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Functional association of poly(ADP-ribose) polymerase with DNA polymerase α–primase complex: a link between DNA strand break detection and DNA replication

Françoise Dantzer, Heinz-Peter Nasheuer[1], Jean-Luc Vonesch[2], Gilbert de Murcia and Josiane Ménissier-de Murcia*

UPR 9003 du Centre National de la Recherche Scientifique 'Cancérogenèse et Mutagenèse Moléculaire et Structurale', Laboratoire correspondant du CEA no. 14, Ecole Supérieure de Biotechnologie de Strasbourg, Boulevard Sébastien Brant, F-67400 Illkirch-Graffenstaden, France, [1]Institut für Molekulare Biotechnologie, Beutenbergstraße 11, D-07745 Jena, Germany and [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Collège de France, BP 163, 67404 Illkirch Cedex, France

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ABSTRACT

Poly(ADP-ribose) polymerase (PARP) is an element of the DNA damage surveillance network evolved by eukaryotic cells to cope with numerous environmental and endogenous genotoxic agents. PARP has been found to be involved *in vivo* in both cell proliferation and base excision repair of DNA. In this study the interaction between PARP and the DNA polymerase α–primase tetramer has been examined. We provide evidence that in proliferating cells: (i) PARP is physically associated with the catalytic subunit of the DNA polymerase α–primase tetramer, an association confirmed by confocal microscopy, demonstrating that both enzymes are co-localized at the nuclear periphery of HeLa cells; (ii) this interaction requires the integrity of the second zinc finger of PARP and is maximal during the S and G2/M phases of the cell cycle; (iii) PARP-deficient cells derived from PARP knock-out mice exhibited reduced DNA polymerase activity, compared with the parental cells, a reduction accentuated following exposure to sublethal doses of methylmethanesulfonate. Altogether, the present results strongly suggest that PARP participates in a DNA damage survey mechanism implying its nick-sensor function as part of the control of replication fork progression when breaks are present in the template.

INTRODUCTION

To maintain DNA integrity in dividing cells specific biochemical pathways have evolved to accurately coordinate the cell cycle transitions; these checkpoints link completion of one phase to onset of the following phase (1,3). Moreover, DNA damage arrests the cell cycle and induces a cellular response allowing DNA repair, ensuring high fidelity of genetic information transmission (2).

In eukaryotes the highly conserved DNA polymerase α–primase complex is responsible for synthesis of short RNA–DNA primers essential for the initiation step of DNA replication. It consists of four distinct subunits: p180 (180 kDa) is the catalytic subunit. The primase is a heterodimer of 48 kDa endowed with catalytic activity; p58 (58 kDa) bears a stimulatory function, p68 (68 kDa) has a tethering function between p180 and the primase (4,5). Components of the replication apparatus may act as sensors of DNA damage to stall replication forks, inducing transcription of DNA damage-inducible genes (6). A defect in the mammalian tumour suppressor gene *p53* abrogates G1 arrest in response to ionizing radiation (7,8) by transcriptional activation of genes like *GADD45* and $p21^{WAF1}$, a cyclin-dependent kinase inhibitor (9–11). Furthermore, DNA damage sensors may also transduce the stress signal. ATM, which is mutated in patients with the heritable disorder ataxia telangectasia (AT), induces signalling through multiple pathways, thereby coordinating acute phase stress responses with cell cycle checkpoint control and repair of ionizing radiation and oxidative damage (12,13). Patients harbouring mutations in *p53* or *ATM* are cancer prone, implicating checkpoint controls in the prevention of genetic instability.

In yeast the catalytic subunit of DNA primase is thought to link the DNA damage response to DNA replication, whilst mutations in the *PRIl* gene failed to delay bud emergence in response to UV irradiation in G1 (14). Another replication block sensor protein is DNA polymerase ε, which is required for the S→M checkpoint. Interestingly, adjacent to the location of checkpoint-deficient mutations DNA polymerase ε encompasses a zinc finger resembling the zinc fingers of PARP involved in binding to single-strand breaks (15), suggesting that both proteins recognize a similar structure in DNA.

* To whom correspondence should be addressed. Tel: +33 388 655 368; Fax: +33 388 655 352; Email: demurcia@esbs.u-strsbg.fr

Poly(ADP-ribose) polymerase is a component of the immediate cellular response to genotoxic stress, playing a critical role in cell recovery from DNA damage (16,17). Purified PARP was shown to suppress *in vitro* replication of SV40 DNA (18) and to inhibit DNA replication by human replicative DNA polymerases α, δ and ε (19). In contrast, Simbulan *et al.* demonstrated that *in vitro* PARP stimulated DNA polymerase α through a physical association (20). The same authors demonstrated that the PARP–DNA polymerase α association was required in differentiation-linked DNA replication (21,22).

