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Structural mechanism of DNA-end synapsis in the non-homologous end joining pathway for repairing double-strand breaks: bridge over troubled ends

Qian Wu [1,2*]

[1]The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom; [2]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

*For correspondence: Qian Wu (q.n.wu@leeds.ac.uk)

Abstract

Non-homologous end joining (NHEJ) is a major repair pathway for DNA double-strand breaks (DSBs), which is the most toxic DNA damage in cells. Unrepaired DSBs can cause genome instability, tumorigenesis or cell death. DNA-end synapsis is the first and probably the most important step of the NHEJ pathway, aiming to bring two broken DNA ends close together and provide structural stability for end processing and ligation. This process is mediated through a group of NHEJ proteins forming higher order complexes, to recognise and bridge two DNA ends. Spatial and temporal understanding of the structural mechanism of DNA-end synapsis has been largely advanced through recent structural and single-molecule studies of NHEJ proteins. This review focuses on core NHEJ proteins that mediate DNA-end synapsis through their unique structures and interaction properties, as well as how they play roles as anchor and linker proteins during the process of “bridge over troubled ends”.

Introduction

Our human body is constantly challenged by the environment we live in, the lifestyle we choose and medical treatments we need. DNA within the cells of our body is the direct target for these challenges, generated from both exogenous toxic sources (e.g. ionizing radiation) and endogenous by-products from cellular functions (e.g. DNA replication stress). The most cytotoxic damage of all are DNA double-strand breaks (DSBs), when both DNA strands are broken in close proximity on their sugar-phosphate backbone causing the loss of local structural connectivity of DNA strands (**Figure 1**). At the same time, this loss of connectivity also provides critical opportunities for physiological genome arrangement and alteration of topological states of DNA strands. Therefore, DSBs are also purposely generated by nuclear enzymes during important cellular processes: V(D)J and class switch recombination for lymphocyte maturation (1,2), meiotic recombination (3), DNA structural untangle by type II topoisomerase (4) and more recently during gene editing such as the CRISPR-Cas9 system (5). Effective and highly controlled DNA repair pathways are essential for maintaining genomic integrity and cellular functions. Unrepaired or mis-repaired DSBs caused by faulty DSB repair can lead to chromosome breakage,

chromosome translocation (which can potentially cause genome instability), cell death, immunodeficiency and tumorigenesis (6–8).

Eukaryotic cells contain two major pathways for repairing DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR). Cell cycle states, chromatin contexts and the DNA-end resection environment all affect the pathway choice between NHEJ and HR (9). In the HR pathway, long 3′ single-stranded DNA (ssDNA) overhangs are generated from extensive resection of DNA ends by nuclease enzymes (e.g. MRE11, DNA2 and EXO1), before pairing with sister chromatids for template-dependent DNA repair in G2 and S phases (10). DNA ends in the NHEJ pathway are protected from this extensive resection and joined back together directly in a template-free manner after a short stretch of end processing. NHEJ functions as the dominant (around 75%) repair pathway for human cells throughout interphase to repair DSBs rapidly, but with less accuracy compared with HR (11,12). The NHEJ discussed here is referred to as classic NHEJ, which exhibits a low degree of DNA-end homology (less than 4bp microhomology) (13). In addition to NHEJ and HR, there are alternative end joining (aEJ) and single-strand annealing (SSA) pathways utilising different sets of proteins to repair DSBs (9,13,14). It is also important to appreciate that DSB repair occurs in the context of chromatin. ATM signaling (especially including oligomerization of 53BP1) plays a major role in promoting DNA ends synapsis via chromatin compaction (15–17).

An efficient NHEJ pathway for two-ended DSBs begins with a stable synapsis of DNA ends, which identifies two “troubled DNA ends”, followed by re-establishment of the local structural connectivity of DNA strands through “bridge over” by NHEJ protein complexes. This mini review focuses on our current understanding of this “Bridge over troubled DNA ends” process in terms of both spatial and temporal perspectives, through studying structures and dynamic assembly properties of core NHEJ proteins.

Mechanism of NHEJ

The NHEJ pathway for two-ended DNA DSBs has three well-defined objectives (**Figure 1**): 1) synapsis of two DNA ends; 2) processing of these ends to make them ligatable; 3) ligation of these ends together. NHEJ proteins achieve these objectives through both enzymatic and non-enzymatic (scaffold) functions at DSB sites. It is clear that these enzymatic functions come from DNA-PKcs (DNA protein kinase catalytic subunit), end-processing enzymes (e.g. Artemis, Werner syndrome helicase (WRN) and DNA polymerases λ and μ) and DNA ligase IV (LigIV) (18). The non-enzymatic functions that are responsible for mediating stable assembly of NHEJ proteins, especially for the end synapsis, are much more complex.

DNA-end synapsis in NHEJ requires NHEJ proteins to be rapidly assembled at DSB sites with specificity, stability and flexibility to ensure complex formation only at the DSB sites, to stabilise two correct DNA ends for ligation. DNA-end synapsis also

allows various DNA-end configurations to be processed by different NHEJ enzymes before ligation (13). NHEJ accessory proteins such as APLF (APTX and PNKP-like factor) (19) and CYREN (cell cycle regulator of NHEJ) / MRI (20,21) can further regulate the stability of NHEJ complex formation. Post-translational modifications (e.g. phosphorylation by DNA-PKcs) (22,23) modulate inter-molecular interactions and play a key role in regulating the stability of DNA-end synapsis. It is still unclear whether all of these proteins are required for all types of DSB ends, or if they are selectively recruited for different types of DSB ends. However, current technological advances in structural and single-molecule studies have started answering spatial and temporal aspects of the DNA-end synapsis carried out, particularly by core NHEJ proteins including Ku (Ku70-Ku80), DNA-PKcs, XRCC4 (X-ray repair cross-complementing protein 4), XLF (XRCC4 like factor), PAXX (PAralog of XRCC4 and XLF) and LigIV (**Figure 1**). These studies aim to identify what the role for each of the core NHEJ proteins is during DNA end synapsis and to establish the binding order of these proteins.

Recent methods used for studying the spatial and temporal properties of DNA end synapsis of NHEJ *in vitro*

Recent and rapid development of cryo-electron microscopy (cryo-EM) equipment and data analysis software has created new opportunity to study spatial properties of DNA end synapsis by NHEJ protein complexes. At the same time, single-molecule methods are also actively developed and well suited to study the temporal property of this process *in vitro*. In order to create a DSB site for single-molecule experiments, three general DNA configurations have been developed so far (**Figure 2**): 1) two long DNA segments (each over 1 kb) linked with a third DNA segment (**Figure 2A**) (24); 2) two short DNA duplex (each below 100 bp) with a hairpin DNA end (**Figure 2B**) (25,26) or without a hairpin DNA end (**Figure 2C**) (27); 3) a long DNA segment (2 kb) with two free DNA ends (27). In all of these cases, blunt-ended DNA was used because these ends cannot be in close proximity without proteins. Contribution of NHEJ proteins towards DNA-end synapsis was determined through either the physical (**Figure 2A**) (24) or chemical measurements from DNA in solution containing purified proteins (**Figure 2B**) (25,26), or *Xenopus laevis* egg extract with NHEJ proteins depleted (**Figure 2C, D**) (27).

