Cell Cycle Features:

Second-end DNA capture in double-strand break repair

How to catch a DNA by its tail

Amitabh V. Nimonkar and Stephen C. Kowalczykowski*

Departments of Microbiology, and of Molecular and Cellular Biology; University of California; Davis, CA USA

*Correspondence to: Stephen C. Kowalczykowski; University of California Microbiology; One Shields Ave; Davis, CA 95616-8665 USA; Tel.: 530.752.5938; Fax: 530.752.5938; Email: sckowalczykowski@ucdavis.edu

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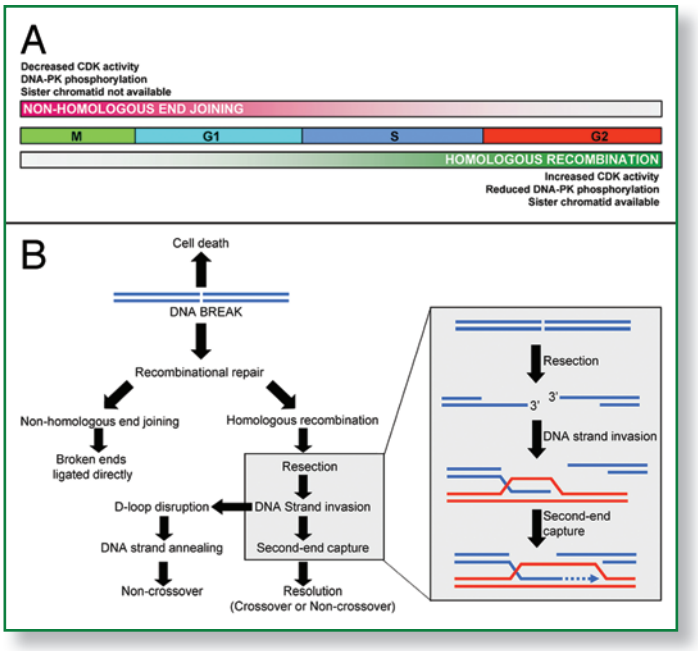

Figure 1. Cell cycle dependent Recombinational repair of double-strand DNA breaks (DSB). (A) Although non-homologous end joining (NHEJ) can occur during all stages of cell cycle (horizontal bar above cell cycle with color gradient from pink (maximum activity) to gray (minimum activity)), it is downregulated during S and G_2. In contrast, homologous recombination (HR) functions primarily during S and G_2 (horizontal bar below cell cycle with color gradient from gray (minimum activity) to green (maximum activity)). The factors that regulate NHEJ and HR are listed on the figure. (B) A DSB, if unrepaired, can lead to cell death (top part). Repair of the break can occur by NHEJ or HR (bottom part). Blue lines: damaged DNA; red lines: donor DNA; broken arrow head: DNA synthesis.

A double-strand DNA break (DSB) represents a potentially lethal form of DNA damage. DSBs are generated upon exposure to ionizing radiation and chemical mutagens. In addition, they arise from replication of imperfect chromosomal DNA, and during meiotic and V(D)J recombination. It is estimated that a normal human cell accurately repairs approximately 50 breaks every cell cycle.[1] How is this achieved? The easiest option is to simply reconnect the broken ends. Non-homologous end joining (NHEJ) achieves this goal and, it has the advantage that it can occur throughout the cell cycle[2] without the need for a template (Fig. 1A). Though effective, NHEJ has two potential pitfalls: first, without a template, incorrectly ligated DSB ends produce chromosomal rearrangements, and second, genetic information is lost if the DNA ends are altered before re-ligation. The alternative is homologous recombination (HR). HR is far more accurate, but is constrained by the requirement for an intact homologous template. It is regulated to occur mainly during S and G_2 stages of cell cycle when the homologous sister chromatid is present[2,3] (Fig. 1A).

The steps involved in the repair of DSBs by HR were elegantly described by Szostak et al.[4] (Fig. 1B). The repair of DSBs starts with resection of the 5′ termini at both ends of the break. This processing is crucial because it generates single-strand DNA (ssDNA) that serves as the substrate for assembly of a RecA/Rad51 nucleoprotein filament that searches for DNA homology. Once found, the processed end invades the homologous DNA to form a recombination intermediate called a joint molecule or displacement loop (D-loop). The invaded strand then primes DNA synthesis using the intact homologous chromosome as a template to restore genetic material lost by resection. Subsequently, the resected second-end pairs, and more DNA synthesis restores the lost genetic information. In the DSB repair (DSBR) model, it was recognized that the second processed end of the DNA break could engage with the joint molecule by two alternative mechanisms: either a second independent invasion or annealing to the displaced strand of the joint molecule. This second-end capture step is critical, because it dictates the outcome of HR: capture leads to formation of double Holliday junctions, which can be resolved to generate crossover (CO) or non-crossover (NCO) products.[4] Alternatively, the D-loop can be disrupted after DNA synthesis from the first processed DNA end, and this end can anneal with the other resected end of the break. This process is termed synthesis-dependent strand annealing (SDSA) and is the primary source of NCO.[5]

In the past year, several papers have converged on aspects of second-end capture,[6-10] refining steps of a key Rad52-dependent annealing process that was identified in earlier studies.[11,12] For more than a decade, it was tacitly assumed that both processed ends would invade the donor dsDNA to form the Holliday junctions. However, prior genetic and biochemical evidence implicated *Saccharomyces cerevisiae* Rad52 as a key protein in this capture event, and one that could anneal ssDNA.[13] Importantly, Rad52 demonstrated the unique ability to anneal ssDNA complexed with replication protein-A (RPA)[12] and in vivo physical analysis established that Rad52 was needed to anneal the second processed end to joint molecule intermediates, where the ssDNA is complexed with RPA.[9]

To elaborate the mechanism of second-end capture, several laboratories examined the pairing of ssDNA to joint molecules formed in vitro.[6-8,10,11] Rad52 was shown to utilize D-loops formed by Rad51

and Rad54 to mediate second-end capture when otherwise inhibitory amounts of RPA were present.[6,10] The product was a "double-D-loop", or complement-stabilized D-loop, whose formation required species-specific interaction with RPA,[6,8] revealing that second-end capture results from annealing and not another invasion event. Similarly, human Rad52 was shown to mediate second-end capture in staged reactions or with synthetic D-loops where the joint molecule was extended by human DNA polymerase η to expose the region homologous to the second-end.[8,10] These studies established clear roles for Rad52-dependent annealing in DSB repair.

Despite the importance of *Rad52* function in unicellular eukaryotes, *rad52*-deficient mammalian cells are not sensitive to ionizing radiation and exhibit only a slightly reduced HR.[14] This difference may be due to the presence of proteins in human cells that are redundant with Rad52 function. In *Ustilago maydis*, a BRCA2 homolog, Brh2, was shown to mediate second-end capture to yield a product similar to one produced by Rad52,[7] although its ability to function with RPA was not fully examined.

Thus, recent findings now provide a function for specific DNA annealing proteins in steps that lead to Holliday junction formation. These Holliday junctions, if unresolved, can cause chromosome segregation problems, but their resolution can lead to chromosome crossovers. The regulation of this process remains a largely open question, but likely sister chromatid cohesion, disruption of joint molecules by the BLM-homologs,[15] and control of Rad52 function by Rad51,[7,16] are involved. Thus, the Rad52-like proteins are the molecular handles used to proverbially grab the DSB by its (ssDNA) tail, an event that is important to complete recombinational DNA repair.

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