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OPINION

Cytoskeletal organization through multivalent interactions

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ABSTRACT

The cytoskeleton consists of polymeric protein filaments with periodic lattices displaying identical binding sites, which establish a multivalent platform for the binding of a plethora of filament-associated ligand proteins. Multivalent ligand proteins can tether themselves to the filaments through one of their binding sites, resulting in an enhanced reaction kinetics for the remaining binding sites. In this Opinion, we discuss a number of cytoskeletal phenomena underpinned by such multivalent interactions, namely (1) generation of entropic forces by filament crosslinkers, (2) processivity of molecular motors, (3) spatial sorting of proteins, and (4) concentration-dependent unbinding of filament-associated proteins. These examples highlight that cytoskeletal filaments constitute the basis for the formation of microenvironments, which cytoskeletal ligand proteins can associate with and, once engaged, can act within at altered reaction kinetics. We thus argue that multivalency is one of the properties crucial for the functionality of the cytoskeleton.

KEY WORDS: Cytoskeletal self-organization, Microtubule-associated protein, Protein avidity, Multivalency, Concentration-dependent off-rates

Introduction

Microtubules and actin filaments, present in virtually every type of eukaryotic cell, are filamentous protein polymers that constitute the major components of the cytoskeleton. Essential functions of the cytoskeleton include establishing cell shape and polarity, as well as driving intracellular transport, cell motility and cell division (Fletcher and Mullins, 2010). Importantly, with their periodic lattice of identical binding sites, cytoskeletal filaments serve as multivalent receptors, rendering them a platform for the association of a plethora of proteins. Typically, electrostatic attraction between filaments and filament-associated proteins provides the basis for the interaction between the filaments, akin to receptors, and their ligands, their interacting proteins (Cooper and Wordeman, 2009). For example, once a protein that is associated with microtubules interacts with a tubulin dimer on the multivalent microtubule surface, it is positioned in close proximity to other, identical binding sites on adjacent tubulin dimers constituting the microtubule lattice. Therefore, for many microtubule-associated proteins (MAPs), it is possible to move between adjacent binding sites without leaving the zone of electrostatic attraction surrounding the microtubule (Fig. 1A). This can result for example in random motion of these MAPs along the surface of the microtubule (Fink et al., 2009; Hinrichs et al., 2012; Kapitein et al., 2008). Importantly, the affinity of a MAP – and hence also its dwell time on the microtubule surface – is further enhanced if multiple microtubule-binding sites are present on the MAP, as demonstrated recently using a synthetic microtubule-binding peptide (Drechsler et al., 2019). This effect, termed avidity (see Box 1), arises whenever a multivalent ligand binds to a multivalent receptor (see Box 2; Fig. 1B).

The most prevalent case of multivalent MAPs are dimers with two identical microtubule interaction sites, each on one constituting monomer. Such divalent dimers comprise for instance, the molecular motors, such as kinesins (Vale, 2003), microtubule crosslinkers, such as the Ase1 (yeast; PRC1 in mammals, MAP65 in plants) (Kapitein et al., 2008; Subramanian et al., 2010), or proteins tracking the dynamic microtubule tips, including members of the end-binding (EB) family (Akhmanova and Steinmetz, 2008). For such divalent ligands, when one interaction site is microtubule bound, the remaining, unbound interaction site is tethered to the microtubule surface and thus displays a strongly increased association rate (compared to the same untethered interaction site diffusing in the surrounding solution). In this Opinion, we will highlight that, due to such local alterations of reaction kinetics, multivalent interactions underlie a range of phenomena as diverse as the exertion of entropic forces between crosslinked filaments independently of molecular motors, long-time stability of filament crosslinking and molecular-motor processivity and autoregulation, as well as spatial sorting of proteins and concentration-dependent protein unbinding. We thus argue that multivalency, which renders the composite of microtubules and MAPs a particular microenvironment that enables reaction kinetics distinct from those of the cytoplasm, is one of the key properties of cytoskeletal structures.

