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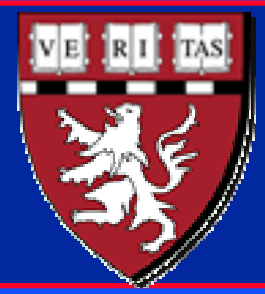

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EFFECTS OF EXOGENOUS MECHANICAL FORCES ON CELLS AND TISSUE

Mechanotransduction Mechanisms

M. Spector, Ph.D.

MECHANOTRANSDUCTION MECHANISMS

Direct Effects on Cells

- **Cell Membrane Strain-Related Mechanisms**
  - Cell wounding
  - Membrane receptor configuration, clustering, and orientation
  - Strain-sensitive (stretch-activated) ion channels
  - Activation of membrane-bound enzymes
  - Alterations in focal adhesion molecules
- **Deformation of the Cytoskeleton (Actin Network)**

MECHANOTRANSDUCTION MECHANISMS

Indirect Effects on Cells (Tissue-Level Effects)

- Compression and Hydrostatic Pressure
- Fluid Flow (Electrokinetic)
- Strain-Related Potentials (Piezoelectric)

CELL WOUNDING

PL McNeil, J. Cell Biol. 137:1 (1997)

- A break in the integrity of the plasma membrane immediately compromises this structure's essential role as a barrier, and this can kill the affected cell.
  - Yet animal cell plasma membranes, unprotected by a cell wall, are highly vulnerable to mechanically induced disruption.
- Moreover, many tissue environments generate and receive "physiological" levels of mechanical force that impose shear, tensile, and compressive stresses on constituent cells.
- Mechanical stress also induces an adaptive response by cells, which must sense and respond to this stimulus.
  - A skeletal muscle experiences during its lifetime a highly variable degree of mechanical load. Its individual myofibers must adapt to this changing mechanical environment, hypertrophying in response to increased load and atrophying in response to decreased load.
- Such changes in tissue architecture are important because they improve mechanical functioning, make economical use of valuable resources, repair or replace injured components, and/or prevent future injury.

CELL WOUNDING UNDER PHYSIOLOGICAL CONDITIONS

Organ (ref.)	Cell Types Investigated	% wounded
Skeletal muscle (29)	Skeletal muscle cells	5-30
Skin (29)	Epidermal cells	3-6
G.I. tract (27)	Epithelial cells	NM
Cardiac muscle (11)	Cardiac myocytes	20
Aorta (46)	Endothelial cells	6.5
Inner ear*	Auditory hair cells	NM

NM: Not Measured

* Unpublished evidence of Dr. M. Mulroy (Medical College of Georgia)

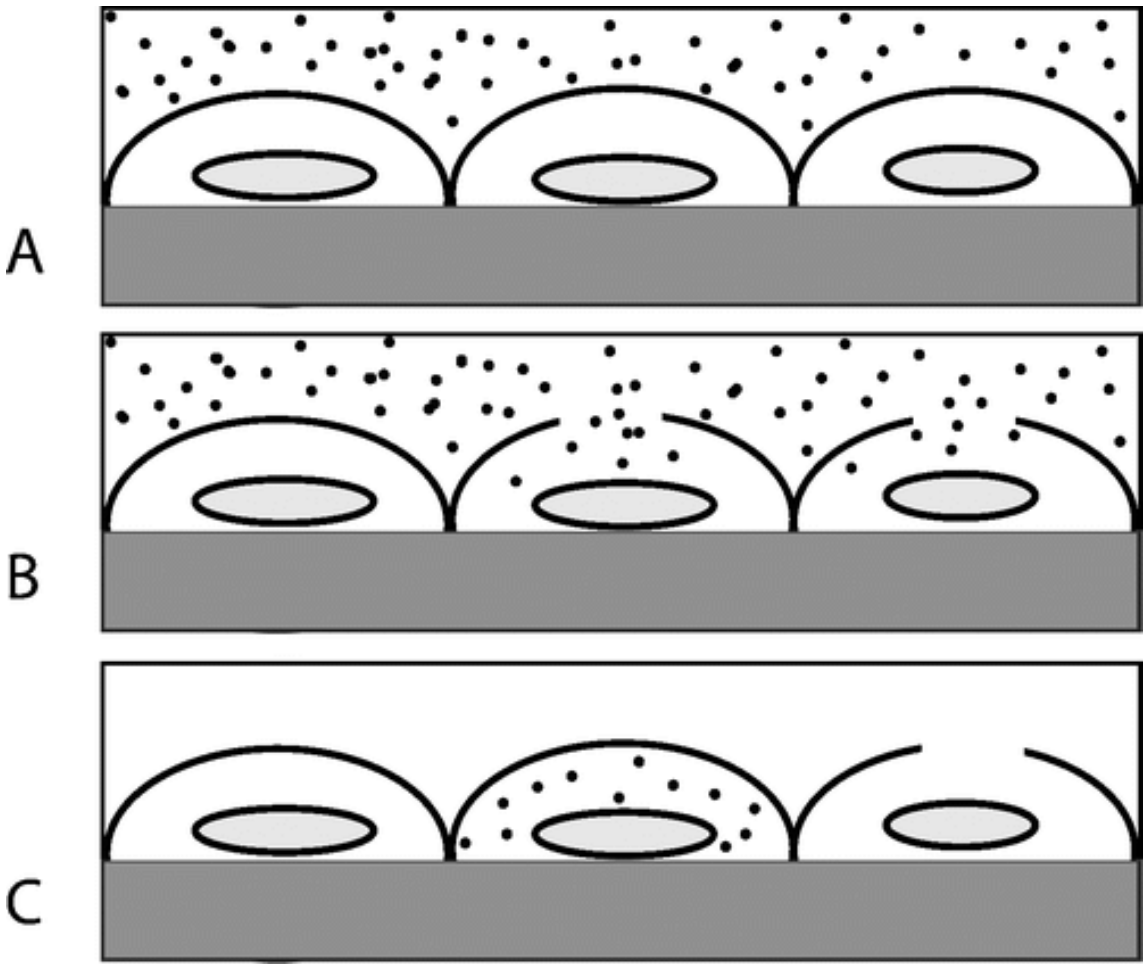
PL McNeil, *J. Cell Biol.* 137:1 (1997)

