Molecular Cell Biology

Intermediate Filaments and Cytoskeletal Forces

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Big Question: How do disease-causing mutations lead to heart failure in humans?

To answer this question, we study cardiac contractility across multiple levels of organization.

- Optical trapping
- Protein expression and purification

Single Molecule Level

Cellular Level

- Stem cell technologies
- CRISPR
- Immunofluorescence
- Traction force microscopy

Macromolecular Assembly Level

Tissue Level

- Microfabrication
- Tissue engineering
- Drug testing

- In vitro reconstitution assays
- Stopped flow kinetics

To find out more, email: greenberg@wustl.edu
Intermediate Filaments
Components of the Cytoskeleton

**Microfilaments**
- Actin

**Microtubules**
- αβ-Tubulin dimer

**Intermediate filaments**
- Various

**Motors**
- Myosins
- Kinesins
- Dyneins

Adapted from Lodish et al., 2012
Introduction

• Present in Metazoa / Animal Cells

• Complex Gene Superfamily
  – 70 in Human Genome

• Specific Expression at Different Times and Places
Intermediate Filament Potential Functions In Vivo

• Mechanical Strength of Cytoplasm

• Help Epithelial Cells Resist Shear Stress - Filaments Connect to Cell-cell Junctions

• Holding the Nucleus in the Center of the Cell

• Signaling Organizers (scaffolds)

• Anchoring Actin Filaments to Focal Adhesions During Cell Migration
Intermediate Filament Structure & Assembly

Highly conserved coiled-coil domain with variable tails.
Intermediate Filament Structure & Assembly

- Can form heterodimers and heterotetramers
- Filaments are apolar (no motors)

A) Monomer

B) Dimer

C) Apolar tetramer (soluble form)

D) Unit Length filament (ULF)
Intermediate Filament Structure & Assembly

ULFs are dynamic while mature IFs are relatively stable.
Intermediate Filaments Physical Properties

Adapted from Alberts et al., 2002
Intermediate Filaments by EM: Filament Unraveling
IFs Need to Dynamically Reorganize in the Cell

Disassembly triggered by phosphorylation (e.g., Cyclin Dependent Kinases) or chaperone binding.
Keratin IF Networks Can Rearrange Within the Cell
FRAP of Vimentin vs. Keratin in One Cell

Left: Vimentin (Green)
Right: Keratin (Red)

10-min time intervals
Filaments are Relatively Stable but the Precursors are Dynamic

Keratin precursors moving 18 microns over 10 minutes
Photoconversion allows the observation of a subset of proteins. Fluorescence is converted from a dark to light state in one region of the cell.

Vimentin ULFs are photoconverted in one area and then become incorporated into other IFs.
IFs Disassemble and are Transported to New Assembly Sites
IF Transport Along Microtubules

Movie 2.

ULF moves along microtubules.

Co-imaging of GFP-ULF (green) and TagRFP-EB3 that decorates microtubules (red). Time-lapse images of GFP-ULF were collected once per second for 1 min using a spinning disc confocal. See legend of Figure 2A for more details.
# Classes of Intermediate Filaments

## TABLE 18-1  The Major Classes of Intermediate Filaments in Mammals

<table>
<thead>
<tr>
<th>Class</th>
<th>Protein</th>
<th>Distribution</th>
<th>Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Acidic keratins</td>
<td>Epithelial cells</td>
<td>Tissue strength and integrity</td>
</tr>
<tr>
<td>II</td>
<td>Basic keratins</td>
<td>Epithelial cells</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Desmin, GFAP, vimentin</td>
<td>Muscle, glial cells, mesenchymal cells</td>
<td>Sarcomere organization, integrity</td>
</tr>
<tr>
<td>IV</td>
<td>Neurofilaments (NFL, NFM, and NFH)</td>
<td>Neurons</td>
<td>Axon organization</td>
</tr>
<tr>
<td>V</td>
<td>Lamins</td>
<td>Nucleus</td>
<td>Nuclear structure and organization</td>
</tr>
</tbody>
</table>

![Desmosomes](image1.png)

![Epithelial cell](image2.png)

![Dense bodies](image3.png)

![Z disk](image4.png)

![Axon](image5.png)

![Nucleus](image6.png)
Vimentin

- All Cells in Early Development
- Cage Around Nucleus
- Interacts with MTs
Keratins

• Expressed in Epithelia
• Keratin Filaments Connect to Desmosome and Hemidesmosomes
• Differentiation of Epidermis includes Production of Massive Amounts of Keratin
• Provides Outer Protection of Skin
• Composes Hair, Nails, Feathers, etc.

