doi:10.1016/j.jmb.2007.05.053 J. Mol. Biol. (2007) 371, 66–78

JMB

Available online at www.sciencedirect.com
ScienceDirect

ELSEVIER

A Dual-nuclease Mechanism for DNA Break Processing by AddAB-type Helicase-nucleases

Joseph T. P. Yeeles and Mark S. Dillingham*

DNA:protein Interactions Unit
Department of Biochemistry
School of Medical Sciences
University of Bristol
University Walk, Bristol
BS8 1TD, UK

*Corresponding author

Nature has devised many strategies for repairing DNA breaks. In homology-dependent pathways, the break is first processed to a 3′-ssDNA overhang that serves as a substrate for DNA strand exchange. Here, we demonstrate a distinct biochemical mechanism for DNA break processing employed by the AddAB class of helicase-nuclease. We show that this enzyme complex contains two active nuclease domains, each of which is dedicated to cleavage of one specific DNA strand. The nuclease activity responsible for cleavage in the 3′→5′ direction is attenuated when the enzyme encounters a recombination hotspot sequence, whereas cleavage in the 5′→3′ direction is unaffected, resulting in the production of recombinogenic 3′-terminated ssDNA tails. Finally, we show that the molecular events that underlie the recognition and response to recombination hotspots can be uncoupled: mutant proteins that are unable to cleave at recombination hotspots retain the ability to form stable complexes with the hotspot sequence.

Keywords: DNA recombination; DNA repair; DNA helicase; RecBCD; AddAB

Introduction

Double-strand DNA breaks are potentially lethal lesions that are formed frequently by a variety of endogenous and exogenous sources, and numerous pathways have evolved for their repair. These pathways can be divided into two major classes depending on the requirement for a homologous donor DNA molecule. In homology-dependent double-strand break repair mechanisms, the DNA lesion is first processed to a 3′-single-stranded (ss) DNA overhang that is bound by a RecA/Rad51 homologue to form a nucleoprotein filament. This promotes repair by catalysing strand exchange with a homologous donor molecule, such as the sister chromatid, that acts as a template for error-free correction in several subsequent enzymatic steps.[1] Alternatively, non-homologous end-joining can repair DNA breaks by re-attaching two DNA ends using end-bridging and ligase activities.[2]

In bacteria, the initiation step for recombinational repair is generally catalysed by an enzyme complex incorporating helicase and sequence-regulated nuclease activities.[3,4] There are two distinct structural classes of helicase-nuclease complex (Figure 1). RecBCD-type enzymes are found mainly in Gram-negative bacteria and are exemplified by the *Escherichia coli* RecBCD enzyme. AddAB-type enzymes (also called RexAB) are found mainly in Gram-positive bacteria such as *Bacillus subtilis*, although there are exceptions.[5] Inactivation of RecBCD or AddAB enzymes results in reduced cell viability and sensitivity to UV irradiation and other DNA damaging agents.[3,6–9] Both classes of enzyme catalyse the same net reaction: a double-strand break is processed to a 3′-terminated ssDNA overhang that is a substrate for RecA nucleoprotein filament formation.[10,11] Accordingly, the two classes of enzyme share several biochemical characteristics. They bind tightly to DNA ends, and employ a processive helicase activity to unwind the DNA substrate into its component single strands. The nascent single strands of DNA are both cleaved until the translocating enzyme encounters a recombination hotspot sequence (Chi), at which point cleavage in the 3′→5′ strand is attenuated. However, several lines of evidence suggest that AddAB and RecBCD

Abbreviations used: ss, single-stranded; ds, double-stranded; ExoI, Exonuclease I.

