Mini Spindles TOG1 Maintains Microtubule Polymerization Rates and Mitotic Spindle Formation

By:
Benjamin Lowe

Senior Honors Thesis
Department of Biology
University of North Carolina at Chapel Hill

April 21, 2017

Approved:

_____

Dr. Kevin Slep, Thesis Advisor

Dr. Stephen Rogers, Reader 1

Dr. Scott Williams, Reader 2

Abstract

Microtubules (MTs) are dynamic polymers that regulate cellular processes including intracellular trafficking and mitotic spindle formation. MT-associated proteins (MAPs) control MT dynamics in space and time in order to ensure proper cellular function. Members of the XMAP215 protein family are conserved MAPs that utilize arrays of αβ-tubulin-binding tumor overexpressed gene (TOG) domains to promote rapid MT polymerization. Higher eukaryotic XMAP215 proteins possess a pentameric TOG array that is structurally diverse despite being positionally conserved across species. Previous studies show that a TOG1-2-5 array is sufficient to promote endogenous MT polymerization rates. Furthermore, multiple studies of XMAP215 family members demonstrate that removing TOG1 and TOG2 from the pentameric array completely abrogates MT growth rates. However, the significance of TOG1 alone in regulating MT dynamics remains uncertain. We investigated TOG1's role in promoting MT polymerization and mitotic spindle formation using the *Drosophila melanogaster (D.m.)* XMAP215 family member Mini Spindles (Msps) as a model. Endogenous Msps was depleted from *D.m.* S2 cells using dsRNA and a Msps construct with TOG1 deleted (ΔTOG1) from the pentameric TOG domain array was expressed. ΔTOG1-expressing cells exhibited dramatically reduced MT polymerization rates in comparison to control cells. Furthermore, ΔTOG1 expression yielded a significantly decreased incidence of normal bipolar spindle phenotypes. These data suggest that TOG1 is critical for proper XMAP215-mediated MT dynamics and mitotic spindle formation. Together, these findings lead to a more comprehensive model of how distinct TOG domains contribute to XMAP215 function. These results also provide a foundation on which to study other TOG-domain containing MAPs.

Introduction

Microtubules (MTs) are cytoskeletal filaments that facilitate many cellular functions including intracellular cargo transport, cell motility, and formation of the mitotic spindle during cell division (1). MTs are polar, hollow, and composed of αβ-tubulin heterodimers. Each MT has a minus-end with α-subunits foremost and a plus-end with β-subunits foremost (2). MTs fluctuate between GTP-dependent polymerization and sudden rapid depolymerization, especially at the plus-end, which is termed dynamic instability (1, 2). MT dynamic instability is vital for maintenance of cellular functions. Thus, regulation of MT dynamics is essential and is carried out by microtubule-associated proteins (MAPs). MAPs control MT dynamics parameters including nucleation, growth, location, and stability (1).

One important class of regulatory MAPs are the plus-end tracking proteins (+TIPs), which preferentially bind to and track MT plus-ends (3). One family within this group of proteins, the XMAP215 family, was first discovered in *Xenopus laevis* and was noted for its plus-end-specific promotion of MT polymerization (4). Proteins orthologous to XMAP215 have been found across eukaryotes (3).

Many studies have focused on the architectural features of the XMAP215 family. Special attention has been placed on tumor overexpressed gene (TOG) domains, the N-terminal domains conserved among the XMAP215 family that bind single tubulin heterodimers. With little exception these domains consist of six HEAT (Huntingtin, elongation factor 3, the PR65/A subunit of protein phosphatase 2A and the lipid kinase Tor) repeats (HRs), which contain conserved residues necessary for tubulin binding (5, 6). Yeast homologs have two TOG domains and dimerize using a C-terminal coiled-coil domain to form a four-TOG domain array (5, 7, 8).

Non-yeast XMAP215 members typically have five sequential TOG domains (a pentameric array) and function as monomers (5, 9).

