24

Binding of Human MCM-BP with MCM2-7 Proteins

Ryoko Kon[1], Shunsuke Kusunoki[1], Naohito Nozaki[2] and Yukio Ishimi[1]
[1]*Ibaraki University, Mito, Ibaraki 351-8511,*
[2]*Kanagawa Dental College, Yokosuka, Kanagawa 238-8580*
Japan

1. Introduction

MCM2-7 proteins play essential roles in DNA replication in eukaryotic cells, probably by acting as a replicative DNA helicase that unwinds DNA duplexes at replication forks (Bell & Dutta, 2002; Forsburg, 2004; Masai et al., 2010). Several lines of evidences suggest that MCM2-7 hexameric complexes assembled on the replication origins are converted to an active form with the assistance of the CDC7, CDC45 and GINS complex (Moyer et al., 2006; Gambus et al., 2006). MCM-BP, which has been identified from human cells as a protein that binds to MCM6 and MCM7 proteins, has amino acids sequences homologous to MCM2-7 (Sakwe et al. 2007). The results in this report indicate that MCM-BP replaces MCM2 in MCM2-7 complex and it binds to a replication origin in HeLa cells, suggesting that the MCM complex containing the MCM-BP may play a role in the initiation of DNA replication. It has also been indicated that down-regulation of MCM-BP affects chromatin binding of MCM4. Recently it has been reported that *Arabidopsis thaliana* ETG1, which has been identified as an E2F target gene, is a homolog of MCM-BP (Takahashi et al., 2008). ETG1 protein is required for efficient DNA replication. Depletion of ETG1 results in inhibition of DNA replication and G2 arrest. Under these conditions, the G2 checkpoint system is induced. The report by Takahashi et al. (2010) indicates that ETG1 is involved in sister chromatid cohesion which is required for post-replicative homologous recombination repair. More recently, it has been reported that *Xenopus* MCM-BP regulates unloading of the MCM2-7 complex from chromatin in the late S phase by interacting with MCM7 (Nishiyama et al., 2011). These evidences suggest a possibility that MCM-BP may interact with the MCM2-7 complex at the replication forks to regulate the chromatin binding of the complex. Such interaction may be required for establishment of the cohesin complex at the forks.

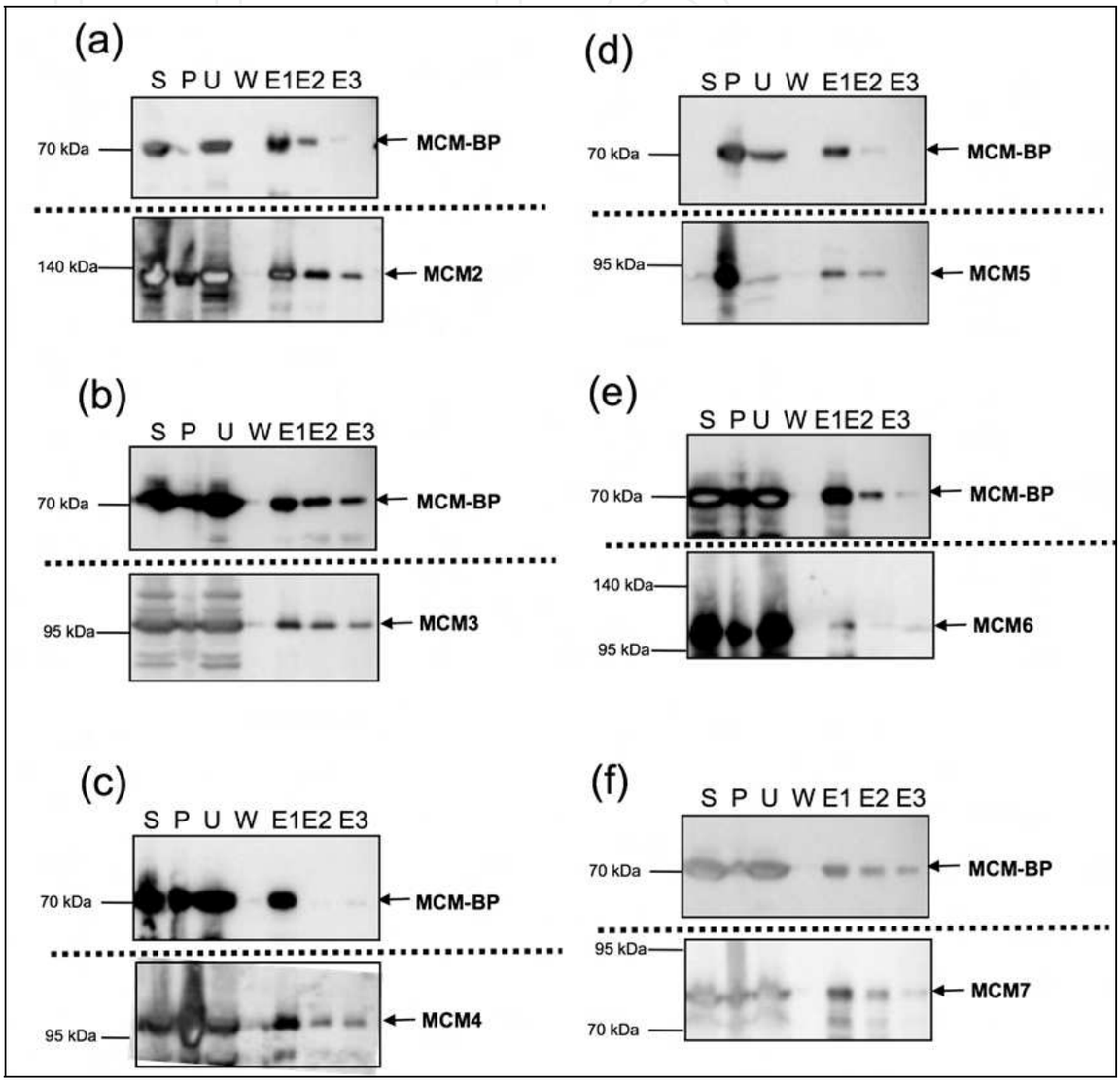
Here we examined biochemical properties of human MCM-BP. First, we found that human MCM-BP can bind all the human MCM2-7 proteins when the MCM-BP and one of the MCM2-7 proteins are co-expressed in insect cells. However, the interaction of MCM-BP with MCM7 was mainly detected when all the MCM2-7 and MCM-BP were co-expressed at the same time. In HeLa cells, MCM-BP was mainly recovered in a Triton-soluble fraction, suggesting that it does not stably bind to chromatin. A small portion of MCM-BP in this fraction was bound to MCM4, MCM5, MCM6 and MCM7 proteins. These results suggest that MCM-BP is not a constituent of pre-RC and it exhibits its functions by interacting with

MCM7. The results are not inconsistent with the notion that MCM-BP may play a role in the dynamics of MCM complex at the forks.

