Recruitment and positioning determine the specific role of the XPF-ERCC1 endonuclease in interstrand crosslink repair

Daisy Klein Douwel, Wouter S Hoogenboom, Rick ACM Boonen† & Puck Knipscheer*†

Abstract

XPF-ERCC1 is a structure-specific endonuclease pivotal for several DNA repair pathways and, when mutated, can cause multiple diseases. Although the disease-specific mutations are thought to affect different DNA repair pathways, the molecular basis for this is unknown. Here we examine the function of XPF-ERCC1 in DNA interstrand crosslink (ICL) repair. We used Xenopus egg extracts to measure both ICL and nucleotide excision repair, and we identified mutations that are specifically defective in ICL repair. One of these separation-of-function mutations resides in the helicase-like domain of XPF and disrupts binding to SLX4 and recruitment to the ICL. A small deletion in the same domain supports recruitment of XPF to the ICL, but inhibited the unhooking incisions most likely by disrupting a second, transient interaction with SLX4. Finally, mutation of residues in the nuclease domain did not affect localization of XPF-ERCC1 to the ICL but did prevent incisions on the ICL substrate. Our data suggest a model in which the ICL repair-specific function of XPF-ERCC1 is dependent on recruitment, positioning and substrate recognition.

Keywords Fanconi anemia; interstrand crosslink repair; nucleotide excision repair; Xenopus egg extract; XPF-ERCC1

Subject Categories DNA Replication, Repair & Recombination; Molecular Biology of Disease

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Introduction

The structure-specific endonuclease XPF-ERCC1 participates in multiple genome maintenance pathways, including nucleotide excision repair (NER), DNA interstrand crosslink (ICL) repair, certain branches of double-stranded break (DSB) repair, and telomere maintenance. Mutations in XPF-ERCC1 have been associated with the genetic disorders Xeroderma pigmentosum (XP), Cockayne syndrome (CS), cerebro-oculo-facio-skeletal syndrome (COFS), Fanconi anemia (FA), and premature aging. These phenotypes are believed to be caused by a defect in one, or several, of the genome maintenance pathways XPF-ERCC1 is involved in, but the molecular basis for this is unknown.

XPF is a structure-specific endonuclease that contains an N-terminal helicase-like domain, a central ERCC4-type nuclease domain, and a C-terminal helix-hairpin-helix (HHH) domain with which it interacts with its cofactor ERCC1 (Fig 1A). Very little is known about the role of the helicase-like domain, but it is important for nuclease activity (Bowles et al, 2012). The function of XPF-ERCC1 in NER, a pathway that removes helix distorting lesions, has been extensively studied (Gillet & Schaer, 2012). After damage recognition by upstream NER factors, XPF-ERCC1 is recruited to the lesion by XPA and excises a short oligo containing the damage in collaboration with another nuclease XPG (Huang et al, 1992; Li et al, 1995; Spivak, 2015). Defects in NER factors are associated with the genetic disease Xeroderma pigmentosum (XP), which is characterized by sunlight sensitivity and skin cancer predisposition. Uniquely among NER factors, deficiency in XPF-ERCC1 not only causes UV sensitivity, but also results in hypersensitivity to ICL-inducing agents, indicating an additional role for this protein in the repair of interstrand crosslinks (De Silva et al, 2000; Kuraoka et al, 2000; Niedernhofer et al, 2004).

ICLs are toxic DNA lesions that covalently link both strands of the DNA together, thereby blocking DNA replication and transcription. ICLs are formed endogenously by products of cellular metabolism, but are also induced at high doses by certain chemotherapeutic drugs. The major pathway of ICL repair is coupled to DNA replication and involves the coordinated action of many DNA repair proteins including the Fanconi anemia (FA) pathway proteins. Mutations in any of the 21 currently known FA genes give rise to Fanconi anemia (FA), a cancer susceptibility disorder characterized by cellular sensitivity to ICL-inducing agents (Kottemann & Smogorzewska, 2013; Dong et al, 2015). Using a Xenopus egg extract-based assay, we and others have recently elucidated a molecular mechanism of replication-coupled ICL repair (Fig EV1; Räschle et al, 2008). This mechanism requires the convergence of
two replication forks at an ICL during DNA replication (Zhang et al., 2015). Both forks initially stall 20–40 nucleotides from the crosslink followed by CMG helicase unloading allowing one fork to approach to within 1 nucleotide of the crosslink (Räsänen et al., 2008; Fu et al., 2011; Long et al., 2011). Dual incisions on either side of the ICL then unhook the lesion from one of the strands. This critical repair step requires the endonuclease XPF (FANCP)-ERCC1, which is recruited to the ICL by the large scaffold protein SLX4 (FANCP), and depends on the activation of the Fanconi anemia pathway by ubiquitylation of the FANC-I-FANCDD complex (Knipscheer et al., 2009; Klein Douwel et al., 2014). After unhooking, a nucleotide is inserted across from the adducted base, followed by strand extension by REV1 and polymerase δ, consisting of REV7 (FANCV) and REV3 (Räsänen et al., 2008; Budzowska et al., 2015; Mamrak et al., 2016). This strand now acts as a template for repair of the opposite strand by HR (Long et al., 2011), leading to fully repaired products.

**Figure 1. Characterization of mutant XPF-ERCC1 complexes.**

A Schematic representation of the domain organization of the XPF protein. Domain boundaries of human and Xenopus laevis XPF are indicated. Relevant mutations of the human protein, and the Xenopus laevis equivalents, are indicated on top and bottom, respectively.

B Superdex 200 gel filtration column elution profile of wild-type XPF-ERCC1 and indicated mutant complexes. Aggregates eluted in the void volume of the column (~45 ml) while the active XPF-ERCC1 heterodimer eluted at ~65 ml. The peak eluting at ~105 ml contains the FLAG peptide used to elute the protein from the FLAG affinity resin. The heterodimer peak was isolated, and proteins were separated on SDS–PAGE and stained with Coomassie blue (inset).

C As in (B) but for different mutant complexes that showed more aggregation.

D Wild-type and indicated mutant XPF-ERCC1 complexes were incubated with a 5’-FAM-labeled stem-loop DNA substrate (10 nM) at room temperature for 30 min. Reaction products were separated on a 12% urea–PAGE gel and visualized using a fluorescence imaging system. Red arrow indicates position of incision by XPF-ERCC1.

E Wild-type and mutant XPF-ERCC1 complexes at various concentrations were incubated with a 5’-FAM-labeled 3’ flap DNA substrate (10 nM) and fluorescent anisotropy was measured. Graphs were fitted to calculate dissociation constants (Kd) as described in the Materials and Methods section. The error bars represent s.d. from three measurements. Experimental replicates are shown in Fig EV2.

