Nanofabrication of Metallic Barriers for Single Molecule Imaging

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Principal Investigator(s): John Brooks Crickard
User(s): David Moraga, Ilayda Korkmaz, Mitchell Woodhouse

Affiliation(s): Molecular Biology and Genetics University: Cornell University

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Contact: jbc287@cornell.edu, dlm345@cornell.edu , ik353@cornell.edu, mvw25@cornell.edu

Research Group Website: https://blogs.cornell.edu/crickardlab/

Primary CNF Tools Used: Zeiss Supra with Nabity system for lithography, CVC SC4500 Even/Odd hour evaporator

Abstract:

DNA curtains are a powerful single-molecule technique that can analyze interactions between proteins and DNA in a high-throughput manner. This approach has transformed single-molecule fluorescence microscopy by combining statistical power with the ability to observe proteins moving along individual DNA strands. While other approaches can achieve this statistical power, they generally lack away to control the organization of DNA within a flow cell. We achieve this by nanofabricating chrome barriers onto microscope slides. This allows the alignment of hundreds of individual DNA molecules that can be visualized using total internal reflection fluorescence microscopy (TIRFM). The addition of fluorescently labelled proteins then allows us to monitor specific binding, protein-protein interactions, and the rate of protein movement along DNA. This powerful approach is made possible by equipment maintained in the center for nanofabrication at Cornell.

Summary of Research:

Our research focuses on the application of the DNA curtain technology. We use this approach to perform visual biochemistry approaches and monitor enzymes that function in DNA repair pathways(Figure 1A and Figure 2AB). Our specific work over the last period has focused on how related DNA motor proteins use translocation activity to facilitate the DNA repair process. Below, I will detail two specific projects that have utilized DNA curtains and our specific interactions with the CNF.

Rdh54 reduces Break induced replication during HR:

Rdh54 is a conserved DNA translocase, known as RAD54B in humans, and is a paralog of Rad54/RAD54L. Rdh54 is semi-redundant with Rad54, but its biological role is still unclear. The dominant

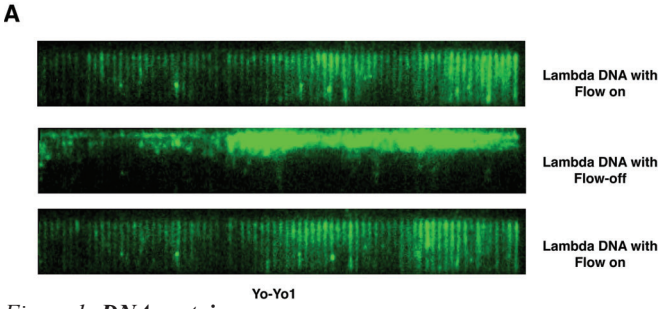

Figure 1: ***DNA curtains*** ***(A)****. Example of DNA curtains. Each green line represents an individual molecule of lambda phage. DNA (48.5 kbp). Flow turns on an off to extend or retract the DNA. The figure is taken from citation E.*

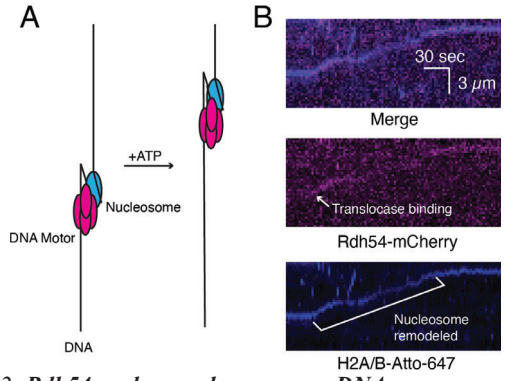

Figure 2: ***Rdh54 pushes nucleosomes on DNA*** ***(A)****. Cartoon illustrating Rdh54 moving nucleosomes.* ***(B).*** *Direct visualization of Rdh54 moving nucleosomes on DNA. This figure is form citation D.*

