Emergence and maintenance of different sized actin filaments in a common pool of building blocks

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ABSTRACT Actin is one of the key structural components of the eukaryotic cytoskeleton that regulates cellular architecture and mechanical properties. Dynamic regulation of actin filament length and organization is essential for the control of many physiological processes including cell adhesion, motility and division. While previous studies have mostly focused on the mechanisms controlling the mean length of individual actin filaments, it remains poorly understood how distinct actin filament populations in cells maintain different size using the same set of molecular building blocks. Here we develop a theoretical model for the length regulation of multiple actin filaments by nucleation and growth rate modulation by actin binding proteins in a limiting pool of monomers. We first show that spontaneous nucleation of actin filaments naturally leads to heterogeneities in filament length distribution. We then investigate the effects of filament growth inhibition by capping proteins and growth promotion by formin proteins on filament length distribution. We find that filament length heterogeneity can be increased by growth inhibition, whereas growth promoters do not significantly affect length heterogeneities. Interestingly, a competition between filament growth inhibitors and growth promoters can give rise to bimodal filament length distribution as well as a highly heterogeneous length distribution with large statistical dispersion. We quantitatively predict how heterogeneity in actin filament length can be modulated by tuning F-actin nucleation and growth rates in order to create distinct filament subpopulations with different lengths.

SIGNIFICANCE Actin filaments organize into different functional network architectures within eukaryotic cells. To maintain different actin network architectures, actin filaments of different lengths are often required. How cells regulate the length heterogeneity in actin filaments is not well understood. Here we show that the modulation of filament growth rates and nucleation rate by binding partners like formin and capping proteins can regulate the emergent length heterogeneity in the filament population and can create distinct sub-populations with different lengths. Our study sheds light into how actin binding proteins can control various aspects of filament size distribution by tuning their concentration or expression levels in the cell.

INTRODUCTION

In eukaryotic cells, actin filament growth and turnover are tightly regulated for coordinating a diverse set of physiological processes including cell motility (1–3), protrusion formation (4–6), endocytosis (7), wound healing (8, 9), synaptic activity (10), cytokinesis (11, 12), and embryonic development (13–16). These different functions often require distinct subpopulations of actin filaments organized into different lengths and architectures (17). Study of size control mechanisms of intracellular structures has been an active area of research. Traditionally, these studies mostly focus on understanding the physical principle and molecular mechanism that give rise to structures of a typical size (18–23), with a relatively narrow size distribution. The origin of size heterogeneity and the mechanisms to create and maintain a heterogeneous population is not well understood. Here we study mechanisms of controlling heterogeneity in length of a population of actin filaments.

The molecular processes underlying actin filament growth have been extensively studied both theoretically and experimentally. Existing models for actin filament growth can be categorized into two main classes according to the availability of monomers (24): growth in an open system where the monomer concentration remains unchanged in time, and growth in a closed system where free monomer concentration decreases as filaments increase in size (keeping the total amount of monomers conserved). Size control mechanisms have been proposed for actin filaments in both the above cases to understand how filaments of a typical size can be achieved (22, 25–27). But, how filaments of different sizes can be obtained is much less understood (28). This is relevant for a cell where actin filaments are found in a wide diversity of lengths. In this study we consider actin filament growth in a closed system, where the monomer pool is limited (29). Though it is not well established if *in vivo* actin systems can be considered to be assembled from a limiting monomer pool, recent studies show that different actin structures in a cell often compete for the same limiting pool of monomeric actin (30, 31). In this study, we use theory and simulations to demonstrate how actin filament length heterogeneity can

be regulated and we demonstrate how distinct actin filament subpopulations may emerge in a limiting monomer pool.

Previous studies have shown that nucleation and polymerization of actin filaments in a limiting monomer pool results in an exponential length distribution at steady-state (24, 32–34), with the standard deviations in length fluctuations as large as the mean. However, the timescale of reaching such a steady-state can be extremely long (order of days) (26, 33, 34) in physiological conditions (e.g., cell volume $\sim 1000\,\mu m^3$ and total monomer concentration $20-100\,\mu M$), making the steady-state size distribution irrelevant for much of the physiologically relevant processes that occur within a timescale of minutes to hours. It has been previously shown that the dynamics of actin length distribution can be segregated into a fast regime of nucleation and growth, and a slow regime of size rearrangement between actin filaments via exchanging monomers (33). In the initial regime, the length distribution dynamics has a convective nature (mean of the distribution increases), while in the later regime the filament lengths undergo a diffusive dynamics (variance increases keeping mean constant) (33) (Supplementary Fig. 1). We are, however, interested in actin length distribution during an intermediate regime where the number of filaments and the mean filament length has reached a steady state. This intermediate regime is achieved within minutes and the resulting length distribution remains approximately invariant over a timescales of hours, making it physiologically relevant. In this regime, the growth of the actin filament population can be regulated by both spontaneous filament nucleation and actin binding proteins.

Here we show that spontaneous nucleation of actin filaments and interactions with specific actin binding proteins (ABPs) can induce significant heterogeneity in actin filament size, persisting for several hours. We specifically study the effects of growth promoting ABPs like formin and growth inhibiting ABPs like capping proteins on the regulation of actin filament length distribution. We show that formin and capping protein concentrations can be tuned to regulate the heterogeneity in actin filament size in the filament population. In particular, we find that the concentrations of formin and capping proteins can be tuned to give rise to a filament population with bimodal size distribution with two distinct subpopulations of long and short actin filaments.

SPONTANEOUS NUCLEATION PROMOTES F-ACTIN LENGTH HETEROGENEITY

We first study the length distribution of actin filaments emerging from spontaneous nucleation in a limiting monomer pool, where the filament number and size both evolve in time. Spontaneous nucleation of actin filaments involves sequential formation of polymerization intermediates, actin dimers and trimers, with the trimers acting as the seed for nucleation (35–37) (Fig. 1a). We consider the growth of filamentous actin (F-actin) via association and dissociation of globular actin (G-actin) from the barbed end (Fig. 1a), with the association and dissociation rates as given in Table 1. For simplicity, we do not consider the nucleotide state of the monomers in the filament and assume that free monomers in the pool are ATP-bound. The dimers and the trimers have a high dissociation rate, making spontaneous nucleation inefficient for nucleating actin filaments. In addition, the actin binding protein profilin is known to suppress spontaneous actin nucleation *in vivo*. However, given that profilin concentration is typically less than half of actin in cells (38), and that formin concentration is relatively low (~1 μM), spontaneous filament assembly may play a significant role. The growth dynamics for the ith filament (i.e., for size > 3 monomers) is described by the following chemical master equation

Table 1: Growth rate values

Growth rate constant	Value	Reference
$K^+_{(2)}$	$35.7\,\mu M^{-1}\,s^{-1}$	(37)
$K^-_{(2)}$	$1.63\times10^8\,s^{-1}$	(37)
$K^+_{(3)}$	$2.18\,\mu M^{-1}\,s^{-1}$	(37)
$K^-_{(3)}$	$1.3\times10^3\,s^{-1}$	(37)
K^+	$11.6\,\mu M^{-1}\,s^{-1}$	(39)
K^-	$1.4\,s^{-1}$	(39)
K^+_C	$12.8\,\mu M^{-1}\,s^{-1}$	(40)
K^-_C	$2\times10^{-4}\,s^{-1}$	(40)
K^+_F	$29.1\,\mu M^{-1}\,s^{-1}$	(40)
K^-_F	$8.1\times10^{-5}\,s^{-1}$	(40)
K^+_{CF}	$1.6\,\mu M^{-1}\,s^{-1}$	(40)
K^-_{CF}	$6.2\times10^{-3}\,s^{-1}$	(40)
$K^-_{CF(2)}$	$6.2\times10^{-3}\,s^{-1}$	(40)
K^+_{FC}	$0.21\,\mu M^{-1}\,s^{-1}$	(40)
K^-_{FC}	$1.8\times10^{-3}\,s^{-1}$	(40)
$K^-_{FC(2)}$	$4.2\times10^{-3}\,s^{-1}$	(40)

