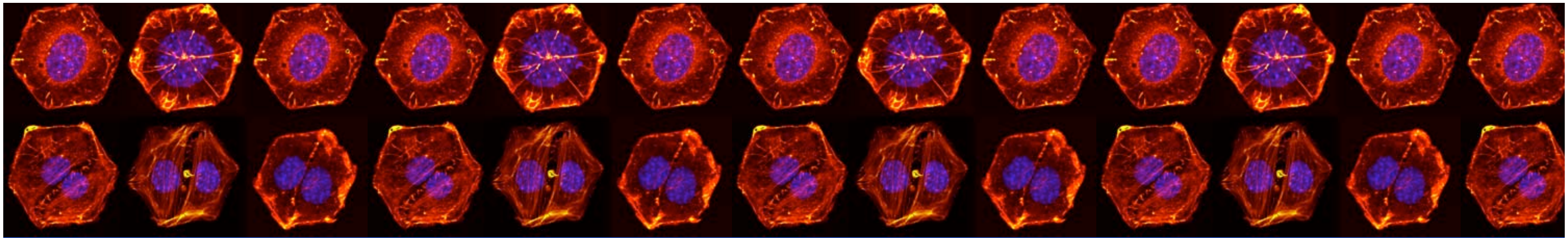
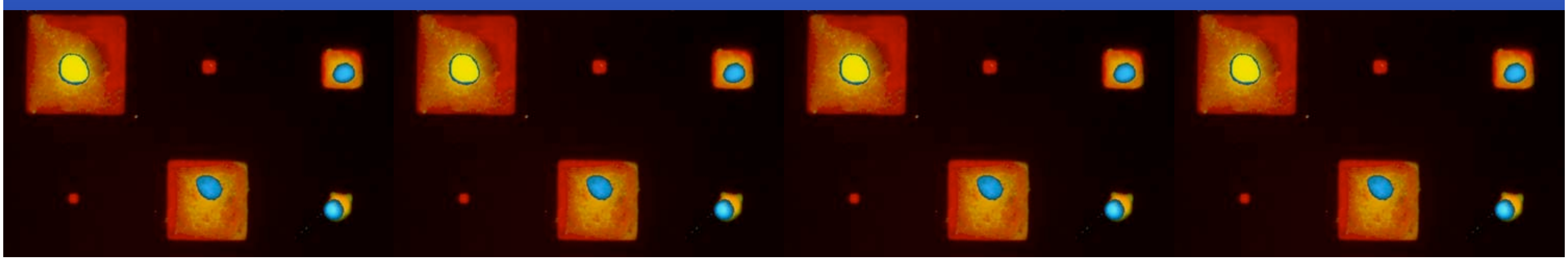


Tensegrity-Based Mechanical Control of Mammalian Cell Fate Switching and Morphogenesis

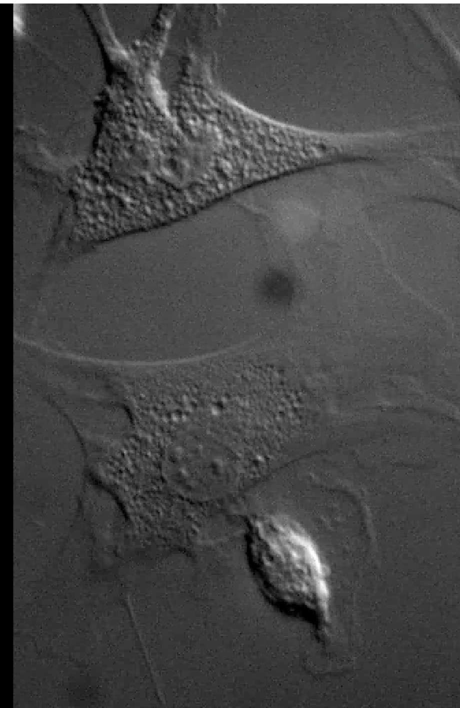
Don Ingber, M.D.,Ph.D.
Judah Folkman Professor of Vascular Biology
Depts. of Pathology & Surgery, Harvard Medical School

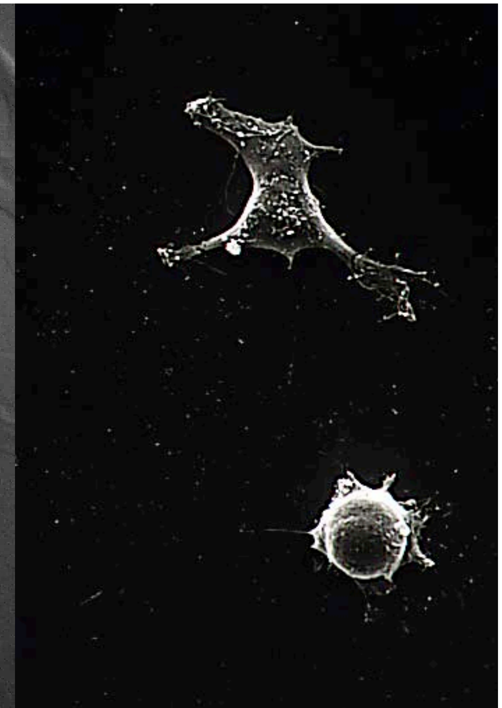
Interim Co-Director, Vascular Biology Program, Children's Hospital Boston

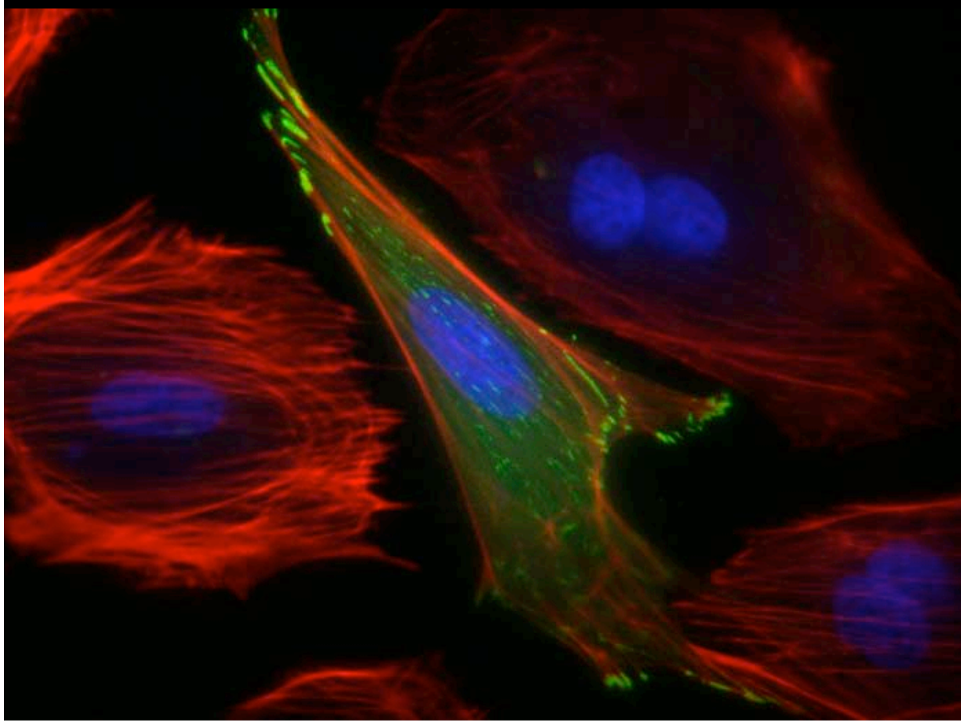
Interim Co-Director, Harvard Institute for Biologically Inspired Engineering



How are living cells and tissues constructed?



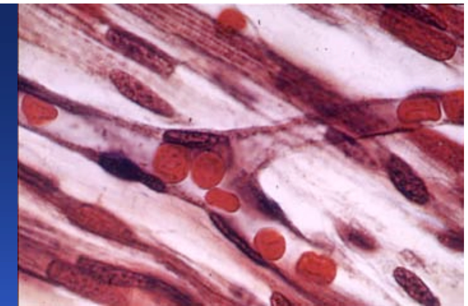


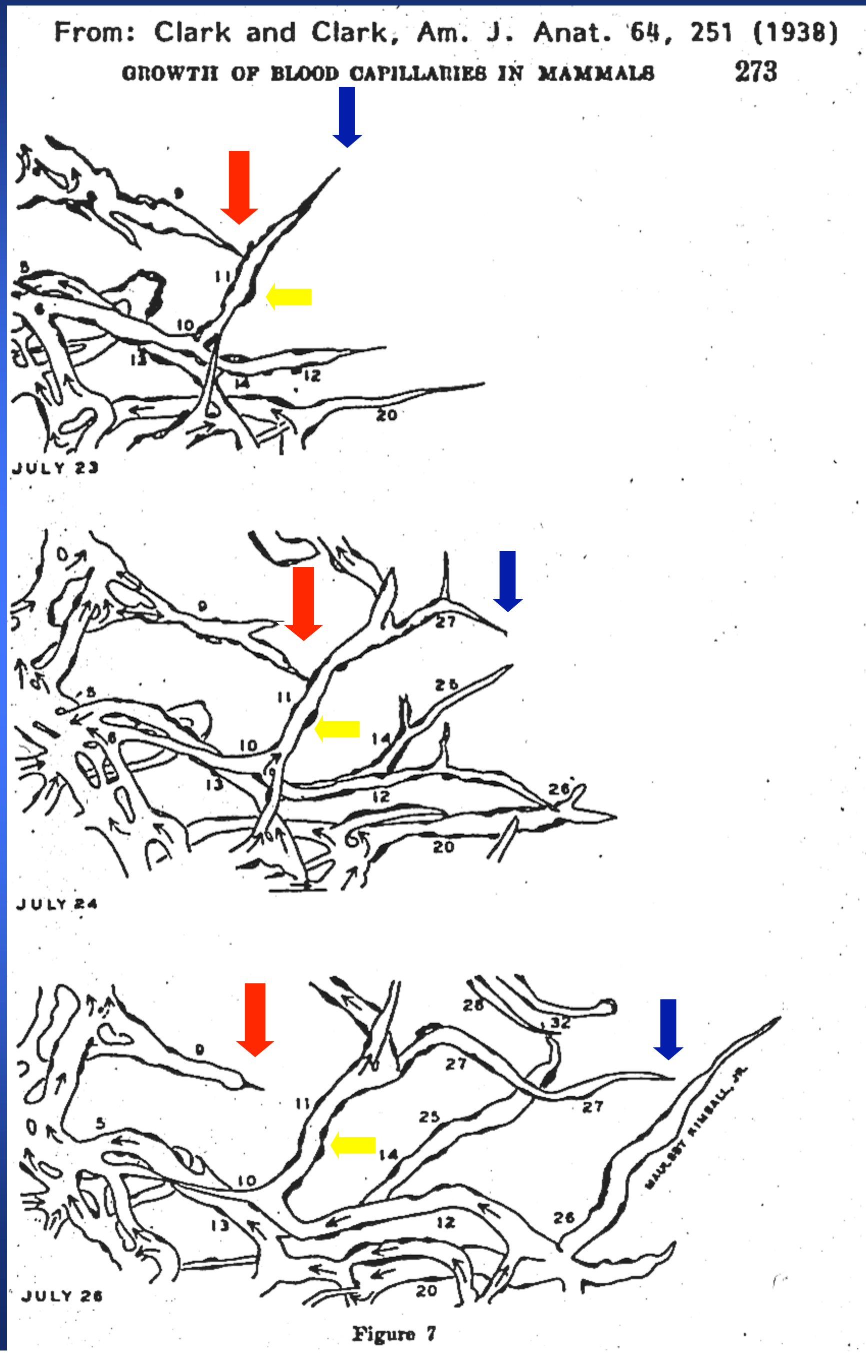


A Linear View of Tissue Development

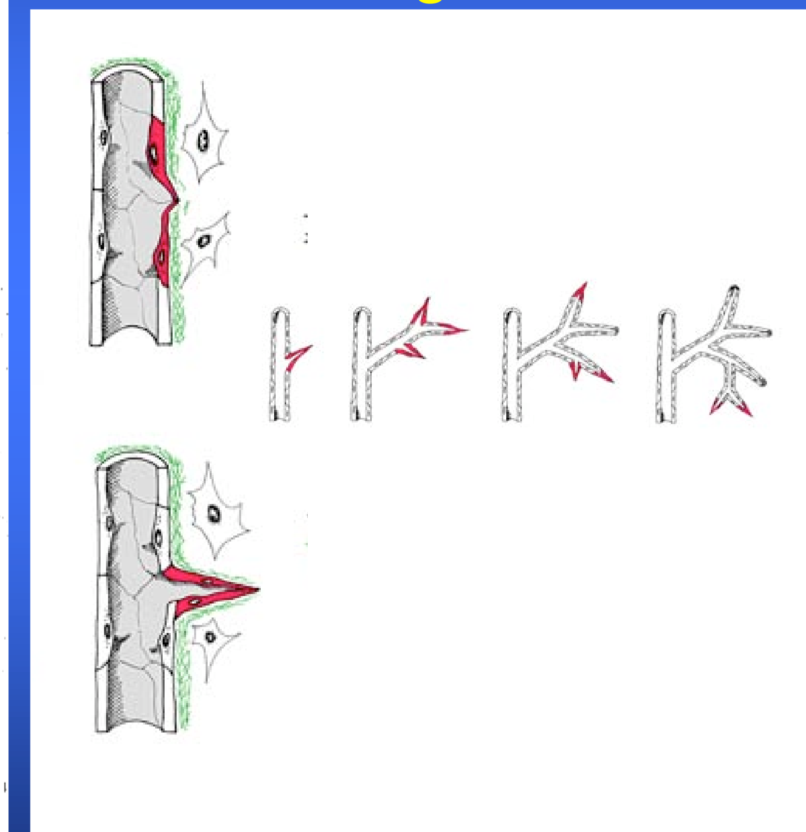
(Tumor Angiogenesis)

Local Control during Angiogenesis

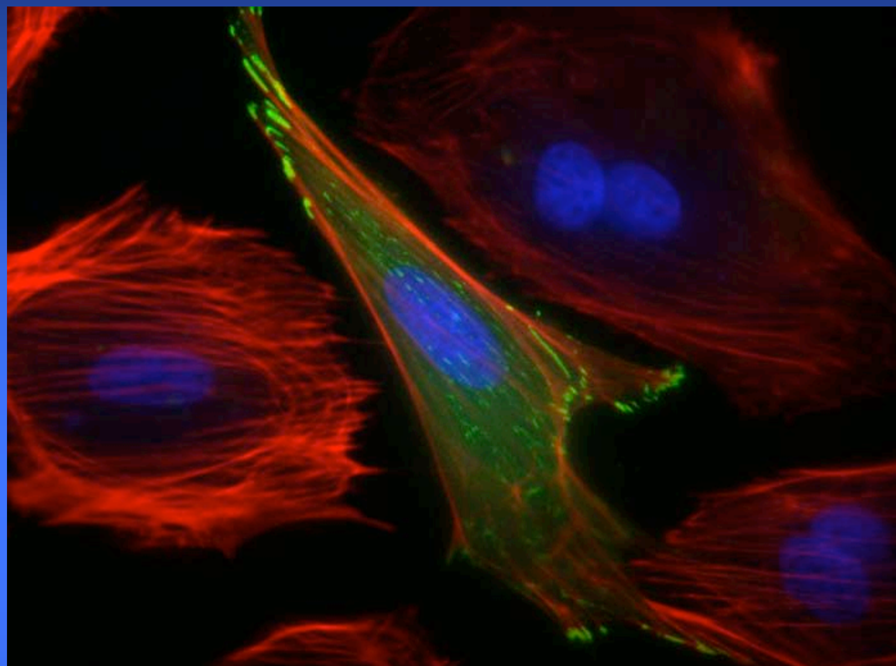


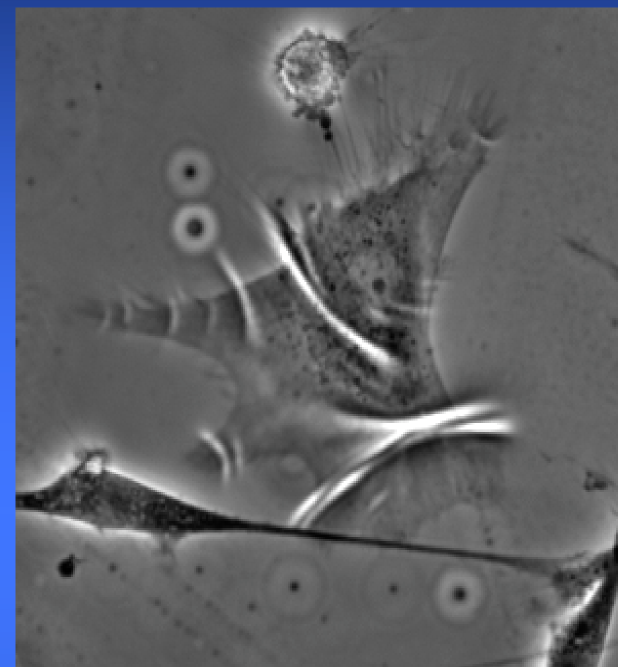


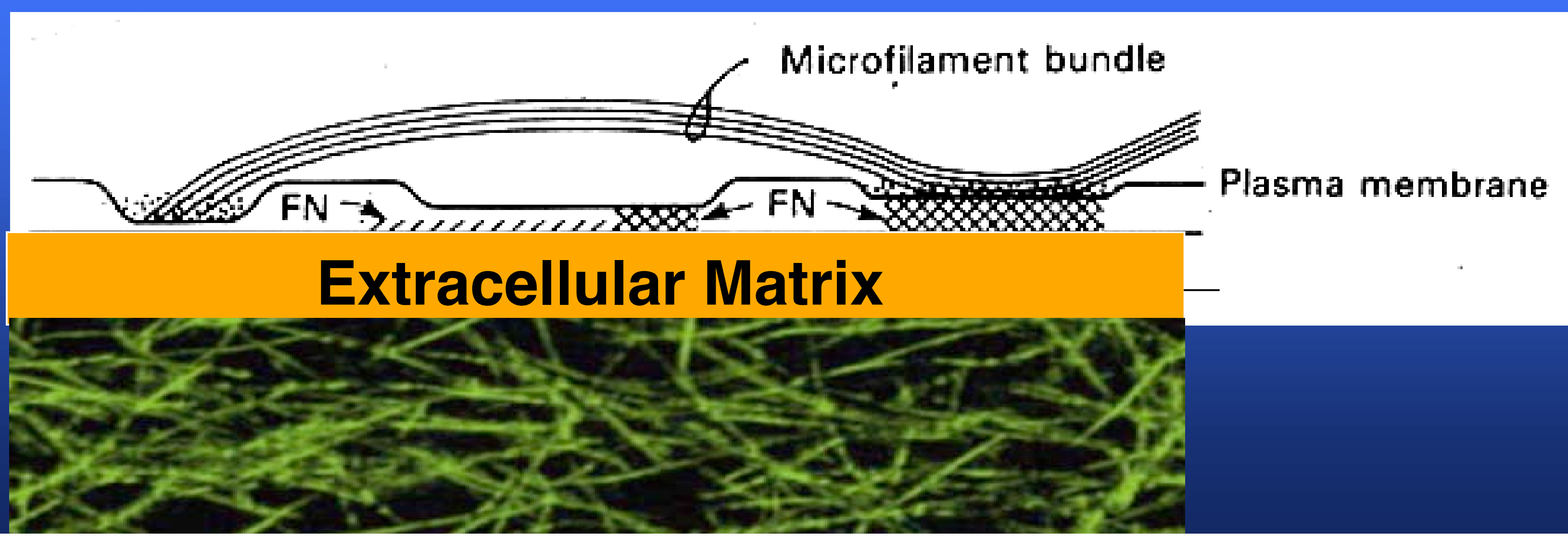
Branching Patterns



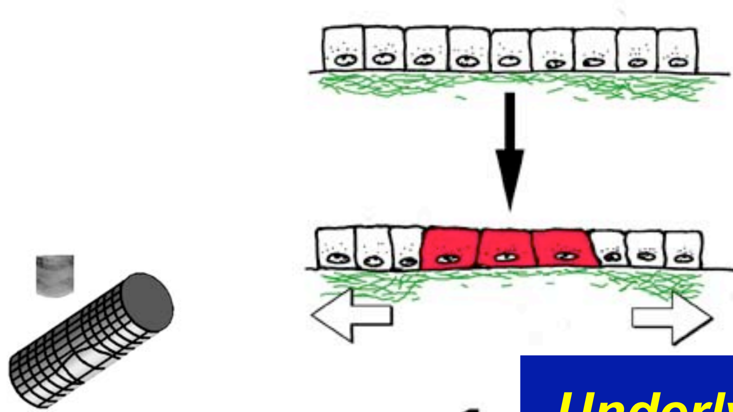
Cells Exert Tension on their Matrix Adhesions

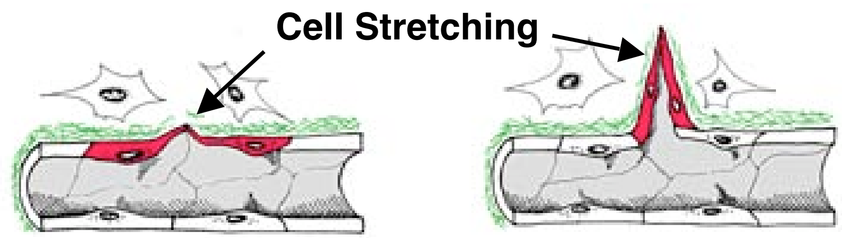


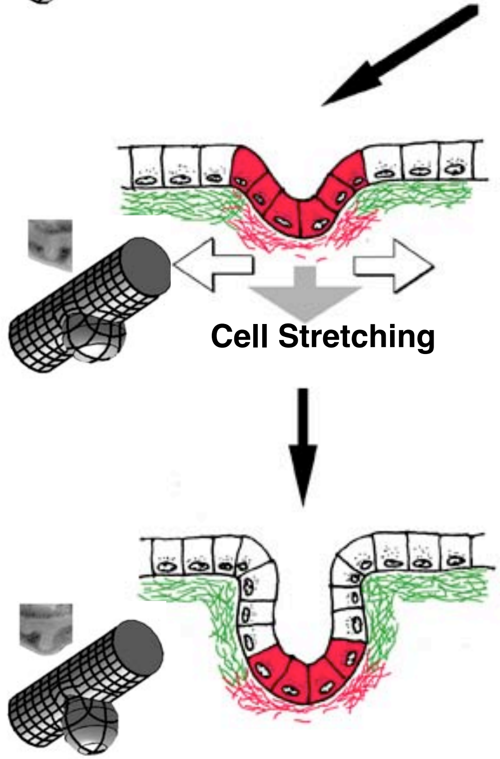




Micromechanical Control of Morphogenesis







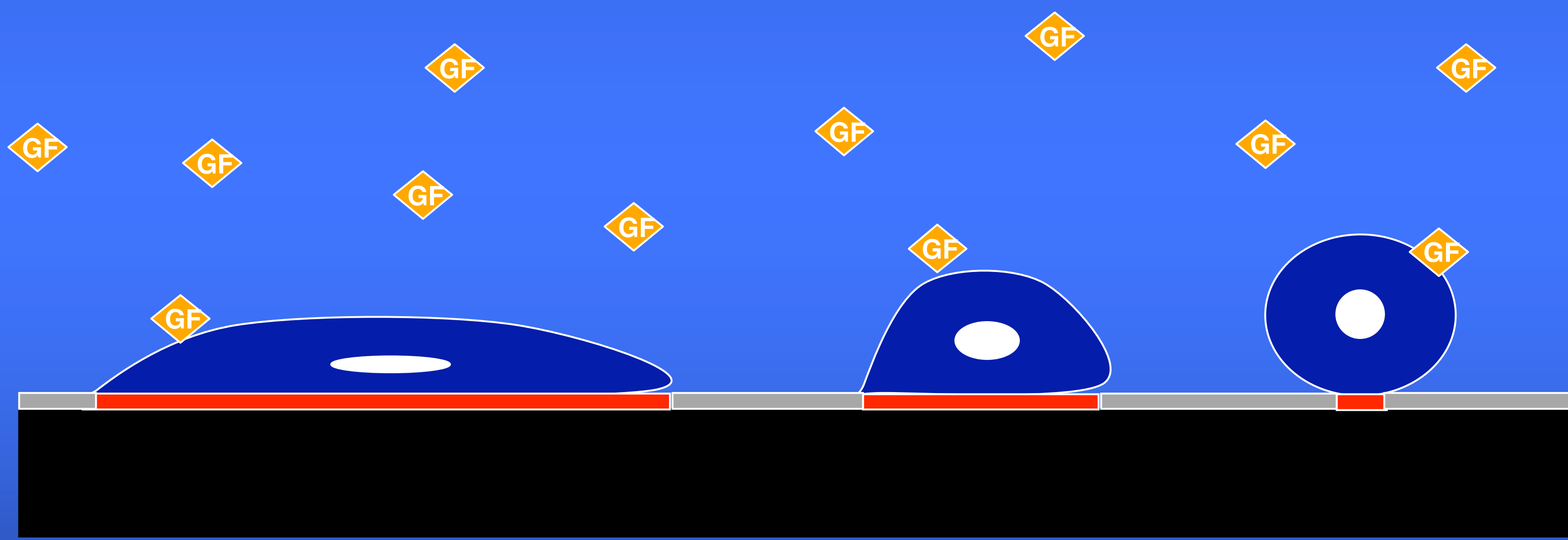
Underlying Hypothesis:

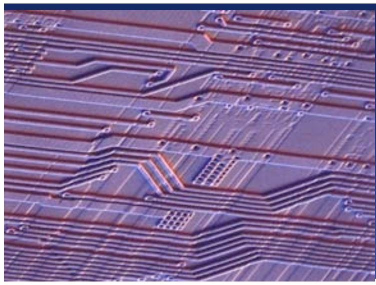
- **ECM remodeling changes LOCAL MECHANICS**
- **Increasing ECM flexibility promotes cell stretching**
- **Tension on adhesion receptors & distortion of the cytoskeleton alters cellular biochemistry**

Localized Growth & Motility

(Ingber et al., *PNAS* 78:3901-5, 1981; Ingber & Jamieson, In: *Gene Expression During Normal & Malig. Differ.* ,1985; Huang and Ingber, *Nature Cell Biol.* ,1999)

Making Cell Distortion an Independent Variable



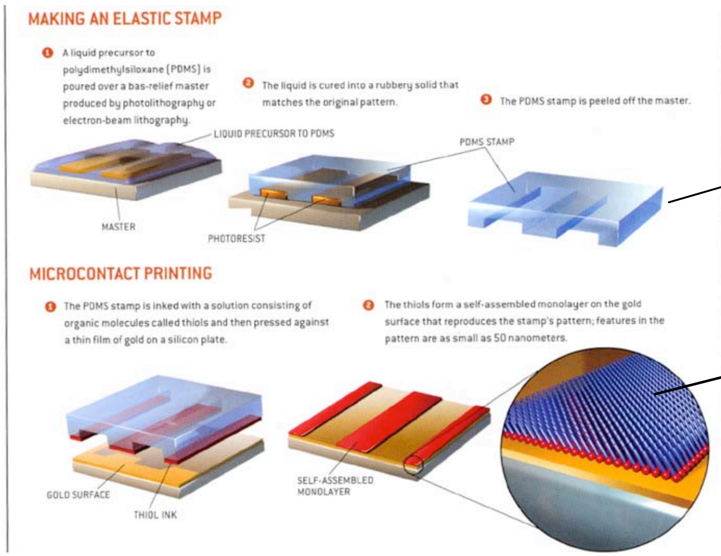


Nanotechnology-Based Microfabrication

(Soft Lithography + Self Assembling Monolayers)

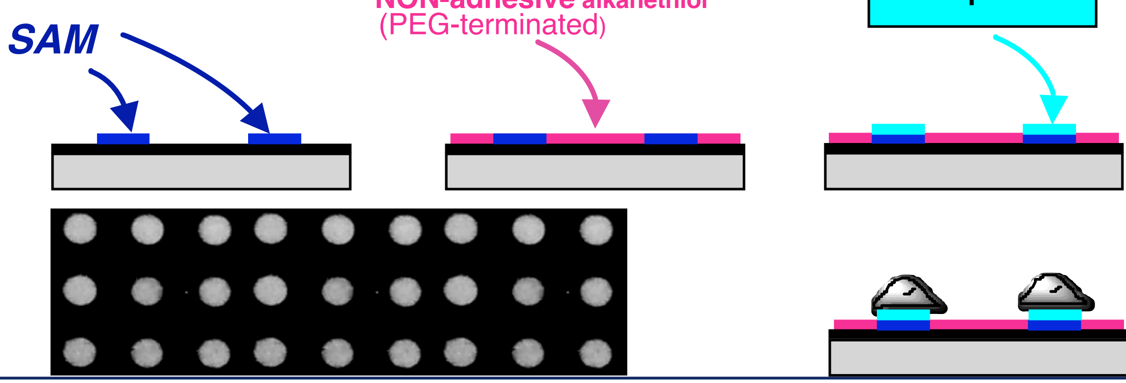
(with George Whitesides, Chemistry Dept. Harvard U.)

