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The structural basis of XRCC1-mediated DNA repair

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Abstract

Scaffold proteins play a central role in DNA repair by recruiting and organizing sets of enzymes required to perform multi-step repair processes. X-ray cross complementing group 1 protein (XRCC1) forms enzyme complexes optimized for single-strand break repair, but participates in other repair pathways as well. Available structural data for XRCC1 interactions is summarized and evaluated in terms of its proposed roles in DNA repair. Mutational approaches related to the abrogation of specific XRCC1 interactions are also discussed. Although substantial progress has been made in elucidating the structural basis for XRCC1 function, the molecular mechanisms of XRCC1 recruitment related to several proposed roles of the XRCC1 DNA repair complex remain undetermined.

Keywords

XRCC1; DNA repair; DNA polymerase beta; DNA ligase 3alpha; single-strand break repair

I: Introduction

DNA lesions are chemically heterogeneous substrates located on the largest cellular macromolecule. Some repair intermediates exhibit increased lability and cell toxicity [1–3]. For these reasons, DNA repair processes invert the typical paradigm in which substrates diffuse to their target enzyme; the repair enzymes instead assemble near their substrate. Furthermore, as a consequence of the complexity of the damage, most repair processes require more than a single enzymatic step. In order to deal with the requirements that result from the nature of the substrate, scaffold proteins play a central role in the DNA damage response. The scaffolding proteins assemble sets of enzymes that facilitate the complete repair process, reducing the likelihood that labile repair intermediates are released leading to further damage. Scaffold proteins that have been identified to play a role in DNA repair have also been found to form heterogeneous complexes, enhancing the capability to deal with the variable nature of the damage. This heterogeneity provides broader capabilities at the expense of efficiency.

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X-ray cross complementing group 1 protein (XRCC1) was the first mammalian gene identified that facilitates protection of the cell from the effects of ionizing radiation [4], and was subsequently shown to provide protection against multiple types of stress including alkylation and UV-induced damage [5–8]. The protein has not been found to possess intrinsic enzymatic activity, but interacts with multiple repair enzymes that are involved in the single-strand break repair (SSBR) pathway [6, 9]. Additional studies support involvement in other repair pathways as well [10–12]. The structural characterization of XRCC1 and its protein and DNA complexes is of central importance for understanding its function, and for evaluating or designing mutations or chemical agents that selectively interfere with these interactions. Such structural data is also extremely useful for elucidating how polymorphisms can influence XRCC1-mediated DNA repair, and thus for providing insight into the rapidly expanding epidemiology database that relates SNPs to cancer and other diseases. Furthermore, structural characterization of repair complexes can provide important insights that connect individual enzymatic activities to the overall repair process. The objective of this review is to summarize available structural information about XRCC1 and its interactions in order to clarify its function.

II: Domain organization

XRCC1 consists of three globular domains connected by two unstructured linker segments of ~ 150 and 120 residues (Figure 1). The XRCC1 N-terminal domain (X1NTD) binds specifically to DNA polymerase β (pol β) [13–15], the C-terminal BRCT domain (X1BRCTb) interacts with DNA Ligase3 α (Lig3 α) [16]. The central domain (X1BRCTa) contains a poly(ADP-ribose) (PAR)-binding motif [17] that mediates recruitment of XRCC1 to polymeric ADP-ribose that forms on PARP-1 after activation resulting from binding to single strand breaks [18]. The two extended interdomain linkers also contain functionally important regions. Linker 1 (XL1) contains a nuclear localization sequence (NLS) [18, 19], and a second Rev1-interacting region (RIR) sequence that recently was shown to interact with the Rev1 C-terminal domain that functions to recruit translesion polymerases [12]. The second linker, XL2, contains a per-phosphorylated motif positioned immediately before its C-terminal BRCTb domain that interacts with the forkhead associated domains (FHA) present in at least three binding partners: polynucleotide kinase phosphatase (PNKP), aprataxin (APTX), and aprataxin- and PNKP-like factor (APLF) [20, 21]. Both linkers are extensively phosphorylated. A catalog of reported phosphorylated residues (PhosphoPlus2, <http://www.phosphosite.org/homeAction.do?sessionId=A21F49C195F29C060F971D672F21232A>) illustrates the strong relationship of linker residue accessibility with phosphorylation (Figure 1). FHA domain binding is dependent on phosphorylation, and multiple phosphorylations are required for submicromolar binding affinity [20]. Linker phosphorylation has been reported to block ubiquitylation and proteasomal degradation of the XRCC1-Lig3 α complex, facilitating its eventual nuclear accumulation [22], but also to be required for efficient polyubiquitylation [23]. This reasons for this apparent inconsistency are unclear. One interesting pattern that emerges from a consideration of these phosphorylation sites is that many of those in XL2 are phosphorylated by constitutively expressed casein kinase 2 (CK2) [22, 24, 25]. In contrast, many of the phosphorylation sites in XL1 are consensus sequences for phosphorylation by

protein kinase C or p38 MAP kinase, both of which are responsive to oxidative stress [26, 27].

In addition to the above interactions, there are numerous reports of XRCC1 interactions with other DNA repair enzymes including several involved in BER [28–32]. PCNA is reported to bind to XRCC1, despite the absence of a consensus P-Box motif [33]. Each of the above interactions has been reported to involve XL1 or the XL1-X1BRCTa region of XRCC1. Other reported interactions include binding to pol τ [34], pol κ [11] and to JWA, a microtubule-associated protein involved in activation of MAPK cascades [35]. In reports supporting these complexes, little information is available on the binding affinities, residues that mediate the interactions generally have not been identified, nor is detailed structural information currently available.

III: N-terminal domain structure, and DNA pol β interaction

There have been several structural characterizations of the XRCC1 N-terminal domain (X1NTD), both in isolation and in complex with the pol β catalytic domain [14, 15, 36]. Although early biochemical characterizations of the XRCC1 pol β -binding domain localized the interacting region on XRCC1 to residues 84–183 [13], both NMR and crystallographic characterizations of this domain have indicated that a globular, pol β -binding domain is formed from residues 2–153 (following removal of Met1 by methionine aminopeptidase [37, 38]. Furthermore, mutations of residues preceding Tyr84, e.g. E69K and F67A, have been reported to abolish the interaction with pol β [39–41]. The study of Kubota et al.[13] determined only that an XRCC1 construct of residues 1–83 did not interact with pol β . Since such a construct would not be expected to adopt the correct globular fold of the N-terminal domain this result is not surprising, but does not indicate that residues prior to 84 are not involved in the interaction. Analysis of the amide chemical shift parameters derived from the NMR study of a construct that included residues 1–183 yields values for residues 153–183 that are in the range expected for a random coil structure, consistent with a lack of interface contacts for this segment (unpublished results).

