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Investigating DNA Repair Processes in Bacteria: Can D. rad PriA load D. rad DnaB onto DNA forks with a leading strand gap?

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Copying Damaged DNA: Can D.rad PriA Load DnaB on to a replication Fork with a leading strand Gap?

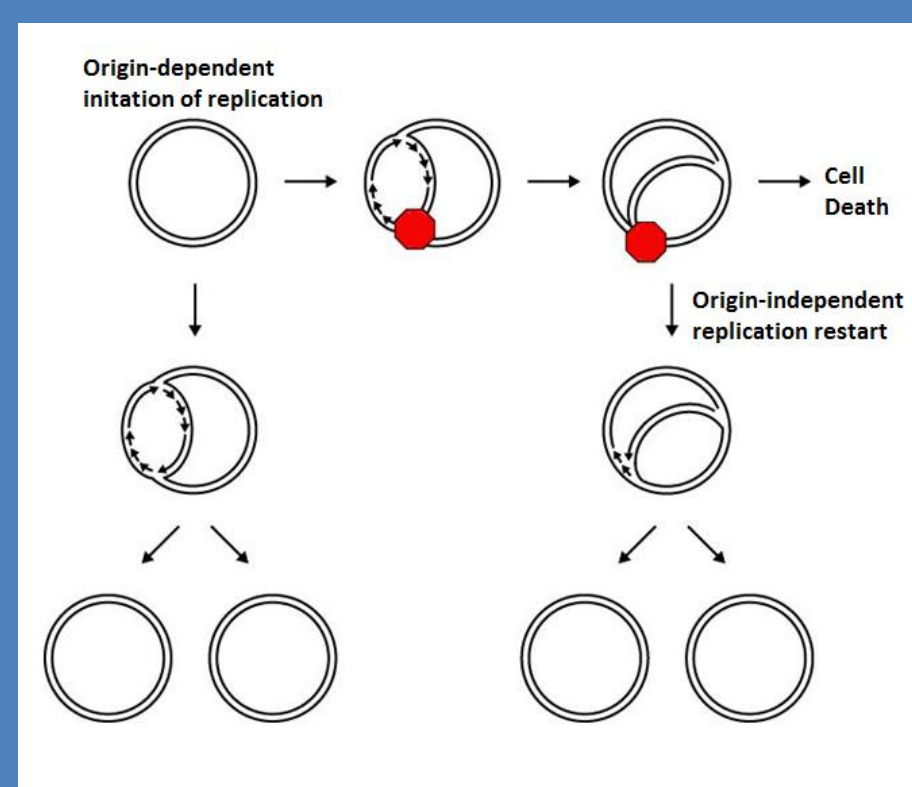
Mike Ryan
Advisor : Dr. Matthew Lopper

Abstract

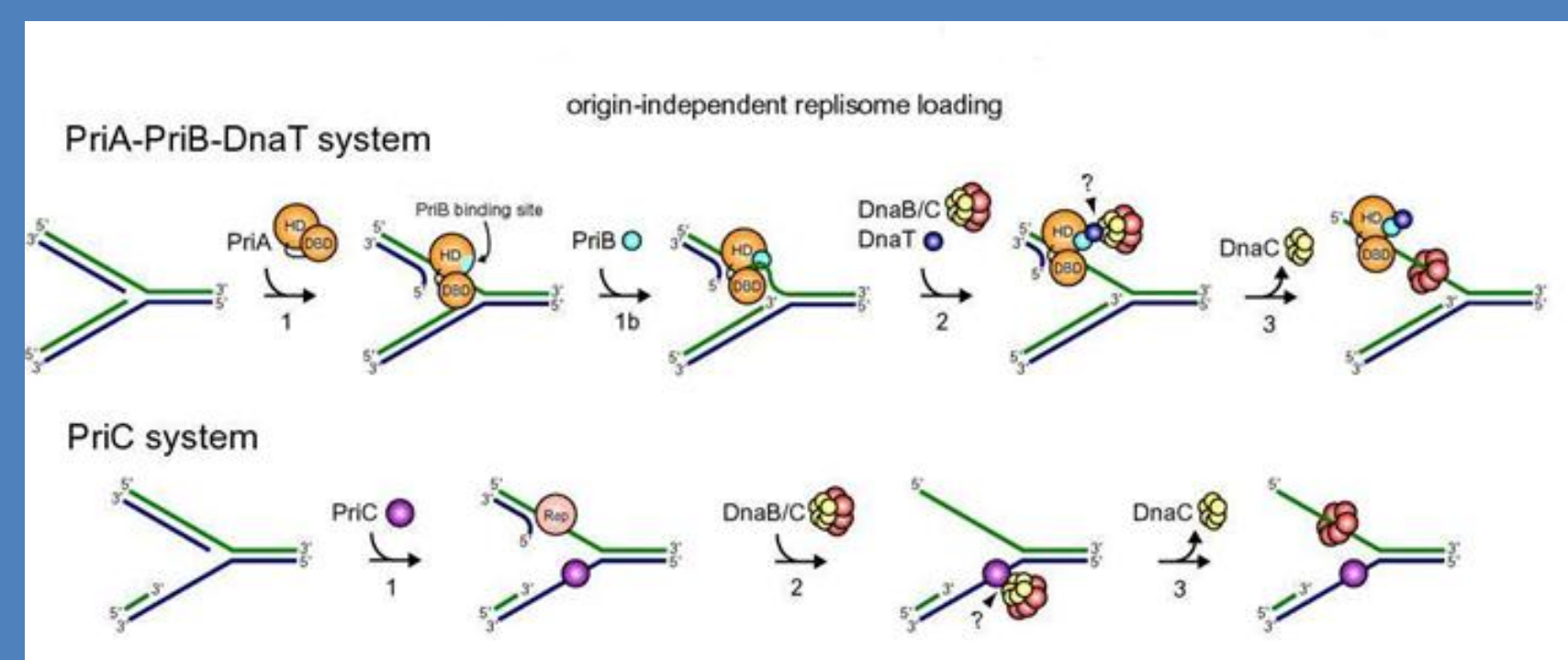
My research focused on the repair and replication of damaged DNA in the *Deinococcus radiodurans* (*D. rad*) bacteria, which is able to survive extreme levels of DNA damage with no detriment to its health because it is very efficient at repairing damaged DNA. In replicating (copying) bacterial DNA, damaged DNA will cause the replication to stop. This requires the DNA replication