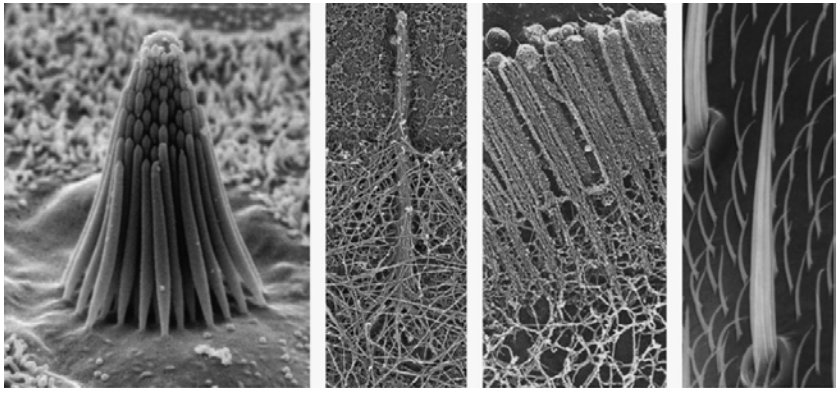
4. the cytoskeleton - fiber bundle models



bathe, heussinger, claessens, bausch, frey [2008]

day	date		topic	notes	material
tue	apr	03	introduction I - cell biology		s01 q01
thu	apr	05	introduction II - cytoskeletal biology, stem cells	n02	s02 l02
tue	apr	10	introduction III - structural mechanics	n03	s03
thu	apr	12	biopolymers I - energy, tension, bending	n04	s04
thu	apr	12	homework I - biopolymers, directed stem cell differentiation	h01	m04
tue	apr	17	biopolymers II - entropy, FJC and WLC model	n05	s05
thu	apr	19	biopolymers III - polymerization kinetics in amoeba	n06	s06 m06
tue	apr	24	cytoskeletal mechanics I - fiber bundle model for filopodia	n07	s07 m07
thu	apr	26	cytoskeletal mechanics II - network model for red blood cells	n08	s08
thu	apr	26	homework II - cytoskeleton, cell mechanics challenges	h02	m10
tue	may	01	cytoskeletal mechanics III - tensegrity model for generic eukaryotic cells	n09	s09 m09
thu	may	03	biomembranes I - micropipette aspiration in white blood cells and cartilage cells	n10	s10
tue	may	08	biomembranes II - lipid bilayer, soap bubble, cell membrane	n11	s11
thu	may	10	biomembranes III - energy, tension, shear, bending	n12	s12
tue	may	15	mechanotransduction I - inter- and intracellular signaling, bone cells	n13	s13
tue	may	15	homework III - micropipette aspiration, final project	h03	m12
thu	may	17	summary and midterm preparation	n14	s14
tue	may	22	midterm		
thu	may	24	mechanotransduction II - electrophysiology in nerve cells	n16	s16
tue	may	29	mechanotransduction III - excitation contraction in skeletal muscle and heart cells	n17	s17
thu	may	31	mechanics of the cell - the inner life	n18	l01 l02
tue	jun	05	final projects - oral presentations	p02	
thu	jun	07	no class		
fri	jun	08	final projects - written projects due	p01	

