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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）染色体の「対称性の破れ」が正常な減数分裂を保障するメカニズムの解明

研究課題名（英文）Mechanisms that promote the correct completion of meiosis by breaking symmetry of chromosomes

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研究成果の概要（和文）：私たちは、線虫の染色体上、交叉の配置から正しい染色体の分配までの経路に働く重要なメカニズムを明らかにしました。シナプトネマ複合体のタンパク質SYP-1とHORMAドメインタンパク質HIM-3は、交叉が非対称に配置されることによって生じる2つのドメインのうち、短い方に特異的にリン酸化を受け、このリン酸化が染色体分離を促進する因子を集めることを明らかにした。
さらに、このリン酸化が短腕への局在が、交叉の数に依存し、交叉が３つ以下の場合には短腕ではなく染色体の全長に局在することを明らかにした。この結果は、グローバルなフィードバック機構が、染色体タンパク質の分配の非対称な局在を促進することを示唆する。

研究成果の学術的意義や社会的意義
Since chromosome segregation in meiosis is critical for fertility and correct development, our work leads to further insight about the possible causes of failure in human meiosis, which is responsible for a significant portion of both infertility and developmental atypicalities in humans.

研究成果の概要（英文）：We achieved the aims of our project by identifying new, important mechanisms in the pathway leading from asymmetric crossover placement to the establishment of cohesion loss strictly on one of two domains on chromosomes of the nematode C. elegans. We showed that the synaptonemal complex central element protein SYP-1 and the HORMA domain protein HIM-3 both become phosphorylated specifically on the shorter of two domains created by the asymmetric placement of a single crossover, and that preventing this phosphorylation leads to failure of downstream recruitment of factors that promote chromosome segregation.
Additionally, we showed that the partitioning of phosphoproteins to the short arm depends globally on the number of crossovers in the nucleus, with partitioning failing when fewer than 4 crossovers are present. This result indicates global feedback mechanisms work to promote asymmetry of chromosome protein recruitment.

研究分野：分子細胞生物学

キーワード：減数分裂　線虫　染色体　シナプトネマ複合体

１．研究開始当初の背景

Meiosis is the specialized cell division that creates haploid cells (sperm and eggs, in the case of animals) from diploid precursor cells. In meiosis, one round of DNA replication is followed by two rounds of chromosome segregation into daughter cells. For these two rounds of division to achieve correct chromosome segregation, the four chromatids must be separated from each other in two discrete steps, meaning that some cohesion must remain between chromosomes after the first division, to keep them together until the second division.

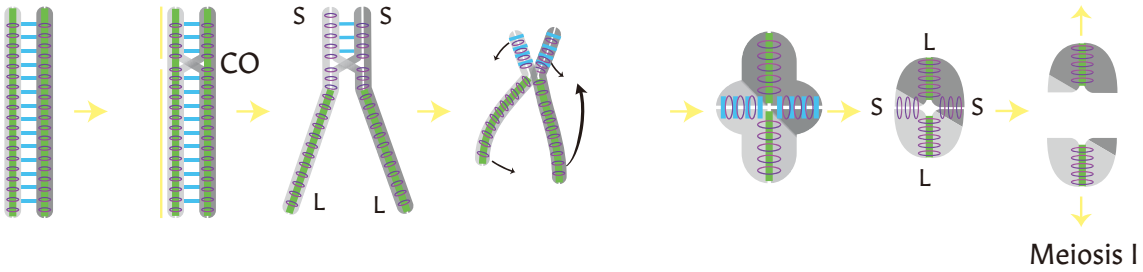

Figure 1: Chromosome dynamics that occur up until the first meiotic division. Green indicates cohesion between sister chromatids.

In most eukaryotes, cohesion between chromosomes is protected at the centromere region, by a protein called Shugoshin. The centromere becomes a domain of “cohesion protection” that enables the two rounds of chromosome segregation to occur. However, many organisms including the nematode *Caenorhabditis elegans* do not have discrete centromere regions; these organisms have evolved different mechanisms to achieve the same aim. In *C. elegans*, a “cohesion protection” domain is established by the following conditions: (1) a single crossover recombination site is established on each chromosome; the number never exceeds 1 and the crossover is almost always positioned either to the right or the left of the chromosome; (2) the crossover divides the chromosome into two segments, called the **short arm** and the **long arm** according to their size; (3) the **short arm** becomes the location of cohesion removal in the first meiotic division (Figure 1); the long arm becomes the location of cohesion protection for the first division. The mechanisms that detect the size of the chromosome segments, as well as establish different functional properties for each segment, are not understood. Despite the narrow species range in which these mechanisms have been found, their elucidation is of great general interest to the fields of genetics and chromosome biology, since they allow a very small-scale local event to affect the large-scale properties of entire chromosomes through the propagation of information.

