Spatial and temporal control of targeting Polo-like kinase during meiotic prophase

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Polo-like kinases (PLKs) play widely conserved roles in orchestrating meiotic chromosome dynamics. However, how PLKs are targeted to distinct subcellular localizations during meiotic progression remains poorly understood. Here, we demonstrate that the cyclin-dependent kinase CDK-1 primes the recruitment of PLK-2 to the synaptonemal complex (SC) through phosphorylation of SYP-1 in C. elegans. SYP-1 phosphorylation by CDK-1 occurs just before meiotic onset. However, PLK-2 docking to the SC is prevented by the nucleoplasmic HAL-2/3 complex until crossover designation, which constrains PLK-2 to special chromosomal regions known as pairing centers to ensure proper homologue pairing and synopsis. PLK-2 is targeted to crossover sites primed by CDK-1 and spreads along the SC by reinforcing SYP-1 phosphorylation on one side of each crossover only when threshold levels of crossovers are generated. Thus, the integration of chromosome-autonomous signaling and a nucleus-wide crossover-counting mechanism partitions holocentric chromosomes relative to the crossover site, which ultimately defines the pattern of chromosome segregation during meiosis I.

Introduction

The production of haploid gametes during meiosis is achieved by a single round of DNA replication followed by two consecutive nuclear divisions. Unique to meiosis is its protracted prophase I, in which chromosomes pair and synapse with their homologues through the formation of a zipper-like protein structure called the synaptonemal complex (SC; Bhalla and Dernburg, 2008; Page and Hawley, 2004). Concomitantly, meiotic recombination initiates with the generation of programmed DNA double-strand breaks (DSBs; de Massy, 2013). A subset of these breaks is repaired to form crossovers, which, together with sister chromatid cohesion, physically link each pair of homologues. During meiosis I, sister kinetochores are attached by microtubules from the same spindle pole (monoorientation), and the cohesion at centromeres is protected from cleavage (Petronczki et al., 2003). These rearrangements of chromosomes then enable the separation of homologues in meiosis I and later sister chromatids in meiosis II.

Polo-like kinases (PLKs) play widely conserved but diverse roles in regulating the complex chromosome behavior during meiosis. The budding yeast Cdc5 is central to crossover recombination and SC disassembly in late pachytene (Argunhan et al., 2017; Clyne et al., 2003; Sourirajan and Lichten, 2008). It also controls the monoorientation of sister kinetochores (Galander et al., 2019) and stepwise loss of cohesin during meiosis (Attner et al., 2013; Brar et al., 2006; Katis et al., 2010). In Caenorhabditis elegans, a meiosis-specific PLK-2 is localized to special chromosomal regions known as pairing centers and promotes homologue pairing and synopsis (Harper et al., 2011; Labella et al., 2011). PLK-2 subsequently relocates to the SC in pachytene and controls DSB formation, SC disassembly, and meiotic chromosome segregation (Harper et al., 2011; Nadarajan et al., 2017; Sato-Carlton et al., 2018). Similarly, mammalian PLK-1 localizes along the SC and functions in SC disassembly (Jordan et al., 2012).

The multifaceted functions of PLKs raise the question of how PLKs are dynamically targeted to distinct structures during meiotic progression. PLKs are recruited to specific subcellular locations using their Polo-box domain (PBD), which binds a phosphorylated protein through a short peptide motif (S-[pS/pT]-P/X; Elia et al., 2003). Thus, generation of these phosphosites by a priming kinase provides an initial layer of PLK regulation. In C. elegans, recruitment of PLK-2 to pairing centers is primed by CHK-2, a meiosis-specific Ser/Thr kinase, which phosphorylates a family of zinc-finger proteins (HIM-8, ZIM-1, ZIM-2, and ZIM-3; Kim et al., 2015) that bind specific DNA sequences enriched at pairing centers (Phillips and Dernburg, 2006; Phillips et al., 2005). PLK-2 localization to pairing centers is also promoted by the nucleoplasmic protein complex HAL-2/3, although the mechanism of its action remains unclear (Roelens et al., 2019; Zhang et al., 2012). Recent work has...
demonstrated that recruitment of PLK-2 to the SC occurs through binding a PBD-binding motif in the C-terminal domain of an SC component, SYP-1 (Nadarajan et al., 2017; Pattabiraman et al., 2017; Sato-Carlton et al., 2018; Fig. 1, A and B). However, the identity of the upstream kinase responsible for SYP-1 phosphorylation is unknown.

In the holocentric C. elegans, as in all eukaryotic species, crossovers partition the chromosome into the short and long arms and distributes key chromosome-associated proteins asymmetrically relative to the crossover site, eventually defining where cohesion will be released during meiosis I (Altendorfer et al., 2020; Martinez-Perez et al., 2008; Nabeshima et al., 2005). While two axis components, HTP-3 and HIM-3, remain associated with all arms of chromosomes (Zetka et al., 1999; Goodyer et al., 2008; Severson et al., 2009), the other axis-associated proteins, HTP-1/2 and LAB-1, become restricted to the long arm of a bivalent (de Carvalho et al., 2008; Martinez-Perez et al., 2008). The SC components in C. elegans (SYP-1, -2, -3, -4, -5, and -6; Colaiacovo et al., 2003; Hurlock et al., 2020; MacQueen et al., 2002; Smolikov et al., 2007; Smolikov et al., 2009; Zhang et al., 2020) and two meiotic RING finger proteins, ZHP-1/2 (Zhang et al., 2018), are reciprocally restricted to the short arm. The short arm ultimately recruits the Aurora B kinase, AIR-2, in maturing oocytes in a manner dependent on Haspin kinase and the cyclin-dependent kinase CDK-1 (Ferrandiz et al., 2018), which plays an essential role in the releasing of sister chromatid cohesion during meiosis I (Kaitna et al., 2002; Rogers et al., 2002). Recent evidence has shown that the N-terminus of HTP-1/2 is required for localization of LAB-1 to the long arm (Ferrandiz et al., 2018), which in turn recruits protein phosphatase 1 (PP1) to antagonize AIR-2 recruitment (de Carvalho et al., 2008; Tzur et al., 2012). Thus, chromosome remodeling and two-step cohesion loss during meiosis are regulated by the interplay and spatial distribution of kinases and phosphatases in C. elegans.

