Poly(ADP-ribose) glycohydrolase promotes formation and homology-directed repair of meiotic DNA double-strand breaks independent of its catalytic activity

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Summary

Poly(ADP-ribosyl)ation is a reversible post-translational modification synthesized by ADP-ribose transferases and removed by poly(ADP-ribose) glycohydrolase (PARG), which plays important roles in DNA damage repair. While well-studied in somatic tissues, much less is known about poly(ADP-ribosyl)ation in the germline, where DNA double-strand breaks are introduced by a regulated program and repaired by crossover recombination to establish a tether between homologous chromosomes. The interaction between the parental chromosomes is facilitated by meiotic specific adaptation of the chromosome axes and cohesins, and reinforced by the synaptonemal complex. Here, we uncover an unexpected role for PARG in promoting the induction of meiotic DNA breaks and their homologous recombination-mediated repair in *Caenorhabditis elegans*. PARG-1/PARG interacts with both axial and central elements of the synaptonemal complex, REC-8/Rec8 and the MRN/X complex. PARG-1 shapes the recombination landscape and reinforces the tightly regulated control of crossover numbers without requiring its catalytic activity. We unravel roles in regulating meiosis, beyond its enzymatic activity in poly(ADP-ribose) catabolism.

Key words: Meiosis, meiotic DNA repair, PARylation, PARG, *C. elegans* germ line

51 Introduction

52 Poly(ADP-ribosyl)ation (PARylation) is an essential post-translational modification involved in
 53 chromatin dynamics, transcriptional regulation, apoptosis, and DNA repair (Koh et al., 2004;
 54 Menissier de Murcia et al., 2003). PARylation is controlled by the opposing activities of PAR
 55 polymerases, PARP1 and PARP2 (PARPs), and PAR glycohydrolase (PARG) (O'Sullivan et
 56 al., 2019; Slade, 2019). The activities of PARPs are crucial for an efficient DNA damage
 57 response, as loss of PARP1 or PARP2 leads to hypersensitivity to genotoxic stress and
 58 impaired spermatogenesis, while the combined deficiencies of PARP1 and PARP2 cause
 59 embryonic lethality (Dantzer et al., 2006; Menissier de Murcia et al., 2003). Likewise, the
 60 PARG knock-out is embryonic lethal and depleted cells become sensitive to ionizing radiation
 61 (IR) and show aberrant mitotic progression (Ame et al., 2009; Koh et al., 2004).

62 Since PARP1/2 double mutants or PARG knock-outs are embryonic lethal in mouse and no
 63 orthologs are present in yeast, our understanding of the roles of PARylation during germ line
 64 development has been limited. *C. elegans* *parg-1/PARG* null mutants are viable and fertile
 65 (Byrne et al., 2016; St-Laurent et al., 2007), allowing us to analyze their function(s) during
 66 gametogenesis. It has been previously shown that *parp-1/-2* and *parg-1* mutants display
 67 hypersensitivity to IR exposure (Dequen et al., 2005; Gagnon et al., 2002; St-Laurent et al.,
 68 2007) however their roles during gametogenesis have remained poorly investigated.

69
 70 In sexually reproducing species, preservation of ploidy across generations relies on meiosis,
 71 a specialized cell division program which promotes the generation of haploid gametes
 72 (Zickler and Kleckner, 1999, 2015). The formation of crossovers (CO) is essential for faithful
 73 chromosome segregation into the gametes (Cao et al., 1990; Sun et al., 1989). Connected
 74 parental homologous chromosomes (also called bivalents) can cytologically be detected in
 75 diakinesis nuclei and are thus a readout for the success of the CO establishment. COs arise

76 by the generation and homologous recombination-mediated repair of programmed DNA
77 double-strand breaks (DSB) effectuated by the evolutionarily conserved topoisomerase VI-
78 like protein Spo11 (Keeney et al., 1997). The activity of Spo11 is tightly regulated to ensure
79 the correct timing, placement, and number of DSBs/COs along meiotic chromosome axes.
80 In *C. elegans*, several factors involved in promoting meiotic DSBs have been identified, and
81 those include MRE-11, HIM-5, HIM-17, DSB-1, DSB-2 and XND-1 (Chin and Villeneuve,
82 2001; Meneely et al., 2012; Reddy and Villeneuve, 2004; Rosu et al., 2013; Stamper et al.,
83 2013; Wagner et al., 2010). Of these, XND-1 and HIM-17 are known to also influence
84 germline chromatin structure (Reddy and Villeneuve, 2004; Wagner et al., 2010). DSB-1 and
85 DSB-2 appear to have roles in maintaining DSB competency throughout early pachytene
86 (Rosu et al., 2013; Stamper et al., 2013). MRE-11 functions both in DSB formation and
87 immediately downstream in end resection (Chin and Villeneuve, 2001); HIM-5 and DSB-2
88 have also recently been shown to couple DSB formation with HR-mediate repair (Macaisne
89 et al., 2018).

90

91 The distribution and number of DSBs and COs also undergoes multiple levels of regulation.
92 In all organisms studied, the number of DSBs exceeds the number of COs, with ratios
93 reaching 10:1 in some cases (Serrentino and Borde, 2012). The excess DSBs use HR-like
94 mechanisms to be repaired with high fidelity, with repair intermediates shunted into non-CO
95 (NCO) outcomes. Importantly, a robust inter-homolog repair bias ensures formation of the
96 obligate CO in the germ cells, which in *C. elegans* occurs even under subthreshold levels of
97 DSBs (Meneely et al., 2012; Rosu et al., 2011; Yokoo et al., 2012). CO interference (Youds
98 et al., 2010; Zickler and Kleckner, 1999) describes the phenomenon whereby CO-committed
99 intermediates influence nearby DSBs to be repaired as NCOs, ensuring that COs are well-
100 spaced across the genome. In *C. elegans*, CO interference is nearly complete, as each

101 chromosome pair receives, in most cases, only one CO (Hillers and Villeneuve, 2003). On
102 the autosomes of the worm, COs occur preferentially on the arms of the chromosomes, away
103 from the gene-rich region in the center of the chromosomes; on the heterochromatic-like X
104 chromosome, there is not gene cluster and COs are more evenly dispersed (Barnes et al.,
105 1995).

106
107 While CO interference explains much about CO distribution in most organisms, some COs
108 are known to arise from an interference-independent pathway. The COs generated through
109 interference-dependent (Class I) and interference-independent mechanisms (Class II) have
110 distinct genetic requirement, driven by MutS-MutL and Mus81 homologs respectively (de los
111 Santos et al., 2003). Genetic evidence suggests that, in *C. elegans*, only Class I COs are
112 present (Kelly et al., 2000; Zalevsky et al., 1999). Nevertheless mutants displaying
113 interference-insensitive COs have been reported (Tsai et al., 2008; Youds et al., 2010),
114 however, these are still dependent on the canonical MSH-5/COSA-1-mediated CO pathway
115 and they can be detected by genetic measurements of recombination (Yokoo et al., 2012).

116
117 CO-repair takes place in the context of the synaptonemal complex (SC), a tripartite
118 proteinaceous structure composed of axial and central elements, arranged as a protein-
119 zipper between each pair of homologs. The SC maintains homolog associations and
120 facilitates inter-homolog exchange of DNA during repair (Colaiacovo et al., 2003). Cross-talk
121 between the SC and COs is essential for modulating recombination. Incomplete synapsis
122 dramatically weakens CO interference and additional COs *per* chromosome can be observed
123 (Libuda et al., 2013; Rosu et al., 2011). Conversely, reduced, but not absent, recombination
124 levels causes premature desynapsis of the chromosome pairs that fail to establish a CO
125 (Machovina et al., 2016; Pattabiraman et al., 2017; Wagner et al., 2010).

Chromosome axis components, which in *C. elegans* include the HORMA-domain proteins HTP-3, HTP-1/2, and HIM-3 (Goodyer et al., 2008; Martinez-Perez and Villeneuve, 2005; Zetka et al., 1999), influence both the abundance of DSBs and the regulation of their repair.

