# Quantitative analysis of micro-biomechanics during morphogenesis.

Lance Davidson, PhD

BioEngineering

## Why Study?

Immediate impact: Understanding the basic science of morphogenesis can identify risk factors underlying birth defects and suggest prevention.

"Dys-morphogenesis" during wound healing and cancer is poorly understood but is the critical consequence of injury and disease.

Tissue engineers need to understand the rules and principles of morphogenesis in order to design novel tissues and materials for tissue regeneration.

# Quantitative analysis of micro-biomechanics during morphogenesis.

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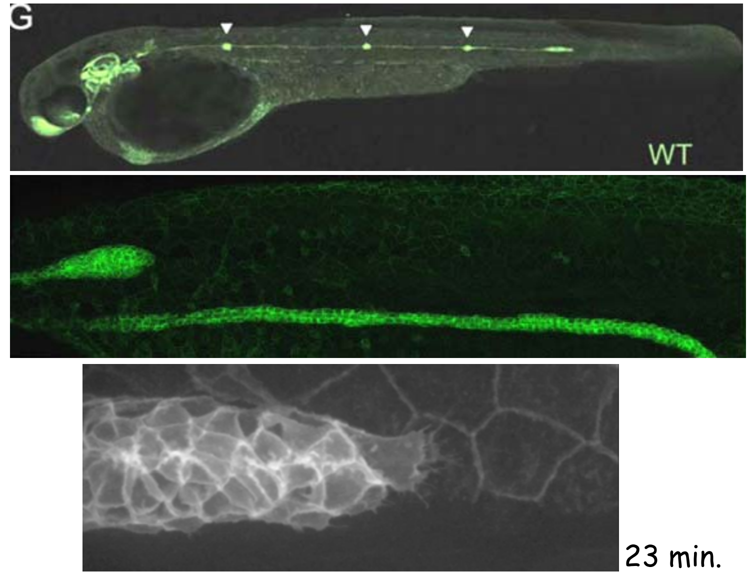
## Intro to frog & mechanics

## Bulk mechanics of C&E:
## Stiffness and Force.

(How bulk mechanics may be regulated on a molecular scale.)

## Where to go next?

## *Directed Cell Migration*

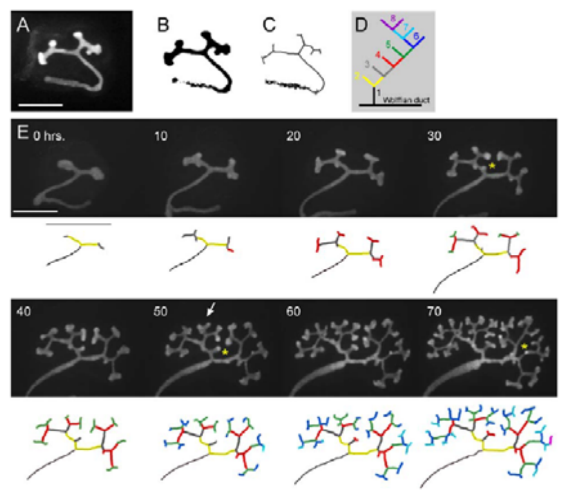


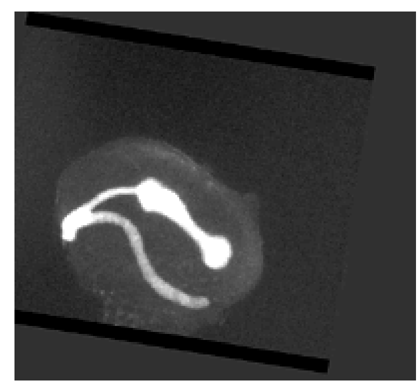

8 hours

23 min.

Haas and Gilmour (2006), Dev Cell, 10: 673+
Migration of lateral line cells (zebrafish)

## *Branching morphogenesis*



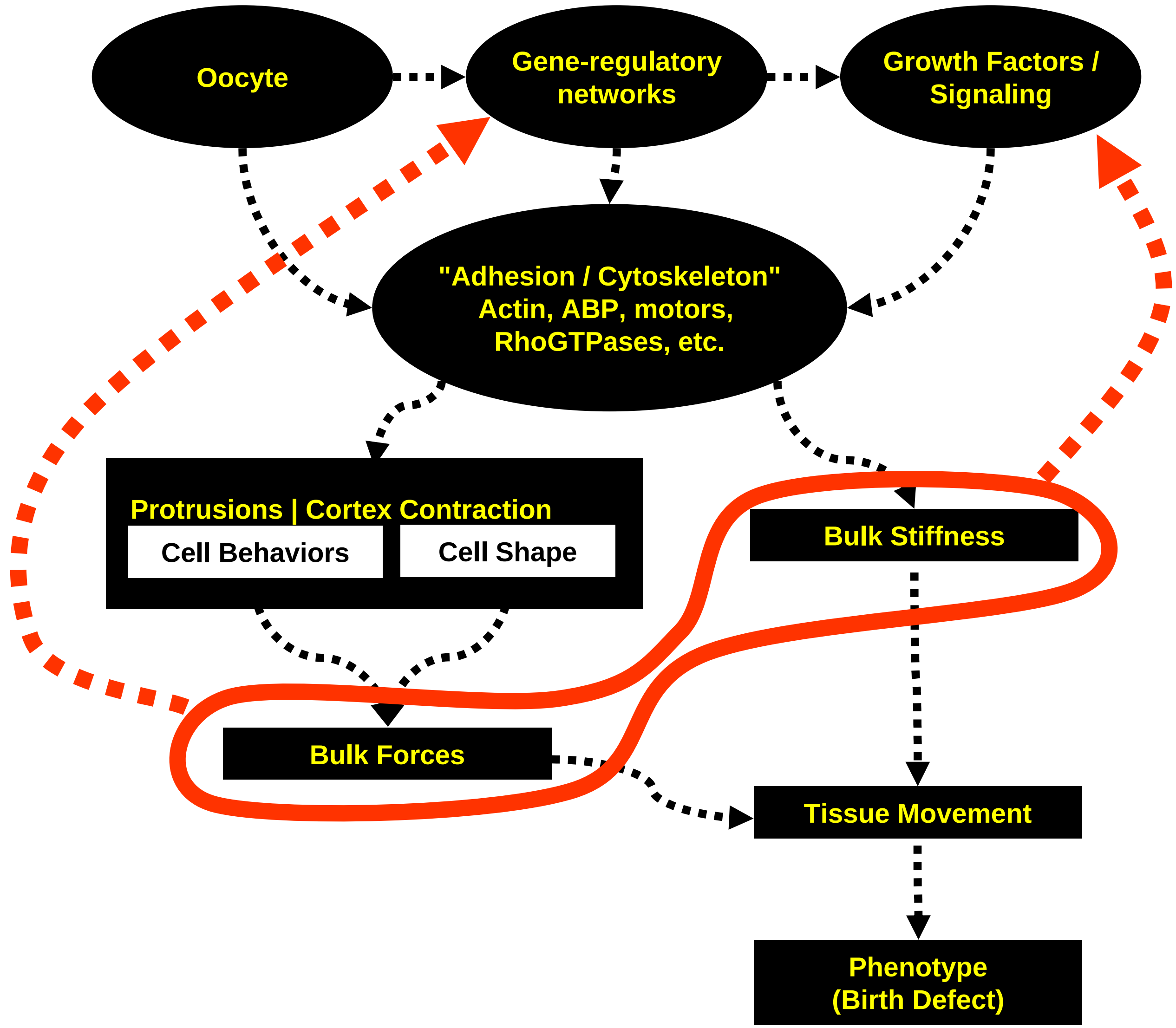



40 hours

Watanabe and Constantinin (2004), Dev Biol, 271: 98+
Renal tubule branching morphogenesis (mouse)

# Three Roles for Mechanics during Embryogenesis:

- Shape tissues
- Maintain robust development
- Provide cues to guide cell fate decisions



**How to study these three roles for mechanics?**

**Our approach is to reverse engineer the physical processes of morphogenesis (convergence and extension) to understand how these pathways are integrated to initiate, control, and carry out morphogenesis.**

**We take an interdisciplinary approach combining:**

- Classical embryology:
  microsurgery, cut-and-paste, explants
- High resolution confocal microscopy:
  observe and analyze cell movements
  and tissue architecture.
- Cell and molecular biology:
  modulate and interrupt cellular processes
- Biophysics and bioengineering:
  measure and manipulate physical properties,
  forces and strains

# Understanding morphogenesis as a machine...

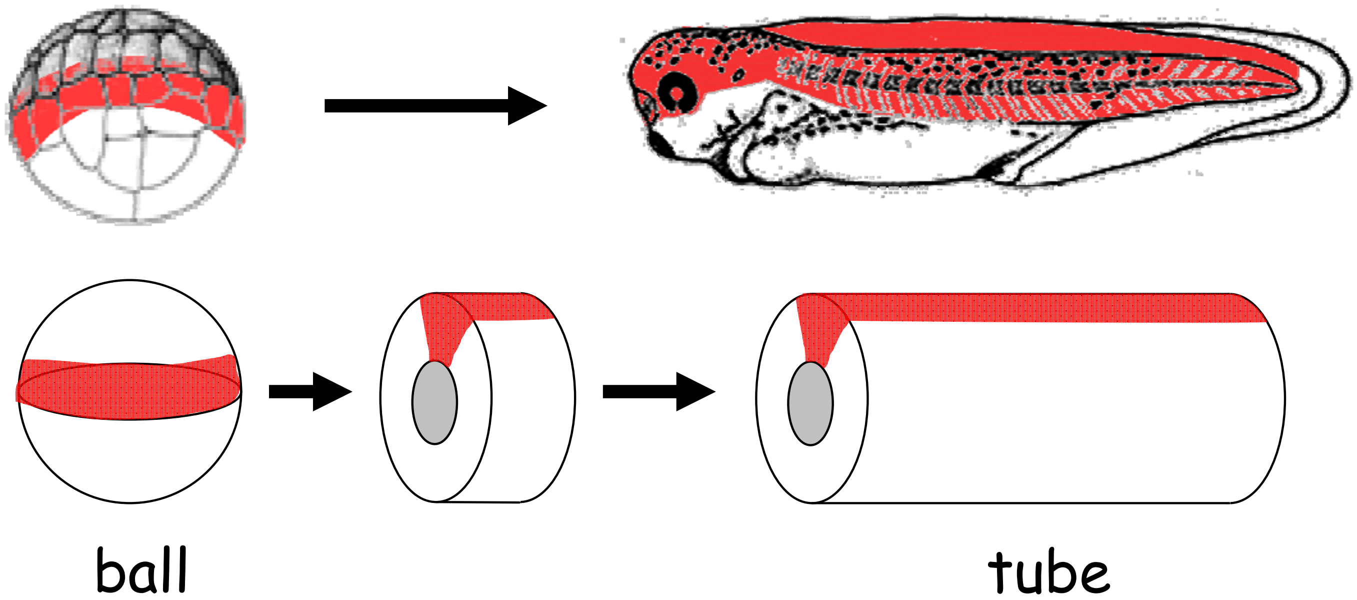
| Process: | Scale: |
| --- | --- |
| Gastrulation | Embryo (~1 mm) |
| ↕ | ↕ |
| Convergence and Extension | Tissue (~100 μm) |
| ↕ | ↕ |
| Mediolateral Cell Intercalation | Cell (~10 μm) |
| ↕ | ↕ |
| Cell Protrusions (traction) | Sub-cellular (~1 μm) |

What are the cell behaviors and forces acting within embryos to bring about morphogenesis?

What is the mechanical "context" that mediates the conversion of local cell behaviors to tissue movement?

What structures, both super-cellular and molecular, are responsible for these mechanics?

