Entropic forces drive contraction of cytoskeletal networks

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The cytoskeleton is a network of interconnected protein filaments, which provide a three-dimensional scaffold for cells. Remodeling of the cytoskeleton is important for key cellular processes, such as cell motility, division, or morphogenesis. This remodeling is traditionally considered to be driven exclusively by processes consuming chemical energy, such as the dynamics of the filaments or the action of molecular motors. Here, we review two mechanisms of cytoskeletal network remodeling that are independent of the consumption of chemical energy. In both cases directed motion of overlapping filaments is driven by entropic forces, which arise from harnessing thermal energy present in solution. Entropic forces are induced either by macromolecular crowding agents or by diffusible crosslinkers confined to the regions where filaments overlap. Both mechanisms increase filament overlap length and lead to the contraction of filament networks. These force-generating mechanisms, together with the chemical energy-dependent mechanisms, need to be considered for the comprehensive quantitative picture of the remodeling of cytoskeletal networks in cells.

Keywords:
cytoskeleton; depletion forces; entropic forces; entropy; filament crosslinkers; force generation; molecular motors

Self-organization mechanisms of filament networks

Networks of microtubules and actin filaments, the two major constituents of the eukaryotic cytoskeleton, provide the interconnected scaffold that supports the three-dimensional shape of a cell. Dynamical remodeling of these networks is essential for cell division, motility, and morphogenesis [1], and is driven by mechanical forces that are generated by two elemental and highly conserved mechanisms: the turnover dynamics of filamentous actin and microtubules as well as the movement of molecular motors along these cytoskeletal filaments. Both, filament turnover dynamics and motor-driven movement require nucleotide hydrolysis for the generation of microscopic forces that drive the cytoskeleton away from equilibrium.

Microtubules and filamentous actin are polar filaments that dynamically self-assemble from α-β-tubulin dimers and actin monomers, respectively. The filament polymerization is tightly coupled to the hydrolysis of the constituent dimer- or monomer-bound nucleotides (GTP in case of microtubules and ATP in case of actin filaments), which yields distinct dynamics of the two filament ends. Nucleotide hydrolysis drives the whole system out-of-equilibrium and thus enables the generation of force. In particular, microtubules stochastically switch between phases of slow growth and rapid shrinkage – a behavior called dynamic instability [2]. When a polymerizing microtubule encounters an obstacle, it generates a force pushing against the obstacle [3]. Additionally, microtubule depolymerization can generate pulling forces on objects that are tethered to the tip of a shrinking microtubule [4, 5]. Similarly, continuous growth at one end of

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an actin filament and its simultaneous depolymerization at the other end leads to a behavior called actin treadmilling, which generates forces necessary for cell motility [6].

Molecular motors associated with microtubules and actin filaments are enzymes that use chemical energy from ATP hydrolysis to generate directed motion along filaments [7]. Most molecular motors form dimers comprised from two force-generating motor domains which bind to a single filament, and cargo-binding tail domains. However, some microtubule-associated motors, for example kinesin-14, have a non-enzymatic secondary microtubule-binding site in their tail domain as their cargo-binding site [8], and other enzymes, such as motors of the kinesin-5 family, form homo-tetramers with motor domains on both sides of the complex. These motor proteins can crosslink two adjacent microtubules and generate directed forces between them, causing filament sliding in bundles of antiparallel microtubules [8, 9]. For parallel microtubules, these motor proteins establish a force balance that leads to stable locking of cross-linked microtubules [8, 10]. In comparison, actin-associated motors of the myosin-II family organize into higher-order structures consisting of multiple dimeric motors, which can crosslink and slide actin filaments relative to each other [11].

Frictional forces accompany the relative motion of bundled filaments. In vivo, isolated filaments and filamentous bundles experience the ubiquitous hydrodynamic drag because they are suspended in the viscous cytosol. The presence of cross-linking proteins and possibly self-interactions of the filaments within bundles can lead to additional frictional forces that are not mediated by the surrounding solvent but are rather reminiscent of conventional macroscopic frictional forces. These frictional forces arise from breaking the bonds between the filament surfaces and the interacting proteins. This friction has been measured between the motor domains of molecular motors and the microtubule, as well as between the binding sites of non-enzymatic microtubule-associated proteins and the microtubule [12, 13]. These frictional mechanisms do not generate forces by themselves; instead friction arises only as a response to forces applied externally, for example by molecular motors or filament dynamics.

