Introduction to Virtual Tissue Modeling of Development and Developmental Diseases Using Compucell3D

Part II

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10th User Training Workshop: Developing Multi-Scale, Virtual Tissue Simulations with CompuCell3D and Tellurium
Hamner Institute, Research Triangle Park, North Carolina
Monday, August 11, 2014

IU Bloomington: [Dr. Dragos Amarie], Dr. Julio Belmonte, Dr. Sherry Clendenon, [Alin Comanescu], [Clayton Davis], Dr. Rita de Almeida, Xiao Fu, [Aaron Dy], [Garth Gast], Dr. J. Scott Gens, [Randy Heiland], [Dr. Susan Hester], [Dr. Mitja Hmeljak], [Dr. Srividhya Jayaraman], [Dr. Roeland Merks], [Dr. Nikodem Poplawski], [Ryan Roper], [Dr. Abbas Shirinifard], Dr. James Sluka, Andre Somogyi, Dr. Maciej Swat, [Dr. Gilberto Thomas], [Ruei Wu], [Benjamin Zaitlen], [Dr. Ying Zhang]. Indiana University, School of Medicine: Dr. Robert Bacallao. University of Houston: Dr. Maria Bondesson, Dr. Jan-Ake Gustafsson, Dr. Catharine McCollum. EPA: Dr. Thomas Knudsen, Dr. Imran Shah, Dr. Nicole Kleinstreuer. University of Michigan: Dr. Santiago Schnell. KUMC: Dr. Charles Little. University College London: Ana S. Dias, Irene de Almeida, Dr. Claudio Stern, University of Dundee: Dr. Mark Chaplain. Moffitt Cancer Center: Dr. Heiko Enderling. CRG Barcelona: Dr. James Sharpe. Cambridge University: Dr. Octavian Voicelescu.

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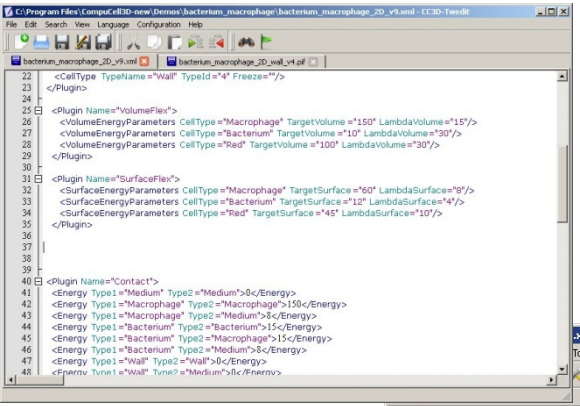
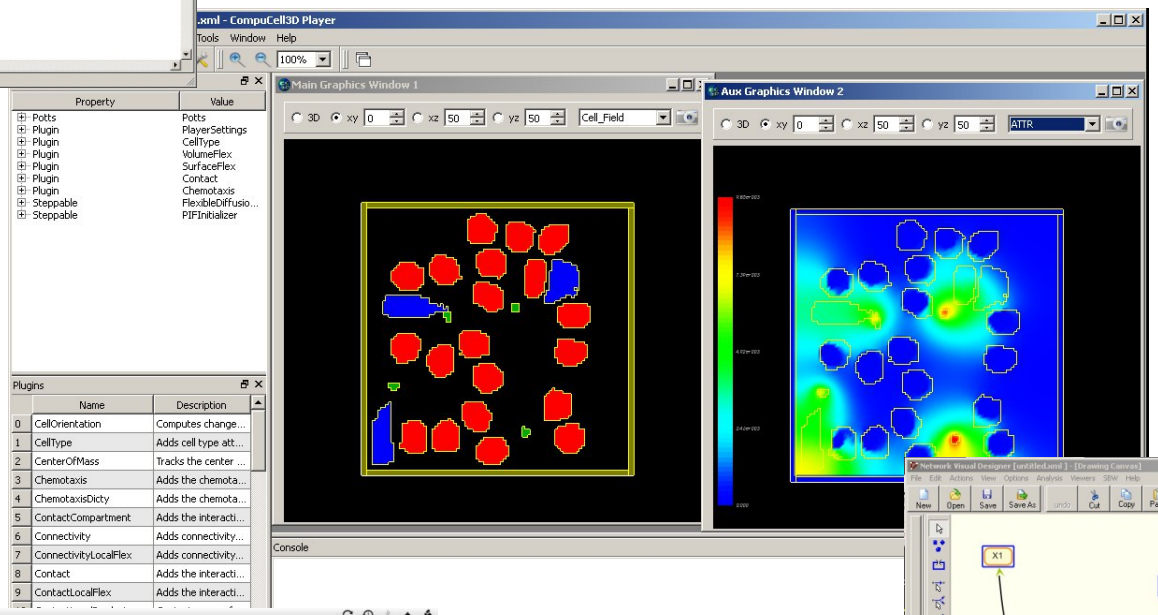
To download software for model building, please visit http://www.compucell3d.org

CompuCell3D - Simulation Environment for Multi-Cell, Multi-Scale Models

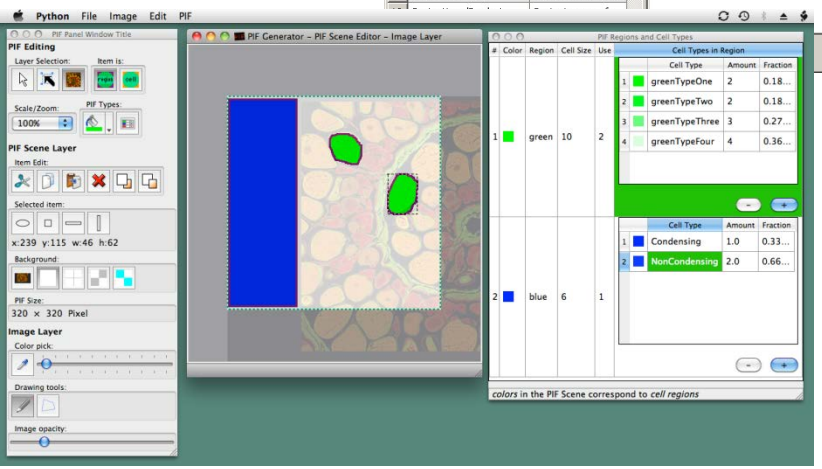


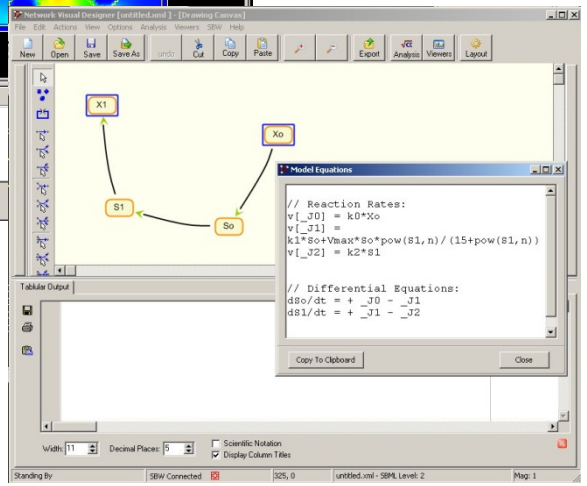
Python and XML model scripting



SBML models (e.g. defined using SBW)

Graphical specification of initial conditions





www.compucell3d.org

GGH Concepts

Objects: Fields, Generalized Cells, Links, Networks

Fields:
Properties: Concentration, Diffusion Constants, Decay Constants
Behaviors: Diffusion, Decay,
Interactions: Reaction, Secretion, Absorption, Advection

Generalized Cells:
Properties: Volume, Polarity, Surface Area, Inertia, Density, Viscosity, Elasticity, Plasticity, Substructure, Adjacency
Behaviors: Motility, Growth, Division, Death
Interactions: Adhesion, Chemotaxis, Differentiation, Secretion, Absorption

Links:
Properties: Length, Target Length, Elastic Modulus, Yield Strain, Target Angles, Bending Moduli
Behaviors: Creation, Destruction, Change of Target Length
Interactions: Exert Forces on Cells, Pulled on By Cells

Networks:
Properties: Values
Behaviors: ODEs, Stochastic Evolution
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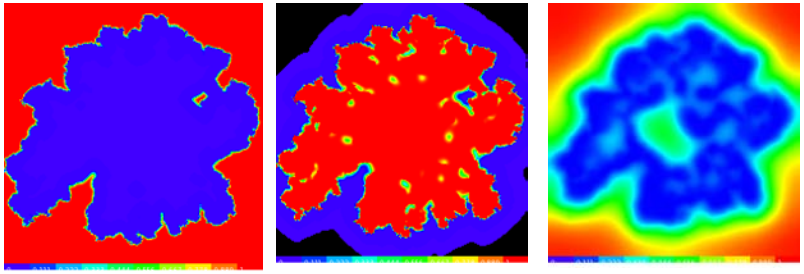

ECM MDE nutrient

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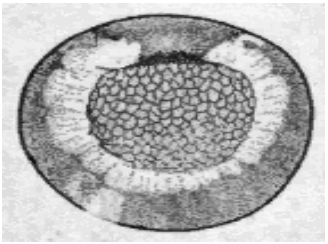

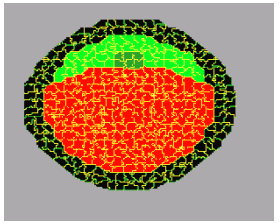

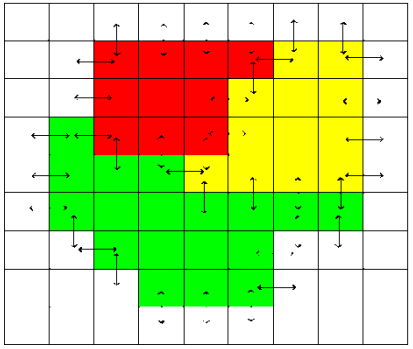

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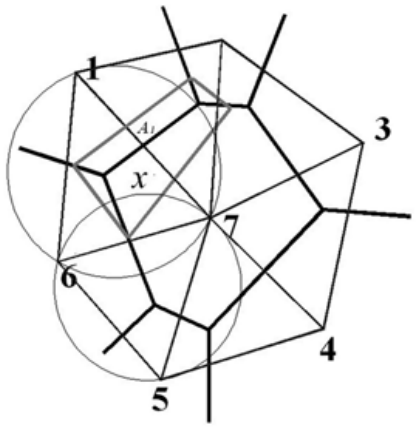

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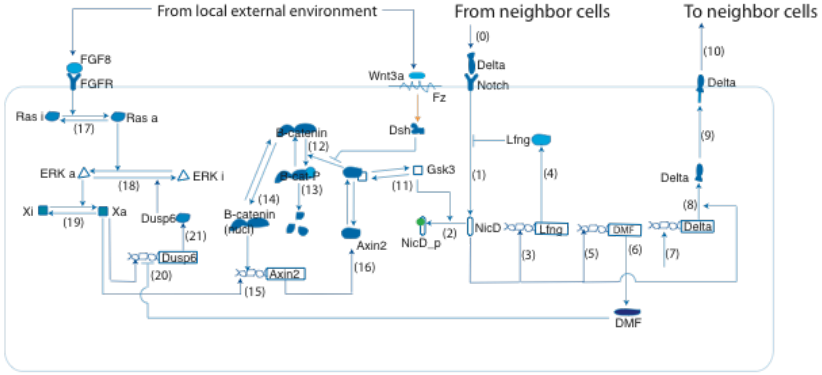
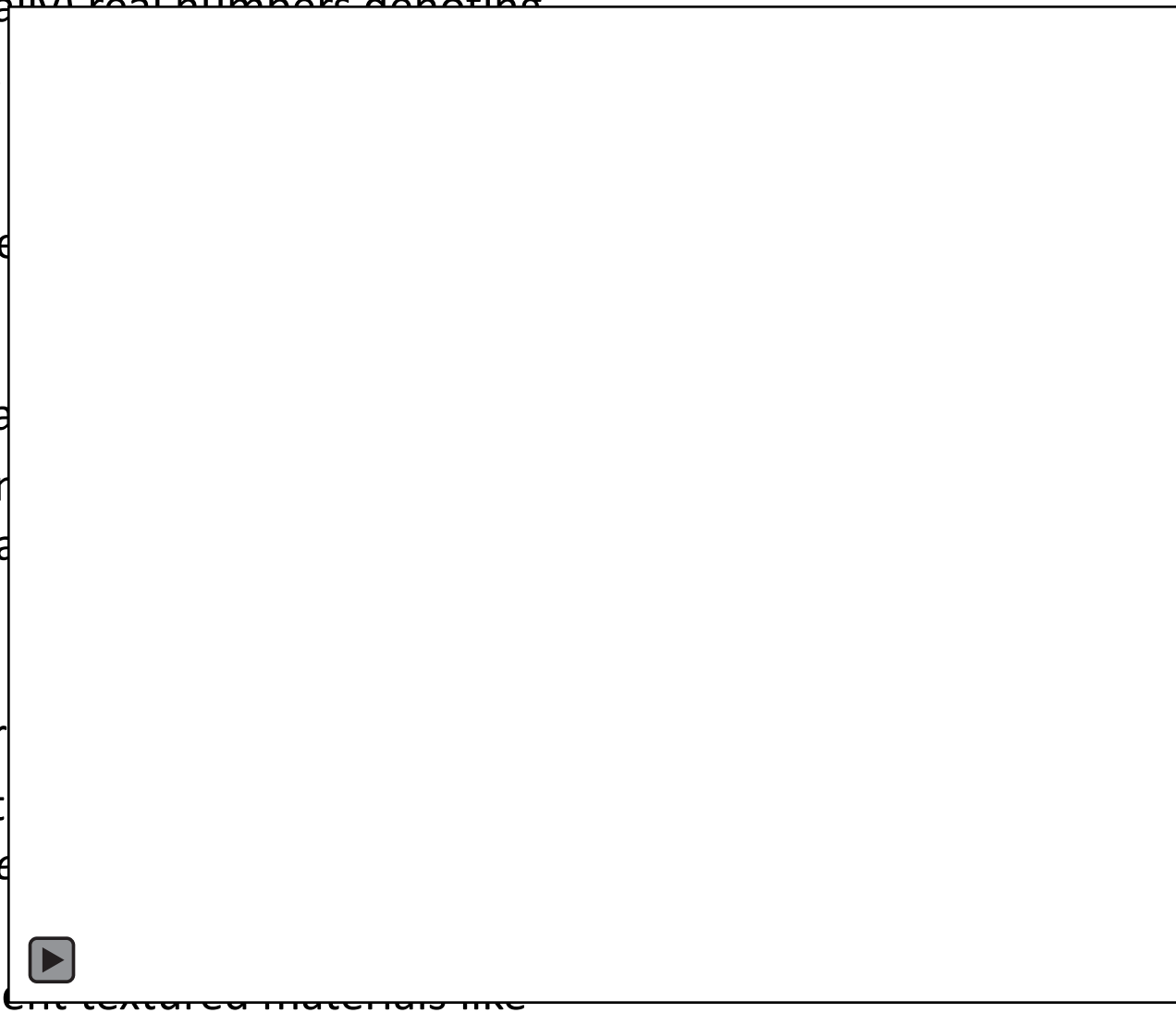


Fields

- A Field is a Lattice of (usually) real numbers denoting
- Fields can represent ECM, charge,…
- Fields and the Cell Lattice notional space (no exclude below.
- Fields may be confined to corresponding to particula
- Fields can be diffusing or r regions and support spatia decay constants.
- Other objects can secrete centers, boundaries or thr
- Other objects can interact interact with each other (e equations).
- Multiple Fields can represent textured materials like fibronectin or collagen Extracellular Matrix.



Field Dynamics

- Most Fields evolve via diffusion, secretion and absorption and cells and by decay.

$$\frac{\partial C(\vec{i})}{\partial t} = D_c \nabla^2 C(\vec{i}) - \gamma_c C(\vec{i}) + S_c\left(\sigma(\vec{i})\right) - A_c\left(\sigma(\vec{i})\right)$$

Diffusion Decay Secretion Absorption

- Sometimes we couple two or more Fields via Reaction-Diffusion Equations of Form:

$$\frac{\partial C_1(\vec{i})}{\partial t} = f(C_1, C_2) + D_{c_1} \nabla^2 C_1(\vec{i}) - \gamma_{c_1} C_1(\vec{i}) + S_{c_1}\left(\sigma(\vec{i})\right) - A_{c_1}\left(\sigma(\vec{i})\right)$$

$$\frac{\partial C_2(\vec{i})}{\partial t} = g(C_1, C_2) + D_{c_2} \nabla^2 C_2(\vec{i}) - \gamma_{c_2} C_2(\vec{i}) + S_{c_2}\left(\sigma(\vec{i})\right) - A_{c_2}\left(\sigma(\vec{i})\right)$$

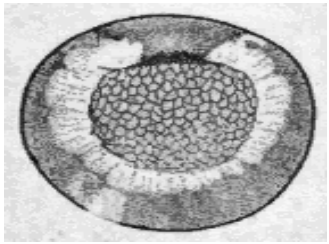
Cells and Compartmental Cells

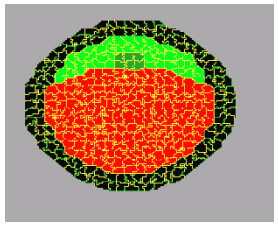
Each Cell has a unique integer Index, σ and consists of all sites on the Cell Lattice containing that Index.

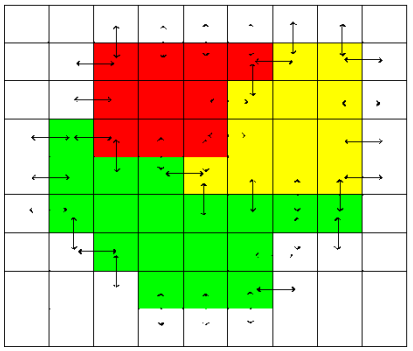
The number of Cell Lattice Sites with Index σ is the Cell's Volume, V.

The number of Lattice Sites with Index σ and, which are next to a Site with a Different Index σ' is the Cell's Surface Area, S.

Each cell also has a Type, τ.







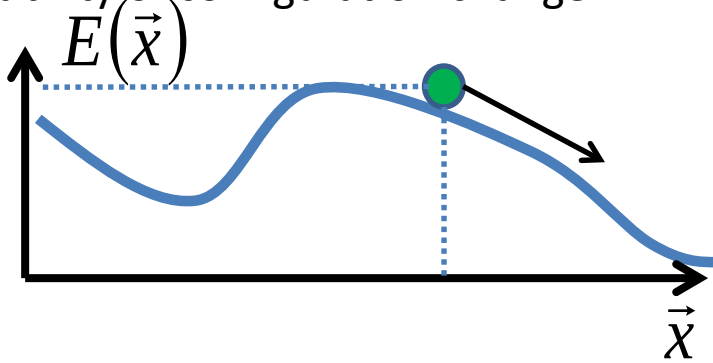
Cell Dynamics

•To simulate the cytoskeleton-driven extension and retraction of cell membranes (including pseudopods, filopodia and lamellipodia). The GGH algorithm tries randomly to extend and retract cell boundaries one pixel at a time.

•At each attempt, it calculates the new configuration Effective Energy and accepts the new configuration according to the Metropolis algorithm: probability of configuration change:

$$P(\Delta E) = e^{-\Delta E / kT}, \Delta E > 0$$

$$P(\Delta E) = 1, \Delta E \leq 0$$



•Result is movement with velocity proportional to the gradient of the Effective Energy, *i.e.*, linear in the applied force.

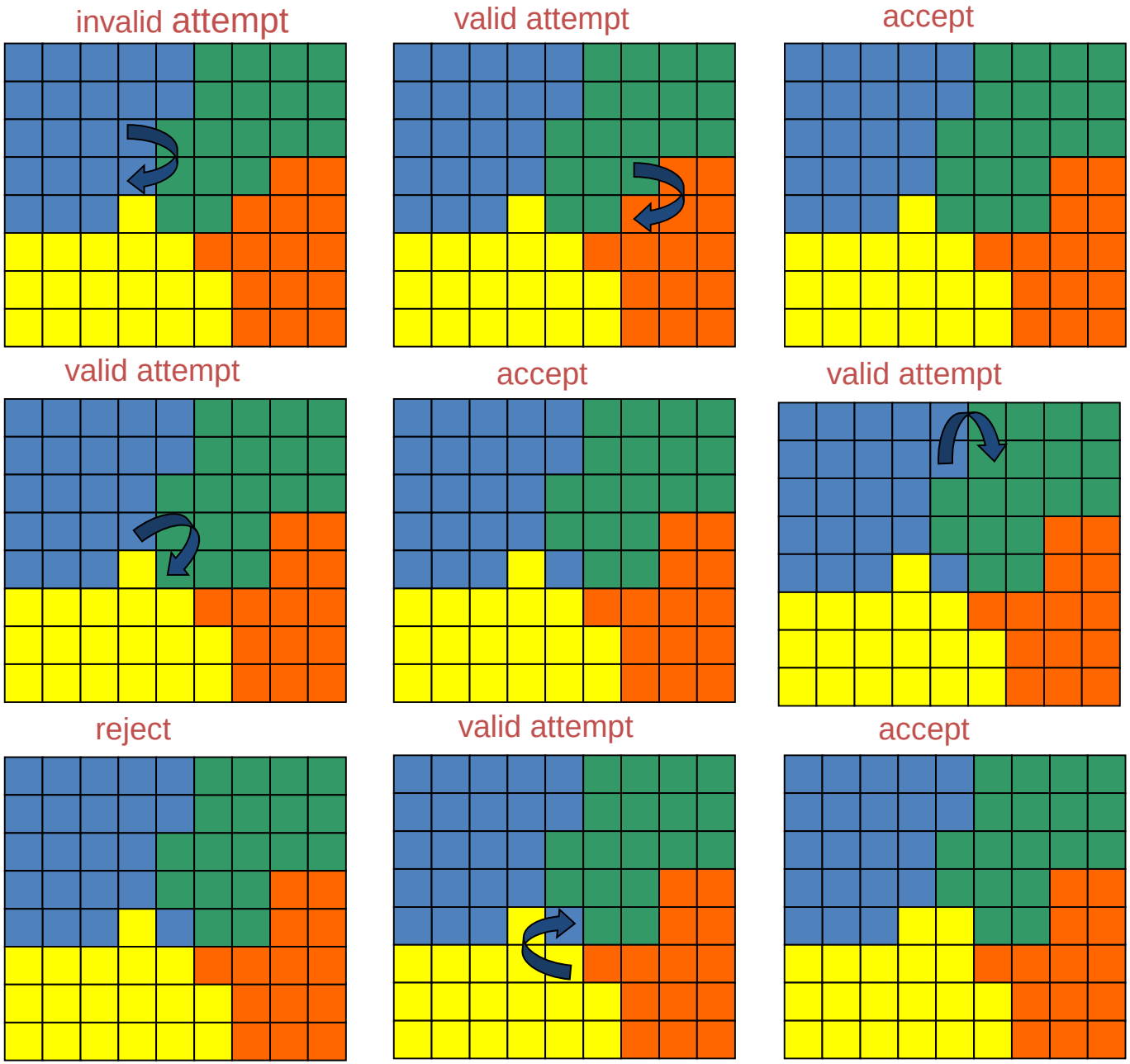
•Method breaks down if $\Delta E / kT$ too large.

