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# Modernizing the Nonhomologous End-Joining Repertoire: Alternative and Classical NHEJ Share the Stage

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## Keywords

classical nonhomologous end joining, alternative nonhomologous end joining, DNA damage response, DNA double-strand breaks, V(D)J recombination, genomic instability

## Abstract

DNA double-strand breaks (DSBs) are common lesions that continually threaten genomic integrity. Failure to repair a DSB has deleterious consequences, including cell death. Misrepair is also fraught with danger, especially inappropriate end-joining events, which commonly underlie oncogenic transformation and can scramble the genome. Canonically, cells employ two basic mechanisms to repair DSBs: homologous recombination (HR) and the classical nonhomologous end-joining pathway (cNHEJ). More recent experiments identified a highly error-prone NHEJ pathway, termed alternative NHEJ (aNHEJ), which operates in both cNHEJ-proficient and cNHEJ-deficient cells. aNHEJ is now recognized to catalyze many genome rearrangements, some leading to oncogenic transformation. Here, we review the mechanisms of cNHEJ and aNHEJ, their interconnections with the DNA damage response (DDR), and the mechanisms used to determine which of the three DSB repair pathways is used to heal a particular DSB. We briefly review recent clinical applications involving NHEJ and NHEJ inhibitors.

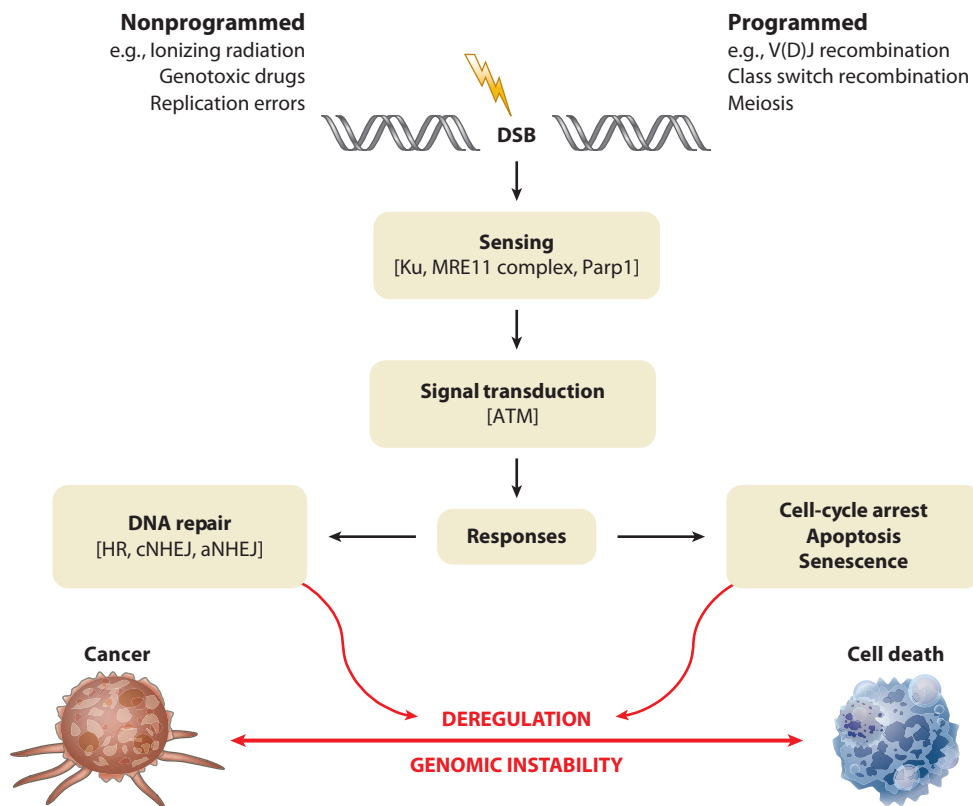
**DSB:** double-strand break

## INTRODUCTION

DNA double-strand breaks (DSBs), although common, are extremely dangerous (**Figure 1**). Unlike most other DNA lesions, DSBs directly threaten genomic integrity by disrupting the physical continuity of the chromosome. Without repair, all genetic material telomeric to the break is lost at the next cell division. A second particular threat posed by DSBs arises from repair mechanisms themselves, which, if not executed properly, possess formidable power to wreak genomic havoc. Misrepair of DSBs can, of course, cause localized sequence alterations and loss of genomic material. Arguably more dangerous, however, is inappropriate joining of the wrong pair of DNA ends. Inappropriate joining can generate interstitial deletions, inversions, and chromosome translocations,

which can initiate neoplastic transformation by a variety of mechanisms. One is through formation of chimeric oncogenes, the classic example being the translocation between chromosomes 9 and 22 in chronic myeloid leukemia (CML), forming the Philadelphia chromosome (86) and creating the novel *bcr-abl* oncogene. Indeed, studies done before cancer genome sequencing became commonplace identified more than 300 gene fusions in human neoplasms, and these are believed to account for approximately 20% of human cancer (83). This number is likely to grow as more cancer genomes are studied in depth.

Complex genomic rearrangements are also initiated by joining two centromere-bearing chromosome fragments together. This initiates a cascade of persistent cycles



**Figure 1**

Schematic of cell responses, and their potential pitfalls, to double-strand breaks (DSBs). Abbreviations: aNHEJ, alternative nonhomologous end joining; ATM, ataxia telangiectasia mutated; cNHEJ, classical nonhomologous end joining; HR, homologous recombination.

of chromosome fragmentation and aberrant rejoining (81), leading to complex deletions/translocations/amplifications termed complications (149). Such complex rearrangements often accompany gross chromosome rearrangements (149). Even more complex genome scrambling arises from a recently described process termed chromothripsis, defined as chromosome shattering and reassembling, which involves multiple simultaneous DSBs and misrepair. Again, cancer-causing lesions can emerge from such catastrophic genomic events (47).

### COMMON LESIONS WITH MANY SOURCES

Given the dire consequences that can attend the failure to properly repair broken DNA ends, it is (at least from the perspective of cancer biologists) unnerving that DSBs occur quite frequently. Endogenous sources of DSBs include fragile sites, errors in DNA metabolism (e.g., replication across single-strand nicks and replication fork collapse), endogenous nucleases, programmed genome rearrangements, physical forces, and reactive oxygen species. Numerous DSBs of exogenous origin, both natural (e.g., cosmic rays, terrestrial background radiation, certain viruses) and man-made (e.g., weapons of mass destruction and diagnostic and therapeutic maneuvers), threaten the genome. The latter category includes diagnostic radiographs, radiation therapy, genotoxic drugs used for cancer therapy, and gene-therapy strategies, which can employ nucleases to induce DSBs in a desired target sequence (27, 45) but which can also cleave unintended (off-target) sequences (92). An interesting estimate of the number of DSBs attending some of these exposures follows: a 10-hour flight from Philadelphia to Paris results in 0.05 DSB per cell; a body CT scan, 0.3 DSB per cell; the Chernobyl accident, 12 DSBs per cell (on average); external beam radiotherapy (typical single dose of 1,800–2,000 mSv), 80 DSBs per cell (32). Clearly, the integrity of our DSB repair systems is tested on an ongoing basis.

### CHALLENGES FACED BY DOUBLE-STRAND-BREAK REPAIR SYSTEMS

Given the foregoing discussion, we can postulate some basic requirements for an effective DSB repair system: (a) high sensitivity, allowing detection of a single DSB, and the ability to do so rapidly enough to allow proper repair before a catastrophic event occurs; (b) a great degree of specificity—the system must detect DSBs but not nicks, mismatches, abasic sites, or interstrand crosslinks, which require distinct repair systems; (c) the ability to repair breaks with a high degree of fidelity (without too much collateral genomic damage); (d) the capacity to repair a variety of different kinds of DNA ends, including those that are not directly ligatable (produced, for example, by certain forms of irradiation and by reactive oxygen species); and (e) the ability to coordinate DSB repair with the physiological state of the cell (e.g., the timing of repair with respect to cell-cycle status).

These requirements are further complicated by genome size, structure, and organization. Mammalian genomes are large, requiring DSB repair systems to possess extraordinary sensitivity (one part per billion) and to be able to consistently detect individual molecular signals (such sensitivity parallels another sensory system, the rod photoreceptor, which can respond to single photons). A second challenge is posed by genomic heterogeneity. The accessibility of a particular DNA sequence varies widely with genomic context and/or physical location. Surveillance mechanisms must reliably detect and repair all DSBs, even those in less accessible locations, such as highly condensed chromatin and specialized subnuclear compartments (although not necessarily with the same rapidity). A third difficulty is raised by the fact that mammalian genomes contain a high proportion of dispersed, highly repetitive DNA sequences. Repair of breaks located in such sequences by homology-directed mechanisms could lead to genome scrambling. These considerations may underlie the evolutionary decision to employ multiple mechanisms for DSB repair and to

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**DNA damage response (DDR):**

multiprotein complex recruited to the DNA break, where it activates signaling pathways that lead to cell-cycle arrest and repair or cell death

**DNA-PK:**

DNA-PKcs + Ku

**DNA-PKcs:**

DNA-dependent protein kinase catalytic subunit

**Ku:** Ku70-Ku80 heterodimer

**ATM:** ataxia

telangiectasia mutated

**MRE11 complex (or**

**MRN):** MRE11-RAD50-NBS1

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exercise careful control over which of these is used to heal a particular DSB at a given time, in a given cell, and at a particular genomic location.

## DOUBLE-STRAND-BREAK SENSING AND THE DNA DAMAGE RESPONSE

Upon sensing a DSB, cells orchestrate a rapid, wide-ranging set of responses that affect many aspects of cellular physiology (e.g., transcription, chromatin remodeling, cell-cycle arrest, senescence, and apoptosis) (32). This DNA damage response (DDR) is mediated by the phosphoinositol-3-kinase-like protein kinases (PIKKs) DNA-PK (DNA-PKcs + Ku), ATM (ataxia telangiectasia mutated), and ATR (ATM and Rad-3 related), and by PARP1 and PARP2, which are members of the poly(ADP-ribose) polymerase (PARP) family (32). DNA-PK and ATM are activated by DSBs, whereas ATR is recruited to stalled replication forks. ATM and the MRE11 complex (consisting of three proteins: MRE11, NBS1, and RAD50) are key players early in DDRs, and an intricate cross talk between these two entities appears to be required for downstream signaling events (104) (**Figure 1**). The MRE11 complex is loaded onto DNA ends and can recruit ATM (70). Cells deficient for MRE11 complex components show defects in ATM activation and in localization of ATM to the sites of breakage (127).

Once activated, ATM and ATR phosphorylate a variety of mediator proteins, which amplify the damage signal by recruiting additional proteins, including additional PIKK substrates (32). Once bound at a DSB, ATM initiates a signaling cascade that regulates DNA repair, cell-cycle checkpoints, and chromatin structure. ATM and ATR regulate many cellular processes, including replication and repair, and the complex network of signaling events integrates the response to DSBs with the replication and metabolic status of the cell, helping to ensure that the appropriate repair pathway is chosen (32). Recent work has implicated these PIKKs in the regulation of additional metabolic pathways (32, 116).

