akirin is required for diakinesis bivalent structure and synaptonemal complex disassembly at meiotic prophase I

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ABSTRACT During meiosis, evolutionarily conserved mechanisms regulate chromosome remodeling, leading to the formation of a tight bivalent structure. This bivalent, a linked pair of homologous chromosomes, is essential for proper chromosome segregation in meiosis. The formation of a tight bivalent involves chromosome condensation and restructuring around the crossover. The synaptonemal complex (SC), which mediates homologous chromosome association before crossover formation, disassembles concurrently with increased condensation during bivalent remodeling. Both chromosome condensation and SC disassembly are likely critical steps in acquiring functional bivalent structure. The mechanisms controlling SC disassembly, however, remain unclear. Here we identify akir-1 as a gene involved in key events of meiotic prophase I in Caenorhabditis elegans. AKIR-1 is a protein conserved among metazoans that lacks any previously known function in meiosis. We show that akir-1 mutants exhibit severe meiotic defects in late prophase I, including improper disassembly of the SC and aberrant chromosome condensation, independently of the condensin complexes. These late-prophase defects then lead to aberrant reconfiguring of the bivalent. The meiotic divisions are delayed in akir-1 mutants and are accompanied by lagging chromosomes. Our analysis therefore provides evidence for an important role of proper SC disassembly in configuring a functional bivalent structure.

INTRODUCTION Meiosis is a specialized cell division that leads to the formation of haploid gametes, which in metazoans are the sperm and egg cells. It involves two cellular divisions after one cycle of replication; in the first (MI), homologous chromosomes segregate away from each other, and in the second (MII), sister chromatids separate

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Abbreviation used SC, synaptonemal complex.
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that physically connects homologous chromosomes in meiotic prophase I to mediate synopsis (Zickler and Kleckner, 1999). The SC is composed of two lateral elements (composed of axis-associated proteins) connected by central region proteins that form a zipper-like structure. This results in a tripartite complex that holds homologous chromosomes together until crossovers are formed. Crossovers then trigger SC disassembly along with condensation, which allows the visual manifestation of the crossover event—the chiasma (Schvarzstein et al., 2010). Thus the SC is a prerequisite for functional bivalent formation; however, it is unclear whether this is due simply to the requirement for synopsis in general or whether the SC and its regulated disassembly directly affect bivalent structure.

In Caenorhabditis elegans late meiotic prophase I, each bivalent is reconfigured around the single off-centered crossover as an asymmetric cruciform structure, containing long and short arms (Schvarzstein et al., 2010). The short arms of the bivalent are the sites at which connections between homologous chromosomes will be removed at MI (Rogers et al., 2002), whereas the long arms maintain associations between sister chromatids until the onset of MI. Thus proper chromosome segregation relies on the restructuring of chromosomes into an asymmetric and tightly condensed cruciform structure. In C. elegans, the most significant condensation occurs during mid to late prophase. Of the three condensin complexes (condensin I, condensin II, and condensin DC), only the condensin II complex is required for the remodeling of the bivalent into the cruciform structure (Chan et al., 2004). Although chromosome condensation and disassembly of the SC occur concurrently, it was shown that these two processes are independent of each other in C. elegans (MacQueen et al., 2002; Colaiacovo et al., 2003; Chan et al., 2004; Smolikov et al., 2007, 2009). Thus any effect SC disassembly might have on the organization of the bivalent is condensin independent. However, it has not been fully tested whether aberrant SC disassembly has implications for bivalent
gonads. High-magnification images of DAPI-stained (blue) and SYP-1– (red, C–L) or HIM-3–stained (red, N–S) nuclei of

FIGURE 1: SC disassembly defects observed in akir-1 mutants are specific to central region proteins. (A) Schematic representation of chromosome behavior in meiosis, highlighting key events in SC disassembly. Chromosomes are in blue. Central region proteins /SYPs are in red, and axis and axis-associated proteins are in green and yellow. (B) Schematic representation of the predicted AKIR-1 protein. The α-helical domain is in blue. The region of the protein deleted in the gk528 mutant allele is indicated (underlined amino acids 1–91, 42% of the protein). Half of the predicted promoter is also removed by gk528 but is not shown here. The red letter H indicates the position of the histidine mutated to proline in the position of the histidine mutated to proline in the rj1 allele. (C–L) akir-1 mutants exhibit SC disassembly defects observed in akir-1 mutants at this stage exhibit similar levels of association of SYP-1 and axes at diakinesis –1 and diakinesis –3.

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structure, as known mutants affecting this process affect only a few of its aspects (Ivanovska et al., 2005; Malmanche et al., 2007; Bhalla et al., 2008; de Carvalho et al., 2008; Martinez-Perez et al., 2008; Sourirajan and Lichten, 2008; Jordan et al., 2009; Peretz et al., 2009; Resnick et al., 2009). This includes mutants affecting the timing of disassembly or altering the subchromosomal pattern of SC proteins, none of which perturb SC function by retaining its ability to connect homologous chromosomes.

To identify new players in chromosome behavior in meiosis, we performed a forward genetic screen in C. elegans, using SC disassembly defects as a functional readout. We identified AKIR-1, a novel C. elegans orthologue of fly and mouse Akirins, a gene family with no previously described role in meiosis. Here we present data suggesting that AKIR-1 supports the efficient removal of SC proteins from the chromosomes. In akir-1 mutants, early meiotic events such as SC assembly and crossover formation occur normally. However, late prophase I events such as SC disassembly, chromosome condensation, and bivalent restructuring are severely perturbed. We show that akir-1 is required to form a compact, cruciform-shaped bivalent structure and acts in both SC-dependent and SC-independent manners to influence chromosome behavior. Thus the akir-1 phenotype shows that aberrant accumulation of SC proteins on chromosomes is linked to disrupted bivalent structure and perturbed meiotic divisions. Together, these data 1) identify the first known role for an akirin gene in meiosis in any organism, 2) indicate a new link between SC disassembly and chromosome structure in meiosis, and 3) suggest that regulated mechanisms exist to prevent aberrant accumulation of SC proteins after their removal from chromosomes during SC disassembly.

RESULTS

Isolation of the worm orthologue of akirin

We isolated akir-1(rj1) in a forward genetic screen aimed at identifying mutants that exhibit defects in SC disassembly (see Materials and Methods). Using a combination of single-nucleotide polymorphism (SNP) mapping (Davis et al., 2005) and genome-wide sequencing techniques, we found that rj1 resulted in a missense mutation (H190P) in E01A2.6. This is an uncharacterized open reading frame (ORF) that is germline expressed (Wang et al., 2009), encoding a protein of 218 amino acids that lacks any known domains (Figure 1B) but is evolutionarily conserved among metazoans. We named E01A2.6 akir-1 to adhere to the nomenclature established in other species (AKIRIN; Macqueen and Johnston, 2009). AKIR-1 has one orthologue in Drosophila, which is 31% identical and 47% similar to the C. elegans protein (MN168211). Vertebrates have two akirin genes, the protein products of which both share homology to the worm protein (∼30% identity and ∼50% similarity; MN001007589, BC082305). The analysis presented here is focused on the meiotic role of worm akirin and does not exclude mitotic or somatic functions for this gene, which are not examined here.

