Dis1/TOG universal microtubule adaptors - one MAP for all?

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Summary

Microtubules play central roles in various cellular processes in eukaryotes. The dynamics and organisation of interphase microtubules and mitotic spindles are dramatically altered during the cell cycle and development. However, the molecular mechanisms underlying this dynamic behaviour remain to be understood. In recent years, a novel family of microtubule-associated proteins (MAPs), the Dis1/TOG family, has emerged as a versatile regulator of microtubule function. These MAPs are highly conserved in eukaryotes from yeasts and plants to humans. The localisation and function of these MAPs are not determined simply by their intrinsic microtubule-binding activity. Instead this family executes its diverse roles by interacting with other regulatory molecules, including microtubule motors and centrosomal proteins. The modular structure of these MAPs may allow them to interact with multiple proteins and thereby be involved in a wide variety of microtubule and spindle functions.

Movies available on-line

Key words: Microtubule, Kinetochore, Spindle, MAP, Msps, XMAP215, Centrosome

Introduction

Microtubules represent one of three cytoskeletal systems found in all eukaryotes and play a wide variety of roles in cell division, cell architecture, cell polarity and intracellular transport. One of the most remarkable features of the microtubule is its dynamic behaviour. Microtubule dynamics and organisation are regulated spatially and temporally in the cell cycle and during development, and an understanding of the underlying mechanisms is central to cell and molecular biology. In addition to intrinsic instability, a class of proteins collectively called microtubule-associated proteins (MAPs), which includes microtubule motors, plays a key role in the regulation of microtubules in vivo (Mandelkow and Mandelkow, 1995). 'Conventional' MAPs were biochemically identified from mammalian brains through co-purification with microtubules some years ago. These are highly expressed mainly in neuronal cells and stabilise microtubules that form the backbone of highly polarised cells. Given the ubiquitous existence and highly dynamic nature of the microtubule in vivo, it is reasonable to assume that MAPs other than these neuronal forms might play an important role in dividing cells. Recently, a highly conserved family of MAPs, the Dis1/TOG family, was discovered in various organisms, including yeasts, animals and plants. These MAPs associate with various microtubule structures in a dynamic way and appear to be involved in a range of different aspects of microtubule function - for example, kinetochore function, spindle formation and cell morphogenesis. Although our knowledge of this new family of MAPs is still relatively patchy, some unified images have already started to emerge.

Here, we review in vivo roles of these MAPs in different microtubule functions across different organisms in relation to their localisation and biochemical properties. We discuss how and why these highly conserved MAPs are used so widely in different aspects of microtubule function.

Identification of the Dis1/TOG family from yeasts and plants to humans

The Dis1/TOG family of MAPs was independently discovered by several groups in a wide variety of organisms, including animals, amoeba, yeasts and plants. It is the only known family of MAPs common to plants and animals. The fact that Dis1/TOG family members were isolated by various approaches - genetics, biochemistry, cell biology and molecular cloning - reflects their multiple cellular roles.

Most members of the family were identified through genetic screens. The *mini spindles* (*msps*) gene in *Drosophila* was originally identified as a mutant that exhibits defective spindle formation (Cullen et al., 1999). In the screen used, lethal mutants that can survive until the larval stage were initially targeted because they are known to be a rich source of cell cycle mutants (Gatti and Baker, 1989). All mutants that affect cell division were further screened by anti-tubulin staining, and the locus was named after the cytological phenotype. As its name indicates, the *msps* mutation abolishes spindle integrity, and smaller additional spindles form in diploid cells. The *Caenorhabditis* member of the family, *zyg-9*, was isolated as one group of the *zyg* (zygote defective) mutants, a collection of temperature sensitive (ts) mutants that lay fertilised eggs that fail to hatch at the restrictive temperature (Hirsh and Vanderslice, 1976). Subsequent studies showed that the requirement for the *zyg-9* gene is strictly maternal and that a loss of ZYG-9 activity affects spindle formation in the meiotic

divisions and the first mitotic division (Wood et al., 1980; Kemphues et al., 1986).

The founding member of the Dis1/TOG family, fission yeast Dis1, was identified through mitotic mutants in a cytological screen of cold sensitive (cs) mutants (Ohkura et al., 1988). A group of mutants showed the so-called dis (defect in sister chromatid disjoining) phenotype. *dis1*$^+$ is one of the three loci that confer this phenotype. In contrast to other members of this family, *dis1*$^+$ is not essential for cell division, and its deletion produces a cs phenotype. This apparent dispensability of the *dis1*$^+$ gene has recently been explained following the identification of a second homologue, *alp14*$^+$ (also known as *mtc1*$^+$), through isolation of mutants that have defects in microtubule function (Garcia et al., 2001) and searches for a *dis1*$^+$ homologue in the fission yeast genome sequence (Nakaseko et al., 2001). *alp14*$^+$ is also dispensable; however, its deletion produces a ts phenotype in clear contrast to the cs phenotypes of *dis1* mutants. More importantly, *dis1 alp14* double mutants are lethal at any temperature, indicating that, unlike other organisms, fission yeast contains two Dis1/TOG homologues: Dis1 plays a major role when the cells are grown at the lower temperature, whereas Alp14 becomes essential at higher temperatures.

