Double-strand breaks induce short scale DNA replication and damage amplification in the fully-grown mouse oocytes

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Abstract

Break-induced replication (BIR) is essential for the repair of DNA double-strand breaks (DSBs) with single ends. DSBs-induced microhomology-mediated BIR (mmBIR) and template-switching can increase the risk of complex genome rearrangement. In addition, DSBs can also induce the multi-invasion-mediated DSB amplification. The mmBIR-induced genomic rearrangement has been identified in cancer cells and patients with rare diseases. However, when and how mmBIR are initiated haven’t been fully and deeply studied. Furthermore, it is not well understood about the conditions for initiation of multi-invasion-mediated DSB amplification. In the G2 phase oocyte of mouse, we identified a type of short scale BIR (ssBIR) using the DNA replication indicator 5-ethynyl-2’-deoxyuridine (EdU). These ssBIRs could only be induced in the fully-grown oocytes but not the growing oocytes. If the DSB oocytes were treated with Rad51 or Chek1/2 inhibitors, both EdU signals and DSB marker γH2A.X foci would decrease. In addition, the DNA polymerase inhibitor Aphidicolin could inhibit the ssBIR and another inhibitor ddATP could reduce the number of γH2A.X foci in the DSB oocytes. In conclusion, our results showed that DNA DSBs in the fully-grown oocytes can initiate ssBIR and be amplified by Rad51 or DNA replication.

Introduction

Nuclear DNA double-strand breaks (DSBs) could be repaired by homologous recombination (HR), non-homologous end joining (NHEJ), single-strand annealing or theta-mediated end joining (CECCALDI et al. 2016; SCHIMMEL et al. 2019). For the HR repair of DSBs, if the resected DNA end invaded into the allelic sequence, the repair process would be mediated by the synthesis-dependent strand annealing (SDSA)(MIURA et al. 2012) or double-holiday junctions (dHJ) (BZYMEK et al. 2010). If the resected DNA end invaded into a non-allelic sequence, it might initiate the non-allelic homologous recombination pathway and produce copy number variant (CNV) (INOUE and LUPSKI 2002; GU et al. 2008; LIU et al. 2012). If the resected DNA end had invaded into a homology or a non-allelic sequence, but the new synthesized DNA single strand couldn’t form dHJ structure or reanneal with the other broken end, it might induce break-induced replication (BIR) (MALKOVA and IRA 2013; KRAMARA et al. 2018).

In yeast, BIR is promoted by the structure-specific endonucleases (SSEs) such as Mus81 and Yen1 (PARDO and AGUILERA 2012). SSEs-mediated DNA incisions at the displacement-loop not only promote the formation of chromosomal nonreciprocal translocations but also initiate the more error-prone replication template switching (LEE et al. 2007; HASTINGS et al. 2009; PARDO and AGUILERA 2012; ANAND et al. 2014; LI et al. 2020), which will increase the risk of complex genomic rearrangement (CGR) (ZHANG et al. 2013; KRAMARA et al. 2018; PELLESTOR and GATINOIS 2018). The BIR-induced template switching is promoted by the deficiency of DNA helicases such as Pif1 or Mph1 (STAFA et al. 2014; SAKOFSKY et al. 2015), indicating that template switching may be caused by the collapse of...
replication fork. Multiple rounds of Fork Stalling and Template Switching (FoSTeS) would create CGRs in genome (LEE et al. 2007; LI et al. 2020). During the template switching, new single-strand DNA end ejected from the collapsed BIR replication fork could initiate a new round of strand invasion and BIR (SMITH et al. 2007). Sequencing results of CGRs showed that the new DNA fragments synthesized by FoSTeS are linked by microhomology sequence, which indicates that the template switching-associated BIR is a microhomology-mediated BIR (mmBIR) (LEE et al. 2007; ZHANG et al. 2009). In human, FoSTeS and mmBIR-mediated CGRs could be formed in both cancer cells (LI et al. 2020) and germline cells (LIU et al. 2017). Genomic data of human family trios indicated that the specific germline CGRs might be formed at the peri-zygotic period and mediated by mmBIRs (LIU et al. 2017). However, it hasn’t been widely studied about the mechanisms of how mmBIRs are formed in the peri-zygotic cells, such as oocytes, spermatoocytes and/or early embryos.

During the homology-mediated strand invasion, it has been reported that the end of the single-strand DNA is not necessary for the strand exchange (WRIGHT and HEYER 2014; PIAZZA et al. 2017; PIAZZA and HEYER 2018; PIAZZA et al. 2021). So the large 3’ overhang generated by DSB end resection might invade into multiple template DNA and form a multi-invasion joint molecule (PIAZZA and HEYER 2019). Notably, if the multi-invasion joint molecules are incised by SSEs, they will produce additional DSB ends and amplify the original DSB damage (PIAZZA and HEYER 2019). In humans, germline de novo mutations (DNMs) tend to gather in a cluster. These clustered DNMs had been proposed to be induced by the single DSB event in oocyte (GOLDMANN et al. 2018). Although the DNMs detected in human germlines are mostly single nucleotide variants (GOLDMANN et al. 2018), it is reasonable to deduce that DSBs in oocytes could amplify DNA damage (DSBs or non-DSBs) and produce clustered DNMs. However, it’s still not well known if and how DNA damage are amplified in oocytes.