Mutation of worm akirin resulted in two phenotypes apparent in late meiotic prophase I: 1) failure in the timely removal of SC proteins from chromosomes, leading to their aggregation, and 2) delay in the condensation of chromosomes (Figure 1, D–G and I–L; diakinesis –3 and –1 are the third and first oocytes, respectively, before conclusion of meiotic prophase I). Both of these phenotypes are associated with an aberrant bivalent structure found in these mutants (Figure 1, D–G, inset). These defects were observed in every gonad dissected from worms containing either one of two akir-1 alleles—rj1 (the point mutant isolated in our screen) and gk528 (a deletion mutant, obtained from the C. elegans Gene Knockout Consortium, Oklahoma Medical Research Foundation)—as well as in 91% of gonads (n = 87) dissected from akir-1(RNAi) worms. The akir-1(rj1) allele contains a histidine to proline substitution in position 190 (Figure 1B). This substitution is expected to result in a severe conformational change by disrupting the predicted α-helical structure of this conserved region (Cole et al., 2008). The akir-1(gk528) deletion removes most of the promoter and coding sequence and is therefore expected to be a null. The molecular nature of the two alleles, their phenotypic similarity, and their phenocopy by RNA interference (RNAi) suggest that they are both loss-of-function alleles.

akir-1 mutants exhibit embryonic lethality that is maternally contributed

Both akir-1 alleles exhibit moderate levels of embryonic lethality (Supplemental Table S1, 21% in akir-1(rj1) and 16% in akir-1(gk528) mutants; p < 0.001, Fisher’s exact test). We also observed a modest increase in the number of males (0.6% in akir-1(rj1) and 0.9% in akir-1(gk528), compared with <0.2% in wild type, significant for akir-1(gk528), p = 0.009, Fisher’s exact test). The contribution to embryonic lethality is maternal, as akir-1(gk528) or akir-1(rj1); fem-2(b245); gk528) mated to wild-type males show similar levels of embryonic lethality to that observed in self-progeny of the hermaphrodite akir-1(gk528) (Supplemental Table S1, p = 0.34, p = 0.19, Fisher’s exact test). Whereas early meiotic events (such as pairing, synapsis, and recombination) are similar in males and females, late meiotic events (such as apoptosis, chromosome condensation, and SC disassembly) are differentially regulated in the two sexes of C. elegans (Shakes et al., 2009). Moreover oocytes, but not sperm, are loaded with maternally contributed proteins, some of which are crucial for development. All of these may account for the maternally contributed embryonic lethal phenotype of akir-1 mutants. We also found a reduction in the number of eggs laid (eggs per worm [average ± SD]: wild type, 256 ± 45; akir-1(rj1), 46 ± 22; akir-1(gk528) 75 ± 24), which is also maternally contributed.

akir-1 is required for the removal of central region proteins, but not lateral element/axis proteins, from chromosomes

SC proteins begin to load onto chromosomes during SC assembly and crossover formation normally. However, late prophase I events such as SC disassembly, chromosome condensation, and bivalent restructuring are severely perturbed. We show that akir-1 is required to form a compact, cruciform-shaped bivalent structure and acts in both SC-dependent and SC-independent manners to influence chromosome behavior. Thus the akir-1 phenotype shows that aberrant accumulation of SC proteins on chromosomes is linked to disrupted bivalent structure and perturbed meiotic divisions. Together, these data 1) identify the first known role for an akirin gene in meiosis in any organism, 2) indicate a new link between SC disassembly and chromosome structure in meiosis, and 3) suggest that regulated mechanisms exist to prevent aberrant accumulation of SC proteins after their removal from chromosomes during SC disassembly.

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However, SYP-1 proteins exhibited aggregated localization (Figure 1, D–G). Quantification of SYP-1 signal intensity suggested that these aggregates contain almost triple the amount of SYP-1 protein observed in wild-type cells (2.9 times SYP-1 signal increase: wild type, akir-1(gk528), n = 24, 42 patches/aggregates, p < 0.001 by the two-tailed Mann–Whitney test, 95% confidence interval). Diakinesis –1 is the position of the last oocyte before fertilization/conclusion of meiotic prophase I, and in wild-type gonads this oocyte consistently lacks any central region/SYP staining (Figure 1H; Nabeshima et al., 2005). Remarkably, SYP-1 disassociation from chromosomes was delayed in akir-1 mutants, as SYP-1 was still present in all diakinesis –1 nuclei although absent from wild-type nuclei (Figure 1, H–L). A large fraction of these aggregates was found to associate with chromosomes (Figure 1M, blue). The extended localization of SYP proteins into diakinesis –1, as well as their aggregation, indicated defects in the removal of SC proteins from chromosomes in akir-1 mutants.

Overall, fewer nuclei were at the stage of diakinesis in akir-1 mutants compared with wild type (wild type, 8.3 ± 1.6; akir-1(1j1), 3.6 ± 1; akir-1(gk528), 6.3 ± 1.6; n = 111, 30, 101 gonads, p < 0.001 for both, Mann–Whitney test). This also correlated with a decrease in the overall size of the gonad in akir-1 mutants (Supplemental Figure S2). Of importance, the reduced number of diakinesis nuclei does not contribute to condensation, and bivalent structure) because other mutants that exhibit reduced numbers of diakinesis nuclei still show wild-type chromosome structure at diakinesis and normal SC disassembly (Supplemental Figure S4). Hence, although we cannot fully rule out the possibility that the reduced number of diakinesis nuclei is a result of slower progression of meiosis in akir-1 mutants (which is specific to the diplotene–diakinesis stage), it is likely not causal to the phenotypes described here. Moreover, the effect of akir-1 on meiosis is unlikely to be due to transcriptional changes of SYP-1, as SYP-1 mRNA levels are not altered in akir-1 mutants (levels normalized to myo-3 control: wild type, 0.89±0.01, and akir-1(1j1), 0.87 ± 0.02 [average ± SD]). This is consistent with the established findings that germline genes are translationally regulated (Merritt et al., 2008) and that transcription is repressed in late meiotic prophase I in C. elegans (Walker et al., 2007), the stage in which the akir-1 mutant phenotypes are observed.

The central region of the SC in C. elegans is composed of four SYP proteins, which exhibit similar localization patterns, interdependent localization, and indistinguishable phenotypes of null mutants (MacQueen et al., 2002; Colaiacovo et al., 2003; Smolikov et al., 2007, 2009). In addition to the SYPs, five axis-associated proteins (HIM-3, HTP-3, HTP-1/2, and LAB-1) localize to the SC throughout meiotic prophase I. By light microscopy, axis-associated proteins exhibit similar localization patterns to central region proteins during pachytene. We hypothesized that the akir-1 mutant would exhibit similar defects in SC disassembly for all four SYP proteins but that loss of AKIR-1 might not disrupt other SC proteins. Indeed, the localization of SYP-2, SYP-3, and SYP-4 showed aggregation upon SC disassembly, identical to that observed for SYP-1 (Supplemental Figure S5 and unpublished data). Containing with antibodies against SYP-1 and SYP-3 revealed that both colocalized to the same aggregates in the akir-1(1j1) mutant (Supplemental Figure S5, M–O). Therefore, all central region proteins mislocalize in akir-1 mutants in a similar manner and are likely to associate with each other in the same aggregate. Unlike central region proteins, the localization of axis-associated proteins in akir-1 mutants was indistinguishable from wild type in both the timing and the pattern of localization. Axis-associated proteins were distributed linearly along the arms of the diakinesis bivalent in akir-1 mutants, unlike the aggregated localization of the SYPs; HIM-3 and HTP-3 associated with both diakinesis bivalent arms (Figure 1, O–P, R, and S, and unpublished data), and LAB-1 and HTP-1/2 (Figure 2, B and D, and unpublished data) associated exclusively with the long arm of the diakinesis bivalent. Thus aberrant SC protein localization is limited to the central region proteins in akir-1 mutants.

AKIR-1 is required for short-arm resolution

A wild-type SC structure, before its disassembly, contains a tripartite organization: SYPs flanked by axial proteins, where SYPs serve as axial connectors (Schild-Prüfert et al., 2011). In C. elegans, disassembly of the SYPs from the short versus the long arm of the bivalent is asymmetric (Nabeshima et al., 2005). SYPs are promptly removed from the long arm upon initiation of SC disassembly starting at late pachytene, whereas they remain associated with the short arm until the transition to diakinesis –1 (for model, see Figure 1A). This progressive asymmetric disassembly process is proposed to secure the resolution of all four chromosomal arms and the formation of a cruciform bivalent.

