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Cdc5p-Dependent Phosphorylation of Bfa1p is Not Sufficient to Trigger Mitotic Exit, but is Required for Dynamic Bfa1p Localization on SPB

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To complete the cell cycle, cyclin-dependent kinase (CDK) activity is inactivated to drive mitotic exit. In budding yeast, mitotic exit is accomplished by the mitotic exit network (MEN). The MEN is a GTPase signaling cascade in which the GTPase activity of Tem1p is inhibited by a heterodimic GTPase activating protein (GAP), Bfa1p/Bub2p complex. Thus, the inhibitory phosphorylation of Bfa1p by Cdc5p, lowering the Bfa1p/Bub2p GAP activity, is suggested to be responsible for delaying mitotic exit in response to diverse checkpoint-activating signals. In this study, we isolated Bfa1p-D8^{M413I} that completely inhibited mitotic exit in the presence of spindle mis-orientation and DNA damage but not in spindle damage, although its GAP activity in combined with Bub2p was markedly impaired. Interestingly, Bfa1p^{M413I} was not phosphorylated by Cdc5p and remained associated with both SPBs throughout cell cycle. This finding, along with the result that wild type Bfa1p also localized onto both SPBs in cells lacking Cdc5p kinase activity, suggests that Cdc5p directs Bfa1p to be displaced from SPB remaining in the mother cells at anaphase onset via phosphorylating Bfa1p. Altogether, it is likely that other mechanisms might exist to inhibit mitotic exit depending on different checkpoint-activating signals, besides the regulation of Bfa1p/Bub2p GAP activity.