A possible molecular mechanism for mechanotransduction at cellular focal adhesion complexes

Jichul Kim1,*
1Independent Researcher, Changwon, Republic of Korea

ABSTRACT  Mechanotransduction at focal adhesion complexes is key for various cellular events. Theoretical analyses were performed to predict a potential role of lipid membranes in modulating mechanotransduction at focal adhesions. Calculations suggested that the size of nanostructural constraints and mechanical pulling applied on lipid membranes affect the generation of cellular traction forces and signaling transduction at focal adhesions. This work provides predictions on how lipid membranes contribute to mechanotransduction at cellular focal adhesions.

INTRODUCTION

Cells interact with their environments and generate traction forces through their adhesive molecular machinery formed across cell membranes. This collective molecular complex, called the focal adhesion (FA), orchestrates a variety of molecules such as adhesive integrin receptors inserted in membranes as well as talins and vinculins in cytoplasmic regions (1). Forces transmitted through these molecules generate biological signals that affect cellular growth, differentiation, migration, and tumor metastasis (2). Therefore, understanding FAs is central for various physiological and pathological processes.

Recent mechanical measurements identified the underlying molecular and biophysical mechanism of FAs from at the cellular level to the single-protein level. For example, the traction force microscopy technique enabled us to measure forces exerted by focal adhesion receptors with different extracellular rigidities (3,4). In addition, rheological methods (5), tension gauge tethers (6), and force sensors using fluorescence resonance energy transfer (FRET) technique (7) identified piconewton-scale mechanics important for the adhesion molecular machinery. Furthermore, single-molecule fluorescent imaging techniques provided information on the molecular length and count at FAs (8,9). More recently, the development of patterned nanolines unveiled how integrin receptors form nanoclusters at adhesion sites (10). Numerical investigations such as finite element simulations also provided predictions on cellular FAs (11,12). Despite the accumulated data and continued theoretical developments, an integrated interpretation on how individual molecular mechanism affects the generation of complex cellular mechanical behaviors and signaling transduction at FAs is still elusive. In addition, the mechanics of lipid membranes was investigated in numerous previous works on the adhesion machinery.

WHY IT MATTERS  Focal adhesion machineries formed across cell membranes orchestrate a variety of signaling and adhesive molecules to function for important cellular physiologies. Although there are evidences that lipid membranes are involved in mechanical transduction at focal adhesions, how the detailed mechanical response of membranes contributes to the process is not identified yet. With many data previously identified, predictions made by theoretical modeling suggest that nonlinear pulling responses of lipid membranes serve as a key factor to interpret mechanotransduction at focal adhesions.
versus force response of membranes contributes to mechanotransduction at FAs is not fully studied yet.

In this work, the combined nanomechanical responses of lipid membranes and talin proteins that incorporate vinculin molecules at FAs were calculated. How the nanoscale extension versus force response of the membrane-talin complex modulates opening, i.e., the activation, of cryptic vinculin binding sites (VBSs) was investigated. Furthermore, by connecting the membrane-talin response to other FA mechanisms, cellular traction forces on elastic substrates were calculated. Overall, this work provides an idea for the integrated FA molecular machinery by emphasizing a crucial role of lipid membranes in modulating mechanotransduction.

MATERIALS AND METHODS

Modeling the mechanical response of lipid membranes at the membrane-talin complex

Among various molecules, two components at FAs were mainly considered in this model: lipid membranes and talins (Fig. 1, a and b) (1). The lipid membrane was described as coarse-grained continuum by directly employing a finite element model introduced in a recent investigation (16). In this model, an energy functional for lipid membranes $\Psi_{\text{membrane}}$ was introduced as in Eq. 1.

$$\Psi_{\text{membrane}} = \int \left(2k_m H^2 + k_K K\right) dA + \int \sigma d\alpha \int dA. \quad (1)$$

The functional was expressed with energies associated with the mean $H$ and Gaussian $K$ curvatures of the surface of membranes (17,18). The mean curvature at a certain point of the surface can be $H = 0.5(h_c \left(\sqrt{1 + h_c^2} \right) + h_r / \left(\sqrt{1 + h_c^2} \right))$, where $h_c$ is the height function of the membrane shape and $r$ is the rotational-symmetric radial function. The first and second derivatives of $h$ with respect to $r$, $h_c$, and $h_r$ were expressed with respect to the parametric coordinate $s[0, 1]$ defined for the arclength of the membrane by using $h_c = h_c(s) r$ and $h_r = h_r(s) r^2 - h_r(s) r^2$, respectively (16). $k_m$ and $k_K$ are the bending modulus and the Gaussian curvature modulus, respectively.

