Cytoskeleton II: Regulation

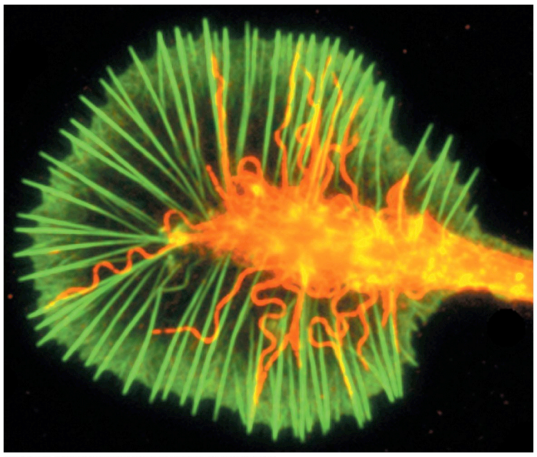


Figure 9-75b *Cell and Molecular Biology*, 5/e (© 2008 John Wiley & Sons)

1

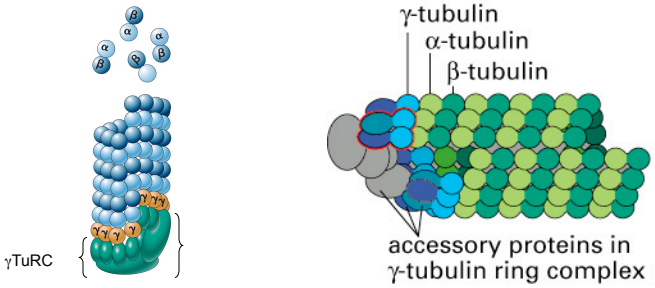
Accessory Proteins

- Filaments (AFs, MTs, IFs) are dynamic and under control of the cell.
- Form higher-order structures (e.g. mitotic spindle).
- Accessory proteins modify these cytoskeletal dynamics.

2

Nucleation by γ-tubulin Protein Complex

- MTOC (centrosome) in animal cells.
- Spindle pole body in yeast.
- γ-tubulin is highly conserved.
- γTuRC accelerates MT formation.
- Nucleation occurs at the (-) end.



3

Nucleation by γ-tubulin Complex

- MT initiation also occurs in cytosol
- e.g. plants.
- MTs nucleate daughter MTs.

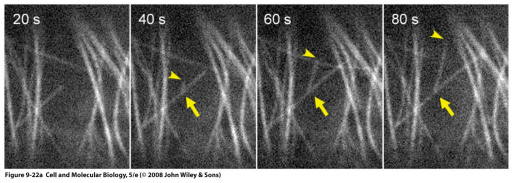
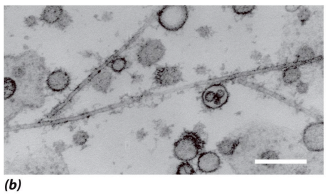
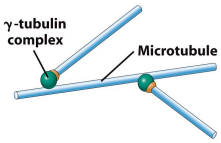


Figure 9-22a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



(b)



(c)

Figure 9-22bc Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

The Centrioles and Centrosome

- Centrosome contains 2 cylindrical centrioles.
- 9 triplets of MTs.
- Centrioles organize PCM.
- Centriole lumen and PCM contain γ-tubulin.
- PCM initiates MT assembly.

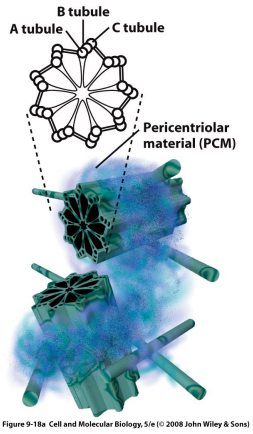


Figure 9-18a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

2 pairs of centrioles after duplication during cell cycle.

