Direct Observation of a Coil-to-Helix Contraction Triggered by Vinculin Binding to Talin

Rafael Tapia-Rojo,[1,*] Alvaro Alonso-Caballero,[1] and Julio M. Fernandez[1,†]

[1] *Department of Biological Sciences, Columbia University, New York, NY 10027, USA*

Vinculin binds unfolded talin domains in focal adhesions, which recruits actin filaments to reinforce the mechanical coupling of this organelle. However, the mechanism by which this interaction is regulated, and its impact in the force transmission properties of this mechanotransduction pathway remain unknown. Here, we use magnetic tweezers force spectroscopy to measure vinculin binding to the talin R3 domain under physiological force loads. For the first time, we resolve individual binding events as a short contraction of the talin polypeptide due to the reformation of the helices in the vinculin-binding sites. This force-dependent contraction dictates the mechanism by which force regulates the talin-vinculin interaction. Force is needed to unfold talin and expose the cryptic vinculin-binding sites; however, the structural contraction triggered by binding introduces an energy penalty that increases with force, defining an optimal binding force at 10 pN. This novel mechanism implies that the talin-vinculin-actin association works in focal adhesions as a negative feedback, which operates to stabilize the force acting on each junction.

* rt2605@columbia.edu

† jfernandez@columbia.edu

I. INTRODUCTION

Cell function relies largely on the ability of cells to interpret their mechanical environment, and respond dynamically to these force cues—mechanotransduction [1–4]. Cells anchor the extracellular matrix and probe their stiffness through focal adhesions, which connect transmembrane integrins with the active cellular cytoskeleton, and regulate the transmission and transduction of force into biochemical regulatory signals [5–7]. Talin connects physically integrins with the actin filaments, and regulates the mechanical response of focal adhesions by forming a complex network of interactions with several other molecular partners [4, 8–11]. Among them, vinculin is particularly relevant, since the 11 vinculin-binding sites distributed along the talin rod are cryptic, and require mechanical unfolding of its helical domains for vinculin to bind [11–14]. Upon binding, vinculin recruits actin filaments, which reinforce the mechanical coupling and increase the strength of the focal adhesion [15–18]. This mechanism has been suggested to operate as a positive feedback; as force across talin increases gradually, its domains unfold and more vinculin molecules bind, increasing actin recruitment and subsequent force transmission [3, 19, 20]. However, vinculin is also required for the stabilization of adhesions under force [21]; hence, it remains unknown how vinculin binding could regulate force application, and control the lifetime of focal adhesions. Each vinculin-actin linkage bears around 2.5 pN of force that contributes to the overall tension of the linkage [21], but vinculin dissociates from talin under excessive force loads [22]. This could suggest that vinculin binding occurs on a restricted force regime, over which each linkage should operate. However, the force-dependency of the talin-vinculin interaction has never been measured, and the mechanism by which force regulates this complex remains unknown.

Here, we use magnetic tweezers force spectroscopy to measure vinculin binding to the talin R3 domain, and investigate how force modulates this interaction. Thanks to the improved resolution of our custom-made setup, we resolve for the first time individual vinculin binding events as a short contraction of the talin polypeptide due to the coil-to-helix transition induced by binding (Fig. 1). The scaling of this contraction with force indicates that vinculin binding is cooperative, and two vinculin molecules bind simultaneously to the unfolded R3 domain. Our experiments reveal a biphasic force dependency of the binding reaction. First, force favors binding by unfolding talin and exposing the cryptic binding sites. However, in order for vinculin to bind, it must do mechanical work against the force to contract the stretched talin polypeptide, which is unfavorable at higher forces. This novel mechanism regulates talin-vinculin interaction, and defines an optimal binding force of 10 pN. By integrating our findings into a minimalistic focal adhesion model, we demonstrate that the talin-vinculin-actin linkage might operate in focal adhesions as a negative feedback, which recruits or dissociates vinculin molecules to stabilize the force on each junction to an optimal value.

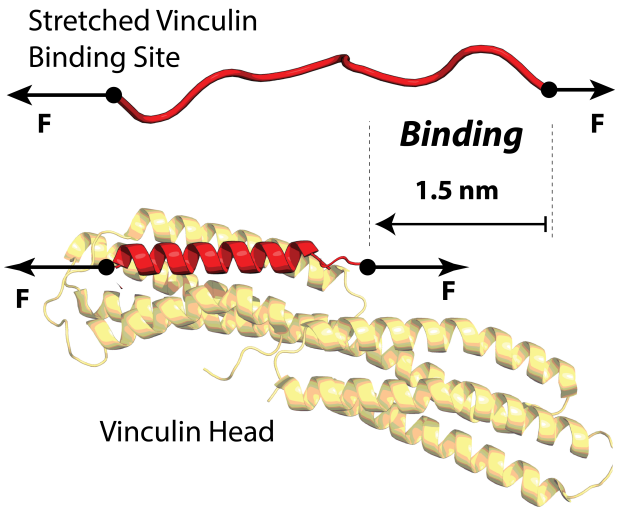

FIG. 1. **Vinculin binding requires the structural accommodation of the talin polypeptide on the vinculin head:** Under force, talin unfolds and the vinculin-binding sites become unstructured polypeptide chains. Upon binding of one vinculin molecule, the binding site helix must reform, which shortens talin by ∼1.5 nm at a force of 9 pN.

