Parp1-Dependent DNA Double-Strand Break Repair in Irradiated Late Pachytene Spermatocytes

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Poly (ADP-ribose) polymerase-1 (Parp1) is a member of nuclear enzymes family involved in to the response to genotoxic stresses, DNA repair, and is critical for the maintenance of genome stability. During gametogenesis, genome stability is essential for inheritance and formation of healthy gametes. The latter involves DNA double-strand break (DSB)-driven pairing of homologous chromosomes in first meiotic prophase. By analysis of DSB repair kinetics in male meiotic prophase cells of homologous recombination (HR) and nonhomologous end joining (NHEJ)-deficient mouse models, we previously demonstrated an interplay between HR and the conventional NHEJ repair pathway. In the current work, we evaluate the relative contribution of Parp1-dependent NHEJ to the repair of ectopic ionizing radiation (IR)-induced DSBs in control and Parp1-inhibited mouse pachytene spermatocytes before and after the completion of meiotic recombination in stages VI–XI. The disappearance of large, exogenous DSB-related γ -H2AX foci was quantified 1 and 8 h after 1 Gy γ -irradiation of control and 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)quinolinone (DPQ) Parp1-inhibited mice. Late pachytene control spermatocytes obtained 8 h after IR had repaired >80% of DSBs observed at 1 h after IR. However, only 64% of DSBs were repaired in late spermatocytes of DPQ-treated (Parp1-inhibited) mice. Thus, it appears that Parp1 contributes to the repair of a fraction of DSBs in late prophase I, providing further insights in DNA repair pathway choreography during spermatogenic differentiation.

Keywords: alternative NHEJ, DNA repair, first meiotic prophase, γ -H2AX, homologous recombination, Parp1, spermatogenesis

Introduction

THE POLY (ADP-RIBOSE) POLYMERASE 1 (Parp1) enzyme is an abundant chromatin-associated protein that has a variety of cellular functions and is implicated in DNA damage signaling and repair, chromatin remodeling, and transcriptional regulation (Chaudhuri and Nussenzweig, 2017; Pazzaglia and Pioli, 2020). Parp1 is contributing to a number of nuclear and cellular functions including DNA repair (Thomas and Tulin, 2013; Chaudhuri and Nussenzweig, 2017; Slade, 2019). Besides being required for base excision repair and its role in other DNA repair pathways, Parp1 is also involved in the alternative nonhomologous end joining (aNHEJ) repair pathway. While some details are still not clear, aNHEJ seems to utilize the synaptic activity of Parp1 and the ligation activity of the XRCC1-DNA ligase III complex (Soni *et al.*, 2014; Kang and Yan, 2018). This alternative pathway of NHEJ can function as a backup to the DNA-PK-dependent classical NHEJ (cNHEJ) pathway, particularly in the absence of Ku or more essential com-

ponents of the cNHEJ pathway or when Parp1 and Ku compete for repair of double-strand breaks (DSBs) (Mansour *et al.*, 2013; Bakr *et al.*, 2016). Although Parp1-knockout mice are viable and fertile with normal life span, they show altered inflammatory processes and are more sensitive to ionizing radiation (IR) and show high levels of chromatid and chromosome aberrations after exposure to genotoxic agents (de Murcia *et al.*, 1997; Wang *et al.*, 1997; Shall and de Murcia, 2000).

Upon IR-induced DSB formation, the histone variant H2AX is phosphorylated at serine 139 in the chromatin surrounding a DSB (Rogakou *et al.*, 1998), leading to microscopically visible DSB-indicating γ -H2AX nuclear foci (Rogakou *et al.*, 1999; Iacovoni *et al.*, 2010). γ -H2AX focus formation and disappearance are widely used as markers reflecting the extent of DSB damage and repair (Rothkamm *et al.*, 2015; Ahmed *et al.*, 2017; Singh *et al.*, 2018). It is of note that a γ -H2AX focus may contain more than one DSB at higher radiation doses (Scherthan *et al.*, 2008; Antonelli *et al.*, 2015).