Tethering of multivalent crosslinkers enables the exertion of entropic forces between microtubules

Microtubule crosslinking, which is essential for organizing antiparallel microtubule structures, such as those in the mitotic spindle or in the yeast interphase microtubule array, has been reported to be mediated by the non-enzymatic crosslinker Ase1 (Loïodice et al., 2005; Mollinari et al., 2002; Yamashita et al., 2005). Yeast Ase1 is a dimeric protein with two microtubule interaction sites through which it moves by diffusion along the microtubule surface (Kapitein et al., 2008). These two binding sites also enable Ase1 to simultaneously bind to two microtubules, which leads to the crosslinking of laterally overlapping microtubules (Janson et al., 2007). When acting collectively in an ensemble, Ase1 crosslinkers can counteract the action of motor proteins (Braun et al., 2011; Janson et al., 2007; Wijeratne and Subramanian, 2018) and exert sliding forces between crosslinked microtubules due to entropic effects (Lansky et al., 2015; Braun et al., 2016). Which principle underpins such a force exertion? Let us consider one molecule in an ensemble of Ase1 molecules crosslinking two microtubules. After one of its binding sites unbinds from one of the microtubules, it remains close to this microtubule, as it is tethered by

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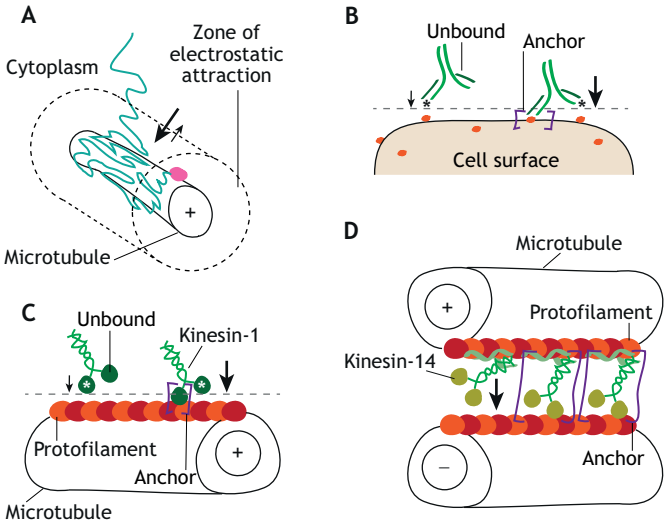

Fig. 1. Consequences of multivalent interactions on ligand–receptor interaction kinetics. (A) Prolonged interaction of filament-associated proteins with polymeric filaments: a ligand protein (pink) enters the zone of electrostatic attraction enveloping the microtubule lattice (dashed lines). Once associated, the ligand protein moves between the available binding sites on the polymeric filament lattice (path of diffusion indicated in teal). Association and dissociation rates are indicated by the black arrows. (B–D) Increased association rates due to protein tethering. (B) Divalent antibodies (green) interacting with a multivalent target, a cell surface (light brown) presenting several identical antigens (orange). When anchored (purple brackets), the antibody binding-sites (indicated by the asterisks) will be kept over prolonged time scales in the vicinity of the target, and thus will exhibit higher association rates to the target antigens. (C,D) Kinesin motor interaction with microtubules. Red and orange units represent the α- and β-tubulin subunits constituting one protofilament of the microtubules (sketched by outlines, orientation indicated by the + and − signs). (C) Divalent, processive kinesin-1 motors interacting with the multivalent microtubule surface. The motor domains (marked with an asterisk) exhibit different association rates to the microtubule surface due to the same principle as explained in B. (D) Ensemble of non-processive kinesin-14 motors crosslinking two microtubules. Anchoring of the motors to one microtubule keeps their motor domains in close vicinity of the second microtubule and leads to an increased association rate.

its other binding site to the second microtubule. Thus, tethering strongly increases the probability of rebinding of the Ase1-binding sites once they become unbound. This consideration can explain why Ase1 is unlikely to leave the overlap (Braun et al., 2011; Lansky et al., 2015), and why the overlap boundary effectively acts as a diffusion barrier for Ase1. Diffusible Ase1 molecules localized in the overlap are thus confined to this region. The confinement of diffusible particles, as described by the ideal gas law, results in an entropic force, which aims to maximize the number of possible states of arrangement of the particles within the confined region (Odde, 2015). Consequently, the two crosslinked microtubules start sliding in the direction increasing their overlap length, analogously to the movement of a piston in a cylinder containing compressed gas. The magnitude of this entropic force is in the order of ∼10 pN (Lansky et al., 2015; Lüdecke et al., 2018; Kučera et al., 2020 preprint), comparable to forces generated by the action of multiple molecular motors, such as, for example, about ten kinesin-5 (Kif11) or kinesin-1 (Kif5) motors (Shimamoto et al., 2015; Furuta et al., 2013) or ∼100 kinesin-14 (Ncd) motors (Lüdecke et al., 2018) (Fig. 2A). When the length of an overlap between two filaments increases, the overall length of the filament pair decreases. Expansion of overlaps between filaments can thus lead to an overall contraction of the filamentous network. This mechanism is likely to also play a role in contractile actin structures, such as the cell cortex, stress fibers or the cytokinetic ring, as shown recently for the cytokinetic-ring-associated protein anillin (Kučera et al., 2020 preprint). Multivalency, thus, can result in the preferential binding of ligands to filament overlaps, which, in turn, leads to their confinement and (given that appropriate structural and geometrical circumstances are provided) the exertion of entropic forces.