Currently, a number of different TOG structures across a wide variety of species have been studied. TOG domains 1 and 2 are structurally similar and have been shown to preferentially bind tubulin in its curved, MT-unbound state (10, 11). TOG3 displays novel architectural features that distinguish it from TOG1 and TOG2, although it is predicted to bind tubulin in a manner consistent with these domains (12). TOG4 has a divergent structure that predicts unique TOG-tubulin interactions, potentially with lattice-incorporated tubulin (13). TOG5 is also structurally distinct, particularly due to a seventh HR, which binds MT-lattice incorporated tubulin (unpublished, Amy Byrnes, UNC Chapel Hill, 14). Together, these studies build a working picture of pentameric XMAP215 proteins: TOG1 and TOG2 are most similar, TOG3 maintains a similar tubulin-binding mechanism to TOGs 1-2, and TOGs 4-5 diverge in structure and are predicted to interact with lateral tubulin heterodimers in the MT lattice. Despite the differences between TOG domains of a single array, individual TOG domains remain highly conserved across species (13).

The XMAP215 family robustly promotes MT plus-end polymerization (4). Other XMAP215 functions including facilitating kinetochore-spindle attachment during mitosis and promoting MT shrinkage have also been elucidated (15). The *Drosophila melanogaster (D.m.)* XMAP215 ortholog Mini Spindles (Msps), for example, was shown *in vivo* to be crucial for proper mitotic spindle formation: Msps depletion corresponded to the formation of small mitotic spindles and a general failure of proper spindle formation (16). Msps also regulates interphase MT dynamics. Depletion of endogenous Msps corresponds to slower MT polymerization rates and an increase in MT pause (17). Despite these and other discoveries, many questions continue

to surround the form and function of XMAP215 proteins. For example, by what mechanism does XMAP215 polymerization activity occur and how does this facilitate proper cell function? One mode of thought asserts that tubulin subunits are added individually and sequentially to growing MTs (18). Opponents of this theory hold that XMAP215 proteins facilitate the formation of a tubulin oligomer, which is added as a whole to a growing MT (19). Related to this is what role each TOG domain plays and how they synergistically facilitate XMAP215 activity. Different TOG domains bind distinct forms of tubulin and this has been shown to take place in a non-cooperative manner, although this has only been tested with yeast TOG domains (10). Before these and other questions can be conclusively answered, more information is needed. In particular, TOG1 must be studied in depth.

A variety of research has contributed to the current understanding of TOG1's structure and function. A study focusing on the TOG1 domain of the yeast XMAP215 ortholog, Stu2, presented its crystal structure bound to yeast tubulin and found that TOG1 binds preferentially to curved tubulin heterodimers found in solution (11). Due to the conservation of TOG domains across species, it is likely that TOG1 from non-yeast XMAP215 proteins will exhibit a similar structure and tubulin-binding preference. Many studies have illuminated the importance of TOG1's ability to bind free tubulin on XMAP215's function. Deleting Stu2 TOG1, for example, reduced MT stability: yeast cells lacking TOG1 activity yielded severely shortened MTs (7). Other studies have investigated TOG1 function as part of broader surveys of pentameric XMAP215 activity. One such *in vitro* assay found that TOG1 and TOG2 make the strongest individual contributions to XMAP215 activity of all TOG domains in the pentameric TOG domain array (20). Mutating key conserved tubulin-binding residues in TOGs 1-2 reduced polymerization rates more than mutations in TOGs 3-5 (20). A study involving mutating TOGs

1-2 both individually and together within a TOG1-4 construct corroborated these results (13). Furthermore, a construct containing TOG1, TOG2, and TOG5 was shown to fully rescue MT growth rates (unpublished, Amy Byrnes, UNC Chapel Hill). A separate experiment showed that rescue capabilities of a TOG1-2 construct is severely limited when the lattice-binding domain is removed (14). These and other studies point to the idea that, with proper MT localization, TOG1 plays a significant role in the regulation of MT growth and function.

