Oocyte Elimination Through DNA Damage Signaling from CHK1/CHK2 to p53 and p63

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ABSTRACT Eukaryotic organisms have evolved mechanisms to prevent the accumulation of cells bearing genetic aberrations. This is especially crucial for the germline, because fecundity and fitness of progeny would be adversely affected by an excessively high mutational incidence. The process of meiosis poses unique problems for mutation avoidance because of the requirement for SPO11-induced programmed double-strand breaks (DSBs) in recombination-driven pairing and segregation of homologous chromosomes. Mouse meiocytes bearing unrepaired meiotic DSBs or unsynapsed chromosomes are eliminated before completing meiotic prophase I. In previous work, we showed that checkpoint kinase 2 (CHK2; CHEK2), a canonical DNA damage response protein, is crucial for eliminating not only oocytes defective in meiotic DSB repair (*e.g.*, *Trip13*^{Gt} mutants), but also *Spo11*$^{-/-}$ oocytes that are defective in homologous chromosome synapsis and accumulate a threshold level of spontaneous DSBs. However, rescue of such oocytes by *Chk2* deficiency was incomplete, raising the possibility that a parallel checkpoint pathway(s) exists. Here, we show that mouse oocytes lacking both p53 (TRP53) and the oocyte-exclusive isoform of p63, TAp63, protects nearly all *Spo11*$^{-/-}$ and *Trip13*$^{Gt/Gt}$ oocytes from elimination. We present evidence that checkpoint kinase I (CHK1; CHEK1), which is known to signal to TRP53, also becomes activated by persistent DSBs in oocytes, and to an increased degree when CHK2 is absent. The combined data indicate that nearly all oocytes reaching a threshold level of unrepaired DSBs are eliminated by a semiredundant pathway of CHK1/CHK2 signaling to TRP53/TAp63.

KEYWORDS checkpoints; meiosis; mouse; oocytes; transducer kinases

OOCYTE development in females begins *in utero*, when primordial germ cells enter and complete early stages of meiosis, including recombination, before arresting perinatally in a stage called dictyate. In the first few days after birth, the oocytes undergo folliculogenesis, in which they become surrounded by flattened granulosa cells (Peters 1969). The resulting "primordial follicles" constitute the finite oocyte pool present in women and female mice of reproductive age (Findlay *et al.* 2015).

Meiocytes have developed mechanisms for minimizing the production of gametes with genetic anomalies such as unrepaired double-strand breaks (DSBs) and meiotic chromosome asynapsis. Mouse oocytes bearing mutations that prevent repair of programmed SPO11/TOPOVIBL-induced DSBs, which are essential for recombination-mediated pairing and synapsis of homologous chromosomes (Baudat *et al.* 2000; Romanienko and Camerini-Otero 2000; Mahadevaiah *et al.* 2001; Robert *et al.* 2016), are eliminated by a DNA damage checkpoint (Di Giacomo *et al.* 2005). The molecular nature of this checkpoint was first revealed as involving signaling of CHK2 to TRP53 and the oocyte-specific TransActivation domain of p63, known as TAp63 (Suh *et al.* 2006; Livera *et al.* 2008), by studies exploiting a hypomorphic allele of *Trip13* (Bolcun-Filas *et al.* 2014). This allele (*Trip13*^{Gt}) causes sterility in both males and females and is useful because it is defective for DSB repair but not synapsis (Li and Schimenti 2007). Deficiency of *Chk2* protected against oocyte loss and restored fertility of *Trip13*$^{Gt/Gt}$ females. *Chk2* also plays a role in the DNA damage checkpoint in spermatocyte meiosis (Pacheco *et al.* 2015).

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Defects in chromosome synapsis during meiotic prophase I also triggers death of most oocytes. There are at least two mechanisms underlying this "synapsis checkpoint." One is meiotic silencing of unsynapsed chromatin (MSUC), a process of extensive heterochromatinization and transcriptional downregulation, which appears to function primarily in situations where only about one to three chromosomes are unsynapsed (Kouznetsova *et al.* 2009; Cloutier *et al.* 2015). A second mechanism pertains to oocytes that are highly asynaptic, in which the silencing machinery is presumably overwhelmed (Kouznetsova *et al.* 2009). Surprisingly, this mechanism is also highly dependent on the DNA damage checkpoint. The mechanistic basis for this is the formation of a threshold level (~10) of SPO11-independent, spontaneously arising DSBs (Carofiglio *et al.* 2013; Rinaldi *et al.* 2017). Approximately 61% of *Spo11*$^{-/-}$ oocytes, which do not form programmed meiotic DSBs and consequently are defective for homologous chromosome synapsis (but do exhibit some nonhomologous synapsis), reach this threshold, leading to depletion of the entire ovarian reserve (primordial oocytes) by a few weeks after birth (Baudat *et al.* 2000; Romanienko and Camerini-Otero 2000). *Chk2* deletion rescued oocyte numbers to ~25% of wild type (WT), indicating that most are either eliminated by an alternative pathway or succumb nonspecifically from a catastrophically high number of DSBs (up to ~100, with an average of ~50/cell) (Rinaldi *et al.* 2017). Similarly, *Chk2* deficiency rescued *Trip13*$^{Gt/Gt}$ oocytes to around one-third of WT levels (Bolcun-Filas *et al.* 2014), raising the possibility that the same CHK2-independent pathway may be active in both cases.

