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NHEJ Often Uses Microhomology: Implications for Alternative End Joining

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Abstract

Artemis and PALF (also called APLF) appear to be among the primary nucleases involved in NHEJ and responsible for most nucleolytic end processing in NHEJ. About 60% of NHEJ events show an alignment of the DNA ends that uses 1 or 2 bp of microhomology (MH) between the two DNA termini. Thus, MH is a common feature of NHEJ. For most naturally-occurring human chromosomal deletions (e.g., after oxidative damage or radiation) and translocations, such as those seen in human neoplasms and as well as inherited chromosomal structural variations, MH usage occurs at a frequency that is typical of NHEJ, and does not suggest major involvement of alternative pathways that require more extensive MH. Though we mainly focus on human NHEJ at DSBs, comparison on these points to other eukaryotes, primarily S. cerevisiae, is informative.

Keywords

Double-strand break repair; lymphoma; chromosomal rearrangements; V(D)J recombination; class switch recombination

Introductory Comments

The majority of pathological and physiological DNA double strand breaks (DSBs) are repaired by nonhomologous end joining (NHEJ) in vertebrates. Following creation of a DSB, a heterodimer of Ku70 and Ku80 (Ku) binds to each broken end allowing recruitment of the relevant nucleases, polymerases, and ligases required to repair the disparate array of end configurations present at a broken end (Fig. 1). The diversity of the components that Ku is able to recruit to DNA ends reflects the mechanistic flexibility required to repair a variety of different lesions (Fig. 2). Importantly, repair by NHEJ is an iterative process with Ku able to recruit factors in any order to each broken end independently. An overview of the NHEJ process has been described in detail elsewhere [1, 2].

Extensive genome-wide sequencing of human cells and tumors has identified many chromosomal deletions in normal and neoplastic cells, as well as translocations in tumor

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cells. Because of this, usage of the term NHEJ has become common in such studies. Understandably, some of the issues of nucleotide loss and terminal microhomology (MH) usage are confusing to genome-wide sequencing scientists who are just now beginning to read and contribute to the DNA double strand break repair (DSBR) field. Within the NHEJ field, we know that over half of NHEJ events in vertebrates have MH. Here we try to clarify our current understanding of nucleotide loss and MH usage in human NHEJ. In order to provide some background to the human work, we also provide a comparison to other eukaryotes, with particular emphasis to yeast (*S. cerevisiae*).

Also, we comment on the debate as to whether there exist two modes of NHEJ, a classical pathway (c-NHEJ) that relies on canonical end-joining factors and an alternative pathway (alt-NHEJ) that functions when a cell is deficient in c-NHEJ components. We, however, favor a substitution model where, if a particular NHEJ factor is missing, another factor can substitute for it, although in a less efficient manner and perhaps relying on more MH to stabilize and annealed intermediate prior to ligation. Under this model there are no separate c-NHEJ or alt-NHEJ pathways, but some proteins of DNA repair, such as the ligases, may have some degree of overlapping function. This ensures redundancy so that a potentially fatal DSB has more than one avenue of repair.

Nucleases of Vertebrate NHEJ

Nucleotide loss occurs at most DSBs in eukaryotic cells, even those events that resolve via homologous recombination (HR). Most DSBs are repaired by NHEJ in metazoan somatic cells. In vertebrate somatic cells, the nucleotide loss that occurs during NHEJ is suspected to be due to the nucleases Artemis and PALF (more commonly called APLF)(Fig. 2, Table 1) [3–11]. In invertebrates and yeast, other nucleases are suspected to play the major role (see below).

Artemis is necessary to repair a subset of DSBs that are caused by ionizing radiation, based on survival assays and γ-H2AX staining [12–15]. For the programmed DSBs of V(D)J recombination, Artemis is required to endonucleolytically open the DNA hairpins at the V, D or J coding ends, and this establishes the location of Artemis at this point of the V(D)J recombination process [16]. Biochemically, we know that Artemis is capable of trimming both 5′ and 3′ overhangs [16–18]. Because Artemis is located at coding ends during V(D)J recombination, we have assumed that Artemis is also well positioned to carry out end trimming functions. It is difficult to prove all facets of this during V(D)J recombination because it is not possible to open the coding ends but then remove Artemis from any possible overhang processing steps. Artemis and DNA-PKcs form a tight complex when they are harvested from human cells [16]. Given the importance of DNA-PKcs for NHEJ, the inferred proximity of Artemis, as part of an Artemis:DNA-PKcs complex, at DNA ends during NHEJ continues to make it a likely cause of much of the nucleolytic end processing.

PALF is a nuclease [10, 11] and several other very nice studies demonstrate that it binds to XRCC4 via the FHA domain in PALF [11, 19–23]. In addition to its role in ligase complex assembly (see Caldecott review) [24] and other potential roles [25] cellular studies using a knockdown of PALF [11] support a role for PALF in nucleolytic end processing. PALF has

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3′ exonuclease activity as well as endonucleolytic activity on single-stranded DNA or on 5′ or 3′ extensions from a duplex DNA end, and PALF is not stimulated by DNA-PKcs [11].