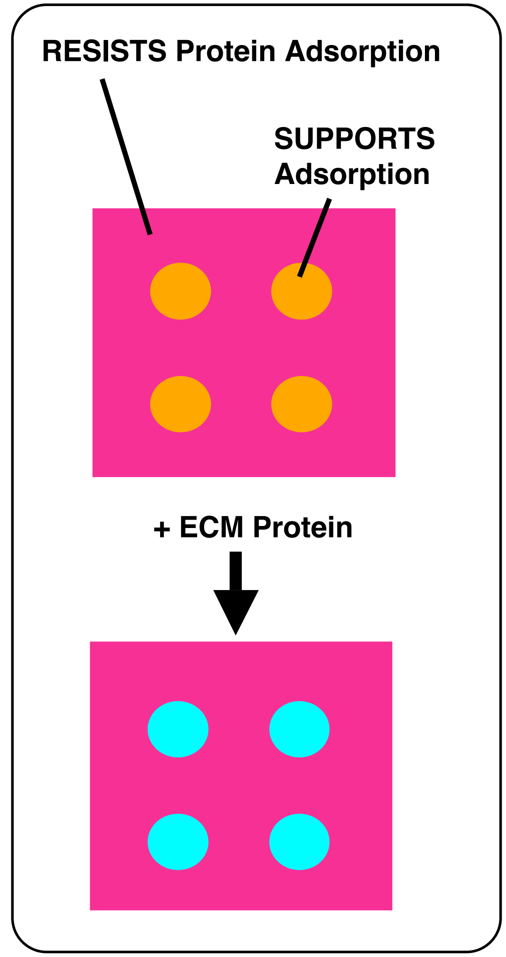
(Singhvi et al., *Science* 1994; Chen et al. *Science* 1997; Chen et al. *Methods Mol. Biol. 2000;* 139: 209-219)



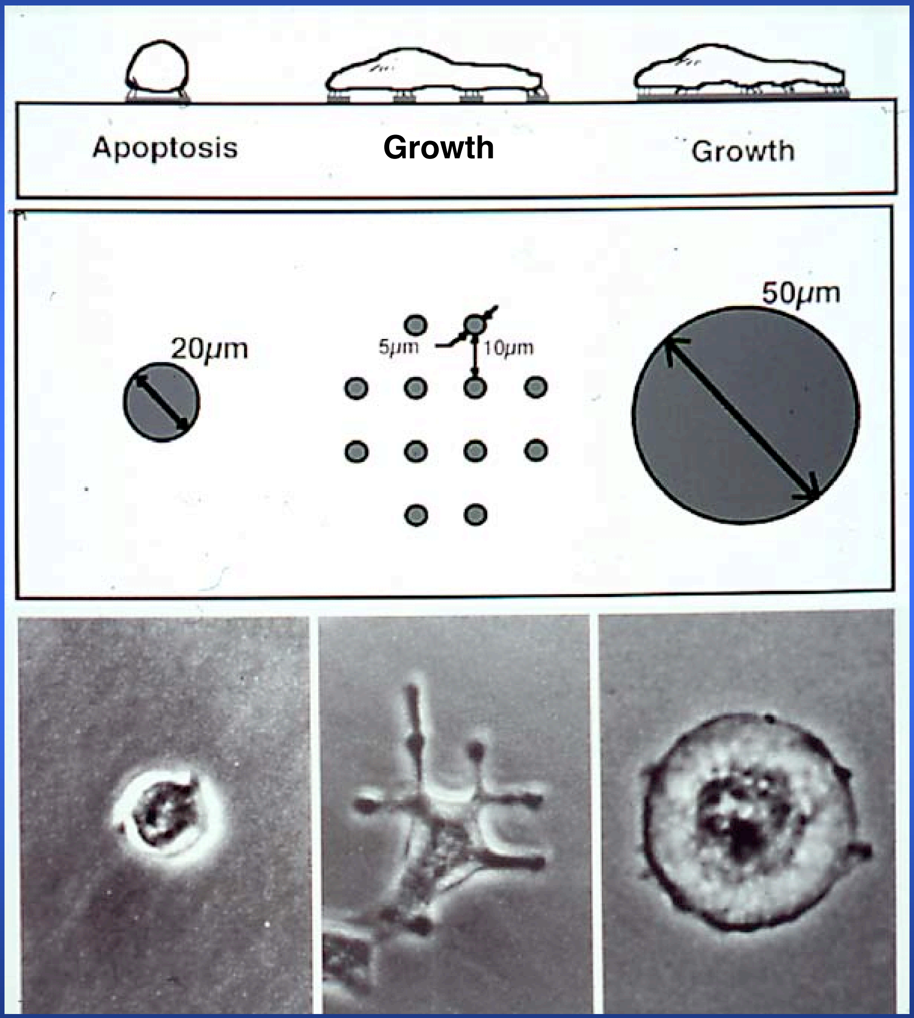
Flexible PDMS Stamp

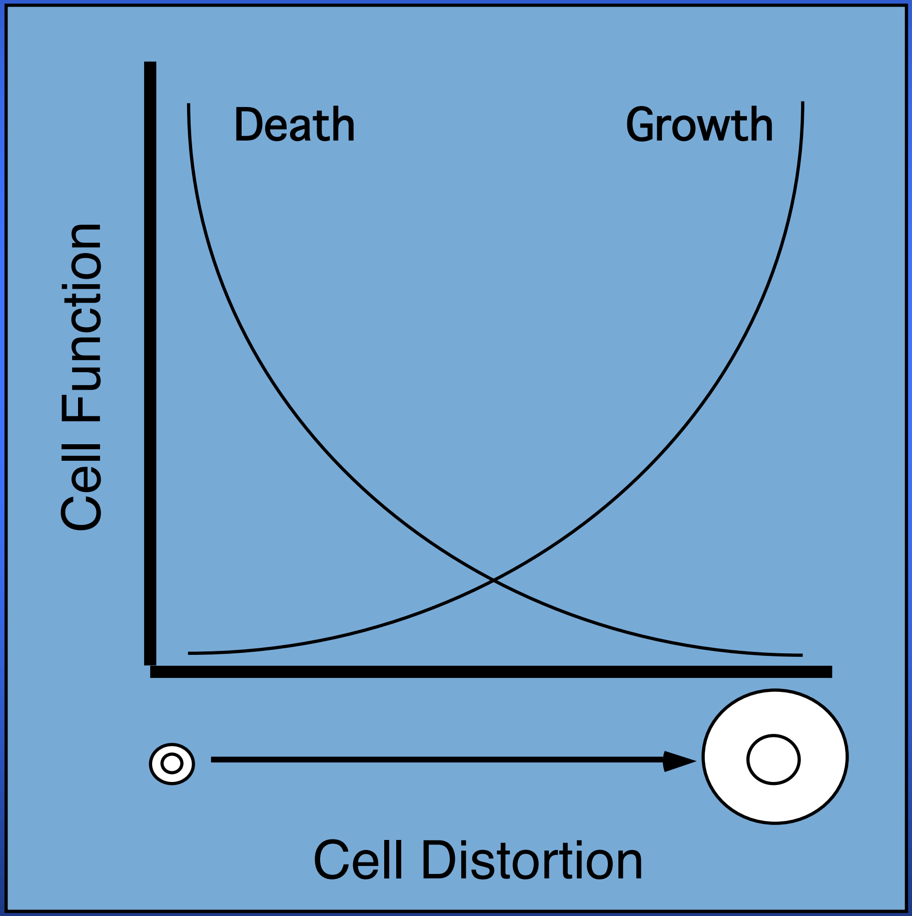
Molecular Self-Assembly on the NANOSCALE





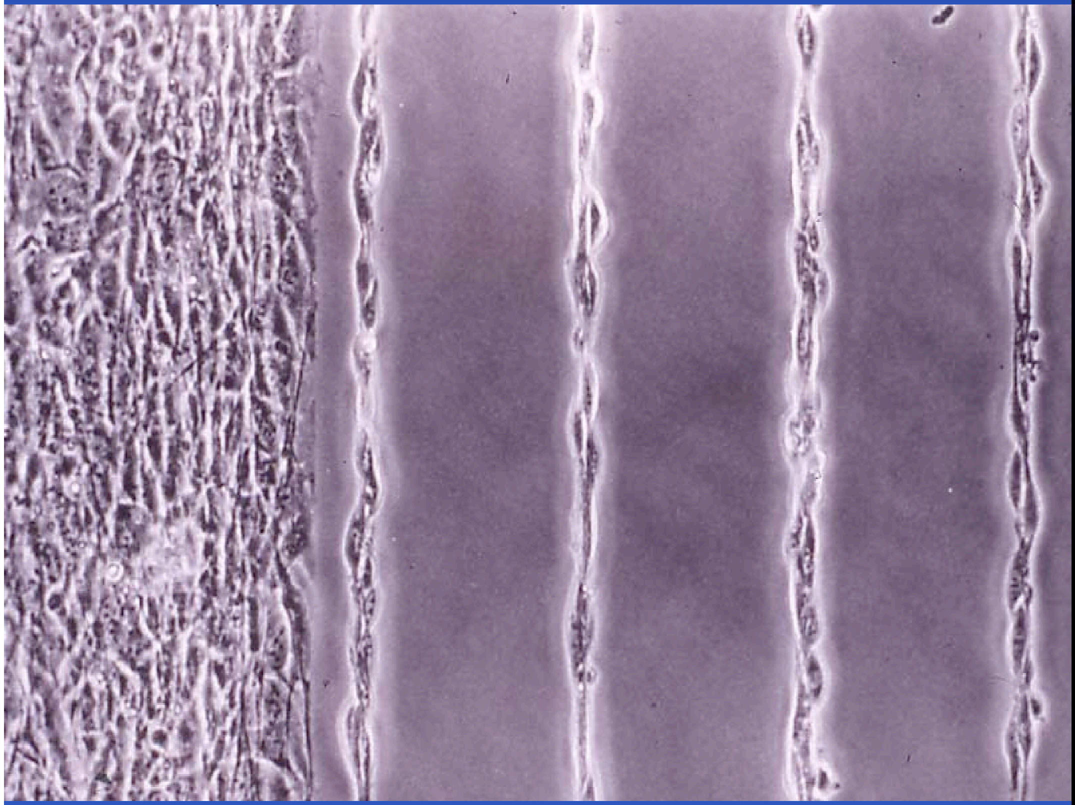
Stretching Cells Makes Them Grow And Rounded Cells Die

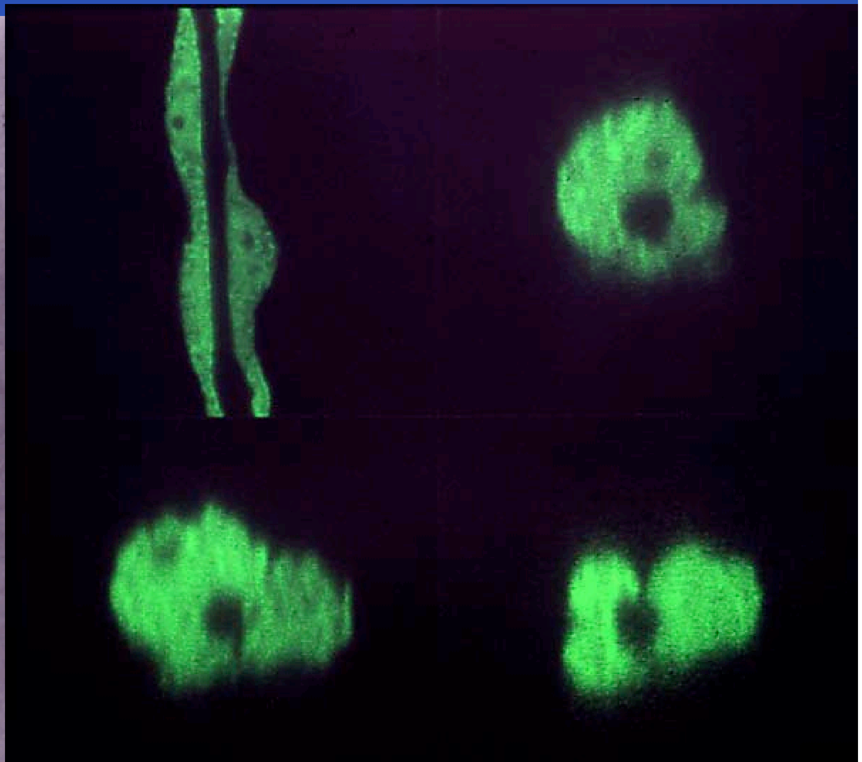




(Singhvi et al. *Science* 1994; Chen et al. *Science* 1997)

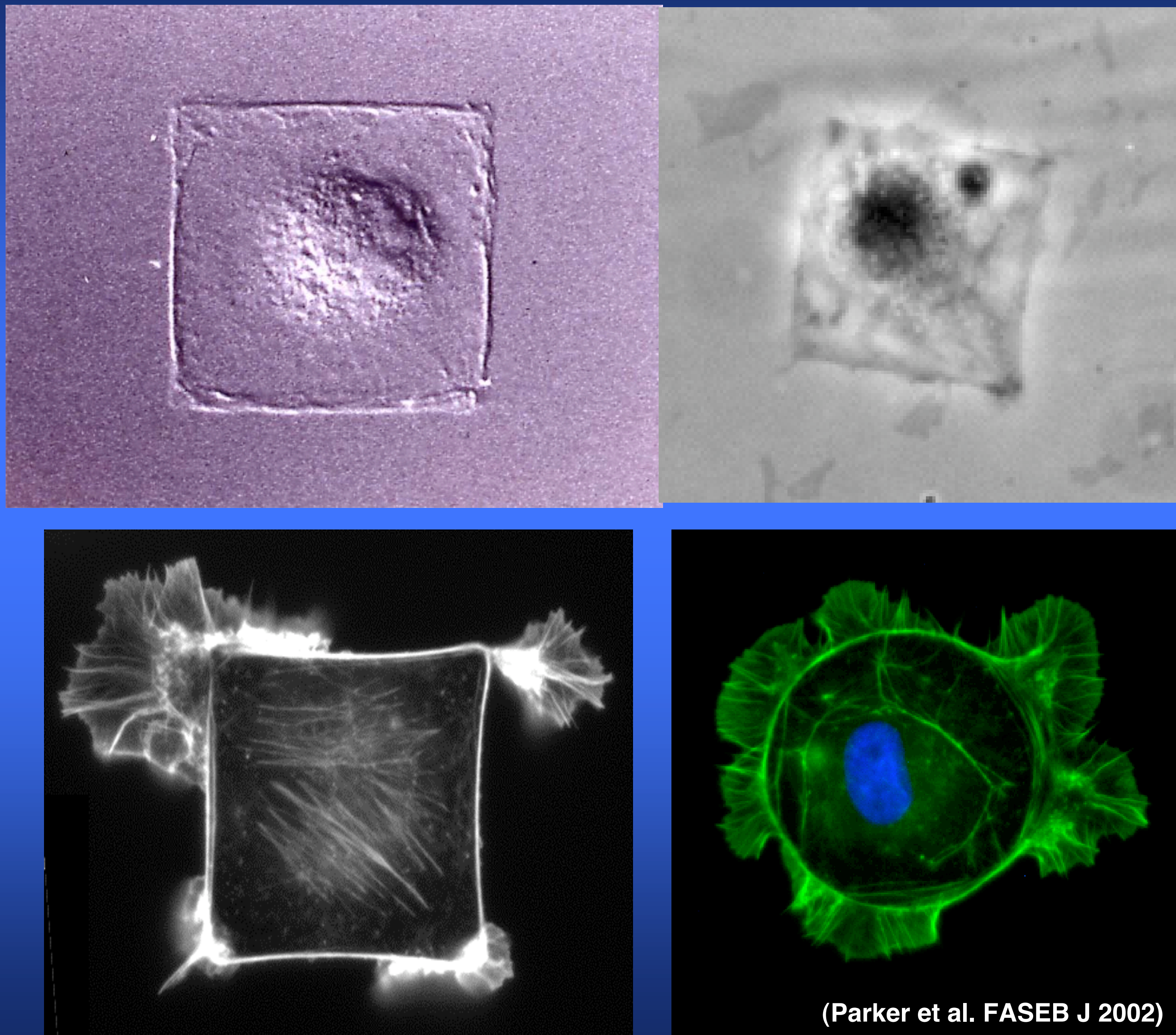
Capillary Blood Vessel Formation In A Dish





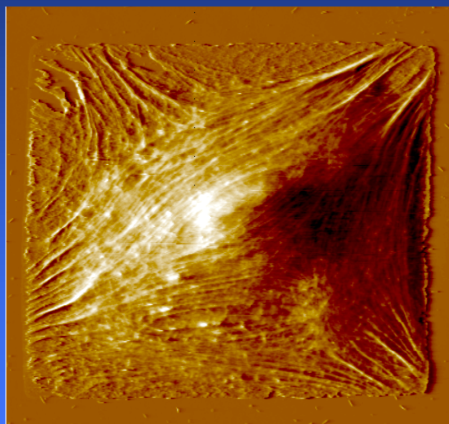
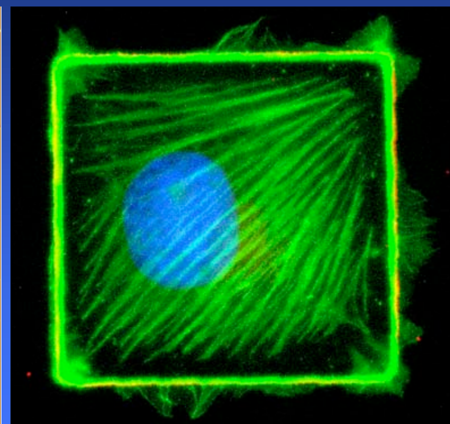
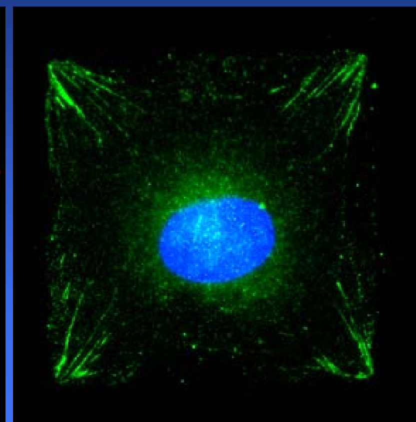
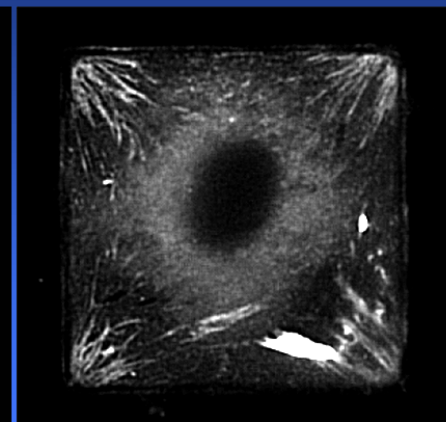
(Dike et al. In Vitro Cell Dev Biol 1999)

Stretch-Dependent Control of Directional Motility



(Parker et al. FASEB J 2002)

Cell Distortion Redirects Molecular Self-Assembly In the Cytoskeleton & Extracellular Matrix

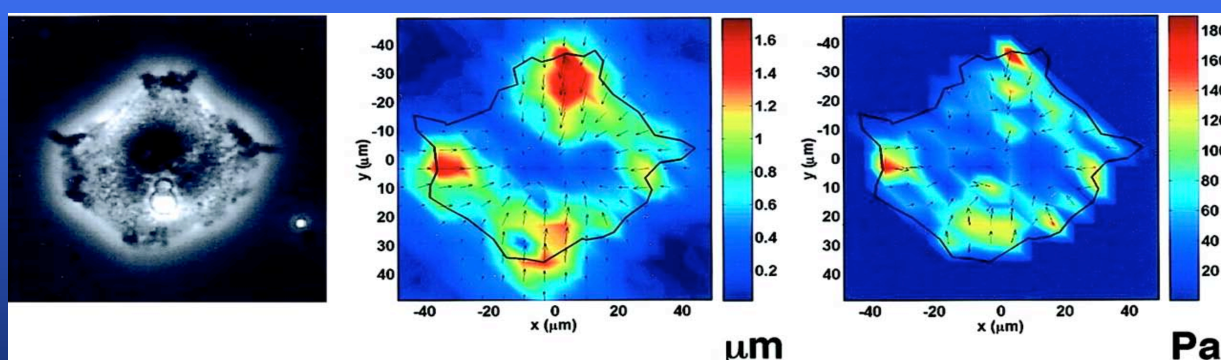
Actin Stress Fibers
(AFM) (F-Actin)

Focal Adhesions
(Vinculin)

Fibronectin
Fibrils

Guided by Localized Tension Application in Cell Corners

TRACTION FORCE MICROSCOPY:



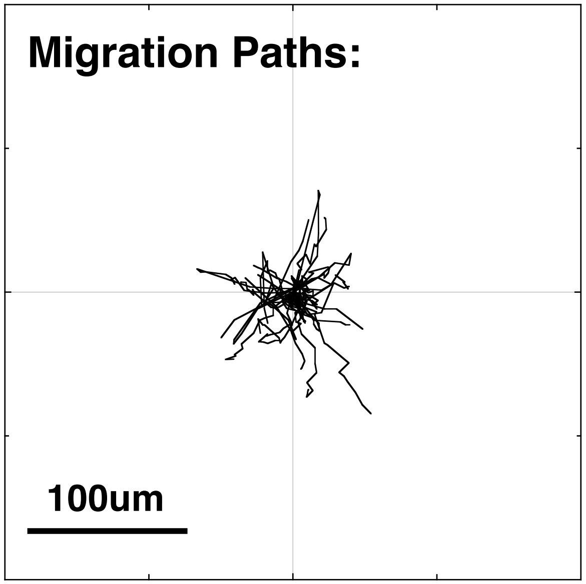
Cell Morphology Strain Map Stress Map

(Parker et al. *FASEB J* 2002; Wang et al., *Cell Cytosk. Motil.* 2002; Brock et al. Langmuir 2003)

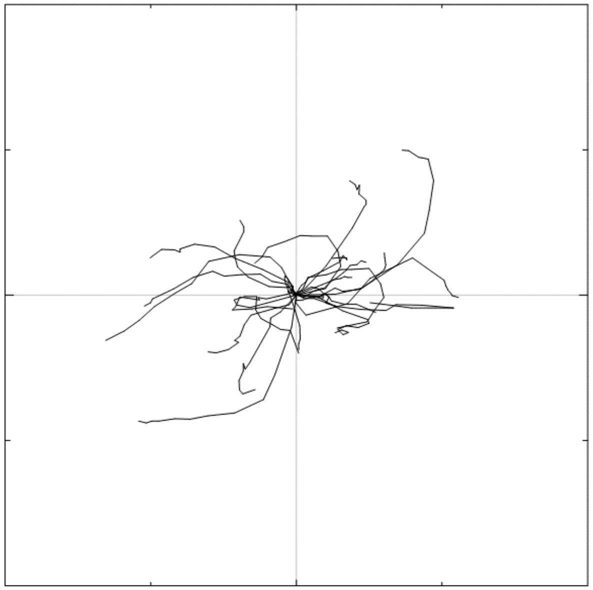
Physical ECM Pattern Governs Directional *Motility*

+ PDGF (NO CHEMICAL GRADIENT!)

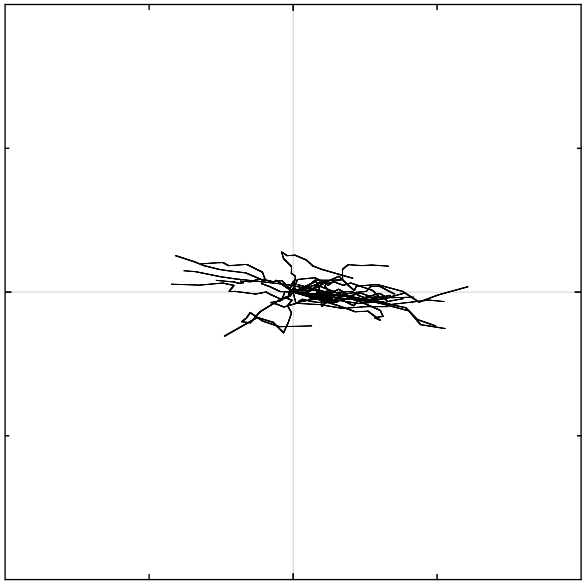
(Xia et al., *FASEB J* 2008)



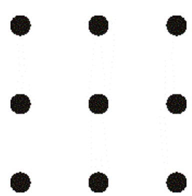
1C-3,3

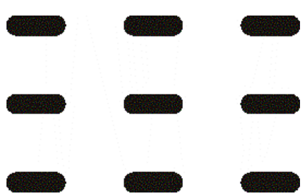


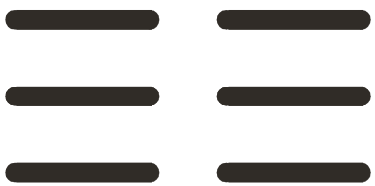
3L-3,3

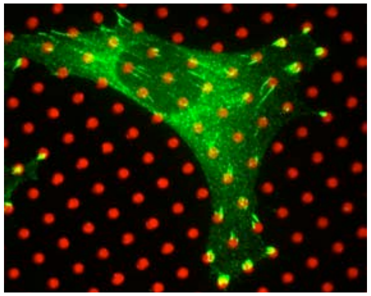


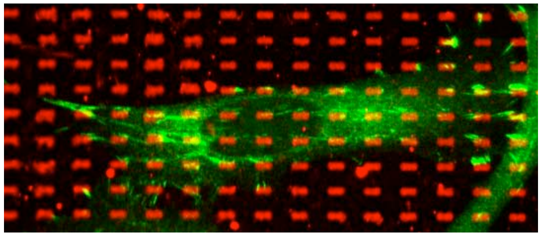
8L-3,3

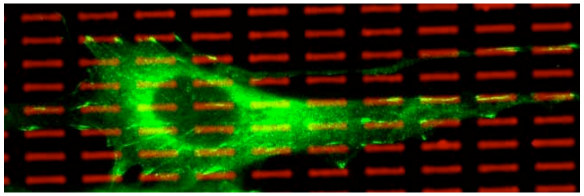




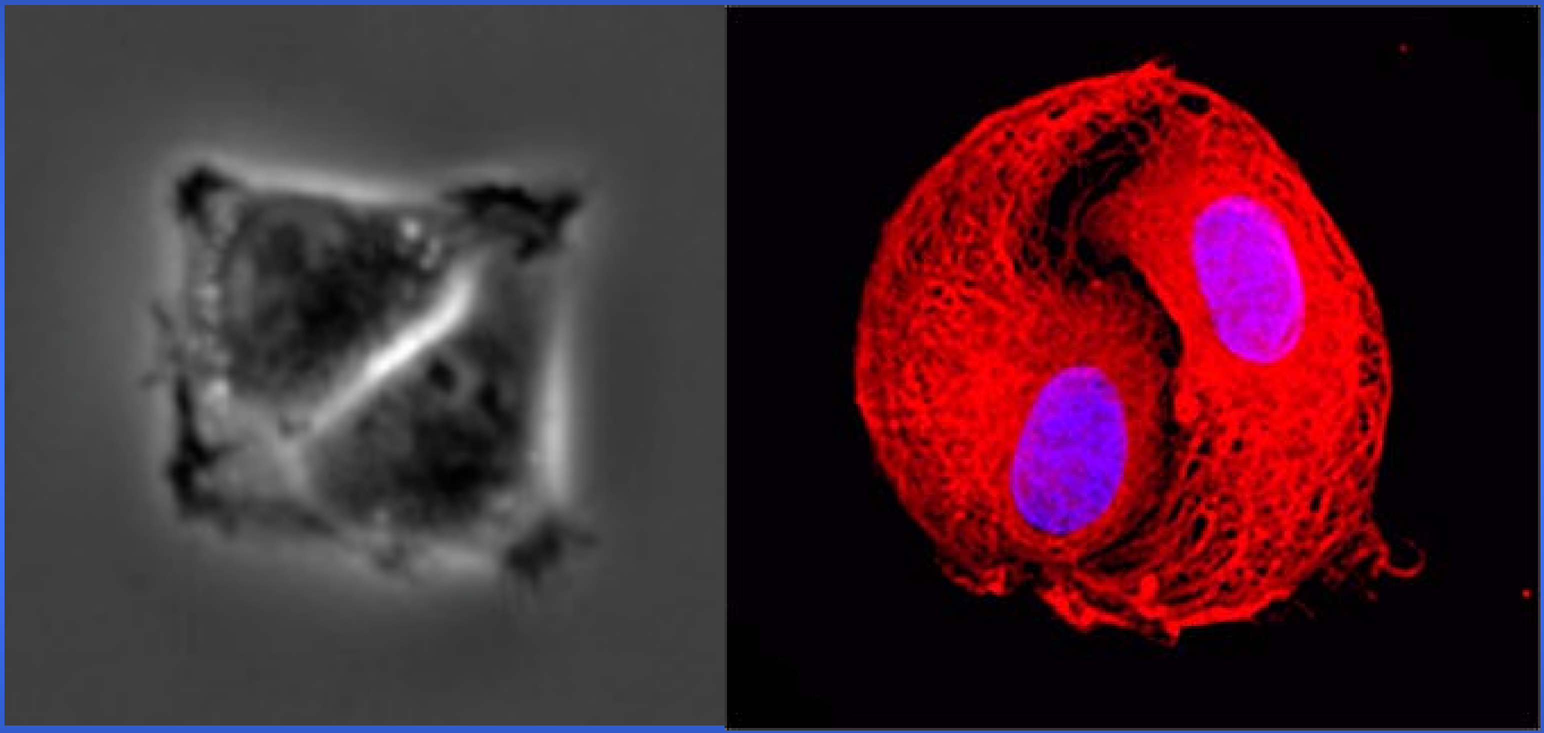








Local Rules & Physical Determinants Govern Pattern Formation

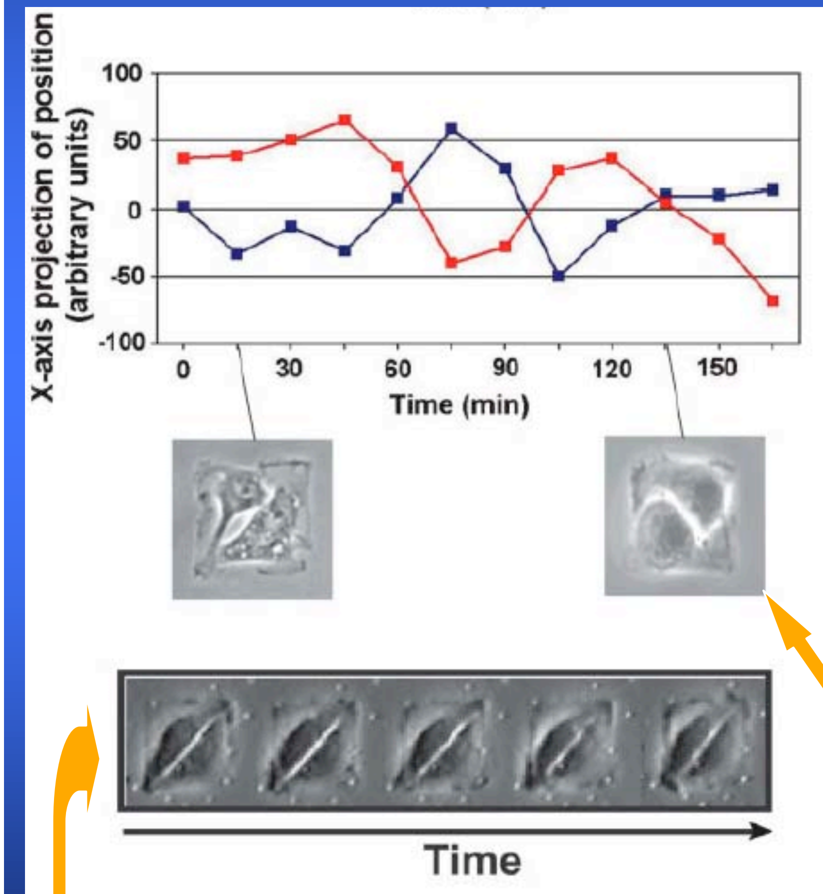


(Brangwynne et al. *In Vitro Cell Dev Biol* 2000;
Huang et al., *Cell Cytosk Motil* 2005)

