



Contents lists available at ScienceDirect



## DNA Repair

journal homepage: [www.elsevier.com/locate/dnarepair](http://www.elsevier.com/locate/dnarepair)

# Mechanism and regulation of incisions during DNA interstrand cross-link repair

Jieqiong Zhang<sup>a</sup>, Johannes C. Walter<sup>a,b,\*</sup>

<sup>a</sup> Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, United States

<sup>b</sup> Howard Hughes Medical Institute

## ARTICLE INFO

### Article history:

Available online xxx

### Keywords:

DNA interstrand crosslink repair  
XPF  
ERCC1  
MUS81  
EME1  
SLX1  
SLX4  
SNM1A  
FAN1  
Fanconi anemia  
FANCI  
FANCD2

## ABSTRACT

A critical step in DNA interstrand cross-link repair is the programmed collapse of replication forks that have stalled at an ICL. This event is regulated by the Fanconi anemia pathway, which suppresses bone marrow failure and cancer. In this perspective, we focus on the structure of forks that have stalled at ICLs, how these structures might be incised by endonucleases, and how incision is regulated by the Fanconi anemia pathway.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

DNA interstrand cross-links (ICLs) are cytotoxic lesions that covalently link the Watson and Crick strands of DNA. From a human health perspective, there are two primary motivations to study ICL repair. First, ICL repair is defective in Fanconi anemia (FA), a human genetic disease caused by biallelic mutations in any one of 16 different FANC genes [7,33,38]. FA is characterized by congenital abnormalities, bone marrow failure, and cancer predisposition. If ICL repair defects indeed cause FA, as is widely believed, understanding how ICL repair normally occurs and why it fails in patients might point the way to a cure for FA. Second, ICL-inducing agents are widely used in cancer chemotherapy. However, cancers almost invariably become resistant to these agents, in some cases due to up-regulation of repair. Novel inhibitors of ICL repair might augment the efficacy of ICL-inducing agents for chemotherapy, although this might also cause enhanced toxicity.

The major ICL repair pathway operating in proliferating cells is coupled to DNA replication [1,56,58,65]. When forks collide with an ICL, repair is initiated through the excision of the ICL from one

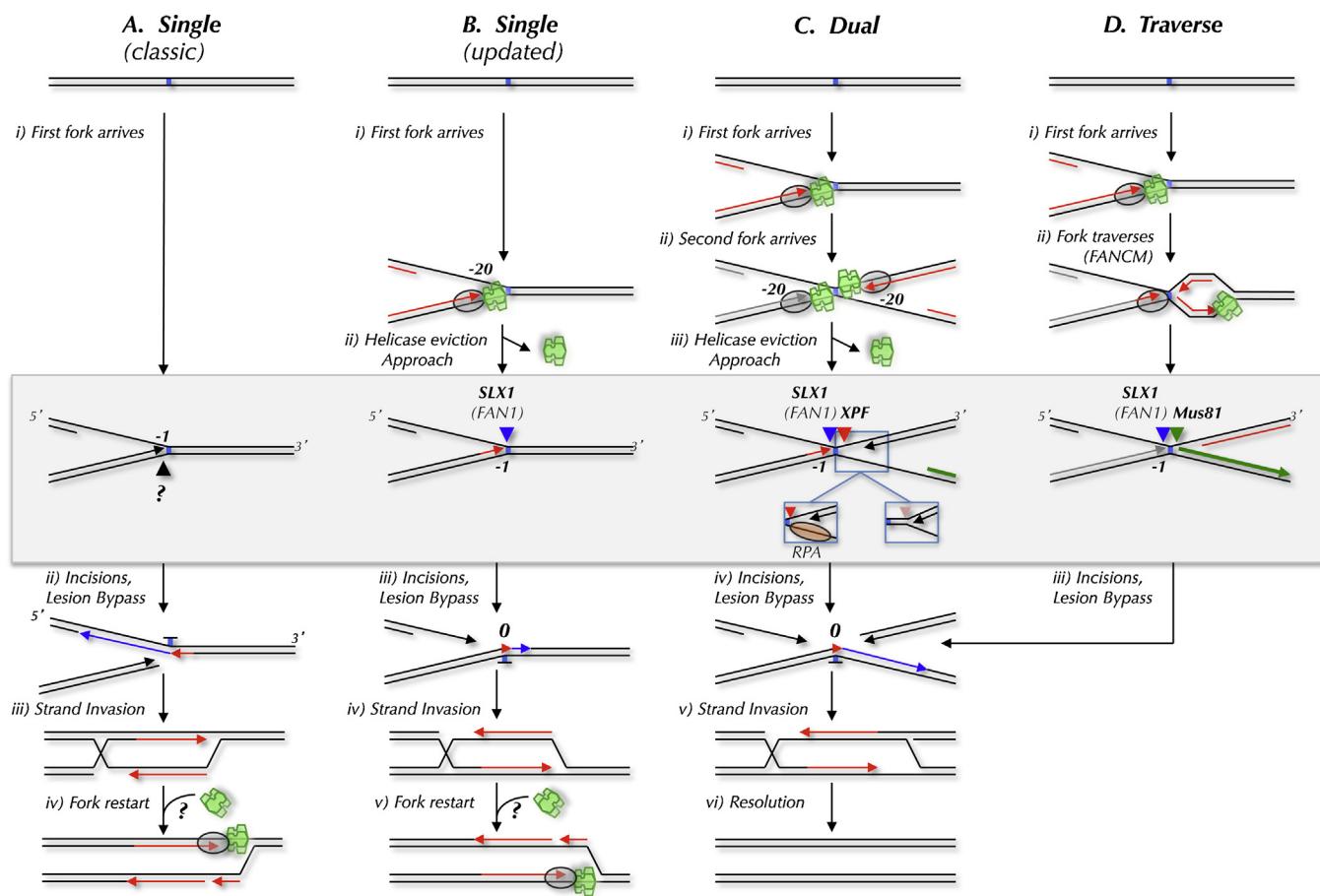
parental strand (Fig. 1A). This releases or “unhooks” one daughter duplex from the ICL, forming a double-stranded DNA break that must subsequently be repaired. ICL repair is thus a rare instance in which stalled replication forks undergo programmed collapse, and recent evidence suggests this process is dependent on the FANC proteins [37]. As such, programmed fork collapse can be regarded as a unique event that distinguishes ICL removal from other forms of DNA repair. To shed light on the mechanisms by which forks are processed during ICL repair, we consider here the possible structures of stalled forks prior to collapse and how diverse endonucleases might act on these structures. We also consider the regulation of fork collapse by the FANC proteins.

## 2. Early models of ICL repair

Genetic analysis has identified four major classes of gene products that confer resistance to ICLs. (1) Structure-specific endonucleases, which recognize and incise specific DNA structures. (2) Translesion DNA synthesis (TLS) polymerases, error prone polymerases that are able to tolerate DNA damage in the template strand. (3) DNA recombinases, proteins that mediate strand exchange during homologous recombination. (4) 16 FANC proteins, which are mutated in FA. In the FA “pathway,” eight “group I” FANC proteins assemble into a core complex that mono-ubiquitylates a

\* Corresponding author.

E-mail address: [johannes.walter@hms.harvard.edu](mailto:johannes.walter@hms.harvard.edu) (J.C. Walter).



