様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２０ 日現在

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研究種目：基盤研究(B)（一般）
研究期間：2021～2023
課題番号：２１Ｈ０２４００

研究課題名（和文）生殖細胞が最適量のDNA二重鎖切断を作りだす分子メカニズムの解明

研究課題名（英文）Elucidation of mechanisms ensuring optimal double-strand break number in meiotic cells

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　13,300,000円

研究成果の概要（和文）：減数分裂前期におけるDNA二重鎖切断の制御機構について、大きな進展を遂げた。まず、ホスファターゼPP4とDNA損傷応答キナーゼATRがC. elegansで二重鎖切断を促進および抑制することを発見した。次に、PP4とATRが共通の基質であるDSB-1タンパク質を介して作用することを発見した。さらに、非リン酸化型DSB-1は過剰活性化され、PP4変異体やdsb-2変異体の二重鎖切断欠陥を補うことができることも明らかにした。これにより、二重鎖切断開始の新たなメカニズムが解明され、DSB-1のリン酸化がDNA切断を防ぐ仕組みをさらに探求するための基盤が築かれた。

研究成果の学術的意義や社会的意義
Our research target DSB-1 (mammalian Rec114) has been under intense investigation in the past few years, and we have used the advantages of C. elegans to understand novel features of how it both promotes and restricts double-strand break activity; our work was published in Elife (2022).

研究成果の概要（英文）：We have made significant progress in understanding how programmed DNA double-strand breaks are regulated in meiotic prophase. First, we found that the phosphatase PP4 and the DNA damage kinase ATR work in opposite directions to promote and suppress double-strand breaks in C. elegans. Second, we found evidence that PP4 and ATR act through a common substrate, the protein DSB-1. DSB-1 (called Rec114 in mammals) is an essential double-strand break cofactor. DSB-1 is hyperphosphorylated in the absence of PP4; conversely, it requires ATR kinase to become phosphorylated. Third, we found that non-phosphorylatable DSB-1 is hyperactive, able to rescue double-strand break defects in PP4 mutants as well as mutants in dsb-2, a paralog of DSB-1. Our results have revealed a new facet of double-strand break initiation, and explained our previous findings in PP4 mutants, and set the stage for continued investigation into how phosphorylation of DSB-1 prevents DNA breaks from occurring.

研究分野：減数分裂

キーワード：減数分裂　C. elegans　染色体　二重鎖切断　リン酸化制御　キナーゼ　ホスファターゼ

様　式　C－１９、F－１９－１（共通）

１．研究開始当初の背景

Proper segregation of chromosomes in meiosis requires that programmed double-strand breaks are created during meiotic prophase. These double-strand breaks (DSBs) are required in some organisms for the pairing of homologous chromosomes. DSBs are also required for initiating recombination between homologous chromosomes, which forms physical connections (chiasmata) that hold chromosomes together so they can segregate to opposite daughter cells in the first meiotic division.

DSBs are also a form of dangerous DNA damage that cells normally try to avoid; however, in meiosis, they are made on purpose. Since they are dangerous, DSB initiation is under very strict regulation. The need to create chiasmata is balanced against the need to avoid unnecessary DNA damage, and a system where “not too many, but not too few” DSBs is created has evolved.

To understand the molecular mechanisms of this system, we build on our previous results showing that the conserved protein phosphatase, PP4, is required for DSBs in *Caenorhabditis elegans*. Without PP4 activity, no DSBs are made; hence, it is likely that a hyperphosphorylated protein substrate of PP4 blocks the creation of DSBs.

２．研究の目的

We aimed to understand the following questions:

1. What is the kinase that antagonizes PP4 (i.e., that phosphorylates this substrate)?
2. What is the DSB-promoting substrate of PP4 phosphatase?
3. How does phosphorylation of this substrate affect DSB formation?

３．研究の方法

1) To identify the kinase that antagonizes PP4, we took a candidate approach based on previous knowledge about PP4. In yeast, PP4 has been shown to mainly dephosphorylate targets of the DNA damage kinases Mec1 and Tel1. The orthologs of these highly conserved proteins in *C. elegans* are ATL-1 (also known as ATR) and ATM-1 (also known as ATM). We therefore combined mutations in these genes with mutations in PP4, reasoning that if hyperphosphorylation of a protein is the cause of loss of DSBs in the PP4 mutant, then reducing phosphorylation by mutating the kinase would restore DSBs.

