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Crosslinking actin networks produces compressive force

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Abstract

Actin has been shown to be essential for clathrin-mediated endocytosis in yeast. However, actin polymerization alone is likely insufficient to produce enough force to deform the membrane against the huge turgor pressure of yeast cells. In this paper, we used Brownian dynamics simulations to demonstrate that crosslinking of a meshwork of nonpolymerizing actin filaments is able to produce compressive forces. We show that the force can be up to several thousand pico-Newtons if the crosslinker has a high stiffness. The force decays over time as a result of crosslinker turnover, and is a result of converting chemical binding energy into elastic energy.

Keywords

actin; crosslinking proteins; endocytosis; mathematical modeling

1 | INTRODUCTION

Polymerization of actin filaments provides the direct driving force for many cellular processes, such as the movement of cells and bacteria (Loisel, Boujemaa, Pantaloni, & Carlier, 1999; Mitchison & Cramer, 1996; Pollard & Cooper, 2009). Actin filaments can also work with myosin motors to power processes like muscle contraction (Rayment et al., 1993; Spudich & Watt, 1971) and cytokinesis (Pollard, 2010; Pollard & Wu, 2010). During clathrin-mediated endocytosis (CME), a flat patch of membrane is deformed toward the cytoplasm to form a vesicle. In yeast cells, this process is opposed by a high turgor pressure of 1–1.5 MPa (Atilgan, Magidson, Khodjakov, & Chang, 2015; Basu, Munteanu, & Chang, 2014; Minc, Boudaoud, & Chang, 2009) and actin network is necessary for successful CME (Kaksonen, Toret, & Drubin, 2006; Mooren, Galletta, & Cooper, 2012). However, actin polymerization-based and motor-mediated force production are unable to meet the force budget required to internalize a vesicle, which is up to ~3,000 pN due to the high turgor

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DATA ACCESSIBILITY

The code used to perform the simulations is available at <https://github.com/ruima86/ActinAroundCylinder>.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

pressure (Dmitrieff & Nédélec, 2015; Lacy, Ma, Ravindra, & Berro, 2018). Assuming each actin filament can generate a force of 1 pN at their polymerizing end (Footer, Kerssemakers, Theriot, & Dogterom, 2007), the maximum force provided by polymerization alone is less than ~200 pN given the number of actin filaments involved in CME is no more than 200 (Berro, Sirotkin, & Pollard, 2010; Sirotkin, Berro, Macmillan, Zhao, & Pollard, 2010). The detachment rate of Myosin-I motors involved in CME significantly increases once the loading force is beyond 2 pN (Laakso, Lewis, Shuman, & Michael Ostap, 2008), suggesting a maximum force of ~600 pN can be produced by motor-filament interaction given the number of Myosin-I motors peaks at ~300 (Sirotkin et al., 2010). Therefore, other force production mechanisms complementary to polymerization and motor-filament interaction need to be investigated.

Actin-binding protein (ABP) fimbrin is an actin filament crosslinker and is the second most abundant protein involved in CME next to actin (Sirotkin et al., 2010). Though fimbrin is a passive ABP that does not convert the chemical energy of adenosine triphosphate (ATP) hydrolysis into mechanical work like molecular motors do, we have shown that its binding chemical energy can be converted into elastic energy upon crosslinking actin filaments (Ma & Berro, 2018). We wondered whether crosslinking may also be able to directly produce force in certain conditions. We hypothesized that an object in the middle of a crosslinked actin meshwork would be subjected to compressive forces. Providing endocytosis is fast, this mechanism could be a nonnegligible source of force production, even if it is transient.