It is possible that there is a division of labor in the endonucleolytic processing by Artemis and PALF during individual NHEJ events. Artemis may be responsible for nucleolytic processing early in the NHEJ process when DNA-PKcs and Artemis are bound at the DSB ends. Later during the processing of the same DNA ends, when the XLF:XRCC4:DNA ligase IV (L4) complex is bound to the ends, the association between PALF and XRCC4 might permit PALF to contribute to end nucleolytic processing. This scheme would be consistent with the stabilization of the XLF:XRCC4:L4 complex by PALF at DNA ends [25].

There are multiple ways for NHEJ to join two DNA ends, and many pairs of ends can be joined without any nucleolytic resection [1]. Therefore, the quantitative decrease in NHEJ may be small when either Artemis or PALF is absent, even though there may be a change in the amount of resection [9, 12–15].

End Processing Components of NHEJ in *Saccharomyces cerevisiae*

The core components of NHEJ [Ku (Yku), XLF (Nej11), XRCC4 (Lif1), and ligase 4 (Dnl4) (Table 1)] are conserved in the eukaryote *S. cerevisiae* (hereafter referred to generally as ‘yeast’). This has allowed us to understand many of the basic principles of NHEJ. The process is largely similar to vertebrates where a heterodimer of Yku70 and Yku80 (Yku) binds to broken DNA ends. Next, Dnl4 and Lif1, together with the haploid-specific Nej1 regulator, are recruited to seal the break [26, 27]. The Mre11:Rad50:Xrs2 (MRX) complex is also recruited to broken ends. The likely function of MRX is to further stabilize and, perhaps, tether the broken ends and to recruit other factors to the break site that act in end processing and checkpoint response [28]. While yeast lacks DNA-PKcs, it would seem that MRX is able to fill many of its roles. Also, the Xrs2 component has an FHA domain that has been shown to interact with Lif1 implicating MRX in Dnl4 recruitment [29]. The binding of Yku and MRX is not mutually exclusive and, most likely, both complexes are present at broken ends. For breaks with compatible ends, such as those generated by restriction endonucleases, these components are sufficient for repair, as the small amount of homology makes the annealed intermediate stable enough for ligation [30, 31].

Contrary to experimental systems, most naturally-arising breaks, such as those from ionizing radiation or oxidative damage will not have compatible ends. The presence of mismatched bases or adducts require, as in humans, end processing of broken DNA ends prior to ligation. Interestingly, yeast lack many of the nucleases involved in NHEJ in humans including Artemis and PALF. One reason for this is that specialized processes, such as V(D)J recombination, evolved later and yeast did not require an enzyme like Artemis specifically for hairpin opening (though the Snm1 nuclease in yeast shares some homology with Artemis, its role appears to be in interstrand crosslink repair [32]). Also, the end-processing factors utilized in yeast are not necessarily NHEJ-specific and have overlap with proteins involved in HR (Table 1).

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In yeast, the MRX complex has the unique role of being integral in both the HR and NHEJ modes of repair and may serve to guide repair towards one or the other. One way it may accomplish this is by controlling the extent of 5′ to 3′ resection that occurs to create 3′ single-stranded DNA (ssDNA) tails. These ssDNA tails are substrates for HR proteins to bind and carryout a homology search for a suitable repair template. Therefore, formation of these tails is a key commitment step to HR and limiting their formation is necessary for repair by NHEJ. Indeed, it has been demonstrated that when a cell is in G1 and a sister chromatid is not available for a repair template, a situation where repair by NHEJ would be preferable, extensive resection does not occur [33, 34].

Recent work has demonstrated that, while the MRX complex possesses endonuclease activity and a 3′ to 5′ exonuclease activity, it is not the nuclease that generates the long 3′ ssDNA tails, but is necessary for the initiation of resection, removing approximately 100 nucleotide (nt) increments of the 5′ strand either in conjunction or concurrently with the Sae2 endonuclease (homolog of human CtIP)[35, 36]. There is also evidence that MRX and Sae2 work together to remove hairpin structures from broken ends that would otherwise prevent repair [37]. (In contrast to the hairpin opening by Artemis in vertebrates, which opens 2 nt 3′ of the hairpin end, the hairpin resolution by MRX and Sae2 is substantially more internal from the hairpin tip, often 20 to 100 bp.) This activity is important for removing degenerate DNA or protein adducts from broken DNA ends and would also reveal more MH that could serve to thermodynamically stabilize an annealed intermediate prior to ligation.

Additionally, stabilization prior to ligation can also be enhanced by removal of terminal mismatches and gap-filling at the broken ends by DNA polymerase IV (Pol4) [38]. Recent work has also demonstrated contribution by Pol3 [39], the catalytic subunit of polymerase delta.

