Biochemical Function of Mouse Minichromosome Maintenance 2 Protein*

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Minichromosome maintenance (MCM) proteins play an essential role in eukaryotic DNA replication and bind to chromatin before the initiation of DNA replication. We reported that MCM protein complexes consisting of MCM2, -4, -6, and -7 bind strongly to a histone-Sepharose column (Ishimi, Y., Ichinose, S., Omori, A., Sato, K., and Kimura, H. (1996) J. Biol. Chem. 271, 24115–24122). Here, we have analyzed this interaction at the molecular level. We found that among six mouse MCM proteins, only MCM2 binds to histone; amino acid residues 63–153 are responsible for this binding. The region required for nuclear localization of MCM2 was mapped near this histone-binding domain. Far-Western blotting analysis of truncated forms of H3 histone indicated that amino acid residues 26–67 of H3 histone are required for binding to MCM2. We have also shown that mouse MCM2 can inhibit the DNA helicase activity of the human MCM4, -6, and -7 protein complex. These results suggest that MCM2 plays a different role in the initiation of DNA replication than the other MCM proteins.

Minichromosome maintenance (MCM) proteins play an essential role in eukaryotic DNA replication. Six MCM proteins (MCM2–7) have been identified from yeast to mammals, and each of them plays a distinct role in DNA replication (reviewed in Refs. 1–3). Several lines of evidence suggest that MCM proteins are required for the initiation of DNA replication (4–6). Consistent with this idea, MCM proteins associate with the chromatin before the onset of DNA replication and detach from the chromatin during DNA replication (7–9). Biochemical analyses have indicated that these six MCM proteins interact. In extracts from Schizosaccharomyces pombe (10), mouse cells (11) and mitotic human cells (12), a complex of approximately 600 kDa, containing all six MCM proteins, has been identified. Based on the molecular mass of each MCM protein, this complex is probably a hexamer containing a single molecule of the six MCM proteins. However, sub-complexes containing MCM2, -4, -6, and -7 or MCM3 and -5 have also been isolated from human cell extracts (13–17). Xenopus egg extracts (18), Schizosaccharomyces pombe (19), and mouse cell extracts (19). Our group recently found that the DNA helicase activity was associated with the human MCM4, -6, and -7 protein complex (20). The 600-kDa complex containing the six MCM proteins is detected in soluble cell extracts; however, the structures of the chromatin-bound MCM protein heterocomplexes (21) remain to be elucidated.

Origin recognition complex (ORC) and CD-C6 protein are both required for the initiation of DNA replication and are necessary for the binding of MCM proteins to chromatin in Xenopus egg extracts (22, 23). CD-C6-dependent loading of MCM proteins onto chromatin (24, 25) and origins (26, 27) has also been observed in Saccharomyces cerevisiae. The genetic interaction between MCM proteins and ORC in S. cerevisiae supports these findings (28, 29). These results suggest that MCM proteins form a complex with ORC and CD-C6 at the replication origin. In S. cerevisiae the number of MCM molecules recovered is much greater than the number of ORC molecules; however, over half of these MCM molecules are not bound to chromatin (24, 30). In addition ORC6 and CD-C6 can be released from the chromatin under conditions where almost all of MCM5 remains associated with the chromatin (24). Therefore, the molecular basis of the interaction between MCM proteins and the chromatin remains to be determined. Here, we report that an amino-terminal portion of MCM2 has an affinity for histone and that MCM2 can inhibit the DNA helicase activity of the human MCM4, -6, and -7 protein complex.