biopolymers

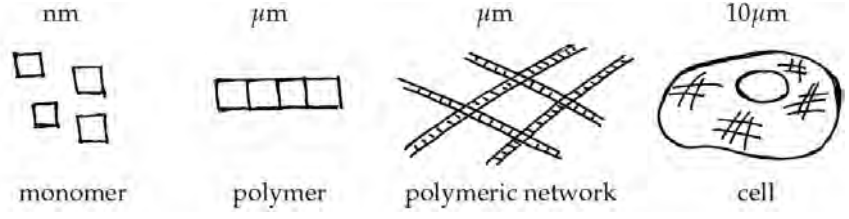
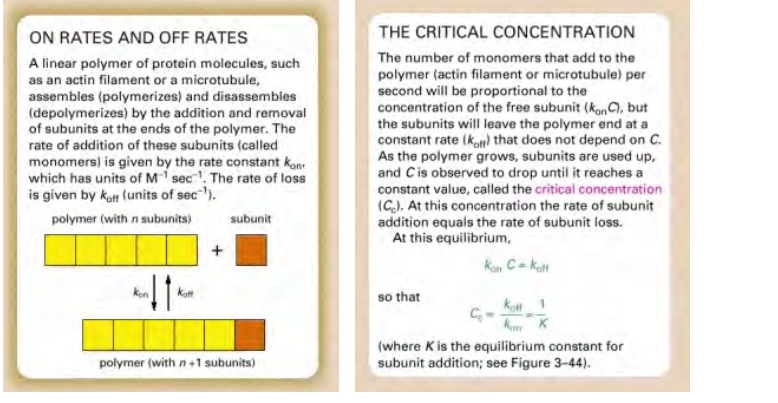
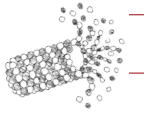


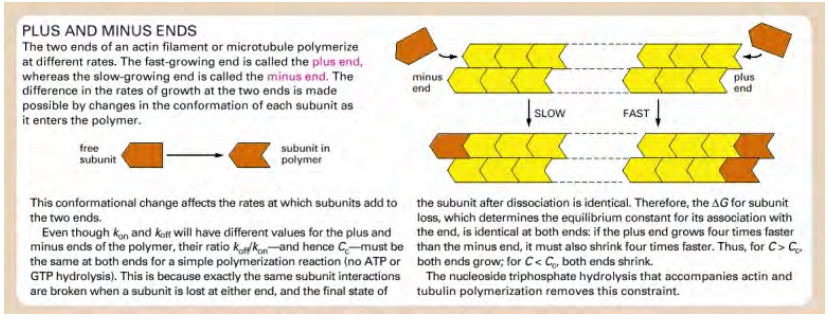
Figure 3.1. Biopolymers. Characteristic length scales on the cellular and sucellular level..

polymerization of actin and tubulin

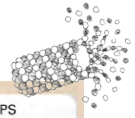


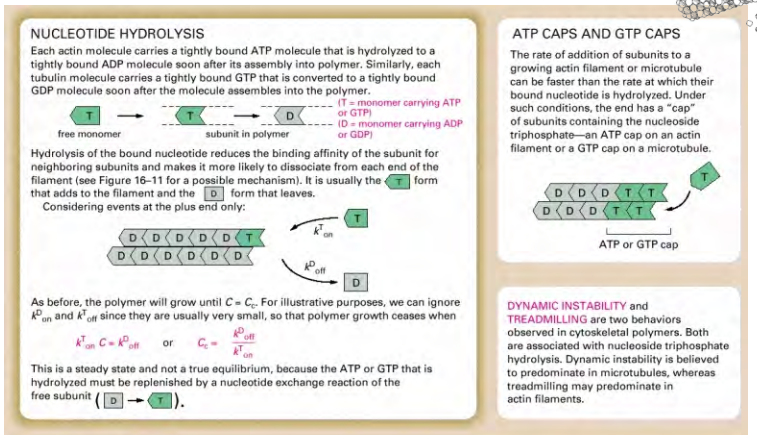
alberts, johnson, lewis, raff, roberts, walter [2002]