**Fig. 1.** Possible mechanisms of replication-coupled ICL repair. Four mechanisms of replication-dependent ICL repair are depicted. The DNA structures acted on by endonucleases in each model are highlighted by a gray box. Incisions are represented by black, blue, red, and green arrowheads. The proposed nuclease(s) that performs each incision is indicated above the arrowhead. (A) The classic ICL repair model, in which a single replication fork collides with the ICL and the leading strand template is incised [50]. (B) The classic model, but taking into account the observation that leading strands initially stall 20 nucleotides from the ICL due to the MCM2-7 complex, and that incision occurs on the lagging strand template [56]. In models A and B, fork restart would require re-loading of the MCM2-7 complex, for which there is no known mechanism. (C) The dual fork convergence model [56]. Left inset, 3' incision substrate if RPA binds the lagging strand template after MCM2-7 removal. Right inset, 3' incision substrate if parental strands re-anneal after MCM2-7 removal. (D) Traverse model [29]. The only difference in the incision substrate in the dual fork and traverse models is the location of the 5' end of the nascent strand on the right side of the ICL (green strand).

heterodimer of two “group II” FANC proteins, FANCI and FANCD2 (the “ID” complex) [2,21,61]. The mono-ubiquitylated ID complex (ID-Ub) is essential for ICL repair [21,37]. The six remaining “group III” FANC proteins fall into the recombinase and nuclease categories. Given the four classes of proteins implicated in ICL repair and the coupling of repair to DNA replication, the following model crystallized several years ago [50,69]. Repair is triggered when a DNA replication fork collides with the ICL (Fig. 1Ai). This creates a substrate for structure-specific endonucleases, which incise the fork, unhooking the cross-link and generating a double-stranded DNA break (DSB) (Fig. 1Aii). The unhooked ICL is bypassed by translesion DNA polymerases (Fig. 1Aiii). Finally, the fork is restored via homologous recombination (Fig. 1Aiii). Although this model accounted for the different gene products implicated in ICL repair and the S phase dependence of repair, it lacked molecular detail. Thus, the precise nature of the DNA intermediates involved remained unclear, making it difficult to understand how the endonucleases and other proteins participate in repair. In addition, it was unknown how the FA pathway promotes repair.

### 3. The dual fork convergence model

More recently, replication-dependent ICL repair was recapitulated in *Xenopus* egg extracts, allowing a more detailed description

of repair intermediates [56]. When a 6 kb plasmid carrying a single, site-specific ICL is incubated in egg extract, a significant fraction of the lesions is repaired in a replication-dependent manner. Repair begins when two replication forks converge on the ICL (Fig. 1Ci and Cii). The 3' ends of both converged leading strands initially stall 20–40 nucleotides from the ICL due to steric hindrance by the MCM2-7 helicase, which translocates along the leading strand template ahead of the polymerase [20]. Upon collision with the ICL, the 5' ends of lagging strands are located 50–300 nucleotides from the lesion, and they subsequently undergo resection. Concurrent with MCM2-7 release from the ICL, one leading strand advances to within 1 nucleotide of the ICL (Fig. 1Ciii; “Approach”). After Approach, the opposing parental strand is incised on either side of the ICL, leading to unhooking of the ICL and formation of a DSB (Fig. 1Civ). In the absence of ID-Ub, incisions are severely impaired and the leading strand remains stuck 1 nucleotide from the lesion [37]. After incisions, the lesion is bypassed in two steps. First, a nucleotide is inserted across from the damaged base by an unknown translesion DNA polymerase (Fig. 1Civ, red arrowhead). The resulting abnormal primer template is then extended by DNA polymerase  $\zeta$  (Fig. 1Civ, blue arrow). Finally, the DSB is repaired via Rad51 dependent strand exchange with the intact sister chromatid [43]. In the *Xenopus* system, a vestige of the ICL remains attached to one parental strand. This observation implies either that the incisions occur very close together, or that the oligonucleotide between

the incisions is processed by a nuclease. Either way, the final adduct is not removed in egg extracts.

Certain features of the dual fork mechanism likely apply to the single fork model (Fig. 1B). Thus, when a single fork strikes an ICL, the leading strand probably also stalls 20 nucleotides from the lesion due to the MCM2-7 footprint (Fig. 1Bi), and incisions are likely to require eviction of the MCM2-7 complex (Fig. 1Bii). Whether incisions occur in the leading (Fig. 1Aiii) or lagging strand template (Fig. 1Biii) of a single stalled fork remains unclear. However we favor the latter scenario because this allows extension past the lesion using the existing replication apparatus (Fig. 1Biii). In addition, as discussed below, it is not obvious which endonuclease would cleave the leading strand template.

#### 4. Merits of the single versus dual fork collision models

Which of the two collision models applies in cells is subject to lively debate [39,45,56]. The single fork mechanism has at least one potential disadvantage. Unlike bacteria [26] and budding yeast [3], metazoans do not contain a known pathway for replicative DNA helicase loading in S phase. Therefore, the restored fork is unlikely to resume synthesis and would have to await the arrival of a converging fork, as seen after HU-induced replication fork collapse [54]. Such a helicase-deficient, stationary fork might be unstable and cause genomic rearrangements. The major criticism of the dual fork model is that it was observed in the context of a small plasmid where two forks inevitably converge on the ICL. *In vivo*, where origins are spaced 100 kb apart and forks move at a rate of 1.5 kb/min [16], most of the time one fork is expected to strike an ICL well before a converging fork arrives. Nevertheless, two forks will converge on an ICL when the lesion is located midway between two origins that fire contemporaneously. Moreover, given that fork processing requires MCM2-7 dissociation, leading strand approach to the ICL, and nuclease recruitment, which together takes at least 30–40 min [20,36,56], there will exist a significant temporal window during which a second fork can arrive before incisions take place. Thus, based on first principles, it seems likely that both single and dual fork collisions will occur at significant frequencies, necessitating pathways to resolve both structures.

#### 5. The traverse model

The single versus dual fork debate recently took an unexpected turn when Seidman and colleagues investigated the encounter of DNA replication forks with fluorescently tagged ICLs *in vivo* using DNA combing [29]. The work showed that single and dual fork collisions each comprise 15–20% of ICL encounters. Surprisingly, in ~60% of cases, DNA replication forks bypass or “traverse” ICLs without unhooking them (Fig. 1Dii). An earlier study inferred that forks generally stall at ICLs, but in this case, the location of ICLs relative to DNA replication tracts was not determined [67]. Traverse requires the translocase activity of FANCM but not other FANC proteins and is very rapid, taking only a few minutes. When FANCM is defective, the frequency of traverse events goes down while single fork collisions go up, indicating that single fork collisions are followed by traverse. It is unclear whether MCM2-7 jumps over the ICL during traverse, a new MCM2-7 molecule is recruited on the other side of the ICL (unlikely given the absence of known S phase MCM2-7 loading mechanisms), or a different DNA helicase loads distal to the ICL. Interestingly, FANCM has been shown to recruit RPA to an ICL in duplex DNA, suggesting it helps to melt DNA in the vicinity of these structures [30], which might facilitate traverse. In summary, it appears that the most common substrate for incisions during ICL repair is an X-shaped DNA molecule, which is generated by some combination of fork traverse and fork convergence events.

