

Cytoskeleton: Regulation

Bio-311

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LECTURE NOTES- download PDF versions

Bibliography

Alberts et al. (2002) Mol. Biol. Of the Cell.

Boal (2008) Mechanics of the Cell

Research papers

Last Lecture- Cytoskeletal Filaments (CFs)

1. Mechanical properties of cytoskeleton
2. Dimensions of the filaments
3. Protofilament bundling vs. single filaments
4. Critical concentration (C_c) of polymerization and nucleation
5. Asymmetric growth of CFs
6. Microtubule dynamics: two-state polymer vs. single state in search and capture

History

- Actin: activating substance when added to myosin extract, caused activation
- Szeged, Hungary 1942: Straub, Szent-Gyorgyi.

Outline

Structure

Regulation of nucleation & polymerization

Side and end-binding to polymers

Severing proteins

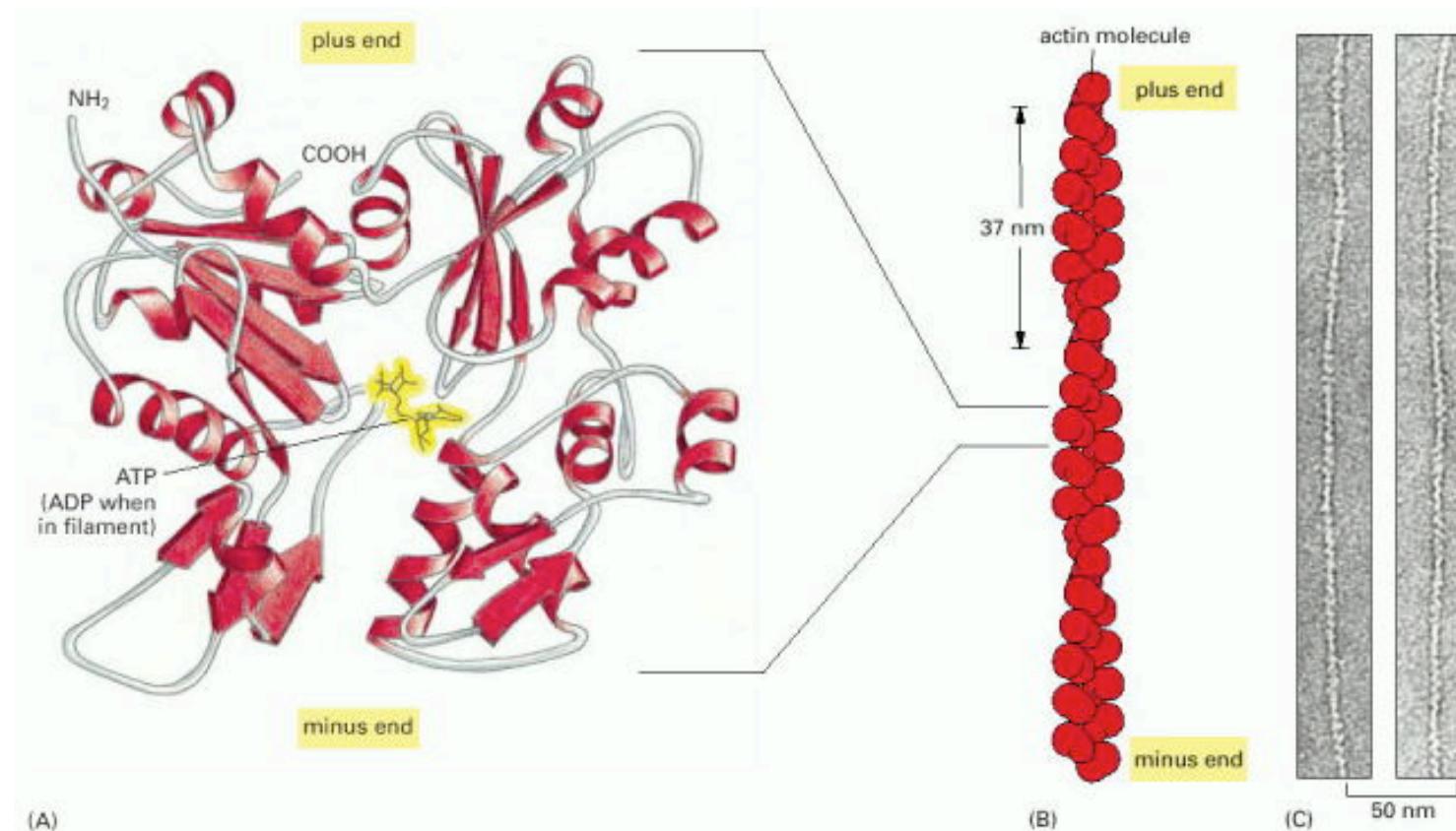
Higher order structure- actin & IF

Attachment to plasma membrane

Extracellular regulation

Protein Structures

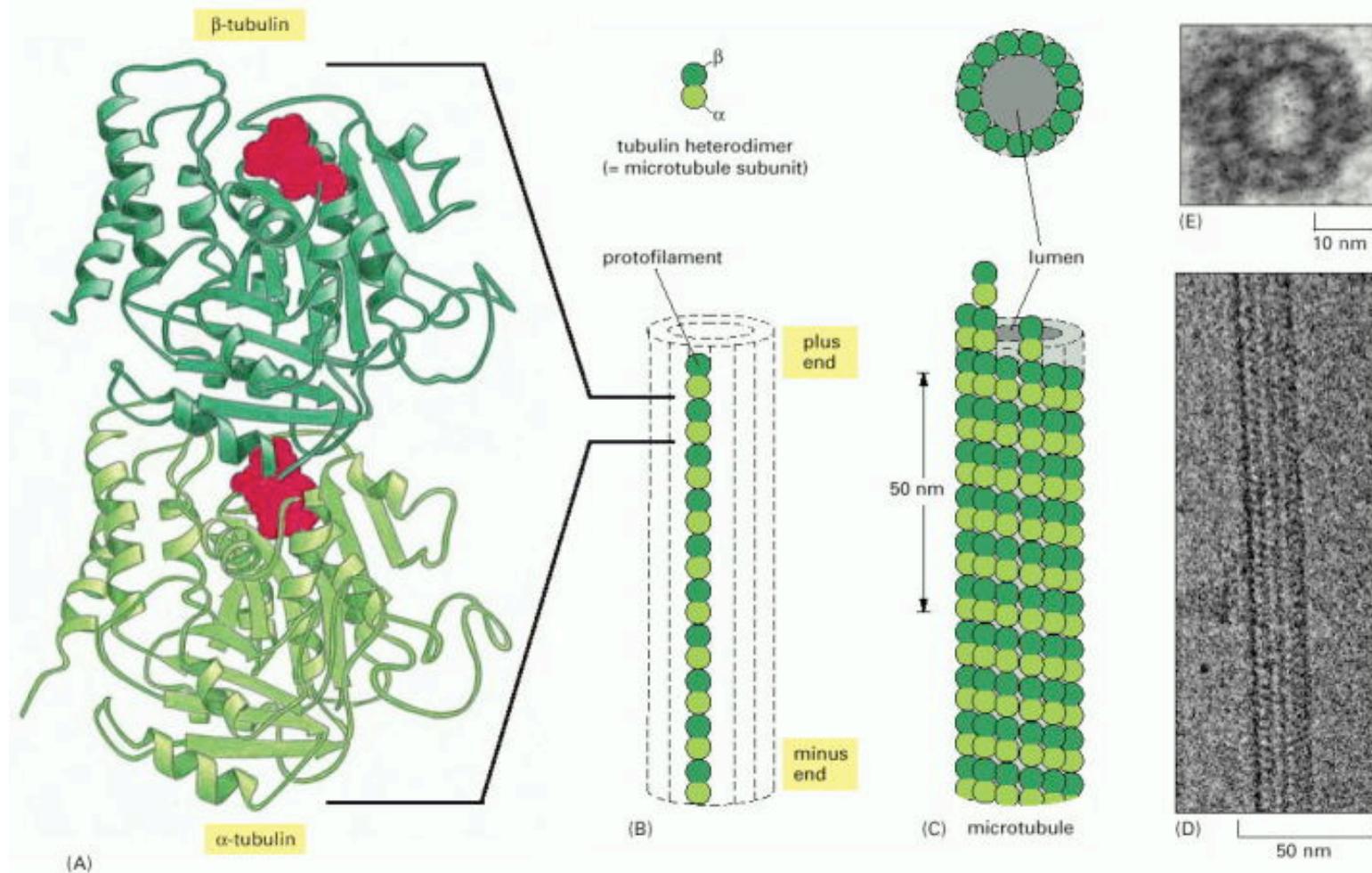
Actin



Actin turn: 37 nm

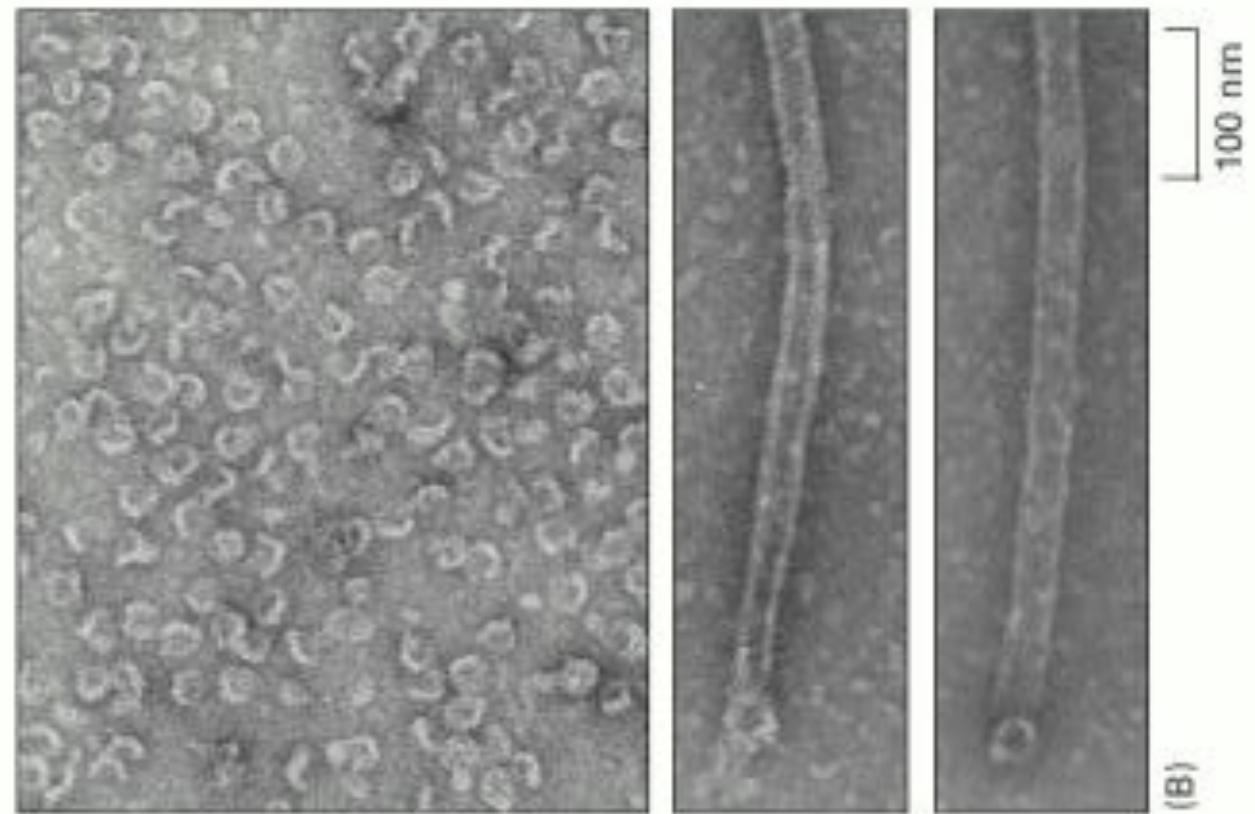
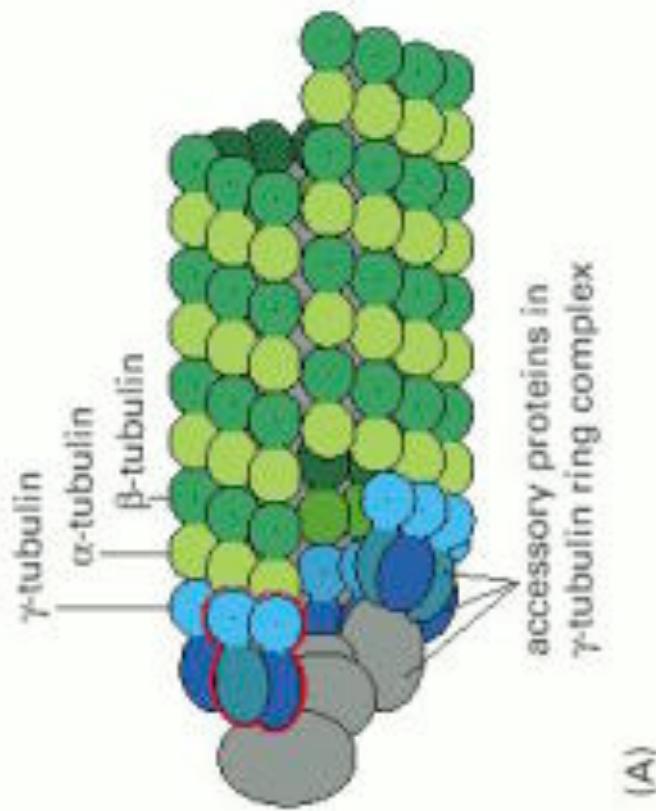
4.7 subunits per turn

Tubulin



Avg. 13 protofilaments. But can range 9-16

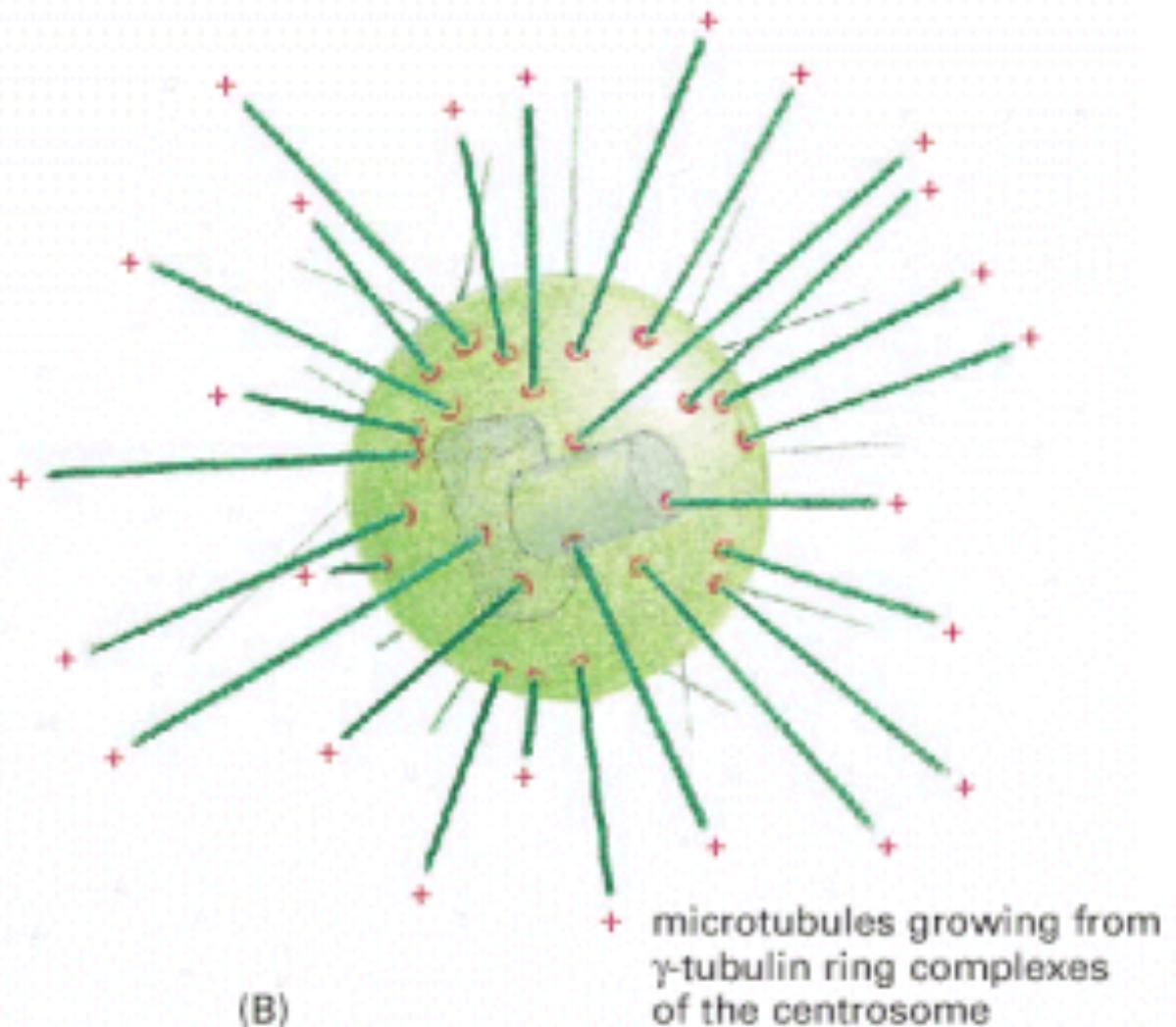
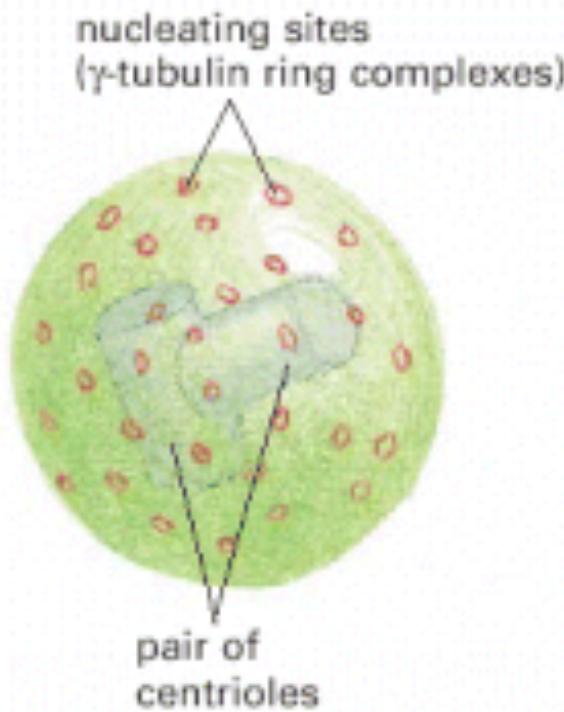
Nucleation Factors- Microtubules



