

# Investigation on the Interaction and Cellular Localization of Mcm-binding protein 1 (Mcb1) and MCM Complex in *Schizosaccharomyces pombe*

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Mini-chromosome maintenance (MCM) is a family of proteins which belongs to AAA<sup>+</sup> ATPase family. *S. pombe* possesses six subunits of Mcm which are known as Mcm2-7. *S. pombe* Mcm2-7 helicase complex has a role in both the initiation of DNA replication and the elongation of the replication fork. A subset of MCM complex proteins associates with *S. pombe* Mcb1. Mcb1 (MCM binding protein 1) is an apparent orthologue of the human MCM-binding protein (MCM-BP). It has been known that Mcb1 is an abundant protein and constitutively present across the cell cycle. It is widely distributed in cytoplasm and nucleoplasm and also bound to chromatin. To date, Mcm subunits have been proved to associate with Mcb1 by immunoprecipitation and mass spectrometry. However, these approaches did not specify the nature of this interaction itself. Recent studies showed that in human and *Xenopus laevis*, MCM-BP interacted with Mcm3-7 but not with Mcm2. In *Arabidopsis thaliana*, MCM-BP interacted with Mcm2-7. Therefore, this study aims to investigate the interaction between fission yeast MCM-BP (Mcb1, SPAC1687.04) and individual *S. pombe* Mcm2-7. Also, based on the previous experiment in our laboratory, the results of Mcm-GFP observation in wild-type Mcb1 showed that Mcm is constitutively localized in the nucleus. Similar result was also observed for Mcb1 temperature sensitive mutants at 25°C. At restrictive temperature, most of Mcm-GFP signal seemed to be dispersed in *mcb1* temperature sensitive mutants, while in wild type, Mcm stayed in the nucleus. Therefore, this study also aims to confirm and investigate the localization of Mcb1<sup>L254P</sup> and its influence to Mcm localization by chromatin fractionation. Through this study, I confirmed individual interaction between *S. pombe* Mcb1 and *S. pombe* Mcm2-7 by *in vitro* binding assay. GST and GST-Mcm2-7 were probed by GST polyclonal antibody and T7-His-Mcb1 was detected using His monoclonal antibody. Western blotting showed that Mcb1 has the ability to bind to all subunit member of MCM complex *in vitro*, even though Mcm4 and Mcm6 had lower interaction affinity to Mcb1 than other Mcm. In subsequent experiment of semi-*in vitro* binding assay, interaction between temperature sensitive mutant of Mcb1 (Mcb1<sup>L254P</sup> and Mcb1<sup>L363P</sup>) and Mcm2-7 were investigated. In contrast to wild type Mcb1, the interaction between mutants Mcb1 and Mcm2-7 was drastically decreased, indicating that the mutated protein of Mcb1 lost the ability to interact with Mcm. As for cell fractionation experiment, the result showed that the majority of Mcb1 existed in soluble fraction. However, the mutated protein of Mcb1 (Mcb1<sup>L254P</sup> and Mcb1<sup>L363P</sup>) was present in soluble and chromatin enriched fraction of cells, indicating clear difference in the cellular localization of wild type and mutated proteins.