The formation and repair of DNA double-strand breaks in mammalian meiosis

Wei Qu, Cong Liu, Ya-Ting Xu, Yu-Min Xu, Meng-Cheng Luo

Programmed DNA double-strand breaks (DSBs) are necessary for meiosis in mammals. A sufficient number of DSBs ensure the normal pairing/synapsis of homologous chromosomes. Abnormal DSB repair undermines meiosis, leading to sterility in mammals. The DSBs that initiate recombination are repaired as crossovers and noncrossovers, and crossovers are required for correct chromosome separation. Thus, the placement, timing, and frequency of crossover formation must be tightly controlled. Importantly, mutations in many genes related to the formation and repair of DSB result in infertility in humans. These mutations cause nonobstructive azoospermia in men, premature ovarian insufficiency and ovarian dysgenesis in women. Here, we have illustrated the formation and repair of DSB in mammals, summarized major factors influencing the formation of DSB and the theories of crossover regulation.

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INTRODUCTION

Meiosis is a specialized form of cell division, that generates haploid gametes from diploid cells, and it is essential for sexual reproduction and evolution. The programmed DNA double-strand breaks (DSBs) are critical in meiosis and the number of DSBs is tightly regulated. Excessive DSBs destabilize the genome, whereas insufficient DSBs impede the crossover (CO) formation, leading to erroneous separation of homologous chromosomes. Many proteins are involved in DSB repair, which generates COs and noncrossovers (NCOs). The CO repair pathway is critical for mammals. First, CO repair promotes the exchange of genetic material between homologous chromosomes. This exchange is essential for the genetic diversity that contributes to biological evolution and adaptation to environment changes. Second, COs establish physical connections between homologous chromosomes. These connections help maintain the balance of forces which ensure that the homologous chromosomes are correctly aligned on the equatorial plate, and pulled apart by the meiotic spindle. Therefore, it is crucial to understand the formation and repair of DSB in mammalian meiosis.

The formation and repair of meiotic DSB in mammals are complex and delicate. These processes include the formation of DSB, the resection of DSB ends, homology search, DNA strand invasion, as well as the stabilization and resolution of the double Holliday junctions (dHJs) as shown in Figure 1.

THE FORMATION OF DSBs

Meiotic recombination is initiated by the programmed induction of DSBs by a topoisomerase-like enzyme SPO11. At the beginning of meiosis, SPO11 binds to DNA strands and generates DSBs. The SPO11 binding requires open chromatin, which is critically mediated by PRDM9, a meiosis-specific histone methyltransferase. PRDM9 contains a N-terminal KRAB domain involved in protein-protein interactions, a SSXRD domain that acts as a transcription repressor in SSX proteins, a SET domain with methyltransferase activity, and a zinc finger domain. This zinc finger domain contains a single proximal zinc finger separated from the rest of the terminal 2H2 zinc finger array comprised of 8 to over 20 fingers. PRDM9 methylates histone H3 at lysine 4 (H3K4) and lysine 36 (H3K36) at surrounding nucleosomes.

SPO11 is an evolutionarily conserved topoisomerase-derived protein, and it is responsible for the formation of most DSBs with the assistance of TopoVIBL during meiosis. SPO11 contains two variants, SPO11α and SPO11β. SPO11α mediates DSB formation on sex chromosomes, while SPO11β mediates DSB formation on autosomes in mammals. SPO11β has an extra exon compared with SPO11α. TopoVIBL, a subunit of TopoVIB, which belongs to the topoisomerase
type IIB family most likely regulates the cleavage of DNA through directly interacting with SPO11.15 Male Top6bl−/− mice were sterile and the formation of DSB was defective in spermatocytes.15 The activity of SPO11-TopoVIBL requires auxiliary protein complex, namely pre-DSB recombinosomes, including IHO1, REC114, MEI1, MEI4, and ANKRD31.18 HORMAD1 localizes along asynapsed axes and recruits IHO1 to establish an platform, on which focal pre-DSB recombinosomes assemble.18 The phenotypes of the Iho1, Mei1, Mei4, and Rec114-deficient mice were similar to those of the Spo11-deficient mice, showing abnormal DSB formation. Therefore, the pre-DSB recombinosomes are essential for meiotic DSB formation in mammals.6,19–22