In performing variational calculations, the Gaussian curvature energy term in Eq. 1 was omitted, based on the Gauss-Bonnet theorem (19). For simplicity, $h_r$ was assumed to be constant in this work (20–23).

In Eq. 1, $dA$ denotes the area element of the membrane.

The functional $\Psi_{\text{membrane}}$ contains another energy term for the area strain to consider in this model: i.e., the number of lipids per a smooth area, in the cell membrane is decreased with stretching. The strain energy density $J_{\text{area}}$ in Eq. 1 can be calculated by integrating the surface tension $\sigma$ vs. area strain $\alpha$ relation from the resting reference strain $\alpha_0$ to the strain under consideration $\alpha$. Two smooth expressions for the surface tension are $\sigma = \alpha_0 \exp(8k_B \alpha / \alpha) \alpha^d$ for $\alpha \leq \alpha_{\text{cross}}$ and $\sigma = K_{\text{app}}(\alpha - \alpha_{\text{cut}}) / \alpha_{\text{cross}}$ for $\alpha > \alpha_{\text{cross}}$, where $\alpha_{\text{cut}}$ and $\alpha_{\text{cross}}$ are the cutoff and crossover strains, respectively (16,24,25). $K_{\text{app}}$ is the apparent area stretching modulus (16,24,25).

The strain is $\alpha = \alpha_{\text{cross}} - \alpha_{\text{cut}}/\alpha_{\text{cross}}$. $K_{\text{cross}}$ is the area of a lipid reservoir, $\alpha_{\text{cross}}$ is the area of the reservoir at the resting reference configuration, $\phi_{\text{cross}}$ is the uniform lipid number density, and $\phi_{\text{cut}}$ is $\phi_{\text{cross}}$ at the resting reference configuration. $K_0$ is the Boltzmann constant, and $T$ is temperature, where $k_B = 1.3806488 \times 10^{-23} \text{ J/K}$ and $T = 300 \text{ K}$ in this work.

Boundary conditions are $\alpha(0, h) = \beta(0), \alpha_{\text{mem-talin}}(0, h) = \beta(0)$, $\alpha(0, h) = \beta(0), \alpha_{\text{mem-talin}}(0, h) = \beta(0)$, and $\alpha(0, h) = \beta(0)$. $E_{\text{mem-talin}}$ is the shared extension of the membrane and the talin. $r_{f_{\text{ca}}}$ defines the radius of a rigid area where clustered integrins are inserted (10,16). $r_{f_{\text{ct}}}$ defines the radius of tented membranes in response to forces on the stiff cluster region (16). The membrane is clamped on the rigid cytoskeleton at the point apart from the center of the cluster by $r_{f_{\text{ch}}}$, whereas it is free to change curvatures within $r_{f_{ct}}$. Image data showing membrane curvatures at FAs may support this parameterization (26,27). Experiments evidenced that the mechanical extension of lipid membranes can be limited in living cells with membrane component (the reservoir region was not plotted). Red arrows indicate the average extension versus force responses obtained by using the Boltzmann function. (d) The average number of activated VBSs versus force calculations for the membrane-talin complex and for the case without the mechanical rigidity of membranes (i.e., talins).

FIGURE 1 The extension versus force curve and the number of activated vinculin binding sites (VBSs) versus force curve for the single membrane-talin complex. (a and b) Illustrations for the unitary adhesion complex composed of one integrin adhesion cluster, lipid membranes, and talin proteins. The membrane is clamped on the rigid cytoskeleton at the point apart from the center of the cluster by $r_{f_{\text{ch}}}$, whereas it is free to change curvatures within $r_{f_{ct}}$. Image data showing membrane curvatures at FAs may support this parameterization (26,27). Experiments evidenced that the mechanical extension of lipid membranes can be limited in living cells with membrane component (the reservoir region was not plotted). Red arrows indicate the average extension versus force responses obtained by using the Boltzmann function. (d) The average number of activated VBSs versus force calculations for the membrane-talin complex and for the case without the mechanical rigidity of membranes (i.e., talins).
the increase of applied forces (16,28). These results suggested that the mobility of lipids in the cell membrane under mechanical deformation is allowed in a confined region. Therefore, the radius $r_{cr}$ was introduced to assume the area of the lipid reservoir for the single membrane-talin complex. The size of the reservoir can be determined by $r_{cr} = 1750 \text{ nm}$ in this work. In this work, $r_{cr} = 1750 \text{ nm}$ was mainly used.

