

The accumulation of MMS-induced single strand breaks in G₁ phase is recombinogenic in DNA polymerase β defective mammalian cells

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ABSTRACT

DNA polymerase (Pol) β null mouse embryonic fibroblasts provide a useful cell system to investigate the effects of alterations in base excision repair (BER) on genome stability. These cells are characterized by hypersensitivity to the cytotoxic effects of methyl methanesulfonate (MMS) and by decreased repair of the MMS-induced DNA single strand breaks (SSB). Here, we show that, in the absence of Pol β , SSB accumulate in G₁ phase cells, accompanied by the formation of proliferating cell nuclear antigen foci in the nuclei. When replicating Pol β null cells are treated with MMS, a rapid phosphorylation of histone H2AX is detected in the nuclei of S phase cells, indicating that double strand breaks (DSB) are formed in response to unrepaired SSB. This is followed by relocalization within the nuclei of Rad51 protein, which is essential for homologous recombination (HR). These findings are compatible with a model where, in mammalian cells, unrepaired SSB produced during BER are substrates for the HR pathway via DSB formation. This is an example of a coordinated effort of two different repair pathways, BER and HR, to protect mammalian cells from alkylation-induced cytotoxicity.

INTRODUCTION

Single strand breaks (SSB) can arise directly or indirectly as normal intermediates of base excision repair (BER). They are induced by both exogenous and endogenous processes, thus posing a continuous threat to genetic integrity. In higher eukaryotes, it is known that SSB repair occurs via two alternative pathways: a DNA polymerase (Pol) β -dependent pathway

(short patch BER) and a proliferating cell nuclear antigen (PCNA)-dependent pathway (long patch BER) [for a review see (1)]. SSB repair is a highly coordinated process and a defect in this ordered chain of enzymatic steps is hampered *in vivo* as testified by the dramatic phenotype of Pol β knocked out mice. Transgenic mice with a homozygous null mutation in Pol β gene are non-viable after birth (2). Pol β mouse defective cells are hypersensitive to MMS and are less efficient in the rejoining of induced SSB (3–6). Pol β has a dual role in the indirect SSB formed during BER: Pol β inserts a single nucleotide at the gap site and repairs the 5'-deoxyribose phosphate terminus (5'-dRP) created by AP endonuclease-1 (APE1). It has been elegantly shown that it is the loss of the 5'-dRP removal by the 5'-dRP lyase activity of Pol β that renders mouse fibroblasts alkylation-hypersensitive (7). Moreover, Pol β mutant mouse cells exhibit increased frequencies of sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) after methyl methanesulfonate (MMS) treatment (4,8).

If SSB are not properly repaired, they may result in double strand breaks (DSB) in replicating DNA. H2AX phosphorylation (γ H2AX) has been found at the sites of DSB in chromosomal DNA (9). Because γ H2AX appears within minutes after ionizing radiation (IR), γ H2AX focus formation is considered to be a sensitive and selective signal for the existence of DSB (10). DSB may be repaired either by direct end-joining of the broken ends (non homologous end joining, NHEJ) or by homologous recombination (HR). Although mammalian cells are presumed to repair DSB predominantly by NHEJ (11,12), accumulating experimental evidence suggests that HR also plays an essential role in the mammalian DSB repair (13–15). One of the central components of HR is Rad51 protein that forms nucleoprotein filament on single-stranded DNA, mediating homologous pairing and strand-exchange reactions between single-stranded DNA and homologous double-stranded DNA (16–19). After DNA damage, Rad51 protein is detected in multiple discrete foci in the nucleoplasm.

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To dissect the mechanism and the consequences of SSB accumulation in the absence of Pol β , we investigated the processing of these intermediates during different phases of the cell cycle. We find that, after MMS treatment, the accumulation of SSB in Pol $\beta^{-/-}$ cells occurs specifically during the G₁ phase of the cell cycle. These unrepaired SSB trigger the formation of chromatin-bound PCNA complex in the cell nuclei. When replicating, Pol β -defective cells are treated with MMS, γ H2AX foci are rapidly formed, indicating that the conversion of BER intermediates into DSB occurred during the S phase. Subsequently, Rad51 relocalizes within the Pol $\beta^{-/-}$ cells nuclei to form foci. These findings indicate that, in the absence of Pol β , the unrepaired SSB produced during BER can act as substrates for the HR pathway to avoid excessive cytotoxicity.

MATERIALS AND METHODS

Cell culture, cell cycle synchronization and cell cycle analysis

SV40 transformed wild-type and Pol β -deficient mouse embryonic fibroblasts (a gift from Dr S. H. Wilson; NIEHS, Research Triangle Park, NC) (3) were cultured in DMEM with glutamax-1 supplemented with different concentrations of foetal calf serum (FCS; see below), penicillin (100 U/ml), streptomycin (100 μ g/ml) and hygromycin (80 μ g/ml) at 34°C in a 10% CO₂ incubator. Wild-type and Pol $\beta^{-/-}$ cells were synchronized in G₁ and S phases by growth in a low serum (0.5%) containing medium for 24 h. They were then incubated in complete medium (10% FCS) in addition to different concentrations of mimosine. In particular, G₁ phase cells were obtained by incubation with 200 μ M mimosine for 16 h and S phase cells by incubation with 100 μ M mimosine for 16 h followed by 6 h recovery in the complete medium. Cells were then trypsinized, fixed, stained with propidium iodide and analysed in an EPICS XL cytofluorimeter (Coulter). The proportion of cells in G₁ and S phase was quantified using the MCYCLE software (Coulter).

