The Molecular Mechanism of Mozart1-Mediated γ-Tubulin Ring Complex Assembly and Cellular Targeting

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Abstract

Microtubule network organization depends on the γ-tubulin ring complex (γ-TuRC), an evolutionarily conserved protein complex composed of multiple subunits (e.g. γ-tubulin, γ-tubulin complex protein (GCP)2-6, Mozart (Mzt)1-2, and NEDD1) in metazoans. While γ-TuRC is known to serve as a template for *de novo* microtubule assembly, the detailed molecular basis underlying γ-TuRC assembly and microtubule organizing centers (MTOCs) targeting remains nuclear. We first report that Mzt1 interacts with different GCPs and forms multi-faceted, structurally-mimetic "modules" that contribute to structural stability and localization specificity of γ-TuRC. By using a multidisciplinary approach, including biochemical studies, X-ray crystallography, and cryo-EM, we demonstrate an intercalative binding mode between Mzt1 and the N-terminal α-helical domains of GCPs in γ-TuRC. Moreover, two of Mzt1 sub-complexes, Mzt1/GCP3-NTD and Mzt1/GCP6-NTD form a "lumenal bridge" and may structurally stabilize the γ-TuRC assembly. This hypothesis is further validated by pull down analysis as removal of Mzt1 binding sites in γ-TuRC reduces pull down of intact γ-TuRC. Additionally, cell-based analyses shows that promiscuous binding of Mzt1 in γ-TuRC controls specific subcellular localization of γ-TuRC and thereby modulates microtubule nucleation and stabilization in fission yeast. We also find a portion of γ-TuRC is recruited to mitotic spindle pole body independent of Mzt1 binding, suggesting the cell cycle-dependent regulation and function of γ-TuRC. Together, our results reveal a microprotein Mzt1-mediated regulatory mechanism for γ-TuRC that temporally and spatially controls of microtubule cytoskeleton formation.

Keywords - γ-tubulin, γ-tubulin ring complex(γ-TuRC), Mozart (Mzt)1, microtubule

Introduction

γ-tubulin ring complex (γ-TuRC) is proposed to promote and regulate microtubule formation in the cell. In current models, γ-TuRC is found to possess a single-turn helical ring like structure with 14 γ-tubulins arranging at the end of the ring which can interact with α/β tubulins to serve as a microtubule nucleation template within cells. After γ-TuRC has been proposed to nucleate microtubule for more than two decades, it is still unclear how γ-TuRC regulates microtubule formation under tightly temporal and spatial control. Although recent cryo-EM structures have revealed the subunit configuration of γ-TuRC assembly, lacking of microtubule nucleation activity of the endogenous γ-TuRC suggests that nucleation activity only occurs after γ-TuRC cellular targeting. Also, due to the low resolution in the cryo-EM structures, the structural details of the non-GCP subunits, known as the functional regulators in γ-TuRC are unclear. To understand how these functional regulators, modulate γ-TuRC activities (e.g. assembly, cellular targeting, microtubule formation), we focus on structural and functional characterization of Mzt1, as (1) the phenotype of Mzt1 depletion in cultured human cells resembles that for γ-tubulin RNAi; namely, a large proportion of monopolar spindle organization of microtubules in mitotic cells, leading to cell cycle arrest, and (2) studies in fission yeast, *C. elegans*, plant and human cells have demonstrated that Mzt1 involves in proper localization of γ-TuRCs to specific MTOCs such as centrosome, spindle pole bodies, basal bodies, or the pre-existing microtubules. Additionally, Mzt1 also has been proposed to participate in the solubilization and oligomerization of reconstituted *S. pombe* and *C. albicans* γ-tubulin-containing sub-complexes *in vitro*, initiating γ-TuRC assembly. Since Mzt1 is known to interact with the N-terminal domains (NTDs) of multiple GCP proteins in many organisms, it has long been argued that whether Mzt1 sub-complexes mediate the assembly or the cellular targeting of γ-TuRC. To examine the function of Mzt1 in γ-TuRC, we therefore carry out biochemical and cell biology analysis to structurally and functionally characterize GCP-NTDs•Mzt1 sub-complexes in γ-TuRC.

Experiments

Bacteria strains, microbiological techniques, plasmids and DNA manipulations, Protein expression and purification, E. coli expression system, Protein crystallization, X-ray data collection and Structure determination, Size-exclusion chromatography, coupled light scattering, Electron microscopy, Imaging processing and two-dimensional reconstruction, Yeast strain construction, Yeast spot-based assay, Yeast cold treatment assay, Immuno-precipitation and Western blot, Imaging *S. pombe* cells by fluorescence microscopy

Results

Here we report that an evolutionarily conserved microprotein, Mozart1 (Mzt1), regulates subcellular targeting and microtubule formation activity of γ-TuRC at different cell cycle stages. Crystal structures of protein complexes demonstrate that Mzt1 promiscuously interacts with the N-terminal domains of multiple γ-tubulin complex protein subunits in γ-TuRC via an intercalative binding mode (Figure 1) [1, 2]. Genetic and microscopy-based analyses show that the promiscuous binding of Mzt1 in γ-TuRC controls specific subcellular localization of γ-TuRC, modulating microtubule nucleation and stabilization in fission yeast (Figure 2) [1]. Moreover, we find Mzt1-independent targeting of γ-TuRC to be crucial for mitotic spindle assembly, demonstrating cell cycle-dependent regulation and function of γ-TuRC [1]. Our findings reveal a microprotein-mediated regulatory mechanism underlying microtubule cytoskeleton formation whereby Mzt1 binding promiscuity confers localization specificity on the multi-protein complex γ-TuRC [1].

