Cell Cycle- and Chromatin Binding State-dependent Phosphorylation of Human MCM Heterohexameric Complexes

A ROLE FOR cdc2 KINASE*

(Received for publication, February 6, 1998, and in revised form, April 21, 1998)

Masatoshi Fujita‡§, Chieko Yamada‡, Tatsuya Tsurumi‡, Fumio Hanaoka§, Kaori Matsuzawa**,
and Masaki Inagaki‡

From the ‡Laboratories of Viral Oncology and §Biochemistry, Research Institute, Aichi Cancer Center, Chikusa-ku, Nagoya, 464, Japan, the **Institute for Molecular and Cellular Biology, Osaka University, Suita, Osaka 565, and the
**Institute of Immunological Science, Hokkaido University, Sapporo 060, Japan

The mammalian MCM protein family, presently with six members, exists in the nuclei in two forms, chromatin-bound and unbound. The former dissociates from chromatin with progression through the S phase. Recently, we have established a procedure to isolate chromatin-bound and unbound complexes containing all six human MCM (hMCM) proteins by immunoprecipitation. In the present study, we applied this procedure to HeLa cells synchronized in each of the G1, S, and G2/M phases and could detect hMCM heterohexameric complexes in all three. In addition, depending on the cell cycle and the state of chromatin association, hMCM2 and 4 in the complexes were found to variously change their phosphorylation states. Concentrating attention on G2/M phase hyperphosphorylation, we found hMCM2 and 4 in the complexes to be good substrates for cdc2/cyclin B in vitro. Furthermore, when cdc2 kinase was inactivated in temperature-sensitive mutant murine FT210 cells, the G2/M hyperphosphorylation of the murine MCM2 and MCM4 and release of the MCMs from chromatin in the G2 phase were severely impaired. Taken together, the data suggest that the six mammalian MCM proteins function and undergo cell cycle-dependent regulation as heterohexameric complexes and that phosphorylation of the complexes by cdc2 kinase may be one of mechanisms negatively regulating the MCM-complex-chromatin association.

The MCM protein family, presently with six members, was originally identified from its involvement in the initiation of DNA replication at autonomously replicating sequences in budding yeast (1–7) and later found to be conserved through eukaryotes (8–16). Although definite functions of the MCM proteins remain largely unknown, they have been implicated in the regulatory machinery allowing DNA to replicate only once during the S phase (17). Although MCM proteins share substantial homology, it is now assumed that the bound form is associated with prereplicative chromatin and released at the time of replication; the soluble form existing abundantly in G2 phase is considered inactive and no longer capable of binding to chromatin. At least in budding yeast, the chromatin regions to which MCM7 binds during the G1 phase contain the replication origins (24). However, details of the mode of MCM-chromatin binding remain unclear. In budding yeast and the Xenopus egg extract system, it has been shown that MCM-chromatin binding is regulated through multiple mechanisms, while MCM binds to chromatin depending on CDC6 and the origin recognition complex (24–27), where the binding is negatively regulated by both S phase and mitotic CDKs (24, 28–30). However, there is so far no direct evidence as to whether these regulators for MCM also function in the mammalian somatic cell cycle.

Whereas the MCM proteins share substantial homology, it has been suggested that each of the six is indispensable for DNA replication in budding yeast (2–7), Xenopus egg extract (31, 32), and mammalian somatic cells (19, 21, 33). On the other hand, physical interactions among MCM proteins have been found in budding yeast (34), Drosophila and Xenopus egg extract (13–15, 29, 31, 32, 35, 36), and murine and human cells (20, 37–41). Recently, we have established a procedure to isolate chromatin-bound and unbound complexes containing all six human MCM (hMCM) proteins by immunoprecipitation with anti-hMCM antibodies (41). However, it remained to be elucidated whether there was any change in the complex formation profile during the cell cycle. In the present study, we therefore applied the immunoprecipitation procedure to synchronized HeLa cells. The results indicate that the six hMCM members may exist as heterohexameric complexes throughout the cell cycle. In addition, it was also found that, depending on the cell cycle and the states of chromatin association, hMCM4 and 2 proteins in the complexes variously change their phosphorylation states. Furthermore, we could show that cdc2 kinase plays a role in the G2/M phase hyperphosphorylation of mammalian MCM2 and 4 and in regulation of the MCM-chromatin association.

