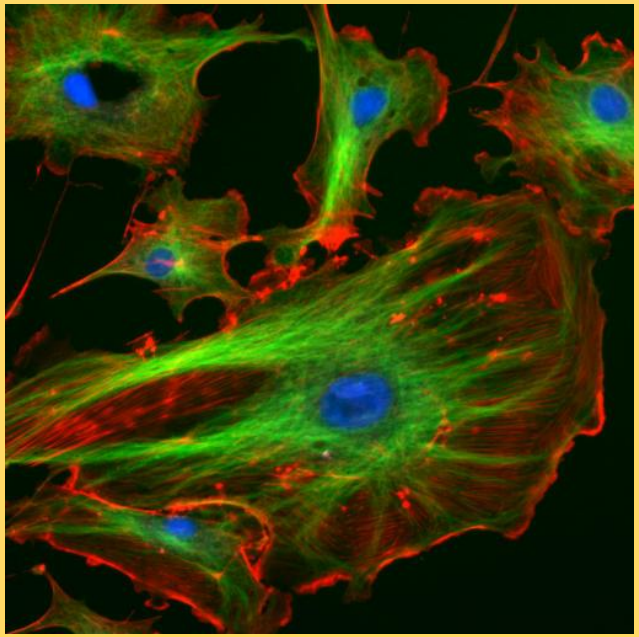
Section 2 : Motion Inside Cells

Lecture 6
The Cytoskeleton : Actin

Roop Mallik, IIT Bombay

BB101, Spring 2023



Resources

- Molecular Biology of the Cell. Alberts, Johnson,Lewis …. Walter
- Physical Biology of the Cell. Phillips, Kondev, Theriot, Garcia
- Mechanics of Motor Proteins and the Cytoskeleton. Jonathon Howard

Ibiology Lecture, J. Theriot

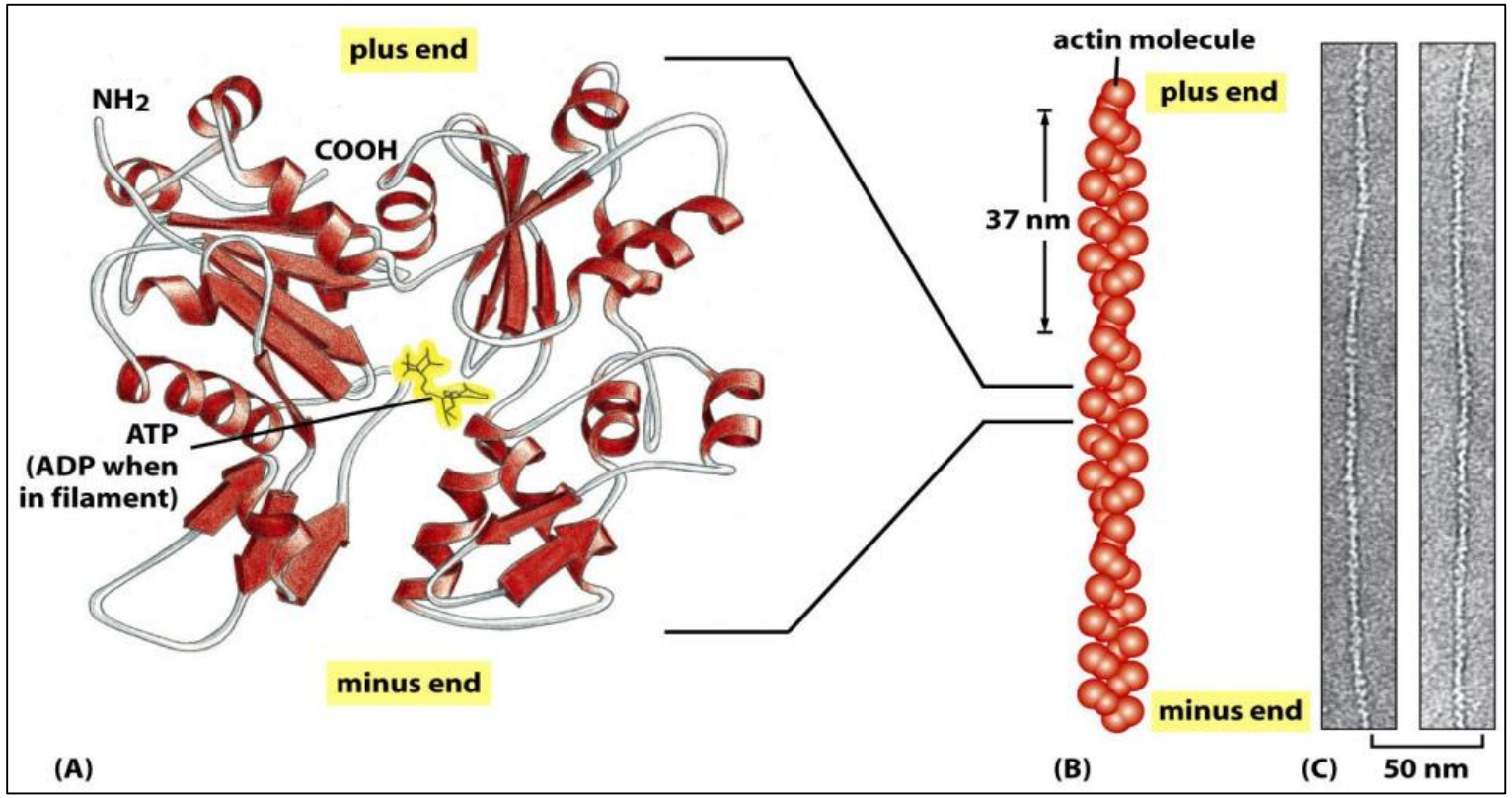
ACTIN DYNAMICS

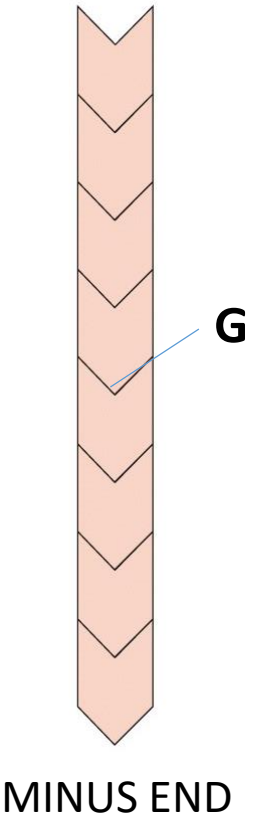
Fluorescent actin in neuron (VIDEO)

Dr. Andrew Moore
Dr. Pedro Guedes-Dias

GLOBULAR ACTIN (G-ACTIN)

FILAMENTOUS ACTIN (F-ACTIN)





G-Actin binds ATP in solution
→ Hydrolyses ATP when it binds to other G-actin subunits
→ Yields energy for making F-Actin (polymerization lowers entropy)