One central mediator of cellular responses is p53, which is activated by DSBs through the kinase activities of ATM and downstream effectors. p53 regulates many potential outcomes, including cell-cycle arrest, apoptosis, and senescence, all of which are responses that appear to minimize the dangers to the cell (or to the organism as a whole) posed by genomic instability (127) (**Figure 1**). The DDR also modifies chromatin in the vicinity of a DSB, including phosphorylation of the histone variant H2AX to form  $\gamma$ -H2AX, which localizes to a large region (~2 Mb in higher eukaryotes) on either side of the break (95). This, in turn, facilitates recruitment of other factors, resulting in assembly of large, multiprotein complexes (32), which may play roles in damage signaling, repair, and holding the DNA ends together, minimizing opportunities for aberrant rearrangements. Activation of the ATM kinase at DSBs also leads to chromatin relaxation, which may be important for allowing access of the repair machinery (151), and represses transcription in the vicinity of the DSB (115).

## THREE SPECIALIZED MECHANISMS FOR REPAIRING DOUBLE-STRAND BREAKS

Given the variety of situations in which DSBs may be formed (e.g., during meiosis, during DNA replication, and in terminally differentiated, nonreplicating cells), the presence of several specialized repair systems is not surprising.

### Homologous Recombination: Template-Directed Repair

Pioneering studies in bacteria and yeast revealed repair pathways that use extensive sequence identities (sequence homology) to template repair. These mechanisms are collectively termed homologous recombination (HR) (129). HR mechanisms are regarded as less error prone than other DSB repair mechanisms because they employ a template to direct repair—the sister chromatid or homolog—so that DSBs or even gaps can be repaired

seamlessly and without the loss of genomic information. A key step in initiation of HR is exonucleolytic resection, which generates long single-stranded tails, the critical intermediates for initiating homologous pairing (129).

### **Classical Nonhomologous End Joining: “Willy-Nilly” End Joining**

By the early 1980s, HR mechanisms were well known and were viewed as safe mechanisms for repairing DSBs, given the requirement for a homologous template to direct repair. This, along with the observation that laboratory strains of *Escherichia coli* lacked the capacity to efficiently join broken DNA ends by ligation (79), made the surprising discovery that mammalian cells efficiently join unrelated DNA fragments together end-to-end in a “willy-nilly” fashion (137). This repair mechanism became known as nonhomologous end joining (NHEJ), although it has now been renamed classical NHEJ (cNHEJ). cNHEJ does not require sequence homology, although very short sequence homologies (microhomologies) of a few nucleotides can appear at the junctions and may help to align ends (110). Extra nucleotides often appear at junctions and arise from a variety of mechanisms (106, 109). cNHEJ appears to be the dominant repair pathway used in mammalian cells and is active throughout the cell cycle, particularly in G0/G1 (111). In the past decade, cNHEJ components and functional end-joining ability have been discovered in phylogenetically distinct prokaryotes, including mycobacteria and *Bacillus subtilis* (8, 118, 136), indicating that cNHEJ is more evolutionarily conserved than initially realized.

### **Alternative Nonhomologous End Joining: Poorly Understood, Less Faithful, and More Dangerous**

The third and most recently discovered category of the DSB repair mechanisms goes by a variety of names: alternative NHEJ (alt NHEJ or aNHEJ), microhomology-mediated end joining (MMEJ), and B (backup)-NHEJ. aNHEJ was discovered in yeast and in mam-

malian cells at approximately the same time as was a backup system capable of repairing DSBs in cells with genetic deficiencies for one (or more) of the factors critical for cNHEJ (21, 64, 73). The subsequent discovery of aNHEJ in cNHEJ-proficient cells, as discussed below, indicated that it is not simply a backup pathway used by cells to allow survival in the absence of cNHEJ.

Three features characterize aNHEJ. First, the junctions generally reveal excessive deletions and frequent microhomologies, although microhomologies are not invariably present. Second, aNHEJ is much less faithful than cNHEJ, as it commonly leads to chromosome translocations (57, 120, 149). Third, aNHEJ, attended by the characteristics described above, occurs in cells deficient for cNHEJ. The molecular events initiating aNHEJ are poorly understood, although both PARP1 [which competes for free DNA ends with the Ku heterodimer of cNHEJ (**Figure 2**), has been implicated in DNA damage sensing (58), and can interact with ATM (2)] and the MRE11 complex appear to play important roles (13). Future studies should clarify whether what we currently term aNHEJ describes a single pathway or is a category containing multiple mechanisms (such as, for example, a distinct pathway that requires microhomologies).

### **MECHANISM OF REPAIR BY CLASSICAL NONHOMOLOGOUS END JOINING**

At first glance, cNHEJ appears relatively straightforward: It joins DNA ends by ligation, without requiring a complicated search for an appropriate homologous repair template. Interesting questions, however, remain. How are ends that are not blunt or self-complementary modified to allow ligation (e.g., ends produced by radiation damage often bear chemical modifications that necessitate considerable processing to render them ligatable)? How are the two ends generated by a particular DSB maintained as a pair to prevent inappropriate joining to other ends that may coexist (many forms of

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**NHEJ:**  
nonhomologous end joining

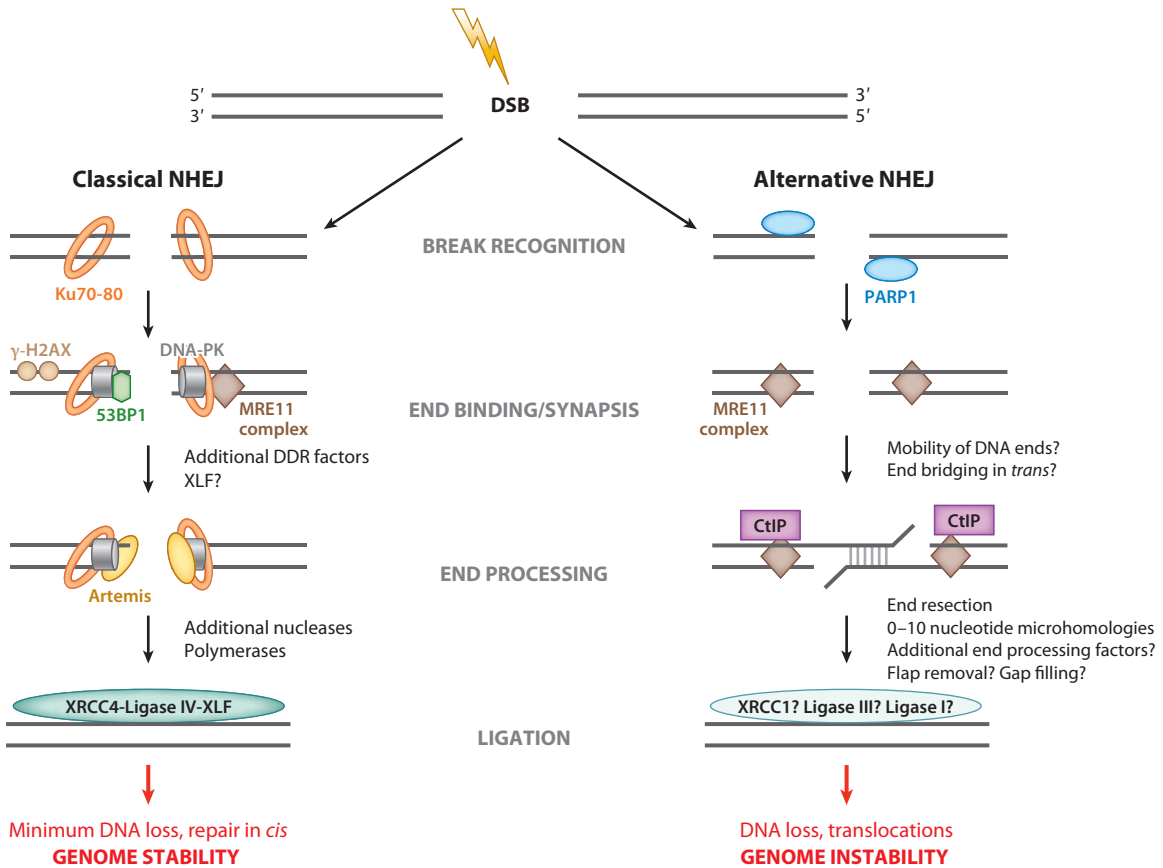
**Classical NHEJ (cNHEJ):** DNA end joining using a defined pathway requiring Ku, DNA-PKcs, Artemis, XRCC4, ligase IV, and Cernunnos/XLF

**Alternative NHEJ (aNHEJ):** DNA end joining independent of the known cNHEJ factors. Translocation prone with the tendency to employ short sequence homologies (microhomologies) to direct joining

**Microhomology:** short stretches (1–10 nucleotides) of DNA sequence identity used to guide repair by cNHEJ and aNHEJ

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**Figure 2**

Classical and alternative nonhomologous end-joining (NHEJ) pathways. Abbreviations: DDR, DNA damage response.

damage produce multiple DSBs per cell)? How are ends specified to undergo repair by cNHEJ instead of HR or aNHEJ? Answers to some of these questions have emerged from numerous studies over the past two decades (**Figure 2**).

### Loading NHEJ Factors onto DNA Ends

Core cNHEJ factors include Ku and the DNA ligase complex XRCC4-ligase IV-XRCC4-like factor (XLF; also called Cernunnos). Core cNHEJ factors are conserved from yeast to mammalian cells, and some prokaryotes also possess NHEJ capability involving Ku and DNA ligase D (an ATP-dependent DNA ligase) (118).

cNHEJ is initiated by the binding of the Ku heterodimer to the broken DNA ends, creating a scaffold for the recruitment of other factors, including DNA-PKcs, XRCC4-ligase IV-XLF, Artemis, and DNA polymerases. Ku is abundant ( $\approx 400,000$  molecules per cell), and has high affinity for DNA with a variety of end structures, including blunt ends, 5' or 3' overhangs, and covalently sealed hairpins (43). Ku70 and Ku80 form a symmetrical, heterodimeric ring that encircles duplex DNA with little direct contact with the DNA backbone or bases (135). Once bound to an end, Ku can translocate along the molecule, allowing multiple Ku heterodimers to load onto linear DNA (43). After ligation, the resulting Ku-DNA complex is extremely stable and theoretically could

be trapped on the DNA molecule after ligation (43). Recent evidence suggests that Ku80 is removed from DNA through a ubiquitin-mediated process (97). Because the DNA-Ku complex is able to recruit many enzymes, it has been difficult to dissect the timing of the recruitment of DNA repair factors to the DNA ends. However, extensive evidence supports the idea that the access is at least partially mediated by a functional DNA-Ku-DNA-PKcs tripartite complex, indicating that the DNA-PK complex must form at DNA ends at an earlier stage and that the enzymes that process and ligate join the DNA repair reaction at a later stage.

### DNA-PKcs

Ku initially interacts with the distal termini of DNA ends (43), protecting them from aberrant end resection and from aNHEJ (see below). Once DNA-PKcs is recruited, Ku translocates inward, by about one helical turn, allowing DNA-PKcs to contact an approximately 10-bp region at both termini (143). Upon binding to the Ku-DNA complex, DNA-PKcs phosphorylates substrates (see below), promoting synapsis of DNA ends and facilitating recruitment of end processing and ligation enzymes (85). Although it is not clear whether end bridging through synapsis is required for full activation of the kinase, Ku is required for targeting DNA-PKcs to DNA breaks and to fully stimulate its kinase activity (85). Kinase activity is important for NHEJ: Eliminating the catalytic activity of DNA-PK sensitizes cells to DSB-inducing agents and blocks repair of recombination activating gene (RAG)-mediated DSBs generated during variable (diversity) joining [V(D)J] recombination (85).

