Biochemical Activities Associated with Mouse Mcm2 Protein*

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Mcm2, a member of the Mcm2–7 protein family essential for the initiation of DNA replication, has several biochemical activities including the ability to inhibit the Mcm4,6,7 helicase. In this study, we characterized the activities associated with Mcm2 and determined the region required for them. It was found that Mcm2 deleted at an amino-terminal portion is able to bind to an Mcm4,6,7 hexameric complex and to inhibit its DNA helicase activity. The same deletion mutant of Mcm2 and the carboxyl-terminal half of Mcm2 were both able to bind to Mcm4, suggesting that the carboxyl-half of Mcm2 binds to Mcm4 to disassemble the Mcm4,6,7 hexamer. Phosphorylation of Mcm2,4,6,7 complexes with Cdc7 kinase showed that the amino-terminal region of Mcm2 is required for the phosphorylation, and it contains major Cdc7-mediated phosphorylation sites. We also found that Mcm2 itself can assemble a nucleosome-like structure in vitro in the presence of H3/H4 histones. The amino-terminal region of Mcm2 was required for the activity where a histone-binding domain is located. Finally, we identified a region required for the nuclear localization of Mcm2. The function of Mcm2 is discussed based on these biochemical characteristics.

All the Mcm2–7 proteins play an essential and distinct role in eukaryotic DNA replication (1–3). They bind to chromatin during late mitosis and the G1 phase and detach from chromatin as DNA replication proceeds. It is suggested that Mcm2–7 proteins bind as a heterohexamer (4–6) with the assistance of Cdc6 and Cdt1 (7–9) to the region where origin recognition complex binds. In the heterohexamer, Mcm4,6,7 proteins form a stable core complex (4, 10–13), and Mcm2 has an affinity to the complex to form a tetramer of Mcm2,4,6,7. An Mcm3/5 deleted at an amino-terminal portion is able to bind to an Mcm4,6,7 hexamer and inhibit the DNA helicase activity. The same deletion mutant of Mcm2 and human (16) Mcm proteins. Mcm2 and Mcm3/5 can inhibit the DNA helicase activity by disassembling the Mcm4,6,7 hexamer into an Mcm2,4,6,7 complex or an Mcm3,4,5,6,7 complex, respectively (13, 17, 18). These results indicate that the assembly of Mcm4,6,7 proteins into a hexamer is crucial for the DNA helicase activity and also suggest that Mcm2, -3, and -5 proteins play a role in regulating the Mcm4,6,7 helicase activity. A Cdc7/Dfb4 kinase that plays an essential role in eukaryotic DNA replication (reviewed in Ref. 19–22) is required for the initiation of DNA replication at each replication origin (23, 24). The substrates of the kinase remain to be determined, but genetic and biochemical evidence suggests that Mcm2 protein is one of the most important (25–36).

We reported that Mcm2 can inhibit the DNA helicase activity of the Mcm4,6,7 complex (17). In the present study, we further analyzed the interplay between Mcm2 and the Mcm4,6,7 helicase and also determined the region in Mcm2 that is required for in vitro phosphorylation with a human Cdc7/ASK1 kinase. In addition, we found that Mcm2 can assemble nucleosome-like structures in vitro, and the activity is related with the histone binding ability of Mcm2.

EXPERIMENTAL PROCEDURES
Preparation of Proteins—Human Mcm4,6,7 hexameric complex was purified from HeLa cells by histone-Sepharose column chromatography and then by glycerol gradient centrifugation as reported (16). For preparing deletion mutants of mouse Mcm2 protein, the Mcm2 gene was treated with a polymerase chain reaction using the following primers; as a forward primer, 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' (nucleotides from the mouse Mcm2 gene are underlined) for starting at amino acid (aa) 22, 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for starting at aa 449. As a reverse primer, 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for starting at aa 97, 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for starting at aa 163, and 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for starting at aa 449. As a reverse primer, 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for starting at aa 282, and 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for ending at aa 282, and 5'-GAGAGAGATATCTTGCCTACCTGCCAGGCC-3' for ending at aa 449 were used. These Mcm2 genes were cloned in a pAceHL-T A vector (Pharmingen) for production of histidine-tagged Mcm2 proteins in a baculovirus expression system. When mouse Mcm2,6,7 complexes were prepared, High5 cells were co-infected with three viruses for producing the histidine-tagged Mcm2 proteins, histidine-tagged Mcm4 and Mcm6 (pAcUW31vector) (15), and Mcm7 proteins (pVL1392 vector). The Mcm2 proteins and Mcm2,4,6,7 complexes were purified by Ni2+ column chromatography, and the Mcm2,4,6,7 complexes were further purified by glycerol gradient centrifugation as reported. A human glutathione S-transferase-huCdc7/myc-ASK complex was purified from High5 cells co-infected with the recombinant baculoviruses (37), and the complex was purified by glutathione-Sepharose chromatography as the manufacturer suggested (Pharmingen). Briefly, cell lysate was mixed with a 1/10 volume of glutathione agarose and incubated for 30

