XMAP215: a key component of the dynamic microtubule cytoskeleton

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Microtubules are essential for various cellular processes including cell division and intracellular organization. Their function depends on their ability to rearrange their distribution at different times and places. Microtubules are dynamic polymers and their behaviour is described as dynamic instability. Rearrangement of the microtubule cytoskeleton is made possible by proteins that modulate the parameters of dynamic instability. Studies using *Xenopus* egg extracts led to identification of a microtubule-associated protein called XMAP215 as a major regulator of physiological microtubule dynamics. XMAP215 belongs to an evolutionarily conserved protein family present in organisms ranging from yeast to mammals. Together with members of the Kin I family of kinesins, XMAP215 and its orthologues form an essential circuit for generating dynamic microtubules *in vivo*.

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In the 1960s and 1970s two technical innovations, electron microscopy and indirect immunofluorescence, together with the discovery of glutaraldehyde fixations, heralded a breakthrough in cell biology. Among other things, these techniques revealed the existence of different filament systems – actin filaments, microtubules and intermediate filaments – extending throughout the cell; the static nature of the techniques themselves suggested a nondynamic 'skeleton' performing a function analogous to the role played by bone in supporting tissues. Thus these filaments were termed the cytoskeleton. In the 1980s, the use of fluorescence recovery after photobleaching (FRAP) [1,2] and other techniques [3,4] allowed measurement of the turnover rates of these cytoskeletal polymers inside cells; such techniques showed a surprisingly high rate of turnover of these polymers. In the case of microtubules, half-lives of 30 s for nonkinetochore microtubules in mammalian and embryonic mitotic spindles were measured [1,5]. Microtubules are polymerized from αβ-tubulin dimers. The purification of tubulin from cells made it possible to analyse microtubule polymerization *in vitro* [6] and demonstrated that the dimer only required GTP to polymerize.

These studies culminated in 1984 in the discovery of dynamic instability, which described the behaviour of the pure microtubule polymer [7–10] (Fig. 1). Microtubules have slow-growing minus ends, which are anchored at microtubule organizing centres such as centrosomes, and fast-growing plus ends, which extend out into the cytoplasm. The isolation of nucleation sites such as centrosomes [11] or axonemes [12] allowed the parameters of plus ends and minus ends to be examined separately. Both microtubule plus ends and minus ends growing under conditions of dynamic instability can transit between growing and shrinking states stochastically [9]. In other words, one can measure the chance that a microtubule will interconvert between growing and shrinking but never predict exactly when this event will occur. The transition from growth phase to shrinkage phase is called 'catastrophe' and the opposite transition is called 'rescue' [9] (Fig. 1b,c). Modelling studies demonstrated that the distribution of microtubules could be defined by the sum total of the parameters of dynamic instability [13]. This unique behaviour suggested interesting models for how microtubule turnover could be used to generate asymmetric patterns of microtubules, such as mitotic spindles [14–16].

These analyses of microtubule behaviour left an outstanding question: under physiological conditions, is microtubule turnover due to dynamic instability? This question was answered by analysis of single microtubule plus ends using video-enhanced differential interference contrast (VE-DIC) microscopy in newt lung cells [17,18]. A more general analysis using *Xenopus* egg extracts – the same material as popularized by the Nobel prize-winning work on the cell cycle [19,20] – allowed comparison of microtubule plus-end parameters between interphase and mitosis. Addition of fluorescently labelled tubulin to the extracts allowed the analysis of the behaviour of individual microtubules under physiological conditions [13,21] (Fig. 2a). These studies showed not only that microtubule turnover *in vivo* is due to dynamic instability but also that dynamic instability changes through the cell cycle: mitotic extracts have a catastrophe rate about 5–10 times that of interphase extracts. These studies demonstrated that dynamic instability exists in physiological conditions, and that it is modulated primarily by adjustment of the catastrophe rate, although changes in the rescue rate were also seen [22]. More recent studies in mammalian cells using GFP-tubulin have confirmed these differences in dynamic instability during the cell cycle [23]. In this review, we will give an overview of the studies of the molecular mechanism of microtubule dynamics in physiological conditions, focusing on the identification of XMAP215 as a key microtubule stabilizer, and the analysis of its role in physiological microtubule dynamics.