A key unsolved question is what the initial trigger is that signals the presence of crossovers to alter chromosome architecture. One of the earliest events that differentiate the long versus short arm is the enrichment of PLK-2 on the SC short arm, which mediates axis remodeling and asymmetric SC
disassembly (Harper et al., 2011; Pattabiraman et al., 2017; Sato-Carlton et al., 2018). PLK-2 can also be recruited to the crossover site (Woglar and Villeneuve, 2018), thus making it an ideal candidate to signal the presence of crossovers. However, the mechanism by which PLK-2 is targeted to the crossover site and the SC during meiotic prophase remains poorly understood.

Here, we demonstrate that CDK-1 directs the recruitment of PLK-2 to the SC through phosphorylation of SYP-1. We also identify mechanisms that prevent precocious association of PLK-2 with the SC in early meiotic prophase to ensure proper axis assembly, homologue pairing, and synopsis. Interestingly, the spatial and temporal control of asymmetric PLK-2 localization is provided by the enrichment of SYP-1 phosphorylation on the short arm, which requires PLK-2 kinase activity itself and threshold levels of crossover designation within the nucleus. Thus, the integration of chromosome-autonomous signaling and a nucleus-wide crossover-counting mechanism drives the spatial partitioning of holocentric chromosomes relative to the chromosome axis is impaired, which is also restored by removal of SYP proteins (Zhang et al., 2012). We observed a similar rescue in localization of PLK-2 in the absence of pairing centers using a strain (ieDf2) that lacks all four pairing center proteins (HIM-8/ZIMs; Harper et al., 2011). In agreement with a recent report (Roelens et al., 2019), PLK-2 was immediately localized along phosphorylated SYP-1 stretches in ieDf2 animals (Fig. 2 A), which has previously been shown to represent intrachromosomal fold-back synopsis within unpaired chromosomes (Harper et al., 2011). Thus, the SC can recruit PLK-2 in the absence of pairing centers. When PLK-2 docking sites were deleted on both pairing centers and the SC by introducing the SYP-1 T452A mutation in the ieDf2 background, PLK-2 was largely detected in the nucleoplasm (Fig. 2 B). Therefore, pairing centers and the SC are the major PLK-2 docking sites available at the onset of meiosis; however, PLK-2 is preferentially targeted to pairing centers in early prophase.

Interestingly, we noticed that many short SC stretches appear in ieDf2; syp-1T452A animals (Fig. 2, B and C), suggesting that the fold-back synopsis in ieDf2 animals might be due to precocious association of PLK-2 with the SC. Indeed, SYP proteins existed as multiple small aggregates and exhibited a delay in forming linear stretches in ieDf2; plk-2 double mutants (Fig. 2 C), supporting the conclusion that uncontrolled action of PLK-2 on the SC causes premature loading of SYP proteins between nonhomologous chromosomes.

Recruitment of PLK-2 to pairing centers is primed by CHK-2–dependent phosphorylation of the pairing center proteins (Kim et al., 2015). In CHK-2–depleted animals, phosphorylation of SYP-1 T452 was diminished in early meiotic prophase, and PLK-2 was found in the nucleoplasm (Fig. S1 C). Nevertheless, robust phospho-signals for SYP-1 T452 were detected on SC stretches later in the germline, which ultimately recruited PLK-2 (Fig. S1 C). Targeting of PLK-2 to pairing centers also depends on the nucleoplasmic HAL-2/3 complex, which functions to promote full activation of CHK-2 and to antagonize the localization and activity of PLK-2 in the nucleoplasm (Roelens et al., 2019; Zhang et al., 2012). In hal-2 mutants, PLK-2 remained largely nucleoplasmic as previously reported (Roelens et al., 2019), and SYP-1 T452 phosphorylation was detected along the SC (Fig. S2 A), which has been shown to assemble onto unpaired chromosome axes (Zhang et al., 2012). Thus, PLK-2 docking sites on the SC are generated independently of CHK-2 and HAL-2/3.

