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Cells deficient in PARP1 show an accelerated accumulation of DNA single strand breaks, but not AP sites, over the PARP1-proficient cells exposed to MMS

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Abstract

Poly(ADP-ribose) polymerase-1 (PARP-1) is a base excision repair (BER) protein that binds to DNA single strand breaks (SSBs) and subsequently synthesizes and transfers poly(ADP-ribose) polymers to various nuclear proteins. Numerous biochemical studies have implicated PARP-1 as a modulator of BER; however, the role of PARP-1 in BER in living cells remains unclear partly due to lack of accurate quantitation of BER intermediates existing in cells. Since DT40 cells, chicken B lymphocytes, naturally lack PARP-2, DT40 cells allow for the investigation of the PARP-1 null phenotype without confounding by PARP-2. To test the hypothesis that PARP-1 is necessary for efficient BER during methylmethane sulfonate (MMS) exposure in vertebrate cells, intact DT40 cells and their isogenic PARP-1 null counterparts were challenged with different exposure scenarios for phenotypic characterization. With chronic exposure, PARP-1 null cells exhibited sensitivity to MMS but with an acute exposure did not accumulate base lesions or AP sites to a greater extent than wild-type cells. However, an increase in SSB content in PARP-1 null cell DNA, as indicated by glyoxal gel electrophoresis under neutral conditions, suggested the presence of BER intermediates. These data suggest that during exposure, PARP-1 impacts the stage of BER after excision of the deoxyribosephosphate moiety from the 5' end of DNA strand breaks by polymerase β .

Keywords

Alkylating agent; N7-methylguanine; AP sites; Base excision repair; single strand breaks; PARP-1

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

1. Introduction

Base excision repair (BER) limits DNA damage formed through spontaneous or oxidative processes associated with endogenous metabolism [1]. Additionally, BER can act upon non-bulky base damage, such as N3-methyladenine, and the depurination product of N7-methylguanine (N7-meG) caused by exposure to mono-functional alkylating agents [2]. With formation of such alkylative damage, entry into BER can proceed with the removal of the adducted base from the DNA strand via spontaneous depurination (e.g., N7-meG) or by the mono-functional methyl purine glycosylase (e.g., N3-methyladenine). The resulting intact apurinic (AP) site is incised by AP endonuclease (APE), thereby generating a single strand break (SSB) with a 5'-deoxyribosephosphate (5'-dRP) terminus. Subsequently, polymerase β (POL β) removes the 5'-dRP moiety and replaces the appropriate nucleotide to the DNA sequence. DNA ligase III α (LIG III α) finally seals the DNA strand to complete this sequence of events, which is commonly referred to as short-patch (SP)-BER. Alternatively, the long-patch (LP)-BER, which consists of a different complement of enzymes, can also operate to remove 5'-dRP residues and ligate DNA. Following the binding of proliferating cell nuclear antigen, POL β or the replicative polymerases δ or ϵ participate in strand displacement synthesis creating a 2 to 8 nucleotide flap that is excised from DNA by flap endonuclease-1 (FEN-1). DNA ligase I subsequently closes the DNA strand [3].

Poly(ADP-ribosyl)ation is a ubiquitous protein modification involved in the regulation of transcription, cell proliferation, differentiation, DNA methylation, and apoptosis [4,5]. Of the 17 human poly(ADP-ribose) polymerase (PARP) enzymes, both PARP-1 and PARP-2 have been proposed to play an important role in DNA single-strand break and base excision repair pathways [4]. In the process of these DNA repair pathways, posttranslation modification believed to limit genotoxic stress is the synthesis and covalent addition of poly(ADP-ribose) (PAR) polymers to acceptor proteins associated with DNA metabolism [4]. These ribosylation reactions are largely attributed to PARP-1, the archetypal member of a diverse family of a proteins capable of such reactions [6]. PARP-1 surveys DNA for strand disruptions, binds to them, and synthesizes PAR polymers, through NAD⁺ consumption, for attachment to itself and other proteins such as histones. While PAR polymers have a transient existence due to degradation by poly(ADP-ribose) glycohydrolase (PARG), ribosylation reactions influence chromatin structure and protein activity. Additionally, charge repulsion causes the dissociation of polyribosylated PARP-1 from DNA with the subsequent cessation of PAR synthesis.

