
SOURCE: Green et al. (2007) Devel Dynam 236: 613-631.

UNDERSTANDING CELL SIGNALING IN A COMPLEX SYSTEM



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

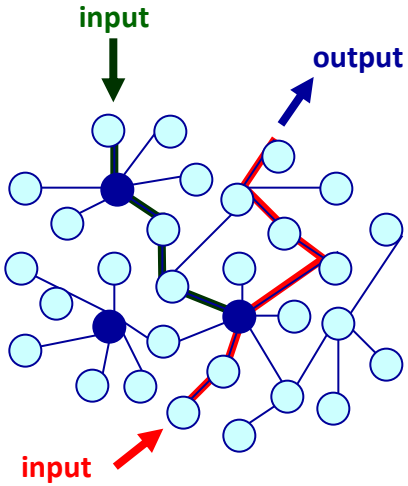


Cellular Response Matrix

patterning
proliferation
apoptosis
differentiation
adhesion
motility
shape
ECM remodeling

SIGNALING NETWORKS

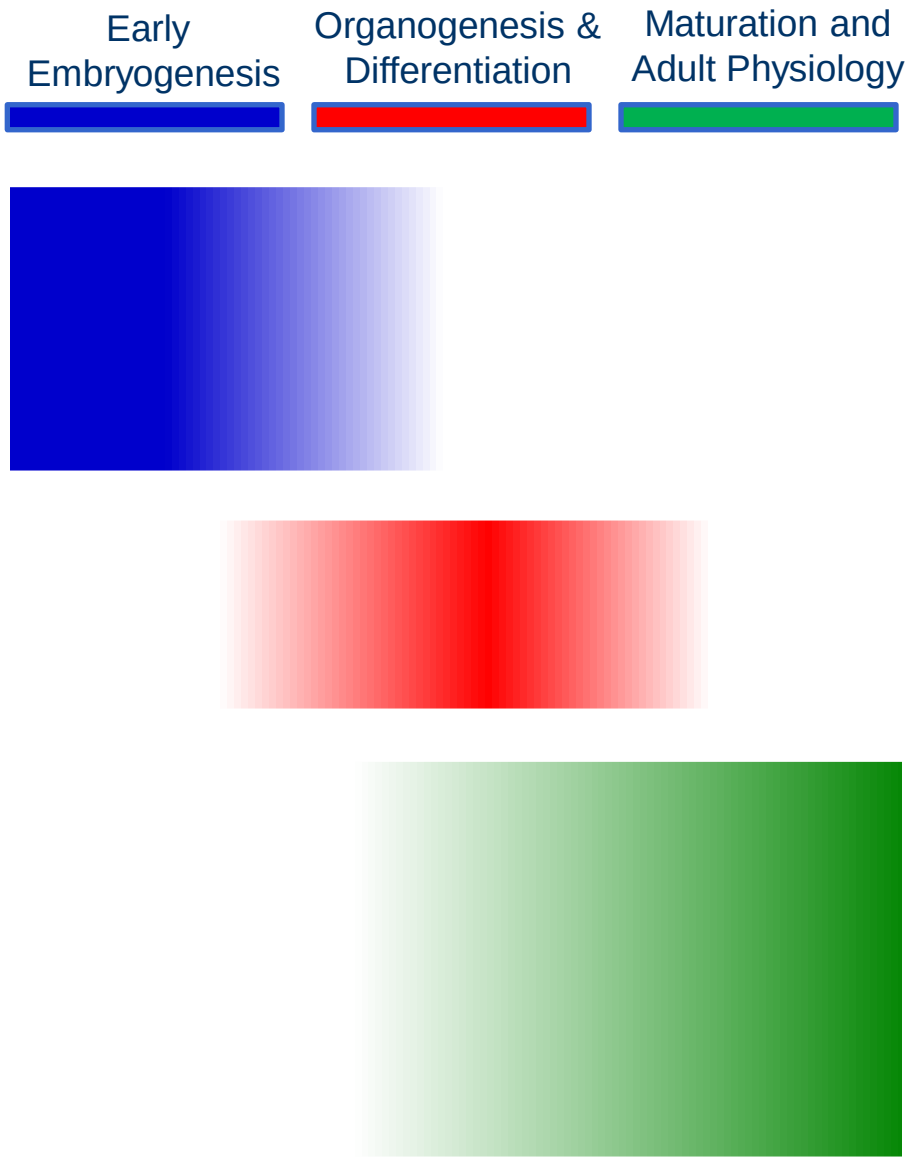
- complex systems have an underlying network structure governing function
- systems-level behavior is ultimately governed by network topology
- network topology refers to size & connectivity (scale-free a general rule)
- this simple example portrays a 32-node scale-free network:



- most nodes have low-degree connectivity
- a few nodes (hubs) have high-degree connectivity
- modular organization integrates information flow
- graceful degradation following perturbation
- Achilles' heel = hubs; vulnerable to targeted attack