Accumulating evidence suggests a permanent or temporary association of PARP with the replication machinery: (i) PARP co-purifies with DNA replication forks (23) and topoisomerase I (24,25); (ii) *in vivo* PARP modifies replication factors such as RP-A (18) and SV40 T antigen (26); (iii) following exposure to low levels of monofunctional alkylating agents inhibition (16,27) or depletion (28,29) of PARP results invariably in G2/M accumulation, presumably reflecting a failure to complete replication, ultimately leading to a mitotic block. Furthermore, in mice lacking PARP proliferating cells are exquisitely sensitive to DNA damaging agents compared with wild-type cells, as measured by: (i) apoptotic cell death of splenocytes exposed to *N*-methyl-*N*-nitrosourea (MNU); (ii) necrosis of the epithelial cells of the small intestine located within the crypts, causing death of PARP-deficient mice by 3 days following 8 Gy γ-irradiation (28). All these data strongly suggest that PARP is a survival factor playing an essential and positive role during DNA damage recovery.

In this work we present evidence that PARP and DNA polymerase α–primase are physically associated in dividing cells, permitting coordination of the initiation of DNA replication with the resolution of replication blocks induced by DNA strand breaks.

MATERIALS AND METHODS

Cell culture

HeLa cells were maintained in DMEM 1000 mg/l glucose medium (Sigma) supplemented with 7% fetal bovine serum (Eurobio) and 0.5% gentamycin (Sigma). Primary fibroblasts (MEFs) were isolated from 13.5 day embryos from homozygous mutant *PARP*$^{-/-}$ and wild-type *PARP*$^{+/+}$ mice as described (28). MEFs were maintained in DMEM 4500 mg/l glucose medium supplemented with 10% fetal bovine serum and 0.5% gentamycin. The cells were grown at 37°C with 5% CO_2.

Immunoprecipitation experiments

HeLa cells (5×10^6) were washed twice with phosphate-buffered saline (PBS) and lysed on ice in 1 ml lysis buffer (20 mM Tris–HCl, pH 8.0, 120 mM NaCl, 0.5% NP40, 0.2 mM phenylmethanesulfonyl fluoride and 4 μg/ml each leupeptin, pepstatin and aprotinin). Solubilized cell lysates (200 μg protein), precleared for 16 h with 15 μl protein A–Sepharose beads (Pharmacia Biotech), were incubated for 2 h at 4°C with either monoclonal antibodies [anti-DNA polymerase α–primase antibodies SJK-132-20 (30), provided by M.Smulson, Georgetown University School of Medicine, Washington, and SJK-237-71 (30), provided by J.Hurwitz, Sloan-Kettering Cancer Center, New York; anti-β-galactosidase clone GAL13, Sigma], a polyclonal anti-PARP antibody or a pre-immune serum. Immunocomplexes were precipitated by addition of 30 μl protein A–Sepharose beads and washed five times in 20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP40 and separated by 10% SDS–PAGE. Proteins were transferred onto nitrocellulose and immunoblotted with appropriate antibodies. Immunoblotting was performed with an enhanced chemiluminescence detection system (Amersham). In *in vitro* experiments 1 μg purified DNA polymerase α–primase and the indicated domains of PARP (1 μg each) were pre-incubated in 200 μl lysis buffer for 1 h on ice and immunoprecipitation was performed as described above using the polyclonal anti-PARP antibody or the pre-immune serum as control.

Confocal laser scanning microscopy

HeLa cells were grown for 24 h on glass coverslips, washed twice with PBS, fixed for 10 min with ice-cold methanol/acetone and washed again with PBS. Cells fixed on coverslips were incubated for 16 h with the first antibodies diluted in PBS, 0.1% Tween, 1% BSA. Dilutions were 1/100 for polyclonal anti-PARP antibody and 1/2 for monoclonal anti-DNA polymerase α p68 (provided by Dr E.Weiss, ESBS, Illkirch, France). After three washes with PBS containing 0.1% Tween, cells were incubated for 3 h with secondary antibodies (FITC-conjugated anti-rabbit IgG and Texas red-conjugated anti-mouse IgG1 respectively, diluted in PBS 1/200). Observations were made with a confocal microscope equipped with an argon/krypton laser and suitable barrier filters (Leica TCS4D, Heidelberg, Germany).

DNA polymerase activity assay

DNA polymerase activity was tested in 60 μl buffer containing 10 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 7.5 mM DTT, 50 μg/ml BSA, 0.5 μg DNase I-activated calf thymus DNA, 25 μM each dATP, dCTP and dGTP, 5 μCi [α-^{32}P]dCTP (3000 Ci/mmol; Dupont NEN). Incorporation of radiolabelled nucleotides was determined by TCA precipitation. DNA polymerase α–primase inhibition was performed in the presence of 10 μl SJK 132-20 or an antibody directed against the DNA polymerase α 68 kDa subunit.