Mammalian oocytes finish the meiotic HR at fetal stage and arrest at the G2-like dictyate stage (it is hereafter referred as G2) before or after birth (MACLENNAN et al. 2015; DALBIES-TRAN et al. 2020). The G2 arrest of oocytes will be maintained for weeks or even tens of years according to different species. When the females are sexually mature, their arrested oocytes will be activated for growing and maturation. For mouse, oocyte needs about two weeks for its growing, maturation and accumulating materials for subsequent embryo development (GOSDEN et al. 1997; LI et al. 2010). When mouse oocytes are fully grown, their transcription activities will be silenced. Meantime their DNA will be condensed and form a ring-like Hoechst-positive staining structure surrounding the nucleolus (SN) (DUMDIE et al. 2018). So these fully-grown oocytes are termed as SN oocytes whereas the growing oocytes are termed as non-SN (NSN) oocytes (TAN et al. 2009). Compared with the fully-grown SN oocytes, NSN oocytes are incompetent for in vitro development. Although about 20% of mouse NSN oocytes could be meiotic matured (BELLONE et al. 2009), they couldn’t bypass the 2-cell block (GODDARD and PRATT 1983) or develop to the blastocyst stage after fertilized with sperms (ZUCCOTTI et al. 1998; BELLONE et al. 2009). However, NSN oocytes in vivo might be developmental competent. They are just smaller in size and can gradually grow to the SN stage (XIAO et al. 2015) to become developmental competent, as long as their
corresponding follicles wouldn’t go to atresia. During the development of oocytes from NSN to SN stage, there is an intermediate stage between the NSN and SN (NSN-SN) (XIAO et al. 2015). These NSN-SN stage oocytes have less condensed chromatin structure and incomplete ring-like Hoechst-positive staining structure surrounding the nucleolus (Figure S1).

For both NSN and SN oocytes, DSBs can be induced in their nuclear DNA by many factors, including endogenous factors (such as reactive oxygen species and aging) (SUBRAMANIAN et al. 2020) and exogenous factors (such as chemotherapeutic drugs and radiotherapy treatments) (CARROLL and MARANGOS 2013; TUBBS and NUSSENZWEIG 2017; WINSHIP et al. 2018; STRINGER et al. 2020). Genomic data of family trio have also indicated that DSBs can form in oocytes and these DSBs might induce both single nucleotide variants and structural variants (DUYZEND et al. 2016; LIU et al. 2017; GOLDMANN et al. 2018). However, it hasn’t been fully analyzed about whether DSBs could be the contributing factor of CGR in the growing NSN and fully-grown SN oocytes. Our previous works had showed that exogenous DSBs could induce the chromatin to be entangled and matted together by Rad51 in the SN oocytes (MA et al. 2019a). In this study we further investigated the features of DSB repair in mouse oocytes, which would be new clues of how CGRs are formed in germ cells and somatic cells.

Experimental procedures

Oocyte isolation and in vitro culture

All of the animal experiments in this study were approved by the ethics committee of Guangdong Second Provincial General Hospital. 8-12 weeks old ICR mice were used for oocyte collection. The large antral follicular oocytes were released directly from ovaries. To distinguish the NSN, SN and shifting NSN-SN oocytes, we preformed the immunofluorescence labeling firstly, and then determined the oocyte stage by observing the Hoechst staining as described in the Introduction part. To block the oocytes from meiotic resumption, all of the manipulations were in the M2 medium (Sigma, M7167) with 2.5 μM Milrinone (MCE, HY-14252).

Treatment of oocytes by molecule compounds

DSBs in oocytes were introduced with Bleomycin at different concentrations (0.1, 0.5, 1, or 10 μM) for 1 h. Oocytes were treated with 100 μM Rad51 inhibitor IBR2 (MCE, HY-103710) to inhibit the Rad51 activity, 100 nM Chek1/2 inhibitor AZD7762 (MCE, HY-10992) to inhibit the DNA damage checkpoint, 2μM Aphidicolin (MCE, HY-N6733) to inhibit the nuclear DNA polymerase activity, and 100 μM ddATP (Apexbio, B8136) to delay the nuclear DNA replication. The control oocytes were treated with DMSO.