In DNA configuration 1, DNA-end synapsis led to the position change of the magnetic bead attached to one end of a DNA segment. Larger vertical extending force (F) was needed to pull two DNA segments apart after synapsis. Position change (Δl) of the magnetic bead was determined as physical measurements (24). In DNA configuration 2 and 3, real-time smFRET (single-molecule Förster resonance energy transfer) can be detected as chemical measurements when Cy-3 (donor) and Cy-5 (acceptor) labelled DNA ends come in proximity during DNA end synapsis (25–27). Values of Time of synapsis ($T_{synapsis}$) were determined in all these conditions.

Mechanism of DNA-end synapsis

Two layers of DNA end synapsis strength have been proposed in the single-molecule studies so far and named stepwise (24): long-range to short-range (27) and flexible to close synapsis (26). Owing to different experimental methods, protein concentrations, running buffer compositions and setup conditions, there is still on-going debate about the exact contribution from each protein towards DNA end synapsis. By integrating all these results together, the core NHEJ proteins with their unique structures can be briefly summarised as “Anchor” and “Linker” proteins for DNA end synapsis. Anchor proteins recognise and bind to DNA ends in high affinity and then recruit linker proteins to bridge two DNA ends (**Figure 3A**).

Anchors

Anchor proteins in the NHEJ pathway are the abundant Ku proteins, which are evolutionally conserved from bacteria to humans (28). Human Ku protein functions as a very stable heterodimer, constituted of Ku70 and Ku80 (**Figure 3B**). Both proteins share similar protein structures that contain an N-terminal vWA (von Willebrand type A-like) domain, central core domain and C-terminal region. Through an extensive dimerization interface contributed from both central core domains, Ku70 and 80 form a ring shape structure with one side of the ring much thicker than the other side (29). The C-terminal regions of Ku70 and Ku80 both contain globular domains connected to the ring structure through flexible linkers (29–31) (**Figure 3B**).

The anchors function of Ku comes from its ability to recognise DNA ends in high affinity (32), hence being the first NHEJ proteins to bind the DNA ends, protecting them from exonucleolytic activity (33,34) and influencing the repair pathway choice for DSBs (35). The inner part of the Ku ring has highly positive electrostatic charges and this, together with the ring structure, allows Ku to achieve a nM range affinity towards DNA ends (29,36). By interacting with the sugar-phosphate backbone of the DNA molecule only, Ku can be anchored at DNA ends in a sequence-independent manner. The thicker side of the ring structure forms a cradle, covering around 14bp of DNA binding, while the other side of the ring contains a large exposed DNA surface (29).

Ku alone is insufficient for mediating DNA end synapsis (24,26,27). After anchoring at the DNA ends the whole Ku protein, particularly the vWA domains, becomes a binding hub for interacting with many NHEJ proteins with various Ku interaction motifs (reviewed extensively in (36)) (**Figure 3B**). Importantly, the C-terminal region of Ku80 is essential for the recruitment of DNA-PKcs to the DNA ends. Even though the last 12 residues in Ku80 were found to be sufficient for binding to DNA-PKcs through pull down experiments (37), the whole C-terminal region of Ku80 could potentially contribute to the recruitment and activation of DNA-PKcs at the DNA ends (36,38–40).

Linkers

One Ku molecule was observed to bind to each DNA end in cells (41). Therefore, NHEJ linker proteins need to bridge two Ku bound DNA ends to stabilise local DNA structure for the following steps. DNA-PKcs, XLF, PAXX and XRCC4 and LigIV contribute to this process (**Figure 3C, D**).

DNA-PKcs

DNA-PKcs is a large, single-chain protein kinase (4128 residues in human), which belongs to phosphatidyl inositol 3-kinase-like serine/threonine kinase (PIKK) protein kinase family (42,43). DNA-PKcs binds to Ku at the DNA end and forms the DNA-PK holoenzyme (44). DNA-PKcs shares a similar domain architecture to ATM and ATR and functions together as three key PIKKs for DNA damage and repair (45). DNA-PKcs is constituted with long HEAT (N-terminal Huntingtin, Elongation Factor 3, PP2 A, and TOR1) repeats followed by a FAT (FRAP, ATM, TRRAP) domain, FRB (FKBP12-rapamycin-binding) domain, kinase domain and FATC (FAT C-terminal) domain. The HEAT repeats form an N-terminal arm structure and circular cradle structures as the main body of DNA-PKcs, while its remaining parts form a head structure sitting opposite the N-terminal arm structure (46) (**Figure 3C**). The Cryo-EM structure of DNA-PK on DNA has shown an extra interaction between the Ku ring structure and the HEAT repeats of DNA-PKcs. Compared with the structure of DNA-PKcs itself, the N-terminal flexible arm structure of DNA-PKcs moves as a gate (**Figure 3D**) for interacting with a DNA bound Ku molecule followed with allosteric conformational change in the kinase domain (38,46–48).

The key kinase function of DNA-PKcs is the autophosphorylation (including residue S2056) which can induce large conformational change and lead to the dissociation of DNA-PKcs from the Ku bound DNA (23,49–51). Mice carrying a catalytic dead DNA-PKcs mutant but not DNA-PKcs null are embryonic lethal because the mutant DNA-PKcs is unable to disassociate from the ends, hence blocking DNA ligation (52). Through interacting with Ku, each side of two DNA ends contains one DNA-PKcs. DNA end synapsis through DNA-PK was observed in atomic force microscopy as well as structural studies using electron microscopy and small angle X-ray scattering (40,53–55). DNA-PKcs functions as a linker protein by bringing two DNA ends close during the processing of binding at DNA ends and mediating autophosphorylation, which is also assisted by LigIV, before releasing from DNA ends (27,56). DNA-PKcs were found to be important as the first step of the DNA synapsis complex (24,27) *in vitro*, yet at the same time concluded to be less important than LX4 in another single-molecule study (26).

XRCC4, LigIV, XLF and PAXX

XRCC4, XLF and PAXX are protein paralogs (57) and share a similar protein fold constituting a globular N-terminal head domain, a coiled-coil structure and flexible C-terminal regions (**Figure 3C**). The NHEJ specific DNA ligase LigIV contains an N-terminal catalytic region, which is conserved among other human DNA ligases, and C-terminal tandem BRCT-domains (BRCT1 and BRCT2), which are unique to LigIV

among the ligases (58). XRCC4 has a long coiled-coil structure, which makes a tight XRCC4 homodimer, with an interaction site specifically binding to the linker region of tandem BRCT domains of LigIV and mediating an extra interaction between BRCT2 domain and the coiled-coil (59,60) (**Figure 3D**). The interaction between XRCC4 and LigIV stabilises the LigIV structure in cells (59,61,62), therefore LigIV is always in the XRCC4-bound form as LigIV-XRCC4 (LX4). XRCC4 without LigIV bound can form tetramers through the interaction of two coiled-coils (63,64) and contribute to DNA end bridging (65). While the catalytic function of LigIV is essential (66), the noncatalytic function of LigIV was also found to contribute to the DNA end synapsis (24,27,56). LX4 was able to bind to Ku without DNA-PKcs at the DNA ends, mediating a flexible synapsis complex to bring DNA ends into a lateral configuration (26).