The more recent crystal structure of the isolated X1NTD (pdb code: 3K77, [38] is qualitatively similar to the earlier NMR structure, with the main variations being in the flexible loops near the C-terminus of the domain. The most significant difference is an interchange in positions of the Phe142 and Tyr136 sidechains, so that in the crystal structure, the Phe142 sidechain is buried in a hydrophobic pocket occupied by Tyr136 in the earlier structure, leaving Tyr136 exposed on the surface of the protein and available for interaction with pol β . A second difference is observed for the sidechain χ_1 value of Cys12 [12]. The crystal structure $\chi_1 \sim 180^\circ$ places the SH sidechain in a buried position where it forms H-bonds with several groups including Trp33, and this conformation is supported by the large chemical shift perturbation of the Trp33NH α resonance that is observed in X1NTD(C12A) [42]. This orientation has important implications for the oxidation rate of XRCC1 [43].

Unexpectedly, the structure of the X1NTD•pol β polymerase domain complex [15] was inconsistent with the previously identified interface based on NMR chemical shift mapping data [14, 36]. In the earlier studies, NMR data were interpreted to indicate that X1NTD

interacts directly with a gapped DNA•pol β complex, making contact with both the C (catalytic/palm) and N (nucleotide binding/thumb) subdomains as well as with the DNA. The crystallographic study indicates that the interaction involves only the N-domain (Figure 2). An overlay of the structures of the X1NTD•pol β polymerase domain and pol β bound to a single nucleotide gapped DNA ligand (Figure 2B) indicates that the X1NTD is located far from the nucleotide gap, where it will not interfere with the conformational changes that have been associated with catalytic activation [44, 45]. As discussed by Cuneo and London [15], these apparently inconsistent models do not arise as a consequence of differences between the solution and crystal state, but rather result from analysis problems of the chemical shift mapping data, which in retrospect can be viewed as consistent with an N-domain specific interaction.

Interactions of DNA repair enzymes with scaffolds or other repair factors frequently utilize specialized domains, e.g. BRCT or FHA, that can target the proteins to specific structures without interfering with function. The linkage of the XRCC1 scaffold to pol β is qualitatively different from the interactions with other binding partners including Lig3 α , PNKP, and APTX as pol β lacks specific binding domains that target it to other structures. Consequently, it becomes more of a challenge to bind pol β without interfering with the complex substrate-induced conformational activation mechanism that determines polymerase fidelity [44, 45]. Although the N-domain-specific interaction represents much less of a perturbation than the multi-faceted interaction proposed earlier [14, 36], it nevertheless constrains the position of the N-domain that functions as a sensor for the correct incoming nucleotide. The perturbation resulting from X1NTD binding is minimized by the fact that the XRCC1-binding motif in pol β utilizes a flexible and highly conserved Val303 loop [36], which may exhibit sufficient mobility to allow N-domain repositioning without dragging the entire X1NTD domain along with it (Figure 2). Thus, the revised pol β •X1NTD binding mode appears well designed to allow N-domain mediated coupling of pol β with XRCC1 without significantly perturbing the complex pol β conformational equilibrium required for polymerase activation.

The structure of the X1NTD•pol β complex provides a useful basis for designing and interpreting the effects of interface-specific mutations that can selectively interfere with this interaction. XRCC1 residues previously identified on the basis of mutagenesis studies to be important for pol β binding are shown in Figure 3 [39]. The sidechains of the interacting residues, as well as a few of the important pol β residues involved in X1NTD binding, are indicated. It is immediately apparent that the residues identified from the earlier mutagenesis studies are all located at the interface. Specific H-bond/salt bridge interactions include those between the XTyr136 hydroxyl and the β Tyr322 carbonyl, the XGlu69 sidechain carboxyl and β Val303 NH, XArg100 and β Asp321 sidechains, and XArg109 and β Glu309 sidechains (where the designations X or β before the residue indicate the protein on which these residues are located). XRCC1 residues XPh67 and XVal86 make hydrophobic contacts with residues in the pol β thumb subdomain, consistent with the use of the F67A and V86R mutations in studies to evaluate the role of the pol β -XRCC1 interaction [40–42, 46, 47]. The direct participation of XTyr136 in pol β binding provides additional consistency with the recent crystallographic structure, since in the earlier structure this residue was buried in

the interior of the protein. However, the structure does not provide insight into the basis for the reported A160S interference with the X1NTD-pol β interaction [48].

IV: The N-terminal disulfide switch

The recent crystallographic studies of the pol β -X1NTD complex also yielded a completely unanticipated result – a second crystal form in which the X1NTD had become oxidized, so that a disulfide bond residue was formed between Cys12 and Cys20. Formation of the disulfide bond is accompanied by extensive structural remodeling of the N-terminal 40 residues of the molecule, most of which do not directly contribute to the pol β interface [15]. However, the N-terminus of the X1NTD moves back toward the pol β interface, where XPro2 makes direct contact with β Val303 (Figure 4A). Other differences are more subtle, including stronger/shorter H-bonding interactions between β Asp321 - XArg100 and β Glu309 – XArg109 backbone atoms. Cysteine switches similar to that discovered in XRCC1 are increasingly recognized as an important basis for the regulation of protein activity, with the bacterial transcription factor OxyR providing a prototypic example [49–51]. In the case of XRCC1, Cys12 is kinetically trapped as a consequence of its low solvent accessibility, so that the redox transition is extremely slow. Consequently, a physiologically practical response is possible only in the presence of a protein disulfide isomerase [43]. We also note that the Cys12 and Cys20 residues involved are only moderately well conserved.

Consistent with the structural data, binding assays indicate significantly greater affinity of pol β for the oxidized form of the domain, X1NTDox [15, 43]. Hence, the redox transition regulates pol β binding affinity. The possibility that this redox transition regulates binding in a physiologically relevant range is supported by the relatively modest affinity of X1NTDred for pol β . Reported K_d values of 300 and 100 μ M [15, 39] are generally consistent with significant but not constitutive binding, although the fractional site occupancy is also dependent on the intranuclear concentrations of the proteins.