Immunofluorescence labeling
To label the endogenous proteins, oocytes were fixed with 4% Paraformaldehyde Fix Solution (Sangon, E672002) at room temperature (RT) for 15-30 minutes. Then in all the following steps, the solutions were made up with PBST (0.1% Tween-20 in PBS). After fixation, oocytes were treated with 0.3% Triton X-100 at RT for 20 minutes. To unmask the antigen epitopes of specific endogenous proteins (Rad51 in this study), oocytes were treated with Quick Antigen Retrieval Solution for Frozen Sections (Beyotime, P0090) at RT for 40 minutes. Then oocytes were washed three times with PBST and blocked with 1% BSA at RT for 1 hour. After that, oocytes were incubated with primary antibodies at 4 ℃ overnight. After washed 5 times with PBST, oocytes were incubated with appropriate secondary antibodies at RT for 2-3 h. Then oocytes were stained with Hoechst for 1 h and observed by the Andor live cell station system. The primary antibodies were: anti-Rad51 (Abcam, ab133534; and Zen Bioscience, 200514), anti-Mitofilin (Proteintech, 10179-1-AP) and anti-γH2A.X (Bioworld, BS4760). The γH2A.X foci numbers and volumes in oocytes were measured or counted by the Fiji software (https://imagej.nih.gov).

EdU labeling

To label the new synthesized DNA, the oocytes were cultured in M2 medium with 10 μM 5-ethynyl-2´-deoxyuridine (EdU) (beyotime, ST067). Then oocytes were fixed with 4% Paraformaldehyde for 15 minutes and permeated with 0.3% Triton X-100 for 15 minutes. After incubation with primary and secondary antibodies, oocytes were treated with the click reaction buffer (beyotime, C0071S) at RT for 1 h. Then oocytes were washed with PBST for 5 times and stained with Hoechst before observation. To compare the EdU signal sizes, we measured the max length of EdU signals with the Fiji software.

Statistic methods

Students’ T test was used for hypothesis test. P-value < 0.01 was recognized as very significant and marked with **; P-value < 0.05 and ≥ 0.01 was recognized as significant and marked with *. P-value ≥ 0.05 was recognized as not significant and marked with ‘ns’.

Results

DSBs induce short scale DNA replication in the SN oocytes

To determine whether DSBs could induce DNA replication in the growing and fully-grown oocytes, EdU was used to monitor DNA replication and Bleomycin was used to induce DSBs. After treatment of 10 μM Bleomycin for 1 h, oocytes were recovered in the Bleomycin-free media for 15 h. After click reaction (HEIN et al. 2008), EdU signals were found in the nuclei of NSN-SN and SN oocytes but not NSN and control oocytes (Figure 1A), indicating that DSBs could induce DNA replication in the NSN-SN and SN oocytes.

As persistent mitochondrial DNA (mtDNA) replication exists in the NSN and SN oocytes (Figure S2), we compared the EdU signal sizes in the nuclei of NSN-SN and SN
oocytes with those in mitochondria by measuring the max lengths of EdU signals. The results showed that the EdU signal sizes in the SN oocyte nuclei and mitochondria were comparable, but the EdU signal sizes in the NSN-SN oocyte nuclei were less than those in the SN oocytes and mitochondria (Figure 1B). As these BIRs in oocytes are short, so we termed them as short scale BIRs (ssBIRs).

By immunofluorescent labeling of the DSB marker γH2A.X, we found that most nuclear EdU signals were connected with the γH2A.X foci (Figure 1C). When DSBs were induced by Bleomycin, the numbers of γH2A.X foci and EdU signals decreased with the decreasing concentration of Bleomycin, but the nuclear EdU sizes showed no significant difference (Figure 1D).

**Rad51 is involved in the oocyte DSB repair**

After 1 μM Bleomycin treatment, oocytes were recovered in the Bleomycin-free media for 0 h, 12 h or 24 h. The numbers of γH2A.X foci significantly decreased in the recovered oocytes as time extension (Figure 2A). In the 24 h oocyte, EdU signals were not totally held together with γH2A.X foci (File S1), indicating that the DSBs could be repaired in the SN oocytes. Similar with our previous results (Ma et al. 2019a), HR repair protein Rad51 could be detected at the γH2A.X foci in the DSB oocytes (Figure 2B). Interestingly, the EdU signals were found just adjacent to the Rad51 foci (Figure 2C). All these combined data indicate that Rad51 is involved in the DSB repair in oocytes.

**DSBs can be amplified in the SN oocytes**

It had been proposed that the multi-invasion joint molecule is established by Rad51 and cleaved by SSEs, and the cleavage of multi-invasion joint molecule can amplify the DNA damage by producing additional DSB ends (Piazza et al. 2017). To test whether Rad51 could amplify the initial DSBs in oocytes, we measured the γH2A.X signal levels in the DSB oocytes treated with Rad51 inhibitor IBR2 (Zhu et al. 2015) (100 μM) or Chek1/2 inhibitor AZD7762 (100 nM). First, oocytes were treated with DMSO (as control), IBR2, AZD7762 or IBR2+AZD7762 for 5 h and induced DSBs by Bleomycin (1 μM). As a result, the γH2A.X signals decreased significantly in the IBR2 or AZD7762-treated oocytes compared to those in the control oocytes (Figure 3A). To further analyze whether Rad51 had amplified the DSBs, oocytes were treated with IBR2 after DSB inducement (Figure 3B). The results showed that the γH2A.X volume increased significantly after Bleomycin removing in the control oocytes, but the increase of γH2A.X volume was suppressed by IBR2 (Figure 3B). These results demonstrated that DSBs can be amplified in the SN oocytes and the DSB amplification is dependent on Rad51.

