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Review Article

Strategies for DNA Interstrand Crosslink Repair: Insights From Worms, Flies, Frogs, and Slime Molds

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DNA interstrand crosslinks (ICLs) are complex lesions that covalently link both strands of the DNA double helix and impede essential cellular processes such as DNA replication and transcription. Recent studies suggest that multiple repair pathways are involved in their removal. Elegant genetic analysis has demonstrated that at least three distinct sets of pathways cooperate in the repair and/or bypass of ICLs in budding yeast. Although the mechanisms of ICL repair in mammals appear similar to those in yeast, important differences have been documented. In addition, mammalian crosslink repair requires other repair factors, such as the Fanconi anemia proteins, whose functions are poorly understood. Because many of these proteins are conserved in simpler metazoans, nonmammalian models have become attractive systems for studying the function(s) of key crosslink repair factors. This review discusses the contributions that various model organisms have made to the field of ICL repair. Specifically, it highlights how studies performed with *C. elegans*, Drosophila, Xenopus, and the social amoeba Dictyostelium serve to complement those from bacteria, yeast, and mammals. Together, these investigations have revealed that although the underlying themes of ICL repair are largely conserved, the complement of DNA repair proteins utilized and the ways in which each of the proteins is used can vary substantially between different organisms. Environ. Mol. Mutagen. 51:646–658, 2010.

Key words: double-strand break; Fanconi anemia; homologous recombination; nucleotide excision repair; translesion synthesis

INTRODUCTION

DNA interstrand crosslinks (ICLs) represent a grave threat to genomic and cellular integrity. They covalently link the two strands of the DNA double helix, thereby blocking replication and preventing crucial proteins involved in transcription and genome maintenance from translocating along the DNA. Because ICLs block essential cellular processes, cells must efficiently detect and remove them to survive. In fact, a single, unrepaired ICL in bacteria or yeast can result in cell death [Lawley and Brookes, 1968; Grossmann et al., 2001], while ~40 ICLs are sufficient to kill repair-deficient mammalian cells [Lawley and Phillips, 1996]. ICLs can also promote genome instability, as error-prone repair of ICLs is known to cause mutations, insertions, and deletions [Jonnalagadda et al., 2005; Richards et al., 2005].

ICLs can be created through the action of exogenous chemicals such as mitomycin C and the nitrogen mustard and cisplatin compounds. These agents are commonly used in chemotherapeutic treatment of certain cancers because of their extreme toxicity in rapidly dividing cell populations (reviewed in [Scharer, 2005]. In addition, endogenous metabolic products such as malondialdehyde, which is formed through lipid peroxidation, may substantially contribute to the endogenous ICL load in many tissues [Niedernhofer et al., 2003]. ICLs may also be created through the action of natural compounds, such as the psoralen-related furocoumarins, which are found at low levels in several edible plants [Scott et al., 1976; Manderfeld et al., 1997]. It should be noted, however, that the current evidence for endogenous ICL formation is mainly indirect and is based on in vitro studies and by inference from mutation spectra (reviewed in [Pang and Andreassen, 2009].

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Because of differences in habitat and diet, organisms are exposed to various types and levels of crosslink-inducing agents. It has been well documented that substantial variation exists in the ability of different organisms (and different tissues within these organisms) to repair ICLs [Lawley and Brookes, 1968; Knox et al., 1991; Lawley and Phillips, 1996]. This variation is likely due, at least in part, to differences in the types of repair pathways used and in their relative robustness in different organisms. Such variation may also be expected to exist in human populations that possess SNPs affecting the activity of different ICL repair proteins. Understanding the molecular basis of this variation will be extremely useful for the design of effective chemotherapeutic treatments.

The major pathways of ICL repair have been characterized in bacteria and yeasts using both molecular genetic and biochemical approaches (reviewed in [Dronkert and Kanaar, 2001; Lehoczky et al., 2007]. The picture that has emerged from these studies is that ICL repair in these organisms involves a combination of proteins that also operate in other DNA repair pathways, including nucleotide excision repair (NER) and homologous recombination (HR). Intensive efforts are currently underway to extend these studies to mammals. However, the existence of additional proteins and repair modules that respond to and repair ICLs in vertebrates adds even more complexity. Specifically, mammals possess a complement of 13 Fanconi Anemia (FA) proteins, whose proposed roles include stabilizing and repairing collapsed replication forks and dealing with ICL-induced damage (reviewed in [Moldovan and D'Andrea, 2009; Rego et al., 2009; Thompson and Hinz, 2009]. To date, the exact functions of many of the FA proteins remain poorly understood, and it is still not clear how the FA network interfaces with other crucial ICL proteins to carry out repair.

Recent genome sequencing projects have revealed that many invertebrates possess key orthologs of mammalian ICL repair proteins (Table 1). These organisms provide an excellent opportunity to study the function of core ICL repair proteins in their native context. Although in many instances these proteins appear to play parallel roles to their mammalian counterparts, there are surprising differences that highlight the multitude of strategies that can be used during ICL repair. This review examines these strategies, with a focus on nonmammalian metazoans and emerging model systems, including *Caenorhabditis elegans*, *Drosophila melanogaster*, *Xenopus laevis*, and *Dictyostelium discoideum*. Throughout the review, important similarities and differences in the ways that these organisms deal with ICLs are highlighted. In addition, selected examples are presented that demonstrate how the information gleaned from these systems may apply to mammals and can reveal unanticipated functions of the ICL repair proteins in other repair pathways, particularly those that deal with DNA double-strand breaks (DSBs).

GENERAL STRATEGIES FOR DNA INTERSTRAND CROSSLINK REPAIR IN BACTERIA AND YEAST

Early research using psoralen-induced crosslinks demonstrated that ICL repair in *Escherichia coli* requires the coordinated action of both NER and HR [Cole, 1973]. Error-free removal of ICLs entails the initial action of the NER proteins $Uvr(A)_2BC$, which nick the DNA on either side of the crosslink [Van Houten et al., 1986] (Fig. 1). Following resection of one of the two DNA strands by the exonuclease activity of Pol I, RecA loads onto the single-stranded DNA and promotes homologous pairing of the undamaged template with the crosslinked DNA molecule [Sladek et al., 1989]. Pol I then catalyzes DNA synthesis across the region containing the crosslink. $Uvr(A)_2BC$ acts again to excise the crosslinked double-stranded DNA fragment and the single-stranded DNA gap is filled by Pol I [Cheng et al., 1991]. In certain situations where HR is not available, as is the case in *recA* mutants or in plasmid-based systems in which no undamaged template is present, a potentially error-prone pathway involving $Uvr(A)_2BC$ and the translesion polymerase Pol II can substitute (Fig. 1) [Berardini et al., 1999].

In principle, the strategies used during ICL repair by the budding yeast, *Saccharomyces cerevisiae*, are similar to those used by *E. coli*. However, two distinct differences exist. First, unlike in bacteria, ICL repair in exponentially growing yeast is associated with DNA DSBs [Jachymczyk et al., 1981]. Second, the type of repair is greatly influenced by the cell cycle. To accommodate these differences, two distinct models, involving at least three DNA repair pathways, have been proposed [Jachymczyk et al., 1981; Grossmann et al., 2001]. These models, though presented mechanistically, are largely based on genetic data. Therefore, the exact sequence of events and the specific roles of many of the proteins involved in each model are still not fully understood.

The first model, which is specific to replicating cells, involves the sequential action of the NER and HR repair pathways. It begins with the stalling of a replication fork at a crosslink (Fig. 2). A DSB intermediate is formed during the initial stages of repair [Magana-Schwencke et al., 1982; Dardalhon and Averbeck, 1995; McHugh et al., 2000], although is unclear whether the break results from endonucleolytic processing of the stalled replication fork or from the fork colliding with a single-strand nick created during initial processing of the crosslink by NER. In support of the latter proposal, several proteins involved in NER, including Rad1/Rad10, Rad2, and the XPD homolog Rad3, are thought to be required for the initial stages of repair of psoralen-induced ICL adducts [Jachymczyk et al., 1981; Miller et al., 1982; Chanet et al., 1985].