Calculations for the membrane model were performed by using the finite element method provided in the previous work (16). In short, the functions $h(s)$ and $r(s)$ in the variational form of the energy functional were parameterized by using the B-spline function, and a system of nonlinear equations obtained from the variational formulation was solved by using the Newton-Raphson method (29). The stationary functions of $\text{Eq. 1}$ was assumed when the Euclidean norm of the difference between two subsequent solution vectors converged to a certain value. In increasing $E_{\text{mem-talin}}$, an estimate for the lipid number density for the $k + 1$ step was calculated from the $k$th step, when the calculated area of the tented membrane in the $k$th step is greater than that in the $k - 1$ step. Here, $k$ is the index for step extensions. The evaluation of $h(s)$ and $r(s)$ functions and numerical integration were performed by using the Gaussian quadrature technique (30). See the previous work for details of the finite element model (16).

### Modeling opening of VBSs in force-bearing talins at the membrane-talin complex

Talin molecules play pivotal roles for mechanical stabilities and transduction at FAs. They are linked to integrin adhesion clusters inserted in the membrane and interact with actin cytoskeletal structures directly or indirectly (7,10,31,32) (Fig. 1, a and b). Recent data measured by using elastic substrates whose stiffness can be variable and FRET tension sensors suggested that the average force applied on the single talin rod is $\sim 4.5 \text{ pN}$ in living cells (7). Therefore, the constant single talin force $F_{\text{talin}} = 4.5 \text{ pN}$ was used in this work. The total number of talin rods connected to one integrin cluster $N_{\text{talin}}$ was calculated from $N_{\text{talin}} = C_{\text{talin}} N_{\text{integrin}}$. The number of integrin pairs $N_{\text{integrin}}$ in the area defined by $r_{\text{cr}}$ can be $N_{\text{integrin}} = (r_{\text{cr}} / r_{\text{integrin}})^2$, where $r_{\text{integrin}}$ is the radius of the area occupied by one integrin pair (33,34). Experimental observations that demonstrated initial adhesion formation without talins (35); minimal and significant variations between the traction forces measured from normal and talin-depleted cells on soft and stiff substrates, respectively (4); and the substrate-rigidity-independent constant force applied on single talin rods (7) together suggested that the number of talins varies with the different level of mechanical stimuli applied on the single membrane-talin complex. Therefore, the number of talin molecules per one integrin pair $C_{\text{talin}}$ was defined as a linear function of the membrane-talin extension in which $N_{\text{integrin}} = 0$ and $N_{\text{talin}} = 3.92$ when $E_{\text{mem-talin}} = 0 \text{ nm}$ and $E_{\text{mem-talin}} = 187 \text{ nm}$, respectively, for $r_{\text{cr}} = 57 \text{ nm}$ (Fig. S1). Here, about four talins interact with the single integrin cluster is consistent with the talin number measured from a nanoscale area of the living cell surface (9). Finally, the force applied on the single membrane-talin complex $F_{\text{mem-talin}}$ can be calculated from $F_{\text{mem-talin}} = F_{\text{mem}} + N_{\text{talin}} F_{\text{talin}}$ (Fig. 1 b).

