MECHANISM AND REGULATION OF HUMAN NON-HOMOLOGOUS DNA END-JOINING

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Non-homologous DNA end-joining (NHEJ) — the main pathway for repairing double-stranded DNA breaks — functions throughout the cell cycle to repair such lesions. Defects in NHEJ result in marked sensitivity to ionizing radiation and ablation of lymphocytes, which rely on NHEJ to complete the rearrangement of antigen-receptor genes. NHEJ is typically imprecise, a characteristic that is useful for immune diversification in lymphocytes, but which might also contribute to some of the genetic changes that underlie cancer and ageing.

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Double-stranded DNA breaks (DSBs; BOX 1) are repaired by two main pathways — homologous recombination and non-homologous DNA end-joining (NHEJ). By comparing the use of these two pathways, we can gain an insight into their biological functions in yeast and multicellular eukaryotes (FIG. 1).

Saccharomyces cerevisiae repairs most of its DSBs using homologous recombination[1], which typically occurs without the loss of genetic information because it essentially involves copying the missing information from one homologous chromosome to the other in a diploid cell. By contrast, NHEJ, which is the more common DSB repair pathway in multicellular eukaryotes, such as mice and humans, involves modifying the two broken ends to make them compatible, followed by rejoining (FIG. 2). A few nucleotides at each end of the DNA break are lost in most instances when NHEJ is used in multicellular eukaryotes[2], and in many instances when it is used in yeast[3]. So, NHEJ is an imperfect process from the standpoint of preserving genetic information.

As well as being imprecise, NHEJ is not essential in diploid yeast — yeast cells that are mutated in components for NHEJ are reasonably viable, and they do not have increased sensitivity to ionizing radiation because they can then rely primarily on homologous recombination. There is some use for NHEJ in the haploid phases of the yeast life cycle, but multicellular eukaryotes are diploid. So, why do multicellular eukaryotes have NHEJ at all, given that their somatic cells are typically diploid and given that homologous recombination is relatively precise?

The biological role of NHEJ

The abundant use of NHEJ in the somatic cells of multicellular eukaryotes could be intrinsic to their genomic organization. The genomes of multicellular eukaryotes, unlike yeast, have a substantial fraction of repetitive DNA. Therefore, the homology-search process for repair of DSBs by homologous recombination is insurmountable when the break occurs in the portion of the genome that is repetitive (about 40% of the human genome). Except during late S, G2 and M phases, when a sister chromatid is optimally positioned physically, homology partners for repetitive regions might be chosen from any of the chromosomes inappropriately. A homologous crossover event arising during DSB repair could result in a chromosomal translocation. So, homologous recombination could be too slow and dangerous in organisms with genomes that have a substantial fraction of repetitive DNA. This might be the primary — and perhaps the only — reason that controls the ratio of homologous recombination and NHEJ.

Cells that are defective for homologous recombination, such as RAD54 and XRCC2 (X-ray cross complementation 2) mutants, show a relatively unchanging

Box 1 | How often do double-stranded DNA breaks occur?

The question of the frequency of spontaneous double-standed DNA breaks (DSBs) is not an easy one to answer experimentally. Recently, several laboratories[95–98] used cytogenetically detectable chromosome breaks as a reflection of the frequency of DSBs. (Chromatid breaks and ROBERTSONIAN TRANSLOCATIONS are also reflections of DSBs, and all of these events are included in the phrase 'chromosomal aberrations'.) The use of this assay as a reflection of DSBs is supported by the fact that mutations in most NHEJ components result in a marked increase in the frequency of chromosome aberrations. With this assay, 5–10% of first-passage primary fibroblasts from mice or humans have a chromosome aberration (mostly chromatid or chromosome breaks)[95,99]. These data are the best estimates of the frequency of DSBs in mammalian cells.

If the results from primary somatic cells apply to dividing cells *in vivo*, the frequency with which mutations in individual cells would arise from DSBs can be estimated. If each DSB takes 24 hours to repair (a high upper limit), then at the end of 70 years of human life, each cell would have about 2,300 imprecise repair sites distributed throughout the genome. This estimate is based on a rolling 9% chromosomal breaks (the most common breakage frequency determined for primary mouse fibroblasts)[95]. Assuming that 5% of the genome is crucial for information content (3% coding, plus 2% extra for local regulatory sequences), then in each diploid cell such a damage incidence would mean that 115 genes in each cell would be mutant. This would be a different 115 genes in each cell. In a genome with an estimated 30,000 genes, 115 mutant genes would correspond to one in every 260 genes being destroyed. Of course, each cell is diploid, so the loss of a given gene is mitigated by the remaining allele. However, the 50% reduction in production of some proteins would compromise function, based on the many examples of HAPLOINSUFFICIENCY in eukaryotic cells. Therefore, the overall function of the organism would be compromised.

These estimates will change as more precise information is obtained. A better estimate for the time it takes to repair a DSB is based on *in vivo* data in yeast and is about 0.5 hours (S. E. Lee and J. E. Haber, personal communication). This would increase the estimate of damaged sites per cell to 5,520, which would correspond to one gene out of every 5.4 genes in the genome. Even factoring in heterozygosity, 3.4% of the genes would be mutated at both alleles after 70 years of accumulated random double-stranded DNA damage/repair sites. Such a high level of DNA damage could contribute to the overall eventual decline of the organism. However, the length of time that it takes to repair a given damage site will probably depend on the chemical nature of the damage. For example, the removal of 3′-phosphoglycolate ends is estimated to require 10 hours (REF. 100).

level of sensitivity to ionizing radiation during the cell cycle[4,5]. This indicates that NHEJ is relatively stable, and that NHEJ enzyme activities might not change significantly during the cell cycle. Interestingly, repair of DSBs by homologous recombination is downregulated during G0, G1 and early S phases in the somatic cells of multicellular eukaryotes[5]. This means that NHEJ is the predominant — if not exclusive — mechanism for the repair of DSBs during G0, G1 and early S phases, and NHEJ continues to repair a minority of breaks during late S and G2 phases. The cell-cycle regulation of homologous recombination in multicellular eukaryotes is ideal in light of the problem of repetitive DNA. The only time that homologous recombination is used in mitotic cells is when the donor (which is the sister chromatid) is directly adjacent. Otherwise, NHEJ is used for repair of DSBs.

In light of the discovery of an important new nuclease for NHEJ[6], this is an ideal time to take a fresh look at how the various components of the NHEJ pathway function together. This review focuses on the actual proteins that are involved in NHEJ repair and on some features of the DNA that is repaired by this process.

ROBERTSONIAN TRANSLOCATION
A type of chromosomal translocation in which two acrocentric chromosomes become linked at their centromeres.

HAPLOINSUFFICIENCY
In a diploid organism, if both alleles are wild type or mutant, then the phenotype can be described as wild type or mutant. If one allele is mutant and the other is wild type, the organism typically appears wild type. However, for some genes, a phenotype arises even in these heterozygotes because half of the amount of the encoded protein is not sufficient. This is known as haploinsufficiency.

How do DSBs arise in mammalian cells?

It is well accepted that ionizing radiation (X-rays and gamma rays) can cause DSBs (as well as nicks) by creating clusters of reactive oxygen species (ROS; BOX 2). Two-hundred million gamma rays that derive from the decay of naturally occurring radionuclides in the earth pass through each of us every hour[7]. Other ROS, such as hydrogen peroxide and superoxide anions, could also potentially cause DSBs. One percent of the oxygen that we breathe is converted into oxidative free radicals — this amounts to more than 3 x 10^{22} free radicals per person every hour[8]. However, it is unclear how ROS cause DSBs. It could be that two independent ROS create nicks that happen to be close enough (<12 base pairs (bp)) to result in a DSB. Or a single ROS might cause free-radical damage on one DNA strand that results in a nick; then, owing to the chain-reaction nature of free-radical reactions, a secondary free radical at the initial damage site might react with the antiparallel DNA strand to cause a DSB (and, perhaps, an interstrand cross link).

Other causes of DSBs are DNA replication across a nick — the nicks might occur in the DNA due to exogenous sources or endogenous ones, such as ROS — and the malfunctioning of, or mis-recognition by, normal DNA-metabolic enzymes. The most clearly documented example of enzyme mis-recognition is the cleavage by the recombination-activating gene (RAG) COMPLEX at sites other than its normal variable (*V*), diversity (*D*) or joining (*J*) sites during *V(D)J* RECOMBINATION in lymphocytes[9–11] (BOX 3). This example is specific to vertebrate lymphocytes. How often more general enzymes, such as TYPE II TOPOISOMERASES, cause DSBs under normal physiological conditions is not clear. But when topoisomerase inhibitors are present, as in chemotherapy, for example, then interruption of the normal type II topoisomerase action can cause DSBs[12].