A focused, in-depth study of the function of a TOG1 domain from a full pentameric TOG domain array *in vivo* is necessary to confirm these data and obtain a more holistic understanding of the mechanism of XMAP215 proteins. As a model system, we focus on the pentameric TOG domain array of the *D.m.* XMAP215 family member, Msps. We pose two primary questions: compared to wild-type Msps, how does removing TOG1 from the pentameric TOG domain array affect interphase MT polymerization, and does TOG1 influence mitotic spindle formation? We hypothesize that deleting TOG1 from the pentameric array will reduce normal interphase MT polymerization rates and increase the proportion of abnormal mitotic spindles. These results would link TOG1 with Msps activity, thus suggesting that TOG1 plays a critical role in regulating proper MT dynamics. Testing this hypothesis is a critical step in deducing the function of TOG1 within the XMAP215 family.

Methods

S2 Cell Culture – D.m. S2 cells were cultured as reported previously (21). Briefly, S2 cells were cultured at 25°C in SF900 growth medium (Gibco). Once confluent, the cells were resuspended by gently flushing them from the bottom of the flask. The cell resuspension was

diluted 1:5 in SF900 media, effectively splitting the cell culture to enable continued growth. This was repeated until around passage 25, at which point new cells were thawed and cultured.

Knockdown and Transfection – S2 cells (~$0.1x10^6$) were added to SF900 medium in a 12-well plate. To deplete endogenous Msps, cells were treated daily for five days with *msps*-directed dsRNA targeting the 5' and 3' UTRs (Table 1). Control cells were treated with scrambled dsRNA (Table 1). On day three, cells were transfected using FuGENE HD (Promega) transfection reagent according to the manufacturer's protocol. Copper-inducible constructs analyzed included a full-length Msps construct (FL) as described by Currie et al. and a Msps construct lacking TOG1 (ΔTOG1), both containing a C-terminal eGFP tag (14). The ΔTOG1 construct was created using the FL construct, KOD polymerase (Novagen), and primers (Table 1) that systematically removed the residues corresponding to TOG1 (6-234). Cells used for analyzing MT polymerization rates were also transfected with an *EB1:EB1-tRFP* construct as described by Rogers et al., which was used as a readout for MT growth rates (22). On day four,

Table 1

Primer	Forward	Reverse
msps 3' UTR dsRNA	5'-GACTATCGTGTCGACTAATACGACTCACTATAGGACTGTGCGCTTCCCGTAGCTA-3'	5'-GACTATCGTGGTACCTAATACGACTCACTATAGGCGCATATAGTTCATGAGGATG-3'
msps 5' UTR dsRNA	5'-GACTATCGTGTCGACTAATACGACTCACTATAGG TGTGAGTAGCGGTCACACTG-3'	5'-GACTATCGTGGTACCTAATACGACTCACTATAGG CATCAGAATTGTGATCCAAGTACC-3'
Scrambled dsRNA	5'-GACTATCGTGTCGACTAATACGACTCACTATAGGAAATTGTAAGCGTTAATATTTTG-3'	5'-GACTATCGTGGTACCTAATACGACTCACTATAGGAACAGTTGCGCAGCCTGAATGG-3'
ΔTOG1	5'-CCTTCACCATGGCCGAGGACACACAGCAGGAAAAGCAAGCGAAGATCG-3'	5'-CGATCTTCGCTTGCTTTTCCTGCTGTGTGTCCTCGGCCATGGTGAAGG-3'

adding $CuSO_4$ to a final concentration of 175 mM induced protein expression. The following day, cells were fixed and stained for phenotypic analysis, or imaged live to analyze MT polymerization rates.

Fixation and Staining – Cells were plated in 2 mL of Schneider's media (Gibco) on dishes coated with Concanavalin A. After 2 hours, cells were washed with PBS and fixed with methanol (-80°C) for 8 minutes at -20°C. Fixed cells were then washed 4x with PBST to remove residual methanol and submerged in blocking buffer (PBST with 0.2% BSA (Fisher Scientific)) for 2-3 hours at 25°C. After blocking, cells were stained for mitotic phenotype analysis. Primary antibodies used included: mouse DM1α (1/1000, Sigma-Aldrich) to target tubulin and guinea pig anti-asterless (1/15,000, G. Rogers, University of Arizona Cancer Center, University of Arizona, Tucson, AZ) to target centrosomes. These were added at the described dilutions and were allowed to incubate overnight at 4°C. The following day, corresponding secondary antibodies with varying fluorophores (Cy3 and Cy5, 1/500, Jackson ImmunoResearch Laboratories) were added to the cells along with DAPI (1/1000, Molecular Probes, Invitrogen) to label DNA. Secondary antibodies were incubated at 4°C for one hour and subsequently washed with PBS. Stained plates were stored at 4°C until imaged.