Previous simulations of crosslinked actin networks have usually treated the filaments as semiflexible polymers and study their viscoelastic properties with different ABPs (Kim, Hwang, Lee, & Kamm, 2009; Müller et al., 2014). The actin filaments in these studies are typically several micrometers long, which is close to the persistence length of actin filaments (~10 μm). Adding ABPs can dramatically alter the morphology and the mechanical properties of the actin network (Kim, Hwang, & Kamm, 2011; Lieleg, Claessens, & Bausch, 2010; Lieleg, Claessens, Heussinger, Frey, & Bausch, 2007). However, the length of actin filaments involved in CME (<200 nm) is much shorter than their persistence length, therefore can hardly bend or twist by thermal fluctuations. Force production mechanisms of actin networks at this short length scale remains unclear. A push-pull model was proposed to explain the pulling force generated by actin network (Carlsson, 2018; Lacy et al., 2018). The idea is that actin polymerization is limited in a circular region with an empty center and the retrograde flow of actin in the periphery caused by polymerization pulls the branched actin filaments in the center, which in turn pull on the membrane. However, in order to meet the high force requirement for CME, simulations of this model either treat the actin network as an elastic continuum of very high rigidity ($>10^4$ Pa) (Carlsson & Bayly, 2014) or very high viscosity (~4 Pa·s μM^{-1}) (Nickaeen, Berro, Pollard, & Slepchenko, 2019; Simon et al., 2019), or consider only the kinetics of actin polymerization and assume a very high polymerization force of actin filament at the growing end (~30 pN) (Mund et al., 2018; Wang & Carlsson, 2017; Wang, Galletta, Cooper, & Carlsson, 2016). The role of ABPs such as crosslinking proteins is usually neglected in these studies.

In this paper, we study the force produced by crosslinking a network of nonpolymerizing actin filaments around an elastic cylinder. We find that the force is compressive and decays

over time due to crosslinker turnover. The magnitude of the force can be up to thousands of pico-Newtons and last tens of seconds if the stiffness of the crosslinker is high (10 pN/nm).

2 | MODEL

In order to mimic the geometry of the actin network around the endocytic patch, we place a total number of $N_{\text{fil}} = 140$ actin filaments around a purely elastic cylinder of radius R and stiffness κ_{cyl} . This differs from our previous study which restrained actin filaments in a cubic geometry free of any obstacle (Ma & Berro, 2018). If a filament penetrates into the cylinder, such that the filament's nearest distance to the cylinder's central axis $D < R$, it experiences a repulsive force $\mathbf{F} = F\mathbf{d}$ with a magnitude F that is proportional to the penetration depth:

$$F = \kappa_{\text{cyl}}(R - D), \quad (1)$$

and $\mathbf{d}$ is the unit vector pointing outward along the axis that defines the nearest distance between the filament and the central axis of the cylinder (Figure 1a).

To model actin filaments and crosslinkers, we use the same model as in Ma and Berro (2018). In summary, each actin filament is modeled as a helical rod with a constant length $L = 135$ nm. As the length of filaments is much shorter than the persistent length, we treat each filament as a rigid body, whose movement is governed by the equation:

$$\Xi_{\text{fric}} \begin{bmatrix} \mathbf{V}_c \\ \Omega \end{bmatrix} = \begin{bmatrix} \mathbf{F}_e \\ \mathbf{T}_e \end{bmatrix} + \begin{bmatrix} \mathbf{F}_s \\ \mathbf{T}_s \end{bmatrix}, \quad (2)$$

where Ξ_{fric} denotes the friction coefficient matrix, $\mathbf{V}_c/\Omega$ denotes the translational/rotational velocity of the filament, $\mathbf{F}_e/\mathbf{T}_e$ denotes the deterministic force/torque generated by the crosslinkers and the elastic cylinder, and $\mathbf{F}_s/\mathbf{T}_s$ denotes the stochastic force/torque due to thermal fluctuations. The friction coefficient matrix includes both translational component ξ^t and rotational component ξ^r . Each component can be further decomposed into contribution in the direction parallel to the central axis $\xi_{\parallel}^t$ and $\xi_{\parallel}^r$ and perpendicular to $\xi_{\perp}^t$ and $\xi_{\perp}^r$.