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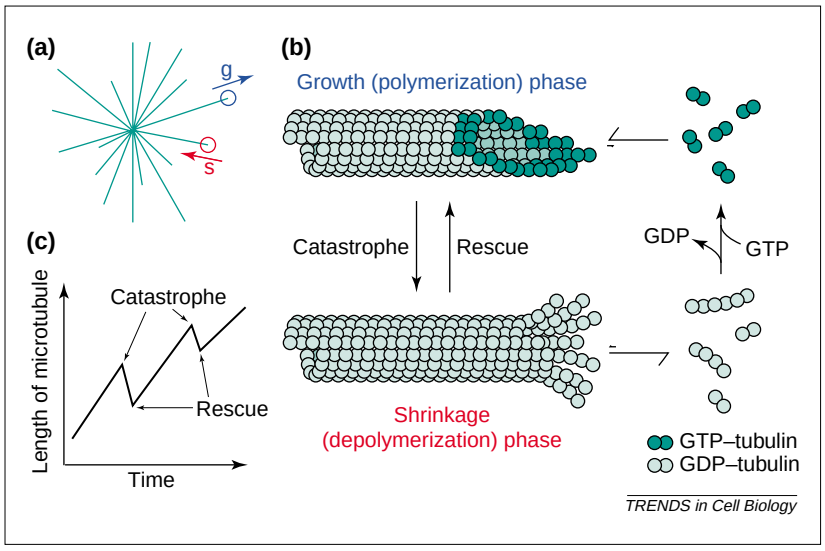

Fig. 1. Dynamic instability of microtubules. (a) Microtubules nucleated from centrosomes (microtubules; green). Growing (polymerizing; blue arrow) and shrinking (depolymerizing; red arrow) microtubules coexist at the same time. (b) Microtubules grow and shrink by polymerization and depolymerizaton of αβ-tubulin dimers at the microtubule ends. GTP-bound tubulin (green) is incorporated at the growing microtubule ends but GTP is hydrolysed to GDP in the lattice after tubulin incorporation into microtubules. During shrinkage phase, GDP-bound tubulin (light green) is released from the ends. The transition from growth phase to shrinkage phase is called catastrophe and the opposite transition is called rescue. Adapted from Refs [15,10]. (c) The transitions between growth phase and shrinkage phase of microtubules occur stochastically. Here, the length of a microtubule is plotted versus time.

XMAP215 as a major factor promoting microtubule polymerization

Because minus ends are anchored at centrosomes, it is thought that most of the morphogenetic properties of microtubules occur in association with the plus ends [14,16]. Minus-end dynamics are also involved in the dynamic properties of microtubule cytoskeletons [24,25], but will not be discussed here. Detailed comparison of the dynamic parameters of microtubule plus ends in cells/*Xenopus* egg extracts and *in vitro* showed that, in physiological conditions, the catastrophe rate and the growth rate were much higher than *in vitro* for a given concentration of tubulin [9,17,18,21]. The importance of this discovery was that it expanded the potential roles of dynamic instability in the generation of asymmetric microtubule-based structures. This stimulated studies to identify proteins that modulate intracellular dynamic instability, and to see how their activities are controlled. Several proteins that bind to the microtubule lattice had been isolated from bovine brain, called microtubule-associated proteins (MAPs). However, the microtubules in neurons are unusually stable, and thus bovine brain MAPs provided little clue as to those proteins that are important for control of microtubule dynamics in proliferating cells.

The first clue to the identity of such a protein came by purification of proteins from *Xenopus* extracts that stimulated the growth rate of microtubules [26]. Such biochemical studies led to the identification of one protein, a 215 kDa protein known as XMAP215 (originally known as XMAP), that stimulated the growth rate of microtubules eightfold [26].

