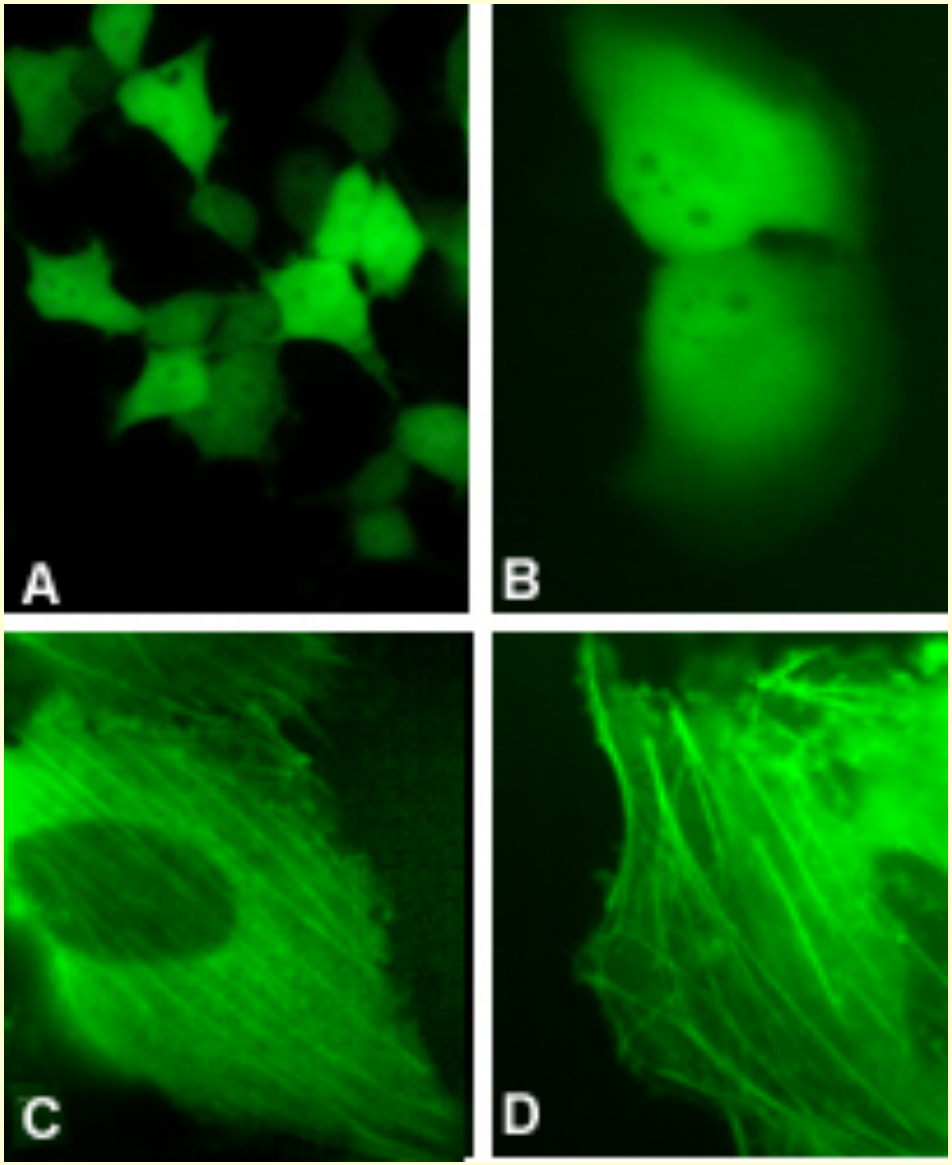


HeLa cells: A+B = GFP; C+D GFP-actin

Genetic modification of cells in culture can aid visualisation of subcellular components.