# Morphogenesis: What is convergent extension and How does it work?




*mechanical* molecules
- motors
- cytoskeleton
- cell adhesion
- extracellular matrix

*mechanical* phenomena
- cell motility / shape change
- cell behaviors
- cellular environment
- force transmission
- tissue deformation

# Thinking about "morphogenesis" as a machine...

**Things we *"watch"*:**

- shape change
- movement and rate
- strain and flow

[do not need to perturb embryos]

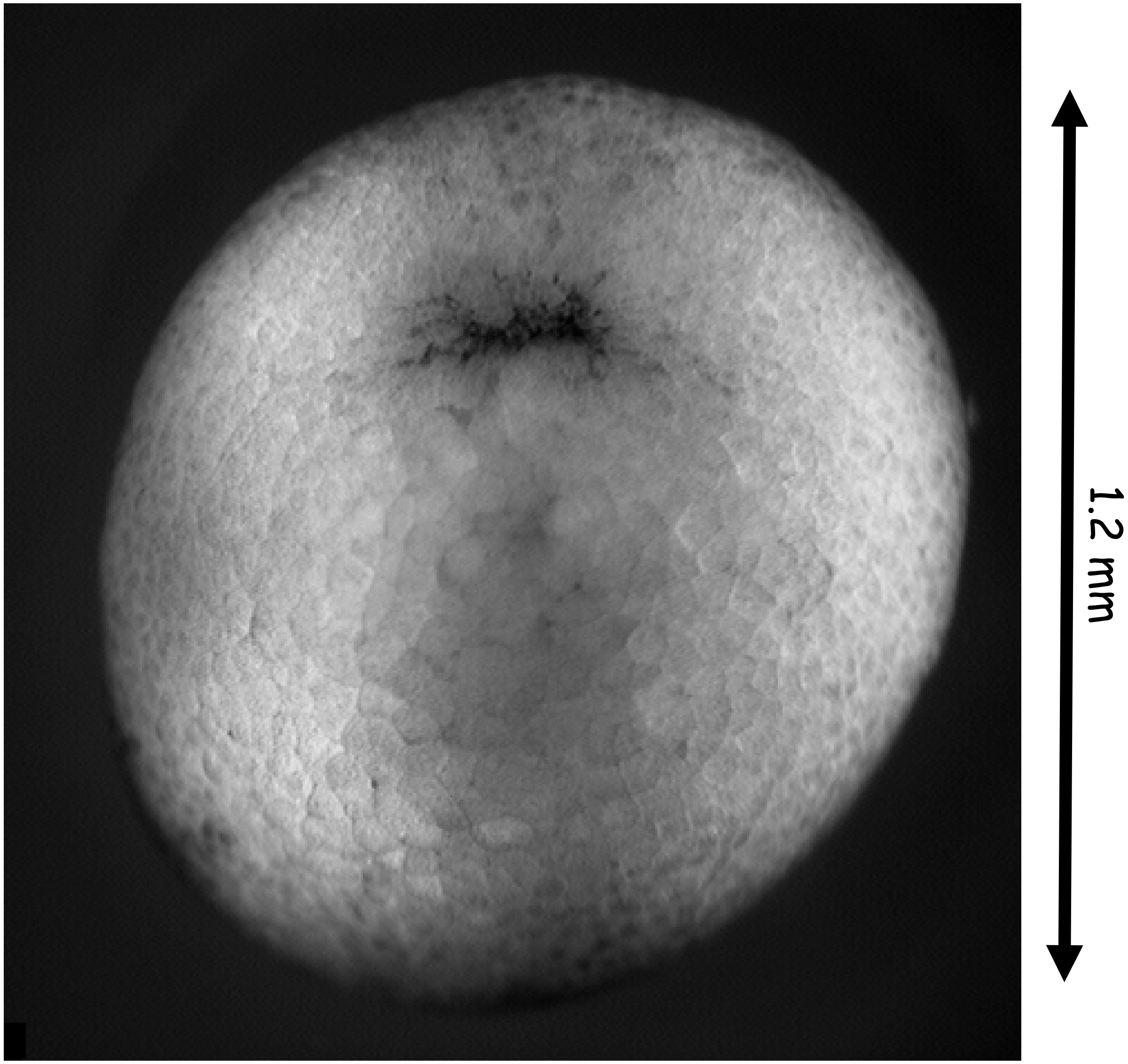
$$\textit{deformation} \sim \frac{\textit{force}}{\textit{resistance}}$$

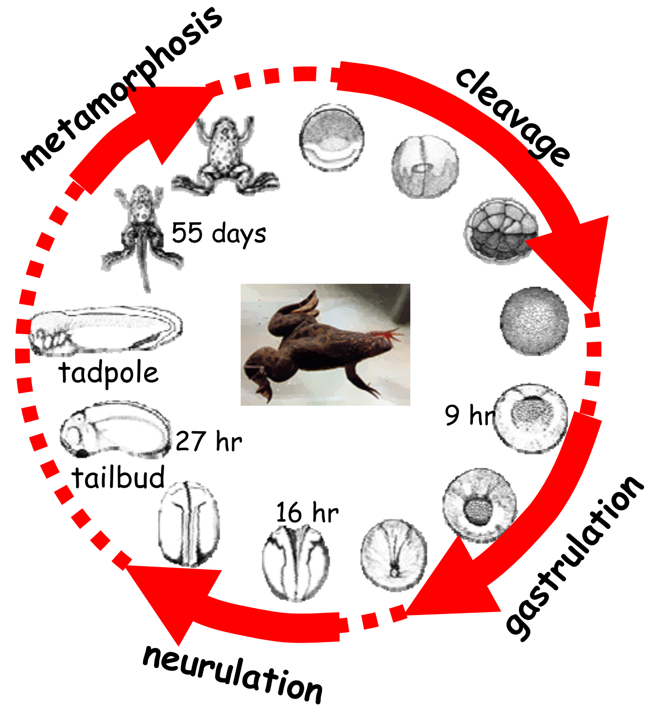
**Things we *"measure"*:**

- force or stress
- stiffness or modulus
- viscosity

[**must** perturb cells or tissues to measure]

# *Xenopus laevis:*
# African Clawed Frog



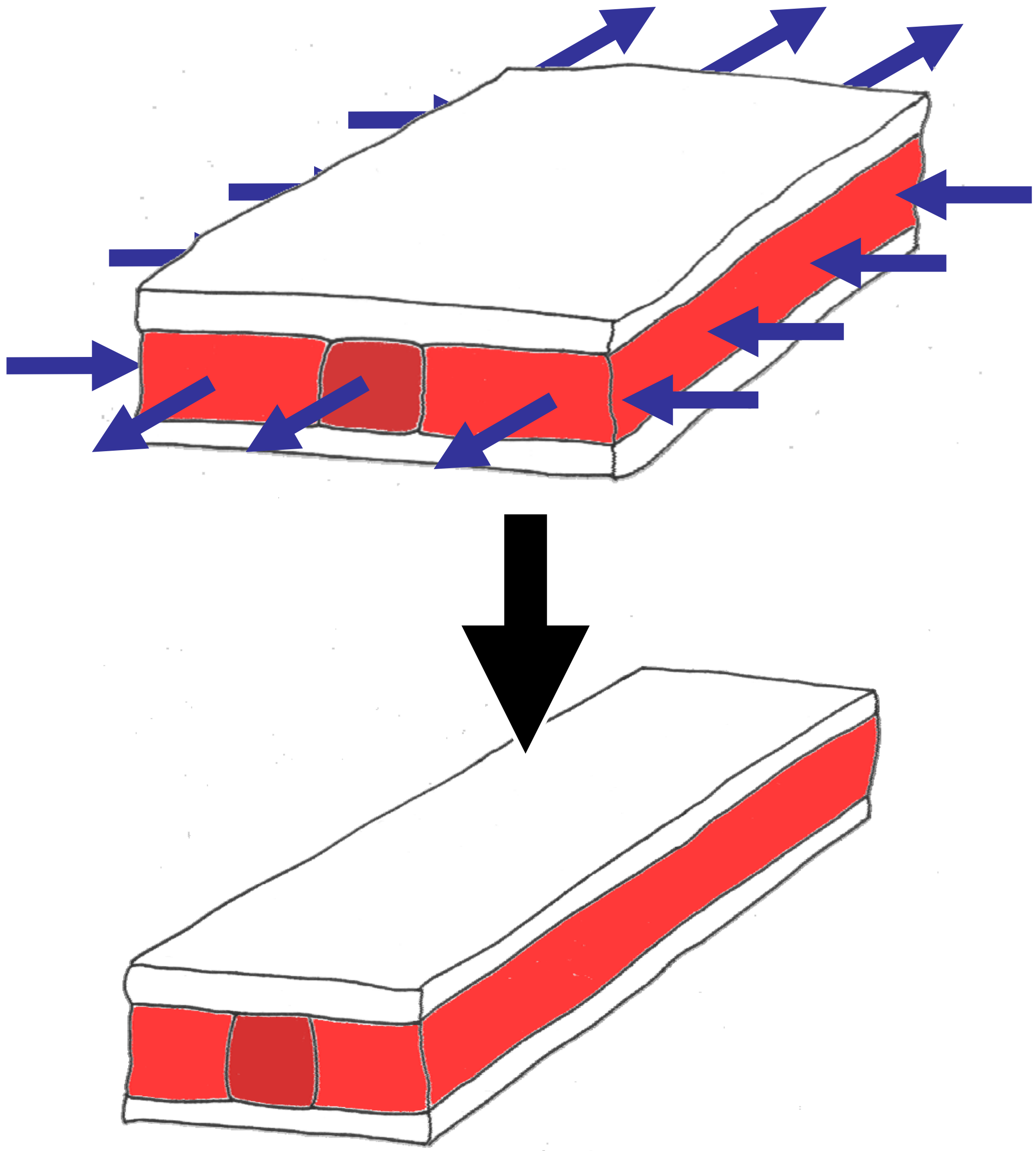



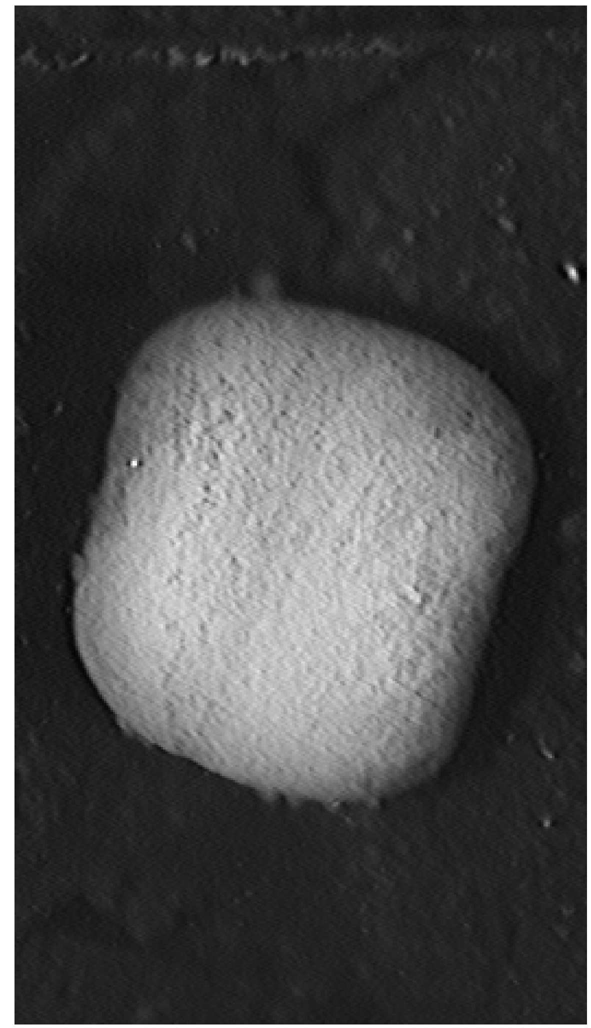

## Advantages of frog embryo:

- Size
- In vitro fert.
- Simple culture conditions
- Microsurgery
- Cell biology
- Vertebrate - tetrapod

14 hours elapsed time
Dave Shook, U. Virginia

**Convergence - lateral tissues move toward the midline (notochord).**
**Extension - tissues extend along the anterior-posterior axis.**