In this article, we focus on alternative mechanisms of force generation in filament networks that increase the system entropy. Entropic forces are independent of the consumption of chemical energy coupled to nucleotide hydrolysis; rather, they harness the thermal energy of the environment in a process that maximizes the entropy of the system. While entropic forces have found ample applications in soft matter physics and materials science [14], their role and relevance in biological systems remains relatively unexplored [15, 16]. Here we discuss two distinct recently published mechanisms leading to the generation of entropic forces in cytoskeletal systems. First, we describe entropic forces that are induced by macromolecular crowding [17]. In the second part, we focus on a mechanism of entropic force generation by non-enzymatic microtubule crosslinkers [18]. In contrast to motor motility and subunit turnover that drives the system away from equilibrium and requires the continuous influx of energy provided by the consumption of chemical fuel, both entropic mechanisms discussed here are equilibrium effects. They are sustained by the inherent tendency of any system to approach the equilibrium state.

Depletion interaction generates sliding forces between filaments

Non-adsorbing polymers induce entropic forces in filament bundles

Colloids and proteins immersed in a suspension of small, non-adsorbing particles (collectively called the depletant) experience effective attractive interactions. This ubiquitous effect, known as the depletion interaction (in physics and materials science) or macromolecular crowding (in biology), is based on the maximization of the total volume available to the depletant molecules [19, 20]. One particular application of this versatile interaction is to induce attractive forces between spherical and rod-like colloids [21]. Likewise, macromolecular crowding agents also induce robust in vitro bundling of biological filaments, even in the absence of any specific crosslinking proteins. Both actin filaments and microtubules were found to spontaneously assemble into bundles upon addition of non-adsorbing polymers [22–26].

The entropic mechanism of the depletion interaction can be understood as follows. The random polymer coils used to induce the attraction – typically poly-ethylene glycol (PEG) or Dextran – can be represented as spheres whose radius is the polymer radius of gyration, R_G. Relationships between the radius of gyration and the molecular weight of both PEG and Dextran are well established in the literature [27, 28]. For instance, 20 kDa PEG has $R_G \approx$ 7 nm. To the first approximation, the polymers can pass through each other and thus behave as an ideal gas of molecules. However, the volume occupied by the biological filaments themselves is not available to the polymers. As a result, the center of mass of each depletant polymer can get no closer than its radius of gyration to the surface of a filament. Thus, individual filaments are surrounded by the cylindrical shell of the "excluded volume," unavailable for the polymers to explore (Fig. 1). Reducing this excluded volume increases the space available to the polymers and their entropy. Since there are many more depletant molecules than filaments, the total entropy of the system is mainly determined by the entropy of the depletant polymers. One way to minimize the excluded volume (and increase the entropy) is to bundle the filaments. In the simplest model of the depletion attraction, the free energy gain for the two overlapping filaments is given by: $\Delta F = -p_o A_{cs} L$ [19]. Here, p_o is the depletant osmotic pressure, A_{cs} is the cross-sectional area, which depends on the geometry of the depleting molecules and filaments, and L is the length of the filament overlap. Clearly, maximizing the overlap length, L, minimizes the system's free energy. The free energy landscape for two overlapping filaments in the presence of the depletant gives rise to the entropic compaction forces. In particular, the entropic force, f acting along the long axis of the bundled filaments, is associated with the change of the bundle overlap length and is given by the partial derivative of the free energy: $f = -\frac{\partial F}{\partial L} = p_o A_{cs}$. Therefore, f is independent of the overlap length, L: once the bundle is formed, the force on the filaments remains constant until the overlap is maximized. This expression implies that the compaction force, f, depends on two parameters. First, it scales linearly with the osmotic