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In male germ cells, at leptotene substage of first meiotic prophase, endogenous DSBs are introduced by SPO11, a topoisomerase II-like endonuclease (Keeney *et al.*, 1997; Baudat *et al.*, 2000). These physiologically induced DSBs contribute to homologous chromosome recognition and are repaired through homologous recombination (HR), which is a highly orchestrated process (Hunter, 2015) that depends on homologous chromosome pairing and synapsis, established during zygotene stage (for review, see Zickler and Kleckner, 2015). Crossovers are formed at least once between each pair of homologous chromosomes during the pachytene stage (Gerton and Hawley, 2005). Two types of DSB-dependent γ -H2AX chromatin foci have been described in spermatocyte nuclei at pachytene stage of meiosis: small γ -H2AX foci that mark the Spo11-related DSBs sites and are located mostly at the synaptonemal complex (SC) and larger γ -H2AX foci (signals) encompassing chromatin loops emanating from the SC that are induced by both unrepaired Spo11-DSBs and IR-induced exogenous DSBs (Chiccheportiche *et al.*, 2007; Ahmed *et al.*, 2018).

Previous analysis of Parp1 expression level in the testes revealed a higher expression in primary spermatocytes relative to other male germ cells (Di Meglio *et al.*, 2003). However, based on its staining intensity, Parp1 expression appears to increase gradually from early to late meiotic prophase spermatocytes (Ahmed *et al.*, 2010b). However, the lack of Parp1 function during oogenesis was found to predispose the female gamete to genome instability and consequently to a spectrum of meiotic defects including incomplete homologous chromosome synapsis or persistent γ -H2AX foci along fully synapsed chromosomes at the late female pachytene stage (Yang *et al.*, 2009). In male severe combined immunodeficiency (SCID) mice, an elevated number of apoptotic pachytene spermatocytes were detected at the stage IV checkpoint of the testicular epithelial cycle, which has been attributed to a role of the cNHEJ pathway during the repair of meiotic DSBs (de Rooij and de Boer, 2003; Hamer *et al.*, 2003). However, at variance, no difference was found in the repair kinetics of IR-induced DSBs in early pachytene spermatocytes from SCID and control mice (Ahmed *et al.*, 2019). In addition, Ku proteins that contribute to cNHEJ are not expressed during early meiotic prophase of primary wild-type spermatocytes (Ahmed *et al.*, 2013). However, investigation of repair kinetics in late spermatocytes of cNHEJ-deficient SCID mice has revealed that about 11% of IR-induced DSBs are not repaired relative to control, indicating that cNHEJ plays a role in the repair of a subset of DSBs in late spermatocytes (Ahmed *et al.*, 2017). Furthermore, Parp1/XRCC1-dependent aNHEJ is active in postmeiotic spermatids (Ahmed *et al.*, 2010a). Thus, the understanding of processes contributing to the repair of exogenous DSB damage during and after completion of meiotic recombination requires more analyses.

Given the higher expression of Parp1 in late spermatocytes (Maymon *et al.*, 2006; Tramontano *et al.*, 2007; Ahmed *et al.*, 2010b), the declining activity of HR to repair DSBs in late pachytene, and the possible role for cNHEJ in repairing a subset of DSBs in these cells, there remains the question whether Parp1-dependent aNHEJ also contributes to this DSB damage repair in late pachytene and diplotene spermatocytes. Here, we addressed this question evaluating the relative contribution of the Parp1-dependent end joining

repair pathway in repairing exogenous irradiation DSBs in late prophase I spermatocytes.