Table 2: Parameter values

Figure	Values
Fig. 1b	$V = 10\,\mu m^3$, $n_f = 4$, $\rho_0 = 1\,\mu M$
Fig. 1c-f	$V = 200\,\mu m^3$, $n_f = 153$, $\rho_0 = 10\,\mu M$
Fig. 2-Fig. 4	$V = 200\,\mu m^3$, $\rho_0 = 10\,\mu M$, $\alpha = 5$

$$\frac{dP^i_n}{dt} = K^+\rho_{\text{av}}P^i_{n-1} + K^-P^i_{n+1} - (K^+\rho_{av} + K^-)P^i_n\,, \qquad (1)$$

where P^i_n is the probability of the ith filament having a size n (in monomer units), K^+ (K^-) is the bare assembly (disassembly) rate, and ρ_{av} is the instantaneous monomer density. The instantaneous monomer density is given by $\rho_{\text{av}} = \rho_0 - \frac{1}{V}\sum_{i=1}^{n_f} n_i$ where ρ_0 is the total actin concentration, V is the system volume, n_i is the size of the ith filament (in monomer numbers), and n_f is the number of actin filaments. The growth of all the filaments can be described by a set of such master equations written according to the polymerization kinetics defined in Fig. 1a. We simulate the stochastic filament assembly dynam-

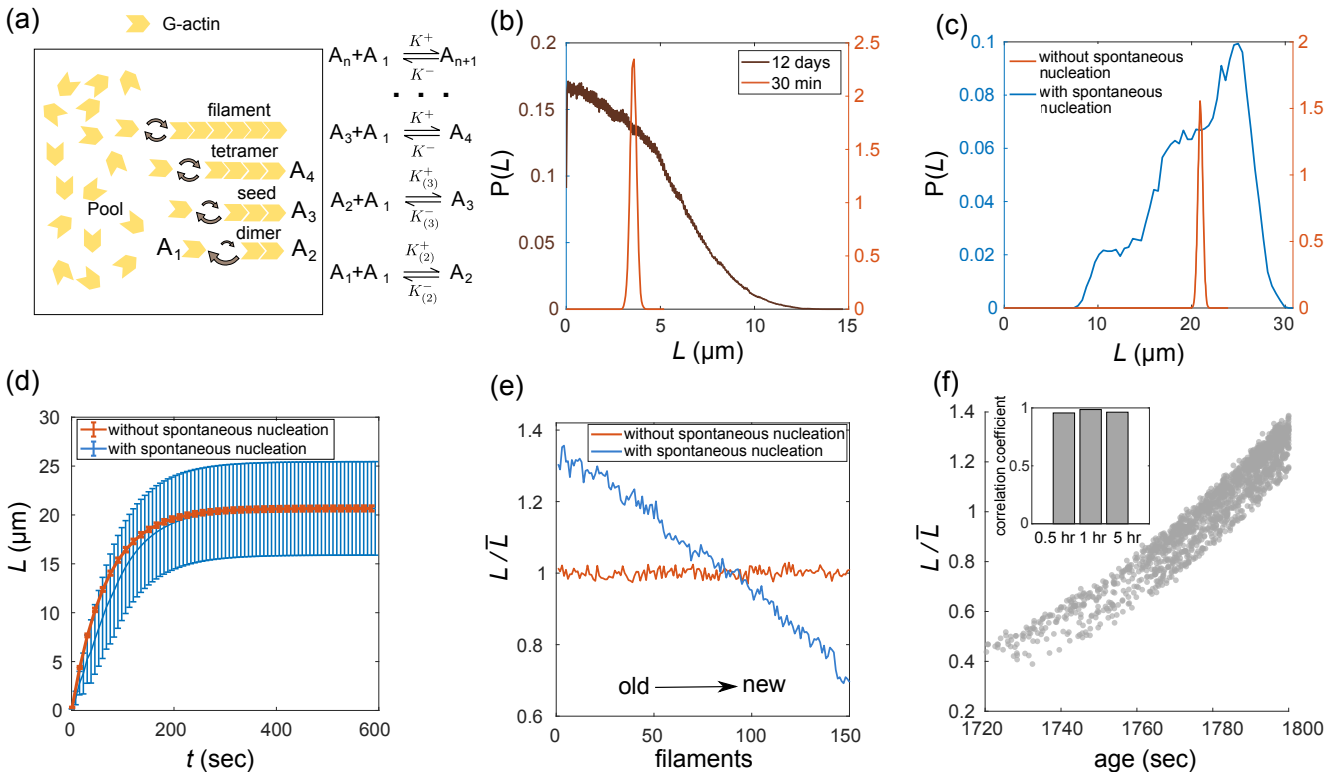

Figure 1: **Spontaneous nucleation promotes heterogeneity in actin filament lengths.** (a) Schematic diagram of spontaneous nucleation and growth of F-actin in a limiting G-actin pool. Here $A_1, A_2, \ldots, A_n$ stand for actin monomer, dimer and n-mer filaments, respectively. The assembly and disassembly rates are defined by the accompanying reaction diagrams. (b) Size distribution of actin filaments growing in a limiting monomer pool without spontaneous nucleation, showing a narrow size distribution at t = 30 min (red), and exponential-like distribution at t = 12 days. (c) The size distribution in the presence of spontaneous nucleation (blue) leads to a significantly broader size distribution as compared to growth of fixed number of F-actin from a limiting monomer pool (red). The broader size distribution originates from the underlying heterogeneity in filament size. (d) Time evolution of the mean filament size, with (blue) and without (red) spontaneous nucleation. (e) Size distribution of filaments, ordered according to their age with '0' being the oldest, i.e. the first filament nucleated. Size is expressed as length (L) divided by mean filament length ($\bar{L}$). There is negligible size heterogeneity in filament population for growth without spontaneous nucleation (blue) as compared filaments growing with spontaneous nucleation (red). (f) Filament length and age are highly correlated in presence of spontaneous nucleation. (inset) The emergent correlation is long-lived and remains almost unaltered for hours. We have used 100 ensembles to produce the size distribution and related statistical quantities. For additional parameter values see Table 1 and Table 2.

ics using the Gillespie's first algorithm (see Supplementary Sec. I and II). The dimerization dynamics is modeled as a deterministic process to avoid large computational cost (see Supplementary Sec. I). To this end, we consider a representative system with total actin concentration 10 μM and volume 200 μm^3. With these parameters, the nucleation process ends within a few minutes and the mean filament size reaches equilibrium in 10-15 min. Over longer timescales, the filaments can only exchange monomers with other filaments via disassembly and assembly, such that the filament population dwells in a long-lived dynamic state.

In the absence of spontaneous nucleation, when there is a fixed number of filaments simultaneously growing from a limiting monomer pool, the resulting length distribution is narrow with a very small coefficient of variation that is maintained over a timescale of 30 min to few hours (Fig. 1b). This early-time length distribution then evolves to become broader, keeping the mean size constant. The distribution eventually becomes exponential over a very long timescale of several days (Fig. 1b). Thus having a large enough G-actin pool can be sufficient to achieve a well regulated size for multiple filaments growing from a shared subunit pool, which is maintained over physiologically relevant timescales of tens of minutes to a few hours. By contrast, the length distribution is significantly broader when the filaments nucleate via spontaneous formation of trimer seeds (Fig. 1c), with all other conditions being the same. The mean length is the same in both cases (Fig. 1d) as it is a property of the pool size and

growth rates. This broadness of length distribution originates from the heterogeneity in size of the filament population (Fig. 1e) and not from temporal fluctuations in individual filament size (see Supplementary Fig. 2).