Basic biochemical features of NHEJ

When DSBs occur in DNA, the biochemical configuration of the broken ends can be very diverse, and they are typically incompatible. So, putting the two ends back together cannot usually be achieved by a simple ligation step.

Single-stranded DNA damage is less problematic because the other anti-parallel strand provides physical integrity and information to direct the accurate repair of the defective strand (by, for example, any of the excision-repair pathways). However, with DSBs, the DNA loses physical integrity and information content on both strands. For the two DNA ends to be processed so that they can be joined, they must be maintained in physical proximity (FIG. 2) — a step known as synapsis. When the two broken DNA ends are brought into proximity, the exact point of joining can occur at any base pair. There is a propensity for the joining to occur at sites that have 1–4 nucleotides that are complementary between the two ends[13]. Therefore, if the DNA sequence at one end is ---TTGGT* (where the dashes represent the rest of the chromosome and the asterisk represents the break terminus), and the other end begins as *cggcc---, then the sequence at the point of

joining has a higher than random probability of being ---TTGGcc---. This means that the 'T' at one end and the 'c' at the other end were removed, and the joining occurred at the two base pairs GG or gg, where the two ends shared these two nucleotides of microhomology.

This preference for homologous nucleotide joining-site selection at the junction arises because this is where the two DNA ends can base pair[13]. This tendency is called microhomology usage. It is not an essential feature, because two DNA ends that do not share microhomology can still be joined at normal efficiencies in mammalian cells[14–16], albeit at tenfold reduced efficiencies in *S. cerevisiae*[17–19]. Rather, the microhomology is a feature that biases the joining into a markedly preferred alignment, relative to numerous alternative alignments. This tendency for microhomology usage is seen in general DNA end-joining and in specialized physiological forms of DSB repair, such as at the coding ends in *V(D)J* recombination[15,16]. The signal-ends in *V(D)J* recombination are typically joined with no loss of nucleotides[20]. This could mean that NHEJ can be precise, and that it becomes imprecise only when precise end-joining is not an option (which would be most of the time for most pathological breaks and the coding ends in *V(D)J* recombination). Alternatively, signal-end precision might also mean that signal-ends are protected by the RAG complex bound at the two signals[21,22]. The latter view is supported by the fact that, in some NHEJ mutant cells, signal-ends become imprecise, even though they are formed at normal efficiency[23].

When the two DNA ends are not aligned at points of microhomology, there is often either excess DNA beyond the point of alignment that must be removed by nucleases, or there are gaps that must be filled using polymerases. We call this the DNA end-processing step. The intriguing feature about this step is that the two DNA ends must be held in proximity to prevent them from diffusing away from each another, and yet the means by which those ends are held must not prevent access by the nucleases and polymerases that process the DNA termini. The last step in restoring chromosomal integrity at a DNA break site is ligation of the DNA strands.

Synapsis: how are the DNA ends held together?

The most intriguing features of NHEJ are steric ones. For example, how are the two DNA ends maintained in proximity, while at the same time allowing access of the nucleases, ligases and polymerases that are necessary to configure the two DNA termini into a ligatable configuration?

The first protein to bind to the ends at a DSB might be Ku. Ku is a heterodimer of Ku70 and Ku86 (Ku86 is also called Ku80, and is actually 83 kDa). Ku is abundant in the cells (estimated at about 4 x 10^5 molecules per cell)[24], and has an equilibrium constant of about 5 x 10^{-10} M for DNA termini[25,26]. Once loaded onto a DNA terminus, Ku can diffuse to internal positions in the absence of NTPs[27,28]. The crystal structure for Ku reveals a form that is ideally suited to its function and biochemical properties[29]. A hole in the Ku protein is just large enough for dsDNA to pass through[29]. This allows primary contact between Ku and the DNA helix at the phosphate backbone in a sequence-independent manner. This TOROIDAL structure of Ku explains why Ku cannot dissociate from a linear DNA molecule that has then been circularized — the Ku protein does not have an end at which to exit[27].

Another protein that binds to DNA ends is a 469-kDa polypeptide known as the DNA-dependent protein kinase catalytic subunit (DNA-PK_{cs})[24]. This serine/threonine kinase is unique because it requires a DNA end as a cofactor to be active. Ku is thought to be about fivefold more abundant than DNA-PK_{cs} (REF. 24), and has a tighter binding to DNA[30]. In the absence of Ku, DNA-PK_{cs} binds to DNA termini with an equilibrium constant of about 3 x 10^{-9} M, and its kinase activity is activated. DNA-PK_{cs} activity is stimulated by Ku when the DNA is longer than 26 bp; otherwise, Ku inhibits DNA-PK_{cs} (REFS 30,31). The dissociation constant of DNA-PK_{cs} for DNA termini improves 100-fold to 3 x 10^{-11} M when Ku is already bound. DNA-PK_{cs}, when bound to a dsDNA end, can be active without Ku[28,31], even under physiological salt conditions[30]. Biochemical and physical studies indicate that Ku and DNA-PK_{cs} do not associate in the absence of a DNA terminus[28]. Low-resolution structures of DNA-PK_{cs} reveal an open central region, the role of which is uncertain[32,33]. It seems that the feature of dsDNA ends

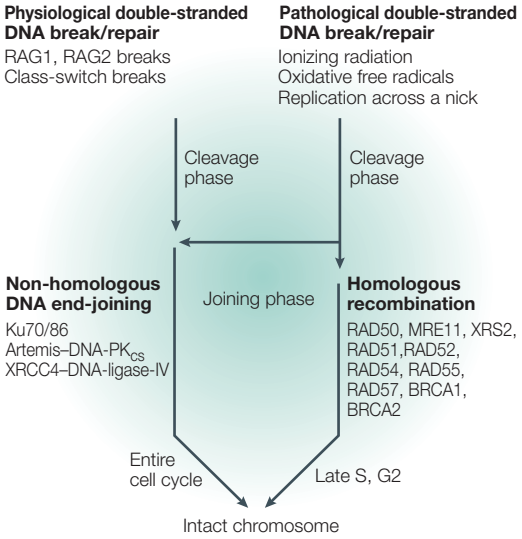


Figure 1 | **Physiological and pathological DNA breaks and their rejoining in vertebrates.** The physiological forms of double-stranded DNA breaks (DSBs) occur in *V(D)J* (variable (diversity) joining) recombination (mediated by recombination-activating gene (RAG)1 and RAG2) and in class-switch recombination in lymphocytes. (During the generation of haploid germ cells (meiosis), DSBs are introduced physiologically, and this is not listed.) One source of pathological breaks are free radicals generated from oxidative respiration. Pathological breaks are also caused by ionizing radiation and by replication across a nick. See BOX 2 for a more complete list of pathological causes of DSBs. BRCA, breast cancer; DNA-PK_{cs}, DNA-dependent protein kinase catalytic subunit; MRE11, meiotic recombination 11; XRCC4, X-ray cross complementation 4; XRS2, X-ray sensitive.

RAG COMPLEX
Immunoglobin heavy and light genes and T-cell-receptor genes are assembled from germline variable- and constant-region gene segments by a DNA recombination process in B and T cells, respectively. These gene rearrangements depend on the expression of recombination-activating genes (*RAG*)*1* and *RAG2*.

V(D)J RECOMBINATION
A specialized form of recombination that assembles the genes that encode lymphocyte antigen receptors from variable (*V*), diversity (*D*) and joining (*J*) gene segments. Double-stranded DNA breaks are introduced between the *V*, *D* and *J* segments and DNA-repair proteins then join the segments together.

TYPE II TOPOISOMERASE
Whereas type I topoisomerases nick one strand and thereby change supercoiling (actually the linking number) in steps of one, type II topoisomerases make a double-stranded DNA break, thereby changing the supercoiling in steps of two.

TOROIDAL
Doughnut-shaped.