Single cell gel electrophoresis (SCGE)

G₁ and S phase synchronized cell cultures were treated with 0.5 mM MMS in the serum-free medium for 15 min. Cells were allowed to repair DNA damage by incubation in complete medium for 1 h. DNA breaks were detected as described previously (20). Briefly, cells were embedded in agarose gel and then spread on a frosted microscope slide. Cells were lysed in 2.5 M NaCl, 10 mM Tris-HCl, 100 mM Na₂EDTA, 1% Triton and 10% dimethyl sulfoxide, pH 10 for 1 h at 4°C. After lysis, the cells were pre-incubated for 20 min at 4°C in the electrophoresis buffer (0.3 M NaOH and 1 mM Na₂EDTA, pH 13.5) and then subjected to alkaline gel electrophoresis (in 300 mA at 4°C for 20 min). After SCGE, cell DNA was stained with ethidium bromide and visualized using fluorescence microscopy. Slides were analysed by a computerized image analysis to quantitate the DNA damage. The tail moment (TM), calculated by multiplying the total intensity of the comet tail by the migration distance from the centre of the comet head, was used as a measure of the DNA damage. The comets were classified into different classes (from 1 to 4)

corresponding to increasing TM values (21). A total of 50 cells for each experimental point were scored blind from two slides.

Immunofluorescence microscopy

Wild-type and Pol $\beta^{-/-}$ cells in G₁ phase were treated with 0.5 mM MMS for 15 min and then allowed to repair for the indicated periods of time. For the detection of chromatin-bound PCNA complex formation, the cells were rinsed twice in phosphate-buffered saline (PBS), fixed in ice-cold methanol for 20 min at -20°C, and then incubated for 30 s with ice-cold acetone to extract the soluble PCNA fraction. Cells were subsequently washed twice with PBS and incubated for 1 h at room temperature with primary anti-PCNA antibody (PC10 from Santa Cruz; 1:50). Subsequently, the cells were washed with PBS and incubated for 45 min with TRIC-conjugated antimouse IgG. After washing three times for 5 min with PBS, cells were mounted with glycerol. No significant signal attributable to secondary antibody alone was detected. The same experimental procedure was used to detect γ H2AX foci. Cells were incubated with the primary anti- γ H2AX antibody (γ H2AX; Trevigen, Gaithersburg, MD) in the ratio 1:100 overnight at 4°C in a humidified incubator, washed with PBS and incubated for 45 min with fluorescein isothiocyanate (FITC)-conjugated antirabbit IgG at 37°C. For the detection of chromatin-bound PCNA complex and γ H2AX positive cells, the dual labelling was performed using the same experimental procedure, which is used for the single labelling of PCNA complex and γ H2AX foci. For the detection of Rad51 foci, an aliquot of the cell suspension was centrifuged onto the clean glass slides at 600 r.p.m. for 10 min, in a cytospin. After cytocentrifugation, the slides were fixed in -20°C methanol for 20 min and then immersed in ice-cold acetone for a few seconds to permeabilize the cells for antibody staining. Following three washes with PBS, the preparations were blocked with 10% normal goat serum (Hyclone Laboratories) in PBS for 1 h, incubated at 4°C overnight with the primary anti-Rad51 antibody (Rad51, Ab1, polyclonal, Oncogene; 1:200), washed in PBS and then incubated for 45 min with FITC-conjugated antirabbit IgG at 37°C.

All the preparations were counterstained with 0.1 mg/ml of 4',6-diamidino-2-phenylindole (DAPI). Coverslips were analysed using a Leica DRMB fluorescence microscope equipped with a charge coupled device camera. The images were processed using the IAS 2000 Delta System software (Adobe, San José, CA).

RESULTS

MMS-induced SSB repair is impaired in G₁-treated Pol β -defective cells

Mouse fibroblasts lacking Pol β present a reduced rate of MMS-induced SSB repair (3–6). To elucidate this mechanism better, MMS-induced SSB repair was analysed in G₁ and S phase synchronized wild-type and Pol $\beta^{-/-}$ cells. A significant enrichment of G₁ and S phase cells (~60–70% of the total cell population) was obtained in both cell lines by serum starvation and mimosine treatment (data not shown). Cells were then treated with MMS (0.5 mM) for 15 min and allowed to repair for 1 h in fresh medium. The level of SSB was measured by alkaline SCGE. Under these conditions, both DNA SSB and

alkali-labile sites (i.e. abasic sites) are detectable. The presence of breaks allows supercoiled loops of DNA to relax and migrate to form a tail, and the fraction of DNA in the tail reflects the frequency of breaks. The number of SSB measured reflects the balance between breaks arising from BER and breaks resealed by the same repair process.

As shown in Figure 1B, Pol β -defective cells treated in G₁ phase, presented a higher level of SSB as compared with wild-type cells immediately after MMS treatment. Since MMS is a

direct-acting mutagen, this is unlikely to reflect differential amounts of induced DNA damage but rather the fast formation and accumulation of SSB in G₁ cells in the absence of Pol β . After a 1 h repair in drug-free medium (Figure 1C), the majority of breaks were repaired in wild-type cells, whereas mutant cells showed an accumulation of SSB.