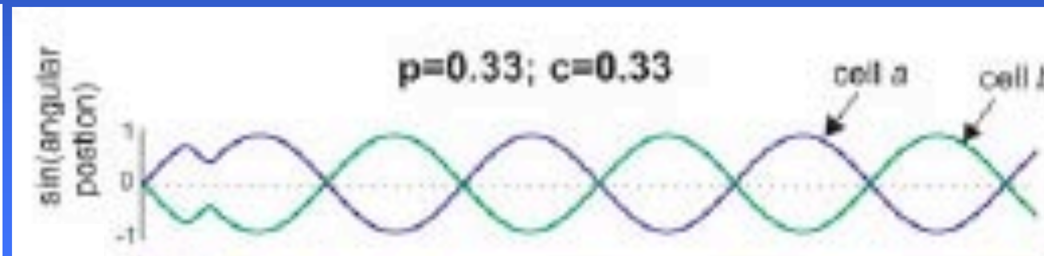
("Symmetry Breaking" in Mammalian Cells)

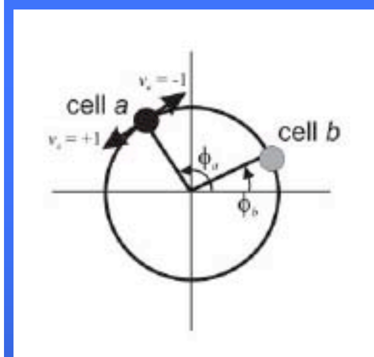
Patterning Predicted by *Whole Cell* Behaviors

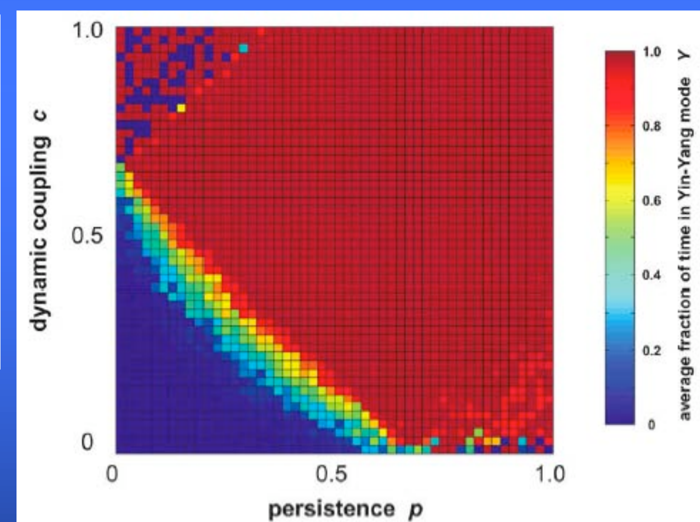
Experimental Results



Computational Model





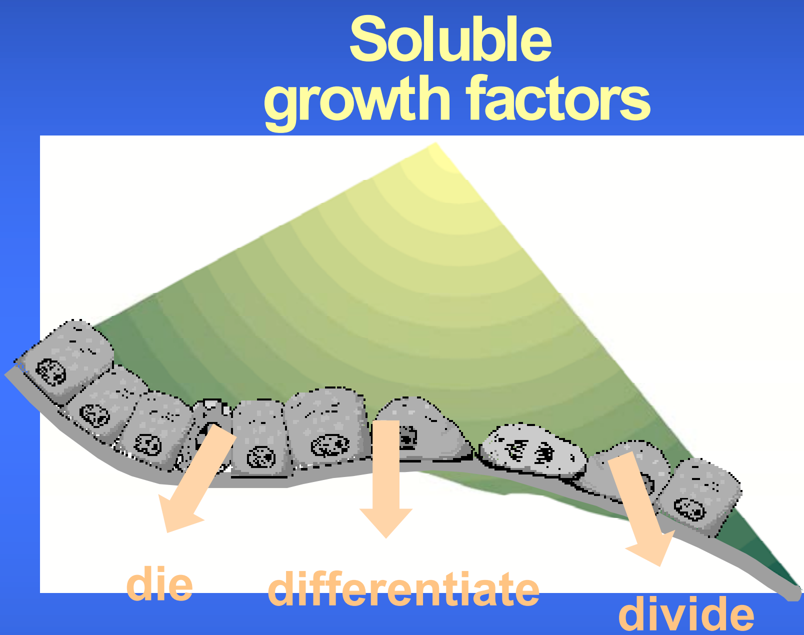


Capillary Endothelial Cell (High Persistence - Yin Yang)

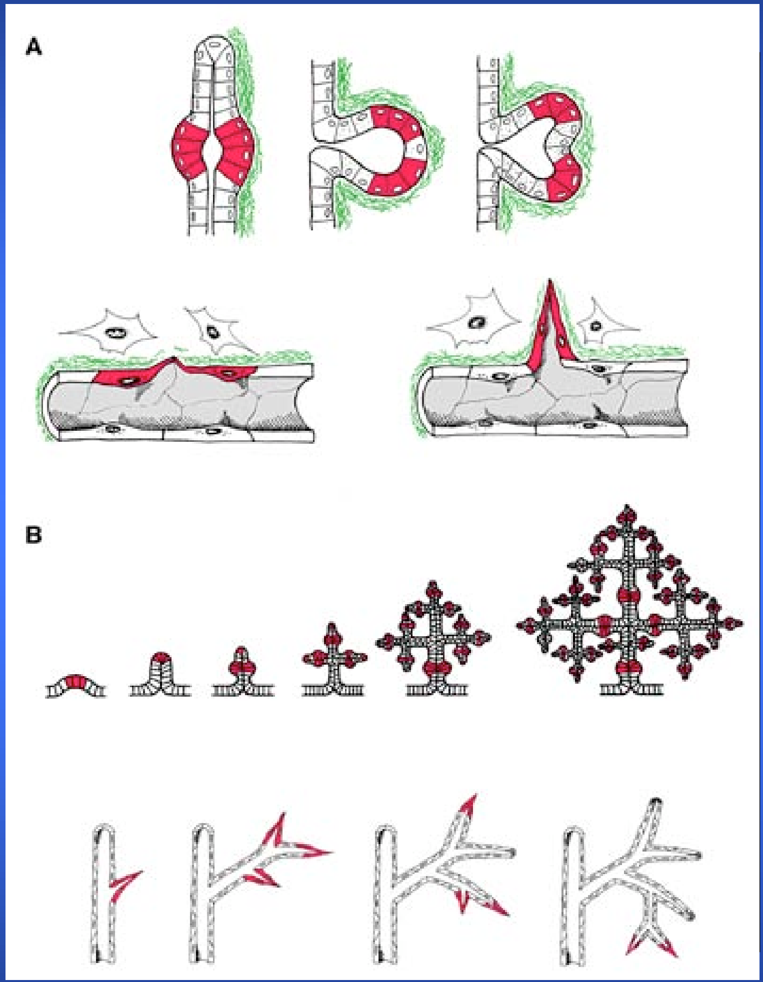
Fibroblast (Low Persistence - No Yin Yang)

(Huang et al., *Cell Motil. Cytosk.* 2005)

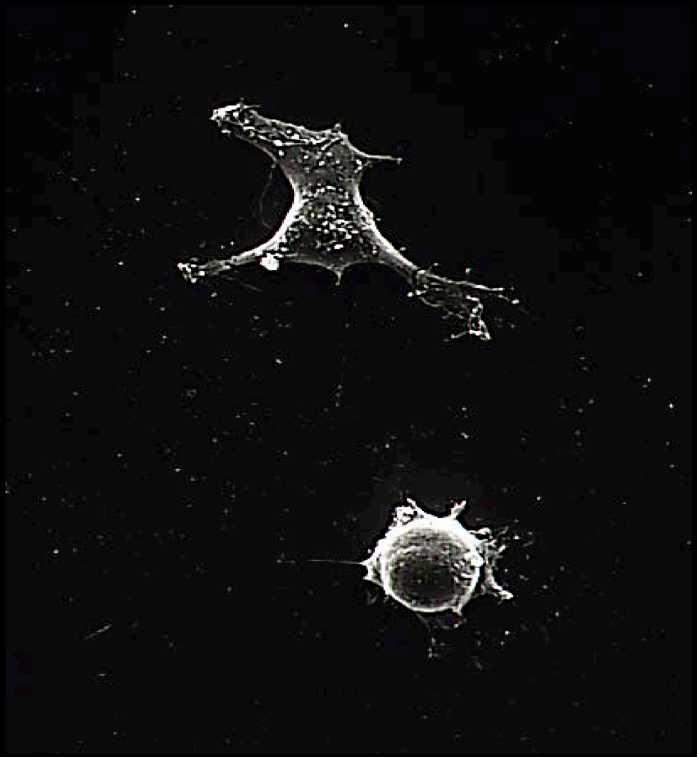
Cell Fate Switching Depends on Physicality of Microenvironment



→ Spatial heterogeneity of cell fates drives morphogenesis



How are Cells Constructed so that they can Sense Force?



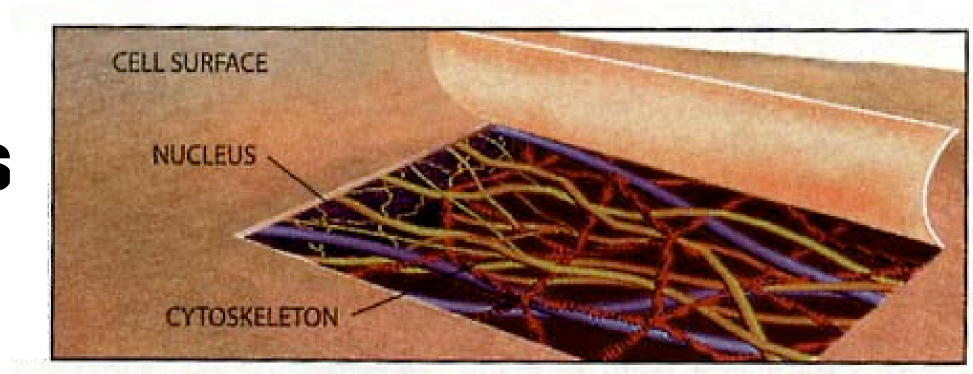
Old View:

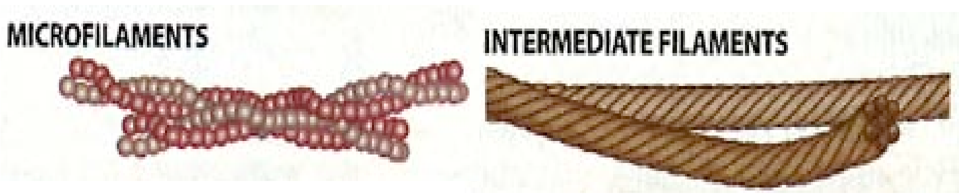
Cells are like Water Balloons

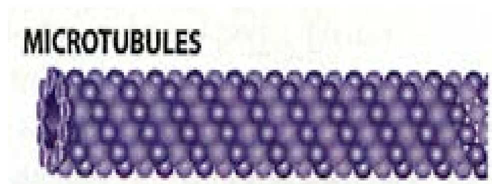
Hypothesis:

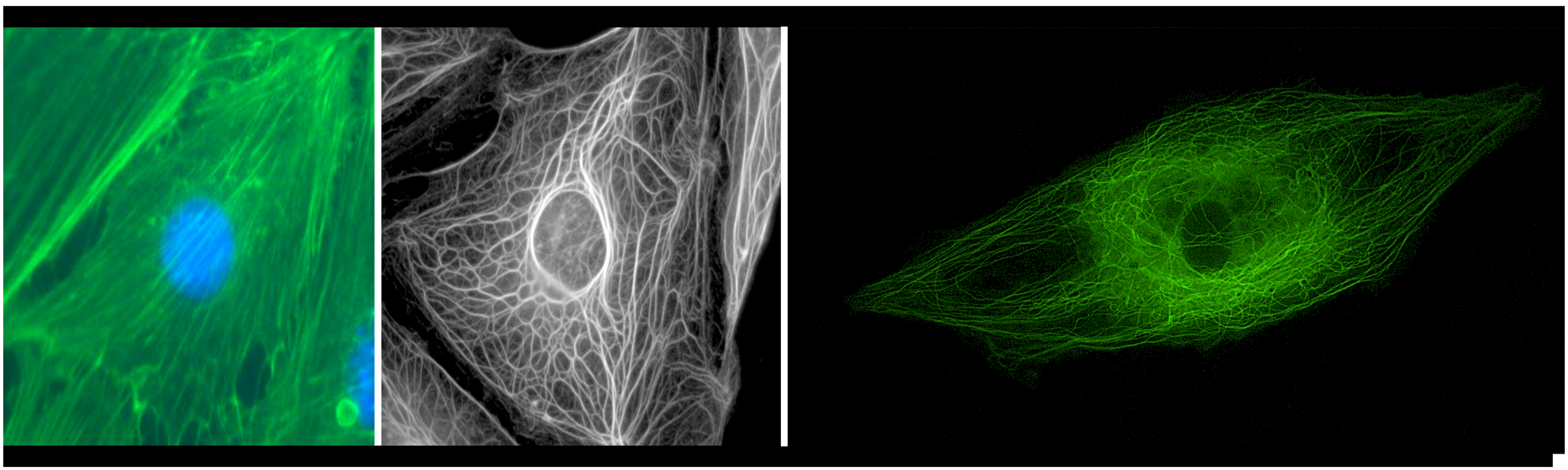
Cells are Built Like Tents

The Molecular Networks of the Cytoskeleton

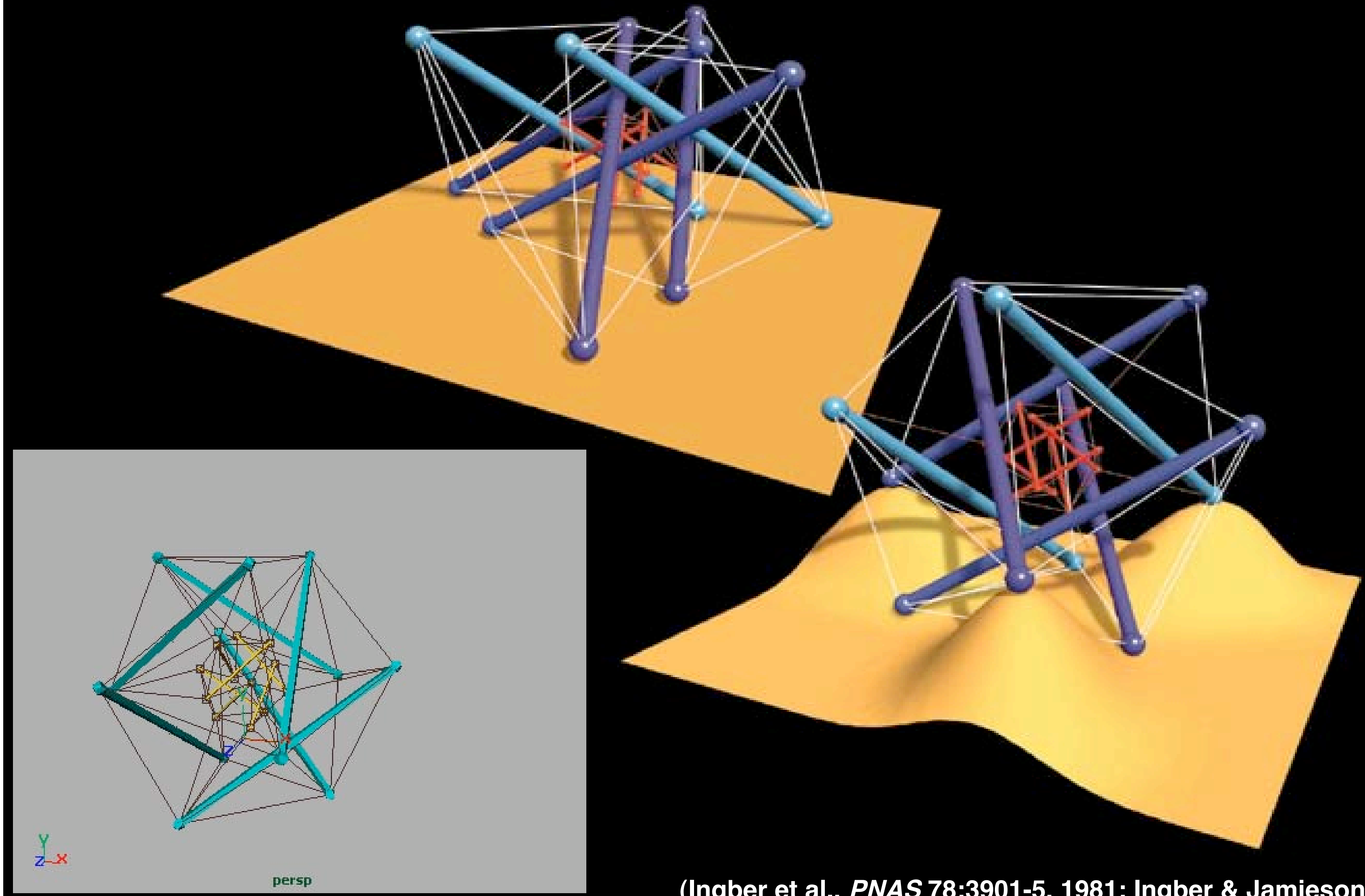








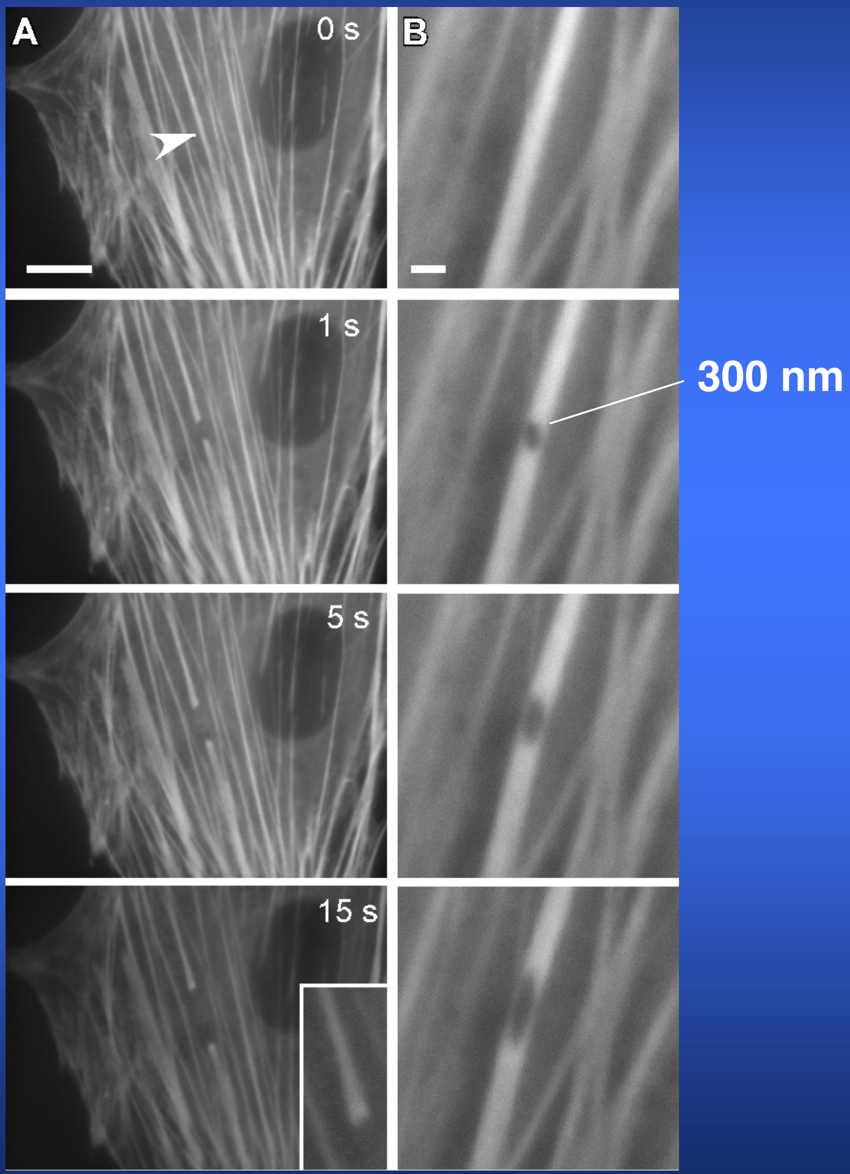
Cellular Tensegrity Model



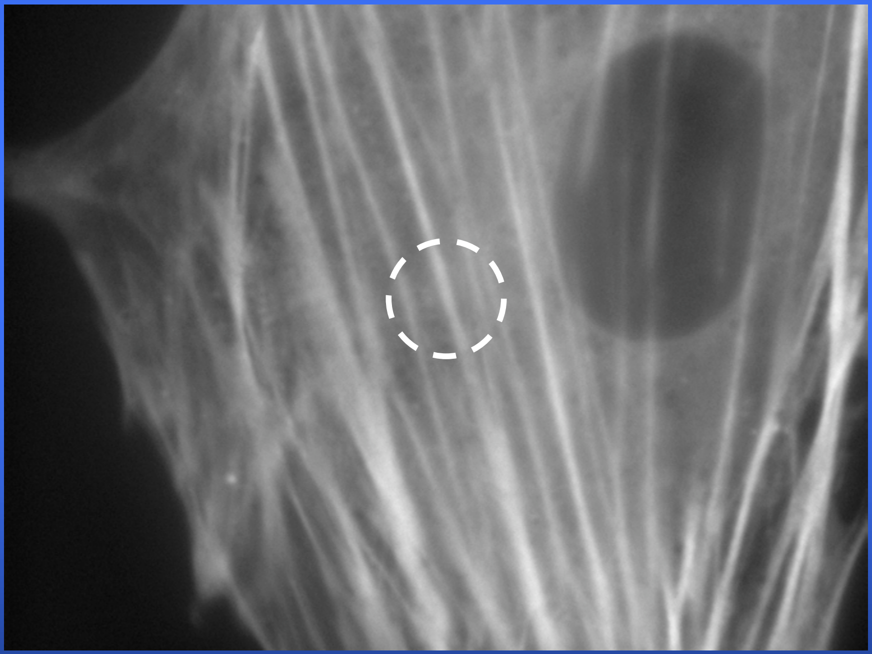
(Ingber et al., *PNAS* 78:3901-5, 1981; Ingber & Jamieson,1985;
Wang et al. *Science* 1993, *PNAS* 2001; Ingber *J. Cell Sci* 1993, 2003)

Resting Tension (Prestress) in Living Stress Fibers Revealed using Laser Nano-Surgery ("tensed cables")

(with Eric Mazur, Dept. Physics, Harvard U.)



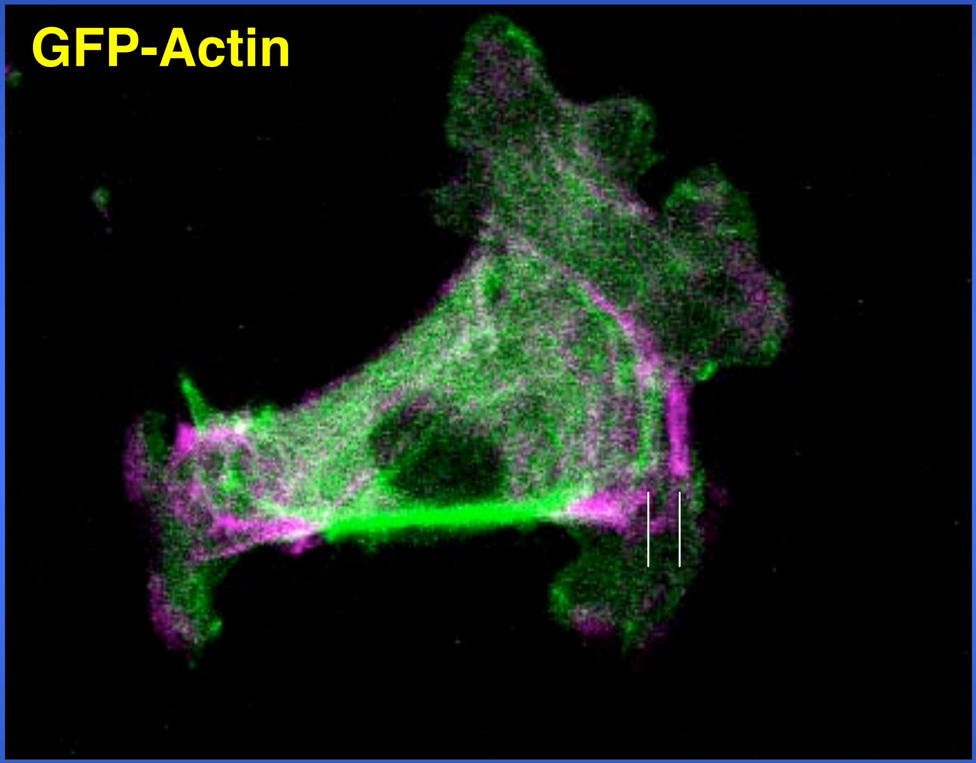
Retraction of a single actin stress fiber in a living cell



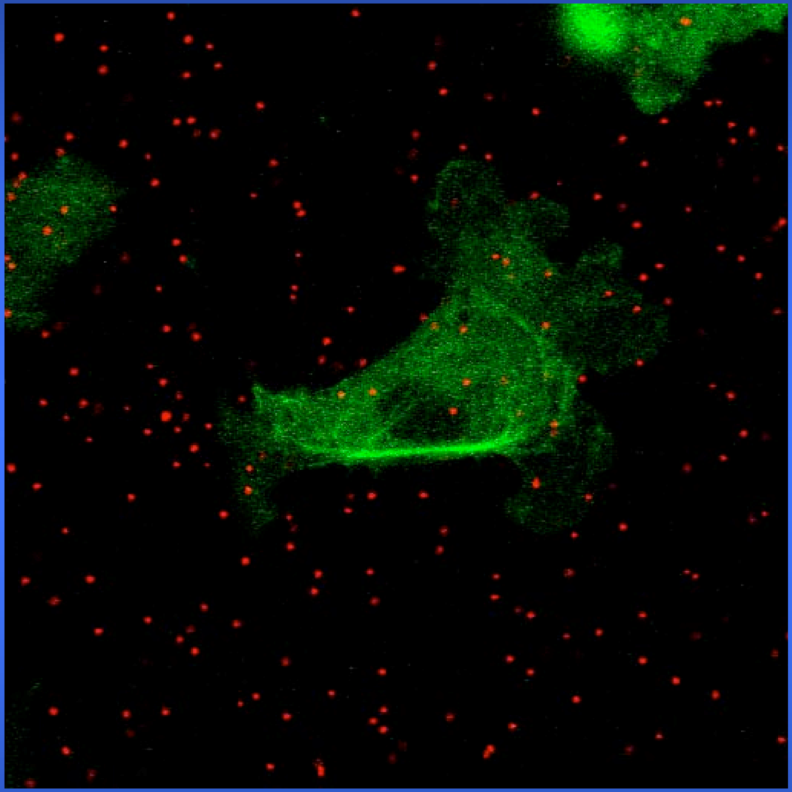
(Kumar et al., *Biophys J.* 2006)

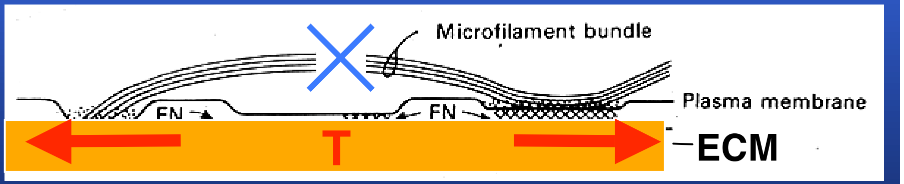
Mechanical Continuity in the Cytoskeleton and ECM

Flexible ECM Substrate



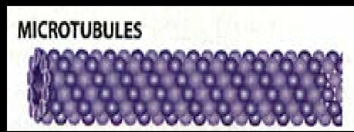
Before Cut: Green
After Cut: Magenta

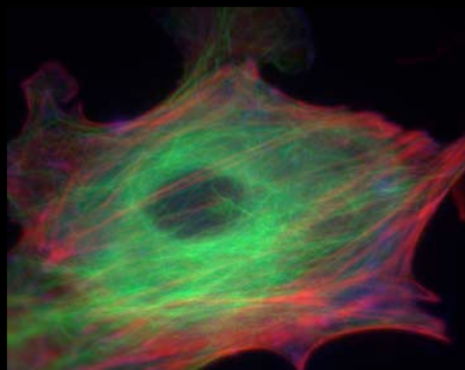




(Kumar et al, Biophys J 2006)

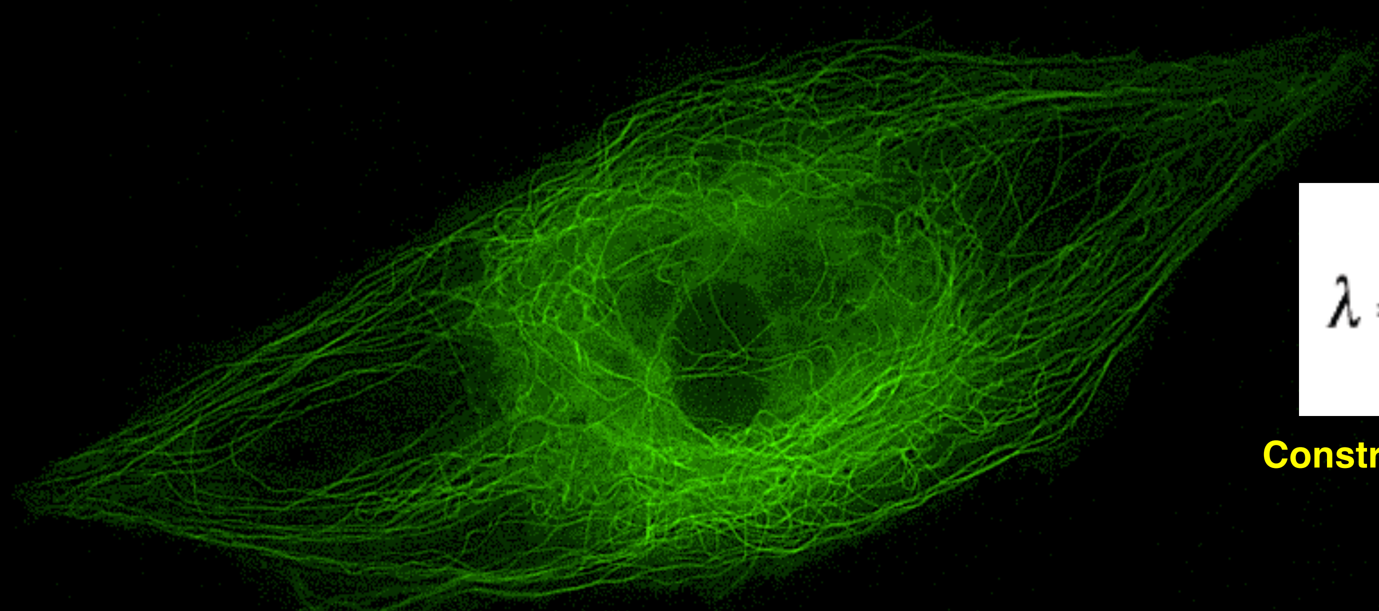
Microtubules are Semi- Flexible Compression Struts





