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SURVEY AND SUMMARY

DNA repair endonuclease ERCC1–XPF as a novel therapeutic target to overcome chemoresistance in cancer therapy

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ABSTRACT

The ERCC1–XPF complex is a structure-specific endonuclease essential for the repair of DNA damage by the nucleotide excision repair pathway. It is also involved in other key cellular processes, including DNA interstrand crosslink (ICL) repair and DNA double-strand break (DSB) repair. New evidence has recently emerged, increasing our understanding of its requirement in these additional roles. In this review, we focus on the protein–protein and protein–DNA interactions made by the ERCC1 and XPF proteins and discuss how these coordinate ERCC1–XPF in its various roles. In a number of different cancers, high expression of ERCC1 has been linked to a poor response to platinum-based chemotherapy. We discuss prospects for the development of DNA repair inhibitors that target the activity, stability or protein interactions of the ERCC1–XPF complex as a novel therapeutic strategy to overcome chemoresistance.

INTRODUCTION

The ERCC1–XPF heterodimer is a 5′-3′ structure-specific endonuclease that is involved in a number of DNA repair pathways in mammalian cells. It is essential for nucleotide excision repair (NER) and has important roles in interstrand crosslink (ICL) repair and double-strand break (DSB) repair. As such it has a key role in the response of cancers to a range of DNA-damaging chemotherapeutics. In the ERCC1–XPF heterodimer, ERCC1 is catalytically inactive and instead regulates DNA– and protein–protein interactions, whereas XPF provides the endonuclease activity and also contains an inactive helicase-like motif and is involved in DNA binding and additional protein–protein interactions.

ERCC1–XPF is essential for NER

UV irradiation-induced cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6,4)-pyrimidone photoproducts (6-4PPs), chemically-induced helix-distorting and bulky DNA lesions are all repaired by NER [reviewed earlier (1)]. *In vivo* NER requires around 30 proteins, but the incision step can be reconstructed *in vitro* with just six core factors, XPC/RAD23B, XPA, RPA, TFIIH, XPG and ERCC1–XPF (2). To complete NER *in vitro*, PCNA, DNA polymerases δ, ε and κ, DNA ligases I and III, RFC, RPA and XRCC1 are also involved. In global genomic NER [GG-NER, reviewed in ref. (3)], DNA damage is recognized by the XPC/RAD23B complex that detects helical distortions rather than the lesion itself. It is thought that XPC/RAD23B binding induces further bending of the DNA, which may act as the trigger for recruiting additional factors to the lesion. For recognition of DNA damage such as CPDs, which only mildly distort DNA, the XPE/UV-DDB complex is also required. In transcription-coupled NER [TC-NER, reviewed in ref. (4)], repair is triggered when RNA polymerase II stalls at a lesion, resulting in recruitment of several proteins including CSA and CSB. Following either of these damage recognition steps, a common repair mechanism proceeds with recruitment of the TFIIH complex, containing XPB and XPD, which possess ATP-dependent helicase activities to unwind DNA around the damage site to form an open complex. XPA and RPA proteins are then recruited to stabilize the NER intermediate. XPA recognizes a helical kink at the damage site (5) and acts as a scaffold for binding to TFIIH, RPA and ERCC1–XPF, whereas RPA binds to ss-DNA. ERCC1–XPF and XPG endonucleases are then recruited to incise the damaged DNA strand 5′ and 3′, respectively, to the lesion. Recruitment of ERCC1–XPF is thought to be mediated by both ERCC1/XPA and XPF/RPA interactions (6,7).

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ERCC1–XPF is involved in DSB repair

Double-strand DNA breaks, induced by ionizing radiation, free radicals and chemotherapeutics, such as cisplatin, mitomycin-C and the topoisomerase inhibitor, etoposide, can be repaired by homologous recombination (HR), or non-homologous end-joining (NHEJ). Although the main HR pathway is error-free, NHEJ involves the ligation of free DNA ends in a Ku70/Ku86-dependent process resulting in error-prone repair, due to addition or loss of bases, or to ligation of the wrong ends. The importance of ERCC1–XPF in DSB repair (DSBR) was initially shown in budding yeast where mutations in RAD10, or RAD1, the yeast orthologues of ERCC1 and XPF, suppressed HR (8). Mammalian cells with mutant ERCC1–XPF are sensitive to DSBs (9) and both the HR and NHEJ pathways for DSBR are attenuated (10–12). The key activity of ERCC1–XPF in both types of DSBR is its ability to remove non-homologous 3′ single-stranded flaps at broken ends before they are rejoined (9). This is achieved in the error-prone RAD52-dependent single-strand annealing (SSA) subpathway of HR (13,14) and in the mechanistically distinct RAD52- and Ku70/Ku86-independent microhomology-mediated end-joining (MMEJ) subpathway of NHEJ [see reference (15) for a review of MMEJ and references (9,16) for the role of ERCC1–XPF].

ERCC1–XPF is involved in ICL repair

ICL repair operates to remove crosslinks induced by chemotherapeutics such as cisplatin, psoralens and mitomycin-C (17). Such lesions are particularly toxic because they prevent helix unwinding and so act as a potent block to transcription and replication. A review of the sensitivity of mammalian NER mutants to ICL agents found that, whereas all NER mutants were more sensitive than the wild-type cells, mutants in ERCC1 or XPF were in general, hypersensitive (17). In eukaryotes, the mechanism of ICL removal depends on the phase of the cell cycle during which the lesion is encountered (18). If incision adjacent to an ICL occurs in G0 or G1 then repair may be completed during this stage. Alternatively, if the ICL persists into S phase, it will be converted into a DSB when it causes replication to stall. Incisions are thought to be made to either side of the crosslink on one DNA strand to unhook the lesion and allow stalled replication complexes to proceed. The DNA is repaired by HR using the newly synthesized strand as template and may use a NER-dependent mechanism to remove the remaining lesion (19). Although the precise mechanism is not known, most models for ICL repair employ an ERCC1–XPF-dependent step (17,18). Importantly, ERCC1–XPF is able to incise to either side of an ICL (20) and, although not the only nuclease involved, ERCC1–XPF has been shown to be required for both S-phase-dependent and -independent ICL repair (21,22).

Non-repair related roles for ERCC1 and XPF

ERCC1–XPF is involved in telomere maintenance and the interactions made with the telomere protein, TRF2, are described in a later section. A role for ERCC1, but not XPF, in mitotic progression has been suggested by the observation that knock-down of ERCC1, but not XPF, in human hepatocellular carcinoma cells caused cell cycle delay and multinucleation (23). This result is not readily reconcilable with the premature polypoidy observed in the livers of both ERCC1 knockout (24) and XPF knockout mice (25) and could result from unrepaired endogenously generated interstrand crosslinks uncoupling the normal relationship between replication and cell division, rather than a non-repair-related role for ERCC1. A similar role in mitosis, but this time for XPF, has been proposed from studies where knocking down XPF in cultured cells led to abnormal nuclear morphology and mitosis (26). XPF was found to interact and co-localize with the kinesin protein, Eg5 (26). Again the effect of XPF knock-down on mitosis could be indirect, resulting from unrepaired endogenous DNA damage rather than a direct role in mitosis. An alternative explanation perhaps made more likely by the observation that Eg5 boosted ERCC1–XPF activity in the standard *in vitro* assay.