•Configurations evolve to satisfy the constraints.

•When constraints conflict, evolve to balance errors.

•CC3D allows users to define their own acceptance functions.

Random Motility Cell Dynamics



Cell Properties/Interactions

- Most biological of Cells and their interactions with each other and with Fields are Encapsulated in the Effective Energy, E.
- E is generally the sum of many separate terms.
- Each term in E encapsulates a single biological mechanism.
- Additional Cell Properties described as **Constraints**.

Energy Terms: Cell Adhesion

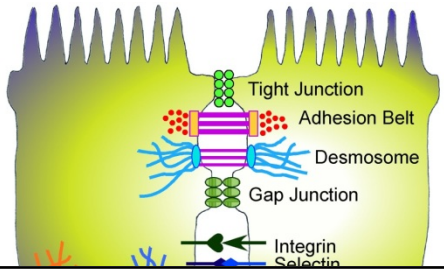
A unit of Cell Boundary (between Adjacent Lattice Sites containing different Indices) has associated Adhesion Energy, J, depending on the Types of the Neighboring Cells:

$$J\left(\tau\left(\sigma\left(\vec{i}\right)\right),\tau\left(\sigma\left(\vec{i}'\right)\right)\right)$$

or the number and types of adhesion molecule on each cell: $f\left(n_j\left(\vec{i}\right),\ldots;n_k\left(\vec{i}'\right),\ldots\right)$

The Total Adhesion Energy, E_{adhesion} is:

$$E_{\text{adhesion}} = \sum_{\vec{i},\vec{i}' \text{ neighbors}} J\left(\tau\left(\sigma\left(\vec{i}\right)\right),\tau\left(\sigma\left(\vec{i}'\right)\right)\right)\left\{1-\delta\left(\sigma\left(\vec{i}\right),\sigma\left(\vec{i}'\right)\right)\right\}$$



or

$$E_{\text{adhesion}} = \sum_{\vec{i},\vec{i}' \text{ neighbors}} f\left(n_j\left(\vec{i}\right),\ldots;n_j\left(\vec{i}'\right),\ldots\right)\left\{1-\delta\left(\sigma\left(\vec{i}\right),\right.$$

Where,

$$\delta\left(\sigma\left(\vec{i}\right),\sigma\left(\vec{i}'\right)\right)=\begin{cases}1, \sigma\left(\vec{i}\right)=\sigma\left(\vec{i}'\right)\\ 0, \sigma\left(\vec{i}\right)\neq\sigma\left(\vec{i}'\right)\end{cases}$$

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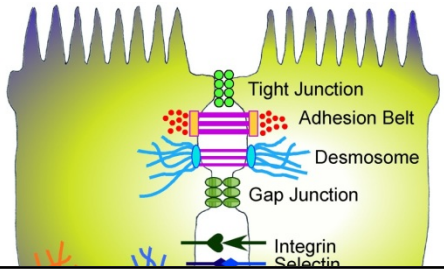

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Energy Terms: Chemotaxis

A Cell attracted or repelled by a chemical is represented by a Chemotaxis or Haptotaxis Effective Energy, E_{chemo}:

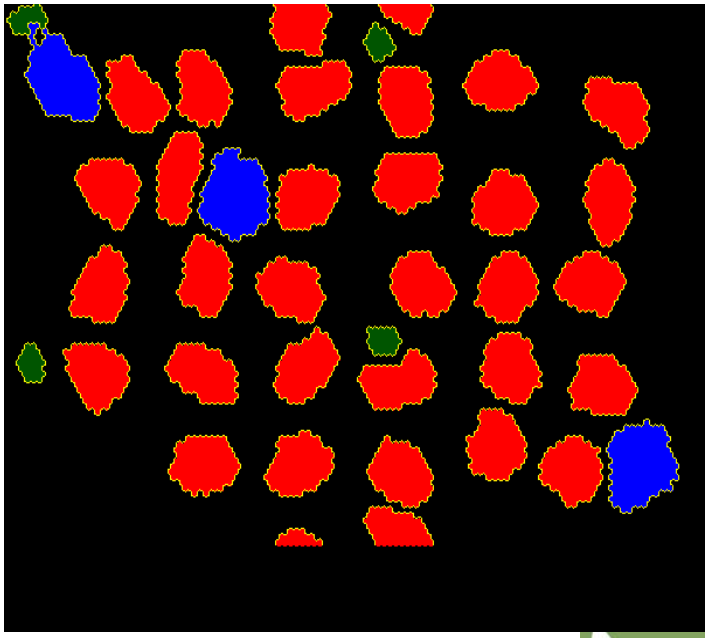
$$E_{\text{chemo}} = \sum_{\vec{i}} \mu\left(\tau\left(\sigma\left(\vec{i}\right)\right)\right) f\left(C\left(\vec{i}\right)\right)$$

μ>0 →chemorepulsion,

μ<0 →chemoattraction

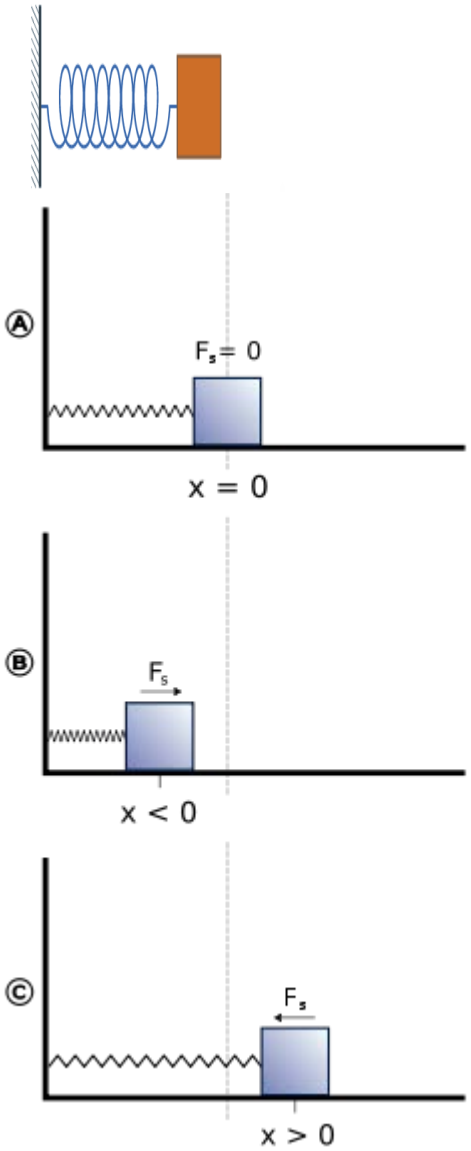
f is the response function of the cell to the chemoattractant

May have many such terms



Constraints

- What is a Constraint?
- A function that pushes a system back towards some predefined state
- *E.g.*
  - A mass on a spring
  - A ball rolling in a bowl



Constraints

- A Constraint is a very convenient method for implementing behaviors via an Effective Energy
- In general, an elastic Constraint has the form:

$$E_{\text{constraint}} = \sum_{\text{objects}} \lambda(\text{object})\left(f(\text{object}) - f_{\text{target}}(\text{object})\right)^2$$

- λ is the Constraint Strength and *f* the Constraint Function. The bigger λ, the smaller the deviations of the behavior of the system from the target
- Because of the Dynamic Behavior of Metropolis Algorithm ANY behavior can be implemented this way

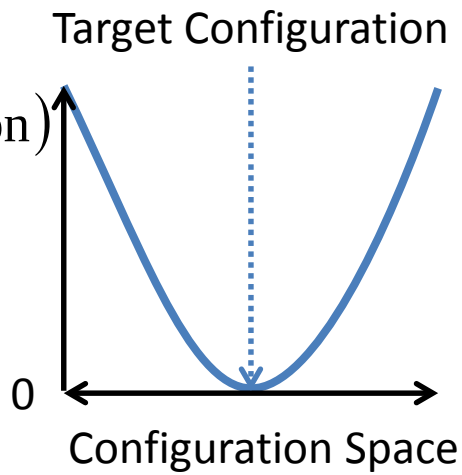
Constraints

- Saw before, the pattern configuration evolves to reduce the Effective Energy at a rate $\left|\nabla E\left(\vec{x}\right)\right|/T$

- For a constraint:

$$E_{\text{constraint}}(\text{configuration})$$

$$E_{\text{constraint}} = \sum_{\text{objects}} \lambda(\text{object})\left(f(\text{object}) - f_{\text{target}}(\text{object})\right)^2$$



- Because the energy function is smooth and has a single minimum, the pattern will evolve from any configuration to try to satisfy the constraint, at a rate proportional to $2\lambda(\text{object})\left(f(\text{object}) - f_{\text{target}}(\text{object})\right)$
- **For multiple incompatible constraints, the selected configuration will be a compromise among the constraints.**

Volume Constraints

- Most Cells (except Generalized Cells representing fluid media) have defined volumes.

$$E_{\text{volume}} = \sum_{\sigma} \lambda_{\text{volume}}(\sigma)\left(V(\sigma) - V_{\text{target}}(\sigma)\right)^2$$

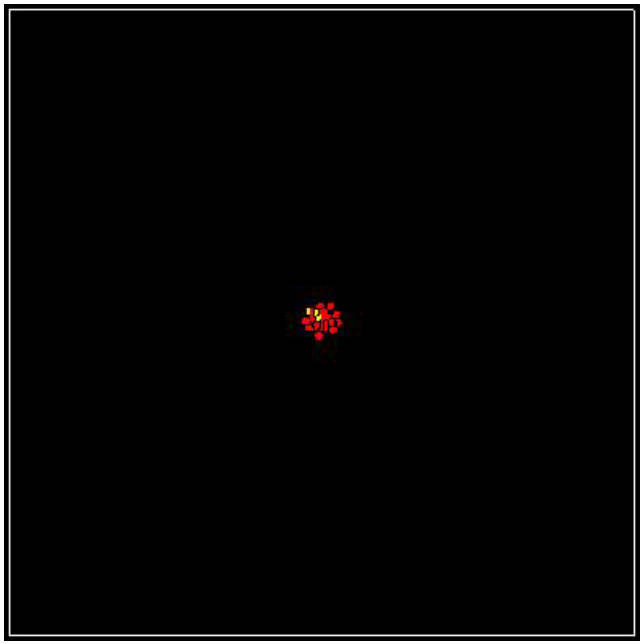
$$\text{Pressure} = 2\lambda_{\text{volume}}(\sigma)\left(V(\sigma) - V_{\text{target}}(\sigma)\right)$$

- *i.e.* the cell obeys the ideal gas law.
- Easy way to implement Cell Growth:

$$\frac{dV_{\text{target}}(\sigma)}{\text{dt}} = f(\text{system state, cell state})$$

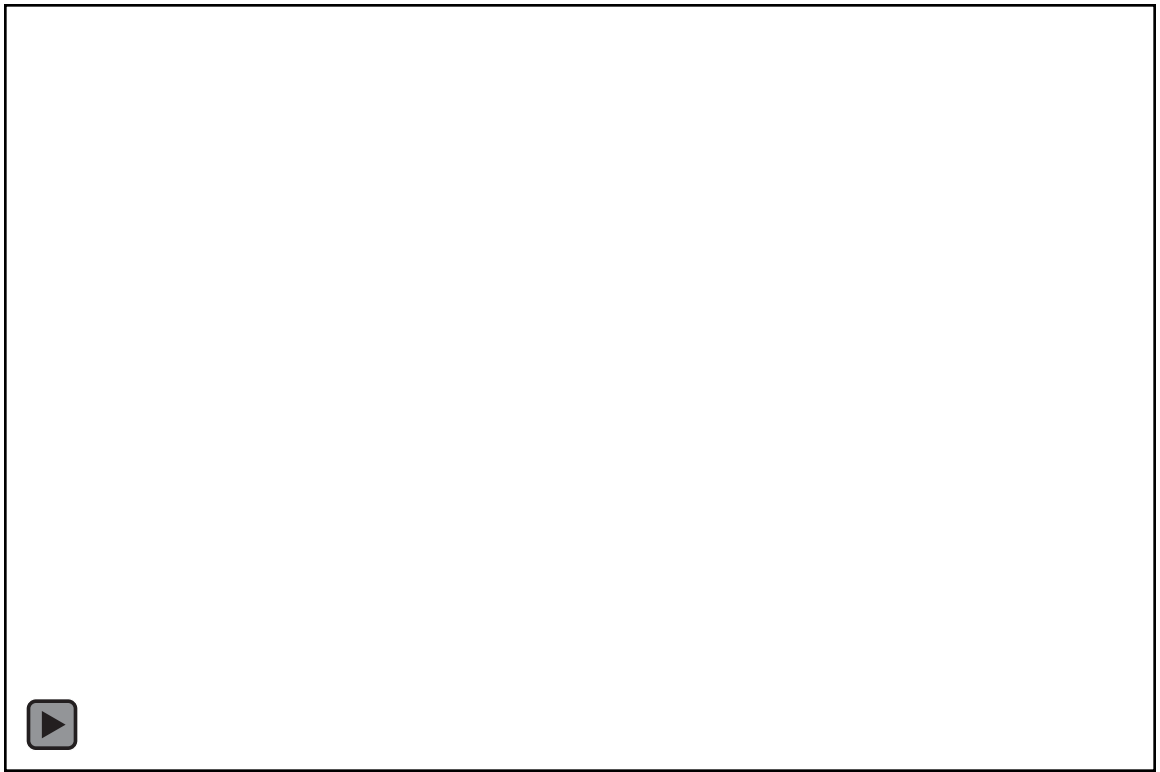
- And Cell Death: $V_{\text{target}}(\sigma) = 0$

The rate of cell disappearance proportional to $\lambda_{\text{volume}}(\sigma)$



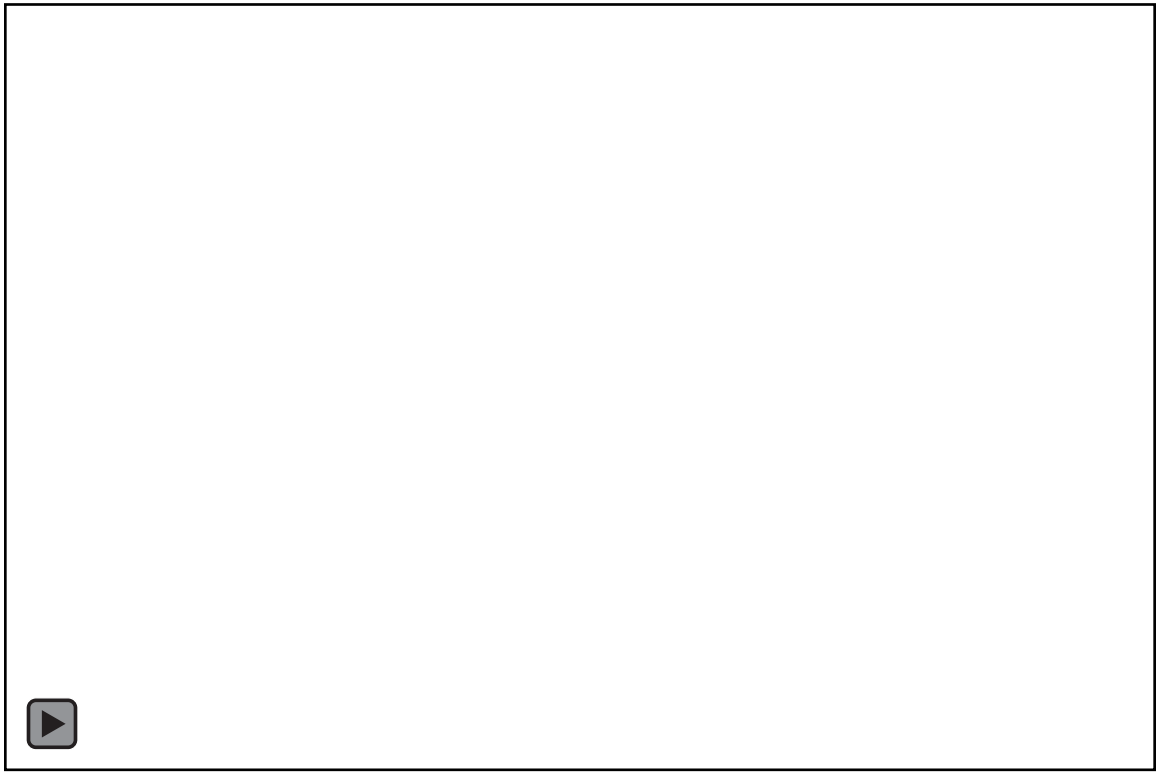
Compartmental Cells

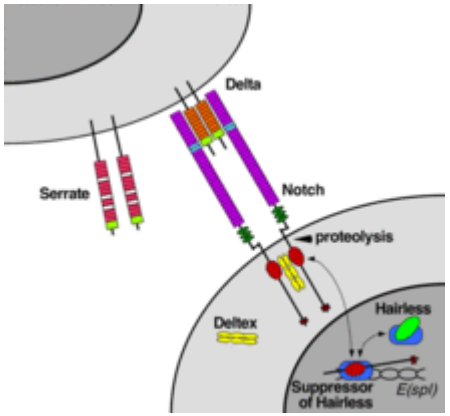
- The Basic CC3D Cell is an isotropic blob
- CC3D allows the division of Cells into compartments called SubCells where each SubCell compartment has a different set of properties
- Example: Gastrulation in chick embryo with convergent extension due to polarized cell-surface properties



Subcell Spatial Modeling Intracellular Fields:

- CompuCell3D can also do subcellular modeling
- *E.g.*, Induction of Planar Polarity Pathway by surface

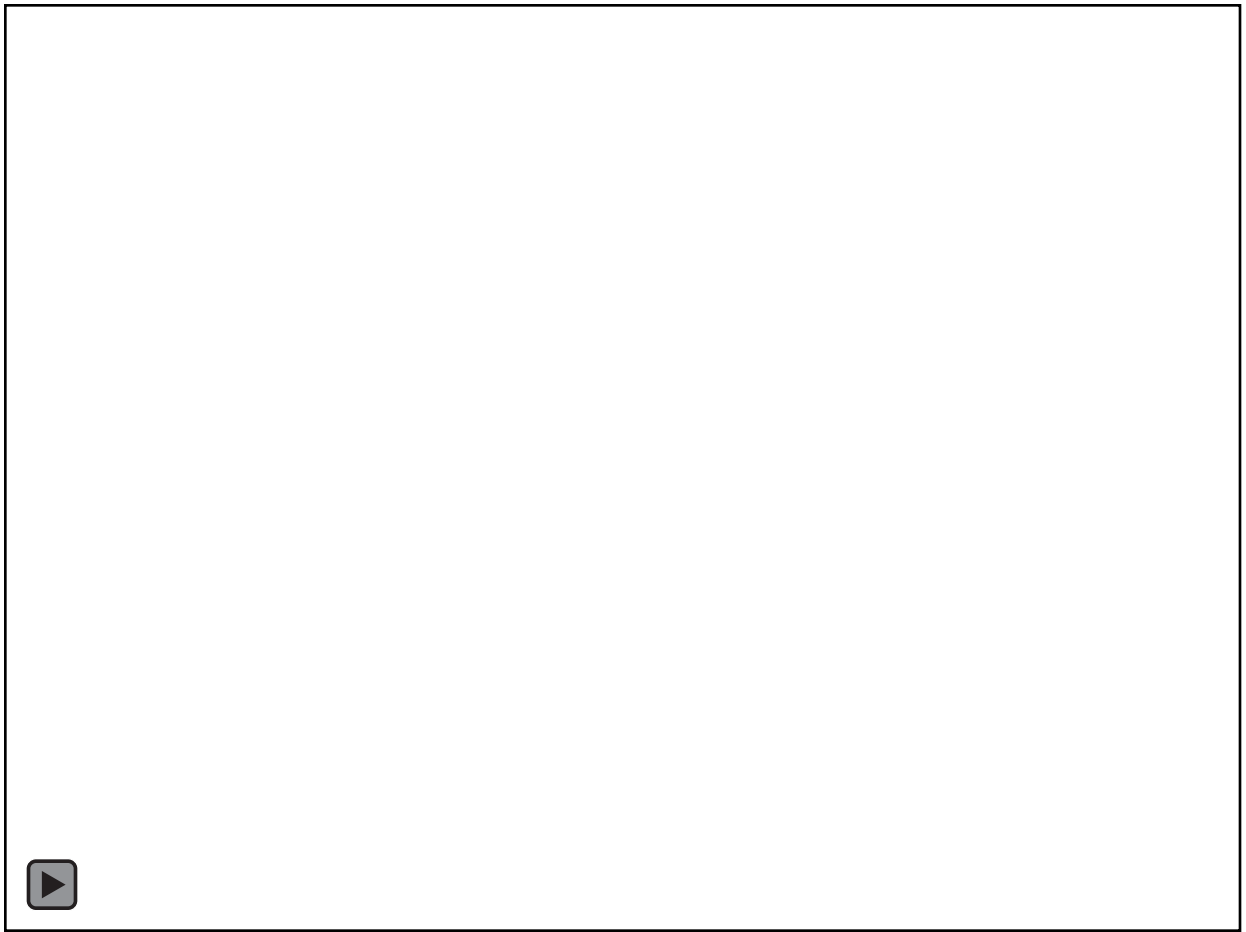




From Wikipedia

Subcell Spatial Modeling Intracellular Fields:

- PAR-2/PAR-6 Polarization by Centromere



Model Components

- Objects/Representations
- Object Properties/Interactions
- Dynamics
- **‘Tweaks’**
- Initial and Boundary Conditions

Tweaks: Mitosis

Set Criterion for Cell Division

When satisfied, divide Cell along an axis (random cell division, oriented cell division)

Assign Cell Lattice Sites in one half of Parent Cell to a New Cell

Decide Properties of New Cell and Parent Cell

Links Describe Elastic and Plastic Materials

Links constrain distances between Anchors

Define the Energy of Link relative to link's Target Length

$$E_{\text{elastic}} = \sum_{\sigma} \sum_{\substack{\mu,\nu=1 \\ \text{neighbors}}}^{m(\sigma)} \lambda_{\text{elastic}}(\sigma,\mu,\nu)\left(\left\|\vec{c}m(\sigma,\mu)-\vec{c}m(\sigma,\nu)\right\| - L_{\text{target}}(\sigma,\mu,\nu)\right)^2.$$

λ_{elastic} is the Young's Modulus of the Solid.