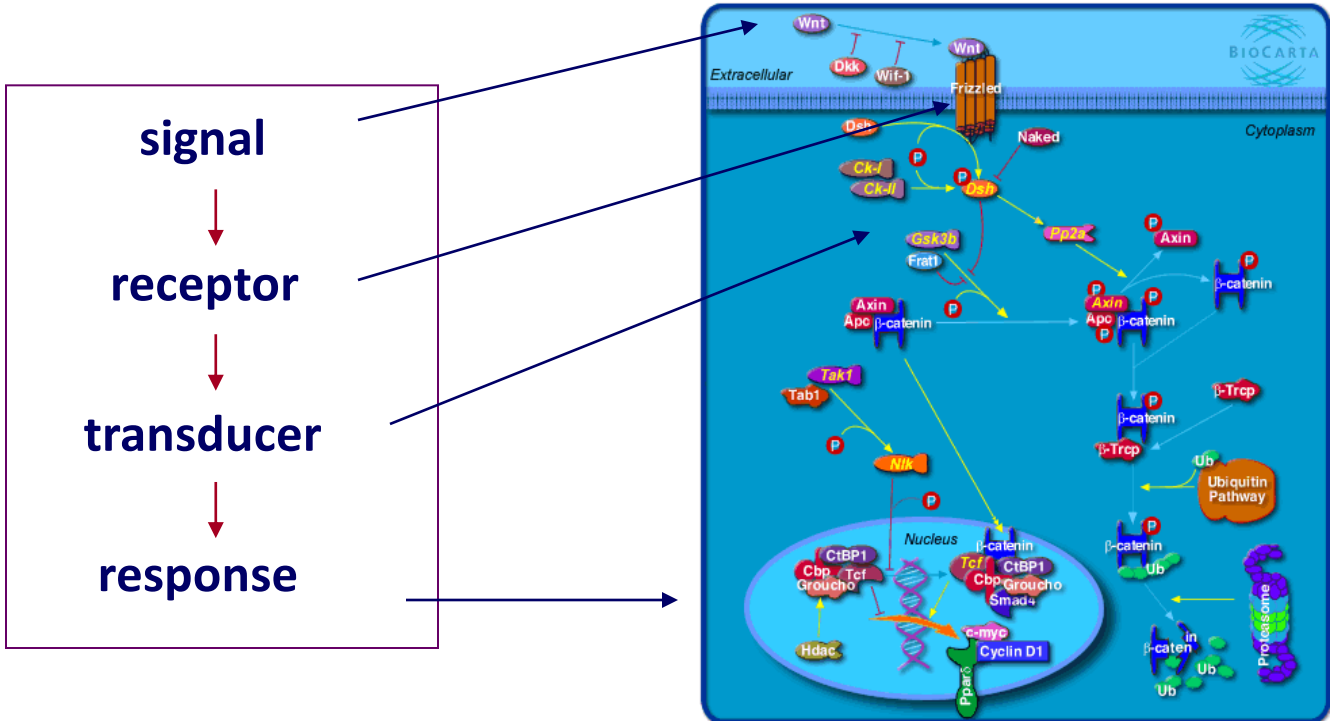
How do the interactions of one or more dysfunctional nodes within a complex genetic network result in structural malformations?

NRC DEVELOPMENTAL SIGNALING PATHWAYS (*circa* 2000)



A TYPICAL DEVELOPMENTAL SIGNALING PATHWAY

- Sequence of steps conducting the flow of molecular regulatory information between cells (e.g., cell-cell communication) or within cells (cellular control).



Wnt signaling pathway

SOURCE: BioCarta

WHAT THESE NETWORKS CONTROL

Core developmental processes

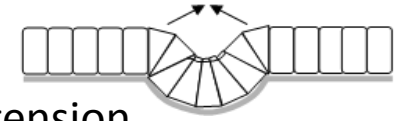
- patterning (sets up future events)
- timing (clocks and oscillators)
- differentiation (cell diversification)
- morphogenesis (tissue organization)

Cellular behaviors

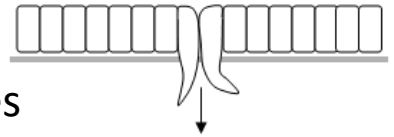
- growth (proliferation)
- death (apoptosis)
- differentiation (function)
- adhesion (DAH)
- shape (geometry)
- motility (cell migration)
- ECM (remodeling)

Morphogenetic movements

- folding
- epiboly



- convergent extension
- branching morphogenesis
- fusion
- cell condensation and sorting
- trans-differentiation
- cavitation
- involution



- tractional forces

CONSERVATION OF CELL SIGNALING

- Many of the molecular components of key cell signaling pathways in embryogenesis are highly conserved.
- Although developmental processes and strategies can differ markedly across species ...
- … the same types of molecules evolved into modular signaling pathways and gene regulatory networks.
- Pathways cross-regulate the activity of one another to control the order and timing of developmental events.

