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

平成　３０　年　　６　月　２７　日現在

機関番号：　１４３０１
研究種目：　基盤研究(B)（一般）
研究期間：　2015～2017
課題番号：　１５Ｈ０４３２８

研究課題名（和文）Investigating phosphoregulation of meiotic recombination using superresolution microscopy

研究課題名（英文）Investigating phosphoregulation of meiotic recombination using superresolution microscopy

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　12,400,000円

研究成果の概要（和文）：本研究期間において我々は、線虫の減数分裂における染色体のリン酸化による制御メカニズムを解析した。我々は、PP4ホスファターゼが減数分裂に必須であることを明らかにし、PP4欠損株を用いたリン酸化解析より、シナプトネマ構造因子のSYP-1のリン酸化サイトを同定した。さらに、我々はSYP-1リン酸化が、染色体分離に必須であること、リン酸型SYP－1は、減数第一分裂期に染色体の分離面となる領域に前もって局在することで、分離面を指定することを明らかにした。

研究成果の概要（英文）：In this grant period we investigated control of meiotic prophase chromosome dynamics by phosphorylation in C. elegans. Our starting point was the phosphatase PPH-4.1, which we had previously identified as a critical regulator of at least 4 critical chromosome dynamics events. We identified SYP-1, a member of the synaptonemal complex, as an important phosphoprotein in meiosis. SYP-1 phosphorylation is required for correct chromosome segregation, acting to specify the region in which chromosome cohesion will be lost in the first meiotic division. Additionally, our data shows that SYP-1 phosphorylation is required to recruit Polo-like kinase to the chromosome central region, as well as to promote the correct distribution of genetic crossovers on chromosomes.

研究分野：　減数分裂においての染色体ダイナミクス

キーワード：　減数分裂　線虫　高解像度顕微鏡　染色体

１．研究開始当初の背景

(1) Sexual reproduction depends on the reductional division of *meiosis*, in which homologous chromosomes of diploid cells recombine and segregate into haploid daughter cells. Human meiosis encounters many difficulties, and errors in meiosis account for a large part of human infertility and birth defects. To understand meiosis, we have used the nematode *C. elegans* as a model, for its ease of genetic manipulation as well as high genomic conservation with all animals, including humans.
(2) Our previous research found that PPH-4.1, a conserved protein phosphatase, was required for multiple, independent processes involving chromosome dynamics in meiotic prophase. At least four essential steps of meiosis: chromosome pairing, homologous chromosome synapsis, recombination initiation, and crossover formation, depend on the activity of PPH-4.1. To identify potential interactors or substrates of PPH-4.1 whose phosphoregulation is important for the success of meiosis, we conducted a phosphoproteomics screen. As a result of this screen, we obtained several candidates for further analysis. Additionally, we performed a screen for new mutations that suppress the lethality of *pph-4.1* mutations, to identify novel interactors our phosphoproteomics screen may have missed. The current research period started from this background and will be reported on hereafter.

２．研究の目的

(1) Our goal was to identify proteins whose regulation by phosphorylation or dephosphorylation is critical for the correct operation of meiosis. PPH-4.1, a phosphatase known to be involved in multiple aspects of chromosome dynamics, was our starting point for this work, since we had shown that it was required for 4 steps in meiotic prophase. Although our initial aim was to identify PPH-4.1 interactors, in fact the identification of novel phosphoproteins could shed light on the molecular mechanisms of meiosis, regardless of whether they interact with PPH-4.1 or not.
(2) Once we had identified such phosphoproteins, our next aim was to determine where they were phosphorylated and how their phosphoregulation is important to their activity.
(3) By identifying novel phosphoregulatory mechanisms in meiosis, we have aimed to understand how chromosomes make “decisions” such as which chromosome to pair with, where and when to initiate recombination, which recombination events should become crossovers, and where chromosomes should separate from each other during cell division.

