The Molecular Contortionist: How Rep Helicase Changes Shape

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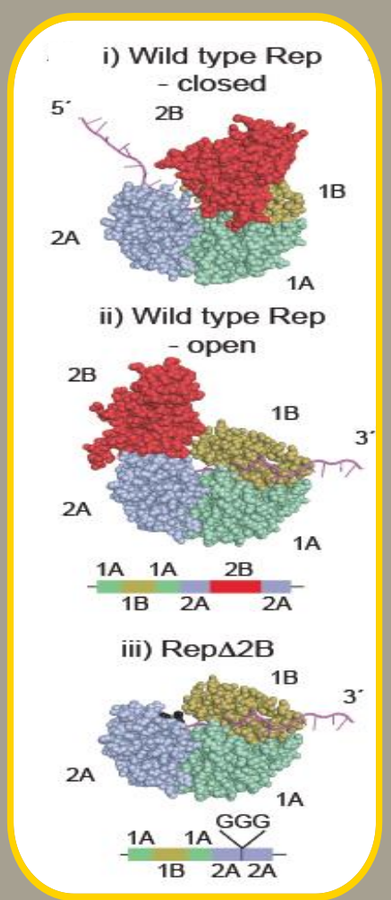
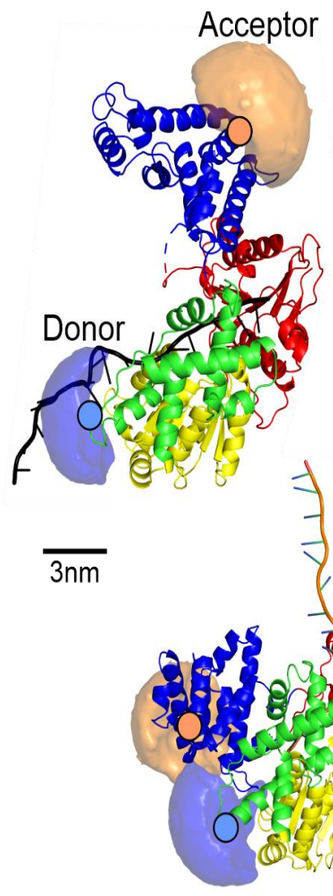
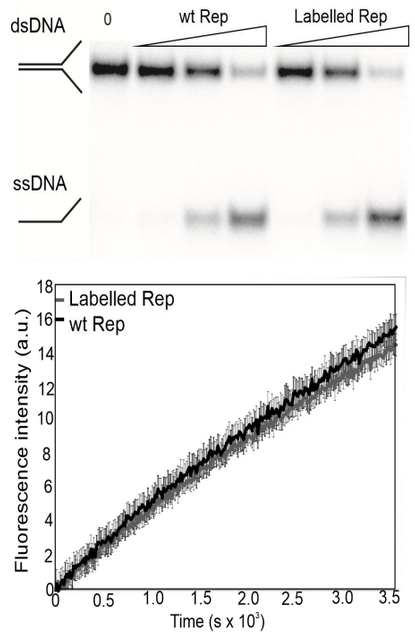
Introduction



- Loyal replication of DNA is critical in maintaining genomic integrity
- The replisome is responsible for DNA replication but is incapable of bypassing blocks to its progress
- Rep is an accessory SF1A DNA helicase that helps the replisome overcome protein–DNA barriers
- Rep contains a highly mobile 2B subdomain that undergoes large rotations
- Monomeric Rep translocates on ssDNA but does not efficiently unwind dsDNA
- Removing 2B domain activates unwinding but abolishes protein displacement and in vivo function
- Previous structural and single-molecule studies suggest multiple Rep conformations, but the full conformational landscape and kinetics are unknown
- How Rep conformational dynamics regulate helicase activity remains unknown