To analyze whether the DSB-induced ssBIR is associated with Rad51, oocytes were treated with DMSO, IBR2, AZD7762 and IBR2+AZD7762 for 5 h and then treated with Bleomycin to induce DSBs. After that the oocytes were recovered from Bleomycin for 6 h. As a result, the γH2A.X volumes in the AZD7762 or IBR2-treated oocytes were significantly decreased (P < 0.01), compared to those in the control oocytes (Figure 4A and 4B). The γH2A.X volumes in the IBR2+AZD7762-treated oocytes were significantly less than those in
the AZD7762-treated oocytes (P < 0.05), but at a same level compared with the IBR2-treated oocytes (Figure 4B). However, the numbers of γH2A.X foci in the IBR2+AZD7762-treated oocytes were significantly less than those in the AZD7762 or IBR2-treated oocytes (P < 0.01) (Figure 4C). With the decreasing numbers of γH2A.X volume or foci, the EdU signals were decreased in the AZD7762, IBR2 and IBR2+AZD7762 treated oocytes compared with those in control oocytes (Figure 4D). These results indicate that the ssBIR could be suppressed by Chek1/2 inhibitor or Rad51 inhibitor.

In addition, we also compared the EdU signal numbers at 6 h after DSB inducement with the γH2A.X focus numbers at 0 h after DSB inducement (Figure 4E). As a result, the numbers of EdU signals were significantly more than those of γH2A.X foci (P < 0.05), which further indicate that the DNA damage are amplified in oocytes.

DNA replication promotes DSB amplification in oocytes

The ssBIR is associated with the DNA replication, but whether DNA replication plays functions on the DNA damage amplification in oocytes is not known. So the DNA replication was inhibited to examine whether the DSB number increased in DSB oocytes. At first, we used DNA polymerase inhibitor Aphidicolin to treat the oocytes, however, Aphidicolin not only blocked the ssBIR in DSB oocytes but also induced additional DSBs in normal oocytes (Figure 5A and 5B), indicating that Aphidicolin is genotoxic to oocytes. So we next chose another DNA replication inhibitor ddATP which can block the mtDNA replication but only delay the nuclear DNA replication. Oocytes were treated with 100 μM ddATP for 5 h and induced DSBs by 0.5 μM Bleomycin for 1 h, and then released from Bleomycin for 12 h. As a result, ddATP could indeed suppress the mtDNA replication but couldn’t fully suppress the nuclear DNA replication in oocytes (Figure 5C and 5D). In addition, the ddATP-treated DSB oocytes had fewer γH2A.X foci than the control oocytes (P < 0.01, Figure 3D), indicating that the delay of nuclear DNA replication can suppress the DSB amplification in the SN oocytes. Moreover, when the DSB oocytes were treated with both ddATP and IBR2, the numbers of γH2A.X foci were further decreased, comparing to those in the DSB oocytes only treated with ddATP (Figure 5C and 5D). This result showed that nuclear DNA replication had promoted the DNA damage amplification in the SN oocytes.

Discussion

In this study, we used mouse oocytes as a model to analyze the DNA DSB repair in late G2 phase cells. The results showed that DSBs in the SN but not NSN oocytes could induce a type of short scale DNA replication termed as ssBIR. The sizes of oocyte ssBIR-induced EdU signals were comparable with that of the mtDNA replication-induced EdU signals, and the length of mouse mtDNA ranges from 16299 to 16301 bp (BAYONA-BAFALUY et al. 2003). So the length of the ssBIR in oocytes should be approximately 10k to 20k bp. Evidence showed that the tract length of gene conversion is about 200–300 bp (MANSAL et al. 2011) which is
less than the length of ssBIR-mediated DNA synthesis in oocytes. On the other hand, ssBIR in the SN oocytes is also different with the classic BIR which would replicate DNA from the breakpoint to the chromosome end or replicate a large genome fragment (Mancera et al. 2008; Ma et al. 2019b).

Besides the DSB-induced DNA replication, DNA replication in G2 oocytes could also be initiated by fusing oocytes with S phase zygotes (Czołowska and Borsuk 2000). In addition, DNA replication could be initiated in unactivated Xenopus egg extracts (Aquiles Sanchez et al. 1995). In mitotic cells, replication stress-associated DNA synthesis could be induced and mediated by Rad52 and endonucleases (Bhowmick et al. 2016). All these results indicated that both G2 and mitotic cells have the competence for DNA synthesis. However, it is still necessary to deeply analyze which factors are essential for the DNA replication origin firing and the BIR initiation in G2/M cells, as well as how to repress these CGR-prone DNA repair.