The helical nature of filaments is explicitly considered by attaching each monomer i of the filament with a unit vector $\mathbf{m}_i$ representing the normal direction of the binding surface with crosslinkers (Figure 1b). A crosslinker is modeled as a combination of one extensional spring and two torsional springs. The extensional energy reads

$$E_{\text{ext}} = \frac{1}{2} \kappa_{\text{ext}} (l - l_0)^2, \quad (3)$$

where κ_{ext} denotes the extensional stiffness, l denotes the length, and l_0 denotes the rest length of the crosslinker. The torsional energy reads

$$E_{\text{tor}} = \frac{1}{2} \kappa_{\text{tor}} (\theta_1^2 + \theta_2^2), \quad (4)$$

where κ_{tor} denotes the torsional stiffness, θ_1 and θ_2 denote the angle spanned between the crosslinker's orientation and the attached monomers' orientation.

A crosslinker can be formed between two monomers in two filaments with a rate constant R_b if (i) the distance between the monomers is less than a critical value d_c and (ii) the occupancy ratio of each filament is less than the maximum value g_{max} . The unbinding rate of a crosslinker depends on the total elastic energy $E = E_{\text{ext}} + E_{\text{tor}}$ via

$$R_u = R_u^0 e^{E/E_c}, \quad (5)$$

where E_c denotes the characteristic energy stored in a crosslinker. In the simulation, crosslinking is realized by randomly selecting N pairs of monomers in two filaments of N_1 and N_2 monomers in length, where N is a random number that obeys the Poisson distribution with an average $N_1 N_2 R_b \Delta t$. For each pair, if they satisfy Conditions (i) and (ii) above, a linker is formed between them. The linker length upon formation can be either longer or shorter than the rest length l_0 .

In addition to the cylinder, we also put an elastic boundary of a cubic geometry with an edge length of 500 nm. The filaments are initially placed around the cylinder and near the upper boundary. We choose a time step $\Delta t = 10^{-5}$ s or smaller to ensure that it is shorter than the relaxation time of a spring so that we can correctly capture the relaxation process. Each simulation is run for 200 s and the parameters are listed in Table 1. A more detailed description of the model can be found in Ma and Berro (2018).

3 | RESULTS

3.1 | Crosslinking actin filaments around a cylinder produces compressive force

We start the simulation with filaments randomly positioned and oriented around the cylinder. Crosslinks rapidly form between filaments such that a meshwork forms around the cylinder (Figure 1c and Movie S1). The elasticity of the crosslinkers tend to pull filaments together. However, the cylinder in the center repels filaments that penetrate into it. As a consequence, the meshwork generates a compressive force on the cylinder. The total compressive force acting on the cylinder is calculated as the sum of the magnitude of the repulsive force experienced by each filament penetrating into the cylinder. Note that the stiffness and the radius of the cylinder have a minor impact on the estimation of the forces (Figure S1). In the following, we investigate how this compressive force depends on the turnover rate and the mechanical properties of crosslinkers.

3.2 | The compressive force generated by a crosslinked actin network decays over time if crosslinkers undergo turnover

If the crosslinking unbinding rate R_u^0 is set to zero, a positive compressive force can persist over the entire simulation, that is, hundreds of seconds (Figure 2a). However, when allowing crosslinkers to turnover after $t = 10$ s by setting their unbinding rate constant R_u^0 to a positive value, the compressive force decays over time (Figure 2b,c). The decay profile has an initial

rapid drop due to sudden breakage of many crosslinkers and is followed by a relatively slow decay that can be fit by an exponential function. The relaxation time of the exponential decay decreases with the unbinding rate R_u^0 . In order to show the decay of the compressive force, we compare the stable force F_s measured at $t = 5$ s when crosslinker turnover is off and the dynamic force F_d measured at $t = 20$ s, after crosslinker turnover has been switched on for 10 s. As the unbinding rate R_u^0 increases, the dynamic force F_d decreases while the stable force F_s remains unchanged, indicating a faster force drop at higher unbinding rate (Figure 2d).

3.3 | The compressive force increases with the extensional stiffness κ_{ext} of the crosslinker and the total number of crosslinkers

To demonstrate the compressive force originates from the elastic interaction between filaments mediated by crosslinkers, we investigate the effect of the extensional stiffness κ_{ext} of the crosslinker on the magnitude and decay time of the compressive force (Figure 3). Both the stable force F_s at $t = 5$ s and the dynamic force F_d at $t = 20$ s increase with κ_{ext} and can be up to 8,000 pN for F_s and 2,000 pN for F_d (Figure 3d). The relaxation time also increases with κ_{ext} . Therefore, a crosslinker with high extensional stiffness is needed to produce large and persistent compressive force.