In this study, we show an unexpected involvement of PARG-1 in promoting DSB and CO formation. We show that PARG-1 functions independently of the known DSB initiation factors and that it cooperates with HIM-5 to regulate global crossover numbers. PARG-1 is detected throughout the germ line and undergoes a progressive recruitment along synapsed chromosomes, culminating in the retraction to the short arm of the bivalent and enrichment at the putative CO sites. In the absence of PARG-1 function, we observe an accumulation of PAR on the meiotic chromosomes, which is suppressed by abrogation of PARP-2 function. We report the association of PARG-1 with numerous key proteins composing the meiosis-specific structure of the SC both by cytological and biochemical analysis. Surprisingly, we found that PARG-1 loading, rather than its catalytic activity, is essential to exert its function during meiosis. Our data strongly suggest that PARG has scaffolding properties which are important for the fine-tuning of meiotic recombination events.

142

143 **Results**

144 **PARG-1 is the main poly(ADP-ribose) glycohydrolase in the *C. elegans* germ line**

The *C. elegans* genome encodes two orthologs of mammalian PARG, PARG-1 and PARG-2 (Bae et al., 2019; Byrne et al., 2016; St-Laurent et al., 2007). Both mutants are hypersensitive to IR exposure and more recently it was shown that *parg-2* is involved in the regulation of HR-dependent repair of ectopic DSBs by influencing the extent of resection upon IR (Bae et al., 2019). To explore possible functional links or redundancies between *parg-1* and *parg-2*, we used CRISPR to engineer *parg-2* null mutations in both the wild type

(WT) and *parg-1(gk120)* deletion mutant backgrounds (Fig.1A). In contrast to mammalian PARG, *C. elegans parg-1* and *parg-2* are largely dispensable for viability (Fig. 1B). However, abrogation of *parg-1*, but not *parg-2* function, led to increased levels of embryonic lethality and segregation of males (which arise from X chromosome nondisjunction (Hodgkin et al., 1979). Screening of *parg-1 parg-2* double mutants did not reveal synthetic phenotypes but recapitulated the *parg-1* phenotypic features, indicating that *parg-2* does not exert prominent roles in an otherwise wild type background and cannot compensate the lack of *parg-1* function (fig. 1B).

To confirm a role of PARG-1 and PARG-2 in PAR catabolism, we investigated PAR accumulation in the mutant animals. Because PAR undergoes a rapid turnover, PAR cannot be detected in wild-type germ lines (Fig. 1C). By contrast, we detected PAR at all stages of meiotic prophase I in *parg-1* mutants. Since PAR accumulation was neither seen in *parg-2* mutants nor further enhanced in *parg-1 parg-2* (Fig.1C), we infer that PARG-1 is the major PAR glyohydrolase in the worm germ line.

Removal of the PAR polymerases *parp-1/-2*, suppressed accumulation of PAR in *parg-1* mutant germ cells (Fig. 1D). Interestingly, we found that while abrogation of *parp-1* function reduced detectable levels to roughly 30%, lack of *parp-2* alone was sufficient to bring PAR staining to background levels. Since both *parp-1(DDR31)* and *parp-2(ok344)* mutant alleles are null, this data suggests that PARP-2 is mainly responsible for the synthesis of PAR during *C. elegans* meiotic prophase I.

Since PAR accumulates at sites of DNA damage in somatic cells (Kaufmann et al., 2017; Mortusewicz et al., 2011), we asked whether the accumulation of PAR in meiotic prophase

nuclei was dependent on the formation of meiotic DSBs. Surprisingly, we found that in the gonads of *parg-1 spo-11* double mutants, in which no programmed DSBs are made, PAR still localized within prophase I nuclei (Fig. S1), indicating that PAR synthesis occurs independently of physiological DNA damage during gametogenesis.

180

PARG-1 forms protein complexes with SC components and its localization requires chromosome axes

To detect PARG-1, we raised a *C. elegans*-specific anti-PARG-1 monoclonal antibody which we used in western blot analysis (Fig. 2A). This antibody confirmed that *parg-1(gk120)* is a null allele. We find expression of PARG-1 in all subcellular compartments in wild-type animals (Fig. 2A), as similarly observed in mammalian mitotic cells (Meyer-Ficca et al., 2004; Ohashi et al., 2003; Winstall et al., 1999). Since localization of PARG is not known in meiocytes, we employed CRISPR to tag the 3' end of the endogenous *parg-1* locus with a GFP-tag. We assessed the functionality of the fusion protein by monitoring PAR accumulation in the gonad, embryonic lethality, and male progeny, none of which showed any differences compared to WT, indicating that PARG-1::GFP is catalytically active and fully functional (Fig. 1B-C and S1). Moreover, western blot analysis employing either anti-PARG-1 or anti-GFP antibodies on fractionated extracts from *parg-1::GFP* worms revealed identical expression as seen with untagged PARG-1 (Fig. 2B), further confirming that the GFP-tag did not affect PARG-1 stability or expression.

196

Immunofluorescence analyses showed that PARG-1::GFP is first detected in pre-meiotic and leptotene/zygotene nuclei and then became progressively enriched along chromosomes throughout pachytene (Fig. 2C). Co-staining with axial proteins HTP-1/HTP-3 and the central SC component SYP-1 (Goodyer et al., 2008; MacQueen et al., 2002; Martinez-Perez and

Villeneuve, 2005) revealed recruitment of PARG-1::GFP onto synapsed chromosomes and its retraction in late pachytene cells to the short arm of the bivalent (Fig. 2D), a chromosomal subdomain formed in response to CO formation. The short arm of the bivalent harbors the chiasma and the central elements of the SC (de Carvalho et al., 2008; Martinez-Perez et al., 2008). Overlapping localization of PARG-1::GFP with both the CO promoting factor COSA-1 and SYP-1 (Fig. 2E) confirmed recruitment of PARG-1 to this chromosomal subdomain, similar to other SC central elements components (Bhalla et al., 2008; Janisiw et al., 2018; Jantsch et al., 2004; Li et al., 2018; MacQueen et al., 2002). In CO-defective *cosa-1* mutant animals, we observed that the initial loading of PARG-1::GFP to the SC was unaffected, but no retraction was observed, confirming that the relocalization of PARG-1 is dependent on bivalent formation (Fig. 2F).

Based on its localization to the SC, we tested whether PARG-1::GFP loading was dependent on chromosome axis or synapsis establishment. Loss of *htp-3*, encoding a HORMA domain-containing protein essential for axis morphogenesis (Goodyer et al., 2008), disrupted PARG-1::GFP localization, resulting in nucleoplasmic accumulation and occasional association with SYP-1-containing polycomplexes (Fig. 2G). By contrast, PARG-1::GFP exhibited linear staining along the chromosome axes in synapsis-deficient *syp-2* mutants (Fig. 2H), where only axial elements are loaded onto the chromosomes (Colaiacovo et al., 2003; Goodyer et al., 2008; Martinez-Perez and Villeneuve, 2005). Thus, we conclude that PARG-1 is recruited to the SC in an HTP-3-dependent manner and its localization changes in response to CO-mediated chromosome remodeling.

Since PARG-1 localizes to chromosome axes and requires HTP-3 for loading, we wondered whether these factors formed protein complexes *in vivo*. To test for their possible association,

we performed immunoprecipitation assays by pulling down HTP-3::GFP (Paix et al., 2015) and proceeded with western blot analysis to detect PARG-1. Robust interaction between HTP-3::GFP and PARG-1 was observed (Fig. 2I). Further, to assess whether PARG-1 establishes physical interactions with additional chromosome axis components as well, we also performed co-immunoprecipitation experiments pulling down HTP-1::GFP and REC-8::GFP, (Crawley et al., 2016; Silva et al., 2014). Western blot showed that PARG-1 co-immunoprecipitated with both HTP-1 and REC-8 (Fig. 2J). Extending these analysis to the central elements of the SC component, we found that PARG-1 could be pulled down with GFP::SYP-3 (Rog and Dernburg, 2015) (Fig. 2I). Together with our localization studies, these physical interactions indicate that PARG-1 is an intrinsic component of the SC.

Loss of *parg-1* suppresses chromosome abnormalities arising from impaired DSB resection

Given PARG-1 recruitment along the SC and enrichment at the presumptive CO sites, we sought to investigate whether synapsis and CO formation are impaired in *parg-1* mutants. Using antibodies directed against HTP-3 and SYP-1 to monitor the establishment of the SC, we observed no difference between the wild type and *parg-1* mutants (Fig. S2A). DAPI-staining of diakinesis nuclei revealed the correct complement of six bodies as in wild-type worms (Fig. S2B). Thus, we infer that *parg-1* is dispensable for synapsis and CO formation.

