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# Behavior of γ-tubulin during spindle formation in *Xenopus* oocytes: requirement of cytoplasmic dynein-dependent translocation

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## Summary

Vertebrate oocytes do not contain centrosomes and therefore form an acentrosomal spindle during oocyte maturation. γ-Tubulin is known to be essential for nucleation of microtubules at centrosomes, but little is known about the behaviour and role of γ-tubulin during spindle formation in oocytes. We first observed sequential localization of γ-tubulin during spindle formation in *Xenopus* oocytes. γ-Tubulin assembled in the basal regions of the germinal vesicle (GV) at the onset of germinal vesicle breakdown (GVBD) and remained on the microtubule-organizing centre (MTOC) until a complex of the MTOC and transient-microtubule array (TMA) reached the oocyte surface. Prior to bipolar spindle formation, oocytes formed an aggregation of microtubules and γ-tubulin was concentrated at the centre of the aggregation. At the late stage of bipolar spindle formation, γ-tubulin accumulated at each pole. Anti-dynein antibody disrupted the localization of γ-tubulin, indicating that the translocation described above is dependent on dynein activity. We finally revealed that XMAP215, a microtubule-associated protein cooperating with γ-tubulin for the assembly of microtubules, but not γ-tubulin, was phosphorylated during oocyte maturation. These results suggest that γ-tubulin is translocated by dynein to regulate microtubule organization leading to spindle formation and that modification of the molecules that cooperate with γ-tubulin, but not γ-tubulin itself, is important for microtubule reorganization.

Keywords: Acentrosomal spindle, Microtubule, Oocyte maturation, Phosphorylation, XMAP215

## Introduction

To extrude a first polar body, a first meiotic spindle is precisely assembled at the surface of the oocyte. Because of their large size and absence of centrosomes, in amphibian oocytes a unique organization of microtubules proceeds to ensure spindle formation during progesterone-induced oocyte maturation (Gard, 1992; Kotani & Yamashita, 2002) as follows: (1) a novel formed microtubule-organizing centre (MTOC) and a transient-microtubule array (TMA) are assembled in the basal regions of the germinal vesicle (GV) at the time germinal vesicle breakdown (GVBD) occurs, (2) the MTOC–TMA complex migrates and transports chromosomes to the oocyte surface after the completion of GVBD, (3) the MTOC–TMA transforms into a compact aggregation of microtubules and chromosomes near the surface of the oocyte, and (4) the aggregation establishes a bipolar spindle axis and elongates microtubules, leading to bipolar spindle formation.

Some molecules are known to be involved in the formation of the MTOC–TMA complex and an acentrosomal bipolar spindle. For example, XMAP230, one of the *Xenopus* microtubule-associated proteins, is required for the assembly of microtubules (Cha *et al*., 1998). XMAP215, a highly conserved microtubule-associated protein among all the eukaryotes, and

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XKCM1, a member of the KinI family of kinesins, antagonistically play a role in the regulation of microtubule dynamics (Becker *et al*., 2003). Cytoplasmic dynein, a minus-end-directed motor protein, and NuMA, a nuclear/mitotic apparatus protein, are required for microtubule assembly in the basal regions of the GV (Becker *et al*., 2003). Upstream of these molecules, maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) regulate the microtubule organization through almost all the process of the spindle formation (Kotani & Yamashita, 2002). Interestingly, XMAP230, XMAP215, dynein and NuMA are good candidates for substrates of MPF and/or MAPK (Shiina *et al*., 1992; Vasquez *et al*., 1999; Becker & Gard, 2000; Addinall *et al*., 2001; Compton & Luo, 1995). Therefore, it seems likely that phosphorylation of the molecules by MPF and/or MAPK regulates microtubule organization during oocyte maturation.

γ-Tubulin is a component of centrosomes in cells (Oakley & Oakley, 1989; Zheng *et al*., 1991; Stearns *et al*., 1991) and plays a central role in the nucleation of microtubules at centrosomes (Joshi *et al*., 1992; Stearns & Kirschner, 1994; Fèlix *et al*., 1994; Oakley, 2000). Although it is thought that γ-tubulin plays an important role during spindle formation in oocytes, little is known about the role of γ-tubulin or its behaviour during oocyte maturation. In *Xenopus* oocytes, localization of γ-tubulin was observed only at select stages of spindle formation (Gard *et al*., 1995), but its sequential localization remains unclear. In mouse oocytes, changes in γ-tubulin distribution have been shown to be consistent with microtubule patterning (Combelles & Albertini, 2001; Meng *et al*., 2004), but the mechanism required for its redistribution is unknown.

In this study, we first observed sequential localization of γ-tubulin during *Xenopus* oocyte maturation, which suggested that γ-tubulin is involved in microtubule organization. Next, we revealed the requirement of cytoplasmic dynein for γ-tubulin translocation, and finally we demonstrated that XMAP215, but not γ-tubulin, is phosphorylated during oocyte maturation.