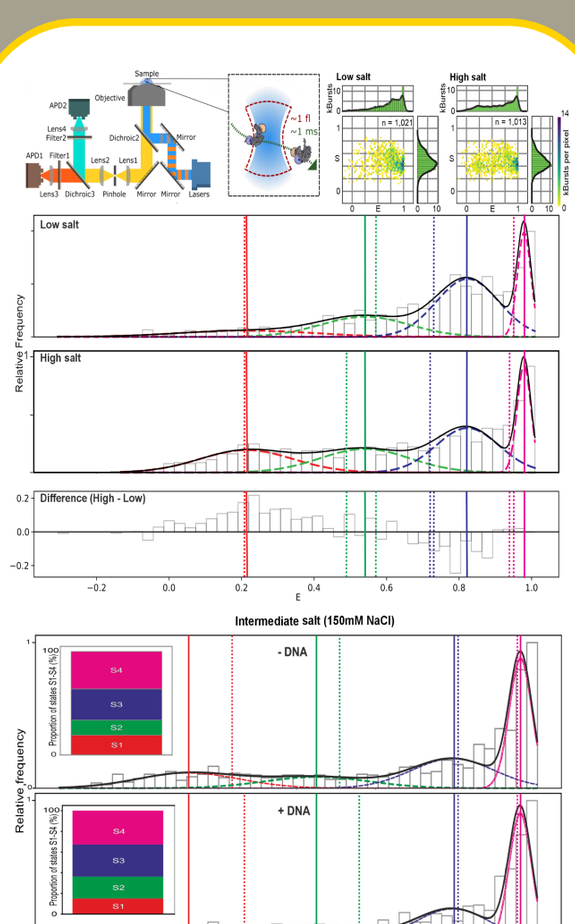
Fluorescently labelled Rep retains WT protein Function





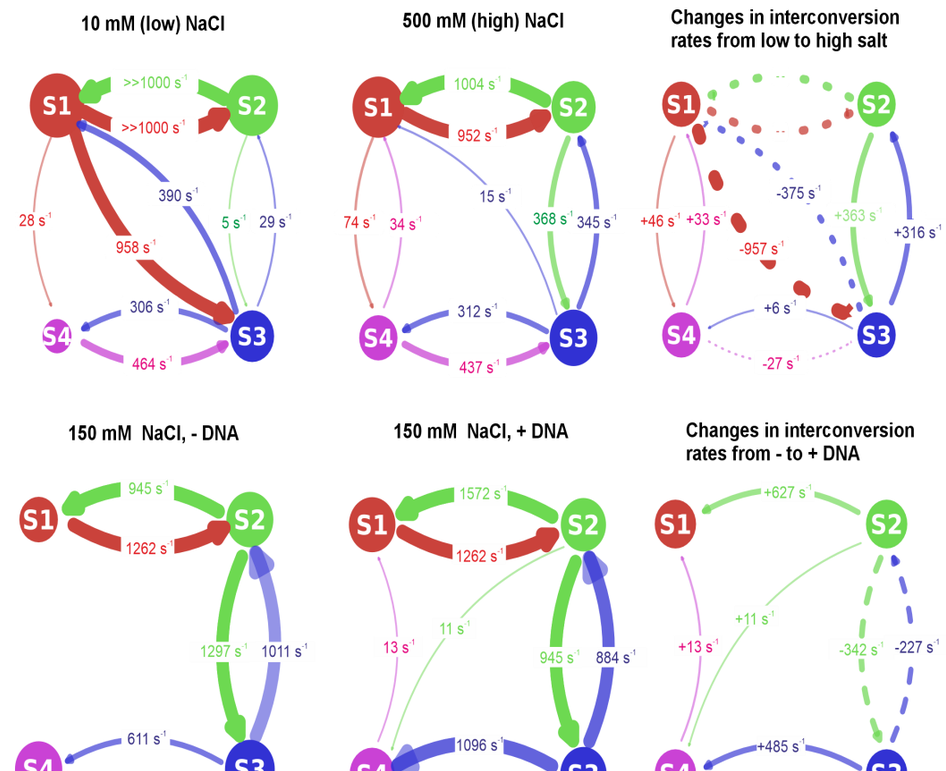
- Labelled Rep unwound 74.6 ±4.7% (±SD) of forked DNA compared to wild type 71.7 ±1.3% in end point assays
- initial velocities: 5.44 ±0.47 vs 5.97 ±0.34 a.u. s^{-1}
- k_{obvs} (272 ±23 vs 299 ±17 a.u. $\mu M^{-1} s^{-1}$)