３．研究の方法

(1) We focused first on the protein SYP-1, a coiled-coil protein that forms part of the central element of the synaptonemal complex, a structure that holds paired chromosomes together and mediates recombination. Our phosphoproteomics analysis showed that SYP-1 was phosphorylated at 12 sites at its C-terminus. The most conserved of these 12 sites was the central threonine in an STP motif, a known consensus site for binding of polo-like kinase (PLK-2 in *C. elegans*). We mutated this single threonine (T452) to Alanine, to assess the requirement for phosphorylation of this residue. This T452A change led to high levels of lethality and chromosome missegregation, showing T452 phosphorylation is critical for meiosis.
(2) We raised antibodies against phospho-T452 of SYP-1 and performed immunofluorescence staining to determine its localization. We found phospho-SYP-1 to localize to the region between the crossover and the nearest telomere; *i.e.*, the “short arm” of the chromosome. Since the short arm of the chromosome in *C. elegans* is the region where cohesion is lost in the first meiotic division, and T452A mutants showed chromosome missegregation, we hypothesized that phospho-SYP-1 was involved in guiding chromosome segregation.
(3) We next analyzed other proteins and protein modifications involved in chromosome segregation by immunofluorescence, and found mislocalization in a large number of cases.
(4) We further analyzed SYP-1 phosphomutant T452A for its ability to undergo crossover recombination by measuring genetic recombination between phenotypic markers. Although lethality was high, the ability to recombine was not lost; in fact, we observed increased recombination in T452A mutants. This indicated that phosphorylation of SYP-1

acted normally to limit the amount of crossover recombination.
(5) To identify proteins other than SYP-1 whose phosphoregulation was important for meiosis, we conducted a suppressor screen for mutations that could allow *pph-4.1* mutant animals to regain fertility. We mutagenized wild-type animals with EMS, creating point mutations, and crossed these animals to *pph-4.1* animals. We observed partial rescue of fertility in 73 F2 offspring carrying two null recessive alleles of *pph-4.1*, indicating that (a) *pph-4.1* is a suppressible mutation, and (b) there are mutations in several candidate genes that may encode PPH-4.1 interactors.
(6) To identify the genes that suppress *pph-4.1*, we have begun analyzing the whole-genome sequence of backcrossed suppressors. Candidate mutations are then re-created with genome editing (CRISPR/Cas9) to verify whether they are capable of suppressing *pph-4.1*.

４．研究成果

(1) Mutation of T452 to alanine led to 40% embryonic lethality due to chromosome missegregation, while SYP-1 loading to chromosomes was unaffected. Additionally, the number of male self-progeny (resulting from nondisjunction of X chromosomes in meoisis) was 6% in these mutants. Together, these results show that phosphorylation of SYP-1 is required for successful meiotic chromosome disjunction.
(2) As expected from the STP sequence motif, phosphorylated SYP-1 could recruit

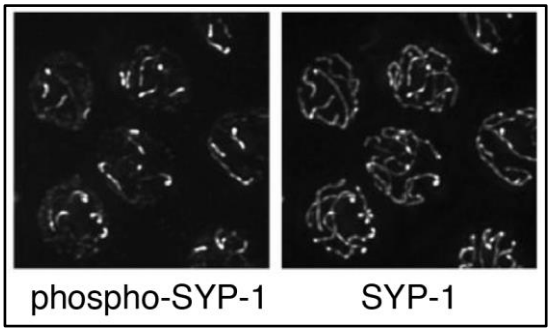

Figure 1. Phospho-SYP-1 (left) localizes to a short subregion of the chromosome, whereas all SYP-1 forms (right) distribute evenly along the entire length.

polo kinase PLK-2 to the chromosome central region, whereas T452A mutants could not recruit PLK-2. Since PLK-2 is required for many aspects of meiotic prophase chromosome dynamics, we conclude that recruiting PLK-2 to chromosomes is a major role played by phospho-SYP-1.
(3) We found SYP-1 phosphorylation concentrates to chromosome "short arms" — domains where cohesion between chromosomes will be lost at meiotic anaphase I — after crossovers are made (**Figure 1**). We showed this localization is dependent on recombination, since *spo-11* mutants, which do not initiate programmed double-strand breaks, have phospho-SYP-1 uniformly distributed along their chromosomes.
(4) Consistent with a role of phosphorylated SYP-1 in establishing the asymmetry of chromosome regions that direct chromosome segregation, the localization of several other critical proteins and protein modifications was

