Branching and capping determine the force–velocity relationships of branching actin networks

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Received 1 June 2012
Accepted for publication 29 November 2012
Published 28 January 2013
Online at stacks.iop.org/PhysBio/10/016004

Abstract
A branching actin network is the major engine that drives cell motility. A measure of the effectiveness of an engine is the velocity the engine is able to produce at a given resistance—the force–velocity relationship. Concave force–velocity relationships consist of a force-insensitive region, indicative of an adaptive response. In contrast, convex force–velocity relationships would reflect a passive response. Even in in vitro experiments, branching actin networks can exhibit both concave and convex force–velocity curves. However, the exact mechanism that can explain both force–velocity curves is not yet known. We carried out an agent-based stochastic simulation to explore such a mechanism. We discovered an emergent behavior of a branching actin network: Upon resistance, it remodels itself by increasing the number of filaments growing in contact with the load. The remodeling is favored by branching events and limited by capping. The force–velocity relationship hinges on the relative time-scale between the intrinsic kinetics of the branching actin network and the loading. Shortly after encountering resistance (∼seconds), the force–velocity relationship of the actin network is always convex, as it does not have enough time to remodel itself. A concave force–velocity relationship requires network remodeling at longer time-scales (∼tens of seconds to minutes) and the faster branching event relative to capping. Furthermore, our model explains the observed hysteresis in the force–velocity relationship of actin networks. Our model thus establishes a unified mechanism that can account for both convex and concave force–velocity relationships observed in branching actin networks.

Introduction
Branching actin networks are the major machinery that drives cell motility. Deciphering the fundamental principle by which the actin network is able to exert force against resistance holds an important key to understanding a number of cellular processes ranging from cell migration (Pollard and Borisy 2003, Fletcher and Mullins 2010) to membrane trafficking (Engqvist-Goldstein and Drubin 2003).

While new factors keep emerging, the essential actin biochemistry has been firmly established by seminal experiments in the past several decades (Pollard and Borisy 2003, Fletcher and Mullins 2010). In migrating cells, individual actin filaments grow from their barbed-ends, pushing against the plasma membrane in the direction of cell movement. Activated by WASP at the membrane, Arp2/3 complex branches off new filaments from the existing filaments at a characteristic angle of 70 degrees. Capping
proteins bind to the barbed-end of the filament preventing its further growth. At the back of the network, actin filaments are severed and depolymerize, providing a fresh actin monomer supply to the front (Pollard and Borisy 2003, Fletcher and Mullins 2010).

As experiments have gathered mounting knowledge of actin biochemistry, the quest of how the biochemistry dictates the mechanical response of branching actin network, however, has not been convincingly addressed. Experiments probing the force–velocity relationship of branching actin networks have been performed (McGrath et al 2003, Marcy et al 2004, Prass et al 2006, Heinemann et al 2011, Parekh et al 2005), but often yielded conflicting results. Migrating cells show an adaptive response exhibiting a concave force–velocity relationship (Prass et al 2006, Heinemann et al 2011). However, the concave force–velocity relationship is typically preceded by a large reduction in velocity to tiny forces before the velocity becomes force-insensitive (Prass et al 2006). The mechanism controlling the concave force–velocity relationship and the initial response to small forces in cells is complicated by other cellular components such as focal adhesions (Gardel et al 2010). To study the exact mechanism that determines the force–velocity relationship of a branching actin network, in vitro experiments with more controllable conditions have been performed. One study measured the velocity of an actin network growing against a constant load force, and the resulting force–velocity relationship was convex (Marcy et al 2004). In a different in vitro experiment, the actin network grew against a flexible cantilever. The load force thus progressively increased as actin polymerization deflected the cantilever, and the network showed a concave force–velocity relationship (Parekh et al 2005). That experiment also showed a hysteresis effect where the velocity of the network was dependent upon the past forces applied to the network.

Because in vitro experiments have demonstrated both convex and concave force–velocity relationships in branching actin networks, it suggests that a branching actin network itself can respond to external forces in both an adaptive and a non-adaptive manner outside of cellular context. However, even within the simplified in vitro setting, it is still unclear how the individual factors of branching actin network dynamics give rise to both the convex and the concave curves. It has previously been proposed that the actin network remodels itself in response to force (Parekh et al 2005), but the nature of such remodeling is largely unknown.

Evidence suggests that actin filaments utilize thermodynamic free energy to add additional monomers to exert force towards the leading edge of the network (Shaevitz and Fletcher 2007). The proposed model for that behavior has been termed the Brownian ratchet (Peskin et al 1993, Mogilner and Oster 1996, 2003). The Brownian ratchet mechanism takes advantage of the asymmetry in the on and off rates of actin monomer binding to an existing filament. Small gaps arise between the actin filaments and the leading edge due to thermodynamic fluctuations. Monomers are able to bind during such fluctuations and push the leading edge forward. The predicted force–velocity curve for a single filament is a convex negative exponential function.

