Published in final edited form as:

Curr Opin Struct Biol. 2010 June ; 20(3): 283–294. doi:10.1016/j.sbi.2010.03.012.

Structural dynamics in DNA damage signaling and repair

J. Jefferson P. Perry^{1,2}, Elizabeth Cotner-Gohara³, Tom Ellenberger³, and John A. Tainer^{1,4}

¹ Department of Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, U.S.A

² The School of Biotechnology, Amrita University, Kollam, Kerala 690525, India

³ Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

⁴ Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Abstract

Changing macromolecular conformations and complexes are critical features of cellular networks, typified by DNA damage response pathways that are essential to life. These fluctuations enhance specificity of macromolecular recognition and catalysis, and enable an integrated functioning of pathway components, ensuring efficiency while reducing off pathway reactions. Such dynamic complexes challenge classical detailed structural analyses, so their characterizations demand combining methods that provide detail with those that inform dynamics in solution. Small angle x-ray scattering, electron microscopy, hydrogen-deuterium-exchange and computation are complementing detailed structures from crystallography and NMR to provide comprehensive models for DNA damage searching, specificity, signaling and repair. Here, we review new approaches and results on DNA damage responses that advance structural biology in the fourth dimension, connecting proteins to pathways.

Introduction

DNA repair networks are essential to life as genomic DNA is incessantly damaged by endogenous metabolites and exogenous toxicants. Chemical modifications of nucleobases by oxidation, deamination, depurination, alkylation, and cross-links, as well as single and double strand breaks in the phosphosugar backbone of DNA occur spontaneously in every cell [1]. Repair of DNA damage classically involves three steps: 1) damage detection, 2) damage removal, and 3) replacement with the correct DNA sequence and chemistry. The excision of damaged DNA generates a 3' primer terminus that can be extended by DNA polymerase using the undamaged strand as a template. A DNA ligase then reseals the broken DNA backbone to complete the repair. One consequence of the find, cut, and fill DNA repair process is the formation of chemically reactive or miscoding intermediates that are as toxic and mutagenic as the original damage. Thus, there is extremely strong genetic selection for both coordination between competing repair pathways and the seamless handoff of repair intermediates to ensure a complete restoration of DNA structure. These

*Correspondence: tome@biochem.wustl.edu and jat@scripps.edu.

These authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

principles are well illustrated by examples of DNA base excision repair (BER) processes [2], including new abasic site endonuclease IV structures, where strain in the enzyme-substrate complex is released upon incision of the DNA backbone, to create a stable product complex for handoff to polymerase and ligase [3•].

DNA repair processes thus provide prototypical systems that enable us to examine how protein structures are intricately connected for the coordination of steps within a pathway or for crosstalk between different pathways. Notably, emerging results indicate that different biological outcomes can be connected to a single, dynamic DNA repair complex. Distinct outcomes occur through modular and transient interactions, conformational switching, and interface exchanges, as exemplified by FEN-1 and ligase interactions with DNA and PCNA [4,5], and by Rad51 interactions with DNA and BRCA2 [6]. To successfully define these dynamic macromolecular assemblies, structural biologists are increasingly employing a combination of methods to measure dynamic motions and alternative conformations that can be mapped onto atomic resolution structures. Here, we outline structural advances and insights for DNA repair complexes with the following examples: 1) progress in using combined methods, 2) lesion recognition and excision for DNA base and nucleotide damage, 3) processing and control of DNA double-strand breaks and ends, and 4) single-strand break responses and repair.

Combined methods for dynamic complexes

Comprehensive biological insights about dynamic ensembles of variant structures come from combining detailed structural information from crystallography and NMR with solution methods, such as small angle x-ray scattering (SAXS) and hydrogen deuterium exchange mass spectrometry (DXMS), plus computational simulations. Macromolecular X-ray crystallography, nuclear magnetic resonance (NMR), and electron microscopy have been our most reliable structural tools, providing information spanning from the nanoscale to the atomic level. These techniques can also provide information about alternative conformational states, but their uses are limited for complexes with functional flexibility and intrinsic disorder. SAXS yields information on flexible macromolecules in solution, including their shapes and assembly states [7]. Recent improvements in SAXS data collection and analysis for biological samples have enabled comprehensive and high-throughput strategies to examine structures of whole pathways and their component interactions in solution, rather than single complexes [8•]. DXMS is advantageous and complementary to SAXS because it reveals conformational changes down to the resolution of single amino acids [9,10•]. An exciting application of DXMS is its combination with crystal structures to define interactions for DNA mismatch repair [10•].

Emerging results suggest that the biological importance of macromolecular dynamics extends from the confines of an enzyme active site to the interactions of large, multi-domain complexes that allosterically regulate enzymatic activity. NMR studies of catalytically active dihydrofolate reductase, an essential enzyme for purine base biosynthesis, indicate that the macromolecular dynamics are specifically tuned to individual steps of the reaction pathway [11•]. Transitions between different enzyme conformations can involve non-native interactions that lower the energy barrier for interconversion between different states [12]. For multi-domain complexes, exemplary results come from the DNA damage response and tumor suppressor protein p53. Combined analyses by NMR, crystallography, and SAXS provided an integrated portrait of p53 and its complexes, informing biological functions [13•]. Importantly, aberrant protein flexibility and destabilization of repair protein frameworks and interactions can cause severe genetic diseases. Thus, structural and biochemical studies of mutations in the multi-domain nucleotide excision repair (NER) helicase XPD associate changes in DNA and ATM binding with cancer, and defects in

framework flexibility or stability with the aging disorders Cockayne Syndrome or Trichothiodystrophy (TTD) [14••]. Similarly, mutations causing the cancer predisposing Nijmegen breakage syndrome cause defects in its interactions with Mre11 and Nbs1, altering the framework for DNA repair [15••,16••].

Exploiting DNA flexibility for damage detection

The structural biology of NER requires concerted assembly of protein complexes, regulated handoffs of DNA repair intermediates, and coordination with replication and transcription. NER excises bulky, helix-distorting lesions from one strand of a damaged DNA duplex: this process requires accurate and efficient detection of structurally diverse modifications to prevent cleavage of the undamaged strand that serves as a template for repair. NER proteins assemble in a step-wise manner at the site of damage to catalyze DNA strand separation and excision of a short (~28 nt) segment containing the damage. The sequential assembly of NER proteins allows damage verification and regulation of NER activity before the committing step of incising the DNA backbone. Two recent structures of DNA damage recognition complexes, representing two NER sub-pathways, have shed light on the initial steps of damage detection [17••].

Yeast Rad4 (mammalian XPC homolog) is a multi-domain protein that binds selectively to the helix-distorting lesions repaired by NER, in a complex with the smaller Rad23 to initiate repair. A crystal structure of the Rad4/Rad23 complex bound to DNA damage mimicked by a thymine-thymine mismatch shows a large DNA structural distortion stabilized by the protein. Rad4 inserts a β -hairpin through the double helix, displacing bases on either strand of the DNA in an extra helical conformation [18]. Surprisingly, the flipped-out thymines on the damaged strand are disordered and not contacted by Rad4. Instead, Rad4 binds the undamaged DNA strand, with its flipped-out thymines lying in a nonspecific binding pocket that could accommodate different sequences. The damaged DNA segment is under wound and the helical axis is offset with respect to the adjacent B-form DNA, creating a bend that extends the Rad4 DNA interaction surface to include adjacent β -hairpin domains and a larger transglycosylase domain. In all, the complex reveals an extensive DNA binding interface that stabilizes the damaged DNA in an energetically unfavorable conformation with the lesion containing bases exposed to solvent. The base pair unstacking that results from a helix-distorting lesion may favor base flipping on the opposite strand, conferring damage-selective binding of Rad4 in preference to undamaged DNA. In this case, DNA damage is sensed indirectly by the flexibility imparted to the undamaged strand in the vicinity of a helix-distorting lesion.

