DNA REPAIR 5 (2006) 566–574

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/dnarepair

Repair of DNA interstrand cross-links: Interactions between homology-dependent and homology-independent pathways

Huyong Zheng[a,b], *Xin Wang*[a], *Randy J. Legerski*[c], *Peter M. Glazer*[d], *Lei Li*[a,c,*]

[a] *Department of Experimental Radiation Oncology, Unit 66, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, United States*
[b] *Capital University of Medical Science, Beijing Children's Hospital, Beijing, China*
[c] *Department of Molecular Genetics, The University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, United States*
[d] *Department of Therapeutic Radiology, Yale University, School of Medicine, New Heaven, CT 06520, United States*

ARTICLE INFO

Article history:
Received 21 October 2005
Received in revised form 29 December 2005
Accepted 16 January 2006
Published on line 29 March 2006

Keywords:
DNA
Damage
Repair
Interstrand
Cross-links
Recombination

ABSTRACT

DNA interstrand cross-links (ICLs) are complex DNA lesions generated by bifunctional alkylating agents, a class of compounds extensively used in cancer chemotherapy. Formation of an ICL covalently links the opposing strands of the double helix and results in severe disruptions of normal DNA functions, such as replication, transcription, and recombination. Because of the structural complexity, ICLs are most likely recognized by a variety of repair recognition proteins and processed through multiple mechanisms. To study the involvement of different repair pathways in ICL processing, we examined a variety of mammalian mutants with distinct DNA repair deficiencies. We found that the presence of ICLs induces frequent recombination between direct repeat sequences, suggesting that the single-strand annealing pathway may be an important mechanism for the removal of ICLs situated within direct repeats. Unlike recombination-independent ICL repair, ICL-induced single-strand annealing does not require the nucleotide excision repair (NER) mechanism. In cells defective in the mismatch repair protein Msh2, the level of recombination-independent ICL repair was significantly increased, suggesting that processing by the mismatch repair mechanism may lead to recombinational repair of ICLs. Our results suggest that removal of ICLs may involve two error-prone mechanisms depending on the sequence context of the cross-linked site.

1. Introduction

Bifunctional alkylating agents have been a major component of cancer chemotherapeutic regimens for over half a century. The bifunctionality of cross-linking agents possesses the unique ability to form DNA interstrand cross-links (ICLs), which appear to be an essential prerequisite for the potent cytotoxicity and antitumor activity of these compounds [1,2]. ICLs define an important class of DNA lesion in which both strands of the double helix are covalently joined by a bifunctional alkylating molecule, thus affecting the integrity of both strands. Covalent linkage between the complementary strands severely disrupts normal DNA function and causes profound cytotoxicity, as strand separation is absolutely essential during DNA replication, transcription, and recombination.

* *Corresponding author*. Tel.: +1 713 792 3424; fax: +1 713 794 5369.
E-mail address: leili@mdanderson.org (L. Li).
1568-7864/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2006.01.010

Mechanisms responsible for ICL removal are poorly defined particularly in mammalian models [1,3]. In budding yeast, mutants in the nucleotide excision repair (NER) and homologous recombination pathways exhibit hypersensitivity to the killing of bifunctional alkylating agents [4–6], suggesting that both pathways participate in ICL repair via a combination of NER and homologous recombination factors [7,8]. Such a mechanism was also reported in studies of ICL repair in *Escherichia coli* [9,10]. In addition to recombinational repair mechanisms, both yeast and *E. coli* also possess recombination-independent mechanisms involving translesion DNA synthesis, which may lead to error-prone removal of ICLs [8,11–14]. However, how cells select a particular repair mechanism for a given ICL is unclear.

In mammals, recognition and processing of ICLs appears to involve several protein factors acting in distinct repair mechanisms. Mammalian mutants deficient in the RAD51 paralogs XRCC2 and XRCC3 are profoundly sensitive to cross-linking agents, suggesting that homologous recombination is critical in cellular resistance against cross-linking agents [15,16]. Biochemical analysis showed that the mismatch repair complex MutSβ is capable of recognizing and providing initial processing of ICLs in mammalian cell extracts [17]. Results from our previous studies demonstrated that the NER pathway, in concert with lesion bypass polymerase(s), constitutes a recombination-independent and mutagenic ICL repair mechanism [18,19]. However, components and mechanisms of recombinational ICL repair pathway(s) and how these pathways contribute to overall ICL repair and mutagenesis remain largely unknown.

Despite the observation that *XRCC2* and *XRCC3* mutants exhibit hypersensitivity to cross-linking agents, there is no direct evidence that the presence of ICLs stimulates recombination between homologous sequences. Previous investigations have shown that ICLs generated by psoralen-tethered triplex-forming oligos are strong inducers of homologous recombination [20]. However, triplex-forming oligos alone were also able to stimulate homologous recombination in an NER-dependent manner [21]. In the study reported here, we investigated whether processing of a defined ICL leads to homologous recombination. We found that ICLs formed between direct tandem repeat sequences induced recombination via the single-strand annealing mechanism in an NER-independent fashion. Moreover, cells defective in mismatch repair displayed increased recombination-independent ICL repair activity, but loss of mismatch repair function had no effect on the single-strand annealing-based ICL removal. This may imply that processing by the mismatch repair mechanism leads to error-free recombinational ICL repair. Collectively, these results implicate multiple pathways in the repair of ICLs and suggest the existence of direct competition among these mechanisms.