Following DSB formation, additional nucleases act to ''unhook'' the crosslink, allowing the crosslinked DNA to swing free from the parental duplex and creating a single-

TABLE 1. Selected Orthologs of ICL Repair Proteins in Model Eukaryotes

H. sapiens	*S. cerevisiae*	*S. pombe*	*C. elegans*	*D. melanogaster*	*X. laevis*	*D. discoideum*	References
Homologous recombination proteins							
RAD51	**Rad51**	**rhp51**		**RAD51**	**RAD51**	**Rad51**	[Lo et al., 2003; Martin et al., 2005]
Fanconi anemia proteins							
FANCD1/BRCA2	None	None	**BRC-2**	**BRCA2**	**BRCA2**	**FncD1**	[Lo et al., 2003; Martin et al., 2005; Zhang et al., 2009]
FANCD2	None	None	**FCD-2**	**FANCD2**	**FANCD2**	**FncD2**	[Collis et al., 2006; Marek and Bale, 2006; Knipscheer et al., 2009; Zhang et al., 2009]
FANCI	None	None	**W02D3.10**	**CG13745**	**FANCI**	**FncI**	[Knipscheer et al., 2009; Youds et al., 2009; Zhang et al., 2009]
FANCJ	None	None	**DOG-1**	None	**BRIP1**	**FncJ**	[Youds et al., 2008; Zhang et al., 2009]
FANCL	None	None	**K01G5.1**	**FANCL**	**FANCL**	**FncL**	[Lambert et al., 2003; Marek and Bale, 2006; Ben-Yehoyada et al., 2009; Meier et al., 2009; Zhang et al., 2009]
FANCM	**Mph1**	**mfh1**	?	**CG7922**	**FANCM**	**FncM**	[Scheller et al., 2000; Sun et al., 2008; Youds et al., 2009; Zhang et al., 2009]
Nucleases							
XPF/ERCC1	**Rad1/Rad10**	**rad16/swi10**	**C47D12.8/F10G8.7**	**MEI-9/ERCC1**	**XPF/ERCC1**	**XPF/?**	[Carr et al., 1994; Yildiz et al., 2004; Liao et al., 2007]
MUS81/EME1	**Mus81/Mms4**	**mus81/eme1**	**MUS-81/?**	**MUS81/MMS4**	**MUS81/EME1**	**Mus81/?**	[Boddy et al., 2000; Liao et al., 2007; Trowbridge et al., 2007]
BTBD12/SLX1	**Slx4/Slx1**	**slx4/slx1**	?	**MUS312/SLX1**	**BTBD12/?**	?	[Yildiz et al., 2002; Coulon et al., 2004; Liao et al., 2007]
SNM1A	**Pso2**	**pso2**	**MRT-1**	**SNM1**	**DCLRE1A**	**Dclre1**	[Lambert et al., 2003; Laurencon et al., 2004; Wu et al., 2004; Hsu et al., 2006; Meier et al., 2009]
Translesion DNA polymerases							
Rev1	**Rev1**	**rev1**	**REV-1**	**REV1**	**REV1**	?	[Larimer et al., 1989; Pothof et al., 2003; Takeuchi et al., 2004; Aslett and Wood, 2006]
pol zeta	**Rev3**	**rev3**	**Y37B11A.2**	**REV3**	**REV3**	**Rev3**	[Cassier and Moustacchi, 1981; Eeken et al., 2001; Sarkar et al., 2006; Zhang et al., 2009]
pol eta	**Rad30**	**eso1**	**POLH-1**	**DNApol-eta**	?	?	[Misra and Vos, 1993; McDonald et al., 1997; Tanaka et al., 2000; Ohkumo et al., 2006]
pol theta	None	None	**POLQ-1**	**MUS308**	?	None	[Boyd et al., 1990; Shima et al., 2004; Muzzini et al., 2008]
pol kappa	None	**dinB**	**POLK-1**	None	**POLK**	None	[Kai and Wang, 2003; Minko et al., 2008]

Shaded boxes correspond to genes that, when mutated, cause sensitivity to ICL-inducing agents. Predicted orthologs that have yet to be identified are indicated by ''?''

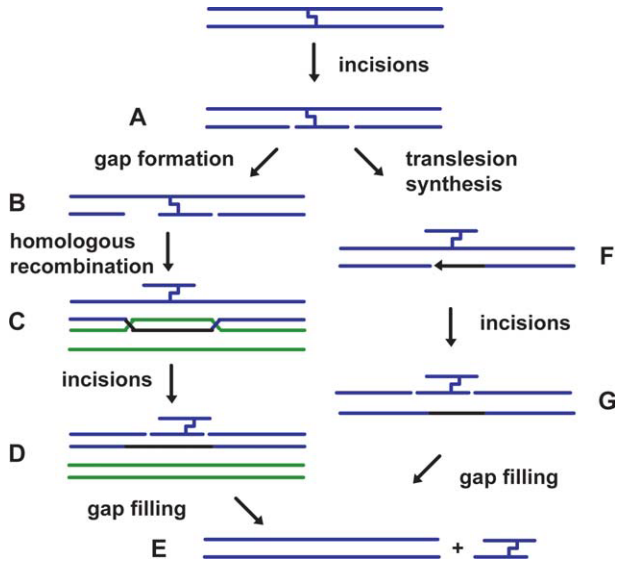

Fig. 1. Strategies for ICL repair in bacteria. (**A**) Excisions are made on either side of the crosslink by the $Uvr(A)_2BC$ endonuclease. (**B**) The exonuclease activity of DNA polymerase I creates a gap on one side of the crosslink, providing single-stranded DNA for RecA loading. (**C**) RecA-mediated strand invasion promotes homologous recombination and synthesis of DNA opposite the crosslink. Holliday junctions are formed and resolved during this process. (**D**) Following homologous recombination, $Uvr(A)_2BC$ again makes incisions flanking the crosslinked oligonucleotide and releases a small piece of DNA containing the crosslink. (**E**) Gap filling by PolI and subsequent ligation completes repair. (**F**) An alternative pathway involving translesion synthesis by DNA polymerase II is invoked if homologous recombination is unavailable. (**G**) Following synthesis across the crosslink, $Uvr(A)_2BC$ nicks the DNA to release the crosslinked DNA and gap filling takes place. References for these models can be found in the text. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

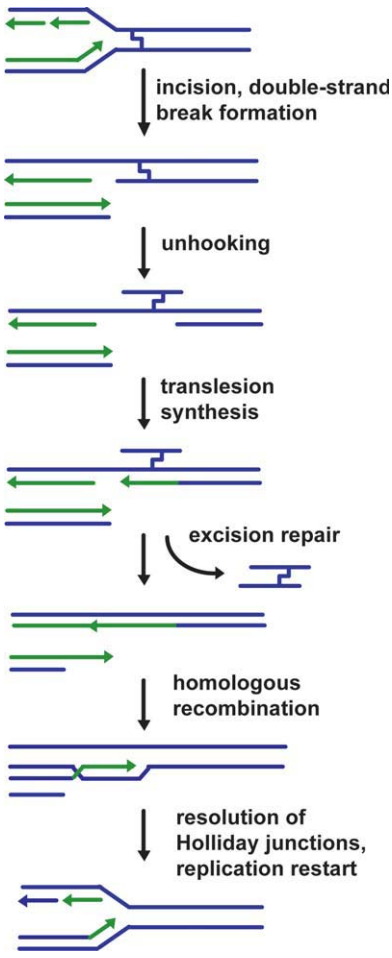

Fig. 2. Possible mechanisms for replication-induced ICL repair. A single replication fork stalls at an ICL. An unknown nuclease(s) catalyzes incision to create a one-ended double-strand break. In mammals, unhooking of the ICL is followed by translesion synthesis by Rev1 and Rev3/Rev7, which extend the parental strand across the lesion. The involvement of translesion synthesis in yeast is uncertain. At an undetermined point, nucleotide excision repair removes the crosslinked oligonucleotide from the intact sister chromatid. The replication fork is rebuilt via invasion of DNA from the one-ended DSB into the intact sister chromatid, followed by synthesis and the resolution of Holliday junctions to form a new fork. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

stranded gap. There is uncertainty as to whether error-prone polymerases are needed during replication-dependent ICL repair in yeast to extend opposite an unhooked ICL [Jachymczyk et al., 1981], as *rev3* mutants lacking the catalytic subunit of translesion DNA polymerase zeta are more sensitive to ICL-inducing agents in stationary phase than during exponential growth [McHugh et al., 2000]. In contrast, exponentially growing *rad51* and *rad52* mutants, which are impaired in HR, are sensitive to a variety of crosslinking agents, demonstrating that HR is critical for repair of the DSB intermediate [Henriques and Moustacchi, 1980; Grossmann et al., 2001; Beljanski et al., 2004]. In the context of ICL repair, HR could be involved in a type of ''replication restart,'' via strand invasion of the one-ended DSB into the homologous duplex and repair using a break-induced replication mechanism [Dronkert and Kanaar, 2001; Davis and Symington, 2004].

The second model of ICL repair in yeast is conceptually similar to the translesion synthesis mechanism known to operate in *E. coli*. It is thought to occur mainly in cells in G1 or in stationary phase, when HR is repressed. It begins with recognition and unhooking of the ICL by the NER machinery [Lehoczky et al., 2007]. Normally, the resulting gap would be filled in by the replicative polymerases delta or epsilon. However, the crosslinked oligonucleotide likely causes the replicative polymerase to stall, resulting in monoubiquitylation of the PCNA sliding clamp on lysine 164 [Garg and Burgers, 2005] and recruitment of the translesion polymerases Rev1 and Rev3/Rev7 (polymerase zeta) to promote translesion synthesis across the unhooked crosslink [Lee and Myung, 2008]. At a later stage, a second set of incisions is made, thereby releasing the crosslinked oligonucleotide and allowing for gap filling and completion of repair.

Several pieces of evidence support this replication-independent repair model. First, ICLs are highly mutagenic, frequently inducing base substitutions and insertions [Barre et al., 1999a]. Second, yeast with mutations in *REV3*, *RAD6*, or *RAD18*, which are all required for error-prone postreplication repair, are sensitive to psoralen [Cassier and Moustacchi, 1981; Henriques and Moustacchi, 1981; McHugh et al., 2000]. The Rad6 protein is an E2 ubiquitin ligase that acts, together with the E3 ligase encoded by Rad18, to monoubiquitylate the K164 residue of PCNA [Waters et al., 2009], which in turn recruits polymerase zeta to the repair site. Third, *rad6* and *rad18* mutants show decreased mutagenesis after treatment with psoralen [Barre et al., 1999b], suggesting that the mutagenic properties of ICLs are largely due to Rad6-dependent postreplication repair.