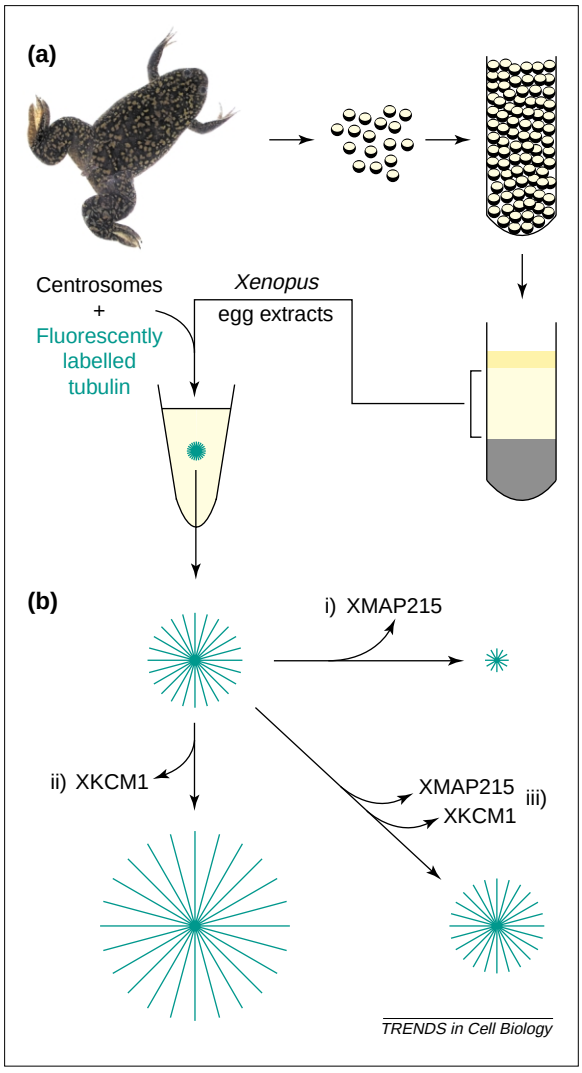

Fig. 2. The analysis of microtubule dynamics using *Xenopus* egg extracts. (a) A schematic protocol to analyse microtubule dynamics in frog egg extracts. Eggs laid by female frogs are collected and then crushed by centrifugation to generate cytosols. Centrosomes and a bit of fluorescently labelled tubulin are added to the extracts to visualize live images of microtubules nucleated from centrosomes. (b) Comparison of microtubule asters in immunodepletion experiments using *Xenopus* egg extracts. Immunodepletion of XMAP215 from the extracts causes decrease of aster sizes and increase of catastrophe frequency (i). By contrast, immunodepletion of XKCM1 decreases catastrophe frequency and increases aster sizes (ii). However, if the activity of XKCM1 is inhibited in XMAP215-depleted extracts, the aster size and the catastrophe frequency go back to the level of control (iii). These observations suggest that XMAP215 and XKCM1 oppose each other to regulate catastrophe frequency in *Xenopus* egg extracts.

Interestingly, XMAP215 has very different effects on microtubule dynamics *in vitro* when compared against brain MAPs, such as MAP2 and tau: XMAP215 strongly stimulates growth rate at plus ends without affecting catastrophe frequency *in vitro* [27]. By contrast, neuronal MAPs have moderate effects on growth rate but strongly suppress catastrophe frequency [28,29]. Immunodepletion of XMAP215 from *Xenopus* egg extracts using specific antibodies demonstrated that it was necessary for growth of microtubules [30]. The depletion of ~60% of

XMAP215 resulted in much shorter and more dynamic microtubules, in both interphase and mitotic extracts (Fig. 2b). The analysis of microtubule dynamics in XMAP215-depleted extracts demonstrated that, surprisingly, XMAP215 suppresses the frequency of catastrophe in both interphase and mitotic extracts [30], probably through its N-terminus [31]. By addition of purified XMAP215 to the depleted extracts, catastrophe frequency was restored to levels observed in control extracts. Therefore, these studies demonstrated that XMAP215 is a key regulator of the catastrophe frequency as well as the growth rate of microtubules in physiological conditions. To date, XMAP215 is the only stabilizing protein that appears to be absolutely required for microtubule growth in *Xenopus* egg extracts.

