

P15 – Single Molecule Studies of Bacterial DNA Remodelling Proteins


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In bacteria, the remodelling of DNA by proteins (often termed nucleoid-associated proteins (NAPs)), plays a major role in the compaction, replication and ultimately the expression of genetic material within cells. Whilst the NAPs from *E. coli* (a model Gram negative bacteria) have been widely studied (1, 2) and their interactions largely understood (3-5), our understanding of comparative proteins in other organisms including those in *B. Subtilis* (a model Gram negative bacteria) remains unclear. Recently, primosomal proteins, DnaD and DnaB from *B. subtilis* have been shown partly by AFM to exhibit novel and opposing DNA remodelling activities (6-8) that are essential to initial DNA replication. The C-domain of DnaD (Cd) appears to bind both supercoiled and linear DNA to form open scaffolds, whereas DnaB laterally compacts the DNA (8, 9). The complexity and putative roles of DnaD and DnaB has limited their detailed study in conventional biological assays; resulting in studies of their interactions with DNA using biophysical techniques such as AFM imaging and single molecule force measurements. Currently, the effects of DNA remodelling upon DnaB binding are being studied by single molecule force pulling experiments. Also, biological cloning techniques are being used to obtain hybrid constructs of DnaD and DnaB by domain swapping to better understand the precise roles of these individual proteins. Further work aims to provide fundamental insights into the remodelling of DNA by such proteins and improve our understanding of processes involved in Gram positive bacterial replication with the hope to identifying new antibacterial drug targets to combat medically relevant Gram positive bacterial infections.

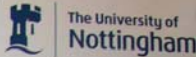
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Single molecule studies of bacterial DNA remodelling proteins

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Introduction

In bacteria, the remodelling of DNA by DNA remodelling proteins and nucleoid-associated proteins (NAPs) plays a major role in processes such as DNA replication and ultimately the expression of genetic material within cells. Whilst the NAPs from *E. coli* (model Gram negative bacteria) have been widely studied (1, 2) and interactions largely understood (3, 4), the understanding of comparative proteins in *B. subtilis* (model Gram positive bacteria) remains unclear. Many important disease causing bacteria are gram positive including *Staphylococci* and *Clostridia*; improving our understanding of processes involved in Gram positive bacterial replication may in the long term help to develop new antibacterial drug targets. Recently DnaD and DnaB primosomal proteins from *B. subtilis* have been shown partly by AFM to exhibit novel, opposing DNA remodelling activities that are essential to initial DNA replication.

Current model for DNA remodelling by DnaD. DnaD is shown (see schematic to the right) as a long Nd domain (green) and a DNA-interacting Cd domain (red). DnaD forms a dimer and at higher concentrations forms filaments and scaffolds via Nd-mediated interactions. Cd binds to DNA and a conformational change (Cd*) shown in yellow reveals a second DNA-dependent oligomerization interface on Cd, causing an accumulation of DnaD molecules on the DNA forming large foci (from ref.5).

DnaB compacts supercoiled DNA laterally. At higher concentrations (2 mg/ml) DnaB causes complete lateral compaction of pBR322 with large protein foci of variable sizes forming along the DNA (see AFM images above). The two duplexes wrapped around each other are clearly indicated by the arrows (from ref.6).

Aims of current study

To obtain biophysical data to help elucidate the precise roles of DnaD and DnaB. Further, to better understand their roles on DNA remodelling at the molecular level.

Biological cloning of hybrid proteins

Two hybrid proteins of DnaD and DnaB were constructed by 'domain swapping' (see figure 1) to investigate in detail the precise roles of DnaD and DnaB.

Controls to DnaB remodeling of Oct4 DNA

The Oct4 (also known as Oct3) is a member of the Pit-Oct-Unc (POU) family of transcription factors which controls gene expression (7). Here, the promoter is amplified from pOCT3-EGFP1 (gift from Dr W. Cui, Imperial College, London) to give a 4,040bp (1.37µm), linear dsDNA sequence and is used for studying DnaB. One end of the DNA is biotinylated and the other end is thiolated for single molecule pulling experiments.

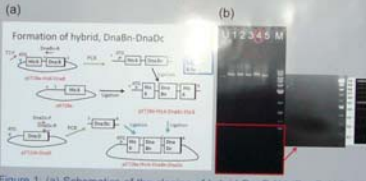


Figure 1: (a) Schematics of the cloning of hybrid DnaD Na (DnaDn) DnaB Cd (DnaBc) in pET28a. (b) 1.2% agarose gel showing undigested (U) and 1-6 colonies of pET28a-BnDc digested by Bam HI and Sac I (expected insert size ~360bp). Circled colony was DNA sequenced and contained the insert.

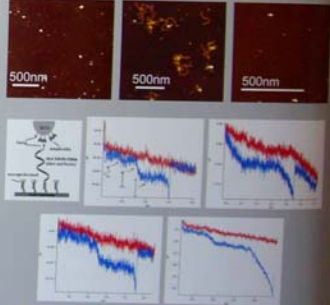


Figure 3: (a) Typical TM-AFM images of DNA containing Oct4 promoter. (b) Schematic of the DNA force experiment and preliminary force-distance curves obtained.

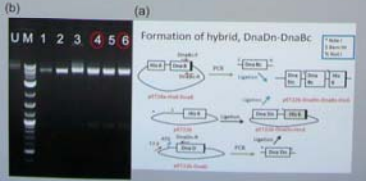


Figure 2: (a) Schematics of the cloning of hybrid DnaD Na (DnaDn) DnaB Cd (DnaBc) in pET28a. (b) 1.2% agarose gel showing undigested (U) and 1-6 colonies of pET28a-BnDc digested by Not I and Not I (insert size ~1kbp). Circled colonies were DNA sequenced to contain the correct insert.

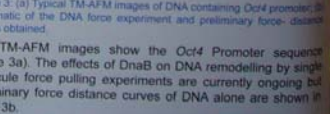




Figure 3: Schematic of the DNA force experiment and preliminary force-distance curves obtained.



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