We examined whether the defects in the disassembly of the SYPs are specific to the short and/or the long arm of the bivalent in akir-1 mutants. After crossover formation, SYP proteins no longer localize along the long arm of the bivalent, whereas LAB-1 and HTP1/2 are retained at this location. Conversely, SYP proteins remain associated along the short arm, whereas LAB-1 and HTP1/2 are no longer associated with this region of the bivalent (de Carvalho et al., 2008; Martínez-Perez et al., 2008). If SYPs were properly removed from the long arm of the bivalent in akir-1 mutants, we would expect that 1) SYP-1 and LAB-1 would localize to distinct domains, and 2) SYP-1 would localize terminally to a crossover marker (since crossovers mark the boarders of the LAB-1/SYP-1 domains at this stage). We found that, as in wild type, SYP-1 and LAB-1 do not colocalize in diakinesis in akir-1 mutants (Figure 2, B and D). Moreover, SYP-1 aggregates acquired terminal (92% of foci, n = 66; Figure 2, F–F) rather than central positioning relative to a crossover marker (ZHP-3, the C. elegans orthologue of the yeast ZIP3, marks crossovers cytologically, forming one focus on each diakinesis bivalent; Jantsch et al., 2004; Bhalla et al., 2008). This indicates that SYP-1 localization is restricted to one side of the crossover normally in akir-1 mutants, which suggests that AKIR-1 is dispensable for removal of SYPs from the long arm of the bivalent.

Analysis of SYP-1 and HIM-3 localization along the short arm of the bivalents revealed that whereas in wild type, SYP-1 localization is no longer observed on this chromosome domain in late diakinesis, the HIM-3 signal is observed overlapping with the borders of the SYP-1 aggregate in 94% of the akir-1–mutant diakinesis bivalents (n = 66; Figure 2G). Thus we hypothesize that in akir-1 mutants SYPs may persist in connecting homologous chromosomes beyond the normal point of SC disassembly, therefore perturbing short-arm resolution.

To examine this hypothesis, we tested whether SYP-1 aggregates were capable of connecting chromosomes in a scenario in which other means of chromosomal association, such as crossovers, were absent. SPO-11 is required for the generation of meiosis-specific double strand breaks (DSBs) and therefore for crossover formation (Dernburg et al., 1998). Its absence results in patchy localization of SYP-1 on the 12 univalents formed, with no restriction to a particular side of the chromosome (Nabeshima et al., 2005; Figure 2I). akir-1(RNAi) in the spo-11(ok79) mutant genetic background resulted in formation of SYP-1 aggregates (Figure 2, K and O). These SYP-1 aggregates in akir-1(RNAi); spo-11(ok79) mutants were
interchromosomal distances

these interchromosomal aggregates significantly decreased the distance between 4',6-diamidino-2-phenylindole (DAPI) bodies

frequently found between pairs of chromosomes (Figure 2, K and O); these interchromosomal aggregates significantly decreased the distance between 4',6-diamidino-2-phenylindole (DAPI) bodies (almost twofold at diakinesis –3; see Table 1 for data and Supplemental Table S2 for statistics). This chromosomal association is dependent on the presence of an aggregate, as 1) chromatin (DAPI stained) bridges were not observed between pairs of chromosomes (Figure 2, K' and O'; the space occupied by the aggregates connecting the bivalents does not contain chromatin); 2) the frequency of associations was reduced as chromosomes progressed through meiosis and aggregates disassociated from chromosomes (percentage of nuclei with aggregation: 85% of diakinesis –3 nuclei, n = 26; 37% of diakinesis –1 nuclei, n = 27; p < 0.001, Fisher's exact test); and 3) the association was dependent on the presence of SYP proteins (akir-1(RNAi); syp-2(ok307); spo-11(ok79) interchromosomal distances were larger than that of akir-1(RNAi); spo-11(ok79) mutants and indistinguishable from that of syp-2(ok307) and spo-11(ok79) single or double mutants (see Table 1 for data and Supplemental Table S2 for statistics). Thus, in akir-1 mutants, SYP-1 aggregates represent a form of SC that retains the property of connecting homologous chromosomes at a stage when this attribute is lost from wild-type oocytes. Of interest, in some species the SC can substitute for lack of chiasmata throughout meiosis, similarly to what is found in akir-1(RNAi); spo-11(ok79) mutants in the diakinesis stage (de la Fuente et al., 2012). These data are consistent with a role for AKIR-1 in the resolution of the short arms of the bivalent, in addition to its role in the timely removal of SC proteins from chromosomes.

AKIR-1 is not involved in chromosome pairing or crossover formation

HTP-1 and -2 affect chromosome pairing in addition to their role in promoting proper SC disassembly (de Carvalho et al., 2008; Martinez-Perez et al., 2008). Therefore, we tested whether AKIR-1 is involved in chromosome pairing during meiotic prophase I. To examine the progression of homologous chromosome pairing, we performed fluorescence in situ hybridization (FISH) for the 5S locus (a central position on chromosome V). Pairing in both wild type and akir-1 mutants was initiated as nuclei entered meiosis (zone 3, transition zone), peaked as nuclei progressed to the pachytene region (zone 4), and was maintained at high levels throughout meiotic prophase I (Figure 3H). There was no statistically significant reduction in meiotic pairing in either of our mutants. These results indicate that AKIR-1 is not required for either the establishment or the maintenance of pairing interactions and that the synopsis observed is homologous (unlike what was found in htp-1 or

FIGURE 2: In akir-1 mutants, SC disassembles aberrantly from the short arm of the bivalent, whereas the long arm is not affected. (A–D) Central region and axis-associated proteins do not colocalize in akir-1 mutants. (E–F') Central region proteins are localized terminally to a marker for crossover site. (G) Tripartite SC in akir-1 mutant diakinesis bivalents. Right, schematic representation of the images. (H–O') SYP-1 aggregates are present in crossover-deficient backgrounds that lack AKIR-1. These aggregates associate pairs of univalents. DAPI-only channels (K, L) show lack of chiasma between the univalents connected by the SYP-1 aggregate. High-magnification images of nuclei of gonads from age-matched wild type (A, A', C, G left, H, L), akir-1(rj1) mutants (F), akir-1(gk528) mutants (B, B', D, G right), akir-1(RNAi) (J, N), spo-11(ok79) (I, M), and akir-1(RNAi); spo-11(ok79) (K, K', O, O') adult hermaphrodites at diakinesis –3 (A–B', E–G, H–K) and diakinesis –1 (C, D, L–O'). All gonads were stained with DAPI (blue or white) and SYP-1 (red). Those in A–D were stained with LAB-1 (green), those in G with HIM-3 (green or white), and those in E and F contained GFP::ZHP-3 fusion protein (green). A' and B' are zoomed-in images of the chromosomes marked by an arrow in A and B (all three channels on the left; antibody-only channel on the right). (F') Quantitative analysis of the various types of localization patterns observed in akir-1(rj1) mutants for ZHP-3 (green) and SYP-1 (red). Images are full projections of a single nucleus (A–F, H–O') or a single bivalent (G). Bars, 1 μm (G), 2 μm (A–F, H–O').

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SC disassembly is coordinated with crossover formation in wild-type nuclei (Nabeshima et al., 2005). Thus defects in SC disassembly could be secondary to perturbations of crossover formation. In addition, a subset of mutants that affect asymmetric disassembly (lab-1 and htp-2) affect crossover formation as well (de Carvalho et al., 2008; Martinez-Perez et al., 2008). We thus performed experiments to examine the possibility that the defects observed in akir-1 mutants were secondary to defects in crossover formation.