The budding yeast member of this family, *STU2*, was identified more directly by a screen aiming to isolate genes that affect tubulin function (Wang and Huffaker, 1997). Extragenic suppressor mutants were isolated from a cs β-tubulin mutant (*stu* stands for suppressors of a tubulin mutation). The suppressing activity of *stu2* is rather complicated, because the cs-rescuing activity of *stu2* is dominant, but *stu2* suppressor mutations on their own show no growth defect. Stu2p is essential for cell division and required for formation of a functional spindle (Severin et al., 2001).

The only known plant gene in the Dis1/TOG family, *mor1* (microtubule organisation gene *1*) in *Arabidopsis*, was identified by a cytological screen for microtubule organisation defects (Whittington et al., 2001). In the screen, chemically mutagenised seedlings were individually tested by immunostaining for an aberrant pattern of cortical microtubules in the epidermal cells of the first leaves. The screen identified two allelic ts *mor1* mutants.

Genetic screens are not the only avenue to lead to the identification of these MAPs. The *Dictyostelium* protein DdCP224 was discovered through an immunoscreen for centrosomal proteins (Graef et al., 2000). Human *ch-TOG* (for colonic and hepatic tumour overexpressing gene) was originally cloned as a gene that is overexpressed in tumour cells. Frog XMAP215 was originally identified as a factor that increases the elongation rate of microtubules in *Xenopus* mitotic egg extract. Subsequently, a 215 kDa protein responsible for this activity was purified and named XMAP215 (Gard and Kirschner, 1987).

The recent progress of genome-sequencing projects has also provided significant insight into the existence of these MAPs. It appears that, except for fission yeast, all eukaryotes contain a single member of the Dis1/TOG family.

Protein structures: conserved repeat motifs

The most characteristic feature of the Dis1/TOG family is an N-terminal repeating structure (Fig. 1). These repeats of ~200 amino acid residues (TOG domains) comprise 2-5 units, depending on the organism. Intriguingly each of these repeats consists of up to five HEAT motifs. HEAT repeats, found in a number of proteins, including the regulatory subunit of the type 2A protein phosphatase, condensin and cohesin subunits and coatomer components, are thought to be protein-protein interaction domains (Neuwald and Hirano, 2000). Because some repeats are degenerate at the primary sequence level, there is a degree of uncertainty over the structure of each protein (Cassimaris et al., 2001).

The family can be divided into three groups. The first group consists of human ch-TOG, frog XMAP215, fruit fly Mini spindles, slime mould (amoeba) DdCP224 and plant MOR1. Members of this group contain five repeats in the N-terminal region and a conserved C-terminal non-repeat domain. A recent study of XMAP215 suggests that these proteins are long thin monomers (Cassimeris et al., 2001). The second group consists of only one known protein, ZYG-9, which has three repeats in the N-terminal region and a C-terminal non-repeat region that has no apparent similarity to the other proteins. The third group consists of yeast proteins, Stu2p, Dis1 and Alp14, which have two repeats in the N-terminal region and a C-terminal non-repeat region. The non-repeat regions in the yeast proteins have little similarity at a primary sequence level but all contain a predicted coiled-coil region, which suggests they have equivalent higher-order structures.

These C-terminal non-repeat regions are likely to be responsible for microtubule-binding and centrosome, spindle pole body (SPB) or kinetochore localisation. In XMAP215 and DdCP224, the region is sufficient to direct centrosome/SPB localisation (Popov et al., 2001; Graef et al., 2000). In Dis1, the C-terminal region is sufficient for microtubule binding and localisation to the SPB and kinetochore (Nakaseko et al., 1996; Nakaseko et al., 2001). In contrast, the significance and function of the repeats that define the Dis1/TOG family are not yet understood. The repeats do not appear to correspond to microtubule-binding motifs or relate to any other particular

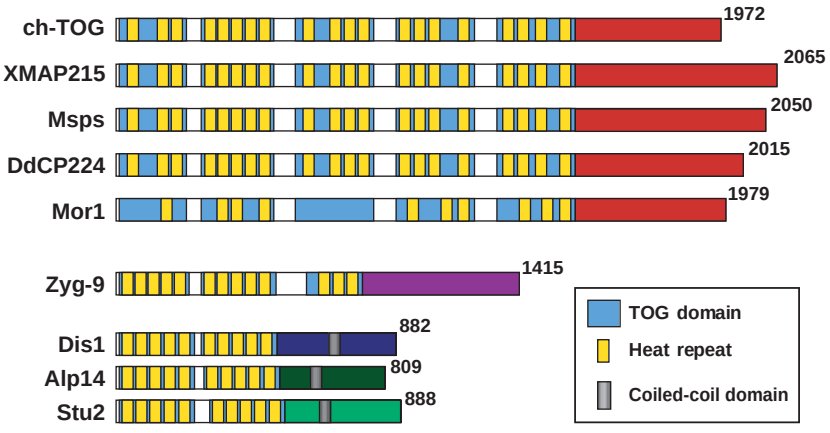

Fig. 1. Architecture of the Dis1/TOG family of MAPs. TOG domains (light-blue boxes), HEAT-repeating units (small yellow boxes) (Neuwald and Hirano, 2000), and coiled-coil regions (small grey boxes) are shown in different members of the Dis1/TOG family from various organisms. The last TOG domain in the first and second groups is very degenerate at the primary sequences (Cassimeris et al., 2001).

function. Given the X-ray structure of HEAT repeats and the diverse function of proteins that possess them (Neuwald and Hirano, 2000; Groves et al., 1999), it is likely that this is the protein-protein interaction domain for each member of the Dis1/TOG family. Depending on different species and biological context, distinct proteins might interact with members of the Dis1/TOG family through the HEAT repeats, which could explain apparent divergent roles of the Dis1/TOG family among species (see below).

