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Structure and function of the DNA ligases encoded by the mammalian *LIG3* gene

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Abstract

Among the mammalian genes encoding DNA ligases (*LIG*), the *LIG3* gene is unique in that it encodes multiple DNA ligase polypeptides with different cellular functions. Notably, this nuclear gene encodes the only mitochondrial DNA ligase and so is essential for this organelle. In the nucleus, there is significant functional redundancy between DNA ligase III α and DNA ligase I in excision repair. In addition, DNA ligase III α is essential for DNA replication in the absence of the replicative DNA ligase, DNA ligase I. DNA ligase III α is a component of an alternative non-homologous end joining (NHEJ) pathway for DNA double-strand break (DSB) repair that is more active when the major DNA ligase IV-dependent pathway is defective. Unlike its other nuclear functions, the role of DNA ligase III α in alternative NHEJ is independent of its nuclear partner protein, X-ray repair cross-complementing protein 1 (XRCC1). DNA ligase III α is frequently overexpressed in cancer cells, acting as a biomarker for increased dependence upon alternative NHEJ for DSB repair and it is a promising novel therapeutic target.

Introduction

DNA ligases play an essential role in maintaining genomic integrity by joining breaks in the phosphodiester backbone of DNA that occur during replication and recombination, and as a consequence of DNA damage and its repair. Three human genes, *LIG1*, *LIG3* and *LIG4* encode ATP-dependent DNA ligases. These enzymes have related catalytic regions that catalyze the same three-step ligation reaction but different flanking domains that mediate protein:protein interactions with different partners (Ellenberger and Tomkinson, 2008).

While almost all eukaryotes have homologs of the *LIG1* and *LIG4* genes, the *LIG3* gene is less widely distributed. Initially, it was thought that the *LIG3* gene was restricted to vertebrates but, with the sequencing of more genomes, it has now been found in about 30% of eukaryotes, including members of 4 of the 6 ancestral eukaryotic groups (Simsek and Jasin, 2011). This distribution suggests that the *LIG3* gene arose relatively early during the evolution of eukaryotes but was not always retained. As eukaryotes became more complex, it was presumably advantageous to have multiple *LIG* genes that encoded a broader repertoire of DNA ligases to participate in the increasing number of specialized DNA transactions, including immunoglobulin gene rearrangements in immune cells, meiosis and germ cell development, the use of poly (ADP-ribose) to signal DNA damage and the

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different DNA repair pathways in proliferating and terminally differentiated cells. Notably, the DNA ligases encoded by vertebrate *LIG3* genes have acquired several conserved accessory domains that flank the core catalytic region during evolution (Simsek and Jasin, 2011). As discussed below, these domains play critical roles in dictating the multiple cellular functions of the DNA ligases encoded by the *LIG3* gene in vertebrate DNA metabolism.

The absence of the *LIG3* gene in yeasts has prevented the use of genetically tractable lower eukaryotes, such as *S. cerevisiae* and *S. pombe*, as models to gain insights into the cellular functions of and interplay between the DNA ligases encoded by the *LIG1*, *LIG3* and *LIG4* genes in higher eukaryotes. Based on the efforts of many laboratories, there is an emerging picture of functional redundancy among the DNA ligases in mammalian cells that is complicated by the *LIG3* gene encoding multiple DNA ligase polypeptides. In this review, we focus on the structure and function of the DNA ligases encoded by the mammalian *LIG3* gene.

LIG3 gene

The human *LIG3* gene is located on human chromosome 17 at q11.2–q12 (Chen et al., 1995; Wei et al., 1995). Unlike the *LIG1* and *LIG4* genes, the *LIG3* gene encodes three or possibly four different DNA ligase polypeptides (Fig. 1). Mitochondrial and nuclear versions of DNA ligase III α are generated in all cells by alternative translation initiation (Lakshmipathy and Campbell, 1999). The DNA ligase III α mRNA open reading frame encodes an N-terminal mitochondrial leader sequence (MLS) that is cleaved off during entry into mitochondria (Fig. 2). Thus, translation initiation at the first ATG of the full-length open reading frame generates mitochondrial DNA ligase III α whereas translation initiation at an internal ATG adjacent to a Kozak consensus sequence generates nuclear DNA ligase III α (Chen et al., 1995; Wei et al., 1995; Lakshmipathy and Campbell, 1999). Since there is no obvious NLS (NLS) within the DNA ligase III α polypeptide, it has been suggested, as shown in Figure 2, that nuclear localization is dependent upon complex formation with a partner protein XRCC1 that does have a NLS (Caldecott, 2003; Parsons et al., 2010). The interaction of DNA ligase III α with XRCC1 and other partner proteins is described below. Prior to the cloning of the human *LIG* genes, biochemical studies had identified a 70 kDa DNA ligase in addition to a 125 kDa DNA ligase I and a 100 kDa DNA ligase III that was designated DNA ligase II (Soderhall and Lindahl, 1975; Tomkinson et al., 1991). Amino acid sequencing of peptides from purified DNA ligase II revealed that this polypeptide was encoded by the *LIG3* gene and is most likely generated by proteolysis of DNA ligase III α during purification (Wang et al., 1994; Chen et al., 1995; Husain et al., 1995). Thus, the confusing nomenclature of the mammalian DNA ligases can be attributed to a purification artifact during attempts to purify and characterize these enzymes.

The *LIG3* gene is ubiquitously expressed at low levels in all human tissues and cells except for the testes where expression levels are about 10-fold higher (Chen et al., 1995; Wei et al., 1995). Further analysis revealed that the elevated levels of expression occur in primary spermatocytes undergoing recombination prior to the first meiotic division and that a distinct mRNA species, DNA ligase III β , is generated by an alternative splicing mechanism that has only been detected in male germ cells (Mackey et al., 1997). The alternative splicing, which replaces the exon encoding the C-terminal 77 amino acids of DNA ligase III α with a novel 17- to 18-amino acid sequence (Fig. 1), begins in early pachytene spermatocytes with DNA ligase III β mRNA detectable throughout pachytene and in round spermatids (Mackey et al., 1997). Since the 5' end of DNA ligase III β mRNA is identical to that of DNA ligase III α mRNA, this transcript is also capable of encoding nuclear and mitochondrial versions of DNA ligase III β by alternative translation (Fig. 1). It should be noted that DNA ligase III β mRNA has not been detected in the ovary (Mackey et al., 1997). This may reflect

differences between oogenesis and spermatogenesis. Furthermore, organisms such as *S. cerevisiae*, that lack a *LIG3* homolog undergo meiosis. Thus, although the expression pattern of DNA ligase III β during spermatogenesis suggests that this enzyme participates in the completion of homologous recombination events that occur during meiotic prophase, this has not yet been definitively demonstrated. Conceivably, DNA ligase III β may be involved in maintaining genomic integrity when histones are replaced with protamines late in the haploid phase of spermatogenesis.

