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Review

Regulation of Single-Strand Annealing and its Role in Genome Maintenance

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Single-strand annealing (SSA) is a DNA double-strand break (DSB) repair pathway that uses homologous repeats to bridge DSB ends. SSA involving repeats that flank a single DSB causes a deletion rearrangement between the repeats, and hence is relatively mutagenic. Nevertheless, this pathway is conserved, in that SSA events have been found in several organisms. In this review, we describe the mechanism of SSA and its regulation, including the cellular conditions that may favor SSA versus other DSB repair events. We will also evaluate the potential contribution of SSA to cancer-associated genome rearrangements, and to DSB-induced gene targeting.

Chromosomal Break Repair by the Single-Strand Annealing Pathway

A chromosomal **double-strand break** (**DSB**; see Glossary) can be generated under several circumstances. DSBs can be directly induced by nucleases, such as topoisomerase II, which is important for untangling chromosomes, as occurs during chromosome condensation [1]. Other cellular processes that require induction of DSBs by nucleases include antibody maturation and meiosis [2,3]. DSBs can also be formed as the result of nucleolytic cleavage of various DNA structures, such as DNA interstrand crosslinks, blocked or reversed DNA replication forks, and three-stranded DNA/RNA hybrids that result from transcription (i.e., R-loops) [4,5]. Furthermore, DSBs can be induced at defined DNA sequences via site-specific endonucleases [6]. Apart from nucleases, clastogenic (i.e., chromosomal breaking) agents, including small molecules and ionizing radiation, can cause cleavage of phosphodiester bonds and result in DSBs. Sources of clastogens include byproducts of cellular metabolism and environmental agents [7]. Additionally, clastogens are commonly applied as anticancer agents, in the form of small molecule chemotherapeutics and ionizing radiation [8]. Thus, characterizing the pathways that repair DSBs is important to understand both genome stability and the cellular response to clastogenic cancer therapeutics.

The focus of this review is the **single-strand annealing (SSA)** DSB repair pathway. SSA involves annealing of homologous repeat sequences that flank a DSB, which causes a deletion rearrangement between the repeats (Figure 1, Key Figure). SSA events have been demonstrated in mammalian cells and in several model organisms including *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Drosophila melanogaster*, and *Caenorhabditis elegans* [9–13]. A study from the Sternberg lab in 1984 provided early evidence for SSA using DNA plasmid substrates harboring homologous repeats transfected into mouse cells, and proposed a model for the steps of this pathway [13]. This model involves a DSB between homologous repeats, followed by DSB **end resection** that generates 3′ ssDNA, which reveals flanking homologous sequences that are annealed together to form a synapsed intermediate (Figure 1). This intermediate is then

Trends

The SSA DSB repair pathway causes a rearrangement between two homologous repeats.

SSA and ALT-EJ are similar, as they both involve an annealed intermediate to synapse a DSB, but show mechanistic distinctions, including differential requirements for RAD52 versus PARP and Polθ.

End resection to generate 3′ ssDNA is a pivotal step of SSA, and is a shared intermediate with HDR and ALT-EJ, but each of these events likely require distinct degrees of end resection. Also, if end resection occurs prior to sister chromatid synthesis, which is the preferred template for HDR, then SSA or ALT-EJ may be required for repair.

The relative role of SSA versus ALT-EJ on repeat-mediated rearrangements is affected by the degree of divergence between the repeats.

Most gene targeting events are mediated by HDR (i.e., RAD51-dependent), however, some targeting approaches appear to involve SSA or ALT-EJ.

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Key Figure

Comparisons between Single-Strand Annealing (SSA) and Other Double-Strand Break (DSB) Repair Events: Homology Directed Repair (HDR), Alternative End-Joining (ALT-EJ), and Canonical Nonhomologous End-Joining (C-NHEJ)