Roles of Dis1/TOG proteins at the plus ends of microtubules

Members of the Dis1/TOG family of MAPs can bind directly to microtubules and have an intrinsic ability to promote microtubule polymerisation (Gard and Kirschner, 1987; Charrasse et al., 1998). Microtubules are dynamic polymers that randomly alternate between an elongation phase and a shortening phase at each end. When purified XMAP215 is added to pure microtubules, it increases the elongation rate dramatically at the plus end of microtubules about tenfold, but at the minus end only about twofold (Gard and Kirschner, 1987; Vasquez et al., 1994). In addition, it also increases the shortening rate and suppresses the frequency of rescue (the transition from shortening to elongation) at the plus end, which produces a population of longer and more dynamic microtubules. Equivalent experiments using purified TOG protein show that it increases the elongation rates about twofold but equally at both ends (Charrasse et al., 1998).

These pure biochemical systems do not fully represent the real activity of this family inside the cell. Depletion of XMAP215 from *Xenopus* egg extract increases the catastrophe frequency at the plus end of microtubules assembled from centrosomes, indicating that XMAP215 has catastrophe-suppressing activity in the extract (Tournebize et al., 2000). This activity, however, appears not to be an intrinsic activity of XMAP215; instead XMAP215 appears to antagonise XKCM1, a major catastrophe-promoting factor in the extract (Tournebize et al., 2000). XKCM1 and its mammalian homologue, Kin I, are kinesin-like proteins that can destabilise microtubules by increasing the catastrophe frequency (Walczak et al., 1996; Desai et al., 1999). How these two activities, namely microtuble stabilisation and catastrophe promotion, are regulated during the cell cycle is not well understood at present. It is the catastrophe-suppressing activity of XMAP215 that promotes microtubule assembly from centrosomes in the extract (Tournebize et al., 2000). In addition, XMAP215 also appears to be required for centrosome-independent aster formation induced by Ran, a ubiquitous GTP-binding protein that is essential for spindle formation (Wilde and Zheng, 1999).

In vitro assembly of spindles in *Xenopus* egg extract requires XMAP215 (Tournebize et al., 2000). Partial depletion of XMAP215 from the extract decreases the size of spindles, and near-complete depletion of XMAP215 prevents spindle assembly all together. Protein truncation analysis of XMAP215 shows that addition of an excess amount of the N-terminal fragment containing part of the HEAT repeat domain but lacking the C-terminal microtubule-binding and centrosome localisation domains can rescue defects caused by the depletion of XMAP215. This suggests that it is the HEAT repeats that directly or indirectly interact with XKCM1 (Popov et al., 2001).

In the nematode *zyg-9* mutant, spindle formation during meiosis is disrupted. Subsequent migration of the pronucleus and rotation of the centrosome-nuclear complex are also disrupted, presumably owing to incomplete astral microtubules. Moreover, the spindle microtubules of the first mitotic division are unusually short in the mutant, and the requirement for *zyg-9* is greatly reduced in later cell divisions, which have shorter spindles (Albertson, 1984; Kemphues et al., 1986). On the basis of these observations and the aforementioned in vitro study of XMAP215, Matthews et al. have suggested that ZYG-9 is required for formation of long microtubules (Matthews et al., 1998).

In budding yeast, a ts *stu2* mutant undergoes metaphase arrest in a spindle-checkpoint-dependent manner. The *stu2*

