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Role and Regulation of Human XRCC4-Like Factor/Cernunnos

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Abstract In mammalian cells, non-homologous end joining (NHEJ) is the major double strand break (DSB) repair mechanism during the G_1 phase of the cell cycle. It also contributes to DSB repair during the S and G_2 phases. Ku heterodimer, DNA PKcs, XRCC4 and DNA Ligase IV constitute the core NHEJ machinery, which joins directly ligatable ends. XRCC4-like factor/Cernunnos (XLF/Cer) is a recently discovered interaction partner of XRCC4. Current evidence suggests the following model for the role of XLF/Cer in NHEJ: after DSB induction, the XRCC4-DNA Ligase IV complex promotes efficient accumulation of XLF/Cer at DNA damage sites via constitutive interaction of the XRCC4 and XLF/Cer head domains and dependent on components of the DNA PK complex. Ku alone can stabilise the association of XLF/Cer with DNA ends. XLF/Cer stimulates ligation of complementary and non-complementary DNA ends by XRCC4-DNA Ligase IV. This activity involves the carboxy-terminal DNA binding region of XLF/Cer and could occur via different, non-exclusive modes: (i) enhancement of the stability of the XRCC4-DNA Ligase IV complex on DNA ends by XLF/Cer, (ii) modulation of the efficiency and/or specificity of DNA Ligase IV by binding of XLF/Cer to the XRCC4-DNA Ligase IV complex, (iii) promotion of the alignment of blunt or other non-complementary DNA ends by XLF/Cer for ligation. XLF/Cer promotes the preservation of 3′ overhangs, restricts nucleotide loss and thereby promotes accuracy of DSB joining by XRCC4-DNA Ligase IV during NHEJ and V(D)J recombination. J. Cell. Biochem. 104: 1534–1540, 2008.

Key words: XLF/Cernunnos; core NHEJ; XRCC4-DNA Ligase IV

Mammalian cells use two major repair pathways to deal with DNA double strand breaks (DSBs): potentially error-prone non-homologous end joining (NHEJ) and error-free homologous recombination (HR). After treatment with ionising radiation (IR), NHEJ constitutes the major DSB repair pathway in the G_1 phase of the cell cycle, whereas HR plays only a minor role. In contrast, in the S and G_2 phases of the cell cycle NHEJ and HR both substantially contribute to the repair of DSBs [Rothkamm et al., 2003]. Depending on the structure and direct ligatability of a DSB, repair by NHEJ does not require sequence homology or can use stretches of 1–6 nucleotides of microhomology [Roth and Wilson, 1986]. In contrast, HR requires sequence homology of approximately 100 base pairs and more, preferably between sister chromatids [Helleday et al., 2007].

NHEJ is initiated by binding of the ring-shaped Ku70/Ku80 heterodimer (Ku) to both ends of a DSB, followed by recruitment of DNA-dependent protein kinase catalytic subunit (DNA PKcs) to the DNA–Ku complexes. Ku and DNA PKcs form the DNA PK complex, which has kinase activity. DNA PKcs mediates tethering of the DNA ends and protects them against degradation and premature ligation. Phosphorylation events within the DNA PKcs molecule regulate the accessibility of DNA ends for further processing and/or ligation. Non-ligatable DSBs generated by IR or radiomimetic drugs like neocarzinostatin, bleomycin, and calicheamicin γ1 must be processed to restore ligatable 3′ OH and 5′ PO_4 termini prior to

Abbreviations used: 3-D, three-dimensional; 53BP1, tumour protein p53 binding protein 1; BRCA1, breast cancer 1, early onset; BRCT, BRCA1 C-terminal; DNA PK, DNA-dependent protein kinase; GST, glutathione S-transferase; H2AX, H2A histone family, member X protein; γH2AX, H2AX phosphorylated at serine 139; MDC1, mediator of DNA damage checkpoint 1 protein; MRE11, meiotic recombination 11 protein; SMC1, structural maintenance of chromosomes 1 protein; XRCC4, X-ray repair complementing defective repair in Chinese hamster cells 4 protein.

