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Report

Oocytes Progress beyond Prophase in the Presence of DNA Damage

Petros Marangos[1,2,3,*] and John Carroll[1,*]
[1]Department of Cell and Developmental Biology, Division of Biosciences, University College London, Gower Street, London WC1E 6BT, UK
[2]Department of Biological Applications and Technology, University of Ioannina, Ioannina 45110, Greece
[3]Department of Molecular Oncology, BSRC "Alexander Fleming," Athens 16672, Greece

Summary

In the female germline, DNA damage has the potential to induce infertility and even to lead to genetic abnormalities that may be propagated to the resulting embryo [1, 2]. The protracted arrest in meiotic prophase makes oocytes particularly susceptible to the accumulation of environmental insults, including DNA damage. Despite this significant potential to harm reproductive capacity, surprisingly little is known about the DNA damage response in oocytes. We show that double-strand breaks in meiotically competent G2/prophase-arrested mouse oocytes do not prevent entry into M phase, unless levels of damage are severe. This lack of an efficient DNA damage checkpoint is because oocytes fail to effectively activate the master regulator of the DNA damage response pathway, ATM (ataxia telangiectasia mutated) kinase. In addition, instead of inhibiting cyclin B-CDK1 through destruction of Cdc25A phosphatase, oocytes utilize an inhibitory phosphorylation of Cdc25B. We conclude that oocytes are the only nontransformed cells that fail to launch a robust G2 phase DNA damage checkpoint and that this renders them sensitive to genomic instability.

Results and Discussion

Mouse Oocytes Enter M Phase in the Presence of DNA Damage

In somatic cells, at the G2 stage, DNA double-strand breaks cause the autophosphorylation and subsequent activation of the master DNA damage checkpoint regulator, ATM (ataxia telangiectasia mutated) kinase [3, 4]. ATM activation is the initial step in the establishment of the G2 checkpoint following DNA damage in the form of DNA double-strand breaks. At the sites of damage, ATM phosphorylates histone H2AX (γH2AX), which forms a platform for the recruitment of the necessary checkpoint and repair factors [5]. The establishment of the checkpoint requires the ATM-mediated activation of checkpoint kinases, Chk1 and Chk2 [6, 7]. Cell-cycle arrest at the G2 stage is a result of a Chk1/Chk2-dependent degradation of the Cdc25A phosphatase [8–10], but in some cases the inhibitory phosphorylation of Cdc25B or Cdc25C [11, 12], leading to a failure to activate cyclin B-CDK1 [9–11].

The few studies that have been undertaken in fully grown oocytes hint at the possibility of a limited DNA damage response [13, 14]. To investigate the DNA damage checkpoint in oocytes, we initially tested the ability of etoposide, a topoisomerase II inhibitor, to cause double-strand breaks as it does in somatic cells [15]. The data verified that treatment with etoposide (5 μg/ml for 3 hr) causes DNA damage as evidenced by the presence of phosphorylated H2AX at Ser139 (γH2AX) [16] (Figure 1A). Having identified that DNA damage can be detected at this concentration, we examined the ability of oocytes to progress into M phase of meiosis I after a 3 hr exposure to increasing concentrations (5–100 μg/ml) of etoposide. Despite the presence of DNA damage at the lower concentrations tested, oocytes were capable of undergoing germinal vesicle breakdown (GVBD) and entering M phase at near normal kinetics (Figure 1B). Etoposide-treated oocytes that undergo GVBD still show a 6-fold greater γH2AX staining intensity compared to controls (see Figures S1A and S1B available online). Thus, the absence of cell-cycle arrest in the presence of DNA damage is likely to be the result of an inadequate DNA damage checkpoint rather than highly efficient DNA repair mechanisms. As the etoposide concentrations increased, the rate and the ability to undergo GVBD were gradually reduced (Figure 1B). In order to achieve an effective G2/prophase arrest, etoposide concentrations at the upper end of the dose response (50–100 μg/ml, 3 hr) were necessary; inducing greater than 80% of oocytes to remain arrested at the G2/prophase stage after 5 hr of culture (Figure 1B).

The ability to enter M phase in the presence of DNA damage increases with prolonged culture (Figure 1C). Even at 100 μg/ml where only approximately 20% of oocytes had undergone GVBD after 5 hr, this increased to over 60% after 20 hr. This recovery from checkpoint arrest was apparently not due to DNA repair because γH2AX staining was present in oocytes that had undergone GVBD (Figures S1C and S1D). More likely, the increase in GVBD may be attributed to "checkpoint adaptation," a mechanism seen in somatic cells that causes the inactivation of the G2 checkpoint despite extensive and irreversible DNA damage [17].