The development of viable *Parp-1* knockout mice provided a model from which subsequent investigations could elucidate the necessity of PARP-1 in DNA repair. Cells from these animals are hypersensitive to alkylating agents and ionizing radiation, suggesting the participation of PARP-1 in BER [7]. Furthermore, mouse embryonic fibroblasts deficient in PARP-1 showed a delayed repair of SSBs caused by methylating agents as determined by a weak alkaline comet analysis [8]. As determined by the comet analysis under strong alkaline conditions, PARP-1 knock-down by siRNA also introduces more persistence of SSBs/alkaline labile sites in human primary fibroblasts and HeLa cells, leading to γ H2AX foci formation [9]. PARP-1 can physically interact with and recruit x-ray cross complementing group 1 (XRCC1) to SSBs [10,11]. Since interactions of XRCC1 with POL β and LigIII α have also been demonstrated, a model has emerged where PARP-1 activity could lead to the formation of a repair complex at SSBs, which consists of XRCC1, POL β , and LigIII α [12,13]. PARP-1 also heterodimerizes with PARP-2, a functional homolog that possesses similar interaction capabilities, but lacks the affinity for SSBs and the capacity for PAR synthesis [4,14]. However, the requirement for PARP-1 in the processing of BER related damage still remains tenuous due to the existence of conflicting observations [8,15]. In addition, it is not well characterized regarding which BER steps are influenced by PARP-1 in living cells. This is partly due to lack of accurate quantitation of base excision repair (BER) intermediates existing in cells using an adequate analysis. In an

attempt to further solidify a requirement for PARP-1 in BER and address which BER process is most affected by PARP-1, we assessed the PARP-1 null phenotype in intact cells. DT40 chicken cells (chicken B lymphocytes) and isogenic PARP-1 null cells were used for this study. Although the chicken genome has major PARP enzymes (e.g., PARP-1, -3, -4, -6, -8, -9, -11, -12, -14, and -16, TIPARP, TNKS, and TNKS2) [16–18], the chicken cells naturally lacks PARP-2, allowing for an investigation without the contribution of this PARP-1 homolog to the genotoxic response [18]. Cell lines were challenged under different MMS exposure scenarios for subsequent evaluation of endpoints, including survival and the accumulation of BER substrates throughout this pathway. We observed an accelerated accumulation of DNA single strand breaks, but not AP sites, in PARP-1-deficient DT40 cells over the PARP1-proficient cells exposed to MMS.

2. Materials and methods

2.1. Culture conditions and dish exposures

The generation of and culture conditions for DT40 and PARP-1 null cells and PARP-1 null cells stably expressing human PARP-1 were described previously [18,19]. For chemical exposure, wild-type (PARP-1 proficient) and mutant DT40 (PARP-1 deficient) cells were seeded into 10 cm dishes with complete medium and allowed to incubate overnight to obtain the desired cell density (1×10^6 /mL). Without changing medium, MMS (Aldrich) dosing solution (100×) was added to the cultures and cells were incubated at 39.5 °C for appropriate time points. After exposure, cells were harvested, washed with cold $1 \times$ PBS, pelleted, and then stored at -80°C until DNA isolation.

2.2. Cytotoxicity assay

Colony formation was determined in medium containing methylcellulose as described previously [19].

2.3. DNA extraction

DNA isolation was performed with modification to the PureGene DNA extraction kit (Gentra Systems Inc., Minneapolis, MN, USA) as described previously [20].

2.4. Immuno-slot blot for ring opened N7-meG

Levels of N7-meG were measured based on the alkaline conversion of the adduct to 2,6-diamino-4-hydroxy-5-*N*-methyl-formamidopyrimidine (roN7-meG) with subsequent immuno-slot blot analysis [21,22].

2.5. AP site assay

AP sites were measured as previously described by aldehyde reactive probe (ARP, Dojindo Molecular Technology, Gaithersburg, MD, USA) labeling and slot blot analysis [23].

2.6. NAD(P)H depletion assay

During continuous MMS exposure, an imbalance in BER for DT40 cell lines was assessed in real-time by a colorimetric assay monitoring intracellular NAD(P)H [19]. NAD(P)H depletion served as a proxy for NAD^+ consumption, an indicator of PARP-1 activation from SSB accumulation [24]. To confirm the activation of PARP-1 during continuous MMS exposure, cells were also co-exposed in the presence of the PARP inhibitor 3-aminobenzamide (3-AB, 10 mM, Sigma).

