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# Mutations to Ku reveal differences in human somatic cell lines

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ABSTRACT

NHEJ (*non-homologous end joining*) is the predominant mechanism for repairing DNA double-stranded breaks in human cells. One essential NHEJ factor is the Ku heterodimer, which is composed of Ku70 and Ku86. Here we have generated heterozygous loss-of-function mutations for each of these genes in two different human somatic cell lines, HCT116 and NALM-6, using gene targeting. Previous work had suggested that phenotypic differences might exist between the genes and/or between the cell lines. By providing a side-by-each comparison of the four cell lines, we demonstrate that there are indeed subtle differences between loss-of-function mutations for Ku70 versus Ku86, which is accentuated by whether the mutations were derived in the HCT116 or NALM-6 genetic background. Overall, however, the phenotypes of the four lines are quite similar and they provide a compelling argument for the hypothesis that Ku loss-of-function mutations in human somatic cells result in demonstrable haploinsufficiencies. Collectively, these studies demonstrate the importance of proper biallelic expression of these genes for NHEJ and telomere maintenance and they provide insights into why these genes are uniquely essential for primates.



## 1. Introduction

The integrity of chromosomes must be maintained in order to ensure survival. This requirement is difficult for cells to sustain since chromosomal DNA is damaged continuously by both exogenous and endogenous agents. Among the many forms of DNA damage that can occur, DNA DSBs (*double-stranded breaks*) are the most dangerous (reviewed by [1,2]). DSBs can occur in response to external stimuli like IR (*ionizing radiation*) and also by exposure to clinical chemotherapeutic agents like bleomycin and etoposide. Moreover, DSBs also arise as a result of natural processes, such as V(D)J and switch recombination, lymphoid-specific processes needed for the maturation of T and B cells (reviewed by [3,4]). Consequently, to ensure their survival, mammals have evolved intricate and efficient mechanisms for the repair of DSBs.

In eukaryotic cells, two major processes are responsible for repair of DSBs, namely HR (*homologous recombination*; reviewed by [5,6]) and NHEJ (reviewed by [2]). HR carries out accurate repair by utilizing a homologous chromosome or an undamaged sister chromatid as a template. In contrast, NHEJ uses no, or very little, sequence homology for repair events that can occur in an error-prone manner. Both pathways are conserved throughout eukaryotic evolution but their relative importance varies between organisms. Simpler organisms like *S. cerevisiae* rely mainly on HR to repair damaged DNA

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while in higher eukaryotes, and particularly in humans, NHEJ is the predominant repair mechanism. This bias is not well understood although it is possible that in the complex human genome where a very small percentage of the DNA actually codes for any protein the errors made by NHEJ are better tolerated. There are at least seven proteins that play important roles in NHEJ: Ku70, Ku86, DNA-PK$_{CS}$ (*DNA*-dependent *p*rotein *k*inase complex *c*atalytic subunit), XRCC4 (*X*-ray *c*ross complementing 4), LIGIV (DNA *lig*ase *IV*), Artemis and Cernunnos/XLF (XRCC4-like *f*actor) (reviewed by [2,7]). In humans, mutations have been described for LIGIV [8,9], Artemis [10] and XLF [11] that cause IR$^S$ (*IR* sensitivity), immune deficiency and/or cancer predisposition. The deleterious phenotypes associated with these mutations substantiate the importance of NHEJ in humans.

Mutations for the other NHEJ factors (Ku70, Ku86, DNA-PK$_{CS}$ and XRCC-4), however, have yet to be described in humans. In particular, even heterozygous mutations for either Ku70 or Ku86 have yet to be documented. Ku is a heterodimeric protein composed of 70 and 86 kDa subunits (Ku70 and Ku86, respectively) and this protein binds to all forms of ds (*d*ouble-stranded) DNA ends in a sequence non-specific manner [7]. The ability to bind virtually all broken dsDNA ends can be explained by the crystal structure of Ku. Ku forms an open, ring-type structure that can be threaded onto a dsDNA end [12]. One side of the ring cradles one face of the DNA while the other side is more open, presumably to allow other NHEJ factors to access the broken DNA end [13,14]. In vertebrates, Ku recruits DNA-PK$_{CS}$ to the sites of DNA damage during DNA DSB repair. The interaction of DNA-PK$_{CS}$ with the DNA-bound Ku heterodimer leads to the formation of the DNA-PK holoenzyme and this complex exhibits a DNA-dependent protein kinase activity that is essential for NHEJ (reviewed by [4]). Cells that lack Ku are IR$^S$, immune deficient and defective for DNA DSB repair (reviewed in [7]). Mice containing targeted disruption in either the Ku70 [15] or Ku86 gene [16,17] show increased sensitivity to IR and a failure to carry out V(D)J recombination. Moreover, the inactivation of the Ku86 gene in the mouse is known to cause growth retardation in cells [18], induce a marked increase in chromosomal aberrations [19–21] and to also cause premature senescence [22]. In summary, mouse models of Ku loss-of-function mutations unequivocally validate the importance of Ku for NHEJ, but a direct demonstration of this is still lacking in human patients.