When many filaments grow against the same load, they share the load. A simple load-sharing mechanism with a fixed number of filaments in contact with the load surface (we termed these filaments as ‘contacting filaments’ from now on) predicted that the force–velocity curve is nonetheless convex (Schaus and Borisy 2008), even though its slope is shallower than that for single filament. A seminal computational study by Carlsson (2003) that solely considers the autocatalytic nature of a branching event could explain the force-insensitive region of the concave force–velocity curve. But without additional negative feedback mechanism, this model is largely incapable of accounting for the convex curve.

More recently, Schreiber et al (2010) suggested that the excluded volume effect of branching actin networks could limit the density of actin filaments at the leading edge, and could be the needed negative feedback that yields the convex force–velocity relationship. Furthermore, the interesting study by Weichsel and Schwarz (2010) suggests that there could exist bistable states for different orientation patterns of actin filaments at the leading edge (−70/0/70 versus −35/35). The two bi-stable states could be responsible for the observed concave and convex force–velocity relationships, and the transition between them could give rise to hysteresis. However, Weichsel and Schwarz’s work does not consider the load-sharing mechanism, and their results are very sensitive to the choice of branching zone width of actin network. Only when the width of branching zone is limited to within the length of two actin subunits (∼5–6 nm from the leading edge) could their model generate the two different orientation patterns of actin network (see below and the supplementary data available from stacks.iop.org/PhysBio/10/016004/mmedia for details).

Given that there is no exact experimental measurement of the branching zone width of actin network, Weichsel and Schwarz’s model remains to be tested. Although the two latter models present a large advance in theory of actin motility and both could account for the concave and convex force–velocity curves, it remains difficult to test their model predictions, and, so far, there is no clear supporting experimental evidence.

The important question remains unexplored both from theory and experimental perspectives: how does the intrinsic biochemical kinetics of branching actin network, e.g. growth, branching, and capping events, dictate its mechanical response—the force–velocity relationship? In this work, we integrate different aspects of a branching actin network and study the emergent behavior from combining these factors. We discover the unique underlying mechanism of force–velocity response of branching actin networks. We used an stochastic simulation method inspired by Weichsel and Schwarz (2010) and Schaust and Borisy (2008). Our model explicitly incorporates the biochemical kinetics of branching actin networks in addition to a load-sharing mechanism. In contrast to the orientation pattern changes as proposed by others (Weichsel and Schwarz 2010), our results show that it is the changes in the number of contacting filaments that account for both convex and concave force–velocity relationships. Furthermore, our model suggests that the balance between growth, branching and capping events controls the ability of a branching actin network to reinforce itself in terms of
in vitro networks. We therefore only focus on the four essential effects without compromising the physical essence of actin to construct the simplest model able to reproduce the observed velocity relationships of branching actin networks, we aimed to discern the physical mechanism governing the force–velocity relationship of branching actin networks.

Model description

To discern the physical mechanism governing the force–velocity relationships of branching actin networks, we aimed to construct the simplest model able to reproduce the observed effects without compromising the physical essence of actin networks. We therefore only focus on the four essential processes of branching actin networks in in vitro conditions. The model concerns with the mechanical actions of the branching actin network without explicit representations of the corresponding proteins. Below, we describe the qualitative features of our model and relegate the detailed simulation method to supplement. Model parameters were estimated based on experiment evidence where possible, and they are listed in table 1 with references.

(1) Filaments grow by adding new monomers to the barbed end of the filaments. When the filament is not in contact with the load, it does not feel the load and, hence, it could grow at its free rate $V_0$. When a filament is in contact with the load surface, the rate of growth follows the Brownian ratchet mechanism, where the growth velocity of the filament is reduced by a Boltzmann factor:

$$B(F) = \exp \left[ -\frac{F \cdot \delta}{k_B T} \right]$$

where $F$ is the force felt by the filament, $\delta$ is the length of an individual actin monomer, $k_B T$ is Boltzmann constant, and $T$ is the absolute temperature.

(2) New filaments are created by branching off the sides of existing filaments. Arp2/3 binds to existing filaments and creates a site for a new filament to grow and generates the characteristic angle in between the original and newly branched filaments (Mullins et al 1998). Branching was modeled as a zero-order reaction, independent of the number of actin filaments, which is consistent with the experiments that suggest WASP/Scar-mediated Arp2/3 activation is the limiting factor of network growth (Pollard et al 2000). The branching angle followed a Gaussian distribution that centers at 70° with a standard deviation of 5° (Mullins et al 1998).

(3) Capping proteins can bind to the tips of actin filaments, preventing them from further elongation. In in vitro conditions, the lifetime of barbed end-bound capping proteins is ~30 min (Schafer et al 1996). This feature is modeled by filament growth stopped once capped. We modeled filament capping as a first-order reaction: the capping rate was proportional to the number of free barbed ends, which is agreement with in vitro experiment measurements (Schafer et al 1996).

