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Wee1B Is an Oocyte-Specific Kinase Involved in the Control of Meiotic Arrest in the Mouse

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Summary

In most species, the meiotic cell cycle is arrested at the transition between prophase and metaphase through unclear somatic signals. Activation of the Cdc2-kinase component of maturation promoting factor (MPF) triggers germinal vesicle breakdown after the luteinizing hormone (LH) surge and reentry into the meiotic cell cycle [1–3]. Although high levels of cAMP and activation of protein kinase A (PKA) play a critical role in maintaining an inactive Cdc2 [4–6], the steps downstream of PKA in the oocyte remain unknown. Using a small-pool expression-screening strategy, we have isolated several putative PKA substrates from a mouse oocyte cDNA library. One of these clones encodes a Wee1-like kinase that prevents progesterone-induced oocyte maturation when expressed in *Xenopus* oocytes. Unlike the widely expressed Wee1 and Myt1, mWee1B mRNA and its protein are expressed only in oocytes, and mRNA downregulation by RNAi injection in vitro or transgenic overexpression of RNAi in vivo causes a leaky meiotic arrest. Ser15 residue of mWee1B is the major PKA phosphorylation site in vitro, and the inhibitory effects of the kinase are enhanced when this residue is phosphorylated. Thus, mWee1B is a key MPF inhibitory kinase in mouse oocytes, functions downstream of PKA, and is required for maintaining meiotic arrest.

Results and Discussion

mWee1b Isolation during a Small-Pool Expression Screening for PKA Substrates

Although PKA involvement in the maintenance of meiotic arrest is widely accepted, it is unclear which PKA substrates are phosphorylated/dephosphorylated during reentry into the cell cycle. To isolate PKA substrates involved in the control of meiotic maturation in mammalian oocytes and to understand the posttranslational steps downstream of PKA, we carried out small-pool expression screening of a mouse oocyte cDNA library (see the Supplemental Experimental Procedures in the Supplemental Data available with this article online). One of the putative PKA substrates isolated by this method codes for a Wee1 kinase homolog (mouse Wee1B, Figure S1, Supplemental Data) similar to a previously isolated clone from human cDNA libraries [7]. A mobility shift of this protein was detected when in vitro-translated [S^{35}]-methionine-labeled mWee1B was incubated with the PKA catalytic subunit (Figure 1A). Similar to *Xenopus* Wee1, which prevents progesterone-induced germinal vesicle breakdown (GVBD) by directly phosphorylating and inhibiting Cdc2 kinase [8, 9], ectopically expressed mWee1B also prevented *Xenopus* oocyte GVBD induced by progesterone treatment in a manner dependent on mRNA concentration Figure 1B). In addition, the Cdc2 inhibitory phosphorylation (phosphotyrosine 15) was not decreased by progesterone treatment when mWee1B mRNA was overexpressed (Figure 1C).

To define the role of mWee1B kinase activity in preventing GVBD, we mutated the lysine residue involved in adenosine triphosphate (ATP) binding in several of the known Wee1-family kinases (K237 in Figure S1A) to methionine [9], and we injected the mRNA of this mutant into *Xenopus* oocytes. The inhibitory effect on progesterone-induced *Xenopus* oocyte maturation could no longer be detected with this kinase-dead mutant (Figure 1D). These findings suggest that mWee1B may be involved in the control of meiotic arrest in mammalian oocytes.

Expression Patterns of Wee1B, Wee1, and Myt1: mWee1B Is a Maternally Expressed Gene

The mammalian Wee1 kinase family, which inhibits CDK activity by phosphorylation of two highly conserved residues, Thr14 and Tyr15 of Cdc2 [10, 11], consists of Wee1 [9, 12] and Myt1 kinases. Because the ortholog XeWee1A protein does not accumulate in stage IV *Xenopus* oocytes [8, 9, 12, 13], Myt1 is considered a major inhibitory kinase of Cdc2 in the *Xenopus* immature oocyte and is required to maintain meiotic arrest. Although the presence of Wee1 was detected in competent mouse oocytes [14, 15], expression of Myt1 has not been reported. To define the expression patterns of the Wee1 kinase family in mouse tissues, we compared the mRNA levels of Wee1, Wee1B, and Myt1 by northern-blot analysis (Figure 2A). Mouse Wee1B was detected only in the ovary, whereas Wee1 was expressed predominantly in the thymus, spleen, kidney, and at lower levels in the ovary and other tissues. These findings are consistent with the expression pattern of *Xenopus* Wee1A kinase, which is present only in the ovary. The cellular patterns of expression of Wee1 and mWee1B mRNAs were further investigated by in situ hybridization. Mouse Wee1B mRNA was detected only in the oocytes at all developmental stages of the follicle including primary, secondary, preantral, and antral follicles with an increase in signal during development (Figures $2B_c$, $2B_d$, $2B_h$, $2B_i$, and $2B_e$). Conversely, Wee1 mRNA was detected only in early developmental stages of oocyte maturation, and oocytes from antral follicles displayed no signal above background (Figures $2B_a$, $2B_b$, $2B_f$, and $2B_g$). Wee1 was also detected in the testis

