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REVIEW

# The Role of Non-Homologous End Joining and Microhomology-Mediated End Joining in Chromosomal Rearrangements

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**Abstract**—Double-strand DNA break (DSB) repair mechanisms vary in their ability to prevent errors during end joining. The joining of DSBs on different chromosomes can result in translocations, potentially leading to tumorigenesis. This review examines the main mechanisms of DSB repair and factors influencing their selection, as well as contribution of these mechanisms to the chromosomal rearrangements in human cells.

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## INTRODUCTION

Double-strand DNA breaks (DSBs) are the most dangerous DNA lesions. They block replication and transcription of damaged DNA, and many genes can be lost during cell division due to chromosomal breaks. Cells employ multiple DSB repair pathways that often overlap for reliability. These pathways differ in the mechanisms involved, repair rate, and protection from errors. The most dangerous error is the joining of DNA ends from different breaks, which can lead to translocations and transformation of normal cells into tumors [1-3]. The contribution of each DSB repair mechanism to the formation of translocations in human cells remains unclear. This review examines the main DSB repair mechanisms, factors influencing DNA repair pathway selection, and their association with chromosomal rearrangements in mammalian cells. Note that other chromosomal rearrangements – large deletions and inversions – have the same mechanisms of formation as translocations, so the discussed pathways apply to them as well.

## GENERAL SCHEME OF DSB REPAIR MECHANISMS

The general scheme of DSB repair pathways in human cells is shown in Fig. 1. The first mechanism is non-homologous end joining (NHEJ), which operates throughout entire cell cycle [4-7]. Alternative end-joining mechanisms that do not require a homology donor, include microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA) [8]. Both MMEJ and SSA use short homologous sequences (microhomologies) on both sides of the break, but differ

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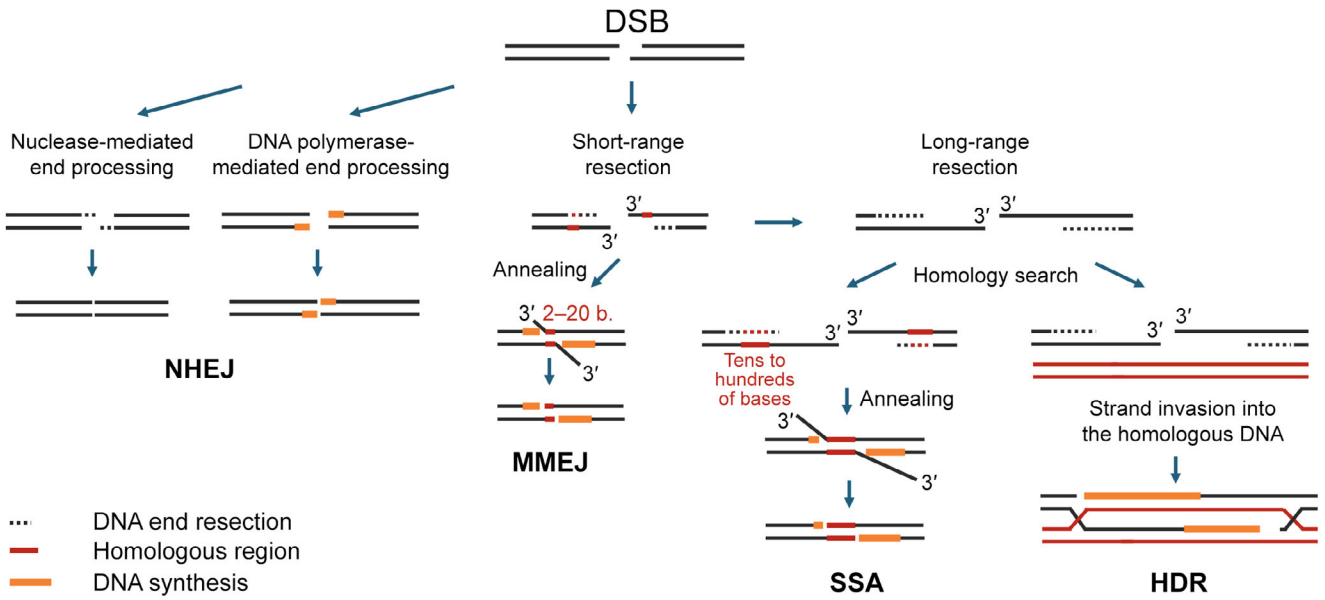


**Fig. 1.** General scheme of DSB repair pathways in human cells: non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), single-strand annealing (SSA), and homology-directed repair (HDR). The final ligation stage for NHEJ, MMEJ, and SSA is not shown; HDR variants resolving Holliday junctions are also not depicted. Orange, newly synthesized DNA; red, homologous regions; dashed lines, nucleotide removal.

in the length of these homologous regions and proteins involved [9]. However, these two pathways lack a mechanism ensuring correct end selection for the DNA ligation, making them error prone. Homology-directed repair (HDR), also called homologous recombination (HR) because it is involved in the crossing over during meiotic recombination [10, 11], is more reliable in preventing chromosomal rearrangements. Because HDR uses a homologous template, typically, sister chromatid, it is active mostly in the S and G2 phases of cell cycle [5]. The use of homology donor prevents incorrect joining of chromosomal ends and formation of translocations. Detailed description of HDR can be found in reviews by Sanchez et al. [11], Sun et al. [12], and Al-Zain and Symington [13].