High Levels of Exposure to Etoposide Activate an ATM/Chk1-Dependent DNA Damage Checkpoint

Having found that oocytes appear not to launch a powerful DNA damage checkpoint, we next sought to define the presence of the major players in the response to DNA damage, namely ATM and Chk1. In this series of experiments, oocytes were exposed to etoposide as for the previous experiment and labeled with antibodies to detect γH2AX and active ATM as determined by monitoring the autophosphorylation of ATM at Ser1981 (ATM-P) [3] (Figures 2A–2C). Exposure to increasing concentrations of etoposide caused an increase in γH2AX as well as ATM activation (Figures 2A and 2B). By correlating the fluorescence staining in Figure 2A with the G2/prophase arrest data from Figure 1B at different etoposide concentrations, we confirmed a positive relationship between ATM/γH2AX and G2 arrest (Figure 2C).

The correlation between ATM staining and the occurrence of G2/prophase arrest suggests that ATM is involved in oocyte checkpoint activation. To test the specificity of the proposed role for ATM, we induced DNA damage in the presence of an

*Correspondence: p.marangos@ucl.ac.uk (P.M.), j.carroll@ucl.ac.uk (J.C.)

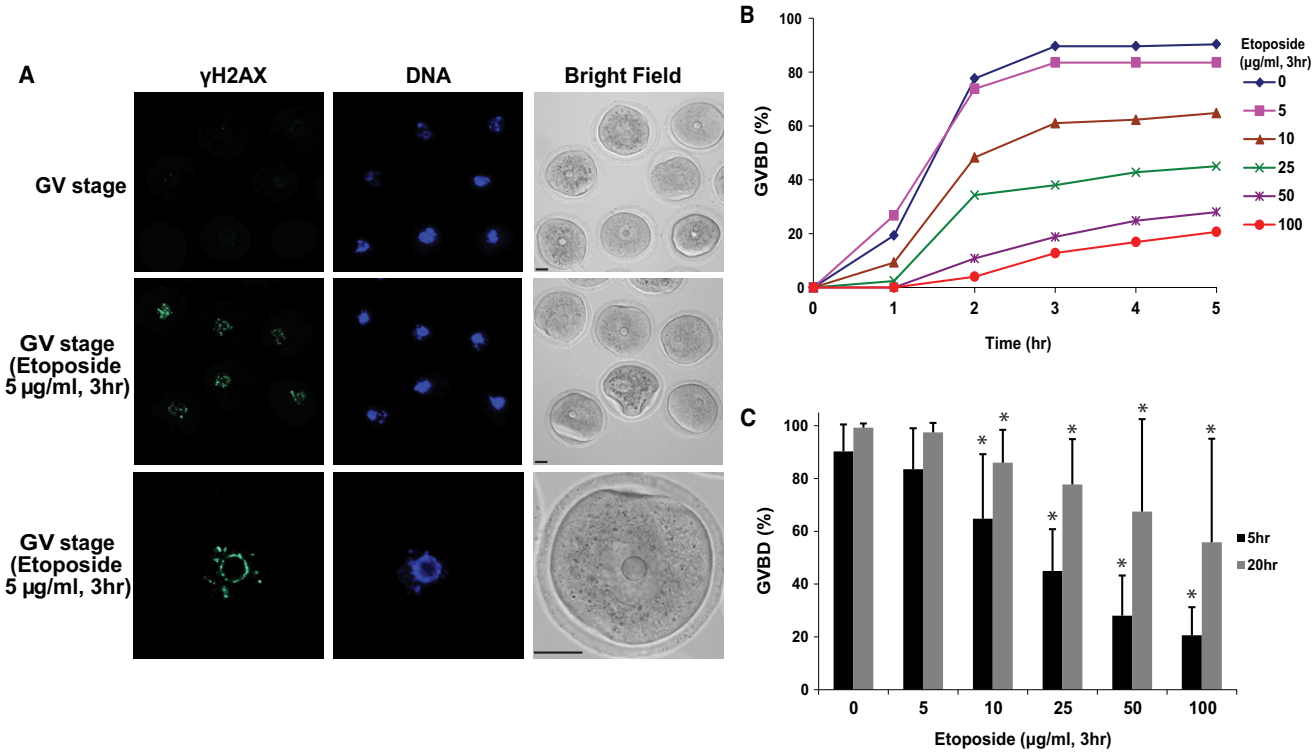

Figure 1. DNA Damage Allows M Phase Entry in Mouse Oocytes

(A) Treatment with 5 μg/ml etoposide for 3 hr causes DNA damage in oocytes. Histone H2AX becomes activated at the sites of damage (γH2AX) and is used as an immunofluorescence marker for the detection of double-strand breaks. Hoechst is used for DNA staining. G2/prophase-arrested oocytes (GV stage) are fixed at the end of treatment with or without 5 μg/ml etoposide. n = 3 experiments. Total oocyte number is ≥20. Scale bars represent 20 μm. The third lane of panels shows a single oocyte in higher magnification.

(B) The effects of increasing concentrations of etoposide on the rate and timing of GVBD. Etoposide treatment is applied during the GV stage. Following a 3 hr treatment, oocytes are released from arrest (release from 3-isobutyl-1-methylxanthine [IBMX]) (T = 0 hr), and the timing of GVBD is monitored. Note that 5 μg/ml of etoposide does not affect the rate and timing of GVBD.