EXPERIMENTAL PROCEDURES

Synthesis of MCM Proteins and Binding to Histone—Six mouse Mcm2 genes, Mcm2 (accession no. D86725) (11), -3 (8), -4 (19), -5 (19), -6 (11), and -7 (31), had been cloned in pBluescript II plasmid (Stratagene, La Jolla, CA) at the EcoRI site, as reported. Truncated forms of the Mcm2 gene were amplified by polymerase chain reaction (PCR) using primers from each site. Forward primers starting from nucleotide (nt) 153 (for deleting Δ amino acid residues 1–34), 236 (Δ1–62), 279 (Δ1–76), 327 (Δ1–92), 399 (Δ1–116), and 536 (Δ1–166) and a reverse primer ending at nt 3323 were used as PCR primers to truncate an amino-terminal region. A forward primer starting from nt 51 and reverse primers ending at nt 3326 (for amino acid residues 1–92), nt 398 (residues 1–116), nt 452 (residues 1–134), nt 509 (residues 1–153), nt 731 (residues 1–227), and nt 896 (residues 1–282) were used to truncate a carboxyl-terminal region of the Mcm2 gene. To construct Mcm2 genes that were deleted from amino acid residues 1–34 or 1–92, an ATG sequence was added to the 5′-ends of the forward primers to create a methionine at the amino terminus of the MCM2 proteins, and stop codons were added to the 3′-end of the reverse primers to produce MCM2 truncated at the carboxyl-terminal region. A forward primer starting from nt 236 and a reverse primer ending at nt 509 were used to construct an Mcm2 gene that encodes amino acid residues 63–153. The resulting DNAs were cloned into pBluescript II SK+ – at the EcoRI site (Δ1–34, Δ1–62, Δ1–76, Δ1–116, Δ1–166, 1–116, and 1–282), between the SalI and EcoRI sites (Δ1–92), or between the EcoRI and BamHI sites (1–92, 1–134, 1–153, 1–227, and 63–153) and purified by anion exchange (QIagen, Hilden, Germany). MCM proteins were synthesized in vitro in the presence of [35S]methionine in a reticulocyte lysate system, as suggested by the manufacturer (TNT®-coupled reticulocyte lysate system, Promega, Madison, WI). The reaction mixture was diluted with 400 μl of 40 mM Hepes, pH 7.5, and 40 mM dithiothreitol and then concentrated to about
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50 µl using a Microcon-10 column (Amicon, Beverly, MA) to remove free [35S]methionine. An aliquot of the sample was diluted to 10 µl with 0.3 M NaCl in buffer A (20 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 1 mM dithiothretiol, 0.1 mM phenylmethylsulfonyl fluoride, and 10% glycerol) containing 0.05% Nonidet P-40 and then incubated with 10 µl of the histone-Sepharose beads in the same buffer for 1 h at 4 °C with vibration. The supernatant was saved after centrifugation, and the beads were washed three times with the above buffer. Proteins bound to the beads were eluted twice with 20 µl of 2 M NaCl in buffer A plus Nonidet P-40. The bound and unbound proteins were electrophoresed in a 10, 15, or 20% polyacrylamide gel (32), and the radioactivity on the gel was analyzed using a Bio-Image Analyzer (Fuji, Tokyo, Japan).

Expression of MCM2-GFP Fusion Protein in HeLa Cells—Truncated forms of the mouse Mcm2 gene were constructed by PCR using forward primers starting from nt 327 (for deleting (Δ) amino acid residues 1–92) and 509 (Δ1–153) and the reverse primer ending at nt 2762. In the cases of Δ1–92 and Δ1–153, ATG sequences were added to the 5′-ends of the forward primers. The native form as well as the truncated forms of the mouse Mcm2 gene were cloned into pgEFP-N1 (CLONTECH, Palo Alto, CA) at the HindIII site (native, Δ1–34) or between the HindIII and EcoRI (Δ1–153) sites, to synthesize MCM2-GFP fusion proteins where the carboxy-terminal ends of the MCM2 proteins were fused to the amino-terminal end of GFP. The cloned DNAs were transfected into HeLa cells using Tfx 20 (Promega). The cells of 105 were grown on coverslips in a 35-mm dish with 2 ml of DMEM supplemented with 5% FCS and antibiotics (100 units/ml penicillin and 50 µg/ml streptomycin) for 1 day. The cloned DNA (1.4 µg) was added to 1 ml of DMEM without FCS and antibiotics, and the solution was mixed well. Tfx 20 (4.2 µl) was then added to this DNA solution (final ratio of Tfx 20: DNA = 2:1). The solution was mixed well and left for 15 min at room temperature. The culture medium was removed from the dish, and the DNA/lipid solution was added. The dish was incubated at 37 °C in a CO2 incubator for 1 h. Two ml of DMEM with 10% FCS and antibiotics was added, and the dish was returned to the incubator. The next day, the medium was replaced with fresh DMEM with 5% FCS plus antibiotics after washing three times with the medium. After 8 h (24 h after transfection), the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature and then permeabilized with 0.5% Triton X-100 in PBS. After washing three times with PBS, cells were stained with 4′,6-diamidino-2-phenylindole. Photographs were taken to Kodak TMY400 film using Zeiss Axioplan with 63× objective.