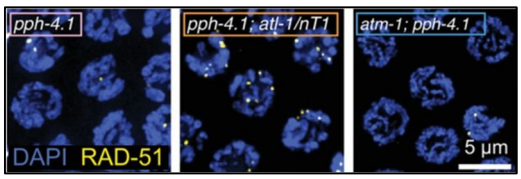

Figure 1

We made double mutants of PP4 with both ATL-1 and ATM-1, and immunostained for RAD-51, a marker of DSBs. We found that while ATM-1 kinase mutants did not rescue DSB formation in PP4 mutants, loss of ATL-1 did restore DSBs to high levels (Figure 1). We therefore concluded that ATL-1 acts in meiosis to suppress DSBs, in opposition to PP4’s promotion of DSBs. This was an unexpected finding, since ATM kinase plays a more active role in meiosis in most other organisms.

2) To identify the DSB-promoting substrate of PP4 phosphatase, we again turned to previous findings in other organisms. It had been previously demonstrated that yeast Rec114 protein

was a necessary cofactor in DSB formation, and a target of Tel1 (ATM) kinase. No information on its dephosphorylation was available, nor were any homologs of Rec114 known in *C. elegans*. However, there were two known proteins, DSB-1 and DSB-2, that had been recently found to be essential for DSBs; further, like Rec114, DSB-1 possessed many conserved instances of the ATM/ATR consensus phosphorylation motif [ST]Q, which made it a strong candidate for playing a Rec114-like role. There are 5 SQ sites in DSB-1, and we used CRISPR to mutate these to alanine, making a non-phosphorylatable version of DSB-1 called *dsb-1(5A)* (Figure

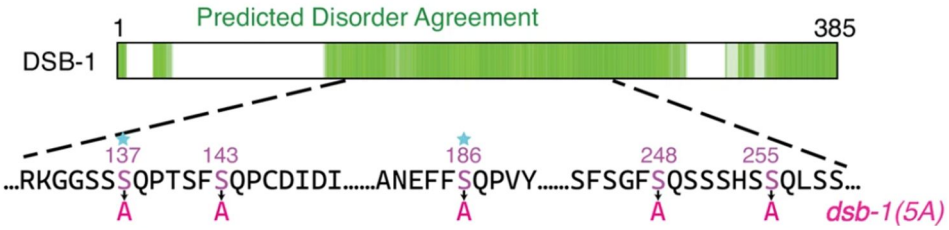

Figure 2

3). To test whether phosphorylation of these SQ sites in particular was a possible explanation for the loss of DSBs in a PP4 mutant, we crossed *dsb-1(5A)* into PP4 mutants and examined DSB activity. We saw that both alone and in combination with PP4, the *dsb-1(5A)* mutant had around twice the DSB level of wild-type cells (Figure 3). Further, while the PP4 mutant is sterile due to the lack of DSBs, we found that introducing *dsb-1(5A)* into PP4 mutants partially rescued this sterility, indicating that these DSBs made in the presence of non-phosphorylatable DSB-1 are functional for chiasma formation. We therefore conclude that the SQ sites in DSB-1 play a functional role in DSB control.

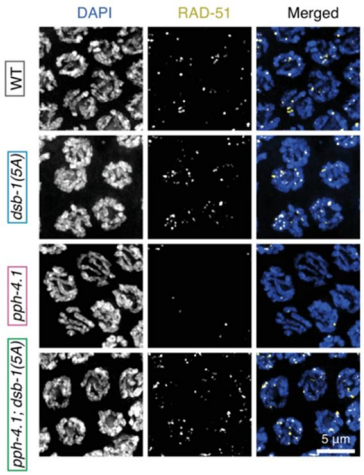

Figure 3

4) Finally, to assess how phosphorylation of DSB-1 reduces DSB formation, we attempted to image DSB-1 in live animals in both a phosphorylated and non-phosphorylated state, by making GFP fusions to both wild-type DSB-1 and to non-phosphorylatable DSB-1(5A). We used CRISPR to fuse GFP to the N terminus of both DSB-1 alleles and performed live imaging to examine its localization. We had hypothesized that loss of DSB-1 phosphorylation may lead to a different localization or abundance of GFP-DSB-1. However, we could detect no significant differences between GFP-DSB-1(wt) and GFP-DSB-1(5A). We hypothesized that hyperphosphorylation of DSB-1 might also affect its localization, so we combined GFP-DSB-1 with PP4 mutants, to create hyperphosphorylated GFP-DSB-1. However, in this combination as well, no difference in GFP-DSB-1 localization was found between wild-type and PP4 mutant backgrounds. We have concluded that localization or protein abundance is likely not the mechanism underlying suppression of DSB formation by phosphorylating DSB-1; we will address ourselves to this question in the future.

4．研究成果

Our main research output for this grant period is the publication “Phosphoregulation of DSB-1 mediates control of meiotic double-strand break activity” published in Elife 11, (2022). This work was performed as a collaboration with two other researcher groups: one, the group of Abby Dernburg (USA) provided reagents such as an inducible degron allele of ATL-1, as well as western blotting showing kinase specificity between ATL-1 and ATR-1; the second, evolutionary biologist Lewis Stevens (UK), provided phylogenetic distance estimates of DSB-1 and its close paralog DSB-2 in nematodes. The work in this paper was also presented as an invited talk at the 2022 Gordon Research Conference on Meiosis (USA) by Peter Carlton.