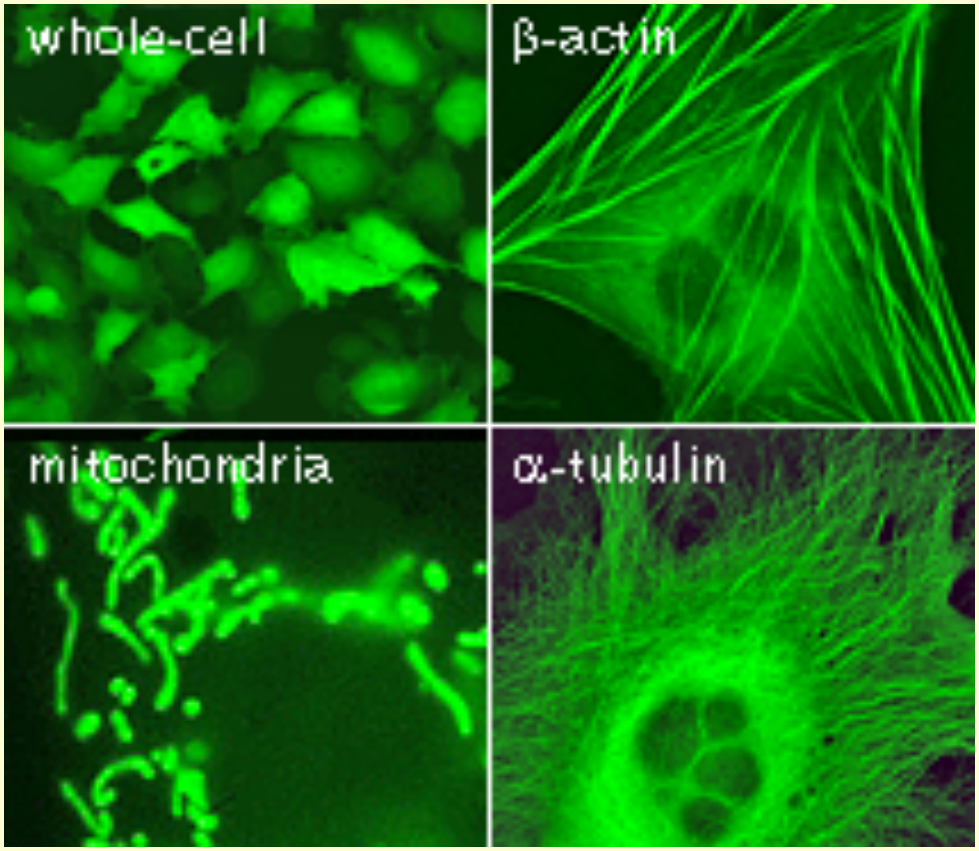
Use of naturally-fluorescent proteins

GFP from *Aquorea victoria*

Transfection is a process by which genes are introduced into mammalian cells in culture by mixing DNA with lipids.

DNA sequence encoding Promoter + GFP

DNA sequence encoding Promoter + GFP fused to Gene X



Using genetic engineering techniques and transfection - any cellular protein can be synthesised as a GFP-'tagged' derivative to reveal information about its position and movement.

http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/press.html

The Nobel Prize in Chemistry 2008

Osamu Shimomura, Martin Chalfie, Roger Y. Tsien

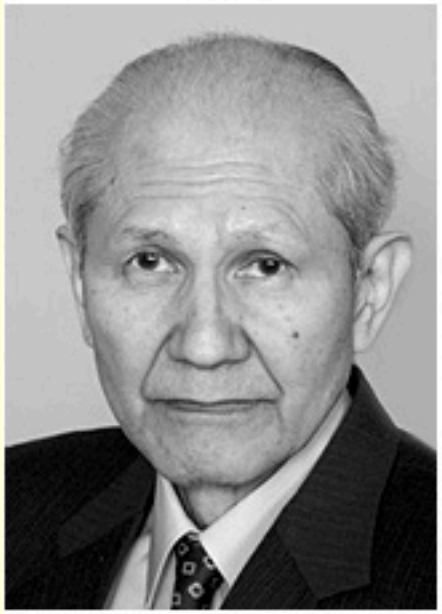


Photo: U. Montan

Osamu Shimomura

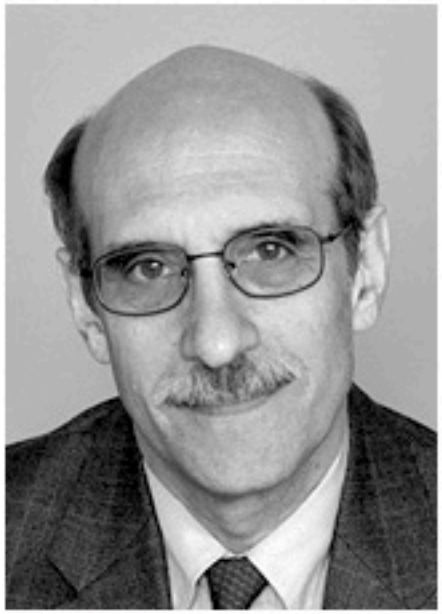


Photo: U. Montan

Martin Chalfie

Photo: U. Montan

Roger Y. Tsien

Module A: Lecture JT1

Lecture JT1: Culturing and Visualizing Cells

Reference. Molecular Cell Biology, Lodish et al, 7th Edition. Chapter 9.1 - 9.3.