2.7. Glyoxal gel electrophoresis assay

To qualitatively assay the extent of SSB formation in genomic DNA from exposed cells, single stranded DNA was fractionated by neutral electrophoresis as previously described with modification [25]. Briefly, equal amounts of DNA (3 – 10 μ g) samples to be compared were first denatured in 1.5 M glyoxal (Fluka), DMSO (50% (v/v); Sigma), and 10 mM sodium phosphate (pH 7) for 1 h at 50°C. Loading buffer, which consisted of 50% glycerol (Fisher), 0.01% bromophenol blue (Sigma), 0.01% xylene cyanol (Sigma), and 10 mM sodium phosphate (pH 7), was added to each sample prior to loading and separation of the DNA fragments on 0.7% agarose gels (Fisher) in 10 mM sodium phosphate (pH 7) for 16 h (30 V) at 4°C. Gels were stained with acridine orange (5 μ g/mL; Fisher) for 1 h and then destained in deionized water for subsequent visualization.

With GGE analysis of DNA from MMS treated DT40 cells, the resulting DNA migration pattern within a gel lane approximated the images normally obtained from the Comet assay. Because of this similarity, our numerical assessment of the GGE experiments was based on image analysis associated with the Comet assay (CometScore version 1.5 from Tritek). We equated the high molecular weight DNA retained above the 23.1 kb marker in the GGE analysis with the high molecular weight DNA retained in the head of the comet [26]. Similarly, the DNA smear produced during GGE represented a comet tail, and the magnitude of DNA migration in both approaches is ultimately predicated by the extent of SSB content. Tail moment was selected to express SSB content revealed by the GGE experiments; this metric was calculated as the product of tail length and percentage of DNA in the tail. Accordingly, a higher tail moment suggested increased DNA damage, in this case SSBs.

2.8. Statistical analyses

Adduct and AP site data were log transformed to approximate linearity. Analysis of covariance (ANCOVA) was then performed to test for differences in the mean intercept and in the slopes of the linear dose-response curves between DT40 and PARP-1 null cells.

3. Results

3.1. Influence of PARP-1 on cell survival during MMS exposure

In this study, DT40 cells and their isogenic PARP-1 null counterparts served as an experimental model to investigate the *in vivo* role of PARP-1 in various aspects of BER. Since they lack PARP-2, DT40 cells allow for the investigation of the PARP-1 null phenotype without confounding by PARP-2 [18]. When challenged with MMS for 10 days, PARP-1 null cells exhibited extreme hypersensitivity to cell killing (Figure 1). The consistency between this observation with previous analyses in vertebrate and mammalian cell models reaffirmed the role of PARP-1 as a survival factor after alkylative stress [8,18]. The hypersensitivity was complemented by ectopic expression of chicken PARP-1 (Figure 1). Therefore, the hypersensitivity of PARP-1 null cells to MMS is due to the lack of PARP-1.

3.2. roN7-meG as an exposure marker

Subsequent experiments aimed to identify any BER defects in PARP-1 null cells, which may allow for the accumulation of repair intermediates that may ultimately elicit alkylation sensitivity. N7-methylguanine is believed to be released from the DNA backbone predominantly by spontaneous depurination [27] at approximately a 60–150 hour half life under physiological conditions [27]; therefore, these lesions have been utilized as a biomarker of exposure [28]. To rule out dissimilar MMS treatments between cell lines, N7-meG, the predominant lesion formed by this methylating agent, served as a biomarker of exposure [2]. Treating genomic DNA from MMS exposed cells with alkaline conditions causes imidazole

ring opening of N7-methylpurines thereby allowing roN7-meG quantitation by an immuno-slot blot technique [22,29]. Over the exposure period, both cells lines showed similar formation of N7-meG with increasing exposure time (Figure 2). These adduct data show that the presence or absence of PARP-1 does not greatly influence the accumulation of base damage, particularly with increased exposure duration. These data confirmed the generation of N7-meG adducts with MMS exposure and provide confidence for the interpretation of subsequent results that PARP-1 status, rather than inconsistent exposure conditions, would be the cause of any phenotypic differences between wild-type and mutant cells. Additionally, the proportional increase in adduct number with exposure time suggests that MMS was stable over this exposure time.