γ-tubulin Ring Complex- stabilizes MINUS end

Centrosome

50x γ -Tubulin Ring Complex, centrioles (basal-bodies)- short microtubules

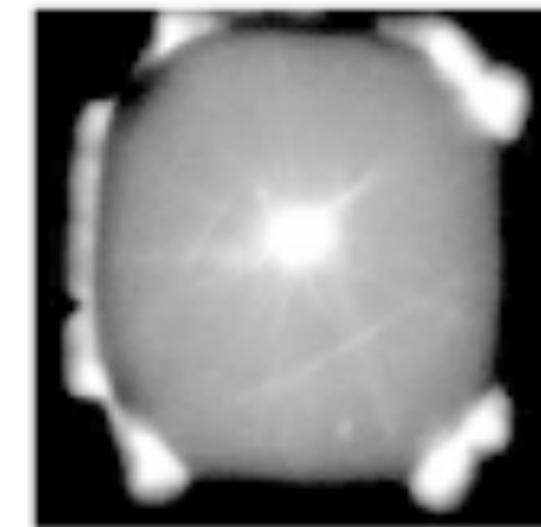


Centering Ability

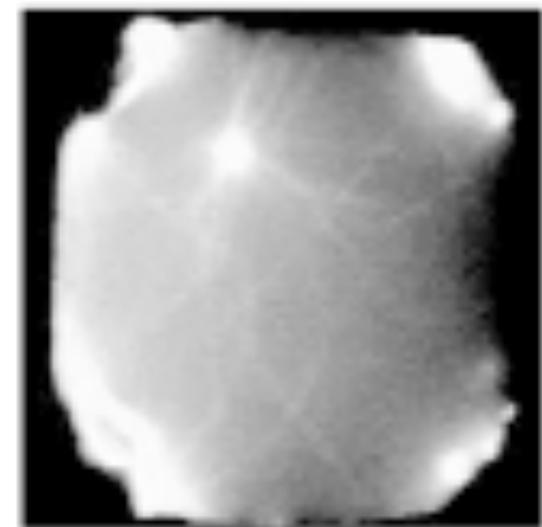
Growing: pushing



Shrinking: pulling

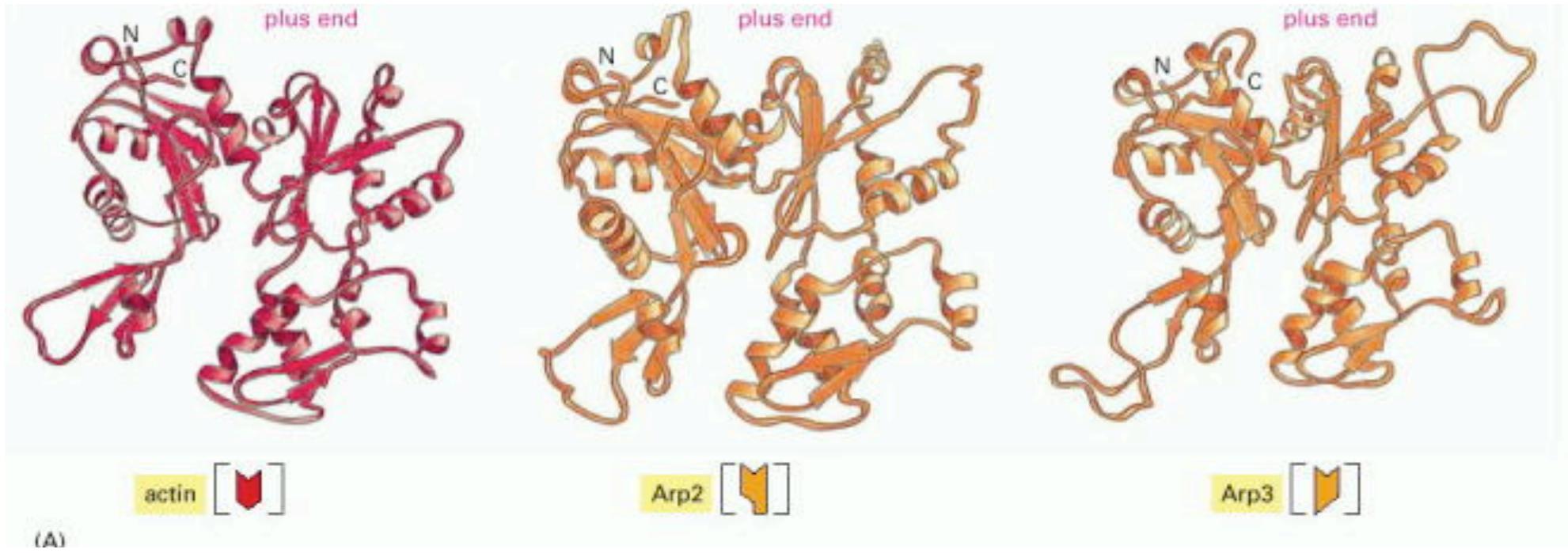


With catastrophes