2. Materials and methods

2.1. Cell lines and tissue culture conditions

SV40-transformed XP mutant fibroblasts were obtained from the National Institute of General Medical Sciences (Camden, NJ) and cultured in minimal essential medium plus 10% fetal bovine serum. HEC59 and its complemented derivative via chromosome 2 transfer (kind gift of Dr. C.R. Boland, UCSD Cancer Center) were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Capan-1 and its complemented derivative 236BRCA2(C-Capan-1) (a kind gift from Dr. M.C. Hung, M. D. Anderson Cancer Center) [22] were also maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Complemented XP2OS (C-XPA) cells were established by a two-step selection scheme. Initially, XP2OS cells transiently transfected with a vector expressing wild-type XPA cDNA (pcDNA3-XPA) were cultured in the presence of G418 (400 mg/ml) for 8 days. Subsequently, the surviving population was exposed to 0.5 J of UVC (254 nm) to eliminate cells lacking XPA expression before single cell colonies were selected. Western blot analyses were used to eliminate potential revertants. A complemented XPF (XP2YO) mutant cell line was similarly constructed. Western blot analysis was performed to ensure that the expression levels of XPA and XPF were similar to those in wild-type cells and to eliminate spontaneous revertants.

2.2. Episomal vector construction and substrate preparation

To construct the pSupFN vector (Fig. 1), p*SupFAR* [21] was digested by EagI to remove the second copy of the *supF* gene, followed by insertion of a 27-base-pair adapter sequence containing a central NheI site. The resulting plasmid contained a single EagI site downstream of the NheI recognition sequence. Subsequently, the second *supF* mutant gene

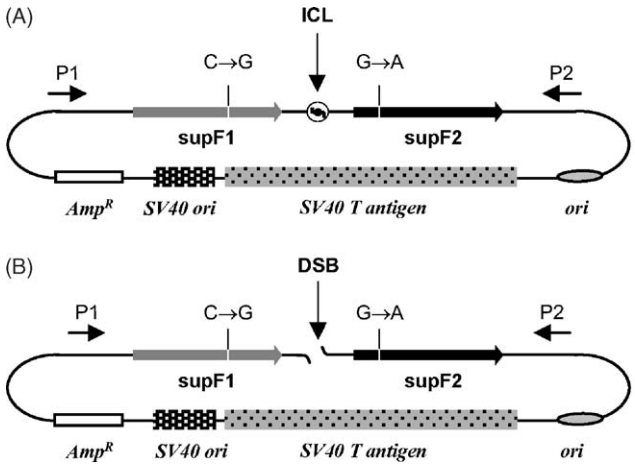

Fig. 1 – Design of the pSupFN vector. The upstream mutant *supF* gene, *supF1*, contains a C-to-G point mutation 77 bases from its start site. The downstream copy of the *supF* gene, *supF2*, contains a G-to-A mutation 22 bases from its start site. The two *supF* genes are separated by a 36-base-pair sequence that contains a single central *NheI* recognition sequence, which allows insertion of the cross-linked oligo (A) or introduction of a single defined DSB (B). P1 and P2 are positions of the PCR primer pair used in analysis of recombination products.

(*supF2*, carrying the G22A mutation) was reinserted at the EagI site in a tandem fashion with the upstream *supF* copy (*supF1*, carrying the C77G mutation), yielding the pSupFN vector.

To prepare cross-linked vector with a single defined ICL, the pSupFN vector was completely digested with NheI, followed by a single cytosine extension of the cohesive end to prevent self-ligation of the ends. Subsequently, a duplex oligo with either a psoralen or mitomycin C (MMC) cross-link was ligated to the vector in vitro and purified via CsCl gradient sedimentation as previously described [18].

2.3. *Luciferase reporter-based recombination-independent ICL repair assay*

A CMV promoter-driven luciferase reporter substrate was generated as previously described [19]. The cross-linked reporter substrate was introduced into cultured cells by FuGENE-6 (Roche Molecular Biochemicals)-mediated transient transfection. Carrier DNA was used to equalize the total amount of plasmid DNA in each experiment. For the luciferase reactivation assay, 0.1–2.5 ng of cross-linked or unmodified control substrate was used for transfections of 1.5×10^5 cells seeded in 35-mm plates. Cells were harvested for the preparation of protein extracts 30 h after transfection. The luciferase activity was determined by using the Luciferase assay system (Promega) and measured on a Moonlight 3010 luminometer (Pharmingen). The linear range of the luciferase assay, in terms of both the amount of transfected DNA and the amount of protein extract used, was established individually for each cell line. A beta-galactosidase expression vector was included in each transfection to normalize transfection efficiency. Each transfection was performed at least three times, and standard deviation was indicated using error bars for each data point.

2.4. *SupF-based recombination assay*

Cross-linked and unmodified plasmid DNA (20 ng) was introduced by FuGENE-6-mediated transient transfection and incubated 48 h to allow processing of the ICLs. A modified Hirt's extraction procedure was used to recover plasmid DNA [23]. Unrepaired original plasmid substrate was removed by DpnI cleavage; therefore, only repaired and replicated DNA substrate was recovered. The plasmid DNA was transformed into *E. coli* MBM7070 (*lacZ*^{Am}). Colonies with functional tRNA suppressor genes able to suppress the *lacZ*^{Am} mutation in MBM7070 were identified as blue colonies capable of metabolizing X-Gal. The blue colonies were normalized against the total number of colonies to yield the recombination frequency.

To analyze the nature of *supF* reactivation, plasmid DNA was extracted from blue colonies for PCR amplification using a pair of primers flanking the *supF* repeats, which produced a 285 base pair product. Single-strand annealing events were identified by a 151-base-pair reduction in the length of the PCR product. DNA sequencing was performed to verify the restoration of the *supF* gene and the deletion at the site of the ICL. Statistical analyses of the luciferase assay and recombination frequency data were performed using Student's two-tailed *t*-test to generate the *P* values.

3. Results

3.1. *Repair of ICLs induces recombination between homologous repeats*

To examine whether repair of ICLs induces recombination between homologous repeats, we modified a *supF* repeat-based episomal vector [21] to allow site-specific insertion of an ICL. As shown in Fig. 1, the resulting pSupFN vector carries two copies of mutant *supF* genes in the form of a tandem repeat. Each copy bears a distinct point mutation at the indicated positions. Therefore, restoration of a wild-type *supF* sequence relies on homologous recombination via either gene conversion or single-strand annealing.