(4) A significant factor in the efficiency of a growing actin network against a load is the ability of the network to share the load across multiple filaments at the leading edge, which has been a recent topic of study (Schaus and Borisy 2008). We implemented a similar load-sharing scheme to Schaus and Borisy (2008) among the filaments in contact with the load surface, i.e. the sum of the load force felt by each contacting filament is in balance with the total load. Specifically, the load force encountered by the $i$th contacting filament is defined as $F_i = \frac{F_{\text{total}} \cos(\theta_i)}{\sum_{i=1}^{N_{\text{contact}}} \cos(\theta_i)}$, where $F_{\text{total}}$ is the total load force, the ‘$i$’ refers the $i$th contacting filament, $\theta_i$ is the orientation angle of the $i$th contacting filament with regard to the normal of the load surface. The sum of $j$ is over all the contacting filaments. As the force is balanced: $\sum_i F_i = F_{\text{total}}$, the contacting filaments work collaboratively to grow against the load. Note that the load forces felt by individual contacting filaments across the leading edge are not equal, because the closer the filament orientation is to the normal of the load surface, the larger share of the load force this filament will feel and, hence, the slower it will grow.

In the simulation, each filament was modeled as a point in a two-dimensional plane representing the filament barbed end. Our model only considers the in vitro experimental conditions, where actin pool is always unlimited. In the principal direction of motion, the plane was bounded by a hard leading edge (representing the load surface), and periodic boundaries in the perpendicular direction. At each time step, calculations were done in the following order. First, the location of the leading edge was determined by the location of the foremost filament. Then, filaments in contact with the leading edge were located. Filaments were next capped and branched, the rates of which were calculated based on the location of the filaments and Poisson statistics. The filaments in contact with the leading edge were neither branched nor capped. Finally, the positions of all the filament tips were updated by the growth rate for each filament in accordance to load-sharing mechanism. We will show in the supplement that the qualitative feature of our results remains intact for three-dimensional case. The optimal load-sharing mechanism is implemented in the simulation if
κ = to force. For (force–velocity curve generated by the initial response of the network ∼ velocity response to force applied at available fromstacks.iop.org/PhysBio/10/016004/mmedia).

on specific choices of model parameters (also see figure S1 timescale ranges from tens of seconds to minutes depending lower than before the application of a load. This recovery and then recovers in a longer timescale reaching a value force at time = force. Figure1(A) of the velocity of a branching actin network against a fixed load. The first set of simulations we ran tested the temporal response force–velocity relationship of actin networks. Results

Our simulations were focused on how the collective properties of a branching actin network influence the ability of the network to grow against a flat surface applying a load force. Fast force–velocity response is always convex

The first set of simulations we ran tested the temporal response of the velocity of a branching actin network against a fixed load force. Figure 1(A) represents a typical example: upon loading force at time = 10 s, the velocity drops almost instantly, and then recovers in a longer timescale reaching a value lower than before the application of a load. This recovery timescale ranges from tens of seconds to minutes depending on specific choices of model parameters (also see figure S1 available from stacks.iop.org/PhysBio/10/016004/mmedia). Taking the velocity at the bottom of the initial response to force response gives a force–velocity relationship for the short time-response of the network. Our model suggests that the force–velocity relationship at short timescale is always convex (figure 1(B)).

Force–velocity relationship at long-time response can be either convex or concave

Running the simulations for an extended amount of time allowed us to study the equilibrium force–velocity relationship. Figure 2(A) shows that we were able to reproduce both convex and concave force–velocity relationships. The only difference between the sets of simulations is the absolute value of the capping and branching rates. Their ratio, and therefore the average number of filaments, was fixed. We hypothesize that the network is able to reinforce itself by bringing more filaments to the leading edge.

Branching actin networks remodel by increasing the number of contacting filaments upon load force

A branching actin network can be visualized as one population of filaments contacting the boundary and another population of filaments trailing the leading edge in reserve. The network reinforces the filaments at the leading edge when trailing filaments grow to reach the leading edge. That remodeling response simply depends on the rate at which trailing filaments are able to catch up to the leading edge. Figure 3 is an explanatory diagram showing a hypothetical branching pattern. The first filament is in contact with the boundary; the second filament branches off the first one, is further back, and serves as a substrate for new filaments. We term the third population of filaments as ‘reserve filaments’ (figure 3(A)). Since these reserved filaments are not in contact with the load surface, they do not feel the load and grow at their free rate faster than the leading edge. Consequently, some of the reserved filaments could catch up to the leading edge. When the capping rate is high, these reserved filaments will get capped and stop growing before reaching the load surface (figure 3(B)). As a result, the number of contacting filaments remains roughly the same upon load force. This situation corresponds to the simple load-sharing mechanism investigated by Schaus and Borisy (2008), in which a fixed number of contacting filaments result in a convex force–velocity relationship. Conversely, when the capping rate is low, these reserve filaments are able to grow into contact with the load surface. Thus, the number of contacting filaments will increase, thereby reinforcing the growth of the leading edge against the load, resulting in a concave force–velocity curve (figure 3(C)).