Live Beating Heart Cell

(Brangwynne et al, *J Cell Biol 2006 with D. Weitz & K. Parker, Harvard U.* & F. Macintosh, Amsterdam)

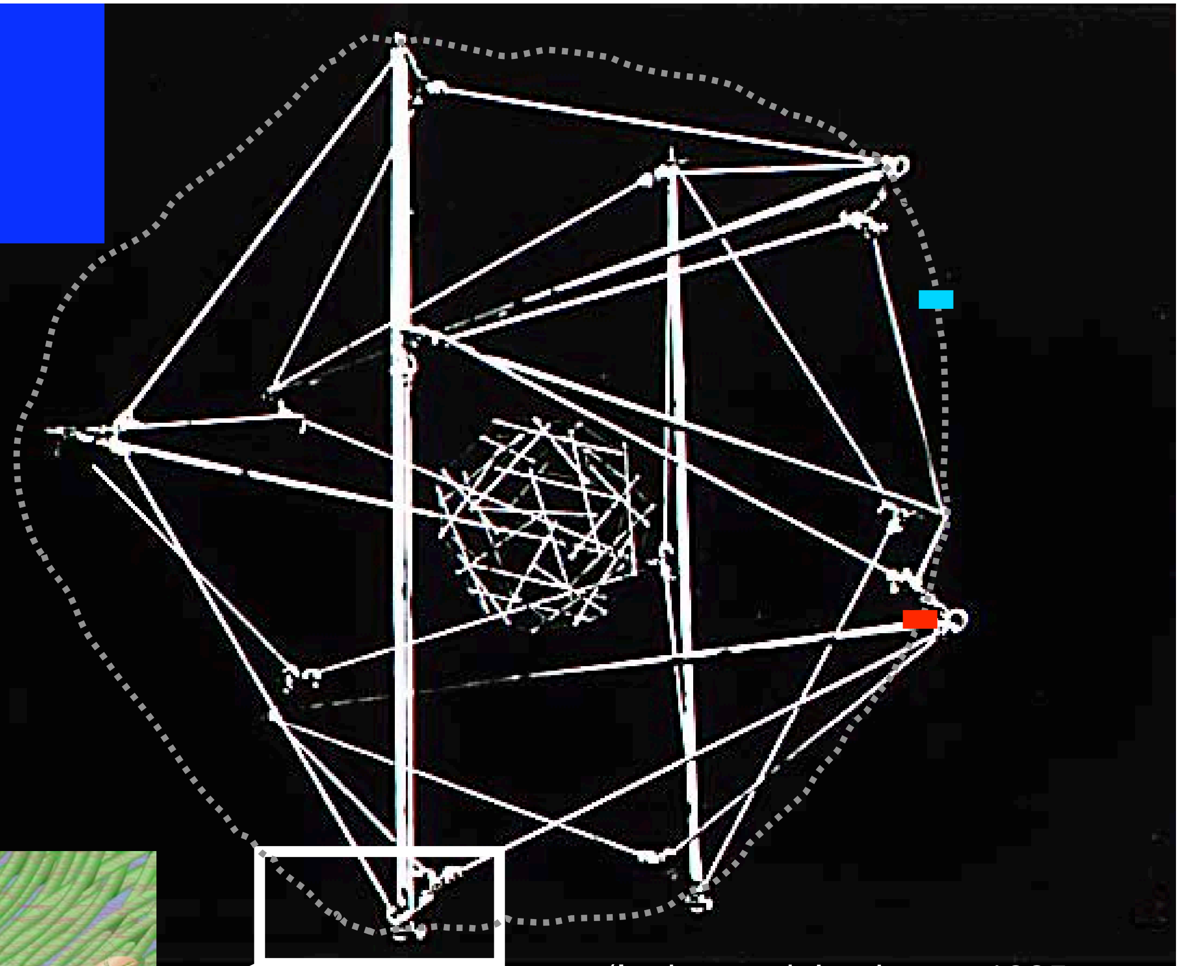


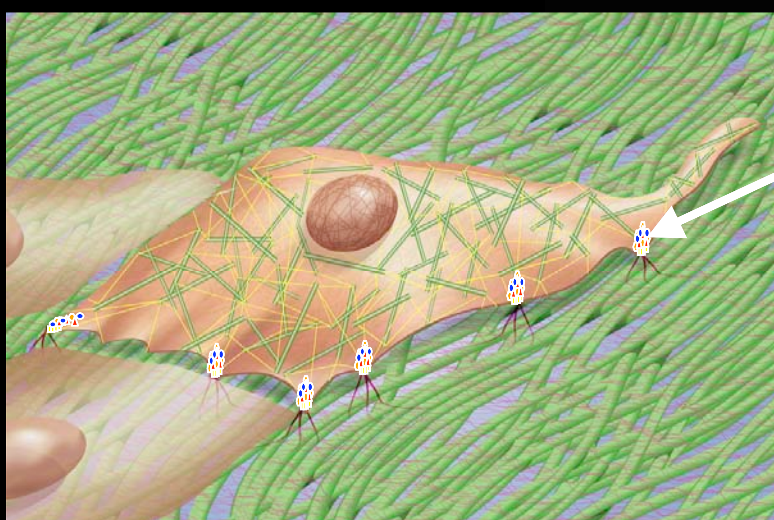
$$\lambda = 2\pi \sqrt[4]{\frac{\kappa}{\alpha}}$$

Constrained Buckling Theory

Curved (Buckled) Microtubules in a Fixed Cell

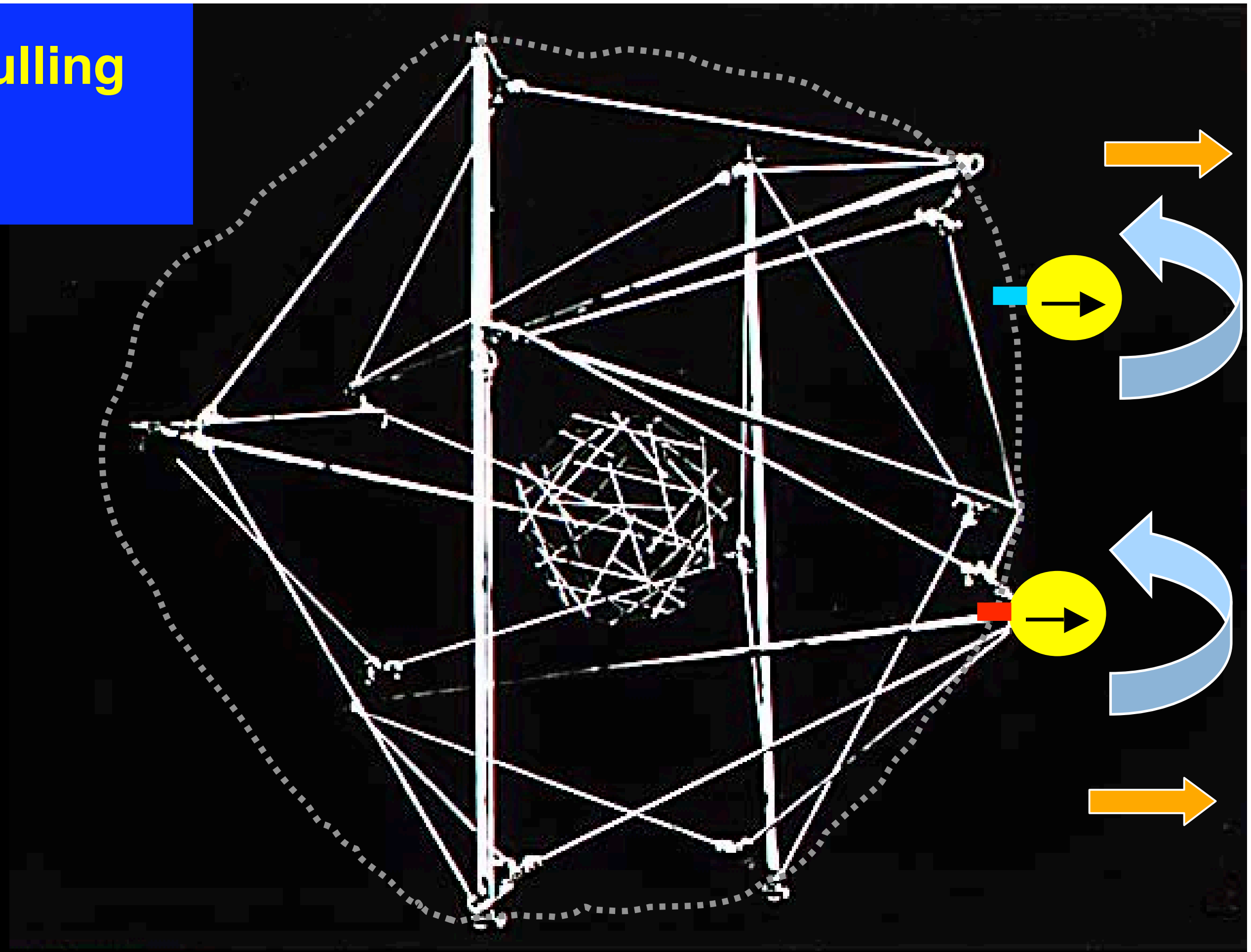
Tensegrity predicts Adhesion Receptors act as Mechanoreceptors

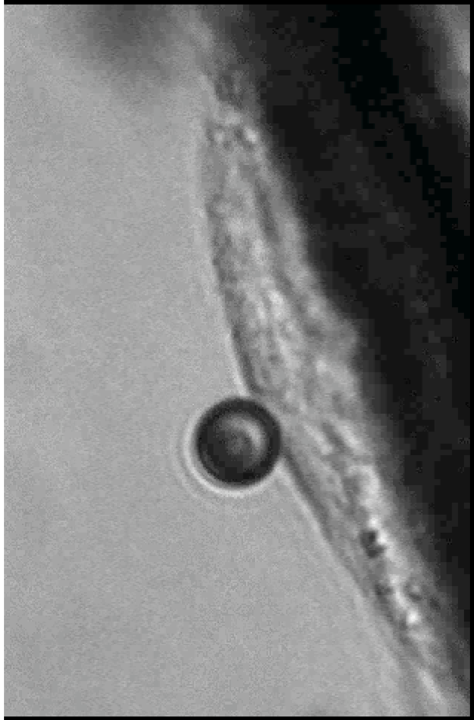




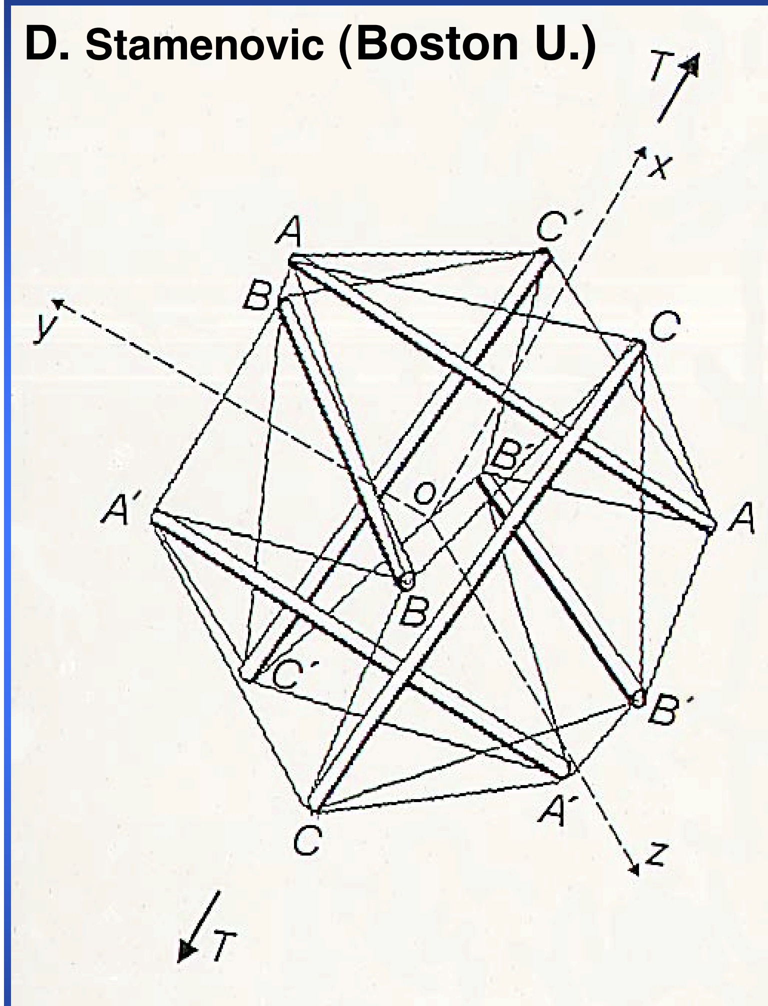
(Ingber and Jamieson, 1985;
Ingber, Curr Opin Cell Biol 1991)

Magnetic Twisting & Pulling Cytometry





Mathematical Tensegrity Model of the Cell



Stamenovic et al. J. Theor. Biol.1996
Coughlin & Stamenovic, J App Mech 1997,1998
Stamenovic & Coughlin, J Theor Biol. 1999
Stamenovic & Coughlin, J. Biomech. Engin. 2000
Wang & Stamenovic, Am J. Physiol Cell Physiol. 2000
Stamenovic,J. Biomech.,2005.

A Priori Predictions now Confirmed:

•Linear relation between Stiffness and *Applied* Stress
(Wang et al., Science 1993; Wang and Ingber, Biophys. J. 1994))

•Cell Mechanics depends on Prestress
(Wang & Ingber, Biophys. J. 1994; Lee et al., Am. J. Physiol. 1997)

•Linear relation between Stiffness and *Prestress*
(Wang et al., PNAS 2001; Wang & Stamenovic, Am. J. Physiol 2002)

•Hysteresivity independent of prestress
(Maksym et al.,Am. J. Phys. 2000;Wang et al., PNAS 2001)

•Quantitative Prediction of Cellular Elasticity
(Stamenovic and Coughlin, J. Biomech. Engineer. 2000)

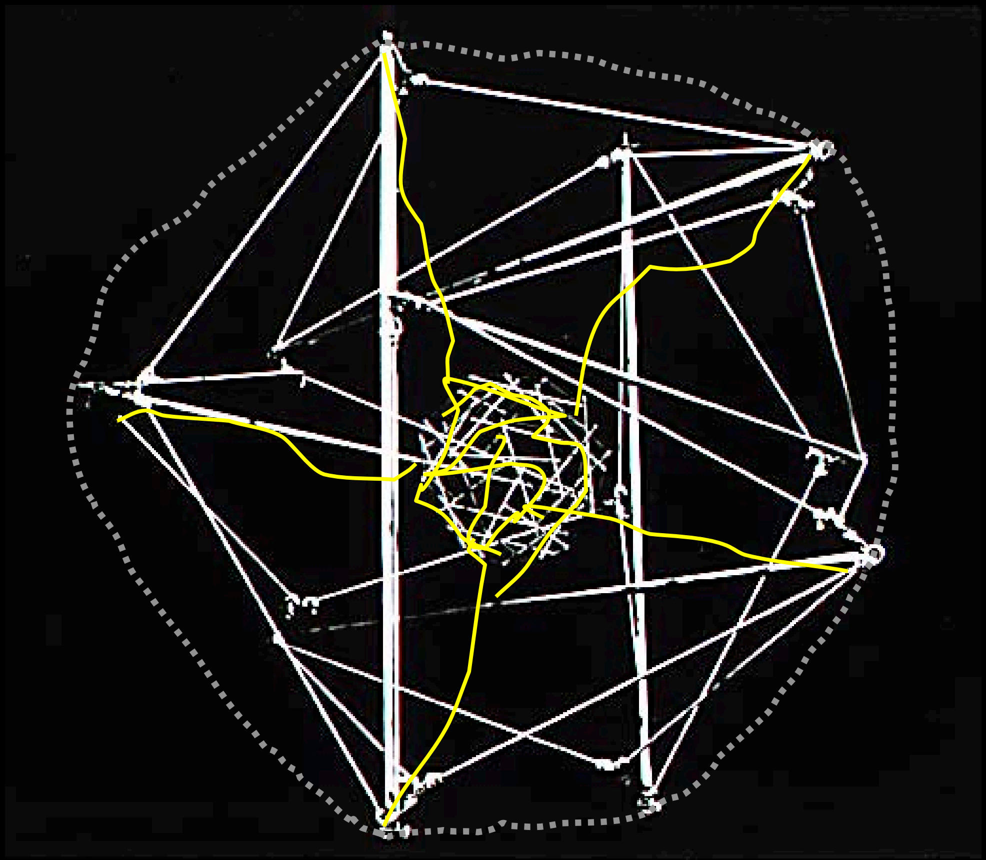
• Prediction of Dynamic Mechanical Behavior
(Sultan et al., Ann Biomed Engin. 2004)

• Mechanical Contribution of Intermediate Filaments to Cell Mechanics
(Wang and Stamenovic, Am J Physiol Cell Physiol, 2000)

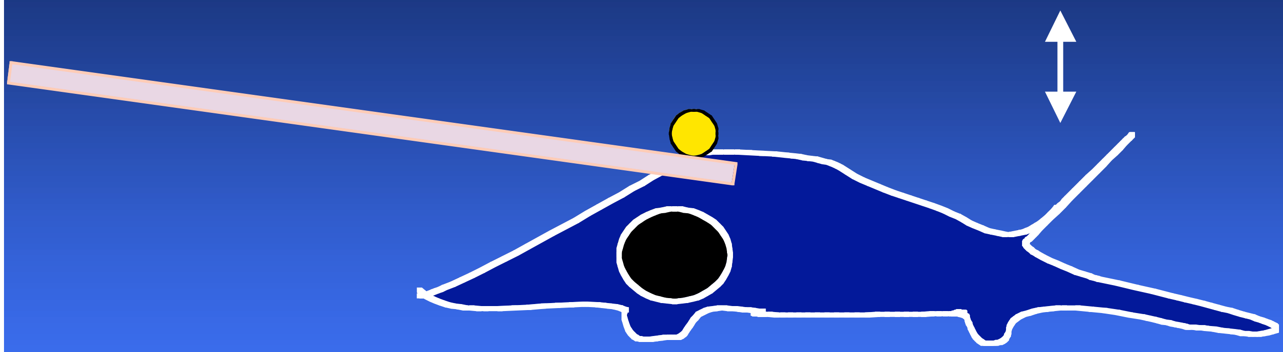
• Microtubules Bear Compression
(Keach et al., 1996; Wang et al., 2001; Hu et al., Bioscience, 2004; Brangwynne et al., J Cell Biol 2006)

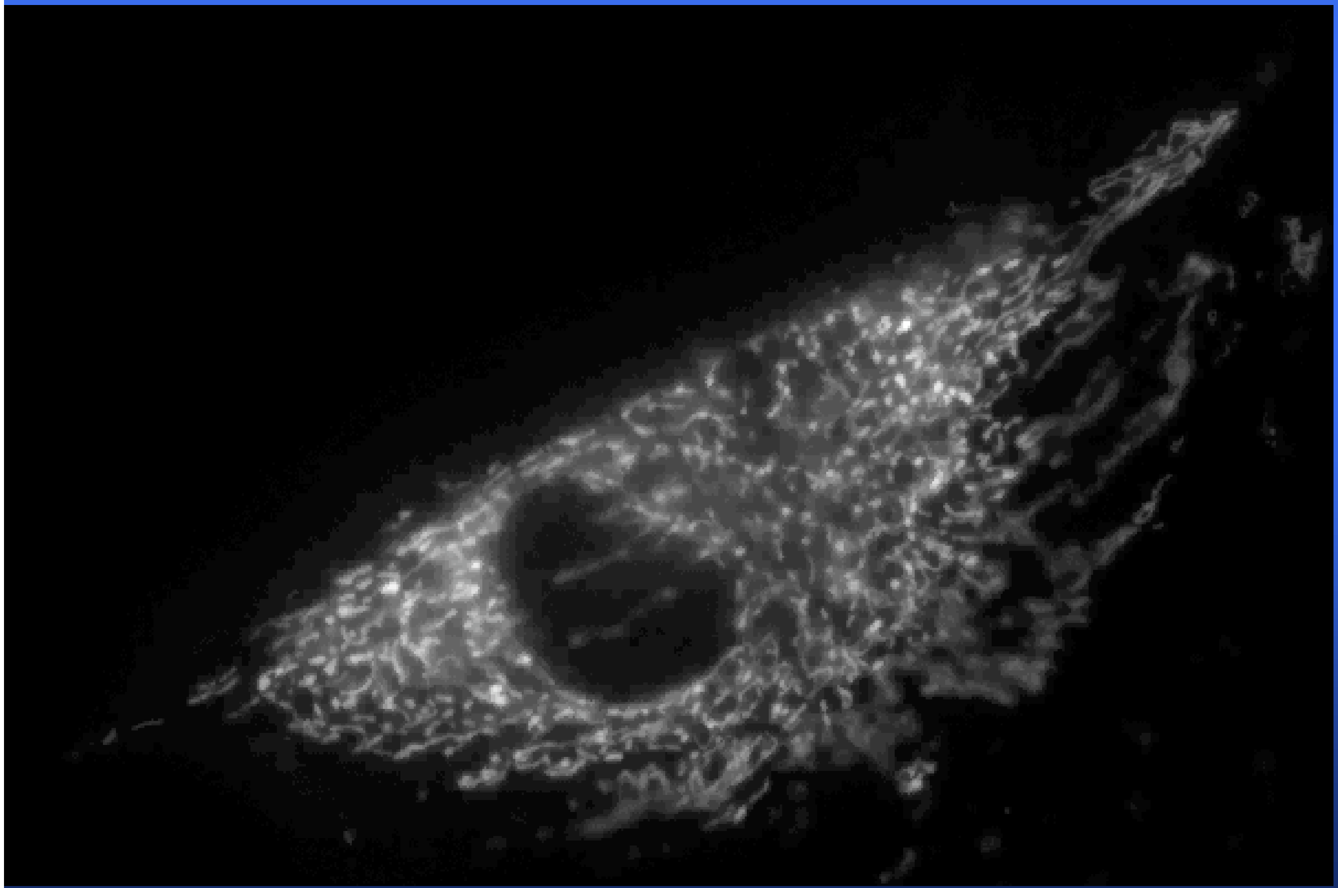
‘Hierarchical’ Tensegrity Model

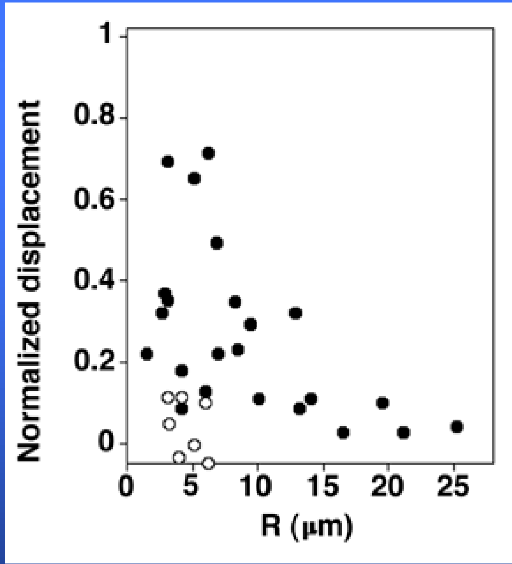
(Cell & Nucleus Connected by Tension Elements)



A local stress can produce DISTANT responses



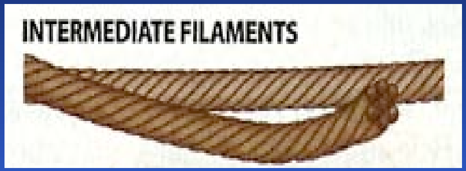




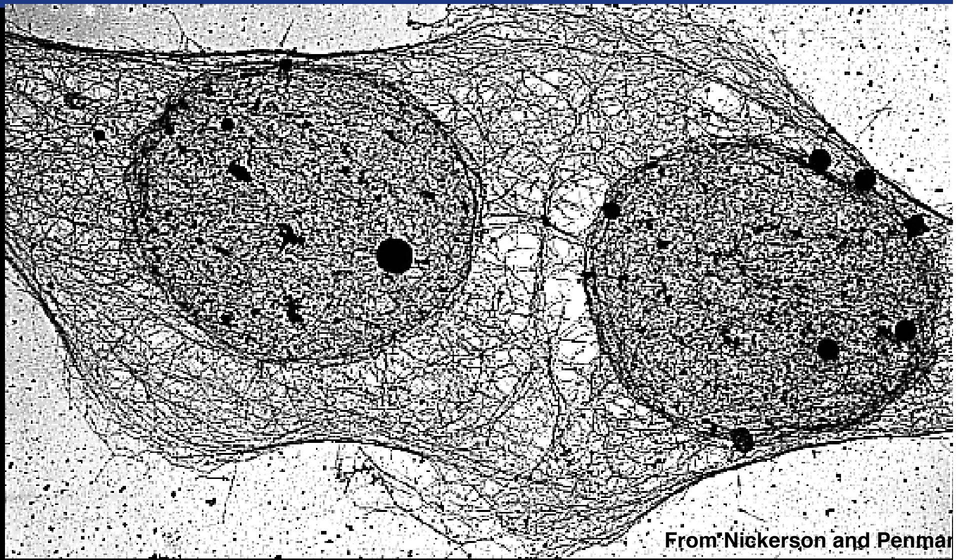
(Wang et al. *PNAS* 2001)

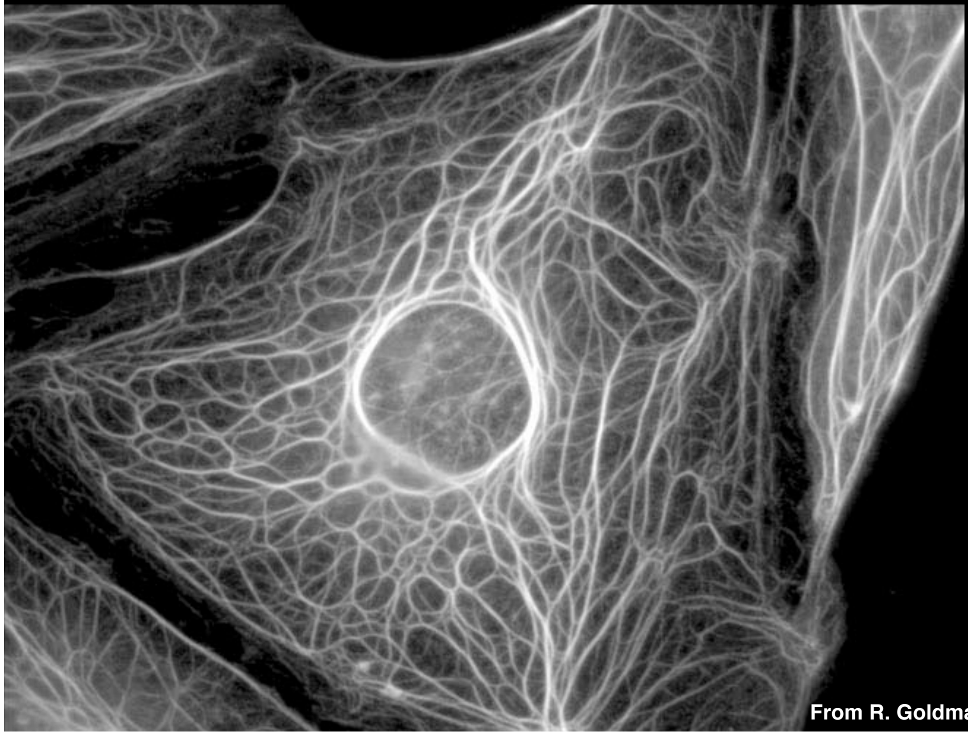
Intermediate Filaments are Suspensory Cables

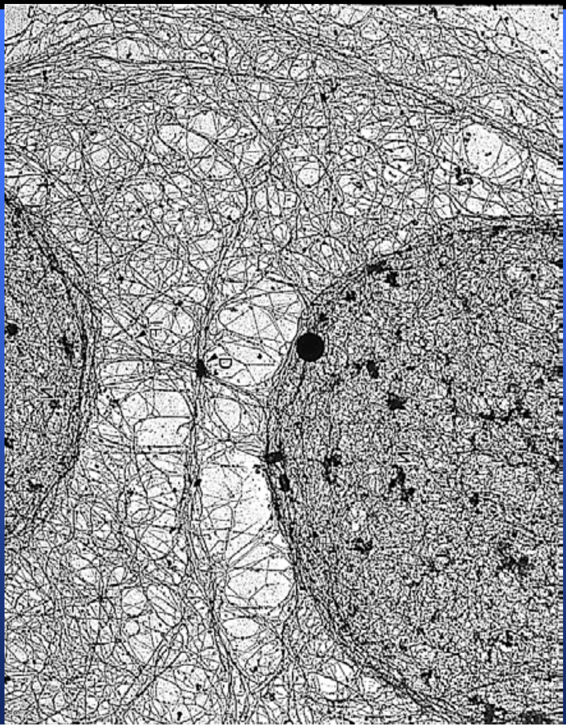
(Maniotis et al. PNAS 1997; Eckes et al. JCS1998)