An alternative mechanism of DNA damage sensing in the NER pathway involves the DDC1-DDC2 dimer, which binds tightly to mildly distorting cis, syn-cyclobutane pyrimidine dimers (CPDs) that are not efficiently recognized by XPC-Rad23. Two crystal structures of DDC1-DDC2 bound to DNA containing either a 6-4 photoproduct or a tetrahydrofuran abasic site show that DDC2 exclusively contacts the DNA using one face of a 7-bladed WD40 repeat domain, with the opposite face of the WD40 domain contacting the DDC1 subunit [17••]. DDC1 consists of 3 WD40 domains and a small α -helical motif, which participate in interactions with DDC2 and the E3 ubiquitin ligase CUL4-RBX1. The DDC1-DDC2-CUL4-RBX1 complex localizes to sites of damage and ubiquitinates XPC and DDC2. Post-translationally modified DDC2 releases the DNA to affect a handoff to XPC-Rad23, and the normal NER pathway subsequently completes repair. This two-step mechanism for sensing damage accelerates the repair of CPDs by overcoming their limited affinity for XPC-Rad23.

In the crystal structure of the DDC1-DDC2-DNA complex, the 6-4 photoproduct is in flipped-out orientation that docks in a shallow binding pocket on the surface of DDC2. This lesion is not extensively contacted by DDC2, consistent with a broad binding specificity for structurally diverse DNA lesions. The adenine bases opposite the 6-4 photoproduct remain stacked within the DNA duplex, participating in hydrogen bonding interactions with DDC2 residues that are inserted into the gap left by the flipped-out 6-4 photoproduct. The DNA backbone is compressed at the step that separates the pyrimidine and pyrimidine nucleosides of the 6-4 photoproduct. This distortion is nicely accommodated by the binding site and could provide selectivity for UV photodimers like the CPD and 6-4 photoproduct. Since binding to DDC2 does not require a distortion of the undamaged DNA strand, this interaction may be better suited for binding to CPD lesions causing minimal destabilization of duplex DNA. In contrast, XPC-Rad23 can accommodate diverse DNA helix-distorting lesions, including bulky nucleobase adducts, which may cause a disadvantage for sensing CPD lesions that are reasonably well accommodated in B-form DNA. Thus, DDC1-DDC2 damage sensing in NER complements XPC-Rad23: mutations in DDC1-DDC2 cause photosensitivity in association with Xeroderma pigmentosum complementation group E.

Transient encounters with DNA detect and select BER substrates

In contrast to helix-distorting DNA lesions processed by NER, the base excision repair pathway (BER) detects and removes single nucleotides having modifications as subtle as a single methyl group addition. Most BER substrates minimally perturb dsDNA structure, yet they can be potent replication blocks because of the stringent templating requirements of replicative DNA polymerases. BER damage detection is further complicated by substrate chemical diversity resulting from oxidative damage, alkylation, and mispairing during DNA synthesis. A corresponding diversity of DNA glycosylases detects and processes different types of modified bases to initiate the BER pathway. Recent crystal structures of the oxidative damage-specific glycosylases OGG1 [19•] and MutM [20••,21••] have revealed the initial steps of selecting 8-oxoG bases in duplex DNA and flipping the modified nucleotide into the active site of OGG1.

Previously reported structures of OGG1 and the bacterial ortholog MutM in complex with a 8-oxoG containing DNA substrate showed a pseudo-Michaelis complex, with 8-oxoG inserted into these enzymes' active site pocket. 8-oxoG has a signature hydrogen bond donor (N9) and acceptor (O8) absent from guanine, and these 8-oxoG substituents interact with OGG1 active site residues to confer lesion selectivity. The substrate nucleotide is secured in a flipped-out orientation that aligns the glycosylic bond for nucleophilic attack by water.

Yet, steps preceding the formation of a Michaelis complex by nucleotide-flipping enzymes have been enigmatic, spawning various mechanistic proposals with enzymes sliding along the DNA helix to interrogate base pairs during the search for damage. However, the kinetics of a DNA scanning process by which a repair enzyme interrogates every base pair, binding and flipping each nucleotide out of the DNA helix, are presumably too slow to keep pace with cell growth and error-free transmission of genetic information. It therefore seemed likely that shortcuts would be needed to efficiently identify damaged nucleotides within a vast excess of undamaged DNA to expeditiously complete repair.

Enter the Verdine lab, with a chemical biologist's toolkit to stabilize transient intermediates in DNA binding and catalysis by repair enzymes. By covalently attaching a tether between a repair enzyme and DNA, and by engineering mutations in both, they have captured the initial encounter of MutM with oxidatively damaged DNA [20••]. The protein-DNA crosslink enables crystallization of complexes with oxidatively damaged and undamaged DNAs in different, matched sequence contexts. The results show a local distortion of the

DNA backbone in complex with MutM that is predictive of an 8-oxoG lesion, providing a means of sensing damage prior to flipping the nucleotide into the enzyme active site. Molecular dynamics simulations support the notion that DNA bending by MutM lowers the energetic barrier to nucleotide-flipping, and that local base pair unstacking induced by the lesion further favors flipping of an oxidatively-damaged nucleotide versus a normal Watson-Crick nucleotide pair. The demonstration that 8-oxoG can be detected in duplex DNA without further exposure by nucleotide flipping is consistent with the free diffusion of MutM along DNA visualized by single molecule techniques. Sliding on DNA speeds up the search for damage, whereas the energetically costly process of nucleotide flipping appears to be reserved for sites of damage.

In a further refinement of their DNA crosslinking strategy, the authors incorporated a photoactivatable guanosine in DNA covalently tethered to MutM [21••]. A crystal of the MutM-DNA complex was exposed to a laser pulse to release the caging group, and the crystal was frozen immediately by plunging into liquid nitrogen. In contrast to structures of human OGG1 cross-linked to guanosine-containing DNA, the uncaged guanosine in complex with MutM rapidly penetrated the active site pocket. However, many of the side chains that contact 8-oxoG in a productive complex are turned away from the extra-helical guanosine in the inactive complex, suggesting nucleotide flipping can precede rearrangement of active site residues that form the Michaelis complex. Similar results have been reported for human OGG1 complexed to the caged guanosine-containing DNA [19•].

This active site cross-linking strategy to trap labile protein–DNA complexes is likely to have general applications, as it was also recently used to define DNA complexes for *Escherichia coli* AlkB and its human homologue ABH2, which repair DNA/RNA lesions via a direct oxidative dealkylation mechanism. These structures show that AlkB squeezes together the two bases flanking the flipped-out damaged nucleotide whereas ABH2 uses a finger residue to intercalate into the DNA duplex [22•].

A repair protein decoy co-opts an alternative pathway of repair

Alkyltransferase-like proteins (ATLs) provide prototypical examples of how a metastable complex can control pathway selection. ATLs share structural motifs with the O(6)-alkylguanine-DNA alkyltransferases (AGT) that catalyze the reversal of DNA alkylation-damage and are important targets for cancer chemotherapy. ATLs can protect cells from DNA alkylation damage [23•], yet surprisingly, these proteins lack an active site reactive cysteine or detectable alkyltransferase activity. Recent structures of fission yeast ATLs in complex with damaged or undamaged DNA have revealed that ATLs instead utilize nucleotide flipping as a molecular switch to activate an alternative pathway of DNA repair. Instead of reversing the damage, the ATL-DNA complex mimics a helix-distorting lesion that is subsequently repaired by the NER pathway. ATLs function at the nexus between BER and NER pathways, and ATL genes have broad phylogenetic distribution in all domains of life, including homologues in sea anemone and ancestral archaea. A human ATL ortholog has not been identified but would have interesting implications for the development of more effective cancer treatments, whereas expressing ATL could protect bone marrow and other sensitive cells during alkylation therapy.