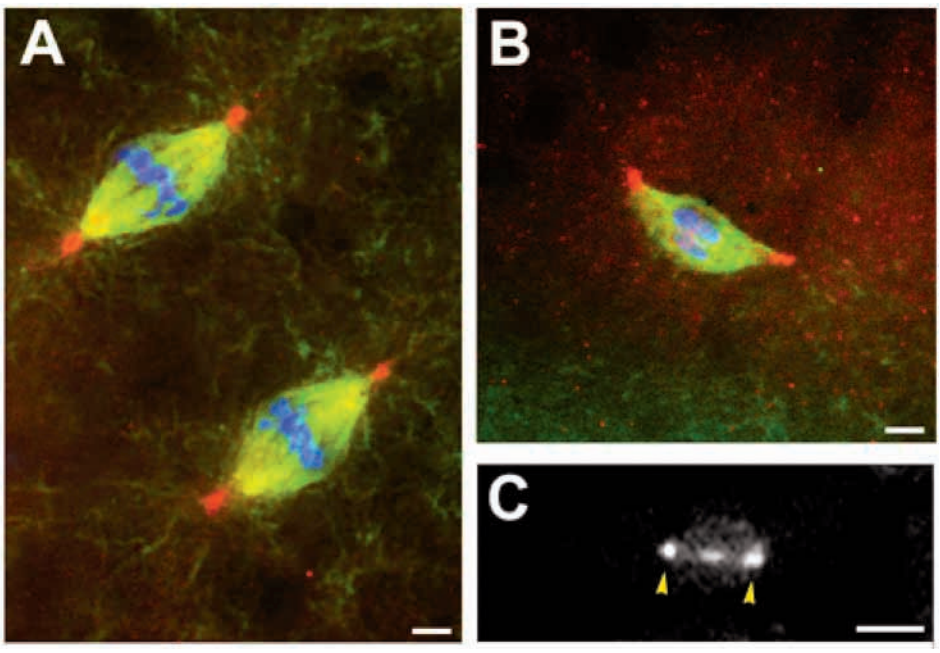

Fig. 2. Universal localisation to spindle poles - centrosomes, acentrosomal poles and SPBs. (A) *Drosophila* Msps localises to centrosomes of mitotic metaphase spindles in *Drosophila* syncytial embryos (red, Msps; green, tubulin; blue, DNA). (B) Msps also localises to acentrosomal spindle poles at female meiosis I (red, Msps; green, tubulin; blue, DNA). (C) Fission yeast Alp14 localises to SPBs (two stronger signals indicated by arrow heads) at mitotic metaphase as well as kinetochores (weaker signals in the centre). Bars, 2 μm.

mutant is also defective in spindle elongation (anaphase B). This defect can be rescued by deletion of the *kip3* gene, which encodes a kinesin-like protein. This suggests that antagonism between the Dis1/TOG family of MAPs and a kinesin-like protein is conserved (Severin et al., 2001)

Roles of Dis1/TOG proteins in kinetochore function

In vitro studies using *Xenopus* extracts revealed that the Dis1/TOG family of MAPs assist the assembly of spindle microtubules by promoting general microtubule polymerisation at the plus end. However, the studies from fission yeast indicate that these MAPs can function in a more restricted way. The loss of Dis1 results in a failure of sister chromatid separation without affecting formation of pole-to-pole spindles and spindle elongation (Ohkura et al., 1988; Nabeshima et al., 1995). Real-time analysis revealed that *dis1* mutants lack a period equivalent to metaphase (Nabeshima et al., 1998). This can be ascribed to defects in formation of pole-to-kinetochore microtubules in this mutant, which leads to the suggestion that Dis1 is specifically required for kinetochore microtubule function but not for the assembly of spindle microtubules in general. Live image analysis using GFP-tagged Dis1 showed that Dis1 localises to mitotic kinetochores, and furthermore chromatin immunoprecipitation (CHIP) demonstrates that it associates with the central core region of centromeres (Nakaseko et al., 2001). Significantly the association is observed only during mitosis. A recent study indicates that Stu2p in budding yeast also localizes to kinetochores and has a similar function (He et al., 2001).

Although the fission yeast homologues of these MAPs, Dis1 and Alp14, play redundant roles in cell viability, the phenotypes of the mutants are not identical. In contrast to deletion of *dis1*, the *alp14* deletion abolishes spindle microtubule assembly as well as formation of bipolar spindles. This suggests that Alp14 promotes general spindle microtubule assembly. Furthermore *alp14* mutants have compromised interphase microtubules: cytoplasmic microtubules are much shorter and misoriented. Therefore Alp14 might play a more general role in assembly of microtubules in vivo.

Probably the most interesting observation is that loss of *alp14* compromises the spindle-assembly checkpoint. This checkpoint prevents sister chromatid segregation and exit from mitosis until all kinetochores are attached to microtubules. Most components (e.g. Mad1, Mad2, Mad3, Bub1 and Bub3) of the spindle checkpoint are conserved through evolution and localise to kinetochores (Amon, 1999). In the presence of microtubule-depolymerising reagents, the *alp14* deletion mutant fails to maintain mitotic arrest, which is reminiscent of spindle-checkpoint-defective phenotypes. Cytological analysis shows that Alp14 protein localises to the kinetochore periphery, and CHIP indicates that it interacts with centromeric DNA. In common with Dis1, this association is cell cycle specific and observed only during mitosis. In the light of checkpoint function, it may be significant that association of Alp14 with the kinetochore is dependent on mitotic spindles, whereas Dis1 appears to localise to kinetochores independently of spindles.

At present it is not clear whether the kinetochore functions of this family are conserved in organisms other than yeasts. There are some reports of kinetochore localisation in other organisms. Immunostaining of DdCP224 revealed microtubule-dependent localisation around kinetochore regions (Graef et al., 2000). In addition, ZYG-9 shows some immunostaining on the periphery of chromosomes during metaphase and accumulates on chromosomes when spindle microtubules are depolymerised (Matthews et al., 1998). Given that *C. elegans* has holocentric chromosomes, this might represent kinetochore localisation. Note that the kinetochore localisation of Alp14 (but not SPB localisation) becomes apparent at mitosis only when live imaging of Alp14-GFP is used. Conventional fixation and subsequent procedures for immunofluorescence microscopy obscure the image at the kinetochore. If we are to know the in vivo localisation and functions of this family, detailed studies are required in other organisms.

Roles of Dis1/TOG MAPs at spindle poles

Although it is not entirely clear how conserved the kinetochore localisation of Dis1/TOG MAPs is, the localisation of these MAPs to the centrosome/SPB is universally observed from yeasts to humans (Fig. 2) (Charrasse et al., 1998; Cullen et al., 1999; Garcia et al., 2001; Graef et al., 2000; Matthews et al., 1998; Nabeshima et al., 1995; Tournebize et al., 2000; Wang and Huffaker, 1997). The localisation to the centrosome/SPB remains even after microtubules are depolymerised, and immunoelectron microscopy indicates that XMAP215 localises to pericentriolar material as well as microtubules (Popov et al., 2001).

