Methods 51 (2010) 336–346

Contents lists available at ScienceDirect

Methods

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

Analyzing the branch migration activities of eukaryotic proteins

Matthew J. Rossi, Olga M. Mazina, Dmitry V. Bugreev, Alexander V. Mazin *

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102-1192, USA

ARTICLE INFO

Article history:
Accepted 12 February 2010
Available online 16 February 2010

Keywords:
Branch migration
Holliday junction
ATPase
Joint molecules

ABSTRACT

The Holliday junction is a key intermediate of DNA repair, recombination, and replication. Branch migration of Holliday junctions is a process in which one DNA strand is progressively exchanged for another. Branch migration of Holliday junctions may serve several important functions such as affecting the length of genetic information transferred between homologous chromosomes during meiosis, restarting stalled replication forks, and ensuring the faithful repair of double strand DNA breaks by homologous recombination. Several proteins that promote branch migration of Holliday junctions have been recently identified. These proteins, which function during DNA replication and repair, possess the ability to bind Holliday junctions and other branched DNA structures and drive their branch migration by translocating along DNA in an ATPase-dependent manner. Here, we describe methods employing a wide range of DNA substrates for studying proteins that catalyze branch migration of Holliday junctions.

1. Introduction

The Holliday junction is a key intermediate in various genetic processes including homologous and site-specific recombination and DNA replication [1]. The Holliday junction is a four-stranded structure that forms during exchange between two homologous DNA molecules. Robin Holliday first predicted this structure in his model of homologous recombination which provided the molecular basis for both gene conversion and crossing-over [2]. The remarkable feature of the Holliday junction is its ability to branch migrate along the DNA axis, in which one DNA strand is progressively exchanged for another.

Branch migration of Holliday junctions may serve several important functions in DNA repair, replication, and recombination. It may extend or shorten the heteroduplex DNA, formed during recombination, affecting the length of conversion tracks and thereby the amount of genetic information transferred between the two DNA molecules [3]. Branch migration may cause dissociation of recombination intermediates and thereby affect the choice between a crossover and non-crossover pathway by which recombination will proceed [4]. An increasing number of genetic and biochemical data indicate that formation and branch migration of Holliday junctions may also play a critical role in restarting stalled replication forks during cell recovery after DNA damage [5–10]. It is thought that the major pathway of DNA replication restart involves unwinding of the stalled replication fork to generate a Holliday junction [5]. Branch migration may also take part in controlling the frequency of recombination exchanges [11].

The Holliday junction is a substrate for proteins which promote its branch migration and for structure-specific nucleases which are responsible for its cleavage and formation of crossover products. Branch migration proteins have been identified in all domains of life [12–15] and have been classified as members of the helicase superfamilies [16]. These proteins rely on their DNA-dependent ATPase activity to translocate along DNA in a manner that drives the branch migration of Holliday junctions. The first branch migration enzymes identified were found also to possess classical helicase activity, i.e. the ability to separate strands of duplex DNA [1]. However, as more branch migration proteins are discovered [17] it appeared that not all of them have a helicase activity [16,18]. Analysis of these proteins required the development of more specific approaches and DNA substrates. Here, we describe the methods our laboratory used to characterize the branch migration activity of the homologous recombination protein Rad54. These assays are applicable for other branch migration proteins regardless of whether they have helicase activity or not.

2. Preparation of oligonucleotide substrates for analysis of branch migration proteins

2.1. Strategy for designing branched DNA oligonucleotide substrates

In earlier works, "non-movable" X-junctions (Holliday junctions), consisting of a central homologous "movable" core flanked by mutually heterologous terminal DNA branches [12] (Fig. 1A), were successfully used to detect the branch migration activities of prokaryotic enzymes (RuvAB, RecG) that have both branch

* Corresponding author. Address: Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, 245 N 15th Street, NCB, Room 10103, Philadelphia, PA 19102-1192, USA. Fax: +1 215 762 4452.
E-mail address: amazin@drexelmed.edu (A.V. Mazin).

