The environmentally-regulated interplay between local three-dimensional chromatin architecture and gene expression

Rashid, F.Z.M.

Summary

When an *Escherichia coli* cell pellet is lysed in a final volume up to 50 times that of the pellet, it produces a solution so viscous that jelly-like droplets can be picked with a pair of tweezers. It is fascinating to envision that this viscous material, concentrated by a factor of 50, is encapsulated within a membrane, and that this capsule is alive. The blueprint of its life is contained within a circular genome 1.5 mm in length that is folded by nucleoid associated proteins (NAPs) and packaged into a phase-separated structure with the cell. The cell faces a delicate challenge: the blueprint of its life must remain compactly packaged to facilitate its segregation into daughter cells prior to cell division, and, at the same time, every locus must remain accessible for the cell to rapidly respond to favourable and stressful environmental stimuli. The NAPs that address this challenge must, therefore, have an architectural property to compact the genome, and be environmentally-sensitive to facilitate genomic response to stimuli. Advances in fluorescence microscopy, chromosome conformation capture (3C)-based techniques, and *in vitro* genome-in-a-box approaches have begun to shed light on the principles of chromosome organisation and dynamics. In this thesis, single-cell fluorescence microscopy, ensemble reverse transcriptase quantitative PCR (RT-qPCR) and 3C-qPCR, and single-molecule Förster resonance energy transfer (smFRET) have been used to provide insights into the intriguing interplay between three-dimensional chromatin structure and gene expression. An experimentally-supported model that consolidates the architectural properties and regulatory functions of NAPs has also been described.

An up-to-date review on the interplay between chromosome organisation and genomic transactions including replication, transcription, and segregation in bacteria has been provided in **Chapter 1**. Chromosome folding and compaction at a molecular level mediated by NAPs, and the modulation of the structure as a result of cross-talk between NAPs, and proteins that regulate NAP function has been described. The hierarchical organisation of bacterial chromosomes has also been addressed in **Chapter 1** and an overview of the impact of architectural NAPs and genome transactions on local and global patterns of chromosome organisation, and how this may be affected by environmental conditions has been provided.

The three-dimensional structure of the prokaryotic chromosome is encoded in its sequence as binding sites of varying strength for the diversity of NAPs expressed by the cell, and as the positions of transcriptional units and associated regulatory elements. This compels that chromosome architecture be studied in the context of

genomic sequence. Hi-C enables it. Hi-C is a chromosome conformation capture based technique that combines proximity ligation with next generation sequencing to detect the physical proximity of chromosomal loci in three-dimensional space. A detailed protocol for Hi-C in prokaryotes has been provided in **Chapter 2**.

In **Chapter 3** the H-NS-regulated, osmosensitive *proVWX* operon of *Escherichia coli* has been used as a model system to demonstrate, for the first time in prokaryotes, that the expression of a transcriptional unit is correlated with its three-dimensional architecture. RT-qPCR was used to report on the transcriptional profile of *proVWX* and verify the roles of H-NS and StpA in regulating the expression of the operon. 3C-qPCR, and live cell FROS were used to show the formation of a loop anchored between the promoter and terminator of *proVWX* under conditions at which the operon is repressed, and the destabilisation of the loop at osmolarity conditions that activate *proVWX*. The results that have been presented in **Chapter 3** also reveal that StpA, often considered a molecular back-up for H-NS, plays physiological roles in a wild-type cell that are distinct from H-NS.

The dynamic organisation of the chromosome and its association with genome processes creates a need for visualizing the genome in a non-perturbing manner. In **Chapter 4**, HI-NESS (H-NS-based indicator for nucleic acid stainings) – a minimally-perturbing DNA labelling dye designed by translationally fusing the DNA binding domain of H-NS to a fluorescent protein – has been described. HI-NESS labels the chromosome in *Escherichia coli* cells that lack H-NS, eukaryotic cells in culture, and in zebrafish embryos.

How the work reported in this thesis forms a foundation for investigating chromosome organisation and dynamics in other bacteria, and in archaea – a domain that is generally regarded as the missing link between bacteria and eukaryotes – has been discussed in **Chapter 5**. In the chapter, a model of how the interplay between chromosome structure and genome transactions may have driven evolution has been proposed.