(C) Oocytes that remain arrested 5 hr after exposure to high concentrations of etoposide eventually (20 hr after exposure) enter M phase (GVBD). The data for the 5 hr group are the same as that in (B). Asterisks on the 5 and 20 hr bars denote a significant difference from nontreated controls (0 μg/ml of etoposide) at the 5 or 20 hr time point, respectively (*$p < 0.0001$, unpaired t test). (B) and (C) show data from five experiments. Total oocyte number is ≥50.

See also Figure S1.

ATM kinase inhibitor (ATMi). Under DNA damage conditions that normally induce the checkpoint in mouse oocytes (50 μg/ml, 3 hr etoposide), the inhibition of ATM alleviates the checkpoint and allows M phase entry (Figure 2D). Thus, when the checkpoint is invoked at high levels of DNA damage, it is mediated via an ATM-dependent pathway.

The most studied downstream effector of the ATM-dependent G2 checkpoint is Chk1 kinase. We found that etoposide leads to a dose-dependent activation of Chk1 through the phosphorylation at Ser317 [7] (Figures S2A–S2C). These data also reveal that Chk1 remains almost completely inactive at 5 μg/ml of etoposide, which likely underlies the reason why a DNA damage checkpoint is not activated under these conditions of DNA damage (Figures S2A–S2C). However, at doses of 100 μg/ml etoposide, there is a major increase in phosphorylated Chk1. The possibility of a direct role of Chk1 in the activation of the oocyte DNA damage checkpoint was tested by examining progression through the G2/M transition in the presence of a dominant-negative form of the kinase (kinase-dead Chk1-D130A; Chk1Δ) [7]. These data show that inhibition of Chk1 kinase during DNA damage leads to oocytes escaping the G2 checkpoint (Figure S2D). Thus, at the levels of DNA damage induced by high concentrations of etoposide, we conclude that it is an ATM/Chk1-dependent checkpoint that is primarily responsible for maintaining the G2/prophase arrest.

Oocytes Are Refractory to DNA Damage due to a Limited Ability to Activate ATM

The presence of an ATM/Chk1 pathway shows that oocytes have a mechanism for sensing DNA damage, yet many oocytes enter M phase despite the presence of DNA damage as indicated by γH2AX staining. The reasons for this lack of fidelity in the induction of the G2 DNA damage checkpoint may be caused by the detector (ATM) not being very sensitive or, alternatively, in a failure of the resultant signal to impinge on the cell-cycle machinery and induce arrest at G2. To test the sensitivity of the detector, we monitored the levels of γH2AX and ATM-P in the nuclei of oocytes and blastomeres at different concentrations of etoposide (Figure 3). To detect early and immediate signs of DNA damage and to avoid saturation of the DNA damage response, we reduced the exposure time to etoposide from 3 hr to 15 min. As in somatic cells [7, 18], blastomeres showed significant activation of ATM following DNA damage. ATM activity was high even at the low levels of etoposide tested (10 μg/ml), and the level of