## Materials and methods

### Animals and oocytes

*Xenopus laevis* was purchased from a dealer (Hamamatsu Seibutsu Kyozai, Shizuoka, Japan). Full-grown immature oocytes at stage VI were isolated with forceps in modified Barth's saline with Hepes (MBS-H) (Cyert & Kirschner, 1988) and induced to mature by incubating in MBS-H with 10 μg/ml of progesterone. Maturing oocytes were fixed in 3.7% formaldehyde/0.2% Triton X-100/0.5 μM taxol in a microtubule assembly buffer (80 mM KPipes, 5 mM EGTA, 1 mM $MgCl_2$, pH 6.8) (Gard, 1991).

### Microinjection

Oocytes were manually isolated with forceps and injected with 20 ng of anti-dynein antibody (70.1, Sigma). For controls, oocytes were injected with 20 ng of anti-GST antibody (Nakahata *et al*., 2003) or anti-His-probe antibody (Santa Cruz Biotechnology). The oocytes were treated with progesterone to induce maturation and fixed at intervals after appearance of a white spot, which indicates the occurrence of GVBD.

### Immunocytochemistry

The fixed oocytes were dehydrated, embedded in paraffin, and cut into 12-μm-thick sections as described previously (Kotani & Yamashita, 2002). Anti-γ-tubulin rabbit antibody (Sigma) and Alexa-488-conjugated secondary antibody (Molecular Probes) were used to observe the localization of γ-tubulin. Cy3-conjugated anti-β-tubulin antibody (TUB 2.1, Sigma) was used to observe microtubules. The oocytes were mounted by using a ProLong Antifade kit (Molecular Probes) and observed under a Bio-Rad MicroRadiance confocal microscope.

### Production of anti-XMAP215 antibodies

To construct a His-tagged protein, the cDNA clone encoding the N-terminus (amino acids 1–560) of XMAP215 was amplified by polymerase chain reaction (PCR) with a 5′-primer (GGAATTCTAATGGGGGATGACAGCGAGTGG) and a 3′-primer (ACGCGTCGACCGCTTTGGCTTTCTTGACAG). The PCR product was digested with *Eco*RI and *Sal*I and ligated into the *Eco*RI/*Sal*I site of pET21. The His-tagged protein was expressed in *Escherichia coli* BL21 and purified by SDS–polyacrylamide gel electrophoresis followed by electroelution in Tris-glycine buffer without SDS, as described previously (Hirai *et al*., 1992). The purified protein was dialysed against 1 mM Hepes (pH 7.0), lyophilized, and injected into two mice to produce polyclonal antibodies, according to the procedures described previously (Yamashita *et al*., 1991). The antibodies (H1 and H2) were each affinity-purified with the antigenic protein electroblotted onto an Immobilon membrane.

### Oocyte extraction, immunoprecipitation and immunoblotting

After injection with 250 μCi of [γ-$^{32}$P]ATP, half the oocytes were treated with progesterone and half were not treated. At 15 h after progesterone treatment, treated mature oocytes and untreated immature oocytes were harvested and extracted as described