EXPERIMENTAL PROCEDURES

Cell Culture and Synchronization—HeLa cells were grown in Dulbecco’s modified Eagle’s medium with 5% fetal calf serum. The cells were synchronized at G2/M phase by treatment with 50 ng/ml nocodazole for 16–18 h. Cells arrested in early S phase were obtained by 2.5 mM hydroxyurea or 15 µM aphidicolin treatment for 18–20 h. G0 HeLa cells were synchronized by treatment with 100 µM mimosine for 6 h and released into fresh medium without mimosine for 14 h. Cells were harvested at the G1/S phase.

** The abbreviations used are: hMCM, human MCM protein; PAGE, polyacrylamide gel electrophoresis; TX-100, modified CSK buffer containing Triton X-100.

* This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Laboratory of Viral Oncology, Research Institute, Aichi Cancer Center, Chikusa-ku, Nagoya 464, Japan. Tel.: 81-52-762-6111; Fax: 81-52-763-5233; E-mail: mfujiita@uichigw.aichi.cc.pref.aichi.jp.

This paper is available online at http://www.jbc.org
cells were obtained as follows. Cells in the log phase were first arrested in early S phase by aphidicolin treatment and then released. After 10–12 h, mitotic cells were collected by mitotic shake-off, replated, and cultured for a further 4–6 h.

FM3A and FT210 cells (42) were maintained at 32 °C in RPMI 1640 buffered with 25 mM Hepes and supplemented with 10% fetal calf serum. For synchronization experiments, the cells (5 × 10^6/ml) were first arrested in early S phase by 15 μM aphidicolin treatment for 16 h at 32 °C and then released to progress through S phase in the presence of 50 ng/ml nocodazole, either at 32 or 39 °C. Triton X-100-soluble fractions and chromatin-bound fractions were processed as in panel A. C, G2/M phase HeLa cells were obtained by treatment with nocodazole for 16–18 h. Triton X-100-soluble fractions were processed as in panel A.

**Immunoprecipitation**—Immunoprecipitation with anti-hMCM7 (hCDC47) or anti-murine MCM3 (mMCM3) antibodies was performed as described previously (41). The immunoprecipitates were boiled in SDS-sample buffer and subjected to 10% (acrylamide 10, bis-acrylamide 0.1) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining or immunoblotting.

**Immunoblotting**—Immunoblotting was performed as described previously (22, 41). The protein blots were incubated with appropriate first antibodies for 1 h at room temperature; purified anti-hMCM7 antibodies with 1 μg/ml (22); rabbit anti-mMCM4 antiserum (20) at 1:500 dilution; rabbit anti-Xenopus MCM2 (XMCM2) antiserum (43) at 1:500 dilution; and anti-edc2-phosphorylated vimentin mouse monoclonal antibody (44; Ref. 44) at 1:3000 dilution of the culture supernatant. The blots were then probed with peroxidase-labeled goat anti-rabbit IgG or anti-mouse IgG antibodies (Zymed) and visualized using the ECL system (Amersham Pharmacia Biotech).

**Phosphorylation**—Orthophosphate or cdc2 Kinase Treatment of the Immunoprecipitates—Immunoprecipitates were resuspended in 25 μl of phosphorylation buffer (50 mM Tris-Cl, pH 7.8, 5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) or kinase buffer (50 mM Tris-Cl, pH 7.5, 10 mM MgCl2, 1 mM EGTA, 1 mM dithiothreitol, 100 μM ATP, 1 mM cAMP, 0.5 mM phenethylsulfonyl fluoride) and incubated with 80 units of λ-phosphatase (New England Biolabs) for 30 min at 30 °C or 50 units of cdc2/cyclin B (New England Biolabs) for 60 min at 30 °C. The reactions were stopped by the addition of 2× SDS-sample buffer and processed for SDS-PAGE.