XLF (also called Cernunnos), with a shorter coiled-coil structure than XRCC4, does not contain a strong interaction site for LigIV as in XRCC4. Instead, it contains a fold-back helix structure that contacts with the N-terminal head domain (67,68) (**Figure 3C**). XLF is recruited to the DSBs through its C-terminal Ku interaction motif binding to an internal site of the Ku80 vWA domain (69,70) (**Figure 3D**). The highly dynamic exchange rate between bound and free XLF and DNA can be stabilised in the presence of XRCC4 (69). Once at the DNA ends, the head domain of XLF interacts with the head domain of XRCC4. As XLF and XRCC4 are both stable homodimers mediated by their coiled-coil domains, their heterodimerisation mediated by their head domains can potentially lead to formation of XLF-XRCC4 proto-filaments. Indeed, crystal structures, electron micrographs, size-exclusion chromatography and native mass spectrometry have all shown the concentration-dependent XLF-XRCC4 filament formation *in vitro* (65,71–73) (**Figure 3D**), with this filament able to mediate DNA bridging (22,25,65,74,75). XLF-XRCC4 filaments were also studied using dual-and quadruple-trap optical tweezers, combined with fluorescence microscopy and observed filament bridging property (74). Super-resolution microscopy studies showed that there were elongated repair structures in U2OS cells, having transiently-expressed XLF and XRCC4 fused with fluorescent tags (25). There is possibility that these long XLF-XRCC4 filaments may represent an *in vitro* artefact, hence further studies are needed to verify whether endogenous XLF and XRCC4 form this elongated repair structure.

One of key questions is how the higher-order complex formation of XRCC4 and XLF can be regulated. The presence of full length LigIV reduces the XLF-XRCC4 filament formation (76). Therefore, there may be a regulatory property to restrain the length of the XLF-XRCC4 complex by blocking the accessibility of some XLF-XRCC4 interaction sites. Single-molecule studies revealed the importance of the XLF-XRCC4 interaction for the final stage of the stability of DNA end synapsis (24,26,77), but the number of XLF required for DNA end synapsis were concluded differently. In the *Xenopus laevis* egg extract system with a long piece of DNA, it was found that only one XLF dimer is needed for DNA end synapsis (77). An interaction model is therefore proposed that one homodimer XLF binds to two LX4 complexes (77). Using purified

proteins with shorter DNA substrates, another single-molecule study showed that XLF enhanced the DNA end-to-end through forming a small patch of XLF-XRCC4 filament with up to three XLF homodimers (26). XLF without a C-terminal for Ku and DNA binding can still maintain function within the DNA synapsis complex in linked DNA configuration (24). Therefore, it is likely that the roles of XLF involved in DNA end bridging are constituted with its multi-interactions with XRCC4, Ku and DNA through both the head domain and C-terminal region (75,78).

PAXX (also called as XLS or C9orf142) is the most recently discovered member of the XRCC4 superfamily in NHEJ, with a short coiled-coil region and no fold back structure (57,79,80) (**Figure 3C**). The head domain of PAXX does not interact with either XLF or XRCC4 (57). PAXX also contains one of the Ku interaction motifs at the C-terminal region and binds to Ku70 instead in the presence of DNA, with this interaction stimulating the LigIV ligation efficiency and promoting Ku accumulation at DNA breaks (57,79–82). Cellular studies have shown redundant scaffold function between PAXX and XLF (83–85). The interaction between PAXX and Ku is important for its function in DNA-end synapsis since the PAXX mutant, which cannot bind to Ku, disrupted the DNA-end synapsis *in vitro* (24) and also destabilised the NHEJ protein assembly *in vivo* (57). Therefore, it is possible that PAXX links two DNA ends through its homodimer structure, with one Ku binding site for each end.

Conclusion

As a template free DNA repair pathway for DSBs, NHEJ provides a rapid solution for cells to fix the damage. During this process, temporarily assembled NHEJ protein complexes bridge over two DNA ends to compensate for the loss of structural connectivity of DNA strands at a damage site. Through Ku protein as an anchor, other core NHEJ proteins (DNA-PKcs, XRCC4, LigIV, XLF and PAXX) bind to the DNA ends spontaneously as linkers and establish a multi-protein-protein interaction network between two DNA ends. Depending on combinations of these proteins, NHEJ can achieve DNA synapsis ranging from low stability to high stability levels. The difference of these stability levels might be to adapt NHEJ for different types of DSB ends. Future research combining cryo-EM structure, single-molecule study and super resolution imaging will enable us to further define and study the property of this DNA end synapsis complex at the DNA ends.

The stability of DNA-end synapsis influences the efficiency and accuracy of NHEJ, which is key for genome stability in cells. Understanding the mechanism of DNA end synapsis in molecular detail will also provide new therapeutic targets for developing small molecules that can be tested in cells for their ability to modulate the function of NHEJ. This will lead to a new direction of enhanced genome editing efficiency and new medical applications, such as more effective cancer treatment through radiotherapy/chemotherapy, as well as overcoming drug resistance.

Perspectives section

(i) DNA repair is a fundamental mechanism which preserves our genomic integrity. It is important to understand how DNA double-strand breaks, the most toxic damage in cells, are repaired through the non-homologous end joining (NHEJ) pathway.
(ii) The DNA end synapsis in the NHEJ pathway is mediated through NHEJ protein complexes bridging over two DNA ends.
(iii) Combining advances in cryo-electron microscopy, single-molecule methods and super-resolution imaging, the structural mechanism of the NHEJ pathway in cells will be revealed in much more detail in the future.

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Competing interest

The Authors declare that there are no competing interests associated with the manuscript.

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Figure legends:

Figure 1: The Non-homologous end joining (NHEJ) pathway for repairing DNA double-strand breaks (DSBs). Exogenous and endogenous damage sources generate DSBs, which can be repaired by NHEJ

and HR (homologous recombination) pathways. There are three general end conditions (synapsis, processing and ligation) during NHEJ. Core NHEJ proteins for mediating DNA-end synapsis are Ku, DNA-PKcs, PAXX, XLF, XRCC4 and LigIV (shown as the zoomed in circle as an interaction network). Each black arrow represents direct protein-protein and protein-DNA interactions.

