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Nonhomologous end joining: A good solution for bad ends

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ABSTRACT

Double strand breaks pose unique problems for DNA repair, especially when broken ends possess complex structures that interfere with standard DNA transactions. Nonhomologous end joining can use multiple strategies to solve these problems. It further uses sophisticated means to ensure the strategy chosen provides the ideal balance of flexibility and accuracy.

1. Introduction

Assaults on the genome can result in various forms of DNA damage with potentially catastrophic consequences. A particularly serious class of damage is that of the double strand break (DSB). DSBs arise during programmed recombination events such as those in V(D)J recombination, but also in response to damaging agents like ionizing radiation (IR) and reactive oxygen species (ROS). DSBs that are misrepaired or left unrepaired cause genomic instability. This in turn has devastating effects at both cellular and organismal levels, including cytotoxicity, accelerated aging, cancer predisposition.

Mammalian cells rely on two major repair pathways to resolve DSBs – homologous recombination (HR) and nonhomologous end joining (NHEJ). Considered the most accurate of DSB repair pathways, HR faithfully replaces lost or damaged sequence via extensive DNA synthesis, using an intact sister chromatid as a template. The need for a sister chromatid and an abundant nucleotide pool restricts HR to the S- and G2-phase of the cell cycle (reviewed in e.g. [1,2]).

In contrast, NHEJ directly rejoins chromosome ends, and is thus not subject to the same requirement for both homology (to use as template) and extensive synthesis, relative to HR. This makes NHEJ available for repair throughout the cell cycle [2]. On the other hand, there are disadvantages to using NHEJ. All other pathways take advantage of redundancy to replace excised damage (a sister chromatid for HR; the intact complementary strand for base excision repair, nucleotide excision repair, and mismatch repair) [3]. The inability to similarly employ sequence redundancy to replace excised damage means the standard DNA transactions – synthesis and ligation – must be made more flexible, and products of repair typically involve deletion. NHEJ thus might not be considered "DNA repair" in the strictest sense.

Compounding the difficulty for NHEJ is the mélange of possible damage at DSBs that could interfere with ligation, and, which must often be excised. DSBs generated by damaging agents are associated with a wide array of different damaged nucleotides, including

Abbreviations: Alt-EJ, alternative end joining; APLF, aprataxin and polynucleotide kinase/phosphatase-like factor; ATM, ataxia-telangiectasia mutated; ATR, ATM-, Rad3-related; APTX, aprataxin; β-Casp, metallo-β-lactamase-associated CPSF Artemis SNM1 PSO2; BER, base excision repair; BRCT, BRCA1 C-terminal; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, DNA protein kinase catalytic subunit; DSB, double-strand break; dRP/AP, deoxyribose-phosphate/apurinic/apyrimidinic; DSB, double strand break; dsDNA, double-stranded DNA; FHA, forkhead-associated; HEAT, Huntington elongation factor 2, A subunit of protein phosphatase 2A and TOR1; HR, homologous recombination; HRDC, helicase and RNase D C-terminal; IR, ionizing radiation; Ku, Ku70/80 heterodimer; MRN, Mre11/Rad50/Nbs1; NAP1, nucleosome assembly protein-1; NHEJ, non-homologous end-joining; PIKK, phosphoinositide 3-kinase-like family of protein kinases; PNKP, polynucleotide kinase/phosphatase; PAR, poly(adenyl-ribose); PARP, PAR polymerase; PBZ, PAR-binding zinc finger; ROS, reactive oxygen species; RQC, RecQ carboxy-terminal; SAP domain, SAF-A/B, Acinus and PIAS domain; SCID, severe combined immunodeficiency; SET, Su(var) Ez and Trithorax; SSBR, single-strand break repair; Tdp1, tyrosyl-DNA phosphodiesterase 1; Tdp2, tyrosyl-DNA phosphodiesterase 2; TdT, terminal deoxyribonucleotidyltransferase; vWa, von Wilebrand; WRN, Werner's Syndrome helicase; XRCC4, X-ray-complementing Chinese hamster gene 4; X4–L4 complex, XRCC4–DNA ligase IV complex; XLF, XRCC4-like factor.

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Table 1
NHEJ associated proteins.

Factor[a]	Activity
Core factors	
XRCC5, XRCC6 (Ku)	Senses DSB break [155] then recruits proteins to break
PRKDC (DNA PKcs)	Tethers broken DNA ends together [22,23] and has kinase activity [19,156]
LIG4 (DNA ligase IV)	Ligase [157]
XRCC4	Obligate ligase subunit [29]
NHEJ1 (XLF)	Promotes end-bridging [50]
Processing factors	
APLF	Histone chaperone [70] 3′–5′ exonuclease, endonuclease [64,65]
PNKP	Removes 3′ phosphates and phosphorylates 5′ hydroxyls [57]
APTX	Removes 5′-adenylate adducts [61]
TDP1	Removes Top I adducts [72], 3′ deoxyribose fragments [76,78,158]
TDP2	Removes Top II adducts [73]
POLM (Pol μ)	Fills in gaps when ends align with no complementarity [82]
POLL (Pol λ)	Fills in gaps when ends are partly complementary [82,83]
DCLRE1C (Artemis)	Endonuclease, 5′–3′ exonuclease [101]
SETMAR (Metnase)	Endonuclease/exonuclease, histone methylase [115]
MRE11/RAD50/NBN (MRN)	3′–5′ exonuclease, endonuclease [119,120]
WRN	3′–5′ exonuclease [159,160] and 3′–5′ helicase [161]