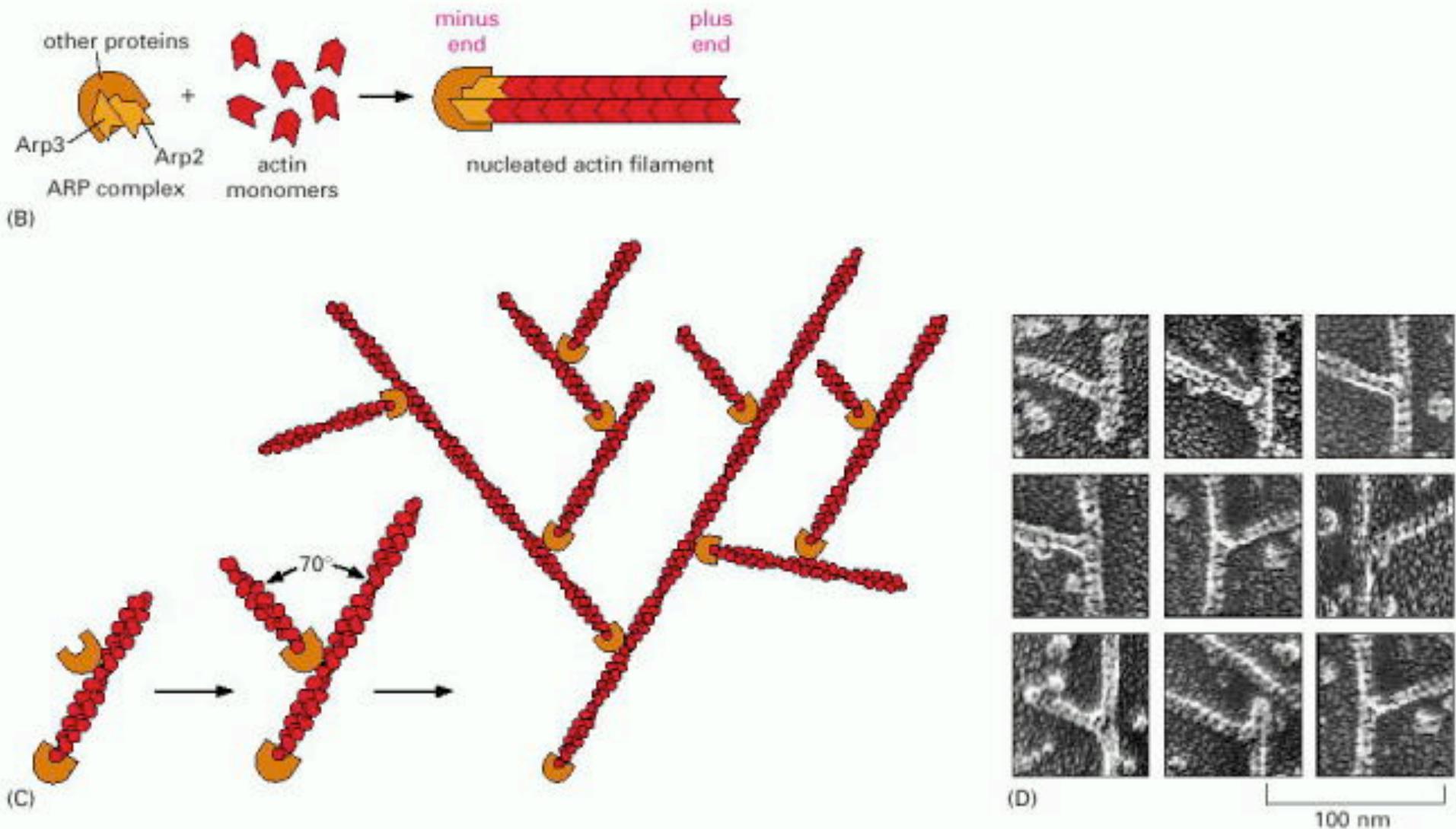
Without catastrophes

Nucleation Factors- Actin related proteins (ARPs)



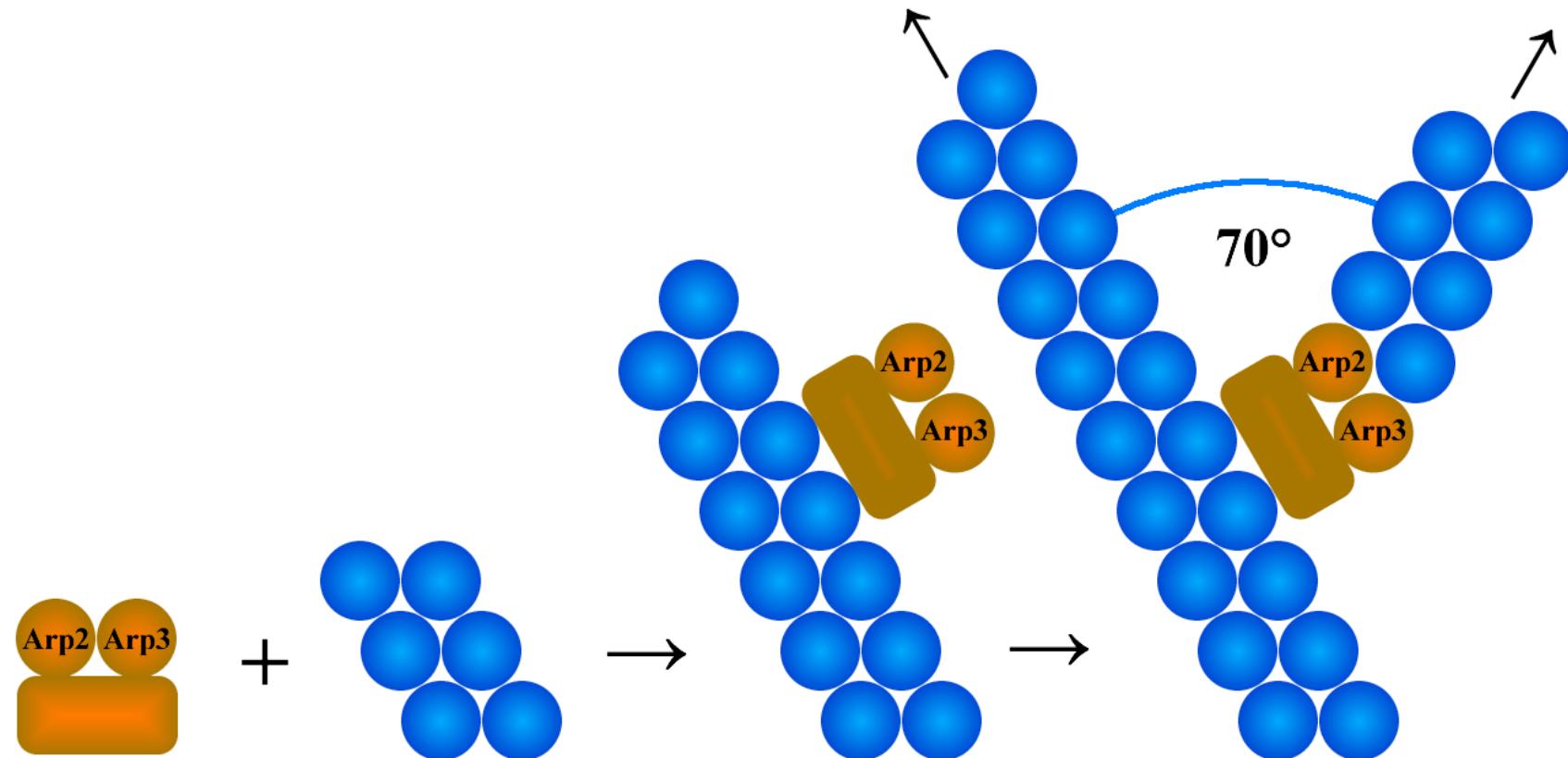
Actin related proteins (ARPs) :Arp 2/3 complex
Minus-end binding, Side-binding

Nucleation and Growth



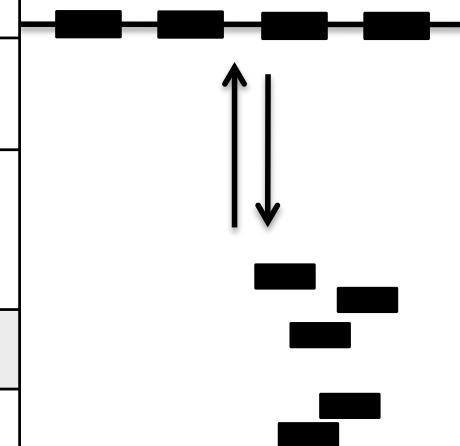
Mullins et al. (1998)