#### 6. Can a single fork trigger ICL repair?

The apparent preponderance of X-shaped structures at ICLs *in vivo* raises the important question of whether a single fork that has not traversed an ICL can trigger repair. Replication of a psoralen ICL in *Xenopus* egg extracts suggested that sometimes, a single fork can trigger incisions, but it was unclear whether the observed incisions led to a productive repair outcome [41]. In mammalian cells, repair of an ICL flanked by a replication roadblock suggested that a single fork can trigger repair [49]. However, the possibility of traverse was not considered and the efficiency of the roadblock was not determined, leaving open the possibility that an X-shaped structure was the substrate for repair. In the future, it will be critical to directly compare the efficiency of processing and repair of single stalled forks, converged forks, and traversed forks.

#### 7. What DNA structures are formed at ICL-stalled forks?

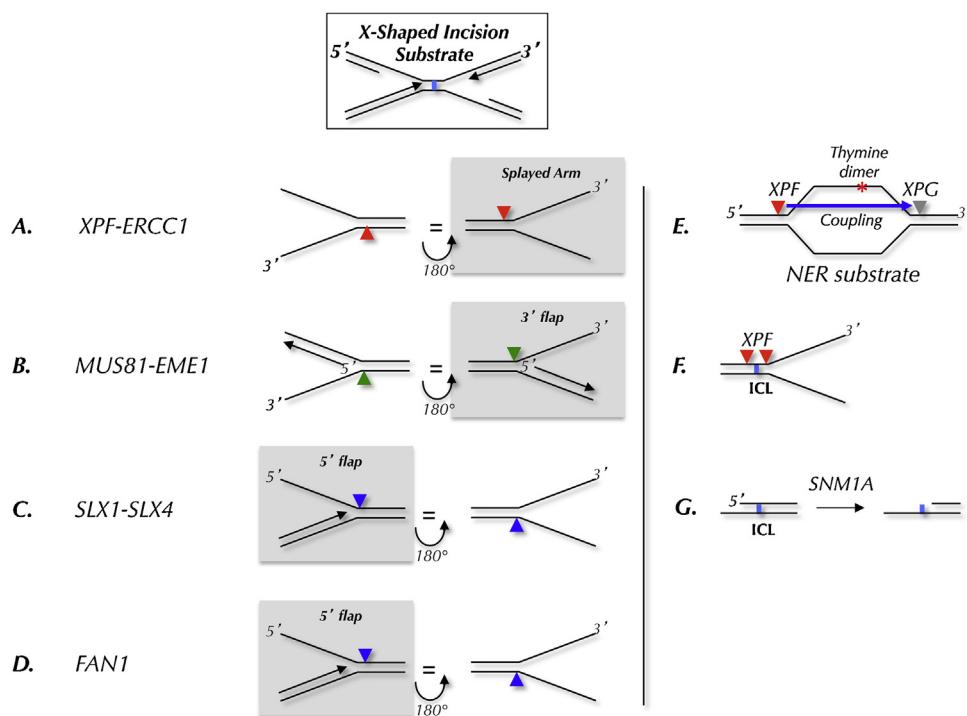
A detailed knowledge of the DNA structures created after forks encounter an ICL is critical to understand the mechanism of incisions. During repair of ICLs in egg extracts, incisions occur only after one leading strand has advanced to within 1 nucleotide of the ICL (−1 position)[56]. Thus, on the 5' side of the ICL (left side in Fig. 1C–D), nucleases act on a replication fork whose leading strand abuts the lesion. The structure on the 3' (right) side is less clear. After MCM2-7 eviction from converged forks [20], the ssDNA between the ICL and the 3' end of the leading strand (located 20–40 nucleotides from the ICL) might not re-anneal due to RPA binding (Fig. 1Ciii, left inset). Since RPA's preferred binding mode involves a 30 nucleotide footprint [70], it is likely to preferentially occupy the lagging strand template where more ssDNA is available. In this case, incisions by a 3' flap endonuclease would likely occur close to the ICL (Fig. 1Ciii, left inset, red arrowhead). Alternatively, after MCM2-7 departure, the parental strands might re-anneal up to the 3' end of the leading strand (Fig. 1Ciii, right inset). In this case, incisions would likely occur near the dsDNA–ssDNA junction (right inset, pink arrowhead), further away from the ICL, but as discussed below, this is not an ideal substrate for 3' flap endonucleases. The structure generated during traverse should be similar to the structure that results when two forks converge. The only possible difference is that if DNA synthesis re-initiates just beyond the ICL, the 5' end of the new leading strand after traverse will be closer to the lesion than at a converged fork (compare green strands in Fig. 1C and D). This would affect which endonucleases are able to cut the structure (see below). For single fork collisions without traverse, the 3' side of the ICL will be double-stranded (Fig. 1A and B), and therefore not recognized by structure specific endonucleases, unless the ICL is distorting, in which case there could be a limited amount of single-stranded DNA 3' to the ICL. Most likely, a diversity of structures exists at ICL-stalled forks, which might help explain the many endonucleases that have been implicated in ICL repair.

#### 8. Endonucleases

Six different nucleases (XPF-ERCC1, MUS81-EME1, SLX1-SLX4, FAN1, SNM1A, and SNM1B) have been implicated in ICL repair. For each nuclease, we discuss the evidence linking it to the incision step, its specificity, and how it might act on the structures shown in Fig. 1.

#### 9. XPF-ERCC1

The best candidate for an incision endonuclease is XPF-ERCC1, which is best known for its role in nucleotide excision repair (NER) [11]. XPF contains an excision repair cross-complementation



**Fig. 2.** Model incision substrates for endonucleases. (A–D) Preferred substrates, as determined in biochemical assays, for the indicated endonucleases. The arrowheads indicate the location of incision. Each substrate is shown in two orientations related by a 180 degree rotation. The left column shows where the nuclease would cut on the 5' side of a converged fork; the right column shows incisions on the 3' side. In each column, the nucleases that are likely to cut the ICL-associated X-shaped structure shown at the top are highlighted by a gray box. (E) Classic nucleotide excision repair (NER) substrate and locations of XPF and XPG incisions. (F) Location of incisions when XPF is presented with an ICL-containing fork-shaped structure. (G) Action of SNM1A on ICL-containing DNA.

group 4 (ERCC4) endonuclease domain and a Helix-hairpin-Helix (HhH) DNA binding domain. ERCC1 contains the same domains but the endonuclease domain has acquired mutations that render it catalytically inactive. The preferred substrate of XPF-ERCC1 is a “splayed arm” structure, which it cuts at the base of the 3' arm, a few nucleotides internal to the DNA duplex (Fig. 2A). XPF-ERCC1 also cuts the 3' flap structure depicted in Fig. 2B, but less efficiently than splayed arms [13], and it does not cut the 3' arm of 5' flap structures (Fig. 2C) [57]. During NER, XPF-ERCC1 helps remove bulky lesions by cutting on the 5' side of an open bubble structure surrounding the lesion (Fig. 2E). XPF-ERCC1 is unique among NER proteins in conferring cellular resistance to ICLs [15,40,51]. Mutations in ERCC1 that disrupt its interaction with XPA, another NER factor, prevent NER but not ICL repair, showing that the two functions of this nuclease can be uncoupled [52]. Mutations in mouse *Ercc1* cause a spectrum of phenotypes reminiscent of Fanconi anemia [28,47,55], and recently, XPF mutations were discovered in Fanconi patients [7,33]. Together, the data strongly suggest that XPF-ERCC1 operates in the FA-dependent ICL repair pathway.

