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

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Chapter 5:
Outlook

Chromosome architecture is closely intertwined with gene transcription. In *Caulobacter crescentus* and *Bacillus subtilis*, the boundaries between self-interacting chromosome interaction domains (CIDs) are mediated by highly-transcribed genes (1, 2). In preliminary studies, the chromosome contact map of *Escherichia coli* treated with Rifampicin to limit transcription shows a loss of chromosome architecture (Figure 5.1). A similar change was also observed for the euryarchaeum *Haloferax volcanii* following treatment with the transcription inhibitory drug actinomycin D (3). In addition, the first chromosome contact maps generated for *Haloquadratum walsbyi*, a slow growing archaeal species that populates hypersaline lakes (4–6) and has low levels of transcription (7), show limited chromosome structure (Figure 5.2).

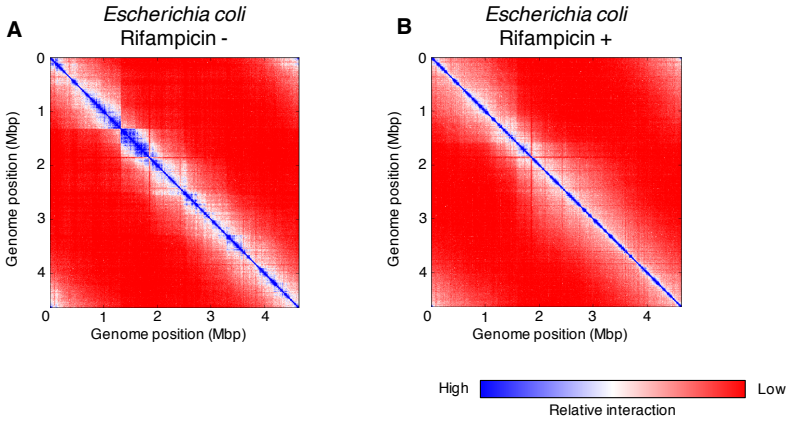

Figure 5.1: Global chromosome organisation in *Escherichia coli* is dependent on active transcription. The chromosome contact profiles of exponentially growing *E. coli* **(A)** show a loss of three-dimensional chromosome architecture upon treatment with Rifampicin **(B)** – a drug that inhibits transcription. **Organism:** *Escherichia coli* MG1655 Δ*endA* (NT331); **3C-based study:** Hi-C (8); **Resolution:** 10 kb; **Growth conditions: Panel A:** LB medium, 37 ºC, exponential phase, **Panel B:** LB medium, 37 ºC, exponential phase followed by a 2-hour long treatment with 100 µg/mL Rifampicin; **Fixation conditions:** 80% cold methanol for 10 minutes followed by 3% formaldehyde for 1 hour; **Restriction enzyme:** PsuI (ThermoFisher Scientific); **Fractionation:** Yes.

The work presented in this thesis builds on the observations of a global interplay between chromosome structure and transcription. It provides evidence of an interplay between local three-dimensional chromosome architecture and gene expression using the *proVWX* operon of *Escherichia coli* that encodes the ProU osmoprotectant transporter as a model system. At *proVWX*, a bridge between the σ^{70}-dependent promoter of the operon and the terminator is associated with a repressed operon. The disassembly of the bridge in response to hyperosmotic stress correlates with an active operon (Chapter 3, this thesis). *proVWX* encodes an ABC transporter (9). ABC transporters form part of the largest group of paralogous

operons/genes that evolved before the divide between archaea and bacteria (10, 11). Evidently, ABC transporters are prevalent in archaea, bacteria, and eukaryotes (11). The model of the interplay between the chromosome structure and gene expression presented for *proVWX* may, therefore, provide clues to potentially conserved regulatory features of several operons and genes across all domains of life. The prevalence of ABC transporters makes the operons and genes that encode these structures an ideal model system to study the evolution of the interplay between local chromatin structure and gene expression.