In addition to the genetic results mentioned above, we have used the structural prediction pipeline AlphaFold2/colabfold to gain structural information about DSB-1, its paralog DSB-2, and a third protein, DSB-3, which is an ortholog of yeast Mei4, known to associate as in a trimer with two copies of Rec114. We generated predictions for the structures of *C. elegans*

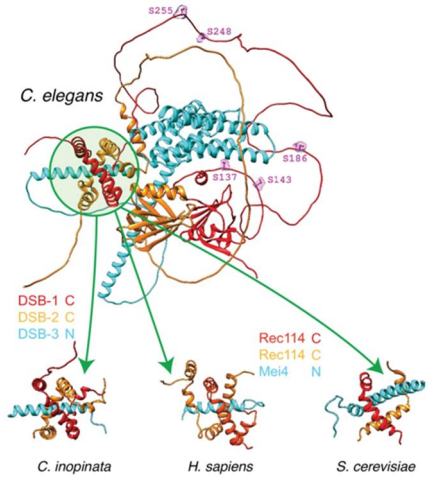

Figure 4

DSB-1/DSB-2/DSB-3 complexes, as well as several other species: the nematode *Caenorhabditis inopinata*, and yeast and human Rec114:Rec114:Mei4 complexes. In all cases, the returned structures shared a novel trimerization domain, consisting of the C terminal alpha-helices of DSB-1 and DSB-2 wrapping around each other, forming a channel into which the N-terminal alpha-helix of DSB-3 is inserted (Figure 4). The agreement of these predictions, despite high sequence divergence, led us to assign high confidence to this trimerization interface. This trimerization domain has since been shown to exist in fact, based on NMR structure determination.

In summary, under this grant we have revealed a major locus of control of programmed double-strand breaks in meiosis, determined the identity of the kinase and phosphatase that regulate DSB-1 through phosphorylation and dephosphorylation, showed the likely functional sites of phosphorylation through mutational analysis, and highlighted a unique structural feature of this conserved trimer complex that has since come under intense study. In addition to making a major contribution to the field of meiosis and recombination, we have laid groundwork for a future productive research program in determining how DSB-1 and DSB-2 promote and inhibit DSBs, leading to optimal control of DSB number in meiosis.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Guo Heyun、Stamper Ericca L、Sato-Carlton Aya、Shimazoe Masa A、Li Xuan、Zhang Liangyu、Stevens Lewis、Tam KC Jacky、Dernburg Abby F、Carlton Peter M	４．巻 11
２．論文標題 Phosphoregulation of DSB-1 mediates control of meiotic double-strand break activity	５．発行年 2022年
３．雑誌名 eLife	６．最初と最後の頁 na
掲載論文のDOI（デジタルオブジェクト識別子） 10.7554/eLife.77956	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Carlton Peter M、Davis Richard E、Ahmed Shawn	４．巻 na
２．論文標題 Nematode chromosomes	５．発行年 2022年
３．雑誌名 Genetics	６．最初と最後の頁 na
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/genetics/iyac014	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計6件（うち招待講演　4件／うち国際学会　0件）

１．発表者名 Peter CARLTON
２．発表標題 Phosphoregulation of DSB initiation by DSB-1
３．学会等名 C. elegans genetics conference
４．発表年 2021年

１．発表者名 Peter CARLTON
２．発表標題 Phosphoregulatory control of meiotic double-strand break initiation
３．学会等名 生殖細胞・減数分裂研究の過去・現在・未来／生殖細胞・減数分裂研究の最前線（招待講演）
４．発表年 2022年

１．発表者名
Peter CARLTON

２．発表標題
Phosphoregulation of Double-Strand Break Initiation in C. elegans

３．学会等名
Gordon Research Conference on Meiosis（招待講演）

４．発表年
2022年

１．発表者名
Peter CARLTON

２．発表標題
第27回ＤＮＡ・組換え・修復ワークショップ

３．学会等名
Phosphoregulation of factors promoting DNA double-strand breaks in meiosis（招待講演）

４．発表年
2023年

１．発表者名
Aya SATO

２．発表標題
第46回日本分子生物学会年会

３．学会等名
線虫DSB-1Rec114のリン酸化制御が減数分裂前期におけるDNA二重鎖切断量を調節する（招待講演）

４．発表年
2023年

１．発表者名
Peter CARLTON

２．発表標題
40th Chromosome Workshop / 21st Nuclear Dynamics Meeting

３．学会等名
Phosphoregulation of meiotic double-strand breaks

４．発表年
2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関			
米国	UC Berkeley			
英国	University of Edinburgh			