Learning Objectives;

Culture of animal cells in the laboratory.

1. **Understand that different types of cells have different potential for mitotic division and growth.**
2. **Know the distinction between a terminally differentiated cell, a stem cell and a cancer cell.**
3. **Understand how cultures of mammalian cells are established and maintained over multiple generations (passages) in a laboratory.**
4. **Know how cultured cells can be genetically modified to study the function of genes and the definition of the term transfection.**

Visualisation of cells by light microscopy

5. **Know the basic operating principles of the light microscope and how it can be used to study mammalian cells.**
6. **Describe how immunofluorescent microscopy is carried out and compare this technique with use of green fluorescent protein to study protein distribution in cells.**
7. **Be able to describe techniques that can demonstrate the dynamics of molecules inside cells.**

BIOSCI 201 Cellular and Molecular Biology

Lecture JT2.

Actin, cell motility and muscle contraction

Lodish et al., 7th ed Chapter 17

Learning Objectives.

1. Become familiar with the structure of actin and the reversible assembly of F-actin.

2. Understand the role played by actin-binding proteins.

3. Understand how actin is critical to the shape of different types of cells and that cell movement involves actin polymerization.

4. Give examples of how intracellular pathogens use actin polymerization for movement

5. Know the basic features of myosin structure and the function of different classes of myosin in different cell types.

6. Understand how actin and myosin II function together during the contraction of skeletal muscle and know how this process is regulated by other actin-binding proteins.

•There are 3 classes of cytoskeletal protein filaments in eukaryotic cells.