2.1 Binding of human MCM-BP with MCM2-7

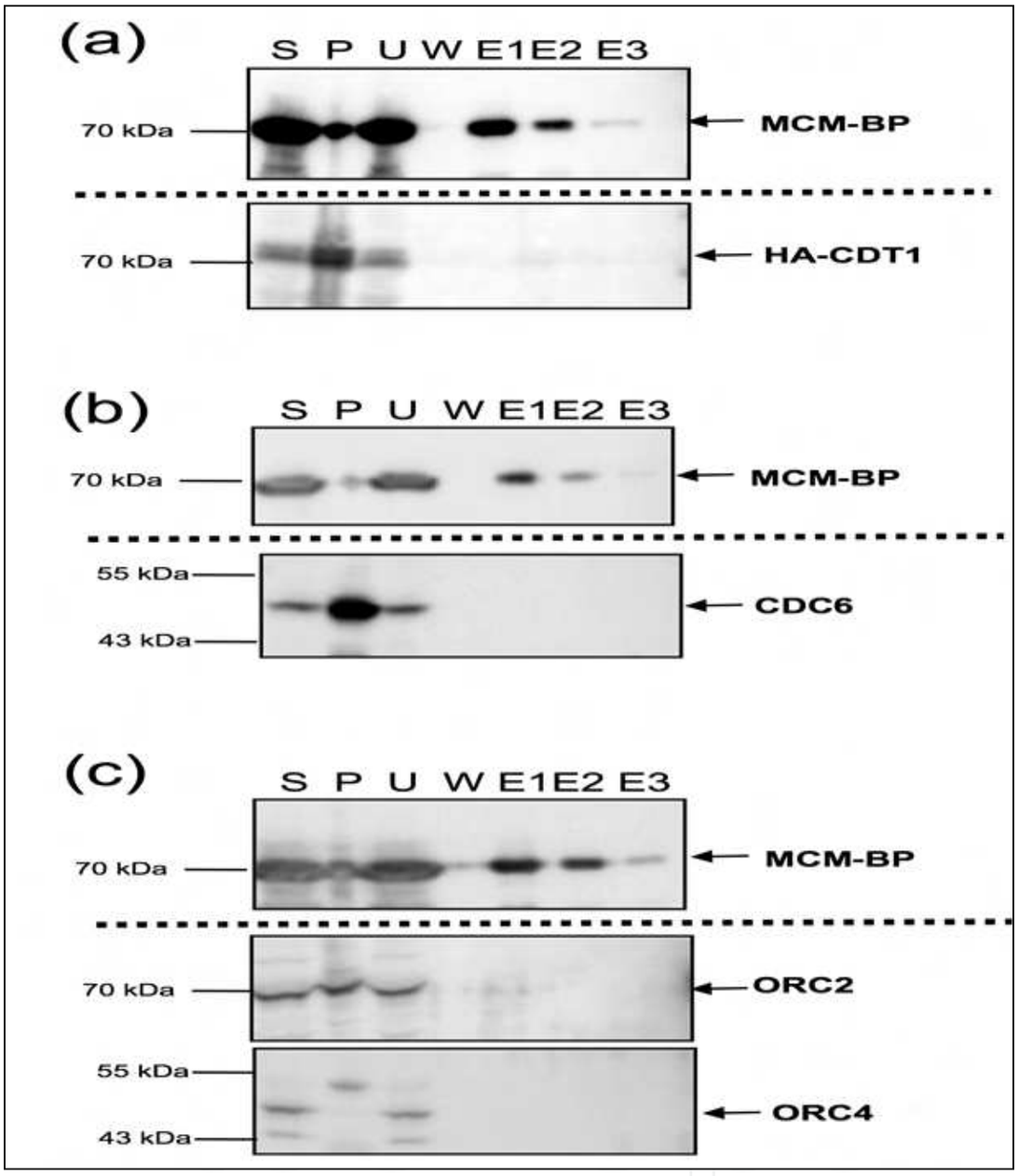
The human MCM-BP gene was synthesized from cDNA of HeLa cells by the RT-PCR method. The sequencing of the cloned gene indicates that an internal deletion of six nucleotides is present in comparison to the gene in Genebank (NM_002388). Thus two amino acids of Cys and Lys at amino acid no. 334 and 335 are deleted. This deleted portion is localized near the center of the protein where the Walker A and B motifs are located at amino acid no. 396-407 and 457-465, respectively. To examine the interaction of the MCM-BP and MCM2-7 proteins, Flag-tagged MCM-BP and one of MCM2-7 were co-expressed in High5 cells and the cell lysate was immuno-precipiated with anti-Flag antibody. We examined whether co-expressed MCM2-7 is co-precipiated with Flag-MCM-BP or not (Fig. 1). In all combinations, precipitation of expressed MCM-BP was confirmed, and all the co-expressed MCM2-7 proteins were co-precipitated. Since the amounts of MCM2-7 proteins present in the Triton-soluble fraction different, it is difficult to compare the strength of their interaction with MCM-BP in this system. However, the results suggest that the interaction between the MCM-BP and MCM2 is as strong as the interactions between the MCM-BP and MCM3-7 proteins. The interactions of MCM-BP with CDT1 and CDC6, which are MCM2-7 loaders, and with ORC2 and ORC4 were examined by using the same experimental system (Fig. 2). Only faint bands of CDT1 and ORC2 were detected in the fractions precipitated with MCM-BP, but co-precipitation with CDC6 and ORC4 were not detected. These results indicate that MCM-BP can specifically and directly interact with the MCM2-7 proteins. These results appear to be in contrast to the interaction of MCM7 with MCM2-6 proteins in that MCM7 mainly interacts with the MCM3 and MCM4 proteins (Numata et al., 2010). Such interaction of MCM7 is consistent with the placement of MCM2-7 proteins in the heterohexameric MCM2-7 complex (Yu et al., 2004).

To further examine the interaction of MCM-BP with the MCM2-7 proteins, all MCM2-7 proteins were co-expressed with MCM-BP in insect cells, and Flag-MCM-BP was precipitated with anti-Flag antibody. Proteins bound to the Sepharose beads were eluted with Flag peptide. In addition to MCM-BP, all the MCM2-7 proteins were detected in the eluted fractions, and the MCM7 protein was dominantly detected in silver-stained gel (Fig. 3). In glycerol gradient centrifugation of the eluted proteins, MCM-BP was mainly recovered in the fractions no. 7-13 (Fig. 4). Purified MCM-BP itself was recovered in fractions no. 9-13 (data not presented), suggesting a possibility that MCM-BP itself mainly forms a dimmer. MCM4/6/7 hexamer is recovered in fractions no. 3-6 under the same conditions. Both MCM-BP and MCM2-7 proteins were also detected in these fractions. Thus, it is assumed that MCM-BP may bind one to several molecules among the MCM2-7 proteins. Sedimentation profile supports the notion that MCM7 is mainly associated with MCM-BP. MCM-BP in the fraction no. 9 of the glycerol gradient was precipitated with anti-Flag antibody (Fig. 5). Although all the MCM2-7 proteins were hardly detected in unbound fractions, only MCM6 and MCM7 proteins were detected in elution fractions. Based on the finding that the direct interaction between MCM6 and MCM7 is not strong (Yu et al., 2004; Numata et al., 2010), all these results suggest that MCM-BP mainly interacts with MCM7 and also with MCM6.