Source data are available online for this figure.
Patient phenotypes linked to specific XPF mutations can be extremely valuable in determining pathway-specific functions. Most patients with a mutation in XPF suffer from a mild form of XP and are deficient in NER. These patients express residual protein and are likely proficient in ICL repair, because they do not show features of FA (Ahmad et al., 2010). In some cases, XPF mutations can lead to much more severe phenotypes. An extreme progeroid syndrome was caused by a mutation in the helicase-like domain of XPF (R153P). This patient suffered from neurological and hematological defects and a cellular sensitivity to UV and ICLs indicating both NER and ICL repair were defective (Niedernhofer et al., 2006). Another patient, with a mutation in the same XPF domain (C236R), presented with phenotypes of XP, but also of CS, such as developmental and neurological abnormalities (Kashiyama et al., 2013). This patient also showed FA-like features and ICL sensitivity suggestive of a defect in ICL repair. In addition, some patients with specific mutations in XPF were diagnosed with Fanconi anemia and showed no signs of XP (Bogliolo et al., 2013). These mutations were mapped to the helicase-like domain (L230P), and the nuclease domain (R689S) of XPF.

To examine what features of XPF-ERCC1 determine its specificity in ICL repair, we employed the Xenopus egg extract system. We monitored both replication-coupled ICL repair and nucleotide excision repair and identified five XPF mutants that are deficient in ICL repair and proficient in NER. Although all of these mutants showed a defect in ICL unhooking, the majority was still efficiently recruited to the ICL. In contrast, mutation of xXPF leucine 219, equivalent to the human leucine 230 mutated in Fanconi anemia, abrogated this ICL localization. This was caused by a defect in interaction with SLX4. We propose there are two interaction sites between XPF and SLX4. We next examined the endonuclease activity of the mutant XPF-ERCC1 complexes. To this end, a fluorescently labeled stem-loop substrate was incubated with increasing concentrations of protein, and reaction products were separated by denaturing urea–PAGE. All XPF-ERCC1 complexes with mutations in the helicase-like domain showed nuclease activity similar to wild-type protein, as seen by the appearance of the incision product (Fig 1D, top two panels, and Fig EV2B). Importantly, this demonstrates that the XEL219R and XEC225R mutant complexes behaved similarly to the wild type on gel filtration (Fig 1B) and the peak containing the heterodimer was isolated and used for further experiments. However, the XEL219R and XEC225R mutant complexes showed an increased aggregate peak and lower heterodimer peak (Fig 1C). Nevertheless, when this heterodimer peak was isolated and rerun on a gel filtration column, it did not aggregate (Fig EV2A).

We next examined the endonuclease activity of the mutant XPF-ERCC1 complexes. To this end, a fluorescently labeled stem-loop substrate was incubated with increasing concentrations of protein, and reaction products were separated by denaturing urea–PAGE. All XPF-ERCC1 complexes with mutations in the helicase-like domain showed nuclease activity similar to wild-type protein, as seen by the appearance of the incision product (Fig 1D, top two panels, and Fig EV2B). Importantly, this demonstrates that the XE R670S complex was slightly reduced in nuclease activity while the XER670S complex showed a more dramatic reduction and was only capable of cutting the substrate at high concentrations (Figs 1D and EV2D). This was not surprising as both mutations are located in the nuclease domain of XPF, and the human equivalent of the R670S mutant has decreased nuclease activity (Enzlin & Schaerer, 2002; Su et al., 2012). We also analyzed the endonuclease activity on a 3′ flap substrate and obtained similar results (Fig EV2C). In conclusion, all mutants except R670S have nuclease activity similar to wild-type protein.

Finally, we analyzed the DNA binding of the mutant XPF-ERCC1 complexes. For this, a fluorescently labeled 3′ flap substrate was incubated with increasing concentrations of XPF-ERCC1 complexes and fluorescence anisotropy was measured to assess binding. All our mutants showed very similar binding curves and the Kd values derived from these curves were comparable to each other and to wild-type XPF-ERCC1 (Figs 1E and EV2E). This indicates that the mutations do not affect DNA binding affinity. To validate our results, we measured fluorescence anisotropy of a mutant XPF-ERCC1 carrying two point mutations in ERCC1 (K247A and K281A) that were previously shown to affect DNA binding (Su et al., 2012). Consistent with this, we found that this XEA2AKA mutant had reduced affinity for DNA (Figs 1E and EV2E).

In summary, we purified six mutant XPF-ERCC1 complexes that are predicted to affect ICL repair. All mutant complexes form stable heterodimers and interact normally with DNA. The nuclease activity
of the mutants is similar to wild type, with the exception of one mutation in the nuclease domain.

**Mutations in helicase-like and nuclease domains abrogate ICL repair**

To investigate the effect of the XPF mutations on ICL repair, we used *Xenopus* egg extracts. This system recapitulates DNA replication-coupled repair of a sequence-specific cisplatin ICL situated on a plasmid template (pICL; Räschle et al., 2008). Moreover, it enables the quantification of repair by the regeneration of a Sapi restriction site that is blocked by the crosslink (Fig 2A). We immunodepleted ERCC1 from egg extract and complemented the repair reaction with wild-type or mutant XPF-ERCC1. Since depletion of ERCC1 leads to equal depletion of XPF (Klein Douwel et al., 2014), we refer to this depletion as an XPF-ERCC1 depletion. Because depletion of XPF-ERCC1 leads to co-depletion of SLX4, we also complemented all the depleted reactions with purified αSLX4 protein unless stated otherwise (Fig EV3A; Klein Douwel et al., 2014). Reactions were stopped at various time points, and DNA repair intermediates were isolated and digested with Sapi to quantify ICL repair. A small fraction of non-crosslinked plasmids is present in pICL preparations leading to a constant background of Sapi digestible replication products. XPF-ERCC1-depleted extracts did not support ICL repair above this background while addition of recombinant wild-type XPF-ERCC1 (XEWT) restored ICL repair (Fig 2B–D; Klein Douwel et al., 2014). XEΔL19R and XEΔC225R, which carry mutations in the helicase-like domain of XPF, did not efficiently rescue ICL repair (Figs 2B and EV3B). We then tested the other two helicase-like domain mutants that were expected to affect the interaction with the BTB domain of SLX4. While addition of XEG314E to XPF-ERCC1-depleted extract supported ICL repair, the deletion mutant XEANSGW was

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**Figure 2. Effect of mutations in XPF-ERCC1 on ICL repair in Xenopus egg extract.**

A Schematic representation of repair of a plasmid containing a site-specific cisplatin ICL (pICL) in *Xenopus* egg extract. The Sapi site that is blocked by the ICL becomes available on one of the replicated molecules after full repair via HR using the sister molecule (Fig EV1). The sister molecule is repaired by lesion bypass, but retains the unhooked ICL that is not removed efficiently in *Xenopus* egg extract (Raschle et al., 2008).

B XPF-ERCC1-depleted (ΔXE) and XPF-ERCC1-depleted extracts complemented with wild-type (XEWT) or indicated mutant XPF-ERCC1 (XEmut) were analyzed by Western blot using α-XPF antibodies (left panel). Line within blot indicates position where irrelevant lanes were removed. These extracts were used to replicate pICL. Replication intermediates were isolated and digested with HincII and Sapi, and separated on agarose gel. Repair efficiency, represented by Sapi digestible replication products, was calculated as described (Raschle et al., 2008) and plotted (right panel).

C, D As in (B) but analyzing different XPF-ERCC1 mutant complexes. Experimental replicates are shown in Fig EV3. Note: repair levels can differ per batch of individually prepared extract or per depletion experiment and can only be compared within an experiment. *, background band.