Many of the genes involved in ICL repair in yeast were originally identified by screening for mutants that were sensitive to *pso*ralen (*pso* mutants) [Henriques and Moustacchi, 1980; Brendel et al., 2003]. The *REV3* (*PSO1*) and *RAD6* (*PSO8*) genes were found in this manner. Another *pso* mutant, *pso2*, appears to act in a pathway separate from both NER and HR. *PSO2* is allelic to *SNM1* [Cassier-Chauvat and Moustacchi, 1988], which was originally identified based on its sensitivity to nitrogen mustard [Ruhland et al., 1981]. The Snm1 protein has 5′ nuclease activity [Li et al., 2005] and contains a conserved β-CASP domain similar to that found in the Artemis endonuclease, which participates in end-joining repair of DSBs during V(D)J recombination [Ma et al., 2002]. Yeast lacking Snm1 are able to carry out the initial incision step of ICL repair and form DSB intermediates following ICL treatment [Magana-Schwencke et al., 1982], demonstrating that Snm1 is required for an uncharacterized downstream processing event (the potential roles of Snm1 are described more fully in the review by McHugh and coworkers, this issue).

In summary, multiple pathways in yeast are involved in ICL repair, including NER, HR, and postreplication repair. However, the epistatic relationships between these pathways are complicated by the fact that proteins within each pathway compete for repair intermediates, and the stage of the cell cycle has a strong influence on availability of key repair factors. Furthermore, specific types of crosslinks are repaired differently in different mutant backgrounds [Beljanski et al., 2004]. Therefore, additional experimental analyses will be required to completely understand the mechanisms of ICL repair in even this ''simple'' eukaryote.

MAMMALIAN ICL REPAIR INVOLVES ADDITIONAL LAYERS OF COMPLEXITY

The mechanisms of ICL repair in mammals are less understood than those in yeast. Nonetheless, several similarities between the two are thought to exist. First, most ICL repair happens during S phase, suggesting that stalled replication forks are likely needed to initiate repair (see the review by Legerski and coworkers in this issue for a thorough description of S phase repair of ICLs). Second, DSBs are formed after passage of ICL-treated mammalian cells through S phase [Akkari et al., 2000], and these breaks require HR for their repair (Fig. 2A, see also the review by Hinz and coworkers in this issue). Third, there exists an error-prone, HR-independent mechanism of ICL repair involving NER and one or more translesion polymerases [Wang et al., 2001; Zheng et al., 2003; Shen et al., 2006].

There are also significant differences between yeast and mammalian ICL repair. Although NER is crucial for the initial steps of repair in yeast, most mammalian NER mutants show only mild sensitivity to ICLs [De Silva et al., 2000, see also the review by Wood and coworkers in this issue). One notable exception is the XPF/ERCC1 complex. Cell lines from mice and human patients lacking either XPF or ERCC1 are exquisitely sensitive to crosslinking agents [Niedernhofer et al., 2004; Clingen et al., 2005]. These observations have led to models involving NER-independent mechanisms of crosslink unhooking, perhaps involving the mismatch repair proteins, and proposals that XPF/ERCC1 is required downstream of unhooking [Bergstralh and Sekelsky, 2008]. Notably, mismatch repair involvement in unhooking may promote relatively error-free processing of crosslinks in contrast to NER-dependent pathways [Shima et al., 2004; Wu et al., 2005].

Another difference is that in yeast, only Rev1 and polymerase zeta, but not polymerase eta (encoded by *RAD30*), partner in an error-prone ICL repair pathway, whereas in mammals, multiple translesion polymerases can act during ICL repair. Genetic and biochemical evidence suggests that mammalian Rev1 and polymerases zeta, eta, theta, and kappa can all promote recombination-independent bypass of ICLs [Zheng et al., 2003; Shima et al., 2004; Shen et al., 2006; Minko et al., 2008, see also the review by Scharer and coworkers, this issue]. The choice of polymerase may depend on the type of ICL adduct and on other factors, which have yet to be characterized.

The most extreme difference in mammalian ICL repair is the requirement for the FA proteins. There are 13 FA genes in vertebrates, named FANCA, B, C, D1, D2, E, F, G, I, J, L, M, and N (reviewed in [Wang, 2007]. Mutation of any of these genes in humans results in FA, a rare disease characterized by bone marrow failure, various developmental deficiencies, and an increased risk of cancer [Tischkowitz and Hodgson, 2003]. Although the precise roles of the FA proteins in ICL repair are still being elucidated, several recent studies support a model in which the FA complex recruits and regulates proteins from other DNA repair pathways when DNA replication is impaired

by fork-blocking lesions [Hussain et al., 2003; Wang, 2007]. For example, FANCD1 is allelic to BRCA2, which encodes a protein required for the recruitment of Rad51 to DSBs during HR [Howlett et al., 2002]. In addition, the FANCN and FANCG proteins interact with BRCA2 [Hussain et al., 2003; Reid et al., 2007]. The FANCJ protein also interacts with BRCA1 [Cantor and Andreassen, 2006], a key regulator of HR repair of DSBs [Scully et al., 2004]. Together, these observations suggest that the FA complex may be important for the process and/or regulation of HR during ICL repair. Furthermore, FANCJ interacts with the mismatch repair complex MutLα, hinting that the FA complex may also recruit mismatch repair proteins to the sites of ICLs [Peng et al., 2007].

Most of the FA proteins, including FANCA, B, C, E, F, G, L, and M, together with two interacting proteins FAAP24 and FAAP100, assemble into a nuclear complex [Rego et al., 2009]. One function of this complex is to monoubiquitylate two additional FA proteins, FANCD2 and FANCI [Meetei et al., 2004; Sims et al., 2007; Smogorzewska et al., 2007]. This reaction is catalyzed by the FANCL E3 ubiquitin ligase, which promotes the interaction of the complex with the E2 ubiquitin ligase Ube2t [Machida et al., 2006]. In spite of the critical nature of this ubiquitylation reaction in ICL repair, the exact function performed by FANCD2/FANCI modification is unclear. The other two FANC proteins, FANCJ and FANCM, have been shown to bind DNA and possess DNA structure-specific unwinding activity [Levitus et al., 2005; Meetei et al., 2005; Gari et al., 2008]. Of note, FANCJ can unwind G-quadruplex DNA [Wu et al., 2008], suggesting that FANCJ, and perhaps other FA proteins, may be important for processing other types of replication-blocking structures in addition to ICLs.

Although recent progress in elucidating the functions of the FA proteins and other key players in ICL repair has been rapid, many fundamental questions remain unanswered. To address these questions, many researchers have turned to other organisms that possess key orthologs of proteins known to be involved in mammalian ICL repair. In the next few sections, several examples are presented that illustrate how these model systems have provided insight into ICL repair mechanisms. In many cases, studies of ICL repair proteins in these systems have accelerated the elucidation of the functions of the corresponding mammalian orthologs. In addition, the studies have revealed that several variations on the central themes of crosslink repair, as originally characterized in bacteria and yeast, have evolved in different organisms.

GENETIC STUDIES OF ICL REPAIR IN *C. elegans*

The nematode *Caenorhabditis elegans* possesses several characteristics that make it an excellent model system for investigating DNA repair processes [Youds et al., 2009]. Many DNA repair studies in *C. elegans* focus on the germline, which is organized both temporally and spatially such that mitotic and meiotic cells can be easily identified. In addition, cytological analysis and immunological studies using germlines isolated from individual animals provide reliable methods to visualize foci of various repair proteins. Finally, young adults can be exposed to various DNA-damaging agents (at a time when eggs are developing) and the survival efficiency of their progeny can be measured to determine sensitivity to the agent.

Because of these advantages, *C. elegans* has proven to be a good system in which to study the core FA proteins. Of the 13 FA proteins, four have clear orthologs in *C. elegans*: BRC-2 (FANCD1/BRCA2) [Petalcorin et al., 2006], FCD-2 (FANCD2) [Collis et al., 2006], DOG-1 (FANCJ) [Youds et al., 2008], and W02D3.10 (FANCI) [Youds et al., 2009]. To date, a functional characterization of W02D3.10 has not been published. Like its mammalian ortholog FANCD2, FCD-2 is monoubiquitylated upon ICL damage, and *fcd-2* mutants are sensitive to cisplatin [Collis et al., 2006]. Also similar to mammals, the FANCD1 ortholog BRC-2 is important for HR repair of DSBs [Martin et al., 2005]. Although sensitivity of *brc-2* mutants to ICL-inducing agents has not been published, such a phenotype is predicted based on its functional conservation.

One of the biggest contributions of *C. elegans* to the ICL repair field came with the report that the nematode *dog-1* gene encodes a FANCJ homolog with $5' \rightarrow 3'$ helicase activity [Youds et al., 2008]. As would be expected, *dog-1* mutants are sensitive to ICL-inducing agents. However, the surprising finding was that mutation of *dog-1* also results in deletions of guanine-rich DNA that can form secondary structures and stall replication forks [Youds et al., 2008]. These results paved the way for the discovery that human FANCJ also unwinds G-rich secondary structures, such as those that are frequently found at trinucleotide repeats and telomeres [Wu et al., 2008]. Therefore, it seems likely that FANCJ recognizes and responds to multiple types of replication fork-blocking lesions.