Far western blotting analysis

Radiolabelled proteins were produced using the *in vitro* TNT lysate coupled Transcription–Translation System (Promega) with 30 μCi L-[^{35}S]methionine (1175 Ci/mmol; Dupont NEN). One microgram of purified DNA polymerase α–primase complex was separated by 8% SDS–PAGE and transferred to nitrocellulose membrane. After renaturation for 16 h at 4°C in 10 ml buffer containing 10 mM Tris–HCl, pH 7.4, 5 mM 2-mercaptoethanol, 0.2% Triton, 0.5% milk powder and 0.25% gelatine the membrane was incubated in 1 ml renaturation buffer containing each radiolabelled polypeptide. The membrane was then washed twice in 10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Tween and autoradiographed.

Cell synchronization

Exponentially growing HeLa cells were maintained for 24 h in medium containing 5 μg/ml aphidicolin (Sigma). Cells were released from the cell cycle block by washing three times with PBS and adding fresh complete medium. At various times after release from the aphidicolin block samples were harvested for

immunoprecipitation and flow cytometry analysis using an Epics Elite (Coulter).

S phase analysis after G1 block release

Mouse embryonic fibroblasts were synchronized in G0 in DMEM containing 0.1% fetal bovine serum for 96 h. Cells were harvested, treated or mock-treated with 150 μM MMS for 30 min at 37°C and released into fresh complete medium. Twenty four hours later cells were pulse labelled with 10 μM 5-bromodeoxyuridine (BrdU) for 1 h and the percentage of cells in S phase was monitored as described (16).

RESULTS

PARP interacts with DNA polymerase α–primase

Previous studies (20) have shown that PARP is physically associated *in vitro* with DNA polymerase α. To assess the existence of this association in living cells HeLa whole cell extracts were immunoprecipitated using two different monoclonal antibodies raised against the DNA polymerase α–primase 180 kDa subunit (30) and the immune complex was subjected to SDS–PAGE. Figure 1A shows that PARP (116 kDa), whose activity was detected by activity blot (31), was immunoprecipitated with a polyclonal anti-PARP antibody (lane 1) and was also specifically co-immunoprecipitated with DNA polymerase α–primase using anti-DNA polymerase α antibodies (lanes 2 and 3) but not with an anti-β-galactosidase antibody as a negative control (lane 4).

Conversely, HeLa whole cell extracts were immunoprecipitated with a polyclonal anti-PARP antibody and the immune complex was assayed for DNA polymerase activity using DNase I-activated DNA as a substrate (Fig. 1B). As expected, DNA polymerase activity was co-immunoprecipitated with PARP. This activity could be neutralized by 50% by adding anti-DNA polymerase α antibodies directed against either the 180 (SJK 132-20) or 68 kDa subunits of DNA polymerase α to the reaction mixture. The same level of inhibition was obtained when aphidicolin was added (5 μg/ml) to the reaction mixture (data not shown). In control experiments DNA polymerase activity was not associated either with the pre-immune serum or with protein A–Sepharose beads. Furthermore, both enzymes were co-immunoprecipitated by specific antibodies to either PARP or DNA polymerase α (data not shown) from lysates of insect cells co-infected with recombinant baculovirus expressing both the p180 large subunit of DNA polymerase α (32,33) and PARP (34).

To exclude the possibility that this association could occur via tightly bound DNA fragments rather than by specific protein–protein interactions the immune complex was assayed for DNA polymerase activity in the absence or presence of DNase I-activated DNA as substrate. We found that DNase I-activated DNA was absolutely required for DNA polymerase activity, suggesting that no DNA co-purified with the PARP/DNA polymerase α–primase complex and consequently that the interaction occurred via direct protein–protein contacts (data not shown).

A possible interaction between PARP and other replication and/or repair enzymes was further investigated by immunoprecipitation using antibodies against replication factor A (RPA) and DNA polymerases β, δ and ε. Under the same experimental conditions PARP was found associated with DNA polymerase β only, in keeping with its potential role in base excision repair (BER) (17).