SSA is a rare repair pathway because it requires extensive homology regions (>20 nucleotides) at both break sites [14, 15]. Therefore, translocations occurring by SSA are uncommon. SSA is discussed in Bhargava et al. [9], Blasiak [16], and Vu et al. [17]. We chose not to describe in detail the HDR and SSA pathways in this review and focused on NHEJ and MMEJ, their molecular mechanisms, and role in the formation of chromosomal translocations.

## NON-HOMOLOGOUS END JOINING (NHEJ)

NHEJ is a dominant DSB repair pathway in vertebrates during the G1 phase, although it is active throughout the entire cell cycle [5, 7, 18]. It is a universal mechanism for joining genomic fragments, including during V(D)J recombination (immunoglobulin and T-cell receptor gene formation). NHEJ had once been considered a simple, linear process: break detection, end retention, processing, and ligation. However, recent data have revealed a more complex organization, with NHEJ protein complexes transiting between multiple functional states [19, 20].

DSBs are recognized by the Ku70/Ku80 (XRCC6/XRCC5, or X-ray repair cross-complementing protein 6/5) complex [21, 22], which is a heterodimer consisting of two subunits with molecular masses of 70 and 80 kDa, respectively, that form a ring-like structure with positively charged amino acids residues inside. Ku70/Ku80 binds to the DNA ends [21, 23], both blunt and overhanging, and recruits the catalytic subunit (DNA-PKcs) of DNA-dependent protein kinase (DNA-PK), forming the DNA-PK holoenzyme [24]. Two break ends, each bound by Ku70/Ku80 and DNA-PKcs, constitute the long-range synaptic complex (LR), which may include additional factors, such as XRCC4 (X-ray repair cross-complementing protein 4), XLF/Cernunnos, and LIG4 (DNA ligase IV). This complex holds the break ends together.

Cryo-electron microscopy has revealed two structural variants of LR, differing in their architecture and protein composition (Fig. 2). In the first of them, dimerization occurs through the interaction of the C-terminal domains of Ku80 with DNA-PKcs in *trans* (referred to as Ku80-mediated LR or domain-swap LR). In the second variant (referred to as XLF-mediated LR), dimerization is mediated by a filament formed by auxiliary factors, such as XLF and two LIG4 complexes