Emergence of filament length heterogeneity can be understood from the interplay between sequential nucleation of filaments and their subsequent growth. After nucleation, filaments start growing with a growth rate proportional to the instantaneous monomer pool density and all filaments keep growing until the monomer pool density has reached the critical value $K^-/K^+ \simeq 0.12\,\mu$M. Thus the filaments that nucleated earlier grow to be larger than the filaments that are nucleated later (see Supplementary Movie. 1). Once the monomer density has reached its critical value, the filaments can only change their size by exchanging monomers between each other. This diffusive process of length rearrangement is very slow for a large actin pool. This leads to the long-lived dynamic state that retains the heterogeneity in filament size. This consequently results in filament length being correlated with their age (Fig. 1f), which is retained for a very long time (Fig. 1f, inset). This age-size correlation is noteworthy because it presents a possible regulatory mechanism where ABPs (such as ADF/cofilin) that interact with filaments in an age-dependent manner (41) (e.g., nucleotide phosphorylation state dependent) can exploit this correlation to build a size-dependent regulation of filament growth. The size heterogeneity decreases (increases) with increasing (decreasing) rate of nucleation, as we have shown by changing dimer production rate (see Supplementary Fig. 2). Thus cellular processes that can affect the nucleation rate can regulate the length heterogeneity of the resulting filament population.

F-ACTIN CAPPING INCREASES FILAMENT LENGTH HETEROGENEITY

We next investigate how the length distribution of actin filaments are regulated by actin binding proteins that inhibit filament growth. Capping proteins (CP) act as F-actin growth inhibitors by binding to F-actin barbed ends and blocking the assembly and disassembly of monomers from that end (Fig. 2a) (42, 43). Here we consider a well studied capping protein, CapZ, modeled as coarse-grained moieties that associate F-actin barbed ends at a rate K_C^+ and dissociate at a rate K_C^-. CapZ-bound filaments will stall growth (K^+, $K^- \to 0$) and remain in the same size until the CapZ protein unbinds.

In presence of capping, the mean filament size decreases (Fig. 2b) with increasing capping concentration (ρ_c). The mean size ($\bar{L}$) is approximately inversely proportional to ρ_c (Fig. 2c-inset). The reduction in average length of F-actin with increasing CapZ concentration cannot be explained without considering the effect of capping proteins on filament nucleation. By inhibiting the growth of the nucleated filaments, the capping proteins induce a slower depletion of the monomer pool and promote nucleation of more filaments (Fig. 2c). The filament density increases approximately linearly with increasing capping concentration (Fig. 2c). This increase in filament abundance decreases the mean length of the filament population. In the absence of spontaneous filament nucleation (i.e., growing a fixed number of filaments), capping proteins can only slow down the growth of the existing filaments. This will lead to the same mean filament length and cannot explain a permanent decrease in average length (Supplementary Fig. 3).

The filament length distribution loses the unimodal nature in the presence of capping proteins by becoming exponential (Fig. 2d), with the coefficient of variation in length approaching 1 (Fig. 2e). This heterogeneity in length does not arise from exchange of monomers between filaments. Rather, the capping proteins slow down the filament rearrangement dynamics in the diffusive growth regime even further (Supplementary Fig. 3). The exponential length distribution arises from the interplay between the initial growth of filaments and the dynamics of capping protein binding to the newly created filaments. In the initial period of growth, the filament length grows almost linearly and the waiting time for capping binding to occur has an exponential distribution (as the binding reaction is a Poisson process, independent of filament length). Thus at high enough capping concentration, the filaments captured by the capping proteins will acquire an exponential length distribution. The capping unbinding rate being slow, this nucleation-growth-capture process will deplete the monomer pool and give rise to a long-lived state with large size heterogeneity and an exponential distribution (Supplementary Movie. 2). The process of nucleation-growth-capture does not preserve the information of filament age in their length (Fig. 2f). The capping proteins bind to all free barbed ends with equal probability regardless of the filament age. Hence the difference in filament length emerging previously from the difference in their age, cannot be retained in the presence of capping. The correlation of size with age progressively diminish with increasing capping concentration (Fig. 2f).

In summary, we show here that growth inhibition by capping enhances heterogeneity in size and we do not see the long-lived state with unimodal size distribution that resulted with spontaneous F-actin nucleation (Fig. 1c). Our results are in good agreement with the experimentally measured dynamics of mean filament length (44, 45), length distribution (45) and filament nucleation (46) in presence of capping proteins.

F-ACTIN GROWTH PROMOTERS MAINTAIN FILAMENT LENGTH HETEROGENEITY

Assembly of actin filaments in cells is enhanced by growth promoting factors such as formins that bind to the barbed end of F-actin, increasing F-actin polymerization rate up to a few folds (47, 48). Formins not only regulate F-actin organization (30) and actomyosin dynamics (49), but also plays important roles in cell motility (50) and cell adhesion (51). While many different types of formins with varied effects on barbed end assembly rate are present in cells, we parametrize our simulations based on mDia1 (40). We model formins