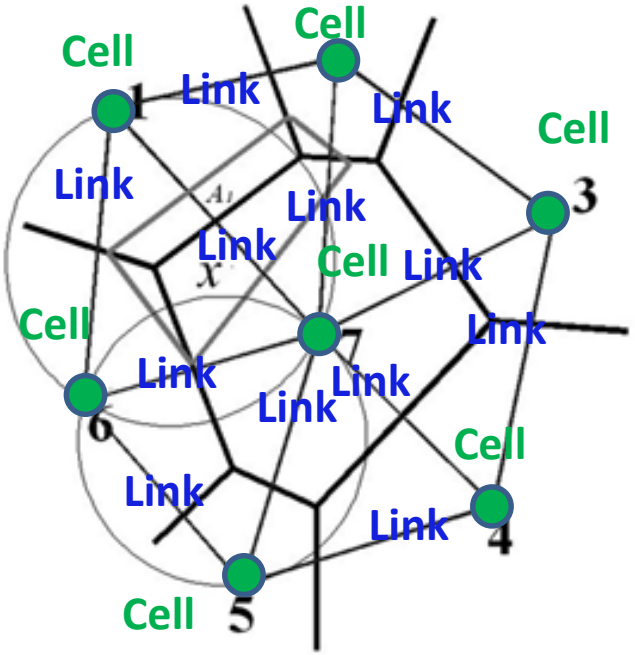
The strain on a link is:

$$\left\|\vec{c}m(\sigma,\mu)-\vec{c}m(\sigma,\nu)\right\| - L_{\text{target}}(\sigma,\mu,\nu)$$

The stress on a link is:

$$\lambda_{\text{elastic}}(\sigma,\mu,\nu)\left(\left\|\vec{c}m(\sigma,\mu)-\vec{c}m(\sigma,\nu)\right\| - L_{\text{target}}(\sigma,\mu,\nu)\right)$$

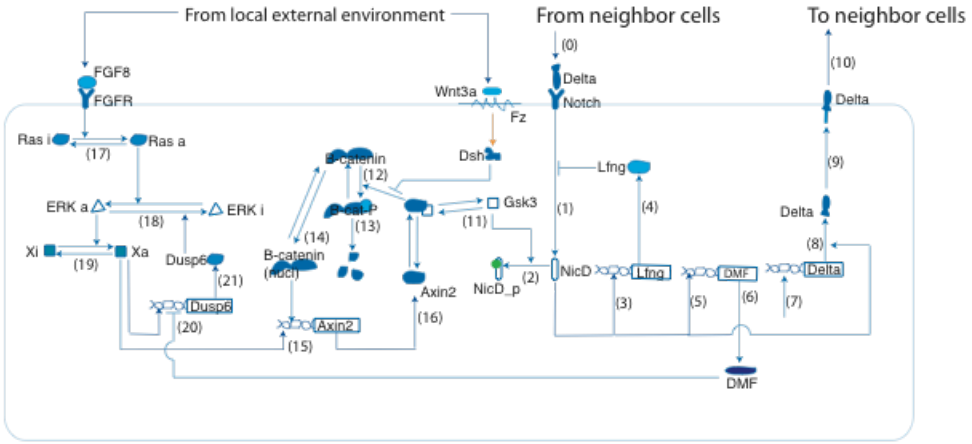
For a plastic material, define a Yield Strain (or Yield Stress at which the links break.



Reaction Kinetic Networks

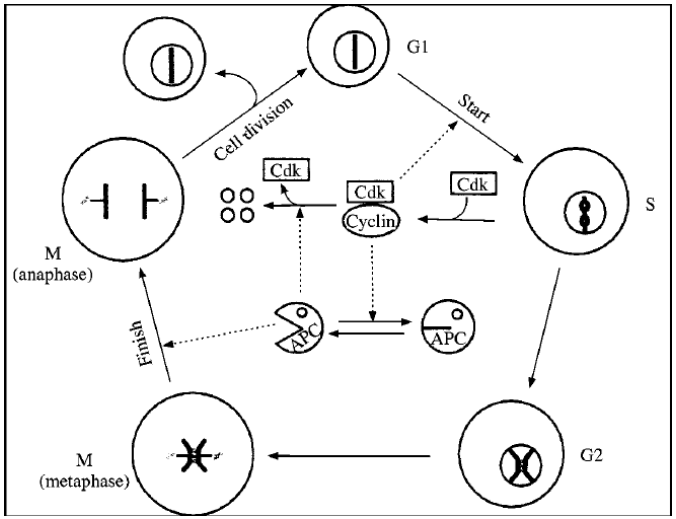
- Can attach one or more coupled RK submodels to any Object
- *E.g.* subcellular functions like Regulatory, Metabolic or Signaling Networks
- *E.g.* global Physics-Based Pharmaco-Kinetic Modeling (PBPK) modeling.
- RK networks can control the properties of other Objects and respond to their states
- RK models can also couple to each other within or between Objects (*e.g.* intracellular *vs.* juxtracrine signaling

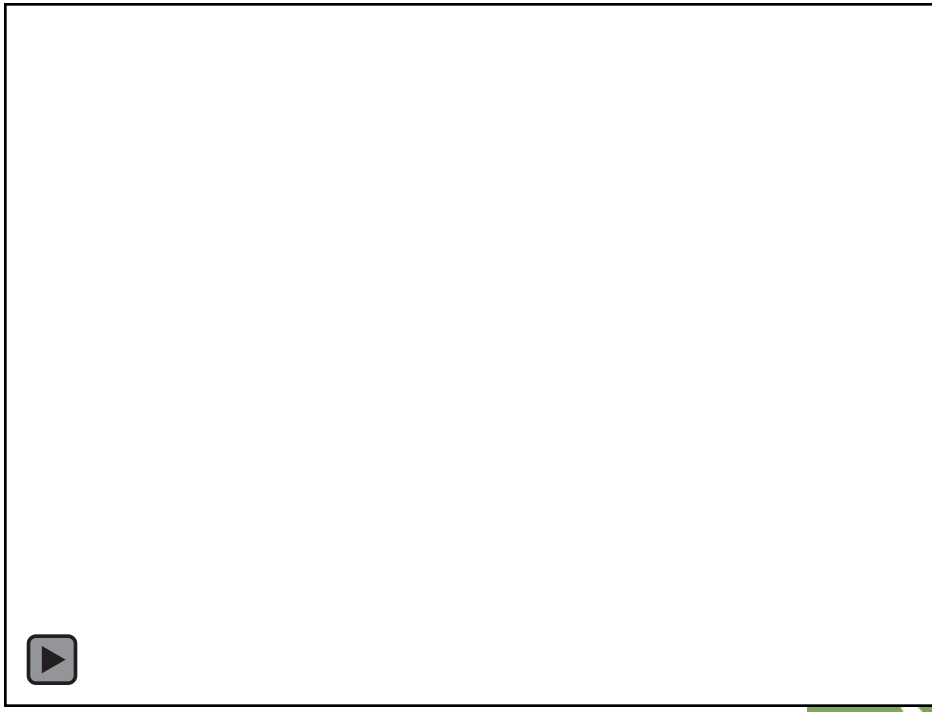
- Examples of Biochemical Kinetics:
  - Cell-Cycle
  - Circadian rhythms
  - Cardiac rhythms
  - cAMP oscillations
  - Delta-Notch patterning
  - WNT pathway
  - FGF pathway
  - Etc…



Subcellular modelling

- Biochemical Kinetics:
  - Cell-Cycle
  - Circadian rhythms
  - Cardiac rhythms
  - cAMP oscillations
  - Delta-Notch patterning
  - WNT pathway
  - FGF pathway
  - Etc…





Model Components

- Objects/Representations
- Object Properties/Interactions
- Dynamics
- ‘Tweaks’
- **Initial and Boundary Conditions**

Initial and Boundary Conditions

- Define Initial Configurations for All Objects and Initial Values for all Internal Variables and Parameters
- Define Boundary Conditions of Fields and Cell Lattice (Periodic or Fixed, Absorbing or Reflecting, Excluded Volumes/No Excluded Volumes…)

Sample Current Applications

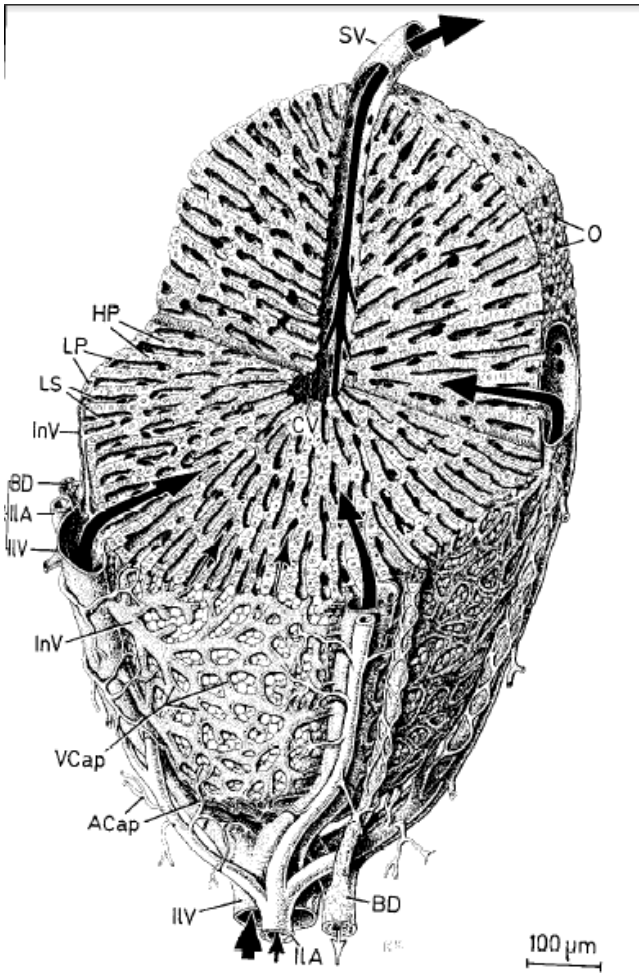
- Angiogenesis and Vasculogenesis (EPA, CWI)
- Vascular Tumor Growth
- Cancer Evolution
- Age Related Macular Degeneration
- **Liver Toxicology (IUB, EPA)**
- Polycystic Kidney Disease (IUB, IUPUI)
- Segmentation (IUB, UCL)

Liver Toxicity

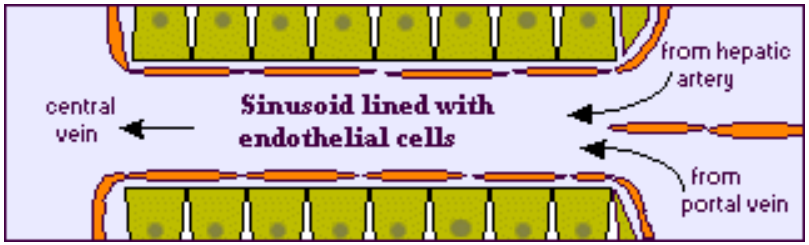
- The liver is the primary metabolizing organ of the body and is the first line of defense against toxins.
- The liver has a high capacity to regenerate.
- *The liver is a "massively parallel device" that presents a unique opportunity for modeling not present in non-parallel organs like the heart.*

- *With EPA and Hamner Institute Collaborators*

Liver Lobule[1]



Liver Sinusoid[2]



1. Human Microscopic Anatomy: An Atlas for Students of Medicine and Biology, R. V. Krstic, Springer-Verlag, 1991 (ISBN 978-3-540-53666-6).
2. http://biology.about.com/library/organs/bldigestliver.htm

Sluka

Acetaminophen
(APAP, Paracetamol)

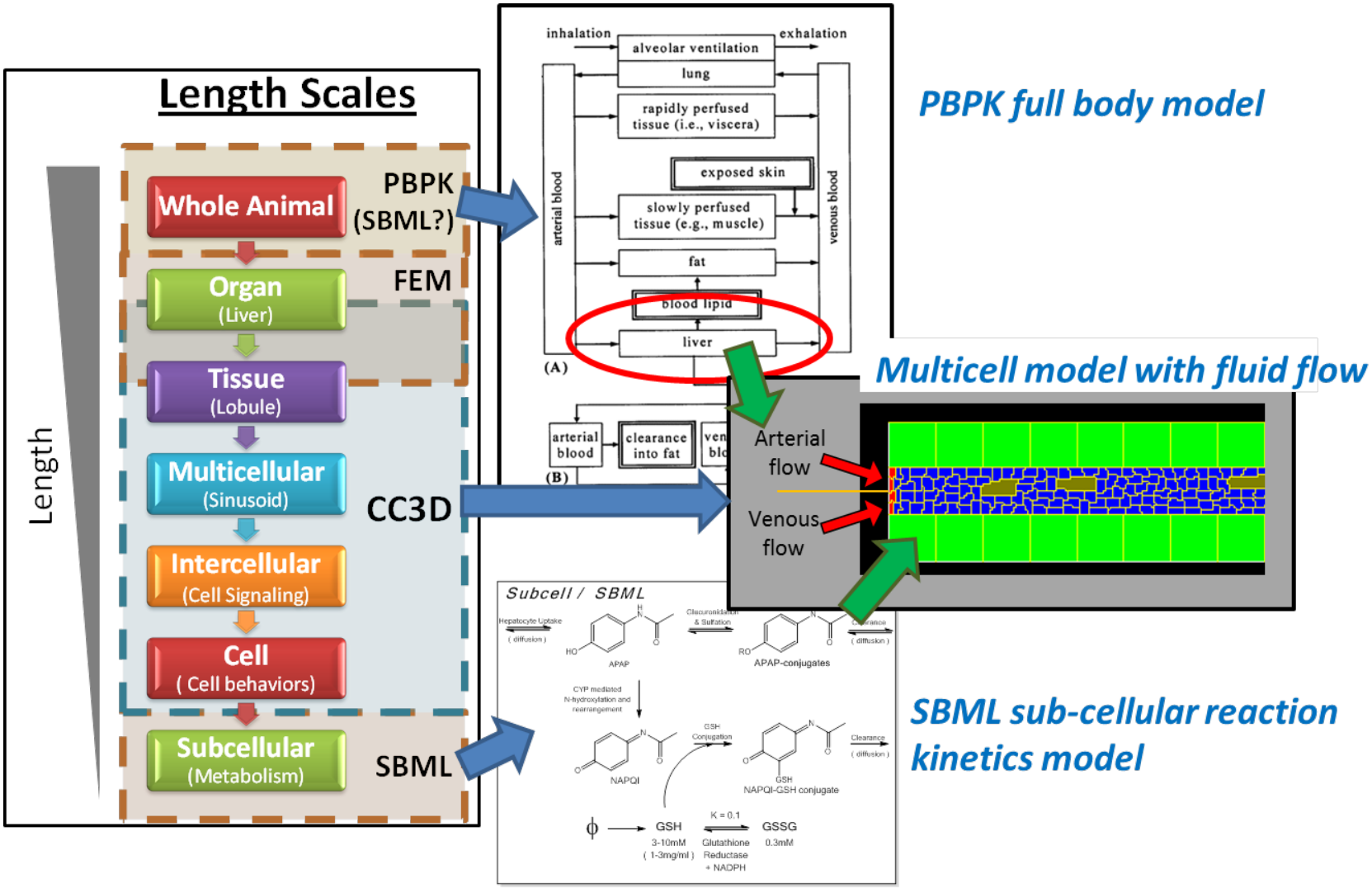
- Widely used over the counter analgesic and fever reducer
- Cyclooxygenase (COX) inhibitor
- Therapeutic index (ratio of toxic dose to therapeutic dose) of just 10 is unusually small for an over the counter medication
- Overdose results in rapid centrilobular necrosis of the liver, which can lead to death
- Leading cause of acute liver failure and accounts for most drug overdoses in the Western world
- Extensively studied in both humans and laboratory animals

- **Liver has huge regenerative capacity after surgical resection**

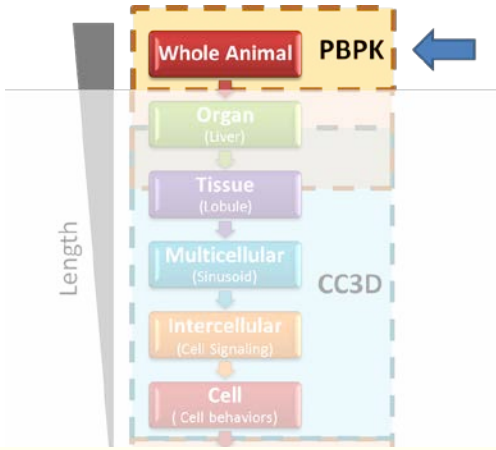
- **Not clear why liver fails to regenerate after acute Acetaminophen poisoning, which kills only a small fraction of hepatocytes**

Sluka

Liver Toxicity Model



Partitioning of APAP

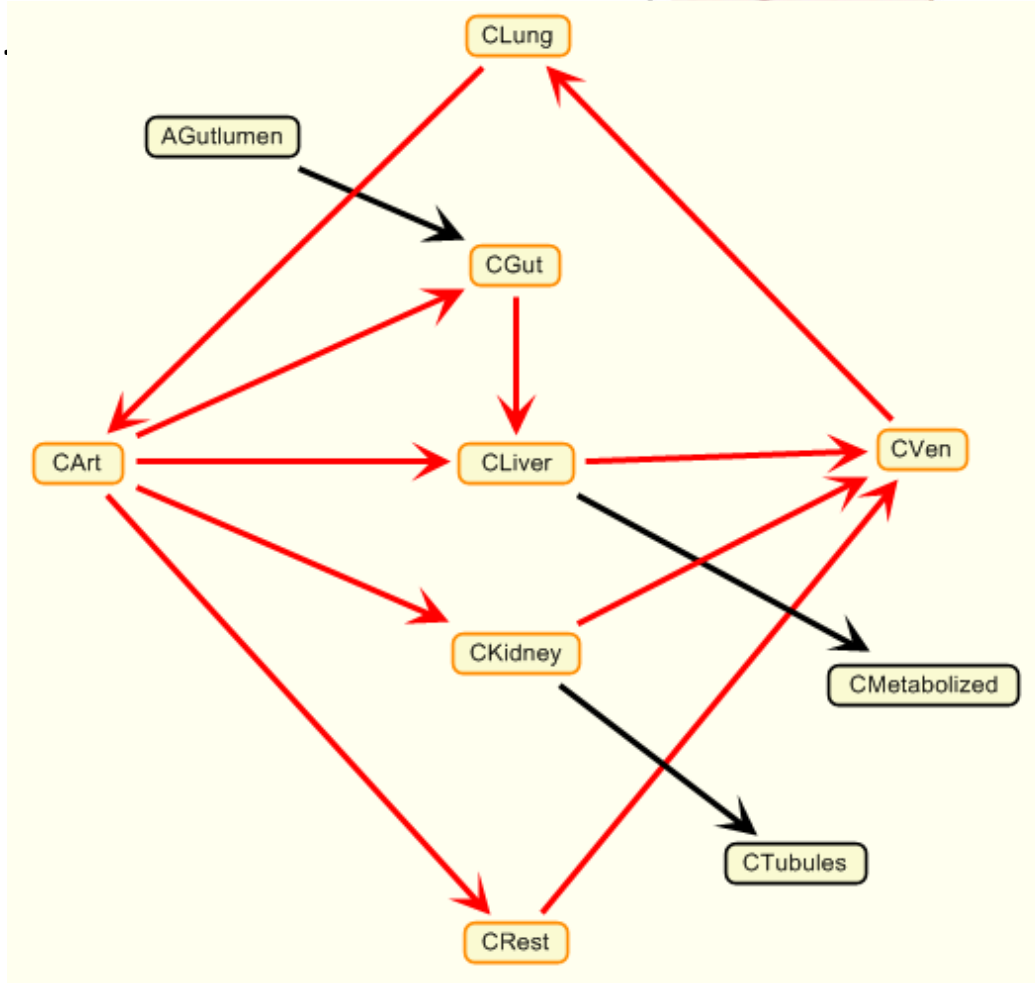


- Model based on published models of Acetaminophen (APAP, Paracetamol) PBPK models.[1]
- Model translated into SBML using SBW [2] tools, particularly Jarnac and JDesigner2.

$$\frac{-dA}{dt} = \frac{Q_{source}}{V_{source}} \times A_{source}$$

Where:

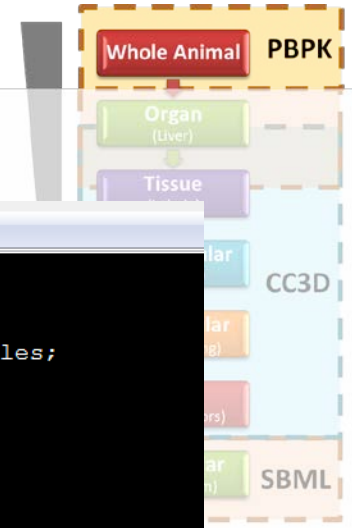
- Q_{source} is a volumetric flow (vol/time)
- V_{source} is the volume of the source compartment
- A_{source} is the amount (mass) of the compound in the source compartment
- Compartments are “well stirred”



1. Wambaugh, J. & Shah, I. Simulating Microdosimetry in a Virtual Hepatic Lobule. PLoS Computational Biology 6, (2010).
2. http://sys-bio.org/

