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Review

Repair Pathway Choices and Consequences at the Double-Strand Break

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DNA double-strand breaks (DSBs) are cytotoxic lesions that threaten genomic integrity. Failure to repair a DSB has deleterious consequences, including genomic instability and cell death. Indeed, misrepair of DSBs can lead to inappropriate end-joining events, which commonly underlie oncogenic transformation due to chromosomal translocations. Typically, cells employ two main mechanisms to repair DSBs: homologous recombination (HR) and classical nonhomologous end joining (C-NHEJ). In addition, alternative error-prone DSB repair pathways, namely alternative end joining (alt-EJ) and single-strand annealing (SSA), have been recently shown to operate in many different conditions and to contribute to genome rearrangements and oncogenic transformation. Here, we review the mechanisms regulating DSB repair pathway choice, together with the potential interconnections between HR and the annealing-dependent error-prone DSB repair pathways.

Trends

Of the four known pathways for repairing DNA DSBs, some evolved towards high-fidelity processes (HR and C-NHEJ), while others are intrinsically mutagenic (alt-EJ and SSA).

Some repair pathways are end resection-independent (C-NHEJ), while others are end resection-dependent (HR, alt-EJ, and SSA). End resection likely plays a key role in dictating DNA repair pathway choice.

Homology-based repair pathways (HR, alt-EJ, and SSA) are competitive and mutually regulated around the RAD51 presynaptic and postsynaptic steps of HR.

Error-prone repair pathways can compensate for the loss of HR. Polυ (an alt-EJ polymerase) is upregulated in HR-deficient cancers: loss of the HR and Polυ-mediated alt-EJ pathways is synthetic lethal.

Mechanisms of DNA DSB Repair

Detection and faithful repair of damaged DNA is essential for genome integrity. Many types of DNA lesions impede replication fork progression, resulting in replication fork collapse and DSB formation with loss of physical continuity of the genome [1]. The repair of DSBs involves four possible mechanisms (Figure 1). The first mechanism is C-NHEJ. In this mechanism, the DSB is repaired by blunt end ligation independently of sequence homology, but requiring many factors such as Ku70/80, DNA-PKcs, and DNA ligase IV (Figure 1A). C-NHEJ can occur throughout the cell cycle but is dominant in G0/G1 and G2 [2,3]. Despite the mutagenicity of C-NHEJ, its fast kinetics has a clear role in protecting genome integrity, notably by suppressing chromosomal translocations, at least for the majority of repair events [4]. Alternatively, the DSB end can be resected, leaving 3′ single-stranded DNA (ssDNA) overhangs. The resected DSB can be repaired by three possible mechanisms: HR, SSA, and alt-EJ. HR predominates in the mid-S and mid-G2 cell cycle phases, where the amount of DNA replication is highest and when the sister template is available [3]. Because HR uses a sister or homologous chromatid for repair, it requires strand invasion mediated by the recombinase RAD51 and the process is typically error-free even though completion of HR often requires error-prone polymerases (Figure 1B) [5]. The resected DSB can also be repaired by mutagenic repair pathways, namely SSA or alt-EJ. SSA mediates end joining between interspersed nucleotide repeats in the genome and involves reannealing of Replication Protein A (RPA)-covered ssDNA by the RAD52 protein. Although this is homology-directed repair, one copy of the repeat and the intervening sequence between the repeats are deleted in the repair product, thus resulting in loss of genetic information (Figure 1C) [5].

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In recent years, the notion of an alt-EJ pathway in addition to C-NHEJ has become more evident [6]. The use of alt-EJ for DNA DSB repair has been described in various cellular contexts, but the mechanistic details of this pathway remain unclear. The use of alt-EJ for repairing DSB has harmful consequences on genomic integrity because of its apparent predilection for joining DSBs on different chromosomes, thereby generating chromosomal translocations and mutagenic rearrangements (Figure 1D) [7,8]. Early evidence for alt-EJ came from studies showing that yeast and mammalian cells deficient in C-NHEJ were still able to repair DSBs via end joining [6]. Further evidence for an alternative end-joining pathway arose from the observation that mice deficient in C-NHEJ still exhibited chromosomal translocations and V(D)J recombination [9]. Molecular characterization of this alt-EJ activity in cells lacking C-NHEJ revealed that the XRCC1/DNA ligase III complex and the PARP1 polyribosylating enzyme were involved [10]. Initially, alt-EJ was considered merely a backup repair pathway for C-NHEJ for end joining of chromosomal DSBs in the context of V(D)J recombination because of its error-prone nature and its original detection only in the absence of C-NHEJ [11]. Subsequent studies, however, have demonstrated that alt-EJ might have a more primary role in repairing endogenous chromosomal DSBs, depending on the biological context [6].