Protein scaffolds for DNA replication and checkpoint activation

Proliferating cell nuclear antigen (PCNA) and Rad9-Rad1-Hus1 (9-1-1) complex are trimeric ring structures acting as sliding clamps critical to DNA replication and repair. These clamp complexes provide exemplary information regarding emergent properties of macromolecular scaffolds that control chemistry, specificity, and coordination of multi-step pathways, via flexible tethering of multiple enzyme partners. The heterotrimeric 9-1-1

complex resembles the PCNA replication processivity factor, and shares some roles and partners with PCNA including FEN-1 and Ligase. Yet, 9-1-1 is not involved in DNA replication and has distinct DNA damage sensing roles, including recruiting and stimulating ATR kinase at DNA damage sites, leading to replication checkpoint and inhibition of cell cycle progression.

Crystal structures of human Rad9-Rad1-Hus1 (9-1-1) independently determined by three laboratories show that human 9-1-1 has a PCNA-like trimeric ring structure but with noticeable asymmetry [24–26••] (Fig. 1). The subunits in both PCNA and 9-1-1 are composed of homologous N- and C-terminal domains linked by an interdomain connecting loop (IDCL); head-to-tail connections between subunits form the trimeric ring.

PCNA subunits form a docking platform for DNA metabolizing enzymes that contain a PCNA-interacting peptide motif (PIP-box) consensus sequence of Q-X-X-[I/L/M]-X-X-[F-F/Y], first identified in DNA polymerases, where PCNA interactions significantly increased processivity of DNA synthesis. Currently, over 50 distinct PCNA-interacting proteins with diverse functions are known in eukaryotes. Crystal structures of a canonical PIP-box bound to PCNA reveal that the p1 Gln side chain binds to PCNA on a region termed the "Q-pocket", whereas the p4 [I/L/M] residue and the p7/8 F-[F/Y] side chains bind into a hydrophobic pocket near the IDCL. In some PCNA partners, including FEN-1 and p21, the residues C-terminal to the PIP-box form an extensive anti-parallel β -strand interaction with the IDCL of PCNA. Interestingly, translesion synthesis (TLS) polymerases also bind to PCNA, but with lower affinities that may allow recruitment to stalled replication forks and TLS activity without interfering with normal DNA replication. TLS PIP-box interactions with PCNA show key differences including altered binding to the Q-motif region through non-canonical PIP-box motifs that explain lower binding affinities [27•].

In the 9-1-1 heterotrimeric ring, differences between subunits are particularly notable in the IDCL/PIP-box binding site. The IDCL/PIP-box binding region of Rad9 is most similar to that of PCNA. The IDCL region is weakly conserved in Rad1, and least conserved in Hus1. This asymmetry may allow the combinatorial assembly of distinctive 9-1-1 repair factor complexes for efficient handoffs of DNA substrates between partner proteins. The FEN-1 PIP-box preferentially binds to Rad1, with lower affinity for Rad9 and Hus1 [26••]. Crystallographic analysis revealed density for the FEN-1 PIP-box bound to the Rad1 subunit, poorly ordered density at the Rad9 interface and no interpretable electron density at the Hus1 interface, demonstrating selectivity in interactions with the 9-1-1 complex. Doré and co-workers also confirmed that FEN-1 binding perturbed the binding of p21 [24••]. However, their results implicate additional interaction interfaces within 9-1-1, as FEN-1 with a deletion of its PIP-box motif still binds to the 9-1-1 complex [24••].

The loading of these clamps onto DNA may be subject to specific and distinctive protein interactions that exploit structural differences in PCNA and 9-1-1 complex subunits. The replication factor C complex opens and loads PCNA onto DNA, whereas a specialized Rad17-RFC complex loads 9-1-1. The 9-1-1 ring likely opens via that Rad9-Rad1 interface, perceived as the weakest interaction [24••,25••]. However, the Hus1-Rad1 interface most closely resembles PCNA and may serve as the opening point, as it is likely to be recognized by RFC [26••]. The ~120 residue C-terminal extension of Rad9 is predicted to be disordered and was removed for crystallization; yet, this region is important for cell cycle-dependent checkpoint activation and, in its phosphorylated form, recruits TopBP1 to sites of DNA damage for activation of the ATR checkpoint kinase. Overall, architectural differences in 9-1-1 and PCNA, particularly the IDCL/PIP-box binding region, provide insights regarding recruiting and assembling partners to enable specific responses to different genotoxic stresses.

Double-strand break (DSB) detection and repair

Structures of the DNA-dependent protein kinase *holo*-enzyme (DNA-PK), which plays an essential role in the non-homologous end-joining pathway of DSB repair in mammalian cells, reveal binding and conformational changes controlling DSB pathway end joining initiation. DNA-PK is a serine/threonine protein kinase that consists of two subunits, the ~4100 amino acid catalytic subunit (DNA-PKcs) and the smaller Ku70/80 heterodimer. This protein complex belongs to the phosphatidylinositol-3-OH kinase (PI(3)K)-related protein family and has been indicated to phosphorylate a significant number of proteins *in vitro*, though as of yet only Ku70/80, XRCC4, Werner syndrome protein (WRN) and Histone H2AX, are clear targets *in vivo* (reviewed in [28]).

DNA-PK's large size allowed determination of a 7 Å cryo-EM structure [29]. Recently a 6.6 Å crystal structure of DNA-PK [30••] - a landmark achievement considering the size and plasticity of the complex - provided added insights into DNA-PK architecture and flexibility. The crystal structure reveals a 160 Å long and 120 Å wide ring-like structure built from α -helical heat repeats, with a central cavity large enough to accommodate dsDNA (Fig. 2A). On top of the ring is a crown that contains the C-terminal kinase domain with associated FAT and C-terminal FAT-C domains, named for the protein families containing this domain (FRAP, ATM, TRRAP). At the base of the ring is an N-terminal opening. A region of the head/crown termed the forehead and residues at the N-terminal gap arch towards each other, creating cradle-like structure, when viewed from the side. Several features of this crystal structure agree with those delineated by EM studies, including a ring-like shape, a head/crown and also potential DNA-binding domain inside of the ring structure.

SAXS analyses of DNA-PK provide insights about major conformational changes that are required for DNA-PK's transient function during the initiation of DSB repair by NHEJ [31••] (Fig. 2B). The SAXS experiments show flexibility in the C-terminal arm of Ku80 (which is not defined in the crystal structure) that recruits and retains DNA-PKcs at DSBs. DNA-PKcs dimerizes through an association with Ku or DNA, and this promotes trans-autophosphorylation. DNA-PK phosphorylation causes a large conformational change that opens the ring and could thereby promote release from DNA ends by modulating the N-terminal gap in the ring shape of the DNA-PK crystal structure.

DNA-PK, along with ATM and ATR checkpoint kinases, phosphorylate the histone H2AX C-terminus in chromatin flanking DNA damage. This phosphorylation establishes a recruitment platform for checkpoint and repair proteins, but the recognition proteins for spontaneous replication associated breaks were unknown. SAXS and crystal structural analyses combined with genetic and biochemical studies of the *Schizosaccharomyces pombe* Brc1, a 6-BRCT-domain protein that resembles mammalian PTIP, identify Brc1 binding to phosphorylated histone as a key chromatin-specific response to replication-associated DNA damage. Such tandem BRCT domains are key molecular readout modules for the assembly of phosphorylation-regulated DNA damage response effectors and signaling complexes. The crystal structure reveals how variable insertion loops sculpt tandem-BRCT phosphoprotein-binding pockets to facilitate unique phosphoprotein-interaction specificities. The collective results also reveal an acidic Brc1 surface suitable to facilitate H2AX histone exchange and remodeling of chromatin structure proximal to damaged DNA by an interaction predicted to compete with histone-DNA binding [32••].

