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Poly(ADP-ribose) polymerase in base excision repair: always engaged, but not essential for DNA damage processing★

Sarah L. Allinson, Irina I. Dianova and Grigory L. Dianov✉

MRC Radiation and Genome Stability Unit, Harwell, Oxfordshire, OX11 0RD, U.K.

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Poly(ADP-ribose) polymerase (PARP-1) is an abundant nuclear protein with a high affinity for single- and double-strand DNA breaks. Its binding to strand breaks promotes catalysis of the covalent modification of nuclear proteins with poly(ADP-ribose) synthesised from NAD^+. PARP-1-knockout cells are extremely sensitive to alkylating agents, suggesting the involvement of PARP-1 in base excision repair; however, its role remains unclear. We investigated the dependence of base excision repair pathways on PARP-1 and NAD^+ using whole cell extracts derived from normal and PARP-1 deficient mouse cells and DNA substrates containing abasic sites. In normal extracts the rate of repair was highly dependent on NAD^+. We found that in the absence of NAD^+ repair was slowed down 4–6-fold after incision of the abasic site. We also established that in extracts from PARP-1 deficient mouse cells, repair of both regular and reduced abasic sites was increased with respect to normal extracts and was NAD^+-independent, suggesting that in both short- and long-patch BER PARP-1 slows down, rather than stimulates, the repair reaction. Our data support the proposal that PARP-1 does not play a major role in catalysis of DNA damage processing *via* either base excision repair pathway.

Base excision repair (BER) is the major pathway for repair of many types of DNA damage, including spontaneously arising lesions, such as uracil and abasic (AP) sites, and the products of base oxidation and alkylation (Lindahl, 1993). The initiating stage of BER is the re-

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✉To whom correspondence should be addressed: Radiation and Genome Stability Unit, Medical Research Council, Harwell, Oxfordshire, OX11 0RD, U.K.; tel. (44) 1235 841 134; fax (44) 1235 841 200; e-mail: g.dianov@har.mrc.ac.uk

Abbreviations: PARP-1, poly(ADP-ribose) polymerase 1; pADPr, poly(ADP-ribose); 8-oxoguanine, 8-oxo-7,8-dihydroguanine; BER, base excision repair; AP site, apurinic/apyrimidinic site, abasic site; rAP, reduced abasic site; APE1, apurinic/apyrimidinic endonuclease 1; dRP, 5′-deoxyribosephosphate; FEN1, flap endonuclease 1; Pol β, DNA polymerase β; dNTPs, deoxyribonucleotide triphosphates; ddNTPs, dideoxyribonucleotide triphosphates; WCE, whole-cell extract.

moval of the modified base by an appropriate glycosylase, generating an AP site. The AP site is a substrate for the AP endonuclease, APE1, which incises the backbone 5′ to the abasic site generating a 3′-hydroxyl and a 5′-deoxyribosephosphate (dRP). In the majority of cases repair then proceeds *via* short-patch BER (Dianov *et al.*, 1992), in which the 5′-dRP is removed through a β-elimination reaction catalyzed by polymerase β (Pol β), that also inserts a single nucleotide to fill the gap (Matsumoto & Kim, 1995). Strand integrity is then restored by the action of DNA ligase (Tomkinson & Levin, 1997). In certain cases, however, the AP site may have been chemically modified such that it is refractory to β-elimination. In this event repair must then continue down the long-patch pathway in which from 2 to 8 nucleotides are inserted into the repair gap *via* a strand displacement mechanism (Frosina *et al.*, 1996). The displaced dRP-containing "flap" is then removed by flap endonuclease 1 (FEN1), allowing ligation to proceed (Klungland & Lindahl, 1997).

The basic enzymology of BER is reasonably well understood and the repair pathways have been reconstituted *in vitro* using purified enzymes (Klungland & Lindahl, 1997; Kubota *et al.*, 1996; Matsumoto *et al.*, 1999; Pascucci *et al.*, 1999). However, recent evidence suggests that the precise mechanism of BER may be more complicated, with an increasing number of proteins having been demonstrated to be associated with the repair process (Wood *et al.*, 2001). One such enzyme, poly(ADP-ribose) polymerase-1 (PARP-1), an abundant nuclear enzyme found in multicellular organisms, is thought to be involved in BER (Dantzer *et al.*, 1999) but its precise role remains controversial. PARP-1 binds tightly as a homodimer to both single- and double-strand breaks. Binding of PARP-1 to a strand break activates its catalytic activity which is to covalently link nuclear proteins with poly(ADP-ribose), pADPr, synthesised from NAD^+. One of the main cellular results of this process is an accumulation of negative charge and concomitant loss of affinity of PARP-1 for the DNA strand break (for review see references Althaus & Richter, 1987; D'Amours *et al.*, 1999).

