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Mutant PriA C-Tev ML346 and its Unwinding DNA Capabilities

Binding and Unwinding Damaged DNA on a Longer Leash

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Abstract

In DNA replication mistakes can happen where the DNA sequences have been damaged. When that happens it causes the process to stall. There's a protein called PriA that recognizes this issue, unwinds the duplex DNA at the fork junction and reloads the replisome which will restart the replication process. PriA has multiple structural domains that cooperate with one another to carry out its functions in binding DNA and unwinding it. These structural domains are a very compact unit when they're all assembled together in the inact protein. The winged helix domain, however, seems to resist this trend. It's connected by a long, flexible tether to the remaining compact structure. This experiment examined the significance of the winged helix domain's long, flexible tether by lengthening the C-terminal tether. We hypothesized it would alter its DNA unwinding capability. Through a helicase assay it was observed that lengthening the C-terminal tether didn't change its capability to unwind duplex DNA.

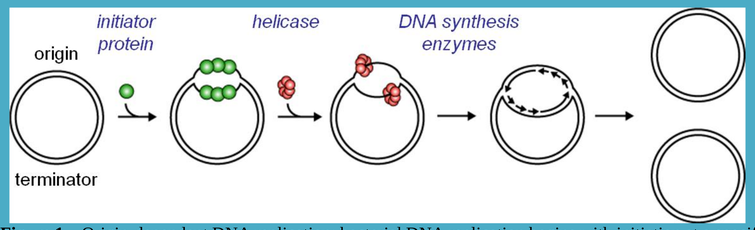

Figure 1 – Origin-dependent DNA replication: bacterial DNA replication begins with initiation at a specific origin on the chromosome in which a helicase separates the two complimentary DNA strands. Then DNA synthesis enzymes, along with the helicase, separate and duplicate the chromosome until there are two separate cells.

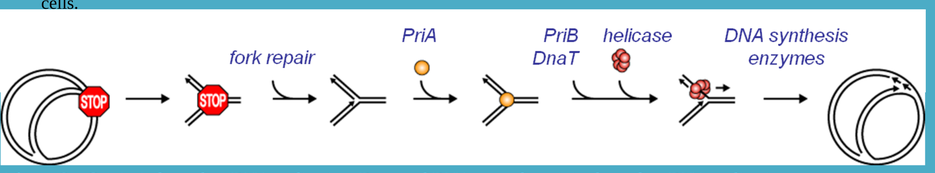

Figure 2 – Origin-independent DNA replication: Damaged DNA stops the origin-dependent DNA replication process, resulting in the dissociation of the DNA replication machinery (replisome) represented by the stop sign. In order for the process to continue the replisome must be reloaded onto the fork. One of PriA major roles is to orchestrate origin-independent replisome loading at the stalled fork. Then PriB and DnaT is recruited to form a complex to signal for the helicase and the DNA synthesis enzymes to reload onto the chromosome and continue the DNA replication process.

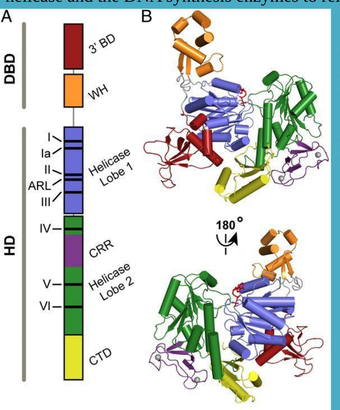

Figure 3 – Domain structure and ribbon diagrams of PriA helicase. (A) Schematic diagram of PriA domain structure. (B) Crystal structure of KpPriA. Domains are colored as in A. ADP (red sticks) is bound within the helicase core, and two Zn^{2+} ions (gray spheres) are bound to the CRR.

The winged helix domain is a DNA binding domain. It contributes to PriA being able to bind onto the stalled replication fork.

Goal

The objective of this project was to connect the role of the winged helix's isolation with PriA's function. We hypothesized that lengthening the C-terminal tether of the winged helix domain, thus increasing its mobility, in PriA will alter Kpn PriA activity. To test this hypothesis, I introduced the mutagenized PriA into the pET28b plasmid, confirmed the insertion, grew and purified the cells, and then performed a helicase assay to determine its capability of unwinding duplex DNA.