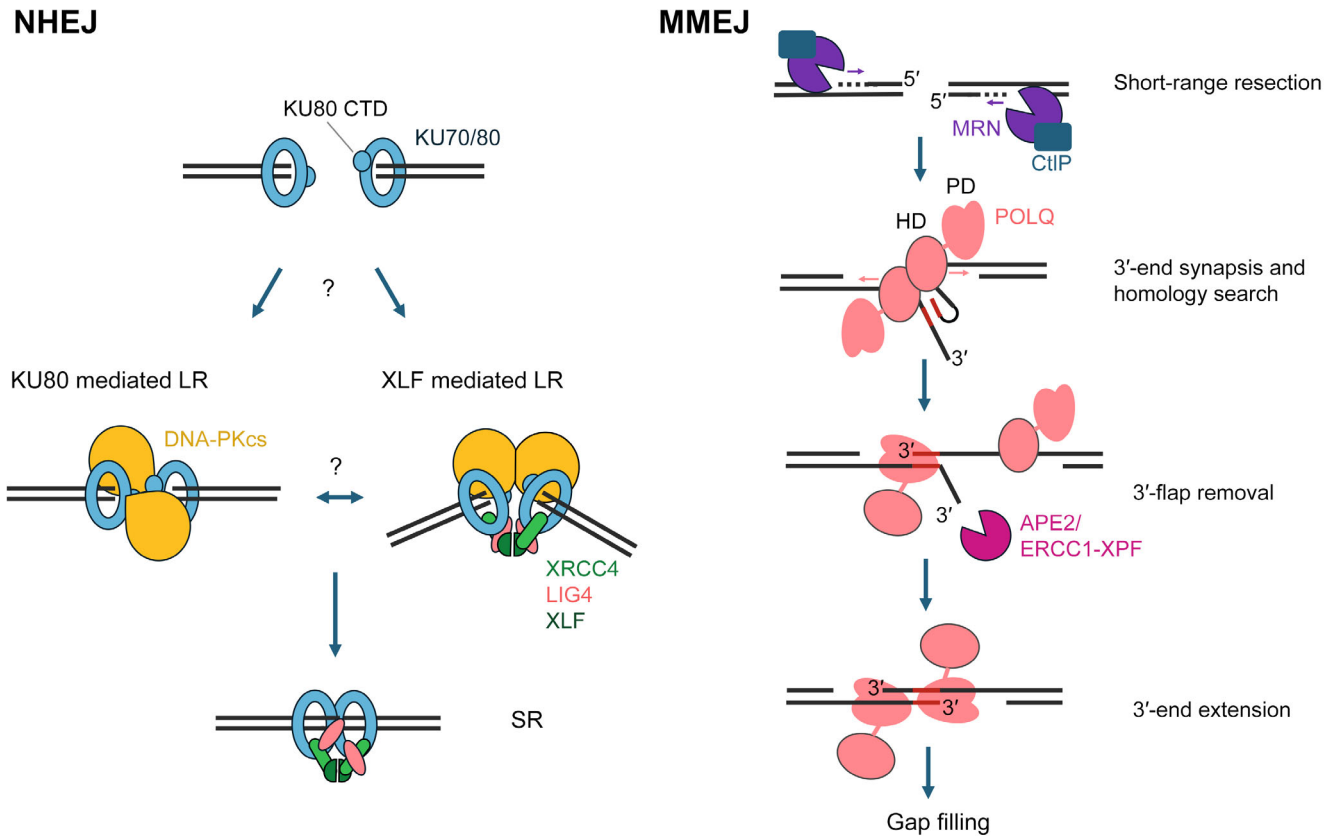


**Fig. 2.** NHEJ and MMEJ pathways of DSB repair in human cells. CTD, C-terminal domain; LR and SR, long- and short-range synaptic complexes, respectively; HD and PD, helicase and DNA polymerase domains of POLQ, respectively. Small arrows indicate the direction of the exonuclease and helicase activities of MRN and POLQ (see the text for explanation).

associated with XRCC4 [25-27]. So far, it remains unclear whether the Ku80- and XLF-mediated LR complexes can interconvert or they form from different monomeric structures of the DNA-PK holoenzyme (described, for example, in Liu et al. [28]), or both scenarios are possible.

The XLF-mediated LR can compact into a short-range synaptic complex (SR) through mutual phosphorylation of two DNA-PKcs molecules, followed by their dissociation from the complex [27, 29]. This brings the break ends sufficiently close for the ligation by LIG4. The organization of the complex appears to be determined by the autophosphorylation of DNA-PK and its interaction with DNA [19, 28].

During the NHEJ-mediated repair, the ends of the DNA break can undergo processing. The nature and extent of DNA processing in NHEJ, as well as additional proteins recruited for this purpose, vary depending on the properties of DSB ends. Blunt ends and compatible overhangs can be ligated directly [30]. However, if the ends contain incompatible overhangs, chemical damage, or modifications, the NHEJ complex employs specialized nucleases and polymerases to prepare the ends for ligation. The major nuclease in NHEJ is DNA cross-link repair 1C protein (ARTEMIS), which possesses both endonuclease and 5′→3′ exonuclease activities [31, 32]. DNA polymerases involved in NHEJ are polymerase μ (POLM) and polymerase λ (POLL) (members of the Pol X family) that can add nucleotides in a template-independent manner in addition to the standard polymerase activity [33].

Using *in vitro* systems, it was shown that incompatible 5′ ends are preferentially trimmed by ARTEMIS [34], while incompatible 3′ ends undergo both degradation by ARTEMIS and extension by POLM [34]. ARTEMIS also resolves the hairpin structures formed at DNA ends during V(D)J recombination; mutations in the ARTEMIS gene lead to severe combined immunodeficiency [35, 36]. Polynucleotide kinase/phosphatase (PNKP) removes phosphate groups from 3′ ends and adds phosphate groups to 5′ ends of DNA. Notably, the presence at the overhangs of complementary nucleotides (microhomology regions of 2-4 nucleotides) facilitates end joining [34]. The processing of break ends results in the appearance of small insertions and deletions (indels) at the repair site [34, 37].

Different NHEJ complexes can include different processing enzymes and activities. ARTEMIS is recruited by the DNA-PK holoenzyme either as a monomer [28] or in the content of the Ku80-mediated LR [19].