Link other filaments & membrane to the nucleus

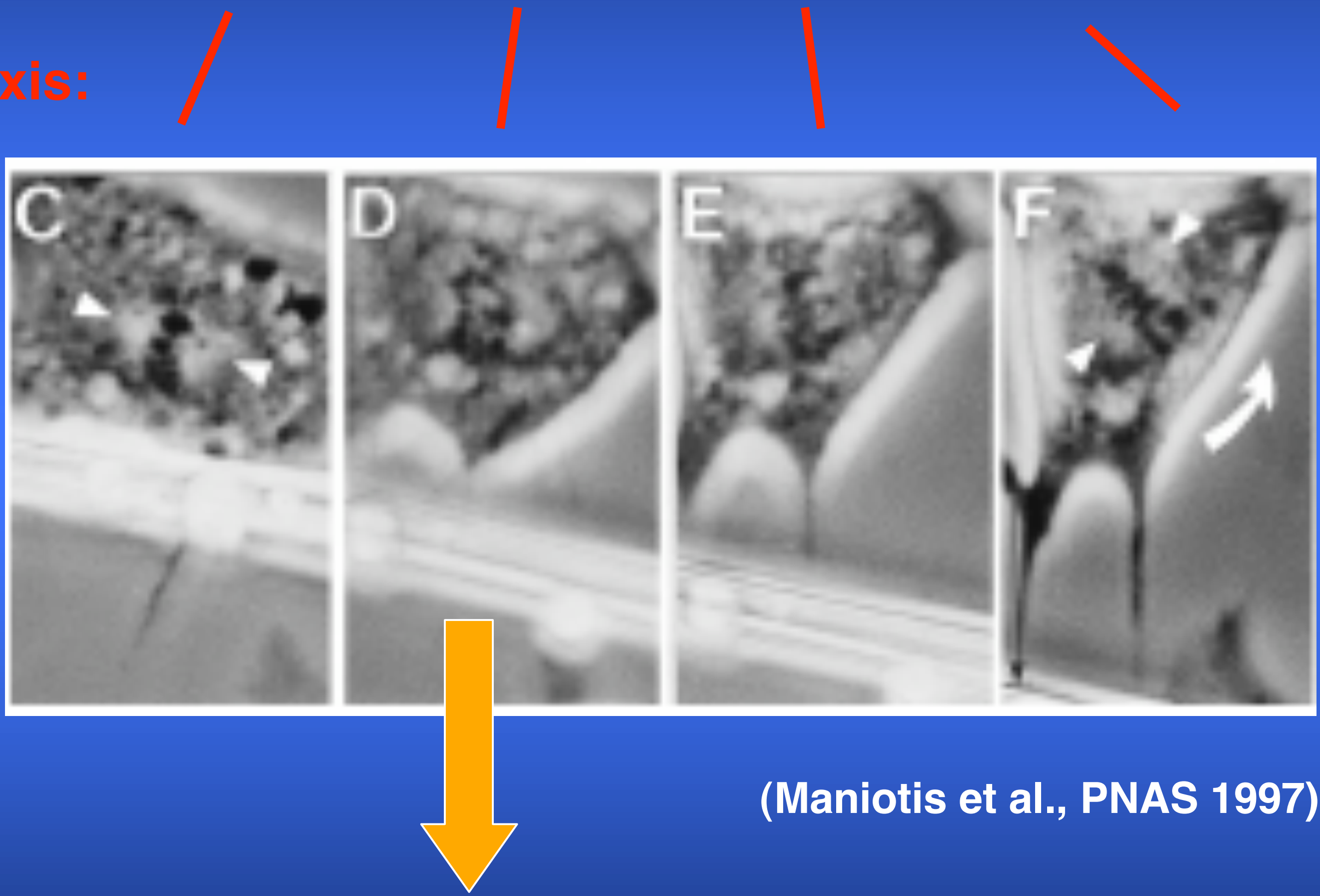






Mechanical Control of the Mitotic Spindle Axis

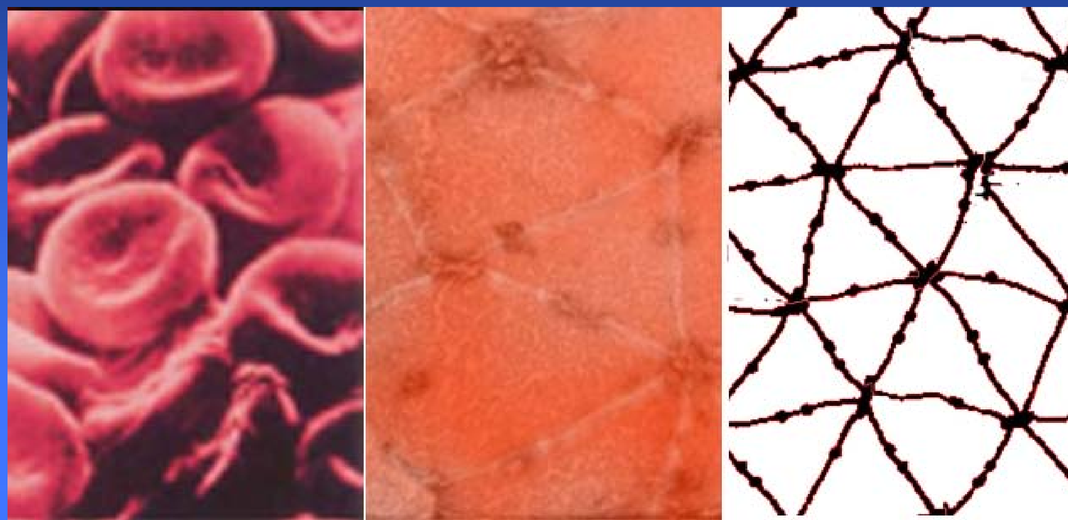
Spindle Axis:



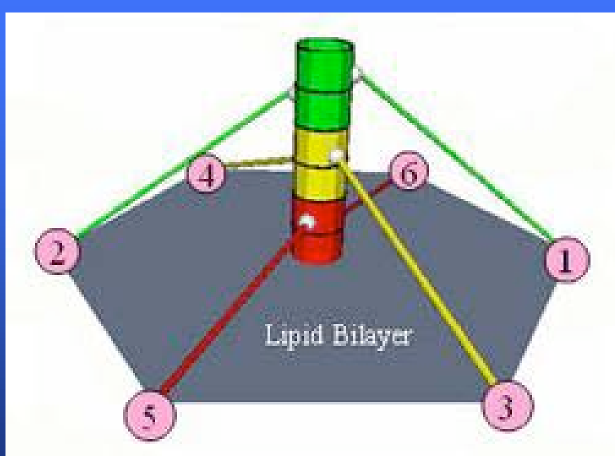
(Maniotis et al., PNAS 1997)

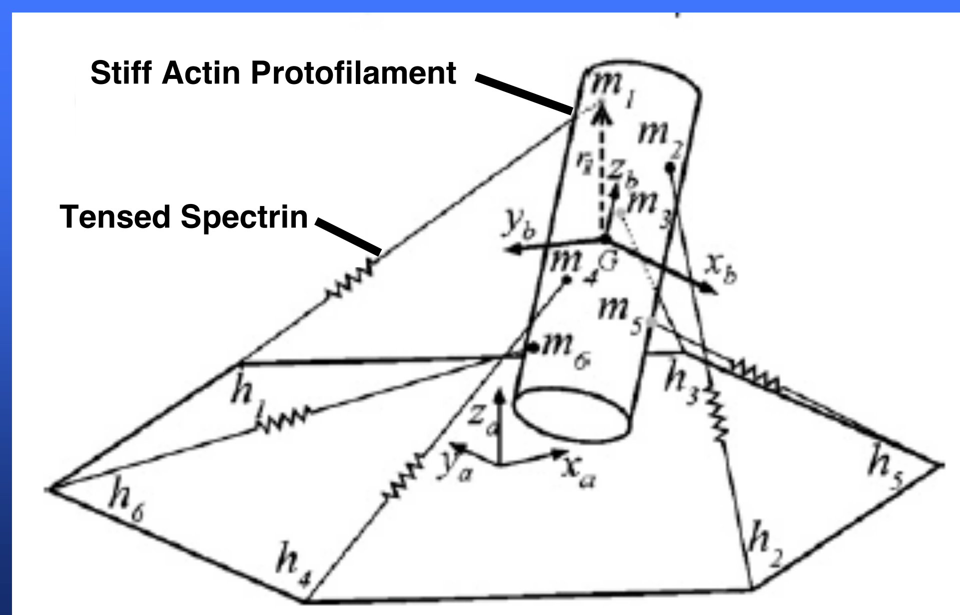
Cortical Membrane as a Prestressed Tensegrity

(Vera et al. *Annals Biomed. Engin.* 2005; with Bob Skelton, UCSD)



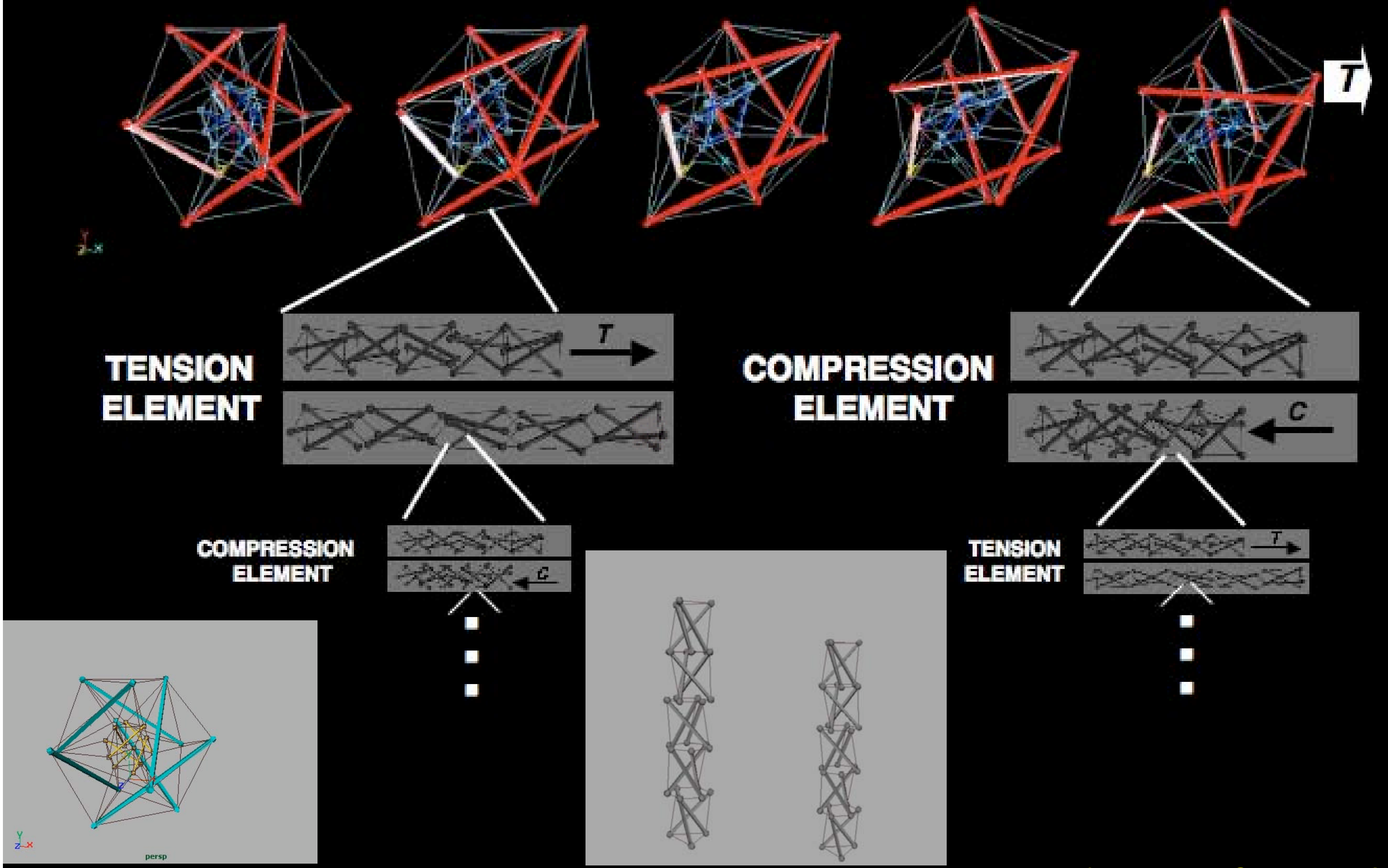
www.jacobsschool.ucsd.edu/news_events/releases/release.sfe?id=484





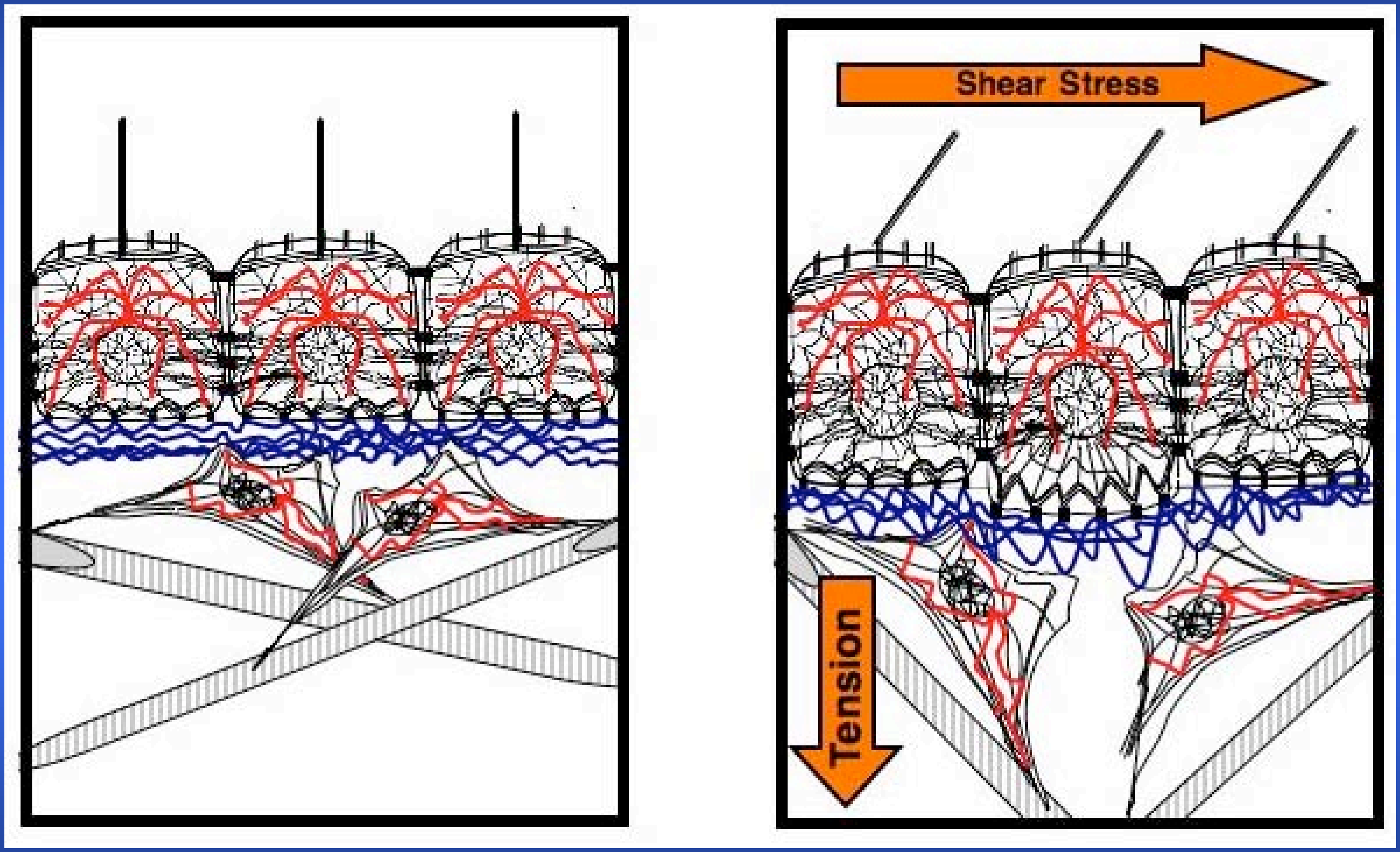
Tensegrity-Based Hierarchical Integration

(computer images by Eddy Xuan, U. Toronto)

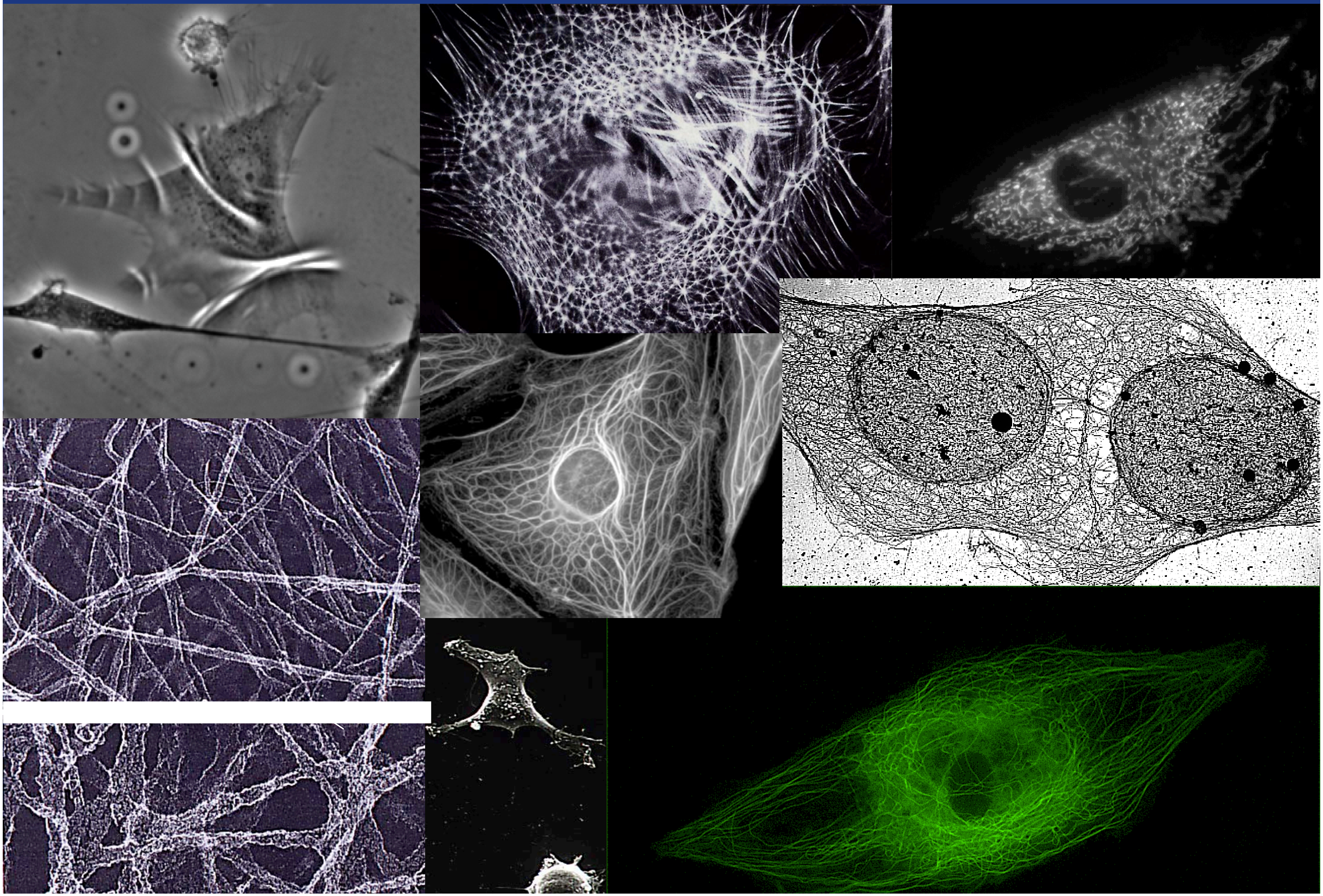


(Ingber, *FASEB J* 2006)

Tensegrity Focuses Force on Molecules in the Extracellular Matrix and Cytoskeleton



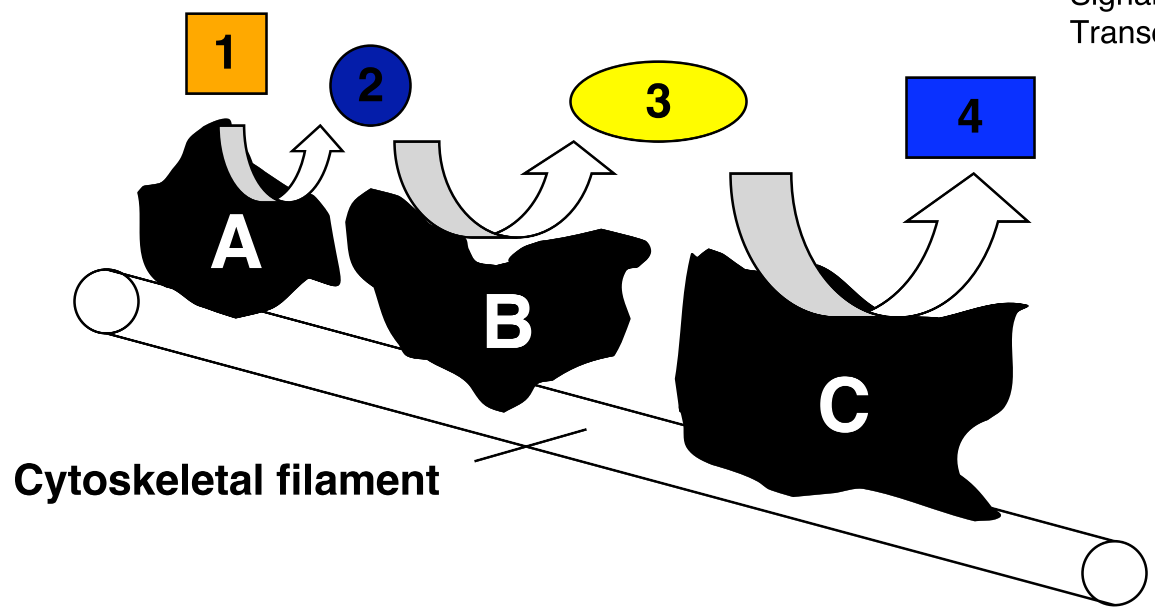
Cytoskeleton is More than a Mechanical Scaffold



Solid-State Biochemistry on Cytoskeletal Scaffolds
(Structure = Catalyst)

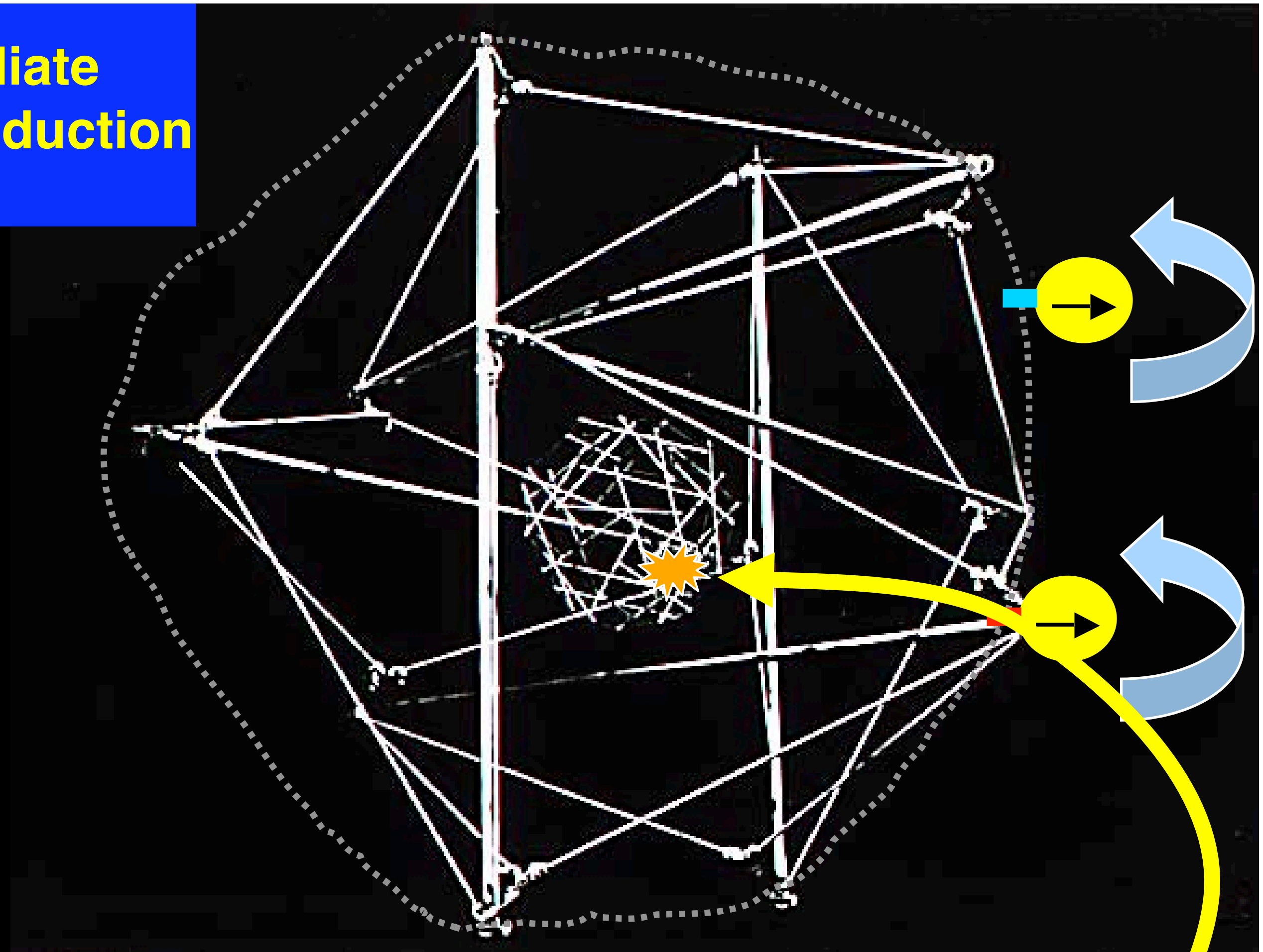
'Channeling' of Chemical Reactions:

DNA replication
RNA Processing
Transcription
Translation
Glycolysis
Signal
Transduction



(Ingber, *Cell* 1993)

Surface Integrins Mediate Mechano-Chemical Transduction

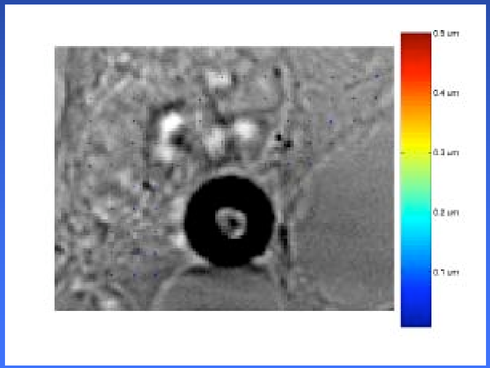


Pulling on "Integrins" Activates Signaling & Gene Transcription

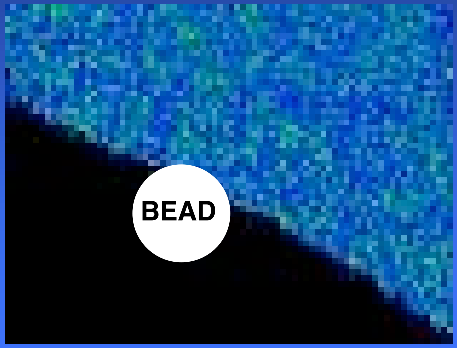
Pulling on Integrins Specifically Activates Ca^{+2} Influx

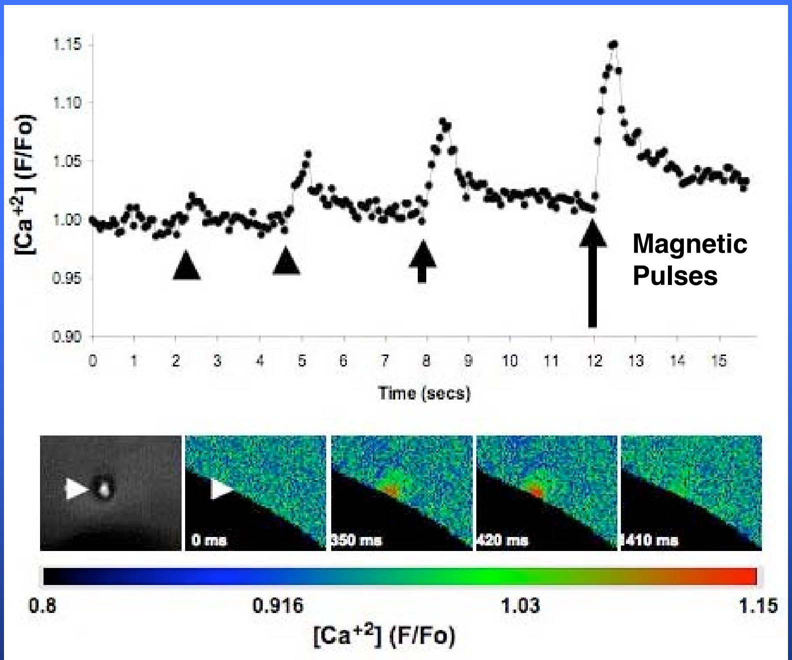
(Time scale < 10 milliseconds) *[Funded by DARPA & NIH]*

Cytoskeletal Strain

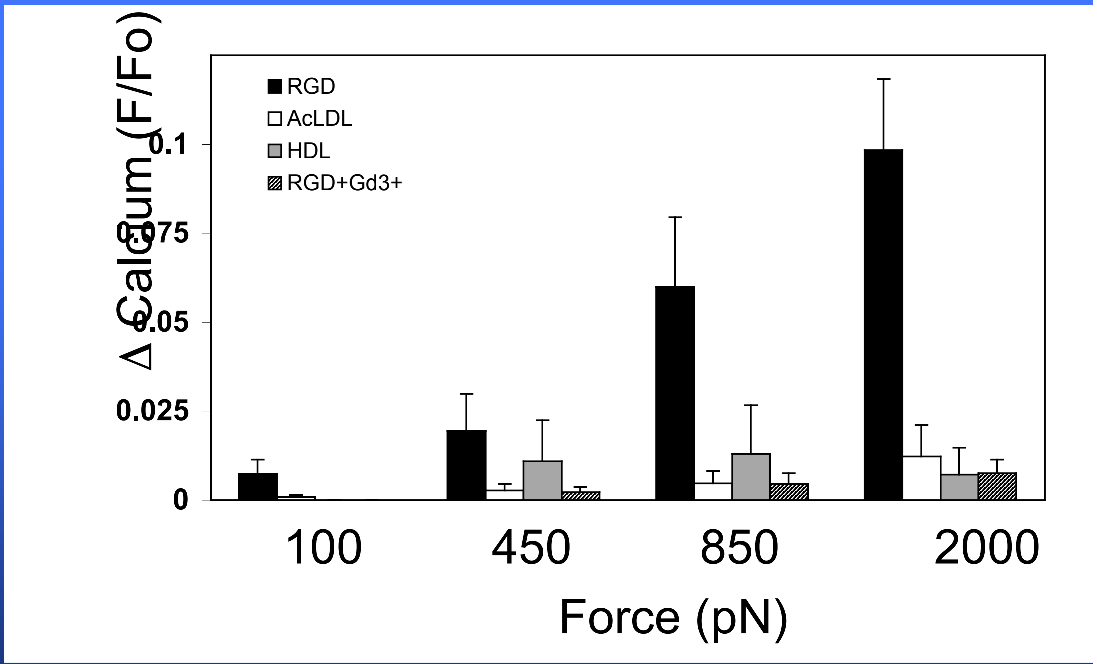


Calcium (FLUO4)





Force Dependence

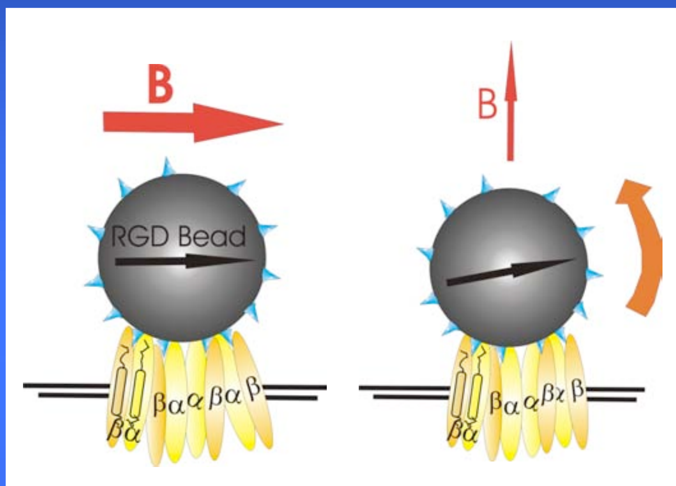


Force & Integrin Dependence

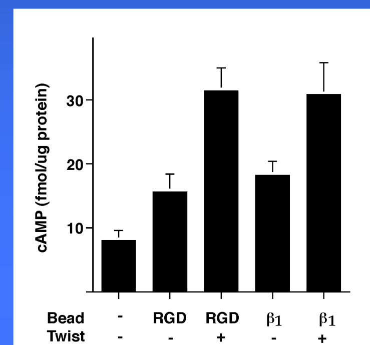
(Matthews et al., in review)

