

# Poly(ADP-RIBOSE) polymerase-1 (Parp-1) antagonizes topoisomerase I-dependent recombination stimulation by P53

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## ABSTRACT

PARP-1 interacts with and poly(ADP-ribosyl)ates p53 and topoisomerase I, which both participate in DNA recombination. Previously, we showed that PARP-1 downregulates homology-directed double-strand break (DSB) repair. We also discovered that, despite the well-established role of p53 as a global suppressor of error-prone recombination, p53 enhances homologous recombination (HR) at the *RAR* $\alpha$  breakpoint cluster region (bcr) comprising topoisomerase I recognition sites. Using an SV40-based assay and isogenic cell lines differing in the p53 and PARP-1 status we demonstrate that PARP-1 counteracts HR enhancement by p53, although DNA replication was largely unaffected. When the same DNA element was integrated in an episomal recombination plasmid, both p53 and PARP-1 exerted anti-recombinogenic rather than stimulatory activities. Strikingly, with DNA substrates integrated into cellular chromosomes, enhancement of HR by p53 and antagonistic PARP-1 action was seen, very similar to the HR of viral minichromosomes. siRNA-mediated knockdown revealed the essential role of topoisomerase I in this regulatory mechanism. However, after I-SceI-meganuclease-mediated cleavage of the chromosomally integrated substrate, no topoisomerase I-dependent effects by p53 and PARP-1 were observed. Our data further indicate that PARP-1, probably through topoisomerase I interactions rather than poly(ADP-ribosyl)ation, prevents p53 from stimulating spontaneous HR on chromosomes via topoisomerase I activity.

## INTRODUCTION

PARP-1 plays fundamental roles in the recruitment and modulation of enzymatic and regulatory factors involved in transcription, DNA replication, repair and recombination [reviewed in (1–3)]. Importantly, PARP-1 catalyses poly(ADP-ribosyl)ation of many of these proteins including itself and dissociates from DNA after auto-modification (4). PARP-1 is enzymatically activated by binding to single-strand breaks (SSBs) and participates in base excision repair (5,6). Given that PARP-1 also recognizes double-strand breaks (DSBs), interacts with Ku70/80, the catalytic subunit of DNA-dependent protein kinase (DNA-PK<sub>cs</sub>), and the RecQ helicase WRN, and regulates the biochemical activities of DNA-PK<sub>cs</sub> and WRN (7–10), several groups investigated potential activities of PARP-1 in DSB repair. Moreover, two recent studies showed that dysfunction of homology-directed DSB repair sensitizes cells to PARP inhibition suggesting that PARP enzymatic activity is required to avoid the accumulation of lesions which are repaired by homologous recombination (HR) (11,12).

DSBs are caused spontaneously during physiological DNA processing in replication, immunoglobulin gene diversification and meiosis and can arise from exogenous DNA-damaging agents, including ionizing radiation or cancer chemotherapeutic agents. The two major pathways of DSB repair are nonhomologous end joining (NHEJ) and HR (13,14). In the NHEJ pathway, Ku70 and Ku80 bind the DSB, followed by recruitment and activation of DNA-PK<sub>cs</sub>, which mediates synapsis and recruits XRCC4 and DNA Ligase IV. In mammalian cells HR requires a protein complex comprising Mre11, Rad50 and Nbs1 for DSB recognition and end resection to yield 3'-ssDNA tails. Subsequent strand exchange between the processed ssDNA and an intact homologous duplex are catalysed by Rad51. This reaction is facilitated by the DNA end-protecting protein Rad52, the DNA-dependent ATPase and SNF2/SWI2 family member Rad54, as

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well as by the Rad51 paralogs Rad51B, Rad51C, Rad51D, Xrcc2 and Xrcc3. The breast cancer related gene product BRCA2 is thought to assist Rad51 filament assembly on ssDNA coated by replication protein A (RPA) (15). BLM and WRN, mutated in Bloom's and Werner's syndrome, respectively, unwind DNA, and WRN additionally exhibits exonucleolytic activity. These enzymes may play a role in resolving aberrantly paired structures, particularly during error-prone Rad51-dependent recombination at stalled replication forks (16).

Waldman and Waldman (17) observed lower frequencies of illegitimate recombination after treatment with an inhibitor of poly(ADP-ribosylation). Consistently, Rudat *et al.* (18) found that overexpression of the mere DNA-binding domain of PARP-1 (PARP-DBD), which causes *trans*-dominant inhibition of poly(ADP-ribosylation), inhibits rejoining of ionizing radiation-induced DSBs, suggesting that PARP-1 counteracts NHEJ when stably bound to DNA strand interruptions. On the other hand, in a recent biochemical study, Audebert *et al.* (19) discovered that PARP-1 provides synapsis activity on a novel route for DSB rejoining that complements the DNA-PK-dependent pathway. In agreement with the latter idea, poly(ADP-ribose) colocalizes with the break sensor Mre11, when nascent opened ends are generated during the coding end resolution stage of V(D)J recombination, but only in DNA-PK<sub>cs</sub>-negative cells (20). High levels of sister chromatid exchange (SCE) in cells from PARP-1<sup>-/-</sup> mice indicated a role for PARP-1 in protecting against excess HR (21,22). Recently, Shibata *et al.* (23) also provided *in vivo* evidence for a role of PARP-1 in suppressing deletion and insertion mutations which accompany chromosomal rearrangements in response to alkylation treatment. Consistently, Schultz *et al.* (24) noticed a hyperrecombination phenotype in cells from PARP-1<sup>-/-</sup> mice as well as after treatment of wild-type cells with an inhibitor of poly(ADP-ribosylation), as indicated by increased nuclear Rad51 foci formation. However, no significant rate change was seen in homology-directed repair of a single DSB after chemical PARP-1 inhibition, although similar treatments caused stimulation of intrachromosomal (25) and extrachromosomal (26) HR as well as homology-directed gene targeting (27). In our previous work, we used a rapid, fluorescence-based recombination assay in order to better discriminate between early DSB repair and subsequent apoptotic processes, which indirectly may enhance DSB repair (28). The results demonstrated a reduction of homology-directed DSB repair frequencies by exogenously expressed PARP-1. On the other hand, using a similar assay, Yang *et al.* (29) did not find evidence for interference of PARP-1 with DSB repair, although enhancement of Rad51 foci formation and SCE in cells from PARP-1<sup>-/-</sup> mice was confirmed. Additionally, delayed S-phase progression of PARP-1<sup>-/-</sup> cells was detected after replication arrest, suggesting a role in the recombinative reactivation of stalled replication forks.

The p53 tumor suppressor protein represents another PARP-1 target and has been established as an antagonist of error-prone HR events (30–35). Accumulating evidence has further demonstrated that p53 regulates HR independently of its transcriptional transactivation, cell cycle control and pro-apoptotic functions. Rather, p53 is likely to interfere with HR through physical interactions with Rad51, Rad54,

RPA, BRCA1, BRCA2, BLM and/or WRN, and the DNA intermediates of homologous exchange. Similarly, p53 was also found to inhibit error-prone NHEJ (36–38). Surprisingly, when applying a specifically designed SV40-based recombination test, we discovered that p53 upregulates recombination within a fragment of the *RARα* breakpoint cluster region (bcr), which comprises two perfect topoisomerase I recognition sequences and is responsive to the topoisomerase I inhibitor camptothecin (39). A functional link between p53 and topoisomerase I had already been suggested by the fact that p53 forms stable complexes with topoisomerase I and enhances topoisomerase I-mediated relaxation of supercoiled DNA (40,41). Mutant analyses showed that members of the *Rad52* epistasis group are involved in the repair of topoisomerase I–DNA complexes, thus indicating a critical role of the homology-directed pathway (42,43).

Based on the findings that PARP-1 interacts with and poly(ADP-ribosylates) p53 and topoisomerase I, which both have been implicated in HR (30–32,39,44–49), we examined the effect of PARP-1 on p53- and topoisomerase I-dependent recombination. For this purpose we adapted and applied our cellular assay systems for analyses of recombination within extrachromosomal plasmid DNA substrates, SV40 minichromosomes and cellular chromosomes (35,39,50). To distinguish possible influences of direct physical interactions from those due to enzymatic activities of PARP-1, we additionally analysed the C-terminally truncated PARP-1 mutant PARP-DBD. We demonstrate that p53 stimulates recombination through topoisomerase I, whereas PARP-1 abrogates this effect, and we define the conditions required for these processes.

## MATERIALS AND METHODS

### Plasmid constructs and topoisomerase I knockdown

The plasmids for extrachromosomal HR measurements were constructed by Sal I insertion of the 0.3 kb *RARα* bcr fragment (39) in both orientations in place of the hygromycin resistance cassette in the pHR-EGFP/3'EGFP plasmid (36). Thus, two plasmids, pHR-EGFP/3'EGFP-*Rarα* fwd and pHR-EGFP/3'EGFP-*Rarα* rev were constructed, with the *Rarα* bcr fragment being localized between two disrupted *EGFP* genes.