However, Stinson et al. [30] found that the removal of 5′ overhangs in *Xenopus laevis* egg extracts was catalyzed by an unidentified 5′→3′ exonuclease (not ARTEMIS), whose activity depended on XLF and XRCC4–LIG4. This exonuclease was present in XLF-mediated LR or SR. There is an emerging consensus that other types of processing occur either in the XLF-mediated LR and SR or exclusively in the SR [19, 30, 38], which is supported by the fact that DNA-PKcs and Ku70/80 protect the ends from most processing enzymes until the SR is formed [30], allowing access for ARTEMIS only [28].

Focusing the majority of processing activities at the SR stage, i.e., when ligation takes place, appears to be an efficient way to minimize mutagenesis during DNA repair, as the processed ends join at the earliest opportunity [39]. Therefore, new data and models challenge the concept of NHEJ as a mechanism prone to indels due to its simplistic nature.

## MICROHOMOLOGY-MEDIATED END JOINING (MMEJ)

The first evidence of an alternative end-joining pathway came from the studies of *Saccharomyces cerevisiae* Ku70 mutants. Even with additional Rad52 mutations, these cells retained their capacity for the repair of DSBs caused by ionizing radiation, albeit this process was accompanied by the formation of deletions [40]. Extracts from bovine thymus also demonstrated the presence of two end-joining pathways: one joining blunt ends with no homology, and another using short identical sequences (microhomologies) [41]. The studies of mammalian cells deficient in NHEJ components confirmed the existence of an alternative pathway, termed alt-NHEJ [42]. It was shown that this mechanism acts as an alternative to NHEJ and HDR, but can also function simultaneously with these pathways. Later, it was renamed MMEJ to emphasize its reliance on microhomology [44]. NHEJ can also use microhomologies of 2-4 nucleotides [34], but this is not obligatory. In this review, the term MMEJ refers specifically to the pathway described below, while NHEJ refers to the canonical mechanism dependent on Ku proteins, DNA-PKcs, and LIG4. Older publications may use the terms alt-NHEJ, a-NHEJ, or a-EJ to describe MMEJ. Due to the involvement of DNA polymerase θ (POLQ), MMEJ is also called TMEJ (theta-mediated end joining) [15, 45].

MMEJ typically begins with the DSB recognition by the MRN complex composed of the MRE11 nuclease, regulatory ATPase RAD50, and NBS1 (Nijmegen breakage syndrome) scaffold protein [46]. MRN is recruited by poly(ADP-ribose) polymerases (PARP1 and PARP2) recognizing the breaks in DNA [47]. Accumulation of MRN at the break sites can occur via liquid-liquid phase separation, driven by the intrinsically disordered domains of MRNIP (MRN complex interacting protein) [48, 49].

After recognition of the break, MRN resects its ends. The activation of MRN requires its phosphorylation by CtIP (CtBP-interacting protein/retinoblastoma-binding protein 8), after which MRN binds to DNA at a distance from the DSB [50, 51]. MRE11 creates nick and then degrades the 5′ end back toward the DSB end due to its 3′→5′ exonuclease activity (Fig. 2) [14, 52]. The PARylation of histones, BRCA1 (BReast CAncer gene 1), and other proteins is crucial for the resection initiation [53-56].

The next step in MMEJ is the search for microhomologies and annealing. If 3′ overhangs contain microhomologies (typically, <20 nucleotides), they can anneal [14, 15]. DNA polymerase θ (POLQ in humans) holds the 3′ ends while the search for the microhomologies takes place. POLQ is a central protein in the MMEJ mechanism in mammals [57, 58]. Its distinctive feature is the presence of the helicase domain [59, 60] that non-specifically binds single-stranded DNA regions, competing with RPA (replication protein A). Due to ATP hydrolysis, the helicase domain threads the protruding 3′ ends of DSBs, displacing RPA proteins [61]. During this process, single-stranded DNA regions can anneal if the microhomology regions are present. It is important to note that the helicase domains of POLQ function as a dimer, thus holding the break ends together [58, 62, 63]. After microhomology annealing, any protruding unpaired 3′ end (3′-flap) is removed. According to different studies, removal of 3′-flaps involves the XPF–ERCC1 complex, APE2 (apurinic/apyrimidinic endonuclease 2) protein, or 3′→5′ exonuclease activity of DNA polymerase δ [43, 64, 65].

POLQ then extends the complementary region via non-processive, error-prone synthesis. This process is accompanied by multiple dissociation/reassociation of POLQ, formation and elongation of hairpins on DNA single strands, etc., resulting in the generation of templated insertions (TINSs) – a hallmark of MMEJ (Fig. 3) [66, 67]. After addition of ~10 nucleotides, POLQ is replaced by the less error-prone and more processive POLδ–PCNA complex [65, 68]. After filling the gaps, the breaks are ligated by LIG1 (replicative ligase) or LIG3 [69, 70].

Since in MMEJ, the DNA ends are held together due to the annealing in two microhomology regions, one of these regions will be lost after DNA repair, as well the DNA fragment between them. These microhomology-mediated deletions and TINSs are used to identify MMEJ events [66, 67].