There is much controversy about the role of PARP-1 in the repair process. PARP-1 null mice are hypersensitive to alkylating agents and irradiation (Ménessier-de Murcia *et al.*, 1997; Wang *et al.*, 1997). Immortalised cells from these animals manifest characteristic features of genomic instability, including delayed rejoining of strand breaks, mixed ploidy and elevated frequencies of SCE and micronuclei formation upon treatment with DNA-damaging agents (Ménessier-de Murcia *et al.*, 1997; Trucco *et al.*, 1998; Wang *et al.*, 1997). Further evidence to suggest a positive role for PARP-1 in BER comes from its ability to interact with other participants in BER including XRCC1 (Caldecott *et al.*, 1996; Masson *et al.*, 1998), and Pol β (Dantzer *et al.*, 2000). In addition, repair of regular AP sites, which is mainly accomplished by short-patch BER, shows a dependence on NAD^+ (Satoh & Lindahl, 1992; Satoh *et al.*, 1993). It has been proposed that PARP-1 binds to the incised intermediate and interferes with the repair process: in the absence of NAD^+ PARP-1 will persist at the strand break and DNA repair will be stalled (Satoh & Lindahl, 1992; Satoh *et al.*, 1993). In support of this model Vodenicharov *et al.* (2000) recently demonstrated efficient, NAD^+-independent repair of single-strand breaks and alkylated bases by extracts of cells from PARP-1 deficient mice.

However, Dantzer *et al.* (2000) reported that cells deficient in PARP-1 show a moderate defect in short-patch repair of AP sites but are almost totally incapable of performing long-patch BER suggesting an active role for PARP-1 in DNA damage processing.

We have recently developed a cell-free assay which allows us to monitor the progress of BER at single nucleotide resolution and to differentiate between the short- and long-patch pathways (Allinson *et al.*, 2001). This assay has been used to study the NAD^+ and PARP-1-

dependence of both short- and long-patch BER of AP-site containing substrates.

MATERIALS AND METHODS

Materials. The 8-oxoguanine-containing synthetic oligodeoxyribonucleotide, purified by high-performance liquid chromatography, was obtained from Midland. [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from NEN Life Science Products. Recombinant human PARP-1 was obtained from Alexis Biochemicals. Mouse 8-oxoguanine-DNA glycosylase (mOGG1) was a gift from Dr. D. Zharkov.

Preparation of substrate. Oligonucleotide 5′-ATATACCGCG**[8-oxo]G**CCGATCAAGCT TATT-3′ (30 pmol) was 5′-end labeled with 100 μCi (33 pmol) of [γ-^{32}P]-ATP and used for construction of a substrate containing a single 8-oxoguanine in circular closed double-stranded DNA as previously described (Dianov *et al.*, 1998). This substrate was converted to AP-site containing substrate by incubating 2 nM substrate with 25 nM OGG1 for 20 min at 37°C in buffer containing 50 mM Hepes/KOH, pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA and 1.5 mM dithiothreitol (DTT).

Cells and extracts. Mouse embryonic fibroblasts derived from normal and PARP-1 knockout mice were kindly provided by Dr. G. de Murcia. Cells were maintained in DMEM medium supplemented with 10% foetal bovine serum and antibiotics. Whole-cell extracts were prepared by the method of Tanaka *et al.* (1992) as modified by Vodenicharov *et al.* (2000) and dialysed overnight against buffer containing 25 mM Hepes/KOH, pH 7.9, 2 mM DTT, 12 mM $MgCl_2$, 0.1 mM EDTA, 17% glycerol and 0.1 M KCl. Extracts were aliquoted and stored at −80°C.

BER reactions. The BER reactions were carried out in a reaction mixture (50 μl) containing 50 mM Hepes/KOH, pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 1.5 mM DTT, 2 mM ATP, 0.4 mg/ml bovine serum albumin (BSA), 25 mM phosphocreatine (di-Tris salt, Sigma), 2.5 μg creatine phosphokinase (type I, Sigma), 8.5% glycerol (Fluka), 20 μM each of the indicated dNTPs or ddNTPs, 100 ng (20 fmol) of ^{32}P-labeled single 8-oxoguanine containing DNA substrate, 400 ng of carrier plasmid DNA (pUC18) and, where applicable, 0.25 mM NAD^+. Reactions were initiated by the addition of whole-cell extract (100 μg) and incubated for the indicated time at 37°C. The reactions were stopped by addition of 40 μl of the reaction mix to 2 μl of 0.5 M EDTA, 2 μl of 10% SDS, and 2 μl of Proteinase K (5 mg/ml). After incubation for 30 min at 37°C, substrate DNA was purified from the reaction mixture by phenol-chloroform extraction and filtered through a Sepharose-G25 spin column equilibrated with 10 mM Tris/HCl, pH 8.0. Filtrates were spin-dried, dissolved in appropriate restriction buffers supplied by the manufacturer and treated with 10–40 units of the indicated restriction endonuclease(s) for 1 h at 37°C. An equal volume of gel loading buffer (95% formamide, 20 mM EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanol) was then added, and following incubation at 90°C for 3 min the products were separated by electrophoresis in a 10% polyacrylamide gel containing 7 M urea in 89 mM Tris/HCl, 89 mM boric acid, and 2 mM EDTA, pH 8.0.

