DNA Polymerase λ Protects Mouse Fibroblasts against Oxidative DNA Damage and Is Recruited to Sites of DNA Damage/Repair*

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DNA polymerase λ (pol λ) is a member of the X family of DNA polymerases that has been implicated in both base excision repair and non-homologous end joining through in vitro studies. However, to date, no phenotype has been associated with cells deficient in this DNA polymerase. Here we show that pol λ null mouse fibroblasts are hypersensitive to oxidative DNA damaging agents, suggesting a role of pol λ in protection of cells against the cytotoxic effects of oxidized DNA. Additionally, pol λ co-immunoprecipitates with an oxidized base DNA glycosylase, single-strand-selective monofunctional uracil-DNA glycosylase (SMUG1), and localizes to oxidative DNA lesions in situ. From these data, we conclude that pol λ protects cells against oxidative stress and suggest that it participates in oxidative DNA damage base excision repair.

Mammalian cells express a large number of DNA polymerases that are considered to differ from one another according to specialized roles in DNA replication, repair, and other essential DNA transactions such as somatic hypermutation (1). Although these enzymes, numbering over two dozen, catalyze a similar chemical reaction, they differ in primary structure and in features of catalytic specificity as measured in vitro (2). In recent years, DNA polymerases have been grouped into categories or families based on primary structure similarities (3), and members of the X family have been assigned specialized roles in DNA repair (4).

DNA polymerase λ (pol λ) is a member of the X family of DNA polymerases, along with DNA polymerase β (pol β) and several other enzymes (4). Pol β has been shown to be important in the cellular defense against DNA base damage by virtue of its capacity to perform steps in base excision repair (BER) (5). For example, mouse embryonic fibroblast (MEF) cells that are deficient in pol β are hypersensitive to the cytotoxic effects of DNA alkylating agents (6), and this appears to be due to the persistence of toxic intermediates of DNA repair that are normally removed by pol β in wild-type cells (7). Biochemical characterizations of pol β and steps in the BER pathway have focused attention on removal of the deoxyribose phosphate group from the BER intermediate after apurinic/apyrimidinic endonuclease (APE) cleavage of the abasic site (8), and this step is known to be important for alkylating agent-induced cytotoxicity in MEFs (9).

DNA polymerase λ is a single polypeptide enzyme consisting of two overall domains, the N-terminal and the C-terminal (10, 32). The latter is similar in size, structure, and enzymatic composition to pol β, including capacity for gap-filling synthesis and deoxyribose phosphate lyase activity on a BER substrate. It has also been shown that pol λ is able to conduct uracil-initiated BER in extracts of MEFs (11), as well as in assays where BER is reconstituted with purified enzymes (12). Nevertheless, there appears to be no requirement for pol λ in the MEF cellular response to DNA alkylating agents, since cells deficient in pol λ showed no hypersensitivity to these agents (13). Thus, the role of pol λ in the cellular BER response to DNA damage is still unclear. Another important feature of pol λ is that it has been implicated in double-strand break repair in mammalian cell extracts (14), whereas pol β has not. Genetic studies of Saccharomyces cerevisiae pol IV first implicated a pol λ like enzyme in double-strand break repair (15, 16). Yet, a similar role in living mammalian cells remains to be confirmed. Finally, the N-terminal domain of pol λ carries no known enzymatic activity; however, this domain contains a BRCT motif that may be involved in targeting the enzyme through protein-protein interactions (10, 14).

In this study, we further examined the role of pol λ in cellular protection against oxidative DNA damage. DNA lesions such as 5-hydroxymethyluracil (hmUra) and 8-oxoguanine are produced by oxidative stress agents (17) and are repaired mainly by the BER pathway. We report here that MEFs with a pol λ gene deletion are hypersensitive to the oxidative agent H2O2 and are even more hypersensitive to the introduction of hmUra into genomic DNA. DNA polymerase λ was found to localize to sites of oxidative damage by in situ analysis of cells, and the requirements for pol λ localization to damage/repair sites was examined.