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joining by the XRCC4-DNA ligase IV complex. These processing activities include nuclease, polymerase, and kinase/phosphatase activities. Ku70/Ku80, DNA PKcs, DNA Ligase IV, and XRCC4 constitute core NHEJ factors, which can join directly ligatable ends [Povirk, 2006; Weterings and Chen, 2008]. This review article discusses the role and regulation of the XRCC4 interaction partner XRCC4-like factor/Cernunnos (XLF/Cer) in NHEJ.

XLF/Cer is the most recent member of the core NHEJ factor group. Patients with mutations in the XLF/Cer gene showed growth retardation, microcephaly, and immunodeficiency. The immunological defects were due to T and B lymphocytopenia and hypogammaglobulinemia of serum IgG and IgA antibodies. They reflect a role of XLF/Cer in V(D)J recombination, particularly in accurate signal join formation, a minor role in the prevention of nucleotide loss at coding joins, and a potential role in class switch recombination. Fibroblasts of XLF/Cer patients or XLF/Cer human knock down cells showed increased sensitivity to IR and to the DSB inducing agent bleocin, impaired NHEJ-dependent random chromosomal plasmid integration in vivo, and impaired DNA end ligation of restriction enzyme-induced DSBs in vivo and in vitro in comparison to XLF/Cer proficient cells. These data indicated a role of XLF/Cer in NHEJ. G_1/S, intra S and G_2/M checkpoint responses after IR and formation of irradiation-induced γH2AX, MRE11, 53BP1 and RAD51 foci were normal in XLF/Cer-deficient fibroblasts [Ahnesorg et al., 2006; Buck et al., 2006]. Homozygous targeted deletion of exons 4 and 5 of the murine XLF/Cer gene in mouse embryonic stem (ES) cells lead to similar defects as observed in XLF/Cer patients: strong reduction of the extent of signal and coding join formation in transient V(D)J recombination assays, radiation sensitivity, an intrinsic DSB repair defect, and increased genomic instability with elevated levels of chromosomal breaks, chromatid breaks and translocations in comparison to wild type ES cells. The mutant ES cells analysed produced a truncated XLF/Cer transcript and could potentially express a truncated XLF/Cer protein. However, immunoblot analysis of cell extracts with a polyclonal anti-XLF/Cer antibody confirmed that the mutant ES cells expressed no or undetectable levels of XLF/Cer protein [Zha et al., 2007].

The human XLF/Cer gene is located on chromosome 2q35. Human XLF/Cer mRNA was ubiquitously expressed and encoded a nuclear protein of 299 amino acids with a theoretical molecular weight of 33 kD. The amino acid sequence of XLF/Cer was conserved to a low degree from yeast to human. However, no XLF/Cer homolog was found in plants and prokaryotes [Ahnesorg et al., 2006; Buck et al., 2006; Callebaut et al., 2006; Hentges et al., 2006; Andres et al., 2007; Li et al., 2008].

INTERACTION PARTNERS OF XLF/CER

In silico sequence alignments, sequence structure comparisons and Hydrophobic Cluster Analysis predicted similar secondary and tertiary structures of XLF/Cer and XRCC4 with an amino-terminal globular head domain followed by an α-helical stalk able to adopt a coiled-coil structure [Ahnesorg et al., 2006; Buck et al., 2006; Callebaut et al., 2006; Hentges et al., 2006; Li et al., 2008]. Co-immunoprecipitation and pull down experiments with differently tagged human XLF/Cer versions suggested that XLF/Cer formed multimers [Ahnesorg et al., 2006; Callebaut et al., 2006; Deshpande and Wilson, 2007]. Size exclusion chromatography, crosslinking experiments, analytical ultracentrifugation, small-angle X-ray scattering, dynamic light scattering and circular dichroism spectroscopy with purified recombinant human XLF/Cer confirmed, that the full-length protein or residues 1–233 formed homodimers in solution [Hentges et al., 2006; Li et al., 2008]. XLF/Cer residues 1–224 also formed trimers and tetramers at high protein concentrations [Andres et al., 2007]. The globular head domain of XLF/Cer alone could not mediate the interaction with full length XLF/Cer in pulldown experiments from yeast extracts with overexpressed, differently tagged human proteins, indicating a role of the remainder of the protein in dimerisation [Deshpande and Wilson, 2007].