Immunofluorescence Imaging and Analysis – Cells undergoing phenotype analysis were imaged using a 100x oil/1.49 objective, a Nikon Eclipse T*i*-E inverted microscope, and a Photometrics CoolSNAP HQ camera (Roper Scientific) controlled by Nikon NIS-Elements AR software. Mitotic cells from fixed plates were imaged using multiple z-stacks. Fiji imaging software was used to categorize cells as bipolar, monopolar, multipolar, or misaligned. Bipolar cells had spindles with 2 poles and proper DNA alignment along the metaphase plate. Monopolar cells had spindles emanating from a single pole. Multipolar cells had spindles emanating from 3+

poles. Misaligned cells had spindles with two poles and DNA scattered away from the metaphase plate. Data was plotted and analyzed using Prism Software.

TIRF Imaging and Analysis – Cells undergoing MT polymerization analysis were imaged using a 100x oil/1.49 objective, a Nikon Eclipse T*i*-E inverted microscope equipped with a motorized total internal reflection fluorescence (TIRF) system (Nikon), and an Andor-Clara Interline camera (Andor Technologies, Belfast, UK) controlled by Nikon NIS-Elements AR software. Cells were live-imaged every 3 seconds for 4 minutes. Ten different MT plus-ends were tracked per cell (via EB1-tRFP) and averaged to provide a mean MT polymerization rate for each cell. Data was plotted and analyzed using Prism Software.

Results

Msps TOG1 is necessary to maintain cellular MT polymerization rates – XMAP215 TOG1 and TOG2 have been shown to play an important role in promoting MT growth (7, 13, 20). However, it is still unclear how these domains individually contribute to Msps activity. To examine the role of TOG1 in promoting MT polymerization, we knocked down endogenous Msps in *D.m.* S2 cells and transfected them with a novel ΔTOG1 (6-234) construct. MT polymerization rates were then measured using EB1 as a readout for MT plus-end polymerization rates. Endogenous Msps knockdown reduced MT polymerization to a rate of 4.8 μm/min, which was rescued by transfection of FL Msps (Fig. 1A). In contrast, ΔTOG1-expression was not able to rescue MT polymerization rates (Fig. 1A). Both ΔTOG1-cells and Msps knockdown cells yielded polymerization rates that were significantly lower ($p < 0.0001$) than cells with FL Msps activity. The ΔTOG1 construct localized to MT plus-ends suggesting that the reduction in MT polymerization rates in ΔTOG1-expressing cells was due to the absence

of TOG1 and not improper protein localization (Fig. 1B). These results demonstrate that removing TOG1 from the Msps TOG domain array abolishes the protein's ability to promote MT polymerization, despite its proper localization to the plus-end tip of MTs.