The specific function of XPF in ICL repair has been slow to emerge. In the absence of XPF or ERCC1, ICL-induced DSBs accumulate and persist, leading to the conclusion that XPF is not required for the first incision of ICL unhooking [15,51]. In contrast, recent results from the Knipscheer laboratory show that in XPF-depleted *Xenopus* egg extracts, forks that have converged on an ICL are not incised [36]. These apparently contradictory results can be reconciled by the proposal that XPF is responsible for initial ICL incision in both systems, but that in cells, forks persisting in the absence of XPF are aberrantly cleaved by MUS81 [68].

Given its preference for the 3' arm of a splayed arm structure (Fig. 2A), XPF-ERCC1 probably cuts on the 3' side of ICL-associated X-shaped structures (red arrowhead in Fig. 1C). XPF's cleavage of 3' splayed arms is dramatically enhanced by RPA binding to the 5' arm [14], as depicted in Fig. 1Cii (left inset). However, when the

leading strand is present at the junction as depicted in Fig. 1Ciii (right inset), XPF may not cut efficiently [57], requiring another solution (see SNM1A section below). In *Xenopus* egg extracts, neither of the two incisions required for unhooking of converged forks occur in the absence XPF-ERCC1, or when XPF-depleted extracts are supplemented with catalytically inactive XPF-ERCC1 [36]. One explanation for this observation is that the 5' incision cannot take place without the 3' incision. A precedent for coupling between dual incisions is observed in NER, where incision by XPG 3' to a lesion requires prior incision by XPF-ERCC1 on the 5' side [62] (Fig. 2E). Another possibility is that XPF performs both incisions. Thus, in the context of a splayed arm structure containing an ICL, purified XPF-ERCC1 cuts on the 5' and 3' sides of the lesion (Fig. 2F [18,40]). It is presently unclear how this activity would be affected by the presence of a fork on the other side of the ICL. In summary, although XPF-ERCC1 is almost certainly the primary 3' incision endonuclease, the precise mechanism and circumstances of its action remain to be elucidated.

## 10. MUS81-EME1

Like XPF, the MUS81-EME1 heterodimer belongs to the XPF/MUS81 nuclease family, with MUS81 contributing the catalytic active ERCC4 domain [11]. MUS81-EME1 greatly prefers 3' flap structures that contain a 5' end within 4 nucleotides of the flap junction (Fig. 2B) [6,10,11]. Mutations in MUS81-EME1 render cells sensitive to ICLs, but not as sensitive as mutations in XPF-ERCC1 [12,68]. This suggests that MUS81-EME1 plays a secondary role in ICL repair. While early experiments in mammalian cells suggested that MUS81 is required to generate ICL-induced DSBs [23], implying a role in incisions, more recent evidence indicates that MUS81 generates DSBs when normal fork processing is disrupted [68]. Consistent with the latter view, depletion of MUS81-EME1 had no effect on the incision of converged forks in *Xenopus* egg extracts

[36]. We propose that MUS81 only acts on a subset of the intermediates generated when forks encounter ICLs. For example, if leading strand synthesis resumes immediately downstream of the ICL during fork traverse, a 5' end is located near the flap junction (Fig. 1D; green arrow). The 5' end disfavors cutting by XPF-ERCC1 [13,57], but creates an ideal substrate for MUS81-EME1. In summary, we propose that the primary role of MUS81-EME1 in ICL repair is to cut on the 3' side of X-shaped structures in which a 5' end abuts the ssDNA–dsDNA junction, possibly during a subset of traverse events.

Importantly, the structure generated during a single-fork collision lacks a 5' end near the junction and therefore the leading strand template should not be cleaved efficiently by MUS81 (Fig. 1A–B). It may also not be cut efficiently by XPF, which is inhibited by the presence of a 3' end at the junction [57]. Thus, no enzyme implicated in ICL repair is well suited to incise the leading strand template of a single stalled fork (Fig. 1A, black arrowhead). These considerations suggest that single forks should be cleaved on the lagging strand template by a 5' flap endonuclease (Fig. 1B, blue arrowhead).

## 11. SLX1

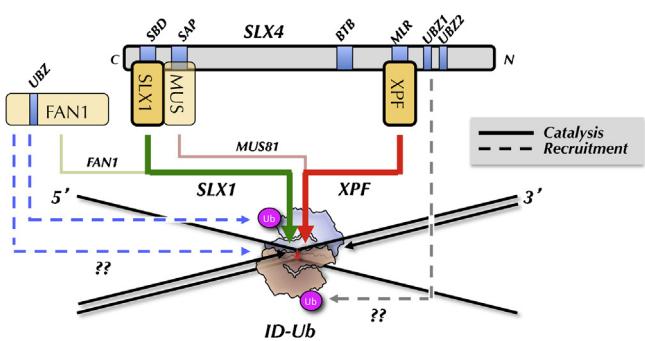
SLX1 is a structure-specific endonuclease containing a UvrC-intron-endonuclease domain (URI) and a PHD-type zinc finger domain [5]. SLX1's activity is dramatically stimulated through interaction with a non-catalytic subunit, SLX4. Homozygous deletion of the SLX1 gene in mice causes sensitivity to ICLs similar to that of MUS81, and rescue of this sensitivity requires the catalytic activity of SLX1 [8]. SLX1-SLX4 is a rather promiscuous endonuclease, in that it cleaves splayed arms, 5' flaps, 3' flaps, and Holliday junctions [19,71]. However, its preferred substrate is a 5' flap, which it cuts at the ssDNA–dsDNA junction (Fig. 2C). In the context of ICL repair, a 5' flap-like structure is generated on the 5' side of the ICL when the leading strand of a stalled fork has been extended to the –1 position (Fig. 1A–D). Based on this and considerations discussed below, we propose that SLX1 is the primary nuclease that cleaves on the 5' side of the ICL.

## 12. Scaffolding by SLX4

Accumulating evidence indicates that SLX4 serves as a master scaffold for incisions. Deletion of SLX4, the binding partner of SLX1, causes much greater sensitivity to ICLs than deletion of SLX1 or MUS81 [8], and it is required for incisions in *Xenopus* egg extracts [36]. Underscoring its importance for ICL repair, SLX4 is a Fanconi gene [34,63]. Strikingly, SLX4 co-precipitates with XPF-ERCC1, SLX1, and MUS81-EME1 [4,17,48,64]. SLX4 binds SLX1 via a helix-turn-helix motif (also referred as SBD: SLX1 binding domain), MUS81 via a SAP motif, and XPF via an MLR motif (Fig. 3). The binding of SLX4 to XPF-ERCC1 and SLX1 is critical for the action of these nucleases in ICL repair, probably by recruiting them to sites of damage [8,12,35,63]. In contrast, the interaction of MUS81 with SLX4 is dispensable for ICL repair [8]. Together, the data indicate that XPF-ERCC1-SLX4-SLX1 represents a core “XESS” complex that incises ICL-associated X-shaped structures (Fig. 3). MUS81 is employed for special scenarios, i.e. when the 3' side of the ICL contains a true 3' flap (Fig. 1D). Importantly, SLX4 appears to be more than just a recruitment platform, since it dramatically enhances XPF nuclease activity toward splayed arm and ICL-containing structures [27]. While SLX1 is probably the primary 5' endonuclease for most situations, the mild ICL-sensitivity of SLX1 mutations suggests its function can be replaced by another endonuclease (next section).