Materials and Methods

Animals and irradiation

The effect of the Parp1 inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)quinolinone (DPQ) on Parp1-dependent damage repair was studied in male, 8- to 10-week-old BALB/c albino mice, a similar model to our previous investigations (Ahmed *et al.*, 2010). Mice were bred at the animal house of Zoology Department, Faculty of Science, Assiut University. Animal experiments were done according to animal welfare rules stated by Assiut University and King Faisal University (KFU/REC 2020/02/07, approved February, 2020). In total, 24 male mice were used in the experiment. Mice were maintained under standard laboratory conditions at ambient temperature (25°C), relative humidity 60%–70%, and a 12 h light/dark cycle. They were allowed to acclimatize for 1 week ahead of the experiment. Mice were fed a standard commercial pellet diet and water and housed in polypropylene cages inside a well-ventilated room.

In brief, mice were intraperitoneally injected with the Parp1 inhibitor DPQ (D5314; Sigma–Aldrich) dissolved in dimethyl sulfoxide (DMSO) or DMSO alone at 10 mg/kg of body weight. Two injections were applied to inhibit Parp1: the first DPQ injection was given 1.5 h before 1 Gy gamma ray whole-body irradiation with a gamma ray source (Elekta, Sweden) at 0.8 Gy/min dose rate. The second injection was given 3.5 h after irradiation. This protocol was followed since it has been useful for DNA repair studies in the mouse testes (Ahmed *et al.*, 2010a). After 1 and 8 h of whole body exposure to a dose of 1 Gy 6 MV gamma rays (four mice per group), mice were sacrificed and one testis was used to prepare meiotic spreads (Muhtadi *et al.*, 2017; Ahmed *et al.*, 2018) and the other was fixed in 10% formalin for 16 h, followed by paraffin embedding for immunohistochemical investigation.

Immunohistochemistry

Paraffin sections (6 μ m) of testes of control and irradiated mice were mounted together on SuperFrost glass slides and dried overnight at 37°C. Sections were dewaxed in xylene, followed by hydration in a graded series of alcohols. The sections were boiled once (each for 10 min) in 0.01 M sodium citrate (pH=6) using a microwave oven (Bio-Rad) and incubated in 0.35% H₂O₂ in phosphate-buffered saline (PBS) for 10 min. Unspecific protein binding was blocked by 5 min incubation in 5% bovine serum albumin (Sigma–Aldrich) and 5% goat serum in PBS. The slides were incubated with the primary antibody (anti- γ -H2AX; Upstate; and anti-Parp1; Abcam; see the Antibodies section) for 1 h at room temperature, followed by washing in PBS and then incubation with the ready-to-use secondary antibody PowerVision poly-anti-mouse/rabbit-HRP (ImmunoVision). Bound antibodies were visualized using 0.3 μ g 3,3-diaminobenzidine (Sigma–Aldrich) in PBS, to which 0.03% H₂O₂ was added. Sections were counterstained with Mayer's hematoxylin (Sigma–Aldrich).

Surface spread preparations

Nuclear spreads of spermatocytes were prepared as previously described (Peters *et al.*, 1998; Ahmed *et al.*, 2010;

Muhtadi *et al.*, 2017). Briefly, a suspension of testis cells was prepared by mincing in MEM- α , followed by a 10-min incubation with a hypotonic buffer (17 mM sodium citrate, 50 mM sucrose, 30 mM Tris-HCl, pH 8.2). After being centrifuged, the pellet was carefully resuspended in a 100 mM sucrose solution and applied over a paraformaldehyde (PFA)-covered glass slide (1% PFA, 0.15% Triton-X-100, pH 9.2–9.5). Slides were kept in a humidified atmosphere in a box for slow drying down. After 1.5 h, the box was opened and the slides were washed in 0.08% photo-flo (Sigma–Aldrich).

Antibodies

The primary antibodies were rabbit polyclonal anti-SCP3 (1:400, ab15092; Abcam), mouse monoclonal anti-phospho-H2AX [Ser139] (1:400, 16-202A; Upstate Biotechnology), and rabbit anti-Parp1 (1:100, Abcam). The secondary antibodies, goat anti-rabbit (Alexa Fluor 488, A-11008) and Texas Red-labeled goat anti-mouse (T6134), were obtained from Jackson ImmunoResearch and applied at a 1:1000 dilution.