Mechanical Control of Gene Transcription

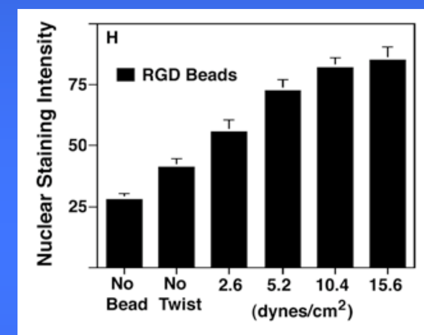
Activation of G_α proteins and cAMP Signaling by Mechanical Force Transmitted Across Integrin Receptors



+ TWIST

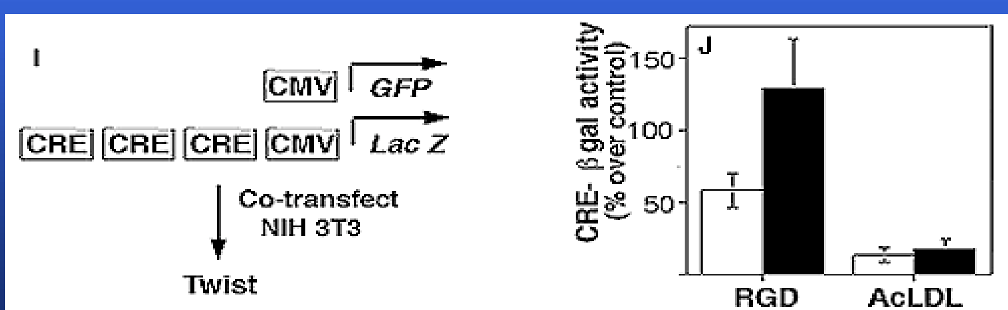


cAMP Levels

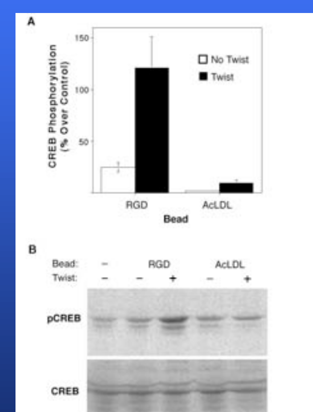


PKA Translocation

Gene Transcription

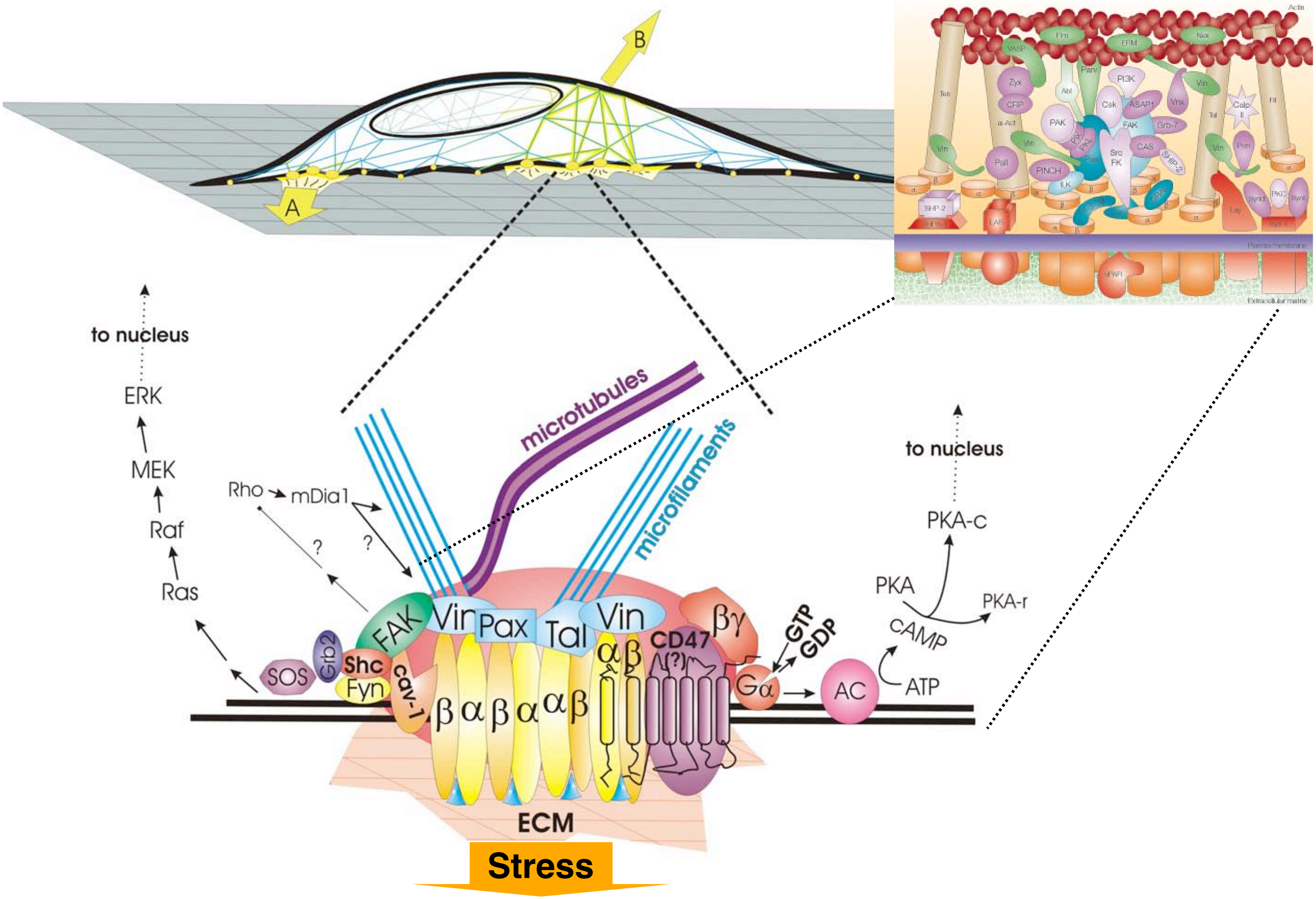


(Meyer et al., *Nature Cell Biol.* 2000)

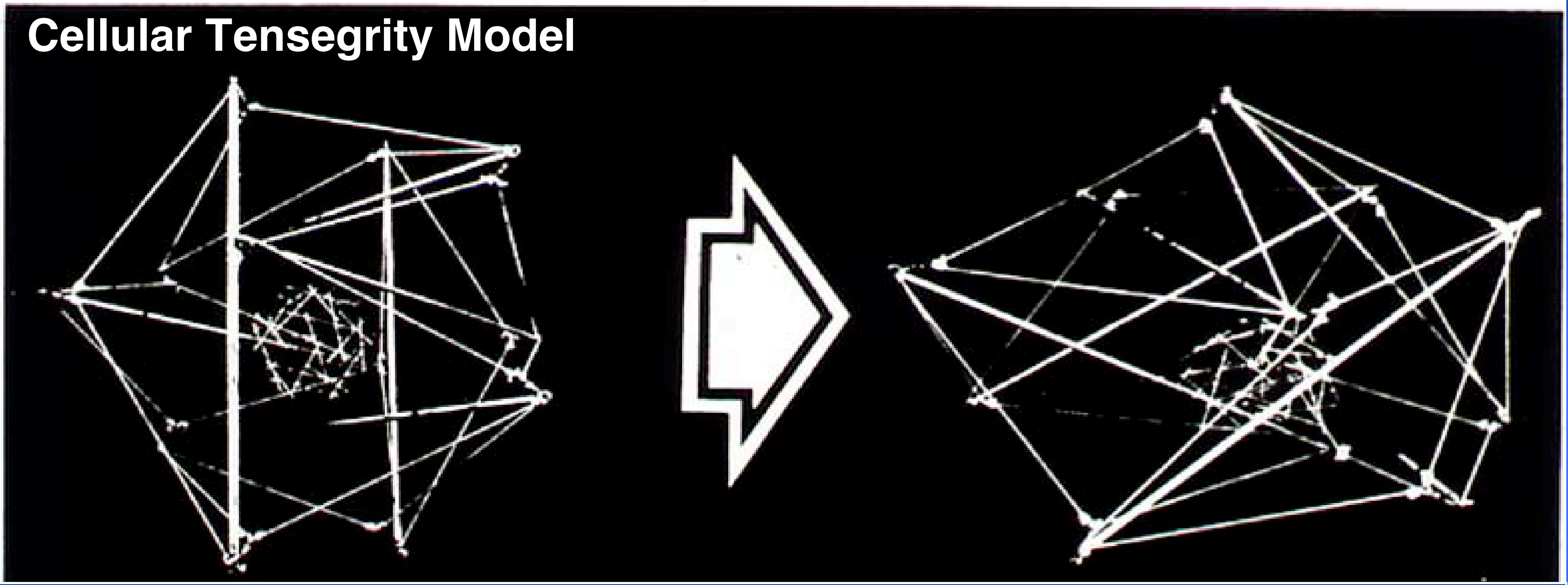


CREB Activation

Focal Adhesion is a Nanoscale Mechanochemical Machine

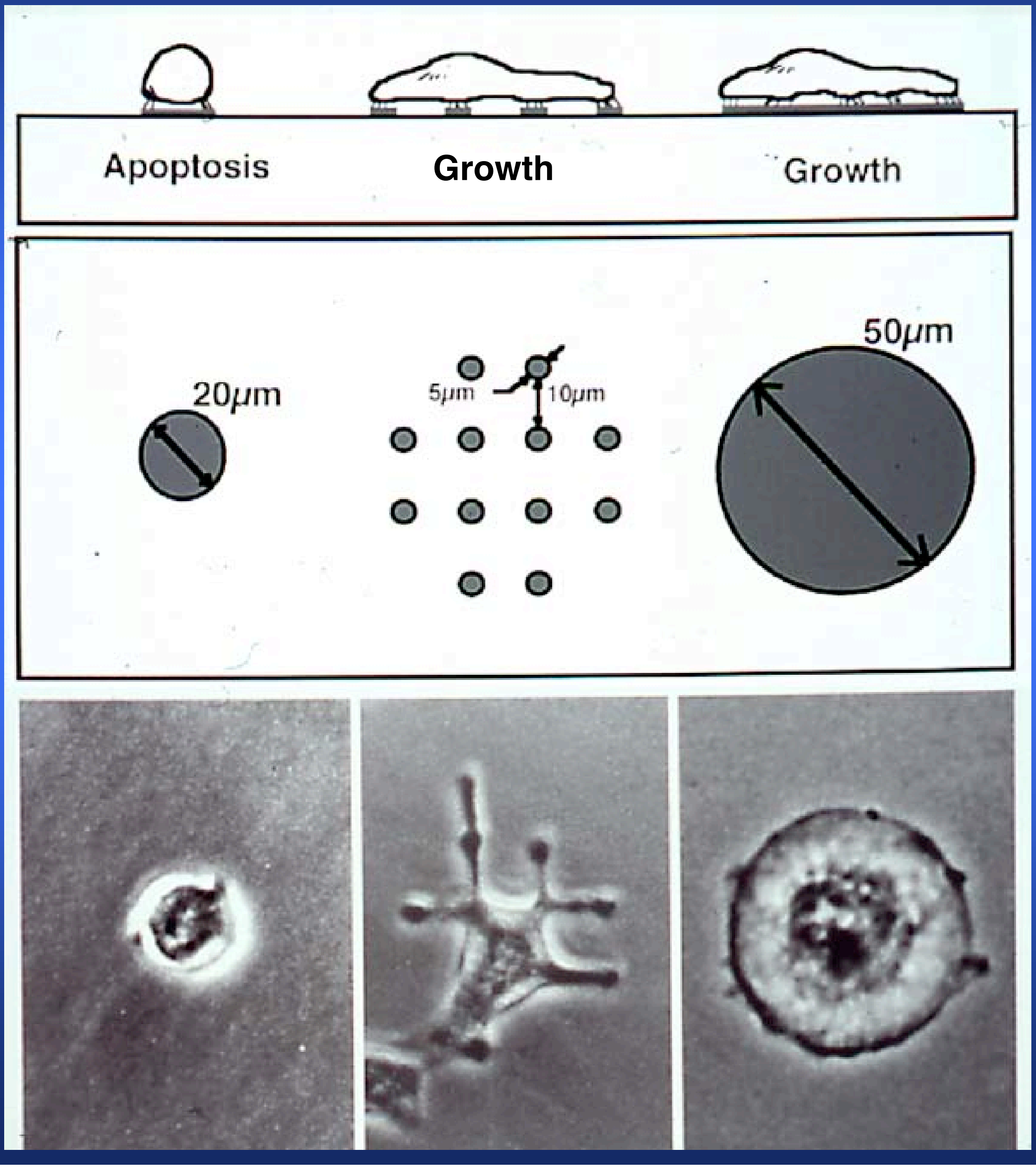


Mechanochemical Transduction

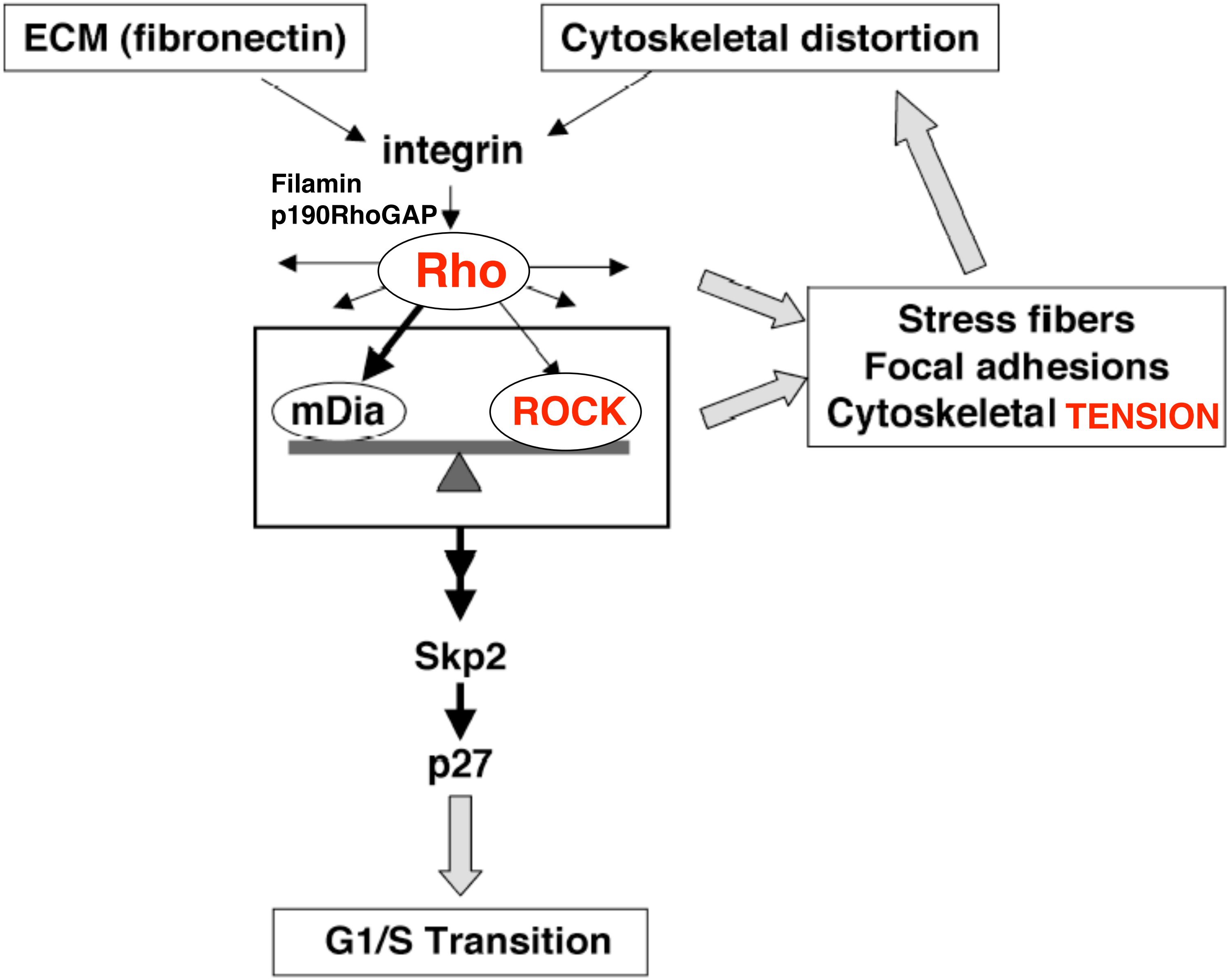


Tensegrity provides a mechanism to *INTEGRATE* both LOCAL and DISTANT structural responses when forces are transmitted through the cytoskeleton

Signal Integration through Cell Distortion: Cells Act Locally, but "Think" Globally

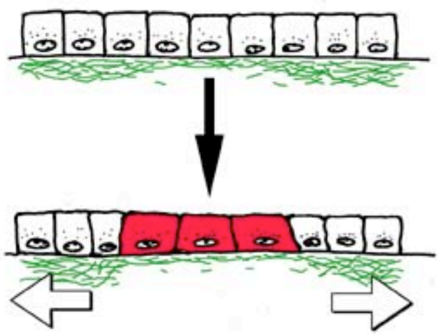


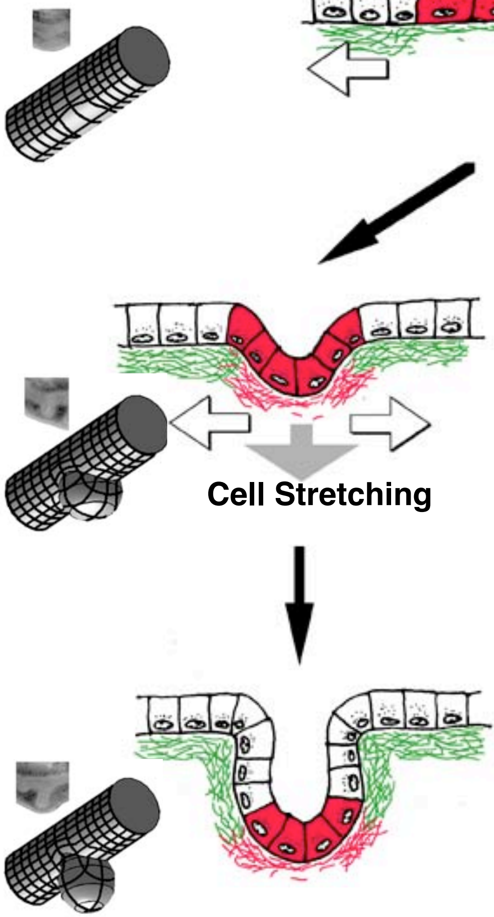
The Small GTPase Rho Mediates Shape Control of Growth

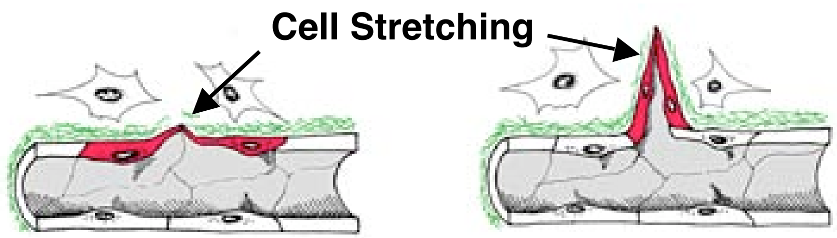


(Huang et al., *Mol. Biol. Cell*, 1998; Huang & Ingber, *Exp Cell Res.* 2002; Numaguchi et al., Angiogenesis 2003; Mammoto et al., *J. Biol. Chem. 2004;* Mammoto et al., *J. Cell Sci. 2007*)

Micromechanical Control of Morphogenesis







Underlying Hypothesis:

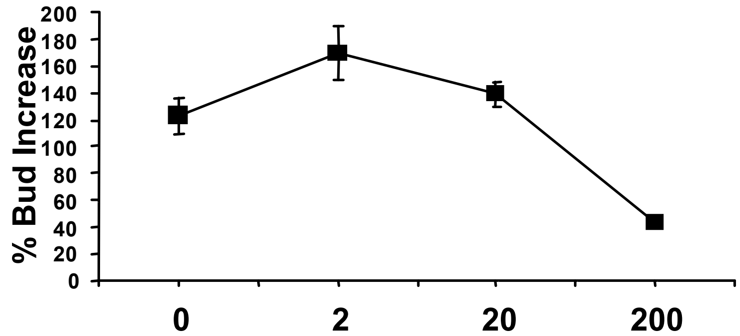
- **ECM remodeling changes LOCAL MECHANICS**
- **Increasing ECM flexibility promotes cell stretching**
- **Tension on adhesion receptors & distortion of the cytoskeleton alters cellular biochemistry**

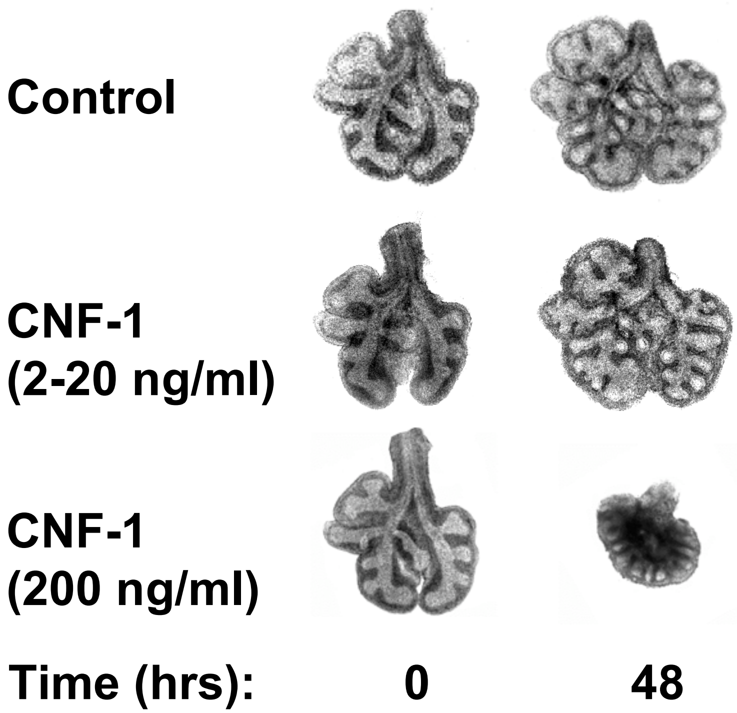
Localized Growth & Motility

(Ingber et al., *PNAS* 78:3901-5, 1981; Ingber & Jamieson, In: *Gene Expression During Normal & Malig. Differ.* ,1985; Huang and Ingber, *Nature Cell Biol.* ,1999)

Developmental Control Requires a Fine Balance of Forces

Bud Inducing Activity

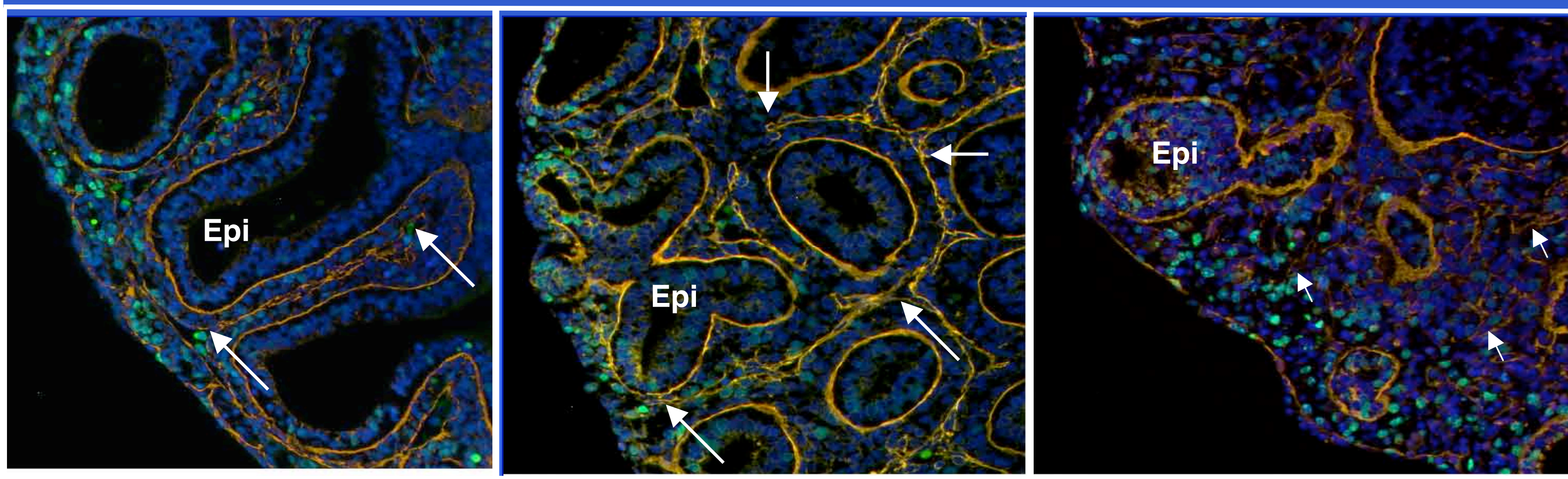




Tension

Tension

Epitheliogenesis & Angiogenesis in Embryonic Lung can be Controlled by Altering Cytoskeletal Tension



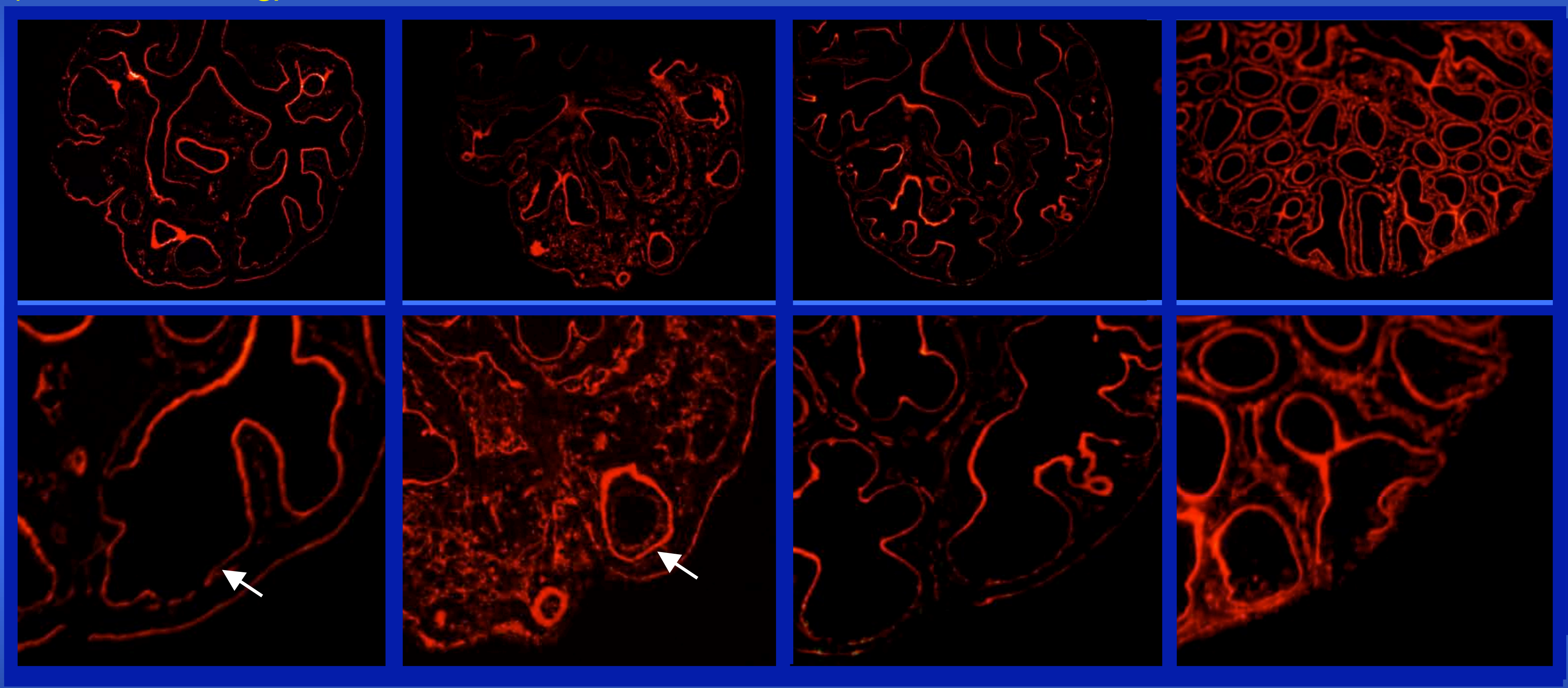
Control | **CNF-1 (20 ng/ml)** | **Y27632 (40 uM)**

↑ **TENSION** | ↓ **TENSION**

(Moore et al, *Dev. Dynamics* 2005)