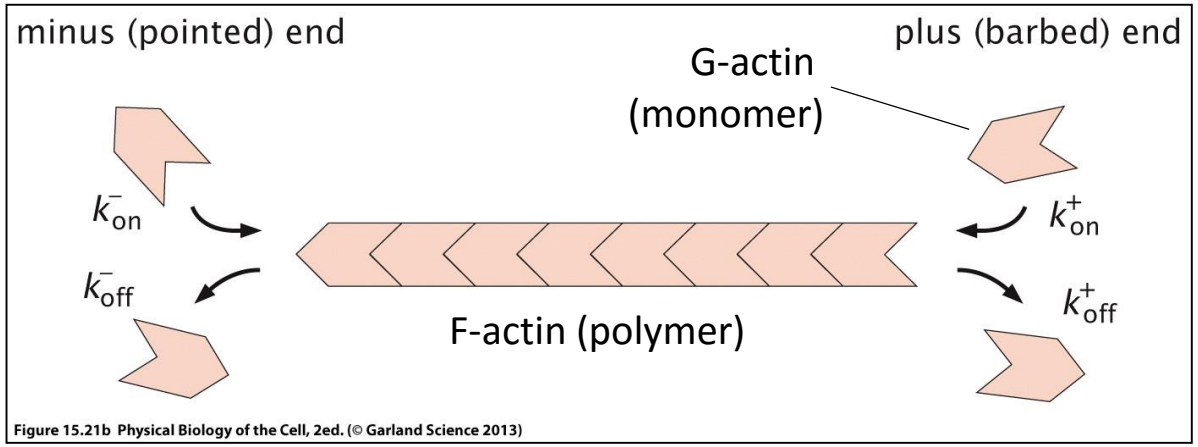


Figure 15.21b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

The time course of actin polymerization in a test tube

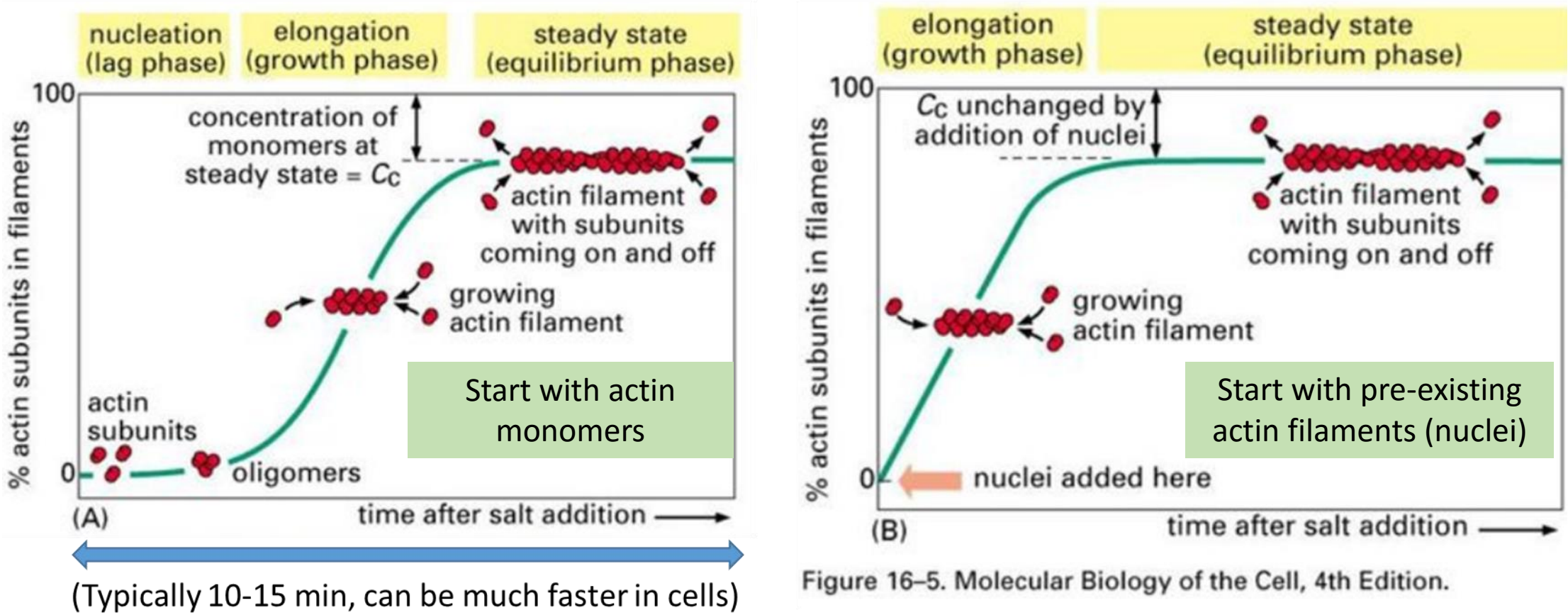
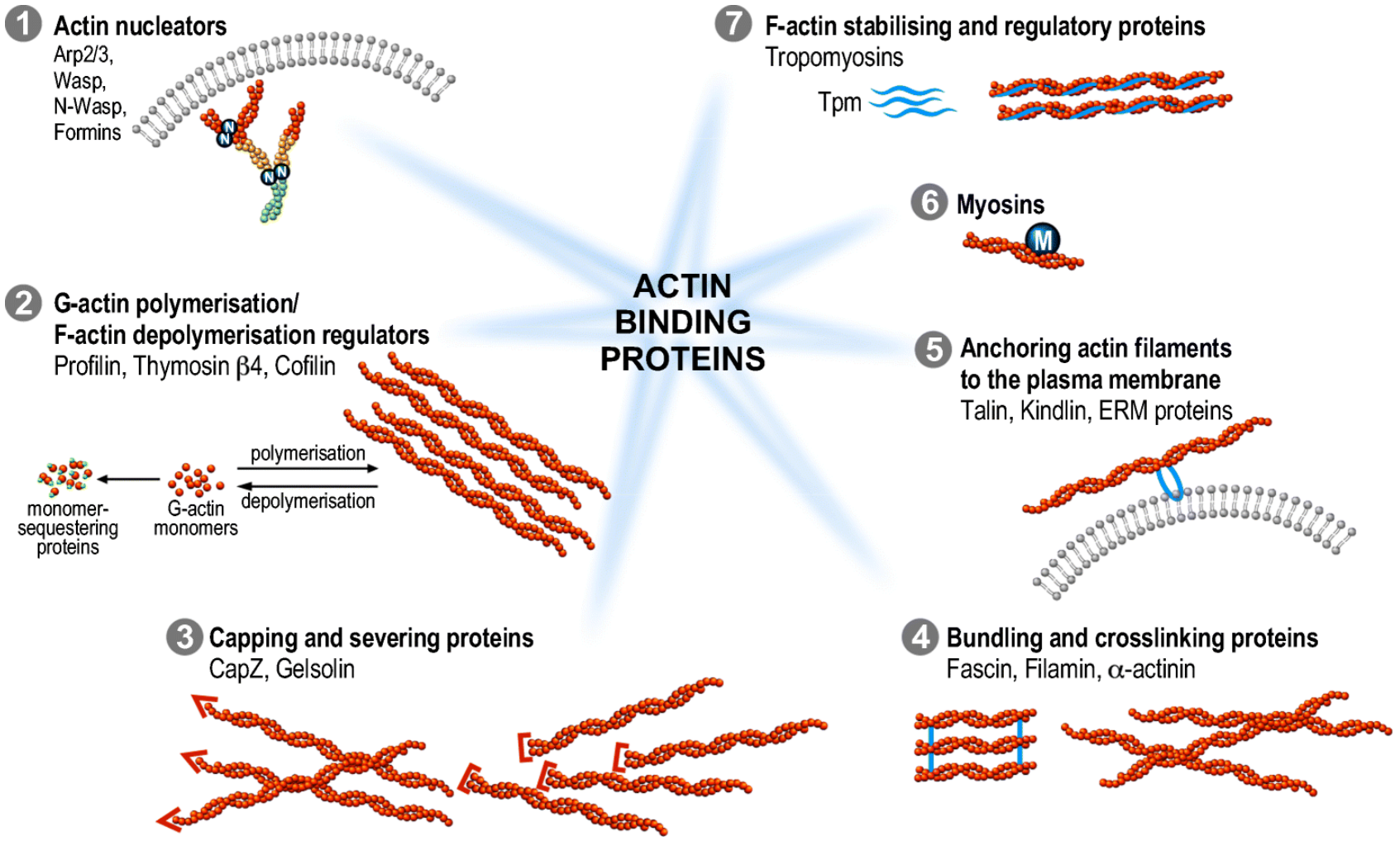


Figure 16–5. Molecular Biology of the Cell, 4th Edition.

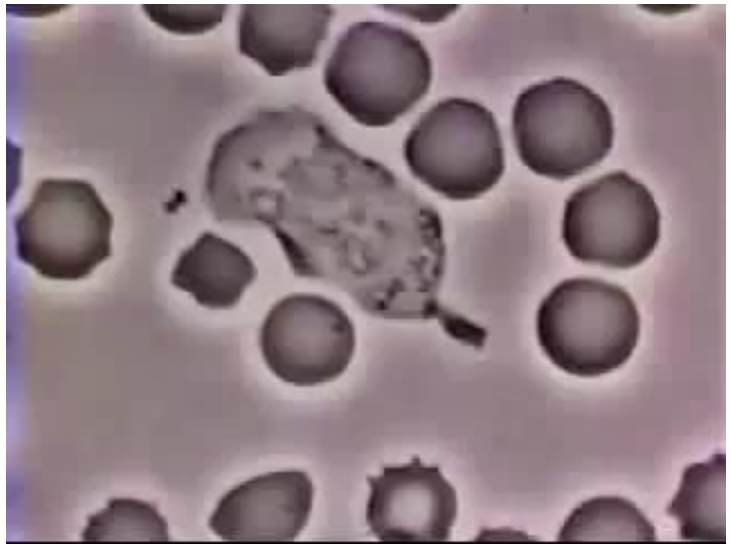
- Polymerization can be started by raising Salt (ion) concentration
- Steady state :- Rate of addition of Monomers to Filaments = Rate of dissociation from Filaments
- Monomer Concentration at Steady State = Critical Concentration of Actin C_C ($K_{ON} * C_C = K_{OFF}$)
- “Lag Phase” if you start with actin monomers. No “Lag phase” if pre-existing actin filaments used
- Suggests how the Cytoskeleton can provide a Local and Rapid response (Actin nucleating proteins)

Making and Breaking Actin for Cell Dynamics



MORE INFORMATION

Crawling of Cells …
Most interesting manifestation of Actin Dynamics



White Blood Cell chasing bacteria

How to study Cell Crawling ?

ibiology Lecture by Julie Theriot

1:19 Actin as a Nanomachine
Actin at Leading edge, rear edge

3:48 Model System
Keratinocytes from Fish Scales

6:26 Fluorescent labelling of Actin and Myosin

7:10
Cycles of Actin based Cell motility

13:26
Adhesion strength affects how cells move (see S. Sen work)

18:48
Myosin chops up actin at rear poles

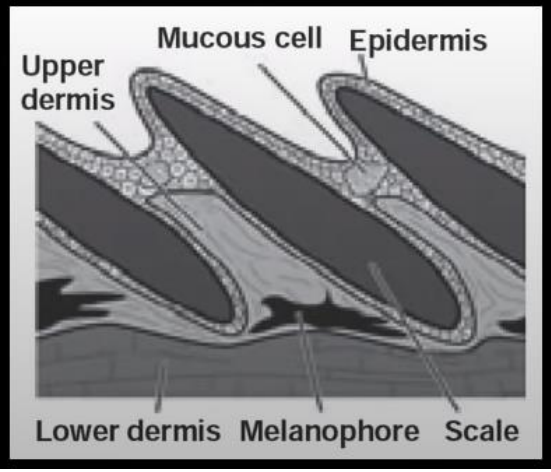
33:16
Neutrophil turning & Myosin asymmetry – “Steering wheel”?