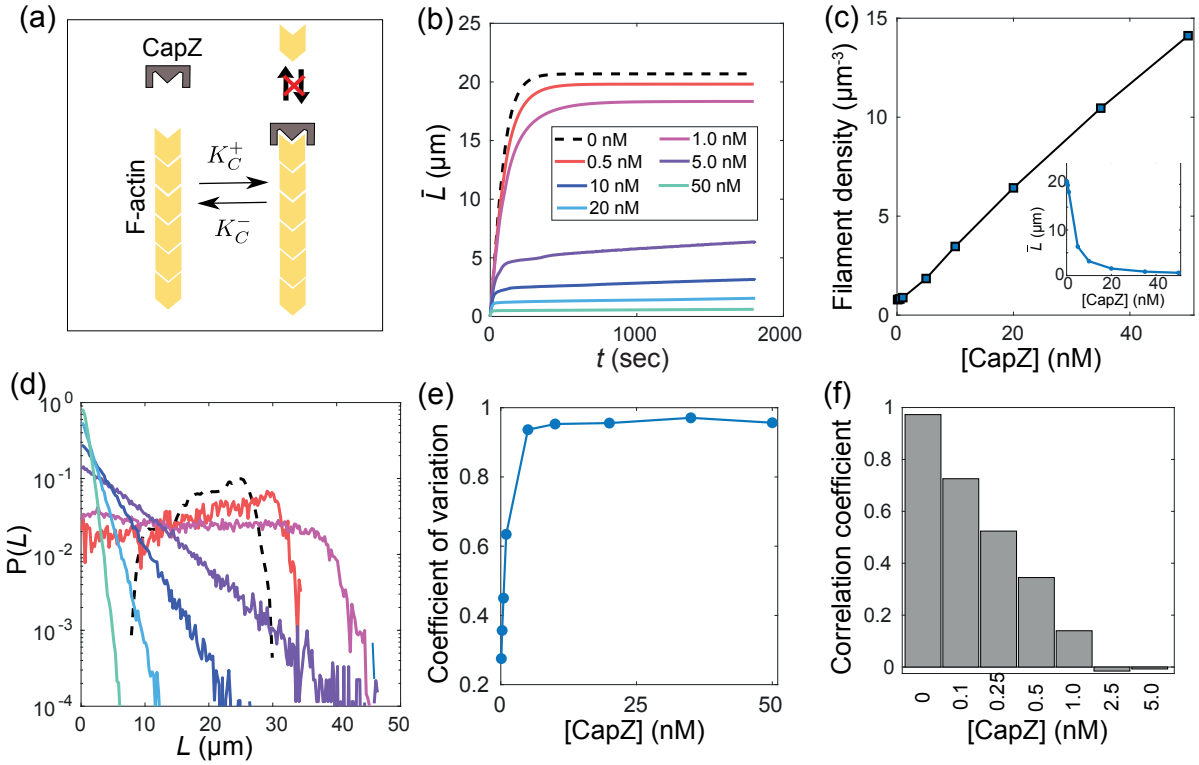

Figure 2: **F-actin growth inhibition and length control by capping proteins.** (a) Schematic diagran showing capping protein (CapZ) binding to F-actin barbed ends. Capping bound filaments do not grow or shrink in size. (b) Average F-actin length vs time for different concentrations of capping proteins. Increasing capping concentration decreases the mean filament size. (c) Filament number density increases with increasing capping concentration as inhibition of F-actin growth leaves excess amount of actin monomers for spontaneous nucleation. (inset) Mean length of F-actin vs capping protein concentration. The mean length being inversely proportional to capping concentration is a consequence of linear increase in filament numbers with capping concentration. (d) F-actin length distribution for different values of capping protein concentration. The size distribution becomes exponential with increasing capping concentration. The color code is same as shown in panel (b). (e) The coefficient of variation (CV) in filament length vs capping concentration, showing that CV increases with increasing capping concentration and saturates close to one as the distribution becomes exponential. (f) The correlation coefficient between filament age and filament length as a function of capping protein concentration. The filament length and age becomes completely uncorrelated in higher capping concentration. We have used 100 ensembles and collected length for the last one minute to produce the size distribution and related quantities. For additional parameter values see Table 1 and Table 2.

as coarse-grained moieties that bind to the barbed end of F-actin at a rate K_F^+ and dissociate at a rate K_F^- (Fig. 3a), with $K_F^- \ll K_F^+$. Formin-bound filaments grow with an enhanced assembly rate αK^+, with $\alpha > 1$ (47, 48). For simplicity, we first neglect the effects of formin-mediated F-actin nucleation. This is a reasonable assumption for some types of formins (e.g., DAAM1, FMNL2 and FMNL3) that have a very low efficiency of nucleating filaments ($\sim 1\%$) and rather promote barbed end elongation at a higher rate (52–54). We discuss the role of formin-mediated nucleation later in this section.

We find that the mean length ($\bar{L}$) of growing actin filaments increase with increasing formin density (Fig. 3b), while eventually saturating at higher values of formin density (Fig. 3c-inset). This saturation of mean length occurs as nucleated filaments remain formin-bound most of the time at high formin density, making the effective actin assembly rate $\sim \alpha K^+$. It is important to note that formins change the critical concentration of actin growth in a concentration-dependent manner (see Supplementary Fig. 4). The enhanced growth of the formin-bound filaments leads to an accelerated depletion of the monomer pool, thereby suppressing spontaneous nucleation. Filament density decreases with increasing formin density, approximately scaling inversely (Fig. 3c).

With increasing formin concentration, filament length distribution shifts to a higher mean but remains unimodal and qualitatively similar to the case without formin (Fig. 3d). At high enough formin density, formins strongly promote growth of the filaments, preserving the unimodality of length distribution without increasing length heterogeneity (Fig. 3d,e and Supplementary Movie. 3). However, the coefficient of

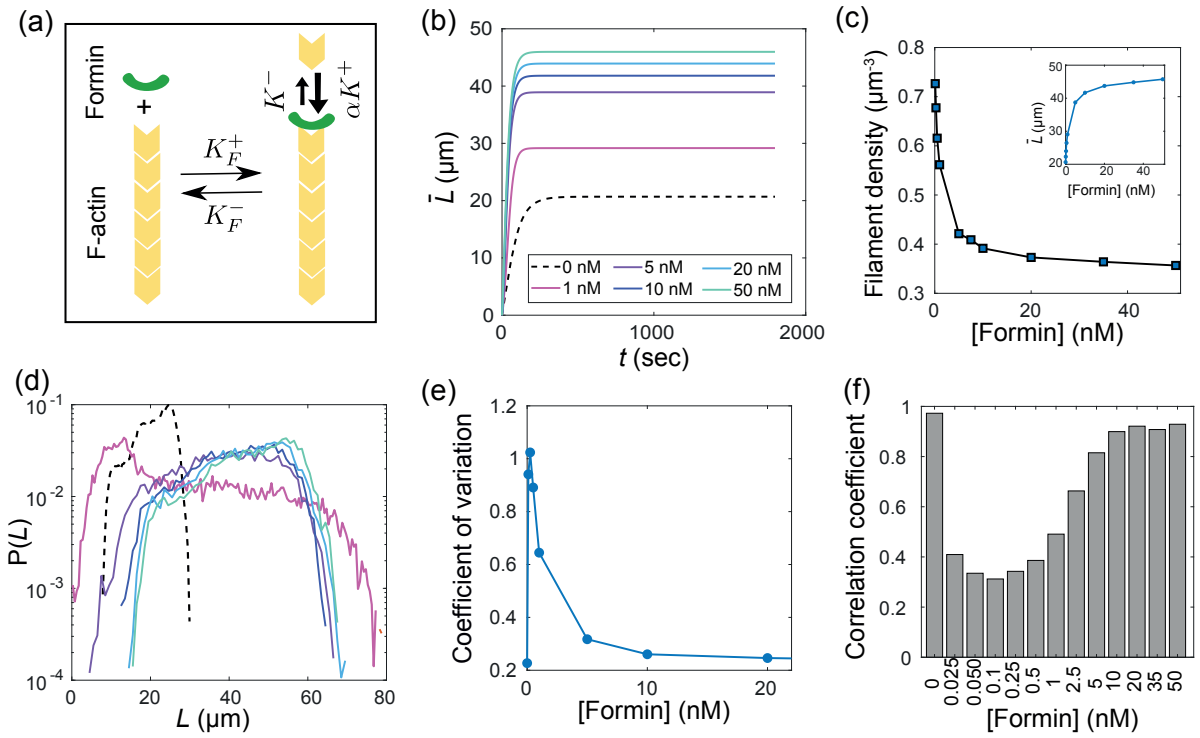

Figure 3: **Role of F-actin growth promoters on length control.** (a) Schematic diagram of formin binding to F-actin barbed ends, leading to an increase in polymerization rate. (b) The mean filament length increases with increasing formin concentration. (c) The filament number density decreases with increasing formin concentration as promotion of filament growth depletes the monomer pool quicker, leaving less monomers for nucleation. (inset) Increasing filament mean length saturates at higher formin density as the filament number density saturates. (d-e) Enhanced polymerization of F-actin by formin retains the unimodality of the filament size distribution but the mean increases. The length heterogeneity is non-monotonic, increasing at lower formin density while decreasing later at higher formin density. The non-monotonic change in coefficient of variation captures the increased heterogeneity in size at small formin concentration. The color code is same as shown in panel-b. (f) The correlation between filament age and length is non-monotonic, with a decrease at an intermediate range of formin concentrations. We have used 100 ensembles and collected length for the last one minute to produce the size distribution and related quantities. For additional parameter values see Table. 1 and Table. 2.

variation in filament length changes non-monotonically with formin density, indicating higher heterogeneity at lower formin concentration (Fig. 3e). This increased heterogeneity in length is caused by the competition between formin-bound and free filaments in absence of enough formins to bind all the filaments that are being nucleated (see Supplementary Fig. 4). This non-monotonicity is also present in the correlation between filament length and age, with a loss of correlation at small formin concentration, while regaining the correlation back at higher concentration of formins (Fig. 3f). The similarity in size-age correlation at high and low formin concentrations arises from the fact that at high formin concentration the actin growth can be effectively represented as growth of only F-actin with an enhanced assembly rate ($\sim \alpha K^+$).