A number of DNA-PKcs substrates have been identified, including Ku, Artemis, XRCC4, ligase IV, and XLF, but phosphorylation of these proteins individually does not seem to affect NHEJ, suggesting functional redundancy between these numerous phosphorylation sites (85). A recent study supports this idea; DNA-PKcs-dependent phosphorylation of XRCC4 and XLF is functionally

redundant and might function to promote XRCC4-XLF complex dissociation (112). Another relevant target of phosphorylation by DNA-PKcs is autophosphorylation of the catalytic subunit itself. Autophosphorylation of DNA-PKcs in *trans* across the DSB in the synaptic complex appears to increase the ability of DNA end-processing enzymes and ligases to access DNA ends, suggesting a structural change of the holoenzyme and/or a dissociation of DNA-PKcs after autophosphorylation (42). The autophosphorylation of two clusters of residues, termed ABCDE and PQR, seems to be an important requirement for regulating DNA end access (85). Interestingly, whereas phosphorylation within the DNA-PKcs ABCDE cluster promotes access to DNA ends, phosphorylation of the PQR cluster inhibits access (85). These results indicate that access of processing enzymes to DNA ends is tightly regulated. This regulation may limit unsafe repair of DNA breaks through, for example, homologous recombination outside the S/G2 phases of the cell cycle, or it may discourage aNHEJ (85, 117).

### End Processing

Aligned, compatible DNA termini that possess a 5' phosphate and a 3' hydroxyl can be directly ligated by the cNHEJ factors described above. However, more complex DNA ends, such as those produced by irradiation or reactive oxygen species, or the hairpin coding ends produced during V(D)J recombination, cannot be directly joined. Therefore, cNHEJ requires additional enzymes to prepare such DNA termini for ligation. One such enzyme is Artemis, discovered as the gene mutated in certain radiosensitive severe combined immunodeficiency (RS-SCID) patients (84). Once the endonuclease activity of Artemis is stimulated by DNA-PK, it carries out hairpin opening (77). Consequently, mice deficient in either Artemis or DNA-PKcs are defective for hairpin opening and accumulate hairpin coding ends during V(D)J recombination (103). Interestingly, RS-SCID patients and *Artemis*<sup>-/-</sup> mice also exhibit

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**RAG:** recombination activating gene

**Variable (diversity) joining [V(D)J]**

**recombination:** process of somatic recombination initiated by the RAG1/2 recombinase by which the V (variable), D (diversity), and J (joining) segments of immunoglobulin genes or T-cell receptor genes are assembled during the development of lymphocytes

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increased sensitivity to DNA-damaging agents, and Artemis is hyperphosphorylated after ionizing radiation (34), indicating an additional role for Artemis in NHEJ. Consistent with these observations, the endonuclease activity of the Artemis-DNA-PK complex seems to be required for removing single-strand DNA overhangs containing damaged nucleotides (68). Processing enzymes, such as WRN (Werner syndrome protein), APLF (aprataxin-and-polynucleotide kinase-like factor), the MRE11 complex, and the BLM (Bloom) helicase, may also participate in cNHEJ (55, 129).

In addition, XRCC4 has been shown to interact with polynucleotide kinase/phosphatase (PNKP), a bifunctional enzyme that phosphorylates 5'-OH termini and dephosphorylates 3'-phosphate termini, therefore providing the correct chemical end groups required for DNA ligation (29). Recent studies have demonstrated that Ku is a 5'-deoxyribose-5-phosphate/abasic or apurinic/aprimidinic (5'-dRP/AP) lyase that excises nucleotide damage near broken ends during cNHEJ (128). This 5'-dRP/AP lyase activity seems specific, as it is restricted to substrates in which excision of an abasic site is required for ligation (128). Interestingly, in the absence of Ku, a near-terminal abasic site is a barrier to aNHEJ, indicating that Ku-dependent cNHEJ is uniquely able to couple 5'-dRP/AP lyase activity to joining (102). cNHEJ is therefore uniquely effective at coupling this end-cleaning step to joining in cells, helping to distinguish this pathway from aNHEJ. Among processing activities used by cNHEJ is the extension of DNA ends by a DNA polymerase, which fills in the gaps at or near the site of a DSB, and template-independent nucleotide addition during resolution of V(D)J recombination intermediates (98). On the basis of amino acid sequence similarity, DNA polymerases in eukaryotes have been categorized into four classes: the A, B, X, and Y families (25). Three members of the pol X family have been associated with mammalian NHEJ: pol  $\lambda$ , pol  $\mu$ , and terminal deoxynucleotidyl transferase (TdT), which all share BRCT (BRCA1 C-terminal) domains essential for complex

formation between the pol X member and core cNHEJ factors at DNA ends (98). TdT is only expressed in cells undergoing V(D)J recombination, where it adds N nucleotides to coding joints, thereby increasing junctional diversity (98). Pol  $\mu$  and pol  $\lambda$  are ubiquitously expressed, and although vertebrate cells that lack pol  $\mu$ , pol  $\lambda$ , or both are not significantly radiosensitive, they could contribute to the quality of repair by NHEJ (98).

## End Joining

The final step in cNHEJ involves the joining of DNA ends by the DNA XRCC4-Ligase IV-XLF complex. Ligase IV has an N-terminal catalytic domain and interacts with the  $\alpha$ -helix of XRCC4 via a region between the two C-terminal BRCT domains (139). Binding of XRCC4 stabilizes DNA ligase IV and stimulates its activity (22, 54). In the presence of Ku, the DNA XRCC4-Ligase IV-XLF complex has the ability to ligate across gaps and to ligate one of the broken strands, even when the other strand is not ligatable (56). Interactions of ligase IV, XRCC4, and XLF with the Ku heterodimer have been described, but it is not clear whether DNA-PKcs is required for the recruitment of these proteins to the DNA ends or whether it simply enhances complex formation and activity (78). Therefore, it is possible that DNA-PKcs and the ligase complex may be recruited to DNA breaks independently rather than in a sequential manner.

In mice, deficiency in ligase IV or XRCC4 leads to embryonic lethality due to p53-dependent cell death of newly differentiated neurons (9, 48, 51). Ku-deficient mice are viable and fertile but also have increased levels of neuronal apoptosis, are small (approximately 50% the size of control littermates), are severely immunodeficient because of an inability to repair V(D)J recombination-associated DSBs, and exhibit premature aging (87, 134, 148). Although both DNA-PKcs- and Artemis-deficient mice are also immunodeficient because of the inability of progenitor lymphocytes to join V(D)J coding ends (103, 108), they lack the more

severe phenotypes observed in Ku-, XRCC4-, and ligase IV-deficient mice. These observations support the idea that proteins such as Artemis and DNA-PKcs, which are not broadly conserved throughout evolution, are dispensable for the repair of a large fraction of DSBs created under physiological conditions.

XLJ/Cernunnos, the most recently identified cNHEJ factor, was discovered simultaneously in patients that display growth retardation, immunodeficiency, and radiosensitivity (23) and by a yeast two-hybrid screen for proteins that interact with XRCC4 (3). XLJ stimulates ligation of noncohesive DNA ends by the XRCC4-ligase IV complex (4, 100). XLJ has additional roles in cNHEJ. For example, XLJ is essential for gap filling by pol  $\lambda$  and pol  $\mu$ , suggesting that it plays a major role in aligning the two DNA ends in the repair complex prior to ligation (4). Recent structural analysis indicates that the XLJ-XRCC4 complex could form extended filament-like structures that facilitate DNA end bridging during cNHEJ (6, 7). These results suggest that the XLJ-XRCC4 complex participates in maintaining the stability of the broken DNA ends during repair. This stabilization function is shared by a number of additional so-called accessory factors (see below) and seems particularly important to completion of safe repair of DNA breaks by cNHEJ, avoiding genome scrambling misrepair events.

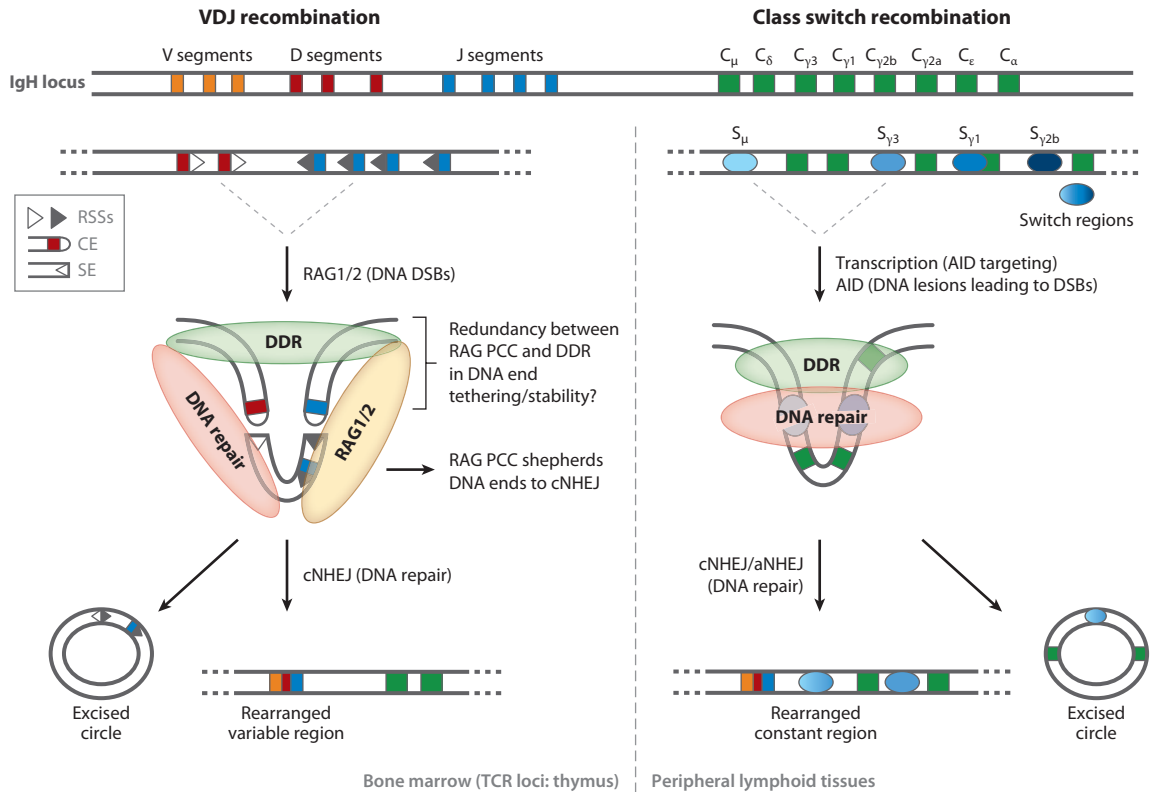
### **ROLE OF THE DNA DAMAGE RESPONSE AND ACCESSORY FACTORS IN CLASSICAL NONHOMOLOGOUS END JOINING**

One key attribute of cNHEJ is its ability to keep track of pairs of ends generated by individual DSBs, discouraging potentially disastrous misrepair events involving ends arising at widely separated genomic locations (**Figure 2**). One obvious way to maintain identification of appropriate end pairs would be to keep them tethered together during end processing and repair. The RAD50/MRE11 complex can bridge DNA ends and may perform this function (127).

