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Review

Functions of the Snf2/Swi2 family Rad54 motor protein in homologous recombination ☆

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ABSTRACT

Homologous recombination is a central pathway to maintain genomic stability and is involved in the repair of DNA damage and replication fork support, as well as accurate chromosome segregation during meiosis. Rad54 is a dsDNA-dependent ATPase of the Snf2/Swi2 family of SF2 helicases, although Rad54 lacks classical helicase activity and cannot carry out the strand displacement reactions typical for DNA helicases. Rad54 is a potent and processive motor protein that translocates on dsDNA, potentially executing several functions in recombinational DNA repair. Rad54 acts in concert with Rad51, the central protein of recombination that performs the key reactions of homology search and DNA strand invasion. Here, we will review the role of the Rad54 protein in homologous recombination with an emphasis on mechanistic studies with the yeast and human enzymes. We will discuss how these results relate to *in vivo* functions of Rad54 during homologous recombination in somatic cells and during meiosis. This article is part of a Special Issue entitled: Snf2/Swi2 ATPase structure and function.

1. Introduction

Homologous recombination (HR) is a complex mechanism to maintain genomic stability. This process uses template-dependent DNA synthesis to repair or tolerate DNA damage, such as DNA double-stranded breaks (DSB), single-stranded DNA gaps, and interstrand crosslinks [1]. HR is intimately linked to DNA replication, as initially elaborated in phage T4 [2], and the function of HR in the recovery of stalled and collapsed replication forks is critical to faithfully duplicate and segregate the genome to daughter cells [1–4]. Moreover, HR is essential for accurate chromosome segregation during the first meiotic division, also generating genetic diversity among the meiotic products in the process [5].

RAD54 is a member of the *RAD52* epistasis group and a core component of the recombination machinery found in all eukaryotes and in some archaea, but not in bacteria [6–8]. The Rad54 protein belongs to a group of the Snf2/Swi2 family of SF2 helicases that share a prominent motor domain which powers ATP-dependent tracking on dsDNA but does not lead to strand separation like classical DNA helicases [9]. The Snf2/Swi2 family of proteins is represented by well-known ATP-dependent chromatin remodelers, such as Snf2/Swi2 or ISWI, but also other proteins that remodel non-chromatin protein–dsDNA complexes such as Mot1, which displaces the TATA-binding protein from DNA [10,11] (see other reviews in this issue of *BBA*). The budding yeast *Saccharomyces cerevisiae* contains 17 Snf2 family proteins, of which 10 function in different aspects of the DNA damage response, including Rad54, Rdh54, Uls1, Rad5, Rad16, Rad26, Ino80, Snf2, Sth1, and Swr1 [7,12].

This review will focus on yeast and human Rad54 proteins, their paralogs, and their function in HR. We will also discuss the yeast Uls1 protein, which appears to have a partially overlapping function with Rad54 and Rdh54. Excellent reviews are available with comprehensive discussions of the HR pathway [13–16], the Rad52 group proteins [17,18], meiotic recombination [5,19], and the Snf2/Swi2 family chromatin remodelers [12,20–22]. We will emphasize the significant novel developments since our last review on Rad54 in 2006 [7] and refer the reader also to other reviews dedicated to the Rad54 protein [6,8]. To distinguish between the yeast and human proteins, we will adhere to the following nomenclature: *S. cerevisiae* Rad54 protein, ScRad54; human RAD54 protein, hRAD54; *S. cerevisiae* Rdh54 protein (also known as Tid1), ScRdh54; and human RAD54B protein hRAD54B. We will use the generic term Rad54 when we refer to both yeast and human Rad54.

2. Homologous recombination — a mechanistic overview

HR can be conceptually divided into three mechanistic stages: pre-synapsis, synapsis, and post-synapsis (Fig. 1) [13–18]. During pre-

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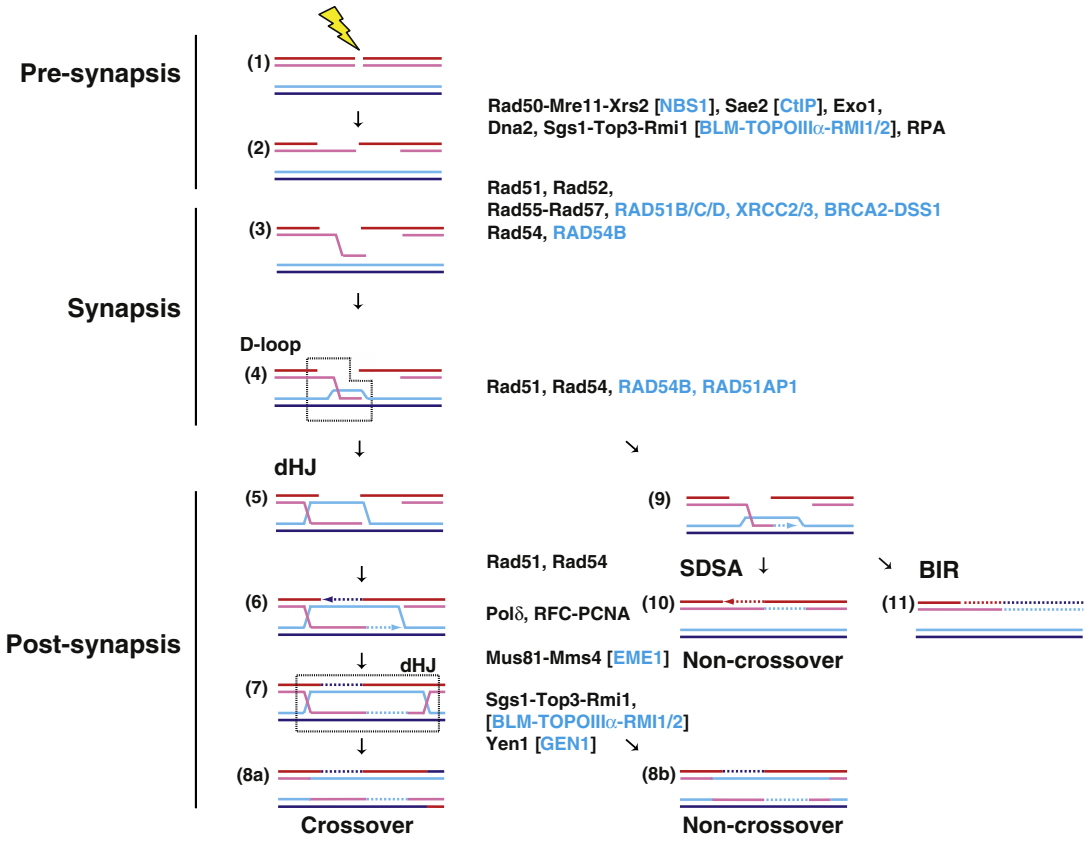