We have previously discovered that the synaptonemal complex protein SYP-1 is phosphorylated, and the phosphorylated form of the protein decorates the short arm of the chromosome in early meiotic prophase. Indeed, this is one of the first visible indications of

the differentiation between the short and long arms, and the significance and effects of SYP-1 phosphorylation are a major focus of our laboratory. We asked whether other chromosome-binding proteins also display similar asymmetric phosphorylation on the short arm, and found a similar phenomenon for the meiotic axis protein HIM-3, a HORMA-domain containing protein required for correct chromosome structure. Our work during this period aims to elucidate the requirements for correct partitioning and the significance of HIM-3 phosphorylation in promoting correct chromosome segregation.

２．研究の目的

We identified three phosphorylation sites on HIM-3 near the *closure motif*, a site known to be involved in interacting with other HORMA-domain proteins. These sites were conserved in HIM-3 proteins from other nematodes, suggesting they may have functional relevance. We decided to test whether phosphorylation of HIM-3 protein at these sites was required for meiosis; whether the phospho-form of the protein had any particular localization on chromosomes that hinted at its function, and which kinase was responsible for its phosphorylation.

３．研究の方法

Our method involves making non-phosphorylatable residues in the proteins we found to be phosphorylated, and analyzing the effects of these mutations on recruitment of proteins known to be involved in chromosome segregation. We use forward genetics (creating mutant alleles by CRISPR/Cas9 editing), high-resolution microscopy of mutants, and genetic interaction analysis.

４．研究成果

1) We found that HIM-3 is phosphorylated on short arms, in a manner very nearly identical to the SYP-1 protein we previously analyzed. However, we found that partitioning of HIM-3 to the short arms occurs somewhat later than that of SYP-1, and that its partitioning is less complete; i.e., some small amount of phosphorylated HIM-3 remains on long arms as well.

To determine whether there was an interaction between phosphorylation of SYP-1 and HIM-3, we examined HIM-3 phosphorylation in non-phosphorylatable SYP-1 mutants. We found that HIM-3 partitioning was abrogated in such mutants, but SYP-1 phosphorylation is still correctly partitioned; that is, partitioning of phosphorylated HIM-3 is downstream of SYP-1. Interestingly, animals without SYP-1 do not show any phosphorylation of HIM-3 at all, showing that phosphorylation of HIM-3 requires synapsis of chromosomes.

We also showed that phosphorylated HIM-3 does not partition in cases where some chromosomes do not receive crossovers. In mutants that receive low crossover numbers, at least 4 crossovers are required to enable partitioning of phospho-HIM-3 to the short arm. This also held true for phosphorylated SYP-1 (*Figure 2*). While crossover-containing chromosomes in nuclei with low crossover numbers did not partition, they did retain all the phosphorylated HIM-3 and SYP-1 in the nucleus. Our results suggest that crossovers increase the affinity of phosphorylated SYP-1 and HIM-3 for chromosomes, and that partitioning is a further process that requires a threshold number of crossovers to occur.

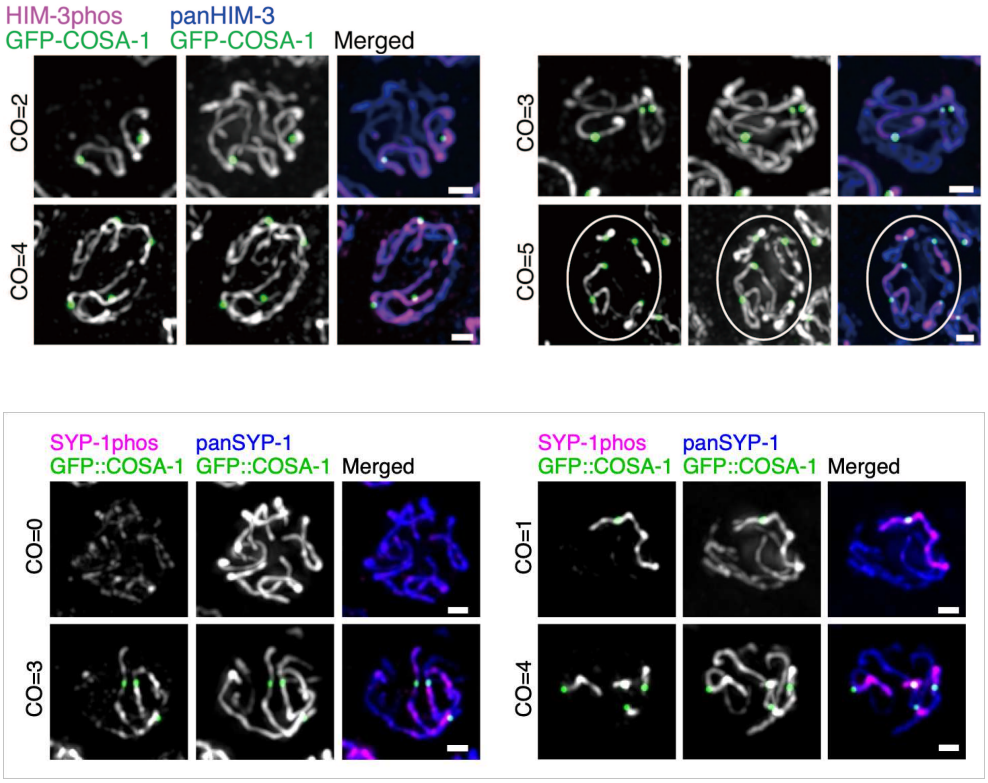

Figure 2: partitioning of phosphorylated HIM-3 (top) and SYP-1 (bottom) only occurs when the number of crossovers in a nucleus is four or greater.

