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**Testing the DNA loop domain model in
*Escherichia coli***

A thesis submitted for the degree of Doctor of Philosophy

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Summary

The ability of DNA to form loops has been employed by evolution in almost every aspect of biology involving DNA, not least the regulation of gene transcription. The biophysical constraints on looping of the DNA polymer at short range (< 300 bp) have been extensively studied, however it is uncertain how the probability of DNA looping decays at longer range. The first part of this thesis presents a quantitative investigation of long range DNA looping both *in vivo* in *E. coli* and *in vitro*. DNA looping is more efficient *in vivo* than measured *in vitro* (by our collaborators) with the technique of Tethered Particle Motion (TPM), and we suggest that DNA supercoiling aids DNA looping *in vivo*. By measuring long-range looping *in vivo* using the two well-characterised looping proteins (the LacI and λ CI repressors) and thermodynamic models of DNA looping, the decay in looping probability is quantified over the range 242–10000 bp. Furthermore this decay is shown to be a property of the DNA tether linking the loop, independent of the nature of the DNA looping protein(s).

Enhancers activate genes at long distance irrespective of position and orientation, so why don't enhancers activate the wrong genes? In other words, what mechanisms drive efficiency and specificity in enhancer-promoter looping? The loop domain model proposes that DNA loops formed by insulators pose a topological barrier that restricts the reach of enhancers to the vicinity of desired target promoter(s). Specifically, the model predicts that two DNA loops in an alternating arrangement should form somewhat mutually exclusively (i.e. they should interfere with one another's formation), whereas nested DNA loops are predicted to assist one another's formation, and side-by-side loops should form independently. In the second part of this thesis, the loop domain model is tested in *E. coli* by combining LacI and λ CI-mediated DNA loops in these different orientations. Accordingly, we quantify DNA looping assistance and interference by fitting experimental data to a statistical-mechanical model, confirming the predictions of the loop domain model. Furthermore, TPM measurements of the same looping constructs support predictions that non-supercoiled DNA *in vitro* should facilitate DNA looping assistance, but not interference. In addition to confirming the loop domain model in *E. coli*, this thesis provides a strong experimental and theoretical foundation for further investigations of enhancer-promoter looping in prokaryotes and eukaryotes, and the relationship between chromatin architecture and gene expression in metazoans.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to David Priest and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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