We introduced cross-linked and unmodified pSupFN DNA into COS-7 cells and allowed 48 h for ICL repair processing to take place. Repaired plasmid DNAs were recovered and analyzed in an *E. coli* indicator strain MB7070 (*lacZ*^{Am}) to reveal the status of the *supF* gene. Based on their ability to suppress amber mutations in the LacZ reporter, pSupFN molecules that had undergone recombination were identified by the resulting blue colonies. The blue colonies were normalized against the total number of colonies to yield the recombination frequency.

As shown (Fig. 2(A and B)), unmodified plasmid DNA exhibited a background recombination frequency of 0.04%. However, psoralen-cross-linked pSupFN DNA exhibited a recombination frequency of 1.57%, a nearly 40-fold increase. Hence, a single defined psoralen ICL, located between the tandomly repeated *supF* genes, was able to induce reactivation of the *supF* sequence, indicating that processing of ICLs strongly facilitated recombination between the *supF* genes.

To ascertain whether the observed recombination could be extended to ICLs in general, a MMC ICL was introduced into the pSupFN vector and similarly tested. We found that the presence of the MMC ICL produced an even stronger stimulation *of supF* recombination, approximately 70-fold over background (4.1% versus 0.06%) (Fig. 2(B)).

ICL-induced homologous recombination could be carried out by two distinct mechanisms. When undamaged homologous sequences are available as a donor, a likely mechanism for error-free ICL repair is conservative homologous recombination that leads to gene conversion. Alternatively, the single-strand annealing mechanism can generate a wild-type *supF* copy, resulting in the deletion of one copy of the homologue along with intervening sequences. To determine the mechanism underlying the observed ICL-induced homologous recombination, we analyzed plasmid DNA isolated from blue colonies using PCR amplification. Three independent analyses of a total of 48 clones showed that plasmids recovered from blue colonies contained only one corrected copy of the *supF* gene; the second copy *supF* and the intervening sequence between the *supF* repeats were absent (Fig. 2C). These findings indicated that single-strand annealing was the predominant mechanism mediating the observed ICL-induced *supF* recombination.

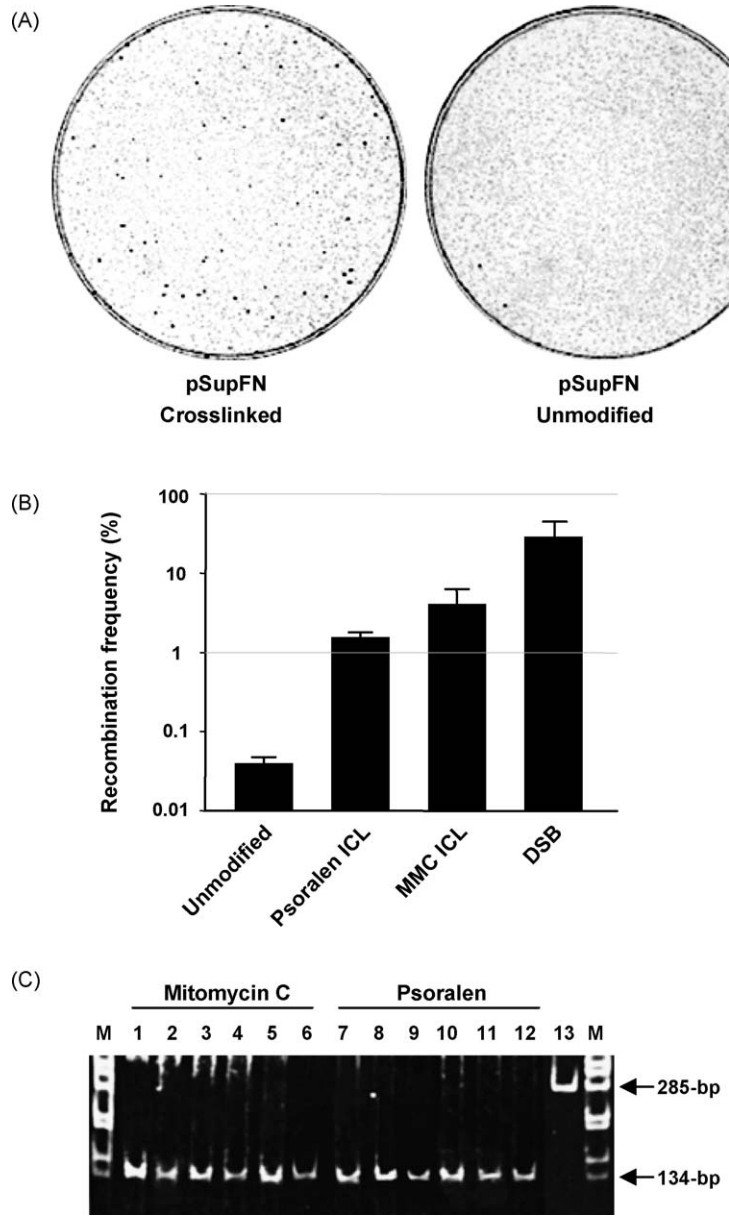

Fig. 2 – ICL-induced *supF* recombination. (A) Blue colonies formed by transforming replicated plasmid DNA recovered from COS-7 cells. Left panel, psoralen cross-linked pSupFN. Right panel, unmodified pSupFN control. Colonies (25,000/plate) were plated on 150-mm plates. (B) Comparison of ICL- and DSB-induced *supF* recombination. Recombination frequency refers to the percentage of blue colonies, derived from no less than 50,000 total colonies. Data values and error bars are derived from three independent experiments. (C) PCR products generated from amplification of plasmids recovered from blue colonies. The DNA samples were resolved on 5% native polyacrylamide gels. Lanes 1–12, PCR products (134 bp) from plasmid recovered from blue colonies. Lane 13, PCR product (285 bp) from the unmodified pSupFN vector.