Each filament is a polymer of protein subunits

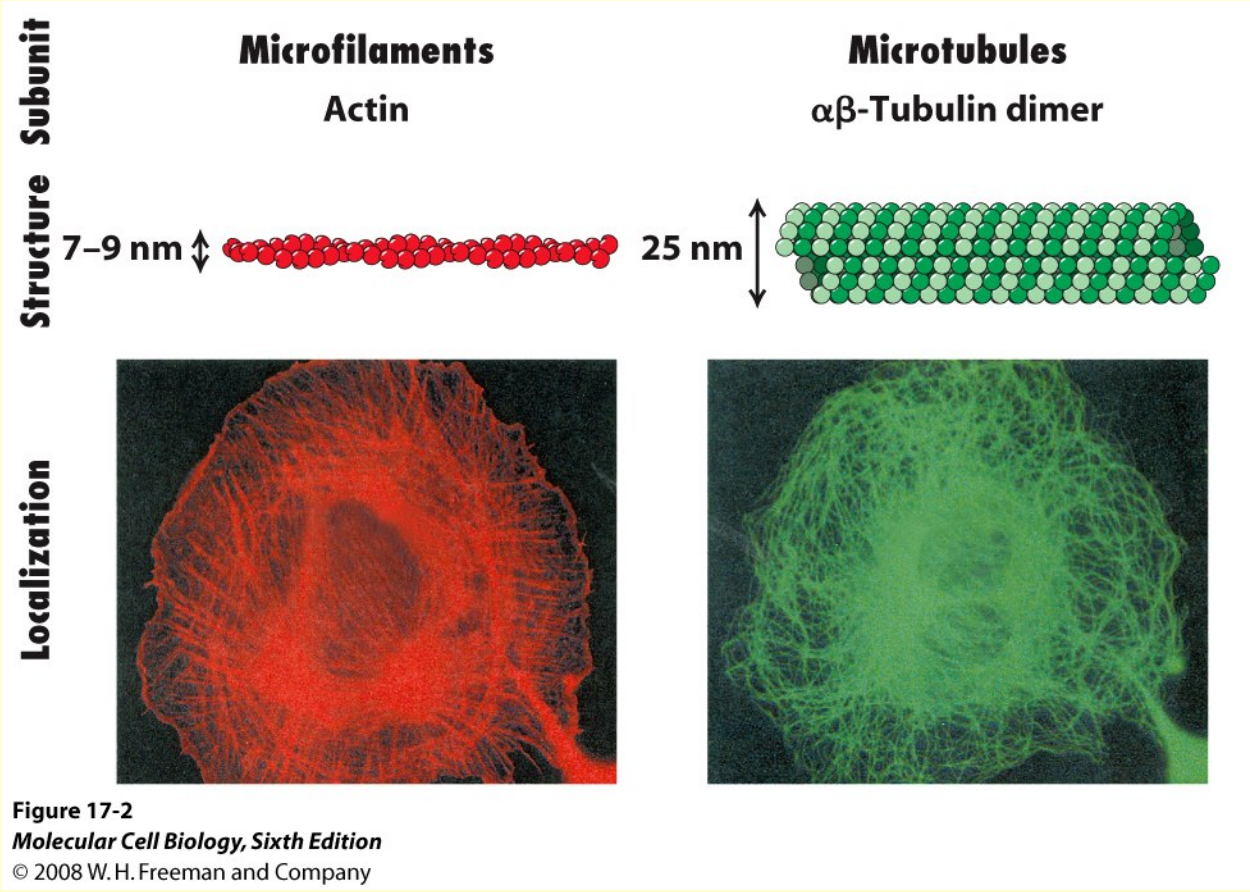


Figure 17-2
Molecular Cell Biology, Sixth Edition

