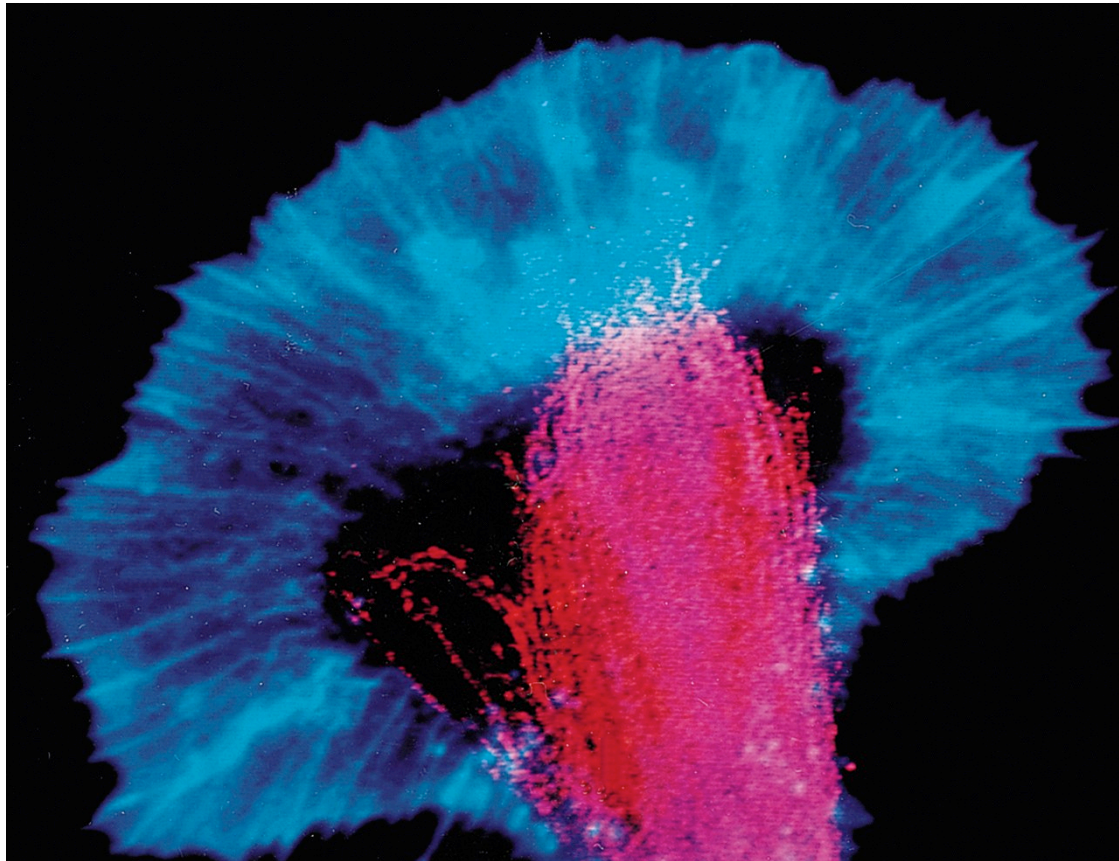


# Cytoskeleton I: Filament Proteins



Chapter 9 Opener Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

# 3 Types

- Actin filaments (AFs, or microfilaments)
- Microtubules (MTs)
- Intermediate filaments (IFs)



*Good summary on page 891.*

# Filament Construction



- Small subunits form filaments.
- Actin and tubulin, compact and globular.
- disassembly, diffusion, reassembly

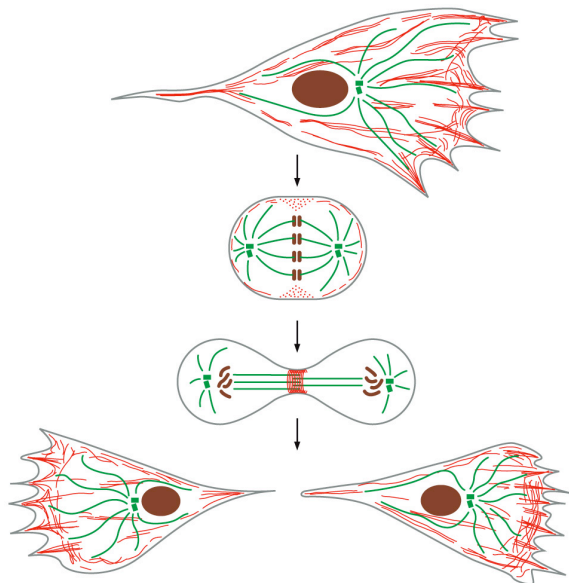
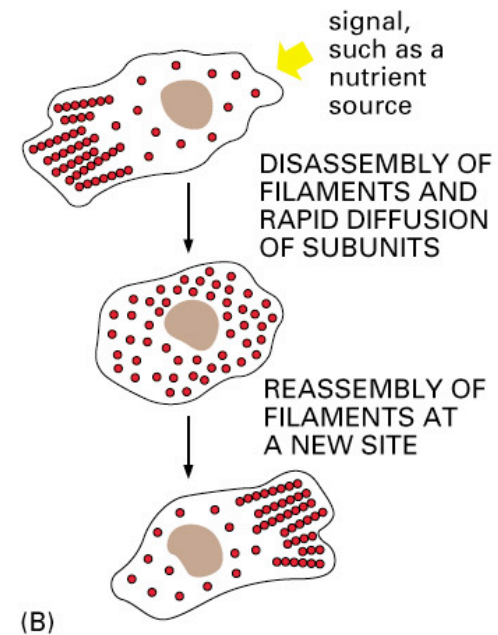
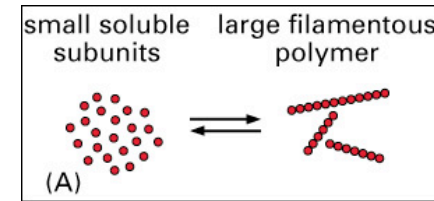


Figure 16-2. Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 16-2. Molecular Biology of the Cell, 4th Edition.

# Filament Construction

- Protofilaments, e.g. MTs
- Weak noncovalent bonds.