Box 1. Avidity

Avidity describes the accumulated affinities (i.e. the accumulated binding strengths of all non-covalent interactions) between a multivalent ligand and a multivalent receptor. Thereby, each binding site of the multivalent ligand has a given affinity to each binding site of the multivalent receptor, characterized by the binding energy. As affinity in general, and thus also the accumulated affinity, depends exponentially on the binding energy, increasing the overall binding energy linearly by summing the individual binding energies of the multiple binding sites will lead to an exponential increase in the avidity. In addition, the structural arrangement of ligand and receptor can further increase the avidity by a mechanism whereby binding of one binding site of a multivalent ligand to a multivalent receptor brings the unbound binding sites of the ligand into close proximity to the unoccupied binding sites of the receptor by ‘tethering’. This proximity strongly increases the association rate of the unbound binding sites of the ligand to the receptor and thus increases the avidity of the interaction. A familiar example of proteins employing this effect are antibodies (Cavacini et al., 1994), which display high avidity for their targets, simply by featuring multiple identical binding sites instead of just one (Fig. 1B).

Box 2. Multivalency in biomolecular interactions

Multivalency is not specific to the cytoskeleton but is a phenomenon omnipresent in biomolecular interactions across scales. An example of a multivalent interaction on a cellular level is cell adhesion, typically mediated by simultaneous interactions between multiple copies of an identical receptor on one of the interacting surfaces and multiple copies of an identical ligand on the other with the adhesion strength strongly increasing with the number of engaged receptor–ligand pairs (Xu and Shaw, 2016). On a subcellular scale, multivalent interactions are essential on both inter- and intra-molecular levels. The consequences of inter-molecular multivalent interactions in the cytoskeleton are discussed in this Opinion. Besides these, multivalent interactions underpin a large spectrum of cellular processes, such as the formation of membrane-free organelles through liquid–liquid phase separation (Hyman et al., 2014) and the clustering of membrane receptors (Banjade and Rosen, 2014). On an intra-molecular level, the multiple non-covalent interactions, such as hydrogen bonds, engaged in protein folding can be considered as an example of the essential role of multivalency in biological systems.

Motor domain tethering is a prerequisite for processive motility on microtubules

An intriguing example of proteins employing multivalency to gain additional functionality is provided by cytoskeletal molecular motors, which achieve processivity, that is, the ability to perform multiple successive steps along their track, by employing locally-increased protein affinities due to motor-domain tethering (Cross, 2016; Hancock, 2016). Molecular motors, such as kinesin-1 (Fig. 1C), typically comprise two identical ATP-binding motor domains that are dimerized through a coiled-coil stalk domain (Vale, 2003; Woehlke and Schliwa, 2000). Kinesin-1 moves directionally on the microtubule surface, in steps that are generated by conformational changes in the microtubule-bound motor domain relative to the stalk (Cross, 2016; Hancock, 2016).

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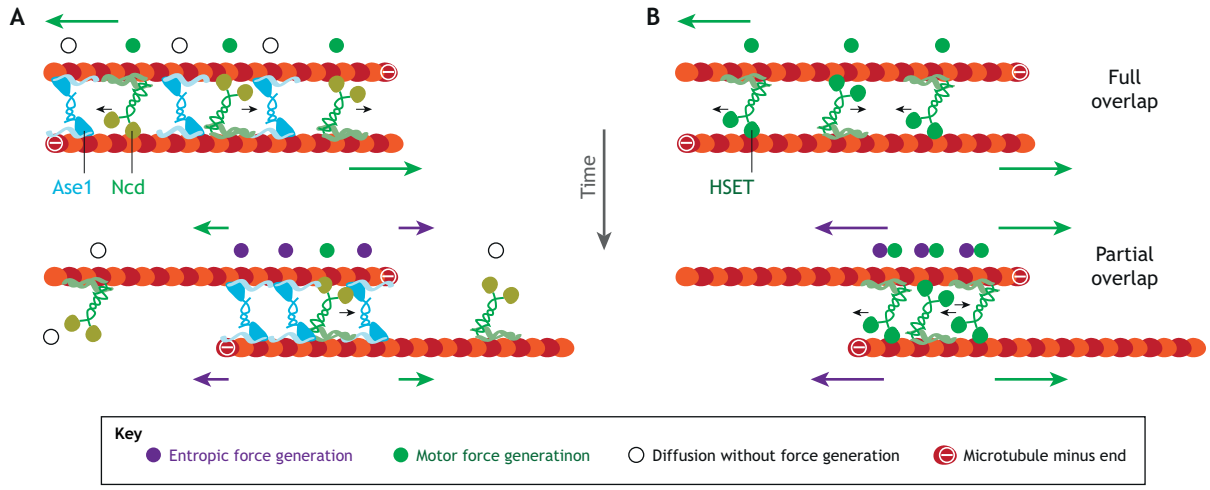

