Phosphorylation of HORMA-domain protein HTP-3 at Serine 285 is dispensable for crossover formation

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Abstract

Generation of functional gametes is accomplished through a multilayered and finely orchestrated succession of events during meiotic progression. In the Caenorhabditis elegans germline, the HORMA-domain-containing protein HTP-3 plays pivotal roles for the establishment of chromosome axes and the efficient induction of programmed DNA double-strand breaks, both of which are crucial for crossover formation. Double-strand breaks allow for accurate chromosome segregation during the first meiotic division and therefore are an essential requirement for the production of healthy gametes. Phosphorylation-dependent regulation of HORMAD protein plays important roles in controlling meiotic chromosome behavior. Here, we document a phospho-site in HTP-3 at Serine 285 that is constitutively phosphorylated during meiotic prophase I. pHTP-3S285 localization overlaps with panHTP-3 except in nuclei undergoing physiological apoptosis, in which pHTP-3 is absent. Surprisingly, we observed that phosphorylation of HTP-3 at S285 is independent of the canonical kinases that control meiotic progression in nematodes. During meiosis, the htp-3(S285A) mutant displays accelerated RAD-51 turnover, but no other meiotic abnormalities. Altogether, these data indicate that the Ser285 phosphorylation is independent of canonical meiotic protein kinases and does not regulate HTP-3-dependent meiotic processes. We propose a model wherein phosphorylation of HTP-3 occurs through noncanonical or redundant meiotic kinases and/or is likely redundant with additional phospho-sites for function in vivo.

Keywords: Caenorhabditis elegans meiosis; HORMA-domain proteins; HTP-3

Introduction

Sexual reproduction relies on the formation of haploid gametes through meiosis, a specialized cell division mechanism that ensures equal distribution of the genetic material in the daughter cells (Zickler and Kleckner 1999, 2015). Faithful chromosome segregation depends on the recognition of the homologous chromosomes (pairing), stabilization of their association through the synaptonemal complex (SC; synopsis), and establishment of chiasmata (recombination; Zickler and Kleckner 1999, 2015). The latter arises from crossover (CO)-dependent repair of programmed double-strand breaks (DSBs), which are generated during meiotic prophase I by the topoisomerase-like enzyme Spo11 (Goodyer et al. 2008). Recent evidence has shown that HTP-3 can be directly regulated by kinases during meiotic progression. For example, Das et al. (2020) observed that ERK/MPK-1 phosphorylates HTP-3 in vitro, however, the actual phosphorylation site remains to be determined.

We have previously shown that par-1, the nematode ortholog of mammalian poly(ADP-ribose) glycohydrolase PARG, is an important factor required to coordinate DSB induction and repair and identified physical interactors of PARG-1 in worms (Janisiw et al. 2020). In a mass spectrometry analysis performed on PARG-1::GFP pull downs, we identified a putative phosphorylated form of HTP-3 at Serine 285, which we further investigated. A phospho-specific HTP-3S285 antibody detected phosphorylation of HTP-3 throughout meiotic prophase I, confirming that this site is phosphorylated in vivo. Phosphorylated-HTP-3S285 localization overlaps with that of total HTP-3 and is independent of meiotic DSBs, synopsis, or COs. Individual removal of canonical meiotic kinases did not alter HTP-3S285 phosphorylation pattern, suggesting that HTP-3 may be phosphorylated at multiple sites and these phospho-sites may function redundantly. Alternatively, S285 may be phosphorylated by multiple kinases in a redundant manner. Preventing HTP-3S285 phosphorylation in vivo does not...
impair axes morphogenesis and recombination, indicating that this site is not essential to successfully achieve chiasma formation. Altogether, our data show that phosphorylation of HTP-3 at Ser285 likely functions in a redundant manner with other phospho-residues that are yet to be identified.