Fluorescent immunostaining and analysis

For immunofluorescent staining, preparations were washed in PBS and incubated for 10 min in PBS, 0.04% Triton-X-100, followed by incubation in blocking solution (10% BSA in PBS for 30 min.). After blocking, primary antibodies (see the Antibodies section) were applied, diluted in blocking solution, for 2 h at room temperature. Then, slides were washed in PBS and incubated with the diluted secondary antibodies (see the Antibodies section) in blocking solution for 1 h at room temperature. Thereafter, cell nuclei were stained with DAPI for 10 min (concentration in H₂O) and mounted in VECTAshield antifade solution (Vector Labs) until inspection.

For immunohistochemistry (IHC) staining, the analysis was carried out in the spermatocytes after identifying epithelial stage according to the method described in detail by Ahmed and de Rooij (2009). Spermatocyte signal intensity measurements were performed using the TissueQuest software (TissueGnostics, Vienna). In brief, the outline of spermatocyte nuclei (blue, DAPI) was determined by the software based on DAPI staining and gray value drop-offs and size of nuclei. Inside the nuclear masks, fluorescence intensities of the green channel were computed for each nucleus and presented as average and standard deviation (SD) of arbitrary gray value units.

To analyze the relative contribution of Parp1-dependent pathway in the repair of exogenously induced DSBs during late prophase I, we studied the kinetics of irradiation-induced large DSB-indicating “L” γ -H2AX foci that emanate from the SC in nuclear spreads as described previously (Chicheportiche *et al.*, 2007; Ahmed *et al.*, 2018). Foci disappearance was studied of control and DPQ-treated mice to determine repair potency. Foci numbers were also scored in pachytene spermatocytes from paraffin-embedded testis sections at defined testicular epithelial stages as described earlier (Ahmed and de Rooij, 2009).

Preparations were viewed with an Olympus epifluorescence microscope (Olympus Tokyo, Japan). Digital gray scale images were recorded using a 63 $\times$ lens and a digital 20MP camera (Leica) and merged using the ImageJ software. Focus counting was performed on the merged RGB images and foci were enumerated in up to 100 well-stained cells from three to four mice per group.

Statistical analysis

The data were analyzed using Student's *t*-test using QuickCalcs software (graphpad.com), and the data are expressed as mean $\pm$ standard error of the mean.

Results

DPQ treatment reduces Parp1-staining intensity in irradiated late spermatocytes

In wild-type mice, Parp1 is expressed in all germ cell types with low staining intensity in early meiotic prophase cells, that is, preleptotene, leptotene, and zygotene spermatocytes (Ahmed *et al.*, 2010b). Nonirradiated control and DPQ-treated (Parp1-inhibited) testes cells failed to display significant differences in Parp1 staining intensity (unpublished observations). However, ionizing radiation (IR) of control mice induced an increase of Parp1 staining intensity in late spermatocytes (late pachytene), whereas irradiated DPQ-treated mice showed a significant reduction in Parp1 staining intensity (Supplementary Fig. S1). As expected, IR exposure also induced DSB-indicating γ -H2AX foci numbers in spread spermatocytes (Fig. 1A–F) and spermatocytes of tissue sections of control and Parp1-inhibited mice (Fig. 1G–L).

Parp1-dependent DNA repair in early pachytene spermatocytes

Next, we determined the average γ -H2AX foci number per spermatocyte (surrogate marker for DSBs) (Mahadevaiah *et al.*, 2001; Chicheportiche *et al.*, 2007; Lobrich *et al.*, 2010; Singh *et al.*, 2018) in testicular sections of irradiated control and Parp1-inhibited mice *in situ* after γ -H2AX IHC staining (Figs. 1G–L and 2A–F). The kinetics of DSB repair was derived from γ -H2AX focus formation and disappearance (Ahmed *et al.*, 2018; Singh *et al.*, 2018) between 1 and 8 h after irradiation in seminiferous epithelial stages I–III, IV–V, VI–IX, and X–XI.