Received for publication, June 15, 2005, and in revised form, July 5, 2005
Published, JBC Papers in Press, July 7, 2005, DOI 10.1074/jbc.C500256200

* This work was supported in part by the Intramural Research Program of the National Institutes of Health, NIEHS. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: pol λ, DNA polymerase λ; pol β, DNA polymerase β; BER, base excision repair; MEF, mouse embryonic fibroblast; APE, apurinic/apyrimidinic endonuclease; hmUra, 5-hydroxymethyluracil; XRCC1, X-ray repair cross-complementing group 1; SMUG1, single-strand selective monofunctional uracil-DNA glycosylase; hmdUrd, 5-hydroxymethyl-2′-deoxyuridine; IB, immunoblot; IP, immunoprecipitation; PARP, poly(ADP-ribose) polymerase; siRNA, small interfering RNA; BisTris, 2-(bis(2-hydroxyethyl)amino)-2-(hydroxyethyl)propane-1,3-diol; GFP, green fluorescent protein.

Printed in U.S.A.
**Experimental Procedures**

**Cell Lines**—Pol β+/+ and pol β−/− MEF cells were cultured as described previously (6). XRCC1+/+ and XRCC1−/− MEFs were a generous gift from Dr. Robert Tebbs (Lawrence Livermore National Laboratory, Livermore, CA) and were grown at 37°C in a 10% CO₂ incubator. The cells were transfected with plasmids pEGFP-N1-dN-XN and was in-frame relative to EGFP at the C terminus of each protein. Single-strand selective nuclease digestion of the host plasmid pEGFP-N1-dN-XN-and pol λ with XhoI and StuI. The resulting fragment was purified and introduced into the plasmid pEGFP-N1-dN-XN between the XhoI and EcoRV sites. The polymerase domain of pol λ, pol λ-POL₂₅₇₄₀, encoding Met1 to Arg₄₄, was prepared by digesting the plasmid pEGFP-N1-dN-XN-pol λ with Xhol and StuI. The resulting fragment was purified and digested into the plasmid pEGFP-N1-dN-XN between the XhoI and EcoRV sites. The polymerase domain of pol λ, pol λ-POL₂₅₇₄₀, encoding Met1 to Arg₄₄, was amplified by PCR from the plasmid pEGFP-N1-dN-XN-pol λ. The PCR primers, 5′-GTCCTGGACGAGCAGCCCTAAGCAGAAGGCG-3′ and 5′-CTCGAGCAGTCTGCAGGAGGTTCG-3′, were designed to incorporate XhoI sites at both termini. The resulting PCR fragment was introduced into the plasmid pEGFP-N1-dN-XN at the XhoI site. Each construct contains a modification in the host plasmid pEGFP-N1-dN-XN and was in-frame relative to EGFP at the C terminus of each protein. Single-strand selective nuclease digestion of the host plasmid pEGFP-N1-dN-XN and pol λ siRNA expression vector was constructed as described previously (21). The pol λ siRNA expression vector was constructed as previously described (21). Pairs of RNA oligonucleotides encoding hairpin RNAs were designed based on four different pol λ gene-specific targeted sequences (position on the pol λ gene is indicated in parentheses): 5′-ATGAGAGCAGCGTCCAGGACGACA-3′ (1259), 5′-GTTGGTCACGATAGGAGGCA-3′ (1439), 5′-GTCCTGGATGAATAGGTA-3′ (779), and 5′-GTCCTGGATGAATAGGTA-3′ (820). The 19-nucleotide target transcript sequence and its reverse complement are shown in Fig. 5. The 19-nucleotide target transcript sequence and its reverse complement are shown in Fig. 5.