XMAP215 antagonizes XKCM1-induced catastrophe in *Xenopus* egg extracts

These immunodepletion experiments suggested that the activity of XMAP215 opposed the activity of catastrophe factors in *Xenopus* egg extracts. So far, two catastrophe factors have been identified: one is Op18/stathmin and the other is XKCM1 [10,32–34]. Op18/stathmin is a small heat-stable protein abundant in many types of cancer cells [35] that was purified as a factor capable of destabilizing microtubules from calf thymus [32,36]. On the other hand, XKCM1 is a *Xenopus* kinesin-related protein and belongs to the internal catalytic domain subfamily (Kin I subfamily for 'kinesin internal') [37,38]. These kinesins are nonconventional in the sense that they use their catalytic domain to induce microtubule catastrophes rather than to translocate along the microtubule lattice [39]. The Kin I subfamily also includes mammalian MCAK (mitotic centromere-associated kinesin) and *Caenorhabditis elegans* MCAK (CeMCAK), which associate with centromeres during mitosis [40,41]. In *Xenopus* mitotic egg extracts, the inhibition of XKCM1 activity decreases catastrophe frequency four times (in high-speed supernatant) [33] to seven times (in low-speed supernatant) [30], whereas >95% immunodepletion of Op18/stathmin causes a roughly two times decrease of catastrophe frequency (in low-speed supernatant) [42]. These results suggest that XKCM1 is the major factor regulating the catastrophe frequency in *Xenopus* egg extracts (Fig. 2b). Indeed, the inhibition of XKCM1 activity in XMAP215-depleted extract can suppress the catastrophe frequency to a level similar to that in nondepleted extracts (Fig. 2b) [30]. Therefore, these studies demonstrate that XMAP215 antagonizes the activity of XKCM1 in the regulation of microtubule dynamics in *Xenopus* extracts.

Reconstitution of physiological microtubule dynamics

The effects of immunodepletions of XMAP215 and XKCM1 on microtubule dynamics in *Xenopus* extracts suggested that the coordinated action of these proteins might explain why microtubules in cells polymerize rapidly and exhibit high rates of catastrophe. This hypothesis was tested by combining these two factors with purified tubulin *in vitro* and assessing whether such a mixture could reconstitute essential features of physiological microtubule dynamic instability [43]. XKCM1 reduced the steady-state length of microtubules, whereas XMAP215 opposed the tendency of purified XKCM1 to decrease the steady-state length of microtubules [43]. This result indicated that XMAP215 alone can oppose the ability of XKCM1 to decrease the steady-state length of microtubules assembled from purified tubulin. Using roughly physiological concentrations of the proteins, microtubules in this reconstituted mixture polymerized at an average rate of 8.7 $\mu m\ min^{-1}$ and underwent 1.06 catastrophe events per minute [43], rates that are similar to those exhibited by mitotic *Xenopus* extracts [13,42]. Thus, this simple three-component mixture recapitulated the essential features of physiological microtubule dynamic instability: fast polymerization and frequent catastrophe rates.

In the three-component mixture, the presence of XMAP215 must partially suppress the catastrophe-promoting activity of XKCM1 to generate the combination of fast polymerization and high catastrophe rates. This partial suppression of XKCM1-induced catastrophes by XMAP215 is central to understanding why the three-component mixture reconstitutes the combination of fast polymerization and high catastrophe rates.
In conclusion, these studies demonstrate that microtubules in the presence of XMAP215 together with XKCM1 have dynamic properties similar to those of microtubules in *Xenopus* egg extracts. They suggest that the essential features of dynamic instability in *Xenopus* extracts could be derived from the sole action of these two factors on tubulin. Recent structural studies have demonstrated that XMAP215 is a long thin molecule and can bind to a single protofilament of microtubules [44]. It has also been reported that XKCM1 binds to a protofilament of microtubules, and that the C-terminus of tubulin is required for the microtubule-destabilizing activity of XKCM1 [45]. XMAP215 might directly inhibit the protofilament binding of XKCM1, or it might interrupt the interaction between XKCM1 and the C-terminus of tubulin. The details of the molecular mechanism allowing XMAP215 to inhibit XKCM1-induced catastrophe remain to be determined.