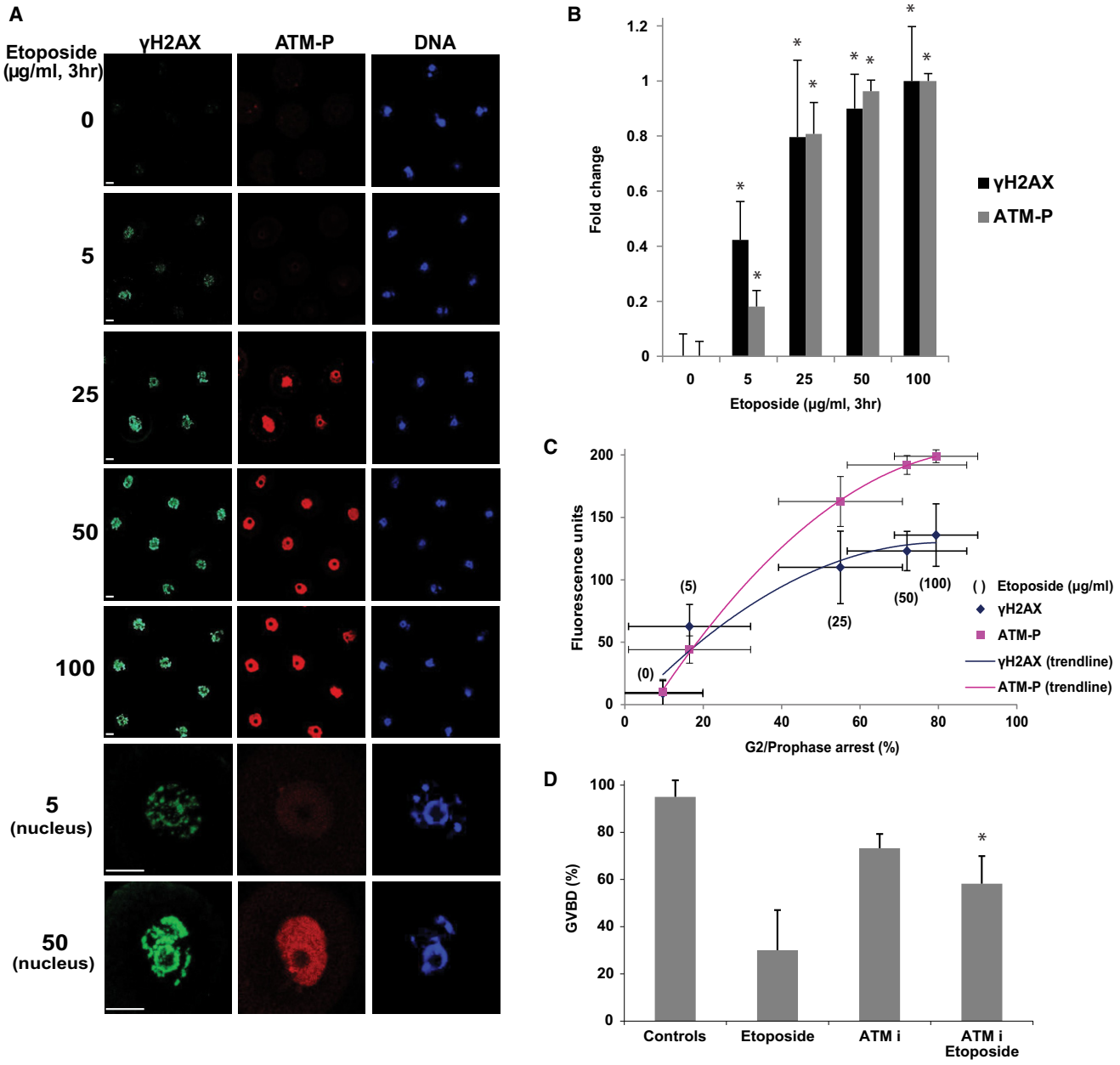

Figure 2. An ATM-Dependent DNA Damage Checkpoint Can Be Established at High Levels of Exposure to Etoposide

(A) Increasing concentrations of etoposide lead to a respective increase in the levels of γH2AX and ATM-P (Ser1981). Oocytes were arrested at G2/prophase at the time of the experiment. Hoechst is used for DNA staining, and primary antibodies against γH2AX and ATM-P have been used for immunofluorescence and confocal imaging. Representative images from three independent experiments with ≥30 oocytes at each concentration are shown. Last two lanes show single oocytes in higher magnification. Scale bars represent 20 μm.

(B) Relationship between etoposide and activation of H2AX and ATM. The data from (A) are presented as normalized fold change. Fold change in ATM and H2AX activation was determined by the formula $F - F_0/F_{max} - F_0$. F is the mean value of each etoposide treatment, and F_0 is the control value where no etoposide is used (0 μg/ml). F_{max} is the highest value of the experiment (exposure to 100 μg/ml etoposide). Error bars indicate SD. Asterisks denote a significant difference from nontreated controls (0 μg/ml of etoposide) (*$p < 0.0001$, unpaired t test).

(C) Graph of data extracted from Figures 1B and 2A to illustrate a positive relationship between levels of G2/prophase arrest and active H2AX and ATM. Fluorescence levels are extracted from (A) and are the same used in (B). Vertical error bars indicate SD of fluorescence levels. The G2/prophase arrest levels and horizontal SD bars are from the 5 hr time point in Figure 1B.

(D) ATM kinase inhibition (10 μg/ml ATMi) allows entry into M phase (GVBD) in the presence of DNA damage (50 μg/ml etoposide, 3 hr). The level of GVBD is determined 5 hr after release from IBMX. ATMi is used for the duration of the experiment. Data are from three experiments; total oocyte number is ≥30. The asterisk denotes a significant difference from non-ATMi-treated oocytes subjected to etoposide-induced DNA damage (*$p < 0.0001$, unpaired t test).

See also Figure S2.

activation continued to increase through the concentration range. In contrast, ATM activation in oocytes remained at basal levels before undergoing an increase at the maximal concentration of 100 μg/ml (Figures 3A–3C). This activation profile revealed that for intermediate concentrations of etoposide (25 and 50 μg/ml), there was a 15- to 20-fold increase in