Indeed, a conserved feature of the MRE11 complex is a zinc-coordinating motif in RAD50 called the RAD50 hook that enables the dimerization of chromatin-associated MRE11 complexes and could provide a flexible link between DNA ends (35, 138). Another plausible means for maintaining appropriate associations between end pairs arising from a single DSB is the assembly of DDR factors over large DNA regions of chromatin on both sides of DNA breaks to form so-called nuclear DNA repair foci. These foci are MRE11- and ATM-dependent, and contain histone H2AX, MDC1, 53BP1, and NBS1 (32). DDR factor deficiencies increase genomic instability, including, most remarkably, the rate of unrepaired chromosomal breaks and translocations. These findings led to the proposal that these large multiprotein complexes, although not strictly required for joining per se, assist in tethering the DNA ends prior to their ligation (53, 89, 145).

### **LESSONS FROM ANTIGEN RECEPTOR GENE REARRANGEMENT AND IMMUNOGLOBULIN CLASS SWITCHING**

Studies of the repair of physiological DSBs generated at specific sites during lymphocyte differentiation (programmed gene rearrangements) have provided valuable insights into NHEJ mechanisms (13, 59, 105). Programmed rearrangements in lymphocytes fall into two categories that employ different mechanisms and are undertaken at different developmental stages. Both are initiated by lymphoid-specific mechanisms that result in DSBs, which are then repaired by non-cell-type-specific NHEJ processes. V(D)J recombination occurs early in development of B and T lymphocytes and is responsible for assembling complete antigen receptor genes from separately encoded germ-line gene segments (**Figure 3**) (59, 105, 113). This process brings together V and J elements in a combinatorial fashion to generate immunoglobulin light chains and the T-cell-receptor  $\alpha$  and  $\gamma$  genes. Immunoglobulin heavy



**Figure 3**

DNA rearrangements at the IgH locus: V(D)J recombination and class switch recombination. Abbreviations: AID, activation-induced cytidine deaminase; CE, coding end; DDR, DNA damage response; DSB, double-strand break; NHEJ, nonhomologous end joining; PCC, postcleavage complex; RAG, recombination activating gene; RSS, recombination signal sequence; SE, signal end; TCR, T-cell receptor.

chains and T-cell-receptor  $\beta$  and  $\delta$  chains require the assembly of V, D, and J gene segments. Combinatorial assembly of the complete antigen receptor genes by choosing from several possible V, D, or J gene segments provides a fundamental mechanism for creating a diverse repertoire of antigen-binding sites in these receptors.

Rearrangement is initiated by the protein products of the RAGs, RAG1 and RAG2, which together constitute a site-specific endonuclease that introduces DSBs adjacent to conserved recognition sites [termed recombination signal sequences (RSSs)] (113). Cleavage at a pair of RSSs (e.g. one adjacent to a V segment and one adjoining a J segment) generates four broken

DNA ends: two blunt signal ends, which terminate in the RSS, and two covalently sealed (hairpin) coding ends. These ends are then joined by cNHEJ in a recombinant configuration, forming a coding joint (the rearranged antigen receptor gene) and a reciprocal product termed a signal joint. Early studies established that efficient joining requires the cNHEJ machinery (13, 59, 105).

The second lymphocyte-specific programmed DNA rearrangement process occurs only in mature B cells and serves to swap the DNA segment encoding the default C-terminal immunoglobulin effector region, which encodes the IgM isotype, for other germ line-encoded effector regions to create

different classes of antibodies (e.g., IgG, IgE, IgA) with different biological properties (**Figure 3**) (5, 125). This process, termed isotype switching or immunoglobulin class switch recombination (CSR), is also initiated by the introduction of DSBs in specific regions, termed switch regions, of DNA adjacent to the effector cassettes. Unlike V(D)J recombination, these DSBs are not precisely site-specific but can occur at a variety of locations along the highly repetitive switch regions. They are also generated by an indirect mechanism that is initiated by the activation-induced cytidine deaminase (AID) (5, 125). Pioneering studies revealed that the DNA ends generated during CSR are also joined by cNHEJ, although there are interesting differences between this process and V(D)J recombination (**Figure 3**) (44, 96, 122), as discussed below.

### The V(D)J Recombinase: A Special Case of End Tethering?

Deficiencies in ATM, H2AX, MDC1, the MRE11 complex, or 53BP1 reduce CSR levels (13). Interestingly, these factors, although present at RAG-mediated DSBs (31), seem to have only modest roles in V(D)J recombination. The most striking example is 53BP1: 53BP1 is important for the repair of AID-dependent DNA breaks, most likely by directly participating in cNHEJ, regulating DNA end resection and long-range DNA end synapsis, and for activation of cell-cycle checkpoints (13). Although 53BP1 is critical for CSR in the context of V(D)J recombination, loss of 53BP1 might be compensated by other accessory proteins. A similar situation is observed in the case of XLF. XLF-deficient animals, unlike other cNHEJ-deficient animals, are not especially immunodeficient (72, 133). Indeed, pro-B cell lines derived from XLF-deficient mice, although ionizing radiation-sensitive, perform nearly normal V(D)J recombination, leading to the speculation that unknown lymphocyte-specific factors/pathways might compensate for XLF function during V(D)J recombination (72).

One such factor could be the (lymphocyte-specific) V(D)J recombinase itself: The RAG-postcleavage complex holds the DNA ends together (1, 61) and shepherds them to the cNHEJ repair machinery (69) (**Figure 3**). Could the recombinase provide a V(D)J recombination-specific end-tethering function that compensates for the lack of 53BP1 or XLF? Additional evidence that supports a role for the V(D)J recombinase in providing RAG-specific end tethering is provided by a more detailed analysis of the effects that deficiencies in various factors involved in DDR or cNHEJ have on the joining of the two types of ends produced by the V(D)J recombinase: the coding and signal ends. The RAG proteins bind much more avidly to signal-end pairs than to coding-end pairs (1, 61), perhaps reflecting the need for coding ends to be accessible for processing by Artemis-DNA-PKcs before ligation. With this in mind, our RAG-tethering hypothesis predicts that signal-joint formation should be more resistant than coding-joint formation to defects in other end-tethering functions. This is indeed the case. Although capable of supporting V(D)J recombination, lymphocytes deficient for certain DDR factors (the MRE11 complex, ATM, and 53BP1) accumulate unrepaired coding ends, which can lead to subsequent chromosomal deletions and translocations (59). Interestingly, disrupting the interaction between XLF and XRCC4 also leads to impaired coding (but not signal)-joint formation, presumably reflecting end-tethering activities of the XLF-XRCC4 complex (112) that can be partially compensated for by signal-end tethering provided by the RAG-postcleavage complex.

The proposed tethering function provided by the RAG-DNA end complex does not obviate the need for additional assistance. Combined deficiencies of ATM, H2AX, or 53BP1 with XLF result in a severe block in lymphocyte development with a significant defect in the repair of RAG-mediated DSBs (75, 90, 144). Combined deficiency of XLF with ATM also leads to defective signal-joint formation and accumulation of signal ends, suggesting that the absence of both cNHEJ and DDR abilities to

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**Class switch recombination (CSR):** process of somatic recombination initiated by the activation-induced cytidine deaminase (AID) enzyme, by which B cells change the production of antibodies from one isotype to another

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tether DNA ends affects joining, even when the ends are supported by a RAG-postcleavage complex (91, 144).

Another example of functional redundancy between cNHEJ and DDR factors comes from the analysis of signal-joint formation in DNA-PKcs- and ATM-deficient lymphocytes. Two recent reports point to overlapping functions of DNA-PKcs and ATM, mediated through their kinase activities, in promoting efficient signal-joint formation and preventing accumulation of signal ends (52). In response to DSBs, DNA-PKcs and ATM phosphorylate a large number of shared substrates, including chromatin-associated factors, cNHEJ factors, and potentially the RAG proteins. It is therefore difficult to attribute the flaws in signal-joint formation in this situation to defective tethering. Nevertheless, it is tempting to speculate that cNHEJ and DDR [and RAG proteins, in the special case of V(D)J recombination] collaborate in maintaining DNA end pairs together to facilitate proper repair and to minimize aberrant end joining (**Figure 3**) (see below for further discussion).

### **BIOLOGICAL CONSEQUENCES OF CLASSICAL NONHOMOLOGOUS END JOINING**

cNHEJ restores the physical continuity of the chromosome, but because it generally causes short deletions and insertions at the junctions, repair often alters the nucleotide sequence information immediately surrounding the repair site. This is a distinct advantage in the case of V(D)J recombination, which seeks to generate junctional diversity that is translated into a diverse repertoire of antigen-binding proteins. Recent gene therapy strategies have taken advantage of the propensity of cNHEJ to make slight modifications at the junctions to inactivate target genes cleaved by sequence-specific nucleases (93).

The nonconservative nature of cNHEJ has led many to refer to it as an error-prone pathway. Nevertheless, cNHEJ generally restores

chromosome integrity without leading to chromosome rearrangements. Given that mammalian genomes contain a great deal of DNA sequence with no known coding function, microscopic alterations produced by repair may often be of no consequence and can be viewed as the price the cell is willing to pay to preserve global genomic integrity. From a genome-wide perspective, we would argue that cNHEJ is not particularly error prone, especially when compared with aNHEJ.

### **MECHANISM OF REPAIR BY ALTERNATIVE NONHOMOLOGOUS END JOINING**

As discussed above, evidence for an alternative NHEJ pathway emerged from investigation of cells deficient for cNHEJ components. Efficient joining of extrachromosomal DNA fragments was observed in cNHEJ-deficient cells (64, 73), as were rare junctions formed by V(D)J recombination at endogenous antigen receptor loci in cNHEJ-deficient mice (12, 17, 18, 80). The structures of these junctions often bear the features (e.g., large deletions, microhomologies, occasional insertions of large DNA segments of unknown origin) described above that have been taken as signatures of aNHEJ, although none of these features is invariably present.

The characteristics of its products imply that aNHEJ involves enzymes promoting end resection, proteins that take advantage of microhomologies (presumably to stabilize paired intermediates), nucleases capable of removing noncompatible 5' and 3' overhangs, and ligation. The factors involved and the mechanism(s) underlying aNHEJ (or even whether it represents a single pathway or multiple pathways) remain unclear. Recent work has implicated the MRE11 complex and CtIP in end resection that facilitates aNHEJ (37, 60, 71, 99, 140, 146), and DNA ligase III appears to promote the ligation step, although there may also be a role for DNA ligase I (13, 15, 119) (**Figure 2**). Recent studies show that PARP1



and DNA ligase III are important for joining mediated by aNHEJ at telomeres lacking the Ku heterodimer and the protection of the full shelterin complex (114). We refer the interested reader to a recent review for more details (13).

Although aNHEJ was initially viewed as merely a backup pathway present only when cNHEJ is disabled, recent studies have revealed that aNHEJ can be surprisingly efficient and that it occurs in cells proficient for cNHEJ (88). Studies using mutant RAG proteins demonstrated that, at least with extrachromosomal substrates, aNHEJ occurs at approximately 10% of the frequency of cNHEJ in both cNHEJ-proficient and cNHEJ-deficient cells (33). CSR mediated by aNHEJ in cNHEJ-deficient cells occurs at roughly 50% of the frequency observed in cNHEJ-proficient contexts (121, 142).