Dissipation of CSK Tension Prevents ECM Thinning And Inhibits Morphogenesis

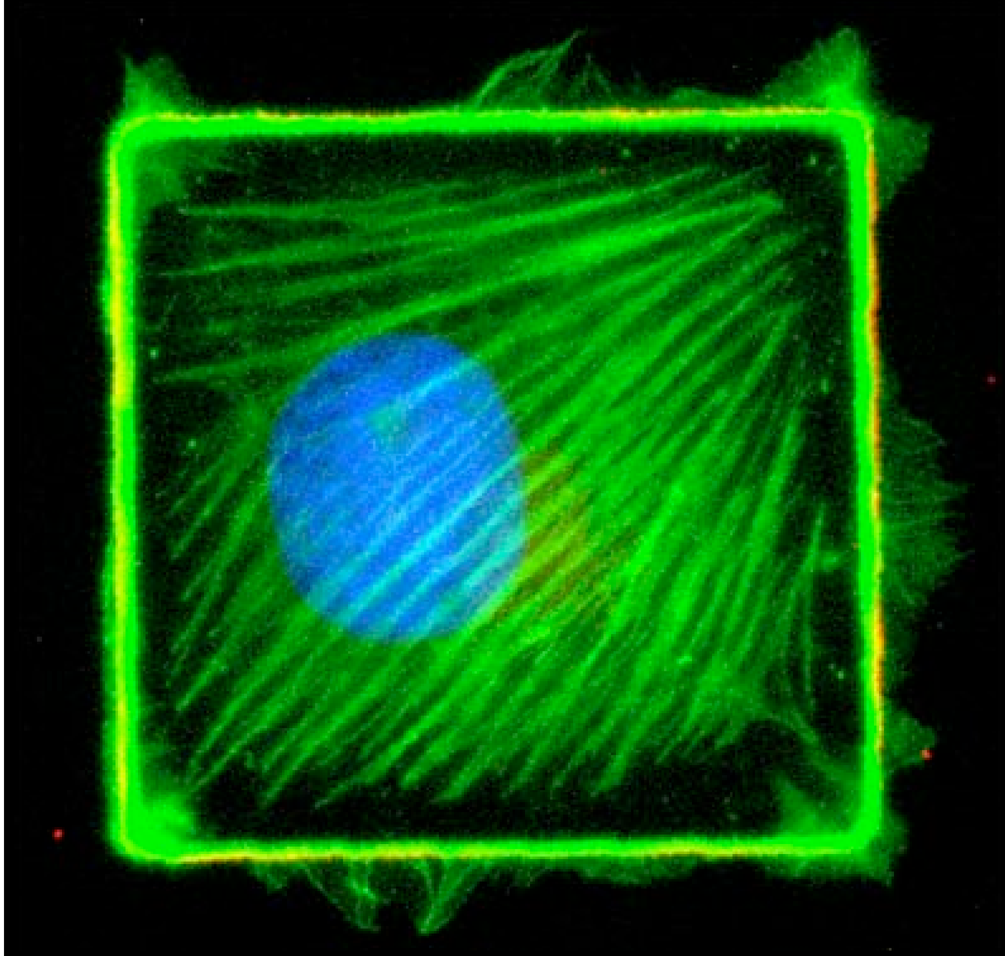
(Laminin Staining)

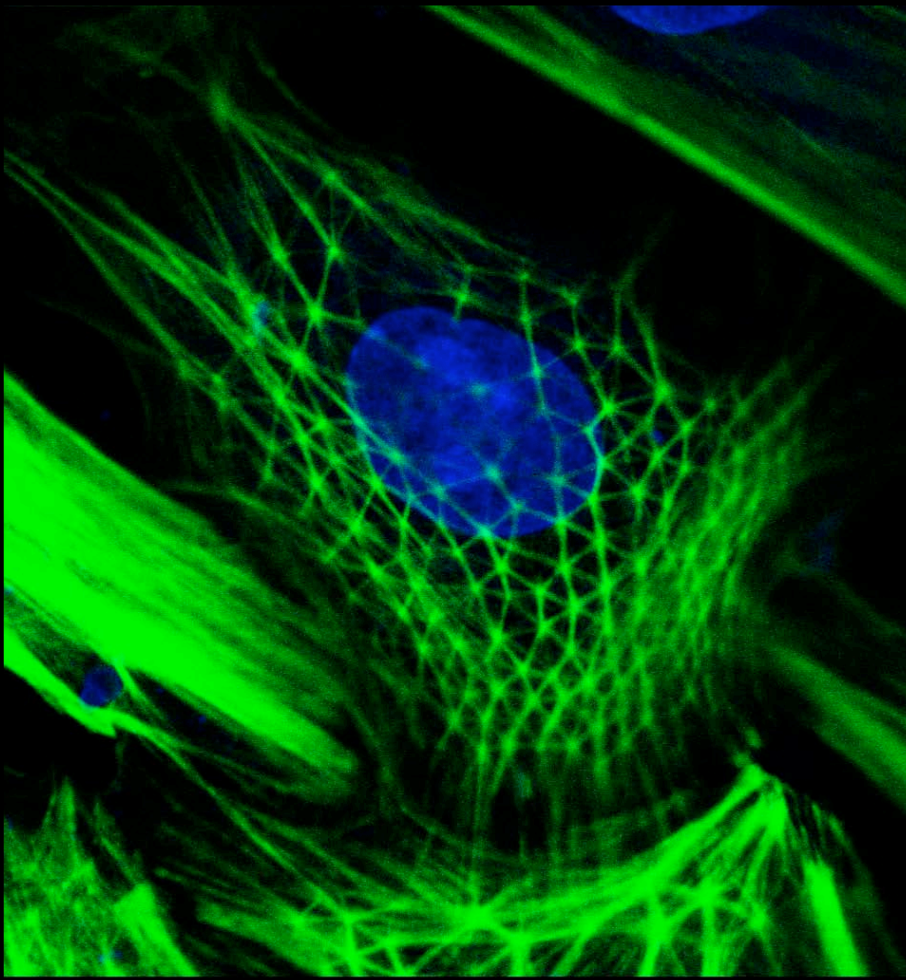


Control Y27632 Rescue CNF-1

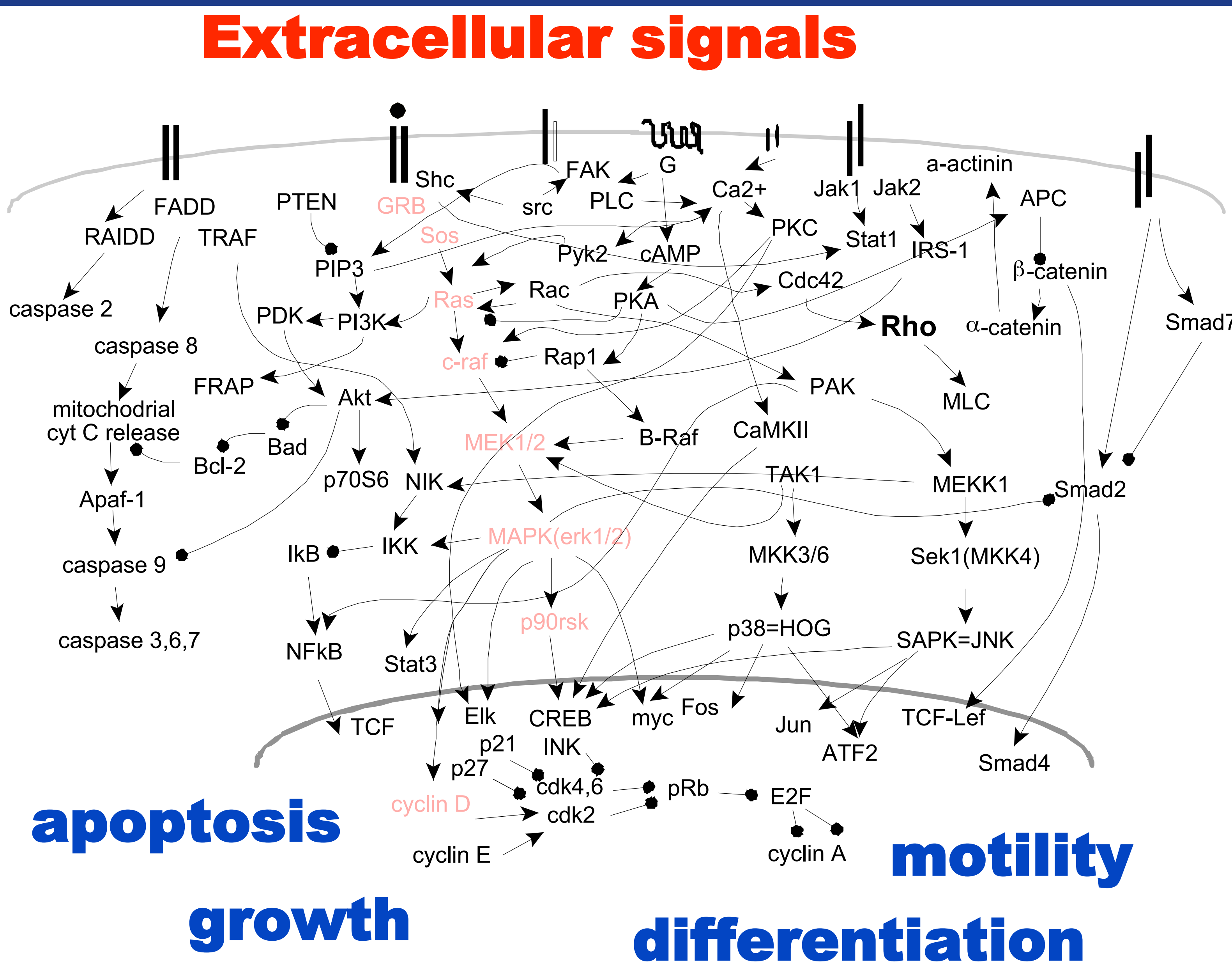
(Moore et al, *Dev. Dynamics* 2005)

Extracellular Matrix & Cytoskeleton not just structural supports
they also are KEY DEVELOPMENTAL REGULATORS
Because they mediate MECHANICAL SIGNALING

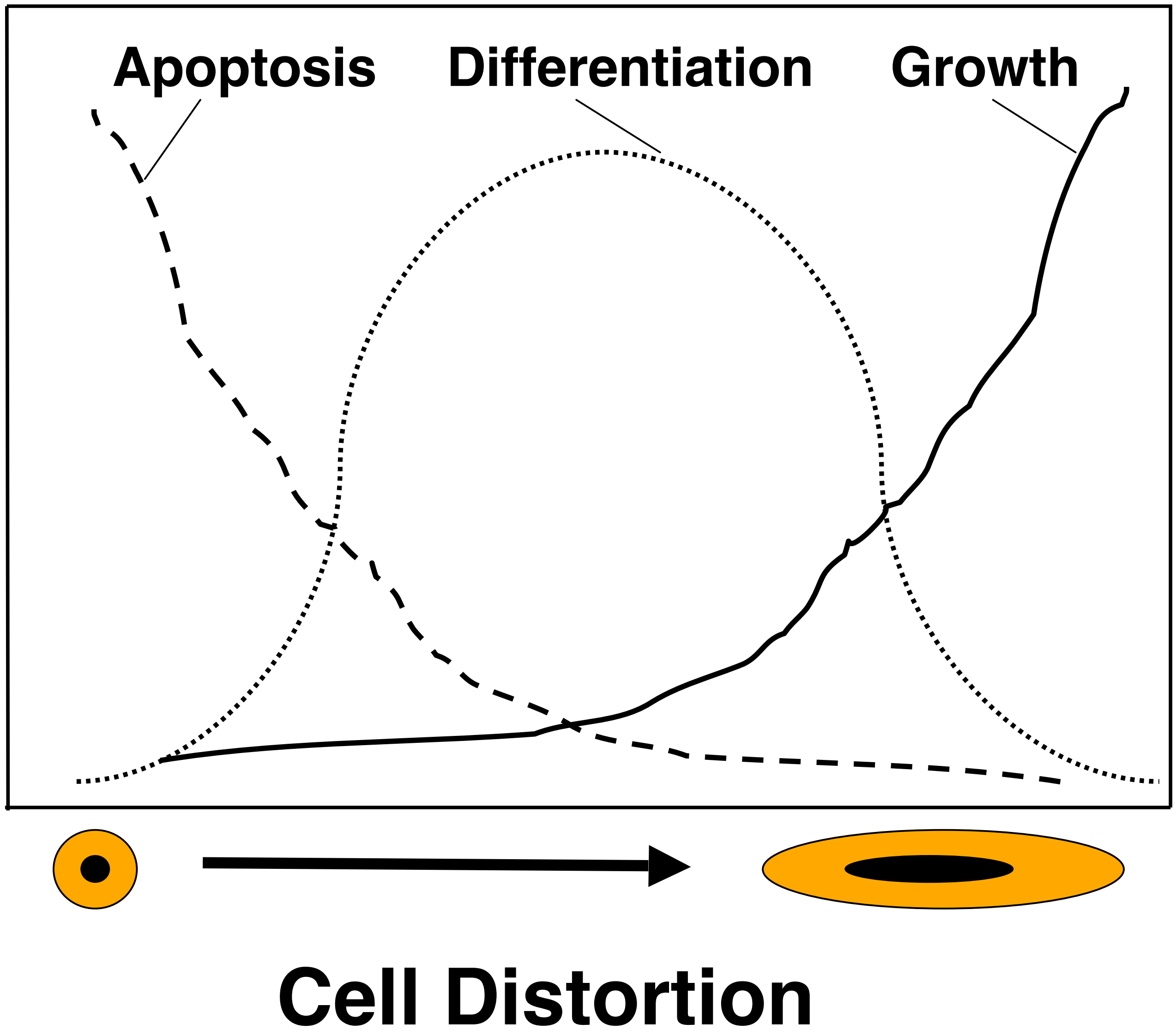




But Where is the Specificity? [SOFTWARE Challenge]



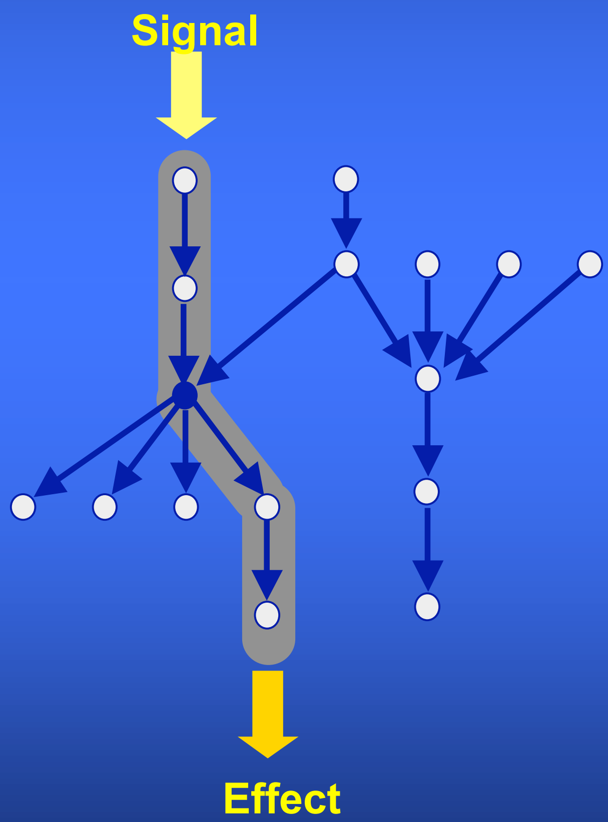
Cell Fate Switching: A Biological "Phase Transition"



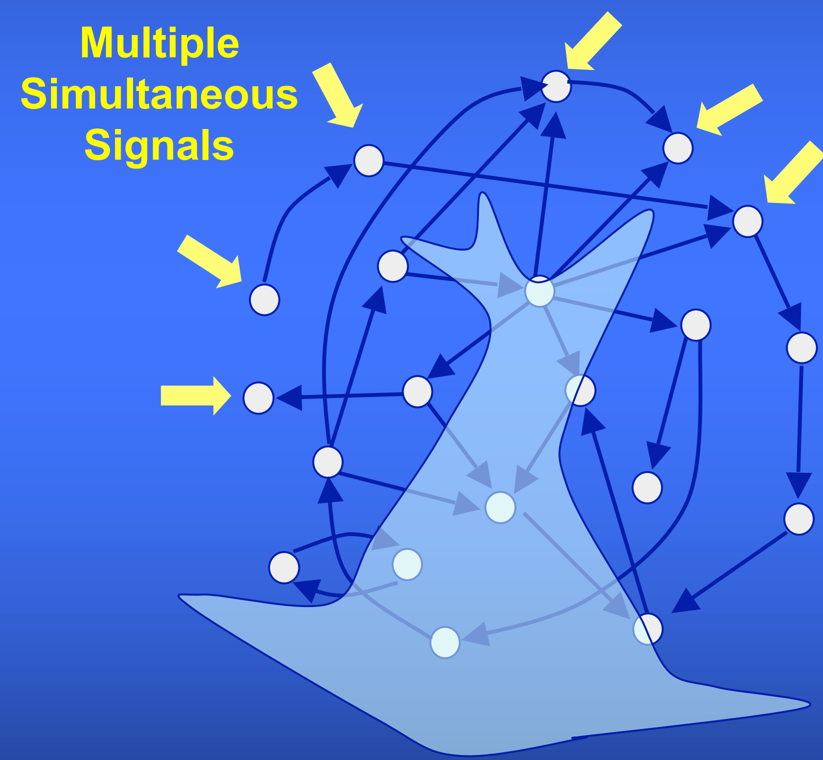
Complex Behaviors are *EMERGENT*

(Distributed vs. Deterministic Regulation)

Pathway view



Network view

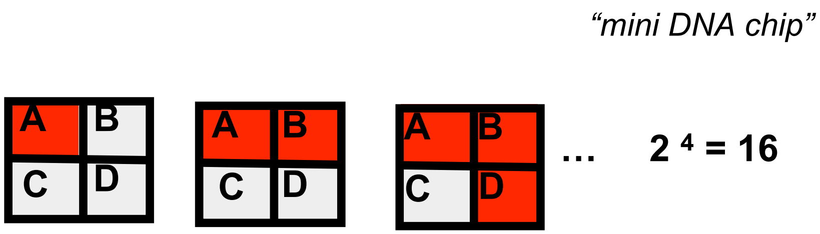


Effect of Collective behavior
(constrained by network interactions)

Stable Functional States Emerge from Dynamic Information Networks

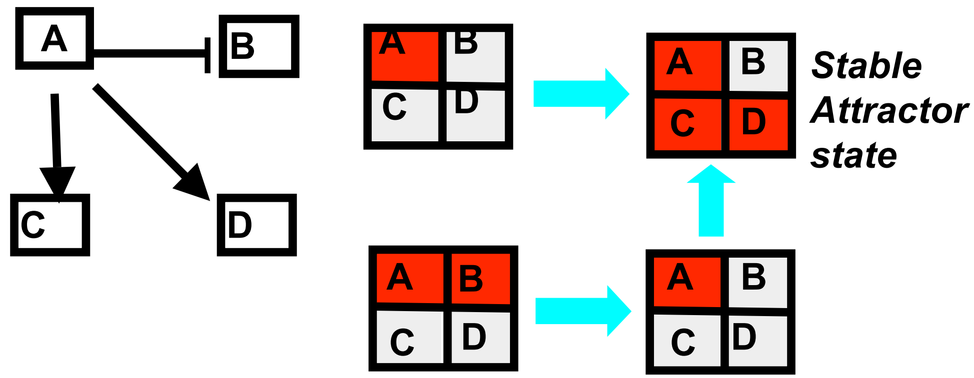
(work from “Complexity Field” by Stuart Kauffman)

If genes were independent:



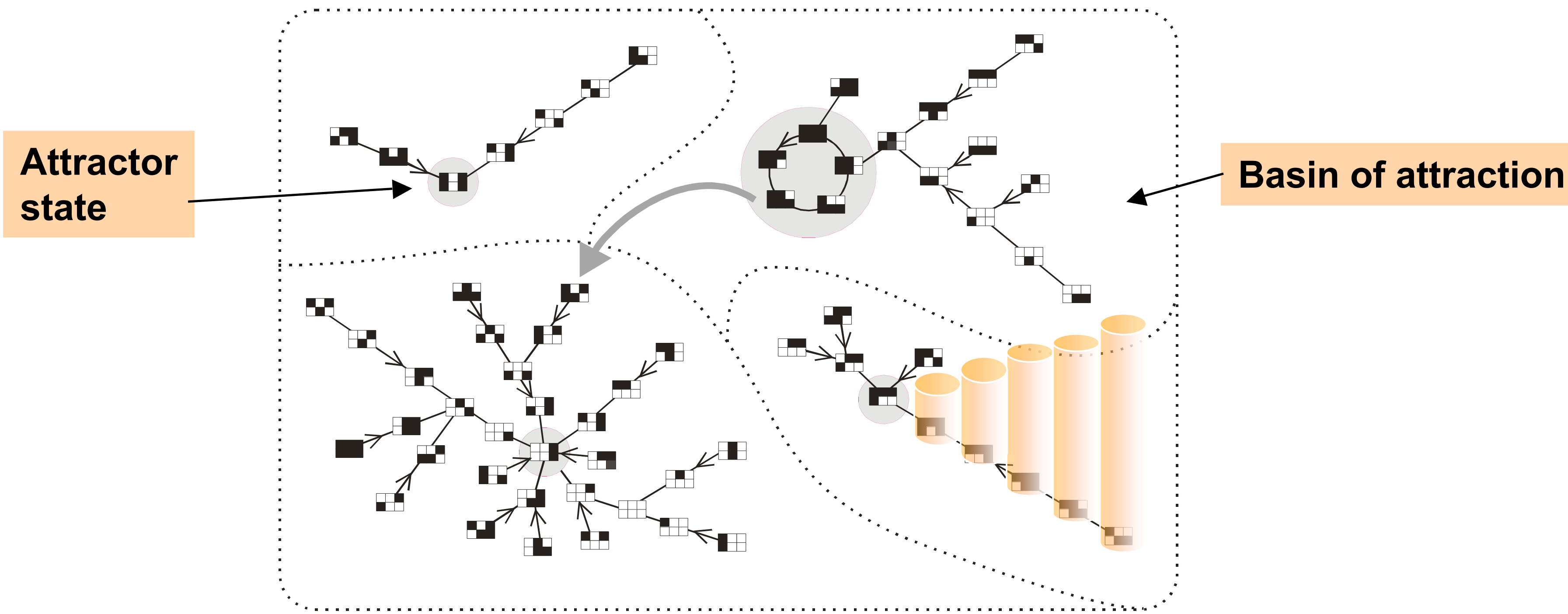
All expression configurations
are equally possible.

If genes are interacting via Logic F(x)s:



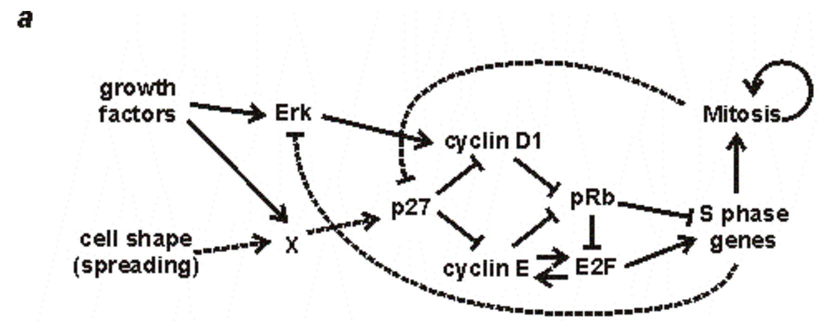
Regulatory interactions CONSTRAIN paths!

Gene activity State Space (for 6-gene network):



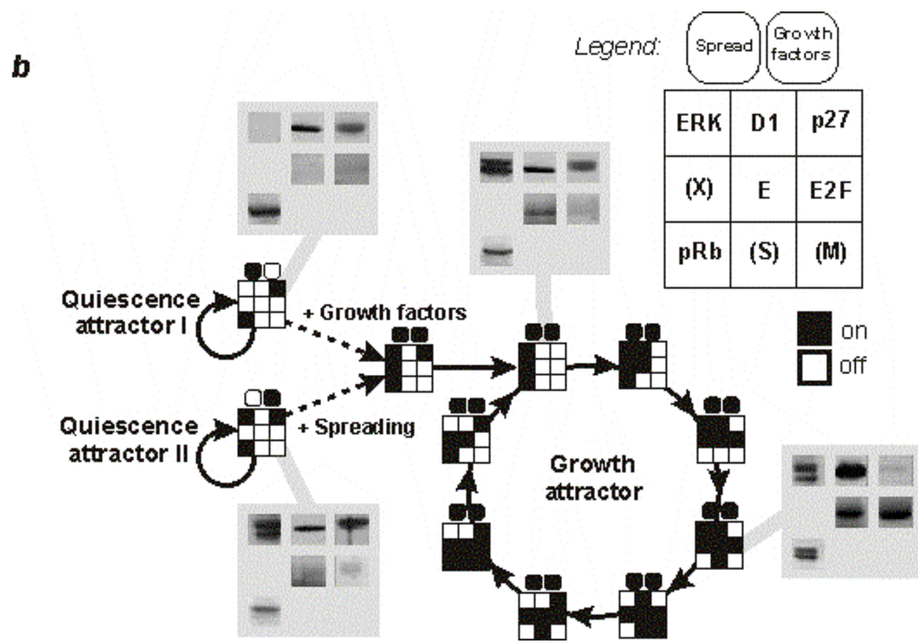
Boolean Network of Cell Cycle Control

a



network element	Erk	D1	p27	(X)	E	E2F	pRb	(S)	(M)
input 1	GF	Erk	(M)	GF	E2F	E	D1	pRb	(M)
input 2	(S)	p27	(X)	spread	p27	pRb	E	E2F	(S)
boolean function	not if	not if	implicat	and	implicat	not if	nand	not if	not if

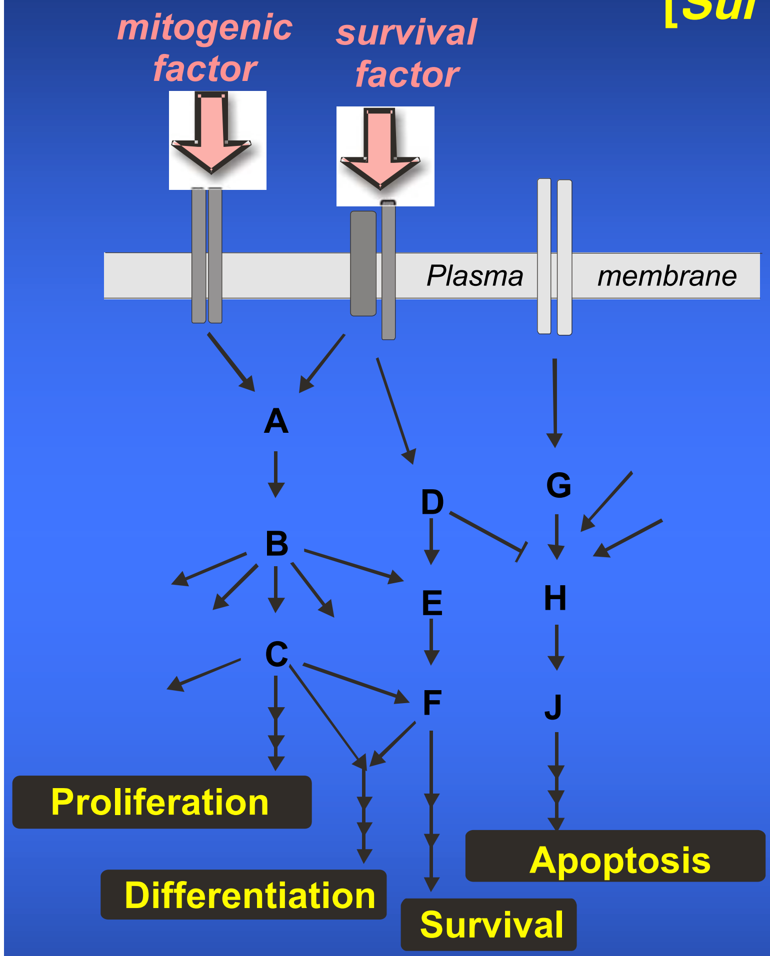
b

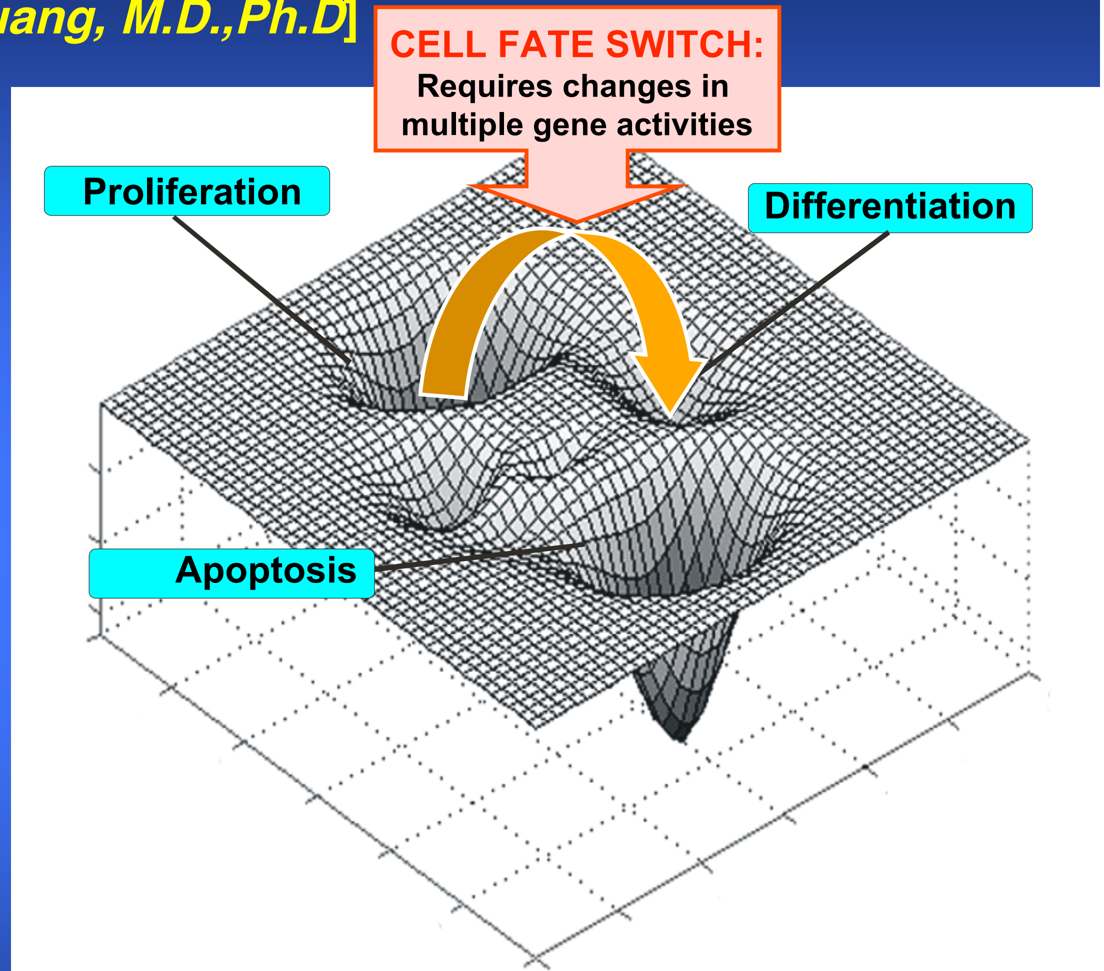


(Huang & Ingber, Exp Cell Res 2000)

Complex Systems Biology:
From Linear Paths to Dynamic Trajectories

[*Sui Huang, M.D.,Ph.D*]





(Huang & Ingber, Exp. Ce l Res. 2000; Ingber, J Cell Sci. Part II 2003)

<u>OLD VIEW</u>:
Linear pathways
No conceptual link to phenotype

<u>NEW VIEW</u>:
Dynamic Networks
Emergent Attractors = Phenotype
('High Dimensional' Attractors)