hypothesis is that Rdh54 reduces Rad51 binding to dsDNA, increasing pools of Rad51 available to promote recombination. However, the role of Rdh54/RAD54B during HR is poorly understood. To improve our knowledge, we investigated the role of phosphorylation in activating Rdh54. Our conclusions from this study are that the effector kinase Rad53 regulates the clustering activity of Rdh54 through a kinase site on the C-terminus of the protein. The kinase activity helps prevent the onset of a mutagenic type of repair, break-induced replication (BIR). This complemented our earlier study, which illustrated that Rdh54 acted to stabilize HR intermediates and provided a novel

mechanism by which Rdh54 improves the fidelity of the strand exchange reaction. Our biochemical studies have been welcomed in the field because of the general challenges in understanding the role of this protein. Studies to understand the biological role of Rdh54 in the template-switching process are ongoing.

The activity of Rad54 as a regulator of crossover/ NCO outcomes:

Rad54 is a Snf2 DNA motor protein that remodels DNA and works with Rad51-ssDNA filaments during recombination to catalyze strand exchange. A wealth of information exists on the biochemistry of Rad54 proteins, and we have identified several novel hypomorphic alleles, which allowed us to connect in vitro observations with tangible in vivo phenotypes. Generally, mutations or deletions of Rad54 have resulted in severe sensitivity to genotoxins, which has made developing more refined models for Rad54 function in vivo difficult. We have generated a genetic tool to dissect Rad54's function in all organisms by identifying and developing these hypomorphic alleles. These mutations slow translocation along DNA and in S. cerevisiae cause elevated genetic crossovers between homologous chromosomes during mitotic growth. A key finding from this study is that Rad54 is likely to improve the fidelity of the repair.

Interactions with CNF:

Our interactions with CNF are limited to the nanofabrication of flow cells, which we use to make flow cells (Figure 3AB). This involved electron beam lithography and chrome deposition to make barriers on the glass slide. These methods require us to train biochemistry and biology students to use the equipment in the cleanroom. These students would not have any interactions with this type of equipment or lithography applications otherwise. This cross-disciplinary training is a part of my research program but is made possible by the CNF.

Conclusions and Future Steps:

In the future we will continue to train students to make microscope slides.

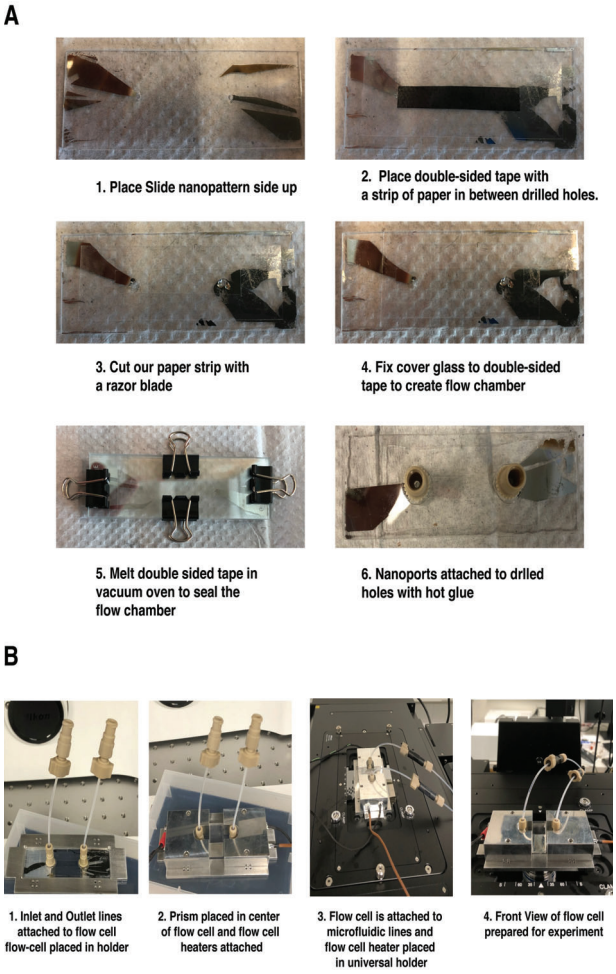

Figure 3: ***Assembly of flow cell for DNA imaging (A)****. Assembly steps for a flow cell.* ***(B)****. Assembly of a flow cell on the microscope. This figure is from citation E.*

References:

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