Here, we tested the possibility that the incomplete rescue of oocytes mentioned above is due to the existence of another pathway either distinct or complementary to that involving CHK2, but which also involves TRP53 and TAp63. Our results indicate that this is indeed the case, and that most *Spo11*$^{-/-}$ and TRIP13-deficient oocytes are ultimately eliminated by the combined activation of TRP53 and TAp63.

Materials and Methods

Mice

Alleles used in this study and their genetic backgrounds were previously described (Bolcun-Filas *et al.* 2014). Comparisons of compound mutants and controls utilized littermates whenever possible, otherwise animals from related parents or different litters from the same parents were used. Animal work was approved by Cornell's Institutional Animal Care and Use Committee, under protocol 2004-0038 to J.C.S.

Histology and follicle quantification

Ovaries were fixed in Bouin's solution, embedded in paraffin, serially sectioned at 6 μm, and stained with hematoxylin and eosin. Follicle identification (Myers *et al.* 2004) and quantification was as described (Bolcun-Filas *et al.* 2014). Graphs and statistical analysis were performed with GraphPad Prism8. Comparisons of follicle numbers across genotypes were performed using an ordinary one-way ANOVA test.

Western blot analysis of protein phosphorylation

Ovaries from postnatal (3–5 day old) mice were collected and divided into control and treatment groups. Treated groups were exposed to 3 Gy of ionizing radiation (IR) as described above and proteins were extracted 3 hr post irradiation. Ovaries from all the females in the litter were dissected and individually frozen while genotyping was performed. Proteins from ovaries of selected genotypes were pooled into groups of four and extracted with lysis buffer containing: 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, protease inhibitors (#11836153001; Complete Mini-Roche), and phosphoprotease inhibitors (#04906845001; PhosSTOP-Roche). Proteins were resolved on 4–20% gradient acrylamide gels (#4561093; Bio-Rad, Hercules, CA), transferred to PVDF transfer membranes (#IPVH00010; Millipore, Bedford, MA) and blocked with 5% BSA or 5% nonfat milk according to the manufacturer datasheet for the corresponding antibody. Membranes were probed with rabbit anti-phospho-Chk1 (Ser345) (1:750, 133D3; Cell Signaling Technology), rabbit anti-p53 (rodent-specific 1:750, D2H90; Cell Signaling Technology), mouse anti-p63 (1:500, CM163A; Biocare Medical), and rabbit anti-DDX4/MVH (1:750, 13840; Abcam).

Data availability statement

Mouse strains that were not obtained from others will be made available upon request. Supplemental Material, Figure S1 is a Western blot that is a biological replicate of Figure 2. Table S1 contains primordial and total follicle counts for the genotypes included in Figure 1. Supplemental material available at figshare: https://doi.org/10.25386/genetics.12092187.

Results and Discussion

To address whether a CHK2-independent pathway exists that can eliminate oocytes bearing unrepaired DSBs, we utilized two mutant models, *Trip13*^{Gt} and a *Spo11* null (*Spo11*$^{-}$). Virtually all *Trip13*$^{Gt/Gt}$ oocytes are eliminated due to failure to repair SPO11-dependent DSBs (Li and Schimenti 2007) by the end of pachynema (Rinaldi *et al.* 2017). *Chk2* deficiency rescued around one-third of these oocytes, and these rescued oocytes gave rise to viable offspring (Bolcun-Filas *et al.* 2014). Although disruption of either of CHK2's downstream phosphorylation targets, TRP53 and TAp63, enabled little or no rescue of *Trip13*$^{Gt/Gt}$ oocytes, *Trip13*$^{Gt/Gt}$ *TAp63*$^{-/-}$ *Trp53*$^{+/-}$ mice exhibited oocyte rescue similar to that of *Trip13*$^{Gt/Gt}$ *Chk2*$^{-/-}$ mice (Bolcun-Filas *et al.* 2014). At the time of that report, double mutants (*TAp63*$^{-/-}$ *Trp53*$^{-/-}$; the former allele ablating the TA domain only) were not assayed for the extent to which they could rescue *Trip13*$^{Gt/Gt}$ oocytes. We hypothesized that the inability to achieve full oocyte rescue in either *Trip13*$^{Gt/Gt}$ *TAp63*$^{-/-}$ *Trp53*$^{+/-}$ or *Trip13*$^{Gt/Gt}$ *Chk2*$^{-/-}$ females was due to one of the following:

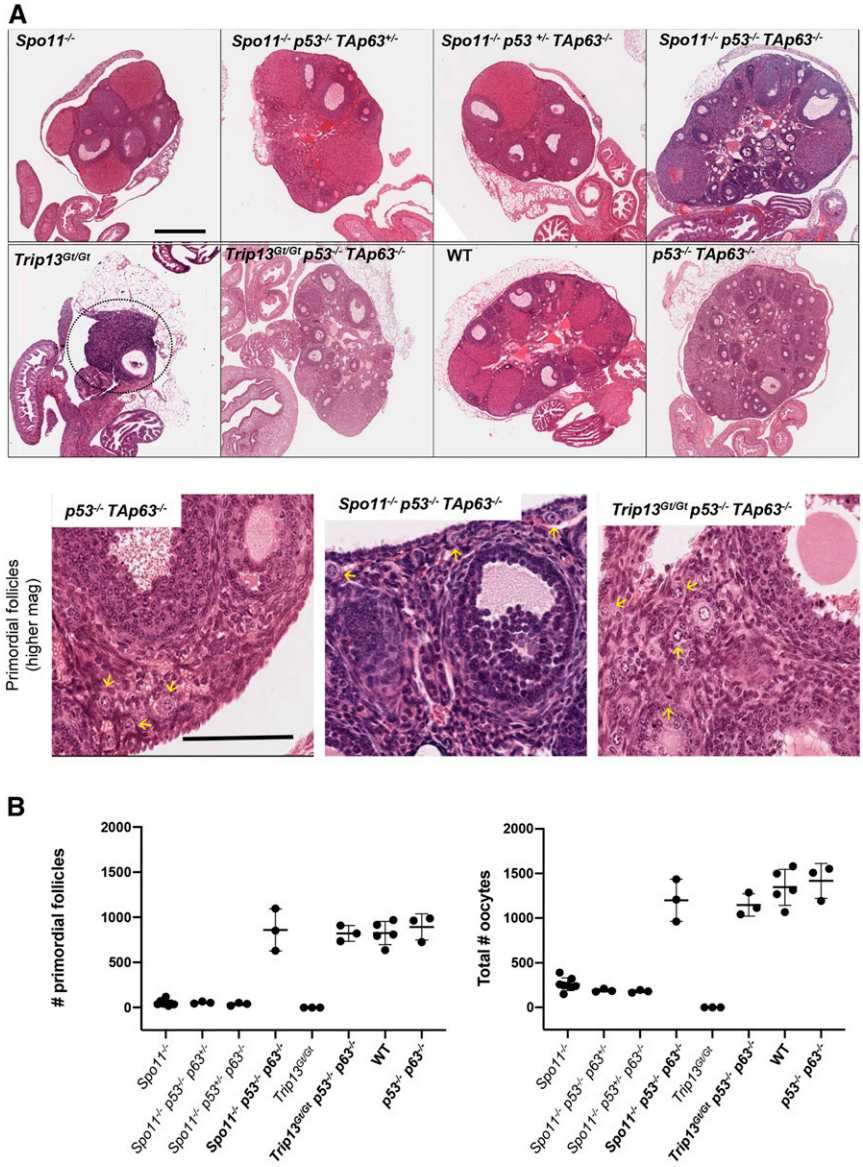

Figure 1 Rescue of SPO11- and TRIP13-deficient oocytes by compound deletion of *p53* and *TAp63*. (A) Hematoxylin and eosin stained ovaries from 2-month-old mice of the indicated genotypes. The top two rows are images are from histological sections through the approximate center of the ovaries. Bar, 500 μm. The dashed circle indicates the residual *Trip13*$^{Gt/Gt}$ ovary. The bottom row shows higher magnification images of selected genotypes. Bar, 100 μm. Yellow arrows indicate examples of primordial follicles. (B) Oocyte quantification. To the left is the quantification of primordial follicles [$P <$ 0.0001 for all oocyte rescued (in bold) genotypes compared to nonrescued genotypes; $P = 0.92$ for oocyte rescued genotypes *vs.* WT and *Trp53*$^{-/-}$ *TAp63*$^{-/-}$ ovaries]. To the right are total oocytes (from all stages of follicles) from individual ovaries ($P < 0.0001$ for all oocyte rescued genotypes compared to nonrescued genotypes; $P = 0.33$ for oocyte rescued genotypes *vs.* WT and *Trp53*$^{-/-}$ *TAp63*$^{-/-}$ ovaries). Oocyte quantification data are presented in Table S1. Genotype abbreviations are as follows: *TAp63* is abbreviated as *p63*; WT, wild type.