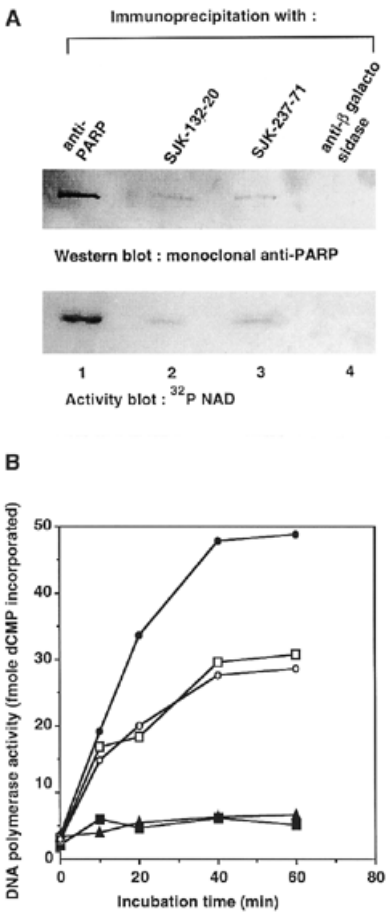

Figure 1. PARP interacts with DNA polymerase α–primase. (**A**) HeLa cell extracts were subjected to immunoprecipitation with anti-PARP (lane 1) or two different anti-DNA polymerase α–primase antibodies (SJK-132-20, lane 2; SJK-237-71, lane 3). An anti-β-galactosidase antibody was used as a negative control (lane 4). Immunoprecipitates were then analysed by immunoblotting using an anti-PARP antibody (top). Immunoprecipitates were also analysed by activity blot (bottom). (**B**) HeLa cell extracts were incubated with anti-PARP (●, □, ○), pre-immune serum (▲) or without antibody as controls (■) and the precipitates were assayed for DNA polymerase activity as described in Materials and Methods. SJK 132-20 (□) and anti-p68 (○) anti-DNA polymerase α–primase antibodies were added to the reaction mixtures to inhibit DNA polymerase α activity.

PARP and DNA polymerase α–primase are co-localized in the HeLa cell nucleus

PARP/DNA polymerase α intranuclear localization was estimated from optical sections obtained using a confocal laser scanning microscope. Figure 2 shows typical PARP and DNA polymerase patterns of doubly stained nuclei observed in proliferating HeLa cells. Panels A and B show confocal images of DNA polymerase α–primase and PARP labelling respectively within the same nucleus. Co-localization (yellow) is observed at the nuclear periphery and in nucleoli; both patterns overlapped within the limits of the procedure. Altogether, these results

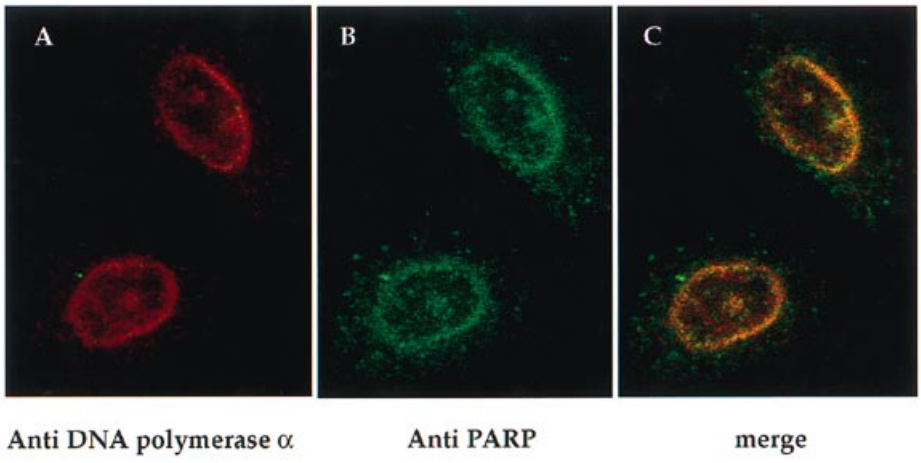

Figure 2. Confocal analysis of PARP and DNA polymerase α double staining in HeLa cells. (**A**) Rhodamine-labelled DNA polymerase α (red); (**B**) fluorescein-labelled PARP (green); (**C**) merged image (regions of overlap are in yellow).

indicate that *in vivo* the two proteins are in close vicinity and are both preferentially present in the nuclear envelope.

The DNA binding domain of PARP interacts with the catalytic subunit of DNA polymerase α–primase

The interacting domains between PARP and DNA polymerase α–primase were mapped using two independent approaches. First, equimolar amounts of purified homogeneous human DNA polymerase α–primase and full-length PARP or PARP functional domains (29 kDa DBD or 40 kDa catalytic domain; Fig. 3A) were pre-incubated *in vitro* on ice. Then an anti-PARP antibody or the pre-immune serum was added to the reaction mixture and the immunoprecipitates were assayed for DNA polymerase α activity. As shown in Figure 3B, DNA polymerase activity co-immunoprecipitated with full-length PARP as well as with the 29 kDa DBD, but not with the 40 kDa catalytic domain or the pre-immune serum.