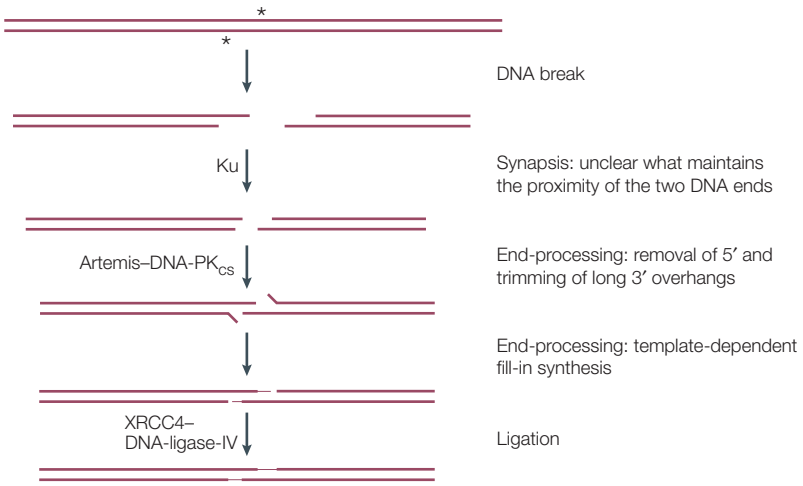


Figure 2 | **Non-homologous DNA end-joining and the proteins involved in vertebrates.** When a double-stranded DNA break (DSB) occurs, the ends must be held in proximity to allow subsequent repair steps to proceed and to align the two ends. This first step can be referred to as synapsis. Ku and the DNA-dependent protein kinase catalytic subunit (DNA-PK$_{cs}$) bind to DNA ends during this initial phase, although it is not clear how the synapsis occurs or what proteins specifically carry out this function. End-alignment can occur if there is terminal microhomology of, typically, 1–4 nucleotides between the two ends. This is an optional aspect, as non-homologous DNA end-joining (NHEJ) occurs regardless of microhomology. End-processing refers to the removal of DNA by the Artemis–DNA-PK$_{cs}$ complex and the filling in of gaps by polymerases. Ligation is the final step, and it requires a ligatable nick on each strand. Ligation in NHEJ is done by the XRCC4 (X-ray cross complementation 4)–DNA-ligase-IV complex.

that stimulates DNA-PK$_{cs}$ is the single-strandedness, which, for a blunt end, would arise from BREATHING of the DNA end[34].

The ability of Ku to inhibit or activate DNA-PK$_{cs}$, depending on the DNA terminus length, raises the question of what lengths are physiologically relevant. If a DSB occurs precisely between two typical nucleosomes spaced 40 bp apart, the DNA length from the broken end to the nucleosome would be only 20 bp. Positioning of the break further from one nucleosome places it closer to the adjacent nucleosome. If the break occurs on the DNA wrapped around a nucleosome, to what extent is the DNA peeled off the nucleosome to permit repair of the DSB? Alternatively, the nucleosome might be repositioned internally.

What holds the two DNA ends together? Several laboratories that have done atomic-force microscopy have pointed out the end-to-end DNA associations seen with Ku alone[28,35], with DNA-PK$_{cs}$ alone[28] or with both Ku and DNA-PK$_{cs}$ present[28,36]. However, it is unclear whether these protein–DNA interactions are the basis for physiological end-to-end association[28]. There is one report which indicated that purified Ku can non-covalently link incompatible DNA ends in a biochemical assay[37]. Another laboratory has reported that Ku has helicase activity[38]. Both of these biochemical observations are laboratory specific. In fact, the helicase observation has been refuted in another report[39]. The Ku end-to-end association seen by atomic-force microscopy has also not been confirmed by electron microscopy[40]. Recently, DNA-PK$_{cs}$ has been shown to provide a low level of synapsis activity[40]. Therefore, DNA-PK$_{cs}$ might be the best candidate for an end-to-end synapsis factor. However, this activity of DNA-PK$_{cs}$ seems to be limited to a very low ionic strength and a temperature of 4°C, and it will be interesting to see what conditions or other factors are important for this property.

Another candidate for holding the two DNA ends together after a DSB is the nucleosome itself. Out of every 200 bp, 160 bp are fully wrapped around the nucleosome. This means that only 40 bp are internucleosomal. However, even active chromatin might be organized (folded) into a 30-nm filament in which the 10-nm 'beads on a string' are further folded. So, a DSB might not allow the ends to diffuse apart.

Processing of two incompatibable broken ends

Pathological and physiological DSBs usually result in incompatible ends. Therefore, the rejoining typically requires nucleases to remove several nucleotides and polymerases to fill in gaps of several nucleotides (FIG. 2). As mentioned, many — but not all — joinings occur at regions of microhomology that are 1–4 nucleotides in length. These are nucleotides that, by chance, are shared between the two ends. In these cases, the excess DNA beyond the point of homology must be removed. Some of these excess single-stranded regions require 5′ nucleases, and others require 3′ nucleases. Pathological breaks generated by ionizing radiation or ROS would also typically generate oxidized nucleotides and sugars. Consequently, the 5′ and 3′ configurations would not be 5′ phosphates or 3′ hydroxyls. Oxidized sugars would often give rise to 3′ phosphoglycolates. There is some indication that polynucleotide kinase participates in the phosphorylation of 5′ ends, where necessary[41]. However, there would still be an important requirement for a nuclease for all but blunt ends. Any nuclease that might trim the 5′ and 3′ overhangs must be able to act on overhangs with non-standard compositions.

A new component gene, called *ARTEMIS*, was identified on the basis of its mutation in patients with human severe combined immunodeficiency (SCID)[42–44]. The gene name was meant to represent the affliction of children with an immunodeficiency; Artemis was the Greek goddess of the protection of children (as well as the goddess of hunting). The function of *ARTEMIS* at that time was not known. However, cells from *ARTEMIS*-null patients have *V*(*D*)*J* recombination features (successful signal-joint formation, but no coding-joint formation) that are indistinguishable from *DNA-PK$_{cs}$*-null mice[42,43].

Subsequently, it was determined that Artemis has nuclease activity[6]. This activity is limited to a 5′ exonuclease activity in the absence of DNA-PK$_{cs}$. However, Artemis and DNA-PK$_{cs}$ form a physical complex both *in vitro* and *in vivo*, and DNA-PK$_{cs}$ can phosphorylate Artemis[6] (FIG. 3). The Artemis–DNA-PK$_{cs}$ complex acts as an endonuclease at both 5′ and 3′ overhangs and trims 5′ overhangs with a distribution of products that has a strong preference for the site that perfectly blunts the end. At 3′ overhangs, by contrast, the overhang is clipped with a preference to leave a four- or

BREATHING
The hydrogen bonds within the Watson–Crick DNA helix are thought to transiently and focally 'melt'. Such breathing varies with the local sequence, particularly the A–T content. The length of DNA, duration and frequency of such sites of breathing has been studied for short test sequences, but is much less certain for longer stretches of DNA.

Box 2 | **Pathological causes of double-stranded DNA breaks**

Ionizing radiation (gamma rays)

Reactive oxygen species
- Superoxide anion
- Hydrogen peroxide
- Hydroxyl radical

DNA replication across a nick

Malfunction of endogenous enzymes
- *V(D)J* recombination (lymphoid cells)
- Class-switch recombination (B cells)
- Type II topoisomerases (especially when inhibitors are present, as in chemotherapy)

five-nucleotide single-stranded overhang. Mutations of Artemis in its β-lactamase fold abolish endonuclease activity, which indicates that this region might be at least partly responsible for the nuclease activity.

The Artemis–DNA-PK$_{cs}$ complex is also very efficient at opening DNA hairpins, with a preferential cutting position of two nucleotides 3′ to the hairpin tip[6]. This is extremely important in the context of *V(D)J* recombination (FIG. 2). The RAG1 and RAG2 proteins of *V(D)J* recombination generate DNA hairpins at the end of the DSB process at the *V*, *D* and *J* segments. The hairpins must be opened before the *V*, *D* or *J* segments can be joined by using the NHEJ pathway. It had been unclear for many years how the DNA hairpins were opened, and the Artemis–DNA-PK$_{cs}$ complex seems to be the exclusive mechanism for doing this. *Artemis*- or *DNA-PK$_{cs}$*-null mice accumulate intermediates of *V(D)J* recombination at the DNA-hairpin step[45]. So the genetic and biochemical data indicate that the Artemis–DNA-PK$_{cs}$ complex accounts for all or nearly all of the hairpin opening in *V(D)J* recombination. The extremely rare clones that arise in mice and in a small subset of human *ARTEMIS* mutants represent a severe reduction in *V(D)J* recombination. In most human *ARTEMIS*-mutant patients, no B or T cells are found in the peripheral blood, indicating that the block in *V(D)J* recombination is complete.

The common structural feature between DNA hairpins and 5′ and 3′ overhangs is single-stranded DNA just up- or downstream of double-stranded DNA, such that the Artemis–DNA-PK$_{cs}$ complex cuts about four nucleotides immediately 3′ to the single-stranded region[6]. The activation of DNA-PK$_{cs}$ requires a double-stranded DNA end. This is ideal for NHEJ because the only time that the dangerous nuclease activity of Artemis–DNA-PK$_{cs}$ is active is when a DSB arises. The joining of two DNA ends does not require long-range activation of proteins, because all of the enzymatic action that is essential is within a very local region.