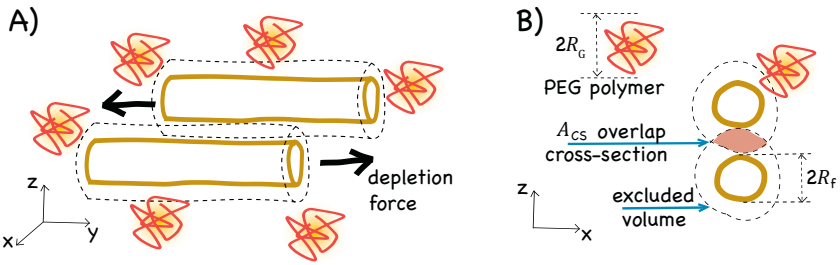

Figure 1. Schematic of depletion-induced filament bundling. The presence of a depletant in solution induces attractive interactions between filaments. **A:** A bundle maximizes its overlap length due to the depletion force, which acts on both filaments in the axial direction. **B:** Cross-section of a two-filament bundle demonstrates the overlap cross-section (A_{cs}) between two excluded volume shells, formed around each filament. Indicated length scales (depleting particle, radius of gyration, R_G; filament radius, R_f) influence the magnitude of the depletion force.

pressure of the depletant, p_o. Additionally, the compaction force is related to the cross-section A_{cs}, which in turn depends on the lateral inter-filament distance in the bundle (as illustrated in Fig. 1B). The spacing between individual filaments is determined by the interplay of attractive and repulsive interactions [29]. In solutions of biological filaments, repulsion is dominated by the electrostatic interactions between charged protein surfaces. Thus, the cross-sectional area A_{cs} (and the compaction force) exhibit strong dependence on the ionic strength of the surrounding solution.

Measuring depletion-induced compaction forces in bundled microtubules

Recent experiments have directly measured the depletion-induced compaction forces in bundled microtubules [17]. Briefly, two microtubules were attached to micron-sized silica beads and manipulated with time-shared optical tweezers in order to form a bundle. The magnitude of the compaction force, f, was measured by analyzing the thermal motion of optically trapped beads. Measured values of f ranged from 0.02 to 0.2 pN, depending on the depletant (polymer) concentration and the ionic strength of the solution. As theoretically predicted, the depletion-induced force scaled linearly with polymer concentration, and was independent of the bundle overlap length. Increasing the ionic strength yielded larger f. The relationship was non-linear and appeared to reach saturation at high concentrations of screening counterions. Furthermore, decreasing the ionic strength below a certain threshold suppressed bundle formation. In this limit, the repulsive electrostatic interactions overpowered the depletion attraction (see Fig. 5 in [30]). The force on a particular filament doubled when the bundle contained three filaments instead of two. In other words, the depletion interaction is pairwise additive. While the simple depletion interaction theory qualitatively explains all the experimental results, its prediction overestimates the magnitude of the compaction force. Taking into account the brush-like surface of the microtubules along with a more detailed calculation of the electrostatic repulsion, the theoretical predictions come closer to the experimentally measured values.

Despite relatively weak compaction forces, depletion-induced bundles maximize their overlaps on the timescale of several seconds. The dynamics of this compaction process can be explained by assuming that the frictional force resisting the sliding of a microtubule pair is dominated by weak hydrodynamic interactions. This explains the relatively rapid maximization of bundle overlaps even with sub-piconewton forces as well as the diffusive behavior of fully overlapping bundles [31]. However, increasing the depletant concentration increases the adhesion force, and above a critical value the filaments within a bundle become arrested on experimental time scales. This strong-coupling limit was studied for actin filaments [30]. It was found that actin filaments within a depletion-induced bundle exhibit solid-like friction that is fundamentally different from the weak hydrodynamic drag found in microtubule bundles. Frictional forces between bundled actin filaments can reach tens of piconewtons, and can be explained by a model that assumes that helical actin filaments intercalate with each other, thus effectively forming a number of bonds that are connected in series. Sliding of filaments requires that these bonds are simultaneously broken and a microscopic mechanism by which this takes place has been proposed [30]. Intriguingly, such friction interactions are suppressed in microtubules since their surface is coated with a disordered amino-acid sequence known as E-hooks. It has been suggested that these act as a polyelectrolyte brush which makes the molecular features of the filament surface inaccessible and thus eliminates the solid-like sliding friction.