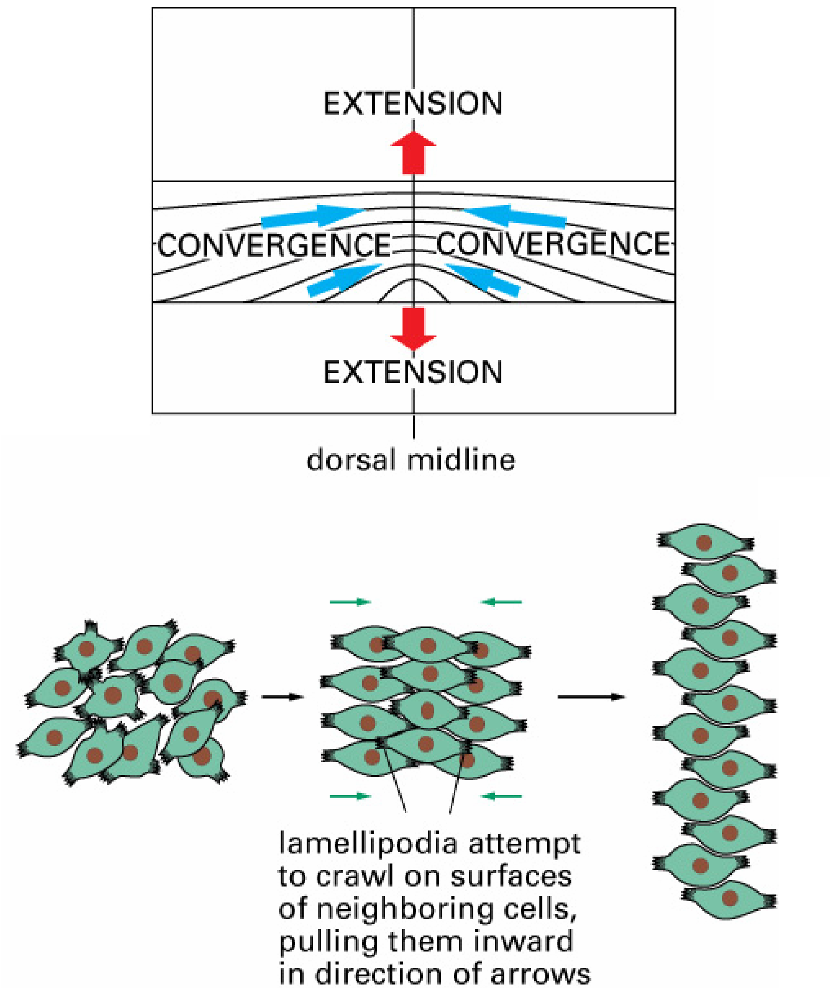




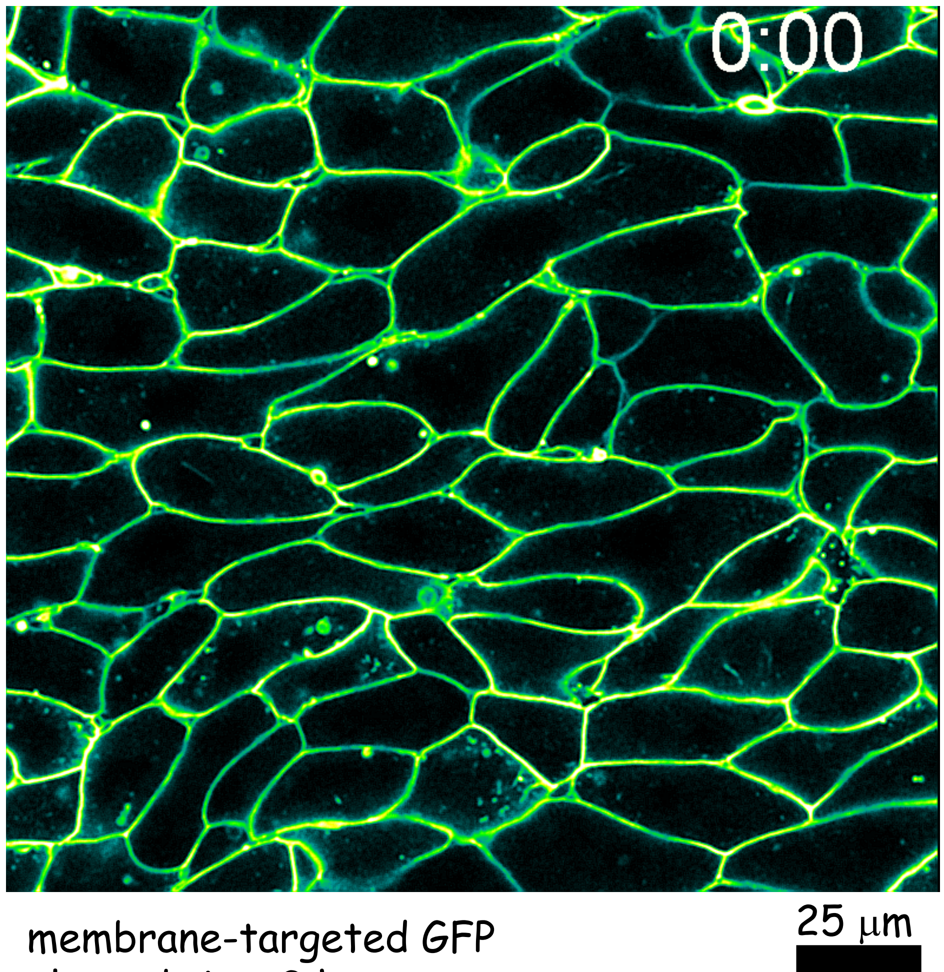
5 hours elapsed time

# Things we "watch"...

**Elongation of the embryo is driven by mediolateral cell-intercalation (an example of directed cell migration).**




Molecular Biology of the Cell, 4th Edition.




membrane-targeted GFP
elapsed time 3 hours.
(Sagar Joshi)

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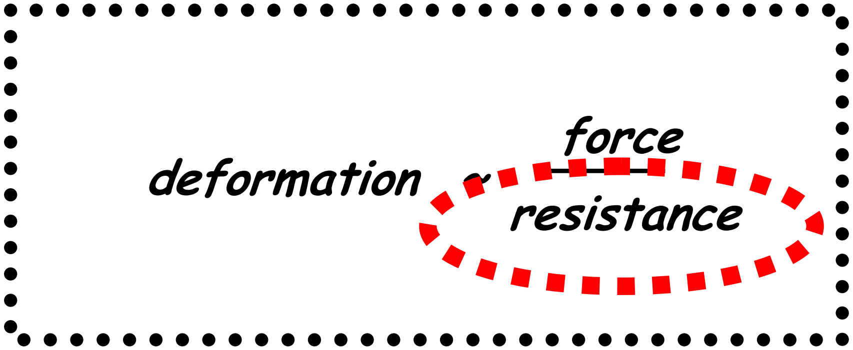
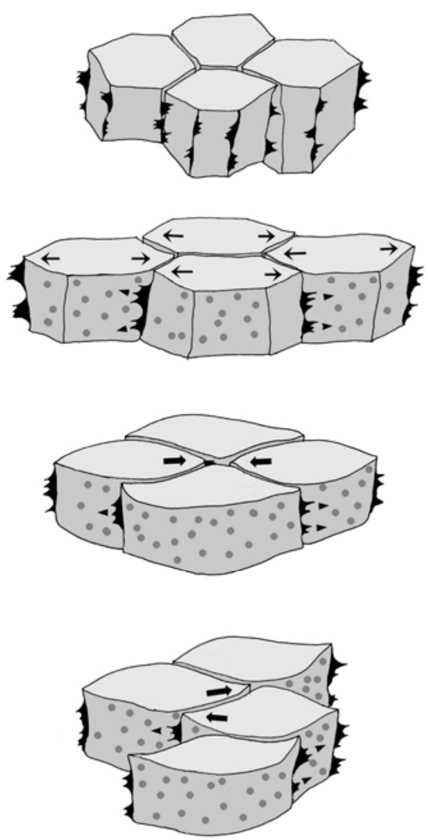
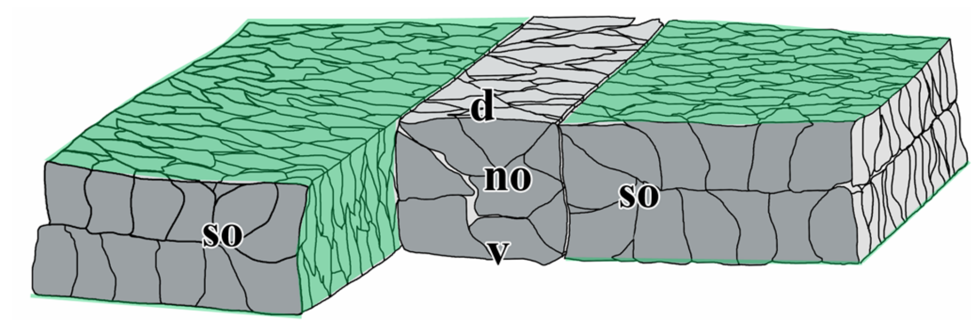
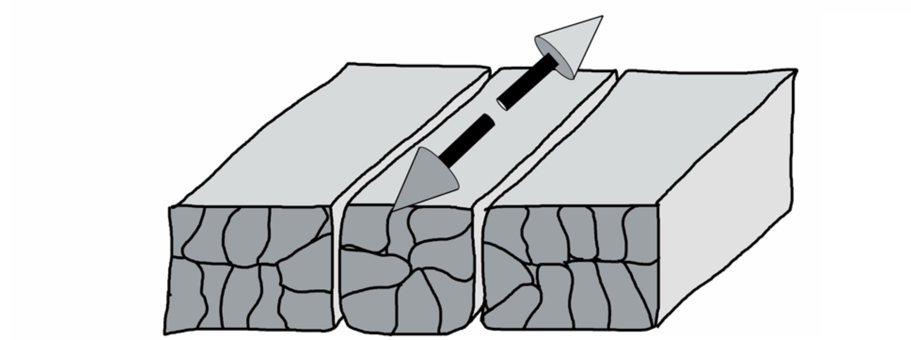
# Understanding morphogenesis as a machine...




**Sources of resistance?**
Structural: eg. notochord
Extracellular matrix
Cytoskeleton

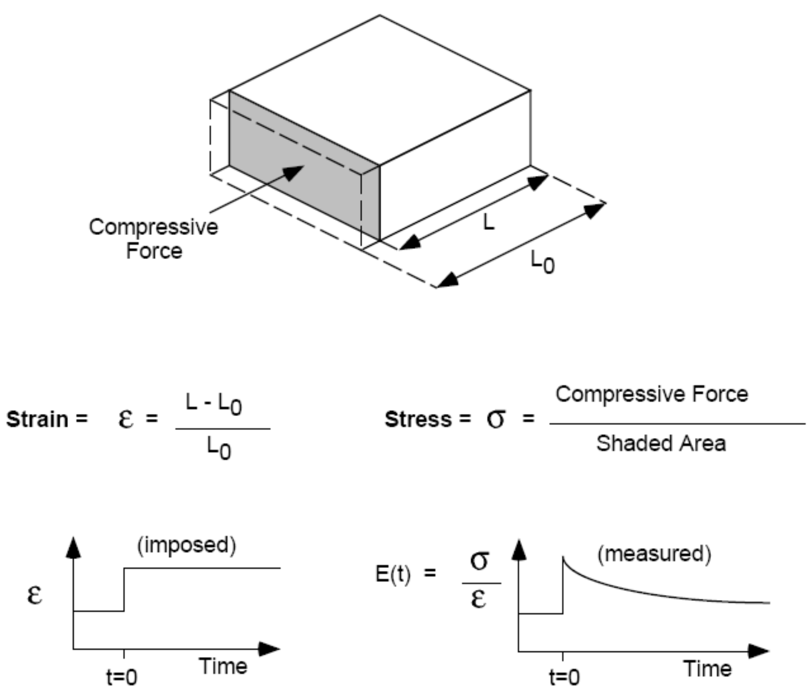


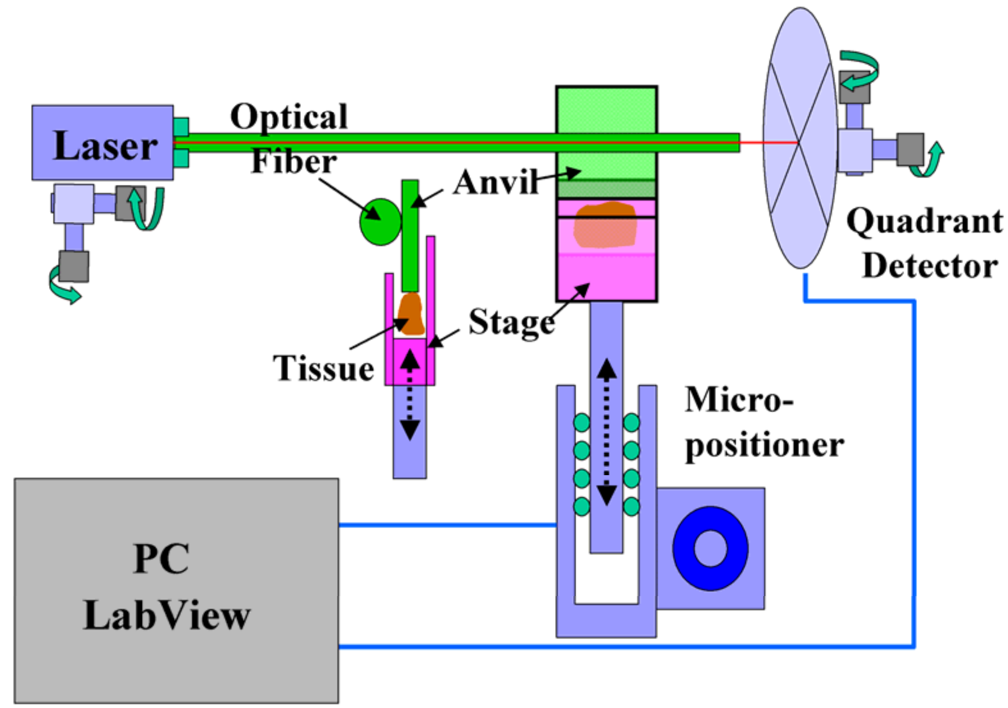


# *Measuring Resistance*:

# nanoNewton Force Measurement:
## *Unconfined uniaxial compression*



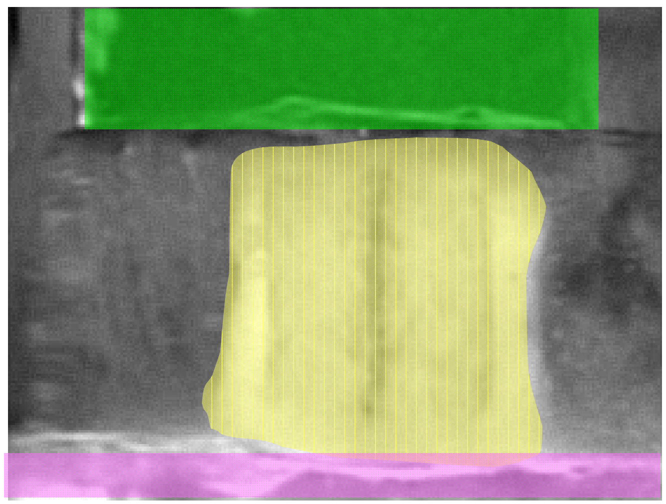




Koehl (1990), Seminars in Dev. Biol.

