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Single Molecule Force Analysis of RecBCD

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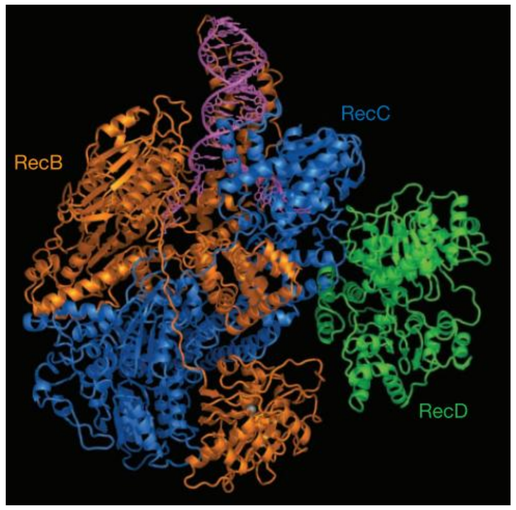
Introduction

Breaks in double-stranded DNA (dsDNA) if unrepaired are lethal events. Breaks can arise from many sources. In eubacteria, dsDNA breaks are restored beginning with the RecBCD family of enzymes.



RecB – unwinds 3’ ⟶ 5’
RecD – unwinds 5’ ⟶ 3’

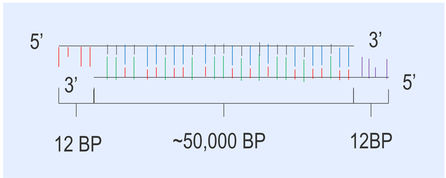
RecC – recognizes X
X = 5’ – GCTGGTGG – 3’

Abstract

Objective: The aim of this study is to understand how the enzyme RecBCD converts Adenosine Triphosphate or ATP (chemical energy) into DNA unwinding (mechanical work). By exerting an opposing force on the enzyme, we hope to also find the stall force of RecBCD (the necessary force for it to stop unwinding DNA).
Methods: By using an optical trap, we are able to isolate a single molecule of λ-DNA bound to a bead. At the same time, due to the nature of the enzyme, a bead-bound RecBCD molecule is attached to the DNA.
Results & Future Directions: Thus far, procedures to efficiently biotinylate λ-DNA have been developed. By similar methods, biotinylated RecBCD has been attached to a streptavidin coated microsphere. The DNA-bead and enzyme-bead subunits will be then brought together to create a complex. Since calibration of the apparatus has already been done, rates of unwinding relative to an exerted force will be determined next at the single molecule level.

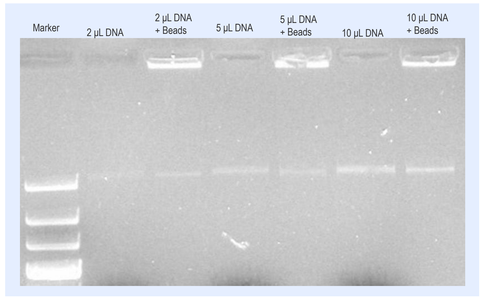
λ -DNA to Bead Binding

λ-DNA is a single molecule of DNA composed of approximately 50,000 base pairs. Each length of the double-stranded molecule has a non-identical 12 base pair overhang.

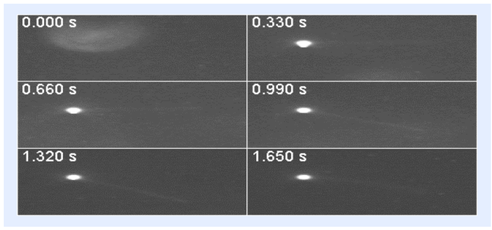


To one end of the DNA molecule, a biotinylated oligonucleotide is ligated.
This gives us, essentially, a biotinylated λ-DNA molecule.

Also used in our experiments is a streptavidin coated bead. The association of streptavidin to biotin is very strong.



