Figure S1.

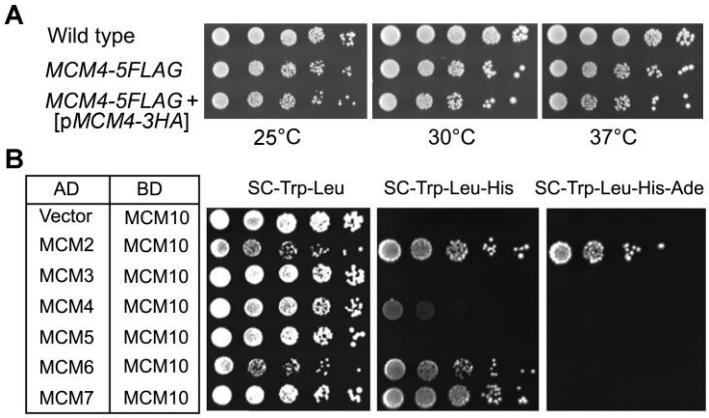

Figure S1- Related to Figure 1.

(A) Growth assays of the yeast strains used in purification of the DH Mcm2-7 complexes. 5-fold serial dilution of log phase cells were spotted on the plates and incubated for 3 days at the indicated temperatures before being photographed.

(B) Mcm10 shows positive interactions with Mcm2, 4, 6 and 7 in a yeast two-hybrid assay (Y2H). *MCM2-7* were cloned into pGADT7 and introduced to AH109 strain in the presence or absence of a pBGKT7 plasmid expressing the indicated Mcm10 alleles. Yeast two hybrids were carried out as in Table S1.

Figure S2.

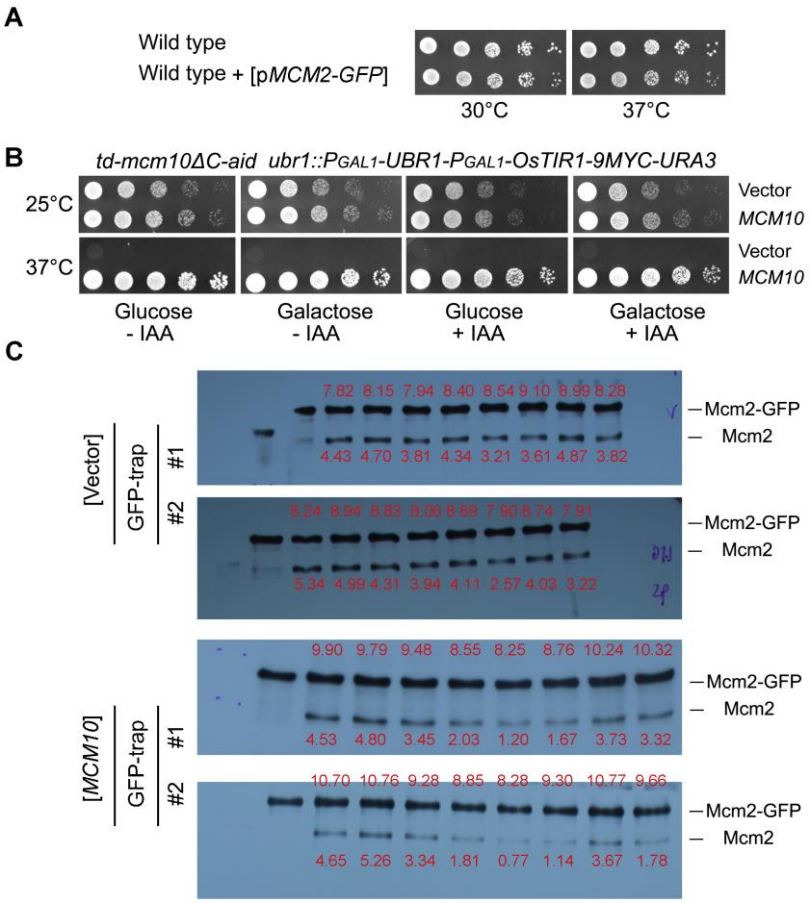

Figure S2- Related to Figure 2.

