Functional characterization of the MCM8-9 helicase complex in HR repair

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Introduction

Homologous recombination (HR) is a conserved biological process that ameliorates the effects of genotoxic stress by faithfully removing DNA double-strand breaks and repairing damaged replication forks. The repair DNA synthesis step in HR copies information from a homologous DNA template and therefore serves a pivotal role in maintaining sequence fidelity during DNA damage and replication fork repair processes. Previous studies have implicated DNA polymerase (Pol) **δ** in the mediation of repair DNA synthesis. However, which DNA helicase functions with Pol **δ** is unknown.

Emerging evidence has implicated MCM8-9 in HR and is largely supported by cellular MCM8-9 knockout data via Crispr-Cas9 reflecting substantial HR repair deficiency (*1,2*). Importantly, mutations in the MCM8-9 complex are also associated with increased infertility and cancer risk (*3*).

MCM8 and MCM9 are members of the minichromosome maintenance family of proteins including the hetero-hexameric DNA replicative helicase MCM2-7 complex (*4,5*). However, unlike their protein family members, MCM8 and MCM9 appear to have no known function in DNA replication; but rather, are implicated in HR, given their direct interaction with various HR proteins.

Abstract

HR is a highly intricate, multi-stage process that is catalyzed by the RAD51 recombinase protein. RAD51 binds single stranded DNA (ssDNA) derived from the nucleolytic processing of DNA lesions to form a highly ordered nucleoprotein filament called the presynaptic filament capable of searching for DNA homology in a donor chromatid and mediating its invasion to generate the displacement loop (D-loop) intermediate.

Recently, a protein complex consisting of MCM8 and MCM9 proteins has been shown to interact with RAD51 and its cellular depletion leads to impaired HR repair. As such, the MCM8-9 complex is indispensable for HR efficiency.

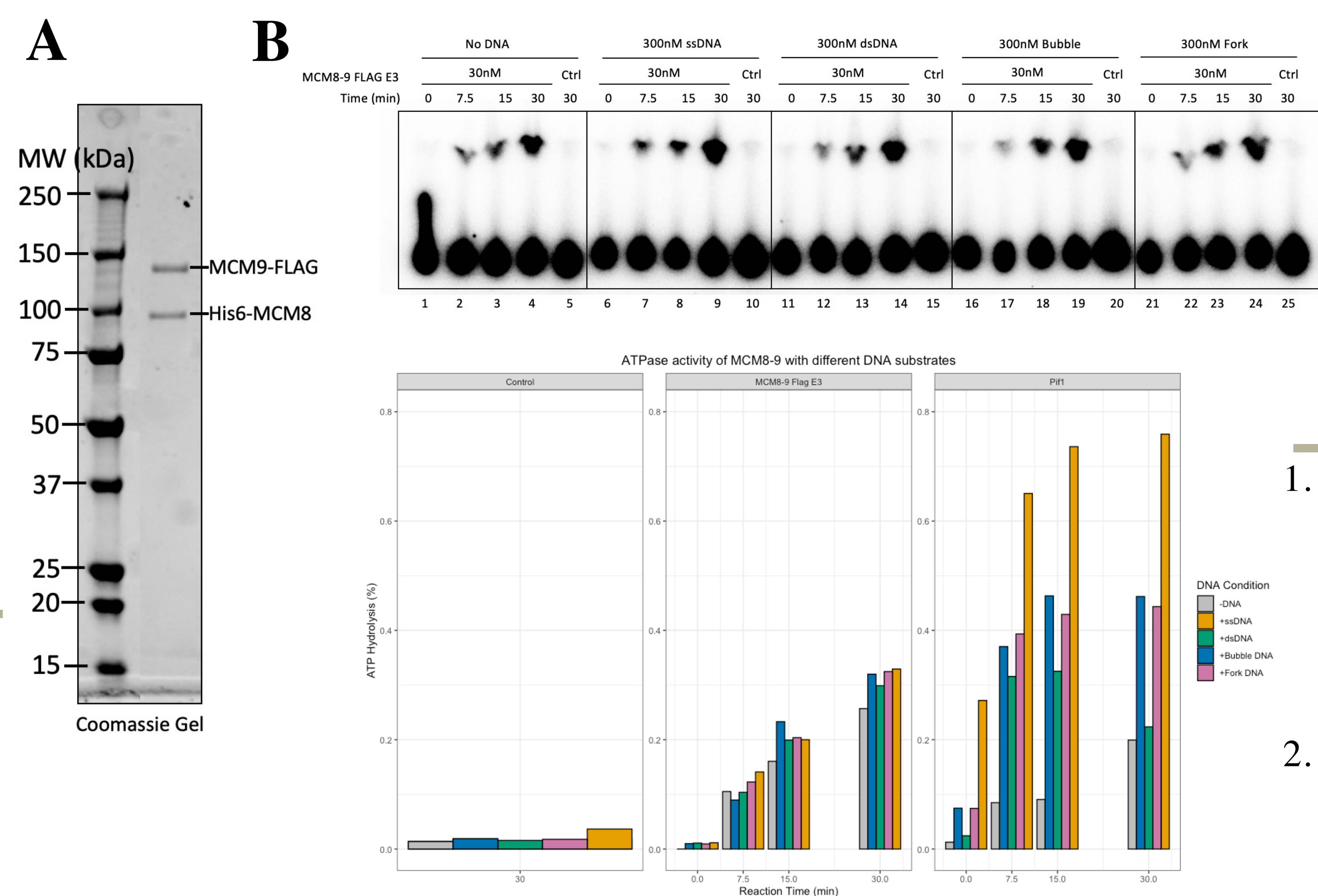
Here, we purify the MCM8-9 complex to near homogeneity and demonstrate its functional ability to hydrolyze ATP and unwind various DNA substrates through in vitro ATPase and helicase assays.