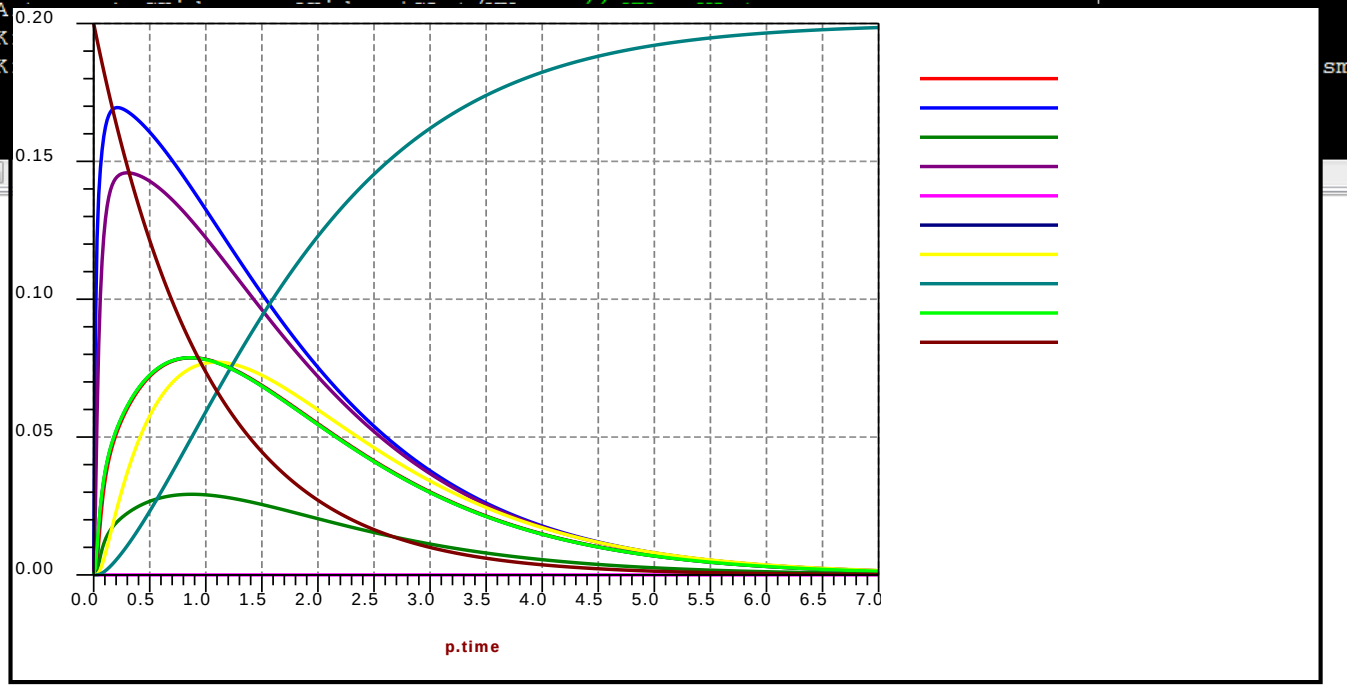
Whole Body Scale: PBPK

(System Biology Workbench-Jarnac)



APAP_GSH_Liver_Metab.jan | Wambaugh_vLiver_PBPK_based.jan

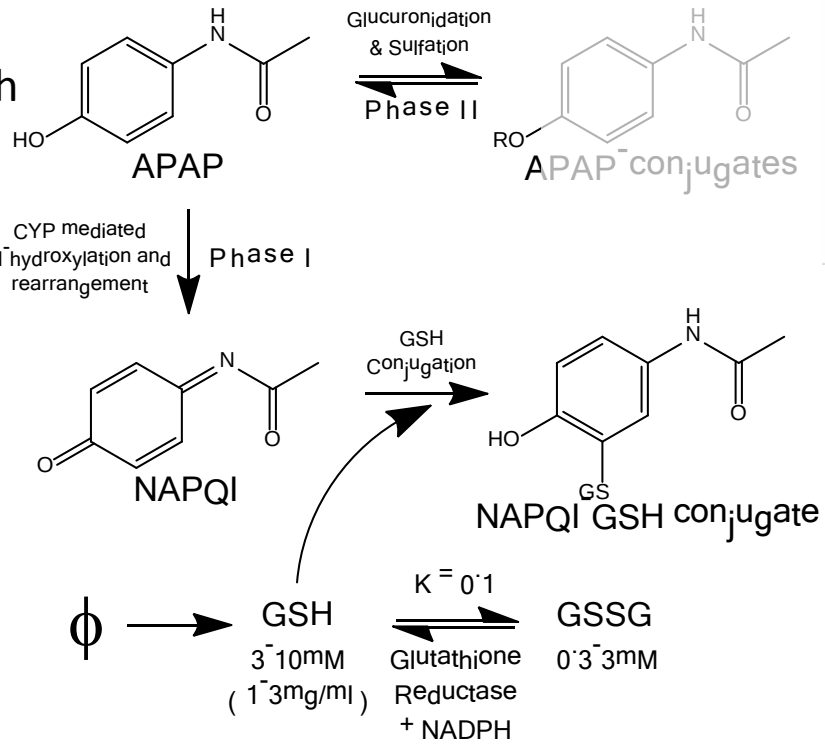
```
19 p = defn PBPK_model
20
21 vol compartment;
22 var CArt, CGut, AGutlumen, CLung, CVen, CRest, CLiver, CMetabolized, CKidney, CTubules;
23
24 J1:  CArt     -> CGut;     QGut*CArt/VVen;     // VVen=VArt
25 J2:  AGutlumen->CGut;     kGutabs*AGutlumen;
26 J3:  CLung    -> CArt;     QCardiac*CLung/VLung;
27 J4:  CVen     -> CLung;    QCardiac*CVen/VVen;
28 J5:  CArt     -> CRest;    QRest*CArt/VVen;    // VVen=VArt
29 J6:  CRest    -> CVen;     QRest*CRest*Ratioblood2plasma/KRest2plasma/Fraction_unbound_plasma/VRes
30 J7:  CArt     -> CLiver;   QLiver*CArt/VVen;   // VVen=VArt
31 J8:  CLiver   -> CMetabolized; CLmetabolism*CLiver/Kliver2plasma/Fraction_unbound_plasma/VLiver;
32 J9:  CGut     -> CLiver;   QGut*CGut/VGut;
33 J10: CLiver   -> CVen;     (QLiver+QGut)*CLiver*Ratioblood2plasma/Kliver2plasma/Fraction_unbound_pl
34 J11: CA
35 J12: CK
36 J13: CK
37
38 end;
39
```

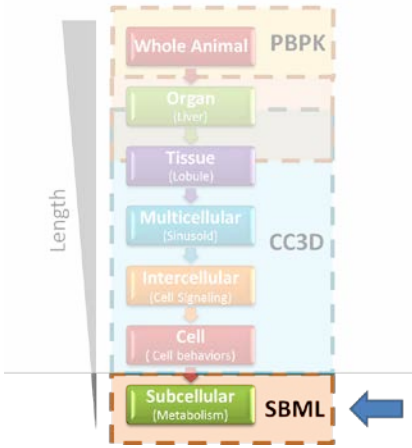


Sluka

APAP Metabolism and Glutathione Model

- Acetaminophen (*APAP*) metabolized in the liver in both Phase I and Phase II reactions.
- High APAP doses deplete hepatocyte Glutathione (*GSH*) levels, leading to hepatocyte death
- Glutathione:
  - Acts as a RedOx buffer of the cell's cytosol
  - Antioxidant, reactive oxygen species (ROI) and electrophile scavenger
  - Cofactor for several enzymes



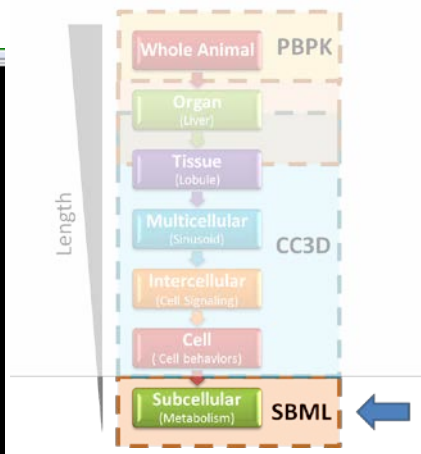


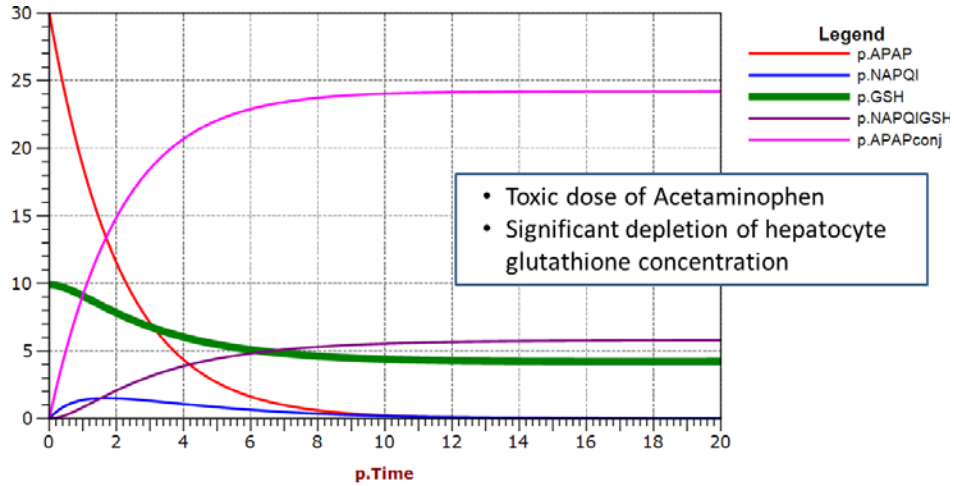
Sluka

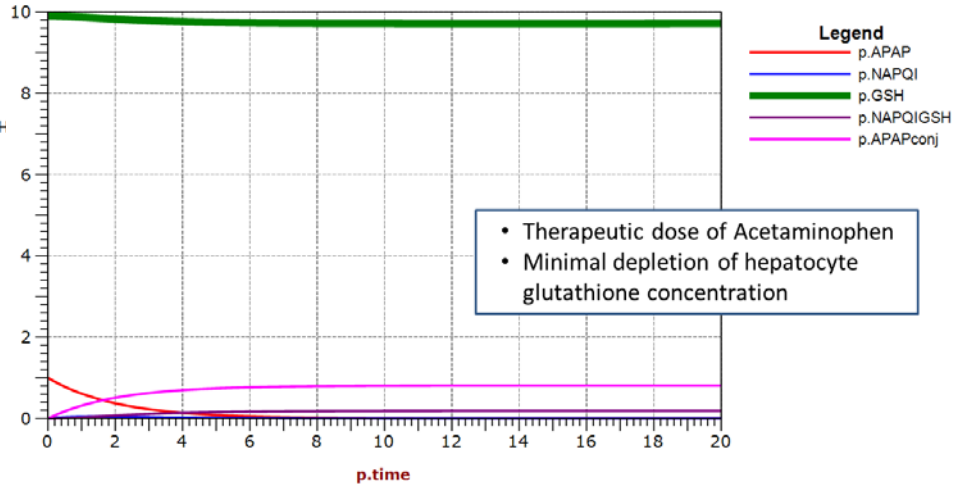
Reaction Kinetic Model

```
//   Kgsh            Instance of first order rate constant              SBO:0000049
//-----------------------------------------------------------------------------------

p = defn ApapGshConjModel
var APAP, NAPQI, GSH, NAPQIGSH, APAPconj;
ext X1;
J0:         APAP -> NAPQI;       Vmax_2E1_APAP*APAP/(Km_2E1_APAP + APAP);
J1: NAPQI + GSH -> NAPQIGSH;     kNapqiGsh*NAPQI*GSH;
J2:          $X1 -> GSH;         kGsh*(GSHmax-GSH);
J3:         APAP -> APAPconj;    Vmax_PhaseIIEnz_APAP*APAP/(Km_PhaseIIEnz_APAP + APAP);
end;
```

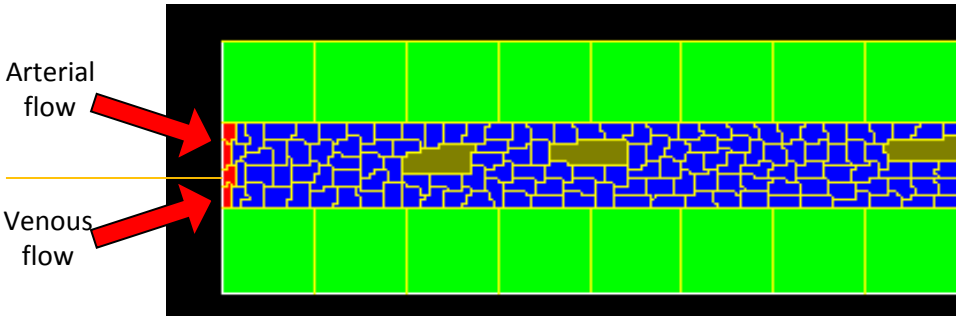


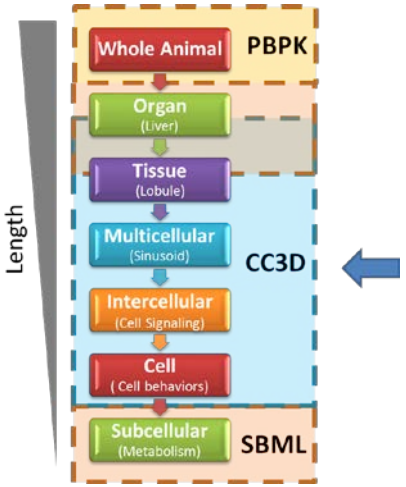




Sluka

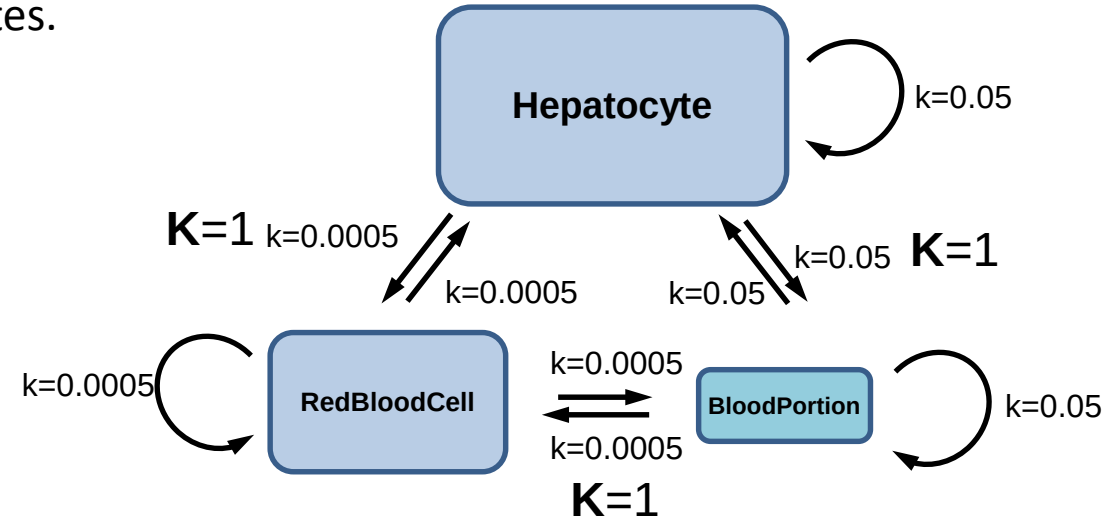
Liver Sinusoid Model: Blood Flow and Transport Model





- Hepatocytes (top & bottom)
- Red Blood Cells (RBCs, dark green)
- Serum Portions (blue)
- Blood "source" cells (red)

- RBCs and Serum Portions carry a load of Acetaminophen that diffuses into/out of the serum, RBCs and hepatocytes.



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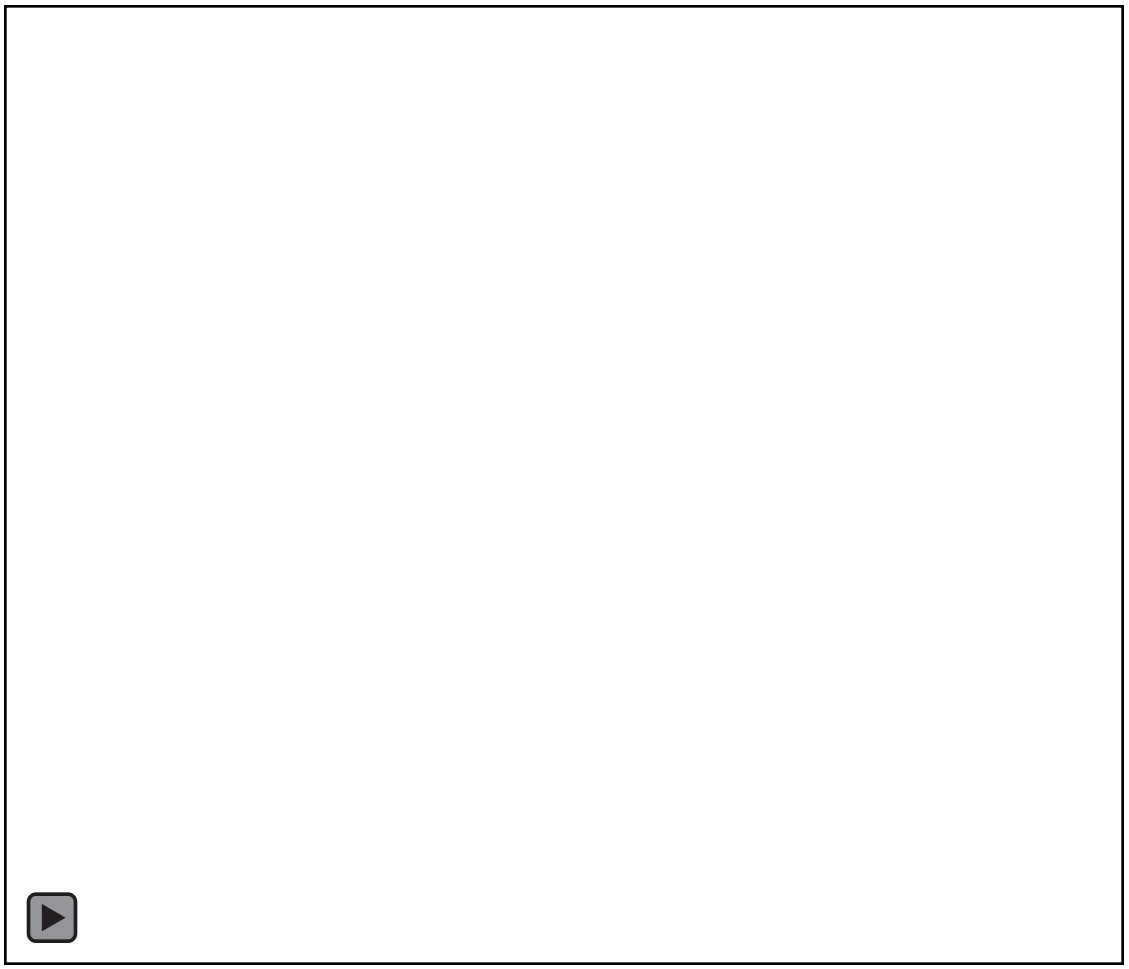
Liver Sinusoid Model: Blood Flow and Molecule Diffusion and Transfer

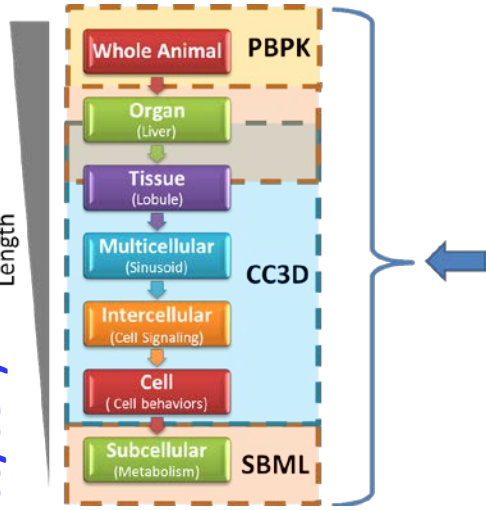
Cell field

APAP "Concentration"



Time scale: 4,000 MC = 1 simulated second

Load Field (APAP mass/cell)



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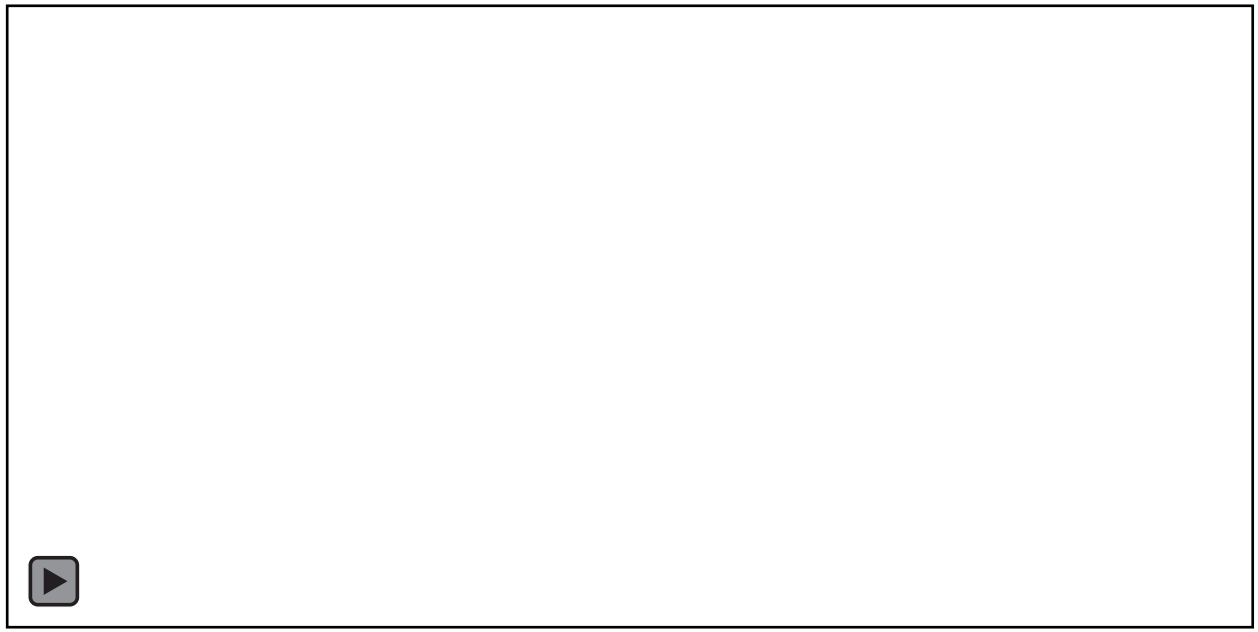
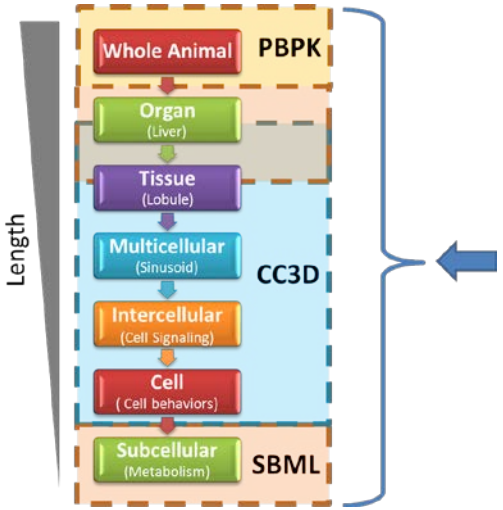
Virtual Tissue Simulation of APAP Metabolism, Clearance and Toxicity