**Immunoblotting of Cyclin B and α-Tubulin**—Immunoblotting of cyclin B and α-tubulin was performed as described previously (41).
tase inhibitors and subjected to immunoprecipitation with anti-hMCM7 or anti-mMCM3 antibodies. In these experiments, the washing buffer was changed to 200 mM NaCl TET buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% Triton X-100, 200 mM NaCl) containing phosphatase inhibitors. \(^{32}P\) radiography was performed with a Bio-Imaging analyzer BAS2000 (Fuji, Japan).

**RESULTS**

**Six hMCM Members Participate in Heterohexameric Complexes throughout the Cell Cycle, whether Bound to Chromatin or Not**—Triton X-100-extractable fractions prepared from HeLa cells synchronized in G1, S, and G2/M phase, and DNase I-released fractions from G1 and S phase cells were subjected to immunoprecipitation with anti-hMCM7 antibodies, and the precipitates were subjected to SDS-PAGE followed by silver staining (Fig. 1) and to Western blotting with antibodies against each of the hMCMs (data not shown except for hMCM2 and 4 in Fig. 2; see also Ref 41). All six hMCM proteins were detectable in the precipitates of all the fractions tested. On the other hand, the hMCM2 and 4 proteins variously changed their electrophoretic mobility depending on the cell cycle and the state of chromatin association. Because such changes in the mobility could be due to phosphorylation, we treated the immunoprecipitates with \(\lambda\)-phosphatase, by which hMCM2 and 4 showing aberrant mobility disappeared and the levels of p125 hMCM2 and p97 hMCM4 concomitantly increased. Mobility of the other hMCM proteins was not changed, and treatment of the precipitates with phosphatase buffer alone did not alter the mobility of any hMCM proteins (data not shown). As we reported previously (22, 41), hMCM7 resolved into a doublet on SDS-PAGE, by an unknown mechanism. The results after \(\lambda\)-phosphatase treatment demonstrated that in all the fractions tested, anti-hMCM7 antibodies co-precipitated the other hMCMs with hMCM7 with roughly equivalent stoichiometry (Fig. 1). Therefore, we suggest that the six hMCM members may exist as heterohexameric complexes throughout cell cycle, whether bound to chromatin or not.

**hMCM2 and 4 Proteins in the Complexes Undergo Complicated Phosphorylation Depending on the Cell Cycle and the State of Chromatin Association**—As described above, the hMCM4 protein in the complexes was found to variously change its electrophoretic mobility depending on the cell cycle and the state of chromatin association (Figs. 1 and 2A). In the G1 cells, most of the chromatin-bound hMCM4 migrated to the same position as that of dephosphorylated hMCM4, whereas most of the soluble hMCM4 displayed slower mobility. In the S phase cells, although the migration pattern of the soluble hMCM4 was unchanged compared with that in G1 phase cells, the migration pattern of the chromatin-bound hMCM4 was obviously changed; the forms showing slower mobility were increased. In the G2/M phase cells, most of the hMCM4 displayed the slowest mobility (apparent molecular mass was \(\sim 115 \text{ kDa}\)). All of these extra bands disappeared with \(\lambda\)-phosphatase treatment. Although an unchanged mobility pattern does not always mean an unchanged phosphorylation state, these data suggest the following for the phosphorylation of hMCM4 in hMCM complexes: (1) in the G1 phase, most of the chromatin-bound hMCM4 was in the underphosphorylated form while most soluble hMCM4 is in the more phosphorylated form; (2) during progression from G1 to S phase, although the phosphorylation state of the soluble hMCM4 is apparently unchanged, the chromatin-bound form becomes more extensively phosphorylated; and (3) most of the hMCM4 in G2/M phase is in the hyperphosphorylated form. The buffer used for these immunoprecipitation studies contained ATP to facilitate chromatin-complex binding and complex stability, and the chromatin-bound complexes were solu-
bibilized by incubation with DNase I at 25 °C (41). Therefore, it is possible that the observed phosphorylation pattern of hMCM4 is modified in vitro. We therefore repeated the experiments in the presence of phosphate inhibitors and a kinase inhibitor and in the absence of ATP, and we solubilized the bound complexes by salt extraction on ice. Although the hMCM heterohexameric complexes are partially disrupted under such conditions (41), the same results for hMCM4 phosphorylation were obtained (data not shown).