## 13. FAN1

Fanconi Associated Nuclease 1 (FAN1) contains a UBZ4-type ubiquitin binding domain, a SAP DNA binding domain, and a



**Fig. 3.** Model for incisions. ID-Ub binds directly to an ICL in such a manner as to sequester the bottom parental strand while exposing the top parental strand. ID-Ub also recruits the SLX4-SLX1-MUS81-XPF-ERCC1 complex to the ICL, possibly via a direct interaction between the UBZ domains of SLX4 and the ubiquitin of the ID complex (dashed, gray arrow). XPF is the primary enzyme that incises 3' to the ICL (thick red arrow), while SLX1 promotes the 5' incision (thick green arrow). MUS81 performs a specialty role, cutting on the 3' side in cases where the 5' end of the leading strand on the same side abuts the ICL (pink arrow; shown in Fig. 1D and 2B). FAN1 is recruited to ICLs via two independent mechanisms, one of which requires ID-Ub (dashed blue arrows). FAN1 cuts on the 5' side of the ICL (light green arrow), and/or it might function downstream of incisions, during the HR step (not shown).

PD-D/E(X)K nuclease motif, placing it in the same nuclease superfamily as XPF and MUS81 [39]. Like SLX1, FAN1 prefers 5' flap structures. However, unlike SLX1, which cleaves at the ssDNA–dsDNA junction, FAN1 cleaves four nucleotides 3' to the branch point (Fig. 2D) [39,42,45,60]. FAN1 also exhibits robust 5' → 3' exonuclease activity of 5' recessed DNA ends, nicks, or gaps. FAN1 gene knockdown or knock-out selectively sensitizes cells to ICL-inducing agents, although not to the same extent as disruption of the FA pathway [39,42,45,60,66,73,76]. FAN1 and FA pathway mutations are not epistatic, suggesting distinct roles in ICL repair [73,76]. Indeed, FANC mutations cause much greater damage-induced chromosomal instability than FAN1 mutations [66,73,75]. Consistent with these observations, FAN1 and FANC mutations cause distinct clinical phenotypes, the former being associated with karyomegalic interstitial nephritis, a form of chronic kidney disease instead of FA [66,76]. Together, the data indicate that FAN1 participate in ICL repair, but via a distinct and secondary role relative to the FA pathway.

The function of FAN1 in ICL repair remains enigmatic. ICL-induced γH2AX foci still form in the absence of FAN1, leading to the proposal that FAN1 might function in the HR step of ICL repair, downstream of incisions [45]. However, γH2AX foci are caused by DNA damage other than dsDNA breaks [54], leaving open the possibility that FAN1 is required for incisions. When FAN1 is depleted from *Xenopus* egg extracts, there is no detectable repair or incision defect [36], but this might be due to redundancy with other nucleases such as SLX1. In the future, SLX1-FAN1 redundancy should be examined using biochemical and genetic ICL repair assays. A reasonable working hypothesis is that FAN1 plays a secondary role in cleaving the 5' side of ICL-associated X-shaped structures (Fig. 3; light green line).

## 14. SNM1 family nucleases

SNM1 nucleases (SNM1A, SNM1B, and SNM1C) belong to the β-CASP subfamily of metallo-beta-lactamases, which are DNA processing enzymes [9]. The single SNM1 family member in yeast, Pso2, exhibits singular sensitivity to ICLs but not other damaging agents. In vertebrates, SNM1A and SNM1B mutants are selectively sensitive to ICLs, with SNM1A showing the greatest sensitivity [9,31]. In contrast, SNM1C/Artemis mutants are not sensitive to ICLs but to ionizing radiation, consistent with this protein's role in non-homologous end-joining. While SNM1A and B are reported to

exhibit non-epistasis with homologous recombination, translesion DNA synthesis, and FANC genes in chicken cells [31], there are hints of an epistatic relationship with XPF in mammalian cells [68]. Thus, although SNM1A and B appear to participate in ICL repair, whether they function in the FA-dependent pathway remains uncertain.

Given its greater sensitivity to ICLs, functional complementation of yeast *pso2* mutants [24], and more robust nuclease activity [59], we will focus on SNM1A. Unlike the endonucleases discussed above, SNM1A functions as a 5' → 3' exonuclease with no obvious role in incisions [24,25]. Consistent with this, *pso2* mutants in yeast still form ICL-induced dsDNA breaks [22,46]. Interestingly, SNM1A can use its 5' → 3' exonuclease activity to digest one DNA strand a few nucleotides past an ICL (Fig. 2G [68]). This suggests that as long as an incision is made on the 5' side of an ICL (e.g. by SLX1 or FAN1), SNM1A might be able to complete the unhooking reaction without the need for 3' endonucleolytic cleavage by XPF or MUS81. Exonucleolytic degradation by SNM1A therefore might replace the 3' incision in cases where the 3' side of the ICL is not ideal for incision by XPF or MUS81 (as in Fig. 1Ciii, right inset). Another possible role for SNM1A is that after 5' and 3' incisions have occurred, SNM1A reduces the oligonucleotide between the incision points to a mono-adduct that can be bypassed by translesion DNA polymerases.

## 15. Regulation of ICL processing

How does the FA pathway regulate incisions [37]? Upon exposure of cells to ICLs, the ID-Ub complex forms DNA damage foci that overlap with several DNA repair factors, including Rad51 and BRCA1, suggesting it binds to sites of damage [65]. Consistent with this idea, during repair of an ICL in egg extracts, ID-Ub localizes to the ICL immediately before incisions take place, as measured by chromatin immunoprecipitation [36]. Moreover, FANCI and FANCD2 bind preferentially to a variety of branched DNA structures [32,44,53,74], and the crystal structure of FANCI with DNA suggests that the ID complex should be able to accommodate an ICL-associated X-shaped structure (Fig. 3 [32]). Thus, ID-Ub likely binds directly to ICLs, allowing it to exert local control over the process of incisions (Fig. 3).

Several connections between the ID complex and the incision machinery have now been observed. First, the recruitment of FAN1 to damage foci in cells requires ubiquitylated FANCD2, as well as the UBZ domain of FAN1 [39,42,45,60]. On the other hand, FAN1 and the FANC genes are not epistatic [73,76], and a FAN1 mutant disrupting the UBZ domain is functional for ICL resistance [76]. These data suggest that although FAN1 can be recruited to ICLs via ID-Ub, this is not essential in some cases, perhaps due to another, independent recruitment pathway (Fig. 3, dashed blue arrows). The connection between ID-Ub and XPF is more compelling. Most importantly, in *Xenopus* egg extracts, the binding of SLX4 and XPF to ICLs during repair requires ubiquitylated FANCD2 [36]. This observation is consistent with the earlier finding that SLX4 damage localization in cells depends on the SLX4 UBZ motif and ubiquitylated FANCD2 ([72], but see [35]). Although the molecular details remain unclear, the data strongly suggest that ID-Ub recruits the XESS complex to sites of damage.