In this study, the DSB-induced EdU signals were generally adjacent to the Rad51 foci, indicating that 3’-overhangs bound with Rad51 hadn’t been fully exchanged with the template DNA. The partial strand exchange in the SN oocytes might be caused by two reasons: the condensed DNA configuration or the sequence difference between the template and broken DNA. As microhomology sequences had been detected in the breakpoints of most CGRs, partial strand exchange might be caused by the invasion of broken DNA ends to the microhomology regions in the SN oocytes (Figure 6A).

Rad52 has been proven to mediate BIR in the S phase of mammalian cells (Sotiriou et al. 2016), whereas Rad51 is essential for the BIR in yeast (Davis and Symington 2004). In this study and our previous work (Ma et al. 2019a), Rad51 inhibitors RI-1 and IBR2 could reduce the number of BIR events in the SN oocytes, indicating that BIR in the late G2 phase cells might be associated with Rad51. However, as the Rad51 inhibitor decreases the numbers of not only EdU signals but also γH2A.X foci, so it is still not known whether Rad51 directly participates in the ssBIR in the SN oocytes.

It had been reported that the Aphidicolin and Hydroxyurea-induced replication stress could lead to the formation of rare CNVs in mammalian cells (Arlt et al. 2009; Arlt et al. 2011; Arlt et al. 2012). In this study Aphidicolin could fully inhibit the ssBIR in the SN oocytes, indicating that DNA polymerase α and/or δ participate in the DSB-induced DNA replication. Aphidicolin could not only inhibit DNA replication but also induce additional DSBs. However, it is not known whether these Aphidicolin-induced DSBs are associated with DNA polymerases. One possibility is that the Aphidicolin-induced DSBs in G2 phase are also potential factors of rare CNV formation.

Although the direct evidence of Rad51-mediated multi-invasion is absent, the DSB numbers have been amplified in oocytes. The DSB amplification could be suppressed by Rad51 inhibitor, Chek1/2 inhibitor or DNA replication inhibitor ddATP in oocytes. These results might suggest that the DSB amplification is induced by not only multi-invasion (Piazza and Heyer 2019) (Figure 6B) but also DNA replication-associated template switching. Similar to the multi-invasion model, multiple rounds of template switching could also form the multi-invasion-like joint molecule which might amplify the DSBs by endonucleases (Figure 6C). All these speculations deserve a further study.
Data Availability

The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures and supplemental files. Supplemental Material available at figshare: https://doi.org/10.25386/genetics.14339222.

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Conflict of interest

None declared.

Literature Cited

Anand, R. P., O. Tsaponina, P. W. Greenwell, C. S. Lee, W. Du et al., 2014 Chromosome rearrangements via template switching between diverged repeated sequences. Genes Dev 28; 2394-2406.

Aquiles Sanchez, J., D. R. Wonsey, L. Harris, J. Morales and L. J. Wangh, 1995 Efficient plasmid DNA replication in Xenopus egg extracts does not depend on prior chromatin assembly. J Biol Chem 270; 29676-29681.

Arlt, M. F., J. G. Mulle, V. M. Schaibley, R. L. Ragland, S. G. Durkin et al., 2009 Replication stress induces genome-wide copy number changes in human cells that resemble
polymorphic and pathogenic variants. Am J Hum Genet 84: 339-350.

Arlt, M. F., A. C. Ozdemir, S. R. Birkeland, T. E. Wilson and T. W. Glover, 2011 Hydroxyurea induces de novo copy number variants in human cells. Proc Natl Acad Sci U S A 108: 17360-17365.

Arlt, M. F., T. E. Wilson and T. W. Glover, 2012 Replication stress and mechanisms of CNV formation. Curr Opin Genet Dev 22: 204-210.

Bayona-Bafaluy, M. P., R. Acin-Perez, J. C. Mullikin, J. S. Park, R. Moreno-Loshuertos et al., 2003 Revisiting the mouse mitochondrial DNA sequence. Nucleic Acids Res 31: 5349-5355.

Bellone, M., M. Zuccotti, C. A. Redi and S. Garagna, 2009 The position of the germinal vesicle and the chromatin organization together provide a marker of the developmental competence of mouse antral oocytes. Reproduction 138; 639-643.

Bhowmick, R., S. Minocherhomji and I. D. Hickson, 2016 RAD52 Facilitates Mitotic DNA Synthesis Following Replication Stress. Mol Cell 64: 1117-1126.

Bzymek, M., N. H. Thayer, S. D. Oh, N. Kleckner and N. Hunter, 2010 Double Holliday junctions are intermediates of DNA break repair. Nature 464; 937-941.

