How to afford such a scientific question?

Is force-dependent vinculin binding to talin in live cells a crucial step in anchoring the actin cytoskeleton to focal adhesions ?

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MBC - Chapter 19

TABLE 19–1 Anchoring Junctions

Junction	Transmembrane adhesion protein	Extracellular ligand	Intracellular cytoskeletal attachment	Intracellular adaptor proteins
Cell–Cell				
Adherens junction	Classical cadherins	Classical cadherin on neighboring cell	Actin filaments	α-Catenin, β-catenin, plakoglobin (γ-catenin), p120-catenin, vinculin
Desmosome	Nonclassical cadherins (desmoglein, desmocollin)	Desmoglein and desmocollin on neighboring cell	Intermediate filaments	Plakoglobin (γ-catenin), plakophilin, desmoplakin
Cell–Matrix				
Actin-linked cell–matrix junction	Integrin	Extracellular matrix proteins	Actin filaments	Talin, kindlin, vinculin, paxillin, focal adhesion kinase (FAK), numerous others
Hemidesmosome	$\alpha_6\beta_4$ Integrin, type XVII collagen	Extracellular matrix proteins	Intermediate filaments	Plectin, BP230

Is force-dependent vinculin binding to talin in live cells a crucial step in anchoring the actin cytoskeleton to focal adhesions ?

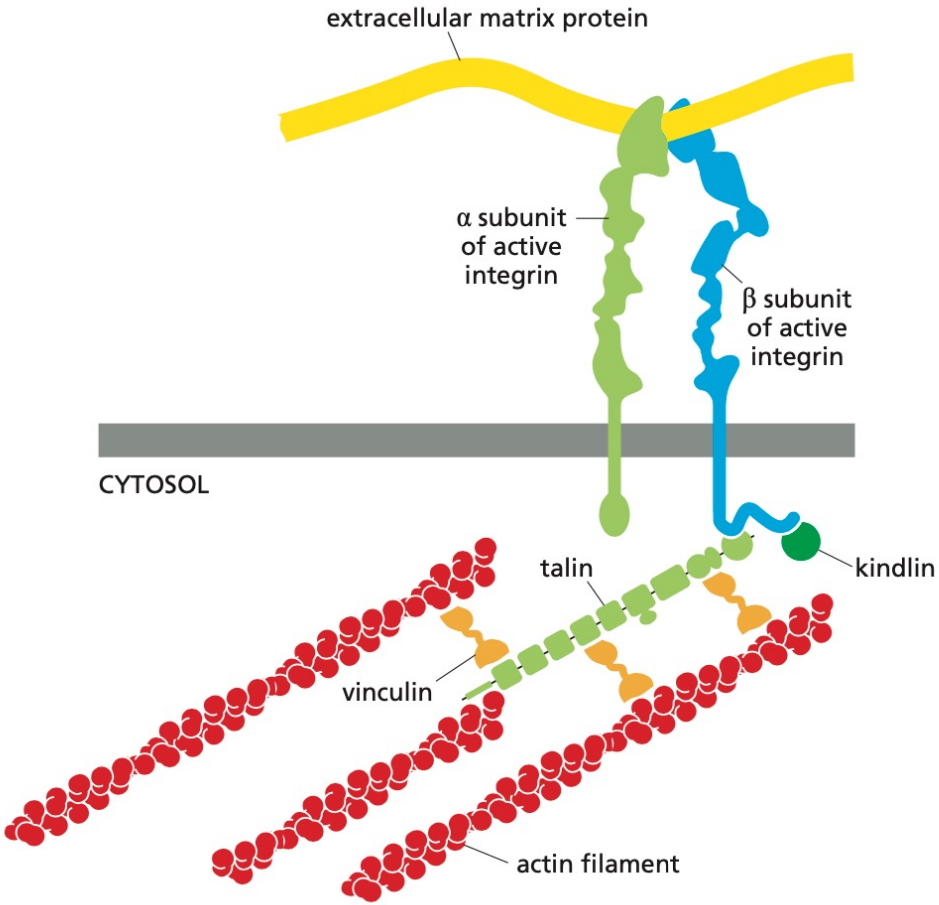


Figure 19–55 The subunit structure of an active integrin molecule, linking extracellular matrix to the actin cytoskeleton. The N-terminal heads of the integrin chains attach directly to an extracellular protein such as fibronectin; the C-terminal intracellular tail of the integrin β subunit binds to adaptor proteins that interact with filamentous actin. The best-understood adaptor is a giant protein called talin, which contains a string of multiple domains for binding actin and other proteins, such as vinculin, that help reinforce and regulate the linkage to actin filaments. One end of talin binds to a specific site on the integrin β subunit cytoplasmic tail; other regulatory proteins, such as kindlin, bind at another site on the tail.

Integrins exist in two major activity states

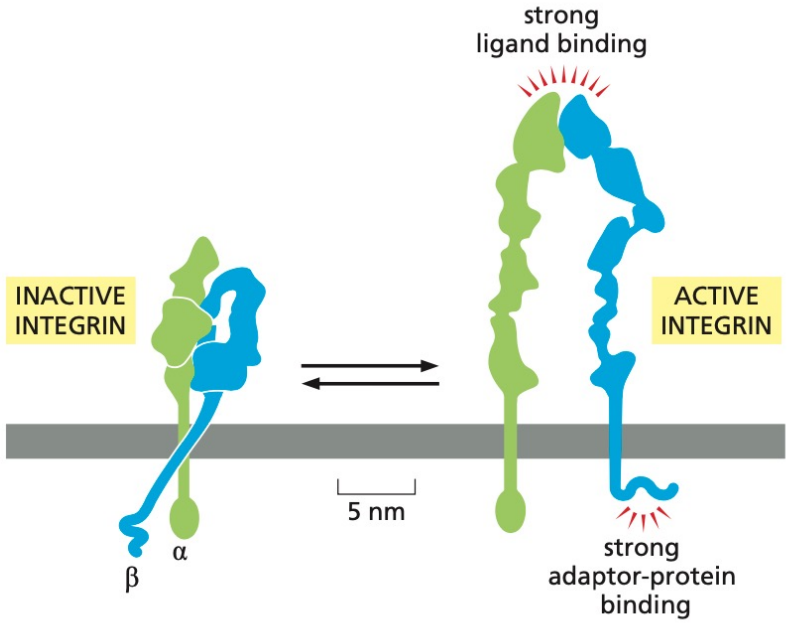


Figure 19–57 Integrins exist in two major activity states. Inactive (folded) and active (extended) structures of an integrin molecule, based on data from x-ray crystallography and other methods.