Microhomology Usage at Human Chromosomal Deletion Junctions and Translocation Sites

In human lymphoid cells, most DNA ends have nucleotide loss for lengths of 1 to 15 bp [40]. The average loss is ~5 bp, but this varies substantially depending on the sequence at the DNA end [40]. MH usage refers to the propensity for the two DNA ends undergoing joining to align themselves at short (typically, 1 to 3 nt) regions of homology between the two ends (e.g., a ‘right’ DNA end terminating with CC might align and join with a ‘left’ end with CC at the terminus, such that only one of the two sets of CC remains in the final junction). In V(D)J recombination in human cells, when TdT is not present, 1 or 2 nts of MH is used at nearly 60% of coding joins [40]. This illustrates that MH usage is an intrinsic part of NHEJ. When TdT is present during V(D)J recombination, MH usage drops markedly, presumably because TdT additions to either or both of the two DNA ends will suppress any annealing by MH that is intrinsic to the ends [40]. Moreover, the TdT additions themselves can generate MH that assists in a more rapid ligation of the ends. We have referred to this latter possibility as polymerase-generated MH [1], and such additions are not possible to identify based on simple inspection of the junctional sequences. A lower level of

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such polymerase-generated MH may occur due to polymerase mu, and perhaps even polymerase lambda [16–18].

Similar to V(D)J recombination, it appears that at least 90% of immunoglobulin class switch recombination joining occurs by NHEJ, based on the relative rate of class switching in wild-type versus NHEJ mutant cells [41]. When NHEJ components are missing, a slower process that is more reliant on MH joins these ends. However, it is not clear that this is a separate pathway or rather NHEJ that substitutes other proteins (e.g., DNA ligase I or III in place of IV) when one or more NHEJ components are missing [42]. Likewise, some types of DNA end configurations do not seem to require all NHEJ components. For example, DNA-PKcs is not required for V(D)J signal joints [43, 44] or in some other cases [45])(Fig. 3).

Importantly, human chromosomal deletions and translocations do not appear to rely on MH usage at the junctions in major way [46–48]. In human lymphoid malignancies, many chromosomal breaks arise at a time when TdT is present, and this naturally suppresses MH usage. However, even junctions formed without any apparent contribution by TdT appear to have relatively little MH usage. When the MH usage is present, it is usually limited to 1 or 2 bp. This feature appears to extend to chromosomal deletions and translocations in non-lymphoid cells and even in normal (non-neoplastic) cells, where MH usage is also relatively infrequent and limited in length [49].

Some studies of human chromosomal structural variation may have incorrectly understood the distinction between NHEJ and alternative end joining [50]. It is easy to understand how such confusion could arise, given that the name, MMEJ, might be interpreted to lay claim to all events that have even 1 bp of MH. The potential for confusion is made greater if someone new to this area interprets the letters, NH, in the NHEJ acronym to mean the absence of even terminal MH (rather than the actual intention to distinguish NHEJ from homologous recombination). As stated above, most NHEJ events use some short length (usually 1 or 2 bp) of MH [1].

Implications for Alternative End Joining

An aspect of NHEJ that is often debated is whether the end-joining events that occur in cells defective for a key NHEJ component occur via a separate alternative pathway (alt-NHEJ) (sometimes referred to as alt-EJ, micro-single-strand annealing (micro-SSA), or microhomology-mediated end joining (MMEJ)), distinguished from so-called classical NHEJ (c-NHEJ) that relies on canonical factors to join broken ends efficiently (Fig 4). Over the years, several labs have interpreted their results to mean that there is a distinct alt-NHEJ pathway (reviewed most recently in [51]). Characteristics of alt-NHEJ include events that occur independently of Ku and/or DNA ligase IV, extensive use of MH, and a propensity for repair that results in genome rearrangements. Is this evidence enough, however, to conclude that a separate, bona fide end-joining pathway exists and that the joins are not merely result of NHEJ using other available enzymes that complete joining less efficiently and with more errors?

Some work has raised the possibility that alt-NHEJ might be responsible for a disproportionate amount of end joining at human chromosomal DSBs, particularly

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chromosomal translocations, with DNA ligase III being particularly important for proposed alt-NHEJ events [52, 53]. However, a more recent study finds no effect of a ligase III knockout on Ig class switch recombination or the Ig-myc chromosomal translocation [54]. Moreover, in humans, sequences of chromosomal events (translocations and inherited chromosomal variations) indicate that NHEJ is the likely pathway by which the majority of these DSBs are repaired, though a subset of joining events are difficult to assign with certainty to a specific joining pathway. As we have already discussed, it does not appear that an alt-NHEJ pathway acts at the physiologically relevant V(D)J and class switch recombination processes as there is little reliance on long MH (> 4bp), an apparent requirement of alt-NHEJ. Also, repair that results in deletions or translocations does not seem to rely on MH usage either. Therefore, the repair appears to occur by c-NHEJ only.

Since DNA DSBs are repaired in a much less specialized manner by NHEJ in yeast compared to the breaks generated by V(D)J recombination, can we rule out an alt-NHEJ pathway here as well? Use of 1–3 bp of homology to align broken ends is commonly observed in yeast, though use of greater than 5 bp also occurs [55, 56]. That greater homology use occurs in the absence of Yku is not necessarily surprising as greater stabilization of the broken ends would be required [57]. Therefore, what many call alt-NHEJ may be engaged when a thermodynamically suitable annealing template is unable to form for a sufficiently long time interval to permit Pol4 and Nej1:Lif1:Dnl4 to further stabilize and seal the break. The MRX complex, likely in conjunction with Sae2, is present to clip off approximately 100 nucleotides to provide more substrate to locate a suitable annealing template with more choices of microhomology and of greater length. If these events occur in G1, where resection mechanisms appear to be limited, and Yku is still able to bind stably to the broken ends, Pol4 and Nej1:Lif1:Dnl4 have another chance to ligate the top and/or bottom strand of the DSB before more extensive resection mechanisms drive the process towards homology-directed repair.