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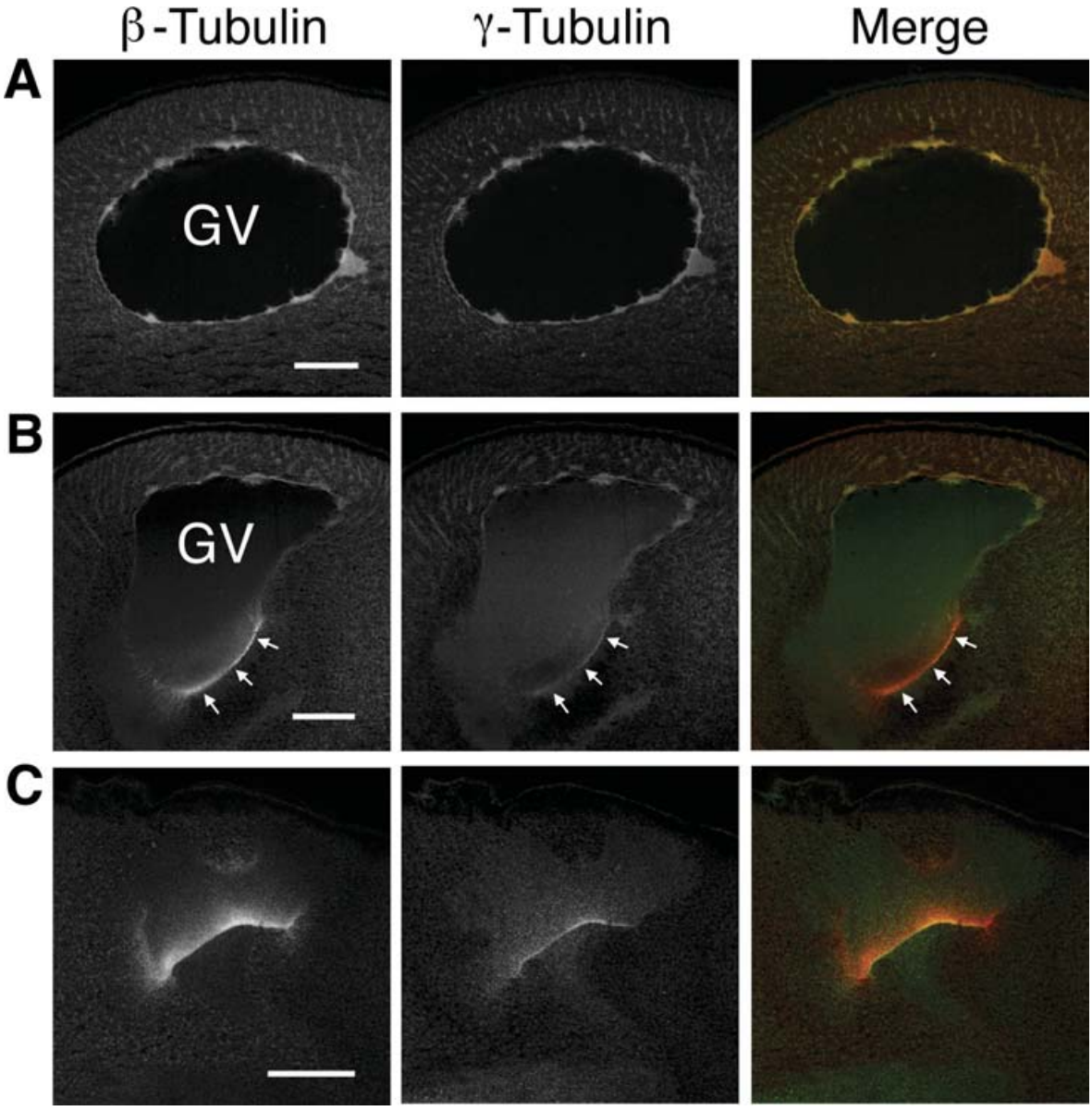


**Figure 1** Localization of γ-tubulin during MTOC–TMA formation. Oocytes were stained with anti-β-tubulin antibody (β-Tubulin) and anti-γ-tubulin antibody (γ-Tubulin). Merged images (Merge) are also shown. β-Tubulin, red; γ-tubulin, green in merged images. (*A*) Full-grown immature oocytes arrested at the G2/M border. GV, germinal vesicle. (*B*) Oocytes undergoing germinal vesicle breakdown (GVBD). The arrows indicate the disk-shaped microtubule-organizing centre (MTOC) formed in the basal region of the GV. (*C*) Migration of a complex of MTOC and transient microtubule array (TMA) towards the oocyte surface 0–15 min after appearance of a white spot. Scale bars represent 100 μm.

previously (Kotani & Yamashita, 2002). Anti-XMAP215 (H1) antibody was used to immunoprecipitate XMAP215, and anti-XMAP215 (H2) antibody was used to immunoblot XMAP215. Anti-γ-tubulin rabbit antibody (Sigma) and anti-γ-tubulin mouse antibody (Sigma, GTU-88) were used to immunoprecipitate and immunoblot γ-tubulin, respectively. Immunoprecipitation and immunoblotting were performed as described previously (Nakahata *et al*., 2003). The phosphorylated proteins were visualized with a Fuji BAS2000 image analyser.

## Results

### Localization of γ-tubulin during *Xenopus* oocyte maturation

To gain an insight into the molecular mechanisms of spindle formation during oocyte maturation, we first observed the localization of γ-tubulin during the process of spindle formation. In full-grown immature oocytes, γ-tubulin composed a part of the microtubule array surrounding the GV (Fig. 1A), as previously

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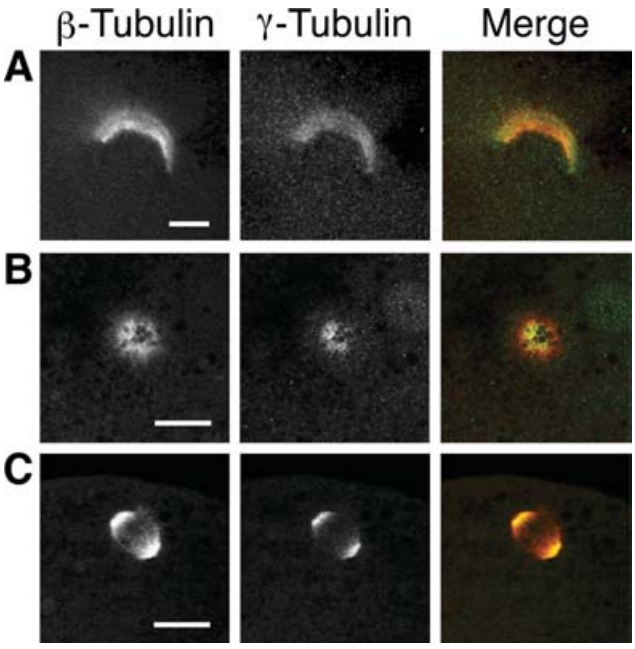