While depletion-based effects are most easily understood for a simple mixture of filaments and non-adsorbing polymers, they are ubiquitous and occur whenever there is a large concentration mismatch between the two components in either protein or colloidal mixture. For example, bundling of microtubules and actin filaments was observed in concentrated solutions of non-specific proteins – bovine serum albumin (BSA) [32] and ovalbumin [33], respectively. It follows that the depletion should also be relevant for the cytoskeleton in cells, where highly concentrated spherical proteins can act as depletants and thus drive the bundling of both microtubules and actin filaments. Because the attractive forces add along the entire contour length, the depletion forces will be much more pronounced for long filaments when compared to other spherical structures.

Diffusible crosslinkers induce directed sliding of crosslinked filaments

Confinement of diffusible crosslinkers generates entropic forces

Complementary to the depletion-based mechanism described above, entropic compaction forces can also be generated due to the confinement of diffusible molecules. For example,

confined gas particles reconfigure into a state of higher entropy by maximizing the number of accessible states (or sample volume). The change in entropy thus generates a force, which manifests itself as pressure in three dimensions. If one of the container walls was allowed to move (such as a piston) or the container became flexible (such as an air balloon), the pressure would cause the volume to increase (through directed movement or deformation). Two essential requirements for such a mechanism of force-generation are that particles are confined and that diffusive motion allows the constituent particles to explore all accessible states.

In cells, confinement of molecules and proteins is a ubiquitous phenomenon that can fundamentally alter the particle dynamics. For example, the motion of certain proteins confined by their interaction with microtubules can be restricted to one dimension, along the longitudinal axis of the microtubule [34]. This confinement is believed to be due to electrostatic interactions between the negatively charged microtubule surface and positively charged patches on the microtubule-associated protein. Importantly, this interaction has relatively low energy barriers between the neighboring binding sites along the microtubule, allowing for effective one-dimensional diffusion of the molecules along the microtubule backbone. The energy for dissociation of such proteins from the microtubule into solution is lower than the energy associated with hopping between neighboring binding sites along the microtubule (Fig. 2) [35]. For proteins with two identical microtubule-binding sites that crosslink two overlapping microtubules, the unbinding energy becomes even higher, thus making unbinding less likely. For such molecules, not only unbinding to solution, i.e. letting go with both binding sites, but also diffusing out of the overlap region onto a single microtubule, i.e. letting go with one binding site, is energetically unfavorable. Therefore, the ends of the microtubule overlaps constitute diffusion barriers. The diffusible crosslinkers, once bound in between two microtubules, are preferentially confined to the overlap region. This confinement is manifested by higher crosslinker density in the overlap regions compared to single microtubules, as has been observed in experiments [35].

In symmetrical crosslinkers, each of the two identical binding sites has the same affinity for microtubules. Nevertheless, for any given crosslinker concentration in solution, the rate of binding of a crosslinker's single binding site to a microtubule is lower than the rate of binding of its second binding site to the second microtubule in an overlap region. This difference arises because any overlap-localized crosslinker, unbound with one of its binding sites, will be held in close proximity to the unbound microtubule by its interaction with the other microtubule, leading to an increase in the apparent local concentration of crosslinkers. To describe how crosslinkers unbind from an overlap, we hypothesize that there are two possibilities: after unbinding of one of its binding sites it can either (i) diffuse away from the overlap region along the other microtubule or (ii) unbind also from the second binding site and leave the overlap into solution. A crosslinker is retained in the overlap if it rebinds to the first microtubule before either (i) or (ii) happens. The efficiency of crosslinker confinement in the overlap thus depends on the interplay between the rate of the crosslinker diffusion along the microtubule lattice and the rates of crosslinker binding and unbinding.