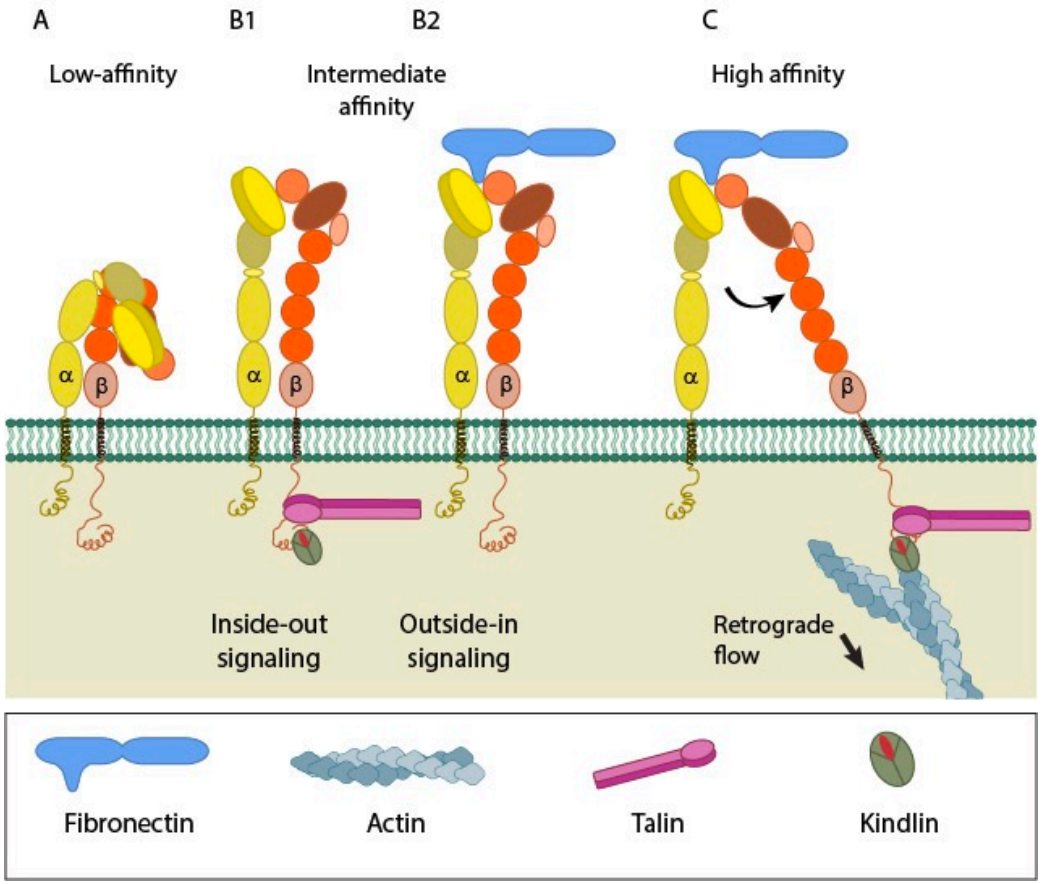
Inside-out signaling versus Outside-in signaling

Outside-in signaling

Ligand binding to external domain causes conformational changes that increase ligand affinity, modify protein-interaction sites in the cytoplasmic domains and thence the resulting signals.

Inside-out signaling

Signals received by other receptors foster the binding of talin and kindlin to cytoplasmic end of the integrin β subunit, at sites of actin polymerization.