The localisation of these proteins to spindle poles is not limited to the centrosome/SPB-containing poles. Female meiotic spindles commonly lack centrosomes in organisms such as *Drosophila*, *Xenopus* and mouse (McKim and Hawley, 1995). Live image analysis using *Drosophila* showed that it lacks a discrete microtubule-organising centre (MTOC), and microtubules apparently grow from chromatin and eventually form a bipolar spindle. Surprisingly Msps protein localises to these acentrosomal poles. This is a significant finding, because no protein, including γ-tubulin, has been shown to localise to the acentrosomal poles in *Drosophila*.

The mechanism for localisation of Msps to acentrosomal poles has been characterised in detail. It requires at least two other proteins, D-TACC (*D*rosophila-transforming acidic coiled-coil-containing) and the Ncd motor (Cullen and Ohkura, 2001). D-TACC associates and colocalises with Msps at the acentrosomal poles and is required for localisation of the protein at the poles. Indeed, loss of D-TACC produces a spindle defect identical to that of the *msps* mutants. The Ncd motor is a kinesin-like minus-end-directed microtubule motor involved in spindle formation at female meiosis (Hatsumi and Endow, 1992; Matthies et al., 1996). Ncd is required for efficient localisation of Msps to the poles, but D-TACC can localise to the poles in an Ncd-independent manner. Given that the Ncd motor localises along spindle microtubules, it could transport Msps to the poles, whereas D-TACC could anchor it there (Cullen and Ohkura, 2001). It would be of interest to know which region of Msps interacts with these proteins.

The involvement of D-TACC in Msps localisation was also demonstrated in mitosis of syncytial embryos (Lee et al., 2001). In syncytial embryos of *Drosophila*, loss of D-TACC greatly decreases the accumulation of Msps at centrosomes,

and the centrosomal microtubules appear to be destabilised. When D-TACC is overexpressed, both D-TACC and Msps, but not γ-tubulin, accumulate on and around the centrosomes, and the centrosomal microtubules appear stabilised. This microtubule-stabilising effect requires Msps activity. In human cultured cells, TACC3, one of three D-TACC homologues, is capable of recruiting TOG when it is overexpressed, which indicates that the interaction between Msps and D-TACC is conserved (Lee et al., 2001).

Loss of Msps disrupts structural and functional integrity of the mitotic spindle in larval neuroblasts (Cullen et al., 1999). A typical defect is that, in addition to a normal bipolar spindle, one or more extra bipolar spindles are formed in diploid cells, but processes such as microtubule assembly, pole focusing and bipolarity itself are not affected. In some cases, one spindle pole appears to split into two. A female sterile allele of *msps* in fact shows tripolar spindles; again other aspects, such as microtubule assembly and pole focusing, are unaffected (Cullen and Ohkura, 2001). Considering the difference between the mechanisms of spindle formation at mitosis and female meiosis, these defective phenotypes seem remarkably similar.

The microtubule-assembly-promoting activity of XMAP215 is sometimes referred to as plus end 'specific' (Gard and Kirschner, 1987). This is rather misleading, because the study clearly shows that it promotes microtubule assembly at the minus end as well. A loss of such a localised microtubule-stabilising activity at spindle poles may cause destabilisation of spindles, leading to the defective phenotype seen in *Drosophila* mutants. Alternatively, given that a mutant comprising the C-terminal half of XMAP215 is reported to have microtubule-bundling activity (Popov et al., 2001), loss of bundling activity at the poles might contribute to the spindle abnormality.

Roles of Dis1/TOG MAPs in cell morphogenesis

Microtubules form not only the spindle during mitosis but also a highly organised cytoskeleton during interphase and in differentiated postmitotic cells in eukaryotes. Appropriate regulation of the dynamics and organisation of these microtubules is crucial for cell morphogenesis and polarity. Not surprisingly the Dis1/TOG MAPs are also implicated in cell morphogenesis.

alp14 in fission yeast was originally identified as a cell shape mutant (*alp* stands for *al*tered *p*olarity) (Radcliffe et al., 1998). Fission yeast is a cylindrical cell that grows only at the ends. Cytoplasmic microtubules run from the central region to the tips of the cell along the long axis, their plus ends being located at the tips (Drummond and Cross, 2000; Tran et al., 2001). In this yeast, as in other eukaryotes, microtubules are crucial for growth polarity. In the *alp14* mutant, cytoplasmic microtubules are short and do not reach the tips, which results in branched or bent cells (Garcia et al., 2001). In contrast, *dis1* mutants show no defects in cell morphology. However, it is likely that a role of Dis1 in cell morphology is hidden by Alp14, since the *dis1 alp14* double deletion mutant shows severe morphological abnormalities, and overproduction of Dis1 induces alteration of growth polarity (Garcia et al., 2001; Nakaseko et al., 1996; Nakaseko et al., 2001). The fact that both proteins localise to cytoplasmic microtubules during interphase is consistent with a function in cell morphology. However, there is a significant difference in the modes of interaction of these proteins with microtubules: Dis1 localises along cytoplasmic microtubules, whereas Alp14 can be seen as particles that constantly move along the microtubules (see below) (Nabeshima et al., 1995; Garcia et al., 2001; Nakaseko et al., 2001).