Role of profilin – Formin is known to increase the assembly rate of F-actin in presence of profilin proteins that bind to actin monomers to create a profilin-actin (PA) pool (55). PA selectively takes part in the assembly of formin-bound filaments with higher assembly rate. In the above, we did not explicitly consider profilin dynamics and implicitly assumed that formin-bound filaments incorporate PA to increase their assembly rates. We also assumed that spontaneous nucleation can proceed in presence of profilin. These assumptions are reasonable since actin-profilin ratios in cells are typically larger than 2:1 (38), leaving enough free monomers to spontaneously nucleate actin filaments. To validate our assumptions, we explicitly modeled profilins to study the role of profilin-actin on F-actin length control (Supplementary Material). We found that in the presence of small concentrations of profilin (actin-profilin concentration ratios 5:1 and 2:1), the effect of formin on F-actin length distribution remains qualitatively similar (see Supplementary Fig. 5). In the absence of Formin or capping proteins, profilin reduces F-actin length heterogeneity (Supplementary Fig. 10).

Role of Formin-mediated nucleation – To test the role of formin-mediated nucleation on F-actin length distribution, we explicitly modeled irreversible dimer formation (either two G-actin or one G-actin and one profilin-actin) by formins at a rate K_{FN} (see Supplementary section. *II* for details). At lower formin concentrations and smaller formin-mediated nucleation rate (K_{FN}), we found an increase in mean filament length with a reduced nucleation of filaments. At larger K_{FN} and higher formin concentration, formins may act as potent nucleators by reducing mean filament length by enhancing nucleation (see Supplementary Fig. 6 & 7). These results are in good agreement with the recently reported role of formin in limiting actin filament length in an *in vitro* assay (56). Overall, our results (Fig. 3) predict the effect of growth promotion by formins in the emerging length heterogeneity in filament population. Aside from the case of inefficient nucleators, these results may also be relevant *in vivo* where formin-mediated nucleation often requires other co-factors or nucleation promoting factors. In the absence of such cofactors, formins only play an essential role in barbed end elongation. For mDia1 the co-factor required for nucleation is adenomateous polyposis coli (APC) and without APC, mDia1 hardly nucleates new filaments in presence of profilin (57).

COMPETITION BETWEEN FORMIN AND CAPPING RESULTS IN BIMODAL F-ACTIN LENGTH DISTRIBUTION

In previous sections, we studied how F-actin growth inhibitors and growth promoters maintain filament length heterogeneity and retain unimodility of length distribution. Here we study their combined effect on filament growth and length control. Formin and capping proteins both compete for the F-actin barbed end and this competition was previously thought to be exclusive (58). Recent studies (40, 59) have uncovered the interaction between free formins (F) and capping bound to filaments (BC) and vice-versa. When bound to a barbed end, formin and capping proteins can form a ternary complex, referred to as BFC or BCF depending on the order of the complex formation (Fig. 4a). For instance, capping protein binding to a formin-bound barbed end forms a BFC complex, while formin binding to a capping-bound barbed end forms a BCF complex. These complexes can disassemble in two different ways leaving the barbed end either formin-bound or capping-bound (Fig. 4a). The disassembly rate of this complex is larger than the very small disassembly rate of capping and formin from the barbed end (Table 1). Thus this interaction enables actin filaments to switch from a capping-bound non-growing state to a formin-bound fast growing state.

By varying the amount of formin and capping proteins, we find four distinct types of F-actin length distributions that emerge via the interaction between F-actin, CapZ and formin (Fig. 4b). When formin density is considerably higher than the capping protein density, the resulting length distribution is unimodal (Fig. 4c). Here, the formin proteins outcompete the effect of capping and retain unimodality of size distribution. We find the characteristic exponential length distribution emerging from capping-induced inhibition of F-actin growth, but this effect fades away in the presence of even small amount of formin (Fig. 4b). In the region where capping concentration is much higher than formin we find that the filament population has a high amount of heterogeneity in length and the coefficient of variation is large ($CV > 1$) (Fig. 4b and Fig. 4e). These fat-tailed length distributions often exhibit power-law tails that eventually decay exponentially (Fig. 4e, Supplementary Fig. 8). This broadness in length distribution appears where amount of capping is higher than formin but it is noteworthy that capping alone cannot produce such high amount of heterogeneity in size and the competition between capping and formin plays a role in promoting this large heterogeneity in length.

When formin and capping concentrations are comparable, we see the emergence of a bimodal size distribution, where the filament population has two clearly segregated subpopulations of small and large filaments (Fig. 4b and Fig. 4d). The bimodal size distribution is long-lived, being stable without any significant changes over a timescale of tens of minutes to tens of hours (Fig. 4d). The origin of the bimodality lies in the early time segregation of filaments either by growth promoters in the subpopulation of large filaments or by growth inhibitors in the subpopulation of small filaments (Fig. 4f-g and Supplementary Movie. 4). These segregated subpopulations are stable over a timescale of many hours, as the monomer pool reaches critical concentration when it is in equilibrium with the filaments. After this initial period of growth, formin and capping proteins cannot significantly alter the filament size distribution, as in this long-lived diffusive growth regime a small filament cannot grow fast to become large even if it is formin-bound. In addition to modulating the nature of length distributions, the mean length of the filament population can also be tuned by changing formin and capping concentrations (Fig. 4h). Increase in formin concentration and decrease in capping concentration increases the mean filament size as expected from the results of the previous sections.

As discussed in the previous section, we tested the validity of our assumption of neglecting the role of profilin and formin-mediated nucleation by explicitly modeling these effects. Our results show that the qualitative nature of the filament length phase diagram does not change in the presence of profilin and formin-mediated nucleation, such that we can obtain both bimodal and fat-tailed length distributions (see Supplementary Fig. 5 & 9).

DISCUSSION

Actin filaments organize into many different competing structures in cells which may consist of F-actin of distinct lengths. For example, in the fission yeast, three different actin structures (actin cables, rings and patches) have been observed to compete for a shared monomer pool (30). These differ-

