A microtubule dynamics reconstititutional convention

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In vitro reconstitution is the fundamental test for identification of the core components of a biological process. In this issue, Moriwaki and Goshima (2016. J. Cell Biol. https://doi.org/10.1083/jcb.201604118) reconstitute all phases of microtubule dynamics through the inclusion of five key regulators and demonstrate that Polo kinase activity shifts the system from an interphase mode into an enhanced mitotic mode.

Microtubules are cytoskeletal structures that serve as tracks for motor-based intracellular transport and underlie the organization of biological apparatuses, including the mitotic spindle, cilia, and the phragmoplast. In vivo, microtubules are highly dynamic and interconvert between phases of polymerization, pause, and depolymerization. In vitro, microtubules are dynamic, but their behavior poorly mimics that observed in living cells. Identifying the regulators responsible for tuning in vitro microtubule dynamics to match the in vivo behavior has been a quest in the reconstitution field. In a new study published in this issue, Moriwaki and Goshima have now identified the five key components that are necessary for the recapitulation of all three phases of microtubule dynamics in vitro.

Nearly a century ago, mitotic spindle dynamics were observed. Determining the mechanisms that underlie these movements has remained a topic of active research. A key advance occurred in 1967 when Inoué and Sato (1967) used polarization microscopy to observe dynamic linear elements in the spindle. What were these linear elements made out of and how did they change their length? The discovery that colchicine disrupted the spindles positioned the field to test whether XMAP215, identified, the field was now positioned to test whether XMAP215, and Kinesin-13 (Kinoshita et al., 2001). With the inclusion of these MAPs, microtubule dynamics had been reconstituted, but there was one catch: the rates observed in vitro and the percentage of time microtubules spent in each phase ( polymerization, depolymerization, and pause) did not correlate well with in vivo observations. Were there other factors required to regulate and tune dynamic instability? Enter the microtubule-associated proteins (MAPs).

Two key MAP families were identified that promoted microtubule polymerization and depolymerization. In 1987, Gard and Kirschner (1987) purified XMAP215 and characterized its ability to potentiate microtubule polymerization. In 1999, Desai et al. (1999) identified Kinesin-13 family members as microtubule depolymerization factors. With these factors now in hand, the Hyman laboratory set out to reconstitute microtubule dynamics using purified tubulin, XMAP215, and Kinesin-13 (Kinoshita et al., 2001). With the inclusion of these MAPs, microtubule dynamics started to approach in vivo rates, but the limited sampling of the microtubule pause state in vitro suggested that yet another factor was required to stabilize microtubules in the pause state.

Key steps forward included the identification of the CLIP-associating protein (CLASP) family that promotes the microtubule pause state (Akhmanova et al., 2001; Sousa et al., 2007), as well as a microtubule plus end tracking complex involving EB1 and SLAIN2/Sentin that recruits XMAP215 and CLASP to growing microtubule tips (van der Vaart et al., 2011; Li et al., 2012). With these molecular machines identified, the field was now positioned to test whether XMAP215, Kinesin-13, CLASP, Sentin, and EB1 could collectively reproduce microtubule dynamics in vitro. Moriwaki and Goshima (2016) have now addressed this challenge. Given the extent to which these regulators had been characterized in Drosophila melanogaster cell culture, the authors decided to use purified...
Drosophila homologues for their in vitro analyses. This would enable them to compare and contrast cellular microtubule dynamics in wild-type and factor-depleted cultures with the effects of all or a subset of these MAPs on in vitro microtubule dynamics. They purified Drosophila XMAP215Msps, Kinesin-13Klp10A, CLASP$^{Mas/Or}$, and Kinesin-13Klp10A, promote polymerization, pause, and depolymerization, respectively. Although these regulators engage the microtubule directly, EB1 and Sentin recruit CLASP$^{Mas/Or}$ and XMAP215Msps to polymerizing microtubule plus ends and affect their activity. (B) Microtubule dynamics diamond graphs showing in vitro microtubule behavior without (left) and with (right) Plk$^\text{Polo}$ treatment. The black diamond graph at left is overlaid on the purple graph at right and shown in dark gray for comparison.

As cells transition from interphase into mitosis, the parameters of microtubule dynamic instability change. The polymerization rate of astral microtubules increases, as does the catastrophe frequency, whereas the rescue frequency decreases (Belmont et al., 1990; Rusan et al., 2001). If Moriwaki and Goshima (2016) had identified the core components required for dynamic instability, would there be other MAPs required to establish the mitotic parameters, or could there be a factor that modified the activity of the core five regulators? Although mitotic MAPs play key roles in this process, Moriwaki and Goshima (2016) inquired whether a mitotic kinase could modulate the activity of their reconstituted system. The prime candidate was the kinase Cdk1 that had been shown in cells to trigger the transition from interphase microtubule dynamics to mitotic microtubule dynamics (Verde et al., 1990). When the authors incubated Cdk1 and ATP with their five MAPs, Kinesin-13Klp10A and XMAP215Msps were phosphorylated, but these posttranslational modifications had little effect on microtubule dynamics in
their reconstituted system. Perhaps Cdk1 was acting upstream of another mitotic kinase that directly drove the mitotic transition. Their next candidate was Polo kinase (Plk1). In vitro, Plk1 phosphorylated Kinesin-13Klp10A, Sentin, and CLA. These observations suggested that Plk1 is involved in the regulation of microtubule dynamics. Their reconstituted system paralleled the way the parameters also changed in cells transitioning from interphase to mitosis. Collectively, this strongly suggests that these five components are indeed the core regulators of microtubule dynamic instability and that Polo kinase tunes the system for mitosis.

Moriwaki and Goshima (2016) have established a foundation upon which additional regulators of microtubule dynamics can be investigated. Of note, whereas the seven parameters for dynamic instability have been reconstituted, they do not perfectly align with in vivo rates and frequencies. Differences are caused, in part, by experimental constraints: not all components could be analyzed at physiological concentrations, crowding agents were not present, and there were likely limits imposed by protein activity as well as in vitro environmental constraints. Clearly limiting is the fact that only five MAPs were present. Adding higher order complexity through the addition of other regulators will be key for future studies. As the complexity of the reconstituted system grows and synergistic effects of components are observed, it will be important to approach the system with quantitative rigor and to systematically titrate components. What is the future for microtubule dynamics reconstitution? Key areas to explore include the identification and mechanistic analysis of phosphorylation sites on MAPs, analysis of tubulin posttranslational modifications on the system’s behavior, and the addition of other motors and MAPs (augmin, katanin, tetrameric kinesins, etc.). Incorporating other biological systems into the fray will also be a key trajectory: how do dynamic microtubules engage targets such as membrane anchor points or kinetochores, and how do cytoskeletal cross-linkers affect the concerted dynamics of the microtubule and actin network? Many researchers are actively tackling these questions, but the groundwork laid to date by numerous laboratories, including the work by Moriwaki and Goshima (2016), will help direct these efforts.

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