(a)
S P U W E1E2 E3
70 kDa
MCM-BP
140 kDa
MCM2
(b)
S P U W E1E2 E3
70 kDa
MCM-BP
95 kDa
MCM3
(c)
S P U W E1E2 E3
70 kDa
MCM-BP
95 kDa
MCM4
(d)
S P U W E1E2 E3
70 kDa
MCM-BP
95 kDa
MCM5
(e)
S P U W E1E2 E3
70 kDa
MCM-BP
140 kDa
95 kDa
MCM6
(f)
S P U W E1 E2 E3
70 kDa
MCM-BP
95 kDa
70 kDa
MCM7

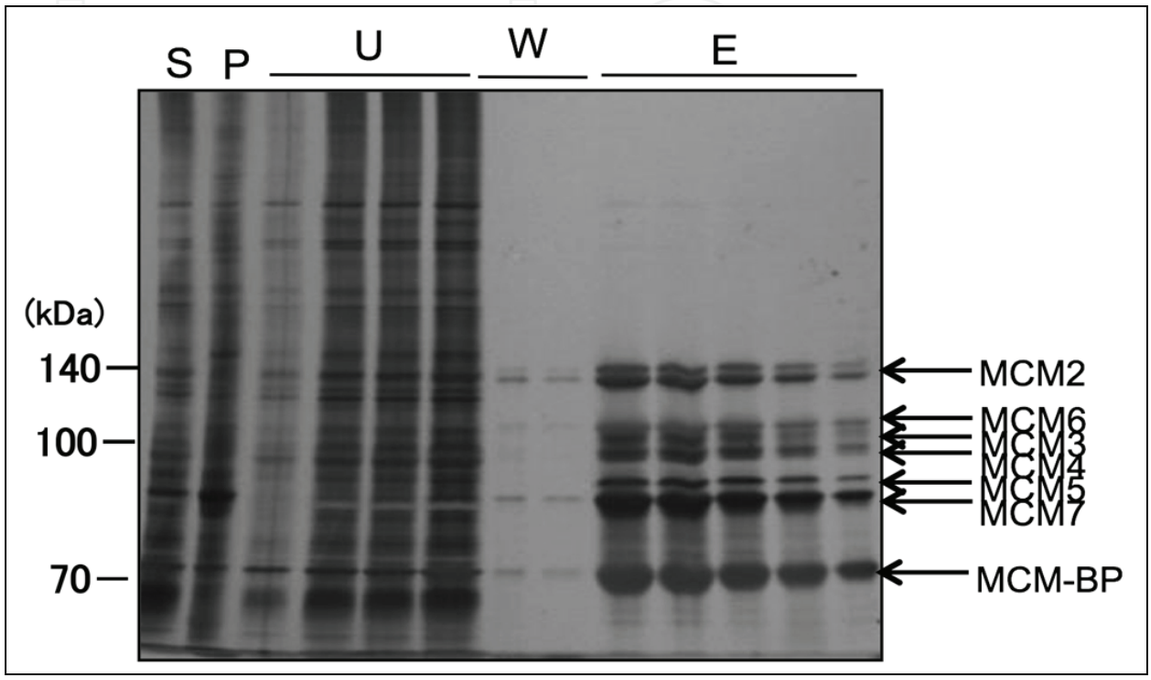
(a-f) High5 cells (7x10^6 cells) were co-infected with the viruses expressing the MCM-BP protein (0.25 ml of viral stock solution) and MCM2-protein (a) (0.25 ml of viral stock solution), MCM3 (b), MCM4 (c), MCM5 (d), MCM6 (e) or MCM7 (f) for 2 days. The cells were suspended in a 500 μl of lysis buffer consisting of 10 mM Tris-HCl (pH 7.5), 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM sodium phosphate buffer, 10 mM $Na_4P_2O_7$, and protease inhibitors (Pharmingen, BD, San Jose, CA). The mixture was incubated for 40 min on ice, and insoluble components were separated by centrifugation at 40,000 rpm (TLS55; Beckman, Fullerton, CA) for 40 min at 4°C. Supernatant of Triton-soluble (S) was recovered, and the precipitate was suspended with 100 μl of lysis buffer to obtain Triton-insoluble (P) fraction. The recovered supernatant (200 μl) was mixed for 1 h at 4°C with anti-Flag antibody (2.5 μg) and then protein G-Sepharose (20-30 μl) (Amersham Biosciences, Piscataway, NJ, USA) was added. The solution was mixed overnight at 4 °C. After spin, proteins unbound to the Sepharose beads were recovered (U). The beads were washed 10-12 times with 200 μl of phosphate-buffered saline (PBS) containing 0.1% Triton X-100, and supernatant after the final spin was recovered (W). The proteins bound to the beads were eluted three times with 20-30 μl of elution buffer (0.1 M glycine, pH 2.5 and 0.15 M NaCl) (E1, E2 and E3). These eluates were neutralized by adding 1/10 volume of 2 M Tris-HCl, pH 8.0. Proteins in the obtained fractions were separated by SDS-polyacrylamide gel electrophoresis. After the proteins in the gel were transferred to Immobilon-P transfer membrane (Millipore, Bedford, MA, USA), the membrane was incubated for 1 h at room temperature with a blocking buffer (EzBlock, ATTO, Tokyo, Japan) diluted by three-fold with TBS (50 mM Tris-HCl, pH7.9, 150 mM NaCl) plus 0.1% Triton X-100; it was then incubated overnight at 4 °C with 0.5-1 μg/ml of 1st antibodies in the diluted blocking buffer or culture supernatant of hybridoma cells producing anti-MCM-BP antibody (Nakaya et al., 2010). After washing the membrane with TBS containing Triton X-100, it was incubated for 2 h at 27°C with 2nd antibody conjugated with horseradish peroxidase (BioRad, Hercules, CA, USA). After washing, the membrane was incubated with SuperSignal West Pico Maximum Sensitivity Substrate (Pierce, Rockford, IL, USA), and chemiluminescent signals were detected by Light-Capture (ATTO). Anti-MCM2, -MCM3, and –MCM4 antibodies were prepared as reported (Nakaya et al., 2010). Anti-MCM5 (Santa Cruz, Bio, sc-165995), anti-MCM6 (Santa Cruz, Bio, sc-9843), anti-MCM7 (Santa Cruz Bio, sc-9966), and anti-Flag (Sigma, F-3165) antibodies were purchased. In the experiments (a-f), one filter was proved with anti-MCM-BP antibody (top) and the other was proved with anti-MCM2-7 antibodies (bottom). Due to over-loading, MCM2 bands in S and U fractions were not fully detected in (a). In (d), a band of MCM-BP in S fraction was hardly detected by unknown reason.