RAD-51 is a strand-exchange protein required for DSB repair, acting downstream of DSB formation (Alpi et al., 2003). RAD-51 forms foci on meiotic prophase I chromosomes (Colaiacco et al., 2003), which are a good indicator of the levels of DSBs in the C. elegans germline (Mets and Meyer, 2009). Increases in the levels of RAD-51 foci frequently correlate with defects in repair of DSBs through homologous recombination (MacQueen et al., 2002; Colaiacco et al., 2003; Carlton et al., 2007; Smolikov et al., 2007, 2008, 2009). We counted the RAD-51 foci per nucleus in the gonads of akir-1 mutants compared with wild type and found no significant increase at the regions where DSB repair occurs (zones 5–7; Figure 3, A–E, and Supplemental Table S3). Moreover, as in wild type, RAD-51 foci are not found in diplote and diakinesis. Zones 4 and 5 show a slight decrease in RAD-51 foci; however the level of DSBs is high enough to support proper crossover formation (see later discussion). Apoptosis levels are somewhat elevated in akir-1 mutants compared with wild type (wild type, 3.72, n = 109; akir-1(gk528), 4.98, n = 125; p = 0.002, Mann–Whitney test). However, this increase is much lower than those observed in synapsis-defective mutants (3.5-fold increase for syp-2(ok307) to 13.15, n = 71, Mann–Whitney test). Therefore, it is likely that DSB repair is proficient in akir-1 mutants.

In akir-1 mutants, chromosomes still form bivalent-like, albeit highly abnormal, structures at diakinesis, as the number of DAPI-stained bodies observed in diakinesis is indistinguishable from wild type (wild type, 5.9 ± 0.3; akir-1(g1), 5.8 ± 0.5; akir-1(gk528), 5.9 ± 0.3; n = 41, 45, 38 nuclei, p = 0.52, p = 0.61, Mann–Whitney test). Nevertheless, the pattern of SC disassembly can be influenced by the number of crossovers (Martinez-Perez et al., 2008), and diakinesis bivalents are not always an indication of a mature crossover (Saito et al., 2009). Therefore, we examined whether the number of crossovers is altered in akir-1 mutants. We tested this by two methods: the cytological analysis of ZHP-3, a protein that localizes to the sites of crossovers (sex per nucleus; Bhalla et al., 2008); and the direct examination of crossover frequencies using SNPs. We observed no difference in the number of ZHP-3 foci in akir-1 mutants compared with wild type (Figure 3, F and G; akir-1(g1)/+, 5.9 ± 0.7; akir-1(g1), 5.6 ± 0.8; n = 37, 60 nuclei, p = 0.12, Mann–Whitney test). Moreover, akir-1(gk528) mutants show no change in crossover frequencies (Table 2) compared with wild type. These data indicate that as in wild type, homologues are being held by crossovers in akir-1 mutants. Taken together, our analysis shows that AKIR-1 acts downstream from crossover formation and is likely not involved in early aspects of prophase I chromosome dynamics.

**akir-1 is required for proper diakinesis bivalent structure independent of condensin**

A major event taking place in late prophase I is chromosome condensation. Chromosome morphology in akir-1 mutants resembles that of wild type until diakinesis (Supplemental Figures S2A and S3; DAPI). As nuclei enter diakinesis, their bivalents exhibit two structural defects: 1) atypical diakinesis bivalent shape (Figures 1, D–G, inset, and 4, A and B) and 2) elongated chromosome arms (Table 3 and Figure 4, A and B).

In C. elegans, bivalent remodeling around a single crossover results in a cruciform structure with a small gap in the interface between the arms (Figure 4A, top). In akir-1 mutants, diakinesis bivalents acquired an atypical shape (Figure 4A, bottom), including defects in the size of the gap region (Figure 4B). Half of the diakinesis bivalents of akir-1 mutants acquired an aberrant cruciform shape (Figure 4A; p < 0.0013, Fisher’s exact test, compared with wild type). This included an atypical arm positioning with detached axes for both homologues, a morphology that was never observed for wild-type diakinesis bivalents. In many cases this resulted in very little interface shared between each of the chromosome pairs, with only a thin chromatin thread connecting the two homologues. Moreover, diakinesis bivalents with wild-type cruciform structure frequently exhibited an enlarged gap region, almost twice as long in these mutants compared with wild type (Figure 4B, right). Because these structural defects are present in the region occupied by the SYB-1 aggregate, this suggests that SYB-1 aggregation may be a cause for this aspect of the diakinesis bivalent structural defect. To test this

**TABLE 1: Distance between DAPI-staining bodies measured at diakinesis –1 and –3.**

|                      | Average (μm) | Fold over wild type | n  |                      | Average (μm) | Fold over wild type | n  |
|----------------------|--------------|---------------------|----|----------------------|--------------|---------------------|----|
| Wild type            | 0.19         | —                   | 60 |                      | 0.23         | —                   | 60 |
| akir-1(RNAi)         | 0.3          | 1.6                 | 60 |                      | 0.29         | 1.3                 | 60 |
| akir-1(RNAi), spo-11 | 0.8          | 4.2                 | 60 |                      | 0.42         | 1.8                 | 60 |
| syp-2                | 1.37         | 7.2                 | 90 |                      | 1.1          | 4.8                 | 84 |
| spo-11               | 1.18         | 6.2                 | 114|                      | 0.8          | 3.5                 | 102|
| syp-2, spo-11        | 1.13         | 5.9                 | 72 |                      | 0.77         | 3.3                 | 72 |
| syp-2, akir-1        | 1.26         | 6.6                 | 84 |                      | 0.86         | 3.7                 | 84 |
| akir-1(RNAi), syp-2  | 1.12         | 5.9                 | 72 |                      | 0.66         | 2.9                 | 72 |

All pairwise comparisons between wild type, akir-1, and akir-1; spo-11 and all other mutants is statistically significant (Mann–Whitney test; see Supplemental Table S2 for p values). n is the number of distances measured (Materials and Methods).
Although length in effects or are independent of SC function. We examined chromosome these defects are simply the outcome of earlier SC disassembly de

**TABLE 2:** Crossover frequencies are not altered in **akir-1** mutants.

|                        | Nonrecombinant chromosomes | Recombinant chromosomes | Map distance (cM) |
|------------------------|----------------------------|-------------------------|-------------------|
| Wild type              | 133                        | 61                      | 31.4              |
| **akir-1**<sub>(gk528)</sub> | 114                        | 50                      | 30.5              |

Snip-SNP analysis revealed no significant change in crossover frequencies on chromosome II in **akir-1** mutants compared with wild type (Fisher’s exact test, \( p = 0.42 \)). SNPs used were pkP2101 and uCE2-2131.

**TABLE 2:** Crossover frequencies are not altered in **akir-1** mutants.
### FIGURE 4: In akir-1 mutants, chromosome structure is affected.

akir-1 mutants exhibit altered chromosome structure; 50% of bivalents lack the typical cruciform structure (A), increase in the size of the gap between the chromosomal arms (B), and increase in diakinesis bivalent length. (A) Chromosomes were stained with axial marker (HIM-3) and imaged at diakinesis –1. All three channels are shown on the left, DAPI only in the middle, and the antibody-only channel on the right. “Other” represents bivalents that do not belong to any of the categories indicated that were hard to score due to their positioning in the nucleus. (B, left) Schematic representation of a diakinesis bivalent, with wild type expected configuration on the left and mutant on the right. The gap is indicated with an arrow, and only forward-facing diakinesis bivalents were analyzed. (B, right) Length of the region between homologous chromosomes is in blue, the length of bivalents, which are longer and contain a larger gap. (B, right) Schematic representation of a diakinesis bivalent, with wild type expected configuration on the left and mutant on the right. The gap is indicated with an arrow, and only forward-facing diakinesis bivalents were analyzed. (B, right) Length of the region between homologous chromosomes is in blue, the length of bivalents, which are longer and contain a larger gap.