3:48 Julie Theriot Lecture

Model System to Study Cell Crawling : Keratinocytes from Fish Scales

Hypsophrys nicaraguensis

Fish epidermal cell - keratocyte



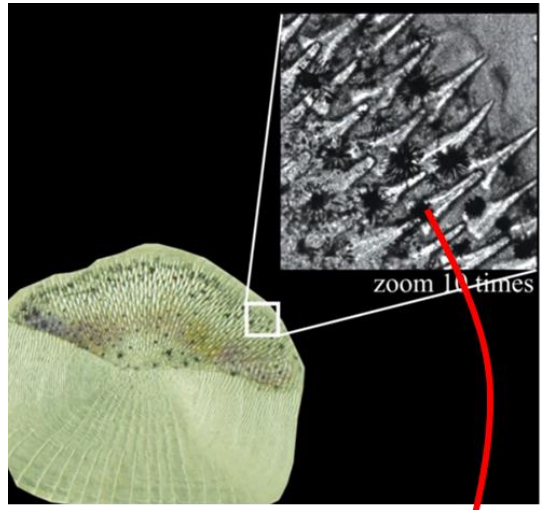
Greg Allen

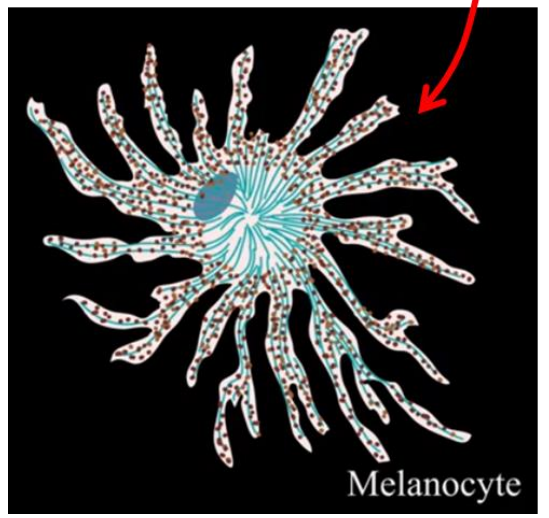
DIGRESSION

Fish Scale Melanocytes

Study Microtubule Motors

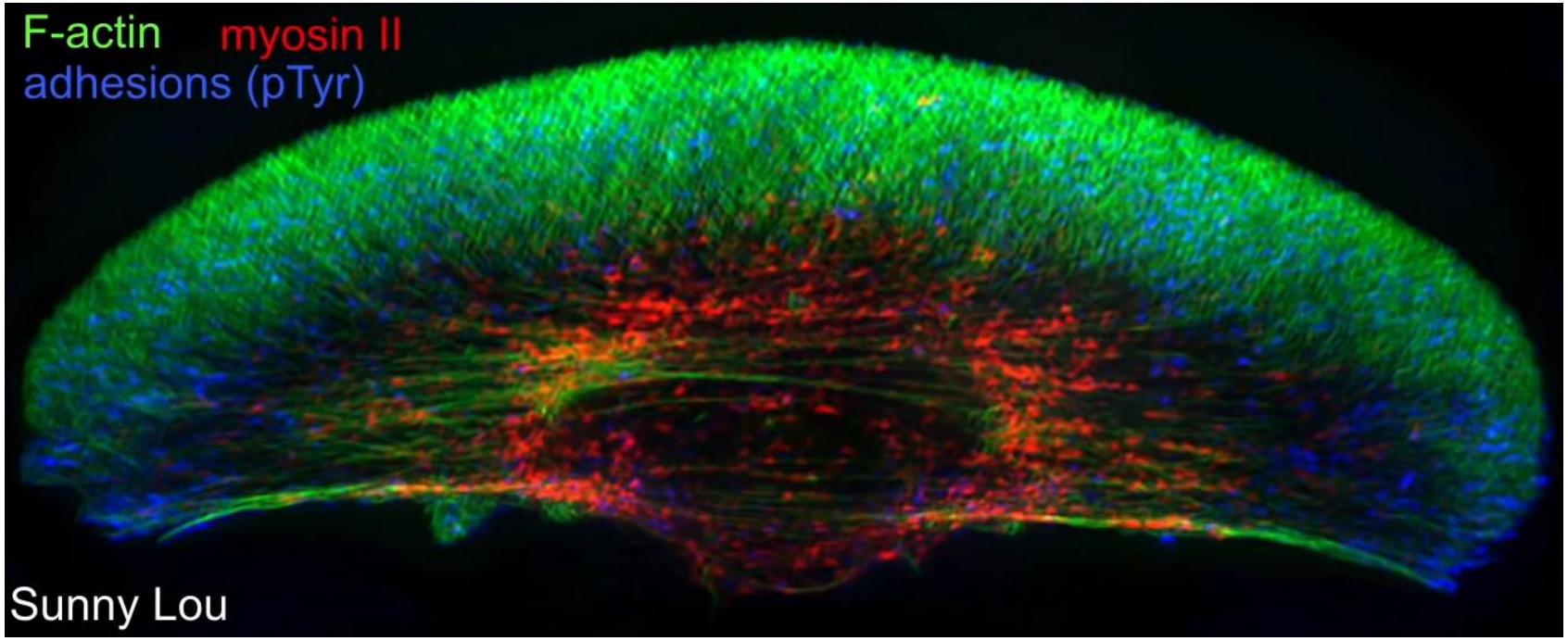
Video by Minhajuddin group

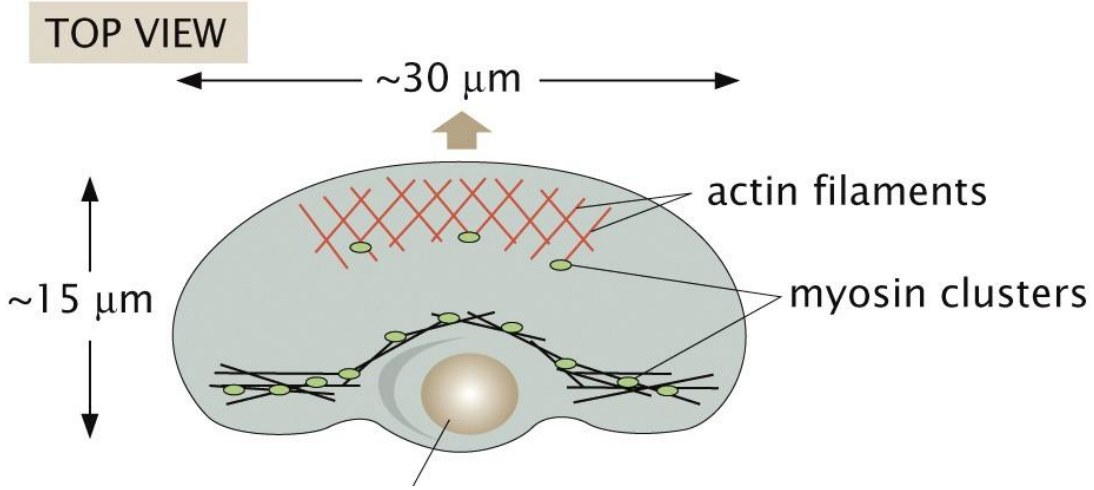




6:26 Julie Theriot Lecture

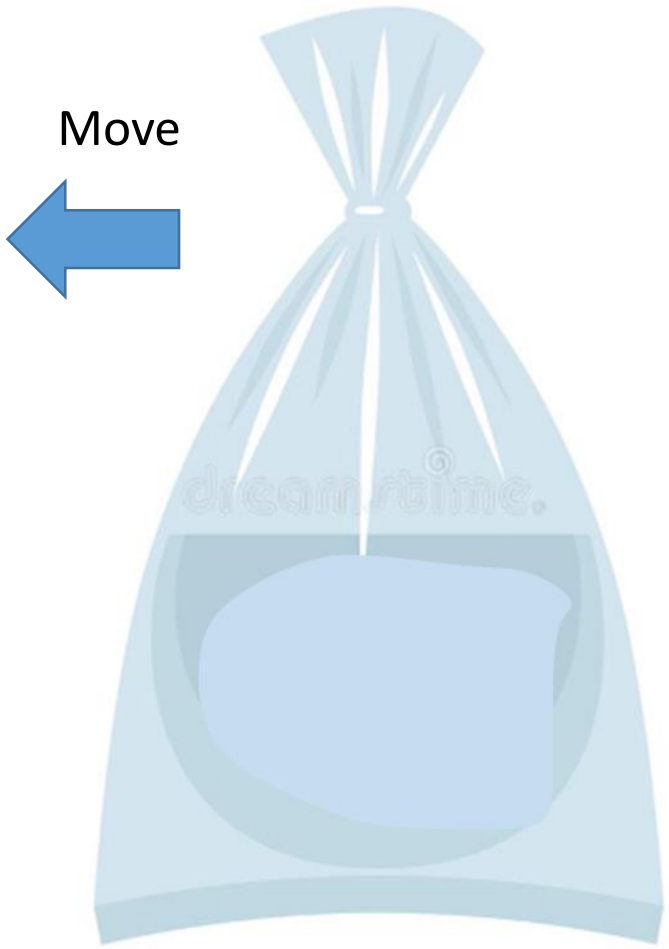
Fluorescent labelling of Actin and Myosin





How can a bag filled with water move on its own ?