Fig. 1. Double-strand break repair by homologous recombination. Homologous recombination can be conceptually divided into three stages: pre-synapsis (1–2), synapsis (3–4), and post-synapsis (5–11). The *Saccharomyces cerevisiae* proteins identified to function at the individual stages are listed in black, the human proteins with alternate nomenclature are listed in blue in square brackets and human proteins not found in yeast are listed in blue without brackets. Three different pathways emanate from the D-loop intermediate (4), the product of DNA strand invasion by the Rad51-ssDNA filament. The dHJ (double Holliday junction; steps 5–8) pathway engages both ends of the DSB to ultimately form a double Holliday junction intermediate (dHJ), which can be resolved into crossover and non-crossover products. SDSA (synthesis-dependent strand annealing; step 10) retracts the invading strand after DNA synthesis on the target duplex to anneal the newly synthesized strand with the single strand resulting from resection of the second end, leading to localized conversion without crossover. BIR (break-induced replication; step 11) was proposed to assemble a replication fork at the D-loop to copy the entire chromosome arm distal to the DSB site leading to long gene conversion events.

synapsis, the DNA damage is processed to generate ssDNA, onto which Rad51 can assemble the pre-synaptic filament. The Rad51-ssDNA filament performs homology search and DNA strand invasion, the key steps during HR that define synapsis. During post-synapsis, HR is completed by several pathways that entail DNA synthesis and different modes to resolve recombination-mediated junction intermediates. The double Holliday Junction pathway (dHJ in Fig. 1) is of particular interest, because it can lead to crossovers, which are essential for meiotic recombination [5,19]. In somatic (vegetative) cells, the predominant recombinational repair pathway appears to be Synthesis-Dependent Strand Annealing (SDSA in Fig. 1), as it inherently avoids crossover and thus the possibility of deleterious genome rearrangements. Lastly, Break-Induced Replication (BIR in Fig. 1) appears restricted to conditions of one-sided DSBs, where the second DSB end is missing, for example after breakage of the replication fork.

Recent genetic and biochemical work defined two independent DSB resection pathways in budding yeast controlled by Exo1 and Sgs1-Top3-Rmi1 with Dna2, respectively, which function after the initial processing by Rad50-Mre11-Xrs2 in conjunction with Sae2 (for review [23]). Similar pathways exist in humans (see Fig. 1). The resulting 3′ overhanging ssDNA is bound by the ssDNA binding protein, RPA, which removes potential secondary structure in the ssDNA. Since RPA has exceptionally high affinity to ssDNA, it interferes with binding of Rad51. This impediment is overcome by so-called mediator proteins, Rad52 and Rad55–Rad57 in yeast, which allow the formation of Rad51-ssDNA filaments on RPA coated ssDNA. In humans, the breast and ovarian cancer tumor suppressor BRCA2 and its associated factor DSS1 perform mediator function, and the RAD51 paralogs, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 have been implicated as well [24–26]. The Rad51 filament performs homology search and DNA strand invasion. Extension of the invading 3-OH end marks the onset of post-synapsis. In yeast, the primary DNA polymerase for D-loop extension appears to be DNA polymerase delta with its cofactors PCNA/RFC [27]. In vertebrates, genetic and biochemical data suggest an involvement of the translesion polymerase Pol eta [28,29].

In the dHJ pathway, the displaced strand of the D-loop anneals to the second DSB end through the action of Rad52 protein [30], to form a junction intermediate that has the potential to mature into a dHJ upon ligation of all strand interruptions. Such junction intermediates, including also nicked and partial dHJs, can be cleaved to crossover or non-crossover products by specific endonucleases, such as Yen1 (human GEN1) or Mus81–Mms4 (human MUS81–EME1) [31,32]. Alternatively, the two junctions of a dHJ can be migrated towards each other to result in a hemi-catenane that can be dissolved into non-crossover products by the combined action of Sgs1 helicase with the type IA topoisomerase Topo3 and the specificity factor Rmi1 (human BLM-TOPOIIIα-RMI1/RMI2 [33]). In the second post-synaptic pathway, the DNA synthesis-extended strand of the D-loop can be extricated to anneal with the second DSB end, in a process called Synthesis-Dependent Strand Annealing (Fig. 1). Lastly, in the absence of a second DSB end, Break Induced Replication establishes a replication fork at the D-loop to initiate long-range DNA synthesis to copy the entire distal