(1) the number of DSBs was so high that elimination of most oocytes occurred in a checkpoint-independent fashion; (2) residual TRP53 activity in the *Trip13*$^{Gt/Gt}$ *TAp63*$^{-/-}$ *Trp53*$^{+/-}$ mice sufficed to trigger apoptosis in many oocytes; and/or (3) a parallel checkpoint pathway is active in *Chk2*$^{-/-}$ oocytes.

To test these possibilities, we first assessed the ovarian reserve in *Trip13*$^{Gt/Gt}$ *Trp53*$^{-/-}$ *TAp63*$^{-/-}$ mice. Remarkably, the numbers of primordial and later-stage oocytes in the triple mutants were indistinguishable from WT (Figure 1, A and B). This result indicates that essentially all *Trip13*$^{Gt/Gt}$ oocytes are eliminated by checkpoint signaling to TRP53 and TAp63, thereby eliminating hypothesis 1, but supporting hypothesis 2. This result is also consistent with hypothesis 3, implying that another pathway or kinase is signaling to these two effector proteins.

Next, we tested whether the incomplete rescue of *Spo11*$^{-/-}$ oocytes by *Chk2* deletion is also potentially a consequence of checkpoint signaling to TAp63 and TRP53 via a different transducer. Accordingly, we bred mice that lacked either or both of these proteins in the context of *Spo11* deficiency. Oocyte numbers in *Spo11*$^{-/-}$ mice that were also homozygous for mutations in either *Trp53* or *TAp63* and heterozygous for a mutation in the other (*Trp53*$^{-/-}$ *TAp63*$^{+/-}$ and *Trp53*$^{+/-}$ *TAp63*$^{-/-}$) were indistinguishable from *Spo11* nulls; nearly the entire oocyte reserve was depleted after 2 months of age, as is characteristic for *Spo11* deficiency (Di Giacomo *et al.* 2005). However, homozygosity for both *Trp53* and *TAp63* dramatically restored oocyte numbers to WT levels (Figure 1, A and B). It is unclear why heterozygosity for either *Trp53* or *TAp63* in the context of nullizygosity for the other gene failed to rescue any *Spo11*$^{-/-}$ oocytes unlike *Trip13*$^{Gt/Gt}$ oocytes, but we can speculate that other factors could play a role. These include strain background, enhanced recognition by DNA damage

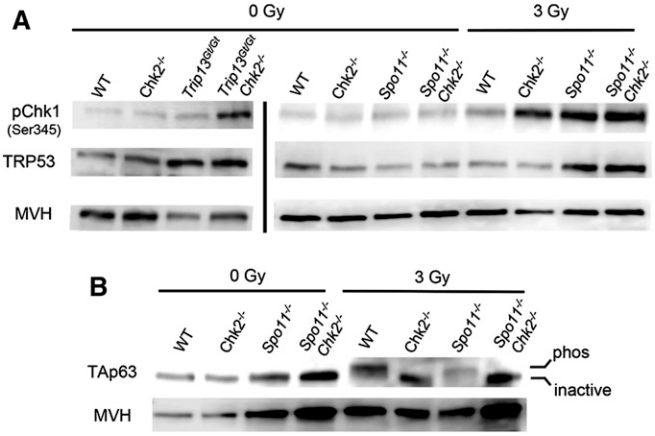

Figure 2 Increased CHK1 activation and p53 stabilization, but not TAp63 activation, in CHK2-deficient oocytes. (A) CHK1 phosphorylation in oocytes is stimulated by induced or meiotic DSBs. Shown are Western blots probed with indicated antibodies. Each lane contains total protein extracted from four ovaries (postnatal day 3–5) that were either exposed or not to 3 Gy of ionizing radiation (IR). Ovaries were harvested for protein extraction 3 hr post-IR. The blots on the left, separated by a vertical bar from those on the right, were from a different blot and different protein samples and mice. The same two blots (left and right) were stripped and reprobed sequentially with the three antibodies. A biological replicate is shown in Figure S1. Note that the decreased MVH levels in *Trip13*$^{Gt/Gt}$ ovaries is due to reduction in oocytes. (B) Activation of the TAp63 isoform is dependent on DNA damage and CHK2 signaling, not asynapsis. Shown is a Western blot probed sequentially for TAp63 and the germ cell marker MVH. Each lane contains protein extracted from ovaries as described in A. An upward shift in the band indicates the presence of the active (phosphorylated) *vs.* inactive TAp63. A biological replicate is shown in Figure S1.