Rad50 and Mre11 are essential effectors and signaling proteins for DSB repair and homologous recombination that are conserved in archaea, fission and budding yeasts, and higher metazoans. The associated eukaryotic Nbs1 binds to phosphoproteins during

activation of DNA damage-induced cell cycle checkpoints. The Mre11-Rad50-Nbs1 (MRN) complex forms a multi-purpose DNA tether that can directly bridge severed DNA ends and chromatin domains, as initially suggested by the elongated architecture of the complex [33]. Structural analyses of the DSB ATPase Rad50 and mismatch repair ATPase MutS furthermore provide insights into ABC-ATPase superfamily functions [34]. Recent structures of Mre11-DNA complexes reveal how Mre11 dimers can distinguish a DSB with two ends from a one-ended DSB that results when a replication fork encounters a single-stranded break in the DNA template [15••]. The locations of Mre11 mutations that are associated with DNA damage sensitivity or a loss of Nbs1 binding suggest that Nbs1 binds across the back face of the Mre11 dimer, opposite to the DNA-binding face (Fig. 3A). In this position on Mre11, the Nbs1 subunit could distinguish a symmetric Mre11 dimer bound to a two-ended DSB from the asymmetric Mre11 dimer predicted at a collapsed replication fork with a single-ended break.

The long awaited architecture and a key interaction surface of Nbs1 have now been characterized by protein crystallography and SAXS. These studies reveal an elongated, disjointed arm-like Nbs1 conformation [16••,35] with FHA-BRCT-BRCT tandem domains (Fig. 3B) that are connected through a C-terminal linker to motifs that interact with Mre11 and ATM. These Nbs1 structural features suggest how the MRN complex may coordinate DSB repair and checkpoint signaling through interactions with ATM, MDC1, and Ctp1 (CtIP/Sae2). Nbs1 mutations associated with Nijmegen breakage syndrome (NBS) cause a predisposition to cancer. Approximately 90% of NBS patients harbor a frame shifting mutation (657del5) that creates a bi-cistronic message encoding a smaller N-terminal construct and a larger C-terminal construct initiating at a cryptic start site. This mutation thereby separates the FHA and BRCT domains onto separate polypeptides, effectively uncoupling the phosphoregulatory interactions of Nbs1 from its interactions with Mre11 and ATM/Tel1. Structural analysis suggests that the decreased abundance and stability of the C-terminal construct may result from solvent exposure of a large hydrophobic patch that is buried in the wild-type Nbs1 protein. Two additional missense mutations, R215W that is linked to NBS and L150F that is linked breast cancer, occur in structurally characterized Nbs1 regions. R215W appears to decrease protein stability while the L150F mutation likely decreases the BRCT phosphoprotein binding, analogous to some mutations of the breast cancer susceptibility protein BRCA-1. Genetic, biochemical, and structural analyses of the Nbs1-Ctp1 co-complex determined that Nbs1 recruits phosphorylated Ctp1 to DSBs, via binding of the Nbs1 FHA domain (Fig. 3B), and this interaction was critical for DSB repair in yeast. Importantly, this tethering of Ctp1 to a flexible Nbs1 arm suggests a dynamic mechanism for restricting DNA end processing and homologous recombination activities of Ctp1, to the immediate vicinity of DSBs that are bound by the MRN complex (Fig. 3C).

Replication restart and recombination typically involve the four-stranded Holliday junction whose migration and resolution in bacteria involves the RuvABC DNA translocase-resolvase complex. Wild-type and mutant high-resolution RuvB structures in complex with nucleotide plus SAXS results showed how AAA+ ATPases couple nucleotide binding and hydrolysis to interdomain conformational changes and asymmetry within the RuvB hexamer [36]. Now EM structures of wild-type and mutant RuvA/RuvB/Holliday complex reveal a dumbbell-like, tripartite, bendable, structure consistent with a butterfly-like movement to allow RuvC resolvase access to the junction center. The EM results reveal an intriguing similarity to the architecture of SV40 large T antigen, which belongs to the same AAA+ family as RuvB [37•].

Dynamic recognition of DNA strand breaks by ligases

DNA backbone nicks and breaks are created directly through DNA damage or indirectly as intermediates in DNA repair and replication pathways. DNA strand breaks are recognized and sealed by DNA ligases, ubiquitous enzymes expressed in all organisms. Crystal structures plus NMR and SAXS results on viral, bacterial and mammalian ligases at different stages of the ligation reaction show that conserved ligase catalytic domains undergo large rearrangements during the three-step DNA end joining reaction. In the initial enzyme adenylation step, the OB-fold domain (OBD) domain packs tightly against the active site of the nucleotidyl transferase (NTase) domain to orient ATP for adenylation of an active site lysine residue [38•]. During the subsequent steps, the NTase and OBD domains together with the DNA binding domain (DBD) completely encircle the DNA substrate to sequester the ends of DNA and expose them to interactions with many conserved residues in the ligase active site [39]. The DNA-dependent steps require a different set of residues from the OBD to engage the DNA substrate, requiring a 180° rotation between the initial ATP-bound state and the subsequent DNA-bound conformation of the OBD module [39].

Recent NMR spectroscopy examined both interdomain and intradomain flexibility of a minimal DNA ligase from *Chlorella virus* (ChVLig) [38•]. Two previous crystal structures of adenylated ChVLig showed that the NTase and OBD domains adopt an open, extended conformation without DNA that has substantial mobility between NTase and OBD domains in solution by NMR [38•]. However, the average degree of closure between the NTase and OBD domains in solution is intermediate between those of crystal structures determined in the presence or absence of a nicked DNA substrate. The enzyme closes further, approaching the clamped DNA-bound conformation, when phosphate anions are added to mimic nicked DNA 5'-phosphate. Collectively, these results suggest that ChVLig continually samples open and clamped conformations, and this equilibrium shifts towards the clamped conformation when 5'-phosphate is bound to the active site [38•]. A related conformational selection mechanism of DNA binding is used by the lac repressor, which samples many conformations in the absence of DNA or the presence of a noncognate DNA sequence but adopts a single conformation in the presence of its target DNA sequence [40]. Similar dynamic conformational ensembles of DNA ligases function in the recognition of DNA breaks.

Flanking the conserved catalytic core of mammalian DNA ligases are N- and C-terminal protein interaction domains that are linked to the core by flexible tethers. This flexibility may enable the temporal control of DNA end joining activities within dynamic repair complexes that assemble at sites of damage. For example, DNA ligase IV (LigIV) is recruited to double strand breaks through an interaction between its C-terminal tandem BRCT domains and XRCC4. The crystal structure of the tandem BRCT domains of human LigIV bound to XRCC4 reveals an extensive binding interface in LigIV that is composed of the linker between the BRCT domains and the C-terminal BRCT domain (Fig. 4) [41]. The newly identified interaction between the C-terminal BRCT and XRCC4 is essential for the stable interaction *in cellulo*, though it is not seen in the homologous complex Lif1p/Lig4p from *S. cerevisiae* nor is it required for the interaction of purified human LigIV/XRCC4 [41]. Electron microscopy of the full-length complex revealed that the extensive interactions between XRCC4 and the tandem BRCT of LigIV seen in the crystal structure do not impinge on the conformational heterogeneity of the LigIV catalytic domains [42]. The flexibility in the catalytic domains may allow LigIV to repair diverse types of DNA breaks and/or increase its processivity by allowing the NTase and OBD domains to catalyze the enzyme adenylation reaction while remaining tethered to the break through XRCC4 (Fig. 4). Multiple catalytic cycles without dissociation from the DNA would allow a single ligase molecule to seal both strands of a DNA DSB.

Combined EM and crystallographic results on archaeal systems from which PCNA-mediated handoffs were originally proposed [4] extend our understanding of ligase-PCNA and polymerase-PCNA interactions and handoffs. Pyrococcus ligase-PCNA complex with nicked DNA (PfuLig-PCNA-DNA) suggests how the PCNA provides a platform for the sequential recruitment of replication factors [43•]. Pyrococcus PCNA structures furthermore reveal the B DNA polymerase (PfuPol) PIP box plus a second interaction site which likely acts in switching between the polymerase and exonuclease modes [44•].