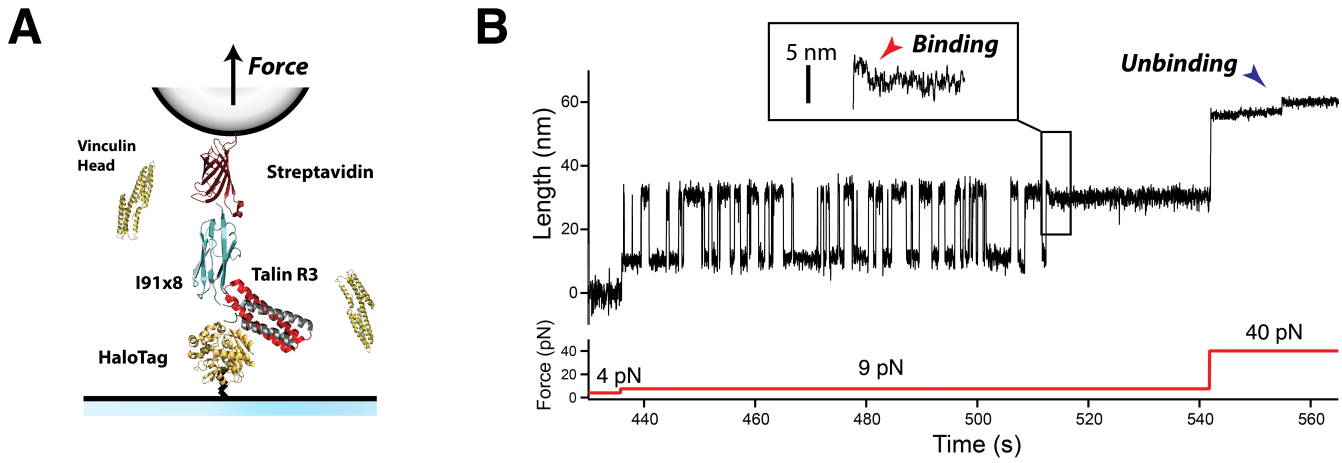

FIG. 2. **Real time detection of the coil-to-helix contraction induced by vinculin binding:** (A) Schematics of a magnetic tweezers experiment for detecting vinculin binding events. We engineer a (R3 IVVI)-$(I91)_8$ protein construct, flanked by a HaloTag, for covalent tethering to a glass coverslip, and a biotin, for anchoring to streptavidin-coated superparamagnetic beads. Physiological-level forces in the pN range are applied through a magnetic field gradient created either by a pair of permanent magnets or a magnetic head. The experiment is done in presence of vinculin head, and the extension changes due to folding or binding are measured with nm resolution. (B) Magnetic tweezers recording showing individual vinculin binding events. The R3 domain folds and unfolds in equilibrium at 9 pN, yielding extension changes of ∼20 nm. In the presence of 20 nM vinculin head, these dynamics eventually stop due to vinculin binding. The event is resolved as a 3 nm contraction the unfolded polypeptide is observed due to the reformation of the α-helices in the vinculin-binding sites (red arrow, inset). The complex dissociates at high force, showing 3 nm steps (blue arrow).

II. RESULTS

A. Real Time Detection of Vinculin Binding to Talin

In order to isolate individual vinculin binding events, we work with the talin R3 domain, a four helix bundle located in the N-terminal region of the talin rod (Fig. S1) [13]. This domain has two vinculin-binding sites, and unfolds under low mechanical loads [19, 20]. Hence, it is ideally placed to play a key role in talin activation by force, recruiting a cluster of vinculin molecules at weak forces, which would amplify the mechanical coupling, and contribute to the maturation of the focal adhesion. In particular, we work with the R3 IVVI mutant, which has a higher mechanical stability that is useful to amplify the mechanical signature for vinculin binding, while its vinculin-binding sites are intact [13]. We use our custom-made magnetic tweezers to apply physiological forces to single talin molecules in the presence of vinculin head, and measure its extension changes in real time with nm resolution. Our molecular construct contains the R3 IVVI domain followed by eight repeats of titin I91, flanked by a HaloTag enzyme for covalent tethering to the glass surface, and a biotin for anchoring to a superparamagnetic bead (Fig. 2A; see SI for methods). Forces between 0.1 and 120 pN are applied with sub-pN resolution by generating a magnetic field with a pair of permanent magnets, or a magnetic head [23, 24].

Figure 2B shows our single molecule assay for vinculin binding detection. Starting from folded talin at 4 pN, we increase to 9 pN, where the R3 IVVI domain exhibits reversible folding/unfolding dynamics (see [24], and Fig. S2 for characterization of this domain). The unfolding/folding transitions appear as ascending/descending ∼20 nm changes in talin extension, due to the transition between the folded state and an unstructured polymer. Previous force spectroscopy studies, showed that vinculin binding blocks talin refolding [22]. Our experiments confirm this observation; in the presence of 20 nM vinculin head, talin folding dynamics cease after few seconds, and the protein is locked on its unfolded state. However, the improved resolution of our instrument and the use of a constant force, allows us to observe the binding event as a short contraction of the talin polypeptide that always precedes the arrest of the folding dynamics (Fig. 2B; inset, red arrow). This contraction indicates that vinculin binding induces a conformational change on unfolded talin, likely the coil-to-helix transition required for vinculin to firmly bind its substrate (Fig. 1). This bound state can be reversed by a high force pulse, where the opposite transition occurs, and ∼3 nm upward steps are observed (blue arrow) after which talin recovers its ability to fold (Fig. S3).