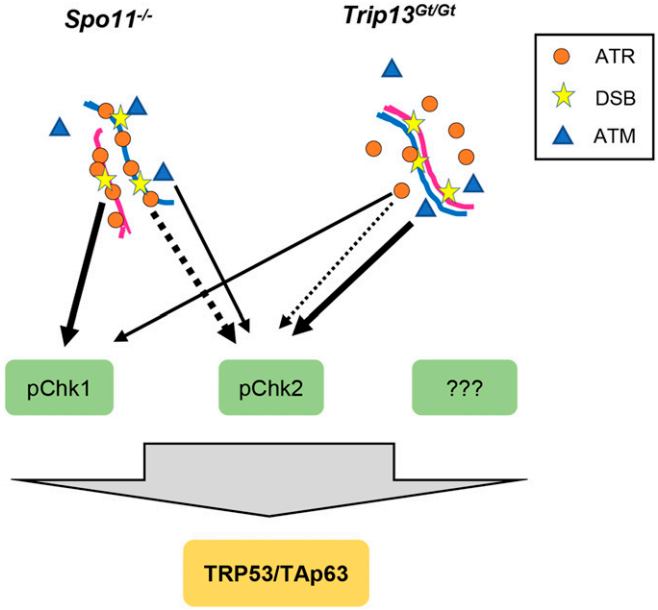

Figure 3 Model of checkpoint signaling in mouse oocytes. We propose that all DSB damage signaling in oocytes requires activation of TRP53 and TAp63 for complete oocyte elimination. The dashed lines represent non-canonical phosphorylation of CHK2 by ATR, and the thickness of all lines represents the relative amounts of activation in the two indicated mutant situations. We propose that in highly asynaptic *Spo11* mutant oocytes, the "preloading" of ATR as part of the MSUC response leads it to play a larger role in signaling to CHK1 and CHK2 than under situations in which DSBs occur on synapsed chromosomes.

sensors of spontaneous DSBs on asynapsed chromosomes (*Spo11*$^{-/-}$) *vs.* meiotically induced DSBs on synapsed chromosomes (*Trip13*$^{Gt/Gt}$), or greater availability of DNA damage signaling factors in *Spo11*$^{-/-}$ oocytes stimulated by the MSUC response.

These experiments indicate that unrepaired meiotic DSBs, when present at levels above the threshold to trigger their elimination (Rinaldi *et al.* 2017), ultimately cause DNA damage signaling to both TRP53 and TAp63. Additionally, we conclude that one or both of these proteins can be activated not only by CHK2, but also another kinase. In our previous studies, we suggested that the apical kinase ATM, which when activated by DSBs typically phosphorylates CHK2, is not essential for the meiotic DNA damage checkpoint (Bolcun-Filas *et al.* 2014). This conclusion was based on the observation that many *Atm*$^{-/-}$ oocytes, which have extensive DSBs due to ATM's role in negatively regulating SPO11 (Lange *et al.* 2011), are eliminated in a CHK2-dependent manner. We proposed (Bolcun-Filas *et al.* 2014) that the related kinase ATR (ataxia telangiectasia and Rad3 related) might activate CHK2 in oocytes similar to irradiated mitotic cells (Wang *et al.* 2006), which in turn would phosphorylate TAp63 and TRP53. Since ATR primarily activates CHK1, albeit most notably in the context of damage at DNA replication forks, we speculated that CHK1 can trigger death of DSB-bearing oocytes by activating TRP53 in the absence of CHK2. TRP53 is a known target of CHK1 (Shieh *et al.* 2000; Ou *et al.* 2005), and studies have shown that CHK1 can be activated in response to DSBs either in an ATM-dependent (Flaggs *et al.* 1997; Maréchal and Zou 2013) or ATM-independent (Flaggs *et al.* 1997; Balmus *et al.* 2012) manner. Recombinant CHK1 has also been reported to phosphorylate TRP63 *in vitro* (Kim *et al.* 2007).