**DNA Constructs**—The plasmid pEGFP-N1-dN-XN was modified from pEGFP-N1 (Clontech, Mountain View, CA) by deletion of the origin of replication. An insertion of the pol λ-NotI site between BglII and AgeI sites at the multiple cloning site. The plasmid pEGFP-N1-dN-XN, containing full-length pol λ cloned between the XhoI and NotI sites of pEGFP-N1-dN-XN-pol λ, was constructed similarly to the method previously described (20). The BRCT domain of pol λ, pol λ-BRCT₁₋₇₄₄, encoding Met1 to Arg₄₄, was prepared by digesting the plasmid pEGFP-N1-dN-XN-pol λ with XhoI and StuI. The resulting fragment was purified and introduced into the plasmid pEGFP-N1-dN-XN between the XhoI and EcoRV sites. The polymerase domain of pol λ, pol λ-POL₂₅₇₄₀, encoding Met1 to Arg₄₄, was amplified by PCR from the plasmid pEGFP-N1-dN-XN-pol λ. The PCR primers, 5′-GTCCTGGACGAGCAGCCCTAAGCAGAAGGCG-3′ and 5′-CTCGAGCAGTCTGCAGGAGGTTCG-3′, were designed to incorporate XhoI sites at both termini. The resulting PCR fragment was introduced into the plasmid pEGFP-N1-dN-XN at the XhoI site. Each construct contains a modification in the host plasmid pEGFP-N1-dN-XN and was in-frame relative to EGFP at the C terminus of each protein. Single-strand selective nuclease digestion of the host plasmid pEGFP-N1-dN-XN and pol λ siRNA expression vector was constructed as described previously (21). The pol λ siRNA expression vector was constructed as previously described (21). Pairs of RNA oligonucleotides encoding hairpin RNAs were designed based on four different pol λ gene-specific targeted sequences (position on the pol λ gene is indicated in parentheses): 5′-ATGAGAGCAGCGTCCAGGACGACA-3′ (1259), 5′-GTTGGTCACGATAGGAGGCA-3′ (1439), 5′-GTCCTGGATGAATAGGTA-3′ (779), and 5′-GTCCTGGATGAATAGGTA-3′ (820). The 19-nucleotide target transcript sequence and its reverse complement are shown in Fig. 5. The 19-nucleotide target transcript sequence and its reverse complement are shown in Fig. 5.

**Cell Sensitivity Assays**—Cell extracts were prepared by mixing 1 mg of total extract protein and 0.7 mg of rabbit non-immune IgG, anti-pol λ polyclonal, or anti-SMUG1 polyclonal antibody with 25 μg of MEFS cell extracts at 37°C in a 25-mm Tris, pH 7, 60 mM NaCl, 2 mM dithiothreitol, 0.1% Nonidet P-40, 1 mg/ml bovine serum albumin, and 10% (v/v) peroxi-

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RESULTS

DNA Polymerase λ−/− Cells Are Hypersensitive to Oxidative DNA Damage-causing Agents—MEFs that are pol λ-deficient by virtue of homozygous gene deletion were examined for sensitivity to DNA damaging agents. These pol λ null cells were not significantly more sensitive to methylmethane sulfonate than an isogenic wild-type line developed in parallel (data not shown). This is in contrast to the clear methylmethane sulfonate hypersensitivity observed with pol β null cells (6). DNA polymerase λ null cells, however, exhibited slightly more hypersensitivity to H$_2$O$_2$ than pol β null cells (Fig. 1A). This observation encouraged us to examine the sensitivity of pol λ null and isogenic wild-type cells to hmdUrd. Use of this nucleoside allows introduction of a common oxidative base lesion into genomic DNA without exposing cells to an oxidative stress agent in the culture medium (24): hmdUrd is taken up, converted into the 5-hydroxymethyl-2′-deoxyuridine triphosphate, and incorporated into genomic DNA during replication. The hmdUrd lesion appears to be innocuous unless BER is initiated by SMUG1, which recognizes hmUra in genomic DNA and removes it, initiating the BER process (25, 26). We found that pol λ null cells were strongly hypersensitive to hmdUrd (Fig. 1B), and this effect was somewhat stronger than that observed previously with pol β null cells (7). The hmdUrd hypersensitivity of the pol λ null cells could be complemented by recombinant expression of mouse pol λ to a level similar to that found in the isogenic wild-type cell line (Fig. 1, C and D). Thus, stable transfection of pol λ null cells with an expression vector for mouse pol λ and then selection of cells with resistance to hmdUrd allowed us to isolate two cell
Pol λ Participates in Repair of Oxidative DNA Damage