In addition to the FA genes, three other genes with apparent roles in processes crucial to ICL repair in *C. elegans* have been described. The first, *polq-1*, is orthologous to Drosophila *mus308* [Muzzini et al., 2008], which encodes translesion DNA polymerase theta. *Polq-1* acts epistatically with *brc-1* (encoding *C. elegans* BRCA1) but in a parallel pathway to *fcd-2* for the repair of nitrogen mustard-induced ICLs. A second gene, *hel-308*, is the ortholog of Drosophila *mus301* and appears to operate in the same pathway as *fcd-2* for ICL repair, most likely involving HR [McCaffrey et al., 2006; Muzzini et al., 2008]. One model consistent with these results is that in *C. elegans*, ICLs are processed by either HR or translesion syn-

thesis, with the FCD-2-dependent pathway responsible for HR-related functions and BRC-1/POLQ-1-mediating translesion synthesis.

Finally, a *C. elegans* ortholog of *SNM1*, named MRT-1, was recently described [Meier et al., 2009]. Biochemical studies of MRT-1 indicate that it is a processive 3′ → 5′ exonuclease. This contrasts with the 5′ exonuclease activity displayed by yeast Snm1 and human SNM1A [Li et al., 2005; Hejna et al., 2007]. Mutations in *mrt-1* that abolish exonuclease activity cause extreme sensitivity to crosslinking agents but not ionizing radiation. In addition, MRT-1 possesses an N-terminal OB2-fold similar to that found in the telomere-related protein POT1 [Meier et al., 2009]. MRT-1 is required for telomerase function in *C. elegans* [Ahmed and Hodgkin, 2000], raising the possibility that it might process DNA structures common to both telomeres and ICL repair intermediates.

DROSOPHILA *MUS* MUTANTS REVEAL INTERACTIONS BETWEEN ICL AND OTHER DNA REPAIR PATHWAYS

Like *C. elegans*, Drosophila has provided a genetically tractable system to identify genes that are involved in ICL repair. The Drosophila genome possesses recognizable orthologs corresponding to four of the FA complementation groups: *Fancd2*, *Fancl*, *Fancm* [Marek and Bale, 2006], and *Fancd1/brca2* [Lo et al., 2003]. As might be expected, the fly BRCA2 protein is required for HR repair of DSBs both in mitosis and meiosis [Brough et al., 2008; Klovstad et al., 2008], although there are no published data regarding sensitivity to crosslinking agents. Knockdown of Drosophila FANCD2 or FANCL by RNA interference results in sensitivity to a number of crosslinking agents, and depletion of FANCD2 also causes chromosome fusions and defects in the S-phase checkpoint [Marek and Bale, 2006]. As is the case in mammals, FANCL is responsible for the ubiquitylation of FANCD2. Thus, a minimal FA complex exists in flies, but whether it performs an analogous function to the FA complex in mammals remains to be determined.

In the 1970s and 1980s, several groups performed genetic screens to identify mutants defective in the repair of the monofunctional alkylating agent methane methylsulfonate and nitrogen mustard [Boyd et al., 1976, 1981; Mason et al., 1981; Snyder and Smith, 1982; Leonhardt and Boyd, 1993]. These studies were extended by screening a collection of EMS-induced mutations originally isolated by Charles Zuker [Laurencon et al., 2004]. Many of the mutagen-sensitive (*mus*) mutants isolated in these screens were sensitive to both agents, suggesting that they were defective in general DNA repair or checkpoint functions. However, several mutants specifically sensitive to nitrogen mustard were isolated, including *mus115*, *308*, *321*, *322*, and *323*. To date, only two of the genes affected in the nitrogen mustard-sensitive mutants have been cloned. One of these, *mus322*, is orthologous to yeast *SNM1*. Sequencing of the *mus322* locus from several independent mutant stocks revealed mutations in the β-lactamase domain [Laurencon et al., 2004], suggesting that, as in yeast and nematodes, the enzymatic activity associated with this domain is critical for the protein's function in ICL repair [Li and Moses, 2003].

The first nitrogen mustard-sensitive specific mutant identified in Drosophila, *mus308*, has been the subject of several studies [Boyd et al., 1990; Oliveri et al., 1990]. The *mus308* gene encodes an ortholog of mammalian DNA polymerase theta [Seki et al., 2003]. It contains a polymerase domain similar to *E. coli* Pol I at its C-terminus and an N-terminal helicase-like domain. Polymerase theta protein purified from flies and human cells possesses both error-prone polymerase and ATPase activities, but unwinding activity has not yet been demonstrated [Seki et al., 2003; Pang et al., 2005]. The role of polymerase theta in ICL repair is apparently conserved among metazoans. As described earlier, *C. elegans polq-1* mutants are sensitive to ICL-inducing agents, and *chaos1* mutant mice lacking polymerase theta display heightened genomic instability in the form of micronuclei in reticulocytes, a phenotype that is exacerbated by mitomycin C treatment [Shima et al., 2003, 2004].

To date, the role of Drosophila polymerase theta in ICL repair is still unclear. Two possibilities can be envisioned. First, pol theta may function as a translesion polymerase that can bypass ICLs, equivalent to the role of pol zeta in yeast. This hypothesis is consistent with the fact that *mus205* mutant flies, possessing mutations in pol zeta, are not sensitive to nitrogen mustard [Henderson et al., 1987; Laurencon et al., 2004]. Alternatively, polymerase theta could be involved in specialized repair of DSBs that are formed as intermediates during ICL repair. Supporting this model, bone marrow cultures from POLQ −/− mice are sensitive to ionizing radiation and bleomycin [Goff et al., 2009]. In addition, we have recently demonstrated that Drosophila polymerase theta plays an important role in DNA ligase IV-independent end-joining repair of DSBs, and this role is particularly vital in the absence of Rad51-mediated HR (our unpublished data). Therefore, polymerase theta might be responsible for repairing particular types of DSBs that are poor substrates for HR pathways.

Studies using Drosophila also identified a conserved ICL repair protein, MUS312, which appears to play an important role in the repair of DSBs created during crosslink processing. As in mammals, flies with mutations in either *mei-9* (the XPF homolog) or *ercc1* are sensitive to nitrogen mustard [Yildiz et al., 2004; Radford et al., 2005]. Several years ago, a third protein that interacts with MEI-9 and ERCC1, named MUS312, was identified [Yildiz et al., 2002]. *mus312* mutants are more sensitive

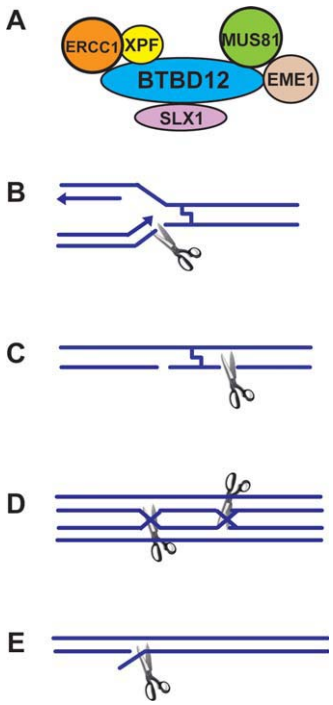

Fig. 3. Potential roles for the BTBD12 complex in ICL repair. (**A**) Schematic showing how BTBD12 acts as a scaffold for structure-specific nucleases. BTBD12 interacts with the XPF/ERCC1 and MUS81/EME1 heterodimeric nucleases. In addition, it stimulates the Holliday junction cleavage activity of SLX1. (**B**) Following the stalling of a replication fork at an ICL, one or more nucleases (including Mus81) found in the BTBD12 complex may cut the opposite 3′ end of the nascent leading strand to create a one-ended double-strand break. (**C**) The Xpf/Ercc1 heterodimer may participate in the unhooking of interstrand crosslinks. (**D**) SLX1 in complex with BTBD12 may catalyze the cleavage of Holliday junctions that form during homologous recombination repair of ICL-induced double-strand breaks. (**E**) The Xpf/Ercc1 heterodimer may cut 3′ flaps that remain after homologous recombination or single-strand annealing repair of ICL-induced double-strand breaks. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to nitrogen mustard than *mei-9* mutants, although double mutants behave like *mus312* single mutants, suggesting that MUS312 has MEI-9-independent functions in ICL repair. Recently, a mammalian ortholog of MUS312, named BTBD12, was identified and shown to be involved in ICL repair [Andersen et al., 2009; Fekairi et al., 2009; Munoz et al., 2009; Svendsen et al., 2009]. BTBD12, when complexed with the SLX1 nuclease [Fricke and Brill, 2003], acts as a canonical Holliday junction resolvase [Fekairi et al., 2009; Svendsen et al., 2009]. It also interacts with multiple structure-specific endonucleases, including MUS81/EME1 and XPF/ERCC1.