Given the 5′ and 3′ overhang endonuclease cleavage activity of the Artemis–DNA-PK$_{cs}$ complex, is there a requirement for any other nucleolytic activities in NHEJ? Neither Artemis nor DNA-PK$_{cs}$ seems to be present outside of vertebrates. Therefore, NHEJ in plants, yeast and invertebrate animals must all rely on nuclease activities other than the Artemis–DNA-PK$_{cs}$ complex. It is clear that *ARTEMIS*-null humans and mice are highly sensitive to ionizing radiation, similarly to *DNA-PK$_{cs}$*-null mice[42,43,45]. If another nuclease is involved in vertebrate NHEJ, its contribution cannot be great enough to compensate for the ionizing radiation sensitivity that is observed in *ARTEMIS*- or *DNA-PK$_{cs}$*-null mammals. The RAD50–MRE11–NBS1 COMPLEX has been proposed to be a component of NHEJ in yeast and all other eukaryotes, on the basis that MRE11 (meiotic recombination 11) has 3′ nuclease activity[46–48]. However, the genetic evidence in mammals calls such a proposal into question. *V(D)J* recombination, which requires intact NHEJ for its joining phase, is normal in Nijmegen breakage syndrome (*NBS1*)-null cells or cells that are homozygous for shortened alleles of *RAD50* (REFS 49–51). The 5′ and 3′ overhang endonuclease activity of the Artemis–DNA-PK$_{cs}$ complex means that there is no need for another nuclease, and the radiation sensitivity of the *ARTEMIS*- and *DNA-PK$_{cs}$*-null mammals indicates that there might not be another efficient NHEJ nuclease. Nevertheless, it is difficult to be certain that there is no other nuclease in the absence of a complete set of pairwise genetic knockouts.

Is there a requirement for a polymerase in NHEJ? It would be assumed that a polymerase would make NHEJ more efficient by allowing gaps to be filled in during the joining of the two ends. However, NHEJ mechanistic schemes that do not require a polymerase for at least some NHEJ events could be proposed. For example, the Artemis–DNA-PK$_{cs}$ nuclease activity could simply resect DNA until the two DNA ends were compatible as blunt ends or with one- or two-nucleotide compatible overhangs. A knockout of the *S. cerevisiae POL4* gene affects the composition of nearly half of all junctional additions[19]. Therefore, the Pol4 protein participates in a fraction of gap fill-in during DNA end-joining in *S. cerevisiae*. The corresponding polymerase in multicellular eukaryotes has not yet been identified, although a physical interaction between the Ku–DNA–XRCC4–DNA-ligase-IV complex with polymerase-μ (pol-μ) has been reported[52]. In this regard, it is interesting that Pol4 and pol-μ are both Pol-X family members. There are at least 15 polymerases in mammalian cells[53], and genetic and biochemical data will be required to determine which are involved in NHEJ of multicellular eukaryotes.

Ligation at DSBs

There are two DNA ligases in *S. cerevisiae* (DNA ligases I and IV)[54], and three in mammalian cells (DNA ligases I, III and IV). A genetic knockout of DNA ligase IV in *S. cerevisiae* indicated that it is responsible for joining DSBs[54–56]. Concurrently, DNA ligase IV in mammalian cells was implicated as the ligase for DSB repair on the basis of its physical association with XRCC4 (REFS 57,58), which was shown in radiation-sensitivity studies to be important for NHEJ[59–62].

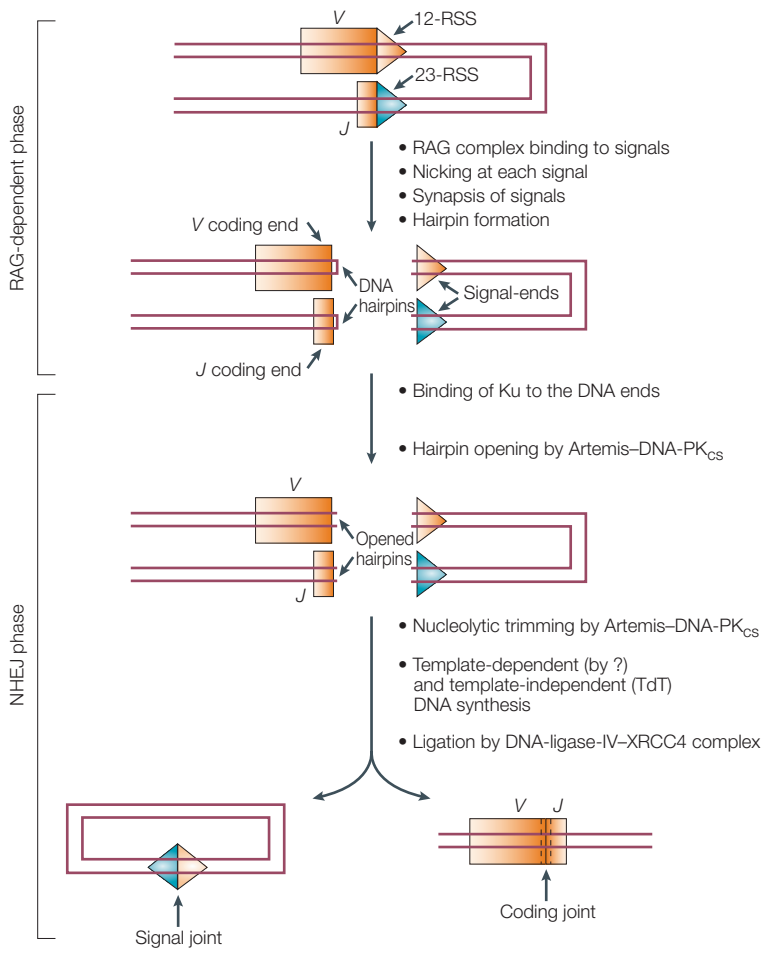
Confirmation that DNA ligase IV is the DSB repair ligase in mammalian cells required a genetic knockout. In one study, the two DNA ligase IV alleles were knocked

RAD50–MRE11–NBS1 COMPLEX
This complex is involved in homologous recombination, along with other proteins such as RAD51, RAD52, RAD54, RAD55, RAD57, XRCC2, XRCC3 and possibly BRCA1 and BRCA2. Patients who are mutant for *NBS1* (known as *XRS2* in yeast) have Nijmegen breakage syndrome, which includes microcephaly, increased malignancy and chromosomal instability. Mice with hypomorphic alleles of *Rad50* have normal *V(D)J* recombination[49–51], which indicates that this complex does not have a primary role in this pathway.

Box 3 | **Overview of *V(D)J* recombination**

V(D)J (variable (diversity) joining) recombination is a specialized double-stranded DNA break (DSB) and rejoining pathway which generates the exon that encodes the variable domain for immunoglobulins and T-cell receptors. Joining events in each pre-B cell and pre-T cell are the basis of the antigen-specific immune system of vertebrates. The RAG proteins, which generate the DNA breaks, are expressed only in early lymphoid progenitors. The ends are joined in a new configuration by the non-homologous DNA end-joining (NHEJ) pathway, which is present in all eukaryotic cells for purposes of general DSB repair, and which is used in vertebrate lymphocytes for rejoining the RAG-induced breaks into a new genomic configuration.

The recombination-activating gene (RAG)-dependent (DNA cleavage) phase begins when the RAG1, RAG2 and high-mobility group (HMG)1 proteins form a complex that binds at the recombination signal sequences (RSS, depicted as filled triangles) adjacent to each *V*, *D* or *J* element (depicted as filled rectangles). The RAG complex nicks the DNA at each signal. Two signals are brought into synapsis in accordance with the 12/23 rule, which simply means that a 12-RSS recombines better with a 23-RSS than it does with another 12-RSS. The 3′OH at each nick is then used as the nucleophile to attack the antiparallel strand at each site to generate a DNA hairpin at each *V*, *D* or *J* element. The Ku protein binds to some or all of the four DNA ends, perhaps thereby displacing the RAG complex. The Artemis–DNA-PK$_{cs}$ (DNA-dependent protein kinase catalytic subunit) complex, which is recruited to the DNA ends by Ku, opens the hairpinned *V*, *D* or *J* ends. The Artemis–DNA-PK$_{cs}$ complex then acts as an endonuclease (and perhaps Artemis acts as an exonucleolease) to trim the *V*, *D* or *J* ends to variable extents, thereby contributing to junctional diversity. If terminal deoxynucleotidyl transferase is present, as it is in most lymphoid progenitors, then template-independent DNA synthesis contributes to the junctional diversity.