Recent studies have revealed MMEJ as a preferred DNA repair mechanism in mitosis [71-73].

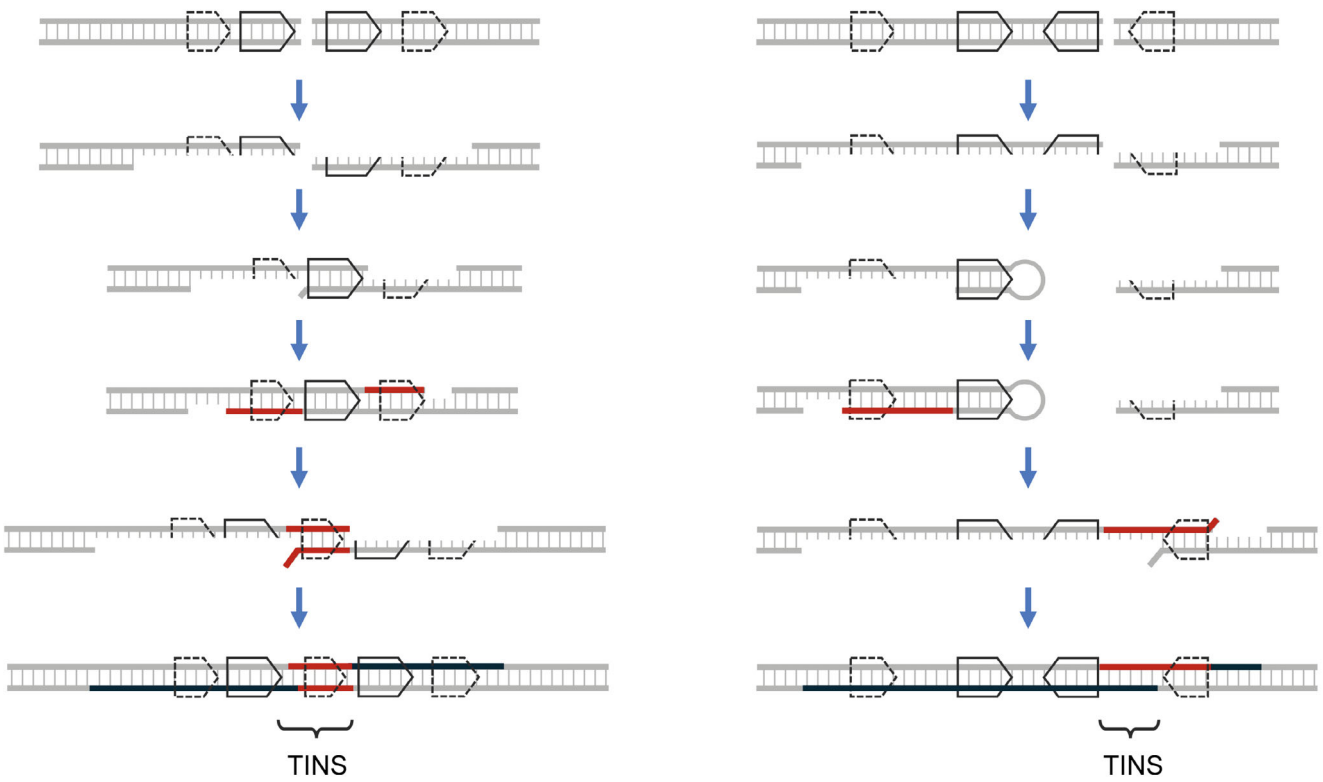


**Fig. 3.** Two examples of MMEJ mechanisms leading to the formation of templated insertions (TINSs). The choice of the mechanism depends on the presence and orientation of homology regions (direct or inverted repeats shown in boxes). Newly synthesized DNA is shown in (initially synthesized) and blue (synthesized after secondary annealing of DNA ends).

It was found that MMEJ is frequently involved in rearrangements at common fragile sites, which are prone to replication fork stalling and underreplication. As a result, these sites are prone to DNA breaks that are repaired by MMEJ during mitosis [74, 75].

Therefore, MMEJ serves as a backup for DSBs unrepaired by NHEJ or HDR, but its involvement results in the formation of deletions in the repaired regions. For this reason, the activity of MMEJ is low in normal cells. However, tumor cells often have impaired DNA repair pathways, making MMEJ the principal mechanism of DNA repair in these cells, which also makes POLQ a potential therapeutic target in the antitumor therapy [76-78].