Figure 2: DNA configurations used in current single-molecule studies for DNA-end synapsis in NHEJ. **A)** Two linear dsDNA segments (1510 bp each) (one linked to a magnetic bead, one immobilised to a glass coverslip) are connected through a third DNA segment (690 bp). Black arrows indicate the vertical extending force (*F*, pN) that is applied to the DNA. Distances (Δl, μm) the magnetic bead moved were measured in the presence of various purified NHEJ proteins (24); **B)** Two short DNA segments (85 and 74 bp) are labelled with Cy5 and Cy3 respectively. The Cy5 labelled DNA was immobilised. The Cy3 labelled DNA has a hairpin DNA end (25,26). FRET values were measured in the presence of various purified NHEJ proteins. **C)** Two DNA duplex are both 100 bp. One is immobilised and labelled with Cy3, while another one has Cy5 on each end (27); **D)** A 2kb DNA segment with two DNA ends labelled with Cy3 and Cy5 (27). FRET values in **C)** and **D)** were measured in the presence of egg extract with specific NHEJ protein depleted (27).

Figure 3: Structures of core NHEJ proteins involved in NHEJ end-synapsis. **A)** Anchor and linker proteins at DNA ends. **B)** Crystal structure of DNA bound Ku (PDB code: 1JEY) (29), Nuclear magnetic resonance (NMR) structures of C-terminal globular domain of Ku80 (PDB code: 1Q2Z) (31) and C-terminal globular domain of Ku70 (PDB code: 1JJR) (86). Ku70 (light green), Ku80 (green) and DNA (black) are labelled. **C)** Protein structures of individual linker proteins: crystal structure of DNA-PKcs (PDB code: 5LUQ) (46) with rainbow colour as N-terminus in blue and C-terminus in red. The Structure of Ku80 C-terminal region is not shown here. The head structure, circular cradle and N-terminal arm structure are indicated; crystal structures of XRCC4 (blue, PDB code: 1FU1) (64), XLF (pink, PDB code:2QM4) (68) and PAXX (turquoise, PDB code: 3WTD) (57). The head domain and coiled-coil structure are indicated. The C-terminal flexible regions of XRCC4, XLF and PAXX were not included in constructs of these crystal structures. **D)** Protein structures of linker proteins in complex. Cryo-EM structure of DNA bound DNA-PK (PDB code: 5Y3R) (47). DNA-PKcs (grey), Ku (green) and DNA (black) are indicated. Crystal structure of the Ku-DNA complex bound with XLF peptide (pink) (PDB code: 6ERG) (70). Crystal structures of the catalytic domain of LigIV (yellow) before (PDB code: 3W5O) (87) and after binding to DNA (PDB code: 5BKG) (88). N-terminus of LigIV and DNA are indicated. XRCC4 bound with the BRCT domains of LigIV (PDB: 3II6) (60). Part of crystal structure of XLF-XRCC4 filament shown in both cartoon and surface representations (PDB code: 3W03) (73).

Reference

1. Gellert M. V(D)J recombination: RAG proteins, repair factors, and regulation. Annu Rev Biochem. 2002;71:101–32. https://doi.org/10.1146/annurev.biochem.71.090501.150203
2. Chaudhuri J, Alt FW. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. Nat Rev Immunol. 2004 Jul;4(7):541–52. https://doi.org/10.1038/nri1395
3. Dresser ME. Meiotic chromosome behavior in Saccharomyces cerevisiae and (mostly) mammals. Mutat Res Mol Mech Mutagen. 2000 Jun;451(1–2):107–27. https://doi.org/10.1016/s0027-5107(00)00043-9
4. Adachi N, Suzuki H, Iiizumi S, Koyama H. Hypersensitivity of Nonhomologous DNA End-joining Mutants to VP-16 and ICRF-193. J Biol Chem. 2003 Sep 19;278(38):35897–902. https://doi.org/10.1074/jbc.M306500200
5. Doudna JA, Charpentier E. Genome editing. The new frontier of genome

engineering with CRISPR-Cas9. Science. 2014 Nov;346(6213):1258096. https://doi.org/10.1126/science.1258096
6. van Gent D, Hoeijmakers J, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. Nat Rev Genet. 2001 Mar;2(3):196–206. https://doi.org/10.1038/35056049
7. Hoeijmakers JHJ. Genome maintenance mechanisms for preventing cancer. Nature. 2001 May 17;411(6835):366–74. https://doi.org/10.1038/35077232
8. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet. 2001 Mar ;27(3):247–54. https://doi.org/10.1038/85798
9. Scully R, Panday A, Elango R, Willis NA. DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat Rev Mol Cell Biol. 2019 Jul **20**, 698–714. https://doi.org/10.1038/s41580-019-0152-0
10. Symington LS. Mechanism and regulation of DNA end resection in eukaryotes. Crit Rev Biochem Mol Biol. 2016 May;51(3):195–212. https://doi.org/ DOI: 10.3109/10409238.2016.1172552
11. Mao Z, Bozzella M, Seluanov A, Gorbunova V. Comparison of nonhomologous end joining and homologous recombination in human cells. DNA Repair (Amst). 2008 Oct;7(10):1765–71. https://doi.org/10.1016/j.dnarep.2008.06.018
12. Her J, Bunting SF. How cells ensure correct repair of DNA double-strand breaks. J Biol Chem. 2018 Jul 6;293(27):10502–11. https://doi.org/10.1074/jbc.TM118.000371
13. Pannunzio NR, Watanabe G, Lieber MR. Nonhomologous DNA end-joining for repair of DNA double-strand breaks. J Biol Chem. 2018 Jul;293(27):10512–23. https://doi.org/10.1074/jbc.TM117.000374
14. Chang HHY, Pannunzio NR, Adachi N, Lieber MR. Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol. 2017;18(8):495–506. https://doi.org/10.1038/nrm.2017.48
15. Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Löbrich M, et al. ATM Signaling Facilitates Repair of DNA Double-Strand Breaks Associated with Heterochromatin. Mol Cell. 2008;31(2):167–77. https://doi.org/10.1016/j.molcel.2008.05.017
16. Difilippantonio S, Gapud E, Wong N, Huang C-Y, Mahowald G, Chen HT, et al. 53BP1 facilitates long-range DNA end-joining during V(D)J recombination. Nature. 2008 Nov 27;456(7221):529-33; https://doi.org/10.1038/nature07476
17. Zimmermann M, de Lange T. 53BP1: pro choice in DNA repair. Trends Cell Biol. 2014 Feb;24(2):108–17. https://doi.org/10.1016/j.tcb.2013.09.003
18. Lieber MR. The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway. Annu Rev Biochem. 2010;79(1):181–211. https://doi.org/10.1146/annurev.biochem.052308.093131
19. Grundy G, Rulten S, Zeng Z, Arribas-Bosacoma R, Iles N, Manley K, et al. APLF promotes the assembly and activity of non-homologous end joining protein complexes. EMBO J. 2013 Jan 9;32(1):112-25 https://doi.org/10.1038/emboj.2012.304
20. Hung PJ, Johnson B, Chen B-R, Byrum AK, Bredemeyer AL, Yewdell WT, et al. MRI Is a DNA Damage Response Adaptor during Classical Non-homologous End Joining. Mol Cell. 2018 Jul;71(2):332-342.e8. https://doi.org/ 10.1016/j.molcel.2018.06.018