NER deficiency disorders

Inherited defects in human NER genes result in the rare syndromes xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy. Whereas XP is considered a repair syndrome, CS and trichothiodystrophy are regarded as transcription syndromes (1). Diagnostic features of XP are dry scaly skin, abnormal pigmentation patterning in sun-exposed areas and severe photosensitivity, resulting in >1000-fold increased risk of developing UV-induced skin cancers (27). In 20–30% of XP patients, there is also progressive neurological degeneration, emphasizing the importance of NER in repair of endogenous DNA damage (1). CS patients are also photosensitive, but do not exhibit pigmentation abnormalities, or an increased cancer risk (1,27). CS patients also show developmental defects and neurological symptoms (1). In XP, GG-NER is always defective and TC-NER may also be affected, whereas in CS, TC-NER is lost, but GG-NER is retained (1,27).

Characterization of the *ERCC1* (28) and *XPF* genes (29,30) made possible the identification of mutations in XP patients. Mutations in the *ERCC1* or *XPF* genes can result in the even rarer XF-E syndrome (31). Patients show characteristics of XP and CS, but also exhibit additional neurologic, hepatobiliary, musculoskeletal and haematopoietic symptoms (31). In addition to a complete loss of TC- and GG-NER, cells derived from XF-E patients also show hypersensitivity to ICL agents due to the additional role of ERCC1–XPF in ICL repair (31). This distinguishes the XF-E syndrome from either XP, CS or combined XP/CS (31).

Patients with ERCC1–XPF mutations

Only two patients with *ERCC1* mutations have been observed: one (XP202DC) harbouring a Lys226X nonsense mutation with a IVS6-26G-A splice mutation, a second (165TOR) with a Gln158Stop mutation inherited from the mother and a Phe231Leu mutation from the

father (32,33). *XPF* mutations have been characterized in 14 patients, 9 harbour an Arg799Trp mutation (32). This is proposed to be situated in an interaction domain between the XPF nuclease and ERCC1 central domains (34). An Arg153Pro mutation in the helicase-like domain may disrupt protein–protein interactions resulting in XF-E syndrome (31). Other mutations observed are Pro379Ser and Arg589Trp, both in the helicase-like domain (32). Although it is yet to be shown for any of the XPF mutations that they actually disrupt specific protein–protein interactions, there is evidence that the Arg153Pro XF-E mutation results in the protein failing to reach the nucleus, probably due to misfolding (35). The locations of *ERCC1* and *XPF* mutations resulting in amino acid substitutions are shown in Figure 1.

ERCC1 is a target to overcome chemoresistance

ERCC1–XPF is required for the repair of DNA damage caused by many chemotherapeutics, including the commonly used platinum compounds, such as cisplatin (36). Testicular cancers have very low levels of ERCC1 and are effectively treated by cisplatin (37). High expression of ERCC1 has been linked with poor responses to chemotherapy in numerous cancer types, including non-small cell lung cancer, squamous cell carcinoma and ovarian cancer (38–45). Although it has not been linked to altered ERCC1 expression, the T variant of a silent *ERCC1* polymorphism at codon 118 was predictive of poor survival for cisplatin-treated non-small cell lung cancer patients (46). The same association has not been seen consistently in other studies and the role of particular *ERCC1* alleles needs further clarification [for review, see ref. (36)].

Increased levels of ERCC1 mRNA have been reported in melanoma and ovarian cancer cell lines in response to cisplatin-induced DNA damage (47,48). In ovarian cancer cells, increased levels of the transcriptional activators, c-fos and c-jun, were involved in the cisplatin response (48). Treatment of melanoma cell lines with cisplatin resulted in increased phosphorylation of the extracellular signal-regulated kinase (ERK) (49). The MAPK pathway also has an important role in the regulation of ERCC1 expression by epidermal growth factor in human hepatoma cells (50). Importantly, MAPK pathway-dependent increased levels of both ERCC1 and XPF proteins have been demonstrated after cisplatin treatment of melanoma cells (47). This led us to propose that inhibition of ERCC1–XPF could be used to overcome chemoresistance in many cancers. To demonstrate this, we used a mouse xenograft model of melanoma to show that, whereas ERCC1-proficient xenografts were resistant to cisplatin treatment, isogenic ERCC1-deficient melanoma xenografts could be cured by just two cisplatin treatments (51).

We now review the known protein–protein and protein–DNA interactions made by the ERCC1–XPF complex to better understand its mechanistic role in DNA repair and consider which of these interactions might be targeted in order to overcome chemoresistance.

THE ERCC1–XPF STRUCTURE-SPECIFIC ENDONUCLEASE

The ERCC1–XPF complex is a structure-specific endonuclease which cleaves DNA at ds- to ss-junctions, nicking the ds-DNA on the 5′ strand, two nucleotides from the junction (52). This structure-specificity was first demonstrated for RAD10-RAD1, the yeast orthologues of ERCC1 and XPF (53). ERCC1–XPF is active in *in vitro* endonuclease assays utilizing a variety of substrates, including stem–loops and structures with 3′ overhangs (52). The minimum loop size required for cleavage is 6 nt, but the preference is for larger, 22–40 nt loops (52,54).

Domain architecture of the ERCC1–XPF complex

The ERCC1 protein is thought to have arisen from a gene duplication of XPF in the eukaryotic lineage (55). In Archaebacteria only XPF is present, forming homodimers with each protein containing nuclease and HhH_2 domains. Archaeal XPF monomers interact primarily through HhH_2 domains, with an additional interaction through the β5 strand of the nuclease domains (34). In addition to

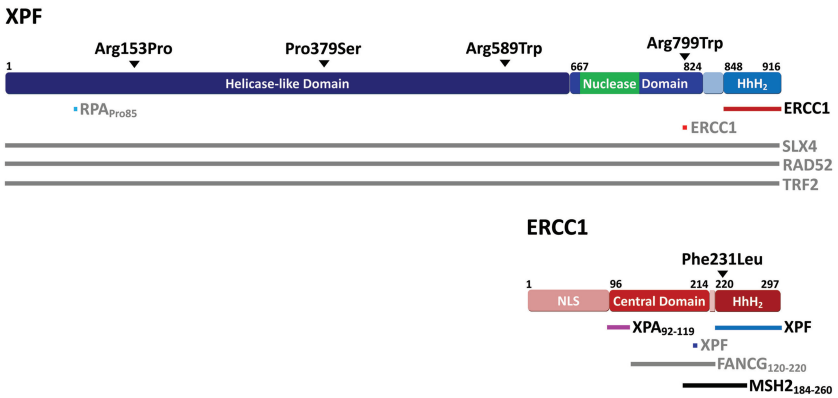

Figure 1. Domain architecture of ERCC1 and XPF proteins. The active site within the XPF nuclease domain is shown as a green box. Confirmed protein–protein interacting regions are mapped and identified with black text; undefined or unconfirmed protein–protein interactions are identified by grey text. Amino acid substitution mutations identified in XP or XF-E patients are also indicated. The same colour scheme shown here to identify the protein domains is used in all the figures. NLS, putative nuclear localization signal.

nuclease and C-terminal HhH_2 domains, mammalian XPF also contains an N-terminal helicase-like domain, but lacks residues essential for helicase activity (56). An overview of the ERCC1 and XPF domains is shown in Figure 1. The ERCC1 protein differs from XPF in that it contains a catalytically inactive central domain and lacks the helicase-like domain. ERCC1 mediates DNA binding and many of the protein–protein interactions of the ERCC1–XPF complex. The HhH_2 domains of ERCC1 and XPF show a high degree of conservation (55,57). In human XPF, the second HhH motif lacks the characteristic GhG hairpin, instead being replaced by a short three residue β-turn, nevertheless XPF still adopts a canonical HhH_2 folded structure (57).