### Table 1: Summary of Diakinesis Bivalent Analysis

| Structure | Wild type | akir-1(gk528) | akir-1(rj1) |
|-----------|-----------|---------------|-------------|
| Cruciform-Front View | 57% | 30% | 27% |
| Cruciform-Side View | 31% | 11% | 23% |
| Detached Axis | 12% | 9% | 149 |

### akir-1 mutants are largely proficient at AIR-2 and P-H3 recruitment, as well as at mitogen-activated protein kinase activation

The last events in meiotic prophase I involve oocyte maturation, the transition from diakinesis and metaphase of meiosis I. Oocyte maturation is supported by the activation of mitogen-activated protein kinase (MAPK) via its dephosphorylation (Miller et al., 2001). Concurrently, in diakinesis –1 oocytes, AIR-2 is recruited to chromosomes (Schumacher et al., 1998; Rogers et al., 2002) to mediate histone H3 phosphorylation (P-H3; Hsu et al., 2000) on the sister chromatids of the short arm of the bivalent, the site of cohesion removal at MI (mid–bivalent region; Nabeshima et al., 2005; de Carvalho et al., 2008). We examined whether these events occur properly in akir-1 mutants.

In akir-1 mutants, AIR-2 and P-H3 localized to the mid–bivalent region, as observed in wild type (Figure 6, A–J). However, in contrast to wild-type nuclei (Figure 6, D and G), P-H3 failed to colocalize with the chromosome-associated SYP-1 in akir-1 mutants (Figure 6, E, F, and H–J). In these chromosomes, P-H3 was positioned in close proximity to the chromosomal arms, whereas SYP-1 was found further away from them (Figure 6J). The activated form of MAPK was recruited to chromosomes (Schumacher et al., 1998) on the sister chromatids of the short arm of the bivalent, the site of cohesion removal at MI (mid–bivalent region; Nabeshima et al., 2005; de Carvalho et al., 2008). We examined whether these events occur properly in akir-1 mutants.

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oocytes, n = 60; Figure 6K) as well as akir-1 mutants (72% of diakinesis –1 oocytes, n = 97; Figure 6L), indicating proficient oocyte activation. All mutant diakinesis –1 oocytes contained SYP aggregates and condensation-impaired bivalents. Therefore, SC disassembly and condensation defects are not caused by the inability to activate oocytes in akir-1 mutants. Of interest, whereas no diakinesis –1 oocytes in wild-type worms lacked P-H3 staining (n = 53), a significant fraction of akir-1 mutant oocytes had no P-H3 staining (diakinesis –1 oocytes: akir-1(rj1), 25%; akir-1(gk528), 10%; n = 57, 58 oocytes; p < 0.001, p = 0.028, Fisher's exact test). These numbers are similar to the percentage of oocytes that do not show MAPK activation in akir-1 mutants.

**AKIR-1 is required for proper meiotic divisions**

akir-1 mutants exhibit increased embryonic lethality, which could be due to errors in the meiotic divisions or to postmeiotic events. Such errors could include defects in the formation of the meiotic spindle. To test this, we performed live imaging, as well as examined fixed samples, of akir-1(rj1) mutants containing histone H2B fused to mCherry (to visualize chromosomes) and α-tubulin fused to GFP (TBA-2, to visualize the spindle; Wignall and Villeneuve, 2009). akir-1(rj1) mutants formed a distinctive meiotic spindle (Figure 7, B and D; normal spindle: MI, 98%; MII, 100%; n = 46, 44 fertilized oocytes) with no apparent deviations (p = 1, Fisher's exact test) from the wild-type spindle structure (Figure 7, A and C; normal spindle: MI, 99%; MII, 98%; n = 69, 43 fertilized oocytes). Embryonic lethality may result from defects in the segregation of chromosomes at either one, or both, of the meiotic divisions. As expected, no segregation defects were observed in wild-type fertilized oocytes during both meiotic divisions (Figure 7, E and G; n fertilized oocytes at MI, MII = 28, 21). However, akir-1 mutants exhibited a high frequency of aberrant chromosomal segregation patterns at MI (Figure 7, F and F', and Supplemental Table S4; akir-1(rj1), 23%, n = 28, 31 MI fertilized oocytes, wild type vs. mutants, p = 0.001, Fisher's exact test) and MII (Figure 7, H and H', and Supplemental Table S4; akir-1(rj1), 33%, n = 21, 24 MII fertilized oocytes, wild type vs. mutants, p = 0.004, Fisher's exact test). These defects included lagging chromosomes, misaligned chromosomes, and, to a much lesser extent, condensation defects. No chromosome bridges were observed. Because both divisions are similarly impaired in akir-1 mutants, this could be attributed to defects in sister chromatid cohesion. However, defects in loading the cohesin complex are probably not the cause for these phenotypes, since immunostaining for REC-8, one of the meiotic-specific cohesin proteins (Pasierbek et al., 2001; Severson et al., 2009), localized to gonadal nuclei in akir-1 mutants indistinguishably from wild-type nuclei (Supplemental Figure S8).

The aberrant chromosomal segregation patterns may explain the ~20% of embryonic lethality and the very weak Him phenotype (indicative of X chromosome nondisjunction) in the akir-1 mutants. However, measurements of chromosome nondisjunction (Supplemental Table S5) indicate that surviving embryos do not show high levels of nondisjunction (this assay cannot exclude low levels and/or few chromosomes involved in nondisjunction). It is possible that the lagging and misaligned chromosomes observed do not result in

| Diakinesis –3 | Length (μm) of a diakinesis bivalent (n) | Diakinesis –1 | Length (μm) of a diakinesis bivalent (n) | Fold change over wild type |
|---------------|----------------------------------------|---------------|----------------------------------------|---------------------------|
| Wild type     | 2.7 (48)                               | 3.18 (48)     | NA                                     |                          |
| akir-1(RNAi)  | 4.08 (48)                              | 4.45 (48)     | 1.5, 1.4                               |                          |
| hcp-6(mr17)   | 3.11 (48)                              | 3.81 (48)     | 1.2, 1.2                               |                          |
| akir-1(RNAi); hcp-6(mr17) | 4.49 (48) | 6.31 (48) | 1.6, 2.0 |                          |
| dpy-28(s939)  | 2.81 (42)                              | 3.27(42)      | 1.0, 1.0                               |                          |
| akir-1(RNAi); dpy-28(s939) | 3.93 (54) | 4.91 (48) | 1.4, 1.5 |                          |

Length of a diakinesis bivalent is an accumulative length of both arms (for details of measurement see Materials and Methods). All values are statistically significant from each other (Mann-Whitney test p < 0.001), with the following exceptions: wild type to dpy-28(s939) and akir-1(RNAi) to akir-1(RNAi); dpy-28(s939), which are not significantly different.