It is worth noting that the regulatory mechanism of DSB formation on sex chromosomes is different from that on autosomes. ANKRD31 is highly and uniquely expressed in meiotic cells and appears between pre-leptotene and early-pachytene, which promotes DSB formation on the pseudo-autosomal region (PAR) of X and Y chromosome.23 ANKRD31 accumulates on the asynapsed chromosome axes, especially on the PAR of spermatocytes.23 The loss of ANKRD31 caused infertility in male mice and accelerated declining of fertility in female mice.23 Mo-2 arrays have been considered as one of the important factors responsible for regulating DSB formation on PAR.24 Mo-2 arrays are 20-kb minisatellites, with a 31-bp repeat localized on PAR of sex chromosomes and the noncentromeric ends of chromosomes 4, 9 and 13.24 Mo-2 arrays can recruit ANKRD31, which further recruits IHO1 and REC114 to DSB hotspot sites to form DSB.23,24 The formation of DSB mediated by Mo-2 arrays is independent of PRDM9, and heterochromatin histone modifications are enriched at the position of mo-2 arrays at the onset of meiosis.24

**Figure 1:** The formation and repair process of DSB in mammals. DSB: double-strand break.

**FACTORS INFLUENCING THE FORMATION OF DSBs**
A sufficient number of DSBs are advantageous to ensure the pairing/synthesis of homologous chromosomes. However, excessive DSBs are harmful because irreparable DSBs undermine the integrity of the genome.2 Therefore, sophisticated machinery modulates the number of DSBs in mammals.

**SPO11 dosage**
The expression level of SPO11 determines the number of DSBs. SPO11β is mainly expressed in leptotene, and SPO11α is expressed in pachytene/diplotene spermatocytes.16 Doubled SPO11β expression increased the number of DSB by about 25%, whereas half SPO11β expression decreased the amount of DSBs by 20%–30%, indicating that the transgene dosage of SPO11 is critical for the number of DSBs.25,26
**Chromosome length**

Meiotic DSBs are not randomly distributed, as random distribution leads to a higher risk of mis-segregation for smaller chromosomes.27 Studies have shown that accessory DSB proteins, including REC114 and MER2, preferentially bind to short chromosomes and exist for a longer time to ensure that the short chromosomes have enough DSBs for faithful separation of homologous chromosomes in yeast.28 It should be noted that large chromosomes better tolerate fewer DSBs, and they are less vulnerable to entanglement than small chromosomes in mammals.29 Compared with the X chromosome, the Y chromosome seems to be relatively resistant to tangling.30

**Synaptonemal complex**

As a sufficient number of DSBs are needed to support the normal synopsis of chromosomes, vice versa the synapsed state of chromosomes affects DSB formation. Without synopsis, DSBs will persist on unsynapsed segment of chromosomes until pachynema.31-33 This phenomenon may be related to HORMAD1 and pre-DSB recombinosomes. When homologous chromosomes are not fully synapsed or asynapsed, HORMAD1 serves as a platform to continuously recruit pre-DSB recombinosomes to the chromosome to promote DSB formation.18 When the homologous chromosomes are completely synapsed, HORMAD1 and pre-DSB recombinosomes dissociate from the chromosome to prevent overproduction of DSB.18 Moreover, synaptonemal complex promotes IH01 depletion from synapsed chromosome axes.18