We also vary the maximum occupancy ratio g_{max} of crosslinkers to see the effect of the total number of crosslinkers on the compressive force (Figure 4). When g_{max} is lowered to 0.03, the total number of crosslinkers is reduced to ~ 110 , the actin filaments do not form a crosslinked network that surrounds the cylinder but tend to diffuse away, and the compressive force vanishes (Figure 4d). As g_{max} is increased to 0.06, actin filaments are loosely connected and they can generate a small compressive force on the cylinder. At $g_{\text{max}} = 0.125$, a stable crosslinked network can form with a uniform distribution of actin filaments around the cylinder. Both the magnitude and the relaxation time of the compressive force increase with the total number of crosslinkers.

3.4 | The torsional stiffness κ_{tor} of the crosslinker has a weak effect on the compressive force

With increasing torsional stiffness κ_{tor} of the crosslinker, both the stable force F_s and the dynamic force F_d remain almost flat until $\kappa_{\text{tor}} = 18$ pN · nm (Figure 5). The stable force F_s then increases with κ_{tor} , but the dynamic force F_d shows the opposite trend. This is likely due to the fact that with increasing κ_{tor} , the torsional energy stored in crosslinkers increases. Once crosslinker turnover is switched on, the stored elastic energy is rapidly released to relax the stress and a higher κ_{tor} leads to a higher effective unbinding rate of the crosslinkers, since it increases exponentially with the stored energy (Equation 5).

3.5 | The helicity of actin filaments does not impact the compressive force

To further confirm the compressive force arises primarily from the extensional stiffness of the crosslinker that pulls filaments together but not from the torsional stiffness, we run simulations with filaments of different helical pitches (Figure 6). A filament with a pitch number n_p has an angular increment $\varphi = (1 + (1/n_p))\pi$ between its two consecutive subunits

counting counter-clockwise from the pointed end to the barbed end. The additional phase π is due to the fact that actin filaments are composed of two strands of subunits facing opposite directions from each other. We only consider n_p of odd numbers, such that monomer i and monomer $i + n_p$ have the same orientation but are in different strands. A natural actin filament has a pitch number $n_p = 13$. Our simulations show that both the magnitude and the relaxation time of the compressive force are very similar for filaments with different pitch numbers (Figure 6d).

4 | DISCUSSION

In this paper, we have studied the compressive force produced by crosslinked actin filaments. We showed that a crosslinked actin network generates force if the filaments are placed around an elastic cylinder. The force is compressive and decays over time as a result of crosslinker turnover. The magnitude and persistent time of the force strongly depends on the extensional stiffness κ_{ext} of the crosslinkers but weakly on their torsional stiffness κ_{tor} . More than a 1000 pN of force can be produced by crosslinkers with an extensional stiffness $\kappa_{\text{ext}} = 10$ pN/nm and last for tens of seconds. As formation of a clathrin-coated pit is fast (~ 10 s), this force could be a significant contribution to the force required for endocytosis, especially when compared with the force that can be produced by the polymerization of the same number of actin filaments (~ 150 pN) (Kaksonen et al., 2006; Lacy et al., 2018). However, the force we have shown here is a compressive force, which plays a role of line tension but does not contribute to the directed force needed to pull a membrane inward. This line tension might help deform the membrane through a buckling mechanism (Lenz, Crow, & Joanny, 2009). A rough estimation by dimensional analysis of the buckling line tension F_{buckle} for a flat membrane patch with a base radius of $r = 50$ nm (Kukulski, Schorb, Kaksonen, & Briggs, 2012) and bending rigidity of $\kappa = 2000 k_B T$ (Dmitrieff & Nédélec, 2015) leads to $F_{\text{buckle}} \sim (\kappa/r) = 160$ pN. Therefore, the compressive force generated by the crosslinked actin network is around an order of magnitude larger than the force required to buckle a flat membrane. However, this estimation of the buckling force totally neglects the contribution of turgor pressure, therefore is likely to be largely underestimated.