[a] *HUGO* gene nomenclature: *XRCC5,XRCC6* (Ku80, Ku70), X-ray repair complementing defective repair in Chinese hamster cells 5/6; *PRKDC*, protein kinase, DNA-activated, catalytic polypeptide; *LIG4*, ligase IV, DNA, ATP-dependent; *XRCC4*, X-ray repair complementing defective repair in Chinese hamster cells 4; *NHEJ1* (XLF), nonhomologous end-joining factor 1; *APLF*, aprataxin and PNKP like factor; *PNKP*, polynucleotide kinase 3′-phosphatase; *APTX*, aprataxin; *TDP1*, tyrosyl-DNA phosphodiesterase 1; *TDP2*, tyrosyl-DNA phosphodiesterase 2; *POLM*, polymerase mu; *POLL*, polymerase lambda; *DCLRE1C* (Artemis), DNA cross-link repair 1 C; *SETMAR* (Metnase), SET domain and mariner transposase fusion gene; *MRE11/RAD50/NBN*, meiotic recombination 11 homolog/RAD50 homolog/nibrin (Nbs1); *WRN*, Werner syndrome.

oxidized nucleotides and nucleotide fragments [4–6]. Intermediates in V(D)J recombination possess hairpin-terminated breaks [7], and alignment of pairs of ends regardless of source often result in mismatches, gaps and flaps. Proteins may also occlude DSB ends, like the covalently adducted products of aborted topoisomerase activity [8], or non-covalently associated chromatin proteins.

Thus, damage at DSBs presents a particularly onerous challenge to NHEJ. To resolve this challenge NHEJ employs a series of core factors that work to sense a DSB, align ends, and act as a scaffold for a host of processing factors that facilitate removal (and sometimes even replacement) of ligation-blocking damage (discussed in Section 2) (Table 1). We suggest that together this complex acts as a multi-protein machine, and is capable of employing an amalgam of the of strategies used by other repair pathways, including (1) damage tolerance, (2) targeted, short patch excision, and (3) long patch excision (discussed in Section 3). Having these multiple strategies at its disposal increases NHEJ's flexibility, allowing it to resolve DSBs with diverse end structures. However, determining which strategy is used is critical, as it both impacts the fidelity and efficiency of DSB repair.

2. NHEJ is a multi-protein machine

The ability to employ several strategies for repair implies an organized, multi-protein machine capable of dynamic control of DNA end metabolism. This machine starts with the assembly of a core complex, capable of recognizing broken ends and – most importantly – aligning ends together such that strand break termini are presented appropriately to the factors that must act on them. Notably, the core-assembly includes a pathway-specific ligase complex, which aside from its essential role it catalyzing the final step in NHEJ also provides an important non-catalytic scaffolding function.

The core assembly then recruits processing factors (Table 1). As argued above, diversity in end structure implies a need for a wide variety of end processing factors, and the list of such factors implicated in NHEJ is still growing. The list includes, not surprisingly, an array of end-cleaning enzymes shared with base excision repair/single strand break repair (BER/SSBR). Several nucleases and polymerases have also been implicated in NHEJ. However, unlike the end cleaning enzymes, nucleases and polymerases are more often uniquely suited to act in the context of a DSB and thus, are less often shared with BER/SSBR.

We discuss below the contribution of factors within the NHEJ machine as well as some gross structural features (a more detailed review of NHEJ factor structures can be found elsewhere in this issue). We emphasize how factors interact with each other and DNA substrate, as these interactions will determine how the machine is assembled as well as how the machine controls access to ends.

2.1. Core factors

The core machinery for NHEJ is typically considered to include the Ku heterodimer (Ku 80/70), the DNA dependent protein kinase catalytic subunit (DNA-PKcs), DNA Ligase IV, XRCC4, and the XRCC4-like factor (XLF, or Cernunnos).

2.1.1. Ku

Ku is a heterodimer of 70 and 83 kD subunits (Ku70, Ku80). The subunits have a similar domain organization [9,10] (Fig. 1A, upper panel), but N- and C-terminal domains especially have diverged in function. An extended C-terminal domain in Ku80 possesses the primary interface for promoting interaction with DNA-PKcs [9,11,12], while Ku70 has a C-terminal SAP domain with DNA binding activity [13]. The N-terminal domain (vWA domain) of Ku70 has acquired enzymatic activity, (see below), while Ku80's vWA domain interacts with APLF [14]. Finally, the central heterodimerization domains of both Ku subunits promote interaction with XLF [15].

Ku loads on ends by threading them through a central channel formed by the heterodimer [16] (Fig. 1A, lower panel). This binding mechanism explains its high specificity for ends and, once bound, both its ability to remain stably bound [17] and translocate internally on the DNA fragment [18].

2.1.2. DNA-PKcs

DNA-PKcs is a 460 kD kinase specifically recruited to, and its kinase activity dependent on, Ku-bound DNA ends [19]. It is related to two other kinases also implicated in the DNA damage response – Ataxia Telangiectasia mutated (ATM) and Ataxia Telangiectasia Related (ATR) [20]. DNA-PKcs forms a ~150 angstrom wide C clamp-like shape (Fig. 1B, lower panel) composed largely of HEAT repeats, with the kinase domain and Ku interacting region (Fig. 1B, upper panel) located as a "crown" opposite the clamp's gap [21] (Fig. 1B, lower panel). DNA-PKcs and Ku together form a very stable complex that remains tethered to the end [22,23] and blocks access of other factors – nucleases, polymerases, and even ligase IV – in the absence of kinase activity [23,24]. As discussed below (Section 3.4) phosphorylation of DNA-PKcs, either autophosphorylation [25] or by ATM [26], plays a key role in regulating access to ends during NHEJ.