As previously reported (21), change in mobility because of phosphorylation during the cell cycle was also observed for hMCM2 in the complexes. In this case, phosphorylated forms displayed faster mobility on SDS-PAGE (Figs. 1 and 2). The changing pattern of the hMCM2 phosphorylation appeared to be essentially similar to that of hMCM4.

Identification of cdc2/cyclin B as a Regulator of Mammalian MCM2 and 4 Hyperphosphorylation in G2/M Phase—As an initial step to analyze complex phosphorylation, we focused on the G2/M phase hyperphosphorylation of the MCM2 and 4 proteins, leading to their remarkable mobility shift. One possible candidate for the responsible kinase(s) is cdc2/cyclin B, which is maximally active in late G2/M phase. As shown in Fig. 3, it was found that recombinant cdc2/cyclin B phosphorylated in vitro the hMCM2 and hMCM4 in the hMCM complexes immunoprecipitated from S phase HeLa cells and promoted a shift in their mobility. The positions of the shifted bands were almost the same as those of hMCM2 and 4 in the G2/M phase (see also Figs. 1C and 2). Mobility of the other hMCMs was not changed by the treatment. These data show that hMCM2 and hMCM4 proteins in the hMCM complexes are excellent substrates for cdc2/cyclin B in vitro.

We examined the in vivo role of cdc2/cyclin B in G2/M hyperphosphorylation of mammalian MCM2 and 4 using murine cdc2 kinase temperature-sensitive mutant FT210 cells and their parental FM3A cells (42, 45–47). Cells were arrested at early S phase by aphidicolin, and then released at a permissive (32 °C) or a nonpermissive temperature (39 °C) in the presence of nocodazole to prevent cells entering the next G1 phase. Analysis of the DNA content and the mitotic index showed that there were no obvious differences in cell cycle progression among FM3A cells, and FT210 cells cultured at a permissive temperature; approximately 70–80% of the cells were arrested in early S phase, 4 h after release, they were in mid S phase; at 8 h, they were in late S/G2 phase; and at 12 h, they were arrested in M phase (Fig. 4). However, when FT210 cells were cultured at a nonpermissive temperature, although they similarly reached late S/G2 phase 8 h after release, even at 12 h there were few mitotic cells (Fig. 4), in agreement with the previously reported G2-arrest phenotype (45–47).