CELL WOUNDING

PL McNeil, *J. Cell Biol.* 137:1 (1997)

- Plasma membrane disruption is a common, normal, and therefore biologically important event in many tissues.
- Specialized adaptations prevent disruption-induced cell death and protect cells from incurring this common injury
  - an active, exocytotic mechanism rapidly reseals the membrane
  - specialized cytoskeletal proteins provide mechanical reinforcement
- Mechanotransduction occurs when growth factors (*e.g.*, FGF) and other regulators are released through disruptions.
- Signals such as Ca^{2+} enter through the breach in the membrane
- Since mechanical stress predates cell-cell signal molecules as an important biological stimulus, it may be that Ca-regulated exocytosis evolved originally as a resealing mechanism, and FGF may, by similar reasoning, be an evolutionary primitive autocrine signal.

PL McNeil, *Ann Rev Cell Dev Biol* 19:697(2003)



Detection of resealed plasma membrane disruptions.

(*A*) A membrane impermeant probe (*black dots*) is present in the fluid bathing cells. (*B*) Two cells experience a plasma membrane disruption that allows probe entry into cytosol. (*C*) After washing away of the probe, only the cell (*middle position*) that suffered and survived a plasma membrane disruption is labeled. The dead cell (*right position*), that failed to reseal, remains unlabeled. In vivo probe removal can be accomplished by vascular perfusion with saline lacking the probe, or, if the probe is a non-native component of the external milieu, elimination can occur naturally via the urine.

Healing of a Cell Wound

Disruption-elicited exocytosis facilitates the spontaneous resealing of the membrane by reducing membrane tension (maintained by the cytoskeleton)

Figure removed for copyright reasons.
Source: Figure 1b in McNeil, P. L. and M. Terasaki. "Coping with the inevitable: how cells repair a torn surface membrane." ***Nature Cell Biol*****. 3:E124 (2001)**

Healing of a Cell Wound

The "Patch Hypothesis."
Entry of Ca^{++} through the disruption stimulates a rapid, massive vesicle-vesicle fusion that results in a vesicle patch of the breach in the membrane.

Figure removed for copyright reasons.
Source: Figure 1c in McNeil, P. L. and M. Terasaki. "Coping with the inevitable: how cells repair a torn surface membrane." ***Nature Cell Biol*****. 3:E124 (2001)**

Diagram removed for copyright reasons.

Deformation-Induced Lipid Trafficking

A: A lung epithelial cell prestretch.

B: Stretching of the cell triggers exocytotic fusion of cytoplasmic vesicles with the plasma membrane.

C: The result is an increase in plasma membrane surface area.

D: The cell can now be stretched beyond its previous breaking point.

PL McNeil, Crti Care Med 31(8) Suppl:S496 (2003)

Diagram removed for copyright reasons.

Damage Sensing

Ca2+ can enter and growth factors can exit through a disruption. In consequence, a response is elicited in the wounded cell itself (e.g., cfos expression) and possibly in neighboring cells (e.g., through released growth factors).

PL McNeil, Crit Care Med 31(8) Suppl:S496 (2003)

ELECTROPORATION

http://www-fp.mcs.anl.gov/ccst/research/reports_pre1998/comp_bio/electroporation/

- Cell membranes are largely composed of amphiphilic lipids which self-assemble into highly insulating structures and thus present a large energy barrier to transmembrane ionic transport.
- The lipid matrix can be disrupted by a strong external electric field leading to an increase in transmembrane conductivity and diffusive permeability.
- These effects are the result of formation of aqueous pores in the membrane, which also alter the electrical potential across the membrane.
- These events are encountered in practice, both by design and by accident.
- Electroporation of cell membranes is used as a tool in injecting drugs and DNA into the cell.
- Electroporation is also the basic mechanism of tissue injury in high-voltage electric shock.