21. Arnoult N, Correia A, Ma J, Merlo A, Garcia-Gomez S, Maric M, et al. Regulation of DNA repair pathway choice in S and G2 phases by the NHEJ inhibitor CYREN. Nature. 2017 Sep 20;549(7673):548-552. https://doi.org/10.1038/nature24023
22. Roy S, Andres SN, Vergnes A, Neal JA, Xu Y, Yu Y, et al. XRCC4's interaction with XLF is required for coding (but not signal) end joining. Nucleic Acids Res. 2012 Feb;40(4):1684-94. https://doi.org/10.1093/nar/gkr1315
23. Jette N, Lees-Miller SP. The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. Prog Biophys Mol Biol. 2015 Mar;117(2–3):194–205. https://doi.org/10.1016/j.pbiomolbio.2014.12.003
24. Wang JL, Duboc C, Wu Q, Ochi T, Liang S, Tsutakawa SE, et al. Dissection of DNA double-strand-break repair using novel single-molecule forceps. Nat Struct Mol Biol. 2018 Jun;25(6):482-487. https://doi.org/10.1038/s41594-018-0065-1
25. Reid D, Keegan S, Leo-Macias A, Watanabe G, Strande N, Chang H, et al. Organization and dynamics of the nonhomologous end-joining machinery during DNA double-strand break repair. Proc Natl Acad Sci. 2015 May 19;112(20):E2575-84. https://doi.org/10.1073/pnas.1420115112
26. Zhao B, Watanabe G, Morten MJ, Reid DA, Rothenberg E, Lieber MR. The essential elements for the noncovalent association of two DNA ends during NHEJ synapsis. Nat Commun. 2019;10(1):3588. https://doi.org/10.1038/s41467-019-11507-z
27. Graham T, Walter J, Loparo J. Two-Stage Synapsis of DNA Ends during Non-homologous End Joining. Mol Cell. 2016 Mar 17;61(6):850–8. https://doi.org/10.1016/j.molcel.2016.02.010
28. Downs JA, Jackson SP. A means to a DNA end: the many roles of Ku. Nat Rev Mol Cell Biol. 2004 May 1;5(5):367–78. https://doi.org/10.1038/nrm1367
29. Walker JR, Corpina RA, Goldberg J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature. 2001 Aug 9;412(6847):607–14. https://doi.org/10.1038/35088000
30. Zhang Z, Hu W, Cano L, Lee TD, Chen DJ, Chen Y. Solution structure of the C-terminal domain of Ku80 suggests important sites for protein-protein interactions. Structure. 2004 Mar;12(3):495–502. https://doi.org/10.1016/j.str.2004.02.007
31. Harris R, Esposito D, Sankar A, Maman JD, Hinks JA, Pearl LH, et al. The 3D solution structure of the C-terminal region of Ku86 (Ku86CTR). J Mol Biol. 2004 Jan 9;335(2):573–82. https://doi.org/10.1016/j.jmb.2003.10.047
32. Blier PR, Griffith AJ, Craft J, Hardin JA. Binding of Ku protein to DNA. Measurement of affinity for ends and demonstration of binding to nicks. J Biol Chem. 1993 Apr 5;268(10):7594–601.
33. Liang F, Jasin M. Ku80-deficient Cells Exhibit Excess Degradation of Extrachromosomal DNA. J Biol Chem . 1996 Jun;271(24):14405–11. https://doi.org/10.1074/jbc.271.24.14405
34. Mimitou EP, Symington LS. Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. EMBO J. 2010 Oct;29(19):3358–69. https://doi.org/10.1038/emboj.2010.193
35. Shibata A, Jeggo P, Lobrich M. The pendulum of the Ku-Ku clock. DNA Repair (Amst). 2018 Nov;71:164-171. https://doi.org/10.1016/j.dnarep.2018.08.020