Far-Western Blotting of H3 Histone Fusion Protein—The histone H3 gene of HeLa cells was cloned by reverse transcription-PCR (RNA LA PCR Kit, Takara, Tokyo). The H3 histone gene was cloned into pTrxFus vector (Invitrogen, NV Leek, Netherlands), and the DNA sequence was determined using a DNA sequencing kit (Dye Termination Cycle Sequencing Ready Reaction, Perkin-Elmer). The amino acid sequence of H3 histone, deduced from the nucleotide sequence, was the same as the published sequence (accession no. X0090) (33). Truncated forms of the H3 histone gene were cloned into an HLTA vector between the KpnI and BamHI sites of the pTrxFus vector. Thioredoxin-H3 fusion proteins were expressed in transformed Escherichia coli. The fusion proteins were electrophoresed on a 15% polyacrylamide gel and then transferred to a membrane (Immobilon-P, Millipore, Bedford, MA). The proteins on the membrane were visualized by staining with 0.1% Ponceau S in 1% acetic acid for 10 min. After destaining with water, the membrane was incubated in 0.05% Tween 20 in PBS for 2 h and then incubated in PBS containing 5% calf serum and 0.5% bovine serum albumin (34). The membrane was incubated with the [35S]methionine-labeled amino-terminal fragment (amino acid residues 1–282) of the mouse MCM2 in the same solution for 2 h at room temperature. After washing with PBS, the radioactivity on the membrane was measured using the Bio-Image Analyzer.

Production of MCM2 Protein in Insect Cells—The full-size mouse Mcm2 gene was cloned into an HLT-A vector between the EcoRI and NotI sites (PharMingen, San Diego), and recombinant baculovirus encoding histidine-tagged Mcm2 gene was prepared as the manufacturer suggested. Soluble His-MCM2 protein was recovered after lysis of the virus-infected SF9 cells. The extracts were mixed with nickel nitritolacti-acid-agarose beads, and the beads were washed with 50 mM sodium phosphate, pH 6.0, 0.3 M NaCl, and 10% glycerol in the presence of 1% Triton X-100. Proteins bound to the beads were eluted with 0.3 M imidazole in 50 mM sodium phosphate, pH 6.0, 0.3 M NaCl, and 10% glycerol. The partially purified His-MCM2 protein was then loaded onto a histone-Sepharose column in 0.3 M NaCl, and the fusion protein was eluted by 2 M NaCl (18). Purified His-MCM2 protein was diluted to 0.2 M NaCl and then concentrated with a Centricon-30 column (Amicon).

DNA Helicase Assay and Protein Analysis—The MCM protein fraction that contains mainly MCM4, -6, and -7 proteins was purified from HeLa cells by histone H3/H4-Sepharose column chromatography and then by glycerol gradient centrifugation (20). DNA helicase activity was measured by displacement of 17-mer oligonucleotides annealed to M13mp18 DNA. Proteins were electrophoresed in 10% polyacrylamide gels containing SDS, or 4% or 5% polyacrylamide gels in 50 mM Tris-HCl and 50 mM glycine, pH 8.8 (35), and then stained with silver. SV40 T Antigen Signal—A plasmid (pSV78-T-lacZ) encoding the SV40 T antigen nuclear localization signal (PPKKKKRKV) was kindly provided by M. Tanaka (Mitsubishi Kasei Institute of Life Sciences). An HindIII site was created at the carboxy end of a fragment containing the signal by PCR, and the resultant HindIII fragment (5′-AGCTT-ACC/ATG/GCA/AAG/ATG/CCT/CTT/AAG/AAAG/AAAG/AAAG/GTC/ GAG/AA-3′) containing the signal was inserted into a construct producing the truncated form (Δ1–153) of mouse MCM2 protein that had been fused with GFP. Slash indicates frames of translated amino acids. As the result, a peptide (MAKIPPKKKRKVE) was added to the amino terminus of the fusion protein.