We next addressed whether loss of *parg-1* would impact the formation and processing of recombination intermediates by analyzing the dynamic behaviour of the recombinase RAD-51, which forms discrete chromatin-associated foci with a distinct kinetics of appearance and disappearance (Alpi et al., 2003; Colaiacovo et al., 2003), (Fig. 3A-B). While in wild-type worms we see a progressive increase of RAD-51, peaking in early-mid pachytene (zone 3)

251 and disappearing by late pachytene (zone 6), in *parg-1* mutants, we observed the delayed
 252 formation of RAD-51 foci with progressive accumulation at the pachytene stage. RAD-51 foci
 253 formation was entirely suppressed by SPO-11 removal, suggesting specific abnormalities in
 254 the induction and/or processing of meiotic DSBs rather than spontaneous or unscheduled
 255 damage arising during mitotic replication (Fig. 3A-B).

256
 257 Since RAD-51 foci appeared with delayed kinetics, we wanted to investigate whether PARG-
 258 1 might have a role in the regulation of DSB formation. Since tools to directly quantify meiotic
 259 DSBs are presently not available in *C. elegans*, we took advantage of a genetic epistasis
 260 analysis to determine if *parg-1* has a role in DSB formation. In diakinesis nuclei, DSB
 261 resection-defective mutants, such as *com-1/CtIP/Sae2* and *mre-11(iow1)/Mre11*, display
 262 massive chromatin clumps and occasional chromosome fragments that arise from aberrant
 263 repair of meiotic DSBs. Accordingly, these clumps and fragments are fully suppressed in the
 264 DSB-devoid *spo-11* mutants (Chin and Villeneuve, 2001; Penkner et al., 2007; Yin and
 265 Smolikove, 2013). Similarly, in the *com-1; parg-1* and *parg-1; mre-11(iow1)* double mutants,
 266 we found that the vast majority of diakinesis nuclei contained twelve intact univalents (Fig.
 267 3C-D). These results are consistent with a role for PARG-1 in DSB induction, but could also
 268 reflect a function for *parg-1* in targeting breaks to alternative repair pathways.

269
 270 To distinguish between these possibilities, we exposed the aforementioned double mutants
 271 to gamma irradiation (IR) to ectopically induce DSBs. We reasoned that if *parg-1* mutants
 272 were defective solely in DSB induction, the breaks induced by IR should restore the aberrant
 273 chromosome morphology typical of *com-1* and *mre-11*. By contrast, if *parg-1* has a role in
 274 repair pathway utilization, the IR-induced breaks would still be shunted into an alternative
 275 pathway and the appearance of DAPI bodies would remain unchanged after IR exposure.

276 Diakinesis nuclei of irradiated *com-1*; *parg-1* reverted to the *com-1*-like (chromosome
277 clumping-fusion) phenotype, supporting a putative role for PARG-1 in DSB induction. By
278 contrast, *parg-1*; *mre-11(iow1)* were indistinguishable from non-irradiated controls, indicating
279 that PARG-1 may also influence DNA repair pathway choice when *mre-11*, but not *com-1*,
280 function is compromised (Fig. 3C-D). Together, these results suggest an involvement of
281 PARG-1 in promoting both the formation and repair of meiotic DSBs.

282

283 **PARG-1 augments the formation of meiotic DSBs and interacts with MRE-11**

284 To further explore PARG-1's putative involvement in promoting DSBs, we tested its ability to
285 genetically interact with mutations that are impaired in DSB induction. We combined the
286 *parg-1(gk120)* deletion with two hypomorphic *him-17* alleles and with a *him-5* null mutation
287 that reduce, but do not completely eliminate, SPO-11-dependent DNA breaks (Meneely et al.,
288 2012; Reddy and Villeneuve, 2004). Consistent with published results, we observed that
289 these single mutants displayed reduced numbers and delayed formation of RAD-51 foci
290 (Meneely et al., 2012; Reddy and Villeneuve, 2004). The number of RAD-51 foci was further
291 diminished in-both *parg-1*; *him-5* and *parg-1*; *him-17* double mutants (Fig. S3A). The defects
292 in RAD-51 filament formation in *parg-1*; *him-17* and *parg-1*; *him-5* double mutants were
293 correlated with defective loading of pro-CO factors such as HA::RMH-1, GFP::MSH-5 and
294 OLLAS::COSA-1 (Fig. 4B-C and S4) as we would expect for mutations that impair DSB
295 formation. Analysis of diakinesis nuclei revealed an extensive lack of chiasmata (Fig 4D) and
296 enhancing embryonic lethality (Fig S3B) in the double mutants as expected from the defects
297 in CO repair. Loading of RMH-1, MSH-5 and COSA-1, as well as bivalent formation, were
298 largely, although not completely, rescued by IR exposure (Fig. 4A-D and S4), further
299 corroborating that the lack of COs was due to impaired DSB formation. Abrogation of PARG-
300 1 function also exacerbated the CO defect observed in both young (day #1) and old (day #2)

301 *dsb-2* mutants (Fig. S3C), which display an age-dependent loss in the proficiency to induce
302 DSBs (Rosu et al., 2013). These results indicate that loss of *parg-1* function impairs a
303 parallel, *him-17*-, *him-5*, and *dsb-2*-independent pathway for DSB induction.

304

305 To further interrogate PARG-1 function in DSB formation, we next sought to investigate the
306 interplay between PARG-1 and DSB-promoting factors. To this end, we assessed the
307 localization of the pro-DSB factors HIM-5::3xHA, HIM-17::3xHA, DSB-2 and XND-1 in *parg-1*
308 mutants. We observed no gross defects in localization compared to the controls (Fig. S5A-D),
309 which suggests that PARG-1 is not required for the loading of these pro-DSB factors.

310 Conversely, PARG-1::GFP loading appeared normal in *him-5*, *dsb-2*, and *him-17* (null and
311 hypomorph alleles) mutant backgrounds. The only difference compared to wild type is the
312 lack of retraction of PARG-1::GFP to the short arm of the bivalent, which is a consequence of
313 the lack of COs caused by these mutations (Fig. S5E) similar to *cosa-1* mutations (described
314 above). Given the synergistic phenotypes observed in the double mutants and the lack of
315 defects in the loading/expression of DSB-promoting proteins, we conclude that PARG-1
316 supports formation of DSB *via* alternative pathway(s) to the known pro-DSB factors in *C.*
317 *elegans*.

318

319 It has been previously shown that the axial component HTP-3 promotes the formation of
320 meiotic DSBs in worms possibly through its interaction with the MRN/X complex factor MRE-
321 11, known to be involved in DSB formation (Goodyer et al., 2008; Hayashi et al., 2007; Yin
322 and Smolikove, 2013). Since we already showed an interaction between HTP-3::GFP and
323 PARG-1 (Fig. 2I), we now wanted to address if this extended to an association with MRE-11.
324 Western blot analysis for PARG-1 on GFP pull downs performed with the *mre-11::GFP*
325 transgene (Reichman et al., 2018) also showed coimmunoprecipitation (Fig. 4E). This

326 suggests that the PARG-1-mediated activity in promoting meiotic DSBs may intersect the
327 HTP-3-MRE-11 axis.

328

329 **PARG-1 and HIM-5 modulate crossover numbers**

330 While the loading of pro-CO factors was largely rescued in the irradiated *parg-1; him-5*
331 double mutants, over half of the diakinesis nuclei still displayed univalents (Fig. 4D),
332 indicating substantial, yet incomplete, restoration of chiasmata. The dose employed in our
333 irradiation experiments (10 Gy) sufficed to fully elicit bivalent formation in *him-5, spo-11*,
334 *parg-1 spo-11* and *spo-11; him-5* (Mateo et al., 2016), (Fig. 4D). Therefore, we conclude that
335 additional CO execution steps are defective in *parg-1; him-5*. Importantly, this phenotype was
336 not observed in *parg-2; him-5* double mutants, in which the number and structure of DAPI
337 bodies resembled *him-5* single mutants both before and after exposure to IR (Fig. S6). We
338 thus confirmed that the recombination defects observed in *parg-1; him-5* are specific to
339 impaired *parg-1* function.

340

341 The SC is a dynamic structure that responds to the presence or absence of (as yet
342 unidentified) CO intermediates in the nucleus. When COs are made, they stabilize the SC in
343 *cis*. In genetic backgrounds with reduced DSB induction, such as those described above, the
344 chromosome pairs lacking a CO undergo desynapsis at a late pachytene stage, whereas in
345 mutants that completely lack COs, homologs remain fully synapsed but the SC subunits are
346 more labile (Machovina et al., 2016; Pattabiraman et al., 2017). Given the partial rescue of
347 chiasmata formation in *parg-1; him-5* double mutants after IR and the localization of PARG-1
348 to the SC, we sought to determine if CO designation and SC dynamics are decoupled by
349 simultaneous loss of both HIM-5 and PARG-1 functions.