Data information: (B–D) #: Sapi fragments from contaminating uncrosslinked plasmid present in varying degrees in pICL preparations.

Source data are available online for this figure.
defective in ICL repair (Figs 2C and EV3C). This suggests that this region is important for ICL repair, likely through mediating interaction with SLX4. Finally, XPF-ERCC1-depleted extracts were supplemented with the nuclease domain mutants XE5670S and XE5767F. Both mutants were unable to restore ICL repair (Figs 2D and EV3B). These results show that specific residues in the helicase-like domain and the nuclease domain of XPF-ERCC1 are required for ICL repair.

**ICL repair-deficient XPF mutants are proficient in NER**

To determine whether the XPF mutations specifically affect ICL repair, we investigated their activity in nucleotide excision repair (NER). During NER, the endonucleases XPF-ERCC1 and XPG make incisions on either side of a lesion, creating a gap that is subsequently filled in. This gap-filling DNA synthesis is called unscheduled DNA synthesis (UDS) to differentiate it from the semi-conservative DNA synthesis that takes place during replication. UDS can be measured on UV-damaged plasmids incubated in a high-speed supernatant Xenopus egg extract and used as a readout for NER activity (Fig 3A; Shivji et al, 1994; Gaillard et al, 1996). To this end, we incubated non-damaged or UV-damaged plasmid in a non-replicating Xenopus egg extract in the presence of 32P-dCTP. The DNA was subsequently isolated and linearized, and the products were separated on an agarose gel (Fig 3B). While a UV-damaged DNA was subsequently isolated and linearized, and the products showed clear incorporation of 32P-dCTP indicative of UDS, a non-damaged plasmid only showed some background incorporation, probably due to nicks created during plasmid preparation (Fig 3B, lanes 1 and 2). To confirm that the UDS on the UV-damaged template is a result of the repair, we directly monitored cyclobutane pyrimidine dimers and showed that Xenopus egg extract is capable of removing these lesions (Fig EV4C). To further validate that the unscheduled DNA synthesis is caused by NER, we showed that UDS is strongly reduced after depletion of NER factors PCNA as well as XPA (Fig EV4D and E). In addition, we found that immunodepletion of XPF (Fig EV4A) strongly reduced UV-dependent UDS (Fig 3B, compare lanes 2 and 4, and Fig 3C). The slight increase in UDS compared to the non-damaged plasmid is likely caused by an incomplete depletion of XPF-ERCC1 or other repair mechanisms present in the extract. Addition of wild-type XPF-ERCC1 (XEWT) to XPF-depleted extracts fully rescued UDS, while addition of a catalytically inactive XE668A mutant did not support UDS (Fig 3B, compare lanes 5 and 10, Figs 3C and EV4B). This shows that XPF-ERCC1 is required for UDS in Xenopus egg extract. We then complemented an XPF-ERCC1-depleted extract with the XPF-ERCC1 mutants and found that all mutants were able to rescue the NER defect (Fig 3B and C). This observation was especially striking for the XER670S mutant whose nuclease activity on model DNA templates was strongly reduced (Fig 1D). This finding is consistent with a previous report in which the human equivalent of this mutant was able to make NER incisions, although there was a difference in the position of the incisions compared to the wild-type protein (Su et al, 2012).

In summary, we identified three mutations in the helicase-like domain (XE219R, XE225R and XEANSGW) and two in the nuclease domain (XE5670S and XE5767F) that are defective in ICL repair, while supporting functional NER. Strikingly, this separation of function is achieved by mutations in two different domains of XPF-ERCC1.
Separation-of-function mutations in XPF specifically affect ICL incisions

To determine the mechanism underlying the specific inhibition of ICL repair in these mutants, we examined which step in ICL repair was affected. We previously showed that XPF is required for ICL unhooking (Klein Douwel et al., 2014). However, XPF could also have additional roles downstream, for example, in HR, that might be affected by our mutations (Bergstralh & Sekelsky, 2008). To directly monitor unhooking incisions that take place on the parental strand, we pre-labeled pICL with $^{32}P$-dCTP using nick translation and replicated it in *Xenopus* egg extract. Replication intermediates were linearized and separated on a denaturing agarose gel. At early times, the parental strand migrates as a large X-structure, while after crosslink unhooking during repair, it is converted to a linear molecule and arms (Fig 4A; Knipscheer et al., 2009). The decline of the X-shaped structures and the accumulation of the lines are a direct readout of unhooking incisions. In XPF-ERCC1-depleted extract (Appendix Fig S1), the X-structures persist and the appearance of linear structures is greatly reduced (Fig 4B–D and Appendix Fig S1B–D), indicating the unhooking incisions are blocked (see also Klein Douwel et al., 2014). Addition of wild-type XPF-ERCC1 (XE$^{WT}$) rescues this incision defect (Fig 4B–D and Appendix Fig S1B–D) whereas the helicase-like domain mutants XE$^{L219R}$ or XE$^{C225R}$ did not (Fig 4B and Appendix Fig S1B). The XE$^{G314E}$ mutant did not inhibit incisions, while the XE$^{NSGW}$ mutant caused a strong reduction in ICL unhooking (Fig 4C and Appendix Fig S1C). This is consistent with our earlier observation that the point mutant is functional in ICL repair while the deletion mutant is not. Finally, we found that the nuclease domain mutants XE$^{R670S}$ and XE$^{S767F}$ were unable to support efficient incisions (Fig 4D and Appendix Fig S1D). These results show that all our separation-of-function mutants are defective in ICL unhooking, which explains their inability to support ICL repair.

The XPF$^{C225R}$–ERCC1 mutant complex is not recruited to the ICL

A possible explanation for why our mutants were not able to support ICL unhooking is that they are not recruited to the site of damage. To test this, we examined XPF recruitment by chromatim immunoprecipitation (ChIP). We replicated pICL in extract depleted of XPF-ERCC1 and supplemented with wild-type or mutant XPF-ERCC1 (Appendix Fig S2A), and performed chromatim immunoprecipitation with XPF antibodies at various time points. An unrelated precipitated DNA was recovered and amplified by quantitative PCR with primers specific to the ICL region or to pQuant (Fig 5A). Using this assay, we recently showed that XPF is specifically recruited to the ICL at the time of unhooking incisions (Klein Douwel et al., 2014). The exact timing of recruitment can vary as a result of the immunodepletion procedure. We first examined the most N-terminal mutant XE$^{L219R}$ and found that, in contrast to the wild-type protein, recruitment of this mutant to the ICL was completely blocked (Fig 5B). In contrast, the XE$^{C225R}$ complex, containing a mutation just six residues further downstream, was recruited to the ICL as efficiently as the wild-type protein (Fig 5C and Appendix Fig S2B). We then examined the XE$^{NSGW}$ mutant and found that it was recruited normally to the ICL (Fig 5D and Appendix Fig S2C). This is striking, because this region was shown to be important for the interaction between XPF and SLX4 (Andersen et al., 2009) and we have previously shown that SLX4 is important for the recruitment of XPF to the ICL. Lastly, we examined the recruitment of the nuclease domain mutants XE$^{R670S}$ and XE$^{S767F}$. Both mutants were recruited...
to the ICL as efficiently as the wild-type protein (Fig 5E and F and Appendix Fig S6D).

