(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Guo Heyun
Thesis Title	Regulation of DNA double-strand breaks during meiotic prophase in the nematode *C. elegans*		

(Thesis Summary)

Proper chromosome segregation during meiosis depends on connections called chiasmata between homologous pairs of chromosomes, that allow chromosomes to orient in opposite directions in the first meiotic division. Chiasmata, in turn, are created by the repair of programmed double-strand breaks as crossovers between homologous chromosomes. Meiotic double-strand breaks are catalyzed by the conserved endonuclease Spo11. During meiotic prophase, the level of double-strand breaks must be under strict regulation, since too many breaks can lead to genome instability and apoptosis, while having too few breaks risks not having sufficient repair intermediates to be repaired as crossovers, leading to aneuploidy through chromosome missegregation.

The candidate has shown in this thesis that in the nematode *Caenorhabditis elegans* (*C. elegans*), meiotic DSB levels are tuned by the phosphorylation and dephosphorylation of a conserved protein, DSB-1. DSB-1 is a distant homolog of the protein Rec114, which is found in most eukaryotes, and is known to play a critical role in double-strand break regulation. The candidate found that DSB-1 phosphoregulation occurs via the opposite roles respectively played by the the DNA damage kinase ATL-1 (mammalian ATR), and the serine/threonine protein phosphatase PPH-4.1 (mammalian PP4). By quantitatively measuring the number of double-strand breaks in mutant strains with both ATL-1 and PPH-4.1 mutations, the candidate demonstrated that PPH-4.1 counteracts the anti-DSB activity of ATL-1, and thus promotes double-strand break initiation to ensure sufficient crossover formation for proper chromosome segregation. Moreover, by using CRISPR to create non-phosphorylatable mutant versions of DSB-1, the candidate has demonstrated that reducing the phosphorylation of DSB-1 greatly increases double-strand break levels. Non-phosphorylatable DSB-1 was also shown to rescue the double-strand break initiation defects found in mutants lacking PPH-4.1, providing strong evidence that DSB-1 is a direct substrate of dephosphorylation by PPH-4.1. Moreover, in addition to increasing the levels of double-strand breaks, non-phosphorylatable DSB-1 mutants also rescue the homologous pairing defects previously found in *pph-4.1* mutants, providing evidence for the hypothesis that homologous pairing is strengthened by DSBs in *C. elegans.*

In addition, the candidate has analyzed a paralog of DSB-1, termed DSB-2, whose loss was previously shown to lead to drastic reduction of meiotic double-strand breaks as animals age. The candidate demonstrates that loss of DSB-2, as well as increasing maternal age, both independently increase the phosphorylation of DSB-1, providing a potential explanation of the lowered double-strand break levels in older animals. Significantly, the abovementioned non-phosphorylatable mutations of DSB-1 also rescue the reduced meiotic DNA break levels found in animals that lack DSB-2 activity, indicating that the nonphosphorylatable DSB-1 allele is hyperactive, and does not depend on DSB-2.

Taken together, the candidate's work demonstrates that PPH-4.1 phosphatase, ATL-1 kinase and DSB-2 work together with DSB-1 to ensure sufficient levels of double-strand breaks for robust chromosome inheritance in sexual reproduction, while not creating too many breaks which could potentially lead to deleterious outcomes.

(Form 2)

（Thesis Evaluation Summary）

We hereby report that the candidate named above has duly presented and defended her work on the regulation of double-strand breaks in meiosis in the model system *Caenorhabditis elegans* via phosphorylation and dephosphorylation of the conserved protein DSB-1, both in writing and orally in her thesis defense.

The candidate has analyzed genetically modified strains that contain mutations in predicted phosphorylation sites of DSB-1, in combination with the kinase (ATL-1) and phosphatase (PPH-4.1) which the candidate also has demonstrated act to regulate the activity of DSB-1. Using results from diverse methods such as quantitative fluorescence imaging to count sites of double-strand breaks, western blotting to demonstrate the phosphorylation state of DSB-1, and genetic epistasis analysis of population statistics, a coherent model has emerged in which the phosphorylation state of DSB-1 is a major regulator of double-strand break levels. High levels of DSB-1 phosphorylation lead to inhibition of double-strand breaks, whereas low levels of DSB-1 phosphorylation tend to increase break levels. The process of double-strand break control is critically important for sexual reproduction, and the candidate has identified a novel and likely highly conserved mechanism of its regulation. Furthermore, the thesis points to new lines of research that will help to further clarify the molecular mechanisms of double-strand break regulation. This work is expected to be very well-received and make a strong contribution to the field of meiosis.

The written thesis, and its oral presentation and defense, substantiates the candidate's extensive and deep knowledge of life sciences, demonstrates expert research capability in the field of genetics and cell biology, and presents new discoveries that contribute substantially to the understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently and contains ample references, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference.

On August 3rd, 2022, the PhD thesis oral examination was held. The candidate delivered a presentation of approximately 45 minutes, followed by another 45 minutes of questioning by the committee members and by several attendees. The questioning was rigorous and probed both the candidate's understanding of her own work as well as her ability to place her results in a larger biological context. During the question/answer period the candidate acquitted herself well, demonstrating her understanding of the field and her ability to extrapolate from her own results to larger questions in life science. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.
(Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) : mm dd , yyyy