Molecular basis of actin dynamics in cell migration

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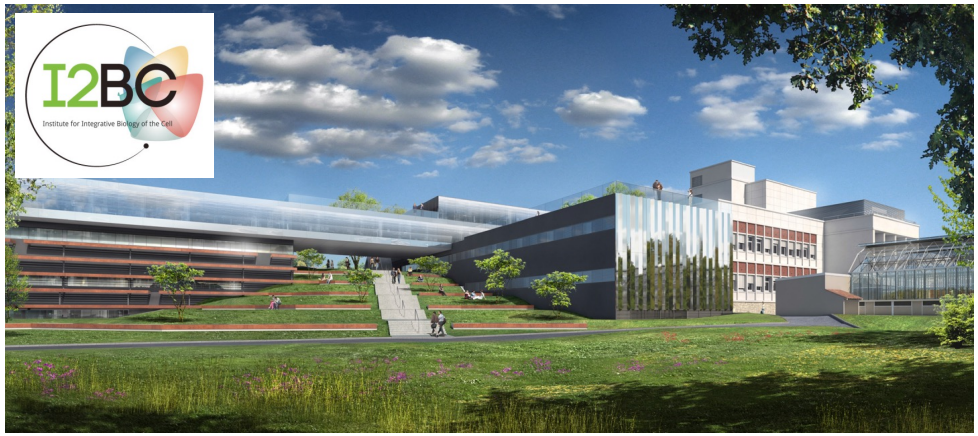

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    1.2. Cells from the immune system cross the endothelium to eliminate bacteria during wound healing
    1.3. Cancer cells migrate in a collective manner to invade tissues

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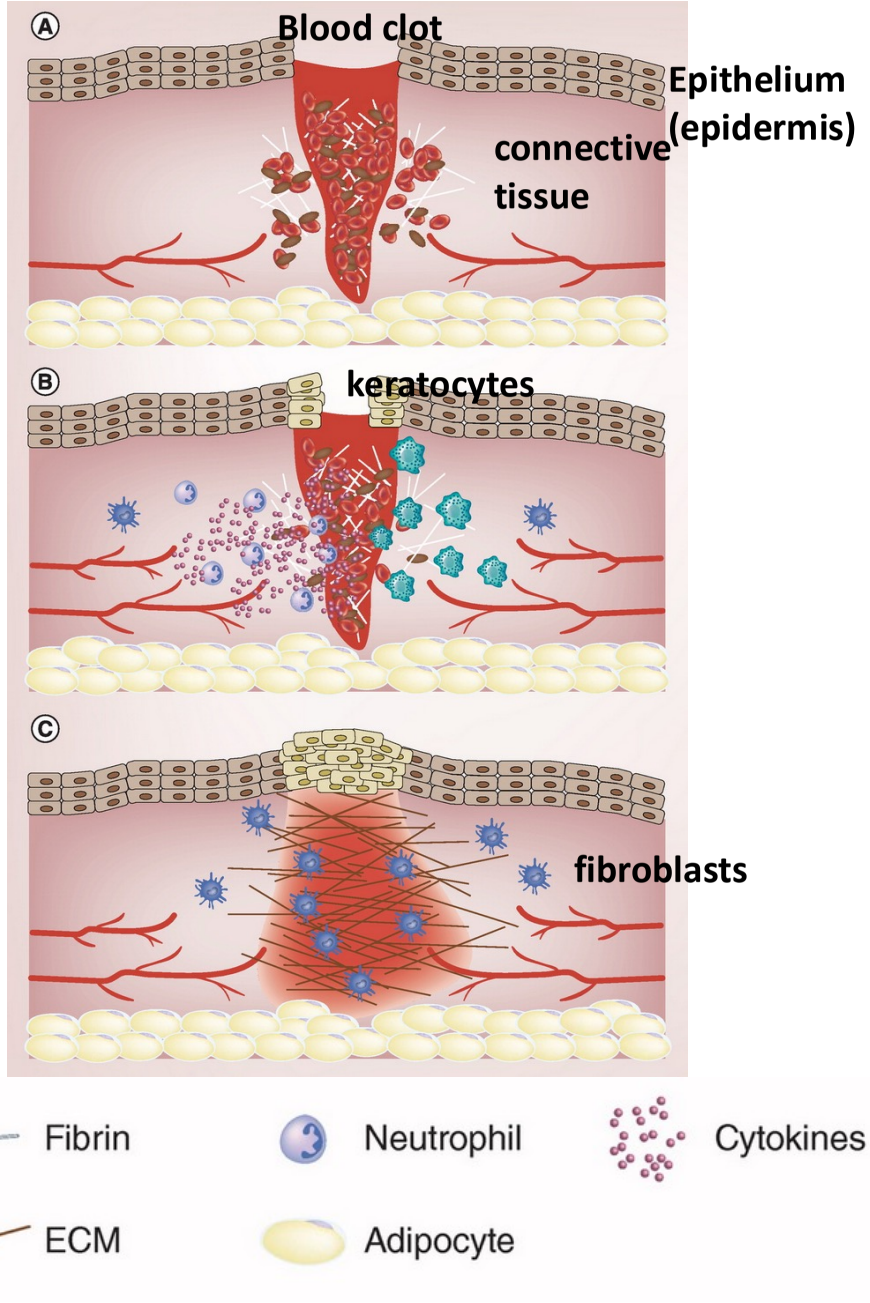
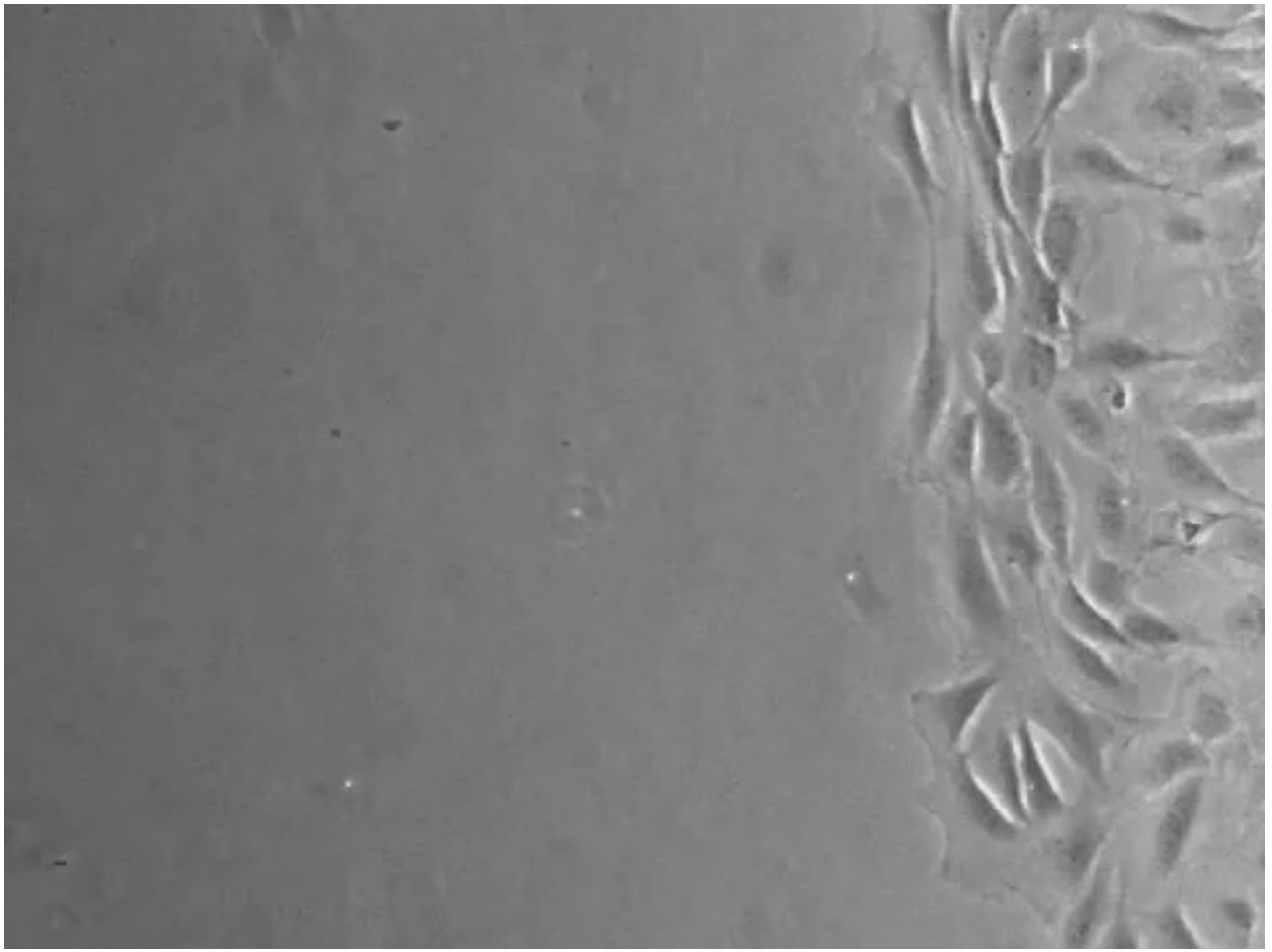
1. Why do cells migrate ?

1.1. Wound healing involves fibroblast migration

(A) During the first stages of wound healing, platelets are recruited to the open wound and deposit fibrin (which serves as a preliminary extracellular matrix) to arrest bleeding. **(B)** During the next stages of wound healing, immune cells including neutrophils followed by macrophages are recruited to the wound and clear dead tissue and debris in preparation for healing. New blood vessels sprout around the site. Fibroblasts are recruited to the site in anticipation of scar formation. Keratinocytes begin to migrate to cover the cutaneous wound surface. **(C)** Finally, during the remodeling phases of wound healing, the keratinocytes have covered the site. Below the fibroblasts deposit new extracellular matrix replacing the fibrin plug, which is then remodeled to form the final scar. New blood vessels are pruned and nerves begin to regenerate to the site.

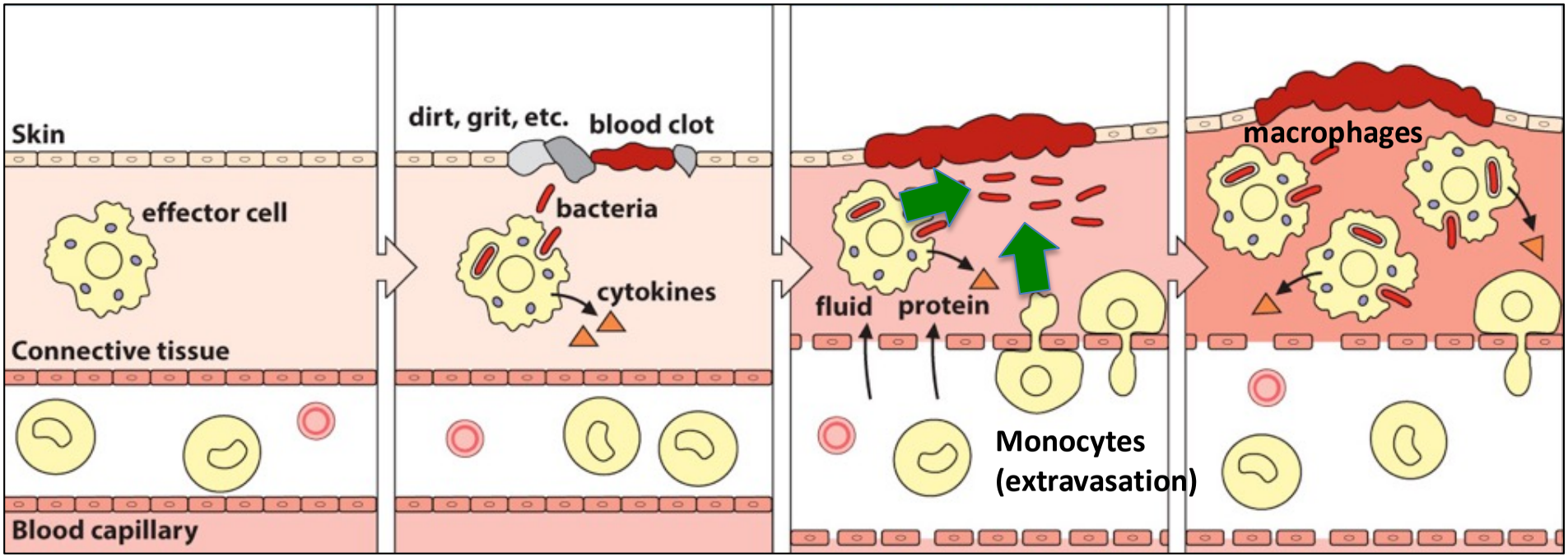


1.1. Wound healing involves fibroblast migration



Migration of fibroblasts in vitro in response to an artificial wound

1.2. Cells from the immune system cross the endothelium to eliminate bacteria during wound healing



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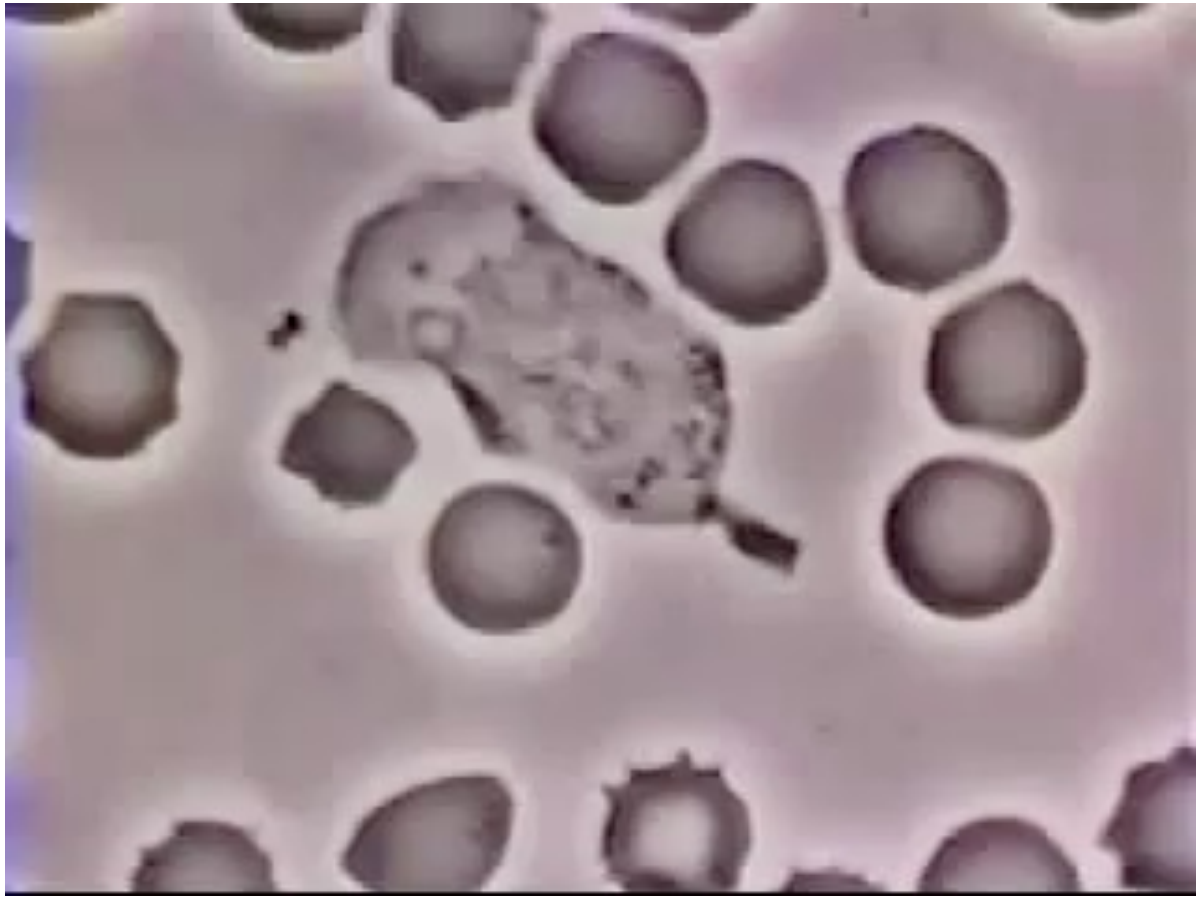
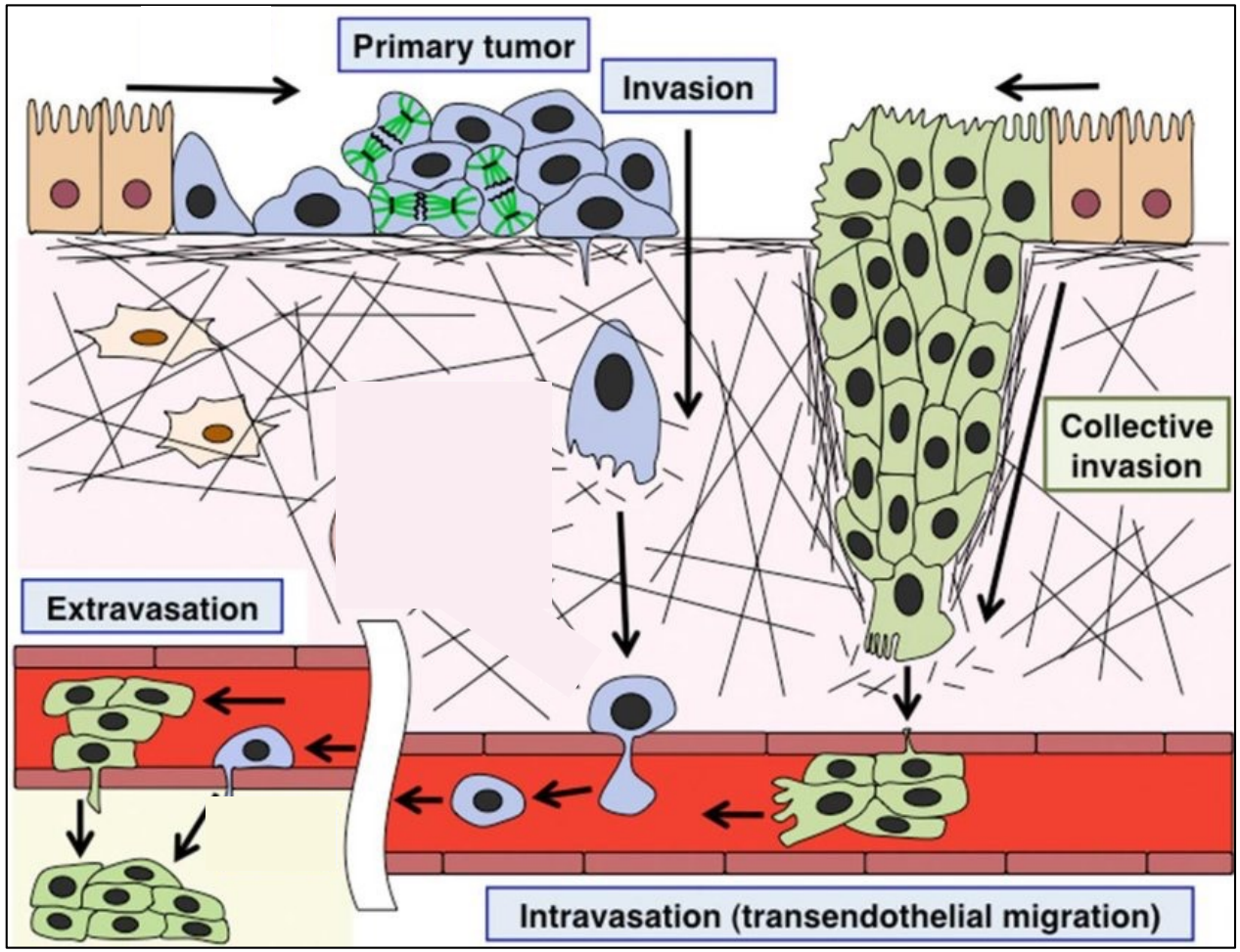


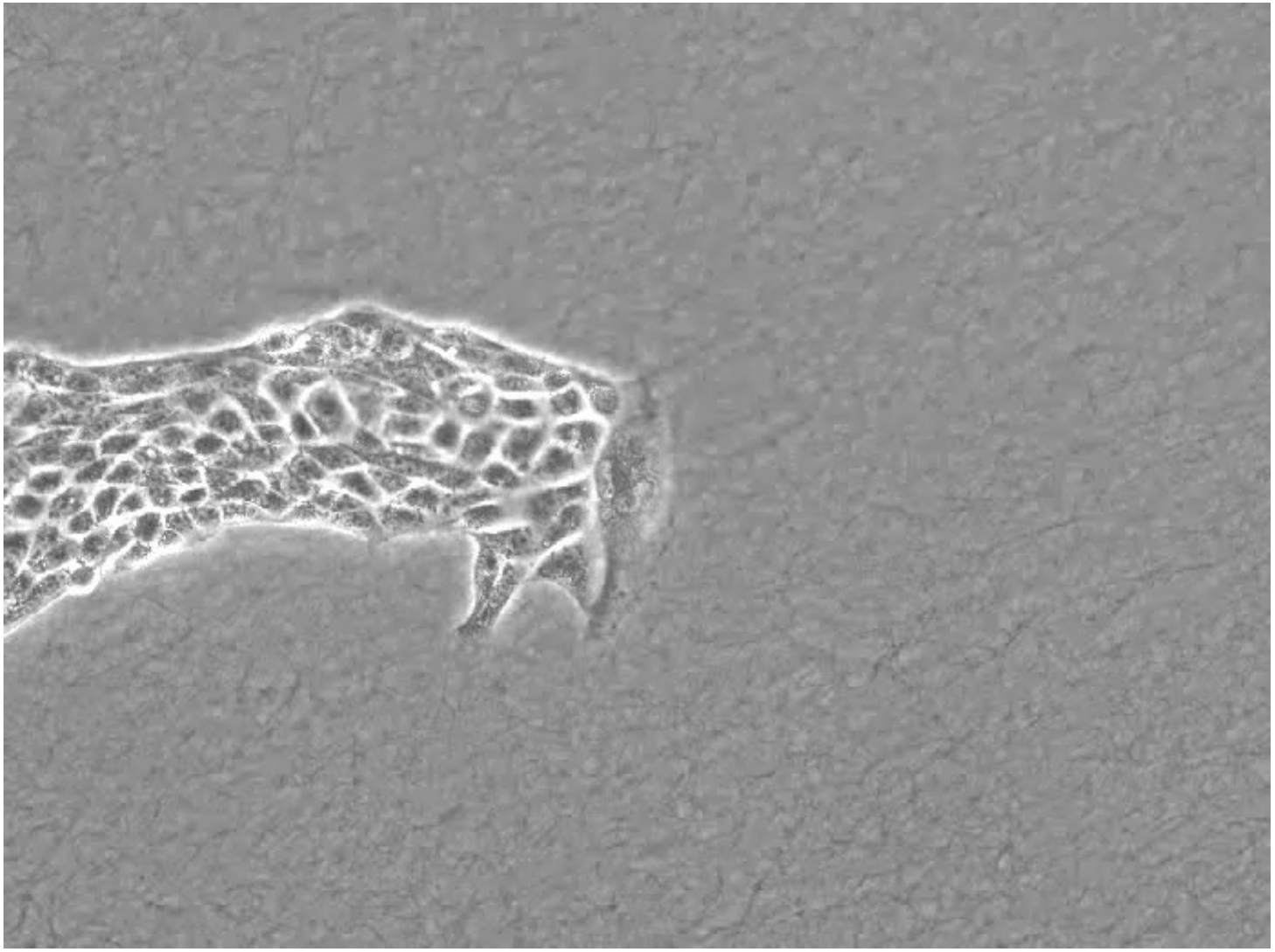
Migration of a macrophage in vitro

1.3. Cancer cells migrate in a collective manner to invade tissues



Metastasis

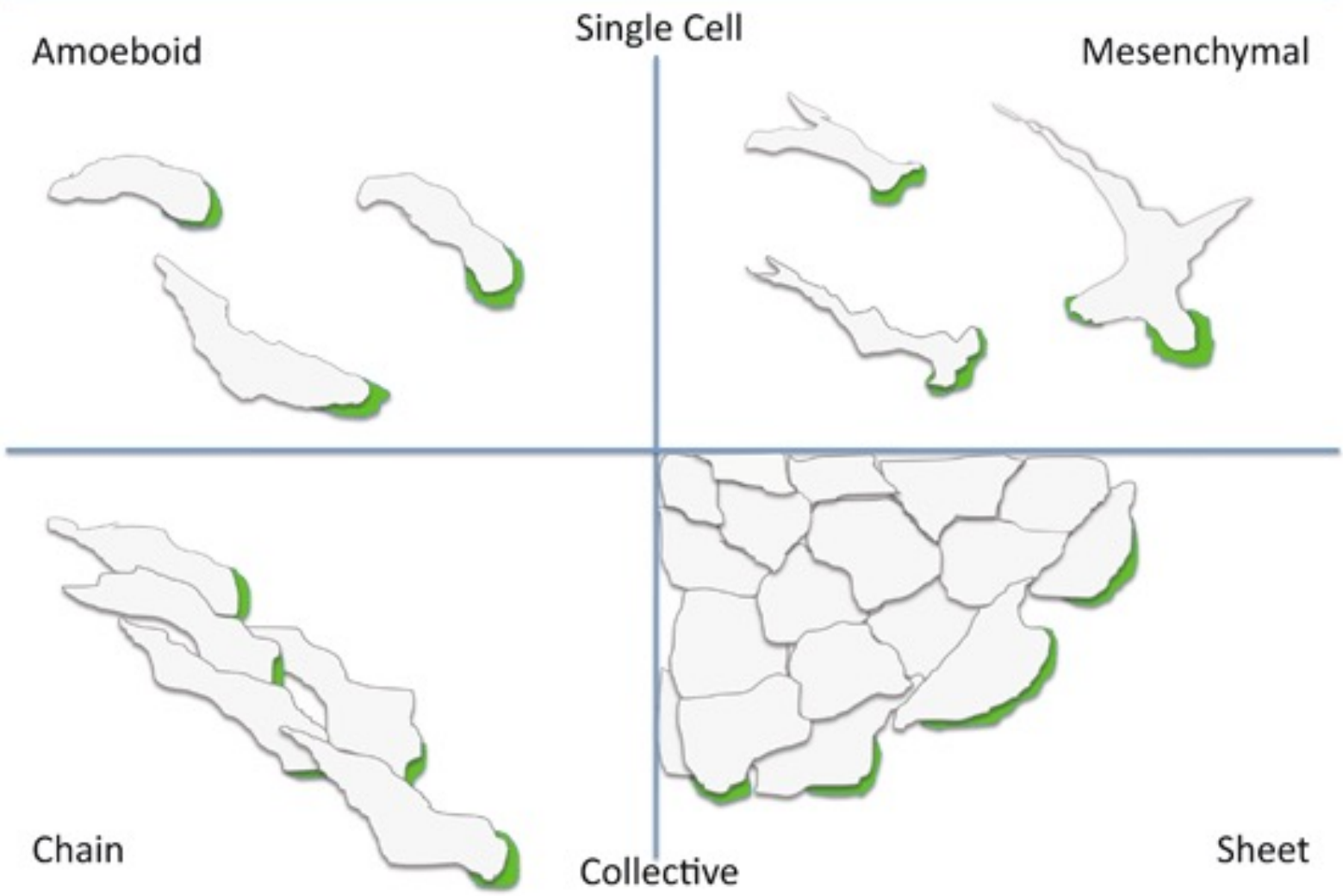
1.2. Cancer cells migrate in a collective manner to invade tissues



