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Article

Loading of Meiotic Cohesin by SCC-2 Is Required for Early Processing of DSBs and for the DNA Damage Checkpoint

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Summary

Background: Chromosome segregation and the repair of DNA double-strand breaks (DSBs) by homologous recombination require cohesin, the protein complex that mediates sister chromatid cohesion (SCC). In addition, cohesin is also required for the integrity of DNA damage checkpoints in somatic cells, where cohesin loading depends on a conserved complex containing the Scc2/Nipbl protein. Although cohesin is required for the completion of meiotic recombination, little is known about how cohesin promotes the repair of meiotic DSBs and about the factors that promote loading of cohesin during meiosis.

Results: Here we show that during *Caenorhabditis elegans* meiosis, loading of cohesin requires SCC-2, whereas the cohesin-related complexes condensin and SMC-5/6 can be loaded by mechanisms independent of both SCC-2 and cohesin. Although the lack of cohesin in *scc-2* mutants impairs the repair of meiotic DSBs, surprisingly, the persistent DNA damage fails to trigger an apoptotic response of the conserved pachytene DNA damage checkpoint. Mutants carrying an *scc-3* allele that abrogates loading of meiotic cohesin are also deficient in the apoptotic response of the pachytene checkpoint, and both *scc-2* and *scc-3* mutants fail to recruit the DNA damage sensor 9-1-1 complex onto persistent damage sites during meiosis. Furthermore, we show that meiotic cohesin is also required for the timely loading of the RAD-51 recombinase to irradiation-induced DSBs.

Conclusions: We propose that meiotic cohesin promotes DSB processing and recruitment of DNA damage checkpoint proteins, thus implicating cohesin in the earliest steps of the DNA damage response during meiosis.

Introduction

The maintenance of genome integrity requires the accurate partitioning of chromosomes during cell division and the timely repair of damage accumulated on the DNA. The cohesin complex, which mediates sister chromatid cohesion (SCC) by physically tethering together sister chromatids, is a key player in these two events [1, 2]: it holds together sister chromatids from the completion of S phase until their segregation in anaphase, and it is also required for the repair of DNA double-strand breaks (DSBs) by homologous recombination, a process by which the broken chromatid is repaired using its intact counterpart as a repair template. Intersister recombination is the main mechanism of DSB repair in postreplicative cells, and it requires the recruitment of cohesin around DSB sites and the reestablishment of SCC [3–6]. Loading of cohesin to chromatin during S phase, and also in response to DSBs in postreplicative cells, depends on a conserved complex composed of the Scc2/Nipbl and Scc4 proteins [7–10]. In mitotic yeast cells, Scc2 is also involved in the loading of two cohesin-related complexes, condensin and the SMC-5/6 complex [11, 12], which also associate with chromatin to promote chromosome segregation and DSB repair.

Cohesin is also required to promote chromosome segregation [13] and DSB repair [14, 15] during meiosis, the specialized cell division program that produces haploid gametes from diploid germ cells. In contrast to somatic cells, most meiotic DSBs, which are purposely created to initiate meiotic recombination, are not repaired using the sister chromatid as a template but rather the homologous chromosome [16, 17]. This type of repair leads to the formation of interhomolog crossover events, which together with SCC are required to ensure correct homolog segregation. Interestingly, SCC is locally relaxed around crossover sites [18], suggesting that the interplay between DSB repair and SCC may be differently regulated during mitosis and meiosis. Exactly how cohesin affects the repair of meiotic DSBs is not well understood.

In addition to its direct role in DNA repair, cohesin also participates in the regulation of checkpoints that are activated by the presence of DNA damage in somatic cells. In mammalian cells, the cohesin subunits Smc1 and Smc3 are phosphorylated by the master checkpoint kinase ATM, and cells that express a nonphosphorylatable version of Smc1 are defective in the activation of the intra-S phase checkpoint [19, 20]. Cohesin is also required for the DNA damage checkpoint in postreplicative cells, and this checkpoint function of cohesin is independent of its role in mediating SCC [21]. A conserved DNA damage checkpoint, known as the pachytene checkpoint, also monitors the efficient repair of meiotic DSBs and induces apoptosis when DSBs are not timely repaired [22]. Although some of the key components of mitotic DNA damage checkpoints, such as the DNA damage sensor 9-1-1 complex [23] and the ATM and ATR kinases, are also required for the pachytene DNA damage checkpoint [24, 25], whether cohesin participates in this meiotic DNA damage checkpoint is not known.

Here we have investigated the involvement of cohesin in the repair of meiotic DSBs and in the activation of the pachytene DNA damage checkpoint in the *Caenorhabditis elegans* germline. We show that meiotic cohesin is loaded by SCC-2 and that in the absence of meiotic cohesin, recombination intermediates accumulate extensively but fail to trigger the apoptotic response of the pachytene checkpoint. Furthermore, we demonstrate that meiotic cohesin is required for early DSB processing and for the efficient recruitment of DNA damage sensors, thus implicating cohesin in the early events of the meiotic DNA damage response.

Results

Isolation of *C. elegans scc-2* Mutants

In order to identify genes required for correct meiotic chromosome morphogenesis, we performed a genetic screen designed to isolate mutants with cytological defects in their

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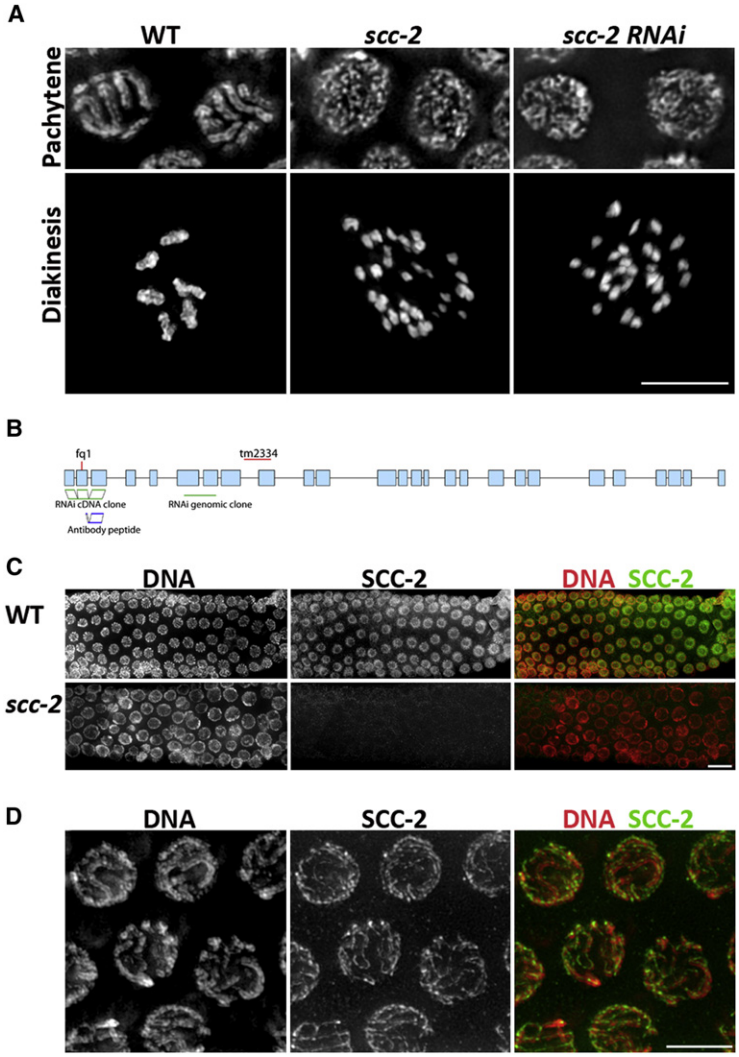