To determine whether this association also exists *in vivo*, in a second approach HeLa (H29-11) cells constitutively expressing the human PARP 29 kDa domain as well as the parental cell line (HpECV) were used (16). The proteins were identified by Western blot analysis using monoclonal anti-PARP antibody C1,9 (17; Fig. 3C). Lane 4 shows the typical pattern of cell line H29-11 expressing the recombinant 29 kDa DBD and also containing full-length endogenous PARP. Both proteins were immunoprecipitated with anti-PARP antibody (lane 3) and with SJK 237-71 (lane 2), but not with a non-specific anti-β-galactosidase antibody (lane 1). PARP was immunoprecipitated with SJK 237-71 in HeLa cells, as already reported in Figure 1A. Thus both *in vitro* and *in vivo* PARP contacts DNA polymerase α–primase through its 29 kDa DBD. In crude lysates obtained from the parental line HpECV no protein migrating at a molecular weight of 29 kDa was co-immunoprecipitated (Fig. 3C lane 5).

To assess the integrity of the PARP zinc fingers in this interaction ^{35}S-radiolabelled PARP functional domains were synthesized *in vitro* and used in Far western blot analysis. Purified human DNA polymerase α–primase tetramer was separated by SDS–PAGE, transferred to nitrocellulose membrane and, after renaturation of the proteins, hybridized. As shown in Figure 3D, the 180 kDa catalytic subunit of DNA polymerase α–primase bound to the full-length PARP as well as the 46 kDa DBD, whereas the 40 kDa C-terminal catalytic domain did not interact under these conditions. Interestingly, the two mutated forms of the 46 kDa DBD failed to interact with the 180 kDa catalytic subunit of DNA polymerase α–primase, strongly suggesting that integrity of the second zinc finger, at least, is required. From this Far western blot experiment we also concluded that the interaction is not mediated by DNA.

PARP/DNA polymerase α–primase interaction occurs in a cell cycle-dependent manner

To test whether PARP/DNA polymerase α–primase association is cell cycle regulated HeLa cells were blocked by aphidicolin at the G1/S boundary of the cell cycle; following release cell cycle progression was determined by flow cytometric analysis (Fig. 4A). p180 and PARP were present throughout the cell cycle (Fig. 4B and C respectively). Cell lysates from different stages of the cell cycle were immunoprecipitated with the anti-DNA polymerase α–primase antibody SJK 132-20. The immune complex was immunoblotted with both monoclonal antibody directed against the 180 kDa subunit of DNA polymerase α–primase and anti-PARP antibody (Fig. 4D and E respectively). Although DNA polymerase α–primase was efficiently immunoprecipitated throughout the cell cycle, PARP was found associated with DNA polymerase α–primase only during the S and G2 phases of the cell cycle, and not during G1 phase.

DNA polymerase activity is reduced in cell lysates from PARP-deficient cells derived from PARP knock-out mice

To evaluate the functional significance of the PARP/DNA polymerase α–primase interaction total DNA polymerase activity in cellular lysates from embryonic fibroblasts derived from either wild-type *PARP*$^{+/+}$ or PARP knock-out mice (*PARP*$^{-/-}$) was measured in the presence of nicked DNA. The same amount of the large subunit p180 of DNA polymerase α–primase was present in both extracts. As shown in Figure 5, DNA polymerase activity was reduced by 50% in *PARP*$^{-/-}$ cellular lysates compared with lysates obtained from parental *PARP*$^{+/+}$ cells,