The identification of this three-component system suggests a mechanism for control of microtubule dynamics through the cell cycle: subtle modulation of the activity of either XKCM1 or XMAP215 could change the dynamic properties of the microtubules. Indeed, *in vitro* analysis suggests that the dynamics

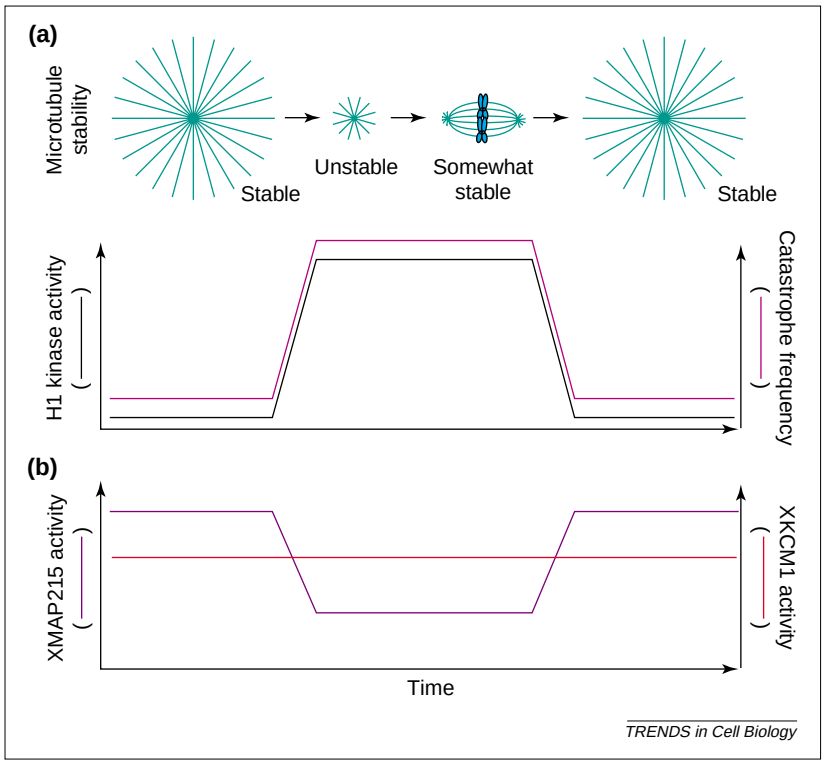

Fig. 3. Microtubule dynamics change during the cell cycle. (a) The change of microtubule stability during the cell cycle in *Xenopus* egg extracts. In interphase, microtubules are relatively stable and show low catastrophe frequency. During mitosis, the catastrophe frequency is increased and microtubules become unstable and more dynamic although the partial stabilization of microtubules occurs in the spindle. (b) A model of microtubule dynamics regulation by XMAP215 and XKCM1 during the cell cycle. XKCM1 seems to be active throughout the cell cycle so the activity of XMAP215 might be regulated in cell cycle-specific manner. In this model, the activity of XMAP215 to inhibit catastrophe is higher in interphase than that in mitosis although XMAP215 still has an activity to regulate catastrophe frequency in mitosis.

of the microtubules is very sensitive to changes in concentrations of either protein. It is likely that other regulatory proteins (e.g. Op18, other MAPs) make this three-component mixture more robust and less sensitive to subtle changes in protein concentrations. Analysis in *Xenopus* extracts suggests that XKCM1 is active throughout the cell cycle. Thus, a plausible mechanism for modulation of dynamic instability is through cell-cycle changes in the activity of XMAP215 (Fig. 3). Supporting evidence for such a model is that XMAP215 is hyperphosphorylated in mitosis [26] , and phosphorylation by Cdc2 kinase *in vitro* alters XMAP215 function [46].