The hypothesis that the network reinforces itself by increasing filaments growing to the leading edge would suggest that increasing the capping rate would result in a more convex-like force–velocity relationship. We theoretically test this hypothesis by obtaining the snapshots of the evolution of actin network growth against the load. Figure 2(B) reveals that the filaments increases near the load surface as they encounter the load resistance. For a given load force, the increase in the

Figure 1. The force–velocity relationship of branching actin network at short timescale. (A) A characteristic time trace of the velocity response to force applied at ~10 s. (B) The convex force–velocity curve generated by the initial response of the network to force. For (A) and (B), the simulations were run with capping rate κ = 1/s/filament and branching rate λ = 200 s⁻¹.

not otherwise mentioned. In the discussion, we will further study the effects of a sub-optimal load-sharing mechanism on the force–velocity relationship of actin networks.

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Figure 2. The increase in the number of contacting filaments dictates the convex and the concave force–velocity relationships at long timescale. (A) The change of the force–velocity curves from convex to concave is continuous upon the reduction of capping rates. (B) Snapshots of the evolution of actin network growth against a constant load force 6.8nN. The low capping rate is 3 per sec per filament; and the high capping rate is 7 per sec per filament. The ‘t’ refers the time after the application the load force. The blue lines represent the actin filaments, the red solid line represents the load surface, and the black dash line denotes the position of one actin subunit length away from the load surface (the contacting zone). For illustration purposes, only 1/10 of the filaments are shown in each snapshot. (C) The number of contacting filaments increases upon load force, where the capping rates are kept the same as in (B). For (A)–(C), the ratio of branching rate versus capping rate is kept fixed as λ/κ = 200 filaments. The error bars in (A) and (C) represent the standard deviation estimated from 10 simulations.

contacting filaments is rather limited when the capping rate is high, whereas it becomes more drastic in the low capping region.

Moreover, our calculations in figure 2(A) corroborate this hypothesis: by increasing the capping rate, the force–velocity curve continuously changes from concave to convex shapes. Note that the inter-conversion between the concave and the convex force–velocity relationships can be continuously tuned by actin biochemistry, e.g., capping rates. This model prediction is in contrast to the proposal by Weichsel and Schwarz (2010) that predicts an abrupt bistability-like switch. We will further compare our model with others in the discussion section (see below).

The level of reinforcement is further attested by the number of contacting filaments as the function of load force for different capping rates. Figure 2(C) shows that, for the same set of capping rates as in figure 2(B), the number of contacting filaments increases with increasing force for all cases. Note that the cases with lower capping rates generate a larger increase before peaking and stalling. This is because the branching rate is zeroth order, where the rate is independent of the number of filaments, whereas the capping rate is first order and increases with the number of available filaments. Before reaching the peak, the network is, in some sense, branching dominated. On the other hand, increases in force increase the number of filaments in contact with the leading edge, so do the reserved filaments deriving from these contacting filaments. As the capping event is the first order reaction, it will increase as well. When the effective capping rate becomes faster than the branching rate for these reserved filaments,
the reinforcement will get attenuated and, hence, the number of contacting filaments will drop off. It is also of note that the specific filaments in contact with the leading edge are constantly changing. When the capping effect dominates over branching, the leading edge velocity is no longer sufficient to provide the turnover to sustain the higher number of filaments. Approximately at the peak of figure 2(C), the network begins to stall in the force–velocity curves in figure 2(B).

Branching actin network remodeling could account for the observed hysteresis effect

The accumulation of contacting filaments is also able to explain some of the hysteresis observed in actin networks. As seen in figure 2(C), the number of contacting filaments increases with increasing force. Subjecting the network to a large force and subsequently releasing that force should leave excess filaments in contact with the leading edge.

Figure 4(A) shows the velocity of a simulation where the network pushed against low force for the first third of the run, followed by high force in the middle third, and finally the original low force. Here, figure 4(B) serves as the simulation input. That is comparable to the experiment in Parekh et al. (2005). The velocity initially shoots back up in response to the reduced force, but it rapidly decays back to the initial equilibrium force similar to Carlsson (2003). The reduction in the load force speeds up the growth rate of all the contacting filaments at the leading edge. Due to the angle dependence of the load sharing for individual filaments, the speed-up of growth rates is heterogeneous across the contacting filaments. As a result, some filaments grow faster while staying in contact with the load, and slower growing filaments slide off the leading edge and get capped. Ultimately, the number of contacting filaments relaxes back to the velocity corresponding to the original force and completely loses its memory of the previous loading force. That is, there is no sustained ‘memory’.

On the other hand, figure 4(C) shows that the same force-reversal input (figure 4(B)) can yield the sustained hysteresis, if we incorporate a factor that causes the actin filaments to stick to the leading edge (see supplementary data for details available from stacks.iop.org/PhysBio/10/016004/mmedia).

In this scenario, a larger load force results in more filaments contacting the load surface. Due to the adhesive interaction between the filament tips and the load surface, these contacting filaments will tend to stay with the load. Consequently, the number of contacting filaments holds up, even when the network growth velocity speeds up as the load force is reduced. Thus, it is the combined effect of the ‘sticky’ load surface and the load-induced increase in the contacting filaments that gives rise to the observed hysteresis. While we do not know the exact nature of such an interaction between the filament tips and the load surface, actin tethering has been theoretically proposed (Mogilner and Oster 2003) and has some experimental evidence (Giardini et al 2003, Co et al 2007, Svitkina 2007).