DNA ligase polypeptides encoded by the *LIG3* gene

The related catalytic regions of the human DNA ligases contain three domains, a DNA binding domain (DBD), a nucleotidyl transferase domain (NTase) and an oligonucleotide/oligosaccharide-fold binding domain (OBD) (Ellenberger and Tomkinson, 2008). Similar to DNA ligase I, the DNA ligase III polypeptide adopts a flexible, extended conformation in the absence of DNA (Cotner-Gohara et al., 2010). When it engages a DNA nick, the domains of the catalytic region contact and encircle the nicked DNA in a compact, closed clamp structure shown in Figure 3A. (Cotner-Gohara et al., 2010). The protein architecture and conformation of the catalytic domains in this structure are remarkably similar to those of the DNA ligase I catalytic domains bound to nicked DNA despite these polypeptides only sharing 21% amino acid identity (Cotner-Gohara et al., 2010).

A unique feature of the DNA ligases encoded by the *LIG3* gene compared with the other human DNA ligases is an N-terminal zinc finger (ZnF) (Fig. 1). This ZnF is structurally related to the pair of ZnFs at the N-terminus of poly (ADP-ribose polymerase 1 (PARP1) that facilitate binding of PARP1 to DNA breaks and other abnormal DNA structures (Mackey et al., 1999). Although the DNA ligase III ZnF also binds to DNA breaks, it is not required for nick ligation but does enable the enzyme to efficiently join nicked DNA at high salt concentrations (Mackey et al., 1999). Interestingly, the DNA ligase III ZnF and DBD cooperate to form a nick-binding module with the NTase and OBD forming a second nick-binding module (Cotner-Gohara et al., 2008). These two modules have different DNA binding properties with ZnF/DBD module being more tolerant of different nick structures, including gaps, whereas the NTase/OBD module preferentially binds to ligatable nicks (Cotner-Gohara et al., 2008). Based on these properties and biophysical studies, a jackknife model for the ligation of DNA nicks by DNA ligase III has been proposed (Cotner-Gohara et al., 2008; Cotner-Gohara et al., 2010). In this model, the ZnF/DBD module acts as a nick sensor that initially engages DNA breaks with the DNA ligase III polypeptide in an extended conformation (Fig. 3B). If the nick is ligatable, the DNA ligase III polypeptide undergoes a conformational change with NTase/OBD displacing the ZnF/DBD module and then forming the compact, closed clamp structure with the DBD around the DNA nick (Fig. 3B).

Among the human DNA ligases, DNA ligase III has the most robust intermolecular DNA joining activity (Chen et al., 2000). This activity is not only dependent upon the ZnF but also involves key residues within the DBD (Cotner-Gohara et al., 2008; Cotner-Gohara et al., 2010). It has been proposed that the NTase/OBD and ZnF/DBD modules each engage a DNA end (Cotner-Gohara et al., 2010), enabling DNA ligase III to juxtapose and ligate the DNA ends. Alternatively, it is possible that intermolecular ligation involves an interaction *in trans* between the ZnF and DBD of two DNA ligase III molecules, each bound to a DNA end.

At the C-terminus of DNA ligase III α is a breast cancer susceptibility protein (BRCA) 1-related C-terminal (BRCT) domain (Bork et al., 1997). This domain, which is about 100 amino acids long, has been found in many proteins involved in DNA repair and the DNA

damage response, and is often involved in protein:protein interactions (Bork et al., 1997). In the case of nuclear DNA ligase III α , the BRCT domain interacts with the DNA repair protein, X-ray repair cross-complementing protein 1 (XRCC1) (Fig. 2A), an interaction that is described in more detail below. As a result of the alternative splicing event, the majority of the BRCT domain is missing from DNA ligase III β , which does not interact with XRCC1. The short additional amino acid sequence at the C-terminus of DNA ligase III β acts as a NLS (Fig. 1).

Protein partners of DNA ligase III

XRCC1

XRCC1 was the first DNA ligase III-interacting protein identified (Caldecott et al., 1994). These proteins form a stable, constitutive complex in the nucleus (Caldecott et al., 1995). The human *XRCC1* gene was cloned based on its ability to complement the DNA damage sensitive phenotypes of a mutant Chinese Hamster Ovary cell line, EM9, that is sensitive to DNA alkylating agents and ionizing radiation, and has an increased frequency of spontaneous sister chromatid exchanges (Thompson et al., 1990). XRCC1 contains two BRCT domains, one of which, the C-terminal BRCT2 domain, interacts with the C-terminal BRCT of DNA ligase III α forming a heterodimer (Fig. 2A). The BRCT2 domain of XRCC1 and the BRCT domain of DNA ligase III α are also capable of forming homodimers. Recent structural studies have shown that residues adjacent to the XRCC1 BRCT2 domain also contribute to the interface with the DNA ligase III α BRCT domain, thus providing a mechanism by which formation of the DNA ligase III α :XRCC1 heterodimer is favored over formation of homodimers (Cuneo et al., 2011).

Since the steady state levels of DNA ligase III α are reduced in *xrcc1* cell lines and these levels are restored to normal levels by expression of wild type XRCC1 but not a mutant version with amino acid changes in the BRCT2 domain that disrupt the interaction with DNA ligase III α (Caldecott et al., 1995; Taylor et al., 2000a), it appears that XRCC1 is required for the stability of nuclear DNA ligase III α . Moreover, since XRCC1 has a NLS (Masson et al., 1998), it is possible that DNA ligase III α , which lacks a NLS, must interact with XRCC1 in the cytoplasm for transport into the nucleus. Recently, it has been shown that phosphorylation of XRCC1 by a cytoplasmic form of casein kinase 2 is required for nuclear accumulation of XRCC1 and the stabilization of nuclear DNA ligase III α (Parsons et al., 2010). Based on these studies, it has been suggested that XRCC1 and DNA ligase III α form a complex in the cytoplasm that is protected from ubiquitin-mediated by phosphorylation of XRCC1 and targeted to the nucleus via the NLS of XRCC1 (Fig. 2C). It is likely that the DNA ligase III α polypeptide with the N-terminal MLS will also interact with XRCC1 in the cytoplasm (Fig. 2A). We envision that the activity of the DNA ligase III α MLS predominates over that of the XRCC1 NLS, resulting in targeting of the complex to mitochondria. Then we predict that the MSL-dependent folding of DNA ligase III α as it passes through the mitochondrial membrane disrupts the interaction with XRCC1 (Fig. 2B).

Although XRCC1 has no known catalytic activity, it does interact with a large number of DNA repair proteins (Caldecott, 2003; Ellenberger and Tomkinson, 2008). This has led to the suggestion that XRCC1 is a scaffold protein that co-ordinates the activities of DNA repair enzymes, enhancing the efficiency of DNA repair. In support of this idea, XRCC1 and DNA ligase III α have been identified as subunits of different multiprotein complexes, including complexes that catalyze the repair of oxidized base lesions (Luo et al., 2004; Das et al., 2006).