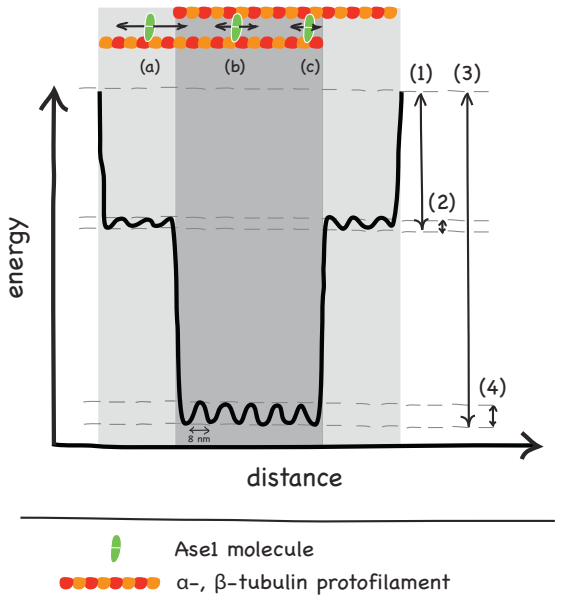

Figure 2. Microtubule overlap as a potential energy well for diffusible crosslinkers. Schematic of a hypothetical energy landscape of Ase1 binding to a microtubule protofilament. Experiments show that Ase1 molecules are diffusible on single microtubules as well as in microtubule overlaps, with diffusion being slower in the overlap regions. Ase1 molecules were shown to have higher affinity for the microtubule overlaps as compared to the single microtubules, which leads to their confinement in the overlaps. This evidence suggests that the overlap region can be understood as a potential energy well for Ase1, with relatively low energy barriers between the individual binding sites along the microtubule enabling the hopping of Ase1 molecules from one binding site to another driven by thermal energy of the environment, but impeding the Ase1 from leaving the overlap region.

Until recently, diffusible crosslinkers have been regarded as mere friction generators regulating the action of molecular motors by slowing down the motor-driven sliding of filaments [35, 36]. New experimental and theoretical findings, however, revealed that filament crosslinkers also have an alternate role as generators of compaction forces [18, 37, 38]. Such is the case for the microtubule crosslinker Ase1 from the Ase1/MAP65/Prc1 family, which is found in antiparallel microtubule overlap regions in the mitotic spindle [39]. Ase1 molecules diffuse one-dimensionally along the microtubule driven by the thermal energy of the environment. This situation is analogous to particles of a gas diffusing in a container. Similar to the confined gas, Ase1 molecules generate pressure on the overlap ends thus tending to maximize filament overlap length. The pressure of confined crosslinkers can be described by the ideal gas law in one dimension $FL = nk_BT$, where L is length, F is one-dimensional pressure, or force, n is the number of crosslinkers in the overlap, and k_BT is the Boltzmann

factor. This formula shows that the force generated by the confined Ase1 molecules increases linearly with the number of molecules in the overlap. Moreover, for a given number of Ase1 molecules the force decreases hyperbolically with the increasing length of the overlap region. The maximum force generated by this mechanism is theoretically predicted – and experimentally confirmed – to be on the order of 1 pN for an overlap in which all the binding sites are fully occupied by the crosslinkers [18].

Entropic forces exerted by confined diffusible crosslinkers maximize the overlap length of overlapping filaments

The force as predicted by the ideal gas law cannot be harnessed entirely because of the friction of the Ase1–microtubule interfaces. Microscopically, this friction is due to the fact that the relative motion of the surfaces of the crosslinkers and the microtubule is both a collective and an activated process, which stems from the finite flexibility of the Ase1-mediated linkages (Fig. 3) [18]. We hypothesize that the flexibility is not only due to an elasticity of the Ase1 molecule itself but also due to the Ase1 molecule being displaced from its ideal binding position on the microtubule. For a filament to move, multiple crosslinkers have to hop simultaneously between the neighboring binding sites. Thus, the activation barrier for the hopping of multiple crosslinkers is the sum of the individual activation barriers leading to an exponential decrease in the rate of crossing such a barrier. Thus, the friction scales exponentially with the number of crosslinkers. When the number of crosslinkers exceeds a few hundreds, the frictional forces become prohibitively large and suppress the entropic compaction forces.

Experimental results demonstrated that the entropic force nevertheless overcomes the friction when tens to hundreds of cross-linkers are confined within the microtubule overlap. In this regime, the maximum entropic forces generated by crosslinkers are predicted by mathematical modeling and experimentally confirmed to be in the range of several piconewtons [18]. Whenever two microtubules encounter each other and form a partial overlap, the compaction force will drive the directed motion of the two microtubules relative to each other in the direction of increasing overlap. In the absence of other forces, this sliding will continue until the two microtubules fully overlap. We note that this mechanism is solely driven by entropy and does not rely on prestraining the system, i.e. the mechanism does not require compression of the cross-linkers that would later relax and drive microtubule sliding. Nor does this force generation rely on changes in enthalpy. Contrary to Brownian ratchets, which are driven by binding energy, the mechanism discussed here does not require the binding of new cross-linkers from solution. Instead, it is purely the channeling of the random thermal motion of the environment that drives the system to a state with a higher "volume" and thus a higher number of possible configurations. The associated increase in disorder of the crosslinkers with respect to the available binding sites, described by an increase in entropy, is harnessed in a way that aligns the microtubule pair, i.e. makes it more ordered. The two microtubules constitute a minimal component of a microtubule network. The effect of this mechanism on the entire network is a maximization of all possible overlaps, leading to contraction (Fig. 4).