Activation of integrins by intracellular signaling

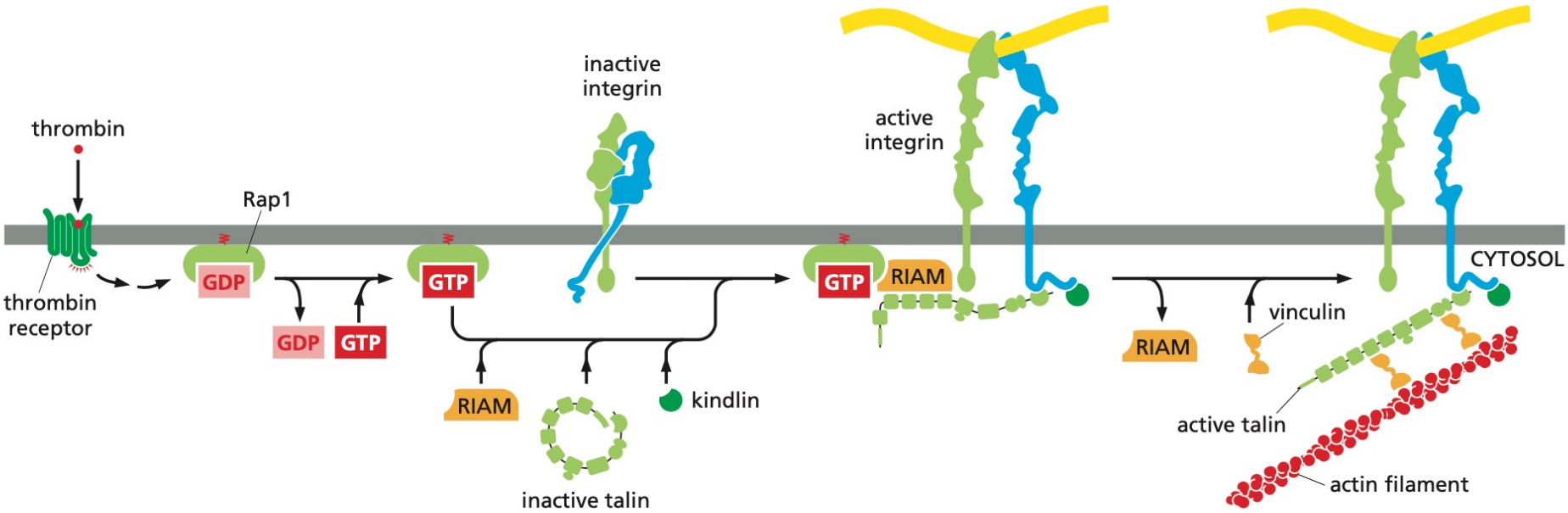
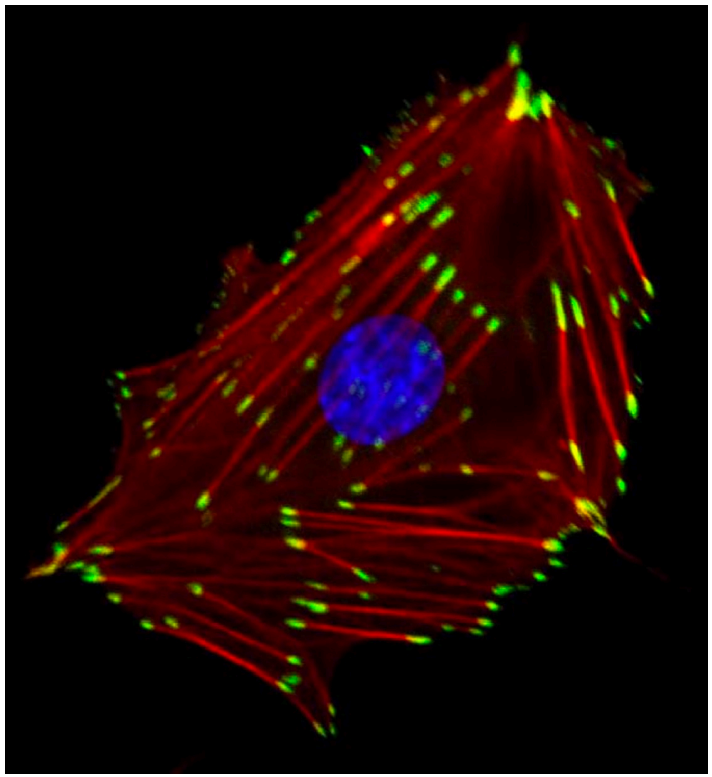
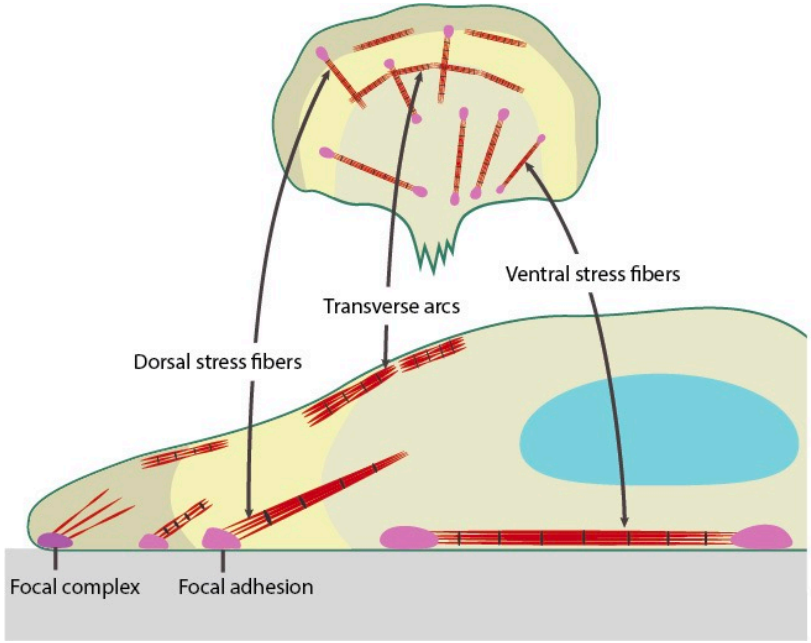


Figure 19–58 Activation of integrins by intracellular signaling. Signals received from outside the cell can act through various intracellular mechanisms to stimulate integrin activation. In platelets, as illustrated here, the extracellular signal protein thrombin activates a G-protein-coupled receptor on the cell surface, thereby initiating a signaling pathway that leads to activation of Rap1, a member of the monomeric GTPase family. Activated Rap1 interacts with the protein RIAM, which then recruits talin to the plasma membrane. Together with another protein called kindlin, talin interacts with the integrin β chain to trigger integrin activation. Talin then interacts with adaptor proteins such as vinculin, resulting in the formation of an actin linkage (see Figure 19–55).

Talin regulation depends in part on an interaction between its flexible C-terminal rod domain and the N-terminal head domain that contains the integrin-binding site. This interaction is thought to maintain talin in an inactive state when it is free in the cytoplasm. When talin is recruited by RIAM to the plasma membrane, the talin head domain interacts with a phosphoinositide called $PI(4,5)P_2$ (not shown here, but see Figure 15–28), resulting in dissociation of the rod domain. Talin unfolds to expose its binding sites for integrin and other proteins.

Focal adhesions





A REF52 fibroblast plated on a collagen-coated glass coverslip reveals focal adhesions stained for vinculin (green), F-actin (red), showing stress fibers and the nucleus labeled in blue.Image was kindly provided by David Graham.

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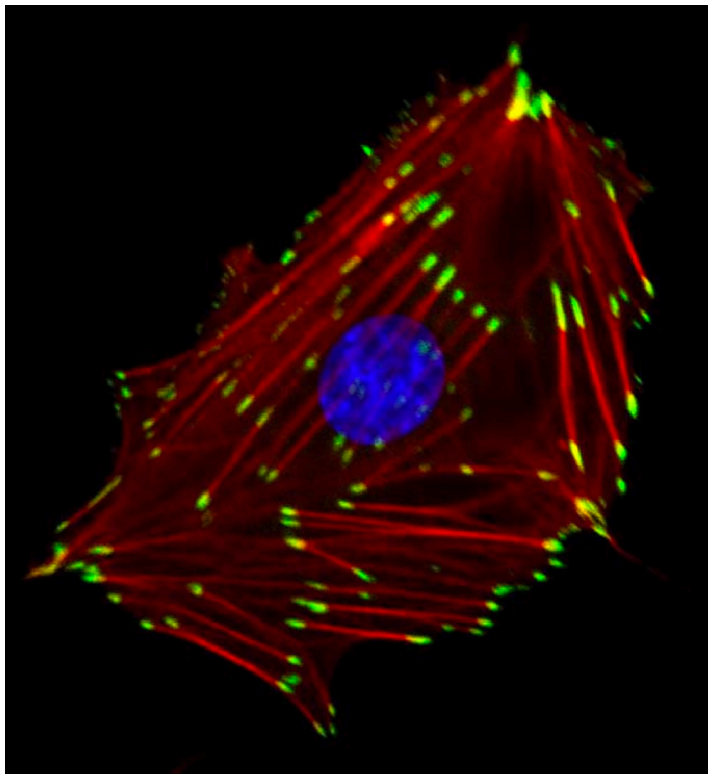
Focal adhesions