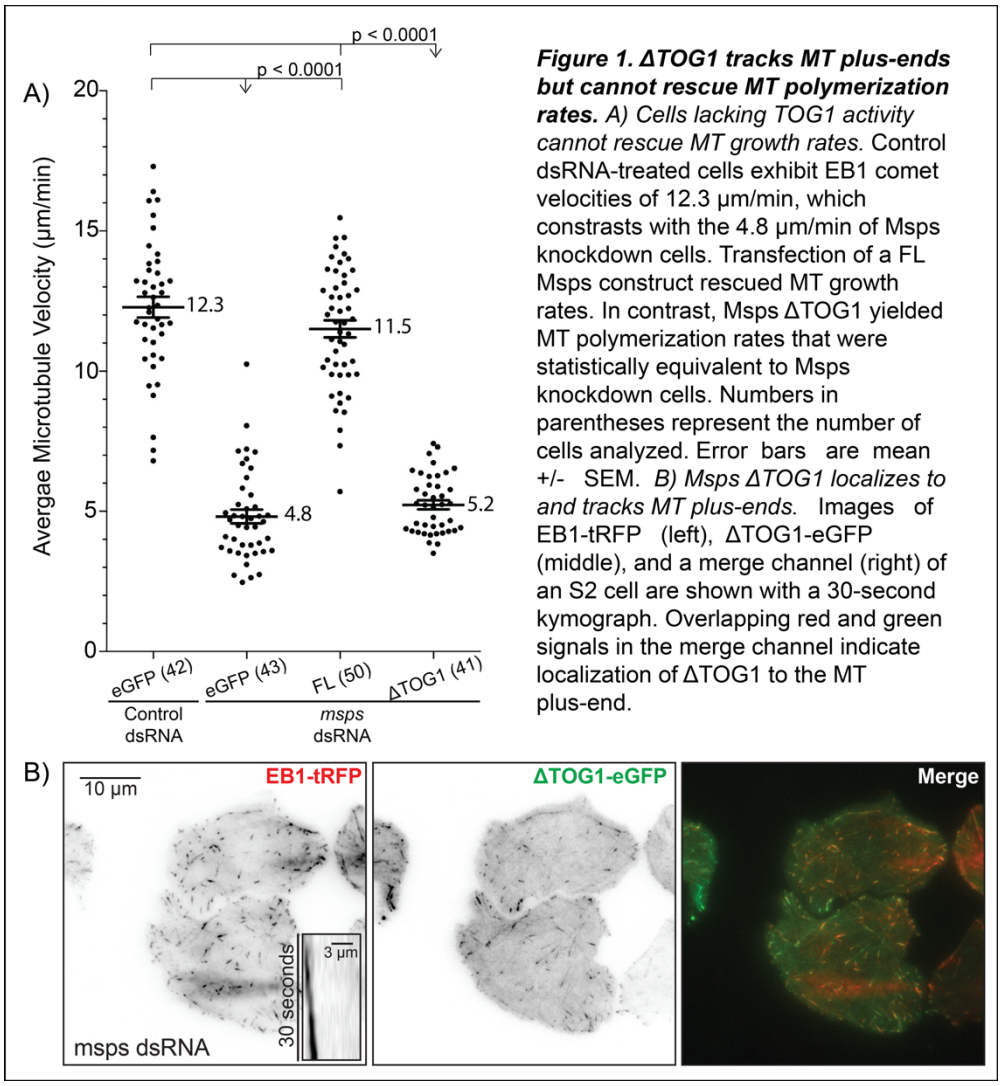

Figure 1. ΔTOG1 tracks MT plus-ends but cannot rescue MT polymerization rates. *A) Cells lacking TOG1 activity cannot rescue MT growth rates.* Control dsRNA-treated cells exhibit EB1 comet velocities of 12.3 µm/min, which constrasts with the 4.8 µm/min of Msps knockdown cells. Transfection of a FL Msps construct rescued MT growth rates. In contrast, Msps ΔTOG1 yielded MT polymerization rates that were statistically equivalent to Msps knockdown cells. Numbers in parentheses represent the number of cells analyzed. Error bars are mean +/- SEM. *B) Msps ΔTOG1 localizes to and tracks MT plus-ends.* Images of EB1-tRFP (left), ΔTOG1-eGFP (middle), and a merge channel (right) of an S2 cell are shown with a 30-second kymograph. Overlapping red and green signals in the merge channel indicate localization of ΔTOG1 to the MT plus-end.