In contrast, the distribution of MMS-treated S phase cells according to the TM was very similar in normal and Pol β ^{-/-} cells (Figure 2), indicating that Pol β does not play a major role

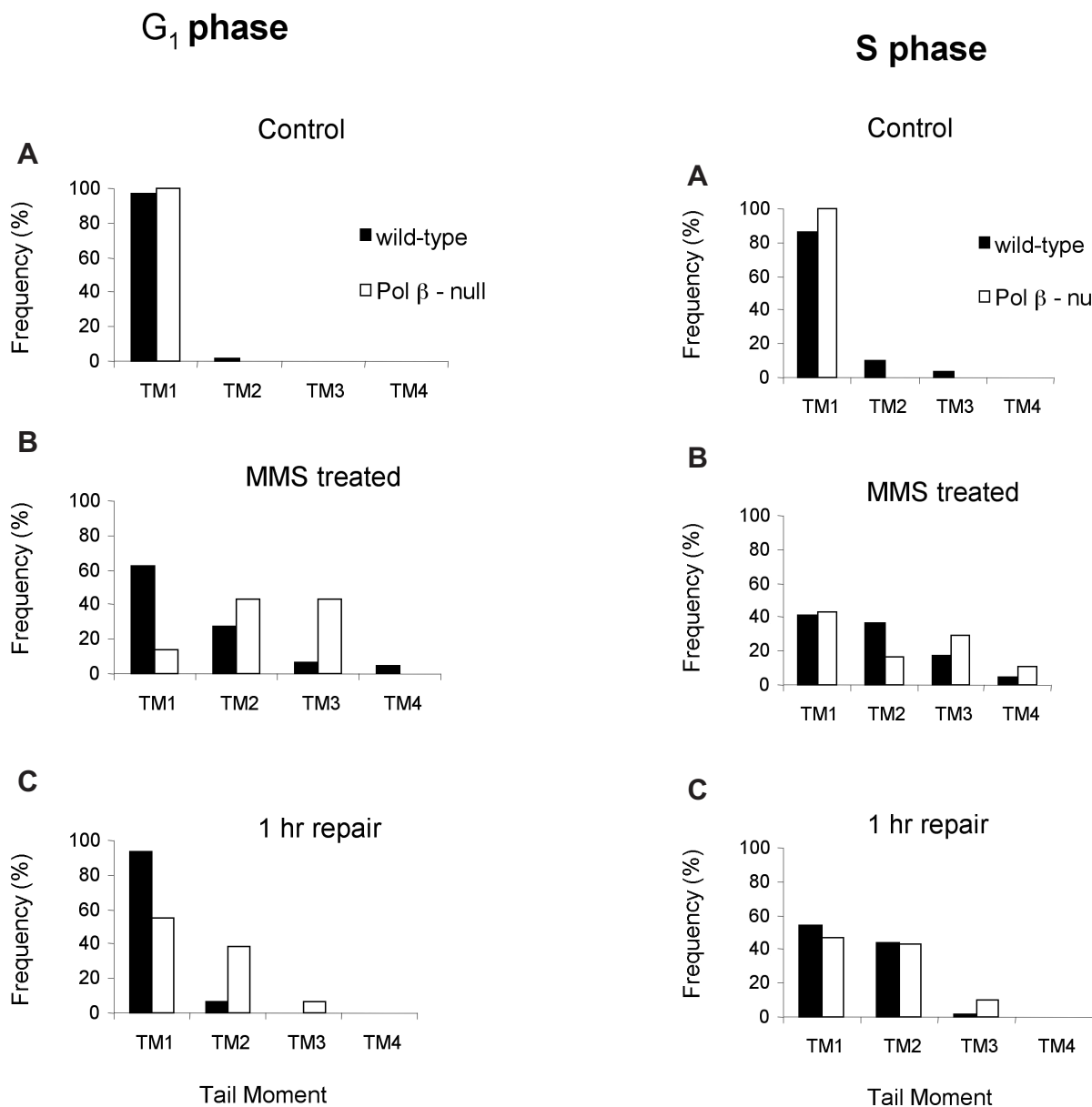


Figure 1. Effect of the lack of Pol β on MMS-induced DNA SSB repair during G₁ phase. Comet assay was performed in wild-type and Pol β ^{-/-} cells, synchronized in G₁ phase, after 15 min treatment with 0.5 mM MMS followed by 1 h repair in complete medium. The TM of 50 comets per experimental point was measured by computerized image analysis and the comets were classified in different classes (TM1-4) according to TM values. (A) Heterogeneity in DNA repair in G₁ phase untreated cells, (B) in G₁ phase MMS treated cells and (C) in G₁ phase cells after repair.

Figure 2. Effect of the lack of Pol β on MMS-induced DNA SSB repair during S phase. Comet assay was performed in wild-type and Pol β ^{-/-} cells, synchronized in S phase, after 15 min treatment with 0.5 mM MMS followed by 1 h repair in complete medium. The TM of 50 comets per experimental point was measured by computerized image analysis and the comets were classified in different classes (TM1-4) according to TM values. (A) Heterogeneity in DNA repair in S phase untreated cells (B) in S phase MMS treated cells and (C) in S phase cells after repair.

in the fast repair reactions occurring in S phase cells. It is of note that MMS treatment (Figure 2B) of the S phase Pol β null cells resulted in a mild shift of the TMs to the right, in comparison with wild-type cells, indicating that DNA was fragmented to a slightly higher and more varied degree. At 1 h post-treatment (Figure 2C), both cell lines showed a persistence of DNA breaks but the yield of unrepaired breaks was similar in the two cell lines. The comet assay allows the detection of both SSB and DSB. Therefore, it is possible that the unrepaired breaks observed after the 1 h repair of S-phase-treated cells are DSB formed by the progression of the replicative fork in the presence of MMS-induced lesions and/or repair intermediates.