Previous evidence has shown that defects in nuclear reorganization and homologue pairing in hal-2 mutants are rescued by removal of SYP proteins (Zhang et al., 2012). We reasoned that this might be due to the loss of PLK-2 binding to the SC such that PLK-2 is then free to localize to pairing centers, restoring the nuclear reorganization required for homologue pairing. To test this hypothesis, we combined the null allele of hal-2 with the T452A mutation in SYP-1 and examined the localization of PLK-2. Strikingly, bright PLK-2 foci were detected at the X chromosome pairing center in hal-2; syp-1T452A mutants (Fig. 3 A), which largely rescued the pairing of X chromosomes (Fig. 3, A and B). In hal-2 mutants, loading of HTTP-1/2 onto the chromosome axis is impaired, which is also restored by removal of SYP proteins (Zhang et al., 2012). We observed a similar rescue in chromosomal localization of HTTP-1/2 in hal-2; syp-1T452A.
mutants throughout the germline (Fig. 3 C), indicating that a key function of the HAL-2/3 complex is to prevent precocious interactions between the SC and PLK-2 in early prophase. However, PLK-2 was rarely detected at the autosomal pairing centers in hal-2; syp-1T452A mutants, and the SYP-1 T452A mutation only partially restored the crescent-shaped nuclear morphology characteristic to the leptotene/zygotene stage (Fig. 3 A). We attribute this to the requirement of HAL-2/3 in full activation of CHK-2 (Roelens et al., 2019). Phosphorylation of HIM-8 by CHK-2 occurs more robustly than for the ZIM proteins at the autosomal pairing centers (Kim et al., 2015). Thus, the X chromosome pairing centers are more likely to recruit PLK-2 when CHK-2 activity is reduced in hal-2; syp-1T452A mutants. The SYP-1 T452A mutation also did not rescue the failure to form crossovers in hal-2 mutants (Fig. S2, B and C), consistent with the requirement of CHK-2 in generation of meiotic DSBs (MacQueen and Villeneuve, 2001). Taken together, our results demonstrate that constrained PLK-2 activity at the pairing centers is essential for proper axis assembly, homologue pairing, and synapsis.

**Asymmetric enrichment of SYP-1 phosphorylation requires the kinase activity of PLK-2 and threshold levels of crossover designation**

Phosphorylation of SYP-1 T452 is robustly detected upon meiotic entry in the absence of PLK-2 (Sato-Carlton et al., 2018), indicating that PLK-2 is not the kinase responsible for the initial SYP-1 T452 phosphorylation. However, the phospho-signal for SYP-1 was significantly weakened on chromosome arms after crossover designation in plk-2–null animals, suggesting that PLK-2 is necessary for enforcing its own binding to the SC (Sato-Carlton et al., 2018). To test whether PLK-2 kinase activity is required, we generated a worm strain expressing a kinase-dead form of PLK-2 (K65M; Link et al., 2018). The K65M mutation was semi-dominant, as only ∼30% of embryos from the heterozygous
animal were viable (Fig. S2 D), suggesting that the presence of PLK-2K65M may interfere with the ability of PLK-1 to compensate for PLK-2 functions (Harper et al., 2011; Labella et al., 2011). Animals homozygous for the plk-2K65M mutation exhibited more aggravated defects, further reducing the embryo viability to \( \sim 6\% \). The kinase-dead PLK-2 localized normally to the nuclear envelope in the premeiotic region and to pairing centers in early prophase (Fig. 4 A). However, the kinase-dead PLK-2 precociously spread to SC stretches in early pachytene (Fig. S3, A and B), hinting that PLK-2 kinase activity is required for sustaining its association with the pairing centers. Consistent with the results from plk-2-null animals, the initial phosphorylation of SYP-1 T452 was robustly detected in plk-2K65M mutants (Fig. S3 C). However, this phospho-signal was reduced from chromosome arms after crossover designation, and both SYP-1 phosphorylation and PLK-2 were concentrated at crossover sites in plk-2K65M animals (Fig. 4, A and B; and Fig. S3 C). Thus, we conclude that PLK-2 kinase activity is required to enforce asymmetric phosphorylation of SYP-1 T452.

Enrichment of PLK-2 to the SC short arm depends on crossover formation, and this is important for chromosome remodeling required for homologue separation during meiosis I (Nadarajan et al., 2017; Pattabiraman et al., 2017; Sato-Carlton et al., 2018). Thus, we investigated how SYP-1 T452 phosphorylation is regulated by crossover designation. In crossover-defective cosa-1 mutants (Yokoo et al., 2012), SYP-1 T452 phosphorylation persisted along the SC, and yet PLK-2 was not recruited (Fig. S3 D). We also examined SYP-1 phosphorylation in dsb-2 mutants, in which formation of meiotic DSBs is substantially reduced, leading to crossover designation only on a few chromosomes (Rosu et al., 2013). Consistent with PLK-2 localization in dsb-2 mutants (Pattabiraman et al., 2017), phosphorylation of SYP-1 T452 was detected along chromosomes harboring COSA-1, while it was lost from chromosomes that had failed to designate crossovers (Fig. 5...
Interestingly, we observed that the asymmetric appearance of PLK-2 and SYP-1 phosphorylation correlates with the number of crossover-designated sites within each nucleus. While most nuclei with two to three COSA-1 foci exhibited symmetric distribution of SYP-1 phosphorylation along the chromosome length (87% in nuclei with two COSA-1; 58% in nuclei with three COSA-1), the majority of nuclei with four to five COSA-1 foci showed asymmetric SYP-1 phosphorylation on one side of each crossover (56% in nuclei with four COSA-1; 69% in nuclei with five COSA-1; Fig. 5, B and C). Thus, signals from crossover-designated sites are transmitted in cis to enrich for SYP-1 phosphorylation, and asymmetric remodeling of chromosomes requires a threshold level of crossover designation within the nucleus.

