A 3.2 Å structure of the PriA/PriB/replication fork complex reveals mechanistic insight into bacterial DNA replication restart

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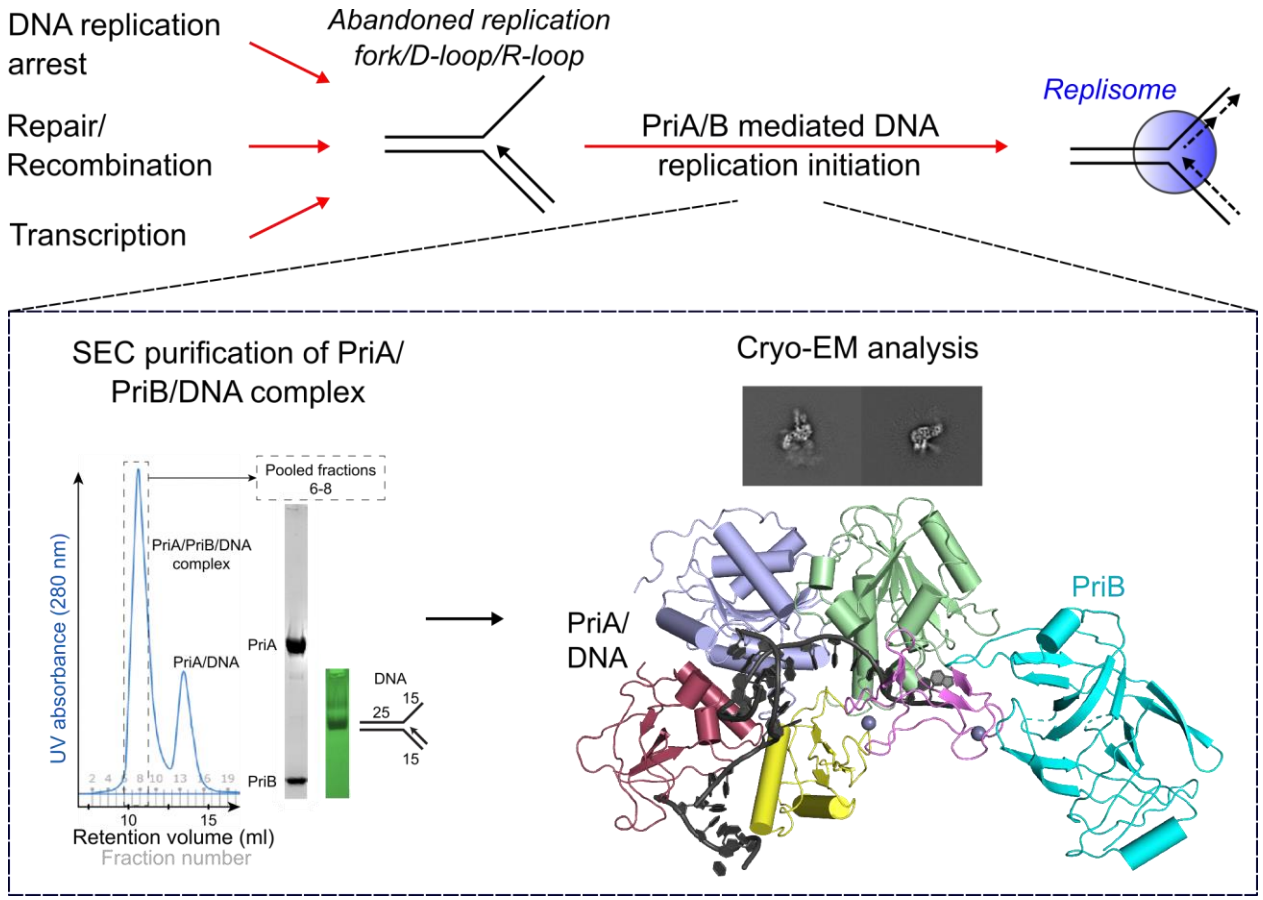
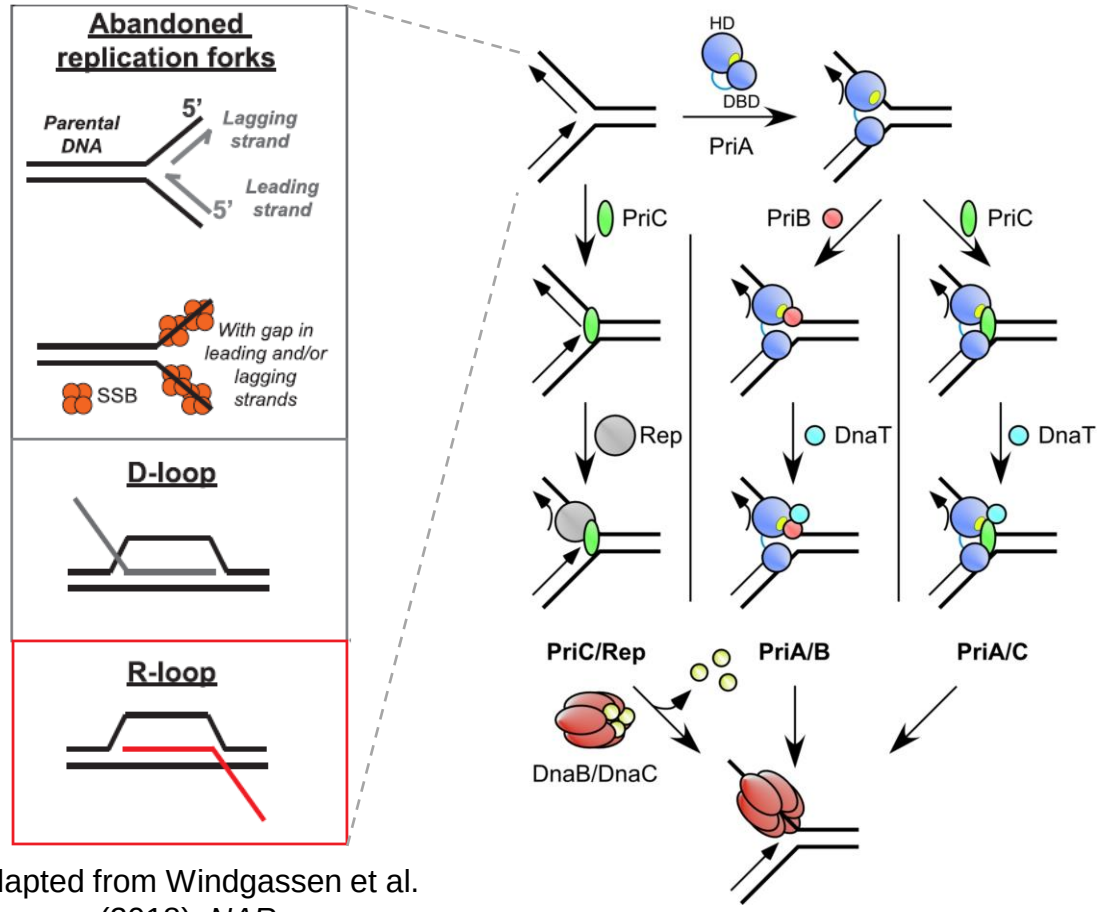