‘TOOLKIT GENES’

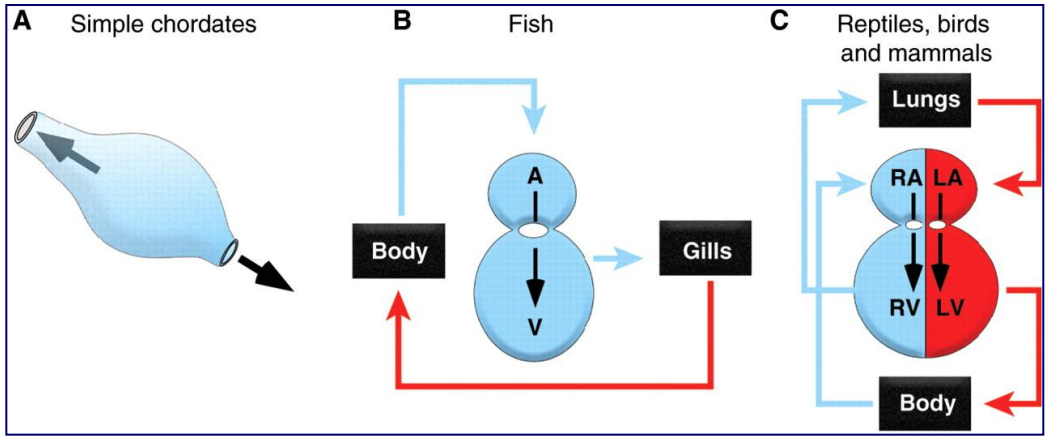
- Conservation of cell signaling implies a fundamental strategy of how molecular information is used.

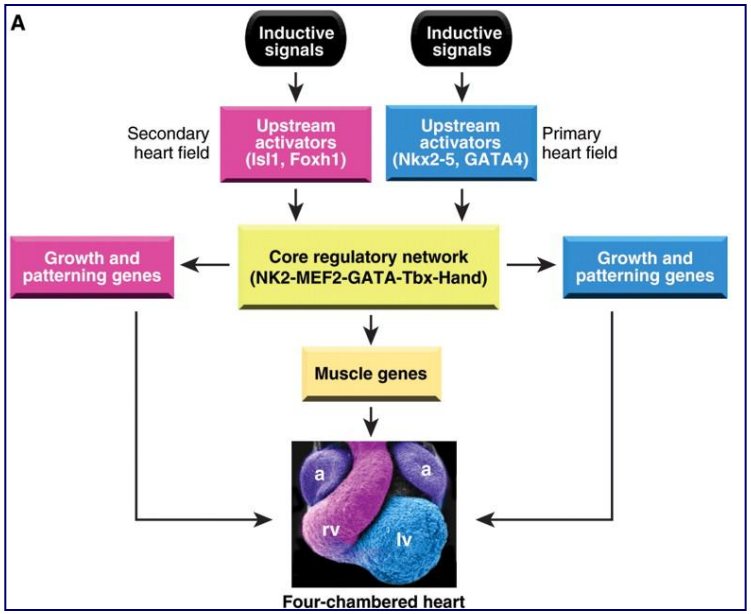
- ‘Toolkit genes’ appear to play the same role across phyla, and in all vertebrate species from zebrafish to humans.

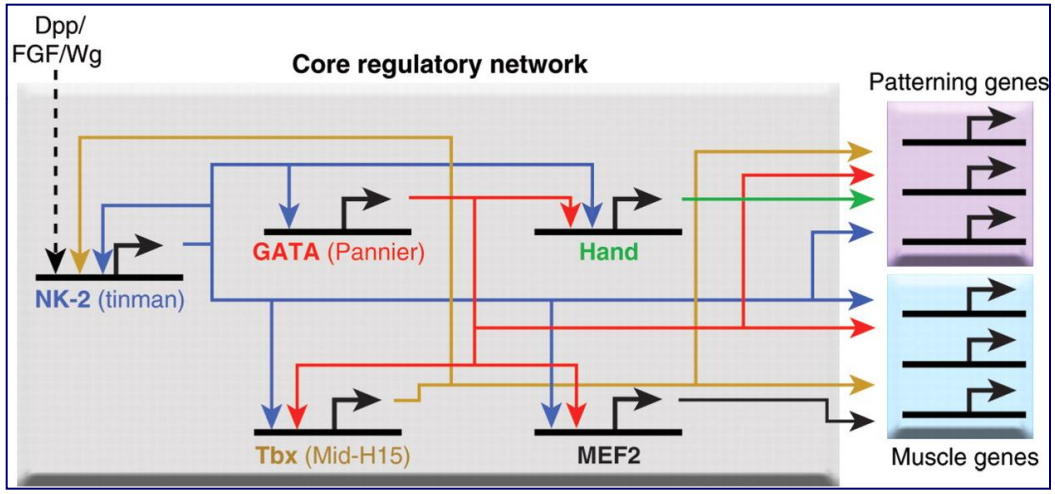
- Best examples are transcription factors:

  Pax6 → eye development
  Nkx/tinman → heart development
  Hox genes → axial patterning
  Hes-1 → molecular clocks

CO-OPTING GENES INTO PATTERNING SYSTEMS: heart







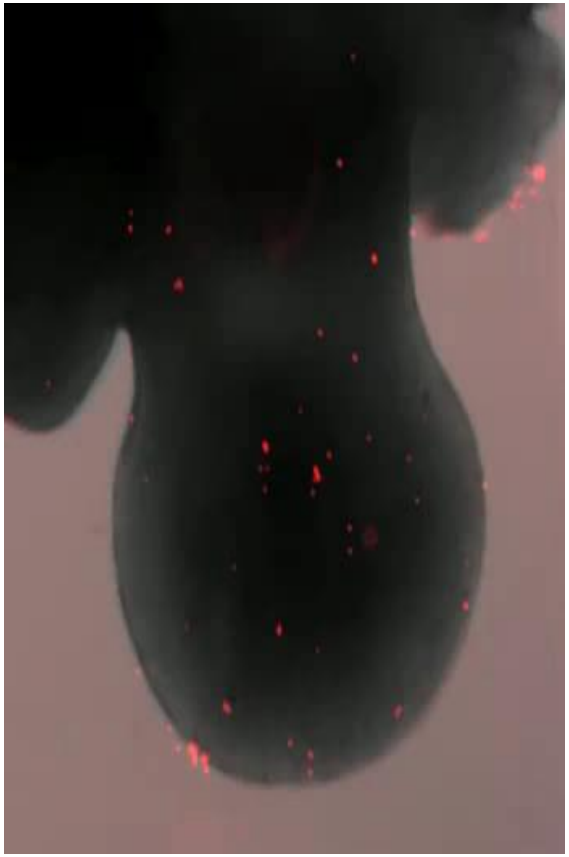
ancestral kernel
(core – primitive condition)

↓

co-opted genes
(modularity – advanced condition)

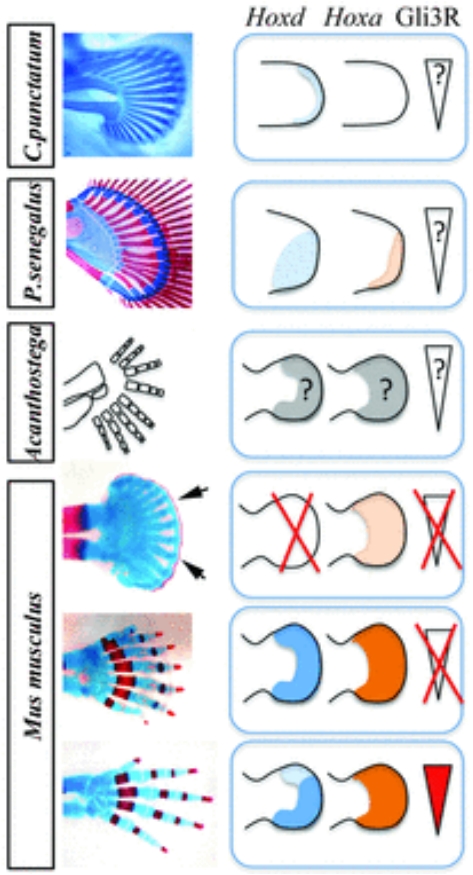
SOURCE: Olson (2006) Science 313, 1922-1927.