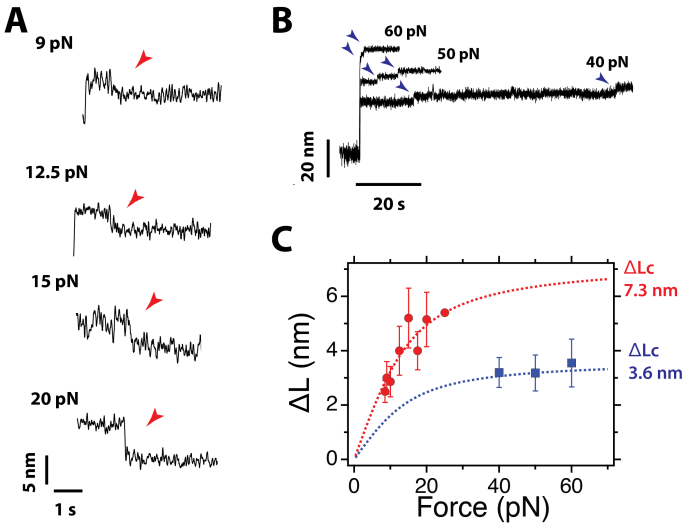

FIG. 3. **Vinculin binding contracts unfolded talin:** (A) Averaged recordings of the binding contractions at different pulling forces. The magnitude and duration of the contraction depend on the force. Traces averaged from >10 recordings. (B) Unbinding steps at different pulling forces. Two ∼3 nm steps are observed, after which talin recovers its ability to refold. (C) Average step sizes for the binding contractions (red) and unbinding steps (blue) measured as a function of the pulling force. The binding contraction scales with force following the FJC polymer model with a contour length of 7.3 nm, which agrees with the simultaneous formation of the two α helices of the vinculin-binding sites. The steps of unbinding have half that contour length, indicating that they correspond to the unraveling of a single binding site helix. Error bars are the SEM; data collected over 35 molecules, 156 binding steps, and 501 unbinding steps.

B. Cooperative Binding of Vinculin to Talin R3 Domain

Our measurements demonstrate that the mechanical fingerprint for vinculin binding is a contraction of a few nanometers of the talin polypeptide. Structural studies have determined that the vinculin-binding sites are amphipathic six-turn α-helices, buried in the core of talin domains by extensive hydrophobic interactions [25]. Upon binding, this helix is inserted intimately into vinculin head, which displaces the initial interaction between the head and tail of vinculin, present in its autoinhibited state.[26]. This helical structure is required for vinculin recognition and binding, which might explain the need for the structural contraction we observe upon binding.

Figure 3A shows averaged recordings of individual vinculin binding events measured at different forces. The size of the contraction induced by binding increases with force, which is an expected observation in the transition from a random-coiled chain to a compact structure. Interestingly, in all our experiments we observe a single contraction event, sufficient to form the talin-bound state, even though the R3 domain has two vinculin-binding sites. By contrast, when dissociating mechanically at forces above 40 pN, we resolve in most cases two distinct steps with an extension of ∼3 nm (Fig. 3B). In some traces, we observe only a single unbinding step, likely because the first one occurred too fast to be resolved, since two unbinding steps are required for complete dissociation (Fig. S4 and S5). An analysis of the unbinding kinetics confirm that a fraction of unbinding events occurs within the resolution limit of our instrument (Fig. S5).

This evidence suggests that two vinculin molecules bind simultaneously to unfolded talin, while each vinculin unbinds independently. The magnitude of the binding and unbinding events scales with force following the freely-jointed chain (FJC) model for polymer elasticity [27]. We measure the size of the binding contraction as a function of force (Fig. 3C, red circles), and fit its dependence to the FJC model, obtaining a contour length change of $\Delta L_c = 7.33 \pm 0.69$ nm (red dashed line). Plotting the same FJC fit with half that contour length results in a complete agreement with the steps measured for unbinding (Fig. 3C; blue). This indicates that the talin sequence sequestered by each binding contraction is twice that liberated by each unbinding step. Given that each vinculin-binding site contain 19 residues, and that the extension of the formed helix is about 3.5 nm [13, 26], the expected contour length change for each coil-to-helix transition is 3.7 nm, in agreement with our measurements. Together, our observations confirm that each binding contraction corresponds to the simultaneous reformation of two vinculin-binding helices, while each unbinding step is the uncoiling of a single helix.

From this evidence, it remains uncertain which is the binding pathway followed by vinculin to reach the talin bound state. One of the possible scenarios could involve repeated fluctuations between the coil and helix states in talin, with a first-hitting binding mechanism upon encounter of the appropriate substrate conformation. However, an

