www.sciencemag.org/cgi/content/full/science.1199211/DC1

Supporting Online Material for

Protein Tyrosine Kinase Wee1B Is Essential for Metaphase II Exit in Mouse Oocytes

Jeong Su Oh, Andrej Susor, Marco Conti*

*To whom correspondence should be addressed. E-mail: contim@obgyn.ucsf.edu

Published 31 March 2011 on *Science* Express
DOI: 10.1126/science.1199211

This PDF file includes:

Supporting Online Material

Materials and Methods

Oocyte collection and microinjection

GV oocytes were obtained from the ovaries of 3-5-week-old C57BL/6 mice (Charles River) 46-48 hours after injection of 5IU of pregnant mare's serum gonadotrophin (PMSG, Calbiochem). Oocytes were placed in M2 medium (Millipore) supplemented with 200μM of 3-isobutyl-1-methylxanthine (IBMX, Sigma) to prevent GVBD, and maintained at 37ºC. For long-term incubation, oocytes were cultured in M16 medium (Millipore) at 37ºC in 5% CO_2 atmosphere. To obtain MII oocytes, 5IU of human chorionic gonadotrophin (hCG, Sigma) was administered 48 hours after PMSG injection. Oocytes were microinjected as described previously (*8*). Approximately 10pl of the mRNA solution containing 100-500ng/μl was injected per oocyte. After injection, oocytes were washed and cultured in IBMX-free M16 medium for 20 hours. Parthenogenetic activation of MII oocytes was achieved by washing oocytes into Ca^{2+}-free media containing 10mM $SrCl_2$ (Sigma). The oocytes were observed and imaged on an inverted microscope (DMI 4000B; Leica).

Plasmids, morpholinos and preparation of mRNAs

Constitutively active (CA)-CaMKII, which is a C-terminal deletion of wild-type CaMKIIα (*S1, S2*), was kindly provided by T. Lorca; GFP-cyclin B and GFP-Δ90cyclin B were gifts from K. Jones (*S3*). All Wee1B constructs were made as previously described (*7*). mRNAs for microinjection were made *in vitro* using mMESSAGE mMACHINE kit (Ambion). The mRNAs were polyadenylated, purified and dissolved in nuclease-free water. Morpholinos were designed as described previously (*8*).

RT-PCR

Total RNAs were extracted from oocytes or embryos using RNeasy Micro kit (Qiagen) followed by reverse transcription (RT) using Sensiscript RT kit (Qiagen). PCR was performed as described previously (*7*).

Immunoblotting and kinase assay

Oocytes were washed in phosphate buffered saline (PBS) containing 1% polyvinylpyrrolidine (PVP) and frozen in SDS sample buffer. Western blotting was performed using antibodies against Wee1B, α-tubulin (Abcam), Emi2 (Santa Cruz), cyclin B (Abcam), securin (Abcam), phospho-tyrosine (Abcam), Cdc2 (Santa Cruz), or phospho-tyrosine 15 Cdc2 (Santa Cruz). Wee1B antibodies were raised against the N-terminus of Wee1B expressed in *E.coli*. H1 and myelin basic protein kinase assays were performed as previously described (*7*). For CaMKII kinase assay, wild-type or mutant Wee1Bs were overexpressed in HEK293 cells or E.coli and purified by immunoprecipitation or affinity chromatography, respectively. Purified Wee1B proteins were incubated with CaMKII for 10 min at 30°C in a kinase buffer containing 20mM Tris-HCl (pH7.5), 20mM $MgCl_2$ and 100mM ATP in the presence of 2mM $CaCl_2$, 2.4mM calmodulin and 500 μCi/ml γ-^{32}P]ATP. The reaction was stopped by addition of

2X SDS sample buffer and boiling for 3 min. Samples were subjected to SDS-PAGE followed by either coomassie staining or blotting to PVDF membrane. The gel or membrane was analyzed by either autoradiography or immunoblotting with Wee1B antibodies.

Immunofluorescence imaging

Oocytes were fixed in 4% paraformaldehyde in PBS/PVP for 1 hour at room temperature and permeabilized for 15 min in 0.1% Triton X-100 in PBS at room temperature. Oocytes were stained with anti-mouse acetylated α- tubulin antibody (Sigma) overnight at 4 ºC. After washing three times with PBS containing 1mg/ml BSA, the oocytes were incubated for 1 hour with an Alexa Fluor-594 conjugated goat polyclonal anti-mouse IgG (Molecular probes) followed by incubation with DAPI and FITC-conjugated Lens culinaris agglutinin (LCA). For chromosome spreads, oocytes were placed in hypotonic solution (1% sodium citrate) for 20 minutes and fixed by methanol: glacial acetic acid (3:1). DAPI was used for chromosome staining. Immunostaining was visualized using an inverted confocal microscope (TCS SP5; Leica) with 63×objective.