All experiments were repeated at least 3 times and phosphorimages of representative gels are shown. Gels were analysed using Quantity One software (Bio-Rad).

RESULTS

Substrate characterization

A single AP site-containing closed circular DNA substrate was generated from 8-oxoguanine-containing substrate by treatment with 8-oxoguanine-DNA glycosylase (OGG1). The substrate DNA was designed so that the

AP site and an upstream ^{32}P label were located within a 59-mer *Hin*dIII restriction fragment (Fig. 1A). The AP site was positioned within one of two *Hae*III recognition sequences, effectively blocking *Hae*III cleavage at this site until its repair. The substrate was carefully characterized prior to use. Treatment of the substrate with *Hin*dIII generated only a 59-mer labeled DNA fragment (Fig. 1B, lane 1), confirming that the lyase activity of OGG1 had not cleaved the AP site. The presence of the AP site was verified by treatment of the substrate with both *Hin*dIII and APE1, which generated the expected 47-mer fragment (Fig. 1B, lane 2). Finally, double digestion of the substrate with both *Hin*dIII and *Hae*III yielded a 52-mer fragment, confirming that the presence of the AP site did indeed block cleavage of one of the *Hae*III sites (Fig. 1B, lane 3). Restoration of this *Hae*III site by repair would result in a 48-mer fragment being generated by *Hin*dIII/*Hae*III double digestion.

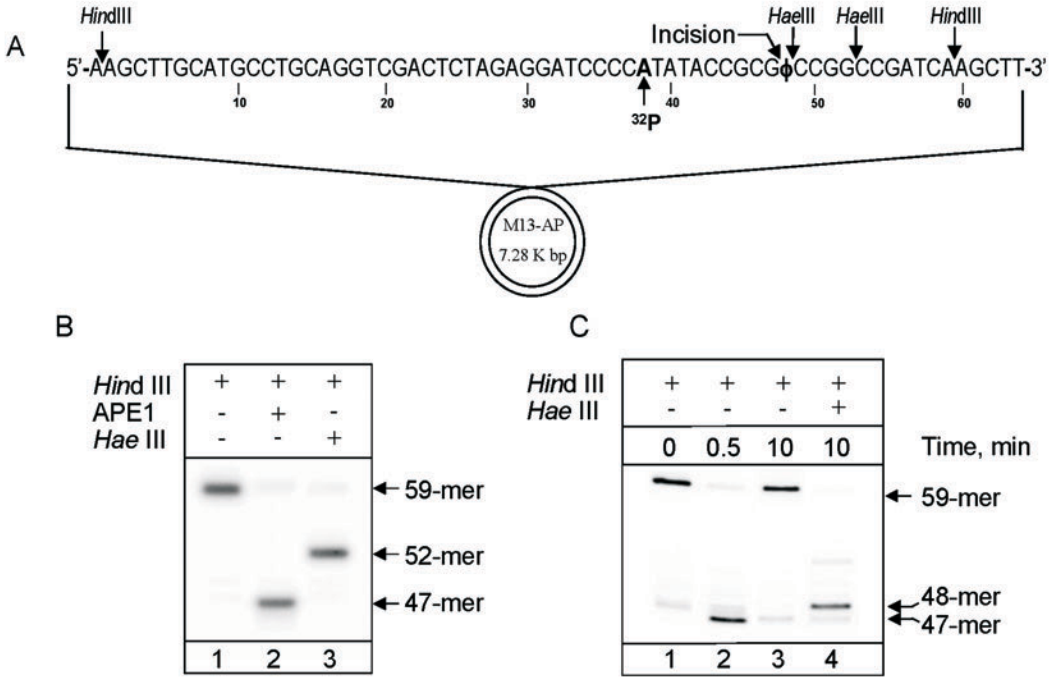

Figure 1. Substrate design and characterisation.