Figure 1. *scc-2* Mutants Display Cytological Defects during Meiosis

(A) Partial projections of pachytene nuclei from the indicated genotypes stained with DAPI. Bottom panel shows full projections of diakinesis nuclei stained with DAPI that demonstrate separation of sister chromatids and the presence of small chromatin bodies that could represent chromosome fragments. Note that not all DAPI-stained bodies can be seen individually as a result of some overlap in the projections. *scc-2* RNA interference (RNAi) was performed at 25°C. Scale bar represents 5 μm.

(B) Diagram of the predicted structure of the *C. elegans scc-2* gene; exons are indicated as blue boxes, whereas introns are represented as thin black lines. The position of the early STOP codon caused by the *fq1* mutation and the region removed by the *tm2334* deletion are indicated above the diagram. Regions used to create two different RNAi vectors, as well as the region against which an antibody was raised, are indicated below the diagram.

(C) Staining of pachytene nuclei from wild-type (WT) germlines and *scc-2(fq1)* mutants with anti-SCC-2 antibodies and DAPI. Scale bar represents 10 μm.

(D) Pachytene nuclei from wild-type germlines stained with anti-SCC-2 antibodies following the extraction of soluble protein with a postfixation triton wash. This treatment reveals a fraction of SCC-2 associated with the axial element of meiotic chromosomes. Scale bar represents 5 μm.

germlines (see Supplemental Experimental Procedures available online). Worms homozygous for the *fq1* mutation displayed cytological defects that were suggestive of meiotic defects in SCC. First, while DAPI staining of wild-type pachytene nuclei revealed highly organized, thick chromosomal tracks, representing paired homologs, pachytene nuclei in *fq1* mutants appeared disorganized with predominantly thin chromatin tracks (Figure 1A). Second, most diakinesis oocytes in *scc-2(fq1)* mutants displayed between 15 and 20 DAPI-stained bodies, which is consistent with partial separation of sister chromatids, whereas in some oocytes the presence of 24 DAPI-stained bodies demonstrated full separation of sisters (Figure 1A). Furthermore, many oocytes also contained small chromatin masses that could represent chromosome fragments (Figure 1A). Although *fq1* homozygous mutants arising from heterozygous mothers were viable, they produced 100% dead embryos, suggesting that the viable *fq1* homozygous mutants were rescued by maternal contribution of mRNA and/or protein.

Using single nucleotide polymorphism (SNP) mapping [26], we located the *fq1* mutation to a region in the left end of chromosome II containing the *pqn-85* gene, the *C. elegans* ortholog of *scc2* [27]. Sequencing of the *pqn-85* gene (referred to as *scc-2* from here) in *fq1* mutants revealed the presence of a single C-to-T substitution at position 382 of the cDNA, which is predicted to create an early STOP codon after 127 amino acids of the 2203 amino acids thought to be present in SCC-2 (Figure 1B). We then used three different approaches to verify that the C382T mutation that we identified in *scc-2* is responsible for the phenotypes observed in *fq1* mutants. First, a complementation test between *fq1* and a strain carrying a 515 bp deletion (*tm2334*) in *scc-2* demonstrated that these two mutations affect the same gene. Second, immunostaining experiments showed that the SCC-2 protein accumulates strongly in wild-type pachytene nuclei but not in *scc-2(fq1)* mutant germlines, suggesting that the *fq1* mutation is a strong loss-of-function or a null allele of *scc-2* (Figure 1C). Interestingly, the extraction of soluble nuclear protein demonstrated a fraction of SCC-2 associated with the axial element of meiotic chromosomes in wild-type germlines (Figure 1D). Finally, we knocked down *scc-2* function by RNA interference (RNAi) using two different vectors (Figure 1B) and obtained worms with cytological defects in their germlines identical to those observed in *fq1* mutants (Figure 1A). Taken together, these results confirm that the *fq1* mutation impairs the function of *C. elegans scc-2* and that SCC-2 is required to promote normal meiotic chromosome organization.

SCC-2 Is Required for Meiotic Loading of Cohesin but Not Condensin or the SMC-5/6 Complex

Given that our initial visualization of meiotic chromosomes in *scc-2(fq1)* mutants suggested defects in SCC and that Scc2 is required for cohesin loading in yeast and vertebrate mitotic cells [7–10], we tested the loading of cohesin subunits (SMC-1, SMC-3, and the meiosis-specific kleisin REC-8) in *scc-2(fq1)* germlines. Whereas control germlines demonstrated normal loading of the three cohesin subunits to the axial element of meiotic chromosomes, no staining was detected in the meiotic chromosomes of *scc-2(fq1)* mutants or in *scc-2* RNAi worms

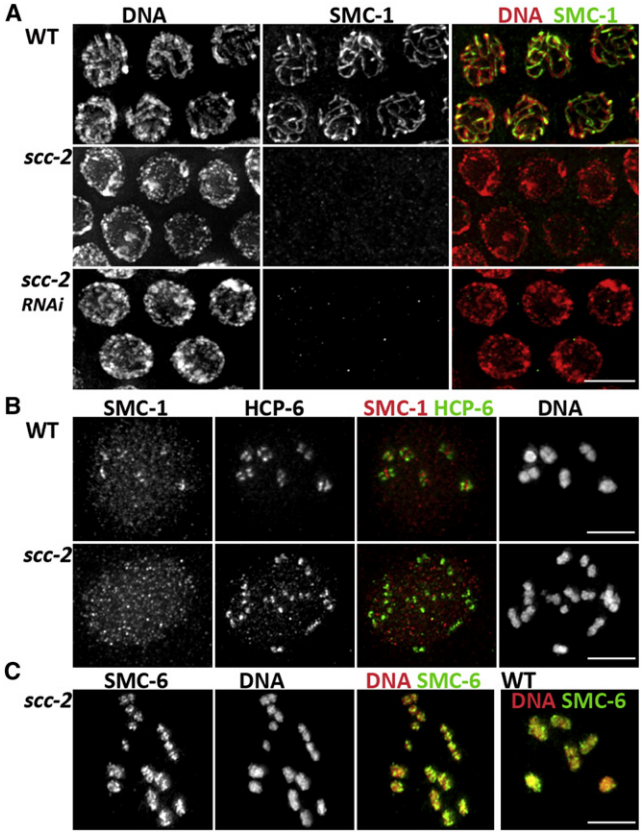

Figure 2. SCC-2 Promotes Loading of Meiotic Cohesin

(A) Pachytene nuclei from WT, *scc-2(fq1)* mutants, and *scc*-2 RNAi (performed at 25°C) stained with anti-SMC-1 antibodies and DAPI.