Because it acts as a scaffold for the binding of several nucleases (Fig. 3), it is currently unclear what the exact function of BTBD12/MUS312 might be in ICL repair, or whether it might act at numerous points during the repair process. One possibility is that BTBD12, in combination with XPF/ERCC1, resolves Holliday junctions that are formed during HR repair of ICL repair intermediates. This model is supported by the observation that Drosophila *mus312* and *mei-9* mutants have greatly reduced levels of meiotic crossovers [Yildiz et al., 2002], the formation of which requires the action of a Holliday junction resolvase. However, the *mei-9*^{12} mutant, in which MEI-9 fails to interact with MUS312, is not sensitive to nitrogen mustard, making this scenario less likely in flies. BTBD12 could also recruit XPF/ERCC1 to cleave flaps created during noncrossover repair of DSBs [de Laat et al., 1998]. Alternatively, or in addition, BTBD12, in combination with its nuclease partners, might create the incisions required for DSB formation or unhooking early in ICL repair. Interestingly, mammalian BTBD12, but not fly MUS312 or yeast Slx4, possesses two UBZ4 domains that may interact with ubiquitylated proteins [Fekairi et al., 2009]. Thus, one can imagine a scenario in which BTBD12 recruits both structure-specific nucleases and monoubiquitylated FANCD2/FANCI to sites of ongoing ICL repair, bringing several components needed for efficient processing of crosslinks together in one complex.

A XENOPUS EXTRACT SYSTEM PROVIDES MECHANISTIC INSIGHT INTO REPLICATION-DEPENDENT ICL REPAIR

Many of the models for ICL repair described thus far are based largely on genetic data. Furthermore, studies performed using mammalian cell extracts are not specific to S-phase [Li et al., 1999; Mu et al., 2000; Bessho, 2003], which is when the majority of crosslinks are likely repaired. To address these caveats, two independent groups recently published a cell-free system for ICL repair that used extracts from *Xenopus laevis* eggs [Raschle et al., 2008; Ben-Yehoyada et al., 2009]. In a study from Johannes Walter's lab, plasmids with single, helix-distorting nitrogen mustard ICLs were used, whereas plasmids containing mitomycin C-like ICLs were used by Jean Gautier and coworkers. The plasmids were incubated in egg extracts under conditions where a single round of DNA replication could occur. In both systems, full repair of about 15% of the ICLs was observed. Interestingly, Raschle et al. found that repair was entirely dependent on DNA replication, whereas Ben-Yehoyada et al. observed a substantial contribution of replication-independent repair. Although this discrepancy might be explained by the fact that mitomycin C-like ICLs distort the DNA helix to a much greater extent than nitrogen mustard ICLs, the different results obtained using the two systems beg further experimentation.

One strength of the Xenopus system is the amount of mechanistic detail that it provides. Using electron microscopy, Raschle et al. demonstrated that replication proceeds bidirectionally around the plasmid until two

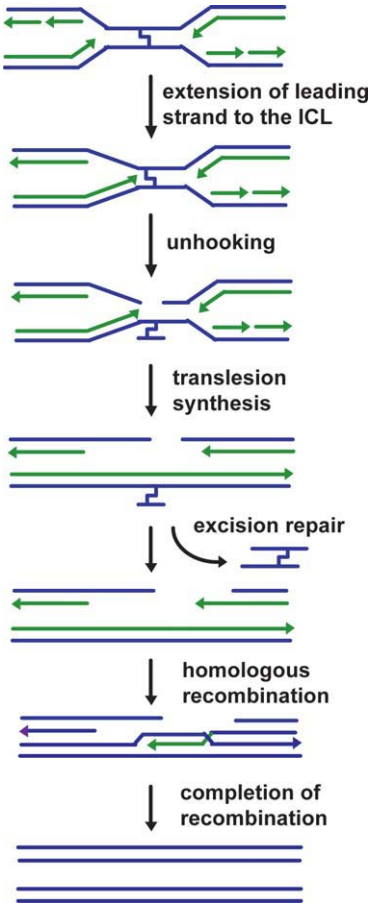

Fig. 4. A model for ICL repair involving stalling of two replication forks at a crosslink. Both forks initially stall 20–40 nucleotides from the lesion, and one leading strand is eventually extended to within several nucleotides of the ICL. Following unhooking of the ICL, translesion synthesis proceeds using the nascent leading strand as a template. The resulting two-ended double-strand break is most likely repaired through homologous recombination from the intact sister chromatid. At some point during repair, nucleotide excision repair proteins may remove the crosslinked oligonucleotide. The model is based on data published in Raschle et al. [2008]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

replication forks converge on the crosslink (Fig. 4). Detailed analysis of reaction intermediates revealed that the leading strands of each fork stall ~20–40 nucleotides away from the ICL for a period of time, after which one of the leading strands is extended to within a couple of nucleotides from the crosslink. After another delay, unhooking of the crosslink releases the two parental DNA strands, and translesion synthesis across from the crosslink occurs. Immunodepletion of REV7 [Raschle et al., 2008] and sequencing of cloned repair products [Ben-Yehoyada et al., 2009] were used to demonstrate that polymerase zeta is likely involved in the translesion synthesis. Presumably, HR is also required for the completion of repair, but this was not addressed in either of these studies. Recently, Walter's group further used its system to show that ubiquitylated FANCD2-FANCI is required for two of the early steps of replication-dependent ICL repair [Knipscheer et al., 2009]. In the absence of these proteins, nucleolytic incisions near the ICL and translesion DNA synthesis across the lesion did not occur, suggesting that one function of the FA network of proteins is to promote multiple enzymatic reactions during ICL repair that occurs in S phase.

One of the most unexpected findings to emerge from the Xenopus studies is that translesion synthesis can proceed using a nascent DNA strand as a template, instead of a parental strand as postulated in many previous models [Raschle et al., 2008]. As a consequence, ICL unhooking need not occur before the initiation of translesion synthesis, meaning that the polymerases required to extend past the crosslink site can be recruited before the separation of the two parental strands. Therefore, current models of replication-dependent ICL repair will need to be adjusted to accommodate these new findings.

STUDIES WITH DNA DAMAGE EXTREMOPHILES: THE SLIME MOLD ENTERS THE FRAY

One recent example that illustrates the power of using new model systems to study ICL repair involves the social amoeba, *Dictyostelium discoideum*. Dictyostelium is a soil-dwelling extremeophile that is likely exposed to high concentrations of a number of agents that induce ICLs. Like the bacterium *Deinococcus radiodurans*, it is resistant to high doses of ionizing radiation and ICL-inducing agents [Zhang et al., 2009]. Quite surprisingly, Dictyostelium with mutations in genes orthologous to either FANCE or REV3 are only mildly sensitive to cisplatin (Table 1), whereas mutation of XPF causes severe sensitivity [Zhang et al., 2009]. Therefore, it appears that this organism has evolved an excision nuclease-based mechanism of repairing ICLs that is highly dependent on XPF, but not NER or translesion synthesis.

Because mice with mutations in XPF are also extremely sensitive to cisplatin [Tian et al., 2004], Dictyostelium may provide an excellent opportunity to investigate the function(s) of XPF/ERCC1 in ICL repair and its potential roles in other repair pathways. Furthermore, studies with this organism may provide clues about the mechanisms by which human cancers develop cisplatin resistance [Olaussen et al., 2007].

CONCLUSIONS

Clearly, the use of model organisms to study the various DNA repair pathways and proteins involved in ICL repair has proven quite fruitful. However, many basic questions still remain. Exactly how do the FA genes interface with proteins from other DNA repair pathways to

modulate ICL repair? What is the function of the SNM1 nuclease and what ICL intermediate(s) does it recognize? How does translesion polymerase theta promote resistance to crosslinking agents? Do different translesion polymerases act during the repair of specific crosslink adducts or does substantial functional redundancy exist?

Model metazoans such as *C. elegans* and Drosophila, with their extensive sets of molecular and genetic tools, will likely continue to be front-line participants in studies designed to address these questions. In addition, complementary ''in vivo'' biochemical approaches, such as those performed using the Xenopus egg extract system, can be used both to confirm and refute various aspects of current ICL repair models. Finally, investigations using organisms with radically different habitats and life histories, such as Dictyostelium and other extremophiles, may allow the elucidation of additional novel approaches to ICL repair. As more genomes continue to be sequenced and other model systems are developed, our understanding of the number and variety of approaches used by different organisms to repair ICLs will deepen. Moreover, in the context of medicine, studies using model organisms may translate into an increased understanding of the multiple ways in which cancer cells can evolve resistance to ICL-inducing drugs, thereby allowing the development of more effective approaches to chemotherapy.

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REFERENCES

Ahmed S, Hodgkin J. 2000. MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*. Nature 403:159–164.

Akkari YM, Bateman RL, Reifsteck CA, Olson SB, Grompe M. 2000. DNA replication is required to elicit cellular responses to psoralen-induced DNA interstrand cross-links. Mol Cell Biol 20:8283–8289.

Andersen SL, Bergstralh DT, Kohl KP, LaRocque JR, Moore CB, Sekelsky J. 2009. Drosophila MUS312 and the vertebrate ortholog BTBD12 interact with DNA structure-specific endonucleases in DNA repair and recombination. Mol Cell 35:128–135.

Aslett M, Wood V. 2006. Gene ontology annotation status of the fission yeast genome: Preliminary coverage approaches 100%. Yeast 23:913–919.

Barre FX, Asseline U, Harel-Bellan A. 1999a. Asymmetric recognition of psoralen interstrand crosslinks by the nucleotide excision repair and the error-prone repair pathways. J Mol Biol 286:1379–1387.