Materials and Methods

Protein expression and purification: Affinity-tagged human MCM8 and MCM9 were co-expressed and co-purified from *Trichoplusia ni* insect cells using the baculovirus expression system.

ATPase Assay: Purified MCM8-9 was incubated with a reaction mixture that contained unlabeled and radiolabeled P^{32}-ATP at 37°C for a specified time range. The quenched reaction was developed on a TLC plate and imaged on a phospho-imager.

Helicase Assay: A concentration gradient of purified MCM8-9 was incubated with a reaction mixture that contained a P^{32} radiolabeled double-stranded DNA substrate at 37°C for 30 minutes. The quenched reaction was resolved on an 8% native TAE gel, developed, and imaged on a phospho-imager.



Results

1. We demonstrate optimal co-expression and co-purification of the human MCM8-9 helicase complex from *Trichoplusia ni* insect cells utilizing the baculovirus expression system.
2. From the ATPase assay, our purified MCM8-9 retains ATPase activity as it can hydrolyze ATP in the presence of different DNA substrates.
3. Through the helicase assay, we show robust unwinding activity of MCM8-9 on different double-stranded DNA substrates.

Conclusion

Our results provide the initial foundation for obtaining pure and enzymatically active human MCM8-9 to carry out our downstream in vitro assays to address the complex's functional role during the DNA synthesis step of HR repair.

Furthermore, these activity assays will also allow us to introduce potential protein regulatory factors of MCM8-9 and quantify the level of stimulation or inhibition of the ATPase or helicase activity of MCM8-9.

Lastly, the purification regime will serve as a template for when we need to express and purify mutant and/or truncation constructs of MCM8-9 to conduct additional mapping and or separation of function experiments.

C

Fork dsDNA

MCM8-9 - - 25 50 100 [nM]

1 2 3 4 5

Bubble dsDNA

MCM8-9 - - 25 50 100 [nM]

1 2 3 4 5

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