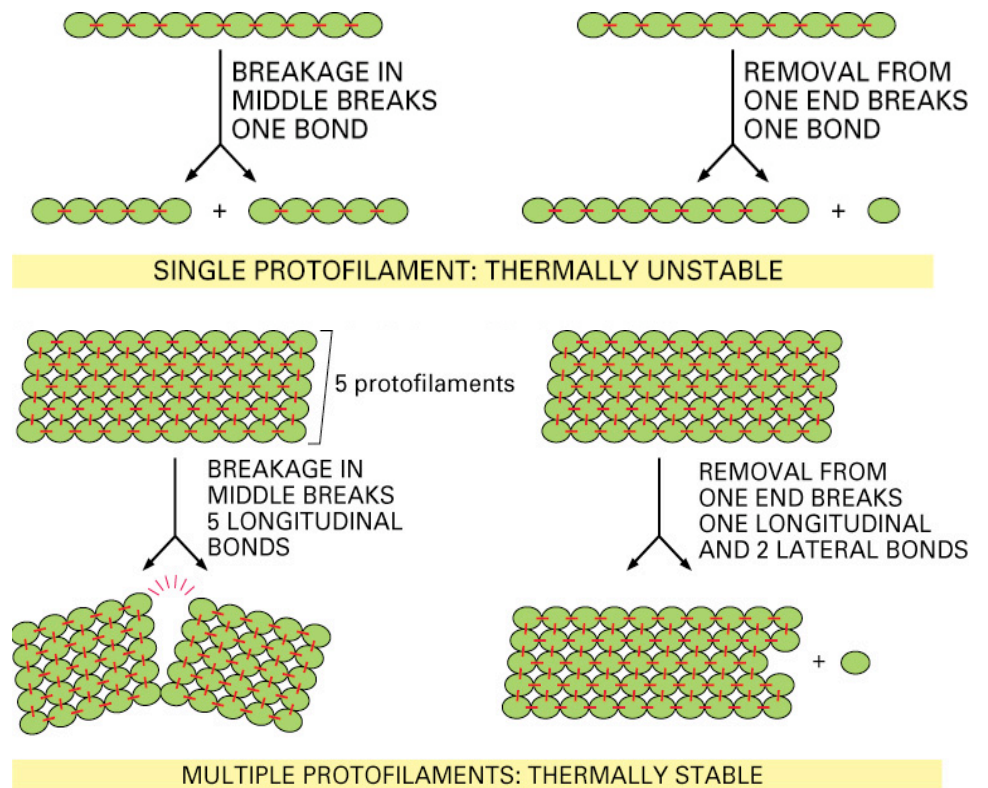


Figure 16-3 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

# Nucleation

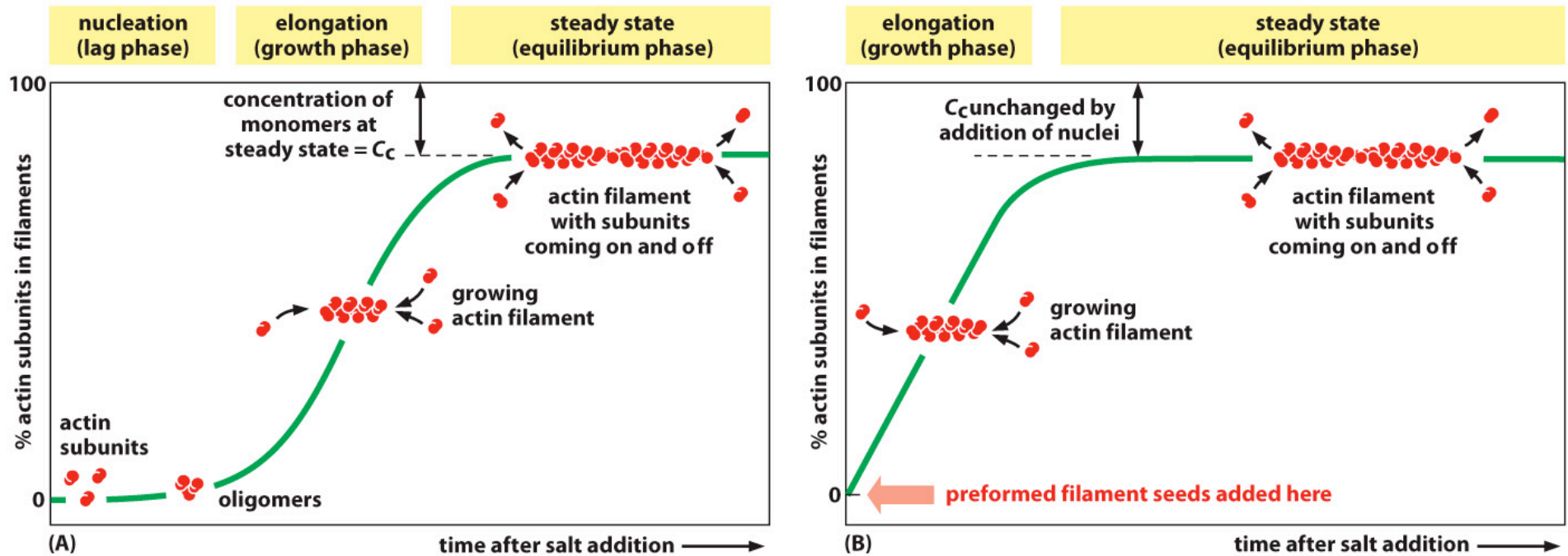
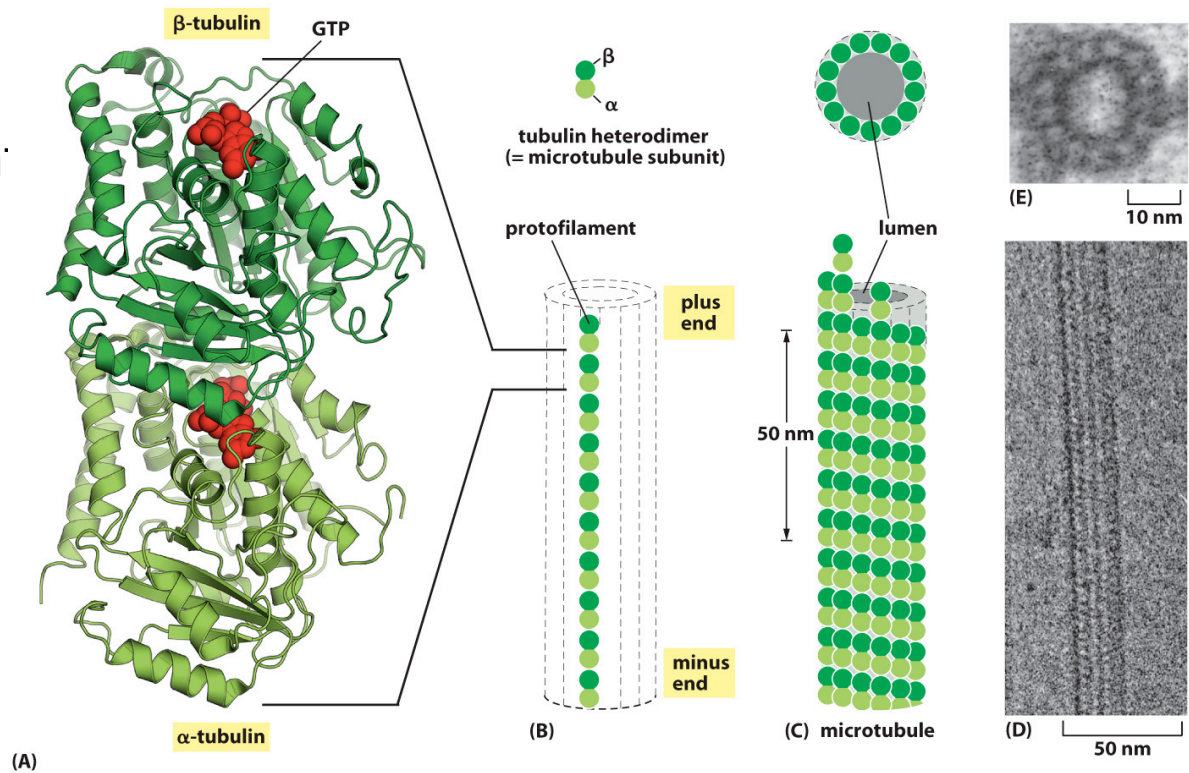


Figure 16-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Tubulin



- Heterodimer = 2 different proteins, but considered “1 subunit” of tubulin
- each binds GTP; hydrolyzed at only 1 site
- 13 protofilaments
- “plus” and “minus” end