Fig. 1. Interaction of MCM-BP with MCM2-7 proteins



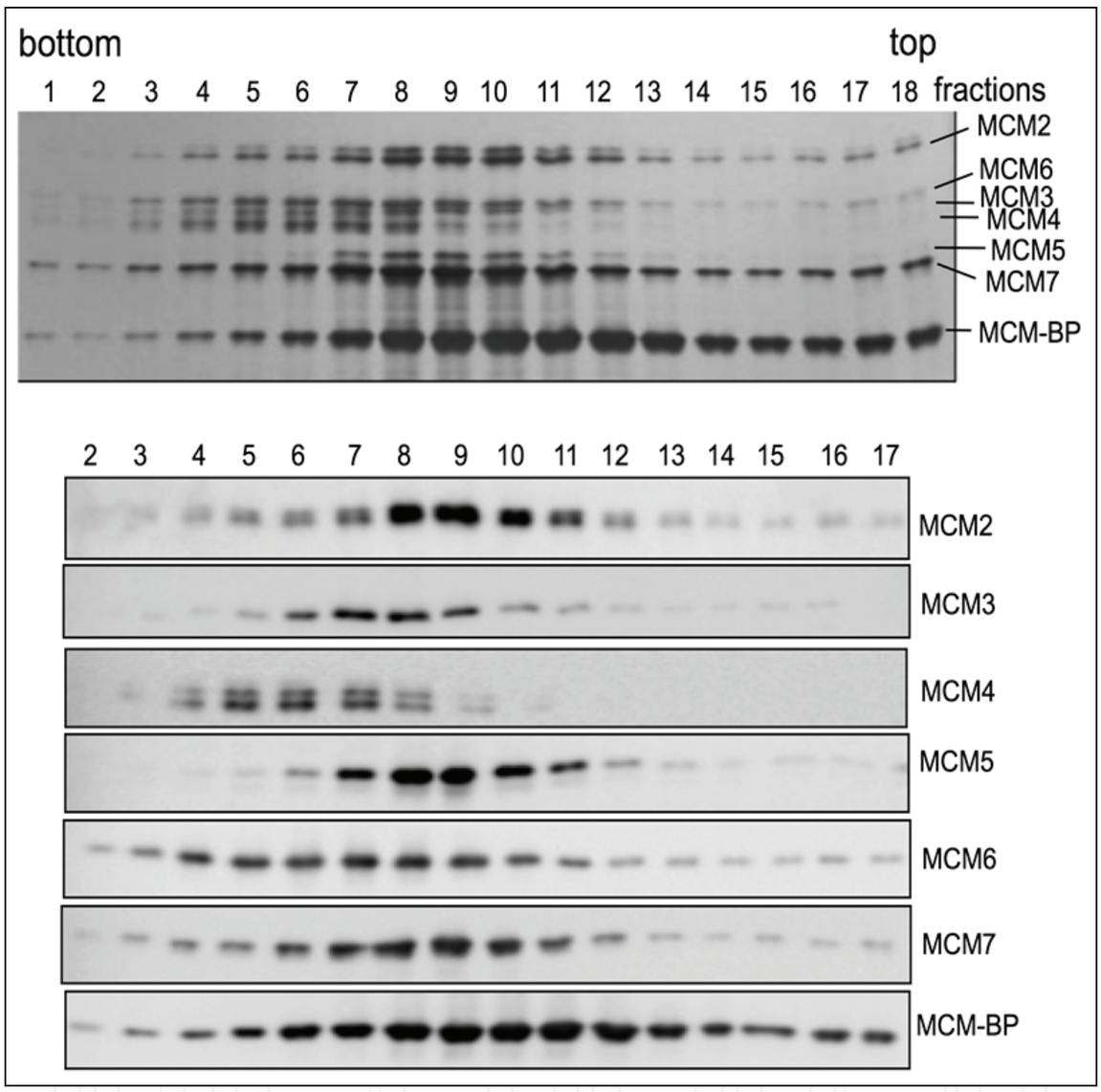
Human MCM-BP-Flag was co-expressed with human CDT1 (a), CDC6 (b) and ORC2 and 4 (c) in High5 cells and the cells were fractionated into Triton-soluble (S) and -insoluble (P) fractions. Proteins in the Triton-soluble fraction were immuno-precipitated with anti-Flag antibody. Unbound proteins (U) and those contained in the final wash (W) were recovered. In addition to the proteins eluted from the beads (E1, E2 and E3), proteins in other fractions were examined by immuno-blotting. In the experiments (a-c), one filter was proved with anti-MCM-BP antibody (top) and the other was proved with anti-HA(CDT1, Santa Cruz Bio. sc-7392), CDC6(Santa Cruz Bio. sc-8341), and ORC2, ORC4(Santa Cruz Bio. sc-20634) antibodies, (bottom).

Fig. 2. Interaction of MCM-BP with MCM loader proteins



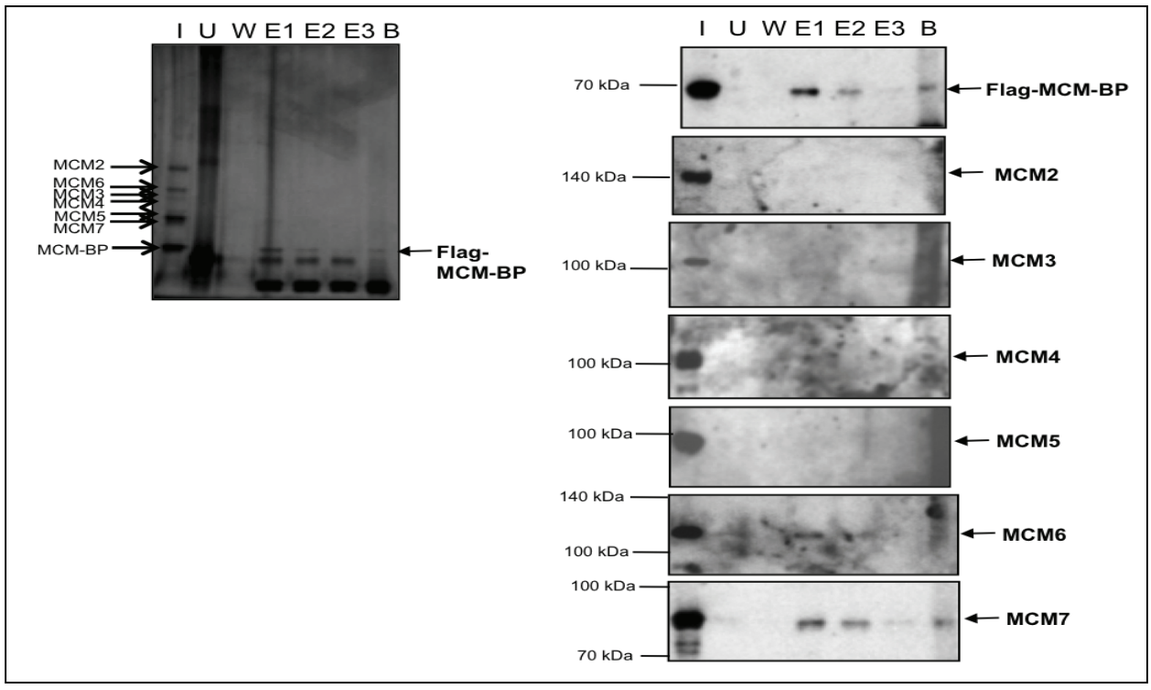
High5 cells (3x10^7 cells) were co-infected with recombinant baculoviruses producing Flag-MCM-BP (0.5 ml of stock solution), MCM2-MCM7(His) (0.5 ml), MCM3-MCM5(His) (1 ml) and MCM4(His)-MCM6 (0.5 ml). The infected cells (9x10^7 cell in total) were lysed with lysis buffer (4.5 ml) and fractionated into Triton-soluble (S) and -insoluble (P) fractions. Flag-MCM-BP in the Triton-soluble fraction was loaded onto a column of anti-Flag antibody beads (0.3 ml, Sigma, St. Louis, Missouri, USA)). Unbound proteins were collected (U). Supernatant after washing was recovered (W). Proteins bound to the beads were eluted by incubating with a buffer (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl) containing Flag peptide (50 μg/ml, Sigma) (E). Proteins in these fractions were electrophoresed and they were stained with silver. Bands corresponding to MCM2-7 and MCM-BP proteins are indicated at the right.

Fig. 3. Preparation of MCM-BP-binding MCM2-7 proteins



Proteins eluted from anti-Flag antibody column were fractionated by glycerol gradient centrifugation (Ishimi, 1997). Proteins were loaded onto a linear gradient of 15% to 30% glycerol and centrifuged at 36,000 rpm for 14 h in TLS55 rotor (Beckman). Aliquots of the obtained fractions (no. 1-18) were loaded on SDS-polyacrylamide gel. After electrophoresis, proteins were stained with silver (top). Positions of MCM2-7 and MCM-BP were indicated at the right. Proteins of MCM2-7 and MCM-BP in these fractions (no. 2-17) were detected by immuno-blotting using the specific antibodies (bottom). Two bands are detected for MCM4, which is probably due to degradation of the protein.

