Mcm proteins that play an essential role in eukaryotic DNA replication are phosphorylated in vivo, and cyclin-dependent protein kinase is at least in part responsible for the phosphorylation of Mcm4. Our group reported that the DNA helicase activity of Mcm4,6,7 complex, which may be involved in initiation of DNA replication, is inhibited following phosphorylation by Cdk2/cyclin A in vitro. Here, we further examined the interplay between mouse Mcm4,6,7 complex and cyclin-dependent kinases and determined the sites required for the phosphorylation of Mcm4. Six Ser and Thr residues, in all, were required for the phosphorylation. Inhibition of Mcm4,6,7 helicase activity by Cdk2/cyclin A was largely relieved by introducing mutations in these residues of Mcm4. Anti-phosphothreonine antibodies raised against one of these sites reacted with Mcm4 prepared from HeLa cells at mitotic phase but did not bind to those at G1 and G1/S, suggesting that this site is mainly phosphorylated in the mitotic phase. Mcm4,6,7 complex purified from HeLa cells at the mitotic phase exhibited a low level of DNA helicase activity, compared with the complexes prepared from cells at other phases. These results suggest that phosphorylation of Mcm4 at specific sites leads to loss of Mcm4,6,7 DNA helicase activity.

A number of replication initiation sites that are present in the genome of eukaryotic cells are utilized in a temporal order during the DNA synthesis (S) phase of the cell cycle. Reinitiation of DNA replication is usually prevented, and only a single round of DNA replication is performed in a cell cycle. This regulation of DNA replication is called replication licensing. The initial role in the initiation of DNA replication is critical for the regulation of the replication (reviewed in Ref. 30). Previous studies have shown that cyclin-dependent protein kinases and geminin, a small protein that plays a role in loading Mcm proteins onto chromatin, function as a licensing factor (31, 32). To contribute to the negative regulation of DNA replication by inactivating Cdt1 (7, 32).

All of the members of the Mcm2–7 protein family contain DNA-dependent ATPase motifs in the central domain (33). Partly consistent with this notion, we (34–36) and Lee and Hurwitz (37, 38) observed that Mcm4,6,7 proteins form a hexameric complex and function as a DNA helicase in vitro. This DNA helicase activity is not processive under standard conditions of DNA helicase assay, but it is processive when a tailed substrate was used for the DNA helicase assay (38). These results, together with in vivo findings (39, 40) that suggest that Mcm proteins are involved in elongation of DNA replication, raise the possibility that Mcm4,6,7 helicase or Mcm2–7 complex is responsible for replication fork movement (41–43). Mcm proteins are phosphorylated in vivo and in vitro (44–49), and Cdc6 protein was overproduced in yeast cells (13–15) and when cyclin-dependent protein kinase was inactivated in yeast cells (16–19). In Drosophila (20), and in human cells (21). The kinase inhibits the assembly of preinitiation complex at the replication origin (19, 22, 23). One of the targets of this negative regulation is Cdc6 protein. In Saccharomyces cerevisiae the phosphorylation of Cdc6 by cyclin-dependent kinase leads to its degradation (24). In mammalian cells, phosphorylation of Cdc6 by the kinase causes exclusion of the protein from the nucleus (25–27). It has been also shown that cyclin-dependent kinase can exclude Mcm4 from the nucleus in yeast (28, 29).

Thus, the cyclin-dependent protein kinase that plays an essential role in the initiation of DNA replication is critical for the regulation of the replication (reviewed in Ref. 30). In addition to cyclin-dependent kinase, geminin (31), which accumulates during the S and G2 phases, appears to contribute to the negative regulation of DNA replication by inactivating Cdt1 (7, 32).