Analysis of the crystal structure of human XLF/Cer homodimer comprising residues 1–233 or 1–224 at 2.3 or 2.5 Å resolution confirmed that its overall structure resembled the structure of the XRCC4 homodimer (Fig. 1) [Andres et al., 2007; Li et al., 2008]. In both XLF/Cer and XRCC4 homodimers, interactions between the two monomers involved formation of a coiled-coil structure by the α-helical stalks and interactions between the coiled-coil region of

one monomer with the head domain of the partner monomer [Junop et al., 2000; Li et al., 2008]. In comparison to XRCC4, XLF/Cer contained an additional α-helix (α_A) at its amino-terminus in the globular head domain. The coiled-coil region of XLF/Cer was much shorter than the long, extended coiled-coil region of XRCC4 and the angle between the globular head domains and the coiled-coil was larger in XLF/Cer (130°) than in XRCC4 (85°). Most strikingly, the part of XLF/Cer carboxy-terminal to the coiled-coil region comprised a loop that reversed the direction of the polypeptide chain towards the head domain, followed by α-helices α_E and α_F. These two α-helices encircled the coiled-coil and stabilised the dimer in this way. The carboxy-terminal of these α-helices (α_F) contacted the head domain of the same monomer through hydrogen bonds. In the XLF/Cer homodimer, the most amino- and carboxy-terminal α-helices (α_A and α_F) acted as wedges to position the globular head domains away from the coiled-coil (Fig. 1) [Andres et al., 2007; Li et al., 2008].

Human XLF/Cer co-fractionated with a proportion of cellular XRCC4-DNA Ligase IV after size exclusion chromatography at 200 kD, interacted with XRCC4 in yeast two hybrid assays and with XRCC4 and DNA Ligase IV in pulldown and co-immunoprecipitation analyses. In yeast two hybrid and pull down studies from yeast extracts with overexpressed tagged human proteins, XLF/Cer and XRCC4 interacted via their globular head domains. The XLF/Cer-XRCC4 interaction was more salt sensitive