350

351 In unirradiated *him-5* mutant worms, the sole absence of a CO on chromosome X caused its
 352 extensive desynapsis in late pachytene nuclei (Fig. 5A, C), recapitulating previous
 353 observations (Machovina et al., 2016). By contrast, in *parg-1; him-5* double mutants, the
 354 majority of nuclei showed full synapsis (Fig. 5A-C), in agreement with the fact that de-
 355 synapsis is not triggered when CO establishment is fully abrogated (Machovina et al., 2016;
 356 Pattabiraman et al., 2017). In support of this interpretation, we show that nuclei containing
 357 fully synapsed chromosomes displayed no COSA-1 loading in unirradiated *parg-1; him-5*
 358 double mutants (Fig. 5D). Immunostaining for H3K4me2, a histone modification that shows
 359 specific enrichment on the autosomes, but not on the X chromosome (Reuben and Lin,
 360 2002), further revealed that the X chromosome was fully synapsed in *parg-1; him-5* doubles,
 361 consistent with the lack of a CO and in stark contrast to *him-5* mutants (Fig. 5C).

362
 363 We next wanted to address whether SC stabilization and CO formation are coordinated in the
 364 *parg-1; him-5* double mutants after irradiation, where six COSA-1 foci were observed (Fig.
 365 5B) but univalents resulted (Fig. 4D). For this analysis, we undertook a time course analysis.
 366 In the *him-5* single mutant, 10 Gy of IR is sufficient to both rescue COSA-1 loading and to
 367 suppress X chromosome desynapsis as observed both 8 hr and 17 hr post-IR, as shown
 368 previously (Machovina et al., 2016). No defects were observed in the *parg-1* single mutants.
 369 In *parg-1; him-5*, COSA-1 foci numbers were also largely rescued at 8 hr post-IR and
 370 remained steady at 17-hours post-IR (Fig 5B). However, synapsis levels started to decline at
 371 8 hr post-IR and were further reduced at 17-hours after irradiation (Fig. 5A). Strikingly, a
 372 substantial number of nuclei exhibited desynapsis, yet they showed the full complement of
 373 six COSA-1 foci (8 hr= 52% and 17 hr= 74.3%) (Fig. 5D-E), a situation never described in
 374 other meiotic mutants. COSA-1 foci were never associated with unsynapsed chromosome(s)
 375 at the observed time points. The fact that these nuclei contain six COSA-1 foci, as in wild-

376 type animals, suggests that some chromosomes might bear additional COSA-1 marked CO
377 events. These results revealed that the global regulation of CO-mediated DNA repair is
378 profoundly perturbed in the absence of PARG-1 and HIM-5 functions.

379

380 To further characterize the defects in *parg-1; him-5* mutants, we examined the meiotic
381 progression marker phospho-SUN-1^{S8} (Penkner et al., 2009). In wild-type animals, SUN-1^{S8}
382 is phosphorylated in leptotene/ zygotene and dissipates at mid- pachytene (Woglar et al.,
383 2013). The lack of DSBs or impaired homologous recombination-mediated repair trigger
384 retention of phospho-SUN-1^{S8} at the nuclear envelope until the late pachytene stage (Woglar
385 et al., 2013). In DSB-defective mutants, but not in mutants with impaired recombination
386 (such as *cosa-1*), delayed removal of phospho-SUN-1^{S8} is rescued by exogenous DSB
387 induction (Machovina et al., 2016; Rosu et al., 2013; Stamper et al., 2013; Woglar et al.,
388 2013). Since *parg-1; him-5* double mutants appear to carry defects in both DSB induction and
389 repair, we analyzed phospho-SUN-1^{S8} localization before and after IR exposure to assess
390 whether these phenotypes could be uncoupled by phospho-SUN-1^{S8} dynamics.

391 *parg-1* mutants displayed mild prolongation of phospho-SUN-1^{S8} staining (Fig. S6),
392 consistent with the delayed accumulation of RAD-51 foci (Fig. 3A and S3). *him-5* and *parg-1*;
393 *him-5* mutants showed comparable, prolonged phospho-SUN-1^{S8} staining under
394 unchallenged growth conditions, consistent with defective DSB induction and recombination.

395 While IR exposure fully suppressed the persistence of phospho-SUN-1^{S8} in the *him-5* as
396 expected, it only mildly suppressed it in *parg-1; him-5* (Fig. S7). The inability of IR to
397 suppress phospho-SUN-1^{S8} accumulation further reinforces the conclusion that lack of both
398 PARG-1 and HIM-5 impairs both meiotic DSB formation and repair.

399

400

401 **PARG-1 shapes the recombination landscape and reinforces CO interference**

402 Given the involvement of *parg-1* in regulating not only DSB formation, but also homology-
 403 mediated repair, we investigated the recombination frequency in different genetic intervals on
 404 chromosome I and V by monitoring SNP markers in Bristol/Hawaiian hybrids, which allowed
 405 us to assess both CO numbers and their position (Hillers and Villeneuve, 2009). We found a
 406 striking increase of COs in the central regions of both chromosomes (Fig. 6A-B), where COs
 407 are usually absent in the wild type (Lim et al., 2008). In addition, double and triple COs were
 408 observed, albeit at a low frequency. These results revealed that impaired *parg-1* function
 409 impacts the global levels and distribution of COs and weakens CO interference in *C. elegans*.
 410

411 **PARG-1 catalytic activity is dispensable for meiotic functions**

412 We next sought to investigate whether PARG-1 catalytic activity is necessary to exert its
 413 function during meiosis. To this end, we generated a *parg-1* “catalytic-dead” mutant (referred
 414 to as *parg-1(cd)* hereafter) using CRISPR to mutate two glutamates in the catalytic domain
 415 (E554, 555A). These amino acids are conserved throughout evolution and were shown to be
 416 essential for PARG activity *in vitro* in both mammals and nematodes (Mortusewicz et al.,
 417 2011; Patel et al., 2005; St-Laurent et al., 2007). Immunostaining analysis in *parg-1(cd)* and
 418 *parg-1(cd)::GFP* revealed accumulation of PAR on meiotic chromosome axes as in *parg-*
 419 *1(gk120)* null mutants, indicating that also *in vivo* E554-E555 are necessary for PAR removal
 420 (Fig. S8). PARG-1^{CD}::GFP was expressed and loaded in meiocytes (Fig. 7A) but displayed
 421 prolonged localization along the chromosomes in late pachytene cells, where PARG-1
 422 normally is retained mostly at the short arm of the bivalent in control animals (Fig. 2).
 423 Western blot analysis showed that the overall levels of both PARG-1^{CD}::GFP and untagged
 424 PARG-1^{CD} were indeed increased, ruling out possible artifacts due to the addition of GFP

(Fig. 7B). The blots were also probed with anti-PAR antibodies and this confirmed that PAR accumulates in strains with compromised glycohydrolase activity (Fig. 7B).

To assess whether the catalytic activity of PARG-1 was required for the induction and/or repair of meiotic DSBs, we analyzed the *parg-1(cd); him-5* double mutants as described above. Offspring viability was only mildly reduced compared to *him-5* mutants (Fig. 7C). This indicates robust establishment of chiasmata in contrast to the *parg-1(gk120); him-5* double mutants. Similarly, X chromosome desynapsis (Fig. 7D-E) and its consequent nondisjunction (Fig. 7F) were rescued to the same extent upon IR exposure in both *parg-1(cd); him-5* and *him-5* mutants, unlike the lack of rescue in *parg-1(gk120); him-5* (Fig. 5A). These results suggest that PARG-1 loading onto chromosomes and/or a non-catalytic function of PARG-1 are essential to avert recombination defects in the absence of HIM-5. This interpretation was reinforced by the observation that the simultaneous removal of *parp-1* and *parp-2* did not rescue CO formation in *parg-1(gk120); him-5* mutants (Fig. 7F), indicating that CO defects are independent of PAR. Thus, we conclude that the glycohydrolase activity of PARG-1 is not required to promote induction of meiotic DSBs and their homologous recombination-mediated repair.