Talin rods convert mechanical inputs into biological protein interactions. Opening of VBSs in force-bearing talins is one major event for mechanotransduction at FAs (36). A statistical description was introduced to predict the average number of activated VBSs with respect to the continuous application of forces to the membrane-talin complex. Previous experimental results from living cells demonstrated the gradient of the mechanical extension applied within the single talin rod, i.e., larger extensions near the head of the talin and smaller extensions near its tail region (7). This result suggested to assume sequential stretching of the talin rod from the head to the tail region with the continuous mechanical extension. The single talin rod has 11 cryptic VBSs (37). Therefore, by assuming a 4.5 nm unitary extension for 62 helices, the 11 extension values for opening of VBSs were defined as follows for the single talin rod: 18, 27, 40.5, 49.5, 54, 121.5, 148.5, 162, 207, 225, and 261 nm. Then, smooth $N_{\text{state}}$ reference states can be obtained by interpolating discrete the number of activated VBSs versus extension data as shown in Fig. S2. With the 51 nm resting length of the talin (8,38) and the neck linker length up to 20 nm (37), the 332 nm total length when the 11 VBSs are opened by the constant force $F_{\text{talin}} = 4.5 \text{ pN}$ in this model is consistent with measured data obtained by using magnetic tweezers (5). By employing the Boltzmann function and assuming that the total energy of the membrane-talin complex for the $i$th state is $G_i = G_i - E_{\text{mem-talin}} L_i^{\text{ref}}$, the probability of finding the $i$th state can be formulated as in Eq. 2 (39,40).

$$P_i = \frac{\exp\left(\frac{-\beta G_i^{\text{ref}}}{C_0}\right)}{\sum_{j=1}^{N_{\text{states}}} \exp\left(\frac{-\beta G_j^{\text{ref}}}{C_0}\right)} = \frac{1}{\sum_{j=1}^{N_{\text{states}}} \exp\left[\frac{\beta}{C_0} \sum_{n=0}^{N_{\text{states}}} G_i - F_{\text{mem-talin}} L_i^{\text{ref}}\right]}.$$ (2)

Here, $G_i$ and $L_i^{\text{ref}}$ are the internal energy and the reference extension for the $i$th state, respectively. $G_i$ was calculated by integrating the force versus extension response of the membrane-talin complex (or the complex without the membrane rigidity) from the zero extension to $L_i^{\text{ref}}$. The constant $\beta$ was obtained from $\beta = 1/(k_B T)$. From Eq. 2, the average number of activated VBSs $N_{\text{VBS}}$ can be written as follows:

$$N_{\text{VBS}} = \sum_{i=1}^{N_{\text{states}}} N_{\text{VBS}}^{i} P_i,$$ (3)

where $N_{\text{VBS}}^{i}$ is the number of activated VBSs for the $i$th state (see Fig. S2, orange curve). Finally, the average number of activated VBSs at the single membrane-talin complex can be obtained by multiplying $N_{\text{VBS}}$ and $N_{\text{talin}}$. Similarly, the average extension of the membrane-talin complex $E_{\text{mem-talin}}$ can be calculated from Eq. 4.

$$E_{\text{mem-talin}} = \sum_{i=1}^{N_{\text{states}}} L_i^{\text{ref}} P_i,$$ (4)

$N_{\text{state}} = 100$ was used to provide enough smoothness for the calculated Boltzmann’s function curves.

### Modeling cellular traction forces from the mechanical response of the membrane-talin complex

To investigate how the membrane-talin response contributes to the generation of cellular traction forces, the stiffness of extracellular substrates was modeled as in Eq. 5 by directly following previous investigations (Fig. 3, a and b) (3,4,41).

$$k_{\text{substrate}} = \frac{9 F_{\text{traction}}}{(4E_s)}.$$ (5)
Here, $k_{\text{substrate}}$ is the Young's modulus of substrates. $F_{\text{traction}}$ is the traction force to the substrate-horizontal direction applied on the area defined by the radius $r_s$. The normal component of the force was not considered in this work. $F_{\text{traction}}$ can be calculated from $F_{\text{traction}} = N_{\text{mem-talin}} C_g \frac{r_{ct}^2}{C_2}$, where $N_{\text{mem-talin}} = \frac{r_s^2}{r_{ct}^2}$ (or $N_{\text{mem-talin}} = 1$ when $r_s = r_{ct}$) is the number of the membrane-talin complex in the adhesion area. $C_g$ is the geometric coefficient to account for the tilt of the molecular complex with respect to the substrates (see Fig. 3 b). It might be possible that the level of membrane-talin deformation affects the tilt angle of the complex. However, for simplicity, a constant $C_g$-value was used in this work. $E_s$ is the lateral extension of substrates, and a condition $E_s = L_m - C_g E_{\text{mem-talin}} \geq 0$ should be satisfied. Here, $L_m$ is the size of serial stretching of the substrate and the membrane-talin complex to the surface-horizontal direction. External mechanical stimuli such as shear stresses and intercellular mechanical interactions, as well as forces generated from the cellular interior region, can affect the mechanics at FAs (42,43). In this work, the contraction of acto-myosins was assumed as the main force generating mechanism. Therefore, the $L_m$ parameter is associated with the size of actomyosin contraction to the substrate-horizontal direction. Experimental data without external mechanical stimuli revealed that the inhibition of myosin II motor activities significantly reduced the magnitude of traction forces (4,44). Without full consideration on dynamic effects, this model assumed quasistatic configurations of FAs as similarly investigated in the experiment (4). All calculations and analyses were performed by using MATLAB (The MathWorks, Natick, MA). Parameter values used in this study are summarized in Fig. S3 and Table 1.