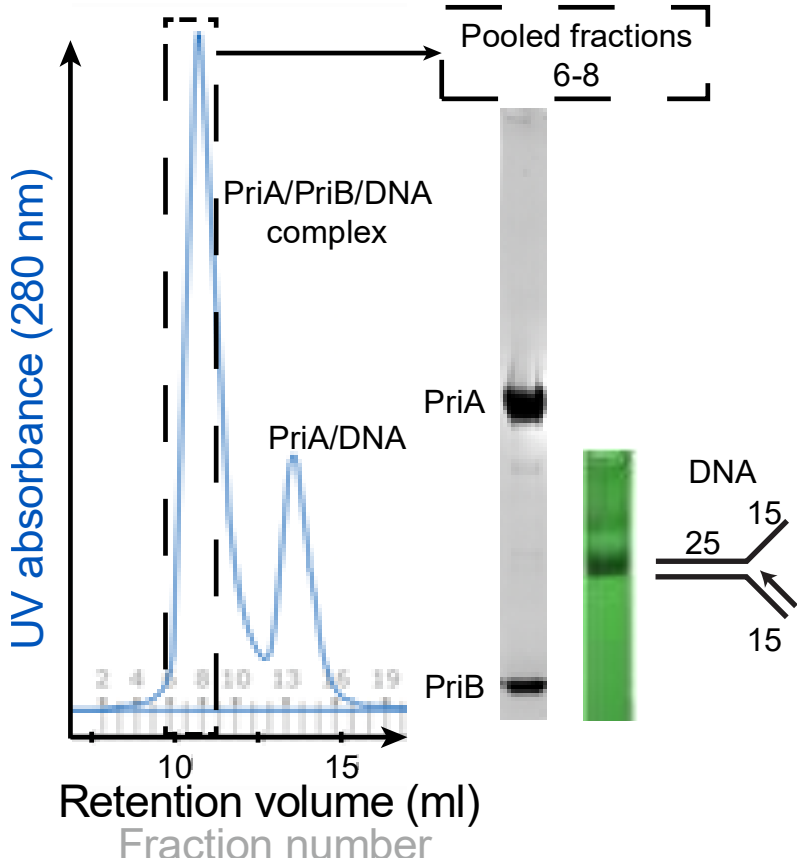
DNA replication restart is an essential process in bacterial cells