It is possible that without Yku bound to the ends, the kinetics of the MRX complex clipping off ends is changed and therefore occurs more readily. This may account for the larger deletions observed at break junctions that have been attributed to a separate pathway. Furthermore, without Ku, Lif1-Dnl4 recruitment may be impaired, which may also account for a lesser dependence on Dnl4 with DNA ligase I, Cdc9, likely having a greater role in ligating the break, as it does in SSA. However, some effect of Dnl4 in alt-NHEJ is not surprising as the MRX complex can interact with Lif1 through Xrs2. This perspective, then, suggests a substitution model over alt-NHEJ operating in yeast as well.

One of the suggested criteria for alt-NHEJ is that it can act independently of L4. Indeed, it has been demonstrated that residual end-joining can occur even in the complete absence of L4 in both yeast and mammalian cells [58–61]. However, as ligation is such a specialized process, it is not surprising that DNA ligase I (and likely DNA ligase III in mammals) would be involved and that the joining, especially at incompatible ends with limited MH, would be less efficient.

In reality, the phenomenon of alt-NHEJ may only be the effect of a less efficient NHEJ pathway adapted to the loss of a major repair protein. If we examine Table 1, where the

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main repair proteins of NHEJ are divided between the three major phases of NHEJ, (1) break recognition and recruitment, (2) end processing, and (3) ligation, we see that the number of proteins available for step (1) and (3) is limited. For example, there are only a finite number of DNA ligases in the cell; therefore, if ligation occurs in the absence of ligase IV, ligase I or ligase III must be utilized. For step (2), however, there are a number of proteins that can potentially act in end processing when one or both of the DNA ends is too complex to allow simple religation or to compensate for the deficiency of another protein. The more complex the break, the more enzymes needed to repair it (Fig. 3).

Furthermore, NHEJ is an iterative process, with each end of a DSB being acted on independently, likely by different proteins and in a different order depending on the physical configuration of each end [2, 62]. Given that the result of repair is similar (e.g., repair with small to limited MH), we feel the evidence is consistent with a single NHEJ pathway with multiple enzymes at its disposal as opposed to several different alt-NHEJ pathways being available. If one of the major NHEJ proteins is deficient, the alternative components could compensate if the cell is to survive, but this comes at a cost of lower repair efficiency and a greater likelihood of genome rearrangements.

Caveats of DNA Repair Studies to Consider

Three key elements of studying the genetics and molecular biology of DSB repair in vivo are to (1) create one or more DSBs, (2) remove one or more repair genes and (3) have a reliable assay to measure the effect. The available parameters for each of these elements are vast. Here, we briefly discuss some of the caveats to consider.

Using gene knockouts to study DNA repair pathways, while providing a great deal of knowledge, can be problematic. A major issue is that many of the genes involved in repair, *MRE11* for example, are pleiotropic (i.e., multiple roles), making it difficult to apply a specific phenotype to the deleted gene. Furthermore, it has been shown recently that particular knockouts generated for experimental use acquire predictable secondary mutations, demonstrating an ability to adapt to deficiencies [63]. It is clear that when a particular pathway is deficient for a key protein that other DNA repair enzymes can substitute for that protein. Therefore, referring to this phenomenon as a separate pathway may not be accurate.

Further dissection of DNA repair pathways would require the use of missense mutants to examine the effect of a particular protein being present, yet defective for a specific function. For example, recent work in yeast has shown that when the catalytic domain of Dnl4 is mutated, but the protein is still present to bind and recruit other factors, that DNA ligase I takes on a greater role in ligating broken ends, albeit in a less efficient manner [64]. Such work supports the model that, rather than an alternative pathway being engaged, a protein with capable function substitutes for the deficiency.

Another choice faced when studying DSBR is whether to examine spontaneously occurring or induced DSBs. If it is the latter, then how are the breaks to be induced? Use of restriction endonucleases or meganucleases (e.g. I-SceI or HO) have the advantage of being able to generate a DSB in a specific location in a large number of cells. However, the ends lack the

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complexity that would be expected for physiological DSBs created by chemicals, radiation, or free radicals. Also, the ends often have 2–6 nt of shared homology at the very ends of the break, allowing for more efficient annealing and ligation.

In contrast, ionizing radiation (IR) generates DSBs that may be more physiological in nature. However, these breaks can occur anywhere in the genome. Also, DSBs are only a subset of the damage that occurs when cells are exposed to IR and the diversity of those DSBs can be quite complex, requiring many different enzymes to process the ends prior to ligation. Available evidence indicates that the breaks induced by site-specific nucleases and those generated by IR have different molecular requirements for repair [62]. Thus, the method chosen to induce breaks can greatly influence repair outcome.

