Review

Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP

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The molecular mechanisms by which microtubule-associated proteins (MAPs) regulate the dynamic properties of microtubules (MTs) are still poorly understood. We review recent advances in our understanding of two conserved families of MAPs, the XMAP215/Dis1 and CLASP family of proteins. *In vivo* and *in vitro* studies show that XMAP215 proteins act as microtubule polymerases at MT plus ends to accelerate MT assembly, and CLASP proteins promote MT rescue and suppress MT catastrophe events. These are structurally related proteins that use conserved TOG domains to recruit tubulin dimers to MTs. We discuss models for how these proteins might use these individual tubulin dimers to regulate dynamic behavior of MT plus ends.

Microtubule dynamics

Microtubules (MTs) are dynamic protein polymers used to move and organize cellular components for processes such as cell division, membrane trafficking and cell morphogenesis. The α- and β-tubulins form obligate αβ-heterodimers (tubulin dimers) that assemble in a head-to-tail fashion and form protofilaments with distinct polarity (Figure 1) [1,2]. MTs are composed of approximately 13 protofilaments, which associate laterally in parallel and produce a rigid, hollow 25 nm diameter tube (Figure 1) [3]. An essential property of MTs is the ability to grow and shrink at their ends. Transitions between these states are termed catastrophe and rescue. In a catastrophe event, the MT ceases growth and transitions into an explosive shrinkage phase in which protofilaments peel off from the MT plus end [1]. In a rescue event, a MT stops shrinkage and reverts to a state of MT assembly. These growth and shrinkage states are accompanied by dynamic conformational changes in the structure of the MT plus end. MT protofilaments at MT ends are straight structures during MT growth and curved structures that peal outwards during MT shrinkage (Figure 1) [4]. Cycles of GTP binding and hydrolysis in β-tubulin provide a conformational switch that helps to drive assembly and disassembly states in the polymer (Figure 1) [3]. These dynamic states are highly sensitive to the local concentration of soluble dimer at the MT plus end [2,5]. For instance, MTs shrink uniformly at low concentrations of tubulin, whereas high concentrations of tubulin promote MT assembly and rescue events, and inhibit catastrophe [5]. At physiological concentrations of tubulin, pure MTs display dynamic instability *in vitro*, in which MTs in the same population exhibit assembly and disassembly, and undergo switch-like transitions stochastically [5].

In vivo, a set of conserved MT regulatory proteins bind along the MT lattice or at MT ends and control almost every aspect of MT dynamic behavior [1]. These regulatory proteins modulate in space and time the dynamics and organization of MTs, creating MTs with distinct properties in certain regions of the cell or during certain phases of the cell cycle. Despite the extensive study, little is known about how regulators affect MT dynamics at the molecular and structural levels. The molecular details of the MT plus end itself are poorly understood [2]. For instance, there is continuing debate in the field about what alternative MT lattice arrangements might exist at the plus end, and how GTP-GDP states of tubulin contribute to MT plus end regulation and structure [2,6–8]. A recent study showed that the +TIP protein EB1/Mal3 binds *in vitro* to MTs containing GTPγS (a GTP analog) but not to GDP or GMPCPP MTs, providing evidence that these EB proteins normally localize to the MT plus end by recognizing a conformation of tubulin dictated by its guanine nucleotide γ-phosphate binding site [9]. Although many proteins on MT plus ends have been identified, how they modulate the structure of an MT plus end and/or the state of the guanine nucleotide in tubulin is generally not known.

We present the current understanding of two related families of MT regulatory proteins, the XMAP215/Dis1 and cytoplasmic linker-associated proteins (CLASP). XMAP215/Dis1 proteins have been implicated primarily as an accelerator of MT assembly, whereas CLASP proteins are involved in promoting MT rescue and suppressing MT catastrophe. These MAPs, which are conserved from yeasts and plants to man, have essential cellular functions *in vivo*, regulating MT dynamics in the mitotic spindle and interphase MT [10,11]. Recent studies have shown that to regulate MT dynamics, these proteins use TOG domains to bind to soluble tubulin dimer [12–16]. How presentation of individual tubulin dimers to the MT plus end modulates MT growth or shrinkage remains an open and highly interesting question. We compare and contrast these structurally related proteins, and propose

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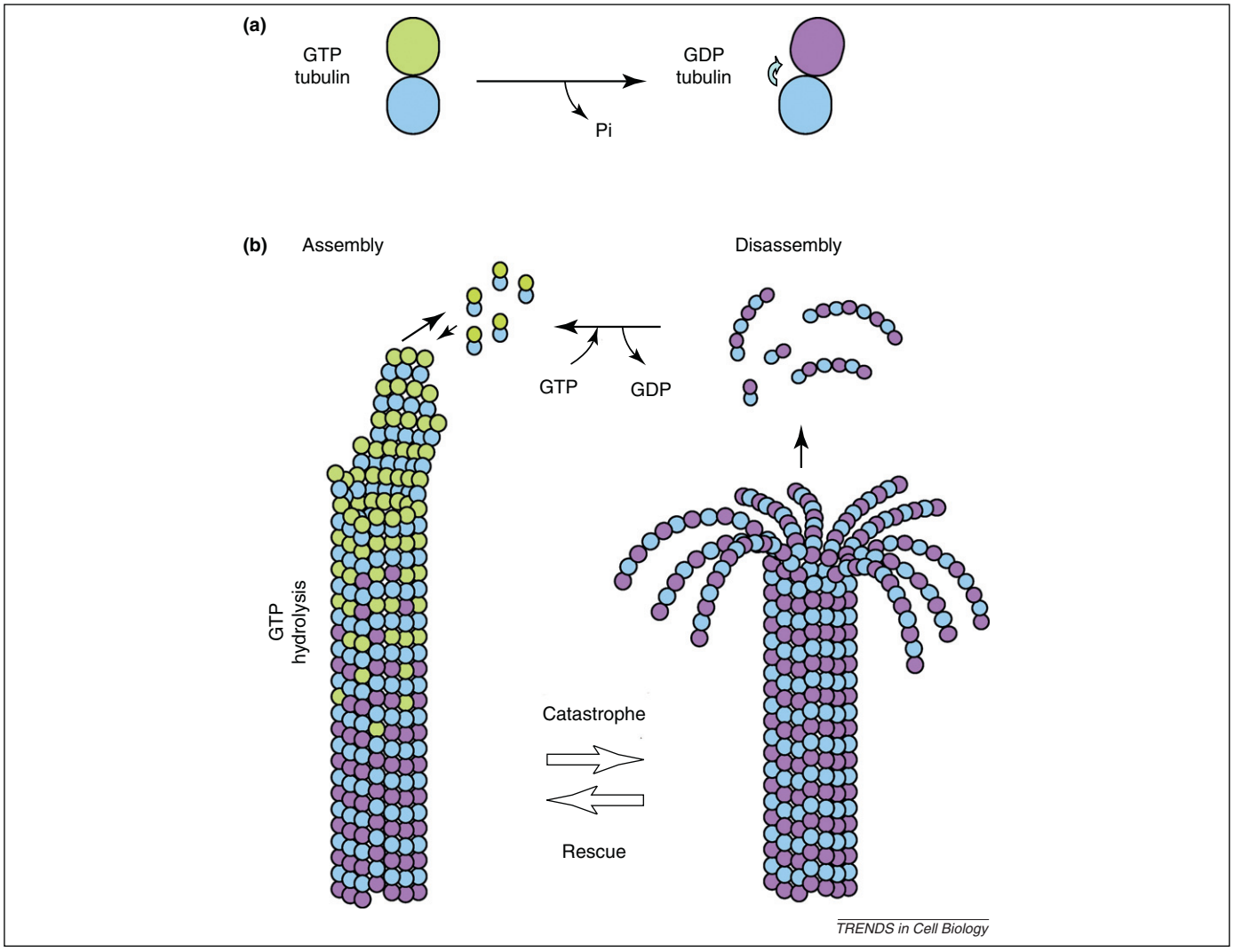