Plasmids pPARP31, pPARP6 (51,52) and pCMV-p53 (BD Biosciences Clontech, Heidelberg) direct overexpression of full-length PARP-1, the DNA-binding domain of PARP-1, and p53, respectively, have been described previously. DNA-modifying enzymes were purchased from New England Biolabs, Frankfurt/Main.

To generate SV40 genomes carrying a 398 bp fragment from the 3'-untranslated region (3'-UTR) of the PARP gene without topoisomerase sequences (53), we transferred the HindIII fragment after blunt end formation from the *PARP* 3'-UTR into the SmaI site within the Cla linker of shortened SV40 genome derivatives (39). The resulting vector set with a pUC-SV40-Cla, a pUC-SV40-tsVP1(196Y)-Cla and a pUC-SV40-tsVP1(290T)-Cla derivative carried the foreign sequence in the same orientation. Foreign sequences and insertion sites were verified by use of the ABi PRISM Big Dye Ready Reaction Cycle Sequencing kit and an ABi 377 sequencer.

To generate the plasmid pSUPER-TopoI, which directs the synthesis of siRNAs targeting topoisomerase I mRNA (54) the following oligonucleotides were synthesized (Thermochemicals, Ulm): AR-TopoI10-fwd, 5'-GATCCCCAGCAGAGGAAGTAGCTACGTTCAAGAGACGTAGCTACTTCC-TCTGCTTTTTTGGAAA-3'; AR-TopoI10-rev, 5'-AGCTTTTCCAAAAAGCAGAGGAAGTAGCTACGTCTCTTGAA-CGTAGCTACTTCTCTGCTGGG-3'. After annealing and 5'-terminal phosphorylation the oligonucleotides were ligated into the BglIII/HindIII digested pSUPER plasmid. In parallel, the following two sets of oligonucleotides were synthesized, cloned and tested under the conditions described above: AR-TopoI9-fwd, 5'-GATCCCCAGCTTGATGAAGCTGG-AAGTTCAAGAGACTTCCAGCTTCATCAACTGTTTTTGGAAA-3'; AR-TopoI9-rev, 5'-AGCTTTTCCAAAAACAG-TTGATGAAGCTGGAAGTCTCTTGAAGTCCAGCTTC-ATCAACTGGGG-3' and AR-TopoI11-fwd, 5'-GATCCCC-GCAGCCCGAGGATGATCTTTTCAAGAGAAAGATCAT-CCTCGGGCTGCTTTTTTGGAAA-3'; AR-TopoI11-rev, 5'-AGCTTTTCCAAAAAGCAGCCCGAGGATGATCTTTCT-CTTGAAGATCATCCTCGGGCTGCGGG-3'. Whereas pSUPER plasmid containing AR-TopoI11-fwd/AR-TopoI11-rev showed no knockdown activity, both plasmids with AR-TopoI9-fwd/AR-TopoI9-rev and AR-TopoI10-fwd/AR-TopoI10-rev were positive. pSUPER-TopoI with AR-TopoI10-fwd/AR-TopoI10-rev was the most effective and was, therefore, employed in further experiments. To verify RNA interference under recombination assay conditions (see below), we electroporated KMV(HR-EGFP/3'EGFP) cells (36) with 15 µg pSUPER-TopoI (AR-TopoI10-fwd/AR-TopoI10-rev) DNA plus empty vector, p53 expression plasmid and/or PARP-1 expression plasmid. Subsequently, cells were returned to growth conditions for 48–72 h and topoisomerase I protein expression was analysed by western blotting. In these different cell types, topoisomerase I was downregulated to 40–50% of the protein expression levels in control cells correspondingly transfected with pSUPER plus empty vector, p53 expression plasmid, and/or PARP-1 expression plasmid (mean values from two to four western blots as calculated from topoisomerase I-specific band intensities after normalizing with actin or tubulin-specific band intensities).

### Mammalian cell culture, cell cycle profiles and viral DNA synthesis

For SV40-based recombination assays LMV, LMV-PARP, LMV-p53her and LMV-p53her/PARP cell lines, described in Süsse *et al.* (28), were used. Briefly, LMV is derived from the parental line LLC-MK<sub>2</sub> from rhesus monkey (*Macaca mulatta*) kidney, stably expressing the estradiol-inducible transcriptional transactivation factor Gal4ERVP. LMV-PARP cells additionally express PARP-1 via the Gal4ERVP-responsive plasmid pGC-PARP-1, LMV-p53her cells express the estradiol-responsive wild-type p53 fusion protein p53her, and LMV-p53her/PARP cells express both PARP-1 and p53her. These cell lines were cultivated at 37°C in DMEM supplemented with 10% foetal calf serum (FCS) (PAA Laboratories, Pasching). Estradiol-like substances were removed from FCS and used in combination with phenol-red free medium for the propagation of cell

cultures before analysis (28). During recombination measurements cultures were incubated in the presence of 1 µM β-estradiol (Sigma, München). CV1 and COS1 cells were propagated for the production of virus particles and for the determination of plaque forming units (PFUs) as described previously (50).

For fluorescence-based recombination experiments we utilized KMV cells, which had been subcloned from the leukemia cell line K562 for stable Gal4ERVP expression (36). Additionally, we applied KMV cells with chromosomally integrated pHR-EGFP/3'EGFP recombination plasmid, named KMV(HR-EGFP/3'EGFP) (36). For maintenance, the growth medium RPMI 12/1 supplemented with 10% FCS and 2% L-Glutamine (Biochrom AG, Berlin) was used. The cell cultures used in this work were free from *mycoplasma* contamination.

KMV(HR-EGFP/3'EGFP) cells were analysed for cell cycle progression and for apoptosis 72 h after transfection and cultivation under the conditions of the recombination assay. According to our published protocol (55), we harvested cells by centrifugation which was followed by fixation, propidium iodide staining and FACS<sup>®</sup> analysis.

To measure *de novo* SV40-DNA synthesis, we labelled cells on 60 mm dishes with 30 µCi of [<sup>3</sup>H]thymidine for 1 h at the indicated times after infection, purified viral genomes, quantified [<sup>3</sup>H]thymidine incorporation and calculated rates of viral DNA synthesis as described previously (39).