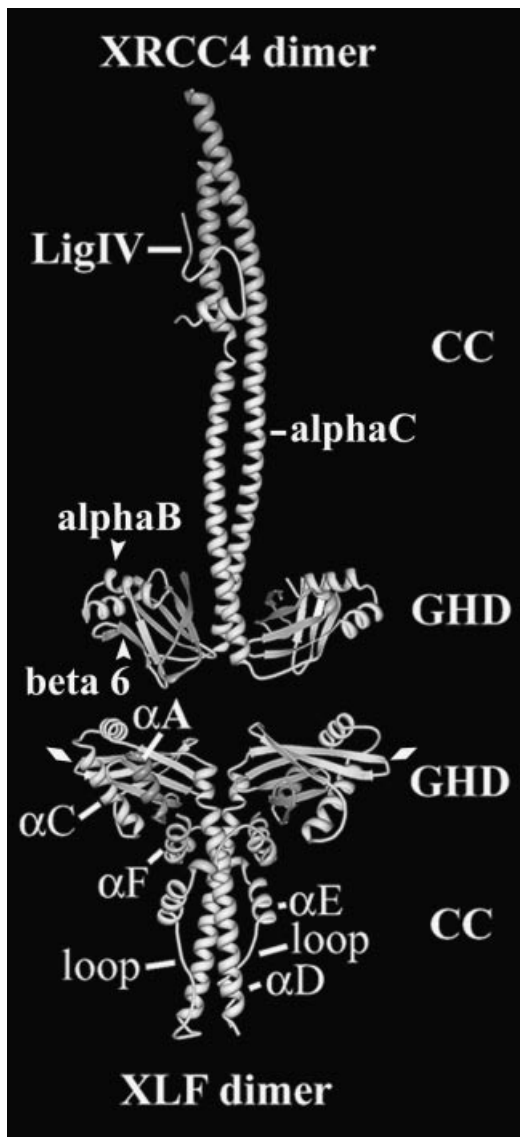

Fig. 1. Human XRCC4-DNA Ligase IV and XLF/Cernunnos (XLF/Cer) complexes. 3-D models of the XRCC4 homodimer (residues 1–213) in complex with DNA Ligase IV BRCT-linker polypeptide (Lig IV, upper half) and of the XLF/Cer homodimer (residues 1–233, lower half). XRCC4 and XLF/Cer homodimers both contained globular head domains (GHD) and coiled-coil regions (CC). The coiled-coil of XLF/Cer was much shorter than the extended coiled-coil of XRCC4. The region carboxy-terminal to the coiled-coil in each XLF/Cer subunit reversed direction towards the head domain via a loop region (indicated in both subunits for clarity) and the carboxy-terminal α-helices α_E and α_F encircled the coiled-coil. α-helices α_A and α_F acted as wedges that position the globular head domains of XLF/Cer away from the coiled-coil, resulting in a larger angle between each head domain and the coiled-coil in XLF/Cer in comparison to XRCC4 [Andres et al., 2007; Li et al., 2008]. XRCC4 and XLF/Cer interacted via their globular head domains in yeast two hybrid and pulldown studies [Deshpande and Wilson, 2007]. The second set of β-strands in the globular head domain of XRCC4 (beta 6 is the middle one of three β-strands in this structure) and α-helices α_A and α_C in the globular head domain of XLF/Cer were predicted interaction surfaces [Li et al., 2008]. Mutational analysis identified the following residues as crucial for the interaction of XRCC4 with XLF/Cer in vitro: K63 and K65 near the amino-terminus of α-helix alphaB and K99 near the carboxy-terminus of β-strand beta 6 in the head domain of XRRC4 (approximate positions of mutations are indicated by arrow heads in one subunit). Mutation of L115 in the β-strand 6–7 loop in the head domain of XLF/Cer abolished the interaction between XLF/Cer and XRCC4 in vitro. The approximate position of L115 is indicated by diamonds in both subunits for clarity [Andres et al., 2007]. The exact mode of interaction between the head domains of XLF/Cer and XRCC4 remains to be analysed. Several experimental approaches suggested a 2:2:1 stoichiometry of the XLF/Cer-XRCC4-DNA Ligase IV complex. Whether and how XLF/Cer directly interacts with DNA Ligase IV in the XRCC4-DNA Ligase IV complex has to be elucidated by future research. Structures of XRCC4-DNA Ligase IV and XLF/Cer complexes were obtained from Protein Data Bank (PDB ID: 1ik9, Sibanda et al. [2001] and PDB ID: 2qm4, Li et al. [2008], respectively, http://www.pdb.org) and visualised with Protein Workshop [Moreland et al., 2005].

than the XRCC4-XRCC4 interaction, indicating the involvement of ionic interactions [Ahnesorg et al., 2006; Callebaut et al., 2006; Deshpande and Wilson, 2007; Lu et al., 2007]. In Surface Plasmon Resonance studies, immobilised XLF/Cer and XRCC4 in solution interacted with an affinity of 7.8 μM. The conserved surface regions between XLF/Cer and XRCC4 orthologues were analysed with respect to a favourable energy change, when buried in a protein–protein interaction. This analysis identified the second set of ß-strands in the head domain of XRCC4 and α-helices α_A and α_C in the head domain of XLF/Cer as putative protein-protein interaction sites (Fig. 1) [Li et al., 2008]. Mutational analysis identified the following residues as crucial for the XLF/Cer-XRCC4 interaction in vitro: Residues K63 and K65 near the amino-terminus of α-helix alphaB and residue K99 near the carboxy-terminus of β-strand 6 in the second set of β-strands in the XRCC4 head domain. Mutation of L115 in the second set of β-strands of the head domain of XLF/Cer abolished the interaction of XLF/Cer with XRCC4 in vitro (Fig. 1) [Andres et al., 2007]. The role of XLF/Cer α-helices α_A and α_C in mediating the XLF/Cer-XRCC4 interaction has to be investigated by further mutational analyses. Co-immunoprecipitation experiments with endogeneous human proteins showed that the interaction between XLF/Cer and XRCC4 did not require the presence of DSBs, of DNA or of DNA Ligase IV. However, the presence of DNA Ligase IV strongly enhanced this interaction [Wu et al., 2007].