CDK-1 is responsible for SYP-1 phosphorylation at T452

We next sought to identify the upstream kinase responsible for the phosphorylation of SYP-1 T452. Docking sites for PLKs often overlap with consensus phosphorylation motifs for CDKs (Archambault and Glover, 2009), and SYP-1 T452 is indeed an excellent substrate for recombinant human CDK-1 in vitro (Fig. S1 A). Thus, we hypothesized that a CDK might phosphorylate SYP-1 to prime the recruitment of PLK-2 to the SC.

Recent evidence has shown that CDK-1 controls the recruitment of AIR-2 to the short arm (Ferrandiz et al., 2018), raising the possibility that CDK-1 might play a role in chromosome remodeling upon crossover designation. Thus, we tagged the endogenous CDK-1 with a small epitope Ollas at its C-terminus and examined its expression by immunofluorescence. Consistent with its potential functions in the germline, CDK-1 was robustly expressed in nuclei at all stages of meiotic prophase (Fig. S4 A). We first tested its significance by RNAi. The knockdown efficiency was determined to be >85% by comparing the lysates of RNAi-treated worms to serially diluted control samples in a Western blot (Fig. S4 B). Animals treated with cdk-1 RNAi

Figure 4. The kinase activity of PLK-2 is required for enrichment of SYP-1 T452 phosphorylation along chromosomes that have designated crossovers. (A) Composite immunofluorescence images of whole gonads dissected from wild-type and plk-2K65M hermaphrodites and stained for PLK-2::3Flag. Scale bar, 50 µm. (B) Immunofluorescence images of late pachytene nuclei from wild-type and plk-2K65M mutants showing PLK-2::3Flag (red), COSA-1 (white), and SYP-1 pT452 (green) staining. Scale bar, 5 µm.
displayed fewer enlarged nuclei in the premeiotic region, a phenotype characteristic of endoduplication after failed mitotic entry (Edgar and Orr-Weaver, 2001), and defects in chromosome condensation in diplotene (Fig. S4 C). Importantly, knockdown of cdk-1 completely abolished SYP-1 T452 phosphorylation (Fig. S4 C), demonstrating that CDK-1 is required for the phosphorylation of SYP-1 T452.

We also employed the auxin-inducible degradation (AID) system (Zhang et al., 2015) to confirm the requirement for CDK-1 in SYP-1 phosphorylation. To this end, we inserted the AID tag at the N-terminus of endogenous CDK-1 in a genetic background that expresses the plant F-box protein TIR1 from the germline-specific sun-1 promoter (Fig. 6 A). Self-progeny of this worm strain was largely viable (98% egg viability; Fig. S2 D), indicating that the AID tag does not interfere with CDK-1 functions. Consistent with the RNAi results, fewer enlarged nuclei were observed in the premeiotic region of CDK-1-depleted germlines (Fig. S4, D and E). Depletion of CDK-1 did not alter the recruitment of PLK-2 to pairing centers (Fig. S5, A and B), nor did it affect synopsis and crossover formation (Fig. 6 B), as germlines lacking CDK-1 displayed the requisite six COSA-1 foci on average in late pachytene and six DAPI-staining bodies at diakinesis (Fig. 7, A–C). However, phosphorylation of SYP-1 T452 was completely eliminated in CDK-1-depleted animals (Fig. 6 C), corroborating the conclusion that CDK-1 is responsible for phosphorylating SYP-1 at T452.

CDK-1 is required for targeting PLK-2 to the SC short arm and for chromosome remodeling

As a consequence of the failure to phosphorylate SYP-1 T452 in CDK-1-depleted germlines, PLK-2 was unable to localize to the SC even after crossover designation (Fig. 7 A and Fig. S5, A and B).
In addition, PLK-2 was no longer observed at the nuclear periphery in the premeiotic region of these animals (Fig. S5 A), indicating that CDK-1 is also responsible for targeting PLK-2 to the nuclear envelope, similar to the PLK-1 regulation in mitotic prophase (Linder et al., 2017; Martino et al., 2017). Interestingly, recruitment of PLK-2 to crossover sites was also abolished when CDK-1 was depleted by auxin (Fig. 7 A), while this was not observed in *cdk-1* RNAi-treated animals (Fig. S5 C). We attribute this to a stronger CDK-1 depletion by the AID system and conclude that CDK-1 primes the recruitment of PLK-2 not only to the SC, but also to the crossover site.

Recruitment of PLK-2 to the SC short arm is essential for SC disassembly and axis remodeling in late prophase (Harper et al., 2011; Sato-Carlton et al., 2018). In the wild type, HTP-1/2 was removed from the short arm in diplotene (Martinez-Perez et al., 2008), whereas HIM-3 and HTP-3 remained on all chromosome arms (Goodyer et al., 2008; Zetka et al., 1999). In CDK-1-depleted animals, however, HTP-1/2 persisted on the short arm (Fig. 7, C and D), and SC disassembly was delayed until diakinesis (Fig. S5 D). Thus, CDK-1 is required for targeting PLK-2 to the SC during meiotic prophase, which drives the asymmetric remodeling of holocentric chromosomes. While chromosomes were continually compacted in the wild type to eventually yield the cruciform-shaped bivalent structure, chromosomes in CDK-1-depleted oocytes appeared diffuse and lacked distinct bivalent structure in diakinesis (Fig. 7, C and E). This is distinct from the phenotypes observed in *syp-1T452A* animals and likely reflects the additional requirement of CDK-1 in chromosome condensation (Bazile et al., 2010).