(B) Diakinesis oocytes from wild-type worms and *scc-2(fq1)* mutants stained with anti-SMC-1 and anti-HCP-6 (condensin II) antibodies and counterstained with DAPI.

(C) Diakinesis oocytes from *scc-2(fq1)* mutants and wild-type worms stained with anti-SMC-6 antibodies. Scale bars represent 5 μm. See also Figures S1 and S2.

(Figure 2; Figures S1A and S1B). Importantly, cohesin subunits accumulated extensively in the mitotic nuclei and the meiotic S phase nuclei that precede the start of meiotic prophase (Figure S1C), demonstrating that the lack of cohesin staining in pachytene chromosomes of *scc-2(fq1)* mutants was not caused by a lack of expression of cohesin subunits at earlier stages. By late pachytene, SMC-1 started accumulating inside the nucleus of both wild-type and *scc-2(fq1)* mutant germlines, demonstrating that SMC-1 is normally expressed at this stage (Figure S1D), but chromatin-associated SMC-1 was only seen in wild-type oocytes (Figure 2B). Furthermore, synaptonemal complex (SC) assembly was completely abrogated in *scc-2 (fq1)* mutants (Figures S2A and S2B), as previously observed in situations where meiotic SCC is not established [28, 29]. These results demonstrate that cohesin subunits are not loaded to meiotic chromosomes in *scc-2(fq1)* mutants.

In mitotic cells of *Saccharomyces cerevisiae*, Scc2 has also been proposed to promote the loading of other SMC complexes that play roles in chromosome structure and DNA repair, namely condensin and the SMC-5/6 complex [11, 12]. Therefore, we tested whether the loading of condensin II and the SMC-5/6 complex, which have been previously shown to associate with meiotic chromosomes in *C. elegans* [30–32], was affected in *scc-2(fq1)* germlines. In contrast to cohesin, both condensin II (visualized using anti-HCP-6 antibodies [32]) and the SMC-5/6 complex are clearly loaded onto meiotic chromosomes in the germlines of *scc-2(fq1)* mutants. In fact, the intensity of condensin II and SMC-5/6 staining in the diakinesis chromosomes of *scc-2(fq1)* oocytes was very similar to that observed in wild-type controls (Figures 2B and 2C). Furthermore, the condensin I component DPY-28 [30, 33] also displayed a similar staining pattern in the meiotic nuclei of wild-type and *scc-2(fq1)* mutant germlines (Figure S2C). Although we could not determine precisely how much of the DPY-28 signal overlapped with DNA given that we do not see evidence that in *scc-2(fq1)* mutants, DPY-28 overlaps any less with DNA than in wild-type pachytene nuclei, we think it likely that some loading of condensin I may take place in the absence of SCC-2.

In summary, SCC-2 is required for the meiotic loading of cohesin, but substantial loading of both condensin and the SMC-5/6 complex occur in the absence of SCC-2.

scc-2 Mutants Are Competent for SPO-11-Dependent DSB Formation but Defective in Their Repair

We next investigated how a lack of chromosome-bound cohesin impacts on the formation and repair of meiotic DSBs that are normally formed to initiate meiotic recombination. By following the staining pattern of the RAD-51 recombinase, which binds to single stranded DNA produced upon resection of DSBs [34], we observed that *scc-2(fq1)* mutants displayed an extensive accumulation of recombination intermediates during meiosis. In the mid and late pachytene region of *scc-2(fq1)* germlines, nuclei not only showed much higher numbers of RAD-51 foci than wild-type controls but also showed the presence of elongated RAD-51 structures (Figure 3), which suggested an accumulation of anomalous recombination intermediates. In order to determine whether these recombination intermediates were initiated by meiotic DSBs, which are created by the topoisomerase-like protein SPO-11, we analyzed RAD-51 staining in the germlines of *spo-11; scc-2* double mutants. While we observed an average of 8.95 RAD-51 foci per nucleus in zone 5 of *scc-2* mutant germlines, the same region in *scc-2; spo-11* double mutants displayed an average of 0.67 RAD-51 foci per nucleus (Figure 3B), demonstrating that over 90% of the RAD-51 signals detected in the meiotic region of *scc-2(fq1)* mutants are produced by SPO-11. The remaining SPO-11-independent RAD-51 foci may have originated during S phase, because SCC has been proposed to facilitate the repair of replication-associated DNA damage [35]. These results show that the repair of meiotic DSBs is severely impaired in *scc-2* mutants, consistent with previous reports that meiotic cohesin is required for the proper repair of SPO-11 DSBs [14, 15].

Meiotic Cohesin Is Required for the Apoptotic Response to Accumulated DNA Damage

The efficient repair of meiotic DSBs is monitored by a conserved DNA damage checkpoint that triggers apoptosis when unrepaired recombination intermediates persist in late pachytene nuclei [22, 36]. Given the extensive accumulation of RAD-51 intermediates present in the pachytene nuclei of *scc-2(fq1)* mutants (Figure 3), we expected to see an increase of apoptotic corpses in these germlines. Surprisingly, levels of apoptosis in *scc-2(fq1)* germlines were similar to those observed in wild-type controls, whereas *syp-1* mutants, which are defective in synaptonemal complex formation and also accumulate recombination intermediates [37], showed a clear