We conclude that all mutants, except for XE<sup>L219R</sup>, are recruited normally to the site of damage, suggesting the defect in incisions observed for these mutants is due to a defect in proper positioning of the nucleosome within the repair complex.

XPF leucine 219 is part of the major binding site between XPF and SLX4

To determine why XE<sup>L219R</sup> is not recruited to the ICL, we examined recruitment of both SLX4 and XPF to the ICL by ChIP. When we supplemented an XPF-ERCC1-depleted reaction with XPF-ERCC1 only, and not SLX4, XPF was not recruited to the ICL (Figs 6A and EV5A; and Klein Douwel et al, 2014). Supplemented SLX4 bound to the ICL and rescued the recruitment of wild-type XPF-ERCC1 (Figs 6A and EV5A; and not of the XE<sup>L219R</sup> mutant complex (Figs 6A and EV5A–C). These results show that a single point mutations can abrogate XPF recruitment and strongly suggest that this is caused by a defect in the direct interaction with SLX4.

To confirm this, we co-expressed FLAG-tagged XE<sup>WT</sup> and XE<sup>L219R</sup> with His-tagged SLX4 in Sf9 insect cells, immunoprecipitated XPF, and examined co-precipitation of SLX4. His-SLX4 was enriched after with His-tagged SLX4 in Sf9 insect cells, immunoprecipitated XPF, and SLX4 was examined co-precipitation of SLX4. His-SLX4 was enriched after immunoprecipitation of wild-type XPF-ERCC1 (Figs 6A and EV5A; and not of the XE<sup>L219R</sup> mutant complex (Figs 6A and EV5A–C). These results show that a single point mutations can abrogate XPF recruitment and strongly suggest that this is caused by a defect in the direct interaction with SLX4.

To confirm this, we co-expressed FLAG-tagged XE<sup>WT</sup> and XE<sup>L219R</sup> with His-tagged SLX4 in Sf9 insect cells, immunoprecipitated XPF, and examined co-precipitation of SLX4. His-SLX4 was enriched after immunoprecipitation of wild-type XPF-ERCC1, but not XE<sup>L219R</sup>. ERCC1, indicating this mutant does not bind SLX4 (Figs 6B and EV5D). These findings indicate that XPF’s leucine 219 is essential for the interaction between XPF and SLX4 and therefore required for the recruitment of XPF to the site of damage.

Two domains in SLX4 have been implicated in the interaction between SLX4 and XPF. The previously mentioned BTB domain and the MUS312/MEI9 interaction-like, or MLR, domain (Fig 6C; Fekairi et al, 2009; Kim et al, 2013). To further investigate the importance of the MLR domain for the interaction with XPF, we purified xISLX4<sup>WT</sup> and xISLX4<sup>AMLR</sup>. In contrast to wild-type SLX4, the AMLR mutant was not able to bind XPF from *Xenopus* egg extract (Fig 6D). This shows that the MLR domain of SLX4 acts as the major interaction site with XPF, which is in line with previous reports in human cells (Kim et al, 2013). Based on our data, this domain most likely interacts with leucine 219 of XPF.

Finally, we set out to examine the role of the interaction between the SLX4 BTB domain and XPF. The hsG325E mutation in XPF abrogates the interaction between XPF and the BTB domain of SLX4 (Andersen et al, 2009). We found that the XE<sup>G314E</sup> and XE<sup>ANSGW</sup> mutants were both able to interact normally with full-length SLX4 (Figs 6B and EV5D). This is consistent with previous reports showing that the BTB domain is not essential for SLX4 and XPF interaction (Kim et al, 2013; Guervilly et al, 2015). One explanation for these observations is that this interaction is transient and can only be observed in the absence of the major interaction site involving the MLR domain. To be able to study this, we cloned and purified the SLX4 BTB domain alone and examined the interaction with XPF using size exclusion chromatography. However, even using an excess of BTB domain protein, we did not observe an interaction with XPF-ERCC1 (Fig EV5E). This indicates that this is not a high-affinity interaction site.

These findings, together with previous reports, support a model in which XPF and SLX4 interact through two binding sites (Fig 7). The first consists of the MLR domain of SLX4 and XPF leucine 219, and possibly a region around this residue. This is a high-affinity binding site that is responsible for the recruitment of XPF via SLX4.
Mutations in XPF-ERCC1 affect several DNA repair pathways and can cause multiple diseases likely due to differential inhibition of these pathways. Using Xenopus egg extracts, we have examined how certain mutations in XPF inhibit ICL repair, while maintaining proficient nucleotide excision repair. We have characterized five separation-of-function mutations that reside in the helicase-like and nuclease domains of XPF. While all these mutants are defective in ICL unhooking, this is caused by different mechanisms. The nuclease domain mutants are normally recruited to the ICL and most likely affect interactions with the DNA template or specific protein–protein interactions important for substrate recognition (Fig 7). The helicase-like domain mutants are part of a dual interaction site with SLX4. XPF’s leucine 219 is part of a high-affinity interaction site that interacts with the MLR domain of SLX4, while deletion of residues 312–315 of XPF disrupts a transient second interaction site with the BTB domain of SLX4 (Fig 7 and Table 1).

We have previously shown that XPF-ERCC1 is recruited to the site of damage by SLX4 (Klein Douwel et al, 2014). A specific residue in the helicase-like domain of XPF has been implicated in the interaction with SLX4 (Yildiz et al, 2002; Andersen et al, 2009). A glycine to glutamic acid mutation at residue 325 of human XPF abrogated the interaction with SLX4 in a yeast two-hybrid assay (Andersen et al, 2009). This yeast two-hybrid assay was performed with C-terminal deletion mutants of SLX4 and the interaction with XPF was pinpointed to the BTB domain. However, these mutants lacked the MLR domain which has also been implicated in the interaction with XPF (Kim et al, 2013). In our hands, the equivalent mutation in xXP, G314E, did not abrogate ICL repair or recruitment. Moreover, a deletion mutant in which this glycine and three additional residues around it were removed (XPFΔG314E) interacted

**Discussion**

Leucine 219 in the helicase-like domain of XPF is essential for the interaction of XPF with the MLR domain of SLX4. This interaction mediates the recruitment of XPF to an ICL. Residues 312–315 transiently interact with the BTB domain of SLX4 and are required for the incisions of an ICL by XPF. Arginine 670 and serine 767 in the nuclease domain of XPF are crucial for the recognition of the ICL substrate.

**Figure 6.** XPF leucine 219 is part of the major interaction site between XPF and SLX4.

A pICL was replicated in XPF-ERCC1-depleted (ΔXE) extract or in XPF-ERCC1-depleted extract supplemented with wild-type XPF-ERCC1 only (+XEWT), wild-type XPF-ERCC1 and SLX4 (+XESXEWT), or XPFΔG314E-ERCC1 and SLX4 (+XEG314EXEWT, see Fig EV5A). Samples were taken at the indicated times and immunoprecipitated with α-XPF (left panel) or α-SLX4 antibodies (right panel). Co-precipitated DNA was isolated and analyzed by quantitative PCR using ICL or pQuant primers. The qPCR data were plotted as the percentage of peak value with the highest value set to 100%.