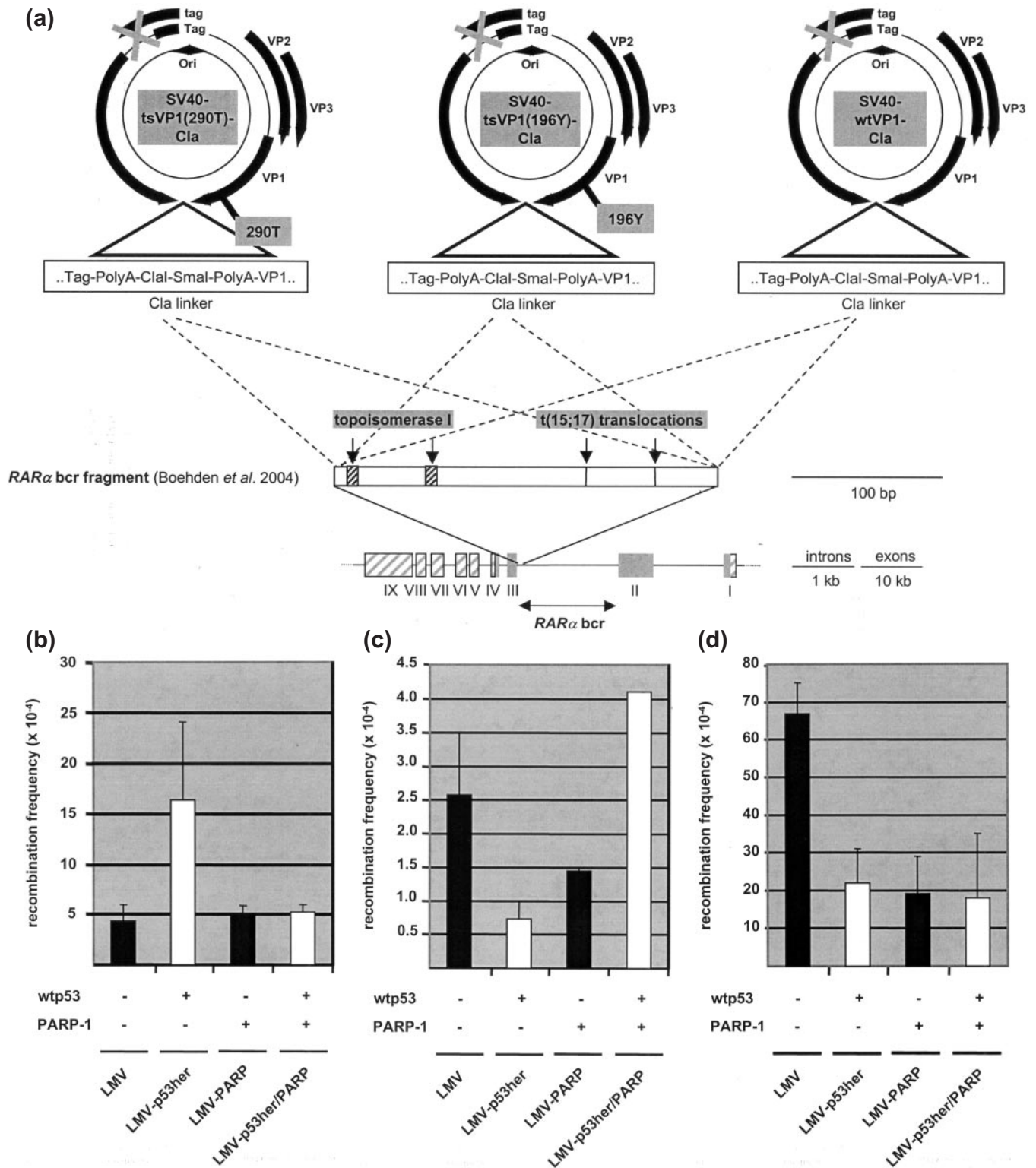
### Recombination assays

SV40-based recombination experiments, including plaque assays for the quantitative determination of reconstituted viral particles, were performed and evaluated exactly as detailed in Boehden *et al.* (39). To obtain recombination frequencies, the ratios between the values from double infections with tsVP1-SV40 mutants and from control infections with the same infectious units of wtVP1-SV40 were determined for each single assay condition. This procedure served to normalize recombination frequencies, in order to exclude rate deviations caused by growth regulatory effects with the individual cell line used, differences in cell lethality, and alterations in virus propagation related to the specific DNA element inserted and/or alterations of transcriptional and translational activities. During recombination assays, the two tsVP1-SV40 mutants and the control wtVP1-SV40 virus carried the same foreign sequence of interest.

To quantify EGFP reconstitution as a measure of chromosomal recombination frequencies KMV(HR-EGFP/3'EGFP) cells were electroporated at 200 V and 1050 mF with a total amount of 40 µg plasmid DNA. For p53, PARP-1 and PARP-DBD expression 5 µg of plasmid DNA were included each and replaced by the corresponding empty vectors in controls. For experiments involving pSUPER-TopoI we used 15 µg of the plasmid mediating RNA interference. For experiments with targeted cleavage of the chromosomally integrated HR-EGFP/3'EGFP recombination substrate, 5 µg of pCMV-I-SceI were also included. After electroporation cells were cultivated for 72 h at 37°C. Subsequently, cells were analysed by flow cytometry by use of a FACS<sup>®</sup> Calibur Calibur FACScan (Becton & Dickinson, Heidelberg) using the 488 nm laser line for excitation in combination with the filters used for green (F11) and orange (F12) fluorescence detection.

One hundred thousand living cells were analysed each to distinguish between EGFP-positive and -negative cells by the diagonal gating method in the F11/F12 dot plot (36,56). To determine the individual expression levels of EGFP in the presence or absence of p53, PARP-1, PARP-DBD and/or topoisomerase I, each experiment included a cotransfection

with a control plasmid, which carried wild-type *EGFP* at the acceptor position. The fraction of EGFP-positive cells was individually determined and was used to normalize each single recombination frequency, to exclude rate deviations related to growth regulatory effects, differences in growth, cell lethality, transcriptional and translational



activities (36). The statistical significance of differences was determined using Student's *t* test for unpaired samples.

To assess recombination with extrachromosomal substrate DNA, KMV cells were transfected with Lipofectamine™ 2000 Reagent (Invitrogen, Karlsruhe) and 4 µg of total plasmid DNA. The recombination plasmids pHR-EGFP/3'EGFP-Rarαfwd and pHR-EGFP/3'EGFP-Rarαrev were applied at 0.5 µg each. For p53, PARP-1 and PARP-DBD expression, 0.5 µg of plasmid DNA were included each and substituted by the corresponding empty vectors in controls. After cultivation for 48 h at 37°C expression of the reactivated EGFP was measured via FACS® cytometry as described above.

### Western blot analysis

Immunoblot analysis was done essentially as described in Akyüz *et al.* (36). Briefly, total cell extracts were prepared from KMV or KMV(HR-EGFP/3'EGFP) cells 48 h after transfection under recombination assay conditions. Proteins were separated on 8% polyacrylamide–SDS gels and transferred to Immobilon-P Transfer Membranes (Millipore, Schwalbach). The presence of topoisomerase I was revealed by the human antiserum Scl-70 (1:5 000; Topogen, Port Orange) followed by incubation with peroxidase-conjugated secondary antibody (Biomol Rockland, Vienna, Austria). For western blot analyses of PARP-1 and PARP-DBD we used goat polyclonal IgG N-20 (1:10 000; Santa Cruz, Heidelberg), for human p53 we applied the monoclonal antibody DO1 (1:10 000; Calbiochem, Bad Soden) and anti-mouse and anti-goat IgG peroxidase-conjugated secondary antibodies, respectively (Biomol Rockland, Vienna; Dianova/Jackson Immuno, Hamburg). To verify equal loading, tubulin or actin were detected by goat serum I-19 (1:1 000; Santa Cruz, Heidelberg) and the mAb DM1A (1:1 000; abcam, Cambridge, MA), respectively. Western blot signals were visualized by enhanced chemiluminescence (SuperSignal West Dura; Pierce/Perbio Science, Bonn).

## RESULTS

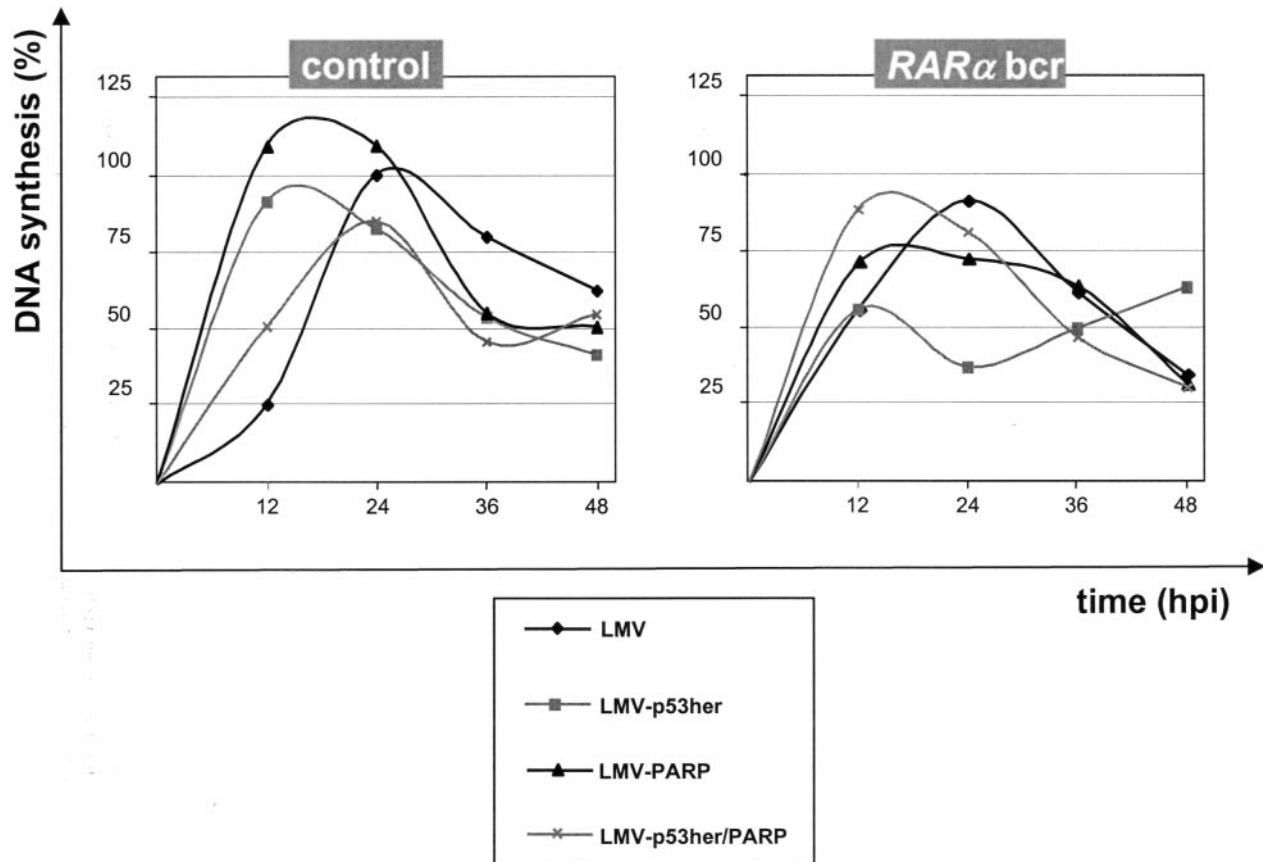
### Effect of PARP-1 on the p53-dependent regulation of recombination between SV40 minichromosomes