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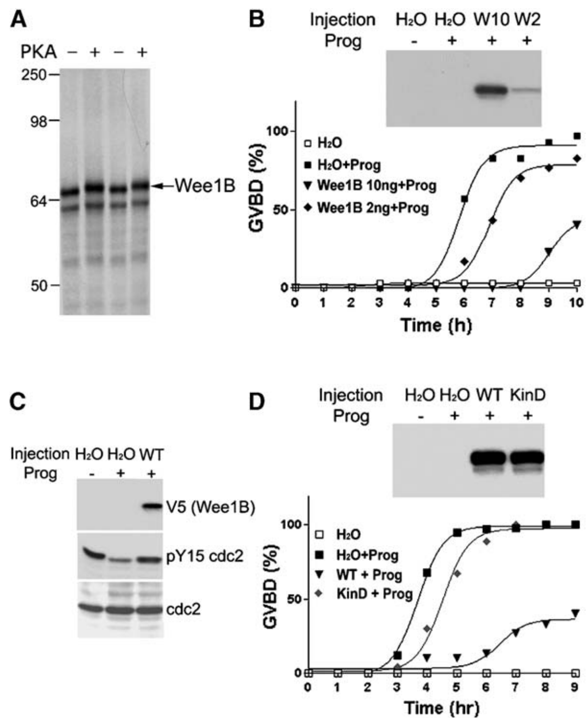

Figure 1. Isolation and Characterization of a Clone Coding for a Wee1-like Kinase

(A) PKA phosphorylation causes a mobility shift of the protein encoded by the mWee1B clone. Isolated mWee1B cDNA was translated in vitro with [^{35}S] methionine, and the reaction mixture was incubated with (+) or without (−) a PKA catalytic subunit. The samples were then subjected to SDS-PAGE with 8% SDS-PAGE gel, and bands were detected by autoradiography. The migrations of protein size markers are shown on the left (kDa).

(B) Mouse Wee1B inhibits progesterone-induced GVBD in *Xenopus* oocytes. Indicated amounts of in vitro-synthesized V5-tagged-mWee1B mRNA (for W10, 10 ng mRNA; for W2, 2 ng mRNA) or H_2O were injected in stage IV *Xenopus* oocytes. Sixteen hours after injection, oocytes were treated with 500 nM progesterone (Prog) to induce oocyte maturation. The percentage of GVBD was determined by scoring the oocytes for a spot on the animal pole at different times after the addition of progesterone. The expression of the mWee1B protein was confirmed by western-blot analysis with a V5 antibody.

(C) Inhibition of GVBD by mWee1B is associated with maintenance of Cdc2 phosphorylation. The Tyr 15 phosphorylation state of Cdc2 and the total amount of Cdc2 protein in the mWee1B-injected or H_2O-injected *Xenopus* oocytes was determined by western-blot analysis with the phosphorylation-specific (pY15 Cdc2) or Cdc2 antibodies 8 hr after progesterone treatment.

(D) The kinase activity of mWee1B is required for inhibition of progesterone-induced GVBD in *Xenopus* oocytes. Wild-type (WT) or kinase-dead mutant (KinD) mWee1B mRNAs were injected into *Xenopus* oocytes (10 ng/oocyte). After 16 hr incubation, oocytes were treated with 500 nM progesterone to induce maturation. The number of oocytes displaying a white spot on the animal pole was scored at different times after the addition of progesterone. The expression levels of tagged wild-type and mutated mWee1B proteins were compared by western blot with the V5 antibody.

by northern-blot analysis and in situ hybridization, whereas mWee1B was not present (Figure 2A and Figure S2), unlike that reported in humans, where Wee1B is expressed in the testis [7]. This suggests that mWee1B does not play a role in mouse male meiosis. Conversely, Myt1 mRNA was detected in most tissues including the ovary and the testis, albeit at low levels (Figure 2A and Figure S3). This restricted expression of mWee1B was confirmed by RT-PCR with granulosa cells and oocytes. Mouse Wee1B was detected only in oocytes, whereas Wee1 and Myt1 were present in granulosa cells (Figure 2C). Finally, mouse expressed sequence tag (EST) database searches showed that mWee1B sequences are retrieved only in cDNA pools of ovary, GV oocytes, unfertilized eggs, and two-cell-stage embryos. All these data strongly suggest that the *Wee1B* gene is expressed exclusively in oocytes and zygotes.