Branching of Actin

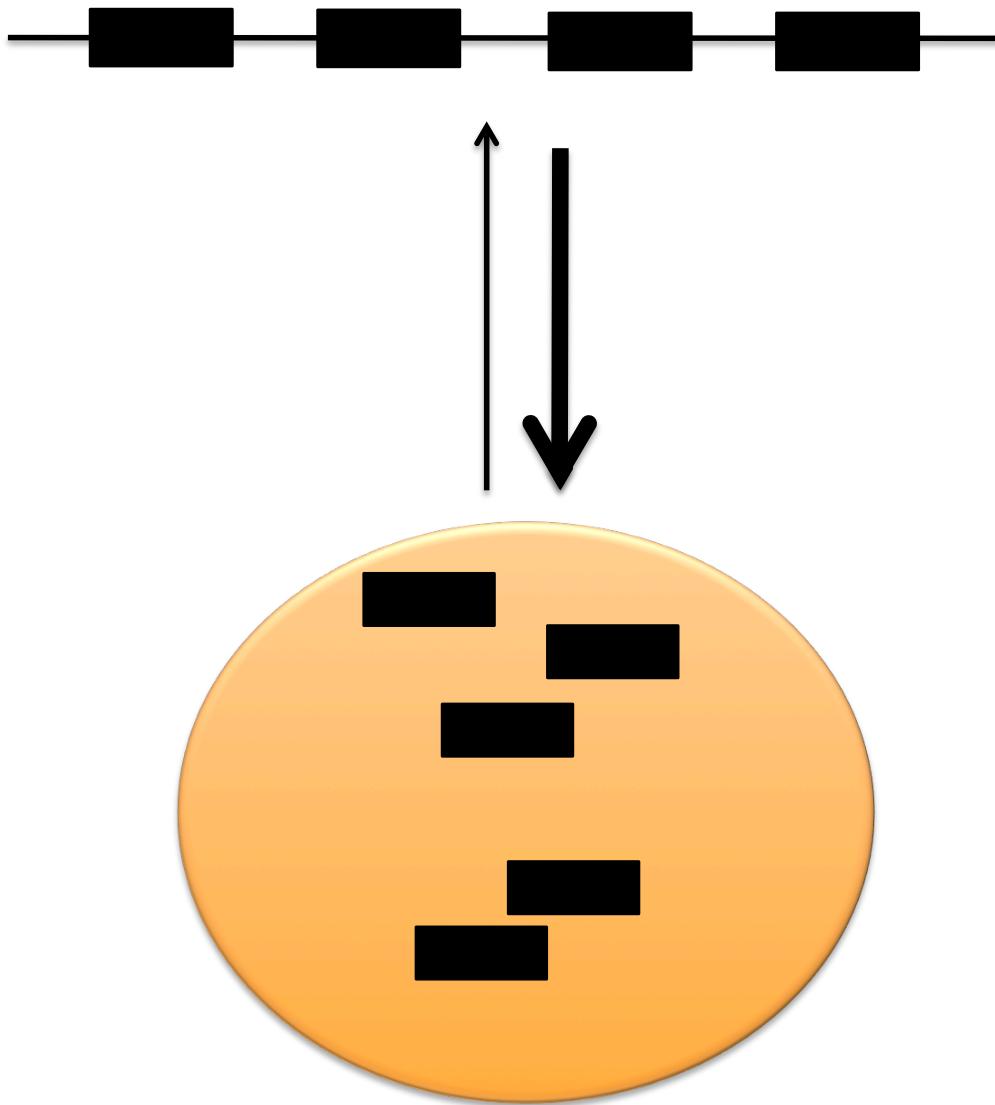


Polymerization Modification by Drugs

Drug	Effect	Source
Actin		
Latrunculin	Actin monomer stabilization	Latrunculia
Phalloidin	Actin filament stabilization	Amanita
Cytochalasin	Actin filament stabilization, end-capping	
Microtubule		
Taxol	Microtubule filament binding	
Colchicine, colcemid	Tubulin sub-unit binding	Autumn crocus
Vinblastine, vincristine	Tubulin sub-unit binding	Vinca rosea
Nocodazole	Tubulin sub-unit binding	Yew tree



Regulation of Growth by Sequestration

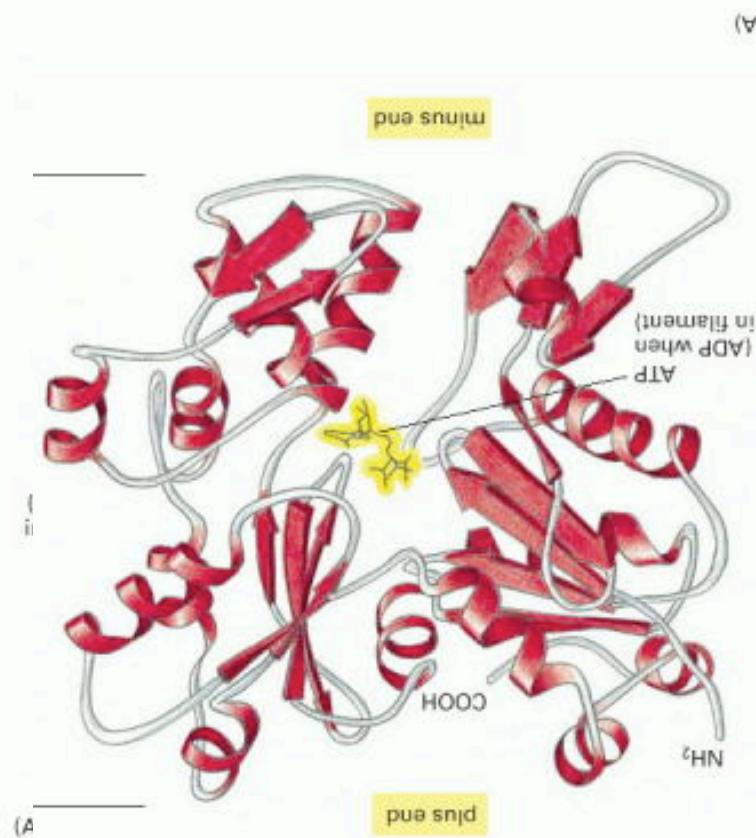
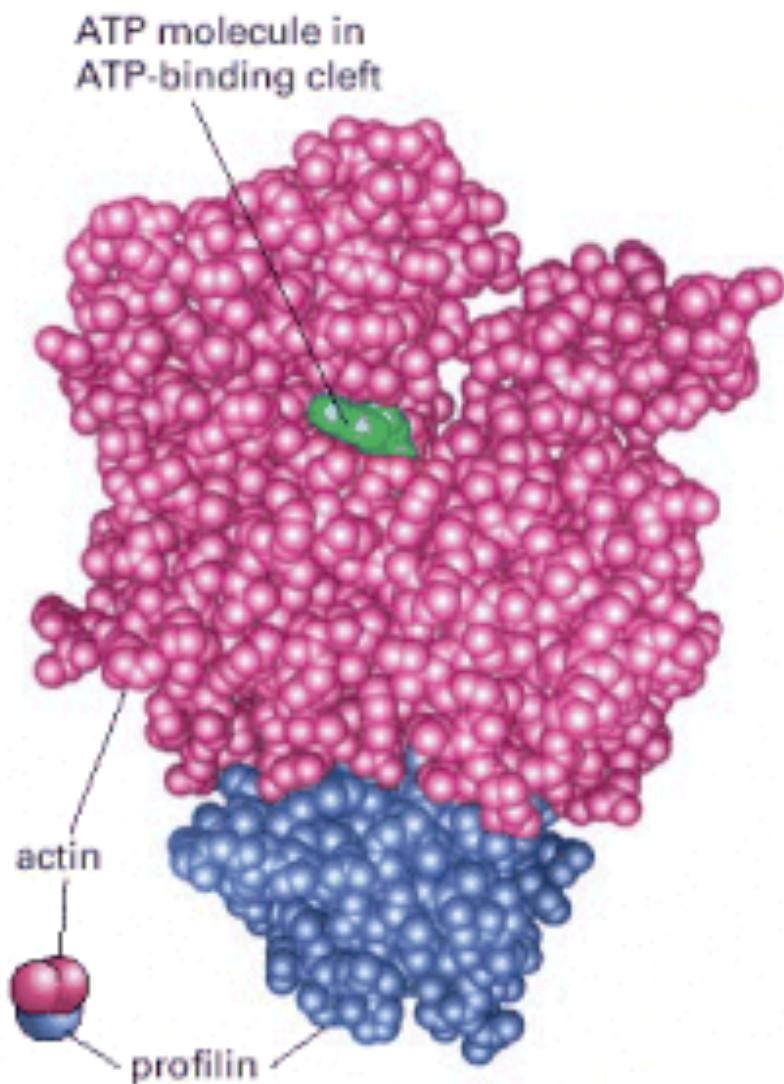


g-Actin abundance: 50–200 μM
 $C_c < 1 \mu\text{M}$ (in vitro)

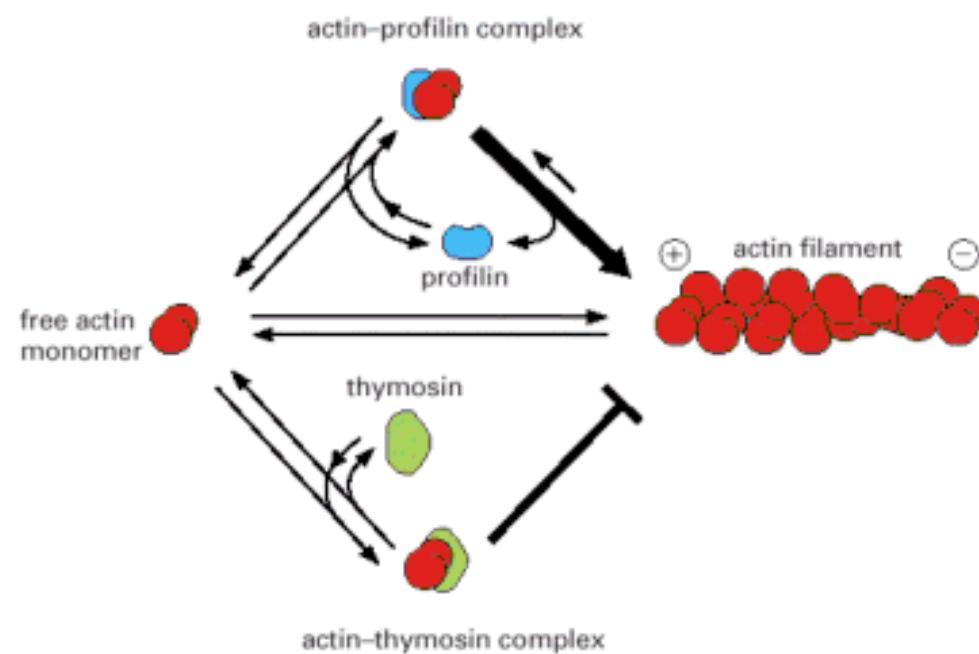
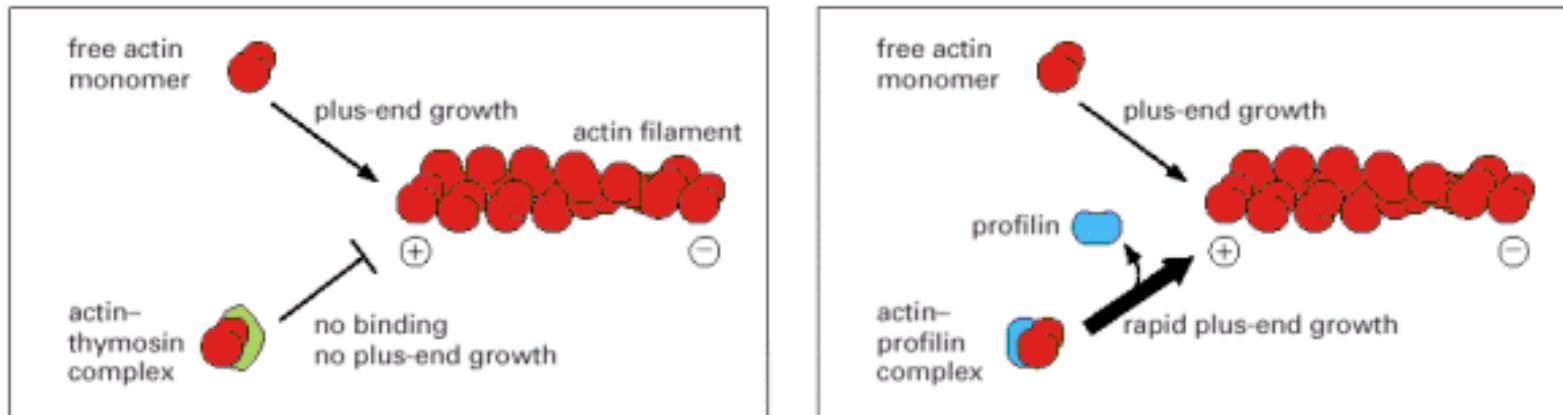
Thymosin