**TABLE 4:** Chromosome length measured at diakinesis –1 and diakinesis –3.

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embryos with nondisjunction since they are repaired before the completion of each meiotic division. If this is so, meiotic lagging/misaligned chromosomes will either halt or delay the meiotic divisions, which allows the repair of their misalignment. To test this, we analyzed the timing of both meiotic divisions using live imaging. Our findings indicate that both MI and MII are about twice as long in akir-1 mutants as in wild type (A–H) but not with condensin I/I' mutants (I–P). SYP-1 aggregates are formed and fail to disassemble at the end of meiotic prophase I in akir-1 mutants but not in condensin mutants. High-magnification images of stained nuclei in gonads from age-matched wild type (A, E, I, M), akir-1(RNAi) mutants (B, F, J, N), condensin II mutants hcp-6 (C, G), and condensin I/I' mutants dpy-28 (K, O), akir-1(RNAi); hcp-6 (D, H) and akir-1(RNAi); dpy-28 (L, P). Diakinesis –3 (A–D, I–L), diakinesis –1 (E–H, M–P). Nuclei are stained with DAPI (blue) and SYP-1 (red) and HIM-3 (green). Images are full projections of a single nucleus. Bars, 2 μm.

**DISCUSSION**

Acquiring proper diakinesis bivalent structure is a prerequisite for accurate chromosome segregation in meiosis, as shown by many studies demonstrating that altered chromosome condensation perturbs progression through meiosis. Our findings now provide an additional mechanism for maintaining proper bivalent structure: through analysis of a novel meiotic gene, akir-1, we demonstrate a role for SC disassembly in restructuring the diakinesis bivalent. Our studies identify a previously unknown meiotic role for the conserved metazoan akirin gene family, specifically for akir-1 in *C. elegans*. akir-1 is the first gene identified to play a role in the resolution of the short chromosome arm and is an important new component of the mechanisms regulating SC disassembly and chromosome condensation.

**AKIR-1 is essential for proper diakinesis bivalent structure in late prophase**

In *C. elegans*, condensin II–dependent bivalent condensation acts at the diplotene-to-diakinesis transition, concurrent with but independent of SC disassembly (MacQueen et al., 2002; Colaiacco et al., 2003; Chan et al., 2004; Smolikov et al., 2007, 2009). Our data show that AKIR-1 acts in a distinct pathway from that of condensin to modulate chromosome structure in meiosis. The condensation defects observed in akir-1 mutants are, however, milder than those observed for condensin II null mutants, in which diakinesis bivalent structure cannot even be detected. In addition, since most akir-1 mutant chromosomes eventually condense at the exit from prophase I, AKIR-1’s role in condensation seems to be restricted to late meiotic prophase I. The identification of this distinct pathway involving AKIR-1 adds a new layer of regulation to chromosome behavior at this important meiotic stage.

**AKIR-1 regulates short-arm resolution through its role in central region disassembly**

Proteins involved in SC disassembly have been identified in numerous model organisms (Ivanovska et al., 2005; Malmanche et al., 2007; Bhat et al., 2008; de Carvalho et al., 2008; Martinez-Perez et al., 2008; Sourirajan and Lichten, 2008; Jordan et al., 2009, 2012; Peretz et al., 2009; Resnick et al., 2009). Unfortunately, mutations in these genes lead to multiple meiotic defects in addition to disrupting SC disassembly. Thus it is possible that that the role of these proteins in SC disassembly is indirect. Alternatively, it may be a reflection of a biological program in which pathways controlling other key aspects of meiosis are coopted to regulate SC disassembly. AKIR-1 seems to fit this latter model, being a protein with multiple roles in meiosis.

The disassembly of SC proteins in *C. elegans* occurs through distinct mechanisms in the short versus the long arm of the bivalent. AKIR-1 is the only known player in the process of short-arm resolution, which is mechanistically distinct from the asymmetric disassembly processes occurring on both arms. We propose a role for AKIR-1 in preventing the accumulation of SYP proteins on chromosomes once SC disassembly is initiated and SYPs are removed from the long arm of the diakinesis bivalents. Our data agree with a model in which AKIR-1 prevents SYPs that have unloaded from the long arms of the chromosomes from reassociating with SYPs still bound to the short arms. This process is crucial for disabling the SYPs from connecting homologues while they are still associated with chromosomes in diakinesis. In akir-1 mutants, SYPs bind and form aggregates on the short arm of the diakinesis bivalent, delaying arm resolution. We propose that this accumulation of SYPs pushes the chromatid in different directions, expanding the gap in the interface between the arms and distorting the structure of the bivalent. When central region proteins are removed from chromosomes before fertilization, the region normally bound by the SYPs appears as a widened gap, resulting in aberrant diakinesis bivalent structure, which may account for the meiotic division defects observed in the mutants.

**The interplay between central region disassembly and diakinesis bivalent structure**

Our results point to an interplay between chromosome restructuring and SC disassembly, two key events in late prophase I. We suggest that AKIR-1 modulates chromosome structure in late meiotic prophase I by 1) regulating chromosome condensation (length) in an
meiotic progression defects, it would be hard to explain why over, if the phenotypes we observe were an outcome of general disassembly, as mutants with a more severe reduction in the number of diakinesis nuclei in meiosis prophase I progression is a reduced number of diakinesis nuclei in mutant oocytes are activated and able to mature. The only alteration in meiotic prophase I progresses normally. The kinetics of meiotic events such as pairing, DSB formation and repair, loading of SC proteins, and clustering and dispersal of chromosomes are all unaffected in mutants. Moreover, staining for active MAPK indicates that clustering and dispersal of chromosomes are all unaffected in mutants. AKIR-1 could affect condensation directly, which in turn affects SC disassembly indirectly. In this case, that would be the first demonstration of a protein affecting SC disassembly through its role in condensation, reflects the versatile functions of this conserved protein family. The data we present here represent an important step forward, identifying the worm AKIR-1 orthologue and demonstrating a novel function for this protein in meiosis. This role, outside transcription, raises the possibility that AKIR-1’s functions in meiosis might involve transcription as well. We believe it likely, however, that the meiotic and somatic mechanisms of action of AKIRIN proteins are distinct and that AKIR-1 does not act in transcription in the worm germline. Several results support this conclusion. First, in the C. elegans germline, protein expression is generally regulated translationally and not transcriptionally (Merritt et al., 2008). Second, transcription is repressed at diakinesis, the stage in which akir-1 mutants exhibit their strongest phenotype. Third, the level of SYP-1 transcript is no different in akir-1 mutants and wild type. Finally, other studies have shown that proteins acting as transcription factors in somatic tissues adopt different roles in meiosis (e.g., HIM-8; Phillips et al., 2005). The further investigation of the meiotic and somatic functions of akirin in both C. elegans and other model organisms will be required for a complete understanding of the complex biological roles played by this conserved protein family.

SC- and condensin-independent manner and 2) supporting cruciform shape by promoting proper short-arm resolution in an SC-dependent manner. The current view is that condensation is not required for SC disassembly in C. elegans (Chan et al., 2004). Therefore it is likely that the two functions of AKIR-1 in chromosome structure are independent of each other. An alternative hypothesis is that AKIR-1 could affect condensation directly, which in turn affects SC disassembly indirectly. In this case, that would be the first demonstration of a protein affecting SC disassembly through its role in condensation in C. elegans. Regardless of the molecular role AKIR-1 plays in meiosis, our findings reveal an important role for AKIR-1 in meiotic chromosome behavior and forge a link between proper SC disassembly and acquiring a functional bivalent structure at diakinesis.

Meiotic prophase I progression and AKIR-1
Whereas meiotic divisions are delayed in akir-1 mutants, meiotic prophase I progresses normally. The kinetics of meiotic events such as pairing, DSB formation and repair, loading of SC proteins, and clustering and dispersal of chromosomes are all unaffected in akir-1 mutants. Moreover, staining for active MAPK indicates that akir-1 mutant oocytes are activated and able to mature. The only alteration in meiotic prophase I progression is a reduced number of diakinesis nuclei in akir-1 mutants. However, this is likely not causal to SC disassembly, as mutants with a more severe reduction in the numbers of diakinesis nuclei have no defects in SC disassembly. Moreover, if the phenotypes we observe were an outcome of general meiotic progression defects, it would be hard to explain why akir-1 mutation affects specifically all four SYPs without affecting all other SC proteins.

