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The Role of Cdc7 Kinase and Mcm10p in the Initiation of Eukaryotic DNA Replication

Joon-Kyu Lee

Department of Biology Education, Seoul National University, Seoul 151-742

The initiation of eukaryotic DNA replication is a multistep process that commences with the binding of the origin recognition complex (ORC) to replication origins. During the G1 phase of cell cycle, several proteins such as Cdc6/Cdc18, Cdt1, and Minichromosome maintenance (Mcm) complex are sequentially recruited to origin DNA in an ORC dependent manner to form the pre-replicative complex.Upon entering S phase, DNA replication is initiated by the activation of pre-replicative complex, which leads to the binding of Cdc45, subsquent unwinding of replication origin, and the recruitment of replication fork machinery. From these components of pre-replicative complex, Mcm complex is a major candidate of replicative helicase, which is required for the initiation and elongation steps during DNA replication. Previous studies in *Saccharomyces cerevisiae* suggested an essential role of two S-phase promoting kinases, the S-phase cyclin dependent kinase (Cdk) and Dbf4-Cdc7 kinase complex during the initiation of DNA replication. Although *in vivo* targets of the S-phase Cdk are unclear, Dbf4-Cdc7 kinase complex appeared to play an essential role for the activation of pre-replicative complex by phosphorylating several target proteins such as Mcm complex. To study the mechanism of the activation of pre-replicative complex, we have examined the phosphorylation and the activation of Mcm complex by Cdc7 kinase complex. From the study using *Schizosaccharomyces pombe* as a model system, we have found that the phosphorylation of Mcm complex *in vitro* by Cdc7 kinase complex required Cdc23 (Mcm10) protein, which was another essential factor for the initiation and elongation steps of eukaryotic DNA replication. Although Dfp1-Hsk1 kinase complex (*S. pombe* homologue of Dbf4-Cdc7) efficiently phosphorylated *S. pombe* Mcm2 protein alone, Mcm complex containing all six subunits was hardly phosphorylated by Dfp1-Hsk1 kianse in the absence of Cdc23 protein. The addition of Cdc23 protein of stoichiometric level at the same condition stimulated the phosphorylation level of Mcm complex up to ten folds, and Mcm2 and Mcm4 subunits in Mcm complex were major targets of the phosphorylationby the kinase at this reaction condition. Cdc23 protein interacted directly with both the Mcm complex and Dfp1-Hsk1 kinase complex by selectively binding to the Mcm4/6/7 subunits and Dfp1 protein, respectively. The N-terminus of Cdc23 protein was found to interact with Dfp1 and was essential for phosphorylation of Mcm complex. To map the essential domain of Cdc23 protein for *in vivo* function, we have generated truncated derivatives of Cdc23 protein and examined the interactions with Mcm complex and Dfp1-Hsk1 kinase, phosphorylation stimulatory activity, and in vivo activity for cell survival. Inerestingly, all truncated proteins that complemented the temperature-sensitive phenotype of *cdc23*mutant cells at restrictive temperature also stimulated the phosphorylation of Mcm complex, implying that this stimulatory activity might be a critical role of Cdc23p *in vivo*. These results together with the genetic and biochemical properties suggested that Cdc23 protein participates in the activation of pre-replicative compelx by recruiting the Dfp1-Hsk1 kinase and stimulating the phosphorylation of the Mcm complex. Cdc23 protein that has a highly asymmetric, elongated structure in solution also interacted with many other replication factors such as origin recognition complex and DNA polymerase alpha-primase. These properties suggested that Cdc23 protein may act as a scaffold protein for the assembly of the pre-replicative complex and replication fork amchinery. Although we have successfully phosphorylated Mcm complex *in vitro* in this study, we have not detected activation of the DNA helicase activity of the Mcm complex or alterations of its structure after phosphorylation by Dfp1-Hsk1 kinase. Additional factors might be important for the activation of DNA helicase activity of Mcm complex. Possible mechanisms of the activation of Mcm DNA helicase activity will be discussed.