The oxidized N-terminal domain, X1NTDox, is further stabilized by formation of an N-terminal carbamate adduct, so named since it involves the *imino* acid proline, which is hydrogen bonded to Arg7, Ser44, and Lys129 (Figure 4B). Such adducts are unusual but not unprecedented; a similar N-terminal carbamate adduct is present in bacterial thymidylate synthase [52]. Such adducts form due to the nonenzymatic reaction of CO₂ with amines [53–55] and although ubiquitous, only become significant in structures for which adduct formation significantly enhances stability. Nevertheless, carbamate adducts play critical roles in many protein systems, including CO₂ transport by hemoglobin [56] and CO₂ fixation by RUBISCO [57]. NMR studies demonstrate that oxidized X1NTD, containing a Cys12-Cys20 disulfide bond, forms a dynamic structure which is then stabilized by selection of the carbamate adduct (Figure 4C) [43]. This selection is facilitated by the accumulation of positive charges near the N-terminus.

Horton et al. [42] recently evaluated *Xrcc1*^{−/−} mouse fibroblast cells expressing an oxidation-blocked form of XRCC1, the C12A mutant. Among other deficiencies, over-accumulation of cellular poly(ADP-ribose) after DNA base damage was consistent with

disruption of the interaction between pol β , XRCC1, and PARP-1. These results are consistent with functionally significant oxidation of intra-nuclear XRCC1.

VI: An RIR motif suggests involvement in trans-lesion synthesis

The first XRCC1 linker domain, XL1, is characterized by a ^1H - ^{15}N HSQC spectrum that is typical of intrinsically disordered proteins [12]. This region of the protein has been reported to mediate interactions with a number of other proteins, several of which are involved in DNA repair. Rev1 is a Y-family polymerase capable of translesion synthesis and is a component of the Fanconi anemia core complex [58, 59]. The function of Rev1 appears to depend more critically on its role as a scaffold for the recruitment of other trans-lesion polymerases than on its intrinsic catalytic activity, which involves the preferential insertion of cytosine across a variety of DNA lesions [60]. The Rev1 scaffold utilizes a small C-terminal domain that contains a specific binding surface for pol ζ (Rev7/Rev3), as well as a second binding surface that interacts with Rev1 interacting regions (RIRs) present in other translesion polymerases [61–65]. The RIR binding motif identified in pol κ , pol η and pol ι , involves a short α -helical segment with sequence: X-X-X-F-F-Y-Y-K-Y-Y. The primary requirements for the Y residues is that they not include helix unfriendly proline or glycine residues, while the requirement for K is apparently not absolute [62], but has been included in the definition by some researchers [64, 66]. Binding assays indicate that the Rev1 C-terminal domain exhibits a strong preference for DNA pol κ , which has a high capability for extending mispaired nucleotides, and a lower affinity for pol η and pol ι , both of which are characterized by K_d values in the low micromolar range [12].

The first XRCC1 linker domain, XL1, contains a pair of sequential phenylalanine residues that are not accompanied by the other residues that form the consensus PIP-box motif [33]. After finding only low affinity for PCNA, we evaluated the possibility that this region of the linker might fulfill the requirements of the RIR motif, and studied its interaction with the Rev1 C-terminal domain. NMR structural studies of an XRCC1 peptide containing the putative RIR motif confirmed that XRCC1 binds to the Rev1 C-terminal domain in a manner similar to that determined for the RIR peptides of pol η and pol κ [12] (Figure 5). Dissociation constants determined using both NMR and fluorescence methods indicate that the interaction of the XRCC1 RIR peptide with the Rev1 C-terminal domain falls into the low micromolar range, similar to values obtained for RIR peptides from pol η and pol ι , but significantly weaker than the value for pol κ [12]. The position of this RIR motif near the N-terminal pol β binding domain suggests that one possible function of such an interaction would involve the recruitment of pol β for potential trans-lesion synthesis activity. This interpretation is consistent with the significant literature supporting the translesion synthesis capabilities of pol β for dealing with cisplatin adducts [67–72] as well as other DNA lesions [70, 73–76]. The reported inverse association between XRCC1 expression and cisplatin sensitivity is consistent with recruitment of pol β as a bypass polymerase [77, 78].

From a structural standpoint we note that, as summarized earlier, pol β lacks the specialized recruitment domains found in most other polymerases, and thus lacks a convenient structural handle for recruitment. Therefore, XRCC1-mediated pol β recruitment can provide a

convenient handle for pol β recruitment. This region of the linker may also mediate recruitment of pol β for other DNA repair pathways.

VII: The nuclear localization signal

The presence of a nuclear localization signal in the first linker, XL1, was initially demonstrated by Masson et al., [18], and proposed to include residues 239–266. The corresponding sequence contains a set of basic residues that typify the segments shown to interact with importin α as part of the classical nuclear import pathway [79]. Nevertheless, this sequence lacks the consensus K(K/R)X(K/R) sequence more generally present in mono- and bipartite NLS [80]. Hirano et al. [81] showed that fusion of an extended segment of XRCC1 containing the NLS (residues 239–403) with aprataxin could overcome a nuclear pore defect that interfered with aprataxin transport. They subsequently determined that the minimum XRCC1 NLS sequence required to facilitate nuclear localization extended beyond residue 266 by an additional 10 amino acids [19]. We recently characterized the complex of importin α with the XRCC1 NLS crystallographically, and our results are consistent with the results of the Kiriya study [19]; Kirby et al., submitted).

It is believed that phosphorylation regulates nuclear targeting of classical nuclear localization signal (NLS)-bearing cargos by modulating their affinity for cellular transporters of the importin (IMP) superfamily [82]. Kubota et al. [83] reported that nuclear localization of XRCC1 is dependent on phosphorylation by casein kinase II (CK2). More recently, Parsons et al. [22] suggested that CK2 phosphorylation stabilizes XRCC1 by interfering with ubiquitylation and proteasomal degradation, indirectly promoting nuclear accumulation. Interestingly, the NLS region on linker 1 contains two sequences: T²⁵⁷PS and S²⁶⁶PS, which have been implicated in phosphorylation-dependent nuclear uptake mediated by Importin7 [84]. However, these sequences are not conserved in mouse XRCC1, making their functional significance unclear.

VIII: X1BRCTa – mediating PAR-dependent XRCC1 recruitment

The X1BRCTa domain is the most highly conserved region of the XRCC1 molecule, surviving in plants such as *A. thaliana* that lack an N-terminal domain, and in *D. melanogaster* that lacks the second, C-terminal BRCTb domain [85]. The solution structure of X1BRCTa determined by NMR spectroscopy was reported by Nagashima, Hayashi and Yokoyama, pdb code 2D8M, however no associated publication is available. The domain adopts a typical BRCT fold, consisting of a short, central β -sheet formed from four parallel β -strands, flanked by several helices (Figure 6). Interestingly, the reported structure contains a glutamine at position 399, and thus corresponds to the R399Q polymorphism rather than to the predominant wild-type sequence. One of the most striking features of BRCTa is its high pI of 10.2 (Figure 1B). This basic characteristic is consistent with its reported role in mediating the interactions with PAR-modified PARP1 [86]. Pleschke et al. [17] identified a PAR-binding consensus sequence in X1BRCTa (Figure 6). However, Li et al. [87] have reported that a mutation outside of this region, K369A, abolished PAR binding. The basis for this inconsistency is at present unclear, and the result that a single lysine residue would be of

sufficient importance to exert such a large effect on the affinity for this polymeric ligand is surprising.

