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# Reprint of "The clinical impact of deficiency in DNA non-homologous end-joining"☆

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ABSTRACT

DNA non-homologous end-joining (NHEJ) is the major DNA double strand break (DSB) repair pathway in mammalian cells. Defects in NHEJ proteins confer marked radiosensitivity in cell lines and mice models, since radiation potently induces DSBs. The process of V(D)J recombination functions during the development of the immune response, and involves the introduction and rejoining of programmed DSBs to generate an array of diverse T and B cells. NHEJ rejoins these programmed DSBs. Consequently, NHEJ deficiency confers (severe) combined immunodeficiency – (S)CID – due to a failure to carry out V(D)J recombination efficiently. NHEJ also functions in class switch recombination, another step enhancing T and B cell diversity. Prompted by these findings, a search for radiosensitivity amongst (S)CID patients revealed a radiosensitive sub-class, defined as RS-SCID. Mutations in NHEJ genes, defining human syndromes deficient in DNA ligase IV (LIG4 Syndrome), XLF-Cernunnos, Artemis or DNA-PKcs, have been identified in such patients. Mutations in XRCC4 or Ku70,80 in patients have not been identified. RS-SCID patients frequently display additional characteristics including microcephaly, dysmorphic facial features and growth delay. Here, we overview the clinical spectrum of RS-SCID patients and discuss our current understanding of the underlying biology.



## 1. Introduction

A DNA double strand break (DSB) is a critical lesion promoting cell death if unrepaired or genomic rearrangements if misrepaired. While ionising radiation (IR) is renowned for its avid ability to induce DSBs, around 10–20 DSBs arise endogenously per cell per day. Perhaps surprisingly, cells also create DSBs, termed programmed DSBs, during developmental processes such as immune development and meiosis. Significantly, while the goal of DSB repair in somatic cells is to maintain genomic integrity and stability, the end-point of the developmental processes involving programmed DSB formation is to create genetic diversity. Amazingly despite this basic difference, the same DSB pathway is exploited to maintain genomic stability in the face of DNA damage induced DSBs and to create diversity during immune development, although the underlying processes encompass tweaks to achieve the distinct goals. The major DSB repair pathway in mammalian cells is DNA non-homologous end-joining (NHEJ). Homologous recombination (HR) also functions to rejoin DSBs but is limited to situations where a sister chromatid, which serves as the source of the homologous sequence, is present. HR, in fact, has a more significant role in promoting recovery from replication fork stalling or collapse, a process which may not involve DSB formation at all or possibly a DSB with one end. HR also functions during meiosis, which represents another situation when a sister chromosome is present. The goal of this review is to focus on the clinical manifestation of defects in NHEJ in patients. Since NHEJ functions during immune development, we will focus on the immunodeficiency observed in NHEJ-defective patients. However, NHEJ deficiency can also confer additional developmental abnormalities, including microcephaly. We aim to couple our current understanding of the underlying biology with the clinical manifestation. We will focus on the impact in patients but call upon studies using cultured cells and mouse models, as well as biochemical and structural biology approaches to provide additional insight. Since most (but not all) of the NHEJ proteins are essential for embryonic viability, the mutational changes observed in patients are frequently

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hypomorphic (they are not fully inactivating and allow residual protein function). We use the standard convention and describe such patients as being deficient in the protein; complete loss of protein function (caused by a null mutational change) results in a patient defective for the protein under discussion.

## 2. The process of NHEJ

Current insight into the NHEJ mechanism will be covered elsewhere in this DNA Repair Special Issue. Other detailed reviews are also available [1–3]. Here, we provide only a brief overview encompassing points relevant for considering NHEJ-deficient patients. NHEJ is initiated by binding of the Ku heterodimer to double stranded (ds) DNA ends (Fig. 1). The rapid end-binding of Ku, which arises in part from its avid capacity to bind DNA ends coupled with its high abundancy, provides prompt protection of the DNA ends from unwanted nucleolytic degradation; Ku end-binding additionally recruits the DNA-PK catalytic subunit (DNA-PKcs) generating the DNA-PK complex. DNA-PK is a phosphatidylinositol 3-kinase (PI3K)-like kinase with a kinase domain in the C-terminus [4]. Studies in vitro have demonstrated that Ku has the capacity to translocate inwards forming multimeric complexes on the DNA molecule [5]. There is, however, no evidence that this occurs in vivo

