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RuvA is a Sliding Collar that Protects Holliday Junctions from Unwinding while Promoting Branch Migration

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The RuvAB proteins catalyze branch migration of Holliday junctions during DNA recombination in *Escherichia coli*. RuvA binds tightly to the Holliday junction, and then recruits two RuvB pumps to power branch migration. Previous investigations have studied RuvA in conjunction with its cellular partner RuvB. The replication fork helicase DnaB catalyzes branch migration like RuvB but, unlike RuvB, is not dependent on RuvA for activity. In this study, we specifically analyze the function of RuvA by studying RuvA in conjunction with DnaB, a DNA pump that does not work with RuvA in the cell. Thus, we use DnaB as a tool to dissect RuvA function from RuvB. We find that RuvA does not inhibit DnaB-catalyzed branch migration of a homologous junction, even at high concentrations of RuvA. Hence, specific protein–protein interaction is not required for RuvA mobilization during branch migration, in contrast to previous proposals. However, low concentrations of RuvA block DnaB unwinding at a Holliday junction. RuvA even blocks DnaB-catalyzed unwinding when two DnaB rings are acting in concert on opposite sides of the junction. These findings indicate that RuvA is intrinsically mobile at a Holliday junction when the DNA is undergoing branch migration, but RuvA is immobile at the same junction during DNA unwinding. We present evidence that suggests that RuvA can slide along a Holliday junction structure during DnaB-catalyzed branch migration, but not during unwinding. Thus, RuvA may act as a sliding collar at Holliday junctions, promoting DNA branch migration activity while blocking other DNA remodeling activities. Finally, we show that RuvA is less mobile at a heterologous junction compared to a homologous junction, as two opposing DnaB pumps are required to mobilize RuvA over heterologous DNA.

Keywords: DNA replication; DNA recombination; Holliday junction; branch migration; RuvA

Introduction

DNA recombination functions in *Escherichia coli* to repair damaged DNA and rescue stalled replication forks.[1] During recombination, a DNA strand is paired with its homolog from a different duplex in a reaction catalyzed by RecA working in concert with other proteins.[1] This creates a four-way DNA structure, also called a Holliday junction, which is processed by the RuvABC proteins.[2] The RuvA protein initially binds the Holliday junction, and then recruits RuvB protein rings to opposite sides of the junction.[3,4] RuvB is a molecular motor that uses the energy derived from ATP binding and hydrolysis to drive unidirectional movement of the four-way junction.[5] The RuvAB complex then recruits RuvC to the junction.[6] RuvC is a nuclease that cleaves the Holliday junction, thereby resolving it into two duplex DNAs.[7]

RuvAB has been studied by biochemical and structural techniques. RuvA binds as a tetramer or octamer to a Holliday junction. The RuvA protein has acidic pins that inhibit binding to double-stranded DNA, thereby targeting the protein to Holliday junctions.[8–11] RuvB is a hexameric ring

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Abbreviation used: ssDNA, single-stranded DNA.

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protein with a central channel wide enough to encircle double-stranded DNA.[11–13] RuvB alone does not bind Holliday junction DNA under physiological conditions, but after RuvA binds the Holliday junction, RuvA facilitates the assembly of two RuvB rings onto opposite sides of the RuvA-junction (Figure 4(a)).[14–16] The two RuvB rings are thought to function in concert as DNA pumps that drive branch migration of the Holliday junction.[15]

DnaB functions in DNA replication and is a member of the class of ring-shaped helicases.[17–19] DnaB is the primary replicative helicase of *E. coli*, and unwinds the parental duplex to provide single-stranded DNA (ssDNA) for the replicative polymerases.[20] The DnaB hexamer encircles ssDNA while translocating along it, pumping the strand through the central channel.[21–24] Upon encountering a forked duplex structure, the second DNA strand cannot fit into the central channel of DnaB, and therefore the continued advance of DnaB along the original strand forces its separation from the second DNA strand.[24,25] It is thought that DnaB, like other hexameric helicases, may act at the replication fork to unwind parental DNA in this manner.[26–28]

The DnaB hexamer can also operate in a mode in which it encircles both strands of duplex DNA. In this mode, the DnaB does not unwind the DNA.[24] However, DnaB actively translocates along the duplex while encircling two DNA strands as it powers branch migration of Holliday junctions.[29] Although this reaction is very efficient *in vitro*, the *in vivo* role for DnaB-catalyzed branch migration is unclear. In summary, DnaB unwinds DNA when encircling one DNA strand, and drives DNA branch migration while encircling two DNA strands.