Figure 1. Assembly and disassembly of dynamic microtubules (MTs). (**a**) Conformational change of αβ-tubulin accompanying GTP hydrolysis. In the GTP state (β-tubulin in green), α and β tubulin monomer interfaces result in a 'straight' tubulin dimer. In the GDP state, the αβ-tubulin dimer interface is curved by 5° (arrow), leading to a 'bent' tubulin dimer. (**b**) Structural changes at MT plus ends. During MT assembly, MT plus ends form a sheet-like group of straight protofilaments. GTP-tubulin dimers (green) assemble on the ends, forming a cap of GTP-tubulin. GTP hydrolysis over time converts GTP-tubulin in the lattice to GDP-tubulin (note that the extent of the GTP cap is not known). In the MT disassembly phase, GDP-tubulin protofilaments curl and peel off the MT plus ends. The transitions between growth and shrinkage states are termed catastrophe and rescue.

some working models for how these proteins function. The study of these TOG-domain proteins represents a promising avenue into understanding new mechanisms used to regulate MT polymers.

XMAP215/Dis1 proteins promote MT assembly

XMAP215/Dis1 proteins have conserved roles in promoting the assembly of MTs. These proteins include: *Saccharomyces cerevisae* Stu2, *Schizosaccharomyces pombe* Alp14 and Dis1, *Arabidopsis thaliana* MOR1, *Caenorhabditis elegans* Zyg9, *Drosophila melanogaster* MiniSpindles (MSPS) and human ch-TOG [17–22]. XMAP215/Dis1 orthologs localize to MT plus ends, MT organizing centers (MTOCs), kinetochores and, to varying degrees, along MT lattices (Figure 2a and b). *In vivo* studies show that these proteins generally promote the rate of MT assembly. In general, loss of function leads to interphase MTs that are short and exhibit reduced growth rates and increased frequencies of MT catastrophe and pause events [17,20,21,23–25]. As illustrated by the name of the *Drosophila* mutant minispindles, knockdown of these proteins often leads to small or abnormally organized spindles and short astral MTs [20,26,27]. At kinetochores, these proteins are needed for regulation of kinetochore-MT attachment [17,28]. Stu2, for instance, has been shown to participate in the formation of MTs from the kinetochore to facilitate the attachment of kinetochores to the spindle [29].

XMAP215 was identified as a factor in *Xenopus* extracts that promotes MT assembly [30]. *In vitro* studies with recombinant XMAP215 show that these molecules bind directly to growing MT plus ends and accelerate MT plus end assembly roughly tenfold (Figure 2 h and i) [14,31]. Each XMAP215 molecule associates with the growing MT plus end only transiently and, during this time, it helps to polymerize 25 tubulin dimers onto the MT end before disassociating [14]. Although XMAP215 was initially hypothesized to bind preformed tubulin oligomers and load them onto MT ends [32], several lines of evidence demonstrate that each XMAP215 can bind only one tublin dimer at a time. XMAP215 and Stu2 can also catalyze the reverse reaction, MT depolymerization, at low concentrations of

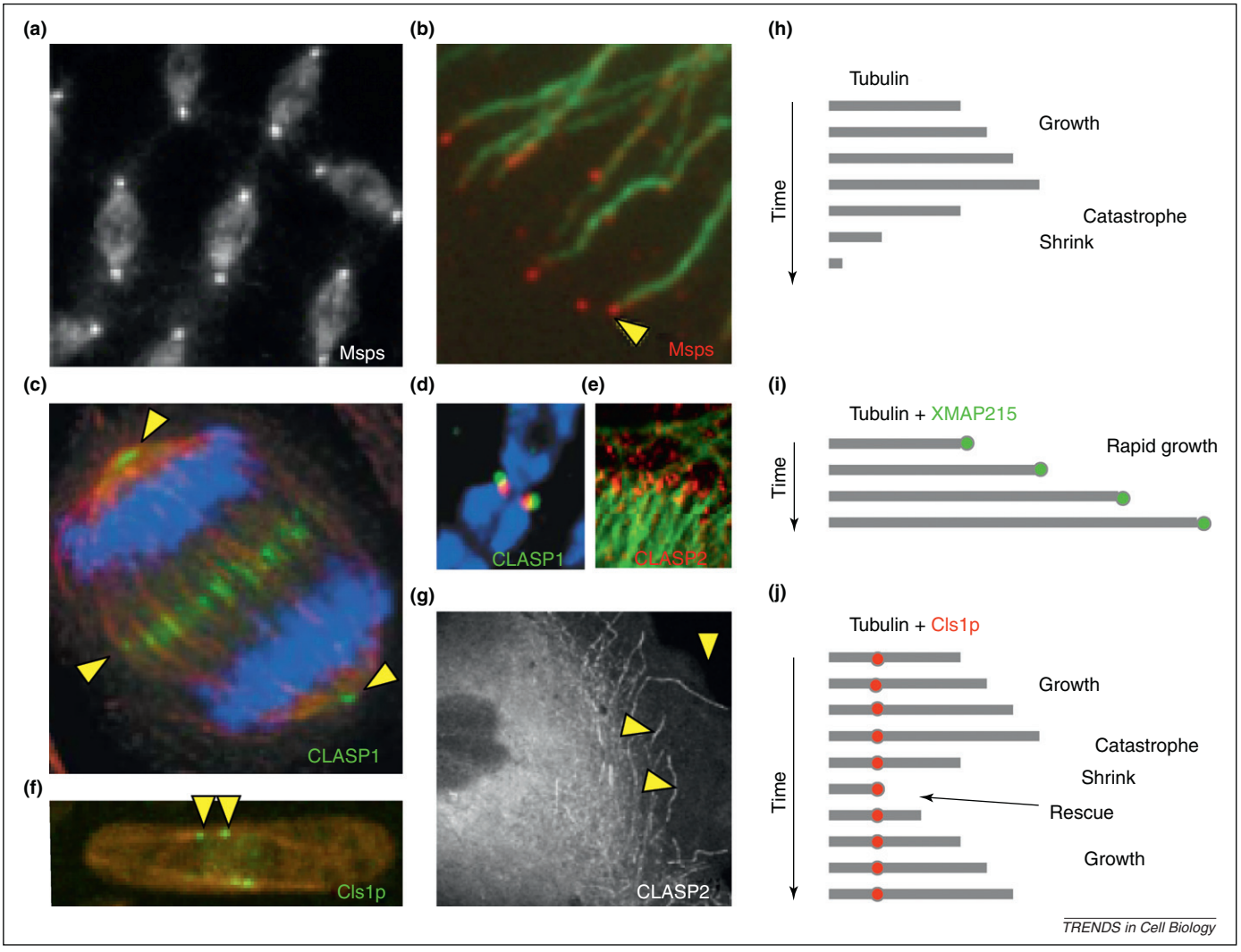

Figure 2. Localization and activities of XMAP215/Dis and CLASP proteins. (**a**) *Drosophila* Msps (XMAP215/Dis1) on spindle MTs and poles. (**b**) *Drosophila* Msps on interphase MT plus ends (Msps, red arrowhead; tubulin, green). (**c**) Human CLASP1 on the anaphase spindle, at the spindle midzone MTs and poles (arrows: CLASP1, green; MT, red; DNA, blue). (**d**) Human CLASP1 at the kinetochore on a metaphase chromosome (CLASP, green; DNA, blue; ACA centromere marker, red). (**e**) Human CLASP2 staining at interphase MT plus ends near the plasma membrane (CLASP, red; MT, green). (**f**) Fission yeast CLASP Cls1p in clusters on interphase MT bundles near the nuclear envelope (Cls1p, green; MT, red). (**g**) Human CLASP1 on the lattice of interphase MTs near the leading edge of the cell (arrowhead). Images in **(a–g)** are reproduced with permission from [40]. (**h**) Scheme showing the dynamic behavior of pure MTs (gray lines). (**i**) XMAP215 (green) at the MT plus end accelerates MT assembly and leads to formation of long MTs. (**j**) *S. pombe* CLASP Cls1p (red) binds to the MT lattice and promotes local MT rescue, preventing MTs from shrinking completely [24,48,53,61,63].

tubulin (added to MTs stabilized with a non-hydrolysable GTP analog, GMPCPP) and were identified biochemically as MT depolymerases [33,34]. Thus, XMAP215 and its orthologs may act catalytically as MT polymerases that promote the reversible assembly of single tubulin dimers onto MT plus ends.