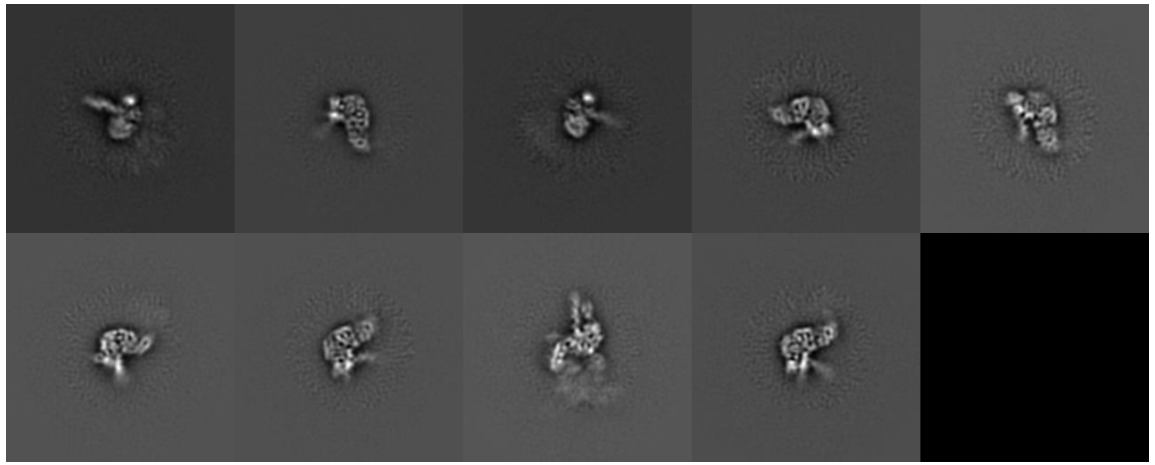
- Structure-specific method of DNA replication initiation
  - Replication arrest → abandoned replication forks
  - Repair/recombination → D-loops
  - Transcription → R-loops
- PriA/PriB pathway is thought to be preferred
- Mechanisms of PriB and DnaT remain poorly understood