(A) Schematic presentation of the AP site-containing substrate. The AP site is indicated by ϕ. The sites of cleavage by the restriction enzymes, *Hin*dIII and *Hae*III and the position of the ^{32}P label are shown. The site of incision by AP endonuclease is indicated with an arrow. **(B)** Characterisation of the AP site-containing substrate. Digestion of the AP substrate with *Hin*dIII (lane 1), *Hin*dIII and APE1 (lane 2) and *Hin*dIII and *Hae*III (lane 3). Products were analysed by electrophoresis in a 10% denaturing polyacrylamide gel. **(C)** Rapid cleavage of substrate by APE. Reaction mixtures (50 μl) contained 100 ng of AP site-containing substrate DNA, 100 μg of PARP-1$^{+/+}$ WCE and 0.25 mM NAD^+ and were incubated at 37°C for the indicated times. Reactions were quenched by addition of 2 μl of 0.5 M EDTA and 2 μl of 10% SDS. For the 0 min time point the EDTA and SDS were added to the WCE immediately prior to addition of the substrate. The substrate was purified, treated with 20 mM $NaBH_4$ to stabilize the AP sites to electrophoresis and then digested with either *Hin*dIII (lanes 1–3) or *Hin*dIII and *Hae*III (lane 4). Products were analysed as in Fig. 1B.

Initial experiments showed that the activity of AP endonuclease in all cell extracts used was so high that all the substrate was cleaved at the AP site before the earliest measurable time point of 30 s (Fig. 1C, lane 2). Therefore, in practice any 59-mer observed in the *Hin*dIII digests was due to fully repaired reaction products, as confirmed by conversion of this

59-mer to 48-mer on treatment with *Hae*III (Fig. 1C, lanes 3 and 4). Thus, repair progress could be monitored by accumulation of 59-mer in the *Hin*dIII digests, and for simplicity all the remaining figures show only the *Hin*dIII digests although double digests were carried out in each case.

NAD^+ is required for repair of abasic sites through both short- and long-patch BER pathways

Whole-cell extracts used in our assay are nearly completely depleted of NAD^+ (Vodenicharov *et al.*, 2000) and thus suitable to study the effect of NAD^+ on the repair process. The stimulatory effect of NAD^+ on short-patch BER, involved in repair of DNA base damages and regular AP sites, is well established, and it is widely accepted that NAD^+ stimulates release of PARP-1 from the complex with nicked DNA (D'Amours *et al.*, 1999). However, little is known on the effect of NAD^+ on long-patch BER, which is involved in repair of AP sites that are resistant to β-elimination, and, therefore, cannot be processed *via* short-patch BER.

Whole cell extracts derived from normal mouse embryonic fibroblasts (PARP-$1^{+/+}$ WCE), supplemented or non-supplemented with NAD^+, were incubated with the regular AP site-containing substrate (Fig. 2). Although, as previously discussed, APE1 cleavage of the substrate was very rapid, in the absence of NAD^+ repair was relatively slow, proceeding to 84% completion, as judged by accumulation of the 59-mer fragment following *Hin*dIII digestion, only after 60 minutes' incubation (Fig. 2A, lanes 1 to 3). When NAD^+ was added to the reaction mix, the rate of the repair reaction was greatly increased, with 97% of the substrate being completely repaired within 30 min (Fig. 2A, lanes 4 to 6), and an approximately 4-fold increase in maximal repair rate (Fig. 2B).

Similar experiments were performed with the substrate containing a reduced AP site (rAP). To confirm that our substrate containing a rAP site was repaired only through long-patch BER, we first carried out the repair reaction under conditions allowing only short-patch synthesis (Allinson *et al.*, 2001). Briefly, this involves limiting the pool of nucleotides available for repair synthesis to dGTP only, with all other nucleotides being present as dideoxyribonucleotide triphosphates (ddNTP). Following insertion of the first nucleotide (dGTP), repair may only pro-

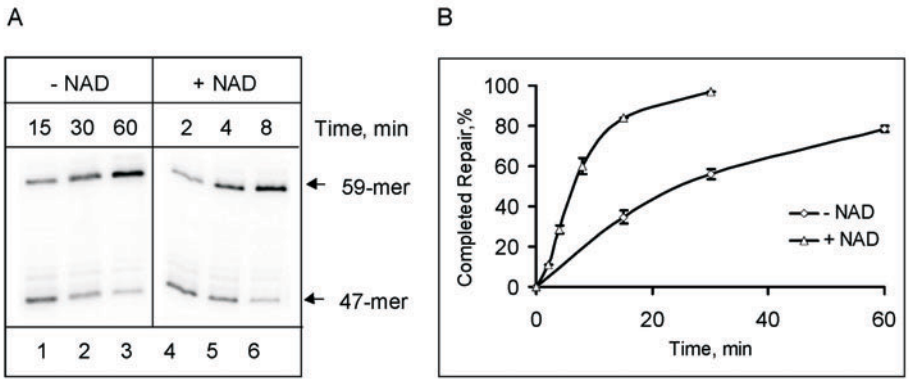

Figure 2. Repair of a regular AP site-containing substrate by PARP-$1^{+/+}$ cell extracts.