Fig. 2. Tethering enables the exertion of motor and entropic forces between microtubules. (A) In full microtubule overlaps (top panel), molecular motors, such as the kinesin-14 Ncd cause sliding of anti-parallel microtubules against each other (green arrows). When microtubules start to slide apart (bottom panel), Ase1 crosslinkers are retained in the shortening partial overlap and collectively exert an entropic force which acts in the direction of increasing the overlap length (purple arrows) and thus counteracts the motor-driven force. Unlike Ase1, Ncd motors are not retained in the shortening overlap, leading to a decrease in the Ncd-generated force. A stable overlap is established when motor force and the entropic force are in equilibrium. The stepping direction of the motors is indicated by black arrows. The mode of force generation is color-coded in circles above each molecule. (B) Human kinesin-14 HSET is retained in the shortening overlaps, and thus combines in itself the ability to generate both a sliding force acting in the direction of decreasing overlap length (green arrows) and an entropic force acting in the direction of increasing overlap length (purple arrows).

Thereby, motor movement is governed by the enzymatic ATP hydrolysis cycle, with different nucleotide states corresponding to different conformations and different affinities of the motor domains for the microtubule surface (Cross, 2016; Hancock, 2016). Crucial for motor processivity is that the unbound motor domain always remains tethered to the microtubule, leading to a strongly increased binding rate relative to the binding rate of an untethered motor domain from solution (Hancock, 2016). The resulting high affinity increases the probability of the unbound motor domain binding to the microtubule before the bound motor domain unbinds, thereby decreasing the probability of the motor detaching from its track (Mickolajczyk and Hancock, 2017).

Processivity is a measure of the average number of steps a motor performs per one instance of association with a microtubule and can vary from thousands of steps for super-processive motors (Soppina et al., 2014; Varga et al., 2009) to only a single step for non-processive motors (deCastro et al., 2000; Fink et al., 2009). Nevertheless, transport by non-processive motors, which individually unbind after each step, can become processive when the motors team up into ensembles. As an example, non-processive kinesin-14 motors can drive processive motility when as few as two or three motors are connected to an artificial DNA scaffold (Furuta et al., 2013). The likely explanation of this observation is that these motors, which are temporarily bound to the microtubule, serve as tethers for the temporarily unbound motors (Fig. 1D). Indeed, linking multiple non-processive kinesin-14 motors to a cargo is a strategy employed by, for instance, plant cells to drive processive cargo transport by non-processive motors (Jonsson et al., 2015). Similarly, motor tethering is likely to influence the association rates of oppositely directed processive motors (such as kinesin-1 and cytoplasmic dynein) attached to the same cargo, potentially tuning cargo directionality in bidirectional transport systems (Ohashi et al., 2019).