Profilin

Structural Basis for Regulation



Profilin & Thymosin



PROFILIN COMPETES WITH THYMOGIN FOR BINDING TO ACTIN MONOMERS
AND PROMOTES ASSEMBLY

Actin regulators- constitutive and inducible

Thymosin

- cytoplasmic

Profilin

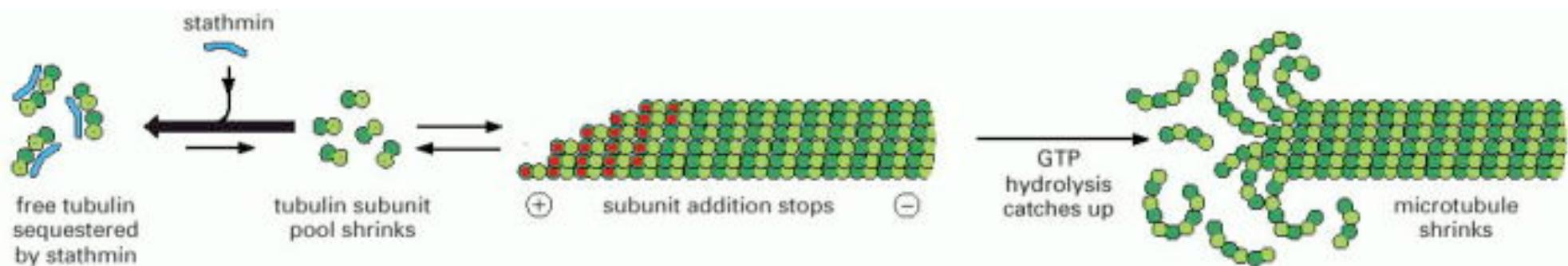
- membrane bound
- phosphorylation

Tubulin Sequestration

Stathmin + 2 $\alpha\beta$ -Tubulin \rightarrow Complex

$$V_g \approx k_{on} * \alpha\beta\text{-Tub}$$

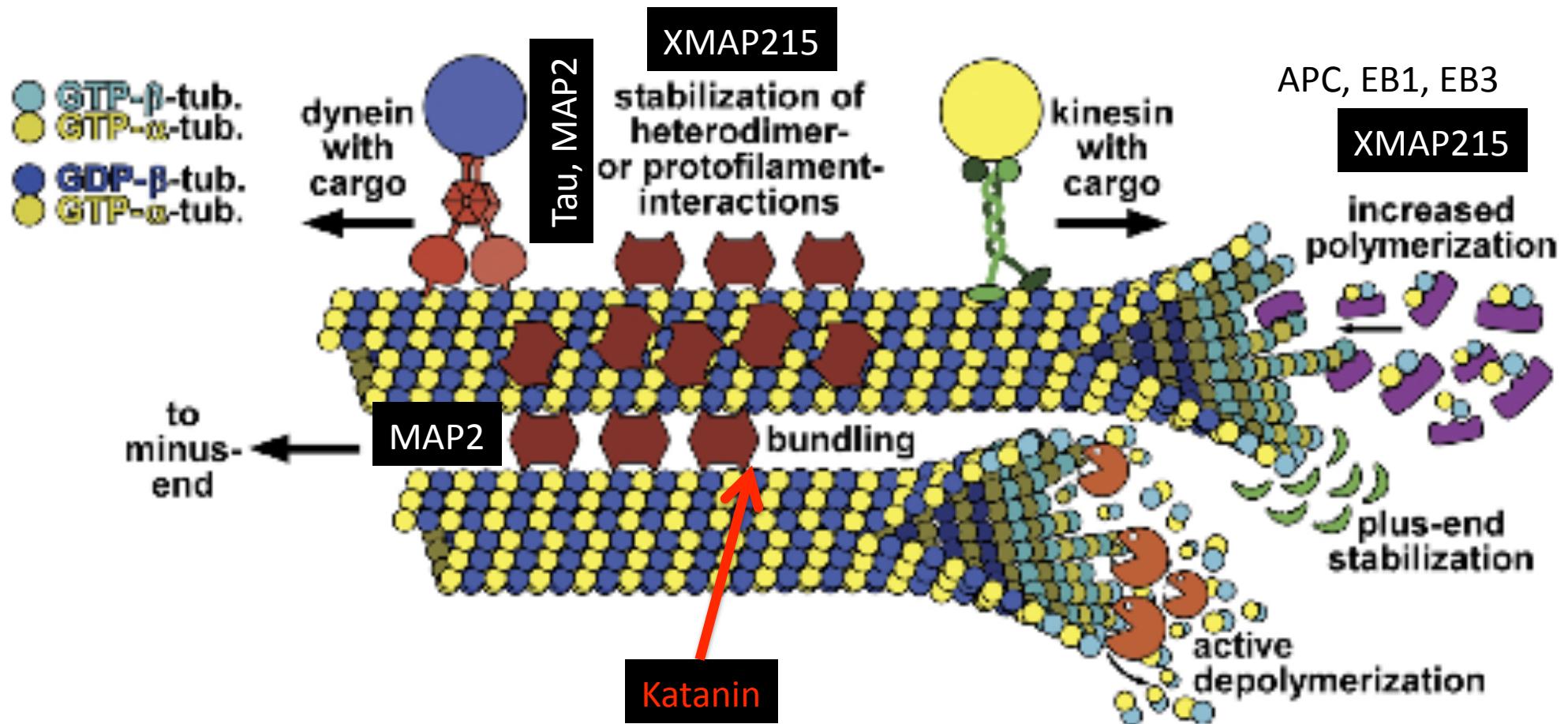
f_{cat} increase



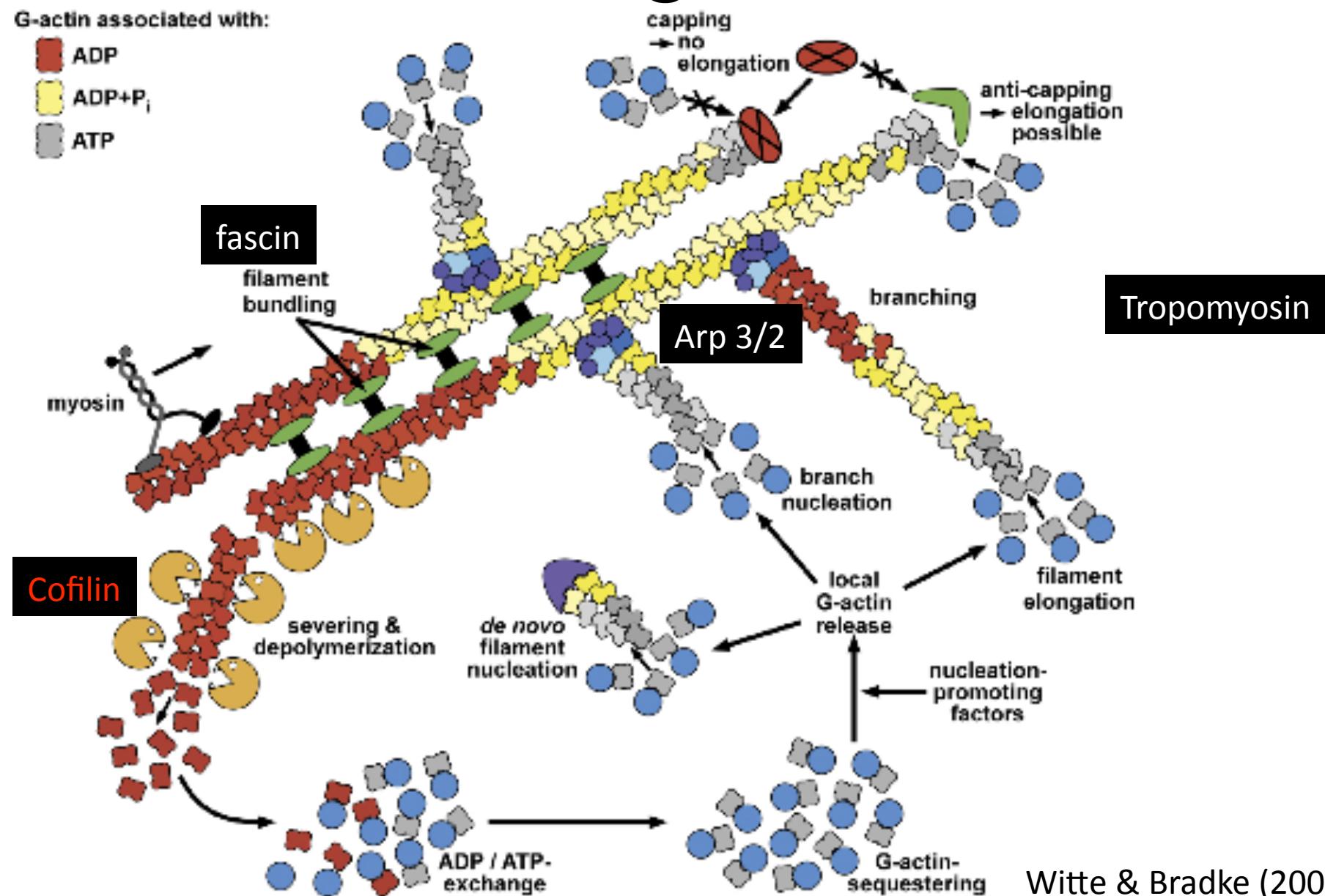
Polymerisation Modifying Factors

- Tip-binding
- Side-binding

Microtubule Associated Proteins (MAPS)

