# Introduction to Cell Adhesion and Focal Adhesions

**Focal adhesions (FAs) are specialized, dynamic structures** that enable cells to **anchor** to the extracellular matrix (ECM) and **communicate biochemical and mechanical signals** across the cell membrane.

They play essential roles in cell migration, differentiation, and mechanotransduction—the process by which cells sense and respond to mechanical signals. These adhesions are a major research area in biophysics due to their role in mechanobiology and cellular force transmission (Geiger et al., 2009).

"The term **sensing** is used metaphorically and refers to those **environmental features that can exert measurable effects on cell dynamics, function and fate** following specific modulation"

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**Physics of adherent cells**

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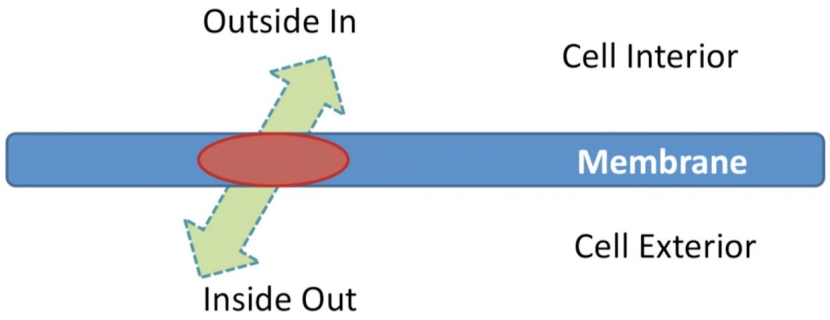
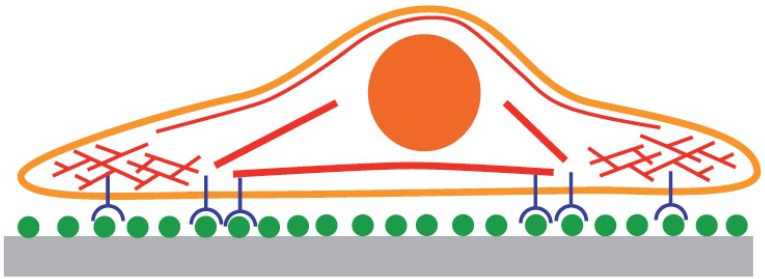
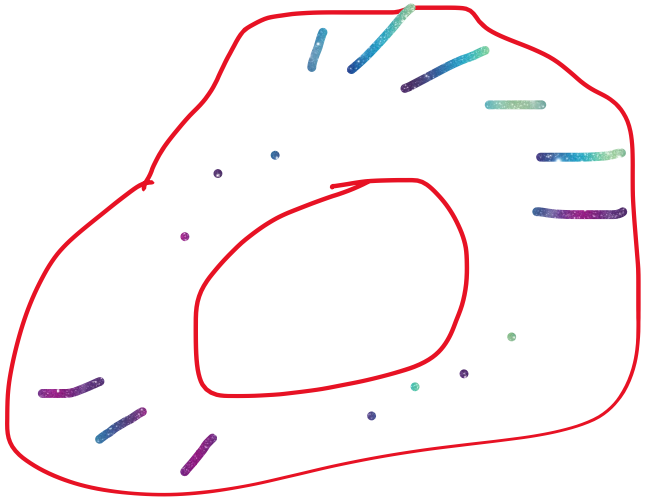
# Focal adhesions mediate bi-directional signaling





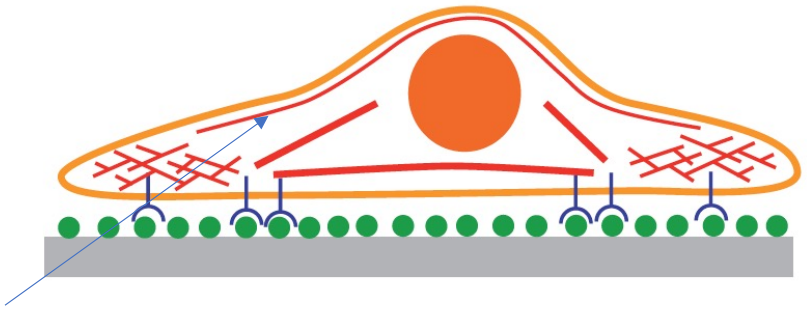
FA are made by more than 100 different proteins

Areas are spreaded : (0.5-1.0) $\mu m^2$
(3-10) $\mu m^2$

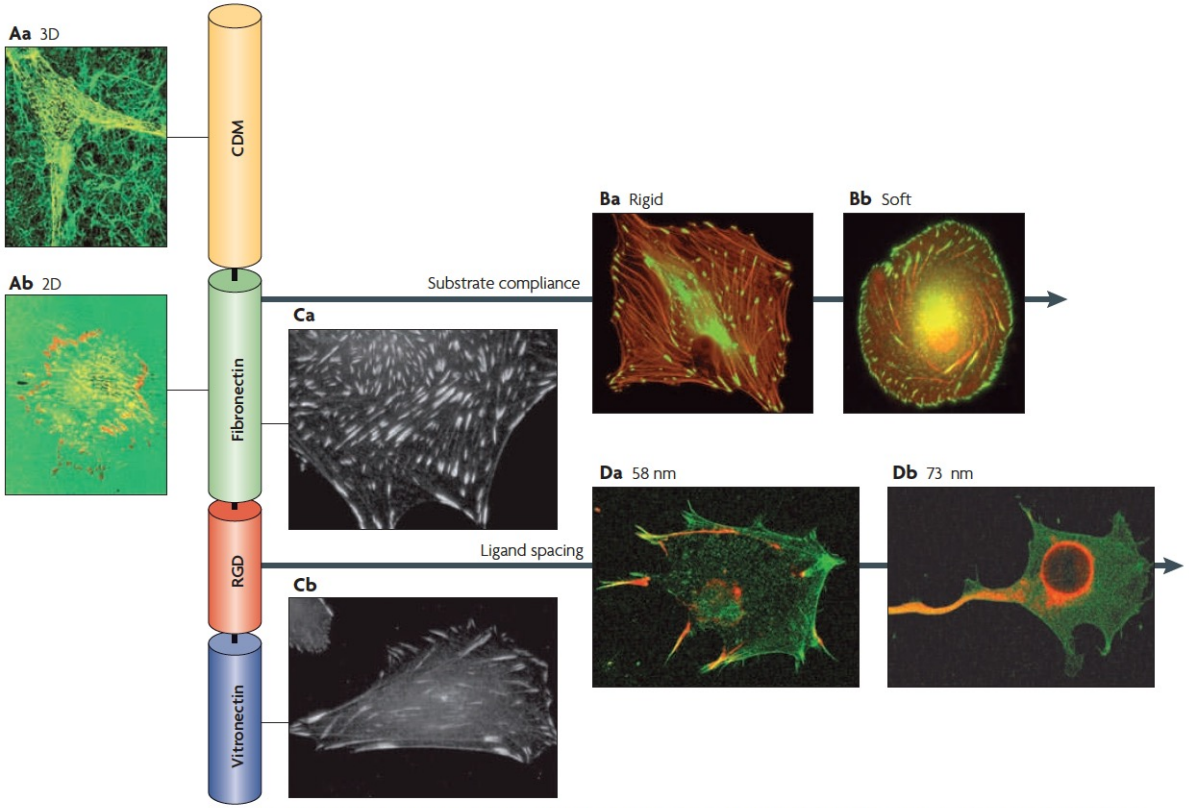
**Role of Focal Adhesions in Cell Movement**:

Focal adhesions act as signaling hubs that allow cells to sense and respond to mechanical stimuli from their environment.
They provide traction forces that enable cells to migrate by dynamically forming and disassembling during movement.

On one side, they collect the cell membrane to the cytoscheleton while, on the other side, anchor to the extracellular matrix



Underneath the plasma membrane is the **actin cortex**, a relatively thin (100 nm) dynamic layer of cross-linked actin filaments whose mechanical properties dominate the elastic response in reaction to deformations of the cell. On the other side, cells adhere to the extracellular environment.

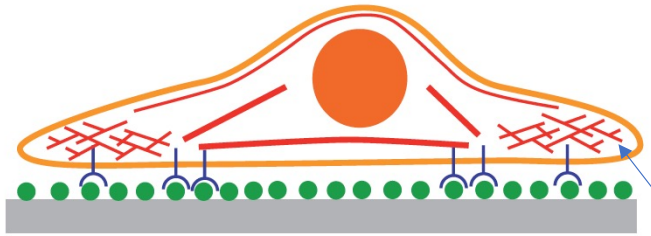



Adhesion is induced only if it is promoted by specific molecular signals that are found on the substrate.

The specificity of cell-matrix adhesion is implemented by transmembrane adhesion receptors (in humans, these are mainly the 24 variants of the integrin family), which bind to complementary ligands of the extracellular matrix (including collagen, fibronectin, vitronectin, and laminin).

The early stages of cell adhesion and spreading can be strongly determined by viscoelastic processes, e.g., the deformation of the rim of the developing contact region. Later stages are more strongly determined by remodeling of the cytoskeleton and the establishment of localized sites of specific adhesion.