(A) Figure 16-42 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Actin



- Monomer
- ATP
- “plus” and “minus” end

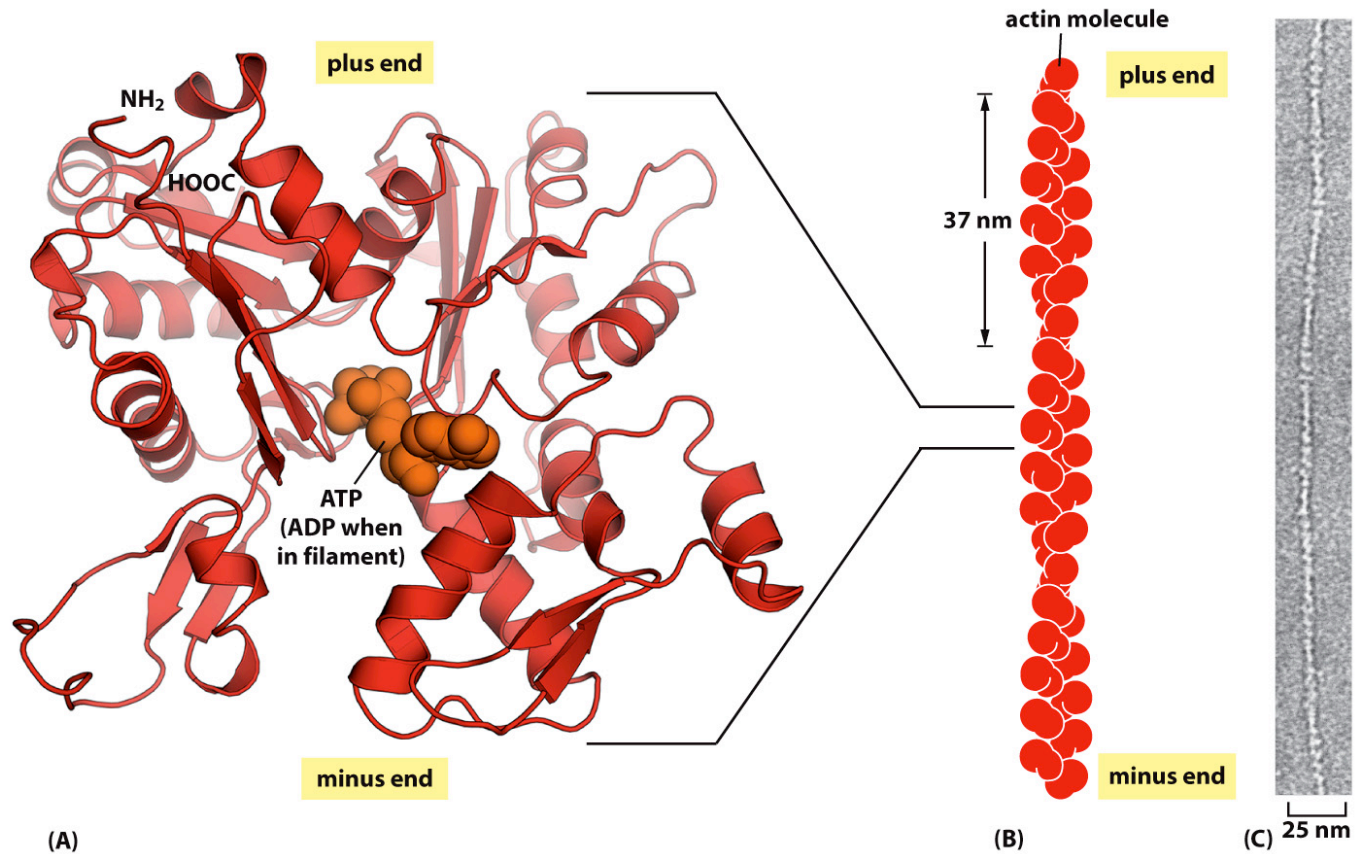


Figure 16-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Treadmilling and Dynamic Instability



- Growth / shrinkage of filament proteins.
- Actin and tubulin catalyze hydrolysis of ATP or GTP.
- “T” or “D” form indicates if triphosphate or diphosphate form exists.
- ATP / GTP caps.
- Critical concentration ( $C_c$ ): where subunit addition equals subunit loss.

*See pp. 902-903.*

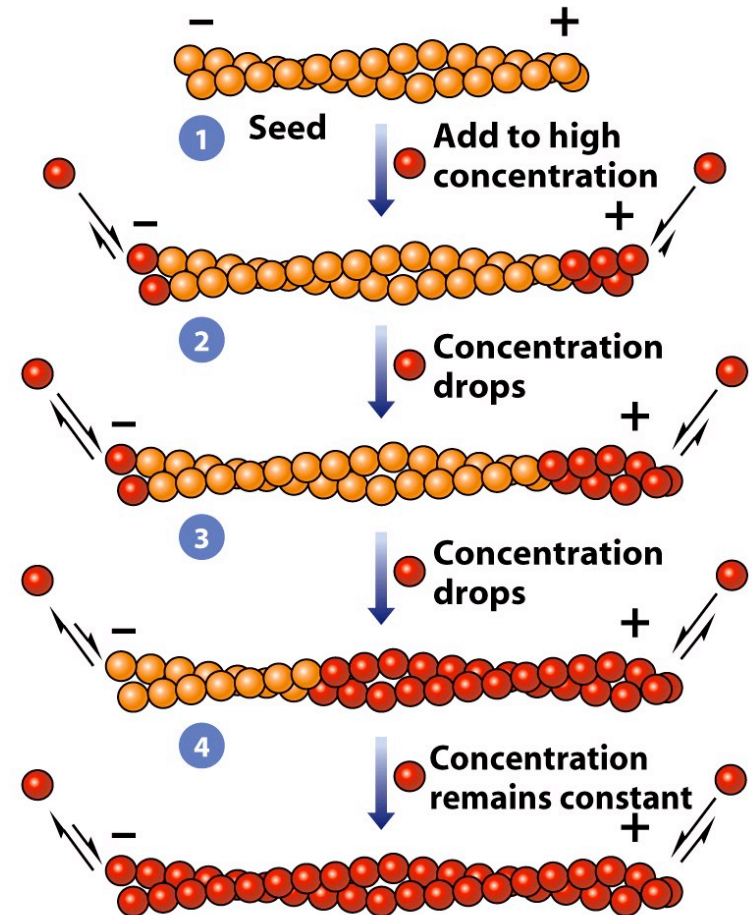


# Treadmilling – Actin Filaments

Experiment:

1. Filaments added to ATP-actin.
2. [ATP-actin] high, addition occurs at both ends.
3. [ATP-actin] drops, addition greater at plus end.
4. Steady state.