In this study, we have neglected actin dynamics but focused on the force production mechanism by passively crosslinking actin filaments. The force comes from perturbing an initially highly crosslinked actin network with an elastic cylinder, rather than from a perturbation of shearing the actin network, as most of previously actin simulations did. This perturbed state is out of equilibrium and the decay of the force is essentially the relaxation process for the system to reach a new equilibrium. Crosslinker turnover is essential for the redistribution and relaxation of the stress. It has been shown that the viscoelastic property of the actin network also depends on how fast the external perturbation is applied relative to the unbinding rate of the crosslinker (Kim et al., 2011; Müller et al., 2014). If crosslinkers are rapidly recruited to the actin network and actin polymerization is considered on top of the crosslinker turnover, then the system will be driven out of equilibrium by the fast actin polymerization, and the newly polymerized actin filaments, that is, increased actin density, might compensate for the reduced force due to crosslinker unbinding at constant actin density. Investigating the compressive force in the presence of turnover for both actin and

crosslinkers will determine whether this force production process can be sustained over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Model of a crosslinked actin network around an elastic cylinder. (a) Illustration of the model. A repulsive force is applied to filaments that penetrate into the cylinder. (b) Actin filaments are modeled as a helical rod with the arrows representing the orientation of the monomers' binding surface with crosslinkers. Each crosslinker is modeled as a combination of one extensional spring and two torsional springs. (c) Snapshots of a typical simulation using the reference parameter values (Table 1). Each filament is depicted as a blue line. For clarity crosslinkers are not plotted. The deformed part of the cylinder (green) represents the largest cylindrical region without any filaments penetrated in

FIGURE 2.

The total force decays over time when crosslinkers are allowed to turn over. (a-c) Temporal evolution of the total force for force-torque-free unbinding rate $R_u^0 = 0/\text{s}$ in (a), $R_u^0 = 0.01/\text{s}$ in (b) and $R_u^0 = 0.1/\text{s}$ in (c). The red curve represents the average over 10 simulations and the shaded region (green) spans the SD . The orange curve is the exponential fit $a e^{-(t-10)/\tau} + b$ to the red curve from $t = 10 \text{ s}$ before which crosslinker turnover is switched off such that $R_u^0 = 0/\text{s}$ for all conditions. Inset labels show the relaxation time τ and the force $F_0 = a + b$ extracted from the exponential fitting. (d) The stable force F_s measured at $t = 5 \text{ s}$ (blue) and dynamic force F_d measured at $t = 20 \text{ s}$ for different unbinding rates R_u^0

FIGURE 3.

The total force generated by crosslinked actin networks strongly depends on the extensional stiffness of the crosslinker. (a-c) Temporal evolution of the total force for extensional stiffness $\kappa_{\text{ext}} = 0.1 \text{ pN/nm}$ in (a), $\kappa_{\text{ext}} = 1 \text{ pN/nm}$ in (b) and $\kappa_{\text{ext}} = 10 \text{ pN/nm}$ in (c). The red curve represents the average over 10 simulations and the shaded region (green) spans the *SD*. The orange curve is the exponential fit $a e^{-(t-10)/\tau} + b$ to the red curve from $t = 10 \text{ s}$ before which crosslinker turnover is switched off such that $R_u^0 = 0/\text{s}$ for all conditions. Inset labels show the relaxation time τ and the force $F_0 = a + b$ extracted from the exponential fitting. (d) The stable force F_s measured at $t = 5 \text{ s}$ (blue) and dynamic force F_d measured at $t = 20 \text{ s}$ for different extensional stiffness of the crosslinker κ_{ext}

FIGURE 4.