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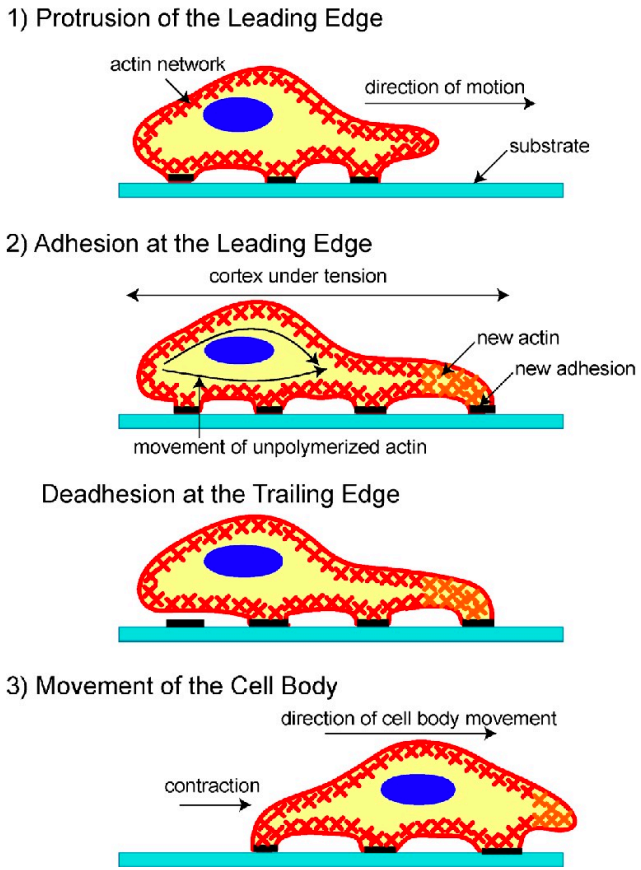

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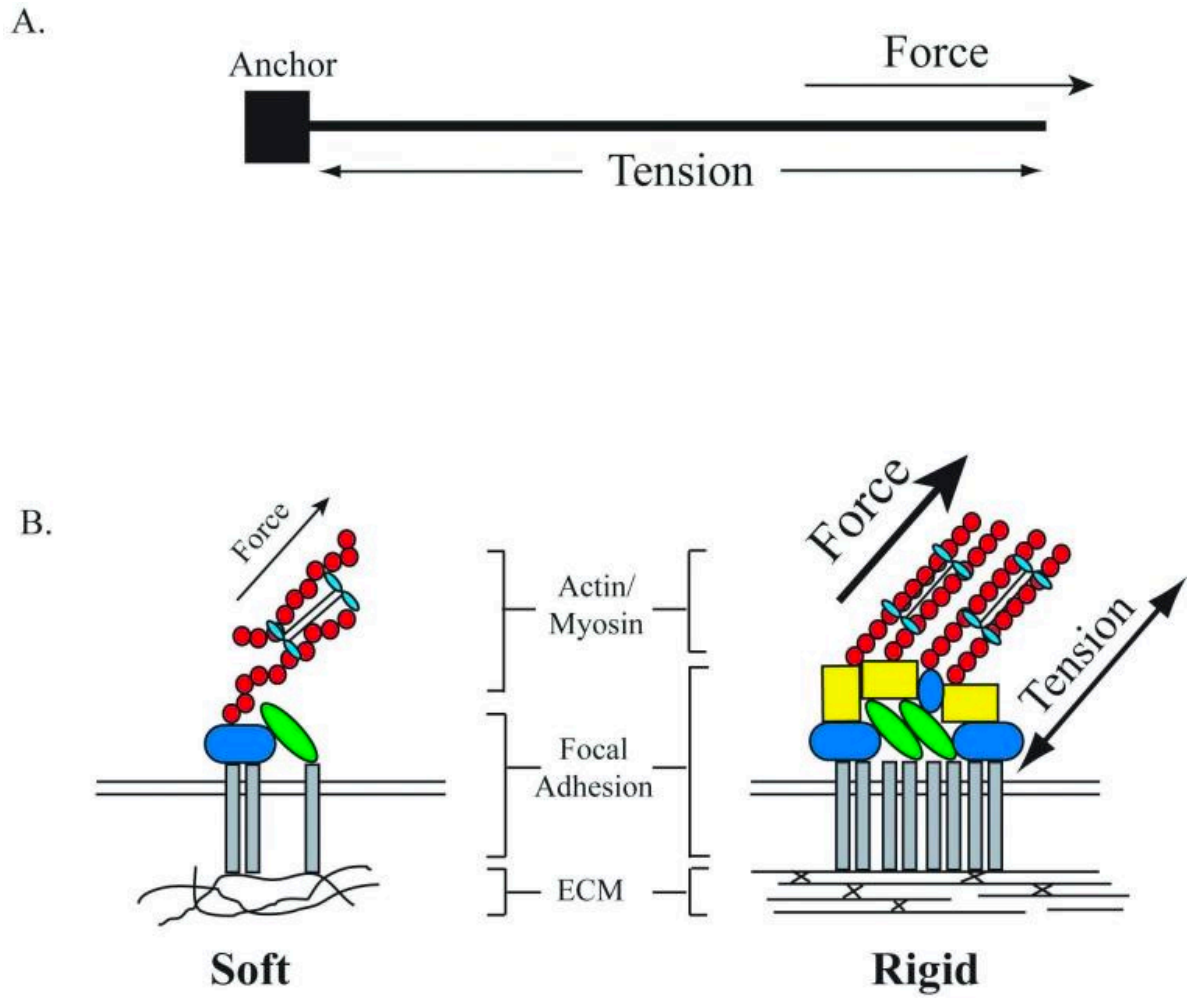
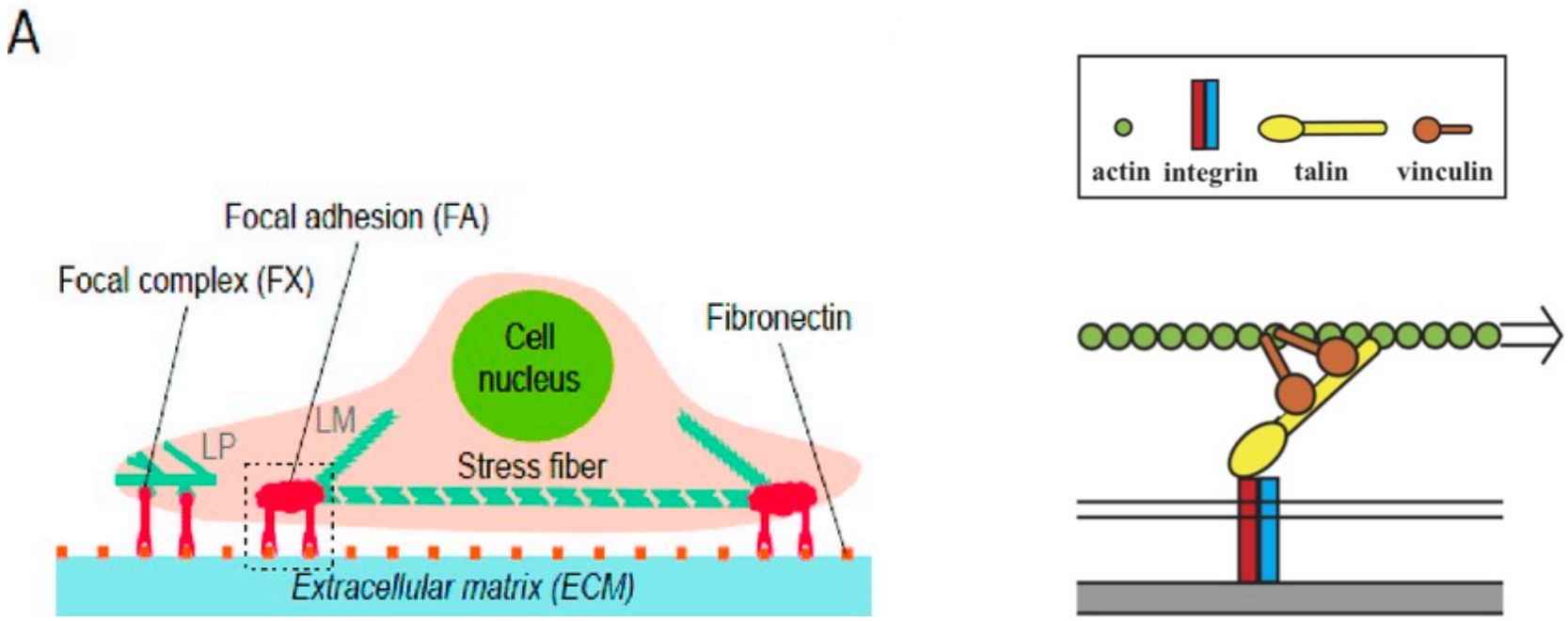
Focal adhesion