**Fig. 2.** DNA polymerase λ repairs hmUra lesions *in vitro*. A, *in vitro* BER time course of hmUra lesion with cell extract from wild-type (lanes 1–3) or pol λ−/− (lanes 4–6) primary MEF cells. BER reaction mixtures were incubated for 10 (lanes 1 and 4), 30 (lanes 2 and 5), or 60 min (lanes 3 and 6) and analyzed as described under “Experimental Procedures.” Photographs of autoradiograms, after denaturing PAGE illustrating the incorporation of [32P]dCMP into DNA, are shown. The position of the ligated BER product (35-mer) is indicated. B, ligated 35-mer BER products in lanes 3 and 6 from A were scanned by a PhosphorImager and quantified using ImageQuant software. Quantified BER products (measured in PhosphorImager units) were plotted for both the pol λ+/+ and pol λ−/− extract. C, cell extracts from pol λ+/+ and pol β−/− primary MEFs were preincubated with preimmune serum (lanes 1–4) or pol λ neutralizing antibody (lanes 5–8) as described. After preincubation, hmUra-containing 35-base pair duplex DNA was added, and *in vitro* BER was initiated. Repair reactions continued for 10 (lanes 1 and 5), 15 (lanes 2 and 6), 30 (lanes 3 and 7), or 60 min (lanes 4 and 8). Photographs of the autoradiogram after denaturing PAGE are shown, and the position of the ligated BER product (35-mer) is indicated. D, results of reconstituted BER with purified enzymes, illustrating repair of hmUra. *In vitro* BER time course reactions were reconstituted with 20 nm SMUG1, 10 nm APE, 200 nm DNA ligase I, and 100 nm pol λ (lanes 1–3) or 10 nm pol β (lanes 4–6). BER reaction mixtures were incubated for 10 (lanes 1 and 4), 30 (lanes 2 and 5), or 60 min (lanes 3 and 6) and analyzed as described. Photographs of autoradiograms after denaturing PAGE are shown, and the position of the ligated BER product (35-mer) is indicated.

lines with an expression level of pol λ similar to that of wild-type cells. Analysis of these transfected cells revealed a protection by pol λ against hmdUrd-induced cytotoxicity, whereas cells transfected with an empty vector showed no such complementation (Fig. 1C). Additionally, in a series of experiments not shown, introduction of human pol λ into the pol λ null cells by expression vector transfection failed to produce any complementation of the hmdUrd hypersensitivity.

Next, we explored siRNA-mediated down-regulation, an alternate approach toward developing a pol λ deficiency in MEFs. Since both pol β and pol λ appear to participate in repair of hmUra lesions in MEFs, we examined hmdUrd cytotoxicity in cells containing reduced expression of both these polymerases. For these experiments, pol β null MEFs were stably transfected with a pol λ-siRNA-producing vector. Two clonal cell lines were eventually obtained with ~60% reduction in pol λ level (Fig. 1F), and the effect of this reduction in pol λ on hmdUrd sensitivity was examined. These pol λ knockdown cells were more sensitive to hmdUrd than were cells transfected in parallel with an empty vector and used as a negative control (Fig. 1E).

We conclude from these results with MEFs that a deficiency in pol λ is associated with hypersensitivity to oxidative stress and to an oxidized base, hmUra, in genomic DNA.

**DNA Polymerase λ Mediates Repair of hmUra in Vitro**—Earlier, we had shown that pol λ is capable of mediating a back-up role to pol β in MEF extract mediated BER of uracil-DNA (11). In view of the results on hmdUrd hypersensitivity described above, we wished to verify that pol λ is able to perform extract mediated BER of hmUra-DNA. Results of BER assays with cell extracts and an oligonucleotide DNA substrate carrying the hmUra lesion are shown in Fig. 2. DNA polymerase λ was capable of a role in extract mediated BER as revealed by pol λ null cell extracts when compared with wild-type cell extracts (Fig. 2, A and B). In addition, pol λ was able to mediate repair in an extract from pol β null cells, as revealed by antibody inhibition (Fig. 2C). Finally, using a BER system reconstituted with purified human SMUG1, APE, pol λ or pol β, and DNA ligase I, we found robust repair of the hmUra containing substrate in reactions with either polymerase (Fig. 2D). This was reminiscent of earlier work by others (12) showing that purified pol λ is able to mediate BER of uracil-DNA.