MDCK cells moving in a collective manner

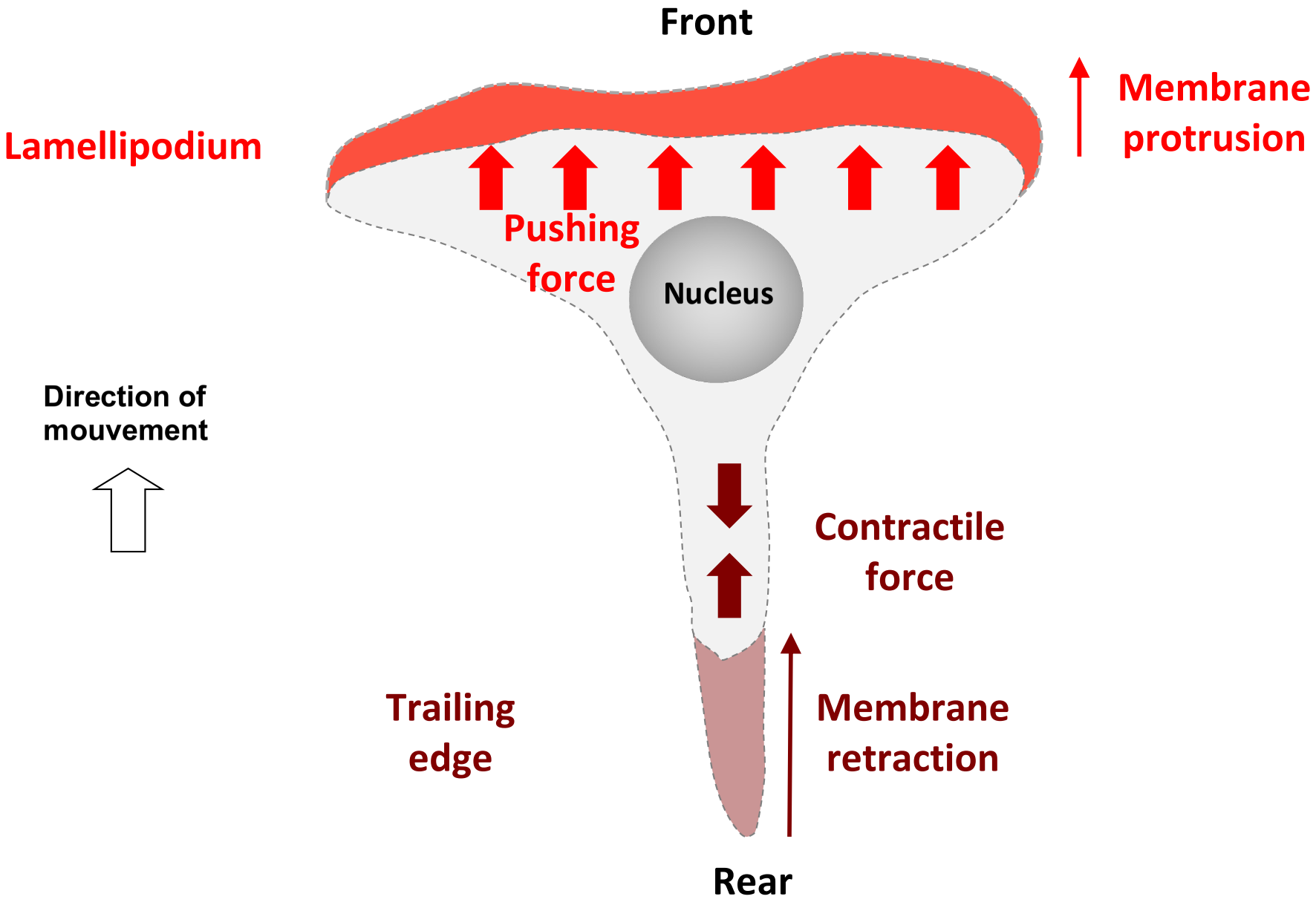
2. Anatomy of migrating cells

2.1. Modes of cell migration

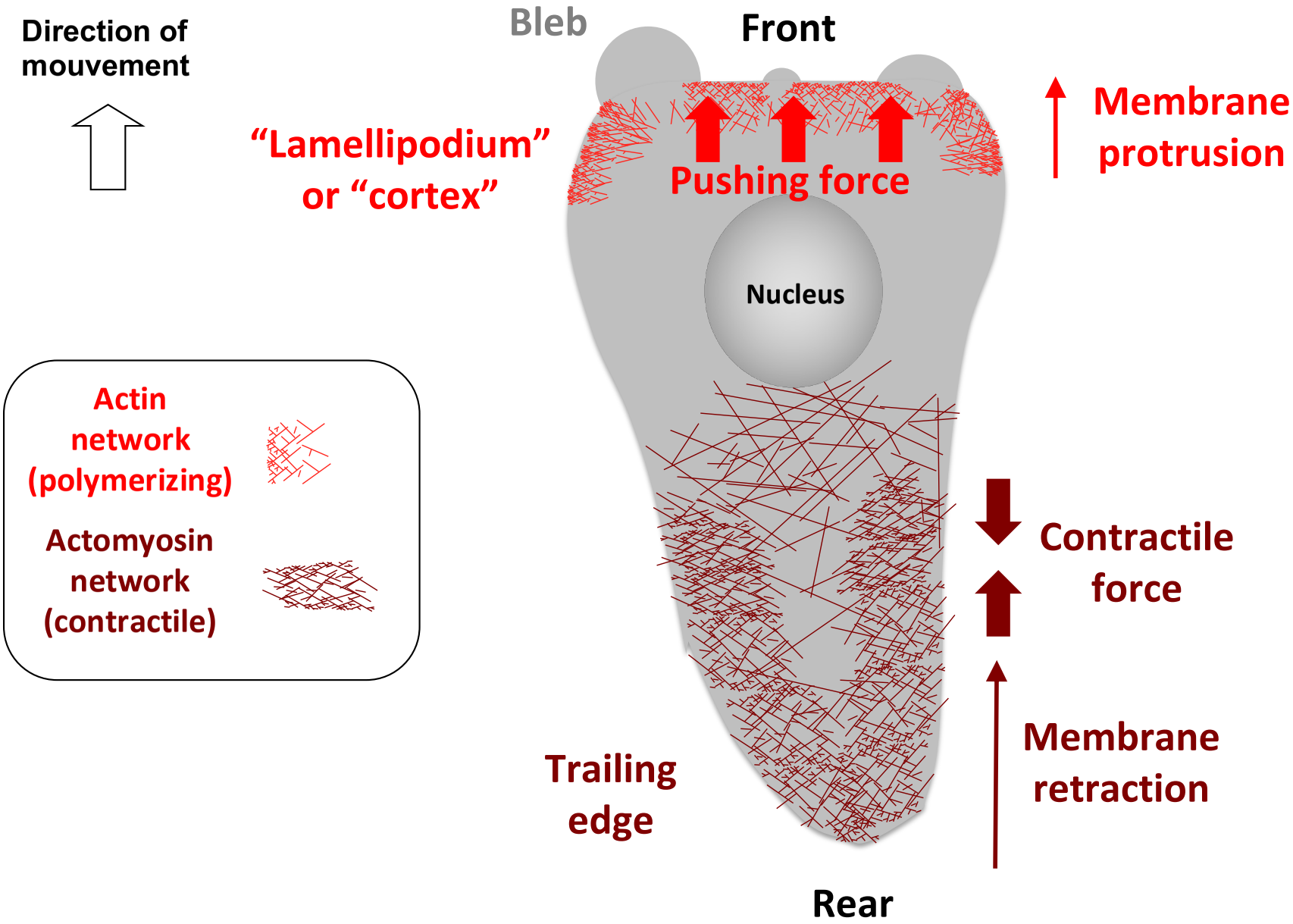


The migrating front is in green

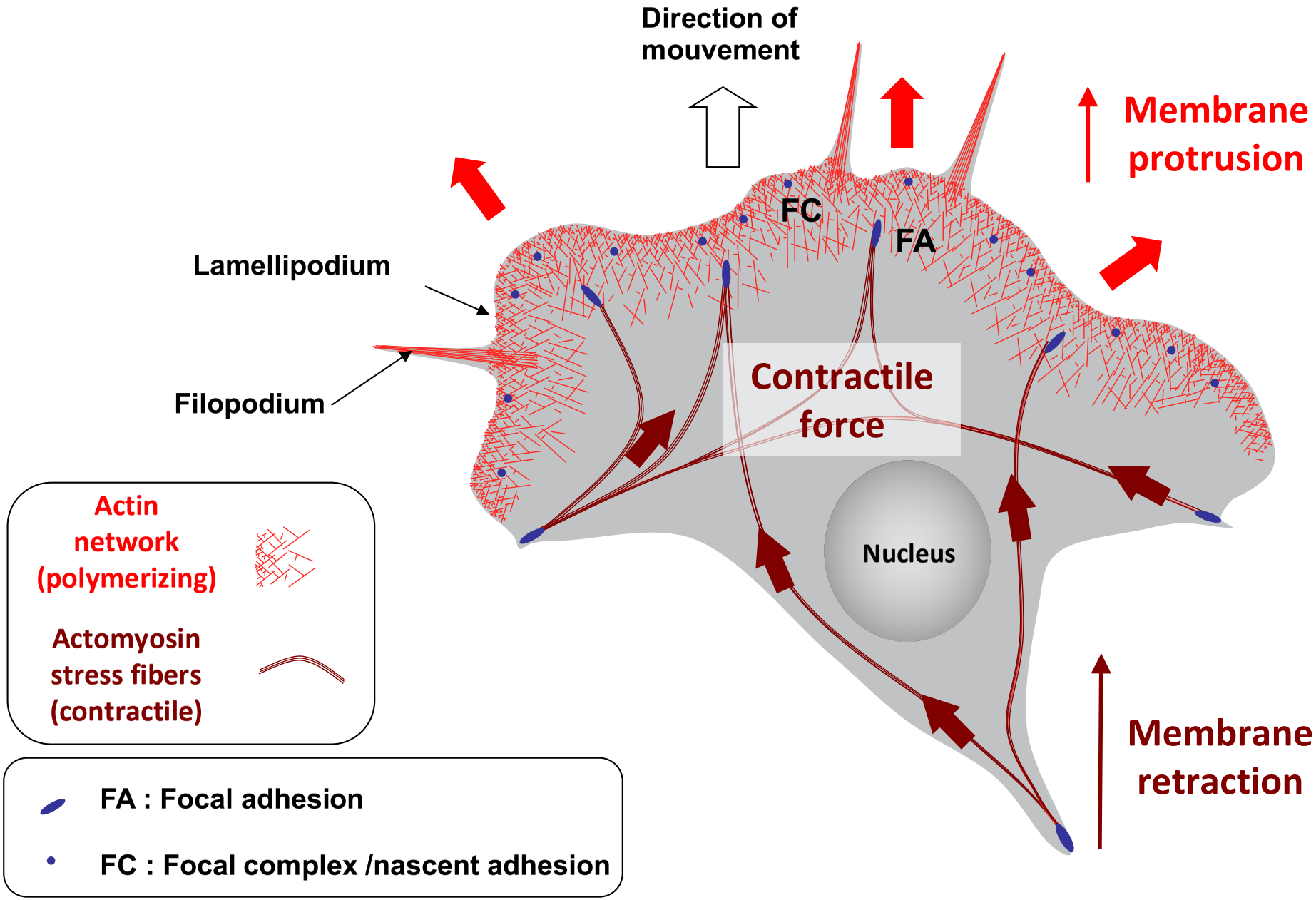
2.2. Common features in migrating cells: intracellular forces drive membrane protrusion at the front and membrane retraction at the rear



2.3. Amoeboid cells



2.4. Mesenchymal cells

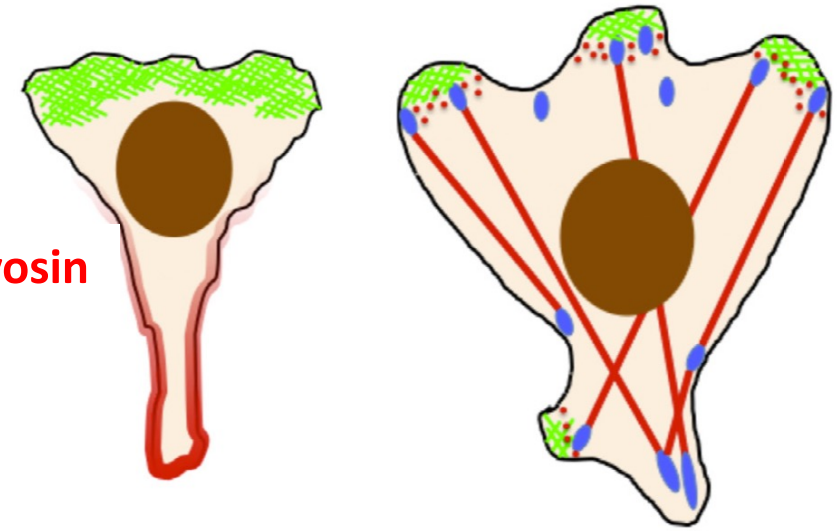


2.5. To remember: comparison between amoeboid and mesenchymal cell migration

Polymerizing actin

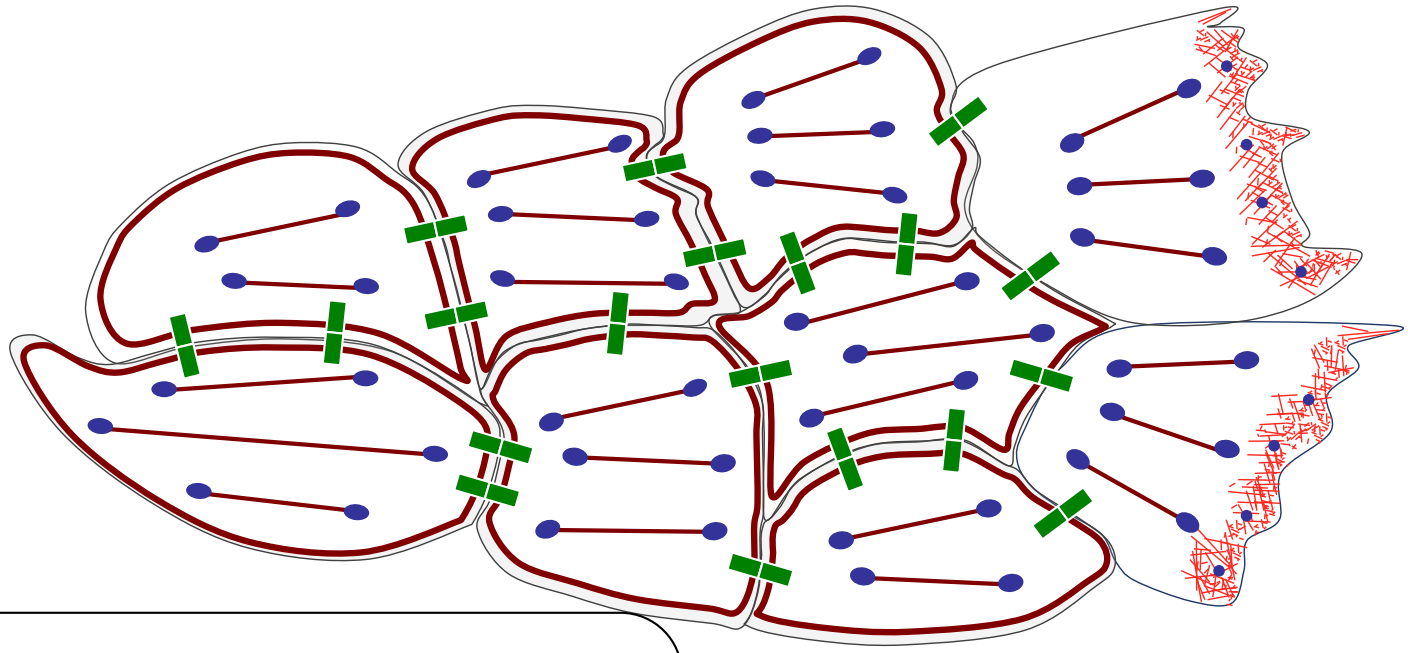
Contractile Actomyosin

Focal adhesions



	Amoeboid	Mesenchymal
Migration speed	Fast, ~ 10 μm/min	Slow, < 1 μm/min
Polarity	Well-defined front and rear	Multiple, competing lamellipodia
Adhesion	Relatively weak, mostly intercellular	Strong, mostly ECM with well-defined adhesion complexes
Migration mechanics *in vivo*	Sqeezing through pores in matrix/stroma	Traction via adhesion to ECM, matrix degradation as necessary
Organization of action cytoskeleton	Thick dendritic actin network at the cell front; elsewhere, cortical actomyosin mediates contractility beneath the plasma membrane	Dendritic F-actin in lamellipodia; acto-myosin minifilaments mediate contractility behind the leading edge(s) and form thick stress fibers attached to focal adhesions

2.6. Collective migration



Actin network (polymerizing)

Actomyosin cortex (contractile)

Actomyosin stress fibers (contractile)

FA : Focal adhesion

FC : Focal complex /nascent adhesion

Cell-cell adhesion (adherens junctions)

3. Mechanism of force generation in the lamellipodium of migrating cells

3.1. Actin polymerization produces force for movement

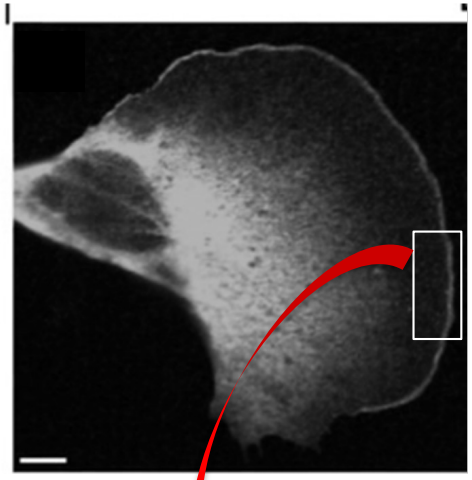
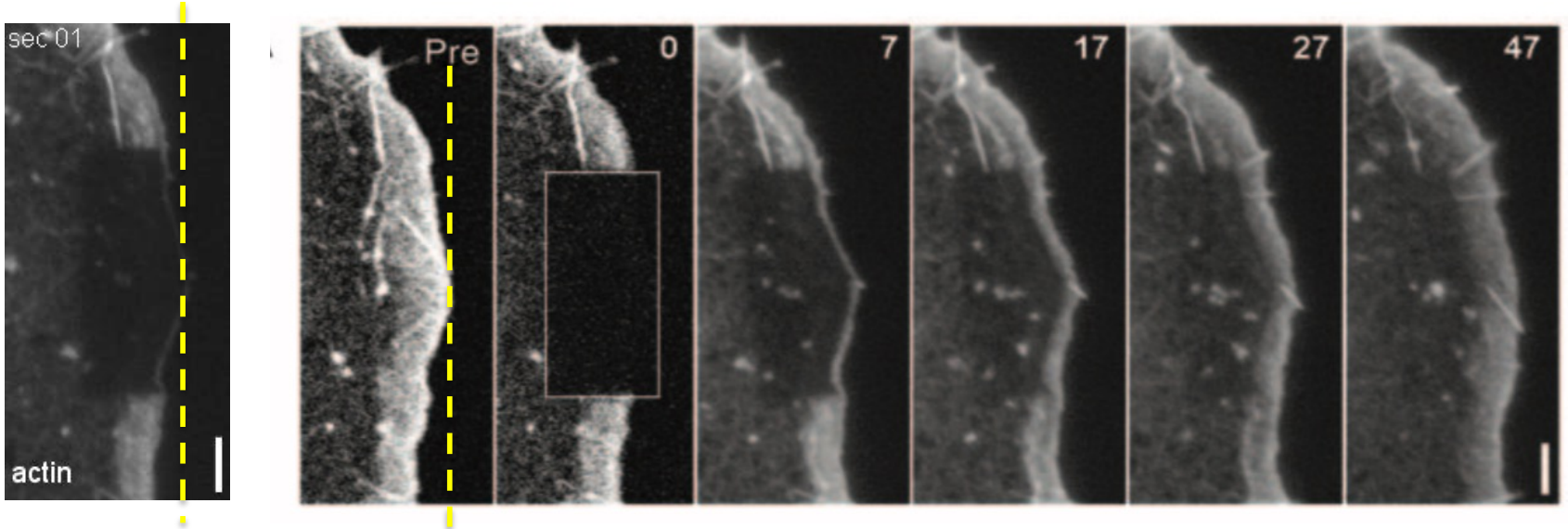
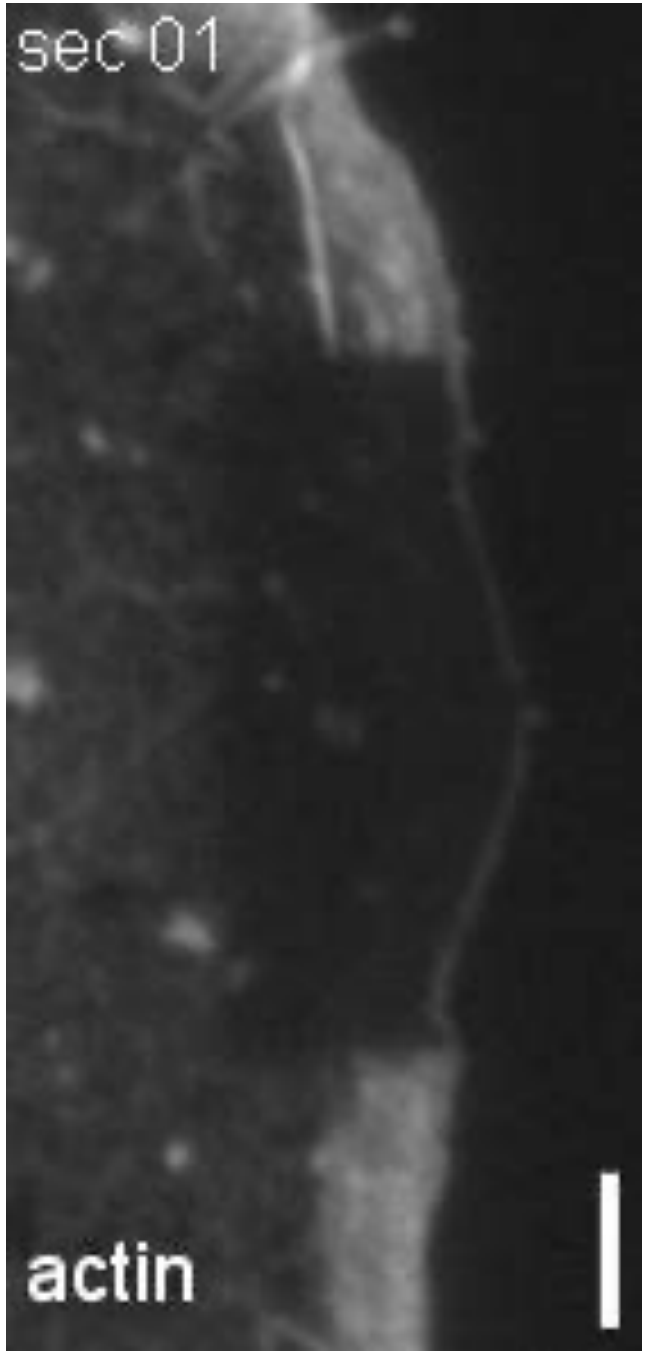
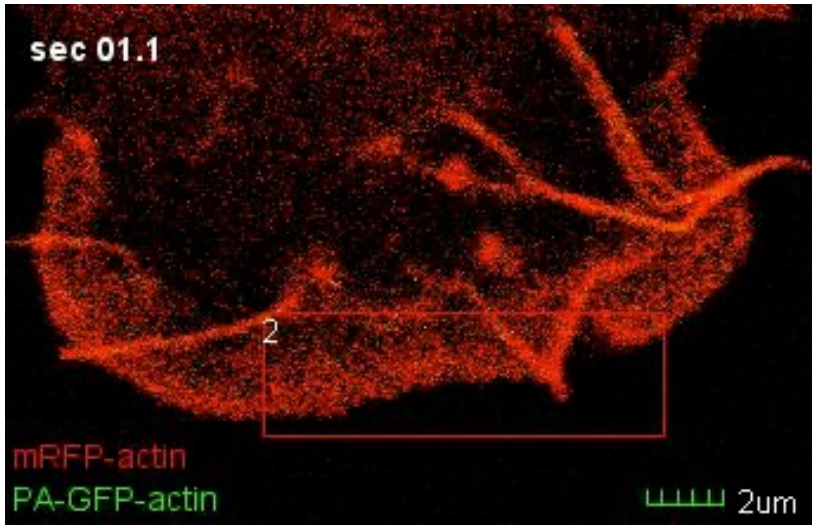
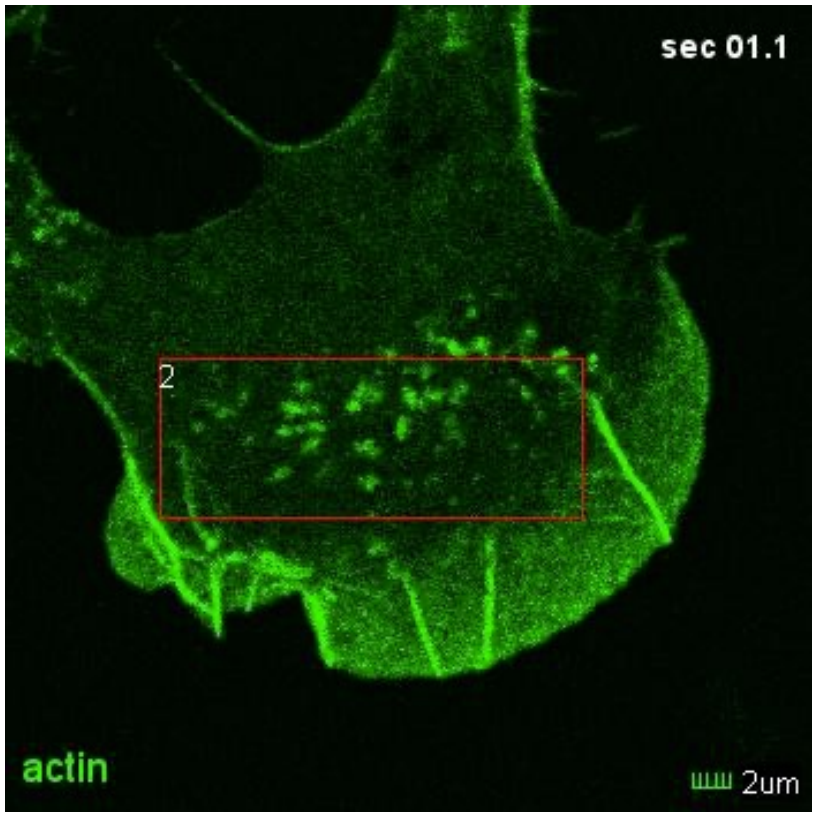


Photo-bleaching of lamellipodial actin in a migrating cell (Wang, 1985, Lai et al, 2008)









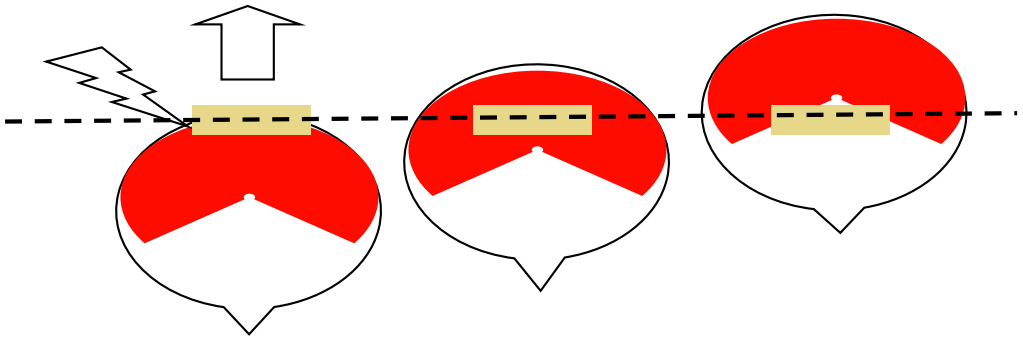
Co expression of
RFP-actin
+
photoactivable
GFP-actin

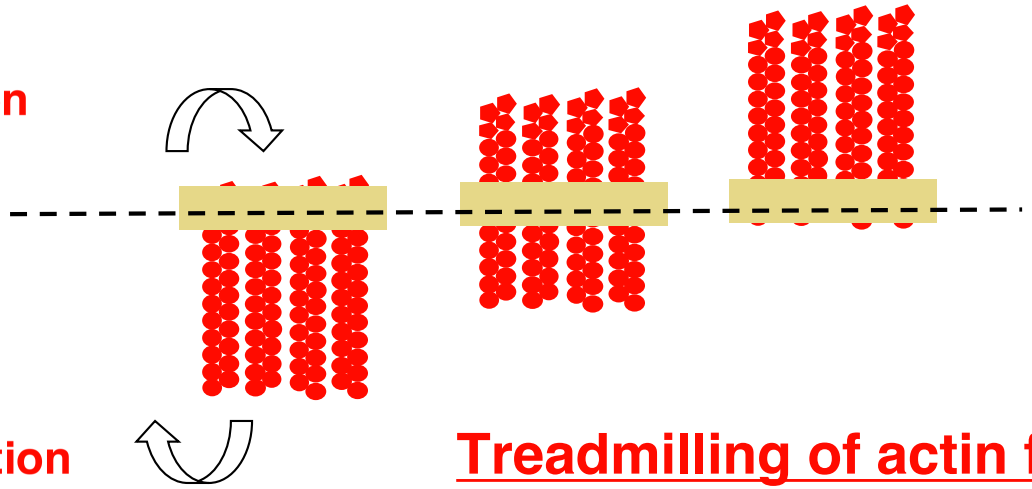
FRAP of the central part of the cell

Lai et al, 2008

3.1. Actin polymerization produces force for movement

Photo-bleaching of lamellipodial actin in a migrating cell (Wang, 1985)



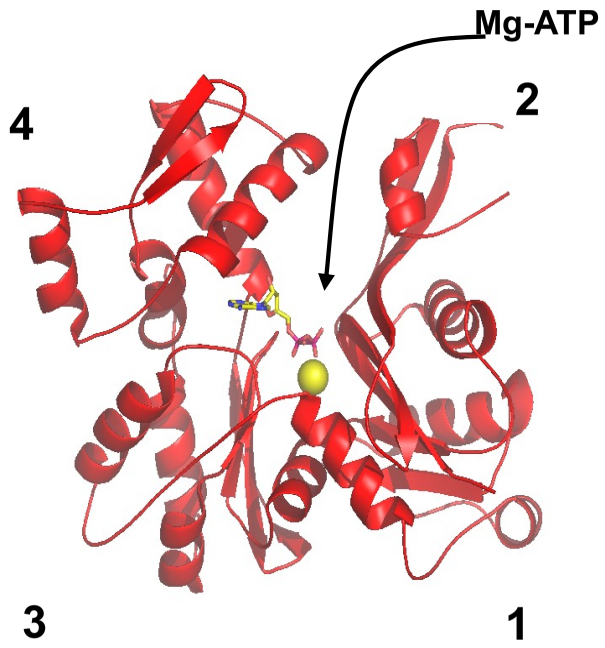


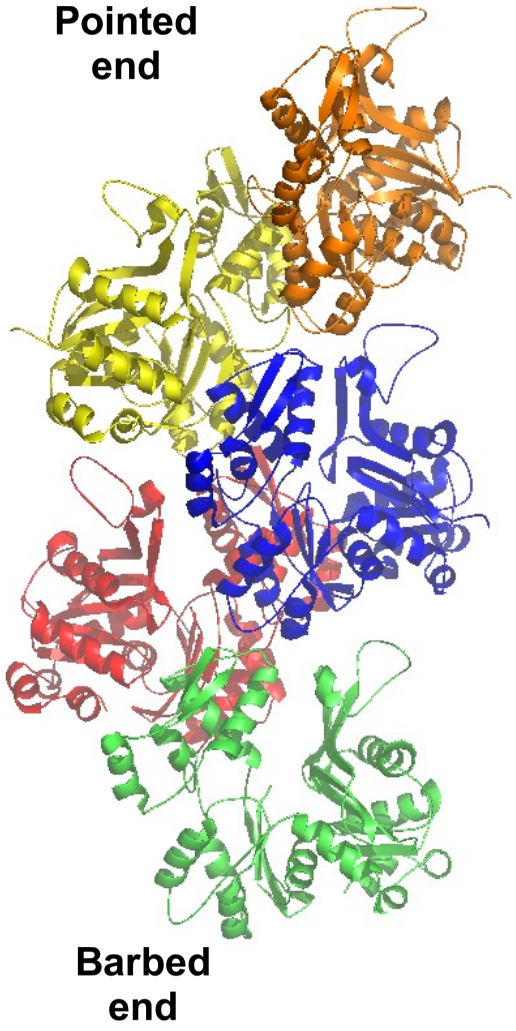
Treadmilling of actin filaments ?

3.2. Molecular basis of actin polymerization

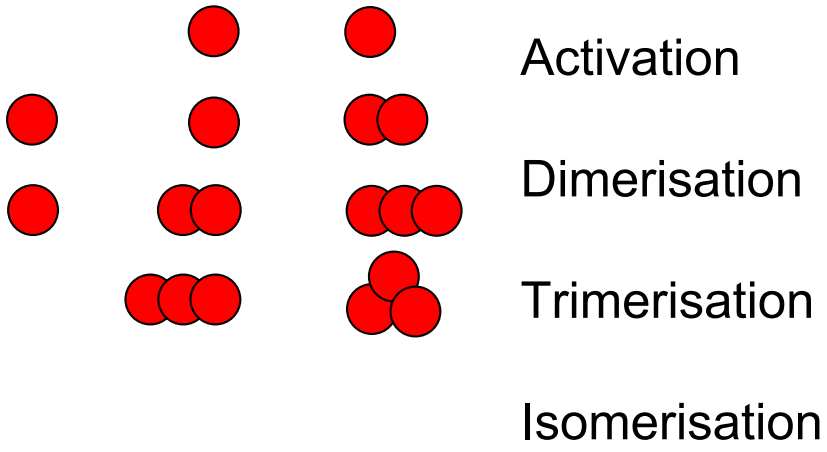
3.2.1. Actin

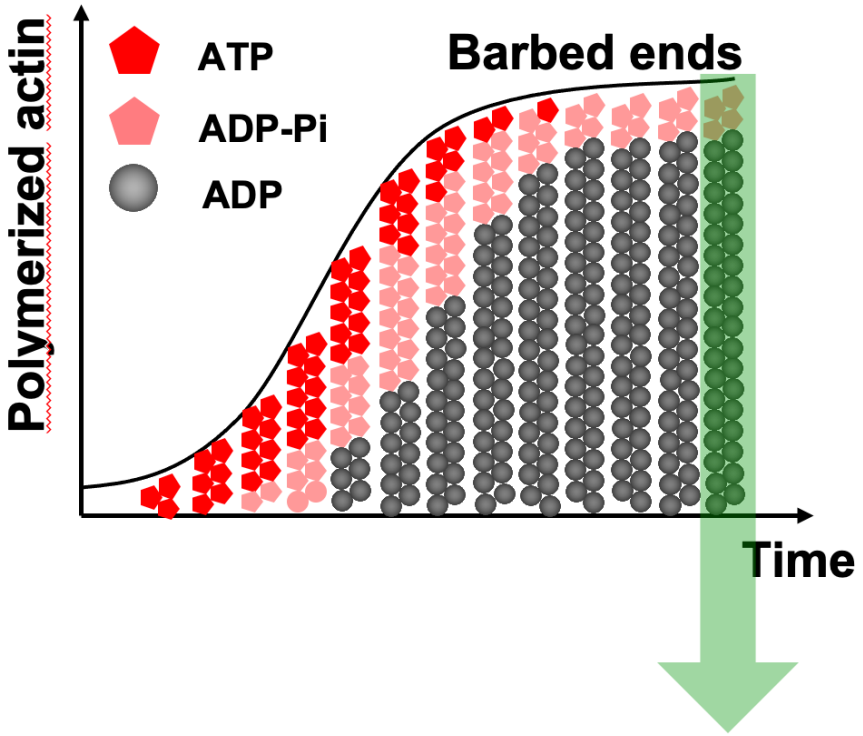
1. Actin is a conserved eukaryotic globular protein of 42 kDa
2. Actin polymerises into polarised helical filaments
3. Actin binds 1 molecule of ATP