These findings assign the defect in SSB rejoining of Pol β null cells to DNA repair that occurs during the G₁ phase, strengthening the notion that Pol β operates during a pre-replicative BER.

Chromatin-bound PCNA complex formation is triggered by MMS damage in Pol β null cells

It is known that the chromatin-bound PCNA complex formation is triggered by DNA damage in quiescent cells in G₁ and G₂ phases. This occurs following treatment with various DNA damaging agents like UV light (22–24), X-rays (25), alkylating agents (26) and hydrogen peroxide (26,27). We asked whether the accumulation of SSB in G₁ Pol β null cells

could also be revealed by PCNA complex formation. Pol β -proficient and deficient cell lines synchronized in G₁ phase were treated with MMS, and PCNA foci were analysed by immunofluorescence. In untreated cells, the chromatin-bound PCNA was barely detectable (Figure 3A and B). In Pol $\beta^{-/-}$ cells, the PCNA complex appeared to form rapidly during treatment, since the cells fixed immediately after 15 min incubation with MMS (no recovery time) showed an intense chromatin-bound PCNA foci staining (Figure 3B and C). In contrast, wild-type cells did not show any PCNA staining immediately after treatment (Figure 3C). The higher level of SSB in G₁ Pol $\beta^{-/-}$ cells is therefore associated with chromatin-bound PCNA complex formation. The analysis of PCNA immunostaining at different post-treatment incubation times showed that PCNA foci also appeared in wild-type cells but at later times as compared with Pol $\beta^{-/-}$ cells. In both cell lines, the percentage of PCNA foci reached a peak at 15 min after MMS treatment and then gradually declined (Figure 3C).

These data indicate that, following MMS damage, PCNA foci accumulate in Pol β -defective cells, consistent with the accumulation of SSB in G₁ phase cells.

γ H2AX foci are induced in MMS-treated Pol β -defective cells

Since G₁-treated Pol $\beta^{-/-}$ cells present a higher level of SSB when compared with wild-type cells, we raised the question