PARP1

Proteins that bind to the DNA ligase III α subunit of the nuclear DNA ligase III α :XRCC1 complex have been identified (Fig. 4). For PARP1, these interactions involve both DNA ligase III α and XRCC1 (Caldecott et al., 1996; Masson et al., 1998; Leppard et al., 2003; Della-Maria et al., 2011). PARP1 is an abundant nuclear protein that has been implicated in many different DNA transactions, including the repair of single- and double-strand breaks (SSBs and DSBs) (Ame et al., 2004). During the repair of SSBs, PARP1 binds to the breaks via its N-terminal ZnFs. This activates the NAD-dependent polymerase activity of PARP1, resulting in the synthesis of ADP-ribose polymers on PARP1 itself and other chromatin proteins (Ame et al., 2004). Both XRCC1 and DNA ligase III α preferentially bind to poly (ADP-ribosylated) PARP1 *in vitro* and their recruitment to DNA damage sites *in vivo* is dependent upon poly (ADP-ribose) synthesis (Masson et al., 1998; Schreiber et al., 2002; Leppard et al., 2003; Okano et al., 2003; Okano et al., 2005). It has been suggested that the DNA ligase III α ZnF may enable DNA ligase III α :XRCC1 and associated proteins to find and engage SSBs in the presence of negatively charged poly (ADP-ribose) polymers generated by PARP1 (Leppard et al., 2003).

hMre11:hRad50:Nbs1—As with PARP-1, both DNA ligase III α and XRCC1 interact with the hMre11:hRad50:Nbs1 complex (Fig. 4) (Della-Maria et al., 2011) that plays a multifunctional role in the cellular response to DSBs, including end resection (D'Amours and Jackson, 2002; Paull and Lee, 2005; Haber, 2008). This generation of 3' single strands is a key early step in hRad51-dependent recombinational repair and is also involved in a poorly defined, minor alternative non-homologous end-joining (NHEJ) pathway. hMre11:hRad50:Nbs1 and DNA ligase III α :XRCC1 associate in a DNA damage-dependent manner in cells that are deficient in the major DNA-dependent protein kinase (DNA PK)-dependent NHEJ pathway (Della-Maria et al., 2011). Furthermore, these complexes process and join DNA ends *in vitro* generating deletions and utilizing microhomologies at the repair sites, both of which are characteristics of the alternative NHEJ pathway (Nussenzweig and Nussenzweig, 2007; Della-Maria et al., 2011). Based on these results, it appears that hMre11:hRad50:Nbs1 and DNA ligase III α :XRCC1 act together in the alternative NHEJ pathway.

Tyrosyl phosphodiesterase 1 (TDP1)—This enzyme, which removes the covalently-linked topoisomerase I (topo I) peptide from aborted topo I-DNA complexes and participates in the repair of SSBs generated by ionizing radiation (Plo et al., 2003; El-Khamisy et al., 2005; El-Khamisy et al., 2007), appears to interact exclusively with DNA ligase III α (Fig. 4) (El-Khamisy et al., 2005). Protein complexes immunoprecipitated with Tdp1 antibody, which contain DNA ligase III α , XRCC1 and PNK, are active in the repair of 3'-tyrosyl DNA substrates (El-Khamisy et al., 2005). Although these complexes are presumably involved in the repair of the nuclear genome, a fraction of Tdp1 is localized in mitochondria and participates in mitochondrial base excision repair (Das et al., 2010), raising the possibility that the interaction between Tdp1 and DNA ligase III α contributes to repair in this organelle. Notably, mutations in the *TDP1* gene are responsible for the inherited neurodegenerative disease, spinocerebellar ataxia with axonal neuropathy-1 (El-Khamisy et al., 2005). Thus, the nuclear and/or mitochondrial repair pathways involving DNA ligase III α and Tdp1 appear to play a key role in maintaining the viability of terminally differentiated neuronal cells. This is discussed in more detail in the section below.

NEIL1 and NEIL2—The NEIL DNA glycosylases, which also have AP lyase activity, remove oxidatively damaged DNA bases. These proteins also appear to interact exclusively with DNA ligase III α (Wiederhold et al., 2004; Das et al., 2006). The interaction of the C-terminal region of DNA ligase III α with either NEIL1 or NEIL2 (Fig. 4) is one in a network

of protein:protein interactions that links DNA ligase III α :XRCC1 with NEIL1 or NEIL2, polynucleotide kinase 3' phosphatase and DNA polymerase β in complexes that repair oxidative base lesions via an AP endonuclease-independent mechanism (Wiederhold et al., 2004; Das et al., 2006). As with Tdp1, NEIL1 and NEIL2 are present in mitochondria as well as nuclei (Hu et al., 2005; Mandal et al., 2012).

DNA polymerase γ —XRCC1 is absent from mammalian mitochondria suggesting that the mitochondrial version of DNA ligase III α has different protein partners in this organelle. Indeed, an interaction between DNA ligase III α and the mitochondrial DNA polymerase, Pol γ , has been identified (De and Campbell, 2007). This interaction, which involves the central catalytic region of DNA ligase III α (Fig. 4), presumably co-ordinates the activities of these enzymes during mitochondrial DNA replication and repair.

Cellular functions of DNA ligases encoded by the *LIG3* gene

Following the identification of the interaction between DNA ligase III α and XRCC1 (Caldecott et al., 1994), it was assumed that DNA ligase III α participated in base excision repair and the repair of SSBs with XRCC1 and that mutational inactivation of the *LIG3* gene would result in the same phenotype as *xrcc1* mutant cells. However, expression of a mutant version of XRCC1, which did not interact with DNA ligase III α and, as a consequence, did not increase the steady state levels of nuclear DNA ligase III α , complemented the DNA damage sensitivity of cycling *xrcc1* mutant cells (Taylor et al., 2000a). These studies demonstrated that XRCC1, which is present at higher levels than DNA ligase III α (Leppard et al., 2003), functions independently of DNA ligase III α in nuclear DNA repair. Furthermore, studies by the Campbell laboratory showed that, although XRCC1 was not detectable in mitochondria, the reduction of DNA ligase III α levels by siRNA disrupted mitochondrial function, indicating that the mitochondrial version of DNA ligase III α functions in mitochondrial DNA metabolism independently of XRCC1 (Lakshmiopathy and Campbell, 2000; Lakshmiopathy and Campbell, 2001).