Ericksson et al., 2009
Desmin

• Expressed in Muscle
• Elastic Elements to Prevent Over-stretching
• Connects / Aligns Z lines
• Mutations Lead to Myopathies

Goldfarb et al., 2009
Neurofilaments

• Neurofilament H, M, L Copolymer
• Prevent Axon Breakage
• Diseases with Clumps of Neurofilaments
  – Superoxide dismutase model for ALS
  – Clumps are secondary, not causative

Immunostain for neurofilaments showing clumps (green arrows)
Lamins

- Square Lattice on Inner Surface of Nuclear Membrane
- Mutations Cause Accelerated Aging Diseases
  - Progerias – dominant mutations
- Important Roles in Mechanosensing

17 Years Old
Intermediate Filaments Mediate Mechanosensing

Stem cells will differentiate to different lineages based on the stiffness of the substrate.

Forces are transmitted from the extracellular matrix to lamins in the nucleus, leading to changes in DNA transcription.

Engler et al., 2006
The Nuclear Lamina Senses Mechanical Forces
The Nuclear Lamina Senses Mechanical Forces

Figure 18-51
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The Nuclear Lamina Senses Mechanical Forces
Cytoskeletal Polymer Dynamics
Cytoskeletal Polymers Self Assemble *In Vitro*
General principles of macromolecular assembly

1. Large structures assemble from subunits
2. Specificity comes from multiple weak bonds on complementary surfaces
3. Subunits in symmetrical structures have identical or quasi-equivalent bonds between subunits
   • Helices
   • Icosahedrons
4. New properties emerge along assembly pathways
5. Regulation is imposed at multiple steps along pathways
Advantages of assembling large structures from small protein subunits

- Allows synthesis of error-free components; errors in protein synthesis occur ~1/3000 residues, so most small subunits are free of errors
- Quality control: defective subunits can be discarded
- Subunits are easy to recycle
- Multiple modes of regulation are available
Thermodynamics of Polymerization

- Add/Lose Subunits Only at Ends
- ON Rate = $k_+ c_1 N$
- OFF Rate = $k_- N$

$c_1 =$ Concentration of Monomers
$N =$ Concentration of Filament Ends

If the OFF Rate > ON Rate, get disassembly.
If the ON Rate > OFF Rate, get assembly.

The transition from disassembly to assembly occurs when the ON Rate=OFF Rate. This is the critical concentration, $C_c$.

Simple algebra shows that $C_c = k_- / k_+$
Steady-state Concentrations of Polymer & Monomer

Adapted from Lodish et al., 2012
Nucleators (e.g., Arp2/3, gamma-tubulin) reduce the energetic barrier to nucleation.

Adapted from Lodish et al., 2012
Directionality of Polymerization

In this model, there is no preference for adding to/removing from either end of the filament.

In intermediate filaments, the addition of ULFs is non-directional.

In actin filaments and microtubules, monomers are added directionally.
Polymerization Occurs Directionally in AF and MTs

Directionality is due to conformational changes in actin and microtubules associated with nucleotide hydrolysis.
Directionality of Polymerization

Nucleotide binding and hydrolysis changes the conformation of the monomer, leading to asymmetric binding interfaces.

Addition occurs preferentially at the plus end. The critical concentration is lower at the plus end than the minus end.