Branching zone width affects orientation patterns but not force–velocity relationship

It is generally believed that new filament branching in actin networks occurs in a narrow zone near the membrane (Atilgan et al 2005, Pollard et al 2000), although there is no quantitative measurement on the exact width of such an active Arp2/3 complex-enriched zone. It has been suggested that the restriction of filament branching at the membrane may have a role in the geometric organization of the network (Schaus et al 2007, Atilgan et al 2005). We ran simulations to test if restricting the area where new filaments could branch would change the predictions of our simulations. We restricted the branching of new filaments to a zone of distance L away from, but not at, the leading edge while keeping all the other model parameters the same as those in figure 2(B). Here, the distance L ranges from 2 to 40 actin subunit lengths, corresponding to 5.4–108 nm. Figure 5 represents a typical example. It shows that such spatial restriction of branching events does not significantly change the qualitative behavior of the force–velocity relationship as compared to the unrestricted case (figure 2(B)).

In addition, our model was only able to reproduce the orientation patterns predicted by Weichsel and Schwarz (2010) for very small branching zones. When we limited the branching zone within a distance of 2 actin subunit lengths (5.4 nm)
Figure 4. Hysteresis in force–velocity relationships of branching actin network. (A) Without ‘sticky’ load surface, a typical simulation result in a transient hysteresis effect. (B) The time-dependent load force as the model input used in both (A) and (C). A typical simulation with ‘sticky’ load surface, i.e. filaments reaching the leading edge stuck to the leading edge. For (A)–(C): the capping rate is 1/s/filament, the branching rate is 200 s⁻¹.

from the load surface as in Weichsel and Schwarz’s model, we saw the similar orientation pattern changes upon increasing load forces they reported (figures S2(A)–(C) available from stacks.iop.org/PhysBio/10/016004/mmedia). This calculation confirms both results of Weichsel and Schwarz (2010) and the prediction by Atilgan et al (2005). But when the branching zone is increased to even 4 actin subunit lengths (∼10.8 nm), the network only displays (−70/0/70) as the stable orientation pattern (figure S2(D) available from stacks.iop.org/PhysBio/10/016004/mmedia). In fact, when we artificially start the simulation with the orientation of actin filaments as (−35/35), it quickly transforms and converges to the orientation pattern of (−70/0/70) (figure S3 available from stacks.iop.org/PhysBio/10/016004/mmedia). Without the extremely limited branching zone from the load surface, the (−70/0/70) orientation pattern thus appears to be the only stable pattern.

Importantly, figures 5 and figures S2 and S3 (available from stacks.iop.org/PhysBio/10/016004/mmedia) suggest that although limiting the width of the branching zone could alter the orientation pattern of the actin network, it did not significantly affect the qualitative behavior of the force–velocity relationship. This result leads us to the conclusion that, instead of orientation pattern, the most important factor in defining the force–velocity relationship is the number of filaments at the leading edge. Our conclusion is therefore in contrast to that by Weichsel and Schwarz (2010), and can be experimentally tested.

The balance between capping and branching events dictates actin network remodeling

Our results suggest that the actin network remolds itself by changing the number of filaments in contact with the leading edge. It is such remodeling that determines the shape of the force–velocity relationship. As figure 2 shows, the balance between the branching and capping rates controls the nature of the remodeling. To obtain a systematic and quantitative understanding of the rate dependence of the actin force–velocity relationship, we carried out a phase diagram study for the capping and branching rates (figure 6). We used the force at which the velocity drops by 50% ($f_{1/2}$) as a measure of the length of the force-insensitive region in the force–velocity curves. Based on the force–velocity relationships figure 2(B), $f_{1/2}$ can thus serve as a proxy for how concave the curve is: the more concave, the larger the $f_{1/2}$ is. Figure 6 shows the calculated $f_{1/2}$ values at each parameter value that constitutes our principle prediction:
Figure 6. The calculated phase diagram on the dependence of force–velocity relationships of branching actin network on branching rate and capping rate. For each pair of branching rate and capping rate, we quantitatively calculated the $f_{1/2}$ from the force–velocity curves, averaged over 20 simulations. The color bar represents the calculated $f_{1/2}$ for a variety of branching and capping rates. The cases with concave force–velocity curves are labeled with white stars. Decreasing the capping rate and increasing the branching rate serve to generate more filaments, which shift $f_{1/2}$ rightward, meaning a more concave curve.

smaller branching rates lead to smaller $f_{1/2}$ and more convex like force–velocity curves; smaller capping rates result in larger $f_{1/2}$, more concave like curves and, hence, more adaptive load response. It is known that, in contrast to the in vitro condition where its lifetime is $\sim$30 min (Schafer et al 1996), the lifetime of the capping proteins bound to F-actin barbed ends is $\sim$1 s during cell migration (Miyoshi et al 2006). The much shorter lifetime of capping proteins attenuates the potency of capping events, which confers a more adaptive load response in accordance to the model prediction. Although the model focuses on the in vitro scenario, this prediction does open up a window for understanding the regulation of capping proteins in cell motility, highlighting the biochemical control over the mechanical response of branching actin network both in vivo and in vitro.