Cell field

APAP Concentration

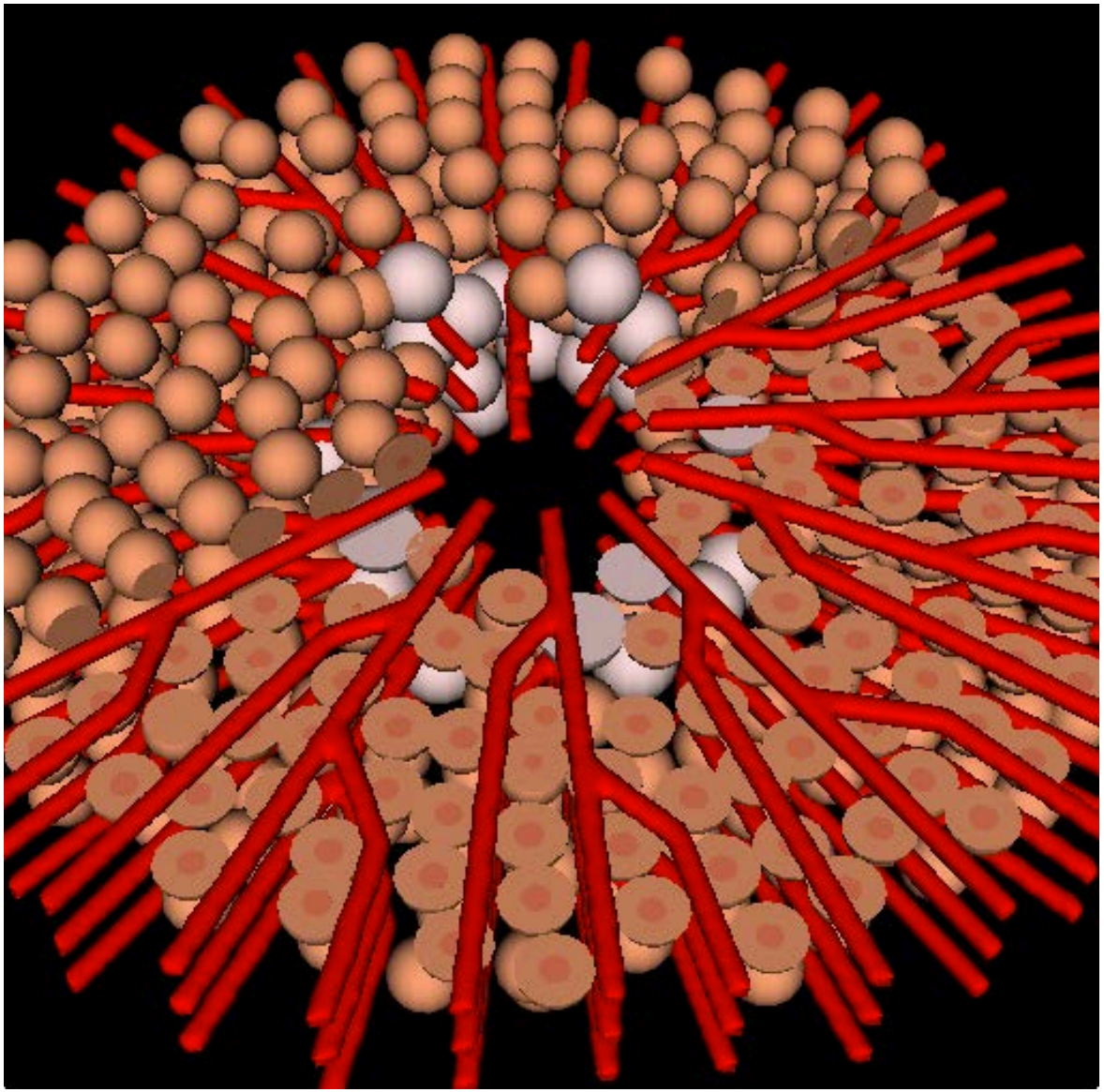




Time scale: 4,000 MC = 1 simulated second

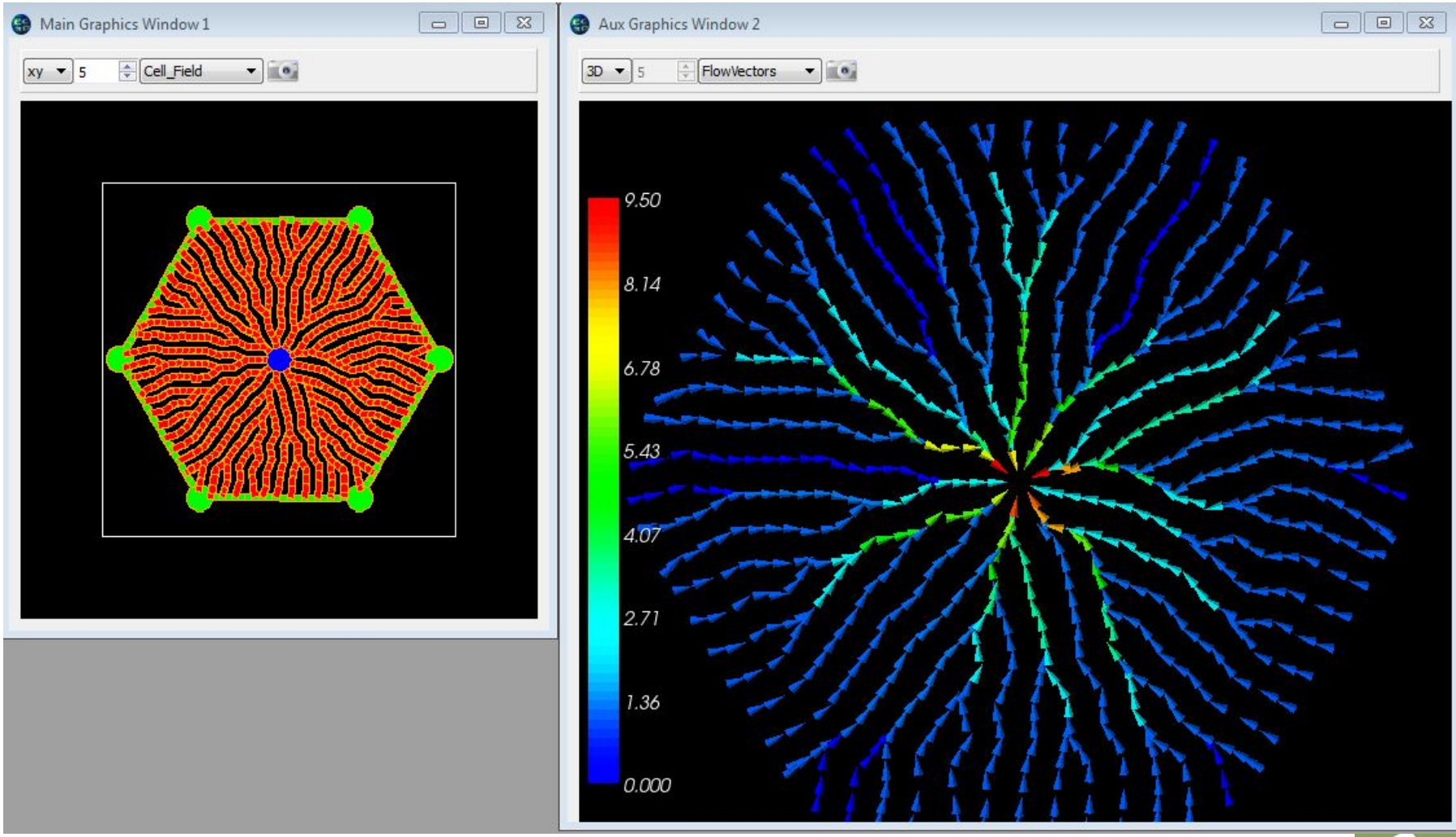
Sluka

Alternative Visualization: Model Cutaway



Sluka, Heiland

3D CC3D Model of Flow in a Section of a Lobule



Sample Current Applications

- Angiogenesis and Vasculogenesis (EPA, CWI)
- Vascular Tumor Growth
- Cancer Evolution
- Age Related Macular Degeneration
- Liver Toxicology (IUB, EPA)
- **Polycystic Kidney Disease (IUB, IUPUI)**
- Segmentation (IUB, UCL)

Polycystic Kidney Disease

ADPKD - Autosomal dominant polycystic kidney disease:

- Adult onset progressive loss of renal function due to accumulation of cysts.
- Most common genetic cause of end-stage renal disease.
- ADPKD accounts for 2 billion tax dollars per year.

Associated mutations are known

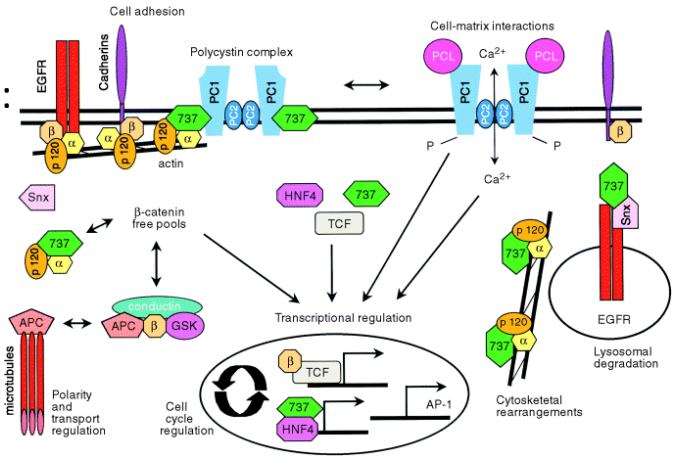
- Polycystin-1 mutations: responsible for 80% of ADPKD.
- Polycystin-2 mutations: responsible for 15% of ADPKD.

Link between mutations and presentation of disease unknown:

Key Questions:

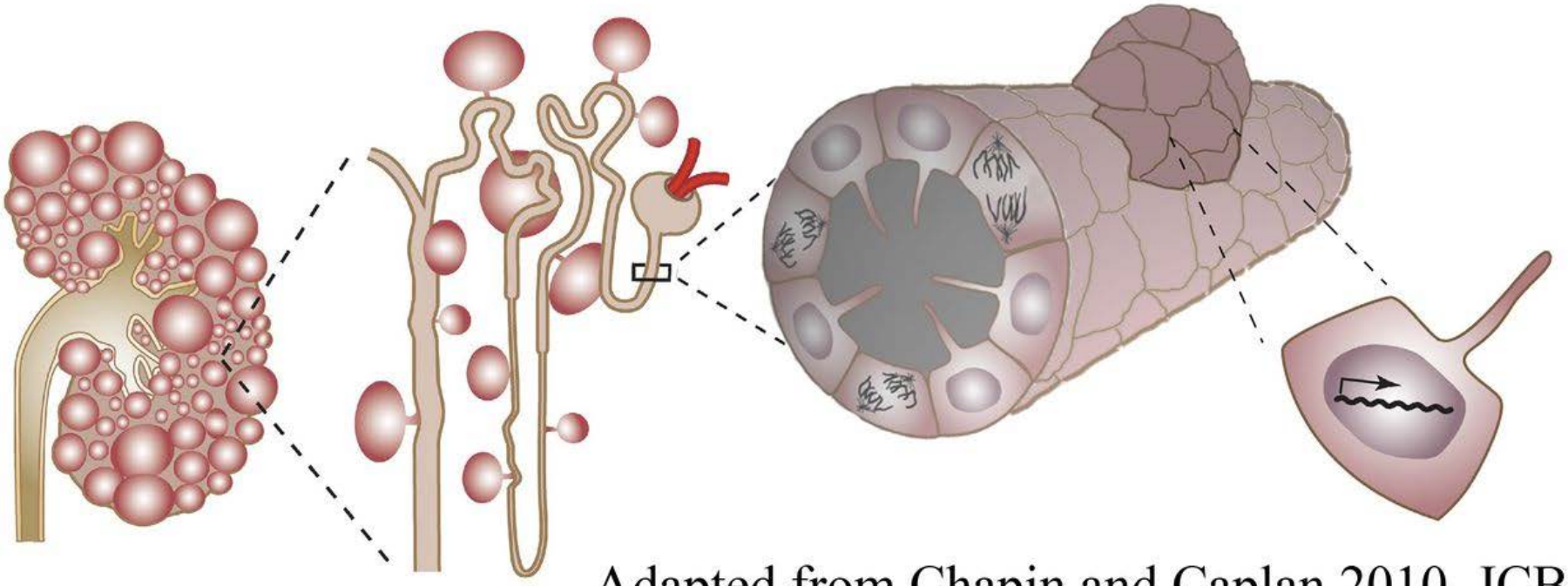
- **How do PC1 and PC2 mutations result in epithelial dis-regulation in kidney tubules?**
- **What is the mechanism of cyst formation from epithelial tubules?**

No therapeutic options exist to ameliorate the disease course.



Kidney International (1999) **55**, 1187–1197

Model of Polycystic Kidney Disease



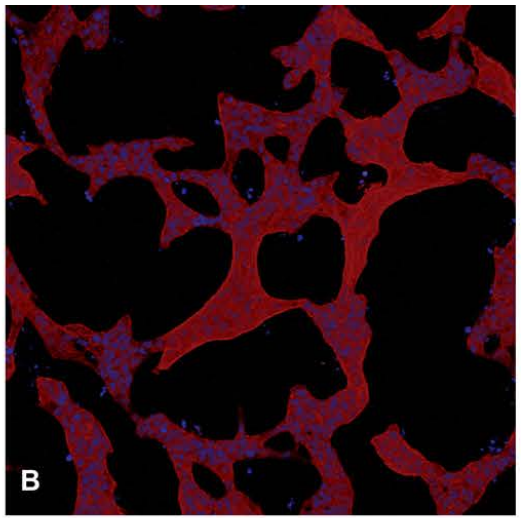
Adapted from Chapin and Caplan 2010. JCB

- 85% of cases caused by Polycystin-1 mutation
- Cad-8 present during development only
- Cad-N present in normal renal cells
- Cad-8 injection (1 cell) is sufficient to induce PKD

Bacallao

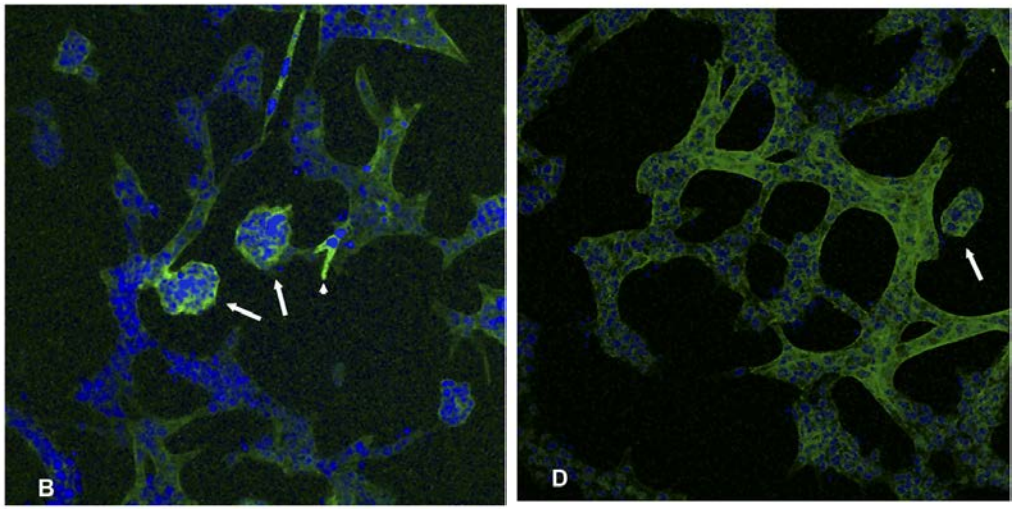
In Vitro Ectopic Cad-8 Induces Cysts

Normal Tubules form in 3D collagen matrix culture of HK-2 cells



HK-2 cells plated at 100,000 cells/cm^2 after 14 d. Composite extended focus fluorescent image set with phalloidin staining of F-actin (red) and Hoescht, blue staining of nuclei. Field of view ~ 1 mm.

Cysts form under the same conditions when HK-2 cells are transfected with Cadherin-8



HK-2 cells 48 h after adenovirus microinjection with cadherin 8 and tet trans-activating adenovirus. Fluorescence (green) staining for cadherin 8 (left) and N-cadherin (right) with Hoescht 33342 (blue) staining of nuclei. Cystic outgrowths (arrows) and a small stalk (arrowhead) express cadherin 8. Tubules and cysts both express N-cadherin (arrow). Field of view ~ 1 mm.

Bacallao

Developing PCKD Cadherin Hypotheses to Test

- What cell behaviors change with ectopic expression of cadherin-8?
- Cadherins play at least two roles—in cell-cell adhesion and contact inhibition:

  1) Change cell adhesion. Could:

     1.1) Increase adhesion strength

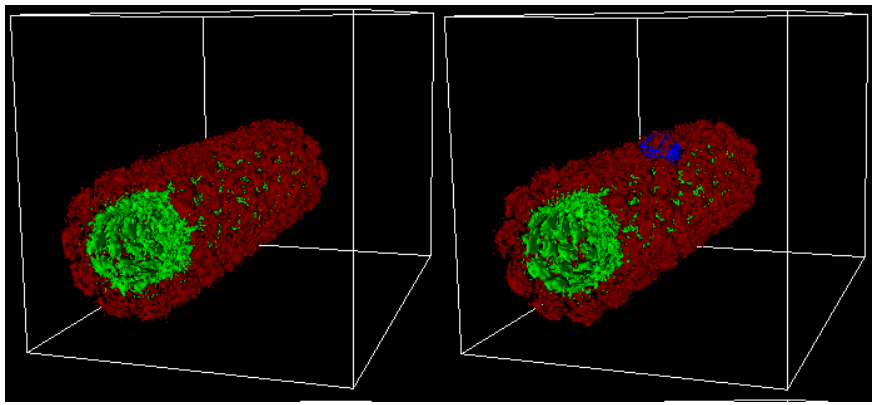
     1.2) Decrease adhesion strength

  2) Deregulate contact-inhibition proliferation:

     - Higher Threshold for Contact Inhibition of Proliferation

Cyst Model Mechanisms

- Epithelial Nephrocytes, Lumen, Surrounding Supporting Medium
- Contact-induced Cell Polarity and Adhesion
- Cell Growth and Proliferation with Contact Inhibition of Growth
- Secretion of ECM at apical surface of polarized cells
- Initial Condition:
  - Single Cell in Medium
  - Tubule with single transformed cell



Belmonte

Hypotheses to Test

1) Change cell adhesion:

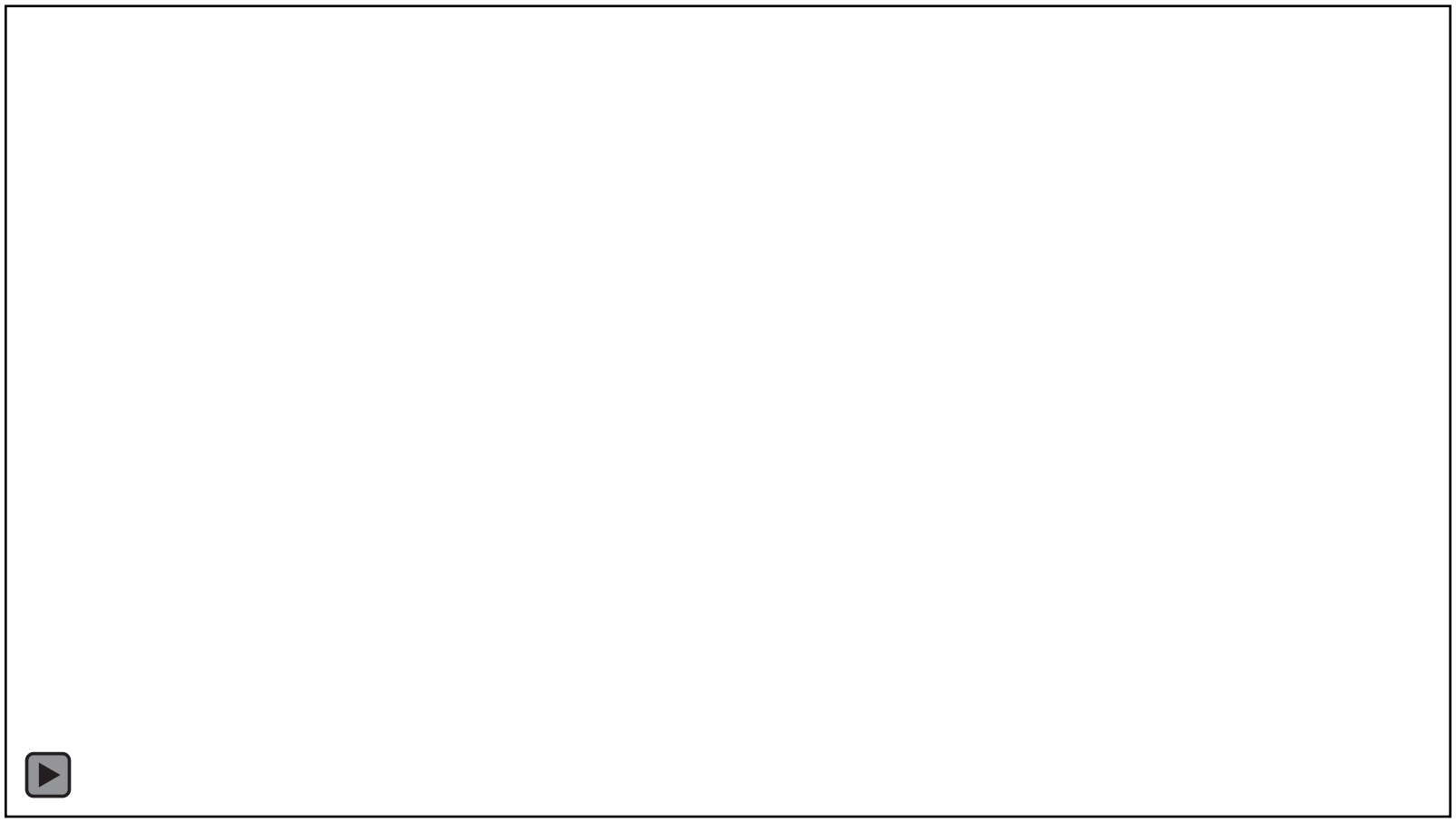
    1.1) Increase in adhesion strength

    1.2) Decrease in adhesion strength

2) Deregulate contact-inhibition:

