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Role of Budding Yeast Cdc5 in Cell Division: Spatial and Temporal Regulation of M Phase Progression by Polo-Like Kinase

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Members of the polo subfamily of protein kinases have been identified in various eucaryotic organisms from budding yeast to mammals and appear to play pivotal roles in cell division and proliferation. Polo-like kinases (Plks) include mammalian Plk1, Snk, and Fnk/Prk, *Xenopus laevis* Plx1, *Drosophila melanogaster* polo, *Schizosaccharomyces pombe* Plo1, and *Saccharomyces cerevisiae* Cdc5 [1]. Budding yeast *S. cerevisiae* has become a excellent model system to study the function of the Plk because the organism contains only one Plk gene *CDC5* whereas mammals express at least three different Plks; Plk1, Snk, and Fnk. Among these, Plk1 and Cdc5 functions during M phase but Snk and Fnk seems to act at early stage of cell division prior to M phase. In addition to a high degree of sequence similarity in the kinase domain, polo kinases contain a strikingly conserved motif termed "polo-box" in the noncatalytic C-terminal domain. It is apparent from genetic and biochemical analyses that polo kinases regulate diverse cellular events at various stages of M phase, including centrosome maturation, bipolar spindle formation, activation of Cdc2 through Cdc25C phosphatase at at the G_2/M transition, DNA damage checkpoint adaptation, activation of the anaphase-promoting complex, and cytokinesis in various eucaryotic systems [1]. The roles of these kinases are likely to be conserved among evolutionarily distant eucaryotic cells. It was demonstrated that murine Plk is a functional homolog of *S. cerevisiae* Cdc5 and that the polo box is required for the ability of Plk to functionally complement the *cdc5-1* defect by targeting the catalytic activity of the enzyme to specific subcellular location [2]. Without impairing kinase activity, conservative mutations in the polo box abolish the ability of Cdc5 to functionally complement the defect associated with a *cdc5-1* temperature-sensitive mutation, to localize to the spindle pole body (SPB) and cytokinetic neck filaments, and to induce elongated cells with ectopic septin ring structures. Consistent with the polo box-dependent subcellular localization, the C-terminal domain of Cdc5, but not its polo box mutant, is sufficient for subcellular localization, and its overexpression appears to inhibit cytokinesis [7]. These data provide evidence that the polo box is required to direct Cdc5 to both SPB and mother-bud neck through binding to components of specific subcellular locations. To provide new insights into the function of budding yeast polo kinase Cdc5p, novel temperature-sensitive *cdc5* mutants were generated by mutagenizing the C-terminal domain. At a semipermissive temperature, the *cdc5-3* mutant exhibited a synergistic bud elongation, an indicative of G2/M arrest, and growth defect with loss of *HSL1*, a component important for

normal G2/M transition. Loss of *SWE1*, which inactivates the budding yeast Cdk1 homolog Cdc28p by inhibitory phosphorylation, suppressed the *cdc5-3 hsl1*Δ defect, suggesting that Cdc5p functions at a point upstream of Swe1p. Thus, Cdc5p contributes to the activation of the Swe1p-dependent Cdc28p/Clb pathway at G2/M transition [4,5]. Intact polo-box of Cdc5 is essential to localization at mother-bud neck containing septin filament. Overexpression of the C-terminal domain of Cdc5 (cdc5ΔN), but not the corresponding polo-box mutant, resulted in connected cells. These cells shared cytoplasm with incomplete septa, and possessed aberrant septin ring structures. Moreover, depletion of Cdc5 function leads to an arrest in cytokinesis. Provision of additional copies of endogenous *CDC5* remedied this phenotype, suggesting a dominant-negative inhibition of cytokinesis [6]. The polo-box-dependent interactions between Cdc5 and septins (Cdc11 and Cdc12) suggest that direct interactions between cdc5ΔN and septins regulate cytokinetic pathways after completion of chromosome separation. Bbp1p, isolated as a polo-box interacting protein by a yeast two-hybrid screen, localizes to the periphery of the central plaque of the SPB and plays an important role in SPB duplication. Similarly, Cdc5p localized to the cytoplasmic periphery of the SPB. *In vitro* binding studies showed that Cdc5p interacted with the N-terminal domain of Bbp1p (Bbp1pΔC). In addition, Bbp1p was required for proper localization of Cdc5p to the SPB. The C-terminal coiled-coil domain of Bbp1p (Bbp1p(243-385)), which is crucial for both the homodimerization and the SPB localization, could target the localization-defective Cdc5pΔC to the SPB. Consistent with this observation, expression of CDC5ΔC-BBP1(243-385) under *CDC5* promoter control partially complemented the cdc5Δ defect. These data suggest that Bbp1pΔC interacts with the polo-box domain of Cdc5p, and this interaction is critical for the subcellular localization and mitotic functions of Cdc5p [3]. Thus, Bbp1 and septins provide a spatial compartment of Cdc5 functions by mediating the polo-box-dependent localization of Cdc5 at both SPB and cytokinetic neck filament where Cdc5 timely coordinates various stages of M phase including G2/M transition, mitotic exit, and cytokinesis.

1. Glover, D.M., Hagan, I.M., and Tavares, A.A. (1998). Polo-like kinases: a team that plays throughout mitosis. *Genes Dev.* **12**, 3777-3787.
2. Lee KS, Sukgil Song, Erikson RL. (1999) The polo-box-dependent induction of ectopic septal structures by a mammalian polo kinase, plk, in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.*(USA). **96**(25):14360-14365.
3. Park CJ, Sukgil Song, Giddings TH Jr, Ro HS, Sakchaisri K, Park JE, Seong YS, Winey M, Lee KS. (2004) Requirement for Bbp1p in the Proper mitotic functions of Cdc5p in *Saccharomyces cerevisiae*. *Mol Biol Cell.* **15**(4):1711-23.
4. Park CJ, Sukgil Song, Lee PR, Shou W, Deshaies RJ, Lee KS. Loss of CDC5 function in *Saccharomyces cerevisiae* leads to defects in Swe1p regulation and Bfa1p/Bub2p-independent cytokinesis. (2003) *Genetics*. **163**(1):21-33.
5. Sukgil Song, Philip Lee, John Lippincott, Hyunsoo Ro, Rong Li, and Kyung S. Lee. (2002) Proper function of septin complex requires Bni5 localization at bud neck filaments. (2002) *Mol Cell Biol.* **22**(19):6906-20.

6. Sukgil Song and Kyung S. Lee. (2001) A novel function *of Saccharomyces cerevisiae CDC5* in Cytokinesis. *Journal of Cell Biology* **152**: 451-470.
7. Sukgil Song, Grenfell TZ, Garfield S, Erikson RL, Lee KS. (2000) Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol. Cell. Biol.* **20**(1):286-298.