(A) Kinetics of repair of AP site in the absence (lanes 1–3) and presence (lanes 4–6) of NAD^+. Reaction mixtures (50 μl) contained 100 ng of substrate DNA, 100 μg of PARP-$1^{+/+}$ WCE and, where applicable, 0.25 mM NAD^+. Reactions were incubated at 37°C for the indicated time periods. The substrate DNA was subsequently purified and then treated with *Hin*dIII. Products were analysed as in Fig. 1B. **(B)** Comparative plots for repair progress in the presence and absence of NAD^+ are shown, and the amount repaired is calculated as the percentage of 59-mer present after *Hin*dIII cleavage. Error bars show the standard deviation.

ceed to completion *via* removal of the deoxyribose phosphate moiety by the dRP lyase activity of polymerase β and subsequent ligation (i.e., *via* the short-patch mechanism). If long-patch repair is involved, then a second nucleotide (ddNTP) will be inserted and further repair synthesis/ligation will be blocked, resulting in accumulation of a 49-mer product following *Hin*dIII hydrolysis of the substrate DNA. Under these conditions the regular AP site was efficiently repaired (Fig. 3, lanes 1 and 2), but repair of the rAP substrate was unable to proceed beyond the polymerase step (Fig. 3, lanes 3 and 4). As expected, repair terminated following the incorporation of ddNTPs, forming a non-processable species which was subject to subsequent slight nucleolytic degradation (seen as <47-mers (Fig. 3, lanes 3 and 4)).

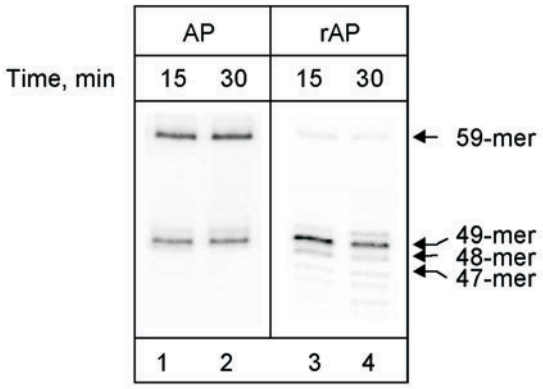

Figure 3. Repair of normal and reduced AP site-containing substrates under "short-patch" conditions.

Reaction mixtures containing 100 ng of substrate DNA, 100 μg of PARP-$1^{+/+}$ WCE, 0.25 mM NAD^+ and 20 μM dGTP, ddATP, ddCTP and ddTTP were incubated at 37°C for the indicated time periods. The substrate DNA was subsequently purified and then treated with *Hin*dIII. Products were analysed as in Fig. 1B.

Having confirmed that the rAP substrate could only be repaired *via* the long-patch pathway, we then applied this substrate to studying long-patch BER by PARP-$1^{+/+}$ WCE both in the presence and absence of NAD^+. Under these conditions slightly reduced rates of repair were observed relative to the corresponding rates for the AP substrate. However, the stimulation of repair rate by NAD^+ was of a similar magnitude (6-fold) to that previously observed for a regular AP-site containing substrate (Fig. 4).

PARP-1 reduces the efficiency of both short- and long-patch BER

While the data above showed that NAD^+ is able to stimulate both short- and long-patch repair to approximately equal extents, we were unable to conclude whether PARP-1 plays a

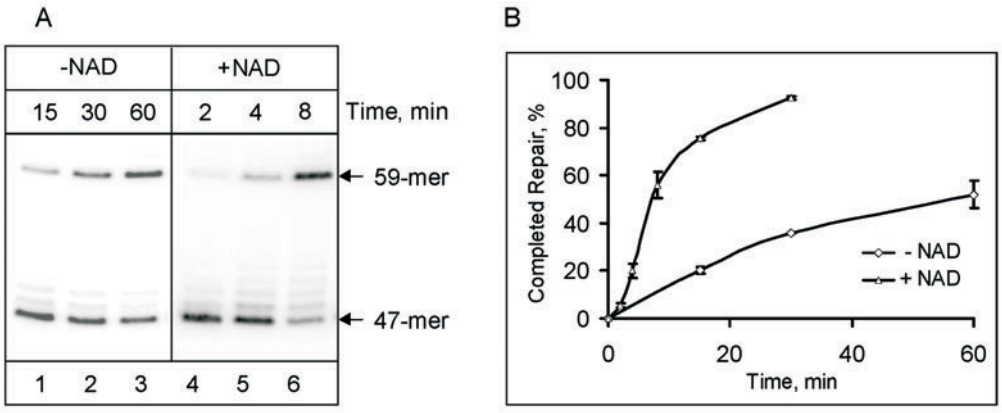

Figure 4. Repair of a reduced AP site-containing substrate.