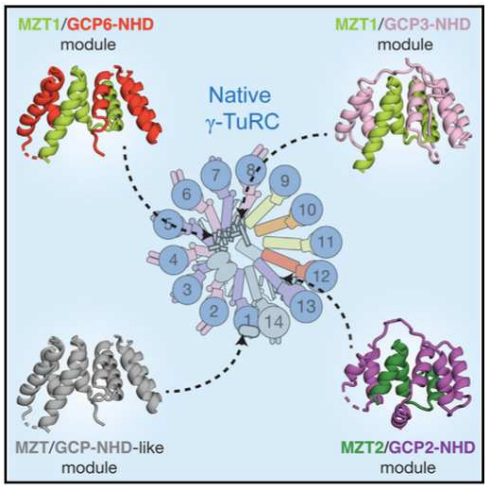

Figure 1. Schematics of the native γ-TuRC. Cartoon representation of Mzt1 complexes. Locations of each complex in the native γ-TuRC are indicated.

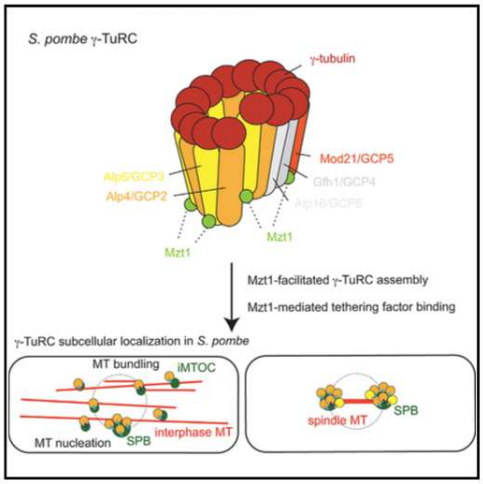

Figure 2. A schematic model for how the microprotein Mzt1 regulates γ-TuRC targeting to MTOCs. Schematic models of the assembly of γ-TuRC modulated by Mzt1, which controls the subcellular localization of γ-TuRC. Mzt1-dependent and Mzt1-independent γ-TuRC localization at MTOCs. During interphase, recruitment of γ-TuRC to SPBs and iMTOCs in a Mzt1-dependent fashion is facilitated by binding of Mzt1 to Alp6-NTD and Mod21-NTD. During mitosis, γ-TuRC is targeted to SPBs via Mzt1-dependent and Mzt1-independent pathways.

Discussion

In this study, we conducted a multidisciplinary approach, including biochemistry, structural biology (X-ray crystallography and cryo-EM), yeast genetics and cell based analysis to study the structure and function of Mzt1 protein in γ-TuRC. As Mzt1 belongs to a microprotein family duo to its small molecular weight, the structures of Mzt1 in complex with different GCP-NTDs provide examples of how a microprotein interacts with its binding partners. Our data reveal three distinct structurally-mimetic sub-complexes, termed Mzt "modules", can provide both structural stabilization and cellular targeting regulation for γ-TuRC. Furthermore, using *S. pombe* as model organism, we propose a model of how microtubule array formation is regulated by γ-TuRC at various MTOCs. Currently centrosomin motif 1 (CM1) is the only protein sequence found in the γ-TuRC tethering factors and is proposed to recruit γ-TuRC to MTOCs. It is unclear how CM1 in tethering factors recruits γ-TuRC to different MTOCs. Moreover, whether other binding motifs exist and facilitate different γ-TuRC cellular targeting remains unknown. Based on our results, we propose that γ-TuRC can be recruited to MTOCs through a Mzt1-dependent and -independent manners by MTOC-specific receptors.

Highlights

1. Mzt1 interacts with multiple γ-TuRC subunits via an intercalative binding mode.
2. Mzt1/GCP3-NTD and Mzt1/GCP6-NTD occupy the γ-TuRC luminal bridge.
3. Promiscuous binding of Mzt1 to γ-TuRC modulates γ-TuRC assembly and targeting.
4. Mzt1 regulates specific subcellular localization of γ-TuRC
5. γ-TuRC Prossesses founctional heterogeneity, modulating microtubule formation.

References

1. **Huang, T.L.**, Wang, H.J., Chang, Y.C., Wang, S.W., Hsia, K.C. (2020) Promiscuous binding of microprotein Mozart1 to γ-tubulin complex mediates specific subcellular targeting to control microtubule array formation. *Cell Rep.* 30;31(13):107836.
2. Wieczorek, M.*, **Huang, T.L.***, Urnavicius, L.*, Hsia, K.C., Kapoor, T.M. (2020) MZT proteins form multi-faceted structural modules within the γ-tubulin ring complex. *Cell Rep.* 30;31(13):107791 (equally contributed*)