AKIR-1 and transcription
AKIRIN encodes an evolutionarily conserved protein with a somatic role in transcription through its interaction with DNA-binding proteins, including chromatin remodelers and transcription factors (Goto et al., 2008; Komiya et al., 2008; Nowak et al., 2012). This raises the possibility that AKIR-1’s functions in meiosis might involve transcription as well. We believe it likely, however, that the meiotic and somatic mechanisms of action of AKIRIN proteins are distinct and that AKIR-1 does not act in transcription in the worm germline.

MATERIALS AND METHODS
Strains
All C. elegans strains were cultured under standard conditions at 20°C (Brenner, 1974). Bristol N2 worms were used as the wild-type background, and Hawaiian CB4856 wild-type worms were used for SNP mapping in the process of cloning akir-1(rj1). The following mutations and chromosome rearrangements were used (McKim et al., 1993; Dernburg et al., 1998; MacQueen et al., 2002; this work):

| Strain | Mutation        | Genotype                        |
|--------|-----------------|--------------------------------|
| LGI:   | akir-1(rj1)     | hT2[bli-4(e937) qIs48] (I,III) |
| LGIII: | dpy-28(s939)    |                                |
| LGIV:  | spo-11(ok79)    |                                |
| LGV:   | syp-1(me17)     | capg-2(tm1833)                  |
| LGVI:  | syp-2(ok307)    |                                |
| LGVII: | hcp-6(mr17)     |                                |

The following transgenic lines were used:

- UV7 unc-119(ed33) III; [tlts2[pie-promoter::GFP::zhp-3 + unc-119(+)]] (Bhalla et al., 2008)
- OD57 unc-119(ed33); lts37 [pAA64: pie-1p::mCHERRY::his-58 + unc-119 (+)]; lts25 [pAZ132; pie-1p::GFP::tba-2 + unc-119 (+)] (McNally et al., 2006)
located at the −1-cM position on chromosome I. In addition, deficiency analysis pointed to rj1 being located in the region encompassed by sDf4. Genome-wide sequencing indicated that four genes in this region contained nonsynonymous mutations, using methodology previously described (Rose et al., 2010). The presence of the rj1 mutation was verified by Sanger sequencing. These four genes were examined for prophase I cytological phenotypes of any existing allele and/or RNAi-mediated depletion for these ORFs (RNAi was performed as in Timmons et al. [2001], except that 1 mM isopropyl-β-D-thiogalactoside was used). Both the gk528 allele and RNAi for E01A2.6 phenocopied the rj1 phenotype. Neither RNAi or allele analyses to any of the other genes exhibited a meiotic prophase I phenotype. RNAi for akir-1 was performed using pl4440, to which the entire predicted E01A2.6 cDNA was cloned. The rj1 allele contains a histidine-to-proline substitution at position 190.

The gk528 allele was generated by the C. elegans Reverse Genetics Core Facility at the University of British Columbia (Vancouver, Canada), which is part of the International C. elegans Gene Knockout Consortium. It contains a 678-base pair deletion including exon 1 and extending halfway into exon 2 of open reading frame E01A2.6. The deletion also removes most of the predicted promoter and 42% of the coding region. The gk528 deletion is expected to remove a small ORF (E01A2.9) with unknown function nested in the first intron of the gene. Because gk528 does not exhibit more severe phenotype compared with its RNAi or the rj1 allele, E01A2.9 is likely not involved in meiosis or may not be a functional gene. akir-1(gk528) exhibits the same phenotype as akir-1(rj1) and akir-1(rj1)/akir-1(gk528) (Figure 1), supporting the algorithm that both strains contain alleles of the same gene. Moreover, the phenotype caused by akir-1(RNAi) was indistinguishable from the phenotype caused by the two akir-1 alleles.

Both rj1 and gk528 are recessive akir-1 alleles (DAP1- and SYP-1–stained germlines of rj1/+ and gk528/+ hermaphrodites were identical to wild-type germlines). We showed that these two alleles exhibit indistinguishable phenotypes based on all assays to be described. However, the penetrance of some of the phenotypes was significantly higher for the point-mutant allele (rj1) compared with the deletion allele (gk528). This includes embryonic lethality (21 vs. 16%, p = 0.05, Fisher’s exact test, n = 456, 755), percentage of aggregates as significant (30 vs. 24, p = 0.01, Mann–Whitney test, n = 108, 108), reduction in P-H3 staining (25 vs. 10%, p < 0.04, Fisher’s exact test, n = 57, 58), number of oocytes in diakinesis (3.3 vs. 4.6, p < 0.001, Mann–Whitney test, n = 30, 24). This may indicate that our point-mutant allele is not a complete loss of function but exhibits some recessive gain-of-function properties. However, this does not change our main conclusions, as the phenotypes are consistent and of similar degree between the two alleles. The present results suggest that akir-1(gk528) is a null and akir-1(rj1) is close to being a null. Both of the mutant phenotypes (SC disassembly and diakinesis bivalent structure defect) were evident in every oocyte and diakinesis bivalent examined in all mutant genotypes.

**Characterization and isolation of akir-1 alleles**

We isolated the rj1 allele in a forward genetic screen using ethane methyl sulfonate as a mutagen. Because meiotic mutants will develop into healthy adults that produce many inviable eggs, we screened for mutants that exhibit a maternally rescued embryonic-lethal phenotype. We then performed a secondary, cytology-based screen to identify those mutants in which SC disassembly was specifically affected. This screen used high-resolution imaging techniques to identify mutants with aberrant SC localization at diakinesis via immunostaining for SYP-1. The mutation was linked to a SNP

| MI | Duration (min) | n | Duration (min) | n |
|----|----------------|---|----------------|---|
| Wild type | 6.66 | 4 | 11.88 | 6 |
| akir-1(rj1) | 14.53 | 8 | 20.95 | 10 |

**TABLE 5:** Timing of meiotic divisions in wild type and akir-1 mutants, based on live imaging.

**Germ cell apoptosis**

Germ cell corpses were scored in adult hermaphrodites 20 h post-L4 as in Kelly et al. (2000). Statistical comparisons between genotypes were performed using the two-tailed Mann–Whitney test, 95% confidence interval.

**Crossover recombination frequencies**

Meiotic crossover recombination frequencies were assayed using SNP markers as in Hillers and Villeneuve (2003). akir-1 was introduced into the Hawaiian strain CB4856 by six outcrosses. Because of the embryonic lethality observed for akir-1(gk528) mutants and
the unsuccessful mating of homozygote males, recombination frequencies were assayed on homozygotes generated by mating akir-1(gk528) hermaphrodites from the Hawaiian strain CB4856 background to akir-1(gk528)/ht2(zli-4[e937]pIs48) males from the Bristol (N2) background. The F2 generation of hermaphrodites that were homozygous for akir-1(gk528) and heterozygous for SNP markers were analyzed. The presence of homozygous alleles of akir-1(gk528) was verified by PCR. The SNPs used were pkP2101 and uCE2-2131 as in Davis et al. (2005).

Nondisjunction frequencies using restriction fragment length polymorphism assay

Meiotic nondisjunction frequencies were assayed using the restriction fragment length polymorphism (RFLP) assay developed by Severson et al. (2009). We modified this assay by using nested PCR to increase the reliability of the amplification. Primers used for primary PCR, GACGGAGAATGAGATTCTGACC and GCTCCG-TGTCGCTTTCGTCAG. Primers used for secondary PCR, CG-GCTCGTCCTTATGAAACGGA and GTGAGCCCCTTAAATCCA.