It remains to be tested whether all members of the XMAP215/Dis1 protein family act as polymerases or have other activities. For instance, *S. pombe* has two related proteins in this family: Alp14 and Dis1 [17,35]. Alp14 appears to promote assembly of tubulin dimers at MT plus ends like XMAP215 (Al-Bassam and Chang, unpublished observations). Dis1 functions at kinetochores and spindles for chromosome segregation and is targeted to kinetochores by binding to the Ndc80 complex [36]. Recent studies show that Dis1 is localized along the MT lattice in interphase cells, and appears to function in bundling MTs in the spindle and interphase arrays [37]. *S. cerevisae* Stu2 has been shown to interact with the EB1 and CLIP-170 orthologs [38]. In addition, many XMAP215/Dis1 proteins have been shown to function at MTOCs in a complex with transforming acidic coiled-coil (TACC) proteins [38–42]. TACC proteins, which have been identified as partners of XMAP215/Dis1 proteins in yeast to human cells, target XMAP215/Dis1 proteins to centrosomes or SPBs. At MTOCs, XMAP215-TACC complexes increase the number and length of centrosomal MTs during mitosis, possibly by stabilizing or anchoring MT minus ends [39,42,43]. These complexes are activated during mitosis by Aurora A kinase phosphorylation on TACC [42,44,45] and by Ran-dependent nuclear import in yeast [46]. Whether XMAP215 functions as a MT plus end polymerase at the MTOC or contributes some other function, for instance at MT minus ends with TACC, remains to be characterized (Figure 3a).

CLASP promotes MT rescue

CLASP proteins have been implicated in stabilizing subsets of MTs. CLASP family members include *S. cerevisae* Stu1, *S. pombe* Cls1, *A. thaliana* CLASP, *C. elegans* Cls2, *D. melanogaster* MAST/orbit, and human CLASP1 and

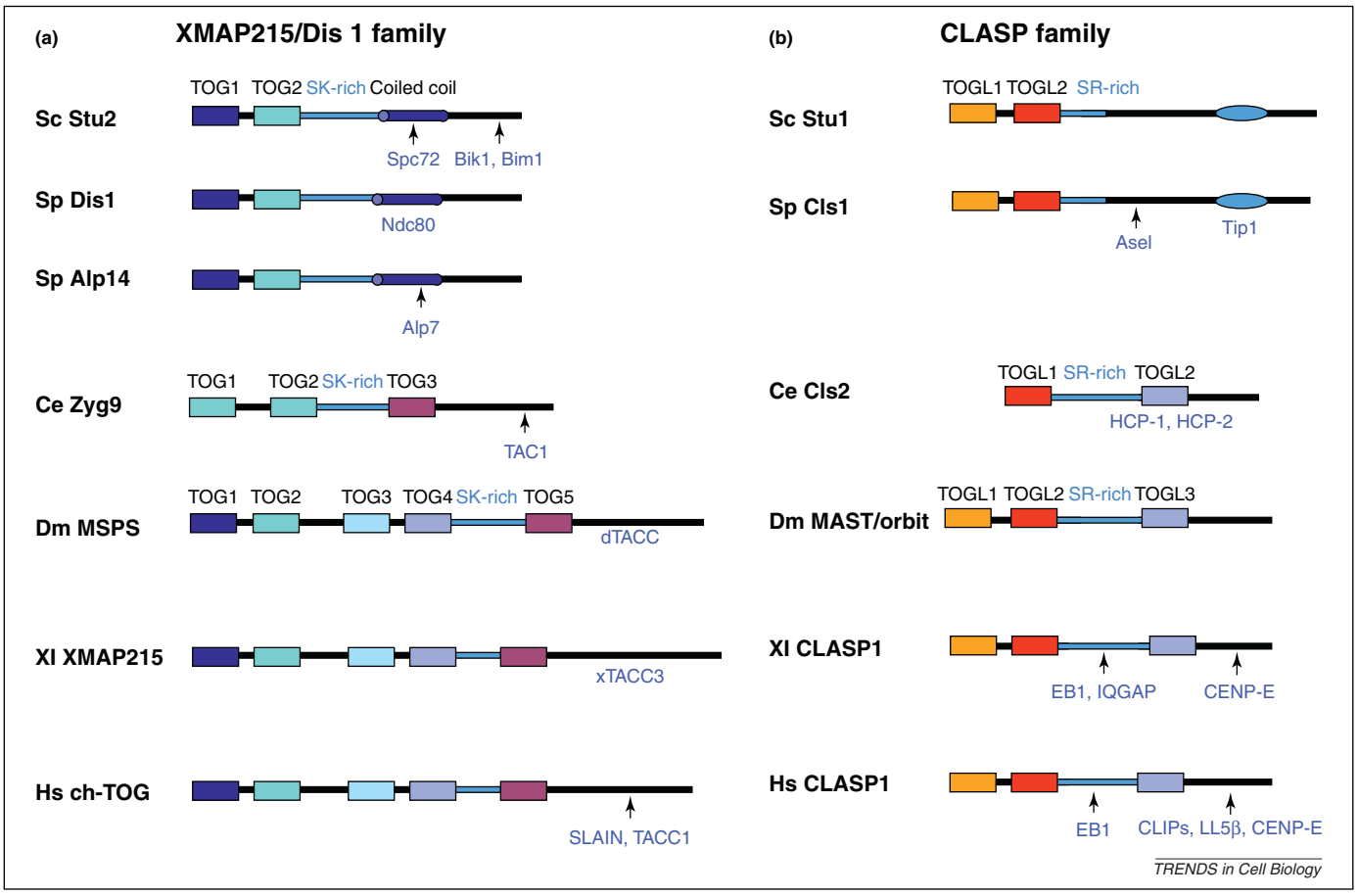

Figure 3. **Domain organization of XMAP215/Dis1 and CLASP families from yeast, worms and mammals and their binding partners**. (**a**) XMAP215/Dis1 proteins contain conserved TOG domains and an SK-rich domain. Domain organization of yeast orthologs *S.cereviae* Stu2, *S.pombe* Dis1 and Alp-14 with two TOG domains, *C. elegans* Zyg9 with three and *D. melanogaster* MSPS, *Xenopus laevis* XMAP215 and human ch-TOG with five. All molecules contain regions with stretches of sequences rich in serine, glycine and lysine (SK-rich domains). TOG domains are colored on the basis of the conserved phylogenetic classes from sequence alignments (Figure 5): TOG1 class, blue; TOG2 class, cyan; TOG3 class, sky blue; TOG4 class, purple; TOG5 class, maroon. Protein binding partners (blue) described in the text are shown below each protein, with arrows denoting approximate binding sites. An absence of an arrow denotes an interaction in which binding domains have not been mapped. (**b**) CLASP proteins contain conserved TOG-Like (TOGL) domains and SR-rich domains. Similar to (a), domain organization of *S. cerevisae* Stu1 and *S. pombe* Cls1 with two TOGL domains, *C. elegans* Cls2 with two TOGL domains and *D. melanogaster* MAST/orbit, human and *X. laevis* CLASP1 with three TOGL domains. All molecules contain regions with stretches of sequences rich in serine, proline and arginine (SR-rich domains). TOGL domains are colored on the basis of the conserved phylogenetic classes from sequence alignments (Figure 4): TOGL1 class, orange; TOGL2 clas, red; TOGL3 class, purple. Protein binding partners (blue) described in the text are shown below each protein with arrows denoting approximate binding sites based on studies described in the text. The absence of an arrow denotes an interaction in which the interacting domain has not been mapped.