B Wild-type and mutant FLAG-XPF-ERCC1 are co-expressed with His-SLX4 in Sf9 insect cells. Cells were lysed and XPF was immunoprecipitated via the FLAG-tag antibodies (left panel) or SLX4 antibodies (right panel). Line within blot indicates position where irrelevant lanes were removed. *, background band.

C Purified wild-type FLAG-SLX4 and FLAG-SLX4ΔMLR were added to Xenopus egg extract. SLX4 was immunoprecipitated via the FLAG-tag. Samples were analyzed by Western blot using α-FLAG and α-XPF antibodies. Line within blot indicates position where irrelevant lanes were removed.

D Purified wild-type FLAG-SLX4 and FLAG-SLX4ΔMLR were added to Xenopus egg extract. SLX4 was immunoprecipitated via the FLAG-tag. Samples were analyzed by Western blot using α-FLAG and α-XPF antibodies. Line within blot indicates position where irrelevant lanes were removed.

Source data are available online for this figure.
normally with SLX4. Based on these observations, we suggest that the interaction site between the BTB domain of SLX4 and residues 312–315 of XPF is a minor interaction site. This is consistent with our data showing that the isolated BTB domain does not interact strongly with XPF and with results reported by Guervilly et al. that show only a slight decrease in XPF binding after mutation of the SLX4 BTB domain (Guervilly et al., 2015). However, this interaction is important because the XPFL219R-ERCC1 mutant complex is defective in ICL unhooking and repair (Figs 2 and 4). Therefore, we propose that this transient interaction site is important for activation of XPF-ERCC1 by ensuring correct positioning onto its substrate. Interestingly, SLX4 has been shown to stimulate XPF-ERCC1 activity on model substrates (Hodskinson et al., 2014) which could be mediated through this interaction. Although we cannot exclude that this ICL repair defect of the XPFLANISGW-ERCC1 mutant is caused by a different mechanism, the fact that this mutant completely overlaps with a previously identified interaction site strongly supports this explanation.

We and others have shown that the MLR domain of SLX4 is essential for the interaction with XPF (Fig 6 and Kim et al., 2013), but it was not known which site on XPF was involved in this interaction. We now show that the XPF leucine 219 to arginine mutant is defective in binding to SLX4 indicating this is the site that interacts with the MLR domain. Further examination of the residues surrounding leucine 219 is required to better characterize this interaction site. Notably, our ChIP results indicate that the cysteine at position 225 is not required for the interaction. We further show that the XPFL219R-ERCC1 mutant complex is defective in ICL repair but not in NER. This shows that the interaction with SLX4 is specific to the role of XPF-ERCC1 in ICL repair. This is consistent with the fact that patients with an L230P mutation suffer from FA and not XP. While previously it was assumed that poor stability of the XE323OP protein was causing the FA phenotype, these data suggest that a functional defect, namely impaired interaction with SLX4, may cause, or contribute to, the disease.

Interestingly, the XPFCL225R-ERCC1 mutant does affect ICL recruitment but is still defective in ICL repair. Possibly this mutant does not prevent binding to SLX4, but does affect the interaction site in a way that it cannot properly position XPF for incisions. Two patients have been identified carrying the C236R (xlC225R) mutation, both show Cockayne syndrome (CS) phenotypes, while only one patient additionally shows a Fanconi anemia phenotype. The Cockayne syndrome phenotype is thought to be caused by a specific defect in transcription-coupled nucleotide excision repair (TC-NER). While mutations in XPF are not expected to specifically affect TC-NER, because it acts downstream in the NER pathway where the transcription-coupled and global NER pathways have come together, a CS phenotype has been observed previously in patients with mutations in XPF (Kashiyma et al., 2013). Our data show that the xIC225R mutation prevents ICL repair, but does not affect NER. However, Xenopus egg extracts are transcription incompetent and we may therefore not identify a defect in TC-NER. Why only one of the patients carrying the C236R mutation presents with a clear FA phenotype is currently unclear. Possibly the other patient has an additional mutation or specific genetic background that neutralizes the ICL repair defect.

In addition to the helicase-like domain mutants, we found two separation-of-function mutants in the nuclease domain of XPF. Arginine 670 is located within the active site of xIXPF and mutating it to a serine severely reduces nuclease activity as was shown by us and others (Bogliolo et al., 2013). Nevertheless, our data indicate that the XEP670S mutant can still support NER to wild-type levels. This is in line with previous data showing the human equivalent, hS689S, can incise an NER substrate (Enzlin & Schärer, 2002; Staresinic et al., 2009; Su et al., 2012). Interestingly, the human mutant protein did show a shift in incision position, suggesting the residue is not directly involved in catalysis, but contributes to the proper orientation of the active site onto the DNA substrate (Su et al., 2012). This aberrant positioning is apparently not detrimental for NER but does prevent ICL repair in our assays. This is a likely explanation as the DNA template for incision differs in both repair pathways. Moreover, it is supported by the identification of a patient with the hS689S mutation that suffers from FA, but not XP (Bogliolo et al., 2013).

The S767F mutation is also located in the nuclease domain and structure predictions based on the crystal structure of the nuclease domain of archaeal XPF in complex with DNA, indicating that it could be involved in protein–DNA interaction (Newman et al., 2005). In our experiments, this mutant shows a mild reduction in nuclease activity, is proficient in NER but largely deficient in ICL repair. We propose that, like the arginine 670, this residue is important in positioning the active site specifically on an ICL template likely by direct contact with the DNA. We did not observe reduced DNA binding affinity for these mutants most likely because XPF-ERCC1 contains multiple DNA interacting domains and it was shown that mutation of at least two of those is required to reduce this affinity (Su et al., 2012).

XPF-ERCC1 is essential for the repair of ICLs induced by chemotherapy agents, such as derivatives of cisplatin and nitrogen

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**Table 1. Summary of features of different XPF mutations.**

| Mutation        | Slx4 binding | Nucleolytic activity | DNA binding | NER | ICL repair | Unhooking | Recruitment to ICL | Slx4 binding |
|-----------------|--------------|----------------------|-------------|-----|------------|-----------|---------------------|--------------|
| XPFCL219R       | ++           | +                    | ++          | --  | --         | ++        | N.D                 | N.D          |
| XPFCL225R       | ++           | +                    | +           | --  | --         | ++        | N.O                 | N.D          |
| XPFANISGW       | N.A.         | N.A.                 | ++          | ++  | --         | --        | N.D                 | N.D          |
| XPER670S        | N.A.         | N.A.                 | ++          | ++  | --         | --        | N.D                 | N.D          |
| XPS767F         | N.A.         | N.A.                 | ++          | ++  | --         | --        | N.D                 | N.D          |

Abbreviations are as follows: +, normal; -, absent or defective; NA, not applicable; ND, not determined.
mustards (Kirschner & Melton, 2010). Moreover, high expression of ERCC1 has been associated with poor response to chemotherapy in many cancers and could be a potential target to overcome resistance (McNeil & Melton, 2012). A better understanding of the ICL repair function of XPF-ERCC1 could potentially lead to the design of ICL-specific inhibitors that could be beneficial in cancer treatment.