Carroll, J., and P. Marangos, 2013 The DNA damage response in mammalian oocytes. Front Genet 4: 117.

Ceccaldi, R., B. Rondinelli and A. D. D'Andrea, 2016 Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol 26; 52-64.

Czolowska, R., and E. Borsuk, 2000 Induction of DNA replication in the germinal vesicle of the growing mouse oocyte. Dev Biol 223; 205-215.
Dalbies-Tran, R., V. Cadoret, A. Desmarchais, S. Elis, V. Maillard et al., 2020 A Comparative Analysis of Oocyte Development in Mammals. Cells 9.

Davis, A. P., and L. S. Symington, 2004 RAD51-dependent break-induced replication in yeast. Mol Cell Biol 24: 2344-2351.

Dumdie, J. N., K. Cho, M. Ramaiah, D. Skarbrevik, S. Mora-Castilla et al., 2018 Chromatin Modification and Global Transcriptional Silencing in the Oocyte Mediated by the mRNA Decay Activator ZFP36L2. Dev Cell 44: 392-402 e397.

Duyzend, M. H., X. Nuttle, B. P. Coe, C. Baker, D. A. Nickerson et al., 2016 Maternal Modifiers and Parent-of-Origin Bias of the Autism-Associated 16p11.2 CNV. Am J Hum Genet 98: 45-57.

Goddard, M. J., and H. P. Pratt, 1983 Control of events during early cleavage of the mouse embryo: an analysis of the '2-cell block'. J Embryol Exp Morphol 73: 111-133.

Goldmann, J. M., V. B. Seplyarskiy, W. S. W. Wong, T. Vilboux, P. B. Neerincx et al., 2018 Germline de novo mutation clusters arise during oocyte aging in genomic regions with high double-strand-break incidence. Nat Genet 50: 487-492.

Gosden, R., J. Krapez and D. Briggs, 1997 Growth and development of the mammalian oocyte. Bioessays 19: 875-882.

Gu, W., F. Zhang and J. R. Lupski, 2008 Mechanisms for human genomic rearrangements. Pathogenetics 1: 4.

Hastings, P. J., G. Ira and J. R. Lupski, 2009 A microhomology-mediated break-induced replication model for the origin of human copy number variation. PLoS Genet 5: e1000327.
Hein, C. D., X. M. Liu and D. Wang, 2008 Click chemistry, a powerful tool for pharmaceutical sciences. Pharm Res 25: 2216-2230.

Inoue, K., and J. R. Lupski, 2002 Molecular mechanisms for genomic disorders. Annu Rev Genomics Hum Genet 3: 199-242.

Kramara, J., B. Osia and A. Malkova, 2018 Break-Induced Replication: The Where, The Why, and The How. Trends Genet 34: 518-531.

Lee, J. A., C. M. Carvalho and J. R. Lupski, 2007 A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell 131: 1235-1247.

Li, L., P. Zheng and J. Dean, 2010 Maternal control of early mouse development. Development 137: 859-870.

Li, Y., N. D. Roberts, J. A. Wala, O. Shapira, S. E. Schumacher et al., 2020 Patterns of somatic structural variation in human cancer genomes. Nature 578: 112-121.

Liu, P., C. M. Carvalho, P. J. Hastings and J. R. Lupski, 2012 Mechanisms for recurrent and complex human genomic rearrangements. Curr Opin Genet Dev 22: 211-220.

Liu, P., B. Yuan, C. M. B. Carvalho, A. Wuster, K. Walter et al., 2017 An Organismal CNV Mutator Phenotype Restricted to Early Human Development. Cell 168: 830-842 e837.

Ma, J. Y., X. Feng, X. Y. Tian, L. N. Chen, X. Y. Fan et al., 2019a The repair of endo/exogenous DNA double-strand breaks and its effects on meiotic chromosome segregation in oocytes. Hum Mol Genet 28: 3422-3430.

Ma, J. Y., L. Y. Yan, Z. B. Wang, S. M. Luo, W. S. B. Yeung et al., 2019b Meiotic chromatid recombination and segregation assessed with human single cell genome sequencing.
data. J Med Genet 56; 156-163.

MacLennan, M., J. H. Crichton, C. J. Playfoot and I. R. Adams, 2015 Oocyte development, meiosis and aneuploidy. Semin Cell Dev Biol 45; 68-76.

Malkova, A., and G. Ira, 2013 Break-induced replication: functions and molecular mechanism. Curr Opin Genet Dev 23; 271-279.

Mancera, E., R. Bourgon, A. Brozzi, W. Huber and L. M. Steinmetz, 2008 High-resolution mapping of meiotic crossovers and non-crossovers in yeast. Nature 454; 479-485.

Mansai, S. P., T. Kado and H. Innan, 2011 The Rate and Tract Length of Gene Conversion between Duplicated Genes. Genes (Basel) 2; 313-331.