Finally, the XRCC4 (X-ray cross complementation 4)–DNA-ligase-IV complex ligates the two DNA ends. The signal-ends are also joined together by the XRCC4–DNA-ligase-IV complex. The signal end-joining does not require the Artemis–DNA-PK$_{cs}$ complex, although abnormalities of signal joints can be found in cells that are defective for this complex.

out in a diploid human pre-B-cell line[63]. The cells became X-ray sensitive and could no longer carry out *V(D)J* recombination. Overexpression of DNA ligase I or III could not compensate for the absence of DNA ligase IV. In another study, DNA ligase IV was knocked out in mice[64]. The mice failed to develop lymphocytes with rearranged antigen receptors, and cells from them were X-ray sensitive.

The mouse knockout of *XRCC4* is identical to that of DNA ligase IV (REF. 65). XRCC4 seems to stabilize and enhance the activity of DNA ligase IV by interacting with it to form a physical complex[57,66,67]. XRCC4 readily forms a homodimer[68] or a homotetramer[69]. However, the native XRCC4–DNA-ligase-IV complex can purify as a heterotrimer (two XRCC4 molecules and one DNA ligase IV molecule), rather than as a higher-order complex[70]. Because XRCC4 alone can form a homotetramer, it has been speculated that the stoichiometry of the XRCC4–DNA-ligase-IV complex might be four XRCC4 molecules with two DNA ligase IV molecules (or two XRCC4 molecules and one DNA ligase IV molecule)[71].

The XRCC4–DNA-ligase-IV complex does not seem to form a stable complex with DNA, but it does so with a Ku–DNA complex[72,73]. On the basis of this, Ku might recruit the ligase complex, much as it recruits the Artemis–DNA-PK$_{cs}$ nuclease complex[6]. Consistent with the biochemical evidence for Ku–DNA interaction with the ligase complex are genetic data from a double-null mouse of *Ku86* and the DNA ligase IV gene[74]. In this mouse, the lethality of being DNA-ligase-IV null is overcome when the mouse is also null for *Ku86*. This is consistent with the possibility that — in a mouse that fails to recruit the ligase — the genetic loss of that ligase is inconsequential.

Regulation of NHEJ

The NHEJ system is ideally configured for responding to DSBs. If the density of Ku protein in a typical nucleus is calculated and a random distribution is assumed, then the average distance between two Ku molecules is only 4–6 times the Ku diameter. This means that there is a Ku molecule within 4–6 molecular diameters of

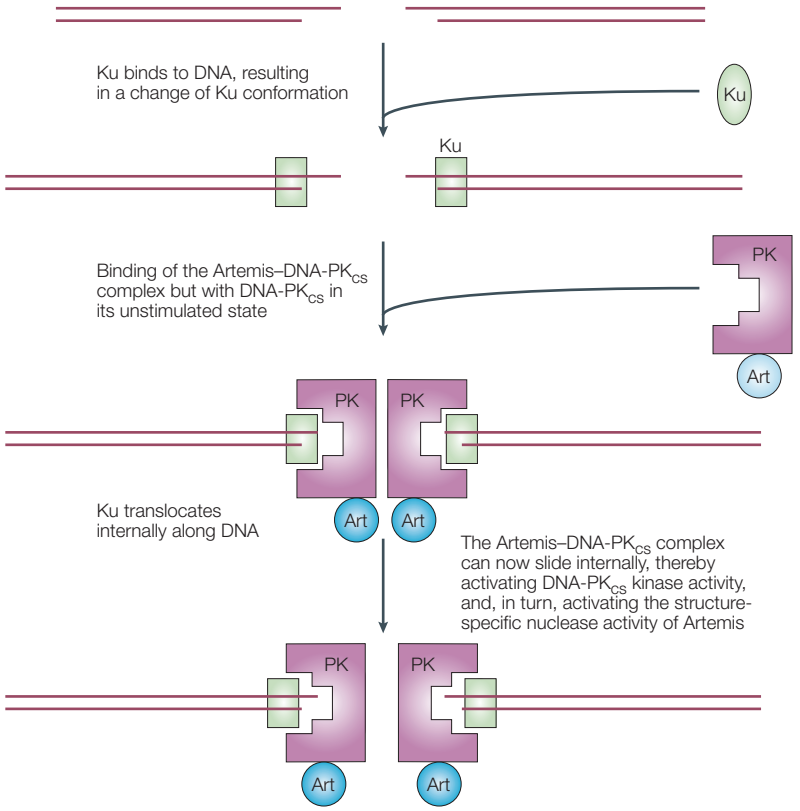


Figure 3 | **The Artemis–DNA-PK$_{cs}$ complex in NHEJ.** Ku loads on to DNA at a double-stranded DNA end. This results in a change in the conformation of Ku because now the Ku–DNA complex binds to the DNA-dependent protein kinase catalytic subunit (DNA-PK$_{cs}$) with an affinity that is 100-fold higher than the affinity of DNA for DNA-PK$_{cs}$ directly. *In vivo*, Artemis is in complex with DNA-PK$_{cs}$. So, it is likely that the recruitment of DNA-PK$_{cs}$ to the Ku–DNA end also recruits Artemis with it. After DNA-PK$_{cs}$ contacts the Ku–DNA-end complex, it becomes active as a protein kinase. Artemis is a key phosphorylation target of this kinase activity. This allows the Artemis–DNA-PK$_{cs}$ complex to be active as an endonuclease. Art, Artemis; PK, DNA-dependent protein kinase catalytic subunit; NHEJ, non-homologous DNA end-joining.

any potential DSB. So, early DSB detection by Ku is unlikely to be a rate-limiting step, especially given the high affinity of Ku for DNA ends[26,30]. Once Ku is bound, the affinity of DNA-PK$_{cs}$ (and potentially of the Artemis–DNA-PK$_{cs}$ complex) is markedly increased to 3 x 10^{-11} M (REFS 30,75). The contacting of DNA-PK$_{cs}$ with the DNA DSB provides the essential cofactor needed for DNA-PK$_{cs}$ function as a serine/threonine protein kinase[24]. We have already discussed how this 'alarm system' serves the cell mechanistically by allowing the activation of Artemis by phosphorylation[6].

How does activated DNA-PK$_{cs}$ affect the cell in other ways? This is still an active area of investigation, with many interesting findings that have yet to be fitted into a broader context. It seems that DNA-PK$_{cs}$ phosphorylation of itself at several sites causes the disassembly of the DNA–Ku–DNA-PK$_{cs}$ complex[76]. Mutation of one of these DNA-PK$_{cs}$ autophosphorylation sites has been reported to be defective in complementing ionizing-radiation sensitivity[77], raising the possibility that the inability of the DNA-PK$_{cs}$ molecule to dissociate from the DNA ends inhibits subsequent steps of NHEJ, such as ligation.

The actions of activated DNA-PK$_{cs}$ on Artemis and on itself represent local effects that influence the NHEJ mechanism. Are there more distant effects on the cell? Many proteins are phosphorylated *in vitro* by DNA-PK$_{cs}$ (REF. 24), and there is a growing list of proteins for which isolated reports argue for phosphorylation by DNA-PK$_{cs}$. It is too early to determine which of these is physiologically important. Does activated DNA-PK$_{cs}$ halt the cell cycle or trigger apoptosis? The answers to these questions are not yet clear, and they might depend on the number of DSBs. In yeast, one DSB is enough to cause a decrease in viability[78], but the DSB dose–response relationship in the cells of multicellular eukaryotes is less clear.

How is the decision made as to whether NHEJ or homologous recombination is used to repair a DSB? This is also an area of active investigation, and a comprehensive model cannot yet be formulated. Early *in vivo* work raised the possibility that there is competition between these two pathways, because elimination of Ku resulted in increased homologous recombination[79–81]. However, mutations in components of the XRCC4–DNA-ligase-IV complex increase homologous recombination in mammalian cells, which is contrary to the competition models in which Ku should suppress homologous recombination[82]. Moreover, in *S. cerevisiae*, ligase mutants do not result in suppression of homologous recombination[83]. These studies have raised the possibility that NHEJ is the initial pathway that is attempted and, when this fails, homologous recombination might take over[82,83]. In multicellular eukaryotes, the hand-off from a failed NHEJ event to the homologous-recombination pathway would require that the cells be in late S or G2 phase, when homologous recombination is active. A potential role for Werner's protein (WRN) has been indicated in the possible 'hand-off' of an unsuccessful NHEJ event to the homologous-recombination pathway[84,85].

Pathological effects of NHEJ defects

Inherited defects in NHEJ account for about 15% of human SCID, and nearly all of these are null mutations of *ARTEMIS*[6,42–44]. These patients are also sensitive to ionizing radiation.