### Is aNHEJ a Distinct Repair Pathway?

Does aNHEJ operating in cNHEJ-deficient cells simply substitute a missing cNHEJ factor with another enzyme borrowed from another repair pathway? According to this view, aNHEJ is simply a substitution variant of cNHEJ (74). This hypothesis predicts that removal of different cNHEJ factors should affect the nature of the products generated by aNHEJ because each aNHEJ pathway would be unique in its means of overcoming the deficiency of a particular cNHEJ factor. For instance, Ku deficiency might affect end resection much more drastically than XRCC4 or ligase IV deficiency. This is, however, not the case. Instead, both Ku and XRCC4 deficiency lead to similar junctions that contain large deletions and microhomologies (57). Furthermore, the substitution model predicts that deficiency of multiple factors should influence the nature of the aNHEJ reaction (efficiency, structures of junctions, etc.). Instead, immunoglobulin CSR occurs rather efficiently in the combined absence of Ku and ligase IV, and produces identical junctions (14, 16). Furthermore, DNA-end-shepherding-deficient RAG mutants revealed

that aNHEJ is a robust pathway even in cNHEJ-proficient cells (33). Additional support for aNHEJ as a bona fide pathway rather than a variant of cNHEJ is the discovery of aNHEJ in *E. coli*, which lacks components of cNHEJ, such as Ku (30), suggesting that aNHEJ might have preceded cNHEJ in evolution. Together, these findings provide strong evidence that aNHEJ is a pathway in its own right, with components (and perhaps functions) distinct from those of cNHEJ.

If aNHEJ is a distinct pathway, does it have a particular biological function? aNHEJ may simply provide a fail-safe mechanism for repair of chromosomal breaks. This would have the advantage of preserving large swaths of the genome between a DSB and the telomere, which would otherwise be lost upon cell division. The disadvantages, however, appear significant: loss of DNA from ends because of extensive exonucleolytic processing and frequent chromosome translocations (120, 142, 149), indicating that these mechanisms are truly error prone. Indeed, recent biochemical studies indicate that end resection uncoupled from HR occurs in extracts from mitotic cells, raising the intriguing possibility that one physiological function of aNHEJ may be to heal chromosomes broken during mitosis (94).

### aNHEJ is Error Prone on a Genome-Wide Scale

cNHEJ-deficient mice (whether they lack Ku80, XRCC4, ligase IV, DNA-PKcs, or even Artemis) that are also deficient for p53 invariably develop pro-B cell lymphomas harboring oncogenic chromosomal translocations involving the IgH and c-Myc loci (or N-Myc in the case of Artemis deficiency), all of which are catalyzed by aNHEJ and harbor microhomologies at the breakpoints (145). These observations not only supported the existence of an error-prone aNHEJ pathway, but also gave rise to the concept that the more high-fidelity cNHEJ pathway acted as a tumor suppressor (38, 50, 107) by promoting faithful joining (**Figure 2**). One interpretation of these results is that,



in the presence of functional p53, cells bearing unrepaired DSBs are (mostly) eliminated; deactivating p53 allows the cells to survive long enough for the ends to be repaired by (presumably slower) aNHEJ.

Cultured cells deficient for either XRCC4-ligase IV or Ku exhibit the same translocation frequency and breakpoint junction characteristics, supporting a role for aNHEJ as the major pathway to translocation formation (14, 120, 142). Analyses of chromosomal translocation junctions in human tumors also revealed several features, such as microhomologies and extensive end resection, and led to the suggestion that aNHEJ forms translocations (126, 147). Indeed, most chromosome translocations in both cNHEJ-proficient and cNHEJ-deficient cells appear to be generated by aNHEJ (14, 120, 142). This is supported by the observation that their frequency is reduced in the absence of DNA ligase III (119), one of the newly identified components of the aNHEJ pathway.

Why is aNHEJ so error prone? The foregoing discussion suggests two possibilities. The propensity of aNHEJ to generate genome rearrangements could reflect defects in stabilizing or tethering pairs of DNA ends. During repair of DSBs by cNHEJ, DNA ends are likely maintained in close proximity by both cNHEJ and DDR factors, therefore promoting repair *in cis*. Such a role has been illustrated for Ku, as in its absence DNA ends undergo long-range movements within the nucleus (123). A corresponding function may be lacking in aNHEJ. Additionally, aNHEJ appears to repair DSBs with slower kinetics than cNHEJ *in vivo* (142), increasing the chances that more than one DSB is present concomitantly and therefore increasing the chances of repairing the wrong DNA ends *in trans* (in the case of chromosomal translocations).

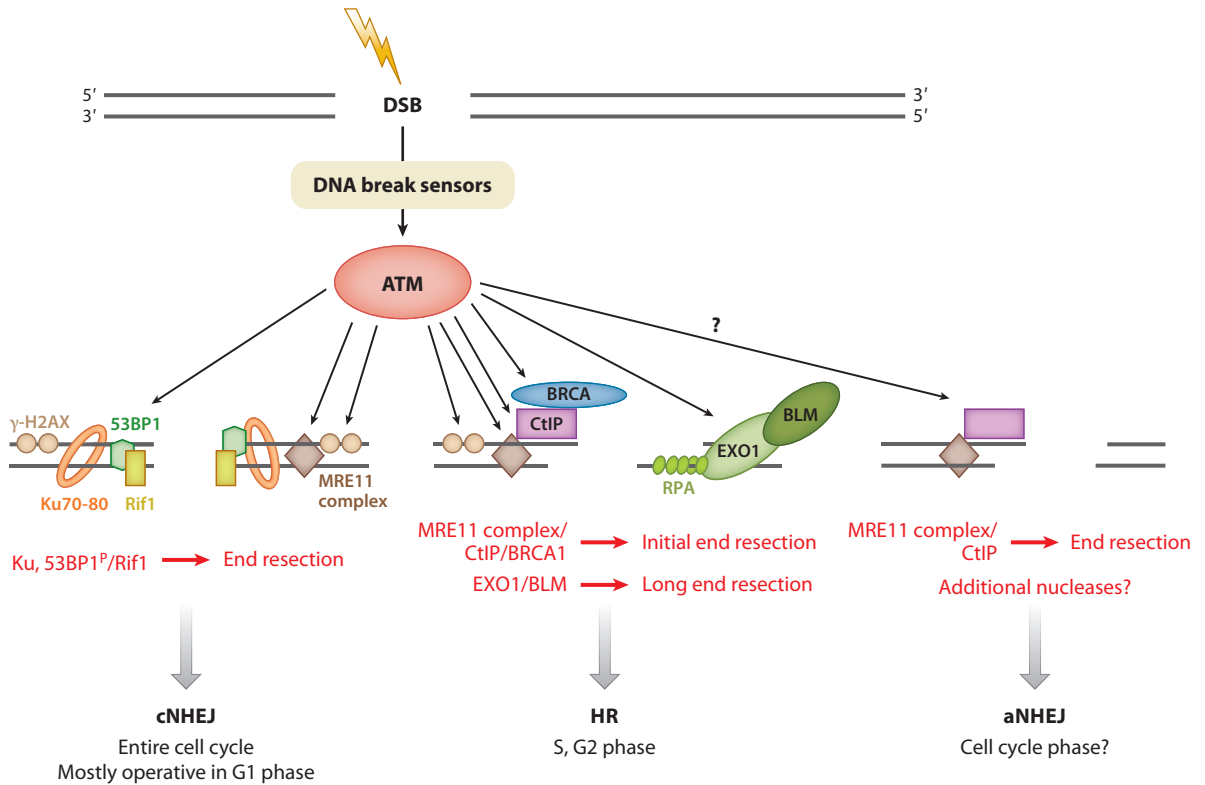
### REPAIR PATHWAY CHOICE: A CRITICAL REGULATORY DECISION

At least three pathways are, in principle, available to repair a particular DSB. Indeed,

these pathways can compete or collaborate in repair (65, 89, 101, 120). Given that the three pathways have quite different outcomes, one would expect that mechanisms exist to control the choice of repair pathway used under certain sets of conditions. For example, HR mechanisms predominate during S/G<sub>2</sub>, when a sister chromatid template is available (129). cNHEJ, in contrast, operates throughout the cell cycle (65) (**Figure 4**). cNHEJ may be the default pathway in noncycling mammalian cells (129). In budding yeast, DNA ends are first available to cNHEJ. As nuclease-mediated resection proceeds, long single-stranded tails are generated that can only be joined via HR (49). Recent work indicates that the choice between HR and cNHEJ in replicating or G<sub>2</sub>/M phase yeast, and in mammalian cells, is regulated by end resection (62, 63). In *S. cerevisiae*, resection of broken ends is greatly reduced in noncycling cells, favoring cNHEJ (129).

The initial phase of end resection is limited to fairly short stretches, with as few as 20 bp processed (33, 37, 132), and is carried out by the MRE11 complex and CtIP, making the ends available for aNHEJ. Indeed, both the MRE11 complex and CtIP have been implicated in aNHEJ (37, 60, 71, 99, 140, 146) (**Figure 2; Figure 4**). In a second phase of end resection, the BLM helicase and exonuclease 1 generate the long single-stranded tails required to initiate HR (129) (**Figure 4**). At this stage, the long single-stranded DNA ends become poor substrates for binding by Ku, and cells appear to be committed to HR (129), although this commitment might be reversed if the tails are trimmed by nucleases.

The presence of robust aNHEJ activity in mammalian cells suggests that mechanisms may exist to limit use of (translocation-prone) aNHEJ (33, 64, 120, 121, 142). Recent data reveal a role for 53BP1 in limiting access of nucleases to DNA ends, promoting cNHEJ (19, 20, 24, 39, 40, 114) (**Figure 4**). This end protection depends upon phosphorylation of 53BP1 by activated ATM, promoting recruitment of Rif1 (28, 41, 46, 150) (**Figure 4**). In the absence of Rif1 (or 53BP1), DSBs generated during



**Figure 4**

End resection as a determinant of double-strand break (DSB) repair pathway choice. Abbreviations: ATM, ataxia telangiectasia mutated; HR, homologous recombination; NHEJ, nonhomologous end joining.

immunoglobulin CSR are extensively resected and are not repaired by cNHEJ, leading to persistent chromosome breaks, genomic instability, and repair by homology-based pathways (19, 20, 38, 141). End joining of deprotected telomeres due to the removal of members of the shelterin complex can also occur by aNHEJ in the absence of 53BP1, in a process dependent on DNA ligase III and PARP1 (114). Thus, 53BP1 appears to be one of the factors responsible for modulating pathway choice between aNHEJ and cNHEJ.

Additional levels of pathway choice control appear to be incorporated in the handling of certain physiological DSBs. For example, during V(D)J recombination, the RAG proteins take some responsibility for restricting repair of the broken ends to cNHEJ, limiting their access to HR and aNHEJ (33, 69). This may

reflect a carefully orchestrated handoff from the RAG-DNA end complex to the cNHEJ machinery. In agreement with this notion, certain mutations in the C terminus of RAG2 destabilize the RAG-DNA end complex and allow increased aNHEJ (33, 36, 69). This is accompanied by genomic instability and accelerated lymphomagenesis in the absence of p53 (36), generating tumors bearing a complex landscape of translocations, deletions, inversions, and duplications (82). A second example is provided by telomeres: The shelterin complex appears to provide telomeres with an extra level of protection against both cNHEJ and aNHEJ (114).