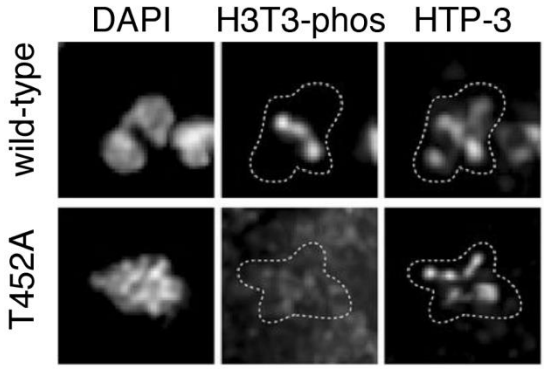

Figure 2. H3T3-phos in wild-type chromosomes (top) localizes to the short arm between homologs, where it can direct the localization of AIR-2. In T452A mutants of SYP-1, however, H3T3-phos is lost. Axis protein HTP-3 (right) is not affected. Chromosomes are visualized with DAPI (left).

disrupted in T452A nonphosphorylatable mutants of SYP-1. In particular, the Aurora B kinase (AIR-2 in *C. elegans*), which phosphorylates cohesin, fails to localize to the region between paired homologs in late meiotic prophase when SYP-1 cannot be phosphorylated. Since AIR-2 relies in part on phosphorylation of histone H3 threonine 3 (H3T3-phos), we examined the localization of this modified histone in T452A mutants. Unlike wild-type chromosomes, H3T3-phos was not localized to chromosomes in T452A mutants (**Figure 2**). We hypothesized that this loss of H3T3-phos could be due to the phosphatase PP1, which is recruited by the chromosome axis protein HTP-1, becoming mislocalized to the short arms. We examined HTP-1 staining and found that it indeed localized improperly to the short

arms in SYP-1 T452A mutants. Thus, SYP-1 phosphorylation and its usual localization to short arms influences the binding location of several other proteins that direct proper chromosome segregation.
(5, summary) These findings represent a large step forward in our understanding of how phosphoregulation of chromosomes influences their behavior. The mechanism by which *C. elegans* chromosomes causes the long arms to retain chromosome cohesion, while losing it on the short arm, has previously not been understood. While our research does not uncover the initial cue that senses the length of these domains, we do show that PLK-2 kinase localization to the short arm, most likely by its binding to SYP-1, is required to achieve correct localization of factors (H3T3-phos, AIR-2, and PP1 in complex with HTP-1) that decide where and when chromosome disjunction occurs. Since other recent studies have shown that SYP-1 and the other components of the synaptonemal complex are highly dynamic and mediate long-distance communication along the chromosome, it is plausible that phosphorylation influences the dynamics of this complex in ways that allow length to be sensed and chromosome functional properties to be modified in a length-dependent manner. Our ongoing research is focused on determining the effect of phosphorylation of SYP-1 on its dynamics.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　1　件）

(1) Sato-Carlton, A., Nakamura-Tabuchi, C., Chartrand, S.K., Uchino, T., and Carlton, P.M. (2017). Phosphorylation of the synaptonemal complex protein SYP-1 promotes meiotic chromosome segregation. J. Cell Biol. 217(2)：555-
DOI: 10.1083/jcb.201707161
（査読有り）

〔学会発表〕（計　2　件）

(1) IPR international seminar on
“Genome stability and instability in mitotic and meiotic cells”
Title: Partitioning of synaptonemal complex phosphorylation promotes meiotic chromosome segregation in *C. elegans*
Speaker: CARLTON, Peter Mark
Date: 2018-04-10
Location: Osaka, Japan

(2) 第 35 回染色体ワークショップ
Title: SYP-1 のリン酸化制御が減数分裂期染色体を「短い方」と「長い方」に分けて分離する
Speaker: CARLTON, Peter Mark
Date: 2017-12-20~12-22
Location: 愛知県、西尾市

〔その他〕
ホームページ等

https://www.carltonlab.org
https://github.com/pmcarlton

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