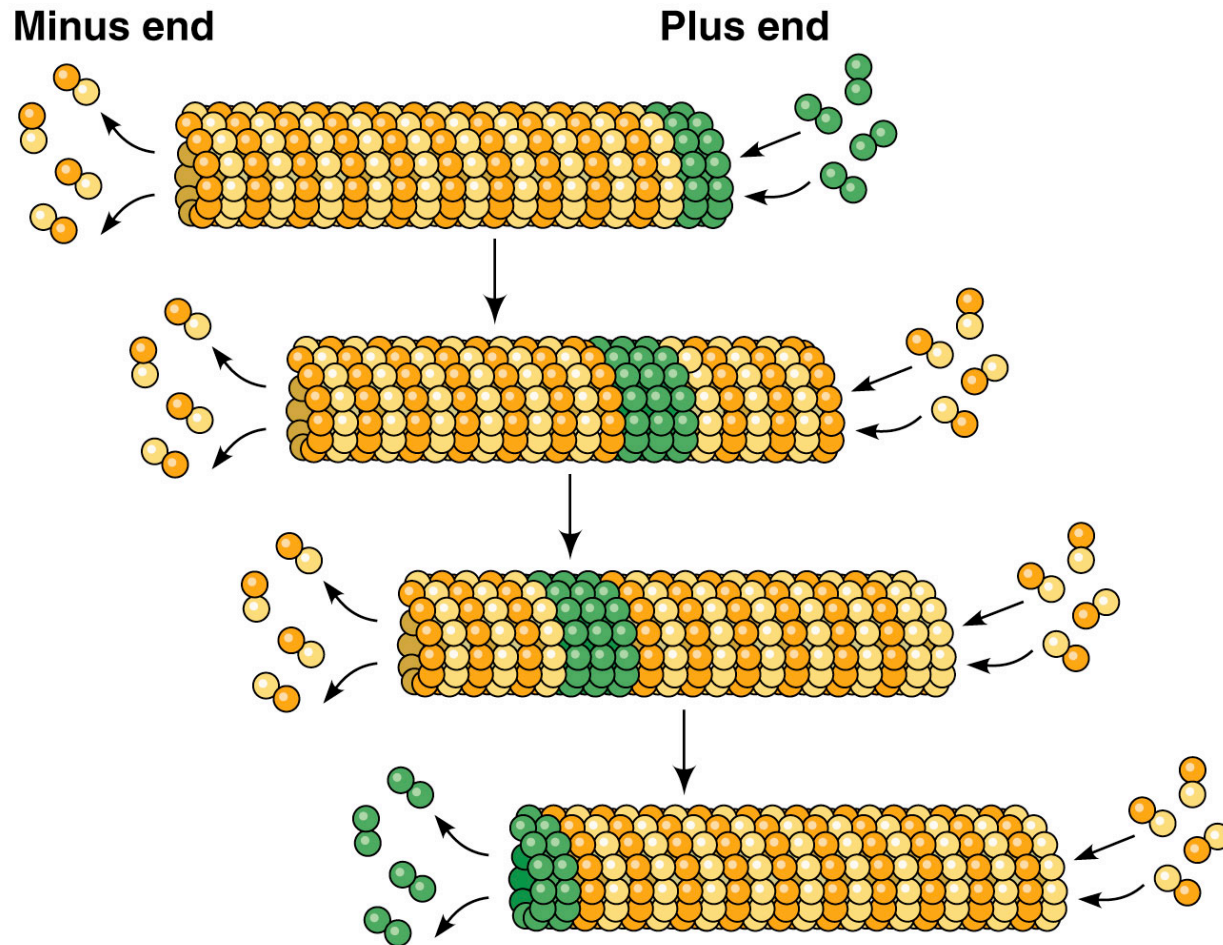
*Treadmilling*



*Nucleation is not a factor here since pre-formed filaments were added to actin solution*

Figure 9-46b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

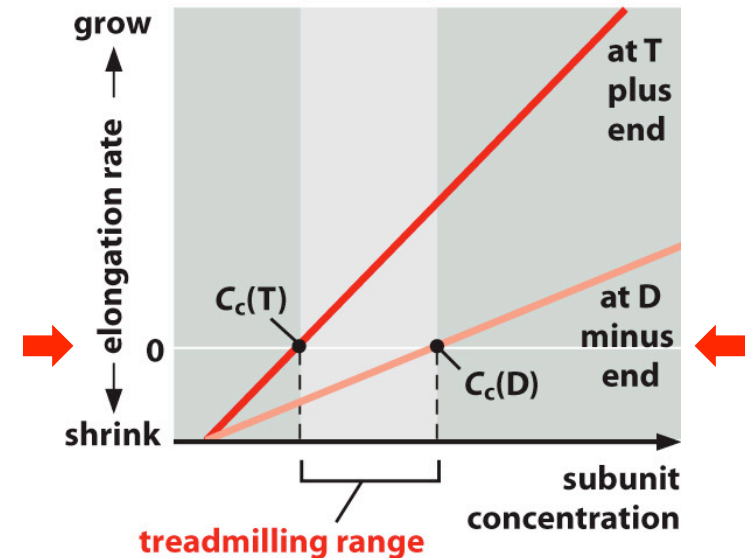
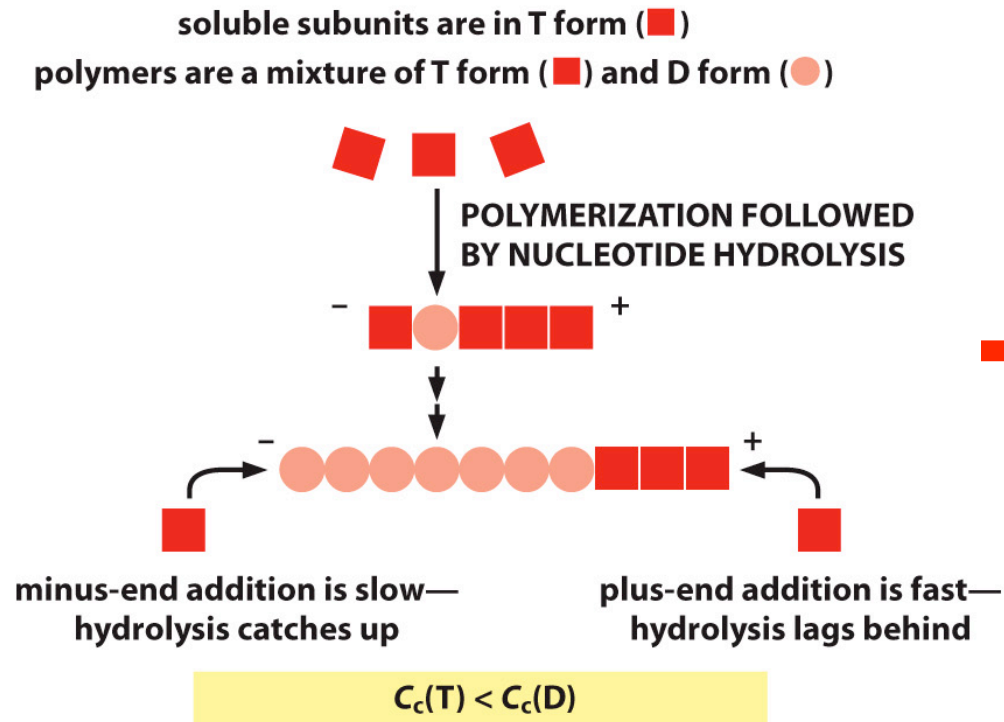
# Treadmilling – Microtubules



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# Treadmilling

- Rate of hydrolysis and  $C_c$
- Linear relationship of [subunit] vs. elongation rate