Zhou, Kim, and Davidson (2009) Development

# Measuring *Resistance*:

# nanoNewton Force Measurement:
## *Unconstrained uniaxial compression*

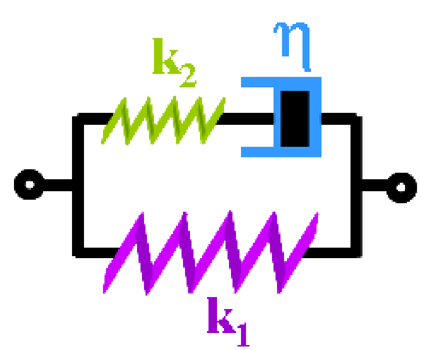


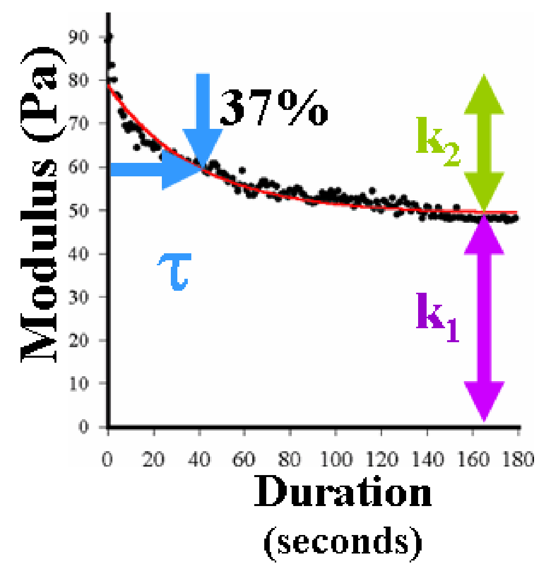
3 minutes unconfined compression

### Standard Linear Solid Material

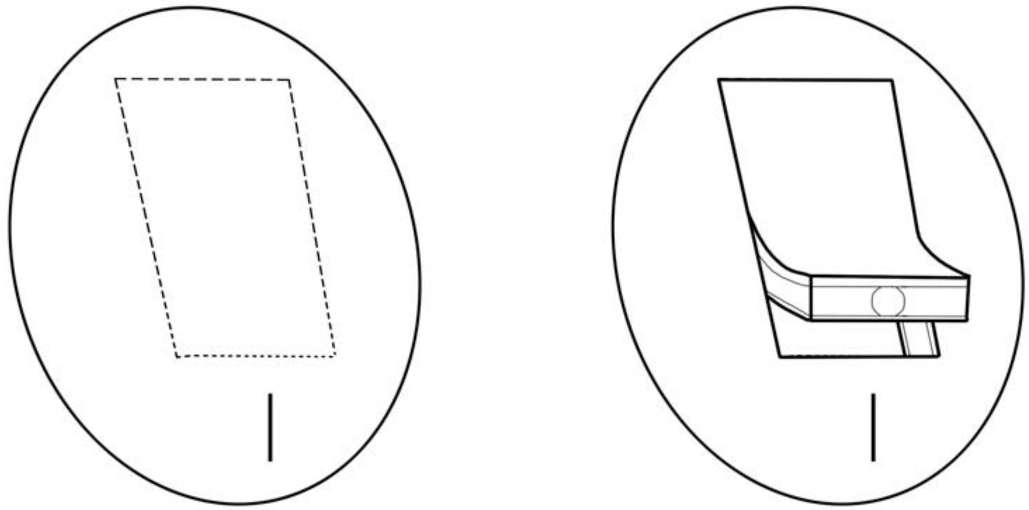
$$E(t) = k_1 + k_2\, e^{-t/\tau}$$

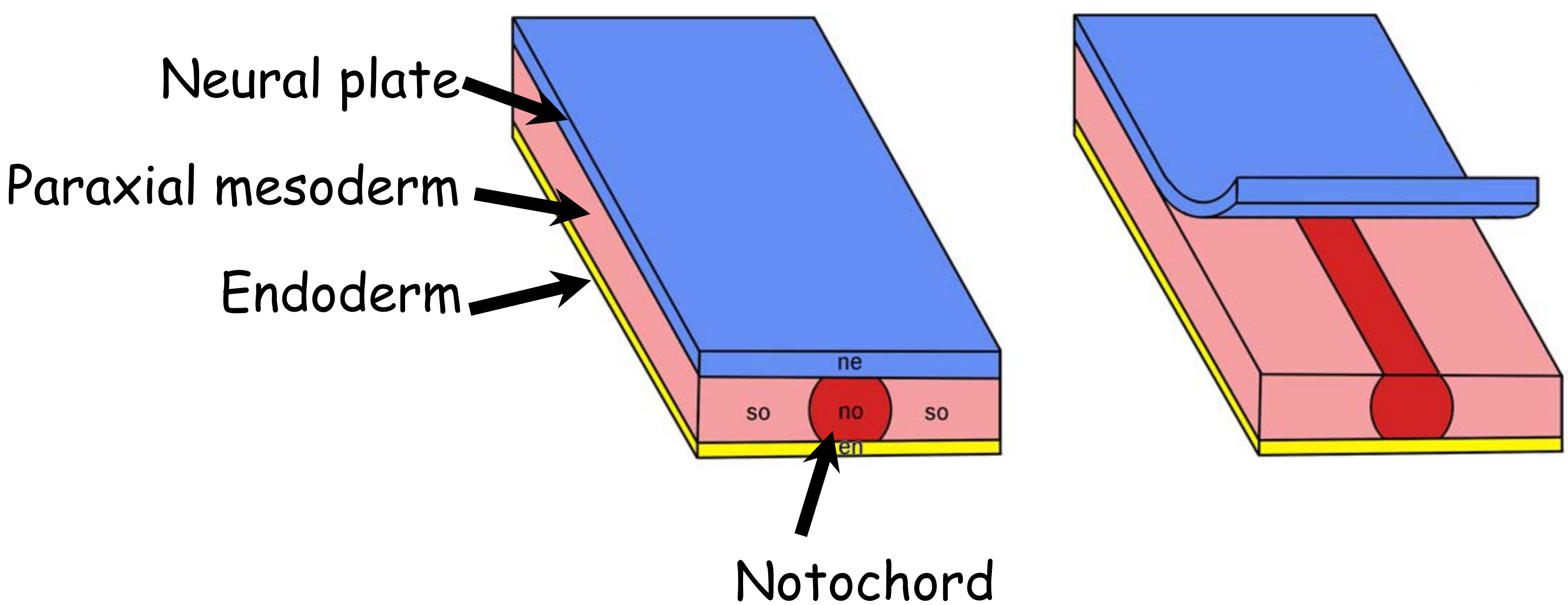
$$\tau = \eta / k_2$$



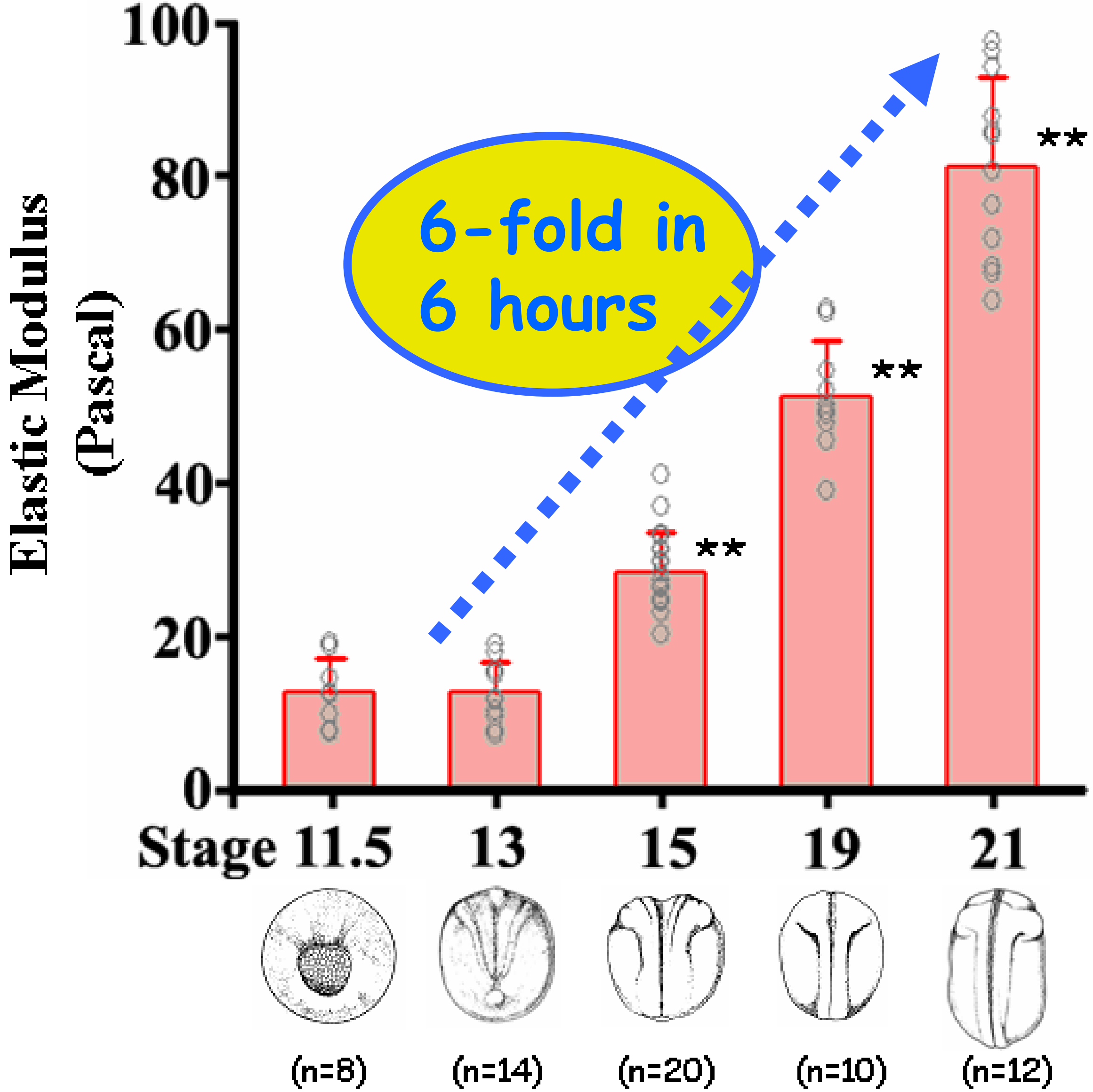



# Basic Microsurgery: How to make a Dorsal Isolate.





# Dorsal Tissue Stiffness Increases with Stage




| Tissue Type | Modulus |
|---|---|
| Achilles' tendon | 310 Mpa |
| Spinal cord | 89 kPa |
| Thyroid cancer[a] | 45 kPa |
| Breast tumor | 4 kPa |
| Kidney | 2.5 kPa |
| Brain | 260–490 Pa |
| Lymph node | 120 Pa |
| Mammary gland | 160 Pa |
| Fat | 17 Pa |

Levental et al (2007) Soft Matter.