Why has a system as elaborate as the FA pathway evolved to regulate incisions? We propose this regulation arose to deal with the complexity inherent in ICL-associated X-shaped structures. In principle, these structures could be cleaved by structure-specific endonucleases in any one of the four arms that meet at the ICL. While several combinations of cuts are possible, the only one that yields a productive outcome involves incising the same parental strand on either side of the lesion (Fig. 3, red and green arrows), or incising on the 5' side of the ICL and using an exonuclease to degrade past the ICL. We propose that ID-Ub has at least two functions. First, it recruits the XESS complex to the lesion to promote

5' and 3' incisions of one parental strand. Second, it embraces the ICL in such a manner as to suppress incisions on the other parental strand. Ultimately, to understand how ID-Ub controls incisions, the reaction will have to be reconstituted with purified components and a crystal structure of the ubiquitylated ID complex with an appropriate X-shaped structure will be needed. However, already one can envision that ID-Ub sequesters the lower strand of the X-shaped structure to protect it from cleavage (Fig. 3). It is important to note that the X-shaped DNA structure is not symmetrical, since a 3' end abuts the ICL on just one side. This would allow the ID complex to interact differentially with the two parental strands.

## 16. Outlook

It is now clear that a signature event in ICL repair is the ID-Ub-dependent incision of stalled replication forks. The discovery of the XESS complex lays the foundation to understand how these incisions are carried out and regulated. Going forward, it will be critical to re-examine the specificities of all the relevant endonucleases on DNA substrates that better mimic the various structures predicted to exist at ICL-stalled DNA replication forks (Fig. 1A–D), including with distorting and non-distorting ICLs. This should help clarify which endonuclease is employed under which circumstances. A more ambitious goal is to reconstitute ID-Ub-dependent incisions with defined components so that we may precisely define the function of the Fanconi anemia pathway. These biochemical approaches will have to be complemented with incision assays under physiological conditions. Finally, it will be critical to understand why deficiencies in two endonucleases (FAN1 versus XPF-ERCC1-SLX4) that appear to resolve the same type of DNA damage cause such different clinical phenotypes. As we address these important issues, nature will undoubtedly serve up more surprises.

## Acknowledgements

We thank KJ Patel and Puck Knipscheer for communicating unpublished results. We thank Agata Smogorzewska, Orlando Scherer, and Puck Knipscheer for feedback on the manuscript. The work on ICL repair in the Walter Laboratory is funded by NIH grant HL098316 and a grant from the Fanconi anemia research foundation.

## References

- [1] Y.M. Akkari, R.L. Bateman, C.A. Reifsteck, S.B. Olson, M. Grompe, DNA replication is required To elicit cellular responses to psoralen-induced DNA interstrand cross-links, *Mol. Cell. Biol.* 20 (2000) 8283–8289.
- [2] A.F. Alpi, P.E. Pace, M.M. Babu, K.J. Patel, Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCI, and FANCI, *Mol. Cell* 32 (2008) 767–777.
- [3] R.P. Anand, S.T. Lovett, J.E. Haber, Break-induced DNA replication, *Cold Spring Harb. Perspect. Biol.* 5 (2013), a010397.
- [4] S.L. Andersen, D.T. Bergstrahl, K.P. Kohl, J.R. LaRocque, C.B. Moore, J. Sekelsky, *Drosophila* MUS312 and the vertebrate ortholog BTBD12 interact with DNA structure-specific endonucleases in DNA repair and recombination, *Mol. Cell* 35 (2009) 128–135.
- [5] L. Aravind, E.V. Koonin, SAP – a putative DNA-binding motif involved in chromosomal organization, *Trends Biochem. Sci.* 25 (2000) 112–114.
- [6] S.A. Bastin-Shanower, W.M. Fricke, J.R. Mullen, S.J. Brill, The mechanism of Mus81-Mms4 cleavage site selection distinguishes it from the homologous endonuclease Rad1-Rad10, *Mol. Cell. Biol.* 23 (2003) 3487–3496.
- [7] M. Bogliolo, B. Schuster, C. Stoepker, B. Derkunt, Y. Su, A. Raams, J.P. Trujillo, J. Minguillon, M.J. Ramirez, R. Pujol, et al., Mutations in ERCC4, encoding the DNA-repair endonuclease XPF, cause Fanconi anemia, *Am. J. Hum. Genet.* 92 (2013) 800–806.
- [8] D. Castor, N. Nair, A.C. Declais, C. Lachaud, R. Toth, T.J. Macartney, D.M. Lilley, J.S. Arthur, J. Rouse, Cooperative control of holliday junction resolution and DNA repair by the SLX1 and MUS81-EME1 nucleases, *Mol. Cell* 52 (2013) 221–233.
- [9] E. Cattell, B. Sengerova, P.J. McHugh, The SNM1/Pso2 family of ICL repair nucleases: from yeast to man, *Environ. Mol. Mutagen.* 51 (2010) 635–645.
- [10] A. Ciccia, A. Constantinou, S.C. West, Identification and characterization of the human mus81-eme1 endonuclease, *J. Biol. Chem.* 278 (2003) 25172–25178.