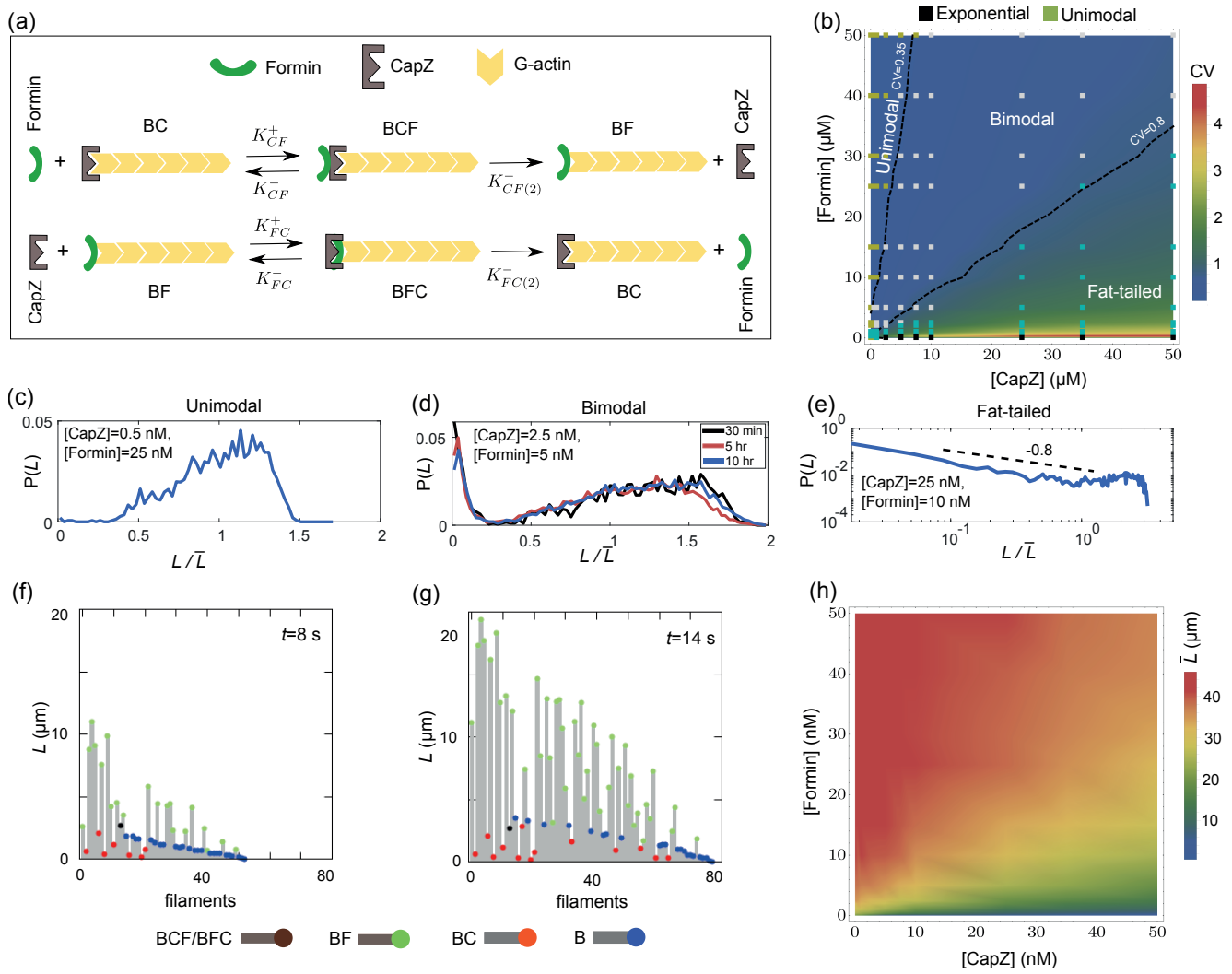

Figure 4: **Emergence of bimodal F-actin length distribution by competition between formin and capping proteins.** (a) Schematic diagram of the interaction between actin, formin and capping proteins. The capping-formin complex may form in two different ways, either by formin binding to a capping-bound barbed end (BC) or vice-versa, with different rates of association and dissociation. (b) A state-diagram showing the different F-actin length distributions resulting at different values of formin and capping protein concentration. Four qualitatively different distributions emerge: Unimodal, exponential, bimodal and fat-tailed distribution. The fat-tailed length distribution has very broad power-law tails and a large heterogeneity in size. (c-e) Representative plots for the three types of length distributions (left to right): (c) unimodal, (d) bimodal and (e) fat-tailed distributions computed at the indicated values of formin and capping protein concentrations. The bimodal length distribution (at [CapZ]=2.5 nM and [Formin]= 5 nM) remains almost unchanged over a long timeperiod spanning minutes to tens of hours. (f-g) Segregation of filaments during early growth period into subpopulations of large and small sized filaments by formin and capping, respectively. Shown here are the filament length distributions at (f) t = 8s and (g) t = 14s. The parameters used are [CapZ]=4 nM and [Formin]= 5 nM. (h) Mean filament length as a function of formin and capping protein concentrations. Increasing formin (capping) concentration increases (decreases) the mean length of the filament population. Additional parameter values are given in Table 1 and Table 2.

ent types of actin structures may require a heterogeneous population of actin filaments to form and function properly. Heterogeneity in actin filament length has been found to play significant roles in determining the morphological (60) and mechanical (45) properties of actin organizations and their dynamics (61). However, it is not well understood how actin filaments regulate heterogeneity in length in order to maintain different actin network organizations that may require long and short filaments. Here we develop a stochastic model of actin filament assembly with spontaneous nucleation of

filaments and interactions with a limiting pool of binding partners. Our results show that spontaneous nucleation can induce significant length heterogeneity in the filament population. This heterogeneity in length is an emergent property of spontaneous nucleation that cannot result from the growth of a fixed number of filaments from specialized nucleators (26, 27). In addition, spontaneous nucleation results in a strong age-size correlation in the filament population.

Filament length heterogeneity and distribution can be controlled by tuning the interaction between F-actin and its growth promoters and inhibitors. We find that strong growth inhibition leads to increased length heterogeneity within physiologically relevant timescales. In this case, length-independent growth inhibition by capping proteins reduces the age-size correlation in the filament population and a complete loss of correlation is seen at higher concentration of capping proteins. Our results agree well with *in vitro* studies reporting exponential length distribution and decreasing mean filament size with increasing capping concentration (45). Promotion of growth by formin reveals a non-monotonic behaviour in filament length distribution with increasing formin concentration. The heterogeneity in length increases for small concentration of formin (i.e., weak promotion of growth) due to a competition between formin-bound and formin-free filaments. This heterogeneity goes away at higher concentration of formins when there are enough formin to bind all the filaments. Thus, a strong promotion of growth does not result in any significant change in length heterogeneity and retains the unimodality of length distribution.

Motivated by recent *in vitro* studies reporting the interactions between formin and capping proteins (40, 59), we study actin filament growth in presence of capping and formin. We find that formin and capping concentrations can be tuned to regulate the heterogeneity in length in the filament population. We find a bimodal length distribution in a regime where the concentration of formin and capping proteins are comparable such that the effect of their competition is enhanced. In this regime, filaments are captured by formin and capping during early growth, leading to the formation of two subpopulations of long and short filaments. Bimodal size distribution may originate from an underlying bistability due to autocatalytic growth of single filaments (27), as observed *in vitro* for microtubules growing in the presence of Kip3 motors (62). However, the bimodal size distribution we report here does not originate from any underlying bistability in the filament growth dynamics and cannot be understood from a single-filament growth description. Here bimodality is an emergent collective effect that emerges from the modulation of filament growth by its binding partners (promoter or inhibitor). This mechanism for maintaining subpopulations of short and long actin filaments may play an important role in assembling distinct actin network organizations in the cortex (63, 64).

We show that explicit modeling of profilin in physiologically relevant abundance does not alter the qualitative results but changes the overall size of the formin-captured subpopulation of filaments (see Supplementary Fig. 5). Taking formin-mediated filament nucleation into account alters the dependence of filament length on formin concentration depending on the formin's nucleation efficiency. But the qualitative nature of the different phases do not change. Interestingly, while formin retains heterogeneity and capping proteins increase it, profilin (without formin or capping) is able to reduce filament length heterogeneity by limiting nucleation (see Supplementary Fig. 10). Due to the large variety in formin proteins and the complex interplay between actin, profilin and formin, the effect of formin on actin filament growth remains poorly understood. A thorough exploration of the different aspects of actin filament growth in the presence of profilin, formin and capping is beyond the scope of the current study and will be pursued in future work.