Dimerization of ERCC1 and XPF occurs through their HhH_2 domains

The key protein–protein interaction of ERCC1 and XPF is the dimerization of their hydrophobic C-terminal regions to form a stable heterodimer through the double helix–hairpin–helix motifs in their HhH_2 domains (57,58). It is thought that during protein folding XPF acts as a scaffold for ERCC1 and that ERCC1 may be unable to fold correctly *in vitro* in the absence of XPF (57). Without dimerization it was conventionally thought that neither protein was stable and each was rapidly degraded due to aggregation following exposure of their hydrophobic interaction regions (57,59). However, recent siRNA experiments have indicated that, although XPF protein levels were decreased when ERCC1 was knocked down, the converse was not true (60). There is no catalytic activity in the absence of dimerization. Indeed, although the catalytic domain is within XPF and ERCC1 is catalytically inactive, ERCC1 remains indispensable for activity of the complex (57).

What residues are essential for dimerization?

The ERCC1 and XPF HhH_2 domains have a 1534Å^2, predominantly hydrophobic, interacting surface (57). Each domain forms five core α-helical structures (XPF: H1, 849–853; H2, 860–868; H3, 873–877; H4, 881–887; H5, 891–903. ERCC1: H1, 233–240; H2, 247–257; H3, 260–265; H4, 268–272; H5, 280–288), with ERCC1 forming an additional α-helical structure in its N-terminus (ERCC1, 226–229) (59). In both ERCC1 and XPF the H1 and H2 helices constitute the first HhH motif, with H4 and H5 constituting the second motif (57). From cross-saturation techniques, XPF residues from Gln849 to Ala906 appear to interact with ERCC1 residues Arg234 to Leu294 (59).

Two residues essential for interaction are XPF Phe905 and ERCC1 Phe293, which anchor the two proteins together (Figure 2). ERCC1 Phe293 positions into a 280Å^2 hydrophobic pocket on XPF (57). This interaction is protected by ERCC1 Leu294 which locks Phe293 in position (57). In mutational studies, deletion of ERCC1 Phe293 resulted in abolition of dimerization and enzyme activity (29,61). A reciprocal arrangement exists for the XPF Phe905 residue, which positions into a 220Å^2 hydrophobic pocket on ERCC1, although no mutational studies have been performed on this XPF residue (57). In the human patient 165TOR, ERCC1 encoded by the allele with the Gln158Stop mutation cannot form active protein due to its inability to heterodimerize. The second allele from this patient, with the Phe231Leu mutation, does produce functional protein. Reduced binding affinity for XPF Phe905, due to the Phe231Leu mutation in the ERCC1 interaction pocket, could explain the reduced levels of ERCC1–XPF complex and moderate sensitivity to UV and crosslinking agents observed in cells from this patient (33).

How does DNA binding by the HhH_2 domains influence endonuclease activity?

It has been proposed that the ERCC1–XPF HhH_2 domains form two independent binding sites to complex with ss-DNA (57,58). This interaction is thought to be necessary for the proper orientation of ERCC1–XPF at the ds- to ss-DNA junction (52). Tripsianes *et al.* (57) monitored chemical shift perturbations upon DNA binding and found that both hairpin regions of ERCC1 contacted DNA, proposing that residues Val245 and Asn246 of the first HhH motif and Gly276, Leu277, Gly278 and Lys281 of the second hairpin interact with DNA. Under their experimental conditions, Tripsianes *et al.* (57) could not detect DNA interaction by XPF. Similarly, Tsodikov *et al.* (58) proposed DNA contacts to be made by ERCC1 residues Lys243 and Lys247 of the first and Gly276 and Gly278 of the second HhH domains. In contrast, however, they proposed XPF makes DNA interactions via Gly857, Lys861 and Gly889. They showed that the recombinant ERCC1–XPF HhH_2 domain complex binds with 6-fold preference to two ss-DNA strands over ds-DNA and measured the binding affinity (Kd) to be 0.2 μM (58). Interestingly, Su *et al.* showed that recombinant ERCC1–XPF protein harbouring ERCC1 Lys247Ala and Lys281Ala mutations had a 2-fold reduced DNA-binding affinity and was inactive *in vitro*, whereas XPF harbouring Lys861Ala and Arg864Ala mutations had a 1.6-fold reduction in DNA-binding affinity and retained *in vitro* activity (62). Furthermore, *in vivo* only the ERCC1 double mutant resulted in a mild NER defect, suggesting that defects in DNA binding of the ERCC1–XPF complex can be partly overcome by other NER proteins (62). Das *et al.* utilized an XPF HhH_2 homodimer and demonstrated that this can bind ds-DNA and form a stable complex with ss-DNA (63). They showed that upon binding of two 10-nt ss-DNA fragments to the homodimer, chemical shift perturbations were observed for XPF residues between Lys861 and Val870 and proposed that Lys861, Arg864, Ser865, His868, His869 and Asn890 were directly involved in making DNA contacts (63). In addition, they showed that the bases are orientated away from the HhH_2 domain with the exception of one base that orientates into a 140Å^2 pocket in the XPF HhH_2 domain (63). This pocket is formed due to an altered conformation for Lys861 and Asn890 upon DNA binding and proposed hydrogen bond formation with the side chain of Asn890 (63).

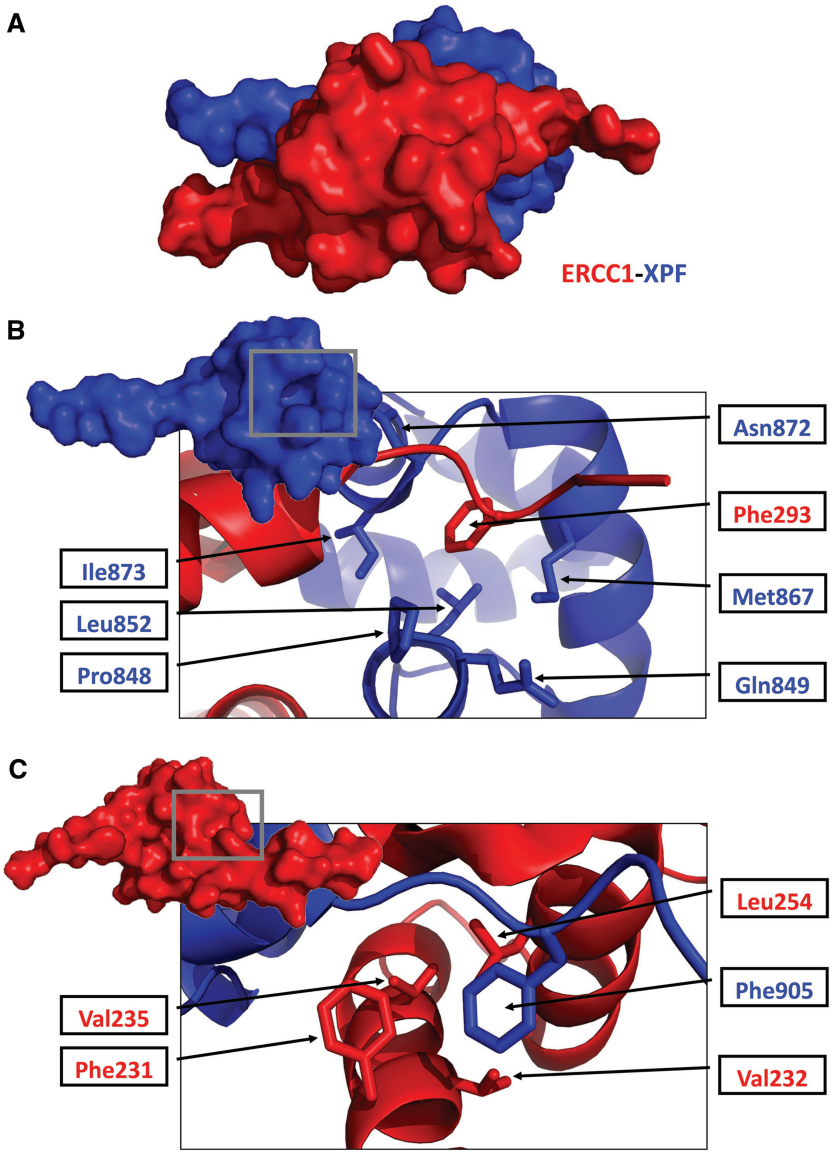

Figure 2. Interaction of ERCC1 and XPF through their HhH_2 domains. (**A**) Heterodimer of the HhH_2 domains of ERCC1 (red) and XPF (blue). (**B**) Expanded cartoon representation of the region boxed on XPF, identifying key interacting residues in the XPF pocket for ERCC1 Phe293. (**C**) Expanded cartoon representation of the region boxed on ERCC1, identifying key interacting residues in the ERCC1 pocket for XPF Phe905. Figure created using PyMOL v0.99 with the ERCC1–XPF HhH_2 domain crystal structure (PDB code 2A1J) (58).