Single-molecule FRET reveals that rep exhibits four states



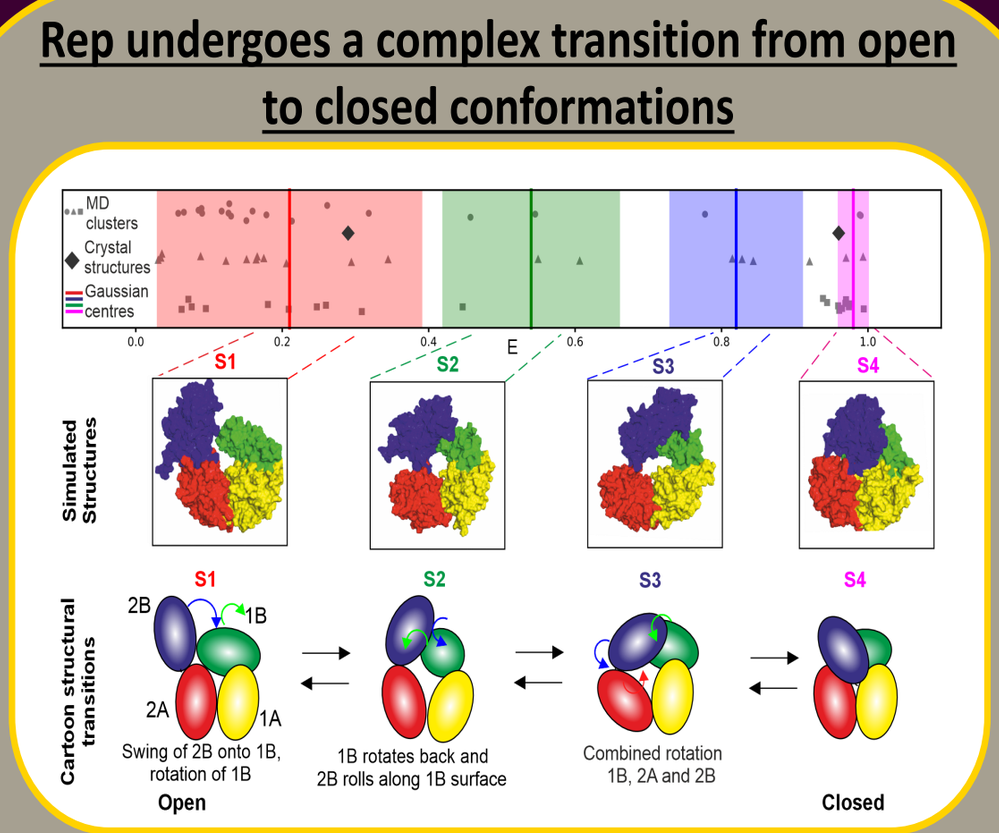
- Single-molecule FRET reveals four interconverting Rep conformations (S1–S4)
- S1 and S4 correspond to open (low FRET) and closed (high FRET) states
- Two previously hidden intermediate states (S2 and S3) are resolved
- Salt concentration tunes the relative populations of all four states
- DNA binding redistributes state populations, reducing the open state and stabilising intermediate conformations

Rep undergoes dynamic transitions over a timescale range of sub-milliseconds to seconds



- Rep dynamically interconverts between four states over sub-ms to second timescales
- Transition rates are tuned by salt concentration
  - DNA binding reduces conformational flexibility and reshapes kinetics

Rep undergoes a complex transition from open to closed conformations



- Rep closes via a multi-step pathway, not a single 2B swing as previously thought
- Intermediate states reflect distinct domain rearrangements
- Coordinated motions of multiple subdomains drive closure
- The 1B subdomain may sterically hinder 2B motion, preventing premature closure in the absence of DNA

Scan here for an animation of the states that Rep occupies and the transitions between them

Conclusions

- Rep explores a landscape of multiple interconverting conformations rather than switching between a single open and closed state
- Salt concentration tunes this landscape, biasing Rep towards more open or closed conformations before DNA binding
- DNA binding reduces conformational flexibility, stabilising intermediate states
- The 1B subdomain may act as a regulatory element, preventing premature closure enabling DNA capture
- This flexibility supports a general model in which accessory helicases exploit conformational plasticity to locate and displace protein–DNA obstacles
- Further work to identify states responsible for DNA unwinding and nucleoprotein block removal and active oligomeric states to follow