Fig. 4. Binding of MCM-BP with MCM2-7 proteins



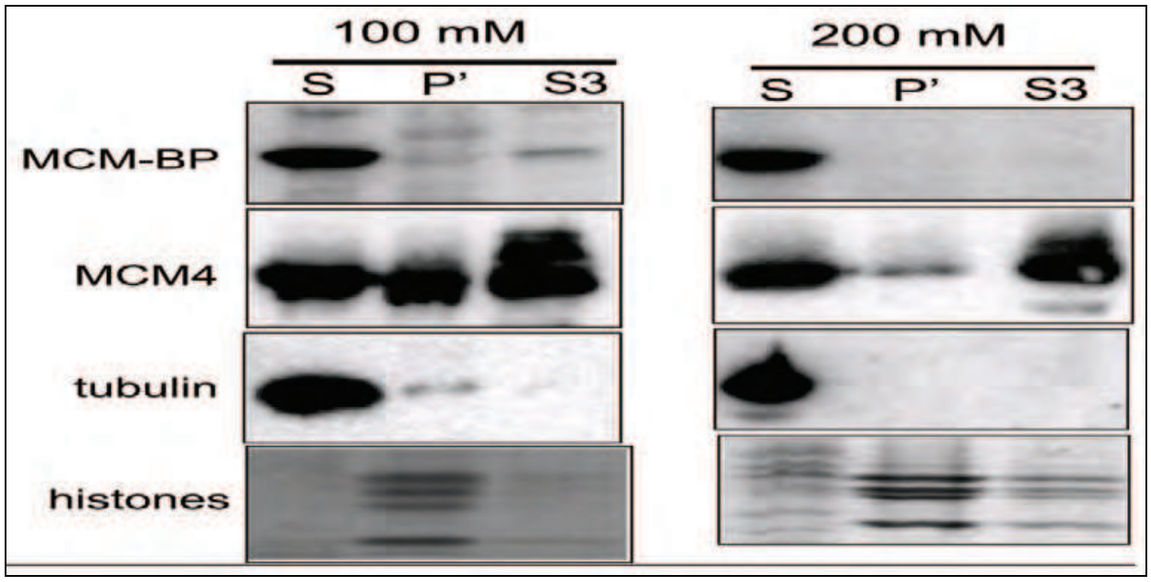
Proteins (I) in the fraction no. 9 in Fig. 4 were precipitated with anti-Flag antibody. After proteins unbound to the beads (U) and those in the supernatant of the final wash (W) were recovered, those bound to the beads were eluted three times by incubating with elution buffer (0.1 M glycine, pH 2.5 and 0.15 M NaCl) (E1, E2 and E3). Proteins remaining in the eluted beads were eluted by boiling in SDS-sample buffer for electrophoresis (B). Proteins in these fractions were electrophoresed and stained with silver (left), and MCM-BP and MCM2-7 were detected by immuno-blotting (right).

Fig. 5. Binding of MCM-BP with MCM6 and MCM7 proteins

2.2 Chromatin-binding of human MCM-BP

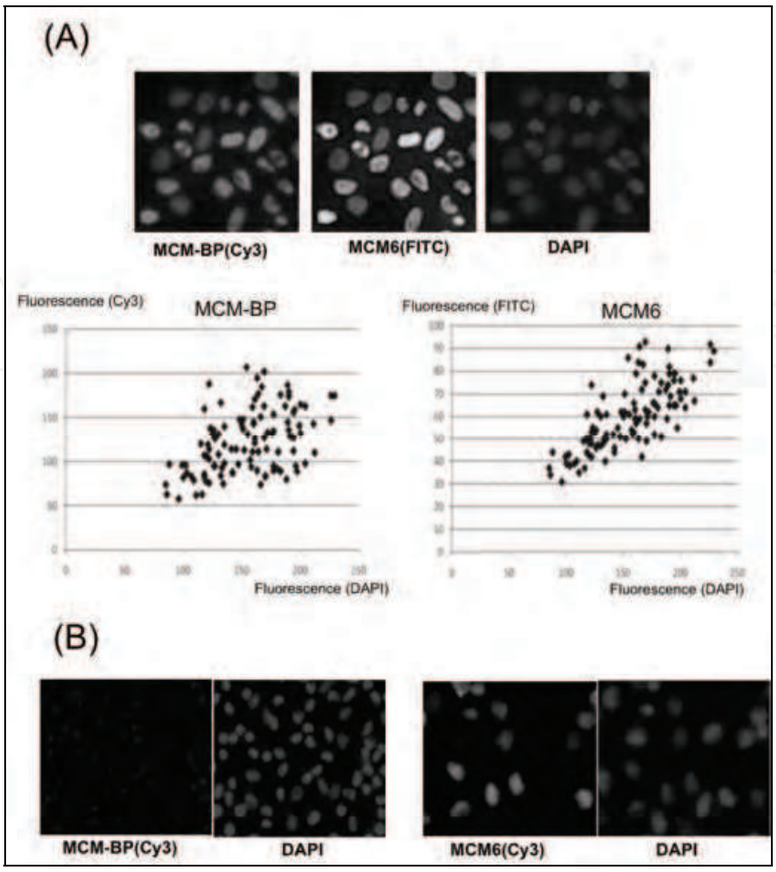
To examine whether MCM-BP binds with chromatin or not, logarithmically growing HeLa cells were lysed in a buffer containing Triton X-100 and 100 mM NaCl. After Triton-soluble nucleoplasmic and cytoplasmic proteins were recovered, nuclear DNA in the Triton-insoluble fraction was digested with DNase I. The DNase I-soluble fraction contained a small portion of histones, and DNase I-insoluble fraction contained a large portion of histones (Fig. 6). Proteins in these three fractions were examined by immuno-blotting. MCM4 protein distributed almost evenly into these three fractions. In contrast, MCM-BP was almost exclusively recovered into the Triton-soluble fraction and only a small portion of MCM-BP was detected in the DNase I-soluble chromatin fraction. When NaCl concentration in lysis buffer was increased to 200 mM, however, MCM-BP was not detected in the DNase I-soluble chromatin fraction, suggesting that a small portion of MCM-BP loosely associates with chromatin. To examine cellular localization of MCM-BP, logarithmically growing HeLa cells were stained with anti-MCM-BP antibody (Fig. 7A). The antibody almost exclusively stained nuclei, similarly to the antibody against MCM6. The intensities of the staining with anti-MCM-BP antibody appeared to be differ among cells. When the fluorescence of DAPI-staining and the anti-MCM-BP antibody staining was quantified, there was a weak

correlation between these two intensities (Fig. 7A), suggesting that MCM-BP accumulates in nuclei from the G1 to G2 phases. A similar increase in the level of MCM6 protein during progression of cell cycle was detected. When proteins that do not stably bind to the nuclear structure including chromatin were extracted with a buffer containing Triton X-100, only half of the extracted cells strongly reacted with anti-MCM6 antibody (Fig. 7B). Probably this is due to the fact that the amounts of chromatin-bound MCM6 decrease during late S and G2 phases. In contrast, the fluorescence signal with anti-MCM-BP antibody was hardly detected in the Triton-extracted HeLa cells, and a small area in nuclei that may be nucleolus was faintly stained. These results, which are consistent with the immuno-blotting data, indicate that MCM-BP does not stably bind to chromatin during the cell cycle.



For biochemical fractionation of HeLa cells, the cells were lysed at 2 × 10^6 cells per 100 μl in modified CSK buffer (10 mM Pipes, pH 6.8, 100 mM NaCl, 1 mM $MgCl_2$ and 1 mM EGTA) containing 0.1% Triton X-100, 1 mM ATP, proteinase inhibitors (Pharmingen) (solution A) and placed on ice for 15 min. On the right, NaCl concentration in the lysis buffer was increased to 200 mM, as indicated at the top. The cell suspension was centrifuged (5,000 rpm for 5 min in a microcentrifuge), and its supernatant was saved (S). Recovered precipitate was suspended in solution A and centrifuged. The precipitate was suspended in a volume of solution A to yield 4 × 10^6 cells per 100 μl (P) and then incubated with DNase I (Takara, Tokyo, Japan) at 200 μg/ml at 30 °C for 15 min, and then soluble (S3) and insoluble (P') fractions were recovered after centrifugation. The insoluble materials (P') were suspended in a volume of solution A to yield 4 × 10^6 cells per 100 μl. The proteins in these fractions were electrophoresed and analyzed by immuno-blotting. MCM-BP, MCM4 and tubulin proteins in these fractions were analyzed. Distribution of histones was shown at the bottom.