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Experimental Procedures

**Purification of Mouse Mcm4,6,7 Complex—Site-directed mutagenesis of the Mcm4 gene was conducted using the QuickChange site-directed mutagenesis kit (Stratagene).** The oligonucleotide 5'-GTCCCCGGCATCGCCCCAGGCCCAGAC-3' was used as primer to introduce a change from Ser to Ala at amino acid 7, 5'-GAGGAAACGAGTGGCCACCC-
AATCGGAG-3' was used for the change at amino acid 32, and 5'-GAAGTGTTAGGGACCCCACTCCGTAGAAGCAGGCCC-3' was used for the change at amino acid 109. The oligonucleotide 5'-ATGTCGGCGCGTCCGATGTGCCCGAGGAGCCGACCTG-3' was used as the primer to change both Ser-2 and Thr-7 to Ala, 5'-GAGCCGGAGGGATCTGCGACCGCCACTCCCTTGAAAGT-3' was used for the change at amino acid 19, and 5'-CTACTGCATATGGCCACCCGAGGAGCCGACCTG-3' was used for the change at residue 53. The mutated Mcm4 gene was cloned into a pAcUW31 vector (Pharmingen) in which the Mcm6 gene had been cloned (36). All of the mutated sites in Mcm4 were confirmed by nucleotide sequencing. High 5 insect cells were co-infected with recombinant baculoviruses expressing proteins Mcm4 and Mcm6 and virus expressing Mcm7 protein (pVL1392 vector). The Mcm4,6,7 complex was purified by nickel column chromatography and then by glycerol gradient centrifugation without histone H3/H4 column chromatography (36).

**DNA Helicase Activity**—A 17-mer oligonucleotide (5'-GGTTTC-CAGCTGACAG-3') was labeled at the 5'-end with polynucleotide kinase in the presence of [γ-32P]ATP and then annealed to M13 DNA. The annealed oligomer (5 fmol) was incubated at 37 °C for 30 min with Mcm4,6,7 complex in 50 mM Tris-HCl, pH 7.9, 20 mM β-mercaptoethanol, 10 mM magnesium acetate, 10 mM ATP, and 0.5 mg/ml bovine serum albumin. The reaction was terminated by adding 0.2% SDS, and an aliquot was electrophoresed on a 12% acrylamide gel in Tris borate/EDTA. The labeled oligomer in the gel was detected by using a BioImage analyzer (FLA2000, Fuji).

**Preparation of Anti-phosphothreonine Antibodies**—Antiserum against phosphothreonine at amino acid 19 of mouse Mcm4 was obtained by immunizing rabbits with a synthetic peptide of NH2-SRRGRV(phospho-T)PTQSLRSEC-COOH conjugated with keyhole limpet at the carboxyl terminus. Anti-phosphothreonine antibodies were first purified by phosphopeptide column chromatography. The serum was loaded onto a phosphopeptide column that had been prepared by binding the peptide with CNBr-activated Sepharose, and the antibodies were eluted with 0.1 M glycine (pH 2.5) and 0.15 M NaCl. The eluted solution was neutralized and dialyzed against phosphate-buffered saline. Then, the purified antibodies were loaded onto a non-phosphopeptide column. The pass-through fraction was concentrated and used as anti-phosphothreonine antibodies. The specificity of binding of the purified antibodies was confirmed by enzyme-linked immunosorbent assay.

**Preparation of Other Proteins**—Human Cdk2/cyclin A was purified as reported (50), and the protein concentration of the purified protein was 150 μg/ml. Human Cdc2 (His)/cyclin B (Myc) was purified from High 5 cells co-infected with baculoviruses containing these two genes by nickel-nitrilotriacetic acid affinity column chromatography (these viruses were kindly provided by R. A. Laskey). The concentration of the kinase activity of the purified Cdk2/cyclin B was similar to that of the Cdk2/cyclin A. The kinase activity was measured by the ability that phosphorylates H1 histone as reported (50).