**Figure 2** Localization of γ-tubulin during the first meiotic spindle formation 15–60 min after appearance of a white spot. Oocytes were stained with anti-β-tubulin antibody (β-Tubulin) and anti-γ-tubulin (γ-Tubulin). Merged images (Merge) are also shown. β-Tubulin, red; γ-tubulin, green. (*A*) Late MTOC–TMA complex. (*B*) Compact aggregation of microtubules prior to bipolar spindle formation. (*C*) Bipolar spindle formation. Scale bars represent 20 μm.

reported (Gard, 1994). At the onset of GVBD, γ-tubulin accumulated at the basal region of the GV, on which a transient-microtubule array (TMA) also assembled simultaneously (Fig. 1B; arrows). This MTOC–TMA complex migrated towards the oocyte surface after the completion of GVBD. While the MTOC–TMA complex migrated, γ-tubulin remained localized on the disk-shaped MTOC, which was positioned at the minus ends of the microtubules (Fig. 1C). At a late stage of MTOC–TMA, as it reached at the oocyte surface, γ-tubulin co-localized with a wide range of microtubules (Fig. 2A). Prior to bipolar spindle formation, a compact aggregation was organized and γ-tubulin was accumulated at the centre of the aggregation (Fig. 2B). During spindle formation, γ-tubulin was first distributed along microtubules, but it was later concentrated at each pole after elongation of microtubules (Fig. 2C). These results suggest the importance of γ-tubulin during the entire process of microtubule organization.

### Disruption of γ-tubulin assembly by the anti-dynein antibody

We have shown that γ-tubulin is translocated by cytoplasmic dynein towards the microtubule minus ends, resulting in the assembly of microtubules to mitotic asters in cell-free extracts (Kotani & Yamashita, 2005). In addition, dynein has been shown to be required for MTOC–TMA formation in *Xenopus* oocytes (Becker *et al*., 2003). Thus, it is thought that dynein also translocates γ-tubulin during oocyte maturation, resulting in MTOC–TMA formation. To verify this possibility, we prevented dynein activity by an anti-dynein antibody, which reacts with the dynein intermediate chain and inhibits dynein function (Heald *et al*., 1997). Injection of 20 ng of anti-dynein antibody disrupted MTOC–TMA formation (Fig. 3A), as previously reported (Becker *et al*., 2003). In these oocytes, γ-tubulin was not assembled at the basal regions of the GV but was distributed around the surface of the nucleoplasm (Fig. 3A, arrows; compare with Fig. 1B, C). Anti-dynein antibody also inhibited the formation of a microtubule aggregation in 44% of the oocytes (data not shown), and even in the 56% of the oocytes in which microtubule aggregation formed a functional bipolar spindle was not formed (Fig. 3B). In these oocytes, γ-tubulin remained distributed around the disordered microtubule array instead of accumulating at the poles (Fig. 3B; compare with Fig. 2C). In contrast, control antibodies did not affect the localization of γ-tubulin and microtubule organization (data not shown). These results indicate that γ-tubulin assembly at the basal regions of the GV at the onset of GVBD and accumulation at the poles during spindle formation are dependent on dynein activity. Conversely, our results suggest that disruption of γ-tubulin assembly by anti-dynein antibody results in failure of microtubule organization in both MTOC–TMA and spindle formations.

### Phosphorylation of XMAP215, but not γ-tubulin, during oocyte maturation

In budding yeast, γ-tubulin is phosphorylated predominantly during G1 phase and this modification may be important for microtubule organization (Vogel *et al*., 2001). XMAP215 contributes to the assembly of microtubules in cooperation with γ-tubulin (Popov *et al*., 2002; Usui *et al*., 2003) and is phosphorylated by MPF *in vitro* (Vasquez *et al*., 1999). We next examined whether γ-tubulin and XMAP215 were phosphorylated during progesterone-induced oocyte maturation. Incorporation of [γ-$^{32}$P]ATP in XMAP215 immunoprecipitated from mature oocytes but not immature oocytes (Fig. 4) indicated that XMAP215 was phosphorylated during oocyte maturation. In contrast, lack of labelling of γ-tubulin immunoprecipitated from both immature and mature oocytes (Fig. 4) showed that γ-tubulin was not phosphorylated.