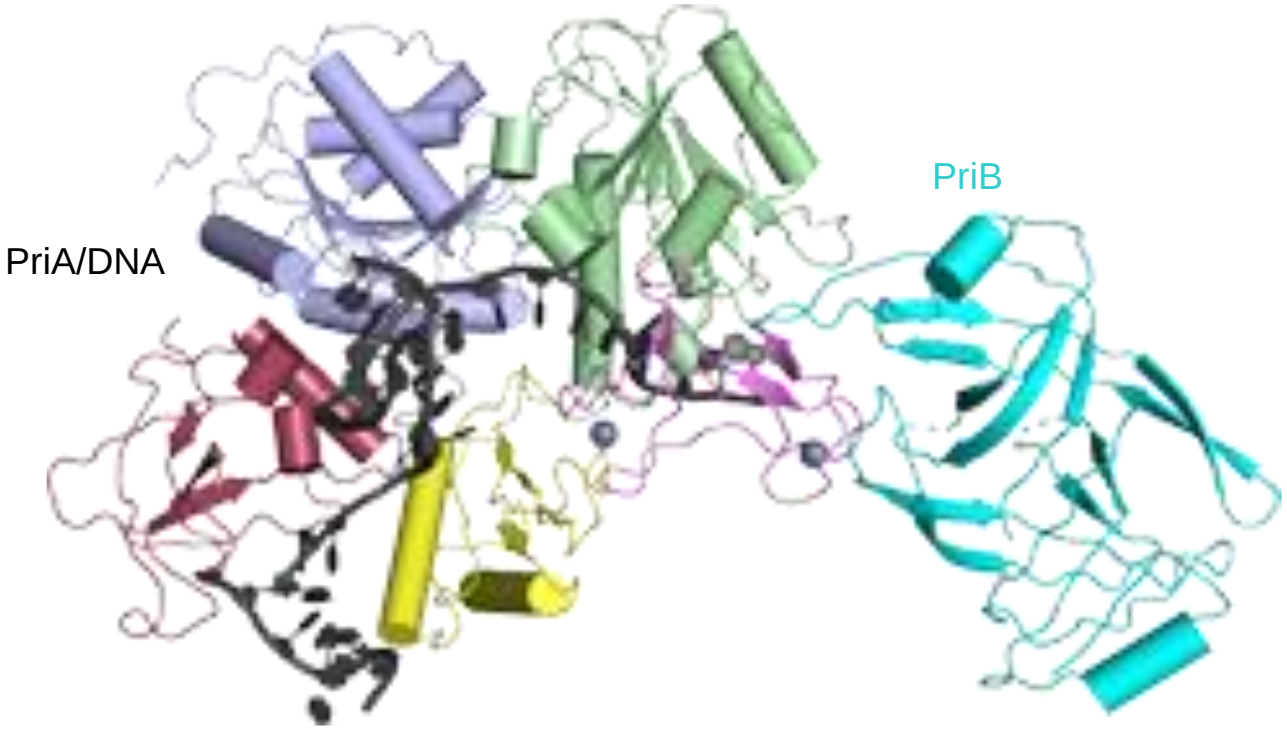
Adapted from Windgassen et al. (2018), *NAR.*