Miura, T., Y. Yamana, T. Usui, H. I. Ogawa, M. T. Yamamoto et al., 2012 Homologous recombination via synthesis-dependent strand annealing in yeast requires the Irc20 and Srs2 DNA helicases. Genetics 191; 65-78.

Pardo, B., and A. Aguilera, 2012 Complex chromosomal rearrangements mediated by break-induced replication involve structure-selective endonucleases. PLoS Genet 8; e1002979.

Pellestor, F., and V. Gatinois, 2018 Chromoanasynthesis: another way for the formation of complex chromosomal abnormalities in human reproduction. Hum Reprod 33; 1381-1387.

Piazza, A., and W. D. Heyer, 2018 Multi-Invasion-Induced Rearrangements as a Pathway for Physiological and Pathological Recombination. Bioessays 40; e1700249.

Piazza, A., and W. D. Heyer, 2019 Homologous Recombination and the Formation of Complex Genomic Rearrangements. Trends Cell Biol 29; 135-149.
Piazza, A., P. Rajput and W. D. Heyer, 2021 Physical and Genetic Assays for the Study of DNA Joint Molecules Metabolism and Multi-invasion-Induced Rearrangements in S. cerevisiae. Methods Mol Biol 2153: 535-554.

Piazza, A., W. D. Wright and W. D. Heyer, 2017 Multi-invasions Are Recombination Byproducts that Induce Chromosomal Rearrangements. Cell 170: 760-773 e715.

Sakofsky, C. J., S. Ayyar, A. K. Deem, W. H. Chung, G. Ira et al., 2015 Translesion Polymerases Drive Microhomology-Mediated Break-Induced Replication Leading to Complex Chromosomal Rearrangements. Mol Cell 60: 860-872.

Schimmel, J., R. van Schendel, J. T. den Dunnen and M. Tijsterman, 2019 Templated Insertions: A Smoking Gun for Polymerase Theta-Mediated End Joining. Trends Genet 35: 632-644.

Smith, C. E., B. Llorente and L. S. Symington, 2007 Template switching during break-induced replication. Nature 447; 102-105.

Sotiriou, S. K., I. Kamileri, N. Lugli, K. Evangelou, C. Da-Re et al., 2016 Mammalian RAD52 Functions in Break-Induced Replication Repair of Collapsed DNA Replication Forks. Mol Cell 64: 1127-1134.

Stafa, A., R. A. Donnianni, L. A. Timashev, A. F. Lam and L. S. Symington, 2014 Template switching during break-induced replication is promoted by the Mph1 helicase in Saccharomyces cerevisiae. Genetics 196: 1017-1028.

Stringer, J. M., A. Winship, N. Zerafa, M. Wakefield and K. Hutt, 2020 Oocytes can efficiently repair DNA double-strand breaks to restore genetic integrity and protect offspring health. Proc Natl Acad Sci U S A 117; 11513-11522.
Subramanian, G. N., J. Greaney, Z. Wei, O. Becherel, M. Lavin et al., 2020 Oocytes mount a noncanonical DNA damage response involving APC-Cdh1-mediated proteolysis. J Cell Biol 219.

Tan, J. H., H. L. Wang, X. S. Sun, Y. Liu, H. S. Sui et al., 2009 Chromatin configurations in the germinal vesicle of mammalian oocytes. Mol Hum Reprod 15: 1-9.

Tubbs, A., and A. Nussenzweig, 2017 Endogenous DNA Damage as a Source of Genomic Instability in Cancer. Cell 168: 644-656.

Winship, A. L., J. M. Stringer, S. H. Liew and K. J. Hutt, 2018 The importance of DNA repair for maintaining oocyte quality in response to anti-cancer treatments, environmental toxins and maternal ageing. Hum Reprod Update 24; 119-134.

Wright, W. D., and W. D. Heyer, 2014 Rad54 functions as a heteroduplex DNA pump modulated by its DNA substrates and Rad51 during D loop formation. Mol Cell 53; 420-432.

Xiao, S., F. E. Duncan, L. Bai, C. T. Nguyen, L. D. Shea et al., 2015 Size-specific follicle selection improves mouse oocyte reproductive outcomes. Reproduction 150; 183-192.

Zhang, C. Z., M. L. Leibowitz and D. Pellman, 2013 Chromothripsis and beyond: rapid genome evolution from complex chromosomal rearrangements. Genes Dev 27; 2513-2530.

Zhang, F., C. M. Carvalho and J. R. Lupski, 2009 Complex human chromosomal and genomic rearrangements. Trends Genet 25; 298-307.

Zhu, J., H. Chen, X. E. Guo, X. L. Qiu, C. M. Hu et al., 2015 Synthesis, molecular modeling, and biological evaluation of novel RAD51 inhibitors. Eur J Med Chem 96; 196-208.