Modular structures and functions of PARP-1 domains

Combined methods reveal DNA-induced, functional conformational changes for the flexible, multi-domain DNA nick sensor, Poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 recruits multiple repair factors, including DNA ligase III, to DNA single-stranded breaks through the formation of ADP-ribose polymers. SAXS studies of the PARP-1 N-terminus reveal a flexible structure even when bound to DNA, which may facilitate the recruitment of multiple repair factors into a dynamic and malleable repair complex [45•]. The structures of all six PARP-1 domains have been determined individually, but the mechanism by which these domains work together for damage recognition and signaling is not well understood [46–48]. Yet, DNA binding by two N-terminal zinc finger domains in PARP-1 is required for activation of the enzyme, stimulating the C-terminal catalytic domain up to 500-fold. This stimulation may occur through dimerization and/or conformational changes of PARP-1 upon DNA binding. A recently identified zinc ribbon domain (Zn3) within the N-terminal DNA-binding region is required for DNA-dependent catalytic activity, but not for DNA binding activity *per se*, suggesting a role for the Zn3 domain in mediating the stimulation of PARP activity [47,48]. This domain could also interact with other domains of PARP-1 to stimulate enzymatic activity. In support of this hypothesis, the Zn3 domain is able to stimulate catalytic activity *in trans*, [47,48]. SAXS studies of a PARP-1 N-terminal fragment containing Zn3 reveal DNA-induced conformational changes that may underlie the observed stimulation of catalytic activity by this domain [45•]. In an alternative proposal, the Zn3 domain, which forms a dimer in the crystal structure but not in the solution NMR structure of the domain, may promote auto-modification of PARP-1 through its dimerization [47,48]. Though an N-terminal fragment containing the Zn3 domain is a monomer in the presence or absence of DNA, as demonstrated by SAXS, the full-length enzyme may dimerize upon DNA binding [45•]. In support of this hypothesis, kinetic analyses suggest that PARP-1 functions as a catalytic homodimer.

Polynucleotide kinase prepares DNA ends for repair

Polynucleotide kinase (PNK) functions in the BER and NHEJ pathways, employing dual 5'-kinase and 3'-phosphatase catalytic activities to create the 5'-phosphate and 3'-hydroxyl termini necessary for ligation of DNA breaks. PNK comprises three domains: an N-terminal FHA domain that binds to phosphorylated forms of XRCC1 and XRCC4 to target the enzyme to BER and NHEJ, a phosphatase domain, and a kinase domain [49]. The two catalytic domains interact through an extensive interface, apparently fixing the conformation with both active sites on the same face of the enzyme [49]. Consistent with this idea, the two catalytic domains adopt a compact globular structure in solution [50•]. The close proximity of the two active sites precludes simultaneous binding of substrates to both active sites [50•]. The 3'-phosphatase activity is faster than the kinase activity towards the 5'-hydroxyl of the same DNA substrate. This kinetic difference ostensibly prevents the generation of nicks with a 5'-phosphate and 3'-phosphate, which could be 5'-adenylated by DNA ligases to create an unrepaired abortive ligation intermediate that is cytotoxic and/or mutagenic, as illustrated by the neurological disorder ataxia oculomotor apraxia-1.

Besides having different kinetic properties, the two catalytic domains of PNK exhibit different substrate specificities: the promiscuous phosphatase domain requires just 3 nucleotides adjacent to the 3'-phosphate end, whereas the preferred substrate of the kinase domain has at least 8 base pairs of dsDNA next to a 5'-hydroxyl recessed by at least 3 nucleotides [49]. The PNK crystal structure reveals a basis for the kinase catalytic selectivity, including an active site structurally and electrostatically complementary to a recessed 5'-terminus [49]. Mutations in a positively-charged patch near the active site cleft enhance selectivity towards an internal 5'-hydroxyl over a blunt-ended 5'-hydroxyl, indicating that residues in this region bind to the dsDNA on the 5' side of the nick [50•]. Mutations in a second positively charged patch, on the opposite side of the catalytic aspartic acid, reduce the requirement for an internal 5'-end over blunt ends, suggesting that this region binds to the 3' side of the nick [50•]. Furthermore, SAXS experiments support this model of DNA binding [50•]. Interestingly, the fit of the models to SAXS data was significantly improved by including just two biochemically-relevant restraints, a technique likely useful for defining other flexible complexes.

Conclusions and perspectives

DNA repair structural biology initially largely focused on structures of DNA complexes that helped elucidate damage detection, removal, and replacement. In concert with biochemical and genetic results, these structures have revealed general themes for the recognition of damaged bases: sequence-independent DNA recognition motifs, minor groove reading heads for initial damage recognition, and nucleotide flipping from the major groove into active-site pockets for high specificity of base damage recognition and removal. However, new themes are emerging, as evident from the recent structures of endonuclease V, which initiates a major new base-repair pathway for nitrosative deamination. An endonuclease V wedge motif acts as a minor groove–damage sensor and separates DNA strands at the lesion while the deaminated adenine is rotated ~90 degrees into a recognition pocket ~8 Å from the catalytic site providing a novel recognition for base damage [51•]. Other new structures are also identifying new motifs, which distinguish functions despite structural similarity, as nicely shown for a (6-4) photolyase structure that provides a comparative framework for DNA repair photolyases and clock cryptochromes [52•]. For bulky lesion repair, structural analyses of motifs and domains for the XPD and XPB helicases point to functionally important conformational changes that expand our understanding of dynamic complexes connecting DNA damage responses [14•,53].

As we look from proteins to pathways, we are discovering functional stutters and stops regulating repair pathway progression. Collective results show that toxic and mutagenic DNA repair intermediates are sequestered during multi-step reactions through damage binding and efficiently coordinated handoffs. However, the molecular feedback among DNA damage detection, repair effector assemblies, and signal propagation remains enigmatic. Yet, collective findings already implicate probable regulation candidates: strain-dependent activation, scaffolding of signaling molecules within the repair complex, flexible tethering to control local concentrations, strain release to hold intermediate products for handoffs by interface exchange, and conformational sculpting that shapes complexes for subsequent interactions. The observed biologically important changes in shape and assembly are often mediated by intermolecular allosteric events with discrete structural consequences that might be subject to control with small molecule binders, as dysregulation of repair complexes has links to tumorigenesis, rapid aging and neurological disease.

Overall, the resulting deeper understanding of the structural biology of DNA repair pathways and networks from combined methods is becoming a classic example of how pieces of the science puzzle can come together, to give a collective picture. Importantly, this

picture impacts a quantitative understanding that is predictive of complex biological responses. Furthermore, the newly discovered families of SUMO-Targeted Ubiquitin Ligases (STuBLs) and SUMO-like domains (SLDs) [54,55•] appear poised to reveal new dimensions and levels of control to this emerging integrated portrait of DNA damage responses key to a predictive understanding of biology. We can now expect, however, that unraveling the structural chemistry and physics of repair pathway mechanisms and connections will depend on measurements of detailed interactions and conformations, combined with flexibility and dynamics. The results outlined here already promise not only specific insights, but also furthermore robust technologies and approaches generally applicable toward achieving high-resolution data and solution ensemble information in many systems, to successfully join detailed structures to biological outcomes.