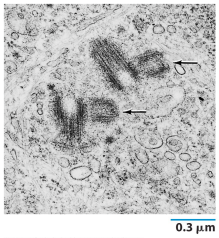


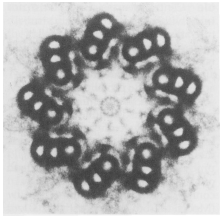
Figure 9-18b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Karp. Cell and Molecular Biology

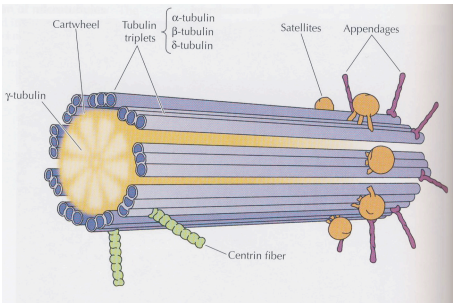
5

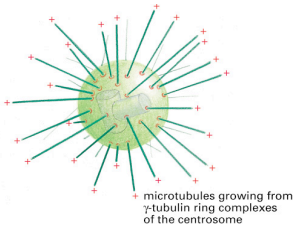
The Centrioles and Centrosome

- Centrosome near nucleus.
- (-) ends of MTs are anchored.
- (+) ends of MTs emanate in astral configuration.



0.1 µm





Cooper and Hausman. The Cell: A Molecular Approach

6

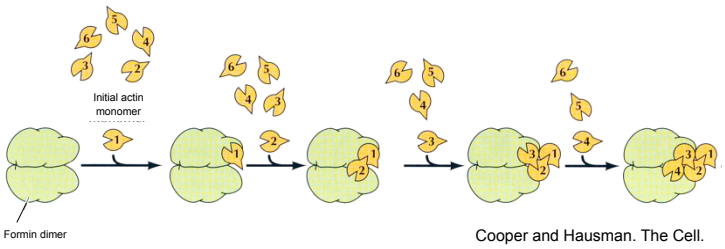
Nucleation of AFs

- Cell periphery (cortex), where density of AF proteins is highest.
- Location related to AF function (cell shape, movement).
- Facilitated by ABPs (including ARPs).

7

Initiation of AFs (unbranched)

- Initiation by *formin*, an ABP
- Correct alignment for polymerization
- e.g. filaments of muscle cells.



Cooper and Hausman. The Cell.

8

Initiation of AF Branches

- Cell periphery
- Arp2/3 produces extensive branching.
- Complex contains 7 proteins.
- Binds at (-) end.
- 70º favourable angle.

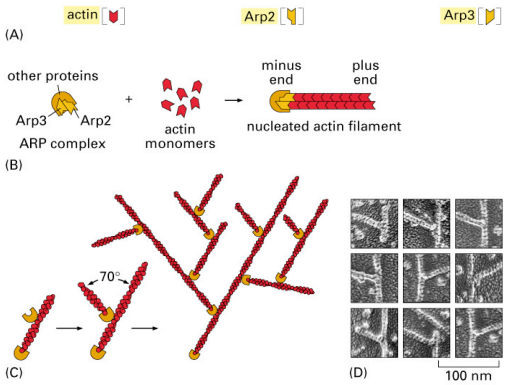


Figure 16–28 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

9

Control of Subunit Pools

- Both actin and tubulin are maintained in the cytosol at high concentrations.
- Concentration can exceed C_c.
- Accessory proteins may sequester unused subunits (*sequestering proteins*).
- Sequestered proteins are not hydrolyzed.

10

Thymosin Sequesters Actin

- Thymosin sequesters, but profilin recruits monomers.
- Thymosin makes polymerization *less favourable*.
- Profilin *competes* with thymosin and promotes assembly.

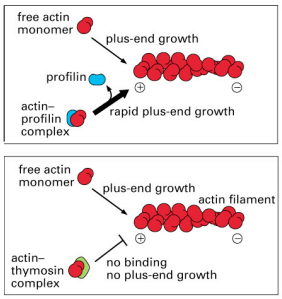


Figure 16–30 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

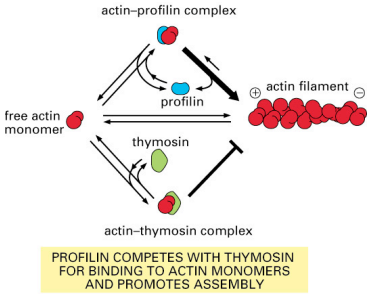


Figure 16–30 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

11

Stathmin Sequesters Tubulin

- Stathmin binding *prevents* polymerization.
- Decreases effective [tubulin].
- Promotes dynamic instability (catastrophe).