**DNA Polymerase λ Co-immunoprecipitates with SMUG1**—In light of the dependence on pol λ for cellular resistance to hmdUrd, we wished to explore the possibility that pol λ and the DNA glycosylase for hmUra-DNA, SMUG1, may cooperate through a protein-protein interaction. Results from co-immunoprecipitation experiments with purified SMUG1 and pol λ are shown in Fig. 3. Initially, we examined an extract from pol λ+/+ MEFs to see if co-immunoprecipitation of pol λ and SMUG1 could be observed. Both enzymes were co-immunoprecipitated with the respective antibody (Fig. 3, A and B). To determine whether pol λ and SMUG1 directly interact with each other, these precipitation reactions were repeated with purified pol λ and SMUG1 protein. SMUG1 was co-precipitated with antibody to pol λ (Fig. 3C) and conversely the antibody to SMUG1 co-precipitated pol λ (Fig. 3D). Negative controls in these experiments failed to reveal any nonspecific precipitation of either protein. Since the BRCT domain of pol λ has been hypothesized to participate in protein-protein interactions, we evaluated whether this domain was responsible for the interaction between SMUG1 and pol λ. As seen in Figs. 3E and F, a truncated version of pol λ lacking the BRCT domain co-precipitated with SMUG1 when an antibody against pol λ was used. The inverse precipitation reaction using SMUG1 antibody also co-precipitated these proteins. We conclude from these experiments that pol λ and SMUG1 directly interact allowing the
enzymes to be co-immunoprecipitated, either from wild-type cell extract or a mixture of purified enzymes.

**DNA Polymerase λ Is Recruited to Sites of Laser-induced Oxidative DNA Damage**—The strategy for studying localization of enzymes involved in oxidative DNA damage repair in living mammalian cells has previously been established (20). Cells expressing GFP-tagged SMUG1 or pol λ were subjected to near-UV light irradiation in the nucleus to produce oxidative base damage, single-strand DNA breaks, and double-strand DNA breaks. The GFP-tagged enzymes were followed by confocal microscopy, as described previously (20). We used this system to examine localization of SMUG1 and pol λ to sites of damage/repair and obtained similar results with both HeLa cells and MEFs. Initially we observed that SMUG1 co-localized to sites of damage/repair (Fig. 4A). Also, SMUG1 accumulation at damage/repair sites was independent from pol λ expression, since SMUG1 localization was not impaired in a pol λ null background (Fig. 4B). However, the kinetics for SMUG1 foci formation were altered in the absence of pol λ. While SMUG1 foci rapidly dissipated after 20 s in wild-type cells, pol λ null cells retained SMUG1 foci for the 300 s duration of the study (Fig. 4B).