To determine the expression of mWee1B protein, we produced a mouse Wee1B-specific antibody against the C terminus of the protein (Figure S1A) and carried out western-blot analysis with GV- and MII-stage mouse oocytes. Mouse Wee1B protein was detected in GV- and MII-stage oocytes (Figure 2D$_a$). A second antibody raised in rat also detected a protein of the correct size in both transfected cells and oocytes (Figure 2D$_b$).

Downregulation of mWee1B by RNAi Induces GVBD in Mouse Oocytes

To probe the function of mWee1B during oocyte maturation, a strategy of RNA interference was used to downregulate mWee1 expression. If mWee1B were to play an essential role in cAMP-mediated meiotic arrest, downregulation of its expression would cause meiotic resumption. A fusion construct of green fluorescence protein (GFP) and hairpin dsRNA corresponding to the amino terminus of the *mWee1B* sequence was generated [16]. This hairpin construct was injected into eggs to generate transgenic mice, and nine female and two male founders were obtained. Nine founders were used to generate F1 females that were injected with pregnant mare serum gonadotropins (PMSG) at 22–24 days of age. Oocytes were collected in medium supplemented with cilostamide (10 μM), and maturation was scored after 24 hr. In oocytes where PDE3A phosphodiesterase is absent or is inhibited with the specific compound, cilostamide, PKA activity is maintained at high levels by the increased cAMP, thus preventing maturation in vitro and in vivo [17]. Under this condition of meiotic block, more than 25% of the oocytes collected from 12 transgenic females from nine founders were no longer in GV and progressed to GVBD or polar-body extrusion (Figures 3A and 3B) although differences were observed among different founders (15%–35%). A decrease in the amount of endogenous *mWee1B* mRNA was detected by RT-PCR (Figure 3C) and in situ hybridization (Figure S5). Together with the expression of GFP protein, this finding documented that the hairpin was successfully expressed in these oocytes (Figures 3A$_c$ and 3A$_f$). Conversely, Myt1 mRNA expression was not decreased in the knockdown oocytes. An assay of pools of five oocytes randomly selected from the different groups confirmed an increase in Cdc2-kinase activity after the knockdown of mWee1B (Figure 3D).

When mWee1B hairpin RNA was injected in vitro into either wild-type oocytes maintained in meiotic arrest with cilostamide or into PDE3A$^{-/-}$ mouse oocytes,