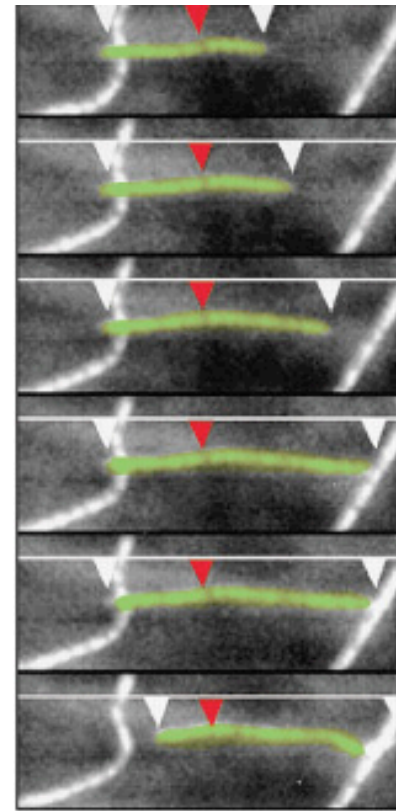
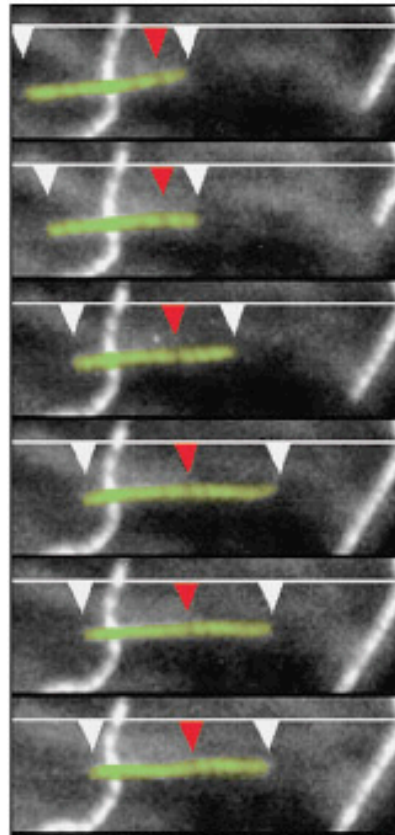
For  $C_c(T) < C < C_c(D)$   
 treadmilling occurs


Figure 16-14 Molecular Biology of the Cell 6e (© Garland Science 2015)