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1 The abbreviations used are: ASK, activator of S phase kinase; aa, amino acid; GFP, green fluorescent protein; NTS, nuclear-targeting sequence.
min at 4 °C. Then the agarose was washed with phosphate-buffered saline, and the human glutathione S-transferase-Cdc7/myc-ASK complex was eluted with 5 mM glutathione in 50 mM Tris-HCl, pH 8.0.

**In Vitro Phosphorylation**—The Mcm2,4,6,7 complexes (40–400 ng) were incubated with various amounts of the huCdc7/ASK complex at 30 °C for 30 min in a 20-μl reaction mixture consisting of 40 mM Hepes-KOH, pH 8.0, 40 mM potassium glutamate, 1 mM EGTA, 8 mM magnesium acetate, 2 mM dithiothreitol, 0.5 mM EDTA, 0.1 mM ATP, and

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**FIG. 1. Inhibition of Mcm4,6,7 hexamer formation by Mcm2.** Mcm4,6,7 hexameric complex (100 ng) was incubated with full-size Mcm2 (150 ng) or with increasing amounts (75, 150, and 300 ng) of a mutant Mcm2 that was deleted at the amino terminus (1–162) (A) or a carboxyl-half of Mcm2 (B) and then analyzed in native acrylamide gel. Mcm complexes were detected with anti-Mcm4 antibodies. The presence or absence of added Mcm2 was indicated at the top. At the bottom (C), a summary of the results is shown with several motifs in the Mcm2 protein.

**FIG. 2. Inhibition of Mcm4,6,7 helicase activity by Mcm2.** A. Mcm4,6,7 complex (100 ng) was examined for DNA helicase activity in the presence of two different amounts (25 and 50 ng) of Mcm2 mutants (Δ1–162, 1–282). The position of the released 17-mer is indicated. At the bottom (B), a summary of the results is shown.
0.01% Triton X-100 in the presence of $[\gamma^{32}P]ATP$. The phosphorylated proteins were analyzed in 10% polyacrylamide gel containing SDS. The same sample was digested with lysyl endopeptidase in the presence of 0.1% SDS and analyzed in 15–25% polyacrylamide gel.

**Synthesis of Mcm proteins and Binding to Mcm4-Beads—** Each of the mouse Mcm2 and Mcm6 genes was cloned in pBluescript II SK plasmid (Stratagene). Truncated forms of the Mcm2 gene were amplified by polymerase chain reaction using primers from each site. An ATG sequence was added to the 5’-end of the forward primers to create a methionine at the amino terminus of the Mcm2 proteins. Site-directed mutagenesis of the Mcm2 gene was conducted using the QuickChange site-directed mutagenesis kit (Stratagene). The oligonucleotide 5’-GTCAGTACACGGTGCAAGCCAACTTGTACTGGCCG-3’ was used as a primer to introduce changes from Cys to Ala at positions 329 and 332 in a putative zinc finger motif. The resulting DNAs were cloned into pBluescript II SK. The mutated sites in the Mcm2 gene were cloned into pEGFP-N1 (CLONTECH) to synthesize Mcm2-GFP fusion proteins where the carboxyl-terminal end of the Mcm2 protein was fused to the aminoterminal end of GFP. The cloned DNAs were transfected into mouse L cells using a CalPhos Maximizer transfection kit (CLONTECH). The cells (5 × 10^4) were grown in 8-well chambers with 250 μl of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum for 1 day. The cloned DNA (1.5 μg) was mixed with CalPhos Maximizer, calcium solution, and Hepes-buffered saline and left for 15 min at room temperature. The DNA solution was added to the cells in culture medium. The dish was placed at 37 °C in a CO₂ incubator for 5 h. The DNA solution was removed, growth medium was added, and the dish was returned to the incubator. After 2 days, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline for 30 min at room temperature. After three washes with phosphate-buffered saline, the fluorescence in the cells was observed by Olympus AX80 and photographs were taken.