Finally, we tested the importance of the HIM-3 phosphorylation sites by changing them to a non-phosphorylatable residue (Alanine) or to phospho-mimetic residues (D or E). Although the effect on viability (and thus the success of meiotic division) was negligible, in all cases, there was a significant effect on the localization of SYP-1. SYP-1 normally departs the long arm before chromosomes condense in preparation for division; however, in our HIM-3 mutants, SYP-1 remained abnormally associated with chromosomes until the division itself. Thus, our observations add to the growing evidence that redundant mechanisms ensure cleavage of cohesin on short arms at meiosis I even in the absence of asymmetric disassembly of the SC.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　4件／うちオープンアクセス　4件）

１．著者名 Nono Masanori、Kishimoto Saya、Sato-Carlton Aya、Carlton Peter Mark、Nishida Eisuke、Uno Masaharu	４．巻 30
２．論文標題 Intestine-to-Germline Transmission of Epigenetic Information Intergenerationally Ensures Systemic Stress Resistance in C.?elegans	５．発行年 2020年
３．雑誌名 Cell Reports	６．最初と最後の頁 3207～3217.e4
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.celrep.2020.02.050	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 Takemoto Kazumasa、Imai Yukiko、Saito Kenji、Kawasaki Toshihiro、Carlton Peter M.、Ishiguro Kei-ichiro、Sakai Noriyoshi	４．巻 16
２．論文標題 Sycp2 is essential for synaptonemal complex assembly, early meiotic recombination and homologous pairing in zebrafish spermatocytes	５．発行年 2020年
３．雑誌名 PLOS Genetics	６．最初と最後の頁 1-1
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pgen.1008640	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 Billmyre KK, Doebley A-L, Spichal M, Heestand B, Belicard T, Sato-Carlton A, Flibotte S, Simon M, Gnazzo M, Skop A, Moerman D, Carlton PM, Sarkies P, Ahmed S	４．巻 15
２．論文標題 The meiotic phosphatase GSP-2/PP1 promotes germline immortality and small RNA-mediated genome silencing	５．発行年 2019年
３．雑誌名 PLOS Genetics	６．最初と最後の頁 e1008004
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pgen.1008004	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 Peter M. Carlton, Aya Sato-Carlton	４．巻 36
２．論文標題 線虫・ショウジョウバエの減数分裂における染色体分離・染色体の出会いと別れのダイナミクス	５．発行年 2018年
３．雑誌名 実験医学	６．最初と最後の頁 149-156
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Sato-Carlton Aya、Nakamura-Tabuchi Chihiro、Li Xuan、Boog Hendrik、Lehmer Madison K.、Rosenberg Scott C.、Barroso Consuelo、Martinez-Perez Enrique、Corbett Kevin D.、Carlton Peter Mark	4．巻 16
2．論文標題 Phosphoregulation of HORMA domain protein HIM-3 promotes asymmetric synaptonemal complex disassembly in meiotic prophase in Caenorhabditis elegans	5．発行年 2020年
3．雑誌名 PLOS Genetics	6．最初と最後の頁 e1008968
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pgen.1008968	査読の有無 有
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〔学会発表〕　計5件（うち招待講演　3件／うち国際学会　3件）

1．発表者名 Peter Carlton
2．発表標題 Synapsis-dependent phosphorylation at the C- terminus of HORMA domain-containing axial element protein HIM-3
3．学会等名 EMBO Meiosis Meeting（国際学会）
4．発表年 2019年

1．発表者名 Peter Carlton
2．発表標題 Partitioning of synaptonemal complex phosphorylation promotes meiotic chromosome segregation in C. elegans
3．学会等名 タンパク研シンポジウム （大阪大学　タンパク質研究所）international seminar on “Genome stability and instability in mitotic and meiotic cells”（招待講演）（国際学会）
4．発表年 2018年

1．発表者名 Peter Carlton
2．発表標題 Partitioning of synaptonemal complex phosphorylation promotes meiotic chromosome segregation
3．学会等名 日本放射線影響学会第61回大会（招待講演）
4．発表年 2018年

１．発表者名 Peter Carlton
２．発表標題 Partitioning of synaptonemal complex phosphorylation promotes meiotic chromosome segregation
３．学会等名 第41回日本分子生物学会年会（招待講演）
４．発表年 2018年

１．発表者名 Peter Carlton
２．発表標題 Partitioning of synaptonemal complex phosphorylation promotes meiotic chromosome segregation
３．学会等名 2018 Asia-Pacific C. elegans meeting（国際学会）
４．発表年 2018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