**Synchronization of HeLa Cells**—HeLa cells were cultured in minimum Eagle's medium supplemented with 5% calf serum at 37 °C. The cells were synchronized at G2/M by adding nocodazole at 50 ng/ml for 18 h. HeLa cells at G2/M phase were prepared by incubating them with 1 mM hydroxyurea for 18 h. For G1 synchronization, the following procedures were employed. HeLa cells were treated with 1 mM hydroxyurea for 11 h. After being washed, the cells were incubated in the presence of nocodazole for 12 h. Mitotic cells were collected from the surface by shaking the bottle, and the collected cells were cultured for 6 h without nocodazole. The cellular proteins were isolated by chromatography on histone-Sepharose column chromatography and then by glycerol gradient centrifugation (34, 35). Usually, 1–2 × 107 cells were used for the purification. An Mcm4,6,7 complex purified from HeLa cells at the G2/M phase was treated with 12 units of lambda protein phosphatase (New England BioLabs) for 30 min at 30 °C under the conditions recommended by the manufacturer.

**RESULTS**

**Requirement of an Amino-terminal Region of Mcm4 for DNA Helicase Activity of Mcm4,6,7 Complex**—A, recombinant mouse Mcm4,6,7 complexes containing wild-type Mcm4 (WT) and two mutant Mcm4 deleted at the amino terminus (Δ1-35 and Δ1-112) were examined for DNA helicase activity. Increasing amounts (3, 6, 12, 25, and 50 ng) of these complexes were added to reaction mixtures that contained 17-mer oligonucleotide annealed to M13 DNA. The proportion of displaced oligonucleotide was calculated in the reaction containing the highest amount of the complex, and values are indicated at the bottom. B, the three Mcm4,6,7 complexes (45 ng) were incubated with increasing amounts (0.4, 7.5, and 37 ng) of Cdk2/cyclin A, and phosphorylated peptides were analyzed on SDS-polyacrylamide gel after digestion with lysyl endopeptidase. The phosphorylated amino-terminal peptide of Mcm4 are indicated (Mcm4P), and the number of phosphates incorporated into the peptide at the highest dose of the kinase is shown at the bottom.
these 35 amino acids play an important role in phosphorylation with Cdk2/cyclin A. However, we showed that deletion of up to 112 amino acids from the amino terminus was required for the almost complete loss of phosphorylation in Mcm4 (50). The DNA helicase activity of these mutant Mcm4,6,7 complexes was examined (Fig. 1A). Deletion of 35 amino acids resulted in a 5-fold reduction in the DNA helicase activity of the Mcm4,6,7 complex, and the removal of 112 amino acids resulted in an ~10-fold reduction. Thus, the amino-terminal portion of Mcm4 is required for the DNA helicase activity of the mouse Mcm4,6,7 complex. In contrast, single-stranded DNA-dependent ATPase activity was not affected by the deletion of this portion (data not shown).

**Sites of Mcm4 Required for Phosphorylation by Cdk2—**Fig. 2A shows the amino acid sequence in the amino-terminal region of mouse Mcm4. There are six Ser-Pro (SP) and five Thr-Pro (TP) sites (11 in total) that are potentially phosphorylated by the cyclin-dependent kinases. First, we mutated all three consensus sites (amino acids 7, 32, and 109) of the kinase to alanine in this region and prepared a mutant Mcm4,6,7 complex containing the mutagenized Mcm4 (Fig. 2B). After phosphorylating the mutant complex with Cdk2/cyclin A, proteins in the complexes were digested with lysyl endopeptidase to analyze phosphorylation in the amino-terminal region of Mcm4 (Fig. 2C). As the amounts of Cdk2/cyclin A in the reaction increased, the incorporated radioactivity enhanced and the phosphorylated bands shifted upward; these changes were similar to those in the wild-type complex. We introduced a fourth mutation at each amino acid 3, 19, or 53 in the triple mutant of Mcm4 (7/19/32/53/109m) and prepared three more mutant Mcm4,6,7 complexes. The phosphorylation of the two complexes containing Mcm4 mutated at 3/7/32/109 and 7/19/32/109 was similar to that of wild-type complex. In the complex containing Mcm4 mutated at 7/32/53/109, the slowest migrating band was not detected, but the incorporation of $^{32}$P was slightly increased. When the Mcm4,6,7 complex containing Mcm4 mutated at five sites (7/19/32/53/109) was phosphorylated with the kinase, however, only a low level of phosphorylation was observed. The phosphorylation of a mutant complex containing Mcm4 mutated at six sites (3/7/19/32/53/109) showed almost no phosphorylation in this region of Mcm4. Thus, these three Ser-Pro sites (3, 32, and 53) and three Thr-Pro sites (7, 19, and 109) are required for phosphorylation of Mcm4 in Mcm4,6,7 complex by Cdk2/cyclin A. The results also suggest that three sites (3, 19, and 53) among them play an important role in the phosphorylation.