Zhou, Kim, and Davidson (2009) Development

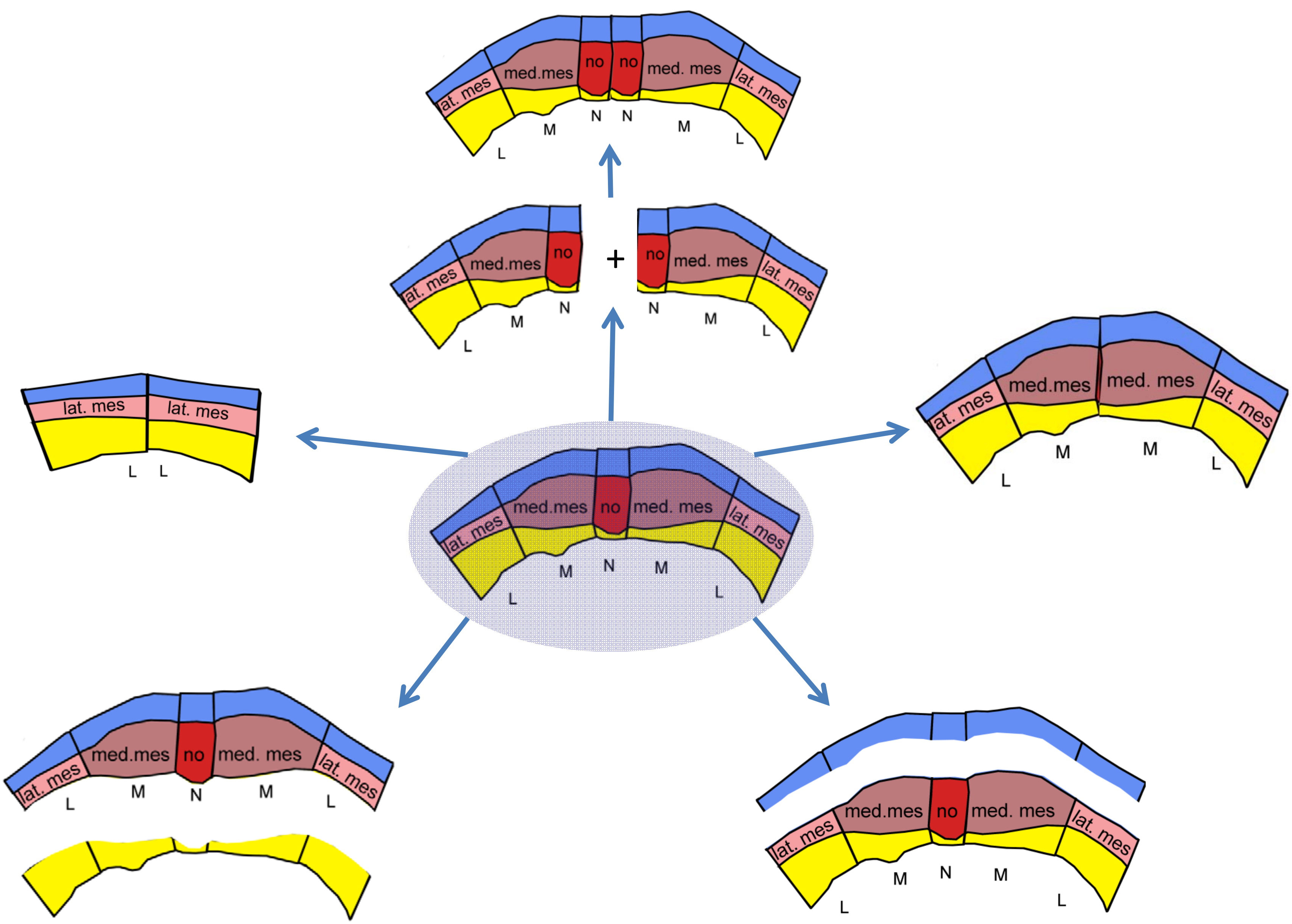
## What are the sources of stiffness?

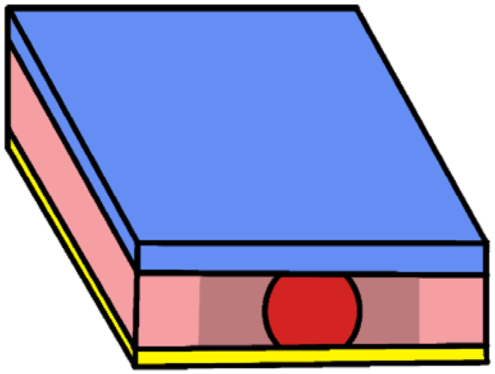
**Structural -** Notochord

**Extracellular matrix** - fibronectin, laminin, fibrillin, ...

**Cytoskeleton** - actin, myosin, microtubules, ...

# Microsurgery to Create Various "Structures"



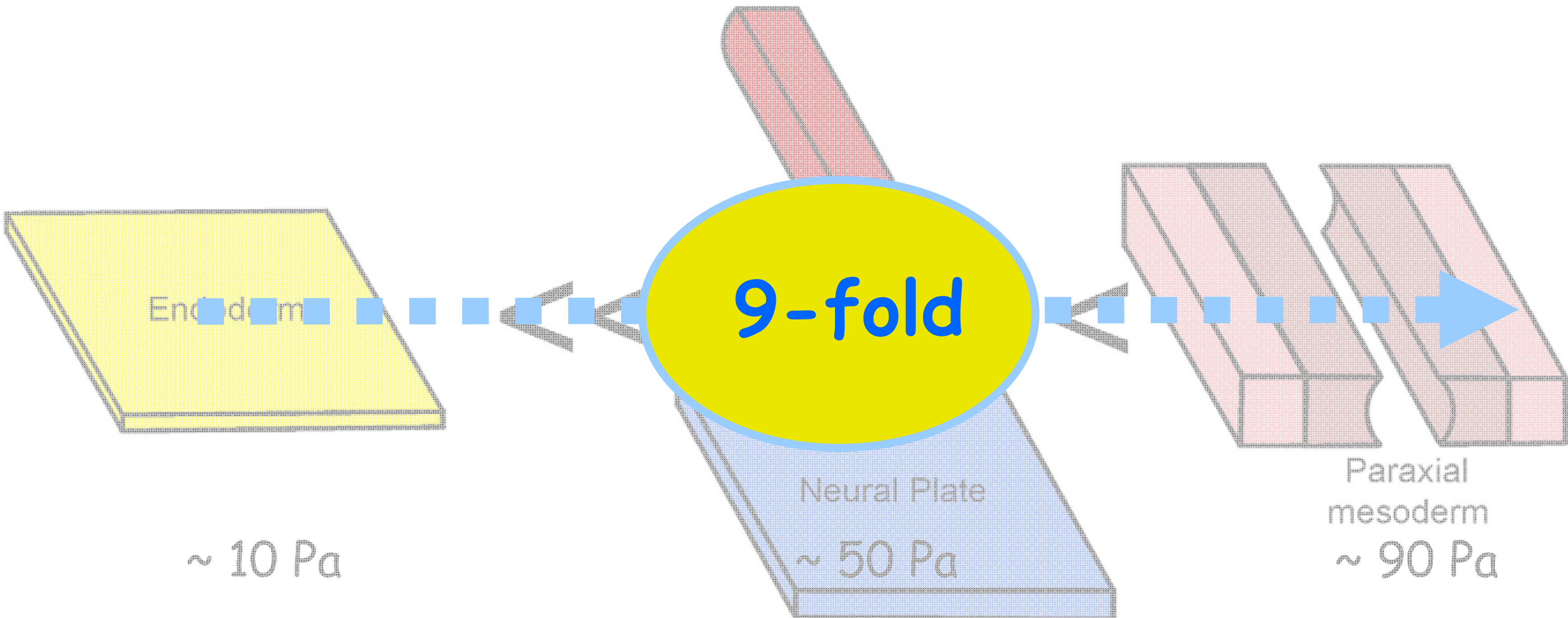



# Spatial Differences in Tissue Stiffness

(before differentiation)

~ may provide positional information

~ can direct cell fate choices
Engler ... Discher (2006), Cell
Farge (2003), Current Biology




Zhou, Kim, and Davidson (2009)
Development

# What is the mechanical "context" that mediates the conversion of local cell behaviors to tissue movement?

**What are the sources of stiffness?**

**Structural** - ~~Notochord~~, ...

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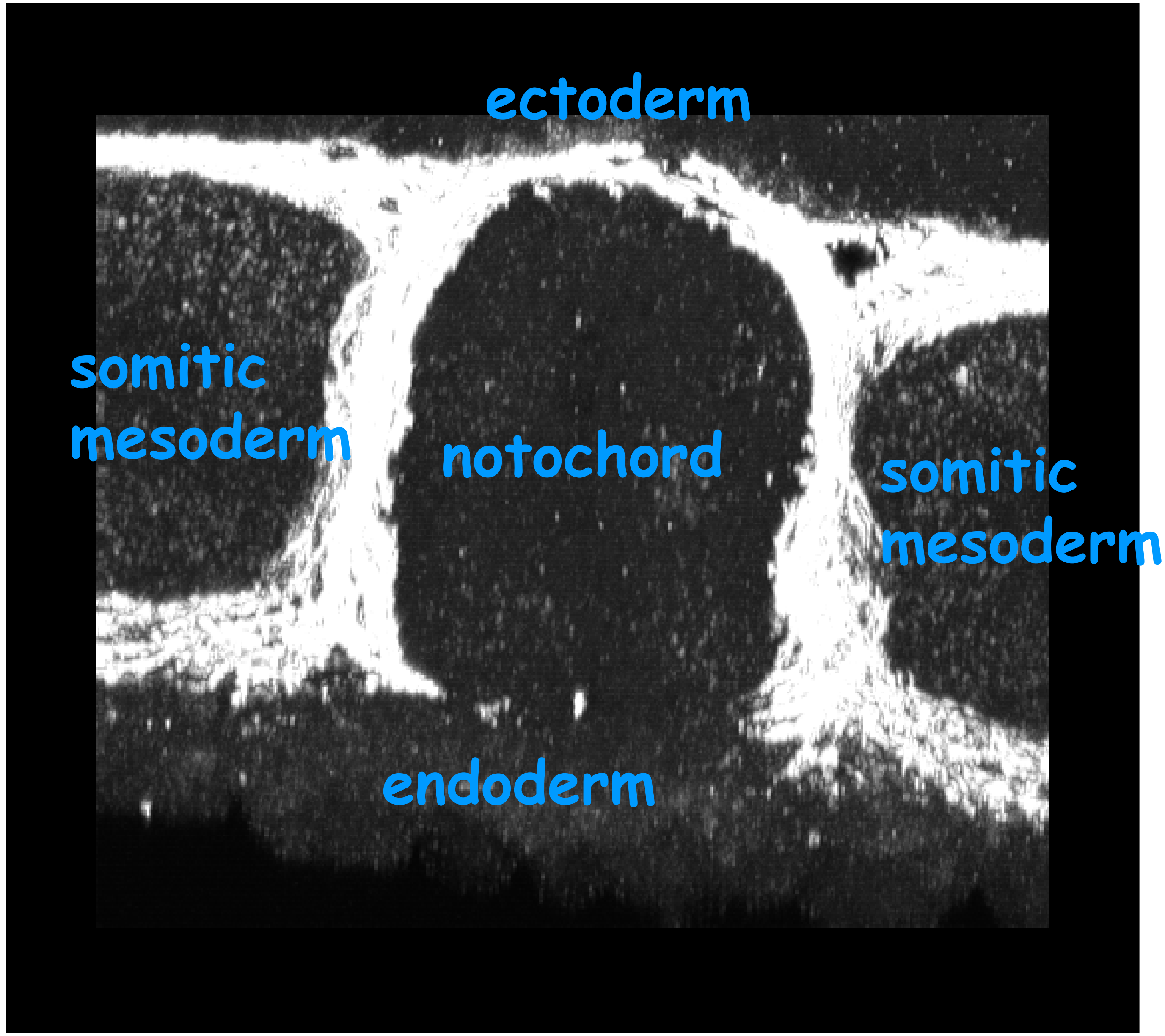
# ECM is a good candidate...Fibronectin Fibrils Are Localized to All Tissue Interfaces.

- fibrils form at endoderm/mesoderm tissue boundaries

- fibrils cleared from notochord

- all mesodermal cells are in direct contact with fibrils




Davidson et al. (2004) Dev. Dyn.