Materials and methods
Caenorhabditis elegans genetics and viability assays
The Bristol N2 C. elegans strain (Brenner 1974) was used as the wild-type control. The htp-3(S285A) was generated by CRISPR/Cas9 genome editing by SUNY Biotech. Silent mutations encoding for an HfA1 restriction site were included for screening purposes. The strains generated by CRISPR/Cas9 were outcrossed to wild-type N2 worms at least twice before use. All strains were maintained at 20°C under standard conditions for all experiments unless otherwise indicated. Viability and male progeny assessment were performed on single animals plated as L4 and then moved onto fresh NG plates every 24 h for 3 days. Dead embryos/total embryos were scored 24 h after the mother had been moved and the presence of males was evaluated 3 days later. Strains used for this study were:

CA1199: unc-119(ed3) III, iet538 [sun-1::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)::IV.
TY5308: htp-3(tm3655) I/hT2 [bli-4(e937) let-7(q782) qs48] (I,III).
PHX3069: htp-3(jyb3069[S285A]) I.
AV590: cos-1(tm3298) III/qC1 [dpv-19(e1259) glp-1(q339)] III.
AV106: spo-11(ok79) V/nt1 [unc-5(n754) let-7] (IV, V).
AV276: spo-2(ek307) V/nt1 [unc-5(n754) let-7(m435)] (IV, V).
RB1583: pik-2(ok1936) I.
ATG330: chk-2[+$q41[chk-2::AID] ] V, iet538 IV.
YKM295: GFP::cosa-1 II, AID::cdk-1 III, iet538 IV.
YKM110: cdk-2::AID::3xFLAG I, GFP::cosa-1 II, iet538 IV.
YKM388: cdk-2::AID::3xFLAG I, AID::cdk-1 III, GFP::cosa-1 II, iet538 IV.
NSV363: atm-1(ek186) I, atl-1(tm853) V/nt1 [unc-5(n754) let-7 qs50] (IV, V).
RB1562: him-5(ok1896) V.
VC172: cep-1(ek138) I.
NSV228: brc-1(ddr41) III.

RNAl experiments and auxin treatment
RNAl experiments were conducted by feeding L4 staged animals on HT115(DE3) bacteria expressing dsRNA of the relevant target gene (chk-1) for 48 h until dissection. NGM plates containing 1-mM 5-Hydroxyindole-3-acetic acid (Sigma) dissolved in absolute ethanol (+auxin) were used for auxin-induced degradation of cdk-1, cdk-2, and chk-2. The control plates (-auxin) were poured in the same way with the addition of an identical volume of ethanol without auxin. Given that presence of auxin inhibits bacterial growth, a saturated OP50 culture was concentrated 5 x before being spotted onto NGM plates. Plates were left to dry overnight at room temperature before L4 animals were plated and then dissected 24 h later.

Immunostaining and images acquisition
About 20–24 h post-L4 stage animals were dissected in 15 µl of 1xPBS and fixed with an equal amount of 2% PFA (diluted in 1xPBS from a 16% stock) for 5 min at room temperature. A 24 x 24 coverslip was gently applied and slides were submerged in liquid nitrogen for freeze-crack. Samples were placed in methanol at –20°C for 5 min and then washed thrice for 5 min at room temperature in 1xPBS with 0.1% Tween.

Blocking was performed by leaving the slides for 1 h at room temperature in 1% BSA (dissolved in 1xPBS with 0.1% Tween), followed by primary antibody treatment overnight at 4°C in a humid chamber. The following day, slides were washed in 1xPBS with 0.1% Tween thrice for 10 min each and secondary antibodies were left in incubation on the slides for 2 h at room temperature in the dark. After 3 washes in the dark at room temperature for 10 min each, a 60 µl drop of DAPI (2 µg/ml) was placed onto the samples, allowed to stain for 1 min in the dark, and then washed for at least 20 min in 1xPBS with 0.1% Tween. A drop of 12 µl of Vectashield was placed onto the samples and a 22 x 22 coverslip was then sealed using nail polish.

The primary antibodies used in this study were: rabbit anti-pHTP-3 S285 (this study, absorbed against htp-3[S285A] mutant, 1:500), guinea pig anti-HTP-3 (Kim lab, 1:750), rabbit anti-SYP-1 (Janiszew et al. 2020, 1:1,500), rabbit anti-RAD-51 (this study, 1:1,300), and mouse anti His-tag (Sigma, 1:3,000).

Images were acquired with an upright fluorescence microscope Zeiss AxiosImager.Z2 equipped with a Hamamatsu ORCA Flash 4.0, sCMOS sensor camera, using UPlanSapo 100 x/1.4 Oil objective with Z-stacks at 0.24 µm thickness. Images were deconvolved with ZEN 3.0 Blue software (Zeiss), using “constrained iterative” algorithm at maximum strength.