We also examined the phosphorylation of Mcm4 by Cdc2/cyclin B using the same set of mutant complexes. As shown in Fig. 3, the wild-type and mutant Mcm4,6,7 complexes were incubated with Cdc2/cyclin B, and the extent of phosphorylation was examined by measuring the gel-mobility shift of Mcm4. Mcm4 bands that shifted upward were detected in the presence of larger amounts of the kinase when the wild-type complex or the triple mutant complex (7/32/109m) was phosphorylated. Such a mobility-shifted band appeared to be decreased in the four kinds of complexes (7/32/109m, 7/19/32/109m, 7/32/53/109m, and 7/19/32/53/109m). In the mutant 3/7/19/32/53/109m, such shifted bands were not detected. Thus, the six mutated sites are required for phosphorylation of Mcm4 by Cdc2/cyclin B in the Mcm4,6,7 complex. The results appear to be essentially similar to those obtained with Cdk2/cyclin A.

**Relationship between Phosphorylation of Mcm4 and DNA Helicase Activity—**We reported that phosphorylation of Mcm4 in human and mouse Mcm4,6,7 complex is associated with the inhibition of Mcm4,6,7 helicase activity (50) (Fig. 4). To further understand this relationship, the mutant Mcm4,6,7 complex containing six mutations in Mcm4 (3/7/19/32/53/109m), which had become resistant to phosphorylation by Cdk2/cyclin A (Fig.
2), was examined for DNA helicase activity in the presence of Cdk2/cyclin A (Fig. 4). The mutant complex was much more resistant to the inhibition of DNA helicase activity by the kinase. This result indicates that the phosphorylation of these six amino acids in Mcm4 is mainly responsible for the inhibition of Mcm4,6,7 helicase by the kinase. We do not know why the DNA helicase activity of the mutant complex containing six mutations in Mcm4 is slightly inhibited by the kinase. It is possible that phosphorylation at other sites in Mcm4 or in Mcm6 are involved.

**Correlation between Phosphorylation of Mcm4 and Lowered Level of Mcm4,6,7 Helicase Activity** — Mcm4 is heavily phosphorylated at the mitotic phase of the cell cycle. It has been shown using a temperature-sensitive mutant of Cdc2 kinase that Cdc2/cyclin B complex is mainly responsible for the phosphorylation of Mcm4 in mouse FM3A cells (47). We synchronized HeLa cells at G2/M by treating them with nocodazole, and we purified Mcm4,6,7 complex from the cells. Chromatin-unbound proteins were prepared from the cells, and Mcm4,6,7 complex was purified from this fraction by histone-Sepharose column chromatography and then by glycerol gradient centrifugation. The SDS-polyacrylamide gel used for electrophoresis of the purified Mcm4,6,7 complex is shown in Fig. 5A. Mcm4 was shifted upward and was detected at a position between Mcm2 and Mcm6 as a broad band, which was also determined by immunoblotting analysis using anti-Mcm4 antibodies (data not shown). Phosphatase treatment of the purified mitotic Mcm4,6,7 complex resulted in a mobility shift of Mcm4 protein from these positions to almost the same position as the Mcm4 purified from logarithmically growing HeLa cells, indicating that phosphorylation causes mobility-shift of Mcm4. The DNA helicase activity of the Mcm4,6,7 complex prepared from the cells at G2/M phase was measured (Fig. 5B). As compared with the Mcm4,6,7 complex that was purified from logarithmically growing HeLa cells, the Mcm4,6,7 complex purified from the cells at G2/M exhibited a much lower level of DNA helicase activity. These results indicate that phosphorylation of Mcm4 at higher level is associated with the inhibition of the DNA helicase activity of the purified Mcm4,6,7 complex. We measured DNA helicase activity of the Mcm4,6,7 complex prepared from HeLa cells at G2/M after treatment with phosphatase, but the activity was not enhanced by the treatment (data not shown). It is possible that irreversible structural change in the Mcm4,6,7 hexamer may occur by the phosphorylation of Mcm4.