CLASP2 [47–52]. A prominent conserved function of CLASP is at the mitotic spindle [50,53–55]. Loss of CLASP activity causes a variety of severe mitotic defects, often resulting in the collapse of spindles into a monopolar spindle, in which the chromosomes are located at the middle of the aster [54,56]. CLASP localizes to the outer periphery of kinetochores, where it promotes the stability and growth of kinetochore MTs (Figure 2d) [54,57]. In *C. elegans*, the CLASP ortholog Cls-2 is targeted to kinetochores via CENP-F-like proteins HCP-1/2 [58]. At the mammalian kinetochore, CLASP1 binds CENP-E and has been shown in early mitosis to be in a complex with the kinesin Kif2b, which promotes kinetochore movement and MT turnover, and in a separate complex in metaphase with astrin that promotes MT stability [59,60]. During anaphase, CLASP localizes to spindle midzone MTs, where it stabilizes these overlapping MTs and contributes to cytokinesis (Figure 2c) [53,54,56].

CLASPs mediate the selective stabilization of interphase MTs. In animal cells, CLASPs are commonly seen on the growing MT plus ends (Figure 2e) [52], as well as on the MT lattice and other locations (see below). Knockdown of mammalian CLASP2 causes a decrease in MT density at the periphery of the cell and a decrease in the frequency of MT rescue and pauses, but little change in general MT growth or shrinkage rates [61]. CLASPs are implicated in the attachment of MT plus ends to cortex at the leading edge of fibroblasts (Figure 2e) [62]. In migrating epithelial cells, CLASP accumulates on the lattices of persistently growing MTs that extend into the leading edge of these migrating cells (Figure 2g) [63]. In *Drosophila* macrophages and neuronal growth cones, CLASP stabilizes MT bundles that contribute to directional migration, as well as cell–cell repulsion [64,65]. In plants, CLASP mediates the attachment of the MT lattice to the cortex [49]. CLASPs are also located at MTOC structures [52]. Notably, in migrating mammalian cells, a subset of CLASP molecules localizes to the Golgi apparatus and are responsible for organization of MTs emanating from the Golgi apparatus, which contributes to asymmetric distribution of MTs [66].

In addition to binding MT lattices directly, CLASPs are targeted to MT plus ends as well as to diverse cellular compartments through their associations with other proteins in their C-terminal halves, including ase1/PRC1 (MT bundles and spindle midzone), CENP-E and F (kinetochores), LL5β (patches at the plasma membrane) and GCC185 (Golgi apparatus) (Figure 3b) [48,59,62,63,66]. CLASP is recruited to MT plus ends through its interactions with the +TIP proteins EB1 and CLIP-170, which both contribute to plus end binding by binding to different regions of the molecule [52,61,67]. CLASP binds to EB proteins via an EB interaction domain (S-X-I-P motif) near its central MT lattice-binding domain [68]. In migrating epithelial cells, the localization of CLASP to MT plus ends and MT lattice is spatially regulated by GSK3β kinase, which phosphorylates CLASP at multiple sites near these domains. Differential phosphorylation at these sites may influence whether CLASP binds to MT lattice or plus ends [67].

The function of CLASP in promoting MT rescues is perhaps most clear in the fission yeast *S. pombe* [48]. The sole fission yeast CLASP ortholog cls1/peg1 localizes primarily on the MT lattice, at regions of MT anti-parallel bundling at spindles and in small clusters (of about 13 molecules) in the middle of interphase MT bundles through an interaction with the MT bundling protein ase1/PRC1 (Figure 2f). Genetic studies show that cls1p is responsible for practically all the local rescue events within both the mitotic spindle and interphase MT bundles. In *cls1* mutant cells, MT rescue is not observed, while other MT dynamic parameters are not affected. When over-expressed, cls1p accumulates along MTs and causes dramatically increased rescue frequency [48].

In vitro studies with recombinant *S. pombe* cls1p show that it decreases the frequency of MT catastrophes, and increases the frequency of MT rescues (Figure 2j) [15]. It binds to the MT lattice directly without diffusing and does not track growing MT plus ends. MT rescue events occur at sites on the MT where Cls1 molecules are present in high concentration. Thus, these results together with genetic *in vivo* results show that cls1p is a MT rescue-promoting factor.

Although *S. pombe* cls1p is the only CLASP extensively characterized *in vitro* thus far, the proposed function of MT rescue is fully consistent with the effects seen with CLASPs in animal cells. If bound to the MT plus end, CLASP might promote sustained growth by causing repeated rescue events that immediately reverse catastrophes [54,61]. At MTOCs and the *trans*-Golgi network, CLASPs may help to stabilize small segments of MTs by binding near the minus ends, thereby making them resistant to complete depolymerization every time they undergo catastrophe. Thus, CLASP can be regarded as a movable clamp that can locally promote rescue on specific portions of dynamic MTs.

TOG domains bind to soluble tubulin dimer

XMAP215/Dis1 and CLASP family proteins share the highest degree of conservation in their N-termini, which contain 250 residue sequence repeats, termed tumor over-expressed gene (TOG) domains, named after the human ortholog ch-TOG [12]. In XMAP215/Dis1 proteins, the number of TOG domains varies from two in yeast (Alp14, Dis1, Stu2), three in worms (Zyg9), and five in flies, plants and mammals (Dm MSPS, Xl XMAP215, Hs ch-TOG; Figure 3a). CLASP family proteins contain TOG-like (TOGL) domains that share weak sequence homology with XMAP215/Dis1 family TOG domains. Yeast CLASP orthologs (Sc Stu1, Sp Cls1) contain a minimum of two