(A) Growth assays of the yeast strains used in the MCM DH splitting assay. Introduction of a second copy of MCM2 with GFP tag does not interfere with normal cell growth.

(B) The growth of the Mcm10 depletion strains transformed with an empty vector or wild type *MCM10* was examined by spotting on the indicated media at either 25 ℃ or 37 ℃. See Figure 2C for Mcm10 depletion.

(C) Raw quantification data of *in vivo* MCM DH split assay shown in Figure 2E. To ensure the signals are within the linear range, immunoblots with different exposure were quantified by Quantity One (Biorad) as indicated on the films.

Figure S3.

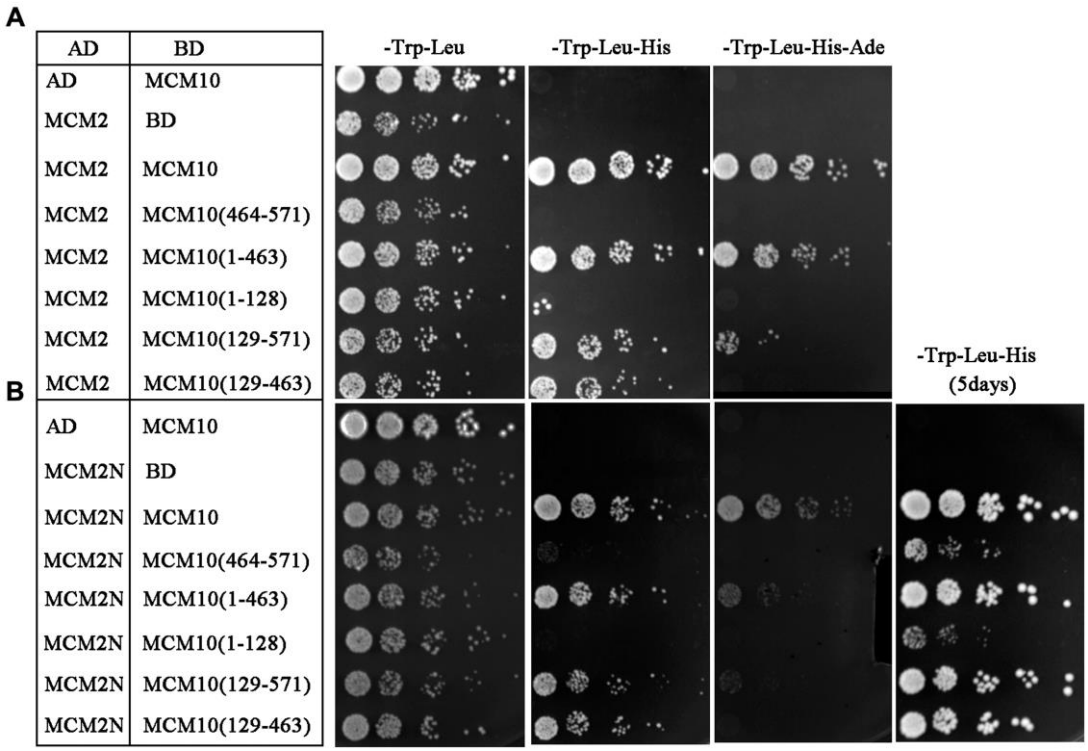

Figure S3- Related to Figure 3. Mcm10 is able to directly bind the N-termini of Mcm2, 4, and 6, which associate each other at the interface of MCM DH *in vitro*.

(A, B) Similar to Mcm2 full length (A), Mcm2N (a.a. 1-390) (B) showed robust interaction with full length and truncations of Mcm10 in a yeast two-hybrid assay (Y2H).

