UKE Paper of the Month August 2022

Mechanism of AAA+ ATPase-mediated RuvAB-Holliday junction branch migration

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ABSTRACT:

The Holliday junction is a key intermediate formed during DNA recombination across all kingdoms of life. In bacteria, the Holliday junction is processed by two homo-hexameric AAA+ ATPase RuvB motors, which assemble together with the RuvA-Holliday junction complex to energize the strand-exchange reaction. Despite its importance for chromosome maintenance, the structure and mechanism by which this complex facilitates branch migration are unknown. Here, using time-resolved cryo-electron microscopy, we obtained structures of the ATP-hydrolysing RuvAB complex in seven distinct conformational states, captured during assembly and processing of a Holliday junction. Five structures together resolve the complete nucleotide cycle and reveal the spatiotemporal relationship between ATP hydrolysis, nucleotide exchange and context-specific conformational changes in RuvB. Coordinated motions in a converter formed by DNA-disengaged RuvB subunits stimulate hydrolysis and nucleotide exchange. Immobilization of the converter enables RuvB to convert the ATP-contained energy into a lever motion, which generates the pulling force driving the branch migration. We show that RuvB motors rotate together with the DNA substrate, which, together with a progressing nucleotide cycle, forms the mechanistic basis for DNA recombination by continuous branch migration. Together, our data decipher the molecular principles of homologous recombination by the RuvAB complex, elucidate discrete and sequential transition-state intermediates for chemo-mechanical coupling of hexameric AAA+ motors and provide a blueprint for the design of state-specific compounds targeting AAA+ motors.

STATEMENT:

Molecular motors are complex devices composed of many different parts that consume energy to perform various cellular activities. In short, molecular machines transform energy into useful work. Understanding the mechanistical aspects underlying these motors begins with generating a detailed description of their overall architecture and atomic organization. However, to uncover the core mechanisms energizing these motors it is essential to decode all of the molecular dynamics in atomic detail.

Our work reveals the architecture, complete functional cycle and the mechanism of a RuvAB branch migration complex, a molecular motor which is essential for DNA recombination in bacteria. To determine the individual steps of this process, we used cutting-edge time-resolved cryo electron microscopy to observe the motor's machinery in slow motion. This work reveals the critical role of substrate-disengaged RuvB subunits, whose highly coordinated motions control the nucleotide cycle in the RuvB hexamer. We show that the nucleotide cycle is a spatiotemporal continuum of conformational changes through which RuvB AAA+ ATPase motors convert the chemical energy retained in ATP to a lever action. As AAA+ ATPase motors are essential elements to perform various biological processes our work uncovers a fundament mechanistic principle present in all organisms, which could be used as a blueprint to

understand similar molecular motors. Furthermore, the RuvAB system might serve as a target system to generate a novel generation of antibiotics in the future.

BACKGROUND:

This work was performed at the Institute of Structural and Systems Biology, Center of Experimental Medicine, University Medical Center Hamburg-Eppendorf, located at CSSB-Centre for Structural Systems Biology in Hamburg, in the group of Thomas Marlovits. The research of the institute studies the structural basis for assembly, regulation, and function of molecular machines involved during infection of important human pathogens through a multidisciplinary approach. This work is part of the PhD thesis of Jiri Wald, who joined the group of Thomas Marlovits in 2013 and continues in the group as Senior Scientist (PostDoc).