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Review of the PhD thesis submitted by Julia Dłużewska, MSc.

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

The doctoral dissertation by the PhD candidate, Ms. Julia Dłużewska, outlines the research carried out at the Laboratory of Genome Biology, Faculty of Biology of the Adam Mickiewicz University, under the supervision of Prof. Piotr Ziółkowski. Ziółkowski's team is well-recognized for their work on deciphering various aspects related to the meiotic recombination in plants and Ms Dłużewska decided to specifically focus on the role of MSH2 protein in this process. This put her in a perfect position to perform and present her study in the broader context, provided by the collective research done within this group. Accordingly, she structured her thesis as a collection of thematically related papers, which she co-authored. It includes three articles published in peer-reviewed journals: a review, a protocol and an original research paper, as well as one manuscript, also describing the results of original research, which has been deposited in the preprint server bioRxiv. This collection is preceded by the theoretical Introduction written by Ms Dłużewska and includes her comments on the individual papers. Additionally, the authorship statements signed by the papers' co-authors are included, indicating their individual contributions.

As a reviewer, I assessed this doctoral dissertation in terms of fulfilling the three necessary criteria, which are: 1) presenting general theoretical knowledge in the discipline of biological sciences by a PhD candidate 2) presenting an original solution to the scientific problem and 3) demonstrating the ability to conduct independent scientific research. Below is my opinion on these aspects.

Assessment of the general theoretical knowledge in the discipline of biological sciences presented by the PhD candidate

Meiosis is a highly conserved cell division process, crucial for sexually-reproducing organisms. Accordingly, there has been a huge interest in dissecting the mechanisms and factors involved in the meiotic recombination, associated with it. Still, there are substantial gaps in our understanding of this process. Also, while the molecular players involved in some stages of the meiotic recombination are well described in yeasts, which are single-cellular organisms, the repertoire of proteins engaged in similar – but not necessarily identical processes in plants, is far less recognized. For all these reasons, covering the current state of knowledge regarding meiotic recombination and the factors influencing spatial distribution of crossover in a concise but comprehensive way was not a trivial task. In my opinion, Ms. Dłużewska managed this task very well. First, in the Introduction to her thesis, she briefly outlined the necessary facts and unknowns. Moreover, the review paper *"Where to cross over? Defining crossovers sites in plants"*, of which Ms. Dłużewska is one of the leading co-authors, is a fount of knowledge regarding the current research on this subject. I very appreciate that it nicely combines the results collected using various cell models (e.g. mammals, yeasts and plants), highlighting the similarities and differences between them. It also points to some contradictory findings and the still-existing gaps, thus indicating the possible directions for future research. It should be also emphasized that this review paper covers over 200 references, which makes it a valuable resource itself. While reading, I have a habit of underlining the text which I find new, important or worth to remember and I must admit that I underlined a large part of this article. As confirmed by the authorship statements, Ms Dłużewska substantially contributed to writing this review. Also, as I outline below, she proved her deep knowledge of molecular biology by planning, conducting and interpreting a well-designed series of experiments, described in the PhD thesis. **Therefore, I have no doubts that Ms. Julia Dłużewska possesses the theoretical knowledge in biological sciences. Moreover, her in-depth knowledge regarding meiotic recombination and the modern molecular biology techniques, is distinguishable.**

Assessment of the demonstrated solution to a scientific problem and the ability to conduct an independent research

Previously, Ziółkowski and colleagues identified the "heterozygosity juxtaposition effect" in meiotic recombination, where the crossovers form preferentially in the polymorphic parts of the chromosome, while the neighboring homozygous regions are depleted in them. The crossover