Isolated failures in the physiological forms of double-stranded DNA breakage, such as *V(D)J* recombination, might result in chromosomal translocations that yield follicular lymphomas, and similar events could occur for CLASS-SWITCH RECOMBINATION in the case of sporadic Burkitt's lymphoma[86]. Regarding cancers in non-lymphoid cells, it is not yet clear how many mutations in tumour-suppressor genes, for example, are due to DSBs. Small deletions with some of the features of NHEJ account for 5–15% of mutations in p53 (M.R.L., unpublished observations).

There are several intriguing observations that relate defects in NHEJ to accelerated senescence. Cells that lack Ku seem to senesce in culture more quickly[87–89]. (It is interesting in this regard that Ku and WRN physically interact[39,90], and there is one report which showed that WRN and DNA-PK$_{cs}$ interact[91].) Mice that are mutant in either component of the DNA-ligase complex (XRCC4 or DNA ligase IV) show defects in *V(D)J* recombination[64,65],

CLASS-SWITCH RECOMBINATION
This is the process by which the immunoglobulin heavy-chain isotype changes from IgM to IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 or IgE in humans (and similar isotypes in mice and other vertebrates). It involves a double-stranded DNA break (DSB) at the switch regions (which are repetitive, G-rich and several kilobases in length) at the immunoglobulin heavy-chain locus. The DSBs are rejoined by some of the components of the non-homologous end-joining pathway.

just as human pre-B cells do[63]. However, the mice die during the final days of gestation. It is not yet clear why they die, but they show an increased apoptotic death of neurons at distinct locations at specific times during gestation. The reasons why some cells die and others do not is unclear. Interestingly, mice deficient for Ku70 show a depletion of enteric neurons[92]. Presumably, this apoptotic cell death is triggered by the inability to repair DSBs.

As mentioned earlier, the imprecision of NHEJ might contribute to normal ageing. Whether mutations in NHEJ contribute to accelerated ageing could depend on the type of enzyme activity that is missing. The various *NHEJ*-null mutants are heterogeneous for reasons that probably relate to the biochemistry of the NHEJ pathway[74]. That is, the lack of a ligase leaves the Artemis–DNA-PK$_{cs}$ nuclease present to cause unchecked rounds of degradation. By contrast, the lack of the Artemis–DNA-PK$_{cs}$ nuclease still leaves the XRCC4–DNA-ligase-IV complex, and the amount of DNA loss might not be as severe.

Therapeutically, inhibitors of NHEJ could be of great value as radiation sensitizers or in combination chemotherapy strategies. There are several inhibitors of DNA-PK$_{cs}$, at least one of which is being tested in cellular assay studies for its effectiveness in combination chemotherapy[93]. Clinically, the stratification of patients on the basis of their NHEJ pathway efficiency might allow the identification of those patients who have difficulty tolerating the normal doses of therapeutic radiation that are required to cure some cancers. Therefore, insights into the NHEJ pathway are likely to have a substantial clinical effect in the near future.

Concluding remarks

We have discussed some of the current developments that are central to the repair of DSBs by the NHEJ pathway. Regarding the mechanistic aspects, it will be important to establish which — if any — polymerases participate in NHEJ. If all of the central components for NHEJ have been identified, then reconstitution of these proteins in purified form would allow a more complete functional analysis of the interrelationships of the components, and a more in-depth analysis of how, for example, synapsis of the DNA ends is achieved. From a regulatory standpoint, DNA-PK$_{cs}$ is at least the principal component for the 'local' regulation of the NHEJ machinery. A greater understanding of the relationship between phosphorylation by DNA-PK$_{cs}$ of itself, of Ku, of Artemis, and possibly of other NHEJ components, will be crucial for understanding the details of the individual steps.

Is there a layer of NHEJ regulation that affects the cell beyond the local regulation by DNA-PK$_{cs}$? For NHEJ at a single DSB in a cell, it will be interesting to learn whether there is any slowing or halting of the cell cycle. For more abundant DSBs or for lesions that require more time to repair, it will be interesting to understand how this is integrated with other cellular processes, such as DNA replication or transcription[94].

During late S and G2 of the cell cycle, NHEJ and homologous recombination are both active. Is the choice of pathway during this time random or somehow orchestrated? Integretion of NHEJ with homologous recombination will be another interesting area. Is the WRN–Ku interaction part of such a link?[39,90] Early indications indicate that it might be[84,85].

Finally, reconstitution of NHEJ will facilitate the testing of inhibitors or modulators of NHEJ. These might be useful modulators in cancer, but also in non-neoplastic proliferation disorders. So, although the framework for NHEJ is progressing quickly, there is a vista of interesting basic questions and medical applications that lies ahead.

1. Haber, J. E. *In vivo* biochemistry: physical monitoring of recombination induced by site-specific endonucleases. *BioEssays* **17**, 609–620 (1995).
2. Roth, D. & Wilson, J. in *Genetic Recombination* (eds Kucherlapapti, R. & Smith, G. R.) 621–653 (American Society for Microbiology, Washington DC, 1988).
3. Critchlow, S. E. & Jackson, S. P. DNA end-joining: from yeast to man. *Trends Biochem. Sci.* **23**, 394–398 (1998).
4. Cheong, N., Wang, X., Wang, T. & Iliakis, G. Loss of S-phase-dependent radioresistance in irs-1 cells exposed to X-rays. *Mut. Res.* **314**, 77–85 (1994).
5. Takata, M. *et al.* Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J.* **17**, 5497–5508 (1998).
Determines the division of labour for the repair of DSBs at different times during the cell cycle.
6. Ma, Y., Pannicke, U., Schwarz, K. & Lieber, M. R. Hairpin opening and overhang processing by an Artemis:DNA-PKcs complex in *V(D)J* recombination and in nonhomologous end joining. *Cell* **108**, 781–794 (2002).
Describes the nuclease activity of Artemis and of the Artemis–DNA-PK$_{cs}$ complex.
7. National Radiation Protection Board. *Living with Radiation* (Reading, England, 1986).
8. Chance, B., Sies, H. & Boveris, A. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**, 527–603 (1979).
9. Raghavan, S. C., Kirsch, I. R. & Lieber, M. R. Analysis of the *V(D)J* recombination efficiency at lymphoid chromosomal translocation breakpoints. *J. Biol. Chem.* **276**, 29126–29133 (2001).
10. Marculescu, R., Le, T., Simon, P., Jaeger, U. & Nadel, B. *V(D)J*-mediated translocations in lymphoid neoplasms: a functional assessment of genomic instability by cryptic sites. *J. Exp. Med.* **195**, 85–98 (2002).
11. Lewis, S. M., Agard, E., Suh, S. & Czyzyk, L. Cryptic signals and the fidelity of *V(D)J* joining. *Mol. Cell. Biol.* **17**, 3125–3136 (1997).
12. Kirsch, I. R. (ed.) *The Causes and Consequences of Chromosomal Translocations* 277–309 (CRC, Ann Arbor, 1993).
13. Roth, D. B. & Wilson, J. H. Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. *Mol. Cell. Biol.* **6**, 4295–4304 (1986).
14. Gu, H., Förster, I. & Rajewsky, K. Sequence homologies, N sequence insertion and J_H gene utililization in $V_HD_HJ_H$ joining: implications for the joining mechanism and the ontogenetic timing of Ly1 B cell and B-CLL progenitor generation. *EMBO J.* **9**, 2133–2140 (1990).
15. Gerstein, R. M. & Lieber, M. R. Coding end sequence can markedly affect the initiation of *V(D)J* recombination. *Genes Dev.* **7**, 1459–1469 (1993).
16. Gerstein, R. M. & Lieber, M. R. Extent to which homology can constrain coding exon junctional diversity in *V(D)J* recombination. *Nature* **363**, 625–627 (1993).
17. Tsukamoto, Y., Kato, J. & Ikeda, H. Silencing factors participate in DNA repair and recombination in *S. cerevisiae*. *Nature* **388**, 900–903 (1997).
18. Wu, X., Wilson, T. E. & Lieber, M. R. A role for FEN-1 in nonhomologous DNA end joining. *Proc. Natl Acad. Sci. USA* **96**, 1303–1308 (1999).
19. Wilson, T. & Lieber, M. R. Efficient processing of DNA ends during yeast nonhomologous end joining: evidence for a DNA polymerase β (Pol4)-dependent pathway. *J. Biol. Chem.* **274**, 23599–23609 (1999).
Describes the genetic evidence for a specific polymerase in NHEJ.
20. Lieber, M. R., Hesse, J. E., Mizuuchi, K. & Gellert, M. Lymphoid *V(D)J* recombination: nucleotide insertion at signal joints as well as coding joints. *Proc. Natl Acad. Sci. USA* **85**, 8588–8592 (1988).
21. Agrawal, A. & Schatz, D. G. RAG1 and RAG2 form a stable postcleavage synaptic complex with DNA containing signal ends in *V(D)J* recombination. *Cell* **89**, 43–53 (1997).
22. Hiom, K. & Gellert, M. Assembly of a 12/23 paired signal complex: a critical control point in *V(D)J* recombination. *Mol. Cell* **1**, 1011–1019 (1998).
23. Lieber, M. R. *et al.* The defect in murine severe combined immune deficiency: joining of signal sequences but not coding segments in *V(D)J* recombination. *Cell* **55**, 7–16 (1988).
24. Anderson, C. W. & Carter, T. H. in *Molecular Analysis of DNA Rearrangements in the Immune System* (eds Jessberger, R. & Lieber, M. R.) 91–112 (Springer, Heidelberg, 1996).
25. Falzon, M., Fewell, J. & Kuff, E. L. EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single- to double-strand transitions in DNA. *J. Biol. Chem.* **268**, 10546–10552 (1993).
26. Mimori, T. & Hardin, J. A. Mechanism of interaction between Ku protein and DNA. *J. Biol. Chem.* **261**, 10375–10379 (1986).
27. deVries, E., vanDriel, W., Bergsma, W. G., Arnberg, A. C. & vanderVliet, P. C. HeLa nuclear protein recognizing DNA termini and translocating on DNA forming a regular DNA-multimeric protein complex. *J. Mol. Biol.* **208**, 65–78 (1989).