1046-2023/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2010.02.010

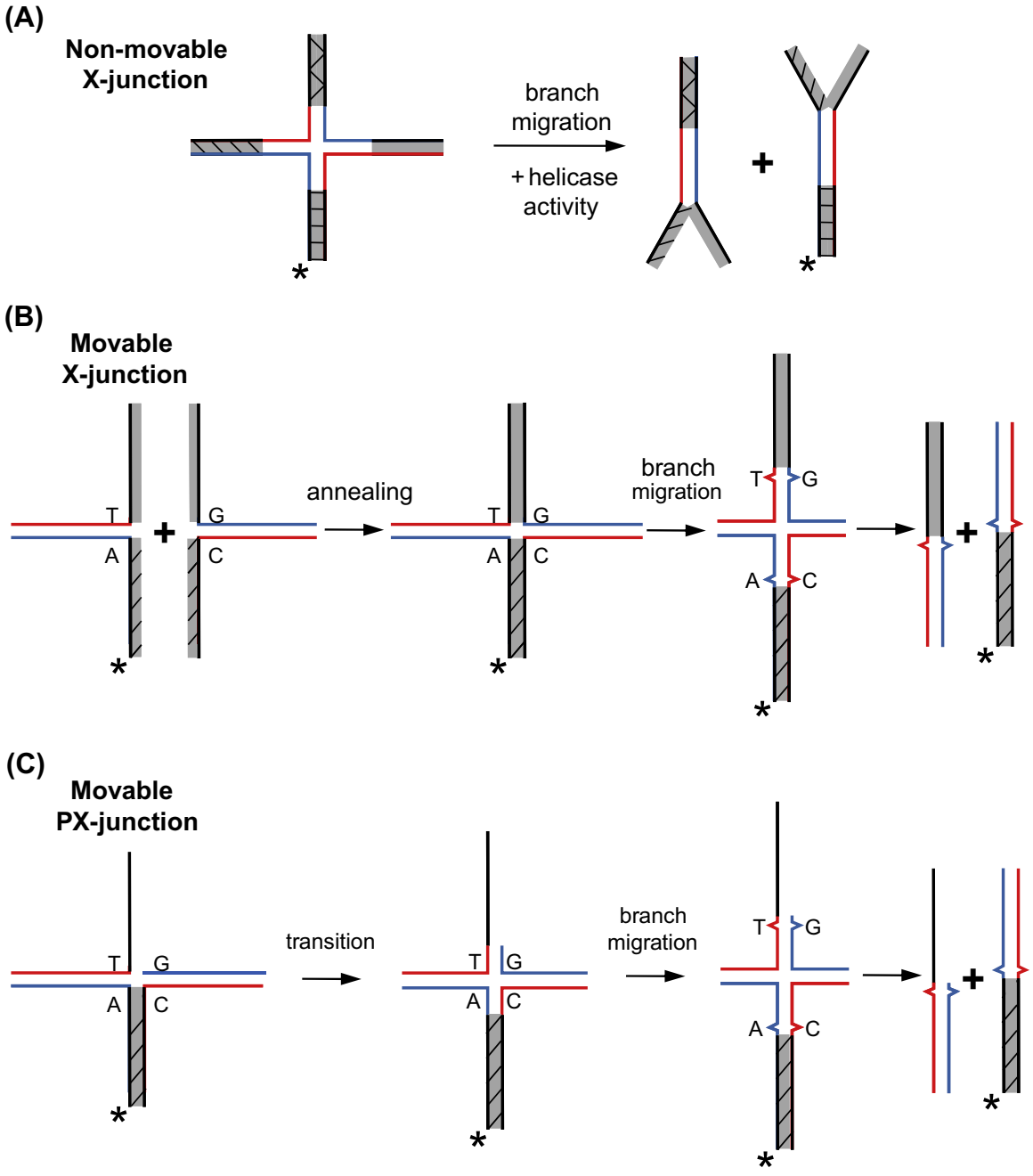

Fig. 1. Construction of oligonucleotide substrates to test the branch migration activity of proteins. (A) Both branch migration and DNA helicase activity are required to disrupt a non-movable X-junction. (B) The movable X-junctions contain a mismatch to block spontaneous branch migration and are formed by annealing of two forked DNA intermediates. (C) The branch migration of a PX-junction requires an additional step, the transition from a three-stranded to four-stranded structure. Shaded regions denote heterologous DNA terminal branches. The asterisk indicates ^{32}P-label at the DNA 5′-end.

migration and helicase activities [1]. However, these substrates were unsuitable for detecting the branch migration activity of Rad54, which does not have canonical helicase activity [19]. We therefore constructed "movable" oligonucleotide substrates in which two of the four terminal DNA branches were mutually homologous [17]. This allowed the crossover point to move freely by branch migration to the end of the DNA molecule, resulting in complete separation of the two DNA duplexes without a need for helicase activity (Fig. 1B). Here, we describe the protocols for constructing a series of branched DNA substrates including the X-junctions, PX-junctions (partial Holliday junctions), and movable replication forks.

Oligonucleotide substrates can be prepared through the annealing of two simple DNA intermediates (forked, tailed, or ssDNAs) (Fig. 1B). When constructing substrates with four DNA arms (X-junctions, PX-junctions), it is important that two of the opposing arms of the predicted branched DNA molecule are non-homologous relative to each other, while the remaining two are homologous (Fig. 1B and C shaded). Without this orientation, it would be impossible to prepare forked DNA intermediates. To block spontaneous branch migration in a 4-stranded reaction [20,21] at least one base pair heterology must be introduced in the two homologous duplexes involved in branch migration (Fig. 1B). The substrates used to study the three-stranded branch migration reaction, where ex-