- [11] A. Ciccia, N. McDonald, S.C. West, Structural and functional relationships of the XPF/MUS81 family of proteins, *Annu. Rev. Biochem.* 77 (2008) 259–287.
- [12] G.P. Crossan, L. van der Weyden, I.V. Rosado, F. Langevin, P.H. Gaillard, R.E. McIntyre, F. Gallagher, M.I. Kettunen, D.Y. Lewis, K. Brindle, et al., Disruption of mouse Slx4, a regulator of structure-specific nucleases, phenocopies Fanconi anemia, *Nat. Genet.* 43 (2011) 147–152.
- [13] W.L. de Laat, E. Appeldoorn, N.G. Jaspers, J.H. Hoeijmakers, DNA structural elements required for ERCC1-XPF endonuclease activity, *J. Biol. Chem.* 273 (1998) 7835–7842.
- [14] W.L. de Laat, E. Appeldoorn, K. Sugasawa, E. Weterings, N.G. Jaspers, J.H. Hoeijmakers, DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair, *Genes Dev.* 12 (1998) 2598–2609.
- [15] I.U. De Silva, P.J. McHugh, P.H. Clingen, 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.
- [16] K.E. Duderstadt, R. Reyes-Lamothe, A.M. van Oijen, D.J. Sherratt, Replication-fork dynamics, *Cold Spring Harb. Perspect. Biol.* 6 (2014), pii: a010157.
- [17] S. Fekairi, S. Scaglione, C. Chahwan, E.R. Taylor, A. Tissier, S. Coulon, M.Q. Dong, C. Ruse, J.R. Yates, P. Russell 3rd, et al., Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases, *Cell* 138 (2009) 78–89.
- [18] L.A. Fisher, M. Bessho, T. Bessho, Processing of a psoralen DNA interstrand cross-link by XPF-ERCC1 complex in vitro, *J. Biol. Chem.* 283 (2008) 1275–1281.
- [19] W.M. Fricke, S.J. Brill, Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3, *Genes Dev.* 17 (2003) 1768–1778.
- [20] Y.V. Fu, H. Yardimci, D.T. Long, A. Guainazzi, V.P. Bermudez, J. Hurwitz, A. van Oijen, O.D. Scharer, J.C. Walter, Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase, *Cell* 146 (2011) 931–941.
- [21] I. Garcia-Higuera, T. Taniguchi, S. Ganeshan, M.S. Meyn, C. Timmers, J. Hejna, M. Grompe, A.D. D'Andrea, Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway, *Mol. Cell* 7 (2001) 249–262.
- [22] K.F. Grossmann, A.M. Ward, R.E. Moses, *Saccharomyces cerevisiae* lacking Ssn1, Rev3 or Rad51 have a normal S-phase but arrest permanently in G2 after cisplatin treatment, *Mutat. Res.* 461 (2000) 1–13.
- [23] K. Hanada, M. Budzowska, M. Modesti, A. Maas, C. Wyman, J. Essers, R. Kanaar, The structure-specific endonuclease Mus81-Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks, *EMBO J.* 25 (2006) 4921–4932.
- [24] A. Hazrati, M. Ramis-Castelltort, S. Sarkar, L.J. Barber, C.J. Schofield, J.A. Hartley, P.J. McHugh, Human SNM1A suppresses the DNA repair defects of yeast pso2 mutants, *DNA Repair (Amst.)* 7 (2008) 230–238.
- [25] J. Hejna, S. Philip, J. Ott, C. Faulkner, R. Moses, The hSNM1 protein is a DNA 5'-exonuclease, *Nucleic Acids Res.* 35 (2007) 6115–6123.
- [26] R.C. Heller, K.J. Marians, Replisome assembly and the direct restart of stalled replication forks, *Nat. Rev. Mol. Cell Biol.* 7 (2006) 932–943.
- [27] M.R. Hodkinson, J. Silhan, G.P. Crossan, J.I. Garaycochea, S. Mukherjee, O.D. Scharer, K.J. Patel, Mouse Slx4 is a tumour suppressor that stimulates the activity of the nuclease Xpf-Erc1 in DNA crosslink repair, *Mol. Cell* 54 (2014), in press.
- [28] K.T. Hsia, M.R. Millar, S. King, J. Selfridge, N.J. Redhead, D.W. Melton, P.T. Saunders, DNA repair gene Ercc1 is essential for normal spermatogenesis and oogenesis and for functional integrity of germ cell DNA in the mouse, *Development* 130 (2003) 369–378.
- [29] J. Huang, S. Liu, M.A. Bellani, A.K. Thazhathveetil, C. Ling, J.P. de Winter, Y. Wang, W. Wang, M.M. Seidman, The DNA translocase FANCM/MHF promotes replication traversal of DNA interstrand crosslinks, *Mol. Cell* 52 (2013) 434–446.
- [30] M. Huang, J.M. Kim, B. Shiotani, K. Yang, L. Zou, A.D. D'Andrea, The FANCM/FAAP24 complex is required for the DNA interstrand crosslink-induced checkpoint response, *Mol. Cell* 39 (2010) 259–268.
- [31] M. Ishiai, M. Kimura, K. Namikoshi, M. Yamazoe, K. Yamamoto, H. Arakawa, K. Agematsu, N. Matsushita, S. Takeda, J.M. Buerstedde, et al., DNA cross-link repair protein SNM1A interacts with PIAS1 in nuclear focus formation, *Mol. Cell. Biol.* 24 (2004) 10733–10741.
- [32] W. Joo, G. Xu, N.S. Persky, A. Smogorzewska, D.G. Rudge, O. Buzovetsky, S.J. Elledge, N.P. Pavletich, Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway, *Science* 333 (2011) 312–316.
- [33] K. Kashiyama, Y. Nakazawa, D.T. Pilz, C. Guo, M. Shimada, K. Sasaki, H. Fawcett, J.F. Wing, S.O. Lewin, L. Carr, et al., Malfunction of nuclease ERCC1-XPF results in diverse clinical manifestations and causes Cockayne syndrome, xeroderma pigmentosum, and Fanconi anemia, *Am. J. Hum. Genet.* 92 (2013) 807–819.
- [34] Y. Kim, F.P. Lach, R. Desetty, H. Hanenberg, A.D. Auerbach, A. Smogorzewska, Mutations of the SLX4 gene in Fanconi anemia, *Nat. Genet.* 43 (2011) 142–146.
- [35] Y. Kim, G.S. Spitz, U. Veturi, F.P. Lach, A.D. Auerbach, A. Smogorzewska, Regulation of multiple DNA repair pathways by the Fanconi anemia protein SLX4, *Blood* 121 (2013) 54–63.
- [36] D. Klein Douwel, R.A.C.M. Boonen, D.T. Long, A.A. Szypowska, M. Raschle, J.C. Walter, P. Knipscheer, XPF-ERCC1 acts in unhooking DNA interstrand crosslinks in cooperation with FANCD2 and FANCN/SLX4, *Mol. Cell* 54 (2014), in press.
- [37] P. Knipscheer, M. Raschle, A. Smogorzewska, M. Enioi, T.V. Ho, O.D. Scharer, S.J. Elledge, J.C. Walter, The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair, *Science* 326 (2009) 1698–1701.
- [38] M.C. Kottemann, A. Smogorzewska, Fanconi anaemia and the repair of Watson and Crick DNA crosslinks, *Nature* 493 (2013) 356–363.
- [39] K. Kratz, B. Schopf, S. Kaden, A. Sendoel, R. Eberhard, C. Lademann, E. Cannavò, A.A. Sartori, M.O. Hengartner, J. Jiricny, Deficiency of FANCD2-associated nuclelease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents, *Cell* 142 (2010) 77–88.
- [40] 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.
- [41] C. Le Breton, M. Hennion, P.B. Arimondo, O. Hyrien, Replication-fork stalling and processing at a single psoralen interstrand crosslink in Xenopus egg extracts, *PLoS ONE* 6 (2011) e18554.
- [42] T. Liu, G. Ghosal, J. Yuan, J. Chen, J. Huang, FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair, *Science* 329 (2010) 693–696.
- [43] D.T. Long, M. Raschle, V. Joukov, J.C. Walter, Mechanism of RAD51-dependent DNA interstrand cross-link repair, *Science* 333 (2011) 84–87.
- [44] S. Longerich, J. San Filippo, D. Liu, P. Sung, FANCI binds branched DNA and is monoubiquitinated by UBE2T-FANCL, *J. Biol. Chem.* 284 (2009) 23182–23186.
- [45] C. MacKay, A.C. Declais, C. Lundin, A. Agostinho, A.J. Deans, T.J. MacArtney, K. Hofmann, A. Gartner, S.C. West, T. Helleday, et al., Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2, *Cell* 142 (2010) 65–76.
- [46] N. Magana-Schwencke, J.A. Henriques, R. Chanet, E. Moustacchi, The fate of 8-methoxysoralen photoinduced crosslinks in nuclear and mitochondrial yeast DNA: comparison of wild-type and repair-deficient strains, *Proc. Natl. Acad. Sci. U. S. A.* 79 (1982) 1722–1726.
- [47] J. McWhir, J. Selfridge, D.J. Harrison, S. Squires, D.W. Melton, Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning, *Nat. Genet.* 5 (1993) 217–224.
- [48] I.M. Munoz, K. Hain, A.C. Declais, M. Gardiner, G.W. Toh, L. Sanchez-Pulido, J.M. Heuckmann, R. Toth, T. Macartney, B. Eppink, et al., Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair, *Mol. Cell* 35 (2009) 116–127.
- [49] K. Nakanishi, F. Cavallo, L. Perrouault, C. Giovannangeli, M.E. Moynahan, M. Barchi, E. Brunet, M. Jasin, Homology-directed Fanconi anemia pathway cross-link repair is dependent on DNA replication, *Nat. Struct. Mol. Biol.* 18 (2011) 500–503.
- [50] L.J. Niedernhofer, A.S. Lalai, J.H. Hoeijmakers, Fanconi anemia (cross)linked to DNA repair, *Cell* 123 (2005) 1191–1198.
- [51] L.J. Niedernhofer, H. Odijk, M. Budzowska, E. van Drunen, A. Maas, A.F. Theil, J. de Wit, N.G. Jaspers, H.B. Beverloo, J.H. Hoeijmakers, et al., The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks, *Mol. Cell. Biol.* 24 (2004) 5776–5787.
- [52] B. Orelli, T.B. McClendon, O.V. Tsodikov, T. Ellenberger, L.J. Niedernhofer, O.D. Scharer, The XPA-binding domain of ERCC1 is required for nucleotide excision repair but not other DNA repair pathways, *J. Biol. Chem.* 285 (2010) 3705–3712.
- [53] W.H. Park, S. Margossian, A.A. Horwitz, A.M. Simons, A.D. D'Andrea, J.D. Parvin, Direct DNA binding activity of the Fanconi anemia D2 protein, *J. Biol. Chem.* 280 (2005) 23593–23598.
- [54] E. Petermann, M.L. Orta, N. Issaeva, N. Schultz, T. Helleday, Hydroxyurea-stalled replication forks become progressively inactivated and require two different RAD51-mediated pathways for restart and repair, *Mol. Cell* 37 (2010) 492–502.
- [55] J.M. Prasher, A.S. Lalai, C. Heijmans-Antoniissen, R.E. Ploemacher, J.H. Hoeijmakers, I.P. Touw, L.J. Niedernhofer, Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient Ercc1<sup>-/-</sup> mice, *EMBO J.* 24 (2005) 861–871.
- [56] M. Raschle, P. Knipscheer, M. Enioi, T. Angelov, J. Sun, J.D. Griffith, T.E. Ellenberger, O.D. Scharer, J.C. Walter, Mechanism of replication-coupled DNA interstrand crosslink repair, *Cell* 134 (2008) 969–980.
- [57] K. Rodriguez, Z. Wang, E.C. Friedberg, A.E. Tomkinson, Identification of functional domains within the RAD1-RAD10 repair and recombination endonuclease of *Saccharomyces cerevisiae*, *J. Biol. Chem.* 271 (1996) 20551–20558.
- [58] A. Rothfuss, M. Grompe, Repair kinetics of genomic interstrand DNA cross-links: evidence for DNA double-strand break-dependent activation of the Fanconi anemia/BRCA pathway, *Mol. Cell. Biol.* 24 (2004) 123–134.
- [59] B. Sengerova, C.K. Allerston, M. Abu, S.Y. Lee, J. Hartley, K. Kiakos, C.J. Schofield, J.A. Hartley, O. Gileadi, P.J. McHugh, Characterization of the human SNM1A and SNM1B/Apollo DNA repair exonucleases, *J. Biol. Chem.* 287 (2012) 26254–26267.
- [60] A. Smogorzewska, R. Desetty, T.T. Saito, M. Schlabach, F.P. Lach, M.E. Sowa, A.B. Clark, T.A. Kunkel, J.W. Harper, M.P. Colaiaco, et al., A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair, *Mol. Cell* 39 (2010) 36–47.
- [61] A. Smogorzewska, S. Matsuoka, P. Vinciguerra, E.R. McDonald 3rd, K.E. Hurov, J. Luo, B.A. Ballif, S.P. Gygi, K. Hofmann, A.D. D'Andrea, et al., Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair, *Cell* 129 (2007) 289–301.
- [62] L. Staresinic, A.F. Fagbemi, J.H. Enzlin, A.M. Gourdin, N. Wijgers, I. Dunand-Sauthier, G. Giglia-Mari, S.G. Clarkson, W. Vermeulen, O.D. Scharer, Coordination of dual incision and repair synthesis in human nucleotide excision repair, *EMBO J.* 28 (2009) 1111–1120.
- [63] C. Stoepker, K. Hain, B. Schuster, Y. Hilhorst-Hofstee, M.A. Rooimans, J. Steltenpool, A.B. Ooster, K. Eirich, E.T. Korthof, A.W. Nieuwint, et al., SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype, *Nat. Genet.* 43 (2011) 138–141.
- [64] J.M. Svendsen, A. Smogorzewska, M.E. Sowa, B.C. O'Connell, S.P. Gygi, S.J. Elledge, J.W. Harper, Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair, *Cell* 138 (2009) 63–77.