**Discussion**

The dynamic localization of PLK-1 during mitosis is largely controlled by the interaction between the PBD within PLK-1 to
a short peptide motif that has been phosphorylated by CDK-1 (Archambault and Glover, 2009). Here, we demonstrate that this general paradigm also applies to PLK-2 targeting during meiosis in *C. elegans*. Previous work using RNAi had shown a requirement for CDK-1 in germ cell mitosis; however, it did not reveal its functions during meiotic prophase (Boxem et al., 1999). We have now established that CDK-1 primes the recruitment of PLK-2 to the nuclear envelope in the premeiotic region as well as to crossover sites and the SC in pachytene, thereby driving the chromosome remodeling essential for proper chromosome segregation during meiosis I (Fig. 8).

Our evidence shows that two major PLK-2 docking sites are generated in early meiotic prophase: (1) the SC, which is primed by CDK-1-dependent phosphorylation of SYP-1, and (2) the pairing centers, which are primed by CHK-2-dependent phosphorylation of HIM-8/ZIMs (Kim et al., 2015). Interestingly,
PLK-2 is preferentially targeted to the pairing centers, where it acts locally to drive homologue pairing and synapsis (Harper et al., 2011; Labella et al., 2011). Uncontrolled action of PLK-2 on the SC is deleterious, as exemplified in iedF2 and hal-2 mutants, leading to erroneous loading of SYP proteins between unpaired chromosome axes (Harper et al., 2011; Roelens et al., 2019; Zhang et al., 2012). Remarkably, mutating the PBD-binding motif on SYP-1 rescues PLK-2 targeting to the pairing centers in hal-2 mutants, which restores HTP-1/2 loading, homologue pairing, and synapsis (Fig. 3). Therefore, combined with its requirement for full activation of CHK-2 (Roelens et al., 2019), the HAL-2/3 complex ensures the recruitment of PLK-2 to the pairing centers by preventing premature association of PLK-2 with chromosome axes (Harper et al., 2011; Labella et al., 2011). Uncontrolled action of PLK-2 might phosphorylate SYP-1 and reinforce its own docking sites after crossover formation, similar to the self-priming of PLK-1 recruitment to the central spindle in late mitosis (Neef et al., 2007). However, SYP-1 T452 does not conform to the consensus motif for PLK phosphorylation, and PLK-2 was unable to phosphorylate SYP-1 T452 in our in vitro kinase assays (data not shown). Therefore, we think it more likely that PLK-2 activity is required to preserve SYP-1 phosphorylation through an indirect mechanism affecting the turnover of SYP proteins and/or by sustaining the activity of CDK-1 through phosphorylating its upstream regulators, such as CDC25 phosphatases or WEE1 kinases (Gheghiani et al., 2017; Watanabe et al., 2004).

Our evidence corroborates the previous findings that crossover-designated sites transmit a signal to recruit PLK-2 and stabilize the SC in cis. This change in the dynamic state of the SC has been shown to depend on PLK-2 (Machovina et al., 2016; Nadarajan et al., 2017; Pattabiraman et al., 2017), which retains SYP-1 phosphorylation on chromosomes harboring crossovers. We have now shown that the symmetry breaking of SYP-1 phosphorylation and PLK-2 recruitment requires a threshold level of crossover designation within the nucleus. In nuclei with sub-threshold crossovers, SYP-1 phosphorylation is retained along the length of chromosomes that have designated crossovers, while it is lost from chromosomes without crossovers. On the other hand, SYP-1 phosphorylation and PLK-2 are enriched...
on the SC short arm when sufficient numbers of crossovers (more than four) are generated (Fig. 5 B). We speculate that crossover-designated recombination intermediates nucleate and compete for limiting factors (e.g., PLK-2) to stabilize the SC in a chromosome-autonomous manner. Sub-threshold crossovers would recruit high levels of such SC-stabilizing factors, which then spread along the entire length of chromosomes by re-inforcing SYP-1 phosphorylation. As previously suggested (Pattabiraman et al., 2017), this may also deplete the soluble pools of PLK-2 or SYP proteins below the critical concentration required to maintain the SC on chromosomes without crossovers, leading to their desynapsis (Machovina et al., 2016). As crossover designation arises above the threshold, the concentration of SC-stabilizing factors recruited to each chromosome will be decreased. Then the self-reinforcing mechanism for PLK-2 targeting and SYP-1 phosphorylation might yield a faster equilibrium and their accumulation on the SC short arm.

An alternative explanation for the asymmetric enrichment of SYP-1 phosphorylation is the presence of an opposing phosphatase that antagonizes PLK-2 and dephosphorylates SYP-1 on the long arm. PPI is recruited to the long arm by HTP-1/2 and LAB-1 to antagonize the recruitment of AIR-2 in maturing oocytes (de Carvalho et al., 2008; Ferrandiz et al., 2018). Thus, we explored the possibility that PPI might be responsible for dephosphorylating SYP-1 from the long arm upon crossover designation. However, both SYP-1 phosphorylation and PLK-2 localization exhibited the asymmetric appearance relative to the crossover-designated site in lab-1(tm791) or PPI.GSP-1/2-depleted animals (data not shown), ruling out the contribution of PPI to symmetry breaking in pachytene. Taken together, our evidence suggests that differentiation of the short versus long arms is controlled by intrinsic properties of PLK-2, which is targeted to chromosomes harboring crossovers and whose effects depend on the number of crossover-designated sites within the nucleus. Thus, the integration of nucleus-wide and chromosome-autonomous signaling partitions the holocentric chromosome relative to the crossover site, which ultimately defines the pattern of chromosome segregation during meiosis I (Fig. 8).