Generation of anti-phospho-HTP-3 S285 and anti-RAD-51 antibodies
Phospho-specific antibodies against S285 in HTP-3 were produced by immunizing rabbits with the synthetic peptide CNFLEDIEYFPSPGR (Genscript) in which the cysteine was added for conjugation to KLH (keyhole limpet hemocyanin). Polyclonal phospho-HTP-3 S285 antibodies were purified and then further absorbed against htp-3(S285A) mutant worms to reduce background. The specificity of the antibody was assessed by immunofluorescence where lack of staining was observed in htp-3(S285A) mutants but not in WT animals.

The synthetic peptide CQSAQASRQKKSQDEQRAADQA corresponding to the amino acids 40–59 of C. elegans RAD-51 isoform (including the underlined cysteine required for conjugation to KLH) was used to perform 4 immunization rounds in 2 rabbits (Genscript). RAD-51 polyclonal antibodies were separately affinity purified from raw sera of both animals against the same synthetic peptide employed as the immunogen. The specificity of the antibody was assessed by immunostaining of spo-11 mutants in which, unlike WT control animals, detection of RAD-51 foci was abrogated due to the absence of physiological DSBs.

Immunoprecipitation and sample preparation for mass spectrometry
Nuclear extracts from parg-1::GFP and untagged WT animals were produced as detailed in Silva et al. (2014). One milligram of pooled nuclear-soluble and chromatin-bound fractions were used for GFP immunoprecipitations by employing agarose GFF-traps (Chromotek), which had been pre-equilibrated in Buffer D (20% glycerol, 0.2 mM EDTA pH 8, 150 mM KCl, 20 mM Heps-KOH (pH 7.9), and 0.2% Triton X-100, supplemented with protease inhibitor cocktail (Roche)). Incubation of beads with the extracts was carried out over night at 4°C on a rotating shaker. The following day, the beads were spun down and washed extensively in Buffer D (without Triton X-100) before an equal amount of 2 x Laemmli buffer was added. Samples were boiled for 10 min and...
then the immunoprecipitated complexes were separated on a 4–12% acrylamide gel.

For mass spectrometry analysis, agarose beads were resuspended in 30 μl elution buffer (2 M urea, 50 mM ammonium bicarbonate), disulfide bonds reduced with 10 mM dithiothreitol for 30 min at room temperature, and then alkylated with 25 mM iodoacetamide for 15 min in the dark. After quenching with another 5 mM dithiothreitol, 150 ng of trypsin (Trypsin Gold, Promega) was added followed by 90-min incubation at room temperature in the dark. The supernatant without beads was transferred to a new tube and another 30 μl of elution buffer was added to the beads. The supernatants without beads were combined, diluted to 1 M urea concentration, and another 150 ng of trypsin was added before incubation at 37°C in the dark overnight. The digest was stopped by the addition of trifluoroacetic acid to a final concentration of 1%, and the peptides were desalted using C18 Stagetips (Rappsilber et al. 2007).

Peptides were separated on an Ultimate 3000 RSLC nano-flow chromatography system (Thermo-Fisher), using a precolumn for sample loading (Acclaim PepMap C18, 2 cm × 0.1 mm, 5 μm, Thermo-Fisher), and a C18 analytical column (Acclaim PepMap C18, 50 cm × 0.75 mm, 2 μm, Thermo-Fisher), applying a segmented linear gradient from 2% to 35% and finally 80% solvent B (80% acetonitrile, 0.1% formic acid; solvent A 0.1% formic acid) at a flow rate of 230 nl/min over 120 min. Eluting peptides were analyzed on a Q Exactive HF Orbitrap mass spectrometer (Thermo Fisher), which was coupled to the column with a nano-spray ion-source using coated emitter tips (New Objective).

Raw data were processed using the MaxQuant software package (version 1.5.5.1; Tynerova et al. 2016) and the Uniprot C. elegans reference proteome (www.uniprot.org), as well as a database of most common lab contaminants. The search was performed with full trypsin specificity and a maximum of 2 missed cleavages at a protein and peptide spectrum match false discovery rate of 1%. Carbamidomethylation of cysteine residues was set as fixed, phosphorylation (serine, threonine, or tyrosine), oxidation (methionine), and N-terminal acetylation as variable modifications. Carbamidomethylation of cysteine residues was set as fixed, Label-free quantification and the “match between runs” feature were activated—all other parameters were left at default. The spectrum supporting phosphorylation of pHTP-3S285 was validated manually.