To further understand the relationship between the phosphorylation of Mcm4 and the level of Mcm4,6,7 helicase activity, we raised anti-phospho antibodies against Thr at amino acid 19 of Mcm4, which plays a role in the phosphorylation by cyclin-dependent kinase *in vitro* (Figs. 2 and 3). Mcm proteins were purified from HeLa cells synchronized at various stages of the cell cycle, and Mcm4 in the purified fraction was examined for binding with the anti-phosphothreonine antibodies. As shown in Fig. 6A, the anti-phosphothreonine antibodies reacted with Mcm4 from HeLa cells at the mitotic phase but did not react with Mcm4 from cells at G1 and G1/S, indicating that phosphorylation of Thr-19 mainly occurs at the G2/M in HeLa cells. The Mcm4,6,7 complex purified from these synchronized HeLa cells were examined for DNA helicase activity (Fig. 6B). Hexameric protein complex formation of the purified Mcm4,6,7 complex was confirmed by native gel electrophoresis. Compared with the Mcm4,6,7 complex from cells in G1 and G1/S, the complex from cells in G2/M showed much weaker activity. These results show a correlation between phosphorylation at amino acid 19 of Mcm4 and the lowered level of DNA helicase activity of the purified Mcm4,6,7 complex.
In this study, we determined the sites in Mcm4 that are required for phosphorylation by the cyclin-dependent kinases in vitro. One of these sites was indeed phosphorylated in vivo, as was determined by using anti-phosphothreonine antibodies. We demonstrated that the phosphorylation of specific sites in the amino-terminal region of Mcm4 leads to inactivation of Mcm4,6,7 helicase. The correlation between the Mcm4 phosphorylation and the lowered level of DNA helicase activity was also observed when DNA helicase activity was compared among Mcm4,6,7 complexes prepared from HeLa cells at various stages of the cell cycle. These results suggest that one of the roles of the Mcm4 phosphorylation by the cyclin-dependent kinase is to inactivate the DNA helicase activity of the Mcm4,6,7 complex that is probably involved in DNA replication in vivo.

Mcm proteins are phosphorylated in vivo. The mobility in SDS gel of the Mcm4 that was prepared from HeLa cells at the G2/M was greatly retarded, suggesting that several sites in Mcm4 are phosphorylated at G2/M in vivo. Fujita et al. (47) have shown that Cdc2/cyclin B is mainly responsible for the phosphorylation of Mcm4 at the G2/M in mouse FM3A cells. In Xenopus, phosphorylation of Mcm4 by the cyclin-dependent kinase has been reported by several groups (45, 46, 48, 49). Hendrickson et al. (46) have shown that Cdc2/cyclin B is mainly responsible for the phosphorylation of Mcm4 at the mitotic phase, and Pereverzeva et al. (49) have indicated that a kinase(s) in addition to Cdc2/cyclin B are involved in the phosphorylation. The function of the Mcm4 phosphorylation is not clear, but it may inhibit the binding of Mcm complexes to chromatin (46, 48). Conversely, it is suggested that the phosphorylation of Mcm4 at mitotic phase is required for binding of the Mcm complex to chromatin at the G1 phase (49). Here, we suggested another function of the Mcm4 phosphorylation. We determined the specific sites in the amino-terminal region of mouse Mcm4 that are required for phosphorylation by Cdk2/cyclin A in vitro. The same sites in Mcm4 appeared to be required for the phosphorylation by Cdc2/cyclin B in vitro, suggesting that both Cdk2/cyclin A and Cdc2/cyclin B have similar specificity of substrate recognition. In addition to three consensus sites for these kinases in the amino-terminal region of Mcm4, we identified two sites of SerPro(SP) and one site of ThrPro(TP) in this region that are required for the phosphorylation with Cdk2/cyclin A and Cdc2/cyclin B. One of these sites (Thr-19) was phosphorylated mainly at the G2/M phase in vivo.
human HeLa cells, which was determined by using anti-phosphothreonine antibodies. It is tempting to speculate that this site is phosphorylated by Cdc2/cyclin B at G2/M in vivo, but further experiments are needed to test this notion. The anti-phosphothreonine would be useful to analyze the mechanism of the Mcm4 phosphorylation.