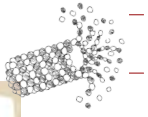


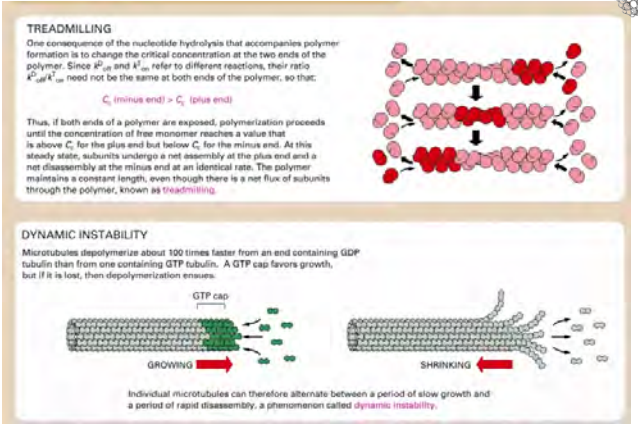
alberts, johnson, lewis, raff, roberts, walter [2002]



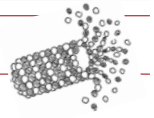


alberts, johnson, lewis, raff, roberts, walter [2002]





alberts, johnson, lewis, raff, roberts, walter [2002]



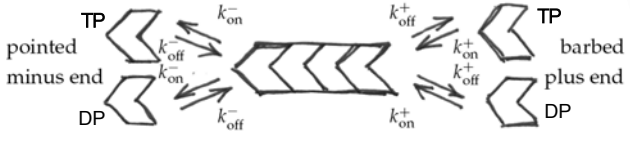
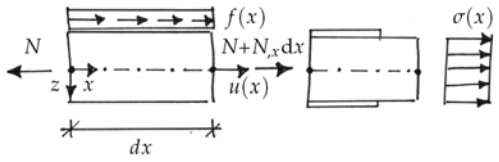


Figure 3.4: Eight rate constants for polymerization capture and release in non-symmetric actin and microtubules filaments.

	k^+_{on} [1/(μMs)]	k^+_{off} [1/s]	k^-_{on} [1/(μMs)]	k^-_{off} [1/s]	C^+_{crit} μM	C^-_{crit} μM
ATP-actin	11.60	1.40	1.30	0.80	0.12	0.62
ADP-actin	3.80	7.20	0.16	0.27	1.90	1.70
GTP-tubulin	8.90	44.00	4.30	23.00	4.90	5.30
GDP-tubulin	0	733	0	915	n/a	n/a

Table 3.1: Measured rate constants of actin filaments and microtubules

axial deformation - tension



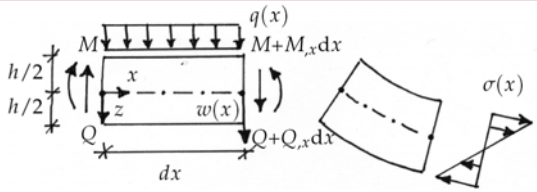
$EA\,u_{,xx} + f = 0$ with EA ... axial stiffness

cross section area $A = \pi r^2$

	r	A	E	EA
microtubule	12.5 nm	491 nm^2	1.9·10^9 N/m^2	93·10^{-8} N
intermediate filament	5.0 nm	79 nm^2	2.0·10^9 N/m^2	15·10^{-8} N
actin filament	3.5 nm	39 nm^2	1.9·10^9 N/m^2	7·10^{-8} N

Table 3.1: Axial stiffness EA of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

transverse deformation - bending



$q = EI\,w_{,xxxx}$ with EI ... bending stiffness

for circular cross sections $I = \pi r^4 / 4$

	r	I	E	EI
microtubule	12.5 nm	19,175 nm^4	1.9·10^{9}N/m^2	364·10^{-25}Nm^2
intermediate filament	5.0 nm	491 nm^4	2·10^{9}N/m^2	10·10^{-25}Nm^2
actin filament	3.5 nm	118 nm^4	1.9·10^{9}N/m^2	2·10^{-25}Nm^2