Kim et al. [88] recently demonstrated preferential X1BRCTa binding by PAR containing at least seven ADP-ribose units, corresponding to a K_d of 20 nM, while the affinity for the ADP-ribose monomer was reported to be in the millimolar range. This dramatic difference between the affinity for the polymer and the monomeric units has also been observed for the APLF PAR-binding Zinc Finger (PBZ) domains. Each of the two domains in APLF is reported to bind PAR with sub-nanomolar affinity [89]. In contrast, NMR studies of the interaction of ADP-ribose and 2'-O- α -d-ribofuranosyladenosine with the APLF PAR-binding Zinc Finger (PBZ) domains yielded K_d values of ~ 10 mM [90]. Estimating the length of an ADP-ribose unit as ~ 12 Å on the basis of available NAD crystal structures, the seven units could extend for ~ 84 Å, substantially longer than the longest dimension of ~ 40 Å for X1BRCTa. This suggests that PAR may wrap around the X1BRCTa structure, interacting with multiple ADP-ribose recognition motifs on the surface of the domain. This type of binding might allow the X1BRCTa domain to move more easily along the PAR polymer following a process of partial dissociation and binding.

Reports dealing with the structural basis for XRCC1 recruitment by PARP1 have been inconsistent regarding the extent to which it is PAR-dependent. Masson et al. [18] indicate that PARP1 auto-modification augments but is not essential for binding, and Beernink et al. [91] reported evidence for a direct interaction between the X1BRCTa domain and the PARP1 BRCT domain, consistent with binding in the absence of PARylation. Alternatively, a recent study of the interaction of X1BRCTa with various PARP1 constructs revealed no significant affinity for any of these [92]. Consistent with this result, Kim et al. [88] have concluded that the XRCC1 interaction with PARP-1 is PAR-dependent, and that there are few if any direct contacts between PARP-1 and XRCC1. The latter results also are consistent with reports that XRCC1 recruitment is strictly dependent on PARP-1 automodification [86].

Elucidation of the role of X1BRCTa has been facilitated by non-conservative mutations of core hydrophobic residues Leu360, Ile361, Trp385 and Cys389 [85, 93, 94]. All of these hydrophobic residues are buried in the interior of the domain. Analysis of the fractional solvent exposure with the program VADAR [95] gives values of 0.0 (Leu360), 0.0 (Ile361), 0.03 (Trp385), and 0.0 (Cys389). As might be anticipated, the C389A mutation is the least perturbing, while the more structurally perturbing L360D/I361D double mutation causes a major functional loss, consistent with the expected structural disruption. The C389Y mutation has been reported to reduce XRCC1 expression [47]. *In silico* mutagenesis indicates significant steric clashes resulting from this mutation (unpublished results). As discussed by Kubota and Horiuchi [93], these mutations are designed to be destabilizing, although it is unclear to what extent each of these mutations will produce local or global unfolding of the domain. As a general observation, it is preferable to evaluate function by mutating surface residues that selectively perturb specific interactions, as discussed above for the X1NTD, rather than introducing destabilizing mutations, since the unfolded regions may introduce additional structural and functional perturbations. Wei et al. [23] recently showed that XRCC1 containing the (L360D,I361D) double mutation was polyubiquitinated, and interpreted this result in terms of a loss of PAR binding by X1BRCTa. However, since

these mutations are predicted to significantly destabilize the folded structure, and since polyubiquitylation is part of the cellular response for proteasomal degradation of unfolded proteins [96], it is possible that this observation results from a protein unfolding effect, rather than from a more specific effect on PAR binding.

IX: Phosphorylated FHA binding motif

The second XRCC1 linker, XL2 contains a multiply phosphorylated motif shown to interact with the forkhead associated (FHA) domains that are present in at least three binding partners: aprataxin (APTX), polynucleotide kinase phosphatase (PNKP), and aprataxin and PNKP-like factor (APLF) [20, 24, 97, 98]. These enzymes play an important role in the resolution of DNA lesions with problematic ends. PNKP has two enzymatic activities: a 3'-phosphatase that prepares the substrate for polymerization and/or ligation, and a 5'-kinase that prepares the substrate for ligation [99, 100]. APTX corrects DNA ligation errors by removing the 5'-AMP adduct [101, 102]. APLF exhibits 3'-exonuclease as well as limited endonuclease activity, but its roles in non-homologous end joining have been more thoroughly investigated than its roles in single-strand break repair [103, 104]. The position of the phosphorylated FHA binding sequence immediately preceding the C-terminal BRCTb domain which interacts with Lig3 α (Figure 1) suggests that the end processing reactions catalyzed by these FHA domain-containing enzymes are coordinated with Lig3 α mediated DNA ligation. The structure of the PNKP FHA domain in complex with the phosphorylated FHA binding sequences from XRCC1 and XRCC4 have been determined crystallographically [20, 105]. In the XRCC1 study, the observed FHA complex included the peptide segment: Y⁵¹⁵AG(pS)(pT)DEN⁵²² (Figure 7). However, isothermal titration calorimetric (ITC) binding studies indicated that a second FHA domain could simultaneously interact with a downstream sequence of phosphorylated residues: (pT)⁵¹⁹DEN(pT)DSEE⁵²⁷ to form a ternary complex [20]. It is at present unclear how the 1:2 XRCC1:FHA₂ complex would accommodate the overlapping XRCC1 sequences. A more recent study of the interaction of full length phosphorylated XRCC1 with the PNKP FHA domain obtained a lower K_d value and a 1:1 stoichiometry [106]. The crystal structure of the complex of the PNKP FHA domain with a phosphorylated XRCC4 peptide includes the sequence: Y²²⁹DES(pT)DE, which is very similar with that observed in the XRCC1 complex [105]. In the XRCC4 complex, Asp²³⁴ forms a salt bridge with Arg44, substituting for the interaction with phosphoSer518 in the XRCC1 complex.