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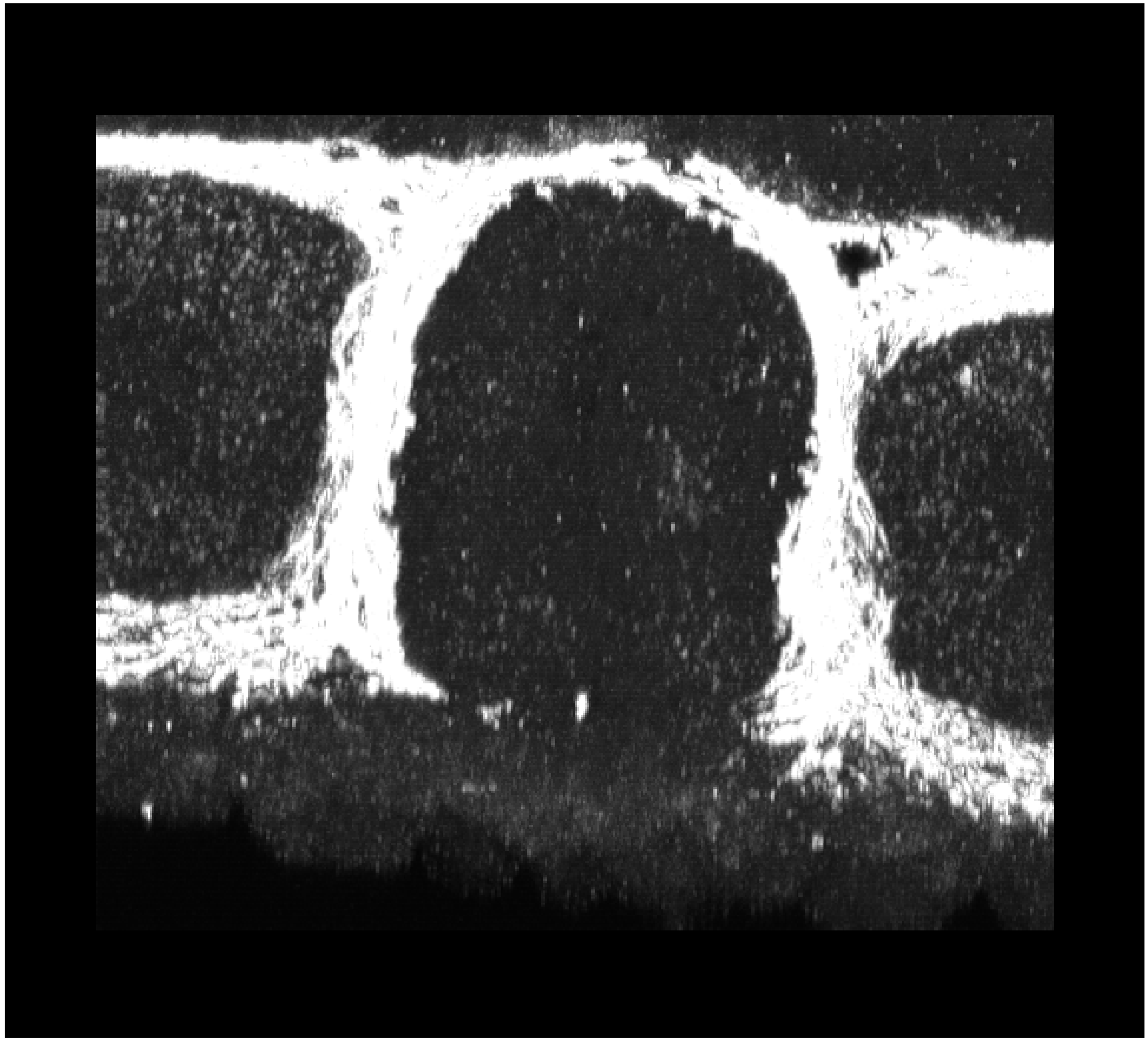
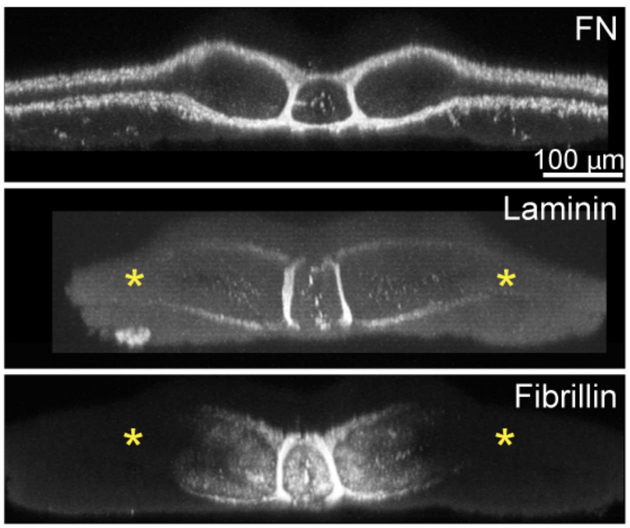
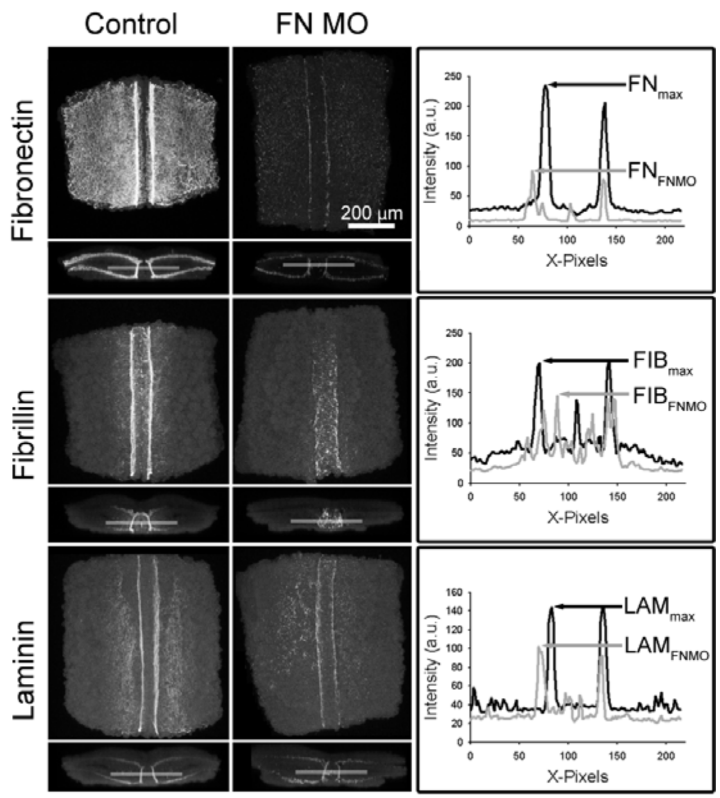


Davidson et al. (2004) Dev. Dyn.

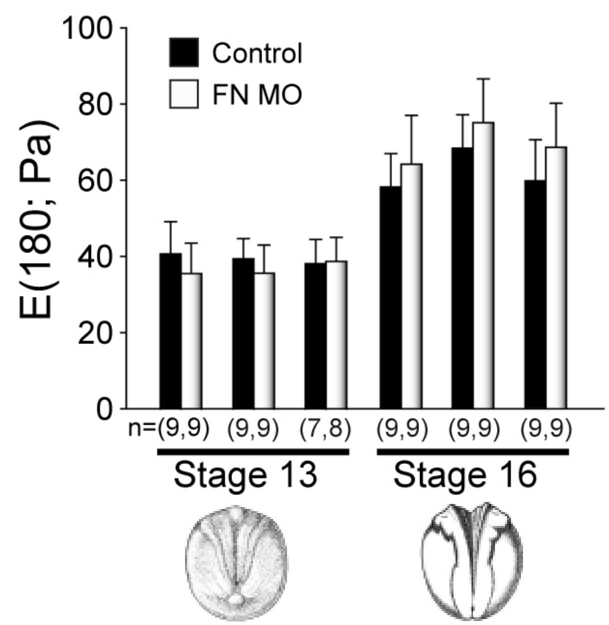
# Other ECM fibrils assemble around dorsal axial tissues during gastrulation and elongation...




At stage 16



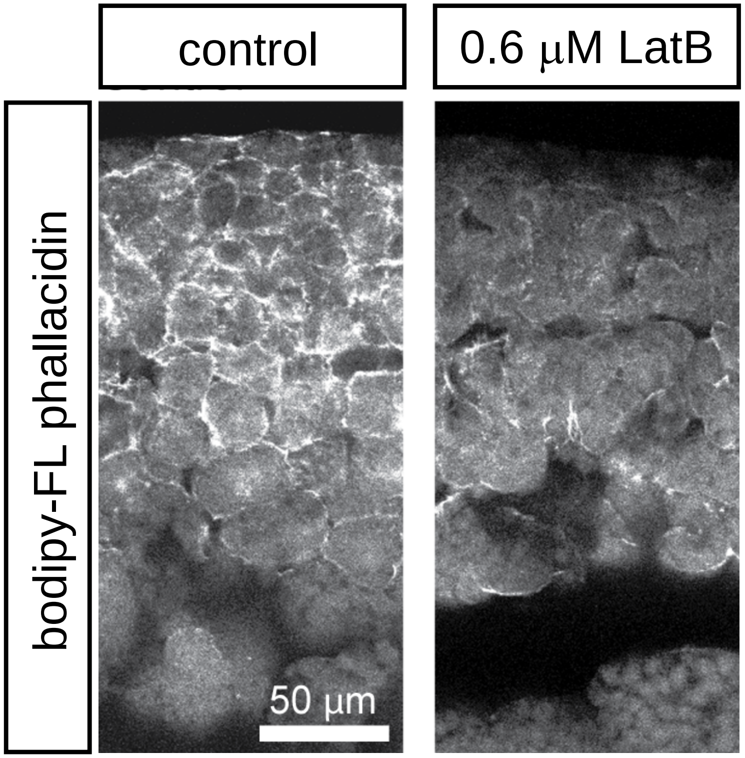

... but contributes little to stiffness.



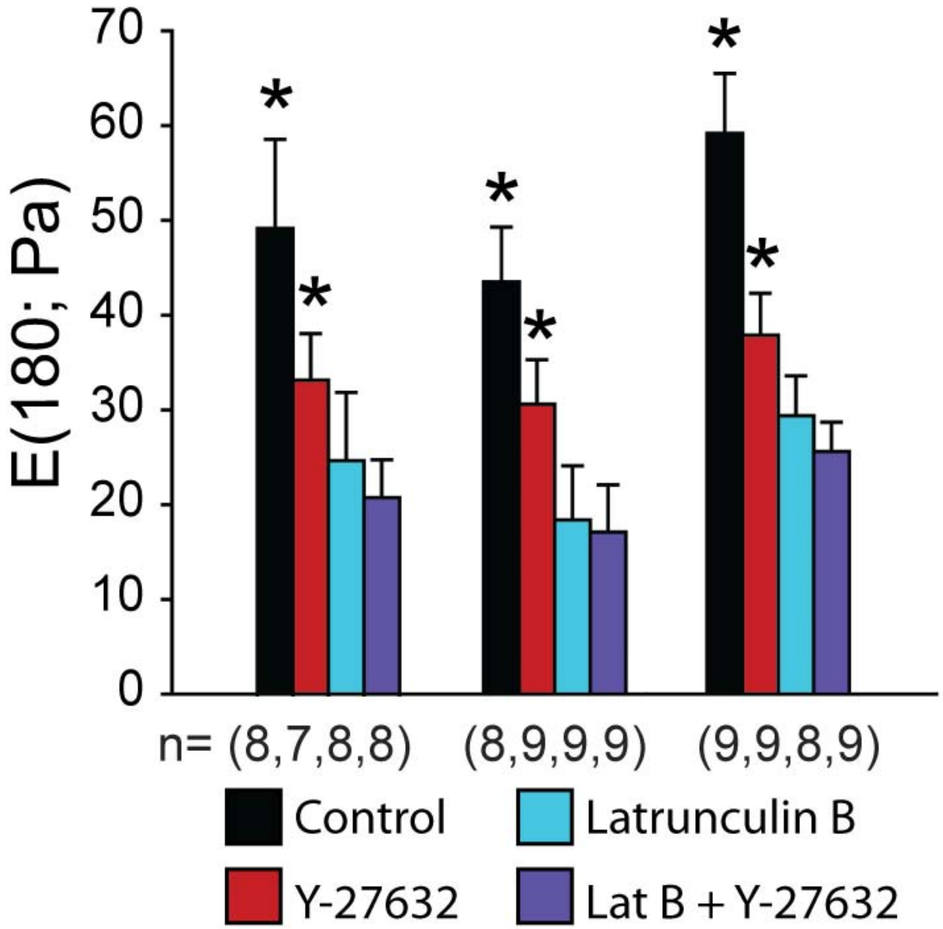

Zhou, Kim, and Davidson (2009) Development

# Actomyosin controls stiffness

De-polymerizing F-actin or reducing Myosin II activity reduces stiffness




At stage 16




Zhou, Kim, and Davidson (2009) Development

*Significantly different from column to right, $P < 0.05$

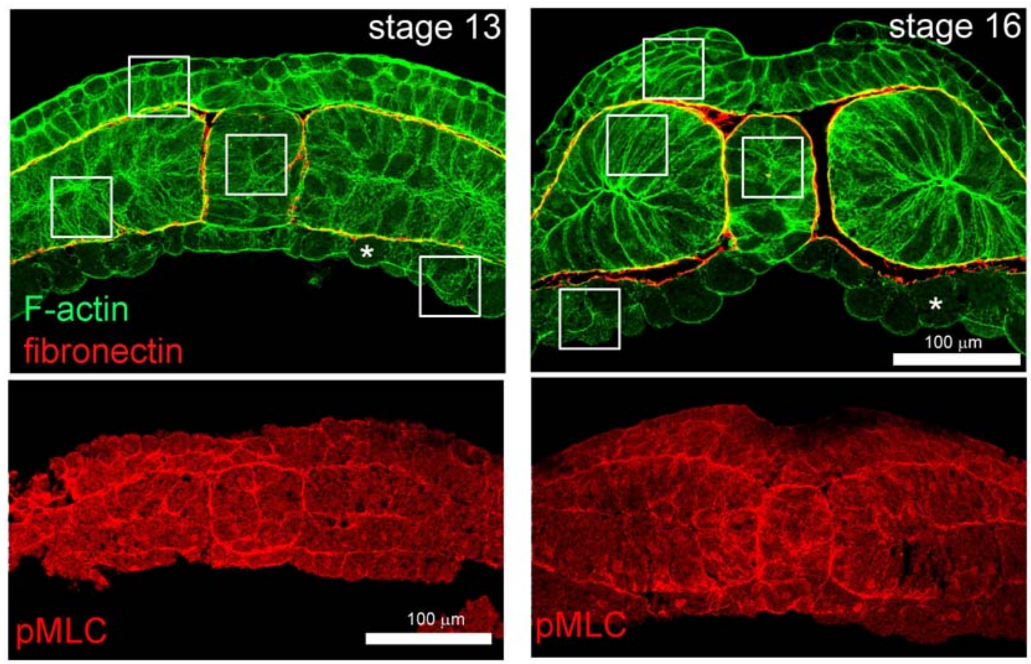
# What are the sources of stiffness?