Purification of PriA/PriB/DNA fork and Cryo-EM analysis



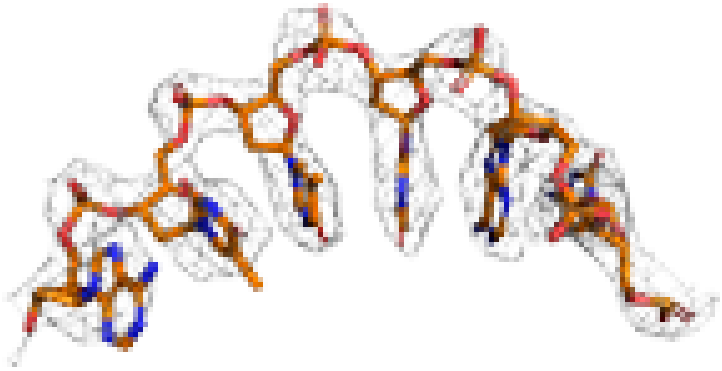


3.2 Å Cryo-EM structure of PriA/PriB/DNA fork resolves ssDNA path

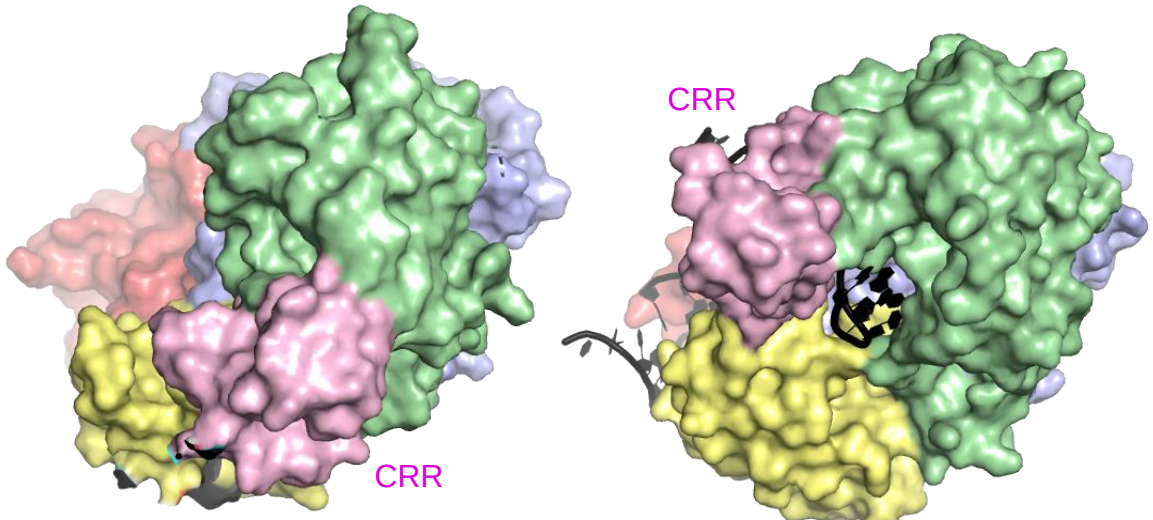


- Resolved portions of both parental and leading strand dsDNA, 8 nucleotides of lagging strand ssDNA

- PriA (colored by domain) and PriB dimer (cyan) resolved bound to DNA (black)
- PriB is bound to PriA’s cysteine-rich region (purple)