RESULTS

Binding of MCM2 Protein with Histone—Among the six MCM proteins that were detected in 0.2 M NaCl-soluble HeLa whole cell extracts, MCM protein complexes containing MCM2, -4, -6, and -7 proteins bound strongly to a histone-Sepharose column (16). These four MCM proteins from mouse FM3A cells also bound to the histone-Sepharose column (data not shown). Each of the six mouse MCM proteins was synthesized in vitro in the presence of [35S]methionine using a reticulocyte lysate system (Fig. 1A). The most slowly migrating bands of each MCM protein synthesized corresponded to full-size products, since the electrophoretic mobilities of these bands were coincident with those of native MCM proteins. The ability of the MCM proteins to bind histone was examined (Fig. 1B). Each of the labeled MCM proteins was incubated with histone-Sepharose beads at 0.3 M NaCl, and bound proteins were eluted from the Sepharose by increasing the NaCl concentration to 2 M. Approximately 90% of the recovered MCM2 was detected in the bound fraction, but the other five MCMs did not bind to the histone-Sepharose under these conditions (less than 10% was recovered in the bound fraction). These results suggest that MCM2 is mainly responsible for the strong binding of MCM2,
-4, -6, and -7 complexes to the histone-Sepharose. This conclusion was supported by the finding that the elution of MCM2 from the histone H3/H4-Sepharose column was slightly retarded compared with that of the MCM4, -6, and -7 (16).

To determine the region of MCM2 responsible for binding to histone, the histone binding activity of the truncated forms of MCM2 was examined. First, MCM2 proteins that were deleted externally from the amino terminus were synthesized, and histone binding was examined (Fig. 2). A large portion (76%) of the recovered native MCM2 was detected in the bound fraction. Deleting the amino-terminal region (amino acid residues 1–62) did not affect the binding with histone. Smaller proteins were also synthesized using this MCM2 construct (Δ1–62), which may result from the initiation of protein synthesis from methionines at a downstream region in this system. These smaller proteins did not bind the histone-Sepharose. Deleting amino acid residues 1–76 decreased the binding, since almost equal amounts of MCM2 were recovered in the unbound and bound fractions. Almost no binding was detected in MCM2 deleted amino acid residues 1–92, 1–116, and 1–166.

Next, the MCM2 proteins, deleted externally from the carboxyl terminus, were synthesized, and histone binding was examined (Fig. 3). A large portion (approximately 75%) of the MCM2 proteins recovered from the bound fractions contained the amino-terminal region amino acid residues 1–153, 1–227, and 1–282. However, the MCM2 protein containing amino acid residues 1–134 showed a decrease in binding activity (55% was recovered in the bound fraction), and the MCM2 protein containing amino acid residues 1–92 and 1–116 lost the binding activity. These results suggest that the region of amino acid residues 63–153, which contains a high concentration of charged amino acids, is required for binding to histone (Fig. 4). Consistently, the MCM2 fragment from amino acid residues 63

**Fig. 2.** Histone binding activity of MCM2 deleted from the amino terminus. DNA constructs that delete the amino-terminal region of MCM2 were prepared as described under "Experimental Procedures." Native and truncated forms of MCM2 were synthesized in vitro and examined for binding to histone-Sepharose, as described in Fig. 1. Unbound (lane 2), bound (lanes 3 and 4), and total proteins (lane 1) were analyzed in 10% SDS-polyacrylamide gels. The largest protein bands, indicated with arrows, were probably full-size products of the constructs. The number of amino acid residues deleted are indicated at the top.