DNA double strand breaks (DSBs) have been observed in cells treated with DNA cross-linking agents. These DSBs may be produced as intermediates during ICL repair, or, alternatively, they may arise as a result of processing of replication forks blocked by an ICL [7,24]. To test whether the observed ICL-induced single-strand annealing was mediated by the formation of DSBs, we examined DSB-induced single-strand annealing in the pSupFN vector system. To mimic a DSB, the pSupFN vector was linearized at the same *Nhe*I site where the site-specific ICL was placed in the experiments described above. A single dCTP residue was filled-in at the restriction ends to prevent direct religation. After passage through COS-7 cells, recovered pSupFN plasmid was introduced into the *E. coli* reporter strain to reveal the recombination frequency. As shown in Fig. 2(B), the site-specific DSB yielded a recombination frequency of $28.8 \pm 5.16\%$, a dramatic increase over what was observed for either psoralen or MMC ICLs (1.57% and 4.1%, respectively). This result suggested that processing of

ICLs may not lead to their efficient conversion into DSBs such as those generated by ionizing radiation or in vivo restriction enzyme digestion.

3.2. *ICL-induced single-strand annealing is suppressed by the NER mechanism but unaffected by BRCA2 deficiency*

Our previous studies have suggested the existence of a recombination-independent ICL repair pathway that relies on a combination of NER and lesion bypass mechanisms [18,19], in which processing by NER is an essential and early step. The XPA protein is a critical factor in both global and transcription-coupled NER pathways, and loss of XPA completely abolishes NER function.

To determine whether NER is also required in the recombinational repair of ICLs as assayed by the *SupF* reactivation assay, we tested an XPA mutant cell line (XP2OS) for its ability to carry out psoralen ICL-induced single-strand annealing. We found (Fig. 3(A)) that the XPA mutant had an ICL-dependent recombination frequency of 0.55%. However, complementation of the XPA mutant, via stable integration of an XPA cDNA expression vector (Fig. 3(C)), decreased the recombination frequency to 0.18%, indicating that NER suppressed the ICL-induced recombination. To confirm this result, we performed the same experiment but with an MMC-mediated ICL substrate. Again, restoration of the NER pathway reduced ICL-induced single-strand annealing, (Fig. 3(B)).

To further confirm these findings, we examined a second NER mutant, XP2YO, which lacks 5′ NER incision activity due to a mutated *XPF* gene. The results (Fig. 3(D and E)) obtained with both psoralen- and MMC-cross-linked pSupFN again showed that complementation of the NER deficiency in XPF cells significantly reduced ICL-induced single-strand annealing. Collectively, these results indicated that ICL-induced single-strand annealing is independent of the NER mechanism and is likely in direct competition with the NER-dependent ICL repair mechanisms.

The Brca2 protein is a critical factor in homologous recombination. Biochemical studies showed that Brca2 stimulates Rad51 nucleation at a dsDNA–ssDNA junction [25]. Mutations in the BRCA2 gene render cells sensitive to DNA damaging agents, including interstrand cross-linking agents [26]. To test if the ICL-induced *single-strand annealing* requires the participation of Brca2, we examined the human Capan-1 mutant, which carries the 6174delT truncation mutation [27]. As shown (Fig. 3(G)), the ICL-induced *single-strand annealing* was not affected by Brca2 deficiency, as no difference could be observed between Capan-1 and its complemented derivative. Therefore, it is unlikely that the ICL-induced *supF* recombination was mediated by Holliday junction-dependent homologous