Fig. 6. Chromatin-binding of MCM-BP

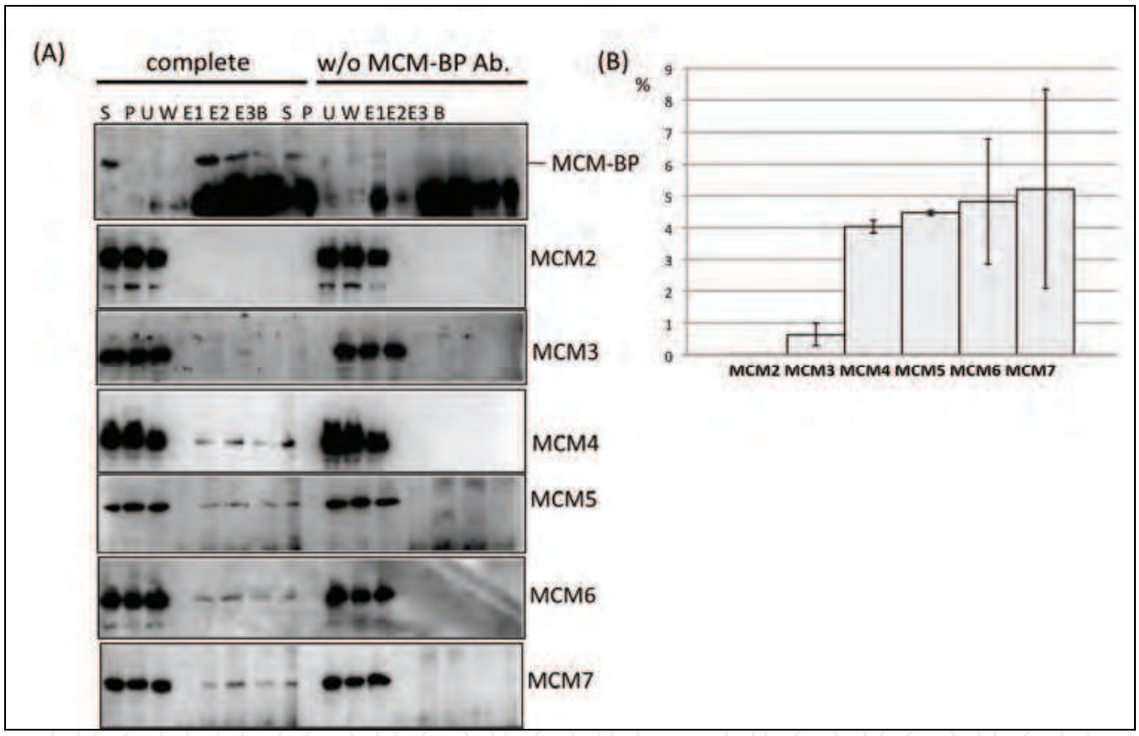


(A) Logarithmically growing HeLa cells were fixed by incubation with 4% paraformaldehyde in PBS for 5 min at room temperature, and then permeabilized and blocked by incubation with 0.1% Triton X-100, 0.02% SDS and 2% nonfat dried milk in PBS for 1 h at room temperature. The fixed cells were incubated at 4°C with anti-MCM-BP mouse antibody (culture supernatant of hybridoma cells) and anti-MCM6 goat antibody in the blocking solution. Cells were washed with the blocking solution and then incubated with Cy3-conjugated anti-mouse antibodies (Jackson Immuno-Research, West Grove, PA, USA) and FITC-conjugated anti-goat antibodies (Jackson Immuno-Research) for 1.5 h at 37°C in the blocking solution. Washed cells were stained with 2 μg/ml DAPI for 15 min at room temperature. After washing with PBS, cells were mounted in 90% glycerol and 10% PBS solution containing 1,4-diazabicyclo[2,2,2]-octane (DABCO, Sigma) (2.3%) and observed using fluorescence microscopy (BZ9000, KEYENCE, Japan). The levels of the fluorescence were measured (bottom). The values in each cell are plotted on a graph where DAPI fluorescence level is shown as a vertical line and Cy3 or FITC fluorescence level is horizontal line. (B) For extraction of chromatin-unbound proteins, HeLa cells were extracted by incubating with a buffer (10 mM PIPES, pH6.8, 0.1 M NaCl, 0.3 M sucrose, 3 mM $MgCl_2$ and 0.5 % Triton X-100) for 10 min at room temperature before fixation. After fixation, the extracted cells were stained with anti-MCM-BP antibody or with anti-MCM6 antibody. Fluorescence (Cy3) from the second antibody (Cy3-conjugated anti-mouse antibody and Cy3-conjugated anti-goat antibody) and from DAPI was detected.

Fig. 7. Cellular localization of MCM-BP

2.3 Binding of MCM-BP with MCM2-7 proteins in HeLa cells

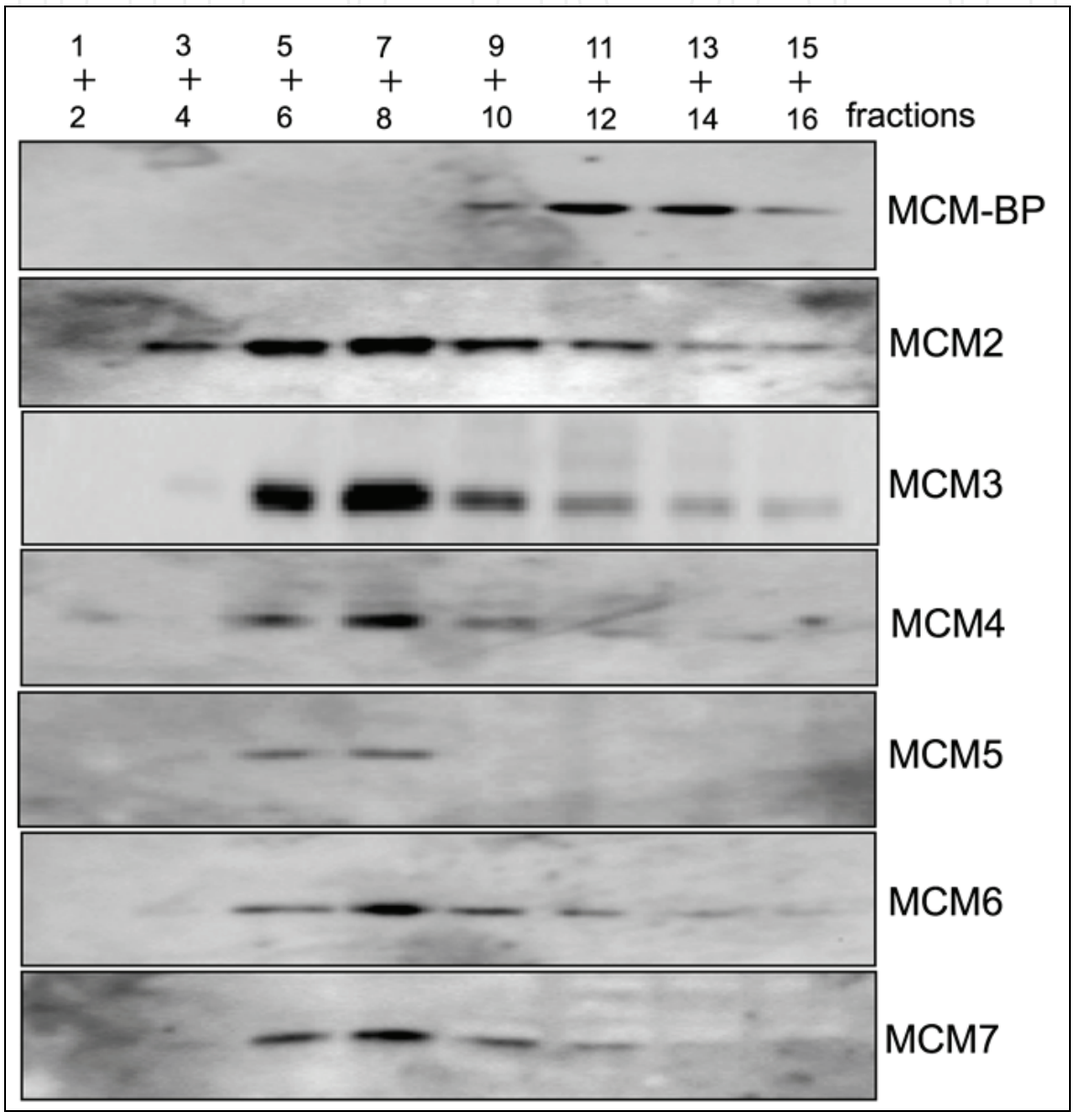
Since it was found that MCM-BP was almost exclusively recovered in Triton-soluble fraction, we examined whether MCM-BP in the fraction binds with MCM2-7 proteins. It is known that considerable amounts of MCM2-7 were present in the nucleoplasm and they can be extracted with buffer containing Triton X-100. When MCM-BP in the Triton-soluble fraction was immuno-precipitated with the anti-MCM-BP antibody, MCM-BP was detected in the elution fractions (Fig. 8A). Among the MCM2-7 proteins, MCM4, MCM5, MCM6 and MCM7 were detected in the elution fractions. But MCM2 was not detected and MCM3 was only slightly detected in these fractions. Quantification of the data indicates that similar portions of MCM4-7 proteins were recovered in the elution fractions (Fig. 8B). As a control experiment, immuno-precipitation was performed in the absence of anti-MCM-BP antibody. Neither MCM-BP nor MCM2-7 was detected in the elution fractions.