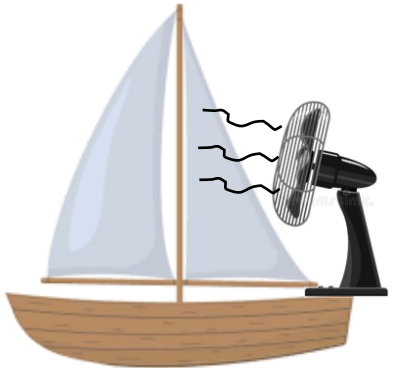
Move



1) What if we add some “active material” inside …

2) Must grab & generate force/reaction against a substrate …

3) Must let go on the other side …



Cell Crawling …

1. Know which way you want to move (front versus back)
2. At the front :
   i. Extend towards the front
   ii. Attach to the outside and push back
3. At the back : Detach from the outside and retract

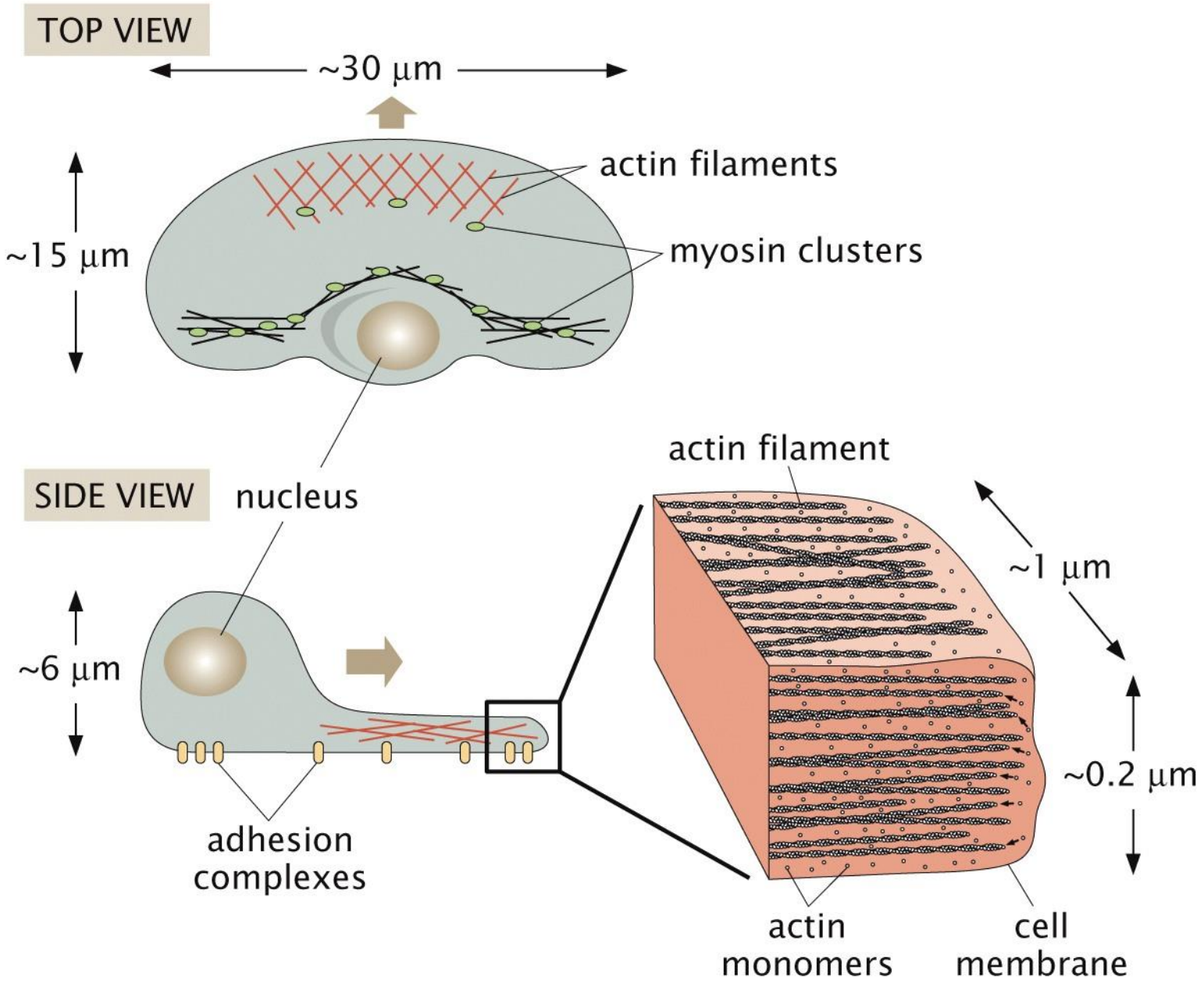
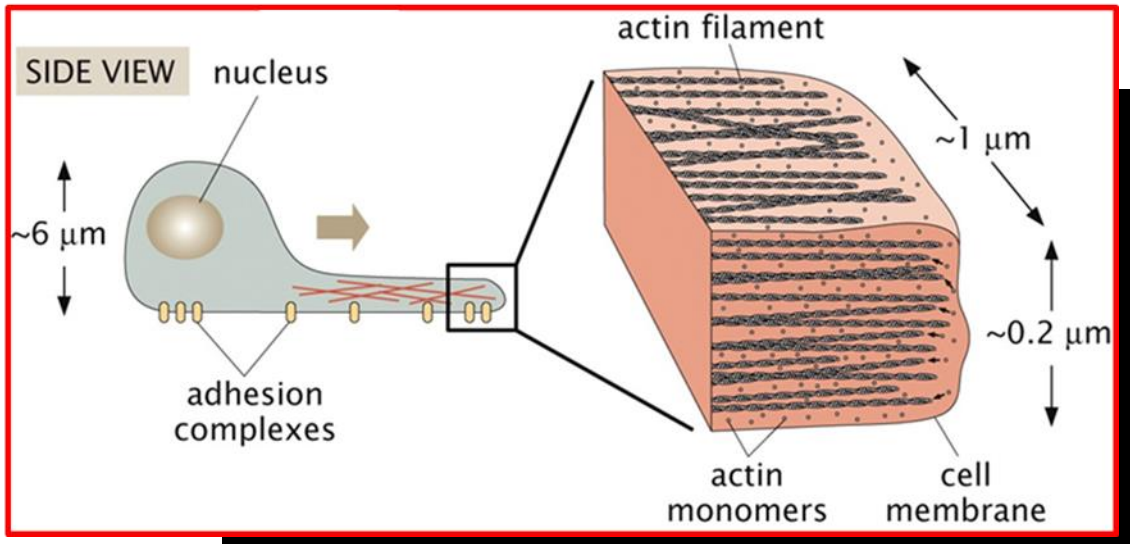
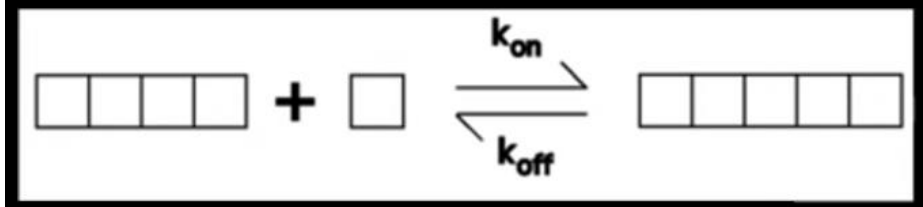