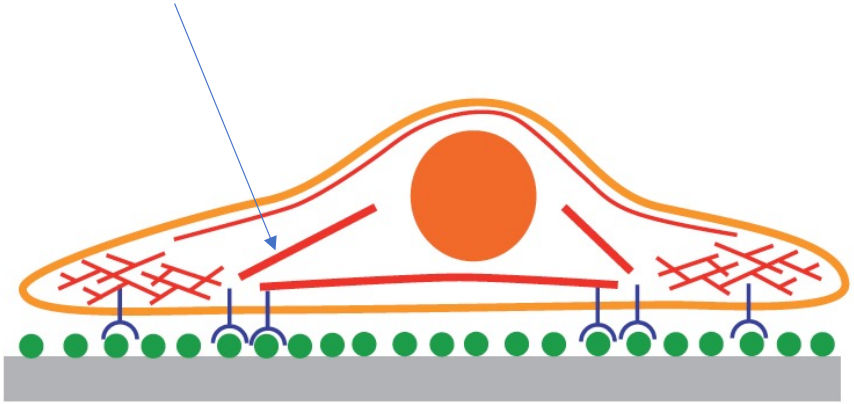
During the **remodeling** process, **the actin system is organized into additional networks** extending throughout the cytoplasm.
Because these networks are cross-linked, the **actin cytoskeleton provides the cell with elastic restoring forces that resist shear deformations** and is thus essential in determining the shape, stability, and mechanical response of cells.
While the volume of a cell tends to stay constant during adhesion and spreading, the surface can increase by up to 50%, which occurs via the flattening of the undulated membrane as well as by the addition of new lipid material (Gauthier et al., 2011).



The main mechanism that leads to outward expansion of the plasma membrane and thus to the development of a contact area with the substrate is the **rapid polymerization of an actin network at the cell periphery** (lamellipodium). Lamellipodia grow through the elementary processes of actin filament polymerization, branching, capping, and cross-linking. The exact organization of the lamellipodium varies as a function of cell type, motility state, and external signals.

One of the most important aspects of **lamellipodia growth** is its **force-velocity relation**, for which conflicting experimental evidence exists.

Other types of actin structures that develop in cell adhesion are **bundles and networks that are contractile** due to the action of molecular motors that tend to slide actin filaments relative to each other. If the filaments are sufficiently anchored to their surroundings, they can no longer move; thus, instead of motion, tension is developed in the actin bundles or network by the forces exerted by the molecular motors. In adhesive cells, this is mainly achieved by the molecular motor protein myosin II, which organizes into myosin minifilaments that typically contain only dozens of nonskeletal myosins II molecules. The most prominent myosin-based contractile structures in adhesion-dependent cells are **stress fibers.**

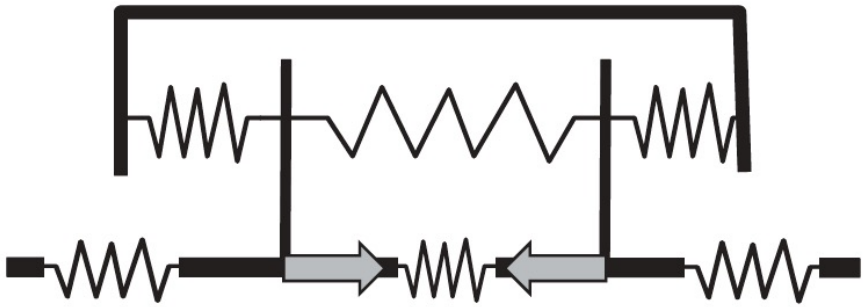


Stress fibers are thought to serve as the main sources of cellular forces that are exerted on the substrate, since their end points are often found at large adhesion sites that correlate with large forces.

The **lamellipodium** and **stress fibers** are actin assemblies that create pushing and pulling forces, respectively; hence, they are the two main force-generating mechanisms for cells that adhere to flat substrates.

Although its effect is rather indirect, the **plasma membrane** plays an important role in this context.

Apart from acting as host for the transmembrane receptors from the integrin family, it also controls the polymerization of the lamellipodium and the contraction of the stress fibers by triggering biochemical signals that regulate these processes (Ridley, 2011).
Equally important, the plasma membrane plays an important role in the overall force balance in the cell, since its tension and curvature elasticity provide the counterforces to actin-generated forces that tend to extend and deform the membrane. An imbalance in these forces is especially important in cell migration.



To first order, the **lamellipodium** can be depicted as a **compressed spring** that pushes outward on the cell membrane and inward on the focal adhesion.
The **stress fibers** appear as **stretched springs** that pull inward on the adhesion.

The fluid nature of the **plasma membrane** means that it **is only indirectly involved in force generation**. Transmission of forces and, in particular, the sensitivity to shear requires a solid like structure.

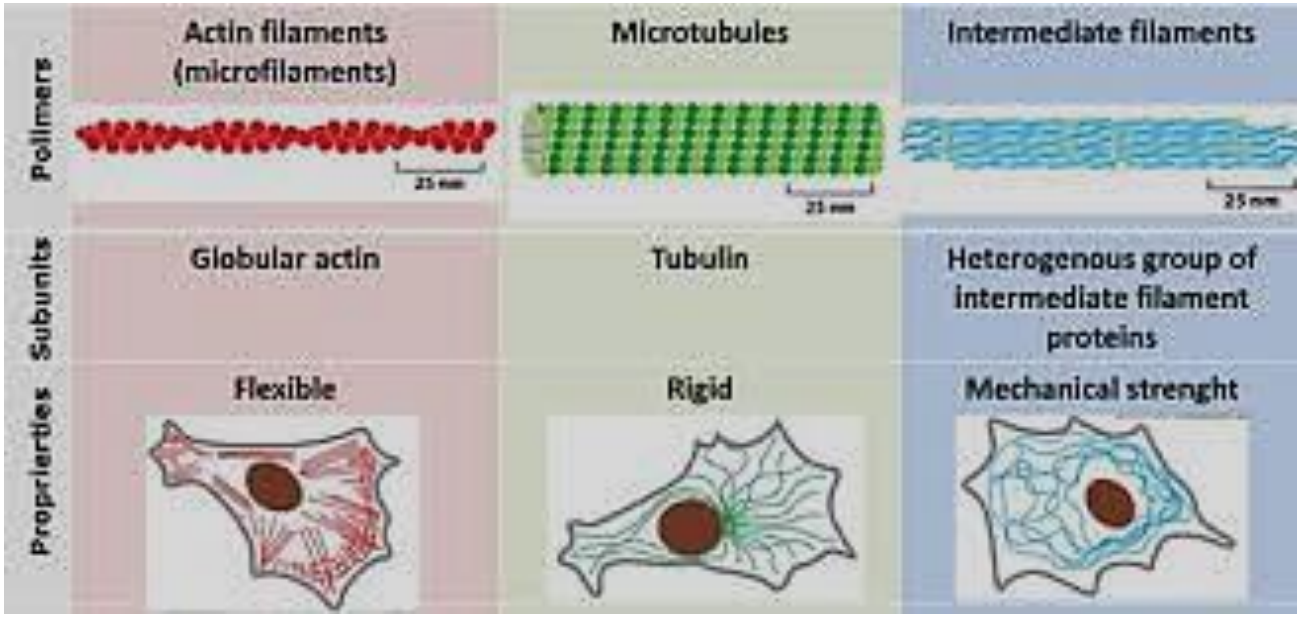
In cells, the structural elements that give the cell its shape integrity and its ability to respond to and to transmit forces reside in the **cytoskeleton**.
In addition to this role, the cytoskeleton is also important in anchoring organelles such as the Golgi apparatus in their place in the cell, in determining the organized changes that take place during cell division, in regulating the imbalance of internal forces that results in cell motion, and in providing a scaffold for signaling processes inside cells.

All three types of cytoskeletal polymer filaments form as helical assemblies of subunits / protofilaments, which self-associate, using a combination of end-to-end and side to side protein contacts.
**Subunit self association and growing** are based on two types of reactions:

•Polymerization / depolymerization
•ATP or GTP hydrolysis

Moreover, filaments dynamics and networking are regulated by accessory proteins.
The stability and mechanical properties of each type of filaments depend on differences in the structure of the subunits and the strength of the attractive forces between them.
The three types of cytoskeletal "polymers" are held together by weak noncovalent interactions, which means that **their assembly and disassembly can occur rapidly**, without covalent bonds being formed or broken.
In contrast, many biological polymers—including DNA, RNA, and proteins—are held together by covalent linkages between their subunits.

In terms of **force-generating processes during cell adhesion**, the most important player is the the actin cytoskeleton. Such forces are **balanced** over the **sites of adhesion**.