Table 3.2: Bending stiffness of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

concept of persistence length

- stiffer filaments are straighter ∝ bending stiffness EI
- cooler filaments are straighter ∝ inverse temperature kT

$$A = \frac{EI}{kT} \quad \text{...} \quad \text{persistence length}$$

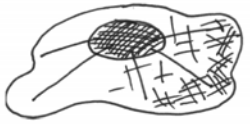
	r	E	EI	$A = [EI]/[kT]$
microtubule	12.5 nm	1.9·10^{9}N/m^2	364·10^{-25}Nm^2	8.800 mm
intermediate filament	5.0 nm	2·10^{9}N/m^2	10·10^{-25}Nm^2	0.240 mm
actin filament	3.5 nm	1.9·10^{9}N/m^2	2·10^{-25}Nm^2	0.048 mm

Table 3.6: Persistence lengths of major consituents of cytoskeleton at room temperature: microtubules, intermediate filaments and actin filaments

from molecular level to cellular level

assuming we know the mechanical properties of the individual filaments, what does that actually tell us about the assembly of filaments that we find in the cell?

- could we then predict the **stiffness of the overall assembly**?
- how does the filament microstructure affect **cytoskeletal properties**?
- how can we calculate the **macroscopic network properties** from the individual microscopic filament properties?



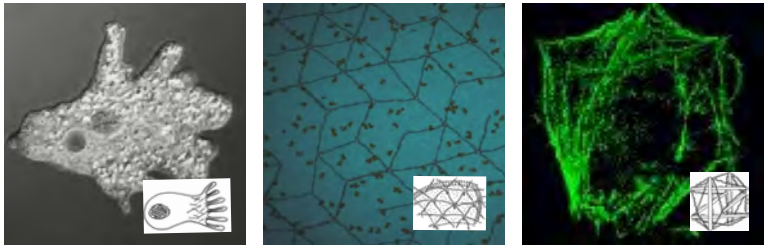
elements of the cytoskeleton
microtubules
intermediate filaments
actin filaments

Figure 4.1: The cytoskeleton provides structural stability and is responsible for forces during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

mechanics of the cytoskeleton

three examples

- **fiber bundle model** for filopodia
- **network model** for red blood cell membranes
- **tensegrity model** for generic cell structures



microstructural arrangement of actin

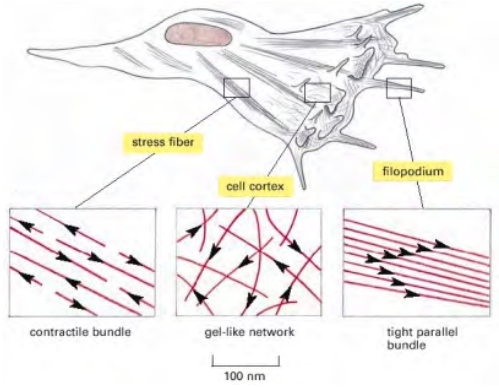


Figure 4.2.1. A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. The cell cortex underlies the plasma membrane. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments.

alberts, johnson, lewis, raff, roberts, walter [2002]

filopodia

filopodia are **thin dynamic cytoplasmic projections** composed of **tight bundles of long actin filaments** extending from the leading edge of migrating cells. sometimes, the name filopodia is used to describe all different kinds of cytoskeletal protrusions including thick filopodia, cell feet, and amoebae pseudopods. **filopodia contain actin filaments cross-linked into bundles by actin-binding proteins such as fimbrin**. many types of motile cell such as fibroblasts or keratinocytes use filopodia for **cell locomotion**. filopodia at the leading edge of a migrating cell seem to explore the extracellular matrix and surfaces of other cells. once they have identified appropriate targets, they **form focal adhesions** linking the cell surface to the substratum further down the migratory pathway. the contraction of stress fibers then retracts the rear of the cell and the cell crawls forwards.

