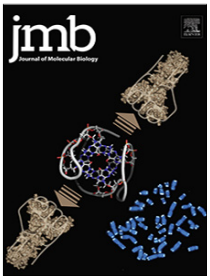
Research Article



Novel insights into ATP-Stimulated Cleavage of branched DNA and RNA Substrates through Structure-Guided Studies of the Holliday Junction Resolvase RuvX

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Abstract

Much of our understanding of the homologous recombination (HR) machinery hinges on studies using *Escherichia coli* as a model organism. Interestingly enough, studies on the HR machinery in different bacterial species casts doubt on the universality of the *E. coli* paradigm. The human pathogen *Mycobacterium tuberculosis* encodes two Holliday junction (HJ)-resolvase paralogues, namely RuvC and RuvX; however, insights into their structural features and functional relevance is still limited. Here, we report on structure-guided functional studies of the *M. tuberculosis* RuvX HJ resolvase (MtRuvX). The crystalline MtRuvX is a dimer in the asymmetric unit, and each monomer has a RNAse H fold vis-à-vis RuvC-like nucleases. Interestingly, MtRuvX also contains some unique features, including the residues essential for ATP binding/coordination of Mg^{2+} ions. Indeed, MtRuvX exhibited an intrinsic, robust ATPase activity, which was further accentuated by DNA cofactors. Structure-guided substitutions of single residues at the ATP binding/Mg^{2+}coordination sites while markedly attenuating the ATPase activity completely abrogated HJ cleavage, indicating an unanticipated relationship between ATP hydrolysis and DNA cleavage. However, the affinity of ATPase-deficient mutants for the HJ was not impaired. Contrary to RuvC, MtRuvX exhibits relaxed substrate specificity, cleaving a variety of branched DNA/RNA substrates. Notably, ATP hydrolysis plays a regulatory role, rendering MtRuvX from a canonical HJ resolvase to a DNA/RNA non-sequence specific endonuclease, indicating a link between HJ resolvase and nucleic acid metabolism. These findings provide novel insights into the structure and dual-functional activities of MtRuvX, and suggest that it may play an important role in DNA/RNA metabolism.

Introduction

The Holliday junction (HJ) and other branched DNA intermediates arise in cells during homologous recombination (HR), repair of double-strand breaks and strand regression at stalled replication forks.[1–5] Several studies have demonstrated that the resolution of this all-important DNA intermediate is crucial for the successful completion of HR, DNA repair and chromosome segregation.[5–8] The HJ intermediates are resolved by a group of highly specialized structure-selective endonucleases called the Holliday junction resolvases (denoted as HJRs).[2,6,7] The HJRs cleave the HJ intermediate by symmetrically nicking two strands of the same polarity at positions close to the junction point.[2,6,7,9–12]

Structural and evolutionary relationships among HJRs suggest that their functions have evolved independently from three distinct structural folds,