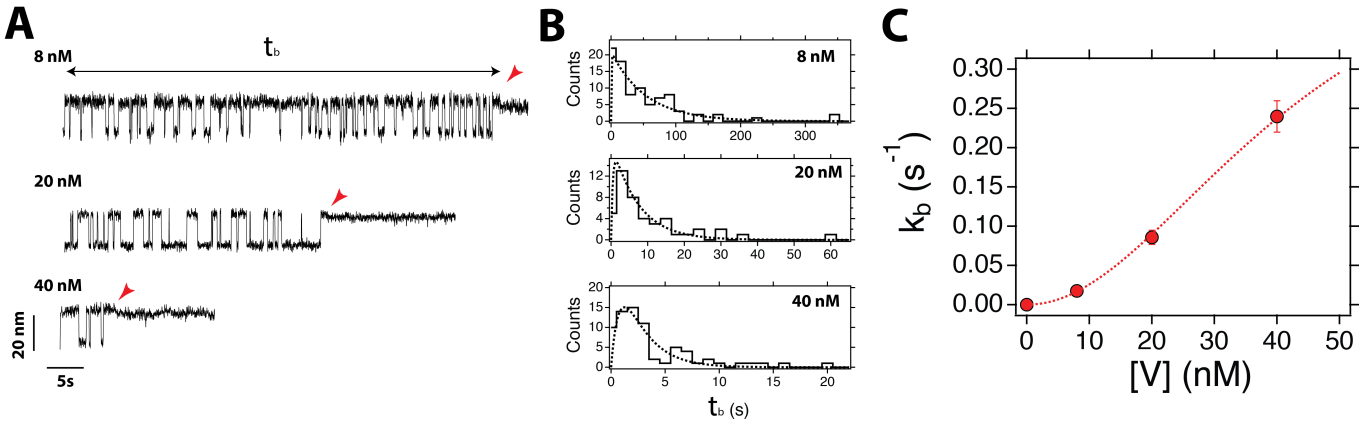

FIG. 4. **Stoichiometry of vinculin binding to talin:** (A) Magnetic tweezers recordings of talin in presence of 8, 20, and 40 nM vinculin at a force of 9 pN. The waiting time for vinculin binding (t_b) can be measured at the single molecule level, as the time taken since the probe force is set, until the contraction is observed and the hopping dynamics stop. (B) Distribution of vinculin binding times at different concentrations, and at a force of 9 pN. The shape of the distribution follows a single molecule enzymatic model, where two vinculin molecules bind simultaneously to unfolded talin. (C) Concentration dependency of the rate of vinculin binding. This dependency follows a second order Hill-like equation, which indicates a cooperative binding reaction. The maximum velocity of formation of the complex is the unfolding rate of talin.

interesting observation from our single molecule recordings is that binding is not instantaneous, but occurs as a slow relaxation, which, at 9 pN, takes as long as 500 ms, and that accelerates with force (Fig. S5). This suggests that vinculin binding requires a maturation process, perhaps initiated by a first recognition of key residues on the unfolded talin polypeptide, followed by a sequential contraction and reformation of the helices. However, the rationale for the force-dependency observed in this maturation process remains inconclusive.

The simultaneous nature of the vinculin binding event suggests a cooperative binding mechanism that should introduce a non-linear concentration dependency of the binding kinetics. From the perspective of single molecule enzyme kinetics [28, 29], this process can be modeled as:

$$\mathrm{F} \underset{\mathrm{k_F}}{\overset{\mathrm{k_U}}{\rightleftharpoons}} \mathrm{U} + 2\mathrm{V} \xrightarrow{k^0_{on}} \mathrm{U}_b,$$

where F stands for folded talin, U for unfolded talin, $\mathrm{U_b}$ for the bound state, and V for the vinculin head domain. This process is governed by three kinetic rates: k_U and k_F are the unfolding and folding rates of talin, and k^0_on is the pseudo-first-order rate constant, which depends on the concentration of vinculin as $k^0_\mathrm{on} = [\mathrm{V}]^2 k_\mathrm{on}$, since two molecules are required to acquire the talin-bound state. Experimentally, we measure the waiting time (t_b) to observe the talin-bound state (Fig. 4A), and the binding rate $k_\mathrm{b} = 1/t_\mathrm{b}$ can be derived analytically as (see SI):

$$\frac{1}{t_\mathrm{b}} = k_\mathrm{b} = \frac{k_\mathrm{U}[\mathrm{V}]^2}{[\mathrm{V}]^2 + K_\mathrm{M}}, \tag{1}$$

where $K_\mathrm{M} = (k_\mathrm{U} + k_\mathrm{F})/k_\mathrm{on}$. This expression is the single molecule analogous to a second-order Hill equation. Interestingly, in this case, the unfolding rate of talin plays the role of the maximum velocity of the reaction.

Figure 4A shows vinculin binding trajectories at 9 pN, and different vinculin concentrations. The waiting time t_b is determined for each recording as the time spanned between the beginning of the 9 pN probe pulse, and the contraction event after which talin dynamics stop. Figure 4B shows the distributions of waiting times at each concentration, fitted to the expression derived from the kinetic model (see SI). Vinculin binding kinetics are governed by two competing timescales, the folding/unfolding dynamics of talin, and the concentration-dependent on-rate k^0_on. At low concentrations, the on-rate is much slower than talin folding kinetics, and the process is rate-limited by vinculin association. However, as the concentration increases, both opposing processes become comparable, and we observe a peaked distribution; binding cannot occur faster than talin unfolds. Unfortunately, it is not possible to carry out experiments at higher vinculin concentrations, since binding occurs so fast that the fingerprint for vinculin binding is lost.

From these distributions, we determine the biding rate k_b, which has a quadratic dependence with the concentration, as described by Hill equation (Fig. 4C). This nonlinearity demonstrates the cooperative character of the vinculin

binding reaction. Fitting our data to Eq. 1 we obtain $k_{\mathrm{U}} = 0.52 \pm 0.13$ s^{-1}, and $K_{\mathrm{M}} = 1938 \pm 624$ nM^2. The value of the unfolding rate agrees with that measured from talin folding trajectories (Fig. S2). From K_{M}, we obtain that half the maximum binding velocity occurs at 44 nM, and that the on-rate at 9 pN is $k_{\mathrm{on}} \approx 6 \times 10^{-4}\mathrm{s}^{-1}\mathrm{nM}^{-2}$. Our observations are in agreement with previous evidence that revealed that vinculin binds talin with an affinity in the nanomolar range [26, 30]. Those experiments reported values between 3 and 30 nM, but were calculated using biochemical assays where vinculin was left to interact with isolated vinculin-binding sites helices. However, the physiological affinity of vinculin to talin must be understood as a force dependent quantity, which, as we demonstrated here, triggers structural changes on the binding substrate that should depend strongly on the tension applied to talin.