X: The BRCTb domain

Caldecott and coworkers first demonstrated that XRCC1 forms a strong complex with DNA ligase3 α (DL3 α) that is mediated by interactions between the C-terminal BRCT domains on both proteins [16, 38]. From a genomics perspective, the importance of this interaction is supported by the presence of a nuclear localization signal in the β but not the α form of Lig3 [107]. Thus, nuclear import of Lig3 α is apparently dependent on its interaction with XRCC1 and XRCC1-mediated nuclear transport. The XRCC1 BRCTb domain was the first BRCT domain for which detailed structural characterization was available [108]. This structure revealed that X1BRCTb can form a homodimer, with the interface involving its N-terminal residues, particularly those on helix α 1. An NMR solution structure of the Lig3 α BRCT

domain also concluded that the structure was dimeric [109]. Based on modeling of the Lig3 α BRCT domain and the conservation of residues involved in interface binding, it was proposed that a structurally analogous L3BRCT:X1BRCTb interface would mediate the interaction between the two proteins. Nevertheless, until recently there were no examples of protein-protein interactions that involve two directly interacting hetero-BRCT domains, and the structural isolation and characterization of an L3BRCT:X1BRCTb complex proved to be elusive [110].

These problems were recently resolved by Cuneo et al. [38], who determined that crystallization of the L3BRCT:X1BRCTb heterodimer appeared to be limited by highly favorable lattice interactions supporting crystallization of the X1BRCTb:X1BRCTb homodimer. This limitation was overcome by introducing a non-conservative Y574R mutation into the murine X1BRCTb domain (corresponding to Y576R in human XRCC1). XRCC1 polymorphisms have been characterized by Berquist et al. [47], and the Y576S polymorphism at this position does not significantly affect function, indicating that Tyr576 can be mutated without significantly affecting function. Introduction of a non-conservative Y574R mutation into the mouse X1BRCTb domain was then found to allow crystallization of the heterodimeric L3BRCT:X1BRCTb, and structural data for both homodimers was also obtained. Consistent with the original proposal of Zhang et al. [108], both of the homodimers as well as the L3BRCT:X1BRCTb heterodimer utilize the same interface for binding, which involves residues in the N-terminal region of the domain and in α -helix 1 (Figure 8). This result indicates that under some conditions the homodimers may be present in the cell. Use of a common interface poses fundamental questions regarding the relative fractions of homo- and hetero-dimers that are present under various cellular conditions and compartments.

Cuneo et al. [38] were able to resolve the basis for selection of the X1BRCTb:L3BRCT heterodimer over the homodimers by noting that a portion of the XL2 linker immediately preceding X1BRCTb could interact with L3BRCT in the heterodimer, while a similar interaction in the homodimer was not present. The crystal structure of a tetramer containing two L3BRCT: X1BRCTb heterodimers is shown in Figure 9. Both X1BRCTb molecules include linker residues from Glu528-Glu536 that interact with L3BRCT, enhancing the relative stability of the heterodimer. The contribution of this linker to heterodimer stability was supported by melting curves indicating that the melting temperature of the heterodimer containing the additional linker segments was increased by 7.7°C. Involvement of the linking residues in heterodimer formation places the Lig3 α recognition domain immediately after the phosphorylated FHA binding sequence (PFBS) that mediates interactions with the FHA domains of PNKP, APTX, and APLF. This suggests that Lig3 α may be the preferred ligating enzyme in repairs involving modified DNA termini that require these additional factors as part of the repair process.

XI: Direct DNA binding by XRCC1

There are multiple reports of the interaction of XRCC1 and its component domains with DNA [14, 23, 47, 106, 111–115], however no structural data for the corresponding DNA complexes is available. A common finding is a lack of significant affinity for double-

stranded DNA (dsDNA), but relatively high affinity for nicked, gapped, or blunt-ended DNA. Mani et al. [111] reported a large effect of nicked and gapped DNA on the intrinsic (tryptophan) fluorescence of XRCC1, and obtained K_d values of 65 and 34 nM, respectively. Each of the three globular domains of XRCC1 contains two tryptophan residues, one of which is completely buried and one with 10–15% solvent exposure, so the fluorescence response apparently supports participation of at least one globular domain in mediating the DNA interaction. It also has been reported that the affinity of XRCC1 for DNA is inversely related with the degree of phosphorylation [106, 115].

The most detailed data supporting a direct DNA-XRCC1 interaction was reported by Marintchev et al. [14], using gel shift and NMR studies to demonstrate a direct interaction of the X1NTD with a gapped DNA ligand. Although dissociation constants were not reported, a bound form was detected in the gel shift assay at 10 nM concentration. The NMR amide chemical shift mapping data is, however, somewhat more ambiguous, revealing fairly small, non-localized shift perturbations, some of which were suggested to result from changes in salt concentration. Residues proposed to mediate DNA binding include Arg109 and Tyr136, which have subsequently been found to mediate pol β binding (Figure 3), as well as Ser91 and Ser97 located near the pol β interface. Based on the structural results summarized above, it appears that the DNA interaction and the pol β interaction involve interface contacts with significant overlap, so that both could not occur simultaneously. This result is also consistent with the modeled structure of the X1NTD•pol β •DNA complex shown in Figure 2B. A preliminary NMR study of the interaction of X1NTD with a double hairpin DNA model for single nucleotide gapped DNA that we performed failed to provide evidence of chemical shift perturbations. In summary, we have had difficulties confirming the interaction of gapped DNA with X1NTD and additional studies are required to confirm this interaction and to determine the mode of binding.

The BRCTa domain presents a more likely candidate for interaction with DNA due to its high pI value (Figure 1), and indeed it has previously been demonstrated that PAR can compete with DNA for common histone binding sites [116, 117], consistent with the possibility that DNA could interact with the PAR-binding motif. Nevertheless, preliminary chemical shift mapping studies of this interaction using a double hairpin model for gapped DNA similarly failed to indicate significant shift changes.

At this time, data supporting the interaction of gapped and nicked DNA with the X1NTD requires further confirmation and characterization. We are aware of no mutagenesis data allowing more specific identification of the DNA-interacting regions of XRCC1, nor is there any structural data for a DNA•XRCC1 complex.

XII: XRCC1 polymorphisms

Mohrenweiser et al. [118] have identified and quantified nine polymorphisms in XRCC1, including three common substitutions: Arg194Trp, Arg280His, and Arg399Gln (Table 1). The first two are located in linker XL1 between the N-terminal pol β -binding domain and BRCTa (Figure 1), and the third is on the surface of BRCTa, within the identified PAR binding motif (Figure 6). Extensive epidemiological studies, not reviewed here, have been

reported relating these polymorphisms to the incidence of various cancers and other diseases. The availability of structural information for XRCC1 complexes provides a basis for analyzing the effects of some of these polymorphisms, and in principle could be used in the design of epidemiologic studies to test specific hypotheses.