3.2.2. Actin nucleation and polymerization

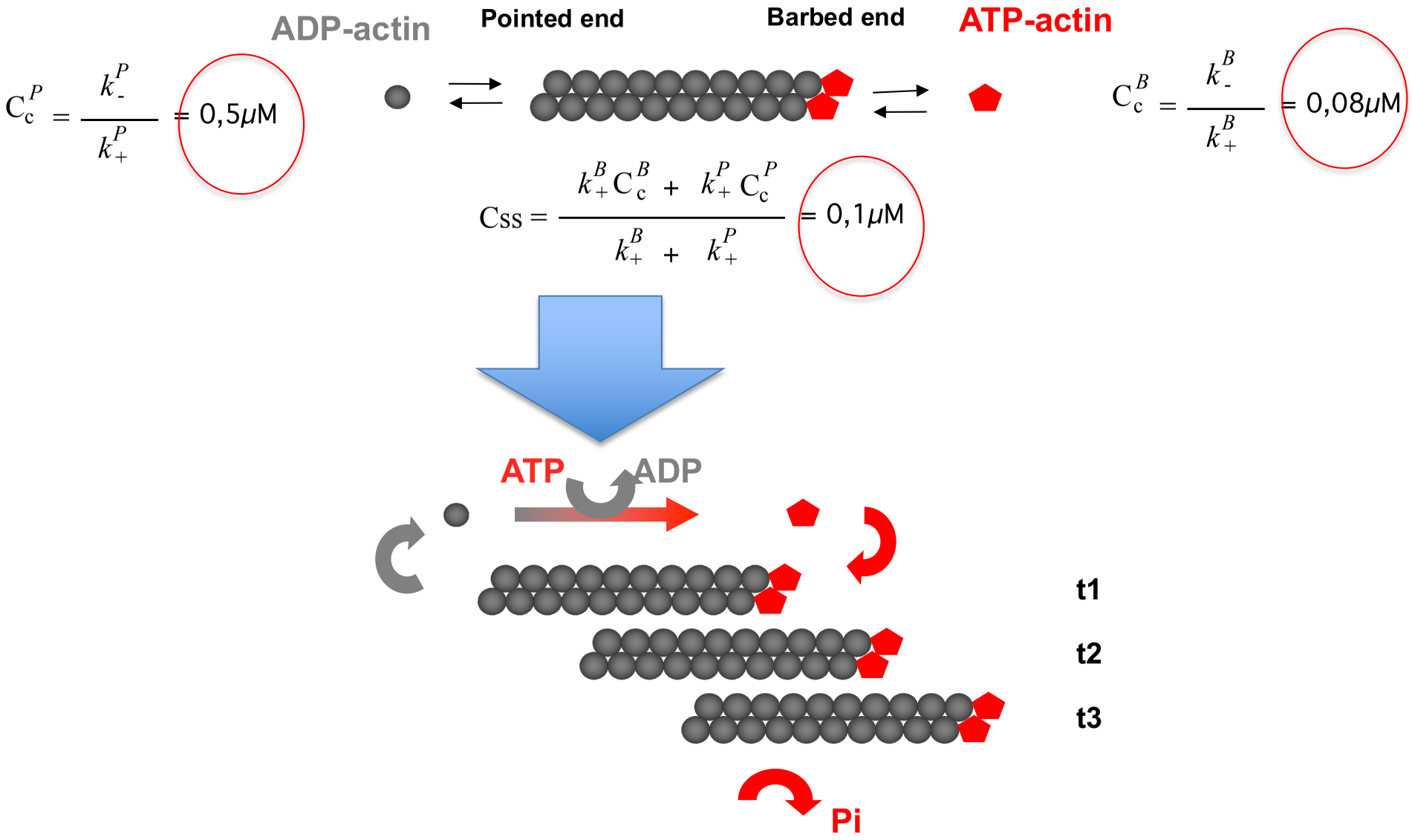




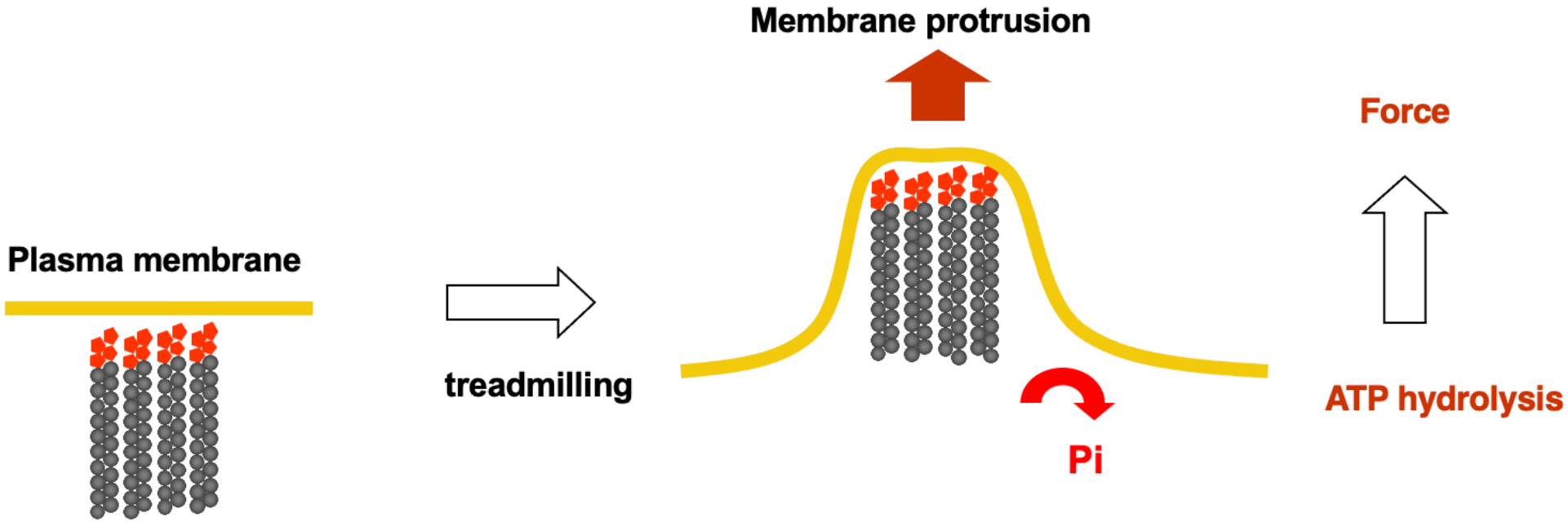
In cells, the actin cytoskeleton is maintained at equilibrium / steady state

3.2.3. Actin dynamics at steady state

(forget the equation…)



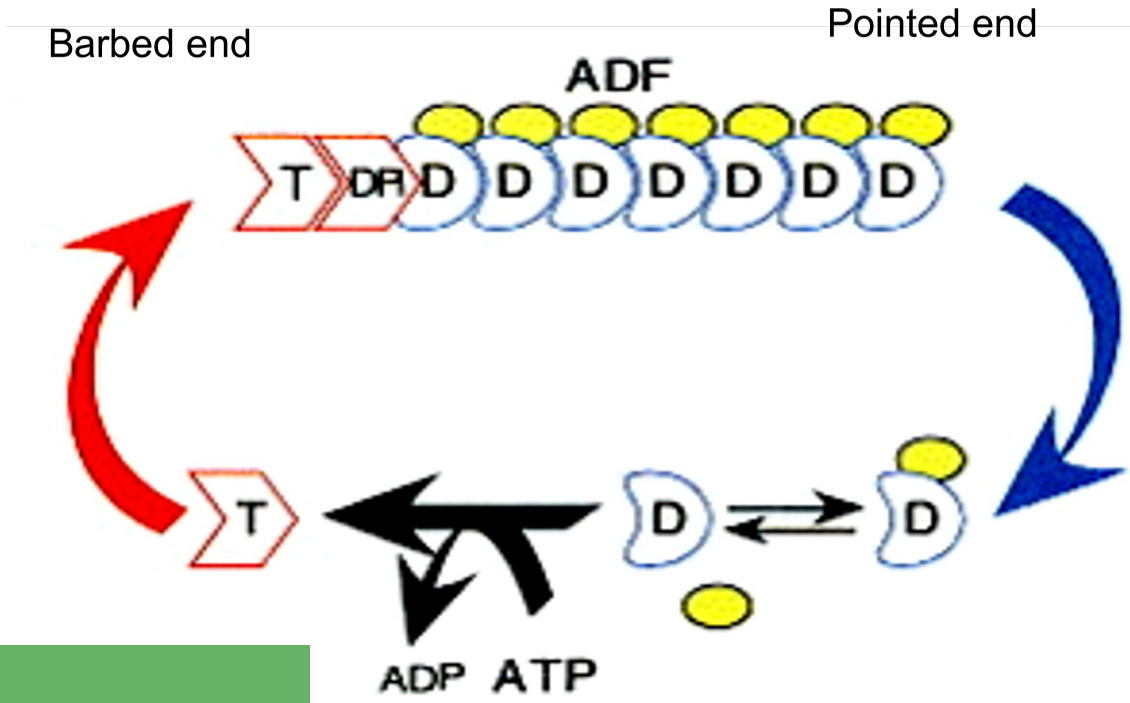
3.2.4. Actin treadmilling is too slow to account for the speed of migrating cells



However the treadmilling is too slow to account for the rapid actin based processes in living cells

3.2.5 Regulation of actin treadmilling

3.2.5.1. ADF (Actin Depolymerizing Factor) depolymerises actin filaments

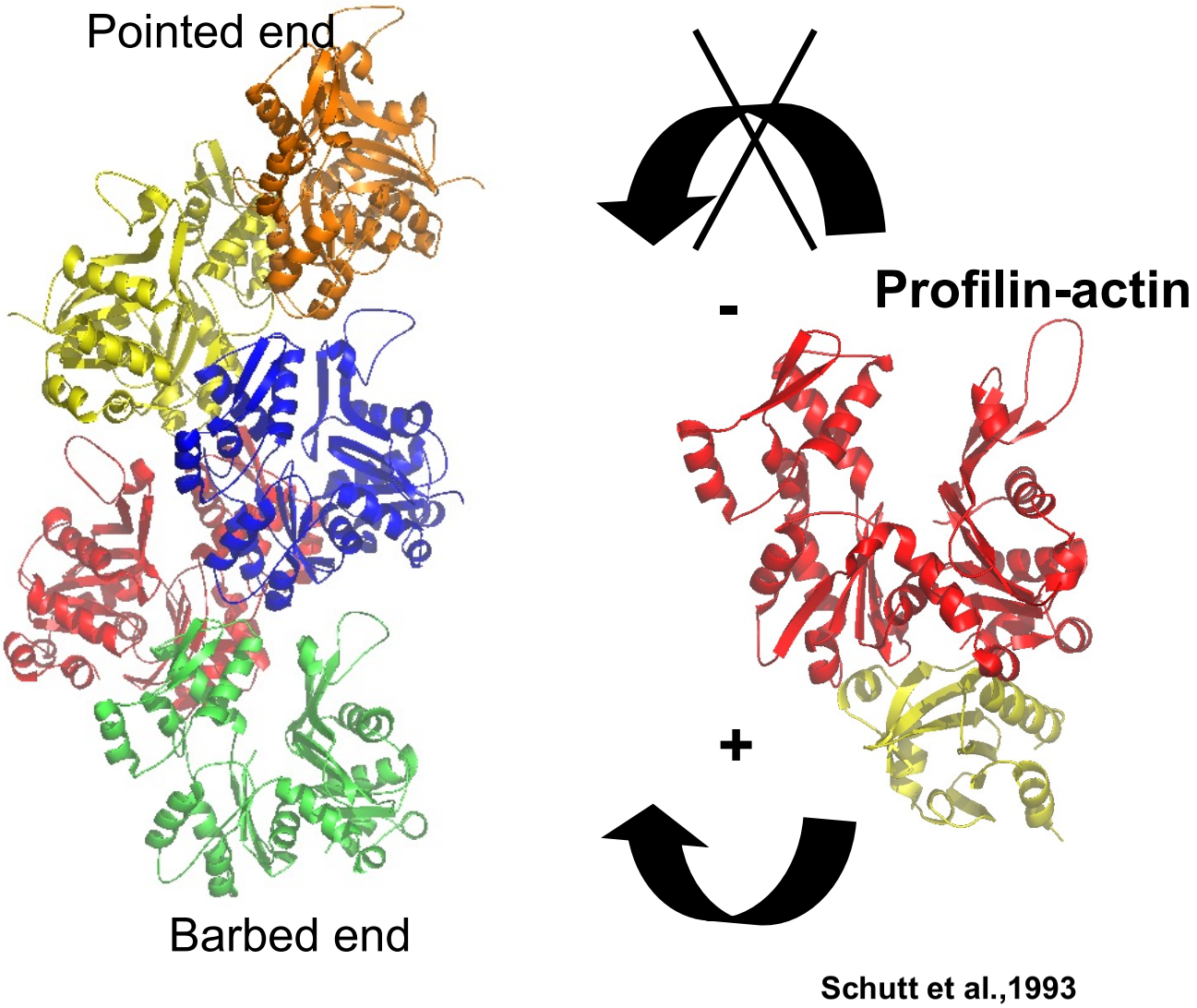


Css ↗

Pantaloni et al, 2001

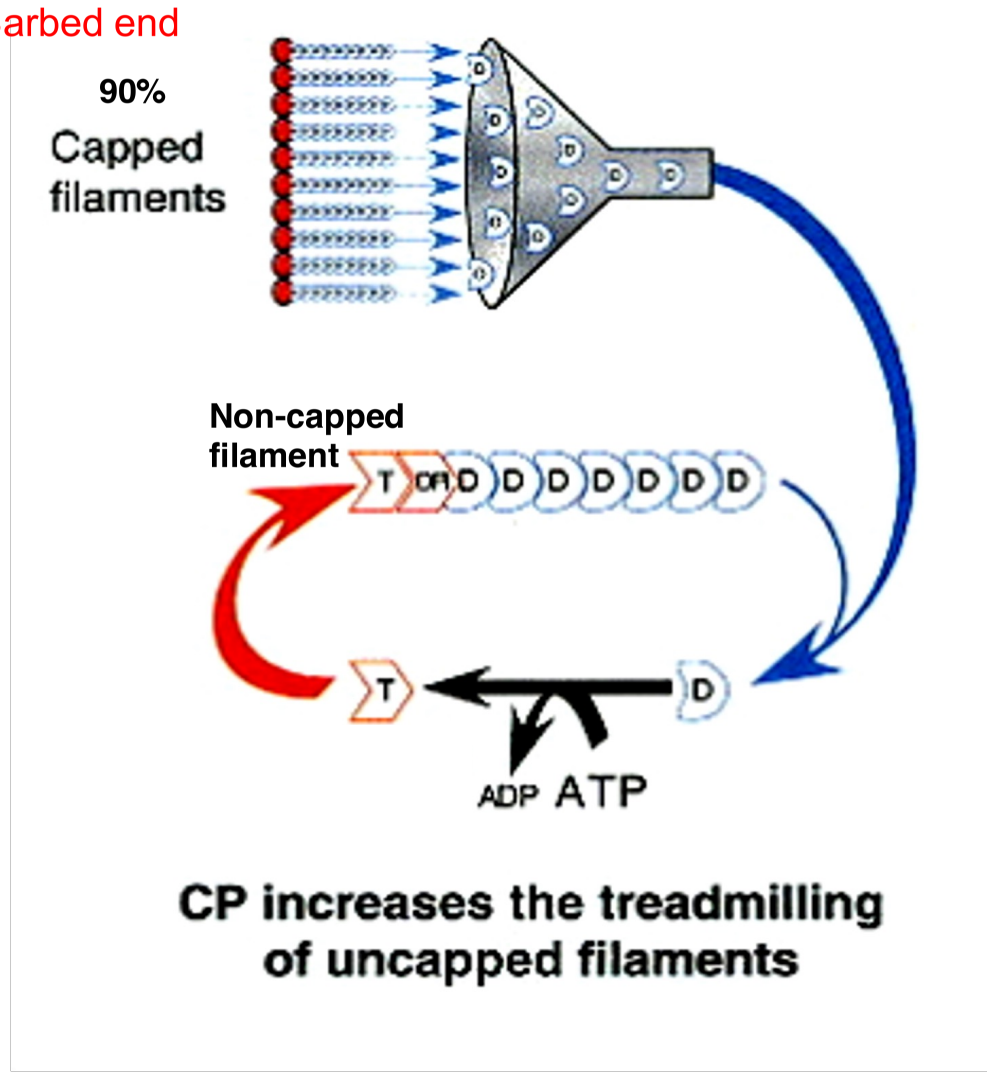
ADF (Actin Depolymerizing Factor) : depolymerises actin filaments from the pointed end to increase the concentration of monomeric actin at steady state which finally enhances barbed end elongation.

3.2.5.2. The profilin-actin complex assembles exclusively at the barbed end



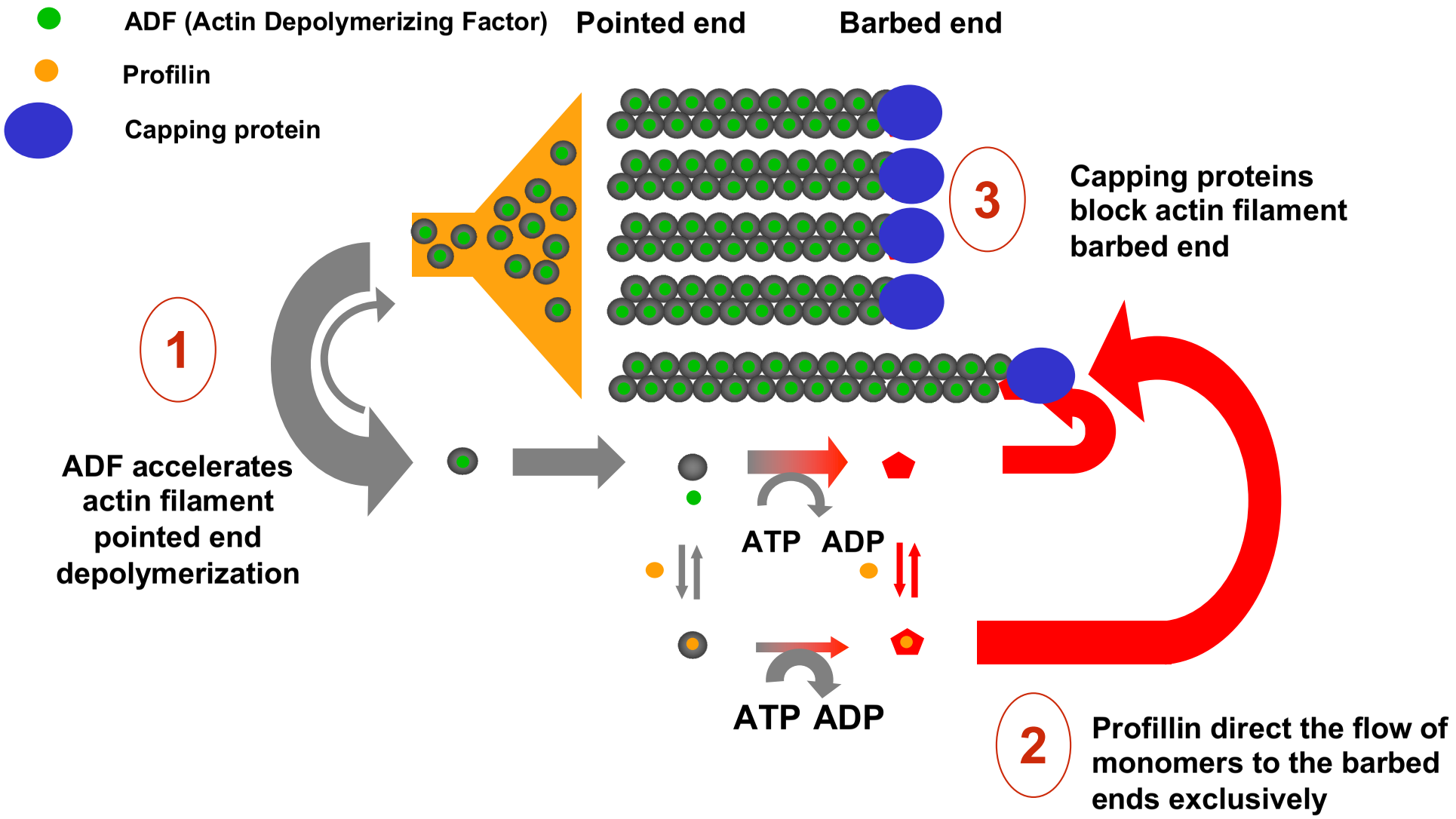
Schutt et al.,1993

3.2.5.3. Capping proteins (CP) block actin barbed ends …



… to favour the growth of non capped filaments

3.2.5.4. To remember: regulation of actin treadmilling



From Le Clainche and Carlier, 2008

3.2.5.4. To remember: regulation of actin treadmilling

ADF (Actin Depolymerizing Factor) : depolymerises actin filaments from the pointed end to increase the concentration of monomeric actin at steady state which finally enhances barbed end elongation.

Profilin : forms a complex with monomeric actin that assembles exclusively at the barbed end

Capping proteins : Block the majority of actin filament barbed end to favour the growth of non capped filaments

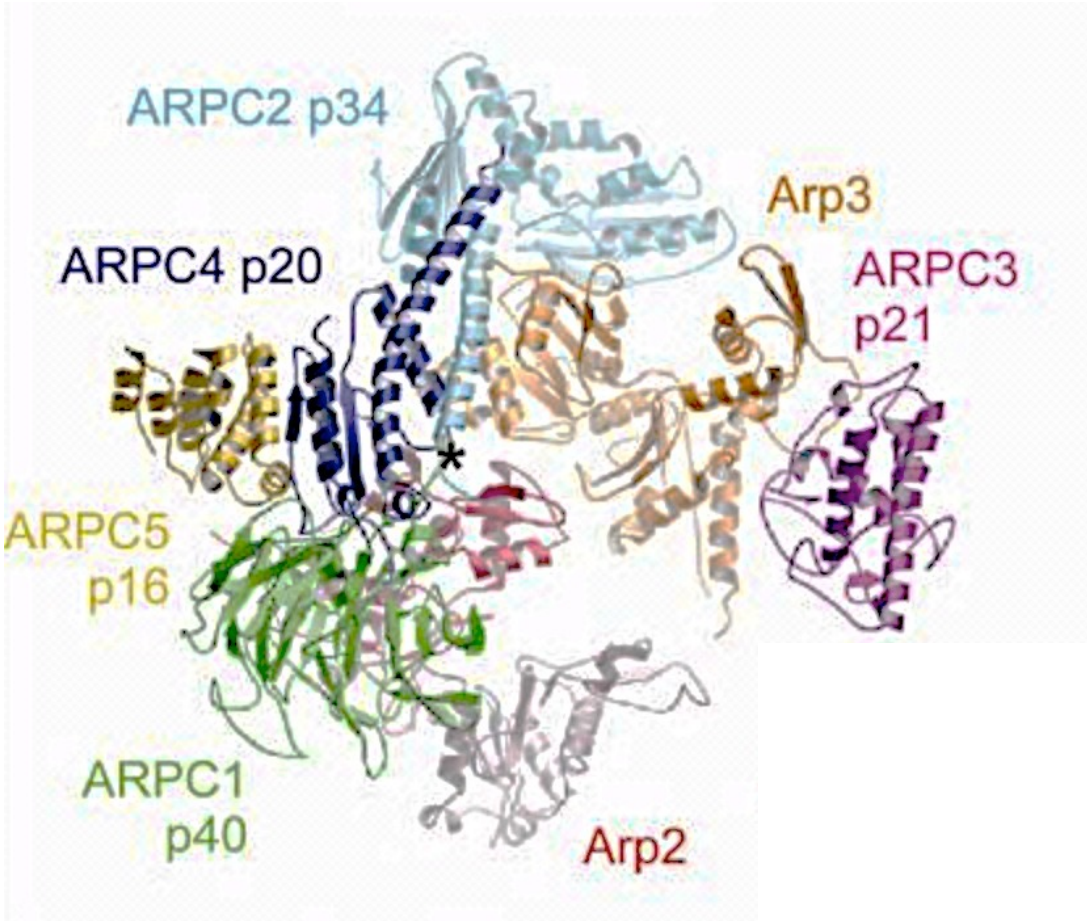
Problem : ultimately capping proteins block the system

Cells need to generate new barbed ends continously to balance the activity of capping proteins

3.2.6. Nucleation of actin filaments in the lamellipodium of migrating cells ?

3.2.6.1. The Arp2/3 complex is a 7 subunit protein complex associated with the lamellipodial actin network

Structure

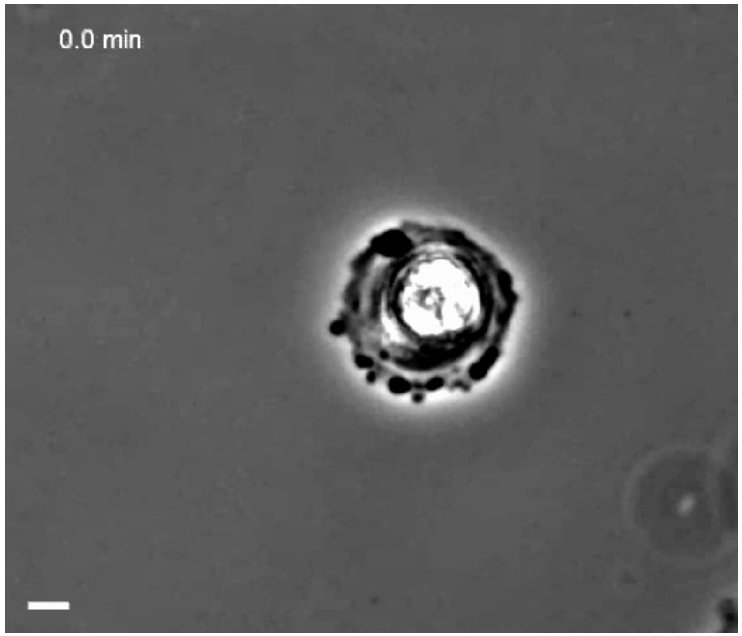


3.2.6.1. The Arp2/3 complex is a 7 subunit protein complex associated with the lamellipodial actin network

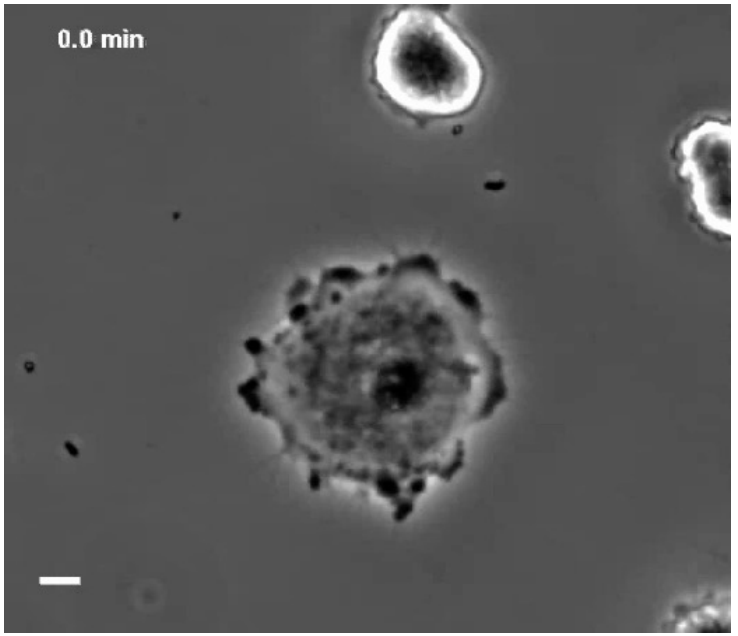
Localisation

3.2.6.2. The Arp2/3 complex is necessary for lamellipodium extension

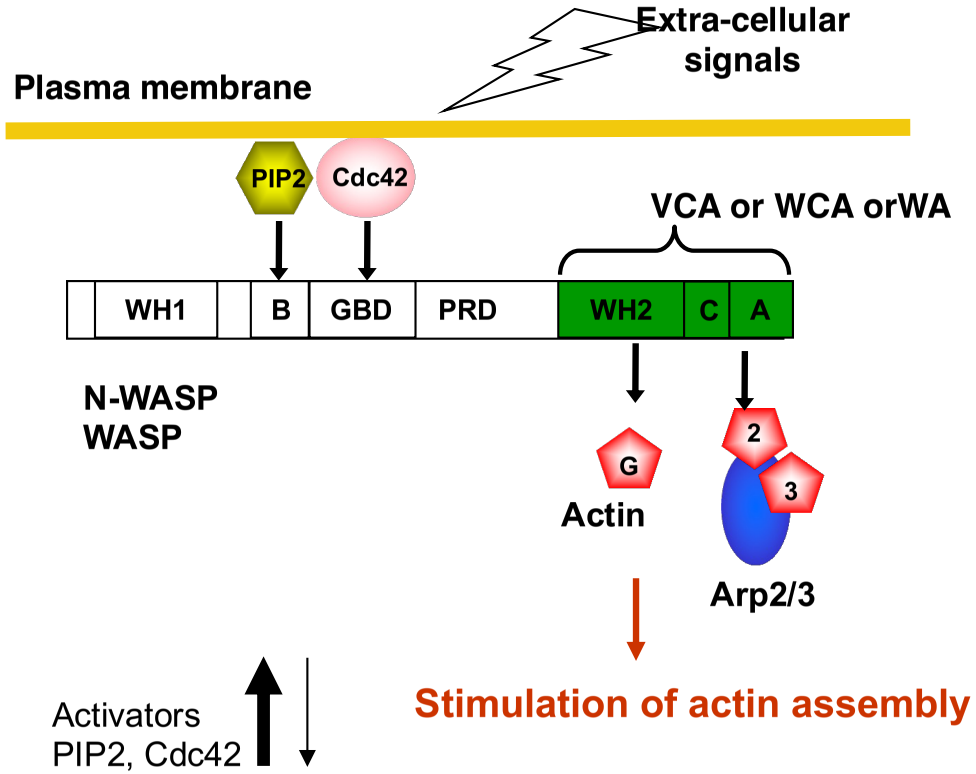
WT

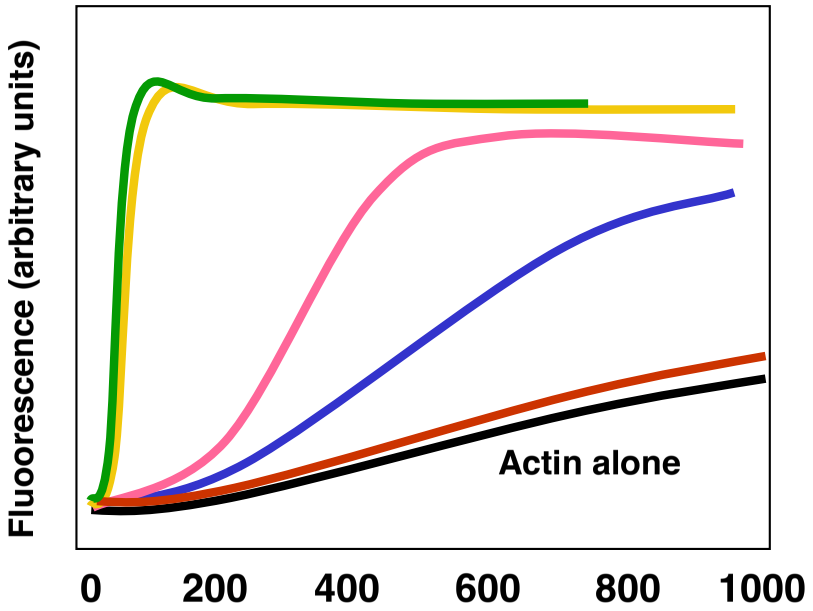


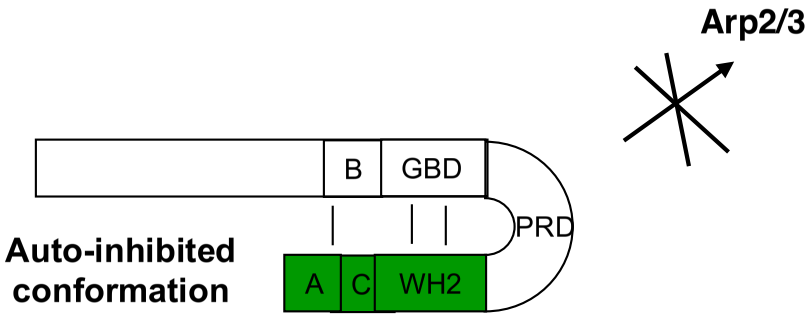
ARPC3-/-



3.2.6.3. The Arp2/3 complex stimulates actin polymerization in response to a signalling cascade



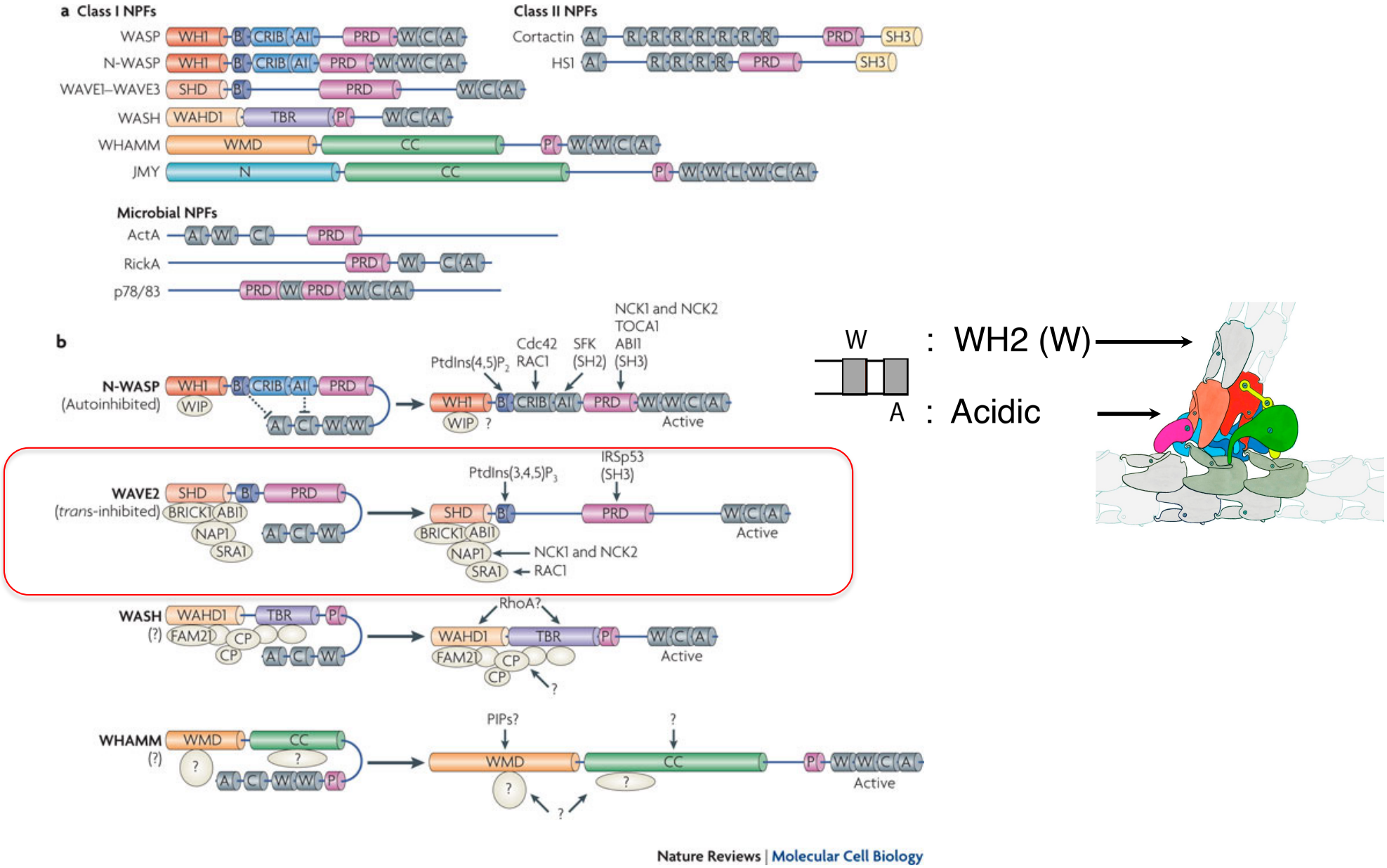




- Actin alone
- Actin + Arp2/3
- Actin + Arp2/3 + N-WASP
- Actin + Arp2/3 + N-WASP+ Cdc42
- Actin + Arp2/3 + N-WASP+ Cdc42 + PIP2
- Actin + Arp2/3 + VCA