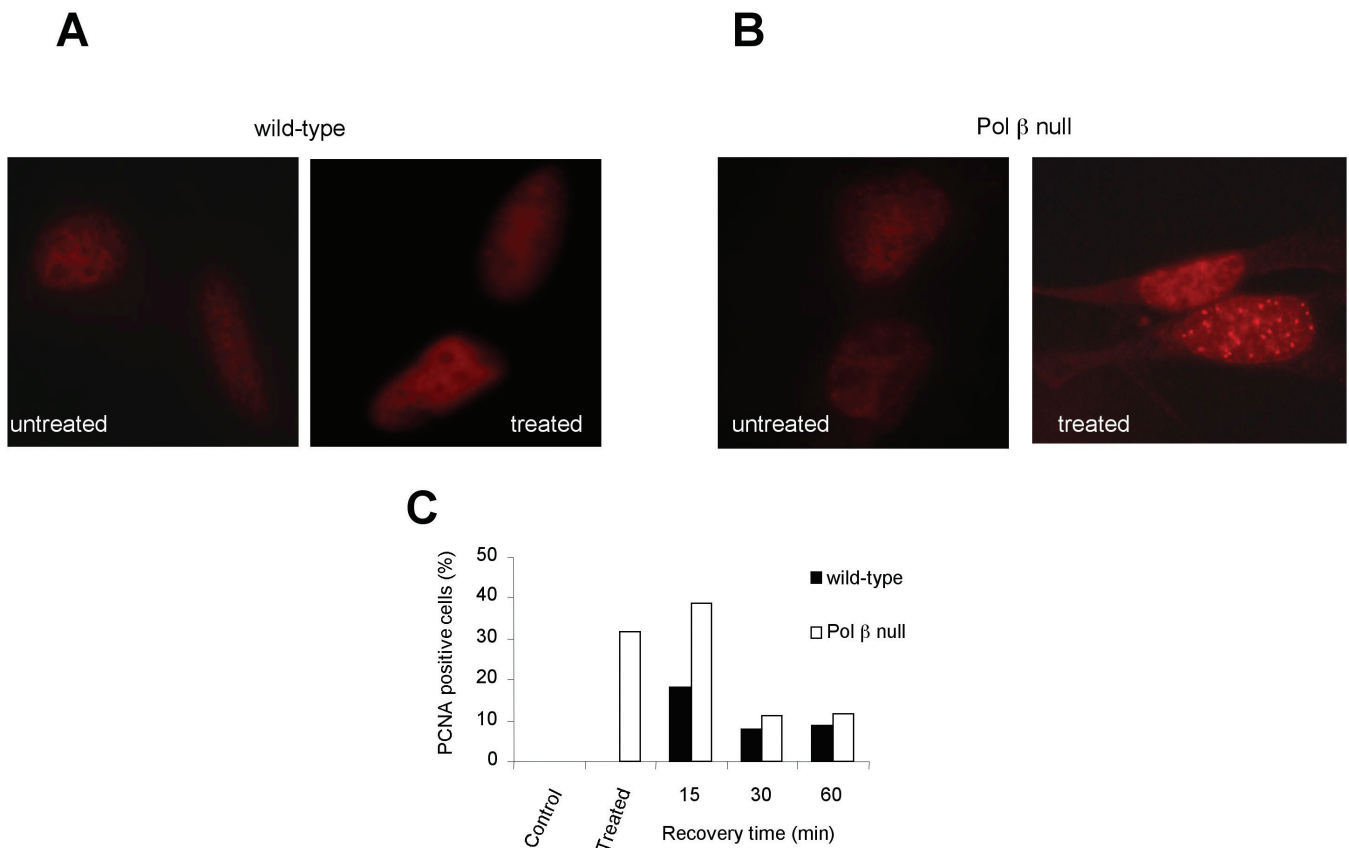


Figure 3. Immunofluorescence analysis of chromatin-bound PCNA in Pol β proficient and deficient cells after MMS treatment. Wild-type and Pol $\beta^{-/-}$ cells, synchronized at G₁ phase, were treated with 0.5 mM MMS for 15 min and then allowed to repair for the indicated periods of time. Cells were fixed in cold methanol and then in cold acetone to extract the soluble PCNA fraction. The spatial distribution of PCNA foci is shown in representative nuclei of wild-type (A) and Pol $\beta^{-/-}$ (B) cells under high magnification. The histogram (C) reports the percentage of PCNA foci. Cells with at least 5 foci were counted as PCNA positive cells.

which is the biological effect of the accumulation of these repair intermediates when replication occurs. We then examined the effects of MMS treatment on cell cycle of Pol β -proficient and deficient cells. Proliferating cells were treated for 1 h with 1 mM MMS and then washed to remove MMS from the culture medium. In agreement with the previous studies (6,8), at 12 h post-treatment in Pol $\beta^{-/-}$ cells, the proportion of S phase increased significantly (37% increase), whereas a mild effect was observed on the cell distribution of wild-type cells (18% increase of S-phase cells) (data not shown). The accumulation of unrepaired SSB in the absence of Pol β might determine a delay in the progression through the cell cycle.

Next, γ H2AX focus formation was investigated in proliferating MMS-treated cells. Histone H2AX is rapidly phosphorylated in the chromatin microenvironment surrounding a DNA DSB, where it recruits repair and checkpoint protein complexes (28). As shown in Figure 4, the kinetics of γ H2AX foci formation showed rapid DSB formation in Pol β -defective cells immediately after MMS treatment (0.5 mM for 15 min), whereas very few foci were detectable in wild-type cells. These foci gradually declined during repair time (Figure 4B). Moreover, the double staining with γ H2AX and PCNA revealed that the γ H2AX foci were at most localized in the nuclei of S phase cells (data not shown). In order to detect γ H2AX foci in wild-type cells, doses higher than those used