A model for the structure of ERCC1–XPF bound to DNA

Das *et al*. proposed a model for ERCC1–XPF whereby the ds- and ss-DNA binding of the ERCC1–XPF HhH_2 domains position the complex at the ds- to ss-DNA junction, incorporating the nucleotide-binding pocket that they mapped onto the XPF HhH_2 domain in the XPF HhH_2 homodimer crystal structure (63). In their model, the ERCC1 central domain does not make contact with DNA as shown by Tsodikov *et al*. (58,64). In addition, the non-cleaved DNA strand winds around the back of the ERCC1–XPF HhH2 domain before contacting the nucleotide-binding domain and the model does not show how the central and HhH_2 domains of ERCC1 are connected.

Bowles *et al*. (54) showed in an *in vitro* endonuclease assay that ERCC1–XPF may have a sequence-specific preference for nucleotides immediately surrounding the cleavage site. As a result, they have also proposed a model, whereby XPF contains a nucleotide-binding pocket, but instead, although there is no evidence in support, they suggest that this may reside in the nuclease domain, rather than in the XPF HhH_2 domain as shown above by Das *et al*. (63). Their very schematic model shows the helicase domain inside the loop of the stem–loop substrate DNA. The minimum loop size for ERCC1–XPF enzymatic activity is six nucleotides (52,54). Based upon our homology model, the narrowest part of the XPF helicase-like domain would require a minimum loop size of ~12 nt to be accommodated, so this model seems unlikely. In addition, in their model the ERCC1 central and XPF HhH_2 domains do not make the DNA contacts shown by Tsodikov *et al*. (58,64).

The alternative model that we propose takes into account all the known protein–protein and protein–DNA interactions by ERCC1 and XPF (Figure 3). Unlike the other models, our model also has the C-terminal end of each ERCC1 and XPF domain in close proximity to the N-terminal end of the next domain in the sequence. Similar to the other models, we show the ERCC1 HhH_2 domain making contact with ds-DNA, however after the DNA strands have separated, we additionally show the ERCC1 central domain also making contact with ss-DNA. With regards to XPF, instead of a nucleotide-binding domain on the XPF central domain as suggested by Bowles *et al.* (54), we show the nucleotide-binding domain to be present in the HhH_2 region of XPF as demonstrated by Das *et al.* (63). We further propose that this is localized adjacent to the XPF nuclease domain and so may act to present the DNA backbone towards the XPF nuclease domain for catalysis. Our predicted structure of the XPF helicase domain has a clamp-like structure and, although there is no evidence to indicate how it is positioned with respect to the rest of the protein, we have shown it clamped around the nuclease domain with its RPA-binding site in a suitable position to contact RPA bound to the non-damaged DNA strand.

Do ERCC1 and XPF interact through regions other than their HhH_2 domains?

It is unclear whether the XPF nuclease domain interacts with the central domain of ERCC1, similar to the nuclease domain interactions observed with Archaebacterial XPF (58). In several studies stable interactions between these domains did not form, although transient interactions cannot be excluded (58,61). In support of this notion, the XPF patient carrying the Arg799Trp mutation exhibited 5-fold decreased NER activity due to ERCC1–XPF instability. Based on sequence alignments to Archaeal Hef (similar to human XPF), Nishino *et al.* (34) mapped the Arg799Trp mutation to the middle of the β5 strand of the XPF nuclease domain, proposing an interaction with the ERCC1 central domain (34). Further evidence is required to prove the existence of this interaction in human ERCC1–XPF.

Inhibition of ERCC1–XPF dimerization as a target for drug discovery

ERCC1–XPF interaction through their HhH_2 domains is an obligate requirement for a stable ERCC1–XPF complex and so is essential for catalytic activity. Development of small molecule inhibitors of the HhH_2 domain interaction would be expected to sensitize cells to chemotherapeutics whose DNA-damaging effects are repaired by ERCC1–XPF-dependent pathways. Given the high affinity [we have estimated the Kd to be 5 nM by surface plasmon resonance assay (E. M. McNeil, M. Wear, M. Walkinshaw and D. W. Melton, unpublished observations)] and the hydrophobic nature of this interaction, it will be considerably more difficult to block than an enzyme active site. However, the successful development of the p53/MDM2 interaction inhibitor, nutlin, demonstrates what can be achieved (65). Mutagenesis studies indicate that disruption of the ERCC1 Phe293 interaction with XPF is sufficient to prevent complex formation (29,61). Furthermore, availability of an ERCC1–XPF HhH_2 domain crystal structure (PDB code 2A1J) (58) provides an attractive first step for rational drug design programmes.

Are human XPF homodimers of functional significance?

XPF HhH_2 interaction domains form homodimers *in vitro* mimicking the homodimerization of Archaeal XPF (66). In mixtures of ERCC1 and XPF HhH_2 domains at ambient temperatures, the heterodimer is the predominant form, but at temperatures >50°C ERCC1 HhH_2 domains aggregate leaving XPF HhH_2 domains to homodimerize. It has been proposed that XPF homodimers may act as an inactive storage complex in cells to protect against the risks of non-specific DNA cleavage (59). However, if human XPF does form homodimers *in vivo* it must be at very low levels because, in ERCC1-deficient cells, or after ERCC1 siRNA knock-down, only low levels of XPF are found (55,60).

The XPF nuclease domain

The nuclease site of the ERCC1–XPF complex has been mapped to XPF residues 681–751, it contains a $V/IERKX_3D$ motif conserved between XPF protein family members and binds Mg^{2+} or Mn^{2+} as a metal co-factor (67). The presence of the metal is not necessary for DNA binding and does not alter to the DNA-binding affinity.

Key residues for catalytic activity

Active site mutations identified Asp687, Asp715, Lys727 and Asp731 as essential for catalytic activity, whereas mutation of residues Arg689 and Arg726 resulted in residual activity (67). Asp687, Glu690, Asp715 and Glu725 have all been directly implicated in metal binding (67). Enzlin and Schärer proposed that residues Asp687 and Asp715 coordinate metal ion binding and residue Asp731 may function to activate a water molecule to act as a nucleophile for phosphodiester bond hydrolysis (67). Mutation of residues Glu690 and Glu725 led to reduced nuclease activity in the presence of Mg^{2+} but not Mn^{2+}, suggesting an altered active site incompatible with the greater stringency requirements for Mg^{2+} compared with Mn^{2+} binding (67). This suggests that the biologically relevant metal co-factor is Mg^{2+}, but in *in vitro* ERCC1–XPF endonuclease assays, Mn^{2+} is the preferred metal (54,58). Although an Arg689Ala mutation resulted in residual nuclease activity *in vitro,* activity was retained *in vivo* but with a shifted incision pattern, suggesting that this residue may be involved in correct positioning of the substrate in the active site for incision (62). Positioning of key amino acids in the nuclease site of XPF is shown in Figure 4.