The 200 kD XLF-XRCC4-DNA Ligase IV complex identified by size exclusion chromatography could accomodate one Ligase IV molecule (103 kD) and one to two XRCC4 and XLF/Cer molecules (38 and 33 kD, respectively). Interaction site mapping studies suggested that residues 180–213 in the extended coiled-coil region of XRCC4 interacted with the linker region between the carboxy-terminal BRCT domains of DNA Ligase IV. Crystallographic analysis of the complex between an XRCC4 fragment (residues 1–213) and the DNA Ligase IV BRCT-linker polypeptide at 2.3 Å resolution revealed that a single Ligase polypeptide interacted asymetrically with residues 173–195 in both subunits of an XRCC4 dimer (Fig. 1). This lead to local unwinding of the coiled-coil in the XRCC4 dimer [Sibanda et al., 2001]. In solution, the XRCC4-DNA Ligase IV complex also had 2:1 stoichiometry [Modesti et al., 2003]. Sequence alignments showed that key residues in the DNA Ligase IV interaction region of human XRCC4 were conserved in human XLF/Cer. They were located in the intervening sequence between two segments of the left-handed, parallel coiled-coil in XRCC4 and in the region of α-helix α_E in XLF/Cer (Fig. 1) [Sibanda et al., 2001; Callebaut et al., 2006; Li et al., 2008]. However, yeast two hybrid analyses as well as co-immunoprecipitation and pull down experiments with overexpressed human XLF/Cer and overexpressed human XRCC4 or DNA Ligase IV from human 293T, insect cell or yeast extracts suggested that XLF/Cer bound readily to XRCC4, but not or only weakly to DNA Ligase IV [Deshpande and Wilson, 2007; Lu et al., 2007]. In vitro, XLF/Cer fragment (residues 1–224) did not interact with a carboxy-terminal fragment of DNA Ligase IV comprising the tandem BRCT domains. However, XLF/Cer fragment bound to XRCC4 in the absence or presence of DNA Ligase IV carboxy-terminal fragment. The interaction between XRCC4 with the DNA Ligase IV carboxy-terminal fragment was also readily detected [Andres et al., 2007]. This indicated that XLF/Cer lacking the carboxy-terminal 75 residues, but comprising the conserved DNA Ligase IV interaction region, did not interact with the DNA-Ligase IV BRCT domains or with the BRCT-linker polypeptide. Additionally, structural studies showed that folding and twisting of α-helices α_E and α_F back onto α_D of the opposing XLF/Cer dimer subunit made the DNA Ligase IV interaction region inaccessible [Andres et al., 2007; Li et al., 2008]. The following findings indicated that XLF/Cer might interact with both components of an XRCC4-DNA Ligase IV complex: the presence of DNA Ligase IV or XRCC4 enhanced the interaction of XLF/Cer with XRCC4 or DNA Ligase IV, respectively, in co-immunoprecipitation experiments [Lu et al., 2007; Wu et al., 2007]. Binding of the BRCT-linker polypeptide of DNA Ligase IV to the XRCC4 dimer had no impact on the structure of the globular head domain of XRCC4, which mediated the interaction with XLF/Cer [Sibanda et al., 2001; Deshpande and Wilson, 2007]. Finally, cellular fractionation assays after treatment of human knock down or control cells with the DSB inducing agent calicheamicin γ1 showed that the intact XRCC4-DNA Ligase IV complex and DNA PKcs were required for the efficient

recruitment of XLF/Cer to damaged chromatin. DNA PKcs governed the efficient accumulation of XRCC4-DNA Ligase IV on damaged chromatin [Wu et al., 2007]. In hamster cell lines, XRCC4 enhanced the accumulation of overexpressed human XLF/Cer at laser-induced DSB tracks by decreasing the exchange rate of XLF/Cer at sites of DNA damage. Recruitment of XRCC4 and of overexpressed human XLF/Cer to laser-induced DSB tracks in hamster cell lines required Ku, but not DNA PKcs. Ku also interacted with XLF/Cer in a DNA-dependent manner after immunoprecipitation of overexpressed XLF/Cer from human cell extracts and in pull-down assays with purified components. Ku promoted the association of XLF/Cer with shorter double stranded DNA fragments in vitro, to which XLF/Cer alone could not bind. These results suggested that Ku stabilised binding of XLF/Cer to DNA ends [Mari et al., 2006; Yano et al., 2008].