DNA replication and repair of base lesions and SSBs

Recent conditional gene targeting approaches have shown that the *LIG3* gene is essential because of the mitochondrial function of DNA ligase III α (Gao et al., 2011; Simsek et al., 2011b; Arakawa et al., 2012). Furthermore, cells lacking nuclear DNA ligase III α did not exhibit sensitivity to a variety of DNA agents that *xrcc1* mutant cells are sensitive to (Gao et al., 2011; Simsek et al., 2011b). Using shRNA to knockdown expression of the other DNA ligases, it was concluded that DNA ligase I plays the predominant role in excision repair and the repair of SSBs in the nucleus (Gao et al., 2011). There are, however, conflicting reports regarding the contribution of DNA ligases I and III α to DNA repair. For example, mouse embryonic fibroblasts, either deficient in or lacking DNA ligase I, activity do not exhibit DNA damage sensitivity (Bentley et al., 1996; Bentley et al., 2002; Harrison et al., 2002) whereas human DNA ligase I-deficient fibroblasts are sensitive to DNA damage, in particular DNA alkylation (Teo et al., 1983a; Teo et al., 1983b; Barnes et al., 1992). Differences in DNA repair between mouse and human cells, the expression levels of DNA ligase I and DNA ligase III α and the degree of redundancy between DNA ligase I and DNA ligase III α in different cell types may underlie these apparently contradictory observations. In support of this idea, it has been shown recently that the relative stoichiometry of DNA ligases I and III α differed between the mouse striatum and cerebellum and that these differences correlated with BER efficiency and triplet repeat stability in these tissues (Goula et al., 2012).

Based on the compelling evidence linking DNA ligase I with DNA replication (Ellenberger and Tomkinson, 2008), it was surprising that *lig1* null mouse cells were viable (Bentley et

al., 1996; Bentley et al., 2002). Studies in chicken DT40 cells have shown that nuclear DNA ligase III α is essential for DNA replication in the absence of DNA ligase I and may even contribute to DNA replication in the presence of DNA ligase I (Arakawa et al., 2012). This appears to also be the case in mammalian cells as both DNA ligase III α and XRCC1 are required for proliferation in cells with reduced levels of DNA ligase I. In addition, there is increased association of DNA ligase III α and XRCC1 with chromatin and co-localization with replication in DNA ligase I-deficient cells (Chalony et al, 2012). At the present time, it is unclear how DNA ligase III α is targeted to replication forks. One possible mechanism is recruitment to single-strand interruptions in the lagging strand via an interaction of the DNA ligase III α :XRCC1 complex with poly (ADP-ribosylated) PARP1 (Masson et al., 1998; Schreiber et al., 2002; Leppard et al., 2003; Okano et al., 2003; Okano et al., 2005). Alternatively, the DNA ligase III α :XRCC1 complex may be recruited via an interaction between XRCC1 and PCNA (Fan et al., 2004). In base excision repair (BER), there are different subpathways involving either DNA ligase I or DNA ligase III α (Caldecott et al., 1996; Frosina et al., 1996; Cappelli et al., 1997; Levin et al., 2000). DNA ligase III α -dependent short-patch BER is thought to be a housekeeping repair pathway that acts on the entire genome whereas replication-associated BER appears to occur via DNA ligase I-dependent long-patch BER (Ellenberger and Tomkinson, 2008). Similarly, in nucleotide excision repair, there is a DNA ligase III α -dependent pathway that operates in cycling and non-cycling cells and a DNA ligase I-dependent pathway that operates in S phase cells (Moser et al., 2007). The results of recent studies are challenging this simple model, in which DNA ligase I-dependent excision repair is only active in proliferating cells. As mentioned previously, post-mitotic tissues with higher levels of DNA ligase I have increased BER activity and triplet repeat stability (Goula et al., 2012). Furthermore, DNA ligase I appears to be the predominant DNA ligase active in XRCC1-mediated DNA repair even in non-dividing cells (Gao et al., 2011; Katyal and McKinnon, 2011). It should, however, be noted that mouse embryonic fibroblasts, either lacking or having reduced DNA ligase I activity, do not exhibit increased sensitivity to DNA damaging agents (Bentley et al., 2002; Harrison et al., 2002). This suggests that DNA ligase III α may be able to effectively substitute for DNA ligase I in nuclear excision repair in certain cell types. At the present time, the identity of the DNA ligase(s) that completes DNA mismatch repair is not known.

Repair of DSBs

The majority of DSBs are repaired by the major NHEJ pathway involving DNA PK and DNA ligase IV (Ellenberger and Tomkinson, 2008). As mentioned above, there is evidence for a DNA ligase III α -dependent alternative NHEJ pathway that makes a minor contribution to DSB repair in cells with a functional DNA PK-dependent NHEJ pathway (Wang et al., 2005; Corneo et al., 2007; Yan et al., 2007; Xie et al., 2009; Simsek et al., 2011a). The alternative NHEJ pathway is predominantly responsible for chromosomal translocations (Simsek et al., 2011a) and, in accord with the DNA ligase III α ZnF being critical for intermolecular ligation *in vitro* (Taylor et al., 2000b; Cotner-Gohara et al., 2008; Cotner-Gohara et al., 2010), deletion of the DNA ligase III α ZnF significantly reduces the frequency of translocations (Simsek et al., 2011a). Interestingly, XRCC1 does not appear to be required for alternative NHEJ making this the first nuclear DNA repair pathway in which DNA ligase III α appears to function independently of XRCC1 (Boboila et al., 2012). DNA ligase III α is overexpressed in a significant fraction of cancer cell lines (Chen et al., 2008; Sallmyr et al., 2008). Notably, this overexpression is indicative of increased activity of the DNA ligase III α -dependent alternative NHEJ pathway and an increased dependence on this pathway for the repair of DSBs in these cells (Sallmyr et al., 2008; Tobin et al., 2012a; Tobin et al., 2012b). Cells with this DNA repair abnormality, which has been detected in samples from patients with therapy-resistant forms of chronic myeloid leukemia and breast cancer, can be selectively targeted by inhibiting both DNA ligase III α and PARP1 (Tobin et

al., 2012a; Tobin et al., 2012b). These preclinical studies suggest that DNA ligase III α is a potential therapeutic target, in particular in cancers that have failed frontline therapy. There are, however, concerns about the potential toxic effects of DNA ligase III inhibitors on normal tissues and cells because of the essential role of this enzyme in mitochondria. At the present time, the identity of the DNA ligase(s) that completes recombinational repair of DSBs is not known.

Role of the *LIG3* gene in neuronal cells

As mentioned above, defects in the DNA ligase III α -interacting protein TDP1 have been identified as the cause of the hereditary neurodegenerative disease, spinocerebellar ataxia with axonal neuropathy 1 (El-Khamisy et al., 2005). In addition, defects in two other DNA repair proteins, aprataxin and polynucleotide kinase phosphatase, that interact with the DNA ligase III α :XRCC1 complex have been identified as the causes of ataxia-oculomotor apraxia 1 (Moreira et al., 2001; Ahel et al., 2006) and a recently discovered disease characterized by microcephaly, early onset-intractable seizures and developmental delay (Shen et al.), respectively. TDP1, aprataxin and PNKP are all involved in the cleaning up of termini at SSBs both in nuclei and mitochondria (Rass et al., 2007; Das et al., 2010; Sykora et al., 2011; Mandal et al., 2012; Tahbaz et al., 2012). Aprataxin removes adenylate groups from 5' phosphate DNA termini, generated by abortive ligation (Ahel et al., 2006) whereas PNKP phosphorylates 5' hydroxyl termini and dephosphorylates 3'-phosphate termini (Karimi-Busheri and Weinfeld, 1997; Karimi-Busheri et al., 1998). These studies suggest that cells of the nervous system are more sensitive to the loss of proteins involved in the repair of single strand breaks than other cell types. This may be because terminally differentiated neuronal cells lack some of the DNA repair mechanisms that are active in proliferating cells and/or because of the high levels of active oxidative metabolism in the CNS that generate excessive DNA damage (Barzilai, 2007; Chen et al., 2007).