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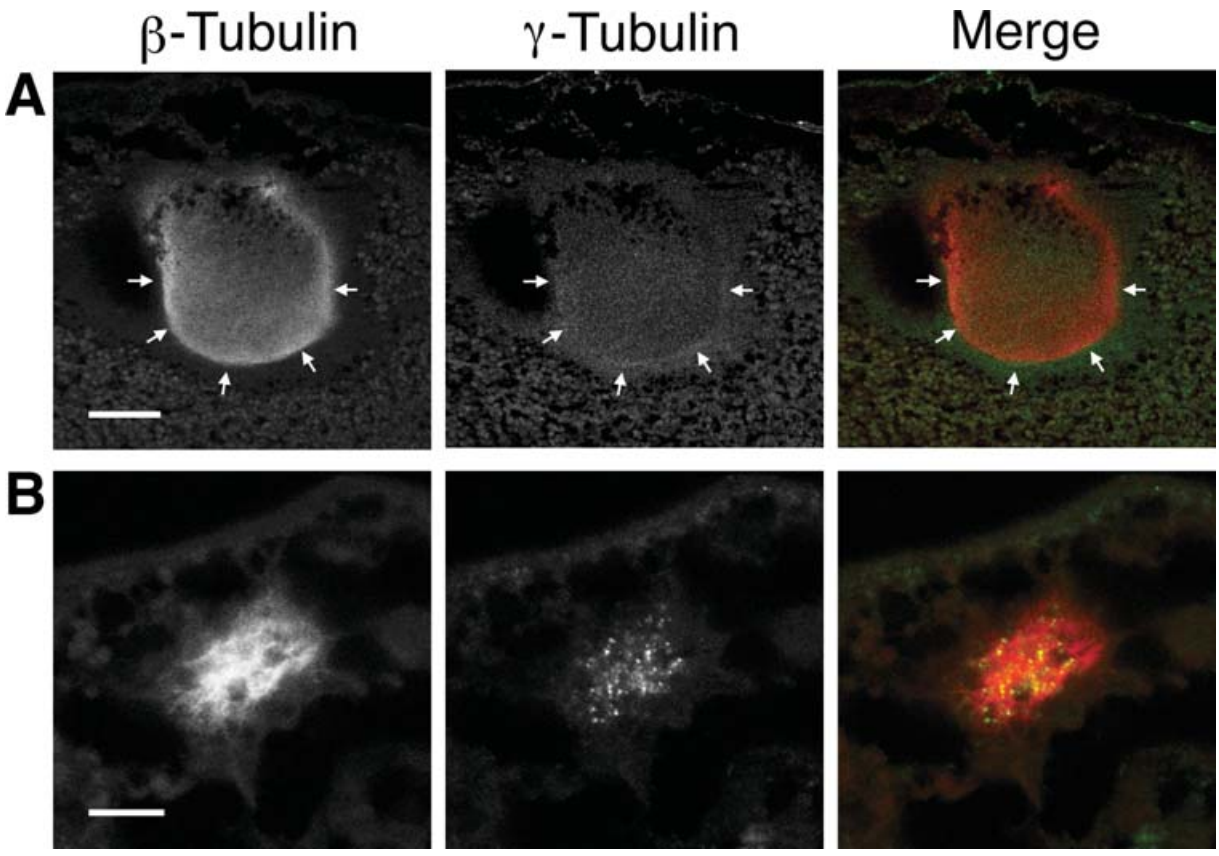


**Figure 3** Effect of anti-dynein antibody on γ-tubulin localization. Oocytes that had been injected with anti-dynein antibody were stained with anti-β-tubulin antibody (β-Tubulin) and anti-γ-tubulin (γ-Tubulin). Merged images (Merge) are also shown. β-Tubulin, red; γ-tubulin, green. (*A*) Oocytes completing GVBD 0–30 min after the appearance of a white spot. At this time, control oocytes formed a TMA–MTOC complex and the complex migrated to the oocyte surface (Fig. 1C). Instead, in these oocytes, microtubules were assembled from the surface of the nucleoplasm, on which γ-tubulin was localized (indicated by arrows). (*B*) Organization of a microtubule aggregation at the oocyte surface 30–60 min after the appearance of a white spot. At this time, control oocytes formed a bipolar spindle (Fig. 2C). Instead, in these oocytes a disordered microtubule array was assembled and γ-tubulin remained distributed around the microtubule array. Scale bar represents 50 μm in (*A*) and 10 μm in (*B*).