filopodia and lamellipodia

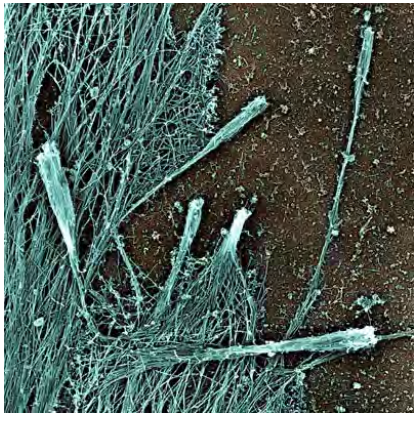


Figure 4.2.2. Filopodia and lamellipodia are actin-rich protrusions important for cell motility. This electron micrograph shows exaggerated filopodia with club-like shape. Filopodia are filled with bundled actin filaments which were born in and converged from the lamellipodial network.

czech, svitkina, yang [2007]

filopodia and other fiber bundels of F-actin

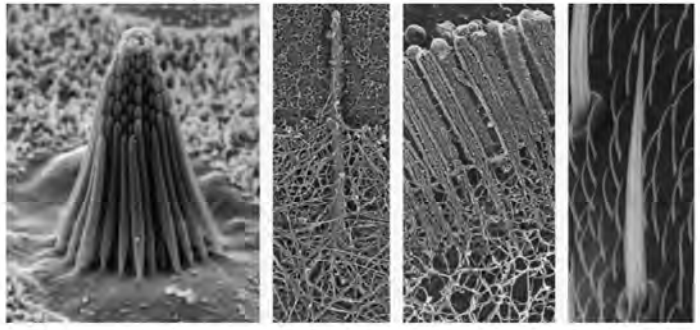


Figure 4.2: Fiber bundles of F-actin. Ciliary bundle from the sensory epithelium of a bullfrog saccule consisting of stereocilia, filopodium protruding from the lamellipodium of a mouse melanoma cell, epithelial microvilli, and drosophila neurosensory micro- and macrochaete bristles.

bathe, heussinger, claessens, bausch, frey [2008]

assembly of crosslinked actin filaments

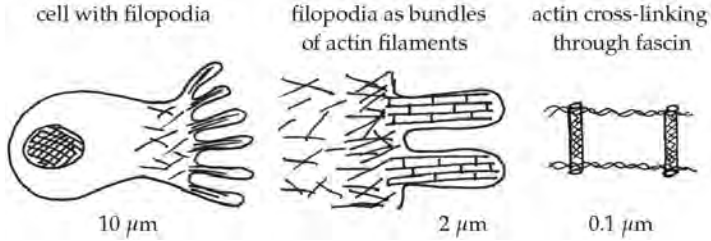
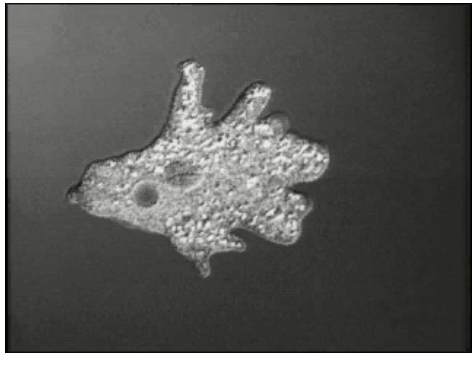


Figure 4.3. Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth.

filopodia are very thin structures approximately 0.2 um in diameter. they can easily extend up to 2.0um. they typically polymerize and depolymerize at rates of approximately 10 um/min. the mechanical properties of filopodia play an essential role in various different physiological processes, including hearing, cell migration, and growth. despite their importance to cell function, the structural architecture responsible for their overall mechanical behavior remains largely unknown.

pushing the envelope



this single-celled amoeba crawls around by using actin polymerization to push out pseudopods, or false feet, to explore new territory. at the same time, organelles move in complex patterns within the cell.

alberts, johnson, lewis, raff, roberts, walter [2002]

pushing the envelope

simplified model for cell locomotion

- protrusion ... polymerization at the leading edge of the cell
- attachment ... formation of focal adhesions to link the cell to the surface
- retraction ... contraction of stress fibers to retract the rear of the cell