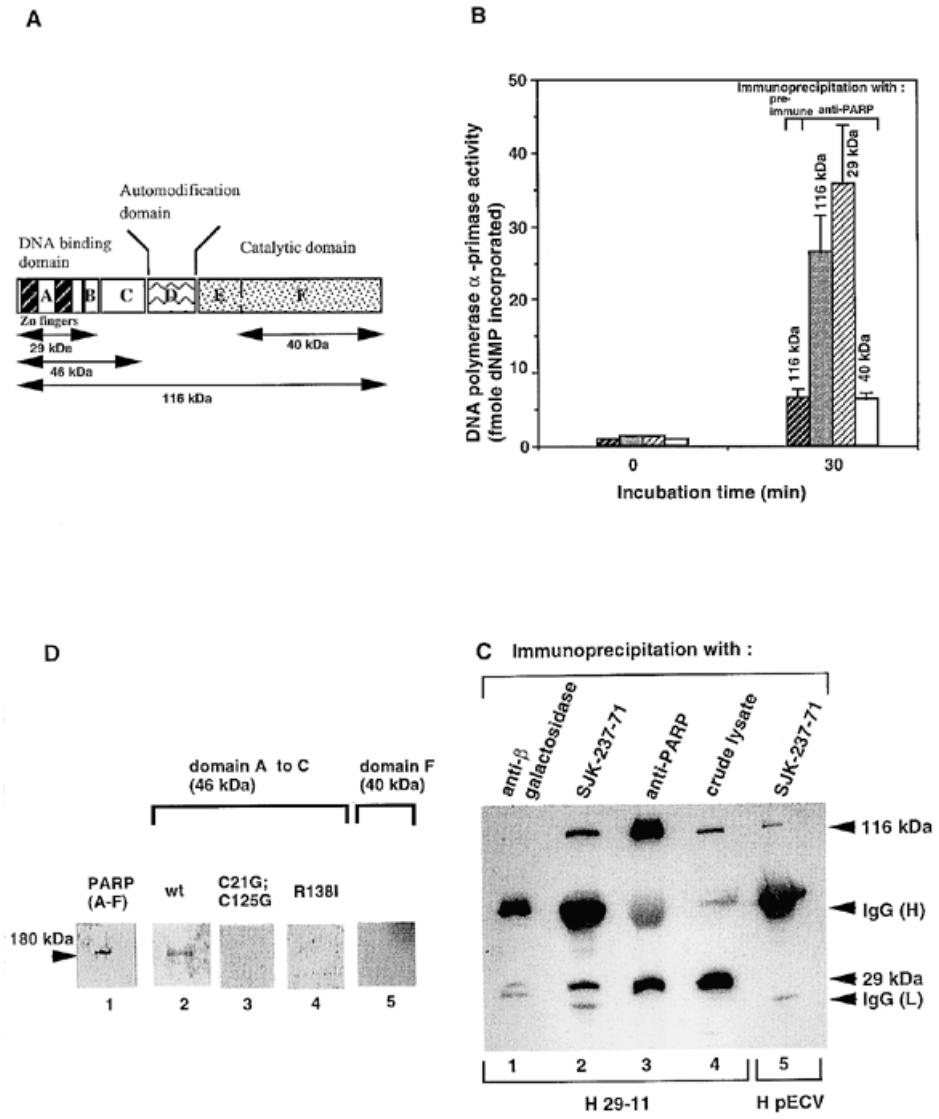

Figure 3. The DNA binding domain of PARP interacts with the catalytic subunit of DNA polymerase α–primase. (**A**) Modular organization of the human PARP molecule. (**B**) Purified DNA polymerase α–primase was incubated with PARP (116 kDa) or the purified domains of PARP (29 and 40 kDa). Immunoprecipitation was then performed with anti-PARP and the precipitates were assayed for DNA polymerase α activity at 0 and 30 min. Immunoprecipitation with the pre-immune serum was performed as a negative control. (**C**) H29-11 cell lysates were immunoprecipitated with an anti-β-galactosidase antibody as a negative control (lane 1), anti-DNA polymerase α–primase antibody SJK 237-71 (lane 2) or anti-PARP antibody as positive control (lane 3). Aliquots of 10 µg H29-11 crude lysate were loaded as controls (lane 4). HpECV cell lysates were immunoprecipitated with anti-DNA polymerase α–primase SJK 237-71 (lane 5). Proteins were then analysed by immunoblotting with anti-PARP. IgG(H), immunoglobulin G heavy chain; IgG(L), immunoglobulin G light chain. (**D**) Far western blotting analysis. Purified DNA polymerase α–primase was separated by SDS–PAGE, transferred to nitrocellulose membrane and hybridized with ^{35}S-radiolabelled full-length PARP (lane 1), wild-type 46 kDa domain (lane 2), the double point mutant C21G C125G (lane 3), the single point mutant R138I (19) (lane 4) or the 40 kDa catalytic domain (lane 5).

suggesting that PARP may stimulate the active replicative complex *in vivo*.

The rate of ongoing S phase is retarded in PARP-deficient cells following DNA damage

The involvement of PARP in progression of the replication fork following DNA damage was monitored by the ability of cells to progress into S phase after exposure to sublethal doses of MMS. MEFs derived from *PARP*$^{+/+}$ and *PARP*$^{-/-}$ mice were synchronized by serum starvation. Immediately following release into fresh medium some cells were exposed to MMS, whereas other cells were left untreated; 24 h later they were all pulse labelled with BrdU (Fig. 6). When untreated only 28% of *PARP*$^{-/-}$ cells had entered S phase compared with 40% of the parental cells, suggesting that they have been delayed in the cell cycle. Following exposure to a sublethal dose of MMS, progression of *PARP*$^{+/+}$ MEFs into S phase was not affected, however, cells lacking PARP showed reduced progression through S phase, since only 21% of cells had entered the S phase. Therefore, *PARP*$^{-/-}$ cells have a reduced capacity to replicate their genome under DNA damage conditions.

