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The *Methanothermobacter thermoautotrophicus* MCM Helicase is Active as a Hexameric Ring

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Replicative DNA helicases play an essential role during chromosomal DNA replication by separated the two strands of the duplex DNA providing the single-stranded (ss) DNA for the polymerase. In bacteria, the replicative helicase is the DnaB protein which forms hexameric rings (1, 2). The eukaryotic minichromosome maintenance (MCM) complex is a family of six related polypeptides (Mcm2-7), each of which is essential for cell viability. *In vivo* and *in vitro* studies have shown that in addition to forming a heterohexamer revealed the existence of several additional MCM complexes (3). It was shown that the Mcm4,6,7 heterotrimer forms hexameric rings (4) that posses DNA helicase activity. The enzyme also forms double-hexamers in the presence of forked DNA substrate (5).

The structure of the archaeal MCM complex is unclear. The MCM homologues of *Sulfolobus solfataricus* (6-8), *Archaeoglobus fulgidus*, *A. pernix* (9) and *Thermoplasma acidophilum* (10) form hexamers in solution. The *Methanothermobacter thermautotrophicus* (Mth) enzyme appears to form dodecamers (11-13), and a dodecameric structure was also suggested by the crystal structure of the N-terminal portion of the protein (14) and by an electron microscopic (EM) analysis of the full-length enzyme (15-17). However, other EM reconstruction studies of the *M. thermautotrophicus* enzyme showed hexameric (18), heptameric (19), open rings (15) and filamentous structures (20). Those conflicted structures of Mth MCM are summarized in Fig. 1.

The conflicting data regarding the structure of the *M. thermautotrophicus* MCM hinder progress in elucidating the mechanism of enzyme action during initiation and elongation at the replication fork. The knowledge of the active form of the enzyme is needed as to form a working and testable hypothesis regarding the helicase activity during the initiation and elongation phases. One would expect, for example, that a hexamer would work differently than a filament. Therefore, a study was initiated to determine the

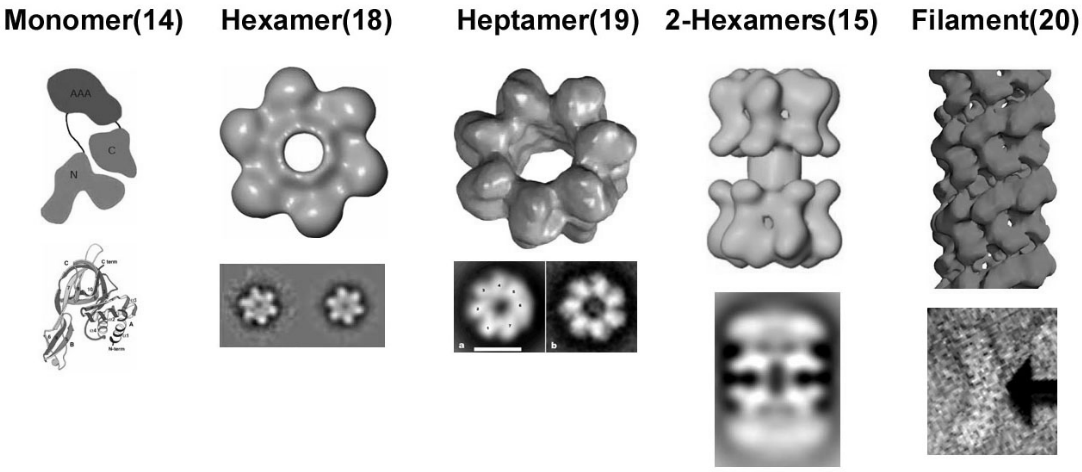

Figure 1. Structural polymorphism of MthMCM

active form of the enzyme using different substrates and experimental conditions. Using a combination of biochemical and structural analysis it is shown here that the *M. thermoautotrophicus* MCM helicase is active as a hexamer. The scientific impact of this result on DNA replication model is discussed.

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