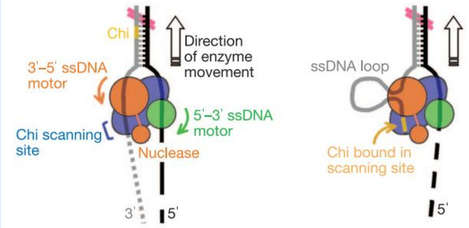
This gel shows the binding of DNA to Beads. Since beads are unable to migrate through a gel, any DNA bound to them would stay in the well. You can tell there is a significant drop in DNA content when you compare it to a lane with the same concentration of free DNA.



This is a montage of images that display use of the optical trap to capture a bead-bound λ-DNA molecule. The bead is the fluorescent white circle. Behind it is a faint gray line.

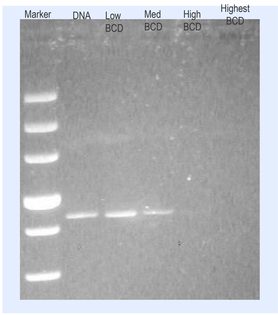
Bead Binding of RecBCD

RecBCD is a single enzyme composed of three subunits, RecB, RecC, and RecD. RecC is a structural subunit, while RecB and RecD both demonstrate motor function. RecB also demonstrates nuclease activity. We attach biotin to RecC *in vivo*.



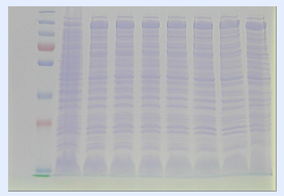
RecC is also responsible for the identification of χ. χ is a series of bases reading 5’ – GCTGGTGG – 3’. Before χ , RecB degrades the 3’-strand of DNA. Upon recognizing χ, the nuclease activity of RecB switches to the other strand of the DNA molecule.

For our experiments, we bind RecBCD to a streptavidin-coated microsphere via the RecC subunit. This is the ideal spot as it does not interfere with the motor functions of RecB and RecD. Once bound to beads, we assay for helicase/nuclease activity.

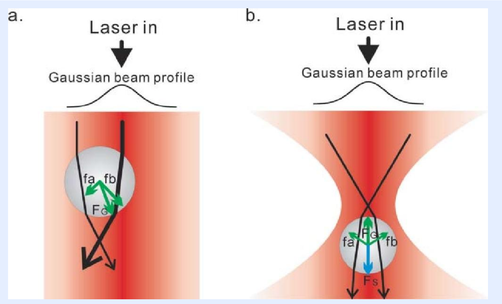


This gel demonstrates the nuclease activity of RecBCD. Various concentrations of BCD were added to a linearized plasmid of dsDNA.

This is a protein gel demonstrating the overexpression of RecB-$^{(biotin)}$C-D.

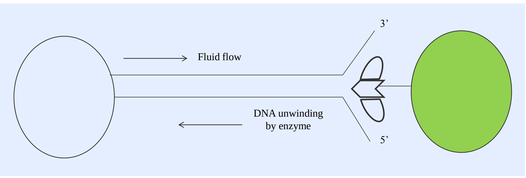


The Optical Trap



The optical trap is a function of the particle-like properties of light. The momentum of light exerts many forces on the bead,. Propagation of light through the particle also causes various directional forces on the particle.
The law of conservation of momentum then requires the bead to undergo an equal and opposite momentum change.

Future Directions



Once we have purified active, biotinylated RecBCD , we plan on using the optical trap to determine stall forces and unwinding forces of the enzyme.
For better visualization, RecBCD will also be purified with various tags on the RecC subunit including the fluorescent protein mCherry, and a SNAP tag.
By use of similar protocols, we plan on comparing the force-velocity curves of RecBCD to RecBC.

References

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Singleton, Martin R. and Dillingham, Mark S., Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. Nature Publishing Group 1, (2004).

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