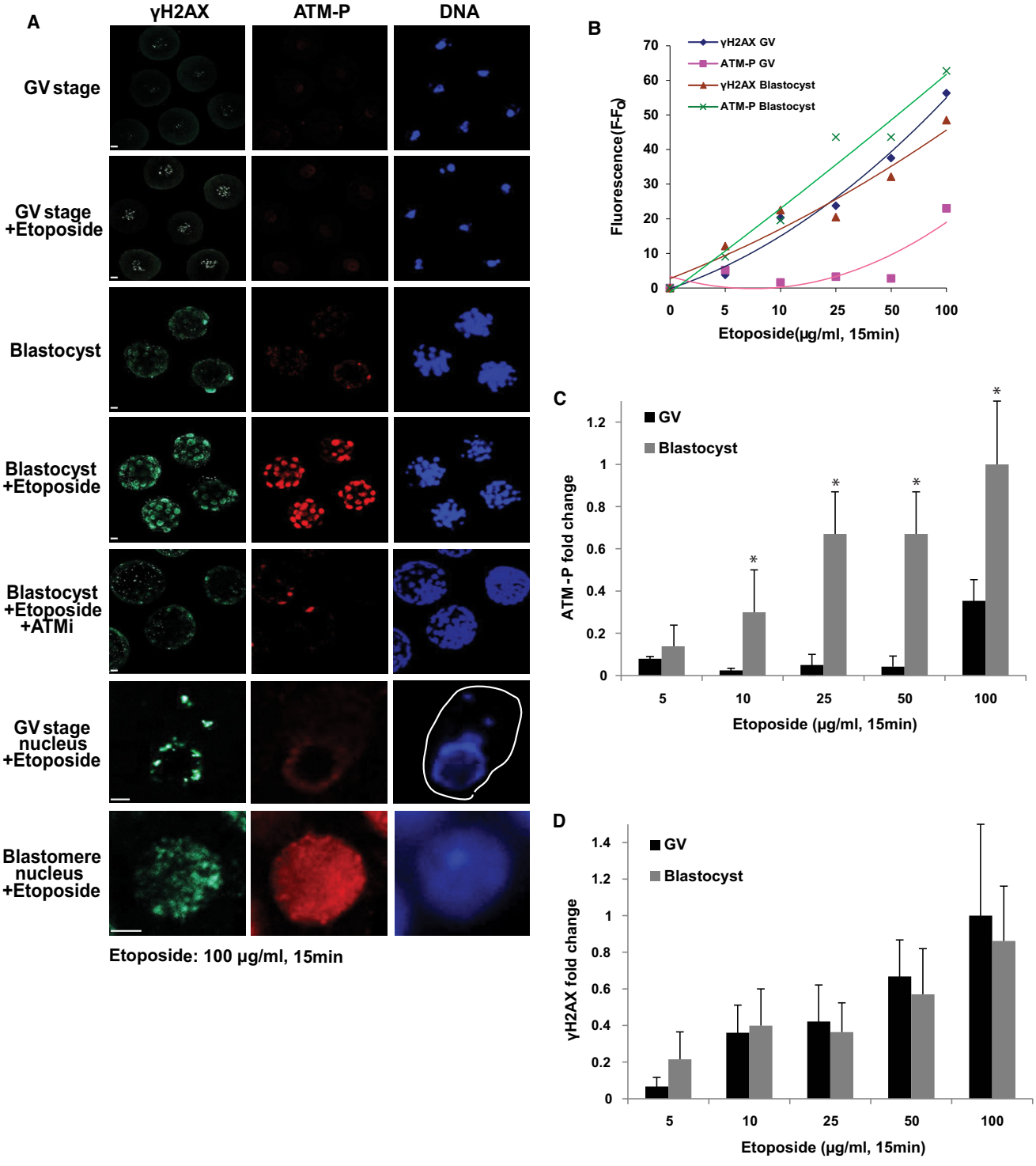

Figure 3. The Insensitivity of the DNA Damage Checkpoint in Oocytes Is Due to Reduced ATM Activity

(A) Oocytes and blastocysts (blastomeres) were treated with 100 μg/ml etoposide for 15 min and examined under similar conditions for activation of ATM and H2AX. ATMi abolishes detection of active ATM, verifying the specificity of the ATM-P (Ser 1981) antibody. Note that the intensity of staining for both markers of DNA damage increases with increasing concentration of etoposide. n = 3 experiments. Total oocyte number is ≥30. Total blastocyst number is ≥20. Scale bars represent 20 μm. Last two lanes show single nuclei in higher magnification. White shape denotes the boundaries of the oocyte nucleus. Scale bars represent 5 μm.

(B) The increase in immunolabeling for active H2AX and ATM at increasing concentrations of etoposide. Plot is of raw fluorescence data obtained by measuring pixel intensity of nuclear area of oocytes and individual blastomeres at different concentrations of etoposide. Data extracted from (A) correspond to the 100 μg/ml etoposide treatment in (B). We used the formula $F - F_0$ (F, F_0 measured as in Figure 2). This formula was used to remove nonspecific staining (ATMi shows that basal ATM staining is nonspecific), which allows for a better comparison of the different cell types. All the identifiable blastomeres of different blastocysts were measured.

the activation of ATM in cells of the blastocysts compared to oocytes. This relative difference was 3-fold at a concentration of 100 μg/ml, as there is an increase in ATM activity in oocytes at this concentration (Figures 3B and 3C).