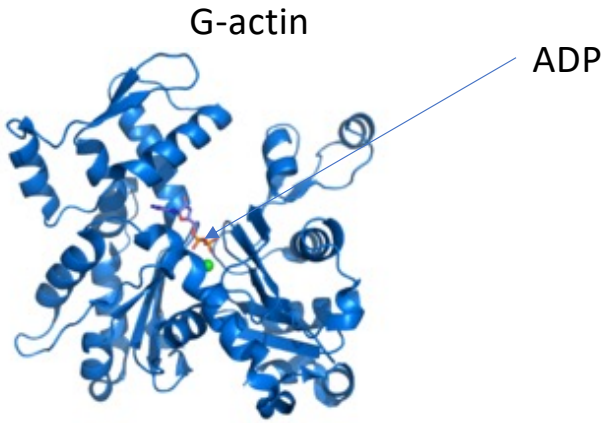



Actin (in both monomeric and polymeric forms) comprises between 5% and 10% of the protein in eukaryotic cells and is of great importance in cell structure and motility.

# Growth of actin polymers

In contrast to self-assembling, equilibrium polymerization, these are catalyzed by the **binding of adenosine triphosphate (ATP) to monomeric (globular) actin (G actin).** While many synthetic polymers are nonpolar, **actin polymers are chiral** with each macromolecule comprising two helical, interlaced strands of monomeric subunits. The two-filament assembly is thus polar so that the **two ends are therefore not equivalent**; hence polymerization rates at one end are not necessarily equal to those at the other.

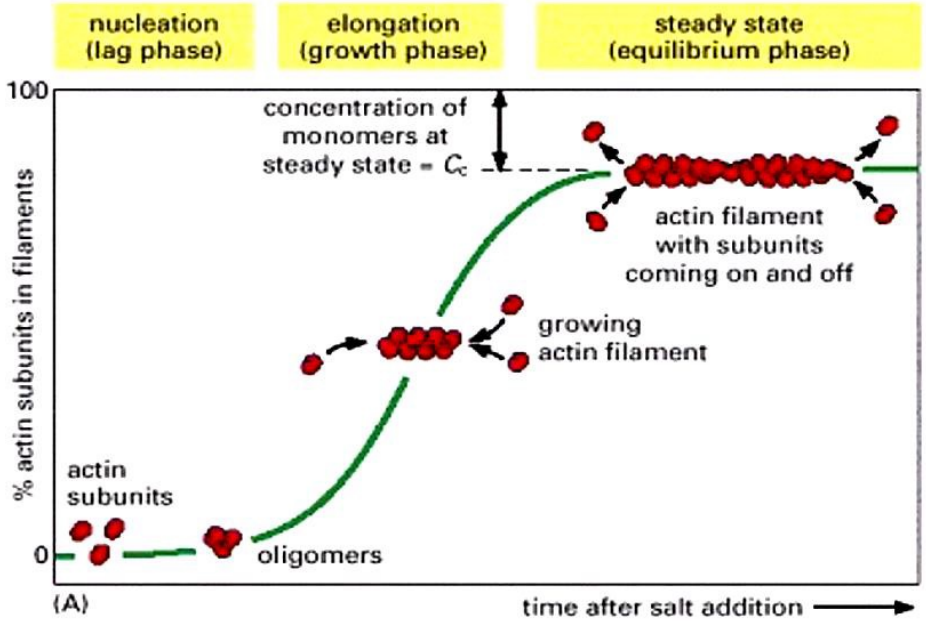
**Actin polymerization is therefore a polar, energy-consuming, nonequilibrium Process.**




The energy released by ATP hydrolysis is used to modify the conformations of actin, that is bound to ATP in its lowest energy state. The **resulting conformational changes** result in; in the case of actin, hydrolysis destabilizes polymerization at **increased or decreased bonding of the molecules to other molecules;** in the case of actin, hydrolysis destabilizes polymerization at its plus end.

# Growth of actin polymers

The nonequilibrium nature of actin polymerization in cells is related to the conformational changes in the monomers that are catalyzed by ATP;
G-actin monomers bound to ATP join the plus end of the actin polymer. Within a time of about **2 s**, however, ATP is hydrolyzed to form ADP which reduces the binding strength of the monomers in the chain, thus destabilizing the polymer.
There is therefore a nonequilibrium competition between growth and shrinkage of the polymer.
Note that, in solution, the G-actin monomers that have dissociated from the chain can dissociate from ADP and reassociate with ATP to rejoin the polymer; this turnover makes the process highly dynamic.




Three phases:
- Nucleation,
- Growth,
- Equilibrium.

Since the actin polymer is polar due to its double helical structure, the growth and shrinkage at the + and - ends is different

**Passive polymerization and active polymerization**

**Passive polymerization** (Equilibrium polymer)

Consider only the polymerization/depolymerization, ignoring that actin and tubulin are nucleotide triphosphatases, i.e. ignore the free energy derived from ATP/GTP hydrolysis.

**Single strand** vs **double strand** models –allow to understand the average length and stability of filaments in cell.

**Passive polymerization**, single strand model :
(demo annex 9.1, Howard)

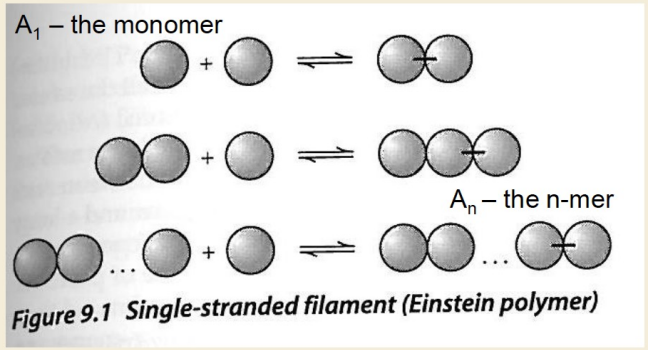


*Figure 9.1 Single-stranded filament (Einstein polymer)*

Howard

Individual subunit-addition reaction:

$$A_n + A_1 \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} A_{n+1}$$

equilibrium constant $K_{eq}$ :

$$K_{eq} = \frac{[A_{n+1}]}{[A_n] \cdot [A_1]}$$

dissociation constant K:

$$K = 1 / K_{eq} = \frac{k_{off}}{k_{on}}$$

Assume identic dissociation constants K for all reactions:

$$K = \frac{[A_1]\,[A_1]}{[A_2]}$$

$$K = \frac{[A_1]\,[A_2]}{[A_3]}$$

$$K = \frac{[A_1]\,[A_n]}{[A_{n+1}]}$$

[An] – concentration of polymers with n-mers.

Exponential distribution

verifies ←

$$[A_n] = K \exp\left(-\frac{n}{n_0}\right)$$

$n_0$ – ct related to $[A_1]$

the association or dissociation energy itself must be the same at either end since, although the monomers are asymmetric at the two ends, the molecular bonds that are formed are the same:

$K^+_{off} / K^+_{on} = K^-_{off} / K^-_{on}$

for such equilibrium polymers there is no state in which one end is growing and the other is shrinking; the polymer either grows or shrinks from both ends—albeit with different on and off rates for the two ends of polar chains. However, it does not apply for the polymerization of actin in cells.

**Passive polymerization**, single strand model :

The average length, $n_{av}$ of the polymer filaments for the single stranded model increases slowly with the total concentration $[A_t]$

Even when $[A_t] >> K$ the average length of the single-stranded filaments is still modest:

$$n_{av} \cong \sqrt{\frac{[A_t]}{K}}$$

Ex: for $[A_t] = 100$ K , the average length of the polymer $n_{av} = 10$ only !

But the cytoskeleton filaments are thousands subunits length. This would require $[A_t] > 10^6 K$ !

Considering the concentrations of actin and tubulin:

*Actin*, total concentration $[A_t] \sim 200$ uM, and the dissociation constant K= 0.1 uM $n_{av} = sqrt(2000) \approx 45$
*Tubulin* $[A_t] \sim 20$ uM vs K~ 10 uM ----- $n_{av} \approx 1.4$

Conclusion: The single stranded, passive model fails because, i.e. it does not fit the reality!
The single-strand predicts short filaments, mainly because of the assumption:
"same K" a monomer can decrease the free energy of the system equally well by associating with another monomer as it can by the associating with a long polymer.