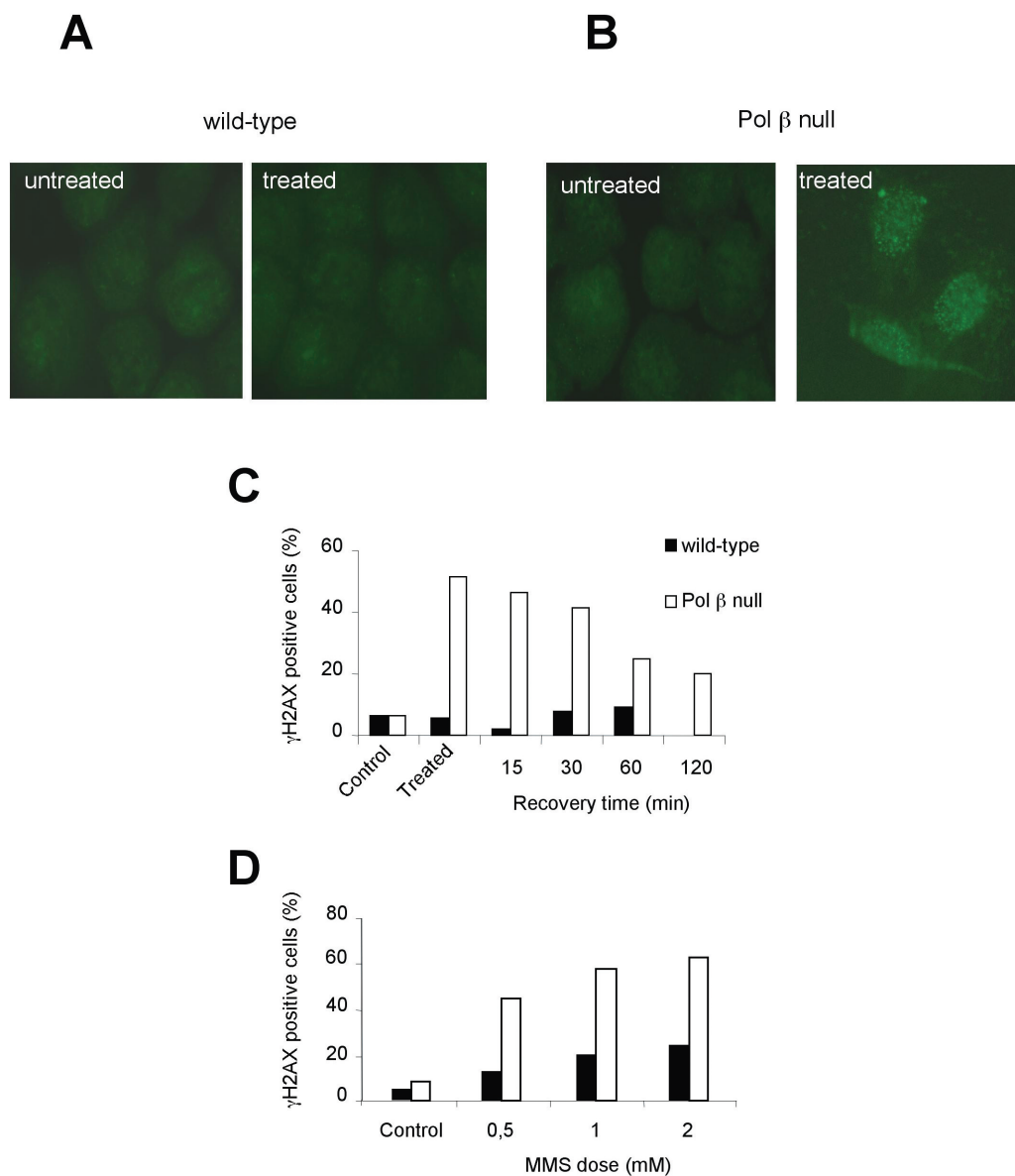


Figure 4. Immunofluorescence analysis of γ H2AX foci in Pol β proficient and deficient cells after MMS treatment. Wild-type and Pol $\beta^{-/-}$ cells were treated with MMS and γ H2AX foci analysis was performed. The spatial distribution of γ H2AX foci is shown in representative nuclei of wild-type (A) and Pol $\beta^{-/-}$ (B) cells under high magnification. The histogram (C) reports the percentage of γ H2AX foci after cell treatment with 0.5 mM MMS for 15 min and different repair times as indicated. The histogram (D) reports the percentage of γ H2AX foci after cell treatment with increasing MMS doses as indicated. Cells with at least 10 foci were counted as γ H2AX positive cells.

for Pol β null are required (1–2 mM MMS) (Figure 4D). When the cells treated in this dose range were examined by pulse-field gel electrophoresis (PFGE) DSB formation was confirmed, but owing to the lower sensitivity of this technique, DSB were only detected in Pol β null cells (data not shown).

These results strongly suggest that, in the absence of Pol β , the accumulation of SSB in the G₁ phase increases the likelihood of DSB formation during replication fork progression. DSB, so generated, are a signal for the phosphorylation and consequently the formation of the γ H2AX foci. This phenomenon occurs also in wild-type cells and might explain the recombinogenic potential of MMS.

Rad51 foci are induced in MMS-treated Pol β -defective cells

The formation of γ H2AX foci in Pol β defective cells prompted us to analyse the Rad51 protein. It is well known that Rad51 protein, which is essential for HR, relocalizes within the nucleus, in response to a variety of DNA damaging treatments, to form distinct foci that represent sites where repair reactions take place (29,30).

Rad51 focus positive cells were observed in proliferating populations of Pol $\beta^{-/-}$ cells at early times after MMS treatment (2 h) (Figure 5C). The increase in focus positive cells observed in wild-type proliferating cells was less pronounced and appeared later in time (Figure 5C). In both cell lines,

the percentage of Rad51 positive cells reached a maximum at 8 h post-treatment and then declined to background values within 24 h.

The presence of Rad51 foci in wild-type cells indicates that after MMS treatment recombination occurs. The elevated or accelerated assembly of Rad51 foci in Pol $\beta^{-/-}$ cells is consistent with the involvement of unrepaired SSB in recombination events by DSB formation.

DISCUSSION

SSB repair in Pol β mutant cells

The clearest defects detected so far in Pol β mutant cells are the hypersensitivity to MMS and the reduced rate of MMS-induced SSB repair (3–6). Another mutant cell system shares these same features: the XRCC1 mutant rodent cells. XRCC1 protein plays a major role in SSB repair by interacting with multiple SSB repair partners, including PARP-1, Pol β , PCNA, DNA ligase III, APE-1 and polynucleotide kinase [reviewed in (31)]. Pol β is also known to interact with several proteins, largely overlapped to XRCC1 partners (32,33) and with XRCC1 itself (34,35). Biochemical and molecular modelling experiments suggest that the XRCC1–Pol β complex surrounds the SSB and helps recruiting the correct repair pathway (36). The striking similarity between the spectrum of