A similar comparison of the fold change of γH2AX levels does not reveal any significant difference in activation between oocytes and blastomeres (Figures 3B and 3D). It is possible that in oocytes H2AX becomes phosphorylated independently of ATM, as has been shown in the liver and kidneys of ATM knockout mice and in pachytene germ cells [19, 20]. It is unlikely that the difference in ATM activity is caused by the asynchronous nature of the cell cycle in individual blastomeres, because the levels of total and activated ATM have been shown to be similar in asynchronous and G2 cells [21]. Therefore, limited ATM activation appears to be one of the main reasons for the insensitivity of the G2 DNA damage checkpoint in oocytes. Possible reasons for this limited ATM activity could be low levels of expression of ATM, a possibility that is supported by monitoring total ATM in oocytes as compared to growing oocytes and blastocysts (Figure S3). The absence in G2/prophase oocytes of extracellular signal-regulated kinase (ERK) 1/2-dependent ATM activation could also be involved [22]. In addition, ATM activity is known to be influenced by chromatin structure [3], and G2/prophase-arrested oocytes are known to have a distinct chromatin configuration [23] and constitutively active histone deacetylases [24], either of which may limit the response to DNA damage (Figure S4).

Inhibition of Cdc25B, and Not Degradation of Cdc25A, Is Responsible for the Establishment of the Oocyte DNA Damage Checkpoint

We then investigated downstream of ATM to determine whether the DNA damage pathway is coupled to the cell-cycle machinery. In somatic cells, the establishment of the G2 checkpoint requires the phosphorylation of Cdc25A by Chk1 that targets the phosphatase for degradation through the action of SCF (Skp1-Cullin-F box) ligase [9, 10]. We found that Cdc25A is stable in oocytes even after the extended DNA damage protocol (100 μg, 3 hr) that induces high levels of ATM activation (Figure 4A). Furthermore, in an effort to directly phosphorylate Cdc25A, we overexpressed Chk1, but despite this treatment being very effective at inducing G2 arrest (Figure S2E), Cdc25A levels remained unaffected (Figure S2F). These observations and the fact that a G2 DNA damage checkpoint can be induced at high levels of DNA damage (Figures 1B, 2, and 4A) suggest that cell-cycle regulators other than Cdc25A must be involved. A strong candidate is the vital for female meiosis Cdc25 isoform, Cdc25B [25]. Inhibition of Cdc25B through phosphorylation at Ser323 by protein kinase A (PKA) is responsible for the physiological G2/prophase arrest in fully grown oocytes [26, 27]. We tested the possibility that rather than Cdc25A degradation, checkpoint activation requires phosphorylation-dependent inhibition of Cdc25B. We found that increasing concentrations of etoposide lead to a respective rise in the inhibitory phosphorylation levels of Cdc25B at Ser323. Ser323 phosphorylation is low following treatment with 5 μg/ml etoposide but

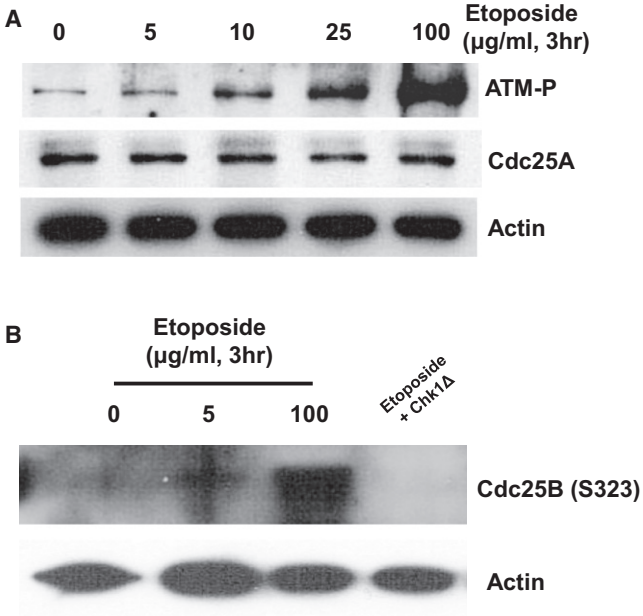

Figure 4. The Oocyte DNA Damage Checkpoint Is Induced by Inhibition of Cdc25B rather than Degradation of Cdc25A

(A) Increasing concentrations of etoposide cause a respective increase in the activation of ATM (ATM-P) but do not cause Cdc25A degradation.

(B) Cdc25B Ser323 phosphorylation coincides with high concentrations of etoposide and therefore establishment of the DNA damage checkpoint. Ser323 phosphorylation is Chk1 dependent because Chk1 inactivation, through the use of Chk1Δ, leads to the inhibition of phosphorylation (100 μg/ml etoposide). For this experiment, IBMX, which is used to sustain PKA activity and therefore maintain oocytes in G2/prophase arrest, is replaced by roscovitine, a CDK1 inhibitor, in order to keep oocytes arrested without PKA-dependent Cdc25B phosphorylation. Samples (50 oocytes/sample) for western blotting were collected immediately after treatment. Actin was used as loading control. n = 2 experiments.