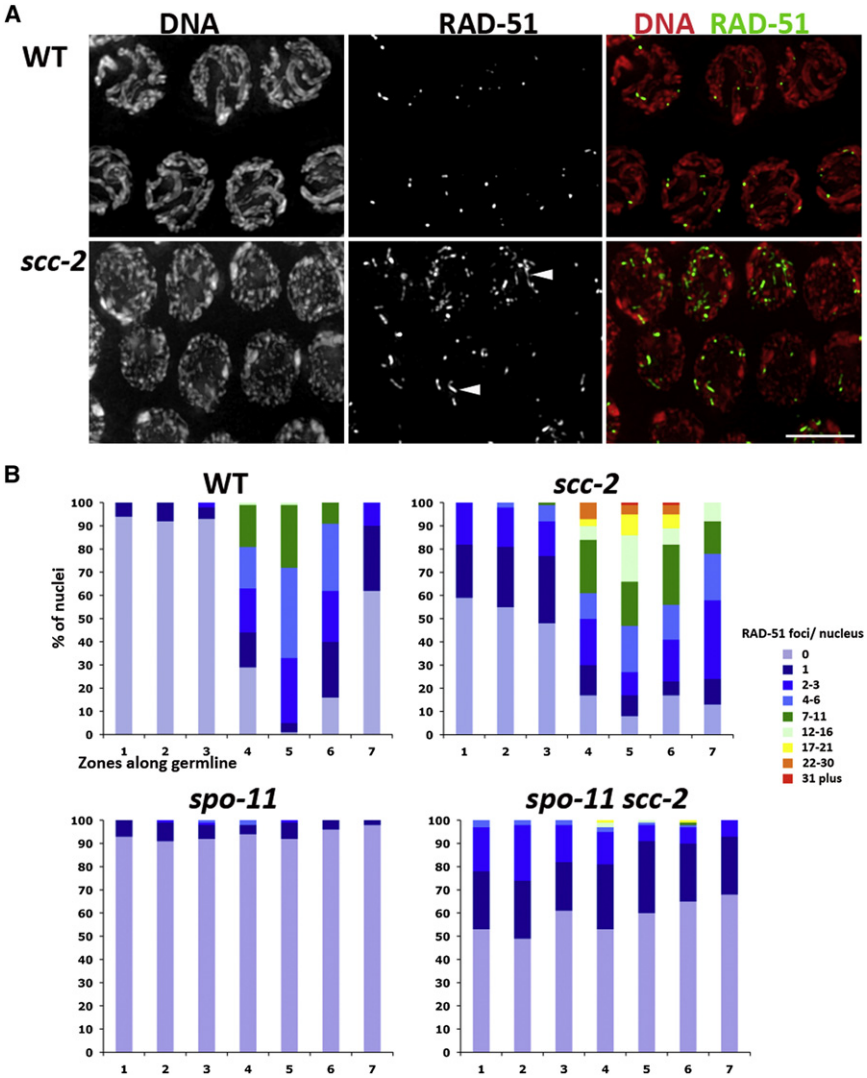

Figure 3. SPO-11-Dependent Intermediates Accumulate in *scc-2* Mutants

(A) Pachytene nuclei stained with anti-RAD-51 antibodies and DAPI. Arrowheads point to elongated RAD-51 structures seen only in *scc-2(fq1)* mutants. Scale bar represents 5 μm.

(B) Quantification of RAD-51 foci from whole-mounted germlines in WT, *scc-2(fq1)*, *spo-11 (ok79)*, and *scc-2(fq1); spo-11(ok79)* mutants. Each germline was divided into seven equal-size regions, with regions 4–7 representing early to late pachytene. The x axis indicates the seven regions along the germline, whereas the y axis indicates the percentage of nuclei with a given number of RAD-51 foci (as indicated in the color key). At least 100 nuclei were scored per zone in each genotype.

increase in apoptotic corpses (Figure 4A; Figure S3A). We next investigated apoptosis levels in germlines of *scc-3(ku263)* mutants, which also lack meiotic cohesin and show accumulation of RAD-51 intermediates at late pachytene [28, 38, 39]. Similarly to *scc-2(fq1)* mutants, apoptotic levels remained low in *scc-3(ku263)* germlines (Figure 4A). The pachytene DNA damage checkpoint also induces apoptosis in response to γ irradiation; however, we failed to detect increased apoptosis levels in *scc-2(fq1)* and *scc-3(ku263)* mutants following γ irradiation (Figure 4A). These results demonstrate that both *scc-2(fq1)* and *scc-3(ku263)* mutants fail to trigger apoptosis in response to the accumulation of SPO-11-dependent recombination intermediates and to DSBs produced by γ irradiation. This defective response to DNA damage could be explained by a requirement of cohesin in the activation of the DNA damage checkpoint or in the apoptotic machinery. The level of apoptosis that we detected in *scc-2(fq1)* and *scc-3(ku263)* mutants was similar to the level seen in wild-type controls, which is known to be independent of the DNA damage checkpoint and is thought to represent the culling of excess meiotic nuclei [36]. These observations suggest that the core apoptotic machinery remains functional in the absence of meiotic cohesin but that activation of the pachytene checkpoint by persistent DNA damage may be deficient in *scc-2* and *scc-3* mutants.

Reduced Levels of Meiotic Cohesin Impair DSB Repair but Not the Apoptotic Response to DNA Damage

We decided to further test the idea that cohesin is required for the pachytene DNA checkpoint by studying the induction of apoptosis in situations where meiotic cohesin levels were reduced but not absent. Depletion of SCC-2 by feeding *scc-2* RNAi at 20°C often resulted in germlines with reduced, but detectable, levels of cohesin in pachytene nuclei, suggesting that SCC-2 had been partially depleted in these germlines. In all cases where *scc-2* RNAi induced a visible reduction of cohesin levels, we also detected a dramatic increase of RAD-51 foci in pachytene nuclei (Figure 4B), suggesting that normal levels of cohesin are important for the repair of SPO-11 DSBs. Furthermore, these germlines also showed elongated RAD-51 structures similar to those that we observed in *scc-2(fq1)* mutants (Figure 4B). *scc-2* RNAi germlines with reduced cohesin levels also displayed reduced synapsis (Figure S3B), which would be expected to impair the timely repair of SPO-11 DSBs. However, the presence of elongated RAD-51 structures, which are not observed in SC-deficient mutants, suggests that the reduction in cohesin is an important contributor for the accumulation of RAD-51 intermediates in the partial *scc-2* knockdown germlines. These results are in agreement with the recent finding that DSB repair is defective in mitotic yeast cells in which cohesin is reduced to 30% of the levels present in wild-type cells [40].