From
Rohatgi et al, 1999
Egile et al, 1999

3.2.6.4. Among the multiple NPFs (Nucleating Promoting Factors), WAVE activates Arp2/3 in the lamellipodium



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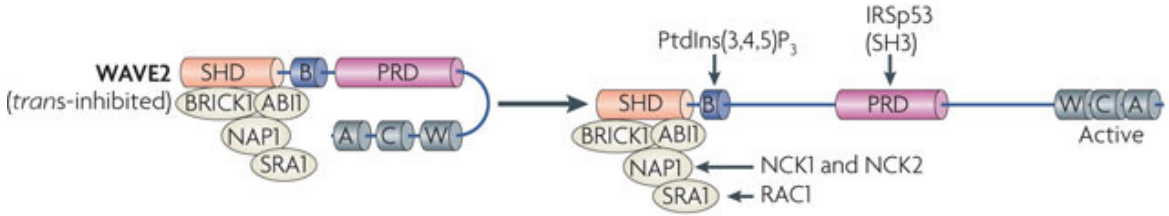
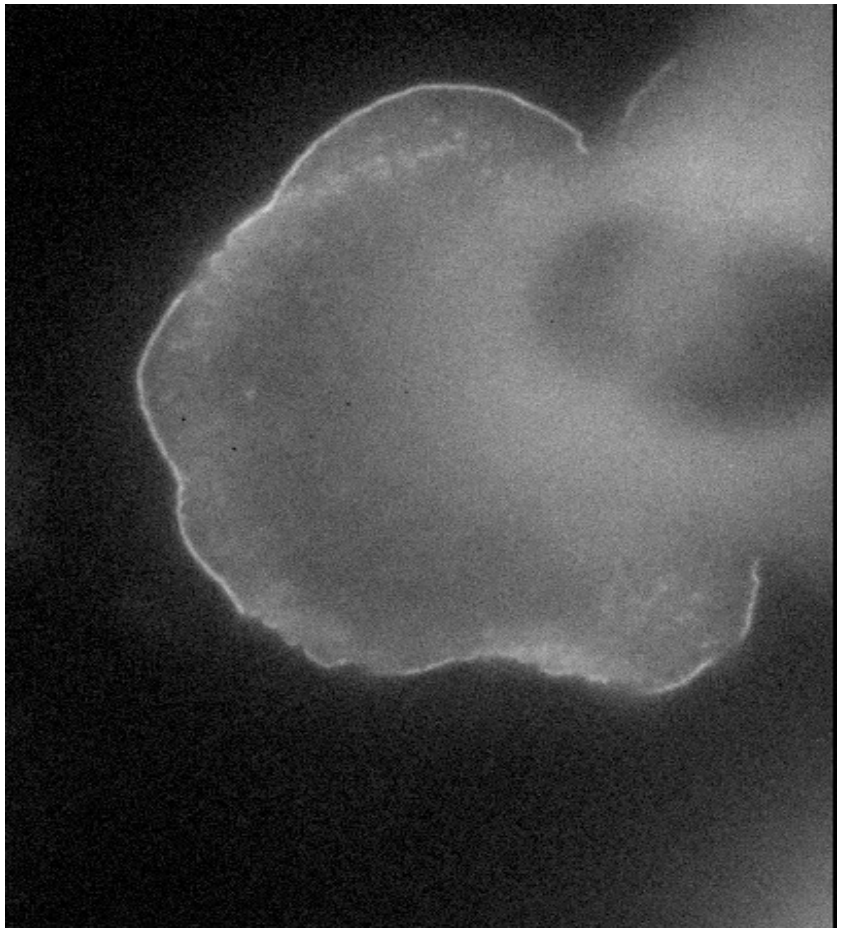


GFP-WAVE

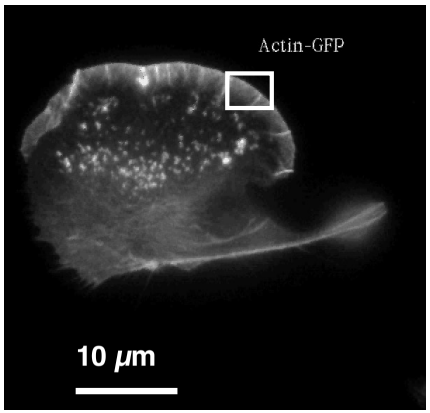


Hahne et al., FEBS lett. 2001.

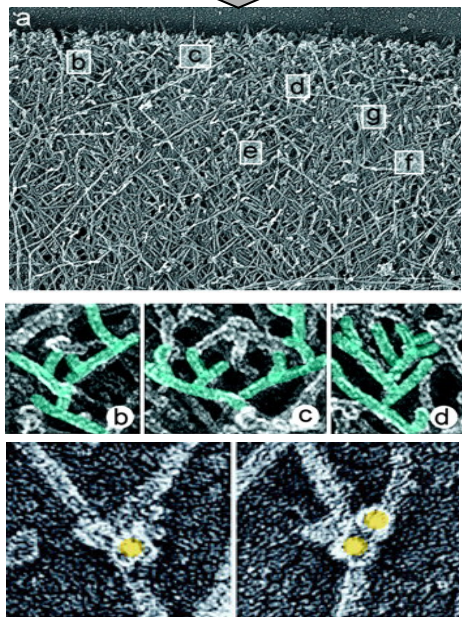
3.2.6.5. The Arp2/3 complex generates (nucleates) new filaments by branching pre-existing ones

In vivo

Migrating cell (GFP-actin)



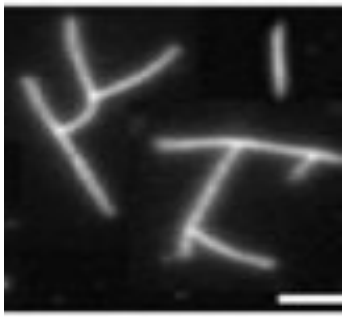
Branched actin network



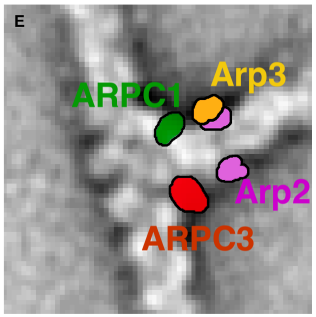
Immuno-gold labelling of Arp2/3 (EM)

From Svitkina et al, 1999

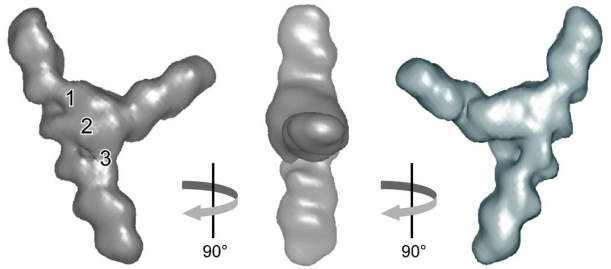
In vitro (Arp2/3 + VCA + actin)

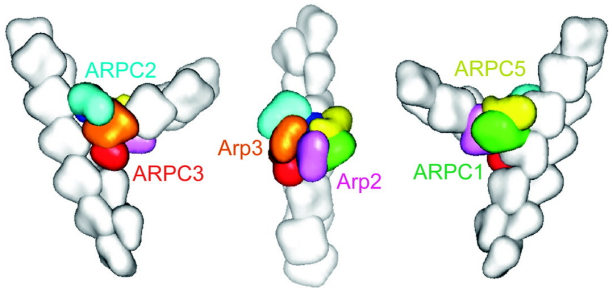


Fluorescence (Rhodamine-phalloidin)



Cryo-EM + Fluorescence of Arp2/3 subunits





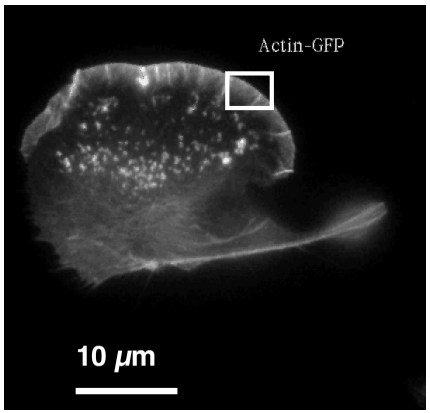
Reconstitution from Cryo-EM

From Egile et al, 2006

3.2.6.5. The Arp2/3 complex generates (nucleates) new filaments by branching pre-existing ones

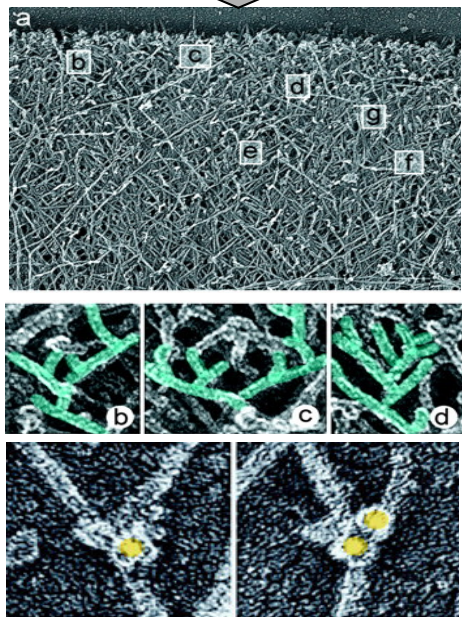
In vivo

Migrating cell (GFP-actin)



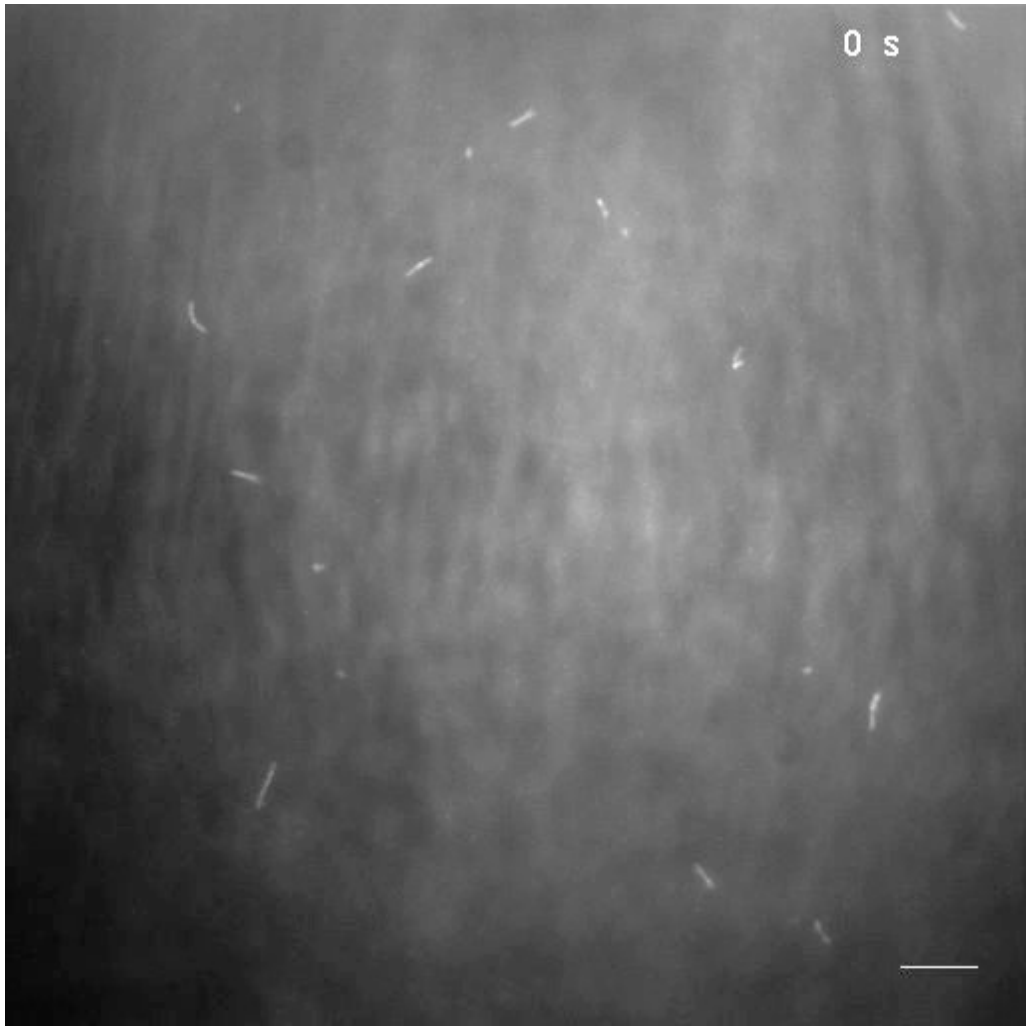
Branched actin network

Immuno-gold labelling of Arp2/3 (EM)



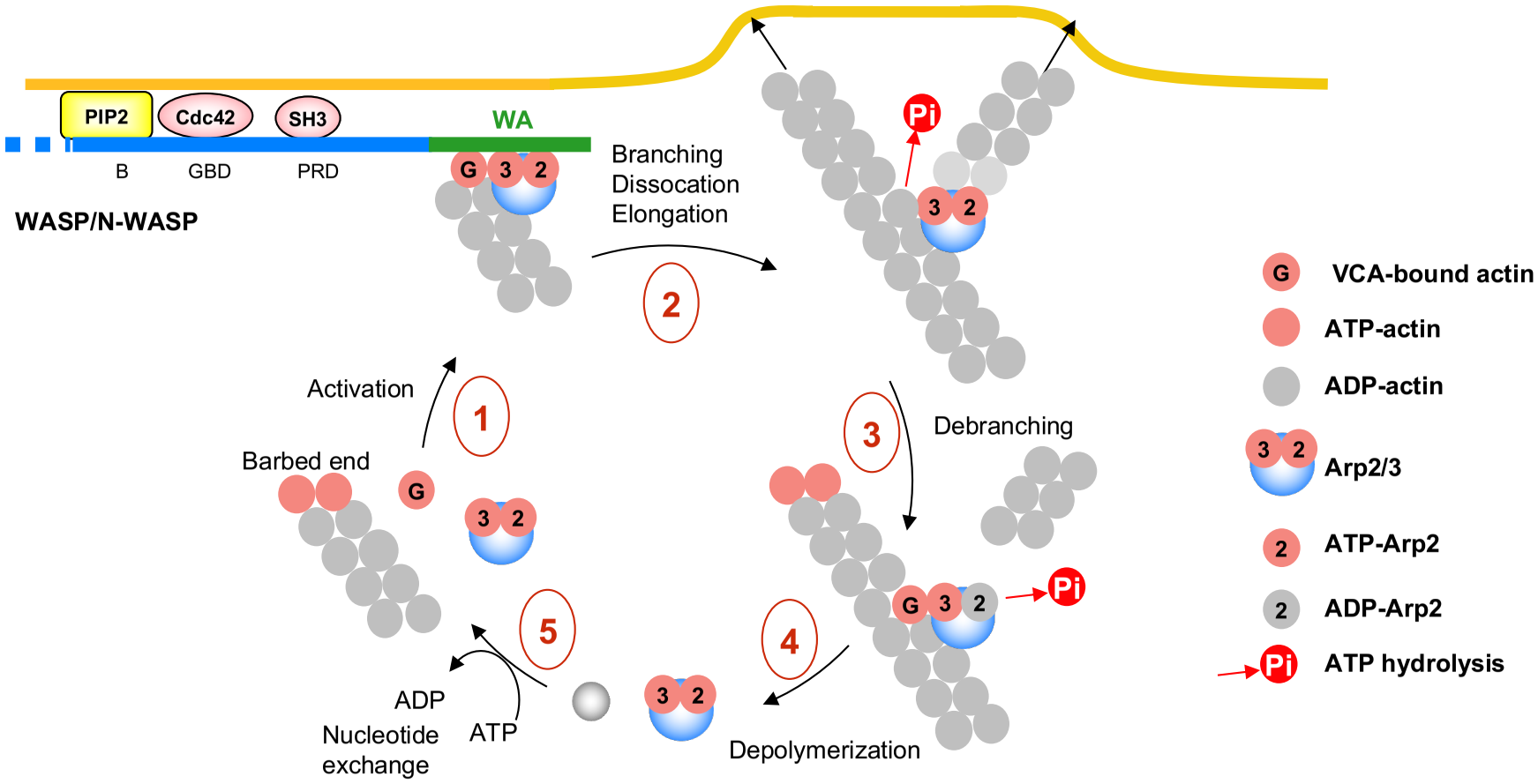
From Svitkina et al, 1999

In vitro (Arp2/3 + VCA + actin)



From Pernier et al 2016

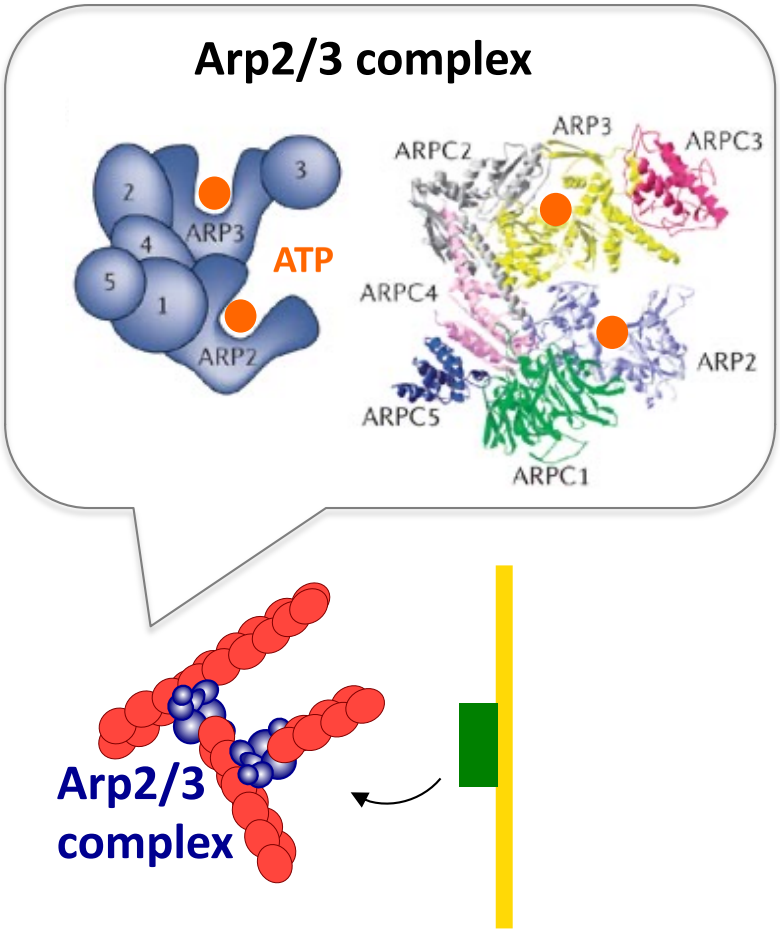
3.2.6.5. Model of the cycle of actin filament branching-debranching

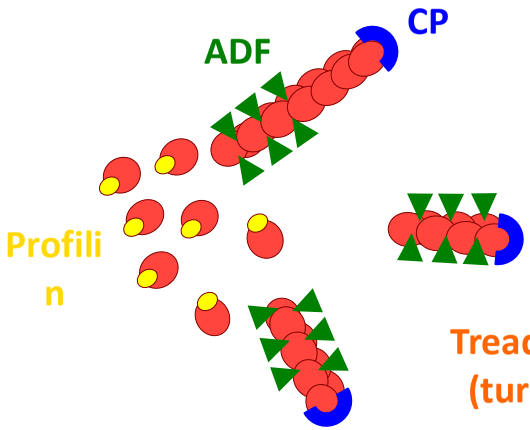


3.2.6.7. Summary

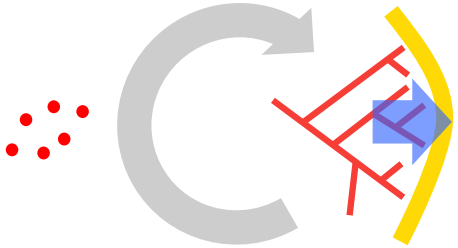
To produce force
against a membrane…

… cells initiate an actin network …





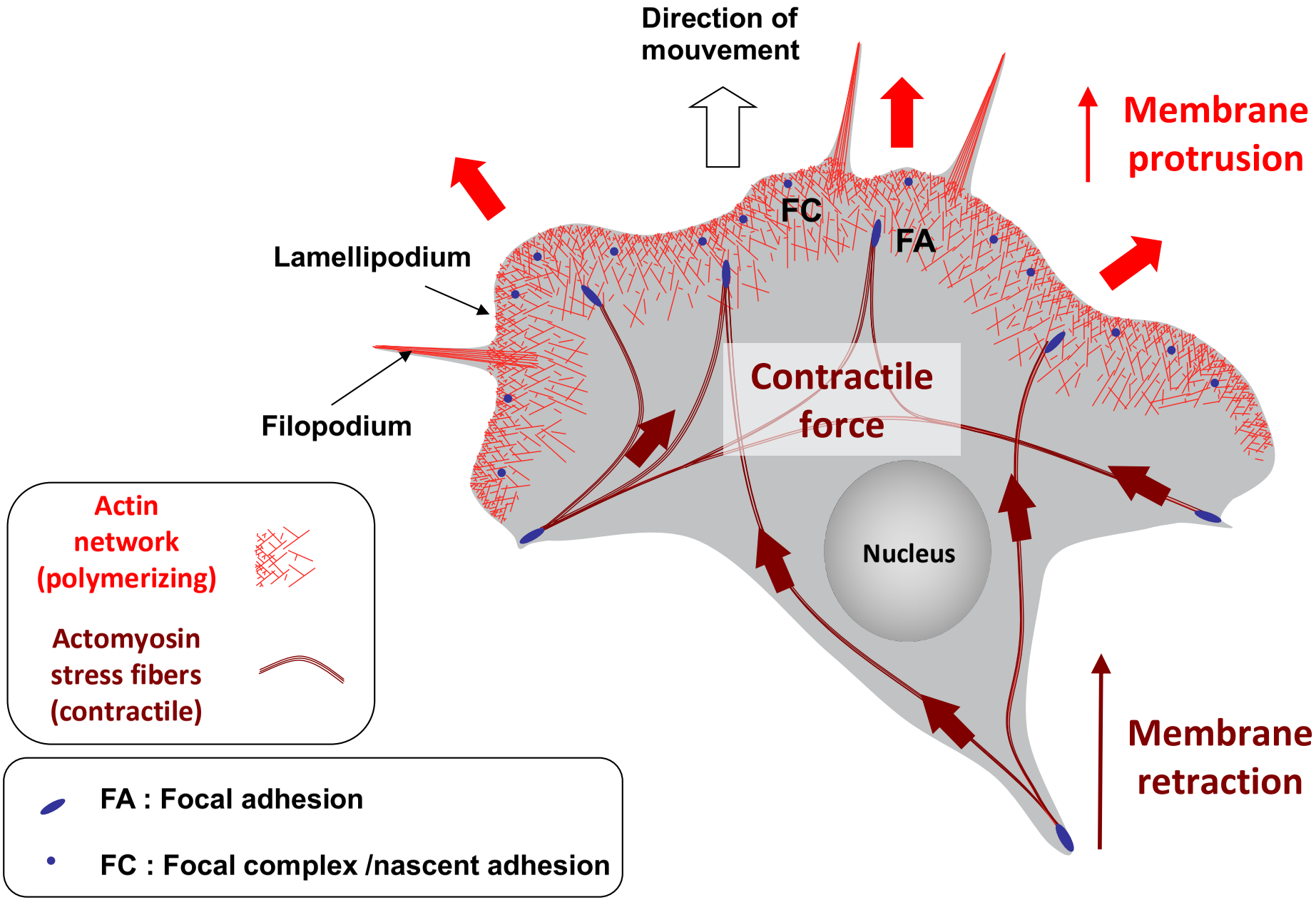
Treadmilling
(turnover)



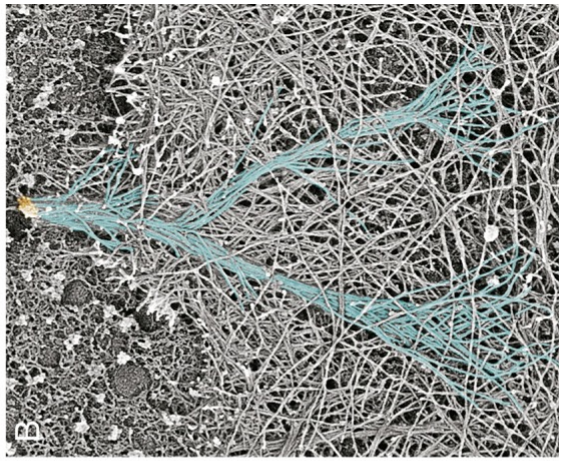
… and accelerate
its turnover

4. Mechanism of force generation in filopodia of migrating cells

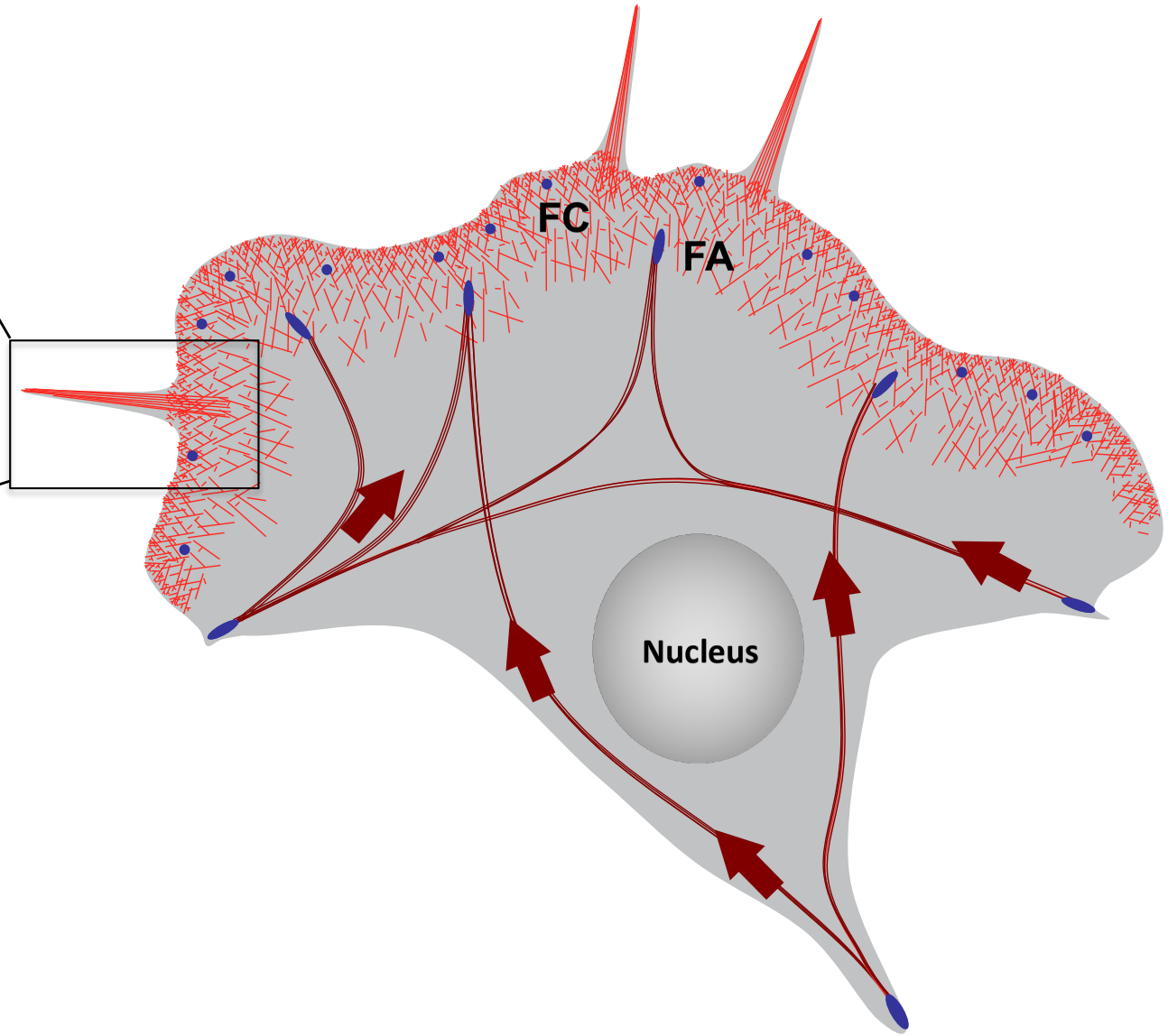
4.1. Filopodia



4.1. Filopodia



Filopodium observed in electron microscopy (TEM). The actin filaments that belong to the filopodium are highlited in blue



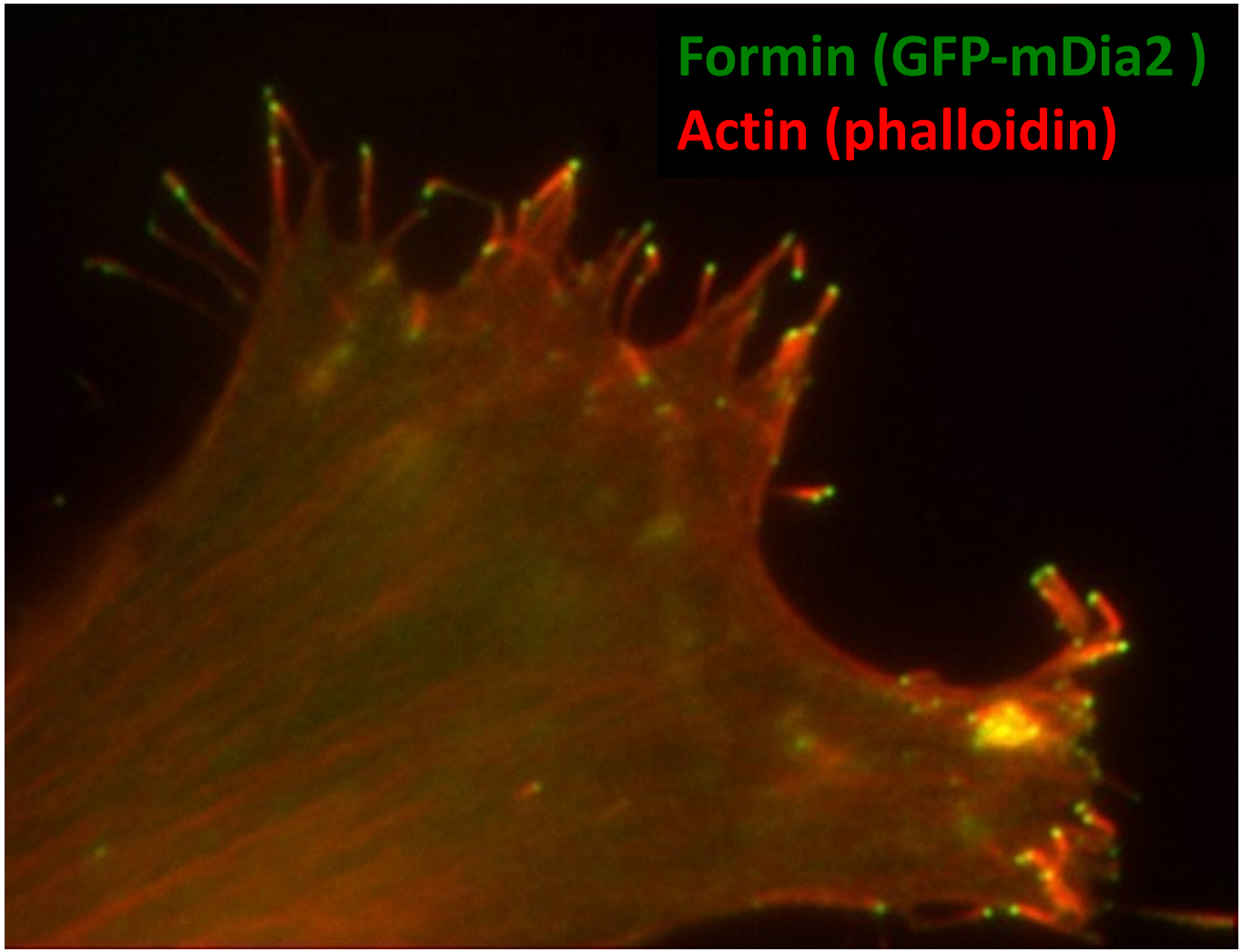
Actin network (polymerizing)

Actomyosin stress fibers (contractile)