Although p53 has been established as a surveillance factor that downregulates HR in a DNA sequence-independent manner, we observed recently a stimulatory effect of p53 on recombination within a 0.3 kb DNA fragment from the

*RARα* bcr (33–35,39). There has been controversy about the functional links of PARP-1 to p53 (31,57–63) and about the possible role of PARP-1 in homology-directed DNA repair (24,28,29). We therefore applied our previously described SV40-based assay (39,50) in order to examine recombination under conditions where p53 exerts a positive or a negative regulatory effect. The SV40-based assay relies on the genetic exchange between SV40 genomes with different *VPI*-mutations causing temperature sensitivity (Figure 1a). Virus particles were produced at the permissive temperature (32°C) and DNA recombination was tested after double infection of target cells at the non-permissive temperature (39°C). Genetic reconstitution of wild-type *VPI* (wtVPI) virus genomes was monitored by plaque assays at 39°C using supernatants from the doubly infected cultures. Plaque formation after wtVPI-SV40 infection was scored in parallel in order to normalize PFUs from coinfection experiments. For the analysis of *RARα* bcr-dependent recombination we generated virus particles from shortened SV40 genome variants [SV40-tsVPI(290T)-Cla, SV40-tsVPI(196Y)-Cla, SV40-wtVPI-Cla] with the 0.3 kb *RARα* bcr sequence cloned into a synthetic ClaI restriction site adjacent to the mutations causing temperature sensitivity (Figure 1a).

To address whether PARP-1 plays a role during recombination between SV40 minichromosomes, we made use of a representative set of primate cell lines conditionally expressing exogenous PARP-1 in a wild-type p53-positive or -negative background, respectively (28). The parental line LMV represents an LLC-MK<sub>2</sub> derivative expressing the estradiol-inducible transcription factor Gal4ERVP, which directs expression of PARP-1 from pGC-PARP-1 plasmid. LMV subclones with stably integrated pGC-PARP-1 expression plasmid (LMV-PARP), with pSV53her plasmid for constitutive expression of the estradiol-responsive fusion protein containing wild-type p53 and the estradiol-binding domain of the human estrogen receptor (LMV-p53her), and with both plasmids (LMV-p53her/PARP) have been characterized previously with respect to protein expression levels, transcriptional, cell cycle regulatory and pro-apoptotic responses (28). Consistent with our earlier findings (39), expression of wild-type p53 in LMV-p53her as compared with LMV cells caused a 3.8-fold stimulation ( $P = 0.034$ ) of recombination between SV40 genomes carrying the *RARα* bcr fragment (Figure 1b). Similar recombination frequencies were measured for LMV and LMV-PARP cells, i.e. no major influence of PARP-1 on *RARα* bcr-dependent recombination was

**Figure 1.** Influence of PARP-1 on recombination in the SV40-based assay. **(a)** Principle of the recombination test. Temperature sensitive SV40 mutants [SV40-tsVPI(290T)-Cla, SV40-tsVPI(196Y)-Cla] used for recombination measurements (50) carry the *RARα* bcr fragment within the ClaI site of a synthetic ClaI linker (39). Perfect topoisomerase I recognition sequences and *t*(15;17) translocation sites found within the *RARα* bcr fragment in acute promyelocytic leukemia patients are marked. The genomic localization of the fragment within the *RARα* bcr is indicated (squares, exons, black lines, introns, coding regions, grey, UTRs, striped). Virus particles were amplified at the permissive temperature of 32°C and used for doubly infecting cells with the *RARα* bcr variants of SV40-tsVPI(290T)-Cla and SV40-tsVPI(196Y)-Cla at 39°C. SV40-wtVPI-Cla virus reconstitution by recombination was scored by reinfection and plaque assays at 39°C. Control infections with the SV40-wtVPI-Cla variant served to calculate recombination frequencies [(PFUs coinfection)/(PFUs SV40-wtVPI-Cla infection × fraction of doubly infected cells)]. Mean recombination frequencies and SD from two to four independent quadruplicate measurements are shown graphically. **(b)** Recombination at the *RARα* bcr fragment. Recombination assays were performed by doubly infecting LMV (–wtp53, –PARP-1), LMV-p53her (+wtp53, –PARP-1), LMV-PARP (–wtp53, +PARP-1) and LMV-p53her/PARP (+wtp53, +PARP-1) cells (28) using SV40-tsVPI(290T)-Cla, SV40-tsVPI(196Y)-Cla, and SV40-wtVPI-Cla derivatives comprising the *RARα* bcr fragment. **(c)** Recombination between SV40 genomes without foreign sequence. Basal recombination frequencies in LMV, LMV-p53her, LMV-PARP and LMV-p53her/PARP cells were determined by use of the control SV40 variants SV40-tsVPI(290T), SV40-tsVPI(196Y) and SV40-wtVPI. **(d)** Recombination between SV40 genomes at the 3'-UTR of the *PARP* gene. Recombination assays were performed by doubly infecting LMV, LMV-p53her, LMV-PARP and LMV-p53her/PARP cells using SV40-tsVPI(290T)-Cla, SV40-tsVPI(196Y)-Cla and SV40-wtVPI-Cla derivatives comprising a fragment from the *PARP* 3'-UTR without topoisomerase I sites (53).



**Figure 2.** DNA synthesis. SV40 DNA replication was determined in LMV (closed diamonds), LMV-p53her (grey squares), LMV-PARP (closed triangles) and LMV-p53her/PARP (grey crosses) cells after infection with control SV40 and with the derivative comprising the *RARα bcr* fragment, respectively. [<sup>3</sup>H]thymidine incorporation was determined at various times postinfection (hpi). To calculate relative DNA synthesis rates the maximum rate for SV40 without foreign sequence in LMV cells 24 hpi was defined as 100%.

detected. Most interestingly, however, we did not see recombination enhancement in LMV-p53her/PARP cells suggesting that PARP-1 counteracts p53-mediated stimulation of recombination between SV40 minichromosomes carrying the *RARα bcr* subregion. In sharp contrast to the results obtained with *RARα bcr* SV40 variants, recombination between SV40 genomes without foreign sequence was repressed by wild-type p53 in LMV-p53her cells (3.5-fold,  $P = 0.008$ ) (Figure 1c). LMV-PARP cells showed a 1.8-fold reduced recombination frequency. A downregulatory effect of PARP-1 was not detected in LMV-p53her/PARP cells, possibly due to the formation of DSBs by apoptotic processes which overcome anti-recombinogenic activities of p53 and PARP-1 (28). Possibly, this effect is seen only at specific response sequences or at low basal recombination frequencies, i.e. below  $5 \times 10^{-4}$ . Figure 1d shows additional control data involving a genomic sequence, which is similarly sized as the *RARα bcr* fragment (0.4 kb) and displays recombination-promoting features (recombination frequency for LMV:  $67 \times 10^{-4}$ ), but lacks strong topoisomerase I recognition sites. The results depicted in this figure confirm that wild-type p53 in LMV-p53her represses rather than stimulates recombination, when topoisomerase I sites are missing in the foreign DNA element (3.1-fold,  $P = 0.025$ ). PARP-1 expression in LMV-PARP cells caused

a 3.5-fold downregulation. Expression of both p53 and PARP-1 in LMV-p53her/PARP cells repressed recombination 3.7 fold.