(A) (left) Logarithmically growing HeLa cells were lysed, and Triton-soluble (S) and -insoluble (P) fractions were obtained. MCM-BP in the Triton-soluble fraction was immuno-precipitated with anti-MCM-BP antibody bound to protein G beads. After proteins in the final wash were recovered (W), those bound to the beads were eluted three times by incubating with elution buffer (0.1 M glycine, pH 2.5 and 0.15 M NaCl) (E1, E2, and E3). Proteins bound to the beads after elution were eluted by boiling in SDS-sample buffer (B). MCM-BP and MCM2-7 proteins in these fractions were detected by immuno-blotting. (right) The same experiment was performed without addition of anti-MCM-BP antibody. (B) The levels of immuno-precipitated MCM2-7 proteins are shown with error bars by dividing the total chemiluminescence values detected in the E1-3 and B fractions by those in S and P fractions.

Fig. 8. Binding of MCM-BP with MCM2-7 proteins in HeLa cells

When proteins in the Triton-soluble fraction were fractionated by glycerol gradient centrifugation, all the MCM2-7 proteins were recovered at fraction no. 5-8 (Fig. 9). It is probable that the MCM2-7 hexameric complex is one of major components in these fractions. MCM-BP was mainly recovered at fraction no 10-14; the positions are comparable to those where MCM-BP purified from over-expressed insect cells is recovered (data not presented). Thus, it is suggested that MCM-BP is mainly present in a form free from MCM2-7 proteins in nucleoplasm and only a small portion of MCM-BP binds MCM4-7 proteins (Fig. 8).



Proteins in the Triton-soluble fraction from HeLa cells were fractionated by glycerol gradient centrifugation (36,000 rpm for 14 h). After fractionation into 16 fractions, pairs of neighboring fractions were combined. Proteins in the combined fractions were concentrated and they were electrophoresed. MCM-BP and MCM2-7 were detected by immuno-blotting.

Fig. 9. MCM-BP does not stably bind to MCM2-7

3. Discussion

We presented data indicating that human MCM-BP can bind all of human MCM2-7 proteins when both are expressed in insect cells but it most strongly interacts with MCM7. It is possible that the central MCM domains are involved in the interaction of MCM-BP with MCM2-7 proteins. Consistently, it has been reported that *Xenopus* MCM-BP binds to the conserved MCM box in MCM7 (Nishiyama et al., 2011). In HeLa cells, endogenous MCM-BP protein was present in nuclei, and its amount increased during cell cycle. MCM-BP was almost exclusively recovered in a Triton-soluble fraction of HeLa cells and a small portion of MCM-BP in this fraction was bound with MCM4-7 proteins. As to chromatin binding of MCM-BP, a faint band was detected in DNase I-soluble chromatin fraction. When salt concentration in lysis buffer was increased from 100 mM to 200 mM, however, the MCM-BP band was not detected in the chromatin fraction. Under these conditions, MCM4 protein was still detected in the chromatin fraction. Thus, MCM-BP does not stably bind to DNA as MCM2-7 do in HeLa cells. These results suggest that MCM-BP, which is mainly present in nucleoplasm, plays a role in the S and G2 phases by interacting mainly with MCM7. We also found that the purified MCM-BP did not exhibit DNA helicase activity and did not show single-stranded DNA binding activity (data not presented). Consistent with the published results (Sakwe et al., 2007), MCM-BP did not significantly inhibit DNA helicase activity of the MCM4/6/7 hexamer (data not presented).

Recently, it has been reported that MCM-BP mainly interacts with MCM7 in *Xenopus* egg extracts (Nishiyama et al., 2011). It is suggested that MCM-BP plays a role in unloading of MCM2-7 complex from chromatin in late S phase. The results presented here are not inconsistent with the proposed function of MCM-BP. It has also been reported that MCM-BP is required for cohesion of replicated chromosomes in *Arabidopsis* and human cells (Takahashi et al., 2010). At the DNA replication forks, reorganization of cohesion complexes on chromosomes should occur, since they must encounter a large protein complex of DNA replication proteins that is required for replication fork progression (Uhlmann, 2009). The coordination of DNA replication fork movement and cohesion re-establishment may occur at the forks. Slowing down of MCM2-7 helicase movement or depletion of the MCM complex from the forks may be required for the cohesion re-establishment. It is possible that the interaction of MCM-BP with MCM7 is involved in the regulation of replication fork progression. Our data show that a small portion of MCM-BP binds with MCM4-7 proteins in nucleoplasm. These complexes may be generated from the interaction of MCM-BP and MCM proteins at the forks or on replicated DNA.

Recently, we comprehensively searched proteins that can interact with human RPA in insect cells (Nakatani et al., 2010). RPA plays an essential role in DNA replication by stabilizing the unwound single-stranded DNA region and assembling various replication proteins at the replication forks. In addition of MCM3-7, CDC45, TIPIN, Claspin and cyclin-dependent kinases, MCM-BP was found to interact with RPA among 30 proteins examined. This finding may support the role of MCM-BP in regulation of DNA replication fork movement. As mentioned above, it is probable that MCM helicase may be displaced from the replication forks or replicated DNA by active processes for regulation of DNA replication progression. Several factors could be involved in the displacement of MCM complex at the forks. Cyclin-dependent kinase that plays an essential role in preventing re-replication of DNA phosphorylates MCM4 during the S phase. MCM4 bound to chromatin is specifically phosphorylated with the kinase during the S phase, and MCM4 at G2 and M phases was

highly phosphorylated with the kinase (Fujita et al., 1998). MCM4/6/7 DNA helicase activity was inhibited by the phosphorylation with CDK (Ishimi & Komamura-Kohno, 2001). MCM4 phosphorylation with CDK is stimulated under DNA replication checkpoint conditions (Ishimi et al., 2003). Thus MCM4 phosphorylation with CDK may be one of the reactions of the kinase to prevent re-replication of DNA. Consistently, the addition of excess cyclin-dependent kinase causes detachment of the MCM complex from chromatin to inhibit DNA replication in a replication system using *Xenopus* egg extracts (Hendrickson et al., 1996). Thus, it is possible that phosphorylation of MCM4 on chromatin may be involved in detachment of the MCM complex from the replication forks or DNA.