We also examined the requirements for pol λ foci formation. DNA polymerase λ was also able to form foci at laser-irradiated sites (Fig. 4C), and its localization required the N-terminal domain of the protein (Fig. D). Although the laser treatment used in this study produces oxidative base damage, single-strand DNA breaks and double-strand DNA breaks, we hypothesize that pol λ accumulates at sites of oxidative base damage. In data not shown, we observe that the accumulation of pol λ is enhanced in the presence of RO-19-8022, a photosensitizer that increases production of oxidized bases after irradiation (20). Additionally, we examined accumulation at double-strand DNA breaks. HeLa cells were pretreated with bromodeoxyuridine to allow for its incorporation into genomic DNA. Upon laser irradiation of these cells, energy from the near-UV light is absorbed by bromine and released to break nearby phosphodiester bonds and produce double-strand DNA breaks (27). Using these conditions, we did not observe an increase in formation of pol λ foci (data not shown), suggesting that pol λ accumulation does not increase when the number of double-strand DNA breaks increases. However, we cannot exclude the possibility that small increases in recruitment of GFP-tagged pol λ or endogenous pol λ may not have been detectable in this study. The dependence upon XRCC1 and PARP activity was also evaluated for formation of pol λ foci. DNA polymerase λ recruitment was identical in a background of either wild-type or XRCC1−/− MEFs (Fig. 4, E and F). Similarly, treatment of HeLa cells with the inhibitor 1,5-
with the 405-nm laser. D and shares key repair related characteristics with pol forms of oxidative DNA damage BER has been long suspected protection against the cytotoxic and mutagenic effects of DNA damage, BER is considered the predominant DNA repair pathway to sites of damage/repair in living cells. Previous studies with pol λ-deficient mammalian cells and are also the first demonstration of the localization of pol λ to sites of oxidative repair in living cells. Previous studies with pol λ−/− mouse embryonic stem cells (13) and pol IV null S. cerevisiae (30) failed to reveal H_2O_2 hypersensitivity in null cells. This may be due to the different cell types studied, thereby reflecting differential responses to oxidative stress. Additionally, these earlier studies generated oxidative DNA damage with ~10-fold higher concentrations of H_2O_2 than used here. These higher concentrations are known to lower the efficiency of aldehydic DNA lesion production in mammalian cells, in contrast to the optimum concentrations of H_2O_2 employed in our study to produce these lesions (31).

It is likely that pol λ exerts its protective effect against oxidative DNA damage by conducting steps in BER. The hypersensitivity of pol λ null cells to oxidative damaging agents indicates that the role of pol λ is not fully complemented by pol β or the other DNA polymerases expressed in these cells. This suggests that there is a specialized role for pol λ in oxidized lesion repair. Yet, pol β also appears to function in hmUra-DNA repair, since pol β null cells are hypersensitive to hmUrd (7), and pol β is capable of mediating BER of hmUra-DNA in the cell extract. The mechanism underlying the dependence on pol λ, even in the presence of strong pol β expression, remains to be elucidated. Similarly, although pol λ and pol β share many features, the requirements for their respective recruitment to sites of damage/repair in living cells are different. Finally, we were interested in the observation that the N-terminal domain of pol λ was required for localization to sites of dihydroisoquinoline under conditions that inhibited PARP failed to alter pol λ recruitment (data not shown). These results indicate that requirements for pol λ localization to sites of damage/repair were different from those observed for pol β localization, which required both XRCC1 and PARP activity (20).

**DISCUSSION**

Mammalian cells are constantly exposed to endogenous and exogenous reactive oxygen species that are capable of modifying DNA. While cells have developed several mechanisms for protection against the cytotoxic and mutagenic effects of DNA damage, BER is considered the predominant DNA repair pathway for combating the detrimental effects of oxidized base lesions. Several subpathways of BER have been identified that are generally divided into two main categories: single nucleotide BER and long patch BER. Typically, BER begins with a lesion-specific DNA glycosylase that cleaves the N-glycosidic bond between the damaged base and deoxyribose. In the case of some oxidative damage glycosylases, strand incision often accompanies base removal, whereas in other cases the glycosylase product is cleaved by APE, to produce a single-strand break containing BER intermediate. Gap tailoring and DNA synthesis ultimately generate the nicked DNA intermediate that is ligated to complete repair. DNA polymerase β has been shown to have a role in protection of MEFs against DNA alkylating agents.