FA : Focal adhesion

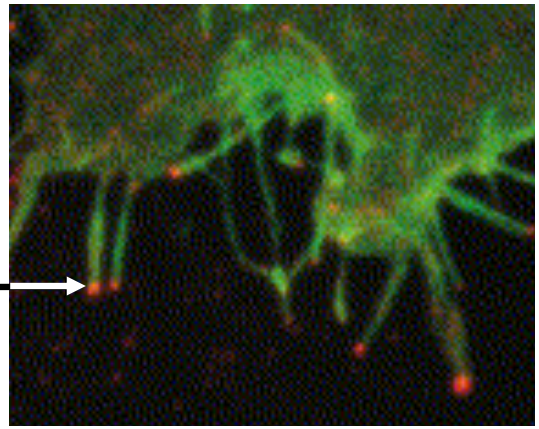
FC : Focal complex /nascent adhesion

4.2. Proteins from the formin family drive membrane protrusion at the tip of filopodia

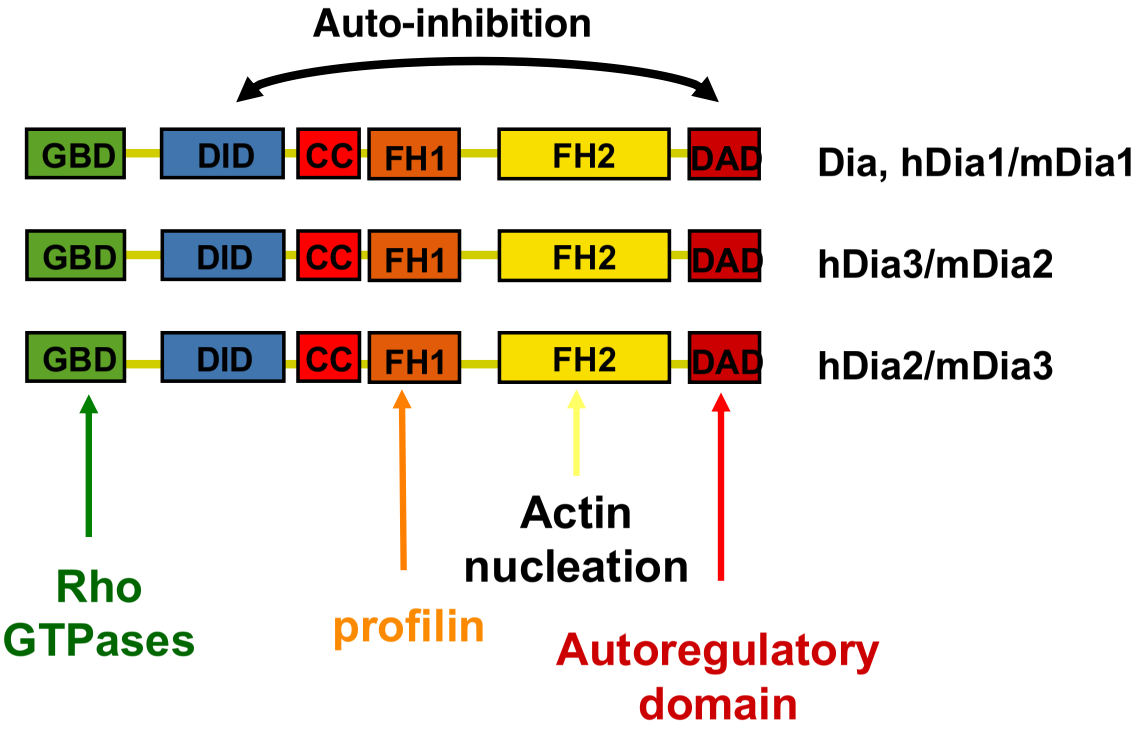


4.2. Domain organization of formins

Filopodia, cell polarity, endosome movement, cytokinesis

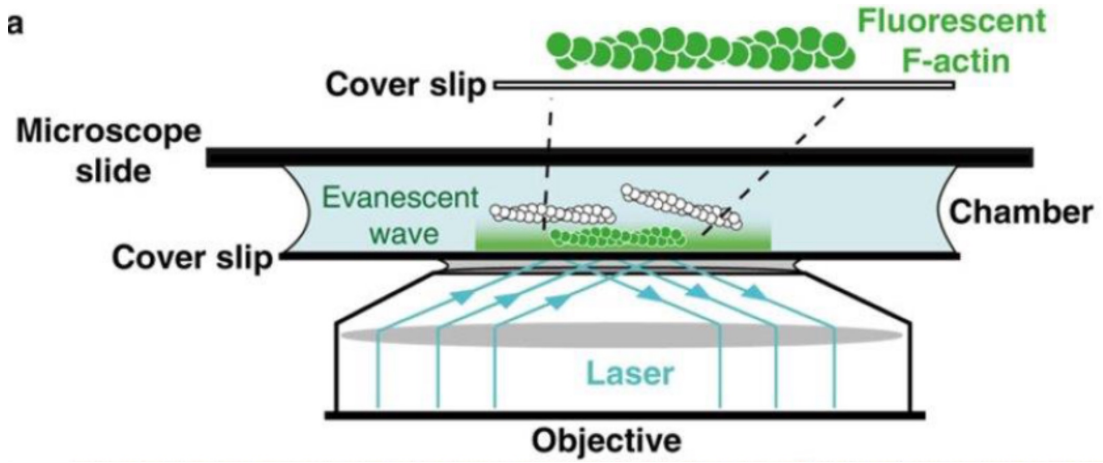


Filopodia
(Faix et Grosse, 2006)

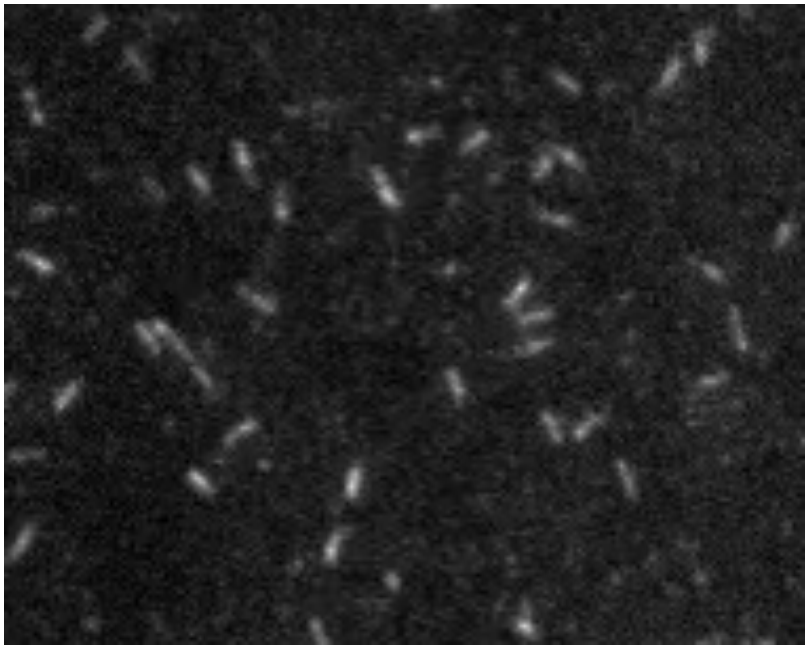


GBD	: GTPase Binding Domain
DID	: Diaphanous Inhibitory Domain
CC	: Coiled-coil
FH1	: Formin Homology 1
FH2	: Formin Homology 2
DAD	: Diaphanous Auto-regulatory Domain

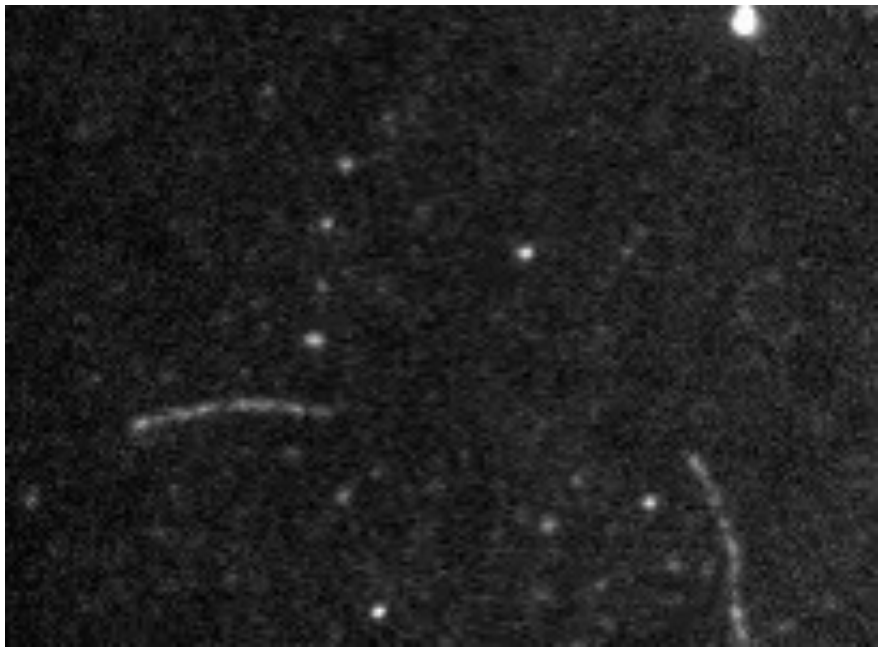
4.3. Formins elongate actin filaments in the presence of profilin



FH1-FH2



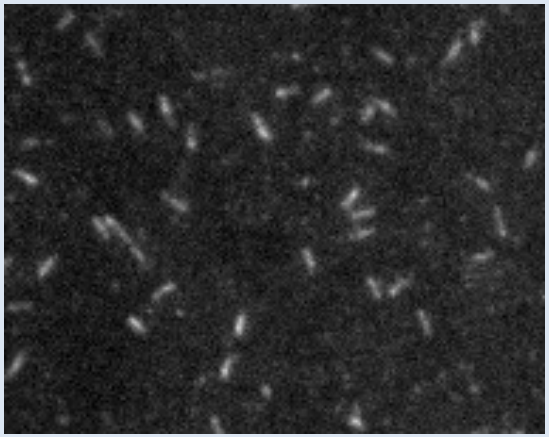
FH1-FH2 + Profilin

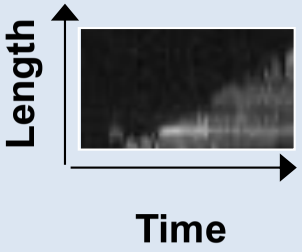


From Le Clainche et al, 2010

4.3. Formins elongate actin filaments in the presence of profilin

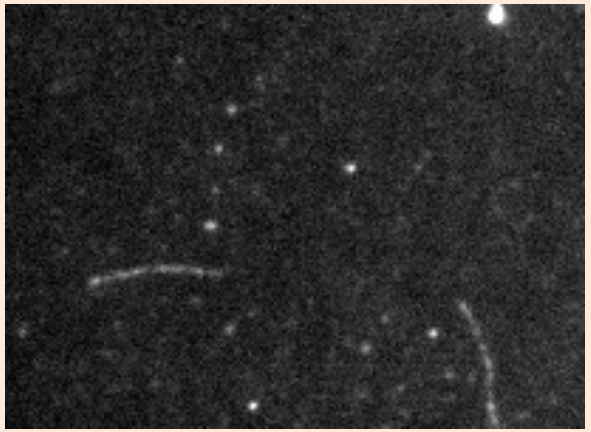
FH1-FH2

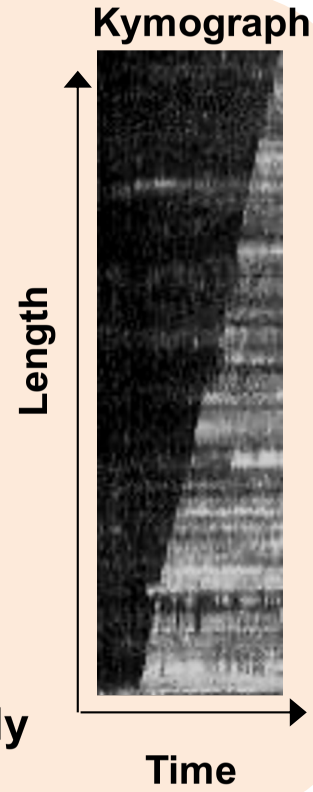




Nucleation

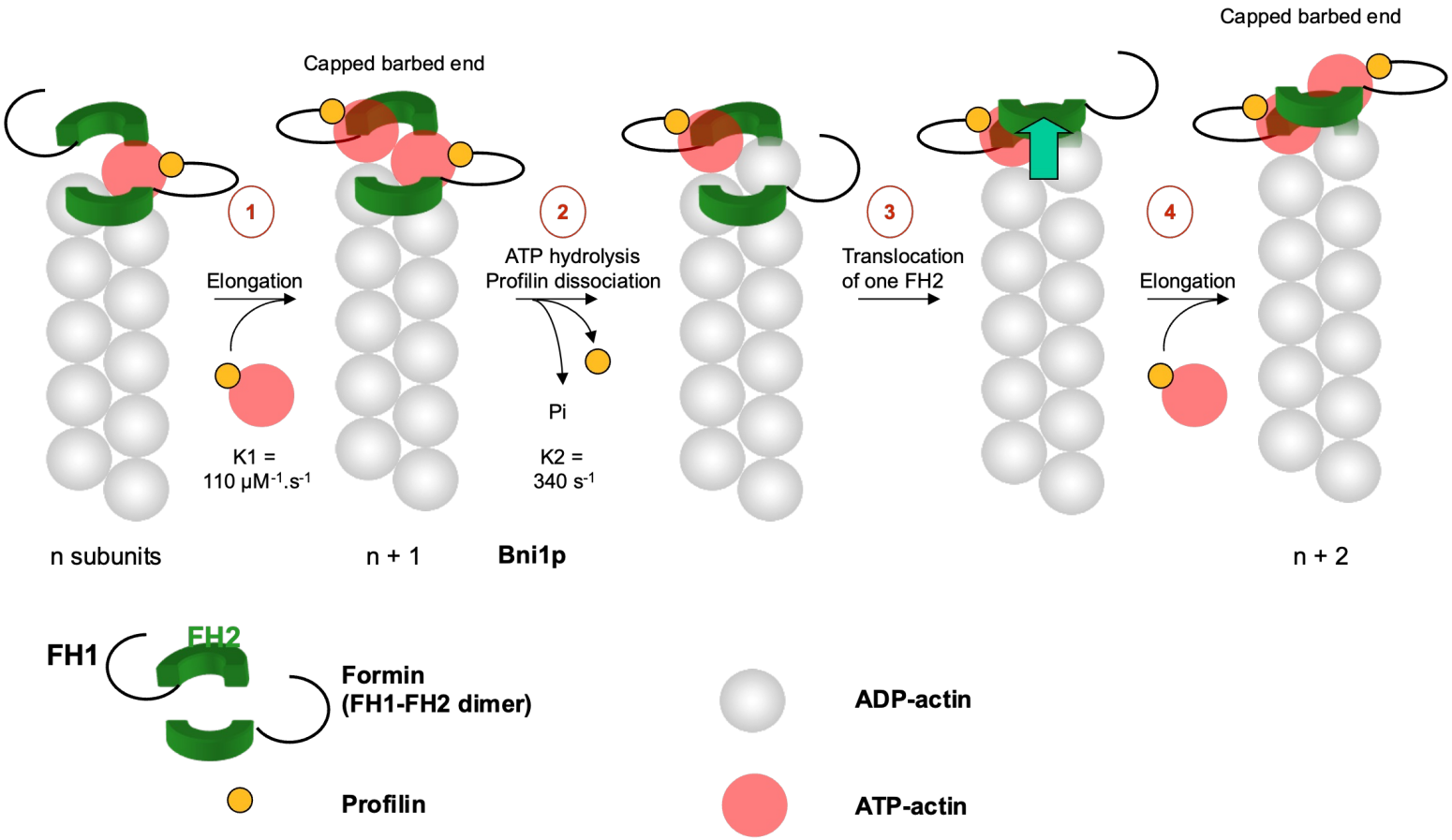
FH1-FH2 + Profilin



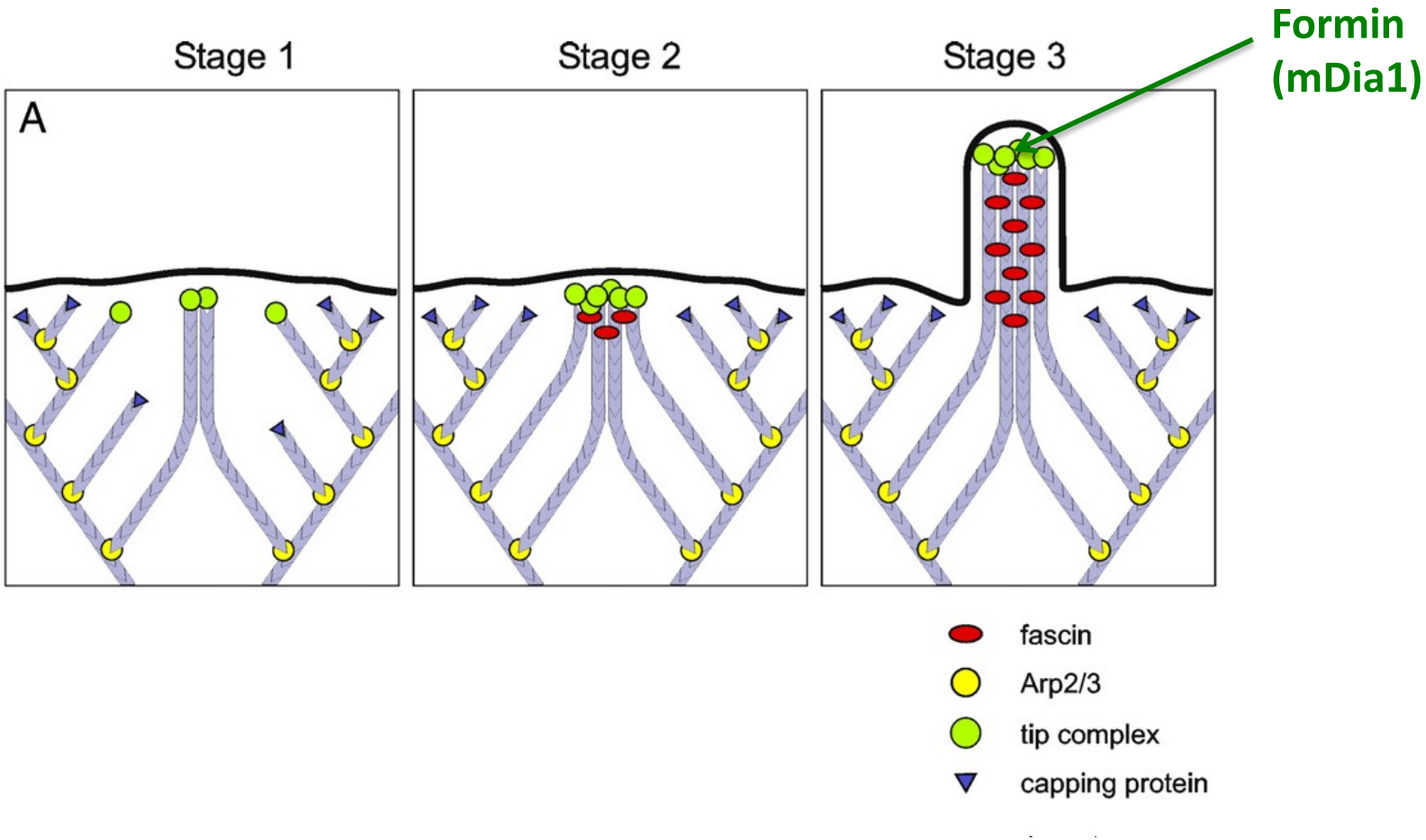


Nucleation
+ fast processive assembly

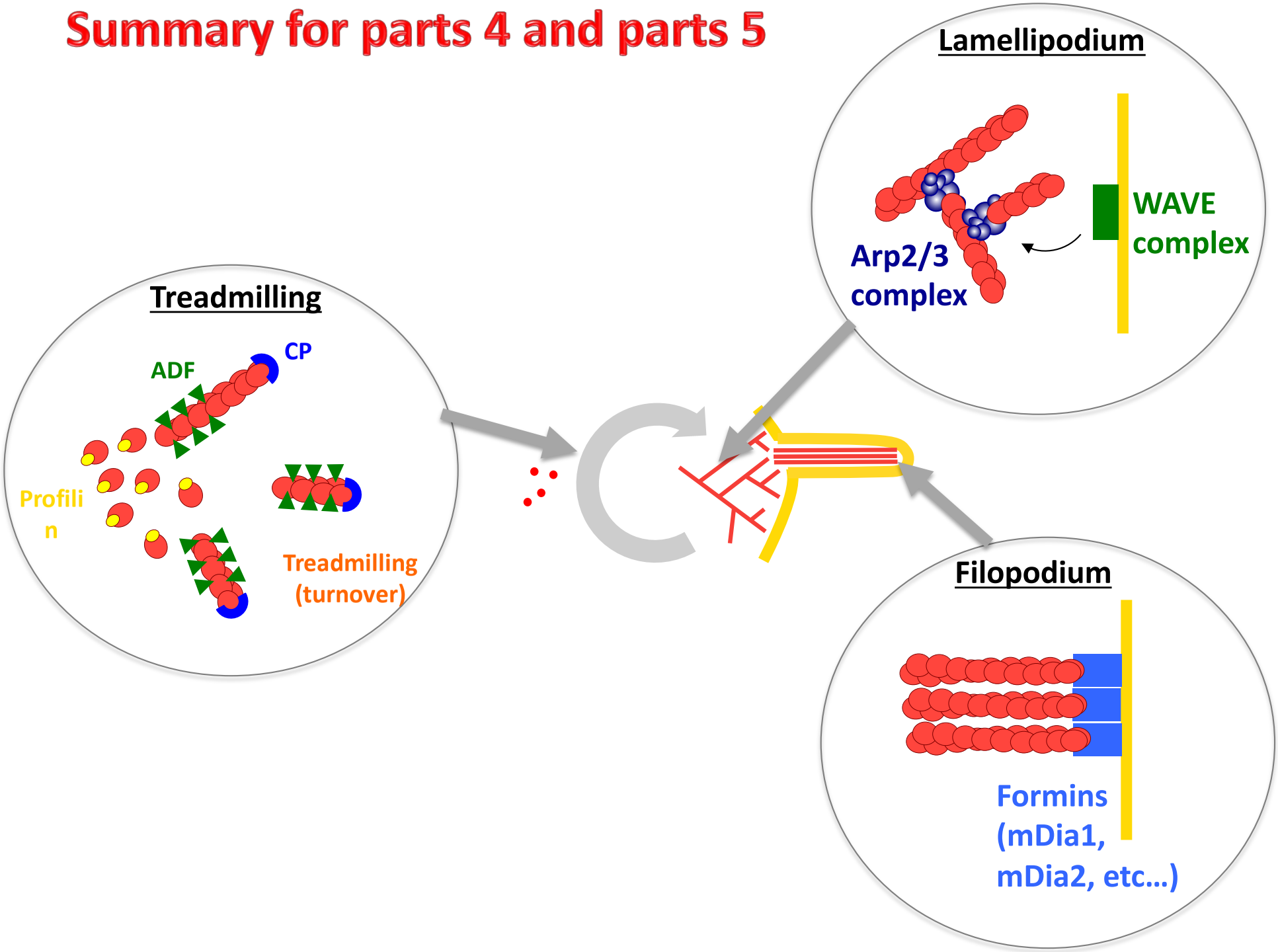
4.4. Formins accelerate the elongation of actin filaments in a processive manner (formin remains associated to actin during elongation)



4.4. To remember: mechanism of filopodia formation



Summary for parts 4 and parts 5



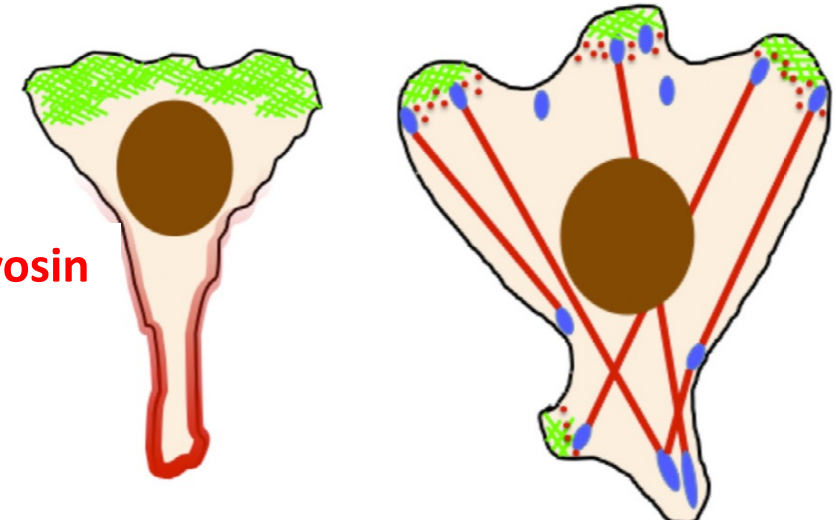
5. Actomyosin networks in cell migration

5. Actomyosin networks in cell migration

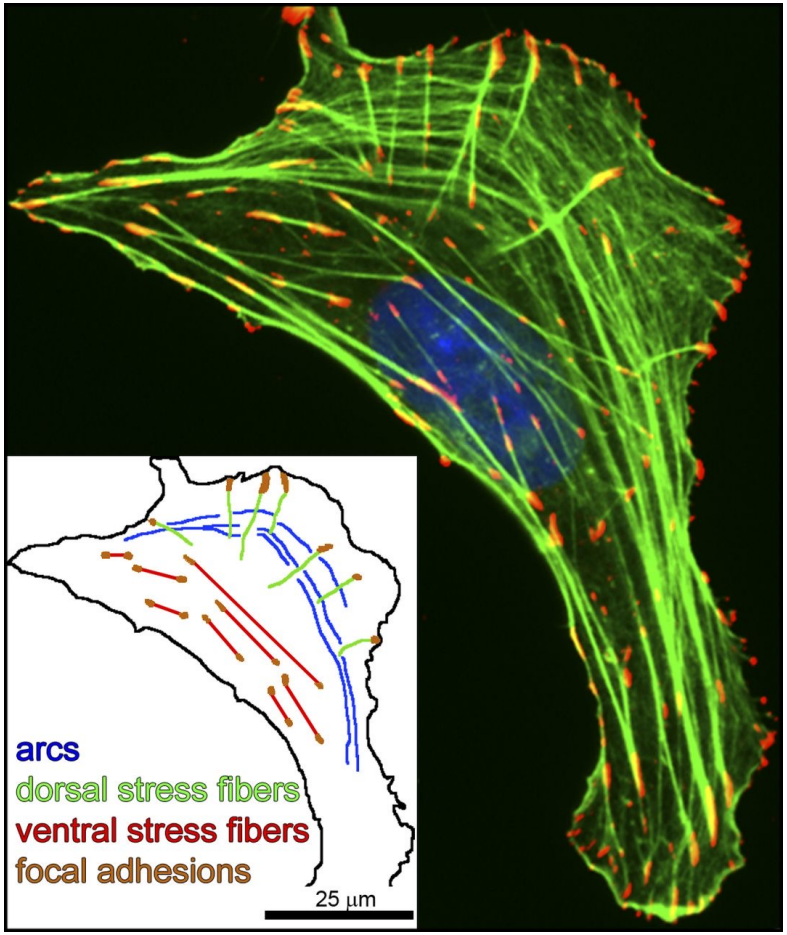
Polymerizing actin

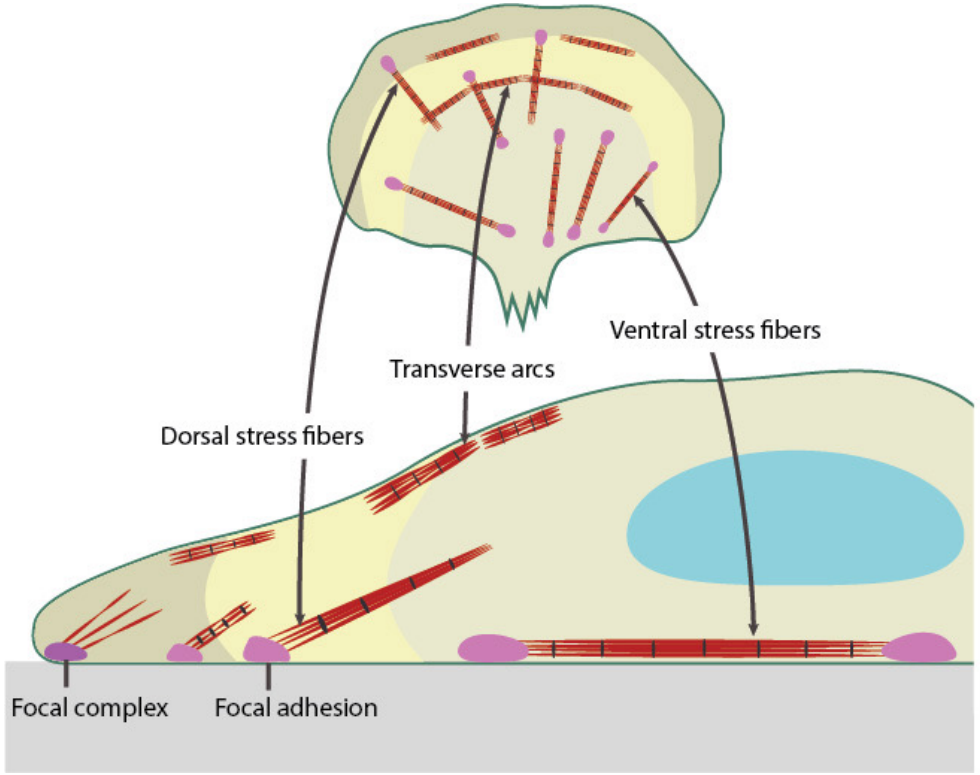
Contractile Actomyosin

Focal adhesions

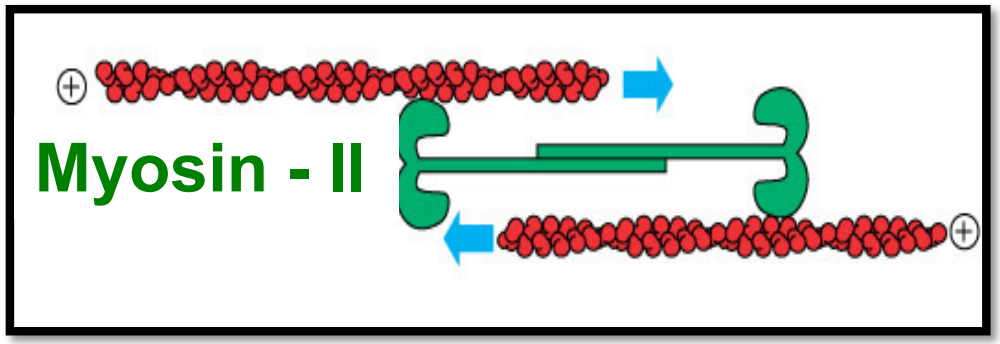


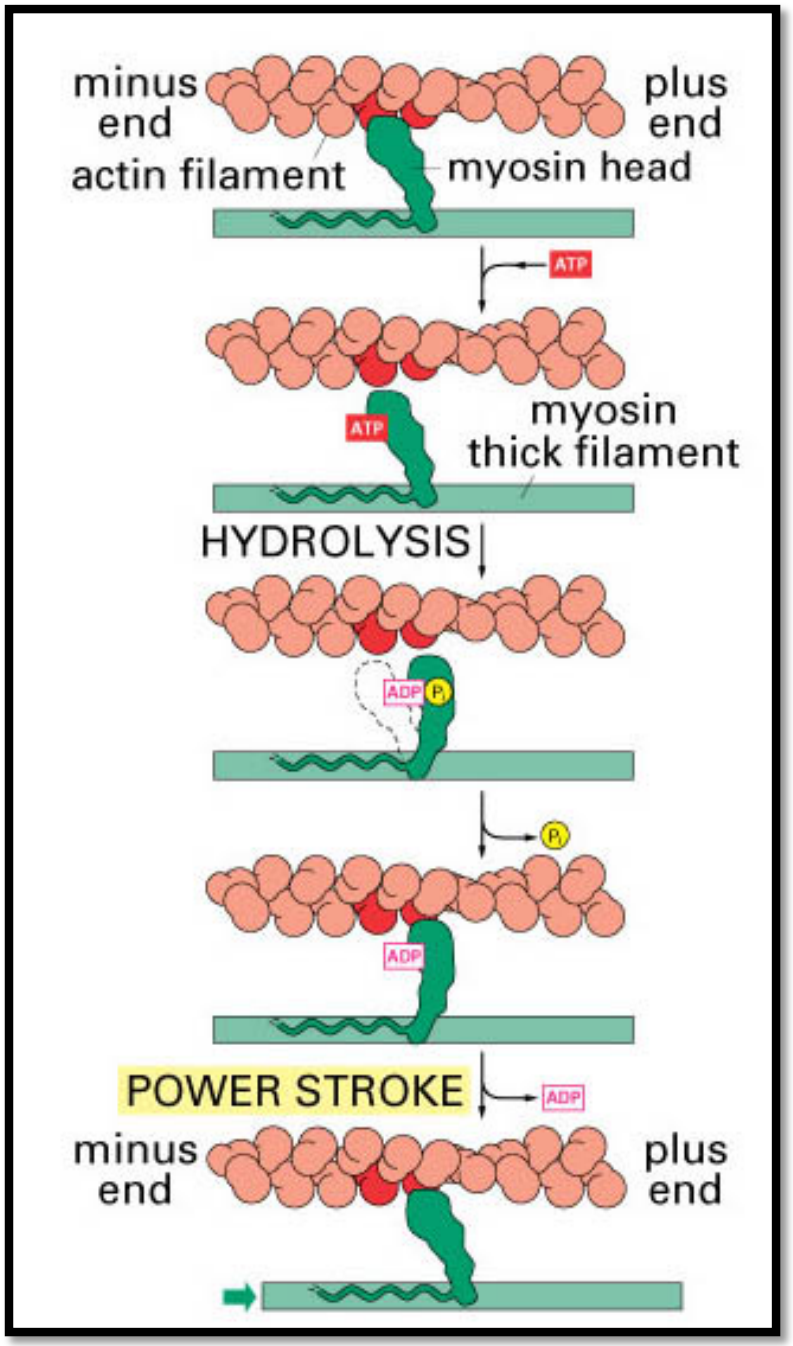
5.1. Classification of the stress fibers in migrating cells