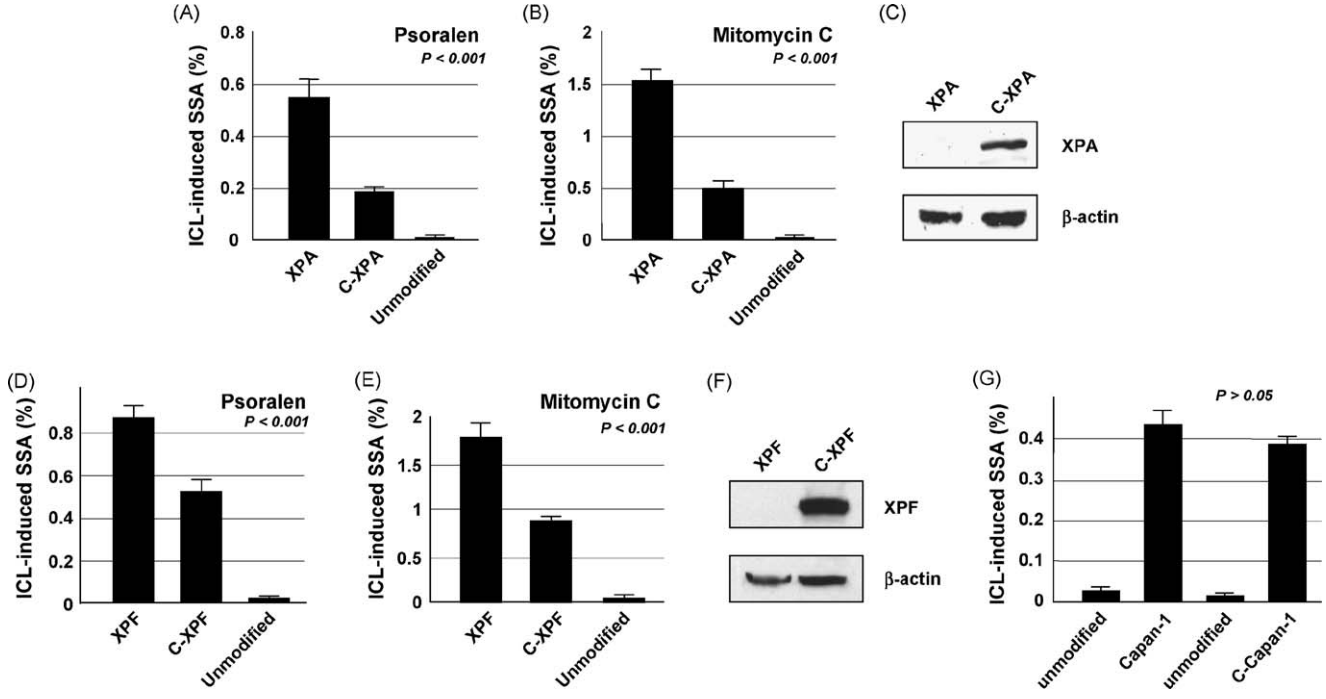

Fig. 3 – ICL-induced single-strand annealing in NER and BRCA2 mutant cell lines. (A) ICL-induced single-strand annealing in XPA(XP2OS) and complemented XPA (C-XPA) cells transfected with psoralen-cross-linked pSupFN vector and of XPA cells transfected with unmodified pSupFN control. (B) MMC-cross-linked pSupFN tested as in (A). (C) Immunoblotting of cell lysates prepared from XPA and C-XPA cells with an affinity-purified anti-XPA antibody. (D) ICL-induced single-strand annealing in XPF (XP2YO) and complemented XPF (C-XPF) cells transfected with psoralen-cross-linked pSupFN vector and of XPF cells transfected with an unmodified pSupFN control. (E) MMC-cross-linked pSupFN tested as in (D). (F) Immunoblotting of cell lysates prepared from XPF and C-XPF cells with an anti-XPF antibody. (G) ICL-induced single-strand annealing in the BRCA2 deficient Capan-1 cells and complemented Capan-1 cells. Data points and error bars are derived from four independent experiments.

recombination. This result further supports that removal of the ICL between the supF genes was likely through the single-strand annealing mechanism.

3.3. *Recombination-independent ICL repair in mismatch repair mutant cells*

Cells defective in certain components of the mismatch repair machinery exhibit reduced survival upon exposure to cross-linking agents compared to monoadduct-forming agents [28,29]. Biochemical studies have suggested that mismatch repair proteins (MutSβ) are capable of recognizing ICLs and initiating its processing [17]. Therefore, it is plausible that recognition and processing by mismatch repair factors may act to divert ICL repair to a recombination-dependent mode. This hypothesis predicts that in the absence of mismatch repair function, recombination-independent ICL repair would be elevated.

Previously, we have established a reporter reactivation-based assay to measure repair of ICLs in the absence of homologous recombination [18,19]. In this assay, a single psoralen or MMC ICL is introduced downstream of a CMV promoter and upstream of the initiation ATG of a firefly luciferase reporter plasmid. The presence of an ICL at such a location constitutes an absolute block to luciferase gene transcription. Thus, removal of the ICL can be monitored by monitoring expression of the luciferase gene. Since undamaged sequence homologous to the reporter plasmid is not available from chromosomal DNA, repair of ICLs is carried out in a recombination-independent and error-prone fashion [19].

To achieve accurate quantification of ICL repair, we normalized the luciferase activity from cells transfected with cross-linked plasmid against that of cells transfected with an unmodified plasmid. The resulting ratio (recombination-independent ICL repair activity) effectively controls for variations in transfection efficiency, gene transcription, and reporter protein accumulation from different cell lineages, allowing us to compare ICL repair between different cell lines.

We examined a human mismatch repair mutant, HEC59, and compared it to a derivative cell line complemented via chromosomal transfer [30,31]. As shown (Fig. 4(A)), lack of Msh2 (HEC59) resulted in a noticeable increase in recombination-independent ICL repair activity with both psoralen and MMC ICLs. Thus, it appears that mismatch repair plays a direct role in ICL processing and that its involvement appears to lessen the contribution of the NER-dependent, recombination-independent ICL repair.

To determine whether the reduced recombination-independent ICL repair activity in HEC59 cells was due to a gain in homology-dependent ICL repair, which may include the ICL-induced single-strand annealing pathway, we introduced psoralen- and MMC-cross-linked pSupFN plasmid into the HEC59 mutant and its complemented derivatives and examined the recombination frequency in these cells (Fig. 4(B)). Complemented HEC59 cells exhibited no significant changes in ICL-induced single-strand annealing frequency compared to mutant cells. This result suggested that Msh2 plays only a minimum role, if any, in ICL-induced single-strand annealing, which implies that an unknown damage recognition and processing mechanism is responsible for the ICL-induced single-strand annealing.

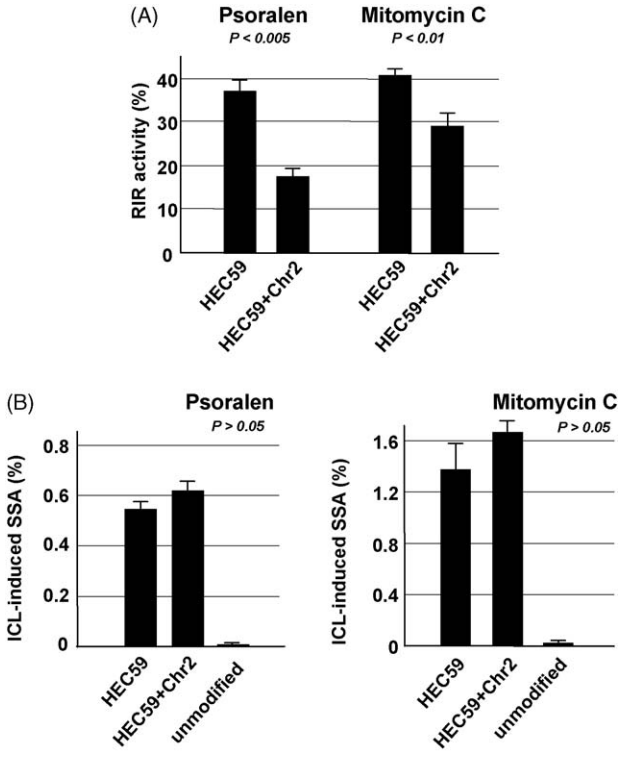

Fig. 4 – ICL repair in the mismatch repair mutant HEC59. (A) Recombination-independent ICL repair activity of psoralen- and MMC-cross-linked luciferase reporter in the HEC59 and complemented mutant (HEC59 + Chr2). (B) Psoralen- and MMC ICL-induced single-strand annealing in the HEC59 mutant. Background recombination levels were obtained from unmodified pSupFN vector. Data points and error bars are derived from three independent experiments.