Irradiated early to mid-pachytene spermatocytes of stages I–V testis tubules displayed similar average DSB-indicating γ -H2AX foci numbers in control and DPQ-treated mice (Figs. 2G and 3A). This suggests that Parp1 plays no role in the repair of endogenous and exogenous dsDNA damage in early prophase I spermatocytes up to the mid-pachytene stage, agreeing with the view that HR being the major repair pathway in the early prophase I substages.

Reduced DNA repair in late pachytene spermatocytes of Parp1-inhibited mice

The investigation of γ -H2AX DSB foci in irradiated late pachytene spermatocytes after completion of crossing over, that is, in seminiferous stages VI–XI, revealed a Parp1 inhibition-dependent significant increase in the average number of γ -H2AX DSB foci/spermatocyte 1 h after IR exposure relative to the control (Fig. 3A), indicating that Parp1/Parp1-dependent aNHEJ is contributing to fast DNA repair. Likewise, Parp1-inhibited late pachytene spermatocytes of epithelial stages VI–VIII and IX–XI 8 h after IR also displayed a significant increase ($p < 0.01$) in the average numbers of persistent foci (3.7 ± 0.16 foci per cell [fpc]) in stages VI–VII late pachytene spermatocytes

FIG. 1. Meiotic recombination-related γ -H2AX foci in spread pachytene spermatocytes of control (con; A–C) and DPQ-treated mice (DPQ; D–F). eP spermatocytes show numerous γ -H2AX (red) foci in their chromatin (A, D). Persistent spontaneous large (L) γ -H2AX foci (examples arrowed) in contact with the SC (green) of IP spermatocytes (B, E). Numerous foci are observed in meiotic spreads of 1 Gy irradiated of IP spermatocytes of control (C) and DPQ-treated mice (F) 1 h after γ -IR. DNA was counterstained DAPI (blue). Representative images of testes tissue sections (G–L; different epithelial stages are shown) 1 h after γ -irradiation stained for DSB marker γ -H2AX (DAB, brown) by IHC of control (G–I) and DPQ-treated mice (J–L). Sex chromosomes (XY body) show strong γ -H2AX fluorescence (red). Magnification bar: 10 μ m. DAB, 3,3-diaminobenzidine; DPQ, 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)quinolinone; DSB, double-strand break; eP, early pachytene; IHC, immunohistochemistry; IR, ionizing radiation; IP, late pachytene; SC, synaptonemal complex. Color images are available online.

FIG. 2. γ -H2AX staining of testicular sections of 1 Gy γ -irradiated control (A–C) and DPQ-treated mice (D–F) 8 h after IR. Representative images from various epithelial stages showing γ -H2AX foci (dark brown; DAB IHC) and XY bodies (large dark blob) in spermatocytes of stages IV–V (A, D), VI–VII (B, E), and X–XI (C, F). Representative images of individual pachytene spermatocytes (G) with XY body (large dark area) and DSB foci from different epithelial stages of control (Con) and DPQ-treated mice as indicated, 8 h after irradiation. Color images are available online.

(IP) and 5.1 ± 0.15 fpc in stages IX–XI IP, respectively, while control stages VI–VIII IP displayed 2.3 ± 0.12 fpc and 3.1 ± 0.12 fpc in IP of stage IX–XI tubules.

The kinetics of foci disappearance (repair) between 1 and 8 h was significantly ($p=0.04$) reduced in stages VI–VIII IP spermatocytes from 78% in control to ~66% in Parp1-inhibited mice (Fig. 3C). In stages IX–XI IP, the average number of large γ -H2AX foci was reduced from 68.3% in control to 55.4% in Parp1-inhibited mice (Fig. 3C). This reduction is significant ($p=0.004$), indicating that in late

pachytene spermatocytes Parp1 contributes to the repair of about 10% of exogenously (IR) induced DSB foci during 8 h following a genotoxic insult.