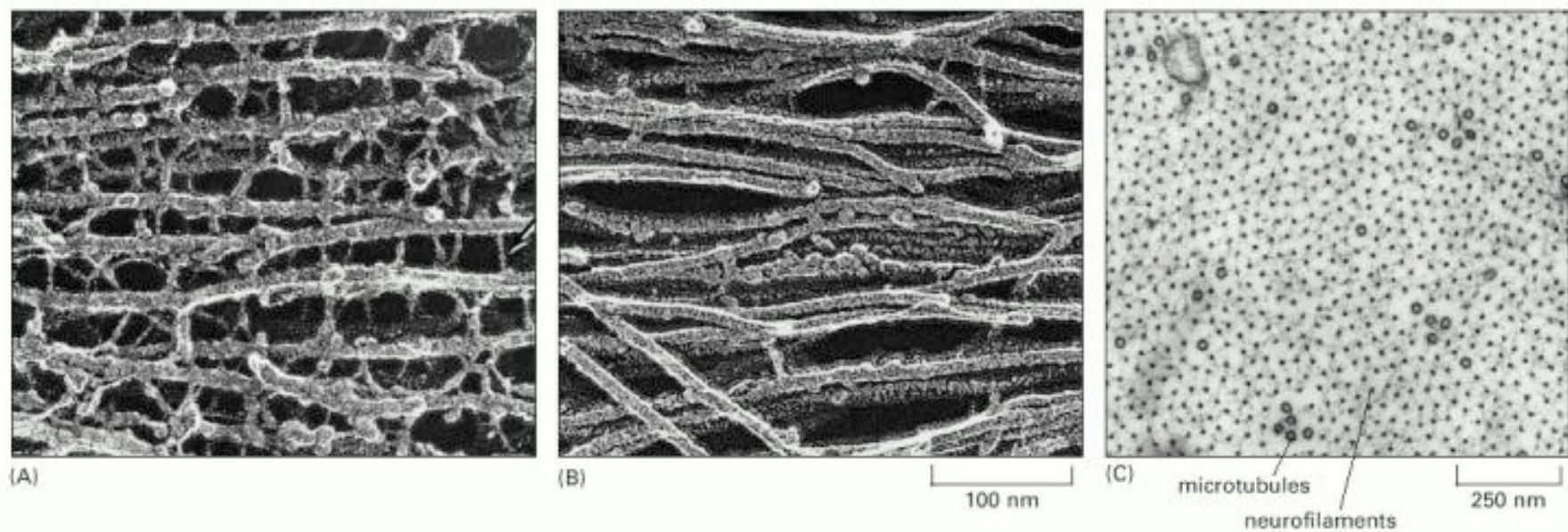


Actin Regulation



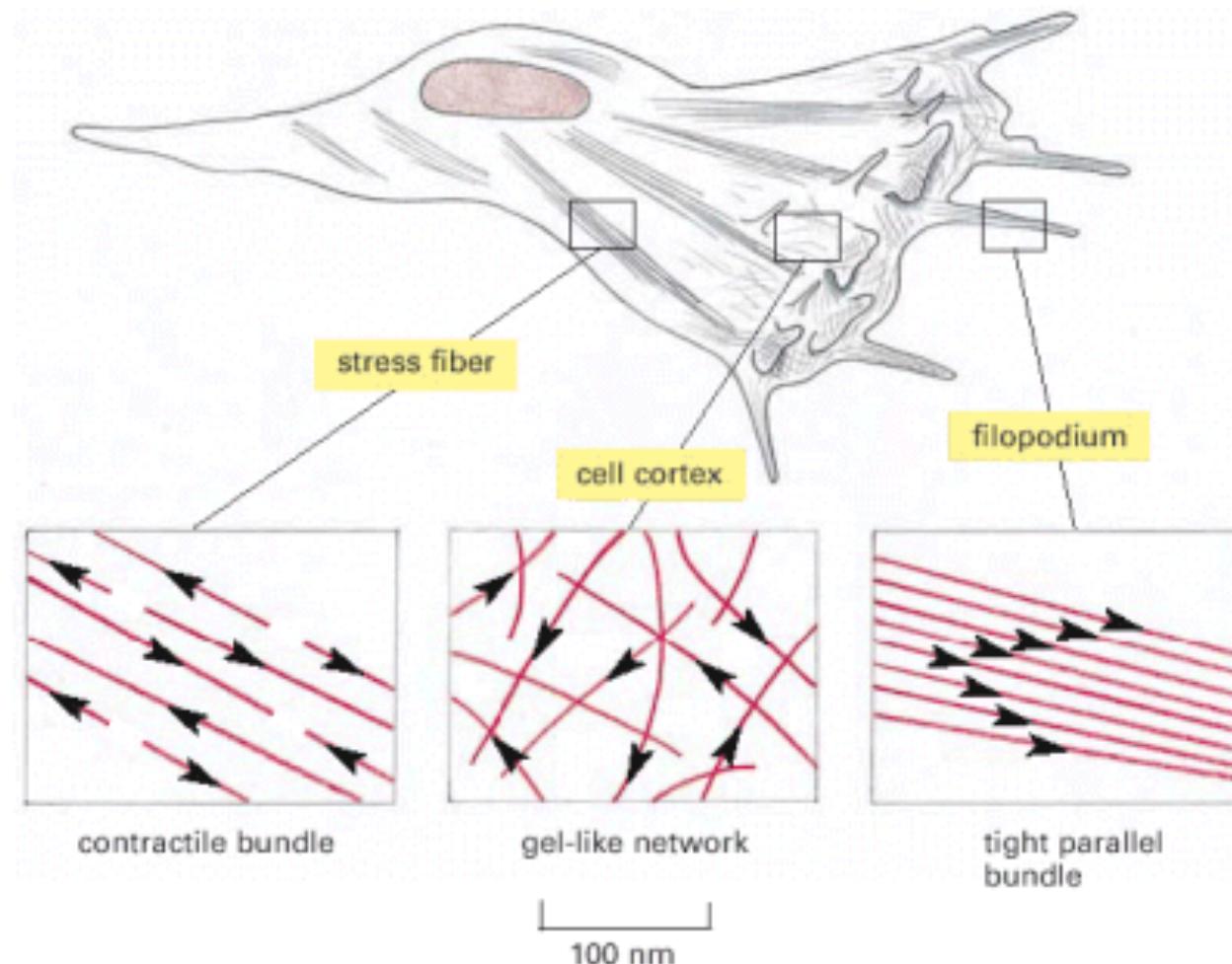
Higher Order Structure-IFs

Neurofilaments in axon- EM



Cross Section
of Axon

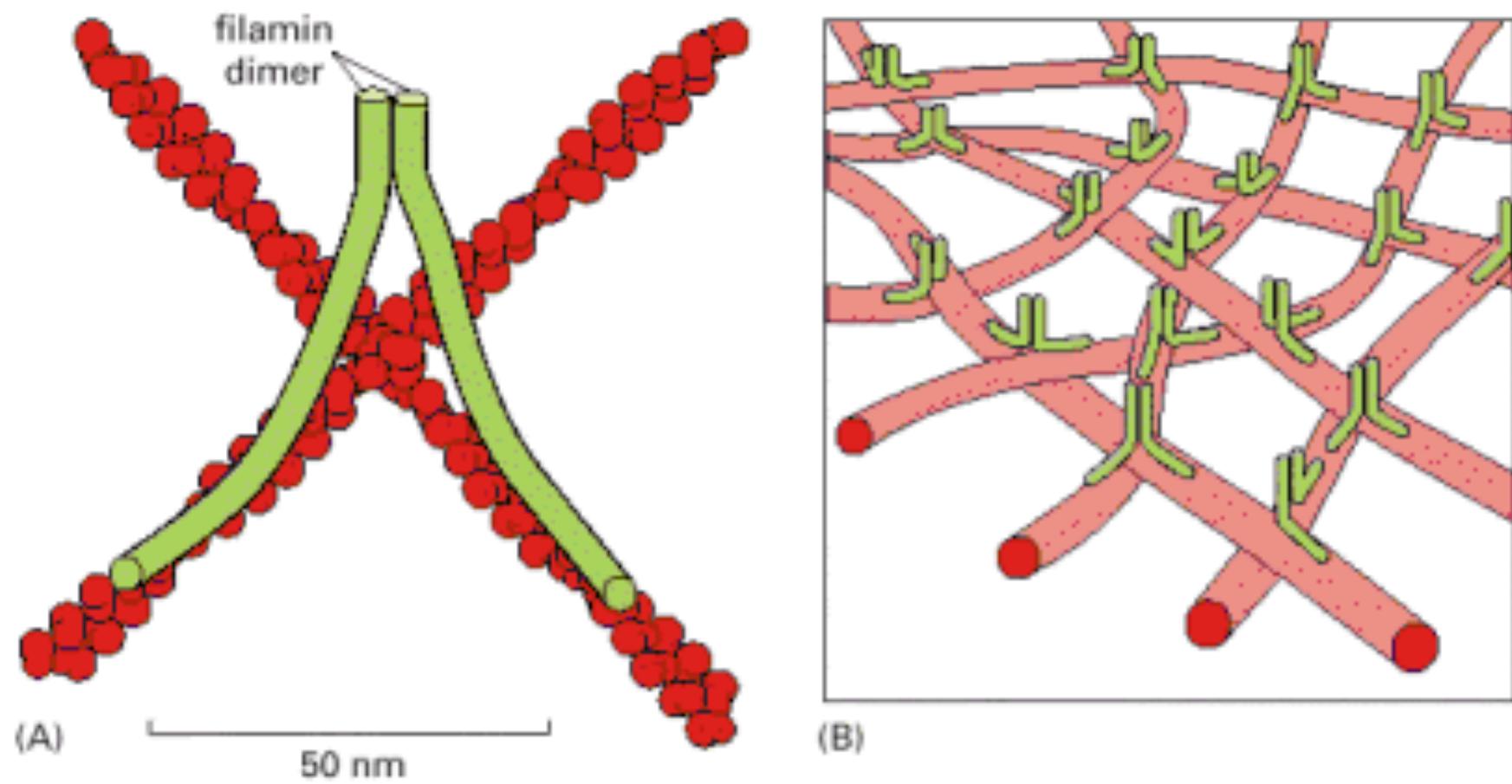
Higher-order structure- Actin



bundling proteins
Fimbrin, Villin

gel-forming proteins
 α -actinin, filamin

Actin Gel



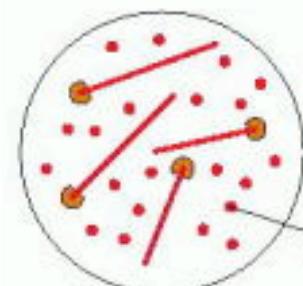
Changing Cell Shape by Actin Regulation

CapZ

Ca²⁺, gelsolin

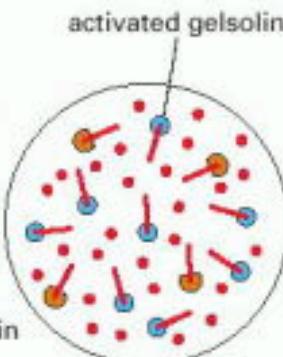
PIP2xGIs,Cz

Filamin, α -actinin,
fimbrin



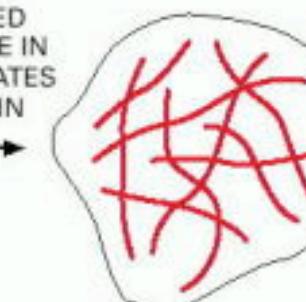
actin filaments capped
by capping protein in
unactivated blood
platelet

SIGNAL-MEDIATED
Ca²⁺ INFLUX
ACTIVATES
GELSOLIN

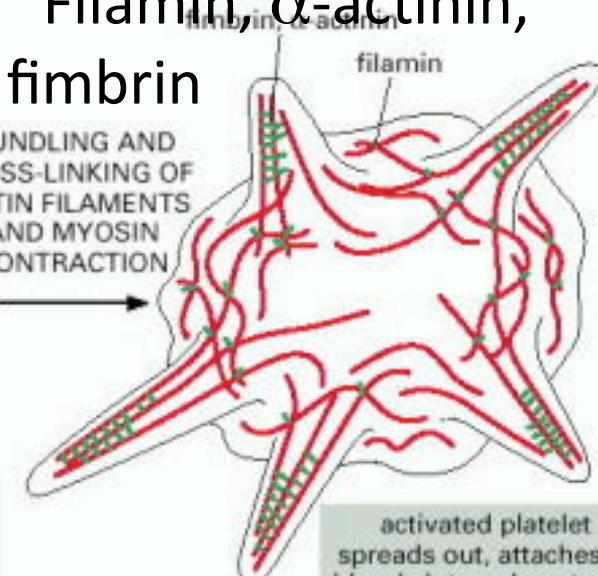


severed actin filaments