TOG1 activity helps maintain normal, bipolar spindle formation during mitosis – As the absence of TOG1 leads to an inability of Msps to maintain interphase MT polymerization rates, we next investigated the effects of TOG1 deletion on mitotic spindle formation, a more complex process that requires Msps (16). To test if removing TOG1 from the Msps pentameric array also affects mitotic spindle formation, we analyzed mitotic spindle phenotypes in control and ΔTOG1-expressing S2 cells. Control mitotic cells were 52.4% bipolar, with misaligned and multipolar spindles accounting for the majority of abnormal spindle phenotypes (Fig. 2). Msps knockdown yielded a considerably reduced percentage of bipolar spindles with a concomitant increase in misaligned and monopolar spindle phenotypes. Transfection of FL Msps rescued

mitotic spindle phenotypes, with a nearly identical proportion of bipolar cells (Fig. 2). Cells expressing the ΔTOG1 construct yielded a diminished proportion of bipolar spindle phenotypes on par with Msps knockdown cells. This data suggests that ΔTOG1 mitotic cells are much less likely to have a bipolar spindle than cells expressing FL Msps.

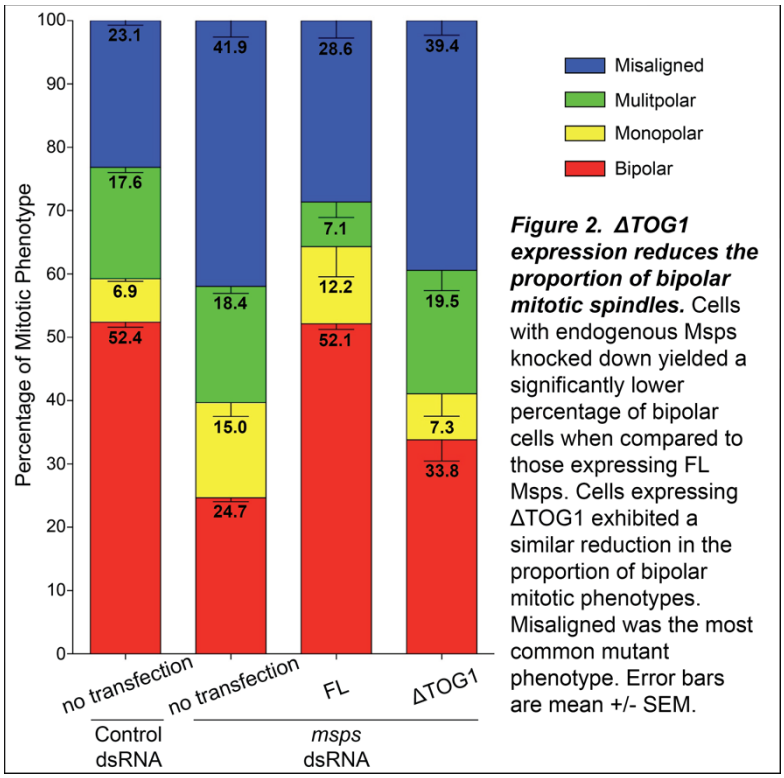

Figure 2. ΔTOG1 expression reduces the proportion of bipolar mitotic spindles. Cells with endogenous Msps knocked down yielded a significantly lower percentage of bipolar cells when compared to those expressing FL Msps. Cells expressing ΔTOG1 exhibited a similar reduction in the proportion of bipolar mitotic phenotypes. Misaligned was the most common mutant phenotype. Error bars are mean +/- SEM.

Discussion

Knockdown of Msps and its XMAP215 orthologs decreases MT growth rates (17, 20). Thus, the reduced MT polymerization rates we observed in Msps-depleted cells is consistent with previous XMAP215 analyses. Significantly, our results also showed that deleting TOG1 from the Msps array could not rescue MT growth rates. Specifically, expression of the ΔTOG1 construct led to a drastic reduction of MT polymerization rates in comparison to cells with FL Msps activity. The failure of this truncated protein to rescue MT growth rates was statistically equivalent to a complete lack of the Msps protein. As hypothesized, these results indicate that TOG1 is required for Msps-mediated MT polymerization activity. We also conclude that the other TOG domains cannot compensate for loss of the first TOG domain including TOG2, which has been shown to be important for XMAP215 activity and, based on its similarity to TOG1,

would be predicted to be the best candidate to offset the negative impact of TOG1 deletion (13, 20).