Total cell lysates, Triton X-100-extractable fractions, and nuclear pellets were prepared from these cells. First, the total cell lysates were immunoblotted with monoclonal antibody 4A4 specific for cdc2-phosphorylated vimentin (44) (Fig. 5A). The data showed cdc2 activation to occur around the G2 phase (8 h after release) and become maximum in the M phase (12 h after release) in FM3A cells, and FT210 cells cultured at a permissive temperature. However, in FT210 cells cultured at a nonpermissive temperature, virtually no activation of cdc2 was detected, as expected. Then the Triton X-100-extractable and the nuclear fractions were immunoblotted with anti-mMCM4 antibodies (Fig. 5B). In addition to the main band of mMCM4, the antibodies also recognized two bands showing different mobility in FM3A cells, and FT210 cells cultured at a permissive temperature; one was observed in the chromatin-bound form in the S phase cells and displayed slower mobility, and the other was observed in the soluble form in the G2/M phase cells and displayed the slowest mobility. Both of these two bands disappeared with phosphatase treatment, and the former was undetectable in G1 phase cells (data not shown). These data show that mMCM4 also has at least two counterparts of the isoforms because of phosphorylation observed for hMCM4, the S phase- and chromatin-bound form-specific phosphorylated form and the hyperphosphorylated form in the G2/M phase. Interestingly, the latter became almost completely undetectable when FT210 cells were cultured at a nonpermissive temperature (Fig. 5B). Similarly, immunoblot analysis with anti-XMCM2 antibodies demonstrated the presence of hyperphosphorylated mMCM2 showing fastest mobility in the G2/M phase cells and its disappearance in cdc2-inactivated FT210 cells (Fig. 5C).

We further examined the phosphorylation of the mMCM proteins in G2/M phase FT210 cells by metabolic labeling with orthophosphate. Cells arrested in early S phase were released in phosphate-free medium supplemented with nocodazole either at a permissive or a nonpermissive temperature. After 8 h, [32P]orthophosphate was added and the cells were labeled for a further 2 h. Even in the absence of phosphate, the cells reached...
G2 phase 8 h after the release as usual (data not shown). The Triton X-100-extractable fractions prepared from the labeled cells were subjected to immunoprecipitation with anti-hMCM7 or anti-mMCM3 antibodies, and the immunoprecipitates were analyzed by SDS-PAGE followed by silver staining, radiography or immunoblotting. In these experiments, the washing buffer was changed to one containing 200 mM NaCl. Under such conditions, the hMCM heterohexameric complexes are partially disrupted (41), making it easier to detect each mMCM protein on SDS-PAGE; namely, anti-hMCM7 antibodies precipitated mMCM7, 6, 4, and 2, whereas anti-mMCM3 did mMCM3 and 5 (Fig. 6, A and B). In the precipitates from G2/M FT210 cells cultured at a permissive temperature, mMCM2 showing the fastest mobility and predominantly labeled by 32P was detected (Fig. 6A). However, it was remarkably decreased at a nonpermissive temperature (Fig. 6A). However, the slow migrating mMCM2 was still labeled by 32P at a nonpermissive temperature (Fig. 6A). Western blot analysis clearly showed the presence of hyperphosphorylated mMCM4 showing the slowest mobility at a permissive temperature and its disappearance at a nonpermissive temperature, although its detection by silver staining or radiography was difficult because of its overlapping with mMCM6 (Fig. 6A). Similar to the situation for mMCM2, mMCM4 was also labeled by 32P even at a nonpermissive temperature (Fig. 6A). mMCM3 and 6 were also labeled by 32P at a permissive temperature, and seemingly, the rate of the phosphorylation was not changed at a nonpermissive temperature (Fig. 6, A and B). However, further analyses are required to establish conclusively whether cdc2/cyclin B phosphorylates mMCM3 or 6 in vivo or not. For mMCM5 and 7, virtually no incorporation of 32P was found (Fig. 6, A and B). These in vivo data with FT210 cells, together with the above described in vitro data, indicate that cdc2/cyclin B plays an indispensable role in G2/M hyperphosphorylation of mammalian MCM4 and MCM2 proteins. On the other hand, the data from the labeling experiments suggest that mMCM2, 3, 4, and 6 are also phosphorylated at G2/M phase by kinase(s) other than cdc2.