See also Figure S2.

increases dramatically at high levels of DNA damage (100 μg/ml etoposide) (Figure 4B). Therefore, the oocyte G2 checkpoint activated by high concentrations of etoposide appears to be caused by inhibitory phosphorylation of Cdc25B at Ser323. We then tested whether phosphorylation of Cdc25B was dependent on Chk1 activation by inducing DNA damage when Chk1 is inhibited by expression of Chk1Δ. Inhibition of Chk1 completely abolishes Cdc25B phosphorylation (Figure 4B). Thus, although it is formally possible that phosphorylation of all Cdc25 isoforms may contribute [6, 11, 12], our data show that an ATM/Chk1-dependent inhibitory phosphorylation of Cdc25B is involved in the activation of the G2 checkpoint in oocytes following extended DNA damage.

We have described how fully grown G2/prophase-arrested mouse oocytes respond to DNA damage. Surprisingly, we find that the oocyte, despite its central role in propagation of the species, is capable of detecting DNA damage but has a greatly damped ability to establish a G2 checkpoint. Oocytes can therefore progress from G2/prophase and enter the

(C and D) Data from (B) presented as normalized fold change of ATM-P (C) and H2AX (D). Fold change is measured as in Figure 2 (ATM F_{max}, blastomeres exposed to 100 μg/ml; H2AXγ F_{max}, GV stage oocytes exposed to 100 μg/ml). Error bars indicate SD. Asterisks denote a significant difference from the oocyte (GV) value for the same concentration of etoposide (*$p < 0.0001$, unpaired t test).

See also Figures S3 and S4.

meiotic divisions, despite the presence of substantial levels of DNA damage. It is unclear why oocytes show a marked reduction in the ability to launch a DNA damage response, including ATM activation and checkpoint establishment through degradation of Cdc25A. The differences may represent a conflict between normal meiotic function and the ability to establish an ATM-mediated G2 DNA damage checkpoint, as well as a modified importance of Cdc25 isoforms in meiosis and mitosis [25, 26]. It is possible, however, that the oocyte is capable of repairing minor DNA damage during the prolonged G2/prophase arrest and the lengthy meiotic M phases, prior to embryonic development. Alternatively, it is possible that DNA damage triggers follicular atresia leading to oocyte degeneration [28]. Nevertheless, the G2 DNA damage checkpoint deficiency in meiosis raises concerns regarding the mammalian oocyte's susceptibility to DNA damaging insults. A major issue is in the potential impact of assisted reproductive technologies where it may be expected that exposure to light [29], increased free radical generation [29], and potential contaminants in culture media [30] could serve to increase DNA damage. The ability to progress through meiosis while carrying such damage will almost certainly lead to embryos with decreased developmental potential.

Supplemental Information

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2012.03.063.