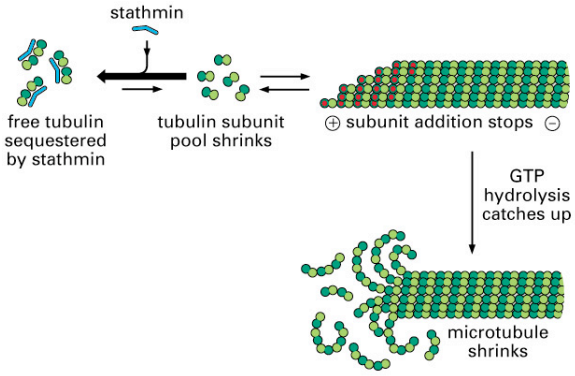


Figure 16–31. Molecular Biology of the Cell, 4th Edition.

12

MT-Associated Proteins (MAPs)

- Several binding domains.
- Length of projecting domain determines packing of MTs.
- MAP2 (neuronal cell bodies); *tau* (axons).
- *tau* and Alzheimer's Disease (*tau* unable to bind to MTs).
- Poorly soluble (hyperphosphorylated) *tau* may induce neurodegeneration.

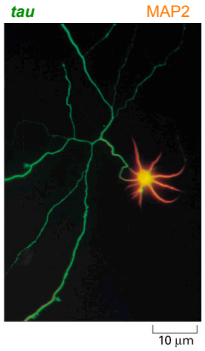
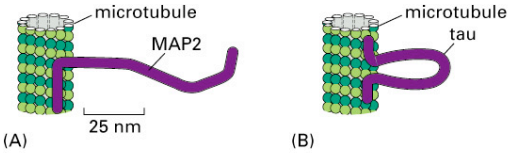


Figure 16–32. Molecular Biology of the



AF Binding Proteins

Cofilin destabilizes AFs

- binds to side of proteins.
- binding induces mechanical stress.
- treadmilling, turnover.
- cell locomotion.

Tropomyosin stabilizes AFs

- binds to side of proteins.
- muscle contraction.

14

Modifications at AF Ends

- "Capping"
- Recall elongation slower at (-) end.
- e.g. CapZ (+), elongation occurs only at (-) end.
- e.g. tropomodulin (-) in muscle contraction.

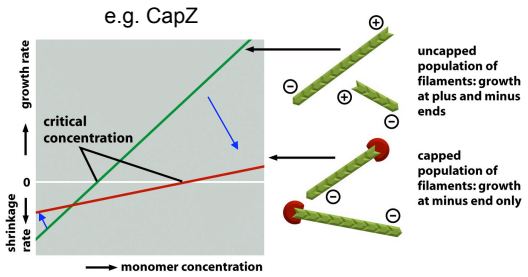


Figure 16-43 *Molecular Biology of the Cell* (© Garland Science 2008)

Modifications at MT Ends

- Capping in MTs has dramatic effect on dynamic instability.

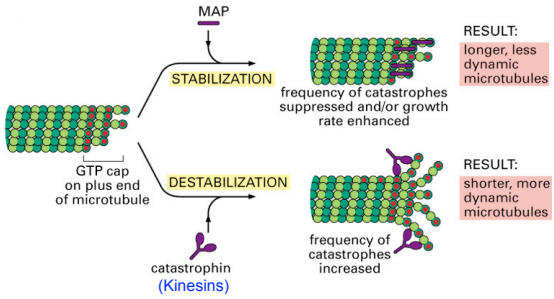


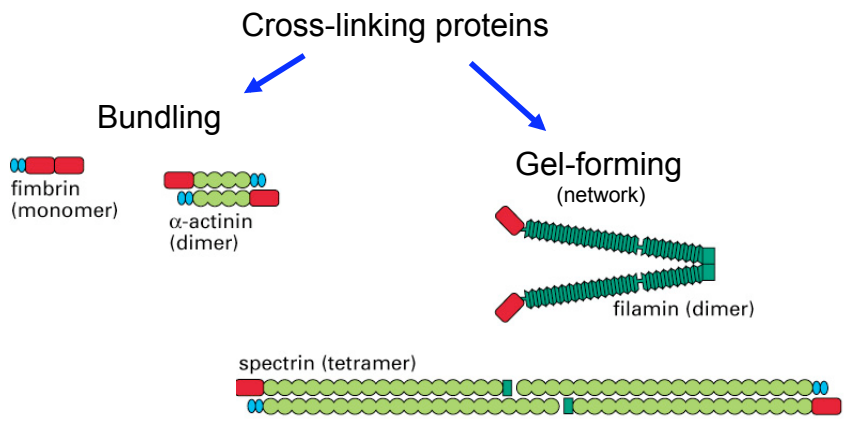
Figure 16–36 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Cross-Linking Proteins and AFs