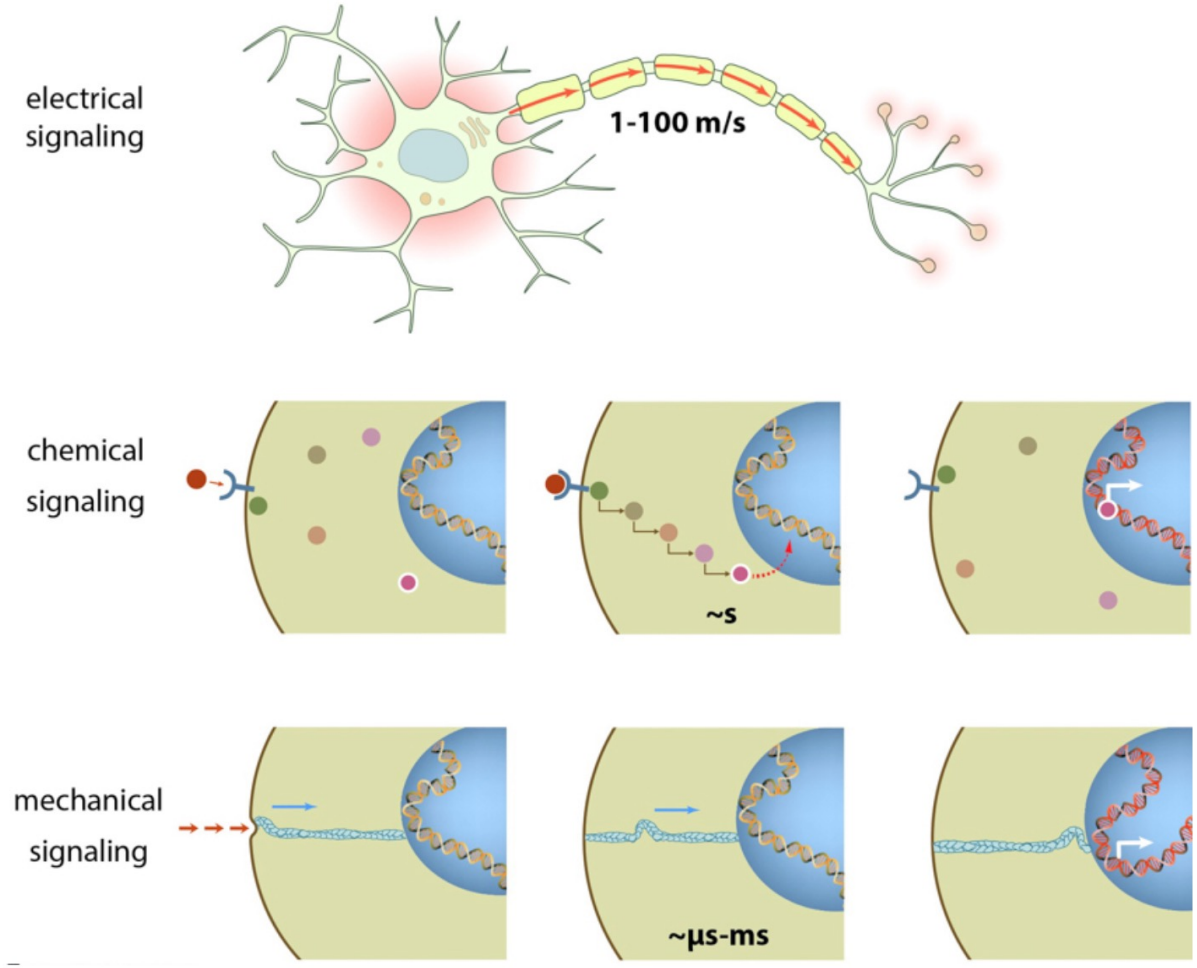


Schematic of focal adhesions organization.



(A) The cell adheres to the extracellular matrix (ECM) by means of adhesions. Fibronectin, a ligand presents on the ECM, allows the cell to adhere through nascent adhesions. In the lamellipodium (LP) the adhesions are in the focal complexes stage, where they can then mature in the lamella (LM) into focal adhesions. This happens in response to traction forces applied on the adhesions which are connected to stress fibers. The ventral stress fibers connect one focal adhesion to another and the dorsal stress fibers link the focal adhesions to the actin network. (B) Focal adhesions are composed of transmembrane integrins which bind to the fibronectin on the ECM. Integrins are connected inside the cell to actin stress fibers through talin. There is also proteins, such as vinculin or paxilin, which enhance the basic mechanical link of integrin with actin filaments. (Hoffmann and Schwarz, 2013)

How do cells communicate?