**Two-stranded model**
(demo annex 9.2, Howard)

Actin filaments and microtubules are multi-stranded, and multi-stranded filaments are inherently longer.
In a two-stranded filaments model, there are two different classes of bonds, one within the strands and one between the strands --- there are two different nuclei A2* and A2** and three different dissociation constants: K, K1 and K2, with K << K1, K2

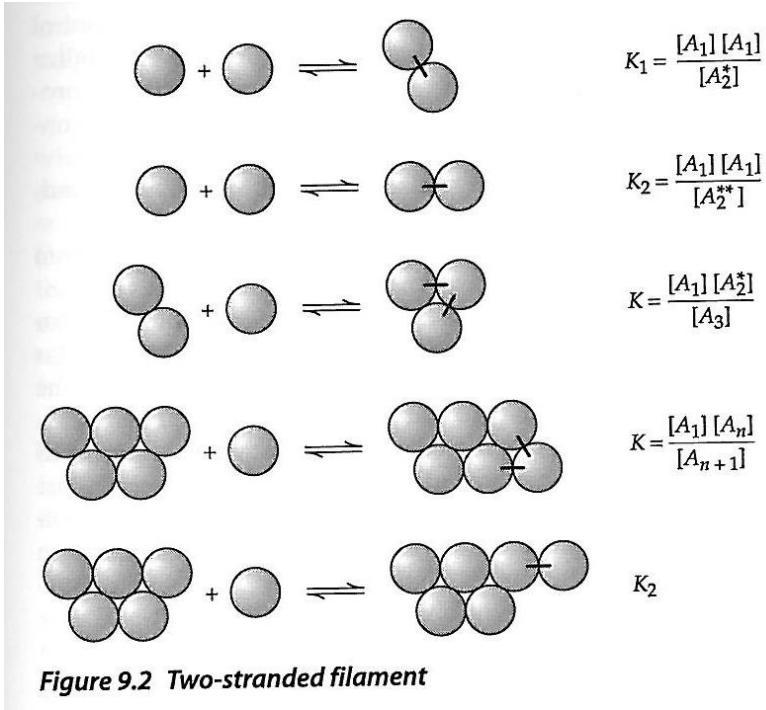


*Figure 9.2 Two-stranded filament*

**The average length, $n_{av}$, of the two-stranded filaments:**

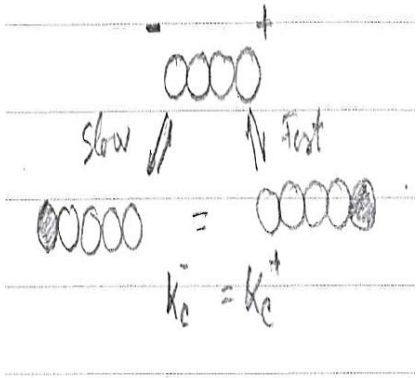
$$n_{av} \cong \sqrt{\frac{K_1}{K}} \sqrt{\frac{[A_t]}{K}}$$

Ex for **actin**: $[A_t]$ = 200 uM>> K= 0.1 uM, K1=K2= 0.1 M
-$n_{av} \approx 4.5 \times 10^3$, corresponding to a 12.4 um long actin filament!

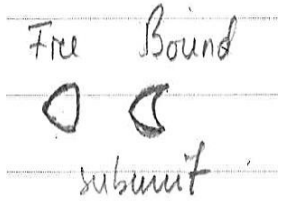
The two-stranded model predicts filament lengths consistent with the polymer length in the cell. Two stranded filaments are longer than single stranded filaments because the ends of a two stranded filament are energetically unfavorable, so there will be only a low concentration of them at equilibrium.

**Polar filaments –plus and minus ends**

Although the rates at the two ends might be different, the critical concentrations must be the same.
The end with faster kinetics is referred to plus end, while the other end is called the minus end.
An important constraint on passive polymerization (equilibrium polymer) is that the critical concentration must be the same at both its ends.




Although the rates at the two ends might be different, the critical concentrations must be the same.
The end with **faster kinetics** is referred to **plus end**, while the **other** end is called the **minus end.**




The difference in the rates of growth at the two ends is made possible by changes in the conformation of each subunit as it binds another subunit i.e. enters the polymer.

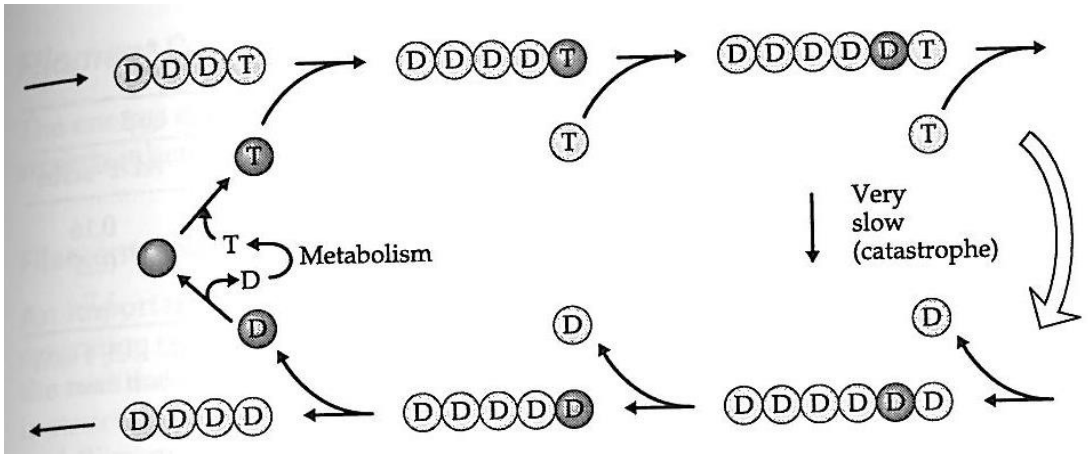
**ATP hydrolysis that accompanies actin polymerization removes this constraint.**

## Active polymerization

Cytoskeletal filaments are not at equilibrium in cells --- fast addition and subtraction of subunits
Nonequilibrium requires an energy source ---- hydrolysis of ATP (actin) and GTP (tubulin)

**The polymerization cycle for actin / tubulin**

**Polymerization and hydrolysis are coupled**:
-ATP Hydrolysis is catalyzed by polymerization
-The exchange of NTP for NDP is catalyzed by depolymerization




During the polymerization process, ATP that is bound to G-actin is hydrolyzed to ADP that is bound to F-actin. The hydrolysis reaction occurs on the F-actin subsequent to the polymerization reaction in two steps:
cleavage of ATP followed by the slower release of inorganic phosphate (Pi).
As a result, at high rates of filament growth a transient cap of ATP-actin subunits exists at the ends of elongating filaments, and at steady state a stabilizing cap of ADP+Pi-actin subunits exists at the barbed ends of filaments.
Cleavage of ATP results in a highly stable filament with bound ADP+Pi, and release of Pi destabilizes the filament. Thus, these two steps of the hydrolytic reaction provide potential mechanisms forregulating the monomer-polymer transition.

# Growth of actin polymers: dynamical model

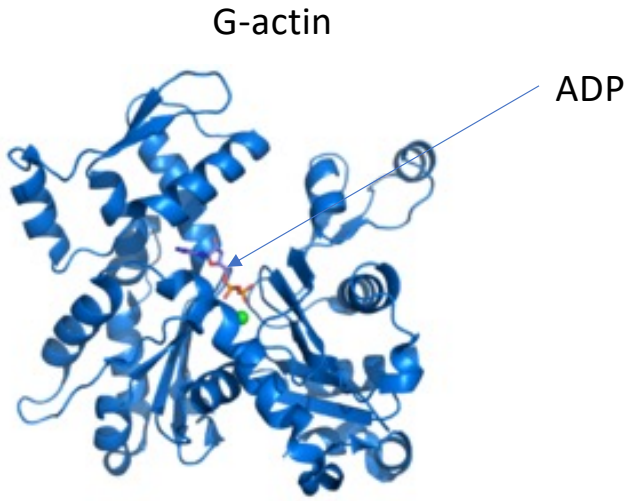


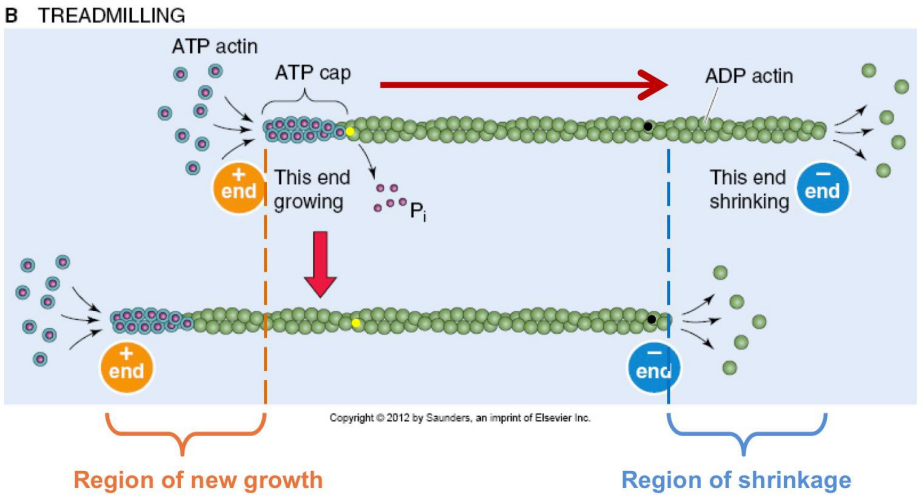

*Table 11.1 Rate constants for actin polymerization and depolymerization*

| Rate constant or equilibrium constant | Plus end | | Minus end | |
|---|---|---|---|---|
| | ATP-actin | ADP-actin | ATP-actin | ADP-actin |
| $k_{on}$ ($\mu M^{-1} \cdot s^{-1}$) | 11.6 | 3.8 | 1.3 | 0.16 |
| $k_{off}$ ($s^{-1}$) | 1.4 | 7.2 | 0.8 | 0.27 |
| $K_c$ | 0.12 | 1.9 | 0.6 | 1.7 |

The plus ends have lower critical concentration Kc than the minus ends. $K_c^- > K_c^+$