to be restarted in order for replication to be completed and cell death avoided. In most bacteria the proteins that function to restart DNA replication at points of DNA damage are fairly well conserved from bacteria to bacteria; however the *D. rad* bacteria lacks many of those proteins. I investigated the interactions between the proteins in this pathway that *D. rad* bacteria has, resulting in a clearer understanding of how these proteins interact in the *D. rad* replication restart pathway.

Replication Restart

- DNA is fragile, causing replication to become stalled at points of damage.



- Cells have a way of resuming replication in a process called replication restart.



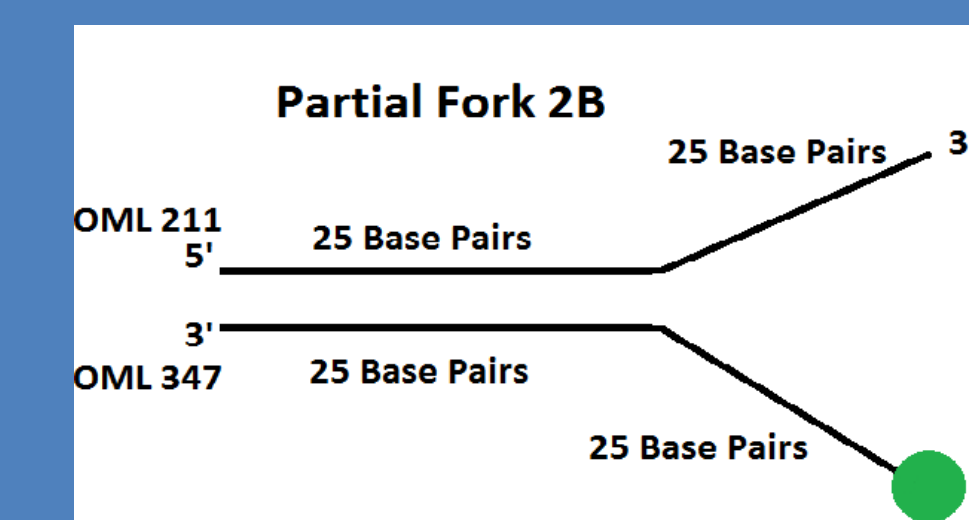
- In most bacteria there are two different replication restart mechanisms depending on the type of DNA fork.