**Fig. 3.** Histone binding activity of MCM2 deleted from the carboxyl terminus. MCM2 proteins that were deleted from the carboxyl terminus and a fragment of 63–153 residues were synthesized in vitro and examined for binding to histone-Sepharose. Unbound (lane 2), bound (lanes 3 and 4), and total proteins (lane 1) were analyzed by 10% (for 1–282), 15% (for 1–227), or 20% (for others) SDS-polyacrylamide gel electrophoresis. The number of remaining amino acid residues in the truncated MCM2 are indicated at the top.

**Fig. 4.** The region of MCM2 required for binding to histone. The results in Figs. 2 and 3 are summarized. The structures of truncated MCM2 proteins are shown; the DNA-dependent ATPase domain and two candidates of nuclear localization signals (NLS) are indicated. The histone binding activity of the MCM2 protein is indicated at the right by +/−, and binding of intermediate strength is indicated by (+). The region required for binding to histone is enlarged at the bottom, where basic amino acid residues are underlined and acidic residues are overlined.
A Region of H3 Histone Required for the Binding with MCM2 Protein—Our group reported that MCM protein complexes containing MCM2, -4, -6, and -7 specifically bind to a histone H3-Sepharose column (16). To identify the region(s) of H3 histone responsible for the binding to MCM2, various deletion mutants of H3 histone were produced as fusion proteins, and the binding of these H3 histones to the fusion proteins were shown; the amino acid residue numbers are indicated, and the binding activity of the fusion proteins with MCM2 is also indicated on the right by +/−.

Requirement of an Amino-terminal Region of MCM2 Protein for Nuclear Localization—Mouse MCM2 proteins are localized in nuclei when they are expressed in COS cells (11), suggesting that MCM2 contains a nuclear localization signal. Two regions that may be required for the nuclear localization of MCM2 were found in the amino-terminal region; one region is amino acid residues 18–34 where a bipartite-type nuclear localization signal is present, and another region is amino acid residues 118–152 where positively charged amino acid residues are highly concentrated (Fig. 4). To determine the region required for nuclear localization of MCM2, two DNA constructs producing deletion mutants of MCM2 proteins as GFP fusion proteins were prepared and transfected into HeLa cells (Fig. 6). Full size MCM2 (Fig. 6A) as well as the MCM2 that was deleted from the amino terminus to amino acid residue 92 (Fig. 6B) were localized to the nucleus. However, the mutant MCM2 deleted from the amino terminus to amino acid residue 153 was not concentrated in the nucleus; they were localized uniformly throughout the cells (Fig. 6C). A nuclear localization signal from SV40 T antigen was ligated to the mutant MCM2-GFP fusion protein (Δ1–153). This fusion protein was localized to the nucleus (Fig. 6D). These results suggest that the second region from amino acid residues 118 to 153 is capable of localizing MCM2 to nucleus.

Inhibition of the DNA Helicase Activity by MCM2—We reported the results suggesting that the human MCM protein...
complex containing MCM4, -6, and -7 has both DNA helicase and DNA-dependent ATPase activities (20). When MCM protein complexes were fractionated by glycerol gradient centrifugation, these activities were detected in the 350-kDa fraction, where MCM2 protein was almost absent, but a main peak of the four MCM proteins was detected at a position of 230 kDa (Fig. 7, A and C) (20). When MCM proteins in the gradient fraction were electrophoresed in native agarose gel, two major complexes of approximately 450 and 600 kDa were detected (Fig. 7B). The amounts of the 600-kDa complex in the gradient fractions were almost proportional to the DNA helicase activity (Fig. 7, B and C). These results suggest that MCM4, -6, and -7 form a hexamer and function as a DNA helicase, as previously suggested (20). Two complexes of similar sizes were also detected on SDS-polyacrylamide gel after protein cross-linking (20). Correlation between the amounts of the 600-kDa complex and DNA helicase activity was more clearly observed when the complex was detected on SDS-polyacrylamide gel after protein cross-linking, which is probably due to the fact that composition of MCM complexes is slightly changed during native gel electrophoresis. The results in Fig. 7 also indicated that DNA helicase activity was mainly detected in the fractions lacking MCM2. Therefore, it was possible that MCM2 protein can inhibit the DNA helicase activity by the MCM4, -6, and -7 protein complex.