*There is no net change in length of T or D ends at their respective  $C_c$*

# Treadmilling behaviour – MT

time 0 sec



time 89 sec   
2  $\mu$ m



*Cell injected with rhodamine-labelled tubulin*



# Dynamic Instability – MTs

- [Tubulin] within critical values
- GTP cap
- Continuous transitions

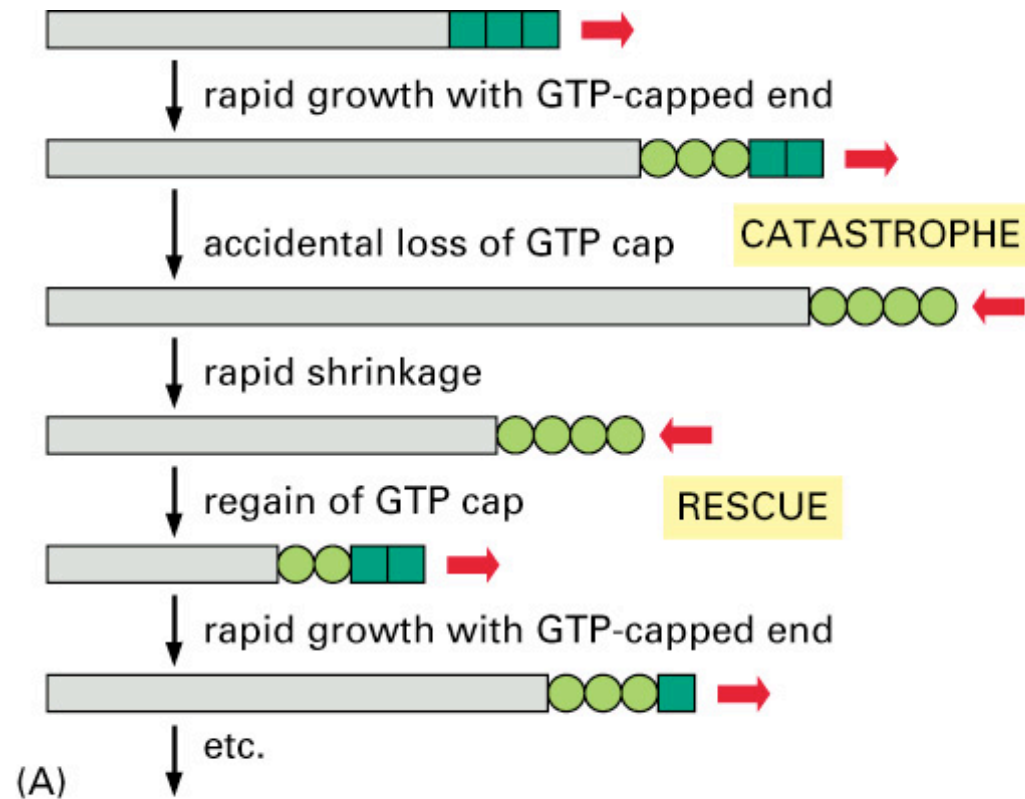


Figure 16–11 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

# Dynamic Instability – MTs

- Hydrolysis affects conformation

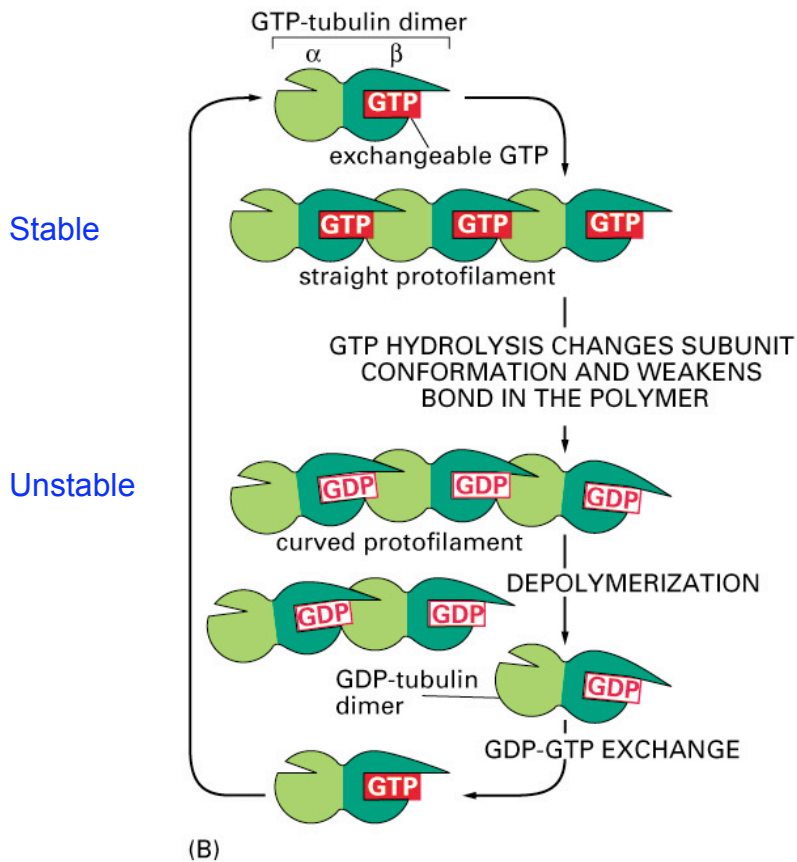


Figure 16–11 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

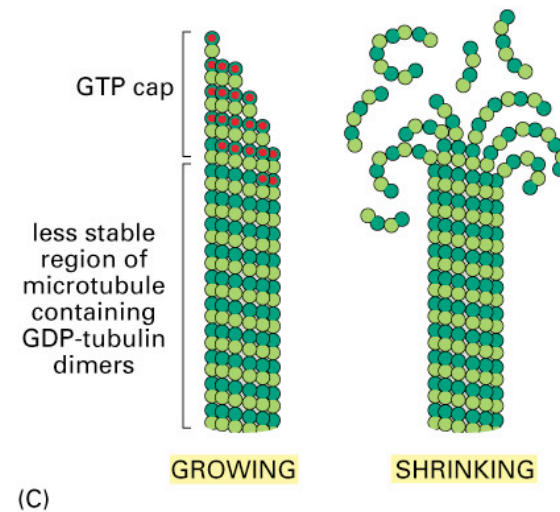
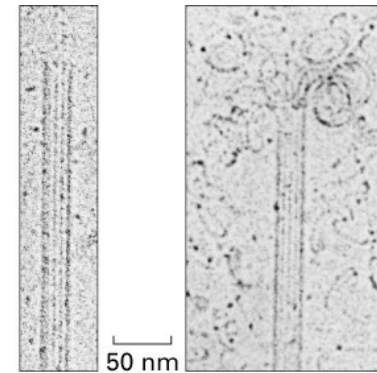
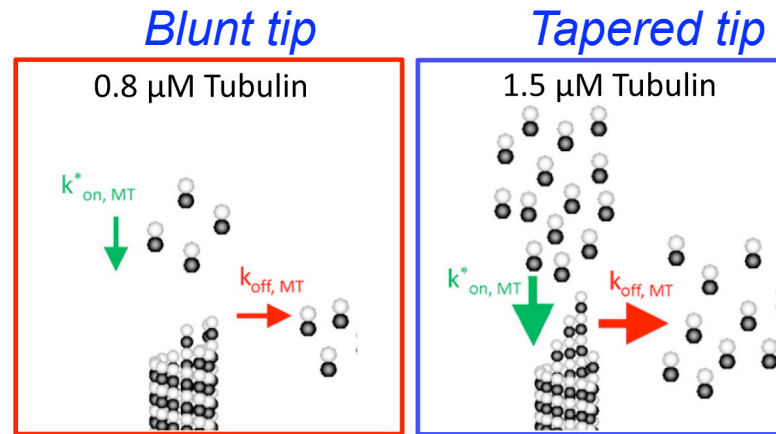


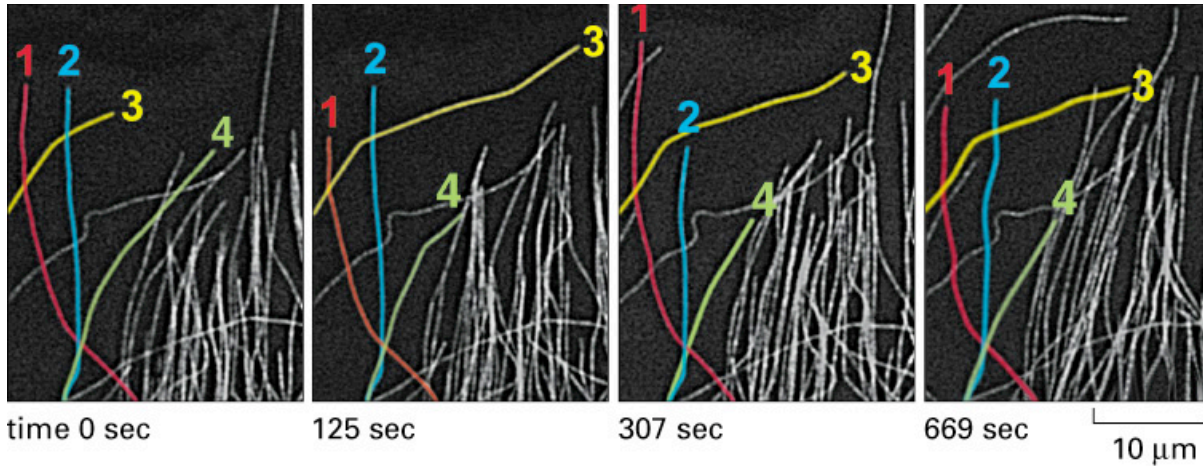
Figure 16–11 part 3 of 3. Molecular Biology of the Cell, 4th Edition.

# Kinetics Dependent on [tubulin]



	<i>Previously</i>	<i>Gardner et al., 2011</i>
$k_{on,MT}$	$\sim 5 \mu M^{-1}s^{-1}$	$\sim 58 \pm 4 \mu M^{-1}s^{-1}$
$k_{off,MT}$	Constant	Concentration Dependent
Kinetics	<p>Slow Kinetics</p>	<p>Rapid Tip-State Dependent Kinetics</p>

# Dynamic Instability – MTs



*Epithelial cell,  
Rhodamine-  
labelled tubulin*

Dyn. Inst.



Figure 16-12. Molecular Biology of the Cell, 4th Edition.

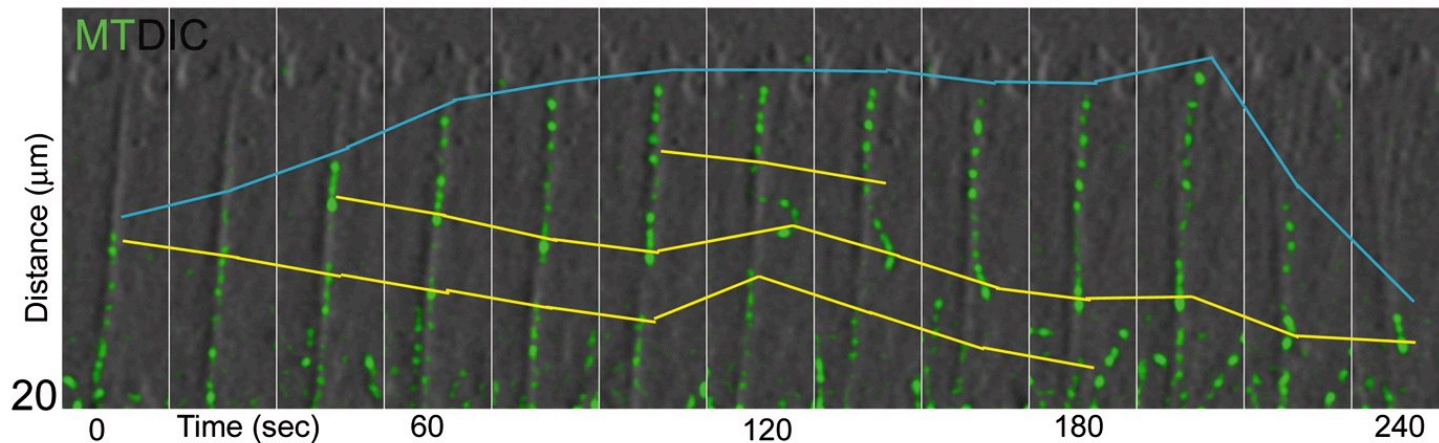


Figure 9-27 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

*Growth cone of neuron*

# Treadmilling and Dynamic Instability

What is the purpose?



Constant ATP consumption. Is it worth it?

- Spatial and temporal flexibility with high turnover.
- Fastest way to grow filaments without nucleation.
- Exploration for attachment sites and remodelling.

# Intermediate Filaments

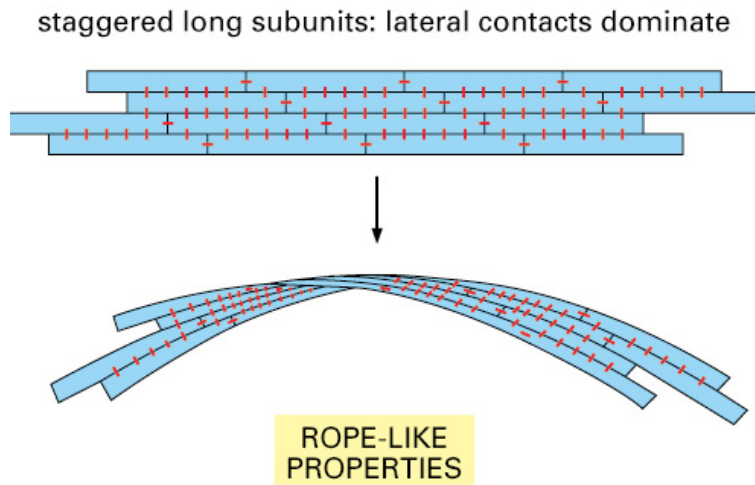


Figure 16-4. Molecular Biology of the Cell, 4th Edition.