    - Higher Threshold for Contact Inhibition of Proliferation

Ectopic Cadherin Increases Cell Adhesion



Increased Adhesion does not disrupt tubule

Hypotheses to Test

1) Change cell adhesion:

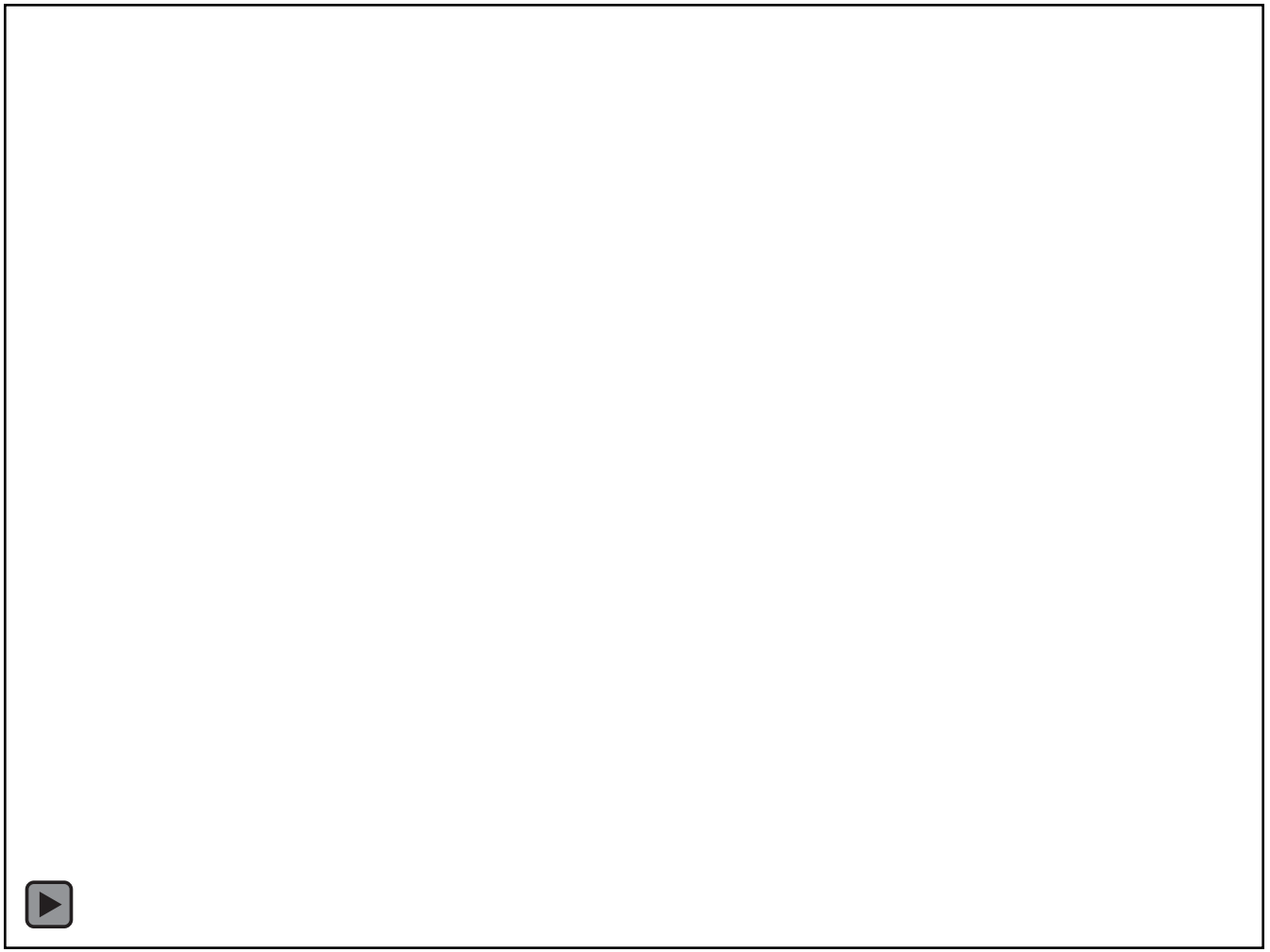
   ~~1.1) Increase adhesion strength~~

   1.2) Decrease adhesion strength

2) Deregulate contact-inhibition:

   - Increase proliferation rate

Ectopic Cadherin Decreases Cell Adhesion



Decreased Adhesion Produces Cysts

Hypotheses to Test

1) Change cell adhesion:

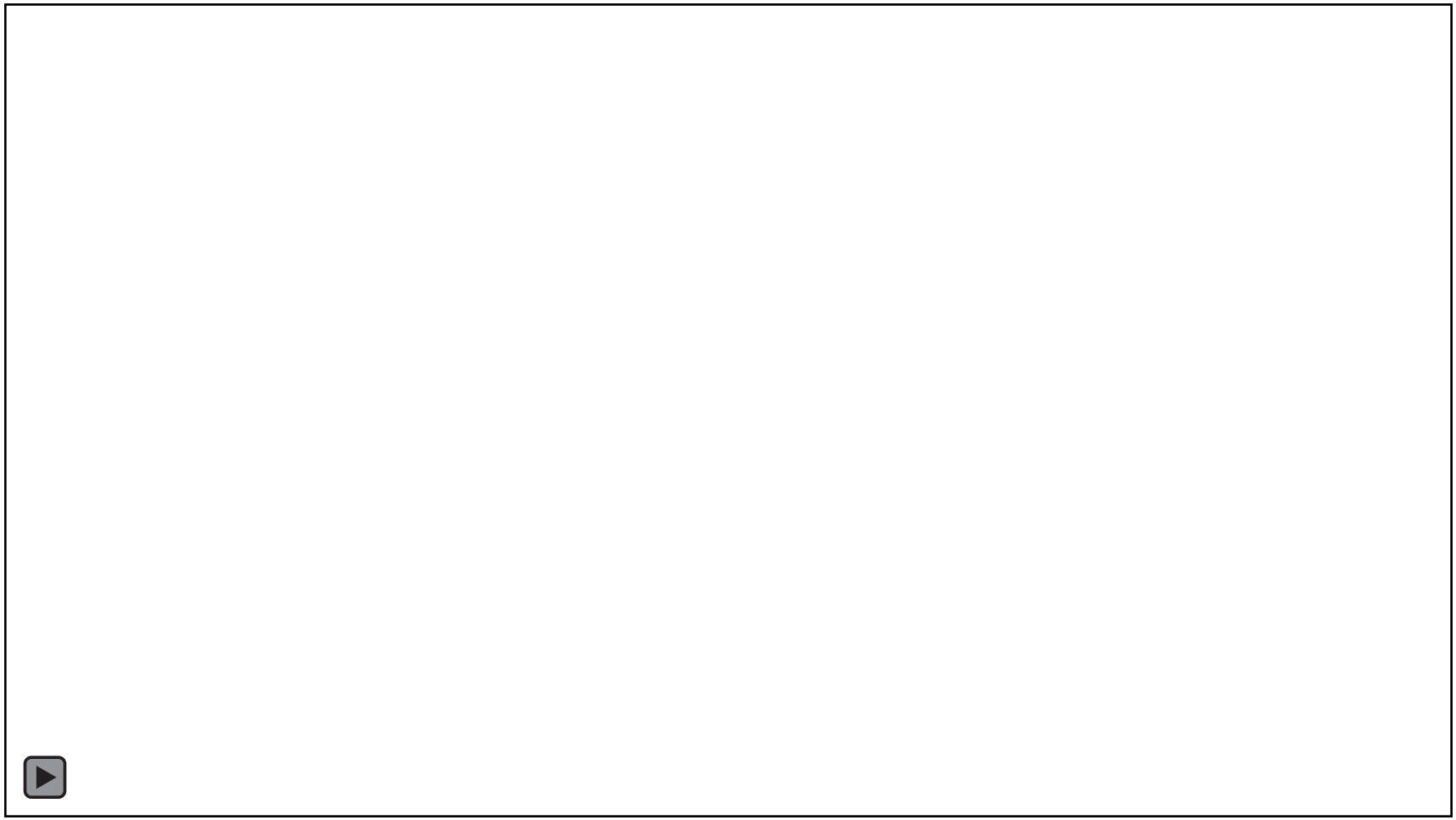
~~1.1) Increase adhesion strength~~

1.2) Decrease adhesion strength

2) Deregulate contact-inhibition:

Higher Threshold for Contact Inhibition of Proliferation

Ectopic Cadherin Increases Threshold for Contact Inhibition of Proliferation



Reduced Contact Inhibition Produces Cysts

Belmonte

Hypotheses to Test

1) Change cell adhesion:

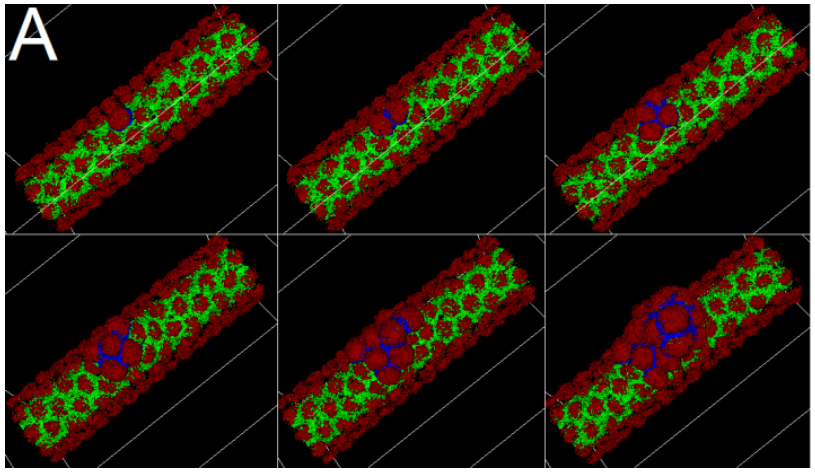
~~1.1) Increase adhesion strength~~

1.2) Decrease adhesion strength

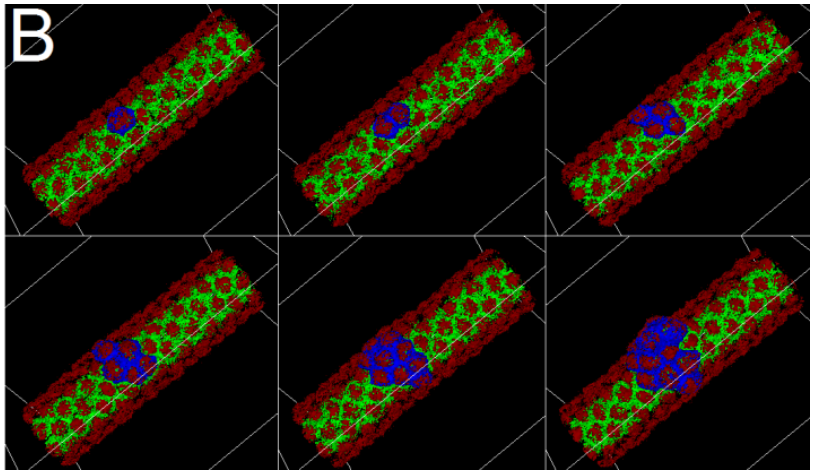
2) Deregulate contact-inhibition:

Higher Threshold for Contact Inhibition of Proliferation

Differences between Reduced Adhesion and Reduced Contact Inhibition

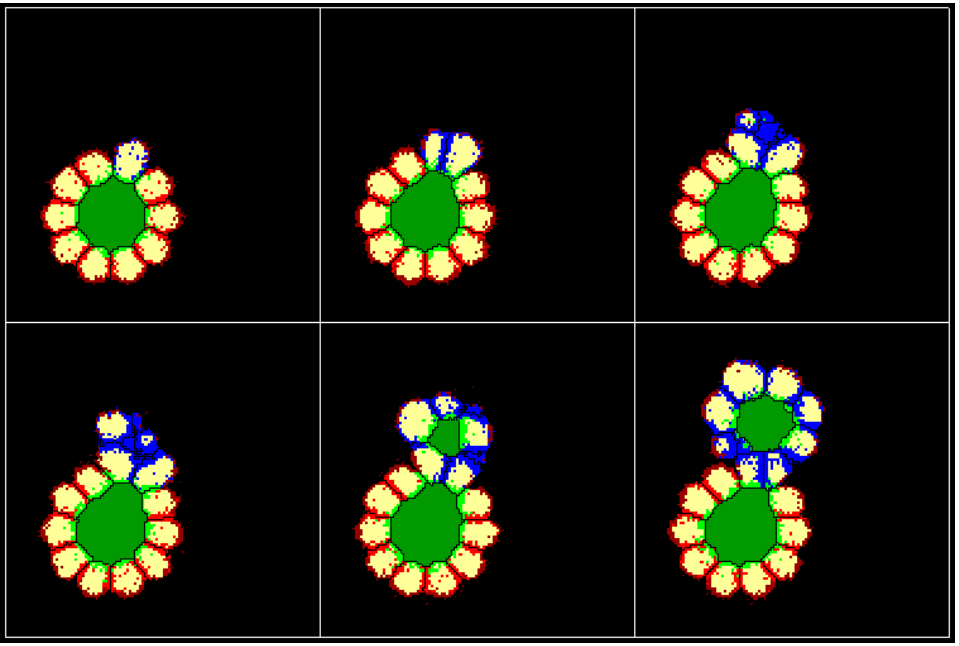


(A)Reduced cell adhesion leads to proliferation of perturbed cells outside the renal tubule



(B) Disruption of contact-inhibition leads to lateral spread of cells before cyst formation

Differences between Reduced Adhesion and Reduced Contact Inhibition

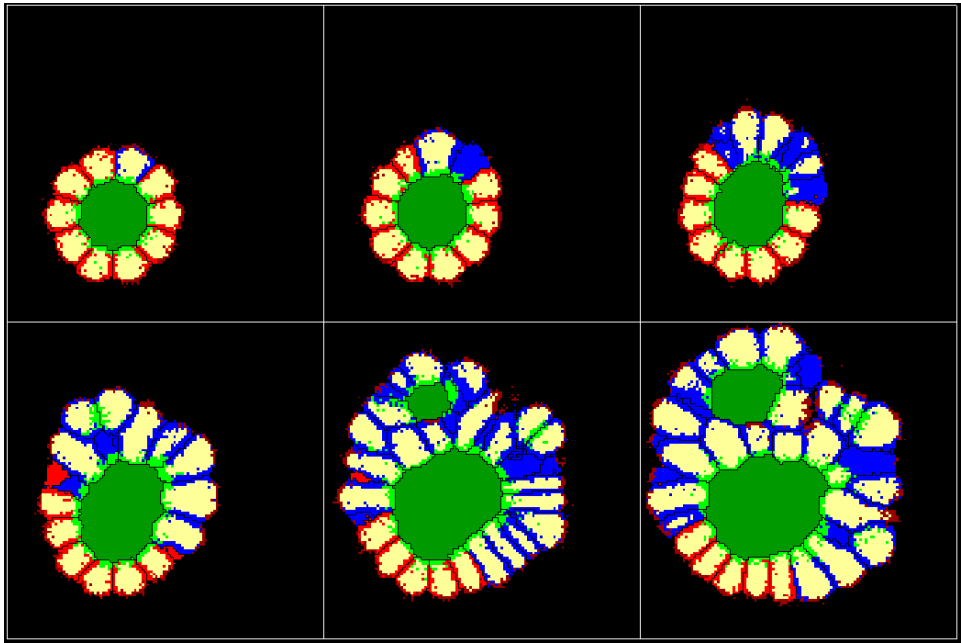


←Reduced cell adhesion

Cad-8 cells "pop" out and cyst forms outside tube

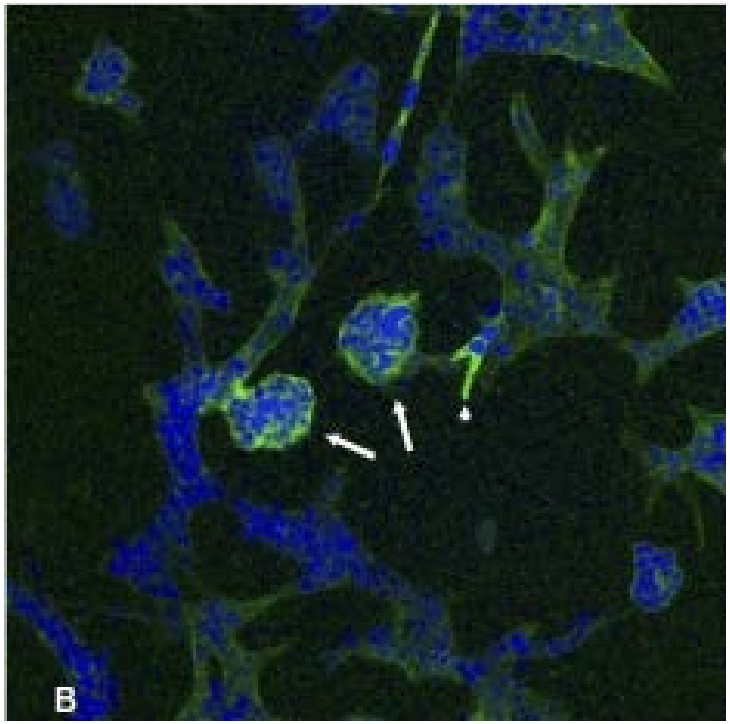
Impaired Contact Inhibition→

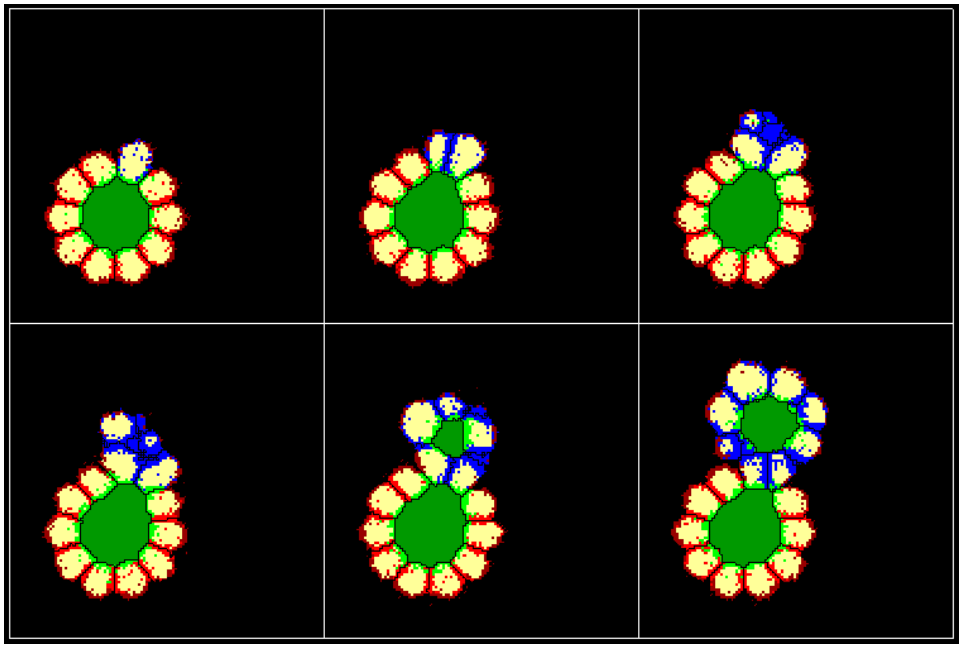
Cad-8 cells remain on the tube and cyst buckles from tube



Belmonte

Experiment vs. Simulation





- *Immune fluorescence images of cysts (arrows) stained for cadherin- 8 (green)*
- The predicted dynamics and shape of cysts from reduced adhesion simulations match the experiment better than for impaired contact inhibition

Belmonte

Hypotheses to Test

1) Change cell adhesion:

~~1.1) Increase adhesion strength~~

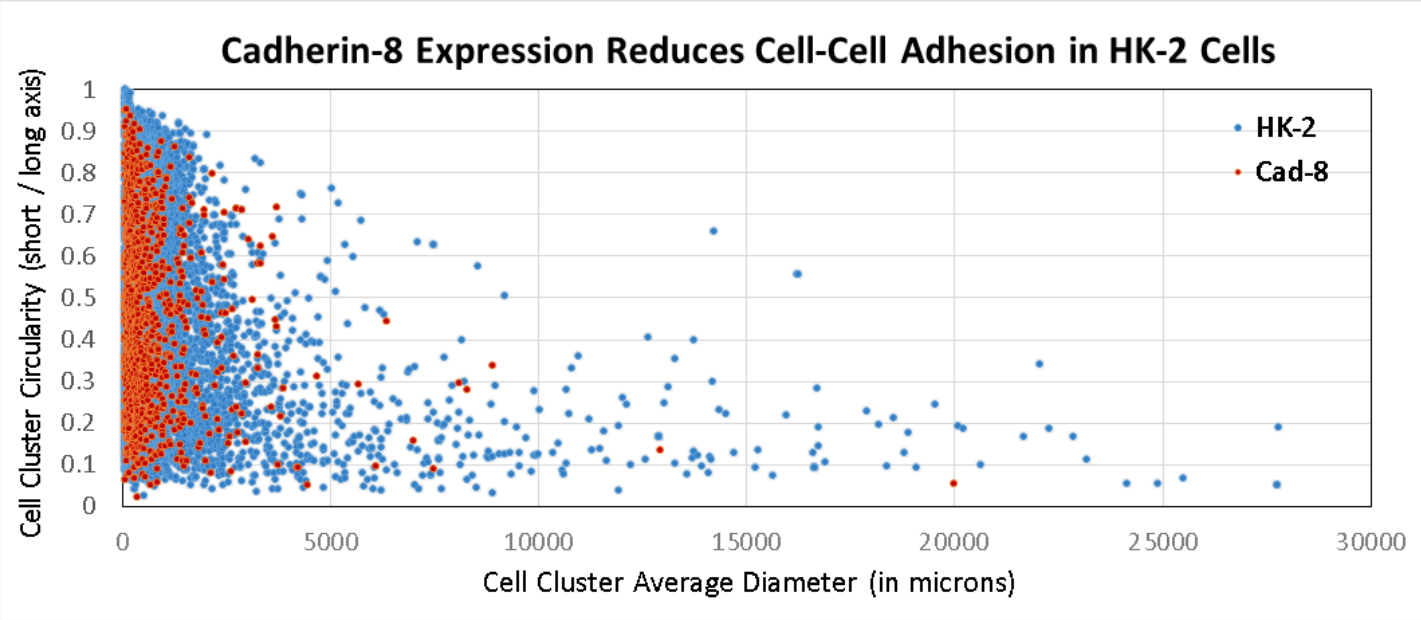
1.2) Decrease adhesion strength

2) Deregulate contact-inhibition:

~~Higher Threshold for Contact Inhibition of Proliferation~~

Experimental Validation of Adhesion Reduction Hypothesis

Hanging Drop Culture of normal and Cad-8 transformed HK-2 Cells

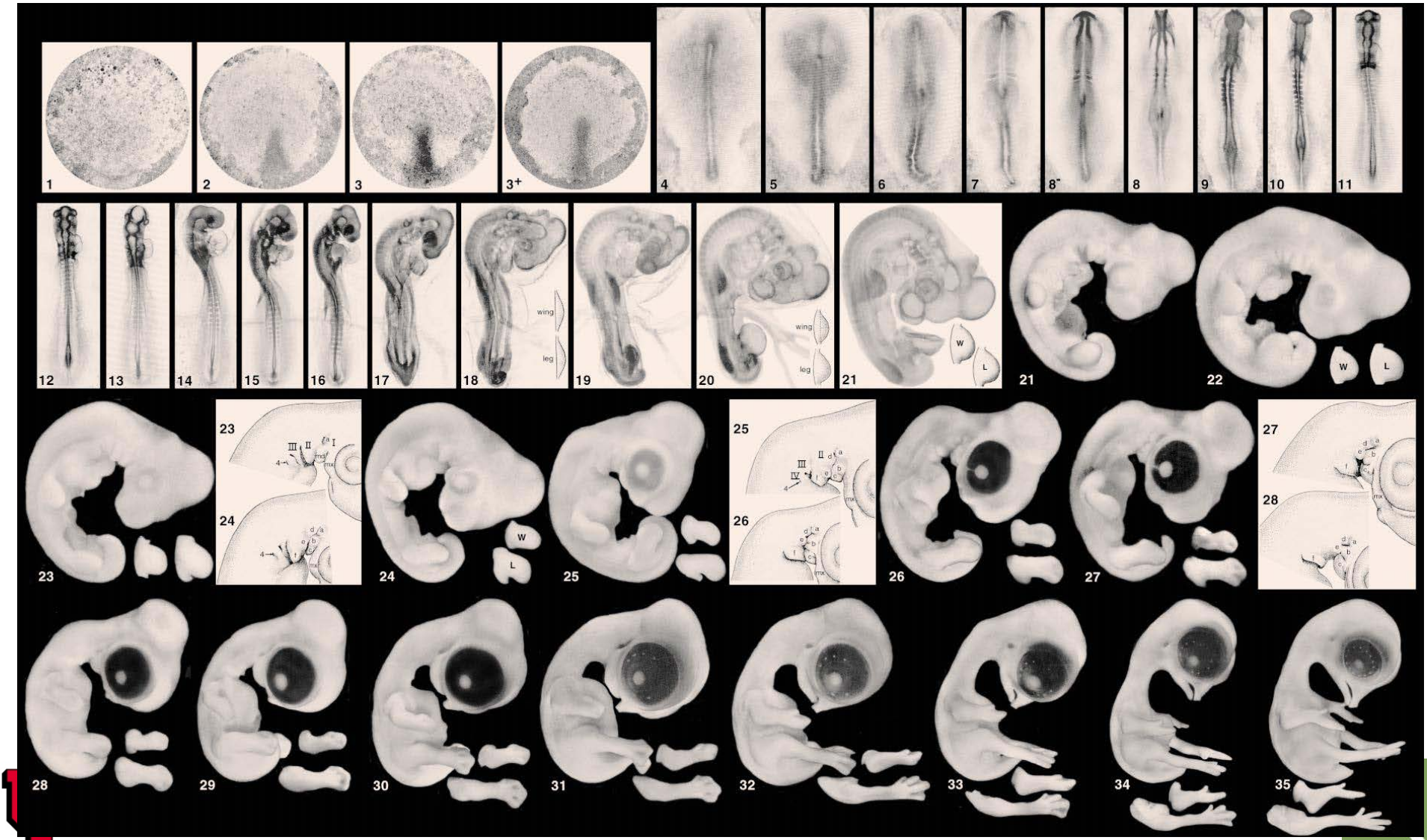


Bacallao, Clendenon

Sample Current Applications

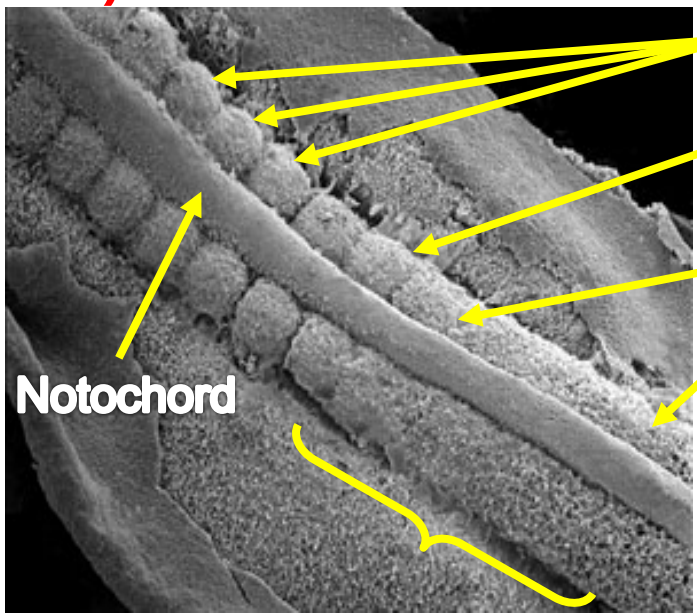
- Angiogenesis and Vasculogenesis (EPA, CWI)
- Vascular Tumor Growth
- Cancer Evolution
- Age Related Macular Degeneration
- Liver Toxicology (IUB, EPA)
- Polycystic Kidney Disease (IUB, IUPUI)
- **Segmentation (IUB, UCL)**

Somitogenesis: Two Cautionary Stories with the Same Ending



Somitogenesis

Anterior (head)



Somites

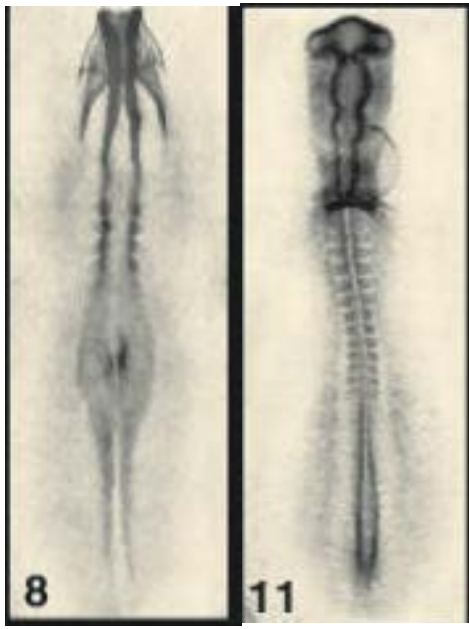
Forming somite

Older cells more anterior

Younger cells more posterior

Posterior (tail)

Presomitic mesoderm (PSM)



HH staged chick embryos, fixed (Wiley)

Regulation (Intra-cell), Inter-cell communication (Feedback), Cell Behaviors, build tissue structure

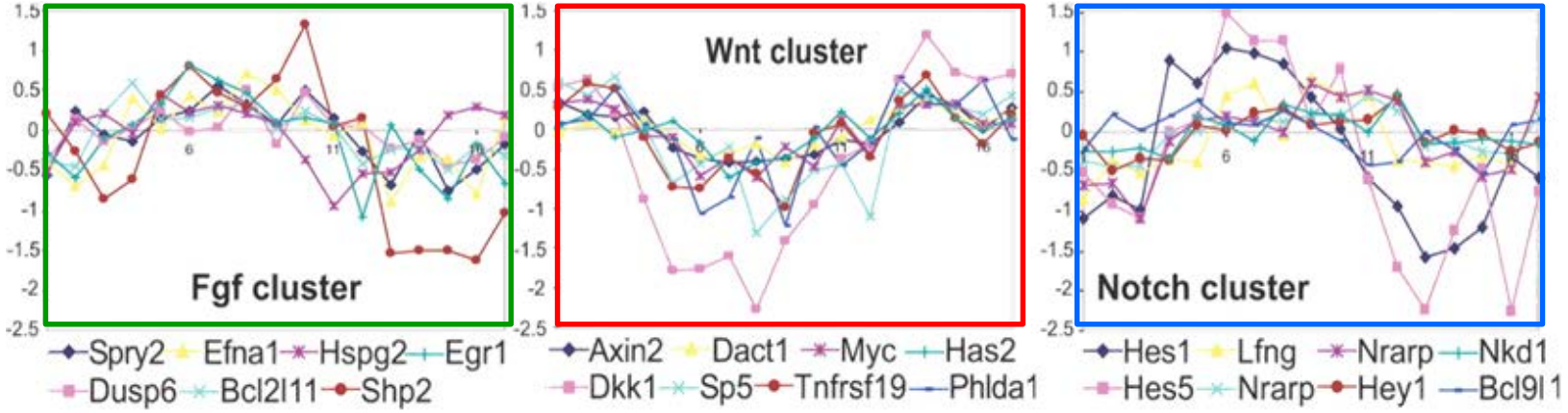
Key Questions

How do molecular signals integrate with changes in cell behavior to create repeated segmental pattern?

Why is segmentation robust to molecular and environmental perturbations?

Somites with a Clock

Key Regulatory Genes Oscillate in three Clusters



Dequeant *et al.* 2006 (microarray time series of mRNA in mouse)

A→P gradient of Retinoic Acid (differentiation inducer)
P→A gradient of FGF8, Wnt3a and BMP4 (differentiation inhibitors)

Tissue elongates and somites differentiate sequentially one at a time A→P

Different phases of the three clusters correlate with graded expression of adhesion and repulsion molecules

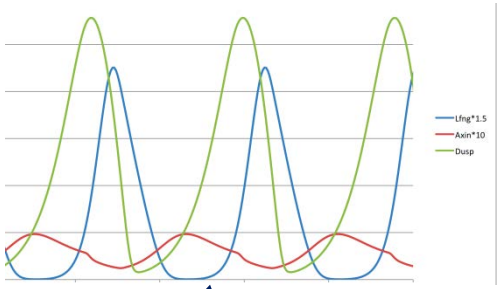
Cells in forming somites epithelialize

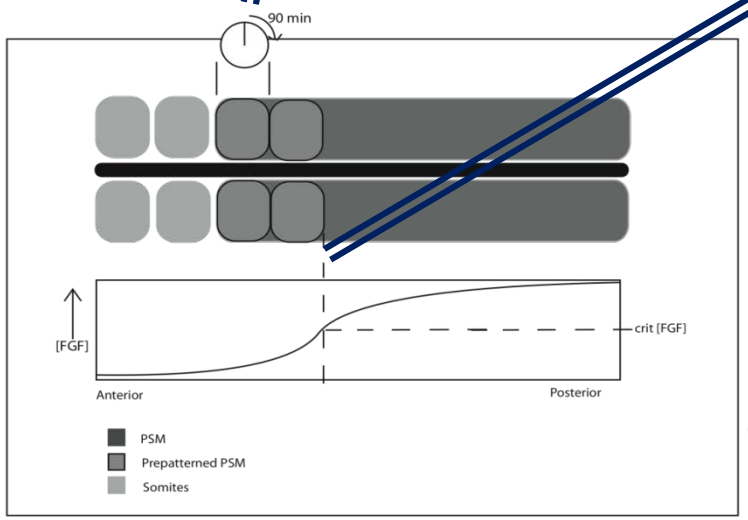
Glazier JA, Zhang Y, Swat M, Zaitlen B, Schnell S. (2008) **Coordinated action of N-CAM, N-cadherin, EphA4, and ephrinB2 translates genetic prepatterns into structure during somitogenesis in chick.** *Curr Top Dev Biol.* **81:** 205-247.

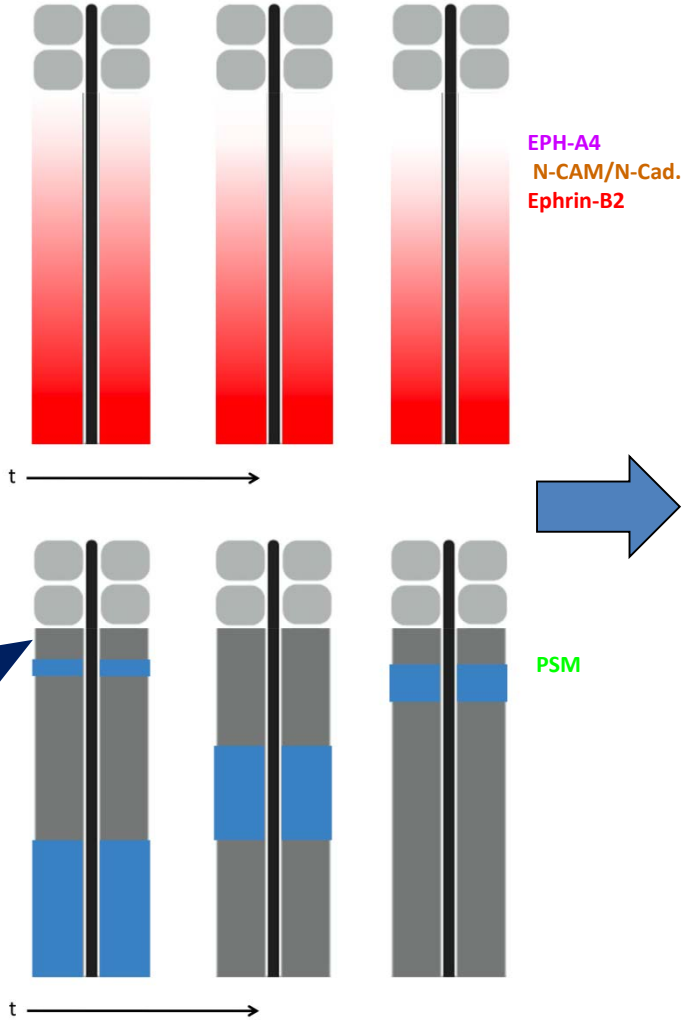
Hester SD, Belmonte JM, Gens JS, Clendenon SG, Glazier JA. (2011) A Multi-cell, Multi-scale Model of Vertebrate Segmentation and Somite Formation. *PLoS Computational Biology* DOI: 10.1371/journal.pcbi.1002155

Clock and Wavefront

- Graded expression along AP axis, FGF8, Wnt3a, retinoic acid
- Stripes of gene expression move P→A in tissue
- Oscillating gene expression - *segmentation clock*
- To translate into structure, **assume key is observed regulation of adhesion molecules**
- **Neglect Epithelialization and ECM to begin with**

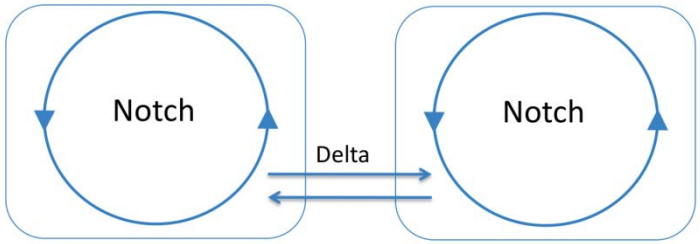




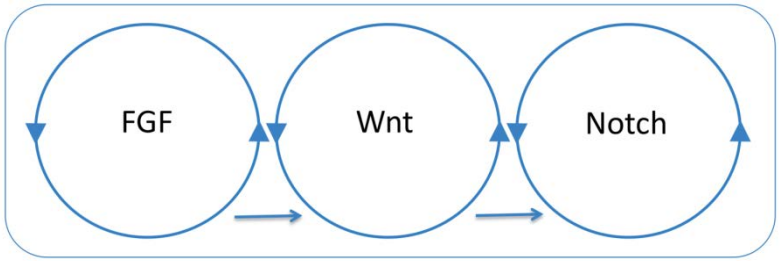


Cooke and Zeeman, 1976; Baker and Schnell, 2006; Aulehla et al., 2008

Not Easy to Put it All Together…



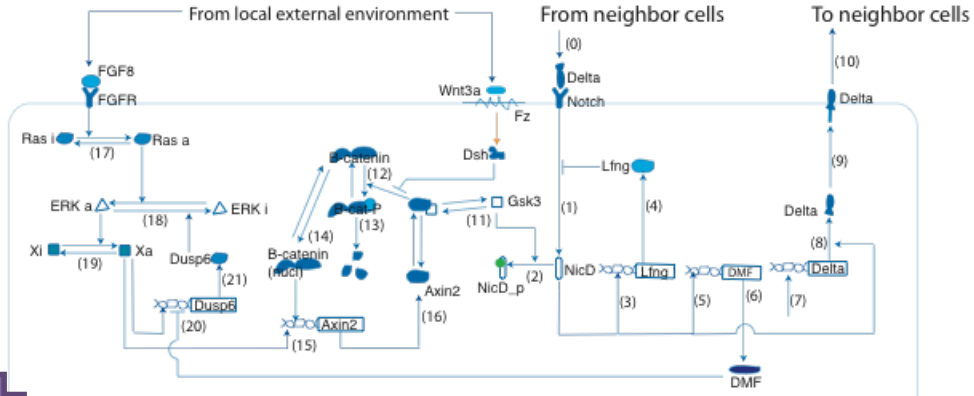
Lewis (2000, 2003)



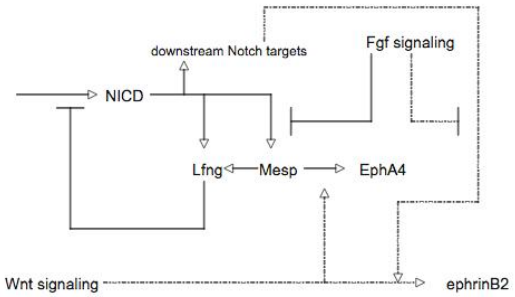
Goldbeter and Pourquié (2008)

Intercell Communication (Feedback)

Intracell Communication (Regulation)

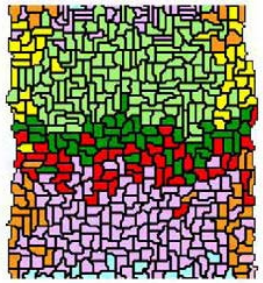


Hester-Belmonte-Glazier Segmentation Clock (2010)



Hester-Belmonte-Glazier Readout Network (2010)

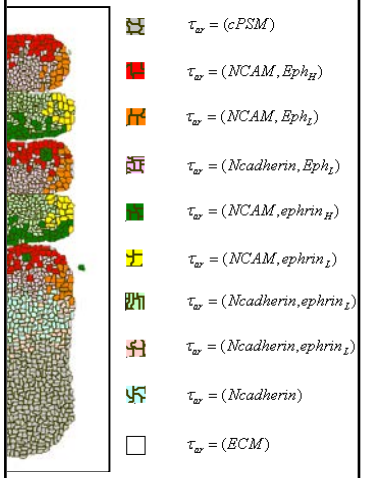
Readout



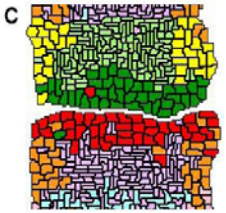
Cell Differentiation, Differential Adhesion and Motility

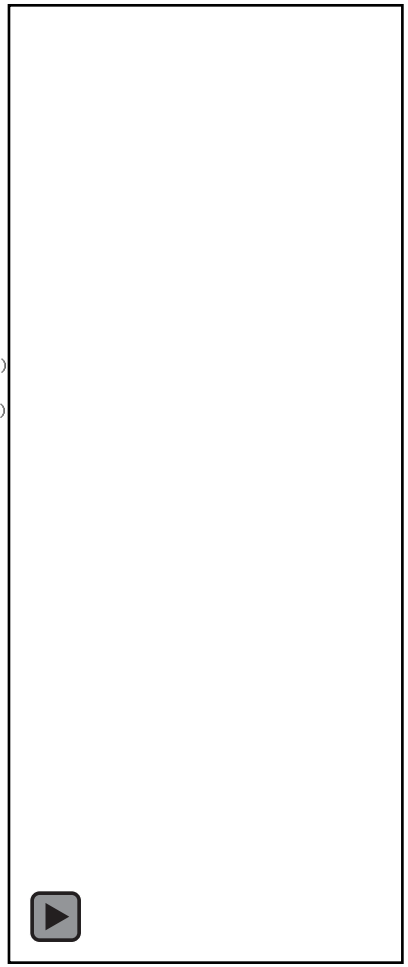
Clock and Wavefront Controlled Adhesion Works

reement



reement





Cell Type

FGF8

Lnfg

(Species Variation Works Too)

Robustness to Random Initial Phase of Oscillators in Tailbud

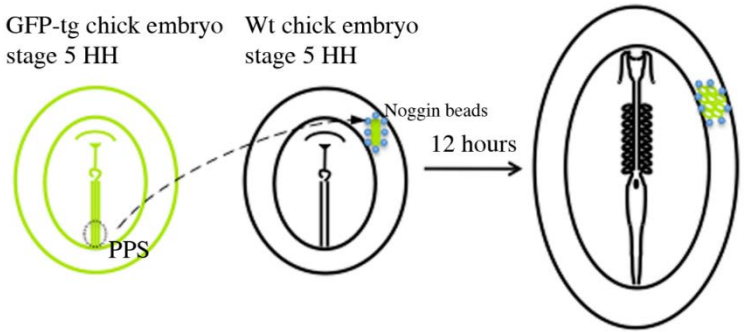
Hester SD, Belmonte JM, Gens JS, Clendenon SG, Glazier JA. A Multi-cell, Multi-scale Model of Vertebrate Segmentation and Somite Formation. **PLoS Computational Biology** (2011) DOI: 10.1371/journal.pcbi.1002155

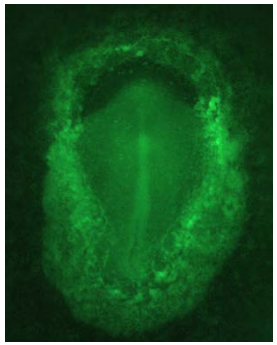
So We Are Done…

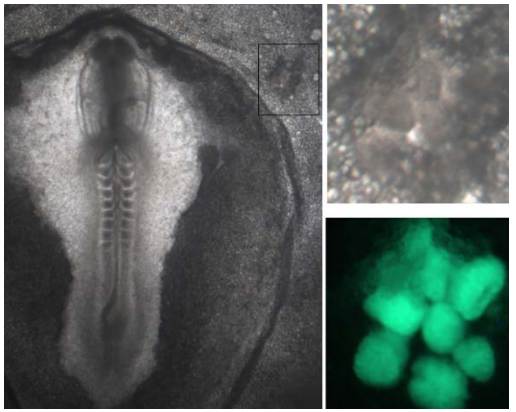
But…

Experiments Get in the Way…

Experiments by Dr. Claudio Stern (UCL) show that Noggin induces Somites to form in non-somite-forming Posterior Primitive streak tissue
These Extopic Somites function as somites normally when grafted







No clock:
Key somitic clock genes (Wnt, Notch/Delta and FGF, Hairy1, Hairy2 and LFng) **do not oscillate** before or during ectopic somite formation

9h

No wavefront:
Somites form simultaneously, not sequentially
Presomitic marker Dapper-1 not expressed

Dias AS, de Almeida I, Belmonte JM, Glazier JA, Stern CD (2014), Somites without a clock. *Science* **343**: 791-795.

