Methods 51 (2010) 329–335

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Review Article

Promotion and regulation of homologous recombination by DNA helicases

Sierra Colavito, Rohit Prakash, Patrick Sung *

Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, 333 Cedar St., SHM-C130, New Haven, CT 06520-8024, USA

ARTICLE INFO

Article history:
Accepted 9 February 2010
Available online 13 February 2010

Keywords:
Helicase
Homologous recombination
DNA repair
Rad51

ABSTRACT

In eukaryotes, homologous recombination (HR) provides an important means to eliminate DNA double-stranded breaks and other chromosomal lesions. Accordingly, failure in HR leads to genomic instability and a predisposition to various cancer types. While HR is clearly beneficial for genome maintenance, inappropriate or untimely events can be harmful. For this reason, HR must be tightly regulated. Several DNA helicases contribute to HR regulation, by way of mechanisms that are conserved from yeast to humans. Mutations in several HR-specific helicases e.g. BLM and RECQ5, are either associated with cancer-prone human syndromes or engender the cancer phenotype in animal models. Therefore, delineating the role of DNA helicases in HR regulation has direct relevance to cancer etiology. Genetic, cytological, biochemical, and other analyses have shown that DNA helicases participate in early or late stages of HR, to disrupt nucleoprotein filaments that harbor the Rad51 recombinase or dissociate the D-loop intermediate made by Rad51, or to prevent undesirable events and/or minimize potentially deleterious crossover products. Moreover, the ensemble that harbors BLM and topoisomerase IIIα can dissolve the double-Holliday junction, a complex DNA intermediate generated during HR, to produce non-crossover products. These regulatory pathways function in parallel to promote the usage of the genome-preserving synthesis-dependent strand annealing HR pathway or otherwise suppress crossover formation.

1. Introduction

DNA double-strand breaks (DSBs) occur frequently in cells as the result of replication fork collapse, endogenous chromosome damage, or upon exposure to ionizing radiation and mutagenic chemicals. Unrepaired or misrepaired DSBs result in genomic instability and genetic alterations which can lead to cell death or transformation [1]. Cells have developed two distinct mechanisms for the repair of gratuitous DSBs. The non-homologous end joining (NHEJ) pathway is particularly important during the G1 and early S phases of the cell-cycle, and repair by this pathway usually involves only a limited amount of DNA end processing. Since homologous recombination (HR) typically utilizes the intact sister chromatid to guide the repair process, it is active mostly during the S and G2 phases of the cell-cycle. HR is dependent upon extensive processing of the DSB ends in a manner that yields 3′ ssDNA tails (see below). These DSB repair pathways also differ in the extent to which they are conservative. NHEJ often entails the gain or loss of nucleotides and is thus an error-prone pathway. Whereas, especially when the sister chromatid is used as the information donor, HR is largely an error-free means of repair. Herein, we focus on the helicases that regulate HR to ensure that the desirable outcome of genome preservation is achieved.

After DSB formation, nucleolytic processing of the ends results in a pair of 3′ ssDNA tails, which attract the recombinase protein Rad51, leading to the assembly of an extended, right-handed helical Rad51 filament, commonly referred to as the presynaptic filament [2] (Fig. 1). With the aid of one of several accessory factors, such as the Swi2/Snf2-related DNA motor protein Rad54 [3], the presynaptic filament conducts a search for a homologous DNA sequence, then invades the latter to form a DNA joint called the displacement loop, or D-loop. The D-loop can be resolved by one of several means, with different consequences. In the canonical double-strand break repair (DSBR) pathway, DNA synthesis initiated from the primer terminus of the D-loop serves to enlarge the structure, allowing capture of the second end of the break, resulting in a DNA intermediate that harbors a double-Holliday junction (dHJ) (Fig. 2A). The dHJ is cleaved by a specialized endonuclease called HJ resolvase, to yield a mixture of crossover and non-crossover products (Fig. 2A, [4] reviewed in [2,5]). Alternatively, the synthesis-dependent strand annealing (SDSA) pathway, through the employment of a specialized DNA helicase, resolves the D-loop structure to generate exclusively non-crossover recombinants (Fig. 2B). In the third pathway, the dHJ intermediate is "dissolved" via the combined action of a DNA helicase and topoisomerase to yield exclusively non-crossover recombinants (Fig. 2C). Since crossover HR is prone to producing chromosome aberrations, such as arm translocations, mitotic cells mainly employ the latter two non-crossover pathways in processing HR