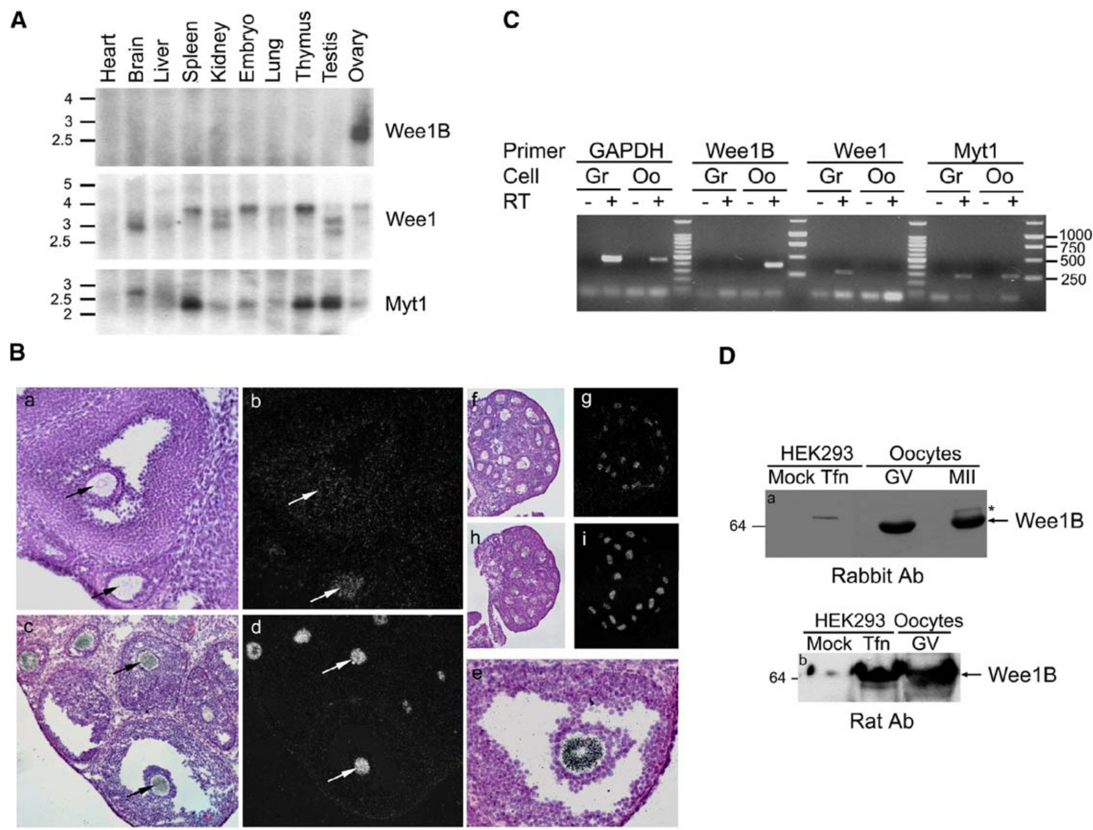

Figure 2. Expression of Wee1, Wee1B, and Myt1 in Mouse Tissues

(A) Northern-blot analysis of Wee1-kinase-family mRNA expression in mouse tissues. Mouse-tissue RNA blot was hybridized with the [^{32}P]-labeled mWee1B, Wee1, and Myt1 cDNA probes, respectively. Numbers at left refer to the migration of DNA standards (kb).

(B) In situ hybridization was performed with Wee1 (B_a, B_b, B_f, and B_g) and mWee1B (B_c, B_d, B_h, B_i, and B_e) RNA in the mouse ovary section. ($B_{a–e}$) are from PMSG-treated mouse ovaries (22 days old), and ($B_{f–i}$) are from 10-day-old immature mouse ovaries. Arrows mark the cytoplasm of oocytes in the bright- and dark-field micrographs. Hybridization with sense RNAs produced no localized signal (data not shown). The following magnifications were used: for ($B_{a–d}$), 200×; for ($B_{f–I}$), 40×; and for (B_e), 600×.

(C) The expression of Wee1-kinase-family mRNAs was determined by RT-PCR in the mouse ovary and oocyte. Approximately 5 ng RNA from granulosa cells (Gr) and total RNA from oocyte lysate corresponding to six oocytes (Oo) were used for RT-PCR. The sizes of the PCR products are 480 bp for GAPDH, 380 bp for mWee1B, 300 bp for Wee1, and 270 bp for Myt1.

(D) Western-blot analysis of mWee1B protein in mouse oocytes. Transfected HEK293 cell lysate (1 μg for blot a, 10 μg for blot b) or total oocyte lysate from mouse GV or MII oocytes (300 oocytes per lane) were prepared and fractionated on 8% SDS-PAGE. Gels were then transferred to the PVDF membrane and probed with affinity-purified mWee1B antibodies raised in rabbits (D_a) or rats (D_b). The construct transfected in HEK293 cells had an additional 45 amino acids compared to the native mWee1B as a result of V5 tagging at the C terminus. Asterisks indicate possible phosphorylated forms of mWee1B in the MII stage of mouse oocytes.

which remain arrested in the meiotic prophase even after isolation, approximately 30% resumed meiosis I and extruded a polar body (Figure S4). These data suggest that mWee1B acts downstream of PKA in maintaining the blockade of oocyte maturation. Several contributing factors may explain why downregulation of mWee1B in vitro or in vivo does not produce meiotic maturation in all oocytes. One possibility is that the RNAi produces variable downregulation averaging a 50% reduction in mWee1B mRNA and that the mWee1B protein has a long half-life. Indeed, preliminary observations suggest that mWee1B is more stable than Wee1 when expressed in frog oocytes (S.J.H., unpublished data). Another possibility is that the turnover of Tyr15 phosphorylation is slow in oocytes in the absence of an active Cdc25 phosphatase responsible for dephosphorylation and activation of Cdc2. Thus, a decrease in the activity of the mWee1 kinase is reflected only in a partial decrease in phosphorylation of Cdc2, unless Cdc25 is activated at the same time. Indeed, if PKA phosphorylates Cdc25 and promotes 14-3-3 binding, Cdc25 should be largely inactive in PDE3A$^{-/-}$ oocytes. Finally, it is also possible that a loss of mWee1B activity is compensated by the presence of Wee1 or Myt1 kinase. Wee1 kinase immunoreactivity has been observed in mouse oocytes, even though a decline has been reported in competent oocytes [15], and Myt1 mRNA is detectable in GV oocytes (Figure 2C). Little increase in Myt1 expression was detected in oocytes where mWee1B is downregulated (Figure 3C).