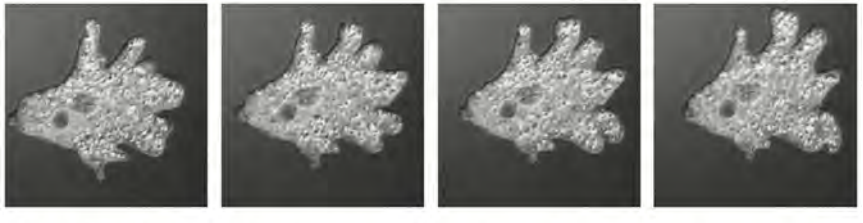


Figure 4.4: Single-celled amoeba crawling around by using actin polymerization to push out pseudopods to explore new territory. Organelles move in complex patterns within the cell,

alberts, johnson, lewis, raff, roberts, walter [2002]

pushing the envelope

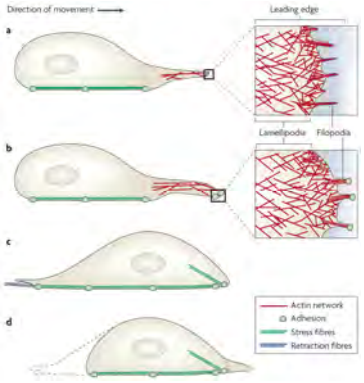


Figure 4.2.3. Cell migration is dependent on different actin filament structures. Motility is initiated by an actin-dependent protrusion of the leading edge, which is composed of lamellipodia and filopodia. **a|** These protrusive structures contain actin filaments, with elongating barbed ends orientated towards the plasma membrane. **b|** During cellular extension, new adhesions with the subtratum are formed under the leading edge. **c|** Next, the nucleus and the cell body are translocated forward through actomyosin based contraction forces that might be mediated by focal adhesion-linked stress fibers, which also mediate the attachment to the substratum. **d|** Then, retraction fibers pull the rear of the cell forward, adhesions at the rear of the cell disassemble and the trailing edge retracts.

mattila & lappalainen [2008]

pushing the envelope - filament force

$F_{\text{fil}} \doteq F_{\text{mem}}$ critical force $F_{\text{crit}} = \dfrac{\pi^2 EI}{L_{\text{crit}}^2}$ thus $F_{\text{fil}} = \dfrac{\pi^2 EI}{[2L]^2} = \dfrac{\pi^2 EI}{4L}$

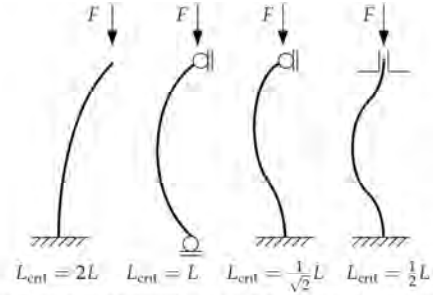


Figure 4.5: The four Euler buckling modes. As the buckling length L_{crit} decreases from $L_{\text{crit}} = 2L$ to $L_{\text{crit}} = 1/2\,L$ from left to right, the critical buckling force $F_{\text{crit}} = \pi^2 EI / L_{\text{crit}}^2$, or rather the resistance to buckling, increases.

pushing the envelope - membrane force

$F_{\text{fil}} \doteq F_{\text{mem}}$

- membrane forces acting on a cylinder of radius r_{fil}

$F_{\text{mem}} \approx 5\, r_{\text{fil}} \dfrac{\text{pN}}{\text{nm}}$

- radius r_{fil} of the filopodium

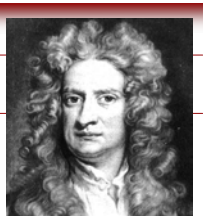
$A_{\text{fil}} = \pi r_{\text{fil}}^2$

$A_{\text{act}} = n\ \pi r_{\text{act}}^2$ $\qquad A_{\text{fil}} = A_{\text{act}}$ thus $r_{\text{fil}} = \sqrt{n}\, r_{\text{act}}$