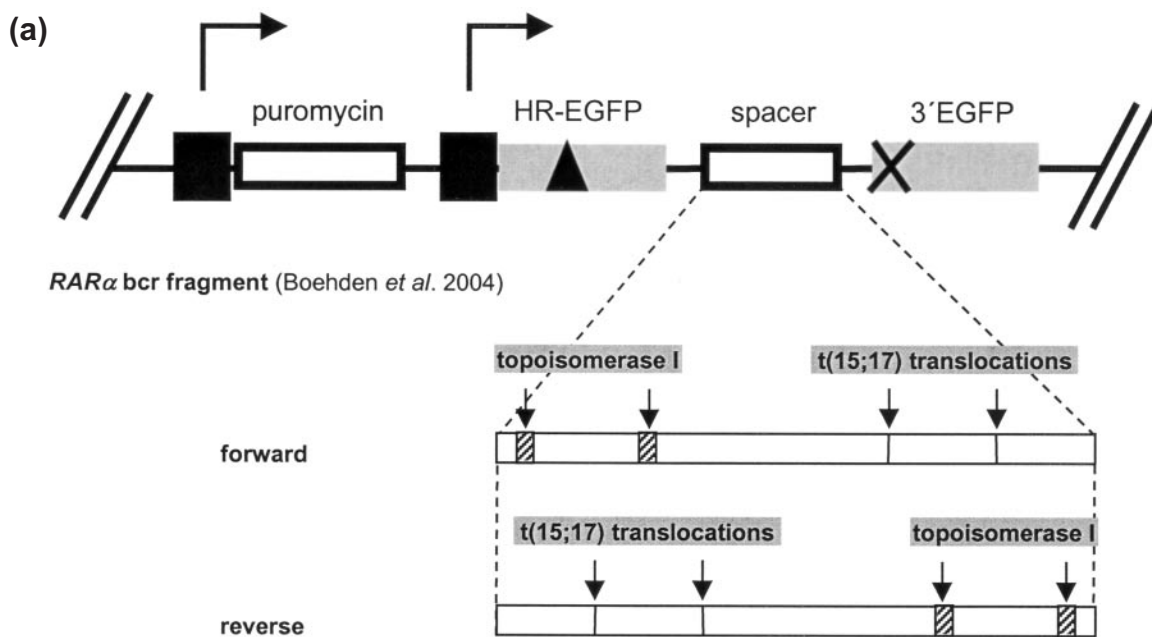
#### Effect of PARP-1 and p53 on viral DNA synthesis

Since replication arrest had been shown previously to stimulate recombination between SV40 minichromosomes (64,65), the impact of p53 and PARP-1 on *RARα bcr*-dependent DNA synthesis was examined. We measured [<sup>3</sup>H]thymidine incorporation into viral DNA at various times after infection (Figure 2). As reported previously (39), *de novo* DNA synthesis was slightly reduced by wild-type p53 (LMV-p53her), both for SV40 genomes without foreign sequence (control) and for *RARα bcr* comprising derivatives. Compared with LMV, expression of PARP-1 in LMV-PARP and LMV-p53her/PARP cells did not cause any major changes in the [<sup>3</sup>H]thymidine incorporation pattern compared with the control SV40 and the *RARα bcr*-variant. However, we cannot fully exclude a minor influence of replication on the recombination activities determined. Nevertheless, effects on recombination frequencies by PARP-1 expression in a wild-type p53-positive or -negative background failed to show a close correlation with changes in DNA replication rate.

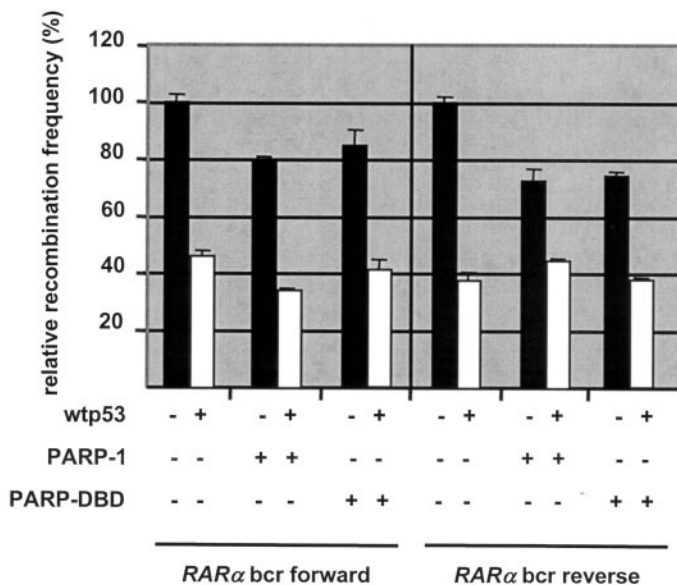
**p53 and PARP-1 downregulate recombination in extrachromosomal plasmid substrates**

To further delineate the influence of p53 and PARP-1 in HR at the *RARα* bcr subregion, we investigated possible

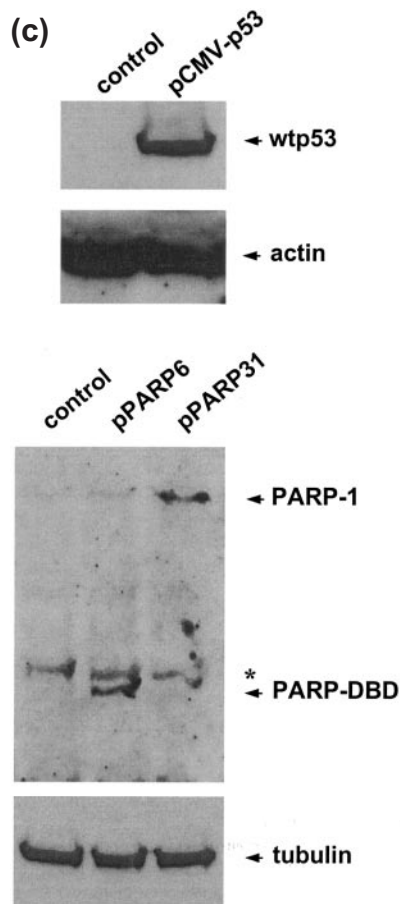
SV40-specific effects. For this purpose, we constructed plasmids pHR-EGFP/3'EGFP-*RARα*wd and pHR-EGFP/3'EGFP-*RARα*rev, which allow assessment of HR between mutated *EGFP* genes (*HR-EGFP* and 3'*EGFP*) in the vicinity



(b)



(c)



of the *RAR $\alpha$*  bcr fragment (Figure 3a). This enabled us to use the fluorescence-based assay (36) which had successfully been used to study p53 and PARP-1 in DSB repair (28). To quantify recombination frequencies in this transient transfection assay, *EGFP* reconstitution is monitored, i.e. the ratios between green fluorescent cells and the total number of cells in the population are determined by flow cytometry. In order to relate cellular recombination events to the fraction of successfully transfected cells, we performed cotransfections with wild-type *EGFP* control plasmid parallel to each recombination experiment. We also wanted to exclude possible indirect effects related to DNA structure and, therefore, inserted the *RAR $\alpha$*  bcr fragment between *HR-EGFP* and *3'EGFP* in both orientations (Figure 3a).

To study homology-directed repair as a function of p53 activity, we lipofected KMV cells with the recombination plasmids pHR-EGFP/3'EGFP-*RAR $\alpha$*  fwd or pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev and pBS control plasmid or pCMV-p53. pCMV-p53 directs expression of wild-type p53 in KMV cells, which are derived from the parental line K562 with homozygously deleted p53 (36). Surprisingly, KMV cells ectopically expressing p53 (Figure 3c) showed a reduction in recombination frequency by 54% rather than an increase (Figure 3b). Having shown that PARP-1 counteracts p53-dependent recombination increases at the *RAR $\alpha$*  bcr in the SV40-based assay, we investigated the role of PARP-1 with the episomal substrates pHR-EGFP/3'EGFP-*RAR $\alpha$*  fwd and pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev. To separate the enzymatic PARP-1 function from its DNA-binding activity we also tested the effect of overexpression of the mere DNA-binding domain of PARP-1 (PARP-DBD), which is devoid of enzymatic activity and causes *trans*-dominant inhibition of poly(ADP-ribosylation) (51,52). For this purpose, KMV cells were not only transfected with one of the recombination plasmids together with or without pCMV-p53 but also with plasmids pPARP31 or pPARP6 which direct expression of PARP-1 and PARP-DBD in KMV cells, respectively (Figure 3c) (28). For PARP-1 and PARP-DBD expression in the absence of p53, we observed a reduction by 21% ( $P = 0.000$ ) and 16% ( $P = 0.030$ ), respectively, with pHR-EGFP/3'EGFP-*RAR $\alpha$*  fwd as a recombination substrate, which was similar to the reduction of 27% ( $P = 0.000$ ) and 26% ( $P = 0.000$ ) with pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev (Figure 3b). In the presence of p53, expression of PARP-1 or PARP-DBD did not induce any additional, significant changes in recombination frequency at the given

protein/DNA substrate ratios (28). Equivalent results were obtained with a similarly sized 0.4 kb fragment from the late region of the SV40 genome containing one topoisomerase I recognition sequence and a 1.3 kb hygromycin resistance cassette with multiple topoisomerase I recognition sequences (see below), thereby excluding a major regulatory influence from the specific DNA sequence (data not shown). Taken together, both p53 and PARP-1 as well as the PARP-DBD diminished HR activities with plasmid substrates independently of DNA sequence content, length and orientation between the substrates for homologous exchange.