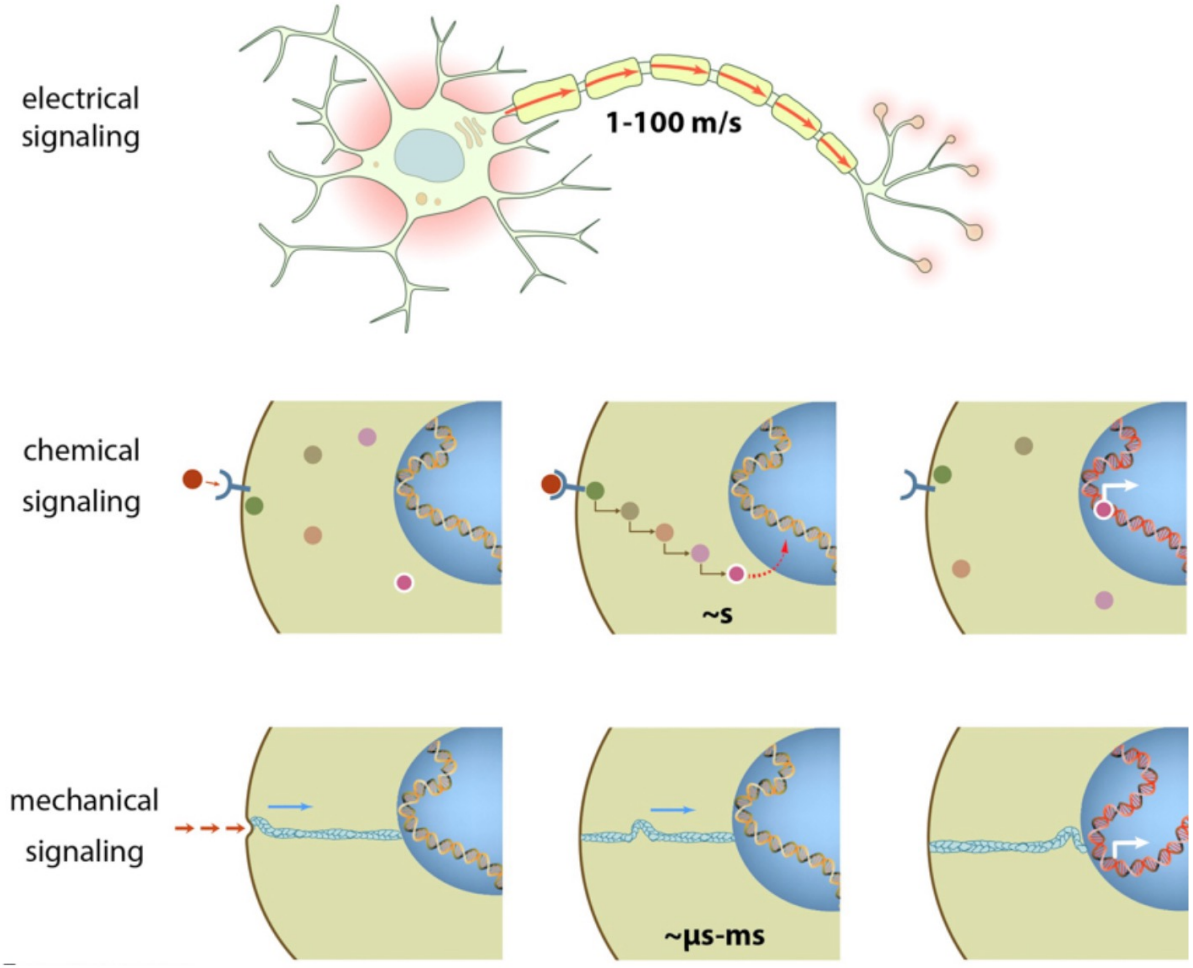
Cells sense their environment through distinct mechanisms and subcellular structures. These allow cells to produce and detect a variety of signal types, from soluble molecules or chemicals, to electrical current and even mechanical cues that are transmitted via the push and pull of biomolecules.

Mechanosignaling can often trigger cellular signaling processes much faster than a purely chemical means of activation.

Mechanical signaling refers to a process where a signal is triggered by a mechanical force such as shear stress or a pull/push applied to biomolecules.

Usually, application of such force would induce a conformational change in the protein/receptor, thus exposing functional domains to the environment.

Mechanosignaling can often trigger cellular signaling processes much faster than a purely chemical means of activation. This is illustrated by the mechanical stimulation of focal adhesions in smooth muscle cells in which Src was activated in less than 0.3s. This rapid response was in contrast to the slow (12 second) response that followed chemical activation.



Cells sense their environment through distinct mechanisms and subcellular structures. These allow cells to produce and detect a variety of signal types, from soluble molecules or chemicals, to electrical current and even mechanical cues that are transmitted via the push and pull of biomolecules.

Talin is a tension sensor at cell–matrix junctions.