Preferential polymerisation of actin at the (+) end

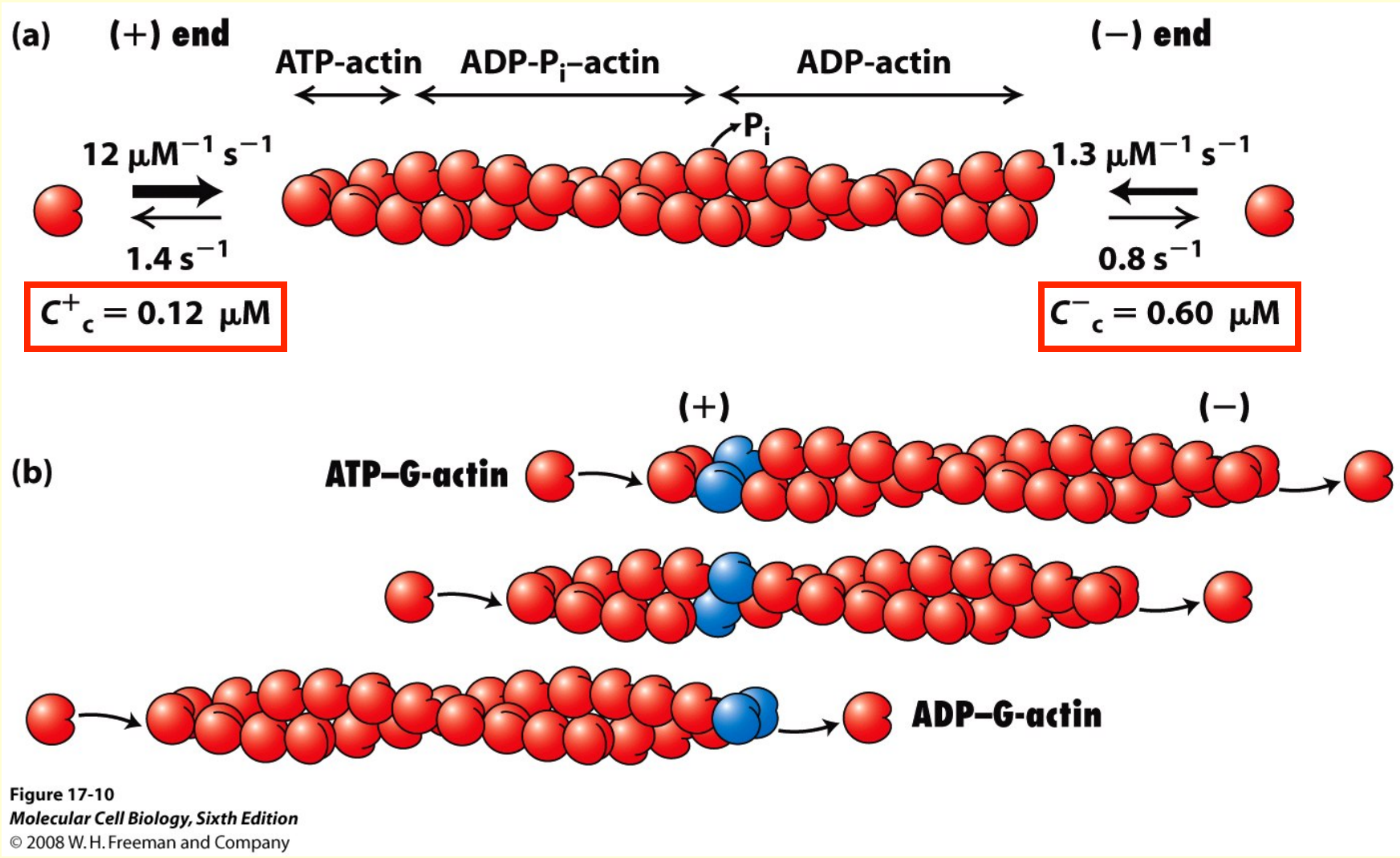
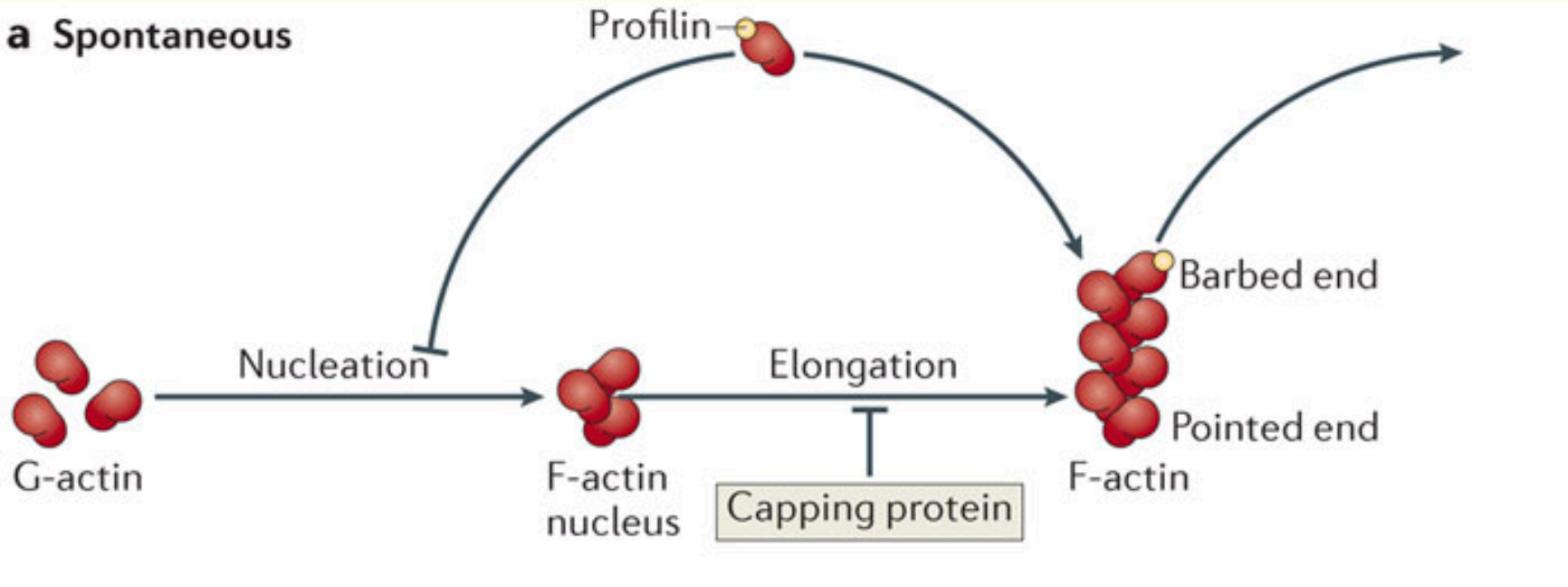