## THE CHOICE OF DSB REPAIR MECHANISM

DSB repair pathways have been traditionally viewed as independent molecular processes. However, DNA repair can start via one pathway and end via another. For example, the switch between the mechanism can occur after recognition of the break ends, as the binding of Ku70/80 does not prevent the recruitment of MRN and initiation of end resection via MMEJ or HDR [51, 79, 80], while recognition of the break by the MRN complex can lead to the NHEJ-mediated DNA repair [81, 82]. MMEJ, SSA, and HDR share many similar steps; for example, they all start with the MRN recruitment and end resection [83].

Despite the possibility of switching between the DNA repair pathways, there are factors that predetermine realization of particular mechanisms. The key factor appears to be the end resection. Many proteins affect the choice of the repair mechanism by modulating the extent of resection. Blunt ends or compatible overhangs are optimal for NHEJ, while incompatible ends require processing by nucleases/polymerases [37, 84]. Extended single-stranded ends poorly bind Ku70/80 [85]. MMEJ requires overhangs for the microhomology search. Therefore, once resection is initiated, the cells choose between HDR, SSA, or MMEJ.

Resection proceeds in two stages: short-range resection (tens to hundreds of nucleotides) by MRN followed by the long-range resection (hundreds to thousands of nucleotides) by EXO1 and DNA2. Short-range resection can occur even when the ends are blocked by proteins or secondary structures; it starts with a nick introduced at distance from the break, after which DNA is degraded toward the break. Long-range resection is faster, but more sensitive to obstacles. Short-range resection is sufficient for MMEJ, while HDR and SSA require long-rejection resection [14, 28, 83, 86].

Resection of DNA ends occurs during the S and G2 phases of cell cycle and favors MMEJ, SSA, and HDR. Cyclin-dependent kinases CDK1 and CDK2 phosphorylate CtIP, thus activating MRN and promoting resection [87, 88]. Post-translational modifications of repair proteins play an important role in the regulation of resection. Thus, ataxia telangiectasia mutated (ATM) kinase activated by DSBs, phosphorylates H2AX histone, resulting in the recruitment of DNA repair proteins to the break. Ubiquitination of H1 and H2A histones by ubiquitin ligases RNF8 and RNF168 also signals for the repair protein recruitment [81, 89].

53BP1 (p53-binding protein) and BRCA1 are key regulators of resection. Both are recruited to the chromatin regions containing histones ubiquitinated by RNF8/RNF168 [81, 89]. 53BP1 inhibits resection and directs DNA repair toward NHEJ [90, 91]. 53BP1 binds most DSBs by default, even in the G2 phase [92-94]. Its antagonist BRCA1 modifies CtIP, BLM, WRN, and EXO1, thus stimulating resection [95-97]. BRCA1 displaces 53BP1 during the S and G2 phases and recruits the SMARCAD1 chromatin remodeling complex [18, 85, 98-101]. The removal of ubiquitinated nucleosomes disrupts the binding between 53BP1 and DSBs and facilitates resection of the break ends [18, 85, 98-101]. Recent data suggest that the state of chromatin influences the choice of DNA repair pathway mechanism, e.g., euchromatin favors NHEJ over MMEJ compared to heterochromatin [14, 102].

There is a mechanism that limits NHEJ in the S and G2 phases. The micropeptide CYREN (a member of a family of small regulatory peptides named by analogy with microRNAs) binds Ku70/80, thus restricting its participation in the DSB repair [103, 104]. Finaly, NHEJ can also occur “instantly,” without involvement of ATM, RNF8, RNF168, or protein complexes responsible for the choice of DNA repair mechanism [105].

The choice of MMEJ is strongly facilitated by PARP1 [106]. Its activity varies during the cell cycle, with the highest and lowest activities observed in the S and G1 phases, respectively [107]. PARP1 recruits MRN to DSBs [108], thus promoting resection. As a result, PARP1 inhibits NHEJ by competing with Ku70/80 for DNA binding and reducing the affinity of Ku70/80 for DNA (since the affinity of these proteins to the single-stranded DNA formed by resection is lower) [109]. PARP1 also inhibits the long-range resection, which shifts DNA repair toward MMEJ [106, 110, 111].

As mentioned above, MMEJ is a preferred DSB repair pathway in mitosis, when NHEJ and early stages of HDR are inhibited [90, 112, 113]. Polo-like kinase 1 (PLK1), which controls cell entry to mitosis, phosphorylates RHINO (Rad9, Hus1, Rad1 Interacting Nuclear Orphan). RHINO is accumulated during mitosis; its major function, together with the 9-1-1 complex (Rad9, Hus1, Rad1), is triggering of ATR signaling and cell cycle arrest in response to the replication stress and DNA damage [114, 115]. However, it was shown recently that phosphorylated RHINO recruits POLQ to DSBs for MMEJ activation independently of PARP1 [106, 116-119].