**Materials and Methods**

**Protein expression and purification**

His-tagged hsERCC1 was cloned into pDONR201 (Life Technologies). FLAG-tagged xISXF was cloned into pFastBac1 (Life Technologies) and in pDONR201. The XPF-ERCC1 (L219R, C225R, G314E, ANSGW, R670S, D668A, and S767F) and ERCC1 mutation (K247A/K281A) were introduced in pDONR-XPF using QuikChange site-directed mutagenesis protocol. Baculoviruses were produced using the BAC-to-BAC system (xISXFWT), or the BaculoDirect system (hsERCC1 and xISXFΔTTT) following manufacturer’s protocol (Life Technologies). Proteins were expressed in suspension cultures of SF9 insect cells by co-infection with His-hsERCC1 (or His-hsERCC1K247A/K281A) and FLAG-xISXF (or FLAG-xISXF mutants) viruses for 72 h. Cells from 750 ml culture were collected by centrifugation, resuspended in 30 ml of lysis buffer (50 mM K$_2$HPO$_4$ pH 8.0, 500 mM NaCl, 0.1% NP-40, 10% glycerol, 0.4 mM PMSF, 1 tablet/50 ml Complete Mini EDTA-free Protease Inhibitor Cocktail (Roche), 10 mM imidazole), and lysed by sonication. The soluble fraction obtained after centrifugation (40,000 × g for 40 min at 4°C) was incubated for 1 h at 4°C with 1 ml of Ni-NTA-agarose (Qiagen) that was pre-washed with lysis buffer. After incubation, the beads were washed using 50 ml of wash buffer (50 mM K$_2$HPO$_4$ pH 8.0, 300 mM NaCl, 0.1% NP-40, 10% glycerol, 0.1 mM PMSF, 10 μg/ml aprotonin/leupeptin, 20 mM imidazole). The xISXF-hsERCC1 complex was eluted in elution buffer (50 mM K$_2$HPO$_4$ pH 8.0, 300 mM NaCl, 0.1% NP-40, 10% glycerol, 0.1 mM PMSF, 10 μg/ml aprotonin/leupeptin, 250 mM imidazole). The eluate was diluted with FLAG-wash buffer I (20 mM K$_2$HPO$_4$ pH 8.0, 200 mM NaCl, 0.1% NP-40, 10% glycerol, 0.4 mM PMSF) and incubated for 1 h at 4°C with 500 μl of anti-FLAG M2 affinity gel (Sigma) that was pre-washed with FLAG-wash buffer I. After incubation, the beads were washed with 30 ml of FLAG-wash buffer I, and subsequently with 30 ml of GF buffer (25 mM Hepes pH 8.0, 200 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol). The xISXF-hsERCC1 complex was eluted in 3 ml of GF buffer containing 100 μg/ml 3 × FLAG peptide (Sigma). The protein was then loaded onto a HiLoad 16/60 Superdex 200 pg gel filtration column (GE Healthcare) equilibrated in GF buffer. Fractions containing the xISXF-hsERCC1 heterodimer were eluted between 60 and 70 ml (Enzlin & Schärer, 2002), pooled, and concentrated with an Amicon Ultra-4 centrifuge filter unit, 30 kDa (Merck Millipore). Protein was aliquoted, flash-frozen, and stored at −80°C.

Increasing concentrations of protein were incubated with 10 nM of a 3’ flap DNA substrate containing a 5’-fluorescent FAM label (see Nuclease assay). The reaction was incubated with annealing buffer (25 mM Hepes pH 8.0, 15% glycerol, 0.1 mg/ml BSA, 2 mM CaCl$_2$) in a 384-well plate (kBioscience) for 1 h at room temperature, and fluorescent anisotropy was measured on a SpectraMax i3 (Molecular Devices). The data were fitted using Origin 8.5 to the equation $y = a + b/x + x/y$, where $x$ is the protein concentration, $y$ is the fluorescence anisotropy, and $k$ is the $K_d$ value.

**DNA replication and repair assay in Xenopus egg extracts**

DNA replication and preparation of Xenopus egg extracts (HSS and NPE) were performed as described previously (Walter et al., 1998; Tutter & Walter, 2006). Preparation of plasmid with a site-specific cisplatin ICL (pICL), and ICL repair assays were performed as
described (Räschle et al, 2008; Enou et al, 2012). Briefly pICL was incubated with HSS for 20 min, following addition of two volumes of NPE (t = 0) containing $^{32}$P-$\alpha$-dCTP. Aliquots of replication reaction (4–10 µl) were stopped at various times with ten volumes of Stop Solution II (0.5% SDS, 10 mM EDTA, 50 mM Tris pH 7.5). Samples were incubated with RNase (0.13 µg/µl) followed by proteinase K (0.5 µg/µl) for 30 min at 37°C each. DNA was extracted using phenol/chloroform, ethanol-precipitated in the presence of glycogen (30 mg/ml), and resuspended in 5–10 µl of 10 mM Tris pH 7.5. ICL repair was analyzed by digesting 1 µl of extracted DNA with HincII, or HincII and SapI, separation on a 0.8% native agarose gel, and quantification using autoradiography. Repair efficiency was calculated as described (Knipscheer et al, 2012).

**Unscheduled DNA synthesis**

The assay to monitor unscheduled DNA synthesis (UDS) in *Xenopus* egg extract was adapted from Gaillard et al (1996). A 6.25-µl reaction containing 2.5 µl HSS and 6 ng/µl non-treated or UV-C-irradiated (350 µJ/m²) pControl was supplemented with 5 mM MgCl$_2$, 0.5 mM DTT, 4 mM ATP, 40 mM phosphocreatine, 0.5 µg creatine phosphokinase, and 80 µCi/ml $^{32}$P-$\alpha$-dCTP (3,000 Ci/mmol). Reactions were incubated at room temperature for 2 h and stopped by addition of ten volumes of Stop Solution II (0.5% SDS, 10 mM EDTA, 50 mM Tris pH 7.5). Samples were incubated with proteinase K (0.5 µg/µl) for 30 min at 37°C. DNA was extracted using phenol/chloroform, ethanol-precipitated in the presence of glycogen (30 mg/ml), and resuspended in 5–10 µl of 10 mM Tris pH 7.5. Extracted DNA (2 µl) was digested with HincII and separated on a 0.8% native agarose gel. The gel was stained with SYBR GOLD (Fisher) and subsequently dried and quantified using autoradiography. The background signal from non-treated plasmid was subtracted. To compare the levels of UDS between experiments, the background signal from non-treated plasmid was set to 100%. This is because the efficiency of UDS can differ between extract preparations and depletions.