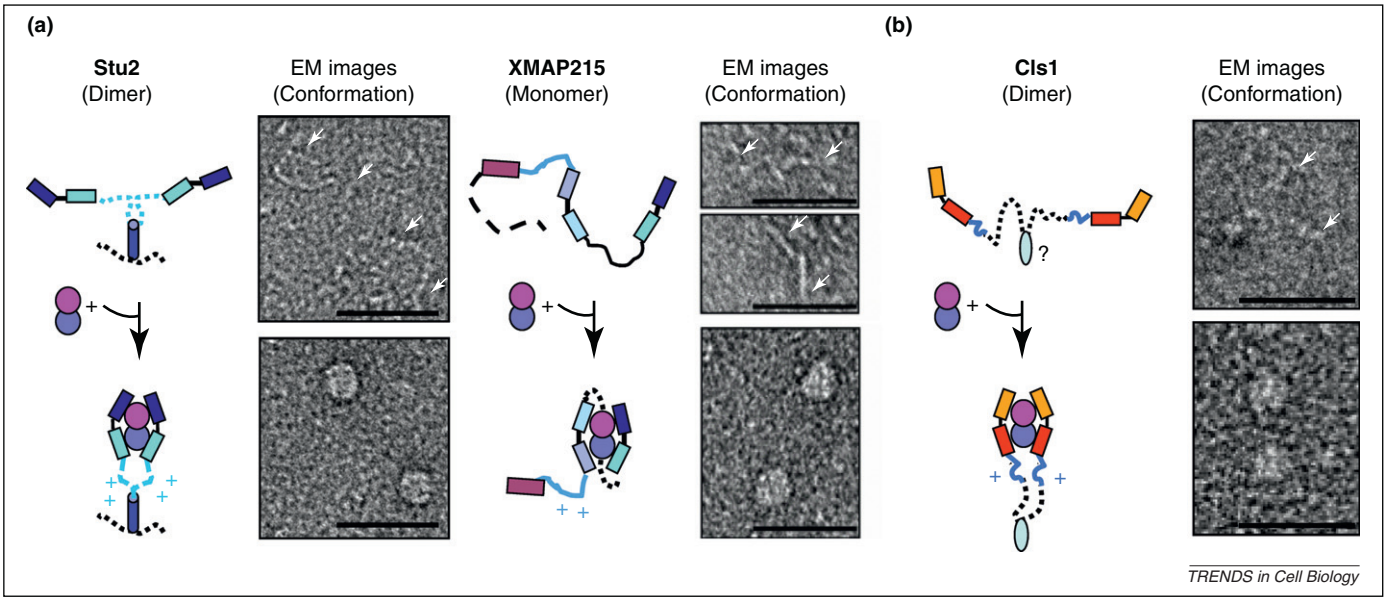

Figure 4. XMAP215/Dis1 and CLASP proteins bind to a tubulin dimer to form a globular complex. (**a**) XMAP215/Dis1 molecules wrap around soluble tubulin dimers with their TOG domains to form globular complexes. Models for tubulin binding are shown on the left, and electron microscope images are shown on the right (Stu2 or XMAP215 alone, above; tubulin complex, below). Yeast Stu2 is a homodimer with two sets of TOG domains that wrap around a single tubulin dimer, while *X. laevis* XMAP215 is a monomer with two internal halves, each consisting of two TOG domains that interact with a single tubulin dimer. Electron microscope images of XMAP215 and Stu2 are reproduced with permission from [12,14]. White arrows denote the open conformation of XMAP215 and Stu2 molecules. (**b**) *S. pombe* CLASP Cls1 is a homodimer that wraps around soluble tubulin dimers with two sets of TOGL domains. The model for Cls1 dimer binding to the tubulin dimer is shown on the left; electron microscope images are shown on the right (Cls1 alone, above; cls1-tubulin complex, below). Electron microscope images of Cls1 were reproduced with permission from [15]. White arrows denote the open conformation of XMAP215 and Stu2 molecules.

conserved TOGL domains, while fly and vertebrate CLASP orthologs (Ce Cls2, Dm MAST/orbit, Xl and Hs CLASP1) contain an additional TOGL domain in their C-terminal half, which cannot be identified in yeast CLASP orthologs [15,69].

We aligned the sequences of TOG/TOGL domains from diverse XMAP215/Dis1 and CLASP family orthologs (Supplementary Figure 1). Phylogenetic analysis indicates that the XMAP215/Dis1 and CLASP families share common evolutionary origins in their N-termini (Figure 4a) and that their conserved TOG domains can be classified into distinct groups. For example, all TOG1 domains from XMAP215/Dis1 family proteins form a single class that is distinct from TOG2 domains.

A key function of the XMAP215 TOG and CLASP TOGL domains is to bind to soluble tubulin dimers [12–15]. The TOG domains of XMAP215, Stu2 and cls1p have been shown to bind soluble (non-polymerized) tubulin dimers with high affinity, but do not bind the tubulin dimers polymerized in the MT lattice. This indicates that the TOG domains can recognize features of tubulin when it is soluble but not when it is incorporated into the MT lattice [14,15].

One reason why the vertebrate XMAP215/Dis1 and CLASP proteins are larger and contain more TOG domains than the yeast orthologs is because vertebrate proteins are monomers whereas the yeast proteins function as dimers [12,14]. The *S. cerevisiae* XMAP215 ortholog Stu2 is a homodimer that contains two sets of two TOG domains; it binds soluble tubulin dimer with a stoichiometry of two Stu2 molecules to one tubulin dimer [12]. Interactions between a C-terminal 100 residue coiled coil are responsible for dimerization (Figure 3B). In contrast, XMAP215, containing five TOG domains, is a monomer that binds soluble tubulin dimers with a stoichiometry of one XMAP215 per tubulin dimer [14]. Thus, mammalian XMAP215/Dis1 orthologs contain two internal halves, each consisting of a pair of TOG domains (TOG1-2 and TOG 3-4). However, the role of the fifth TOG domain in mammalian orthologs remains unclear. *S. pombe* Cls1p resembles Stu2, as it also forms a homodimer that binds to a single tubulin dimer [15]. Although the oligomeric states of other CLASP orthologs have not been measured for purified recombinant proteins, fluorescence correlation spectroscopy suggests that mammalian CLASP2 is monomeric when diffuse in the cytoplasm [70]. The presence of a C-terminal TOGL domain suggests that the mammalian CLASPs may bind to tubulin as a monomer through two tubulin-binding halves of TOGL domains, similar to mammalian XMAP215/Dis1 (Figure 3b).

Electron microscope images of purified negatively stained complexes reveal how these related proteins clutch soluble tubulin dimers with their TOG domains [12,14,15]. In the absence of tubulin, both Stu2p and Cls1p dimers are elongated molecules with thin domains and flexible linkers. With addition of soluble tubulin dimer, the TOG domains of both proteins appear to wrap around the outer length of the tubulin dimer to form a globular complex (Figure 4a). The overall conformation of Cls1p and Stu2p tubulin complexes are highly similar (Figure 4). In both Cls1p-tubulin and Stu2p complexes, two identical sets of TOG domains (TOG1 and TOG2) contact a tubulin dimer. It is not known which TOG domain binds to which tubulin, or if each TOG in the pair binds to a different face of tubulin. Although XMAP215 molecules are monomers, they are similar to Stu2p in flexibility and wrap around a tubulin dimer to form a similar globular complex [14]. At this level, the striking similarity between the XMAP215 and CLASP proteins provides strong evidence that these are related proteins that use TOG domains to bind to tubulin dimers.

Crystal structures combined with mutational analysis define the TOG domain binding site for soluble tubulin dimer. Structures of three TOG domains from XMAP215/Dis1 proteins show that they contain a highly conserved α-helical fold consisting of six conserved Huntington, elongation factor 2, phosphatase A2, TOR PI-3 kinase (HEAT) repeats [13,16]. Unlike other HEAT repeat proteins, which have curved solenoid-like structures, TOG domains form flat paddles with wide thin edges. The wide edges of a TOG paddle are composed of HEAT repeat α-helices and the narrow edges are composed of loops that connect those helices (Figure 5b, left panel). Two groups of residues are highly conserved in all TOG domains (Supplementary Figure 1): (i) hydrophobic residues buried between neighboring HEAT repeats α-helices that stabilize the overall structure of the domain (Supplementary Figure 1); (ii) five short intra-HEAT repeat loops (T1 – T5) on one narrow edge, comprising the most conserved surface of the domain (Figure 5b and c). Mutations of the strictly conserved residues in these loops abolish binding to the tubulin dimer, indicating that these five loops comprise the TOG domain tubulin-binding site [16].

Sequence comparisons in the tubulin-binding loops within the TOG domains begin to explain functional differences between TOG domains. Some residues in these tubulin-binding loops are strictly conserved across all classes (Figure 5c, purple), whereas others are conserved only among different classes. These class-specific variations may modulate the affinity of each TOG domain for tubulin. For instance, in mammalian XMAP215/Dis1 orthologs, the composition of class-conserved residues in these tubulin-binding loops suggests that TOG1 and TOG2 bind with high affinity, while TOG3, TOG4 and TOG5 domains bind with lower affinity.