Deinococcus Radiodurans

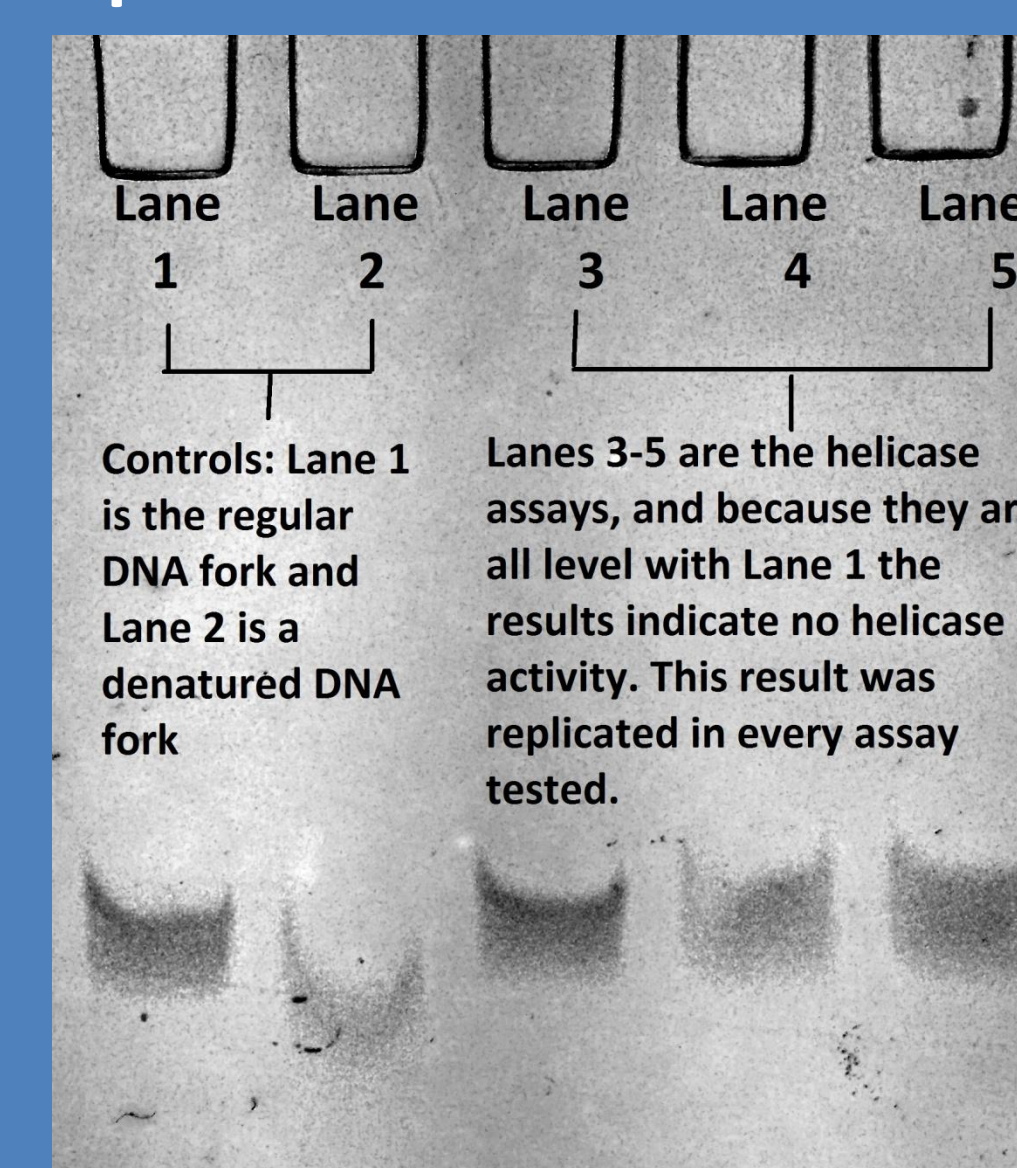
- *D. rad* can survive hundreds of double stranded DNA breaks.
- The ability to survive high levels of DNA damage, indicates that it might have a very proficient replication restart pathway.
- *D. rad* lacks most proteins involved in replication restart such as DnaT, DnaC, PriB, or PriC. (The *E. coli* version is shown to the left)
- *D. rad* PriA has structural differences from PriA proteins found in other bacteria

Process

- Produce and purify the required components.
 - *D. rad* PriA, SSB, and DnaB
 - The DNA fork



- Helicase assays were run to test for DNA winding. Unwound DNA indicates an active helicase.
- We visualized the results of these helicase assays using gel electrophoresis



Results

- No helicase activity was observed in any of the experiments that included all combinations of PriA, SSB, and DnaB.

Conclusions

- Combinations of *D. rad* PriA, SSB and DnaB did not unwind a DNA fork with a leading strand gap.

Future Work

- Test for additional cofactors and isozymes.
- Characterize the structure of *D. rad* PriA .
- Further testing *D. rad* DnaB for helicase activity.