- Formation of higher-order structures.
- Spacing of 2 actin binding domains of cross-linking protein determines type of structure.
- 2 major groups: bundling and gel-forming.

17

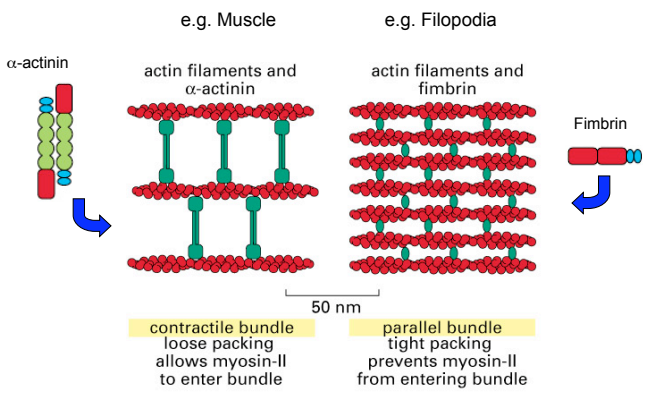
Cross-Linking Proteins and AFs



Red = actin binding domains

18

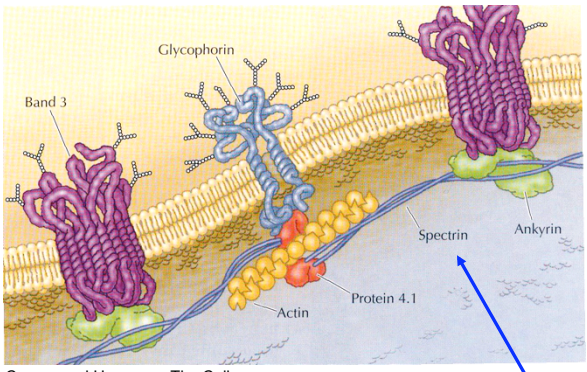
Actin Bundling Proteins



19

Gel-forming Proteins

Red blood cell membrane



Cooper and Hausman. The Cell.

- See also Fig. 10-41 in Alberts.
- Forms 2-D network.
- Gives RBC membrane flexibility.

spectrin (tetramer)

20

Gel-forming Proteins

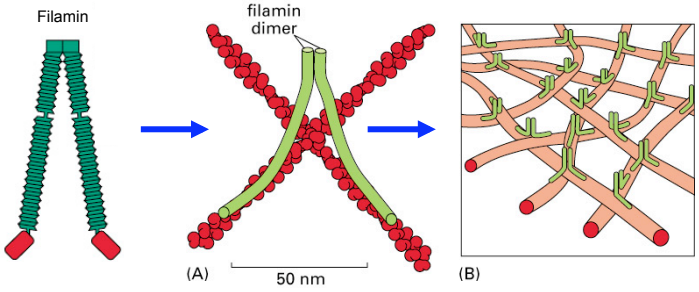


Figure 16–42. Molecular Biology of the Cell, 4th Edition.

e.g. lamellipodia in locomotion

21

Changes in Cell Shape During Embryonic Development

- Vertebrate embryo.
- Folding of neural tube during nervous system development.
- Cell height – MTs
- "Rolling" tube – AFs.