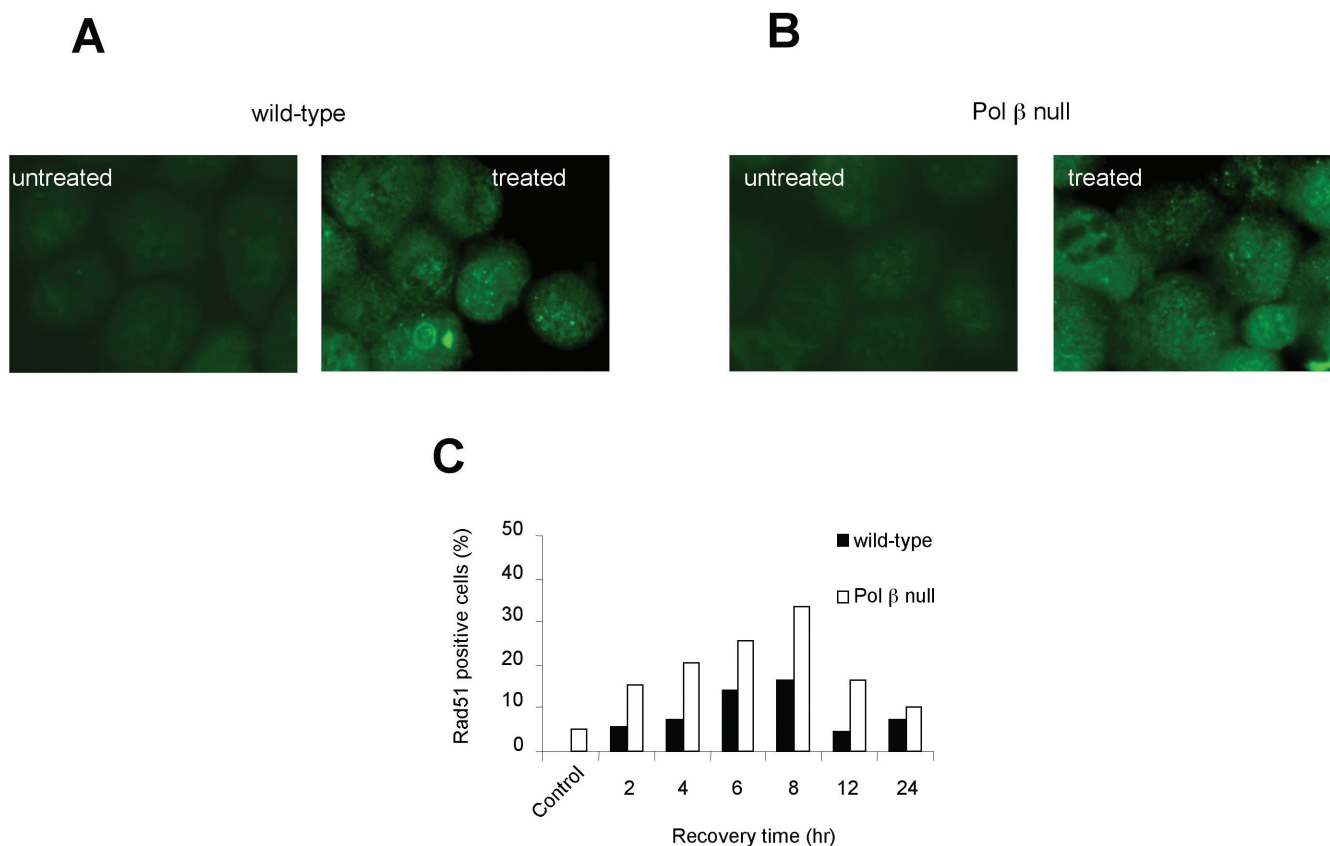


Figure 5. Immunofluorescence analysis of Rad51 foci in Pol β proficient and deficient cells after MMS treatment. Wild-type and Pol $\beta^{-/-}$ cells were treated with 0.5 mM MMS for 15 min and then allowed to repair for the indicated periods of time. The spatial distribution of Rad51 foci is shown in representative nuclei of wild-type (A) and Pol $\beta^{-/-}$ (B) cells under high magnification. The histogram (C) reports the percentage of Rad51 foci. Cells with at least 10 foci were counted as Rad51 positive cells.

Table 1. Genetic instability of XRCC1 and Pol β defective cell lines

Phenotype	XRCC1 mutant ^a	Pol β mutant ^b
Sensitivity to MMS	Highly increased	Increased
Sensitivity to H ₂ O ₂	Increased	No difference
Sensitivity to IR	Slightly increased	No difference
SSB repair	Reduced rate after IR, EMS, MMS	Reduced rate after MMS
DSB repair	Reduced rate after X-rays	Reduced rate after MMS
SCE	Increased spontaneous and induced SCE	Increased induced SCE
CA	Increased spontaneous and induced CA	Increased induced CA
Mutation frequency (MF)	Increased spontaneous and induced MF	Increased induced MF

^aData from (31).^bData from (4,53,54).

altered functions in Pol β and XRCC1 mutant cells (Table 1) is therefore not surprising. It should also be mentioned that cells defective in the DNA nick sensor protein, PARP-1, share several features with Pol β /XRCC1 mutants (reviewed in 37). Interestingly, recent data suggest that PARP-1 plays a role as a surveillance protein for a stalled BER intermediate and an important regulator factor in BER sub-pathway choice (38,39). The extensive work of characterization of XRCC1-dependent SSB repair as a function of cell cycle by Caldecott and co-workers (40) has allowed the identification of different SSB repair pathways that operate in different phases of the cell cycle: a rapid SSB repair that appears to operate throughout interphase (likely overlapped with the so-called short patch BER) and an S/G₂ specific SSB repair. It has been speculated that this second pathway, which is coordinated with DNA replication, employs long-patch BER proteins like PCNA, Fen 1 and DNA ligase I (41). In this study, we show that Pol β has a specific role in the rapid SSB repair that occurs in G₁ phase and also requires an XRCC1–Lig3a interaction (40). In contrast, we show that S phase-treated cells repair SSB efficiently, independent of Pol β . On the basis of these data, we hypothesize that the second XRCC1-dependent pathway, which operates specifically in S/G₂, does not involve Pol β but replicative DNA polymerases. This model gives a possible explanation for the more severe phenotype of XRCC1 defective cells as compared with Pol β mutant cells (see Table 1): XRCC1 is an essential component of SSB repair in all phases of the cell cycle, whereas DNA polymerases other than Pol β operate during replication-associated SSB repair. It is therefore the defect in the S/G₂ specific XRCC1-dependent (Pol β independent) SSB repair that accounts for the higher genetic instability of XRCC1 defective cells as compared with Pol β defective cells after DNA damage.