The parameters of the assay can also make the results of an experiment difficult to interpret. Very nice work was recently done demonstrating that if a mammalian cell line is first irradiated and then transfected with a linearized plasmid, greater MH is used in repairing the plasmid than if the cells were not irradiated prior to transfection [65]. Similar results were observed in yeast. One interpretation of this would be that irradiated cells preferentially repair breaks, even those not induced by radiation, by an alt-NHEJ pathway. However, another explanation would be that the DSBs created by radiation, and the large number of free DNA ends introduced upon transfection of the linearized plasmid by cationic lipids (e.g., Lipofectamine), titrates away NHEJ factors such as Ku. Lower levels of Ku would require other factors to substitute and this may lead to, as discussed above, increased use of MH to stabilize an annealed intermediate prior to ligation. Distinguishing between these possibilities would be difficult.

Finally, for brevity, the contributions of chromatin and signaling have not been discussed in this review, but it appears signaling plays a role in end processing as well. Studies in yeast, for example, suggest that Rad9 (53BP1) can phosphorylate Rad53 (CHK2) in a Mec1 (ATR) -dependent manner, leading to degradation of Exo1, thus inhibiting resection [66, 67]. Work with mammalian systems has also demonstrated that 53BP1 may inhibit resection, thus favoring NHEJ [68]. Interestingly, Mec1 can also stimulate Sae2 (CtIP) activity, suggesting a checkpoint-controlled balance of end processing [69].

Final Comments

Inspection of junctional nucleotide loss and MH usage in human neoplastic and non-neoplastic chromosomal structural changes is informative for guiding thinking about the contribution of NHEJ in shaping the genome. NHEJ often uses terminal MH, and more extensive MH usage is seen in circumstances where NHEJ components are missing (experimental systems and extremely rare natural mutations).

Undoubtedly, the debate of whether there is a formal alternative end joining pathway versus mere substitution of ‘second string’ components for the ‘first string’ ones will continue. We favor the latter, but we acknowledge that this may be a minority opinion within the NHEJ field.

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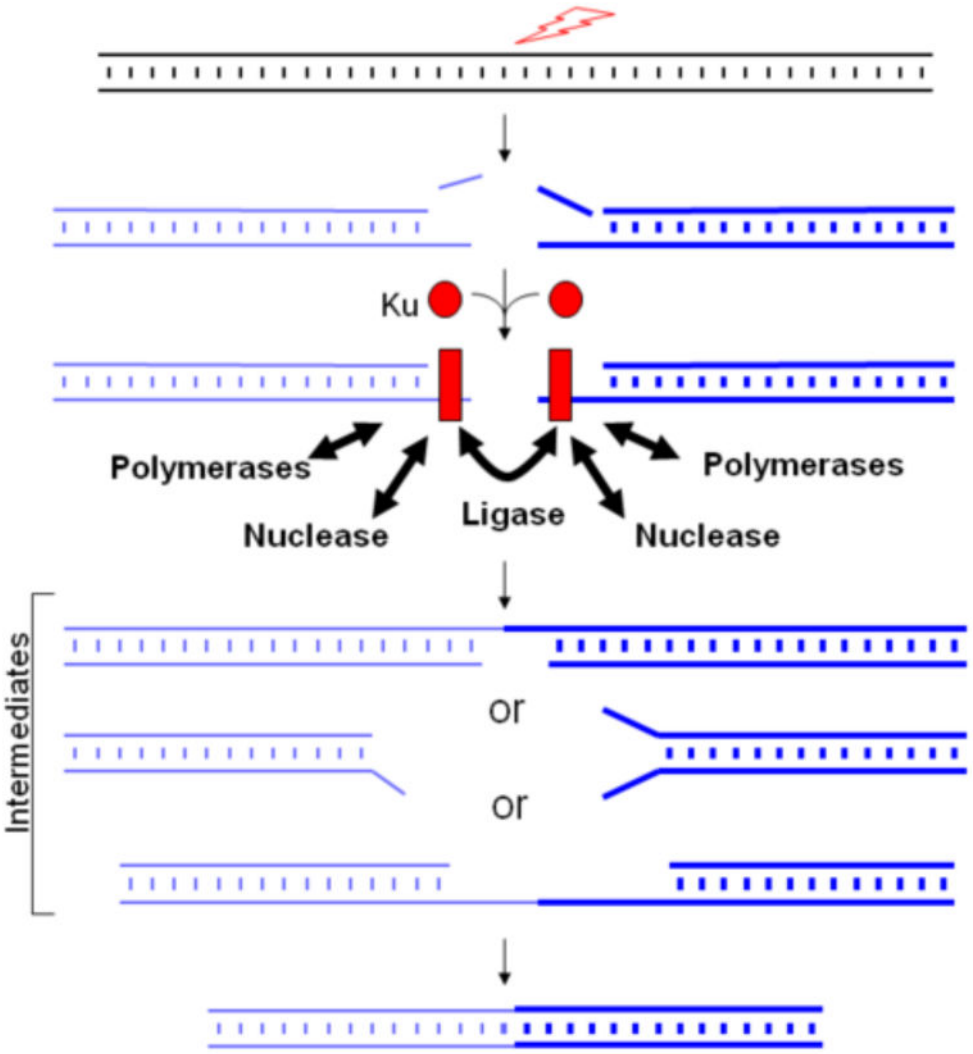

