Identification of novel BRCA2-binding proteins that are essential for meiotic homologous recombination

Jingjing Zhang

Department of Chemistry and Molecular Biology

University of Gothenburg

2021

UNIVERSITY OF GOTHENBURG

Identification of novel BRCA2-binding proteins that are essential for meiotic homologous recombination

Jingjing.zhang@gu.se

ISBN 978-91-8009-166-4 (PRINT)
ISBN 978-91-8009-167-1 (PDF)
http://hdl.handle.net/2077/67213
Printed in Gothenburg, Sweden 2021
Printed by STEMA SPECIALTRYCK AB

For CD village

Identification of novel BRCA2-binding proteins that are essential for meiotic homologous recombination

Jingjing Zhang

Department of Chemistry and Molecular Biology, University of Gothenburg, SE-40530 Gothenburg, Sweden

Abstract

Meiotic recombination is a molecular process in which the induction and repair of programmed DNA double-strand breaks (DSBs) creates genetic exchange between homologous chromosomes and thus increases genetic diversity and ensures chromosome segregation.

Breast cancer susceptibility gene 2 (*BRCA2*) is a potent cancer suppressor and is required for DSB repair by homologous recombination (HR) in mitosis. However, due to the embryonic lethality of the *Brca2* knockout (KO) mice, the role of BRCA2 in meiotic HR is less well defined.

In our work, we have identified two novel meiosis-specific proteins, MEILB2 (meiotic localizer of BRCA2) and BRME1 (BRCA2 and MEILB2-associating protein 1) that form a ternary complex with BRCA2 and shed light on BRCA2 and its co-factors' roles during meiotic HR.

In *Meilb2* KO male mice, the localization of the recombinases RAD51 and DMC1, which catalyze the homology-directed repair of DSBs, is almost totally abolished, leading to errors in meiotic DSB repair and subsequent sterility. Moreover, MEILB2 binds directly to BRCA2 and is responsible for BRCA2 localization at the meiotic DSBs.

BRME1 functions as a stabilizer of MEILB2 by binding to the α-helical N-terminus of MEILB2 and preventing MEILB2 self-association. In *Brme1* KO mice, the BRCA2-MEILB2 complex is destabilized, leading to defects in DSB repair, homolog synapsis, and crossover formation. Persistent DSBs in *Brme1* KO spermatocytes reactivate the somatic-like DNA-damage response (DDR), which repairs DSBs but cannot complement the crossover formation defects. Further, MEILB2-BRME1 is activated in many human cancers, and somatically expressed MEILB2-BRME1 impairs mitotic HR.

Finally, we solved the crystal structure of the MEILB2-BRCA2 complex and showed that disruption of the MEILB2-BRCA2 interface compromises the recruitment of both MEILB2 and BRCA2 to recombination sites in mouse spermatocytes, thus demonstrating their inter-dependent localization mechanism in meiosis.

Taken together, our results show that the meiotic BRCA2 complex plays a central role during meiotic HR, and its misregulation is implicated in human infertility, miscarriage, and cancer development.

Keywords: meiosis, DSB, cancer, BRCA2, HR, MEILB2, BRME1, RAD51, DMC1, DDR

ISBN 978-91-8009-166-4 (PRINT)
ISBN 978-91-8009-167-1 (PDF)

Papers discussed

This thesis is based on the following studies, referred to in the text by their Roman numerals.

*Equal contribution
†Corresponding author

I. A meiosis-specific BRCA2 binding protein recruits recombinases to DNA double-strand breaks to ensure homologous recombination.
Jingjing Zhang*, Yasuhiro Fujiwara*, Shohei Yamamoto, Hiroki Shibuya†
Nature Communications. 2019.10:722

II. The BRCA2-MEILB2-BRME1 complex governs meiotic recombination and impairs the mitotic BRCA2-RAD51 function in cancer cells.
Jingjing Zhang*, Manickam Gurusaran*, Yasuhiro Fujiwara*, Kexin Zhang, Meriem Echbarthi, Egor Vorontsov, Rui Guo, Devon F Pendlebury, Intekhab Alam, Gabriel Livera, Martini Emmanuelle, P Jeremy Wang, Jayakrishnan Nandakumar, Owen R Davies, Hiroki Shibuya†
Nature Communications. 2020.11:2055

III. Structural basis of BRCA2 recruitment to meiotic recombination sites.
Devon F. Pendlebury*, Jingjing Zhang*, Ritvija Agrawal, Hiroki Shibuya† and Jayakrishnan Nandakumar† (Manuscript)

Papers not included in this thesis:

Distinct TERB1 Domains Regulate Different Protein Interactions in Meiotic Telomere Movement.
Jingjing Zhang, Zhaowei Tu, Yoshinori Watanabe, Hiroki Shibuya†
Cell reports. 2017. 21(7):1715-1726

Telomeric double-strand DNA-binding proteins DTN-1 and DTN-2 ensure germline immortality in *Caenorhabditis elegans*.
Io Yamamoto, Kexin Zhang, Jingjing Zhang, Egor Vorontsov, Hiroki Shibuya†
Elife. 2021. 10:e64104

Table of Content

Introduction

1. Mitosis and meiosis

The cell cycle has two major phases – interphase, which is the preparatory phase for cell division, and M phase, which is the division phase, where M stands for either mitosis or meiosis (CP Leblond, 1998).

Mitosis occurs in somatic cells, where replicated sister chromatids are divided into two genetically identical daughter cells. The major functions of mitosis are organism growth and replacing damaged cells (MT Hayashi, 2013).

Meiosis, in contrast, occurs exclusively in reproductive cells and involves two successive cell divisions, meiosis I and meiosis II. During meiosis I, homologous chromosomes are paired, recombined, and divided into two daughter cells, whereas in meiosis II, sister chromatids are divided to produce genetically different haploid germ cells (the sperm or egg). This procedure increases genetic diversity (D Zickler, 2016).

The characteristic events during meiosis are the pairing and recombination of homologous chromosomes in prophase I, which ensure proper crossover (CO) formation as well as accurate chromosome segregation of homologous chromosomes in subsequent processes (**Fig. 1**).

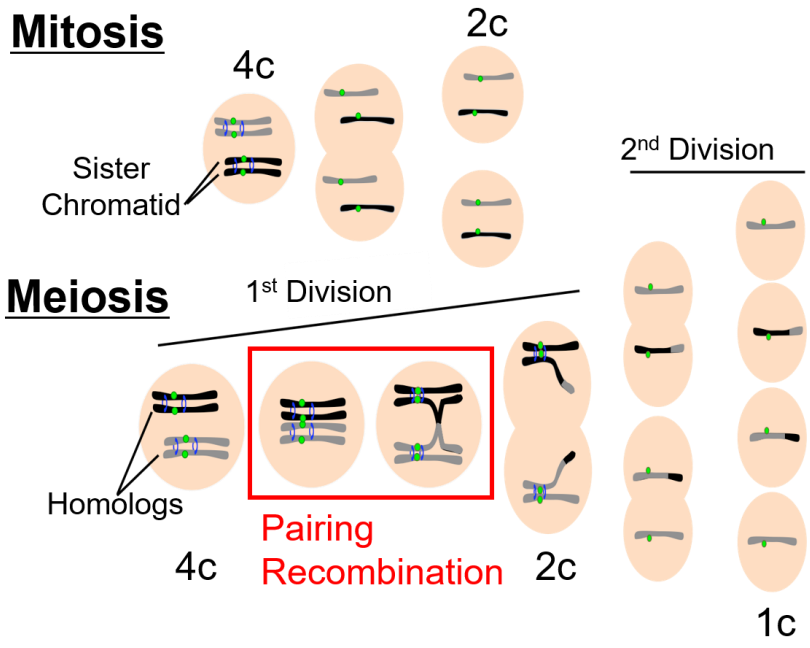

Figure 1. Mitosis and meiosis.

During mitosis, replicated sister chromatids are divided into two daughter cells. In contrast, during meiosis I, homologous chromosomes are paired, recombined, and divided into two daughter cells. During meiosis II, sister chromatids are divided without being replicated, thus producing haploid germ cells (the sperm or egg).

2. DNA damage and repair pathways

DNA preserves and transmits genetic information across generations (A Ciccia, 2010). However, DNA is constantly being attacked by endogenous factors (e.g., hydrolytic and oxidative reaction) and exogenous agents (e.g., UV radiation, ionizing radiation, and mutagenic chemicals). The DNA lesions formed from these insults can lead to errors during DNA replication and transcription, which result in cell cycle arrest, cell death, aging, cancer, and genetic diseases. Therefore, the proper repair of DNA damage is essential for the maintenance of genomic integrity (A Ciccia, 2010) (SP Jackson, 2009).

To counteract the threats caused by DNA damage, at least five major DNA repair pathways can be activated to specifically repair different types of DNA lesions (**Table 1**). Notably, some lesions can be recognized by multiple repair pathways (T Helleday, 2008). Thus, efficient DNA repair can occur either in a damage-specific or in a collaborative manner in order to protect DNA integrity and promote survival (A Ciccia, 2010) (SS Ambekar, 2017).

Table 1: DNA damage and repair pathways

DNA repair pathway	Damaging agents	Examples
Mismatch repair (MMR)	DNA replication errors	A-G mismatches, insertions, deletions
Base excision repair (BER)	Reactive oxygen species, X-rays, alkylating agents, spontaneous chemical reactions	Oxidation (8oxoG), uracil, single-strand breaks
Nucleotide excision repair (NER)	UV radiation and polycyclic aromatic hydrocarbons	Pyrimidine dimers, bulky adducts, intra-strand crosslinks
Non-homologous end joining (NHEJ) and Homologous recombination (HR)	Ionizing radiation, anti-tumor agents	Double strand breaks, inter-strand crosslinks

3. DNA double-strand break (DSB) repair pathways

Among the aforementioned DNA damages, DSBs are extremely toxic and are the most complicated to repair. As **Table 1** illustrates, the two major pathways, homologous recombination (HR) and non-homologous end-joining (NHEJ) are responsible for repairing DNA DSBs (A Tubbs, 2017) (A Shibata, 2017) (R Ceccaldi, 2016).

3.1 NHEJ

NHEJ is considered to be a relatively simple pathway because it does not rely on any template and can remain active throughout the entire cell cycle. When the NHEJ machinery encounters DNA damage, the broken ends are trimmed through a series of protein complexes, including Ku70/80 heterodimer, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and the nuclease Artemis. Eventually, the compatible ends are sealed by XRCC4 DNA ligase 4. Although this pathway is effective, it is inherently prone to generating mutations (I Brandsma, 2012) (**Fig. 2**).

3.2 HR

In contrast, HR uses the sister chromatid as the repair template to ensure the high-fidelity transmission of the genetic information. However, HR can only take place when sister chromatids are present. Therefore, HR only occurs in the S and G2 phases of the cell cycle.

During HR, the proteins that encounter the damaged DNA are the MRE11-RAD50-NBS1 (MRN) complex and C-terminal-binding protein-interacting protein (CtIP). These complexes function together to resect the broken ends of the damaged double-stranded DNA and generate 3′ single-stranded DNA (ssDNA) overhangs (EP Mimitou, 2009). The ssDNA is first coated by the replication protein A (RPA) complex, which is an ssDNA binding protein. Subsequently, the RAD51 recombinase replaces RPA and forms nucleoprotein filaments on the ssDNA with the help of BRCA2 (RB. Jensen, 2010) (W Zhao, 2015). These RAD51 nucleoprotein filaments then perform the homology search and catalyze DNA strand exchange between sister chromatids in order to carry out the homology-directed repair (**Fig. 2**).

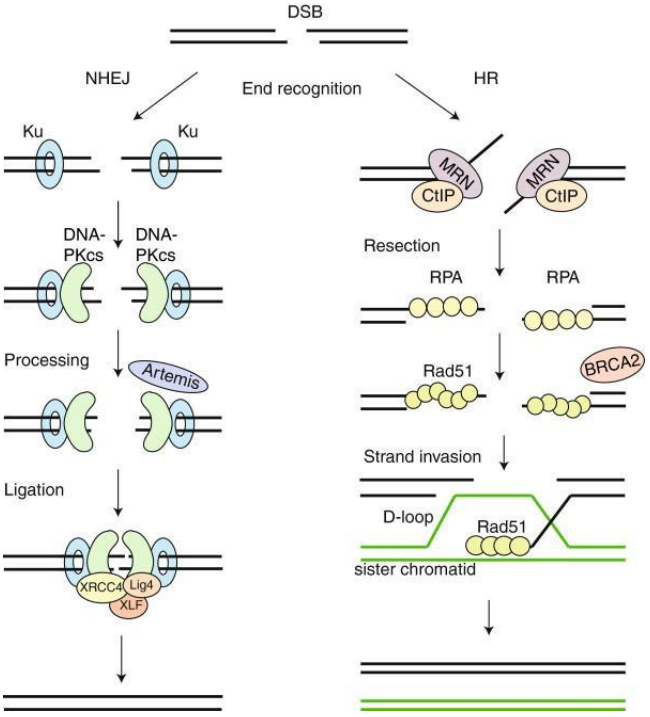

Figure 2. NHEJ and HR.

During NHEJ, the broken ends are recognized by the Ku70/80 heterodimer and recruited DNA-PKcs and Artemis, finally ligated by XRCC4-DNA ligase IV-XLF ligation complex. In contrast, the HR pathway starts with resection of the broken ends by the MRN-CtIP complex to generate ssDNA. The ssDNA is coated by RPA and subsequently replaced by RAD51 recombinase with the help of BRCA2. These RAD51 nucleoprotein filaments mediate strand invasion using sister chromatids. Finally, the extension of the displacement loop (D-loop) and the capture of the second end leads to the repair of the lesion. This figure is adapted from (Inger Brandsma, 2012).

3.3 Balance between NHEJ and HR

Because both NHEJ and HR contribute to the repair of DNA DSBs, regulatory factors must be involved to determine which pathway should be used.

One of the main factors is the cell cycle phase, and, as mentioned above, HR only functions in S/G2 after DNA replication because the sister chromatid is necessary as the repair template, whereas NHEJ can repair DSBs throughout the entire cell cycle (LS Symington, 2011).

The second factor that has been well studied is the DSB end structure. As an example, in S phase, because of the lack of a partner for the DSB end, one-ended DSBs are mainly repaired by HR. In contrast, in G2 phase, when two-ended DSBs are induced by ionizing radiation, 70% of the DSBs will be rapidly repaired by NHEJ (A Shibata, 2017) (JR Chapman, 2012).

In addition, an antagonistic relationship has been found between p53-binding protein 1 (53BP1) and breast cancer susceptibility gene 1 (BRCA1) regarding the choice of the DSB repair pathways. Phosphorylation of 53BP1 by ataxia telangiectasia mutated (ATM) promotes the recruitment of the effector molecule RIF1 to the DSB site, which blocks the loading of the CtIP-BRCA1 complex and thus inhibits DNA resection and drives the repair through the NHEJ pathway. In turn, BRCA1-CtIP removes 53BP1 and Ku70/80 from the break site and facilities the end resection by the MRN complex through the HR pathway. It has been shown that loss of 53BP1 can rescue the viability in *Brca1*-deficient mice (L Cao, 2009) (JR Chapman, 2012).

During early meiotic prophase I, NHEJ is inhibited and HR ensures the repair of DSBs in order to ensure genetic exchange between homologous chromosomes. However, a recent study showed that NHEJ can be activated in response to exogenously induced DNA damage during the late stages of meiotic prophase I. This finding provides new insights into the transitions between the two different DSB repair pathways in mouse meiotic prophase I (A Enguita-Marruedo, 2019).

3.4 DDR

The DNA damage response (DDR) is a signal transduction pathway that senses DNA damage and activates a series of protein kinases. When DSBs occur, the first regulator that engages in this pathway is the MRN complex, which forms an activation loop together with ATM for detecting the damaged DNA. Deficiency in any component of the MRN complex leads to defects in ATM signaling (BJ Lamarche, 2010). ATM and Rad3-related (ATR) are activated upon the activation of ATM and functions together with DNA-PKcs to carry out the second wave of phosphorylation. Thus, the downstream checkpoint kinases CHK1 and CHK2 act as the key regulators of the inhibition of the CDC25 phosphatases. This in turn suggests the connection between DNA damage signaling and cell cycle regulation (AM Heijink, 2013).

Here, the following three distinct cell cycle checkpoints are activated upon the detection of DNA damage; the G1/S checkpoint, which prevents cells from entering S phase and precludes the replication of damaged DNA. The intra-S checkpoint, which delays and inhibits the ongoing replication of damaged DNA, and the G2/M checkpoint, which prevents cells from entering into mitosis (AM Heijink, 2013).

In addition, chromatin modification is one of the key events detected after DNA damage, where the histone variant H2AX becomes phosphorylated at its C-terminal Ser-139 residue by the ATM, ATR, and DNA-PK kinases to form γH2AX. Subsequently, chromatin

modification stabilizes the interactions among other DNA damage signaling proteins such as 53BP1, BRCA1, and NBS1 at the break site (A Grabarz, 2012) (K Rothkamm, 2015).

4. Meiotic HR

Meiotic recombination is the most important feature of meiosis. During meiotic HR, abundant DSBs are intentionally introduced by the activation of the meiosis-specific endonuclease SPO11 (S Keeney, 2006) (B de Massy, 2013) (I Lam, 2014). For the quick repair of the abundant meiotic DSBs, the coordinated action of the RAD51 recombinase and its meiosis-specific paralog DMC1 is required (DL Pittman, 1998) (A Shinohara, 2004) (J Dai, 2017) (**Fig. 3**).

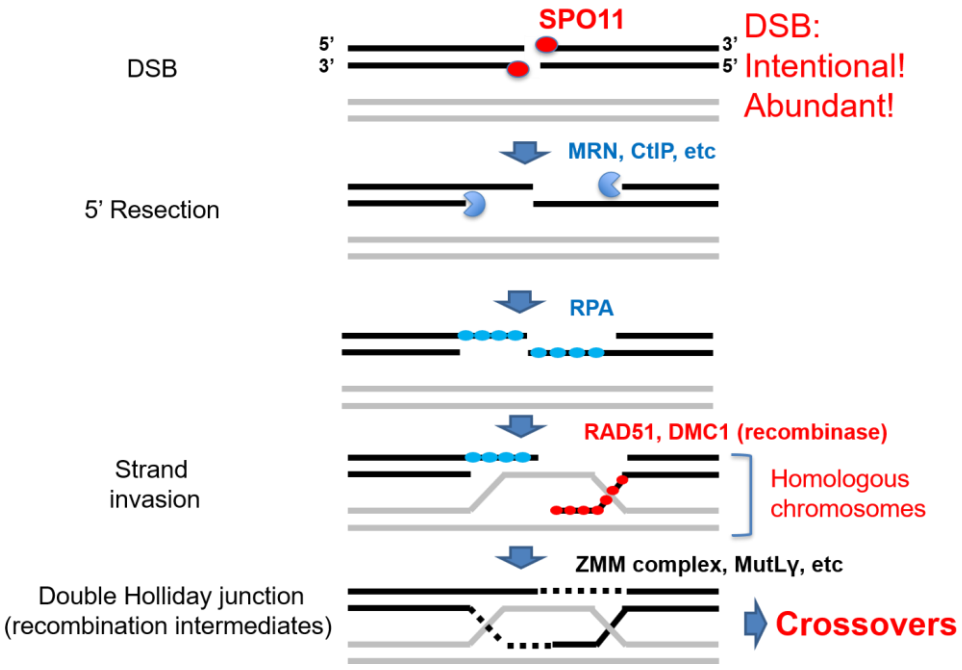

Figure 3. Meiotic HR.

Meiotic programmed DSBs are abundantly distributed throughout the genome (about 300 per nucleus in mice) and are introduced by the activation of the meiosis-specific endonuclease SPO11. The MRN complex resects the 5' ends to produce 3'-ssDNA overhangs. The ssDNA overhangs are recognized by RPA and subsequently replaced by the RAD51 recombinase and its meiosis-specific paralog DMC1. The coordinated action of these two distinct recombinases mediates strand invasion between the homologous chromosomes, but not sister chromatids, and promotes the formation of double Holliday junctions (dHJs). Finally, dHJs are resolved yielding COs.

4.1 Mitotic vs. Meiotic HR

Meiotic and mitotic HR share many common features; for example, MRN, RPA, and RAD51 are all involved in both processes. However, there are three major differences (**Table 2**). First, homologous chromosomes are used as the repair template in meiotic HR instead of the sister chromatid that is used in mitotic HR. Second, the ultimate goal of meiotic HR is to form beneficial product COs, which are essential for increasing genetic diversity and for ensuring accurate chromosome segregation during anaphase I, while the purpose of mitotic HR is to repair DNA damages (KP Kim, 2010) (H Guillon, 2015). Third, DSBs are intentionally introduced in meiosis by the meiosis-specific endonuclease SPO11 and are

distributed throughout the genome, while in mitosis DSBs are usually generated by accident (S Keeney, 1997) (J Lange, 2016).

Table 2: Summary of the differences between mitotic HR and meiotic HR

	Mitotic recombination	Meiotic recombination
Repair template:	•Sister chromatid	•Homologous chromosomes
Function/ consequence:	•Repair DNA damage	•Bivalent formation •Increase genetic diversity •Chromosome segregation
DSB initiation:	•Accident (UV, Genotoxins, DNA replication error)	•Intentional
	•Few (∼50 per cell)	•Abundant (∼300 per cell)

4.2 The synaptonemal complex

The synaptonemal complex (SC) is a meiosis-specific and evolutionarily conserved structure that is required for the stabilization of synapses between homologous chromosomes and that further facilitates homology-directed recombination and CO formation (J Fraune, 2012). The ladder-like structure of the SC is composed of two lateral elements (LEs), also known as axial elements (AEs) (before chromosome synapsis), which are held together by transverse filaments (TFs) and a number of central elements (CEs) (**Fig.4**).

In mice, the major protein components of the AEs/LEs are cohesin complexes as well as SYCP3 and SYCP2. *Sycp3*-deficient males are sterile, with failure to form complete SCs and chromosomal synapsis. Meanwhile, the absence of SYCP3 negatively affects the loading of HR proteins, such as RAD51 and RPA. SYCP2 binds to SYCP3 through a conserved coiled-coil domain and forms heterodimers with SYCP3 both *in vitro* and *in vivo*. *Sycp2* mutant males are also infertile due to failure to form AEs/LEs and chromosomal synapsis (M Spindler, 2019) (L Yuan, 2000) (F Yang, 2006) (K Winkel, 2009).

The TF protein SYCP1 plays a central role in SC assembly, and the interaction between SYCP1 and SYCP2 has been confirmed in both yeast two-hybrid (Y2H) assay and immunoprecipitations (IP) using meiotic cell extracts. Further, SYCP2 acts as a linker between SYCP1 and SYCP3 by directly binding to SYCP3, and SYCP1 is crucial for recruiting the CE protein components SYCE1, SYCE2, SYCE3, TEX12, and SIX6OS1 in sequential order (A Castro, 2005) (A Hernández-Hernández, 2016) (L Gómez-H, 2016).

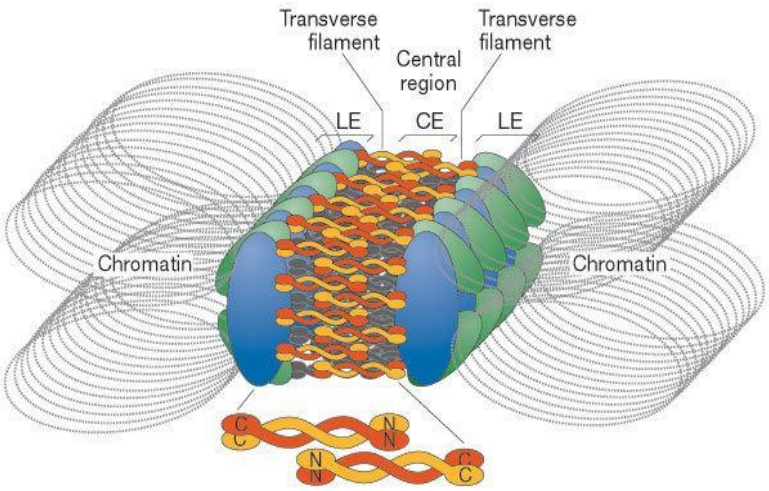

Figure 4. The synaptonemal complex.

The SC is a protein structure formed by LEs (SYCP2 and SYCP3), CEs (SYCE1, SYCE2, SYCE3, TEX12, and SIX6OS1), and TFs (SYCP1). This figure is adapted from (A Castro, 2005), which was originally adapted from (SL Page, 2004).

4.3 Chromosomal events of meiotic prophase I

During meiotic prophase I, chromosome organization consists of 4 different stages – leptotene, zygotene, pachytene, and diplotene – that are defined by SC dynamics and homologous axis morphogenesis (N Hunter, 2015).

Leptotene starts with the loading of AEs along the length of sister chromatids, and this is coincident with DSB formation. During zygotene, homologous chromosomes start to synapse and the SC begins to form. The presence of a complete SC determines the entering of pachytene, while COs appear at the end of pachytene. Finally, during diplotene, the SC is disassembled and homologs become compacted and connected by chiasmata (N Hunter, 2015) (**Fig. 5**).

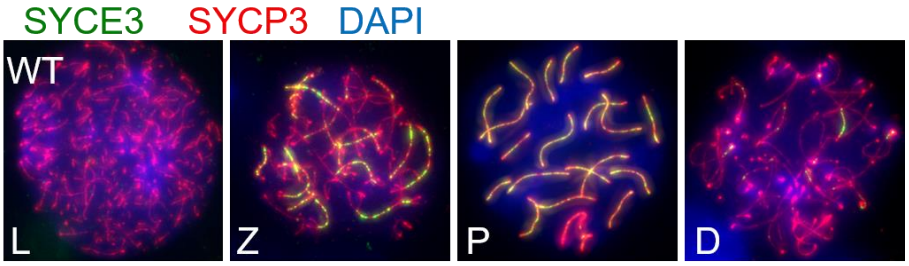

Figure 5. Chromosomal events of meiotic prophase I.

Spermatocytes stained for AEs/LEs (SYCP3, red), CEs (SYCE3, green, but appears yellow because of signal overlap), and DAPI. Leptotene (L), zygotene (Z), pachytene (P), and diplotene (D). This figure is adapted from **Paper II**.

4.4 Key players and steps during meiotic HR

4.4.1 SPO11 and the formation of DSBs

It has been well studied that DSB formation during meiotic recombination in mice is catalyzed by the meiosis-specific endonuclease SPO11 and its binding partner TOPOVIBL. *Spo11-/-* spermatocytes exhibit synapsis defects, including incomplete synapsis as well as partner switches, which are indicators of nonhomologous synapsis. Moreover, the localization of recombinases RAD51 and DMC1 is abolished due to the loss of DSB induction. Germ cells undergo massive apoptosis resulting in infertility in both genders of *Spo11-/-* mice (F Baudat, 2013). In addition, TOPOVIBL is required for meiotic DSB formation and is essential for spermatocyte and oocyte development (T Robert, 2016).

SPO11 activity requires the coordinated involvement of other pro-DSB factors, for instance, meiosis-specific 4 homolog (MEI4), REC114, and IHO1. *Mei4-/-* mice exhibit deficiency in DSB formation and homologous synapses (R Kumar, 2010). Moreover, MEI4 forms a complex with REC114 and IHO1 in mouse spermatocytes, and *Rec114-/-* and *Iho1-/-* mice show similar phenotypes as *Mei4-/-* mice (R Kumar, 2018) (M Stanzione, 2016). Additionally, ANKRD31, HORMAD1, and IHO1 function in organizing those pro-DSB factors on meiotic chromosomes (M Boekhout, 2019) (F Papanikos, 2019) (K Daniel, 2011) (M Stanzione, 2016). All these studies suggest that SPO11-TOPOVIBL as well as its partner proteins are essential for the induction of meiotic DSBs.

4.4.2 The MRN complex and end processing

DNA end resection is a prerequisite for producing the extensive 3’-ssDNA overhangs that provide a platform for recruiting ssDNA-binding proteins. Multiple factors are involved in this process, including the MRN complex, CtIP, exonuclease 1 (EXO1), DNA replication helicase/nuclease 2 (DNA2), and Bloom syndrome protein (BLM) (F Baudat, 2013) (T Liu, 2014) (N Hunter, 2011) (F Zhao, 2020).

The MRN complex consists of three molecules – MRE11, RAD50, and NBS1. MRE11 functions as a catalytic subunit with both endonuclease and exonuclease activities (H Tsubouchi, 1998). RAD50 contains a coiled-coil domain, which is important for tethering the complex to free DNA ends and for regulating MRE11’s endonuclease/exonuclease activities (M Chansel-Da Cruz, 2020). NBS1 is a multifunctional protein that is involved in DNA damage signaling through the activation of the ATM kinase and by recruiting CtIP to DSBs through its FHA domain (F Zhao, 2020). In addition, CtIP plays indispensable roles in promoting resection and embryonic development. It works as the interacting partner of the MRN complex and is required for catalyzing the 5’-3’ DNA resection (F Polato, 2014). Upon initiation of MRN-CtIP–mediated end resection, EXO1, DNA2, and BLM carry out their nuclease and helicase activities and are responsible for the extensive end resection (BJ Lamarche, 2010) (T Liu, 2014) (F Zhao, 2020).

4.4.3 The RPA complex, SPATA22, and MEIOB

The resected 3’-ssDNA is rapidly bound by the trimeric RPA complex to prevent degradation and secondary structure formation (MS Wold, 1997). The RPA heterotrimer consists of three different subunits – RPA1 (70 kDa), RPA2 (32 kDa), and RPA3 (14 kDa). Because of the embryonic lethality of the mutant mice, the role of the RPA heterotrimer in meiotic HR has remained unknown. However, a recent study using testis-specific *Rpa1* conditional knockout (cKO) mice showed that RPA complex is essential for the loading of RAD51 and DMC1 as well as the proper CO formation in mouse spermatocytes. In addition, the stability of RPA2

and RPA3 depends on RPA1. Taken together, RPA1 appears to function in both recombinase recruitment and CO formation during meiotic HR (B Shi, 2019).

MEIOB was identified from the meiotic chromatin-associated protein screening, and it contains an OB-fold domain with homology to one of the RPA1 OB-folds. MEIOB can thus directly bind to ssDNA. *Meiob-/-* mice exhibit a reduction in both RAD51 and DMC1 focus counts, synapse defects, and unrepaired DSBs with the persistence of γH2AX and RPA signals, consequently leading to impairment of CO formation and infertility in both genders (B Souquet, 2013) (M Luo, 2013). SPATA22 forms an obligate complex with MEIOB and *Spata22* deficient mice phenocopies *Meiob* deficient mice. The localization of MEIOB and SPATA22 at sites of meiotic recombination is interdependent, and they are recruited to DSBs as a complex (S La Salle, 2012) (S Ishishita, 2014).

Moreover, the MEIOB-SPATA22 complex physically associates with the RPA heterotrimeric complex. However, the localization of RPA does not require both MEIOB and SPATA22, and RPA can still form foci in the absence of either MEIOB or SPATA22. Furthermore, both MEIOB and SPATA22 foci are still present in *Rpa1* cKO mice (Y Xu, 2017) (B Shi, 2019). These findings suggest that the RPA complex and MEIOB-SPATA22 complex independently bind to ssDNA while there is a protein-protein interaction between these two complexes.

In addition to their canonical function of 3'-ssDNA binding at DSBs, RPA, MEIOB, and SPATA22 might be involved in the stabilization of the D-loop seeing that these complexes colocalize with each other and form bright foci in late zygotene and early pachytene, where DSBs are gradually repaired and matured into D-loops (M Luo, 2013). MEIOB and SPATA22 may also contribute to the second-end capture after removing 3'-flaps and thus result in dHJ (B Souquet, 2013).

4.4.4 RAD51, DMC1, and D-loops

RAD51 and its meiosis-specific paralog DMC1 are members of the RecA family, which form nucleoprotein filaments that carry out the homology search and strand invasion and that result in the production of D-loop structures (DL Pittman, 1998) (A Shinohara, 2004) (DK Bishop, 1994). After stabilization of the D-loop and DNA synthesis, an intermediate is formed that is called single-end invasion (SEI) (N Hunter, 2001). Further processing of the SEI in subsequent repair steps generates two distinct products, non-crossovers (NCOs) and COs. The synthesis-dependent strand annealing (SDSA) pathway contributes to most of the NCOs, where the invading strand is displaced from the D-loop to dissolve the SEI and is annealed to the complementary DNA sequences on the other end of the DSB. Finally, the process ends with the formation of NCOs (N Hunter, 2001). Alternatively, during the CO-forming pathway, the SEI can be stabilized to capture the second 3' DSB end, which results in the formation of a dHJ. The resolution of the dHJ yields COs that contribute to the formation of chiasmata (A Schwacha, 1995). dHJ resolution can also result in NCO formation depending on the cleavage orientation of each HJ (N Hunter, 2001) **(Fig.6)**.

The recruitment of RAD51 and DMC1 to the meiotic DSB site requires specific modulators or mediators to overcome the inhibitory effect of the heterotrimeric RPA (A Shinohara, 2004) (JS Martinez, 2016). Several factors function as modulators of RAD51 and DMC1. In the mouse model, the HOP2-MND1 heterodimer can physically interact with both RAD51 and DMC1 recombinases and can stimulate their activity to form D-loops. Notably, this stimulatory effect in the case of DMC1 can be up to 35-fold. In the absence of HOP2-MND1, homology search and strand invasion will be delayed, leading to inter-homolog recombination, synapsis defects, and meiotic arrest (GV Petukhova, 2005).

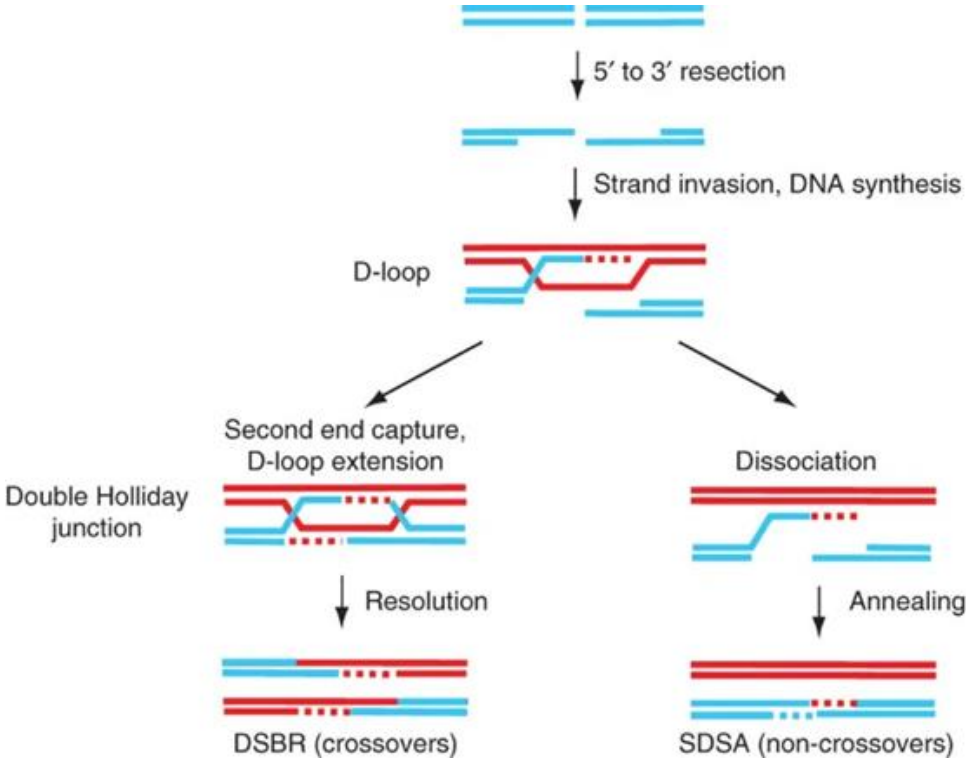

Figure 6. Pathways of D-loop resolution.

After forming the D-loop, the joint molecule will proceed through either dHJ or SDSA. In dHJ (the left branch), the second DSB end is captured to form dHJ which is then resolved into COs or NCOs. Alternatively, in SDSA (the right branch), the newly synthesized strand is dissociated from the template and annealed to the other end. Followed gap-filling and ligation yields NCOs. This figure is adapted from (DV Bugreev, 2010).

4.4.5 MLH1 and crossover formation

The final product of the meiotic HR is the formation of COs, which are essential for increasing genetic diversity and for establishing the physical connections between homologous chromosomes that ensure proper segregation during meiosis I. Errors in CO formation will result in aneuploidy, which can lead to infertility, miscarriages, or birth defects, for instance, Down syndrome (N Hunter, 2015) (TT Saito, 2017).

Two different classes of COs have been identified, namely class I and class II. The majority of COs (90–95% in mice) belong to class I, which are regulated mainly by the ZMM proteins and MutLγ complex. Class I COs were first identified in budding yeast, and the deletion of the ZMMs caused defects in SC and Class I CO formation (N Hunter, 2015) (S Gray, 2016).

The ZMMs consist of the following proteins and complexes. ZIP1 is the TF protein in budding yeast, and SYCP1 in mammals is believed to be an ortholog of ZIP1 based on functional similarities (FAT de Vries, 2005). ZIP2 (MZIP2 in mammals), ZIP4 (TEX11 in mammals), and SPO16 form a trimeric complex and function in the stabilization of the joint molecules (K Arora, 2019) (F Yang, 2015) (Q Zhang, 2018) (Q Zhang, 2019). ZIP3 (RNF212 in mammals), an E3 ligase, can catalyze SUMO conjugation and is required for the localization of other ZMMs (A De Muyt, 2014) (A Reynolds, 2013). MER3 (HFM1 in

mammals) is required to complete synapsis and to ensure the correct number of COs (MF Guiraldelli, 2013). Finally, the MutSγ components MSH4 and MSH5 are involved in stabilizing dHJ intermediates (SS de Vries, 1999) (B Kneitz, 2000).

The MutLγ complex acts downstream of ZMM proteins, consists of MLH1 and MLH3, and their endonuclease activities are essential for the resolution of dHJ intermediates. Because they perfectly localize at Class I CO sites in a number of organisms, therefore, MLH1 and MLH3 are the most commonly used markers for Class I CO (S M Baker, 1996) (SM Lipkin, 2002) (N Hunter, 1997) (S Santucci-Darmanin, 2002).

Besides, other proteins such as HEI10, CNTD1, PRR19 and CDK2 are also engaged in Class I CO formation regulation (differentiation and maturation) (H Qiao, 2014) (JK Holloway, 2014) (A Bondarieva, 2020). In addition to Class I COs, a small subset of COs (5–10% in mice), called Class II COs, are generated through the MUS81-MMS4 pathway (EK Schwartz, 2012), while the detailed molecular mechanism underlying the biogenesis is less understood compared to that of Class I COs.

From a global perspective, three principles have been discovered for governing COs – assurance, interference, and homeostasis. CO assurance provides at least one CO to each homologous chromosome, interference ensures that the COs are widely spaced with other COs on the same chromosome, and homeostasis ensures that the number of COs is independent of DSB numbers (M Shinohara, 2008) (TT Saito, 2017).

5. BRCA2

BRCA2 was first identified in 1995 and has been the subject of intensive research over the past 25 years (R Wooster, 1995). This is because *BRCA2* acts as a potent cancer suppressor gene, and germline mutations in *BRCA2* predispose to a variety of adult and pediatric cancers in humans. During mitosis, the primary function of BRCA2 is thought to be the repair of DSBs by the HR pathway (A Fradet-Turcotte, 2016). As described earlier, BRCA2 facilitates the loading of the RAD51 recombinase through direct binding to both ssDNA and RAD51 (RB Jensen, 2010) (G Chatterjee, 2016). In addition to RAD51, other interacting partners of BRCA2 have also been identified, including DSS1, which is essential for the stability of BRCA2 and for delivering BRCA2-RAD51 to RPA-bound ssDNA (J Li, 2006) (W Zhao, 2015), BRCA1, which functions upstream of BRCA2 in response to DNA damage (F Zhang, 2009), and PALB2, which acts as a linker between BRCA1 and BRCA2 and is essential for BRCA2 functioning in HR (B Xia, 2006).

However, due to the embryonic lethality of *Brca2-/-* mice, the role of BRCA2 in meiosis has been relatively poorly studied (K Gudmundsdottir, 2004), although some breakthroughs have been made in recent years. BRCA2 is initially expressed during spermatogenesis, specifically during meiotic prophase (F Connor, 1997). BRCA2 is then thought to colocalize with BRCA1 and RAD51 on the meiotic chromosome axis (J Chen, 1998). Moreover, deletion of the COOH-terminal domain of BRCA2 exhibits no apparent meiotic defects (KA McAllister, 2002). *Brca2*-null mice carrying a human BAC with the human *BRCA2* gene show spermatocytes arrested in early prophase I with unrepaired DSBs, incomplete synapses, and reduced numbers of RAD51 foci (SK Sharan, 2004).

Despite these previous findings, the detailed mechanism of BRCA2 in DSB repair and meiotic recombination remains poorly understood.

6. BRCA2 and sporadic cancers

While the mutation of *BRCA2* is a major cause of familial breast cancer cases, the misregulation of BRCA2-interacting proteins is thought to be a cause of sporadic cancers (H Daum, 2018). For example, depletion of the BRCA2 binding protein DSS1 leads to hypersensitivity to DNA damage (J Li, 2006), and overexpression of SYCP3 in somatic cells impairs the function of BRCA2 in mitotic HR by forming a complex with BRCA2 (N Hosoya, 2011). Further, overexpression of *EMSY* impairs the BRCA2-RAD51 pathway specifically in sporadic cancers (I Cousineau, 2011). These results highlight the role of BRCA2 interactors in the development of sporadic cancers, thus necessitating the identification of BRCA2 cofactors to fully understand the role of BRCA2 as a cancer suppressor.

Results

Paper I

MEILB2 is a novel regulator of meiotic HR

In this paper, we used an *in vivo* electroporation technique to express GFP-fusion proteins in mouse testes in order to examine the subcellular localization of meiotically upregulated genes of unknown function. One of the candidates, HSF2BP, showed punctate signals along the chromosome axes. We renamed this protein as meiotic localizer of BRCA2 (MEILB2). By reverser transcription (RT) PCR, we confirmed the germ cell-specific mRNA expression of *Meilb2*.

MEILB2 is a 338 amino acid protein that is composed of an N-terminal coiled-coil domain and a C-terminal armadillo repeat domain. Consistent with the localization of the GFP-fusion protein, endogenous MEILB2 showed a characteristic localization pattern specifically in early prophase I in which punctate signals formed along the chromosome axes. These MEILB2 foci colocalized with known meiotic HR factors, such as RPA2 and SPATA22, proving that MEILB2 is a novel meiotic HR protein.

Disruption of *Meilb2* results in synapsis defects and abrogates the localization of recombinases RAD51 and DMC1

To address the function of MEILB2, we generated *Meilb2* KO mice. We observed a smaller testis size in KO compared with WT littermates, and no mature sperm was found in the KO epididymis. Further, TUNEL assay detected numerous apoptotic cells at the periphery of the *Meilb2-/-* seminiferous tubules, suggesting that the germ cells were eliminated during the progression of meiotic prophase I.

Moreover, by staining for the CE marker SYCE3 that marks the synapsed chromosome axes, we found that *Meilb2-/-* spermatocytes are arrested in the zygotene stage with incomplete synapsis. Surprisingly, the localization of the RAD51 and DMC1 recombinases is largely abolished in *Meilb2-/-* spermatocytes, leading to errors in meiotic DSB repair.

MEILB2 binds to BRCA2 and recruits BRCA2 to sites of meiotic recombination

We conducted a comprehensive Y2H screening using a mouse testis cDNA library, and BRCA2 was repeatedly identified as a MEILB2 binding protein. We also identified the MEILB2-binding domain (MBD) within BRCA2, which is necessary and sufficient for the interaction with MEILB2. We confirmed this specific interaction between MEILB2 and BRCA2-MBD by both Y2H and GFP pull-down assay.

Furthermore, by overexpressing the GFP-BRCA2-MBD construct in mouse testes, we found that BRCA2-MBD formed punctate foci along the chromosome axis, which colocalized with the known meiotic HR makers. Notably, this localization of BRCA2-MBD was completely abolished in *Meilb2* KO, which suggests MEILB2 functions as a localizer of BRCA2 in meiotic HR through the direct protein-protein interaction.

Paper II

BRME1 interacts with MEILB2 and forms a ternary complex with MEILB2-BRCA2

We identified BRME1 (BRCA2 and MEILB2-associating protein 1) by performing the MEILB2 Y2H screening. We also mapped the MEILB2 binding-domain (MBD) within BRME1. Y2H analysis confirmed that BRME1-MBD was indeed interacting with MEILB2. The FLAG pull-down assay from cultured cells co-expressing FLAG-tagged BRME1 truncations ($^{FLAG-}$BRME1) with MYC-tagged MEILB2 (MEILB2^{-MYC}) also showed that BRME1-MBD is necessary and sufficient to interact with MEILB2.

In addition, we expressed the GFP-tagged BRCA2 ($^{GFP-}$BRCA2) truncations together with $^{FLAG-}$BRME1 and MEILB2^{-MYC} to perform the GFP pull-down assay. When all three proteins were co-expressed, we could detect the formation of a $^{GFP-}$BRCA2-MEILB2^{-MYC}-$^{FLAG-}$BRME1 ternary complex. In addition, BRME1-MBD and BRCA2-MBD were sufficient for the formation of the ternary complex with MEILB2. In conclusion, MEILB2 mediates the BRCA2–BRME1 interaction through its C- and N-termini, respectively. Moreover, the reciprocal BRCA2 and BRME1 IP from mouse testis extracts provided further evidence for the existence of BRCA2-MEILB2-BRME1 ternary complex *in vivo.*

BRME1 is a meiosis-specific protein and a stabilizer of MEILB2

Similar to *Meilb2*, we observed germ cell-specific expression of *Brme1*, and endogenous BRME1 formed dotty foci colocalized with the DSB marker RPA2. To address the function of BRME1, we generated *Brme1* KO mice by CRISPR-Cas9. We found that in *Brme1-/-* spermatocytes both signal intensity and foci numbers of MEILB2 were reduced. Consistent with this, the protein level of MEILB2 was also decreased in *Brme1-/-* spermatocytes proving that BRME1 functions as a stabilizer of MEILB2 *in vivo*. Further, by collaborating with Dr. Davies lab, we purified recombinant MEILB2-BRME1 complex *in vitro* and showed that BRME1 functions as a stabilizer of MEILB2 by binding to the α-helical N-terminus of MEILB2 and preventing MEILB2 self-association.

BRME1 is essential for normal meiosis progression, DSB repair, homologous synapsis, CO formation, and male fertility

From the histological analysis, we found smaller testis size in *Brme1-/-* mice in comparison with WT, and spermatids were absent. Mature spermatozoa were also missing in the epididymis.

Consistent with the reduction of MEILB2, we observed attenuated signal intensity of GFP-BRCA2-MBD in *Brme1-/-* spermatocytes. As the downstream proteins, the foci numbers of both RAD51 and DMC1 were also significantly reduced in *Brme1-/-* spermatocytes. Moreover, we found the persistence of RPA2 and γH2AX staining until late prophase I (diplotene), which implied that the DSBs remained unrepaired. Further, various synapsis defects were found, and these were mainly in the pachytene stage. As a consequence, CO formation, seen by the staining of MLH1, was abrogated in some of the homologous pairs, which led to the appearance of univalent chromosomes misaligned in metaphase I. In addition, TUNEL assay showed two rounds of cell death in *Brme1-/-* testes, either in pachytene or metaphase I, suggesting that most of the germ cells were removed by synapsis and spindle assembly checkpoints, respectively.

Reactivation of the somatic-like DDR pathway

As mentioned above, RPA2 and γH2AX foci persisted until the diplotene stage in *Brme1-/-* spermatocytes. As an intriguing discovery, at late-pachytene, RAD51 foci, but not DMC1

foci, reaccumulated globally at DSB sites in *Brme1-/-* spermatocytes and colocalized with the remaining γH2AX foci. This phenomenon likely reflects the reactivation of the somatic-like DDR in late-pachytene, which only involves RAD51 but not DMC1 (A Enguita-Marruedo, 2019), suggesting that there is a stage-specific transition from meiotic to somatic-like DDR pathway in *Brme1-/-* spermatocytes.

MEILB2 and BRME1 inhibit mitotic HR

In addition to their meiotic roles, we also provided insights into the somatic roles of MEILB2 and BRME1. First, we showed that MEILB2 is able to localize to mitotic DSBs when overexpressed in somatic cancer cells. Second, this localization of MEILB2 on mitotic DSBs depended on its ability to bind to endogenous BRCA2. Third, BRME1 also localized to mitotic DSBs when overexpressed with MEILB2. Most importantly, the overexpression of MEILB2 and BRME1 in somatic cancer cells impaired the activation of the mitotic HR pathway. The inhibitory function of MEILB2 required its BRCA2-binding activity. These results suggest that MEILB2 and BRME1 are potential oncogenes, whose overexpression impairs mitotic HR.

Paper III

Disruption of the MEILB2-BRCA2 interface compromises the recruitment of both MEILB2 and BRCA2 to recombination sites in mouse spermatocytes

We illustrated that the localization of BRCA2 at meiotic recombination sites depends on the MBD of BRCA2 (**Paper I**). Furthermore, in **Paper II**, mouse MEILB2$^{87\text{-end}}$ binding to BRCA2^{MBD} resulted in a unique heterocomplex. In this paper, by collaborating with Dr. Nandakumar lab, we demonstrated that human MEILB2$^{83\text{-end}}$ -BRCA2^{MBD} formed a 4:2 stoichiometric heterocomplex. By analyzing the crystal structure, we have identified amino acid residues essential for MEILB2-BRCA2 interaction, such as D2242 and D2265 in BRCA2 and W136, D272, D330, and R204 in MEILB2. By introducing point mutations to these residues and examining the subcellular localization of mutant proteins, we have concluded that the BRCA2 and MEILB2 interaction is indispensable for the localization of both MEILB2 and BRCA2 in mouse spermatocytes, proving their interdependent localization mechanism.

Concluding remarks

BRCA2 is the most well-known cancer suppressor gene. People carrying mutations in *BRCA1/2* are estimated to have a lifetime risk of 65%–80% for developing breast cancer, and up to 20%–45% of them develop ovarian cancers (H Daum, 2018). Around 1 in 400 people have a *BRCA2* mutation that can be inherited from either parent, which largely increases the risk of both ovarian and breast cancers (N Petrucelli, 2010).

Despite BRCA2 being important from clinical perspectives, the regulatory mechanisms of BRCA2 are still poorly understood especially in meiosis. Specifically, the embryonic lethality of the gene knockout limits the study of BRCA2 in meiotic HR. Therefore, it is essential to identify the BRCA2-interacting proteins.

In working to determine the roles of BRCA2 and its co-factors, we identified MEILB2 (**Paper I**) and BRME1 (**Paper II**) as meiosis-specific BRCA2-binding proteins. Through the functional analysis of MEILB2 and BRME1, we have acquired deeper insights into the functions of BRCA2 during meiotic HR.

First, we found that the localization of BRCA2, as well as the downstream recombinases RAD51 and DMC1, are abolished in *Meilb2-/-* spermatocytes. These findings not only demonstrated how BRCA2 is recruited to sites of meiotic HR, but also defined the uniqueness of MEILB2 in meiotic HR. This meiosis-specific pathway may ensure the efficient and rapid repair of abundance meiotic DSBs. Clinically, mutations in the *MEILB2/HSF2BP* gene have recently been found in three homozygous carriers that suffer from infertility (Z Tang, 2017) (BV Halldorsson, 2019).

Second, our data showed that BRCA2-MEILB2-BRME1 formed a ternary complex that is required for the normal progression of meiosis. Further, a novel 4:2 human MEILB2$^{83\text{-end}}$-BRCA2^{MBD} complex was identified, thus suggesting a synergistic mechanism for the recruitment of both proteins to meiotic DSBs. These findings have significantly broadened our vision of how BRCA2 is involved in meiotic HR and how BRCA2 is molecularly modified for its meiosis-specific purpose.

Third, overexpressing MEILB2 and BRME1 in mitotic cancer cells disrupted the intrinsic BRCA2 function by binding directly to the protein and further blocking the RAD51 foci formation. Thus, the misregulation (upregulation/overexpression) of MEILB2 and BRME1 could potentially contribute to the sporadic cancer development, as reported for the other BRCA2 interacting proteins. Therefore, it will be useful to examine the expression level of MEILB2 and BRME1 in human cancers and its correlation with the cancer malignancy, in order to investigate the possibility of these meiotic BRCA2 interactors being potential cancer drivers. These works can provide better diagnoses and potential treatments for sporadic cancers.

Finally, the ongoing and further research conducted by both our group and our collaborators, including the crystal structural analysis of the ternary BRCA2-MEILB2-BRME1 complex as well as the analysis of *Brca2* mutant mice lacking the MBD coding region, will provide more information regarding the molecular regulation of BRCA2 and its interactors. These findings will have multidisciplinary impacts in both cancer and reproductive biology fields.

Acknowledgements

To my supervisor Hiroki, for your kind and detailed supervision.

To Marc and Peter, for your kind support and encouragement throughout my study.

To my husband Ke and my parents, for all the love and support during the hard times.

To the girls in my lab: Kexin, Shuwen, Nena, and Io, for the friendship, laughter, and tears!

To the girls in my life here: Agata, Cecilia, Anto, Mara, and Cat, for all the happy times!

To all the supervisors in CMB: Per, Julie, Anne, Margit, Markus, Beidong, Anders, Jonas, Jeanette and Marie for providing such a great scientific environment.

To the administrative and technical staff: Leif, Catarina, Valida, Peter, Bruno, Lars, Haamid, Emilia, thank you all for your help and support.

To Ranjan, Mario, Delaney, Dimitra, Emmelie, Ying, Sima, Yash, Josefin, Carmen, Benedicta, Ali, Azadeh, Christina, Amit, Michelle, Johanna, Hanna, and all the people in CMB, thank you for the wonderful time that we have had together.

Reference

Abrahan Hernández-Hernández, Sergej Masich, Tomoyuki Fukuda, Anna Kouznetsova, Sara Sandin, Bertil Daneholt, Christer Höög. (2016). The central element of the synaptonemal complex in mice is organized as a bilayered junction structure. *J Cell Sci*, 129(11):2239-49.

Akira Shinohara and Miki Shinohara. (2004). Roles of RecA homologues Rad51 and Dmc1 during meiotic recombination. *Cytogenet Genome Res*, 107(3-4):201-7.

Alberto Ciccia, Stephen J. Elledge. (2010). The DNA damage response: making it safe to play with knives. *Mol. Cell*, 40, 179–204.

Amélie Fradet-Turcotte, Justine Sitz, Damien Grapton, Alexandre Orthwein . (2016). BRCA2 functions: from DNA repair to replication fork stabilization. *Endocr Relat Cancer*, 23(10):T1-T17.

Anastasiia Bondarieva, Kavya Raveendran, Vladyslav Telychko, H B D Prasada Rao, Ramya Ravindranathan, Chrysoula Zorzompokou, Friederike Finsterbusch, Ihsan Dereli, Frantzeskos Papanikos, Neil Hunter, Attila Tóth . (2020). Proline-rich protein PRR19 functions with cyclin-like CNTD1 to promote meiotic crossing over in mouse. *Nat Commun*, 11(1):3101.

Anastazja Grabarz, Aurélia Barascu, Josée Guirouilh-Barbat, and Bernard S Lopez. (2012). Initiation of DNA double strand break repair: signaling and single-stranded resection dictate the choice between homologous recombination, non-homologous end-joining and alternative end-joining. *Am J Cancer Res*, 2(3): 249–268.

Andrea Enguita-Marruedo, Marta Martín-Ruiz, Eva García, Ana Gil-Fernánde , María Teresa Parra, Alberto Viera, Julio S Rufas, Jesús Page . (2019). Transition from a meiotic to a somatic-like DNA damage response during the pachytene stage in mouse meiosis. *PLoS Genet*, 15(1):e1007439.

Anna Castro and Thierry Lorca. (2005). Exploring meiotic division in Cargèse. *EMBO Rep*, 6:821-825.

Anne Margriet Heijink, Małgorzata Krajewska, Marcel A T M van Vugt. (2013). The DNA damage response during mitosis. *Mutat Res*, 750(1-2):45-55.

Anthony Schwacha and Nancy Kleckner. (1995). Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell*, 83(5):783-91.

Anthony Tubbs and André Nussenzweig. (2017). Endogenous DNA damage as a source of genomic instability in cancer. *Cell*, 168, 644–656.

April Reynolds, Huanyu Qiao, Ye Yang, Jefferson K Chen, Neil Jackson, Kajal Biswas, J Kim Holloway, Frédéric Baudat, Bernard de Massy, Jeremy Wang, Christer Höög, Paula E Cohen, Neil Hunter. (2013). RNF212 is a dosage-sensitive regulator of crossing-over during mammalian meiosis. *Nat Genet*, 45(3):269-78.

Arnaud De Muyt, Liangran Zhang, Tristan Piolot,Nancy Kleckner,Eric Espagne,and Denise Zickler. (2014). E3 ligase Hei10: a multifaceted structure-based signaling molecule with roles within and beyond meiosis. *Genes Dev.*, 28(10): 1111–1123.

Atsushi Shibata. (2017). A. Regulation of repair pathway choice at two-ended DNA double-strand breaks. *Mutat Res.*, 803, 51–55.

Baolu Shi, Jiangyang Xue,Hao Yin,Rui Guo,Mengcheng Luo,Lan Ye,Qinghua Shi,Xiaoyan Huang,Mingxi Liu,Jiahao Sha ,P. Jeremy Wang. (2019). Dual functions for the ssDNA-binding protein RPA in meiotic recombination. *PLoS Genet*, 15(2): e1007952.

Benoit Souquet, Emilie Abby,Roxane Hervé, Friederike Finsterbusch, Sophie Tourpin, Ronan Le Bouffant,Clotilde Duquenne, Sébastien Messiaen, Emmanuelle Martini, Jacqueline Bernardino-Sgherri, Attila Toth, René Habert. (2013). MEIOB Targets Single-Strand DNA and Is Necessary for Meiotic Recombination. *PLoS Genet*, 9(9): e1003784.

Bernard de Massy. (2013). Initiation of meiotic recombination: how and where? Conservation and specificities among eukaryotes. *Annu Rev Genet*, 47:563-99.

Bin Zhang, Zhenghui Tang, Lejun Li, Lin-Yu Lu. (2020). NBS1 is required for SPO11-linked DNA double-strand break repair in male meiosis. *Cell Death Differ*, 27(7):2176-2190.

Bing Xia, Qing Sheng, Koji Nakanishi, Akihiro Ohashi, Jianmin Wu, Nicole Christ, Xinggang Liu, Maria Jasin, Fergus J Couch, David M Livingston . (2006). Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell*, 22(6):719-729.

Bishop, D. K. (1994). RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. *Cell*, 79(6):1081-92.

Bjarni V Halldorsson, Gunnar Palsson, Olafur A Stefansson, Hakon Jonsson, Marteinn T Hardarson, Hannes P Eggertsson, Bjarni Gunnarsson, Asmundur Oddsson, Gisli H Halldorsson, Florian Zink, Sigurjon A Gudjonsson , Michael L Frigge. (2019). Characterizing mutagenic effects of recombination through a sequence-level genetic map. *Science*, 363(6425):eaau1043.

Brandon J Lamarche, Nicole I Orazio, and Matthew D Weitzman. (2010). The MRN complex in Double-Strand Break Repair and Telomere Maintenance. *FEBS Lett.*, 584(17): 3682–3695.

Burkhard Kneitz, Paula E. Cohen, Elena Avdievich,Liyin Zhu,Michael F. Kane, Harry Hou, Jr.,Richard D. Kolodner, Raju Kucherlapati, Jeffrey W. Pollard, and Winfried Edelmann. (2000). MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. *Genes Dev.*, 14(9): 1085–1097.

C P Leblond, M El-Alfy. (1998). The eleven stages of the cell cycle, with emphasis on the changes in chromosomes and nucleoli during interphase and mitosis. *Anat Rec*, 252(3),426-43.

D L Pittman, J Cobb, K J Schimenti, L A Wilson, D M Cooper, E Brignull, M A Handel, J C Schimenti. (1998). Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. *Mol Cell*, 1(5):697-705.

Denise Zickler, Nancy Kleckner. (2016). A Few of Our Favorite Things: Pairing, the Bouquet, Crossover Interference and Evolution of Meiosis. *Semin Cell Dev Biol*, 54,135–148.

Dmitry V. Bugreev, Roberto J. Pezza, Olga M. Mazina, Oleg N. Voloshin, R. Daniel Camerini-Otero, and Alexander V. Mazin. (2010). The resistance of DMC1 D-loops to dissociation may account for the DMC1 requirement in meiosis. *Nat Struct Mol Biol*, 18(1): 56–60.

Eleni P Mimitou, Lorraine S Symington. (2009). DNA end resection: many nucleases make light work. *DNA Repair (Amst)*, 8, 983–995.

Erin K Schwartz, William D Wright, Kirk T Ehmsen, James E Evans, Henning Stahlberg, Wolf-Dietrich Heyer. (2012). Mus81-Mms4 functions as a single heterodimer to cleave nicked intermediates in recombinational DNA repair. *Mol Cell Biol*, 32(15):3065-80.

F Connor, A Smith, R Wooster, M Stratton, A Dixon, E Campbell, T M Tait, T Freeman, A Ashworth. (1997). Cloning, chromosomal mapping and expression pattern of the mouse Brca2 gene. *Hum Mol Genet*, 6(2):291-300.

Fang Yang, Rabindranath De La Fuente, N. Adrian Leu, Claudia Baumann, K. John McLaughlin, P. Jeremy Wang. (2006). Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. *J. Cell Biol*, 173:497-507.

Fang Yang, Sherman Silber, N Adrian Leu, Robert D Oates, Janet D Marszalek, Helen Skaletsky, Laura G Brown, Steve Rozen, David C Page, P Jeremy Wang. (2015). TEX11 is mutated in infertile men with azoospermia and regulates genome-wide recombination rates in mouse. *EMBO Mol Med*, 7(9):1198-210.

Federica Polato, Elsa Callen,Nancy Wong, Robert Faryabi, Samuel Bunting, Hua-Tang Chen,Marina Kozak, Michael J. Kruhlak,Colleen R. Reczek, Wen-Hwa Lee,Thomas Ludwig,Richard Baer,Lionel Feigenbaum, Stephen Jackson and André Nussenzweig. (2014). CtIP-mediated resection is essential for viability and can operate independently of BRCA1. *J Exp Med*, 211(6): 1027–1036.

Fei Zhao, Wootae Kim, Jake A. Kloeber & Zhenkun Lou . (2020). DNA end resection and its role in DNA replication and DSB repair choice in mammalian cells. *Experimental & Molecular Medicine*, 52:1705–1714.

Femke A.T. de Vries, Esther de Boer, Mike van den Bosch, Willy M. Baarends, Marja Ooms,Li Yuan Jian-Guo Liu, Albert A. van Zeeland,Christa Heyting,and Albert Pastink. (2005). Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. *Genes Dev.*

Feng Zhang, Jianglin Ma, Jiaxue Wu, Lin Ye, Hong Cai, Bing Xia, Xiaochun Yu. (2009). PALB2 Links BRCA1 and BRCA2 in the DNA-Damage Response. *Curr Biol*, 19(6):524-9.

Frantzeskos Papanikos, Julie A J Clément, Erika Testa, Ramya Ravindranathan,Ian R Adams, Jiri Forejt, Marco Barchi, Bernard de Massy, Attila Toth. (2019). Mouse ANKRD31 Regulates Spatiotemporal Patterning of Meiotic Recombination Initiation and Ensures Recombination between X and Y Sex Chromosomes. *Mol Cell*, 74(5):1069-1085.

Frédéric Baudat, Yukiko Imai, Bernard de Massy. (2013). Meiotic recombination in mammals: localization and regulation. *Nat Rev Genet*, 14(11):794-806.

Galina V Petukhova, Roberto J Pezza, Filip Vanevski, Mickael Ploquin, Jean-Yves Masson, R Daniel Camerini-Otero. (2005). The Hop2 and Mnd1 proteins act in concert with Rad51 and Dmc1 in meiotic recombination. *Nat Struct Mol Biol*, 12(5):449-53.

Gouri Chatterjee, Judit Jimenez-Sainz, Thomas Presti, Tiffany Nguyen, and Ryan B. Jensen. (2016). Distinct binding of BRCA2 BRC repeats to RAD51 generates differential DNA damage sensitivity. *Nucleic Acids Res.*, 44(11): 5256–5270.

Hagit Daum, Tamar Peretz, Neri Laufer. (2018). BRCA mutations and reproduction. *Fertil Steril*, 109(1):33-38.

He´le`ne Guillon, Fre´de´ric Baudat,Corinne Grey, R. Michael Liskay, and Bernard de Massy. (2015). Crossover and noncrossover pathways in mouse meiosis. *Molecular Cell*, 20(4):563-573.

Hideo Tsubouchi and Hideyuki Ogawa. (1998). A Novel mre11 Mutation Impairs Processing of Double-Strand Breaks of DNA during Both Mitosis and Meiosis. *Mol Cell Biol*, 18(1): 260–268.

Huanyu Qiao, H B D Prasada Rao, Ye Yang, Jared H Fong, Jeffrey M Cloutier, Dekker C Deacon, Kathryn E Nagel, Rebecca K Swartz, Edward Strong, J Kim Holloway, Paula E Cohen, John Schimenti, Jeremy Ward, Neil Hunter. (2014). Antagonistic roles of ubiquitin ligase HEI10 and SUMO ligase RNF212 regulate meiotic recombination. *Nat Genet*, 46(2):194-9.

Hunter, N. (2011). Double duty for Exo1 during meiotic recombination. *Cell Cycle*, 10(16):2607-9.

Inger Brandsma, Dik C Gent. (2012). Pathway choice in DNA double strand break repair: observations of a balancing act. *Genome Integr*, 3(1):9.

Inger Brandsma, Koichi Sato, Sari E van Rossum-Fikkert, Nicole van Vliet, Esther Sleddens, Marcel Reuter, Hanny Odijk, Nathalie van den Tempel, Dick H W Dekkers, Karel Bezstarosti, Jeroen A A Demmers, Alex Maas, Joyce Lebbink, Cl. (2019). HSF2BP Interacts with a Conserved Domain of BRCA2 and Is Required for Mouse Spermatogenesis. *Cell Rep*, 27(13):3790-3798.

Isabel Lam, Scott Keeney. (2014). Mechanism and regulation of meiotic recombination initiation. *Cold Spring Harb Perspect Biol*, 7(1):a016634.

Isabelle Cousineau, Abdellah Belmaaza. (2011). EMSY overexpression disrupts the BRCA2/RAD51 pathway in the DNA-damage response: implications for chromosomal instability/recombination syndromes as checkpoint diseases. *Mol Genet Genomics*, 285(4):325-40.

J Chen, D P Silver, D Walpita, S B Cantor, A F Gazdar, G Tomlinson, F J Couch, B L Weber, T Ashley, D M Livingston, R Scully. (1998). Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol Cell*, 2(3):317-28.

J Kim Holloway, Xianfei Sun, Rayka Yokoo, Anne M Villeneuve, Paula E Cohen. (2014). Mammalian CNTD1 is critical for meiotic crossover maturation and deselection of excess precrossover sites. *J Cell Biol*, 205(5):633-41.

J Li, C Zou, Y Bai, D E Wazer, V Band & Q Gao. (2006). DSS1 is required for the stability of BRCA2. *Oncogene*, 25(8):1186-94.

J Ross Chapman, Martin R G Taylor, Simon J Boulton. (2012). Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell*, 47(4):497-510.

Jieqiong Dai, Oleg Voloshin, Svetlana Potapova, and R. Daniel Camerini-Otero. (2017). Meiotic Knockdown and Complementation Reveals Essential Role of RAD51 in Mouse Spermatogenesis. *Cell Rep.*, 18(6): 1383–1394.

Johanna Fraune, Sabine Schramm, Manfred Alsheimer, Ricardo Benavente. (2012). The mammalian synaptonemal complex: protein components, assembly and role in meiotic recombination. *Exp Cell Res*, 318(12):1340-6.

Juan S. Martinez, Catharina Von Nicolai, Taeho Kim, Åsa Ehlén, Alexander V. Mazin, Stephen C. Kowalczykowski, Aura Carreira. (2016). BRCA2 regulates DMC1-mediated recombination through the BRC repeats. *PNAS*, 113(13):3515-3520.

Julian Lange, Shintaro Yamada, Sam E. Tischfield, Jing Pan, Seoyoung Kim, Xuan Zhu,Nicholas D. Socci, Maria Jasin, and Scott Keeney. (2016). The landscape of mouse meiotic double-strand break formation, processing and repair. *Cell*, 167(3): 695–708.

Kai Rothkamm, Stephen Barnard, Jayne Moquet, Michele Ellender, Zohaib Rana, Susanne Burdak-Rothkamm. (2015). DNA damage foci: Meaning and significance. *Environ Mol Mutagen*, 56(6):491-504.

Kanika Arora and Kevin D Corbett. (2019). The conserved XPF:ERCC1-like Zip2:Spo16 complex controls meiotic crossover formation through structure-specific DNA binding. *Nucleic Acids Res.*, 47(5): 2365–2376.

Karoline Winkel, Manfred Alsheimer, Rupert Ollinger, Ricardo Benavente. (2009). Protein SYCP2 provides a link between transverse filaments and lateral elements of mammalian synaptonemal complexes. *Chromosoma*, 118(2):259-67.

Katrin Daniel, Julian Lange, Khaled Hached, Jun Fu, Konstantinos Anastassiadis, Ignasi Roig, Howard J. Cooke, A. Francis Stewart, Katja Wassmann, Maria Jasin, Scott Keeney & Attila Tóth. (2011). Meiotic homologue alignment and its quality surveillance are controlled by mouse HORMAD1. *nature cell biology*, 13(5):599-610.

Katrin Gudmundsdottir, Alan Ashworth. (2004). BRCA2 in meiosis: turning over a new leaf. *Trends Cell Biol*, 14(8):401-4.

Keun P. Kim, Beth M. Weiner,Liangran Zhang, Amy Jordan, Job Dekker, and Nancy Kleckner. (2010). Sister cohesion and structural axis components mediate homolog bias of meiotic recombination. *Cell*, 143(6): 924–937.

Kimberly A McAllister, L Michelle Bennett, Chris D Houle, Toni Ward, Jason Malphurs, N Keith Collins, Carol Cachafeiro, Joseph Haseman, Eugenia H Goulding, Donna Bunch, E Mitch Eddy, Barbara J Davis, Roger W Wiseman. (2002). Cancer susceptibility of mice with a homozygous deletion in the COOH-terminal domain of the Brca2 gene. *Cancer Res*, 62(4):990-4.

L Yuan, J G Liu, J Zhao, E Brundell, B Daneholt, C Höög. (2000). The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Molecular Cell*, 5(1):73-83.

Laura Gómez-H, Natalia Felipe-Medina, Manuel Sánchez-Martín, Owen R Davies, Isabel Ramos, Ignacio García-Tuñón, Dirk G de Rooij, Ihsan Dereli, Attila Tóth, José Luis Barbero, Ricardo Benavente, Elena Llano, Alberto M Pendas. (2016). C14ORF39/SIX6OS1 is a constituent of the synaptonemal complex and is essential for mouse fertility. *Nat Commun*, 7:13298.

Liu Cao, Xioaling Xu, Samuel F Bunting, Jie Liu, Rui-Hong Wang, Longyue L Cao, J Julie Wu, Tie-Nan Peng, Junjie Chen, Andre Nussenzweig, Chu-Xia Deng, Toren Finkel. (2009). A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. *Mol Cell*, 35(4):534-41.

Lorraine S Symington, Jean Gautier. (2011). Double-strand break end resection and repair pathway choice. *Annu Rev Genet*, 45:247-71.

Makoto T. Hayashi, Jan Karlseder. (2013). DNA damage associated with mitosis and cytokinesis failure. *Oncogene*, 32(39), 4593–4601.

Marcello Stanzione, Marek Baumann, Frantzeskos Papanikos, Ihsan Dereli, Julian Lange, Angelique Ramlal, Daniel Tränkner, Hiroki Shibuya, Bernard de Massy, Yoshinori Watanabe, Maria Jasin, Scott Keeney , Attila Tóth. (2016). Meiotic DNA break formation requires the unsynapsed chromosome axis-binding protein IHO1 (CCDC36) in mice. *Nat Cell Biol*, 18(11):1208-1220.

Marie Chansel-Da Cruz, Marcel Hohl, Ilaria Ceppi, Laëtitia Kermasson, Laurence Maggiorella, Mauro Modesti, Jean-Pierre de Villartay, Talia Ileri, Petr Cejka, John H J Petrini, Patrick Revy . (2020). A Disease-Causing Single Amino Acid Deletion in the Coiled-Coil Domain of RAD50 Impairs MRE11 Complex Functions in Yeast and Humans. *Cell Rep*, 33(13):108559.

Marie-Christin Spindler, Sebastian Filbeck, Christian Stigloher & Ricardo Benavente. (2019). Quantitative basis of meiotic chromosome synapsis analyzed by electron tomography. *Scientific Reports*, 9(1), 16102.

Mengcheng Luo, Fang Yang, N Adrian Leu, Jessica Landaiche, Mary Ann Handel, Ricardo Benavente, Sophie La Salle, P Jeremy Wang. (2013). MEIOB exhibits single-stranded DNA-binding and exonuclease activities and is essential for meiotic recombination. *Nat Commun*, 4:2788.

Michel F Guiraldelli, Craig Eyster, Joseph L Wilkerson, Michael E Dresser, Roberto J Pezza. (2013). Mouse HFM1/Mer3 is required for crossover formation and complete synapsis of homologous chromosomes during meiosis. *PLoS Genet*, 9(3):e1003383.

Michiel Boekhout, Mehmet E Karasu, Juncheng Wang, Laurent Acquaviva, Florencia Pratto, Kevin Brick, Diana Y Eng, Jiaqi Xu, R Daniel Camerini-Otero, Dinshaw J Patel, Scott Keeney. (2019). REC114 Partner ANKRD31 Controls Number, Timing, and Location of Meiotic DNA Breaks. *Mol Cell*, 74(5):1053-1068.

Miki Shinohara, Steve D Oh, Neil Hunter, Akira Shinohara. (2008). Crossover assurance and crossover interference are distinctly regulated by the ZMM proteins during yeast meiosis. *Nat Genet*, 40(3):299-309.

N Hunter, N Kleckner. (2001). The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. *Cell*, 106(1):59-70.

N Hunter, R H Borts. (1997). Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. *Genes Dev*, 11(12):1573-82.

Nancie Petrucelli, Mary B Daly, Gerald L Feldman. (2010). Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2. *Genet Med*, 12(5):245-59.

Neil Hunter. (2015). Meiotic Recombination: The Essence of Heredity. *Cold Spring Harb Perspect Biol*, 7(12):a016618.

Noriko Hosoya, Miyuki Okajima, Aiko Kinomura, Yoshihiro Fujii, Takashi Hiyama,Jiying Sun,Satoshi Tashiro, and Kiyoshi Miyagawab. (2012). Synaptonemal complex protein SYCP3 impairs mitotic recombination by interfering with BRCA2. *EMBO Rep.*, 13(1): 44–51.

Phang-Lang Chen, Chi-Fen Chen, Yumay Chen, Jun Xiao, Z. Dave Sharp, and Wen-Hwa Lee. (1998). The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *PNAS*, 95 (9) 5287-5292.

Qianting Zhang, Jingchen Shao, Heng-Yu Fan & Chao Yu . (2018). Evolutionarily-conserved MZIP2 is essential for crossover formation in mammalian meiosis. *Communications Biology*, 1:147.

Qianting Zhang, Shu-Yan Ji, Kiran Busayavalasa, Chao Yu. (2019). SPO16 binds SHOC1 to promote homologous recombination and crossing-over in meiotic prophase I. *Sci Adv*, 5(1):eaau9780.

R Wooster, G Bignell, J Lancaster, S Swift, S Seal, J Mangion, N Collins, S Gregory, C Gumbs, G Micklem. (1995). Identification of the breast cancer susceptibility gene BRCA2. *Nature*, 378(6559):789-92.

Rajeev Kumar, Cecilia Oliver, Christine Brun, Ariadna B Juarez-Martinez, Yara Tarabay, Jan Kadlec, Bernard de Massy. (2018). Mouse REC114 is essential for meiotic DNA double-strand break formation and forms a complex with MEI4. *Life Sci Alliance*, 1(6):e201800259.

Rajeev Kumar, Henri-Marc Bourbon, Bernard de Massy. (2010). Functional conservation of Mei4 for meiotic DNA double-strand break formation from yeasts to mice. *Genes Dev*, 24(12):1266-80.

Raphael Ceccaldi, Beatrice Rondinelli, Alan D D'Andrea. (2016). Repair Pathway Choices and Consequences at the Double-Strand Break. *Trends Cell Biol*, 26, 52–64.

Ryan B Jensen, Aura Carreira, Stephen C Kowalczykowski. (2010). Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature*, 467(7316):678-83.

S Keeney, C N Giroux, N Kleckner. (1997). Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell*, 88(3):375-84.

S Keeney, M J Neale. (2006). Initiation of meiotic recombination by formation of DNA double-strand breaks: mechanism and regulation. *Biochem Soc Trans*, 34(Pt 4):523-5.

S M Baker, A W Plug, T A Prolla, C E Bronner, A C Harris, X Yao, D M Christie, C Monell, N Arnheim, A Bradley, T Ashley, R M Liskay. (1996). Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat Genet*, 13(3):336-42.

Sabine Santucci-Darmanin, Sophie Neyton, Françoise Lespinasse, Anne Saunières, Patrick Gaudray, Véronique Paquis-Flucklinger. (2002). The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. *Hum Mol Genet*, 11(15):1697-706.

Sandra S. de Vries, Esther B. Baart,Marleen Dekker, Ariaan Siezen,Dirk G. de Rooij, Peter de Boer,and Hein te Riele. (1999). Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. *Genes Dev.*, 13(5): 523–531.

Satoshi Ishishita, Yoichi Matsuda, Kazuhiro Kitada. (2014). Genetic evidence suggests that Spata22 is required for the maintenance of Rad51 foci in mammalian meiosis. *Sci Rep*, 4:6148.

Scott L Page, R Scott Hawley. (2004). The genetics and molecular biology of the synaptonemal complex. *Annu Rev Cell Dev Biol*, 20:525-58.

Shrinivas Sudhir Ambekar, Shanta Shrisel Hattur and Prajakta Budha Bule. (2017). DNA: Damage and Repair Mechanisms in Humans. *Glob J Pharmaceu Sci*, 3(2): 555613.

Shyam K Sharan, April Pyle, Vincenzo Coppola, Janice Babus, Srividya Swaminathan, Jamie Benedict, Deborah Swing, Betty K Martin, Lino Tessarollo, Janice P Evans, Jodi A Flaws, Mary Ann Handel. (2004). BRCA2 deficiency in mice leads to meiotic impairment and infertility. *Development*, 131(1):131-42.

Sophie La Salle, Kristina Palmer, Marilyn O'Brien, John C Schimenti, John Eppig, Mary Ann Handel. (2012). Spata22, a novel vertebrate-specific gene, is required for meiotic progress in mouse germ cells. *Biol Reprod*, 86(2):45.

Stephen Gray, Paula E Cohen. (2016). Control of Meiotic Crossovers: From Double-Strand Break Formation to Designation. *Annu Rev Genet*, 50:175-210.

Stephen P Jackson, Jiri Bartek. (2009). The DNA-damage response in human biology and disease. *Nature*, 461(7267):1071-8.

Steven M Lipkin, Peter B Moens, Victoria Wang, Michelle Lenzi, Dakshine Shanmugarajah, Abigail Gilgeous, James Thomas, Jun Cheng, Jeffrey W Touchman, Eric D Green, Pam Schwartzberg, Francis S Collins, Paula E Cohen. (2002). Meiotic arrest and aneuploidy in MLH3-deficient mice. *Nat Genet*, 31(4):385-90.

Suzanne A Hartford, Rajanikant Chittela, Xia Ding, Aradhana Vyas, Betty Martin, Sandra Burkett, Diana C Haines, Eileen Southon, Lino Tessarollo, Shyam K Sharan. (2016). Interaction with PALB2 Is Essential for Maintenance of Genomic Integrity by BRCA2. *PLoS Genet*, 12(8):e1006236.

T Robert, A Nore, C Brun, C Maffre, B Crimi, H-M Bourbon, B de Massy . (2016). The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. *Science*, 351(6276):943-9.

Takamune T Saito, Monica P Colaiácovo. (2017). Regulation of crossover frequency and distribution during meiotic recombination. *Cold Spring Harb Symp Quant Biol.*, 82:223-234.

Thomas Helleday, Eva Petermann, Cecilia Lundin, Ben Hodgson, Ricky A Sharma. (2008). DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer*, 8(3):193-204.

Ting Liu and Jun Huang. (2014). DNA End Resection: Facts and Mechanisms. *Genomics, Proteomics & Bioinformatics*, 126-130.

Weixing Zhao,Sivaraja Vaithiyalingam,Joseph San Filippo, David G. Maranon,Judit Jimenez-Sainz, Gerald V. Fontenay, Youngho Kwon, Stanley G. Leung, Lucy Lu, Ryan B. Jensen, Walter J. Chazin,Claudia Wiese,and Patrick Sung. (2015). Promotion of BRCA2-dependent Homologous Recombination by DSS1 via RPA Targeting and DNA Mimicry. *Mol Cell*, 59(2): 176–187.

Wold, M. S. (1997). Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu Rev Biochem*, 66:61-92.

Yang Xu, Roger A Greenberg, Ernst Schonbrunn, P Jeremy Wang. (2017). Meiosis-specific proteins MEIOB and SPATA22 cooperatively associate with the single-stranded DNA-binding replication protein A complex and DNA double-strand breaks. *Biol Reprod*, 96(5):1096-1104.

Zefang Tang, Chenwei Li , Boxi Kang, Ge Gao, Cheng Li, Zemin Zhang. (2017). GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res*, 45(W1):W98-W102.