If this hypothesis is true, CHK1 would be activated in response to DSBs present in oocytes. To test this, we examined levels of CHK1 phosphorylated at Ser345 (pCHK1; indicative of the active form) and TRP53 (which is stabilized by phosphorylation) in various genotypes of neonatal (3–5 days postpartum) ovaries, and also in ovaries exposed to 3 Gy of IR. This level of IR induces ~40 DSBs, as measured by RAD51 foci (a proxy for DSBs) on meiotic chromosomes of oocytes (Rinaldi *et al.* 2017). By way of comparison, DSB repair-defective *Trip13*$^{Gt/Gt}$ have ~65 RAD51 foci persisting abnormally on synapsed pachytene cells (Rinaldi *et al.* 2017). Since ovaries of mutant animals have variable numbers of oocytes, we used the germ-cell-specific marker MVH as a loading reference for the amount of protein corresponding to oocytes in each sample. Ovaries were harvested at 3 hr postirradiation. In unirradiated ovaries, there was no apparent difference between repair-proficient genotypes (WT; *Chk2*$^{-/-}$; *Spo11*$^{-/-}$; *Spo11*$^{-/-}$ *Chk2*$^{-/-}$) in the levels of pCHK1 or TRP53 (Figure 2A). Both unirradiated *Trip13*$^{Gt/Gt}$

and irradiated WT ovaries had slightly elevated pCHK1, with the former also having a marked increase in TRP53 (note MVH levels for intersample comparisons). Interestingly, CHK1 phosphorylation was markedly higher in irradiated *Chk2*$^{-/-}$ and unirradiated *Trip13*^{GtGt}/*Chk2*$^{-/-}$ ovaries (Figure 2A and Figure S1). This implies that the ATM and/or ATR kinases have a higher propensity to activate CHK2 than CHK1 in response to DSBs in meiocytes, but that CHK1 becomes a more prominent target in the absence of CHK2, and is able to trigger a TRP53/TAp63 response that results in apoptosis or eventual DSB repair (Bolcun-Filas *et al.* 2014).

Interestingly, IR also caused a marked increase of pCHK1 in *Spo11*$^{-/-}$ oocytes compared to WT (Figure 2A and Figure S1). Levels of TRP53 were also higher in IR-treated *Spo11*$^{-/-}$ ovaries, but the presence or absence of CHK2 had no consequence (Figure 2A). One possible explanation is that repair of IR-induced DSBs by intersister recombination is inhibited in *Spo11* mutants, because unsynapsed chromosome axes retain HORMAD1/2 proteins that prevent such repair (Carofiglio *et al.* 2013; Rinaldi *et al.* 2017). In contrast, *Chk2*$^{-/-}$ oocytes would retain intersister repair ability, and thus either delay or minimize signaling to TRP53. A second possible cause of increased pCHK1 in irradiated *Spo11*$^{-/-}$ oocytes is that asynapsed chromosomes are more susceptible to IR-induced DNA damage than synapsed chromosomes (as in WT and *Chk2*$^{-/-}$ oocytes). A final possibility is that the presence of ATR on asynapsed chromatin (Turner *et al.* 2004, 2006) (Perera *et al.* 2004; Cloutier *et al.* 2016) facilitates DNA damage signaling to CHK1 under conditions of unrepaired DSBs. This implies that ATR is not only involved in MSUC, but also retains its function as a key component of the DSB repair machinery (Widger *et al.* 2018).

As discussed earlier, there is evidence for two processes that can trigger death of oocytes progressing through meiosis: MSUC (which functions when only a few chromosomes are asynapsed) and spontaneous DSBs, when there is extensive asynapsis as in *Spo11*$^{-/-}$ oocytes. While the experiments above revealed that TRP53 is not activated in unirradiated *Spo11*$^{-/-}$ oocytes, it remained possible that activation of TAp63 could be induced by MSUC or extensive asynapsis. As we previously showed, CHK2 is required for IR-induced phosphorylation of TAp63 (Figure 2B) (Bolcun-Filas *et al.* 2014), which leads to the conversion of the inactive dimerized to the active tetramer form of TAp63 (Deutsch *et al.* 2011). However, we found no evidence for activation (phosphorylation) of TAp63 in unirradiated *Spo11*$^{-/-}$ ovaries (Figure 2B).

In summary, we have shown that mouse oocytes with unrepaired DSBs or extensive asynapsis are culled by a DNA damage response funneling through TRP53 and p63. Some, but not all of the damage signaling to these proteins is transduced by CHK2, and we provide evidence that CHK1 can also perform this function (see model in Figure 3). The relative contributions of these transducer kinases in meiotic DNA damage responses is unclear. Even though the essential nature of CHK1 in embryonic and premeiotic germ cell development (Abe *et al.* 2018) complicates analyses, CHK1 conditional mutagenesis and depletion experiments indicate that this kinase plays a role in modulating cell cycle progression in spermatocytes during meiotic prophase I (Abe *et al.* 2018), and in oocytes at the G2/M checkpoint (Chen *et al.* 2012). A key remaining question is whether CHK1 and CHK2 are the sole direct responders for TRP53 and TRP63, or if another transducer kinase(s), such as casein kinases 1 or 2, function in parallel (Figure 3).