A description of mutations is also lacking for DNA-PK$_{CS}$. DNA-PK$_{CS}$ is an ~460 kDa polypeptide and its C-terminus contains sequence homology to the catalytic domains of the proteins of the PIKK (*p*hosphatidyl inositol 3-*k*inase-like *k*inase) family (reviewed by [4]). DNA-PK$_{CS}$ is the product of the *scid* (severe *c*ombined immune *d*eficiency; *prkdc*/XRCC7) gene and the loss of this gene results in defects in DNA DSB repair, immune deficiency and IR$^S$ in mice, Arabian horses and Jack Russell terriers. The only human cell line known to lack DNA-PK$_{CS}$ is M059J [23]. This cell line, which was isolated from a malignant glioma, lacks DNA-PK$_{CS}$ activity due to defective mRNA turnover associated with a frameshift mutation in the *prkdc* gene [24]. Importantly, this mutation appears to have been generated during propagation of the glioma during cell culture and was absent in the patient and from the tumor from which it was derived [23], emphasizing again the complete lack of human patients with DNA-PK mutations.

Besides NHEJ, an additional important role for the DNA-PK complex is in the protection of telomeres, the end structures of chromosomes (reviewed by [25]). Interestingly, all three components of the DNA-PK complex – Ku70, Ku86 and DNA-PK$_{CS}$ – play some role in the protection of telomeres [21,26–29]. Moreover, MEFs (*m*ouse *e*mbryo *f*ibroblasts) from DNA-PK$_{CS}^{-/-}$ mice display a significant increase in chromosome fusions, even though the actual length of telomeres is not altered [29]. Ku70$^{-/-}$ and Ku86$^{-/-}$ MEFs also show elevated chromosome end fusions [26–28]. These observations suggest that Ku is directly involved in a telomere capping function and indeed Ku has been physically located at telomeres by biochemical studies [21,30]. The role of Ku in actual telomere length maintenance is, however, less clear. There are conflicting reports of Ku-defective mice showing telomere shortening [21] and also telomere elongation [28]. Also, deletion of both alleles of Ku in *Arabidopsis thaliana* causes telomere elongation with no apparent telomere fusions [31]. The effect of Ku or DNA-PK$_{CS}$ mutations on telomere function in human patients is not known.

In order to better understand the roles Ku and DNA-PK play in NHEJ and telomere maintenance in humans, our laboratory used gene targeting to functionally inactivate the Ku86 locus in the human adenocarcinoma somatic tissue culture cell line, HCT116 [32]. The null cell lines were not viable, a finding that was unexpected given the existence of many other Ku86-null organisms. Moreover, the derivative human cell lines heterozygous for Ku86 showed significant haploinsufficient phenotypes, with defects in cell proliferation, IR$^S$, elevated levels of p53, polyploidy, shortened telomeres and elevated levels of GCRs (*g*ross *c*hromosomal rearrangements) [33]. All of these phenotypes suggested that, if anything, Ku86 played an even more critical role in NHEJ and telomere maintenance in humans than in other mammals. Importantly, most of these phenotypes have been independently confirmed by laboratories utilizing Ku70 antisense DNA [34], Ku86 antisense DNA [35], Ku86 cRNA [36], or Ku86 RNAi [37] approaches to reduce Ku expression in a variety of human somatic cell lines. Recently, however, Uegaki et al. have reported that heterozygous inactivation of Ku70 and Ku86 resulted in human cell lines that did not show haploinsufficient phenotypes [38]. These studies were carried out in the pre-B leukemic cell line, NALM-6 [39,40]. The reasons for the discrepancies between these two studies are not known. A likely possibility was that the discrepancies might be due to the different cell lines utilized, especially since NALM-6 has been reported to be greatly up-regulated for HR ([41,42]; reviewed by [43]), and thus, presumably, less sensitive to mutations in NHEJ genes. To address this issue, we have generated Ku70 and Ku86 heterozygous mutations in both HCT116 and NALM-6 cell lines and have compared the resulting lines side-by-each. These studies have revealed that while there are some important differences, especially related to radiation sensitivity, the overall effects of these mutations are quite similar for all cell lines.