**DNA Helicase Assay and Mcm Complex Formation—** A 17-mer oligonucleotide (5’-GTTTTCCCAGTCACGAC-3’) was labeled at the 5’-end with polynucleotide kinase in the presence of $[\gamma^{32}P]ATP$ and then annealed to M13 DNA. The annealed oligomer (2.5–5 fmol) was incubated at 37 °C for 30 min with an Mcm4,6,7 complex in the presence of absence of Mcm2 protein in 50 mM Tris–HCl, pH 7.9, 20 mM 2-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 Bio-Image Analyzer (FLA2000, Fuji). To analyze the effect of Mcm2 on the complex formation of Mcm4,6,7 proteins, increasing amounts of Mcm2 proteins were incubated with an Mcm4,6,7 complex for 30 min at 37 °C in 50 mM Tris–HCl, pH 7.9, 20 mM 2-mercaptoethanol, 5 mM ATP, 5 mM MgCl₂, and 0.01% Triton X-100, and the mixture was analyzed under non-denaturing conditions by native 5% acrylamide gel electrophoresis. The proteins in the gel were transferred to a nitrocellulose membrane after incubation in 49 mM Tris–HCl, pH 6.8, 38 mM glycine, and 0.25% SDS at 80 °C for 1 h, and Mcm4 on the membrane was detected by using anti-Mcm4 antibodies.

**Nucleosome Assembly Reaction—** Plasmid DNA (pSV01EP, 100 ng) was incubated with topoisomerase I in 10 mM creatine phosphate (sodium salt), pH 7.8, 4 mM ATP, 7 mM MgCl₂, 0.4 mM dithiothreitol, 25 μg/ml creatine phosphokinase, 400 μg/ml bovine serum albumin, and 20 mM potassium phosphate for 15 min at 37 °C. Indicated amounts of...
Mcm2 protein were mixed with H3/H4 histones (300 ng) in the same buffer as described above for 15 min. These two solutions were combined, and the reaction was further run for 45 min. After purification, DNA was electrophoresed in 1% agarose gel and stained with ethidium bromide.

RESULTS

Interaction between Mouse Mcm2 and Mcm4,6,7 Complex — We reported that Mcm2 can inhibit the DNA helicase activity of an Mcm4,6,7 hexamer by disassembling the hexamer into an Mcm2,4,6,7 tetramer (17). Mcm4,6,7 proteins mainly formed a hexameric complex or else a trimeric complex, as was determined by native acrylamide gel electrophoresis followed by immunoblotting using anti-Mcm4 antibodies (Fig. 1A). In contrast, the amino-terminal half (1–448) of Mcm2 protein did not show any disassembly activity (Fig. 1B). Next, the effect of mutant Mcm2 proteins on the DNA helicase activity of the Mcm4,6,7 hexamer was examined (Fig. 2). Mcm2 deleted at the amino-terminal end (1–162) showed inhibition of DNA helicase activity similar to the full-sized Mcm2 protein (17). However, an amino-terminal fragment (1–282) of Mcm2 did not show inhibitory activity. These results indicate that the amino-terminal region of Mcm2 is dispensable not only for disassembly of the Mcm4,6,7 hexamer but also for the inhibition of the DNA helicase activity of the Mcm4,6,7 hexamer.

From pull-down experiments, we found that Mcm2 protein mainly interacts with Mcm4 among Mcm4, -6, and -7 proteins.2

