Differential Control of Mitotic Exit in Budding Yeast in Response to the Different Type of Perturbation

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After chromosomes have been properly segregated at the end of anaphase, mitotic cyclin-dependent kinases (CDKs) become inactivated for mitotic exit to initiate cytokinesis and entry into a new round of cell cycle. Mitotic exit is best characterized in budding yeast *Saccharomyces cerevisiae*. In budding yeast, mitotic exit is achieved through the activation of a signal cascade known as the mitotic exit network (MEN) that antagonizes mitotic CDKs. In order to maintain genomic integrity, the exit from mitosis is delayed in response to various perturbations such as DNA damage, spindle disruption, and spindle misorientation. The small G-protein Tem1 is the key component of MEN at its upstream and its GTP/GDP switch for GTPase activity has been believed to decide the exit from mitosis. Lte1 possesses a guanine-nucleotide exchange factor (GEF) domain likely for Tem1. The Bfa1/Bub2 complex is proposed to prevent mitotic exit by stimulating Tem1 GTPase activity as a two-component GTPase-activating protein (GAP). However, recent observations showed that cells may control mitotic exit without either Lte1 or Bfa1/Bub2 GAP activity, obscuring how Tem1 is regulated. Here, we assayed *BFA1* mutants with varying GAP activities combined with Bub2 for Tem1, showing for the first time that Bfa1/Bub2 GAP activity inhibits Tem1 in vivo. Interestingly, different levels of GAP activity were required to prevent mitotic exit depending on the type of perturbation. DNA and spindle damages delay mitotic exit in a GAP-dependent manner, but different levels of GAP activity were required for each. Surprisingly, there is no correlation between the decrease of GAP activity and mitotic arrest induced by misoriented spindles. Instead, reduced interaction between Bfa1 and Kin4 was observed in cells with defect in spindle position checkpoint. Thus, we suggest that the GAP-independent surveillance mechanism of Bfa1/Bub2 controls mitotic exit for misaligned spindle.[1]

We observed increased mitotic spindle misorientation in *Δbfa1* cells. In addition, in cells overexpressing Bfa1, astral microtubules became remarkably elongated and stable, frequently curling around the cell cortex. Strikingly, the unusual microtubule dynamics by Bfa1 overexpression is suppressed by Kar9 overexpression. Since astral microtubule dynamics is crucial for spindle positioning and regulated in part by microtubule plus-end interacting proteins such as Kar9, these results suggest that Bfa1 can communicate with microtubule dynamics as well as monitor spindle orientation. On the other hand, we recently reported that Bfa1 disappearance from the mother-bound spindle poles acts to fine-tune the timing of MEN activation on anaphase onset. Interestingly, in the significant fraction of cells lacking Kar3, a minus-directed motor, Bfa1 still was

present on both spindle poles during anaphase, demonstrating the link of Bfa1 localization with microtubule dynamics and spindle positioning. Now we are examining whether microtubule-bud cortex interactions may control the localization of Bfa1 to trigger mitotic exit.[2]

The tight link between the mechanism governing spindle orientation and mitotic exit in budding yeast would be of particular interest since the regulatory mechanism for mitotic exit in budding yeast would help to understand how spindle position is monitored and coupled with mitotic exit for asymmetric divisions in eukaryotes.

References

[1] J. Kim, S. Jang, and K. Song *Mol. Biol. Cell*, **19**, 4328, 2008.

[2] F. Monje-Casas and A. Amon *Dev. Cell*, **16**, 132, 2009.