Calicheamicin γ1 treatment of human cells induced the DNA PKcs-dependent phosphorylation of XRCC4 and XLF/Cer. However, the interaction between XRCC4-DNA Ligase IV and XLF/Cer or their recruitment to the damaged chromatin did not depend on their phosphorylation by DNA PKcs. XLF/Cer was not required for the recruitment of XRCC4-DNA Ligase IV or Ku to the damaged chromatin, the activation of DNA PKcs by autophosphorylation or the phosphorylation of XRCC4 after DSB induction [Wu et al., 2007]. Additionally, GST-tagged, purified XLF/Cer did not interact with DNA PKcs, MRE11, RAD50, or SMC1 in nuclear extracts of human cells in the absence of DSB inducing agents and in the presence of ethidiumbromide [Ahnesorg et al., 2006].

Current evidence from size exclusion chromatography, interaction and cellular fractionation studies and crystallographic analyses suggests a 2:2:1 stoichiometry of the XLF/Cer-XRCC4-DNA Ligase IV complex [Sibanda et al., 2001; Modesti et al., 2003; Ahnesorg et al., 2006; Callebaut et al., 2006; Hentges et al., 2006; Deshpande and Wilson, 2007; Lu et al., 2007; Wu et al., 2007; Li et al., 2008]. The presence of DNA Ligase IV enhanced the interaction between XLF/Cer and XRCC4 [Wu et al., 2007]. This suggests the following model: XLF/Cer interacts via its globular head domains with the head domains of XRCC4 in the XRCC4-DNA Ligase IV complex. Upon binding to XRCC4, XLF/Cer might interact directly with DNA Ligase IV via a protein region different from its conserved DNA ligase IV interaction region. Li et al. [2008] considered formation of XLF/Cer-XRCC4 heterodimers via a hybrid coiled-coil unlikely due to the low degree of sequence conservation in the coiled-coil regions of both proteins.

ROLE OF XLF/CER IN DNA LIGATION DURING NHEJ

In contrast to wild type cell extracts, extracts of XLF/Cer deficient cells did not promote intermolecular ligation of linearised plasmid DNA with complementary 5′ overhangs [Buck et al., 2006]. In in vitro end joining assays with purified core NHEJ proteins, XLF/Cer specifically promoted ligation of linear double stranded DNA substrates by the XRCC4-DNA Ligase IV complex, but not by T4 DNA ligase [Hentges et al., 2006; Lu et al., 2007; Tsai et al., 2007]. XRCC4 and XLF/Cer bound in vitro to double stranded DNA in a sequence-independent manner not necessarily requiring the presence of free DNA ends. XLF/Cer additionally bound to single stranded DNA. Whereas the interaction of XLF/Cer with XRCC4 was mediated by their head domains, interaction of XLF/Cer with double stranded DNA required the carboxy-terminal 75 amino acids of XLF/Cer. An XLF/Cer mutant lacking the carboxy-terminal 75 amino acids hardly stimulated end joining of blunt or non-compatible DNA ends in vitro in comparison to wild type XLF/Cer [Andres et al., 2007; Deshpande and Wilson, 2007]. XRCC4 assisted the recruitment of DNA Ligase IV to DNA and stimulated end joining by enhancing the adenylation of DNA Ligase IV, a necessary step to obtain ligation-competent DNA Ligase IV. In contrast, XLF/Cer did not stimulate the adenylation of DNA Ligase IV in vitro [Modesti et al., 1999; Hentges et al., 2006; Lu et al., 2007]. These data suggested that XLF/Cer promoted DNA end joining by XRCC4-DNA Ligase IV by generally stabilising the XRCC4-DNA Ligase IV complex on DNA. XLF/Cer could also induce a conformational change in the XRCC4-DNA Ligase IV complex by binding to it and in this way modulate the efficiency and/or specificity of DNA Ligase IV. In vitro studies with purified components by Lu et al. [2007] and Hentges et al. [2006] showed that XLF/Cer stimulated the ligation of DNA