Inhibition of the XPF nuclease domain as a target for drug discovery

Inhibition of the XPF catalytic domain would inhibit all known functions of ERCC1–XPF in DNA repair. The presence of Mg^{2+} coordinated in the active site provides

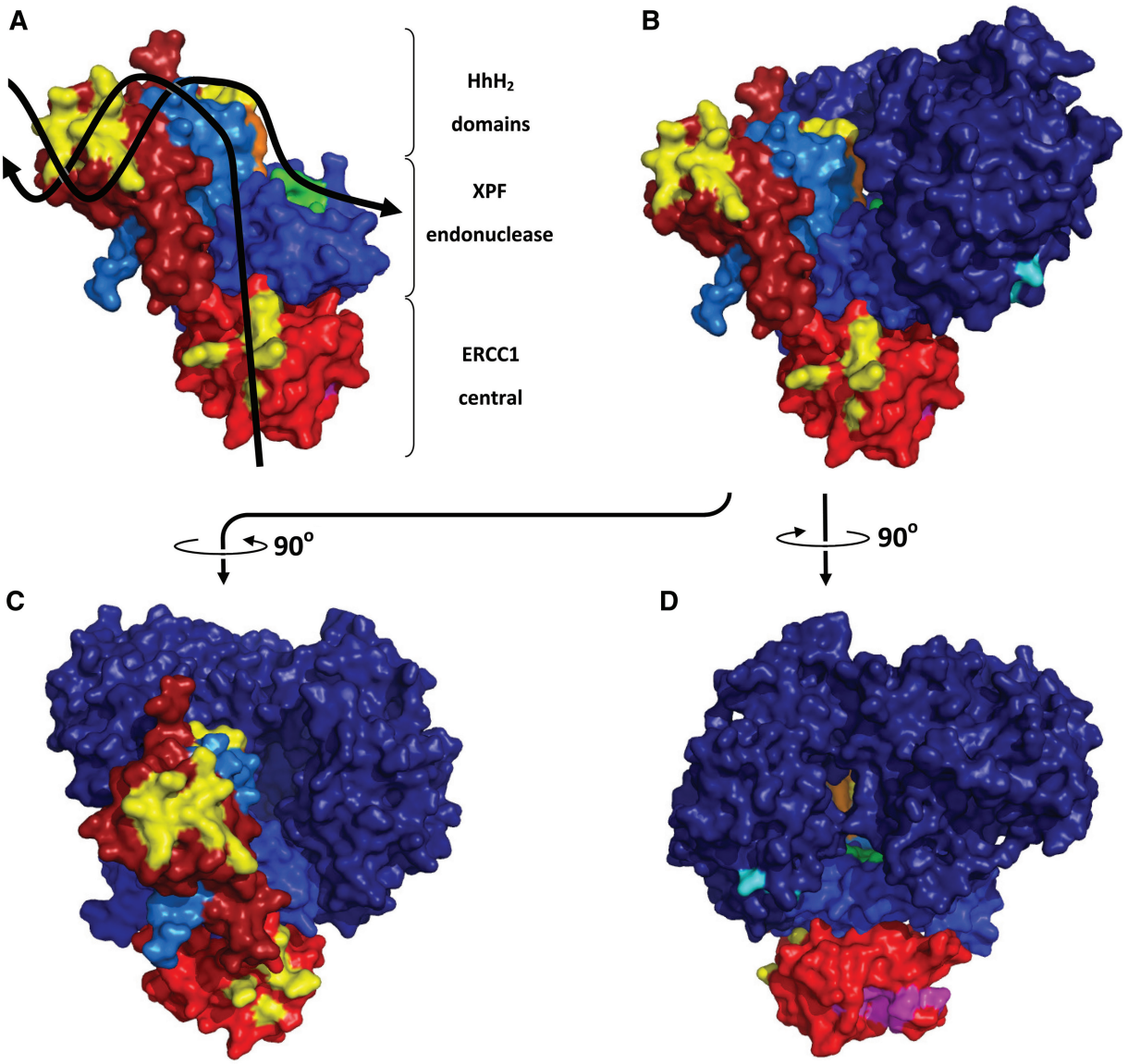

Figure 3. Proposed model for ERCC1–XPF interaction with the DNA substrate. (**A**) Showing the ERCC1 HhH_2 and central domains (red) and XPF HhH_2 and nuclease domains (blue). DNA-binding regions are shown in yellow; the XPF nuclease active site is shown in green; the nucleotide-binding pocket on the XPF HhH_2 domain is shown in orange; the XPA-binding site on the ERCC1 central domain is coloured magenta. The ERCC1 N-terminal region ($ERCC1_{1–98}$) and the XPF domain linking regions (XPF_{666} and $XPF_{825–847}$) are not shown as crystal structures are not available and there is insufficient sequence conservation for homology modelling. (**B**) Same view as in (A), but with the addition of the proposed position of the XPF helicase-like domain and omitting the DNA substrate. The RPA-binding site on the XPF helicase-like domain is shown in cyan. (**C**) As in (B), but with a 90° anti-clockwise rotation of the ERCC1–XPF complex. (**D**) As in (B), but with a 90° clockwise rotation of the ERCC1–XPF complex. Figure created in PyMOL v0.99 using the ERCC1–XPF HhH_2 domain crystal structure (PDB code 2A1J) (58), the ERCC1 central domain crystal structure (PDB code 2A1I) (58) and PHYRE-generated homology models of the XPF endonuclease and helicase-like domains.

an ideal target for metal ion chelation with only weak DNA contacts being made by this domain. There is currently no crystal structure for the human XPF endonuclease domain, although the Archaebacterial XPF crystal structure (PDB code 2BGW) (69) has been used to generate a human homology model that could aid the search for active site inhibitors (Figure 4). Inhibition of the nuclease domain of XPF is, however, problematic due to shared mechanistic activity with closely related nucleases, thus designing the necessary specificity into inhibitors will be challenging. It is of note that drug discovery programmes have been, or are currently being pursued for another structure-specific endonuclease, Flap Endonuclease 1 (FEN1), involved in the final ligation step of NER and base excision repair (BER) (70–72), for Apurinic/apyrimidinic Endonuclease (APE1) required for BER (73,74) and the RAD51 recombinase, involved in HR (75).

The XPF helicase-like domain

The XPF helicase-like domain is related to superfamily-2 helicases, it comprises five subdomains, but lacks critical

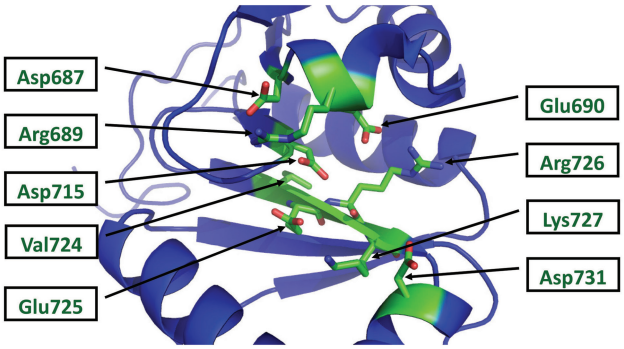

Figure 4. The nuclease domain of XPF. Cartoon representation of XPF identifying amino acids and their side chains. Residues Asp687, Glu690, Asp715 and Glu725 are implicated in metal binding (67). No metal ion has been shown. Figure created using PyMOL v0.99 with a homology model of XPF generated using the Protein Homology/analogY Recognition Engine v2.0 (PHYRE) (68).

residues essential for helicase activity (55,56,76). In the first of the Walker A motifs, usually required for ATP and DNA binding, the GKT consensus is not present (56,77). The second Walker B motif lacks acidic residues present in the DEAD/DExH box motif, meaning that Mg^{2+} is unable to bind and catalytic activity is lost (56,77). Instead, it is thought that the helicase-like domain binds at the junction between ds- and ss-DNA and contributes to substrate specificity (55,76). The presence of leucine-rich motifs indicates a potential role for protein–protein interactions (78,79). An Arg153Pro substitution within this domain resulted in XF-E syndrome, with hypersensitivity to UV-irradiation and ICL agents (31). Although hypersensitivity may have resulted from disruption of protein–protein interactions required for both NER and ICL repair, it is most likely this was due to XPF misfolding resulting from substitution of the basic Arginine residue for the hydrophobic Proline. This is in agreement with the reduction in nuclear ERCC1–XPF levels observed (31).