RESULTS

Nonlinear mechanical responses of lipid membranes modulate the activation of VBSs at the membrane-talin complex

The extension of the membrane-talin complex that resulted from changing forces is shown in Fig. 1 c. In this calculation, $r_{ca} = 57$ nm was used (10), where approximately four talin rods were connected in parallel with the membrane when the rigid cluster was displaced $\sim 187$ nm (9). $r_{ct} = 158$ nm and $r_{ct} = 1750$ nm were used (16). The response of the membrane-talin complex was nonlinear and showed negative extension-force slopes in an intermediate region, i.e., a snap-through instability. The negative stiffness was generated from the response of the membrane component. According to the previous research, the initial sharp curvature change and the accumulation of curvatures to prevent excessive stretching of the membrane area in the higher extension regime is responsible for the generation of this nonlinearity (16). In Fig. 1 c, how the Boltzmann function can generate the average extension versus force response was also evaluated for both the membrane-talin complex and the case without the membrane rigidity (Fig. 1 c, red curves indicated by red arrows). These curves provided comparisons between the characteristic extension values calculated from the analytic model and their thermodynamic average with respect to the applied force. A steep region was identified by using the Boltzmann function for the membrane-talin complex and that overlapped with the center of the negative stiffness region.

The average number of activated VBSs with the applied force was plotted in Fig. 1 d. When calculating the number of activated VBSs without considering the mechanical rigidity of lipid membranes, the value increased gradually in the force regime less than 20 pN (Fig. 1 d, talins). However, the curve was shifted to a higher force regime and transformed into the step-like form by considering the membrane connected in parallel with the talin rods (Fig. 1 d, membrane-talin complex). This result suggested that the deformation of membranes can modulate the activation of VBSs when the FA molecular machinery is mechanically stretched. The modulation of the VBS activation by the membrane demonstrated in Fig. 1, c and d was reminiscent of the mechanism of mechanical switches. Here, $\sim 31.6$ pN force in the steep region was similar to a previous measurement for the activation force of integrin signaling (6). The results in Fig. 1, c and d also demonstrated that the Boltzmann function widely used in interpreting biological signaling systems can be also used for mechanotransduction at FAs (47,48). Furthermore, the results support a notion that if a system shows signaling responses with two distinct states, there is a component that generates the snap-through instability (i.e., a mechanical bistability) (47).
The size of nanostructural constraints applied on lipid membranes modulates the activation of VBSs at the membrane-talin complex

The idea that pulling of cell surface receptors can generate nonlinear nanomechanical responses was recently evidenced experimentally (16). Analyses suggested that the responses can be modulated by the interaction of bilayers with rigid components (16). Therefore, the size of the adhesion cluster and the level of the membrane-cytoskeleton interaction can serve important roles in modulating the mechanical response and the VBS activation at FAs. To test these possibilities, the $r_{ca}$ and $r_{ct}$ values were systematically varied in calculating the extension versus force curve and the activation of VBSs versus force curve for the membrane-talin complex. As shown with black curves in Fig. 2a, increasing $r_{ca}$ resulted in responses that showed more predominant sigmoidal nonlinearity. An initial force peak in the response, i.e., a signature for the generation of the negative stiffness, was shifted to a higher force regime by increasing $r_{ca}$. Shifting of the curve for the VBS activation was also identified, and the force peak of each extension versus force curve approximately overlapped with the steep region of the VBS activation curve. As shown in Fig. 2b, the shapes of the extension versus force curve and the VBS activation versus force curve were also changed by varying $r_{ct}$ from 168 to 190 nm and 225 nm. However, the shifting characteristic shown in Fig. 2a was not significant here. Instead, curves in Fig. 2b demonstrated that the sharpness of the step-like behavior in the activation curve was reduced when the $r_{ct}$ value was increased. In Fig. 2, the responses of the unit complex without the talin molecules connected to the integrin cluster were also plotted (blue curves). Deformed shapes for the membrane component are supplemented in Fig. S4. Additional sensitivity analyses for $r_{ca}$ and $r_{ct}$ are shown in Fig. S5. A sensitivity study for $r_{ct}$ and $r_{ct}$ is shown in Fig. S6.

