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Review

Collaboration and competition between DNA double-strand break repair pathways

Elizabeth M. Kass, Maria Jasin *

Developmental Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

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ABSTRACT

DNA double-strand breaks resulting from normal cellular processes including replication and exogenous sources such as ionizing radiation pose a serious risk to genome stability, and cells have evolved different mechanisms for their efficient repair. The two major pathways involved in the repair of double-strand breaks in eukaryotic cells are non-homologous end joining and homologous recombination. Numerous factors affect the decision to repair a double-strand break via these pathways, and accumulating evidence suggests these major repair pathways both cooperate and compete with each other at double-strand break sites to facilitate efficient repair and promote genomic integrity.

1. Introduction

DNA damage constantly occurs in cells as a result of both environmental and endogenous insults. Double-strand breaks (DSBs), which may arise during the normal course of DNA replication and as a result of exposure to DNA damaging agents, are considered one of the most cytotoxic forms of DNA damage [1]. The ability to accurately repair these breaks is essential for faithful propagation of genetic information. Deficiencies in DSB repair can lead to mutations and chromosomal rearrangements that ultimately may result in genomic instability and tumorigenesis. Therefore, cells have evolved effective mechanisms for the accurate and timely repair of DSBs in DNA.

Two major pathways are involved in the repair of DSBs in eukaryotic cells: non-homologous end joining (NHEJ) and homologous recombination (HR) [2,3]. NHEJ is an efficient pathway that functions throughout the cell cycle and involves the ligation of DNA ends with minimal processing at the site of end joining, while HR, occurring specifically in late S and G2 phases of the cell cycle, utilizes an undamaged homologous sequence as a repair template, preferably the sister chromatid, and is considered a more precise method for repairing DSBs in DNA. This review focuses on the collaboration and competition of the two major pathways of DSB repair in mammalian cells, with an emphasis on factors affecting the decision to repair breaks via HR or NHEJ.

2. Mechanisms of homologous recombination

HR is initiated by resection of DNA ends at the DSB site to yield 3′-single-stranded DNA (ssDNA) overhangs which are capable of invading duplex DNA containing a homologous sequence [4]. Studies in *Saccharomyces cerevisiae* suggest that the MRX complex, encoded by *MRE11, RAD50* and *XRS2* (the ortholog of *NBS1* in mammalian cells), together with the Sae2 protein, is required for the initial end processing step of HR. More extensive processing involves the 5′–3′ exonuclease Exo1 or the combined helicase/nuclease activities of Sgs1/Dna2 [5,6]. The functional counterpart of Sae2 in vertebrate cells is CtIP [7]. The protein product of the breast cancer susceptibility gene *BRCA1* interacts with both MRN and CtIP, and genetic and physical evidence suggests that BRCA1 may be involved in end resection [8–10], although its exact role remains uncertain. There is, however, data supporting a role for mammalian counterparts of Exo1 and Sgs1 in end resection: human EXO1 can resect DNA ends in vitro, and its activity is stimulated by Bloom's syndrome protein (BLM), the Sgs1 ortholog [11]. DNA ends resected by EXO1 and BLM are utilized in subsequent strand exchange reactions.

The 3′-single-stranded DNA overhang generated during end resection is bound by replication protein A (RPA), which is required for the subsequent recruitment of checkpoint and HR proteins such as RAD51 [12]. RAD51, a homolog of the bacterial RecA protein, is a DNA-dependent ATPase that forms nucleoprotein filaments with DNA. In mammalian cells, RAD51 is recruited to DSBs by the protein product of the breast cancer susceptibility gene *BRCA2* [13]. BRCA2 is a large (410kD) protein that binds RAD51 through interactions with a series of eight short conserved repeats termed BRC

* Corresponding author.
E-mail address: m-jasin@ski.mskcc.org (M. Jasin).

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repeats [14,15]. Recent biochemical analysis has shown that one or more BRC repeats stimulate RAD51 nucleoprotein filament formation on ssDNA in the presence of ATP [16,17]. Moreover, structural studies have demonstrated that BRCA2 itself binds to ssDNA [18].

Both BRCA1 and BRCA2 mutant cells are defective for HR [19,20]. BRCA2 appears to interact with BRCA1 via the BRCA2 "partner" PALB2; mutations in PALB2 that disrupt binding to either BRCA1 or BRCA2, as well as clinically relevant mutations in BRCA1 which abrogate binding to PALB2, result in decreased levels of HR [21–24]. Additionally, BRCA2 forms a complex with DSS1, a conserved 70 amino acid protein required for DNA damage-induced RAD51 foci formation, and presumably HR, in mammalian cells [18,25,26]. In yeast, BRCA2 (as well as BRCA1 and PALB2) is not present; therefore, other proteins such as Rad52 assist in loading Rad51 onto ssDNA [12].

Once recruited to the DSB, RAD51 catalyzes strand exchange during which ssDNA invades homologous duplex DNA forming a displacement loop (D-loop). Recently solved crystal structures of *Escherichia coli* RecA-ssDNA and RecA-heteroduplex filaments have shed new light on how RAD51 may facilitate strand exchange [27]: RecA-bound ssDNA is stretched globally but maintains a B-DNA-like conformation locally in base triplets; this unusual structure favors Watson–Crick type base pairing during homology sampling with the complementary strand in a destabilized donor duplex DNA.