- [65] T. Taniguchi, I. Garcia-Higuera, P.R. Andreassen, R.C. Gregory, M. Grompe, A.D. D'Andrea, S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51, *Blood* 100 (2002) 2414–2420.
- [66] J.P. Trujillo, L.B. Mina, R. Pujol, M. Bogliolo, J. Andrieux, M. Holder, B. Schuster, D. Schindler, J. Surralles, On the role of FAN1 in Fanconi anemia, *Blood* 120 (2012) 86–89.
- [67] D. Vare, P. Groth, R. Carlsson, F. Johansson, K. Erixon, D. Jensen, DNA interstrand crosslinks induce a potent replication block followed by formation and repair of double strand breaks in intact mammalian cells, *DNA Repair* 11 (2012) 976–985.
- [68] A.T. Wang, B. Sengerova, E. Cattell, T. Inagawa, J.M. Hartley, K. Kiakos, N.A. Burgess-Brown, L.P. Swift, J.H. Enzlin, C.J. Schofield, et al., Human SNM1A and XPF-ERCC1 collaborate to initiate DNA interstrand cross-link repair, *Genes Dev.* 25 (2011) 1859–1870.
- [69] W. Wang, Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins, *Nat. Rev. Genet.* 8 (2007) 735–748.
- [70] M.S. Wold, Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism, *Annu. Rev. Biochem.* 66 (1997) 61–92.
- [71] H.D. Wyatt, S. Sarbajna, J. Matos, S.C. West, Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells, *Mol. Cell* 52 (2013) 234–247.
- [72] K.N. Yamamoto, S. Kobayashi, M. Tsuda, H. Kurumizaka, M. Takata, K. Kono, J. Jiricny, S. Takeda, K. Hirota, Involvement of SLX4 in interstrand cross-link repair is regulated by the Fanconi anemia pathway, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 6492–6496.
- [73] K. Yoshikiyo, K. Kratz, K. Hirota, K. Nishihara, M. Takata, H. Kurumizaka, S. Horimoto, S. Takeda, J. Jiricny, KIAA1018/FAN1 nuclease protects cells against genomic instability induced by interstrand cross-linking agents, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 21553–21557.
- [74] F. Yuan, J. El Hokayem, W. Zhou, Y. Zhang, FANCI protein binds to DNA and interacts with FANCD2 to recognize branched structures, *J. Biol. Chem.* 284 (2009) 24443–24452.
- [75] B.B. Zhou, P. Chaturvedi, K. Spring, S.P. Scott, R.A. Johanson, R. Mishra, M.R. Mattern, J.D. Winkler, K.K. Khanna, Caffeine abolishes the mammalian G(2)/M DNA damage checkpoint by inhibiting ataxia-telangiectasia-mutated kinase activity, *J. Biol. Chem.* 275 (2000) 10342–10348.
- [76] W. Zhou, E.A. Otto, A. Cluckey, R. Airik, T.W. Hurd, M. Chaki, K. Diaz, F.P. Lach, G.R. Bennett, H.Y. Gee, et al., FAN1 mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair, *Nat. Genet.* 44 (2012) 910–915.