We then utilized *scc-2* RNAi germlines with reduced cohesin staining and accumulated DNA damage to investigate whether apoptosis was induced when meiotic cohesin levels were reduced. We performed *scc-2* RNAi in worms expressing CED-1::GFP, a marker for apoptotic nuclei [41], and before quantifying apoptosis levels, we divided these germlines into two classes: those without visible SMC-1 staining and those with reduced SMC-1 levels. Because the complete lack of SMC-1 staining triggered by *scc-2* RNAi was more efficiently

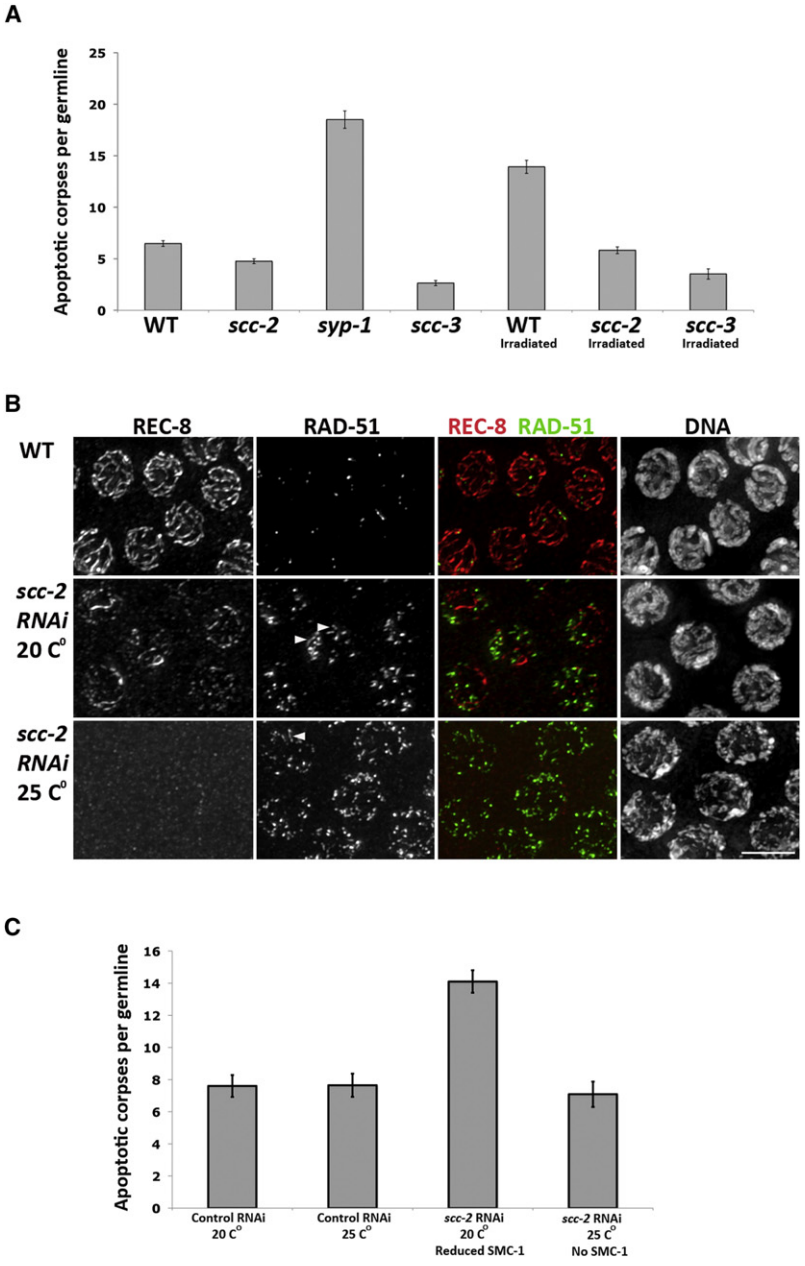

Figure 4. Meiotic Cohesin Is Required for the Apoptotic Response of the DNA Damage Checkpoint

(A) Quantification of SYTO 12-labeled apoptotic corpses in the indicated genotypes. Error bars indicate standard error of the mean (SEM). The number of germlines scored per genotype were: WT (120), *scc-2(fq1)* (95), *syp-1(me17)* (69), *scc-3(ku263)* (73), irradiated WT (53), irradiated *scc-2(fq1)* (88), and irradiated *scc-3(ku263)* (69). Scoring of apoptotic corpses following γ irradiation was performed 20 hr after treatment of young adult worms (18 hr post L4) with 75 Gy.

(B) Pachytene nuclei from WT controls and worms treated with *scc-2* RNAi at 20°C and 25°C stained with anti-REC-8 and anti-RAD-51 antibodies and counterstained with DAPI. Arrowheads point to the presence of elongated RAD-51 structures. Scale bar represents 5 μm.

(C) Quantification of CED-1::GFP-labeled apoptotic corpses following *scc-2* or control RNAi at 20°C and 25°C in germlines that were also costained with anti-SMC-1 antibodies to determine the amount of chromosomal cohesin present in each germline. Statistical analysis demonstrates that although apoptosis levels are significantly different between control RNAi at 20°C and *scc-2* RNAi at 20°C with reduced SMC-1 levels ($p = 2 \times 10^{-7}$), apoptosis levels are not different between control RNAi at 25°C and *scc-2* RNAi at 25°C with no visible SMC-1 staining ($p = 0.29$). Twenty germlines were scored per genotype. Error bars indicate SEM. See also Figure S3.

achieved at 25°C than at 20°C, we also performed control RNAi experiments at both temperatures. Whereas RNAi controls at both 20°C and 25°C displayed similar levels of apoptosis to those previously seen in wild-type worms stained with SYTO 12, *scc-2* RNAi germlines with reduced SMC-1 staining showed a marked increase of apoptosis (Figure 4C). In contrast, germlines with no SMC-1 staining displayed levels of apoptosis similar to RNAi controls, despite the extensive accumulation of RAD-51 foci (Figures 4B and 4C). Therefore, although reduced levels of cohesin are enough to induce apoptosis in response to DNA damage, once cohesin levels are severely reduced, apoptosis is no longer induced. These findings reinforce the idea that meiotic cohesin might be required for the functionality of the pachytene DNA damage checkpoint.

Cohesin Is Required for Loading of the 9-1-1 Complex to Persistent Recombination Intermediates

Prompted by the lack of an apoptotic response in *scc-2* and *scc-3* mutants, we decided to directly investigate whether activation of the pachytene checkpoint was defective in these mutants. We reasoned that because cohesin is bound to chromatin, a lack of cohesin might impair early checkpoint events that take place directly at damage sites. Therefore, we investigated whether binding of DNA damage sensors, which recognize errors and promote the activation of signaling cascades that include the ATM and ATR kinases [22], were deficient in the absence of meiotic cohesin. The conserved PCNA-like 9-1-1 complex is one such sensor [23], and its function is required for inducing apoptosis in response to DNA damage in the *C. elegans* germline [25]. We monitored the recruitment of the 9-1-1 complex in *scc-2* and *scc-3* mutants by using a GFP reporter fused to HUS-1, a 9-1-1 complex component [25]. Despite the large accumulation of DNA damage present in both mutants, the levels of HUS-1 foci in *scc-2(fq1)* and *scc-3(ku263)* germlines were very similar to those seen in wild-type controls, in which DNA damage does not accumulate (Figure 5; Figure S4A). Conversely, *syp-1* mutants, in which apoptosis levels are increased in response to accumulated recombination intermediates (Figure 4A), displayed extensive accumulation of HUS-1::GFP foci (Figure 5; Figure S4A). These results demonstrate a clear failure to load the 9-1-1 complex to persistent SPO-11-dependent recombination intermediates in *scc-2* and *scc-3* mutants.