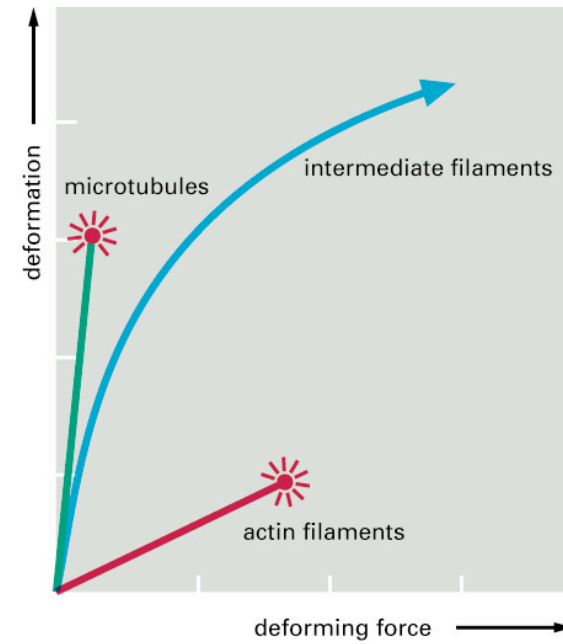
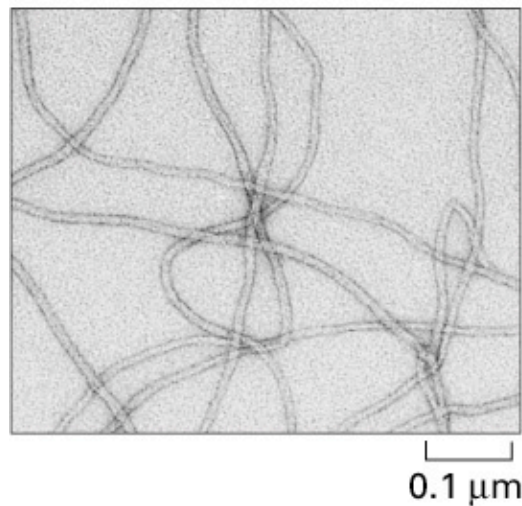


Figure 16-17. Molecular Biology of the Cell, 4th Edition.



# Intermediate Filaments

- (A,B) Dimer formation.
- (C) Tetramer formation.
- (D) Tetramer-tetramer association.

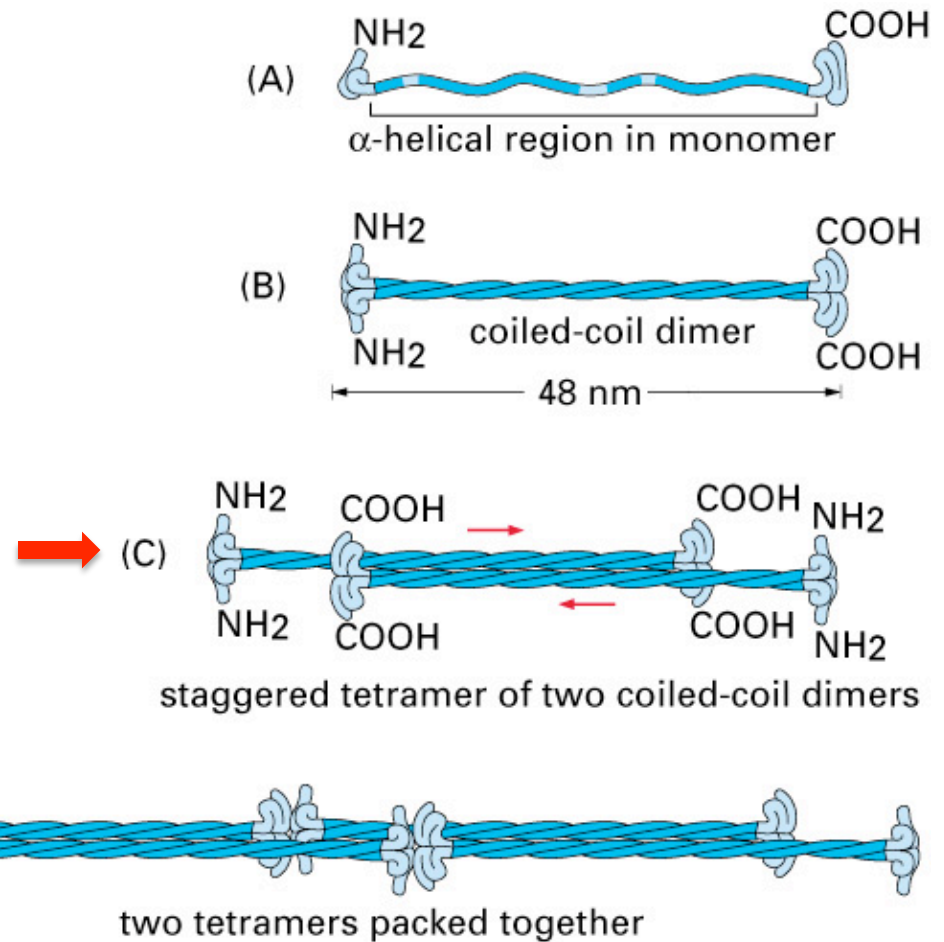


Figure 16-16 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

# Intermediate Filaments

- Tetramers packed into array of 16 dimers in cross-section.
- Rope-like appearance.
- Formation by spontaneous interaction.
- Disassembly likely regulated by phosphorylation.