## NONHOMOLOGOUS END JOINING IN THE CLINIC

Intensive study of the various properties and mechanisms of both cNHEJ and aNHEJ have

spurred some important clinical applications. The observation that cNHEJ often repairs breaks with local disruption of DNA sequences at the joining site has inspired strategies for cNHEJ-mediated gene disruption. One application currently in clinical trials transduces T cells from HIV-infected individuals with a zinc-finger nuclease designed to cleave within the coding region of the *CCR5* gene, which encodes a coreceptor for the HIV virus, with the resulting NHEJ creating *CCR5*-disrupted cells that are resistant to infection (93).

Our knowledge of NHEJ mechanisms also informs new therapeutic strategies against cancer. Genomic instability is characteristic of many cancer cells and is thought to provide fuel for rapid evolution of subclones, which can then be selected for invasiveness, metastatic potential, and drug resistance (76). Recent genomic analyses of 489 ovarian tumors have revealed defects in HR in half of them (26), suggesting that the progenitor cells may have exclusively employed NHEJ to repair DSBs. These data suggest that inhibition of cNHEJ

(or aNHEJ) might provide a therapeutic approach. Indeed, a DNA ligase IV inhibitor impedes tumor progression in mouse cancer models (124). On the basis of the role of PARP in DNA damage sensing, PARP inhibitors are being used in clinical trials for therapy of cancers with lesions in BRCA1/2 (67). Additional rationale for the use of PARP inhibitors is provided by the recent discovery that increased aNHEJ (which, as discussed above, might involve PARP1) in peripheral blood lymphocytes is correlated with an increased risk of breast cancer (66), suggesting that these patients may be genetically predisposed toward utilization of this (dangerous) repair pathway. There is increasing evidence for downregulation of cNHEJ and upregulation of aNHEJ in a variety of human tumors (10, 11, 130, 131). Thus, aNHEJ may present a useful therapeutic target, depending upon on what its normal function(s), if any, might be. Continued study of NHEJ mechanisms and regulation of pathway choice control should provide new insights that can be translated into the clinic.

### FUTURE ISSUES

1. What are aNHEJ's components (other than PARP1, MRE11/CtIP, and ligase III)? What are the mechanisms of aNHEJ-mediated translocation? Is aNHEJ relevant to physiological, biological, or evolutionary processes? Is aNHEJ involved in tumor onset, progression, and/or therapy resistance? Is aNHEJ composed of a single defined pathway or multiple defined pathways?
2. How is the aNHEJ versus cNHEJ and HDR pathways choice regulated?
3. What are the mechanisms regulating chromosomal DSB repair in *cis* (intrachromosomal) and in *trans* (translocational)?
4. How does DDR mechanistically and functionally contribute to DSB repair by cNHEJ and aNHEJ? What are the detailed mechanisms of DDR-mediated synapsis?
5. Is V(D)J recombination a special case of DSB repair? How does the RAG recombinase regulate DSB repair and pathway choice during V(D)J recombination?
6. It is important to translate the knowledge of the DNA repair mechanism into the clinic.

### DISCLOSURE STATEMENT

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## LITERATURE CITED

1. Agrawal A, Schatz DG. 1997. RAG1 and RAG2 form a stable postcleavage synaptic complex with DNA containing signal ends in V(D)J recombination. *Cell* 89:43–53
2. Aguilar-Quesada R, Munoz-Gamez JA, Martin-Oliva D, Peralta A, Valenzuela MT, et al. 2007. Interaction between ATM and PARP-1 in response to DNA damage and sensitization of ATM deficient cells through PARP inhibition. *BMC Mol. Biol.* 8:29
3. Ahnesorg P, Smith P, Jackson SP. 2006. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* 124:301–13
4. Akopiants K, Zhou RZ, Mohapatra S, Valerie K, Lees-Miller SP, et al. 2009. Requirement for XLF/Cernunnos in alignment-based gap filling by DNA polymerases lambda and mu for nonhomologous end joining in human whole-cell extracts. *Nucleic Acids Res.* 37:4055–62
5. Alt FW, Zhang Y, Meng FL, Guo C, Schwer B. 2013. Mechanisms of programmed DNA lesions and genomic instability in the immune system. *Cell* 152:417–29
6. Andres SN, Modesti M, Tsai CJ, Chu G, Junop MS. 2007. Crystal structure of human XLF: a twist in nonhomologous DNA end-joining. *Mol. Cell* 28:1093–101
7. Andres SN, Vergnes A, Ristic D, Wyman C, Modesti M, Junop M. 2012. A human XRCC4-XLF complex bridges DNA. *Nucleic Acids Res.* 40:1868–78
8. Aravind L, Koonin EV. 2001. Prokaryotic homologs of the eukaryotic DNA-end-binding protein Ku, novel domains in the Ku protein and prediction of a prokaryotic double-strand break repair system. *Genome Res.* 11:1365–74
9. Barnes DE, Stamp G, Rosewell I, Denzel A, Lindahl T. 1998. Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Curr. Biol.* 8:1395–98
10. Bentley J, Diggle CP, Harnden P, Knowles MA, Kiltie AE. 2004. DNA double strand break repair in human bladder cancer is error prone and involves microhomology-associated end-joining. *Nucleic Acids Res.* 32:5249–59
11. Bentley J, L'Hote C, Platt F, Hurst CD, Lowery J, et al. 2009. Papillary and muscle invasive bladder tumors with distinct genomic stability profiles have different DNA repair fidelity and KU DNA-binding activities. *Genes Chromosomes Cancer* 48:310–21
12. Blackwell TK, Malynn BA, Pollock RR, Ferrier P, Covey LR, et al. 1989. Isolation of *scid* pre-B cells that rearrange kappa light chain genes: formation of normal signal and abnormal coding joins. *EMBO J.* 8:735–42
13. Boboila C, Alt FW, Schwer B. 2012. Classical and alternative end-joining pathways for repair of lymphocyte-specific and general DNA double-strand breaks. *Adv. Immunol.* 116:1–49
14. Boboila C, Jankovic M, Yan CT, Wang JH, Wesemann DR, et al. 2010. Alternative end-joining catalyzes robust IgH locus deletions and translocations in the combined absence of ligase 4 and Ku70. *Proc. Natl. Acad. Sci. USA* 107:3034–39
15. Boboila C, Oksenyh V, Gostissa M, Wang JH, Zha S, et al. 2012. Robust chromosomal DNA repair via alternative end-joining in the absence of x-ray repair cross-complementing protein 1 (XRCC1). *Proc. Natl. Acad. Sci. USA* 109:2473–78
16. Boboila C, Yan C, Wesemann DR, Jankovic M, Wang JH, et al. 2010. Alternative end-joining catalyzes class switch recombination in the absence of both Ku70 and DNA ligase 4. *J. Exp. Med.* 207:417–27
17. Bogue MA, Jhappan C, Roth DB. 1998. Analysis of variable (diversity) joining recombination in DNA-dependent protein kinase (DNA-PK)-deficient mice reveals DNA-PK-independent pathways for both signal and coding joint formation. *Proc. Natl. Acad. Sci. USA* 95:15559–64

18. Bogue MA, Wang C, Zhu C, Roth DB. 1997. V(D)J recombination in Ku86-deficient mice: distinct effects on coding, signal, and hybrid joint formation. *Immunity* 7:37–47
19. Bothmer A, Robbiani DF, Di Virgilio M, Bunting SF, Klein IA, et al. 2011. Regulation of DNA end joining, resection, and immunoglobulin class switch recombination by 53BP1. *Mol. Cell* 42:319–29
20. Bothmer A, Robbiani DF, Feldhahn N, Gazumyan A, Nussenzweig A, Nussenzweig MC. 2010. 53BP1 regulates DNA resection and the choice between classical and alternative end joining during class switch recombination. *J. Exp. Med.* 207:855–65
21. Boulton SJ, Jackson SP. 1996. *Saccharomyces cerevisiae* Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. 15:5093–103
22. Bryans M, Valenzano MC, Stamato TD. 1999. Absence of DNA ligase IV protein in XR-1 cells: evidence for stabilization by XRCC4. *Mutat. Res.* 433:53–58
23. Buck D, Malivert L, de Chasseval R, Barraud A, Fondaneche MC, et al. 2006. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* 124:260–62
24. Bunting SF, Callen E, Wong N, Chen HT, Polato F, et al. 2010. 53BP1 inhibits homologous recombination in *Brca1*-deficient cells by blocking resection of DNA breaks. *Cell* 141:243–54
25. Burgers PM, Koonin EV, Bruford E, Blanco L, Burtis KC, et al. 2001. Eukaryotic DNA polymerases: proposal for a revised nomenclature. *J. Biol. Chem.* 276:43487–90
26. Cancer Genome Atlas Res. Netw. 2011. Integrated genomic analyses of ovarian carcinoma. *Nature* 474:609–15
27. Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, et al. 2011. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res.* 39:e82
28. Chapman JR, Barral P, Vannier JB, Borel V, Steger M, et al. 2013. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol. Cell* 49:858–71
29. Chappell C, Hanakahi LA, Karimi-Busheri F, Weinfeld M, West SC. 2002. Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. *EMBO J.* 21:2827–32
30. Chayot R, Montagne B, Mazel D, Ricchetti M. 2010. An end-joining repair mechanism in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 107:2141–46
31. Chen HT, Bhandoola A, Difilippantonio MJ, Zhu J, Brown MJ, et al. 2000. Response to RAG-mediated V(D)J cleavage by NBS1 and  $\gamma$ -H2AX. *Science* 290:1962–65
32. Ciccia A, Elledge SJ. 2010. The DNA damage response: making it safe to play with knives. *Mol. Cell* 40:179–204
33. Corneo B, Wendland RL, Deriano L, Cui X, Klein IA, et al. 2007. Rag mutations reveal robust alternative end joining. *Nature* 449:483–86
34. Dahm K. 2007. Functions and regulation of human Artemis in double strand break repair. *J. Cell. Biochem.* 100:1346–51
35. de Jager M, van Noort J, van Gent DC, Dekker C, Kanaar R, Wyman C. 2001. Human Rad50/Mre11 is a flexible complex that can tether DNA ends. *Mol. Cell* 8:1129–35
36. Deriano L, Chaumeil J, Coussens M, Multani AS, Chou Y, et al. 2011. The RAG2 C-terminus suppresses genomic instability and lymphomagenesis. *Nature* 471:119–23
37. Deriano L, Stracker TH, Baker A, Petrini JH, Roth DB. 2009. Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. *Mol. Cell* 34:13–25
38. Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, et al. 2000. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 404:510–14
39. Difilippantonio S, Gapud E, Wong N, Huang CY, Mahowald G, et al. 2008. 53BP1 facilitates long-range DNA end-joining during V(D)J recombination. *Nature* 456:529–33
40. Dimitrova N, Chen YC, Spector DL, de Lange T. 2008. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 456:524–28
41. Di Virgilio M, Callen E, Yamane A, Zhang W, Jankovic M, et al. 2013. Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 339:711–15
42. Dobbs TA, Tainer JA, Lees-Miller SP. 2010. A structural model for regulation of NHEJ by DNA-PKcs autophosphorylation. *DNA Repair (Amst.)* 9:1307–14