2 Y. Ishimi, unpublished results.
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In Vitro Phosphorylation of Mcm2,4,6,7 Complex with Cdc7—A number of reports indicate that Mcm2 protein is a good substrate of Cdc7 kinase in vitro (25–37). We incubated a purified Mcm2,4,6,7 complex with the human Cdc7/ASK kinase complex (Fig. 4, A and B). Consistent with the previous studies, Mcm2 of full-size was specifically phosphorylated with the kinase in the Mcm2,4,6,7 complex under these conditions. To map the region in Mcm2 that is required for the phosphorylation with the Cdc7/ASK kinase, we constructed four Mcm2 proteins deleted at the amino-terminal region and then purified Mcm2,4,6,7 complexes containing these Mcm2 (Fig. 4A). The Mcm2 deleted at amino acids 1–44 showed an unexpected mobility in SDS-gel. We incubated these complexes with Cdc7/ASK kinase (Fig. 4, B and C). In contrast to full-size Mcm2, all the three mutant Mcm2 proteins in which the amino-terminal region was deleted to different extents (Δ1–62, Δ1–162, and Δ1–448) were hardly phosphorylated by the kinase (Fig. 4B). To further map the region required for the phosphorylation, we prepared two additional deletion mutants of Mcm2 (Δ1–21 and Δ1–44) and phosphorylated the Mcm2,4,6,7 complexes containing them with the Cdc7 kinase (Fig. 4C). A slight decrease in the phosphorylation was detected in the Mcm2 deleted at 1–21, and a relatively distinct decrease was detected in the Mcm2 deleted at 1–44. The results indicate that the amino-terminal region (1–62) of mouse Mcm2 plays a crucial role in the phosphorylation by Cdc7/ASK kinase (Fig. 4D). Phosphorylated full-size Mcm2 appears as a single band in Fig. 4B, whereas it appears as two bands in Fig. 4C and Fig. 5A. Comparison of number of incorporated phosphates in these experiments suggests that a higher level of phosphorylation leads to generation of an extra band of phosphorylated Mcm2 on SDS-gel.

To address the issue of whether the amino-terminal region of Mcm2 contains major Cdc7-mediated phosphorylation sites, the phosphorylated full-sized Mcm2 in Mcm2,4,6,7 complex was digested with lysyl endopeptidase to analyze the phosphorylation in the amino-terminal region of Mcm2 (Fig. 5). The digestion generated a fragment of amino acids, 1–150 of Mcm2 (see Fig. 8A), that was detected as a 45-kDa band in SDS-gel (Fig. 5B). Incorporation of 32P into full-sized Mcm2 and into the amino-terminal fragment in the same sample was quantified in several experiments where the phosphorylation was carried out at the different levels of Cdc7 kinase; the results are shown in Fig. 5C. When one molecule of phosphate was incorporated into full-size Mcm2, one molecule of phosphate was recovered in the amino-terminal fragment of Mcm2, indicating that Cdc7 kinase phosphorylates only the amino-terminal region. When 3 mol of phosphate were incorporated into full-size Mcm2, however, two molecules were recovered in the amino-terminal region, suggesting that one mol of phosphate is incorporated into the region other than the amino terminus. These results and those in Fig. 4 suggest that the amino-terminal region of Mcm2 plays a role in the phosphorylation in the region other than the amino-terminal region in addition to the role as the phosphorylation sites.

Nucleosome Assembly Activity of Mcm2 Protein—We determined the histone binding activity in the amino-terminal region of Mcm2 (17). The Mcm2 in Mcm complexes specifically bound to histone H3 among four core histones. To elucidate the physiological role of the histone binding activity, we measured the nucleosome assembly activity of mouse Mcm2 protein (Fig. 6). The histone H3/H4, instead of four core histones, was used for the nucleosome assembly assay (38). If nucleosome-like
structures were formed on circular DNA, negative supercoils of DNA would be generated in this system. Thus, the recovery of negatively supercoiled form-I DNA suggests that the plasmid DNA is fully assembled into nucleosome-like structures. Full-sized Mcm2 was able to stimulate the nucleosome assembly in vitro, similar to Nap-1, which showed nucleosome assembly activity in vitro (39), although the level of the activity in Mcm2 was lower than that in Nap-1 (Fig. 6A). We also determined the region in Mcm2 that is required for the activity by preparing several deletion mutants (Fig. 6, A and B). The results indicated that the deletion of 162 amino acids from the amino terminus abolished the nucleosome assembly activity (Fig. 6A). However, the deletion of 62 amino acids from the terminus did not perturb the activity, and the removal of 96 amino acids reduced the activity (Fig. 6B). Thus, the region spanning amino acids 63–162 at the amino-terminal end of Mcm2 is required for the activity, although the amino-terminal region (1–282) of Mcm2 was not sufficient for the activity (Fig. 6A). The region 63–162 almost overlapped with the histone H3 binding domain of Mcm2 identified previously. Thus, it is suggested that Mcm2 stimulates nucleosome assembly in vitro by interacting with histone H3/H4 through the histone H3 binding domain.