**Structural** - ~~Notochord~~

~~**Extracellular matrix** - fibronectin, laminin, fibrillin, ...~~

**Cytoskeleton** - actin, myosin, ~~microtubules~~, ... *

* however, actomyosin levels alone are not likely to be responsible for stage-to-stage variation in stiffness...

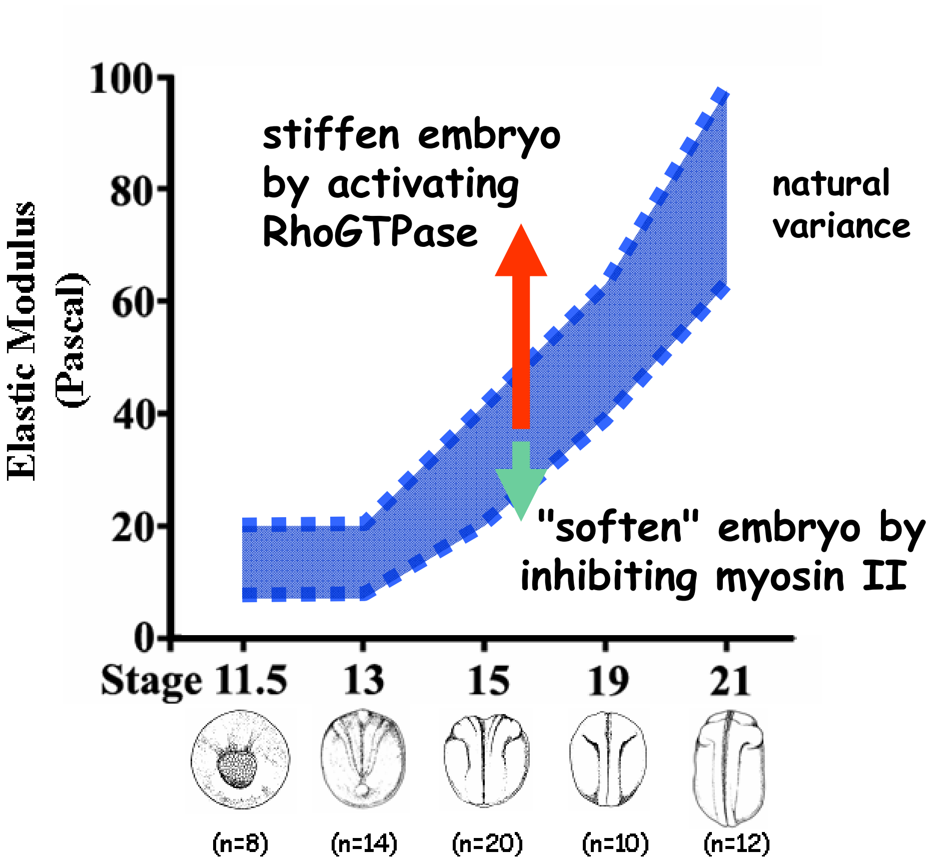


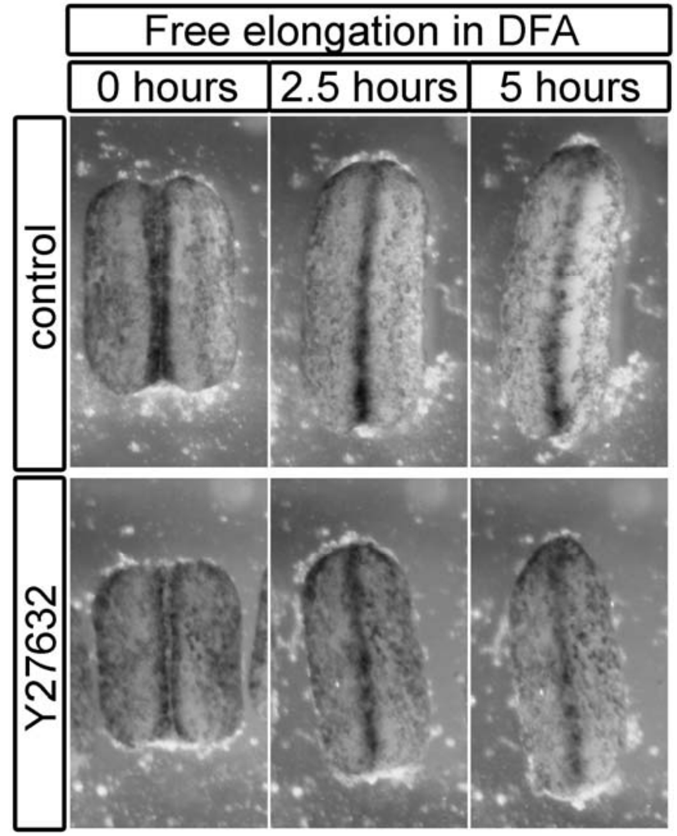
# More Puzzling Observations:

**Rate of elongation is constant even as embryos stiffen 6-fold.**

**Embryos with moderately reduced stiffness develop normally.**

*(Most treatments that stiffen embryos result in gastrulation defects.)*

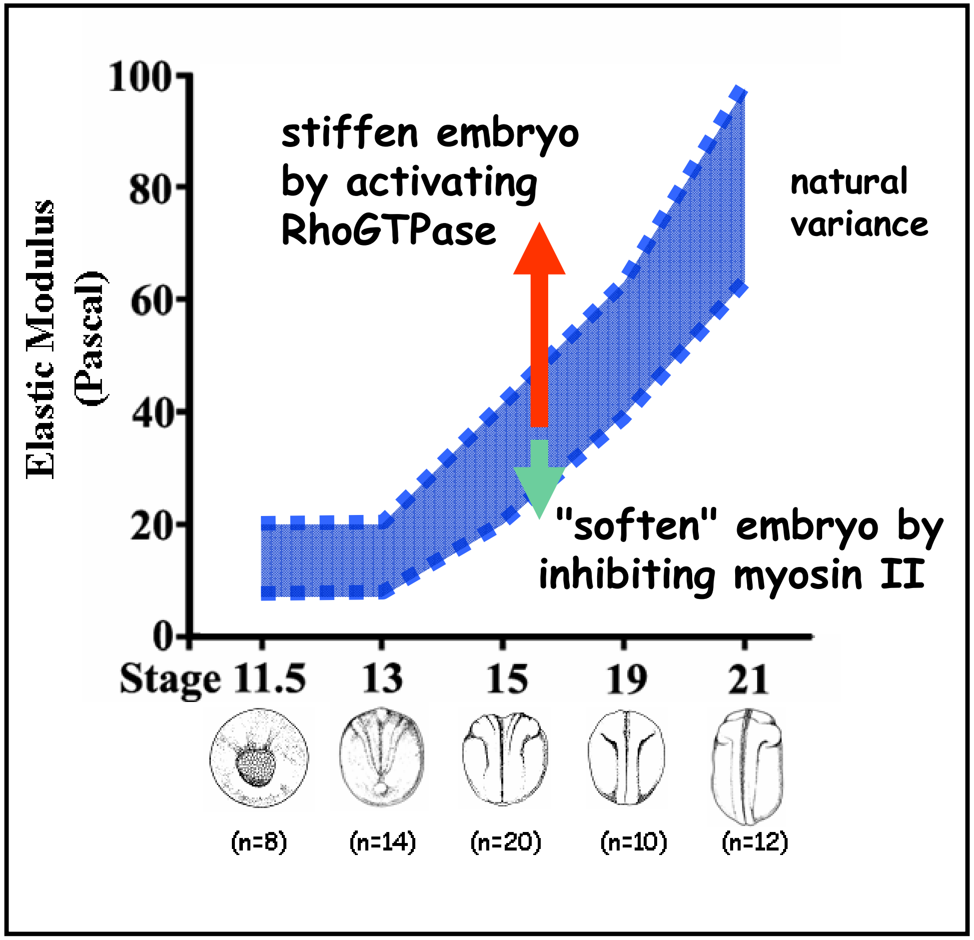



$$\text{deformation} \sim \frac{\text{force}}{\text{resistance}}$$

Jian Zhou

# Summary: Molecular/Genetic Control of Mechanics




$$\textit{deformation} \sim \frac{\textit{force}}{\textit{resistance}}$$

*1: Changes in stiffness during early development and contributions of different tissues to embryo stiffness.*

Large differences in mechanical properties among stages (~6 fold) & germ layers (~9 fold).

*2: Natural variation in tissue mechanics among embryos.*

Variation in mechanical properties among embryos.
(~2 fold variation in apparent stiffness)

Variation in force-production.
(~2 fold variation in maximum stress)

*3: Molecular factors that control embryo stiffness can also regulate force production.*

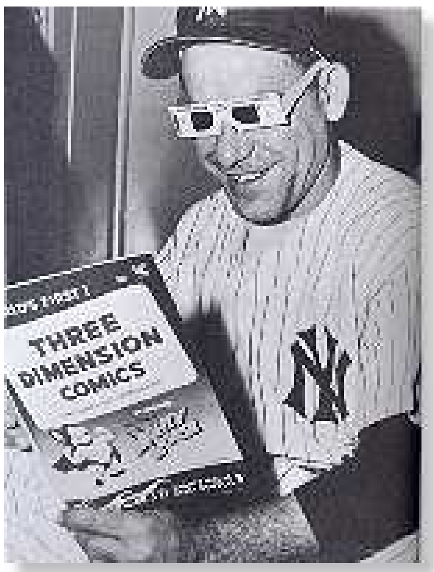
Actomyosin regulates tissue stiffness and force-production and can be modulated by RhoGTPases.

Actin polymerization and myosin activity (*).
(> 6 fold variation in stiffness)

*4: (<u>Something</u> about force generation)* Punctuated actin contractions during C&E and regulation by non-canonical Wnt signaling pathway.

How can I control these "machines" to build stuff?

"You can observe a lot by watching"
- Yogi Berra



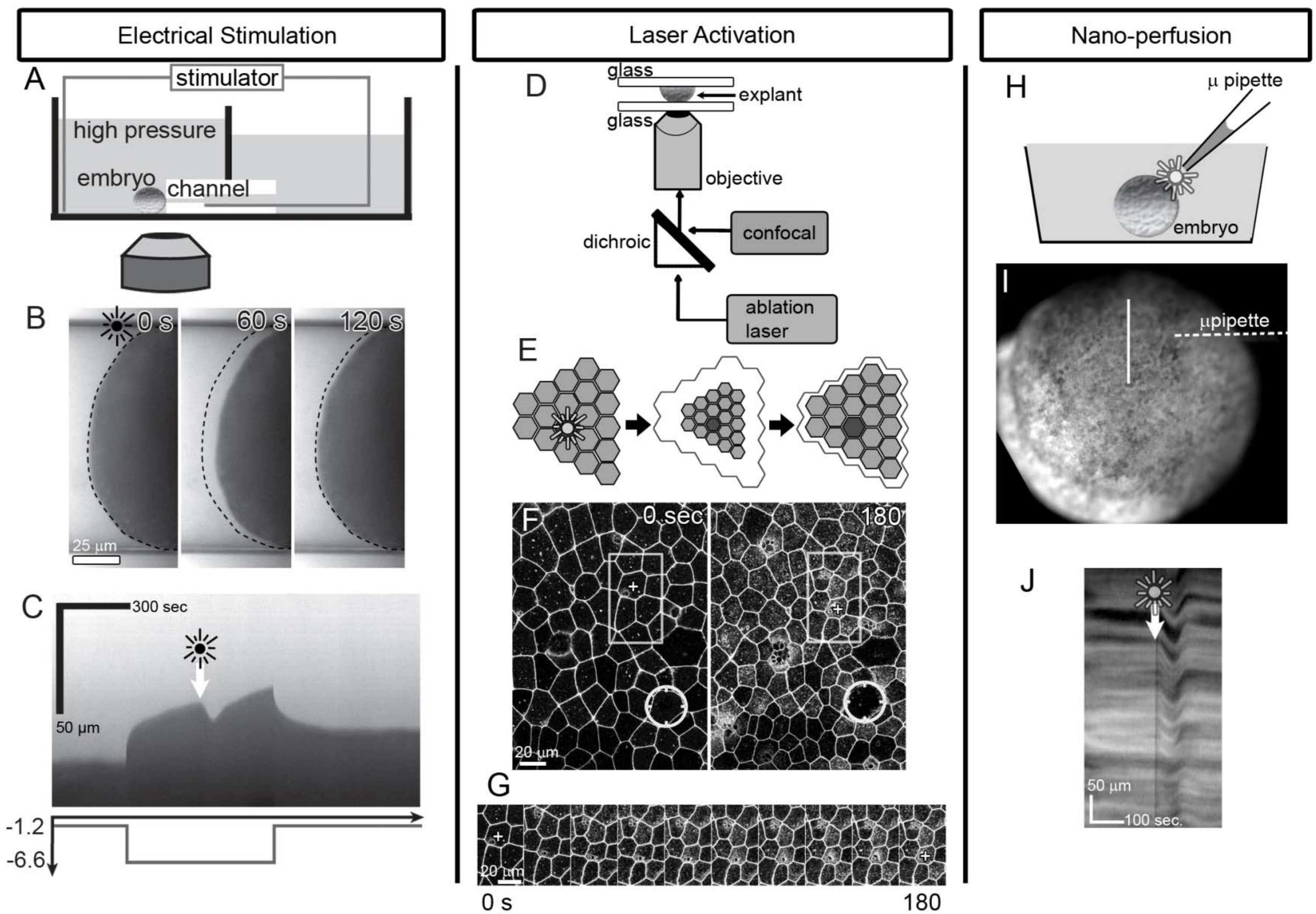

neurulation

gastrulation

organogenesis

Mechanics of Induced-Apical Constriction in Epithelia.