Interestingly, neural-specific inactivation of the *XRCC1* and *LIG3* genes have different effects on the developing nervous system (Lee et al., 2009; Katyal and McKinnon, 2011). Neuronal cells lacking *XRCC1* are hypersensitive to DNA damaging agents that generate SSBs as are neuronal cells lacking either Tdp1 or aprataxin. However, the neuropathology resulting from the loss of *XRCC1* function is more severe than that caused by loss of either Tdp1 or aprataxin. This presumably reflects the central role of *XRCC1* in the repair of all nuclear SSBs whereas Tdp1 and aprataxin are only required for specific subsets of SSBs. These studies are consistent with the conclusion that DNA ligase I is the predominant activity in the *XRCC1*-dependent repair of SSBs in nuclear DNA (Gao et al., 2011). While neural inactivation of *XRCC1* resulted in a seizure-like phenotype after about 3 months, neural inactivation of *LIG3* had a more severe effect, with ataxia evident after two weeks and death within three weeks. The neuropathology induced by loss of DNA ligase III α function was markedly different than that induced by loss of *XRCC1* with the neuronal cells lacking DNA ligase III α exhibiting mitochondrial defects. Thus, it appears that *XRCC1*-dependent repair of nuclear SSBs plays a critical neuroprotective role whereas neuropathologies associated with loss of DNA ligase III α function are due to mitochondrial dysfunction (Katyal and McKinnon, 2011). While the contribution of DNA ligase III α to nuclear DNA metabolism may vary depending on cell-type and growth status, it plays an essential and unique role in mitochondrial DNA metabolism. It is possible that the neuropathology resulting from defects in Tdp1, aprataxin or PNKP may be due, at least in part, to reduced DNA ligase III α -dependent repair of mitochondrial DNA.

Concluding Remarks

The mammalian *LIG3* gene encodes distinct DNA ligase polypeptides that participate in nuclear and mitochondrial DNA metabolism. Recent studies have shown that the *LIG3* gene is essential for cell viability because mitochondrial DNA ligase III α is required for mitochondrial function. There is a significant functional redundancy between DNA ligases I and III α in nuclear DNA replication and repair. Further studies are needed to characterize the role of DNA ligase III α in DNA replication in cells that are deficient in DNA ligase I activity. DNA ligase III α is the predominant activity involved in generating chromosomal translocation via its participation in an alternative NHEJ pathway. Notably, DNA ligase III is frequently overexpressed in a significant fraction of cancer cells, resulting in increased activity of the alternative NHEJ pathway. Initial studies with DNA ligase III inhibitors indicate that cancer cells with this DNA repair abnormality can be selectively targeted. Further studies are needed to elucidate the role of DNA ligase III β , which is generated by an alternative splicing mechanism detected in male germs cells, in meiotic recombination and/or germ cell development.