- force exerted by the cell membrane on the filopodium cylinder

$F_{\text{mem}} \approx 5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}$

pushing the envelope - critical length



Newton's third law: actio = reactio

$F_{\text{fil}} \doteq F_{\text{mem}}$

$F_{\text{fil}} = \dfrac{\pi^2 EI}{[2L]^2} = \dfrac{\pi^2 EI}{4L}$ $\qquad F_{\text{mem}} \approx 5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}$

$\dfrac{\pi^2 EI}{4L_{\text{crit}}^2} = 5\sqrt{n}\, r_{\text{act}} \dfrac{\text{pN}}{\text{nm}}$ thus $L_{\text{crit}} = \dfrac{\pi}{2}\sqrt{\dfrac{EI}{5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}}}$

$E = 1.9 \cdot 10^9\ \text{N/m}^2 = 1.9\ \text{GPa}$ $\qquad r_{\text{act}} = 3.5\ \text{nm}$ moment of inertia I

- loose assembly
- tightly crosslinked

case I - loosely assembled actin filaments

$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{EI}{5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}}}$$

moment of inertia I

$$I = n\, I_{\text{act}} \qquad \text{with} \qquad I_{\text{act}} = \frac{\pi\, r_{\text{act}}^4}{4}$$

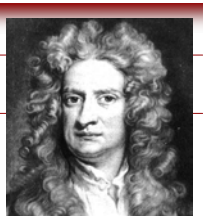
$$E = 1.9 \cdot 10^9\ \text{N/m}^2 = 1.9\ \text{GPa} \qquad r_{\text{act}} = 3.5\ \text{nm}$$

$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{1.9 \cdot 10^9\,\text{N/m}^2 \cdot n\,\pi/4\,[3.5 \cdot 10^{-9}]^4\text{m}^4}{5\sqrt{n}\; 3.5 \cdot 10^{-12}\,\text{N}}} \approx 0.17769\,\mu\text{m}\; n^{1/4}$$

$$n = 30\ \text{filaments} \qquad L_{\text{crit}} = 0.416\,\mu\text{m}$$

much too low - disagrees with observations of 2um

case II - tightly crosslinked actin filaments



$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{EI}{5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}}}$$

moment of inertia I

$$I = \frac{\pi\, r_{\text{fil}}^4}{4} = n^2\,\frac{\pi\, r_{\text{act}}^4}{4} \qquad \text{with } r_{\text{fil}} = \sqrt{n}\, r_{\text{act}}$$

$$E = 1.9 \cdot 10^9\ \text{N/m}^2 = 1.9\ \text{GPa} \qquad r_{\text{act}} = 3.5\ \text{nm}$$

$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{1.9 \cdot 10^9\,\text{N/m}^2 \cdot n^2\,\pi/4\,[3.5 \cdot 10^{-9}]^4\text{m}^4}{5\sqrt{n}\; 3.5 \cdot 10^{-12}\,\text{N}}} \approx 0.17769\,\mu\text{m}\; n^{3/4}$$

$$n = 30\ \text{filaments} \qquad L_{\text{crit}} = 2.278\,\mu\text{m}$$

better model - agrees with observations of 2um

assembly of crosslinked actin filaments

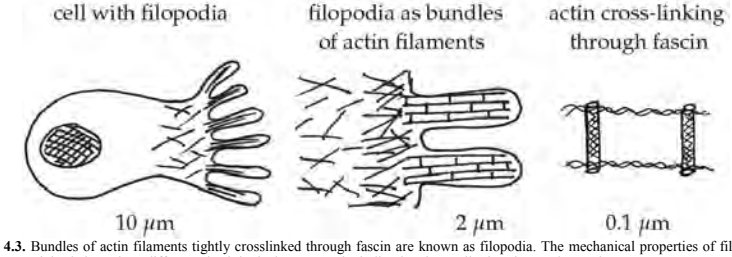


Figure 4.3. Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth.