Role of End Resection in DSB Repair Choice

Given that three of the pathways diverge at the early step of end resection and have different outcomes (Figure 1), it is likely that end resection dictates pathway choice and repair outcome [12]. The initial phase of end resection, called 'end clipping', is carried out by the structure-specific nuclease MRE11 and CtIP. In this phase, a relatively small number of base pairs (i.e., 20 bp in mammalian cells or 100–300 bp in yeast) are processed, making the DNA ends available for alt-EJ [13,14]. In the second phase of end resection called 'extensive resection', helicases and exonucleases (i.e., DNA2, BLM, WRN, CtIP, and EXO1) generate long stretches of ssDNA, thereby committing the cells to HR or SSA [12,15].

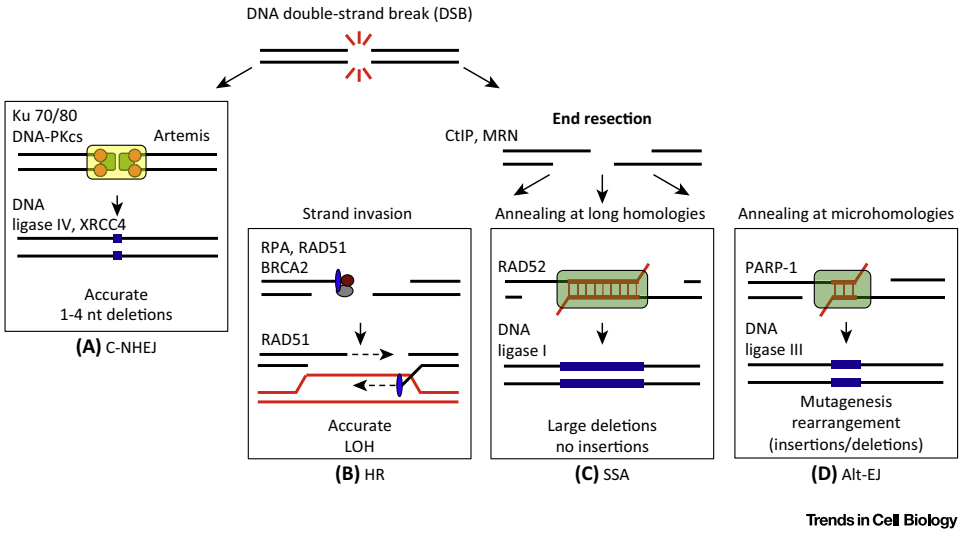

Figure 1. Four Approaches to Repair DNA Double-Strand Breaks (DSBs). (A–D) The repair of DNA DSBs relies primarily on whether DNA end resection occurs. When resection is blocked, repair through C-NHEJ is favored. However, when DNA resection occurs, three pathways (HR, alt-EJ, and SSA) can compete for the repair of DSBs. Indeed, there are two layers of competition for the repair of DSBs. Initially at the stage of end resection, C-NHEJ competes with the resection-dependent pathways. Secondly, once resection has occurred, HR, alt-EJ, and SSA can compete for the repair. Notably, each of the four repair pathways lead to different genetic outcomes (LOH, deletions, insertions) and the fidelity of the repair mechanism is mentioned for each pathway. Abbreviations: nt, nucleotides; LOH, loss of heterozygosity; C-NHEJ, classical nonhomologous end joining; HR, homologous recombination; alt-EJ, alternative end joining; SSA, single-strand annealing.