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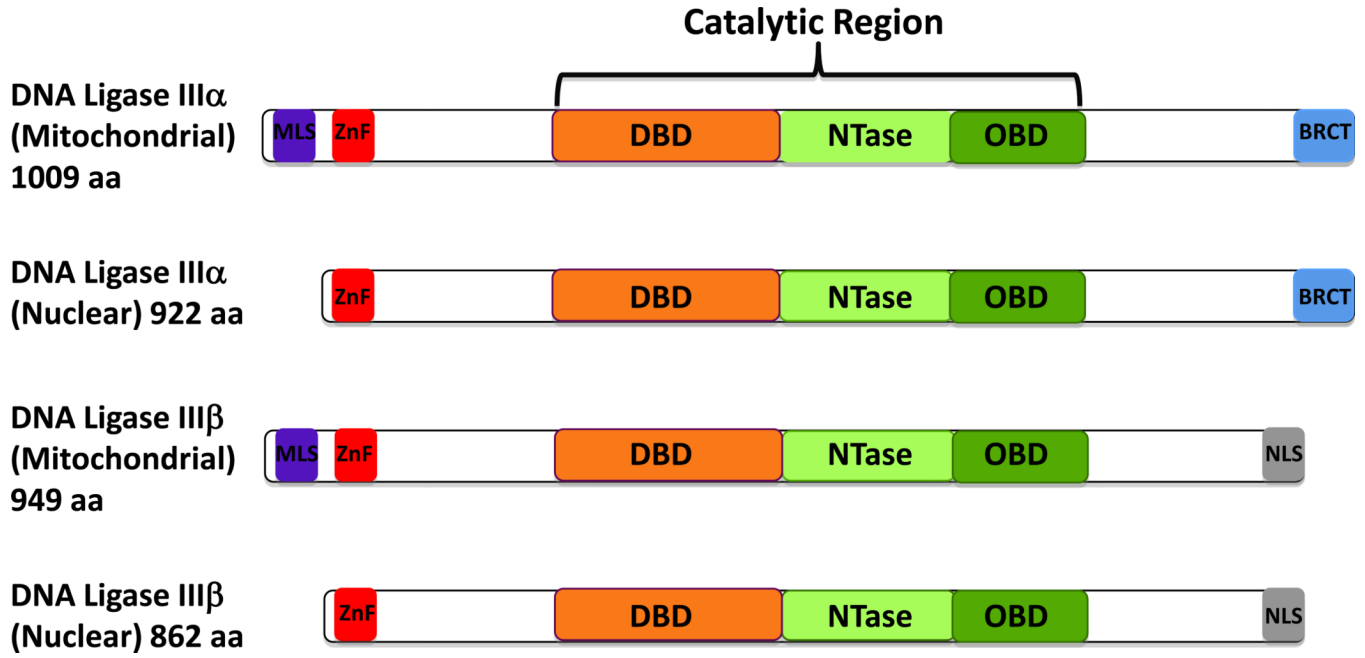
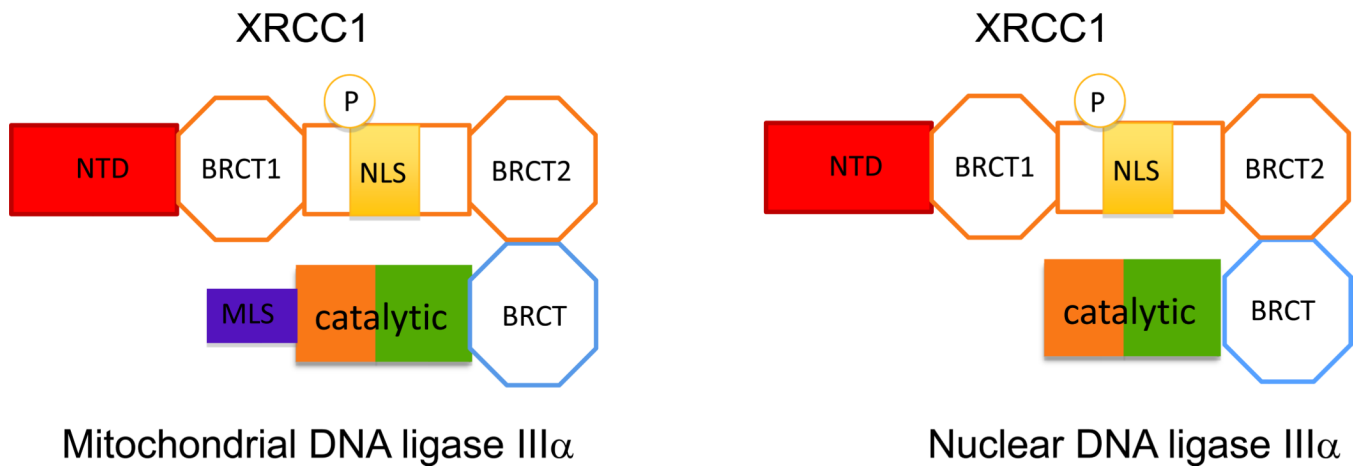
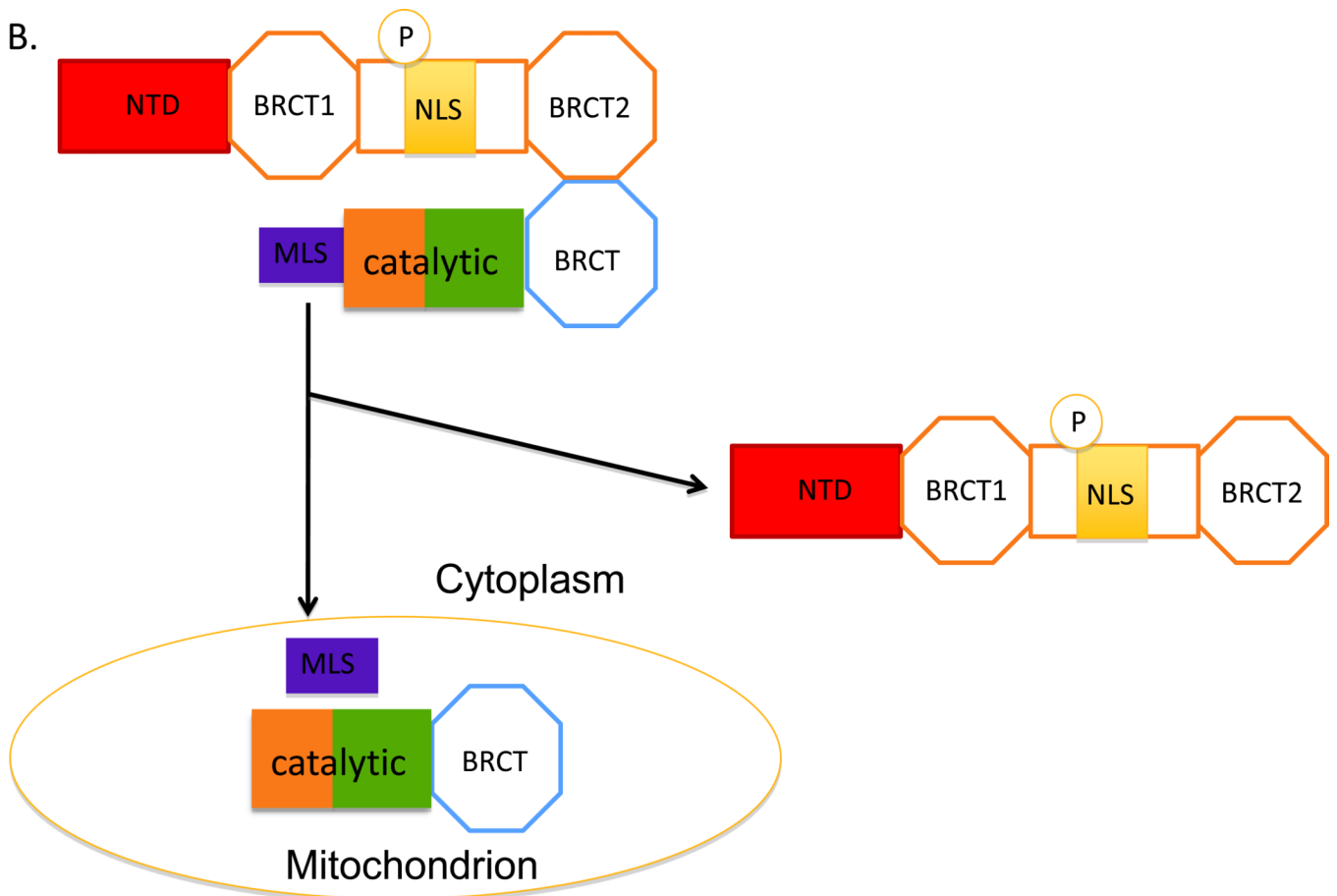


Figure 1. DNA ligases encoded by the *LIG3* gene
 The four DNA ligase polypeptides encoded by the mammalian *LIG3* gene are shown schematically. The positions of the zinc finger (ZnF, red) and the catalytic region, which contains the DNA binding domain (DBD, light brown), nucleotidyl transferase domain (NTase, light green) and oligonucleotide/oligosaccharide-fold binding domain (OBD, dark green), are indicated. Mitochondrial and nuclear versions of DNA ligase III α are generated by alternative translation initiation. The position of the mitochondrial leader sequence (MLS, purple) is indicated. An alternative splicing event that occurs in male germ cells replaces the C-terminal BRCT domain (BRCT, blue) of DNA ligase III α with short amino acid sequence that functions as a NLS (NLS, grey) in DNA ligase III β .

A.



B.



C.