E-mail address of the corresponding author: Mark.Dillingham@bristol.ac.uk

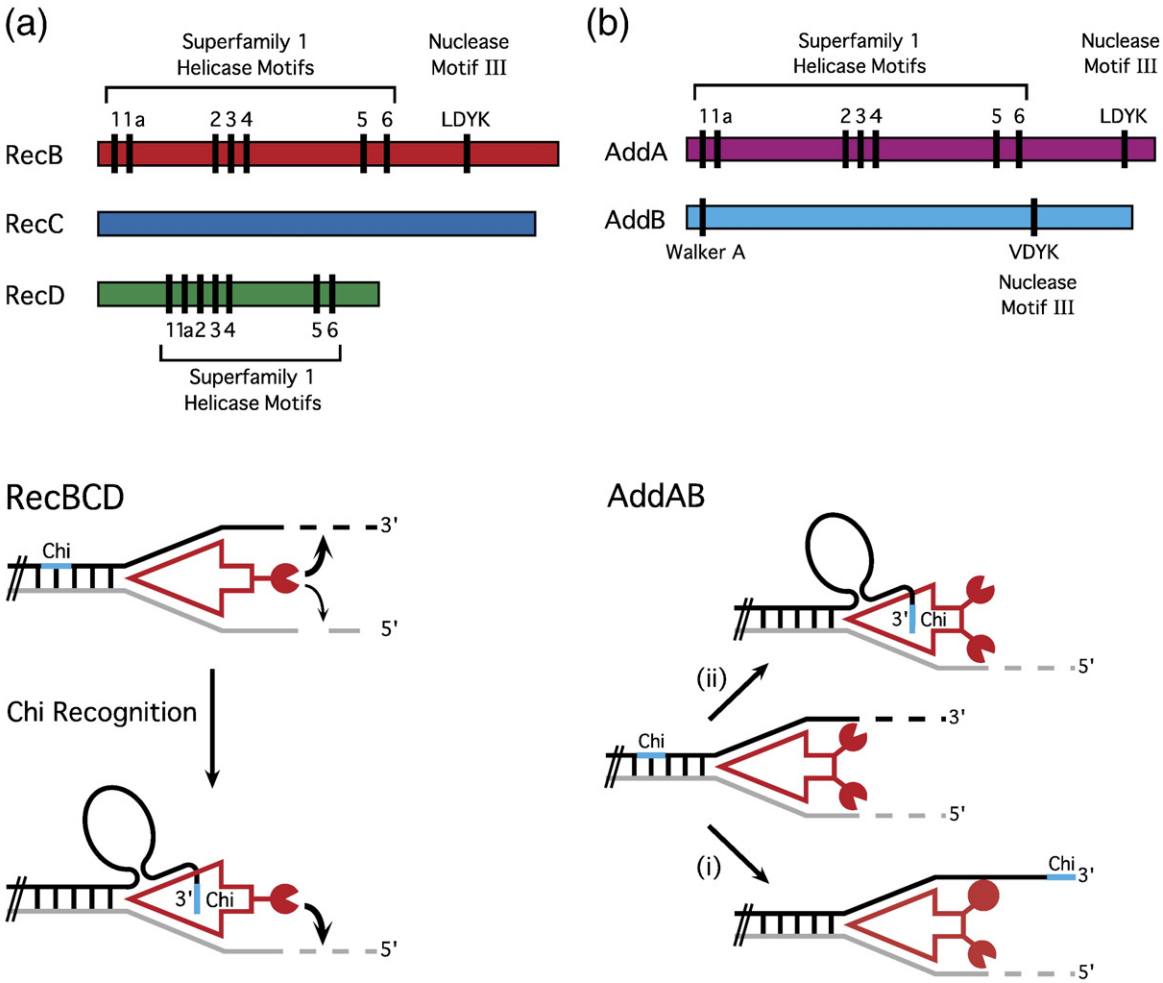

Figure 1. Domain organisation and proposed mechanisms for the AddAB and RecBCD classes of helicase-nuclease. (a) Top panel: Primary structure diagram of the RecB, RecC and RecD components of RecBCD enzyme. The positions of conserved helicase motifs and nuclease motif III containing an essential aspartate residue are shown. Bottom panel: As RecBCD translocates to the left, it cleaves both emerging ssDNA strands with a single nuclease domain. Following recombination hotspot (Chi) recognition, protection of the 3′-terminated strand downstream of Chi is achieved by tight binding of the Chi-containing ssDNA before it reaches the nuclease domain. (b) Top panel: Primary structure of the AddA and AddB polypeptides. The position of conserved helicase motifs, and a Walker A motif are shown. Two nuclease motifs, containing essential aspartate residues that were mutated in these studies, are also shown. Bottom panel: Dual nuclease hypothesis for AddAB enzyme. AddAB enzyme contains two homologous nuclease domains. It has been postulated that each nuclease domain may be specifically responsible for cleavage of one DNA strand prior to Chi-recognition.[3,4] Following Chi-recognition, the 3′→5′ nuclease activity is strongly down-regulated. Protection of the 3′-terminated strand downstream of Chi may be achieved either by (i) inactivating, or (ii) preventing access to, the appropriate nuclease domain.

employ different mechanisms to achieve the same goal. Inspection of the primary structures of each class of helicase-nuclease complex suggests a different domain organisation (Figure 1). The heterotrimeric RecBCD-type enzymes contain two Superfamily 1 (SF1) helicase motors located in the RecB and RecD subunits[12] and a single nuclease domain in RecB.[13] In distinct contrast, AddAB enzymes are composed of only two different polypeptide chains and appear to contain just a single helicase motor (located in the AddA subunit) and two homologous nuclease domains (one located at each of the AddA and AddB C-terminal regions). The two enzyme complexes also recognise different Chi sequences; AddAB recognises the pentameric sequence 5′-AGCGG, whereas RecBCD responds to the octameric sequence 5′-GCTGGTGG.[10]

Of particular interest for the present study is the apparent difference in nuclease domain organisation. Biochemical data have shown that all nuclease activities of the RecBCD complex are associated with a single nuclease domain that resides in the C-terminal region of RecB protein.[13,14] It is proposed that, before Chi-recognition, this nuclease domain can access both nascent ssDNA strands as they emerge at the rear of the translocating complex, although it is observed that the 3′→5′ strand is cleaved more vigorously than the 5′→3′ strand (Figure 1(a)).[11] Following Chi recognition, the 3′→5′ strand is thought to be sequestered within the com-