## IMPACT OF DSB FORMATION ON THE CHOICE OF DNA REPAIR MECHANISM

DSBs can appear in DNA for a variety of reasons. Endogenous DSBs primarily occur during replication, often in actively transcribed regions due to collisions between the replication forks and RNA polymerase or increased level of DNA damage [120-124]. Induction of DSBs can be programmed in certain cell types. For instance, lymphocytes express RAG nuclease and activation-induced cytidine deaminase (AID) for the initiation of V(D)J recombination, class-switch recombination, and somatic hypermutation [125, 126]. In germ cells, SPO11 forms DSBs for meiotic recombination [127-129]. Dysfunction of topoisomerase 2 (TOPO2) can also cause DSBs. TOPO2 decatenates replicated chromosomes and relieves supercoiling [130] by introducing transient DSBs in DNA molecule to pass another DNA segment through [131, 132]. TOPO2 is a dimer; each monomer forms temporary covalent bond with a DNA end in a DSB [133]. If TOPO2 is nonfunctional, religation fails, and the break becomes permanent [134, 135]. TOPO2 inhibitors are used in chemotherapy to induce accumulation of DSBs in rapidly dividing cancer cells and cell death [136-138].

The mechanism of DSB induction affects the structure of break ends and, therefore, the choice of DNA repair pathway [139, 140]. For example, this choice depends on the type of ionization particles causing the breaks in DNA: high-energy particles (e.g., in carbon-ion therapy) induce multiple DNA damage, with DSBs surrounded by single-strand breaks and base damage, which hinders Ku70/80 binding and favors resection-dependent pathways. In contrast, lower-energy photon-based radiotherapy causes DSBs that can be directly ligated by NHEJ [15].

DNA breaks induced by the replication stress, the so-called one-ended DSBs, are typically repaired by HDR [141-143]. If unrepaired in the S or G2 phases, they are repaired mostly by MMEJ (see above) [74, 106].

The presence of covalently linked protein adducts at the DSB ends can also affect the process of DNA repair (as in the case of inhibition by TOPO2). The repair of such DSBs requires removal of the DNA-TOPO2 complex by proteases or nucleases. Nucleolytic removal involves MRN, which cleaves off the protein

adduct together with the DNA fragment, thus initiating resection of the break ends [144]. In the case of proteolytic removal, tyrosyl-DNA phosphodiesterase 2 (TDP2) cleaves the TOPO2-DNA bond. The access of TDP2 to this bond requires denaturation or partial proteolysis of TOPO2. The change in the TOPO2 conformation is provided via sumoylation by the SUMO ligase ZATT (Znf protein associated with TDP2 and TOP2), while proteolytic cleavage is catalyzed by proteasome or metallonuclease SPRTN [144-147]. After the action of TDP2, the ends become available for NHEJ or other pathways; however, such complex mechanism of DNA end processing delays the repair.

Different DNA repair mechanisms occur at different rates, which affects the risk of errors during end joining [148, 149]. Slower repairs increase the chance of ends drifting apart and encountering another break, thus promoting translocations [150]. This may explain why the TOPO2 inhibitor therapy is associated with the emergence of secondary leukemias featuring chromosomal translocations. Etoposide-treated cells often show DNA break ends outside the chromosomal territories, which is a prerequisite for chromosomal translocations [151, 152]. It was found that the ends of etoposide-induced breaks are more mobile than those of radiation-induced breaks [152, 153].

## CONTRIBUTION OF NHEJ AND MMEJ TO THE FORMATION OF TRANSLOCATIONS

The debates continue whether it is canonical NHEJ or alternative mechanism (MMEJ) is the primary driver of chromosomal translocations in mammals [154-158]. Numerous studies using various methodological approaches have yielded conflicting results.

Early studies of cells from leukemia patients have shown that translocations are often formed with the involvement of microhomologies at the break junctions [154]. Inhibition or immune depletion of NHEJ proteins in tumor cell extracts did not abolish the joining of DNA fragments, and sequencing of linked DNA fragments revealed the use of microhomologies, implicating MMEJ in the translocation formation [154].

Another approach to evaluate the contribution of NHEJ and MMEJ in the formation of translocations is comparing DNA repair events in wild-type and NHEJ-deficient cell lines. Simsek and Jasin [155] and Weinstock et al. [159] used embryonic stem cells from *Xrcc4*$^{-/-}$ and *Ku70*$^{-/-}$ transgenic mice containing two transgenic cassettes with the I-SceI meganuclease site. Transient expressed I-SceI induced DNA breaks, while the fusion of two cassettes made cells antibiotic-resistant, so colonies growing on antibiotic-containing media were counted and sequenced. It was found that microhomologies (≥4 nucleotides) were used in ~25% of rearrangements. Experiments were performed in both NHEJ gene-expressing and NHEJ gene-deficient cells. In *Ku70*$^{-/-}$ or *Xrcc4*$^{-/-}$ cells, the frequency of antibiotic-resistant (translocation-containing) colonies was higher, suggesting that in the absence of NHEJ, translocations form via MMEJ and that MMEJ is more prone to erroneous joining [155].