Discussion

We have proposed a simple mechanism where branching actin networks remodel against a load force. The model shows that the initial response of branching actin networks to loading always gives a convex force–velocity relationship (figure 1). On longer time scales, smaller capping rates and larger branching rates generate more concave force–velocity relationships (figures 2 and 6).

A number of recent theoretical studies have focused on how to explain both the convex and concave force–velocity relationships for branching actin networks (Weichsel and Schwarz 2010, Schreiber et al 2010). Likewise, the nature of hysteresis effects observed in experiment (Parekh et al 2005) remains a subject of inquiry. Multiple attempts have been made to explain the stall force of individual actin filaments, but the stall force of a network of cooperating actin filaments is poorly understood Mogilner (2009). Our simulations yielded a stall force of approximately 2–3 pN/filament (see supplementary data available from stacks.iop.org/PhysBio/10/016004/mmedia), which is in close agreement with the reported value of $1.7 \pm 0.8$ pN/filament (Heinemann et al 2011). Our reported stall force per filament provides evidence that actin networks use close to optimal force sharing.

Branching governs the remodeling capacity of branching actin network upon load force

The importance of the number of growing filaments at the boundary determining how the network responds to load force has previously been suggested for branching actin networks (Parekh et al 2005, Carlsson 2001, 2003), and for bundled filaments such as actin (Tsekouras et al 2011) and microtubules (van Doorn et al 2000, Mogilner and Oster 1999). In the context of our model, bundled filaments (actin or microtubule) qualitatively correspond to the case of a very low branching rate and/or a high capping rate. According to our calculation, although the number of contacting filament in this case increases with the load force, the reinforcement is limited (the two lower curves in figure 2(C)). Consequently, the resulting force–velocity curve is always convex, consistent with the previous findings (Tsekouras et al 2011, Mogilner and Oster 1999, van Doorn et al 2000). Our model therefore suggests that a branching event is essential in yielding a concave force–velocity relationship of actin network growth against load (figure 6).

Capping provides the necessary negative feedback to account for both concave and convex force–velocity relationships

It should be noted that a similar conclusion was also reached by the Carlsson (2003) model. Carlsson suggested that actin networks with autocatalytic branching would continually increase the number of filaments at the boundary leading to force-independent velocities. Although branching events are autocatalytic as demonstrated in experiments (Goley and Welch 2006), the Carlsson (2003) model by itself can only account for the force-insensitive region of force–velocity curves; an additional negative feedback would be necessary to limit the increase in branch density predicted by the model to produce the inevitable reduction in velocity at high forces. Moreover, the Carlsson (2003) model predicts a transient hysteresis effect that does not correctly reproduce the sustained hysteresis observed in experiments (Parekh et al 2005).

In contrast to Carlsson’s model, our model has a built-in negative feedback mechanism. The capping rate is a first-order reaction of the number of free filament barbed ends while the branching rate is constant, independent of the number of filaments. As the number of contacting filaments increases with the load force, so does the capping rate. That effect limits the total number of contacting filaments. As such, our model explains both the concave and the convex force–velocity curves without resorting to an additional mechanism (figure 2).
our model can explain the sustained hysteresis in the force–velocity relationship (figure 4(C)) (Parekh et al 2005).

Our model does not preclude any other negative feedback mechanism limiting the density of actin filaments. It is likely that increased filament density would lead to excluded-volume effects at large forces (Schreiber et al 2010). Any external negative feedback mechanism would limit the length of the force-sensitive region of the force–velocity curve. It is important to note that, due to the exponential term in equation (1), even a relatively small change in filament density would lead to a large change in velocity. A doubling of the number of filaments, \(N\), would lead to an approximately \(\exp\left(\frac{F}{k_BT}\right)\)-fold increase in the velocity. A surprising feature we observed in our simulation was a substantial reduction in network velocity to extremely small forces (<200 pN, see supplementary figure S4 available from stacks.iop.org/PhysBio/10/016004/mmedia). This reproduces the effect seen in Prass et al (2006), which cannot be reproduced by Schreiber et al (2010). We hypothesize that network velocity has to be reduced by a sufficient amount before the reserved filaments are able to catch up to the leading edge. When the leading edge is moving close to the growth rate of individual filaments, the reserved filaments are unable to catch up anyway. When the leading edge is sufficiently slowed by the opposing force, trailing filaments are able to reach the leading edge and the leading edge velocity stabilizes.