DnaB and RuvB have several mechanistic features in common. For example, DnaB and RuvB are both hexameric ring proteins that encircle DNA.[2] Furthermore, both DnaB and RuvB utilize ATP binding and hydrolysis to unwind DNA with 5′ to 3′ polarity.[24,30–32] DnaB and RuvB also displace proteins bound to DNA,[29,33] and they both drive branch migration of Holliday junctions.[15,29] Finally, two DnaB rings can bind to opposite sides of a Holliday junction, and work in concert to drive branch migration of an extended heterologous junction, like RuvB.[34–36]

Biochemical studies of RuvA in the past have been performed with RuvA in conjunction with its cellular partner, RuvB. The biochemical action of the RuvAB complex is thus well studied. However, some intrinsic properties of RuvA during its action are unclear, as it is most often studied in conjunction with RuvB. This leaves unanswered a number of questions of how RuvA functions. For example, how does RuvA bind tightly to Holliday junctions, yet become activated to move during branch migration? Previous proposals suggest that RuvB must mobilize RuvA bound to a Holliday junction, and that this action is mediated *via* specific protein–protein interaction between RuvB and RuvA.[10] However, it is difficult to study the process that mobilizes RuvA using only RuvA and RuvB, since the activities of these two proteins are dependent upon one another.[2] Unlike RuvB, DnaB does not require RuvA for activation. Thus, we investigated how RuvA moves at a Holliday junction by using DnaB in conjunction with RuvA. In this study, DnaB is used as a tool to study how RuvA functions, since these two proteins do not function together *in vivo*.

There is an additional advantage to using DnaB with RuvA to study RuvA function. RuvB rings bind to opposite arms of a RuvA-bound Holliday junction in either of two orientations (Figure 4(a)). Thus, it is difficult to target RuvB loading to a particular junction arm. In contrast, DnaB loads onto junction-arm DNA only if a 5′single-strand extension (5′ tail) is attached to a particular junction arm. Thus, unlike RuvB, one DnaB hexamer can be loaded onto a particular junction arm by specifically adding a 5′ loading tail.

We find that RuvA binds tightly to Holliday junction DNA and blocks DnaB-catalyzed unwinding of a Holliday junction. Unwinding activity is blocked even when two DnaB hexamers act in concert. However, RuvA does not block DnaB-catalyzed branch migration of a homologous Holliday junction. Hence, RuvA does not need specific protein activation by RuvB to mobilize in the direction of branch migration. We present evidence that suggests that RuvA slides along the Holliday junction during DNA branch migration, but not during DNA unwinding. Interestingly, two DnaB pumps are needed to power migration of RuvA over heterologous DNA, an action that fits nicely with the physiological architecture of two RuvB pumps straddling the RuvA protein at a Holliday junction.

Results

Homologous and heterologous Holliday junction DNA substrates used in this study

Holliday junctions in the cell are usually homologous, as RecA normally pairs DNA strands of the same sequence to create the junction. By homologous, we mean that the DNA arms contain complementary sequences before and after branch migration. Figure 1(a) shows a homologous Holliday junction substrate used in this study. Note that the substrate is not completely homologous, otherwise the Holliday junction is unstable and can migrate spontaneously. Thus, the homologous substrate used here bears a small degree of heterology (5 bp) to stabilize the structure and render it amenable to experimentation. In the reaction shown in Figure 1(a), the Holliday junction branch-point migrates a distance of 45 bp, of which 40 are complementary, while five are non-complementary.

Heterologous junctions contain DNA sequence that is non-complementary, and the DNA arm will therefore become unpaired after branch migration