**Results and discussion**

**HORMA-domain-containing protein HTP-3 is phosphorylated at S285 in vivo**

We have recently shown that in C. elegans, the PARG-1/PARG protein, ortholog to mammalian poly(ADP-ribose) glycohydrolase, localizes along both the central and lateral elements of the SC, and its function is important for the induction of optimal levels of SPO-11-dependent DSBs and to promote their repair through HR (Janisiw et al. 2020). Furthermore, PARG-1::GFP requires HTP-3 to be properly loaded onto the chromosomes and coimmunoprecipitates with factors localizing along the SC (Janisiw et al. 2020).

In an effort to identify novel interactors of PARG-1 in vivo, we performed GFP-immunoprecipitation on PARG-1::GFP animals followed by mass spectrometry analysis (Janisiw et al. 2020). We observed that PARG-1::GFP coimmunoprecipitated with a phosphorylated form of HTP-3 at Serine 285 (Fig. 1, a and b).

To determine whether HTP-3 is phosphorylated at S285 in vivo, we generated a specific phospho-HTP-3S285 antibody and performed immunofluorescence analysis.

Phospho-specific HTP-3S285 was localized to nuclei in meiotic prophase I (Fig. 1c) and the signal was abolished in the htp-3S285A mutant (Fig. 1d, Supplementary Fig. 1), confirming that the antibody is specific to the phosphorylated form of HTP-3S285.

The expression of phospho-HTP-3S285 overlapped with the HTP-3 expression across the distal-proximal axis of the gonad (Fig. 1, c and e). Careful examination of the two localization patterns revealed that at meiosis onset the pHTP-3S285 staining was rather uneven, relative to total HTP-3; most nuclei displayed a patchy localization of pHTP-3S285, with only a subset of them showing the phospho-staining along the whole length of the chromosome (Fig. 1e, transition zone/Early pachytene). Chromosome axes are established upstream to synopsis and therefore lateral elements such as HTP-3 are loaded earlier than the SYP proteins (Colaiácomo et al. 2003; Couteau et al. 2004; Martinez-Perez and Villeneuve 2005; Goodyer et al. 2008). At this stage, the establishment of the SC occurs gradually (Colaiácomo et al. 2003), thus, we hypothesize that phosphorylation of HTP-3S285 might rely upon the completion of synopsis, which would be consistent with the gradual increase in its loading along the chromosomes (Fig. 2). By the early pachytene stage, the pHTP-3S285 staining was no longer distinguishable from the pan-HTP-3 expression except in the cells undergoing physiological apoptotic cell death, where pHTP-3S285 was absent (Fig. 1e, mid/late-pachytene, dotted circle). Thus, we conclude that HTP-3 is phosphorylated at S285 in vivo during meiotic prophase I.
Phosphorylation of HTP-3S285 occurs independently of synapsis, DSBs, and CO establishment

Assembly of the SC central elements depends on upstream axes morphogenesis and cohesins (Pasierbek 2001; Couteau et al. 2004; Goodyer et al. 2008; Severson and Meyer 2014), and it ensues from loading interdependency amongst its components (SYP-1/6). Thus, the removal of a single member of this protein family blocks the establishment of synapsis (MacQueen et al., 2002; Colaiácovo et al., 2003; Smolikov et al., 2009, 2007; Hurlock et al., 2020; Zhang et al., 2020).

Given the gradual recruitment of p-HTP-3S285 in the nuclei at meiosis entry, we wondered whether HTP-3S285 phosphorylation was dependent on early events that occur during meiotic progression, such as the establishment of synapsis and formation of physiological DSBs, respectively. To assess whether phosphorylation of HTP-3 at Ser285 required the establishment of synapsis, we performed immunostaining analysis of pHPT-3S285 in sypr-2 mutants. We observed that pHPT-3S285 was detectable along chromosomes upon loss of sypr-2 indicating that its localization is independent of SC establishment (Fig. 2).

