Crossover control by mismatch detection protein MSH2 in response to the chromosome heterozygosity pattern in *Arabidopsis thaliana*

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Kontrola crossing-over poprzez białko MSH2 wykrywające nieprawidłowo dopasowane pary zasad DNA w odpowiedzi na wzór chromosomowej heterozygotyczności u *Arabidopsis thaliana*

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Scientific profile of the Author

In 2012, I have received high school degree with the major in Biology and Chemistry in Konin. The same year, I have moved to Poznań to start Bachelor studies in Biotechnology at the Adam Mickiewicz University. I continued my education to obtain Master degree with the major in Biotechnology, studies conducted in English. During my Bachelor and Master studies I was involved in the research concerning the use of antisense oligonucleotides in the molecular therapy of myotonic dystrophy. The results of this experimental work were published in Stepniak-Konieczna et al., 2020.

During my studies at the University, I was head of the Synthetic Biology Science Club. Our team successfully took part in two editions of the iGEM, international synthetic biology competition organised by MIT. The "E.co-Factory" project, concerning sugar-induced bacterial promoters, won gold medal and "Best Manufacturing Project" category, and was nominated for the "Best Part Collection". For those achievements, I was awarded with the scholarship for Best Students from the Ministry of Science and Higher Education.

Moreover, I completed internships in various scientific units, including Institute of Bioorganic Chemistry PAS in Poznań (Department of Plant Genomics and Department of Ribonucleoprotein Biochemistry) or Medical University in Poznań (Forensic Science Department and Department of Neurochemistry and Neuropathololology).

In 2017, I have started PhD program in English at Adam Mickiewicz University in the Genome Biology Laboratory, under the supervision of prof. Piotr Ziółkowski. My doctoral project aimed to decipher the role of interhomolog polymorphism in shaping crossover patterning on the chromosome, with the special focus on mismatch repair system. During my PhD studies, I managed to be a co-author of four research articles, two review articles and one handbook protocol. Another research publication, of which I am a first author, is currently under review (Dluzewska et al., 2023). For my research project, I got funding from the Polish National Science Centre (Preludium grant). I was also awarded with START stipend funded by the Foundation for Polish Science and received AMU Foundation scholarship for Best PhD Students.

Abstract

During meiosis, homologous chromosomes pair and reciprocally exchange genetic material in a process called crossover or meiotic recombination. Crossover is important for generating new allele combinations and at least one crossover per bivalent is necessary to ensure proper chromosome segregation in meiosis. Moreover, crossover placement is not random and its numbers are limited – there are only about 2-3 crossovers per chromosome pair per meiosis, regardless of the physical size of the genome.

One of the factors influencing recombination pattern is interhomolog polymorphism. The presence of heterozygous region juxtaposed to homozygous region on the same chromosome, causes a redistribution of crossovers into the polymorphic region. I showed that this heterozygosity juxtaposition effect depends on the activity of MSH2 protein, key element of mismatch repair system. My results demonstrate MSH2 stimulating role in the formation of Class I crossovers. With genome-wide crossover mapping in *msh2* hybrids, I was able to show that recombination is redistributed from highly polymorphic pericentromeres into less polymorphic subtelomeric regions. Genetic interference was not changed in msh2 inbred and hybrid lines.

By using a panel of *Arabidopsis thaliana* fluorescence-tagged lines (FTLs), I showed that recombination landscape is mostly similar between inbred and hybrid lines, however the presence of heterozygous region on an otherwise homozygous chromosome, redirects crossover events into the former. The increase in heterozygous region is mostly observed close to the heterozygous-homozygous regions boarder, whilst decrease in homozygous part spans the entire region. The protocol for FTL use in crossover rate measurements is also included in this dissertation.

Class II crossovers are polymorphism-sensitive and cannot be efficiently formed in heterozygous regions. In hybrids exhibiting only Class II (*fancm zip4* double mutant), crossover scarcity is the reason for reduced fertility. By additionally inactivating MSH2, I was able to increase plant fertility. Moreover, my genome-wide crossover analysis in different mutant contexts, including *msh2 fancm* and *msh2 recq4*, combined with FTL crossover frequency measurements, revealed that MSH2 limits Class II crossovers. Hence, MSH2 has opposite roles in two crossover pathways. In pericentromeric regions, which are much more polymorphic than the rest of the chromosome, MSH2 inactivation was not able to increase Class II crossovers frequency in heterozygous regions. This shows MSH2-independent polymorphism impact on recombination.

Finally, the overexpression of pro-crossover HEI10 caused significant increase in recombination in all tested heterozygosity variants, with a trend of heterozygous regions attracting crossovers still present. I showed, that in *msh2 HEI10-OE* total

recombination is increased because of HEI10 promoting Class I, however no juxtaposition effect is observed. What is more, no HEI10 stimulation is detected in *msh2 fancm zip4 HEI10-OE* variant, proving that HEI10 has no role in Class II.

To sum up, I showed pro-recombination role of MSH2 for Class I crossovers and an antagonistic, anti-recombination role for Class II crossovers. Therefore, this work demonstrates that MSH2 is a master regulator of both crossover pathways, which allows for dynamic regulation of meiotic recombination outcomes, depending on the level and distribution of sequence divergence between homologs.

Key words: meiosis, crossover, polymorphism, MSH2, Arabidopsis

Streszczenie

Podczas mejozy chromosomy homologiczne łączą się w pary i wzajemnie wymieniają materiałem genetycznym w procesie zwanym crossing-over lub rekombinacją mejotyczną. Crossing-over jest ważne dla generowania nowych kombinacji alleli, a co najmniej jedno crossing-over na biwalent jest niezbędne do zapewnienia właściwej segregacji chromosomów w mejozie. Co więcej, rozmieszczenie zdarzeń crossing-over nie jest przypadkowe, a ich liczba jest limitowana – na jedną parę chromosomów podczas mejozy przypada tylko około 2-3 crossing-over, niezależnie od fizycznej wielkości genomu.

Jednym z czynników wpływających na rozmieszczenie rekombinacji jest polimorfizm pomiędzy chromosomami homologicznymi. Obecność regionu heterozygotycznego obok regionu homozygotycznego na tym samym chromosomie powoduje redystrybucję crossing-over do regionu polimorficznego. Pokazałam, że ów efekt zestawienia heterozygotyczności *in cis* (ang. *heterozygosity juxtaposition effect*), zależy od aktywności białka MSH2, kluczowego elementu systemu naprawy nieprawidłowo sparowanych zasad DNA. Moje wyniki wykazują stymulującą rolę MSH2 w tworzeniu crossing-over klasy I. Dzięki całogenomowej analizie crossing-over w mieszańcach z mutacją msh2 wykazałam, że rekombinacja jest redystrybuowana z wysoce polimorficznych regionów okołocentromerowych do mniej polimorficznych regionów przytelomerowych. Interferencja genetyczna nie uległa zmianie w mutancie msh2 w liniach wsobnych i mieszańcowych.

Korzystając z panelu linii *Arabidopsis thaliana* znakowanych fluorescencyjnie (ang. fluorescent-tagged line, FTL) wykazałam, że wzór rekombinacji jest w znacznej mierze podobny między liniami wsobnymi i mieszańcami, jednak obecność regionu heterozygotycznego na w innym wypadku homozygotycznym chromosomie przekierowuje zdarzenia crossing-over do regionu heterozygotycznego. Wzrost rekombinacji obserwuje się głównie w pobliżu granicy pomiędzy regionem heterozygotycznym i homozygotycznym, podczas gdy spadek zdarzeń crossing-over w części homozygotycznej obejmuje cały ten region. Protokół opisujący wykorzystanie FTL w pomiarach częstotliwości rekombinacji również stanowi część mojej rozprawy doktorskiej.

Zdarzenia crossing-over klasy II są wrażliwe na obecność polimorfizmu i z tego powodu nie są wydajnie formowane w regionach heterozygotycznych. W mieszańcach, w których aktywna jest wyłącznie klasa II (podwójny mutant fancm zip4) przyczyną obniżonej płodności jest niedobór crossing-over. Poprzez dodatkową inaktywację MSH2 zwiększyłam płodność roślin. Co więcej, moje całogenomowe analizy crossing-over w tle różnych mutantów, w tym w msh2 fancm i msh2 recq4, w połączeniu z pomiarami częstości rekombinacji przy użyciu FTL ujawniły, że MSH2 ogranicza zachodzenie crossing-over klasy II. MSH2 wykazuje więc przeciwstawne działanie w dwóch szlakach crossing-over.

W regionach około-centromerowych, które są znacznie bardziej polimorficzne niż reszta chromosomu, inaktywacja MSH2 nie spowodowała zwiększenia częstości crossing-over klasy II w regionach heterozygotycznych. To pokazuje, że polimorfizm może mieć również niezależny od MSH2 wpływ na rekombinację.

Ponadto, nadekspresja pro-rekombinacyjnego czynnika HEI10 spowodowała znaczny wzrost rekombinacji we wszystkich testowanych wariantach heterozygotyczności, z wciąż obecną tendencją regionów heterozygotycznych do stymulowania zdarzeń crossing-over. Wykazałam, że w msh2 HEI10-OE rekombinacja jest zwiększona globalnie z powodu promowania klasy I przez HEI10, jednak nie obserwuje się efektu zestawienia heterozygotyczności in cis. Co więcej, w wariancie msh2 fancm zip4 HEI10-OE nie wykryto stymulowania crossing-over przez HEI10, co dowodzi, że HEI10 nie odgrywa żadnej roli w klasie II.

Podsumowując, wykazałam pro-rekombinacyjną rolę MSH2 dla crossing-over klasy I oraz antagonistyczną, anty-rekombinacyjną rolę dla crossing-over klasy II. Moja praca pokazuje, że MSH2 jest głównym regulatorem dla obu szlaków crossing-over, co pozwala na dynamiczną regulację rekombinacji mejotycznej, w zależności od poziomu i rozkładu heterozygotyczności pomiędzy chromosomami homologicznymi.

Słowa kluczowe: mejoza, crossing-over, polimorfizm, MSH2, Arabidopsis

Works included in the dissertation

1. Nadia Kbiri, **Julia Dluzewska**, Ian R. Henderson, Piotr A. Ziolkowski. Quantifying meiotic crossover recombination in Arabidopsis lines expressing fluorescent reporters in seeds using SeedScoring pipeline for CellProfiler. In: Lambing, C. (eds) *Plant Gametogenesis. Methods in Molecular Biology*, 2022, 2484:121-134. Humana, New York, NY; DOI: 10.1007/978-1-0716-2253-7_10

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List of abbreviations

bp	base pair
cM	centimorgan
CO	crossover
Col	Columbia (Arabidopsis accession)
CRISPR/Cas9	clustered regularly interspaced short palindromic repeats, CRISPR-associated protein 9
Ct	Catania (Arabidopsis accession)
dHJ	double Holliday junction
D-loop	displacement loop
DMC1	disrupted meiotic cDNA
DSB	DNA double-strand break
dsRED	Discosoma red fluorescent protein
EXO1	exonuclease 1
FANCD2	FA complementation group D2
FANCM	FA complementation group M
FIGL1	fidgetin 1 like
FTL	fluorescent-tagged line
GFP	green fluorescent protein
GBS	genotyping-by-sequencing
gRNA	guide RNA
H3K4me3	histone 3 lysine 4 tri-methylation
H3K9me2	histone 3 lysine 9 di-methylation
H3K27me3	histone 3 lysine 27 tri-methylation
HCR1	high crossover rate 1
HEI10	homolog of human HEI10 (enhancer of cell invasion no.10)
HET	heterozygous region
HOM	homozygous region
kb	kilobase
Ler	Landsberg erecta (Arabidopsis accession)
Mb	megabase

MER3	ATP-dependent DNA helicase
MLH1	mutL homolog 1
MLH3	mutL homolog 3
MMR	mismatch repair system
MSH2-7	mutS homolog 2-7
MUS81	MMS and UV sensitive 81
MutL	mutator of *mutL*
MutS	mutator of *mutS*
NCO	noncrossover
NHEJ	non-homologous end joining
OE	overexpression
Ph2	Pairing homoeologous 2
PMS1	postmeiotic segregation increased 1
PRDM9	PR/SET Domain 9
rDNA	ribosomal DNA
RECQ4	RECQ helicase
RF	recombination frequency
RPA	replication protein A
SC	synaptonemal complex
SDSA	synthesis-dependent strand annealing
SGS1	slow growth suppressor 1
SHOC1	shortage in chiasmata 1
SNI1	suppressor of NPR1, inducible
SNP	single nucleotide polymorphism
SPO11	sporulation-specific protein 11
TAF4b	TATA-box binding protein associated factor 4b
ZMM	class I crossover pathway, name comes from the first letters of the proteins required for this process in yeast: ZIP (ZIP1, ZIP2, ZIP3 and ZIP4), MER3, and MSH (MSH4 and MSH5)

Thesis outline

Introduction to meiosis

Meiosis, highly conserved cell division process, results in the formation of four haploid gametes from a single parent cell and is crucial for sexual reproduction. After fertilization with male and female gametes, the original ploidy level of the organism is restored. During meiosis, parental DNA is being reshuffled in a process called crossover, which serves to increase the genetic diversity of the offspring and accelerates evolution of organisms (Ohkura).

Meiotic division consists of a single round of DNA replication, followed by two rounds of chromosome segregation. Meiosis can be divided into two stages, meiosis I and II, both further divided into prophase, metaphase, anaphase and telophase. Prophase I includes four steps: leptotene, zygotene, pachytene, diplotene and diakinesis. During pachytene homologous chromosomes pair with each other and reciprocally exchange genetic material, which results in a crossover (Mercier et al.). Crossover (CO) provides physical links between homologous chromosomes (chiasmata) to promote proper chromosome segregation and produces new combination of alleles. Disturbances in crossover formation might result in reduced fertility or even lethality, as chromosome segregation issues and resulting aneuploidy can change the relative dosage of products from genes located on the mis-segregated chromosomes.

The number and chromosomal distribution of COs are tightly controlled - in fact, the number of COs rarely exceeds 3 per bivalent, irrespective of the physical size of the chromosome (Mercier et al.; Dluzewska et al.). The reasoning for the low CO frequency is still unclear, as recombination increases genetic diversity within population by breaking up haplotypes. On the other hand, COs exhibit mutagenic effect by introducing *de novo* mutations (Halldorsson et al.) and can disrupt linkage between beneficial alleles, which might lead to reduced fitness in the offspring (Otto and Lenormand).

Three phenomena control CO designation: crossover assurance, crossover homeostasis and genetic interference. Firstly, crossover assurance is the ability of the cell to ensure at least one CO for each homolog pair - so called obligatory crossover (Shinohara et al.). Secondly, crossover homeostasis causes no change in CO numbers even when substantial variation in DNA double-strand break (DSB) number is induced – the recombination system is buffered against deficits or excesses of DSBs (Rosu et al., 2011; Wang et al., 2015). CO homeostasis has been identified in budding yeast (where 80% reduction in DSBs results in only 15% fewer COs; Martini et al., 2006) and subsequently found in other organisms, including *Drosophila*, *Caenorhabditis elegans* or mouse. In

plants, crossover homeostasis analysis is challenging, due to lack of efficient tools for DSB numbers quantifications. Nevertheless, it has been reported that in *Arabidopsis thaliana* a 46% decrease in meiotic DSBs results in 17% reduction in Class I COs (Xue et al.), whilst in maize, once the obligatory CO is ensured, the CO number is linearly related to the DSB number (Sidhu et al., 2015). For this reason, it seems that CO homeostasis control is less strict in plants. Finally, recombination patterning is influenced by the phenomenon of genetic interference, which is a stress-related force causing non-random placement of COs on the same chromosome . Because of interference, crossovers are distributed further from each other than it would be expected by chance (Sturtevant; Berchowitz and Copenhaver; Zickler and Kleckner). Despite being so important for CO landscape, the molecular basis of these three processes (CO assurance, CO homeostasis, genetic interference) is largely vague.

Meiotic recombination pathways

Meiotic recombination is initiated in the early prophase, by the formation of a DNA double-strand break (DSB; Szostak et al., 1983), catalysed by highly conserved SPO11 transesterase dimer (Fig. 1; Neale & Keeney, 2006). There are around 150-250 DSBs in *A. thaliana* cell per meiosis (Ferdous et al.; Choi, Zhao, Kelly, et al.), whilst only about 5% of them will be resolved as COs, giving ~10 COs per cell (Giraut et al.; Salomé et al.). The majority of DSBs are repaired as noncrossovers (NCOs, nonreciprocal replacement of one DNA sequence with a DNA from a homologous chromosome), which is mediated by DNA helicases that disassemble recombination intermediates and mostly promote process called synthesis-dependent strand annealing (SDSA, Y. Wang & Copenhaver, 2018). In SDSA, DNA sequence around the DSB is replaced with a copy of a homologous DNA template, while maintaining the original configuration of the flanking regions (Fig. 1).

After DNA cleavage, the DSB is further resected resulting in 3’-ssDNA (single-stranded DNA) ends, which are bound by the recombinases RAD51 and DMC1 to mediate inter-homologue strand invasion and generation of a displacement loop (D-loop) (Fig. 1). RAD51 is involved in both, mitotic and meiotic DSB repair, whereas DMC1 is exclusively active at meiosis. As mentioned before, most inter-homolog intermediates are dissolved to NCOs, but some enter second-end capture and generate double Holliday junctions (dHJ) to further be processed by the major recombination pathway, called ZMM or Class I CO pathway (Ziolkowski, 2023).

The ZMM pathway name comes from the first letters of the budding yeast proteins required for this process: ZIP1, ZIP2, ZIP3, ZIP4, MSH4, MSH5, and MER3. In Arabidopsis, the ZMM proteins are SHOC1, HEI10, ZIP4, PTD, MER3, MSH4

and MSH5. Their role is mostly highly conserved among yeast, animals and plants, and have been shown to directly bind to and stabilize DNA recombination intermediates (Lloyd, 2023; Pyatnitskaya et al., 2019). MER3 is a DNA helicase that recognizes and migrates D-loops, MSH4-MSH5 heterodimer (MutSγ complex) stabilizes dHJ structures, SHOC1 and PTD bind both D-loops and dHJs, ZIP4 directly couples meiotic CO formation to synaptonemal complex assembly. HEI10 is an E3 ligase, acting as dosage dependent regulator of CO formation – it initially forms many small foci on synapsed chromosomes, which eventually enlarge and mark the sites of Class I COs (Morgan et al.). HEI10 foci colocalize with MutL complex proteins, MLH1-MLH3, which serve as resolvases of recombination intermediates protected by ZMM (Piotr A Ziolkowski et al.).

ZMM-mediated processing results in formation of 85-90% of all COs during meiosis (in Arabidopsis), therefore the ZMM pathway is considered the major one (Tab. 1, Dluzewska & Ziolkowski, 2021). Class I COs are sensitive to genetic interference – the additional COs on a chromosome are further apart from each other than it would be expected from random distribution. Around 10-15% of COs occur via minor Class II and those are not subject to interference. Here, recombination intermediates are processed mainly by MUS81 endonuclease (Berchowitz et al.), but other proteins like FANCD2 might also work in this pathway to promote COs (Kurzbauer et al.). However, those factors are not meiosis-specific and act also in somatic cells. In fact, the majority of strand invasion events that were not stabilized by ZMM proteins and enter Class II pathway, will be dissolved by DNA helicases and repaired as NCOs via SDSA. Only small fraction of recombination intermediates will be processed by MUS81, however no CO bias is observed in Class II (Fig.1).

Table 1. Comparison of pathways for CO formation in Arabidopsis.

	Class I COs	**Class II COs**
Alias names	Interfering pathway, ZMM pathway	Non-interfering pathway, MUS81 pathway
Main endonuclease	MLH1-MLH3	MUS81
CO Interference	sensitive	insensitive
“Obligatory” CO	yes	no
Share in the total CO number	85%	15%
DSB repair	exclusively as CO	either CO or NCO
Heterozygosity juxtaposition effect	yes	no

Components specific to meiosis	yes	no
Known *trans*-acting factors	HEI10, HCR1	FANCM, REC4Q, FIGL1, SNI1

When homologues chromosomes pair and synapse, their interaction is stabilized in a zipper-like mode by the formation of synaptonemal complex (SC). SC is a proteinaceous structure assembled from two lateral elements (from chromosome axis), each anchoring two sister chromatids and a central element (Page and Hawley). Numerous transverse filaments span the central element and attach lateral elements together in a zipper-like structure. In many species, transverse element of SC is required for crossover formation via the ZMM pathway, and for the occurrence of genetic interference. Longer SC is associated with higher CO numbers, as interference-sensitive crossovers have physically more space to occur on longer SC (Pyatnitskaya et al., 2019; Zhang et al., 2014). Moreover, differences in SC length are also linked with so called heterochiasmy, sex-specific recombination rates. In Arabidopsis, male meiosis experience around 40% more COs than female meiosis and their localisation is most frequent in distal regions, whilst female COs tend to be more common in pericentromeres (Giraut et al.; Lian et al.). All those observations can be explained by the differences in SC length and interference strength (with female meiocytes experiencing stronger interference). Surprisingly, recent study in Arabidopsis show that in the absence of ZYP1 (responsible for forming SC transverse element) chromosomes pair, but do not synapse, and Class I CO formation is even increased. Furthermore, genome-wide analysis revealed that in *zyp1*, heterochiasmy and CO interference is abolished, with the frequent observation of Class I COs close to each other (Capilla-Pérez et al.).

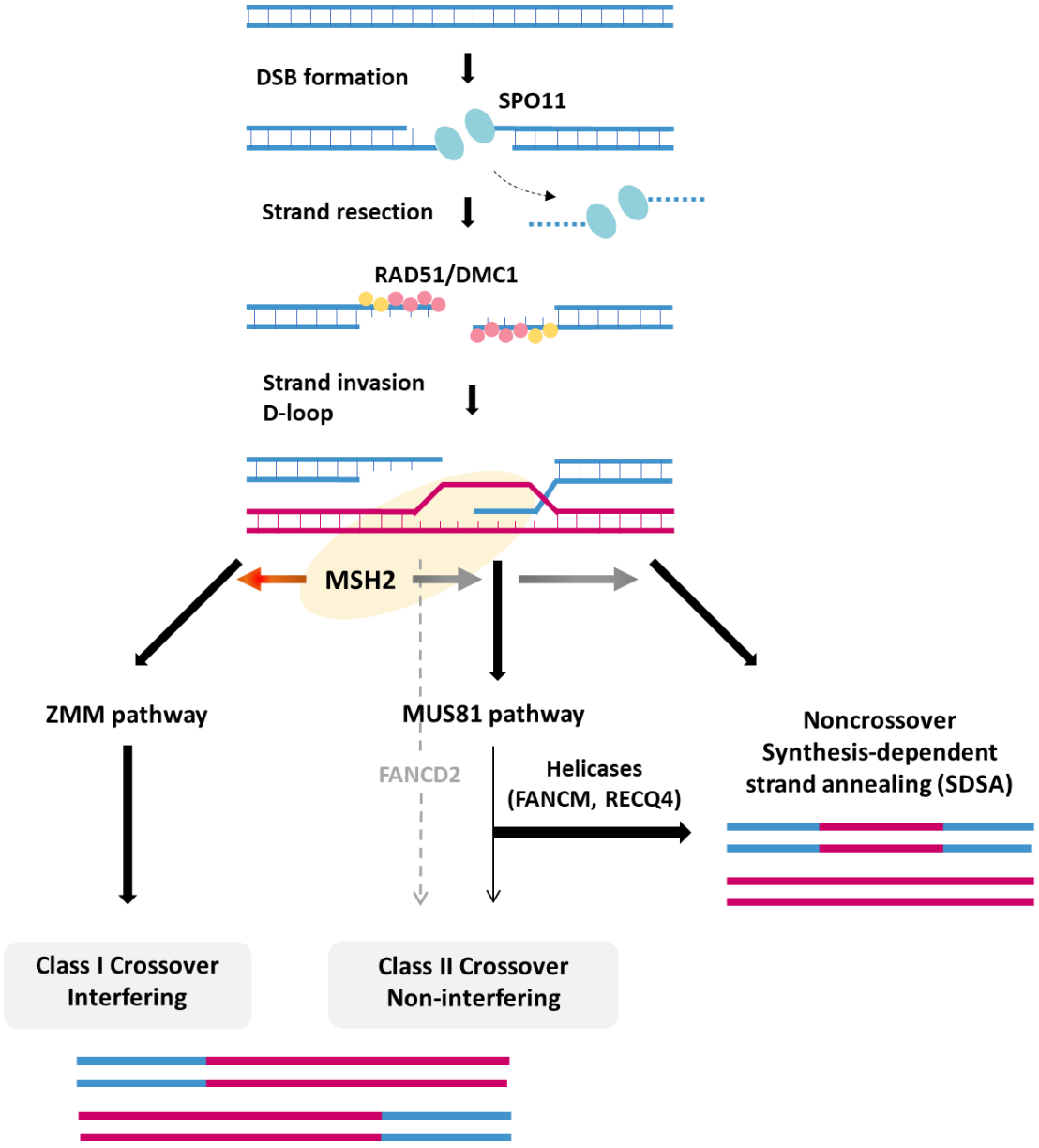

Fig. 1. Meiotic recombination pathways.

SPO11 catalyses the formation of DNA double-strand breaks (DSB) and remains covalently attached to the DNA. Later, strand resection occurs to form 3'ssDNA end, which invades the homologous chromatid - this process is mediated by DMC1 and RAD51. A D-loop structure is formed, that can later be dissolved by a synthesis-dependent strand annealing, resulting in a NCO. Alternatively, second-end capture may occur, leading to dHJ formation, which is protected by ZMM proteins and results in a Class I interfering CO. Most joint molecules that enter Class II are resolved as NCOs by the activity of FANCM and RECQ4 helicases. In dashed grey a possible processing via FANCD2 is also shown. MSH2 affects CO designation by polymorphism recognition (marked in light yellow). Based on Mercier et al. (2015) and Lloyd (2023).

DNA mismatch repair system

In eukaryotes, genome integrity is ensured by a highly conserved DNA mismatch repair system (MMR), which detects and corrects mismatches arising from polymerase errors during replication or from DNA damage (Jiricny). In mammals, the loss of MMR results in the accumulation of DNA mutations and increased predisposition to certain types of cancer, including colorectal cancer, gastrointestinal and endometrial cancer, but it may also be connected with cancers of the breast, prostate, bladder, and thyroid. It is also involved in a UV-B-induced DNA damage response pathway. Moreover, MMR deficiency may lead to resistance to certain chemotherapeutic agents, abnormalities in meiosis and sterility (Li). In plants, inactivation of MSH2, a key player in mismatch recognition, also shows a mutator effect when propagated for a number of generations – in Arabidopsis, fifth-generation *MSH2* mutants showed aberrations in morphology, development, fertility, germination efficiency, seed/silique development, and seed set (Hoffman et al.). Similar morphological and developmental abnormalities were also reported after *MSH2* inactivation in tobacco (Van Marcke and Angenon) and tomato (Sarma et al.). This clearly shows that eukaryotic MSH-homologs display similar MMR-defective phenotypes.

The mechanism of MMR was investigated in details for somatic cells. A mismatch is firstly detected by MutS heterodimer complex, compromising of MSH2 and its partner: MSH3, MSH6 or MSH7 (specific to plants). The MSH2 protein is always present in MutS heterodimer, so its inactivation shuts down the MMR system. MutS complexes differ in their sensitivity to polymorphism substrates: both MutSα (MSH2-MSH3) and MutSβ (MSH2-MSH6) recognize single base mismatches and trinucleotide insertion-deletion loops in the DNA, but MutSβ seems to respond more efficiently to base insertions and deletions larger than three nucleotides (Gupta et al.). MSH2-MSH7 heterodimer, which is specific for plants, exhibits moderate affinity for T/G base-base mismatches (Lloyd et al., 2007). MutS complexes form ring-like structures, resembling sliding clamps, which move on DNA, searching for a mismatch (Fig. 2). A mismatched base pair is encircled by MutS, which next recruits MutL factors to form a mismatch-bound complex. In eukaryotes, different MutL heterodimers can be distinguished, each of which shares MLH1 as a binding partner. In mammals three MutL complexes are active, MLH1-PMS2 (MutLα), MLH1-PMS1 (MutLβ) and MLH1-MLH3 (MutLγ), whilst plants have two, MLH1-PMS1 (MutLα) and MLH1-MLH3 (MutLγ) (Kunkel and Erie). Additionally to its MMR role, MutLγ is meiosis-specific and serves as the main resolvase for Class I COs (Kulkarni et al.; Cannavo et al.). MutL complexes exhibit endonuclease activity and initiate MMR by nicking the daughter strand 5′ from the mismatch. DNA surrounding the mismatch is being later excised by EXO1 exonuclease and the single-stranded gap is stabilized by the replication protein A (RPA). When mismatch is removed, DNA strand is resynthesized by

DNA polymerase and the remaining nick is sealed by DNA ligase to complete the repair process (Ortega et al.; Jiricny).

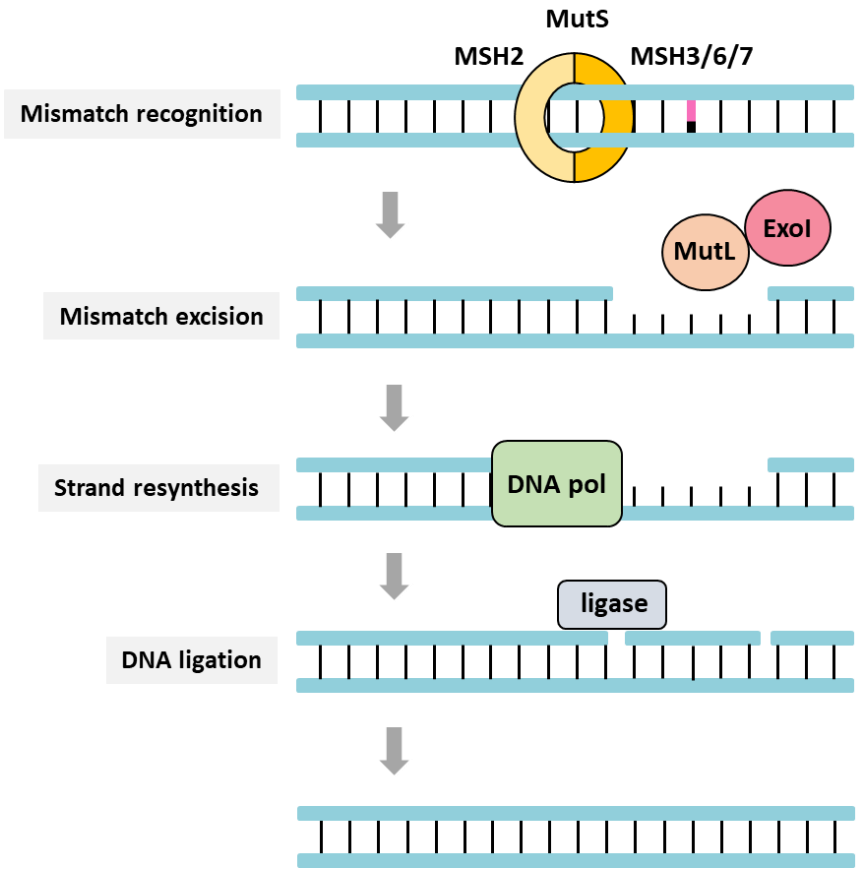

Fig. 2. Mismatch correction by MMR system.

MutS complex scans DNA in a form of a sliding clamp. MutS recognizes mismatched base pair and recruits MutL factors, which together with ExoI remove DNA fragment surrounding the mismatch. Finally, DNA strand is resynthesized by DNA polymerase and the remaining nick is ligated by DNA ligase. Based on Jiricny (2006) and Ortega et al. (2021).

Particular MMR proteins were reported to have a role in meiosis and an anti-recombination activity, due to their ability to identify mismatches and to interfere with the formation of recombination intermediates, activating either helicase-driven DNA unwinding or immediate resolution of the heteroduplex intermediates (Chakraborty and Alani). In addition, an important role of MMR members in meiosis can be proved by looking at the activity of MSH4-MSH5 proteins (MutSγ complex), which act in dHJ stabilization in the ZMM pathway and are components of MutS mismatch repair protein family. However, both of them lack one of two domains responsible for mismatch binding, so their role in MMR itself was lost.

MSH2 in Arabidopsis was previously described as an anti-crossover factor by suppressing recombination between divergent direct repeats in somatic cells or

between homologues from different accessions (Emmanuel et al.; Tam, John B. Hays, et al.). In Arabidopsis Col/Ler hybrid, an 40% increase in recombination was reported after *MSH2* inactivation (Emmanuel et al.). However, this observation was based on a single subtelomeric interval on chromosome 5, in which recombination was calculated with the use of seed-based system (see below "Major findings of work Kbiri et al., 2022"). Hence, it may be risky to exploit findings from one interval to conclude about meiotic recombination patterns throughout the genome. **Indeed, our studies based on genome-wide sequencing of Arabidopsis *msh2* hybrids (crosses between Col/Ler and Col/Ct accessions) revealed Class I COs redistribution upon *MSH2* inactivation, from polymorphic pericentromeres to less polymorphic subtelomeric regions (Blackwell & Dluzewska et al., 2020).** That is why, the increase in subtelomeric interval in *msh2* can be observed, however overall CO numbers are not significantly changed. **On the other hand, we have shown that MSH2 seems to have an opposite role in the formation of Class II COs (Dluzewska et al., 2023). Inactivation of MSH2 leads to a significant increase in Class II COs in *fancm zip4 A. thaliana* hybrids (in which Class I was disabled and Class II is boosted), and this recombination control is driven by polymorphism presence.** MSH2 blocks COs via genetic interaction with both of Class II anti-CO helicases, FANCM and RECQ4 (Dluzewska et al., 2023), probably by recruiting those proteins to mismatch sites. MSH2 interaction with MUS81 to limit its activity is also possible.

In wheat, the inactivation of *MSH2* caused the decrease in chiasma number by 40%, with an increase in chromosome univalents. Therefore, in this context MSH2 appears to have a pro-CO activity in the Class I pathway and the role in obligate CO formation (Ogle et al., 2019). Furthermore, in wheat, the *Ph2* locus, which is important for controlling homoeologous recombination, was recently found to encode MSH7 protein. MSH7 was also shown to have a role in meiotic stabilisation of allopolyploidy (Lloyd et al., 2007; Serra et al., 2021) In tomato, RNAi-mediated *MSH2* silencing affected the progression of male meiosis to a varying degree, with either halt of meiosis at zygotene stage or formation of diploid tetrads. There, MSH2 is also speculated to have an additional role in regulating ploidy stability (Sarma et al.).

Partially contradicting observations on the role of MSH2 in meiotic recombination were found in different species. In budding yeast, a genome-wide tetrad analysis in S288C/SK1 hybrid showed ~20% increase in recombination frequency in *msh2* (Martini, Borde, et al.). More recently, Cooper et al. (2021) reported similar observations - *msh2* mutants displayed an overall increase in COs and a shift of recombination towards more polymorphic regions. Yeast MSH2 and MSH6 were shown to interact with SGS1 (yeast homolog of RECQ helicase) to reject heteroduplex during single-strand annealing (SSA; DSB repair pathway that utilizes tandem repeats flanking the DSB)(Onoda et al.; Sugawara et al.). Indeed,

in recent study utilizing *S.cerevisiae* and *S.paradoxus* hybrids, which are sexually sterile, meiosis-specific inactivation of both *MSH2* and *SGS1* increased hybrid fertility 70-fold (Bozdag et al.). On the contrary, yeast MSH2-MSH3 heterodimer was discovered to stimulate MutLγ factors (MLH1-MLH3) *in vitro*, acting in the resolution of Class I COs, which suggests MSH2 pro-crossover role (Rogacheva et al.).

In mammals, MSH2 plays a role in heteroduplex rejection and correction of mismatches in mitotic cells. However, in *msh2* hybrid mice, the meiotic recombination frequency was not substantially changed (Peterson et al.). Immunolocalization of MSH2-MSH3 complex showed that it localizes to repetitive sequences, suggesting a role of MMR in the maintenance of repeat DNA integrity during mammalian meiosis (Kolas et al.).

All mentioned reports suggest that specific MMR members, mainly MSH2, may act differently, either stimulating or blocking recombination in response to polymorphism, in different organisms, according to a specific genetic context and recombination pathway active.

Factors influencing crossover formation *in cis*

DSB pattern is the first step in deciding about crossover formation sites. In many mammals, the presence of DSB hotspots is mainly determined by PRDM9 meiosis-specific histone methyltransferase, which recognizes defined DNA sequence motifs and methylates nearby nucleosomes to change chromatin accessibility for SPO11 (Paigen and Petkov). However, plants lack PRDM9 and because of that the DSBs hotspots are not associated with any specific motif. Nonetheless, plant DSB hotspots have also been associated with open chromatin regions, which are depleted in nucleosomes, indicating that nucleosomes are physically blocking chromatin accessibility for SPO11 to perform the cut (He et al.; Choi, Zhao, Tock, et al.). In Arabidopsis, SPO11-oligo sequencing has been used to generate DSB maps – DSB density was shown to be correlated with low nucleosome occupancy and low GC content, reduced by around 40% in centromeric regions and enriched in gene promoters and terminators (Choi, Zhao, Tock, et al.).

Chromatin methylation greatly influences recombination patterns and high CO numbers are associated with open chromatin regions. Accordingly, eukaryotic centromeres vastly embedded with heterochromatin are suppressed for meiotic COs. This is important for organisms fertility, as centromere-proximal COs have been associated with chromosome mis-segregation and aneuploidy (Rockmill et al.). Centromeres are surrounded by transposon-dense pericentromeric heterochromatin, which is highly DNA methylated by histone 3 lysine 9

dimethylation (H3K9me2) and DNA methylation in CG and non-CG sequence contexts (Naish et al.). The disruption of *Arabidopsis thaliana* H3K9me2 and non-CG DNA methylation pathways increases meiotic recombination in proximity to centromeres in both hybrid and inbred backgrounds, and likely involves contributions from both the interfering and noninterfering CO repair (Underwood et al.). In *met1*, DNA methyltransferase mutant background, CG context DNA methylation from pericentromeric regions is removed, and, in return, DSB levels are mutually increased (measured with SPO11-oligos). The pericentromeres also show reduced nucleosome occupancy in *met1*, consistent with loss of heterochromatic structure (Choi et al., 2018). In pericentromeric and centromeric regions, both DSBs and COs are positively correlated with open chromatin mark H3K4me3 and DSB formation is positively correlated with H3K27me3 across the entire chromosome (Lambing et al.). Recent study in Arabidopsis showed, that among many genomic and epigenomic features tested, open chromatin status, CHH-context DNA methylation and gene density were enough to explain ~85% of the variation in the CO distribution along chromosome arms (Lian et al.).

Recombination between repetitive sequences might result in unequal DNA distribution or gene loss, potentially being detrimental to the genome stability. Ribosomal RNA genes are arranged in large arrays of repetitive rDNA units, they are transcriptionally active and recruited into nucleolus early in meiosis. This recruitment shields rDNA from acquiring canonical meiotic chromatin modifications and allows only very limited meiosis-specific DSB formation. Any DSBs within rDNA array are repaired with non-homologous end joining (NHEJ), which maintains rDNA integrity during meiosis (Sims et al.).

Another feature affecting CO levels and distribution *in cis* is the state of interhomolog heterozygosity. For a long time it was widely accepted that meiotic recombination predominantly occurs in the highly homozygous regions and is blocked with sequence divergence (Tam et al., 2011b; Zhang et al., 1999). On the other hand, the recombination itself is mutagenic and can introduce new polymorphisms in regions with high CO rates (Arbeithuber et al.). Indeed, recombination rate in the historical populations is highly correlated with sequence diversity along chromosomes (Choi, Zhao, Kelly, et al.; Blackwell et al.; Lian et al.). An example of polymorphism reducing CO numbers is the presence of inversions in heterozygotes – for instance, in Arabidopsis Col/Ler hybrids, a large inversion on the short arm of chromosome 4 blocks COs in this region (Rowan et al.; Lian et al.). Furthermore, COs from ZMM pathway experience a parabolic relationship with SNP density - CO rates increase with sequence divergence up to a certain threshold and later this relationship becomes negative (Blackwell et al.). However, recent studies in Arabidopsis, using EMS-induced SNP markers in inbreds, show that the CO landscape in the inbred tested lines (Col/Col and Ler/Ler) does not differ from the hybrid (Col/Ler) landscape. This suggests that polymorphism density, with the exception of large structural variations, is not a

major determinant of the CO patterning in the genome-wide scale (Lian et al., 2022). At the kilobase scale, Szymanska-Lejman et al. (2023) have shown that in Arabidopsis, hotspots located in SNP-rich regions are more active than neighbouring hotspots at less polymorphic sites. However, COs occur mostly at polymorphism-free sites within each hotspot. The stimulation of a hotspot within polymorphic sites is due to the activity of MSH2 protein, which, as already mentioned, is a key member of MMR, and recognizes polymorphisms between chromosomes.

In 2015 Ziolkowski et al. described the heterozygosity juxtaposition effect. By using recombinant lines, which differ only in the pattern of heterozygosity, not in the expression of trans-acting modifiers, the authors have shown that COs are preferentially formed in the heterozygous (polymorphic) region, when juxtaposed to homozygous region on the same chromosome. At the same time, the homozygous region receives reciprocally less COs, so the total CO number does not change. The process depends on the interfering Class I CO pathway. **My studies proved that the juxtaposition effect relies on MSH2 protein – upon inactivation of *MSH2*, the juxtaposition effect is not observed, as polymorphisms are no longer detected (Blackwell & Dluzewska et al., 2020). In the juxtaposition effect, MutS complexes, compromising of MSH2, recognize a mismatch and direct recombination machinery into polymorphic regions by the recruitment of MutL factors, which are also involved in CO formation by the ZMM pathway (Fig. 3).**

Targeting recombination into polymorphic regions may be especially advantageous for self-pollinating plants, as COs happening in fully homozygous regions do not result in new variability. Selfing organisms might suffer with inbreeding depression, genetically uniform populations and issues with adaptation to the changing environment conditions (Wright et al.). For those reasons, it is beneficial to outcross or at least target CO into polymorphic regions, which might result in the formation of beneficial haplotypes (Ziolkowski, 2023).

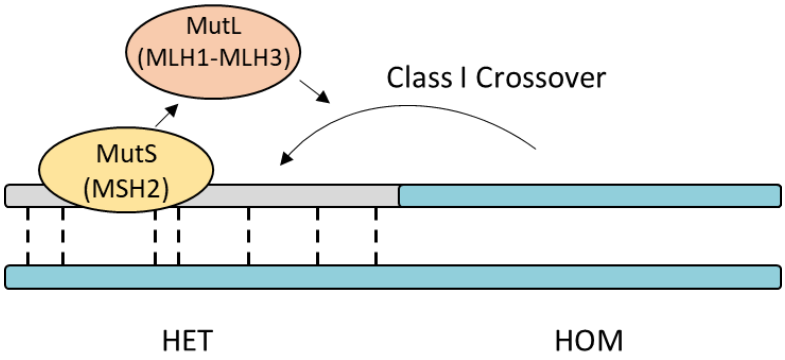

Fig. 3. A proposed model for MSH2-mediated heterozygosity juxtaposition effect.

MSH2 detects polymorphisms (dashed lines) in the heterozygous (HET) region and interacts with other MMR members, compromising MutL complex. This interaction redirects Class I CO machinery into polymorphic region, at the expense of fully homozygous (HOM) region. Based on Blackwell and Dluzewska et al., 2020.

Natural trans-acting modifiers of crossover in Arabidopsis

A number of factors influence CO formation *in trans,* acting globally (Table 1). One of ZMM proteins, a conserved E3 ubiquitin/SUMO ligase HEI10, is a dosage-specific pro-CO factor. Additional copy of HEI0 doubles CO numbers, whilst *HEI10* in a heterozygote state reduces COs – at the same time, recombination distribution itself remains unchanged (Ziolkowski et al., 2017). HEI10 is promoting Class I COs via recently proposed diffusion-mediated coarsening model (Morgan et al.; Durand et al.). In this model, HEI10 initially forms foci along the synaptonemal complex and diffuses along it to eventually form larger foci at the expense of nearby smaller ones. This drives a coarsening process leading to the formation of well-spaced foci, which create a specific context that promotes the formation of Class I COs and protects recombination intermediates from anti-CO factors.

Genetic mapping in Arabidopsis identified TBP-ASSOCIATED FACTOR 4b (TAF4b) as a CO modifier (Lawrence et al.). TAF4b encodes a subunit of the RNA polymerase II general transcription factor TFIID, and its inactivation causes recombination frequency decreases, mostly in subtelomeric regions. Consistent with the role of TFIID in promoting gene expression, RNA-seq of *taf4b* meiocytes identified widespread transcriptional changes, including in genes that regulate the meiotic cell cycle and recombination. TAF4b duplication is associated with acquisition of meiocyte-specific expression and promotion of germline transcription, which act directly or indirectly to elevate crossovers. Another example is SNI1 protein, a component of SMC5/6, which was identified as a negative regulator of MUS81-mediated COs (Zhu et al.). In *sni1*, COs are

elevated in chromosome distal regions but reduced in pericentromeres. Mutations in *SNI1* result in reduced crossover interference and can partially restore the fertility of a Class I CO pathway mutant, which suggests that the SNI1 protein affects noninterfering CO repair. Recently, first Class I COs repressor was identified – HIGH CROSSOVER RATE1 (HCR1) encoding PROTEIN PHOSPHATASE X1 (Nageswaran et al.). HCR1 was shown to interact *in vitro* and *in vivo* with Class I proteins, including HEI10, PTD, MSH5 and MLH1, and its inactivation caused recombination increases in distal chromosome arms.

Meiotic anti-crossover pathways

In *A. thaliana*, COs are greatly outnumbered by DSBs, indicating that many are resolved as NCOs and implying a presence of anti-CO mechanisms. Indeed, in Arabidopsis at least three strong anti-crossover pathways were identified via genetic screens: homolog of the human Fanconi anaemia of complementation group M (FANCM; Crismani et al., 2012; Knoll et al., 2012), RECQ4 (Séguéla-Arnaud et al.) and AAA-ATPase FIDGETIN-LIKE-1 pathway (FIGL1; Fernandes et al., 2018; Girard et al., 2015)

Both FANCM and RECQ4 proteins are DNA helicases, acting in the MUS81-dependent Class II pathway, serving to dissolve recombination intermediates by displacing the invading strand and blocking the formation of a CO. The rejected strand instead can anneal with the other 3′ overhang end of the DSB, leading to NCO formation via SDSA. Arabidopsis contains two RECQ4 orthologs, RECQ4A and RECQ4B, and deletion of both of them is necessary to inactivate protein activity. FANCM helicase acts together with MHF1 and MHF2 partners, which stimulate its DNA-binding activity and targeting to chromatin to promote NCO pathway (Girard, Crismani, et al.). FIGL1 and its partner FLIP counteract DMC1/RAD51-mediated inter-homologue strand invasion to limit Class II COs. FIGL1-FLIP complex interacts with RAD51 and DMC1, and this interaction is evolutionarily conserved in both plants and mammals. In Arabidopsis, knockout of both FIGL1 and FLIP caused changes in CO frequency: ~70% increase in *figl1* and ~30% increase in *flip*, in comparison to wild type (Fernandes et al.).

In Arabidopsis, CO frequency increases in f*igl1* are similar in inbreds (crosses between fully homozygous accessions, here: Col/Col) and hybrids (crosses between accessions polymorphic to each other, here: Col/Ler,). However, in MUS81-controlled COs, sequence divergence between recombination intermediates is affecting FANCM and RECQ4 pathways with different strength. In *fancm* recombination increases (~3-fold) are observed only in inbreds, whilst *recq4*-mediated recombination increases vary in inbreds (~6-fold) in comparison to hybrids (~4-fold; Fernandes et al., 2018). Moreover, it was shown that Class I and Class II display different sensitivity to interhomolog polymorphism, with Class

I being more prone to form COs in heterozygous regions. In a system with *fancm zip4* mutant lines (where only Class II COs are active), which carry different patterns of heterozygosity, Class II COs were formed efficiently only in the homozygous regions (Ziolkowski et al., 2015). Indeed, MUS81-mediated Class II CO formation is fairly similar to somatic recombination, which is more stringent than meiotic recombination when it comes to sequence divergence – in Arabidopsis, a single mutation in otherwise identical 618 bp direct repeat sequence (divergence level of 0.16%), is sufficient to decrease somatic recombination rates approximately threefold (Opperman et al.).

Aims and objectives

The goal of this work is to decipher the role of interhomolog polymorphism on crossover formation. As polymorphism is detected by members of mismatch repair system, I wanted to explore MSH2 impact on recombination in *Arabidopsis thaliana* meiosis.

Hypothesis 1: Crossover relocation into heterozygous region in the juxtaposition effect is dependent on MSH2 and its ability to identify mismatches between recombining chromosomes.

Yeast studies propose that members of mismatch repair system might interact with anti-recombination helicases, blocking Class II pathway. Moreover, previous studies in Arabidopsis demonstrate that two crossover classes differ in their sensitivity to heterozygosity.

Hypothesis 2: Class I and Class II crossover pathways respond differently to interhomolog polymorphism, which affects crossover/noncrossover decision, and this is regulated by MSH2 protein.

To verify our hypotheses, detailed research tasks were formulated:

1. **Optimizing protocol for crossover rate measurements using fluorescent-tagged lines (FTLs).**
   - Preparation of manuscript describing a method for recombination frequency (RF) measurement with seed-based FTL line and CellProfiler software.
2. **Understanding the molecular basis of heterozygosity juxtaposition effect.**
   - Introducing *msh2* mutation into Col/Ct juxtaposition lines using CRISPR/Cas9 system.
   - RF measurements in subtelomeric (*420*) and pericentromeric intervals (*3.9*, *CEN3*) in different genetic backgrounds (wild type, *msh2*, *fancm*, *msh2 fancm*, *fancm zip4*, *msh2 fancm zip4*).
   - Genome-wide analysis of recombination pattern in *msh2* Col/Ct hybrid lines.
   - Analysis of genetic interference in inbred (Col/Col) and hybrid (Col/Ct) lines, in wild type and in *msh2*.
3. **Comparing chromosome-wide recombination landscape in inbred, hybrid and Col/Ct mosaic lines.**
   - Introducing *msh2* mutation into a panel of fluorescent-tagged lines, overlapping upper arm and centromere of Arabidopsis chromosome 3.
   - RF measurements in wild type and in *msh2* in selected FTLs, in inbred, hybrid and Col/Ct mosaic line.

4. **Investigating differences in polymorphism sensitivity between Class I and Class II crossover machineries.**
   - Genome-wide analysis of recombination pattern in Col/Ler hybrid lines: *msh2 fancm*, *msh2 recq4*, *msh2 fancm zip4*.
   - RF measurements of *fancm zip4* and *msh2 fancm zip4* in subtelomeric (*420*) and pericentromeric (*3.9*) intervals, in inbred (Col/Col), hybrid (Col/Ler) and Col/Ct mosaic lines.
5. **Analysing HEI10 activity in promoting Class I crossovers.**
   - Generating *msh2 HEI10-OE* (overexpressor) and *msh2 fancm zip4 HEI10-OE* in Col and Ct accessions.
   - RF measurements of obtained mutant/overexpression variants in juxtaposition lines.

“Quantifying meiotic crossover recombination in Arabidopsis lines expressing fluorescent reporters in seeds using SeedScoring pipeline for CellProfile”

Plant Gametogenesis: Methods and Protocols,

Methods in Molecular Biology, 2022

DOI: https://doi.org/10.1007/978-1-0716-2253-7_10

Major findings of work “Quantifying meiotic crossover recombination in Arabidopsis lines expressing fluorescent reporters in seeds using SeedScoring pipeline for CellProfiler”, Plant Gametogenesis: Methods and Protocols, Methods in Molecular Biology, 2022

The number of meiotic crossovers is quite low, usually not exceeding two or three per chromosome pair. That is why, analysis of many recombination events is necessary to conclude about CO patterning on a chromosome. In plants, CO numbers are commonly calculated using cytological analyses. However, those are challenging, as they require technical expertise. For whole-genome CO analysis, genotyping-by-sequencing (GBS) method is routinely used, but it is laborious, costly and is carried out with the use of external services. Moreover, GBS can be performed only in hybrids, as later bioinformatic analyses utilize polymorphisms present between two lines to map for COs.

For reasons mentioned above, the use of fluorescent-tagged lines (FTLs) for recombination frequency (RF) measurements is gaining popularity, as it allows for fast analysis of thousands of meiosis events. FTLs contain a pair of transgenes encoding fluorescent proteins (either dsRED or GFP), expressed under the control of seed- or pollen-specific promoters (Francis et al.; Wu, Rossidivito, Hu, Berlyand, and R Scott Poethig). The localization of those fluorescent markers indicates specific chromosomal region and their visible segregation in seeds from F2 generation allows for RF measurements in the interval of interest (Fig. 4). Notably, one can use FTLs with markers indicating very different chromosomal regions, e.g. subtelomeric, interstitial or centromeric, and easily calculate RF for all of them (Fig. 5). This allows for tracing CO patterns in different chromosomal contexts, in both inbreds and hybrids.

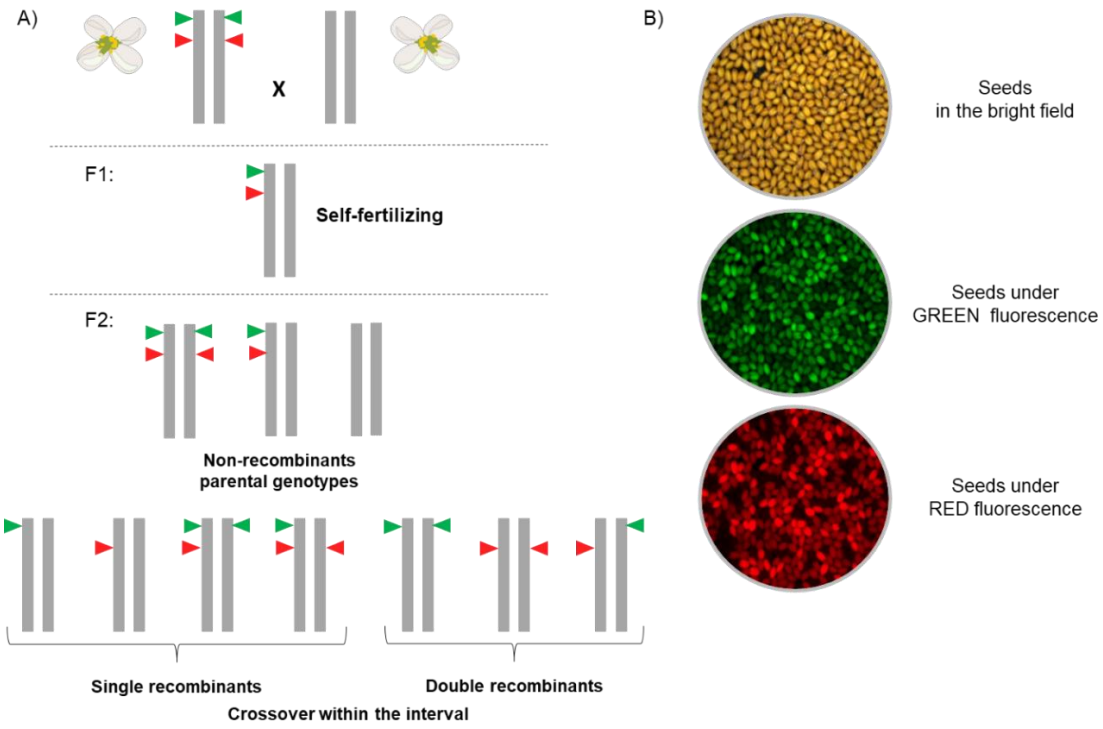

Fig. 4. Recombination frequency measurement using seed-based system in *Arabidopsis thaliana.*

A) FTL with transgenes indicating an interval of interest is crossed with noncolor line. After self-fertilization of F1 plant, F2 offspring seeds are collected and observed under the microscope for the presence or absence of seed-expressed fluorescent proteins. Possible genotypes of F2 seeds are shown: recombinants experience crossover within tested interval, while non-recombinants display parental genotypes. B) Images of Arabidopsis seeds in the bright field, under green and red fluorescence. Adapted from Kbiri et al., 2022.

The method for seed-based RF measurements was first described in Ziolkowski et al. (2015), however no detailed protocol was available until now. Presented manuscript describes the pipeline for RF calculations using CellProfiler software and FTLs developed for *Arabidopsis thaliana* (Poethig et al., 2022; Wu et al., 2015). Our method utilizes images of mono-layered seeds in three channels: in the bright field, UV through RED and UV through GFP filter. CellProfiler calculates the number of all seed objects based on bright field picture and number of fluorescent vs nonfluorescent seeds based on pictures of seeds under red/green fluorescence. The fluorescence intensity values separating nonfluorescent seeds from fluorescent ones are specified by the user from histograms, separately for red and green fluorescent seeds (Kbiri et al., 2022; Fig. 4a). The numbers of green-only, red-only, both colours, no-colour and total seeds are used to measure RF with the following formula: $cM = 100 \times (1 - [1 - 2(N_G + N_R)/ N_T]^{1/2})$, where N_G is the number of green-only fluorescent seeds, N_R is the number of red-only

fluorescent seeds and N_T is the total seed number. Overall, our method allows for fast scoring of approximately 2000 seeds per individual within 5 minutes, giving very reliable and easy to repeat results.

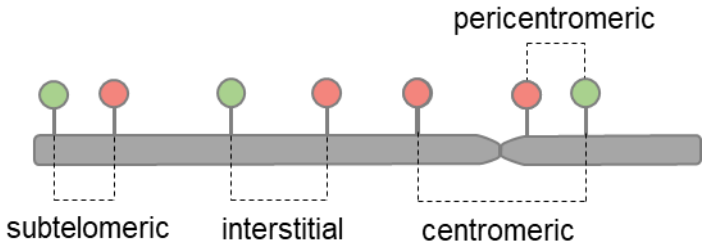

Fig. 5. Examples of possible localizations of fluorescent transgenes indicating diverse chromosomal regions in fluorescent-tagged line (FTL).

Subtelomeric region is located at the chromosome end, interstitial in the chromosome arm, centromeric region spans the centromere, whilst pericentromeric interval indicates a region close to the centromere.

As I'm routinely using the presented method in my experimental work, my role in the preparing of the protocol was to draft and correct the manuscript. Moreover, I was responsible for preparing all figures. CellProfiler method for RF measurements was extensively used in both of the presented below research articles of mine, which constitute part of my dissertation. For instance, I have used *420* subtelomeric interval located on chromosome 3 for the juxtaposition effect analysis – all heterozygosity-homozygosity recombinant lines contained green and red fluorescent markers indicating *420* region, whilst the presence of polymorphisms between recombining chromosomes in this region differ between lines. I was able to measure RF in *420* in different contexts of heterozygosity: HOM-HOM, HET-HET, HET-HOM and HOM-HET, where HOM represents region with full homology between recombining chromosomes, whereas HET indicates heterozygous region (Blackwell & Dluzewska et al., 2020). Moreover, I also calculated RF in eight different FTLs, which overlap upper arm and centromere of chromosome 3. In this instance, the use of FTLs allowed for assessing CO patterning also in the inbred, which is not possible in GBS experiment, as this method utilizes polymorphisms in hybrids to map for COs.

“MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis”

The EMBO Journal, 2020

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Supplementary material

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Major findings of work “MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis”, The EMBO Journal, 2020

In this work we aimed to explore the relationship between sequence diversity, meiotic crossover frequency and mismatch repair in *Arabidopsis thaliana*. For this purpose, we have generated F2 populations from crosses between Col-0 and L*er*-0, Ct-1, Bur-0, Ws-4 and CLC accessions, which are polymorphic to each other. We have utilized genotyping-by-sequencing (GBS) method to generate high-resolution CO maps in F2 hybrid populations (Fig. 6). There, presence of polymorphisms between two accessions allowed to identify crossovers as haplotype switches along the chromosome. After calculating recombination rates and SNP densities genome-wide, we obtained plots for average telomere to centromere crossover distribution, showing a U-shaped pattern. Across all populations, the highest CO levels were observed in the subtelomeres and pericentromeres, with centromeric regions being CO-suppressed (Fig. 7A). Importantly, we observed a parabolic relationship between recombination frequency and SNP density in all populations – CO rate positively correlates with SNP density up to a certain threshold, and higher polymorphism density reduces CO frequency (Fig. 7B). At the local (kilobase) scale, we also observed a significant association of higher SNP density around the CO sites. Using previously generated crossover maps with HEI10 overexpression (*HEI10-OE*; E3 ligase which stimulates Class I COs in a dosage-dependent manner) or with *recq4ab* mutations (where Class II is boosted), or in *HEI10-OE recq4ab* (where both Class I and Class II COs are increased), we were able to show that COs associating with regions of higher SNP density are formed via Class I pathway.

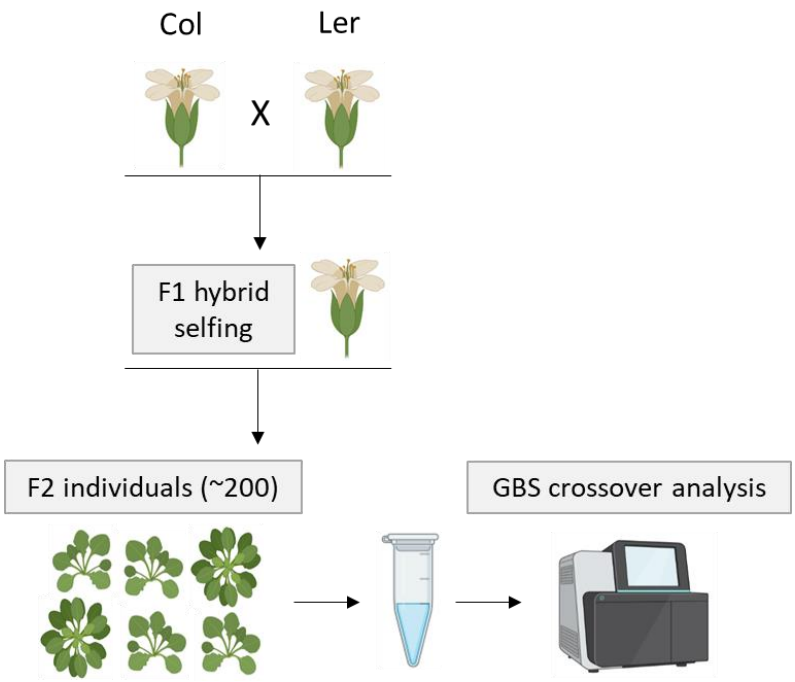

Fig. 6. Crossing scheme used to generate F2 populations for genotyping-by-sequencing CO mapping.

Col plant was crossed to one of polymorphic accessions (here: L*er*) to generate F1 hybrid plants. Those were allowed for self-fertilization to generate F2 population seeds. Leaf material from ~200 individual F2 plants was collected to isolate DNA. DNA samples were used to prepare sequencing libraries for CO mapping by GBS analysis. Figure created with BioRender.com. Adapted from Dluzewska et al., 2023.

Moreover, we introduced *msh2* mutation into L*er*, CLC and Ct accessions and later crossed those with Col *msh2* to obtain hybrid populations, where polymorphism recognition system is inactive. In each *msh2* population sequenced, average crossover numbers did not change significantly, however at the chromosome scale, a significant changes to recombination pattern were observed – *msh2* COs were redistributed from highly polymorphic pericentromeric regions into less polymorphic subtelomeric regions (Fig. 7A). Analysis at the kilobase scale confirmed that in *msh2* the correlation between COs and SNPs was absent.

We further analysed *msh2*-mediated recombination patterning by using fluorescent tagged lines (FTLs). We used *I1b* FTL marking interstitial region on chromosome 1 and show that in *msh2* hybrids a significant CO increase was observed in comparison to the wild type. For centromeric regions analysis, we have used *5.10* interval (marking centromeric region on chromosome 5) and *CEN3* (centromere on chromosome 3). Both of them showed significant CO decreases in *msh2* hybrids in comparison to the wild type. Results obtained from

recombination frequency measurements in FTLs confirmed CO redistribution into subtelomeric regions upon inactivation of MSH2 in hybrids.

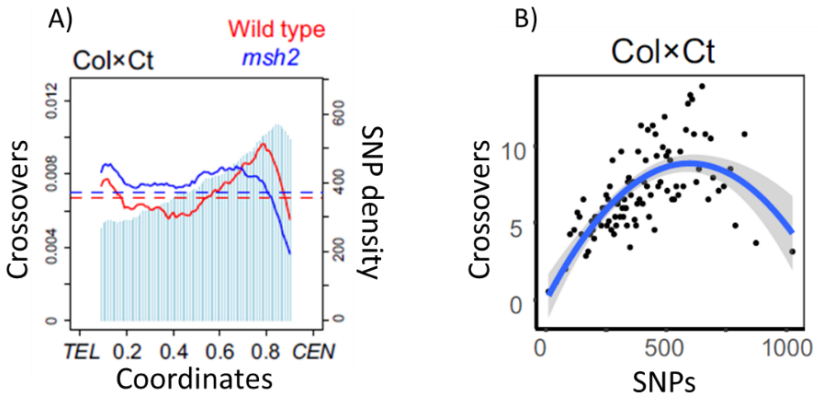

Fig. 7. The relationship between SNP density and recombination pattern in Col/Ct hybrid.

A) Crossovers in wild type (red) and *msh2* (blue), or SNPs (light blue bars) plotted along chromosome arms orientated from telomeres (TEL) to centromeres (CEN), averaged from all five chromosomes. Mean values shown as horizontal dashed lines. B) A parabolic relationship between SNP density and crossover frequency - initially both show a positive correlation, however with the increase in polymorphism density, CO frequency is reduced. Adapted from Blackwell and Dluzewska et al., 2020.

Finally, we examined the juxtaposition effect, where COs are relocated into heterozygous (polymorphic) region at the expense of neighbouring homozygous region. To study the juxtaposition effect, we used Col/Ct Arabidopsis recombinant lines, which, after crossing them together, carry different polymorphism patterns (Fig. 8B). “HOM-HOM” line is Col/Col homozygous through the genome (full inbred), “HET-HET” is Col/Ct through the genome (full hybrid), “HET-HOM” is where the *420* region is Col/Ct heterozygous and the remainder of chromosome 3 is Col/Col homozygous, and “HOM-HET” is where *420* is Col/Col homozygous and the remainder of chromosome 3 is Col/Ct heterozygous. We introduced *HEI10-OE* construct into our recombinant lines and observed a global increase in CO rates, with the heterozygosity juxtaposition effect still present, which proved that this phenomenon is indeed dependent on Class I COs.

As the juxtaposition effect relies on COs being attracted by sequence divergence, we sought to investigate what will happen upon inactivation of the MMR system, which is responsible for polymorphism detection in the cell. For this end, I generated *msh2* CRISPR/Cas9 mutations in all four juxtaposition lines (HOM-HOM, HET-HET, HET-HOM, HOM-HET). I have created a construct carrying a pair of gRNAs targeting 4th exon of *MSH2* for Cas9 cutting. This double cut

causes the deletion of exon fragment and formation of premature STOP codons, which later block translation of the protein. I was able to generate *msh2* deletion mutants for all four juxtaposition lines and measure recombination frequency (RF) in *420* interval for all of them, with the use of seed scoring system (Fig. 8A). We discovered that in *msh2* juxtaposition effect is no longer observed. This shows that MSH2 is required to promote crossovers in heterozygous regions when they are juxtaposed with homozygous regions in Arabidopsis, providing further evidence for a pro-crossover role of MSH2 in regions of higher divergence.

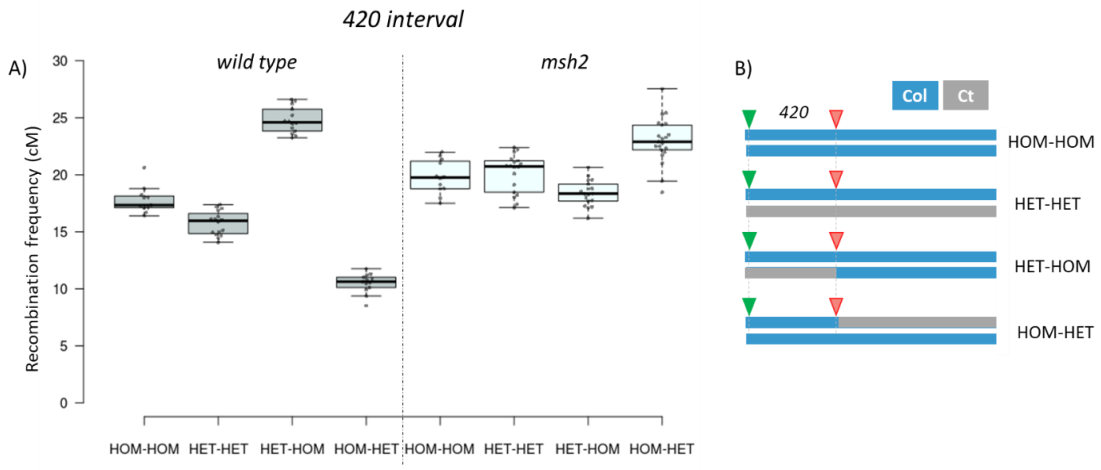

Fig. 8. MSH2 inactivation influences CO distribution in response to interhomolog polymorphism.

A) Inactivation of MSH2 removes juxtaposition effect - recombination frequency measurements in *420* interval in lines with differing heterozygosity patterns: HOM-HOM, HET-HET, HET-HOM, HOM-HET, in wild type and *msh2* genetic background. Each black dot represents recombination frequency measured in a single plant, bold black horizontal lines indicate mean values. B) Schematic representation of Arabidopsis chromosome 3 bivalent obtained from a cross between two recombinant lines, in which part of the chromosome is fixed for Col or Ct genotype. Localization of *420* interval is marked with green and red arrowheads. Based on Blackwell and Dluzewska et al., 2020.

My role in the preparation of the “MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis” publication was to generate F2 population of Col×Ct *msh2* and prepare sequencing libraries of ~300 samples, which allowed for genome-wide CO analysis in Col×Ct hybrid. Moreover, I calculated RF of *CEN3* interval for both wild type and *msh2* in Col×Ct hybrid. As *CEN3* is a pollen-based FTL, I performed flow cytometry experiments to identify fluorescent pollen objects and calculate RF. I have generated CRISPR/Cas9 deletion mutants of *MSH2* in polymorphism-recombinant lines and measured RF in *420* interval for all four of those lines, both in wild type and in

msh2. Moreover, I participated in research design process, data analysis, as well as in writing and correcting the manuscript.

“MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism”

BioRxiv, 2023

Manuscript under review

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“MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism”

Supplementary material

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Major findings of work “MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism”, preprint, manuscript under review, 2023

In the presented manuscript we further explore the relationship between interhomolog polymorphism and crossover (CO) formation. We aimed to decipher MSH2 activity in controlling recombination rates in the context of two different CO formation pathways, Class I and Class II. For this purpose, I generated *fancm zip4* double mutants and *msh2 fancm zip4* triple mutants in Col, L*er* and in juxtaposition lines with differing heterozygosity patterns. In *fancm zip4* double mutant Class I COs are inactivated, whilst Class II action is increased.

We noticed that Col×Ler *fancm zip4* hybrid exhibits severely reduced fertility, because of scarcity of CO events, as polymorphism-sensitive Class II is not able to efficiently form COs in heterozygous regions. However, simultaneous inactivation of *MSH2* increased the fertility of Col×Ler *fancm zip4* hybrids, measured with silique length, seed set and chiasma numbers (Fig. 9A). Therefore, MSH2 limits Class II COs in hybrids, as a result of detection of interhomolog polymorphism. This is in opposition to what we observe for Class I – there, MSH2 stimulates formation of Class I COs in polymorphic regions.

We also analysed recombination landscape in a genome-wide scale. We sequenced F2 individuals of Col×Ler hybrid in different mutant backgrounds, *fancm zip4*, *msh2 fancm*, *msh2 fancm zip4*, *msh2 recq4*, and compared them with profiles for wild type, *msh2* and *recq4* (Fig. 9B). The simultaneous inactivation of *MSH2* together with *FANCM* or *RECQ4* increases recombination rates in pathways controlled by both of mentioned helicases. This again proves MSH2 anti-recombination role in Class II COs, possibly by recruiting FANCM and RECQ2 or by stimulating their activity.

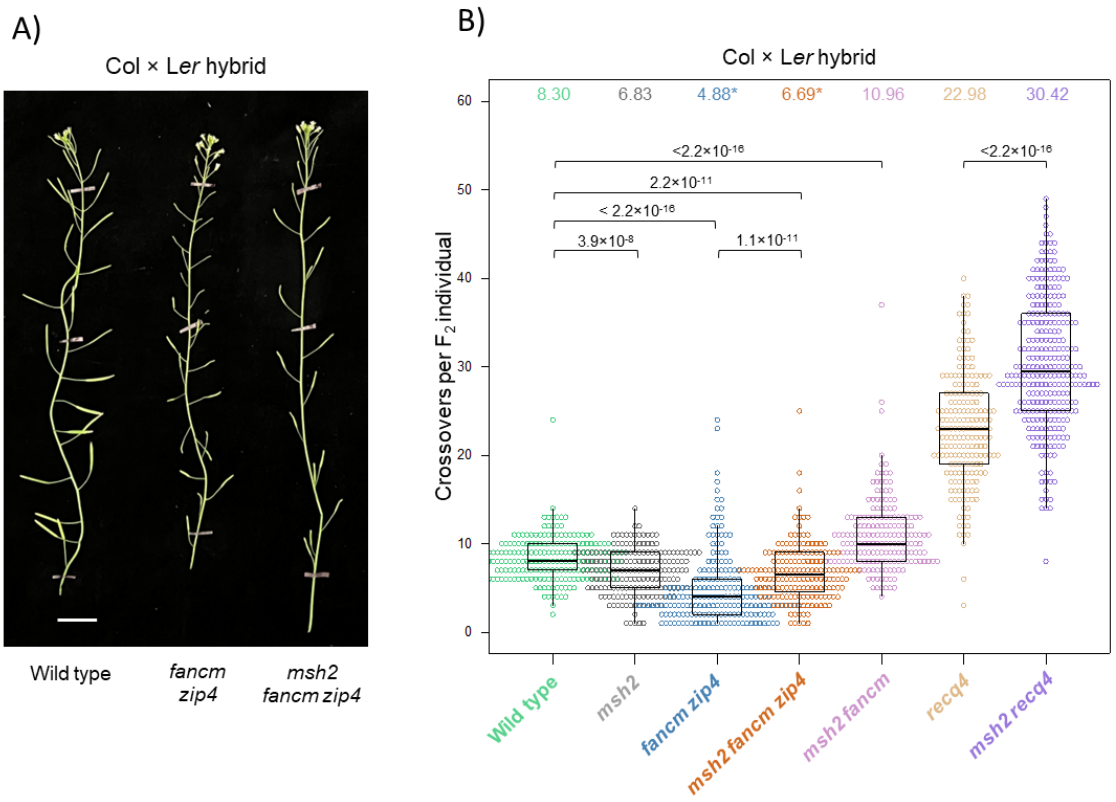

Fig. 9. MSH2 limits Class II crossovers.

A) Main stems of Col×Ler hybrids showing different fertility: wild type, *fancm zip4* and *msh2 fancm zip4.* B) Number of crossovers per F2 individual mapped in the indicated populations. Centre line in the boxplot indicates mean value, which is also shown as a number on top. Adapted from Dluzewska et al., 2023.

We also investigated heterozygosity/homozygosity juxtaposition effect in the context of only Class II being active. I measured recombination frequency (RF) in Col/Ct mosaic lines (HOM-HOM, HET-HET, HET-HOM, HOM-HET), in *420* interval, which spans subtelomeric region of chromosome 3. In *fancm zip4* recombination was increased only if *420* region was homozygous (HOM), whilst in heterozygous regions (HET) RF was very low, which confirms that Class II machinery cannot efficiently form COs, whenever polymorphisms are present. In *msh2 fancm zip4* triple mutant, recombination is increased in HET, but do not reach the level of HOM regions, in which RF for *msh2 fancm zip4* is not changed in comparison to *fancm zip4*. This shows that MSH2 is blocking Class II COs in polymorphic regions, however both the HET-HET and HET-HOM lines in *msh2 fancm zip4* remain colder in *420* than *msh2 fancm zip4* HOM-HOM and HOM-HET, indicating MSH2-indepentent inhibitory effect of DNA polymorphism on Class II.

Moreover, we also checked the impact of MSH2 inactivation on recombination in pericentromeric regions, which are much more polymorphic than subtelomeric *420* interval. I measured RF in Col/Ct juxtaposition lines using two intervals, *CEN3* (pollen-based) and *3.9* (seed-based), which span the centromere of chromosome 3. The inactivation of *MSH2* in *CEN3* and *3.9* caused CO decreases in HET regions. This confirms that in *msh2* hybrids, COs are redistributed from polymorphic pericentromeres

into less polymorphic interstitial regions, hence MSH2 stimulates Class I COs in pericentromeres. In *3.9 fancm zip4* double and *msh2 fancm zip4* triple mutants, I did not observe a significant increase in HET variants upon *MSH2* inactivation. Therefore, we concluded that pericentromeric region of chromosome 3 shows strong MSH2-independent crossover inhibition when Col/Ct heterozygous.

GBS experiments allow for recombination rate analysis only in hybrids, as they exploit SNPs to map for COs. To asses CO rates also in inbreds, I utilized a panel of fluorescent-tagged lines (FTLs), which overlap upper arm and centromeric region of Arabidopsis chromosome 3. Moreover, I introduced *msh2* mutation into all FTLs and crossed them with lines fixed for Col (inbred) or Ct (hybrid) accession, or with lines giving HET-HOM pattern after crossing (where subtelomeric part of chromosome 3 is Ct, whilst the rest of chromosome 3 is Col). This experiment showed that recombination landscape is hardly different between inbreds or hybrids. However, the juxtaposition of heterozygous (HET) and homozygous (HOM) regions causes redistribution of COs into HET. This CO increase in HET was mostly visible near the HET-HOM border, whilst the decrease was observed over the longer section of the homozygous chromosome.

Based on the obtained results we proposed a model for MSH2 polymorphism-driven activity in controlling CO formation (Fig. 10). MSH2 complexes scan heteroduplex DNA in search for a mismatch. In inbreds, which contain full homology between chromosomes, CO formation is mostly determined by chromatin structure. In hybrids, mismatches present across the entire chromosome lead to an even distribution of mismatch-bound MSH2 complexes – this combined with interference, also results in Class I CO distribution mostly determined by chromatin. However, when a heterozygous region is present on an otherwise homozygous chromosome, there's a local saturation of mismatch-bound MSH2 complexes, which stimulate formation of Class I COs in the polymorphic region.

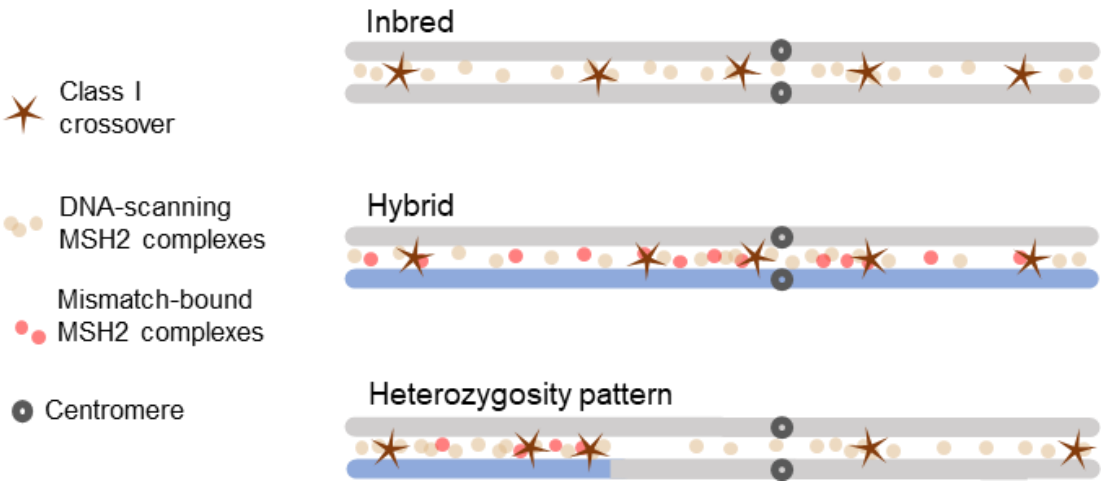

Fig. 10. Model for polymorphism-driven MSH2 activity in crossover formation.

MSH2 complexes scan the interhomolog DNA to find a mismatch. In fully homologous inbreds, MSH2 does not determine crossover formation decision. In hybrids, where mismatches are present along the entire chromosome, MSH2 complexes are bound rather evenly and Class I crossover placement is influenced by genetic interference and chromatin structure. When a heterozygous region is present on an otherwise homozygous chromosome, a local concentration of mismatch-bound MSH2 complexes occur, which stimulate formation of Class I crossovers in the polymorphic region, close to the heterozygous-homozygous region boundary. Adapted from Dluzewska et al., 2023.

I was involved in all stages of publication preparation – experiment design, data collection and analysis, manuscript writing and proofreading. I prepared samples for Col×Ler hybrid GBS experiments: *msh2 fancm*, *fancm zip4*, *msh2 fancm zip4*, *msh2 recq4*. I generated all combinations of mutants and lines with *HEI10-OE*, and measured RF in FTLs or in juxtaposition lines. I conducted flow cytometry experiments for *CEN3* and *I3bc* intervals (for genetic interference analysis). Finally, I analysed fertility of all tested mutants described in the manuscript.

“Where to cross over? Defining crossover sites in plants.”

Frontiers in Genetics, 2018

DOI: https://doi.org/10.3389/fgene.2018.00609

Major findings of work “Where to Cross Over? Defining Crossover Sites in Plants”, Frontiers in Genetics, 2018

In the Dluzewska et al. (2018) review, we aimed to summarise the current knowledge on crossover placement during plant meiosis. The decision concerning location of recombination is important, as COs are usually kept at the low level of one or two per chromosome pair, so a big part of a chromosome might eventually not recombine. A lot of factors affect CO localization, including genetic and non-genetic ones.

We start by describing DNA double strand break (DSB) hotspot regulation. As DSBs are necessary for a CO to occur, they might be considered the first level for defining recombination sites. We discuss chromatin structure, epigenetic marks and local base composition, which affect DSB positioning. The most important factor is probably the accessibility of DNA to SPO11 protein, which catalyses break formation – in the end, DSB occurrence is strongly correlated with nucleosome occupancy. Moreover, we mention other regulatory mechanisms of DSBs, including kinase proteins which respond to DNA damage, or DSB hotspot interference, where likelihood of DSB formation next to already formed DSB on the chromosome is decreased.

Furthermore, we also discuss CO homeostasis, a regulatory pathway, which causes no change in the numbers of COs, even when significant variation in DSB number is induced. Spatial distribution of COs at the chromosome scale is mentioned as well, being influenced by heterochromatin, with strong euchromatin bias. This is connected with epigenetic factors, including DNA methylation. Heterochiasmy is another feature affecting CO localisation – the sex-specific differences in recombination are known to occur commonly in eukaryotes and may be connected with synaptonemal complex length.

Finally, we put a strong focus on the effect of heterozygosity (polymorphism presence between homologous chromosomes) on CO distribution, at the hotspot and chromosome scale. We discuss this issue in the context of mismatch detection system and explain the juxtaposition effect, where heterozygous regions attract COs, which are redistributed there at the expense of homozygous regions. Moreover, we describe sensitivity of CO-forming pathways to polymorphism presence.

Overall, the distribution of COs along a chromosome is a complex process. It can be affected by genetic factors like presence of DSB hotspots, the state of chromatin compaction or epigenetic marks, such as DNA methylation and histone modifications. Interhomolog polymorphism can also have a significant impact on

CO designation. Because of the multitude of issues raised, development of new research methods is required to decipher CO designation sites, as this knowledge is essential for developing novel breeding programs.

My role in the article preparation included manuscript writing and corrections. I was specifically responsible for writing section entitled “Factors influencing spatial distribution of crossovers”, which consists of three paragraphs: Chromosome level, Heterochiasmy and DNA methylation. Furthermore, I was responsible for creating all figures in the article.