Full-length of *MCM2* (A) or its N-terminal fragment (B) was cloned into pGADT7 and introduced to AH109 strain in the presence or absence of a pBGKT7 plasmid expressing the indicated Mcm10 alleles. Yeast two hybrids were carried out as in Table S1.

Figure S4.

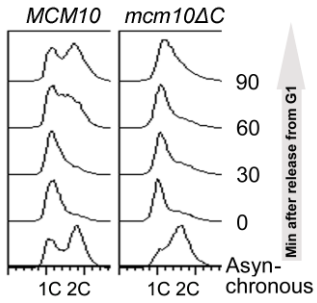

Figure S4- Related to Figure 4.

Cell cycle profiles of the samples used for coimmunoprecipitation experiments of native chromatin fractions in Figure 4.

Cells were synchronized by addition of 7.5 μg/ml of α factor. G1 arrested cells were released by filter washing twice in fresh SC-His medium and continued growth for the indicated time.

Figure S5

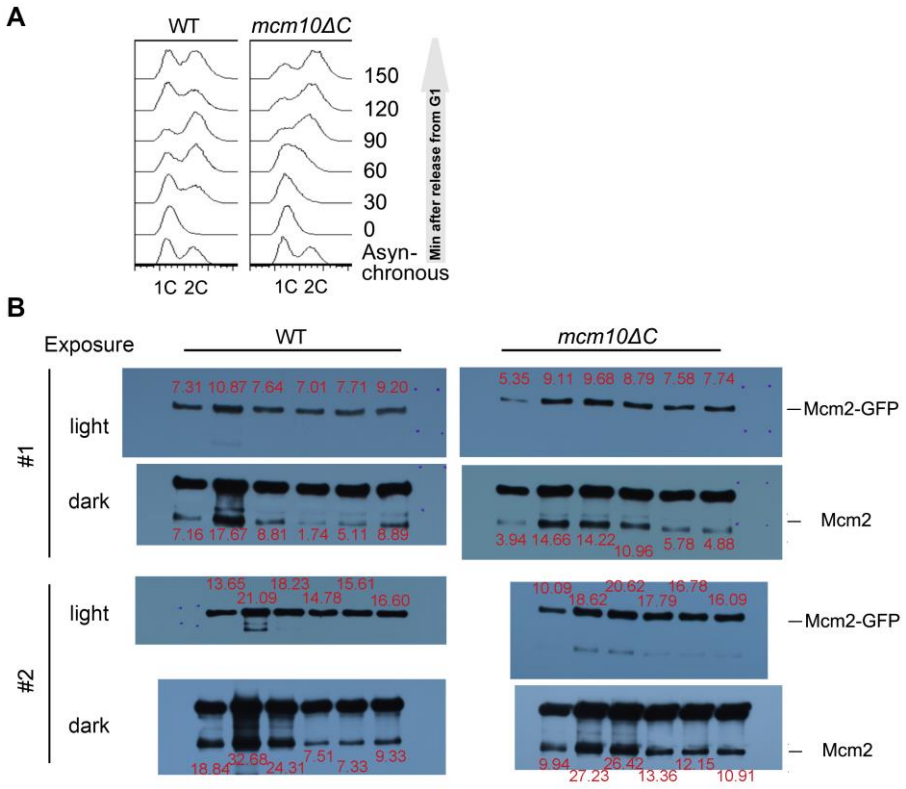

Figure S5- Related to Figure 6.

(A) Representative cell cycle profiles of the cell samples used for double hexamer splitting assay in Figure 6. Cells were collected at the indicated time after released from G1 arrest. Cell cycle profiles were analyzed by flow cytometry.

(B) Raw quantification data of *in vivo* MCM DH split assay shown in Figure 6B. To ensure the signals are within the linear range, immunoblots with different exposure were quantified by Quantity One (Biorad) as indicated on the films.

Table S1. Mcm10 was identified as an interactor of several Mcm2-7 subunits in yeast two-hybrid. Related to Figure 1.