Zuccotti, M., P. Giorgi Rossi, A. Martinez, S. Garagna, A. Forabosco et al., 1998 Meiotic and...
Figure Legends

Figure 1. DNA DSBs induce the short scale DNA replication in the NSN-SN and SN stage oocytes but not NSN oocytes. (A) Oocytes are treated with or without 10 μM Bleomycin for 1 h and then released from Bleomycin for 15 h. EdU signals (green) are detected in the Bleomycin-treated NSN-SN oocytes and SN oocytes. Bar = 20 μm. (B) The EdU signal sizes in the SN stage oocytes are larger than those in the NSN-SN oocytes, but are not significant difference to mtDNA replication. (C) The γH2A.X foci (purple) can be observed beside to or overlapped with the EdU signals. Bar = 10 μm. (D) The EdU sizes induced by different Bleomycin doses have no significant difference. ** represents P < 0.01 and ns represents no significance.

Figure 2. Rad51 participates in the DSB repair in oocytes. (A) DSBs could be repaired in the SN stage oocytes. γH2A.X foci in oocytes are counted at 0, 12, or 24 h after 1 μM Bleomycin treatment for 1 h. (B) Rad51 foci are overlapped with γH2A.X foci at 0 and 24 h after Bleomycin treatment. Rad51 (purple), γH2A.X (green) and DNA (blue). (C) Rad51 foci are adjacent to the EdU signals but not overlapped with them in the Bleomycin-treated oocytes. Rad51 (purple), EdU (green) and DNA (blue). Bar = 10 μm. ** represents P < 0.01.

Figure 3. DNA damage are amplified in oocytes. (A) When oocytes were treated with Chek1/2 inhibitor AZD7762 (AZD), Rad51 inhibitor IBR2 or IBR2+AZD before and during DSB inducement, the numbers of γH2A.X foci are significantly decreased compared to those in the DMSO-treated oocytes. (B) The γH2A.X volume increased 2 h after DSB inducement, and the increase of γH2A.X volume is partially inhibited by IBR2. γH2A.X (purple) and DNA (blue). Bar = 10 μm. ** represents P < 0.01 and * represents P < 0.05.

Figure 4. DSB-induced short scale DNA replication is associated with Rad51 in oocytes. (A) Typical images of EdU signals and γH2A.X foci in the oocytes treated with DMSO, AZD7762 (AZD), IBR2 and IBR2+AZD for 6 h after released from Bleomycin. (B) The total volume of γH2A.X foci is significantly decreased in the AZD or IBR2-treated oocytes compared with that in the DMSO-treated oocytes. (C) The numbers of γH2A.X foci in the IBR2+AZD treated oocytes are less than those in the AZD or IBR2-treated oocytes. (D) The numbers of EdU signals are significantly decreased in the AZD, IBR2 or IBR2+AZD-treated oocytes compared with those in the DMSO-treated oocytes. (E) The numbers of EdU signals at 6 h are significantly increased than those of γH2A.X foci at 0 h after DSB inducement. EdU (green), γH2A.X (purple) and DNA (blue). Bar = 10 μm. ** represents P < 0.01 and * represents P < 0.05.

Figure 5. DNA replication participates in the DNA damage amplification in the SN
oocytes. (A) Aphidicolin (2 μM for 15 hours) induces DSBs in oocytes. The numbers of γH2A.X foci are significantly increased in the Aphidicolin-treated oocytes. APH, Aphidicolin. γH2A.X (red) and DNA (blue). Bar = 10 μm. (B) Aphidicolin blocks the short scale DNA replication induced by Bleomycin. Oocytes were cultured with or without 2 μM Aphidicolin for 12 h after 1 μM Bleomycin treatment for 1 h. EdU (green), γH2A.X (purple) and DNA (blue). Bar = 10 μm. (C) ddATP blocks the mtDNA replication and delay the nuclear DNA replication. Oocytes were cultured with ddATP (100 μM) or with both ddATP and IBR2 (100 μM) for 5 hours. After that, oocytes were treated with 0.5 μM Bleomycin for 1 h and then released from Bleomycin for 12 h. EdU (green), γH2A.X (purple) and DNA (blue). Bar = 20 μm. (D) Compared with the control oocytes, the numbers of γH2A.X foci are less in the ddATP group and further less in the ddATP+IBR2 group. EdU signals in the ddATP and ddATP+IBR2 group oocytes are marked by arrows. ** represents P < 0.01.

Figure 6. Supposed models of DNA DSB repair in the SN oocytes. (A) Partial strand-exchange make Rad51 filament exist beside the newly-synthesized DNA in the SN oocytes. The DNA damage in the SN oocyte can be amplified by both Rad51-mediated multi-invasion (B) and break-induced replication induced template switching (C). The multi-invasion can be suppressed by Rad51 inhibitor IBR2 whereas the template switching can be suppressed by ddATP.