Figure 15.2b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

The Actin network is a Machine. It uses ATP to Generate Force, Do Work …



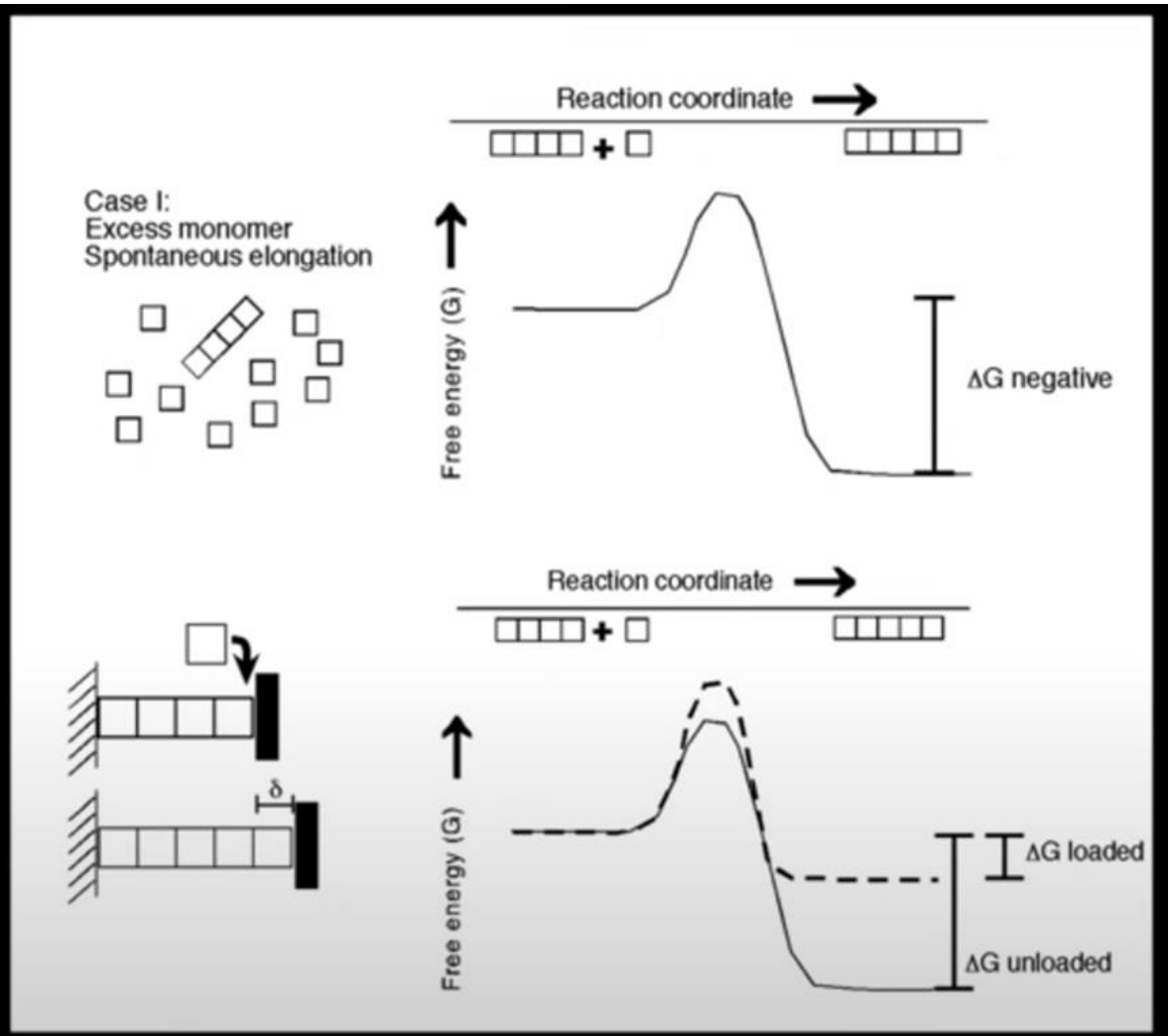
Ibiology Lecture, J. Theriot (Go to 7:30)



$C_{crit} \sim k_{off}/k_{on}$

for actin: $F_{max} \sim 5\text{-}10$ pN

(comparable to myosin or kinesin)