Figure 1. General steps of NHEJ

The lightning arrow indicates ionizing radiation (IR), a reactive oxygen species (ROS), or an enzymatic cause of a DSB. Ku binding to the DNA ends at double-strand breaks (DSBs) improves binding by nuclease, DNA polymerase, and ligase components. Note that Ku is thought to change conformation upon binding to the DNA end, as depicted by its shape change from a sphere to a rectangle. Flexibility in the loading of these enzymatic components, the option to load repeatedly (iteratively), and independent processing of the two DNA ends all permit mechanistic flexibility for the NHEJ process. This mechanistic flexibility is essential to permit NHEJ to handle a very diverse array of DSB end configurations and to join them. In addition to the overall mechanistic flexibility, each component exhibits enzymatic flexibility and multifunctionality. The figure shows that there are many alternative intermediates in the joining process. These intermediates are reflected in a diverse DNA sequences at the junction of the joining process.

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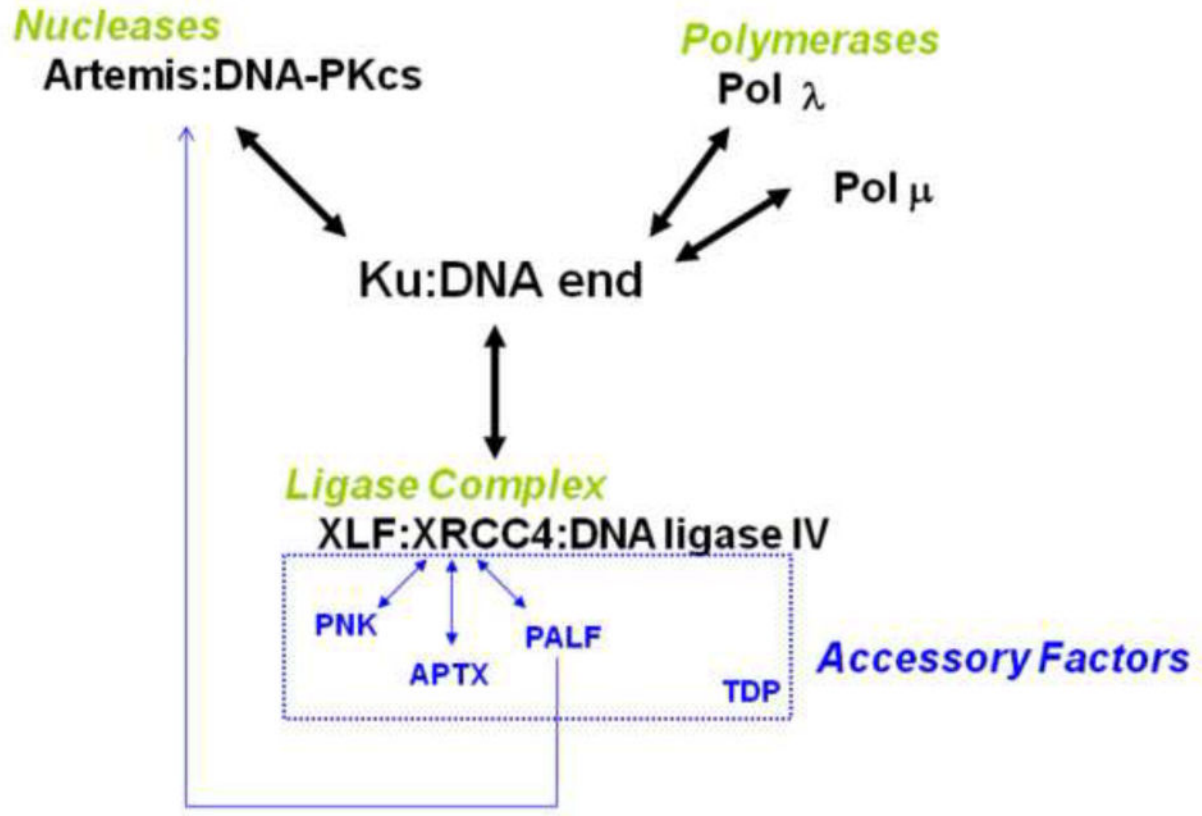

Figure 2. Proteins Involved in Vertebrate NHEJ

The diagram shows known interactions of the proteins documented to participate in the key enzymatic steps of vertebrate NHEJ. (Other proteins may participate in gaining access to the chromatinized DNA or in modulating some of the components shown [70, 71].) XLF, also called Cernunnos, is a component of the DNA ligase IV complex [72, 73]. PNK is polynucleotide kinase. APTX is aprataxin [74]. PALF (Polynucleotide kinase and Aprataxin-like Fork-head-associated) and is more commonly called APLF, based on an alternative order of the same acronym; and TDP is tyrosyl DNA phosphodiesterase (see article in this series by K. W. Caldecott).