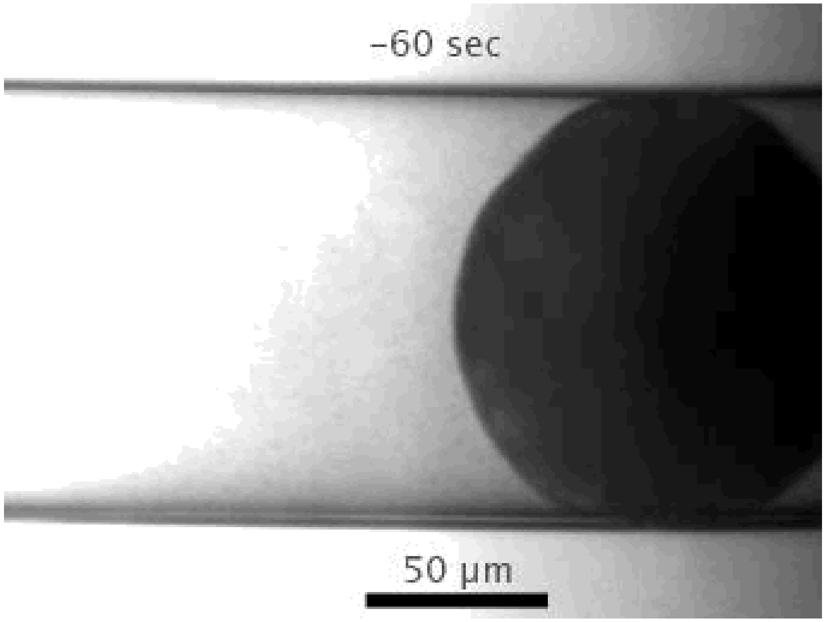
# Mechanics of Induced- Apical Constriction in Epithelia.

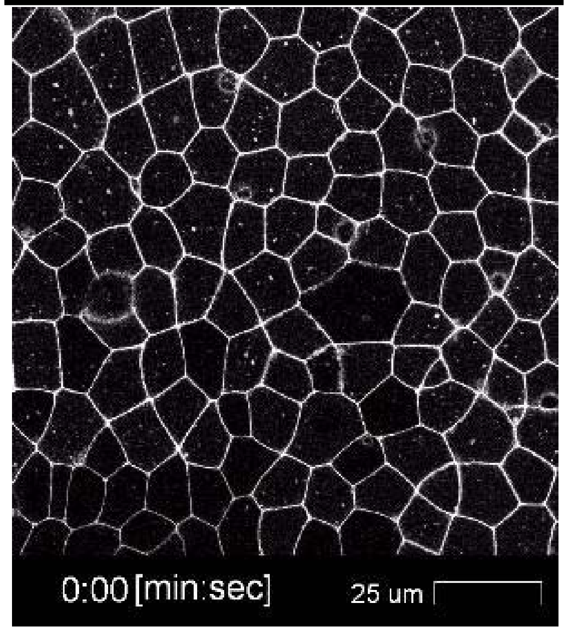


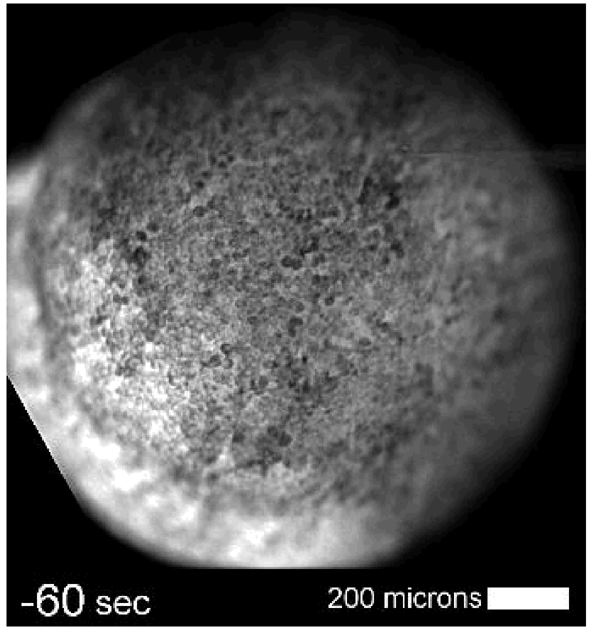

Sagar Joshi and Michelangelo von Dassow

# Mechanics of Induced-Apical Constriction in Epithelia.

| Electrical Stimulation of microaspirated tissue. | Laser Activation | Nano-perfusion of cell lysate |
| --- | --- | --- |

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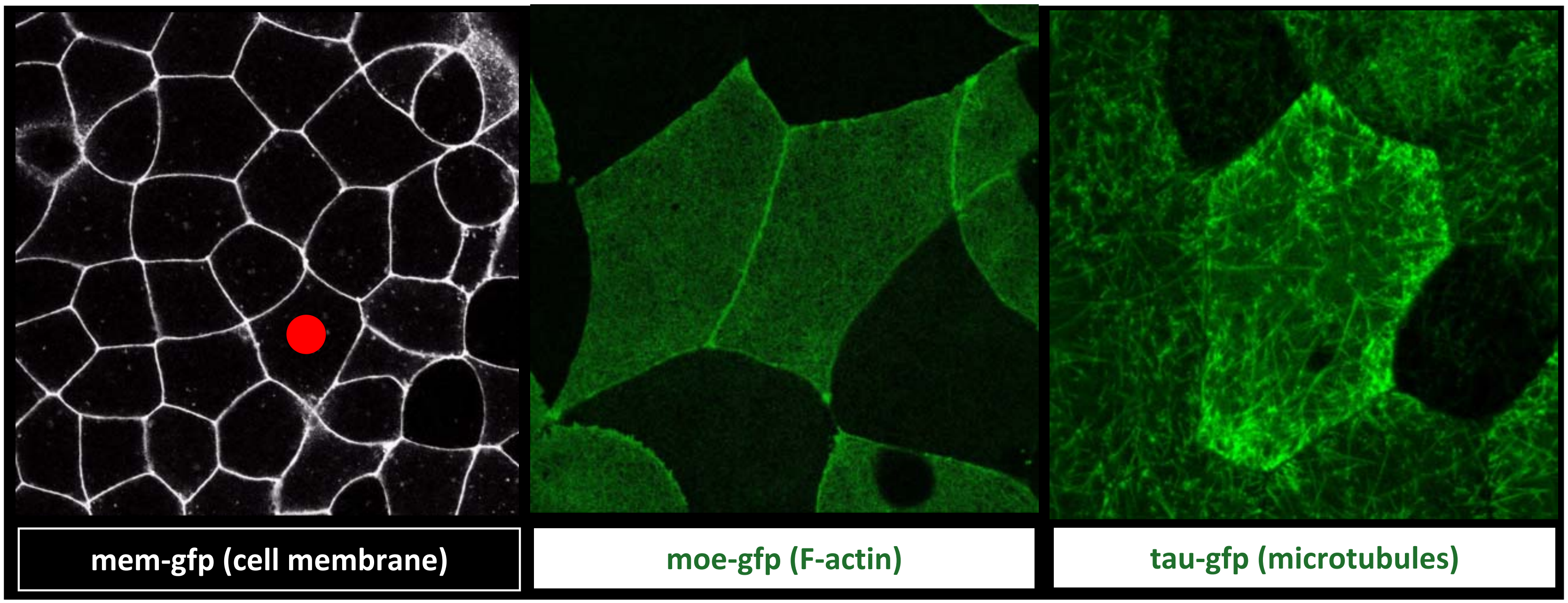
Induced-

# Mechanics of Apical Constriction in Epithelia.

Laser Activation does not produce 'recoil' but stimulates epithelial contraction in surrounding cells. F-actin is rapidly remodeled during contraction.




3 min. elapsed time

Sagar Joshi

# Mechanics of Induced- Apical Constriction in Epithelia.

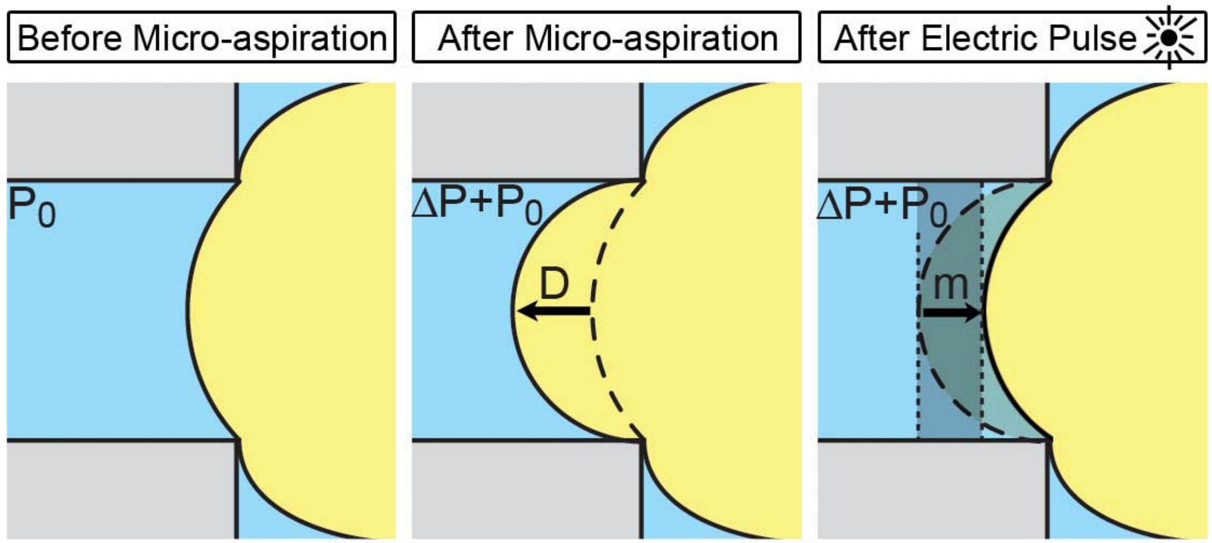
1. Perfusion allows rapid identification of "trigger" factors.

2. Laser Activation allows analysis of molecular motors and signal transduction factors.

3. Stimulation / Microaspiration allows a more sophisticated "muscle-like" mechanical analysis of contracting epithelia.

Work of Contraction
$\approx (\Delta P+P_0)*m$

Stress of Contraction
$\approx \Delta P*m/D$

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[do not need to perturb embryos]

$$\textit{deformation} \sim \frac{\textit{force}}{\textit{resistance}}$$

**Things we *"measure"*:**

- force or stress
- stiffness or modulus
- viscosity

[must perturb cells or tissues to measure]

Why does morphogenesis work so well?

How do molecular mechanisms account for robust movements?

When redundancy fails - how do movements break down?

How can we "control" the morphogenetic machines to build organs?

## Acknowledgements:

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Bard Ermentrout
Sachin Valenkar

(Carnegie Mellon)
Phil LeDuc, Bill Messner, Tony Kim
Gustavo Rohde
Kris Dahl

National Science Foundation
WHERE DISCOVERIES BEGIN

National Institute of Child Health and Human Development (NICHD)

American Heart Association
Learn and Live

BioEngineering