The Dis1/XMAP215 family and microtubule dynamics

Is XMAP215 a special MAP required only for larger frog eggs or is it more generally required for microtubule dynamics? The cloning of XMAP215 revealed that it is a member of a conserved family of proteins [30] (Fig. 4) (see Ref. [47] for review). Members of this protein family were first reported to colocalize with microtubules in the fission yeast, *Schizosaccharomyces pombe*. Fission yeast Dis1 was originally isolated as a cold-temperature-sensitive mutant possessing a defect in sister chromatid separation [48]. The *dis1*$^{+}$ gene encodes a 93 kDa protein that colocalizes with cytoplasmic microtubules, spindles and spindle pole bodies [49]. The human homologue, ch-TOG, was isolated as a gene overexpressed in colon and hepatic cancer cells [50], and TOG protein has also been shown to bind to microtubules and tubulin [51,52]. Sequence analysis has revealed the presence of a ~200-residue repetitive subdomain in the N-terminal region conserved between Dis1 and TOG [53]. This subdomain is conserved in all the members of the Dis1/XMAP215 protein family, and includes HEAT repeats, which correspond to tandemly arranged bihelical structures (Fig. 4) [54]. The identification of other members in the past five years has revealed that this protein family is broadly conserved in organisms ranging from yeast to mammals and plants.

The identification of this orthologous family of proteins begs the question whether their function is conserved. *C. elegans zyg-9* and *Arabidopsis thaliana mor1* mutants have shorter microtubules than the corresponding wild types, as also observed in XMAP215-depleted *Xenopus* egg extracts [55,56]. In mutants and gene disruptants of Mtc1/Alp14, which is another orthologue in fission yeast, few cytoplasmic microtubles are observed [57–59]. These observations suggest that, at a phenotypic level, XMAP215 orthologues are conserved universally for the stimulation and/or for the stabilization of microtubules in eukaryotic cells. The only organism besides *Xenopus* in which microtubule dynamics has seriously been examined is *Saccharomyces cerevisiae*. Kosco and colleagues analysed microtubule dynamics in budding yeast cells and found that depletion of the Dis1/XMAP215 family member, Stu2, produces less dynamic cytoplasmic microtubules, associated with the phenotype of a decrease in the catastrophe frequency [60]. At first glance, this contrasts markedly with XMAP215-depleted *Xenopus* egg extracts. One interpretation of this result is that the activity of Stu2 might be different from that of XMAP215, and Stu2 itself might have the ability to promote catastrophe. Alternatively, other MAPs might be compensating for the absence of Stu2. Interestingly, analysis of the activity of Stu2 suggested that its activity is opposed by the activity of Kip3 [61]. Kip3 has been reported to destabilize microtubules [62], and indeed it is contained within the Kin I subfamily of microtubule-destabilizing kinesins in the phylogenetic tree [61]. Taken together, it seems that, although some of the details can vary, the functions of the XMAP215 family in regulating microtubule behaviour have been conserved through evolution. We believe that a three-component system of Dis1/XMAP215 family, Kin I family and tubulin have coevolved to maintain a highly dynamic state of microtubule plus ends during cell proliferation.

In addition to their essential role in microtubule dynamics, there is evidence in yeasts that the Dis1/XMAP215 protein family might have a second