As noted above, a short, helical segment of linker XL1 that includes Arg194 interacts with the C-terminal domain of the trans-lesion synthesis enzyme Rev1 (Figure 5), and the R194W-substituted RIR peptide was found to exhibit reduced affinity for Rev1 [12]. Based on the possible role of the RIR in the recruitment of XRCC1-pol β for translesion synthesis, this substitution would presumably impact repair pathways utilizing pol β , e.g. repair of cisplatin DNA cross-links. XL1 also contains the nuclear localization signal (NLS), and Arg280 is located several residues after the NLS sequence that interacts with the major NLS binding site on importin α (Kirby et al., submitted). It has been reported that the His280 analog exhibits poorer localization at sites of DNA damage [119].

There have been numerous epidemiological studies relating the common Arg399Gln to various forms of cancer. Nevertheless, a comparison of the SSBR capabilities of cells containing wild-type or R399Q polymorphism found no significant difference between the two alleles, suggesting that the functional loss resulting from the R399Q substitution is minimal [85]. Since the PAR polymer presumably interacts with an extended region on the surface of BRCTa [88], the limited impact of a single, fairly conservative residue substitution is probably not that surprising.

In contrast with the common polymorphisms, there is reason to believe that the rare R560Q polymorphism identified by Mohrenweiser et al. [118] might exert a more significant effect on the interaction with Lig3 α . XArg560 (corresponding to XArg558 in the murine protein) forms salt bridges with L3 α Asp876 and L3 α Asp878 in the X1BRCTb:L3 α BRCT complex (Figure 10A). Even in this case, the homologous substitution would not be predicted to abrogate the interaction but to alter binding affinity.

Sliwinski et al. [120] have identified pol β (K289M) as an independent, early molecular diagnostic marker in breast cancer, reporting a strong association with the Met/Met phenotype. Although this polymorphism may exert its predominant effect on the activity of pol β , the crystal structure of the DNA pol β -X1NTD complex shows that β K289 forms a H-bond with β Gln324, which in turn is H-bonded to the XPro135 carbonyl on XRCC1 (Figure 10B). Thus, the K289M mutation, while not expected to dramatically reduce pol β -XRCC1 affinity, would be predicted to indirectly interfere with this interface. The weak effects of the polymorphisms noted above may prove relatively insignificant in studies utilizing agents that produce significant DNA damage. However, the cumulative effects of such compromised interactions over time might be significant.

XIII: Structural implications for XRCC1 mediated repair of single-strand breaks

PARP1 is thought to be the first responder to a DNA single-strand break, acting as a sensor/recruiter [9, 94, 121]. Damage-activated autoPARylation, which has been reported to result

in polymers containing up to hundreds of ADP-ribose units [122, 123], results in a dramatic physical amplification of the recruitment signal, facilitating accumulation of repair factors containing the PAR-recognition motif. Studies of Mortusewicz et al. [124] indicate that the initial PAR modification produces a chain reaction in which additional PARP1 molecules are recruited based on the interaction of their BRCT domains with the initially-formed PAR polymer, leading to a further enhancement of PAR synthesis. As a result of the polymeric nature of the signal, it presumably does not define a specific spatial relationship of the damage repair machinery to the damage site, but rather brings the repair complex in sufficient proximity to facilitate transfer to enzymes that are able to recognize and bind to the damaged DNA. The PAR-binding BRCTa module of XRCC1 presumably can undergo a series of dissociations and re-associations with the polymer, allowing it to sample a broader region of space until one of the XRCC1-bound enzymes is able to capture the damaged DNA site. However, the recruitment effect of PARylation is apparently self-limiting [124, 125] and this may be a consequence of electrostatic repulsion between PAR and the damaged DNA, or it may result from PARylation of XRCC1 and other proteins in the repair complex [126], which then has the effect of competing with autoPARylated PARP1 for common XRCC1 BRCTa binding sites. Another mechanism for dissolution of the PARP1-XRCC1 complex is the direct recruitment of the PAR degrading enzyme poly(ADP-ribose) glycohydrolase (PARG) by the PAR polymer [127–131]. PARG is also reported to interact directly with XRCC1 [131]. The factors regulating the complex balance between PAR formation and degradation are not currently well understood.

Subsequent to PARP1 recruitment, XRCC1 forms a group of flexible and heterogeneous repair complexes in which the damaged DNA can be traded back and forth among alternate binding partners, several of which are indicated in Figure 11. Each transfer addresses the questions: 1) how well matched is the damaged DNA to the protein binding site? 2) is the damaged DNA a viable substrate? Since there is no deus-ex-machina directing the DNA to the optimal repair enzyme, these questions must often be repeated, so that multiple transfers can take place prior to the appropriate enzymatic response. The damaged DNA is thus trapped by the complex more as a result of the presence of multiple binding partners than by extremely high affinity binding sites, which would ultimately slow down substrate transfer and the repair process. At some point during the repair process, the PAR polymer is hydrolyzed by PARG, releasing PARP1 from the XRCC1 repair complex. PARG is reported to bind to both PARP1 and XRCC1, among other proteins [130, 131]. Although dissociation of a DNA-protein complex is presumably a unimolecular process, the presence of high pI domains such as the X1BRCTa or the pol β lyase domain may facilitate DNA transfers by binding to accessible regions of the DNA and facilitating diffusion-mediated transfer. In this context, the pol β lyase domain is known to have significant affinity for ssDNA, with ability to bind 10 nucleotide stretches [132, 133].

XIV: PAR as a localized concentrator of repair factors

Since in order to deal with variable types of damage XRCC1 needs to interact with alternate binding partners, it would be useful if the repair complex could provide a readily available supply of these alternate binding partners in the vicinity of the complex. From a structural standpoint, PAR probably exhibits similarities to single-strand DNA due to its anionic

composition and flexibility, suggesting the consequent capability to conform to variously structured ssDNA binding sites. The PAR polymer has been found to exhibit structural similarities to both RNA and DNA, since antibodies raised against PAR can recognize RNA and DNA, and vice versa [134, 135]. It also has been demonstrated that PAR is able to compete with DNA for common histone binding sites [116, 117]. This suggests the possibility of a more general capability for weak recruitment of a wide range of repair factors that interact with various forms of damaged DNA. Indeed, the ability of polymerases to interact non-specifically with anionic polymers form the basis for heparin-based purification schemes [136, 137], that in our lab have been useful for pol β purification. The flexibility of PAR should allow it to conform to the bent binding sites that are often involved in binding damaged DNA, and in comparison with dsDNA, PAR is not electrostatically insulated by histones.

