

P056 Regulation of the budding yeast Tem1 GTPase upon spindle mispositioning

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In budding yeast the spindle position checkpoint delays mitotic exit and cytokinesis in case of spindle mispositioning. This occurs through downregulation of the Tem1 GTPase, which activates the Mitotic Exit Network (MEN) that is essential for mitotic exit and cytokinesis.

The two-component GAP Bub2/Bfa1 keeps Tem1 inactive until the spindle is properly oriented. The GAP domain resides on Bub2, whereas Bfa1 is required for Bub2 interaction with Tem1. In addition, we recently found that Bfa1 is a Guanine-nucleotide Exchange Inhibitor (GDI), suggesting that both Bub2 and Bfa1 contribute to Tem1 inactivation.

The GAPs containing a TBC domain, like Bub2, have been recently proposed to downregulate their cognate GTPases through a “dual finger” mechanism, where an arginine and a glutamine residue provided by the GAP are directly involved in GTP hydrolysis. We have characterized bub2 mutant alleles altering the glutamine finger and found that it abolishes the GAP activity and the checkpoint, suggesting that the GAP activity is normally required for activation of the spindle position checkpoint. Consistently, a tem1 mutant where the catalytic glutamine has been changed to leucine is checkpoint deficient.

Altogether, our data suggest that regulation of Tem1 nucleotide-bound state is crucial for preventing mitotic exit and cytokinesis in case of spindle mispositioning.