Acknowledgments

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Literature Cited

Abe, H., K. G. Alavattam, Y. Kato, D. H. Castrillon, Q. Pang *et al.*, 2018 CHEK1 coordinates DNA damage signaling and meiotic progression in the male germline of mice. Hum. Mol. Genet. 27: 1136–1149. https://doi.org/10.1093/hmg/ddy022

Balmus, G., M. Zhu, S. Mukherjee, A. M. Lyndaker, K. R. Hume *et al.*, 2012 Disease severity in a mouse model of ataxia telangiectasia is modulated by the DNA damage checkpoint gene Hus1. Hum. Mol. Genet. 21: 3408–3420. https://doi.org/10.1093/hmg/dds173

Baudat, F., K. Manova, J. P. Yuen, M. Jasin, and S. Keeney, 2000 Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking. Mol. Cell 6: 989–998. https://doi.org/10.1016/S1097-2765(00)00098-8

Bolcun-Filas, E., V. D. Rinaldi, M. E. White, and J. C. Schimenti, 2014 Reversal of female infertility by Chk2 ablation reveals the oocyte DNA damage checkpoint pathway. Science 343: 533–536. https://doi.org/10.1126/science.1247671

Carofiglio, F., A. Inagaki, S. de Vries, E. Wassenaar, S. Schoenmakers *et al.*, 2013 SPO11-independent DNA repair foci and their role in meiotic silencing. PLoS Genet. 9: e1003538. https://doi.org/10.1371/journal.pgen.1003538

Chen, L., S.-B. Chao, Z.-B. Wang, S.-T. Qi, X.-L. Zhu *et al.*, 2012 Checkpoint kinase 1 is essential for meiotic cell cycle regulation in mouse oocytes. Cell Cycle 11: 1948–1955. https://doi.org/10.4161/cc.20279

Cloutier, J. M., S. K. Mahadevaiah, E. ElInati, A. Nussenzweig, A. Tóth *et al.*, 2015 Histone H2AFX links meiotic chromosome asynapsis to prophase I oocyte loss in mammals. PLoS Genet. 11: e1005462 (erratum: PLoS Genet. 11: e1005753). https://doi.org/10.1371/journal.pgen.1005462

Cloutier, J. M., S. K. Mahadevaiah, E. ElInati, A. Tóth, and J. Turner, 2016 Mammalian meiotic silencing exhibits sexually dimorphic features. Chromosoma 125: 215–226. https://doi.org/10.1007/s00412-015-0568-z

Deutsch, G. B., E. M. Zielonka, D. Coutandin, T. A. Weber, B. Schäfer *et al.*, 2011 DNA damage in oocytes induces a switch of the quality control factor TAp63α from dimer to tetramer. Cell 144: 566–576. https://doi.org/10.1016/j.cell.2011.01.013

Di Giacomo, M., M. Barchi, F. Baudat, W. Edelmann, S. Keeney *et al.*, 2005 Distinct DNA-damage-dependent and -independent responses drive the loss of oocytes in recombination-defective mouse mutants. Proc. Natl. Acad. Sci. USA 102: 737–742. https://doi.org/10.1073/pnas.0406212102

Findlay, J. K., K. J. Hutt, M. Hickey, and R. A. Anderson, 2015 How is the number of primordial follicles in the ovarian reserve established? Biol. Reprod. 93: 111. https://doi.org/10.1095/biolreprod.115.133652

Flaggs, G., A. W. Plug, K. M. Dunks, K. E. Mundt, J. C. Ford *et al.*, 1997 Atm-dependent interactions of a mammalian chk1 homolog with meiotic chromosomes. Curr. Biol. 7: 977–986. https://doi.org/10.1016/S0960-9822(06)00417-9

Kim, M.-A., H.-J. Kim, A. L. Brown, M.-Y. Lee, Y.-S. Bae *et al.*, 2007 Identification of novel substrates for human checkpoint kinase Chk1 and Chk2 through genome-wide screening using a consensus Chk phosphorylation motif. Exp. Mol. Med. 39: 205–212. https://doi.org/10.1038/emm.2007.23

Kouznetsova, A., H. Wang, M. Bellani, R. D. Camerini-Otero, R. Jessberger *et al.*, 2009 BRCA1-mediated chromatin silencing is limited to oocytes with a small number of asynapsed chromosomes. J. Cell Sci. 122: 2446–2452. https://doi.org/10.1242/jcs.049353

Lange, J., J. Pan, F. Cole, M. P. Thelen, M. Jasin *et al.*, 2011 ATM controls meiotic double-strand-break formation. Nature 479: 237–240. https://doi.org/10.1038/nature10508