AD	BD	Y2H interaction*
MCM2	empty	-
MCM3	empty	-
MCM4	empty	-
MCM5	empty	-
MCM6	empty	-
MCM7	empty	-
empty	MCM10	-
MCM2	MCM10	++++
MCM2(1-390)	MCM10	++++
MCM2	MCM10(1-463)	++++
MCM3	MCM10	-
MCM4	MCM10	+
MCM4 (1-400)	MCM10	+
MCM5	MCM10	+/-
MCM6	MCM10	+++
MCM7	MCM10	++

* Y2H interaction: "++++" represents growing on SC-L-W-H-A; "+++" represents growing on SC-L-W-H plus 10 mM 3AT; "++" represents growing on SC-L-W-H; "+" or "+/-" represents growing relatively weak on SC-L-W-H.

Table S2. Strains used in this study-Related to Experimental Procedures.

Strain	*Genotype*	Source
BY4741	*MATahis3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Gift from Dr. Junbiao Dai
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Gift from Dr. Junbiao Dai
W303-1a	*MAT*a *trp1-1 ura3-1 his3-11,15 leu2-3,112 ade2-1 can1-100 RAD5*	Gift from Dr. Judith L. Campbell
AH109	*MATa trp1-901 leu2-3,112 ura3-53 his3-200 gal4Δ gal80ΔLYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3 GAL2$_{UAS}$-GAL1$_{TATA}$-ADE2 URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ MEL1*	Clontech
Y187	*MATα ura3-52 his3-200 ade2-101 trp1-901 leu2-3 112 gal4Δ met– gal80Δ URA3::GAL1$_{UAS}$-GAL1$_{TATA}$-lacZ*	Clontech
QY310	*W303 MATa HIS3::MCM2-3HA*	This study
QY317	*W303 MATa KanMX6::MCM10-5FLAG*	This study
QY336	*W303 HIS3::MCM2-3HA KanMX6::MCM10-5FLAG*	This study
QY606	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pMCM10/URA3*	This study
QY713	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pMCM10/URA3 LEU2::MCM2-GFP*	This study
QY715	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pMCM10/URA3 LEU2::MCM4-GFP*	This study
QY793	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pMCM10/URA3 LEU2::MCM4-5FLAG*	This study
QY6129	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pMCM10/HIS3 LEU2::MCM4-5FLAG NatMX::CDC45-3HA*	This study
QY6131	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pmcm10ΔC/HIS3 LEU2::MCM4-5FLAG NatMX::CDC45-3HA*	This study
QY6141	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pMCM10/HIS3 LEU2::MCM4-5FLAG NatMX::CDC45-3HA (p317MCM2-GFP::LYS2)*	This study
QY6142	*BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lys2Δ0 mcm10Δ::KanMX6 pmcm10ΔC/HIS3 LEU2::MCM4-5FLAG NatMX::CDC45-3HA (p317MCM2-GFP::LYS2)*	This study
QY394	*BY4741 KanMX::td-mcm10(1-463)-aid ubr1::P$_{GAL1}$-UBR1-P$_{GAL1}$-OsTIR1-9MYC-URA3*	This study

Table S3. Plasmids used in this study- Related to Experimental Procedures.