28. Yaneva, M., Kowalewski, T. & Lieber, M. R. Interaction of DNA-dependent protein kinase with DNA and with Ku: biochemical and atomic-force microscopy. *EMBO J.* **16**, 5098–5112 (1997).
29. Walker, J. R., Corpina, R. A. & Goldberg, J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* **412**, 607–614 (2001).
Describes the crystal structure of the doughnut-shaped Ku molecule.
30. West, R. B., Yaneva, M. & Lieber, M. R. Productive and nonproductive complexes of Ku and DNA-PK at DNA termini. *Mol. Cell. Biol.* **18**, 5908–5920 (1998).
31. Hammarsten, O. & Chu, G. DNA-dependent protein kinase: DNA binding and activation in the absence of Ku. *Proc. Natl Acad. Sci. USA* **95**, 525–530 (1998).
32. Chiu, C. Y., Cary, R. B., Chen, D. J., Peterson, S. R. & Steward, P. L. Cryo-EM imaging of the catalytic subunit of the DNA-dependent protein kinase. *J. Mol. Biol.* **284**, 1075–1081 (1998).
The first physical image of DNA-PK$_{cs}$.
33. Leuther, K. K., Hammarsten, O., Kornberg, R. D. & Chu, G. Structure of the DNA-dependent protein kinase: implications for its regulation by DNA. *EMBO J.* **18**, 1114–1123 (1999).
A low-resolution X-ray diffraction model of DNA-PK$_{cs}$.
34. Hammarsten, O., DeFazio, L. G. & Chu, G. Activation of DNA-dependent protein kinase by single-stranded DNA ends. *J. Biol. Chem.* **275**, 1541–1550 (2000).
Defines the types of end that activate DNA-PK$_{cs}$.
35. Pang, D., Yoo, S., Dynan, W. S., Jung, M. & Dritschilo, A. Ku proteins join DNA fragments as shown by atomic force microscopy. *Cancer Res.* **57**, 1412–1415 (1997).
36. Cary, R. B. *et al.* DNA looping by Ku and the DNA-dependent protein kinase. *Proc. Natl Acad. Sci. USA* **94**, 4267–4272 (1997).
37. Ramsden, D. A. & Gellert, M. Ku protein stimulates DNA end joining by mammalian DNA ligases: a direct role for Ku in repair of DNA double-strand breaks. *EMBO J.* **17**, 609–614 (1998).
38. Tuteja, N. *et al.* Human DNA helicase II: a novel DNA unwinding enzyme identified as the Ku autoantigen. *EMBO J.* **13**, 4991–5001 (1994).
39. Cooper, M. P. *et al.* Ku complex interacts with and stimulates the Werner protein. *Genes Dev.* **14**, 907–912 (2000).
40. DeFazio, L. G., Stansel, R. M., Griffith, J. D. & Chu, G. Synapsis of DNA ends by DNA-dependent protein kinase. *EMBO J.* **21**, 3192–3200 (2002).
41. Chappell, C., Hanakahi, L. A., Karimi-Busheri, F., Weinfeld, M. & West, S. C. Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. *EMBO J.* **21**, 2827–2832 (2003).
42. Moshous, D. *et al.* Artemis, a novel DNA double-strand break repair/*V(D)J* recombination protein, is mutated in human severe combined immune deficiency. *Cell* **105**, 177–186 (2001).
Identification of Artemis as a defective component in human SCID.
43. Moshous, D. *et al.* A new gene involved in DNA double-strand break repair and *V(D)J* recombination is located on human chromosome 10p. *Hum. Mol. Genet.* **9**, 583–588 (2000).
44. Nicolas, N. *et al.* A human severe combined immunodeficiency condition with increased sensitivity to ionizing radiation and impaired *V(D)J* rearrangements defines a new DNA recombination/repair deficiency. *J. Exp. Med.* **188**, 627–634 (1998).
45. Rooney, S. *et al.* Leaky *scid* phenotype associated with defective *V(D)J* coding end processing in Artemis-deficient mice. *Mol. Cell* **10**, 65–74 (2002).
The *Artemis*-knockout mouse.
46. Paull, T. T. & Gellert, M. The 3′ to 5′ exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. *Mol. Cell* **1**, 969–979 (1998).
47. Paull, T. T. & Gellert, M. Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes Dev.* **13**, 1276–1288 (1999).
48. Paull, T. T. & Gellert, M. A mechanistic basis for the Mre11-directed DNA joining at microhomologies. *Proc. Natl Acad. Sci. USA* **97**, 6409–6414 (2000).
49. Bender, C. F. *et al.* Cancer predisposition and hematopoietic failure in Rad50(S/S) mice. *Genes Dev.* **16**, 2237–2251 (2002).
50. Harfst, E., Cooper, S., Neubauer, S., Distel, L. & Grawunder, U. Normal *V(D)J* recombination in cells from patients with Nijmegen breakage syndrome. *Mol. Immunol.* **37**, 915–929 (2000).
51. Yeo, T. C. *et al. V(D)J* rearrangement in Nijmegen breakage syndrome. *Mol. Immunol.* **37**, 1131–1139 (2000).
52. Mahajan, K. N., Nick McElhinny, S. A., Mitchell, B. S. & Ramsden, D. A. Association of DNA polymerase μ (pol μ) with Ku and ligase IV: role for pol mu in end-joining double-strand break repair. *Mol. Cell. Biol.* **22**, 5194–5202 (2002).
53. Burgers, P. M. *et al.* Eukaryotic DNA polymerases: proposal for a revised nomenclature. *J. Biol. Chem.* **276**, 43487–43490 (2001).
54. Wilson, T. E., Grawunder, U. & Lieber, M. R. Yeast DNA ligase IV mediates non-homologous DNA end joining. *Nature* **388**, 495–498 (1997).
55. Schar, P., Herrmann, G., Daly, G. & Lindahl, T. A newly identified DNA ligase of *S. cerevisiae* involved in RAD52-independent repair of DNA double-strand breaks. *Genes Dev.* **11**, 1912–1924 (1997).
56. Teo, S. H. & Jackson, S. P. Identification of *S. cerevisiae* DNA ligase IV: involvement in DNA double-strand break repair. *EMBO J.* **16**, 4788–4795 (1997).
57. Grawunder, U. *et al.* Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. *Nature* **388**, 492–495 (1997).
58. Critchlow, S., Bowater, R. & Jackson, S. P. Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. *Curr. Biol.* **7**, 588–598 (1997).
59. Taccioli, G. E. *et al.* Impairment of *V(D)J* recombination in double-strand break repair mutants. *Science* **260**, 207–210 (1993).
60. Pergola, F., Zdzienicka, M. Z. & Lieber, M. *V(D)J* recombination in mammalian cell mutants defective in DNA double-strand break repair. *Mol. Cell. Biol.* **13**, 3464–3471 (1993).
61. Giaccia, A. J., Richardson, E., Denko, N. & Stamato, T. D. Genetic analysis of the XR-1 mutation in hamster and human hybrids. *Somat. Cell Mol. Genet.* **15**, 71–79 (1989).
62. Stamato, T. D., Weinstein, R., Giaccia, A. & Mackenzie, L. Isolation of cell-cycle dependent ã-ray sensitive Chinese hamster ovary cell. *Somat. Cell Mol. Genet.* **9**, 165–173 (1983).
63. Grawunder, U., Zimmer, D., Fugmann, S., Schwarz, K. & Lieber, M. R. DNA ligase IV is essential for *V(D)J* recombination and DNA double-strand break repair in human precursor lymphocytes. *Mol. Cell* **2**, 477–484 (1998).
64. Gao, Y. *et al.* A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* **95**, 891–902 (1998).
65. Frank, K. M. *et al.* Late embryonic lethality and impaired *V(D)J* recombination in mice lacking DNA ligase IV. *Nature* **396**, 173–177 (1998).