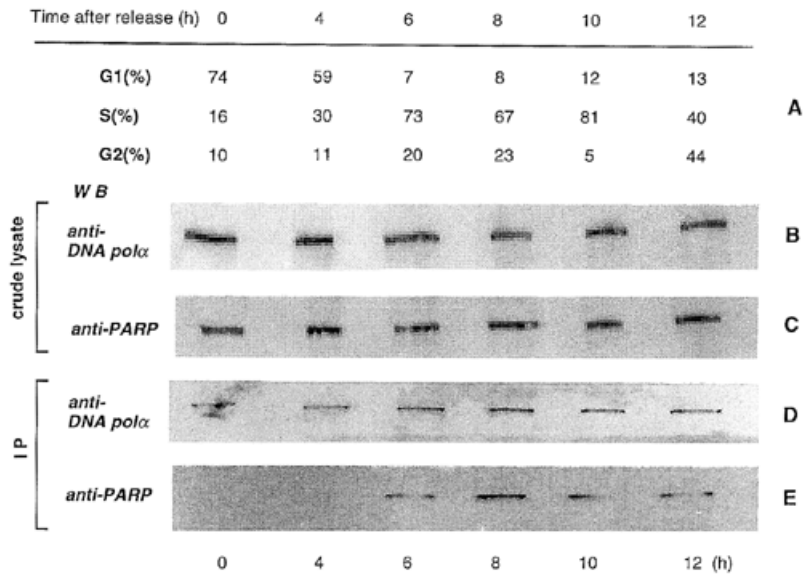

Figure 4. Cell cycle-dependent interaction of PARP with DNA polymerase α–primase. Aphidicolin-arrested HeLa cells were released into fresh medium. Timed samples were monitored for progression through the cell cycle by flow cytometric analysis (**A**). The amounts of p180 DNA polymerase α (**B**) and PARP (**C**) were assessed in lysates. The same samples were immunoprecipitated with anti-p180 and DNA polymerase α (**D**) and PARP (**E**) were revealed by immunoblotting. IP, immunoprecipitation; WB, western blotting.

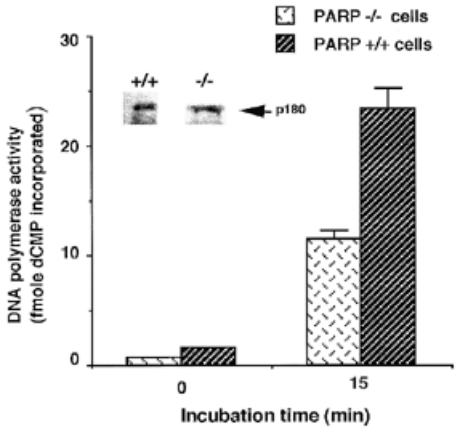

Figure 5. Cell lysates from PARP-deficient cells display a reduced DNA polymerase activity *in vitro*. DNA polymerase activity was assessed at 0 and 15 min on clear lysates from wild-type (*PARP*$^{+/+}$) and mutant (*PARP*$^{-/-}$) mouse embryonic fibroblasts as described under Materials and Methods. Data represent one of three independent experiments. Results represent the average/mean value and standard deviation of three independent experiments. The amount of DNA polymerase α–primase (p180) in the tested samples was revealed by western blot analysis as shown in the insert.

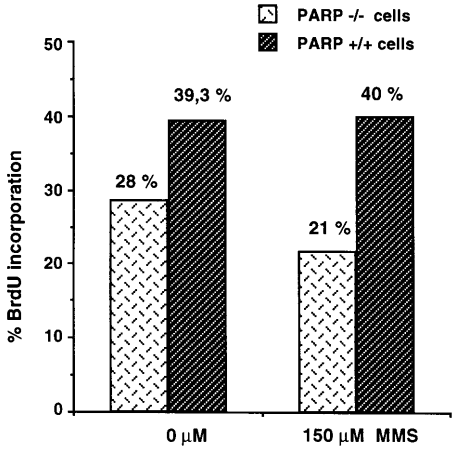

Figure 6. S phase analysis of synchronized *PARP*$^{+/+}$ and *PARP*$^{-/-}$ mouse embryonic fibroblasts. Cells were synchronized in G1 by serum starvation and samples for FACS analysis were taken 24 h following block release with or without 150 μM MMS addition. The percentage of cells in S phase was monitored by BrdU pulse labelling.

DISCUSSION

We have shown previously that in proliferating cells in which PARP was inhibited by overproduction of a dominant negative mutant or in PARP-deficient cells sublethal doses of alkylating agents led to G2/M accumulation, whilst the parental cells were able to progress continuously through the cell cycle. Under these DNA damaging conditions sister chromatid exchanges which occur when replication is blocked by unrepaired lesions increased (16,28,35) and cells underwent apoptosis (16,28). These results were tentatively interpreted in the context of a DNA survey mechanism implicating the nick-sensor function of PARP as part of the control of replication fork progression when breaks are present in the template.