3.3. AP site measurement

AP sites were directly measured to determine whether a PARP-1 deficiency affected the accumulation of these lesions. The number of endogenous AP sites present in DT40 and PARP-1 null cells were similar (Figure 3). Both DT40 and PARP-1 null cells showed equivalent increases in AP site number with MMS exposure (Figure 3). Together, these data suggest that PARP-1 status does not influence AP site accumulation during continuous MMS exposure.

3.4. Determining SSB formation from MMS exposure

With MMS exposure, the accumulation of SSBs as intermediates of BER can lead to PARP-1 overactivation and NAD⁺ consumption with depletion in intracellular NAD(P)H [24]. In DT40 cells, as exposure time increased, levels of intracellular NAD(P)H decreased in a dose dependent manner (Figure 4A). Coexposures to MMS and the PARP inhibitor, 3-AB, protected against depletions in NAD(P)H, confirming PARP-1 activity in response to continuous MMS exposures (Figure 4C). These data suggest the PARP1 activation as an indicator of an imbalanced BER response to DNA alkylation. PARP-1 null cells exposed to MMS resisted a decrease in NAD(P)H of similar magnitude as wild-type DT40 cells treated under similar conditions (Figures 4B and 4C). This observation was expected due to the lack of PARP-1 and -2 activities in the null cells and was consistent with the response previously reported for PARP-1 null mouse embryonic fibroblasts [24]. While the NAD(P)H depletion assay provided an indication of PARP1 activation initiated by SSB formation, we employed a glyoxal-coupled electrophoretic method to visualize strand disruptions in the DNA of PARP-1 proficient and deficient cells exposed to MMS. Since the glyoxal-agarose gel electrophoresis method utilizes neutral conditions for the entire process of the assay, this analysis can avoid artifactual generation of SSBs from alkaline labile sites such as AP sites. When exposed to 1 mM MMS for up to 4 h, DNA from PARP-1 null cells did show greater migration than did DNA from their parental DT40 cells with 3 to 4 h of MMS exposure (Figure 5A). In addition, when cells were exposed to a range of MMS concentrations for 4 h, DNA from PARP-1 null cells migrated to a greater extent than that from wild-type cells, starting at 0.5 mM MMS and as a function of dose (Figure 5B). Use of image analysis software also indicated an increase in DNA damage, as expressed by tail moment, with MMS exposure (Figure 5C). Without exposure, wild-type DT40 and PARP-1 null cells both appeared to have a similar level of SSBs. However, the extent of DNA damage was determined to be statistically greater in PARP-1 deficient cells than in wild-type cells with 0.5 and 1 mM MMS exposures (Figure 5C). These gel data, particularly at high dose and long MMS exposure, provided evidence for SSB formation in PARP-1 null cells, which failed to show a major decrease in NAD(P)H due to a lack of inherent PARP-1 activity (Figures 4B and 4C). These data suggest greater formation of SSBs in DT40 cells exposed to MMS, with PARP-1 null DNA having a higher SSB content, as demonstrated by enhanced DNA migration. Recent study suggested an increase in double strand breaks determined by γ H2AX levels in PARP1 knock-down cultured cells compared with mock-treated cells exposed to H₂O₂ [9]. We could not exclude a contribution of double strand breaks

on the migration of DNA from PARP-1 null cells exposed to MMS by Glyoxal-agarose gel electrophoresis analysis. However, MMS predominantly causes SSBs than double strand breaks even in highly replicating *Saccharomyces cerevisiae* with deficient in either RAD6 or RAD52 [30,31]; therefore, massive DNA strand breaks in PARP-1 null cells caused by MMS is likely due to mostly SSBs.

