Nucleic acid end labels for single molecule atomic force microscopy of DNA:RNA polymerase complexes

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Introduction

The visualisation of DNA-protein complexes by atomic force microscopy (AFM) provides insight into the interactions of proteins with the DNA on a single molecule basis. In the case of *E. Coli* RNA polymerase holoenzyme (RNAP) it is possible to investigate the interactions that occur on DNA templates containing multiple promoter elements. The outcomes of such interactions hold importance in the understanding of naturally occurring elements, such as nested genes.

In the study of DNA-protein complexes, however, AFM is typically not able to distinguish the polarity of the DNA. This limits the ability to study the interactions of more than one globular protein, particularly those similar in size, on the same individual DNA molecule. In studying more than one RNAP transcribing DNA molecules we need to know in which direction the molecules to infer outcomes of transcriptional interference events, which have implications for gene expression. To overcome this problem, the DNA molecules require a polarity label that can be morphologically identified in the AFM but does not significantly affect the system behaviour. Many labels for high resolution microscopy of DNA are protein based or consist of relatively large bulky materials, which can affect AFM sample preparation, as well as being morphologically indistinguishable from other proteins such as RNAP.

A new nucleic acid end-labelling approach

With this is mind, we developed a non-replicative PCR reaction to incorporate a single stranded DNA loop consisting of poly(A) sequence to one end of the DNA template. This loop was clearly distinguishable from the DNA template and RNAP by the AFM (see Figure 1). We also discovered that it had the added advantage for RNAP studies: it stalled an active transcribing RNAP at the end of the template without allowing it to release from the DNA. This enables us to investigate interactions and collision events between two or more RNAPs on single DNA molecules in aligned or convergent configurations.

The non-replicative nature of our initial method meant that recovery of labelled DNA and the efficiency of labelling was low. We have now further extended this approach and designed a new replicative PCR method for the addition of a single stranded DNA end loop label with high efficiency and yield. This method was used to incorporate homo-polynucleotide loops consisting of one of the four DNA bases (A, C, G or T) to the end of double-stranded DNA templates ~620 bp long.

The loop structure was observed with an occurrence greater than 70% for all four of the loop sequences that were incorporated. AFM also confirmed that the DNA recovered after the PCR reaction was the correct fragment through contour length measurements which showed an average length of 207.9±0.7 nm, which gave a base pair rise of 3.3nm, which is in the expected range for B-form DNA. Upon formation of open promoter complexes the loop was easily distinguishable from the RNAP by height and diameter as can be seen in Figure 1.

After transcription elongation approximately 90% of molecules had a RNAP molecule attached to the loop labelled end of the DNA template. This indicates that the loop structure was able to inhibit dissociation of the RNAP after transcription and so therefore inhibit re-association with the promoter.

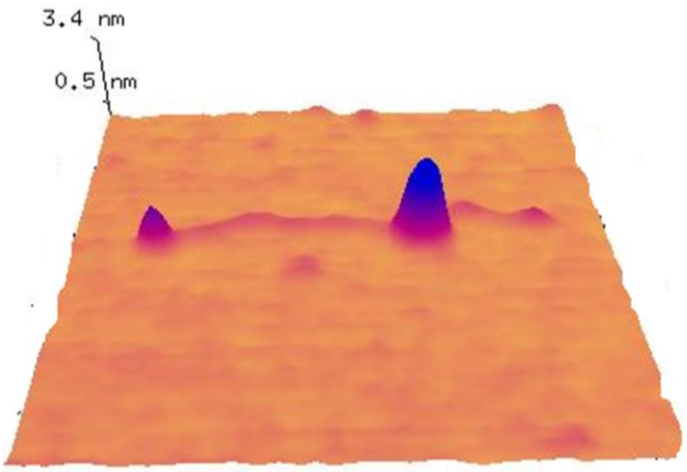

Figure 1: an *E. coli* RNA polymerase open promoter complex (middle) on a 620bp dsDNA template with a 20bp ssDNA end loop label on the left-hand side.

This nucleic acid loop labelling method is allowing us to use AFM to understand more about the fundamental interactions involved between polymerase during transcription. Moreover, it can be applied to the study of many DNA-protein interactions in the AFM, by acting as a fiducial marker.

Publications

Billingsley, D., Bonass, W., Crampton, N., Kirkham, J. & Thomson, N. (2012) Single-molecule studies of DNA transcription using atomic force microscopy. *Phys. Biol.* **9:** 021001.

Billingsley, D., Crampton, N., Kirkham, J., Thomson, N. & Bonass, W. (2012) Single-stranded loops as end-label polarity markers for double-stranded linear DNA templates in atomic force microscopy. *Nucleic Acids Res.* **40:** e99.

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