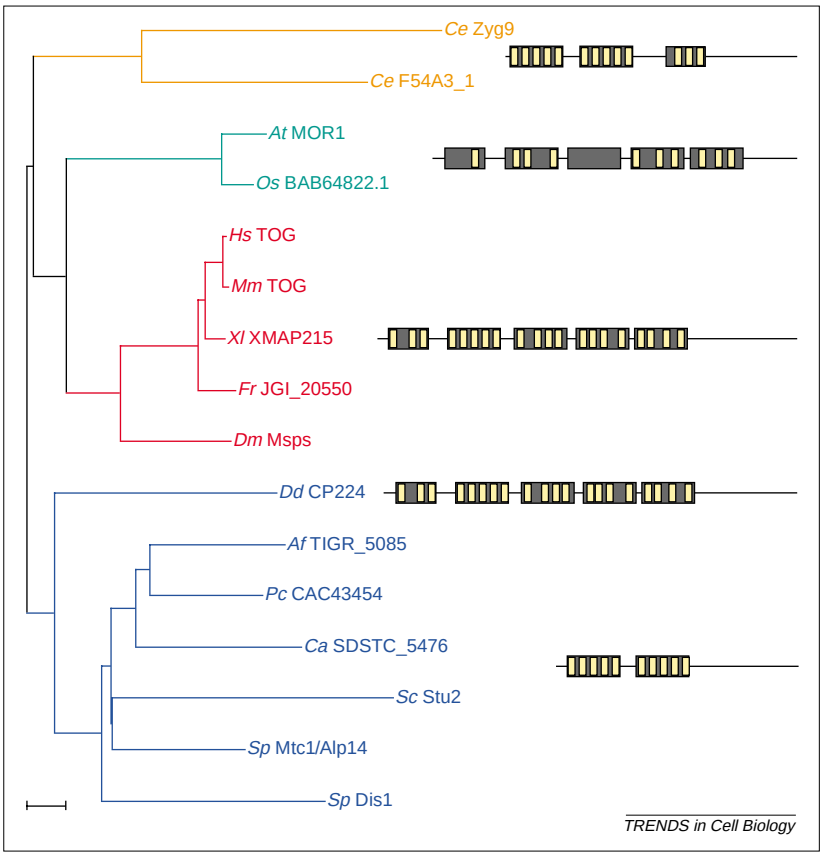

Fig. 4. Phylogenetic analysis and domain analysis of the Dis1/XMAP215 family. The Dis1/XMAP215 family has a characteristic repeat architecture in the N-terminal parts of the proteins, which are composed of the TOG domain (grey boxes), dispersed by HEAT repeats (yellow boxes). This region is thought to be responsible for protein–protein interactions. It is also known that several protein families that are involved in chromosome regulation, such as condensins and cohesins, also contain several copies of HEAT repeats [54]. Depending on the organism, two to five of these units are present in the XMAP215 family members. Whereas the proteins from fungi have only two of the repetitive elements, higher eukaryotes, including plants, have five copies, with the plant sequences containing highly diverged HEAT repeats. The protein architecture from *Dictyostelium* is closely related to the vertebrate and plant homologues. *Caenorhabditis elegans* has two distinct family members of the Dis1/XMAP215 family, with both proteins having three copies of the repetitive element. The third repeat element in *Ce* F54A3_1 is only partially conserved. The C-terminal part of the proteins, which is required for microtubule binding and localization of the centrosome/spindle pole body (SPB) and/or the kinetochore [31,68], shows little conservation between the different subkingdoms. The multiple sequence alignment of the Dis1/XMAP215 family was constructed with Clustal_X [72]; the phylogenetic tree construction was done using the Phylip package [73]. Abbreviations: *Af*, *Aspergillus fumigatus*; *At*, *Arabidopsis thaliana*; *Ca*, *Candida albicans*; *Ce*, *Caenorhabditis elegans*; *Dd*, *Dictyostelium discoideum*; *Dm*, *Drosophila melanogaster*; *Fr*, *Fugu rubripes*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Os*, *Oryza sativa*; *Pc*, *Pneumocystis carinii*; *Sc*, *Saccharomyces cerevisiae*; *Sp*,*Schizosaccharomyces pombe*; *Xl*, *Xenopus laevis*. The scale bar reflects 0.2 substitutions per site.

role in kinetochore activity. Dis1 and Mtc1/Alp14 in *S. pombe* and Stu2 in *S. cerevisiae* clearly localize to kinetochores [58,59,63]. Furthermore, *alp14* mutant cells fail to maintain high histone H1 kinase activity in the presence of microtubule-destabilizing drugs [59], suggesting that Alp14 is a component of the kinetochore-dependent spindle assembly checkpoint [59]. Examination of centromere movement in fission yeast *dis1* and budding yeast *stu2* mutants revealed that dynamic behaviour of sister centromeres during early mitosis requires Dis1/Stu2 function [63,64]. The interplay between kinetochores and microtubule plus ends is complex, making it difficult for detailed interpretation of Dis1/Stu2 function at the kinetochore.

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The mystery at minus ends

Both biochemical and genetic approaches have revealed that the Dis1/XMAP215 family has an essential function in microtubule dynamics at plus ends of microtubules. However, in spite of their essential function at plus ends, the predominant localization of these proteins in many organisms is at the minus ends [65–68]. Domain analysis of XMAP215 suggested that its C-terminal domain is required for targeting to centrosomes [31]. The function of this protein at minus ends remains a mystery. However, a clue has come from the recent identification of centrosomal protein TACC [69] interacting with Dis1/XMAP215 family proteins in *Drosophila* and human [70]. The efficient localization of Dis1/XMAP215 family proteins to centrosomes/spindle poles depends on TACC both in the mitotic and the meiotic spindles in *Drosophila* [70,71]. In addition, in *d-tacc* mutants, the mitotic but not the meiotic spindle shows reduced astral microtubules and becomes shorter than wild-type spindles [70,71]. One possibility is that Dis1/XMAP215 family proteins stabilize minus-end microtubules at spindle poles. However, it is also possible that these proteins when recruited to centrosomes promote efficient nucleation of microtubule plus ends from centrosomes [70].