(A) Reaction mixtures (50 μl) containing 100 ng of substrate DNA, 100 μg of PARP-$1^{+/+}$ WCE, 20 μM dNTPs and, where applicable, 0.25 mM NAD^+ were incubated at 37°C for the indicated time periods. The substrate DNA was subsequently purified and then treated with *Hin*dIII. Products were analysed as in Fig. 1B. **(B)** Comparative plots for repair progress in the presence and absence of NAD^+ are shown and the amount repaired and designations as in Fig. 2B.

positive, accessory role in BER or whether, as proposed by Satoh & Lindahl (1992), its effect is negative and inhibitory. We addressed this question by measuring repair rates for both regular and reduced AP sites in extracts derived from PARP-1-knockout cells (PARP-$1^{-/-}$ WCE). Western blot analysis confirmed the absence of PARP-1 protein in these extracts (not shown). Incubation of the AP site-containing substrate with PARP-$1^{-/-}$ WCE led to complete repair of 87% of the substrate in two minutes with or without NAD^+ (Fig. 5A and B) in comparison to 60% repair in 8 min when incubated with PARP-$1^{+/+}$ WCE in the presence of NAD^+ (Fig. 2). PARP-1 deficient extracts also efficiently repaired the rAP site-containing substrate and repair was likewise NAD^+-independent. Within 8 min 94% was repaired (Fig. 6) in comparison to 56% when incubated with PARP-$1^{+/+}$ WCE in the presence of NAD^+ (Fig. 4).

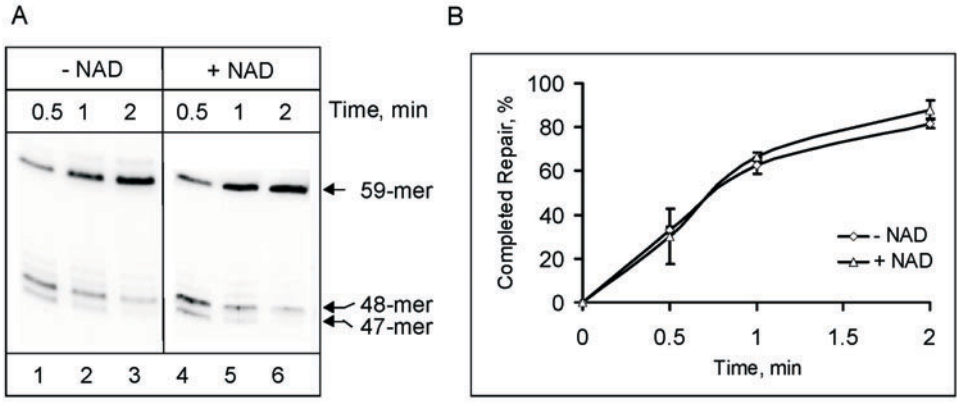

Figure 5. Characterisation of abasic site repair by PARP-1-deficient cell extracts.

(A) Kinetics of repair of AP site in the absence and presence of NAD^+ by PARP-$1^{-/-}$ WCE. Reaction mixtures (50 μl) contained 100 ng of substrate DNA, 100 μg of PARP-$1^{-/-}$ WCE and, where applicable, 0.25 mM NAD^+. Reactions were incubated at 37°C for the indicated time periods. The substrate DNA was subsequently purified and then treated with *Hin*dIII. Products were analysed as in Fig. 1B. **(B)** Comparative plots for repair progress in the presence and absence of NAD^+ are shown, and the amount repaired and designations as in Fig. 2B.

Inhibition of AP-site repair by recombinant human PARP

In order to assess whether the increased rate of repair in $PARP^{-/-}$ WCE reflected a PARP-1 inhibitory effect, purified recombinant PARP-1 was titrated into PARP-$1^{-/-}$ WCE. Since it has been reported (Satoh *et al.*, 1994; Vodenicharov *et al.*, 2000) that PARP-$1^{+/+}$ WCE contains PARP-1 at approximately 1.5–2 μg per mg of extract, we added 30, 150 and 750 ng of PARP-1 to PARP-$1^{-/-}$ WCE and measured the amount of AP substrate repaired. We established that in the presence of NAD^+, PARP-1 had a concentration-dependent inhibitory effect on repair by PARP-$1^{-/-}$ WCE, the major repair intermediate being the 47-mer APE1 incision product (Fig. 7).