A Role for cdc2/cyclin B in Regulation of the Mammalian MCM Protein-Chromatin Association—Using this system, we also examined possible roles of cdc2 kinase in regulation of mammalian MCM-chromatin associations. As expected from previous observations (19–23), Western blot analysis showed that the levels of the chromatin-bound mMCM4 and 7 proteins in the nuclei were decreased during the S phase, becoming minimal in the G2 phase (8 h after release) in FM3A and FT210 cells cultured at a permissive temperature (Fig. 5B). The decrease in the level of the chromatin-bound mMCMs in G2 was also observed in FM3A cells cultured at 39 °C. However, reassociation of the mMCMs to the nuclei was observed in the M phase (12 h after release) in these cells (Fig. 5B). The reason for this reassociation is currently unknown. This unexpected behavior of mMCMs at 39 °C made it difficult to estimate the effect of cdc2 inactivation on mMCM-chromatin association. Nevertheless, the levels of the chromatin-bound mMCMs in G2 phase FT210 cells (8 h after release) cultured at a nonpermissive temperature decreased to a lesser extent than in FM3A cells (Fig. 5B), suggesting that cdc2 kinase plays a role in negatively regulating the mMCM-chromatin association.
DISCUSSION

hMCM Complex Formation and Its Complicated Phosphorylation during the Cell Cycle—Our recent report concerning human cells (41) and two regarding Xenopus egg extracts (31, 32) have provided evidence of the existence of complexes containing all six MCMs. Especially, we have also shown by chromatin immunoprecipitation that chromatin-bound hMCM heterohexameric complexes are found (41). These findings are in agreement with the demonstration that hMCM resides in 500–600 kDa complexes (38–40). However, the problem of whether there is any change in the complex composition during the cell cycle remained. In this regard, native gel electrophoresis analyses of 500–600 kDa MCM complexes in Xenopus and Drosophila eggs showed that they are stably present throughout the cell cycle, although their exact composition was not examined (29, 36). Our present results suggest that the six hMCM members may exist as hexameric complexes throughout the cell cycle, whether bound to chromatin or not. It seems likely that they function and undergo cell cycle-regulation as heterohexameric complexes. The finding that all six XMCM proteins bind to chromatin and are displaced synchronously in a Xenopus egg system (31, 32) is also consistent with this notion. Very recently, it has been reported that hMCM4/6/7 complexes have helicase and ATPase activity, which is inhibited by hMCM2 (48). It remains possible, therefore, that more complicated regulation of the complex composition occurs in specific situations.

Our results also show that the hMCM2 and 4 proteins in the complexes variously change their phosphorylation state depending on the cell cycle and the condition of chromatin association. Therefore, changes in phosphorylation of the hMCM complexes may not induce remarkable rearrangement of the composition, although complex stability seems to differ between bound and unbound forms (41). Hyperphosphorylation of hMCM2 and 4 in the mitotic phase has already been reported (21, 39) and confirmed here. In addition, the present study suggested that, in the G1 phase, unbound hMCM2 and 4 are more phosphorylated than chromatin-bound forms and that, during progression from G1 to S phase, bound forms become phosphorylated. Although investigation of phosphorylation of the other mammalian MCMs during the cell cycle other than in the G2/M phase was not performed in this study, cell cycle-dependent regulation of phosphorylation of mMCM3 has been reported previously; unbound mMCM3 is more phosphorylated than the bound form, and the phosphorylation rate increases during G1-S progression (19). In the present study, mMCM3 was shown to be phosphorylated also in G2/M phase. Mammalian MCM5 (19) and MCM7 in asynchronous cells are not remarkably labeled in vivo by orthophosphate. This was also the case in G2/M cells.

A Role for cdc2/cyclin B in G2/M Hyperphosphorylation of Mammalian MCM2 and 4 and in Regulation of Mammalian MCM Complex-Chromatin Association—Our finding that the G2/M phase hyperphosphorylation of mammalian MCM4 is directed by cdc2/cyclin B is consistent with previous results. hMCM4 and mMCM4 proteins have a cluster of potential target sites for CDK in the N terminus (37, 39), which is conserved through eukaryotes (29, 49). Furthermore, in a Xenopus egg system, XCMC4 was suggested to be hyperphosphorylated by cdc2 kinase in the M phase (29, 49). In addition, the present data also suggest that mammalian MCM2 in MCM complexes is another target of cdc2/cyclin B. It has so far not been clarified whether XMCM2 could be a target of phosphorylation by cdc2 kinase in Xenopus egg extracts, although it has been shown that XMCM3 and 5 are not phosphorylated by cdc2 in vitro (29).