Concluding remarks

Recent studies have revealed that the Dis1/XMAP215 family MAPs are central to microtubule dynamics *in vivo*. They seem to be essential for producing the dynamic properties of microtubules under physiological conditions. They endow microtubules not only with the ability to promote fast polymerization but also with the flexibility to allow high catastrophe rates.

Over the years, many different proteins have been isolated in different systems that influence the dynamic properties of tubulin *in vitro*. What is the role of these proteins in the control of dynamic instability *in vivo*? During the cell cycle, and during various developmental changes, microtubules significantly change their distribution and dynamics. It is likely that the many different microtubule regulators identified interface with the basic three-component system consisting of tubulin, XMAP215 and XKCM1 to modulate microtubule behaviour. Members of the Dis1/XMAP215 family are probably major targets of pathways regulating microtubule dynamics – for example, phosphorylation and dephosphorylation by cell cycle-regulated and developmentally regulated protein kinase/phosphatase systems. We still need to determine how Dis1/XMAP215 family proteins are regulated by the other factors if we are to understand the molecular mechanism of regulation of microtubule dynamics.

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The low-density lipoprotein receptor gene family: a cellular Swiss army knife?

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The low-density lipoprotein receptor gene family is an evolutionarily conserved group of cell-surface receptors produced by mammals and other organisms. Initially thought to be endocytic receptors that mediate the uptake of lipoproteins, recent findings have shown that these receptors have other roles in a range of cellular processes. Among other activities, members of this family act as signal transducers in neuronal migration processes, regulate synaptic plasticity or control vitamin homeostasis. Such multifunctionality is achieved by interaction with diverse cell-surface proteins including glycolipid-anchored receptors, G-protein-coupled receptors and ion channels. Here, we review the molecular interactions of this protein family with other cell-surface proteins that provide specificity and versatility – a versatility that may be reminiscent of a cellular Swiss army knife.

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Endocytic receptors are cell-surface proteins that transport macromolecules into cells through a process known as receptor-mediated endocytosis. This process involves a receptor on the cell surface binding to a specific ligand from the extracellular space, internalizing via specialized regions of the plasma membrane called clathrin-coated pits and moving to an intracellular vesicle (endosome) to discharge its cargo. Thus, the prevailing view has been that endocytic receptors mainly regulate the concentration of ligands in the extracellular fluids and deliver them to cells in need of these metabolites. Now, however, we have widened this view based on findings obtained in a class of endocytic receptors known as the low-density lipoprotein (LDL) receptor gene family [1,2]: the functions of these receptors are much more diverse than originally thought.

Members of this extended gene family can be found in a variety of species ranging from roundworms to insects to vertebrates. There are nine receptors in mammals, all of which share common structural motifs required for receptor-mediated endocytosis (Fig. 1). Their extracellular domains comprise clusters of complement-type repeats (the site of ligand binding) and epidermal growth factor precursor homology domains, which are essential for the pH-dependent release of ligands in endosomes. Their cytoplasmic tails harbor recognition sites for cytosolic adaptor proteins, including motifs that regulate internalization via clathrin-coated pits [3–11] (Box 1).

The prototype of the gene family is the LDL receptor, an endocytic receptor that mediates cellular uptake of cholesterol-rich lipoproteins. Genetic defects in this receptor in humans, rabbits or mice result in an inability to clear lipoproteins from the bloodstream and in massive accumulation of LDL particles in the circulation (hypercholesterolemia) [12]. Because other family members also bind to lipoproteins, roles had been anticipated for the receptors in the regulation of cellular and systemic lipoprotein metabolism [13]. Surprisingly, studies in various experimental systems including knockout mice and patients with receptor gene defects

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