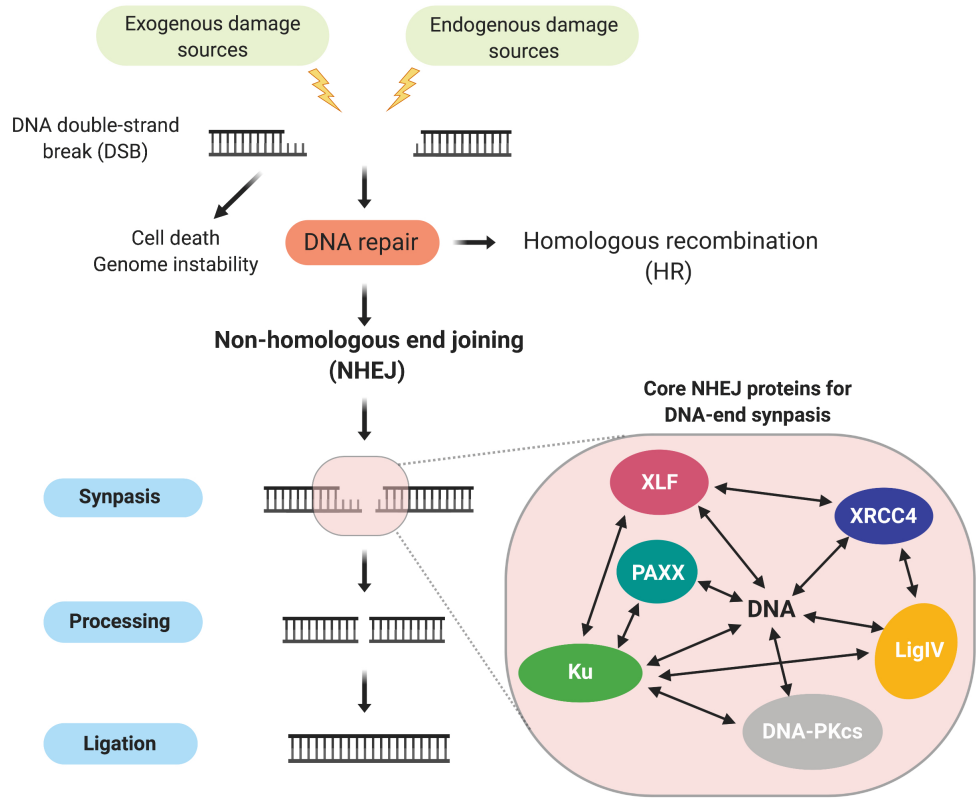
36. Frit P, Ropars V, Modesti M, Charbonnier JB, Calsou P. Plugged into the Ku-DNA hub: The NHEJ network. Prog Biophys Mol Biol. 2019 Mar 6. pii: S0079-6107(18)30251-7. https://doi.org/10.1016/j.pbiomolbio.2019.03.001
37. Gell D, Jackson SP. Mapping of protein-protein interactions within the DNA-dependent protein kinase complex. Nucleic Acids Res. 1999 Sep 1;27(17):3494–502. https://doi.org/10.1093/nar/27.17.3494
38. Wu Q, Liang S, Ochi T, Chirgadze DY, Huiskonen JT, Blundell TL. Understanding the structure and role of DNA-PK in NHEJ: How X-ray diffraction and cryo-EM contribute in complementary ways. Prog Biophys Mol Biol. 2019 Apr 20. pii: S0079-6107(18)30277-3. https://doi.org/10.1016/j.pbiomolbio.2019.03.007
39. Weterings E, Verkaik NS, Keijzers G, Florea BI, Wang S-Y, Ortega LG, et al. The Ku80 Carboxy Terminus Stimulates Joining and Artemis-Mediated Processing of DNA Ends. Mol Cell Biol. 2009 Mar 1;29(5):1134–42. https://doi.org/10.1128/MCB.00971-08
40. Hammel M, Yu Y, Mahaney BL, Cai B, Ye R, Phipps BM, 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. 2010 Jan 8;285(2):1414–23. https://doi.org/10.1074/jbc.M109.065615
41. Britton S, Coates J, Jackson SP. A new method for high-resolution imaging of Ku foci to decipher mechanisms of DNA double-strand break repair. J Cell Biol. 2013 Aug 5;202(3):579-95. https://doi.org/10.1083/jcb.201303073
42. Poltoratsky V, Shi X, York J, Lieber M, Carter T. Human DNA-activated protein kinase (DNA-PK) is homologous to phosphatidylinositol kinases. J Immunol. 1995 Nov 15;155(10):4529–33.
43. Hartley K, Gell D, Smith G, Zhang H, Divecha N, Connelly M, et al. DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. Cell. 1995 Sep 8;82(5):849–56. https://doi.org/10.1016/0092-8674(95)90482-4
44. Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: Requirement for DNA ends and association with Ku antigen. Cell. 1993 Jan;72(1):131–42. https://doi.org/10.1016/0092-8674(93)90057-w
45. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Mol Cell. 2017 Jun;66(6):801–817. https://doi.org/10.1016/j.molcel.2017.05.015
46. Sibanda B, Chirgadze D, Ascher D, Blundell T. DNA-PKcs structure suggests an allosteric mechanism modulating DNA double-strand break repair. Science. 2017 Feb 3;355(6324):520–524. https://doi.org/10.1126/science.aak9654
47. Yin X, Liu M, Tian Y, Wang J, Xu Y. Cryo-EM structure of human DNA-PK holoenzyme. Cell Res. 2017 Nov;27(11):1341–1350. https://doi.org/10.1038/cr.2017.110
48. Sharif H, Li Y, Dong Y, Dong L, Wang W, Mao Y, et al. Cryo-EM structure of the DNA-PK holoenzyme. Proc Natl Acad Sci. 2017 Jul 11;114(28):7367–7372. https://doi.org/10.1073/pnas.1707386114
49. Chen BPC, Chan DW, Kobayashi J, Burma S, Asaithamby A, Morotomi-Yano K, et al. Cell cycle dependence of DNA-dependent protein kinase phosphorylation in response to DNA double strand breaks. J Biol Chem. 2005 Apr;280(15):14709–15. https://doi.org/10.1074/jbc.M408827200

50. Menolfi D, Zha S. ATM, DNA-PKcs and ATR: shaping development through the regulation of the DNA damage responses. GENOME INSTAB. DIS. (2019). https://doi.org/10.1007/s42764-019-00003-9
51. Uematsu N, Weterings E, Yano K, Morotomi-Yano K, Jakob B, Taucher-Scholz G, et al. Autophosphorylation of DNA-PKCS regulates its dynamics at DNA double-strand breaks. J Cell Biol. 2007 Apr 23;177(2):219–29. https://doi.org/10.1083/jcb.200608077
52. Jiang W, Crowe J, Liu X, Nakajima S, Wang Y, Li C, et al. Differential phosphorylation of DNA-PKcs regulates the interplay between end-processing and end-ligation during nonhomologous end-joining. Mol Cell. 2015 Apr 2;58(1):172–85. https://doi.org/10.1016/j.molcel.2015.02.024
53. Spagnolo L, Rivera-Calzada A, Pearl LH, Llorca O. Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. Mol Cell. 2006 May 19;22(4):511–9. https://doi.org/10.1016/j.molcel.2006.04.013
54. DeFazio LG, Stansel RM, Griffith JD, Chu G. Synapsis of DNA ends by DNA-dependent protein kinase. EMBO J. 2002 Jun 17;21(12):3192–200. https://doi.org/10.1093/emboj/cdf299
55. Cary RB, Peterson SR, Wang J, Bear DG, Bradbury EM, Chen DJ. DNA looping by Ku and the DNA-dependent protein kinase. Proc Natl Acad Sci. 1997 Apr 29;94(9):4267–72. https://doi.org/10.1073/pnas.94.9.4267
56. Cottarel J, Frit P, Bombarde O, Salles B, Négrel A, Bernard S, et al. A noncatalytic function of the ligation complex during nonhomologous end joining. J Cell Biol. 2013 Jan 21;200(2):173–86. https://doi.org/10.1083/jcb.201203128
57. Ochi T, Blackford A, Coates J, Jhujh S, Mehmood S, Tamura N, et al. DNA repair. PAXX, a paralog of XRCC4 and XLF, interacts with Ku to promote DNA double-strand break repair. Science. 2015 Jan 9;347(6218):185–188. https://doi.org/10.1126/science.1261971
58. Tomkinson AE, Vijayakumar S, Pascal JM, Ellenberger T. DNA Ligases: Structure, Reaction Mechanism, and Function. Chem Rev. 2006 Feb 1;106(2):687–99. https://doi.org/10.1021/cr040498d
59. Sibanda B, Critchlow S, Begun J, Pei X, Jackson S, Blundell T, et al. Crystal structure of an Xrcc4-DNA ligase IV complex. Nat Struct Biol. 2001 Dec;8(12):1015–9. https://doi.org/10.1038/nsb725
60. Wu P-YY, Frit P, Meesala S, Dauvillier S, Modesti M, Andres SN, et al. Structural and functional interaction between the human DNA repair proteins DNA ligase IV and XRCC4. Mol Cell Biol. 2009 Jun 1;29(11):3163–72. https://doi.org/10.1128/MCB.01895-08
61. Critchlow SE, Bowater RP, Jackson SP. Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. Curr Biol. 1997 Aug 1;7(8):588–98. https://doi.org/10.1016/s0960-9822(06)00258-2
62. Bryans M, Valenzano MC, Stamato TD. Absence of DNA ligase IV protein in XR-1 cells: evidence for stabilization by XRCC4. Mutat Res. 1999 Jan 26;433(1):53–8. https://doi.org/10.1016/s0921-8777(98)00063-9
63. Modesti M, Junop MS, Ghirlando R, van de Rakt M, Gellert M, Yang W, et al. Tetramerization and DNA Ligase IV Interaction of the DNA Double-strand Break Repair Protein XRCC4 are Mutually Exclusive. J Mol Biol. 2003 Nov 21;334(2):215–28. https://doi.org/10.1016/j.jmb.2003.09.031
64. Junop M, Modesti M, Guarné A, Ghirlando R, Gellert M, Yang W. Crystal