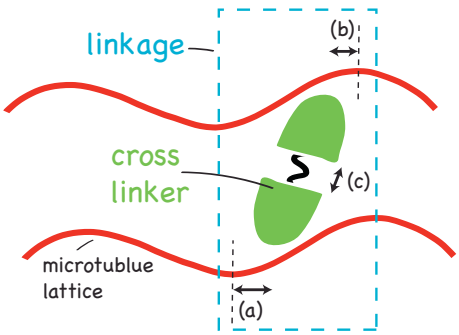

Figure 3. Schematic of a single Ase1-mediated linkage between two microtubules. Numerical simulations suggest that Ase1 mediates a flexible linkage between the two cross-linked microtubules. We hypothesize that the flexibility originates in the displacement of the crosslinker molecule from the center of its binding site on the microtubule (a and b) and the stretching of the crosslinker molecule itself (c).

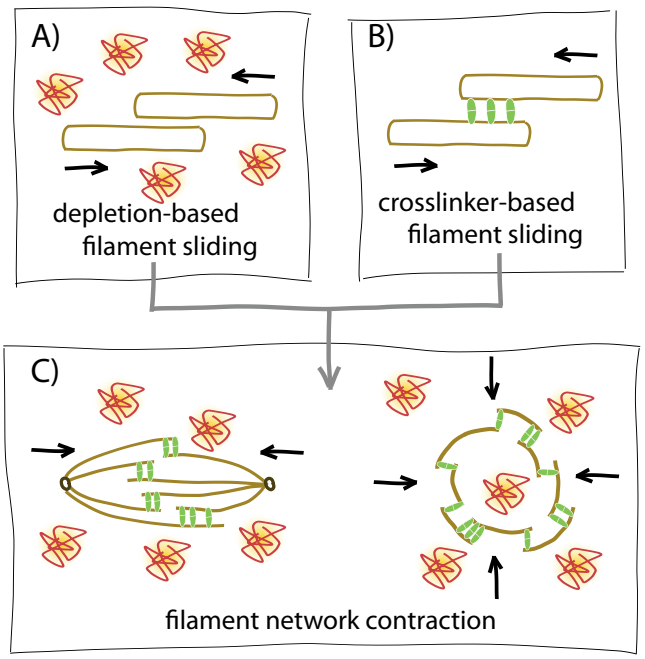

Figure 4. Entropy-based overlap maximization leads to the contraction of filament networks. Two different mechanisms generate entropic forces that maximize the length of overlaps between filaments. Overlap maximization can be induced by macromolecular crowding of the environment (**A**) or by diffusible crosslinkers that are confined in overlaps between filaments (**B**). Maximization of the overlaps between filaments leads to the contraction of the filament network; shown in two hypothetical geometries (**C**). The entropic mechanisms acting on a bipolar array with crosslinked filaments in the central region (left) will generate a force bringing the two poles together. In a ring structure (right), the mechanisms will lead to ring contraction. When coupled with a filament depolymerization mechanism, the entropic effect might lead to a complete closure of the ring.

Entropic forces in the context of intracellular self-organization

Let us now compare the depletion-based and crosslinker-based mechanisms of force generation in filament networks and discuss their implications for network self-assembly. Both mechanisms are purely entropic in origin and lead to the same phenomenon, namely network contraction through relative sliding of partially overlapping filaments. While both mechanisms channel the energy associated with random Brownian motion, the main difference between them is that one requires the presence of diffusible crosslinkers interacting specifically with the filaments, while the other only relies on crowding by unspecific depletant molecules in solution.