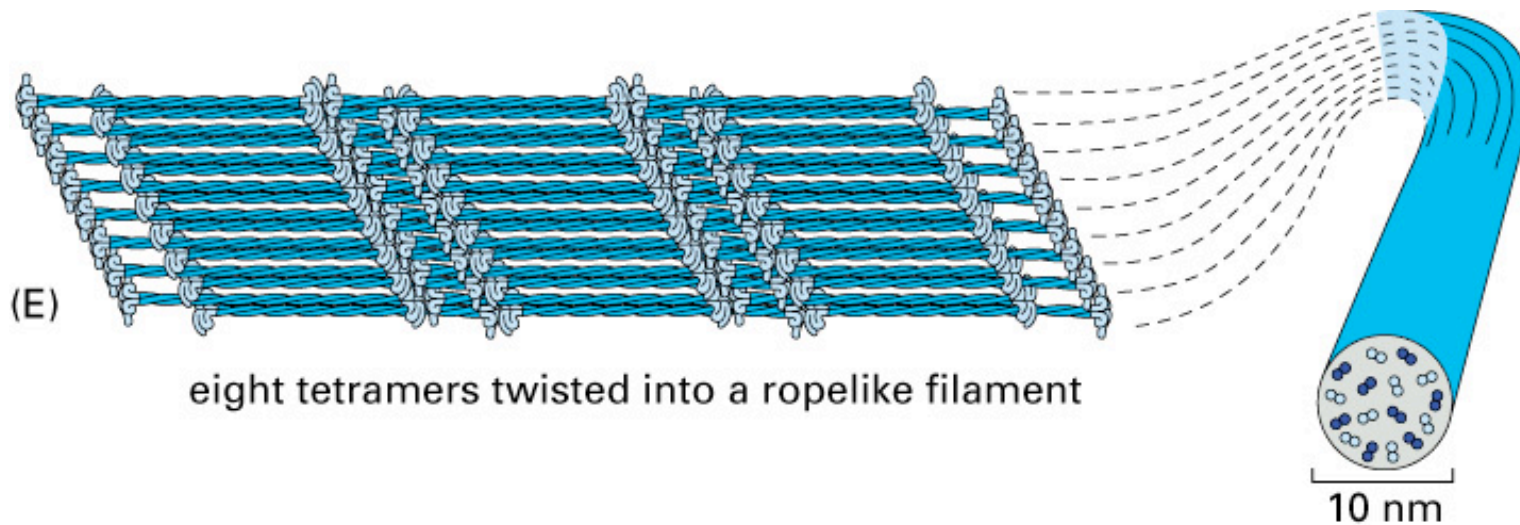


Figure 16-16 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

# IF Types

Epithelial (e.g. keratins)  
Axonal (e.g. neurofilament)

Epithelial (keratins)

- Most diverse family
- Type I and II keratin chains
- Strength in hair, nails

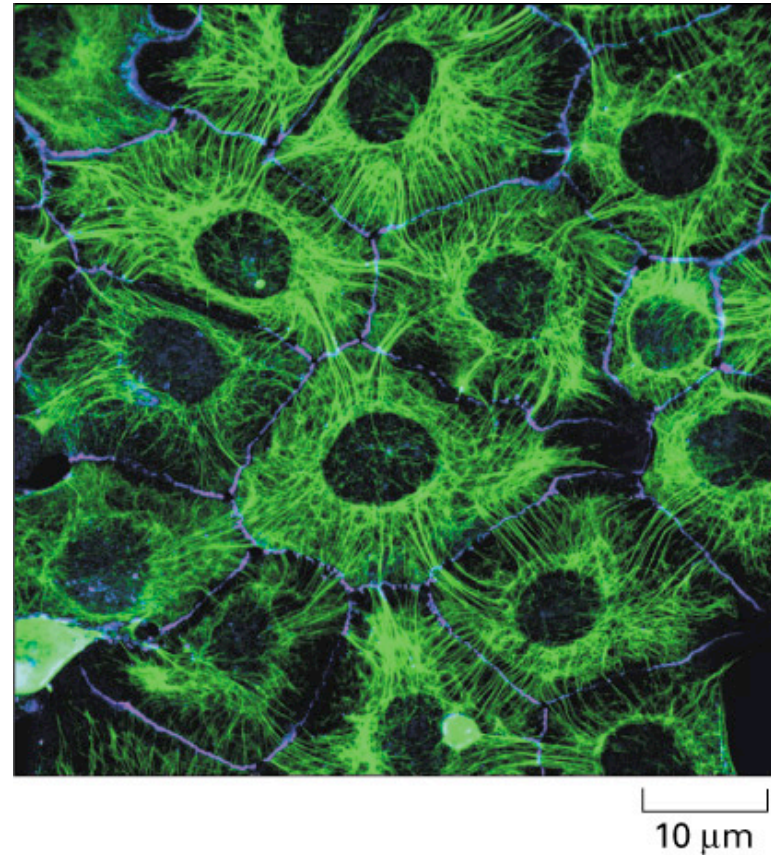


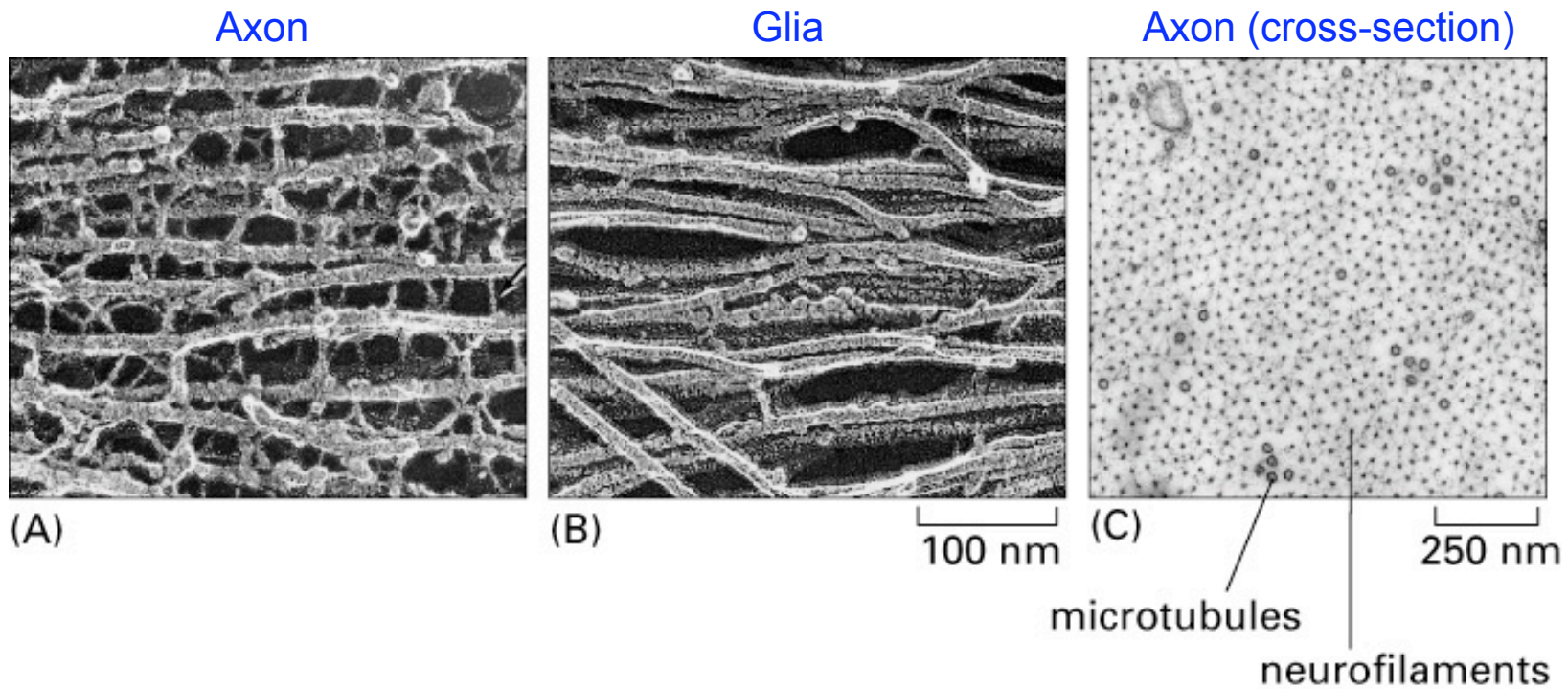
Figure 16–18. Molecular Biology of the Cell, 4th Edition.

# IF Types



## Axonal (neurofilaments)

- Found in central and peripheral axons of vertebrate neurons.
- Type L, M or H.
- Growth, increase axon diameter.



# Things to Consider...

1. Can you differentiate between the processes of treadmilling and dynamic instability?
2. Think about the differences in assembly, growth and shrinkage between each of the 3 filament proteins that we discussed.
3. Where does ATP or GTP fit in?