Here we showed biochemical characteristics of human MCM-BP. The MCM-BP has unique properties of binding capacity with MCM2-7 and high affinity to MCM7 in the experiments using insect cells and it shows weak chromatin-binding ability in HeLa cells. *In vitro* experimental system is required to evaluate the suggested role of MCM-BP at the replication forks.

4. Conclusion

Several lines of evidences suggest that an activated form of MCM2-7 complex functions as a replicative DNA helicase that unwinds duplex DNA at the replication forks. MCM-BP has been identified as a protein that binds to human MCM6 and 7 proteins (Sakwe et al., 2007). It has been suggested that MCM-BP bound to MCM3-7 proteins may play a role in the initiation of DNA replication. Recently, it has been shown that MCM-BP is required for the cohesion of replicated DNA (Takahashi et al., 2010), and it has been suggested that MCM-BP is involved in unloading of the MCM2-7 complex from chromatin at late S phase (Nishiyama et al., 2011). However, biochemical function of MCM-BP is not fully understood. Here, we examined the interaction of human MCM-BP with MCM2-7 in insect cells and in HeLa cells by immuno-precipitation. The results indicate that MCM-BP can bind all of MCM2-7 in insect cells, but it binds most with MCM7. In HeLa cells, MCM-BP was largely detected in a Triton-soluble fraction and only a small portion of MCM-BP was detected in chromatin fraction; the distribution is in contrast to that of MCM2-7. Immuno-precipitation experiment indicates that a small portion of MCM-BP in the Triton-soluble fraction is bound with MCM4, 5, 6 and 7 proteins. These results suggest that MCM-BP in nucleoplasm exhibits its function by interacting mainly with MCM7.

5. Acknowledgement

This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan.

6. References

Bell, S.P. & Dutta, A. (2002) DNA replication in eukaryotic cells. *Annu. Rev. Biochem.* 71, pp. 333-374.

Forsburg, S.L. (2004) Eukaryotic MCM proteins: Beyond replication initiation. *Microbiol. Mol. Biol. Rev*. 68, pp. 109-131.

Fujita, M., Yamada, C., Tsurumi, T., Hanaoka, F., Matsuzawa, K. & Inagaki, M. (1998) Cell cycle- and chromatin binding state-dependent phosphorylation of human MCM heterohexameric complexes. *J. Biol. Chem.* 273, pp. 17095-17101.

Gambus, A., Jones, R.C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R.D. & Labib, K. (2006) GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat. Cell Biol.* 8, pp. 358-366.

Hendrickson, M., Madine, M., Dalton, S. & Gautier, J. (1996) Phosphorylation of MCM4 by cdc2 protein kinase inhibits the activity of the minichromosome maintenance complex. *Proc. Natl. Acad. Sci. USA* 93, pp. 12223-12228.

Ishimi, Y. (1997) A DNA helicase activity is associated with an MCM4, -6, and -7 protein complex. *J. Biol. Chem.* 272, pp. 24508-24513.

Ishimi, Y. & Komamura-Kohno, Y. (2001) Phosphorylation of MCM4 at specific sites by cyclin-dependent kinase leads to loss of Mcm4,6,7 helicase acivity. *J. Biol. Chem.* 276, pp. 34428-34433.

Ishimi, Y., Komamura-Kohno, Y., Kwon, H.-J., Yamada, K. & Nakanishi, M. (2003) Identification of MCM4 as a target of the DNA replication block checkpoint system. *J. Biol. Chem*. 278, pp. 24644-24650.

Masai, H., Matsumoto, S., You, Z., Yoshizawa-Sugata, N. & Oda, H. (2010) Eukaryotic chromosome DNA replication: where, when, and how? *Annu. Rev. Biochem.* 79, pp. 89-130.

Moyer S.E., Lewis P.W. & Botchan M.R. (2006) Isolation of the Cdc45-MCM2-7-GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc. Natl. Acad. Sci. USA* 103, pp. 10236-10241.

Nakaya, R., Takaya, J., Onuki, T., Moritani, M., Nozaki, N. & Ishimi, Y. (2010) Identification of proteins that may directly interact with human RPA. *J. Biochem.* 148, pp. 539-547.

Nishiyama, A., Frappier, L. & Mechali, M. (2011) MCM-BP regulates unloading of the MCM2-7 helicase in late S phase. *Genes Dev.* 25, pp. 165-175.

Numata, Y., Ishihara, S., Hasegawa, N., Nozaki, N. & Ishimi, Y. (2010) Interaction of human MCM2-7 proteins with TIM, TIPIN and Rb. *J. Biochem*. 147, pp. 917-927.

Sakwe, A.M., Nguyen, T., Athanasopoulos, V., Shire, K. & Frappier, L. (2007) Identification and characterization of a novel component of the human minichromosome maintenance complex. *Mol. Cell. Biol.* 27, pp. 3044-3055.

Takahashi, N., Lammens, T., Boudolf, V., Maes, S., Yoshizumi, T., Jaeger, G.D., Witters, E., Inze, D. & Veylder, L.D. (2008) The DNA replication checkpoint aids survival of plants deficient in the novel replisome factor ETG1. *EMBO J.* 27, pp. 1840-1851.

Takahashi, N., Quimbaya, M., Schubert, V., Lammens, T., Vandepoele, K., Schbert, I., Matsui, M., Inze, D. Berx, G. & Veylder, L.D. (2010) The MCM-binding protein ETG1 aids sister chromatid cohesion required for postreplicative homologous recombination repair. *ProsGenet.* 6, e1000817.

Uhlmann, F. (2009) A matter of choice: the establishment of sister chromatid cohesion. *EMBO Rep*. 10, pp. 1095-1102.

Yu, Z., Feng, D. & Liang, C. (2004) Pairwise interactions of the six human MCM protein subunits. *J. Mol. Biol.* 340, pp. 1197-1206.

DNA Replication-Current Advances

Edited by Dr Herve Seligmann

ISBN 978-953-307-593-8
Hard cover, 694 pages
Publisher InTech
Published online 01, August, 2011
Published in print edition August, 2011

The study of DNA advanced human knowledge in a way comparable to the major theories in physics, surpassed only by discoveries such as fire or the number zero. However, it also created conceptual shortcuts, beliefs and misunderstandings that obscure the natural phenomena, hindering its better understanding. The deep conviction that no human knowledge is perfect, but only perfectible, should function as a fair safeguard against scientific dogmatism and enable open discussion. With this aim, this book will offer to its readers 30 chapters on current trends in the field of DNA replication. As several contributions in this book show, the study of DNA will continue for a while to be a leading front of scientific activities.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ryoko Kon, Shunsuke Kusunoki, Naohito Nozaki and Yukio Ishimi (2011). Binding of Human MCM-BP with MCM2-7 Proteins, DNA Replication-Current Advances, Dr Herve Seligmann (Ed.), ISBN: 978-953-307-593-8, InTech, Available from: http://www.intechopen.com/books/dna-replication-current-advances/binding-of-human-mcm-bp-with-mcm2-7-proteins

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen