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## REVIEW

of the doctoral dissertation

by

**Julia Dłużewska, MSc,**

entitled

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

done under the supervision of the University Professor Piotr A. Ziółkowski, PhD, DSc

in the Laboratory of Genome Biology,

Institute of Molecular Biology and Biotechnology, Faculty of Biology

at the Adam Mickiewicz University in Poznań, Poland

(legal basis: art. 187.1-4 and art. 190.3 of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws 2022, item 574, as amended))





## 1. General scope

Julia's doctoral dissertation addresses a fundamental scientific problem linked with various aspects of meiotic recombination, the phenomenon studied for many years, yet, in many ways, still needs to be discovered regarding sufficient understanding. Moreover, its better comprehension is essential both in basic research and agriculture, for example, in obtaining new cultivars of crops. More specifically, the thesis aims to elucidate the role of DNA polymorphism between homologous chromosomes on class I and class II crossover (CO) formation in the model seed plant *Arabidopsis thaliana* (Arabidopsis) in the context of the impact of the DNA mismatch repair (MMR) protein MSH2, which is one of the key players regarding various forms of DNA repair. To address this aim, Julia hypothesises that due to its ability to identify mismatches between recombining chromosomes, MSH2 can be involved in the CO relocation into the heterozygous chromosome regions in the juxtaposition effect. Another hypothesis she formulated postulates that the molecular pathways behind the class I and class II CO events may respond to interhomolog polymorphism differently, affecting the CO vs noncrossover (NCO) repair of double-strand breaks (DSB) with MSH2 being a regulator. Adding the spectrum of advanced methodological approaches and the choice of materials (hybrid, inbred and mosaic lines, various mutants) she used, the topic of this thesis could not be more timely and up to date.

## 2. Formal assessment

The doctoral thesis has 246 pages in total and consists of several sections, the most important of which are: "Abstract" (in English and Polish), "Thesis outline", and "Aims and objectives". These are followed by the printouts of four papers, along with their supplementary materials, constituting the dissertation's core and containing, presenting and discussing its key results. Publication #1 is a chapter recently (2022) published in *Methods in Molecular Biology*, a book series with over 35-year history and a good name in the research community. Publication #2 is an experimental paper published in a high-impact (JCR IF<sub>2020</sub> >11, Average JIF Percentile 91.1) *EMBO Journal*. Item #3 is a preprint of another original paper, currently under review but publicly available in *bioRxiv* (<https://doi.org/10.1101/2023.05.03.539183>). The final paper is a review published in 2018 in a good peer-reviewed journal *Frontiers in Plant Science* (JCR IF<sub>2018</sub> >4, Average JIF Percentile >91.4). Although all of them are multi-author papers, Julia is the first or equal first author in three, which indicates her prominent role. Three of these publications include international (UK, Germany, USA) research collaboration, which is nowadays a very reasonable strategy for doing high-quality science in experimental biology. Although they are relatively recent, some of them (publications #2 and #4) have already attracted numerous citations, which proves the international recognition of the authors and their research. The dissertation ends with "Conclusions" and "References" and the formal author and co-author contribution statements.

It is worth adding that the research in this dissertation was financed by several domestic and international research projects, including the Preludium grant 2020/37/N/NZ2/01226, funded by the National Science Centre Poland, with Julia as its principal investigator. Also, Julia is among the authors of four other publications indexed by Scopus that are listed in this thesis, some published in high-impact journals such as *Nature Communications*, *Proceedings of the National Academy of Sciences of the United States of America*, and *Nucleic Acids Research*. Although not contributing to the dissertation, they highlight Julia's outstanding research activity as a dynamic PhD student and early-stage researcher.



### 3. Substantive assessment of main research findings

The “Thesis outline” is well-written and aims to familiarise the reader with the subject area. It starts with the chapter, which introduces the reader to meiosis, meiotic recombination and crossover assurance, crossover homeostasis and genetic interference – the three phenomena which control CO designation. Then, Julia focuses on molecular details of meiotic recombination and the two CO pathways, i.e. the ZMM-mediated pathway, which is the main CO pathway leading to class I COs and the MUS81 class II CO-related non-interfering pathway, which has a minor (~15%) share in the total number of all CO events. The next chapter provides essential information about key protein components of the DNA MMR system, both in the wider eukaryotic and plant/*Arabidopsis*-specific context, with particular attention to the role of MSH2. The following two chapters overview the *cis*- and *trans*-acting CO modifiers. The final chapter focuses on the three best-known anti-CO pathways in *Arabidopsis*.

The next part provides the reader with the thesis’s main goal, hypotheses and research tasks. They are clearly defined, scientifically and technically ambitious and far-reaching.

Since three of the four papers composing the dissertation have already been assessed by the panels of competent reviewers and editors, there is no point in (re)doing so in detail in this report. Instead, I briefly outline their most prominent findings and conclusions.

In publication #1, Kbiri, Dluzewska and co-workers provided a CO recombination frequency measurement protocol, a detailed, step-by-step version of the method described earlier (<https://doi.org/10.7554/eLife.03708>). This seed-counting-based system utilises genetically modified *Arabidopsis thaliana* plants, so-called fluorescent-tagged lines (FTLs), which carry fluorescent proteins that enable precise analysis of CO occurrence in defined regions of individual chromosomes and diverse genetic backgrounds. Together with the advanced image analysis pipeline *SeedScoring*, this approach allows for counting fluorescent vs nonfluorescent seeds for CO measurement with an accuracy comparable to manual scoring but 20-25× faster. Thus, it provides a robust and high-throughput alternative to technically demanding, arduous and low-throughput cytological analysis of chiasmata distribution and a more expensive, not always (for example, in inbred lines) applicable and usually requiring external service GbS-based approach.

In publication #2, Blackwell, Dluzewska *et al.* explored the link between sequence polymorphism, CO frequency and MMR by genome-wide mapping of CO distribution in wild-type *Arabidopsis* of different genetic backgrounds and defective in mismatch recognition/polymorphism recognition *msh2* mutant in the context of intraspecific sequence diversity. Among the others, they revealed the promotion (to some extent since there is a parabolic relationship between SNP diversity and CO frequency) of the ZMM-mediated COs in the relatively sequence-diverse pericentromeric regions of the wild type. Noticeably, while the number of COs in the *msh2* mutant was comparable, their occurrence was remodelled to the less polymorphic sub-telomeric chromosome regions. Finally, the authors demonstrated and explained by proposing two models of the mismatch binding by MSH2 heterodimers that the CO remodelling occurs due to the heterozygous and homozygous region juxtaposition and depends on the central function of this protein.

The central role of MSH2 in regulating the class I and class II CO formation has been further dissected by Dluzewska *et al.* in publication (preprint in *bioRxiv*) #3. Using a collection of mutants which alter the relative proportions of these two classes of CO events, the authors managed to clarify some not always easy to interpret previous results. They demonstrated that regardless of the MSH2 activity, elevated SNP levels repress the class II CO formation and that MSH2 stimulates the class

I CO formation. This study underlines the central role of MSH2 as a key regulator of meiotic DSB repair, which shapes the CO landscape in connection with interhomolog polymorphism and imposes antagonistic effects on class I and II CO events. Considering the novelty of this research, it can be expected that this preprint will soon appear as a publication in a high-impact journal.

In the fourth and final publication included in the thesis, Dlużewska *et al.* provide a comprehensive review of how eucaryotes, plant organisms in particular, “decide” where to allocate COs to make the best use of them and considering that the number of CO events per chromosome is low and limited. After a general introduction, the authors familiarise the reader with CO in plants and the impact of DSBs on CO distribution. Then, in succession, they focus on the role of recombination initiation hotspots in the context of chromatin structure, transcription factors, local base composition, effects of regulatory circuits on DSB distribution, the evolution of recombination initiation site patterns and the organisation of those sites at the chromosomal scales followed by CO homeostasis. Next, they discuss various factors influencing spatial CO distribution, such as chromosome level, heterochiasmy and DNA methylation. In the final part, the authors focus specifically on the effects of heterozygosity on CO distribution at the chromosomal scale and local/recombination hotspot scale, followed by diverse sensitivity of the two meiotic pathways towards heterozygosity and the impact of the juxtaposition of heterozygous and homozygous chromosome regions. Considering the topic/contents, character and year of this publication, this review would probably serve better as the first, not the last, publication in the thesis.

The results obtained in one methodological and two original papers of the thesis and nine well-defined, thought-over, and justified conclusions clearly show that the general and specific objectives of the doctoral project have been met and yielded many results novel to science regarding various aspects of meiotic recombination and better explaining some seemingly contradictory, earlier data. While conducting her doctoral project, Julia acquired and successfully applied many diverse and often highly advanced methodologies. There is no doubt that her contribution to all four publications is very significant. Though the research she made was essentially fundamental, some of the results may have some future application in developing new crop varieties.

#### 4. Some remarks and questions

Below, I list several comments, for the Author’s convenience, in chronological order (i.e., as they appear in the thesis). In the case of some of them, I would like Julia to address them during her public defence specifically. These have been highlighted **in bold**.

##### Thesis outline

- Page 17: “*Meiosis, highly conserved cell division process, results in the formation of four haploid gametes from a single parent cell*”. – **Is this statement fully justified regarding plants?**
- Page 17: “*Prophase I includes four steps: leptotene, zygotene, pachytene, diplotene and diakinesis*”. – Five, not four.
- Page 26: “*Ribosomal RNA genes are arranged in large arrays of repetitive rDNA units, they are transcriptionally active and recruited into nucleolus early in meiosis*”. – **Does it (see the underlined part of the cited sentence) concern all kinds of rRNA gene categories in eukaryotes?**





– In addition, some 35/45S rDNA loci can be selectively silenced in allopolyploids and interspecific hybrids in a genome-specific manner (nucleolar dominance). Will their integrity during meiosis be maintained as it does in the case of transcriptionally active rDNA loci?

**Publication #4 (Dluzewska et al. 2018, <https://doi.org/10.3389/fgene.2018.00609>)**

- Page 199 in the dissertation/page 5 in the publication: “*However, one should consider significant differences between the structure of Arabidopsis and maize genome: while the first one is very small and compact (0.12 Gb), deprived of transposable elements (TEs) and gene-rich (one gene per 4.5 kb, on average) (The Arabidopsis Genome Initiative, 2000), the other is extremely large (2.3 Gb), TE-rich, with a gene-island organization (single genes separated by very long stretches of non-coding repetitive sequences) (Rodgers-Melnick et al., 2016).*” – **The statement addressing the 2.3 Gb maize nuclear genome as “extremely large” is debatable. Therefore, I ask Julia to discuss it in the context of what is currently known about nuclear genome sizes in angiosperms.**

#### Minor (editorial, language) issues

- The publication year is missing in almost all in-text citations in the “Thesis outline”.
- The abstract in Polish has some trivial language problems (e.g., “na chromosomie” -> “w chromosomie”, “Pokazałam” -> “Wykazałam”, “... z powodu promowania klasy I” -> z powodu promowania crossing over klasy I”, etc.), which is meaningless considering that all of Julia’s results have been or will be published in English.

#### 5. Conclusion

Concluding my review, I emphasise that this is conceptually, methodologically and regarding its outcome, an outstanding doctoral project and dissertation. It is a great achievement of both the Author and her supervisor, Professor Piotr Ziółkowski, at the Adam Mickiewicz University in Poznan. The PhD project involved teamwork in Poznan and fruitful international collaboration. It yielded several significant publications in high-impact journals or reputable book series, demonstrating the impact of this project on the development of plant experimental biology in general and our better understanding of the fundamental yet elusive phenomenon of meiotic recombination in particular. As a PhD student, Julia proved her ability to conduct the research and data analysis and interpretation independently, and her crucial input in this project and thesis is unquestionable. **I do not doubt that the PhD dissertation “Crossover control by mismatch detection protein MSH2 in response to the chromosome heterozygosity pattern in *Arabidopsis thaliana*” by Julia Dłużewska, MSc, fulfils all requirements defined in art. 187 of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws 2022, item 574, as amended). Therefore, with no reservations, I recommend this doctoral dissertation be accepted for public defence. In addition, considering the outstanding scientific level of this doctoral project and thesis, I recommend awarding Julia with a relevant distinction.**