Barre FX, Giovannangeli C, Helene C, Harel-Bellan A. 1999b. Covalent crosslinks introduced via a triple helix-forming oligonucleotide coupled to psoralen are inefficiently repaired. Nucleic Acids Res 27:743–749.

Beljanski V, Marzilli LG, Doetsch PW. 2004. DNA damage-processing pathways involved in the eukaryotic cellular response to anticancer DNA cross-linking drugs. Mol Pharmacol 65:1496–1506.

Ben-Yehoyada M, Wang LC, Kozekov ID, Rizzo CJ, Gottesman ME, Gautier J. 2009. Checkpoint signaling from a single DNA interstrand crosslink. Mol Cell 35:704–715.

Berardini M, Foster PL, Loechler EL. 1999. DNA polymerase II (polB) is involved in a new DNA repair pathway for DNA interstrand cross-links in *Escherichia coli*. J Bacteriol 181:2878–2882.

Bergstralh DT, Sekelsky J. 2008. Interstrand crosslink repair: Can XPF-ERCC1 be let off the hook? Trends Genet 24:70–76.

Bessho T. 2003. Induction of DNA replication-mediated double strand breaks by psoralen DNA interstrand cross-links. J Biol Chem 278:5250–5254.

Boddy MN, Lopez-Girona A, Shanahan P, Interthal H, Heyer WD, Russell P. 2000. Damage tolerance protein Mus81 associates with the FHA1 domain of checkpoint kinase Cds1. Mol Cell Biol 20:8758–8766.

Boyd JB, Golino MD, Nguyen TD, Green MM. 1976. Isolation and characterization of *X*-linked mutants of *Drosophila melanogaster* which are sensitive to mutagens. Genetics 84:485–506.

Boyd JB, Golino MD, Shaw KES, Osgood CJ, Green MM. 1981. Third-chromosome mutagen-sensitive mutants of *Drosophila melanogaster*. Genetics 97:607–623.

Boyd JB, Sakaguchi K, Harris PV. 1990. mus308 mutants of Drosophila exhibit hypersensitivity to DNA cross-linking agents and are defective in a deoxyribonuclease. Genetics 125:813–819.

Brendel M, Bonatto D, Strauss M, Revers LF, Pungartnik C, Saffi J, Henriques JA. 2003. Role of PSO genes in repair of DNA damage of *Saccharomyces cerevisiae*. Mutat Res 544:179–193.

Brough R, Wei D, Leulier S, Lord CJ, Rong YS, Ashworth A. 2008. Functional analysis of *Drosophila melanogaster* BRCA2 in DNA repair. DNA Repair (Amst) 7:10–19.

Cantor SB, Andreassen PR. 2006. Assessing the link between BACH1 and BRCA1 in the FA pathway. Cell Cycle 5:164–167.

Carr AM, Schmidt H, Kirchhoff S, Muriel WJ, Sheldrick KS, Griffiths DJ, Basmacioglu CN, Subramani S, Clegg M, Nasim A, Lehmann AR. 1994. The rad16 gene of *Schizosaccharomyces pombe*: A homolog of the RAD1 gene of *Saccharomyces cerevisiae*. Mol Cell Biol 14:2029–2040.

Cassier C, Moustacchi E. 1981. Mutagen induced by mono- and bi-functional alkylating agents in yeast mutants sensitive to photo-addition of furocoumarins (pso). Mutat Res 84:37–47.

Cassier-Chauvat C, Moustacchi E. 1988. Allelism between pso1–1 and rev3–1 mutants and between pso2–1 and snm1 mutants in *Saccharomyces cerevisiae*. Curr Genet 13:37–40.

Chanet R, Cassier C, Moustacchi E. 1985. Genetic control of the bypass of mono-adducts and of the repair of cross-links photoinduced by 8-methoxypsoralen in yeast. Mutat Res 145:145–155.

Cheng S, Sancar A, Hearst JE. 1991. RecA-dependent incision of psoralen-crosslinked DNA by (A)BC excinuclease. Nucleic Acids Res 19:657–663.

Clingen PH, De Silva IU, McHugh PJ, Ghadessy FJ, Tilby MJ, Thurston DE, Hartley JA. 2005. The XPF-ERCC1 endonuclease and homologous recombination contribute to the repair of minor groove DNA interstrand crosslinks in mammalian cells produced by the pyrrolo[2,1-c][1,4]benzodiazepine dimer SJG-136. Nucleic Acids Res 33:3283–3291.

Cole RS. 1973. Repair of DNA containing interstrand crosslinks in *Escherichia coli*: Sequential excision and recombination. Proc Natl Acad Sci USA 70:1064–1068.

Collis SJ, Barber LJ, Ward JD, Martin JS, Boulton SJ. 2006. *C. elegans* FANCD2 responds to replication stress and functions in interstrand cross-link repair. DNA Repair (Amst) 5:1398–1406.

Coulon S, Gaillard PH, Chahwan C, McDonald WH, Yates JR III, Russell P. 2004. Slx1-Slx4 are subunits of a structure-specific endonuclease that maintains ribosomal DNA in fission yeast. Mol Biol Cell 15:71–80.

Dardalhon M, Averbeck D. 1995. Pulsed-field gel electrophoresis analysis of the repair of psoralen plus UVA induced DNA photoadducts in *Saccharomyces cerevisiae*. Mutat Res 336:49–60.

Davis AP, Symington LS. 2004. RAD51-dependent break-induced replication in yeast. Mol Cell Biol 24:2344–2351.

de Laat WL, Appeldoorn E, Jaspers NG, Hoeijmakers JH. 1998. DNA structural elements required for ERCC1-XPF endonuclease activity. J Biol Chem 273:7835–7842.

De Silva IU, McHugh PJ, Clingen PH, Hartley JA. 2000. Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. Mol Cell Biol 20:7980–7990.

Dronkert ML, Kanaar R. 2001. Repair of DNA interstrand cross-links. Mutat Res 486:217–247.

Eeken JC, Romeijn RJ, de Jong AW, Pastink A, Lohman PH. 2001. Isolation and genetic characterisation of the Drosophila homologue of (SCE)REV3, encoding the catalytic subunit of DNA polymerase zeta. Mutat Res 485:237–253.

Fekairi S, Scaglione S, Chahwan C, Taylor ER, Tissier A, Coulon S, Dong MQ, Ruse C, Yates JR III, Russell P, Fuchs RP, McGowan CH, Gaillard PH. 2009. Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. Cell 138:78–89.

Fricke WM, Brill SJ. 2003. Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes Dev 17:1768–1778.

Garg P, Burgers PM. 2005. Ubiquitinated proliferating cell nuclear antigen activates translesion DNA polymerases eta and REV1. Proc Natl Acad Sci USA 102:18361–18366.

Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. 2008. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. Mol Cell 29:141–148.

Goff JP, Shields DS, Seki M, Choi S, Epperly MW, Dixon T, Wang H, Bakkenist CJ, Dertinger SD, Torous DK, Wittschieben J, Wood RD, Greenberger JS. 2009. Lack of DNA polymerase theta (POLQ) radiosensitizes bone marrow stromal cells in vitro and increases reticulocyte micronuclei after total-body irradiation. Radiat Res 172:165–174.

Grossmann KF, Ward AM, Matkovic ME, Folias AE, Moses RE. 2001. *S. cerevisiae* has three pathways for DNA interstrand crosslink repair. Mutat Res 487:73–83.

Hejna J, Philip S, Ott J, Faulkner C, Moses R. 2007. The hSNM1 protein is a DNA 5′-exonuclease. Nucleic Acids Res 35:6115–6123.

Henderson DS, Bailey DA, Sinclair DA, Grigliatti TA. 1987. Isolation and characterization of second chromosome mutagen-sensitive mutations in *Drosophila melanogaster*. Mutat Res 177:83–93.

Henriques JA, Moustacchi E. 1980. Isolation and characterization of pso mutants sensitive to photo-addition of psoralen derivatives in *Saccharomyces cerevisiae*. Genetics 95:273–288.

Henriques JA, Moustacchi E. 1981. Interactions between mutations for sensitivity to psoralen photoaddition (pso) and to radiation (rad) in *Saccharomyces cerevisiae*. J Bacteriol 148:248–256.

Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD. 2002. Biallelic inactivation of BRCA2 in Fanconi anemia. Science 297:606–609.

Hsu DW, Gaudet P, Hudson JJ, Pears CJ, Lakin ND. 2006. DNA damage signaling and repair in *Dictyostelium discoideum*. Cell Cycle 5:702–708.

Hussain S, Witt E, Huber PA, Medhurst AL, Ashworth A, Mathew CG. 2003. Direct interaction of the Fanconi anaemia protein FANCG with BRCA2/FANCD1. Hum Mol Genet 12:2503–2510.

Jachymczyk WJ, von Borstel RC, Mowat MR, Hastings PJ. 1981. Repair of interstrand cross-links in DNA of *Saccharomyces cerevisiae* requires two systems for DNA repair: The RAD3 system and the RAD51 system. Mol Gen Genet 182:196–205.

Jonnalagadda VS, Matsuguchi T, Engelward BP. 2005. Interstrand cross-link-induced homologous recombination carries an increased risk of deletions and insertions. DNA Repair (Amst) 4:594–605.

Kai M, Wang TS. 2003. Checkpoint activation regulates mutagenic translesion synthesis. Genes Dev 17:64–76.