* Corresponding author. Fax: +1 203 785 6404.
E-mail address: Patrick.Sung@yale.edu (P. Sung).

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doi:10.1016/j.ymeth.2010.02.009

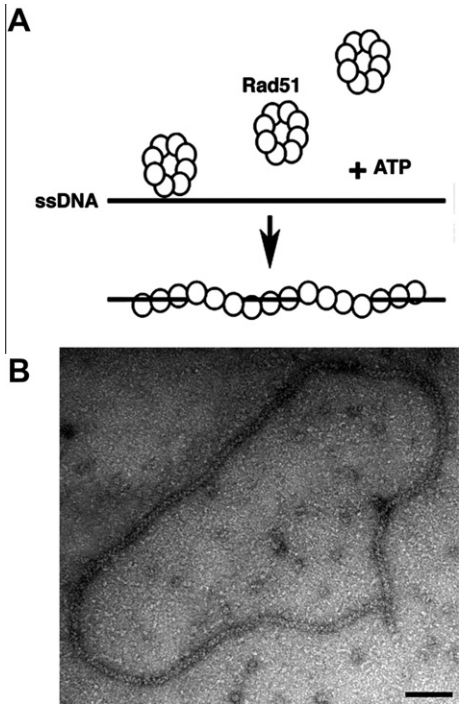

Fig. 1. The Rad51 nucleoprotein filament. (A) Rad51 exists as a seven subunit ring when in solution, but assembles into a right-handed extended helical filament on ssDNA and dsDNA [34]. Formation of the filament requires ATP binding by Rad51, but not ATP hydrolysis [35]. (B) Electron micrograph of the *Saccharomyces cerevisiae* Rad51–ssDNA filament. Scale bar is equal to 200 nm.

intermediates. The DNA helicases and helicase complexes that function to regulate HR are listed in Table 1, and assays for the salient features germane for understanding their mechanism of action are described below.

Table 1
Helicases involved in HR. A listing of the DNA helicases and helicase complexes that function to regulate HR*. The yeast and human orthologues are listed.

Yeast	Human	Biochemical function
Srs [9,10]	RexQ5 [8]	Disrupts the Rad51 presynaptic filament
Mph [11]	FANCM [30]	Dissociates Rad51-made D-loops
Sgs1-Top3	BLM-Topo IIIα [22,23]	Catalyzes dHJ dissolution
	RTEL [14]	Dissociates Rad51-made D-loops

* Even though the WRN helicase has been implicated in HR regulation, its precise role is unknown (reviewed in [28]). Moreover, the *Schizosaccharomyces pombe* Fbh1 helicase and its mammalian orthologue have been shown to regulate HR [31,32] and this has been speculated to stem from an ability of Fbh1 to disrupt the Rad51 presynaptic filament [33].

2. Determination of helicase activity

DNA helicases are motor proteins that use the energy derived from ATP hydrolysis to translocate on DNA, ssDNA in particular. The majority of DNA helicases are able to separate the strands in dsDNA, some are capable of dissociating specific DNA structures, and yet a selected few are endowed with the ability to remove proteins from either ssDNA or dsDNA. In general, all of these functions are coupled to ATP hydrolysis. The studies of DNA helicases typically start with an examination of their ability to unwind duplex DNA or DNA structures, and the results can provide important clues as to their biological function.