DNA polymerase β also has a protective effect against DNA oxidizing agents, but in the absence of pol β, cells continue to show significant survival upon exposure to oxidizing compounds (6, 28). Therefore, the existence of pol β-independent forms of oxidative DNA damage BER has been long suspected (29). Considering that pol λ is biochemically capable of BER (12) and shares key repair related characteristics with pol β, we decided to examine whether pol λ can protect cells against oxidative DNA damage. Initially, we found that MEFs containing a deletion in the pol λ gene were hypersensitive to H_2O_2 and were even more hypersensitive to incorporation of the oxidative lesion hmUra into genomic DNA. Next, we confirmed that pol λ either in cell extract or as a purified enzyme could conduct BER of an hmUra-containing substrate. We also found that pol λ could directly interact with SMUG1, a glycosylase that removes the hmUra-containing substrate. Finally, we found that pol λ was recruited to sites in the nucleus of living cells that were repairing oxidative DNA lesions. These results are the first demonstration of a phenotype for pol λ-deficient mammalian cells and are also the first demonstration of the localization of pol λ to sites of oxidative repair in living cells. Previous studies with pol λ−/− mouse embryonic stem cells (13) and pol IV null S. cerevisiae (30) failed to reveal H_2O_2 hypersensitivity in null cells. This may be due to the different cell types studied, thereby reflecting differential responses to oxidative stress. Additionally, these earlier studies generated oxidative DNA damage with ~10-fold higher concentrations of H_2O_2 than used here. These higher concentrations are known to lower the efficiency of aldehydic DNA lesion production in mammalian cells, in contrast to the optimum concentrations of H_2O_2 employed in our study to produce these lesions (31).

**FIG. 4. Pol λ and SMUG1 form foci at laser-irradiated sites.** Expression (left) and accumulation (right) of GFP-tagged are shown: A, SMUG1 in HeLa cells; C, pol λ in HeLa cells; E, pol λ in XRCC1+/+ MEFs; or F, pol λ in XRCC1−/− MEFs. In each panel, the photograph on the left shows the cell before irradiation, while the photograph on the right shows the cell after irradiation for 20 s (A), 120 s (C), 140 s (E), or 160 s (F), with the arrow pointing to the irradiated site. In A and C, cells were irradiated with a 365-nm laser, while cells in E and F were irradiated with a 405-nm laser. B shows a time course for the accumulation of SMUG1 in pol λ+/+ MEFs (open diamonds) or pol λ−/− MEFs (open squares) after irradiation with the 405-nm laser. D shows a time course for the accumulation of full-length pol λ (open squares), pol λ-BRCT1−174 (open diamonds), or pol λ-POL242–575 (open circles) after irradiation with a 405-nm laser.
damage/repair. Thus, the recruitment to damage/repair sites appeared to be independent of the pol λ-SMUG1 interaction, which did not require the N-terminal domain of pol λ. It is possible that the protein-protein interaction between these two BER enzymes is important for coordination of BER steps, whereas the N-terminal domain of pol λ may target it to complexes assembled at sites of damage/repair.

Acknowledgements—We thank Dr. Rajendra Prasad and Esther W. Hou (NIEHS, National Institutes of Health, Research Triangle Park, NC) for providing purified enzymes and antibodies; Drs. Barbara Bertocci and Claude-Agnès Reynaud (Institut National de la Santé et de la Recherche Médicale, Paris, France) for the truncated pol protein and XRCC1 protein; Drs. Robert S. Tebbs (Lawrence Livermore National Laboratory, Livermore, CA) for XRCC1 and pol protein; and Thomas A. Kunkel (NIEHS, National Institutes of Health, Research Triangle Park, NC) for providing purified enzymes and antibodies; Drs. Barbara Bebenek, Katarzyna Bebenek, and Thomas A. Kunkel (NIEHS, National Institutes of Health, Research Triangle Park, NC) and Luis Blanco (Campus de la Universidad Autónoma de Madrid, Madrid, Spain) for the truncated pol λ protein and the neutralizing antibody against pol λ; Dr. Deborah J. Stumpo and Jesse DeGraff for assistance with primary MEF preparation and mouse breeding; Dr. Bob Petrovich for assistance with preparation of the expression vector; and Jennifer Myers and Dr. William A. Beard for editorial assistance.

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J. Biol. Chem. 2005, 280:31641-31647.
doi: 10.1074/jbc.C500256200 originally published online July 7, 2005

Access the most updated version of this article at doi: 10.1074/jbc.C500256200

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