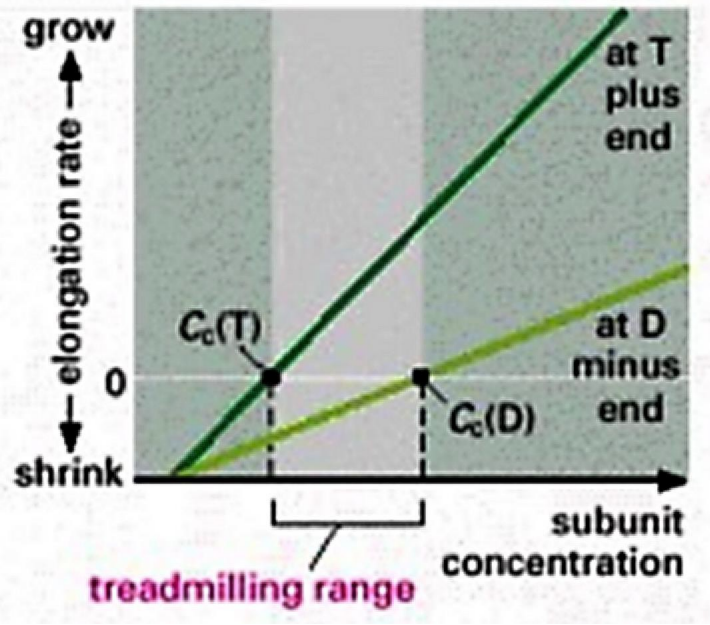
The plus end is expected to grow while the minus end is expected to shrink.

# Actin Filaments: Treadmilling




Boron and Boulpaep, fig. 2-12B





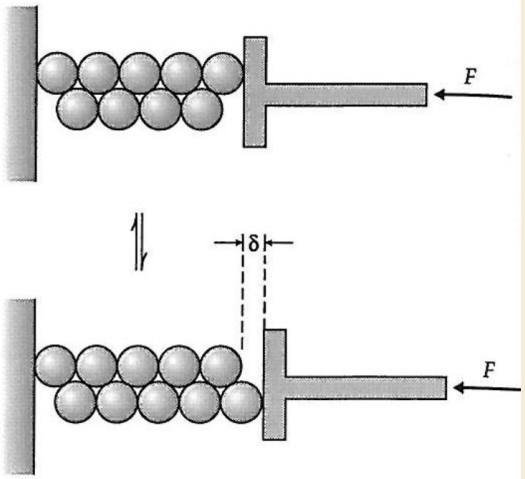

If both ends are exposed, polymerization proceeds until the concentration of free monomer reaches a value K: $Kc^+ < K < Kc^-$

At this steady state, subunits undergo a net assembly at the plus end and a net disassembly at the minus end at an identical rate.

The polymer maintains a constant length, even though there is a net flux of subunits through the polymer, known as **treadmilling.**

**How much force can be generated by polymerization and depolymerization ?**

adding a compressive force opposing the polymerization of a polymer




Polymerization against the force *F* → the mechanical energy of the n+1 mer exceeds that of the n-mer by ***F δ***.

Ex. Actin: δ = 5.5 /2 = 2.75 nm (monomer size / 2 - the number of strands);
Microtubules: δ = 8 /13 ≈ 0.6 nm (monomer size / 13 – number of monom / ring)

At equilibrium in the presence of force, application of Boltzmann's law gives a dissociation constant *K(F)*:

$$K(F) = K_c \exp\left(\frac{F \cdot \delta}{kT}\right)$$

***K(F)*** - the concentration at which there is no net elongation of the filament.

***Kc*** - the critical concentration in the absence of force.

At quilibrium concentration [A1] with force, the system is at equilibrium (i.e. there is no net polymerization) when ***F= $F_{eq}$***:

$$F_{eq} = \frac{kT}{\delta} \ln \frac{[A_1]}{K_c}$$

Ex: for a concentration [A1] of monomer [A1] = 100 Kc → $F_{eq}$ ≈ 7 pN for actin and $F_{eq}$ ≈ 30 pN for microtubule

Some comments:

1. **The equilibrium force is independent of the polymerization mechanism**.
   i.e. does not matter whether the polymer pushes directly on the particle or whether the polymer is coupled to the particle via accessory proteins (e.g. Arp2/3 complex for actin, kinetochore for microtubules).

2. **The maximum force exerted by a real polymer will be less than the equilibrium force.**
   i.e. polymerization might be so slow at higher forces that growth is effectively stalled at forces significantly smaller than the equilibrium force (polymerization stalls at forces < 7 pN for actin and 30 pN for microtubules).

3. **Are the cytoskeletal filaments strong enough to exert forces while polymerizing ?**
   Polymerization-based motility requires that the filaments be in compression, and there is only so much compressive force that a filament can withstand before it buckles. This does not represent a problem until the filaments are not too long (< 1 um ).

The polymerization mechanism can generate sufficiently large forces to account for cellular processes. Both actin and microtubules filaments are rigid enough to support the polymerization forces that are observed in cells.

Another question is: **is the polymerization fast enough** ?

To answer this question, kinetic mechanisms by which polymerization is coupled to force generation should be considered.

It is difficult to envision how the end of a growing or shrinking filament could push or pull on a particle.
e.g. how a depolymerizing polymer could maintain the contact with the particle being pulled ?

A possible answer are the **accessory proteins** which may **connect** the end of **the filament** to **the particle**.
However, experiments in vitro demonstrated that the additional proteins are not a necessary condition for force generation.

This has lead to the idea that in the case of a growing polymer pushing against an opposing force, the particle being pushed must be able to undergo thermal noise sufficiently large to unblock the adjacent filament end and permit subunit addition. → mechanism termed a Brownian ratchet model.

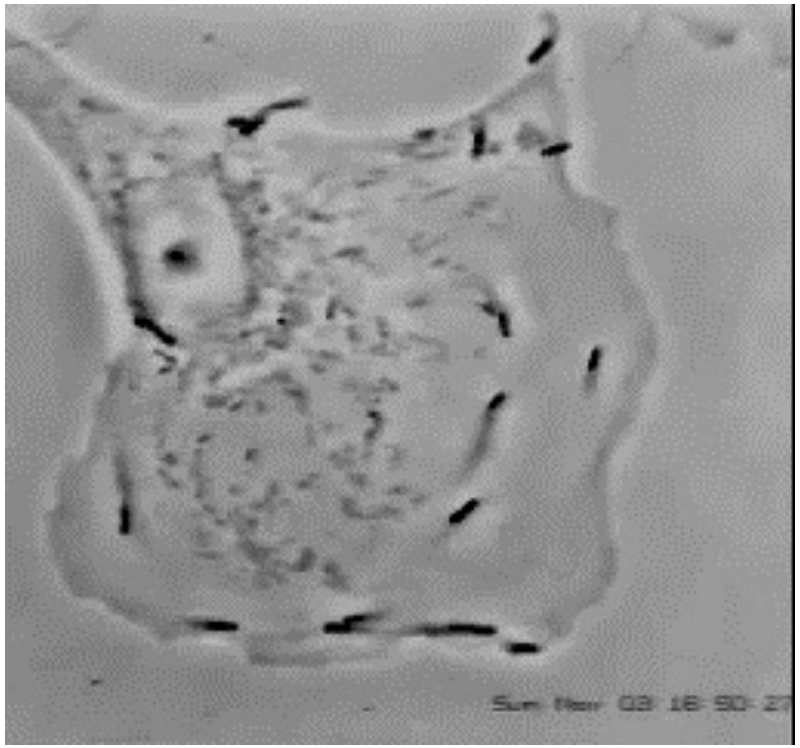
Treadmilling actin filaments in the vicinity of the cell membrane have their motion impeded by the restoring forces (due to surface tension and curvature energy) of the membrane.

However, one important aspect regarding actin networks and bundles in cells is the fact that **these networks are under tension due to the contractile activity of myosin motors.**

In cells, the myosin motors generate **internal forces in the actin network** which are transmitted to its surroundings due to the ‘‘glue’’ the cell produces in the form of proteins that assemble into **focal contacts or focal complexes.**