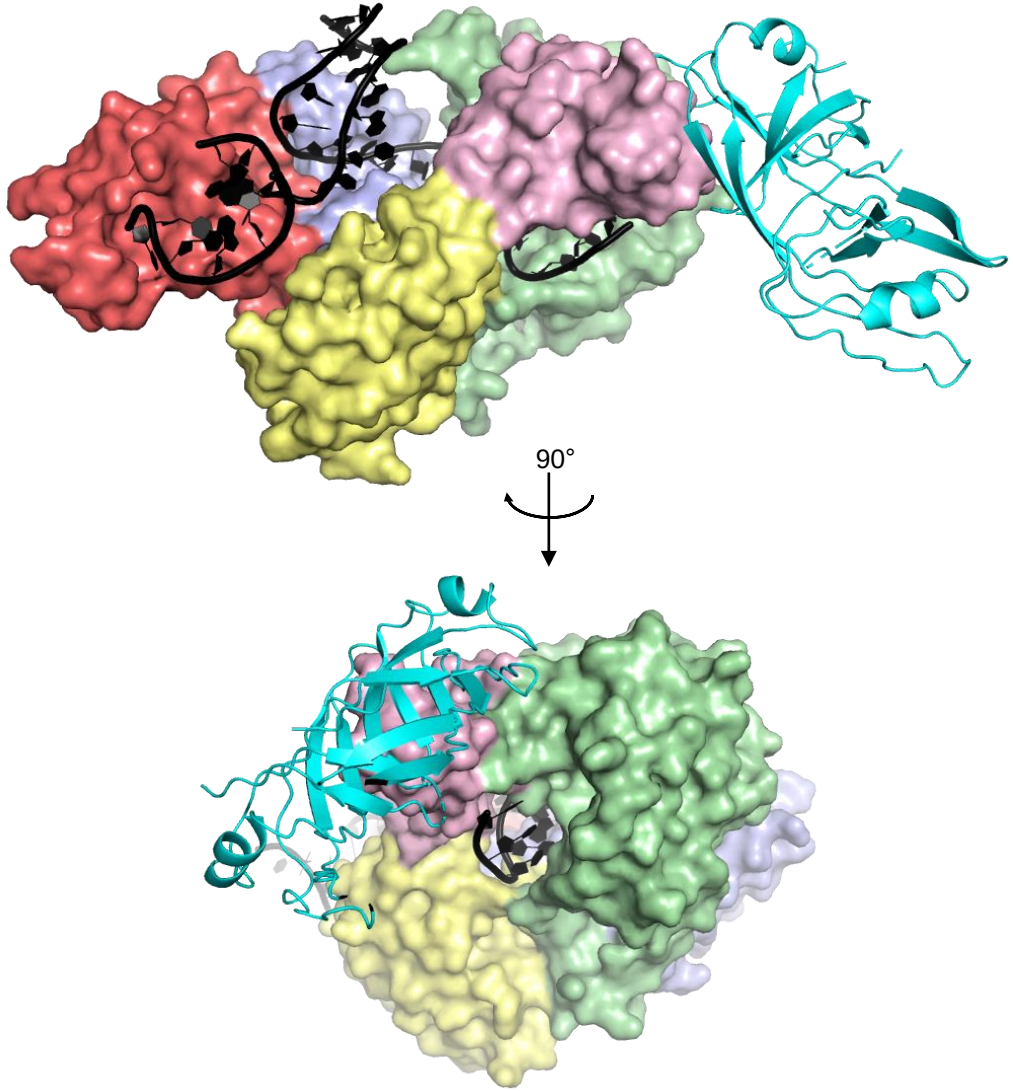


PriA's cysteine-rich region (CRR) undergoes large conformational change to form pore for ssDNA



apo PriA
Windgassen et al. (2018), *PNAS.*
PDB: 6DCR

PriA/PriB/fork Cryo-EM structure



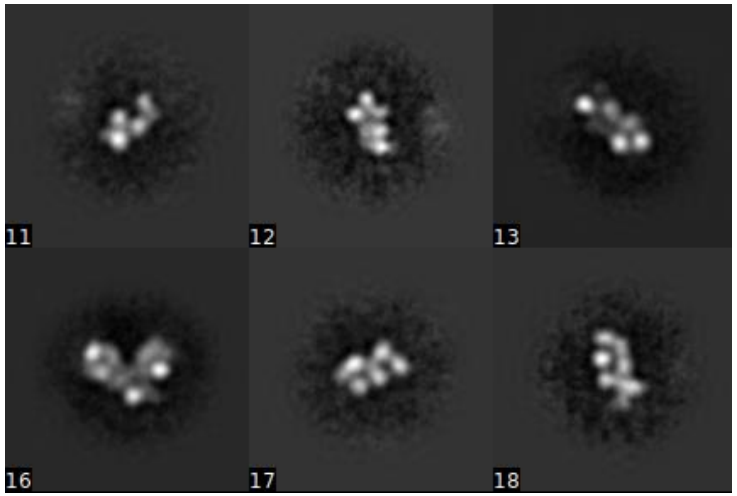
Conclusions, future directions, and acknowledgements

Conclusions:

- Solved a 3.2 Å Cryo-EM structure of PriA/PriB/DNA fork
  - PriB bound to PriA CRR
  - ssDNA resolved in pore formed by CRR conformational change

Future Directions:

- *In vitro/In vivo* validation of the structure
- Add DnaT to the complex:



Negative Stain-EM images of PriA/PriB/DnaT/DNA fork

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NIH National Institutes of Health

Questions?
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