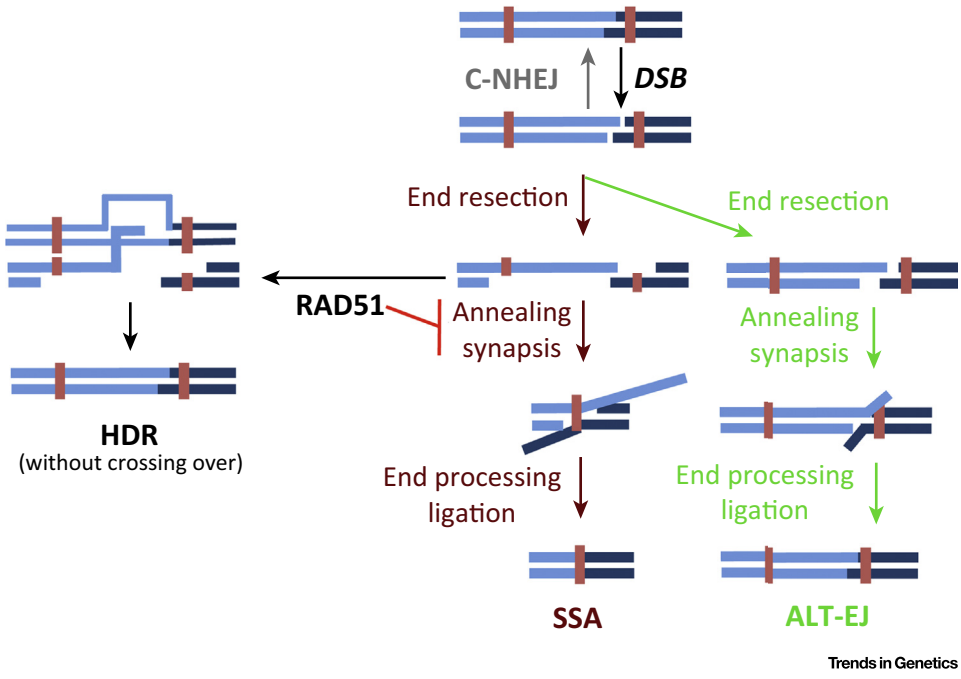

Figure 1. End resection is depicted as a common intermediate of SSA, HDR, and ALT-EJ. In the subsequent steps of SSA, homologous repeats (depicted as rust-colored boxes) anneal to form the synapsis intermediate that is then processed for ligation. The RAD51 recombinase is shown to inhibit SSA, and conversely mediate the strand invasion step of HDR.

processed for ligation, which requires endonucleolytic cleavage of nonhomologous 3′ ssDNA tails, and polymerase filling of any gaps (Figure 1). Studies of individual repair factors have provided evidence for many of these proposed steps of SSA.

Functional analysis of several factors has established that DSB end resection, which refers to the generation of 3′ ssDNA, is a pivotal step of SSA (Figure 1). For example, SSA is dependent on CtIP [14,15], which is a key end resection factor, based on cell biology measurements of ssDNA formation at sites of DNA damage, as well as physical analysis of site-specific endonuclease-generated chromosomal DSBs [16–18]. Conversely, factors that inhibit end resection have been found to suppress SSA, such as the DNA damage response pathway involving H2AX, RNF168, 53BP1, and RIF1 [14,18–21,110]. Beyond these examples, numerous other factors that promote/inhibit end resection have a corresponding effect on SSA [22–37]. Furthermore, this correlation is conserved, since many of the homologs/orthologs of these factors in *S. cerevisiae* affect both SSA and end resection [9,38–41]. A corollary of these findings is that SSA assays can be a useful screening tool for genes and/or small molecules that are candidates for affecting end resection. As one example, bortezomib, a small molecule proteasome inhibitor used for treating multiple myeloma [42], disrupts both SSA and end resection [26].

Glossary

Alternative end-joining (ALT-EJ): a DSB repair event that is independent of the C-NHEJ pathway, and is often mediated by short stretches of homology to bridge the DSB prior to ligation.

Canonical nonhomologous end-joining (C-NHEJ): a major pathway of DSB repair that does not require any homology to bridge the DSB prior to ligation. This pathway is critical for the repair of DSBs that are induced during antibody maturation, particularly V(D)J recombination and class switch recombination.

Double-strand break (DSB): a chromosomal DSB is the cleavage of two phosphodiester bonds on opposing strands that are in close enough proximity to cause loss of the continuous DNA double helix.

End resection: the processing of chromosomal DSB ends to generate 3′ single-stranded DNA (ssDNA).

Gene targeting: the method of editing chromosomal DNA to match the sequence of an exogenous donor, which shares homology with the chromosomal target site. Gene targeting is substantially enhanced by causing a DNA break at the chromosomal target.

Homology-directed repair (HDR): a DSB repair event that involves invasion of a homologous template by at least one strand from the DSB, which templates nascent DNA synthesis to form an extended strand that can form a bridge to the other DSB end. The homologous strand invasion step is catalyzed by the RAD51 recombinase.

Single-strand annealing (SSA): a DSB repair event that uses flanking homology to bridge the DNA lesion, causing a deletion between the repeats.