Figure 17-10
Molecular Cell Biology, Sixth Edition

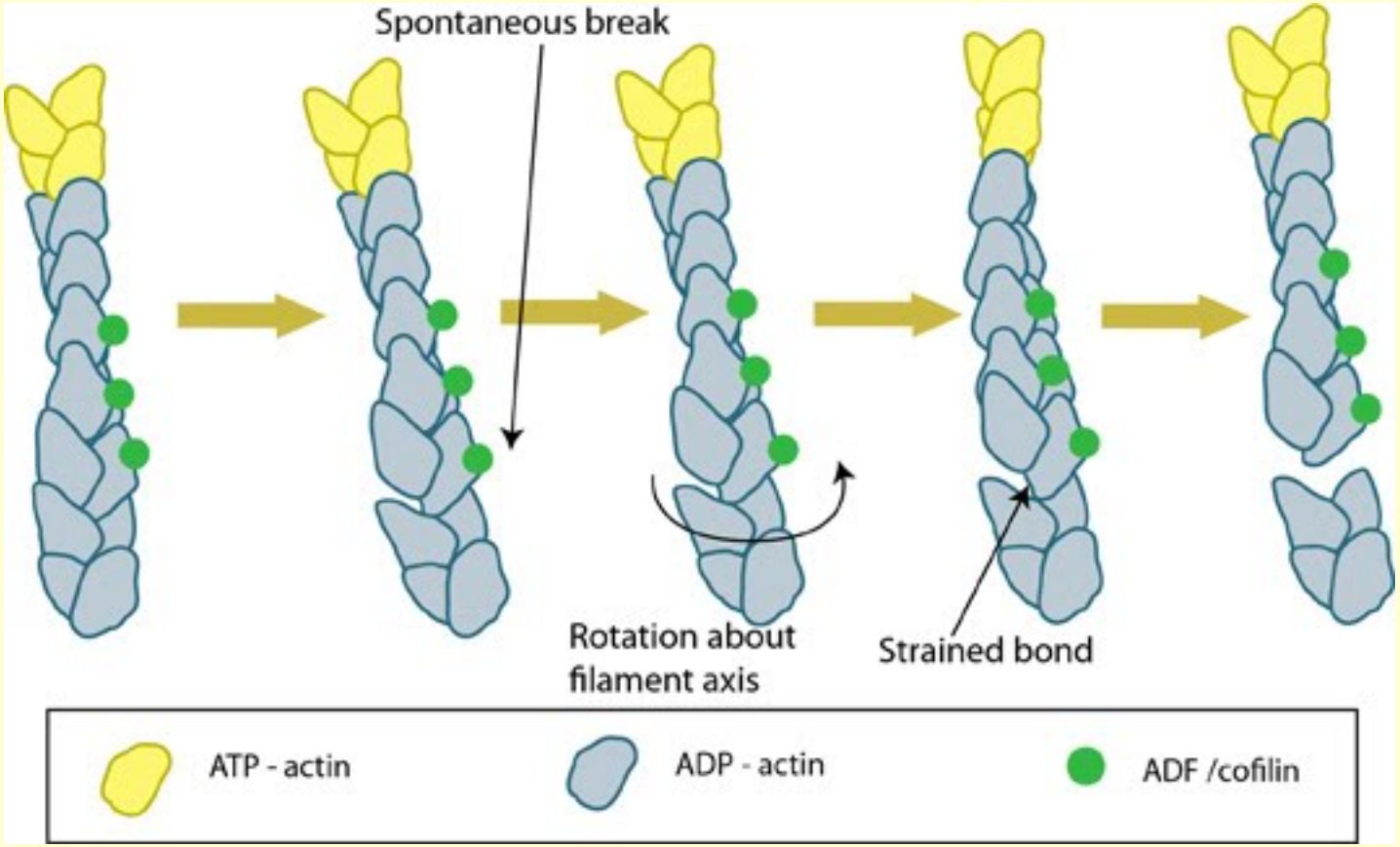


Actin assembly *in vivo* – role of accessory proteins

In the cell actin polymerisation occurs faster than pure actin *in vitro*

In the cell action polymerisation occurs only at the + (or 'barbed' end)

profilin binds ADP-G actin at the opposite side to the cleft and promotes re-charge of ADP –ATP exchange→ polymerisation at the + end and block - end



Cofilin binds to ADP-actin near the (–) ends of a filament causing a twist and then a break, shortening the filament at the (-) end

Together, profilin and cofilin cause treadmilling of the filament