Nucleotide changes

\[ \text{ATP} \rightarrow \text{ADP} \]

Faster (more favorable)

Slower

Dissociation is equally favorable from either end.
Treadmilling

- Can Have Different Critical Concentrations at the Two Ends
- Net Addition at One End, Net Loss at the Other End
Treadmilling: Microtubule Photobleaching Experiment In Vivo

Fluorescent Tubulin Microinjected into Cell as Tracer

Laser Bleaches a Vertical Stripe
Nucleotide Caps

- If the rate of subunit addition > hydrolysis rate, get an NTP cap. This occurs during rapid growth.

- If the rate of subunit addition is similar to the hydrolysis rate, the cap can disappear, leading to depolymerization.
Dynamic Instability in Microtubules

GFP-tubulin in Cells

Pure proteins in vitro
Dynamic Instability in Microtubules

Figure 18-11
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Loss of the GTP cap leads to the release of strain and microtubule catastrophe. This is dynamic instability.
Dynamic Instability in Microtubules

Changes in tubulin structure as it transitions from GTP to GDP bound leads to straining of the microtubule lattice.
In actin filaments, the conformational changes are smaller, meaning that there is less elastic energy stored in the filament and thus less dramatic instability.
Instability Allows for a Dynamic Cytoskeleton

Two-Color Speckle Microscopy

- **Microtubules**
- **Actin**
How do Cells Regulate the Level of Polymerization?

• Total Concentration of Protein
• Covalent Modification of Subunits (e.g., phosphorylation of intermediate filaments)
• Binding of Small Molecules
• Binding of Another Protein (ABPs, MAPs)
Force Generation by the Cytoskeleton
Cytoskeletal Filaments Work Together

Adapted from Lodish et al., 2012
Connections Between Filaments in Neurons

Cortical Region

Deeper Axoplasm

Green = actin filaments
Red = microtubules
Yellow = intermediate filaments
Cyan = crosslinking proteins (plectin)

Klymkowsky 1999
Motors Coordinate Their Activities in Intracellular Transport
Cargoes Navigate a Complex Cytoskeleton

Green = microtubule (MT)
Red = actin filament (AF)

Bead contains both myosin-V (AF motor) and kinesin-1 (MT motor)

Schroeder et al. 2010
Force Generation by the Cytoskeleton

- Molecular motors (e.g., myosins, dynein, kinesins) can generate forces along cytoskeletal filaments.
- Polymerization of cytoskeletal filaments can generate forces.
- Motor proteins and cytoskeletal filaments work together to generate and sense cellular forces.
Cytoskeletal Polymerization Forces in the Axon Growth Cone

Actin
Microtubules
Polymerization Helps Drive Cell Migration

1. Extension
2. Adhesion
3. Translocation
4. De-adhesion and endocytic recycling
Measuring Molecular Forces Using Optical Tweezers

Optical tweezers measure nanometer movements and piconewton forces...

And allow you to play Tetris...
Measuring the Force of Polymerization Using an Optical Trap

Kerssemakers et al., 2006
Measuring the Force of a Single Dynein Using an Optical Trap

Nicholas et al., 2015
Motors Coordinate Their Activities in Intracellular Transport
Bidirectional Transport in Axons
Tug-of-War Leads to Bidirectional Motions of Purified Cellular Cargoes

To increase the efficiency of motors, there are complex regulatory networks involving post-translational modifications of the proteins and associated cargo adaptors.

Hancock 2014
Hendricks et al., 2014
Dynein Forms a Complex with Accessory Proteins
Dynein-Dynactin-BicD (DDB)
Cargo Protein Binding Can Selectively Activate Motors

**Dynein** on Microtubules

**Dynein + Dynactin + BicD2** on Microtubules

McKenney et al., 2014
Microtubules and Motors Coordinate Their Activities to Generate Forces in Mitosis
Microtubules and Motors Coordinate Their Activities to Generate Forces in Mitosis

(a)

(b)
Attachment by kinesin-7; microtubule assembly
Growth
Kinesin-7
(-)
Tethered dynactin-dynein complex
Force from dynein and microtubule depolymerization by kinesin-13, and by kinesin-4 on chromosome arms
Kinesin-4
(+)
Kinesin-13
Shrinkage
Chromosome movement