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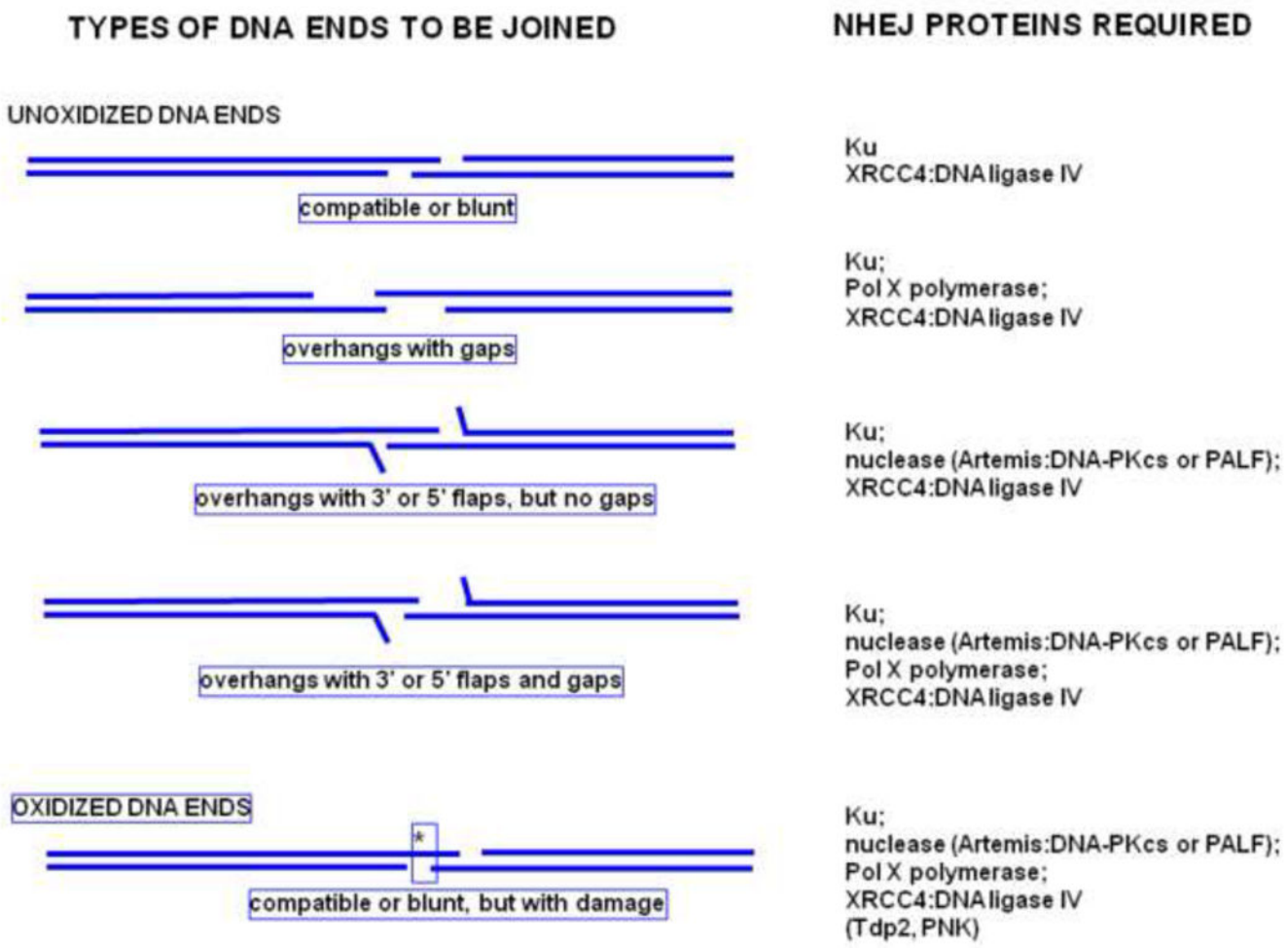

Figure 3. DNA End Configurations Determine Which NHEJ Proteins are Required During NHEJ

The distinctive flexibility required by NHEJ means that it is not easily depicted as a simple linear pathway. If NHEJ were simple enough to depict in a linear pathway, then it would probably not be sufficiently flexible to handle many different DNA end configurations, only a subset of which are illustrated here. Not illustrated is the likely aspect that NHEJ appears to be iterative. The nucleases, polymerases, and the ligase complex can work on either of the two DNA ends independently, iteratively, and in any order until both strands are ligated. Some possible end configurations and corresponding required proteins are listed. The asterisk in the bottom joining diagram represents oxidative base damage.