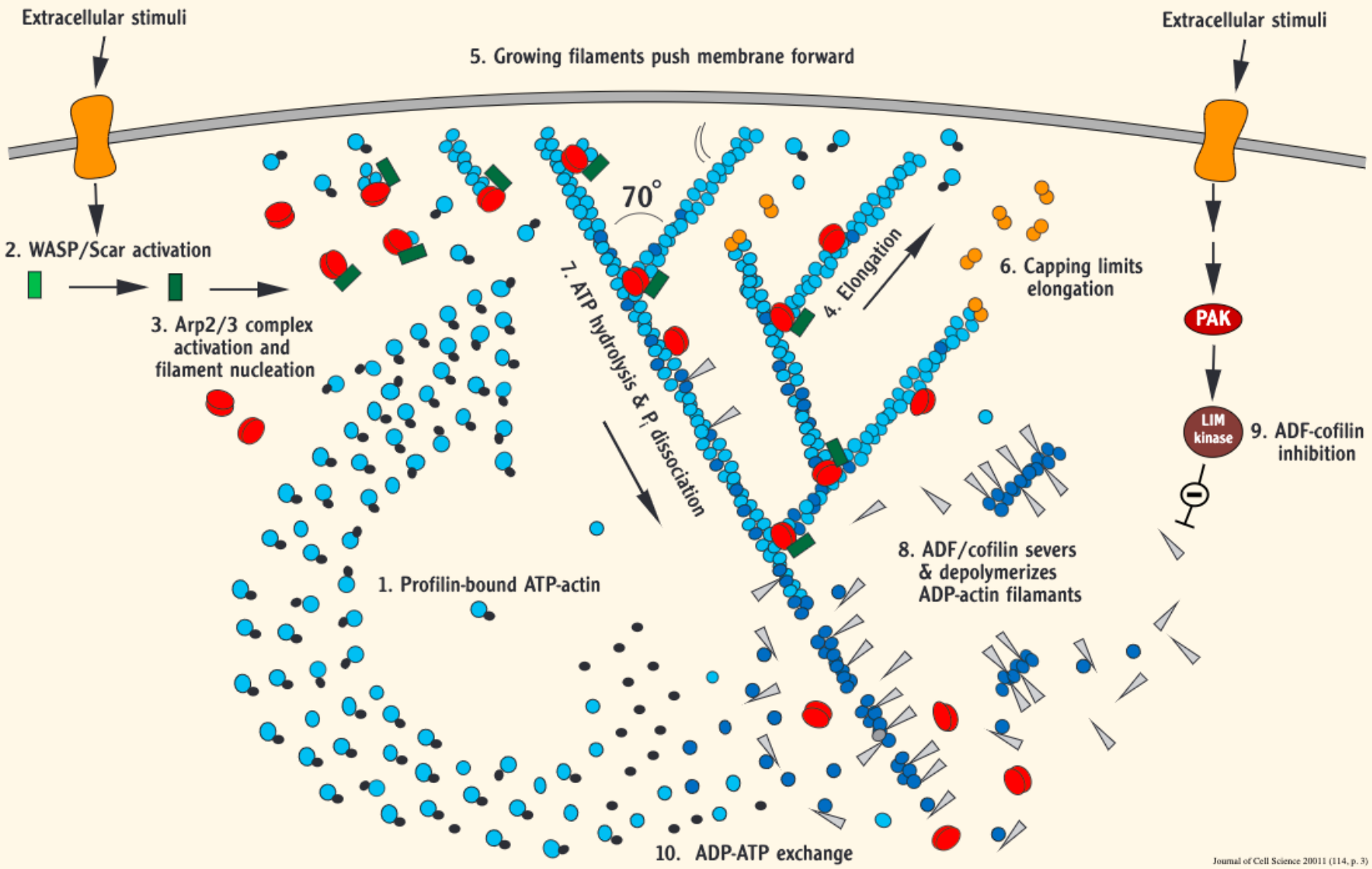


Adapted from Hill & Kirschner, 1982, *Int. Rev. Cytol.* 78: 1-125

Journal of Cell Science

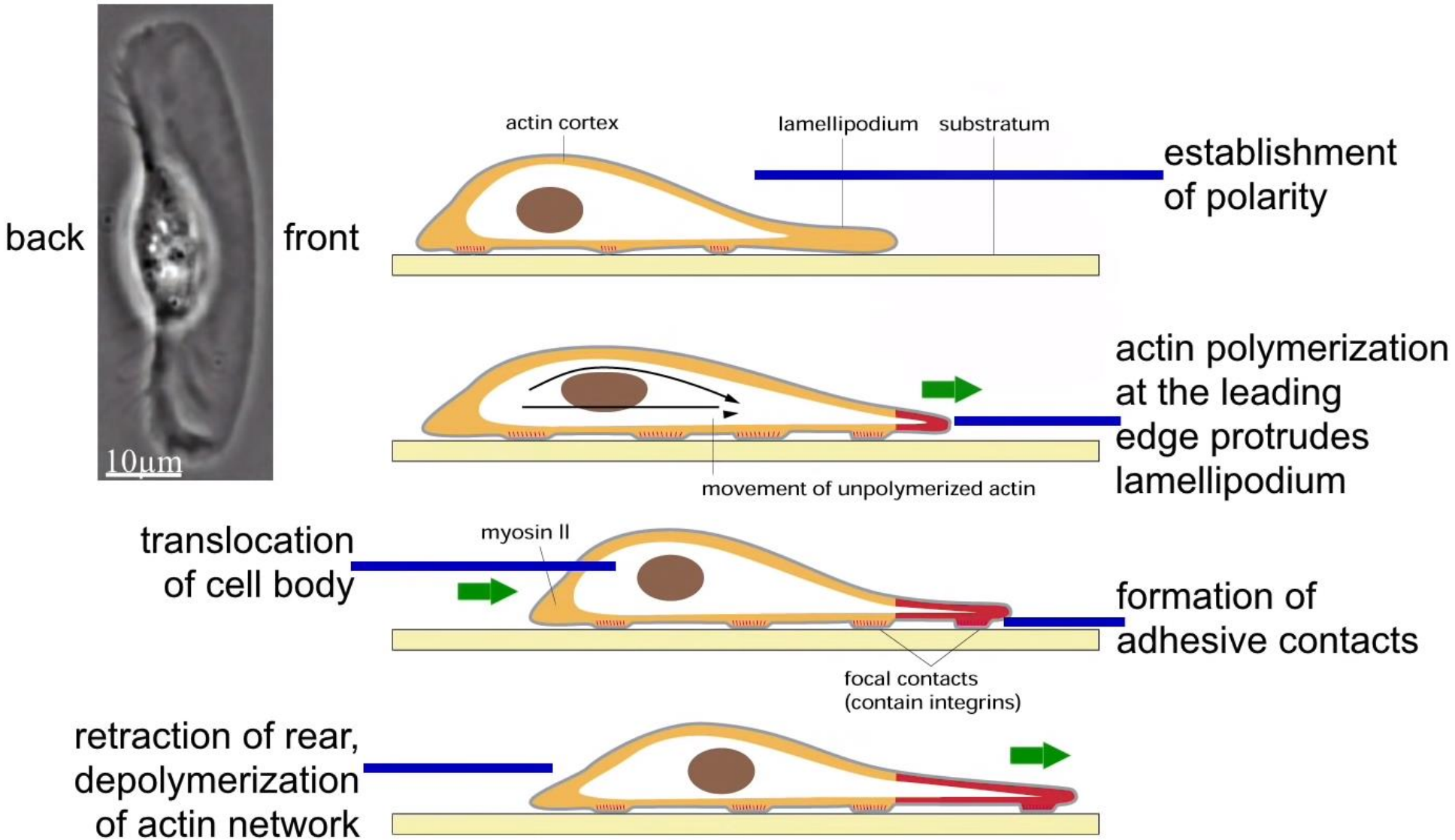
Actin Dynamics

Thomas D. Pollard, Laurent Blanchoin and R. Dyche Mullins

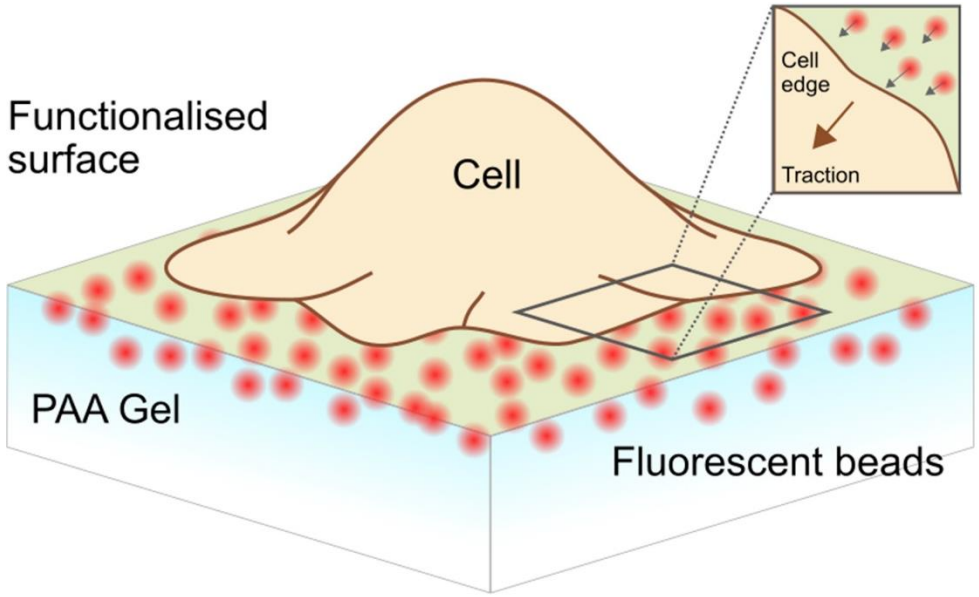


7:10 Julie Theriot Lecture

Cycles of Actin based Cell motility

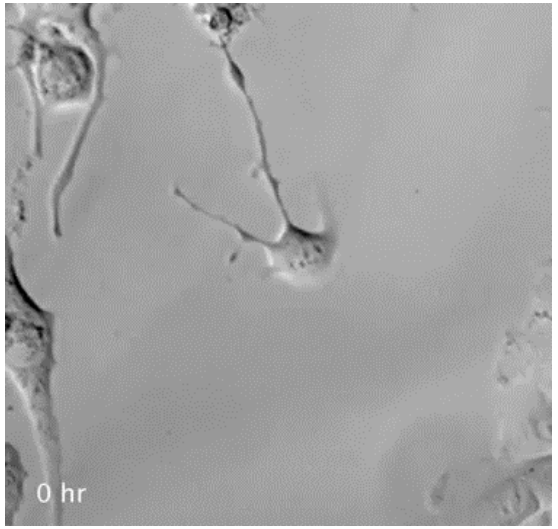


Traction force microscopy, York et al



Traction Force Microscopy of Neurons

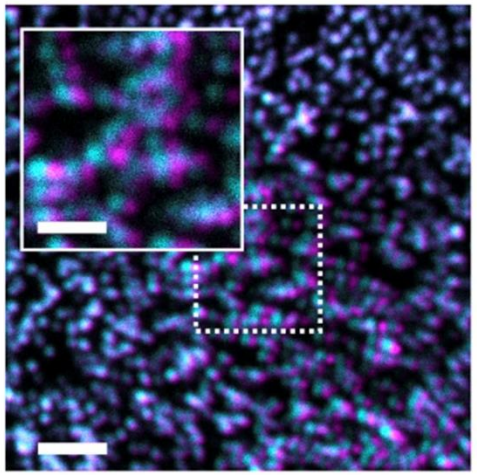
Aurnab Ghose
IISER Pune

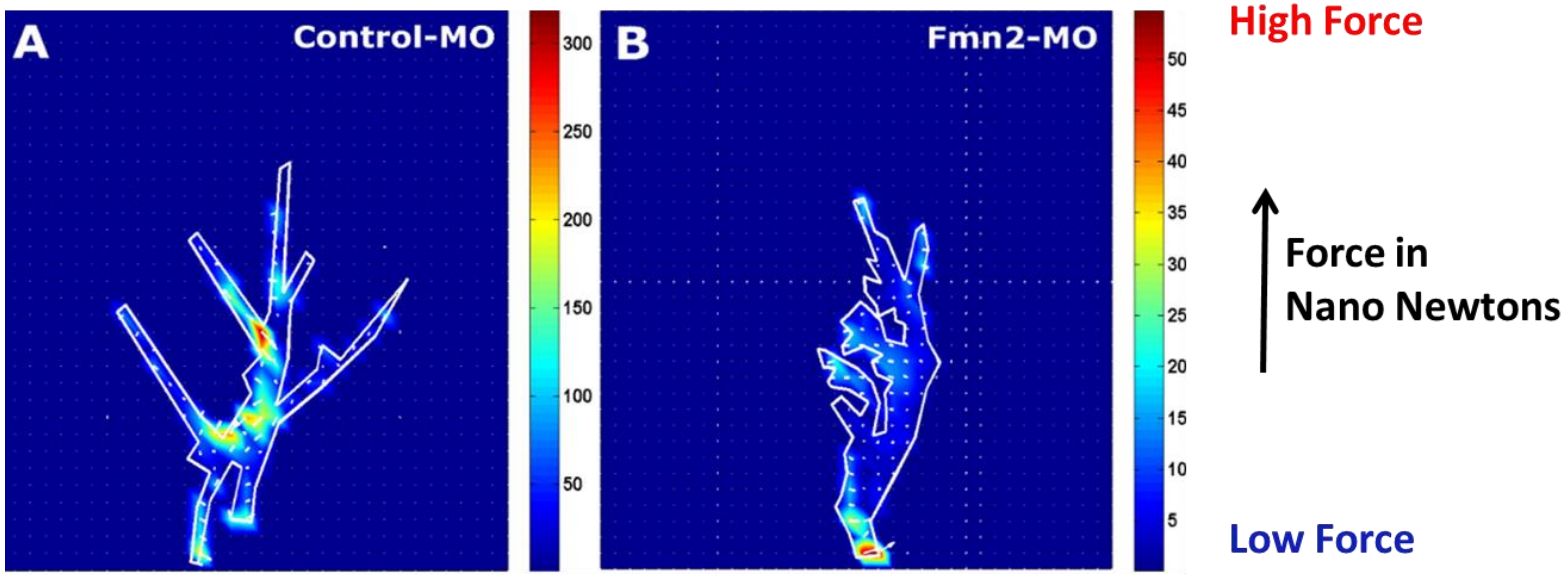


Formin – Protein that promotes elongation of actin filaments

Mutation or reduction in Formin :-
Cognitive dysfunction,
Alzheimer's disease

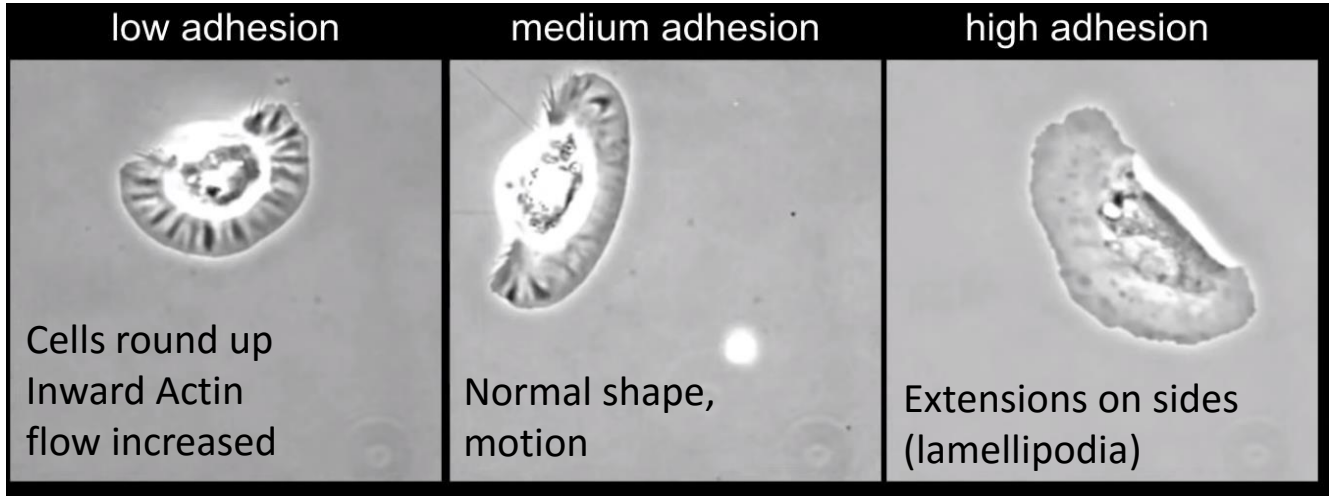
Bead displacement
pre/post force application