**Relative rates of loading and intrinsic kinetics of actin biochemistry dictate the force–velocity relationship**

The majority of our simulations were performed with constant force. However, our model is relevant to both constant and non-constant force because it requires no equilibrium assumptions. Our model showed a relaxation time (tens of seconds to minutes) before the network reaches equilibrium velocity. Changing the force more slowly than this relaxation time would allow the network to continuously adapt to the increased forces and show strong hysteresis effects. Increasing the force substantially faster than the relaxation time would not allow the network to restructure itself leading to constant force type results; such phenomena are captured by the convex force–velocity relationship predicted by our model (see figure 1(B)). An experimentally observed value for this relaxation time could be found, and experiments changing the force pressing against an actin network more slowly than the observed relaxation time could test our model prediction.

Although our predicted rate-dependent force–velocity relationship of branching actin networks holds the similar concept of viscoelasticity, our model is unique in two folds. First, the viscoelasticity of actin network typically refers to an actin network crosslinked by actin binding proteins, wherein it is the mechanosensitive binding/unbinding events of these crosslinkers that give rise to the rate-dependence of mechanical response (Strickler et al 2010). Here, our model suggests that even without these actin crosslinkers, branching and capping events alone can yield a rate-dependent mechanical response. Second, in contrast to lumping the detailed biochemistry into mechanics, our model establishes and identifies the biochemical control (i.e. branching and capping relative to growth) that is responsible for the overall mechanical response.

Figure 2 should be tested experimentally. The relevant biochemical quantities could be manipulated in the vein of Cameron et al (2004). Such an experiment could be performed both with constant force (Marcy et al 2004) and with a constantly increasing force (Parekh et al 2005). We further note that the number of active actin nucleation factors (such as WASP) at the load surface can be negatively regulated by the number of free barbed ends at the leading edge (Akin and Mullins 2008). This additional negative feedback mechanism could limit the effect of increasing the Arp2/3 concentration. Interestingly, capping proteins could increase the growth rate of branching actin networks by promoting more frequent filament nucleation by Arp2/3, funneling actin monomers to the uncapped barbed ends of actin filaments, without affecting the free filament elongation rate (Akin and Mullins 2008). These in vitro studies point to a more intertwined interaction between branching and capping events of actin networks, which will be the future extension of our current model.

The force–velocity relationships have also been explored experimentally with migrating cells in vivo (Prass et al 2006, Heinemann et al 2011, Bohnet et al 2006). Focal adhesions serve as the ‘feet’ of the cell that mediate cell migration (Gardel et al 2010). While actin polymerizes pushing the cell membrane forward, the resilience of cell membrane converts part of the actin polymerization into the retrograde flow of actin network. The retrograde flux engages with and, hence, mechanically stretches focal adhesions (Gardel et al 2010). Due to the mechanosensation of several focal adhesion proteins, such mechanical stretching will further activate protein tyrosine kinases (Sawada et al 2006), which are known to control WASP–Arp2/3–actin polymerization pathways (Serrels et al 2007), and in turn could impact the branching and capping rates. Therefore, to extend our model results to the in vivo case, one needs to consider the time-dependent branching and capping rates, instead of the constants. These time-dependent branching and capping rates will complicate the resulting force–velocity relationship in both short and long timescales (figures 1 and 2).

**Stress stiffening and softening could stem from the intrinsic properties of branching actin network itself**

The more filaments grow against the load, the stronger they will resist against the compression, so will their elastic moduli increase. Our model predicts that there exists a peak in the number of contacting filaments as a function of load force (figure 2(C)). We suggest that, even without the peculiar catch-bond nature of crosslinkers (Stricker et al 2010), the branching actin network by itself is capable of exhibiting the stress stiffening and subsequent stress softening observed in Chaudhuri et al (2007). According to our model, stress stiffening and softening reflect the increase and the decrease in the number of contacting filaments, respectively. Our model predicts that stress softening does not correspond to a typical mechanical collapse of the actin network; as the number of contacting filaments in the stress-softening region reduces to a
value that is still higher than its beginning value, the resulting elastic moduli of the actin network in the stress-softening region are larger than the original value. This model prediction is unique, and importantly, consistent with the experiment observation (Chaudhuri et al. 2007).

Barbed-end branching results in more convex-like force–velocity relationships

From a experimental point-of-view, one cannot strictly rule out the possibility of branching at barbed-ends (Pantaloni et al. 2000), although the side branching mechanism has been clearly demonstrated (Amann and Pollard 2001), in which Arp2/3 complex nucleates filament branches directly from the sides of the pre-existing filaments. So far, our model mainly focuses on the scenario of side branching. As experiments show that the Arp2/3 complex itself constitutes the first subunit of the daughter branch (Rouiller et al. 2008), the branching point should be at least one subunit length away from the leading edge when the first actin subunit adds to the bound Arp2/3 complex. Therefore, in the side branching scenario, branching is not allowed at the leading edge in the model. Our simulations suggest that the width of the branching zone does not qualitatively affect the force–velocity relationship of branching actin network (figure 5).