**DNA damage-responsive phosphoinositide 3-kinase 3-kinase-related kinases**

The DNA damage-responsive phosphoinositide 3-kinase (PI3K) related kinases including ATM, ATR and PRKDC, play important roles in the regulation of DSB in mammals. ATM, as a dimer with no biological activity in undamaged somatic cells, becomes active monomers through intermolecular auto-phosphorylation on serine 1981 after DSB formation.30 The phosphorylated ATM is recruited by the MRE11-RAD50-NBS1 (MRN) complex to the DSB sites and phosphorylates the MRN complex.30 Subsequently, ATM phosphorylates MDC1 and Serine 139 of H2AX.30 Phosphorylation of MDC1 recruits RING-finger ubiquitin ligases RNFL8 and RNFL16.30 RNFL8 ubiquitinates yH2AX, which recruits 53bp1 and BRCA1, thereby spreading the DSB signal.30 ATM can also remove IH01 from the chromosome axes by cooperating with ATR and PRKDC to inhibit DSBs in early pachynema, yet the specific molecular mechanism is still unclear.18

**THE RESECTION OF DSB ENDS**

The ends of DSB lack the 3'-hydroxyl or 5'-phosphate group after DSB formation.10 The MRN complex plays a crucial role in resecting the DSB ends, which is necessary for normal meiosis.34 MRE11 is a homodimer with the dual endonuclease and exonuclease activities.35 The MRN complex cleaves the 5’ strand away from the DSBs through CtIP and the endonuclease activity of MRE11.34 After cleavage, MRE11 generates a short single-stranded DNA (ssDNA) through its 3’ to 5’ exonuclease activity, while EXO1 and DNA2 further resect this end through their 5’ to 3’ exonuclease activity and finally produce a long ssDNA overhang.30

RAD50, as a member of the ATP binding cassette (ABC) superfamily, interacts with two MRE11 monomers, and forms a globular domain that interacts with DNA.35 In the ATP-binding conformation, two catalytical RAD50s block the active sites of MRE11, whereas in the ATP-free conformation, RAD50 dissociates, allowing MRE11 to bind and hydrolyze the DNA phosphodiester backbone.35 NBS1 is the third subunit of the MRN complex and responds to the phosphorylation of CtIP through its FHA and BRCT domains, thereby stimulating the activity of the MRE11-RAD50 core complex through direct physical interactions with MRE11.35 Knockout of each component of MRN complex resulted in embryonic lethality in mice, and Nbs1 knockout in germ cells led to meiotic arrest and male infertility.34

**HOMOLOGY SEARCH AND STRAND INVASION**

The 3’ ssDNA overhangs are produced from resection of the ends of DSB and coated with RPA complex to protect ssDNA and remove secondary structure.36,37 RPA complex is comprised of three subunits of RPA1, RPA2, and RPA3.37 RPA1 is the largest and is mainly responsible for the DNA binding activity of the complex.38 The loss of RPA1 impaired loading of RAP2 and RPA3, and inhibited recruitment of DMC1 and RAD51 to the chromosome.38 MEIOB, a meiosis-specific protein containing the OB domain, forms a highly compact complex with SPATA22.39 The complex binds to ssDNA for recruiting the BRCA2-MEILB2-BRME1 complex.39,40 MEIOB and SPATA22 together facilitate the localization of BRCA2- MEILB2-BRME1 on ssDNA as shown that MEILB2 and BRME1 foci decreased substantially in Meiob−/− mice.41 The BRCA2-MEILB2-BRME1 complex directly recruits RAD51 and DMC1 for binding to ssDNA and replacing RPA.40

After recruited to ssDNA to replace RPA, DMC1 and RAD51 assemble with the ssDNA into nucleoprotein filaments to guide homologous searching and form D-loop structure together with the HO2P-MND1 heterodimer.42 It has been demonstrated that HO2P and MND1 are two key accessory proteins to stimulate the synthesis phase of DNA strand exchange in vitro.43 Most DSBs were repaired in spermatocytes, with intact Hop2 but deleted Mnd1.44 However, DSBs were not repaired and spermatocytes were blocked at the pachytene-like stage in Hop2 knockout male mice.45 These findings indicate that HO2P independently promotes strand invasion in vivo. The invading 3’ ssDNA overhang promotes the synthesis of new DNA; thereafter, the D-loop structure extends to the second end, allowing the capture of the 3’ ssDNA end of the other break. The DNA binding activity of MEIOB may play an essential role during this process.46 Meiob-deletion caused sterility in both genders due to meiotic arrest in zygote or pachytene-like stage.46,47

**THE STABILIZATION AND RESOLUTION OF THE DOUBLE HOLLIDAY JUNCTIONS**

After the resection of 3’ ssDNA flap and end ligation, the DHJs are formed and maintained by multiple proteins.36 The DNA mismatch repair family proteins, MSH4 and MSH5, form the MutSγ complex, which stabilizes the DHJs.47 The MutSγ complex first appeared in zygona as discrete foci on the chromosome axis, reached its maximum number in early pachynema, and declined in mid-pachynema.48,49 Most DSBs were repaired in spermatocytes, with intact Hop2 but deleted Mnd1.44 However, DSBs were not repaired and spermatocytes were blocked at the pachytene-like stage in Hop2 knockout male mice.45 The BRCA2-MEILB2-BRME1 complex directly recruits RAD51 and DMC1 for binding to ssDNA and replacing RPA.40

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In contrast, the symmetric resolution of dHJs results in the formation of NCOs. The formation of CO is regulated by the MRE11-RAD50-NBS1 (MRN) complex to the DSB sites 1981 after DSB formation. The phosphorylated ATM is recruited by the MRE11-RAD50-NBS1 (MRN) complex to the DSB sites and phosphorylates the MRN complex. Subsequently, ATM phosphorylates MDC1 and Serine 139 of H2AX. Phosphorylation of MDC1 recruits RING-finger ubiquitin ligases RNFL8 and RNFL16. RNFL8 ubiquitinates yH2AX, which recruits 53bp1 and BRCA1, thereby spreading the DSB signal. ATM can also remove IH01 from the chromosome axes by cooperating with ATR and PRKDC to inhibit DSBs in early pachynema, yet the specific molecular mechanism is still unclear.18

The presence of a small protein domain and a large protein domain has been observed in the MRN complex, which can bind to DSBs and recruit the MRN complex to the DSB sites.19,20 The phosphorylation of CtIP through its FHA and BRCT domains, thereby stimulating the activity of the MRE11-RAD50 core complex through direct physical interactions with MRE11. Knockout of each component of MRN complex resulted in embryonic lethality in mice, and Nbs1 knockout in germ cells led to meiotic arrest and male infertility.24
complex formed by MLH1/MLH3 as heterodimer is recruited by the MutSy complex on dHJs, it resolves the dHJs via the endonuclease activity. The endonuclease activity of MutLy complex was abolished when the conserved metal binding DQHA(X)2E(X)4E motif of MLH3 was mutated. Besides, EXO1 and MutSy can stimulate the endonuclease activity of MutLy. Replication factor C (RFC) and the proliferating cell nuclear antigen (PCNA) can form a complex with MutLy and MutSy to preferentially cleave DNA with the Holliday junctions. On the other hand, class II crossover is independent of the MutLy or MutSy complex, and is produced by the structure-specific endonuclease MUS81 and EMEl in mammals. 

There are two speculative explanations for the regulatory mechanism of class I crossover and class II crossover. First, MSH4 and MSH5 bind to dHJs, and recruit MLH1 and MLH3 to the majority of the CO sites, while MUS81 is recruited to the remaining CO sites. However, the interaction between MSH4-MSH5 and MUS81 has not been validated. Second, others believe that MSH4-MSH5 and MUS81 belong to two distinct pathways, and MUS81 and MLH1-MLH3 antagonizes each other. It has been proved that human MUS81 can directly bind to dHJs in vitro. When occupying a subset of CO sites, MUS81 prevents MLH1-MLH3 from binding to these sites. These antagonistic effects disappear and MLH1-MLH3 occupies all CO sites in the absence of MUS81. These two models are reasonable hypotheses and need to be further investigated. Currently, BTBD12 has received attention on its potential role in homologous recombination in mammals. BTBD12 interacts with many of the key players such as BLM in both CO pathways. Importantly, MLH1-MLH3 focus numbers increased when BTBD12 was defective. Therefore, BTBD12 may functionally integrate the different CO pathways during mammalian meiosis.