In NIH3T3 mouse fibroblasts, the frequency of translocation increased with the inhibition or knockdown of DNA-PKcs [160]. The results were obtained by *in vivo* microscopy using the cells contained a system for visualization of genomic loci, which allowed to observe I-SceI-induced breaks and their convergence with the following formation of translocations [160]. DNA-PKcs inhibition increased the translocation frequency (as detected by PCR) in human lymphocytes with the integrated CRISPR/Cas9 system for induction of rearrangements between the *MYC* and *IGH* genes [161].

However, the study in human HCT116 and NALM6 cells yielded different results [156]. The breaks were induced by programmable nucleases (ZFN, TALEN, Cas9), and translocations were detected by PCR 48 hours after transfection. The PCR products were cloned into plasmid vectors and sequenced. It was found that most rearrangements occurred without the use of microhomologies, as in the work by Simsek and Jasin [155]. The difference was that in LIG4- or XRCC4-deficient cells, the frequency of translocations decreased, but the number of deletions and the use of microhomologies at the translocation sites increased. The authors concluded that under normal conditions, translocations in human cells are formed mostly by NHEJ, and the differences from the study by Simsek and Jasin [155] may reflect species-specific (mouse vs. human) or methodological variations [156]. For example, during the 48-hour post-transfection period (Ghezraoui et al. [156]), only a fraction of possible translocations was formed, and their frequency was even lower in NHEJ-deficient cells. In contrast, Simsek and Jasin [155] allowed 7-10 days for all possible translocations to occur, so the difference in the translocation frequency in the wild-type cell and cells with mutations in the DNA repair system was the opposite.

Next-generation sequencing (NGS) of translocation junctions has provided deeper insights into the contribution of particular DNA repair mechanisms. Chiarle et al. [162] used mouse cells with integrated I-SceI sites and lentiviral I-SceI expression, followed by massively parallel sequencing of translocation junctions to show that microhomology was used in most translocations. Conversely, based on the results of massively parallel sequencing of Cas9-induced translocation junctions in human cell lines [157],

most translocations occurred without the use of microhomologies (>3 nucleotides), even in the case of DNA-PKcs inhibition.

The studies conducted in 2011-2024, which employed different models, DSB induction methods, and translocation detection techniques, yielded varying results. Currently, it is commonly accepted that both canonical NHEJ and MMEJ are involved in the formation of translocations and normal DNA repair, and the contribution of each pathway depends on the cell type and pathway functionality. A frequent association of translocations with MMEJ – more so than in correct (*cis*) repair [154, 159, 162] – may reflect a slower repair kinetics in MMEJ [163]. Normally, the appearance of a break in DNA should immediately initiate NHEJ [105]. If NHEJ is delayed, the risk of ends drifting apart, getting "lost", and forming translocations increases. Such ends are also more likely to undergo resection and MMEJ; even if Ku70/80 are already bound, this does not preclude the MRN binding and resection [79]. In other words, DNA break ends "getting lost" represent a common prerequisite for both translocation formation and MMEJ-mediated joining. Further NGS studies comparing translocations (*trans* repair) and normal repair (*cis* repair) are needed for better understanding of DNA repair mechanisms.

## CONCLUSION

Cells employ multiple DSB repair mechanisms. The choice of a particular pathway is influenced by the DNA damage type and cell cycle phase and is governed by the repair complex composition and post-translational modifications of histones and repair proteins. Chromosomal rearrangements most commonly occur via NHEJ and MMEJ [3]. NHEJ does not require extensive end processing, so rearrangements typically occur at the break site with minimal indel formation. MMEJ requires resection, while the use of microhomologies inevitably causes deletions. However, MMEJ activation does not necessarily result in chromosomal rearrangements. The key factor in joining the ends from the same break is their tethering until ligation. Both translocations and the bias toward the MMEJ pathway are consequences of end separation. Further research is needed to clarify the contribution of DNA repair pathways to the formation of chromosomal rearrangements.

**Abbreviations**

| | |
|---|---|
| DSB | double-strand break |
| HDR | homology-directed repair |
| LIG4 | DNA ligase IV |
| LR | long-range synaptic complex |
| MMEJ | microhomology-mediated end joining |
| MRN | MRE11–RAD50–NBS1 protein complex |
| NHEJ | non-homologous end joining |
| POLQ | DNA polymerase θ (Pol X family) |
| SR | short-range synaptic complex |
| SSA | single-strand annealing |

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**Ethics approval and consent to participate**

This work does not contain any studies involving human and animal subjects.

**Conflict of interest**

The authors of this work declare that they have no conflicts of interest.

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