structure of the Xrcc4 DNA repair protein and implications for end joining. EMBO J. 2000 Nov 15;19(22):5962–70. https://doi.org/10.1093/emboj/19.22.5962
65. Andres SN, Vergnes A, Ristic D, Wyman C, Modesti M, Junop M. A human XRCC4-XLF complex bridges DNA. Nucleic Acids Res. 2012 Feb;40(4):1868-78. https://doi.org/10.1093/nar/gks022
66. Kaminski AM, Tumbale PP, Schellenberg MJ, Williams RS, Williams JG, Kunkel TA, et al. Structures of DNA-bound human ligase IV catalytic core reveal insights into substrate binding and catalysis. Nat Commun. 2018 Jul;9(1):2642. https://doi.org/10.1038/s41467-018-05024-8
67. Andres SN, Modesti M, Tsai CJ, Chu G, Junop MS. Crystal Structure of Human XLF: A Twist in Nonhomologous DNA End-Joining. Mol Cell. 2007 Dec 28;28(6):1093–101. https://doi.org/10.1016/j.molcel.2007.10.024
68. Li Y, Chirgadze DY, Bolanos-Garcia VM, Sibanda BL, Davies OR, Ahnesorg P, et al. Crystal structure of human XLF/Cernunnos reveals unexpected differences from XRCC4 with implications for NHEJ. EMBO J. 2008 Jan 9;27(1):290–300. https://doi.org/10.1038/sj.emboj.7601942
69. Yano K, Morotomi-Yano K, Wang S-YY, Uematsu N, Lee K-JJ, Asaithamby A, et al. Ku recruits XLF to DNA double-strand breaks. EMBO Rep. 2008 Jan 7;9(1):91–6. https://doi.org/10.1038/sj.embor.7401137
70. Nemoz C, Ropars V, Frit P, Gontier A, Drevet P, Yu J, et al. XLF and APLF bind Ku80 at two remote sites to ensure DNA repair by non-homologous end joining. Nat Struct Mol Biol. 2018/10/05. 2018 Oct;25(10):971–980. https://doi.org/10.1038/s41594-018-0133-6
71. Hammel M, Rey M, Yu Y, Mani R, Classen S, Liu M, et al. XRCC4 Protein Interactions with XRCC4-like Factor (XLF) Create an Extended Grooved Scaffold for DNA Ligation and Double Strand Break Repair. J Biol Chem. 2011 Sep 16;286(37):32638–32650. https://doi.org/10.1074/jbc.M111.272641
72. Ropars V, Drevet P, Legrand P, Baconnais S, Amram J, Faure G, et al. Structural characterization of filaments formed by human Xrcc4-Cernunnos/XLF complex involved in nonhomologous DNA end-joining. Proc Natl Acad Sci U S A. 2011 Aug 2;108(31):12663–8. https://doi.org/10.1073/pnas.1100758108
73. Wu Q, Ochi T, Matak-Vinkovic D, Robinson C V, Chirgadze DY, Blundell TL. Non-homologous end-joining partners in a helical dance: structural studies of XLF-XRCC4 interactions. Biochem Soc Trans. 2011 Oct ;39(5):1387–92. https://doi.org/10.1042/BST0391387
74. Brouwer I, Sitters G, Candelli A, Heerema S, Heller I, Melo de A, et al. Sliding sleeves of XRCC4–XLF bridge DNA and connect fragments of broken DNA. Nature. 2016 Jul 20;535(7613):566–9. https://doi.org/10.1038/nature18643
75. Roy S, de Melo A, Xu Y, Tadi S, Négrel A, Hendrickson E, et al. XRCC4/XLF Interaction Is Variably Required for DNA Repair and Is Not Required for Ligase IV Stimulation. Mol Cell Biol. 2015 Sep 1;35(17):3017–28. https://doi.org/10.1128/MCB.01503-14
76. Ochi T, Wu Q, Chirgadze D, Grossmann G, Bolanos-Garcia V, Blundell T. Structural insights into the role of domain flexibility in human DNA ligase IV. Structure. 2012 Jul 3;20(7):1212–1222. https://doi.org/10.1016/j.str.2012.04.012
77. Graham TGW, Carney SM, Walter JC, Loparo JJ. A single XLF dimer bridges DNA ends during nonhomologous end joining. Nat Struct Mol Biol. 2018

Sep;25(9):877–884. https://doi.org/10.1038/s41594-018-0120-y
78. Bhargava R, Sandhu M, Muk S, Lee G, Vaidehi N, Stark JM. C-NHEJ without indels is robust and requires synergistic function of distinct XLF domains. Nat Commun. 2018;9(1):2484. https://doi.org/ 10.1038/s41467-018-04867-5
79. Xing M, Yang M, Huo W, Feng F, Wei L, Jiang W, et al. Interactome analysis identifies a new paralogue of XRCC4 in non-homologous end joining DNA repair pathway. Nat Commun. 2015 Feb 11;6:6233. https://doi.org/ 10.1038/ncomms7233
80. Craxton A, Somers J, Munnur D, Jukes-Jones R, Cain K, Malewicz M. XLS (c9orf142) is a new component of mammalian DNA double-stranded break repair. Cell Death Differ. 2015 Jun;22(6):890-7. https://doi.org/ 10.1038/cdd.2015.22
81. Tadi S, Tellier-Lebègue C, Nemoz C, Drevet P, Audebert S, Roy S, et al. PAXX Is an Accessory c-NHEJ Factor that Associates with Ku70 and Has Overlapping Functions with XLF. Cell Rep. 2016 Oct;17(2):541–555. https://doi.org/ 10.1016/j.celrep.2016.09.026
82. Liu X, Shao Z, Jiang W, Lee B, Zha S. PAXX promotes KU accumulation at DNA breaks and is essential for end-joining in XLF-deficient mice. Nat Commun. 2017 Jan 4;8. 13816. https://doi.org/ 10.1038/ncomms13816
83. Kumar V, Alt F, Frock R. PAXX and XLF DNA repair factors are functionally redundant in joining DNA breaks in a G1-arrested progenitor B-cell line. Proc Natl Acad Sci U S A. 2016 Sep 20;113(38):10619–24. https://doi.org/ 10.1073/pnas.1611882113
84. Balmus G, Barros A, Wijnhoven P, Lescale C, Hasse H, Boroviak K, et al. Synthetic lethality between PAXX and XLF in mammalian development. Genes Dev. 2016 Oct 1;30(19):2152–2157. https://doi.org/ 10.1101/gad.290510.116
85. Lescale C, Lenden Hasse H, Blackford A, Balmus G, Bianchi J, Yu W, et al. Specific Roles of XRCC4 Paralogs PAXX and XLF during V(D)J Recombination. Cell Rep. 2016 Sep 13;16(11):2967–2979. https://doi.org/ 10.1016/j.celrep.2016.08.069
86. Zhang Z, Zhu L, Lin D, Chen F, Chen D, Chen Y. The three-dimensional structure of the C-terminal DNA-binding domain of human Ku70. J Biol Chem. 2001 Jul 16;276(41):38231–6. https://doi.org/10.1074/jbc.M105238200
87. Ochi T, Gu X, Blundell T. Structure of the Catalytic Region of DNA Ligase IV in Complex with an Artemis Fragment Sheds Light on Double-Strand Break Repair. Structure. 2013 Apr;21(4):672–679. https://doi.org/10.1016/j.str.2013.02.014
88. Kaminski AM, Tumbale PP, Schellenberg MJ, Williams RS, Williams JG, Kunkel TA, et al. Structures of DNA-bound human ligase IV catalytic core reveal insights into substrate binding and catalysis. Nat Commun. 2018 Jul 6;9(1):2642. https://doi.org/10.1038/s41467-018-05024-8