Plasmid	Base plasmid/Genotype	Source
pET28a-MCM2	*kan ^{r}6His- MCM2*	This study
pET28a-*mcm2(1-299)*	*kan^{r} 6His- mcm2 (1-299)*	This study
pET28a-MCM10	*kan^{r} 6His- MCM10*	This study
pGEX-4T-1-MCM10	*amp^{r} GST- MCM10*	This study
pGEX-4T-1-*mcm10(1-128)*	*amp^{r} GST- mcm10 (1-128)*	This study
pGEX-4T-1-*mcm10(1-463)*	*amp^{r} GST- mcm10 (1-463)*	This study
pGEX-4T-1-*mcm6(1-439)*	*amp^{r} GST- mcm6 (1-439)*	This study
pGEX-4T-1-*mcm4(1-471)*	*amp^{r} GST- mcm4 (1-471)*	This study
pGEX-4T-1-*mcm10(464-571)*	*amp^{r} GST- mcm10 (464-571)*	This study
pGEX-6P-1-MCM10	*amp^{r} GST- MCM10*	This study
pRS313-MCM10-5FLAG	*amp^{r}/HIS3 MCM10-5FLAG*	This study
pRS313-*mcm10 ΔN*	*amp^{r}/HIS3 mcm10 Δ(1-128) -5FLAG*	This study
pRS313-*mcm10 ΔC*	*amp^{r}/HIS3 mcm10 Δ(464-571) -5FLAG*	This study
pRS313-MCM10-GBP	*amp^{r}/HIS3 MCM10-GBP*	This study
pRS313- *mcm10 ΔC* -GBP	*amp^{r}/HIS3 mcm10 Δ(464-571)-GBP*	This study
pRS317-MCM2-GFP	*amp^{r}/LYS2 MCM2-GFP*	This study
pGADT7-MCM2	*amp^{r}/LEU2 GAL4-AD-MCM2*	This study
pGADT7-MCM3	*amp^{r}/LEU2 GAL4-AD-MCM3*	This study
pGADT7-MCM4	*amp^{r}/LEU2 GAL4-AD-MCM4*	This study
pGADT7-MCM5	*amp^{r}/LEU2 GAL4-AD-MCM5*	This study
pGADT7-MCM6	*amp^{r}/LEU2 GAL4-AD-MCM6*	This study
pGADT7-MCM7	*amp^{r}/LEU2 GAL4-AD-MCM7*	This study
pGADT7-*mcm2N*	*amp^{r}/LEU2 GAL4-AD-mcm2(1-390)*	This study
pGADT7-*mcm4N*	*amp^{r}/LEU2 GAL4-AD-mcm4(1-400)*	This study
pGBKT7-MCM10	*kan^{r}/TRP1GAL4-BD-MCM10*	This study
pGBKT7- *mcm10 (1-128)*	*kan^{r}/TRP1GAL4-BD-mcm10 (1-128)*	This study
pGBKT7- *mcm10 (1-463)*	*kan^{r}/TRP1GAL4-BD-mcm10 (1-463)*	This study
pGBKT7- *mcm10 (129-571)*	*kan^{r}/TRP1GAL4-BD-mcm10 (129-571)*	This study
pGBKT7- *mcm10 (129-463)*	*kan^{r}/TRP1GAL4-BD-mcm10 (129-463)*	This study
pGBKT7- *mcm10 (464-571)*	*kan^{r}/TRP1GAL4-BD-mcm10 (464-571)*	This study

Supplemental Experimental Procedures

Strains and plasmids

Strains and plasmids used in this study are listed in Supplemental Table S2-S3. Mutants were generated using recombination-mediated cassette exchange (Longtine et al., 1998) or tetrad dissection as previously described (Lou et al., 2008). All constructs were confirmed by DNA sequencing.

Cell synchronization and flow cytometry analysis

7.5 μg/ml of α factor was added for cell synchronization in G1-phase. G1 arrested cells were released by filter washing twice in fresh medium and continued growth for the indicated time. Samples were collected and fixed with 70% ethanol and then processed for flow cytometry using a BD Biosciences FACSVerse machine.

