English Abstract

Bacteria, prokaryotes, are microscopic organisms with extremely long genomes, relative to their cell size. The bacterium *Escherichia coli* is on average 2 µm long, whereas its genome is ~1.5-2 mm, requiring immense condensation to fit inside the confines of this bacterial cell. Such condensation requires strict coordination in order to avoid detrimental effects on growth and survival. *Escherichia coli* K-12 has approximately 4.200 genes which is twice the number of RNA polymerases (RNAP) present, which are required for utilization of the genes present in the cell. This condensation and gene/RNAP ratio require the cell to structure the nucleoid in a way that optimizes RNAP usage.

Danish research has previously shown a relation between the specific genomic position of a gene and the level of expression. Studies has further observed a relation between the genomic position of genes and coordination of gene-expression. These observations suggest that the genome, at least for *Escherichia coli*, is organized in an evolutionary optimized manner. While much work has focused on the role of transcriptional regulatory networks as well as operons in the control of prokaryotic gene expression, there is only scarce knowledge about how the actual arrangement of genes in the three-dimensional structure of the genome influences their expression.

In this study we investigate how the spatial structure of the bacterial genome may impact the coordinated expression of genes by bringing them into spatial proximity. **Part 1** consists of an introduction to the aims of the project and a thorough review of existing literature on the subject.

In **part 2** we present our findings of regular sinusoidal patterns in correlation of pairwise expression and genomic position of genes. We exclude common cellular mechanisms for co-expression. We hypothesise that transcriptional active genes lead to the transcription of spatial proximal genes through recruitment and diffusion of RNAP, termed transcriptional spilling, as a possible mechanism explaining the observed patterns. We support the hypothesis by observing a relation between DNA-DNA interaction of gene pairs and the correlation in pairwise expression.

In **part 3** we couple transcriptional spilling to the observation of RNAP poised at various genomic positions, without associated expression, during optimal growth conditions. These poised RNAP (pRNAP) are hypothesised to be important for quick adaption and regulation of gene expression upon facing changing environments and might possibly provide three-dimensional reservoirs for expression through transcriptional spilling in prokaryotes. We support this hypothesis by investigating the spatiotemporal pRNAP distribution across the genome relative to changing growth conditions. We observe an increase in percentual poising of RNAP according to levels of environmental stress during different growth phases. We further observe a spatial organisation into different groups and observe an increase in correlation of pairwise expression for genes in spatial proximity to these pRNAP reservoirs.

In **part 4** we discuss the possible impact of transcriptional spilling and pRNAP on basic research into evolution as well as for the biotechnical industry, which utilize synthetic biology in production. If transcriptional spilling is prominent, it will underline the importance of spatial genome structure for evolution of microorganisms. In addition, it may explain the conservation of pseudo and phantom-genes, and the low transferability of highly expressed genes, since these will have an impact on gene regulation in a spatially organised genome. With regards to the industry, a model for context dependent gene expression may prove highly useful in the optimization of production yield from genetically modified microorganisms.