Furthermore, individual non-processive motors can become processive when they get tethered to the microtubule via an additional linker protein. An example of this mechanism provides the yeast non-processive kinesin-14 protein Kar3. Kar3 can form a heterodimer with the proteins Vik1 or Cik1, whose shape resembles a kinesin (including a microtubule-binding domain) but which do not generate ATP-dependent motor activity (Allingham et al., 2007). In the resulting heterodimer (Kar3–Vik1 or Kar3–Cik1), Vik1 or Cik1 act as a ‘foothold’ that provides the tethering of the Kar3 motor domain to the microtubule, allowing processive translocation of the complex (Mieck et al., 2015). Likewise, motor tethering has been shown for mammalian cytoplasmic dynein (Grotjahn et al., 2018; Sladewski et al., 2018; Urnavicius et al., 2018). Cytoplasmic dynein is a large complex, which is involved in intracellular transport. Unlike its yeast homolog, which is processive in its dimeric form (Reck-Peterson et al., 2006), purified mammalian cytoplasmic dynein exhibits only limited, mostly diffusible motility (McKenney et al., 2014; Schlager et al., 2014; Trokter et al., 2012). Long-range processive runs of dynein require it to either assemble into multi-motor complexes (Monzon et al., 2018) or associate with other large protein complexes, such as dynactin and cargo adaptors, such as BicaudalD2 (McKenney et al., 2014; Schlager et al., 2014), whereby the dynactin subunit p150 (also known as DCTN1) tethers the motor to the microtubule (Ayloo et al., 2014). Dynactin, furthermore, can bind up to two dimeric dynein molecules that are positioned in such a way that they can both interact with the microtubule (Grotjahn et al., 2018; Sladewski et al., 2018; Urnavicius et al., 2018). Consequently, the mean run length of the dynein–dynactin complex depends on the number of dynein molecules in the complex and can range from ∼5 to 10 µm (Grotjahn et al., 2018; Sladewski et al., 2018; Urnavicius et al., 2018). In other words, one of the roles of dynactin is to act as a scaffold, which tethers one or more motors to the microtubule and thus enhances their interaction with the microtubule surface. Similarly, recent findings demonstrate that the processive kinesin-1 gains enhanced processivity when it is additionally tethered to the microtubule by the mitochondria adaptor protein TRAK1 (Henrichs et al., 2020 preprint). Taken together, the presented examples highlight that tethering of

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multivalent ligand proteins is a general strategy to regulate motor processivity and directionality.

Tethered motor ensembles can exert motor and entropic forces between crosslinked microtubules

Combining the two concepts discussed above, some kinesin-14 motors can simultaneously employ both, motor domain tethering and diffusible molecule confinement, to collectively drive microtubule–microtubule sliding, which comes to a halt when the two microtubules start to slide apart (Braun et al., 2017). Kinesin-14 motors are involved in microtubule organization of the spindle and focusing of the spindle pole (Hepperla et al., 2014; Matthies et al., 1996; Walczak et al., 1997). Their non-processive motor domains typically exhibit interaction times with microtubules on a sub-second timescale (deCastro et al., 2000), whereas their tail domains diffuse along microtubules over tens to hundreds of seconds (Braun et al., 2017). Thus, the motor domains are tethered to the microtubules by the tail domains. The relative positioning between the microtubule-binding sites at their motor and tail domain enables kinesin-14 to crosslink two microtubules (Fink et al., 2009). Some kinesin-14 motors, such as *Drosophila melanogaster* Ncd or *Xenopus laevis* XCTK2 are able to slide microtubules apart until they become fully separated (Fink et al., 2009; Hentrich and Surrey, 2010). To prevent the separation of the crosslinked microtubule pair and to maintain the integrity of the microtubule network, additional factors are necessary, such as the crosslinkers of the Ase1 family discussed above (Braun et al., 2011), or microtubule-sliding molecular motors of opposing directionality, such as kinesin-5 (Hentrich and Surrey, 2010; Tao et al., 2006). Surprisingly, the human kinesin-14 HSET (also known as KIFC1) can slide microtubules while preventing their separation without the need for any additional factors (Braun et al., 2017). Ensembles of HSET motors slide microtubules in a similar manner to other kinesin-14 motors; however, when microtubules begin to slide apart, HSET-driven microtubule sliding decelerates in a feedback loop so that an overlap of finite length is maintained (Braun et al., 2017) (Fig. 2B). What underpins this feedback mechanism? HSET, similar to other kinesin-14s, interacts with the microtubule surface predominantly with its tail domain in a diffusible manner (Braun et al., 2017). When it encounters a microtubule overlap, its motor domain is likely to engage with the second microtubule. Although the dwell time of the motor domain on the microtubule is in the sub-second timescale (Braun et al., 2017), after its unbinding, the motor domain is likely to rapidly rebind because it is positioned close to the microtubule by the long-lasting interaction of the tail domain with the other microtubule. During this fast on–off behavior of the motor domain, the tail domain remains bound to the other microtubule in the crosslinked pair, while diffusing along the microtubule surface. Consequently, in the overlap, an HSET molecule as a whole moves by diffusion, which is slower than that of the tail domain on a single microtubule (Braun et al., 2017). The rapid rebinding of the motor domain also decreases the probability of HSET leaving the overlap, as discussed above for Ase1. Being effectively both diffusible and confined in the overlap, HSET can generate entropic forces that act in the direction of increasing overlap length, counteracting the HSET-generated sliding force when the overlap length decreases, analogously to what is seen with Ase1. This entropic force is not generated by other kinesin-14 motors, such as Ncd or XCTK2, which are not confined in the microtubule overlaps, most likely due to the faster unbinding rate of their tail domain or a slower rebinding rate of their tethered motor domain (Braun et al., 2017).