Whole cell extracts (WCE) and immunoblotting (IB)

WCE of one hundred OD600 units of asynchronized or synchronized cells were prepared by glass bead beating (Mini-Beadbeater-16, Biospec,USA) in lysis buffer {45 mM HEPES, pH 7.2, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 0.2% NP-40, 1 mM PMSF, 2 mM DTT, 1× Protease Inhibitor Cocktail tablet (Roche), 1×PhosSTOP tablet (Roche)}. Protein fractions were separated by SDS polyacrylamide gel electrophoresis (PAGE) and transferred to PVDF membrane. Each protein was probed with the antibody specifically indicated in each figure by western blotting. Antibodies used in this study are: anti-Cdc45 (gift from Dr. Karim Labib); mouse anti-FLAG M2-specific monoclonal antibody (1:2000, Sigma), mouse anti-HA 16B12 (1:1000, Millipore). polyclonal anti-GST (glutathione transferase) (1:1000,ORIGENE), anti-6His antibodies (1:1000, ORIGENE); anti-tubulin(1:10000, MBL) ; Anti-Rad53 (1:1000, Abcam); protein-G-agarose (GE Healthcare), NHS-activated agarose resins (GE Healthcare). HRP-conjugated anti-rabbit or anti-mouse IgG was used as the secondary antibody (1:10 000, Sigma).

Immunoprecipitation (IP)

IP analysis was performed using strains co-expressing the tagged versions of each protein at physiological level as indicated in each figure. IP was carried out basically as described previously (Lou et al., 2008). Input (IN) corresponding to approximately 100 μg total protein, were analyzed in parallel with immunoprecipitates (IP). Proteins were analyzed by mass spectrometry or western blotting using indicated antibodies.

Large scale native chromatin fractionation

The large scale native chromatin fraction was prepared essentially as described (Liang and Stillman, 1997; Sheu and Stillman, 2006) with minor modifications. Briefly, cells (strains QY6129, QY6131) of 150 OD600 units were spheroplasted by incubation with 50 U/ml lyticase (Sigma). Crude extract was prepared by Triton X-100 treatment and fractionized via sucrose cushion in 500 μl of the modified EBX-1 buffer {50 mM HEPES/KOH pH7.5, 150 mM KGlu, 2.5 mM MgOAc, 0.1 mM ZnOAc, 2 mM NaF, 0.5 mM spermidine, 20 mM β-Glycerophosphate, 3 mM ATP, 1 mM DTT, 1 mM PMSF, Protease inhibitor tablets (EDTA free, Roche)}. The supernatant (SN1) contains non-chromatin bound proteins, while the chromatin is in the pellet. Chromatin-bound proteins (SN2) were released by rotation the pellet with EBX-1 buffer supplemented with 200 U/ml of Benzonase (Sigma) and 3 mM ATP for 30 min at 4℃.

For Cdc45-IP and Mcm10-IP, 500 µl of SN2 was mixed with α-HA (Roche) or purified polyclonal antibody against Mcm10 for 1h on ice. Protein G Sepharose was added and incubation continued for 2h with rotation at 4℃. Nonspecific binding proteins were washed out by 1 ml EBX-1 buffer supplemented with 3 mM ATP for three times. The precipitated fraction was analyzed by 8% SDS-PAGE and immunoblots with indicated antibodies.

Sucrose gradient centrifugation

Two litres of G1 or S phase yeast cells (4×10^7 cells/ml) were fractionated as illustrated in Figure 1A by using the EBX-3 buffer {50 mM HEPES/KOH pH7.5, 150 mM KGlu, 2.5 mM MgOAc, 0.1 mM ZnOAc, 2 mM NaF, 0.5 mM spermidine, 20 mM -Glycerophosphate, 3 mM ATP, 1 mM DTT, 1 mM PMSF, Protease inhibitor tablets (EDTA free, Roche)}. 10 ml of SN1 or SN2 was mixed with 250 µl M2 beads for 3h with rotation at 4°C, and washed three times with 5 ml EBX-3 buffer. The bound fraction was eluted 300 µl of 2 µg/µl FLAG peptide. The 600 µl SN1 or SN2 FLAG elution was applied to a 5-30% sucrose gradient in buffer EBX-3. The samples were then centrifuged at 100,000g for 5 h in a SW55 Ti rotor (Beckman Coulter). After centrifugation, the fractions (400 µl each) were collected from the top of the gradient and subjected to SDS-PAGE and immunoblots. Aldlase (158 kDa) and thyroglobulin (669 kDa) were used as size makers.