Klovstad M, Abdu U, Schupbach T. 2008. Drosophila brca2 is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. PLoS Genet 4:e31.

Knipscheer P, Raschle M, Smogorzewska A, Enoiu M, Ho TV, Scharer OD, Elledge SJ, Walter JC. 2009. The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. Science 326:1698–1701.

Knox RJ, Lydall DA, Friedlos F, Basham C, Rawlings CJ, Roberts JJ. 1991. The Walker 256 carcinoma: A cell type inherently sensitive only to those difunctional agents that can form DNA interstrand crosslinks. Mutat Res 255:227–240.

Lambert S, Mason SJ, Barber LJ, Hartley JA, Pearce JA, Carr AM, McHugh PJ. 2003. *Schizosaccharomyces pombe* checkpoint response to DNA interstrand cross-links. Mol Cell Biol 23:4728–4737.

Larimer FW, Perry JR, Hardigree AA. 1989. The REV1 gene of *Saccharomyces cerevisiae*: Isolation, sequence, and functional analysis. J Bacteriol 171:230–237.

Laurencon A, Orme CM, Peters HK, Boulton CL, Vladar EK, Langley SA, Bakis EP, Harris DT, Harris NJ, Wayson SM, Hawley RS, Burtis KC. 2004. A large-scale screen for mutagen-sensitive loci in Drosophila. Genetics 167:217–231.

Lawley PD, Brookes P. 1968. Cytotoxicity of alkylating agents towards sensitive and resistant strains of *Escherichia coli* in relation to extent and mode of alkylation of cellular macromolecules and repair of alkylation lesions in deoxyribonucleic acids. Biochem J 109:433–447.

Lawley PD, Phillips DH. 1996. DNA adducts from chemotherapeutic agents. Mutat Res 355:13–40.

Lee KY, Myung K. 2008. PCNA modifications for regulation of post-replication repair pathways. Mol Cells 26:5–11.

Lehoczky P, McHugh PJ, Chovanec M. 2007. DNA interstrand cross-link repair in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 31:109–133.

Leonhardt EA, Boyd JB. 1993. Identification of a new locus, mus115, in *Drosophila melanogaster*. Mutat Res 301:121–124.

Levitus M, Waisfisz Q, Godthelp BC, de Vries Y, Hussain S, Wiegant WW, Elghalbzouri-Maghrani E, Steltenpool J, Rooimans MA, Pals G, Arwert F, Mathew CG, Zdzienicka MZ, Hiom k, de Winter JP, Joenje H. 2005. The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. Nat Genet 37:934–935.

Li X, Moses RE. 2003. The beta-lactamase motif in Snm1 is required for repair of DNA double-strand breaks caused by interstrand crosslinks in *S. cerevisiae*. DNA Repair (Amst) 2:121–129.

Li L, Peterson CA, Lu X, Wei P, Legerski RJ. 1999. Interstrand cross-links induce DNA synthesis in damaged and undamaged plasmids in mammalian cell extracts. Mol Cell Biol 19:5619–5630.

Li X, Hejna J, Moses RE. 2005. The yeast Snm1 protein is a DNA 5′-exonuclease. DNA Repair (Amst) 4:163–170.

Liao C, Hu B, Arno MJ, Panaretou B. 2007. Genomic screening in vivo reveals the role played by vacuolar H+ ATPase and cytosolic acidification in sensitivity to DNA-damaging agents such as cisplatin. Mol Pharmacol 71:416–425.

Lo T, Pellegrini L, Venkitaraman AR, Blundell TL. 2003. Sequence fingerprints in BRCA2 and RAD51: Implications for DNA repair and cancer. DNA Repair (Amst) 2:1015–1028.

Ma Y, Pannicke U, Schwarz K, Lieber MR. 2002. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell 108:781–794.

Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D'Andrea AD, Dutta A. 2006. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. Mol Cell 23:589–596.

Magana-Schwencke N, Henriques JA, Chanet R, Moustacchi E. 1982. The fate of 8-methoxypsoralen photoinduced crosslinks in nuclear and mitochondrial yeast DNA: Comparison of wild-type and repair-deficient strains. Proc Natl Acad Sci USA 79:1722–1726.

Manderfeld MM, Schafer HW, Davidson PM, Zottola EA. 1997. Isolation and identification of antimicrobial furocoumarins from parsley. J Food Prot 60:72–77.

Marek LR, Bale AE. 2006. Drosophila homologs of FANCD2 and FANCL function in DNA repair. DNA Repair (Amst) 5:1317–1326.

Martin JS, Winkelmann N, Petalcorin MI, McIlwraith MJ, Boulton SJ. 2005. RAD-51-dependent and -independent roles of a *Caenorhabditis elegans* BRCA2-related protein during DNA double-strand break repair. Mol Cell Biol 25:3127–3139.

Mason JM, Green MM, Shaw KES, Boyd JB. 1981. Genetic analysis of X-linked mutagen-sensitive mutants of *Drosophila melanogaster*. Mutat Res 81:329–343.

McCaffrey R, St Johnston D, Gonzalez-Reyes A. 2006. Drosophila mus301/spindle-C encodes a helicase with an essential role in double-strand DNA break repair and meiotic progression. Genetics 174:1273–1285.

McDonald JP, Levine AS, Woodgate R. 1997. The *Saccharomyces cerevisiae* RAD30 gene, a homologue of *Escherichia coli* dinB and umuC, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism. Genetics 147:1557–1568.

McHugh PJ, Sones WR, Hartley JA. 2000. Repair of intermediate structures produced at DNA interstrand cross-links in *Saccharomyces cerevisiae*. Mol Cell Biol 20:3425–3433.

Meetei AR, Yan Z, Wang W. 2004. FANCL replaces BRCA1 as the likely ubiquitin ligase responsible for FANCD2 monoubiquitination. Cell Cycle 3:179–181.

Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, Bier P, Steltenpool J, Stone S, Dokal I, Mathew CG, Hoatlin M, Joenje H, de Winter JP, Wang W. 2005. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. Nat Genet 37:958–963.

Meier B, Barber LJ, Liu Y, Shtessel L, Boulton SJ, Gartner A, Ahmed S. 2009. The MRT-1 nuclease is required for DNA crosslink repair and telomerase activity in vivo in *Caenorhabditis elegans*. EMBO J 28:3549–3563.

Miller RD, Prakash L, Prakash S. 1982. Genetic control of excision of *Saccharomyces cerevisiae* interstrand DNA cross-links induced by psoralen plus near-UV light. Mol Cell Biol 2:939–948.

Minko IG, Harbut MB, Kozekov ID, Kozekova A, Jakobs PM, Olson SB, Moses RE, Harris TM, Rizzo CJ, Lloyd RS. 2008. Role for DNA polymerase kappa in the processing of N2-N2-guanine interstrand cross-links. J Biol Chem 283:17075–17082.

Misra RR, Vos JM. 1993. Defective replication of psoralen adducts detected at the gene-specific level in xeroderma pigmentosum variant cells. Mol Cell Biol 13:1002–1012.

Moldovan GL, D'Andrea AD. 2009. How the Fanconi anemia pathway guards the genome. Annu Rev Genet 43:223–49.

Mu D, Bessho T, Nechev LV, Chen DJ, Harris TM, Hearst JE, Sancar A. 2000. DNA interstrand cross-links induce futile repair synthesis in mammalian cell extracts. Mol Cell Biol 20:2446–2454.

Munoz IM, Hain K, Declais AC, Gardiner M, Toh GW, Sanchez-Pulido L, Heuckmann JM, Toth R, Macartney T, Eppink B, Kanaar R, Ponting CP, Lilley DM, Rouse J. 2009. Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. Mol Cell 35:116–127.

Muzzini DM, Plevani P, Boulton SJ, Cassata G, Marini F. 2008. *Caenorhabditis elegans* POLQ-1 and HEL-308 function in two distinct DNA interstrand cross-link repair pathways. DNA Repair (Amst) 7:941–950.

Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. 2003. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem 278:31426–31433.

Niedernhofer LJ, Odijk H, Budzowska M, van Drunen E, Maas A, Theil AF, de Wit J, Jaspers NG, Beverloo HB, Hoeijmakers JH, Kanaar R. 2004. The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks. Mol Cell Biol 24:5776–5787.

Ohkumo T, Masutani C, Eki T, Hanaoka F. 2006. Deficiency of the *Caenorhabditis elegans* DNA polymerase eta homologue increases sensitivity to UV radiation during germ-line development. Cell Struct Funct 31:29–37.

Olaussen KA, Mountzios G, Soria JC. 2007. ERCC1 as a risk stratifier in platinum-based chemotherapy for nonsmall-cell lung cancer. Curr Opin Pulm Med 13:284–289.

Oliveri DR, Harris PV, Boyd JB. 1990. X-ray sensitivity and single-strand DNA break repair in mutagen-sensitive mutants of *Drosophila melanogaster*. Mutat Res 235:25–31.

Pang Q, Andreassen PR. 2009. Fanconi anemia proteins and endogenous stresses. Mutat Res 668:42–53.

Pang M, McConnell M, Fisher PA. 2005. The Drosophila mus 308 gene product, implicated in tolerance of DNA interstrand crosslinks, is a nuclear protein found in both ovaries and embryos. DNA Repair (Amst) 4:971–982.