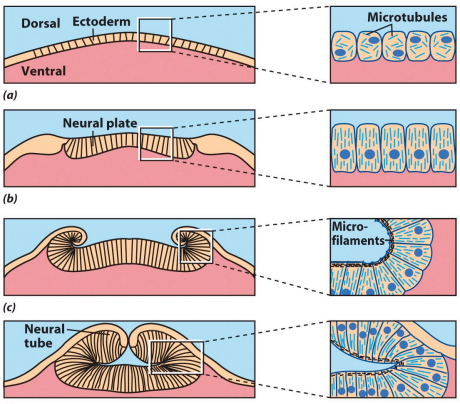


Figure 9-77a-d Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

22

Changes in Cell Shape During Embryonic Development

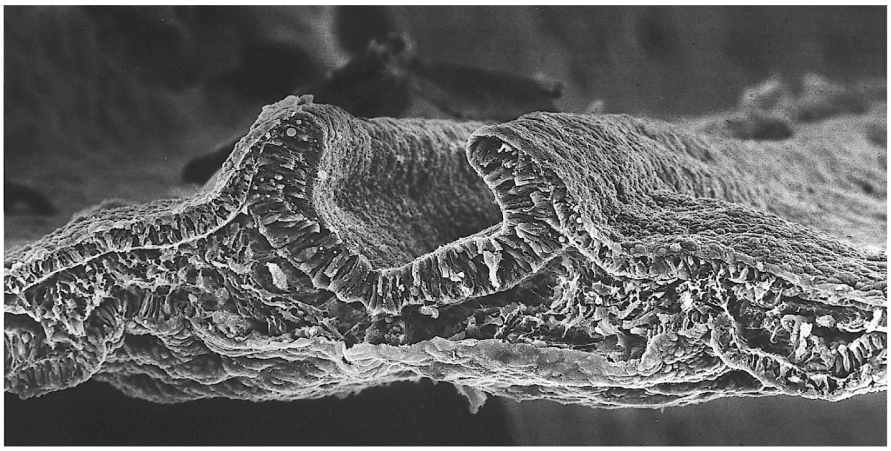


Figure 9-77e Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Induction by Extracellular Signals

- Example, crawling neutrophil.
- Chemical activates WASP...
- Polymerizing filaments push membrane.

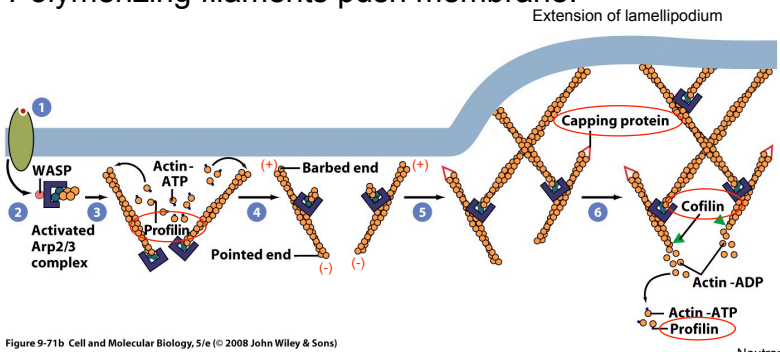


Figure 9-71b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Neutrophil

WASP = Wiskott-Aldrich Syndrome Protein

http://astro.temple.edu/~jbs/courses/2041lectures/neutrophil-js.html

Some sort of extralcellular signal, intracellular affect
Some chemical q, there is a transmembrane protein, which is going to bind this chemical and activate wasp protein, then activate Arp 2/3 complex, which involved in nucleation of actin filaments. profilin is going to step in. Thymsin, which add to subunits and make subunits cannot add to filament. comptete with thymsin, in this way, there is regulation. these two accessory protein (thymsin and profin) help to regulate the common pool of subunits inside the cell. profilin compete with thymsin and is going to help subunits integrate filament, so now we have Arp2/3 nucleation, we have branching, and now we gonna have capping protein. by this time we begin to see the production of this network . that is going to begin pushing out region of plasma membrane. capping protein that is involved would regulate individual filament because filament shouldn't be too long or too big, then it's time to dissemble all that. then cofilin step in and begin to disassemble. that brings actin which now bind to ADP because it's - end and we have hydrolysis to the pool

Sequence of steps

1. Extracellular signal
2. Activation of WASP
3. Nucleation and branching by Arp2/3
4. Promotion of assembly by profilin
5. Elongation reduced by capping proteins
6. Destabilized by cofilin and return of subunits to pool

25

Things to Consider...

1. Think about how each accessory protein affects the stability of AFs and MTs?
2. Many of the dynamic mechanisms that we discussed today do not apply to IFs. Why?

26