substrates with complementary 4 base 3′ overhangs or 2 base 5′ overhangs by XRCC4-DNA Ligase IV alone, whereas Tsai et al. [2007] observed no stimulation of ligation of complementary 4 base 3′ or 5′ overhangs by XRCC4-DNA Ligase IV and DNA PK or Ku. However, under the assay conditions of Tsai et al. [2007] complementary DNA ends were already efficiently joined in the absence of XLF/Cer. Immobilised XLF/Cer stimulated intramolecular ligation, whereas freely diffusible XLF/Cer promoted primarily intermolecular ligation of DNA substrates [Lu et al., 2007]. The extent of stimulation varied between approximately 2- to 200-fold, reflecting differences in assay components and conditions, but also DNA end sequence preferences of XRCC4-DNA Ligase IV in vitro [Hentges et al., 2006; Gu et al., 2007; Lu et al., 2007; Tsai et al., 2007]. At high XLF/Cer to XRCC4-DNA Ligase IV ratios, XLF/Cer even inhibited DNA end joining in vitro, probably due to blocking of DNA ends or inhibitory modulation of DNA Ligase IV activity [Hentges et al., 2006]. In the presence of DNA PK or Ku and XRCC4-DNA Ligase IV, XLF/Cer had a strong stimulatory effect on the intermolecular ligation of non-complementary DNA ends with one or two overhangs and a weaker or no stimulatory effect on the intermolecular ligation of blunt DNA ends [Gu et al., 2007; Tsai et al., 2007]. XLF/Cer required Ku to promote the joining of non-complementary 3′ overhangs by XRRC4-DNA Ligase IV, reflecting a role of Ku in mediating the association of XRCC4-DNA Ligase IV and XLF/Cer with DNA ends [Nick McElhinny et al., 2000; Mari et al., 2006; Tsai et al., 2007; Yano et al., 2008]. Andres et al. [2007] hypothesized that the alternating arrangement of XRCC4-DNA Ligase IV complexes and XLF/Cer dimers on DNA in a protein filament could allow appropriate alignment of blunt or mismatched DNA ends for ligation by a sliding mechanism and could simultaneously stabilise binding of XRCC4-DNA Ligase IV and XLF/Cer to DNA. These authors suggested a stacked head-to-head orientation of XLF/Cer homodimers and XRCC4-DNA Ligase IV complexes instead of an opposite head-to-head orientation as depicted in Figure 1. Additionally, XLF/Cer regulated the extent and mode of nucleotide loss during NHEJ in vivo and in vitro: after transfection of linearised plasmid DNA with blunt ends or non-complementary 3′ overhangs into wild type fibroblasts or XLF/Cer deficient fibroblasts, junctions recovered from wild type fibroblasts were mostly accurate, whereas junctions recovered from XLF/Cer deficient fibroblasts were mostly inaccurate and showed a higher degree of nucleotide loss at the junctions [Buck et al., 2006]. In vitro, XLF/Cer promoted the preservation of 3′ overhangs, but had only a weak or no effect on the preservation of 5′ overhangs. XLF/Cer stimulated the ligation of the 3′ OH end of a 3′ four base overhang to the 5′ PO_4 of a blunt end by XRCC4-DNA Ligase IV, DNA PK or Ku, thereby creating a template for gap filling polymerases [Tsai et al., 2007].

The following model for the function of XLF/Cer in NHEJ is suggested: After DSB induction, the XRCC4-DNA Ligase IV complex promotes efficient accumulation of XLF/Cer at DNA damage sites via constitutive interaction of the XRCC4 and XLF/Cer head domains and dependent on components of the DNA PK complex. Ku alone can stabilise the association of XLF/Cer with DNA ends. XLF/Cer stimulates ligation of complementary and non-complementary DNA ends by XRCC4-DNA Ligase IV. This activity involves the carboxy-terminal DNA binding region of XLF/Cer and could occur via different, non-exclusive modes: (i) XLF/Cer could enhance the stability of the XRCC4-DNA Ligase IV complex on DNA ends due to its ability to bind to double and single stranded DNA, (ii) binding of XLF/Cer to the XRCC4-DNA Ligase IV complex could induce a conformational change of the complex and in this way modulate the efficiency and/or specificity of DNA Ligase IV, (iii) XLF/Cer could promote the alignment of blunt or other non-complementary DNA ends for ligation, for example, via a sliding mechanism involving the formation of DNA-bound protein filaments of XLF/Cer and XRCC4-DNA Ligase IV. XLF/Cer promotes the preservation of 3′ overhangs, restricts nucleotide loss and thereby promotes the accuracy of DSB joining by XRCC4-DNA Ligase IV during NHEJ and the NHEJ-dependent process of V(D)J recombination.

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