66. Herrmann, G., Lindahl, T. & Schar, P. *S. cerevisiae* LIF1: a function involved in DNA double-strand break repair related to mammalian XRCC4. *EMBO J.* **17**, 4188–4198 (1998).
67. Grawunder, U., Zimmer, D., Kulesza, P. & Lieber, M. R. Requirement for an interaction of XRCC4 with DNA ligase IV for wild-type *V(D)J* recombination and DNA double-strand break repair *in vivo*. *J. Biol. Chem.* **273**, 24708–24714 (1998).
68. Mizuta, R., Cheng, H. L., Gao, Y. & Alt, F. W. Molecular genetic characterization of XRCC4 function. *Int. Immunol.* **9**, 1607–1613 (1997).
69. Modesti, M., Hesse, J. E. & Gellert, M. DNA binding of XRCC4 is associated with *V(D)J* recombination but not with stimulation of DNA ligase IV activity. *EMBO J.* **18**, 2008–2018 (1999).
70. Robins, P. & Lindahl, T. DNA ligase IV from HeLa cell nuclei. *J. Biol. Chem.* **271**, 24257–24261 (1996).
71. Junop, M. S. *et al.* Crystal structure of the XRCC4 DNA repair protein and implications for end joining. *EMBO J.* **19**, 5962–5970 (2000).
72. Chen, L., Trujillo, K., Sung, P. & Tomkinson, A. E. Interactions of the DNA ligase IV-XRCC4 complex with DNA ends and the DNA-dependent protein kinase. *J. Biol. Chem.* **275**, 26196–26205 (2000).
73. Nick McElhinny, S. A., Snowden, C. M., McCarville, J. & Ramsden, D. A. Ku recruits the XRCC4–ligase IV complex to DNA ends. *Mol. Cell. Biol.* **20**, 2996–3003 (2000).
74. Karanjawala, Z. E. *et al.* The embryonic lethality in DNA ligase IV-deficient mice is rescued by deletion of Ku: implications for unifying the heterogeneous phenotypes of NHEJ mutants. *DNA Repair* **1**, 1017–1026 (2002).
75. Gottlieb, T. & Jackson, S. P. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell* **72**, 131–142 (1993).
76. Merkle, D. *et al.* The DNA-dependent protein kinase interacts with DNA to form a protein–DNA complex that is disrupted by phosphorylation. *Biochemistry* **41**, 12706–12714 (2002).
77. Chan, D. W. *et al.* Autophosphorylation of the DNA-dependent protein kinase catalytic subunit is required for rejoining of DNA double-strand breaks. *Genes Dev.* **16**, 2333–2338 (2002).
78. Bennett, C. B., Lewis, A. L., Baldwin, K. K. & Resnick, M. A. Lethality induced by a single site-specific double-strand break in a dispensible yeast plasmid. *Proc. Natl Acad. Sci. USA* **90**, 5613–5617 (1993).
79. Pierce, A. J., Hu, P., Han, M., Ellis, N. & Jasin, M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. *Genes Dev.* **15**, 3237–3242 (2001).
80. Fukushima, T. *et al.* Genetic analysis of DNA-PK reveals an inhibitory role of Ku in late S–G2 phase of DNA double-strand break repair. *J. Biol. Chem.* **276**, 44413–44418 (2001).
81. Adachi, N., Ishino, T., Ishii, Y., Takeda, S. & Koyama, H. DNA ligase IV-deficient cells are more resistant to ionizing radiation in the absence of Ku70: implications for DNA double-strand break repair. *Proc. Natl Acad. Sci. USA* **98**, 12109–12113 (2001).
82. Delacote, F., Han, M., Stamato, T. D., Jasin, M. & Lopez, B. S. An XRCC4 defect or Wortmannin stimulates homologous recombination specifically induced by double-strand breaks in mammalian cells. *Nucleic Acids Res.* **30**, 3454–3463 (2002).
83. Frank-Vaillant, M. & Marcand, S. Transient stability of DNA ends allows nonhomologous DNA end joining to precede homologous recombination. *Mol. Cell* **10**, 1189–1199 (2002).
84. Prince, P. R., Emond, M. J. & Monnat, R. J. Loss of Werner syndrome protein function promotes aberrant mitotic recombination. *Genes Dev.* **15**, 933–938 (2001).
85. Saintigny, Y., Makienko, K., Swanson, C., Emond, M. J. & Monnat, R. J. Homologous recombination resolution defect in Werner syndrome. *Mol. Cell. Biol.* **22**, 6971–6978 (2002).
86. Lieber, M. R. in *The Causes and Consequences of Chromosomal Translocations* (ed. Kirsch, I.) 239–275 (CRC Press, Boca Raton, 1993).
87. Nussenzweig, A. *et al.* Requirement for Ku80 in growth and immunoglobulin *V(D)J* recombination. *Nature* **382**, 551–555 (1996).
88. Gu, Y. *et al.* Growth retardation and leaky SCID phenotype of Ku70-deficient mice. *Immunity* **7**, 653–665 (1997).
89. Vogel, H., Lim, D.-S., Karsenty, G., Finegold, M. & Hasty, P. Deletion of Ku86 causes early onset of senescence in mice. *Proc. Natl Acad. Sci. USA* **96**, 10770–10775 (1999).
90. Li, B. & Comai, L. Functional interaction between Ku and the Werner syndrome protein in DNA end processing. *J. Biol. Chem.* **275**, 28349–28352 (2000).
91. Yannone, S. M. *et al.* Werner syndrome protein is regulated and phosphorylated by DNA-dependent protein kinase. *J. Biol. Chem.* **276**, 38242–38248 (2001).
92. Li, G. C. *et al.* Ku70: a candidate tumor suppressor gene for murine T cell lymphoma. *Mol. Cell* **2**, 1–8 (1998).
93. Veuger, S. J., Curtin, N. J., Richardson, C. J., Smith, G. C. M. & Durkacz, B. W. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. *Cancer Res.* (in the press).
94. Rouse, J. & Jackson, S. P. Interfaces between the detection, signaling and repair of DNA damage. *Science* **297**, 547–551 (2002).
95. Karanjawala, Z. E., Grawunder, U., Hsieh, C.-L. & Lieber, M. R. The nonhomologous DNA end joining pathway is important for chromosome stability in primary fibroblasts. *Curr. Biol.* **9**, 1501–1504 (1999).
96. Karanjawala, Z., Murphy, N., Hinton, D. R., Hsieh, C.-L. & Lieber, M. R. Oxygen metabolism causes chromosome breaks and is associated with the neuronal apoptosis observed in double-strand break repair mutants. *Curr. Biol.* **12**, 397–402 (2002).
97. Difilippantonio, M. J. *et al.* DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* **404**, 510–514 (2000).
98. Gao, Y. *et al.* Interplay of p53 and DNA repair protein XRCC4 in tumorigenesis, genomic instability and development. *Nature* **404**, 897–900 (2000).
99. Martin, G. M., Smith, A. C., Ketterer, D. J., Ogburn, C. E. & Disteche, C. M. Increased chromosomal aberrations in first metaphases of cells isolated from the kidneys of aged mice. *Israel J. Med. Sci.* **21**, 296–301 (1985).
100. Bertoncini, C. R. & Meneghini, R. DNA strand breaks produced by oxidative stress in mammalian cells exhibit 3′-phosphoglycolate termini. *Nucl. Acids Res.* **23**, 2995–3002 (1995).

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DNA ligase I | DNA ligase III | DNA ligase IV | MRE11 | *NBS1* | polymerase-μ | *RAD50* | RAD54 | RAG1 | RAG 2 | type II topoisomerase | XRCC2 | XRCC4

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ARTEMIS | DNA-PK$_{cs}$ | Ku70 | Ku86 | p53 | Pol4 | WRN

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