Discussion

PARylation has been extensively studied in the context of the DNA damage response in mitotic mammalian cells, where it facilitates the repair of DNA lesions by promoting both the recruitment of repair factors and mediating local chromatin relaxation around damage sites (Gibson and Kraus, 2012; Gupte et al., 2017; Ray Chaudhuri and Nussenzweig, 2017; Weaver and Yang, 2013). In contrast to PARP1/2, the functions of PARG have been much less investigated due to the lack of a suitable model system, since PARG null mutants are

embryonic lethal in mammals (O'Sullivan et al., 2019). We found that the *C. elegans* PARG-1 regulates DSB induction, in parallel to the so far known HIM-17/HIM-5/DSB-1/DSB-2-dependent routes. Moreover, our data demonstrate that PARG-1 regulates homology-directed repair of DSBs by operating within a functional module with HIM-5 to ensure the efficient conversion of recombination intermediates into post-recombination products, ultimately controlling global CO numbers.

Our cytological analysis, in combination with co-immunoprecipitation assays (Fig. 2), identified PARG-1 as an intrinsic component of the SC, where it is recruited *via* interaction with the chromosome axis protein HTP-3. Studies in mammalian mitotic cells reported nucleoplasmic localization of PARG and robust recruitment onto the DNA lesions induced by laser microirradiation (Kaufmann et al., 2017; Mortusewicz et al., 2011). The association with a meiosis-specific structure such as the SC therefore suggests distinct functional regulation in meiotic cells. Interestingly, PARG-1 retracts to the short arm of the bivalent and becomes enriched with SYP proteins at the presumptive CO sites in late pachytene nuclei (Fig. 2C-D), a localization also described for DNA repair and CO-promoting factors (Bhalla et al., 2008; Janisiw et al., 2018; Jantsch et al., 2004; Li et al., 2018). Nevertheless, abrogation of synapsis did not impair loading of PARG-1 along the chromosomes, a prerogative typically observed for axial rather than central components of the SC (de Carvalho et al., 2008; Goodyer et al., 2008; Martinez-Perez and Villeneuve, 2005; Zetka et al., 1999). This would suggest that PARG-1 may be targeted to both lateral and central elements of the SC or shift from the former to the latter upon CO-mediated chromosome remodeling.

In support of a dynamic model of PARG-1 localization, PARG-1 was found in protein complexes both with HTP-1, HTP-3 and REC-8, all proteins localizing to chromosome axes (Goodyer et al., 2008; Martinez-Perez et al., 2008; Pasierbek et al., 2001), and also with

475 SYP-3, which is a component of the central part of the SC (Smolikov et al., 2007). We believe
 476 that the localization of PARG-1 to the chromosome axes and its interaction with HTP-3 might
 477 hold crucial functional implications for promoting formation of meiotic DSBs. Many axial
 478 proteins, including *C. elegans* HTP-3, have been shown to directly influence abundance of
 479 DSBs during meiosis in several organisms (Goodyer et al., 2008; Kleckner, 2006), and
 480 therefore PARG-1 might exert its pro-DSB functions by operating from within the SC.

481

482 An activity of PARG-1 in promoting meiotic DSB formation by directly regulating pro-DSB
 483 factors is less likely, since the synergistic effects between *parg-1* and *him-17-him-5-dsb-2*
 484 mutants (Fig. 4, Fig. S5) clearly place PARG-1 in a parallel, distinct pathway. Consistently,
 485 expression and localization of PARG-1 and HIM-17, HIM-5 or DSB-2 were not mutually
 486 dependent (Fig. S5). We cannot rule out the possibility that PARG-1 may contribute to DSB
 487 formation by modulating SPO-11 activity or its recruitment to the presumptive DNA break
 488 sites. However, this is an unlikely scenario, since neither defects in bivalent formation nor
 489 RAD-51 loading were observed in *parg-1* mutants (Fig. 3; Fig. S2 and S3), as one might
 490 expect much more severe defects for as general loading problem. An additional argument in
 491 support of a model where interaction with HTP-3 might be key for PARG-1-mediated pro-
 492 DSB function, comes from its co-immunoprecipitation with MRE-11 (Fig. 4E), a proven
 493 interaction partner of HTP-3 (Goodyer et al., 2008). MRE-11 holds important roles in break
 494 resection and in *C. elegans* also break formation across species (Johzuka and Ogawa, 1995;
 495 Yin and Smolikove, 2013). MRE-11 has been invoked as a putative substrate intersected by
 496 HTP-3 function in inducing meiotic breaks (Goodyer et al., 2008). Therefore, PARG-1 might
 497 act together with HTP-3 and MRE-11 to ensure normal levels of breaks.

498

499 Our analysis also revealed that PARG-1, both independently and in combination with HIM-5,
500 plays important roles in the global regulation of meiotic recombination. In fact, *parg-1* mutants
501 show a profoundly perturbed recombination landscape, as distribution of COs displayed a
502 marked shift towards the center of the autosomes (Fig. 6), a chromosome domain normally
503 devoid of COs in wild-type animals (Barnes et al., 1995). This feature has also been
504 observed in mutants with reduced levels bivalent formation or aberrant DSB repair (Jagut et
505 al., 2016; Li et al., 2018; Meneely et al., 2012; Saito et al., 2013; Saito et al., 2009).
506 Moreover, CO interference appeared weakened in absence of *parg-1*, suggesting a
507 diminished stringency in the control of CO numbers.

508

509 The intermediates formed upon abrogation of *parg-1* function are nonetheless fully
510 competent to be processed as COs, as long as HIM-5 function is preserved. In fact, while
511 bivalent formation was fully restored in *parg-1; him-17* double mutants upon IR exposure
512 (Fig. 4D) (arguing for a rescue of reduced DSB levels), diakinesis of irradiated *parg-1; him-5*
513 mutant worms showed only a partial restoration of chiasmata, highlighting a repair defect as
514 well (Fig. 4D). The mutual requirement of PARG-1 and HIM-5 in the reciprocal mutant
515 background suggests the presence of a repair mechanism that relies on these two factors in
516 order to efficiently complete interhomolog recombination repair. Both *him-5* and *dsb-2* exert
517 regulatory functions on DNA repair pathway choice during gametogenesis (Macaisne et al.,
518 2018) and our work also highlights *parg-1* as an important factor operating within such a
519 process. This is also exemplified by our finding that diakinesis nuclei in *parg-1; mre-11(iow1)*
520 display intact, well-shaped univalents both before and after IR exposure, in contrast to the
521 chromatin clumps observed in the *mre-11(iow1)* separation of function mutant (Fig. 3) (Yin
522 and Smolikove, 2013), indicating that PARG-1 can act as a switch in channeling DSB repair
523 into multiple branches.

524 Simultaneous abrogation of *parg-1* and *him-5* function caused much more severe aberrations
525 than just reduced recombination: we found that IR exposure restored COSA-1 loading to the
526 wildtype levels (six foci/nucleus) in pachytene cells (consistent with impaired break
527 formation); nevertheless large portions of chromatin, possibly corresponding to whole
528 chromosome pairs rather than local regions, were devoid of SYP-1/COSA-1 in many of these
529 nuclei. These data indicate that additional COs have been designated on remaining, SC-
530 associated chromosomes (Fig. 5).

531

532 Previous studies in *ex vivo* somatic cells suggested possible functions of PARG that are
533 independent of its catalytic activity or PAR synthesis (Mortusewicz et al., 2011).
534 Our data show that in catalytically impaired *parg-1(cd)* mutants, which consistently
535 accumulate PAR as in *parg-1(gk120)* nulls (Fig. S8), the inactive protein was recruited at
536 higher levels and displayed delayed redistribution along the chromosomes in late pachytene
537 nuclei (Fig. 7A). This is in agreement with reports in mammalian cells showing that PARG^{KD}
538 is recruited to laser-induced microirradiation sites with faster kinetics compared to PARG^{WT}
539 and that this recruitment is only partially dependent on the PARP1 function (Mortusewicz et
540 al., 2011). Strikingly, PARG-1^{CD}::GFP was still capable of promoting chiasmata formation on
541 the autosomes in *him-5* mutants: in fact, the embryonic viability and numbers of DAPI-bodies
542 in *parg-1(cd); him-5* were comparable to *him-5* single mutants before and after IR exposure,
543 and importantly, desynapsis was not observed. This suggests that the loading of PARG-1,
544 rather than its enzymatic activity for PAR removal, was sufficient to induce DSBs and
545 promote efficient bivalent formation in the presence of exogenous DSBs (Fig. 7). This was
546 further corroborated by the fact that in the *parp-1; parp-2; parg-1(gk120); him-5* quadruple
547 mutants, bivalent formation was not rescued, demonstrating that the roles exerted by PARG-
548 1 in promoting DSB induction and meiotic repair are independent of PARylation.