43. Downs JA, Jackson SP. 2004. A means to a DNA end: the many roles of Ku. *Nat. Rev. Mol. Cell Biol.* 5:367–78
44. Dudley DD, Chaudhuri J, Bassing CH, Alt FW. 2005. Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. *Adv. Immunol.* 86:43–112
45. Durai S, Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S. 2005. Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. *Nucleic Acids Res.* 33:5978–90
46. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, et al. 2013. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol. Cell* 49:872–83
47. Forment JV, Kaidi A, Jackson SP. 2012. Chromothripsis and cancer: causes and consequences of chromosome shattering. *Nat. Rev. Cancer* 12:663–70
48. Frank KM, Sekiguchi JM, Seidl KJ, Swat W, Rathbun G, et al. 1998. Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* 396:173–77
49. Frank-Vaillant M, Marcand S. 2002. Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol. Cell* 10:1189–99
50. Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, et al. 2000. Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature* 404:897–900
51. Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, et al. 1998. A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* 95:891–902
52. Gapud EJ, Sleckman BP. 2011. Unique and redundant functions of ATM and DNA-PKcs during V(D)J recombination. *Cell Cycle* 10:1928–35
53. Gostissa M, Alt FW, Chiarle R. 2011. Mechanisms that promote and suppress chromosomal translocations in lymphocytes. *Annu. Rev. Immunol.* 29:319–50
54. Grawunder U, Wilm M, Wu X, Kulesza P, Wilson TE, et al. 1997. Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. *Nature* 388:492–95
55. Grundy GJ, Rulten SL, Zeng Z, Arribas-Bosacoma R, Iles N, et al. 2013. APLF promotes the assembly and activity of non-homologous end joining protein complexes. *EMBO J.* 32:112–25
56. Gu J, Lu H, Tippin B, Shimazaki N, Goodman MF, Lieber MR. 2007. XRCC4:DNA ligase IV can ligate incompatible DNA ends and can ligate across gaps. *Embo J.* 26:1010–23
57. Guirouilh-Barbat J, Rass E, Plo I, Bertrand P, Lopez BS. 2007. Defects in XRCC4 and KU80 differentially affect the joining of distal nonhomologous ends. *Proc. Natl. Acad. Sci. USA* 104:20902–7
58. Haince JF, McDonald D, Rodrigue A, Dery U, Masson JY, et al. 2008. PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *J. Biol. Chem.* 283:1197–208
59. Helmink BA, Sleckman BP. 2012. The response to and repair of RAG-mediated DNA double-strand breaks. *Annu. Rev. Immunol.* 30:175–202
60. Helmink BA, Tubbs AT, Dorsett Y, Bednarski JJ, Walker LM, et al. 2011. H2AX prevents CtIP-mediated DNA end resection and aberrant repair in G1-phase lymphocytes. *Nature* 469:245–49
61. Hiom K, Gellert M. 1997. A stable RAG1-RAG2-DNA complex that is active in V(D)J cleavage. *Cell* 88:65–72
62. Huertas P, Cortes-Ledesma F, Sartori AA, Aguilera A, Jackson SP. 2008. CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature* 455:689–92
63. Huertas P, Jackson SP. 2009. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. *J. Biol. Chem.* 284:9558–65
64. Kabotyanski EB, Gomelsky L, Han J-O, Stamato TD, Roth DB. 1998. Double-strand break repair in Ku86- and XRCC4-deficient cells. *Nucleic Acids Res.* 26:5333–42
65. Kass EM, Jasin M. 2010. Collaboration and competition between DNA double-strand break repair pathways. *FEBS Lett.* 584:3703–8
66. Keimling M, Deniz M, Varga D, Stahl A, Schrezenmeier H, et al. 2012. The power of DNA double-strand break (DSB) repair testing to predict breast cancer susceptibility. *FASEB J.* 26:2094–104
67. Kummar S, Chen A, Parchment RE, Kinders RJ, Ji J, et al. 2012. Advances in using PARP inhibitors to treat cancer. *BMC Med.* 10:25



68. Kurosawa A, Adachi N. 2010. Functions and regulation of Artemis: a goddess in the maintenance of genome integrity. *J. Radiat. Res.* 51:503–9
69. Lee GS, Neiditch MB, Salus SS, Roth DB. 2004. RAG proteins shepherd double-strand breaks to a specific pathway, suppressing error-prone repair, but RAG nicking initiates homologous recombination. *Cell* 117:171–84
70. Lee JH, Paull TT. 2004. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science* 304:93–96
71. Lee-Theilen M, Matthews AJ, Kelly D, Zheng S, Chaudhuri J. 2011. CtIP promotes microhomology-mediated alternative end joining during class-switch recombination. *Nat. Struct. Mol. Biol.* 18:75–79
72. Li G, Alt FW, Cheng HL, Brush JW, Goff PH, et al. 2008. Lymphocyte-specific compensation for XLF/Cernunnos end-joining functions in V(D)J recombination. *Mol. Cell* 31:631–40
73. Liang F, Jasin M. 1996. Ku80-deficient cells exhibit excess degradation of extrachromosomal DNA. 271:14405–11
74. Lieber MR. 2010. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* 79:181–211
75. Liu X, Jiang W, Dubois RL, Yamamoto K, Wolner Z, Zha S. 2012. Overlapping functions between XLF repair protein and 53BP1 DNA damage response factor in end joining and lymphocyte development. *Proc. Natl. Acad. Sci. USA* 109:3903–8
76. Loeb LA. 2011. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat. Rev. Cancer* 11:450–57
77. Ma Y, Pannicke U, Schwarz K, Lieber MR. 2002. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 108:781–94
78. Mahaney BL, Meek K, Lees-Miller SP. 2009. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochem. J.* 417:639–50
79. Malyarchuk S, Wright D, Castore R, Klepper E, Weiss B, et al. 2007. Expression of *Mycobacterium tuberculosis* Ku and Ligase D in *Escherichia coli* results in RecA and RecB-independent DNA end-joining at regions of microhomology. *DNA Repair (Amst.)* 6:1413–24
80. Malynn BA, Blackwell TK, Fulop GM, Rathbun GA, Furlley AJW, et al. 1988. The *scid* defect affects the final step of the immunoglobulin VDJ recombinase mechanism. *Cell* 54:453–60
81. McClintock B. 1941. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 26:234–82
82. Mijuskovic M, Brown SM, Tang Z, Lindsay CR, Efstathiadis E, et al. 2012. A streamlined method for detecting structural variants in cancer genomes by short read paired-end sequencing. *PLoS ONE* 7:e48314
83. Mitelman F, Johansson B, Mertens F. 2007. The impact of translocations and gene fusions on cancer causation. *Nat. Rev. Cancer* 7:233–45
84. Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, et al. 2001. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 105:177–86
85. Neal JA, Meek K. 2011. Choosing the right path: Does DNA-PK help make the decision? *Mutat. Res.* 711:73–86
86. Nowell PC, Hungerford DA. 1960. A minute chromosome in human chronic granulocytic leukemia. *Science* 132:1497–501
87. Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K, et al. 1996. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* 382:551–55
88. Nussenzweig A, Nussenzweig MC. 2007. A backup DNA repair pathway moves to the forefront. *Cell* 131:223–25
89. Nussenzweig A, Nussenzweig MC. 2010. Origin of chromosomal translocations in lymphoid cancer. *Cell* 141:27–38
90. Oksenysh V, Alt FW, Kumar V, Schwer B, Wesemann DR, et al. 2012. Functional redundancy between repair factor XLF and damage response mediator 53BP1 in V(D)J recombination and DNA repair. *Proc. Natl. Acad. Sci. USA* 109:2455–60

91. Oksenykh V, Kumar V, Liu X, Guo C, Schwer B, et al. 2013. Functional redundancy between the XLF and DNA-PKcs DNA repair factors in V(D)J recombination and nonhomologous DNA end joining. *Proc. Natl. Acad. Sci. USA* 110:2234–39
92. Pattanayak V, Ramirez CL, Joung JK, Liu DR. 2011. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. *Nat. Methods* 8:765–70
93. Perez EE, Wang J, Miller JC, Jouvenot Y, Kim KA, et al. 2008. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. *Nat. Biotechnol.* 26:808–16
94. Peterson SE, Li Y, Chait BT, Gottesman ME, Baer R, Gautier J. 2011. Cdk1 uncouples CtIP-dependent resection and Rad51 filament formation during M-phase double-strand break repair. *J. Cell Biol.* 194:705–20
95. Pilch DR, Sedelnikova OA, Redon C, Celeste A, Nussenzweig A, Bonner WM. 2003. Characteristics of  $\gamma$ -H2AX foci at DNA double-strand breaks sites. *Biochem. Cell Biol.* 81:123–29
96. Posey JE, Brandt VL, Roth DB. 2004. Paradigm switching in the germinal center. *Nat. Immunol.* 5:476–77
97. Postow L, Ghenoiu C, Woo EM, Krutchinsky AN, Chait BT, Funabiki H. 2008. Ku80 removal from DNA through double strand break-induced ubiquitylation. *J. Cell Biol.* 182:467–79
98. Ramsden DA. 2011. Polymerases in nonhomologous end joining: building a bridge over broken chromosomes. *Antioxid. Redox Signal.* 14:2509–19
99. Rass E, Grabarz A, Plo I, Gautier J, Bertrand P, Lopez BS. 2009. Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. *Nat. Struct. Mol. Biol.* 16:819–24
100. Riballo E, Woodbine L, Stiff T, Walker SA, Goodarzi AA, Jeggo PA. 2009. XLF-Cernunnos promotes DNA ligase IV-XRCC4 re-adenylation following ligation. *Nucleic Acids Res.* 37:482–92
101. Richardson C, Jasin M. 2000. Coupled homologous and nonhomologous repair of a double-strand break preserves genomic integrity in mammalian cells. *Mol. Cell Biol.* 20:9068–75
102. Roberts SA, Strande N, Burkhalter MD, Strom C, Havener JM, et al. 2010. Ku is a 5'-dRP/AP lyase that excises nucleotide damage near broken ends. *Nature* 464:1214–17
103. Rooney S, Chaudhuri J, Alt FW. 2004. The role of the non-homologous end-joining pathway in lymphocyte development. *Immunol. Rev.* 200:115–31
104. Roos WP, Kaina B. 2012. DNA damage-induced apoptosis: from specific DNA lesions to the DNA damage response and apoptosis. *Cancer Lett.* 332:237–48
105. Roth DB. 2003. Restraining the V(D)J recombinase. *Nat. Rev. Immunol.* 3:656–66
106. Roth DB, Chang X-B, Wilson JH. 1989. Comparison of filler DNA at immune, nonimmune, and oncogenic rearrangements suggests multiple mechanisms of formation. *Mol. Cell Biol.* 9:3049–57
107. Roth DB, Gellert M. 2000. New guardians of the genome. *Nature* 404:823–25
108. Roth DB, Menetski JP, Nakajima PB, Bosma MJ, Gellert M. 1992. V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in scid mouse thymocytes. *Cell* 70:983–91
109. Roth DB, Porter TN, Wilson JH. 1985. Mechanisms of nonhomologous recombination in mammalian cells. *Mol. Cell Biol.* 5:2599–607
110. Roth DB, Wilson JH. 1986. Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. *Mol. Cell Biol.* 6:4295–304
111. Rothkamm K, Kruger I, Thompson LH, Lobrich M. 2003. Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol. Cell Biol.* 23:5706–15
112. Roy S, Andres SN, Vergnes A, Neal JA, Xu Y, et al. 2012. XRCC4's interaction with XLF is required for coding (but not signal) end joining. *Nucleic Acids Res.* 40:1684–94
113. Schatz DG, Swanson PC. 2011. V(D)J recombination: mechanisms of initiation. *Annu. Rev. Genet.* 45:167–202
114. Sfeir A, de Lange T. 2012. Removal of shelterin reveals the telomere end-protection problem. *Science* 336:593–97
115. Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, Janicki SM, Greenberg RA. 2010. ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141:970–81
116. Shiloh Y, Ziv Y. 2013. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nat. Rev. Mol. Cell Biol.* 14:197–210