Acknowledgments

The authors thank Michal Hammel, Lawrence Berkeley National Laboratory, for his helpful comments in the manuscript preparation, and acknowledge support for their efforts to connect DNA repair protein structures to biological networks from the National Cancer Institute program grant Structural Cell Biology of DNA Repair Machines grant CA92584.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature* 2009;461:1071–1078. [PubMed: 19847258]
2. Hitomi K, Iwai S, Tainer JA. The intricate structural chemistry of base excision repair machinery: implications for DNA damage recognition, removal, and repair. *DNA Repair (Amst)* 2007;6:410–428. [PubMed: 17208522]
- 3•. Garcin ED, Hosfield DJ, Desai SA, Haas BJ, Bjoras M, Cunningham RP, Tainer JA. DNA apurinic-apyrimidinic site binding and excision by endonuclease IV. *Nat Struct Mol Biol* 2008;15:515–522. The endonuclease IV structures and mutational analyses provide experimental evidence for strain in BER backbone incision and for stable product complexes that aid handoffs without the release of toxic DNA repair intermediates. [PubMed: 18408731]
4. Chapados BR, Hosfield DJ, Han S, Qiu J, Yelent B, Shen B, Tainer JA. Structural basis for FEN-1 substrate specificity and PCNA-mediated activation in DNA replication and repair. *Cell* 2004;116:39–50. [PubMed: 14718165]
5. Pascal JM, Tsodikov OV, Hura GL, Song W, Cotner EA, Classen S, Tomkinson AE, Tainer JA, Ellenberger T. A flexible interface between DNA ligase and PCNA supports conformational switching and efficient ligation of DNA. *Mol Cell* 2006;24:279–291. [PubMed: 17052461]
6. Shin DS, Pellegrini L, Daniels DS, Yelent B, Craig L, Bates D, Yu DS, Shivji MK, Hitomi C, Arvai AS, et al. Full-length archaeal Rad51 structure and mutants: mechanisms for RAD51 assembly and control by BRCA2. *EMBO J* 2003;22:4566–4576. [PubMed: 12941707]
7. Putnam CD, Hammel M, Hura GL, Tainer JA. X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution. *Q Rev Biophys* 2007;40:191–285. [PubMed: 18078545]
- 8••. Hura GL, Menon AL, Hammel M, Rambo RP, Poole FL 2nd, Tsutakawa SE, Jenney FE Jr, Classen S, Frankel KA, Hopkins RC, et al. Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS). *Nat Methods* 2009;6:606–612. The authors developed an automated, high throughput system and analysis pipeline for SAXS data. Results on 50 samples support the SAXS as a robust technique for efficient throughput that provides information on most samples tested. [PubMed: 19620974]

9. Brudler R, Gessner CR, Li S, Tyndall S, Getzoff ED, Woods VL Jr. PAS domain allostery and light-induced conformational changes in photoactive yellow protein upon I2 intermediate formation, probed with enhanced hydrogen/deuterium exchange mass spectrometry. *J Mol Biol* 2006;363:148–160. [PubMed: 16952373]
- 10••. Mendillo ML, Hargreaves VV, Jamison JW, Mo AO, Li S, Putnam CD, Woods VL Jr, Kolodner RD. A conserved MutS homolog connector domain interface interacts with MutL homologs. *Proc Natl Acad Sci U S A* 2009;106:22223–22228. Deuterium exchange mass spectrometry is used to provide a detailed picture of the functional interface for mismatch repair MutS with its MutL signaling partner. [PubMed: 20080788]
- 11••. Boehr DD, McElheny D, Dyson HJ, Wright PE. Millisecond timescale fluctuations in dihydrofolate reductase are exquisitely sensitive to the bound ligands. *Proc Natl Acad Sci U S A* 2010;107:1373–1378. Nuclear Magnetic resonance studies revealing that the dynamics in *E. coli* dihydrofolate reductase are finely tuned for the intermediates in the catalytic reaction cycle. [PubMed: 20080605]
12. Gardino AK, Villali J, Kivenson A, Lei M, Liu CF, Steindel P, Eisenmesser EZ, Labeikovsky W, Wolf-Watz M, Clarkson MW, et al. Transient non-native hydrogen bonds promote activation of a signaling protein. *Cell* 2009;139:1109–1118. [PubMed: 20005804]
- 13••. Wells M, Tidow H, Rutherford TJ, Markwick P, Jensen MR, Mylonas E, Svergun DI, Blackledge M, Fersht AR. Structure of tumor suppressor p53 and its intrinsically disordered N-terminal transactivation domain. *Proc Natl Acad Sci U S A* 2008;105:5762–5767. Combined NMR, X-ray crystallography, SAXS provided a structural model of p53 in complex with DNA. These results reconcile an EM structure of the p53-DNA complex and demonstrate that the tetramerization of p53 does not occur explicitly through C or N terminal domains. Systematic truncations of p53 defined the requirements for quaternary structure formation. [PubMed: 18391200]
- 14••. Fan L, Fuss JO, Cheng QJ, Arvai AS, Hammel M, Roberts VA, Cooper PK, Tainer JA. XPD helicase structures and activities: insights into the cancer and aging phenotypes from XPD mutations. *Cell* 2008;133:789–800. The combination of biochemistry, mutational analyses, and structures allows the interpretation of disease causing mutants in terms of XPD conformations. [PubMed: 18510924]
- 15••. Williams RS, Moncalian G, Williams JS, Yamada Y, Limbo O, Shin DS, Grocock LM, Cahill D, Hitomi C, Guenther G, et al. Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell* 2008;135:97–109. Combined crystallography, SAXS, and mutational analyses defined a small but biologically critical dimer interface responsible for distinguishing one-ended and two-ended DNA double-strand breaks. SAXS characterizations of structure-based mutants designed to block dimer formation showed they formed dimers in solution and reduced DNA binding. [PubMed: 18854158]
- 16••. Williams RS, Dodson GE, Limbo O, Yamada Y, Williams JS, Guenther G, Classen S, Glover JN, Iwasaki H, Russell P, et al. Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. *Cell* 2009;139:87–99. Combined crystallography, SAXS and proteolysis results reveal the flexibility and detailed interactions for Nbs1. Nbs1 joins the Mre11 nuclease at a DNA double-strand break to the CTIP protein that opens DNA surrounding the break. SAXS results also suggest that the Nbs1 folded core is conserved from *S. pombe* to human Nbs1, and that disease-associated Nbs1 mutations fall into the folded and extended N-terminal FHA-BRCT1-BRCT2 domains defined by SAXS and crystallography. The fused, extended, FHA-BRCT1-BRCT2 domains are flexibly linked to C-terminal Mre11- and ATM-binding motifs. [PubMed: 19804755]
- 17••. Scrima A, Konícková R, Czyzewski BK, Kawasaki Y, Jeffrey PD, Groisman R, Nakatani Y, Iwai S, Pavletich NP, Thomä NH. Structural basis of UV DNA-damage recognition by the DDB1-DDB2 complex. *Cell* 2008;135:1213–1223. This work defines the molecular mechanism by which DDB2 detects lesions refractory to detection by other damage surveillance proteins. [PubMed: 19109893]
18. Min JH, Pavletich NP. Recognition of DNA damage by the Rad4 nucleotide excision repair protein. *Nature* 2007;449:570–575. [PubMed: 17882165]
- 19•. Lee S, Radom CT, Verdine GL. Trapping and structural elucidation of a very advanced intermediate in the lesion-extrusion pathway of hOGG1. *J Am Chem Soc* 2008;130:7784–7785. A new insight into a very late-stage intermediate in the lesion-extrusion pathway, where oxoG is

inserted into the enzyme active site following photodeprotection, but the enzyme and DNA have not yet adjusted to this insertion. [PubMed: 18507380]