Figrue 1


Exogenous damage sources
Endogenous damage sources
DNA double-strand break (DSB)
Cell death
Genome instability
DNA repair
Homologous recombination (HR)
Non-homologous end joining (NHEJ)
Core NHEJ proteins for DNA-end synpasis
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Ligation
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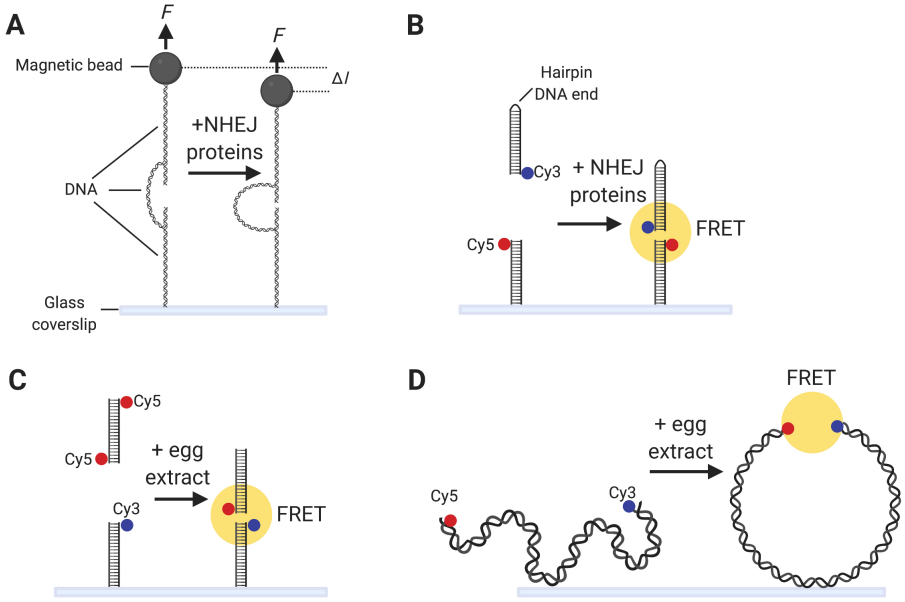
DNA-PKcs

Figure 2


A
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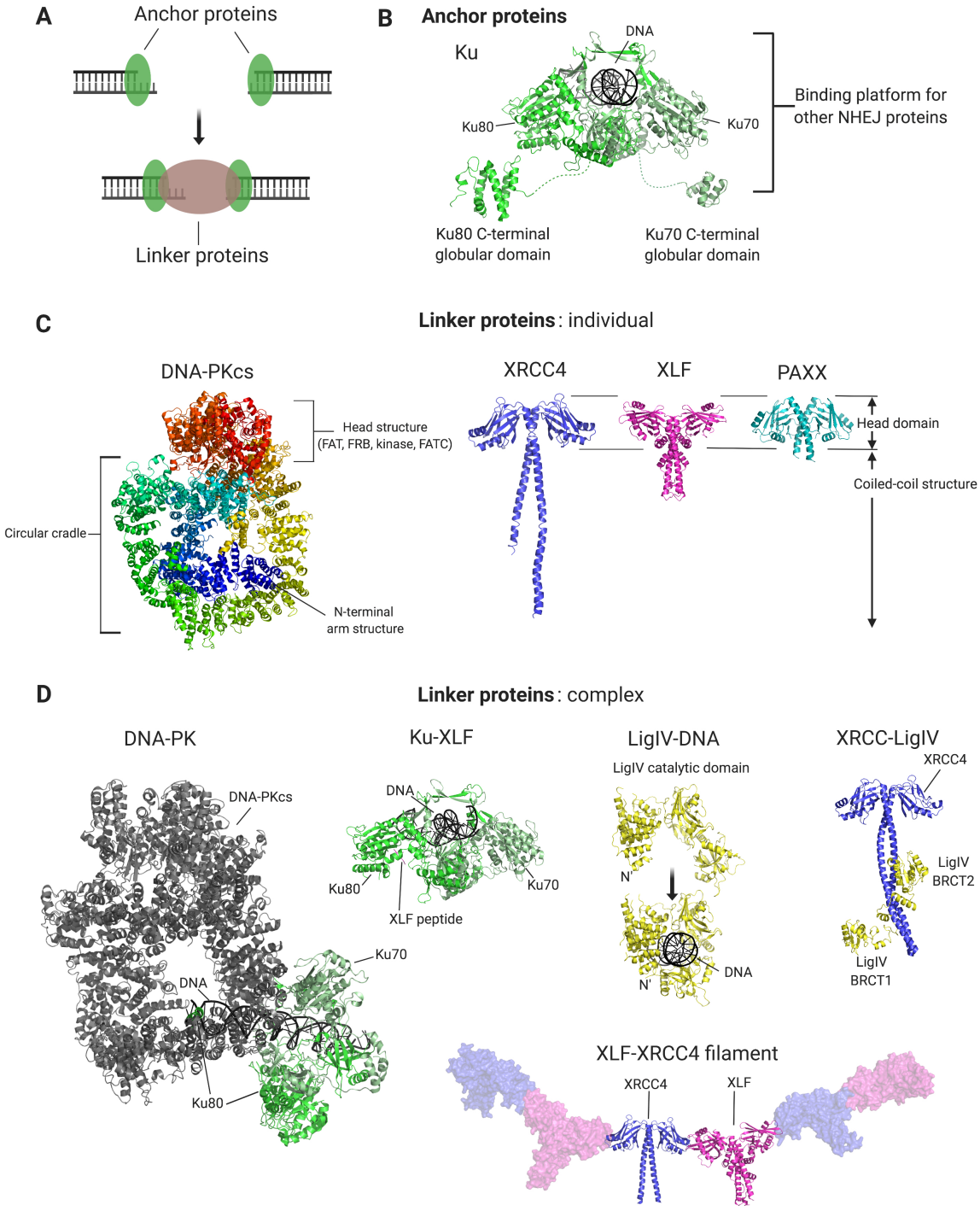
699 Figure 3



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