Li, X. C., and J. C. Schimenti, 2007 Mouse pachytene checkpoint 2 (*Trip13*) is required for completing meiotic recombination but not synapsis. PLoS Genet. 3: e130. https://doi.org/10.1371/journal.pgen.0030130

Livera, G., B. Petre-Lazar, M.-J. Guerquin, E. Trautmann, H. Coffigny *et al.*, 2008 p63 null mutation protects mouse oocytes from radio-induced apoptosis. Reproduction 135: 3–12. https://doi.org/10.1530/REP-07-0054

Mahadevaiah, S. K., J. M. Turner, F. Baudat, E. P. Rogakou, P. de Boer *et al.*, 2001 Recombinational DNA double-strand breaks in mice precede synapsis. Nat. Genet. 27: 271–276. https://doi.org/10.1038/85830

Maréchal, A., and L. Zou, 2013 DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb. Perspect. Biol. 5: a012716. https://doi.org/10.1101/cshperspect.a012716

Myers, M., K. L. Britt, N. G. M. Wreford, F. J. P. Ebling, and J. B. Kerr, 2004 Methods for quantifying follicular numbers within the mouse ovary. Reproduction 127: 569–580. https://doi.org/10.1530/rep.1.00095

Ou, Y.-H., P.-H. Chung, T.-P. Sun, and S.-Y. Shieh, 2005 p53 C-terminal phosphorylation by CHK1 and CHK2 participates in the regulation of DNA-damage-induced C-terminal acetylation. Mol. Biol. Cell 16: 1684–1695. https://doi.org/10.1091/mbc.e04-08-0689

Pacheco, S., M. Marcet-Ortega, J. Lange, M. Jasin, S. Keeney *et al.*, 2015 The ATM signaling cascade promotes recombination-dependent pachytene arrest in mouse spermatocytes. PLoS Genet. 11: e1005017. https://doi.org/10.1371/journal.pgen.1005017

Perera, D., L. Perez-Hidalgo, P. B. Moens, K. Reini, N. Lakin *et al.*, 2004 TopBP1 and ATR colocalization at meiotic chromosomes: role of TopBP1/Cut5 in the meiotic recombination checkpoint. Mol. Biol. Cell 15: 1568–1579. https://doi.org/10.1091/mbc.e03-06-0444

Peters, H., 1969 The development of the mouse ovary from birth to maturity. Acta Endocrinol. (Copenh.) 62: 98–116. https://doi.org/10.1530/acta.0.0620098

Rinaldi, V. D., E. Bolcun-Filas, H. Kogo, H. Kurahashi, and J. C. Schimenti, 2017 The DNA damage checkpoint eliminates mouse oocytes with chromosome synapsis failure. Mol. Cell 67: 1026–1036.e2. https://doi.org/10.1016/j.molcel.2017.07.027

Robert, T., A. Nore, C. Brun, C. Maffre, B. Crimi *et al.*, 2016 The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. Science 351: 943–949. https://doi.org/10.1126/science.aad5309

Romanienko, P. J., and R. D. Camerini-Otero, 2000 The mouse *Spo11* gene is required for meiotic chromosome synapsis. Mol. Cell 6: 975–987. https://doi.org/10.1016/S1097-2765(00)00097-6

Shieh, S. Y., J. Ahn, K. Tamai, Y. Taya, and C. Prives, 2000 The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. Genes Dev. 14: 289–300.

Suh, E.-K., A. Yang, A. Kettenbach, C. Bamberger, A. H. Michaelis *et al.*, 2006 p63 protects the female germ line during meiotic arrest. Nature 444: 624–628. https://doi.org/10.1038/nature05337

Turner, J. M. A., O. Aprelikova, X. Xu, R. Wang, S. Kim *et al.*, 2004 BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. Curr. Biol. 14: 2135–2142. https://doi.org/10.1016/j.cub.2004.11.032

Turner, J. M. A., S. K. Mahadevaiah, P. J. I. Ellis, M. J. Mitchell, and P. S. Burgoyne, 2006 Pachytene asynapsis drives meiotic sex chromosome inactivation and leads to substantial postmeiotic repression in spermatids. Dev. Cell 10: 521–529. https://doi.org/10.1016/j.devcel.2006.02.009

Wang, X. Q., J. L. Redpath, S. T. Fan, and E. J. Stanbridge, 2006 ATR dependent activation of Chk2. J. Cell. Physiol. 208: 613–619. https://doi.org/10.1002/jcp.20700

Widger, A., S. K. Mahadevaiah, J. Lange, E. ElInati, J. Zohren *et al.*, 2018 ATR is a multifunctional regulator of male mouse meiosis. Nat. Commun. 9: 2621. https://doi.org/10.1038/s41467-018-04850-0

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