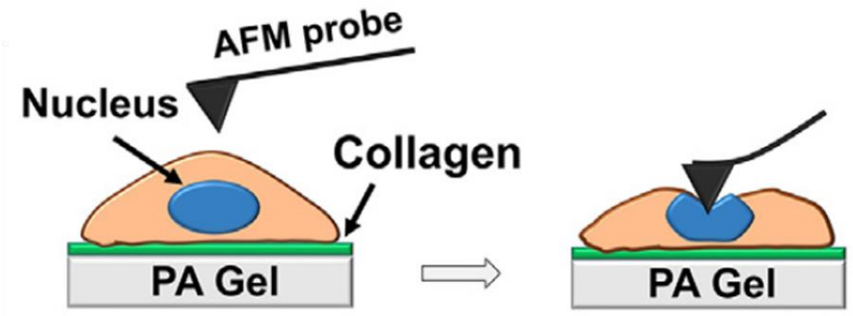
13:26 Julie Theriot Lecture

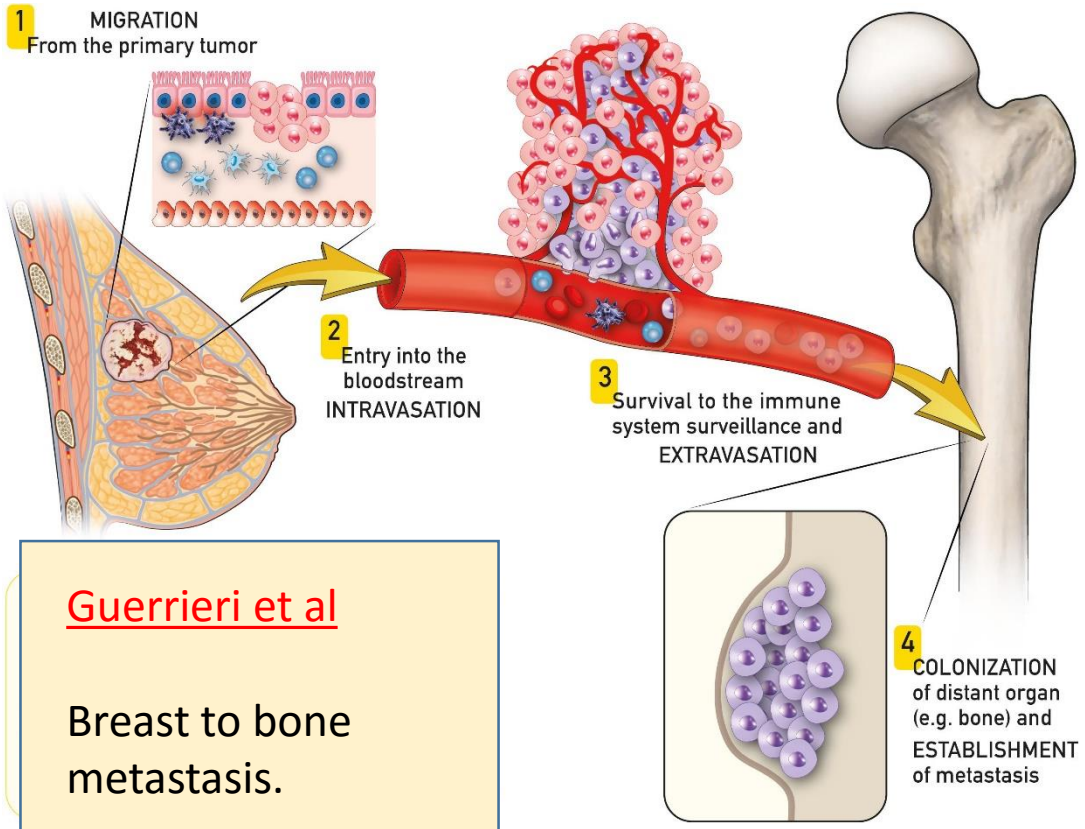
Adhesion strength affects how cells move



WHY THIS IS IMPORTANT – CANCER METASTASIS

Shamik Sen's group at IIT Bombay :-
Cancer Cells soften their nuclei to squeeze through



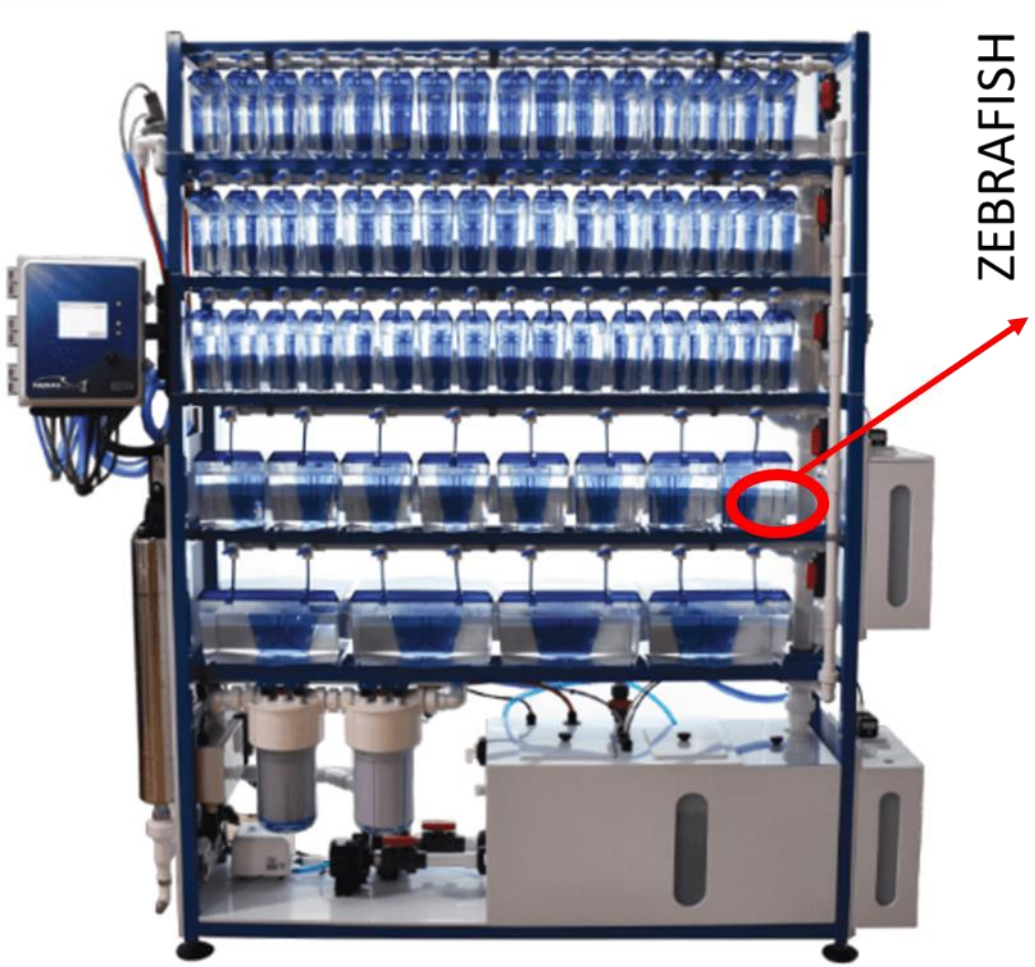


Guerrieri et al

Breast to bone metastasis.

But, studying Cell migration on a Coverslip is not enough

How do cells migrate inside a living animal ??

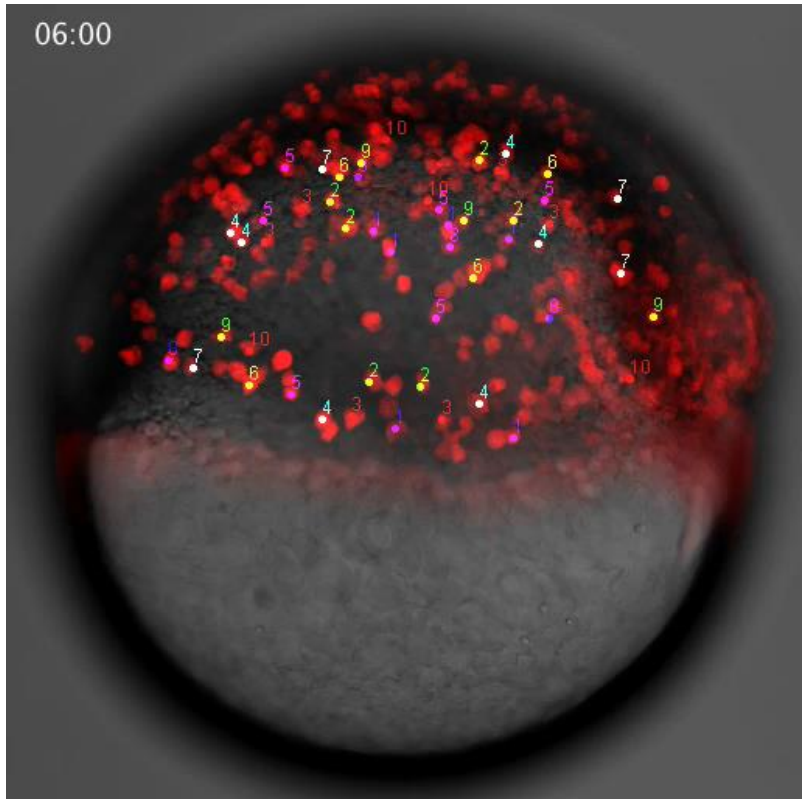


Zebrafish development from the 2 cell stage, for 24 hours, images taken every 2 minutes