To the best of our knowledge, we could not find any report of experimentally observed case of bimodality in actin size distribution in presence of formin and capping, as predicted by our theory. The recent studies on capping-formin interaction (40, 59) are done in flow channels where the monomer pool may not be conserved and data on filament length distribution is not reported. Interestingly, Zigmond *et al* (58) found unimodal length distribution in the presence of 0.5 μM actin, 10 nM capping and 200 nM formin, and this is in good agreement with our prediction that the size distribution will be unimodal when formin concentration is much greater than capping (Fig.4b). Two kinetically distinct (different turnover rates) actin filament subpopulations regulated by arp-2/3 and formin was reported in cortical actin network (65, 66) but the average lengths of these subpopulations was found to be independent of the concentration of the regulators in the simulations (65). The formin mediated filaments were reported to be longer and was found to play a greater role in determining mechanical properties of the cortical network in comparison with the shorter filaments (65). This highlights the significant role that filament length heterogeneity may play in determining the mechanical properties of the cytoskeletal network. The regulatory mechanisms of length heterogeneity in actin filaments in cells should be studied further in both theory and experiments to understand how cells can tune their structural and mechanical by controlling the relative levels of actin binding proteins.

AUTHOR CONTRIBUTIONS

DSB and SB designed and developed the study. DSB carried out simulations and analyzed the data. DSB and SB wrote the article.

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REFERENCES

1. Mogilner, A., and G. Oster, 1996. Cell motility driven by actin polymerization. *Biophysical journal* 71:3030–3045.

2. Olson, M. F., and E. Sahai, 2009. The actin cytoskeleton in cancer cell motility. *Clinical & experimental metastasis* 26:273.

3. Cooper, J. A., 1991. The role of actin polymerization in cell motility. *Annual review of physiology* 53:585–605.

4. Martinez-Quiles, N., R. Rohatgi, I. M. Antón, M. Medina, S. P. Saville, H. Miki, H. Yamaguchi, T. Takenawa, J. H. Hartwig, R. S. Geha, et al., 2001. WIP regulates N-WASP-mediated actin polymerization and filopodium formation. *Nature cell biology* 3:484–491.

5. Vasioukhin, V., C. Bauer, M. Yin, and E. Fuchs, 2000. Directed actin polymerization is the driving force for epithelial cell–cell adhesion. *Cell* 100:209–219.

6. Faix, J., and K. Rottner, 2006. The making of filopodia. *Current opinion in cell biology* 18:18–25.

7. Schafer, D. A., 2002. Coupling actin dynamics and membrane dynamics during endocytosis. *Current Opinion in Cell Biology* 14:76–81.

8. Ajeti, V., A. P. Tabatabai, A. J. Fleszar, M. F. Staddon, D. S. Seara, C. Suarez, M. S. Yousafzai, D. Bi, D. R. Kovar, S. Banerjee, et al., 2019. Wound healing coordinates actin architectures to regulate mechanical work. *Nature physics* 15:696–705.

9. Conrad, P. A., K. A. Giuliano, G. Fisher, K. Collins, P. T. Matsudaira, and D. L. Taylor, 1993. Relative distribution of actin, myosin I, and myosin II during the wound healing response of fibroblasts. *The Journal of Cell Biology* 120:1381–1391.

10. Cingolani, L. A., and Y. Goda, 2008. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nature Reviews Neuroscience* 9:344–356.

11. Pelham, R. J., and F. Chang, 2002. Actin dynamics in the contractile ring during cytokinesis in fission yeast. *Nature* 419:82–86.

12. Yang, H.-C., and L. A. Pon, 2002. Actin cable dynamics in budding yeast. *Proceedings of the National Academy of Sciences* 99:751–756.

13. Martin, P., and J. Lewis, 1992. Actin cables and epidermal movement in embryonic wound healing. *Nature* 360:179–183.

14. Latacha, K. S., M. C. Rémond, A. Ramasubramanian, A. Y. Chen, E. L. Elson, and L. A. Taber, 2005. Role of actin polymerization in bending of the early heart tube. *Developmental dynamics: an official publication of the American Association of Anatomists* 233:1272–1286.

15. Daggett, D. F., C. A. Boyd, P. Gautier, R. J. Bryson-Richardson, C. Thisse, B. Thisse, S. L. Amacher, and P. D. Currie, 2004. Developmentally restricted actin-regulatory molecules control morphogenetic cell movements in the zebrafish gastrula. *Current Biology* 14:1632–1638.

16. Kim, H. Y., and L. A. Davidson, 2011. Punctuated actin contractions during convergent extension and their permissive regulation by the non-canonical Wnt-signaling pathway. *Journal of cell science* 124:635–646.

17. Banerjee, S., M. L. Gardel, and U. S. Schwarz, 2020. The actin cytoskeleton as an active adaptive material. *Annual review of condensed matter physics* 11:421–439.

18. Varga, V., J. Helenius, K. Tanaka, A. A. Hyman, T. U. Tanaka, and J. Howard, 2006. Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nature cell biology* 8:957–962.

19. Howard, J., and A. A. Hyman, 2007. Microtubule polymerases and depolymerases. *Current opinion in cell biology* 19:31–35.

20. Ludington, W. B., L. Z. Shi, Q. Zhu, M. W. Berns, and W. F. Marshall, 2012. Organelle size equalization by a constitutive process. *Current Biology* 22:2173–2179.

21. Marshall, W. F., 2002. Size control in dynamic organelles. *Trends in cell biology* 12:414–419.

22. Erlenkämper, C., and K. Kruse, 2009. Uncorrelated changes of subunit stability can generate length-dependent disassembly of treadmilling filaments. *Physical Biology* 6:046016.

23. Kremneva, E., M. H. Makkonen, A. Skwarek-Maruszewska, G. Gateva, A. Michelot, R. Dominguez, and P. Lappalainen, 2014. Cofilin-2 controls actin filament length in muscle sarcomeres. *Developmental cell* 31:215–226.

24. Edelstein-Keshet, L., and G. B. Ermentrout, 1998. Models for the length distributions of actin filaments: I. Simple polymerization and fragmentation. *Bulletin of mathematical biology* 60:449–475.

25. Erlenkämper, C., and K. Kruse, 2013. Treadmilling and length distributions of active polar filaments. *The Journal of chemical physics* 139:10B625_1.

26. Mohapatra, L., T. J. Lagny, D. Harbage, P. R. Jelenkovic, and J. Kondev, 2017. The limiting-pool mechanism fails to control the size of multiple organelles. *Cell systems* 4:559–567.

27. Banerjee, D. S., and S. Banerjee, 2020. Size regulation of multiple organelles competing for a shared subunit pool. *bioRxiv* .

28. Suarez, C., P. M. McCall, M. L. Gardel, and D. R. Kovar, 2017. When Is "Enough" Enough? *Cell systems* 4:480–482.

29. Goehring, N. W., and A. A. Hyman, 2012. Organelle growth control through limiting pools of cytoplasmic components. *Current Biology* 22:R330–R339.

30. Burke, T. A., J. R. Christensen, E. Barone, C. Suarez, V. Sirotkin, and D. R. Kovar, 2014. Homeostatic actin cytoskeleton networks are regulated by assembly factor competition for monomers. *Current Biology* 24:579–585.

31. Suarez, C., and D. R. Kovar, 2016. Internetwork competition for monomers governs actin cytoskeleton organization. *Nature reviews Molecular cell biology* 17:799–810.

32. Sept, D., J. Xu, T. D. Pollard, and J. A. McCammon, 1999. Annealing accounts for the length of actin filaments formed by spontaneous polymerization. *Biophysical journal* 77:2911–2919.

33. Hu, J., A. Matzavinos, and H. G. Othmer, 2007. A theoretical approach to actin filament dynamics. *Journal of Statistical Physics* 128:111–138.

34. Fass, J., C. Pak, J. Bamburg, and A. Mogilner, 2008. Stochastic simulation of actin dynamics reveals the role of annealing and fragmentation. *Journal of theoretical biology* 252:173–183.