C. Mechanical Force Regulates Vinculin Binding

Force is an essential actor in the interaction between vinculin and talin, being required to unfold talin domains and expose its cryptic sites. Additionally, and as previously reported [22], we have shown that vinculin dissociates at high forces (>40 pN). This process arises likely from the destabilization of the reformed helices with force, which eventually will uncoil, and expel vinculin. In this same sense, the binding reaction should be hampered by force, since the helices reform and contract a polymer that is mechanically stretched. This suggests that force could play a biphasic role in vinculin binding, first by establishing the threshold for talin unfolding, but also by hindering the coil-to-helix contraction as force increases.

We measure the force dependency of the binding reaction over a fixed time-window of 50 s (Fig. 5A), which readily demonstrate the biphasic effect of force on binding. At 8 pN, talin explores the unfolded state with low probability, and vinculin binds with slow kinetics. As we increase the force to 9 and 10 pN, talin unfolds more frequently, which results in faster vinculin binding. However, at 15 and 20 pN, although talin is always unfolded, the bindings kinetics rapidly slow down with force, until binding is blocked above 30 pN.

This negative effect of force on binding arises from the coil-to-helix contraction that is triggered by binding. The talin polypeptide shortens out of the equilibrium extension imposed by force; hence, vinculin binding does mechanical work against the pulling force, and this energy penalty increases steeply with force. We measure the binding probability over the 50 s time-window (Fig. 5B; red circles). Binding only occurs over a force range between 8 and 30 pN; talin unfolding sets the minimum force for binding, but the increase of the mechanical work of binding with force results in a gradual drop of the binding efficiency, until it is forbidden at forces above 30 pN. These two counteracting effects define an optimal binding force of 10 pN.

The mechanical work of binding can be estimated on a first approximation as $\Delta W \approx F \cdot \Delta L(F)$, where ΔL is the force-dependent contraction measured in Fig. 3C. In this regard, we can assume that the on-rate depends on force as $k^0_{\mathrm{on}} = Ae^{-\Delta W(F)/kT}$. From this relation, we can write an analytical expression for the binding probability that incorporates the double effect of force on binding, controlled positively by the unfolding rate k_{U}, but negatively by the on-rate k^0_{on} (see SI for derivation). We use this expression to describe accurately our experimental data (Fig. 5B, dotted line; see Table S1 for parameter list). The agreement between the experimental data and our description confirms the proposed mechanism for the mechanical regulation of the talin-vinculin interaction. Vinculin binding requires a coil-to-helix reformation, which is hampered by force. This, together with the force-induced exposure of the cryptic binding sites, defines the force regime over which vinculin binds. This mechanism can be directly extrapolated to all other talin domains, or to ligand binding reactions that occur under similar conditions. The threshold force for binding is given by the mechanical stability of each helix bundle; for instance, for the WT R3 domain we would expect a reduction of the threshold binding force to 4-5 pN [20]. However, the negative force dependency arises from the entropic penalty of the coil-to-helix transition, and, thus, it is a universal regulatory effect.

III. DISCUSSION

Over the last 30 years, there has been an emphasis in understanding how molecular bonds respond to mechanical forces, a ubiquitous problem in biology. In his seminal 1978 paper, George Bell set the physical basis for the simplest case scenario; mechanical forces tilt the energy landscape in the pulling direction, decreasing linearly the height of the barrier, which results in an exponential decrease of the bond lifetime [31]. This simple theory—and more elaborated analytical corrections that followed—is used as a standard tool for analyzing the lifetime of biological bonds subject to pulling forces [32], and even other biological transitions, such as protein unfolding [33], or force-dependent chemical reactions [34]. In time, more complicated force dependencies have been measured, such as catch bond-like behaviors in the adhesive pili of some bacteria [35], or in the interaction between integrins and fibronectin [36]. However, how molecular interactions behave when force is applied to one of the components of the complex instead of the bond itself remains poorly understood. This situation is of great generality and appears in physiological processes as diverse as