In this work we provide evidence that PARP contacts the replication machinery, both *in vitro* and *in vivo*: (i) PARP and the DNA polymerase α–primase tetramer co-immunoprecipitate using antibodies specific for either PARP or DNA polymerase α and PARP is also immunoprecipitated by an anti-DNA polymerase

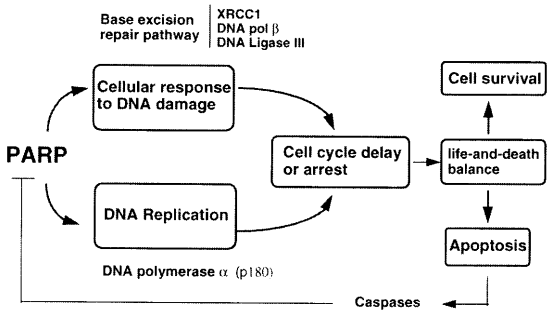

Figure 7. Model for the implication of PARP in the coordination of cell division with the base excision repair pathway. The interaction of PARP with XRCC1 (Masson *et al*., submitted for publication) promotes the recruitment of BER factors and enzymes to the vicinity of DNA strand breaks, thus contributing kinetically to the efficiency of the overall repair reaction, in a time window compatible with cell cycle progression. A delayed or prolonged cell cycle arrest due to the persistence of unrepaired/unreplicated DNA influences the life-and-death balance. Cells challenged with sublethal doses of genotoxins activate the DNA repair network and survive. When apoptosis is the selected option, in the case of saturating levels of damage, ICE family proteases cleave and inactivate PARP as well as a number of other DNA repair/cell survival factors, leading to irreversible commitment of the cell to a death program (40).

β antibody (unpublished data), a DNA polymerase specifically involved in base excision repair (36,37); (ii) both proteins are co-localized at the nuclear periphery, known to be enriched in replication enzymes (38) and factors involved in cell cycle regulation and tumour suppression, like Rb (39); (iii) the PARP DNA binding domain (29 kDa) but not the 40 kDa catalytic domain interacts with the catalytic subunit of DNA polymerase α; (iv) PARP/DNA polymerase α–primase interaction is cell cycle dependent and occurs during the S and G2 phases; (v) in PARP-deficient fibroblasts DNA polymerase activity is decreased by 30–50% compared with the parental cells in the absence of DNA damage, the rate of ongoing S phase being retarded. In addition, our findings suggest that the function of the DNA binding domain of PARP is more complex than initially thought. Besides its role in nick detection (41) and in transfer of the activation signal to the catalytic domain (42), the PARP DNA binding domain binds to several proteins, e.g. XRCC1 (Masson *et al.*, submitted for publication), histones (43), PARP itself (44) and DNA polymerase α (this report). PARP/DNA polymerase α–primase interaction is cell cycle controlled, suggesting that cell cycle-dependent phosphorylation of the p180 or p68 subunits by $p34^{cdc2}$ kinase might modulate protein–protein interaction (46).

Altogether, our results support a model (Fig. 7) in which PARP, as an element of the DNA damage surveillance network, is involved in coordination of cellular responses to DNA damage with the replication apparatus. Owing to its characteristic property to interact cooperatively with DNA, even if it is undamaged, PARP may play a positive regulatory role during initiation of DNA replication under normal conditions (20), perhaps by locally increasing the template concentration (45). Under genotoxic stress conditions the presence of single-strand breaks in the template constitute a strong block for replication (47); in that case their detection by PARP molecules, associated with DNA polymerase α–primase, would immobilize the replication complex at the lesion, thus preventing priming downstream of the damage (48). Moreover, the presence of PARP at the break would ensure rapid recruitment of DNA repair enzymes (Masson *et al*., submitted for publication) to restore DNA continuity, in a time window compatible with normal completion of the cell cycle. As a consequence of persistence of unreplicated sequences containing lesions the cell cycle may be delayed in G2/M, leading to apoptosis (16,28). This replication-linked repair pathway prevents cells from entering mitosis unless their genome has been repaired. To fulfil this function PARP is associated with: (i) factors and enzymes involved in BER, like DNA polymerase β and XRCC1 (Masson *et al*., submitted for publication), which are specifically involved in the major pathway of BER (36,37); (ii) the DNA replication apparatus (20; this work). Following treatment with sublethal doses of DNA damaging agents loss of PARP (and hence PARP activity) increases the cytotoxicity of the DNA damaging agent by considerably delaying the time course of rejoining of DNA breaks, as detected in *PARP*$^{-/-}$ mouse embryonic fibroblasts (Trucco *et al*., submitted for publication). These findings suggest that either the checkpoint mechanisms are not sufficient in *PARP*$^{-/-}$ cells or that coordinated stimulation of DNA repair and DNA replication proteins by PARP is required for efficient repair of damaged DNA.

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