homeostasis is, however not disturbed by this reposition and the total number of crossovers per genome remains unchanged. This observation raised the questions about the possible role of the mismatch repair pathway in controlling crossover formation and its contribution to the juxtaposition pattern. To get deeper insight into the links between heterozygosity and the crossovers, Ms Dłużewska decided to explore the impact of MSH2 protein on recombination in Arabidopsis meiosis. MSH2 is the key member of the mismatch recognition protein hetroduplexes and it indeed seems to be the right candidate for such investigation. Accordingly, Ms Dłużewska, in cooperation with her colleagues, designed and performed a variety of experiments, which allowed her to systematically evaluate the impact of MSH2 protein on the crossover frequency and patterning, with respect to numerous interacting factors, like the presence of polymorphic regions, their localization in the chromosome and the relative position of homozygous and heterozygous regions. She created and analyzed various hybrid and juxtaposition lines derived from Col-0 and several other accessions, carrying fluorescent reporter markers, in which she inactivated *MSH2* gene via CRISPR/Cas9 mutagenesis. Importantly, previous studies demonstrated different effect of local heterozygosity on the class I and class II crossovers, the latter being formed less efficiently in the polymorphic regions. Taking this into account, Ms Dłużewska analyzed the impact of MSH2 on class I and class II crossovers, by producing *msh2* mutants in various genetic backgrounds. For example, *fancm* and *recq4a recq4b* mutants show elevated frequency of class II crossovers due to inactivation of genes encoding DNA helicases with anti-recombination activity, FANCM and RECQ4, respectively. On the contrary, *zip4* mutants are defective in the ZMM pathway, which is involved in the formation of class I crossovers - the most frequent ones. Ms Dłużewska generated and systematically examined various combinations of mutants, along with the appropriate controls, she also introduced variations into the homo- and heterozygosity patterns across the chromosome. She later analyzed plant populations using several complementary approaches, including genotyping-by-sequencing, analysis of fluorescent-tagged lines, flow cytometry analyses and fertility tests. In result she collected an enormous amount of data. Based on the obtained results she demonstrated that MSH2 affects crossover/non-crossover decision during meiosis. Moreover, it exhibits antagonistic role towards class I and class II crossovers. It promotes the formation of class I events in polymorphic regions and is responsible for the heterozygosity juxtaposition effect. On the contrary, MSH2 limits class II crossovers in Arabidopsis hybrids in the pathway controlled by FANCM and RECQ4. These findings allowed her to propose a model, in which MSH2-containing heteroduplexes recognize the mismatches and recruit MutL factors, thus directing the ZMM-pathway recombination machinery into the polymorphic regions. Importantly, the proposed model stays in line with the known stimulating role of MSH2 complexes on ZMM pathway in yeasts. **With that, Ms Dłużewska confirmed her research hypotheses and achieved the aim of her work.**

I would like to stress that I am impressed by the amount of the experimental work carried out by Ms Dłużewska. I also admit her participation in setting up the methods and repeatable protocols, which are indispensable for high quality research. I very much appreciate the care about the details and using multiple alternative approaches to confirm the findings, for example analysis of the crossover frequency using various fluorescent reporter systems and cytological analysis. Basing on the analysis of the authors' contribution statements **I assess, that the role of Ms Dłużewska in performing this study was essential. It allowed her to successfully investigate highly complex process on the molecular level, thus providing new knowledge on the regulation of meiotic recombination. I am also convinced that she proved her skills as an independent researcher through all the stages of the study, from planning the experiments to the interpretation and dissemination of the results. It is worth to mention here that she also managed to obtain partial funding for her research, which is an important achievement at the pre-PhD step of the career.**

I have some questions regarding the discussed topic and I would appreciate if Ms Dłużewska briefly commented on them during the public defense of her thesis.

1. Based on the obtained data you concluded there is some MSH2-independent inhibitory effect of DNA polymorphism on Class II crossovers. What could be the mechanism of this inhibition?
2. In Arabidopsis, MSH2 forms heterodimers with MSH3, MSH6 and MSH7 in vitro, which display different affinities towards small and large mismatches. Do you think that studying the impact of individual heterodimers on meiotic recombination, e.g. by disrupting these genes instead of *MSH2* might bring some new information about the process under study?
3. You proposed that targeting recombination into polymorphic regions may be especially advantageous for self-pollinating plants, ensuring maintaining genetic diversity. What about the outcrossing plants? Does available data allow to perform some comparisons regarding the interaction of mismatch recognition system and meiotic recombination between species? In other words – how universal is the model that you proposed?

Finally, I would like to share some minor criticisms regarding the editorial aspects of the thesis. First, I think that the "*Where to cross over? (...)*" paper should have been the first one presented in the dissertation. I believe it nicely complements the Introduction. Also, chronologically, it was the first part of the dissertation (it was published in 2018), therefore it likely reflects the state-of-knowledge at the very beginning of the research. Having read it first (which I

actually did) would provide a good background for the reader and help to follow the experimental part of the thesis. Second, numerous citations in the Introduction lack the information regarding the publishing year. I found it a bit annoying, since I could not readily assess whether some facts were "new" or already well established in the literature or unambiguously track the correct reference, in case of multiple papers from the same first co-author. Third, the last page of the "*Where to cross over? (...)*" paper is missing. However, I consider these all minor mistakes, which did not really affect the overall high quality of the dissertation and my good impression of it.

Concluding remarks

In my assessment, the thesis presented by Ms Julia Dłużewska fulfills the criteria for a doctoral dissertation pursuant to Art. 187 of the Act of July 20, 2018 Law on Higher Education and Science, hence I am applying to the Scientific Council of the Biological Sciences Discipline, Adam Mickiewicz University in Poznań to proceed to the next steps of the procedure towards awarding Ms Julia Dłużewska the PhD degree. Also, considering the very high quality of the dissertation and the novelty of its findings, I would like to recommend rewarding it with the distinction.

.....Agnieszka Żmieńko.....

Dr hab. Agnieszka Żmieńko, IBCh PAS Professor