Serine 15 of mWee1B Is a Major PKA Phosphorylation Site, and PKA Phosphorylation Increases mWee1B Kinase Activity

Because of its isolation as a putative PKA substrate, we further investigated the possibility that PKA can directly

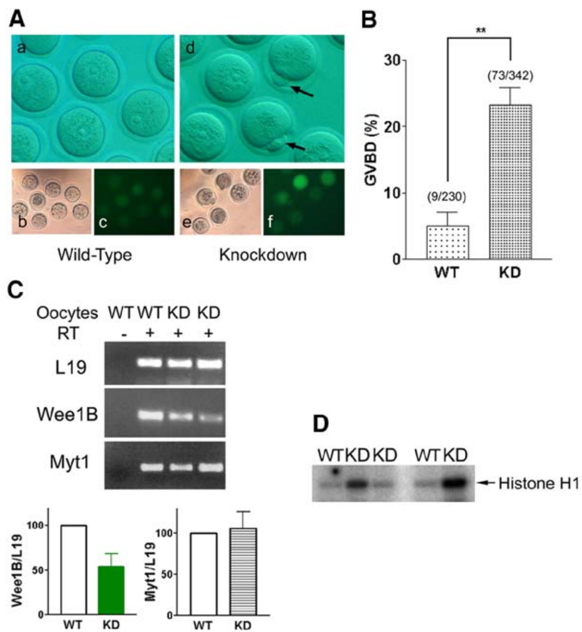

Figure 3. RNAi of mWee1B Induces GVBD in Oocytes

(A) Oocytes from wild-type ($A_{a–c}$) or GFP/mWee1B/RNAi ($A_{d–f}$) mice were collected in 10 μM cilostamide and incubated for 24 hr. GVBD progression and MII stage were scored by loss of GV and emission of the polar body. Arrows indicate the polar body in the MII stage oocytes. Oocytes were monitored for GFP expression with a fluorescence microscope (A_b, A_c, A_e, and A_f) to confirm the expression of the RNAi construct.

(B) Percentage of GVBD and MII stage of oocytes from mWee1B knockdown (KD) and wild-type (WT) mice. GVBD was detected by disappearance of the germinal vesicle, and MII oocytes were counted by extrusion of the polar body after 24 hr incubation with 10 μM cilostamide. Numbers above the bars indicate the number of the GVBD stage and of total isolated oocytes ($p = 0.0061$ with paired t test).

(C) Decrease in the endogenous mWee1B mRNA levels in oocytes from the mWee1B knockdown mice. RT-PCR was performed on pools of six randomly selected oocytes from wild-type or mWee1B knockdown mice with L19, mWee1B, and Myt1 specific primers described in the Supplemental Experimental Procedures. A representative experiment of the three performed is reported. Bars report the average ratio of measurements ± standard error of the mean (SEM) from three separate experiments.

(D) Activity of Cdc2 in wild-type and knockdown mice was measured after 24 hr incubation in cilostamide. The kinase assay followed published procedures [16]. Each lane contains five randomly selected oocytes from two wild-type and three knockdown mice.