The affinity of TOG domains for the tubulin dimer is crucial for function. A recent study found a direct correlation between XMAP215 affinity for tubulin dimer and its MT polymerase activity [71]. The roles of each class of TOG domains in the XMAP215 MT polymerase activity were examined by mutating strictly conserved residues in tubulin-binding loops. Inactivation of all five XMAP215 TOG domains resulted in complete loss of its MT polymerase. Inactivating the high-affinity TOG1-TOG2 domains led to a 75% reduction in both tubulin affinity and MT polymerase activity, whereas inactivating the low-affinity TOG3-TOG4 domains decreased both tubulin binding affinity and MT polymerase by 25%. Although a shortened XMAP215 with only a high-affinity site (TOG1-TOG2) is functional, its MT polymerase activity is lower than that of full-length XMAP215. Thus, both weak and strong tubulin-binding sites are likely to be required for full activity. In compari-

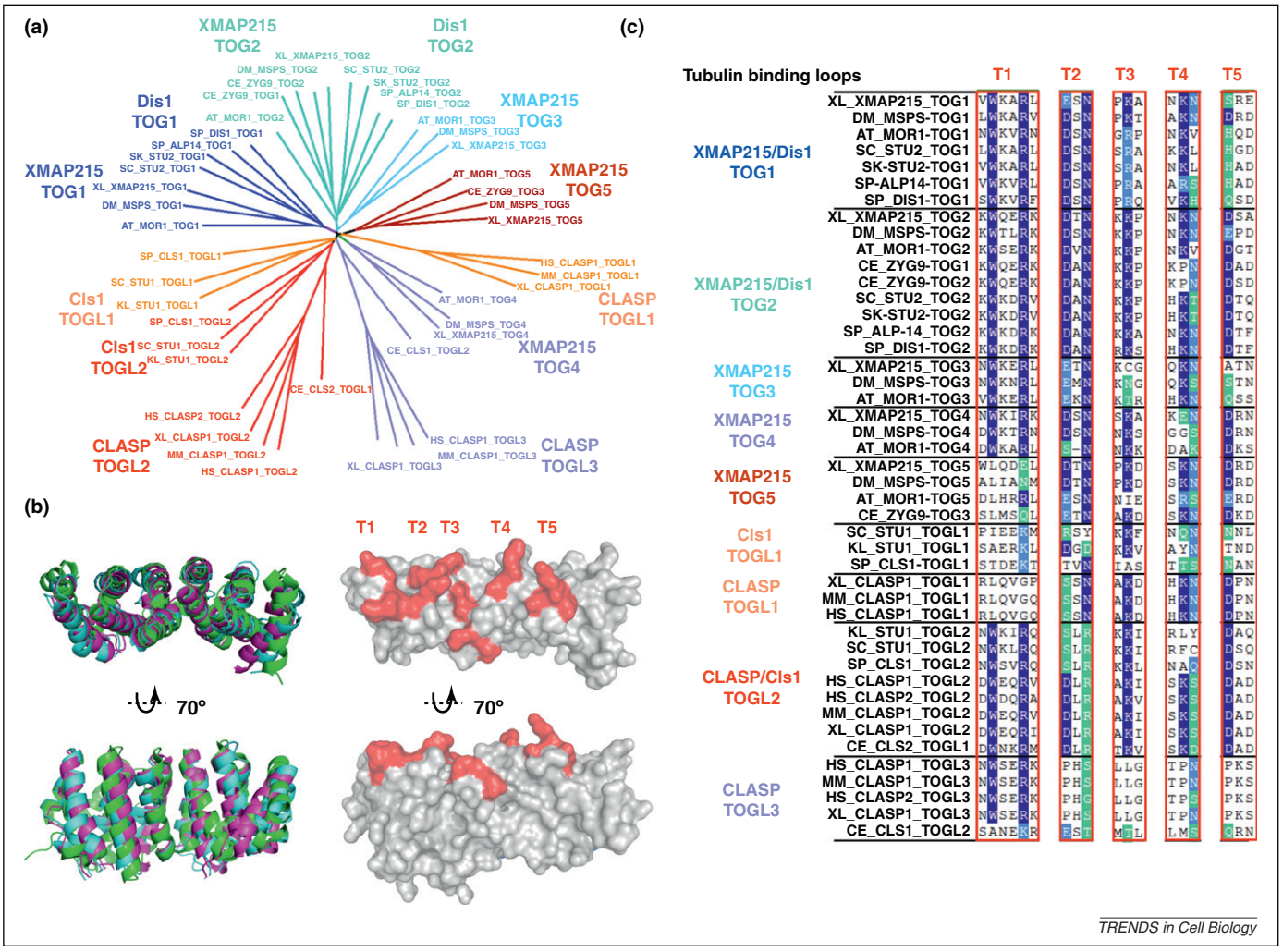

Figure 5. XMAP215/Dis1 TOG and CLASP TOGL domains and the tubulin dimer-binding interface. (**a**) Phylogenetic tree based on the sequence alignment of TOG and TOGL domains: Separate XMAP215/Dis1 family TOG domains and CLASP family TOGL domains from *S. cereviase, Saccharomyces kluyveri, S. pombe, C. elegans, D. melangaster, A. thaliana, X. laevis, Mus musculus* and *Homo sapiens* orthologs were aligned using MUSCLE alignment server (further described in Supplementary Figure 1) [78]. Distance matrices for aligned sequences were used to calculate a phylogenetic tree to classify TOG/TOGL sequences. The tree shows that aligned sequences of TOG and TOGL domains (shown in Supplementary Figure 1) are grouped in conserved phylogenetic classes based on TOG domain position in the protein. This analysis indicates that XMAP215/Dis1 TOG and CLASP family TOGL domain sequences have a common origin. The degree of separation between the different classes describes further divergence in the sequences. The colors of the different branches of the phylogenetic tree are consistent with the coloring scheme of TOG/TOGL domains in Figure 3. The classes are highlighted as follows: XMAP215/Dis1 TOG1, aqua blue; XMAP215/Dis1 TOG2, cyan; XMAP215 TOG3, light blue; XMAP215 TOG4, purple; XMAP215 TOG5, brown; Cls1 TOGL1 and CLASP TOGL1, beige; CLASP/Cls1 TOGL2, orange; CLASP TOGL3, purple. (**b**) Structures of TOG domains and mutational analyses reveal the site for tubulin dimer binding. Top panel: structures of three TOG domains from Zyg9, MSPS and Stu2 TOG domains show that TOG domains have a flat paddle shape with six conserved HEAT repeats [13,16]. The overlaid structures are shown with the narrow side of the paddle overlooking the tubulin-binding loops and the wide side of the paddle overlooking the HEAT repeat helices. Lower panel: sequence conservation and mutational analyses show that TOG domains bind tubulin dimers using intra-HEAT repeat loops T1–T5 (shown in red) in views similar to those in the upper panel. (**c**) Detailed sequences of the tubulin-binding loops in XMAP215/Dis1 TOG domain and CLASP TOGL domain classes. These aligned tubulin-binding loops describe the amino acid variations in the tubulin-binding sites of TOG and TOGL domains. All the tubulin-binding loop (T1–T5) sequences contain strictly conserved residues (purple), moderately conserved residues (blue), and weakly conserved residues (either in cyan or not colored). Although strictly conserved residues are maintained throughout TOG domains, there are class-conserved residues that differ between classes. For example, XMAP215/Dis1 TOG1 and TOG2 classes differ in many residues neighboring strictly conserved residues within T1 and T2; those variations are maintained in each class. Some classes, such as XMAP215 TOG3, XMAP215 TOG4, CLASP TOGL1 and CLASP TOGL3, contain divergent variations in strictly conserved residues, suggesting they may have a weaker affinity for binding the tubulin dimer.

son, yeast XMAP215 orthologs like Dis1 or Stu2 are predicted to have two identical high-affinity sites (TOG1-TOG2) in these homodimeric molecules. It remains to be seen how yeast and vertebrate XMAP215 orthologs compare in their tubulin dimer affinities and MT polymerase activities.