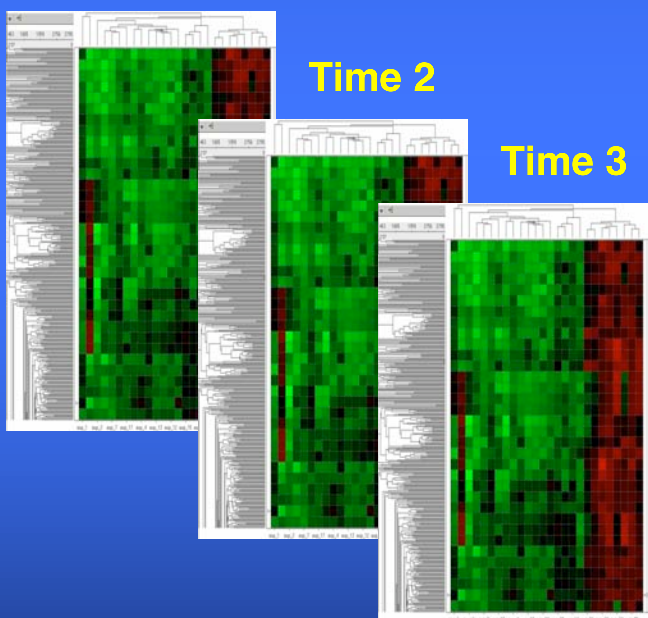
Simultaneous Dynamic Analysis of Whole Gene Array with Genome Expression Dynamics Inspector (GEDI)

Existing Method:

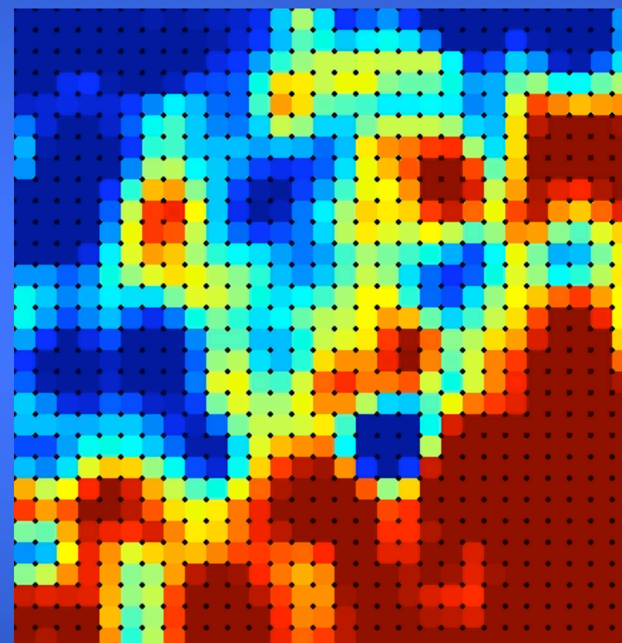
Time 1

Time 2

Time 3



New Method:



HELA Cell Cycle

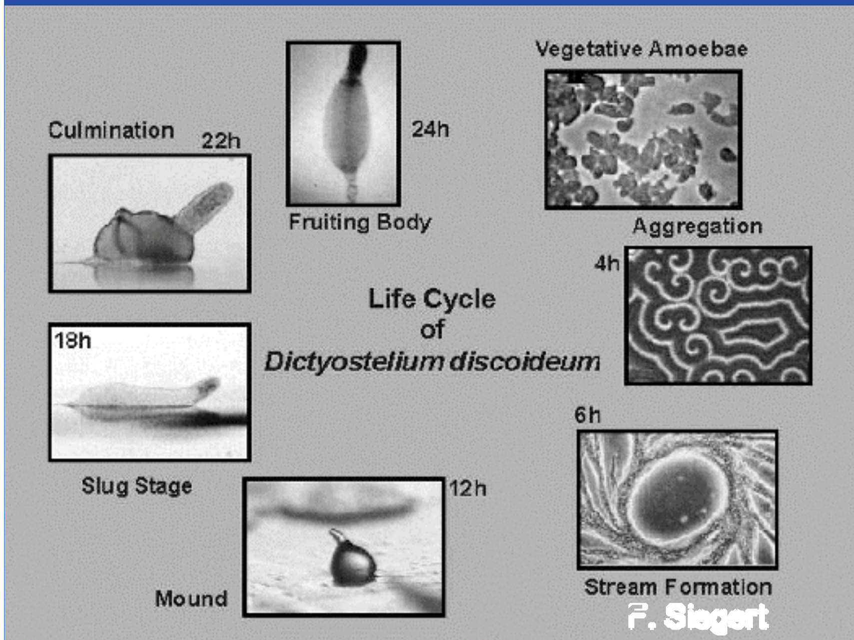
(data from Whitfield et al., *Mol. Biol. Cell* 2002)

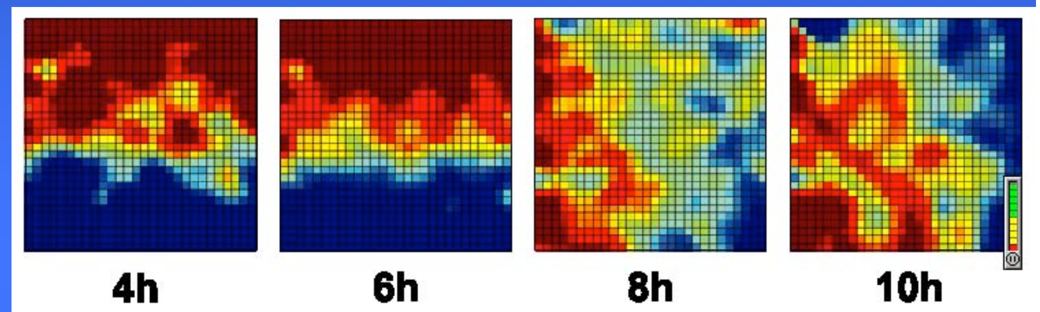
Available at: www.childrenshospital.org/research/ingber/

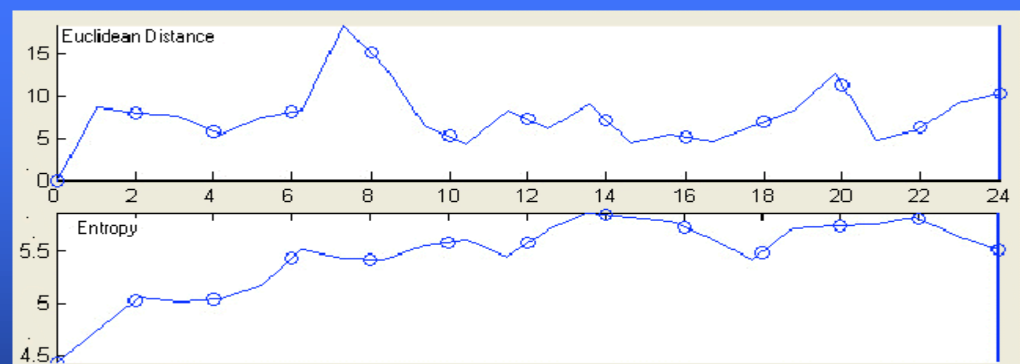
(Eichler, Huang & Ingber, *Bioinformatics* 2003)

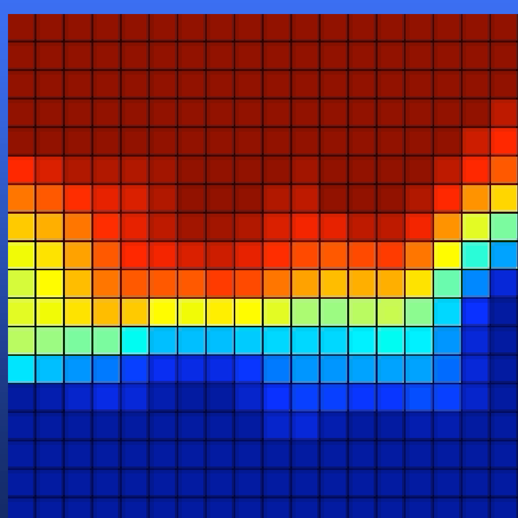
Dictyostelium Development

(data from Van Driessche et al., *Development* 2002)



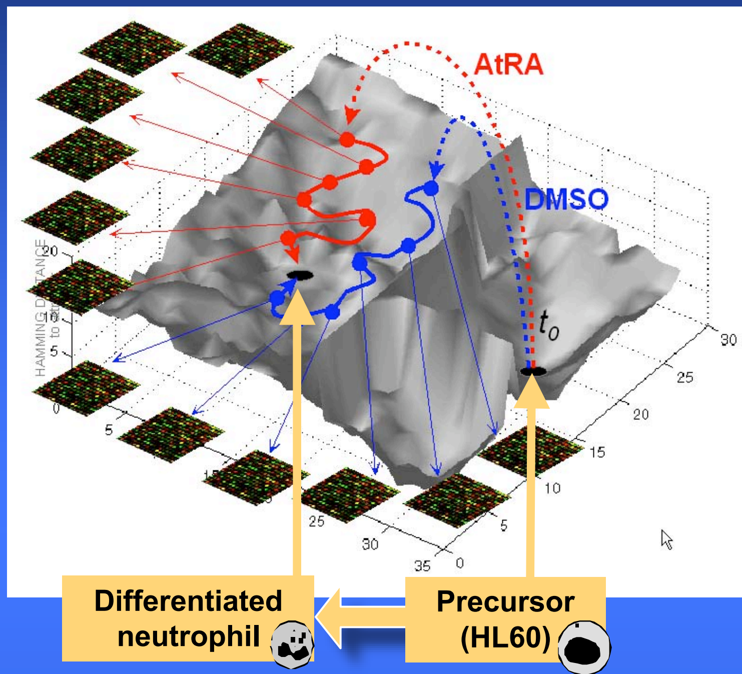






Evidence that Cell Fates are High Dimensional Attractors

(work of Sui Huang and Hannah Chang)



HL60 cells = Promyelocytic Precursor (Stem) Cells

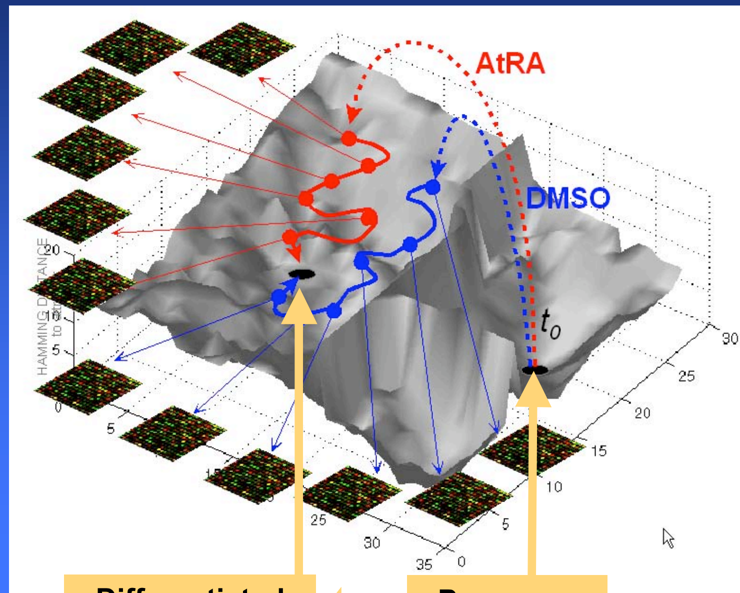
Neutrophil Differentiation was induced by:

1. all-trans Retinoic Acid (AtRA)
   [SPECIFIC HORMONE]

2. Dimethyl Sulfoxide (DMSO)
   [NON-SPECIFIC SOLVENT]

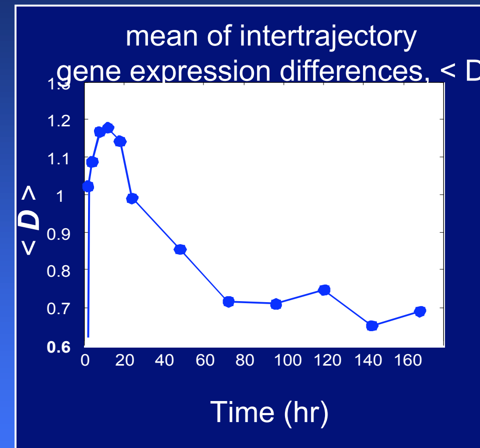
(Huang et al., *Phys Rev Lett* 2005; Chang et al. BMC Bioinform 2006)

Cell Fates are High Dimensional Attractors



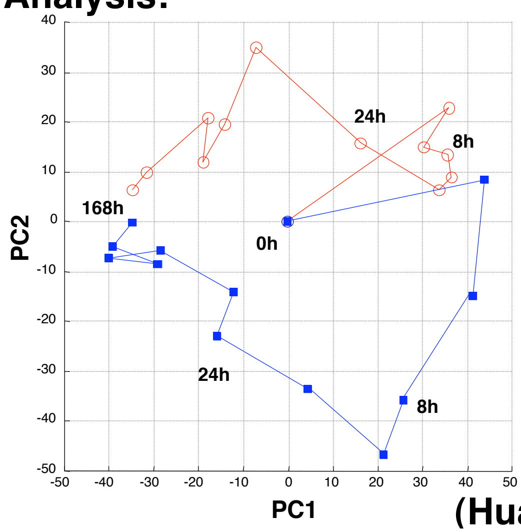
Differentiated neutrophil

Precursor (HL60)

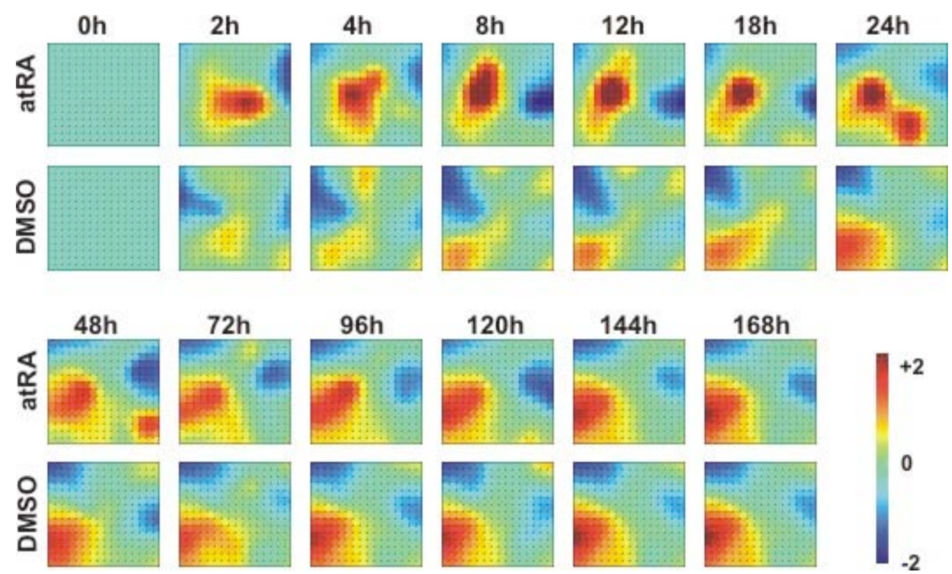


Initial divergence and terminal convergence of the two trajectories in > 70% (2773 genes) of the state space dimensions

PCA Analysis:

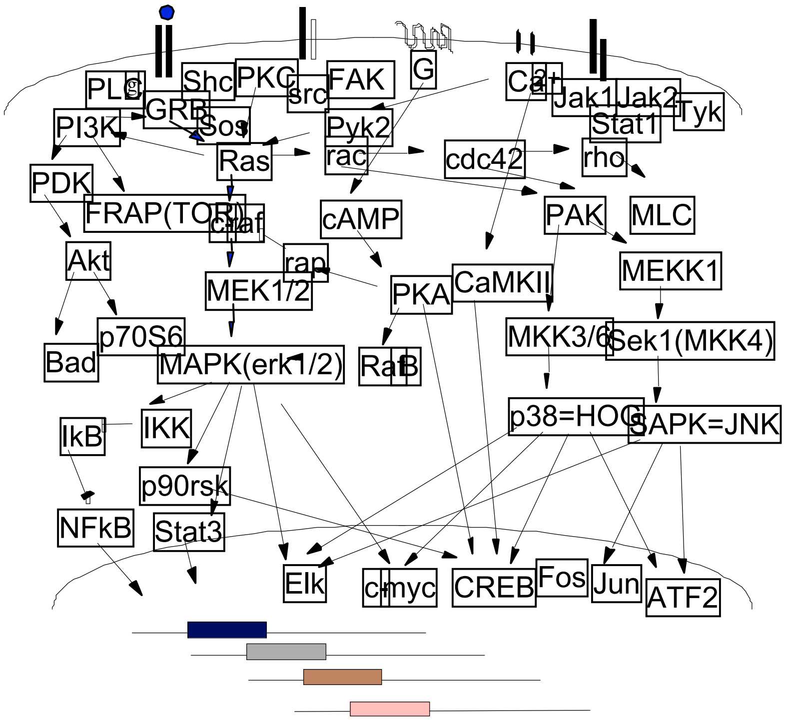


GEDI Analysis:

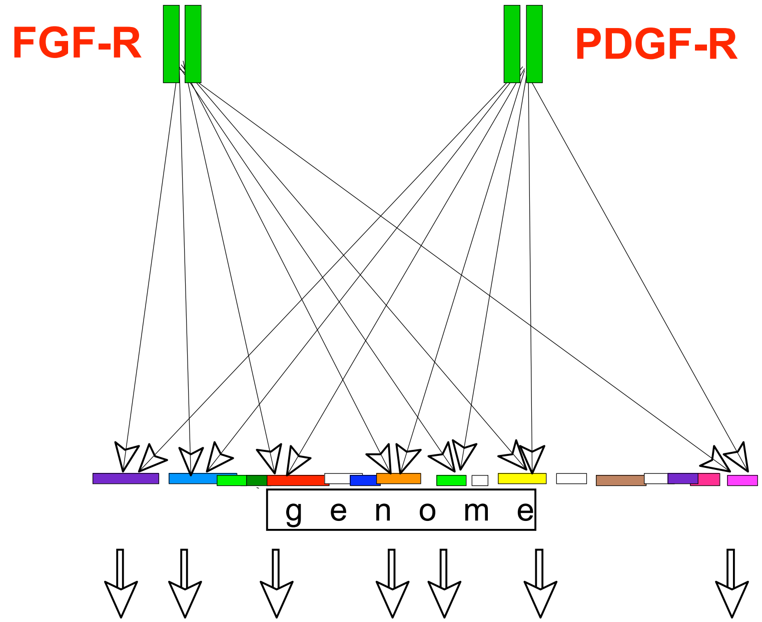


(Huang et al., *Phys Rev Lett* 2005; Chang et al. BMC Bioinform 2006)

Dynamic Networks: Simplicity in Complexity

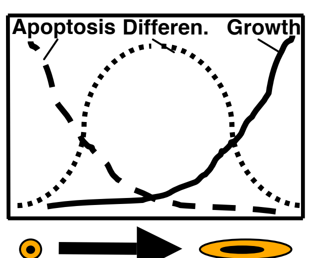


1 CYTOKINE activates > 100 of genes



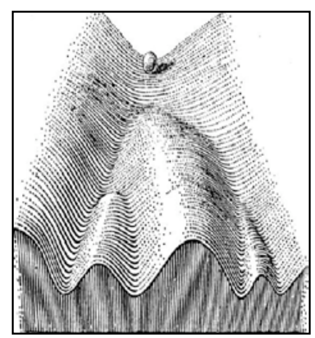
2 different GF RECEPTORS activate same set of 60 genes to induce growth

(Fambrough et al., 1999)



Cell Distortion

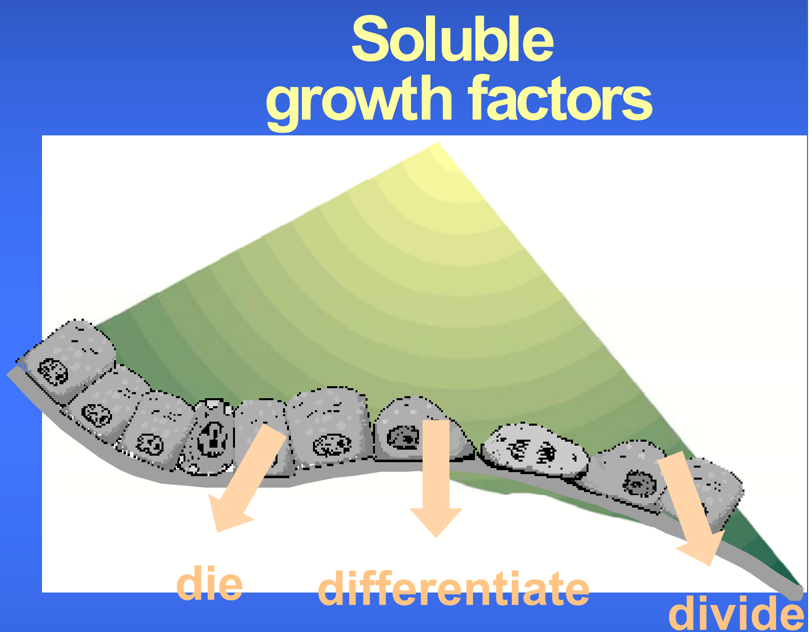
SPECIFIC & NON-SPECIFIC STIMULI produce same cell FATE switches



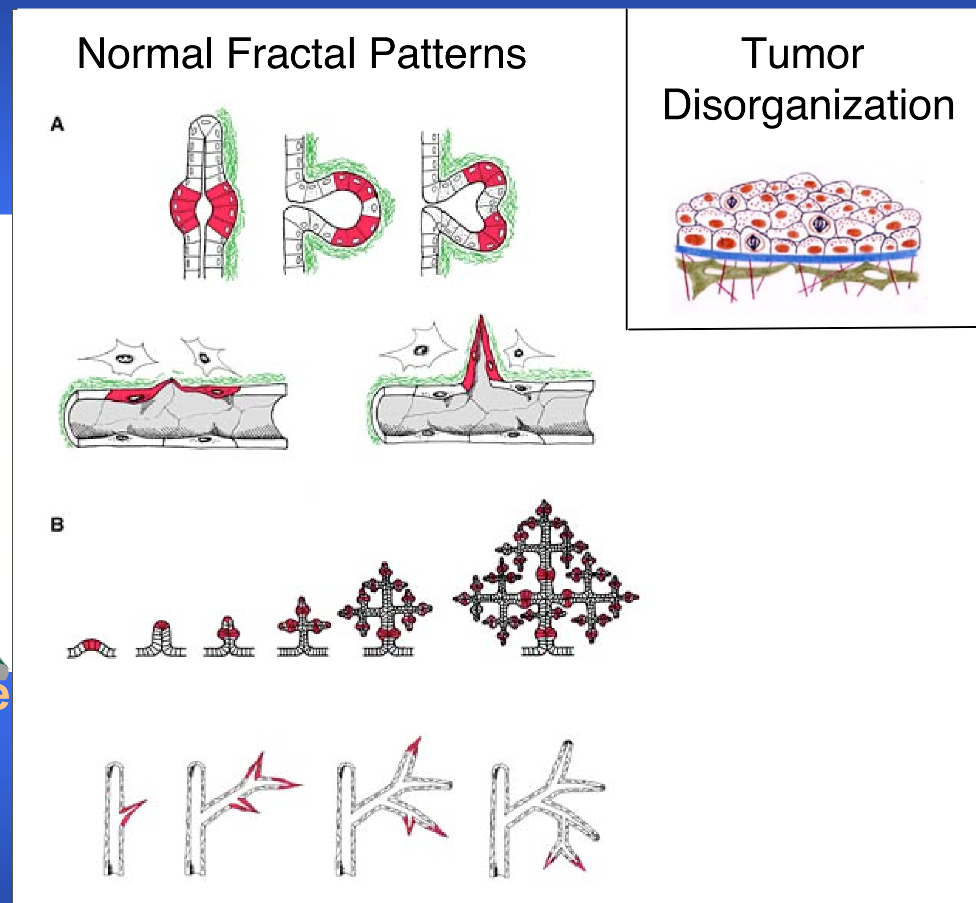
(Waddington, 1956)

MUTUAL EXCLUSIVITY of cell fates in Embryogenesis

Micromechanics and Collective Behavior Govern Pattern Formation



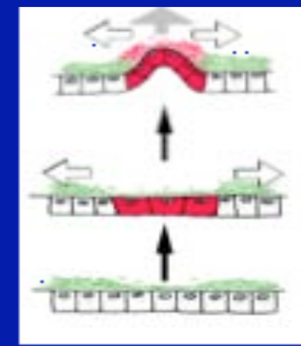
→ Spatial heterogeneity of cell fates drives morphogenesis

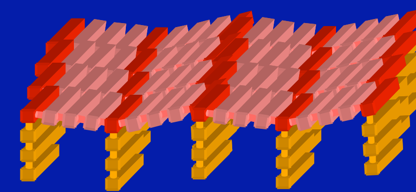


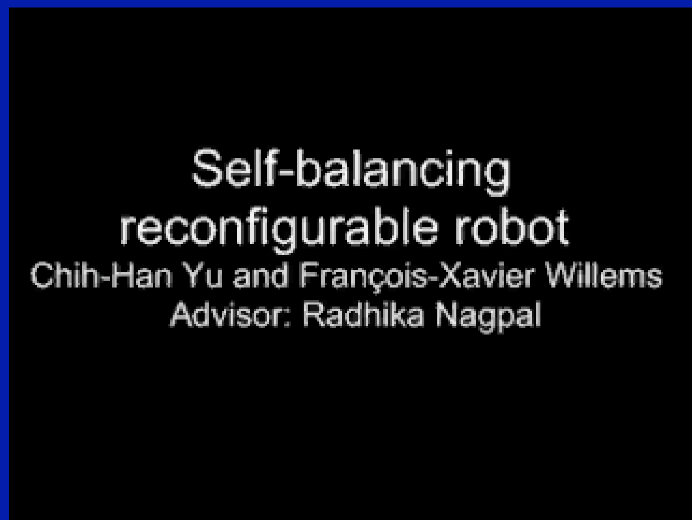
Cell fate switching depends on physicality of microenvironment

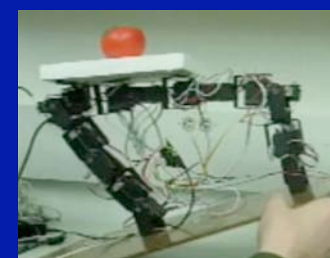
Biomimetic ‘Multicellular’ Robot Swarms

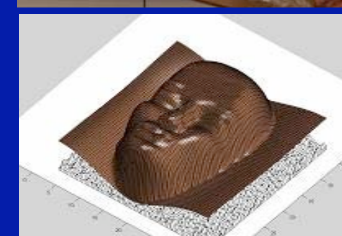
(Radhika Nagpal, SEAS; D. Ingber, HMS) ***[NSF Funded]***





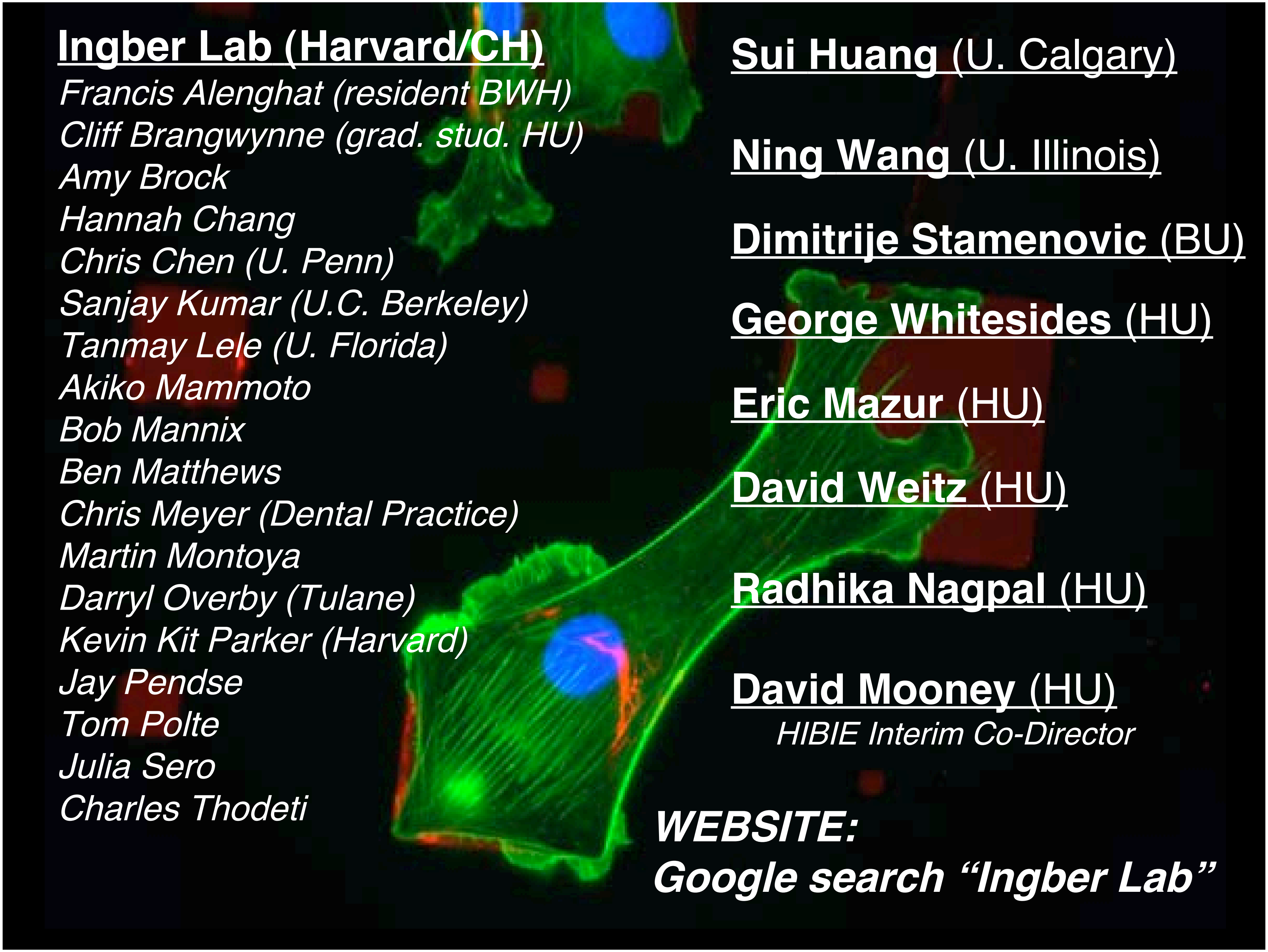






- **Bioinspired Algorithms** for robust and complex shape formation using modular (multicellular) robots
- **Distributed Homeostasis**
  - **Desired shape is described relative to the environment**
  - **Individual robots use local sensing to adapt their behavior**
  - **Inspired by homeostasis in biology and tissue remodeling in response to environment needs**

(Yu et al., *IEEE Intl. Conf. on Intelligent Robots and Systems 2007)*



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