Cellular Mechanobiology

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Lecture Goals

1. To understand common themes and principles of cellular mechanotransduction
2. To appreciate how physical cues can perform a similar role as genetic and chemical cues in governing cell development and behavior
3. To understand current hypotheses on how gene expression is regulated by mechanical stimuli
Mechano- what?

- **Mechanobiology** - the study of how physical forces and changes in cell or tissue mechanics contribute to development, physiology, and disease

- **Mechanotransduction** – the conversion of a physical force to a biochemical response (aka mechanosignaling)

- **Mechanosensing** – when a protein or cellular structure responds to a physical cue to initiate mechanotransduction
Mechanics 101

**Stress** \((\sigma, \text{N/m}^2)\)

\[
\sigma = \frac{F}{A}
\]

**Strain** \((\varepsilon, \text{unitless})\)

\[
\varepsilon = \frac{\Delta L}{L_0}
\]

**Stiffness** \((E, \text{stress over strain, N/m}^2 = \text{Pa})\)

\[
E = \frac{\sigma}{\varepsilon}
\]

**Shear** = Stress that acts parallel to area

**Compression** = pushing force (N)

**Tension** = pulling force (N)

\[ F = \text{force}, \ A = \text{area}, \ L = \text{length} \]
Mechanotransduction in action

All cells are mechanosensitive. Mechanotransduction is ubiquitously implicated in cell and tissue development, physiology, and disease (Atherosclerosis, fibrosis, asthma, osteoporosis, heart failure, cancer)
Mechanotransduction in action:
Blood pressure autoregulation
and coronary artery disease – myogenic tone

Modified from Osol et al., AJP Heart 2002
Mechanotransduction in action: Auditory mechanotransduction and hearing

Beurg et al., J Neuroscience 2006
Mechanotransduction in action: Direction of Cell Fate

Modified from Engler et al., Cell 2006
Mechanotransduction in action:
Bone and tissue remodeling. Barefoot running

Lieberman et al., Nature 2010
Mechanotransduction in action:
Cell Motility – Cancer metastasis

Breast cancer cells expressing GFP coupled to nuclear localization signal

Denais et al., Science 2016
Key concepts in mechanotransduction

**Mechanosensing**
- cells test their environment
- adhesion receptors, membrane proteins probe ECM

**Signal transduction**
- mechanical signal transduced along a linked network
- cytoskeleton is often the force conduit

**Signal integration at nucleus**
- accumulation of signals over time
- chromatin rearrangement, nuclear pore opening

**Cellular response**
- from microseconds to minutes
- cell shape, fate, motility, growth
Mechanotransduction in a representative cell
Cellular model of mechanotransduction: **tensegrity**

- Architectural principle that describes the cytoskeletal architecture and how it responds to changes in force
- Mechanical stability is balanced between –
  - Components in compression (act as bars or struts)
  - Components in tension (act as cables, delineate system spatially)

Buckminster Fuller – *The Montreal Biosphere, a Geodesic dome*

Cytoskeleton in an epithelial cell
Tensegrity in the cell

- Cytoskeletal is "pre-stressed" – balance of tension (actin) and compression (microtubules)
- Breakdown/elongation of cytoskeletal elements allows regulation of cell shape and generation of traction forces
Tensegrity in the cell

• Stiff cytoskeletal network (relative to “soft” cell) allows rapid and long distance force transmission

Eddy Y. Xuan, University of Toronto, Canada
Key concepts in mechanotransduction

- Mechanosensing
  - Signal transduction
    - Signal integration at nucleus
      - Cellular response
Mechanosensing

- Passive (outside-in) – subcellular response to external tension/compression/shear etc
- Active (inside-out) – cell generated forces detect external environment (traction forces, durotaxis)
  - Cells periodically test their environment by pulling on focal adhesions

Modified from Trichet et al., PNAS 2012
Mechanosensing – focal adhesions

Focal adhesion complex
- integrins bind to ECM, bridge gap to an intracellular protein complex
- this complex includes talin/vinculin (and others) at cell periphery, which bind to actin stress fibers

Case and Waterman, Nat Cell Bio 2015
Tension-sensing probes to decode mechanosensation

Grashoff, Nature, 2010

Austen, Nat Cell Bio, 2015
Signal Propagation

- Mechanical signals are propagated via the cytoskeleton, protein-protein interactions and signal transduction cascades.

- Force can:
  - Induce protein conformational change
  - Induce alterations in membrane tension (MSCs)
  - Promote Assembly/turnover of receptor-ligand complexes
  - Cluster molecules together

Robison et al., Science 2016

Modified from Dufort et al., Nat Rev Mol Cell Bio 2011
Signal integration at the nucleus
How does force regulate gene expression?