Mcm proteins are present in cells as a chromatin-bound form as well as an unbound form, and at G2/M phase, almost all of the Mcm proteins are present as the unbound form. They appear to be present mainly as a heterohexameric Mcm2–7 complex either in the chromatin-bound form (51) or the unbound form (52). We purified the Mcm4,6,7 complex from the chromatin-unbound fraction at various stages of the HeLa cell cycle. We used a histone-Sepharose column chromatography by which Mcm2, Mcm3, and Mcm5 proteins, which can be inhibitory to Mcm4,6,7 helicase activity, are removed (35, 37, 53). Then, we compared the DNA helicase activity of these Mcm4,6,7 complexes. The activity of the complex prepared from cells at the G2/M phase was much lower than that prepared from cells in other phases (Figs. 5 and 6). In vitro, phosphorylation of Mcm4 by Cdk2/cyclin A resulted in inhibition of the DNA helicase activity of the mouse Mcm4,6,7 complex (Fig. 4). This inhibition by Cdk2/cyclin A was largely rescued by changing the specific sites in the amino-terminal region of Mcm4 that were required for phosphorylation by Cdk2/cyclin A. These results indicate that phosphorylation at specific sites of Mcm4 by cyclin-dependent kinases can lead to inhibition of the Mcm4,6,7 helicase activity. Related to this point, the Mcm4 bound to chromatin at S phase is more phosphorylated than the protein in unbound form in Xenopus (45) and in HeLa cells (47). The functional significance of the phosphorylation of the chromatin-bound Mcm4 at S phase remains to be determined.

The ATP binding sites in Mcm4,6,7 proteins appear to play a distinct role in the DNA helicase activity of the Mcm4,6,7 hexamer (36). Namely, it was suggested that the ATP binding of Mcm6 is essential for DNA helicase activity but not for single-stranded DNA-dependent ATPase activity and that the ATP binding site of Mcm4 contributes to the binding of Mcm4,6,7 complex to single-stranded DNA. We reported that phosphorylation of Mcm4 by Cdk2/cyclin A moderately affects the single-stranded DNA binding activity (50). The amino-terminal region of Mcm4 was required for the DNA helicase activity of the Mcm4,6,7 complex (Fig. 1A). In this region of Mcm4, there are multiple SerPro(SP) sites that could also act as a binding motif of double-stranded DNA (54). Based on these findings, we consider that this region plays a role in the interaction with DNA during DNA helicase action, and the phosphorylation of Mcm4 by the cyclin-dependent kinase perturbs the interaction to inactivate the DNA helicase activity of the Mcm4,6,7 complex.

Several proteins are involved in the regulation of DNA replication, which occurs once in a cell cycle. The cyclin-dependent kinase plays a central role in the regulation. One important target of the kinase is Cdc6 protein, and the phosphorylation causes degradation or nuclear export of Cdc6 (24–27). However, a significant fraction of Cdc6 protein is present on chromatin throughout the cell cycle of human cells (12, 55). Another important substrate of the cyclin-dependent kinase may be Mcm proteins. The kinase may play a role in the regulation of DNA replication by preventing the loading of Mcm proteins onto chromatin during the S and G2/M phases (19, 22, 46, 56). It is possible that the phosphorylation of Mcm4 at the sites determined here is involved in the detachment of Mcm proteins from chromatin. In this paper, we have suggested another role for the phosphorylation of Mcm4 by cyclin-dependent kinase in the regulation of DNA replication. Namely, Mcm4,6,7 helicase activity may be a target for the negative regulation by the cyclin-dependent kinase.