## Discussion

### Dynein-dependent translocation of γ-tubulin during oocyte maturation

In this study, we first observed localization of γ-tubulin at the sequential stages of *Xenopus* oocyte maturation, in which γ-tubulin was dynamically translocated during the process of spindle formation (Figs. 1, 2). XMAP215 and NuMA localize in the same regions as those in which γ-tubulin localizes – at the base of the MTOC–TMA and at the poles of the spindle (Becker *et al*., 2003) – suggesting that these molecules are involved in supporting the role of γ-tubulin. We previously showed that γ-tubulin plays a role in the translocating and tethering of microtubules to the poles in cooperation with dynein during taxol-induced pole formation in *Xenopus* oocyte extracts (Kotani & Yamashita, 2005). In the present study, we revealed that translocation of γ-tubulin in both MTOC–TMA and spindle formations required dynein activity (Fig. 3). Furthermore, disruption of γ-tubulin assembly by an anti-dynein antibody resulted in defects of microtubule organization in both MTOC–TMA and spindle formations. Although we cannot rule out the possibility that dynein transports other molecules predominantly required for microtubule assembly, it is most likely that γ-tubulin plays a central role in microtubule organization during spindle formation in oocytes, since γ-tubulin is a prerequisite for dynein-dependent microtubule assembly in extracts (Kotani & Yamashita, 2005).

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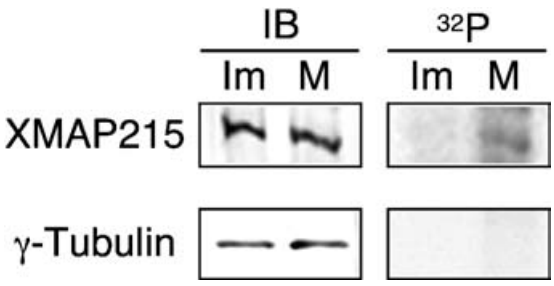


**Figure 4** $^{32}$P-labelling of XMAP215, but not of γ-tubulin, during oocyte maturation. Oocytes were injected with [γ-$^{32}$P]ATP and were treated with progesterone or not treated. The progesterone-treated mature oocytes (M) and non-treated immature oocytes (Im) were extracted and immunoprecipitated with anti-XMAP215 antibody (XMAP215) or anti-γ-tubulin antibody (γ-Tubulin). Immunoblotting (IB) of anti-XMAP215 antibody (upper) or anti-γ-tubulin antibody (lower) showed that similar amounts of proteins were immunoprecipitated from immature and mature oocytes. $^{32}$P-labelling ($^{32}$P) of proteins showed phosphorylation of XMAP215 in mature oocytes but not in immature oocytes and non-phosphorylation of γ-tubulin in both immature and mature oocytes. Similar results were obtained from three independent experiments.

### Modification of XMAP215 and dynein, but not γ-tubulin, during oocyte maturation

The amount of each molecule required for microtubule reorganization, including γ-tubulin, dynein and XMAP215, did not change during *Xenopus* oocyte maturation (data not shown). How, then, is microtubule reorganization regulated during oocyte maturation? It has been shown that MPF and MAPK regulate microtubule organization leading to spindle formation in oocytes (Kotani & Yamashita, 2002; Kotani *et al*., 2001; Verlhac *et al*., 1996). Therefore, phosphorylation of the molecules downstream of MPF and MAPK is important for regulation of changes in microtubules. Thus, it is important to examine whether γ-tubulin is phosphorylated to regulate microtubule assembly during oocyte maturation. In this study, we showed that γ-tubulin was not phosphorylated during oocyte maturation (Fig. 4), indicating that modification of γ-tubulin is not involved in microtubule assembly. In contrast, XMAP215 was phosphorylated during oocyte maturation (Fig. 4). XMAP215 has several sites potentially phosphorylated by MPF and MAPK (Becker & Gard, 2000). In addition, phosphorylation of XMAP215 by MPF alters the microtubule-promotion activity of XMAP215 *in vitro* (Vasquez *et al*., 1999). In oocytes, injection of MPF purified from *Xenopus* eggs induced phosphorylation of XMAP215 at the time GVBD occurred (Gard & Kirschner, 1987). Therefore, phosphorylation of XMAP215 may play an important role in microtubule assembly leading to spindle formation, but its importance *in vivo* has remained unexplored not only in oocytes but also in mitotic cells. Further studies using oocytes will contribute to elucidating the role of phosphorylation of XMAP215 during microtubule organization in mitosis.

In addition to XMAP215, dynein is also phosphorylated during *Xenopus* oocyte maturation (Huang *et al*., 1999). Importantly, dynein phosphorylation starts at the onset of GVBD, at which time γ-tubulin assembles in the basal regions of the GV. Phosphorylation of dynein by MPF results in its release from membranes (Addinall *et al*., 2001). Dynein and phosphorylated factors (possibly dynein itself) are required for microtubule assembly leading to mitotic aster formation *in vitro* (Verde *et al*., 1991). Dynein forms a large complex to focus spindle poles, which consists of dynactin and NuMA (Merdes *et al*., 1996, 2000; Gaglio *et al*., 1996, 1997). Therefore, it is also important that dynactin is phosphorylated at the time GVBD (Huang *et al*., 1999) and that NuMA is potentially phosphorylated by MPF (Compton & Luo, 1995). Taken together, the results suggest that phosphorylation of the components of the dynein complex regulates γ-tubulin translocation, thereby leading to the MTOC–TMA and spindle formations during oocyte maturation. Further studies are needed to test this possibility.