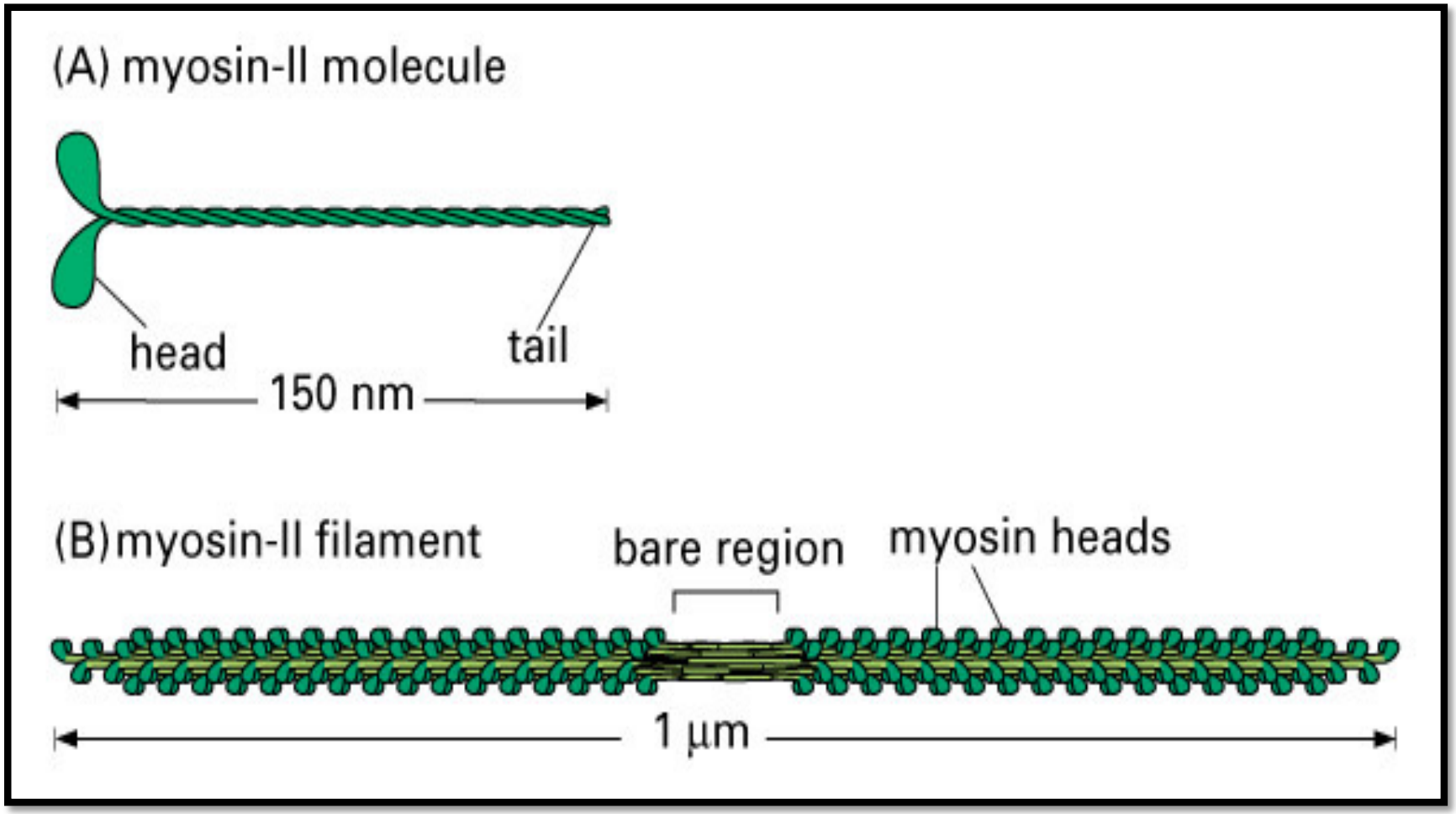


5.2. The motor activity of myosins

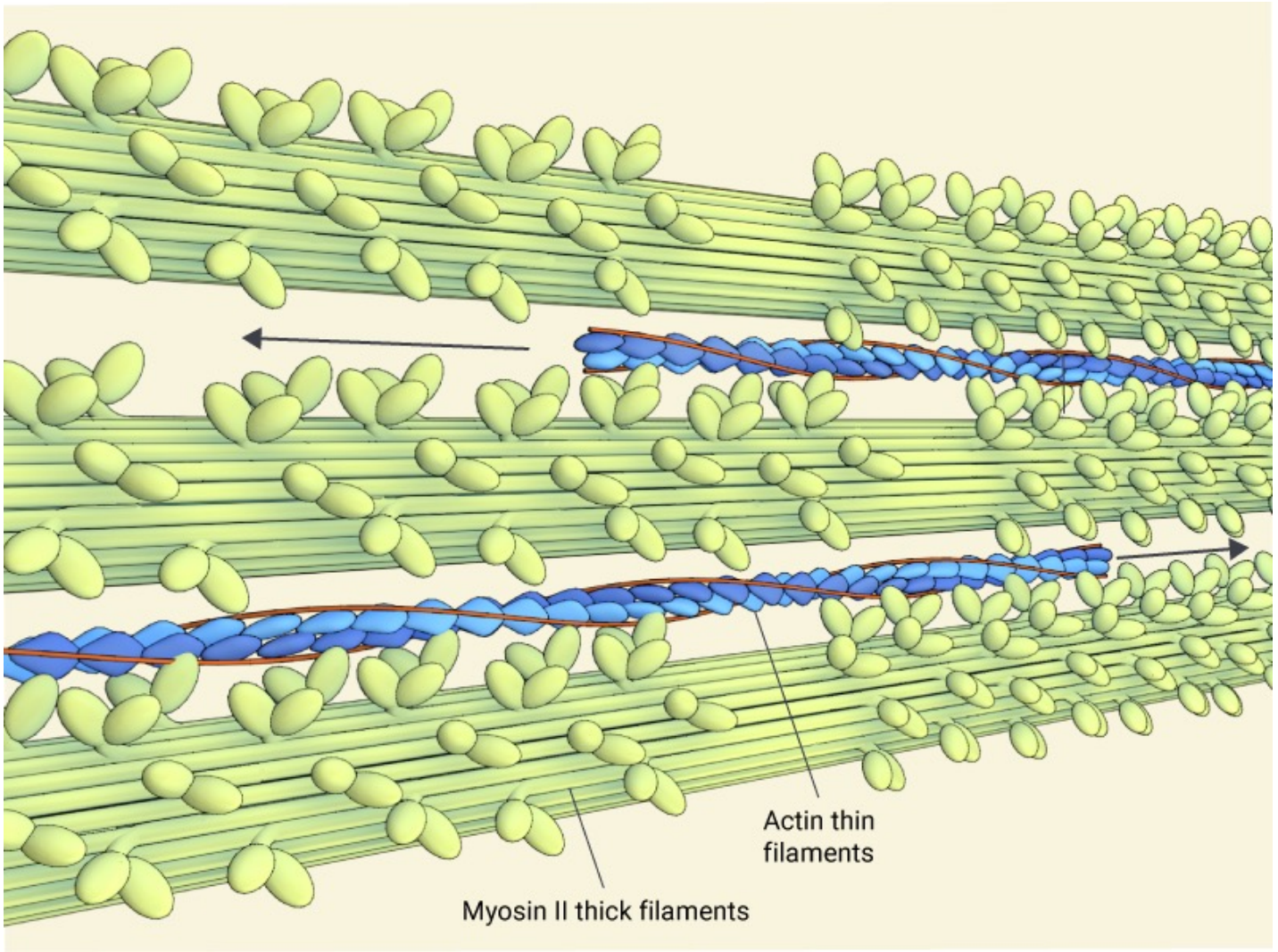




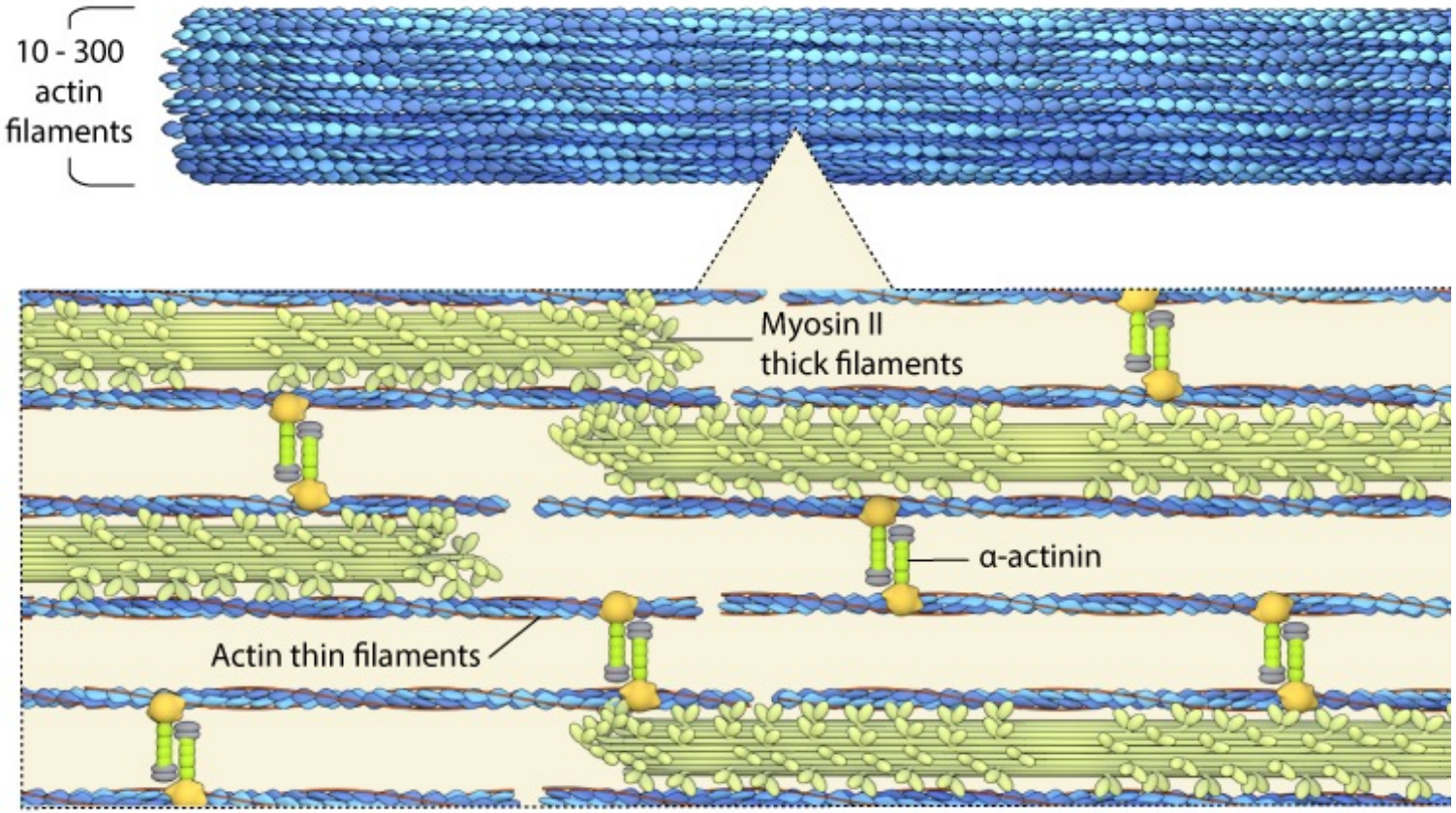
5.3. Myosin-II assembles into bipolar mini-filaments to generate force



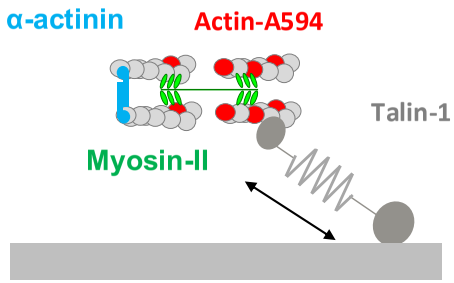
5.4. α-actinin cross-links actin filaments and generates enough spacing for myosin-II to insert and make the fiber contractile

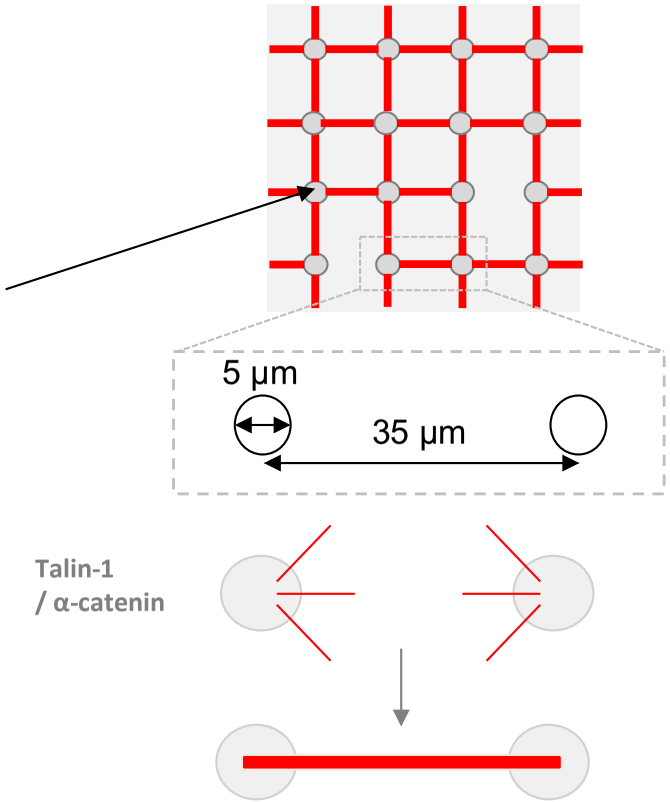


5.4. α-actinin cross-links actin filaments and generates enough spacing for myosin-II to insert and make the fiber contractile



Reconstitution of the self-organisation of tensile actomyosin cables between anchoring points with purified proteins



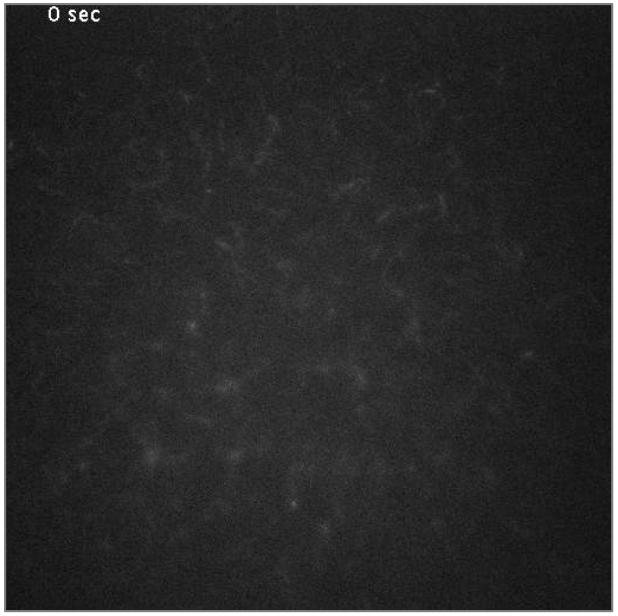


Artificial stress fiber

(Ciobanasu et al. 2014 Nat. comm., Ciobanasu et al. 2015 Nat. Protoc.)

Reconstitution of the self-organisation of tensile actomyosin cables between anchoring points with purified proteins

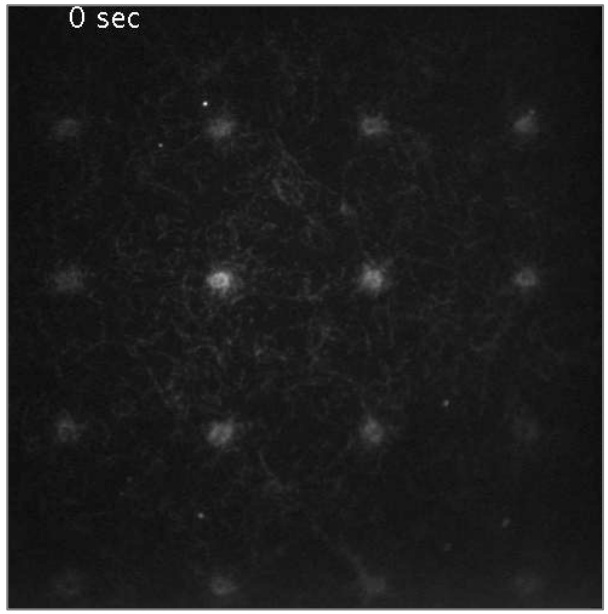
Actin Alexa 594



No Talin in the disks

+

Actin and myosin

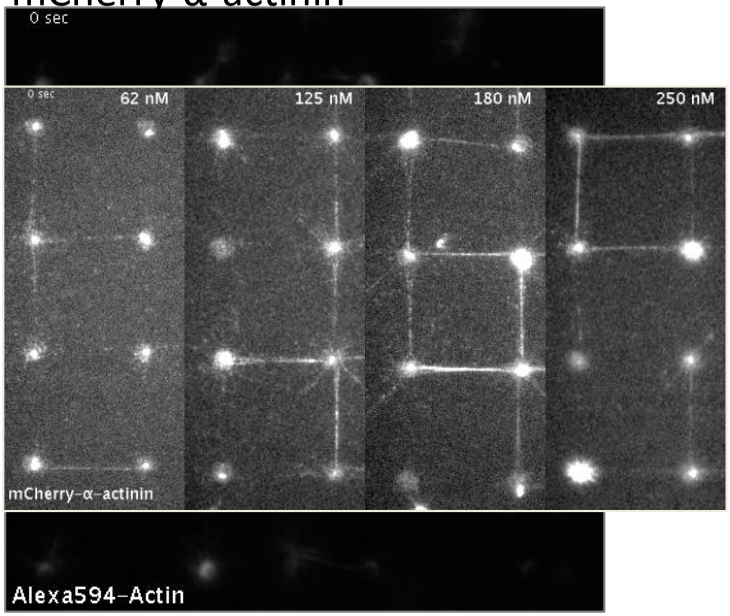


Talin in the disks

+

Actin and myosin

mCherry-α-actinin



Talin in the disks

+

Actin / myosin

+

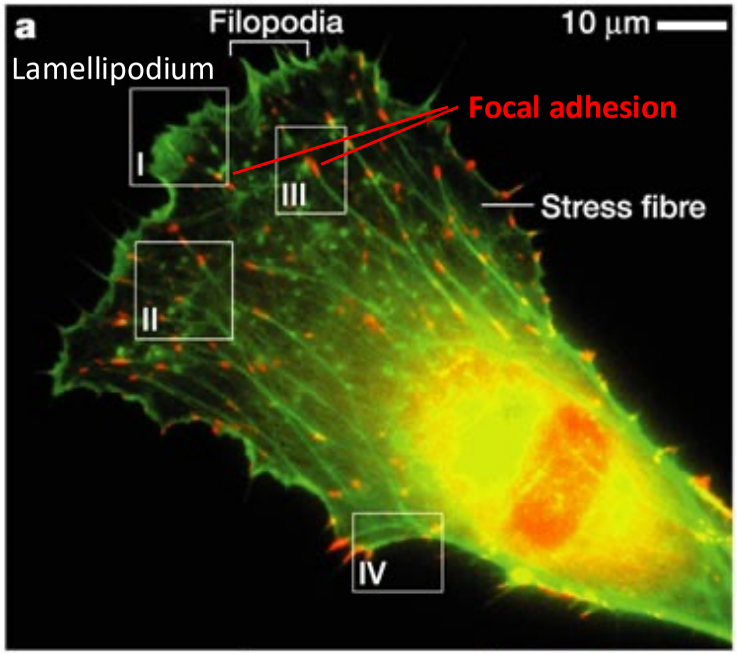
α-actinin

+

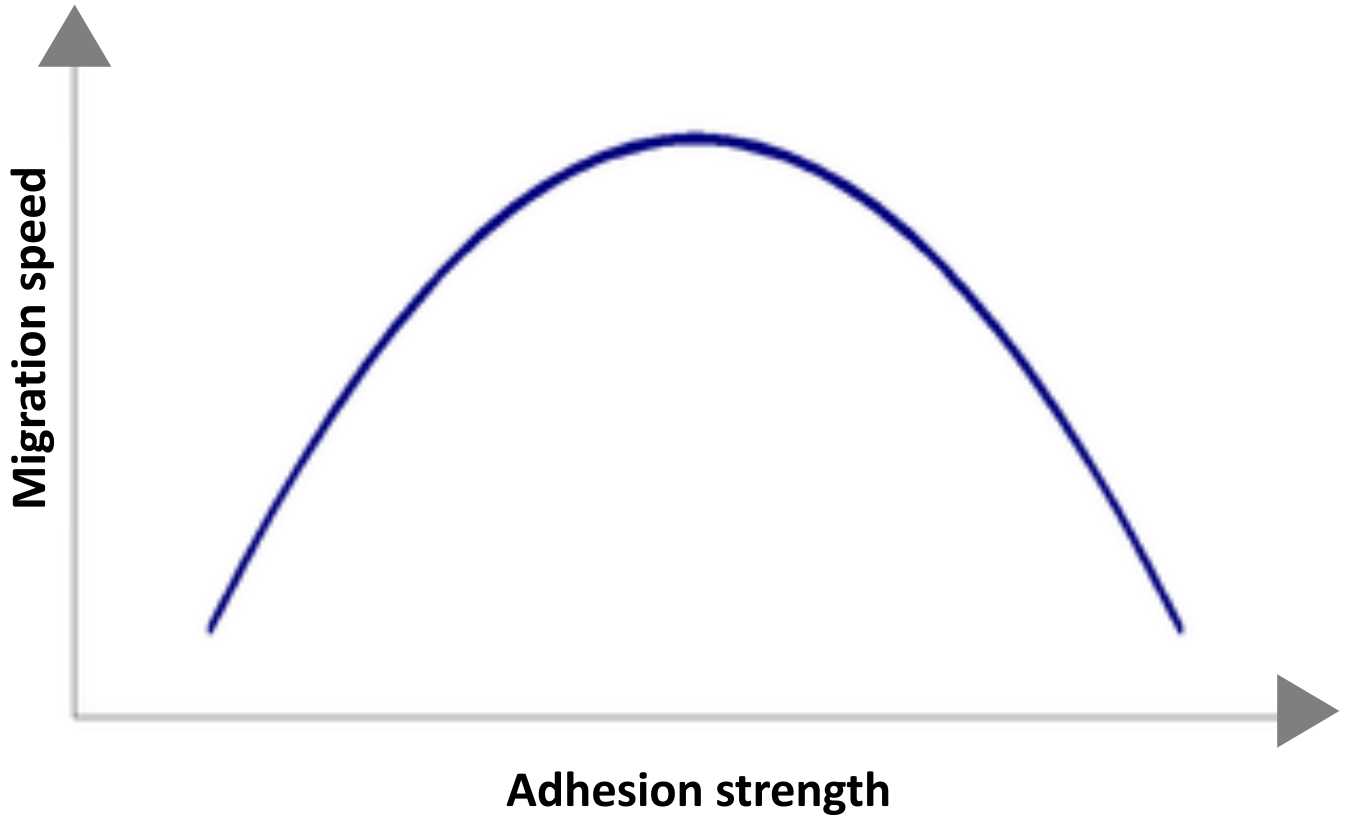
α-actinin

(Ciobanasu et al. 2014 Nat. comm., Ciobanasu et al. 2015 Nat. Protoc.)

6. Cell-matrix adhesion and cell migration

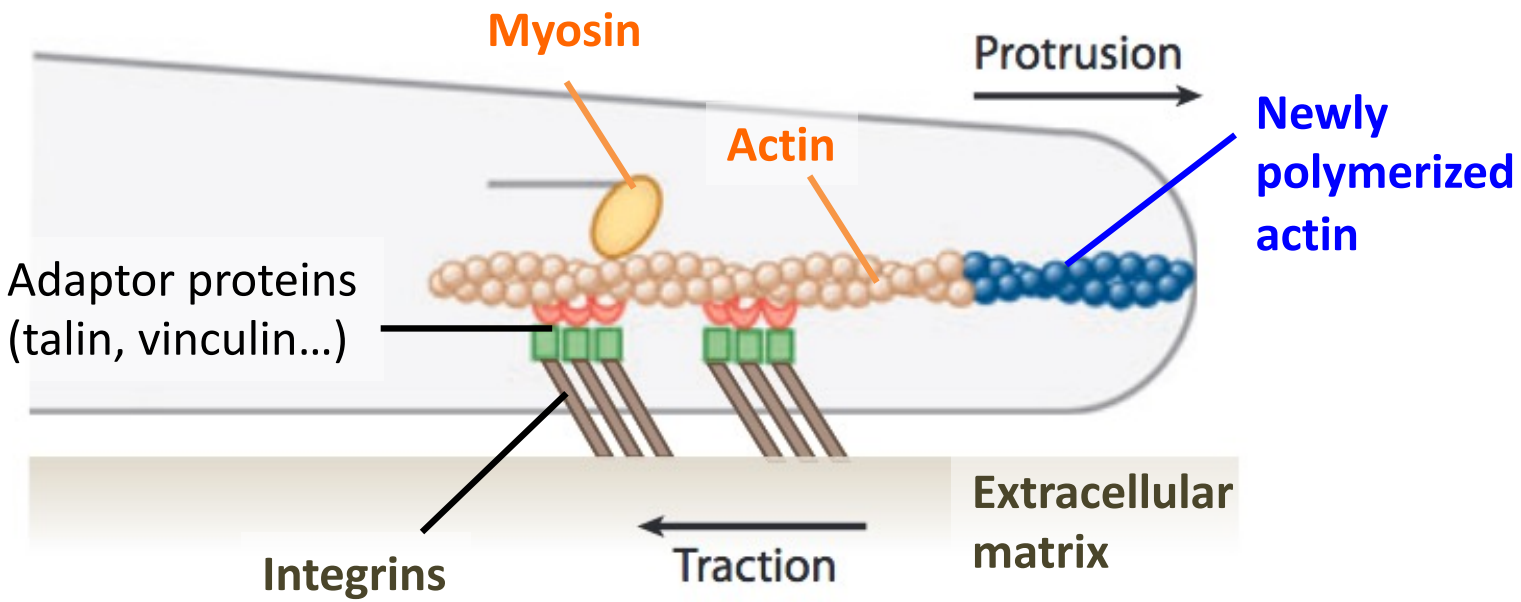


6.1. Relationship between cell-matrix adhesion strength and cell migration speed

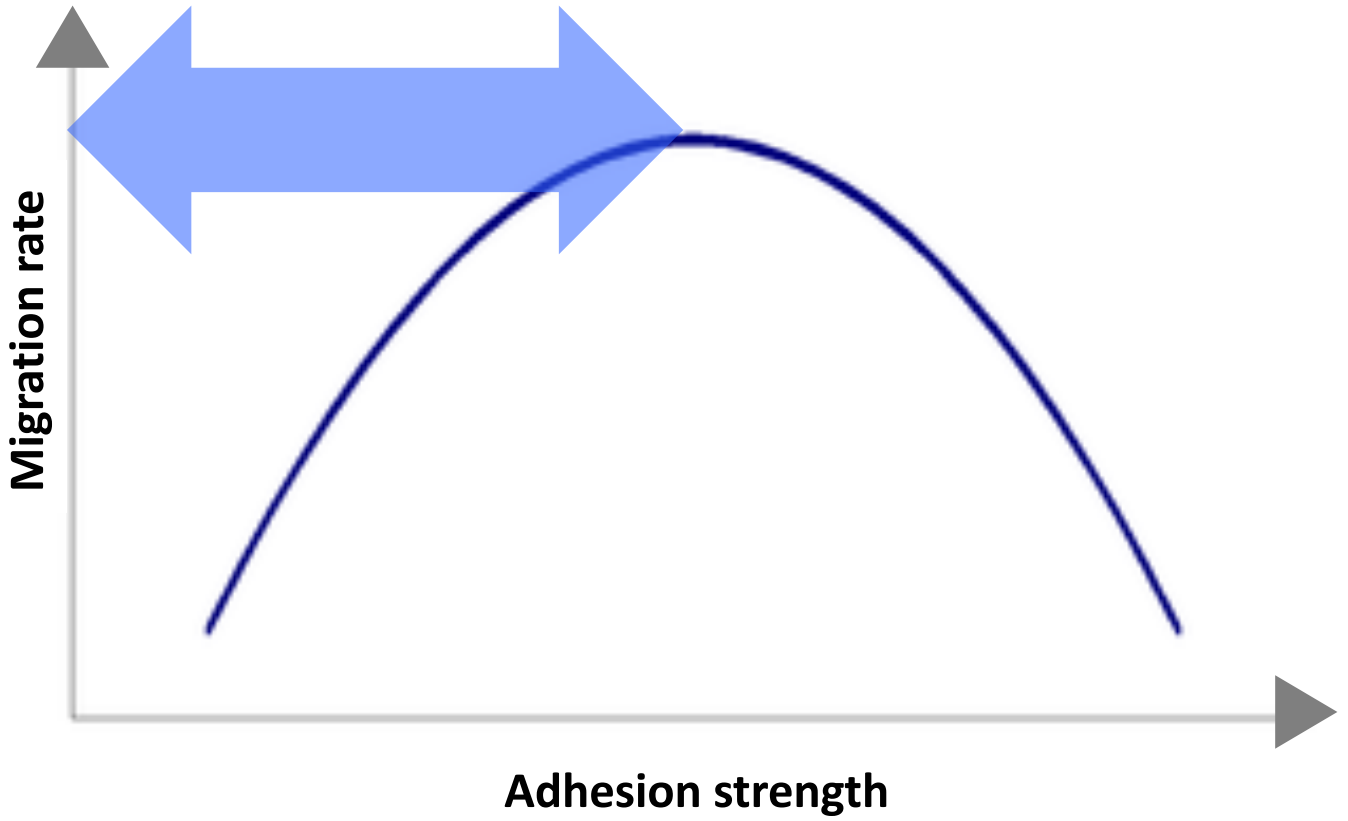


6.2. The molecular clutch concept

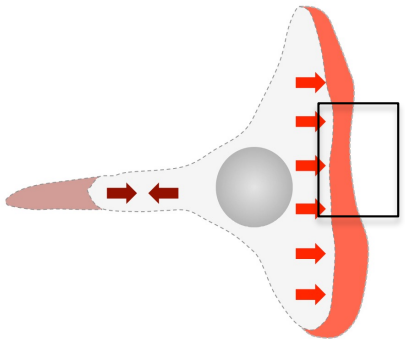
By mechanically coupling the polymerizing and contractile actin cytoskeletons to the extracellular matrix, cell-matrix adhesion act as a molecular clutch that control force transmission