Protein expression, purification

Full length and truncated forms of pGEX4T-1-MCM10, pGEX-4T-1-*mcm6(1-439)*, pGEX-4T-1-*mcm4(1-471)*, pET28a-MCM10, pET28a-MCM2, pET28a-*mcm2(1-299)* constructs used in the biochemical experiments were expressed in E. coli BL21 (DE3) RIL codon-plus (Stratagene) and purified by affinity tags and conventional column chromatography.

Preparation of antibodies and Mcm10 agarose beads

To raise polyclonal antibody specific to Mcm10 or Mcm2, purified full-length protein was used to immunize rabbits. Polyclonal antibodies were affinity purified. Mcm10 beads were prepared by immobilize purified Mcm10 protein to NHS-activated agarose beads as recommended by the manufacturer (GE Healthcare), which was used for efficient *in vitro* pull-down assay.

***In vitro* pull-down assay**

Approximately ten picomoles of each protein were mixed with glutathione-Sepharose 4B (GE Healthcare Life Sciences) or anti-Mcm10 agarosebeads made in this study were mixed in 100 µl of binding buffer (50 mM HEPES-NaOH pH 7.6, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 1 mM PMSF, 1 µg/µl BSA, and 0.1% Triton X-100) and incubated for 1 h at 4℃. The beads were washed at least three times prior to Western blotting and/or Coomassie staining.

Construction of *mcm10* alleles

Viability of various *mcm10* alleles was determined by plasmid shuffling since *MCM10* is an essential gene. Wild type *MCM10* was cloned and expressed in pRS316/*URA3* vector to allow *mcm10Δ* mutants grow. pRS316-*MCM10* plasmid was removed on 5-FOA plates due to its expression of *URA3*, which caused cells toxic to 5-FOA. The ability to support cell growth was tested for various *mcm10* alleles expressed in pRS313/*HIS3* vector under a range of genetic background as indicated in each figure. 5-fold serial dilution of log phase cells were spotted on SC-His plates in the presence or absence of 5-FOA and incubated for 2 days at indicated temperature before photographed.

In vivo GFP trap assay (protein tethering assay)

Interaction between *mcm10* mutants and Mcm2-7 was restored through a tethering strategy using the *in vivo* GFP trap (Qu et al., 2013). In Mcm10-Mcm2-7 tethering experiments, each *mcm10* allele was fused to GBP (GFP binding protein), while the Mcm2 or Mcm4 subunit was tagged with GFP. To ensure specific targeted protein tethering, omission of one of the GFP/GBP pairs was included in all tethering assays as controls.

Yeast two-hybrid assays

Yeast two-hybrid assays with full-length of each subunit of Mcm2-7 constructed in pGADT7 and Mcm10 in pGBKT7 were basically performed as described previously (Lou et al., 2008). A pair of pGBKT7 and pGADT7 derivatives was introduced into *Saccharomyces cerevisiae* AH109 and Y187 cells, respectively. Transformants harboring either pGBKT7 or pGADT7 constructs were selected on synthetic growth medium lacking tryptophan (SC-W) or leucine (SC–L), respectively. The interaction was analyzed by growing on select media lacking histidine (–LWH) or histidine and adenine (–LWHA) at 30 ℃ for 2–3 days.

Supplemental References

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Qu, M., Rappas, M., Wardlaw, Christopher P., Garcia, V., Ren, J.-Y., Day, M., Carr, Antony M., Oliver, Antony W., Du, L.-L., and Pearl, Laurence H. (2013). Phosphorylation-Dependent Assembly and Coordination of the DNA Damage Checkpoint Apparatus by Rad4TopBP1. Molecular Cell 51, 723-736.