phosphorylate mWee1B. Recombinant tagged *mWee1B* was expressed in HEK293 cells, immunoprecipitated with V5 antibody, and incubated with catalytic subunit of PKA. Mouse Wee1B was efficiently phosphorylated by the PKA catalytic subunit (Figure 4A), and this phosphorylation was completely blocked by the PKA inhibitor, H89 (Figure S6). Because Wee1 kinases possess autophosphorylation activity [18], [^{32}P] incorporation into mWee1B was detected in wild-type mWee1B without PKA addition (Figure 4A, lane 4). However, when a kinase-dead mutant of mWee1B was used, the autophosphorylation was abolished, but the PKA phosphorylation was still evident (Figure 4A). Because a major difference in [^{32}P] incorporation was observed between kinase-dead and wild-type mWee1B, we hypothesized that PKA phosphorylation enhances the autophosphorylation of mWee1B. Indeed, when a phosphotyrosine-specific antibody was used, an increase in mWee1B tyrosine phosphorylation was observed in wild-type mWee1B but not in the kinase-dead mutant (Figure 4A, middle panel). The PKA concentration required for half-maximal phosphorylation of mWee1B (IC_{50}) is 75 nM catalytic subunit (Figure S7A). Thus, it is likely that most of mWee1B is phosphorylated in prophase-arrested oocytes because half-maximal inhibition of maturation by PKA occurs at a concentration of approximately 0.1 μM [4].

In order to identify the PKA phosphorylation site of mWee1B, we incubated several maltose binding protein (MBP) fusion proteins with PKA and [γ-^{32}P]ATP. The phosphorylation of the MBP 1–124 region (Figure 4B) indicated that the N terminus of mWee1B contains the major PKA phosphorylation site(s). Although two putative PKA consensus sequences at S15 and T170 were identified, S15 is likely the major phosphorylation site because the 124–546 fusion protein is not phosphorylated by PKA.

Even though it is distinct from the known consensus 14-3-3 binding sequence (RSXS) found in *Xenopus* and mammalian Wee1, mWee1B displays a related sequence at the C terminus (RGTSSV, Figure S1A), opening the possibility of a PKA phosphorylation that is at the carboxyl terminus and promotes 14-3-3 binding. Indeed, some minor PKA phosphorylation could be detected with the C terminus of mWee1B (Figure 4B, 479–556). This phosphorylation was not explored further because we could not detect coimmunoprecipitation of 14-3-3 protein with unphosphorylated or phosphorylated mWee1B when overexpressed in HEK293 cells (data not shown). To confirm whether S15 is the major PKA phosphorylation site, we generated GST-mWee1B fusion proteins containing only the N terminus of mWee1B with point mutations in the putative PKA phosphorylation sites, and we performed an in vitro phosphorylation (Figure 4C). A major decrease in [^{32}P] incorporation was observed after the mutation of S15 to alanine. A similar result was obtained with a S15A mutant of full-length mWee1B (Figure 4D).

The functional consequence of the phosphorylation of S15 was determined by mRNA injection of the phosphorylation mutants of mWee1B in frog oocytes. When the mRNA of the S15D mutant that mimics the phosphorylation state was injected into *Xenopus* oocytes, this mutant inhibited progesterone-induced maturation more efficiently than wild-type mWee1B (Figure 4E). The 15D mutant-enhanced inhibition of oocyte GVBD is due to direct phosphorylation and inhibition of Cdc2 kinase (data not shown). Conversely, inhibition by the S15A mutant did not differ from that of wild-type mWee1B, possibly because progesterone treatment causes a decrease in PKA activity. That mWee1B activity is increased after S15 phosphorylation was further demonstrated with a reconstitution assay. Histone H1 phosphorylation by MII oocyte extracts was determined after incubation with different mWee1B constructs. MII extracts contained dephosphorylated and therefore activated Cdc2/cyclinB complex, which phosphorylates histone H1. The S15D mutant inhibited H1 phosphorylation more potently than wild-type mWee1B (Figure 4F). These results strongly suggest that PKA phosphorylates S15 of