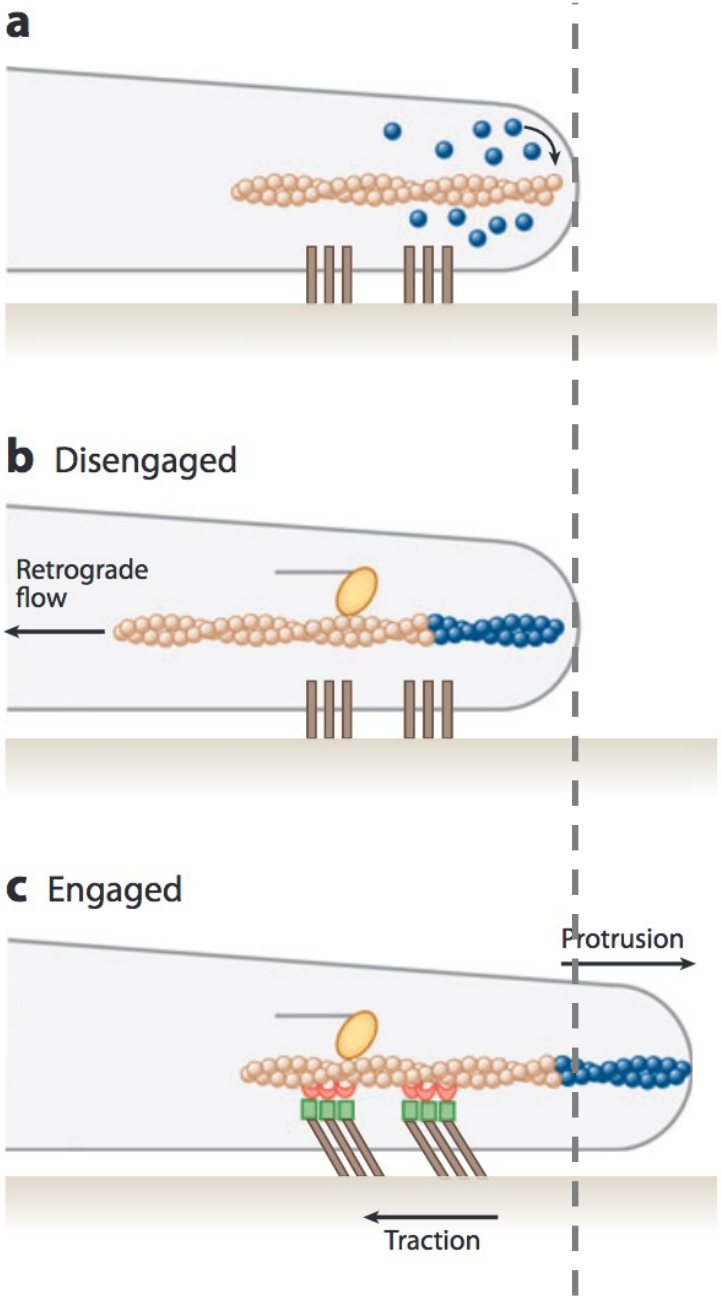


6.3. Relationship between low cell-matrix adhesion and cell migration



6.3. Relationship between low cell-matrix adhesion and cell migration

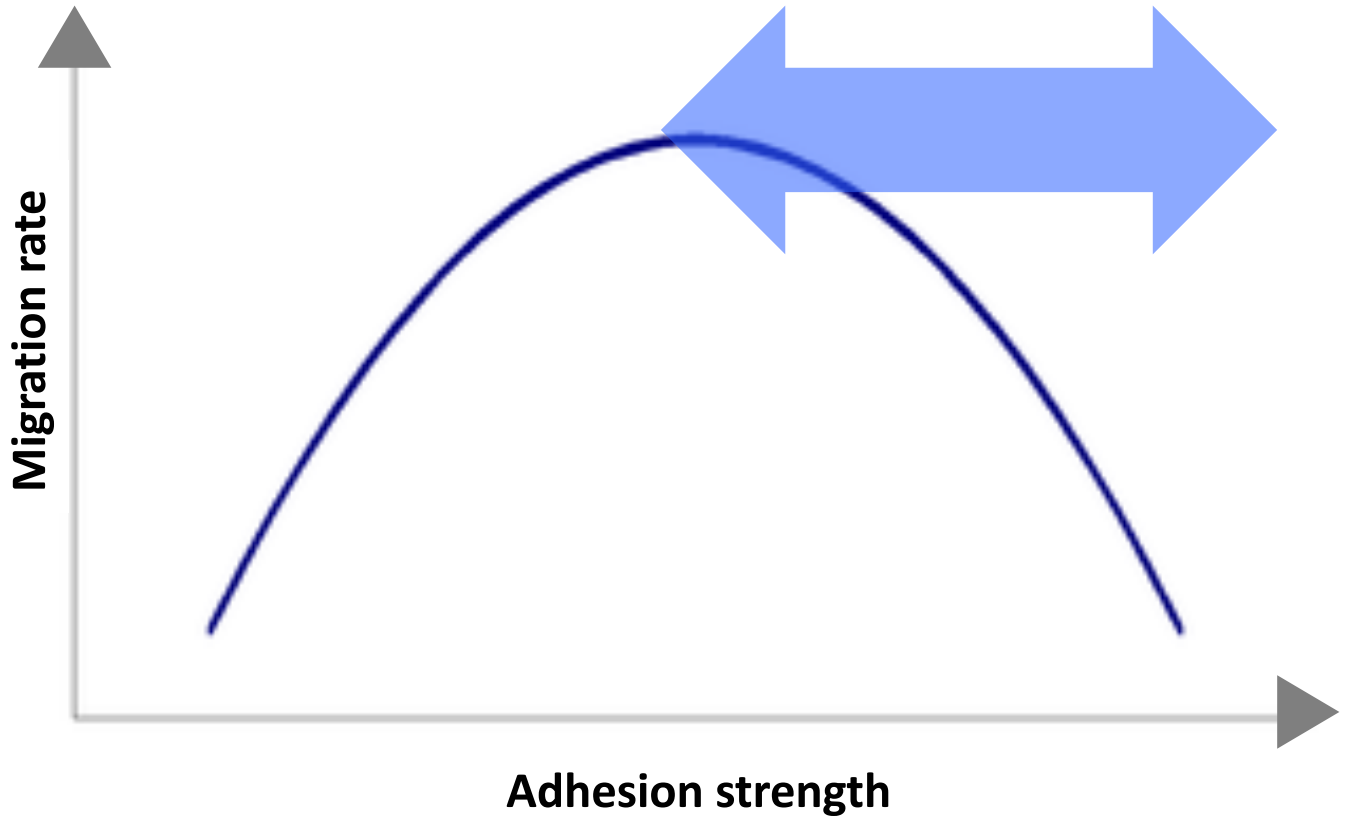




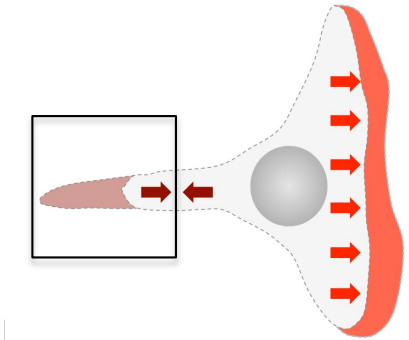
When the molecular clutch is disengaged, the pushing actin cytoskeleton in not anchored, it will not generate force on the membrane but move backwards as a retrograde flow

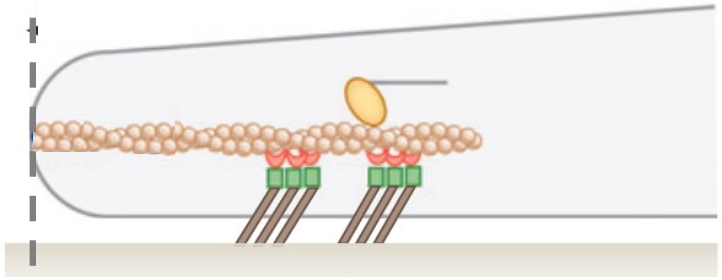
When the clutch is engaged, adhesion anchors the pushing actin cytoskeleton so that protrusion can occur instead of retrograde flow

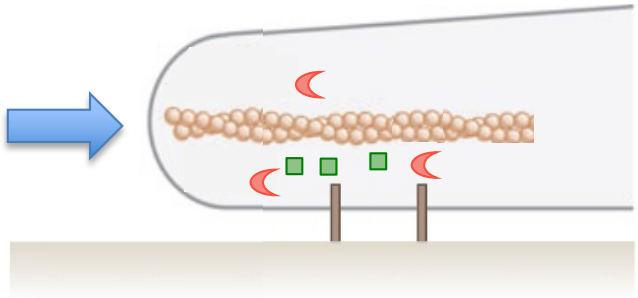
6.4. Relationship between high cell-matrix adhesion and cell migration

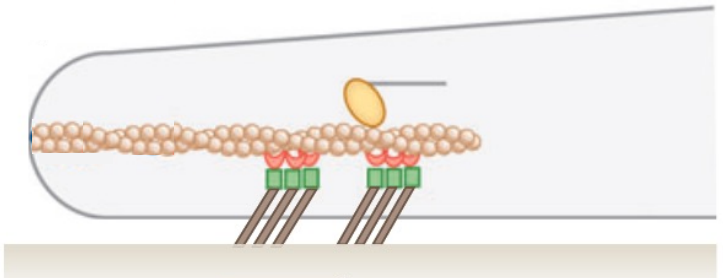


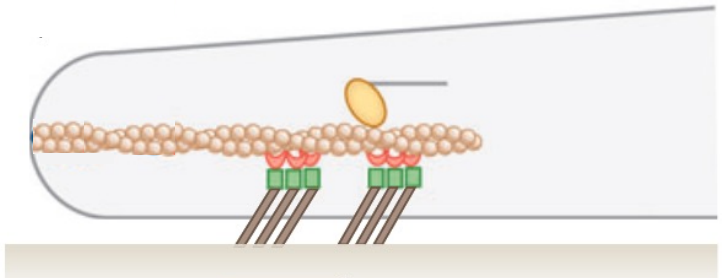
6.4. Relationship between low cell-matrix adhesion and cell migration











At the rear, adhesion detachement is necessary for actin and membrane to retract

At the rear, if adhesion is too strong, the cell does not detach and movement is impossible

6.5. Composition of the molecular clutch

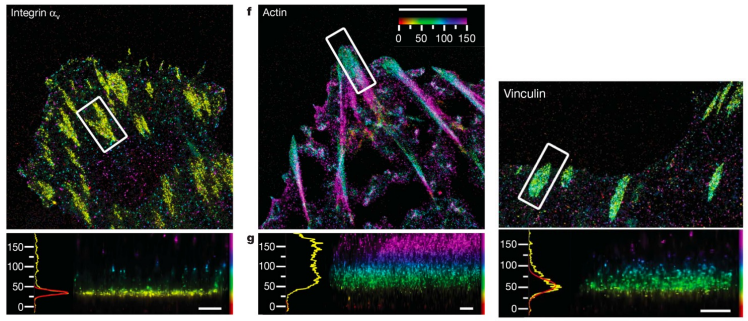
Super resolution 3D imaging of focal adhesions (PALM)

From the top

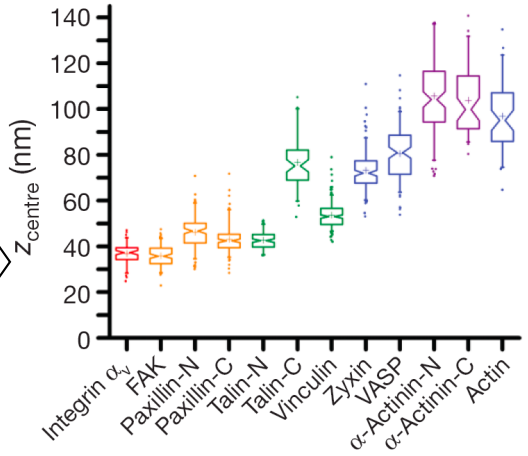
From the side

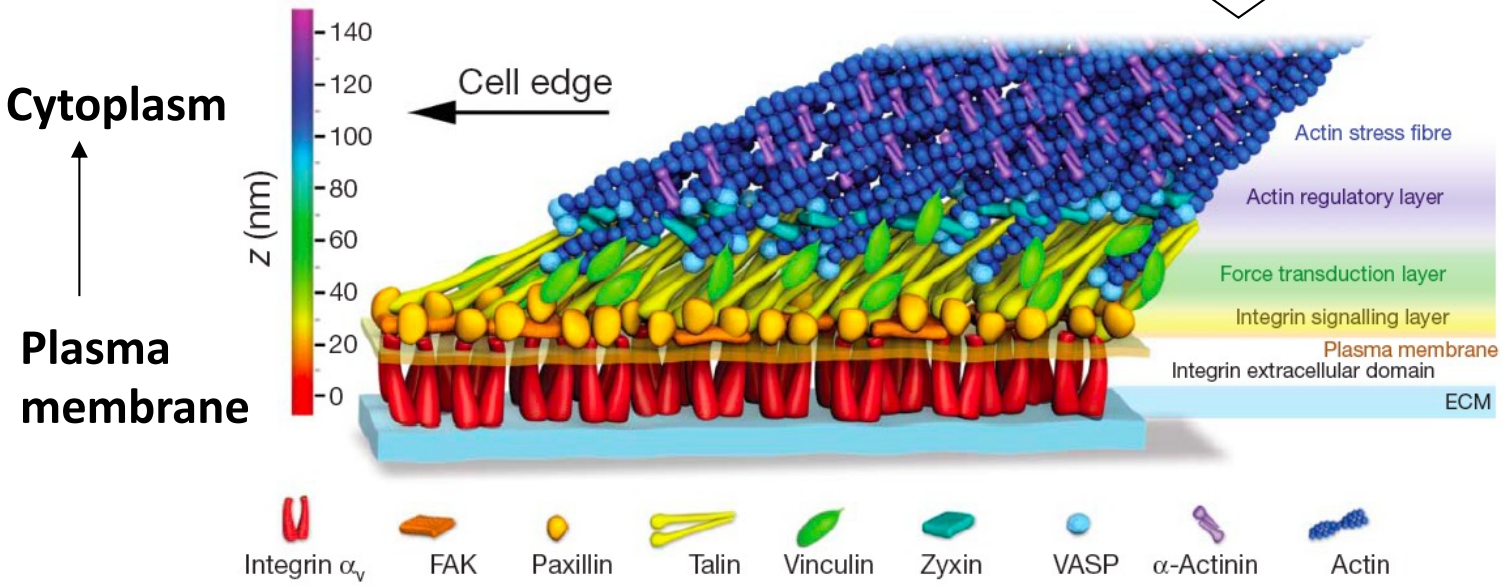
Cytoplasm

Plasma membrane

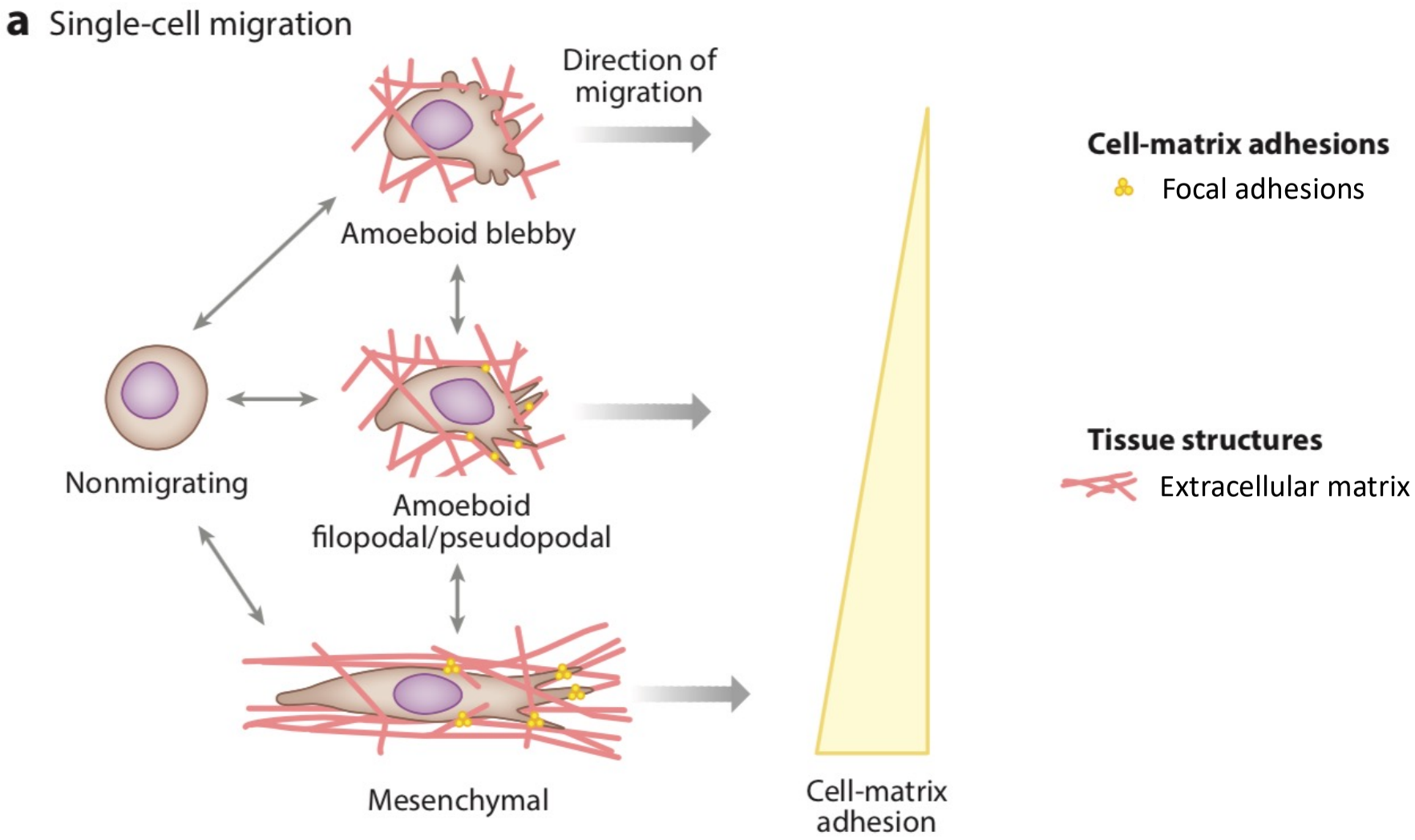


Kanchanawong et al, 2011





6.6. The strength of cell-matrix adhesion dictates the migration mode



7. Role of microtubules in cell migration

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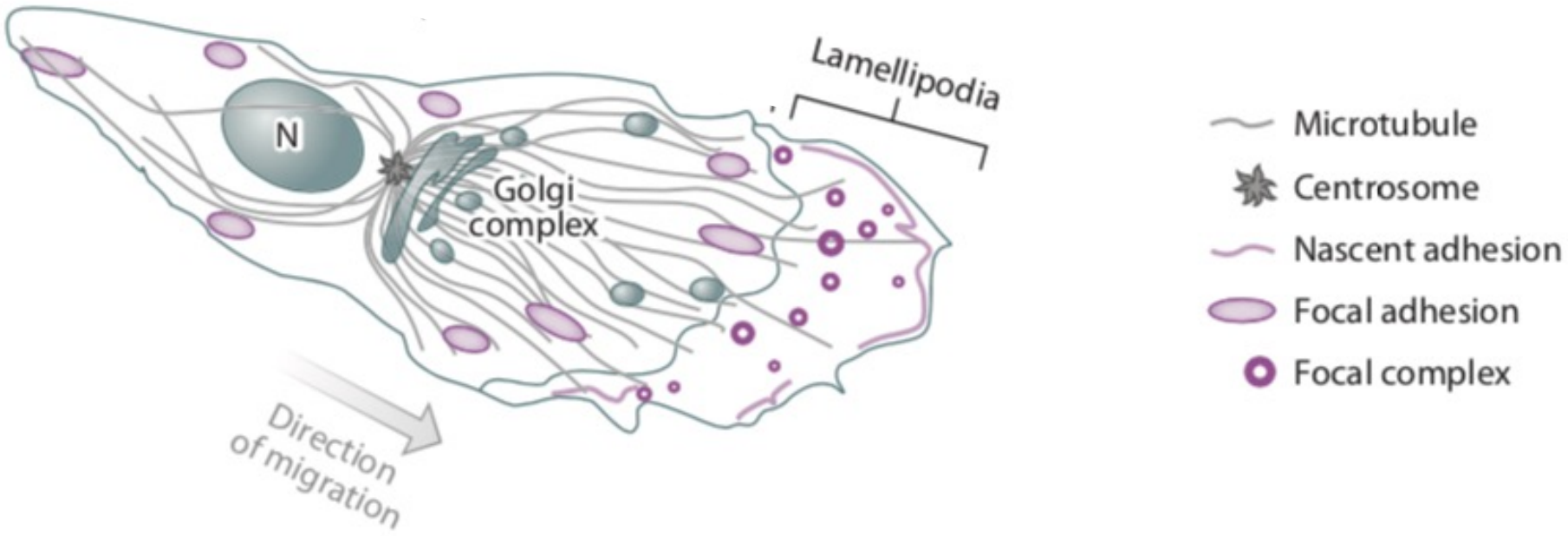


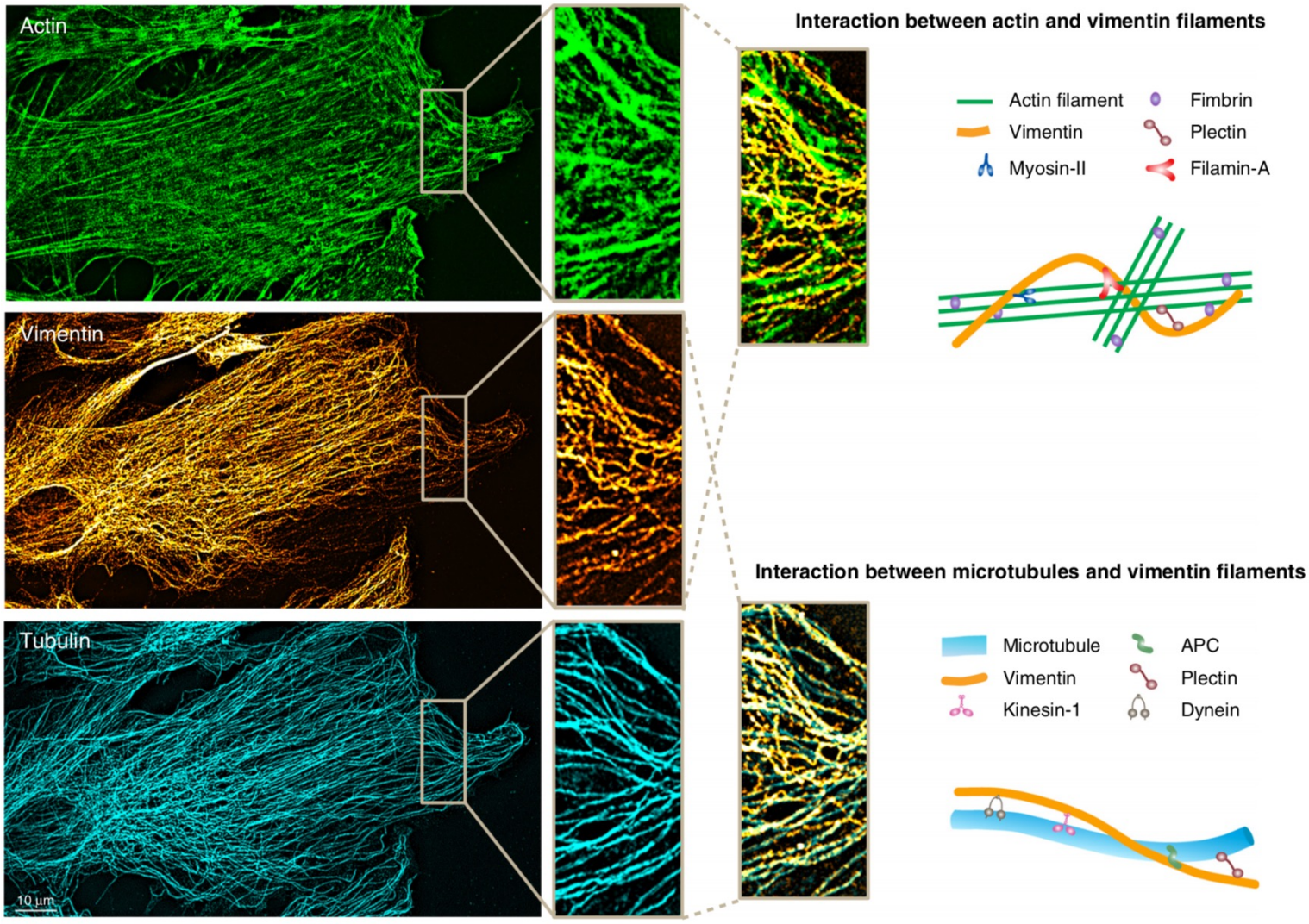
7. Role of microtubules in cell migration

1) Microtubules assembly does not provide force for cell migration

2) Microtubules provide polarity to the cell

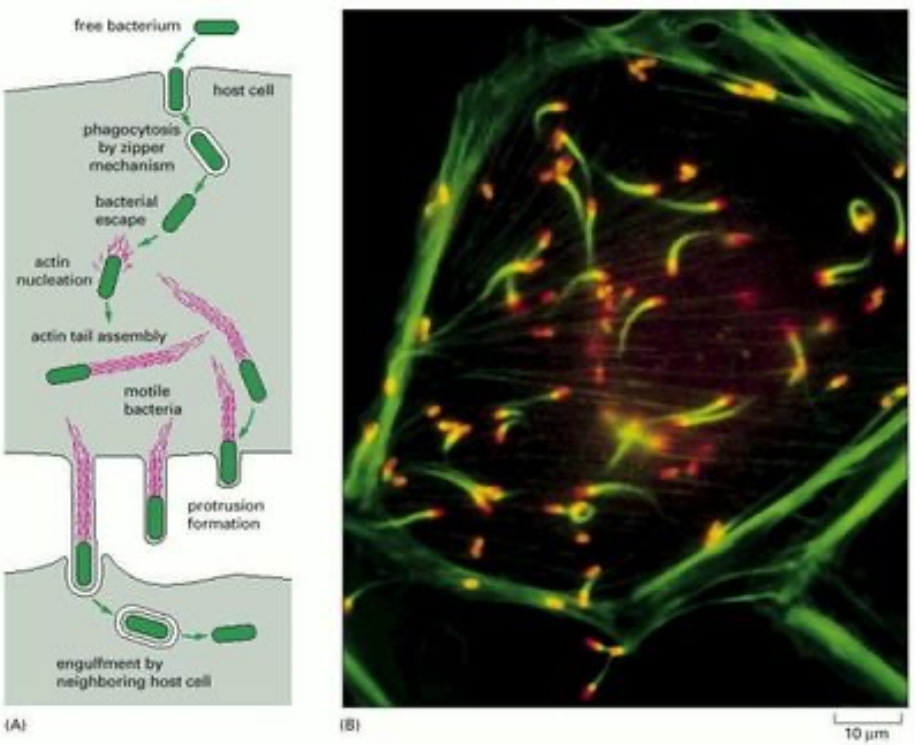
3) Microtubules control adhesion dynamics

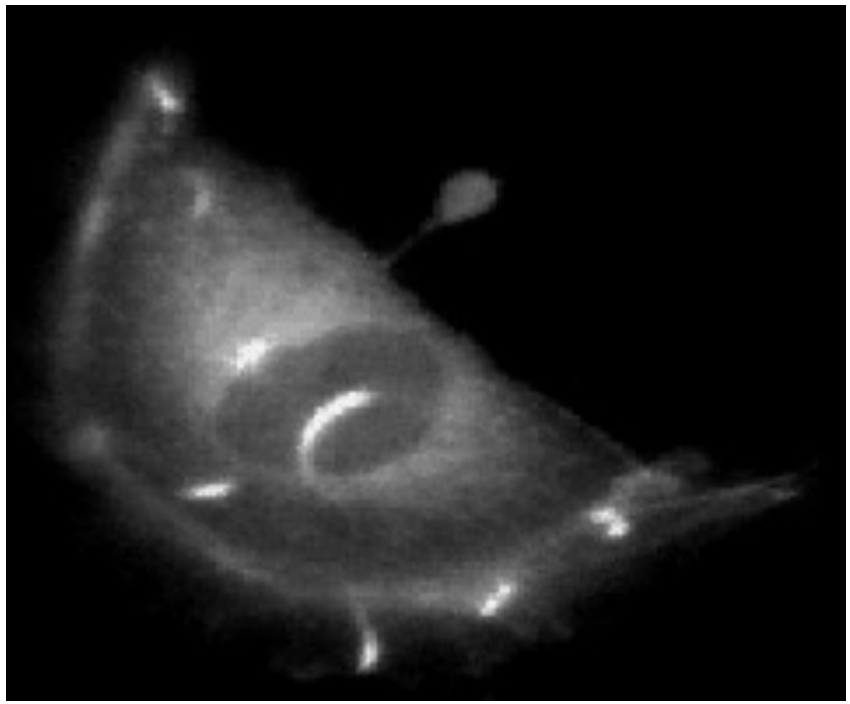
More concepts to unravel in the future because the three cytoskeletons (actin, microtubules, intermediate filaments) interact together in migrating cells



8. Pathogens that hijack the regulators of actin assembly reveal the mechanisms of cellular actin-based motility

Listeria comet tail

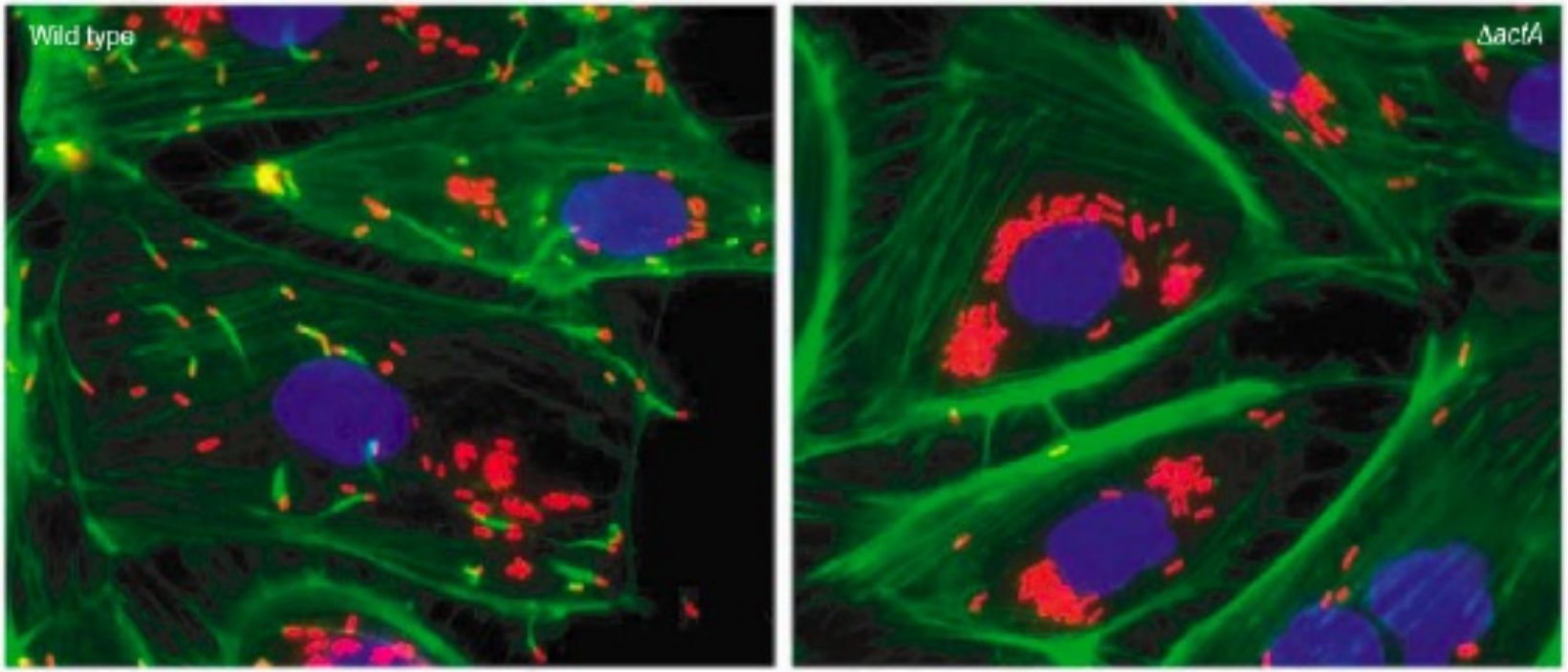


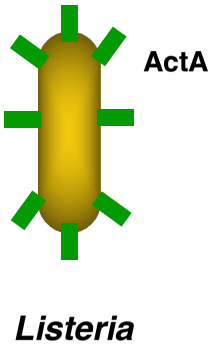


Cell transfected with GFP-actin
and infected by Listeria

Theriot web site

The bacterial protein ActA is necessary for Listeria comet tails

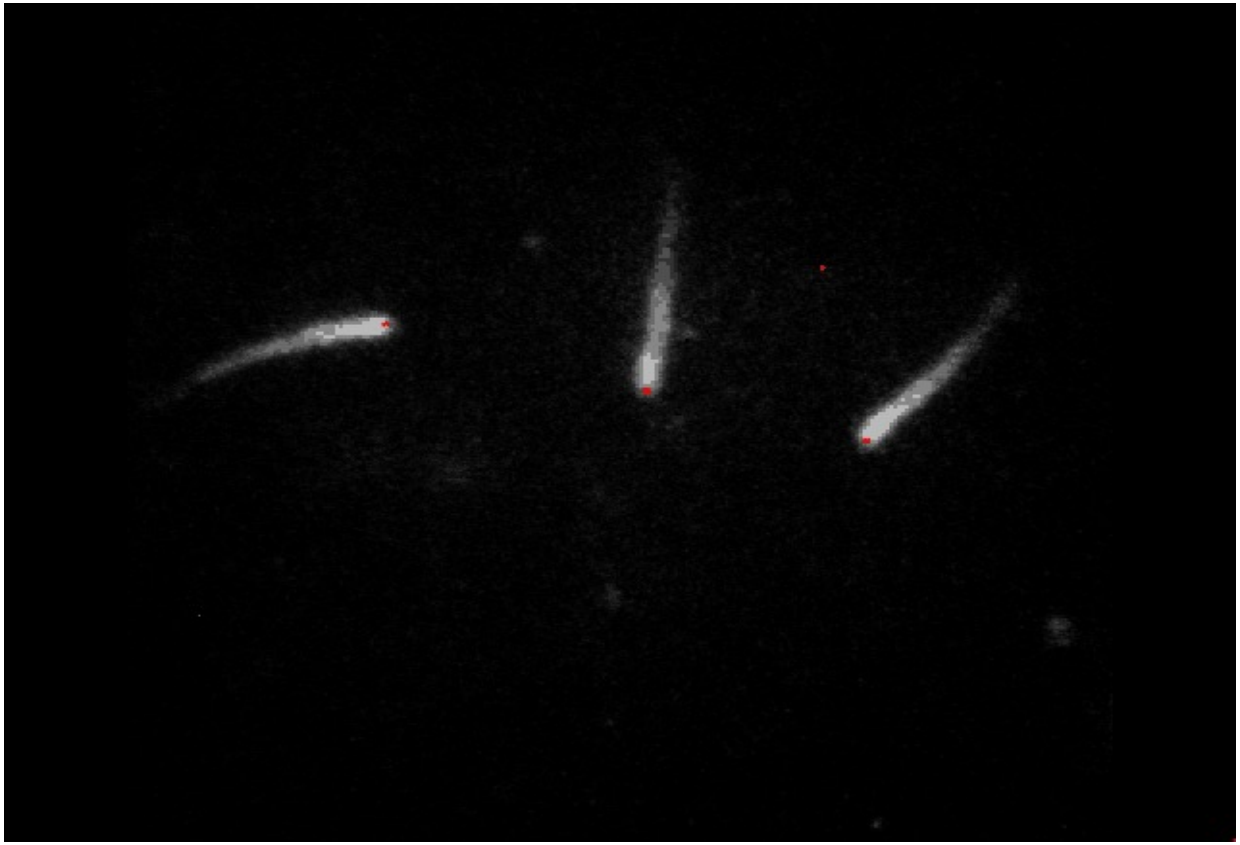


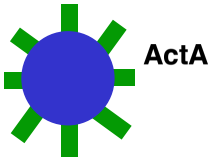


Red : Listeria green : actin filaments blue : nuclei

Kochs et al. Cell 1994. From the group of Pascale Cossart (Institut Pasteur).