4. Discussion

The presence of accessory factors, such as PARP-1, are believed to modulate BER efficiency within cells [32]. Much debate has centered on the significance of PARP-1 in BER, with proponents arguing that PARP-1 causes a positive or negative effect on BER capacity. Early cell free studies suggested that PARP-1 binding to SSBs inhibits repair by denying repair proteins access to damage sites [33,34]. Conversely, the generation of mice deficient in PARP-1 and their exposure to alkylating agents and ionizing radiation established a need for PARP-1 in BER [35–37]. The use of intact cells or cell extracts from such animals produced mixed results, with some studies indicating a requirement for PARP-1 in BER [8,12,38,39], while others showed no need for PARP-1 [15,40]. Other studies, which have employed biochemical or *in vivo* models, have discovered possible roles for PARP-1 within BER [11,13,41–43]. We hypothesized that PARP-1 is necessary for efficient BER during MMS exposure in vertebrate cells. We chronically exposed PARP-1 proficient and deficient DT40 cells to MMS for 10 days as our initial characterization of the PARP-1 null phenotype in this model. With acute MMS exposures, we systematically evaluated aspects of BER to help clarify the significance of PARP-1 within this pathway. Additional endpoint measurements were performed in DT40 cells not challenged by MMS. Since DT40 cells inherently lack PARP-2 [18], a functional homolog of PARP-1, this report is the first characterization of BER in cells lacking both PARP-1 and PARP-2, as well as, the first systematic evaluation covering the entirety of BER function (from base adduction to the presence of SSBs immediately before ligation) within living cells. PARP-1 null cells exhibited a hypersensitive phenotype when chronically exposed to MMS. One could argue about a possibility of indirect results from genomic instability caused by PARP-1 deficiency. However, the hypersensitivity of PARP-1 null cells to MMS was complemented by ectopic expression of human PARP-1. Therefore, these results indicate that the hypersensitivity to MMS is due to the lack of PARP-1. During an acute exposure, both PARP-1 proficient and deficient cells had a similar accumulation of N7-meG and AP sites. However, cells lacking PARP-1 appeared to have a greater extent of SSB formation demonstrated by enhanced DNA migration during electrophoresis. Yet in the absence of MMS exposure, endogenous levels of AP sites were similar between cell lines. Our results strongly suggest that AP sites are excised by a combination of APE1 and polymerase β at a similar extent between PARP-1 proficient and deficient cells under physiological conditions as well as under massive alkylating situation.

From our acute exposure, we detected a slight increase in AP sites at 1 mM MMS for 30 min. When applied to the regression equation ($y = 82.85x$) generated from the measurement of N7-meG adducts in DT40 cells (Figure 2), it was determined that such a cumulative dose had formed 41 N7-meG adducts per 10^6 nucleotides. At this adduct level, 5.4 N3-meA adducts per 10^6 nucleotides could be expected to occur when considering an approximate ratio of one N3-meA to eight N7-meG adducts formed during MMS exposure [44]. These results suggest that at such damage levels, 5'-dRP lesions may already saturate dRP lyase capacity, become uncoupled from the repair apparatus, and then serve as substrates for PARP-1. With PARP-1 binding and NAD⁺ consumption, the ribosylation of histones opens up the local DNA environment and automodification causes PARP-1 to dissociate from DNA, collectively facilitating repair enzyme access to damage sites. The generation of PAR within the vicinity of the SSB could further enhance SP-BER by recruiting the XRCC1, POL β , and LIGIII α repair complex and stimulate LIGIII α by acting as a source of ATP for strand ligation [13,45]. With

high levels of damage and continued PARP-1 binding, intracellular NAD^+ levels may not support the efficient PAR synthesis needed for PARP-1 dissociation from DNA. This scenario could serve as the molecular switch to initiate LP-BER, allowing for the functional interaction between PARP-1 and FEN-1 that stimulates strand displacement synthesis. Previously, the stimulatory effect of PARP-1 on LP-BER was ablated when NAD^+ was added to an *in vitro* system, suggesting that the abortive dissociation of PARP-1 from DNA is critical for LP-BER [46]. Under massive levels of DNA damage, the resulting depletion in NAD^+ would result in necrotic cell death. In contrast, the hypersensitivity of PARP-1 null cells could be explained by the fact that such cells are strictly limited to PARP-1 independent BER, which upon saturation would lead to an accumulation of uncoupled SSBs that are eventually converted to toxic double strand breaks.