549 Altogether, we demonstrate that the catalytic activity and the scaffolding properties of PARG
550 are required for distinct cellular processes. Our study establishes a crucial role of PARG
551 during meiotic prophase I in augmenting induction of meiotic DSBs and regulating their repair
552 via HR in a metazoan model. Further studies are necessary to clarify whether PARG-1
553 recruitment affects the structure of the SC resulting in the modulation of DSB formation and
554 recombination, or whether the presence of PARG-1 along the chromosomes influences the
555 recruitment and dynamic behavior of other factors, which ultimately exert a regulatory role in
556 DSB formation and recombination. Our work highlights the multifaceted aspects of PARG *in*
557 *vivo* not simply as an enzyme mediating the catabolism of PAR, but also as a pivotal factor
558 intersecting multiple functional branches acting during meiosis.

559

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576

577 **Author contributions**

578 NS designed the research and performed most of the experiments with the technical support
579 of EJ; MR, FB and JLY generated some strains, analyzed diakinesis chromosomes, and
580 performed the recombination assay on chromosome I; LPP and AVH analyzed whole
581 genome sequencing data, which initiated the analysis of the catalytic-dead *parg-1* mutants;
582 AB produced the *HA::rmh-1* tagged line; VJ provided logistic, infrastructure, resources and
583 conceptual support; JLY, VJ and NS wrote the manuscript.

584

585 **Declaration of Interests**

586 The authors declare no competing interests.

587

588 **Figure Legends**

589 **Figure 1. PARG-1 is the main ADP-ribose glycohydrolase in *C. elegans*.**

590 **(A)** Schematic representation of *parg-1* and *parg-2* genetic loci. *parg-1* is predicted to encode
591 numerous isoforms and for simplicity only the transcript encoding isoform A is shown. Red
592 lines delineate the position of deletions in the indicated mutant alleles. **(B)** Screening of
593 brood-size, embryonic lethality and segregation of males in indicated genetic backgrounds.
594 Number of embryos scored: WT (1196), *parg-1::GFP* (1389), *parg-1(gk120)* (1200), *parg-*
595 *2(DDR20)* (1304) and *parg-1(gk120) parg-2(DDR20)* (1271). **(C)** Representative images of
596 whole-mount gonads from indicated genotypes showing detection of PAR by
597 immunofluorescence. Scale bar 20 μ m. **(D)** Left: representative pictures of late pachytene
598 nuclei from indicated genotypes showing *parg-1*- and *parg-2*-dependent accumulation of PAR

599 in *parg-1* mutants. Gonads were divided into 7 zones, encompassing the region from the
600 distal tip cell to diplotene. Scale bar 5 μ m. Right: quantification of PAR detected by
601 immunofluorescence. Chart reports average of fluorescence intensity from at least two
602 gonads/genotype. Error bars indicate standard deviation.

603

604 **Figure 2. PARG-1 interacts with SC components and requires chromosome axes for**
605 **proper localization.**

606 **(A)** Western blot analysis of fractionated extracts detects PARG-1 in all subcellular
607 compartments with enrichment in the chromatin-bound fraction. CY= cytosol, NS= nuclear
608 soluble, CB= chromatin bound. GAPDH was used as a loading control of the cytosolic
609 fraction and Histone H3 for the chromatin bound fraction. **(B)** Western blot analysis of
610 fractionated extracts showing similar expression of GFP-tagged and untagged PARG-1. **(C)**
611 Top: PARG-1::GFP localization in a wild-type gonad. Scale bar 30 μ m. Bottom: enlarged
612 insets showing dynamic localization of PARG-1::GFP in different stages of meiotic prophase
613 I. Scale bar 5 μ m. **(D)** Mid- and late-pachytene nuclei of *parg-1::GFP* co-stained for lateral
614 (HTP-1 and -3) and central component (SYP-1) of the SC. Scale bar 5 μ m. **(E)** Late
615 pachytene nuclei showing overlapping localization of PARG-1::GFP with OLLAS::COSA-1.
616 Scale bar 5 μ m. **(F)** Late pachytene nuclei stained for HTP-1, SYP-1 and GFP showing
617 localization of PARG-1 along chromosomes. In *cosa-1* mutants redistribution to the short arm
618 of the bivalent is absent. Scale bar 5 μ m. **(G)** Impaired axes formation in *htp-3* mutants
619 prevents PARG-1::GFP localization. Scale bar 5 μ m. **(H)** PARG-1::GFP associates with HTP-
620 3 in late-pachytene nuclei in absence of synapsis. Scale bar 5 μ m. **(I)** Western blot analysis
621 of endogenous PARG-1 on GFP pull downs performed in *htp-3::GFP* and *GFP::syp-3*
622 strains. Wild-type worms were used as the untagged negative control. **(J)** Western blot

623 analysis of endogenous PARG-1 on GFP pull downs performed in *htp-1::GFP* and *rec-8::*
624 *GFP* strains.

625

626 **Figure 3. Elimination of *parg-1* function suppresses chromosome abnormalities in**
627 **resection-defective mutants.**

628 **(A)** *parg-1* mutants display SPO-11-dependent accumulation of RAD-51 foci. Error bars
629 represent S.E.M. **(B)** Representative examples of cells at different stages of the same
630 genotypes analyzed in A. Scale bar 5 μ m. **(C)** Analysis of diakinesis nuclei in different
631 genotypes before and 27h after exposure to IR. Error bars represent standard deviation.
632 Statistical analysis was performed with non-parametric Mann-Whitney test. *** indicates
633 $p < 0.0001$ and *ns* indicates statistically non-significant differences. **(D)** Representative images
634 of diakinesis nuclei of the same genotypes scored in C. Scale bar 5 μ m.

635

636 **Figure 4. PARG-1 promotes formation of meiotic DSBs and interacts with MRE-11.**

637 **(A)** Schematic representation of the gonad divided into five equal regions spanning transition
638 zone throughout late pachytene, employed for MSH-5 and RMH-1 foci quantification. **(B)**
639 Quantification of GFP::MSH-5 foci in the indicated genotypes before and after IR. Error bars
640 indicate standard deviation. **(C)** Quantification of HA::RMH-1 foci in the indicated genotypes
641 before and after IR. **(D)** Quantification of DAPI-bodies of different genotypes before and after
642 IR exposure. **(E)** Western blot analysis of endogenous PARG-1 on GFP pull downs
643 performed in *mre-11::GFP* and untagged wildtype strains (negative control).

644

645 **Figure 5. PARG-1 and HIM-5 regulate CO numbers.**

646 **(A)** Quantification of synapsis in late pachytene nuclei without IR and at different times after
647 IR exposure, by SYP-1 and HTP-3 co-staining. Only nuclei showing complete colocalization

of HTP-3 and SYP-1 were considered fully synapsed. Quantification was performed in the last seven rows of nuclei before entering the diplotene stage. Error bars indicate S.E.M. **(B)** Quantification of OLLAS::COSA-1 foci formation in late pachytene nuclei in the same genotypes and at the same time points as in A. **(C)** Immunostaining of H3K4me2, HTP-3 and SYP-1 to assess chromosome X synapsis in different genotypes. Scale bar 5 μ m. **(D)** Co-staining of OLLAS::COSA-1 with SYP-1 and HTP-3 shows de-synapsis associated with lack of CO but normal numbers of COSA-1 foci on the remaining chromatin in *parg-1; him-5* double mutants. Scale bar 5 μ m. Arrows indicate examples of unsynapsed regions in nuclei containing six COSA-1 foci. **(E)** High magnification of late pachytene *parg-1; him-5* nuclei after 8h (top) and 17h (bottom) post irradiation of showing normal numbers of COSA-1 foci despite de-synapsis. Arrows indicate desynapsed chromosome regions (presence of HTP-3, absence of SYP-1). Scale bar 1 μ m.

Figure 6. Loss of *parg-1* alters the recombination landscape and weakens CO interference.

(A) Top: schematic representation of the genetic position of the SNPs employed to assess the recombination frequency on chromosome I. PC indicates the position of the pairing center. Middle: recombination frequencies assessed in each of the genetic intervals in wild type and *parg-1* null mutants. Bottom: table displaying number and percentage of single, double and triple crossovers (SCO, DCO and TCO respectively) in both genotypes. *n* indicates number of worms analyzed. **(B)** Same analysis as in A, performed for chromosome V.