### p53 stimulates HR on cellular chromosomes via topoisomerase I and PARP-1 counteracts this stimulatory effect

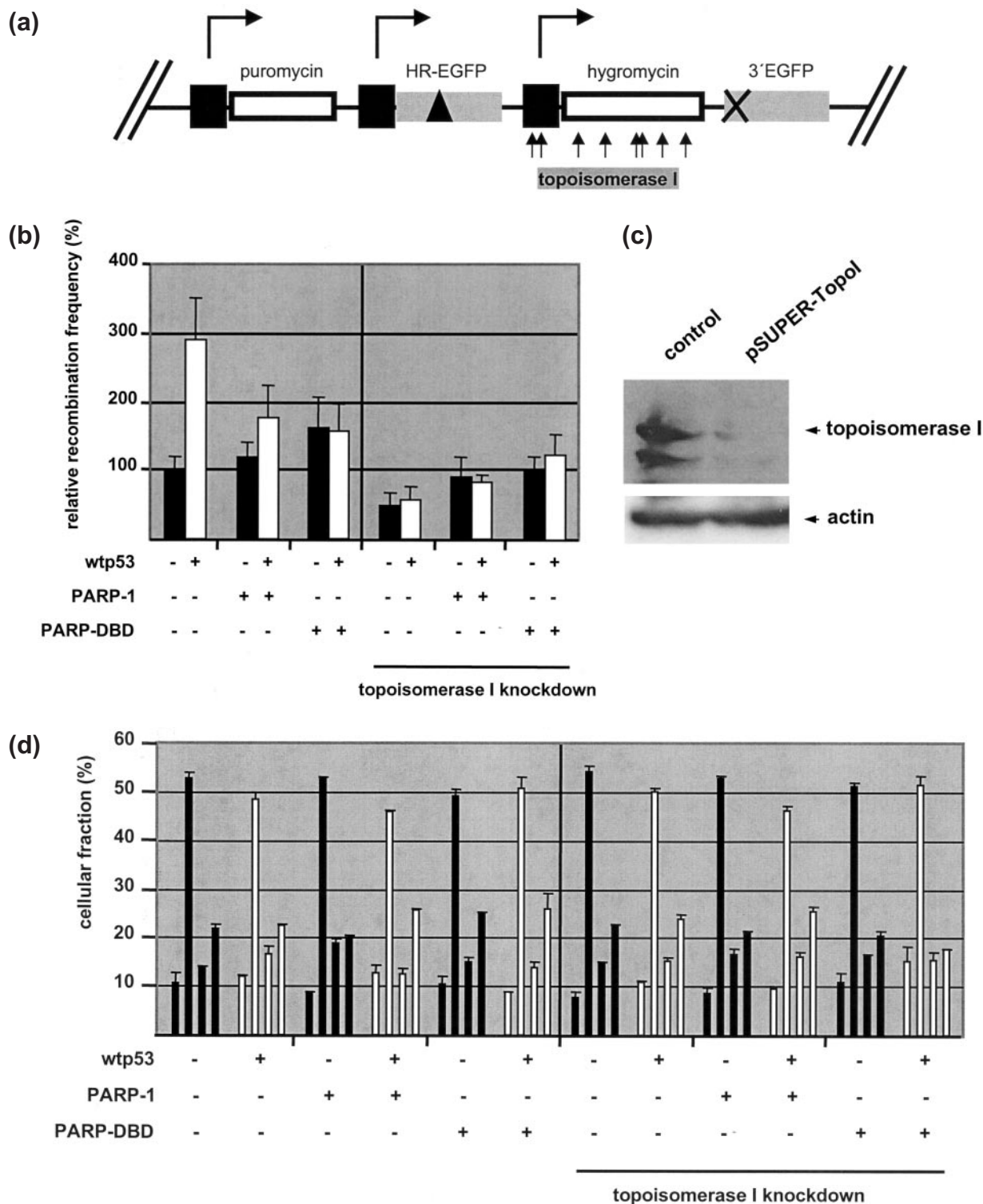
So far, stimulation of recombination by p53 has only been observed with SV40 minichromosomes (39). To clarify whether chromosomal features are critical for this effect, we applied the well-characterized, p53-deficient KMV cell line with a stably integrated HR-EGFP/3'EGFP recombination substrate (36). Within HR-EGFP/3'EGFP, mutated *EGFP* genes are flanking a spacer region consisting of a hygromycin resistance cassette with altogether eight perfect topoisomerase I recognition sequences (Figure 4a). KMV(HR-EGFP/3'EGFP) cells were electroporated with pBS or pCMV-p53 and concomitantly with pSUPER control plasmid or pSUPER-TopoI, which directs the synthesis of siRNAs targeting topoisomerase I mRNA, thereby causing a decrease in topoisomerase I protein level (Figure 4c). Topoisomerase I was silenced previously in HCT116 cells, when using a vector stably expressing siRNA hairpins called pREP4/Top1 (66). p53 expression resulted in a 2.9-fold increase of spontaneous HR ( $P = 0.011$ ; Figure 4b). Downregulation of endogenous topoisomerase I in cells without p53 led to a 51% reduction of the recombination frequency (not significant;  $P = 0.087$ ). Strikingly, knocking down topoisomerase I expression fully suppressed the recombination increase caused by p53 expression (5.1-fold,  $P = 0.002$ ). Next, KMV(HR-EGFP/3'EGFP) cells were additionally transfected with the PARP-1 and PARP-DBD expression plasmids pPARP31 and pPARP6, respectively. In the absence of p53, neither PARP-1 nor PARP-DBD altered recombination frequencies significantly. Reminiscent of the influence of PARP-1 on HR between *RAR $\alpha$*  bcr fragment carrying SV40 genomes, we did not detect any significant p53-mediated recombination

**Figure 3.** Influence of p53, PARP-1 and PARP-DBD expression on recombination with plasmid episomes. (a) Design of the recombination plasmids comprising the *RAR $\alpha$*  bcr sequence. The *EGFP*-based recombination plasmid HR-EGFP/3'EGFP (36) was modified such that the *RAR $\alpha$*  bcr fragment (39) was positioned as spacer between the following DNA elements serving as substrates for homology-directed repair: *HR-EGFP*, an internally mutated *EGFP* gene under the control of the CMV promoter, following a puromycin resistance cassette, and the N-terminally truncated *3'EGFP* gene. Within the spacer region the *RAR $\alpha$*  bcr fragment was inserted in the forward and the reverse orientation to construct plasmids pHR-EGFP/3'EGFP-*RAR $\alpha$*  fwd and pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev, respectively. (b) Recombination after p53, PARP-1 or PARP-DBD expression. KMV cells (36) were subjected to lipofection with the plasmids pBS or pCMV-p53 together with pBS, pPARP31 or pPARP6, for expression of wild-type p53 (*wtp53*), full-length *PARP-1* and *PARP-DBD*, respectively. For determination of recombination frequencies either plasmid pHR-EGFP/3'EGFP-*RAR $\alpha$*  fwd (*RAR $\alpha$*  bcr forward) or pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev was cotransfected (*RAR $\alpha$*  bcr reverse). After lipofection cells were cultivated for 48 h. Reconstitution of EGFP was monitored by FACS<sup>®</sup> analysis and recombination frequencies determined as the fraction of green fluorescent cells within the population of non-fluorescent cells. Individual transfection efficiencies served to normalize each single recombination frequency. Relative recombination frequencies were calculated by taking frequencies in control cells with pHR-EGFP/3'EGFP-*RAR $\alpha$*  fwd or pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev as 100% (absolute values:  $4.3 \times 10^{-2}$  for forward,  $4.2 \times 10^{-2}$  for reverse). Mean values and SEM from six to nine recombination measurements are shown. (c) Protein expression. p53-negative KMV cells were lipofected with pHR-EGFP/3'EGFP-*RAR $\alpha$*  rev together with pBS (control) and pCMV-p53 or with pBS (control), pPARP6, and pPARP31 and total cell extracts prepared after 48 h of continued cultivation. Immunoblotting with the antibody DO1 and goat polyclonal N-20 serum was performed to visualize expression of wild-type p53 (*wtp53*), and *PARP-1* and *PARP-DBD*, respectively. The asterisk marks a band that is detected by N-20 serum independently of the inclusion of expression plasmid.



enhancement on the chromosomally integrated HR-EGFP/  
3'EGFP substrate after cotransfection with pPARP31 or  
pPARP6. Similar observations were also made in cotransfec-  
tion experiments with pSUPER-TopoI. Importantly, FACS®  
analysis of PI stained cells demonstrated that neither topoi-  
somerase I knockdown nor p53, PARP-1 or PARP-DBD

expression had a major influence on cell cycle distribution  
or apoptosis induction under the conditions of the assay  
(Figure 4d). Altogether these data indicated that diminished  
topoisomerase I expression as well as increased PARP-1 and  
PARP-DBD levels counteract spontaneous recombination on  
cellular chromosomes in the presence of p53.