Statistical analysis

Data are representative of at least three independent experiments unless otherwise specified. Values were analyzed by one-way ANOVA or Student's t-test, and $P < 0.05$ was considered statistically significant.

Supplementary References

S1. T. Lorca *et al.*, *Nature* **366**, 270 (Nov 18, 1993).

S2. T. Lorca, A. Abrieu, A. Means, M. Doree, *Biochim Biophys Acta* **1223**, 325 (Sep 29, 1994).

S3. A. Reis, H. Y. Chang, M. Levasseur, K. T. Jones, *Nat Cell Biol* **8**, 539 (May, 2006).

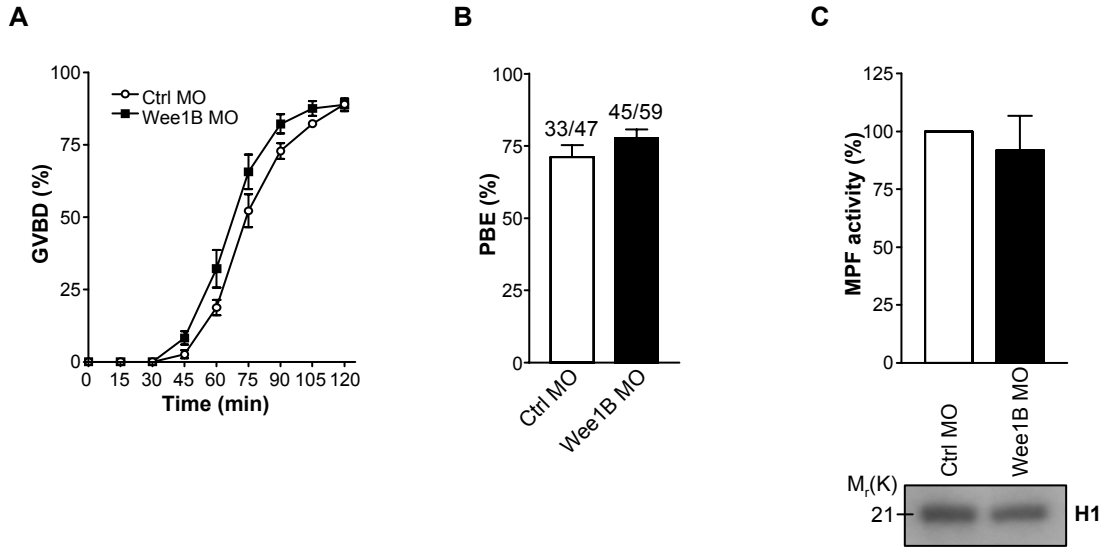

fig S1. Wee1B is dispensable for the establishment of MII arrest. (A-C) GV oocytes injected with control or Wee1B MO were cultured for 24 hours in 200μM of IBMX-containing medium. Because downregulation of Wee1B causes a partial resumption of meiosis (*8*), we used a high concentration of IBMX to maintain meiotic arrest of the oocytes. Oocytes with intact GV were collected and released from IBMX-containing medium by repeated washing. Time course of germinal vesicle breakdown (GVBD) (A), polar body extrusion (PBE) rates (B), and MPF activity of MII-arrested oocytes (C) were measured. Data are the mean ± SEM from three independent experiments. The number of oocytes is reported above the bars. Note that Wee1B knockdown oocytes normally progressed to MII stage, although the timing of GVBD was slightly accelerated.