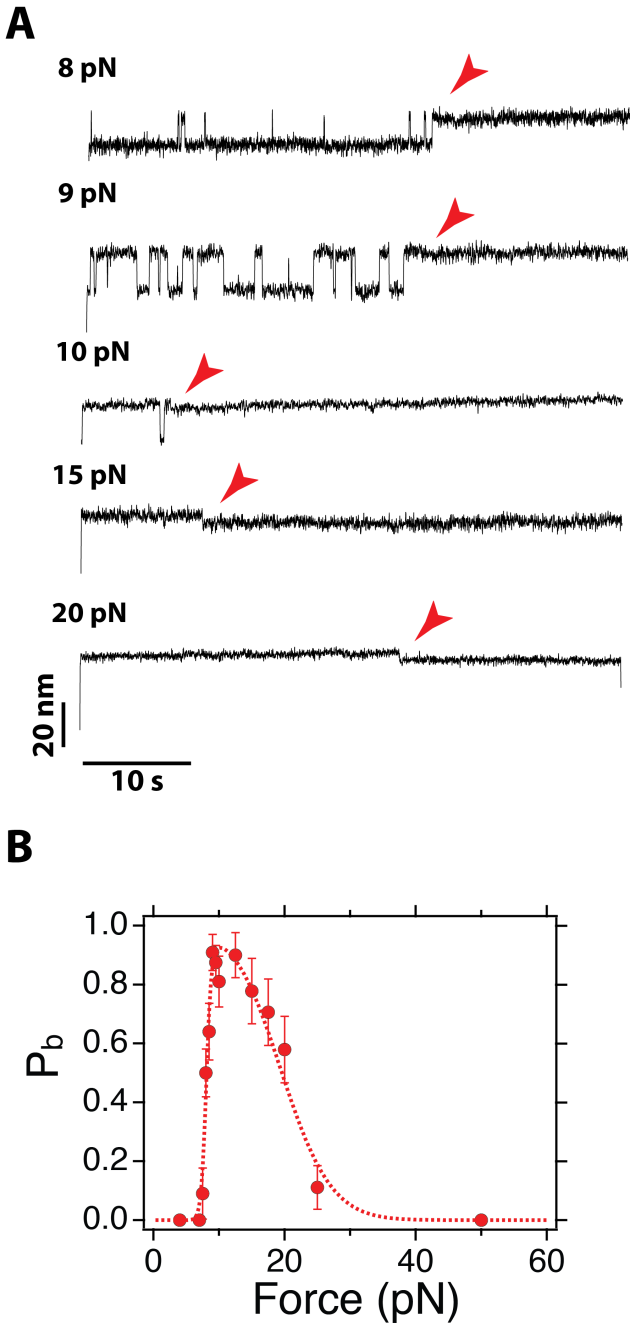

FIG. 5. **Mechanical force regulates vinculin binding:** (A) Typical recordings of vinculin binding at different forces during a 50 s time window and at a concentration of 20 nM. (B) Binding probability measured over a 50 s time window as a function of force. Force has a biphasic effect on binding, favoring it first by unfolding talin, but hampering it due to the energy penalty of the coil-to-helix contraction. The data is described by a simple model based on this mechanism (dashed line). Errors are SEM; data collected over 20 molecules and 259 realizations.

DNA-protein interactions [37], antibody-antigen binding [38], or protein-protein interactions in cellular junctions [3], to name a few.

Here, we have uncovered the molecular mechanism by which force regulates the formation of the talin-vinculin mechanosensing complex. Vinculin binding requires force to uncover its cryptic binding sites in talin, but force hampers this interaction, since binding induces a structural rearrangement on the talin polypeptide. This double effect of force establishes an optimal binding range that could define the mechanical regime over which cell adhesions operate. In order to explore this question, we propose a minimalistic model for a focal adhesion, which accounts

only for the talin-vinculin-actin association. This simple model integrates our measurements with the increased force transmission upon actin recruitment by vinculin, and shows that this interaction defines a negative feedback that stabilizes force in focal adhesions (see SI). In our scheme, an initial force input born by talin triggers unfolding of its bundle domains, promoting vinculin binding. The effect of vinculin is the recruitment of actin filaments, that elevate the force level on this junction, and subsequently increase further talin unfolding and vinculin binding. However, if the force on the linkage increases too much, vinculin binding becomes unfavorable, and the formed talin-vinculin complexes dissociate, decreasing the overall force on the system. Hence, the force dependency of the talin-vinculin interaction defines a mechanical negative feedback, which could explain how vinculin stabilizes force on focal adhesions.

We carry out Monte Carlo simulations on this focal adhesion model, built by a concatenation in series of identical talin domains, with the properties here measured (Fig. S6). The simulations are initiated with an arbitrary force input, which could arise from the mechanical coupling with the extracellular matrix, or the initial actin recruitment by talin. Regardless of the initial condition, the system always works to evolve the force to an stable value of 23 pN. This force is determined by the equilibrium between vinculin binding and unbinding rates, which, interestingly, agrees with that maintained by integrin linkages [36]. In our simulations, we model actin recruitment as an increase in the force on talin by 3 pN upon each binding event, which is about the force measured on each vinculin-actin linkage [21]; however, the system is insensitive to this particular value, as long as there is enough force gain to reach the equilibrium force of 23 pN.

Our simple model overlooks much of the actual complexity of a focal adhesion, which includes a multitude of interacting actors that operate in the different phases of its maturation and function. For instance, while all 13 talin rod domains can unfold under force, they have a hierarchical stability, which suggests the existence of a range of mechanical thresholds for gradual vinculin recruitment [19]. Additionally, there are other molecular partners that bind talin and could regulate its folding properties. For example, RIAM, opposed to vinculin, binds the folded talin bundles, and this interaction could shift unfolding to higher forces and compete with vinculin recruitment [13]. Hence, the integration of all these additional factors could lead to a range of regulatory mechanisms that might explain the extent of mechanical responses observed in celullar adhesions.

In summary, the observation of the coil-to-helix contraction that occurs upon vinculin binding has allowed us to describe how the interaction between vinculin and talin is regulated by force, and which could be its implications for force transmission in focal adhesions. While previous work demonstrated that talin unfolding by force was necessary for force transmission and transduction in focal adhesions [39], how vinculin binding could regulate this cellular process remained an open question. Vinculin transmits forces in focal adhesions [21], and there are at least 11 vinculin sites in each talin molecule. Hence, there is a clear force pathway for the increase in tension along talin, given by a gradual vinculin recruitment. However, it remained unclear how this force level could be regulated, specially since vinculin is an indicator of stable and mature focal adhesions [15, 21]. Our results have demonstrated that the mechanics of the talin-vinculin interaction define a negative feedback by which the mechanical homeostasis of focal adhesions could be maintained to form stable cell adhesions. Hence, our findings provide a first basis by which the talin-vinculin interaction could work in cellular adhesions to regulate force transmission and transduction.