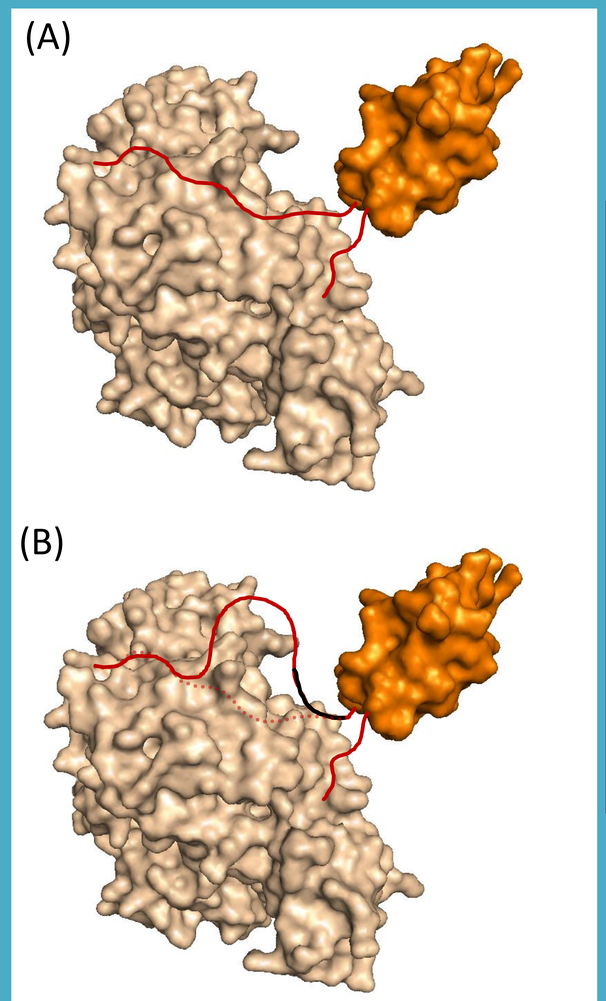

Figure 4 – (A) The winged helix domain, colored orange, of Wild-Type PriA connected to the remaining compact globular protein (B) The predicted elongation of the C-terminal tether of the winged helix domain in PriA

Experimental Methods

- Construction of PriA C-Tev ML346
- Protein Purification
- Helicase Assays

Results

- Construction of the mutant PriA C-Tev ML346 was successful
- Protein purification of mutated PriA showed 90% purity
- Helicase assay results, shown below in Figure 5, showed the PriA C-Tev ML346 unwound duplex DNA about the same as the PriA Wild-Type

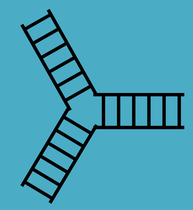

Figure 5 – Fork 2 DNA: This was the DNA that the mutant and wild-type PriA in the helicase assay was performed on. This DNA was used so that we could fast forward through the origin-independent DNA replication restart process to the part where PriA enters.

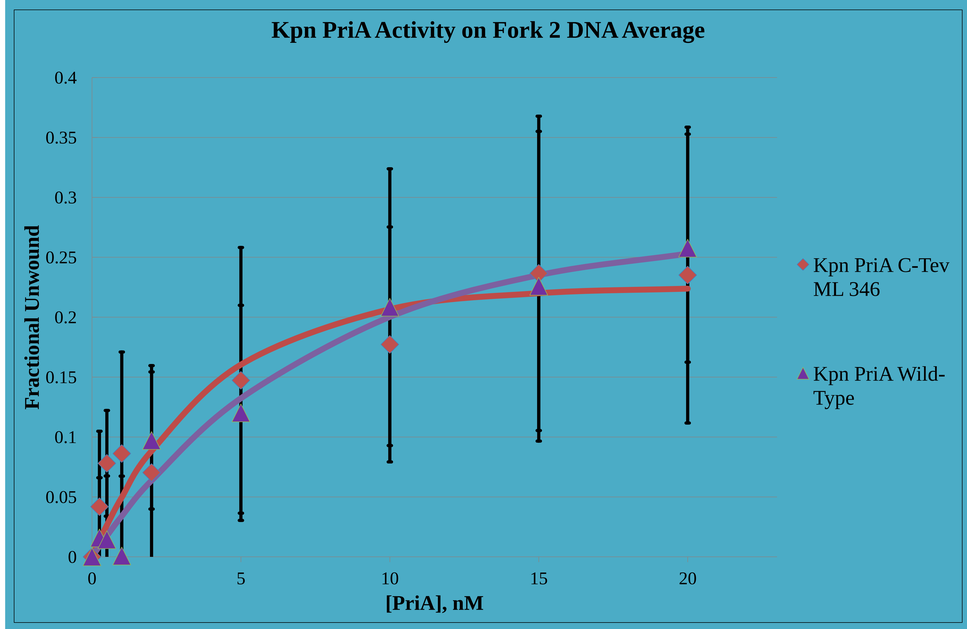

Figure 6 – Kpn PriA C-Tev Activity on Fork 2 DNA data average merged with the Kpn PriA Wild-Type data. When comparing the two it's clear that the mutant PriA follows the same pattern as the wild-type PriA does. This indicates a failed hypothesis: lengthening the C-terminal tether on the winged helix domain in the PriA ML346 didn't alter its Kpn PriA activity.

Conclusion

Lengthening the C-terminal tether of the winged helix domain in the PriA of the ML346 did not have much effect on altering its Kpn PriA activity. When analyzing the structure of PriA it makes sense that it wouldn't change PriA activity. The N-terminal tether is shorter than the C-terminal tether, as seen in Figure 4. So when the C-terminal tether is elongated it ultimately doesn't change the winged helix's position in relation to the rest of the protein. Since the N-terminal tether is keeping that domain the same distance to the remainder of the protein, PriA's capability of unwinding DNA doesn't change.