APLF is directly recruited to PAR by its two C-terminal PAR-binding Zinc Finger (PBZ) domains [89, 90, 138]. It also has recently been reported that the FHA domains of APTX and PNKP exhibit submicromolar affinity for PAR [87], facilitating additional recruitment of these enzymes. DNA Ligase 3 α exhibits weak accumulation at single-strand breaks in the absence of XRCC1 [139], presumably due to PAR binding residues in its Zinc Finger domain [17]. The weakness of the effect suggests a non-specific interaction. Non-specific recruitment may also be the basis of the reported interaction of the PARP-1 BRCT domain with the PAR polymer [124], since the PARP-1 BRCT domain has not been identified as having the PAR-binding motif [17, 140]. The high pI = 9.3 [92] of the PARP1 BRCT domain is consistent with this interpretation. The DNA pol β lyase domain, characterized by a high pI value and ability to bind long stretches of ssDNA [132, 133], is also a potential candidate for non-specific recruitment by PAR. Nevertheless, Kim et al. [88], report that the X1BRCTa domain does not bind to polyadenylate, indicating a limit to the ssDNA – PAR structural analogy.

We emphasize that in order to function optimally, these non-specific binding interactions need to be characterized by low to moderate affinity. The function is to increase the availability of a set of interchangeable parts for the XRCC1 repair complex, rather than to compete with XRCC1 for these enzymes. Moderate affinity binding interactions can facilitate interchange of binding partners before the entire repair complex collapses due to inability to repair a specific defect that requires a binding partner not initially present. A weak localization of other repair proteins, such as the PCNA complex involved in long patch repair, could also facilitate transfer to this pathway when incompletely repaired intermediates become uncoupled from the XRCC1 complex, however there is little evidence for such interactions at the present time.

XV: Involvement of XRCC1 in other DNA repair pathways

Although the role of XRCC1 in single strand break repair has continued to be clarified, its role as a scaffold for organizing base excision repair is less clear. In the BER pathway, DNA glycosylases fulfill a dual role as damage sensors and deglycosylation catalysts. Bifunctional glycosylases also possess lyase activity that results in a strand break. Many glycosylases are reported to exhibit strong product inhibition [141–144]. Consequently, the damaged DNA-

glycosylase complex is expected to persist for a time span that may be sufficient for transfer to the next enzyme in the repair pathway [145]. In various cases, product release by the glycosylase has been demonstrated to be facilitated by AP endonuclease [141, 146] and by SUMOylation [147]. Participation of the XRCC1 complex in BER is supported by several studies [31, 145, 148]. A fraction of the single strand breaks created during BER, possibly corresponding to intermediates that have become uncoupled from the major BER pathway, is recognized by PARP-1, resulting in XRCC1 recruitment and repair by the XRCC1 complex [148] [149]. However, XRCC1 can also be recruited to BER complexes by a PAR-independent signal [150, 151], the structural basis for which remains undetermined.

It has been noted that mammalian DNA glycosylases possess unstructured tails that appear to mediate interactions with other repair enzymes as well as product transfers [30, 152]. There are multiple reports of direct interactions between XRCC1 and various DNA glycosylases, including UNG2 [29], NEIL1 [30]; OGG1 [31], MPG, NTH1, and NEIL2 [32] as well as with AP endonuclease [28]. However recent pull down studies using pol β as bait, that identified XRCC1 and several of its binding partners, failed to identify any of these enzymes [153]. Additional studies are required to determine whether these interactions are dependent on covalent modifications, to more specifically identify the residues that mediate these interactions and to characterize the structures of the complexes that are formed.

The transition from XRCC1-dependent short patch DNA repair to long patch repair is dependent on the nature of the damage [154] and is also influenced by availability of ATP [155]. Damage that is repaired by bifunctional glycosylases is reported to be more susceptible to short patch repair, while damage repaired by monofunctional glycosylases is reported to utilize either pathway. A recent report of the importance of DNA Ligase 1 (Lig1) in SSBR [156] supports the importance of the long patch repair pathway, which utilizes PCNA in complex with DNA Lig1 [157]. Recruitment of PCNA to sites of DNA damage is dependent on XPG [158] and occurs with significantly slower kinetics than recruitment of the XRCC1 complex [159]. It is unclear how to reconcile this difference in localization kinetics with reports of XRCC1-PCNA complex formation [33]. Preliminary NMR studies consistent with only very weak binding (unpublished results), suggest that this interaction may require additional factors or covalent modifications.

The XRCC1-Lig3 α complex also has been shown to play a role in nucleotide excision repair (NER) [10, 11], however the structural basis for XRCC1 recruitment to this pathway is not clear at present. Recent studies report PARP1 recruitment and activation by damaged DNA-binding protein 2 (DDB2), a component of the global genomic NER subpathway [160]. This provides an alternate pathway for PAR-dependent recruitment of the XRCC1 repair complex. Indeed, PAR formation may mediate XRCC1 recruitment in multiple pathways [161].

XVI: Summary and remaining challenges

The fundamental relationship between DNA repair and disease is increasingly appreciated [162, 163]. Structural characterizations of XRCC1 complexes provide critical information required to develop a more complete understanding of the multiple roles of this scaffold

protein in DNA repair. This information can be used to design studies that test specific hypotheses for the functional contributions of these interactions. It also is of value for interpreting the exploding literature relating single nucleotide polymorphisms to cancer and other diseases, and potentially for the design of studies that target particular polymorphisms hypothesized to alter function. The availability of highly sensitive fluorescent antibody-based methods for the detection of protein interactions can be accompanied by a greater prevalence of false positives that in turn can result in a substantial increase in unsuccessful attempts at structural characterization [164, 165]. At the present time, detailed structural characterizations of important XRCC1 interactions particularly for DNA binding, recruitment for BER and NER pathways, and the structural basis for PAR-independent recruitment, significantly lags reports of such interactions limiting the characterization of this important DNA repair complex.

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Abbreviations

BER	base excision repair
CK2	casein kinase 2
Lig1	DNA Ligase 1
Lig3α	DNA Ligase 3 α
L3BRCT	DNA Ligase 3 α BRCT domain
NER	nucleotide excision repair
PAR	poly(ADP-ribose)
PARP-1	poly(ADP-ribose) polymerase 1
SNP	single nucleotide polymorphism
SSBR	single strand break repair
X1BRCTα	XRCC1 BRCT α domain
X1BRCTβ	XRCC1 BRCT β domain
XL1	first linker domain in XRCC1, residues 155–309 in XRCC1
XL2	second linker domain in XRCC1, residues 406–528
XRCC1	X-ray cross complementing group 1 protein
X1NTD	XRCC1 N-terminal domain (residues ~ 1–153)

X1NTDox oxidized form of X1NTD containing a Cys12-Cys20 disulfide bond

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Figure 1.