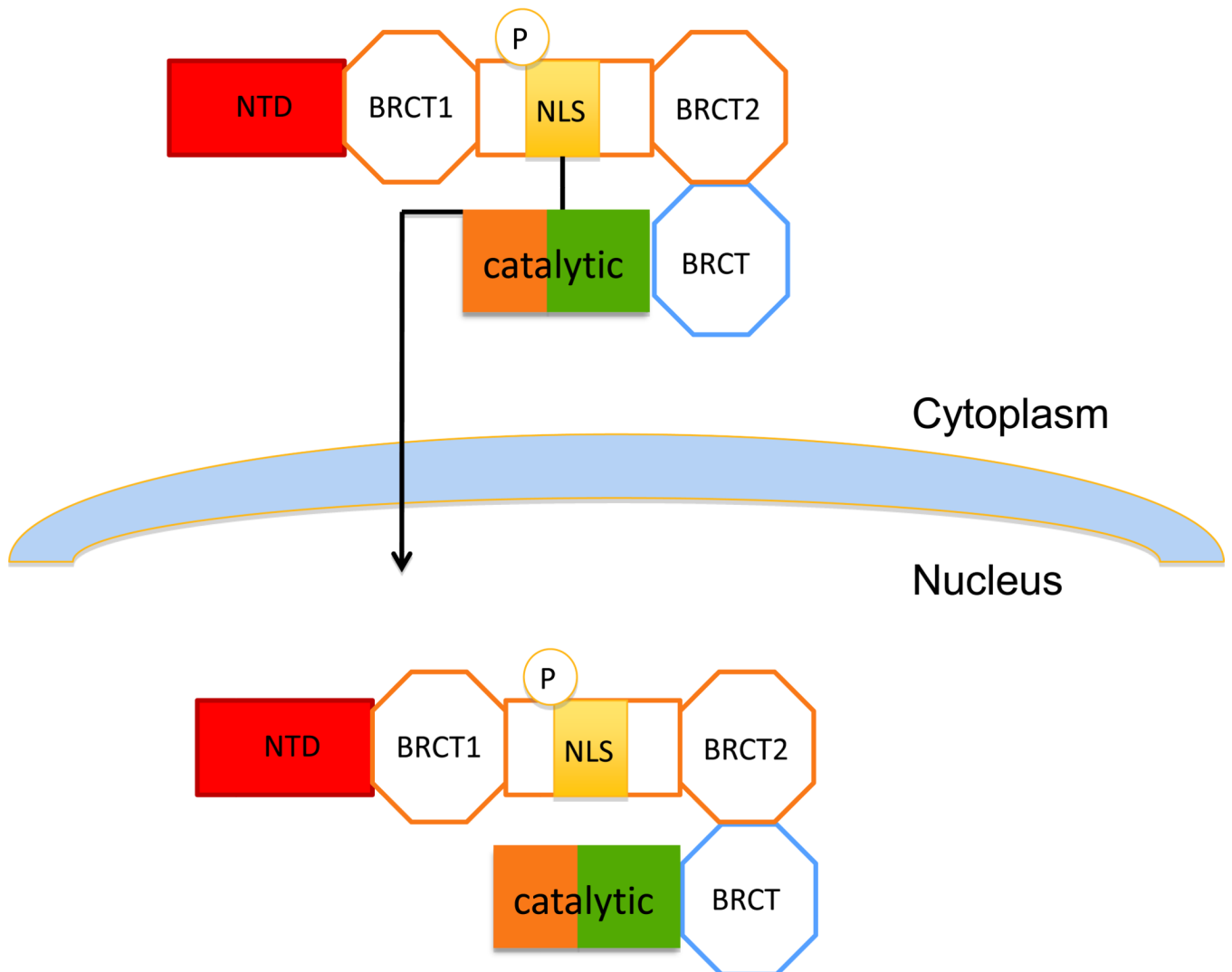
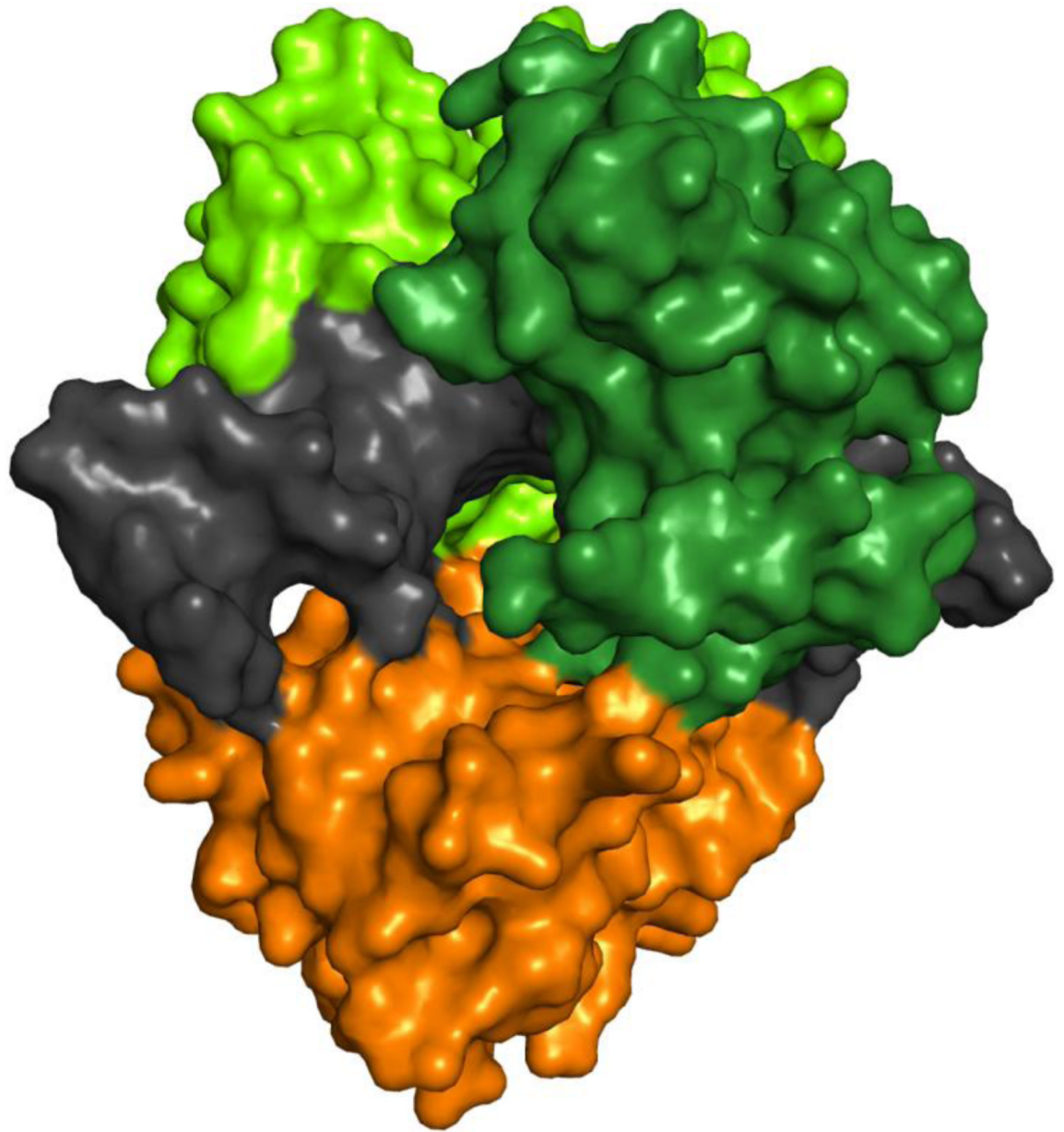


Figure 2. Mitochondrial and nuclear versions of DNA ligase III α .