### Role of p53, PARP-1 and topoisomerase I in DSB repair after targeted cleavage of chromosomal substrate DNA

Having established a p53-topoisomerase I pathway in the regulation of spontaneous recombination events, we were interested in the functional links between p53 and topoisomerase I with respect to homology-directed DNA repair if DSBs were artificially introduced by meganuclease I-Sce I. For this purpose we performed recombination measurements with KMV(HR-EGFP/3'EGFP) cells as shown in Figure 4 and additionally co-electroporated the meganuclease I-Sce I expression plasmid pCMV-I-SceI. HR-EGFP/3'EGFP was designed such that an I-Sce I recognition sequence is positioned within HR-EGFP allowing targeted cleavage of this stably integrated recombination marker gene (Figure 5a) (36). I-Sce I expression caused a 20-fold overall increase of recombination frequencies (Figures 4b and 5b). p53 expression resulted in a 88% decrease of recombination (Figure 5b). In cells without p53, PARP-1 or PARP-DBD expression caused reduction of frequencies by 32 and 21%, respectively. No additional PARP-1-dependent changes were observed in cells electroporated with pCMV-p53. A similar picture emerged in pSUPER-TopoI cotransfection experiments. From these results we conclude that topoisomerase I has no major influence on homology-directed repair once a DSB has been introduced into the target, both in the presence and absence of wild-type p53 or PARP-1.

### DISCUSSION

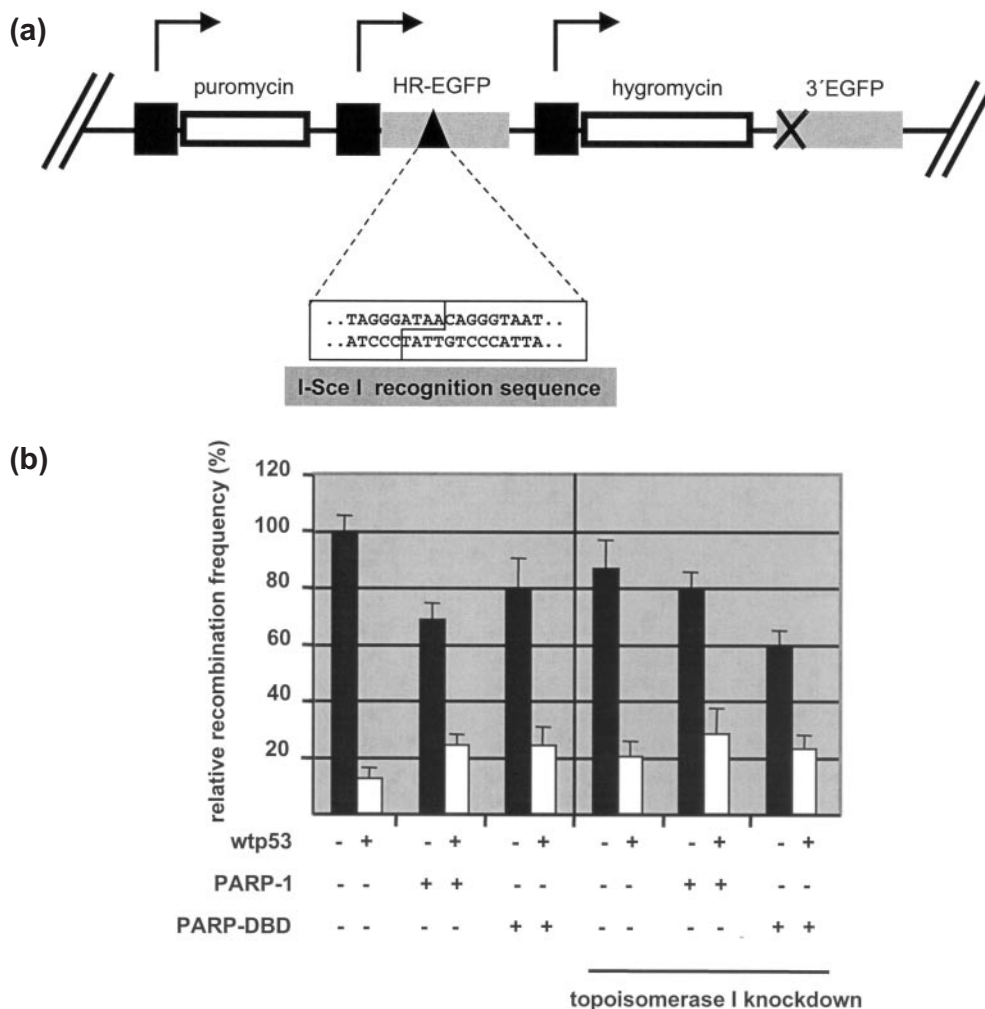
We and others (24,28,29) showed previously that PARP-1 has a negative regulatory role in HR, but it remained unclear whether PARP-1 is involved in homology-directed repair of DSBs, reactivation of stalled replication forks and/or alternative HR pathways. In this work, we showed that PARP-1 expression reduces homology-directed repair of a targeted DSB on chromosomes as well as HR on different extrachromosomal plasmid substrates, i.e. in a manner independent of DNA replication and of specific DNA sequences. PARP-DBD was similarly effective, strongly indicating that the underlying mechanism is binding of the DNA substrate via the ends or secondary structures (67). Supporting this concept, Rudat *et al.* (18) found that overexpression of PARP-DBD inhibits rejoining of DSBs. p53 and

topoisomerase I both represent PARP-1-binding partners and poly(ADP-ribosylation) substrates and play a role in recombination (30–32,35,39,44,45,49). Therefore, we investigated more closely possible links between PARP-1, p53 and topoisomerase I in spontaneous and DSB-triggered HR. Functional interactions between PARP-1 and wild-type p53 may well have been overlooked in previous studies using mutant p53 hamster cells, embryonic stem cells, in which p53 is inactivated due to its cytoplasmic localization or embryonic fibroblasts with p53 gene mutations frequently occurring during early passages (24,29).

Our results from RNA interference experiments firstly demonstrate that p53 can stimulate HR in a manner strictly depending on topoisomerase I, which is consistent with our previous report revealing a similar degree of recombination enhancement by either p53 expression or camptothecin treatment (39). This stimulatory effect was detectable only if the DNA recombination substrate, which carried topoisomerase I recognition sequences in the neighborhood of the exchange site, was localized on viral or cellular chromosomes. This finding could mean that the cooperation between p53 and topoisomerase I depends on chromatin structure. In this regard, it is interesting to note that Rubbi and Milner (68) discovered a role of p53 in regulating chromatin remodelling which may give access to the sites of topoisomerase I action. Topoisomerase I preferentially interacts with supercoiled DNA, which alternatively may be the basis for recruiting the enzyme to DNA in the context of chromatin (69). Recombination enhancement by p53 was no longer seen, when DSBs were introduced within the DNA substrate. Instead, a net decrease of HR was monitored after wild-type p53 expression. Similarly, in the presence of naked plasmid DNA, the role of wild-type p53 in controlling the fidelity of HR dominated over its topoisomerase I-dependent HR upregulation (35,70). These observations strongly suggested that p53 and topoisomerase I cooperate in a pathway increasing the recombinogenicity of the DNA substrate which becomes irrelevant upon availability of highly recombinogenic DNA.

The recombination stimulatory role of p53 might simply be achieved through stimulation of the topoisomerase I relaxation activity to relieve torsional strain (40,41). Another possible explanation has come from biochemical observations made by Grosse and co-workers (46–48). These authors demonstrated that a topoisomerase I–DNA complex can be

**Figure 4.** Recombination on cellular chromosomes after expression of p53, PARP-1 and PARP-DBD, and concomitant knockdown of endogenous topoisomerase I. (a) Structure of the DNA substrate for homology-directed repair in the context of cellular chromosomes. Substrate HR-EGFP/3'EGFP was chromosomally integrated in the p53-negative parental KMV line to yield KMV(HR-EGFP/3'EGFP) cells (36). HR-EGFP/3'EGFP comprises a puromycin resistance cassette, the HR-EGFP cassette, and the 3'EGFP gene (Figure 3). The spacer region contains a hygromycin resistance cassette (SV40 promoter, closed square; hygromycin resistance gene, open square) with eight perfect topoisomerase I recognition sequences. (b) Recombination as a function of p53, PARP-1 and topoisomerase I. KMV(HR-EGFP/3'EGFP) cells were co-electroporated with pBS or pCMV-p53 (*wtp53*) together with pBS, pPARP31 (*PARP-1*) or pPARP6 (*PARP-DBD*) and concomitantly with pSUPER or pSUPER-TopoI (topoisomerase I knockdown) as indicated. Coelectroporations were followed by further cultivation for 72 h. Mean recombination frequencies and SEM were determined from 10 recombination measurements each. For the determination of relative recombination frequencies, frequencies in control cells with pBS were taken as 100% ( $3 \times 10^{-5}$ ). EGFP was typically expressed in 39–57% of the cells electroporated with the 12 different plasmid mixtures. (c) Downregulation of topoisomerase I expression. KMV(HR-EGFP/3'EGFP) cells were electroporated with 40  $\mu$ g of pBS (control) or 15  $\mu$ g of pSUPER-TopoI mediating RNA interference plus 25  $\mu$ g of pBS control plasmid and cultivated for 48 h under conditions of the recombination assay. Endogenous topoisomerase I levels were visualized via immunodetection with the human antiserum Sc1-70. Topoisomerase I from total cellular homogenates appeared as the full-length protein of 100 kDa and as a truncated version. Equal loading was verified by reincubation of the western blot with anti-actin antibodies. (d) Cellular features after topoisomerase I knockdown. Cell cycle distribution and apoptosis were analysed flow cytometrically for propidium iodide stained KMV(HR-EGFP/3'EGFP) cells 72 h after transfection and cultivation under the conditions of the recombination assay. The percentage of cells (cellular fraction) with sub-G1 DNA content (first bar), in G1 (second bar), S (third bar) and G2 phase (fourth bar) are illustrated for cells co-electroporated with pBS or pCMV-p53 (*wtp53*) together with pBS, pPARP31 (*PARP-1*) or pPARP6 (*PARP-DBD*) and concomitantly with pSUPER or pSUPER-TopoI (topoisomerase I knockdown) each. Mean values and SD from two measurements each were calculated.