**Antibodies and immunodepletions**

Antibodies were raised against residues 444–797 of xXP, full-length xERCC1, and residues 825–1,052 of xSLX4. Specificity was confirmed using Western blot (Klein Douwel et al, 2014). XPF-ERCC1 was removed from extract using three rounds of depletion with the xERCC1 serum (HSS and NPE). ERCC1 depletion was described previously (Klein Douwel et al, 2014). For the unscheduled DNA synthesis assay, HSS was depleted using three rounds of depletion with the xXP serum (one volume of PAS was bound to one volumes of anti-serum or pre-immune serum, and added to four volumes of HSS) followed by three rounds of depletion with the xXP serum (one volume of PAS was bound to three volumes of anti-serum or pre-immune serum, and added to five volumes of HSS). Anti-FLAG M2 antibody was purchased from Sigma and His-antibody from Westburg.

**Incision assay**

Incision assay was performed as described in Klein Douwel et al (2014). Briefly, pICL and pQuant were labeled via nick translation. pQuant was added as an internal control to allow accurate calculation of incision efficiency. pICL (225 ng) and pQuant (11.25 ng) were incubated in 1.5 units of NB-BSR DI enzyme (NEB) and 1× NEBuffer 2 for 30 min at room temperature. Subsequently, 11 µl of DNA Polymerase I mix (5 units of DNA polymerase I (NEB), dATP, dGTP, dTTP (0.5 mM each), dCTP (0.4 µM), $^{32}$P-$\alpha$-dCTP (3.3 µM) in 1× NEBuffer 2) was added and this was incubated for 3 min at 16°C. The reaction was stopped with 180 µl of Stop Solution II, treated with proteinase K, and phenol/chloroform-extracted. Excess label was removed using a Micro Bio-Spin 6 Column (Bio-Rad). After ethanol precipitation, the pellet was resuspended in 5 µl of ELB (10 mM HEPES-KOH pH 7.7, 50 mM KCl, 2.5 mM MgCl$_2$, and 250 mM sucrose). The labeled plasmid (pICL) was used in a replication reaction and samples at various times were extracted and digested with HincII. Fragments were separated on a 0.8% alkaline denaturing agarose gel for 18 h at 0.85 Volts/cm, after which the gel was dried and exposed to a phosphor screen. Quantification was performed using ImageQuant software (GE healthcare). The highest value was set at 100% for the X-shape and the linear products.

**Chromatin immunoprecipitation**

Chromatin immunoprecipitation (ChIP) was performed as described (Pacek et al, 2006). Briefly, reaction samples were crosslinked with formaldehyde, sonicated to yield DNA fragments of roughly 100–500 bp, and immunoprecipitated with the indicated antibodies. Protein–DNA crosslinks were reversed and DNA was phenol/chloroform-extracted for analysis by quantitative real-time PCR with the following primers: ICL (5’-AGCCAGATTTTCTCCTC-3’ and 5’-CATGCATTGTCTGCACCTT-3’) and pQuant (5’-TACAAATGTA CGGCCAGCAA-3’ and 5’-GAGTATGAGGGAAACCGGTGA-3’). The values from pQuant primers were subtracted from the values for pICL primers.

**Immunoprecipitations**

Proteins were expressed in adherent cultures of S99 insect cells in 6-well plates by co-infection with His-hsERCC1, His-xSLX4, and FLAG-xXP (or FLAG-xXP mutants) viruses for 72 h. Cells were resuspended in medium and collected by centrifugation, resuspended in 250 µl of lysis buffer (50 mM Tris pH 8.0, 300 mM NaCl, 1% Triton, 4 mM EDTA, 10 µg/ml aprotinin/leupeptin), and lysed by sonication. After centrifugation (20,000 × g for 20 min at 4°C), 200 µl of soluble fraction was incubated for 30 min at 4°C with 8 µl of FLAG M2 beads (Sigma-Aldrich) that were pre-washed with lysis buffer. After incubation, the beads were washed using 2 ml lysis buffer. Beads were taken up in 50 µl of 2× SDS sample buffer and incubated for 5 min at 95°C. Proteins were loaded on SDS–PAGE and visualized by Western blot using respective antibodies. For immunoprecipitations from *Xenopus* egg extract, FLAG-tagged xSLX4 WT or xSLX4 AMLR protein was added to NPE/HSS at a concentration of 5 ng/µl. To each 20 µl extract, 45.5 µl of IP buffer (1×ELB salts, 0.25 M sucrose, 75 mM NaCl, 2 mM EDTA, 10 µg/ml aprotinin/leupeptin, 0.1% NP-40) and 10 µl pre-washed FLAG M2 beads (Sigma-Aldrich) were added. Beads were incubated for 90 min at 4°C and subsequently washed using 2.5 ml IP buffer. Beads were taken up in 30 µl of 2× SDS sample buffer and incubated
for 5 min at 95°C. Proteins were loaded on SDS–PAGE and visualized by Western blot using respective antibodies.

Expanded View for this article is available online.

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Author contributions
DKD and PK designed the experiments. DKD performed the majority of the experiments. WSH performed IP assays from extract and insect cells. RACMB performed some of the ChIP assays. DKD analyzed the data, and DKD and PK discussed the data with the help of the other authors. DKD and PK wrote the manuscript and WSH and RACMB commented on the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.