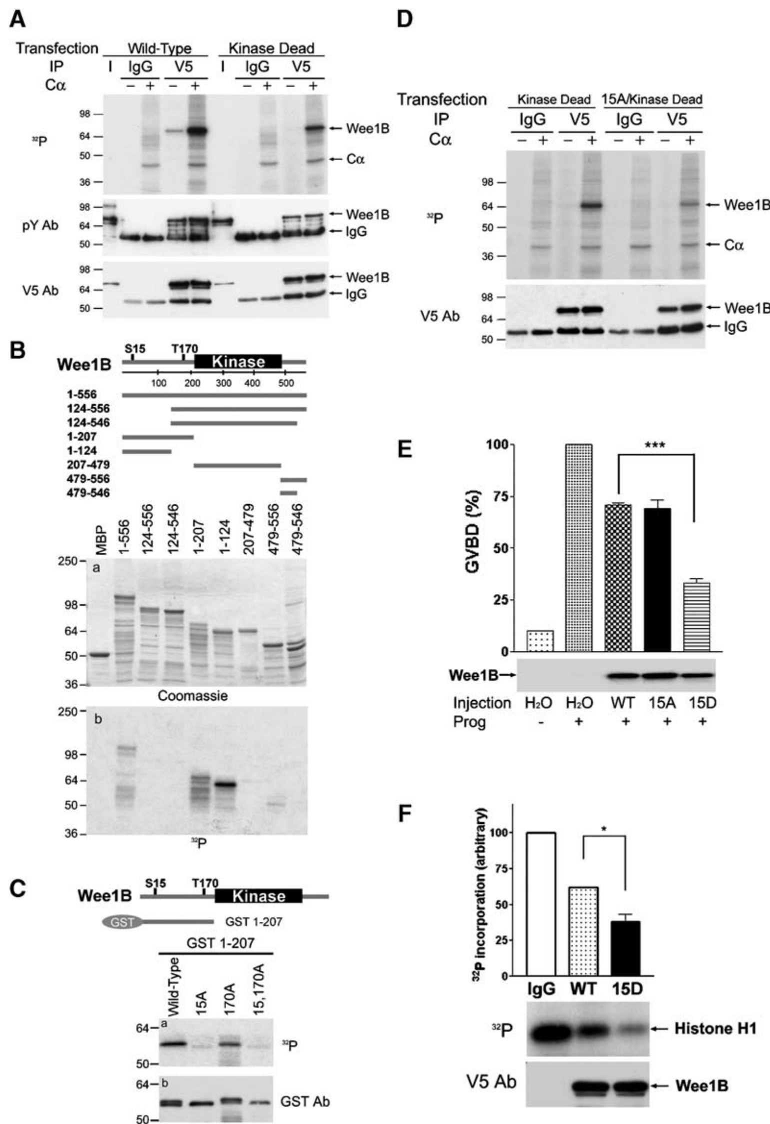

Figure 4. Serine 15 of mWee1B Is Phosphorylated Directly by PKA, and PKA Phosphorylation Increases mWee1B Activity

(A) Wild-type and a kinase-dead mutant of mWee1B were transfected into HEK293 cells, and immunoprecipitation was carried out with IgG or V5 antibodies. The immune complex was incubated with or without the PKA catalytic subunit and 5 μCi [γ-^{32}P]ATP for 30 min, and the phosphorylation state was detected by SDS-PAGE and autoradiography. The first and sixth lanes contain 10% of input for immunoprecipitation to monitor the expression of transfected genes. The tyrosine residue phosphorylation of wild-type and the kinase-dead form of mWee1B were measured by immunoblotting with phosphotyrosine-specific antibodies (PY20). The amounts of loaded protein were determined by western blot with V5 antibodies. A representative experiment of the three performed is reported.

(B) Serine 15 of mWee1B is the major PKA phosphorylation site. A schematic diagram of full length mWee1B and the MBP-fused truncated form is shown. Various MBP-containing truncated forms of mWee1B were generated and expressed in the bacterial system. After purification by affinity chromatography, purified fusion proteins were incubated with PKA catalytic subunits in [γ-^{32}P] ATP for 10 min at 30°C. The radiolabeled fusion proteins were detected by autoradiogram (B_b). The expression and purification of MBP fusion proteins were monitored by Coomassie-blue staining (B_a).

(C) GST-fusion mutants of the mWee1B N terminus were generated and purified by affinity chromatography. The N terminus of mWee1B (amino acid residue 1–207) was constructed to GST fusion protein, and Serine 15 (15A) and threonine 170 (170A) sites were mutated to alanine (15,170A for double mutant). After purification, the fusion proteins were incubated with the PKA catalytic subunit and [γ-^{32}P]ATP for 10 min at 30°C. The radiolabeled fusion proteins were detected by autoradiography (C_a), and the amount of fusion protein and phosphorylation of fusion proteins also were confirmed by mobility shift in the western-blot analysis with GST antibodies (C_b).

(D) Phosphorylation of the wild-type and S15A mutant of mWee1B. Experimental conditions for expression of the mutant and in vitro phosphorylation are the same as those described in Figure 4A.

(E) Effect of Serine 15 mutants of mWee1B on progesterone-induced *Xenopus* oocyte maturation. Five nanograms of mRNA coding for wild-type, S15A, and S15D mutants of mWee1B or H_2O (vehicle) was injected into *Xenopus* oocytes. Twelve hours later, oocytes were treated with 500 nM of progesterone, and meiotic maturation was monitored up to 9 hr. The expression of injected mRNAs was detected by western blot with V5 antibodies. This result is one representative experiment of seven separate experiments performed ($p = 0.0002$ with paired t test).