DISCUSSION

Since the discovery that stimulation of strand break repair by NAD^+ was attributable to the poly(ADP-ribosyl)ation activity of PARP-1 (Satoh & Lindahl, 1992), much evidence suggested that PARP-1 affects BER. Following their initial findings, Satoh and Lindahl proposed a mechanism whereby PARP-1 inhibited the progress of BER by binding to a repair intermediate, protecting it from nucleases and recombination enzymes, but at the same time blocking access by components of the BER machinery (Satoh & Lindahl, 1992). According to this model, mod-

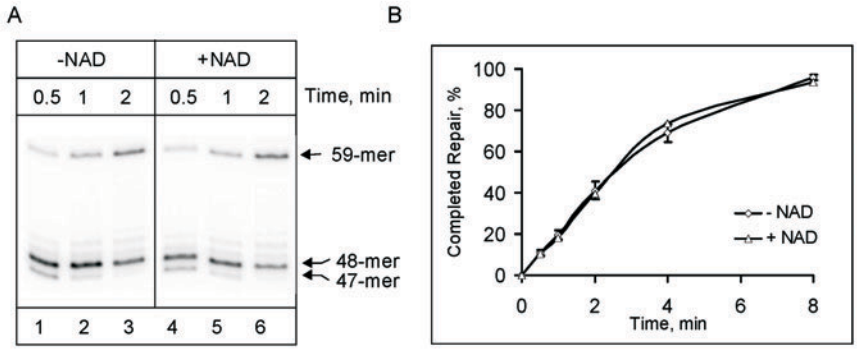

Figure 6. Repair of a reduced abasic site by PARP-1-deficient cell extracts.

(A) Reaction mixtures (50 μl) containing 100 ng of substrate DNA, 100 μg of PARP-1$^{-/-}$ WCE, 20 μM dNTPs and, where applicable, 0.25 mM NAD$^+$ were incubated at 37°C for the indicated time periods. The substrate DNA was subsequently purified and then treated with *Hin*dIII. Products were analysed as in Fig. 1B. **(B)** Comparative plots for repair progress in the presence and absence of NAD$^+$ are shown, and the amount repaired and designations as in Fig. 2B.

ification of PARP-1 with pADPr synthesised from NAD$^+$ leads to its dissociation from the repair site, allowing access of other enzymes. An alternative theory, that PARP-1 plays an active role in recruiting enzymes to the repair site, has since been advanced and supported by the finding that PARP-1 is able to interact with the BER enzymes, XRCC1 (Caldecott *et al.*, 1996; Masson *et al.*, 1998) and Pol β (Dantzer *et al.*, 2000). Experiments carried out with purified recombinant enzymes have shown that PARP-1 is able to stimulate strand displacement synthesis by Pol β but only in the absence of NAD$^+$ (Prasad *et al.*, 2001). Additionally, Dantzer *et al.* (2000) recently reported that extracts from PARP-1$^{-/-}$ cells were significantly inhibited in repair synthesis at AP sites, with the long-patch contribution to repair being particularly affected. However, a contradictory almost simultaneous report by Vodenicharov *et al.* (2000) demonstrated efficient repair by PARP-1$^{-/-}$ WCE of DNA damaged by ionizing radiation or an alkylating agent. This discrepancy of views led us to apply our recently developed *in vitro* DNA repair assay (Allinson *et al.*, 2001),

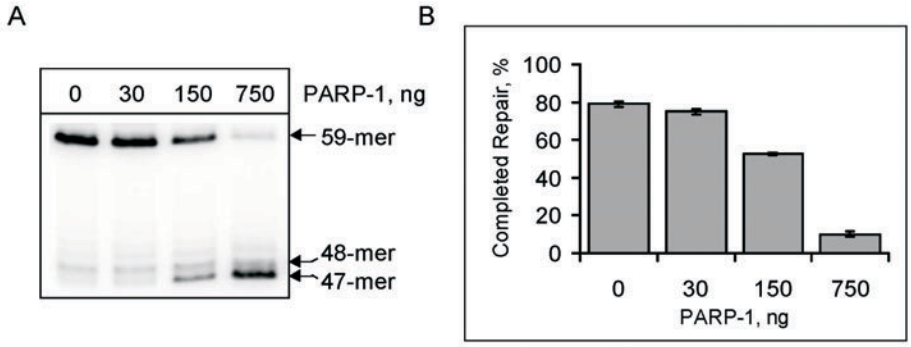

Figure 7. Titration of purified PARP-1 into PARP-1 deficient cell extracts.