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REFERENCES

1. Blow, J. J., and Laskey, R. A. (1988) Nature 332, 546–548
2. Kubota, Y., Minuma, S., Nishimoto, S., Takisawa, H., and Nojima, H. (1995) Cell 81, 601–609
3. Chong, J. P., Mahlbubani, H. M., Kho, C. Y., and Blow, J. J. (1995) Nature 373, 183–187
4. Madine, M. A., Kho, C. Y., Mills, A. D., and Laskey, R. A. (1995) Nature 373, 421–424
5. Maizano, D., Moreau, J., and Mechali, M. (2000) Nature 404, 622–628
6. Nishimoto, H., Lygren, Z., Nishimoto, T., and Nurse, P. (2000) Nature 404, 625–628
7. Tada, S., Li, A., Maizano, D., Mechali, M., and Blow, J. J. (2001) Nat. Cell Biol. 3, 107–113
8. Hu, X., and Newport, J. J. (1998) J. Biol. Chem. 273, 271–281
9. Kelly, T. J., and Brown, G. W. (2000) Annu. Rev. Biochem. 69, 829–880
10. Takisawa, H., Minuma, S., and Kubota, Y. (2001) Curr. Opin. Cell Biol. 13, 695–699
11. Kearsey, S. E., Montgomery, S., Labib, K., and Linndner, K. (2000) EMBO J. 19, 1681–1690
12. Mineday, J., and Stillman, B. (2000) Mol. Cell. Biol. 20, 8602–8612
13. Nishimoto, H., and Nurse, P. (1995) Cell 85, 397–405
14. Jallepalli, P. V., and Kelly, T. J. (1996) Genes Dev. 10, 541–552
15. Petersen, B. O., Lukas, J., Sørensen, C. S., Bartek, J., and Helin, K. (1999) Genes Dev. 13, 1516–1531
16. Broek, D., Bartlett, R., Crawford, K., and Nurse, P. (1991) Nature 349, 388–393
17. Hayles, J., Fisher, D., Woollard, A., and Nurse, P. (1994) Cell 78, 813–822
18. Moreno, S., and Nurse, P. (1994) Nature 367, 236–242
19. Dahmann, C., Diffley, J. F., and Nasmyth, K. A. (1999) Curr. Biol. 9, 1257–1269
20. Sauer, K., Knoblich, J. A., Richardson, H., and Lehner, C. F. (1995) Genes Dev. 9, 1327–1339
21. Itzhaki, J. E., Gilbert, C. S., and Porter, A. C. (1997) Nat. Genet. 15, 258–265
22. Tanaka, T., Knapp, D., and Nasmyth, K. (1997) Cell 90, 649–660
23. Detweiler, C. S., and Li, J. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2384–2389
24. Jallepalli, P. V., Brown, G. W., Muniz-Falconi, M., Tien, D., and Kelly, T. J. (1997) Genes Dev. 11, 2767–2779
25. Saha, P., Chen, J., Thome, K. C., Lawless, S. J., Hou, Z. H., Hendricks, M., Parvin, J. D., and Dutta, A. (1995) Mol. Cell. Biol. 15, 2758–2767
26. Petersen, B. O., Lukas, J., Sørensen, C. S., Bartek, J., and Helin, K. (1999) EMBO J. 18, 396–410
27. Jiang, W., Wells, N. J., and Hunter, T. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6303–6308
Phosphorylation of Mcm4 at Specific Sites by Cyclin-dependent Kinase Leads to Loss of Mcm4,6,7 Helicase Activity
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