filopodia are very thin structures approximately 0.2 um in diameter. they can easily extend up to 2.0um. they typically polymerize and depolymerize at rates of approximately 10 um/min. the mechanical properties of filopodia play an essential role in various different physiological processes, including hearing, cell migration, and growth. despite their importance to cell function, the structural architecture responsible for their overall mechanical behavior remains largely unknown.

pushing the envelope

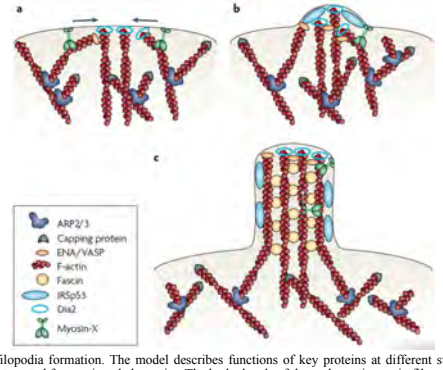


Figure 4.2.4. A working model for filopodia formation. The model describes functions of key proteins at different stages during filopodia formation. **a**| A subset of uncapped actin filaments are targeted for continued elongation The barbed ends of these elongating actin filaments are converged together through the motor activity of myosin-X, leading to the initiation of a filopodium. **b**| When the primary filopodium begins to push the plasma membrane, ISPp53 might further facilitate plasma membrane protrusion by directly deforming or tubulating the membrane. **c**| The incorporation of the actin crosslinking protein fascin in the shaft of the filopodium generates a stiff actin filament bundle. At this stage, myosin-X might localize adhesion molecules to the filopodium tip by barbed-end directed movement and attach the elongating actin filament barbed ends to the plasma membrane.

mattila & lappalainen [2008]

pushing the envelope

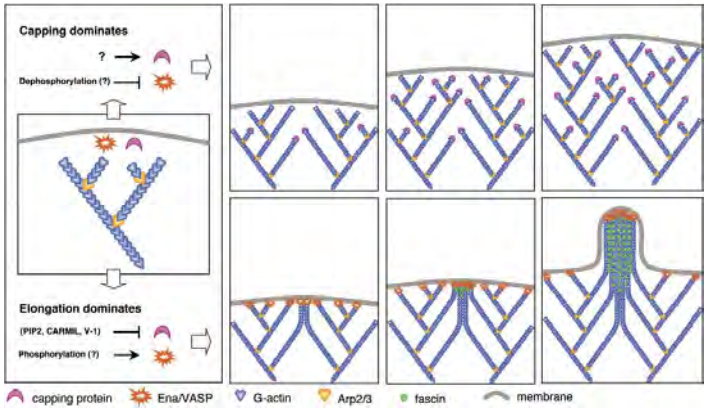


Figure 4.2.5. Model for mechanisms of regulation of lamellipodial versus filopodial protrusion by filament capping.

mejillano, kojima,applewhite, gertler, svitkina, borsy [2004]

visualization of cell locomotion

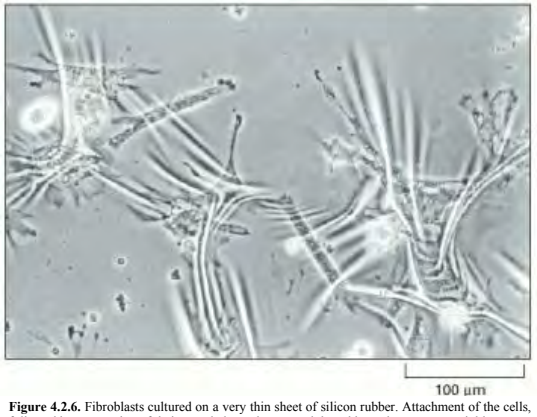


Figure 4.2.6. Fibroblasts cultured on a very thin sheet of silicon rubber. Attachment of the cells, followed by contraction of their cytoskeleton, has caused the rubber substratum to wrinkle.

alberts, johnson, lewis, raff, roberts, walter [2002]