- 20•• Qi Y, Spong MC, Nam K, Banerjee A, Jiralerspong S, Karplus M, Verdine GL. Encounter and extrusion of an intrahelical lesion by a DNA repair enzyme. *Nature* 2009;462:762–766. These studies have characterized the BER protein MutM at the stage of an initial encounter with a damaged nucleobase, revealing that MutM detects a lesion in DNA even at the earliest stages of encounter. [PubMed: 20010681]
- 21•• Qi Y, Spong MC, Nam K, Karplus M, Verdine GL. Entrapment and structure of an extrahelical guanine attempting to enter the active site of a bacterial DNA glycosylase, MutM. *J Biol Chem* 2010;285:1468–1478. Characterization of a MutM complex where an undamaged guanine is being attempted to be presented to its active site, revealing insights into molecular mechanisms of discrimination between oxoG and an undamaged base. [PubMed: 19889642]
- 22• Yang CG, Yi C, Duguid EM, Sullivan CT, Jian X, Rice PA, He C. Crystal structures of DNA/RNA repair enzymes AlkB and ABH2 bound to dsDNA. *Nature* 2008;452:961–965. An active site cross-linking strategy allowed trapping of protein–DNA complexes for *Escherichia coli* AlkB and its human homologue ABH2, which are both weak DNA-binding proteins. The structures defined the basis for these enzymes' distinct damage recognition and substrate preferences. [PubMed: 18432238]
- 23• Tubbs JL, Latypov V, Kanugula S, Butt A, Melikishvili M, Kraehenbuehl R, Fleck O, Marriott A, Watson AJ, Verbeek B, et al. Flipping of alkylated DNA damage bridges base and nucleotide excision repair. *Nature* 2009;459:808–813. Structures of AtI1 in complex with DNA containing large and small alkylated lesions on guanine show how ATL aids the recognition and NER repair of lesions that would otherwise be slow or intractable to repair by damage reversal. [PubMed: 19516334]
- 24•• Doré AS, Kilkenny ML, Rzechorzek NJ, Pearl LH. Crystal structure of the rad9-rad1-hus1 DNA damage checkpoint complex--implications for clamp loading and regulation. *Mol Cell* 2009;34:735–745. Elucidation of the heterotrimeric 9–1–1 complex toroidal structure, and definition of a single enzyme binding site on the complex. [PubMed: 19446481]
- 25•• Sohn SY, Cho Y. Crystal structure of the human rad9-hus1-rad1 clamp. *J Mol Biol* 2009;390:490–502. Structural determination of the 9–1–1 complex and the suggestion that the C-terminal tail of the Rad9 subunit regulates the DNA binding of the complex. [PubMed: 19464297]
- 26•• Xu M, Bai L, Gong Y, Xie W, Hang H, Jiang T. Structure and functional implications of the human rad9-hus1-rad1 cell cycle checkpoint complex. *J Biol Chem* 2009;284:20457–20461. Structural definition of the 9–1–1 complex, and determination of FEN-1 PIP-box binding sites on 9–1–1. [PubMed: 19535328]
- 27• Hishiki A, Hashimoto H, Hanafusa T, Kamei K, Ohashi E, Shimizu T, Ohmori H, Sato M. Structural basis for novel interactions between human translesion synthesis polymerases and proliferating cell nuclear antigen. *J Biol Chem* 2009;284:10552–10560. Definition of the lesion bypass polymerase PIP-box interactions with PCNA, revealing key differences from classical PIP-box interactions observed in the replicative polymerases. [PubMed: 19208623]
28. Meek K, Dang V, Lees-Miller SP. DNA-PK: the means to justify the ends? *Adv Immunol* 2008;99:33–58. [PubMed: 19117531]
29. Williams DR, Lee KJ, Shi J, Chen DJ, Stewart PL. Cryo-EM structure of the DNA-dependent protein kinase catalytic subunit at subnanometer resolution reveals alpha helices and insight into DNA binding. *Structure* 2008;16:468–477. [PubMed: 18334221]
- 30•• Sibanda BL, Chirgadze DY, Blundell TL. Crystal structure of DNA-PKcs reveals a large opening cradle comprised of HEAT repeats. *Nature* 2010;463:118–121. Breakthrough structure for the DNA-PK and ATM classes of repair kinases. The crystal structure allowed the placement of the different functional domains within the extended volume density and comparisons to density volumes defined by electron microscopy and SAXS. [PubMed: 20023628]
- 31•• Hammel M, Yu Y, Mahaney BL, Cai B, Ye R, Phipps BM, Rambo RP, Hura GL, Pelikan M, So S, et al. Ku and DNA-dependent protein kinase dynamic conformations and assembly regulate DNA binding and the initial non-homologous end joining complex. *J Biol Chem* 285:1414–1423. The assembly states of the DNA repair kinase DNA-PK were examined by SAXS in the presence

and absence of DNA. Monomeric and dimeric forms of the kinase subunit suggest how DNA-PK dimers bind to DNA ends and are opened by phosphorylation to release DNA for pathway progression. [PubMed: 19893054]

- 32••. Williams JS, Williams RS, Dovey CL, Guenther G, Tainer JA, Russell P. gammaH2A binds Brc1 to maintain genome integrity during S-phase. *EMBO J.* 2010 SAXS, biochemical, and mutational results were combined with the 1.45 Å resolution crystal structure of the phosphopeptide complex with Brc1. These results show how variable BRCT insertion loops sculpt tandem-BRCT phosphoprotein-binding pockets, to facilitate unique phosphoprotein-interaction specificities, and reveals an acidic DNA-mimicking Brc1 surface. From the collective results, Brc1 binding to phosphorylated histone emerges as a key chromatin specific response to replication-associated DNA damage.
33. Hopfner KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT, et al. The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature* 2002;418:562–566. [PubMed: 12152085]
34. Hopfner KP, Tainer JA. Rad50/SMC proteins and ABC transporters: unifying concepts from high-resolution structures. *Curr Opin Struct Biol* 2003;13:249–255. [PubMed: 12727520]
35. Lloyd J, Chapman JR, Clapperton JA, Haire LF, Hartsuiker E, Li J, Carr AM, Jackson SP, Smerdon SJ. A supramodular FHA/BRCT-repeat architecture mediates Nbs1 adaptor function in response to DNA damage. *Cell* 2009;139:100–111. [PubMed: 19804756]
36. Putnam CD, Clancy SB, Tsuruta H, Gonzalez S, Wetmur JG, Tainer JA. Structure and mechanism of the RuvB Holliday junction branch migration motor. *J Mol Biol* 2001;311:297–310. [PubMed: 11478862]
- 37•. Mayanagi K, Fujiwara Y, Miyata T, Morikawa K. Electron microscopic single particle analysis of a tetrameric RuvA/RuvB/Holliday junction DNA complex. *Biochem Biophys Res Commun* 2008;365:273–278. EM and mutational analyses suggest how RUVAB may open to allow RuvC to access the junction for cleavage. [PubMed: 17981150]
- 38•. Piserchio A, Nair PA, Shuman S, Ghose R. Solution NMR Studies of Chlorella Virus DNA Ligase-adenylate. *J Mol Biol.* 2009 Defined global changes structure and dynamics induced by phosphate interactions with DNA ligase, which cluster around active site, and likely mimic the conformational transitions that occur when ligase-adenylate interacts with the nick 5'-phosphate.
39. Pascal JM, O'Brien PJ, Tomkinson AE, Ellenberger T. Human DNA ligase I completely encircles and partially unwinds nicked DNA. *Nature* 2004;432:473–478. [PubMed: 15565146]
40. Boehr DD, Nussinov R, Wright PE. The role of dynamic conformational ensembles in biomolecular recognition. *Nat Chem Biol* 2009;5:789–796. [PubMed: 19841628]
41. Wu PY, Frit P, Meesala S, Dauvillier S, Modesti M, Andres SN, Huang Y, Sekiguchi J, Calsou P, Salles B, et al. Structural and functional interaction between the human DNA repair proteins DNA ligase IV and XRCC4. *Mol Cell Biol* 2009;29:3163–3172. [PubMed: 19332554]
42. Recuero-Checa MA, Dore AS, Arias-Palomo E, Rivera-Calzada A, Scheres SH, Maman JD, Pearl LH, Llorca O. Electron microscopy of Xrcc4 and the DNA ligase IV-Xrcc4 DNA repair complex. *DNA Repair (Amst)* 2009;8:1380–1389. [PubMed: 19837014]
- 43••. Mayanagi K, Kiyonari S, Saito M, Shirai T, Ishino Y, Morikawa K. Mechanism of replication machinery assembly as revealed by the DNA ligase-PCNA-DNA complex architecture. *Proc Natl Acad Sci U S A* 2009;106:4647–4652. Combined EM and crystallographic results reveal ligase and PCNA enclosed substrate DNA. [PubMed: 19255439]
- 44•. Nishida H, Mayanagi K, Kiyonari S, Sato Y, Oyama T, Ishino Y, Morikawa K. Structural determinant for switching between the polymerase and exonuclease modes in the PCNA-replicative DNA polymerase complex. *Proc Natl Acad Sci U S A.* 2009 Combined EM and crystallographic results suggest a structural mechanism for cooperative switching between polymerase and exonuclease modes.
- 45•. Lilyestrom W, van der Woerd MJ, Clark N, Luger K. Structural and Biophysical Studies of Human PARP-1 in Complex with Damaged DNA. *J Mol Biol.* 2009 The authors have used biochemical, thermodynamic and small-angle X-ray scattering measurements to determine conformational changes are required activate PARP-1, upon binding DNA.