The formation and repair of mammalian DSBs involve a large number of genes and proteins as summarized in Table 1. Knockout of these genes exerts important impact on meiosis.

CHARACTERISTICS OF CO SITES IN MAMMALIAN MEIOSIS

DSBs are predominantly converted to NCOs, with only around 10% converted to COs. Why are there so many DSBs but only a small number of COs formed? Exploring the characteristics of CO sites elicits an important speculation that the chromatin state is more open on CO sites than NCO sites. Thus, DSB hotspots with looser chromatin have a greater chance of forming COs. Second, the designated CO sites are easier to be bound by PRDM9. PRDM9 binding on the template chromosome increases the chance of DSBs being resolved as a CO. Third, CO sites have higher GC content, and DSB hotspots with higher GC content are more likely to be repaired by the CO pathway.

THE THEORIES OF CO REGULATION

Meiotic bet-hedging strategy

Recombination is a "double-edged sword" which generates new alleles to adapt to environmental changes and increases the diversity of organisms. The other hand, excessive recombination impairs genome stability in the organism. COs only happen when benefits outweigh the adverse effects. More COs in gametes are advantageous when the environment changes while fewer COs in gametes are beneficial during environmental stasis. Organisms effectively adapt to environment changes through delicate control of the number of COs.

CO assurance

CO formation is essential for proper chromosome segregation in meiosis. CO assurance refers to needing at least one CO (obligate CO) between each pair of homologous chromosomes. Previous data indicate that even a single DSB is converted to a CO with high efficiency in the Caenorhabditis elegans. However, the theory of CO assurance still needs to be validated in mammals.

CO maturation inefficiency (CMI)

Organisms randomly generate and remove a small subset of COs, at the pre-designated CO sites, during maturation of COs to ensure that only one or two COs occur on a chromosome. This phenomenon is defined as CMI and is only observed in human females. When CMI is abnormally enhanced or diminished, aneuploidy segregation of chromosomes occurs. The existence of this mechanism seems counterintuitive but is of great significance. First, CMI increases the time between two consecutive pregnancies in young women, and reduces the birth rate to ensure that existing children have sufficient resources to survive. Second, CMI interacts with other factors to reduce the fertility of elderly women.

Table 1: Phenotypes of knockout male mice related to the formation and repair of double-strand breaks

| Gene name | Arrest stages | Male phenotype | Reference |
|-----------|---------------|----------------|-----------|
| Prdm9     | Pachytene-like| Infertility    | Mihola et al. |
| Hels      | Pachytene-like| Infertility    | Spruce et al. |
| Hormad1   | Pachytene-like| Infertility    | Stanzione et al. |
| Iho1      | Zygote-pachytene| Infertility    | Stanzione et al. |
| Mei1      | Zygote-like   | Infertility    | Libby et al. |
| Mei4      | Zygote-like   | Infertility    | Kumar et al. |
| Rec114    | Zygote-like   | Infertility    | Kumar et al. |
| Ankrd31   | Pachytene-like| Infertility    | Papanikos et al. |
| Spo11     | Zygote-like   | Infertility    | Baudat et al. |
| Top6b1    | Zygote-like   | Infertility    | Robert et al. |
| Syccp1    | Pachytene     | Infertility    | de Vries et al. |
| Syccp2    | Zygote-like   | Infertility    | Yang et al. |
| Syccp3    | Zygote-like   | Infertility    | Yuan et al. |
| Scre      | Late zygote   | Infertility    | Liu et al. |
| Sycc1     | Pachytene     | Infertility    | Bolcun-Filas et al. |
| Sycc2     | Pachytene     | Infertility    | Bolcun-Filas et al. |
| Sycc3     | Pachytene     | Infertility    | Schramm et al. |
| Tex11     | Anaphase      | Infertility    | Yang et al. |
| Tex12     | Pachytene     | Infertility    | Hamer et al. |
| Six6os1   | Pachytene-like| Infertility    | Gomez et al. |
| Rpa1      | Metaphase to anaphase| Infertility | Shi et al. |
| Meiub2    | Zygote       | Infertility    | Zhang et al. |
| Spata22   | Late zygote-like| Infertility    | La Salle et al. |
| Meiok21   | Zygote/pachytene| Infertility    | Shang et al. |
| Hop2      | Pachytene-like| Infertility    | Petukhova et al. |
| Meiob     | Pachytene-like| Infertility    | Luo et al. |
| Brme1     | Mid-late pachytene| Infertility    | Takemoto et al. |
| Brca2     | Zygote       | Infertility    | Zhang et al. |
| Msh4/5    | Pachytene-like| Infertility    | Kneitz et al. |
| Rn12d12   | Metaphase     | Infertility    | de Vries et al. |
| Mlh1      | Pachytene     | Infertility    | Reynolds et al. |
| Mlh3      | Metaphase     | Infertility    | Edelmann et al. |