The nonlinear mechanical responses and the nanostructural constraints on the membrane-talin complex affect the generation of cellular traction forces at FAs

By using the responses shown in Fig. 1c, traction force versus substrate stiffness responses were generated amounts of the integrin-binding peptide GPen. (f) The number of activated VBSs in the area defined by $r_{f}$. The responses were calculated from the data in the bottom panel of Fig. 2b. Scaled fluorescent intensity ratios between YAPs in the nucleus region and the cytosolic region (with GPen) are also shown. The GPen concentration was 0.05 mM (crosses) and 0.15 mM (diamonds) in the experiments. All measured data were obtained from (4).
TABLE 1 The summary of parameter values

| Parameters                          | Used values                                                                 |
|------------------------------------|------------------------------------------------------------------------------|
| $k_m$ (lipid bilayer bending modulus) (24) | $32 k_BT_a^b$                                                                |
| $\sigma_0$ (lipid bilayer surface tension with the zero strain, i.e., $\alpha = 0$) (16) | $\exp(-10)$ mN/m$^{b1}$                                                    |
| $K_{app}$ (lipid bilayer apparent area stretching modulus) (24) | $275$ mN/m$^{b1}$                                                           |
| $r_{ca}$ (radius of integrin clusters) (10) | $30, 57, 72$ nm$^{c}$                                                        |
| $r_{ct}$ (radius of tented membranes) | $158, 168, 190, 225$ nm$^{d}$                                                |
| $r_{ct}$ (radius of lipid reservoirs) | $1750$ nm$^{e}$                                                              |
| $r_{integrin}$ (radius of the area occupied by one integrin pair) (33,54) | $5$ nm                                                                       |
| $F_{talin}$ (constant force applied on single talins) (?) | $4.5$ pN                                                                    |
| $C_T$ (geometric tilt coefficient) | $0.21^c$                                                                     |
| $L_m$ (size of actomyosin contraction to the substrate lateral direction) | $3.74–60.74$ nm$^c$                                                          |
| $r_s$ (radius of the adhesion area) | $57–1800$ nm$^c$                                                            |

$^a$Sensitivity studies for $k_m$, $\sigma_0$, and $K_{app}$ on the extension versus force response of the membrane are supplemented in Fig. S10.

$^b$With $r_{ca} = 57$ nm, $r_{ct} = 158$ nm, and $r_{ct} = 1750$ nm, the surface tension $\sigma$ of the membrane at $E_{mem-talin} = 188$ nm was $-0.0612$ mN/m (45).

$^c$See Fig S3 for how these parameters were varied.

$^d$These are similar with the $r_{ct}$ and $r_{ca}$-values estimated from the upper surface of living cells (16).

$^e$A sensitivity study for $r_s$ is supplemented in Fig. S6.

$^f$This value assumed for the average tilt of the membrane-talin complex on continuous elastic substrates is smaller than the value measured from elastic tethers linked to integrins and a rigid substrate by a factor of 3 (46).