Materials and methods

C. elegans strains and CRISPR-mediated genome editing

All strains were maintained at 20°C following standard protocols (Brenner, 1974). N2 Bristol was used as the wild-type strain. The following mutations and balancers were used: iaDQ4 IV, hal-2(tm4960) III, dbo-2(me96) II, cosa-1(tm3298) III, plk-2(tm1395) I, lab-1(tm791), hT2 [bli-4(e937) let-7(q782)] qsl48 (I,III), qCI[dp3-19(e1259) glp-1(a339)] II, and ml51[myo-2p::GFP + pes-10p::GFP + F22B7::GFP] IV.

The strain expressing the kinase-dead PLK-2 (K65M) was generated by CRISPR-mediated genome editing as previously described (Dokshin et al., 2018). Young adult YKM490 [plk-2[kim23[plk-2::3FLAG]]] I; mels8 [pie-1p::GFP::cosa-1 + unc-119+] II; iseI8 [sun-1p::TIR1::mRuby::sun-1 3’UTR + Cbr-unc-119+] IV was injected with 16 µM Cas9 protein complexed with 16 µM tracrRNA/cRNA oligos (IDT), pCFJ104 (5 ng/µl; Addgene #19328), pCFJ90 (2.5 ng/µl; Addgene #19327), and an ssDNA oligo (100 ng/µl; IDT) as a repair template. The Ollas tag was inserted at the 3’ end of the endogenous cdk-1 coding sequence with a Gly–Ser linker (GGATCG). The AID tag and a Gly–Gly–Ser–Gly linker (GAGGCTCAGGA) were inserted at the 5’ end of the endogenous cdk-1 in two chunks using ssDNA oligos. F1 progeny were lyzed and screened for successful insertion by PCR, and the correct insertion was validated by sequencing.

For testing the contribution of PPI, we generated a worm strain expressing GSP-1::AID and/or GSP-2::AID by CRISPR. YKM490 [plk-2[kim23[plk-2::3FLAG]]] I; mels8 [pie-1p::GFP::cosa-1 + unc-119+] II; iseI8 [sun-1p::TIR1::mRuby::sun-1 3’UTR + Cbr-unc-119+] IV was injected with 16 µM Cas9 protein complexed with 16 µM tracrRNA/cRNA oligos (IDT), pCFJ104 (5 ng/µl; Addgene #19328), pCFJ90 (2.5 ng/µl; Addgene #19327), and two gBlock fragments (50 ng/µl; IDT) for gsp-1::AID and gsp-2::AID as a repair template. F1 progeny were lyzed and screened for successful insertion by PCR, and the correct insertion was validated by sequencing.

Antibody production

A synthetic phosphopeptide of SYP-1 flanking T452 (SAPLMTStPTLAATRC; Biomatik) was coupled to the maleimide-activated keyhole limpet hemocyanin (KLH, Thermo Scientific) and injected into rabbits (Pocono Rabbit Farm & Laboratory). Polyclonal SYP-1 PT452 antibodies were affinity purified by passing the immune serum through SulfoLink Coupling Resins (Thermo Scientific) coupled to nonphosphopeptide (SAPLMTStPTLAATRC) and subsequently binding to phosphopeptide-coupled resins. Specificities of the antibodies were tested by dot blots using phospho- and nonphosphopeptides, by in vitro kinase assays using recombinant MBP- SYP-1 and CDK-1/cyclin A, and by staining dissected gonads from animals expressing SYP-1T452A.

Protein expression and purification

The full-length open reading frame of SYP-1 was amplified from a C. elegans cDNA library and cloned into the pMAL vector (New England BioLabs) to express maltose-binding protein (MBP)-tagged SYP-1 fused to a 6His tag. To make MBP-SYP-1T452A-6 His, the Thr at residue 452 was mutated to Ala (ACA → GCA) by Q5 mutagenesis (New England BioLabs). Protein expression was induced at 15°C for ~16 h with 50 µM IPTG in Rosetta (DE3) pLysS. Bacterial pellets were resuspended in lysis buffer (PBS,
0 m M NaCl, 20 m M imidazole, and 1 m M DTT) and lysed by
three freeze/thaw cycles and sonication after lysozyme treat-
ment (0.25 mg/mL) on ice for 30 min. After centrifugation at
15,000 rpm (JA-17) for 30 min, the supernatant was incubated
with nickel-nitritrotiacetic acid resins (Qiagen) for 1 h at 4°C. The
protein was further purified by HiTrap SP HP (GE Healthcare)
using a 100 m M to 1 M NaCl gradient elution in PBS with
1 m M DTT. The peak fraction for MBP–SYP-1 was supplemented
with glyc erol (20% final) and snap frozen in liquid nitrogen.

In vitro kinase assays
In vitro kinase assays were performed at room temperature in
20 m M Hepes, pH 7.4, 25 m M KCl, 1 m M MgCl2, and 1 m M DTT
in the presence of 0.2 m M MgATP. 2 µM wild-type and T452A
mutant MBP–SYP-1–His were incubated with 20 U of recom-
binant human CDK-1/cyclin A (Sigma SRP5008), and the re-
actions were stopped at the indicated time points with sample
buffer and analyzed by Western blot.

