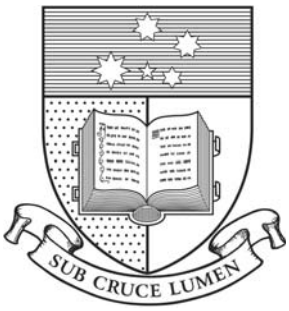

The University of Adelaide
School of Molecular & Biomedical Science
Discipline of Biochemistry

Testing the DNA loop domain model in *Escherichia coli*

A thesis submitted for the degree of Doctor of Philosophy

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Thursday 4th September, 2014

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 λCI 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 λCI-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

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Signed Date

Acknowledgements

I would like to sincerely thank my supervisors Dr Keith Shearwin and Dr Ian Dodd for their years of generous mentorship and support. Two incredibly intelligent, skilled, passionate and kind individuals who demonstrate, every day, why it's worth pursuing this seemingly crazy, yet intensely challenging and rewarding career choice of molecular biological research and teaching. I thank them for giving me the opportunity to work on a project that was so well-designed from the outset. I take with me their passion for discovery and unique perspective on biological systems and look forward to future collaborations.

I would also like to thank the other members of the Shearwin lab for their friendship and willingness to lend a hand. Thanks Iain Murchland, Julian Pietsch, Cui Lun, Nan Hao, Alex Ahlgren-Berg and the many other members I've seen come and go during my 9 year stint!

Thanks to all those within the Biochemistry Discipline and School who work hard to provide such a good learning and teaching environment to which I would happily return in the future.

Thanks Mum and Dad. I would have never been able to make it this far without your unwavering support.

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