A. Two polypeptides are generated from DNA ligase III α mRNA by alternative translation. Mitochondrial DNA ligase III α has an additional N-terminal mitochondrial leader sequence (MLS, purple) in addition to the common catalytic region (brown/green) and C-terminal BRCT domain (BRCT). Since this domain mediates the interaction with the C-terminal BRCT2 domain of XRCC1, it is assumed that both the mitochondrial and nuclear versions of DNA ligase III α will form complexes with XRCC1, which also contains an N-terminal domain (NTD, red), a second BRCT domain (BRCT1) and a nuclear localization domain (NLS, yellow) whose activity appears to be dependent upon phosphorylation (P) of adjacent residues. B. The complex containing mitochondrial DNA ligase III α is targeted to the mitochondria by the MLS. It is assumed that the activity of the MLS is much greater than the activity of XRCC1 NLS. The MLS initiates passage of the DNA ligase III α polypeptide through the mitochondrial membrane and is then removed by proteolysis. Unfolding of the DNA ligase III α polypeptide as it passes through the mitochondrial membrane disrupts the

interaction with XRCC1, leaving free XRCC1 in the cytoplasm. C. The transport of DNA ligase III α lacking the MLS into the nucleus is mediated by the NLS of XRCC1 which binds to the nuclear pore complex, resulting in passage of the XRCC1 into the nucleus.

A



B

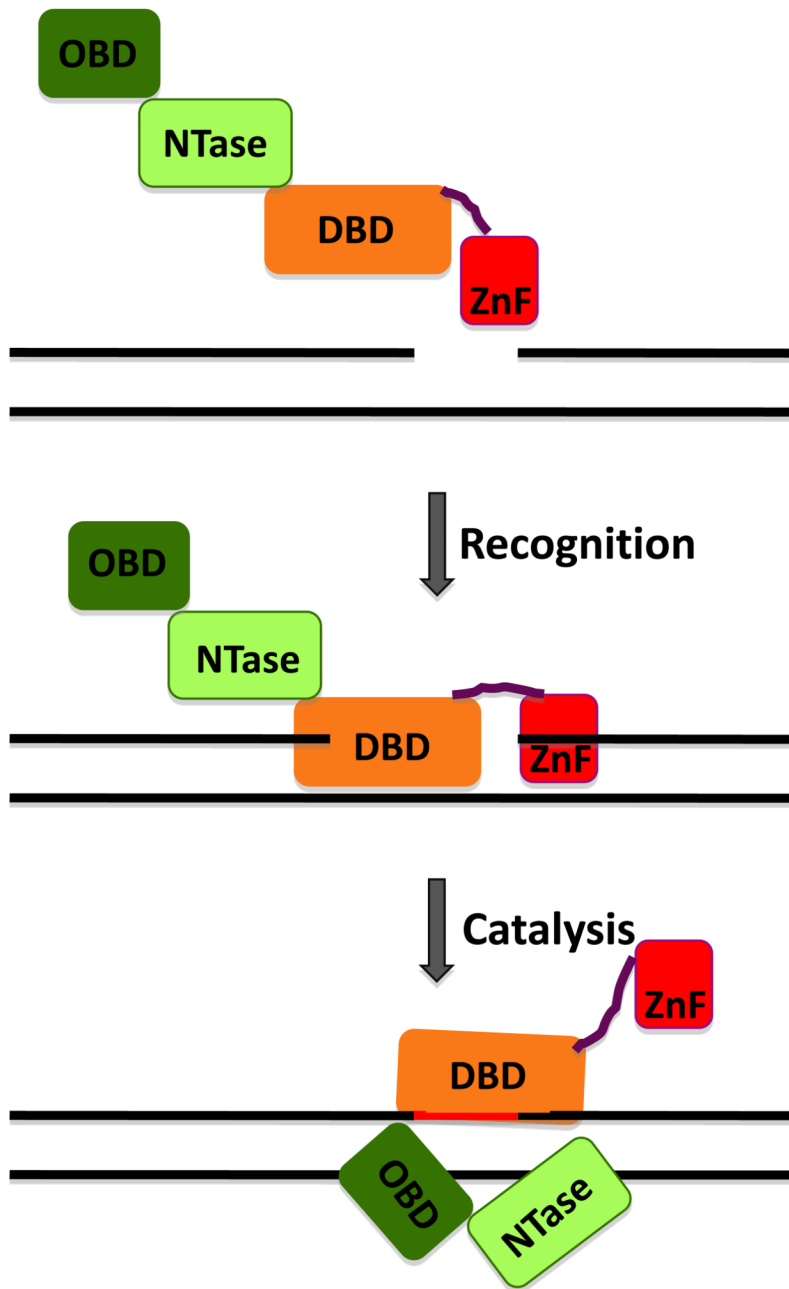


Figure 3. Interaction of DNA ligase III with nicked DNA

A. Space filling model showing the DNA binding (DBD, light brown), nucleotidyl transferase (NTase, light green) and oligonucleotide/oligosaccharide-fold binding domains (OBD, dark green) of DNA ligase III engaging a short oligonucleotide (Black) containing a non-ligatable nick (Cotner-Gohara et al., 2010). The image was made using PyMol (<http://www.pymol.org>). B. A fragment of DNA ligase III encompassing the zinc finger (ZnF, red) and the catalytic region (DNA binding domain, DBD; nucleotidyl transferase domain, NTase; oligonucleotide/oligosaccharide-fold binding domain, OBD) and a nicked DNA duplex are shown schematically (upper panel). The single strand interruption is initially recognized by a combination of the ZnF and the DBD (middle panel). If the nick is ligatable,

the ZnF is displaced by a combination of the NTase and OBD. The DBD, NTase and OBD encircle and ligate the nicked DNA.

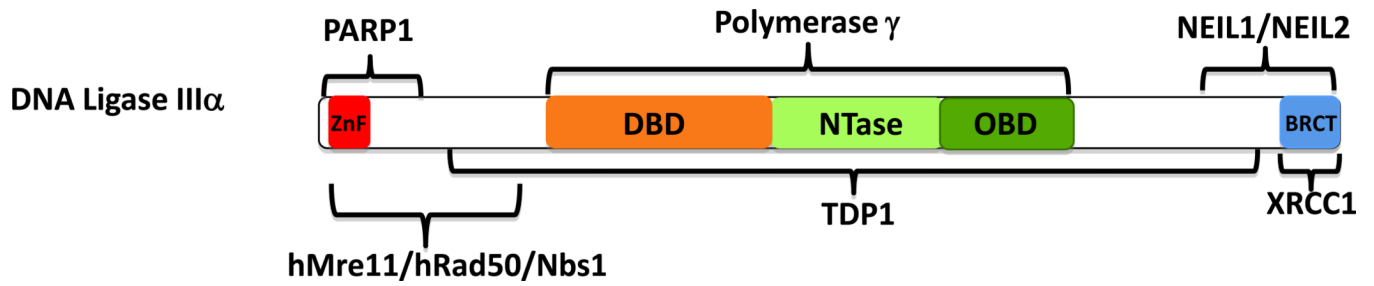


Figure 4. Protein partners of DNA ligase III α

The regions of DNA ligase III α involved in interactions with hMre11/hRad50/Nbs1, NEIL1 and NEIL2, PARP1, TDP1, XRCC1 and mitochondrial DNA polymerase γ are indicated.