**Nuclear Localization of Mcm2—**We showed that the aminoterminal region (1–153) is required for the nuclear localization of mouse Mcm2 when it was expressed in HeLa cells as a GFP fusion protein (41). There are two bipartite-type nuclear-targeting sequences (40) in this region of mouse Mcm2, one at position 18–34 (NTS1) and the other at 132–152 (NTS2). We introduced mutations into these two sequences and examined the effect on the nuclear localization of Mcm2-GFP fusion proteins expressed in mouse L cells (Fig. 7). The mutations in NTS1 did not alter the nuclear localization of the fusion protein, but the mutations in NTS2 significantly perturbed the nuclear accumulation of the fusion protein. The Mcm2-GFP fusion protein mutated at the NTS2 was detected not only in nuclei but also in cytoplasm. When both of the NTS1 and 2 were mutated, the fusion protein was also detected both in nuclei and cytoplasm. These results suggest that the NTS2 plays an important role in the nuclear localization of Mcm2 under these conditions. This conclusion is consistent with the previous observations that the amino-terminal deletion up to amino acids 92 did not affect the nuclear localization of Mcm2 (17).

**DISCUSSION**

In this paper, we analyzed the biochemical activity related to the mouse Mcm2 protein, which includes that to inhibit Mcm4,6,7 helicase and to assemble nucleosomes in vitro. We determined the region in Mcm2 required for the phosphorylation by Cdc7/ASK kinase and the region required for nuclear localization of Mcm2. We localized these activities in Mcm2, as shown in Fig. 8. The results indicated that the carboxyl-half of Mcm2 is required for interacting with Mcm4 as well as for inhibiting the DNA helicase activity of Mcm4,6,7 complex. We showed that the amino-terminal region of Mcm2 where a histone H3 binding domain and a region required for nuclear localization are present is required for the phosphorylation by Cdc7 kinase. In this region, a number of Ser and Thr residues are present in addition to three consensused sites for cyclin-dependent kinase.

Mcm2–7 are all essential for eukaryotic DNA replication, but only the heterohexameric Mcm2–7 complex among several Mcm complexes has the ability to induce DNA replication in the *Xenopus* egg system (12). All the members have a DNA-dependent ATPase motif, but the heterohexameric Mcm2–7 complex shows neither significant ATPase activity or DNA helicase activity (41). In contrast, an Mcm4,6,7 complex as a hexamer showed an ATP-dependent DNA helicase activity in vitro (13, 28).
interacts with Cdc7/Dbf4 in occurs in the Mcm complex (17, 42). Genetically Mcm proteins Mcm2,7 helical complex (16). It has been proposed that a structural change in the Mcm2–7 heterohexamer or a change in protein composition occurs in the Mcm complex (17, 42). Genetically Mcm proteins interact with Cdc7/Dbf4 in Saccharomyces cerevisiae (25, 43). It has been shown that Mcm2 is a good substrate of the kinase in vitro (25–37). Thus, it is probable that Cdc7 kinase is involved in these changes in the Mcm complex. One possibility is that phosphorylation of Mcm2 by the kinase is involved in a structural change of the Mcm2–7 heterohexamer by which the DNA helicase activity that may intrinsically reside in the complex is induced (42). The notion that the heterohexameric Mcm2–7 complex acts as a DNA helicase in vivo is supported by the following findings. First, Mcm proteins appear to migrate on chromosome after the initiation of DNA replication in S. cerevisiae (44). Second, it has been demonstrated that all the Mcm proteins are required for elongation of DNA replication after initiation has occurred (45). In addition, it has been indicated that Mcm proteins mainly form the Mcm2–7 heterohexamer in vivo.