It is very likely that CLASP TOGL domains bind tubulin dimers in a similar manner through analogous residues in the tubulin-binding loops. Mutations of the strictly conserved residues in TOGL1 and TOGL2 domains of *S. pombe* CLASP, Cls1p, cause loss of tubulin dimer binding without affecting MT lattice binding [15]. Multiple mutations in these loops cause a complete loss of Cls1p MT rescue, promoting activity *in vitro* and *in vivo*. CLASP TOGL domains show a higher degree of variation in their tubulin-binding loops than the XMAP15/Dis1 TOG domains (Figure 5c). Some differences in the strictly conserved residues of TOGL1 suggest a weakened affinity for the tubulin dimer (Figure 5c). Although Cls1 TOGL domains form stable complexes with tubulin similar to XMAP215 or Stu2, the detailed on and off-rates have not been measured [12,15]. Differences in TOGL2 tubulin-binding loops (such as T2 of TOGL2) are strictly conserved across CLASP orthologs. It remains to be tested whether differences in key residues in

the TOG/TOGL domains of the CLASP and XMAP215/Dis1 proteins cause different affinities for tubulin binding, and if these affinities underlie differences in protein function.

Binding to the MT lattice

Another key activity of XMAP215/Dis1 and CLASP families is binding to MT lattices. *In vivo*, multiple interactions with MTs and association with other MAPs may contribute further to XMAP215/Dis1 and CLASP localization to MTs [48,61,67]. TOG domains were originally suggested to bind MT lattices but there is little evidence that TOG domains themselves interact directly with MT lattices [14,15]. Domain analysis suggests that direct MT lattice-binding activities are mediated primarily by stretches of positively charged residues immediately C-terminal to the TOG domains in both XMAP215/Dis1 and CLASP families [14,15]. Although these regions are predicted to be mostly unstructured and less conserved in exact sequence, they retain specific features across many species. In XMAP215/Dis1 proteins, MT lattice-binding domains consist of stretches of serine, glycine, and lysine residues (termed S/K-rich domains), whereas MT lattice-binding domains in the CLASP family consist of stretches of serine, proline, and arginine residues (termed S/R-rich domains) [12,15]. As with other MAPs (such as Ndc80 and Dam/DASH kinetochore complexes), these positively charged domains may bind MT lattices by interacting with negatively charged residues at the C-terminus of tubulins [72–75]. In XMAP215, swapping its C-terminus and SK domain with three copies of generic polylysine MT-binding sequence from kinesin-3 (Kif1a/unc104), forms an active MT polymerase [71]. Thus, the MT lattice-binding domains C-terminal to the TOG domains are used primarily for MT localization and are not specifically required for XMAP215/Dis1 polymerase activity.

The MT lattice-binding behavior of XMAP215/Dis1 and CLASP families are quite different, reflecting their different localization patterns *in vivo*. XMAP215 molecules bind MT lattices with very low affinity ($<1\ \mu M$), resulting in molecules that undergo cycles of binding, release and rebinding that lead to rapid diffusion ($0.2\ \mu m/s$) along the MT lattice [14,71]. How XMAP215 localizes on the MT plus end is not known, and we speculate that the molecules diffuse onto the MT and then accumulate at the plus end by recognizing some additional feature of the plus end. This behavior of XMAP215 may allow it to diffuse to and accumulate at the MT plus end, and is similar to other MT-binding proteins that interact with MT lattices via positive charge, such as kinetochore-associated Ndc80 and Dam/DASH complexes [74,75]. Although XMAP215 recognizes MT plus ends *in vitro*, the accumulation of XMAP21 on MT plus ends *in vivo* might be enhanced by interactions with other +TIPs, such as EB1. Indeed, SLAIN has been identified as a bridge between EB1 and ch-TOG, the human XMAP215 ortholog, which may serve to enhance MT plus end binding [76]

By contrast, *S. pombe* CLASP (Cls1p) binds MT lattices with very high affinity: Cls1p molecules remain attached to specific sites along MT lattices without dissociating for minutes, and exhibit a low diffusion coefficient ($0.0015\ \mu m/s$) that is >100-fold slower than most MAPs [15]. Tubulin dimer binding via TOG domains and MT lattice binding via SR or SK domains are not mutually exclusive; hence, XMAP215 and Cls1p can bind to both MTs and tubulin dimer simultaneously, bringing their tubulin dimers to the MT lattice. *In vivo*, multiple interactions with MTs and association with other MAPs and regulatory proteins may contribute to the affinity of XMAP215/Dis1 and CLASP on MTs and other intracellular sites [48,61,67]. For instance, FRAP experiments show that at least a subset of CLASP2 fibroblasts are stable in cortical regions [70]. However, FRAP of CLASP2, specifically on the lattice of MTs in the lamella of eptithelial cells, show very rapid dynamics [63], suggesting that the affinity of this protein for MTs is modulated *in vivo*.

Models for XMAP215 and CLASP mechanisms

We propose working models for how XMAP215 and CLASP families function to regulate MT dynamics. XMAP215 functions as a MT polymerase that accelerates MT polymerization at the growing MT plus end. XMAP215 molecules may diffuse onto the MT due to the low affinity of their SK domains, and accumulate on the MT plus end by recognizing some feature of MT plus ends. We propose two models for how XMAP215 promotes tubulin assembly while persisting at growing MT plus ends (Figure 6a). Model 1: XMAP215 molecules may repeatedly bind and release individual tubulin dimers that are immediately incorporated into the MT plus end. Model 2: XMAP215 may structurally stabilize the MT plus end in a growth phase by stabilizing a polymerized tubulin intermediate at the growing MT plus end and thus prevent the plus end from undergoing small depolymerization events that are observed even in growing MTs [2,77]. In this view, the tubulin dimer held by the TOG domains may be used as an element to stabilize the newly formed MT lattice, and it may or may not be released into the lattice (Figure 5a, Model 2).

We propose that CLASP is a MT rescue-promoting factor. CLASP binds tightly on the MT lattice pre-loaded with a tubulin dimer. When the MT undergoes a catastrophe, the MT plus end depolymerizes until it reaches a site on the MT lattice where CLASP molecules are present in high concentration. These CLASP molecules somehow reverse MT disassembly and mediate rescue. We envision two mechanisms by which CLASP might use its loaded tubulin dimer to accomplish rescue (Figure 6b). Model 1: CLASP may act as an MT polymerase that acts only locally on a depolymerizing MT end, possibly by releasing its bound tubulin dimer to polymerize into the depolymerizing MT lattice and restarting MT assembly. In this model, CLASP is similar to XMAP215 in that it increases the local concentration of tubulin dimer (Figure 6b, Model 1). Model 2: CLASP molecules may halt rapid MT disassembly by locally stabilizing the depolymerizing MT lattice, possibly by preventing protofilament curling (Figure 6b, Model 2). CLASP may use its bound tubulin dimer to transiently stabilize the lattice, and may not need to release it. A variation of Model 2 is that CLASP and its bound tubulin may induce some alternative MT lattice conformation that makes it resistant to MT depolymerization.