Once formed, the D-loop has multiple fates [12]. In the primary pathway in mitotic cells, termed synthesis-dependent strand annealing, the 3′ end in the D-loop is extended by repair synthesis, and then the newly synthesized DNA strand dissociates to anneal to the other DNA end to complete the reaction. If the second DNA end is "captured" by the D-loop, a double Holliday junction forms that can potentially be resolved by several different proteins, including in humans GEN1 and SLX1/SLX4 [4,28]. As double Holliday junction resolution can occur in different ways, crossover and non-crossover products are possible. While crossovers play an important role in facilitating chromosome segregation during meiotic recombination [29], crossovers occurring during mitotic recombination may have serious deleterious effects, including loss of heterozygosity [3]. Proteins that disrupt D-loops or "dissolve" Holliday junctions (such as BLM) suppress mitotic crossovers, thereby decreasing the risk of genomic instability [30].

3. Repair by single-strand annealing

Another repair pathway involving sequence homology, but distinct from HR, is single-strand annealing (SSA) [31]. SSA can occur following end resection if sequence repeats exist on both sides of the DSB. The complementary single strands formed at the repeats then anneal and flaps formed from the annealing reaction are trimmed off, resulting in a loss of sequence between the repeats. Compared to HR, SSA is therefore more mutagenic because it involves loss of genetic information. Proteins identified to promote SSA in mammalian cells and yeast include RAD52 (annealing) and ERCC1 and Rad1/Rad10 (flap endonuclease) [8,32,33].

4. Repair by non-homologous end joining

NHEJ proteins were initially identified through their requirement for resistance to ionizing radiation and V(D)J recombination in the immune system [2]. The first protein in this pathway to bind DNA ends is the Ku70/80 heterodimer (Ku). In mammalian cells, Ku interacts with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and together they may act to synapse the two DNA ends to be repaired [34]. DNA ends are joined by the DNA ligase IV/XRCC4 complex [35]. XRCC4 does not possess any known enzymatic activity but acts as a scaffold that forms interactions with both Ku and DNA and both stabilizes and stimulates the ligase activity of DNA ligase IV [36].

NHEJ can join DNA ends with a number of different structures. As a result, this pathway makes use of a number of processing steps that may include cleavage and gap filling prior to ligation. The nuclease Artemis is recruited to a DSB site by its interactions with DNA-PKcs, and the Artemis/DNA-PKcs complex is able to cleave a variety of damaged DNA overhangs [37]. Cleavage of DNA ends may result in gaps in the DNA that need to be filled in by polymerases involved in NHEJ. Members of the PolX family include polymerases μ and λ, which interact with the Ku:DNA complex via BRCT domains [38], although data in yeast suggests that other polymerase families can substitute [39]. The modification of DNA ends prior to joining by these processing steps can lead to deletions and insertions accounting for the more error-prone nature of NHEJ compared to HR.

The pathway thus described is considered to be the "canonical" NHEJ pathway. However, almost from their initial characterization, cell mutants for the canonical NHEJ factors have been recognized to join DSBs with good efficiency in certain contexts, for example, endonuclease-generated DSBs in plasmid and chromosomal DNA [40,41] and immune system-generated DSBs in mice [42,43]. NHEJ in the absence of the canonical factors is termed alternative NHEJ (alt-NHEJ), but whether this is a distinct pathway is uncertain, although a number of reports have suggested the involvement of a number of other factors (see e.g., [44]). Canonical NHEJ and alt-NHEJ seem to differ by the amount of microhomology at the site of joining. Small sequence microhomologies may help to align broken strands of DNA, but whereas microhomology at breakpoint junctions can occur in canonical NHEJ at frequencies expected by chance, longer microhomologies are over-represented in junctions arising from alt-NHEJ [45].

5. Regulation of repair pathway choice

DSB repair pathway choice is regulated by several factors, including the nature of the lesion and cell cycle phase. Programmed DSBs are channeled into specific repair pathways, e.g., DSBs generated during V(D)J recombination by the RAG proteins are repaired by NHEJ [46], whereas DSBs generated during meiosis by the Spo11 protein are repaired by HR [29]. Several factors may enforce pathway choice for programmed DSBs. For example, Spo11 forms a covalent linkage with DNA and is cleaved off of DNA by the MRX/Sae2 proteins [47]. Moreover, in mouse Ku has been reported to be down regulated early in meiotic prophase, which would presumably lessen NHEJ [48]. Finally, DSBs arising during DNA replication may typically be one ended, requiring HR for repair, as NHEJ of two one-ended DSBs would give rise to translocations [49].

More generally, cell cycle phase is a primary determinant in restricting HR, whereas NHEJ operates throughout the cell cycle [50]. The restriction in HR to the S/G2 phases of the cell cycle makes sense from the standpoint that the primary repair template in mammalian cells is the sister chromatid, which is not present in G1 cells. By contrast, in yeast diploid cells are able to efficiently use the homolog for DSB repair [51,52]. Why might the homolog be used efficiently in yeast for repair but not in mammalian cells? A simple explanation may be that the chance for random collision between homologs is greater in the much smaller yeast nucleus than it is in the mammalian nucleus [53]. That proximity matters is supported by efficient interhomolog recombination in Drosophila, where homologs are actually paired [54].

Cell cycle phase also plays a more active role in regulating HR in that end resection is promoted by cyclin-dependent protein kinases (CDKs). In yeast, CDK activity is required for efficient end