Topics for the class:

What is cell membrane repair?

Why do cells need to actively repair their membranes?

How do cells repair the plasma membrane at the molecular level?

What are the pathologic consequences of defective membrane repair?

Can membrane repair be targeted as a therapeutic approach?
Ca\textsuperscript{2+}-triggered patch model for membrane repair

- Membrane disruption
- \( \downarrow \)
- \( \text{Ca}^{2+} \) entry through the injury site
- \( \downarrow \)
- \( \text{Ca}^{2+} \)-triggering vesicle exocytosis
- \( \downarrow \)
- Vesicle fusion
- \( \downarrow \)
- Patch formation; membrane resealing

*Nature Reviews Molecular Cell Biology 6, 499-505 (June 2005)*
Intracellular vesicles participate in membrane patching following damage

McNeil and Baker, Cell and Tissue Research, 2001
Evidence of membrane resealing in living mammalian cells


Lovering, et al. AJP Cell April 2011 C803-C813
Methods to measure membrane repair

- Dye exclusion/entry into the cell
  - UV laser damage
  - Physical disruption of the membrane
  - Histological analysis of tissues (Evans blue, IgG entry)

- Leak of a biomarker out of the cell
  - Creatine kinase
  - Troponins
  - Lactate dehydrogenase

- Changes in membrane biophysical conditions
Membrane repair is a conserved physiologic process in multiple tissues

<table>
<thead>
<tr>
<th>Organ</th>
<th>Type of mechanical force</th>
<th>Principal cells stressed</th>
<th>Cell wounding (proportion of cells involved)</th>
<th>Reference</th>
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<tr>
<td>Skeletal muscle</td>
<td>Aperiodic, highly variable intensity: shear, stretch</td>
<td>Skeletal muscle cells (myocytes)</td>
<td>Yes (3-20%)</td>
<td>McNeil and Khakee, 1992</td>
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<tr>
<td>Cardiac muscle</td>
<td>Cyclic: shear, stretch</td>
<td>Cardiac myocytes</td>
<td>Yes (25%)</td>
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<td>Skin</td>
<td>Aperiodic, highly variable intensity: shear, stretch, compression</td>
<td>Epidermal cells, Fibroblasts, etc.</td>
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<td>Gastrointestinal tract</td>
<td>Cyclic: shear, stretch</td>
<td>Epithelial cells, smooth muscle cells</td>
<td>Yes, epithelial cells (%) not measured</td>
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<td>Vascular (conducting)</td>
<td>Constant and cyclic:shear</td>
<td>Endothelial cells, smooth muscle cells</td>
<td>Yes, aortic endothelial cells (6.5%)</td>
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<td>Cyclic: stretch</td>
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<td>Alveolar cells (2-30% in mechanically ventilated lung)</td>
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Necessity of membrane repair in eukaryotic cells

Membrane Patch = Cell Survival

? drives membrane repair

Cell Membrane

MG53 drives membrane repair?
Resealing mechanism is dependent on size of membrane disruption

Increasing size of membrane disruption

Membrane tension is a key determinate of cell membrane resealing
Evolution of fusion-based resealing following development of endocytotic apparatus
Skeletal muscle displays a substantial amount of membrane resealing

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Contractile nature of skeletal muscles lead to extensive mechanical stress.
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Cellular mechanism are better understood than the molecular mechanisms.
Originally determined to be a ferlin family protein that was known to be mutated in limb girdle muscular dystrophy (type 2B) and Myoshi myopathy patients, (Nat Genet. 1998 Sep;20(1):31-6).
Muscular dystrophy and compromised membrane repair in dysferlin null mice

Dysferlin function in membrane repair is not clear

Molecular function of dysferlin in membrane repair is still not clear. Does it act as a fusogen for vesicles? Direct effects on remodeling of membrane? A platform to assemble other factors?
Annexin-A5 assembled into two-dimensional arrays promotes cell membrane repair

Bouterm et al. Nature Communications 2, Article number: 270 doi:10.1038/ncomms1270
AHNAK and other proteins interact with dysferlin and modulate membrane repair capacity

Multiple proteins have been shown to be important for the resealing of membranes, however the molecular function of these proteins is not clear.
Other dysferlin interacting proteins linked to membrane repair

Mitsugumin 53 (MG53) is a tripartite motif family protein linked to membrane trafficking during membrane repair.

MG53 knockout mice display myopathy and breakdown of plasma membrane integrity

MG53 is required for translocation of dysferlin to injury sites on the plasma membrane

MG53 also associated with dysferlin to facilitate membrane repair

Han (2011) Skeletal Muscle Vol. 1 Issue 1
Proteins associated with vesicle fusion during endo/exocytosis are also involved in membrane repair.
Reorganization of the cytoskeleton and organelles is required for effective membrane repair.

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Viral overexpression of target genes such as dysferlin

Poloxamer 188 is a long chain co-block polymer that can increase membrane resealing following a number of different types of membrane damage. Has been shown to decrease dystrophic pathology in cardiac muscle.
Recombinant MG53 (rhMG53) can locate to injury sites on the cell membrane

rhMG53 can increase the resealing of muscle and non-muscle cell membranes following various injuries.
Injection of rhMG53 can improve pathology in *mdx* model of muscular dystrophy

Potential mechanism for rhMG53 in membrane resealing