CO-OPTING GENES INTO PATTERNING SYSTEMS: digits



Boot et al. (2008) Nat Met 5: 609

Vogel (2012) Science 338: 1406

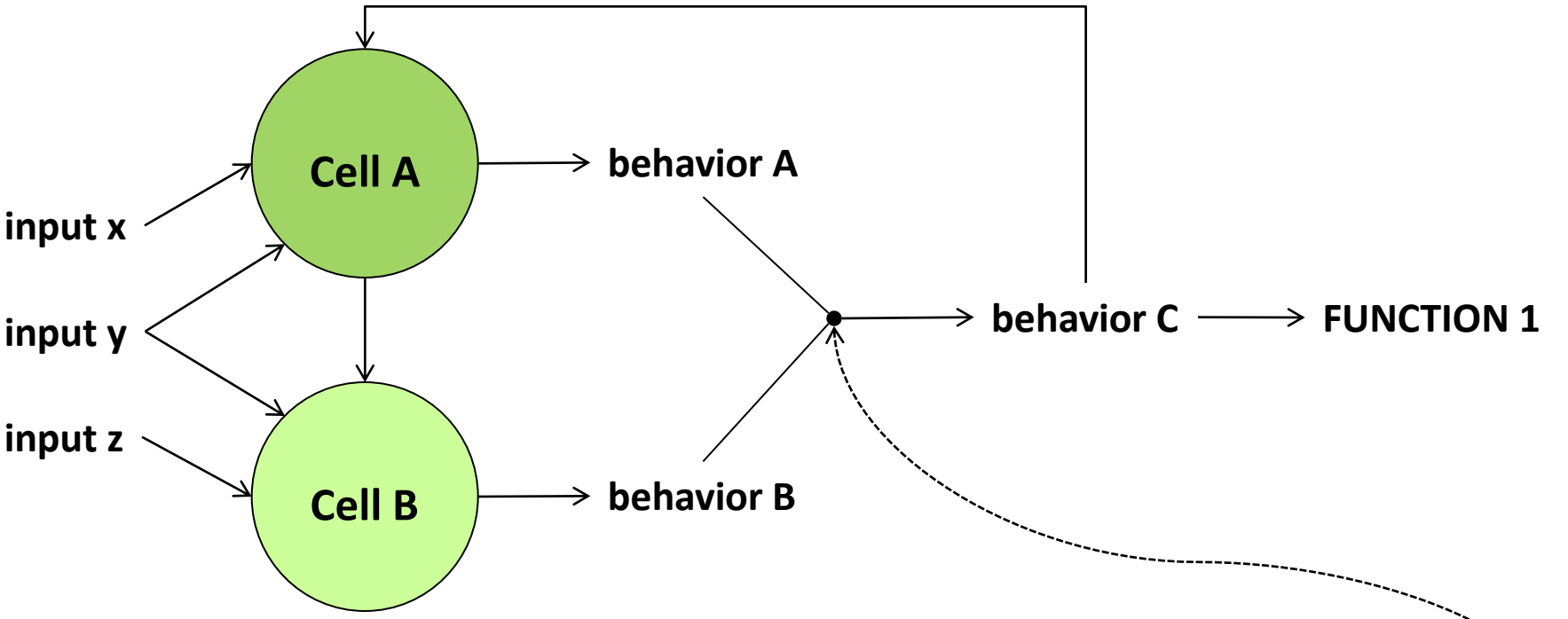


Sheth et al. (2012) Science 338: 1476

CELLULAR DISRUPTION

EMBRYONIC CELL BEHAVIORS	CONSEQUENCES OF DISRUPTION
cell growth & death	incorrect cell number
differentiation & function	missing cell types
cell motility & adhesion	disorganization
clocks & organizers	chaos and ataxia
genetic signals & responses	dysregulation
ECM synthesis & remodeling	loss of mechanical properties