The total force generated by crosslinked actin networks increases with total number of crosslinkers. (a-c) Temporal evolution of the total force for maximum occupancy of crosslinkers $g_{\max} = 0.125$ in (a), $g_{\max} = 0.25$ in (b), and $g_{\max} = 0.5$ in (c). These values of $g_{\max}$ correspond to a total number of crosslinkers of 438, 875, and 1,750, respectively. The red curve represents the average over 10 simulations and the shaded region (green) spans the *SD*. The orange curve is the exponential fit $a e^{-(t-10)/\tau} + b$ to the red curve from $t = 10$ s before which crosslinker turnover is switched off such that $R_u^0 = 0/s$ for all conditions. Inset labels show the relaxation time τ and the force $F_0 = a + b$ extracted from the exponential fitting. (d) The stable force F_s measured at $t = 5$ s (blue) and dynamic force F_d measured at $t = 20$ s (red) for different $g_{\max}$. The x -axis is on a logarithmic scale

FIGURE 5.

The total force generated by crosslinked actin network weakly depends on the torsional stiffness of the crosslinker. (a-c) Temporal evolution of the total force for torsional stiffness $\kappa_{\text{tor}} = 1 \text{ pN} \cdot \text{nm}$ in (a), $\kappa_{\text{tor}} = 10 \text{ pN} \cdot \text{nm}$ in (b), and $\kappa_{\text{tor}} = 100 \text{ pN} \cdot \text{nm}$ in (c). The red curve represents the average over 10 simulations and the shaded region (green) spans the *SD*. The orange curve is the exponential fit $a e^{-(t-10)/\tau} + b$ to the red curve from $t = 10 \text{ s}$ before which crosslinker turnover is switched off such that $R_u^0 = 0/\text{s}$ for all conditions. Inset labels show the relaxation time τ and the force $F_0 = a + b$ extracted from the exponential fitting. (d) The stable force F_s measured at $t = 5 \text{ s}$ (blue) and dynamic force F_d measured at $t = 20 \text{ s}$ for different torsional stiffness of the crosslinker κ_{tor}

FIGURE 6.

The total force generated by crosslinked actin networks is independent from the helicity of filaments. (a-c) Temporal evolution of the total force for filaments of different pitch numbers. The red curve represents the average over 10 simulations and the shaded region (green) spans the *SD*. The orange curve is the exponential fit $a e^{-(t-10)/\tau} + b$ to the red curve from $t = 10$ s before which crosslinker turnover is switched off such that $R_u^0 = 0/s$ for all conditions. Inset labels show the relaxation time τ and the force $F_0 = a + b$ extracted from the exponential fitting. (d) The stable force F_s measured at $t = 5$ s (blue) and dynamic force F_d measured at $t = 20$ s for filaments of different helical pitches

TABLE 1

List of parameters

Symbol	Meaning	Reference value	Varied range
L	Filament length	135 nm	135 nm
b	Filament diameter	6 nm	6 nm
δ	Length elongation of filaments by incorporation of a monomer	2.7 nm	2.7 nm
κ_{ext}	Extensional stiffness of the crosslinker	0.1 pN/nm	0.1–10 pN/nm
κ_{tor}	Torsional stiffness of the crosslinker	10 pN · nm	1–100 pN · nm
R_0	Crosslinker binding rate constant between two unoccupied monomers	1/s	1/s
R_u^0	Force-torque-free unbinding rate of a formed crosslinker	0.1/s	0.001–0.1/s
l_0	Rest length of the crosslinker	10 nm	10 nm
d_c	Reaction distance to build a crosslinker	20 nm	20 nm
E_c	Characteristic energy of a crosslinker	$10 k_B T$	$10 k_B T$
f_{st}	Repulsive force due to steric interaction	100 pN	100 pN
$k_B T$	Thermal energy unit at room temperature	4.1 pN · nm	4.1 pN · nm
r_{min}	Minimum distance between two filaments	6 nm	6 nm
N_{fil}	Total number of actin filaments	140	140
g_{max}	Maximum crosslinker occupancy of a filament	0.25	0.25
η	Viscosity of the medium	10^{-5} pN · s · nm ⁻²	10^{-5} pN · s · nm ⁻²
t	Simulation time step	10^{-5} s	10^{-5} to 10^{-6} s