The lingering question remains: what happens if branching occurs at the barbed-ends of actin filaments? How will branching at the barbed-ends impact the force–velocity relationship? We took the advantage of our model and explored this question. In our model, the essence of branching at the barbed-ends is to allow the branching events directly at the leading edge. Our simulations suggest that such model alternation turns all the force–velocity curves to be convex-like (figure S5 available from stacks.iop.org/PhysBio/10/016004/mmedia). We reason that, the population of ‘reserved filaments’ will reduce if branching occurs at the barbed-ends at the leading edge. Hence, there will not be much less reinforcement of the growth against load as compared to the case of side branching. Consequently, the resulting force–velocity relationships will be more convex.

We further suggest that not only branching rates are essential to account for the force–velocity relationships, but the manner by which the filaments branch also dictates the mechanical response of branching actin network. Conversely, our model could serve as the mechanistic basis to further distinguish the different branching mechanisms: side branching verses barbed-end branching (Pantaloni et al. 2000, Amann and Pollard 2001).

Effectiveness of loading force mechanism

Our model implements a load-sharing mechanism where the contacting filaments collectively share the load across the leading edge. That is, the addition of a new actin monomer is only opposed by a fraction of the total load force pressing on the network. In the context of our model, the load-sharing mechanism is valid as long as Brownian ratchet mechanism holds up. The Brownian ratchet mechanism assumes that the thermal fluctuations between the filament tip and the load surface are significantly faster than the addition of new actin monomers (Mogilner and Oster 1996, 2003, Peskin et al. 1993). Fluctuations must be large enough for a new monomer to fit in the gap between the tip and the load. Smaller and or slower fluctuations would then reduce the efficiency of the mechanism. Experiments have indeed demonstrated that reducing thermal fluctuations by lowering the temperature strongly hinders the efficiency of filament growth (Shaevitz and Fletcher 2007). Thus, it is the thermal fluctuations that buffer between the contacting filament tips and the load surface, providing the flexible interface to accommodate insertions of actin monomers. The separation of time scales also implies that the load force felt by each contacting filament is an average over many fluctuations. Consequently, only the partial load force shared across filaments dictates the network growth rate.

Our results reveal that the actin network growth will reinforce itself against the load after response time (the time it takes for the growth velocity to bounce back, see figure 1). Such reinforcement underscores the basis of the concave force–velocity relationship and, hence, the adaptive response (figures 2 and 3). We further explored how the efficiency of load-sharing mechanism affects such adaptive response (see supplementary data, figure S6 available from stacks.iop.org/PhysBio/10/016004/mmedia). For a large load, our simulation results show that it takes longer and longer time for the filament growth to reinforce against the load as the load sharing efficiency decreases. For instance, whereas it takes ∼10 min for the velocity of actin network to bounce back in the optimal load sharing case (100% efficiency), it takes ∼2 h when the load-sharing efficiency drops to 70%. On the other hand, for a small load, there is no difference in terms of the response time even as the load sharing efficiency drops down to 5%. These results lead us to the conclusion that higher load sharing efficiency will help the reinforcement of actin network growth against large load forces.

The role of the actin filament elasticity in force–velocity relationship

In the model, the actin network was assumed to be a rigid structure that does not buckle nor break down. In reality, there will be many cross-linker proteins that stiffen the actin network. In addition, capping events in our model limited filaments to an average length of less than 1 μm, significantly less than the persistence length of a single actin filament, ∼17 μm (Ott et al. 1993). Consequently, each individual actin filament can be viewed as a rigid rod. The actin filaments in our case are highly branched, which is believed to be much more rigid than its unbranched counterpart. Furthermore, actin filament itself could undergo bending and, subsequently, stiffening under large compression (∼5–10 pN per filament) (Greene et al. 2009). In our simulation, even though the average stalling force per actin filament is ∼1–2 pN, less than that required for inducing filament stiffening, the above study does provide additional layer of adaptive responses especially in the large load region. Moreover, the bending of an actin filament could also direct the biased branching event of the
network in the direction of the larger load (Risca et al 2012). While our model does not describe the bending of individual filament, the biased branching event could be reflected in our model by the enhanced branching rate as the load force increases. According to our model (figure 6), such load-dependent branching rate will provide further reinforcement of actin network growth against load and, hence, more concave force–velocity relationship. In the future, we will further investigate the details of actin elasticity effect on its force–velocity relationship.

Conclusion

The simple physical model shown here gives insight into the emergent behavior of branching actin network remodeling in the presence of a load. In particular, the network velocity dependence upon the number of filaments growing against the leading edge provides a simple mechanical mechanism to explain a number of experimental effects. The ability of actin networks to remodel is controlled by the balance between branching and capping rates. This mechanism can account for both the observed convex and concave force–velocity relationships. Further investigation into actin network properties, both physical and biochemical, that determine how many growing filaments a network is able to recruit to the leading edge will deepen our understanding of actin-based motility.

Acknowledgments

We would like to acknowledge Dr Alex Sodt for his contribution to the code used for the simulations and Andrea Lively for her helpful comments improving the manuscript. We thank Drs Weichsel and Schwarz for sharing their simulation code with us. We also would like to thank Drs Ed Korn, Clare Waterman, and John Hammer III for critical readings and suggestions and Dr Alex Mogilner for his comments. This work is supported by the Intramural Research Program of NHLBI at NIH.

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