The findings obtained with FT210 cells also indicate a role for cdc2 in negative regulation of mammalian MCM-chromatin interaction although we have no direct data as to whether phosphorylation of the MCM complexes by cdc2/cyclin B per se is required for the regulation. The findings are consistent with

\[ \text{M. Fujita and M. Ishibashi, unpublished observations.} \]
results obtained with a Xenopus egg extract system and budding yeast (24, 29). In vitro cdc2/cyclin B treatment of chromatin isolated from the egg extract system induces dissociation of XMCM proteins from chromatin (29). Very recently, it was shown in budding yeast that suppression of mitotic CDK activity by a CDK inhibitor leads to reloading of MCM7 to chromatin fragments containing the origin regions (24). Our data, therefore, demonstrate that cdc2 is an important kinase for the regulation of MCM-chromatin association also in the mammalian somatic cell cycle. The function of cdc2/cyclin B to prohibit rereplication (50, 51) may be achieved partly via its inhibition of MCM-chromatin interaction. This may also be the case in mammalian cells.

Whereas cdc2 kinase is strongly implicated in G2/M hyperphosphorylation of mammalian MCM2 and 4 and in regulation of mammalian MCM complex-chromatin association, our data also suggest that mMCM2, 3, 4, and 6 are phosphorylated at G2/M phase by kinase(s) other than cdc2. However, the biological roles and the responsible kinase(s) are currently unknown.

**S Phase Phosphorylation of Mammalian MCM2 and 4**—Phosphorylation of the chromatin-bound hMCM2 and 4 during the S phase also seems intriguing. The observations are reminiscent of the Xenopus egg system finding that nuclear XMC4 is converted to a partially phosphorylated form in early S phase (49). However, in contrast to the Xenopus system where most nuclear XMC4 is phosphorylated (49), in human cells, only a part of the bound hMCM2 and 4 appeared phosphorylated during the S phase. This might be attributable to differences in the temporal pattern of firing of replication origins between the two; i.e., whereas origins initiate synchronously in the Xenopus embryonic cells, they fire in a temporally staggered fashion in the mammalian somatic cells. S phase phosphorylation might occur only in hMCM complexes associated with chromatin regions that are in the process of being fired. As the S phase phosphorylation of XMC4 seems independent on cdc2 kinase (49), that of MCM4 in mammalian somatic cells may be irrelevant to cdc2 activity. Also, the S phase phosphorylation of hMCM2 and 4 proteins per se does not seem to result in dissociation of the complexes from chromatin, as is the case in the Xenopus egg system (49). Whatever the exact regulation of the phosphorylation of the mammalian MCM complexes during the cell cycle, the complicated regulation and its effects on MCM function will require extensive study for complete understanding.

**Acknowledgments**—We thank Dr. A. Matsukage for helpful discussion and critical reading of the manuscript; Dr. H. Kimura for anti-MCM2 antibodies; Dr. S. Miyake for anti-XMCM2 antibodies; and T. Yoshida and Y. Matsumura for technical assistance.
Cell Cycle- and Chromatin Binding State-dependent Phosphorylation of Human MCM Heterohexameric Complexes: A ROLE FOR cdc2 KINASE
Masatoshi Fujita, Chieko Yamada, Tatsuya Tsurumi, Fumio Hanaoka, Kaori Matsuzawa and Masaki Inagaki

J. Biol. Chem. 1998, 273:17095-17101.
doi: 10.1074/jbc.273.27.17095

Access the most updated version of this article at http://www.jbc.org/content/273/27/17095

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 25 of which can be accessed free at http://www.jbc.org/content/273/27/17095.full.html#ref-list-1