Cooperative interactions on the microtubule surface and spatial sorting of proteins

Some MAPs are intrinsically disordered and bind to the microtubule surface due to charged stretches in their amino acid chains. One example is tau, an unstructured MAP abundant in neurons, which regulates microtubule-based transport and stabilizes microtubules (Dixit et al., 2008; Drechsel et al., 1992; Morris et al., 2011). The interaction of tau with the microtubule surface is mediated by its four binding-repeats (Kellogg et al., 2018). Moreover, interactions of tau with other tau molecules mediated by the N-terminus have been reported (Gamblin et al., 2003). In solution, tau–tau interactions can lead to liquid–liquid phase separation, manifested by the formation of tau droplets (Hernández-Vega et al., 2017). When bound to microtubules at low concentration, single tau molecules diffuse along the microtubule surface (Hinrichs et al., 2012) where they can transiently form multimers (McVicker et al., 2014). At higher tau concentration, micrometer-sized ‘islands’ of stationary tau molecules form reversibly on the microtubule surface within a pool of diffusible tau (Siahaan et al., 2019; Tan et al., 2019). During this process, the dwell time of tau molecules within the islands increases by orders of magnitude compared to that of the individual tau molecules diffusing on the microtubule surface. The islands assemble from nucleation points through the addition of diffusible tau molecules to the stationary tau molecules at the island boundaries (Fig. 3) (Siahaan et al., 2019; Tan et al., 2019). How do the individual tau molecules cooperate to establish such islands of a kinetically distinct tau phase? One explanation is that tau–tau interactions are promoted by the interactions of tau with the microtubule surface. Tau molecules weakly interact with the microtubule surface, where they ‘hop’ rapidly between neighboring binding sites. When a tau molecule encounters the boundary of an island, it establishes an additional contact with the island-associated tau molecules through the proposed tau–tau interaction sites. Within the island, tau molecules are surrounded by other tau molecules, leading to additional interactions that strongly connect these tau

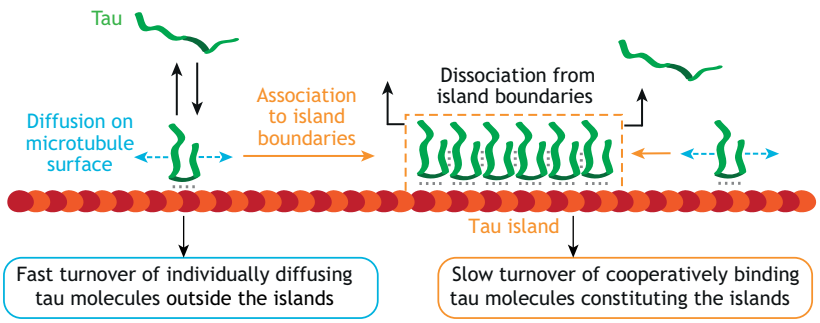

Fig. 3. Multivalency and spatial sorting of proteins. On microtubules, tau islands can form, presumably due to multivalent interactions (indicated by dotted lines; not drawn to scale) of tau with both the microtubule surface and the neighboring tau molecules.

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molecules to the microtubule. This multivalent binding, which is based on the tethering of tau molecules to the microtubule, enhances the affinity of tau for the microtubule surface and can explain the prolonged dwell time of island-associated tau molecules. Conceptually, multivalent ligand proteins can thus increase their affinity for the microtubule surface by collectively forming a super-structure.

Multivalency of the interactions of island-associated tau also explains why islands disassemble predominantly at their boundaries; here, boundary-exposed tau molecules have fewer tau neighbors, resulting in a lower avidity for the microtubule. We thus argue that tethering of multivalent unstructured proteins can give rise to the formation and co-existence of kinetically distinct phases of protein assemblies on the microtubule surface. For example, it has been shown that MAP7, another unstructured MAP, can also form domains on microtubules (Monroy et al., 2018). Crucially, regions of tau and MAP7 on the microtubule surface mutually exclude each other and either differentially recruit or repel kinesin-1 and kinesin-3 motors (Hooikaas et al., 2019; Monroy et al., 2018). Unstructured MAPs can thus spatially sort themselves into regions with distinct kinetic properties and so locally regulate the accessibility of the microtubule for other associated proteins providing the means to locally control transport along microtubules and microtubule stability (Monroy et al., 2020).