Parp1-dependent DNA repair in spread late pachytene spermatocytes

To determine and control the observed DSB numbers at a higher resolution, we next performed nuclear spreads of spermatocytes (Muhtadi *et al.*, 2017) and investigated the

FIG. 3. DNA repair kinetics in spermatocytes from different epithelial stages of irradiated control and DPQ-treated mice 1 and 8 h after IR. **(A)** γ -H2AX foci numbers 1 h after IR in different epithelial stages. While pachytene nuclei in stages IV–V tubuli showed similar average γ -H2AX foci numbers per cell, these were significantly increased in stages VI–XI, indicating a contribution of aNHEJ in DSB repair late in prophase I. **(B)** Parp1-inhibited spermatocytes show significantly more induced γ -H2AX foci numbers 8 h after γ -irradiation. **(C)** Percentages of DSB repair in pachytene spermatocytes of indicated testicular epithelial stages 8 h after IR relative to 1 h after IR. The data shown are derived from up to 100 cells from three mice each; a, significant difference to control at $*p \geq 0.04$ and $**p \geq 0.01$ (t -test). Error bars represent SEM. aNHEJ, alternative nonhomologous end joining; Parp1, poly (ADP-ribose) polymerase-1; SEM, standard error of the mean.

DSB repair kinetics in both groups by enumeration of L- γ -H2AX-foci (Fig. 4A–F) that are a result of DSBs in association with the SC (Chicheportiche *et al.*, 2007; Ahmed *et al.*, 2018). The significant decline of the average γ -H2AX foci numbers in tissue sections of DPQ-inhibited sper-

matocytes relative to control was also detected in γ -H2AX-stained meiotic nuclear spreads (Figs. 1A–F and 4A–F), where the numbers of persistent L- γ -H2AX foci in Parp1-inhibited late spermatocytes (Fig. 4E, F) (6.4 ± 0.28 fpc) were almost twofold relative to untreated control (Fig. 4B, C)

FIG. 4. DNA repair kinetics in nuclear spreads of irradiated pachytene spermatocytes of control (A–C) and DPQ-treated (D–F) mice 8 h after IR. eP (A, D), IP (B, E), and IP/eD (C, F) spermatocytes as indicated. L- γ -H2AX foci numbers are significantly increased in DPQ-treated mice (G) indicating a significantly reduced repair efficiency between 1 and 8 h after IR (H). Sixty cells were counted from three mice per group; a, significant difference to control at $*p \geq 0.025$ and $**p \geq 0.01$ (t -test). eD, early diplotene. Color images are available online.

(3.1 ± 0.1) at 8 h after IR (Fig. 4G). This indicates a highly significant reduction ($p = 0.0006$) of the efficacy of DSB repair from 80% in control to 64% in the Parp1-inhibited late pachytene spermatocytes (Fig. 4H). Altogether, this again supports the conclusion of our tissue analysis above that Parp1 is required to repair a subset of exogenous DSBs in late prophase I spermatocytes.

Discussion

In this study, we have analyzed the contribution of Parp1 to dsDNA damage repair efficacy before and after comple-

tion of crossing over in male first meiotic prophase at the boundary of mid to late pachytene substage after irradiation of control and Parp1-inhibited mouse testes. This approach allowed estimating the relative contribution of the Parp1-dependent aNHEJ repair pathway to the repair of IR-induced exogenous DSBs in these germ cells. Meiotic prophase spermatocytes up to the mid-pachytene substage observed in seminiferous epithelial stages I–V (Ahmed and de Rooij, 2009) failed to display significant differences of γ -H2AX-marked exogenous DSB foci in control and DPQ treated-inhibited mice. Previous analysis of DSB repair kinetics in irradiated *Rad54/Rad54B*^{-/-} HR-deficient mice revealed a

significant reduction of about 37% of DSB repair relative to the irradiated wild type (Ahmed *et al.*, 2010b). Therefore, the HR repair pathway that is predominant during early meiotic prophase also efficiently deals with exogenously induced DSBs. The latter may under certain conditions, for example, lack of endogenous DSBs, even function as substitute for HR hot spots (Liebe *et al.*, 2006; Derijck *et al.*, 2008). Our results, given the limitations of the assay used, suggest that cNHEJ and Parp1-dependent aNHEJ DSB repair pathways are dispensable for repairing endogenous and exogenous breaks in early prophase I spermatocytes before the mid-pachytene stage, where DSB repair is conducted mainly through HR (Ahmed *et al.*, 2010b; Hunter, 2015).