4. Discussion

Removal of DNA ICLs is most likely carried out by multiple repair mechanisms depending upon availability of undamaged homologous sequences, cell cycle stages, and sequence characteristics at the site of the lesion. Our studies suggest that ICLs located between tandem repeats can effectively induce single-strand annealing-based recombination, resulting in elimination of the ICL and deletions at the site of the cross-link. Moreover, ICL-induced single-strand annealing appears to be an NER-independent mechanism and appears to compete directly with the NER-dependent mutagenic ICL repair pathway.

We showed that psoralen-induced and MMC-induced ICLs stimulated *supF* recombination at levels approximately 40- and 70-fold, respectively, seen with an unmodified substrate. Analysis of repaired plasmids revealed loss of intervening sequence along with one copy of the *supF* gene, which

strongly implicated single-strand annealing as the underlying mechanism. The fact that the ICL-dependent *supF* recombination was independent of Brca2 function further support that the ICL-induced single-strand annealing was not mediated by Holliday junction-dependent homologous recombination.

When comparing single-strand annealing induced by ICLs and DSBs, the latter is approximately 7-fold (psoralen) and 20-fold (MMC) higher. We believe this could be attributed to a number of factors. First, several studies showed that DSBs are generated from replication fork collapse when ICLs are encountered [24,32]. In our system, the cross-linked plasmid can undergo SV40 large T antigen-mediated replication in the COS-7 and human cell lines tested. However, the formation of DSBs might be less effective with SV40 large T-mediated replication. Second, DSBs might not be an obligated ICL repair intermediate. The DSBs observed in vivo when cells were exposed to cross-linking agents might be converted from a small portion of susceptible ICL repair intermediates. Third, although DSBs are more susceptible to processing through the single-strand annealing pathway than ICLs, formation of a single DSB on either side of the ICL will not directly result in its uncoupling or removal. However, some endonucleolytic and exonucleolytic processing has been observed around an ICL [33,34], creating single-strand regions on opposite strands of the cross-linked DNA. In the context of tandem repeats, this may allow an alternative single-strand annealing mechanism to remove an ICL without the formation of DSBs (Fig. 5). However, additional studies are required to obtain direct support for such a model.

Our data showed that ICL-induced single-strand annealing was increased by loss of XPA or XPF. This result suggests that the NER mechanism is not required for ICL-induced single-strand annealing. Rather, initial processing by canonical NER incision may compete with the single-strand annealing pathway by directing the ICL repair into the lesion bypass mechanism. The interaction between NER-dependent and NER-independent ICLs repair pathways appears to resemble the competition among DSB repair pathways [35]. Moreover, the observation that XPA was not required for ICL-induced single-strand annealing revealed an important distinction between single-strand annealing induced by ICLs and triplex-forming oligo-tethered ICLs, where repair of the latter does rely on the XPA gene [20,21].

Damaged bases, particularly those adducted by a bifunctional alkylating agent, are incapable of normal hydrogen bonding; thus, these lesions may be recognized as a site of mismatched DNA. Recently, the MutSβ complex was found to recognize psoralen ICLs and along with Ercc1-Xpf to initiate repair processing in vitro [17,36], supporting a role for mismatch repair proteins in the processing of ICLs. In HEC59 cells, we observed that the NER-dependent lesion bypass ICL repair (recombination-independent ICL repair) was elevated by loss of Msh2, suggesting that Msh2-dependent ICL processing may restrict the participation of lesion bypass-based ICL repair. However, Msh2 loss had little impact on ICL-induced single-strand annealing. This result argues that Msh2-dependent ICL processing is not essential for ICL-induced single-strand annealing and perhaps directing ICL repair to an error-free