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**CO interference**
The phenomenon of CO interference of one CO on the chromosome will reduce the probability of another CO occurring within a certain interval nearby. The strength of this interference effect decreases with increasing distance. Interestingly, CO interference appears at the level of the whole chromosome, but is limited to specific domains, indicating that COs communicate at the level of the entire chromosome. The molecular mechanism of CO interference is unclear and needs to be studied.

**CO homoeostasis**
The number of COs remains roughly constant as the number of DSBs change. Homeostatic control is enforced during at least two stages in mice, with one stage being after the formation of early recombination intermediates, and the other stage being the maturation of these intermediates toward COs, and the latter stage may be related with CMI.

**CO patterning**
CO patterning describes that the production of COs is inhibited on the telomeres and centromeres in many species including humans. However, the mouse chromosomes are acrocentric, and male mice have a greater number of COs near the telomeres. The differences may be explained by the sex chromosomes characteristics of mice. Because the PAR is located in the sub-telomere region in mice, there is an obligation for a CO in the PAR of male mice to ensure the normal separation of the X and Y chromosome.

**ABNORMAL FORMATION AND REPAIR OF DSBS CAUSE HUMAN INFERTILITY**
The natality around the world including China is declining, and infertility is one of the main reasons. About 15% of couples have infertility problems, and half of the problems occur in men. So far, mutations in many genes involved the formation and repair of DSBs have been reported to be the cause of spermatocyte arrest.

Three genes involved in the formation of DSB have been reported to be related to human infertility, including PRDM9, SPO11 and MEI1. Two SNPs (G433V and T685R) of PRDM9 have been reported to exist in patients with azoospermia, but not in fertile subjects. The results indicate that these mutations in PRDM9 may be specifically related to male azoosperma. The p.Glu186Lys variant of SPO11 caused meiotic arrest in two brothers from a family in the Middle East. Consistent with its function in mice, the homozygous missense mutation of MEI1 caused two brothers from a consanguineous family to suffer from nonobstructive azoosperma (NOA) and meiosis arrest.

Most of the genes responsible for human infertility are involved in the repair of DSBs during meiosis. The deletion of a glutamate residue in HOP2 (Q201del) in humans damaged the estrogen signal transduction pathway, impaired the size of the follicular pool during fetal development, and caused follicular atresia during puberty, which eventually resulted in ovarian dysgeneis. A homozygous missense mutation (c.106G>A) in DMC1, which co-segregated with NOA and primary ovarian insufficiency (POI) phenotypes in a consanguineous Chinese family has been identified. This single amino acid substitution results in protein misfolding and malfunction of DMC1. STAG3 was first described as a POI gene in 2014, and a homozygous missense variant resulted in POI and NOA in human. The truncation of the C-terminal of the MEIOB (c.1218G>A) caused female POI. A nonsynonymous amino acid mutation (N64I) in MEIOB caused azoosperma. In the process of stabilizing dHJs, the homozygous mutation of MSH4 (P638L and S754L) caused arrest of spermatogenic maturation in human males. Studies have found that missense mutations in TEX11 (V748A) caused male infertility with NOA. It has also been reported that a homozygous RNF212 (c.111dupT) variant caused azoosperma in male siblings. In clinical patients, a homozygous frameshift variant in DNA mismatch repair gene MLH3 (c.3632delA) was also found to cause severe oligozoosperma, leading to male infertility.