Our adhesion mechanism can't work without a clock!
Maybe we were too quick to focus on adhesion variation and neglect polarization and ECM

Dias, de Almeida, Stern

Ectopic Somites Form without Clock or Wavefront

No clock:

Key somitic clock genes (Wnt, Notch/Delta and FGF, Hairy1, Hairy2 and LFng) **do not oscillate** before or during ectopic somite formation

No wavefront:

Somites form simultaneously, not sequentially

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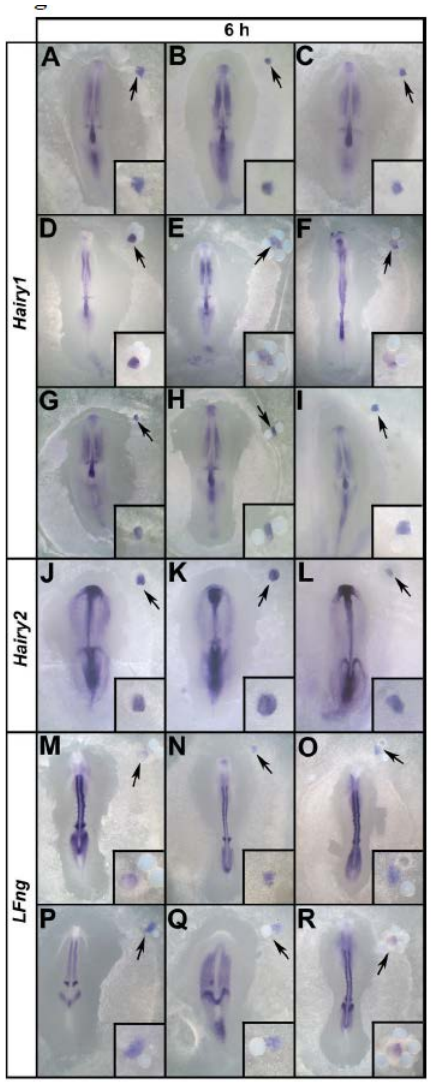
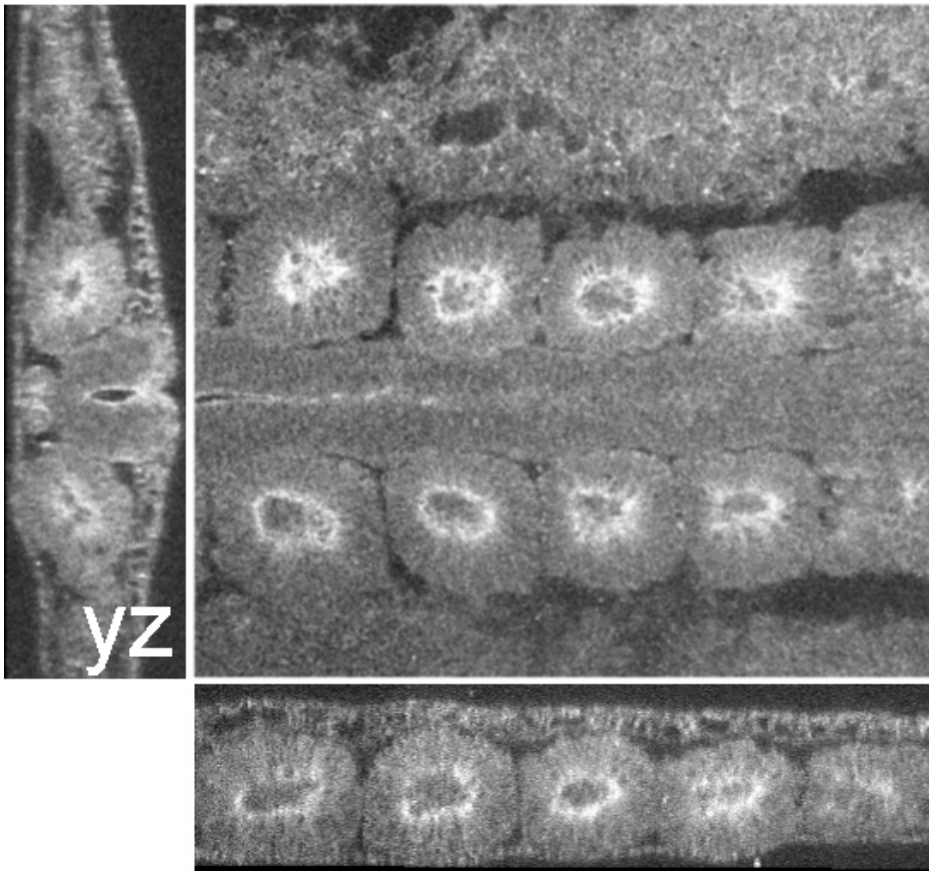
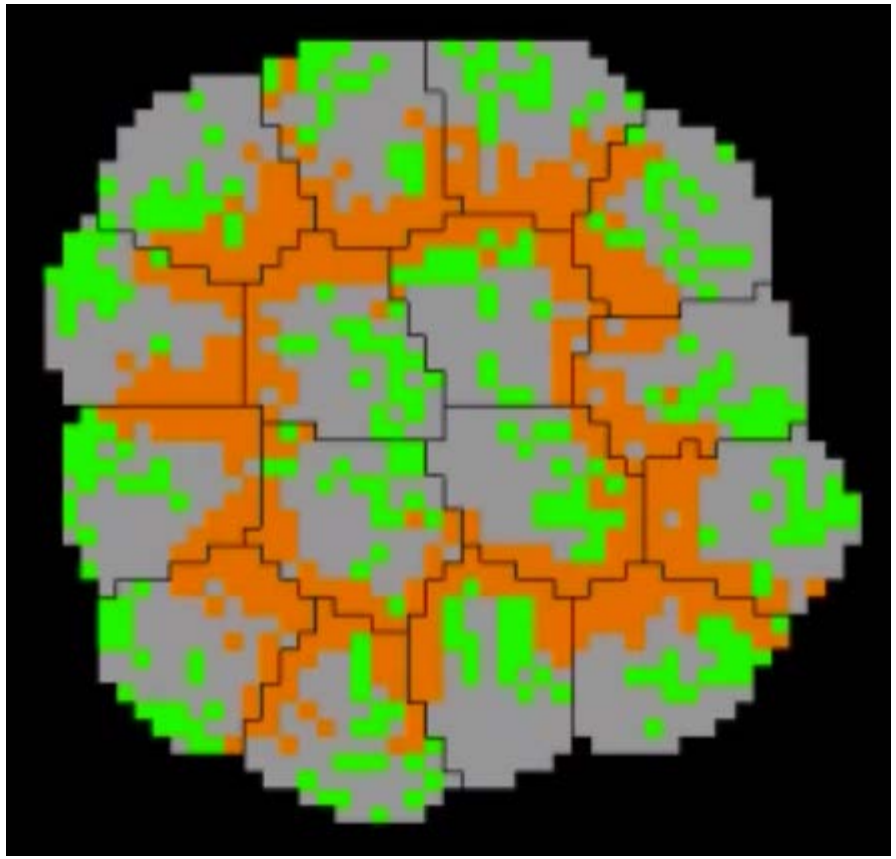


Neglected Cell Epithelialization in Our First Model

Model gradual localization of epithelial markers during normal and induced differentiation

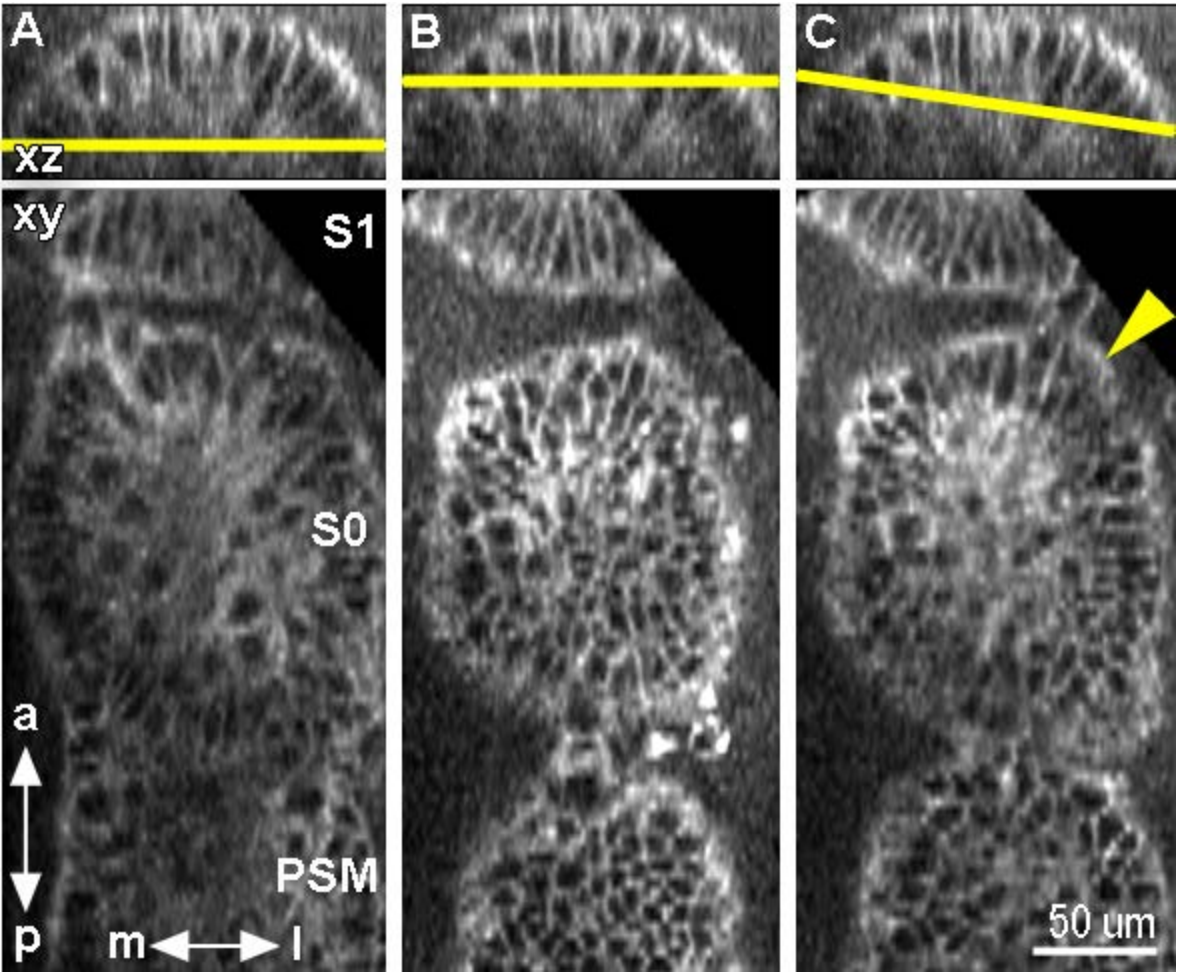


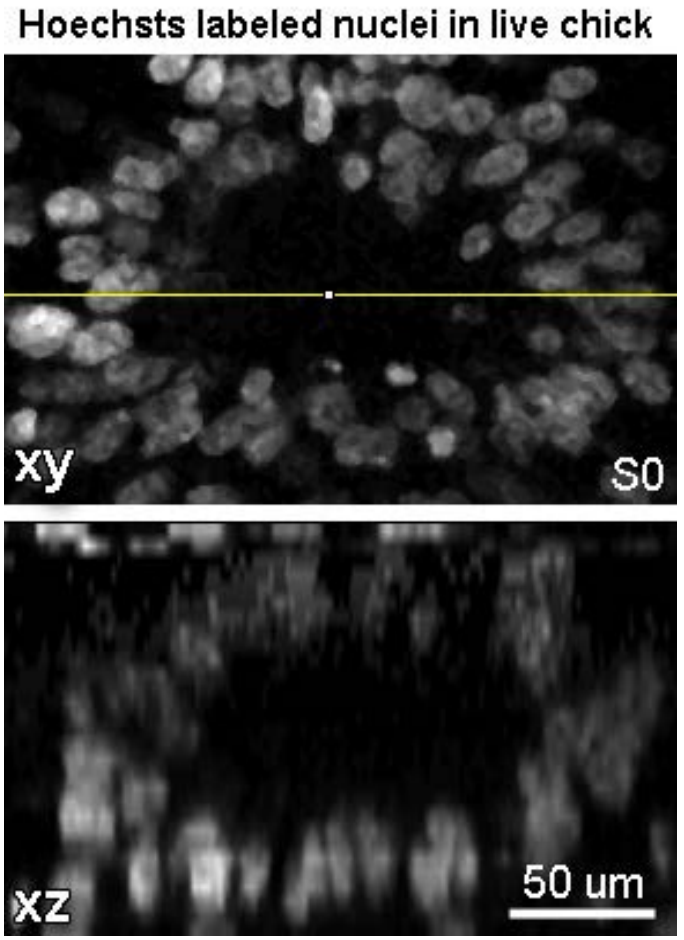


Belmonte, Clendenon

Neglected Cell ECM Secretion in Our First Model

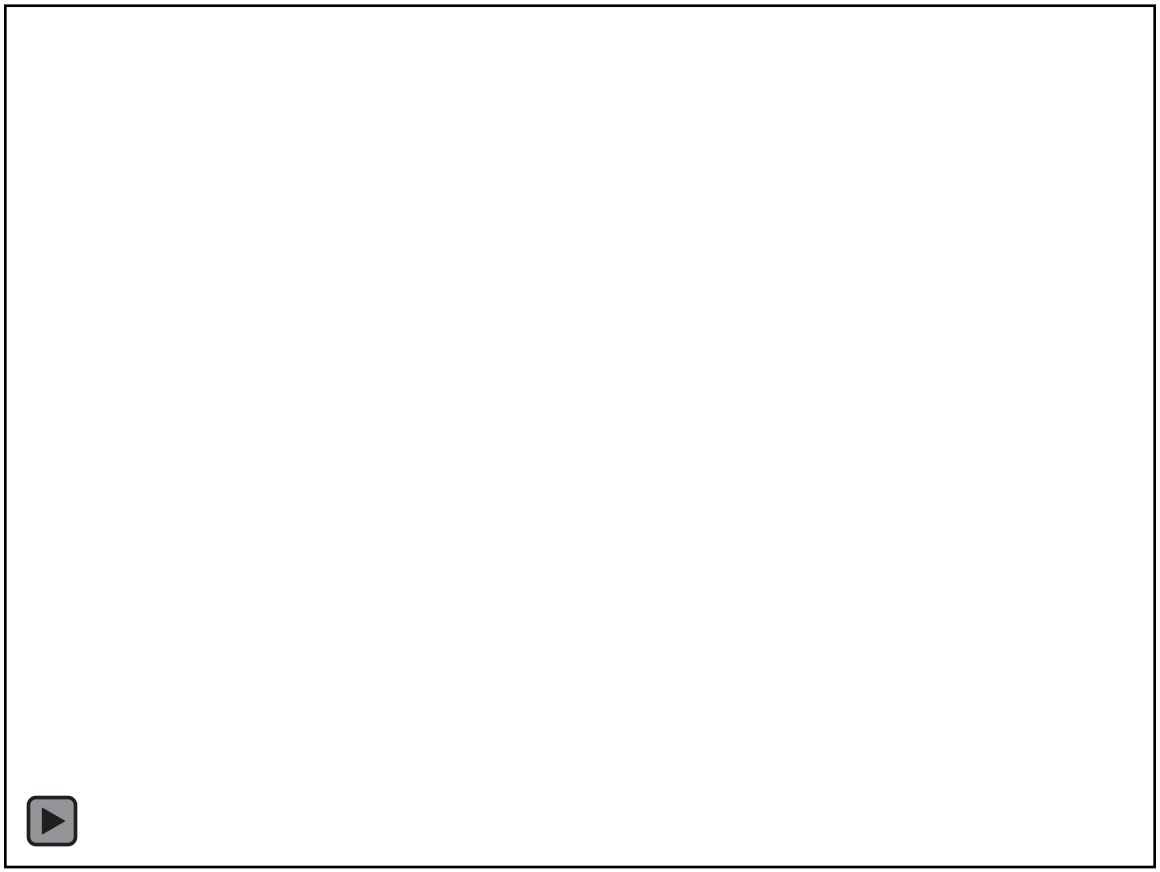
Epithelial cells secrete ECM from apical surfaces





Clendenon

Forming Somites without a Clock via Epithelialization Mechanism

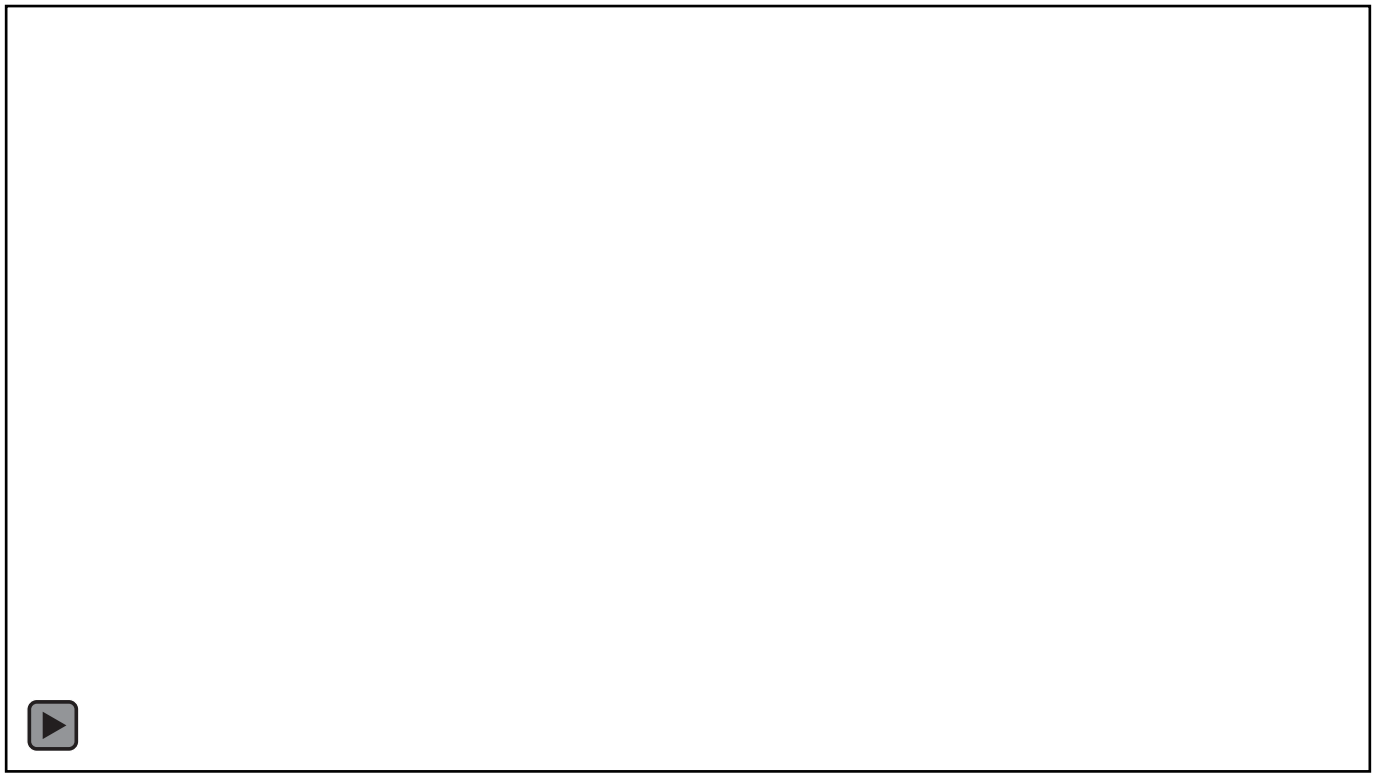


Simultaneous Epithelialization in an extended tissue sufficient to produce uniform sized, regular somites

What Might the Clock and Wavefront Do?

Ectopic Somites lack Anterior and Posterior subcompartments, which the clock mechanism can restore

Somitogenesis via Epithelialization with Wavefront but no Clock is Noisy



Dias AS, de Almeida I, Belmonte JM, Glazier JA, Stern CD (2014), Somites without a clock. *Science* **343**: 791-795.

Inattentional Blindness

Multiscale Virtual Tissue Simulations can connect heterogeneous molecular and cell-level data to predict significant tissue level outcomes

Natural framework for studying developmental processes and failures—angiogenesis disruption, gastrulation, limb growth, liver regrowth and disfunction, polycystic kidney disease…

Often assume that phenomena have **one** explanation.

Faced with a choice of explanations, often prefer the simpler/more elegant option

However, parallel mechanisms are common in biology

Parallel Mechanisms are easy to overlook, easier still when we aren't looking!

The Monster of Troy, 6th century BC (Corinthian, Greek)

www.pinterest.com

www.compucell3d.org