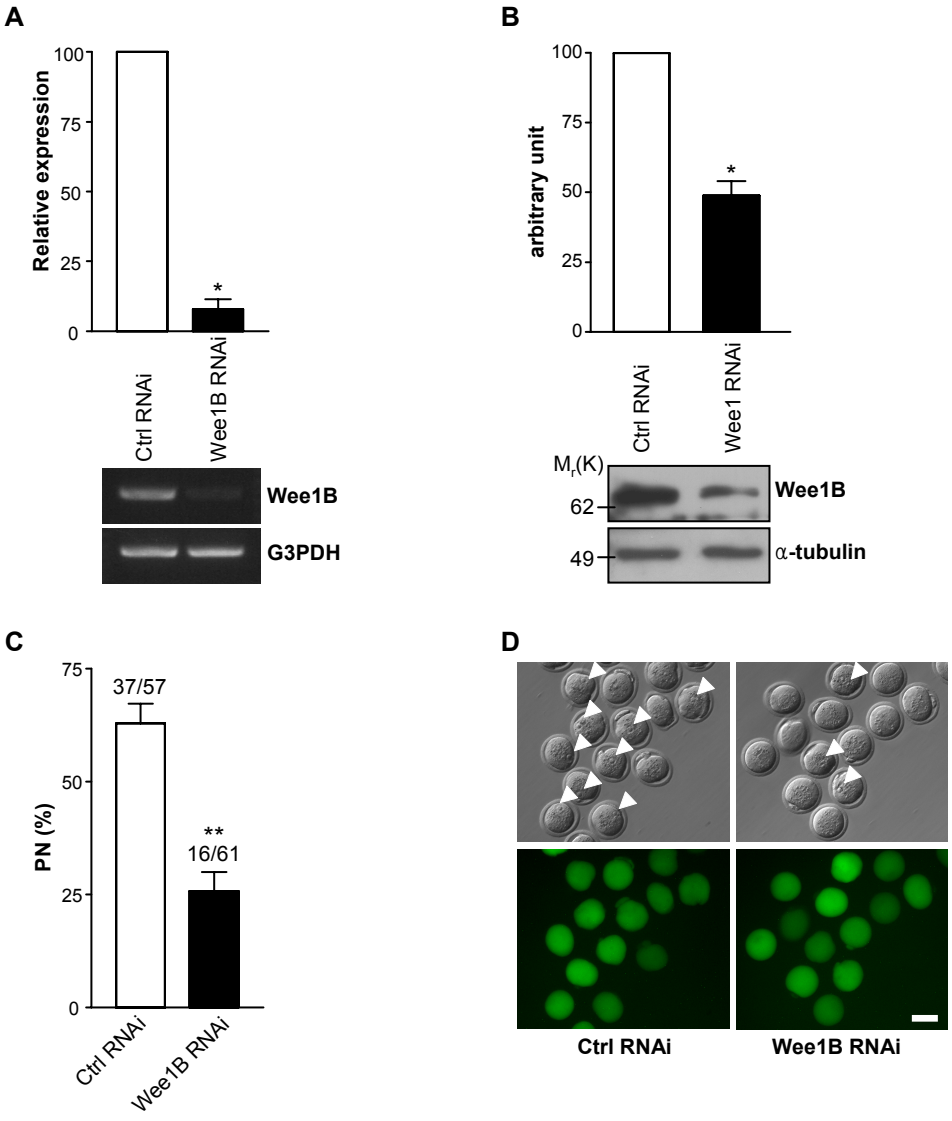

fig S2. RNAi-mediated knockdown of Wee1B during egg activation. GV oocytes were injected with Wee1B RNAi and in vitro matured for 20 hours. RT-PCR (A) and Western blotting (B) of Wee1B knockdown oocytes. Error bar reports the average ratio of measurements ± SEM from three independent experiments. A representative image from three independent experiments is shown. (C) Pronuclear formation of RNAi-mediated Wee1B knockdown oocytes. Representative images are shown in (D). Eggs with pronucleus are indicated by arrows. Bar, 60μm. *p<0.001, **p=0.0036.

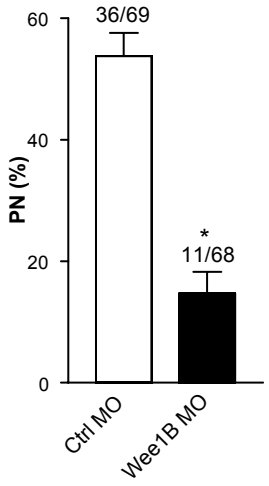

fig S3. In vitro fertilization (IVF) of Wee1B knockdown oocytes. In vitro matured MII oocytes injected with control or Wee1B MO at GV stage were inseminated with sperm, and the pronuclear formation was scored after 8 hours. Data are the mean ± SEM from three independent experiments. *p=0.0017.