46. Ruf A, Menissier de Murcia J, de Murcia G, Schulz GE. Structure of the catalytic fragment of poly(ADP-ribose) polymerase from chicken. *Proc Natl Acad Sci U S A* 1996;93:7481–7485. [PubMed: 8755499]
47. Langelier MF, Servent KM, Rogers EE, Pascal JM. A third zinc-binding domain of human poly(ADP-ribose) polymerase-1 coordinates DNA-dependent enzyme activation. *J Biol Chem* 2008;283:4105–4114. [PubMed: 18055453]
48. Tao Z, Gao P, Hoffman DW, Liu HW. Domain C of human poly(ADP-ribose) polymerase-1 is important for enzyme activity and contains a novel zinc-ribbon motif. *Biochemistry* 2008;47:5804–5813. [PubMed: 18452307]
49. Bernstein NK, Williams RS, Rakovszky ML, Cui D, Green R, Karimi-Busheri F, Mani RS, Galicia S, Koch CA, Cass CE, et al. The molecular architecture of the mammalian DNA repair enzyme, polynucleotide kinase. *Mol Cell* 2005;17:657–670. [PubMed: 15749016]
50. Bernstein NK, Hammel M, Mani RS, Weinfeld M, Pelikan M, Tainer JA, Glover JNM. Mechanism of DNA substrate recognition by the mammalian DNA repair enzyme, Polynucleotide Kinase. *Nucleic Acids Res* 2009;37:6161–6173. SAXS combined with computation defined the shapes, flexibility, and conformations of mPNK complexes. The combined envelopes for DNA complexes and flexible attachment of the FHA domain to the catalytic segment, suggests how flexibility enables mPNK interactions with diverse DNA substrates and protein partners as needed for effective orchestration of DNA end repair. [PubMed: 19671525]
51. Dalhus B, Arvai AS, Rosnes I, Olsen OE, Backe PH, Alseth I, Gao H, Cao W, Tainer JA, Bjoras M. Structures of endonuclease V with DNA reveal initiation of deaminated adenine repair. *Nat Struct Mol Biol* 2009;16:138–143. Structures of endonuclease V with DNA reveal initiation of deaminated adenine repair and provide completely different means of base damage scanning. [PubMed: 19136958]
52. Hitomi K, DiTacchio L, Arvai AS, Yamamoto J, Kim ST, Todo T, Tainer JA, Iwai S, Panda S, Getzoff ED. Functional motifs in the (6–4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. *Proc Natl Acad Sci U S A* 2009;106:6962–6967. The identification and characterization of distinct functional motifs for cryptochrome versus photolyase helps realize the promise of structural biology to objectively inform function. [PubMed: 19359474]
53. Fan L, Arvai AS, Cooper PK, Iwai S, Hanaoka F, Tainer JA. Conserved XPB core structure and motifs for DNA unwinding: implications for pathway selection of transcription or excision repair. *Mol Cell* 2006;22:27–37. [PubMed: 16600867]
54. Perry JJ, Tainer JA, Boddy MN. A SUMO-like role for SUMO and ubiquitin. *Trends Biochem Sci* 2008;33:201–208. [PubMed: 18403209]
55. Prudden J, Perry JJ, Arvai AS, Tainer JA, Boddy MN. Molecular mimicry of SUMO promotes DNA repair. *Nat Struct Mol Biol* 2009;16:509–516. Structural and mutational analyses identify sumo mimicry by SUMO-like domains, which has key functions in DNA repair. [PubMed: 19363481]

Figure 1.

Differential interactions and crystal structure of the 9-1-1 heterotrimeric complex. 9-1-1 has a PCNA-like trimeric ring structure with asymmetry in the IDCL (PDB ID 3G65). Rad9 (green), Rad1 (magenta) and Hus1 (cyan) are shown with hydrophobic pocket region that binds PIP-box motifs (dotted circles), as noted for Rad1 and Rad9 interactions with FEN-1. The flexible connection to the Rad9 120 KDa region with role in DNA binding (dashed green line, lower left) was removed for crystallization.

Figure 2.

Architecture and conformational change in the DNA-PK *holo*-enzyme, as defined by protein crystallography and in solution SAXS analyses. (A) The DNA-PK structure (PDB ID 3KGV) forms a ring-like shape (left) built from α -helical heat repeats, with a head/crown at the top. A side view reveals that the head domain and ends of the arms arch towards each other (right) creating a cradle-like architecture. Head/Crown Kinase domain (yellow) and FAT and FAT-C domains (magenta) have extended HEAT domain arms forming a ring-like structure (green) with a putative DNA binding domain (blue) and a 'forehead' region (dark green). (B) The averaged SAXS volumes for DNA-PKcs (gray) and phosphorylated DNA-PKcs (yellow) each with the crystal structure superimposed, plus representative single

SAXS envelope for DNA-PKcs (gray) and phosphorylated DNA-PKcs (yellow) revealing the large conformational change resulting from phosphorylation. The enclosed cavity in the head region, observed in the crystal structure, is seen visible in the SAXS data as highlighted by darker shading. The bottom panel depicts a cartoon describing proposed conformational changes during autophosphorylation of DNA-PKcs, as revealed by SAXS.

Figure 3.

Nbs1 domain structures and flexibly connected interaction interfaces characterized by combined crystallographic and SAXS analyses. (A) Cancer-causing Mre11 mutations in ataxia telangiectasia-like disorder (ATLD missense mutations W120C and N117S), which result in a loss of Nbs1 binding, suggest that Nbs1 binds across the back face of the Mre11 dimer, opposite the DNA-binding cleft (PDB ID 3DSC). (B) Crystal structure of the N-terminal Nbs1 folded regions (PDB ID 3HUF), revealing FHA domain, (blue), BRCT1 (yellow) and BRCT2 (brown) domains in complex with a phosphorylated Ctp1 peptide (orange) that binds Nbs1 in a surface groove at the distal N-terminal FHA domain. (C) FHA-bound Ctp1 is linked to the Mre11-Rad50 hetero-tetrameric Mre-Rad50 core (labeled

M and R, center) that is bridging a DNA double strand-break through the flexible Nbs1 C-terminus defined by SAXS.

Figure 4.

Proposed mechanism of DNA ligase IV-XRCC4, based on the crystal structure (PDB ID 3II6) and electron micrographs of the complex. DNA ligase IV binds to XRCC4 (pink) through its tandem C-terminal BRCT domains. EM studies indicate that the catalytic domains (DBD, NTase, and OBD) extend away from XRCC4. This flexible connection may allow ligase IV to catalyze both enzyme self-adenylation (A) and DNA end-joining (B) without dissociating from the XRCC4-associated repair complex. Abbreviations: B1 – BRCT domain 1, B2 – BRCT domain 2, HLH – helix-loop-helix.