This family of MAPs also appears to be involved in plant cell morphogenesis (Whittington et al., 2001). *mor1* mutations in *Arabidopsi*s cause ts disruptions of cortical microtubules. At the restrictive temperature, cortical microtubules become shorter through depolymerisation, and their organisation is disrupted. Normal microtubule organisation is rapidly restored after a return to the permissive temperature. Interestingly, in plants, loss of MOR1 appears not to affect formation of mitotic and cytokinetic microtubules. The gene is ubiquitously expressed, and inactivation of MOR1 by temperature shift alters the morphology of any organ examined, at any stage of development; this suggests that MOR1 activity is ubiquitously required throughout development. This alteration of organ morphology is apparently caused by changes in cell morphology not by a change in cell number or pattern.

Although a role for other members of this family in cell morphogenesis has not yet been shown, human TOG and frog XMAP215 are known to localise to cytoplasmic microtubules during interphase in cultured cells (Tournebize et al., 2000). The two genes, together with fly *msps*, are expressed in differentiated post-mitotic cells, such as the adult brain (Gard and Kirschner, 1987; Charrasse et al., 1998; Cullen et al., 1999). How the Dis1/TOG family functions in cell morphogenesis in multicellular organisms is an interesting area to be explored.

Dynamic localisation and protein interaction

An important feature of the Dis1/TOG MAPs is that their function and localisation are not dictated simply by direct interaction with microtubules. Their localisation changes dynamically during the cell cycle and organism development. Dynamic localisation is best highlighted by real-time analysis using GFP-tagged Dis1 and Alp14 proteins in fission yeast (Fig. 3; supplementary data at http://jcs.biologists.org/supplemental/) (Garcia et al., 2001; Nakaseko et al., 2001). During interphase, both proteins localise to interphase cytoplasmic microtubules. In particular, Alp14 dynamically interacts with microtubules as constantly moving particles. At present the mechanisms and significance of such a dynamic localisation are not clear. However, given the fact that *alp14* mutants show compromised interphase microtubules associated with polarity defects, the protein must play a crucial role in maintaining microtubule integrity. Once cells enter mitosis, both Dis1 and Alp14 change their localisation. These two proteins associate with kinetochores as well as SPBs. The restriction of the activities of these MAPs to specific locations and cell cycle stages is likely to be very important for their function.

Such cell-cycle-dependent localisation is also observed in other organisms. In budding yeast and *Dictyostelium*, localisation of Dis1/TOG family members to the centrosome/SPB is observed throughout the cell cycle (Wang and Huffaker, 1997; Graef et al., 2000). In contrast, in cultured human and frog cells, the localisation to centrosomes is

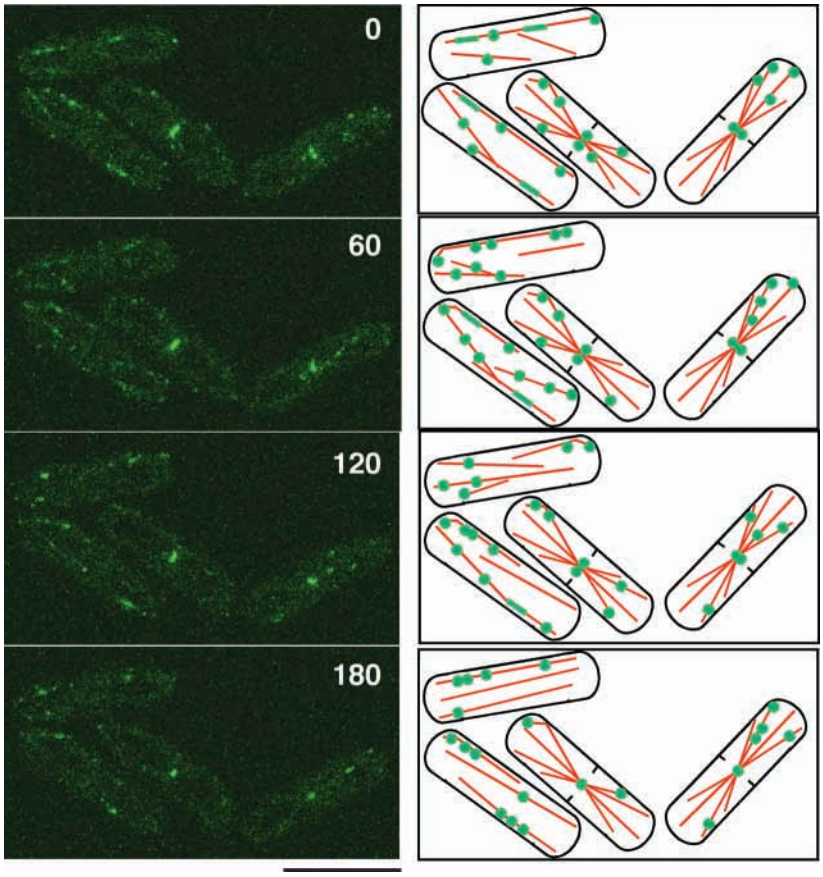

Fig. 3. Dynamic localisation of fission yeast Alp14 during interphase. Four interphase cells, in which two are in late G2 phase (left), whereas the other two (right) are in post-anaphase and G1 phase, are shown. Live images of Alp14-GFP are shown every 60 seconds, and schematic localisation of Alp14 (green), together with microtubules (red), is depicted in the right-hand panels. The movie of this sequence can be found at http://jcs.biologists.org/supplemental/. Bar, 10 μm.