35. Oosawa, F., S. Asakura, et al., 1975. Thermodynamics of the Polymerization of Protein. Academic Press.

36. Tobacman, L. S., and E. D. Korn, 1983. The kinetics of actin nucleation and polymerization. *Journal of Biological Chemistry* 258:3207–3214.

37. Sept, D., and J. A. McCammon, 2001. Thermodynamics and kinetics of actin filament nucleation. *Biophysical journal* 81:667–674.

38. Funk, J., F. Merino, L. Venkova, L. Heydenreich, J. Kierfeld, P. Vargas, S. Raunser, M. Piel, and P. Bieling, 2019. Profilin and formin constitute a pacemaker system for robust actin filament growth. *Elife* 8:e50963.

39. Fujiwara, I., D. Vavylonis, and T. D. Pollard, 2007. Polymerization kinetics of ADP-and ADP-Pi-actin determined by fluorescence microscopy. *Proceedings of the National Academy of Sciences* 104:8827–8832.

40. Shekhar, S., M. Kerleau, S. Kühn, J. Pernier, G. Romet-Lemonne, A. Jégou, and M.-F. Carlier, 2015. Formin and capping protein together embrace the actin filament in a ménage à trois. *Nature communications* 6:1–12.

41. Wioland, H., B. Guichard, Y. Senju, S. Myram, P. Lappalainen, A. Jégou, and G. Romet-Lemonne, 2017. ADF/cofilin accelerates actin dynamics by severing filaments and promoting their depolymerization at both ends. *Current Biology* 27:1956–1967.

42. Wear, M. A., A. Yamashita, K. Kim, Y. Maéda, and J. A. Cooper, 2003. How capping protein binds the barbed end of the actin filament. *Current Biology* 13:1531–1537.

43. Pollard, T. D., L. Blanchoin, and R. D. Mullins, 2000. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annual review of biophysics and biomolecular structure* 29:545–576.

44. Köster, D. V., K. Husain, E. Iljazi, A. Bhat, P. Bieling, R. D. Mullins, M. Rao, and S. Mayor, 2016. Actomyosin dynamics drive local membrane component organization in an in vitro active composite layer. *Proceedings of the National Academy of Sciences* 113:E1645–E1654.

45. Xu, J., J. F. Casella, and T. D. Pollard, 1999. Effect of capping protein, CapZ, on the length of actin filaments and mechanical properties of actin filament networks. *Cell motility and the cytoskeleton* 42:73–81.

46. Akin, O., and R. D. Mullins, 2008. Capping protein increases the rate of actin-based motility by promoting filament nucleation by the Arp2/3 complex. *Cell* 133:841–851.

47. Higashida, C., T. Miyoshi, A. Fujita, F. Oceguera-Yanez, J. Monypenny, Y. Andou, S. Narumiya, and N. Watanabe, 2004. Actin polymerization-driven molecular movement of mDia1 in living cells. *Science* 303:2007–2010.

48. Kovar, D. R., and T. D. Pollard, 2004. Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces. *Proceedings of the National Academy of Sciences* 101:14725–14730.

49. Dehapiot, B., R. Clément, H. Alégot, G. Gazsó-Gerhát, J.-M. Philippe, and T. Lecuit, 2020. Assembly of a persistent apical actin network by the formin Frl/Fmnl tunes epithelial cell deformability. *Nature cell biology* 22:791–802.

50. Yang, C., L. Czech, S. Gerboth, S.-i. Kojima, G. Scita, and T. Svitkina, 2007. Novel roles of formin mDia2 in lamellipodia and filopodia formation in motile cells. *PLoS biology* 5:e317.

51. Krneta-Stankic, V., M. E. Corkins, A. Paulucci-Holthauzen, M. Kloc, A. B. Gladden, and R. K. Miller, 2021. The Wnt/PCP formin Daam1 drives cell-cell adhesion during nephron development. *Cell Reports* 36:109340.

52. Breitsprecher, D., and B. L. Goode, 2013. Formins at a glance. *Journal of cell science* 126:1–7.

53. Vaillant, D. C., S. J. Copeland, C. Davis, S. F. Thurston, N. Abdennur, and J. W. Copeland, 2008. Interaction of the N-and C-terminal autoregulatory domains of FRL2 does not inhibit FRL2 activity. *Journal of Biological Chemistry* 283:33750–33762.

54. Block, J., D. Breitsprecher, S. Kühn, M. Winterhoff, F. Kage, R. Geffers, P. Duwe, J. L. Rohn, B. Baum, C. Brakebusch, et al., 2012. FMNL2 drives actin-based protrusion and migration downstream of Cdc42. *Current Biology* 22:1005–1012.

55. Romero, S., C. Le Clainche, D. Didry, C. Egile, D. Pantaloni, and M.-F. Carlier, 2004. Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis. *Cell* 119:419–429.

56. Zweifel, M. E., L. A. Sherer, B. Mahanta, and N. Courtemanche, 2021. Nucleation limits the lengths of actin filaments assembled by formin. *Biophysical Journal* .

57. Breitsprecher, D., R. Jaiswal, J. P. Bombardier, C. J. Gould, J. Gelles, and B. L. Goode, 2012. Rocket launcher mechanism of collaborative actin assembly defined by single-molecule imaging. *Science* 336:1164–1168.

58. Zigmond, S. H., M. Evangelista, C. Boone, C. Yang, A. C. Dar, F. Sicheri, J. Forkey, and M. Pring, 2003. Formin leaky cap allows elongation in the presence of tight capping proteins. *Current Biology* 13:1820–1823.

59. Bombardier, J. P., J. A. Eskin, R. Jaiswal, I. R. Corrêa, M.-Q. Xu, B. L. Goode, and J. Gelles, 2015. Single-molecule visualization of a formin-capping protein 'decision complex'at the actin filament barbed end. *Nature communications* 6:1–9.

60. e Silva, M. S., J. Alvarado, J. Nguyen, N. Georgoulia, B. M. Mulder, and G. H. Koenderink, 2011. Self-organized patterns of actin filaments in cell-sized confinement. *Soft Matter* 7:10631–10641.

61. Boujemaa-Paterski, R., C. Suarez, T. Klar, J. Zhu, C. Guérin, A. Mogilner, M. Théry, and L. Blanchoin, 2017. Network heterogeneity regulates steering in actin-based motility. *Nature communications* 8:1–13.

62. Rank, M., A. Mitra, L. Reese, S. Diez, and E. Frey, 2018. Limited resources induce bistability in microtubule length regulation. *Physical review letters* 120:148101.

63. Vignaud, T., C. Copos, C. Leterrier, M. Toro-Nahuelpan, Q. Tseng, J. Mahamid, L. Blanchoin, A. Mogilner, M. Théry, and L. Kurzawa, 2021. Stress fibres are embedded in a contractile cortical network. *Nature materials* 20:410–420.

64. Lehtimäki, J. I., E. K. Rajakylä, S. Tojkander, and P. Lappalainen, 2021. Generation of stress fibers through myosin-driven reorganization of the actin cortex. *eLife* 10:e60710.

65. Fritzsche, M., C. Erlenkämper, E. Moeendarbary, G. Charras, and K. Kruse, 2016. Actin kinetics shapes cortical network structure and mechanics. *Science advances* 2:e1501337.

66. Fritzsche, M., R. A. Fernandes, V. T. Chang, H. Colin-York, M. P. Clausen, J. H. Felce, S. Galiani, C. Erlenkämper, A. M. Santos, J. M. Heddleston, et al., 2017. Cytoskeletal actin dynamics shape a ramifying actin network underpinning immunological synapse formation. *Science advances* 3:e1603032.