Two potential mechanisms

- Force-dependent signal transduction
  - Cytosolic signaling cascades, often initiated by forces at the cell periphery, result in the activation/repression of transcription factors that regulate gene expression
  - May or may not include mechano-sensitive channels

- Direct force transmission to the nucleus
  - Force is transmitted via the cytoskeleton to the nucleoskeleton and physically tugs on nucleus to elicit biochemical changes that alter gene expression

These mechanisms are not mutually exclusive and likely each play some role in the mechanoregulation of gene expression
Force-dependent signal transduction:
role of mechanosensitive ion channels

- Channel open probability changes in response to alterations in membrane tension or cytoskeletal strain transmission
- Flux of ions (ex: Ca^{2+}) can affect downstream kinase pathways
- Many of these have only been discovered in the past 5-10 years and are not well understood
- Examples – TRP, Piezo channels

Modified from Xiao and Xu, Curr Biol 2011
Role of mechanosensitive ion channels: direction of cell fate

Trying to differentiate MSCs into glial or neuronal cells. siRNA against Piezo1 was associated with an increase in glial cell fate vs neuronal cell fate and associated glial vs neuronal proteins (ex: GFAP vs Map2)

Pathak et al. PNAS 2014
Role of kinase signaling cascades

- Mechanosensitive proteins deform and subsequent allosteric changes alter downstream kinase signaling
- Examples: focal adhesion associated kinase signaling, YAP/TAZ, titin
Kinase signaling cascades

Gjorevski and Nelson. Cytokine Growth Factor Rev 2009

Dupont et al., Nature 2011
Force transmission to the nucleus

- Direct interactions between the cytoskeleton and nucleoskeleton transmit force to affect nuclear structure/function
- Relatively new field, much to explore

↑ / ↓ gene expression
Force transmission to the nucleus: LINC complex

- **Linkers of the Nucleo- and Cytoskeleton**
- Nesprins 1-4 in the outer nuclear envelope and SUN1/2 in the inner nuclear envelope
- Complete a contiguous route from ECM, to cytoskeleton, to nucleoskeleton, to chromatin

Isermann and Lammerding Current Biology 2016
Force transmission to the nucleus: a role of lamina-associated domains (LADs)?

Force dependent changes in nuclear pore complex?
Chromatin “stretching” regulates gene expression

Modified from Tajik et al., *Nature Materials* 2016

stress applied to integrins propagates through the tensed actin cytoskeleton to the LINC complex and then through lamina–chromatin interactions to directly stretch chromatin and upregulate transcription

Modified from Tajik et al., *Nature Materials* 2016
Strain on the nucleus is sufficient to activate nuclear mechanotransduction

Guilluy et al., Nature Cell Biology 2016
Summary

• Mechanobiology is everywhere
• Cells rely on conserved mechanotransduction principles to achieve diverse results in different organ systems vital to cell health and disease
• Cellular mechanobiology is a burgeoning field
END
Sarcomere shortening drives myocyte contraction

- Troponin – calcium sensor on thin filament
- Titin – sarcomeric spring

Ottenheijm and Granzier, *Physiology*, 2010
Frank Starling – the text book view...

- Increase in myofilament overlap promotes acto-myosin interaction and therefore force (eh not really)
- Stretch increases the Ca sensitivity of the myofilaments. (yes, but how?)

Fukuda et al., Curr Cardiol Rev 2009
Titin strain sensing:

\[ \uparrow \text{strain} \rightarrow \text{structural rearrangements in myosin + troponin} \rightarrow \uparrow \text{Ca sensitivity, } \uparrow \text{force} \]

Myosin mechanosensing:

\[ \uparrow \text{afterload} \rightarrow \text{structural rearrangements in myosin} \rightarrow \uparrow \text{motors recruited} \rightarrow \text{high load contraction} \]
Titin: strain-dependent hypertrophic signaling

Modified from Granzier et al., Circ Res. 2004

LeWinter and Granzier, Circulation 2010
How do stress and strain activate myocyte growth?

1. Nuclear relay hypothesis
2. Force transmission to the nucleus

What are the conduits for force transmission to the nucleus?
Mechanobiology in the Heart

Mechanotransduction is vital for proper cardiac development and for maintaining and tuning the strength of cardiac contraction. Changes in mechanical forces or altered responses to mechanical stress underscore the development of both acquired and inherited heart disease and arrhythmia.
Mechanotransduction in the Heart

What we’ve known for decades/a century:

1. The heart can triple its contractile strength within ms of being stretch, and further increase beating strength over minutes in the face of acute spikes in blood pressure.

2. In the face of chronically elevated blood pressures, the heart profoundly remodels its structure (months, years).

3. Mechanical stress alters the electrical activity of the heart, and can trigger arrhythmia.

*How does this happen? Why should we care?*
Frank-Starling and ANREP effects

- Increased filling of the heart (preload, strain) causes an immediate increase in contractile force
  - Ernest Henry Starling, 1912
  - Exercise, fight or flight, just walking up the stairs

- Increased afterload (elevated arterial BP) causes a further elevation in contractile force
  - Gleb von Anrep, 1912
  - Requires a rise in $[\text{Ca}^{2+}]_i$
Disrupted Ca$^{2+}$ homeostasis drives cardiac hypertrophy and failure


Passier et al., JCI 2000
Teaser - how are mechanical forces transmitted through the myocyte?

• The MT cytoskeleton is required for...
  – Mechanically induced Ca$^{2+}$ release (Iribe et al., Circ Res 2009, Prosser et al., Science 2011)
  – Mechanical signaling between adjacent myocytes (Nitsan et al., Nat Phys 2016)
  – Mechanical stress-induced arrhythmias (Kerr et al., Nat Comm 2015)
The Left Ventricular (LV) Pressure-Volume (PV) Loop

- **PV loop** – stress strain relationship of a single cardiac cycle
- Heart is stretched, generates pressures, and shortens against a resisting load with each beat
- **Preload** – filling pressure at the end of diastole (induces strain)
- **Afterload** – arterial resistance that must be overcome to eject blood (stress)
Cardiac Physiology 101
Sarcomeres structure cardiac muscle