Microtubule nucleation and dynamic instability in interphase fission yeast

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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):

\[ L_{\text{mean}} \approx \frac{v_+}{f_{\text{cat}}} \]  

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 width of ∼3 µm. 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) (see 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 alp7Δ, alp14Δ, and mto1Δ 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 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 mal3Δ is more severe than that in tip1Δ (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.

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.

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