In C. elegans, SC formation takes place independently of induction of SPO-11-mediated DSBs (Dernburg et al. 1998). Programmed DSB induction relies on the catalytic activity of the topoisomerase-like SPO-11, which exerts its function in combination with several cofactors in worms (Reddy and Villeneuve 2004; Wagner et al. 2010; Meneely et al. 2012; Rosu et al. 2013; Stamper et al. 2013; Hinman et al. 2021). Importantly, deletion of htp-3 impairs meiotic break formation, most likely due to its activity in recruiting the MRN/X complex or perhaps a direct role in promoting loading of SPO-11 itself (Goodyer et al. 2008). Previous studies analyzed RAD-51 foci and indirectly posited that induction of DSBs takes place at early meiotic entry (Alpi et al. 2003; Colaiácovo et al. 2003). To determine whether HTP-3
Phosphorylation is triggered by CO formation, we analyzed the localization of pHTP-3'S285 in spo-11 mutants. However, removal of spo-11 did not affect pHTP-3'S285 localization, suggesting that phosphorylation and localization of HTP-3 are independent of physiological DNA damage (Fig. 2).

The cyclin homolog COSA-1/CNTD1 is essential to convert recombination intermediates into mature COs, and its depletion leads to nearly complete lack of chiasmata in both worms and mice (Yokoo et al. 2012; Holloway et al. 2014). To determine whether HTP-3 phosphorylation is triggered by CO formation, we analyzed its localization in the cosa-1 mutants. We did not observe any obvious aberrations in pHTP-3'S285 localization pattern in the cosa-1 mutants (Fig. 2).

Taken together, these results indicate that phosphorylation of HTP-3 at Ser285 is independent of synopsis and COs, and it does not require SPO-11-mediated DSBs, suggesting that this occurs as an early event at meiosis onset, which most likely takes place either contemporaneously or immediately after axes morphogenesis.

Phosphorylation of HTP-3'S285 is highly redundant
Having confirmed the phosphorylation of HTP-3 at Ser285 in vivo, we then wanted to identify the kinase/s driving this modification.
ERK/MPK-1 phosphorylates HTP-3 in vitro (Das et al. 2020), however, the site has not yet been determined. Therefore, we investigated whether HTP-3 S285 is phosphorylated by ERK2. To test this, we performed an in vitro kinase assay using bacterially expressed HTP-3 WT and HTP-3 S285A proteins, employing active ERK2 enzyme (Fig. 3). We observed that the HTP-3 Ser285Ala mutant protein is phosphorylated by active ERK in vitro (Fig. 3a), suggesting that S285 is a phosphor-acceptor for ERK2.

Next, we performed pHTP-3 S285 staining in kinase mutants known to exert roles during meiotic progression in worms. Specifically, we assayed for the involvement of plk-2, which functions partially redundantly with plk-1 and is known to regulate pairing and synapsis (Harper et al. 2011; Labella et al. 2011); chk-1 and chk-2 mediate the DNA damage response and while chk-1 functions more prominently during mitosis, chk-2 promotes several key meiotic processes in worms, including induction of DSBs and licensing of the SC (MacQueen and Villeneuve 2001; Martinez-Perez and Villeneuve 2005; Kim et al. 2015). Finally, atm-1/ATM and atl-1/ATR function in a partially redundant manner to regulate DNA damage response: atm-1 is critical for mitotic replication and null mutants display complete sterility, while atl-1 mutants are viable, suggesting that the ATM-1 function is not essential during germ cell development (Garcia-Muse and Boulton 2005). Overall, we did not observe loss of pHTP-3 S285 phosphorylation in plk-2(ok1936) mutants (Fig. 3b), atm-1(gk186); atl-1(tm853) double mutants (Fig. 3c), upon RNAi-mediated depletion of chk-1 (Fig. 3d, Supplementary Fig. 2), and auxin-induced degradation of CHK-2 using the chk-2::AID; TIR1::mCherry line (Castellano-Pozo et al. 2020, Fig. 3e). Together, these data suggest that pHTP-3 S285 is not phosphorylated by any of these kinases independently.

Recent work has shown that the 2 cyclin-dependent kinases CDK-1/2 perform crucial functions in the C. elegans germline, by promoting phosphorylation of the SC component SYP-1 and by exerting a direct regulatory function on pro-CO factors, thus promoting chiasmata formation (Brandt et al. 2020; Haversat et al. 2021; Zhang et al. 2021). We tested whether CDK1/2 mediates pHTP-3 S285 phosphorylation using a cdk-1/2—AID-tagged lines, in which degradation of CDK-1/2 was elicited by exposure to auxin. We observed that depletion of either cdk-1 (Fig. 4a) or cdk-2 (Fig. 4b), as well as contemporaneous removal of both kinases (Fig. 4c) did not affect the phosphorylation and localization of HTP-3 S285 suggesting that S285 is likely not a substrate of these 2 kinases. Altogether, these results indicate that none of the major kinases that have been shown to exert important roles during meiotic prophase I in the C. elegans gonad regulate phosphorylation of HTP-3 S285, suggesting that this residue may be recognized by a different kinase/s or subjected to a highly redundant phospho-regulation.
Phosphorylation of HTP-3S285 is dispensable for chiasmata formation