Msps depletion not only decreases interphase MT polymerization rates, but also increases the occurrence of abnormal mitotic phenotypes (16). This is corroborated by our mitotic spindle phenotype results, in which endogenous Msps knockdown yielded a much higher proportion of abnormal, non-bipolar mitotic cells when compared to cells expressing FL Msps. ΔTOG1-expressing cells similarly yielded a greater proportion of abnormal spindle phenotypes when compared to FL cells. Thus, we conclude that TOG1 is necessary for Msps to properly regulate mitotic spindle formation. ΔTOG1-expressing cells had a slightly higher percentage of bipolar mitotic cells than Msps knockdown cells (24.7% vs. 33.8%) indicating that Msps retains some functionality regarding mitotic spindle formation even in the absence of TOG1.

Taken together, our results suggest that TOG1 is critical for proper Msps-mediated MT dynamics, confirming our hypothesis. Due to the conservation of individual TOG domains among XMAP215 family proteins, these results can likely be applied to the TOG domain arrays of other XMAP215 family members. However, there are still many more questions to be answered. Some of the questions stemming directly from this study involve the role of TOG2. As stated above, TOG2 is also important for XMAP215 function (13, 20). While we have deduced that TOG1 is a critical component of Msps activity, we do not yet understand the precise role of TOG2. For example, is TOG2 equally important for XMAP215 activity such that full Msps function requires the presence of both TOG1 and TOG2? Or could its effect on Msps function be marginal compared to that of TOG1? Future research focusing on TOG2 will be required to deduce its role in comparison to that of TOG1. This will also help provide answers for some of

the larger questions surrounding XMAP215 proteins, particularly those involving the mechanism by which these proteins promote MT polymerization.

Acknowledgments

I would like to thank Dr. Kevin Slep for allowing me to work in his lab these past two years, providing new questions and ideas, and editing all of my papers, posters, and presentations. I would also like to thank Amy Byrnes for directly mentoring me, including (but not limited to) teaching me basic and advanced lab protocols and skills, helping me to develop my questions, hypotheses, and projects, overseeing my day-to-day work, answering any and all questions I had, and editing all of my papers, posters, and presentations. I am also very appreciative of Rebecca Adikes, Thomas Lane, and Dr. Stephen Rogers for providing extra help and answering miscellaneous questions throughout my time in the lab. Finally, I would like to thank Dr. Amy Maddox and Danielle Spitzer for helping me develop and refine this Senior Honors Thesis and its corresponding presentation.

References

1. Alfaro-Aco, R., & Petry, S. (2015). Building the Microtubule Cytoskeleton Piece by Piece. *The Journal of Biological Chemistry, 290*, 17154-17162.
2. Wade, R. H. (2009). On and Around Microtubules: An Overview. *Molecular Biotechnology, 43*, 177-191.
3. Slep, K. C. (2010). Structural and Mechanistic Insights into Microtubule End-Binding Proteins. *Current Opinion in Cell Biology, 22*, 88-95.
4. Gard, D. L., & Kirschner, M. W. (1987). A Microtubule-Associated Protein from Xenopus Eggs that Specifically Promotes Assembly at the Plus-End. *The Journal of Cell Biology, 105*, 2203-2215.
5. Slep, K., and Vale, R. (2007). Structural Basis of Microtubule Plus-End Tracking by XMAP215, CLIP-170, and EB1. *Molecular Cell 27*, 976-991.
6. Al-Bassam, J., Larsen, N., Hyman, A., and Harrison, S. (2007). Crystal Structure of a TOG Domain: Conserved Features of XMAP215/Dis1-Family TOG Domains and Implications for Tubulin Binding. *Structure 15*, 355-362.