In these models, we note that CLASP and XMAP215 may share similar functions, either as local polymerases or

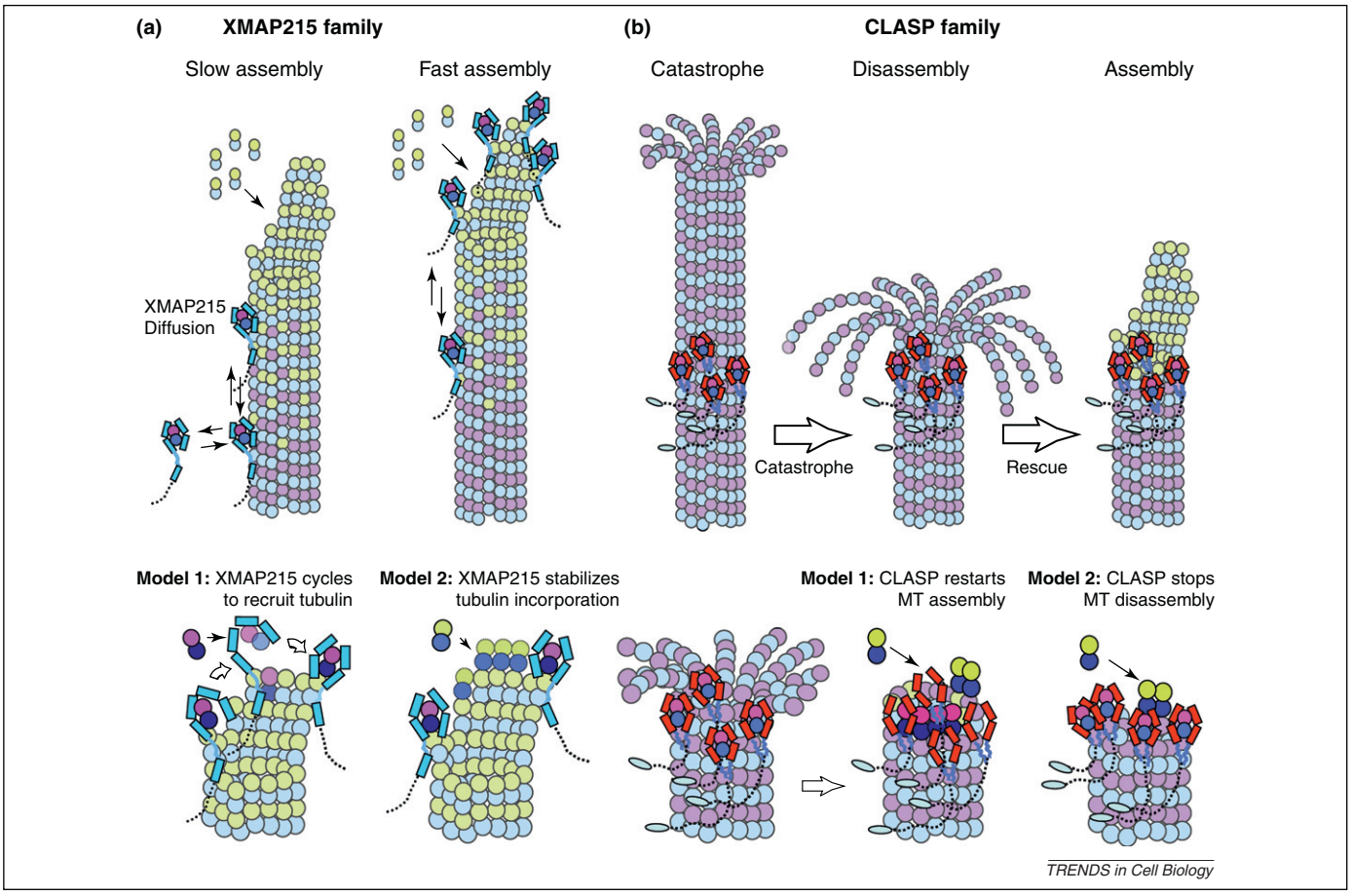

Figure 6. Models for XMAP215/Dis1 and CLASP mechanisms. (a) XMAP215/Dis1 family proteins are MT polymerases. Upper panel: XMAP215 binds tubulin dimers with TOG domains and the MT lattice with its SK domain (left). XMAP215-tubulin complexes bind and diffuse along MT lattices. MT assembly is slow in the absence of the XMAP215–tubulin complex. XMAP215-tubulin complexes accumulate at the MT plus end, which accelerates MT assembly (right). The lower panel shows two models for how XMAP215-tubulin complexes increase MT assembly rate. Model 1, XMAP215 TOG domain cycles to load its bound tubulin dimer at growing MT ends. It promotes multiple cycles of tubulin dimer binding, accompanied by conformational change that release tubulin at MT plus ends. Model 2, XMAP215 stabilizes the assembly conformation of a microtubule by binding and stabilizing polymerized-tubulin conformation (yellow) with its TOG domains. The bound tubulin may be a soluble tubulin dimer or a dimer located at a specific site on the MT, such as the very MT end or seam. The conformation of the tubulin dimer or its nucleotide state while bound to XMAP215 is not known and is shown here as GDP. (**b**) CLASP family proteins promote MT rescues and inhibit MT catastrophes. Upper panel: CLASP binds tubulin dimer with its TOGL domains and binds MT lattices with high affinity with its SR-rich domain (left). CLASP high affinity leads to sites of high concentration along MTs. When a dynamic MT undergoes catastrophe, MT disassembly occurs until the plus end reaches a site of high CLASP concentration (middle). There, CLASP locally promotes rescue events, in which MT depolymerization halts and MT assembly reinitiates (right). The lower panel shows two models of how CLASP molecules induce MT rescue: Model 1, CLASP molecules release their loaded tubulin dimer into the MT plus end and reinitiate polymerization. In this model, CLASP molecules act as local polymerase, similar to model 1 of XMAP215. Model 2, CLASP molecules utilize their loaded tubulin to prevent MT disassembly and restore MT to the assembly phase. During this activity, CLASP molecules may or may not release their bound tubulin. CLASP–tubulin complexes may halt depolymerization, whereas MT assembly may be reinitiated by tubulin dimers polymerizing from solution.

as local stabilizers. One key difference distinguishing these models is whether the TOG domains release the loaded tubulin dimer into the MT lattice.

Concluding remarks

We have just begun to understand how XMAP215/Dis1 and CLASP family proteins utilize tubulin dimers to regulate the dynamic states of MTs. Through analysis of representative proteins in each class, it is clear that these are related protein families that have distinct roles in MT regulation; XMAP215/Dis1 family proteins promote MT assembly, whereas CLASP family proteins promote MT rescues and inhibit catastrophes. A key feature of both of these protein families is that they function by binding to soluble tubulin dimers via TOG domains. There are many questions still remaining about how TOG domains function and how the tubulin dimer is used in MT regulation. A detailed understanding of the interaction between TOG domains and the tubulin dimer will be crucial to dissect the mechanism for how these proteins regulate dynamic MTs. What is the structural basis for why TOG domains bind to the soluble tubulin dimer and not to tubulin dimers polymerized in the lattice? Which TOG domains bind to which tubulin in the dimer, and do a pair of TOG domains bind to different faces on a single tubulin molecule? Some models predict that the TOG domain releases the tubulin dimer at MT plus ends. Do TOG domains release tubulin into the MT lattice? If so, what signals might allow these proteins to 'sense' being at a MT plus end and cause the hand-off of the tubulin dimer to the MT end at the right time and place? Although TOG domains bind to both GTP-tubulin and GDP-tubulin dimers *in vitro* (J.A-B., unpublished observation), whether CLASP and XMAP215 proteins function by somehow promoting or stabilizing the GTP state of tubulin during MT polymerization remains an open question. XMAP215/Dis1 TOG domains and CLASP TOGL domains may affect the incorporation of

tubulin dimers by stabilizing a polymerizing conformation of tubulin at MT plus ends.

A prime tool for future studies of the XMAP215 and CLASP families of proteins will be to exploit the differences and similarities between the two families. Differences in TOG/TOGL tubulin binding and SK/SR domain MT lattice association are likely to underlie the functional differences between these proteins. For instance, variations of the TOG domain tubulin-binding loop sequences may be responsible for different kinetics of tubulin binding and release. Further study of these intriguing proteins will provide crucial understanding of how MT polymers are regulated in cells and provide new conceptual insights into how MTs are regulated at the molecular level.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tcb.2011.06.007.

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