Histidine-tagged mouse MCM2, purified from baculovirus-infected SF9 cells, was added to a DNA helicase reaction containing purified MCM4, -6, and -7 complex (fractions near 350 kDa were pooled). By adding an almost stoichiometrical amount of MCM2, the DNA helicase activity was severely inhibited (only 12% of the activity was detected) (Fig. 8A). In contrast, His-MCM2 had no inhibitory effect on the DNA helicase activity of SV40 T antigen and mouse DNA helicase B (37) (data not shown). His-MCM2 itself did not exhibit DNA helicase activity. As shown in Fig. 8B, a 600-kDa complex was mainly detected in the purified MCM4, -6, and -7 complex, although complexes of smaller molecular mass were also detected, which is probably due to the presence of a small amount of human MCM2 in this fraction. The addition of His-MCM2 prevented the formation of the 600-kDa complex and increased the number of smaller molecular mass complexes. A weak band of about 400 kDa was detected in the presence of His-MCM2 only (data not shown). Therefore, the inhibitory effect of MCM2 on the DNA helicase activity was correlated with the ability of MCM2 to disassemble the 600-kDa complex. To examine this correlation further, the fraction that mainly contains the MCM4, -6, and -7 complex was incubated with or without MCM2 and then the mixture was separated by glycerol gradient centrifugation (Fig. 9). Without adding MCM2, the DNA helicase activity was detected in the fractions 3–5 (near 350 kDa) (Fig. 9C, left) where MCM4, -6, and -7 co-sedimented (Fig. 9A, left), and a 600-kDa complex was mainly detected in native gel (Fig. 9B, left). After incubation with MCM2, however, MCM2, -4, -6, and -7 were mainly detected at the position of 230 kDa (Fig. 9A, right) where a 450-kDa complex was mainly detected in native gel (Fig. 9B, right). The DNA helicase activity was not detected in these fractions (Fig. 9C, right). When MCM2 was incubated in the absence of the MCM4, -6, and -7 complex and then centrifuged, it was detected in a broad region of the gradient fractions, but the peak was detected at fractions 2–4 (data not shown). These results suggest that MCM2 specifically inhibits the DNA helicase activity of MCM4, -6, and -7 by preventing the formation of the hexamer.
to ethanol, 5 mM MgCl2, 5 mM ATP, and 0.01% Triton X-100 for 30 min approximately 7 MCM4, -6, and -7 complex (A shown that co-expressing MCM6 with MCM2 localizes MCM6 proteins are not. In the same experiments, it has also been shown that the nuclear localization signal. Consistently, Kimura et al. (11) have shown that mouse MCM2 proteins, which are expressed in COS cells, are localized to nucleus, but MCM4, -6, and -7 proteins are not. In the same experiments, it has also been shown that co-expressing MCM6 with MCM2 localizes MCM6 to the nucleus. MCM2 can be isolated from cell extracts in a complex with MCM4, -6, and -7 (13–16). Thus, MCM2 may carry MCM4, -6, and -7 to nucleus in vivo.

