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Biochemical analysis of Pfh1, the essential Pif1 family helicase in *Schizosaccharomyces pombe*

Jani Basha Mohammad

Akademisk avhandling

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Fakultetsopponent: Professor, Per Elias,
Medicinsk kemi och cellbiologi, Sahlgrenska akademin, Göteborg, Sverige.

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Author

Jani Basha Mohammad

Title

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Abstract

DNA stores the genetic information of all living organisms, and this information needs to be copied accurately and passed on to each daughter cell when a cell divides. However, the DNA replication machinery often meets obstacles in the genome that cause fork pausing and might result in DNA damage. DNA helicases are motor proteins that unwind duplex DNA structures using the energy from ATP hydrolysis. Helicases can also assist in replication fork progression by resolving obstacles that arise at hard-to-replicate sites such as tightly DNA-bound proteins, R-loops, and DNA secondary structures like G-quadruplexes (G4s). In this thesis, we focused on *Schizosaccharomyces pombe* DNA helicase Pfh1, which is localized in both the nucleus and the mitochondria and belongs to the evolutionary conserved Pif1 helicases. Pfh1 is an accessory replicative helicase, and the goal in this thesis was to gain a better mechanistic understanding of the role of nuclear Pfh1 (nPfh1). Our first aim was to elucidate the role of nPfh1 at G-quadruplex (G4) DNA. Aim two was to understand the function of nPfh1's signature motif. Aim three was to characterize the role of nPfh1 in strand annealing.

Some G-rich sequences can form a four-stranded DNA structure called G4 DNA, and the *S. pombe* genome contains about 450 bioinformatically predicted G4 structures. We selected two of these sequences, one located in the ribosomal DNA region and one located in the telomeric DNA region, and showed that they form inter- and intramolecular G4 structures, respectively. Next, we established a method to express and purify recombinant nPfh1 and demonstrated that nPfh1 binds to and unwinds these structures. In addition, Pfh1 bound to both the ribosomal and telomeric DNA regions *in vivo*, suggesting that Pfh1 can bind and unwind G4 structures *in vivo*. The purified nPfh1 also unwound RNA/DNA more efficiently than DNA/DNA structures, suggesting that nPfh1 has the ability to unwind R-loops *in vivo*. nPfh1 also showed protein displacement activity, suggesting that it can remove tightly bound proteins from DNA. All of these properties of nPfh1 suggest that it is important for fork progression and for preserving genome integrity.

Furthermore, nPfh1 stimulated strand annealing, and this activity did not require ATP hydrolysis. The strand-annealing activity was higher for complementary DNA/DNA compared to RNA/DNA substrates and did not require a DNA overhang. Furthermore, by analyzing Pfh1 truncated variants we demonstrated that the N-terminus region of Pfh1 was mainly responsible for the strand-annealing activity, however the C-terminus region also possessed some strand-annealing activity. Point mutations in the Pif1 signature motif (SM) have been shown to be associated with an increased risk of breast cancer in humans and with inviable *S. pombe* cells. We purified several SM variants and found that the unwinding and protein displacement activities of nPfh1 were dependent on the SM, but not the strand-annealing activity, suggesting that the SM is important for functions that require ATP hydrolysis.

In conclusion, in this thesis we identified nPfh1 as a potent G4 unwinder, and this is the only G4 unwinder identified in *S. pombe* to date. We also provided detailed mechanistic insights into nPfh1 and its different domains, and this has enhanced our understanding of Pfh1's role in maintaining genome integrity.

Keywords
G4, Pfh1, Genome integrity, helicase.

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