Diagram removed for copyright reasons.
See figure "General Model of Mechanical Load Detection and Activation of Mechano-electrochemical Sensory Systems in Cells" in AJ Banes, et al. *Biochem Cell Biol* 73:349 (1995)

Diagram removed for copyright reasons.
See figure "Model for a Cytoskeletally linked Load Deformation Mechanosensory Complex" in AJ Banes, et al. *Biochem Cell Biol* 73:349 (1995)

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Mechanotransduction: All Signals Point to Cytoskeleton, Matrix, and Integrins

FJ Alenghat and DE Ingber

B: Forces applied directly to the cell surface

A: Forces applied through the ECM

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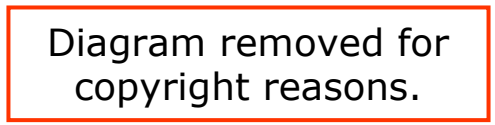
Mechanotransduction: All Signals Point to Cytoskeleton, Matrix, and Integrins
FJ Alenghat and DE Ingber

- **Forces travel to integrin-anchored focal adhesions through matrix attachments or cytoskeletal filaments.**
- **Internally generated tension and forces transmitted through cell-cell contact similarly reach focal adhesions through the cytoskeleton.**
- **Forces concentrated within the focal adhesion can stimulate integrin clustering and induce recruitment of additional cytoskeletal linker proteins (Vin, vinculin; Pax, paxillin; Tal, talin) that connect directly to microfilaments and indirectly to microtubules.**
- **Forces applied to this specialized cytoskeletal adhesion complex also activate integrin-associated signal cascades.**
- **In the case shown, when integrins are mechanically stressed, the complex stimulates Gs-mediated up-regulation of the cAMP cascade through adenylate cyclase (AC), resulting in nuclear translocation of the catalytic subunit of protein kinase A, PKA-c. MEK, mitogen-activated or extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase; GDP, guanosine diphosphate, ATP, adenosine triphosphate.**

Heidemann, Review of the paper by Wang, *et al*. *Sci* 260:1080 (1993)



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See figure "IP_3 Traverses Gap Junctions to Communicate a Load Signal by Ca^{2+} Wave Propagation," in AJ Banes, et al. *Biochem Cell Biol* 73:349 (1995)

F. Guilak
Demonstration of Ca Waves

Diagrams removed for copyright reasons.

Diagrams removed for copyright reasons. See Davies and Tripathi, "Mechanical stress mechanisms and the cell. An endothelial paradigm."
Circ Res 72:239 (1993)

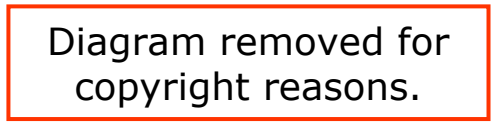
Kinetics of Response Events in Mechnically Loaded Cells

TIME	RESPONSE
Milliseconds to Seconds	Signalling with 20 messengers Mechanically active channels, Ca^{2+}, Na^{+}, K^{+}, H^{+} IP_3, cAMP, cGMP, PGE_2, DAG RTKs, NRTKs G proteins active Signal transduction pathways activated P Signal dampening-modulation
Minutes to Hours	Signalling with kinases (SHC, SOS, GRB2, raf-ras, MEK,ERK) Transcription (c-fos, jun, other TFs) Translation (fos, jun, other TFs, cyclins, CDKs) Structural cytoskeleton Actin, tubulin, other cytoplasmic filament proteins, polymerization Focal adhension rearrangement Other cytoskeletal changes-change in cell shape, nuclear shape
Days, Behavioral	Increased basal stress state to a new equilibrium stress state Contract matrix Migrate, contract Express-degrade matrix Divide-die Adapt all systems to a new equilibrium stress state

Figure by MIT OCW. After Banes et al.

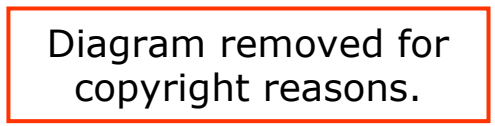
AJ Banes, *et al.*

Autobaric Effects



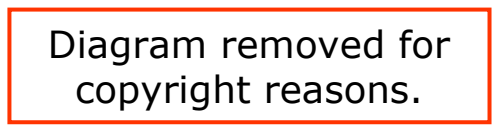
Contraction in response to load allows a cell to change its "stress state"

Parabaric Effects
Direct



Contraction in response to load allows one cell to apply a force to other cell that it is contacting

Parabaric Effects
Indirect



In response to load a cell can contract, and thus deform a substrate thus imparting strain to other cells in the vicinity without directly contacting them

AJ Banes, *et al.*

Apparatus for Stretching Cells on a Flexible Membrane

Five diagrams removed due to copyright reasons.
See Schaffer, et al., "Device for the application of a dynamic biaxially uniform and isotropic strain to a flexible cell culture membrane."
J Orthop Res 12:709 (1994)