ACKNOWLEDGMENTS

We thank Dr. Igor Barsukov from University of Liverpool for sharing the talin plasmid with us. We thank all of the members of the J.M.F. laboratory for the valuable discussions and comments on the manuscript. This work was supported by NIH Grant GG014033. R.T-R. and A. A-C. acknowledge Fundacion Ramon Areces for financial support.

IV. REFERENCES

[1] E. C. Yusko and C. L. Asbury, "Force is a signal that cells cannot ignore," *Molecular Biology of the Cell*, vol. 25, no. 23, pp. 3717–3725, 2014.

[2] P. Roca-Cusachs, V. Conte, and X. Trepat, "Quantifying forces in cell biology," *Nature Cell Biology*, vol. 19, pp. 742–751, 2017.

[3] L. B. Case and C. M. Waterman, "Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch," *Nature Cell Biology*, vol. 17, pp. 955–963, 2015.

[4] P. Kanchanawong, G. Shtengel, A. M. Pasapera, E. B. Ramko, M. W. Davidson, H. F. Hess, and C. M. Waterman, "Nanoscale architecture of integrin-based cell adhesions," *Nature*, vol. 468, pp. 580–584, 2010.

[5] K. Burridge and M. Chrzanowska-Wodnicka, "Focal adhesions, contractility, and signaling," *Annual Review of Cell and Developmental Biology*, vol. 12, no. 1, pp. 463–519, 1996.

[6] J. Eyckmans, T. Boudou, X. Yu, and C. S. Chen, "A hitchhiker's guide to mechanobiology," *Developmental Cell*, vol. 21, no. 1, pp. 35 – 47, 2011.

[7] K. R. Legate and R. Fässler, "Mechanisms that regulate adaptor binding to β-integrin cytoplasmic tails," *Journal of Cell Science*, vol. 122, no. 2, pp. 187–198, 2009.

[8] K. Burridge and L. Connell, "A new protein of adhesion plaques and ruffling membranes.," *The Journal of Cell Biology*, vol. 97, no. 2, pp. 359–367, 1983.

[9] S. J. Shattil, C. Kim, and M. H. Ginsberg, "The final steps of integrin activation: the end game," *Nature Reviews Molecular Cell Biology*, vol. 11, pp. 288–300, 2010.

[10] G. Jiang, G. Giannone, D. R. Critchley, E. Fukumoto, and M. P. Sheetz, "Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin," *Nature*, vol. 424, no. 6946, pp. 334–337, 2003.

[11] R. Zaidel-Bar, S. Itzkovitz, A. Ma'ayan, R. Iyengar, and B. Geiger, "Functional atlas of the integrin adhesome," *Nature Cell Biology*, vol. 9, pp. 858–867, 2007.

[12] A. R. Gingras, W. H. Ziegler, R. Frank, I. L. Barsukov, G. C. K. Roberts, D. R. Critchley, and J. Emsley, "Mapping and consensus sequence identification for multiple vinculin binding sites within the talin rod," *Journal of Biological Chemistry*, vol. 280, no. 44, pp. 37217–37224, 2005.

[13] B. T. Goult, T. Zacharchenko, N. Bate, R. Tsang, F. Hey, A. R. Gingras, P. R. Elliott, G. C. K. Roberts, C. Ballestrem, D. R. Critchley, and I. L. Barsukov, "Riam and vinculin binding to talin are mutually exclusive and regulate adhesion assembly and turnover," *Journal of Biological Chemistry*, vol. 288, no. 12, pp. 8238–8249, 2013.

[14] A. del Rio, R. Perez-Jimenez, R. Liu, P. Roca-Cusachs, J. M. Fernandez, and M. P. Sheetz, "Stretching single talin rod molecules activates vinculin binding," *Science*, vol. 323, no. 5914, pp. 638–641, 2009.

[15] C. Ciobanasu, B. Faivre, and C. Le Clainche, "Actomyosin-dependent formation of the mechanosensitive talin–vinculin complex reinforces actin anchoring," *Nature Communications*, vol. 5, p. 3095, 2014.

[16] A. Carisey, R. Tsang, A. M. Greiner, N. Nijenhuis, N. Heath, A. Nazgiewicz, R. Kemkemer, B. Derby, J. Spatz, and C. Ballestrem, "Vinculin regulates the recruitment and release of core focal adhesion proteins in a force-dependent manner," *Current Biology*, vol. 23, no. 4, pp. 271 – 281, 2013.

[17] J. D. Humphries, P. Wang, C. Streuli, B. Geiger, M. J. Humphries, and C. Ballestrem, "Vinculin controls focal adhesion formation by direct interactions with talin and actin," *The Journal of Cell Biology*, vol. 179, no. 5, pp. 1043–1057, 2007.

[18] H. Hirata, H. Tatsumi, C. T. Lim, and M. Sokabe, "Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions," *American Journal of Physiology-Cell Physiology*, vol. 306, no. 6, pp. C607–C620, 2014.