Mutations in related proteins of synaptonemal complex have also been reported to cause infertility in humans, including SYCP2, SYCP3 and SYCE1. Exome sequencing of infertile males revealed three heterozygous SYCE2 frameshift variants in patients with cryptozoosperma and azoosperma. A 1-bp deletion (643delA) in SYCP3 has been identified in two patients, and the mutation results in a premature stop codon and truncation of the C-terminal, coiled-coil-forming region of the SYCP3 protein, leading to azoosperma with meiotic arrest in human. Mutations in the SYCE1 gene have also been reported in humans; for example, a nonsense homozygous

| Table 2: Meiosis-associated genes that cause human infertility |
|-----------------|-----------------|-----------------|-----------------|
| **Gene name**    | **Clinical phenotype** | **Mutation type** | **Reference** |
| PRDM9           | NOA              | SNPs            | Irie et al.66   |
| SPO11           | NOA              | Missense        | Fakhro et al.67 |
| MEI1            | NOA and ovarian dysgeneis | Missense       | Ben Khelifa et al.68 |
| HOP2            | Ovarian dysgeneis | Deletion of one residue (Glu210) | Zangen et al.69 |
| DMC1            | NOA and POI      | Missense        | He et al.70     |
| MEIOB           | NOA and POI      | Frame-shift deletion and missense | Gershoni et al.75 |
| RNF212          | NOA              | Frame-shift insertion | Krausz et al.76 |
| TEX11           | NOA              | Missense        | Yang et al.77   |
| SYCP2           | NOA              | Frame-shift deletion | Schilt et al.78 |
| SYCP3           | NOA              | Frame-shift deletion | Miyamoto et al.80 |
| SYCE1           | NOA and POI      | Nonsense and splice site homozygous mutation | de Vries et al.81 |
| STAG3           | NOA and POI      | Missense        | Maor-Sagie et al.82 |
| MSH4            | NOA and POI      | Missense and splice homozygous site mutation | Pashaei et al.83 |
| MLH3            | Oligozoosperma   | Frame-shift deletion | Riera-Escamilla et al.73 |

NOA: nonobstructive azoosperma; POI: premature ovarian insufficiency; SNP: single-nucleotide polymorphisms
mutated results in P0I, and homozygous mutations at two different splice sites cause NOA.81–83
Mutations in many genes related to the formation and repair of DSBs have been found to cause human infertility, and a summary is shown in Table 2. Collectively, these data indicate that the establishment and repair of DSBs are indispensable for the maintenance of human fertility.

CONCLUSION
Although numerous studies have revealed the mechanism of formation and repair of DSBs in mammalian meiosis, there are still lots of unanswered questions, especially the mechanism for the regulation of COs. First, in addition to the open chromatin status of CO sites, there should be more potential factors that affect the locations of CO. Future experiments need to be carried out to identify the specificity of CO sites, especially the chromatin environment around them. Second, RNF212 is an important protein that marks CO sites during mid-pachytene.24 RNF212 contains the RING-finger domain, which is an E3 ubiquitin ligase that can catalyze the modification of proteins by ubiquitination of molecules.25 Therefore, the selection of CO sites may be affected by ubiquitination or SUMO modification. Second, considering that the DSB sites have a special motif to recruit PRDM9, whether the CO sites contain a specific motif which recruits CO-related proteins including MutSγ and MutLγ complexes is unclear. Moreover, as the number of DSBs formed in meiotic cells is relatively large compared to the demand for COs, it is interesting to explore the “threshold” of the number of DSBs.

AUTHOR CONTRIBUTIONS
WQ and MCL reviewed the literature, collected data, and wrote the manuscript. CL, YTX, and YMX collaborated in writing, revising, and editing the manuscript. MCL planned this article, designed the concept of this article, helped in literature research and writing the manuscript, and supervised this project. All authors read and approved the final manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

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