specific to mitosis (Charrasse et al., 1998; Tournebize et al., 2000; Popov et al., 2001). Studies in *Drosophila* indicate that this localisation can be developmentally regulated (Cullen et al., 1999). In syncytial embryos, in which cell cycle progression is very rapid (no G1 or G2 phase), Msps localises to centrosomes throughout the cell cycle. However, in cellularised embryos, in which the cell cycle is slowed down dramatically, the protein localises to centrosomes only during mitosis.

Phosphorylation may be in part responsible for this cell-cycle-dependent regulation. The peaks of in vivo phosphorylation of XMAP215 coincide with meiotic or mitotic metaphases (Gard and Kirschner, 1987). Dis1 is also phosphorylated in vivo at potential Cdc2-target sites (Nabeshima et al., 1995). Similarly, TOG is phosphorylated by Cdc2 in vitro, and phosphorylation changes its

Table 1. The Dis1/TOG family in different species

Organisms/name	Number of repeats	Localisation	Function	Interactors
Human (*Homo sapiens*) TOG	5*	M: centrosome, spindle‡ I: cytoplasmic MT/ER	Promote MT assembly at both ends§	MT, tubulin dimer, TACC3, Cyclin B
Frog (*Xenopus laevis*) XMAP215	5*	M: centrosome, spindle I: cytoplasmic MT	Promote MT assembly mainly at the plus end¶, counteract against XKCM1, spindle and aster, MT assembly	MT, XKCM1**, Cyclin B
Fruit fly (*Drosophila melanogaster*) Mini spindles (Msps)	5*	M: centrosome, spindle acentrosomal poles (meiosis) I: centrosome (syncytial) not centrosome (cellularised)	Spindle bipolarity, centrosomal MT assembly	MT, D-TACC, Ncd**
Nematode (*Caenorhabditis elegans*) ZYG-9	3*	M: centrosome, spindle, KC? I: not centrosome	Spindle formation, pronucleus migration, rotation of centrosome/nuclear complex	
Fission yeast (*S. pombe*) Dis1	2	M: SPB, spindle, KC I: cytoplasmic MT	Kinetochore-MT interaction	MT
Alp14/Mtc1	2	M: SPB, spindle, KC I: cytoplasmic MT‡‡	Spindle MT assembly, spindle checkpoint, cytoplasmic MT assembly/cell morphogenesis	MT
Budding yeast (*S. cerevisiae*) Stu2p	2	M: SPB, spindle, KC I: SPB, cytoplasmic MT	Kinetochore-MT interaction, spindle elongation	MT, Spc72p, Kip3p**
Slime mould (Amoeba) (*Dictyostelium discoideum*) DdCP224	5*	M: centrosome, KC I: centrosome, cytoplasmic MT	Centrosome duplication?	
Plants (*Arabidopsis thaliana*) MOR1	5*		Cortical MT assembly/cell morphogenesis	

*The last TOG repeat of the protein is degenerate at a primary sequence level.
‡Abbreviations used in this table: MT, microtubule; ER, endoplasmic reticulum; KC, kinetochore; M, M-phase; I, interphase.
§Purified TOG increases the elongation rate at both ends roughly twofold.
¶Purified XMAP215 increase the elongation rate roughly tenfold at the plus end and roughly twofold at the minus end. It also increases the shortening rate threefold and nearly eliminates rescue.
**No physical interaction was shown.
‡‡As moving particles along cytoplasmic microtubules.

ability to modulate microtubule dynamics (Vasquez et al., 1999).

The multidomain structure of members of the Dis1/TOG family may also contribute to dynamic localisation and function. Truncation studies of XMAP215 indicate that it has at least three separate domains in terms of binding to microtubules (Popov et al., 2001). Each domain contributes differently to how XMAP215 interacts with microtubules in vitro and in vivo. The centrosome-localisation domain is separable from the microtubule-binding domains. In contrast, a truncation study of TOG revealed that the N-terminal region is a microtubule-binding domain whereas the C-terminal region is a tubulin-dimer-binding domain (Spittle et al., 2000). Truncation studies of Dis1 indicate that it consists of a central microtubule-interaction domain, the C-terminal SPB- and kinetochore-localisation domain, and the N-terminal repeat domains, which modulate its localisation in vivo (Nakaseko et al., 1996; Nakaseko et al., 2001).