The 9-1-1 complex is also recruited to meiotic chromosomes following γ irradiation [25]. However, 8 hr post

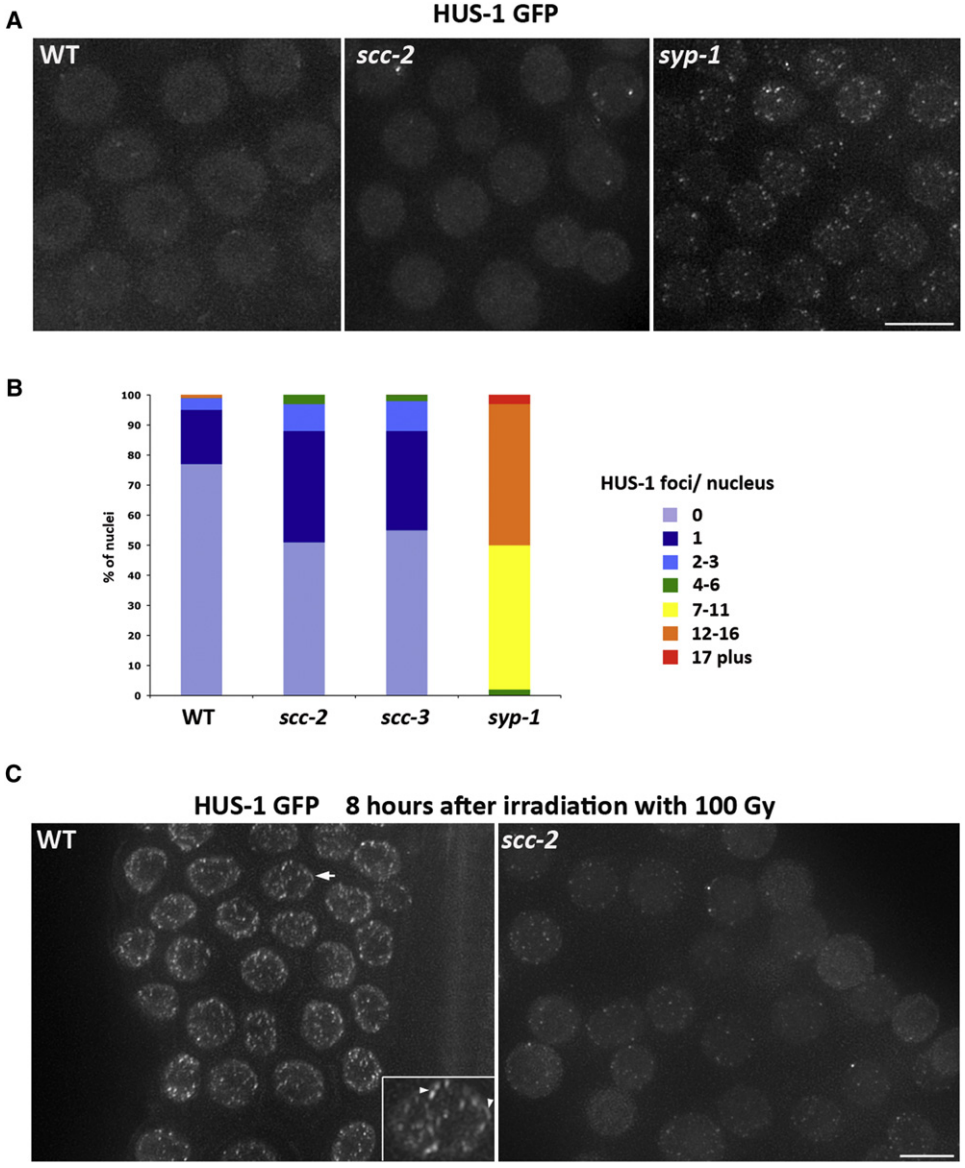

Figure 5. The 9-1-1 Complex Is Not Loaded to Persistent SPO-11 DSBs in *scc-2* and *scc-3* Mutants

(A) Visualization of HUS-1::GFP foci in pachytene nuclei from germlines of WT, *scc-2(fq1)*, and *syp-1(me17)* mutants.

(B) Quantification of HUS-1 foci in pachytene nuclei of the indicated genotypes. One hundred nuclei were scored in WT controls, *scc-2(fq1)*, and *scc-3(ku263)* mutants, and 90 nuclei were scored in *syp-1(me17)* mutants.

(C) Visualization of HUS-1::GFP foci in late pachytene nuclei from germlines of WT and *scc-2(fq1)* mutants 8 hr after the irradiation of young adult worms (18 hr post L4) with 100 Gy. The inset in the WT panel shows a magnification of the nucleus marked with an arrow, and the arrowheads in the inset point to elongated HUS-1::GFP structures. Scale bar represents 5 μm. See also Figure S4.

irradiation with 100 Gy, *scc-2(fq1)* mutants displayed much lower levels of HUS-1 foci than wild-type controls (Figure 5C), despite the extensive accumulation of RAD-51 recombination intermediates (Figure S4B). Furthermore, wild-type germlines showed the presence of elongated HUS-1 signals that were not present in *scc-2(fq1)* mutants (Figure 5C). Thus, meiotic cohesin is also required for efficient recruitment of the 9-1-1 complex following γ irradiation.

The Formation of RAD-51 Filaments Is Delayed in the Absence of Cohesin

An important question arises from the experiments described above: why is loading of the 9-1-1 complex impaired in *scc-2* mutants despite the extensive presence of persistent recombination intermediates? Binding of the 9-1-1 complex to DSBs requires the processing of DSBs to expose regions of single-stranded DNA (ssDNA), as well as ssDNA/dsDNA junctions, which are the preferred binding substrate of the 9-1-1 complex [23]. We tested whether DSB processing was affected by the absence of meiotic cohesin by performing a time course analysis of the formation of RAD-51 filaments, which denote the presence of ssDNA, following the creation of DSBs by γ irradiation. In order to compare the processing of irradiation-induced DSBs between *scc-2(fq1)*, *scc-3(ku263)*, and wild-type controls, we eliminated endogenous DSBs by performing the experiments in the *spo-11* background. We took the first