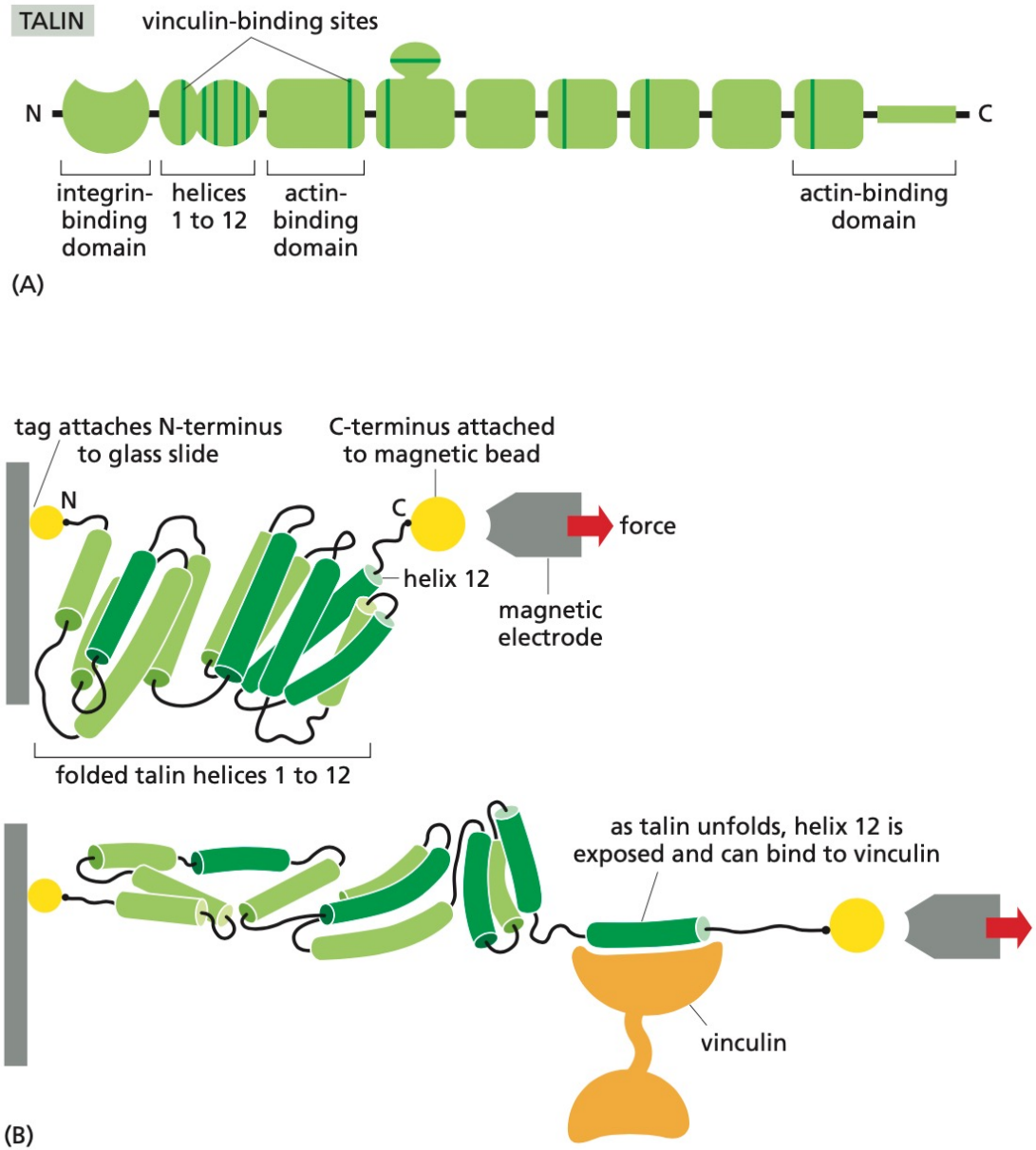
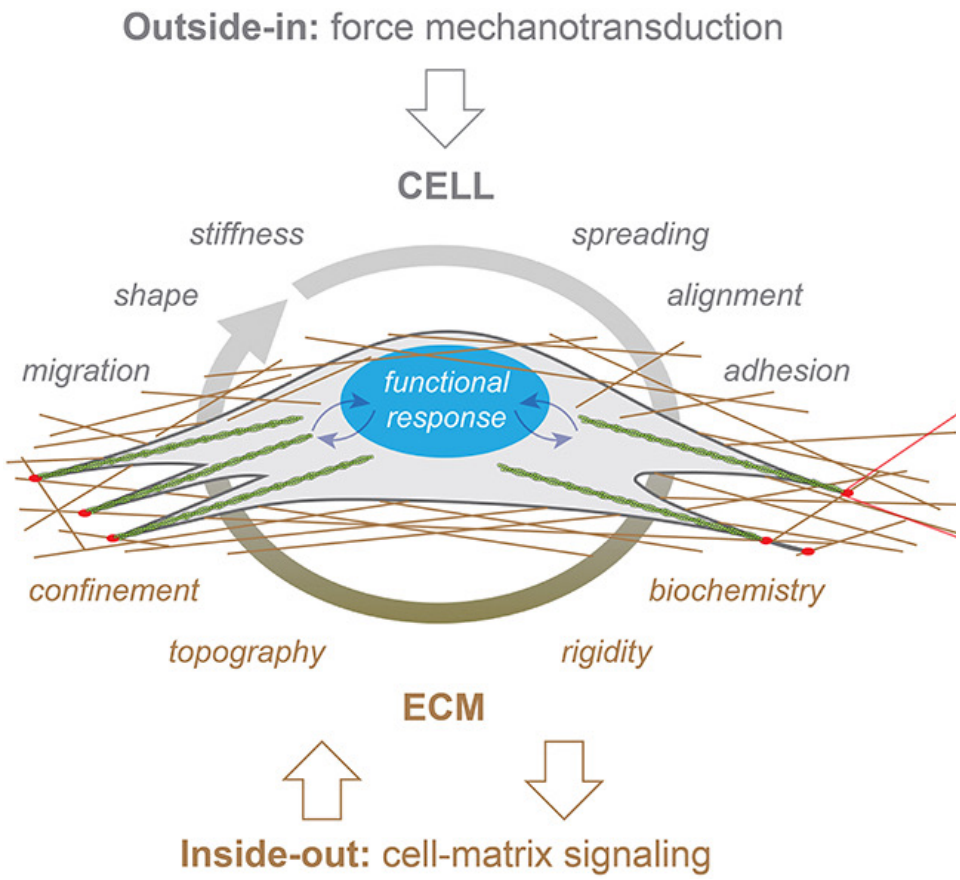


Figure 19–60 Tension across cell–matrix junctions stimulates the local recruitment of vinculin and other actin-regulatory proteins, thereby strengthening the junction's attachment to the cytoskeleton. a fragment of talin containing this domain was attached to an apparatus in which the domain could be stretched, as shown here. the fragment was labeled at its N-terminus with a tag that sticks to the surface of a glass slide on a microscope stage. the C-terminal end of the fragment was bound to a tiny magnetic bead, so the talin fragment could be stretched using a small magnetic electrode. the solution around the protein contained fluorescently tagged vinculin proteins. after the talin protein was stretched, excess vinculin solution was washed away, and the microscope was used to determine if any fluorescent vinculin proteins were bound to the talin protein.

In the absence of stretching *(top)*, most talin molecules did not bind vinculin. When the protein was stretched *(bottom)*, two or three vinculin molecules were bound (only one is

Mechanotransduction



Representation of “outside-in” (in gray) and “inside-out” (in brown) mechanotransduction signals in a cell growing in a three-dimensional (3D) fibrous matrix.

- Focal adhesion (FAs, in red) serve as crucial sites for both outside-in and inside-out mechanotransduction through the recruitment of transmembrane integrins.

- Cells and tissues can sense and react to the modifications of the physico-chemical properties of the extracellular environment (ECM) through integrin-based adhesion sites and adapt their physiological response in a process called mechanotransduction.