Inhibition of the XPF helicase-like domain as a target for drug discovery

The helicase-like domain could be an attractive target for drug discovery, particularly when considering the single Arg153Pro substitution resulted in hypersensitivity to UV and ICL agents (31). It would also be anticipated that inhibition of this domain would result in decreased nuclease activity, because truncated $_{\Delta 95}$ERCC1–$_{\Delta 666}$XPF shows 60-fold reduced activity *in vitro* compared with the full-length complex (58). However, our current understanding of the DNA– and protein–protein interactions is insufficient for a drug screening programme to target this region. In addition, no crystal structures exist for this domain and a homology model would likely be of only limited value due to insufficient sequence homology to other helicases with known crystal structures.

The ERCC1 central domain

The ERCC1 central domain is weakly homologous to the XPF nuclease domain, but has lost the active site residues required for metal binding and catalytic activity (58). Instead, the ERCC1 central domain has acquired the ability to bind DNA and form additional protein–protein interactions, particularly with XPA to recruit the ERCC1–XPF complex for NER. Deletion of the first 91 amino acids of ERCC1 does not affect endonuclease activity, whereas deletion of an additional 11 residues into the central domain results in a loss of activity (29).

Central domain binding to DNA

The ERCC1 central domain surface has a V-shaped groove lined by basic (Arg106, Arg108, Arg144, Arg156) and aromatic (Phe140, Tyr145 and Tyr152) residues, thought to constitute both the DNA binding and XPA interaction domains (58,64). Through chemical shift perturbations the DNA interacting region has been identified as residues Asn99, Ile102, Leu132, Lys213, Ala214 and Gln134 (80). This region binds preferentially to ss-DNA in an orientation-dependent manner, with an 8-fold greater preference for 5′ than 3′ overhangs (58). In low salt conditions, the ERCC1 central domain binds ds-DNA with comparable affinity to ss-DNA (58). However, at physiologically relevant salt concentrations, it has a binding affinity (Kd) of 2.5–10 μM for ss-DNA, whereas binding of ds-DNA is almost undetectable (58,80).

Central domain interaction with XPA

The interaction between ERCC1 and XPA maps to ERCC1 residues 92–119 and XPA residues 59–114 (6,81). In the XPA-binding domain on ERCC1, two motifs are present; a TGGGFI motif essential for binding, and an EEEEEEE motif shown to be contributory, but not essential for interaction (81). Interestingly, the TGGGFI motif was not identified in any other mammalian proteins (64). Binding studies, with a truncated $XPA_{59–93}$ peptide, confirmed the involvement of Gly72, Gly73 and Gly74 in the binding to ERCC1 (64). A second truncated XPA_{67-80} peptide bound stably to $ERCC1_{96-214}$ in a stoichiometric 1:1 ratio, with a binding affinity (Kd) of 0.78 μM (64).

An XPA peptide bound to the hydrophobic V-shaped groove on ERCC1 with the Gly72, Gly73 and Gly74 residues of XPA forming a U-turn in the 1039Å^2 ERCC1 binding site (64). A number of interactions between individual XPA and ERCC1 residues have been described at this binding site, although there are some differences in interpretation of the same crystal structure (64,82,83). There is main chain hydrogen bonding between XPA Gly72, Gly73 and Gly74 and adjacent ERCC1 residues and stacking of aromatic side chains, making the binding site an attractive candidate for small molecule ligands (see below). Furthermore, an XPA Phe75Ala mutation abrogates binding to ERCC1 (64). Tsodikov *et al.* (64) and Tripsianes *et al.* (80) have measured the binding affinity (Kd) of XPA to ERCC1 to be 540 nM and 1 μM, respectively.

Is simultaneous binding of ERCC1 to DNA and XPA possible?

Interaction of XPA and ERCC1 is required for NER activity *in vivo*, confirming its role in recruiting

ERCC1–XPF to DNA damage, but *in vitro* nuclease activity of ERCC1–XPF does not require XPA (64). It is controversial whether ERCC1 is able to bind DNA and XPA simultaneously. Tsodikov *et al.* (64) used fluorescence anisotropy and measured competitive binding of XPA and DNA to the ERCC1 central domain. In contrast, Tripsianes *et al.* (80) monitored chemical shift perturbations upon XPA binding, showing that the strongest response from ERCC1 was in residues Leu139, Phe140 and Phe141 in the deep hydrophobic groove. Other residues important for ERCC1/XPA interaction were Gln107, Asn110, Ser142, Arg144, Asn147 and Arg156 (80). Tripsianes *et al.* (80) concluded that XPA and DNA have distinct binding sites on ERCC1 and suggested that simultaneous binding is possible.

How does the ERCC1–XPF complex associate with XPA?

XPA binds at ds- to ss-DNA junctions and localizes at the junction 5′ to the lesion to recruit ERCC1–XPF (84). However, in light of a model proposed by Das *et al.*, positioning ERCC1–XPF at the DNA junction 5′ to the damage lesion (63), Shell and Chazin argued that, for steric reasons, XPA must instead localize to the 3′ DNA junction (85). For this latter model to be correct and for the ERCC1/XPA interaction to occur, both DNA junctions would need to be in close proximity. Instead, we propose that XPA binds at the 5′ DNA junction and recruits the ERCC1–XPF complex, forcing the DNA junction to advance and so make space for ERCC1–XPF to bind DNA. In support of this, partial unwinding of the DNA junction occurs *in vitro* facilitating XPF cleavage 2 nt upstream of the junction. This process could be driven by a domain rearrangement within ERCC1–XPF upon DNA binding, similar to that known to occur in Archaeal XPF (69).

Mutation of the XPA-binding site of ERCC1 affects NER but does not affect repair of interstrand crosslinks

Endonuclease activity and DNA binding of the ERCC1–XPF heterodimer were not affected by ERCC1 mutations lacking the Asn110, Tyr145 and Tyr152 residues required for interaction with XPA (82). Proteins containing the XPA-binding site mutations expressed in ERCC1-deficient cells failed to associate with XPA and were only able to partially restore UV resistance (82). However, expression of these mutant proteins in ERCC1-deficient cells fully restored resistance to mitomycin-C, cisplatin and ionizing radiation (82), suggesting that the ERCC1/XPA interaction is required for NER, but not for ICL or DSB repair. Given that cisplatin causes mainly monoadducts and intrastrand crosslinks, which are both repaired by NER, in addition to the much less frequent, but more genotoxic, interstrand crosslinks that are repaired by a combination of endonucleolytic cleavage and HR, partial, rather than complete restoration of cisplatin resistance by an ERCC1 protein that cannot interact with XPA would have been anticipated.