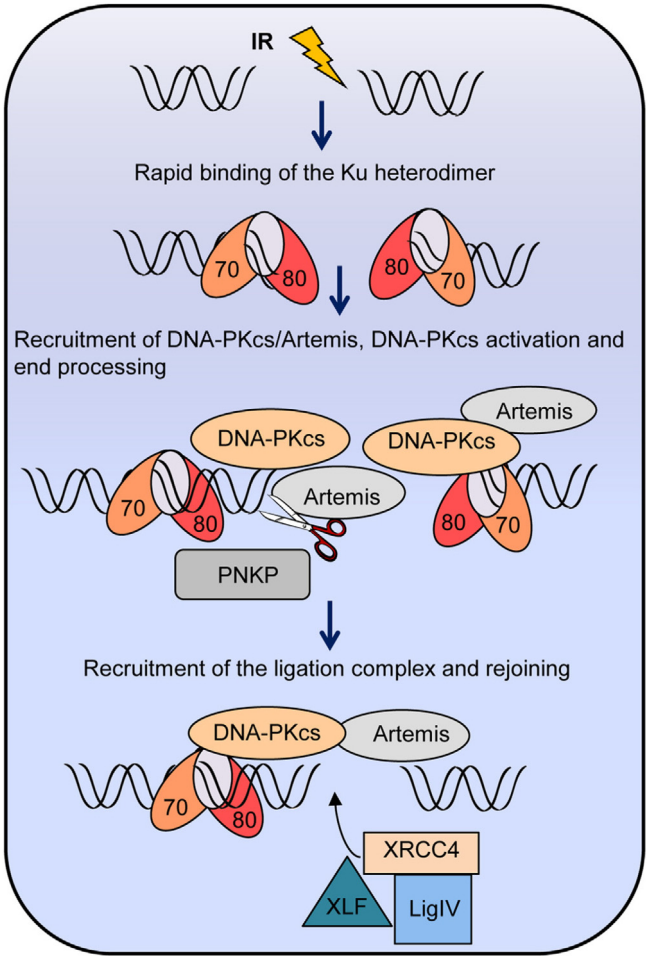


**Fig. 1.** Process of NHEJ. Ku (shown as a 70/80 heterodimer in yellow/red), which has avid DNA end-binding activity and is highly abundant in human cells, rapidly binds DNA ends. DNA-bound Ku recruits DNA-PKcs, which activates DNA-PK activity. DNA-PK undergoes autophosphorylation at specific clusters of sites. DNA-PK promotes steps of end-processing, which can be carried out by PNKP and, for some DSBs, by the nuclease, Artemis. DNA-PK recruits the ligation complex, encompassing DNA ligase IV, XRCC4 and XLF, which carries out the final rejoining step.

and indeed, exploitation of a recent novel approach for high resolution analysis of DNA bound proteins, has demonstrated that in vivo only a single Ku molecule is present at each DNA end (i.e. two molecules at a dsDNA end) [6]. However, DNA-PKcs recruitment may promote inward movement of the single Ku molecule, which may facilitate steps of end-processing. Assembly of DNA-PK on DNA ends activates its kinase activity [7]. Structural studies have shown that Ku is a basket shaped structure with a central core of appropriate dimensions to allow its looping onto ds DNA without necessitating interaction with nucleotide bases [8]. Ku-DNA binding is, therefore, DNA sequence independent. The structure of DNA-PKcs has revealed a flexible ring shaped molecule arising from multiple alpha-helical HEAT repeats (helix-turn-helix motifs), which facilitate bending [9]. The flexible structure and an opening in the ring endows DNA-PKcs with the capacity to fold onto the DNA molecule [10]. It is at this stage of the process that DNA-PK facilitates end-processing steps, which may involve deletion formation or "fill in" regions of ssDNA regions [11]. Ligation requires 3′OH and 5′P ends, which rarely arise following DNA damage, but are generated by a process involving polynucleotide kinase/phosphatase (PNKP). DNA-PK may promote PNKP processing by phosphorylation [12]. End-processing may also require nuclease activities. DNA-PKcs interacts with the structure specific nuclease, Artemis [13]. Artemis has a Metallo-β-lactamase domain at its extreme N-terminus and a closely localised β-Casp domain [14]. The C-terminus appears to be regulatory. Artemis, in contrast to other NHEJ proteins, appears to be uniquely required for the repair of a 15% subset of DSBs, representing those repaired with slow kinetics [15]. Moreover this process has an essential requirement for DNA-PKcs [15]. Artemis also functions to open the hairpin ended intermediates that form during the process of V(D)J recombination in a DNA-PKcs dependent manner (see further details below) [13]. In G2 phase, where HR can also function, Artemis is, as in G1 phase, required for the slow component of DSB repair – however, in G2 this slow repair process represents HR [16]. The available evidence suggests that in G2 phase, there is a defined switch from rejoining by NHEJ to the use of HR in those situations where rapid repair by NHEJ fails to ensue [17]. Thus, we propose a model in which NHEJ initially attempts to repair all DSBs by a "core" process that does not require Artemis processing but if this process stalls, then a switch to promoting resection and repair by HR occurs [17]. In contrast, Artemis is dispensable for HR following replication fork stalling/collapse. However, although Artemis is required for resection, it does not appear to be the nuclease that directly effects resection since it has the wrong polarity. We propose that in G1 phase, a similar hierarchy exists. However, in G1 phase, the slow rejoining process may represent a form of resection-mediated end-joining, such as microhomology mediated end-joining (MMEJ) rather than HR, with resection being less extensive (Lobrich and Jeggo laboratories, unpublished findings). The role of Artemis during V(D)J recombination, where a hairpin end is the barrier to core NHEJ-dependent rejoining, may reflect a similar hierarchy with a function for Artemis in opening the hairpin end [13]. It has been proposed that, at least during V(D)J recombination, DNA-PKcs facilitates Artemis activity by remodelling the DNA end [18]. Whether DNA-PKcs promotes Artemis function at damage-induced DSBs remains to be determined. These in vivo roles of Artemis are consistent with biochemical analysis showing that in the presence of DNA-PK Artemis can cleave hairpin ends and 5′ and 3′ overhangs [13,14].

Finally, DNA-PK plays an important role in recruiting the NHEJ ligation complex, which encompasses XRCC4, DNA ligase IV and XLF [19]. Recent studies also suggest that the ligation complex may promote DNA-PKcs autophosphorylation, and potentially the release of DNA-PK from the end [20]. Nonetheless, it is currently unclear when removal of the DNA-PK complex occurs and whether