[19] A. W. M. Haining, M. von Essen, S. J. Attwood, V. P. Hytönen, and A. del Río Hernández, "All subdomains of the talin rod are mechanically vulnerable and may contribute to cellular mechanosensing," *ACS Nano*, vol. 10, pp. 6648–6658, 07 2016.

[20] M. Yao, B. T. Goult, B. Klapholz, X. Hu, C. P. Toseland, Y. Guo, P. Cong, M. P. Sheetz, and J. Yan, "The mechanical response of talin," *Nature Communications*, vol. 7, p. 11966, 2016.

[21] C. Grashoff, B. D. Hoffman, M. D. Brenner, R. Zhou, M. Parsons, M. T. Yang, M. A. McLean, S. G. Sligar, C. S. Chen, T. Ha, and M. A. Schwartz, "Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics," *Nature*, vol. 466, pp. 263–266, 2010.

[22] M. Yao, B. T. Goult, H. Chen, P. Cong, M. P. Sheetz, and J. Yan, "Mechanical activation of vinculin binding to talin locks talin in an unfolded conformation," *Scientific Reports*, vol. 4, p. 4610, 2014.

[23] I. Popa, J. A. Rivas-Pardo, E. C. Eckels, D. J. Echelman, C. L. Badilla, J. Valle-Orero, and J. M. Fernández, "A halotag anchored ruler for week-long studies of protein dynamics," *Journal of the American Chemical Society*, vol. 138, pp. 10546–10553, 08 2016.

[24] R. Tapia-Rojo, E. C. Eckels, and J. M. Fernández, "Ephemeral states in protein folding under force captured with a magnetic tweezers design," *Proceedings of the National Academy of Sciences*, vol. 116, no. 16, pp. 7873–7878, 2019.

[25] E. Papagrigoriou, A. R. Gingras, I. L. Barsukov, N. Bate, I. J. Fillingham, B. Patel, R. Frank, W. H. Ziegler, G. C. K. Roberts, D. R. Critchley, and J. Emsley, "Activation of a vinculin-binding site in the talin rod involves rearrangement of a five-helix bundle," *The EMBO journal*, vol. 23, pp. 2942–2951, 08 2004.

[26] T. Izard, G. Evans, R. A. Borgon, C. L. Rush, G. Bricogne, and P. R. J. Bois, "Vinculin activation by talin through helical bundle conversion," *Nature*, vol. 427, no. 6970, pp. 171–175, 2004.

[27] M. Doi and S. Edwards, *The Theory of Polymer Dynamics*. Oxford Science Publications, 1986.

[28] B. P. English, W. Min, A. M. van Oijen, K. T. Lee, G. Luo, H. Sun, B. J. Cherayil, S. C. Kou, and X. S. Xie, "Ever-fluctuating single enzyme molecules: Michaelis-menten equation revisited," *Nature Chemical Biology*, vol. 2, no. 2, pp. 87–94, 2006.

[29] S. C. Kou, B. J. Cherayil, W. Min, B. P. English, and X. S. Xie, "Single-molecule michaelis menten equations," *The Journal of Physical Chemistry B*, vol. 109, no. 41, pp. 19068–19081, 2005.

[30] M. D. Bass, B. Patel, I. G. Barsukov, I. J. Fillimgham, R. Mason, B. J. Smith, C. R. Bagshaw, and D. R. Critchley, "Further characterization of the interaction between the cytoskeletal proteins talin and vinculin," *Biochemical Journal*, vol. 362, no. 3, pp. 761–768, 2002.

[31] G. Bell, "Models for the specific adhesion of cells to cells," *Science*, vol. 200, no. 4342, pp. 618–627, 1978.

[32] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, and E. Evans, "Energy landscapes of receptor–ligand bonds explored with dynamic force spectroscopy," *Nature*, vol. 397, no. 6714, pp. 50–53, 1999.

[33] M. Rief, M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub, "Reversible unfolding of individual titin immunoglobulin domains by afm," *Science*, vol. 276, no. 5315, pp. 1109–1112, 1997.

[34] A. P. Wiita, S. R. K. Ainavarapu, H. H. Huang, and J. M. Fernandez, "Force-dependent chemical kinetics of disulfide bond reduction observed with single-molecule techniques," *Proceedings of the National Academy of Sciences*, vol. 103, no. 19, pp. 7222–7227, 2006.

[35] W. Thomas, M. Forero, O. Yakovenko, L. Nilsson, P. Vicini, E. Sokurenko, and V. Vogel, "Catch-bond model derived from allostery explains force-activated bacterial adhesion," *Biophysical Journal*, vol. 90, no. 3, pp. 753 – 764, 2006.

[36] F. Kong, A. J. García, A. P. Mould, M. J. Humphries, and C. Zhu, "Demonstration of catch bonds between an integrin and its ligand," *The Journal of Cell Biology*, vol. 185, no. 7, pp. 1275–1284, 2009.

[37] I. Heller, T. P. Hoekstra, G. A. King, E. J. G. Peterman, and G. J. L. Wuite, "Optical tweezers analysis of dna–protein complexes," *Chemical Reviews*, vol. 114, pp. 3087–3119, 03 2014.

[38] M. Huse, "Mechanical forces in the immune system," *Nature Reviews Immunology*, vol. 17, pp. 679 –690, 07 2017.

[39] A. Elosegui-Artola, R. Oria, Y. Chen, A. Kosmalska, C. Pérez-González, N. Castro, C. Zhu, X. Trepat, and P. Roca-Cusachs, "Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity," *Nature Cell Biology*, vol. 18, pp. 540–548, 2016.