Another possibility is that an Mcm4,6,7 complex is generated from the Mcm2–7 complex, and the phosphorylation of Mcm2 by Cdc7 kinase is involved in the removal of Mcm2,3,5 proteins (17). This possibility is supported by the following findings. First, only the Mcm4,6,7 hexameric complex showed DNA helicase activity among several Mcm complexes including the heterohexameric Mcm2–7 complex that were all prepared in a baculovirus expression system. Second, the genetic evidence that the requirement of Cdc7 kinase was bypassed by mutation of Mcm5 in S. cerevisiae suggests that Mcm5 at least functions as a negative regulator of DNA replication (43). Consistent with this finding, both Mcm2 and Mcm3/5 complex can inhibit Mcm4,6,7 helicase activity in vitro (13, 17, 18). Third, the Mcm4,6,7 proteins form relatively stable complexes of a monomer and a dimer of the 4,6,7 trimer, and Mcm2,3,5 proteins are relatively loosely associated with the Mcm4,6,7 trimer in the Mcm2–7 heterohexamer. This structural information appears to be consistent with the change in the composition of the Mcm complex. It is possible that the ATP binding motif of Mcm2,3,5 proteins is involved in releasing Mcm4,6,7 complex from the heterohexamer at the time when the Mcm complex functions as a DNA helicase. A similar example can be set at the initiation of DNA replication in E. coli where a DnaC protein, which is an ATPase but an inhibitor of DnaB helicase, forms a complex with DnaB and binds to the origin region. In this system, it appears that the ATPase activity of DnaC is required for releasing DnaB at the initiation of DNA replication (46). The second model, that the Mcm4,6,7 complex generated from the Mcm2–7 complex functions as a DNA helicase in vivo, may not be inconsistent with the finding that all the Mcm2–7 proteins are required for elongation of DNA replication as well as initiation (45). Furthermore, the possibility that the Mcm4,6,7 helicase functions in DNA replication is supported by the recent finding that the Mcm4,6,7 complex from S. pombe acts as a processive DNA helicase when a tailed substrate is used for the reaction (47). However, we have no direct evidence for this second model. The present finding indicating that Cdc7/ASK kinase phosphorylates the amino-terminal domain of Mcm2, which is not required for interacting with the Mcm4,6,7 complex, seems to be consistent with the model. However, our results suggest that the region other than the amino-terminal region is also phosphorylated with Cdc7/ASK (Fig. 5). We have another piece of data indicating that other regions of the Mcm2 which include the carboxyl-terminal segment of the protein in the Mcm2–4,6,7 complex are also phosphorylated by the kinase in vitro (data not shown). These phosphorylations at regions other than the amino terminus may modulate the structure of the Mcm complex or influence the interaction of Mcm2 with other components of the Mcm complex. Further efforts are clearly necessary to clarify the mechanism of Mcm function. It also remains to be determined to what extent Mcm helicase is responsible for replication fork movement in vivo (48).

Cdc7/ASK kinase phosphorylated an amino-terminal region of Mcm2 where a histone H3 binding domain and a region required for nuclear localization are present. We do not know the effect of the phosphorylation of Mcm2 by Cdc7/ASK kinase on these two activities. It has been reported that Mcm2 proteins, which are detached from chromatin during the S phase, are more phosphorylated than chromatin-bound Mcm2 proteins (49), suggesting that the phosphorylation of Mcm2 is involved in the detachment of Mcm proteins from chromatin. It has been shown that Cdc2/cyclinB is involved in the phosphorylation of Mcm2 proteins in vivo using a temperature-sensitive mutant of the kinase (50). We observed that the amino-terminal region of Mcm2 is phosphorylated by Cdk2/cyclinA or Cdk2/cyclinE in vitro (data not shown). Thus, both cyclin-dependent kinase and Cdc7 kinase may be involved in the phosphorylation of the amino-terminal region of Mcm2 proteins (34,
In fact, prior phosphorylation of the Mcm2,4,6,7 complex with Cdk stimulated subsequent phosphorylation of Mcm2 by Cdc7 (37). It has been also shown that cyclin-dependent kinase plays a role in the initiation of DNA replication by loading Cdc45 protein at the replication origin (51, 52).

We showed that the region around amino acid 150 (NTS2) is required for nuclear localization of mouse Mcm2. The nuclear localization of Mcm2 may depend on nuclear import of the protein and also on nuclear retention of the protein (53). The results in Fig. 7 suggest that mutation in the NTS2 did not affect the nuclear import but affected the retention of Mcm2 in the nucleus. In S. pombe, both of two regions of amino acids 5–10 and 114–118 are required for nuclear localization of Mcm2 (54). The region near the amino terminus functions as a nuclear localization signal. Finally, we detected in Mcm2 activity that stimulates nucleosome assembly in vitro. The histone binding domain in Mcm2 was required for the activity. Thus, Mcm2 protein may bind with histone H3/H4 and transfer them to DNA to assemble nucleosome-like structures in vitro. The physiological significance of the activity remains to be clarified. It is possible, however, that the activity is involved in chromatin assembly during DNA replication by interacting with parental or newly synthesized histones. Conversely, it may be involved in DNA replication by disassembling nucleosomes to facilitate unwinding of DNA. Recently, it has been reported that Mcm proteins are involved in transcription in vivo (55, 56), and a histone acetyltransferase interacts with the histone H3 binding domain of Mcm2 (57). Mcm proteins may play a role in several chromatin activities in addition to having an essential role in DNA replication.

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