Protein-protein interaction is likely to play a major part in the function and localisation of these MAPs. As mentioned earlier, in the case of *Drosophila* Msps, a physical interaction with D-TACC mediates localisation to centrosomes during syncytial mitosis and to acentrosomal spindle poles in female meiosis (Lee et al., 2001; Cullen and Ohkura, 2001). At least in *Drosophila* female meiosis, efficient localisation also relies on the minus-end-directed motor Ncd (Cullen and Ohkura, 2001). This interaction (involving TOG and TACC3) is conserved in humans. Although D-TACC/TACC3 is not obviously conserved in yeasts, Spc72p in budding yeast might play a role in the SPB localisation of Stu2p. Spc72p physically interacts with Stu2p and localises to the cytoplasmic side of the SPB (Chen et al., 1998). Note that both D-TACC/TACC3 and Spc72p consist of multiple coiled-coil domains (Gergely et al., 2000). One known function of Spc72p is to act as an adaptor for γ-tubulin. A further study is required if we are to determine the role of Spc72p in Stu2p localisation and function. Furthermore, dynamic movements of Alp14 along interphase microtubules and its kinetochore localisation suggest that Alp14 interacts with motor molecules that remain to be identified.

The Dis1/TOG family of MAPs thus appear to interact, directly or indirectly, with a wide variety of molecules. As mentioned earlier, an in vitro study using *Xenopus* extract shows that XMAP215 affects microtubule dynamics by counteracting the catastrophe-promoting activity of XKCM1 (Tournebize et al., 2000). Human TOG has been shown to target cyclin B to microtubules in vitro (Charrasse et al., 2000), but it is not clear whether this is also the case in living cells. Further identification and characterisation of interacting proteins will be crucial to our understanding of the molecular function and regulation of the Dis1/TOG family.

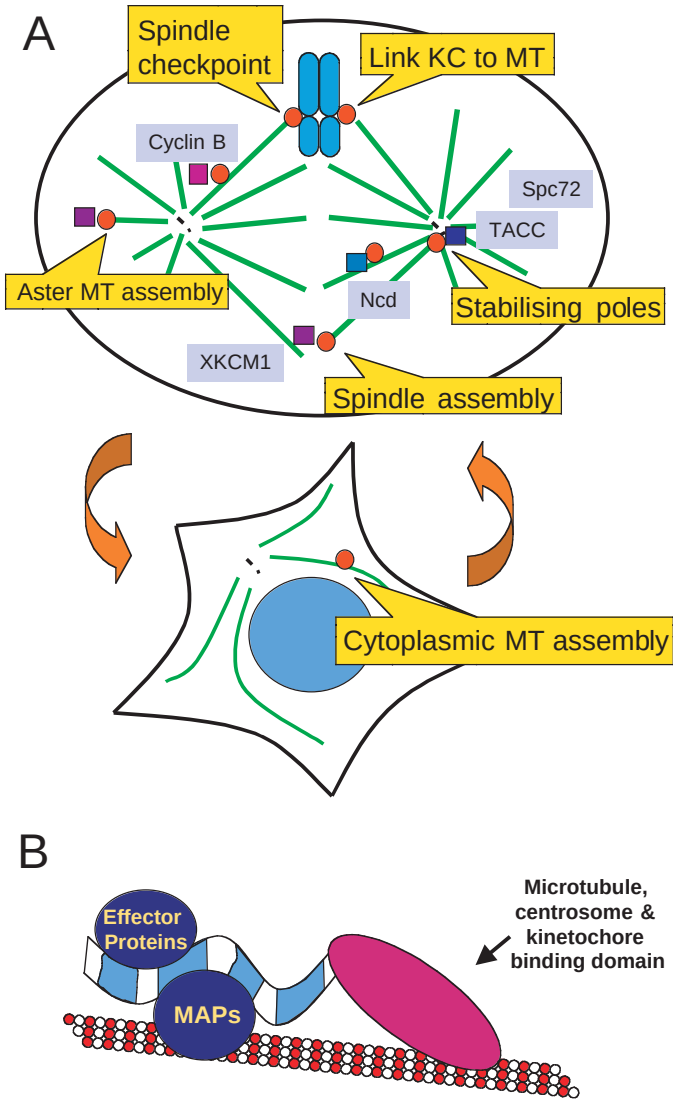

Fig. 4. Multiple roles of the Dis1/TOG family of MAPs. (A) The Dis1/TOG family of MAPs plays crucial roles in multiple microtubule functions (shown in yellow boxes), which are likely to be mediated by their interaction with various proteins (shown in blue boxes). Microtubules, chromosomes, the Dis1/TOG family of MAPs and the interacting proteins are represented by green, blue, red circles and purple squares, respectively. KC, kinetochore; MT, microtubule. (B) A speculative view of the interaction between Dis1/TOG and their binding partners is illustrated. The C-terminal domain (red oval) is interacting with a microtubule (white and red). The N-terminal TOG domains (blue) are also shown interacting with proteins (MAPs and effector proteins)

Conclusion

The Dis1/TOG family is evolutionally conserved from yeasts and plants to humans. It is implicated in various microtubule functions, including microtubule polymer assembly, spindle formation, kinetochore function and cell morphogenesis (Table 1; Fig. 4A). It localises to distinct microtubule structures in a dynamic manner. The localisation and function of members of this family are not dictated solely by an intrinsic microtubule-binding activity but instead are achieved through interactions with various proteins. The repeat structure based on HEAT motifs might allow these proteins to interact with multiple proteins and, by interacting with multiple proteins, they might then be capable of localising to specific places at specific times and modulating microtubule dynamics in a spatially and temporally specific manner (Fig. 4B). The modular nature of the proteins, which allows multiple protein-protein interactions, may have made these MAPs able to adapt to different roles on demand throughout evolution.

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