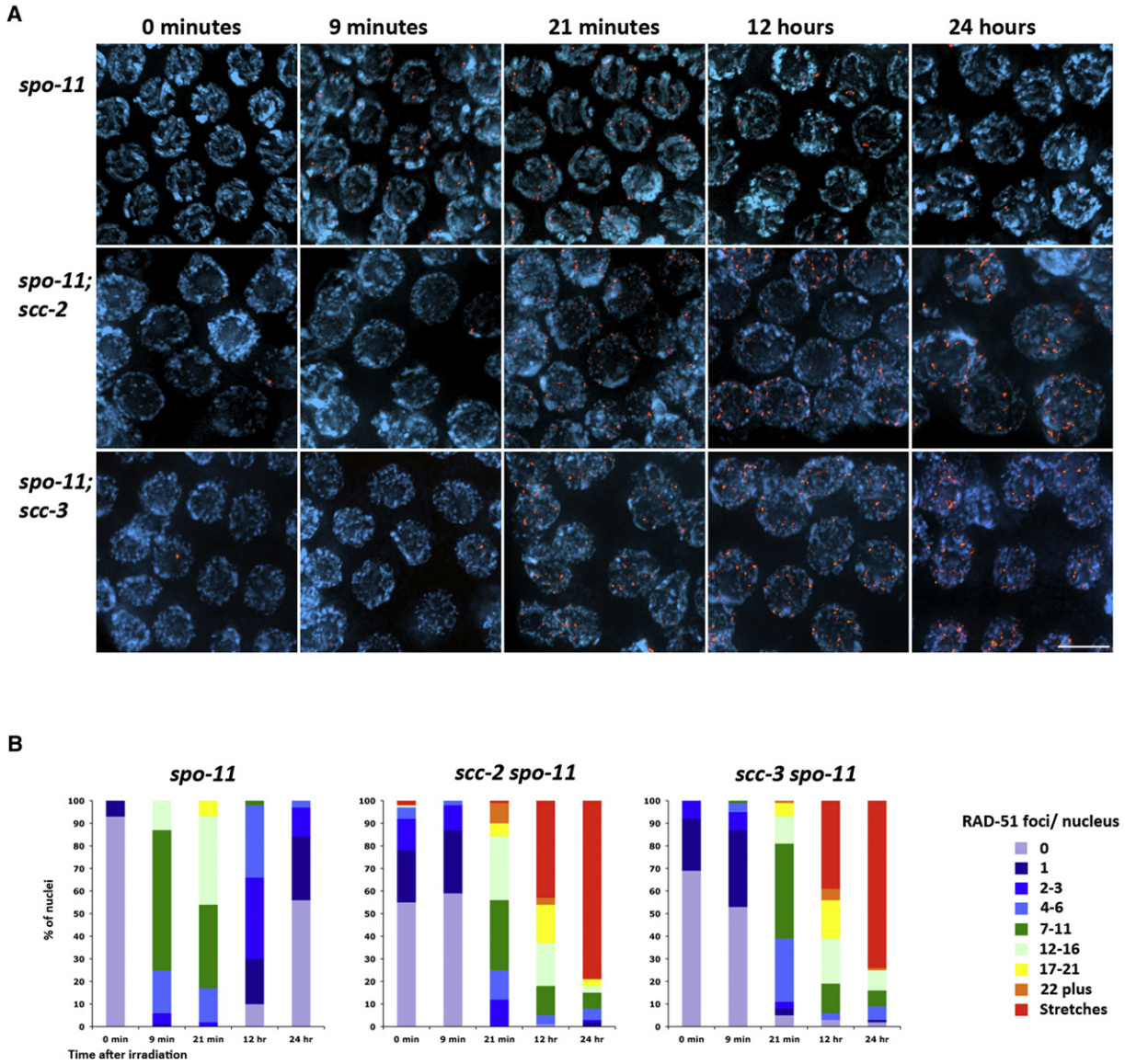

Figure 6. Cohesin Is Required for Early Processing of Irradiation-Induced DSBs

(A) Pachytene nuclei from mutants of the indicated genotypes stained with anti-RAD-51 antibodies and DAPI. Age-matched adult worms (20 hr post L4) were irradiated with 10 Gy. The time at which worms were dissected and fixed after irradiation is indicated on top of each column. Time zero corresponds to control worms before irradiation. Scale bar represents 5 μm.

(B) Quantification of RAD-51 foci from pachytene nuclei at the indicated times post irradiation. The x axis indicates time after irradiation, with T = 0 representing control worms before irradiation, whereas the y axis indicates the percentage of nuclei with a given number of RAD-51 foci (as indicated in the color key). One hundred nuclei were quantified per genotype in each time point.

time point 9 min after irradiating worms with 10 Gy. At this early time point, *spo-11* mutants showed substantially increased levels of RAD-51 foci compared to nonirradiated *spo-11* controls (T = 0), with the average number of RAD-51 foci per nucleus increasing from 0.07 to 8.5 (Figure 6). This demonstrated that after 9 min, many DSBs have been processed enough to promote the loading of RAD-51 to ssDNA. In contrast, the average number of RAD-51 foci per nucleus remained essentially constant between time zero controls and 9 min after irradiation in both *scc-2; spo-11* mutants (0.7 to 0.6) and *scc-3;spo-11* mutants (0.4 to 0.6) (Figure 6). Twenty-one minutes after irradiation, both mutants showed a significant increase in the average number of RAD-51 foci per nucleus (13.6 in *scc-2; spo-11* and 8.1 in *scc-3; spo-11*). These numbers were similar to the average seen in *spo-11* controls at the same time point (10.7), demonstrating that by 21 min, many DSBs have been processed enough to promote RAD-51 loading in the cohesin mutants. The following time points were taken at 12 and 24 hr post irradiation to observe whether DSBs had been repaired. Whereas RAD-51 numbers were clearly reduced in *spo-11* controls, both *scc-2; spo-11* and *scc-3; spo-11* showed extensive accumulation of RAD-51 foci and also elongated RAD-51 filaments (Figure 6), demonstrating that irradiation-induced DSBs are not repaired in a timely manner in the absence of meiotic cohesin. More importantly, the results from the 9 min time point demonstrate that there is a striking failure in the formation of RAD-51 filaments at this early stage in *scc-2; spo-11* and *scc-3; spo-11*

compared to *spo-11* controls. However, by 21 min, similar numbers of RAD-51 foci are present in all three genotypes, demonstrating that the impairment to load RAD-51 following irradiation in *scc-2* and *scc-3* mutants is temporal. Because the formation of RAD-51 nucleoprotein filaments requires that DSBs are resected to expose ssDNA, our results suggest that resection of DSBs, at least in its early stages, is slower in the absence of meiotic cohesin.

Discussion

Our investigations have uncovered an unexpected role for meiotic cohesin in the early processing of DSBs and in the activation of the pachytene checkpoint, showing that cohesin is a key player in the early stages of the meiotic DNA damage response. Furthermore, we have also shown that SCC-2 is required for cohesin loading during meiosis, thus demonstrating that the main mechanism of cohesin loading is conserved between the mitotic and the meiotic cell division programs.