TRANSLATING SPATIAL INFORMATION TO HIGHER LEVELS

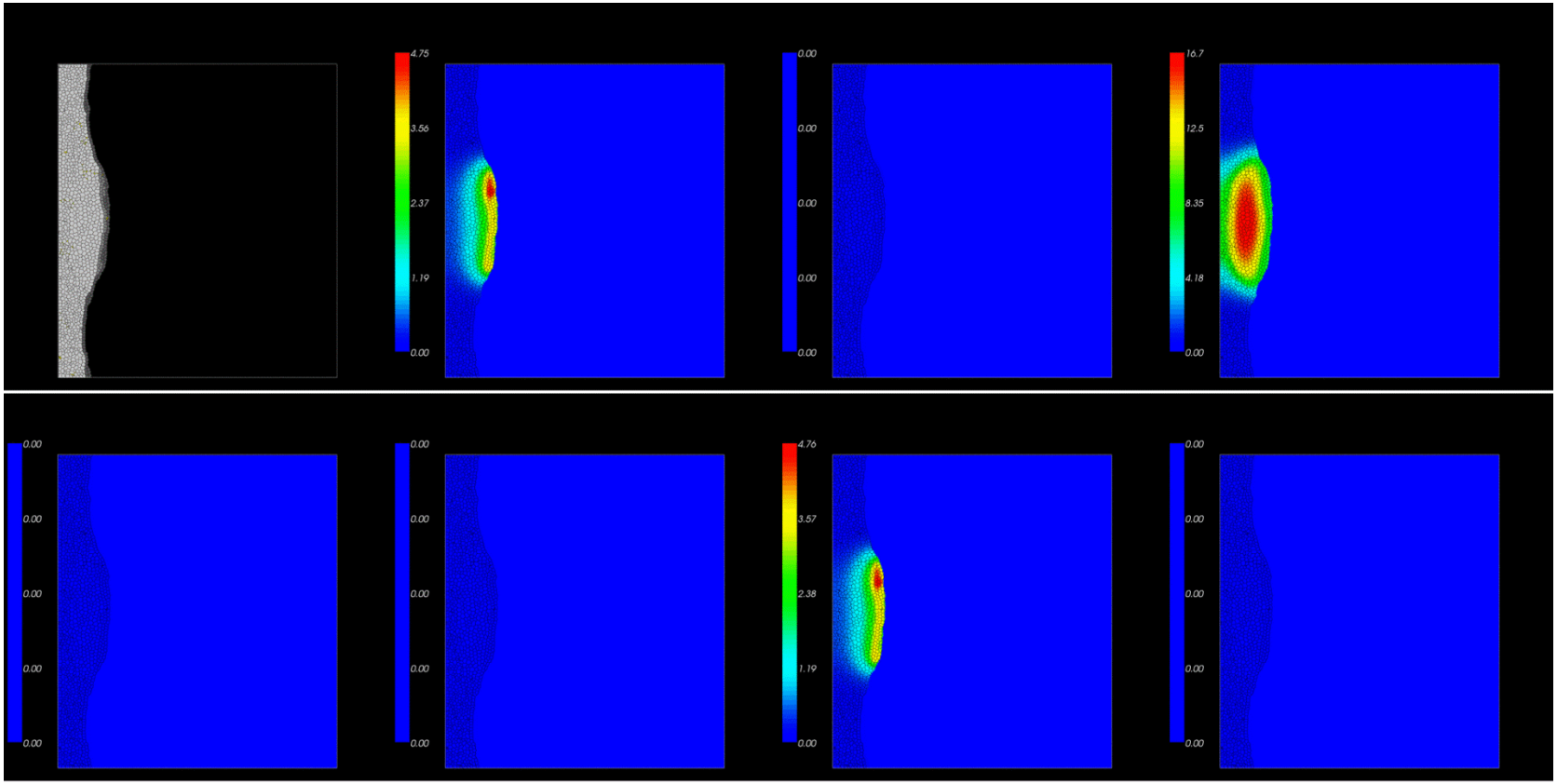


signals ***rules*** ***responses*** ***buffering*** ***outcome***

Capturing this mathematically:

$$\sum_{\sigma}\lambda_V(\sigma)\left(v(\sigma)-V_i(\sigma)\right)^2+\sum_{\sigma}\lambda_S(\sigma)\left(s(\sigma)-S_i(\sigma)\right)^2+\sum_{i,j}J\left(\tau(\sigma(\vec{i})),\tau(\sigma'(\vec{j}))\right)\left(1-\delta\left(\sigma(\vec{i}),\sigma'(\vec{j})\right)\right)-\lambda_{chem}\left(c(\vec{i})-c(\vec{j})\right)$$

VIRTUAL TISSUE MODEL FOR SIGNAL PROPAGATION: limb bud



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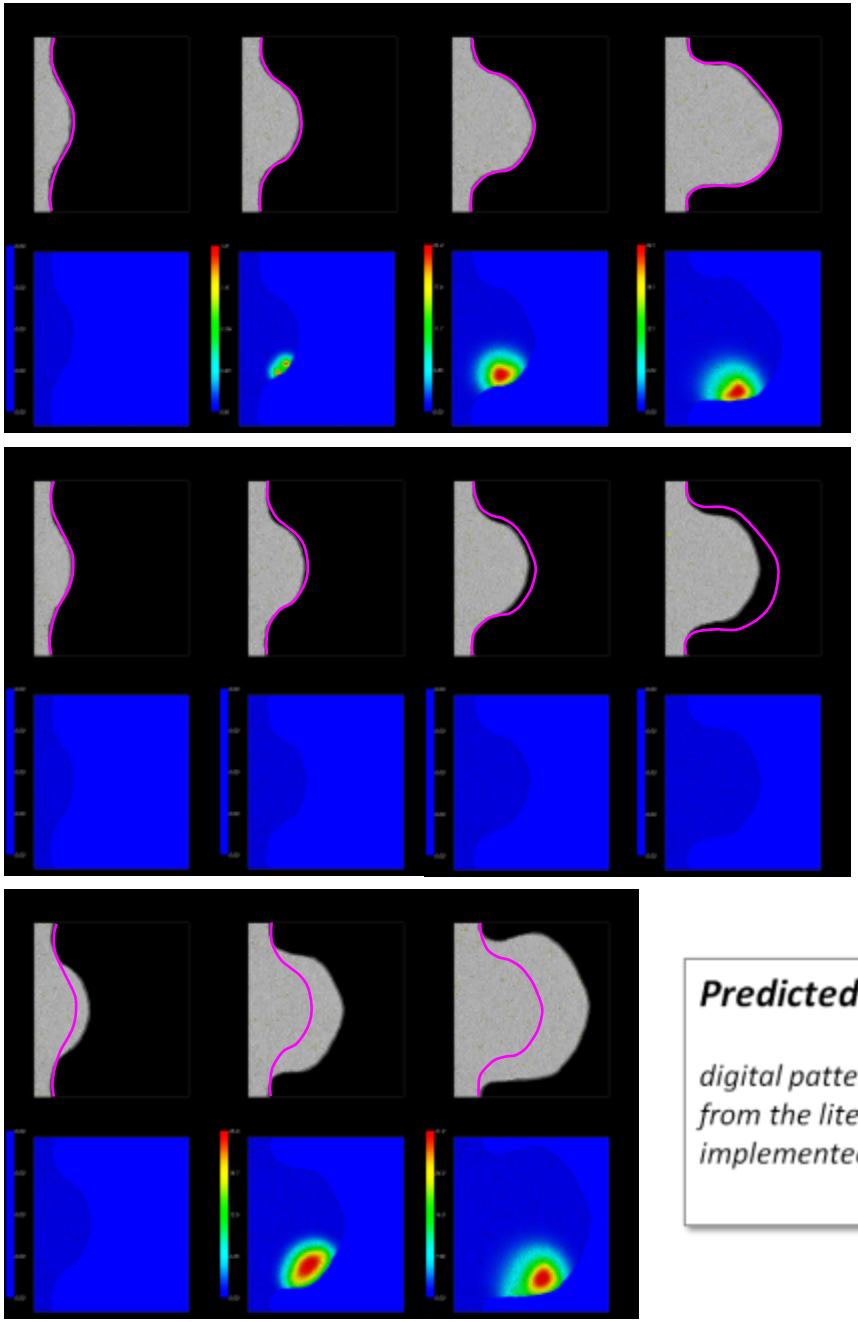
Simulated outgrowth

