Supporting Methods

Hormonal Regimens. Briefly, ovariectomized mice were "primed" for 2 days with 100 ng of E_2. After resting for 2 days, groups of two to three mice were killed 4 days later at different time points after one of the following treatments: (*i*) no treatment (control), (*ii*) one injection of 50 ng of E_2 on the fourth day (E_2), (*iii*) 4 days of 1 mg of P_4 (P_4), or (*iv*) 4 days of 1 mg of P_4 with one injection of 50 ng of E_2 at the same time as the last P_4 injection (P_4E_2).

Preparation of Protein and Total RNA from Uterine Epithelium. For protein extraction, the extraction buffer contained 10 mM Hepes-KOH (pH 7.0), 0.1 M NaCl, 40 mM β-glycerophosphate, 30 mM NaF, 10 mM sodium pyrophosphate, 2 mM Na_3VO_4, and 1 mM PMSF supplemented with protease inhibitor mixture. The beads were washed in 1 ml of washing buffer containing 90 mM Hepes-KOH (pH 7.0), 0.2 M NaCl, 40 mM β-glycerophosphate, 10 mM EDTA, 4 mM EGTA, 1% Nonidet P-40, 30 mM NaF, 10 mM sodium pyrophosphate, 2 mM Na_3VO_4, 1 mM PMSF, and protease inhibitor mixture. Lysates were sonicated and clarified by centrifugation, and equal amounts of protein, measured by BCA protein assay (Pierce, Rockford, IL), were used for each experiment.

Total RNA was isolated from the uterine epithelium by using the guanidinium isothiocyanate method (1) with some modifications. The RNA extraction buffer contained 100 mM Tris•HCl (pH 7.4), 0.1 M NaCl, and 10 mM Ribonucleoside Vanadyl Complex (New England Biolabs, Boston, MA). Approximately 20-30 μg of total RNA was isolated from each group of three mice. The integrity of the total RNA extracted was monitored using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Samples were pooled together for microarray and real-time PCR analysis.

Microarray Experiments. Mouse cDNA microarray chips were obtained from the microarray facility at the Albert Einstein College of Medicine. Each array encompasses 27,396 mouse cDNA sequence-verified clones from Incyte Genomics, the National Cancer Institute, and Integrated Molecular Analysis of Genomes. Microarray analysis

was performed on three separate microarray chips following the standard procedure (2). Briefly, for each hybridization, Cy3-labeled (E_2-treated) or Cy5-labeled (P_4E_2-treated) cDNAs probes were generated by reverse transcription of 100 μg of total RNA through the incorporation of Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotech, Piscataway, NJ). The resulting Cy3- and Cy5-labeled cDNAs were combined, purified, concentrated, and heated at 94°C, followed by the hybridization to a microarray slide overnight at 50°C. After hybridization, the slide was washed at room temperature with 1× SSC/0.1% SDS for 20 min and twice with 0.2× SSC/0.1% SDS for 20 min. The slide was dried and scanned by using a custom-built laser scanner Gene Pix 4000A (Axon Instruments, Union city, CA) at the Albert Einstein College of Medicine microarray facility.

Data Normalization and Data Analysis. An "MA-plot" was used to represent the (R, G) data, where R = red for Cy5 (P_4E_2-treated sample), G = green for Cy3 (E_2-treated sample), and log intensity ratio $M = \log_2 (R/G) = \log_2 R - \log_2 G$ was plotted against mean log intensities $A = \log_2 \sqrt{R \times G} = (\log_2 R + \log_2 G)/2$ for each array. Scale normalization was performed for each array using LOWESS procedure of Microarray package in Bioconductor (www.bioconductor.org). The efficiency of LOWESS normalization was assessed by monitoring M-A plots from each array before and after LOWESS normalization. The normalized data were then back-transformed before further statistical analyses by using the following formulas: $\log Cy5^* = A + \hat{M}/2$ and $\log Cy3^* = A - \hat{M}/2$, where log Cy3* and log Cy5* were the normalized log intensities. Here, $\hat{M}$ represented each of the normalized M values with $\hat{M}$ the LOWESS predicted value for each spot. Average and A were calculated from triplicate arrays at each condition (3).

In our experiment, a criterion that the intensity $A \geq 7$ ($2^7 = 128$) combined with the fold change $M \geq 1$ ($R/G \geq 2$) or $M \leq -0.83$ ($G/R \leq 1.8$) in at the least two of three arrays was used to filter the up-regulated and down-regulated genes. To understand the biological significance of the overall changes in gene expression, Gene Ontology analysis was used

to examine the enrichment functional groups that were further confirmed by searching the PubMed database.

Real-Time PCR. To verify the expression data obtained from the microarray analysis, real-time PCR measurement of MCM transcripts was performed using SYBR Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA). Table 3 lists the primer sequences and amplicon size. Real-time PCR was performed as followed: 95°C for 15 min followed by 40 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. The changes in MCM gene expression were calculated by median threshold cycle (C_T) and then normalized for the housekeeping gene GAPDH transcripts by using the $2^{-\Delta\Delta CT}$ methods (4).

Preparation of a Chromatin-Bound Fraction from the Uterine Epithelial Cells. Chromatin fractionation was performed as described (5) with minor modifications. After the hormone treatments of mice described above, uteri were removed, split longitudinally, and vortexed with Teflon beads in 1 ml of CSK buffer (10 mM Pipes, pH 7.0/100 mM NaCl/300 mM sucrose/3 mM $MgCl_2$/0.5 mM PMSF, supplemented with protease inhibitor mixture) to isolate the uterine epithelia. The beads were washed with 1 ml of CSK buffer with 0.2% vol/vol Nonidet P-40, and the homogenates were incubated on ice for 20 min. The insoluble chromatin-bound fraction and the soluble fraction were separated by centrifugation at 700 × *g* for 5 min at 4°C.

Immunoblotting and Immunohistochemistry (IHC). For Western blotting, equal amounts of protein were separated by SDS/PAGE, transferred to nylon membranes, and probed with appropriate antibodies. Rabbit polyclonal antibody for MCM2 (559542), MCM4 (559544), MCM6 (559546), and MCM7 (559547) were obtained from BD Pharmingen (San Diego, CA); those for MCM3 (sc-9850), Cdc6 (sc-8341), and β-tubulin (sc-9104) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-mouse Cdt1 antibody was a kind gift from Dr. F. Hanaoka (RIKEN). All Western blots were performed with three independently isolated groups of samples. Densitometry for each signal was performed, adjusted for β-tubulin expression as a loading control, and then normalized to the control, hormone-primed, untreated group.

The procedures for IHC were essentially the same as previously described (6). The following goat polyclonal antibodies to MCM2 (sc-9839), MCM3 (sc-9850), MCM6 (sc-9843), and MCM7 (sc-9966) used for IHC came from Santa Cruz Biotechnology. All experiments were replicated at least three times with independent samples and gave similar results. To ensure specificity, the primary antibody to MCM2 (0.1 μg/μl) and MCM3 (0.1 μg/μl) and a 5-fold excess of MCM2 peptide (sc-9839p) and MCM3 peptide (9850p, Santa Cruz Biotechnology) were incubated together, respectively, for 2 h at room temperature before application to the section. Negative controls for MCM2, MCM3, MCM6, and MCM7 also included incubation with normal serum corresponding to the antibody used and omission of the primary antibody. In all cases, these controls were consistently negative.

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