It is well known that the treatment of quiescent cells with UV light (22–24) as well as IR (25), triggers a rapid transition of PCNA from a soluble to an insoluble form, which can be visualized by immunofluorescence. This transition correlates with nucleotide excision repair and BER capacity, respectively. In the case of BER, the PCNA complex formation has been reported to correlate with BER kinetics (42). Our data are in line with these previous findings. The PCNA distribution kinetics closely resemble that of SSB repair, showing a significant increase in PCNA complex formation in Pol β null

cells after MMS damage and a slower decrease as a function of time as compared with wild-type cells. Interestingly, a previous study (43) showed no difference in the PCNA complex formation in Pol β null cells exposed to X-rays as compared with wild-type cells. Altogether, these data indicate that Pol β is the major Pol in pre-replicative SSB repair following alkylation damage but other pathways, which are Pol β independent, are involved in SSB repair after oxidative DNA damage (5).

γ H2AX and RAD51 foci formation in Pol β mutant cells

If cells enter into the S phase with a load of unrepaired SSB, the likelihood of DSB formation is expected to increase. We show for the first time that the repair defect of Pol β null G₁ cells is detectable by using γ H2AX foci analysis. The majority of MMS-induced γ H2AX foci were present in PCNA positive cells and thus these foci are attributed to replication-mediated DNA damage. Conversely, in the case of an agent able to induce direct DSB as IR, these foci are observed in both PCNA positive and negative cells (9). Several studies indicate that γ H2AX foci per cell are proportional to the number of introduced DSB (44,45). A recent study (46) suggests that lesions other than DSB induced by monofunctional alkylating agents might lead to γ H2AX foci formation. Pol β null cells show a significant fraction of γ H2AX nuclear foci in PCNA positive cells immediately after MMS treatment (when wild-type cells show background levels). By using an alternative technique such as PFGE to measure DSB, we were able to confirm the induction of DSB by MMS in Pol β null cells, although MMS doses higher than those used for γ H2AX foci analysis were required to detect an effect. We can thus conclude that MMS-induced γ H2AX foci in Pol β null cells are likely to reflect replication-mediated DSB formation. Moreover, we confirm that γ H2AX foci analysis is the most sensitive assay for DSB formation (45). Almost 50% of γ H2AX foci are still present 2 h after MMS treatment. It is well known that DSB repair presents a biphasic kinetics with a rapid and a slow phase (the bulk of the breaks are repaired within 1–2 h and the remaining breaks are repaired within 5 h) (47). The kinetics of γ H2AX foci disappearance in our cells suggests that MMS-induced DSB are repaired by the ‘slow’ repair system that has been hypothesized to involve HR (48). Repair of DSB may be largely error-free by HR but they are also a potential substrate for NHEJ, which may introduce a large number of mutations (49). It is known that Rad51 is essential for HR and, in response to DNA damage, the protein relocates within the nucleus to form foci (29,30). These foci are thought to represent sites where repair takes place. Rad51 foci assemble at earlier times in Pol β defective cells when compared with wild-type cells. Since Rad51 foci that are formed after DNA damage mark a subset of cells that have entered the HR pathway, we could conclude that BER intermediates, accumulated in the genome as a consequence of a defective BER, could act as substrates for HR by DSB formation.

The presence of γ H2AX foci (at high MMS doses) and of Rad51 foci in MMS-treated wild-type cells deserves a comment. It is known that MMS is recombinogenic (29,50). The detection of γ H2AX foci strongly suggests that MMS is recombinogenic via DSB formation triggered by unrepaired BER intermediates. This same phenomenon would be amplified in the absence of Pol β due to accumulation of unrepaired intermediates.

What is the effect of the switching between BER and recombination for alkylation induced cellular damage? In yeast, it has been shown that BER (Mag-1 initiated) and recombinational repair show a complex genetic interaction that determines alkylation resistance (51,52). It was therefore hypothesized that BER intermediates might be responsible for Mag-1-induced recombination. In mammalian cells, it was shown that MMS induces a dramatic increase in SCE in cells lacking Pol β (4). These cells present relatively more SCE than 3-methyladenine DNA glycosylase (Aag) null and Aag/Pol β null cells, suggesting that unrepaired 5'-dRP lesions can stimulate HR (8). We propose that in Pol β null cells, the accumulation of SSB triggers the HR pathway following DSB formation, thus limiting the cytotoxic effects of alkylation damage. Defects in the recombination pathway are expected to lead to more dramatic effects on alkylation damage-sensitive phenotype than BER defects.

In conclusion, we propose that, in the absence of Pol β , SSB (likely due to persistent 5'dRP residues) accumulate in G₁ phase cells. When these lesions encounter the replication machinery the replication forks collide. A fraction of these events leads to replication-mediated DSB formation (γ H2AX foci formation). The replication-mediated DSB would trigger recombination and activate a checkpoint response. HR will partially compensate for the BER defect to avoid excessive alkylation-induced cytotoxicity.

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