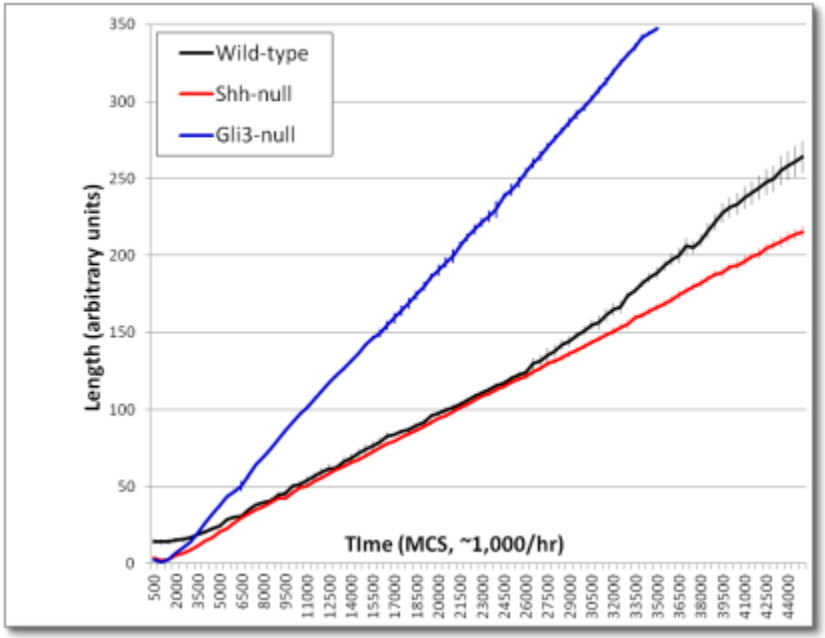
Wild-type

Shh-null

Gli3-null



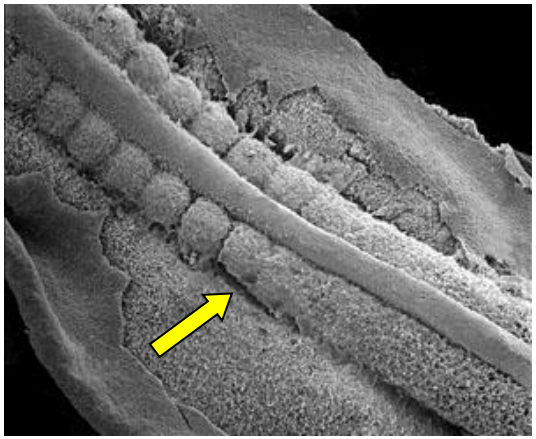
Rate of elongation (n=5)