ActA is sufficient to move beads in cytosolic extracts





Cameron et al., Curr Biol. 1999

Purification of the host factor that polymerizes actin

The assay:

Listeria
+
extract
+
Rhodamine actin
=
Comet tails

Welch et al. Nature 1997

Fractionation of the extract

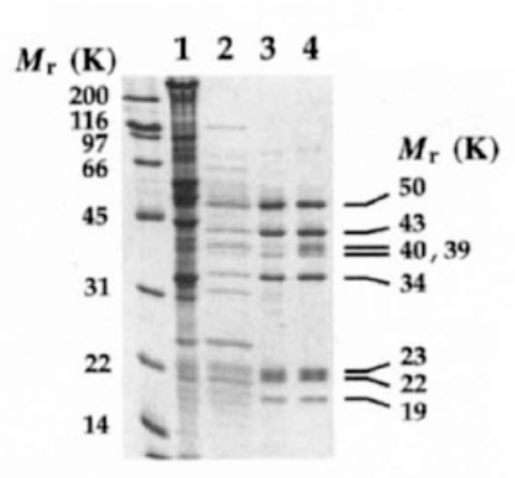
Listeria
+
Inactive fraction
+
Rhodamine actin
=
nothing happens

Listeria
+
Active fraction
+
Rhodamine actin
=
Actin clouds

Clouds instead of comets: the whole process is complex and requires more than one fraction !

Welch et al. Nature 1997

Fractionation of the extract by successive chromatographies

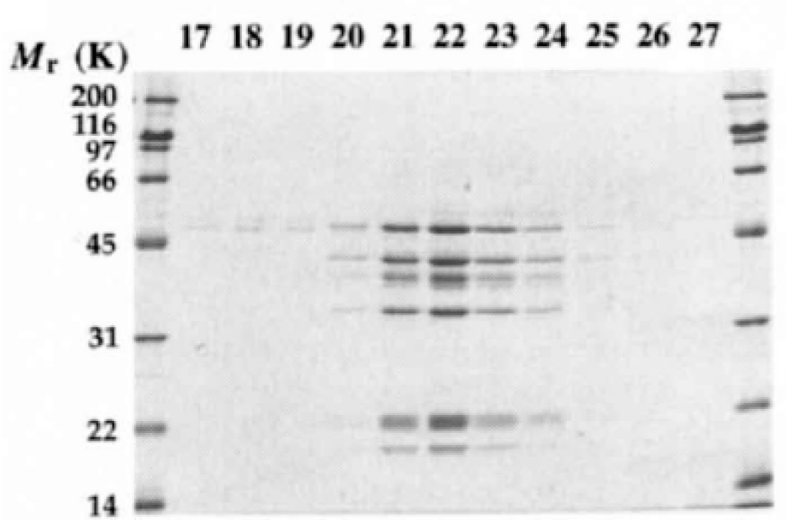


	protein amount (mg)	Specific activity (a.u./mg)
1 complete extract	91	880
2 cation exchange flow-through	7.3	2,200
3 anion exchange peak	1.7	10,600
4 gel filtration	0.6	3,300

Welch et al. Nature 1997

Gel filtration in the last purification step identifies the 7 subunits of Arp2/3

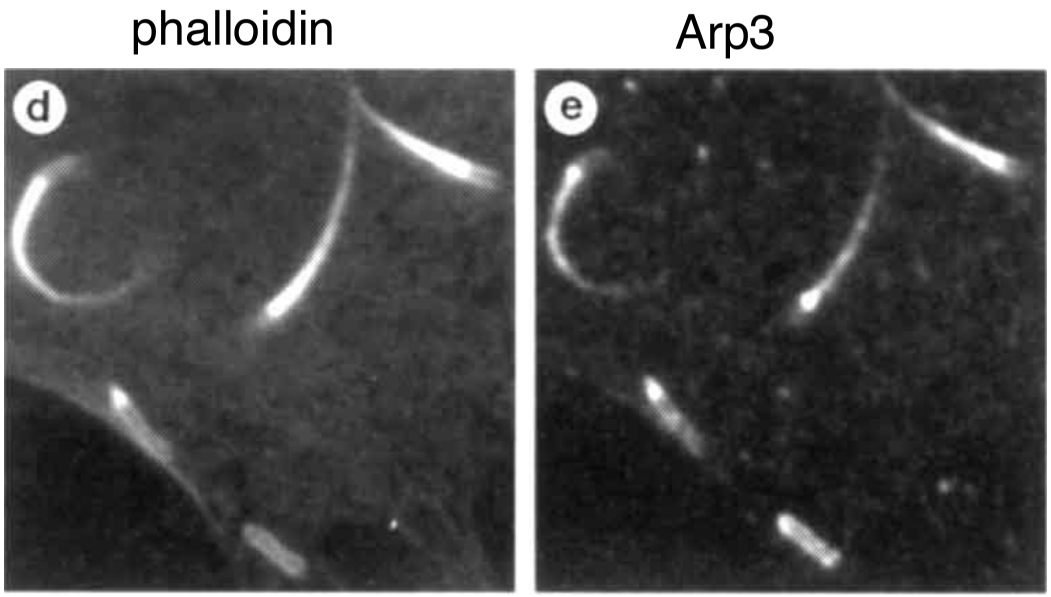
elution fractions



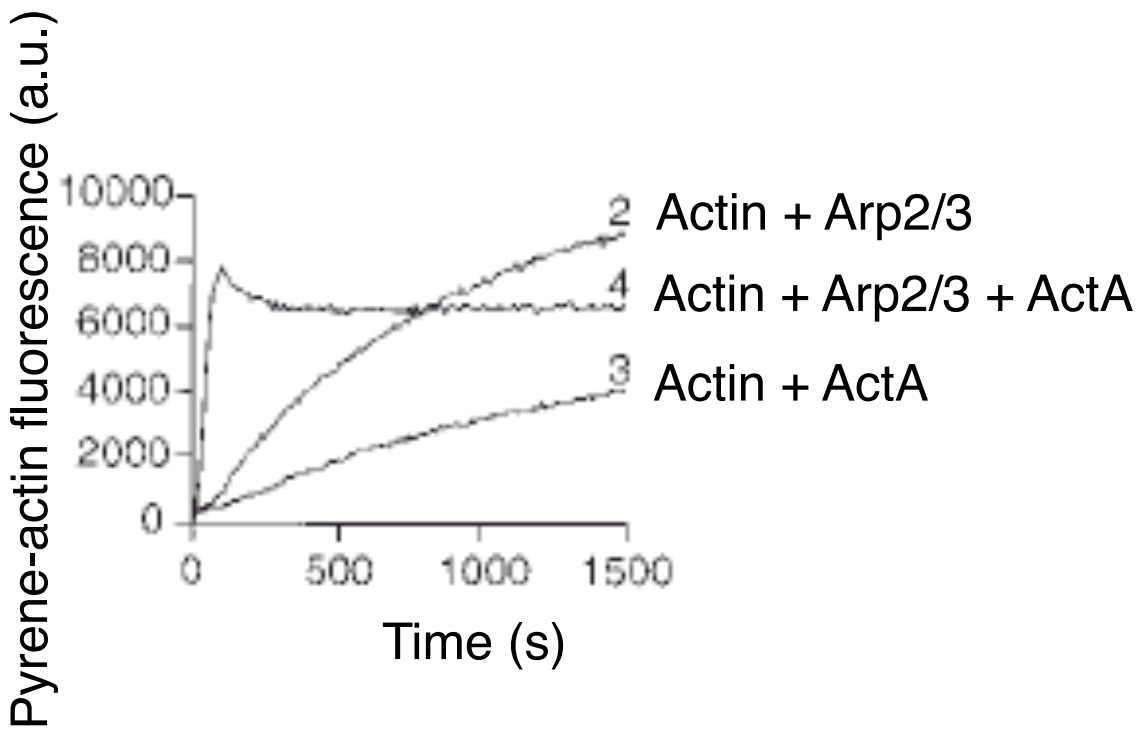
it is a multiprotein complex
identification of the subunits by mass spec
it contains two Actin Related Proteins: Arp2 and Arp3

Welch et al. Nature 1997

Validation: Immunofluorescence on the Listeria induced comet tails

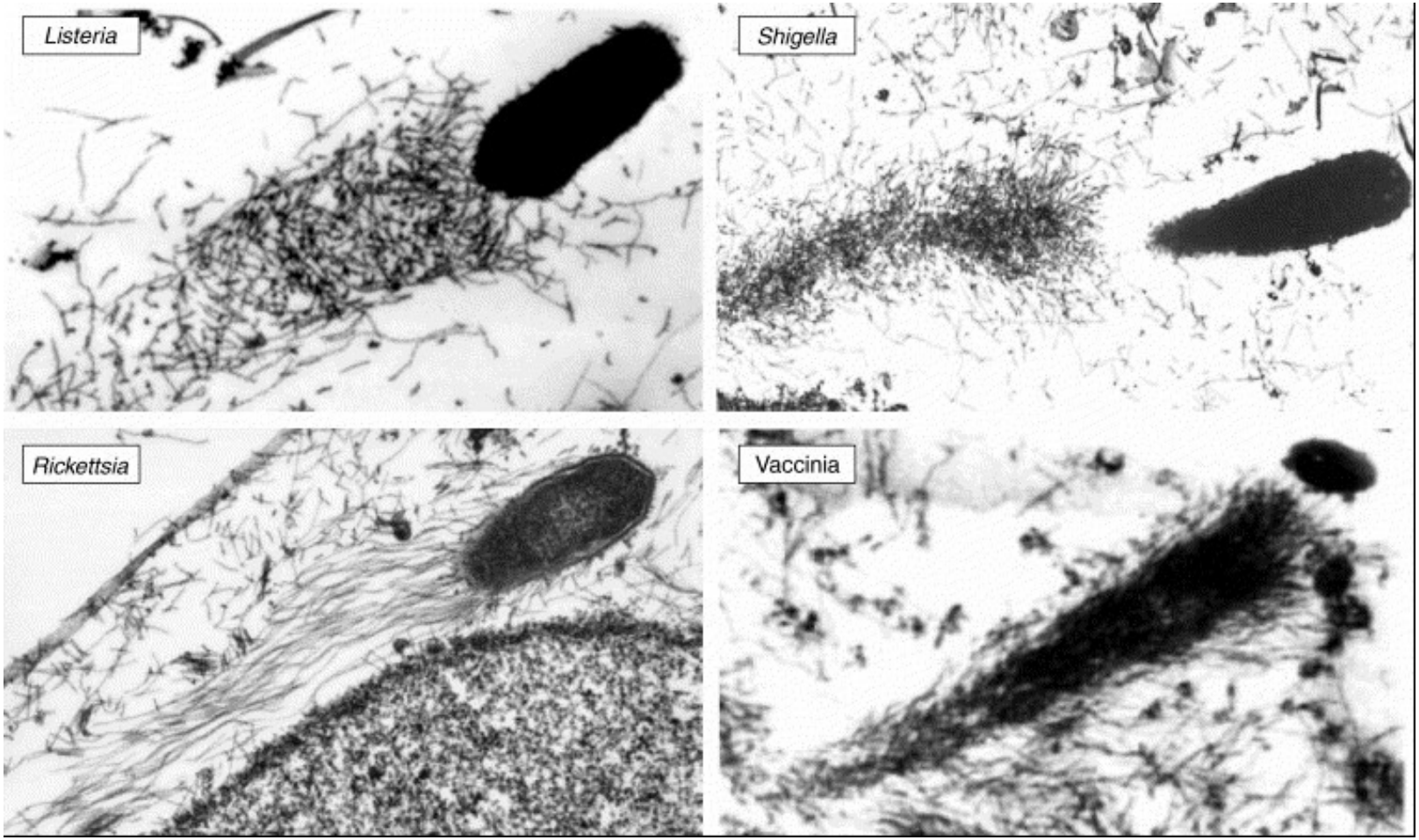


ActA directly activates the Arp2/3 complex



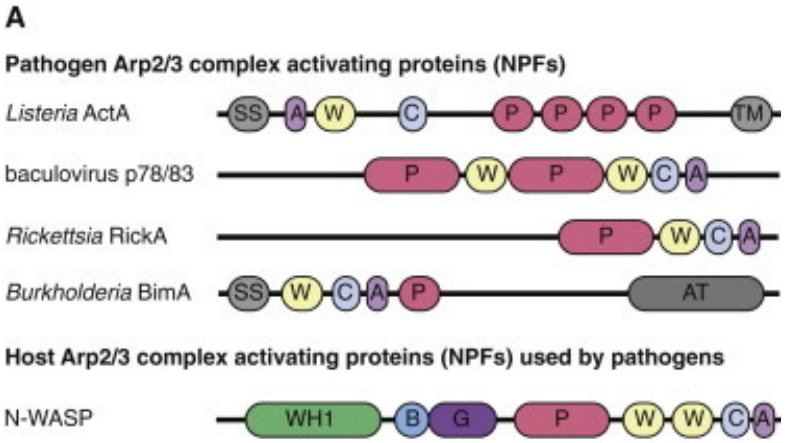
Welch et al. Science 1998

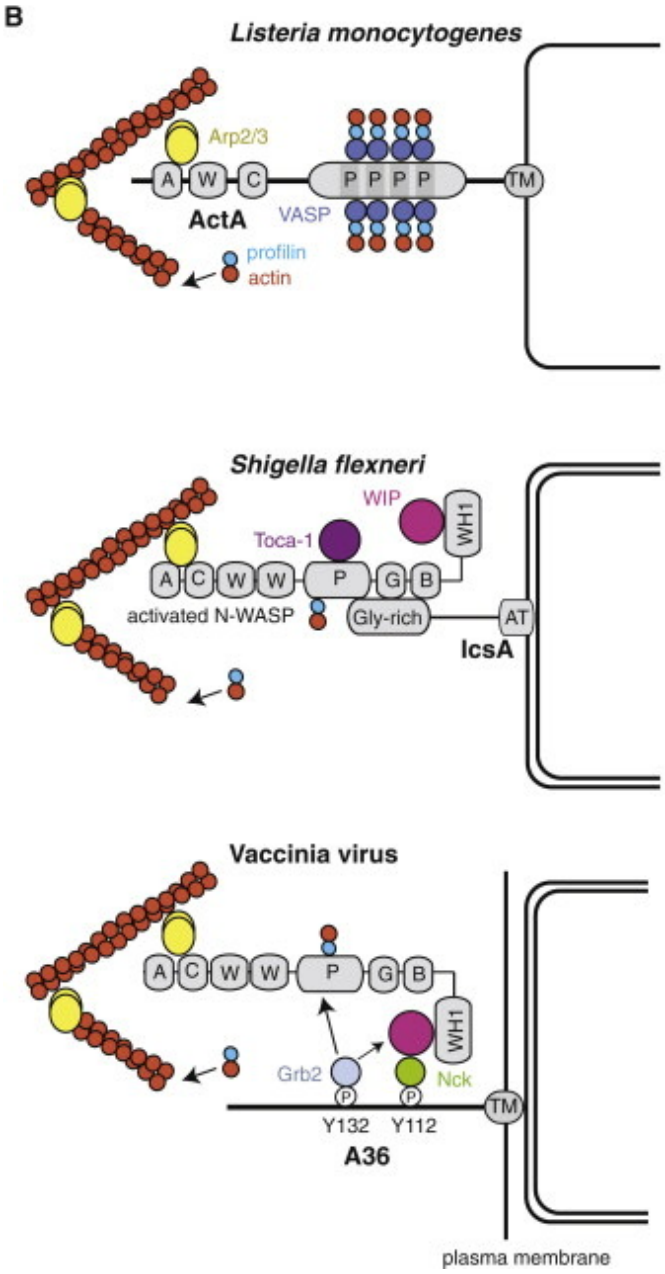
Other pathogens hijack the cellular actin machinery to propel in the cytoplasm



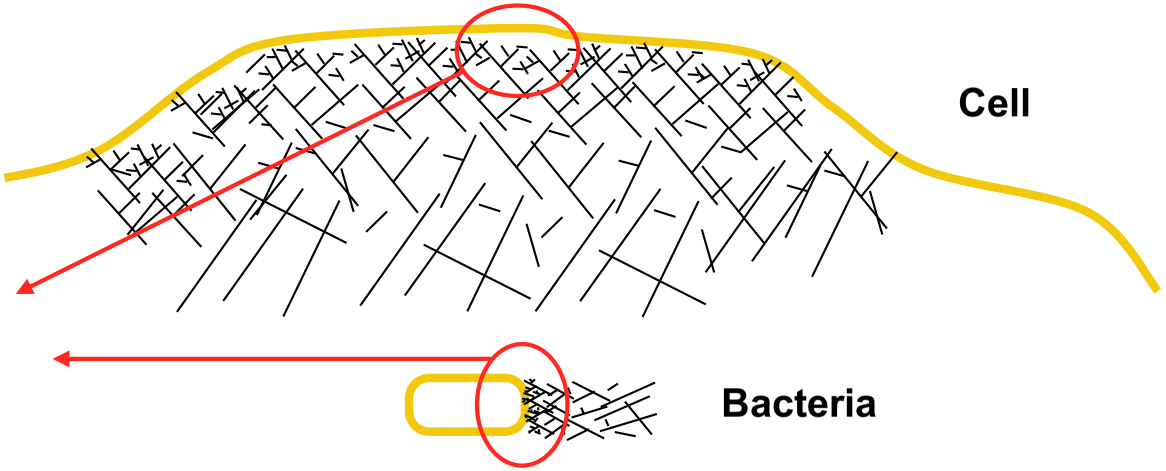
Gouin, Welch and Cosart, 2005

Subversion of Arp2/3 by bacteria and viruses



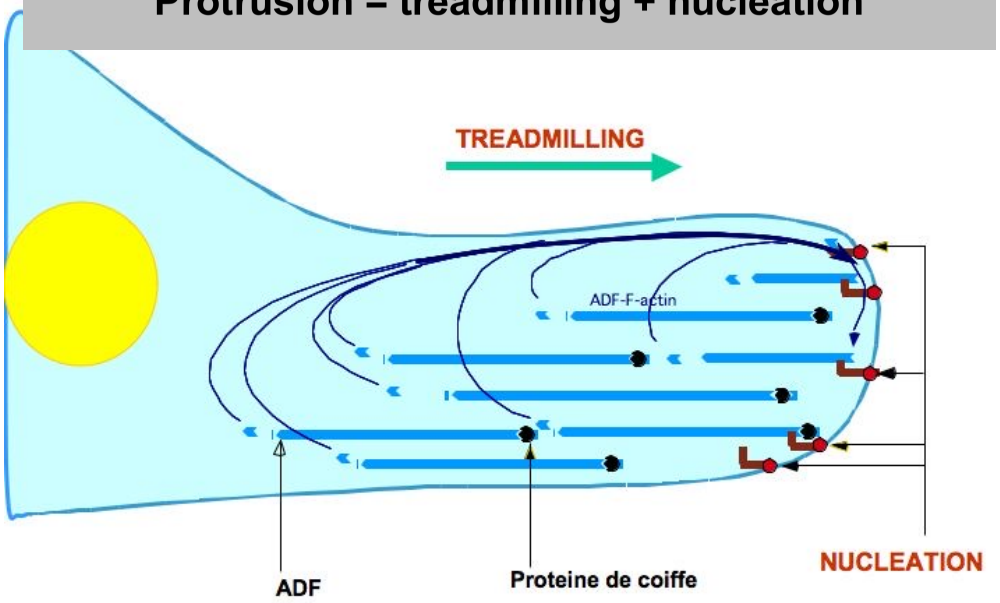


Bacteria propulsion mimicks lamellipodium protrusion

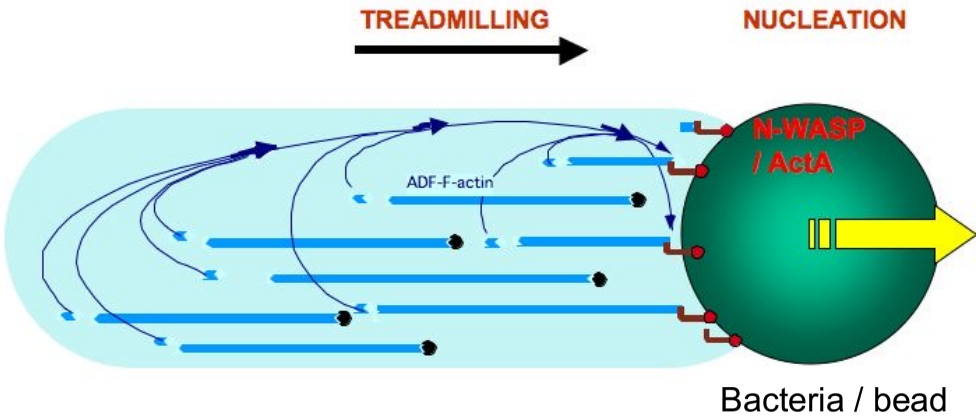


Identical biochemical reactions

Protrusion = treadmilling + nucleation



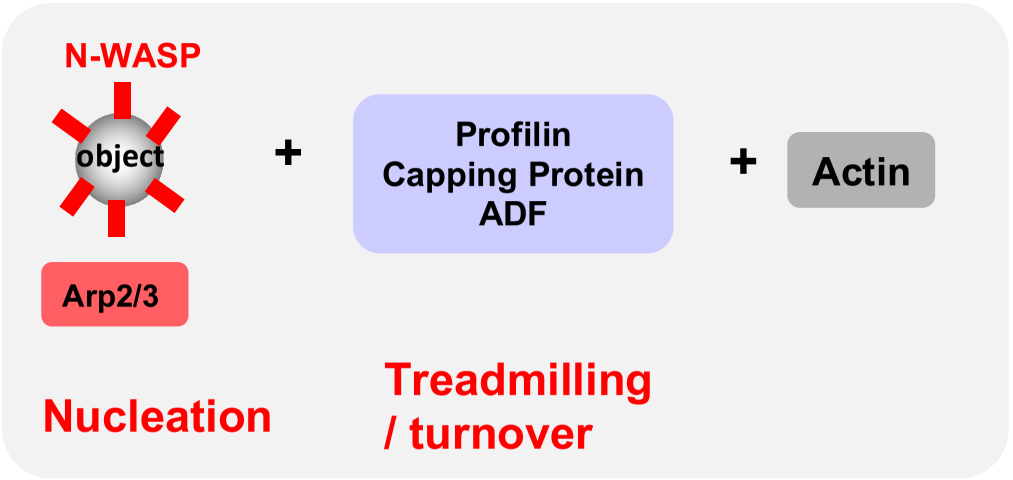
Propulsion = protrusion



Bacteria / bead

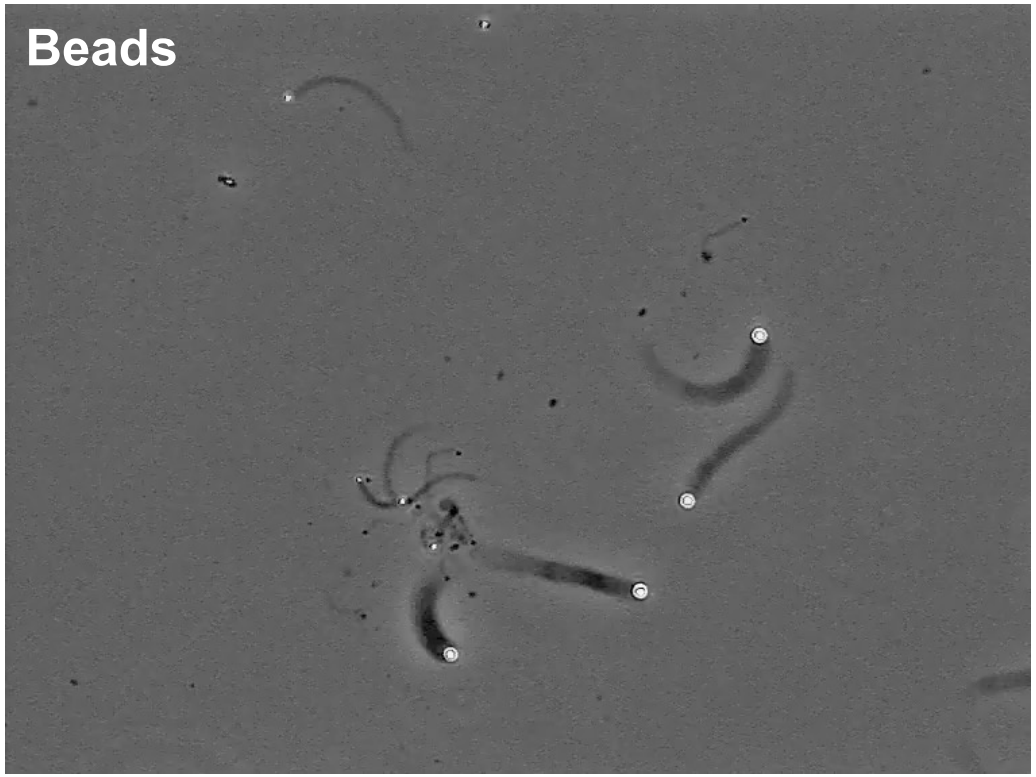
Reconstitution with pure proteins provides the demonstration that we understand the basic principle of bacterial propulsion / lamellipodium protrusion

Reconstitution of actin based motility (1)

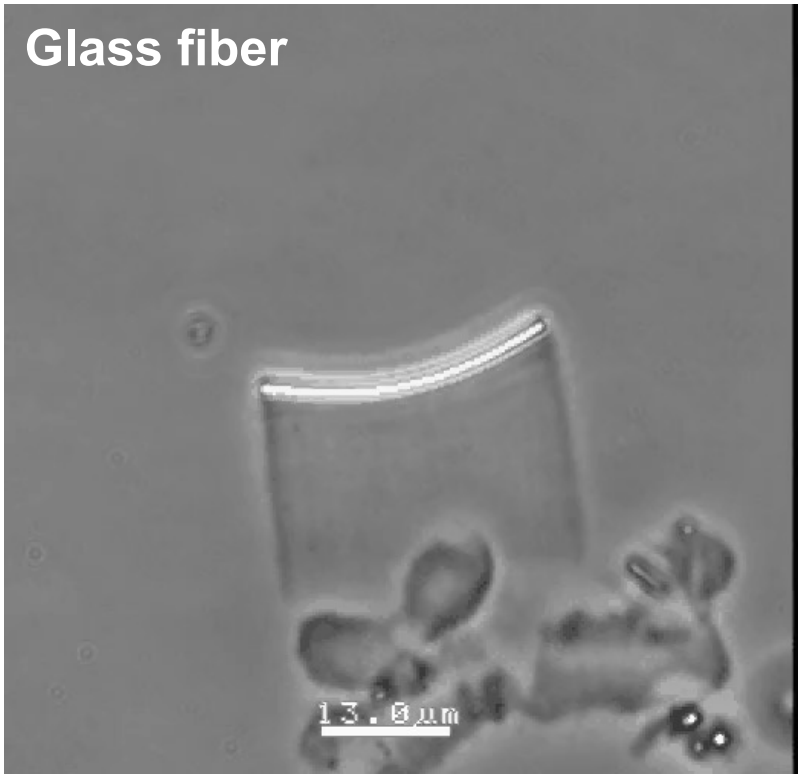


Movement ?

Reconstitution of actin based motility (2)

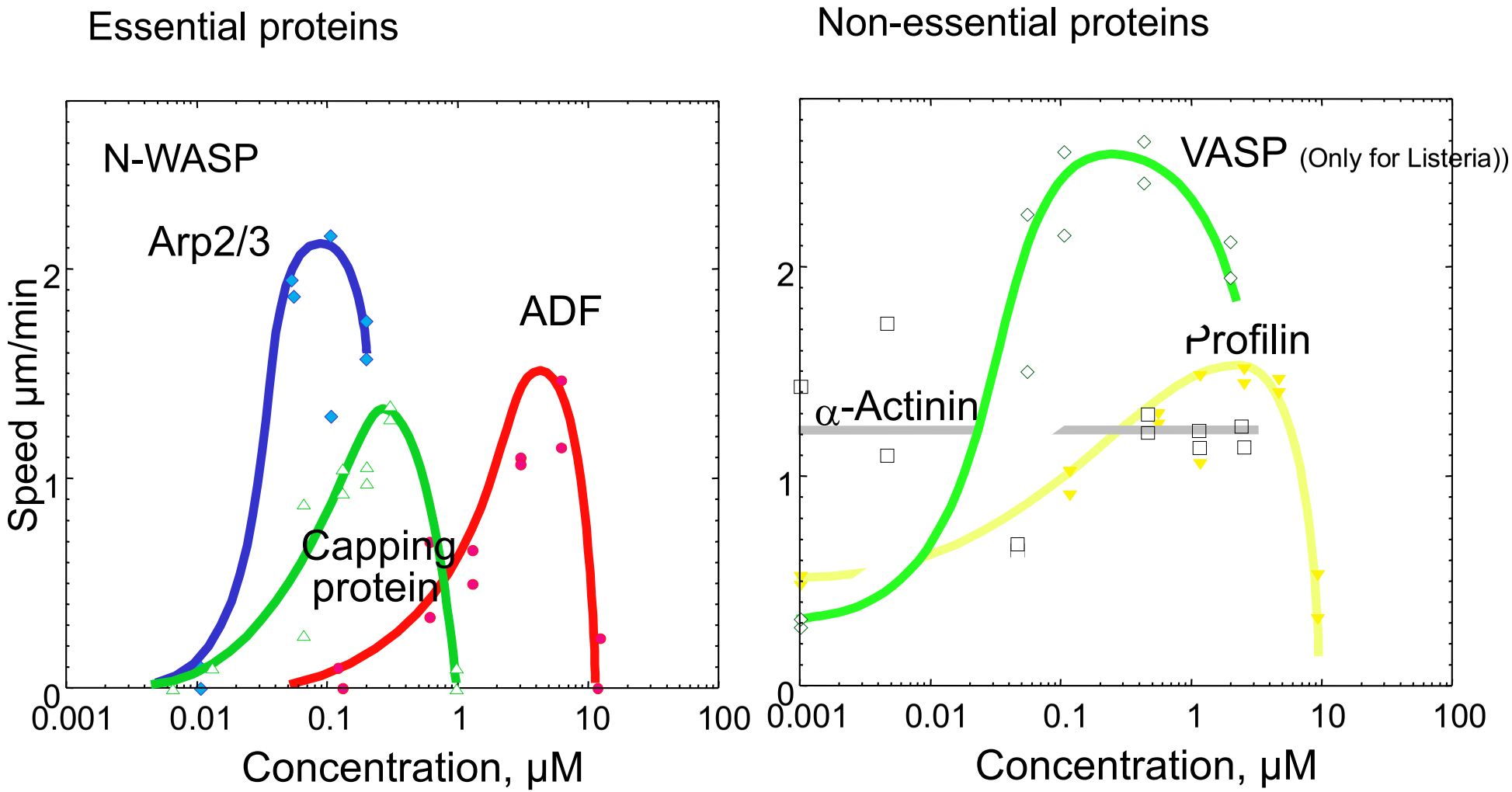


From S. Wiesner et al, 2003



From S. Wiesner

Reconstitution of actin based motility (3)



Loisel et al, 1999