RNAi

cdk-1 RNAi was performed by feeding YKM154 (cdk-1::Ollas III)
animals with the Escherichia coli strain HT115 (DE3), either car-
rying an Ahringer RNAi library clone of cdk-1 (Kamath et al.,
2003) or the empty L4440 vector. Concentrated bacterial cul-
tures were plated onto RNAi plates (NGM, 25 µg/mL carbeni-
cillin, and 1 m M IPTG), left for 1 h at room temperature to dry,
and incubated overnight at 37°C to induce expression. L4 her-
maphrodites were picked onto RNAi plates and left for 48 h
before dissection for immunofluorescence.

Auxin-mediated degradation of CDK-1 and GSP-1/2 (PP1
orthologues)

Auxin-mediated degradation of CDK-1 and GSP-1/2 from the C.
elengas germline was performed as previously described (Zhang
et al., 2015). Briefly, auxin plates were prepared by diluting a
400 m M auxin solution (indole-3-acetic acid in ethanol) into the
NGM, cooled after autoclaving, to a final concentration of 1 m M.
Plates were allowed to dry at room temperature and were stored
at 4°C for up to 1 mo. Plates were spread with the E. coli strain
OP50-1 1 d before use and incubated overnight at 37°C. Young adult
animals with and without the Pnm::TRI::mRuby trans-
gene were picked onto auxin plates and left for 18 h at 20°C before
immunofluorescence. For PPGSP-1/2 depletion, worm strains
expressing GSP-1::AID and/or GSP-2::AID were treated with 1 m M
auxin for 24 h at 20°C before immunofluorescence.

Immunofluorescence

Hermaphrodite germlines were dissected from 24 h post-L4
adults in egg buffer (25 m M Hepes, pH 7.4, 118 m M NaCl,
48 m M KCl, 2 m M EDTA, 5 m M EGTA, 0.1% Tween-20, and
15 m M Na2S) and fixed in 1% formaldehyde before freezing in
liquid nitrogen. Dissected germlines were further fixed in
methanol at –20°C for 1 min and rehydrated with PBS with 0.1% 
Twee n-20. Samples were then blocked with blocking reagent
(Roche 11096176001) for 1 h and incubated with primary anti-
obodies overnight at 4°C. Primary antibodies were used at the
following dilutions in blocking buffer (Roche 11096176001) in
PBS with 0.1% Tween-20: FLAG (mouse, 1:500; Sigma M2), Ollas
(rat, 1:500; Thermo Fisher MA5-16125), SY P-1 (goat, 1:500;
MacQueen et al., 2005), SY P-1 pT452 (rabbit, 1:500), SY P-5
(rabbit, 1:1,000; Hurlock et al., 2020), HTP-3 (guinea pig, 1:500;
MacQueen et al., 2005), HTP-3 (chicken, 1:500; Hurlock et al.,
2020), GFP Booster (1:200; Chromotek gb2AF488), HTP-1/2 (rab-
bit, 1:500; Martinez-Perez et al., 2008), HIM-8 (rat, 1:500; Phillips
et al., 2005), and phospho-HIM-8/ZIMs (rabbit, 1:1,000; Kim
et al., 2015). The following secondary antibodies were pur-
chased from Invitrogen or Jackson ImmunoResearch and used
at 1:200 dilution: donkey anti-rabbit Alexa Fluor 555, donkey
anti-rabbit Alexa Fluor 647, donkey anti-mouse Alexa Fluor
488, donkey anti-mouse Alexa Fluor 555, donkey anti-guinea
pig fluorescein, donkey anti-goat Alexa Fluor 647, and donkey
anti-rat Alexa Fluor 594.

Fixed slides were imaged at room temperature on a Delta-
Vision Elite system (GE Healthcare) with an Olympus 100 × 1.4
NA oil-immersion objective and a scientific complementary
metal-oxide semiconductor camera (PCO). 3D image stacks were
collected at 0.2-µm intervals, processed by iterative deconvolu-
tion (enhanced ratio, 20 cycles), and projected by the Volume
Viewer tool using SoftWoRx suite (GE Healthcare). Composite
images were assembled and colored in Adobe Photoshop.

Statistical analysis

Statistical analysis was performed using Graphpad Prism 8. For
the analysis HIM-8 pairing (Fig. 3B), a χ2 test was used to de-
termine the differences between wild-type and mutant animals
within individual gonad zones. For the analysis of SY P-1 pT452
asymmetry (Fig. 5B), a two-tailed Fisher’s exact test was used to
compare the proportion of nuclei exhibiting either symmetric or
asymmetric SY P-1 pT452 staining. For the analysis of the
number of COSA-1 foci (Fig. 7B), number of DAPI bodies (Fig. S2
C), and number of cell rows in the premeiotic region (Fig. S4 E),
P values were calculated using a two-tailed Mann-Whitney U
test, which is a nonparametric statistical analysis. For all tests
performed, the number of data points (n) is included at the base
of the graph.

Online supplemental material

Fig. S1 shows validation of the SY P-1 pT452 antibody and
SY P-1 pT452 staining in a CHK-2-depleted germline. Fig. S2
shows images and quantification showing the number of
DAPI-staining bodies in diakinesis oocytes from the wild-
type, hal-2, and hal-2; syp-1T452A mutants, and a table show-
ing egg viability and percent males from strains generated in
this study. Fig. S3 shows PLK-2 and SY P-1 pT452 staining in
plk-2Δ65SM and cosa-1 mutants. Fig. S4 shows the level of CDK-
1 knockdown and the abolishment of SY P-1 pT452 staining
upon cdk-1 RNAi treatment. Fig. S5 shows the impact of CDK-
1 depletion on PLK-2 localization and SC disassembly. Table S1 lists the alleles generated in this study. Table S2 lists the crRNAs, repair templates, and prototyping primers for mutant alleles generated in this study. Table S3 lists the worm strains used in this study.