**Figure 5.** Meganuclease I-Sce I-triggered DSB repair on cellular chromosomes as a function of p53, PARP-1 and topoisomerase I. **(a)** Structure of the DNA substrate for meganuclease I-Sce I-triggered DSB repair. Chromosomally integrated HR-EGFP/3'-EGFP substrate contains a recognition sequence (closed triangle) for the rare-cutting I-SceI meganuclease within the HR-EGFP gene to trigger homology-directed DSB repair after I-SceI expression and targeted cleavage (36). **(b)** I-SceI-triggered recombination frequencies. After electroporation with pBS or pCMV-p53 (*wtp53*) and pBS, pPARP31 (*PARP-1*) or pPARP6 (*PARP-DBD*) together with pSUPER or pSUPER-TopoI (topoisomerase I knockdown) KMV(HR-EGFP/3'EGFP) cells were cultivated for 72 h. Additionally, for meganuclease-mediated cleavage of chromosomally integrated HR-EGFP/3'EGFP substrate pCMV-I-SceI was co-electroporated each. Mean values including SEM of relative recombination frequencies are given for 12 measurements each. Recombination frequencies in KMV(HR-EGFP/3'EGFP) cells with pBS control plasmid were taken as 100% ( $5.7 \times 10^{-4}$ ).

recognized by one additional topoisomerase I molecule to form the so-called topoisomerase I double cleavage complex. Release of one cleavage complex together with the oligonucleotide encompassing the DNA lesion creates an ssDNA gap that provides an entry site for a foreign, complementary DNA strand. p53 has a significant stimulatory effect on this double cleavage reaction and on the resulting recombination-mediated repair events.

When p53-mediated HR stimulation was seen on SV40 or cellular chromosomes, coexpression of PARP-1 antagonized this topoisomerase I-dependent effect (summarized in Table 1). SSBs, including those produced by topoisomerase I-DNA complex stabilization, are known to become converted into DSBs at the replication fork (71). Thus, anti-recombinogenic effects of PARP-1 could theoretically stem from its activities in the repair of SSBs (72). However, it has been well documented that p53 inhibits rather than stimulates HR in response to DSB formation at the replication fork,

**Table 1.** Comparative analysis of recombination data for p53 and PARP-1

Recombination substrates at topoisomerase I recognition sequences	Protein expression		
	P53	PARP-1	p53 and PARP-1
<i>RAR</i> α bcr-SV40 minichromosomes	↑ <sup>a</sup>	↔ <sup>b</sup>	↔
Naked plasmid DNA	↓ <sup>c</sup>	↓	↓
Cellular chromosomes	↑	↔	↔
Cellular chromosomes, I-Sce I cleavage	↓	↓	↓

<sup>a</sup>Significant increase of recombination frequency.

<sup>b</sup>No significant change of recombination frequency.

<sup>c</sup>Significant decrease of recombination frequency.

which is why SSB repair activities are unlikely to play a role during the p53- and topoisomerase I-dependent HR enhancement observed in this study (64,73). PARP-1 expression did not have a major influence on the DNA replication pattern or

on S-phase progression under the conditions of the SV40- and *EGFP*-based recombination measurements, respectively, which altogether argues against a critical role of replicational stress in the antagonistic effect of PARP-1 described here.

PARP-1 has also been shown to play a fundamental role in chromatin condensation and decondensation, particularly through *trans*-poly(ADP-ribosylation) of histones and high-mobility group proteins (1,3). Additionally, binding of PARP-1 to DNA is enhanced specifically by histones (74). Therefore, PARP-1 could act on the topoisomerase I pathway via chromatin remodelling activities. However, under conditions of p53-mediated recombination enhancement, but without exogenously expressed p53, PARP-1 did not exert significant recombination regulatory activities. This phenomenon suggests that PARP-1 does not have a direct regulatory influence on topoisomerase I-dependent recombination events in general. Rather PARP-1 appears to revert the effect of p53 on topoisomerase I specifically. These results may be surprising because PARP-1 downregulates DSB repair independently of p53 [(28), this study]. In conclusion, a biochemical activity of PARP-1 that is different from its catalytic activity must be responsible for the modulation of p53/topoisomerase I activities leading to increased recombination.

Major clues to the mechanism underlying the antagonistic role of PARP-1 in p53/topoisomerase I-promoted recombination may come from the results obtained with PARP-DBD. PARP-DBD represents a dominant negative PARP-1 mutant with respect to poly(ADP-ribosylation) (51,52). Ferro and Olivera (75) reported that covalent poly(ADP-ribosylation) inhibits topoisomerase I. On the other hand, Malanga and Althaus (76) saw that poly(ADP-ribose) formation enhances topoisomerase I-mediated break resealing. Consistent with the latter report, it was shown that recruitment of XRCC1 and DNA ligase III by PARP-1 as well as rejoining of DSBs via the novel PARP-1/XRCC1/DNA ligase III-dependent route relies on poly(ADP-ribose) synthesis (5,6,19,77). Given that poly(ADP-ribosylation) is suppressed in PARP-DBD but not PARP-1 expressing cells, these mechanisms cannot explain the anti-recombinogenic effect described in this study, because we did not obtain significantly different results with PARP-1 and PARP-DBD in *EGFP*-based measurements of spontaneous HR on cellular chromosomes. However, mapping studies of the PARP-1 domain interacting with topoisomerase I showed that PARP-DBD is sufficient for topoisomerase I binding (45). Interestingly, p53 contacts the core of topoisomerase I (amino acids 140–484), i.e. a region overlapping with the PARP-1 interactions sites from amino acid 209–400 and 626–765 (45,78,79). Therefore, it is conceivable that PARP-1 competes with p53 for binding to topoisomerase I or even destabilizes p53/topoisomerase I-DNA complexes (Figure 6).

Covalent topoisomerase I-DNA complexes are normally transient but can be stabilized by the chemotherapeutic agent camptothecin (80) and by various DNA lesions (81). p53 increases dissociation of topoisomerase I covalently bound to DNA, which represents the limiting step during topoisomerase I-mediated repair, particularly, when it is trapped on damaged DNA (48). According to a recent report (82), p53 may also trigger proteasomal degradation of topoisomerase I covalently bound to DNA and thereby accelerate complete removal. In contrast, PARP-1 (via the PARP-DBD)

appears to increase the binding affinity of topoisomerase I to substrate DNA and to stimulate the formation of covalent topoisomerase I-DNA complexes (45,79). Therefore, although both factors cause a net increase in topoisomerase I activity (45,46,49), opposing effects of p53 and PARP-1 on topoisomerase I-DNA complex dynamics could be the molecular basis for the direct antagonizing activity in recombination.

Wild-type p53 was demonstrated to recruit topoisomerase I to the genomic DNA in response to DNA damage (41,83,84). Using live cell imaging Yung *et al.* (49) demonstrated that PARP-1 and topoisomerase I colocalize throughout the cell cycle, but in response to DNA damage PARP-1-topoisomerase I interactions are disrupted due to PARP-1 auto-modification. Therefore, topoisomerase I may be connected to different cellular processes by specific complex partners that contact topoisomerase I in a mutually exclusive manner. According to this model, p53 may represent the molecular link to recombinative repair of specific DNA lesions such as complex DSBs which are generated by ionizing irradiation and repaired slowly. PARP-1 may block this activity in undamaged, cycling cells and rather link topoisomerase I to transcription and DNA replication. Any shift of the delicate balance between the different topoisomerase I functions will have major implications for cellular genome stability, because trapped topoisomerase I-DNA complexes initiate illegitimate recombination events with topoisomerase I molecules bound elsewhere in the genome (41,84,85).

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*Conflict of interest statement.* None declared.

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