The direct analysis of DNA base damage and AP sites resulting from MMS exposure showed similar levels of each lesion regardless of PARP-1 status. However, DNA from PARP-1 null cells appeared to have a greater SSB content than that from wild-type cells, as determined by an electrophoretic approach. Such an occurrence is supported by previous observations for increased SSBs/alkaline labile sites in MMS treated cells with inhibited PARP activity, as demonstrated by the strong alkaline Comet assay [47] and delayed repair of SSBs in PARP-1 null cells treated with MMS, as determined by the weak alkaline Comet assay [8]. Interestingly, in our study a difference in the extent of SSB formation was not reflected in AP site numbers, where both cell lines had similar levels. This observation suggests that in both cell lines, AP sites are processed with similar efficiency up to and including their removal by the dRP lyase activity of $\text{POL}\beta$. Subsequently, the resulting intermediates awaiting ligation may be sealed with greater efficiency in wild-type cells than in PARP-1 null cells. Previously, using an *in vitro* DNA repair assay with naked DNA and cell extracts derived from PARP-1 deficient or wild-type murine embryonic fibroblasts, it has been demonstrated that PARP-1 is not required for the efficient processing and rejoining of single-strand interruptions [48]. However, results from *in vitro* experiments particularly regarding a requirement of accessory protein, such as PARP-1 and XRCC1, for the repair of DNA strand breaks need to be interpreted with great caution due to the difference of frequency of DNA damage and the existence of chromatin structure, as documented by a previous report [49]. A lack of both PAR synthesis and the eventual production of PAR degradation products associated with ATP synthesis could explain the SSB repair defect in PARP-1 null cells. Additionally, while demonstrated after hydrogen peroxide exposure, perturbation of the relationship between PARP-1 and PARG decreases SSB repair [50], further suggesting a need for PAR anabolism and break down reactions for the complete repair of SSBs.

In summary, using an isogenic cell system we attempted to link the phenotype of PARP-1 deficient DT40 cells with previous biochemical studies to better define the role of PARP-1 in BER. We conclude that PARP-1 enhances BER *in vivo*, particularly at the late stages during MMS exposure; however, PARP-1 may be dispensable during the processing of certain endogenous BER substrates. We also propose a model in which there is an ordered selection of BER sub-pathways that is predicated on the inverse relationship between intracellular NAD^+ levels and BER substrates. When substrate levels are low, PARP-1 independent SP-BER predominates in lesion processing. As damage levels increase, PARP-1 becomes active in BER to enhance SP-BER. In situations where levels of BER substrates continue to increase, the resulting decrease in NAD^+ levels from PARP-1 overactivation prohibits PARP-1 dissociation from DNA allowing for a switch to LP-BER repair. Together the observations strengthen the positive role of PARP-1 in BER for preventing the accumulation of toxic lesions during chemical exposure.

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Figure 1. Sensitivity of DT40 and PARP-1 null cells to MMS. Survival curves of DT40 (PARP-1 proficient), DT40-derived PARP-1 null cell, and PARP-1 null cells with ectopic expression of hPARP-1 exposed to MMS for 10 days. Each point represents the mean and S.D. (bars) from three independent experiments.

Figure 2.

Measurement of roN7-meG as a marker of MMS exposure. Genomic DNA from DT40 and PARP-1 null cells exposed to 1 mM for up to 4 h was subjected to alkaline conditions to induce a ring-opened form of N7-meG for subsequent immuno-slot blot analysis. Each point represents the mean of four independent measurements. Bars indicate S.D.

Figure 3. Measurement of AP sites in DT40 and PARP-1 null cells exposed to MMS. Genomic DNA from DT40 and PARP-1 null cells exposed to 1 mM for up to 4 h was reacted with ARP for slot blot analysis of AP sites. Each point represents the mean of four independent measurements. Bars indicate S.D.

Figure 4.

Depletion of intracellular NAD(P)H in DT40 and PARP-1 null DT40 cells. NAD(P)H levels in (A) DT40 and (B) PARP-1 null cells continuously exposed to various concentrations of MMS for up to 4 h. NAD(P)H depletion in (C) DT40 and PARP-1 null cells exposed to various MMS concentrations for 4 h in the presence or absence of 3-AB (10 mM).

Figure 5.

Gel electrophoresis analysis of glyoxal denatured DNA from DT40 and PARP-1 null cells exposed to MMS. **(A)** Representative gel showing the migration of genomic DNA from wild-type DT40 (+) and PARP-1 null (-) cells exposed to 1 mM MMS for 1–4 hours. **(B)** Representative gel showing the migration of genomic DNA from wild-type DT40 (+) and PARP-1 null (-) cells exposed to various MMS concentrations for 4 h. **(C)** Comparison of tail moment values as determined by image analysis software between wild-type DT40 and PARP-1 null cells (** $P < 0.01$, * $P < 0.05$, $n = 3$, t test).