Reverse transcription-PCR

To determine the relative levels of SYP-1 expression, reverse transcription (RT)-PCR was performed on mRNA extracted from whole worms in duplicates (RNAeasy kit; Qiagen, Valencia, CA) and subjected to reverse transcription with SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA). For quantitative RT-PCR, SYBR Green I Master Kit was used (Roche, Indianapolis, IN) and read in the LightCycler480 Real-Time PCR System. For each mRNA extracted, three technical controls were made. For syp-1 the following primers were used: gatgaatatctgaccaag and accagactcattg. For myo-3 control the following primers were used: tccggtcatcagaatgag and accacgtcattg.

FISH and time-course analysis of chromosome pairing

The SS FISH probe was generated as in Demburg et al. (1998) from a PCR fragment generated by amplifying C. elegans genomic DNA with the 5′-TACCTGTGGATCGGAGACC-3′ and 5′-CTAAGCTTGCCTGACG-3′ primers. Fragments were labeled with fluorescein-12-dCTP (PerkinElmer, Waltham, MA). For quantitative analysis we defined the long arm of the diakinesis bivalent as the longer halfway through three-dimensional data stacks of whole nuclei (1065 slices/stack), and diakinesis images encompass entire nuclei; both were prepared using softWoRx 5.0.0 and softWoRx Explorer 1.3.0 software (Applied Precision).

Immunostaining and microscopy

Unless stated otherwise, images were collected from all bivalents in each oocyte imaged, to prevent any bias in image analysis. DAPI staining, immunostaining, and analysis of stained meiotic nuclei were performed as in Colaiacovo et al. (2003), except REC-8 staining (Rogers et al., 2002), P-H3 and AIR-2 (Hsu et al., 2000). Primary antibodies were used at the following dilutions: goat α-SYP-1, 1:500 (MacQueen et al., 2002); rabbit α-SYP-2, 1:100 (Colaiacovo et al., 2003); rabbit α-SYP-3, 1:100 (Smolikov et al., 2007); rabbit α-RAD-51, 1:100 (Colaiacovo et al., 2003); rabbit α-HIM-3, 1:500 (Zetka et al., 1999); guinea pig α-HTP-3, 1:500 (Goodyer et al., 2008); mouse α-REC-8, 1:50 (Abcam, Cambridge, MA); rabbit α-LAB-1, 1:300 (de Carvalho et al., 2008); rabbit α-P-H3, 1:400 (Millipore, Billerica, MA); diphosphorylated MAPK, 1:500 (Sigma-Aldrich); and rabbit α-MIX-1, 1:100. The secondary antibodies used were Alexa Fluor 555 anti-rabbit (Invitrogen), fluorescein isothiocyanate α-rabbit, Alexa Fluor 568 anti–guinea pig (Invitrogen), DyLight 594 anti-goat (Jackson Immunochemicals, West Grove, PA), and Cy3 α-mouse (Jackson Immunochemicals), each at 1:500.

For MI and MII analysis, akir-1(rj1) was introduced into the OD57 genetic background (pie-1p::mCHERRY::his-58 and pie-1p::GFP::tba-2). Fertilized oocytes were assessed either by live imaging or after ethanol fixation. MI and MII data for akir-1(gk528) were collected for ethanol-fixed and DAPI-stained fertilized oocytes. Quantitative analysis of the intensity of SYP-1 signals was performed using softWoRx 5.0.0 software (Applied Precision, Issaquah, WA). This was performed under guided model option with a freehand polygon section in all Z-stacks of a particular SYP signal and to multiple gonads from each genetic background. To obtain SYP-1 signal intensity, we subtracted the background of the same image from the SYP-1 signal intensity read.

For time-lapse differential interference contrast (DIC) microscopy, control (pie-1p::mCHERRY::his-58 and pie-1p::GFP::tba-2) and akir-1(rj1) pie-1p::mCHERRY::his-58 and pie-1p::GFP::tba-2 worms were anesthetized in 0.1% tricaine/0.01% tetramisole in M9 and immediately mounted on 2% agarose pads with 8 μl of the anesthetic and covered with a 22-mm square glass coverslip. Tricaine/tetramisole paralyzes body wall movement but does not block several rounds of oocyte maturation and ovulation. Images were captured at room temperature using DIC microscopy and recorded every 3–4 s for up to 44 min using SoftWoRx 5.0.0 software. Movies were compiled at 10–13 frames/s. No photodamage was observed during the course of the experiment.

The images were acquired using the DeltaVision wide-field fluorescence microscope system (Applied Precision) with Olympus 100x/1.40 lenses (except for S3, with 60x lenses). Optical sections were collected at 0.20-μm increments with a CoolSNAP HQ camera (Photometrics, Tucson, AZ) and softWoRx software and deconvolved using softWoRx 5.0.0 software. Pachytene images are projections halfway through three-dimensional data stacks of whole nuclei (15–30 0.2-μm slices/stack), and diakinesis images encompass entire nuclei; both were prepared using softWoRx 5.0.0 and softWoRx Explorer 1.3.0 software (Applied Precision).

Time-course analysis for RAD-51 foci

Quantification of RAD-51 foci was performed for all seven zones composing the premeiotic tip to late pachytene regions of the germ line as in Colaiacovo et al. (2003). The total number of nuclei scored per zone from three gonads each for wild type, akir-1(rj1), and akir-1(rj1) pie-1p::mCHERRY::his-58 are as follows: zone 1, 242; zone 2, 263; zone 3, 213; zone 4, 217; zone 5, 170; zone 6, 169; and zone 7, 137. Statistical comparisons between genotypes were performed using the two-tailed Mann–Whitney test, 95% confidence interval.

Analysis of chromosomal features and distances

Because akir-1 mutants have diakinesis bivalent structure defects (see later discussion), in chromosomes lacking the typical cruciform structure we defined the long arm of the diakinesis bivalent as the longer portion of the chromosome up to the region where the two chromosomes connect and the shorter arm as the remaining portion of each chromosome. Because akir-1 mutant chromosomes are longer than wild type but are confined to the same nuclear space, resolving individual chromosomes was challenging in many cases. We therefore
used HIM-3 as a marker and measured chromosome length by tracing the length of the HIM-3 signal (Tables 3 and 4). For hcp-6/flu17 experiments, worms were grown at 20°C and shifted to 25°C overnight. In these conditions condensation defects were apparent, yet bivalents could be observed. For gap-size analysis (Figure 4B), we analyzed chromosomes only in “face-up position,” in which clear cruciform structures could be resolved via HIM-3 staining. Any other positioning of the chromosomes obscured the gap. RNAi for syp-2 was performed from the L1 developmental stage until 24 h post-L4 on akir-1(gk528)/hT2 (phenotypically wild type) and akir-1(gk528) mutants. We assessed akir-1(gk528)/hT2; syp-2(RNAI) for partial depletion of SYP, as indicated by the appearance of univalents at a low frequency.

We used the following method to measure distances between DAPI bodies: a DAPI body was randomly assigned, and a distance was measured to the closest DAPI body, measuring the smallest distance between the two bodies. This analysis was continued until all DAPI bodies were used for the measurement. No DAPI body was counted more than once. Statistical comparisons between genotypes were performed using the two-tailed Mann–Whitney test.

Length of meiotic stages was measured on gonads from age-matched whole worms ethanol fixed and stained with DAPI. The length of each meiotic stage was defined by measuring the length in micrometres in the middle of the gonad of three-dimensional images. The premeiotic region was defined as extending from the first row after the transition zone with clustered chromosome morphology nuclei. The transition zone was defined by the presence of rows of cells with at least one nucleus with a distinct clustered chromosome morphology. Pachytene was defined as extending from the first row after the transition zone until diplotene. Diplotene was defined as the region in which all rows of nuclei are reduced to two rows of nuclei, until the “bend” region. Diakinesis was defined as the region from the “bend” to the spermatheca.

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