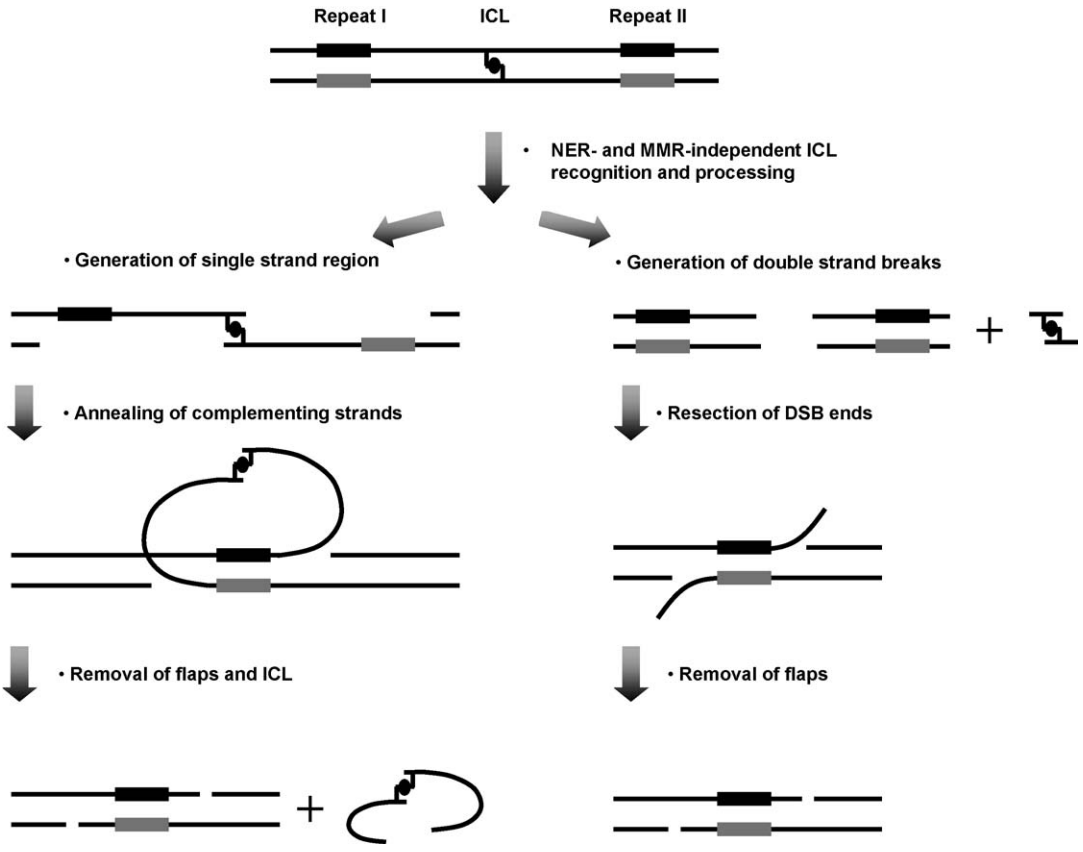

Fig. 5 – Models of ICL-induced single-strand annealing, which result in deletions at the site of an ICL.

repair mechanism mediated by homologous recombination [37].

Our findings provide preliminary evidence that there may be three different mechanisms for ICL processing in mammalian cells: (1) a NER- and lesion bypass-based mutagenic repair pathway; (2) a NER-independent single-strand annealing pathway for ICLs positioned between tandem repeats; and (3) a MutSβ-dependent pathway that perhaps leads to conservative homologous recombination. Continuing efforts in elucidating mechanisms of ICL repair will lead to better understanding of how genetic background affects ICL-induced cytotoxicity and mutagenesis and perhaps facilitate rational design of novel bifunctional alkylating drugs.

Acknowledgements

This work was supported by National Cancer Institute grants CA97175 (L. L.), CA91029 (L. L.), GM54731 (P.M.G.), and CA75160 (R.J.L).