117. Shrivastav M, De Haro LP, Nickoloff JA. 2008. Regulation of DNA double-strand break repair pathway choice. *Cell Res.* 18:134–47
118. Shuman S, Glickman MS. 2007. Bacterial DNA repair by non-homologous end joining. *Nat. Rev. Microbiol.* 5:852–61
119. Simsek D, Brunet E, Wong SY, Katyal S, Gao Y, et al. 2011. DNA ligase III promotes alternative nonhomologous end-joining during chromosomal translocation formation. *PLoS Genet.* 7:e1002080
120. Simsek D, Jasin M. 2010. Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. *Nat. Struct. Mol. Biol.* 4:410–16
121. Soulas-Sprauel P, Le Guyader G, Rivera-Munoz P, Abramowski V, Olivier-Martin C, et al. 2007. Role for DNA repair factor XRCC4 in immunoglobulin class switch recombination. *J. Exp. Med.* 204:1717–27
122. Soulas-Sprauel P, Rivera-Munoz P, Malivert L, Le Guyader G, Abramowski V, et al. 2007. V(D)J and immunoglobulin class switch recombinations: a paradigm to study the regulation of DNA end-joining. *Oncogene* 26:7780–91
123. Soutoglou E, Dorn JF, Sengupta K, Jasin M, Nussenzweig A, et al. 2007. Positional stability of single double-strand breaks in mammalian cells. *Nat. Cell Biol.* 9:675–82
124. Srivastava M, Nambiar M, Sharma S, Karki SS, Goldsmith G, et al. 2012. An inhibitor of nonhomologous end-joining abrogates double-strand break repair and impedes cancer progression. *Cell* 151:1474–87
125. Stavnezer J, Guikema JE, Schrader CE. 2008. Mechanism and regulation of class switch recombination. *Annu. Rev. Immunol.* 26:261–92
126. Stephens PJ, McBride DJ, Lin ML, Varela I, Pleasance ED, et al. 2009. Complex landscapes of somatic rearrangement in human breast cancer genomes. *Nature* 462:1005–10
127. Stracker TH, Petrini JH. 2011. The MRE11 complex: starting from the ends. *Nat. Rev. Mol. Cell Biol.* 12:90–103
128. Strande N, Roberts SA, Oh S, Hendrickson EA, Ramsden DA. 2012. Specificity of Ku's 5' drp/AP lyase promotes nonhomologous end joining (NHEJ) fidelity at damaged ends. *J. Biol. Chem.* 287:13686–93
129. Symington LS, Gautier J. 2011. Double-strand break end resection and repair pathway choice. *Annu. Rev. Genet.* 45:247–71
130. Tobin LA, Robert C, Nagaria P, Chumsri S, Twaddell W, et al. 2012. Targeting abnormal DNA repair in therapy-resistant breast cancers. *Mol. Cancer Res.* 10:96–107
131. Tobin LA, Robert C, Rapoport AP, Gojo I, Baer MR, et al. 2012. Targeting abnormal DNA double-strand break repair in tyrosine kinase inhibitor-resistant chronic myeloid leukemias. *Oncogene* 32:1784–93
132. Truong LN, Li Y, Shi LZ, Hwang PY, He J, et al. 2013. Microhomology-mediated end joining and homologous recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proc. Natl. Acad. Sci. USA* 110:7720–25
133. Vera G, Rivera-Munoz P, Abramowski V, Malivert L, Lim A, et al. 2012. Cernunnos deficiency reduces thymocyte lifespan and alters the T cell repertoire in mice and humans. *Mol. Cell Biol.* 33:701–11
134. Vogel H, Lim DS, Karsenty G, Finegold M, Hasty P. 1999. Deletion of Ku86 causes early onset of senescence in mice. *Proc. Natl. Acad. Sci. USA* 96:10770–75
135. Walker JR, Corpina RA, Goldberg J. 2001. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412:607–14
136. Weller GR, Kysela B, Roy R, Tonkin LM, Scanlan E, et al. 2002. Identification of a DNA nonhomologous end-joining complex in bacteria. *Science* 297:1686–89
137. Wilson JH, Berget PB, Pipas JM. 1982. Somatic cells efficiently join unrelated DNA segments end-to-end. *Mol. Cell Biol.* 2:1258–69
138. Wiltzius JJ, Hohl M, Fleming JC, Petrini JH. 2005. The Rad50 hook domain is a critical determinant of Mre11 complex functions. *Nat. Struct. Mol. Biol.* 12:403–7
139. Wu Q, Ochi T, Matak-Vinkovic D, Robinson CV, Chirgadze DY, Blundell TL. 2011. Non-homologous end-joining partners in a helical dance: structural studies of XLF-XRCC4 interactions. *Biochem. Soc. Trans.* 39:1387–92; Suppl. 2
140. Xie A, Kwok A, Scully R. 2009. Role of mammalian Mre11 in classical and alternative nonhomologous end joining. *Nat. Struct. Mol. Biol.* 16:814–18

141. Yamane A, Robbiani DF, Resch W, Bothmer A, Nakahashi H, et al. 2013. RPA accumulation during class switch recombination represents 5'-3' DNA-end resection during the S-G2/M phase of the cell cycle. *Cell Rep.* 3:138–47
142. Yan CT, Boboila C, Souza EK, Franco S, Hickernell TR, et al. 2007. IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature* 449:478–82
143. Yoo S, Dynan WS. 1999. Geometry of a complex formed by double strand break repair proteins at a single DNA end: recruitment of DNA-PKcs induces inward translocation of Ku protein. *Nucleic Acids Res.* 27:4679–86
144. Zha S, Guo C, Boboila C, Oksenysh V, Cheng HL, et al. 2011. ATM damage response and XLF repair factor are functionally redundant in joining DNA breaks. *Nature* 469:250–54
145. Zhang Y, Gostissa M, Hildebrand DG, Becker MS, Boboila C, et al. 2010. The role of mechanistic factors in promoting chromosomal translocations found in lymphoid and other cancers. *Adv. Immunol.* 106:93–133
146. Zhang Y, Jasin M. 2011. An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. *Nat. Struct. Mol. Biol.* 18:80–84
147. Zhang Y, Rowley JD. 2006. Chromatin structural elements and chromosomal translocations in leukemia. *DNA Repair (Amst.)* 5:1282–97
148. Zhu C, Bogue MA, Lim D-S, Hasty P, Roth DB. 1996. Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. *Cell* 86:379–89
149. Zhu C, Mills KD, Ferguson DO, Lee C, Manis J, et al. 2002. Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. *Cell* 109:811–21
150. Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 2013. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science* 339:700–4
151. Ziv Y, Bielopolski D, Galanty Y, Lukas C, Taya Y, et al. 2006. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat. Cell Biol.* 8:870–76



# Contents

Causes of Genome Instability <i>Andrés Aguilera and Tatiana García-Muse</i> .....	1
Radiation Effects on Human Heredity <i>Nori Nakamura, Akibiko Suyama, Asao Noda, and Yoshiaki Kodama</i> .....	33
Dissecting Social Cell Biology and Tumors Using <i>Drosophila</i> Genetics <i>José Carlos Pastor-Pareja and Tian Xu</i> .....	51
Estimation and Partition of Heritability in Human Populations Using Whole-Genome Analysis Methods <i>Anna A.E. Vinkbuizen, Naomi R. Wray, Jian Yang, Michael E. Goddard, and Peter M. Visscher</i> .....	75
Detecting Natural Selection in Genomic Data <i>Joseph J. Vitti, Sharon R. Grossman, and Pardis C. Sabeti</i> .....	97
Adaptive Translation as a Mechanism of Stress Response and Adaptation <i>Tao Pan</i> .....	121
Organizing Principles of Mammalian Nonsense-Mediated mRNA Decay <i>Maximilian Wei-Lin Popp and Lynne E. Maquat</i> .....	139
Control of Nuclear Activities by Substrate-Selective and Protein-Group SUMOylation <i>Stefan Jentsch and Ivan Psakhye</i> .....	167
Genomic Imprinting: Insights From Plants <i>Mary Gebring</i> .....	187
Regulation of Bacterial Metabolism by Small RNAs Using Diverse Mechanisms <i>Maksym Bobrovskyy and Carin K. Vanderpool</i> .....	209
Bacteria and the Aging and Longevity of <i>Caenorhabditis elegans</i> <i>Dennis H. Kim</i> .....	233
The Genotypic View of Social Interactions in Microbial Communities <i>Sara Mitri and Kevin Richard Foster</i> .....	247
SIR Proteins and the Assembly of Silent Chromatin in Budding Yeast <i>Stephanie Kueng, Mariano Oppikofer, and Susan M. Gasser</i> .....	275

New Gene Evolution: Little Did We Know <i>Manyuan Long, Nicholas W. VanKuren, Sidi Chen, Maria D. Vibranovski</i> .....	307
RNA Editing in Plants and Its Evolution <i>Mizuki Takenaka, Anja Zebrmann, Daniil Verbitskiy, Barbara Härtel, and Axel Brennicke</i> .....	335
Expanding Horizons: Ciliary Proteins Reach Beyond Cilia <i>Shiaulou Yuan and Zhaoxia Sun</i> .....	353
The Digestive Tract of <i>Drosophila melanogaster</i> <i>Bruno Lemaitre and Irene Miguel-Aliaga</i> .....	377
RNase III: Genetics and Function; Structure and Mechanism <i>Donald L. Court, Jianhua Gan, Yu-He Liang, Gary X. Shaw, Joseph E. Tropea, Nina Costantino, David S. Waugh, and Xinhua Ji</i> .....	405
Modernizing the Nonhomologous End-Joining Repertoire: Alternative and Classical NHEJ Share the Stage <i>Ludovic Deriano and David B. Roth</i> .....	433
Enterococcal Sex Pheromones: Signaling, Social Behavior, and Evolution <i>Gary M. Dunny</i> .....	457
Control of Transcriptional Elongation <i>Hojoong Kwak and John T. Lis</i> .....	483
The Genomic and Cellular Foundations of Animal Origins <i>Daniel J. Richter and Nicole King</i> .....	509
Genetic Techniques for the Archaea <i>Joel A. Farkas, Jonathan W. Picking, and Thomas J. Santangelo</i> .....	539
Initiation of Meiotic Recombination: How and Where? Conservation and Specificities Among Eukaryotes <i>Bernard de Massy</i> .....	563
Biology and Genetics of Prions Causing Neurodegeneration <i>Stanley B. Prusiner</i> .....	601
Bacterial Mg <sup>2+</sup> Homeostasis, Transport, and Virulence <i>Eduardo A. Groisman, Kerry Hollands, Michelle A. Kriner, Eun-Jin Lee, Sun-Yang Park, and Mauricio H. Pontes</i> .....	625

## Errata

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