Figure 7. PARG-1 catalytic activity is dispensable for recombination.

(A) Left: immunofluorescence of late pachytene nuclei showing elevated levels of PARG-1^{CD}::GFP versus PARG-1::GFP and delayed redistribution. Right: insets depicting magnified

nuclei from left. Scale bar 10 μ m. **(B)** Western blots with fractionated extracts show higher PARG-1^{CD}::GFP and PARG-1^{CD} abundance compared to controls. Anti-histone H3 was used as loading control for chromatin-bound fraction. Western blot (bottom) confirmed accumulation of PAR in both *parg-1(cd)* and *parg-1(gk120)* null mutants. **(C)** Blocking PARG-1 catalytic activity causes milder synergistic effects when combined with *him-5* mutants in contrast to *parg-1(gk120); him-5*. **(D)** De-synapsis of the X chromosome is fully rescued by IR exposure in *parg-1(cd); him-5* double mutants. Arrows indicate the unsynapsed X chromosome in the indicated genotypes before irradiation. Scale bar 10 μ m. **(E)** Quantification of synapsis by SYP-1 and HTP-3 co-stainings in the indicated genotypes before and after IR exposure. Error bars indicated S.E.M. **(F)** DAPI-stainings (left) and quantification of DAPI-bodies in diakinesis nuclei (right) for the indicated genotypes. Error bars indicate standard deviation. Scale bar 5 μ m.

Methods

Genetics

Worms were cultured at 20°C according to standard conditions. The N₂ strain was used as the wild-type control. We did not notice any significant differences between *him-17(e2707)* and *him-17(e2806)* alleles and the former has been employed for the majority of the experiments unless otherwise indicated. The *parp-1(DDR31)* is a full knock-out generated by CRISPR. A full list of the strains employed for this study are in Table S3.

Screenings

L4 worms were individually plated and moved onto fresh plates every 12 hours for three days. Dead eggs were scored 24 hours after the mother had been removed and male

697 progeny after three days. Embryonic lethality and male progeny were calculated as the
698 fraction of unhatched eggs/total laid eggs and males/total hatched eggs respectively.

699

700 **Cytological procedures and image acquisition**

701 For immunostaining experiments, synchronized worms of the indicated age were dissected
702 and processed as previously described (Janisiw et al., 2018) except for detection of PARG-
703 1::GFP and GFP::MSH-5. Briefly, worms were dissected in PBS and immediately placed in
704 liquid nitrogen. Slides were placed in cold methanol at -20°C for 1' and then fixed with 2%
705 PFA in 0.1M K₂HPO₄ (pH 7.4) for 10' in a humid chamber at room temperature. Samples
706 were then processed as for regular staining. For GFP::MSH-5 detection, worms were
707 dissected and fixed in 2.5% PFA for 2' at room temperature and then freeze-cracked in liquid
708 nitrogen. Slides were placed in absolute ethanol at -20°C for 10' and then washed in 1x
709 PBST. DAPI staining was performed as for normal staining and GFP was directly acquired
710 without employing a primary anti-GFP antibody. For quantification of PAR (Figure 1E),
711 samples were acquired with identical settings and equally adjusted in Fiji. Gonads were
712 divided into seven equal regions from mitotic tip to diplotene entry and a circle of fixed area
713 was employed to assess absolute fluorescence in each nucleus with Fiji as in (Janisiw et al.,
714 2018). Between two and three germlines for each genotype were used for quantification.
715 Number of nuclei scored was (from zone 1 to 7): WT (97, 129, 115, 107, 102, 74, 45), *parg-*
716 *1(gk120)* (129, 136, 140, 129, 130, 96, 82), *parp-1(DDR31)*; *parg-1(gk120)* (93, 113, 123, 129,
717 102, 69, 44), *parp-2(ok344)*; *parg-1* (190, 263, 215, 179, 179, 125, 72), *parp-1(DDR31)*; *parp-*
718 *2(ok344)*; *parg-1(gk120)* (107, 140, 134, 144, 126, 103, 76).

719 For quantification of RAD-51 foci, gonads were divided into seven equal regions from the
720 mitotic tip to the diplotene entry and number of RAD-51 foci was counted in each nucleus.

721 Number of nuclei analyzed is reported in Table S2.

722 Quantification of phospho-SUN-1^{S8} extension was performed as in (Link et al., 2018).
 723 Most images were captured using a Delta Vision system equipped with an Olympus IX-71
 724 microscope and a Roper CoolSNAP HQ2 camera with Z-stack set at 0.25 µm of thickness.
 725 Images in Figure 7D and 7F, were acquired with a Delta Vision system equipped with an
 726 Evolve 512 EMCCD Camera and an upright fluorescence microscope Zeiss AxioImager.Z2
 727 equipped with a Hamamatsu ORCA Flash 4.0, sCMOS sensor camera respectively, using
 728 UPlanSApo 100x/1.4 Oil objective. All images were deconvolved using Softworx (Applied
 729 Precision) except for images in Figure 7F, which are non-deconvolved.

730

731 **Antibodies**

732 The following antibodies at the indicated dilutions were employed for immunolocalization
 733 studies: rabbit polyclonal anti HA (SIGMA, 1:1000), rabbit polyclonal anti OLLAS (Genscript,
 734 1:1500), rabbit polyclonal anti PAR (Trevigen, 1:1000), mouse monoclonal anti GFP (Roche,
 735 1:500), guinea pig polyclonal anti HTP-3 (1:500) (Goodyer et al., 2008), guinea pig polyclonal
 736 anti HTP-3 (1:750) (Y. Kim lab), chicken polyclonal anti SYP-1 (1:500) (Silva et al., 2014),
 737 rabbit polyclonal anti HTP-1 (1:500) (Martinez-Perez et al., 2008), rabbit polyclonal anti RAD-
 738 51 (Novus, 1:10,000), guinea pig polyclonal anti phospho-SUN-1^{S8} (1:750) (Woglar et al.,
 739 2013), rabbit polyclonal anti DSB-2 (1:5000) (Rosu et al., 2013), guinea pig polyclonal anti
 740 XND-1 (1:2500) (Wagner et al., 2010), mouse monoclonal anti H3K4me2 (Millipore, 1:250).
 741 All the secondary antibodies were Alexafluor-conjugated and used at 1:500.

742 The following antibodies at the indicated dilutions were employed in western blot analysis:
 743 rabbit polyclonal anti HA (SIGMA, 1:3000), mouse monoclonal anti HA (Cell Signalling,
 744 1:1000), mouse monoclonal anti PARG-1 (this study, 1:500), chicken polyclonal anti GFP
 745 (Abcam, 1:4000), rabbit polyclonal anti Histone H3 (Abcam, 1:100,000), goat polyclonal anti
 746 actin (Santa Cruz, 1:3000), mouse monoclonal anti Tubulin (Thermofisher, 1:2000), mouse

747 monoclonal anti GAPDH (Ambion, 1:5000). Secondary antibodies were purchased from
748 Thermofisher and were HRP-conjugated.

749

750 **Biochemistry**

751 Fractionated protein extracts were produced as previously described (Silva et al., 2014) and
752 co-immunoprecipitation assays and Western Blot were performed as previously shown
753 (Janisiw et al., 2018). At least 500µg of nuclear extract (pooled nuclear-soluble and
754 chromatin-bound fractions) were used for IPs. Agarose GFP-traps (Chromotek) were
755 employed for pull downs following manufacturer instructions. Buffer D (20mM HEPES pH 7.9,
756 150mM KCl, 20% glycerol, 0.2mM EDTA, 0.2% Triton X-100 and complete protease inhibitor)
757 was used for incubation with beads and washes.