To determine the biological significance of HTP-3S285 phosphorylation, we generated an unphosphorylatable HTP-3 by changing the Serine 285 to Alanine (Supplementary Fig. 3) using CRISPR/Cas9 method. We assessed the hatching rates in the htp-3(S285A) unphosphorylatable mutant worms, as well as monitored the establishment of synopsis and induction/resolution of the recombination intermediates by analyzing RAD-51 dynamics. We found that compared to wild type, there are no defects in either embryonic viability or generation of male progeny (Him phenotype) in the htp-3(S285A) mutant (Fig. 5a), indicating that HTP-3

**Fig. 4.** Phosphorylation of HTP-3S285 does not require cdk-1 and cdk-2. HTP-3S285 phosphorylation in mutant germlines upon loss of cdk-1 (a), cdk-2 (b), or both (c). Scale bar 10 μm.
phosphorylation at this site is not essential to preserve fertility. We also performed viability analysis in worms grown at 25°C, known to induce destabilization of the SC and increased lethality in DNA repair-defective mutants. However, we did not observe any significant differences between WT and htp-3(S285A) mutants (Fig. 5a).

Because removal of pHTP-3(S285) did not cause obvious defects, we wondered whether the phospho-mutant may be sensitive to the lack of factors involved in DSB induction/repair, intersister DSB repair and DNA damage-induced apoptosis. To test this, we generated the htp-3(S285A); parg-1(gk120) and the htp-3(S285A); him-5(ok189) double-mutant backgrounds, respectively (Schumacher et al. 2001; Boulton et al. 2004; Adamo et al. 2008; Meneely et al. 2012; Janisiw et al. 2018, 2020). Lack of parg-1 weakens DSB formation, as its removal from genetic backgrounds bearing suboptimal levels of meiotic breaks exacerbates CO defects. him-5 is important to achieve abundant DSBs and abrogation of its function dramatically impairs the establishment of CO on the chromosome X. Embryonic lethality and male progeny in the htp-3(S285A); parg-1 mutants and wild-type controls. Gonads were divided into 7 equal regions from the mitotic tip to diplotene entry and the number of RAD-51 foci in each nucleus was counted. Bars in the charts indicate mean and standard deviation (ns, nonsignificant. ****P < 0.0001, ***P = 0.0072, **P = 0.047 as calculated by T-test). Insets show representative images of early-pachytene nuclei stained with anti-RAD-51 antibodies in the indicated genotypes. Scale bar 10 μm.

Caenorhabditis elegans brc-1, homolog to mammalian BRCA1, is essential for DSB repair through intersister recombination, and cep-1, homolog to mammalian p53, is involved in DNA damage-induced germ cell death in C. elegans. While single mutants in either brc-1 or cep-1 are viable, double mutants between either of these and genes that affect chiasma formation (e.g. him-14/msh-4) or DSB induction (e.g. him-5) lead to synthetic mutant phenotypes and defects in the resulting progeny (Adamo et al. 2008). Thus, we tested whether there is any synthetic lethal interaction between htp-3(S285A) and brc-1 or cep-1. We observed that the htp-3(S285A); brc-1 and htp-3(S285A) cep-1 double mutants did not differ from the brc-1 and cep-1 single mutants, suggesting that neither intersister-mediated DSB repair nor DNA damage-dependent apoptosis exerts essential functions in htp-3(S285A) phospho-mutant (Fig. 5a).

Additionally, we did not observe any defects in the establishment of synopsis (Fig. 5b) or in the formation of recombination intermediates (Fig. 5c), as indicated by colocalization between panHTP-3 and SYP-1 and comparable number of RAD-51 foci in the htp-3(S285A) mutants as in WT controls. However, we found
Data availability

The data underlying this article are available in the article and in its online supplementary material. All reagents are available upon request.

Supplemental material is available at G3 online.

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Conflicts of interest

None declared.

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