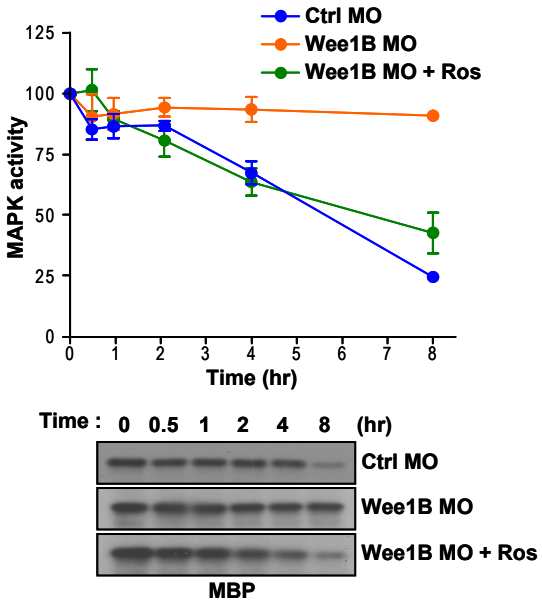

fig S4. MAPK activity of Wee1B knockdown oocytes. GV oocytes injected with Wee1B MO and cyclin B-GFP mRNA were in vitro matured and activated with strontium (see also Fig.3C and D). Oocyte lysates at 0, 0.5, 1, 2, 4, and 8 hours after activation were incubated with [γ-^{32}P]ATP and myelin basic protein (MBP). Phosphorylation was detected by SDS-PAGE and autoradiography. Representative images from two independent experiments are shown.

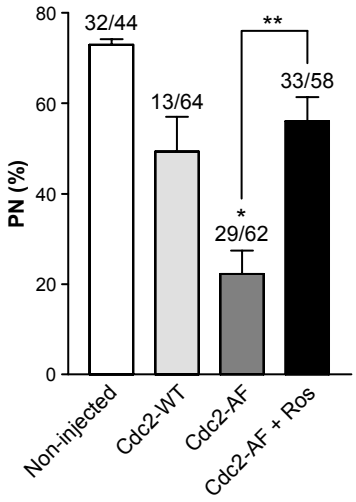

fig S5. Inhibitory phosphorylation of Cdc2 during egg activation. MII oocytes injected with wild-type (Cdc2-WT) or constitutively active Cdc2 (Cdc2-AF) were incubated for 1 hour to allow protein expression and were then activated with strontium for 8 hours in the absence or presence of roscovitine. Data are the mean ± SEM from three independent experiments. *p=0.0144 compared to Cdc2-WT and **p=0.0072.

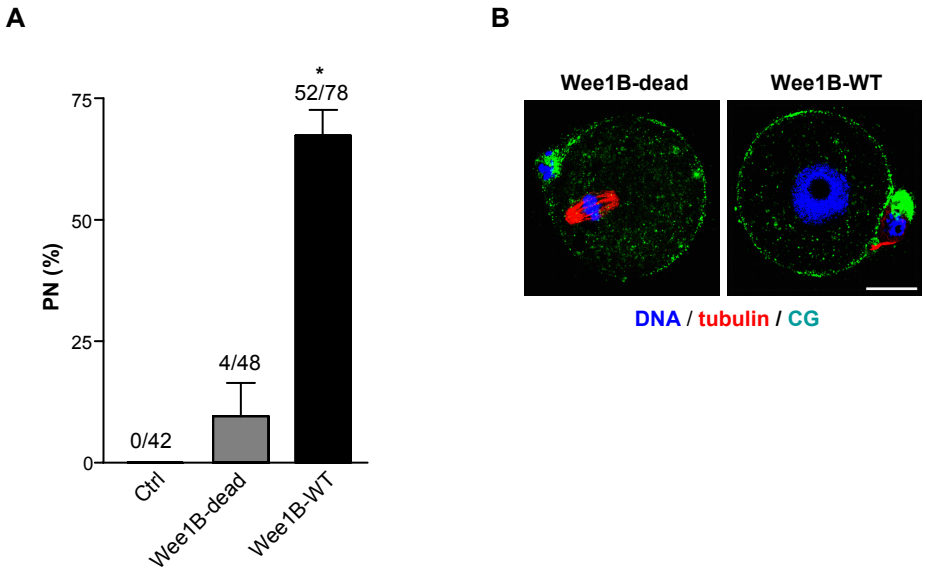

fig S6. Overexpression of Wee1B drives pronuclear formation. (A) MII oocytes were injected with mRNAs encoding wild-type (Wee1B-WT) or catalytically inactive Wee1B (Wee1B-dead) and scored for pronuclear formation after 8 hours. Data are the mean ± SEM from at least three independent experiments. *p<0.0001. (B) Wee1B overexpressed oocytes were stained for tubulin, DNA and cortical granules (CG).