Identification of ERCC1/XPA interaction inhibitors

The non-specific PK-C and CHK1 inhibitor, UCN-01, inhibited NER by causing a reduction in ERCC1 binding to XPA (86). Upon DNA damage and UCN-01 treatment, an accumulation of DNA-bound XPA was observed, but there was a decrease in DNA-bound ERCC1. *In silico* modelling of UCN-01 binding to ERCC1 calculated a binding energy of −4.81 kcal/mol (83). UCN-01 was proposed to bind into the XPA interaction site on ERCC1, disrupting the interaction of Tyr145 and Tyr152 in ERCC1, with several hydrogen bonds stabilizing the UCN-01/ERCC1 interaction (83). An *in silico* screen for potential inhibitors of the XPA interaction site on ERCC1 was performed, but no compounds were investigated for *in vitro* or *in vivo* activity (83).

Inhibition of the ERCC1/XPA interaction is an attractive drug target due to the existence of crystal structures and known inhibitors. Inhibition of this site *in vitro* and *in vivo* has been shown with a synthetic XPA peptide and is also proposed for UCN-01 (64,83). However, an inhibitor of this interaction would only disrupt NER and would not affect the role of ERCC1–XPF in ICL or DSB repair. Thus, synergistic use of an ERCC1/XPA inhibitor with a DNA crosslinking agent, such as cisplatin, would likely only be of limited benefit, although the result for ERCC1 protein that cannot interact with XPA, discussed in the previous section, that fully restores cisplatin resistance to ERCC1-deficient cells may indicate otherwise (82).

Interaction of XPF with RPA

During NER the ERCC1–XPF complex binds to the ss-DNA-binding protein RPA, which protects the undamaged strand, in addition to XPA, but the RPA interaction is less well understood (7,87–89). Using affinity columns, Bessho *et al.* (7) demonstrated that XPA binds ERCC1, whereas interaction with RPA was mediated by XPF. Furthermore, in a pull-down assay ERCC1–XPF bound weakly to DNA and binding was unaffected by XPA addition (87). However, upon adding RPA, the ERCC1–XPF complex was recruited to DNA more efficiently (87). Mutant XPF constructs and a yeast two-hybrid approach showed that XPF binds to the p70 subunit of RPA via its N-terminus (88). In addition, XPF containing a Pro85Ser substitution was unable to interact with RPA, but still interacted with ERCC1 and retained nuclease activity *in vitro*, although the interpretation was complicated by the additional observation that the mutant XPF was mislocalized to the cytoplasm (88). XPF Arg86Ala and Thr89Ala substitutions may also inhibit interaction with RPA (88). Furthermore, addition of RPA increased specificity and activity of ERCC1–XPF cleavage of DNA in *in vitro* endonuclease assays, whereas addition of XPA had no effect (87). When wild-type and Pro85Ser mutant XPF were expressed in XPF-defective CHO UV41 cells, only the control protein fully restored NER ability and UV resistance, suggesting that the XPF/RPA interaction is required for NER (87).

Inhibition of the XPF/RPA interaction as a target for drug discovery

Inhibition of the XPF/RPA interaction may prove to be an effective drug target as RPA has a role in both NER and ICL repair, thus inhibition could potentiate toxicity of

a range of chemotherapeutic agents. However, not enough is known about the interaction site and no crystal structures of interaction exist, so a drug discovery programme based on this target would be premature.

IMPORTANT NON-NER-RELATED INTERACTIONS OF ERCC1–XPF

XPF/SLX4 interaction in ICL repair

Recent research has identified SLX4 (also known as FANCP) as a molecular scaffold for endonucleases SLX1, ERCC1–XPF and MUS81/EME1 to facilitate the processing of branched DNA substrates (90–95). The SLX4 scaffold may also bind additional factors, such as mismatch repair proteins MSH2/MSH3, telomere-binding proteins TRF2/RAP1 and polo-like kinase 1 (91). Depletion of SLX4 induced hypersensitivity to DNA crosslinking agents, but not to UV-induced DNA damage (90,96). In a comparison of SLX4- and ERCC1-deficient mouse embryonic fibroblast (MEF) cell lines with two Fanconi anaemia cell lines (FANCA, FANCC), where there is hypersensitivity to ICLs, the SLX4 and ERCC1 mutants showed the greatest sensitivity and only the ERCC1-deficient cell line also showed hypersensitivity to UV (96).

Size exclusion chromatography indicated two cellular pools of ERCC1–XPF, one associated with the SLX4 complex and presumed responsible for the HR/ICL repair activities of ERCC1–XPF, and the other interacting with XPA and RPA and presumed responsible for NER (90). Direct interaction of the ERCC1–XPF complex with SLX4 has been demonstrated by yeast two-hybrid assays. The interaction is mediated within the N-terminal 669 residues of SLX4, probably through a conserved MLR domain, but the interaction domain on XPF is unmapped (90,91). Expression of SLX4 lacking the SLX1 interacting motif (a C-terminal deletion mutant) in SLX4-deficient MEF cells was sufficient to complement hypersensitivity to mitomycin-C, while expression of SLX4 deficient in the interaction with XPF (N-terminal deletion) was unable to complement mitomycin-C hypersensitivity, indicating the importance of the SLX4/XPF interaction in ICL repair (96). Interestingly, an endonuclease assay, utilizing a DNA stem–loop to determine cleavage specificity of ERCC1–XPF and SLX4/SLX1, showed that SLX1 nicked ds-DNA on the opposite strand to ERCC1–XPF (92).

Inhibition of the XPF/SLX4 interaction as a target for drug discovery

The XPF/SLX4 interaction is an emerging drug target. Disruption would sensitize cells to interstrand crosslinking agents without disrupting the role of ERCC1–XPF in NER. Currently, a drug discovery programme targeting this interaction would be challenging as the XPF/SLX4 interaction site awaits detailed mapping. Furthermore, the relevant crystal structures are unavailable and low SLX4 sequence conservation between species would hamper confidence in homology modelling.

FANCG and the ERCC1 central domain

FANCG-deficient cells are sensitive to ICL agents due to an inability to make a dual incision at the site of a crosslink [for review, see (18)]. In a yeast two-hybrid assay the ERCC1 central domain was reported to interact with FANCG, which forms part of the Fanconi anaemia core complex (97). The ERCC1/FANCG interaction is believed necessary to recruit ERCC1–XPF to the crosslink. Direct interaction of ERCC1 with FANCG could explain how XPF has previously been shown to co-localize with FANCA in cells, presumably through a ternary complex with FANCG (98). Site-directed mutagenesis indicated that the FANCG/ERCC1 interaction occurred through tetratricopeptide repeats (TPR) in FANCG (97). Complementation of FANCG-deficient cells with mutant FANCG proteins indicated that TPR 1, 2, 5 and 6 were all important in correcting sensitivity to the crosslinking agent mitomycin-C (99).

Inhibition of the ERCC1/FANCG interaction site as a target for drug discovery

If the interaction between ERCC1 and FANCG is confirmed, it could represent an attractive and novel drug target that would specifically block the role of ERCC1–XPF in ICL repair. Deletion of ERCC1 or FANCG interacting regions results in sensitivity to crosslinking agents, but it is unknown whether mutation of specific interaction sites on either protein is sufficient to sensitize cells. Further mapping of the interaction site would be necessary before attempting to identify inhibitors of this interaction.

MSH2 interaction with ERCC1

Using a yeast two-hybrid approach, it has been proposed that the mismatch repair protein MSH2 interacts with RAD10, the yeast homologue of human ERCC1, along with several other NER proteins (100). In humans, MSH2 forms a heterodimer with MSH6 (heterodimer known as MutSα), or MSH3 (known as MutSβ) (101). To facilitate repair, MutSβ recognizes an ICL, then in association with ERCC1–XPF, is required for the initial processing and unhooking of the lesion (101). It is thought that the ERCC1/MSH2 interaction, involving ERCC1 residues 184–260, is required for ICL repair and cisplatin resistance in an XPA-independent mechanism (102).