REFERENCES

[1] P.J. McHugh, V.J. Spanswick, J.A. Hartley, Repair of DNA interstrand crosslinks: molecular mechanisms and clinical relevance, Lancet Oncol. 2 (2001) 483–490.
[2] B.A. Theicher, Antitumor alkylating agents, in: Cancer — Principles & Practice of Oncology, fifth ed., Lippincott Raven, 1997, pp. 405–418.
[3] R.J. Legerski, C. Richie, Mechanisms of repair of interstrand crosslinks in DNA, Cancer Treat. Res. 112 (2002) 109–128.
[4] W.J. Jachymczyk, R.C. Von Borstel, M.R.A. Mowat, P.J. Hastings, Repair of interstrand cross-links in DNA of *Saccharomyces cerevisiae* requires two systems of DNA repair: the RAD3 system and the RAD51 system, Mol. Gen. Genet. 182 (1981) 196–205.
[5] R.D. Miller, L. Prakash, S. Prakash, Genetical control of excision of *Saccharoymyces cerevisiae* interstrand DNA cross-links induced by psoralen plus near-UV light, Mol. Cell. Biol. 2 (1982) 939–948.
[6] J.A. Simon, P. Szankasi, D.K. Nguyen, C. Ludlow, H.M. Dunstan, C.J. Roberts, E.L. Jensen, L.H. Hartwell, S.H. Friend, Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of *Saccharomyces cerevisiae*, Cancer Res. 60 (2000) 328–333.
[7] P.J. McHugh, R.D. Gill, R. Waters, J.A. Hartley, Excision repair of nitrogen mustard-DNA adducts in *Saccharomyces cerevisiae*, Nucleic Acids Res. 27 (1999) 3259–3266.
[8] R.B. Greenberg, M. Alberti, J.E. Hearst, M.A. Chua, W.A. Saffran, Recombinational and mutagenic repair of psoralen interstrand cross-links in *Saccharomyces cerevisiae*, J. Biol. Chem. 276 (2001) 31551–31560.
[9] R.S. Cole, Repair of DNA containing interstrand crosslinks in *Escherichia coli*: sequential excision and recombination, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 1064–1068.
[10] R. Cole, S.D. Levitan, R.R. Sinden, Removal of psoralen interstrand crosslinks from DNA of *Escherichia coli*: mechanism and genetic control, J. Mol. Biol. 103 (1976) 39–59.
[11] M. Berardini, W. Mackay, E.L. Loechler, Evidence for a recombination-independent pathway for the repair of DNA interstrand cross-links based on a site-specific study with nitrogen mustard, Biochemistry 36 (1997) 3506–3513.
[12] M. Berardini, P.L. Foster, E.L. Loechler, DNA polymerase II (*polB*) is involved in a new DNA repair pathway for DNA interstrand cross-links *Escherichia coli*, J. Bacteriol. 181 (1999) 2878–2882.
[13] J.R. Nelson, D.W. Lawrence, D.C. Hinkle, Thymine–thymine dimer bypass by yeast DNA polymerase zeta, Science 272 (1996) 1646–1649.
[14] C. Cassier-Chauvat, E. Moustacchi, Allelism between pso1-1 and rev3-1 mutants and between pso2-1 and snm1 mutants in *Saccharomyces cerevisiae*, Curr. Genet. 13 (1988) 37–40.
[15] A.J. Pierce, R.D. Johnson, L.H. Thompson, M. Jasin, XRCC3 promotes homology-directed repair of DNA damage in mammalian cells, Genes Dev. 13 (1999) 2633–2638.
[16] R.D. Johnson, N. Liu, M. Jasin, Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination, Nature 401 (1999) 397–399.
[17] N. Zhang, X. Lu, X. Zhang, C.A. Peterson, R.J. Legerski, hMutSbeta is required for the recognition and uncoupling of psoralen interstrand cross-links in vitro, Mol. Cell. Biol. 22 (2002) 2388–2397.
[18] H. Zheng, X. Wang, A.J. Warren, R.J. Legerski, R.S. Nairn, J.W. Hamilton, L. Li, Nucleotide excision repair- and polymerase eta-mediated error-prone removal of mitomycin C interstrand cross-links, Mol. Cell. Biol. 23 (2003) 754–761.
[19] X. Wang, C.A. Peterson, H. Zheng, R.S. Nairn, R.J. Legerski, L. Li, Involvement of NER in a recombination-independent and error-prone pathway of DNA interstrand cross-link repair, Mol. Cell. Biol. 21 (2001) 713–720.
[20] A.F. Faruqi, M.M. Seidman, D.J. Segal, D. Carroll, P.M. Glazer, Recombination induced by triple-helix-targeted DNA damage in mammalian cells, Mol. Cell. Biol. 16 (1996) 6820–6828.
[21] A.F. Faruqi, H.J. Datta, D. Carroll, M.M. Seidman, P.M. Glazer, Triple-helix formation induces recombination in mammalian cells via a nucleotide excision repair-dependent pathway, Mol. Cell. Biol. 20 (2000) 990–1000.
[22] S.C. Wang, R. Shao, A.Y. Pao, S. Zhang, M.C. Hung, L.K. Su, Inhibition of cancer cell growth by BRCA2, Cancer Res. 62 (2002) 1311–1314.
[23] G. Wang, D.D. Levy, M.M. Seidman, P.M. Glazer, Targeted mutagenesis in mammalian cells mediated by intracellular triple helix formation, Mol. Cell. Biol. 15 (1995) 1759–1768.
[24] I.U. De Silva, P.J. McHugh, P.H. Clingeh, J.A. Hartley, Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells, Mol. Cell. Biol. 20 (2000) 7980–7990.
[25] H. Yang, Q. Li, J. Fan, W.K. Holloman, N.P. Pavletich, The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA-ssDNA junction, Nature 433 (2005) 653–657.
[26] M. Kraakman-van der Zwet, W.J.I. Overkamp, R.E.E. van Lange, J. Essers, A. van Duijn-Goedhart, I. Wiggers, S. Swaminathan, P.P.W. van Buul, A. Errami, R.T.L. Tan, N.G.J. Jaspers, S.K. Sharan, R. Kanaar, M.Z. Zdzienicka, Brca2 (XRCC11) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions, Mol. Cell. Biol. 22 (2002) 669–679.
[27] D.W. Abbott, M.L. Freeman, J.T. Holt, Double-strand break repair deficiency and radiation sensitivity in BRCA2 mutant cancer cells, J. Natl. Cancer Inst. 90 (1998) 978–985.
[28] M.J. Hickman, L.D. Samson, Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 10764–10769.
[29] G. Aquilina, S. Ceccotti, S. Martinelli, R. Hampson, M. Bignami, *N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea

sensitivity in mismatch repair-defective human cells, Cancer Res. 58 (1998) 135–141.
[30] A. Umar, M. Koi, J.I. Risinger, W.E. Glaab, K.R. Tindall, R.D. Kolodner, C.R. Boland, J.C. Barrett, T.A. Kunkel, Correction of hypermutability, N-methyl-N′-nitro-N-nitrosoguanidine resistance, and defective DNA mismatch repair by introducing chromosome 2 into human tumor cells with mutations in MSH2 and MSH6, Cancer Res. 57 (1997) 3949–3955.
[31] M. Koi, A. Umar, D.P. Chauhan, S.P. Cherian, J.M. Carethers, T.A. Kunkel, C.R. Boland, Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-methyl-N′-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation, Cancer Res. 54 (1994) 4308–4312 (erratum appears in Cancer Res. 55 (1) (1995) 201).
[32] T. Bessho, Induction of DNA replication-mediated double strand breaks by psoralen DNA interstrand cross-links, J. Biol. Chem. 278 (2003) 5250–5254.
[33] D. Mu, T. Bessho, L.V. Nechev, D.J. Chen, T.M. Harris, J.E. Hearst, A. Sancar, DNA interstrand cross-links induce futile repair synthesis in mammalian cell extracts, Mol. Cell. Biol. 20 (2000) 2446–2454.
[34] I. Kuraoka, W.R. Kobertz, R.R. Ariza, M. Biggerstaff, J.M. Essigmann, R.D. Wood, Repair of an interstrand DNA cross-link initiated by ERCC1-XPF repair/recombination nuclease, J. Biol. Chem. 275 (2000) 26632–26636.
[35] A.J. Pierce, P. Hu, M. Han, N. Ellis, M. Jasin, Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells, Genes Dev. 15 (2001) 3237–3242.
[36] L. Lan, T. Hayashi, R.M. Rabeya, S. Nakajima, S. Kanno, M. Takao, T. Matsunaga, M. Yoshino, M. Ichikawa, H. Riele, S. Tsuchiya, K. Tanaka, A. Yasui, Functional and physical interactions between ERCC1 and MSH2 complexes for resistance to cis-diamminedichloroplatinum(II) in mammalian cells, DNA Repair 3 (2004) 135–143.
[37] Q. Wu, L.A. Christensen, R.J. Legerski, K.M. Vasquez, Mismatch repair participates in error-free processing of DNA interstrand crosslinks in human cells, EMBO Rep. 6 (2005) 551–557.