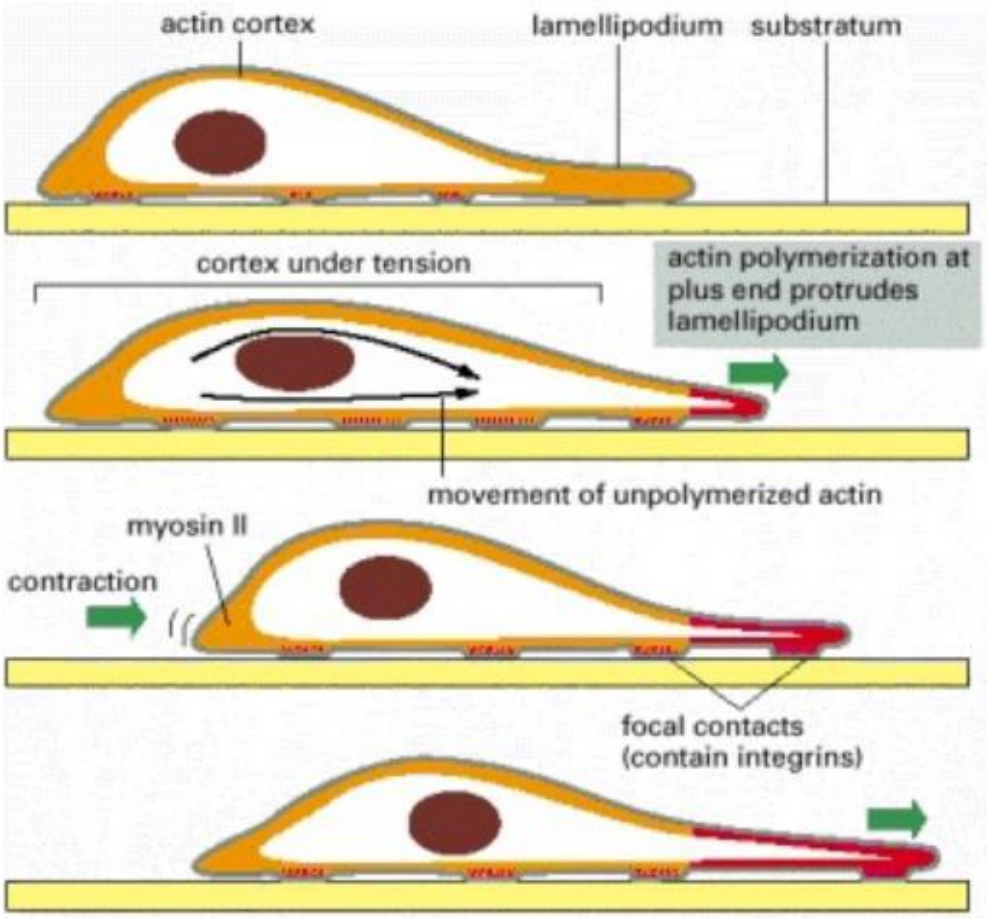
Also in this case, the production of force is a nonequilibrium process that requires energy input via ATP hydrolysis that causes conformational changes in the myosin molecular motors.
**Motor activity** also means that the **cell can exert forces on itself** and this, along with polymerization of actin, plays an important role in **cell motility**.

Example in which actin polymerization generates forces in cells : motility of intracellular viruses and bacteria Listeria monocytogenes (gram positive bacteria) v= 1 um / s



***Example 10.2 The diffusion-limited speed of* Listeria** For a 1-μm-long bacterium being pushed by actin polymerization through water, the diffusion-limited maximum speed is 360 μm/s (= $2D/\delta$, where using $D = 0.5$ $\mu m^2/s$, $\delta = 2.75$ nm), well in excess of the measured speeds of 0.1 to 1 μm/s. This suggests that *Listeria*'s motion may not be diffusion limited. However, if the mobility of a bacterium in cytoplasm is only 1/100 to 1/1000 that in water, the diffusion-limited speed is reduced by a corresponding factor to a value more nearly equal to the observed speeds. If the motion were diffusion limited, the force would equal ~3 pN per filament, by Equation 10.5. This is of the same order of magnitude as the maximum reaction-limited force (Example 10.1). It is interesting that *Listeria* moves at different speeds in different cell types (Dabiri et al., 1990): The three-fold difference could arise from differences in the monomeric actin concentration if the motion were reaction limited, or to differences in "viscosity" (i.e., different mobilities of the bacteria) if the motion were diffusion limited.

## Cells Can Crawl Across A Solid Substratum




**A model of how forces generated in the actin-rich cortex move a cell forward**

The actin-polymerization-dependent **protrusion** and firm **attachment** of a lamellipodium at the leading edge of the cell moves the edge and stretches the actin cortex.
**Contraction at the rear** of the cell propels the body of the cell forward to relax some of the tension (**traction**).
New **focal contacts** are made at the front, and old ones are disassembled at the back as the cell crawls forward.
The same cycle can be repeated, moving the cell forward. The newly polymerized cortical actin is shown in red.

While the response of cells to external forces or other mechanical perturbations can necessitate the disassembly and rebuilding of the actin cytoskeleton, the stable coupling of the cell to the surrounding elastic matrix is due to sites of adhesion called **focal adhesions** that connect the actin cytoskeleton to transmembrane adhesion receptors from the integrin family.

These are then connected, on the extracellular side, to the substrate or extracellular matrix.

The spatial distribution of the adhesion structure of cells is very heterogeneous. It is mainly localized at the cell periphery, because it is strongly coupled to the growth processes of the lamellipodium.

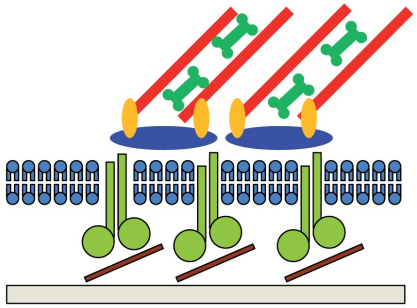
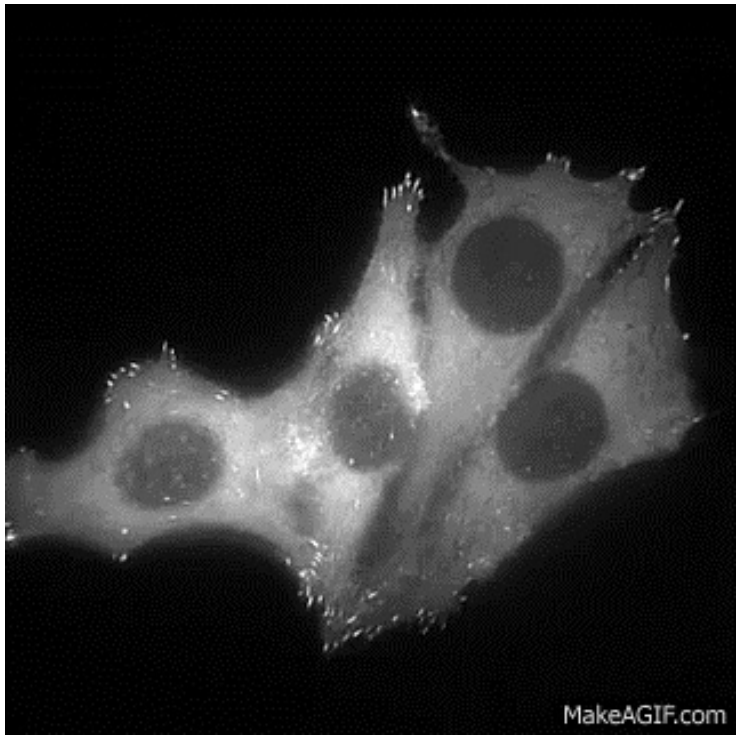


FIG. 7 (color online). Schematic view of a focal adhesion. The transmembrane adhesion receptors from the integrin family (a heterodimer with two subunits) bind to the extracellular matrix (for example, collagen) on the outside and are cross-linked by cytoplasmic proteins such as talin in the inside. Talin binds to actin and this binding is further strengthened by proteins such as vinculin. The contractility of the actin cytoskeleton is determined by the activity of myosin II minifilaments.

Focal adhesions formation during lamellipodial protrusion helps cell spreading.

During lamellipodial protrusion, numerous Fas form along the cell periphery and can be visualized as fluorescent spots (GFP-VASP).
GFP –Green Flurescence Protein
VASP –Vasodilator stimulated phosphoprotein is an actin-associated protein involved in a range of processes dependent on cytoskeleton remodeling and cell polarity.

GFP marks VASP and VASP indicates actin in Fas formation.

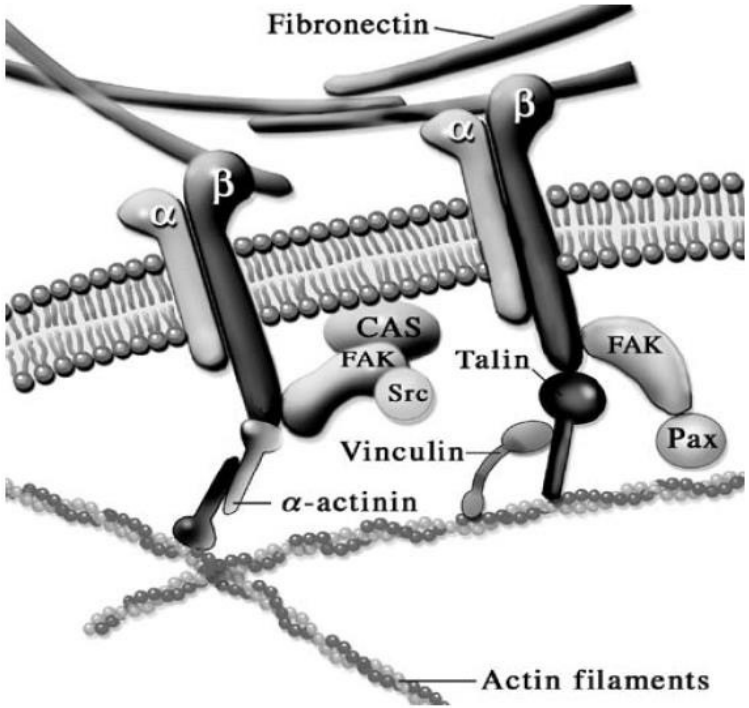


**Focal adhesions** are composed of three main types of components:

1. **Integrins**: Transmembrane receptors that mediate cell-ECM attachment.
    - Integrins form the main link between the ECM and the internal cytoskeleton of the cell.
    - They exist in active (open) and inactive (closed) conformations, with activation often triggered by ECM binding or intracellular signaling.
2. **Adaptor Proteins**: These proteins connect integrins to the actin cytoskeleton and include talin, vinculin, paxillin, and kindlin.
    - **Talin**: Binds directly to integrins and recruits actin, playing a crucial role in force transmission.
    - **Vinculin**: Reinforces the connection between talin and actin, stabilizing focal adhesions under force.
3. **Actin Cytoskeleton**: The internal scaffold that generates forces and helps in cell shape changes.
    - Actin fibers are tethered to focal adhesions, allowing cells to "pull" on their surroundings by contracting actin-myosin bundles (stress fibers).