References
Ahmad A, Enzlin JH, Bhagwat NR, Wijgers N, Raams A, Appledoorn E, Theil AF, Hoeijmakers JH, Vermeulen W, Jaspers NG, Schärer OD, Niedenhofer Lj (2010) Mislocalization of XPF-ERCC1 nucleosome contributes to reduced DNA repair in XP-F patients. PLoS Genet 6: e1000871
Andersen SL, Bergstrøhl DT, Kohl KP, LaRoque J, 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
Bergstrøhl DT, Sekelsky J (2008) Interstrand crosslink repair: can XPF-ERCC1 be left off the hook? Trends Genet 24: 70 – 76
Bogliolo M, Schuster B, Stoepker C, Derkunt B, Su Y, Raams A, Trujillo JP, Enzlin JH, Schärer OD (2012) Repair of cisplatin-induced DNA interstrand crosslinks by a replication-independent pathway involving transcription-coupled repair and translesion synthesis. Nucleic Acids Res 40: 8953 – 8964
Enzlin JH, Schärer OD (2002) The active site of the DNA repair endonuclease XPF-ERCC1 forms a highly conserved nucleosome motif. EMBO J 21: 2045 – 2053
Fekairi S, Scaglione S, Chahwan C, Taylor E, Tissier A, Coulon S, Dong MQ, Ruse C, Yates JR, Russell P, Fuchs RP, McCowan CH, Gaillard PHL (2009) Human SLX4 is a homodimeric resolvase subunit that binds multiple DNA repair/recombination endonucleases. Cell 138: 78 – 89
Friedberg EC (2011) Fluorescence-based incision assay for human XPF-ERCC1: a highly sensitive and specific method for detecting nucleotide excision repair of DNA: the very early history. DNA Repair 10: 668 – 672
Fu Y, Yardimci H, Long DT, Ho TV, Guainazzi A, Bermudez VP, Hurwitz J, van Oijen A, Schärer OD, Walter JC (2011) Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. Cell 146: 931 – 941
Gaillard PH, Martini EM, Kaufman PD, Stillman B, Moustacchi S, Almouzni G (1996) Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. Cell 86: 887 – 896
Gillett LC, Schärer OD (2006) Molecular mechanisms of mammalian global genome nucleotide excision repair. Chem Rev 106: 253 – 276
Guervilly J, Takedachi A, Naim V, Scaglione S, Chawhan C, Lovera Y, Desprès E, Kuraoka I, Kannouche P, Rosselli F, Gaillard P-HL (2015) The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. Mol Cell 57: 123 – 137
Hashimoto K, Wada K, Matsumoto K, Moriya M (2015) Physical interaction between SLX4 (FANCXP) and XPF (FANCQ) proteins and biological consequences of interaction-defective missense mutations. DNA Repair 35: 48 – 54
Hodkinson MRG, Silhan J, Crossan GP, Garaycochea JJ, Mukherjee S, Johnson CM, Schärer OD, Patel KJ (2014) Mouse SLX4 is a tumor suppressor that stimulates the activity of the nucleosome XPF-ERCC1 in DNA crosslink repair. Mol Cell 54: 472 – 484
Huang JC, Svoboda DL, Reardon JT, Sancar A (1992) Human nucleotide excision nucleosome removes thymine dimers from DNA by incising the 22nd phosphodiester bond 5’ and the 6th phosphodiester bond 3’ to the photodimer. Proc Natl Acad Sci USA 89: 3664 – 3668
Kashiyama K, Nakazawa Y, Pilz DT, Guo C, Shimada M, Sasaki K, Fawcett H, Wing JF, Lewin SO, Carr L, Li T, Yoshiura K, Utani A, Hirano A (2013) Malfunction of nucleosome ERCC1-XPF results in diverse clinical manifestations and causes cockayne syndrome, Xeroderma Pigmentosum, and Fanconi Anemia. Am J Hum Genet 92: 1 – 13
Kim Y, Spitz GS, Veturu U, Lach FP, Fuerbacher AD, Smogorzewska A (2013) Regulation of multiple DNA repair pathways by the Fanconi anemia protein SLX4. Blood 121: 54 – 63
Kirschner K, Melton DW (2010) Multiple roles of the ERCC1-XPF endonuclease in DNA repair and resistance to anticancer drugs. Anticancer Res 30: 3223 – 3232
Klein Douwel D, Boonen RACM, Long DT, Szympska AA, Räschle M, Walter JC, Knipscheer P (2014) XPF-ERCC1 acts in unhooking DNA interstrand crosslinks in cooperation with FANCD2 and FANCPSLX4. Mol Cell 54: 460 – 471
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
Dong H, Neberdt DW, Bruford EA, Thompson DC, Joenje H, Vasiliiu V (2015) Update of the human and mouse Fanconi anemia genes. Hum Genomics 9: 32

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

Knipscheer P, Räsche M, Schärer OD, Walter JC (2012) Replication-coupled DNA interstrand cross-link repair in Xenopus egg extracts. Methods Mol Biol 920: 221 – 243

Kochaniak AB, Habuchi S, Loparo JJ, Chang DJ, Cimprich KA, Walter JC, van Oijen AM (2009) Proliferating cell nuclear antigen uses two distinct modes to move along DNA. J Biol Chem 284: 17700 – 17710

Kottemann MC, Smogorzewska A (2013) Fanconi anemia and the repair of Watson and Crick DNA crosslinks. Nature 493: 356 – 363

Kuraoka A, Kobertz WR, Ariza RR, Biggerstaff M, Essigmann JM, Wood RD (2000) Repair of an interstrand DNA cross-link initiated by ERCC1-XPF repair/recombination nuclease. J Biol Chem 275: 26632 – 26636

Li L, Peterson CA, Lu X, Legerski RJ (2005) Cip1 inhibits DNA repair but not PCNA-dependent nucleotide excision repair. Curr Biol 15: 1993 – 1998

Long DT, Räsche M, Jokou V, Walter JC (2011) Mechanism of RAD51-dependent DNA interstrand cross-link repair. Science 333: 84 – 87

Mamrak NE, Shimamura A, Howlett NG (2016) Recent discoveries in the molecular pathogenesis of the inherited bone marrow failure syndrome Fanconi anemia. Blood Rev doi: 10.1016/j.brrv.2016.10.002

McNeill EM, Melton DW (2012) DNA repair endonuclease ERCC1-XPF as a novel therapeutic target to overcome chemoresistance in cancer therapy. Nucleic Acids Res 40: 9990 – 10004

Newman M, Murray-Rust J, Lally J, Rudolf J, Fadden A, Knowles PP, White MF, McDonald NQ (2005) Structure of an XPF endonuclease with and without DNA suggests a model for substrate recognition. EMBO J 24: 895 – 905

Niedernhofer LJ, Odijk H, Budzowska M, van Drunen E, Maas A, Theil AF, de Wit J, Jaspers NJG, Beverloo HB, Hoeijmakers JHJ, Kanar 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

Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, Odijk H, Oostendorp R, Ahmad A, van Leeuwen W, Theil AF, Vermeulen W, van der Horst GTJ, Meinecke P, Kleijer WJ, Vijg J, Jaspers NJG, Hoeijmakers JHJ (2006) A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature 444: 1038 – 1043

Osorio A, Bogliolo M, Fernández V, Barroso A, de la Hoya M, Caldés T, Lasa A, Ramón y Cajal T, Santamaría M, Vega A, Quiles F, Lázaro C, Diez O, Fernández D, González-Sarmiento R, Durán M, Piqueras JF, Marín M, Pujol R, Surrallés J et al (2013) Evaluation of rare variants in the Fanconi anemia gene ERCC4 (FANCC) as familial breast/ovarian cancer susceptibility alleles. Hum Mutat 34: 1615 – 1618

Pacek M, Tutter AV, Kubota Y, Takisawa H, Walter JC (2006) Localization of MCM2–7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication. Mol Cell 21: 581 – 587

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

Shivji MK, Grey SJ, Strausfeld UP, Wood RD, Blow JJ (1994) Cip1 inhibits DNA replication but not PCNA-dependent nucleotide excision repair. Curr Biol 4: 1062 – 1068

Spivak G (2015) Nucleotide excision repair in humans. DNA Repair 36: 13 – 18

Staresinic L, Fagbemi AF, Enzlin JH, Gourdin AM, Wijgers N, Dunand-Sauthier I, Giglia-Mari G, Clarkson SG, Vermeulen W, Schärer OD (2009) Coordination of dual incision and repair synthesis in human nucleotide excision repair. EMBO J 28: 1111 – 1120

Su Y, Orelli B, Madireddy A, Niedernhofer LJ, Schärer OD (2012) Multiple DNA binding domains mediate the function of the ERCC1-XPF protein in nucleotide excision repair. J Biol Chem 287: 21846 – 21855

Tutter AV, Walter JC (2006) Chromosomal DNA replication in a soluble cell-free system derived from Xenopus eggs. Methods Mol Biol 322: 121 – 137

Walter J, Sun L, Newport J (1998) Regulated chromosomal DNA replication in the absence of a nucleus. Mol Cell 1: 519 – 529

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

Zhang J, Dewar JM, Motnenko A, Cohn MA, Walter JC (2013) DNA interstrand cross-link repair requires replication-fork convergence. Nat Struct Mol Biol 20: 242 – 247

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