In summary, we have revealed the behaviour of γ-tubulin during spindle formation in oocytes, including its sequential localization, dependence of its translocation on dynein, and its non-phosphorylation during oocyte maturation. These findings suggest that γ-tubulin plays a central role in spindle formation, translocating in a manner dependent on dynein, and that modification of molecules cooperating with γ-tubulin is involved in the regulation of microtubule organization.

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### References

Addinall, S.G., Mayr, P.S., Doyle, S., Sheehan, J.K., Woodman, P.G. & Allan, V.J. (2001). Phosphorylation by cdc2-CyclinB1

kinase releases cytoplasmic dynein from membranes. *J. Biol. Chem*. **276**, 15939–44.
Becker, B.E. & Gard, D.L. (2000). Multiple isoforms of the high molecular weight microtubule associated protein XMAP215 are expressed during development in *Xenopus*. *Cell Motil. Cytoskeleton* **47**, 282–95.
Becker, B.E., Romney, S.J. & Gard, D.L. (2003). XMAP215, XKCM1, NuMA, and cytoplasmic dynein are required for the assembly and organization of the transient microtubule array during the maturation of *Xenopus* oocytes. *Dev. Biol*. **261**, 488–505.
Cha, B.J., Error, B. & Gard, D.L. (1998). XMAP230 is required for the assembly and organization of acetylated microtubules and spindles in *Xenopus* oocytes and eggs. *J. Cell Sci*. **111**, 2315–27.
Combelles, C.M.H. & Albertini, D.F. (2001). Microtubule patterning during meiotic maturation in mouse oocytes is determined by cell cycle-specific sorting and redistribution of γ-tubulin. *Dev. Biol*. **239**, 281–94.
Compton, D.A. & Luo, C. (1995). Mutation of the predicted $p34^{cdc2}$ phosphorylation sites in NuMA impair the assembly of the mitotic spindle and block mitosis. *J. Cell Sci*. **108**, 621–33.
Cyert, M.S. & Kirschner, M.W. (1988). Regulation of MPF activity *in vitro*. *Cell* **53**, 185–95.
Felix, M.A., Antony, C., Wright, M. & Maro, B. (1994). Centrosome assembly *in vitro*: role of γ-tubulin recruitment in *Xenopus* sperm aster formation. *J. Cell Biol*. **124**, 19–31.
Gaglio, T., Saredi, A., Bingham, J.B., Hasbani, M.J., Gill, S.R., Schroer, T.A. & Compton, D.A. (1996). Opposing motor activities are required for the organization of the mammalian mitotic spindle pole. *J. Cell Biol*. **135**, 399–414.
Gaglio, T., Dionne, M.A. & Compton, D.A. (1997). Mitotic spindle poles are organized by structural and motor proteins in addition to centrosomes. *J. Cell Biol*. **138**, 1055–66.
Gard, D.L. (1991). Organization, nucleation, and acetylation of microtubules in *Xenopus laevis* oocytes: a study by confocal immunofluorescence microscopy. *Dev. Biol*. **143**, 346–62.
Gard, D.L. (1992). Microtubule organization during maturation of *Xenopus* oocytes: assembly and rotation of the meiotic spindles. *Dev. Biol*. **151**, 516–30.
Gard, D.L. (1994). γ-Tubulin is asymmetrically distributed in the cortex of *Xenopus* oocytes. *Dev. Biol*. **161**, 131–40.
Gard, D.L. & Kirschner, M.W. (1987). A microtubule-associated protein from *Xenopus* eggs that specifically promotes assembly at the plus-end. *J. Cell Biol*. **105**, 2203–15.
Gard, D.L., Cha, B.J. & Schroeder, M.M. (1995). Confocal immunofluorescence microscopy of microtubules, microtubule-associated proteins, and microtubule-organizing centre during amphibian oogenesis and early development. *Curr. Top. Dev. Biol*. **31**, 383–431.
Heald, R., Tournebize, R., Habermann, A., Karsenti, E. & Hyman, A. (1997). Spindle assembly in *Xenopus* egg extracts: respective roles of centrosomes and microtubule self-organization. *J. Cell Biol*. **138**, 615–38.
Hirai, T., Yamashita, M., Yoshikuni, M., Lou, Y.H. & Nagahama, Y. (1992). Cyclin B in fish oocytes: its cDNA and amino acid sequences, appearance during maturation, and induction of $p34^{cdc2}$ activation. *Mol. Reprod. Dev*. **33**, 131–40.