2.1. Generation of DNA helicase substrates

A variety of DNA substrates for examining helicase activity can be conveniently generated from oligonucleotides. The most basic substrate consists of radiolabeled DNA that harbors a duplex region and an adjoining 3′ or 5′ ssDNA tail. More intricate structures that resemble HR intermediates, such as the D-loop and Holliday junction, can also be easily prepared. As a general practice, a

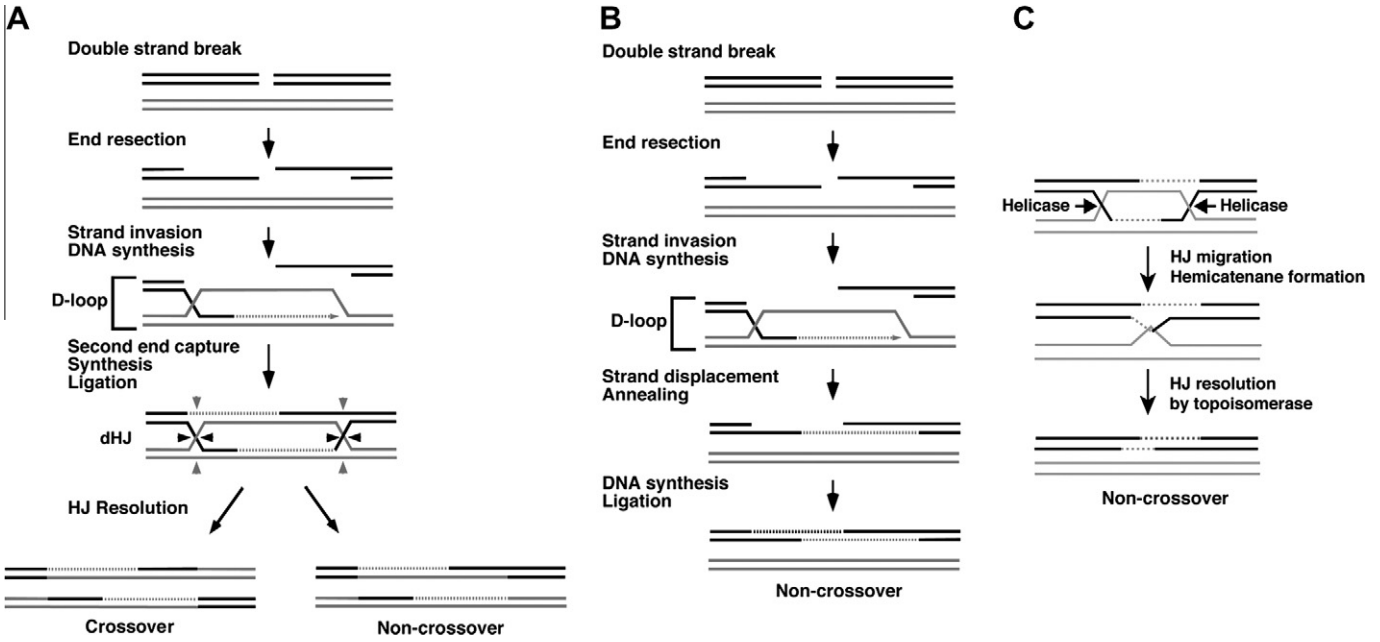

Fig. 2. Pathways of DSB repair. After the DSB occurs, 5′ ends are resected to yield 3′ ssDNA. This serves to recruit repair factors resulting in formation of a nucleoprotein filament which is competent to invade duplex DNA, forming a D-loop structure. (A) After DNA synthesis and D-loop extension, capture of the other side of the break leads to formation of a double-Holliday junction (dHJ) structure. This can be resolved to yield either crossover or non-crossover products. (B) In the SDSA pathway, the invading strand of the D-loop is displaced, and anneals to the other side of the break. DNA synthesis and ligation occur, resulting in the formation of non-crossover products. (C) Certain helicases can migrate the dHJ. The resulting hemicatenane structure can be resolved by a topoisomerase, resulting in non-crossover repair products.