doi:10.1093/jmcb/mjz044
Published online May 24, 2019

Highlight

Microtubule nucleation and dynamic instability in interphase fission yeast

Xin Liang[1,2,*]

[1] Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China
[2] Max-Planck Partner Group, School of Life Sciences, Tsinghua University, Beijing 100084, China

* Correspondence to: Xin Liang, E-mail: xinliang@tsinghua.edu.cn

Microtubules are dynamic cytoskeletal structures essential for shaping cellular architecture and driving cell motility. Their tube-like structure typically comprises 13 protofilaments, each of which is formed of α/β tubulin heterodimers arranged in a head-to-tail manner (Howard, 2001). The formation of microtubules primarily contains two processes. The first process in which soluble dimers start to form a growing microtubule is termed microtubule nucleation (Figure 1A). Spontaneous nucleation, i.e. *de novo* microtubule formation from free dimers, is thermodynamically unfavorable, so cellular microtubules usually nucleate from existing templates (i.e. microtubule organization centers, MTOCs) (Wieczorek et al., 2015) (Figure 1A). The distribution of MTOCs (e.g. the γ-tubulin ring complex) shapes the organization of cytoplasmic microtubules in cells. The rate of nucleation can be regulated by protein molecules associated with MTOCs or involved in microtubule assembly (Wieczorek et al., 2015). In the second process, microtubules show persistent growth but occasionally switch from a relatively slow elongation phase to a rapid shortening phase (i.e. catastrophe) and then switch back (i.e. rescue) (Figure 1A). This behavior is known as microtubule dynamic instability (Mitchison and Kirschner, 1984), which is regulated by microtubule-associated proteins. The mean length (L_{mean}) of cellular microtubules can be estimated by the parameters that describe microtubule dynamic instability (Eq. 1):

$$L_{mean} \cong \frac{v_+}{f_{cat}} \quad (1)$$

where v_+ is the elongation rate (μm·min^{-1}) and f_{cat} is the catastrophe frequency (min^{-1}) (Howard, 2001). Therefore, microtubule dynamics determines how cytoskeletal patterns can be established in cells.

Schizosaccharomyces pombe (i.e. fission yeast) is a great model organism in studying how microtubules are organized in eukaryotic cells. Interphase fission yeast has a polarized shape with a length of ∼10 μm and a width of ∼3 μm. In these cells, cytoplasmic microtubules are organized into several bundles with their plus-ends growing towards the cell tips (along the long axis) and minus-ends located in the middle of the cells (Figure 1B). This arrangement of microtubules is key for establishing and maintaining the cellular polarity of fission yeast. How is this cytoskeletal organization formed? Two new papers from the group of Chuanhai Fu, both appearing in the current issue, address this question by studying microtubule nucleation (Liu et al., 2019) and dynamic instability (Niu et al., 2019) in interphase fission yeast.

Cytoplasmic microtubules in interphase fission yeast are often nucleated from the spindle pole body and/or the nuclear envelop (NE), thought to be the primary sites of interphase MTOCs (i.e. iMTOCs) (but see also Sawin and Tran, 2006). How can microtubules be nucleated from iMTOCs? Previous studies showed that Alp14, a microtubule polymerase (Al-Bassam et al., 2012), promotes microtubule nucleation in fission yeast (Flor-Parra et al., 2018). This nucleation-promoting activity depends on Alp7, a transforming acidic coiled-coil family protein required for the NE localization of γ-tubulin and Mto1, a protein that mediates microtubule nucleation from the γ-tubulin ring complex (Sawin and Tran, 2006). How do Alp7–Mto1 complex and Alp14 work in a concerted manner to regulate microtubule nucleation? To address this question, Liu et al. (2019) analyzed microtubule regrowth in interphase fission yeast. They demonstrated that cytoplasmic microtubules predominantly regrow from the NE, similar to the previous observations using cold treatment (Sawin and Tran, 2006). By analyzing *alp7*Δ, *alp14*Δ, and *mto1*Δ mutants, the authors found that Alp7 and Mto1 are mutually dependent for their normal localizations, while Mto1 is additionally required for microtubule nucleation. Alp14, whose localization on the NE depends on Alp7 and Mto1, contributes to microtubule nucleation and elongation. Based on these results, Liu et al. (2019) proposed a molecular model for how the

Alp7–Mto1 complex and Alp14 act together to promote the regrowth/nucleation of cytoplasmic microtubules from MTOCs on the NE (Figure 1B).

In the elongation process, cytoplasmic microtubules in interphase fission yeast show robust growth and catastrophe often occurs after the microtubules having reached the cell tips (Sawin and Tran, 2006). This is thought to be key in generating microtubules that are long enough so the polarity factors can be delivered to the cell tips (Sawin and Tran, 2006), but how is microtubule catastrophe inhibited to ensure the robust microtubule elongation? Previous studies suggest that two plus-end tracking proteins, Tip1 (CLIP-170) and Mal3 (EB1) (Figure 1B), are both catastrophe preventers (Busch and Brunner, 2004; Sawin and Tran, 2006), but how do they antagonize microtubule catastrophe? To answer this question, Niu et al. (2019) measured the dynamic instability of cytoplasmic microtubules in interphase fission yeast using live-cell imaging. The key finding is that Tip1 stabilizes the tip binding of Alp14 and prevents Klp5, a microtubule catastrophe factor (Garcia et al. 2002), from arriving at the growing plus-ends. In this way, Tip1 could stabilize the persistent elongation of cytoplasmic microtubules, according to Eq. 1, by ensuring a fast elongation rate (v_+) and a low catastrophe frequency (f_{cat}). Therefore, the results in Niu et al. (2019) suggest a new mechanism in which Tip1 plays a key role in regulating the plus-end dynamics of microtubules in interphase fission yeast (Figure 1B).