Parp1 inhibition in mouse spermatogenesis by the potent inhibitor DPQ reduced the staining intensity of Parp1 in late pachytene spermatocytes, the stage when crossing over has been completed. At this stage, 1 h after irradiation, the number of γ -H2AX DSB-indicating foci in Parp1-inhibited late pachytene spermatocytes was significantly elevated by $\sim 10\%$ relative to the control. Previous studies in somatic and germ cells indicated that the absence of the fast cNHEJ repair pathway increased the number of unrepaired breaks at 1 h after IR (Iliakis *et al.*, 2004; Ahmed *et al.*, 2017). Using the same model, irradiated SCID late spermatocytes showed around 30% more DSB foci relative to those of control after 1 h of IR

(Ahmed *et al.*, 2010). In the current model, we have observed a 10% increase in number of foci in Parp1-inhibited spermatocytes at 1 h after IR, which was significantly higher relative to control. Thus, Parp1 activity may, to some extent, contribute to the fast repair of DSBs in late prophase I spermatocytes, such as cNHEJ components do (Fig. 5) (Ahmed *et al.*, 2017).

In late (after mid-pachytene) spermatocytes, the role of HR is declining (Fig. 5), as suggested by a similar DSB foci loss in HR-deficient and wild-type mice (Ahmed *et al.*, 2010b). Data from cNHEJ-deficient SCID mice suggest that this pathway is responsible for the repair of about 30% of DSBs within the first hours after IR (Ahmed *et al.*, 2010b). Earlier studies have also demonstrated the existence of an interplay between HR and cNHEJ in somatic cells (Fukushima *et al.*, 2001; Allen *et al.*, 2002; Kakarougkas and Jeggo, 2014; Bosshard *et al.*, 2019), and a similar interplay has been suggested in the mouse zygote (Derijck *et al.*, 2008). Here, we obtained data that suggest that in addition to the cNHEJ pathway the Parp1-dependent alternative NHEJ pathway contributes to the repair of a subset of exogenous IR-induced DSBs in late pachytene spermatocytes (Fig. 5) that show a declining HR repair activity. Since Parp1 inhibitor treatment seems to affect DNA repair through indirect effects (Flohr *et al.*, 2003; Ma *et al.*, 2012), it may be possible that the observed Parp1 contribution to

FIG. 5. Outline of Parp1-dependent DSB repair during early and late pachytene of mouse meiotic prophase I with respect to NHEJ and HR repair. **(A)** Representative images of pachytene spermatocytes of testicular epithelial stages indicated stained for γ -H2AX DSB damage (small dark brown foci) from mice with (DPQ) and without (Con) Parp1 inhibition. **(B)** Flowchart showing the percentage of relative DSB repair contribution by the respective pathways in late pachytene substage spermatocytes exposed to toxic DSBs. HR, homologous recombination. Color images are available online.

aNHEJ repair of IR-induced DSBs in the G2-equivalent late prophase I spermatocytes could be indirect through its chromatin remodeling activity (Luijsterburg *et al.*, 2016) and/or Parp1 influence on BER and other pathways. Thus, further analyses on the interplay of the different DNA repair pathways and the repair of toxic DSBs in late prophase I chromatin are required.

Authors' Contributions

E.A.A. designed the experiment, conducted practical experiments, analyzed data, prepared figures, and wrote the article. A.M.A. analyzed data, prepared figures, and revised the article. H.S. analyzed data, prepared figures, and revised the article.

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Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

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