Filament sliding propelled by the depletion-induced forces thus provides a more general mechanism, which applies to any two filaments that overlap in the crowded cytoplasm. For any pair of partially overlapping filaments, depletion-induced forces are in the sub-piconewton range and are independent of the overlap length. On the other hand, forces generated by confinement of diffusible crosslinkers are significantly larger, reaching the magnitude of several piconewtons. In the crowded environment of the cytoplasm, these crosslinker-induced forces will be supported by depletion-induced forces of the same directionality. Both of these effects are antagonized by molecular frictional forces that might play an important role in the cytoskeletal dynamics, but systematic studies of these systems have only just begun.

Cytoskeletal filaments are polar structures and many cellular processes rely on the sense of direction, which is defined by the polarity of these filaments. The depletion-based mechanism does not discriminate between parallel or antiparallel overlaps. By contrast, the crosslinker-based mechanism has, in principle, the ability to take advantage of the filament polarity. If the cross-linkers would only bundle, for example, antiparallel filaments, the mechanism would ensure only sliding of antiparallel overlaps, leaving parallel overlaps unaffected.

Entropic forces in the context of cellular force generating mechanisms

In a cell, where other force-generating mechanisms are present, complete network contraction due to entropy-based filament sliding is very unlikely. Rather, entropic forces are just one of many forces that drive the dynamics and structural remodeling of the entire network. The canonical force generators in filament networks are molecular motors and the dynamics of the filaments themselves. Processive molecular motors that take a succession of steps along a filament before unbinding to solution can generate forces of several piconewtons. One example is kinesin-5, which, as a single molecule, can generate forces up to about 1.5 pN, and its collective force increases linearly with the number of motors [40]. Similar scaling of the collective force was reported for non-processive kinesin-14, which generates forces of about 0.5 pN as a single molecule [41]. However, in this case the ensemble of motors was rigidly attached to an artificial cargo, which might increase the efficiency of collective force generation as compared to the native state of the motor, where it is loosely attached to the cargo (which is a second microtubule) by its diffusible tail domain [8]. The dynamics of growing and shrinking cytoskeletal filaments can generate forces of magnitudes similar to the forces generated by molecular motors. Growth of a single microtubule can generate a pushing force of about 2.7 pN [42], whereas its shrinkage can generate pulling forces up to tens of piconewtons, depending on the efficiency of the mechanical coupling to the shrinking microtubule tip [43, 44]. The growth of a small bundle of actin filaments, on the other hand, can be stalled by a relatively small force of about 1 pN [45]. The entropic forces discussed in this review are thus comparable to some of the forces generated by the canonical mechanisms generating forces in filament networks. If the forces within a cellular filament network are balanced, even a small, entropy-based force may be able to tip this balance during network remodeling.

Entropic forces in the self-assembly of the mitotic spindle

The self-assembly of the mitotic spindle provides a good example of such force balance within a filament network. To ensure the integrity of the spindle, contractile (inward) forces must be generated in the spindle midzone to compensate for the expansion (outward) forces generated by microtubule plus-end directed motors, such as kinesin-5. The contractile forces are believed to be generated by the minus-end directed motors, for example dynein or kinesin-14. Forces generated by ensembles of these molecular motors were shown to scale linearly with the number of motors [40, 41]. Linear scaling of the forces with the number of motors suggests that – at constant motor density – these forces decrease with decreasing overlap length. The entropic forces discussed in this paper are weaker, especially when multiple motors are engaged in force generation. However, the magnitude of the entropic forces is either independent of the overlap length (as is the case for the depletion-based mechanism) or even increases with decreasing overlap length (as is the case for the crosslinker-based mechanism). The relevant forces in the mitotic spindle are probably still expected to be mainly molecular motor dependent. However, when these forces are in or close to equilibrium, the entropic forces may play a role in the overall force balance in the network. Apart from being one of the components of the force equilibrium in the microtubule overlaps of the spindle midzone, the entropic forces might thus also function as a "safety brake." If not counterbalanced, molecular motors slide microtubules apart completely [8, 10], which would result in the breakdown of the network. If such motor-driven sliding occurs in the presence of crosslinkers such as Ase1, the overlap ends, which are converging due to the motor force, will increasingly confine the crosslinker molecules and cause an increase in entropic force acting against the motor force. The sliding will stall when the two forces are in equilibrium. This mechanism ensures that microtubules will never slide apart completely, while still allowing sliding elsewhere. The network thus remains fluid but will not break down. Finally, the entropic forces are likely