in Fig. 3 c, middle panel. The traction force responses with and without talins also showed nonlinear mechanical characteristics. The functionality of talin rods is closely related to the number of integrin clusters and myosin II activities at FAs (35,49). Therefore, $r_s$ and $L_m$ were differently assigned for the traction force calculation for the membrane-talin complex and the membrane without talins connected to integrin clusters (Fig. S3). It was remarkable that direct comparisons between the calculations and previously measured data from living cells showed good agreement for both the membrane-talin and membrane-without-talin components (4) (Fig. 3 c, middle panel). As a note, nontrivial mismatches were invoked in the comparison when using same $r_s^*$ and $L_m$-values (see Fig. S7). Similarly, as shown in Fig. 1, c and d, the negative slope region of the force versus stiffness response (Fig. 3 c, middle panel, black) overlapped with the steep region of the curve for the VBS activation (Fig. 3 d, $r_{ca} = 57$ nm). It is well known that the localization of yes-associated proteins (YAPs), the transcriptional regulators within cells, serves as an important indicator for mechanotransduction associated with FAs (4). Therefore, a comparison between the calculation for the VBS activation at the membrane-talin complex and measurements for the nucleus/cytosolic ratio of YAPs was provided in Fig. 3 d ($r_{ca} = 57$ nm and diamonds). There was an agreement for the onset of the VBS activation and the YAP localization to the nucleus region. The comparison demonstrated the membrane-talin complex as an important component in modulating mechanotransduction associated with FAs.

The effect of $r_{ca}$ on cellular traction forces was investigated. For this purpose, the membrane-talin and membrane-without-talin calculations using $r_{ca} = 30$ nm and $r_{ca} = 72$ nm shown in Fig. 2 a were further analyzed to plot the traction force versus substrate stiffness responses in Fig. 3 c, top and bottom, respectively. Here, the $r_s$-value was increased with $r_{ca}$ based on a recent identification that the increase of the size of integrin clusters is positively correlated with that of the FA area (10). In addition, the $L_m$-value was decreased because the increase of $r_{ca}$ results in stiffer membrane-talin complexes (see Fig. S3). Gradual changes in the traction force responses were identified by increasing $r_{ca}$. These changes in the traction forces showed good agreements with living cell measurements obtained by varying the density of fibronectin molecules treated on the surface of substrates (4) (Fig. 3 c). The result suggested a tendency that the increase of the fibronectin density can result in the increase of the integrin cluster size. In Fig. S8, the $r_{ca}$ vs. fibronectin density relation was plotted. Shifting of the VBS activation curve to a high stiffness regime was identified in increasing $r_{ca}$ (Fig. 3 d). Comparisons between the onsets of the VBS activation with different $r_{ca}$-values and the localization of YAPs to the nucleus region with different fibronectin densities showed good agreements.

Investigating how $r_{ct}$ affects the generation of traction forces provided a membrane-based hypothesis for a widely recognized pharmacological method using peptide sequences to modulate the integrin-ligand interaction (50–52). According to the previous investigation, the treatment of the peptide called GPen to inhibit $\alpha_5\beta_3$ without affecting $\alpha_5\beta_1$ integrins reduces the nonlinearity in the force versus stiffness response generated from talin-1-null but talin-2-sufficient cells (4). To reduce the nonlinearity in this model framework, either $r_{ca}$ is decreased or $r_{ct}$ is increased. It was reported that the peptide treatment results in a talin-depleted phenotype (52), and another reported that the dysfunction of talins does not affect the size of adhesion clusters (49). These suggested the investigation of a relation between the effect of the peptide treatment in living cells and the effect of changing $r_{ct}$, not $r_{ca}$, in the mechanical response of the membrane-talin model.

To compare the calculations in Fig. 2 b to traction force versus substrate stiffness measurements from the peptide-treated cells (4), the $r_s^*$ and $L_m$-values were systematically increased with the increase of $r_{ct}$.
Clusters. The relation between membrane without directly affecting the size of integrin peptide can modulate the size of the tented lipid

Experimental data measured at FAs. These include the size of single integrin clusters (10), the integrin activation force (6), the number of talin proteins within a nanoscale area (9), and the traction force versus substrate stiffness responses of living cells (4) (also see Table 1). It was remarkable that by simply assuming pulling of lipid membranes at FAs, those measured data mutually supported their validity. Similar membrane analyses performed for another mechanosensitive system may provide additional support for this work by suggesting that the nanomechanical response of lipid membranes is commonly important for signaling systems mediated by adhesion receptors (57). Overall, with an emphasis on the mechanics of lipid membranes, this work provides an integrated molecular mechanism for the generation of cellular traction forces and signaling transduction at FAS.

SUPPORTING MATERIAL

Supplemental information can be found online at https://doi.org/10.1016/j.bpr.2021.100006.

DECLARATION OF INTERESTS

The author declares no competing interests.

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