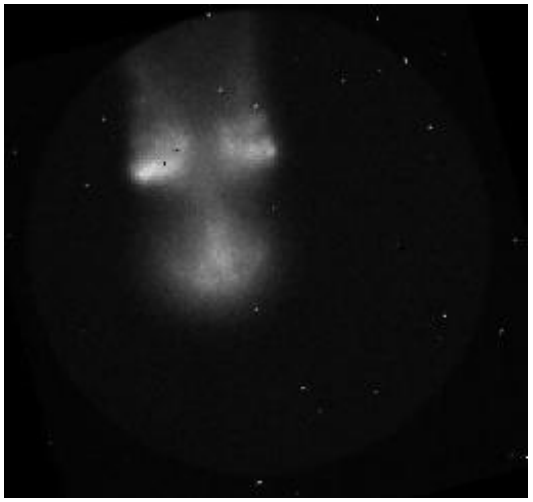


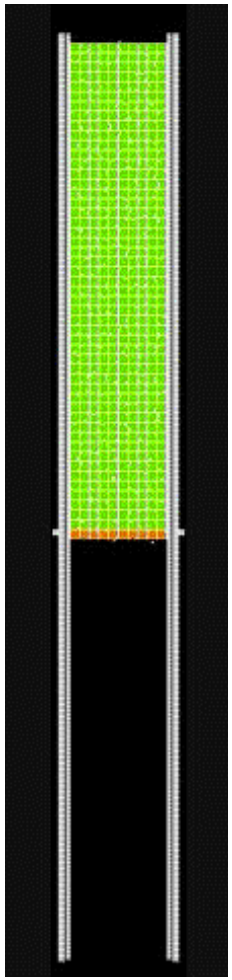
Predicted outcomes

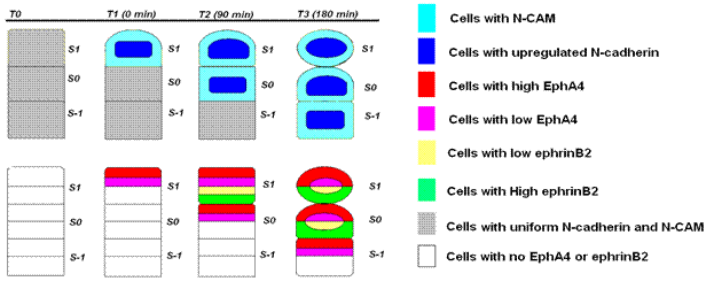
digital patterns inferred from the literature; not yet implemented in the model

VIRTUAL TISSUE MODEL: somite clock & wavefront

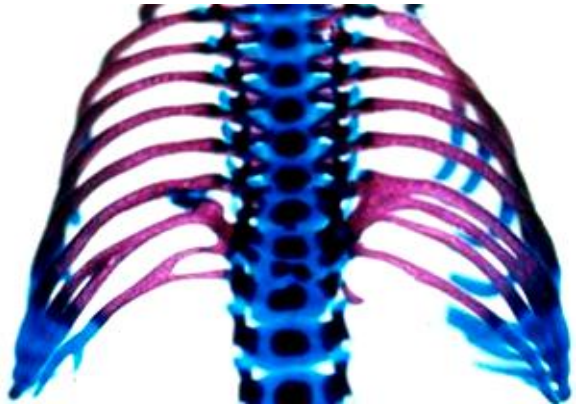








In silico model, CompuCell3D software
SOURCE: Glazier et al. (2008) Cur Top Dev Biol 81:205

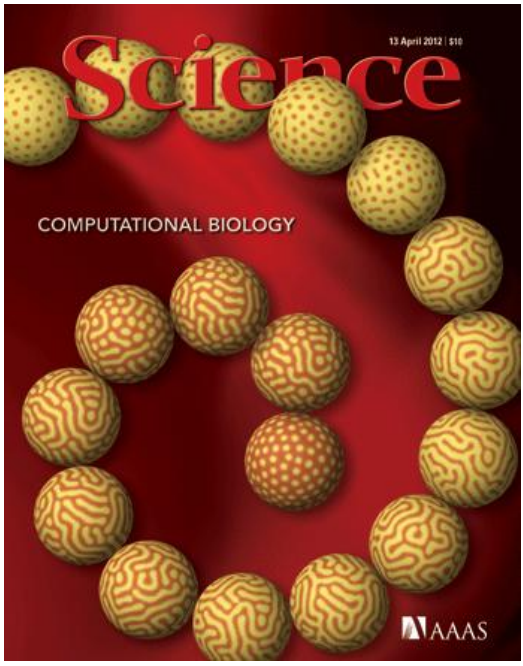


Prenatal exposure, boric acid
SOURCE: John Rogers, EPA

Hes1-EGFP time-lapse (3h) clock-wavefront
SOURCE: Masamizu et al. (2006) PNAS USA 103:1313-18

COMPUTATIONAL SYSTEMS BIOLOGY

- We know a lot about molecular functions that control cellular behavior but far less about **emergence** of systems-level function.
- Emergent properties are those arising from elaborate networks of interactions between molecules and cells comprising the system.
- Bioinformatics provides insight into how the system is wired for a response.
- Mechanistic models needed to understand how cellular changes propagate to higher levels of biological organization.



April 13, 2012

October 12, 2012

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