7. Al-Bassam, J., Van Breugel, M., Harrison, S. C., Hyman, A. (2006). Stu2p Binds Tubulin and Undergoes an Open-To-Closed Conformational Change. *The Journal of Cell Biology, 172*(7), 1009-1022.
8. van Breugel, M., Drechsel, D., and Hyman, A. (2003). Stu2p, the Budding Yeast Member of the Conserved Dis1/XMAP215 Family of Microtubule-Associated Proteins is a Plus End-Binding Microtubule Destabilizer. *The Journal of Cell Biology, 161*(2), 359–369.
9. Cassimeris, L., Gard, D., Tran, P., and Erickson, H. (2001) XMAP215 Is a Long Thin Molecule That Does Not Increase Microtubule Stiffness. *Journal of Cell Science, 114*, 3025-3033.
10. Ayaz, P., Munyoki, S., Geyer, E. A., Piedra, F., Vu, E. S., Bromberg, R., Otwinowski, Z., Grishin, N. V., Brautigam, C. A., Rice, L. M. (2014). A Tethered Delivery Mechanism Explains the Catalytic Action of a Microtubule Polymerase. *eLife, 3*, e03069.
11. Ayaz, P., Xuecheng, Y., Huddleston, P., Brautigam, C. A., Rice, L. M. (2012). A TOG:ab-tubulin Complex Structure Reveals Conformation-Based Mechanisms for a Microtubule Polymerase. *Science, 337*, 857-860.
12. Howard, A. E., Fox, J. C., & Slep, K. C. (2015). Drosophila Melanogaster Mini Spindles TOG3 Utilizes Unique Structural Elements to Promote Domain Stability and Maintain a TOG1- and TOG2-Like Tubulin-Binding Surface. *Journal of Biological Chemistry, 219*(16), 10149-10162.
13. Fox, J. C., Howard, A. E., Currie, J. D., Rogers, S. L., & Slep, K. C. (2014). The XMAP215 Family Drives Microtubule Polymerization Using a Structurally Diverse Tog Array. *Molecular Biology of the Cell, 25*, 2375-2392.
14. Currie, J. D., Stewman, S., Schimizzi, G., Slep, K. C., Ma, A., Rogers, S. L. (2011). The Microtubule Lattice and Plus-End Association of *Drosophila* Mini Spindles is Spatially Regulated to Fine-Tune Microtubule Dynamics. *Molecular Biology of the Cell, 22*, 4343-4361.
15. Al-Bassam, J., & Chang, F. (2011). Regulation of Microtubule Dynamics by TOG-Domain Proteins XMAP215/Dis1 and CLASP. *Trends in Cell Biology, 21*(10), 604-614.
16. Cullen, C. F., Deák, P., Glover, D. M., & Ohkura, H. (1999). Mini Spindles: A Gene Encoding a Conserved Microtubule-Associated Protein Required for the Integrity of the Mitotic Spindle in Drosophila. *The Journal of Cell Biology, 146*(5), 1005-1018.
17. Brittle, A. L., & Ohkura, H. (2005). Mini Spindles, the XMAP215 homologue, suppresses pausing of the interphase microtubules in Drosophila. *The EMBO Journal, 24*, 1387-1396.
18. Brouhard, G., Stear, J., Noetzel, T., Al-Bassam, J., Kinoshita, K., Harrison, S., Howard, J., and Hyman, A. (2008). XMAP215 Is a Processive Microtubule Polymerase. *Cell, 132*, 79-88.
19. Kerssemakers, J., Munteanu, E., Laan, L., Noetzel, T., Janson, M., and Dogterom, M. (2006). Assembly Dynamics of Microtubules at Molecular Resolution. *Nature, 442*(10), 709-712.
20. Widlund, P. O., Stear, J. H., Pozniakovsky, A., Zanic, M., Reber, S., Brouhard, G. J., Hyman, A. A., & Howard, J. (2011). XMAP215 Polymerase Activity is Built by Combining Multiple Tubulin-Binding TOG Domains and a Basic Lattice-Binding Region. *Proceedings of the National Academy of Sciences, 108*(7), 2741-2746.
21. Rogers, S., Rogers, G. (2008). Culture of Drosophila S2 Cells and Their Use for RNAi-Mediated Loss-of-Function Studies and Immunofluorescence Microscopy. *Nature Protocols, 3*(4), 606-611.
22. Rogers, S., Rogers, G., Sharp, J., & Vale, R. (2002). Drosophila EB1 Is Important for Proper Assembly, Dynamics, and Positioning of the Mitotic Spindle. *The Journal of Cell Biology, 158*(5), 873-884.