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Figure S1. Phospho-specificity of the SYP-1 pT452 antibody used in this study, and the analysis of SYP-1 T452 phosphorylation and PLK-2 localization in chk-2 mutants. (A) In vitro kinase assays using human CDK1/cyclin A to phosphorylate MBP-SYP-1 with or without the T452A mutation. Western blot using antibodies against SYP-1 and phosphorylated SYP-1 at T452 is shown. (B) Composite immunofluorescence images of a whole gonad dissected from syp-1T452A mutants showing DNA, SYP-1, and SYP-1 pT452 staining. Scale bar, 50 µm. (C) Composite immunofluorescence images of a whole CHK-2–depleted gonad showing DNA (white), SYP-1 (red), SYP-1 pT452 (green), and PLK-2::3Flag staining. Scale bar, 50 µm.
Figure S2. The SYP-1 T452A mutation does not rescue the failure to form crossovers in hal-2 mutants. (A) Composite immunofluorescence images of a whole gonad from hal-2 mutants showing DNA, SYP-1 pT452, and PLK-2::3Flag staining. Scale bar, 50 µm. (B) Oocyte nuclei at diakinesis from indicated genotypes were stained with DAPI. Scale bar, 3 µm. (C) Graph showing the number of DAPI-staining bodies in oocytes at diakinesis (mean ± SD). Numbers of nuclei scored are indicated (n = 45 for wild type; n = 37 for hal-2; n = 41 for hal-2; syp-1T452A). ns, not significant (P > 0.05) by two-tailed Mann-Whitney test. (D) Table showing the percent viable and male progeny from C. elegans hermaphrodites of indicated genotypes.
Figure S3. Enrichment of SYP-1 phosphorylation on the short arm requires PLK-2 kinase activity and crossover formation. (A and B) Zoomed-in images of nuclei from leptotene/zygotene (A) and early pachytene (B) showing PLK-2::3Flag (red) and phospho-HIM-8/ZIMs (green) staining. Scale bars, 5 µm. (C) Composite immuno-fluorescence images of a whole gonad dissected from the plk-2(K65M) (kinase dead) homozygote animal showing DNA (blue), COSA-1 (yellow), and SYP-1 pT452 staining (white). Scale bar, 50 µm. (D) Late-pachytene nuclei from wild-type and cosa-1(tm3298) mutants were stained for DNA, SYP-1 (red), SYP-1 pT452 (green), and PLK-2 (magenta). Scale bar, 5 µm. Diagrams illustrating the results are shown on the right.
Figure S4. Knockdown of CDK-1 by RNAi abolishes the phosphorylation of SYP-1 at T452 and abrogates PLK-2 targeting to the SC. (A) Composite immunofluorescence image of a full-length gonad dissected from a worm strain expressing CDK-1::Ollas. Scale bar, 50 µm. (B) Western blots showing the knockdown of CDK-1 by RNAi. The level of CDK-1::Ollas in cdk-1(RNAi) worms was compared with serially diluted worm lysates from control animals. Tubulin (TBA-1) was used as a loading control. Molecular weights for both proteins are indicated on the right. (C) Composite immunofluorescence images of a full-length gonad from a cdk-1(RNAi)-treated worm showing DNA (white), SYP-1 (red), and SYP-1 pT452 staining (green). Asterisks indicate the distal tip of the germline. Scale bar, 50 µm. (D) Immunofluorescence images of distal germlines from control and CDK-1-depleted animals showing DNA (blue) and HIM-3 (red) staining. Asterisks indicate the distal tip of the germline, and dotted lines indicate the meiotic entry. Scale bar, 50 µm. (E) Graph showing the number of cell rows in the premeiotic region in control versus CDK-1-depleted germline. Mean ± SD is shown. Numbers of gonads scored are indicated on the bottom (n = 6 for control; n = 7 for CDK-1 depleted). **, P < 0.01 by two-tailed Mann-Whitney test.
Figure S5. Depletion of CDK-1 in the germline by AID system leads to failures in targeting PLK-2 to distinct subnuclear structures. (A) Full-length gonads dissected from control versus CDK-1–depleted animals were stained for DNA and PLK-2::3Flag. Scale bar, 50 µm. (B) Zoomed-in images of the boxed regions in A showing DNA (blue) and PLK-2 (red) staining from control and CDK-1–depleted germlines during leptotene/zygotene. Scale bar, 5 µm. (C) Immunofluorescence of late-pachytene nuclei from wild-type, cdk-1(RNAi), and CDK-1–depleted worms showing SYP-1, PLK-2 (red), and COSA-1 (green). Scale bar, 5 µm. (D) Composite immunofluorescence images of diakinesis nuclei from control versus CDK-1–depleted germline showing DNA (blue) and SYP-1 (red). Scale bar, 5 µm.
There are three tables available online as Word documents. Table S1 lists the alleles generated in this study. Table S2 lists the crRNAs, repair templates, and genotyping primers for mutant alleles generated in this study. Table S3 lists the worm strains used in this study.