(F) Effect of Serine 15 mutant of mWee1B on Cdc2 kinase activity. The kinase assay was carried out with five MII-stage oocytes per lane as a source of activated Cdc2/cyclinB. Immune complexes of wild-type and a S15D mutant of mWee1B were incubated with MII oocyte lysate for 30 min, and incubation continued after the addition of Histone H1 and 5 μCi [γ-^{32}P]ATP for an additional 30 min. After separation on 15% SDS PAGE, the phosphorylation state and amounts of loaded protein were detected by autoradiography (upper panel) or western blot with V5 antibodies (lower panel). A representative experiment of the four performed is reported. The bar graph reports the ratio of [^{32}P] incorporation into Histone H1 and the amount of immune complexes ($p = 0.0195$ with paired t test).

mWee1B, and this phosphorylation causes an increase in mWee1 activity toward Cdc2/cyclinB with consequent enhanced inhibitory effects on oocyte maturation. PKA activation of Wee1B was difficult to detect in this assay paradigm because PKA directly phosphorylates histone H1 and increases the background (data not shown).

The most straightforward hypothesis on how cAMP inhibits the MPF activity is that PKA directly phosphorylates and regulates the Cdc25 phosphatase and Wee1/Myt1 kinase. In agreement with this view, it has been reported that PKA phosphorylates and inhibits the function of Cdc25 through 14-3-3 binding in frog oocytes [19], even though the timing of this phosphorylation is inconsistent with a decrease in PKA activity. Here, we show that mWee1B is a substrate for PKA in vitro and have identified the major phosphorylation site in S15; the site lies within a consensus sequence

for PKA (KKLS). This region is conserved only partially when human and frog sequences are compared. Rather than the PKA consensus, a related sequence is present in human and frog Wee1 orthologs and includes a positively charged R or K upstream of an S. Further experiments are necessary to determine when dephosphorylation of mWee1B occurs and whether the regulation observed in the mouse can be extended to other species. There is precedence for Wee1 activation by phosphorylation. MAP kinase activates XeWee1A via phosphorylation during the first mitotic cell cycle, producing a delay in G2/M [20]. However, the phosphorylation sites and exact mechanisms leading to an increase in the Wee1 kinase activity have not been elucidated. XeWee1A also is positively regulated by Chk1-kinase-dependent phosphorylation on the C terminus of XeWee1 [21]. Thus, in addition to phosphorylations that promote targeting to the proteasome and Wee1 degradation [22], there is an additional mode of Wee1-kinase regulation controlling its activation. It is likely that multiple phosphorylations are required for fine regulation of the function of this kinase that is essential for G2/M transition.

Conclusions

With this report, we demonstrate that mWee1B is a maternally-expressed kinase involved in the phosphorylation of Cdc2 and maintenance of meiotic arrest in oocytes. Mouse Wee1B is exclusively expressed in maturing oocytes, and downregulation of this mRNA causes meiotic resumption under conditions that normally maintain meiotic arrest. Conversely, overexpression of mWee1B delays meiotic maturation in *Xenopus* oocytes, and its kinase activity is required for this inhibition. PKA directly phosphorylates mWee1B in a concentration-dependent manner, at least in vitro. Serine 15 in the N terminus of mWee1B is a major PKA phosphorylation site, and phosphorylation at this site enhances the autophosphorylation activity of mWee1B and its ability to inhibit Cdc2 and oocyte maturation. Thus, mWee1B is likely to be a key regulator of meiotic maturation in mouse oocytes. In view of the expression of mWee1B during MII, further experiments are necessary to explore its function during the first embryonic divisions. These findings solidify the concept that PKA maintains meiotic arrest via dual regulation of the kinase that phosphorylates Cdc2 and the phosphatase that dephosphorylates the same sites. The two limbs of the regulatory loop most likely work in a synergistic fashion to maintain an inactive Cdc2/cyclinB complex.

Supplemental Data

Supplemental Data include Supplemental Results and Discussion, Supplemental Experimental Procedures, and seven supplemental figures and are available with this article online at: http://www.current-biology.com/cgi/content/full/15/18/1670/DC1/.

Acknowledgments

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Accession Numbers

The GenBank accession number for the mouse Wee1B sequence reported in this paper is DQ011691.