MCM protein complexes containing MCM2, -4, -6, and -7 have been purified by histone-Sepharose column chromatography (16). The results of this study suggest that the MCM protein complexes containing these four MCM proteins bind to histone-Sepharose through the interaction of MCM2 protein with histone H3. A major peak of MCM2, -4, -6, and -7 was detected at the position of 230 kDa in glycerol gradient centrifugation (Fig. 7A and Ref. 20), suggesting that they form dimers. However, a 450-kDa complex was mainly detected in the same position, which was analyzed by native agarose gel electrophoresis (Fig. 7B) and also in SDS-polyacrylamide gel electrophoresis after cross-linking (20). When the MCM proteins in the position of 230 kDa were chemically cross-linked and then separated by glycerol gradient centrifugation, the resultant 450-kDa complex, which was detected on an SDS-polyacrylamide gel, sedimented at the position of 230 kDa (data not shown). Therefore, it is probable that MCM2, -4, -6, and -7 detected at the position of 230 kDa form a heterotetramer. A portion of MCM4, -6, and -7 may be separated from MCM2 during elution from the histone-Sepharose column, and the separated MCM4, -6, and -7 mainly form a hexamer of 600 kDa, which sedimented at the position of 350 kDa in glycerol gradient centrifugation (Fig. 7B and Ref. 20). It is possible that two of the separated MCM4, -6, and -7 trimers are assembled into a hexamer during these processes. We previously suggested that the hexamer of MCM4, -6, and -7 exhibits both DNA helicase and ATPase activities (20). Here, we show that MCM2 inhibits both the DNA helicase activity and the formation of MCM4, -6, and -7 hexamers (Figs. 8 and 9). These results support the notion that the MCM2 protein plays a regulatory role in the DNA helicase activity of the MCM4, -6, and -7 complex. By adding MCM2, the 600-kDa hexamer of MCM4, -6, and -7 was converted to the 450-kDa complex containing MCM2, and DNA helicase activity was not detected in this complex (Fig. 9). These results are consistent with the notion that the 450-kDa complex is a heterotetramer consisting of MCM2, -4, -6, and -7. Other smaller complexes of approximately 400 kDa slightly increased in the presence of MCM2 (Fig. 8B), and one of these complexes may be a trimer of MCM4, -6, and -7, which was separated from the hexamer. The system where MCM2 regulates DNA helicase activity of the MCM4, -6, and -7 complex seems to be similar to the α replication system (39). In this system, αP proteins bring dnaB helicase to the α replication origin which is occupied by AO. Since αP proteins inhibit the dnaB helicase activity, they must be removed from the origin with the assistance of the heat-shock proteins DnaJ, DnaK, and GrpE.

MCM2 may be released from the MCM4, -6, and -7 complex to activate its DNA helicase activity at the onset of DNA replication. Cdk2/cyclin and/or Cdc7/Dfh4 protein kinases, both of which are required for the initiation of DNA replication (reviewed in Ref. 40) may phosphorylate MCM2 to remove it from the MCM4, -6, and -7 protein complex. The finding that a mutant of MCM5 (bob-1) bypasses the requirement for Cdc7 kinase in S. cerevisiae (41) seems to be consistent with this notion. The region containing the bob-1 mutation is evolutionarily conserved in MCM2 and MCM4 as well as MCM5. Recently, Lei et al. (42) have reported that MCM2 in addition to MCM3, -4, and -6 can be phosphorylated by Cdc7/Dfh4 in vitro. Since the affinity of MCM2 for histone H3 is high, a structural change of the nucleosome may cause MCM2 to bind H3 histone
in the chromatin, which may concomitantly result in the release of the MCM4,-6,- and -7 protein complex. A third possibility is that newly synthesized H3 histone may remove MCM2 from the chromatin. This correlates with the findings that histone synthesis precedes DNA synthesis during the cell cycle (43).

The region of H3 histone necessary for binding to MCM2 is amino acid residues 26–67 near the amino terminus. This region, which forms an α-helical structure (44) and is involved in histone-DNA binding in the nucleosome (45), is required for cell growth in S. cerevisiae (46). It has been reported that the regions of amino acid residues 1–30 and 50–70 of H3 histone are exposed at the surface of the nucleosome in solution, which was determined by binding of site-specific antibodies of H3 histone to the nucleosome and the chromatin (47). The interaction between MCM2 protein and histone H3 is strong and specific, but the physiological significance of the interaction remains to be clarified. This interaction may be involved in the chromatin binding of MCM protein complexes before the initiation of DNA replication or in a structural change of the nucleosome at the initiation of DNA replication; alternatively, it may be required for the chromatin assembly process during DNA replication.

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