In summary, these two studies reveal local networks of interacting factors that regulate microtubule nucleation and dynamic instability in interphase fission yeast, providing mechanistic insights into understanding of how the cytoplasmic organization of microtubules can be established. These findings also raise many new questions. First, how is the Alp7–Mto1 complex targeted to the NE? Identifying the linker is needed. Second, Alp14 contributes to both microtubule nucleation and elongation, but how are these two activities spatial-temporally coordinated? The molecular role of Alp14 in regulating microtubule nucleation needs to be specified. Third, the microtubule catastrophe phenotype in *mal3*Δ is more severe than that in *tip1*Δ (Busch and Brunner, 2004; Sawin and Tran, 2006), does Mal3 make an additional contribution in inhibiting microtubule catastrophe? The function of Mal3 in this process needs to be clarified. Finally, how does Tip1 stabilize the plus-end binding of Alp14 at molecular level? It would be helpful to measure the tip-binding kinetics of Alp14 at single molecule level using *in vitro* microtubule reconstitution system. Clearly, tackling these questions will not be easy. However, these two new studies published in current issue, together with the previous findings, open the gate to further approach these questions, so we could look forward to the next installments.

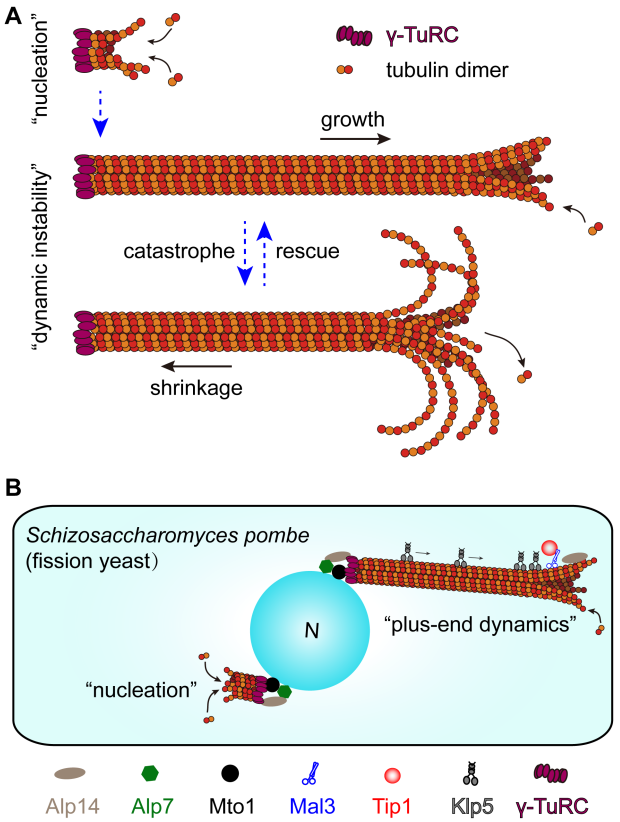

Figure 1 Microtubule nucleation and dynamic instability. (**A**) Schematic of microtubule nucleation and dynamic instability. (**B**) Schematic of microtubule nucleation (lower left microtubule) and dynamic instability (upper right microtubule) in interphase fission yeast.

[The author thanks Yin-Long Song and Wei Chen for their help in preparing the figure and their comments on this manuscript. The author also acknowledges the funding support from the National Natural Sciences Foundation of China (31671389), Max-Planck Partner Group Program and Tsinghua-Peking Joint Center for Life Sciences.]

References

Al-Bassam, J., Kim, H., Flor-Parra, I., et al. (2012). Fission yeast Alp14 is a dose-dependent plus end-tracking microtubule polymerase. Mol. Biol. Cell *23*, 2878–2890.

Busch, K.E., and Brunner, D. (2004). The microtubule plus end-tracking proteins mal3p and tip1p cooperate for cell-end targeting of interphase microtubules. Curr. Biol. *14*, 548–559.

Flor-Parra, I., Iglesias-Romero, A.B., and Chang, F. (2018). The XMAP215 ortholog Alp14 promotes microtubule nucleation in fission yeast. Curr. Biol. *28*, 1681–1691.e4.

Garcia, M.A., Koonrugsa, N., and Toda, T. (2002). Two kinesin-like Kin I family proteins in fission yeast regulate the establishment of metaphase and the onset of anaphase A. Curr. Biol. *12*, 610–621.

Howard, J. (2001). Mechanics of Motor Proteins and the Cytoskeleton. Sunderland, MA: Sinauer Associates.

Liu, W., Zheng, F., Wang, Y., et al. (2019). Alp7–Mto1 and Alp14 synergize to promote interphase microtubule regrowth from the nuclear envelope. J. Mol. Cell Biol. *11*, 944–955.

Mitchison, T., and Kirschner, M. (1984). Dynamic instability of microtubule growth. Nature *312*, 237–242.

Niu, X., Zheng, F., and Fu, C. (2019). The concerted actions of Tip1/CLIP-170, Klp5/Kinesin-8, and Alp14/XMAP215 regulate microtubule catastrophe at the cell end. J. Mol. Cell Biol. *11*, 956–966.

Sawin, K.E., and Tran, P.T. (2006). Cytoplasmic microtubule organization in fission yeast. Yeast *23*, 1001–1014.

Wieczorek, M., Bechstedt, S., Chaaban, S., et al. (2015). Microtubule-associated proteins control the kinetics of microtubule nucleation. Nat. Cell Biol. *17*, 907–916.