Peng M, Litman R, Xie J, Sharma S, Brosh RM Jr, Cantor SB. 2007. The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. EMBO J 26:3238–3249.

Petalcorin MI, Sandall J, Wigley DB, Boulton SJ. 2006. CeBRC-2 stimulates D-loop formation by RAD-51 and promotes DNA single-strand annealing. J Mol Biol 361:231–242.

Pothof J, van Haaften G, Thijssen K, Kamath RS, Fraser AG, Ahringer J, Plasterk RH, Tijsterman M. 2003. Identification of genes that protect the *C. elegans* genome against mutations by genome-wide RNAi. Genes Dev 17:443–448.

Radford SJ, Goley E, Baxter K, McMahan S, Sekelsky J. 2005. Drosophila ERCC1 is required for a subset of MEI-9-dependent meiotic crossovers. Genetics 170:1737–1745.

Raschle M, Knipscheer P, Enoiu M, Angelov T, Sun J, Griffith JD, Ellenberger TE, Scharer OD, Walter JC. 2008. Mechanism of replication-coupled DNA interstrand crosslink repair. Cell 134:969–980.

Rego MA, Kolling FWt, Howlett NG. 2009. The Fanconi anemia protein interaction network: Casting a wide net. Mutat Res 668: 27–41.

Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, Neveling K, Kelly P, Seal S, Freund M, Wurm M, Batish SD, Lach FP, Yetgin S, Neitzel H, Ariffin H, Tischkowitz M, Mathew CG, Auerbach AD, Rahman N. 2007. Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat Genet 39:162–164.

Richards S, Liu ST, Majumdar A, Liu JL, Nairn RS, Bernier M, Maher V, Seidman MM. 2005. Triplex targeted genomic crosslinks enter separable deletion and base substitution pathways. Nucleic Acids Res 33:5382–5393.

Ruhland A, Haase E, Siede W, Brendel M. 1981. Isolation of yeast mutants sensitive to the bifunctional alkylating agent nitrogen mustard. Mol Gen Genet 181:346–351.

Sarkar S, Davies AA, Ulrich HD, McHugh PJ. 2006. DNA interstrand crosslink repair during G1 involves nucleotide excision repair and DNA polymerase zeta. EMBO J 25:1285–1294.

Scharer OD. 2005. DNA interstrand crosslinks: Natural and drug-induced DNA adducts that induce unique cellular responses. Chembiochem 6:27–32.

Scheller J, Schurer A, Rudolph C, Hettwer S, Kramer W. 2000. MPH1, a yeast gene encoding a DEAH protein, plays a role in protection of the genome from spontaneous and chemically induced damage. Genetics 155:1069–1081.

Scott BR, Pathak MA, Mohn GR. 1976. Molecular and genetic basis of furocoumarin reactions. Mutat Res 39:29–74.

Scully R, Xie A, Nagaraju G. 2004. Molecular functions of BRCA1 in the DNA damage response. Cancer Biol Ther 3:521–527.

Seki M, Marini F, Wood RD. 2003. POLQ (Pol theta), a DNA polymerase and DNA-dependent ATPase in human cells. Nucleic Acids Res 31:6117–6126.

Shen X, Jun S, O'Neal LE, Sonoda E, Bemark M, Sale JE, Li L. 2006. REV3 and REV1 play major roles in recombination-independent repair of DNA interstrand cross-links mediated by monoubiquitinated proliferating cell nuclear antigen (PCNA). J Biol Chem 281:13869–13872.

Shima N, Hartford SA, Duffy T, Wilson LA, Schimenti KJ, Schimenti JC. 2003. Phenotype-based identification of mouse chromosome instability mutants. Genetics 163:1031–1040.

Shima N, Munroe RJ, Schimenti JC. 2004. The mouse genomic instability mutation chaos1 is an allele of Polq that exhibits genetic interaction with Atm. Mol Cell Biol 24:10381–10389.

Sims AE, Spiteri E, Sims RJ III, Arita AG, Lach FP, Landers T, Wurm M, Freund M, Neveling K, Hanenberg H, Auerbach AD, Huang TT. 2007. FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. Nat Struct Mol Biol 14: 564–567.

Sladek FM, Munn MM, Rupp WD, Howard-Flanders P. 1989. In vitro repair of psoralen-DNA cross-links by RecA, UvrABC, and the 5′-exonuclease of DNA polymerase I. J Biol Chem 264:6755–6765.

Smogorzewska A, Matsuoka S, Vinciguerra P, McDonald ER III, Hurov KE, Luo J, Ballif BA, Gygi SP, Hofmann K, D'Andrea AD, Elledge SJ. 2007. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. Cell 129:289–301.

Snyder RD, Smith PD. 1982. Mutagen sensitivity of *Drosophila melanogaster*. V. Identification of second chromosomal mutagen sensitive strains. Mol Gen Genet 188:249–255.

Sun W, Nandi S, Osman F, Ahn JS, Jakovleska J, Lorenz A, Whitby MC. 2008. The FANCM ortholog Fml1 promotes recombination at stalled replication forks and limits crossing over during DNA double-strand break repair. Mol Cell 32:118–128.

Svendsen JM, Smogorzewska A, Sowa ME, O'Connell BC, Gygi SP, Elledge SJ, Harper JW. 2009. Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. Cell 138:63–77.

Takeuchi R, Oshige M, Uchida M, Ishikawa G, Takata K, Shimanouchi K, Kanai Y, Ruike T, Morioka H, Sakaguchi K. 2004. Purification of Drosophila DNA polymerase zeta by REV1 protein-affinity chromatography. Biochem J 382 (Part 2):535–543.

Tanaka K, Yonekawa T, Kawasaki Y, Kai M, Furuya K, Iwasaki M, Murakami H, Yanagida M, Okayama H. 2000. Fission yeast Eso1p is required for establishing sister chromatid cohesion during S phase. Mol Cell Biol 20:3459–3469.

Thompson LH, Hinz JM. 2009. Cellular and molecular consequences of defective Fanconi anemia proteins in replication-coupled DNA repair: Mechanistic insights. Mutat Res 668:54–72.

Tian M, Shinkura R, Shinkura N, Alt FW. 2004. Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. Mol Cell Biol 24:1200–1205.

Tischkowitz MD, Hodgson SV. 2003. Fanconi anaemia. J Med Genet 40:1–10.

Trowbridge K, McKim K, Brill SJ, Sekelsky J. 2007. Synthetic lethality of Drosophila in the absence of the MUS81 endonuclease and the DmBlm helicase is associated with elevated apoptosis. Genetics 176:1993–2001.

Van Houten B, Gamper H, Holbrook SR, Hearst JE, Sancar A. 1986. Action mechanism of ABC excision nuclease on a DNA substrate containing a psoralen crosslink at a defined position. Proc Natl Acad Sci USA 83:8077–8081.

Wang W. 2007. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet 8:735–748.

Wang X, Peterson CA, Zheng H, Nairn RS, Legerski RJ, Li L. 2001. Involvement of nucleotide excision repair in a recombination-independent and error-prone pathway of DNA interstrand cross-link repair. Mol Cell Biol 21:713–720.

Waters LS, Minesinger BK, Wiltrout ME, D'souza S, Woodruff RV, Walker GC. 2009. Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. Microbiol Mol Biol Rev 73:134–154.

Wu HI, Brown JA, Dorie MJ, Lazzeroni L, Brown JM. 2004. Genome-wide identification of genes conferring resistance to the anticancer agents cisplatin, oxaliplatin, and mitomycin C. Cancer Res 64:3940–3948.

Wu Q, Christensen LA, Legerski RJ, Vasquez KM. 2005. Mismatch repair participates in error-free processing of DNA interstrand crosslinks in human cells. EMBO Rep 6:551–557.

Wu Y, Shin-ya K, Brosh RM Jr. 2008. FANCJ helicase defective in Fanconia anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability. Mol Cell Biol 28:4116–4128.

Yildiz O, Majumder S, Kramer B, Sekelsky JJ. 2002. Drosophila MUS312 interacts with the nucleotide excision repair endonuclease MEI-9 to generate meiotic crossovers. Mol Cell 10:1503–1509.

Yildiz O, Kearney H, Kramer BC, Sekelsky JJ. 2004. Mutational analysis of the Drosophila repair and recombination gene *mei-9*. Genetics 167:263–273.

Youds JL, Barber LJ, Ward JD, Collis SJ, O'Neil NJ, Boulton SJ, Rose AM. 2008. DOG-1 is the *Caenorhabditis elegans* BRIP1/FANCJ homologue and functions in interstrand cross-link repair. Mol Cell Biol 28:1470–1479.

Youds JL, Barber LJ, Boulton SJ. 2009. *C. elegans*: A model of Fanconi anemia and ICL repair. Mutat Res 668:103–116.

Zhang XY, Langenick J, Traynor D, Babu MM, Kay RR, Patel KJ. 2009. Xpf and not the Fanconi anaemia proteins or Rev3 accounts for the extreme resistance to cisplatin in *Dictyostelium discoideum*. PLoS Genet 5:e1000645.

Zheng H, Wang X, Warren AJ, Legerski RJ, Nairn RS, Hamilton JW, Li L. 2003. Nucleotide excision repair- and polymerase eta-mediated error-prone removal of mitomycin C interstrand cross-links. Mol Cell Biol 23:754–761.