Domain structure of XRCC1. A) Domain structure of XRCC1 showing the positions of the N-terminal domain (NTD), reported REV1 interacting region (RIR), nuclear localization signal (NLS), first BRCT domain (X1BRCTa), phosphorylated FHA binding sequence (PFBS), and second BRCT domain (X1BRCTb). B) Domain pI values, determined from Expasy site: http://web.expasy.org/compute_pi/, and C) reported phosphorylated residues, taken from PhosphositePlus (<http://www.phosphosite.org>).

Figure 2.

Interaction of the XRCC1 N-terminal domain (X1NTD) with pol β. A) Ribbon diagrams showing the complex of the pol β polymerase domain (subdomain color coding: DNA binding (cyan), catalytic (yellow), nucleotide binding (purple)) with X1NTD (green). B) Overlay of the complex shown in A with a pol β bound to a single nucleotide gapped DNA (gray, bound DNA with orange backbone). Figure B indicates the relative positions of X1NTD with pol β-bound gapped DNA.

Figure 3. Residues mediating the binding of pol β N-domain with X1NTD. The figure shows a ribbon diagram of the Val303 loop region of the pol β Nucleotide binding subdomain (purple) in complex with X1NTD (green). X1NTD residues Phe67, Glu69, Val86, Arg100, Arg109, and Tyr136 originally identified to mediate the interaction based on mutagenesis studies [39] are indicated in yellow. All of these residues are located at the pol β: interface.

Figure 4. Oxidation of X1NTD. A) A ribbon diagram showing the oxidized form of the X1NTD (green; the remodeled 40 residues at the N-terminus are yellow) in complex with the pol β N-subdomain (purple). The Pro2, Cys12, and Cys20 sidechains are in cyan. B) Stabilizing interactions of the N-terminal carbamate group with Arg7, Ser44, and Lys129. C) Initial formation of the disulfide bond leads to a dynamic structure that is then stabilized by non-enzymatic recruitment of CO₂ to form the carbamate adduct.

Figure 5. Structure of the complex formed between the XRCC1 RIR motif and the REV1 C-terminal domain. The ribbon diagram shows an overlay of the NMR-derived structure of the XRCC1 RIR peptide [12] with the crystal structure of the REV1 C-terminal domain in complex with Rev7/Rev3 (pdb: 4FJO, Wojtaszek et al., 2012).

Figure 6. Ribbon diagram of BRCTa. NMR-derived solution structure of BRCTa (green, pdb: 2D8M, Nagashima et al., 2006). The PAR-binding region identified by Pleschke et al., [17] is shown in blue. Residues Leu360, Ile361, Trp385, and Cys389 that have been mutated in functional studies are shown in yellow. The reported structure corresponds to BRCTa containing the common R399Q polymorphism (magenta). Additional disordered residues at the terminal positions are not included.

Figure 7.

Complex of the PNKP FHA domain with the XRCC1 FHA-binding peptide. A) The ribbon diagram shows the FHA domain and sidechains for the major interacting residues (green) with the XRCC1 PFBS peptide (cyan): Y⁵¹⁵AG(pS)(pT)DEN (pdb: 2W3O, [20]). The structure also includes two Ca²⁺ ions (magenta) that interact with the phosphorylated residues. The ribbon diagram corresponding to the APTX FHA domain is also overlayed (pdb: 3KT9, [166], gray). Important XRCC1-binding residues are conserved in the APTX FHA domain, with the main difference being substitution of Lys38 for Arg44 (not shown).

Figure 8. BRCT dimer structures. A) Ribbon diagram showing the mX1BRCTb homodimer structure (pdb: 3PC6). The N-terminal loop and α -helices of the domains mediate the dimerization. B) Ribbon diagram of the L3BRCT homodimer (pdb: 3PC7). As with X1BRCTb, the interface similarly involves the N-terminal segment, and the first α -helix. C) Structure of the L3 α BRCT:X1BRCTb heterodimer formed from the two BRCT domains, illustrating the preservation of the interface interaction motif (pdb: 3PC8, [38]). In each structure, the diagrams are color coded from violet (N-terminus) to red (C-terminus).

Figure 9.

Structure of the L3BRCT:X1BRCTb heterodimer. A) The crystal structure contains two L3BRCT:X1BRCTb heterodimers in the unit cell, connected by an additional hydrophobic interface between the pair of X1BRCTb domains at the center (pdb: 3QVG). The X1BRCTb domain in this structure included residues starting at Glu⁵²⁸ which forms an extended segment that interacts with L3BRCT. B) Expanded view of the interaction of the extended X1BRCTb chain with L3BRCT, providing a basis for selecting the heterodimer over the structurally analogous homodimers that lack this additional interaction.

Figure 10.

Structural implications of XRCC1 and pol β polymorphisms. A) The structure of the X1BRCTb :L3αBRCT heterodimer includes salt bridges linking XArg558 with LAsp876 and LAsp878. The rare R558W polymorphism (R560W in hX1BRCTb) (Table 1) is predicted to form a weaker heterodimer structure, possibly resulting in a functional impairment. B) The breast cancer-associated K289M mutation is predicted to exert a small effect on the pol β:X1NTD binding affinity, based on its role in positioning Gln324 which interacts with the Pro135 carbonyl.

Figure 11.

Interaction of the XRCC1 repair complex with damaged DNA. The PARP-1 sensor is extensively autoPARylated, providing a large recruitment signal that interacts with the XRCC1 BRCTa domain or other PAR recognition motifs, and weakly with several other proteins that bind to damaged DNA. The DNA substrate is traded among enzymes until it can be acted upon. Alternate XRCC1 binding partners or other repair factors (gray) may accumulate based on non-specific affinity for PAR, so that they are waiting in the wings if needed.

Table 1XRCC1 and pol β polymorphisms^a

Position	Common residue	Variant residue	Allele frequency
72	Val	Ala	0.03
161	Pro	Leu	0.005
173	Phe	Leu	0.005
194	Arg	Trp	0.13
280	Arg	His	0.03
309	Pro	Ser	0.01
399	Arg	Gln	0.24
560	Arg	Trp	0.01
576	Tyr	Ser	0.01
Pol β			
8	Gln	Arg	0.01
137	Arg	Gln	0.006
242	Pro	Arg	0.005
289	Lys	Met	

^a Adapted from Table 2 of Mohrenweiser et al. [118]. The pol β (K289M) polymorphism discussed by Sliwinski et al. [120] has been included since it is located near the XRCC1 interface.