Conclusions

1. FTL-based seed scoring is an important tool for fast and accurate recombination frequency measurements in the interval of interest. The system allows for crossover rate assessment in both hybrid and inbred lines.
2. MSH2, a key factor of mismatch-recognition in the cell, is responsible for detecting mismatches and small insertions/deletions between recombining chromosomes and by that affects crossover/non-crossover decision during meiosis. MSH2 does not affect global recombination rates, but triggers crossover redistribution from less polymorphic distal regions towards polymorphism-dense pericentromeres.
3. Recombination landscape is mostly similar between inbred and hybrid lines. However, the presence of heterozygous (HET) region juxtaposed to homozygous (HOM) region on the same chromosome, causes the redistribution of crossovers into regions with higher polymorphism density. The specific increase of Class I crossovers is observed close to the HET-HOM regions border, but crossover decreases span the entire homozygous region.
4. **MSH2 exhibits a pro-recombination role in the formation of Class I crossovers in polymorphic regions.** MSH2 is responsible for the heterozygosity juxtaposition effect.
5. Class I and Class II differ in their sensitivity to polymorphism, with Class II being unable to efficiently form crossovers in polymorphic regions.
6. **MSH2 exhibits antagonistic role in the formation of Class I vs Class II crossovers. MSH2 limits Class II crossovers in *A. thaliana* hybrids**, in pathways controlled by both RECQ4 and FANCM DNA helicases.
7. Polymorphism inhibits Class II in MSH2-dependent, as well as in MSH2-independent manner. Higher polymorphism density regions, e.g. pericentromeres, inhibit recombination with stronger efficacy.
8. Genetic interference is stronger in hybrids than in inbred lines. The inactivation of MSH2 does not influence interference values.
9. HEI10 increases frequency of Class I crossovers and has no role in Class II. *HEI10-OE msh2 fancm zip4* line has similar recombination frequency to *msh2 fancm zip4*.

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Contribution statements

AUTHOR'S CONTRIBUTION STATEMENT

I hereby declare that this PhD thesis incorporates material in a form of jointly-authored publications. My contribution is as follows:

1. Nadia Kbiri, Julia Dluzewska, Ian R. Henderson, Piotr A. Ziolkowski. **Quantifying Meiotic Crossover Recombination in Arabidopsis Lines Expressing Fluorescent Reporters in Seeds Using SeedScoring Pipeline for CellProfiler**. *Plant Gametogenesis in Methods in Molecular Biology*, 2022; 2484:121-134. DOI: 10.1007/978-1-0716-2253-7_10

   Contribution: I contributed to writing the manuscript and preparing all the figures. I also shared funding.

2. Alexander R Blackwell[1], Julia Dluzewska[1], Maja Szymanska-Lejman, Stuart Desjardins, Andrew J. Tock, Nadia Kbiri, Christophe Lambing, Emma J. Lawrence, Tomasz Bieluszewski, Beth Rowan, James D. Higgins, Piotr A. Ziolkowski*, and Ian R. Henderson*. **MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis**. *The EMBO Journal*, 2020; 39:e104858; DOI:10.15252/embj.2020104858
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   Contribution: I contributed to research design and writing of the manuscript. I prepared the libraries for GBS sequencing of Col×Ct in *msh2* background. I generated *msh2* mutants with CRISPR/Cas9 in juxtaposition lines and measured recombination frequency (RF) in *420* interval of those lines in *msh2* and wild type genetic background. I measured *CEN3* crossover frequency via flow cytometry in the wild type and *msh2* Col/Ct hybrids.

3. Julia Dluzewska, Wojciech Dziegielewski, Maja Szymanska-Lejman, Monika Gazecka, Ian R. Henderson, James D. Higgins, Piotr A. Ziolkowski. **MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism**. *BioRxiv*, 2023.05.03.539183. DOI: 10.1101/2023.05.03.539183, manuscript under review

   Contribution: I was involved in all stages of manuscript preparation - research design, data collection and analysis, manuscript writing and correction. I prepared samples for Col×Ler hybrid GBS experiments: *msh2 fancm*, *fancm zip4*, *msh2 fancm zip4*, *msh2 recq4*. I generated all combinations of mutants and *HEI10-OE*, and measured RF in FTLs or in juxtaposition lines. I conducted flow cytometry experiments for *CEN3* and *I3bc* intervals (for genetic interference analysis). Finally, I analysed fertility of all tested mutants described in the manuscript. I also shared funding.

4. Julia Dluzewska[1], Maja Szymanska[1], Piotr A. Ziolkowski. **Where to cross over? Defining crossover sites in plants.** *Frontiers in Genetics*, 2018; 9:609, DOI: 10.3389/fgene.2018.00609
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Poznań, 09.05.2023

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“Quantifying meiotic crossover recombination in Arabidopsis lines expressing fluorescent reporters in seeds using SeedScoring pipeline for CellProfile”

Co-Authors contribution statements

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Nadia Kbiri, Julia Dluzewska, Ian R. Henderson, Piotr A. Ziolkowski. **Quantifying Meiotic Crossover Recombination in Arabidopsis Lines Expressing Fluorescent Reporters in Seeds Using SeedScoring Pipeline for CellProfiler**. *Plant Gametogenesis in Methods in Molecular Biology*, 2022; 2484:121-134. DOI: 10.1007/978-1-0716-2253-7_10

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.....

“MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis”

Co-Authors contribution statements

AUTHORSHIP CONTRIBUTION STATEMENT

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Alexander R Blackwell[1], Julia Dluzewska[1], Maja Szymanska-Lejman, Stuart Desjardins, Andrew J. Tock, Nadia Kbiri, Christophe Lambing, Emma J. Lawrence, Tomasz Bieluszewski, Beth Rowan, James D. Higgins, Piotr A. Ziolkowski*, and Ian R. Henderson*. **MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis**. *The EMBO Journal*, 2020; 39:e104858; DOI:10.15252/embj.2020104858
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09.05.2023 Maja Szymańska-Lejman

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Alexander R Blackwell[1], Julia Dluzewska[1], Maja Szymanska-Lejman, Stuart Desjardins, Andrew J. Tock, Nadia Kbiri, Christophe Lambing, Emma J. Lawrence, Tomasz Bieluszewski, Beth Rowan, James D. Higgins, Piotr A. Ziolkowski*, and Ian R. Henderson*. **MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis**. *The EMBO Journal*, 2020; 39:e104858; DOI:10.15252/embj.2020104858
[1] - joint first authors
* - joint corresponding authors

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Nadia Kbiri**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I contributed to writing of the manuscript and calculated recombination frequency of *HEI10-OE* in the juxtaposition lines.

Date and Signature

2023/05/05

Nadia Kbiri

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Alexander R. Blackwell[1], Julia Dluzewska[1], Maja Szymanska-Lejman, Stuart Desjardins, Andrew J. Tock, Nadia Kbiri, Christophe Lambing, Emma J. Lawrence, Tomasz Bieluszewski, Beth Rowan, James D. Higgins, Piotr A. Ziolkowski*, and Ian R. Henderson*. **MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis**. *The EMBO Journal*, 2020; 39:e104858; DOI:10.15252/embj.2020104858
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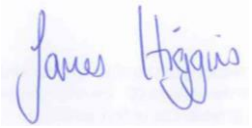
* - joint corresponding authors

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**James D. Higgins**
Affiliation	Department of Genetics and Genome Biology, University of Leicester, Leicester, UK
Contribution Description	I prepared and analysed meiotic chromosome spreads for Col×Ler and Col×CLC hybrids, in wild type and *msh2* genetic backgrounds. I performed co-immunostaining of MSH2 and ASY1 in wild type and in *msh2*, and co-immunostaining of MSH2 and MSH4 in wild type.

Date and Signature

06/05/2023 

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* - joint corresponding authors

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Ian R. Henderson**
Affiliation	Department of Plant Sciences, University of Cambridge, UK
Contribution Description	I designed the research and supervised the experiments. I analysed the sequencing data and contributed to figures presented in the article. I wrote and revised the manuscript. I also shared funding.

Date and Signature

6th May 2023

……

AUTHORSHIP CONTRIBUTION STATEMENT

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Alexander R Blackwell[1], Julia Dluzewska[1], Maja Szymanska-Lejman, Stuart Desjardins, Andrew J. Tock, Nadia Kbiri, Christophe Lambing, Emma J. Lawrence, Tomasz Bieluszewski, Beth Rowan, James D. Higgins, Piotr A. Ziolkowski*, and Ian R. Henderson*. **MSH2 shapes the meiotic crossover landscape in relation to interhomolog polymorphism in Arabidopsis**. *The EMBO Journal*, 2020; 39:e104858; DOI:10.15252/embj.2020104858
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of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Piotr A. Ziółkowski**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I designed the research and supervised the experiments. I analysed the sequencing data and contributed to figures presented in the article. I wrote and revised the manuscript. I also shared funding.

Date and Signature

6.05.2023 

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“MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism.”

Co-Authors contribution statements

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Julia Dluzewska, Wojciech Dziegielewski, Maja Szymanska-Lejman, Monika Gazecka, Ian R. Henderson, James D. Higgins, Piotr A. Ziolkowski. **MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism**. *BioRxiv*, 2023.05.03.539183. DOI: 10.1101/2023.05.03.539183, manuscript under review

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Wojciech Dzięgielewski**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I analysed genome-wide sequencing results for Col×L*er* hybrids (*msh2 fancm*, *fancm zip4*, *msh2 fancm zip4*, *msh2 recq4*). I reviewed and approved final manuscript.

Date and Signature

10.05.2023 Wojciech Dzięgielewski

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Julia Dluzewska, Wojciech Dziegielewski, Maja Szymanska-Lejman, Monika Gazecka, Ian R. Henderson, James D. Higgins, Piotr A. Ziolkowski. **MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism**. *BioRxiv*, 2023.05.03.539183. DOI: 10.1101/2023.05.03.539183, manuscript under review

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Maja Szymańska-Lejman**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I generated CRISPR/Cas9 *zip4* mutant in L*er*. I also corrected and approved final manuscript.

Date and Signature

09.05.2023 Maja Szymańska-Lejman

AUTHORSHIP CONTRIBUTION STATEMENT

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Julia Dluzewska, Wojciech Dziegielewski, Maja Szymanska-Lejman, Monika Gazecka, Ian R. Henderson, James D. Higgins, Piotr A. Ziolkowski. **MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism**. *BioRxiv*, 2023.05.03.539183. DOI: 10.1101/2023.05.03.539183, manuscript under review

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Ian R. Henderson**
Affiliation	Department of Plant Sciences, University of Cambridge, Cambridge, UK
Contribution Description	I contributed by providing protocols for the analysis of sequencing data. I revised and approved final version of the manuscript.

Date and Signature

6th May 2023

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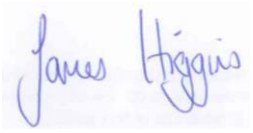
of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**James D. Higgins**
Affiliation	Department of Genetics and Genome Biology, University of Leicester, Leicester, UK
Contribution Description	I prepared chromosome spreads and counted chiasmata for Col×Ler hybrids (wild type, *fancm zip4*, *msh2 fancm zip4*).

Date and Signature

06/05/2023 

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of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Piotr A. Ziółkowski**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I designed the research and supervised the experiments. I analysed the sequencing data and contributed to the preparation of figures presented in the article. I wrote and revised the manuscript. I also shared funding.

Date and Signature

10/05/23 .....

“Where to cross over? Defining crossover sites in plants.”

Co-Authors contribution statements

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Julia Dluzewska[1], Maja Szymanska[1], Piotr A. Ziolkowski. **Where to cross over? Defining crossover sites in plants.** *Frontiers in Genetics*, 2018; 9:609
DOI: 10.3389/fgene.2018.00609
[1] - joint first authors

of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Maja Szymańska-Lejman**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I contributed to writing the manuscript by preparing sections: "General information about crossover in plants" and "Impact of DSB on crossover distribution".

Date and Signature

09.05.2023 Maja Szymańska-Lejman

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the publication

Julia Dluzewska[1], Maja Szymanska[1], Piotr A. Ziolkowski. **Where to cross over? Defining crossover sites in plants.** *Frontiers in Genetics*, 2018; 9:609
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of which I am a co-author, has been included in the doctoral thesis of Julia Dłużewska.

Co-Author Name	**Piotr A. Ziółkowski**
Affiliation	Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
Contribution Description	I created the concept of the work, contributed to writing and revising the manuscript and participated in preparation of figures. I also shared funding.

Date and Signature

6.05.2023 

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