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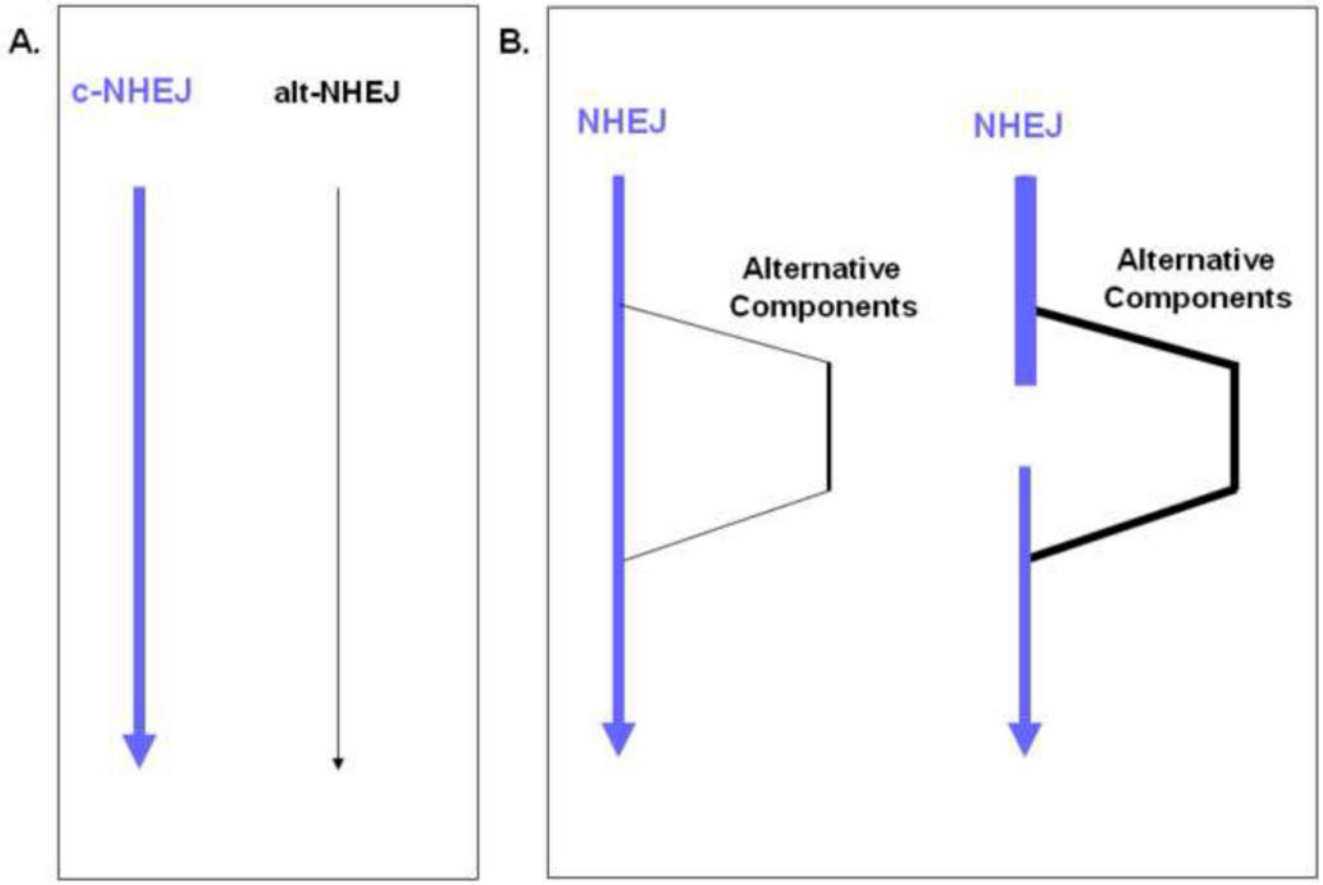

Figure 4. Two models of NHEJ

A. In one conceptualization, two separate NHEJ pathways exist, classical NHEJ (c-NHEJ) and alternative NHEJ (alt-NHEJ) with the latter occurring independently of c-NHEJ components and relying on increased lengths of microhomology (MH) and for a higher percentage of the junctions. B. In a conceptualization that we favor, there is only one NHEJ pathway with alternative components able to substitute for some of the canonical NHEJ components. In WT cells, the alternative components are infrequently used because the c-NHEJ components are most efficient. But if a cell is deficient in a major NHEJ factor, it must utilize the alternative components to complete the join (e.g., L1 or L3 in place of L4), but perhaps with reduced kinetic efficiency. Note that when an NHEJ component is missing, incomplete intermediates of joining may accumulate, as indicated by the thicker blue line.

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Table 1

List of known end joining components in humans and yeast

Homologues between humans and yeast are listed across from each other in the two main columns of the table.

	Main DNA Repair Proteins Utilized in NHEJ		**Alternative DNA Repair Proteins that can Substitute[1]**	
	H. Sapiens	***S. cerevisiae***	***H. Sapiens***	***S. cerevisiae***
Break recognition and recruitment	Ku70/80 DNA-PKcs	Yku70/80 N/A Mre11:Rad50:Xrs2 (MRX)		
End processing				
Nucleases *(and helicases)*	Artemis PALF (APLF)	N/A N/A *MRX*	*MRN* CtIP FEN1 Exo1 *BLM, WRN*	Sae2 Rad27 Exo1 *Sgs1* Dna2
Polymerases	Polλ, Polμ TdT	Pol4 N/A Pol3		
Ligation	XLF:XRCC4:DNA Ligase IV	Nej1:Lif1:Dnl4	Ligase I Ligase III	Cdc9 N/A

“N/A” indicates that there is no known homologue between the two organisms. For purposes of NHEJ, pol mu and pol lambda serve the analogous role as Pol4 in yeast. (The POLX family in humans includes pol beta, pol mu, pol lambda, and TdT, and in yeast includes only Pol4.)