Concentration-dependent unbinding of multivalent proteins

As described above, the collective binding of multiple (often individually weak) binding sites on a ligand to a multivalent receptor increases the accumulated binding strength and leads to prolonged association times of the ligand (or ensembles of ligands) with the receptor. Nevertheless, under certain conditions, multivalently bound ligands can also be released from their receptor on rather short time scales. For example, rapid unbinding can occur when the ligand molecules are present at high concentration in the surrounding solution. The reason for this phenomenon again can be found in the nature of multivalent binding (Graham et al., 2011). Although apparently strongly bound to the receptor, the individual binding sites of a multivalent ligand undergo continuous cycles of unbinding and rebinding to the receptor. During these short-term dissociation events, the contact between the individual binding sites is transiently lost. With no ligand in solution, the most likely outcome is that the ligand will stay strongly engaged with the receptor because it is tethered to the receptor by its remaining binding sites. With increasing concentration of ligand in solution, however, it becomes increasingly probable that a transiently vacated binding site on the receptor will get occupied by an interaction site of a ligand from solution, which then, by sequentially occupying the individual receptor-binding sites, one at a time, can replace the originally receptor-bound ligand (Graham et al., 2011) (see Box 3).

Indeed, in tau islands on microtubules, concentration-dependent unbinding rates result in decreased dwell times of individual, multivalently bound tau molecules (Siahaan et al., 2019). While the dwell time of tau in the island is in the order of 1000 s with no tau in solution, it drops to only ∼10 s with a tau concentration of 100 nM in solution. This increased unbinding does, however, not result in the disassembly or destabilization of the islands, because tau molecules are merely exchanged, so that the total number of island-constituting molecules stays constant. Cells might use such rapid turnover for example to alter the regulatory roles of the islands by exchanging presently bound tau molecules for modified (e.g. phosphorylated) ones (Planel et al., 2008). Notably, this switch would occur without disassembling and re-assembling the islands.

Similarly, for divalent microtubule crosslinkers, concentration-dependent unbinding also applies (Lansky et al., 2015). As described above, Ase1 can bind to two microtubules where an Ase1 dimer with one temporarily unbound binding site continues to be tethered between the two microtubules by the binding site of its partner, allowing for rapid re-binding. Thus, when their concentration in solution is low, Ase1 molecules exhibit a low off-rate from microtubule overlaps. This off-rate increases with increasing Ase1 concentration in solution (Lansky et al., 2015). As is the case for tau, this leads to an exchange of bound molecules, but not a decrease in their overall numbers. This exchange thus does not reduce the collective capacity of Ase1 for a stable crosslinking of microtubules and the exertion of entropic force (Lansky et al., 2015). Analogously to tau, the turnover of Ase1 may be used by cells to rapidly exchange Ase1 in the stable, long-lasting, overlaps for distinct Ase1 molecules; for example, those that are phosphorylated and thus might have different bundling- or force-generating properties. Indeed, Ase1 localization to the spindle midzone and the stability of the midzone itself, have been shown to be phospho-regulated (Khmelinskii et al., 2009).

Finally, the decrease of the run-lengths of kinesin-1 motors observed with increasing motor concentrations in solution (Telley et al., 2009) is likely also a direct consequence of concentration-dependent unbinding rates. The run length of kinesin-1 is given by its dissociation rate from microtubules. Here, kinesin-1 motors from the solution that bind to the microtubule might compete with the unengaged motor domain of the microtubule-bound motor for the next binding site on the microtubule lattice and thus effectively increase the dissociation rate of the motor as discussed above for the case of Ase1 and tau. The consequent sensitivity of the motor run-lengths to the motor concentration in solution could be exploited by