- Due to their critical localization at the cell-ECM interface, transmembrane integrins are mediators of bidirectional signaling, playing a key role in **“outside-in”** and **“inside-out”** signal transduction.

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Focal Adhesion Kinase as a Regulator of Cell Tension in the Progression of Cancer

Robert W. Tilghman and **J. Thomas Parsons***
Department of Microbiology, University of Virginia Health System, Charlottesville, VA 22908 USA

Abstract

Growing evidence indicates that critical steps in cancer progression such as cell adhesion, migration, and cell cycle progression are regulated by the composition and organization of the microenvironment. The adhesion of cancer cells to components of the microenvironment and the forces transmitted to the cells via the actinomyosin network and the signaling complexes organized within focal adhesions allow cancer cells to sense the local topography of the extracellular matrix and respond efficiently to proximal growth and migration promoting cues. Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase that is over expressed in a variety of cancers and plays an important role in cell adhesion, migration, and anchorage-dependent growth. In this review, we summarize evidence which implicate FAK in the ability of cells to sense and respond to local forces from the microenvironment through the regulation of adhesion dynamics and actinomyosin contractility, and we discuss the potential roles of FAK as a mechanosensor in the progression of cancer.

Keywords

Focal adhesion kinase; tension; mechanotransduction; cell migration

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Paradigm	Techniques	Controls

Paper 01

Am J Physiol Cell Physiol 306: C607–C620, 2014.
First published January 22, 2014; doi:10.1152/ajpcell.00122.2013.

Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions

Hiroaki Hirata,[1,2] Hitoshi Tatsumi,[3] Chwee Teck Lim,[1,4] and Masahiro Sokabe[1,2,3]

[1]*Mechanobiology Institute, National University of Singapore, Singapore;* [2]*Cell Mechanosensing Project, International Cooperative Research Project/Solution-Oriented Research for Science and Technology, Japan Science and Technology Agency, Nagoya, Japan;* [3]*Department of Physiology, Nagoya University Graduate School of Medicine, Nagoya, Japan; and* [4]*Department of Biomedical Engineering and Department of Mechanical Engineering, National University of Singapore, Singapore*

Submitted 3 May 2013; accepted in final form 19 January 2014

Hirata H, Tatsumi H, Lim CT, Sokabe M. Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions. *Am J Physiol Cell Physiol* 306: C607–C620, 2014. First published January 22, 2014; doi:10.1152/ajpcell.00122.2013.—Mechanical forces play a pivotal role in the regulation of focal adhesions (FAs) where the actin cytoskeleton is anchored to the extracellular matrix through integrin and a variety of linker proteins including talin and vinculin. The localization of vinculin at FAs depends on mechanical

eton in fibroblasts (17, 34, 65). The talin-mediated link between the actin cytoskeleton and clustered integrin is broken repeatedly by a small force of ~2 pN generated by the retrograde flow of actin filaments (34). On the other hand, the integrin-actin cytoskeleton linkage is strengthened when a mechanical force is loaded to it (7, 61). The strengthened linkage can sustain much larger forces (~20 pN), which

Scientific question?

Hirata H, Tatsumi H, Lim CT, Sokabe M. Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions. *Am J Physiol Cell Physiol* 306: C607–C620, 2014. First published January 22, 2014; doi:10.1152/ajpcell.00122.2013.—Mechanical forces play a pivotal role in the regulation of focal adhesions (FAs) where the actin cytoskeleton is anchored to the extracellular matrix through integrin and a variety of linker proteins including talin and vinculin. The localization of vinculin at FAs depends on mechanical forces. While in vitro studies have demonstrated the force-induced increase in vinculin binding to talin, it remains unclear whether such a mechanism exists at FAs in vivo. In this study, using fibroblasts cultured on elastic silicone substrata, we have examined the role of forces in modulating talin-vinculin binding at FAs. Stretching the substrata caused vinculin accumulation at talin-containing FAs, and this accumulation was abrogated by expressing the talin-binding domain of vinculin (domain D1, which inhibits endogenous vinculin from binding to talin). These results indicate that mechanical forces loaded to FAs facilitate vinculin binding to talin at FAs. In cell-protruding regions, the actin network moved backward over talin-containing FAs in domain D1-expressing cells while it was anchored to FAs in control cells, suggesting that the force-dependent vinculin binding to talin is crucial for anchoring the actin cytoskeleton to FAs in living cells.

Experimental paradigm

Hirata H, Tatsumi H, Lim CT, Sokabe M. Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions. *Am J Physiol Cell Physiol* 306: C607–C620, 2014. First published January 22, 2014; doi:10.1152/ajpcell.00122.2013.—Mechanical forces play a pivotal role in the regulation of focal adhesions (FAs) where the actin cytoskeleton is anchored to the extracellular matrix through integrin and a variety of linker proteins including talin and vinculin. The localization of vinculin at FAs depends on mechanical forces. While in vitro studies have demonstrated the force-induced increase in vinculin binding to talin, it remains unclear whether such a mechanism exists at FAs in vivo. In this study, using fibroblasts cultured on elastic silicone substrata, we have examined the role of forces in modulating talin-vinculin binding at FAs. Stretching the substrata caused vinculin accumulation at talin-containing FAs, and this accumulation was abrogated by expressing the talin-binding domain of vinculin (domain D1, which inhibits endogenous vinculin from binding to talin). These results indicate that mechanical forces loaded to FAs facilitate vinculin binding to talin at FAs. In cell-protruding regions, the actin network moved backward over talin-containing FAs in domain D1-expressing cells while it was anchored to FAs in control cells, suggesting that the force-dependent vinculin binding to talin is crucial for anchoring the actin cytoskeleton to FAs in living cells.

Reagents

MATERIALS AND METHODS

Cell culture. Human foreskin fibroblasts (HFFs) and HeLa cells were cultured in Dulbecco's modified Eagle's medium (Sigma Chemical, St. Louis, MO) supplemented with 10% fetal bovine serum (Nipro, Osaka, Japan) at 37°C in 5% CO_2. For immunofluorescence experiments, HFF cells were grown for 15 h on glass coverslips or elastic silicone (polydimethylsiloxane elastomer) chambers (Strex, Osaka, Japan), which were precoated with 100 μg/ml fibronectin (Sigma Chemical). In some cases, cells were treated with 100 μM blebbistatin (Toronto Research Chemicals, North York, Canada) or 40 μM Y-27632 (Calbiochem, San Diego, CA) for 30 min.

Chamber slides