RAD52 interaction with XPF

ERCC1–XPF and RAD52 are important for single-strand annealing (SSA), a DNA DSBR mechanism that involves annealing homologous single-stranded ends to bridge DSBs (103). Direct physical interaction has been demonstrated between ERCC1–XPF and RAD52 *in vitro* (104). It is thought that the interaction promotes cleavage of 3′ overhangs allowing processing of non-homologous ends for repair (103). XPF interacts with the N-terminal DNA-binding region of RAD52 in a DNA-independent manner (104). RAD52 forms a heptamer around DNA and when a 6:1 ratio of RAD52:XPF is reached, the endonuclease activity of ERCC1–XPF increases 3-fold.

Super-stoichiometric amounts of RAD52 inhibit XPF activity, presumably by binding to DNA and inhibiting ERCC1–XPF recruitment (104).

TRF2 interaction with ERCC1–XPF in telomere maintenance

ERCC1–XPF is involved in telomere maintenance and it is thought that this occurs by its interaction with the TRF2 complex independent of DNA binding (105). To protect telomeres from being recognized as DSBs, 3′ overhangs at the ends of chromosomes, known as G-strands, loop back and insert into duplex DNA to form t-loops (105). TRF2 promotes t-loops and associates with ERCC1–XPF at telomeres (105). In TRF2-deficient cells, a partial loss of telomeres and associated 3′ overhangs was observed that was dependent on ERCC1–XPF expression (105,106). Overhangs are retained in ERCC1-deficient cells following inhibition of TRF2, suggesting that ERCC1–XPF is the main nuclease responsible for 3′ overhang cleavage and is inhibited by TRF2 (105).

Only a minor fraction (~1%) of ERCC1–XPF complexes with TRF2, whereas other proteins essential for NER did not associate, suggesting an NER-independent mechanism for ERCC1–XPF in telomere maintenance (105). As no direct interaction of ERCC1–XPF to TRF2 has been demonstrated, the interaction may occur through a tertiary complex. TRF2 can interact with SLX4 independently of XPF and it is therefore possible that interaction with XPF is through the SLX4 scaffold (91,92).

It has also been proposed that XPF may have a nuclease-independent role in negatively regulating TRF2-mediated control of telomeres (107). Mutant XPF proteins with nuclease site mutations deficient in endonuclease activity still localized with TRF2 and were able to complement TRF2-mediated telomere shortening in XPF-deficient cells, with activity similar to that of wild-type XPF (106,107). However, nuclease activity of XPF is required for TRF2 binding to telomeric DNA, suggesting the mechanism of negative regulation of TRF2 by nuclease inactive XPF is due to ternary complex formation with TRF2, likely via SLX4 and inhibiting DNA binding (108).

REQUIREMENTS FOR AN ERCC1–XPF DRUG DISCOVERY PROGRAMME

Production of recombinant ERCC1–XPF protein

For a drug discovery programme, significant quantities of recombinant ERCC1–XPF would be required. Expression of full-length ERCC1–XPF in *Escherichia coli* leads to predominantly aggregated protein, whereas only small quantities of soluble protein can be purified from expression in Hela cells or baculovirus-infected Sf9 insect cells (58,67). Expression of a truncated ERCC1–XPF complex in *E.coli* is possible in greater yields using $_{\Delta 95}$ERCC1–$_{\Delta 666}$XPF, lacking the ERCC1 N-terminus and the XPF helicase-like domain (58). Tsodikov *et al.* (58) showed that this truncation is active in an endonuclease assay, having the same structure-specificity as the full-length protein, but with a 60-fold slower reaction rate. In contrast, Bowles *et al.* (54) reported that recombinant ERCC1–XPF lacking the helicase-like domain is inactive *in vitro*, although this may be due to expression of ERCC1–$_{\Delta 640}$XPF containing an additional acidic linker on XPF that may inhibit the active site or bind DNA. Consequently, Bowles *et al.* (54) proposed that the endonuclease activity of truncated ERCC1–XPF observed by Tsodikov *et al.* may be non-specific nuclease activity. However, in agreement with Tsodikov *et al.*, our results show that $_{\Delta 95}$ERCC1–$_{\Delta 666}$XPF has the characteristic structure-specific nuclease activity (E. M. McNeil, and D. W. Melton, unpublished observations). We believe that expression of truncated ERCC1–XPF could be sufficient for a drug discovery programme, unless specific protein–protein interactions of the XPF helicase-like domain are being targeted.

An *in vitro* endonuclease assay for ERCC1–XPF

For a drug discovery programme, a convenient *in vitro* ERCC1–XPF endonuclease assay suitable for high-throughput screening of compounds is required. Previously, an assay based upon specific cleavage of a radioactively labelled stem12–loop22 oligonucleotide has been used routinely (58). Recently, this assay has been modified to incorporate a fluorescein label at the 5′-end and a quencher at the 3′-end of the stem [ref. (54) and E. M. McNeil, and D. W. Melton, unpublished observations]. In the presence of recombinant ERCC1–XPF, the stem–loop is cleaved 2 bp 5′ of the ss- to ds-DNA junction. ERCC1–XPF has a preference for the DNA sequence situated around the cleavage site, suggesting the presence of a T-/U-binding pocket on XPF (54). However, because ERCC1–XPF does not have a damage recognition role and is recruited to the site of DNA damage by other proteins, the biological significance of this preference is unknown.

Favoured ERCC1–XPF targets for a drug discovery programme

Developing inhibitors against the ERCC1–XPF complex will be challenging. The most tractable target is the XPF endonuclease site itself, due to lower affinity for the DNA substrate than the affinity of the various protein–protein interactions involved in the different repair roles of ERCC1–XPF. Inhibition of this site would block all the known functions of ERCC1–XPF needed for the repair of chemotherapy-induced DNA damage. However, the lack of a crystal structure for this domain of human XPF and the existence of a number of endonucleases with similar divalent cation-based cleavage mechanisms will complicate the search for compounds of the desired specificity. The only other target whose inhibition would prevent all repair roles of ERCC1–XPF is the interaction domain needed for heterodimer formation. The large hydrophobic surface area of the interaction domain makes this a formidable target, but a single amino acid deletion in this region does completely block the activity (29,61).

Inhibition of other protein–protein interactions made by the complex could also be tractable to a drug discovery

programme. For instance, if there is a requirement to enhance sensitivity to a chemotherapeutic that causes lesions repaired exclusively by NER, then targeting the ERCC1/XPA interaction site should be considered and has the advantage of existing crystal structures and drug and peptide inhibitors. If instead the requirement is to block repair of just ICLs, then inhibitors of ERCC1/XPF interactions with SLX4 or RAD52 may also be effective therapies, but these interactions are not yet sufficiently well understood to be the direct focus of drug discovery programmes.

CONCLUSION

The ERCC1–XPF complex is essential for nucleotide excision repair and its important role in a variety of other key repair pathways, such as ICL repair and DSBR, is increasingly being understood. This makes this protein a particularly attractive target to overcome the resistance of cancer cells to a range of important chemotherapeutic agents. To facilitate the development of ERCC1–XPF inhibitors we have described the mechanism of heterodimerization and the DNA binding and nuclease activity of the ERCC1–XPF complex. We have also reviewed the known protein–protein interactions made by ERCC1–XPF that are essential for nucleotide excision repair, or for its role in other repair pathways and we have proposed novel approaches for drug design to overcome chemoresistance.

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