758

759 **Generation of anti-PARG-1 antibody**

760 To generate the mouse monoclonal anti PARG-1(2D4) antibody, the cDNA encoding for
761 residues 1-350 of *C. elegans* PARG-1 (isoform A) was generated by gene synthesis (IDT)
762 and then cloned into pCoofy31 in frame with a C-ter 6xHis tail. The resulting plasmid was
763 expressed in *E. coli* BL21 cells according to standard procedures and 1 mg of purified protein
764 was used to immunize three mice in the “in-house” monoclonal antibody facility at Max Perutz
765 Laboratories ([https://www.maxperutzlabs.ac.at/research/facilities/monoclonal-antibody-](https://www.maxperutzlabs.ac.at/research/facilities/monoclonal-antibody-facility)
766 [facility](https://www.maxperutzlabs.ac.at/research/facilities/monoclonal-antibody-facility)). Raw sera were screened by western blot employing extracts produced from WT,
767 *parg-1(gk120)* and *parg-1::GFP* worms in order to identify immunoreactive bands against
768 PARG-1. Spleen cells from one mouse were fused with myeloma cells to generate hybridoma
769 cell lines and mixed clones were successively diluted to gain monoclonal line 2D4, from
770 which the antibody was harvested. Antibody specificity was assessed by western blot, where

an immune reactive band of the expected Mw of approximately 90kDa in WT but not in *parg-1* mutant worms was detected (Fig.2A).

Irradiation

Irradiation assays were performed as previously described (Janisiw et al., 2018). For quantification of synapsis and OLLAS::COSA-1 foci number in late pachytene nuclei, worms were dissected at the indicated time after irradiation and quantification was performed in the last seven rows of nuclei before diplotene entry. For quantification of HA::RMH-1 and GFP::MSH-5 in Figure 4, worms were dissected 8h post-IR and gonads from transition zone to late pachytene were divided into five equal regions and number of foci/nucleus was assessed. For diakinesis analysis, worms were dissected 24-27 hours post irradiation. The dose employed for all irradiation experiments was 10 Gy.

Number of nuclei analyzed for each condition are reported in Table S1 and S2.

CRISPR-Cas9 genome editing

Generation of tagged or mutated lines was performed as previously described (Janisiw et al., 2018). Briefly, to tag endogenous locus of *parg-1*, GFP was amplified by PCR with primers carrying 25 base pairs of homology to the left and right side of the STOP codon of *parg-1* gene. To generate the PARG-1^{E555,556A} catalytic dead mutant, a synthetic ultramer (IDT) was employed, in which we included silent mutations to produce an Alu I restriction site for screening purposes. The mutations were generated in both WT and *parg-1::GFP* genetic backgrounds. To elicit a full knock-out of *parg-1*, we employed two sgRNAs targeting the beginning and the end of the gene. The *him-17::3xHA* and *him-5::3xHA* were generated by employing synthetic DNA ultramers (IDT) and N2 worms were injected. All the tagged lines carried a 5x-Gly linker between the tag and the coding region. The *parg-1^{gk120}* line carries the

796 same deletion present in the VC130 strain, which we generated in both WT and CB4856
797 strains. All the lines generated by CRISPR were outcrossed to WT worms at least twice
798 before use.

799

800 **Recombination Assay**

801 The recombination landscape was assessed following the same strategy as in (Hillers and
802 Villeneuve, 2003), by exploiting different Dra I digestion pattern of SNPs present in the Bristol
803 and Hawaiian genetic backgrounds. Briefly, *parg-1(gk120)* and *parg-1(cd)* mutations were
804 generated by CRISPR in both the N2 (Bristol) and CB4856 (Hawaii) strains. Bristol/Hawaiian
805 F1 hermaphrodite hybrids carrying the indicated mutations were backcrossed to Bristol males
806 carrying a tdTomato fluorescent reporter expressed in the soma in order to monitor
807 recombination frequency in the oocytes. The relevant regions containing the SNPs for
808 chromosomes I and V in the indicated genetic intervals were amplified by PCR and the
809 products digested with Dra I to monitor recombination patterns. Data presented in Figure 6
810 refer to the total number of worms analyzed in independent replicates.

811

812 **Supplementary Figure Legends**

813 **Figure S1. PAR accumulates in absence of endogenous DSBs.**

814 Immunostaining analysis showing accumulation of PAR in *parg-1* mutants even in absence of
815 SPO-11-induced DSBs. *parg-1::GFP* does not accumulate PAR, indicating functionality of the
816 fusion protein. Scale bar 10 μ m.

817

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820

821 **Figure S2. PARG-1 is dispensable for synapsis and CO formation.**

822 **(A)** Staining of SYP-1 and HTP-3 shows no abnormalities in *parg-1(gk120)* mutants
823 compared to wild-type (WT) animals. **(B)** Quantification of DAPI-bodies (left) and
824 representative examples (right) of diakinesis nuclei in the indicated genotypes. Scale bar 2
825 μm .

826

827 **Figure S3. Abrogation of *parg-1* function causes reduced amounts of RAD-51 foci in**
828 ***him-5* and *him-17* mutants.**

829 **(A)** Quantification of RAD-51 foci throughout the germline in the indicated genotypes (top)
830 and representative images of mid-pachytene nuclei stained with DAPI and anti RAD-51
831 (bottom). Error bars indicate S.E.M. Statistical analysis was performed using non-parametric
832 two-tailed Mann-Whitney test. ($***p < 0.0001$; $**p = 0.0036$). **(B)** Removal of *parg-1* causes
833 synthetic lethality in DSB-defective mutants. **(C)** The CO defects in *dsb-2* mutants are
834 exacerbated by lack of PARG-1.

835

836 **Figure S4. Loss of PARG-1 impairs loading of pro-CO factors in *him-5* and *him-17***
837 **mutants.**

838 **(A)** Late pachytene nuclei of different genotypes stained for GFP (MSH-5) before and after
839 IR. **(B)** Late pachytene nuclei of different genotypes stained for HA (RMH-1) and OLLAS
840 (COSA-1) before and after IR Scale bar 10 μm .

841

842 **Figure S5. PARG-1 and pro-DSB factors display a mutually independent loading.**

843 **(A)** Viability and male progeny assessment revealed full functionality of *him-5::3xHA* and
844 *him-17::3xHA* tagged lines compared to respective mutant backgrounds. **(B)** HIM-5 shows
845 normal loading in *parg-1* mutant germlines. Scale bar 20 μm . **(C)** HIM-17 and XND-1 do not

display loading abnormalities in *parg-1* mutants. Scale bar 20 μ m. **(D)** *parg-1* is not required for DSB-2 loading. Scale bar 20 μ m. **(E)** Loading of PARG-1 is not dependent on *him-5*, *him-17* and *dsb-2*. Scale bar 20 μ m.

849

850

Figure S6. Loss of *parg-2* does not cause synthetic phenotypes with *him-5*.

(A) Quantification of DAPI-bodies in diakinesis nuclei of the indicated genotypes before and after IR exposure. **(B)** Representative images of diakinesis nuclei of the indicated genotypes stained with DAPI. Scale bar 5 μ m.

855

Figure S7. IR does not fully suppress accumulation of pSUN-1^{S8} in *parg-1*; *him-5*

double mutants.

(A) Quantification of pSUN-1^{S8}-positive nuclei cell rows in the indicated genotypes before and after IR exposure. **(B)** Representative whole-mount gonads of the indicated genotypes before and after irradiation, stained for pSUN-1^{S8} and DAPI. Scale bar 30 μ m.

861

Figure S8. PARG-1^{E554,555A} is a “catalytic-dead” mutant of PARG-1.

Left: whole-mount germlines of the indicated genotypes stained with anti-PAR antibodies and DAPI. Right: PAR staining. Scale bar 20 μ m.

865

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B

Genotype	Brood-size	Embryonic Lethality	Male Progeny
WT	240	0.5%	0%
<i>parg-1::GFP</i>	278	0.14%	0%
<i>parg-1(gk120)</i>	240	3.9%	4.5%
<i>parg-2(ddr20)</i>	261	0.3%	0%
<i>parg-1(gk120)</i> <i>parg-2(ddr20)</i>	254	2.8%	3.8%

A

RAD-51 foci quantification

B

C

Quantification of DAPI bodies in diakinesis nuclei

D

A **B** **C** **D** **E**

A

Total Genetic Interval 45 cM

Recombination frequency along Chromosome I

Ch. I	SCO	DCO	TCO
WT (n= 152)	58/58 (100%)*	0 (0%)*	0 (0%)*
<i>parg-1(gk120)</i> (n= 215)	88/98 (89.8%)*	6/98 (6.1%)*	4/98 (4.1%)*

*= calculated over the total number of recombinant animals

B

Total Genetic Interval 35 cM

Recombination frequency along Chromosome V

Ch. V	SCO	DCO	TCO
WT (n= 193)	62/62 (100%)*	0 (0%)*	0 (0%)*
<i>parg-1(gk120)</i> (n= 377)	143/159 (89.9%)*	15/159 (9.4%)*	1/159 (0.6%)*

*= calculated over the total number of recombinant animals