Example . Vinculin Binding Stretching  Activates Single TalinRod Molecules

Science 323, 638 (2009); Armando del Rio, et al.




This work demonstrates how stretching of Talin induces a biochemical effect (Vinculin binding)

Known:

a. Mechanical forces increase the accumulation of vinculin at the focal adhesions

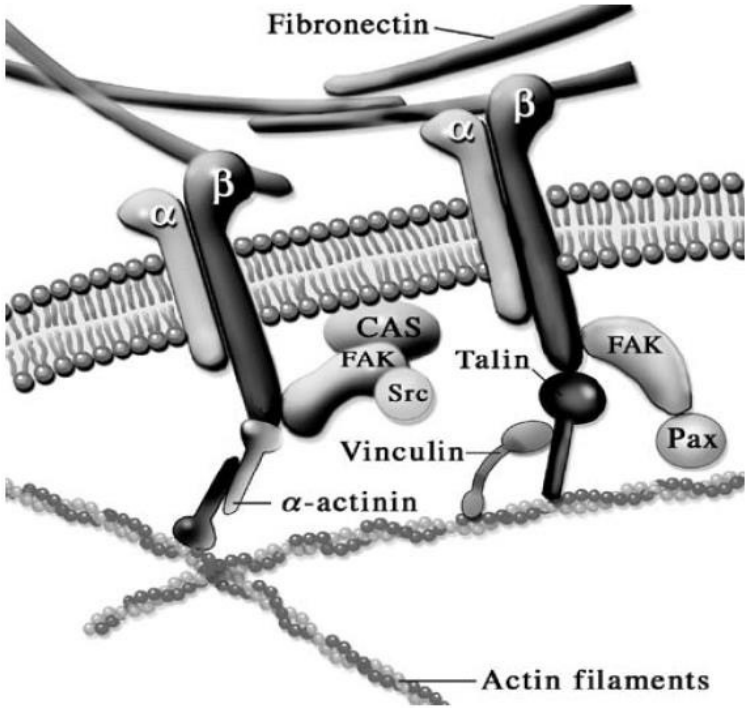
b. Talin can bind to the actin cytoskeleton

Hypotesis:

a, b suggest that force induced by actomyosin contraction could stretch the talin rod exposing talin binding sites to the  vinculin head.

Example . Vinculin Binding Stretching  Activates Single TalinRod Molecules

Science 323, 638 (2009); Armando del Rio, et al.




Focal adhesions not only anchor cells but also act as mechanosensors, converting mechanical cues into biochemical signals in a process known as **mechanotransduction**. When external forces are applied to a cell, focal adhesions sense these forces through integrins, which trigger signaling cascades within the cell.

- **External Force Transmission**: When ECM stiffness increases, focal adhesions can grow and recruit more proteins, thereby strengthening their attachment.
- **Internal Force Generation**: Actomyosin contractility generates forces within the cell that are transmitted outward through focal adhesions, affecting how the cell responds to mechanical changes in the ECM.

# Control of Mechanotransduction by **Molecular Clutch Dynamics**

Alberto Elosegui-Artola, Xavier Trepat, and Pere Roca-Cusachs, Trends in Cell Biology, 2018

The linkage of cells to their microenvironment is mediated by a series of bonds that dynamically engage and disengage, in what has been conceptualized as the molecular clutch model. Whereas this model has long been employed to describe actin cytoskeleton and cell migration dynamics, it has recently been proposed to also explain mechanotransduction (i.e., the process by which cells convert mechanical signals from their environment into biochemical signals). Here we review the current understanding on how cell dynamics and mecha-notransduction are driven by molecular clutch dynamics and its master regu-lator, the force loading rate. Throughout this Review, we place a specific emphasis on the quantitative prediction of cell response enabled by combined experimental and theoretical approaches.

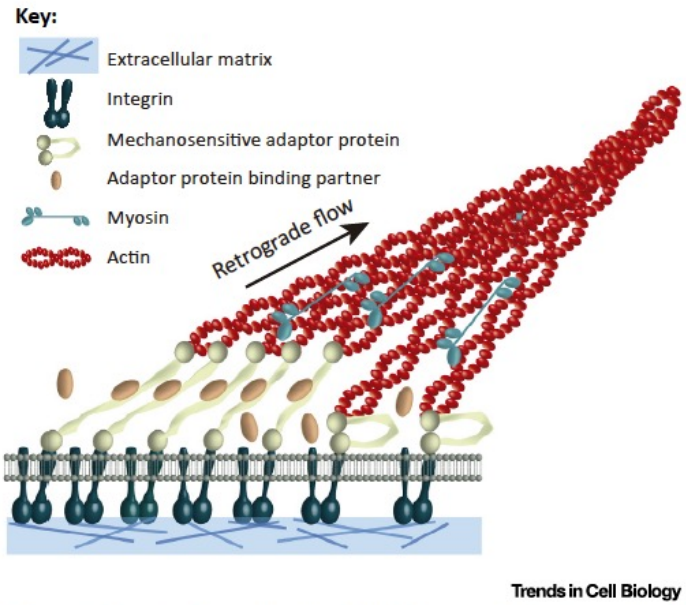



Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

The **molecular clutch model** describes how focal adhesions control the connection between the actin cytoskeleton and the ECM, allowing cells to move in a controlled and efficient manner.

- **Clutch Engagement and Slippage**: In this model, the "clutch" is a molecular linkage that can dynamically engage or disengage between the actin cytoskeleton and integrins bound to the ECM.
    - **Engagement**: When engaged, the clutch transmits forces generated by actin polymerization to the ECM, allowing the cell to "push" against its environment.
    - **Slippage**: When the load becomes too high, the clutch disengages (or slips), releasing some of the tension and allowing the cell to reset.

## Glossary

**Adaptor proteins:** term usually employed to refer to the proteins linking actin to either integrins or cadherins in cell–matrix or cell–cell adhesion complexes, respectively.

**Binding/unbinding rates:** for a given binding event [such as an integrin–extracellular matrix (ECM) bond], this is the inverse of the average time required to bind/unbind the bond, respectively. Binding occurs at zero force, whereas unbinding rates depend on the force applied to the bond.

**Catch bond:** more precisely defined as a catch–slip bond, a catch bond is a bond in which unbinding rates decrease with applied force up to a given threshold, and then increase. Catch bonds thus have an optimal stability (minimum unbinding rate) when a specific value of force is applied to the bond. Importantly, this concept can also be applied to molecular events other than unbinding, such as protein unfolding.

**Contractility:** ability of a cell to contract its actin cytoskeleton via myosin motors. In a situation with very low cell adhesion, contractility would power fast retrograde flows. In a context of high adhesion, contractility is transmitted to the substrate, leading to cell–matrix (or cell–cell) force transmission.

**Durotaxis:** directional cell migration towards areas of increased substrate rigidity.

**Frictional slippage:** regime with low cell–matrix adhesion, in which transient clutch engagement is unable to significantly slow retrograde flow.

**Load and fail/stick–slip:** regime with high cell–matrix adhesion, in which simultaneous engagement of several clutches leads to repeated cycles of progressive buildup of force, followed by complete disengagement and force release.

**Loading rate:** in units of force/time, rate at which applied force increases for a given clutch or clutch ensemble.

**Load and fail/stick–slip:** regime with high cell–matrix adhesion, in which simultaneous engagement of several clutches leads to repeated cycles of progressive buildup of force, followed by complete disengagement and force release.