Huang, C.F., Chang, C.B., Huang, C. & Ferrell, J.E. (1999). M phase phosphorylation of cytoplasmic dynein intermediate chain and $p150^{Glued}$. *J. Biol. Chem*. **274**, 14262–9.
Joshi, H.C., Palacios, M.J., McNamara, L. & Cleveland, D.W. (1992). γ-Tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. *Nature* **356**, 80–3.
Kotani, T. & Yamashita, M. (2002). Discrimination of the roles of MPF and MAP kinase in morphological changes that occur during oocyte maturation. *Dev. Biol*. **252**, 271–86.
Kotani, T. & Yamashita, M. (2005). Overexpression of truncated γ-tubulins disrupts mitotic aster formation in *Xenopus* oocyte extracts. *Biochem. J*. **389**, 611–17.
Kotani, T., Yoshida, N., Mita, K. & Yamashita, M. (2001). Requirement of cyclin B2, but not cyclin B1, for bipolar spindle formation in frog (*Rana japonica*) oocytes. *Mol. Reprod. Dev*. **59**, 199–208.
Meng, X.Q., Fan, H.Y., Zhong, Z.S., Zhang, G., Li, Y.L., Chen, D.Y. & Sun, Q.Y. (2004). Localization of γ-tubulin in mouse eggs during meiotic maturation, fertilization, and early embryonic development. *J. Reprod. Dev*. **50**, 97–105.
Merdes, A., Ramyar, K., Vechio, J.D. & Cleveland, D.W. (1996). A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. *Cell* **87**, 447–58.
Merdes, A., Heald, R., Samejima, K., Earnshaw, W.C. & Cleveland, D.W. (2000). Formation of spindle poles by dynein/dynactin-dependent transport of NuMA. *J. Cell Biol*. **149**, 851–61.
Nakahata, S., Kotani, T., Mita, K., Kawasaki, T., Katsu, Y., Nagahama, Y. & Yamashita, M. (2003). Involvement of *Xenopus* Pumilio in the translational regulation that is specific to cyclin B1 mRNA during oocyte maturation. *Mech. Dev*. **120**, 865–80.
Oakley, B.R. (2000). γ-Tubulin. *Curr. Top. Dev. Biol*. **49**, 27–54.
Oakley, C.E. & Oakley, B.R. (1989). Identification of γ-tubulin, a new member of the tubulin superfamily encoded by *mip*A gene of *Aspergillus nidulans*. *Nature* **338**, 662–4.
Popov, A.V., Severin, F. & Karsenti, E. (2002). XMAP215 is required for the microtubule-nucleating activity of centrosomes. *Curr. Biol*. **12**, 1326–30.
Shiina, N., Moriguchi, T., Ohta, K., Gotoh, Y. & Nishida, E. (1992). Regulation of a major microtubule-associated protein by MPF and MAP kinase. *EMBO J*. **11**, 3977–84.
Stearns, T., Evans, L. & Kirschner, M. (1991). γ-Tubulin is a highly conserved component of the centrosome. *Cell* **65**, 825–36.
Stearns, T. & Kirschner, M. (1994). *In vitro* reconstitution of centrosome assembly and function: the central role of γ-tubulin. *Cell* **76**, 623–37.
Usui, T., Maekawa, H., Pereira, G. & Schiebel, E. (2003). The XMAP215 homologue Stu2 at yeast spindle pole bodies regulates microtubule dynamics and anchorage. *EMBO J*. **22**, 4779–93.
Vasquez, R.J., Gard, D.L. & Cassimeris, L. (1999). Phosphorylation by CDK1 regulates XMAP215 function *in vitro*. *Cell Motil. Cytoskeleton* **43**, 310–21.
Verde, F., Berrez, J.M., Antony, C. & Karsenti, E. (1991). Taxol-induced microtubule asters in mitotic extracts of

*Xenopus* eggs: requirement for phosphorylated factors and cytoplasmic dynein. *J. Cell Biol*. **112**, 1177–87.

Verlhac, M.H., Kubiak, J.K., Weber, M., Gerard, G., Colledge, W.H., Evans, M.J. & Maro, B. (1996). Mos is required for MAP kinase activation and is involved in microtubule organization during maturation in the mouse. *Development* **122**, 815–22.

Vogel, J., Drapkin, B., Oomen, J., Beach, D., Bloom, K. & Snyder, M. (2001). Phosphorylation of γ-tubulin regulates microtubule organization in budding yeast. *Dev. Cell* **1**, 621–31.

Yamashita, M., Yoshikuni, M., Hirai, T., Fukada, S. & Nagahama, Y. (1991). A monoclonal antibody against the PSTAIR sequence of $p34^{cdc2}$, catalytic subunit of maturation-promoting factor and key regulator of the cell cycle. *Dev. Growth Differ.* **33**, 617–24.

Zheng, Y., Jung, M.K. & Oakley, B.R. (1991). γ-Tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. *Cell* **65**, 817–23.