(A) Reaction mixtures (50 μl) containing 100 ng of AP substrate DNA, 100 μg of PARP-1$^{-/-}$ WCE, 20 μM dNTPs, 0.25 mM NAD$^+$ and the indicated amount of PARP-1 were incubated at 37°C for 2 min. The substrate DNA was subsequently purified and then treated with *Hin*dIII. Products were analysed as in Fig. 1B. **(B)** Repair efficiency on addition of varying amounts of PARP-1. The amount repaired and designations as in Fig. 2B.

measuring the number of repair events rather then incorporation of labeled dNTPs during repair, to further examine the participation of both PARP-1 and pADPr synthesis in the short- and long-patch BER sub-pathways.

We found that repair of both regular and reduced AP sites was stimulated 4–6-fold by the presence of NAD^+, with the major repair intermediate being the nicked AP site (Fig. 2, 47-mer). This result suggested that PARP-1 is able to efficiently displace APE1 from the repair site and may therefore have to be incorporated into the 'passing-the-baton' model of base excision repair (Wilson & Kunkel, 2000). According to this model, repair proteins sequentially pass repair intermediates from one enzyme to another without leaving the repair site unattended at any time. It is also suggested that after incision of an AP site, APE1 remains bound to it and is substituted by DNA polymerase β. However, our results clearly indicate that PARP-1 always binds to the nicked AP site before Pol β and must be released from the site for repair to continue. The delayed repair in the absence of NAD^+ observed in our experiments most probably results from spontaneous dissociation of PARP-1 from the nicked site.

Surprisingly, we found that the repair rates for both an AP and an rAP site were actually increased in PARP-$1^{-/-}$ WCE relative to those for PARP-$1^{+/+}$ WCE complemented with NAD^+ (Figs. 5 and 6), suggesting that PARP-1 actively slows down the progress of both BER sub-pathways even in the presence of NAD^+. This was further confirmed by the titration of purified recombinant PARP-1 into PARP-$1^{-/-}$ WCE complemented with NAD^+. We found that addition of PARP-1 to PARP-$1^{-/-}$ WCE led to a decrease in repair rate (Fig. 7).

The repair rate of both substrates by PARP-$1^{-/-}$ WCE was unaffected by NAD^+, in agreement with the results obtained by Vodenicharov *et al.* (2000). Moreover, the rate-limiting step of the repair process in the two different extracts is different. In PARP-$1^{+/+}$ extracts, repair is delayed after APE1 incision and is presumably limited by the accessibility of the repair site to Pol β due to the presence of PARP-1. In PARP-$1^{-/-}$ extracts, the major repair intermediate is the product of insertion of a single nucleotide at the repair site (Fig. 6, 48-mer). For a regular AP site, it is likely that the rate-limiting step in PARP-$1^{-/-}$ extracts is the removal of the dRP moiety, because for repair of an AP site in the absence of PARP-1, dRP removal was reported to be the rate-limiting step (Srivastava *et al.*, 1998).

It was initially puzzling as to why our results should contrast so sharply with those of Dantzer *et al.* (2000). However, their experimental approach was based on quantitating nucleotide incorporation rather than monitoring of actual repair rates. Sanderson & Lindahl (2002) recently demonstrated reduced levels of FEN1 in PARP-$1^{-/-}$ cell extracts. They proposed that if the repair patch size is increased for long-patch repair in PARP-$1^{+/+}$ extracts *versus* PARP-$1^{-/-}$ extracts, then more nucleotides will be incorporated per repair event and repair will appear more efficient in PARP-$1^{+/+}$ extracts.

It is, however, unquestionable that PARP-1 is somehow involved in the mechanism of BER. If this involvement does not improve the efficiency of repair, then why should such a NAD^+ depleting process occur? Vodenicharov *et al.* (2000) have speculated that the hypersensitivity of PARP-$1^{-/-}$ mice and cells to ionizing radiation and alkylating agents may be due to greatly reduced levels of poly(ADP-ribosyl)ation of other cellular targets (Vodenicharov *et al.*, 2000), rather than a defect in base excision repair *per se*. This could also explain why PARP-$1^{-/-}$ cell lines recapitulate the genomic instability effects of PARP inhibitors (Oikawa *et al.*, 1980) or expression of a dominant negative mutant (Kupper *et al.*, 1995; Molinete *et al.*, 1993; Schreiber *et al.*, 1995). PARP-1 and poly(ADP-ribosyl)ation reactions have been implicated in a wide range of cellular processes such as transcriptional

control, chromatin re-modelling and signalling (reviewed in de Murcia & Shall, 2000). Our data lend further support to the theory that the main role of PARP-1 in BER does not lie in direct catalysis of DNA damage processing, but instead in linking repair and these processes.

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