Box 3. Concentration dependence of multivalent unbinding

A monovalent ligand that interacts with a monovalent receptor binds at a rate that is dependent on the ligand concentration in solution. By contrast, the unbinding rates are independent of the ligand concentration in solution – as described in many textbooks. This assumption, however, no longer holds true for multivalent ligands and receptors. In these cases, an individual ligand binding site, which has just dissociated from a binding site on the receptor, is held in close proximity to this (or another) binding site on the receptor due to tethering via the other interaction sites. When there is a low concentration of ligand in solution, this tethering will allow rapid rebinding, which leads to an overall prolonged interaction time of the multivalent ligand with the multivalent receptor. Interestingly, with increasing ligand concentration in solution, it becomes increasingly likely that the interaction sites of free ligands will engage with the temporarily vacated binding sites on the multivalent receptor. Thus, in a gradual process, the successive occupation of the binding sites on the receptor (for our considerations the periodic binding sites on polymeric cytoskeletal filaments) can lead to the stepwise displacement of the entire receptor-engaged multivalent ligand. Thus, although at first glance it is seemingly counterintuitive, the unbinding rate of multivalent ligands from multivalent receptors necessarily increases with increasing ligand concentration in solution. This means that receptor-bound multivalent ligands unbind and turn over faster the more soluble ligands are diffusing in the surrounding solution. This phenomenon has formerly been described experimentally (Graham et al., 2011) as well as theoretically (Sing et al., 2014) for DNA-binding proteins that interact with periodic binding sites on DNA, and is discussed in this Opinion for multivalent proteins interacting with cytoskeletal filaments.

the cell to fine-tune motor-mediated intracellular transport or to effectuate different motor behaviors in cell types differing in concentrations of a particular motor.

Taken together, the regulation of the rates of protein unbinding from cytoskeletal filaments by altering the protein concentration in solution enables cells to modify ligand protein turnover without altering the interaction affinities of the proteins themselves; for example, by enzymatically modifying (e.g. phosphorylating) the interacting proteins. Thus, by providing an alternative to enzymatic, posttranslational modifications of proteins, multivalency allows cells to modulate the unbinding of multivalent ligands from cytoskeletal filaments.

Conclusions

As discussed here, tethering of multivalent proteins to polymeric cytoskeletal filaments results in altered reaction kinetics, which can lead to prolonged association times of ligand proteins to the filaments. The generation of entropic forces, the long-term stability of filament crosslinking, the processivity of molecular motors and their auto-regulation and the spatial sorting of proteins, as well as their concentration-dependent unbinding rates from cytoskeletal filaments, are outcomes of this effect. Conceptually, interactions between multivalent ligand proteins with cytoskeletal filaments can be divided into a number of different categories: (1) multivalent ligand proteins (featuring at least two binding sites) that bind to polymeric filaments, for example most kinesins; (2) multivalent ligand proteins (featuring at least two binding sites) that crosslink at least two polymeric filaments, for example Ase1; (3) combinations of these, for example HSET; and (4) unstructured ligand proteins that cooperatively bind to filaments, such as tau. Furthermore, additional combinations of these categories are possible; kinesin-14 homodimers and kinesin-5 homotetramers divalently bind to one microtubule, and, simultaneously, bind to a second microtubule, thereby crosslinking the two (Braun et al., 2009; Hentrich and Surrey, 2010; Kapitein et al., 2005). In addition, tau molecules, which, as discussed above, cooperatively form tau islands (Siahaan et al., 2019; Tan et al., 2019), can also individually bind to microtubules in a multivalent manner through their four binding repeats (Kellogg et al., 2018); this might be necessary for other roles of tau, such as to crosslink microtubules or to influence their dynamics (Wang and Mandelkow, 2016).

As exemplified above, due to their multivalency, cytoskeletal filaments constitute a distinct microenvironment for their associated proteins. When assembled into large complex structures such as the mitotic spindle, these microenvironments can overlap and merge into three-dimensional super-structures. It is worth noting, that being clearly distinct from the rest of the cytoplasm, these super-structures share some characteristics with membrane-free intracellular compartments. Namely, certain proteins can partition from the surrounding cytoplasm into the super-structure, leading to a local increase in protein concentration, which results in the local alteration of their reaction kinetics. Similarly, liquid phases, which, on the molecular level, are likewise established through dynamic interactions between multivalent proteins (Hyman et al., 2014), provide microenvironments distinct from the remaining cytoplasm, which can concentrate molecules in a confined space and locally facilitate specific reactions (Alberti, 2017). As discussed in this Opinion, we suggest that, analogously, the local microenvironments established by multivalent interactions between cytoskeletal filaments and MAPs enable reaction kinetics distinct from the cytoplasm and lead the emergence of a wide range of phenomena instituting the internal organization of cells.

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Competing interests

The authors declare no competing or financial interests.

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