Meiotic Loading of SMC Complexes by SCC-2

The Scc2/Scc4 complex is responsible for cohesin loading in somatic cells of all studied organisms [7, 10, 27], and it has also been proposed to be the main loader of condensin and SMC-5/6 in budding yeast mitotic cells [11, 12]. We have shown that although loading of meiotic cohesin is clearly impaired in the absence of SCC-2, substantial levels of both condensin II and the SMC-5/6 complex are loaded in the absence of SCC-2. Although these results do not rule out that SCC-2 may promote some level of condensin II and/or SMC-5/6 loading during meiosis, they clearly demonstrate that SCC-2-independent mechanisms for loading these two SMC complexes must exist during meiosis. Importantly, the efficient loading of condensin II and SMC-5/6 onto meiotic chromosomes in *scc-2* mutants, in which cohesin is not detected, also demonstrates that the loading of these two SMC complexes does not require cohesin.

Meiotic Cohesin and Early Processing of DSBs

We have made two important observations that reveal the involvement of cohesin in the early processing of DSBs. First, RAD-51 loading onto irradiation-induced DSBs is delayed by several minutes in both *scc-2* and *scc-3* mutants and second, loading of the 9-1-1 complex to damage sites, which requires processing of DSBs [23], is severely impaired in the absence of meiotic cohesin. Processing of DSBs is thought to involve two steps: first the MRX (Mre11, Rad50, Xrs2/Nbs1) complex and Sae2/CtIP cooperate to remove a small oligonucleotide, which results in the formation of an intermediate containing a short track of ssDNA, and then the exonuclease Exo1 and/or the helicase Sgs1 process this intermediate to generate long tracks of ssDNA [34, 42–44]. These tracks of ssDNA are bound by the recombinase RAD-51 to form nucleoprotein filaments that can search for a homologous DNA duplex. The role of Sae2/CtIP in the early processing of DSBs is clearly conserved because CtIP is required for DNA resection in human cells [45] and for the processing of SPO-11 DSBs in both *Arabidopsis* and *C. elegans* [46, 47]. Because we have observed that *scc-2* and *scc-3* mutants display a delayed loading of RAD-51 to irradiation-induced DSBs, this could imply that the first steps of DSB resection, those requiring Sae2/CtIP, are specifically affected by a lack of cohesin. However, it is unlikely that cohesin simply promotes Sae2/CtIP binding to DSBs, because in *C. elegans Sae2/CtIP* mutants, the RAD-51 protein completely fails to load onto SPO-11 DSBs [46], whereas RAD-51 is loaded to SPO-11 DSBs in *scc-2* and *scc-3* mutants, demonstrating that some resection must occur in these mutants. In fact, we observed that in *scc-2* mutants, both SPO-11 and irradiation-induced DSBs eventually led to the formation of long RAD-51 filaments at late pachytene, suggesting that resection may be still taking place at this stage, which could lead to hyperresected DSBs. This is in agreement with observations made at the *HIS4:LEU2* hotspot in yeast, which demonstrated the accumulation of hyperresected molecules during meiotic prophase in *rec8* mutants [14]. Thus, cohesin might be an important regulator of DSB resection during meiosis, with a previously unappreciated role in the timely execution of the initial steps of DSB processing.

Meiotic Cohesin and the Pachytene DNA Damage Checkpoint

We have shown that neither the accumulation of SPO-11-dependent recombination intermediates nor the induction of DSBs by γ irradiation elicit an apoptotic response of the pachytene checkpoint in *scc-2* or *scc-3* mutants. By examining the early steps of the pachytene checkpoint, we have observed that loading of the damage sensor 9-1-1 complex is severely impaired in the absence of meiotic cohesin. The localization of the 9-1-1 complex to sites of DNA damage is required for activation of the ATR kinase [48], a major regulator of the DNA damage response in somatic cells that is also required for activation of the pachytene DNA damage checkpoint in *C. elegans* [24]. We propose that the absence of meiotic cohesin impairs loading of the 9-1-1 complex to sites of DNA damage, which in turn prevents the activation of ATR (ATL-1 in *C. elegans*), thus rendering the pachytene checkpoint unable to signal the presence of persistent DNA damage.

How might meiotic cohesin promote loading of the 9-1-1 complex to sites of DNA damage? One possible explanation arises from our observation that meiotic cohesin is required for early DSB processing. Studies in human cells have established that CtIP-dependent resection of DSBs is required for ATR activation [45]. Furthermore, whereas the formation of DSBs in human cells initially leads to the activation of ATM, the progression of DSB resection potentiates, in a length-dependent manner, the activation of ATR [49]. Similarly, *C. elegans* mutants lacking the worm homolog of CtIP accumulate meiotic DSBs that fail to trigger an ATR-dependent apoptotic response of the pachytene checkpoint [46], suggesting that DSB resection is also required for ATR activation during meiosis. We have observed that *scc-2* and *scc-3* mutants are defective in both the activation of the pachytene DNA damage checkpoint and in the early processing of DSBs. Therefore, it is possible that these two events are mechanistically linked, such that meiotic cohesin is required to process DSBs in a manner that facilitates binding of the 9-1-1 complex to sites of persistent DNA damage.

Alternatively, meiotic cohesin may be required to promote binding of the 9-1-1 complex to damaged chromatin independently of its role in early DSB processing. Studies in yeast and vertebrate somatic cells have demonstrated an intricate connection between cohesin and the DNA damage response. In postreplicative yeast cells, DSB repair requires the loading of cohesin around the break site, and this damage-induced loading of cohesin depends on checkpoint proteins such as Tel1 (ATM), Mec1 (ATR), and Rad9 (a component of the 9-1-1 complex) [5, 6, 50]. In vertebrate cells, cohesin is also recruited

to sites of DNA damage [51, 52] and phosphorylation of Smc1 and Smc3 by ATM are required for the integrity of the intra-S phase checkpoint [19, 20, 52]. Cohesin is also required for the DNA damage checkpoint in postreplicative mammalian cells, where it promotes the recruitment of the Chk2-activator 53BP1 to damage sites [21]. Whether cohesin is recruited to sites of persistent damage during meiosis is not currently known, but we have shown that the cohesin loader SCC-2 localizes to the axial element of meiotic chromosomes during pachytene and that cohesin is required for activation of the pachytene checkpoint. Thus, recruitment of cohesin to damage sites may occur as part of the meiotic DNA damage response, and this event may be required for the efficient loading of the 9-1-1 complex.

In conclusion, our findings have revealed the involvement of meiotic cohesin in DSB processing and in the recruitment of the 9-1-1 complex to sites of damage, two key events of the DNA damage response. Whether the impaired recruitment of the 9-1-1 complex is a direct consequence of the defects in early DSB processing that we have observed in *scc-2* and *scc-3* mutants will require further investigation.

Experimental Procedures

Standard methods were used for maintenance of *C. elegans* strains, EMS mutagenesis, immunostaining, RNAi, irradiation experiments, and quantification of apoptosis and HUS-1::GFP foci. Detailed methods are provided in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.07.007.

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