to support the initial formation of microtubule overlaps, for example in the spindle midzone. As soon as two microtubule tips (growing from the opposite poles of the spindle) encounter each other in the midzone, the depletion-based mechanism will facilitate a quick formation of an overlap. This initial overlap formation will induce the binding of Ase1 molecules, which locate preferentially in microtubule overlaps. It has been shown in vivo and in vitro that the distance between the surfaces of microtubules, where Ase1 and its human homolog PRC1 are localized, is about 35 nm [46, 47]. For the depletion-based force generation to work, surface-to-surface separation of the filaments should be less or equal to the depletants' diameter. It is thus likely that even after the initial contact between the microtubule tips has been established and crosslinkers started binding into the overlap, cellular structures larger than 30 nm will still act as a depletant supporting the Ase1-generated microtubule sliding. The two mechanisms of entropic force generation thus have the potential to jointly start pulling the microtubules toward each other to increase the overlap length, supporting the minus-end-directed molecular motors.

Entropic forces in the self-assembly of cellular and biomimetic structures

Entropic forces provide a complementary mechanism to force generation by molecular motors also in other contexts, for example in driving the closure of filament rings such as the cytokinetic contractile ring (Fig. 4). As unregulated motor-driven sliding of filaments would proceed until the crosslinked filaments separate completely [8, 9], molecular motors would have to be regulated in a way that stops the sliding once overlaps that correspond to minimal ring diameters are formed. By contrast, entropic-based mechanisms work toward the maximum overlap length, resulting in a stop of the filament sliding once a minimal ring diameter is reached. If combined with a filament depolymerizing mechanism, they could thus drive the full constriction of filamentous rings. Indeed, it has been shown that the constriction of acto-myosin rings depends on the depolymerization of actin filaments [48] but does not require the motor activity of myosin [48, 49]. However, the detailed mechanism of constriction remains a matter of debate [50]. Moreover, as entropic forces always tend to maximize the length of the overlap between the filaments, the mechanisms discussed here could also drive the focusing of polar filamentous structures in the absence of pole-organizing centers, or assist the contraction of stress fibers. Finally, similar entropic mechanisms need not be restricted to one-dimensional filamentous structures, but may also assist the self-assembly of two-dimensional and three-dimensional structures, for example during the fusion of lipid membranes. For example, the presence of depletants alters the phenomenology and behavior of self-organized active gels. The mixture of microtubule filaments and kinesin motor clusters forms aster-like structures with motors localized at the poles [51, 52]. Addition of the depleting polymers switches the system phenomenology from contractile asters to extensile active gels, where motors are localized uniformly along the length of microtubule bundles [31, 53].

Conclusions

The canonical force-generating mechanisms in filamentous networks – the activity of molecular motors and the dynamics of cytoskeletal filaments – are sustained by the consumption of chemical energy. By contrast, we describe two cases of purely entropic forces that are driven by the thermal energy of the environment. This energy can be channeled into directed sliding of partially overlapping filaments toward increased overlap lengths. Entropic force generation is mediated either by crowding agents surrounding the filaments or by diffusible crosslinkers confined in the filament overlaps. Presence of either depletant or diffusible crosslinkers maximizes the filament overlap lengths, which subsequently causes contraction of the network. Both of the force-generating mechanisms discussed in this paper are likely to affect the self-organization of diverse cellular structures, since the cytoplasm is a crowded environment and the diffusivity and confinement of crosslinkers are omnipresent phenomena in cells. In vitro experiments and mathematical modeling suggest that the magnitude of entropic forces is smaller than the forces generated by processive molecular motors such as kinesin-5 [40], kinesin-1 [54], and dynein [55]. However, they are in the range of the forces produced by single filament dynamics [42, 56] and non-processive molecular motors of the kinesin-14 family [41]. In cells, where all of these force generators are present, the entropic mechanisms can supplement the stronger force generation mechanisms driven by chemical energy or can be the factor that tips the balance when opposing forces are close to equilibrium. The entropic force-generating mechanisms discussed here can thus play an important role in the self-organization of filamentous networks and should be accounted for when comprehensive descriptions of the dynamics of filamentous networks are formulated.

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