Bacillus subtilis BsParB compaction of non-specific lambda-DNA in the presence and absence of CTP in single-molecule flow-stretching experiments

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Abstract

Chromosomes are carefully organized within cells, and faithful chromosome segregation during cell division is fundamentally important for all living organisms. In bacteria, the *parABS* partitioning system is central to chromosome segregation and plasmid partitioning. ParB is a DNA-binding protein that specifically recognizes *parS* DNA sites near the replication origin and consists of an N-terminal domain (NTD), DNA-binding domain, and C-terminal domain (CTD). Importantly, ParB spreads 10-20 kb from *parS* sites flanking the region, which promotes the recruitment of additional ParB molecules to associate with neighboring DNA, forming higher-order nucleoprotein complexes that are essential for faithful chromosome segregation. Proposed spreading models are one-dimensional filamentation along DNA, bridging and condensing DNA, and lateral sliding of a ParB-CTP clamp. Recent paradigm-shifting research shows that ParB is a novel CTPase enzyme and utilizes CTP hydrolysis to induce self-dimerization of ParB through its NTD. This creates a clamp that encircles DNA for a sliding model along DNA, but this alone does not fully explain ParB spreading.

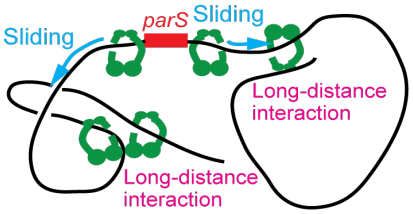

Figure 1. A hypothetical ParB spreading model. ParB with CTP-bound state slides away from the *parS* site in short distances. Longer distance interactions are mediated by ParB-ParB or ParB-DNA interactions

Methods:

- Using purified wild-type *Bacillus subtilis* ParB (BsParB) and CTPase mutant BsParB, we conducted single-molecule DNA flow-stretching experiments on bacteriophage lambda DNA (12 nucleotide overhangs on both ends) without *parS* sites.
- The lambda DNA was annealed with complimentary oligos possessing biotin and digoxigenin and was bound on one end to surface-passivated glass slides via a neutravidin-biotin linkage, and fluorescent anti-digoxigenin quantum dots (Qdots) were tagged on the other end.
- BsParB was added into the flowcell with and without CTP, and DNA-BsParB compaction events on stretched lambda DNA were imaged using total internal reflection fluorescence (TIRF) microscopy.

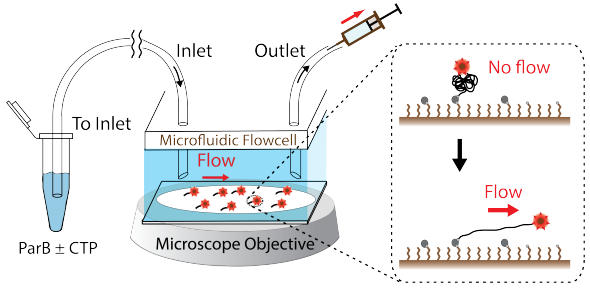

Figure 2. Schematic for DNA flow-stretching experiment. Left shows the flowcell setup. Right shows one DNA end annealed to biotin (gray dot) for passivation to neutravidin and the other end annealed to Qdot (red dot)

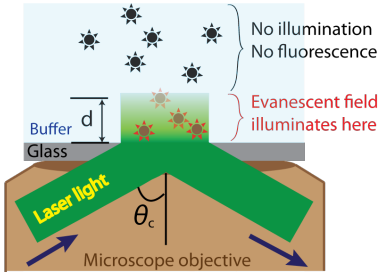

Figure 3. Evanescent field generated by 532 nm laser and visualized by TIRF microscopy

Open Question:

Is there any effect of CTP on the non-specific lambda DNA compaction rates by ParB DNA-binding protein?

Results:

- BsParB at 50nM concentration compacts non-specific lambda DNA without *parS* sites.
- For the nonspecific DNA compaction, the presence of CTP is not required.

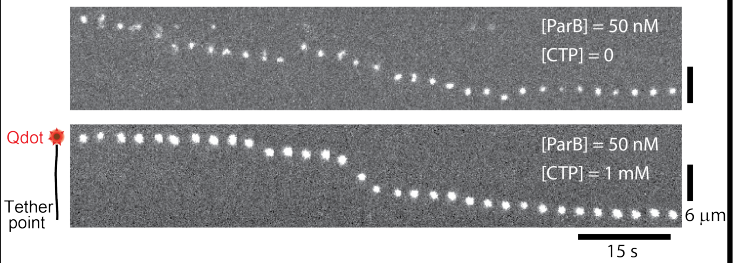

Figure 4. Kymograph showing a motion montage of Qdot moving in response to non-specific DNA compaction by BsParB protein. Scale includes time on the horizontal axis and distance on the vertical axis

Future Work:

More experiments are needed to ascertain rates of compaction and potential differences between rates with and without CTP. Future experiments will study BsParB compaction on specific *parS* containing lambda DNA both in the presence of and without CTP, to compare compaction between non-specific lambda DNA and that with specific *parS* sites.

References

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