capped mostly by Ca²⁺-activated
gelsolin and some capping
protein

SIGNAL-MEDIATED
SLOW RISE IN
PIP₂ ACTIVATES
GELSOLIN



gelsolin and capping
protein removed, and rapid
actin filament growth
from many short fragments



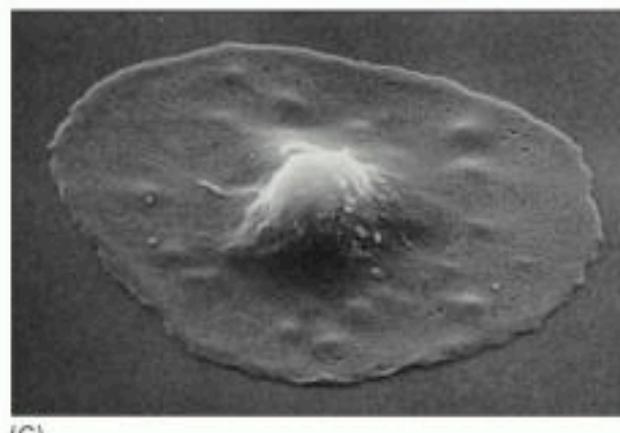
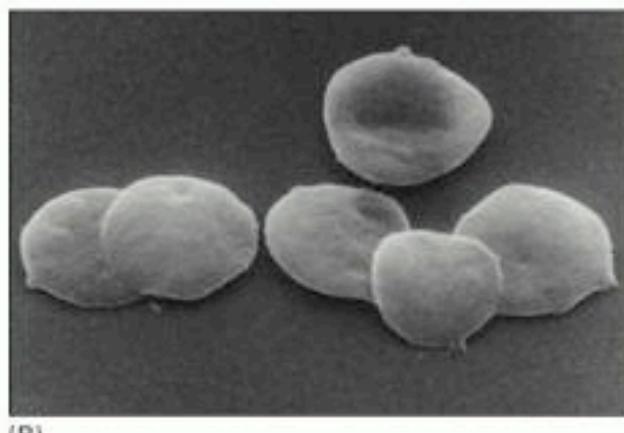
activated platelet
spreads out, attaches to
blood clot, and contracts

Unactivated
platelet

Activated platelet

Clotting (thrombin),
Contact with edge
of damaged blood
vessel

Actin severing, uncapping, elongation and cross-linking



2 μ m



Clotting (thrombin),
Contact with edge
of damaged blood
vessel

Attachment to Plasma Membrane

- Actin- side-binding Ezrin-Radixin-Moesin
(ERM mutation- neurofibromatosis)

Extracellular Regulation

- Rho GTPase
- WASP