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References

1. Jacquet, P., Adriaens, I., Buset, J., Neefs, M., and Vankerkom, J. (2005). Cytogenetic studies in mouse oocytes irradiated in vitro at different stages of maturation, by use of an early preantral follicle culture system. Mutat. Res. *583*, 168–177.
2. Tease, C. (1983). X-ray-induced chromosome aberrations in dictyate oocytes of young and old female mice. Mutat. Res. *119*, 191–194.
3. Bakkenist, C.J., and Kastan, M.B. (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature *421*, 499–506.
4. Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L.V., Kolettas, E., Niforou, K., Zoumpourlis, V.C., et al. (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature *444*, 633–637.
5. Paull, T.T., Rogakou, E.P., Yamazaki, V., Kirchgessner, C.U., Gellert, M., and Bonner, W.M. (2000). A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr. Biol. *10*, 886–895.
6. Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Mol. Cell *40*, 179–204.
7. Gatei, M., Sloper, K., Sorensen, C., Syljuäsen, R., Falck, J., Hobson, K., Savage, K., Lukas, J., Zhou, B.B., Bartek, J., and Khanna, K.K. (2003). Ataxia-telangiectasia-mutated (ATM) and NBS1-dependent phosphorylation of Chk1 on Ser-317 in response to ionizing radiation. J. Biol. Chem. *278*, 14806–14811.
8. Bassermann, F., Frescas, D., Guardavaccaro, D., Busino, L., Peschiaroli, A., and Pagano, M. (2008). The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell *134*, 256–267.
9. Busino, L., Donzelli, M., Chiesa, M., Guardavaccaro, D., Ganoth, D., Dorrello, N.V., Hershko, A., Pagano, M., and Draetta, G.F. (2003). Degradation of Cdc25A by beta-TrCP during S phase and in response to DNA damage. Nature *426*, 87–91.
10. Mailand, N., Falck, J., Lukas, C., Syljuâsen, R.G., Welcker, M., Bartek, J., and Lukas, J. (2000). Rapid destruction of human Cdc25A in response to DNA damage. Science *288*, 1425–1429.
11. Boutros, R., Lobjois, V., and Ducommun, B. (2007). CDC25 phosphatases in cancer cells: key players? Good targets? Nat. Rev. Cancer *7*, 495–507.
12. Darzynkiewicz, Z., Traganos, F., and Wlodkowic, D. (2009). Impaired DNA damage response—an Achilles' heel sensitizing cancer to chemotherapy and radiotherapy. Eur. J. Pharmacol. *625*, 143–150.
13. Bradshaw, J., Jung, T., Fulka, J., Jr., and Moor, R.M. (1995). UV irradiation of chromosomal DNA and its effect upon MPF and meiosis in mammalian oocytes. Mol. Reprod. Dev. *41*, 503–512.
14. Mailhes, J.B., Marchetti, F., Phillips, G.L., Jr., and Barnhill, D.R. (1994). Preferential pericentric lesions and aneuploidy induced in mouse oocytes by the topoisomerase II inhibitor etoposide. Teratog. Carcinog. Mutagen. *14*, 39–51.
15. Wu, C.C., Li, T.K., Farh, L., Lin, L.Y., Lin, T.S., Yu, Y.J., Yen, T.J., Chiang, C.W., and Chan, N.L. (2011). Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide. Science *333*, 459–462.
16. Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., and Bonner, W.M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. *273*, 5858–5868.
17. Yoo, H.Y., Kumagai, A., Shevchenko, A., Shevchenko, A., and Dunphy, W.G. (2004). Adaptation of a DNA replication checkpoint response depends upon inactivation of Claspin by the Polo-like kinase. Cell *117*, 575–588.
18. Bartek, J., and Lukas, J. (2007). DNA damage checkpoints: from initiation to recovery or adaptation. Curr. Opin. Cell Biol. *19*, 238–245.
19. Koike, M., Mashino, M., Sugasawa, J., and Koike, A. (2008). Histone H2AX phosphorylation independent of ATM after X-irradiation in mouse liver and kidney in situ. J. Radiat. Res. (Tokyo) *49*, 445–449.
20. Turner, J.M., Mahadevaiah, S.K., Fernandez-Capetillo, O., Nussenzweig, A., Xu, X., Deng, C.X., and Burgoyne, P.S. (2005). Silencing of unsynapsed meiotic chromosomes in the mouse. Nat. Genet. *37*, 41–47.
21. Pandita, T.K., Lieberman, H.B., Lim, D.S., Dhar, S., Zheng, W., Taya, Y., and Kastan, M.B. (2000). Ionizing radiation activates the ATM kinase throughout the cell cycle. Oncogene *19*, 1386–1391.
22. Wei, F., Xie, Y., Tao, L., and Tang, D. (2010). Both ERK1 and ERK2 kinases promote G2/M arrest in etoposide-treated MCF7 cells by facilitating ATM activation. Cell. Signal. *22*, 1783–1789.
23. De La Fuente, R. (2006). Chromatin modifications in the germinal vesicle (GV) of mammalian oocytes. Dev. Biol. *292*, 1–12.
24. Ma, P., Pan, H., Montgomery, R.L., Olson, E.N., and Schultz, R.M. (2012). Compensatory functions of histone deacetylase 1 (HDAC1) and HDAC2 regulate transcription and apoptosis during mouse oocyte development. Proc. Natl. Acad. Sci. USA *109*, E481–E489.
25. Lincoln, A.J., Wickramasinghe, D., Stein, P., Schultz, R.M., Palko, M.E., De Miguel, M.P., Tessarollo, L., and Donovan, P.J. (2002). Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. Nat. Genet. *30*, 446–449.
26. Oh, J.S., Han, S.J., and Conti, M. (2010). Wee1B, Myt1, and Cdc25 function in distinct compartments of the mouse oocyte to control meiotic resumption. J. Cell Biol. *188*, 199–207.
27. Pirino, G., Wescott, M.P., and Donovan, P.J. (2009). Protein kinase A regulates resumption of meiosis by phosphorylation of Cdc25B in mammalian oocytes. Cell Cycle *8*, 665–670.
28. Soleimani, R., Heytens, E., Darzynkiewicz, Z., and Oktay, K. (2011). Mechanisms of chemotherapy-induced human ovarian aging: double strand DNA breaks and microvascular compromise. Aging (Albany NY) *3*, 782–793.
29. Schultz, R.M. (2007). Of light and mouse embryos: less is more. Proc. Natl. Acad. Sci. USA *104*, 14547–14548.
30. Kastrop, P.M., de Graaf-Miltenburg, L.A., Gutknecht, D.R., and Weima, S.M. (2007). Microbial contamination of embryo cultures in an ART laboratory: sources and management. Hum. Reprod. *22*, 2243–2248.