HOW DO CELLS MOVE INSIDE AN EMBRYO AS IT IS DEVELOPING? "… an optimal range of developmental stage-specific cell sizes appears necessary for collective cell migration to correctly position cells in space and time to shape an amorphous ball of blastoderm into an embryo…"

Sreelaja Nair.
Biosciences &
Bioengineering.
IIT Bombay

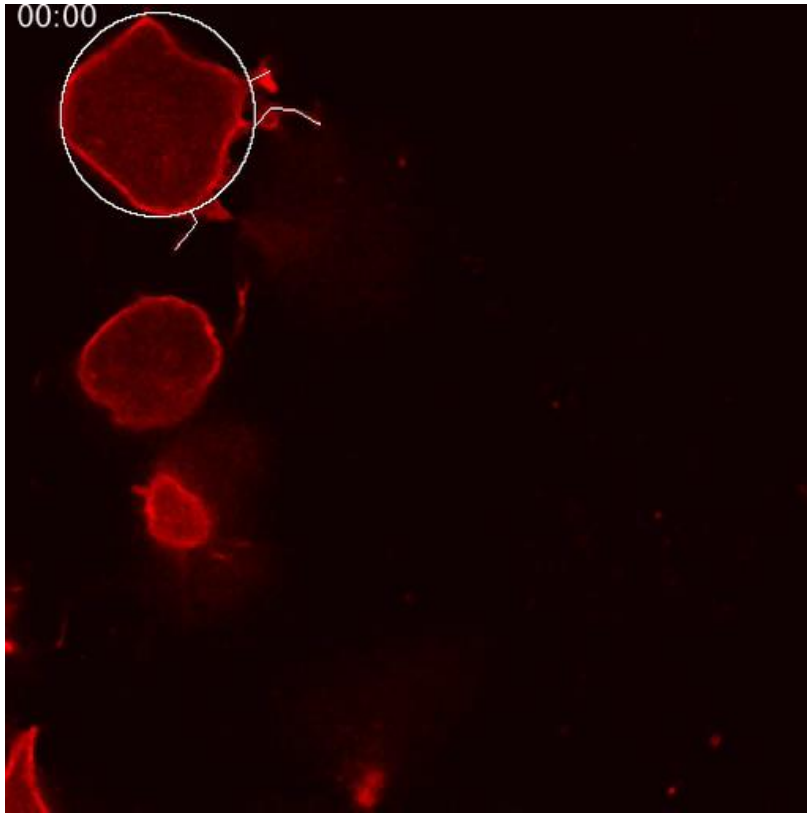
Triveni Menon

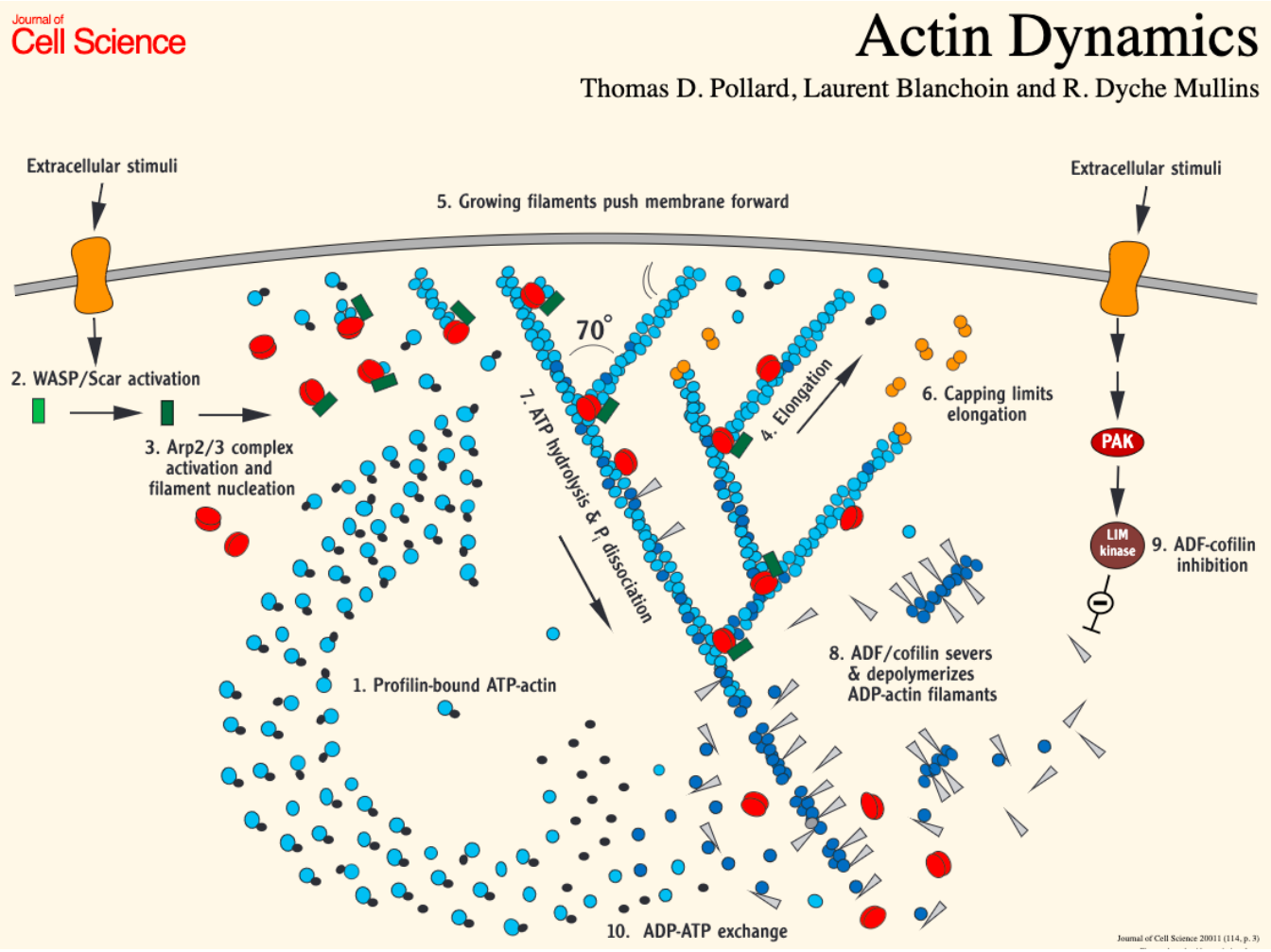


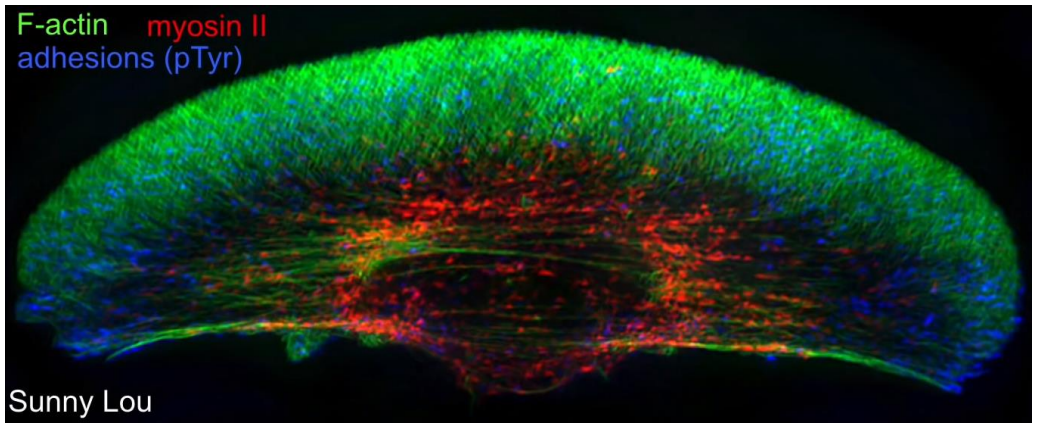
Link
Menon et al, 2019

← Movie 7

Movie 12 →







Conclusion

How do you move if you are inside a Bag.

Cycles of Actin Polymerization and Depolymerization allow Cells to extend their front and detach from the rear

These Cycles are controlled by the Myosin motor and many other proteins that attach to Actin

This cycle can be quite different in different kinds of cells, depending on their function

Strongly recommended

[ibiology Lecture by Julie Theriot]

1:19 Actin as a Nanomachine
3:48 Keratinocytes from Fish Scales
6:26 Fluorescent labelling of Actin and Myosin
7:10 Cycles of Actin based Cell motility
13:26 Adhesion strength affects how cells move
18:48 Myosin chops up actin at rear poles
33:16 Neutrophil turning & Myosin asymmetry