**Loading rate:** in units of force/time, rate at which applied force increases for a given clutch or clutch ensemble.

**Molecular clutch:** link between actin and an ECM ligand (or a neighboring cell) which can be bound (engaged) or unbound. Usually assumed to represent the serial link between actin, an individual

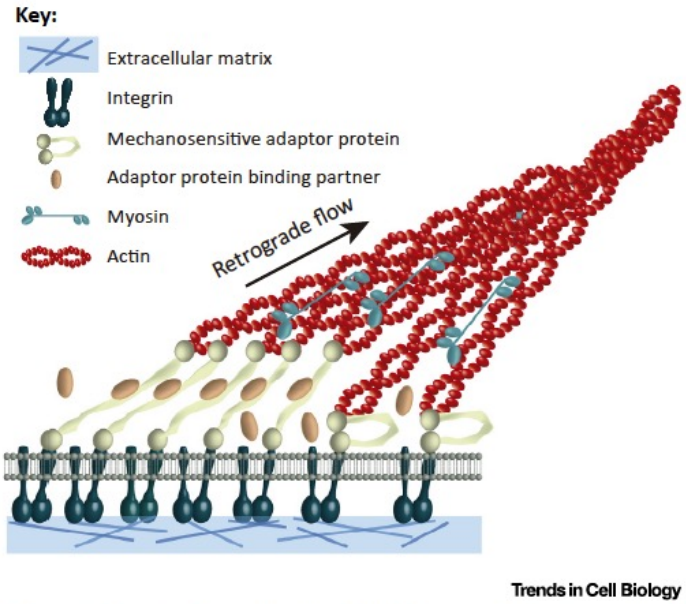



Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

The molecular clutch model helps explain why cells respond differently depending on the stiffness of the ECM:

**On Stiff Substrates**: The clutch remains engaged for a longer period, resulting in stronger force transmission and larger, more stable focal adhesions.

**On Soft Substrates**: The clutch disengages more quickly, leading to weaker force transmission, smaller adhesions, and different migration behavior.

**Dynamic Regulation of Clutch Components**

1. **Talin Stretching and Vinculin Binding**: Talin undergoes conformational changes when stretched, exposing binding sites for vinculin, which stabilizes the link between integrins and actin.
2. **Actin Flow Rate and Focal Adhesion Lifetime**: Actin polymerization rate and retrograde flow (backward movement) can influence clutch engagement and disengagement dynamics, directly affecting focal adhesion lifetime and maturation.

**Implications of the Molecular Clutch Model**

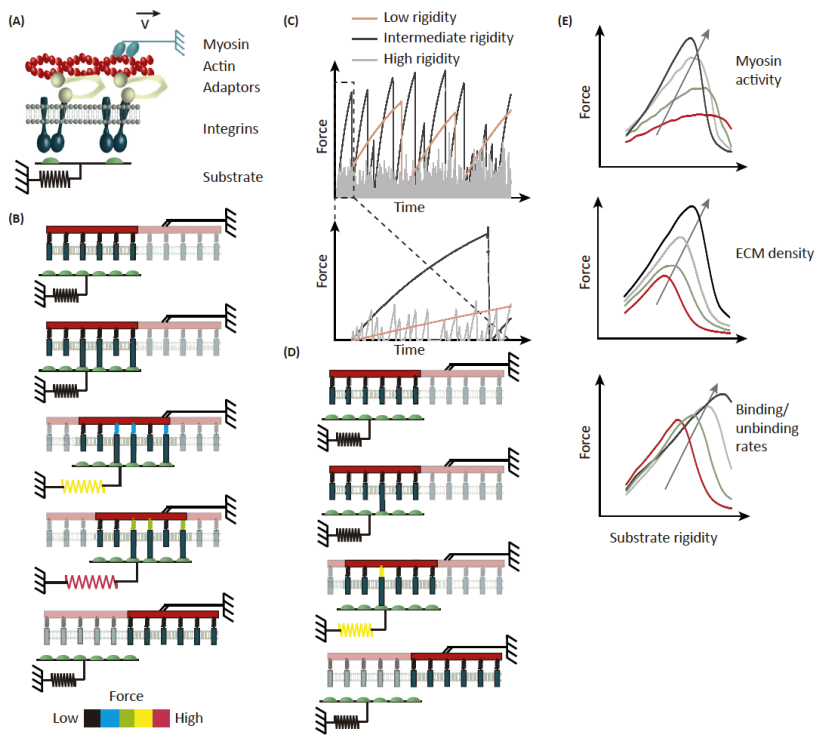
The molecular clutch model highlights the balance between ECM rigidity and cytoskeletal dynamics, explaining why cells exhibit different behaviors on different substrates. This balance is crucial in processes such as:

- **Cell Migration**: Efficient clutch engagement and disengagement allow cells to move directionally.
- **Cancer Metastasis**: Cells with dysregulated focal adhesion dynamics may migrate more aggressively, aiding in metastasis.
- **Stem Cell Differentiation**: Stem cells can sense ECM stiffness and differentiate accordingly, with stiffness affecting clutch engagement.

## Applications and Importance of Understanding Focal Adhesions

Studying focal adhesions and the molecular clutch model provides insight into many biological and medical applications:

- **Tissue Engineering**: Understanding focal adhesion dynamics helps in designing biomaterials that mimic natural ECM properties, guiding cell behavior in engineered tissues.
- **Cancer Research**: Targeting focal adhesion components, such as integrins or talin, has therapeutic potential in limiting cancer cell migration and invasion.
- **Stem Cell Therapy**: Manipulating ECM properties can direct stem cell fate decisions, aiding in regenerative medicine.




Trends in Cell Biology

Figure 2. Force Transmission through the Molecular Clutch. (A) Cartoon summarizing the fundamental elements of the system. (B) From top to bottom, sequence of events in a typical 'load and fail' cycle of a molecular clutch (rectangles represent integrins). As clutches bind, myosin contractility deforms the substrate, building force on the substrate and each bound clutch. At some point, force leads to bond destabilization, all clutches disengage, and the cycle starts again. (C) Typical plots of force exerted versus time for molecular clutches on low, intermediate, and high rigidity. (D) From top to bottom, sequence of events in a typical 'frictional slippage' cycle of a molecular clutch, observed on a high-rigidity regime. As a clutch binds, myosin contractility builds force very quickly due to the high rigidity, leading to clutch disengagement before others have time to bind. This limits overall force transmission to the substrate. (E) Clutch model predictions of average force transmission to the substrate as a function of substrate rigidity. Top, middle, and bottom graphs show the changes in the curve induced by increasing myosin activity, increasing ECM ligand density, and simultaneously increasing binding and unbinding rates, respectively. Abbreviation: ECM, Extracellular matrix.

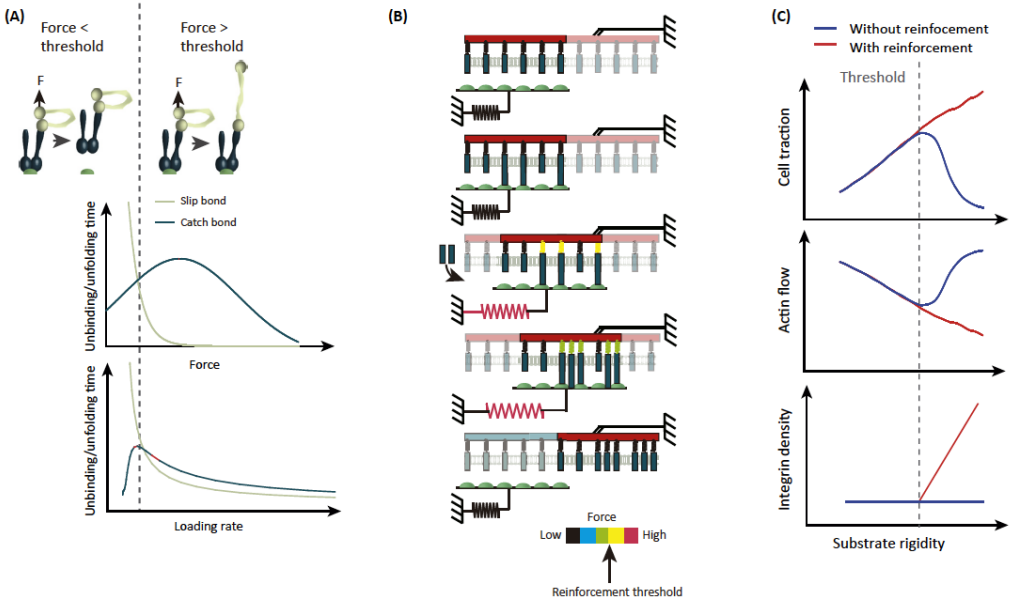



Figure 3. Force Transduction through the Molecular Clutch. (A) Bottom, effect of either a constant force or a constant force loading rate on the average times required for protein unfolding or bond unbinding. Typical curves for a slip or catch bond are shown. Top, expected effect on a system in which force is applied to a serial link between a molecule that unfolds as a slip bond (such as talin) and a bond that unbinds as a catch bond (such as an integrin–fibronectin bond). Unbinding occurs first when force is below the threshold, and unfolding (and subsequent mechanotransduction) occurs first when force is above the threshold. (B) From top to bottom, sequence of events in a typical 'load and fail' cycle of a molecular clutch, including mechanotransduction (reinforcement) events. As clutches bind and force builds, some clutches surpass the threshold force required for mechanotransduction, leading to the recruitment of additional integrins. This increases the number of bound clutches, reducing the force applied per clutch, delaying the failure of the system, and increasing average force transmission. (C) Examples of predicted force/rigidity curves in the presence and absence of reinforcement. Reinforcement only affects force transmission above a threshold in rigidity, which corresponds to the loading rate threshold from (A). Then, the increase in integrin recruitment prevents the reduction in force (and increase in actin flows) normally expected in a molecular clutch system.