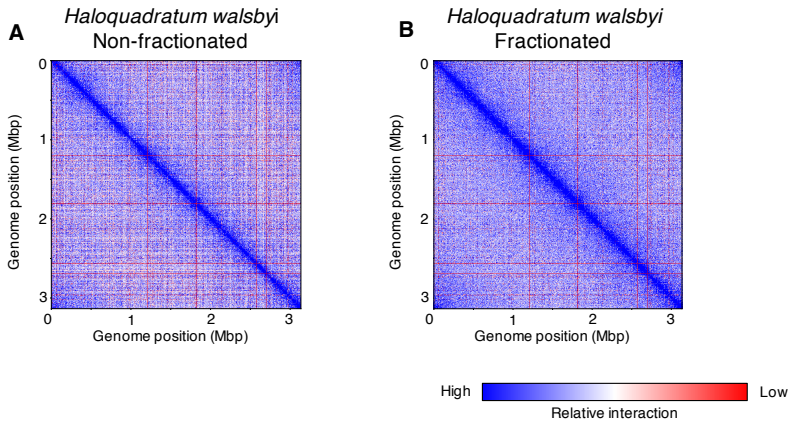

Figure 5.2: The chromosome contact profiles of *Haloquadratum walsbyi*, a slow-growing archaeon with reduced transcription shows limited chromosome architecture. Organism: *Haloquadratum walsbyi* HBSQ001; **3C-based study:** Hi-C; **Resolution:** 10 kb; **Growth conditions:** 37 ºC, stationary phase, standing culture; **Fixation conditions:** 5% formaldehyde, 25 ºC, 3 hours; **Restriction enzyme:** PsuI (ThermoFisher Scientific); **Fractionation: Panel A:** No, **Panel B:** Yes.

ProVWX orthologs are encoded in the genomes of *Salmonella enterica* serovars and *Shigella* species, the latter of which are evolved from *Escherichia* (12). The three genera share identity in the *proVWX* nucleotide sequence, encode H-NS, and exhibit an activation of *proVWX* in response to hyperosmotic stress (13–15). However, *Salmonellae*, in particular, differ in the expression of the operon. Due to limited studies, a similar statement cannot be made for *Shigella*, yet. Indeed, in *Salmonella enterica* serovar Typhimurium, relative expression decreases across the operon from *proV* to *proW* and to *proX* (16), in contrast to the transcriptional profile across *E. coli proVWX* (Chapter 3, this thesis). In *Salmonella enterica* serovar Typhi, a -1 frameshift in *proV* has rendered ProV non-functional (17), nevertheless, the compensation of the absence of ProV by its paralogues in Δ*proV* mutants of *Shigella sonnei* (14) hints that the transport of osmoprotectants by the ProWX in *Salmonella* Typhi may not be entirely abolished. *Salmonella* Typhi could,

therefore, carry a partially cryptic *proVWX* operon. The detailed transcriptional profile of *proVWX*, and the three-dimensional structure of the operon at various osmotic stress conditions in *Salmonella* and *Shigella* – as described in this thesis for *Escherichia coli* – remains to be investigated. Such a study is expected to provide a starting point to highlight principles of H-NS-mediated structural regulation of osmosensitive operons. The ATP-binding cassette (the ATPase) is the most evolutionarily conserved of the ATPase, transmembrane transporter, and substrate binding subunits of ABC transporters (18). A bioinformatic survey of the cross-domain distribution of the ProV ATPase can be used to assemble a phylogenetic tree of the distribution of ProV and ProVWX transporter orthologs. Such a survey will reveal targets to systematically investigate the structural regulation of *proVWX* in organisms that may or may not carry H-NS-like proteins – the integral regulatory NAP of the operon (19–22). The study may lead to the discovery of other architectural transcription regulators that directly detect and respond to osmotic stress.

Salmonella enterica encodes three osmoprotectant transporters: ProP (23), ProU (15), and OsmU (24). ProP is a proton symporter of the major facilitator superfamily of permeases (25). ProU and OsmU are ABC transporters (9, 24). In contrast to *proVWX*, the operon encoding OsmU is arranged as *osmYXWV*, where *osmV* encodes the ATPase, *osmW* and *osmY* encode the polypeptides for the heterodimeric transmembrane transporter, and *osmX* encodes the periplasmic substrate binding protein (24). A functional *osmYXWV* system allows *Salmonella* to survive hyperosmotic stress in a *proP- proVWX-* background, following a lag phase that may last up to 12 hours (24). The *osmYXWV* operon triggers interest owing to its conservation in several species of the *Enterobacteriaceae* family at a nucleotide level and members of the phylum Euryarchaeota at a translational level (24). Furthermore, *Chromobacterium violaceum* of the *Neisseriaceae* also carries an *osmYXWV* ortholog that shares nucleotide sequence similarity with the *osmYXWV* of *Enterobacteriaceae* (24). However, the operon occurs in a chromosomal region that shares no similarity with the genetic neighbourhood of *osmYXWV* in *Enterobacteriaceae*, indicating that the operon may have been acquired by horizontal transfer in *C. violaceum* (24). A comparison of the transcriptional (RNA-Sequencing, and RT-qPCR), structural (3C-based studies), and NAP binding (Chromatin immunoprecipitation) profiles of archaeal and bacterial *osmYXWV* operons will reveal conserved features of the structural regulation of genes/operons during osmotic stress response.

The interdependency of chromosome structure and gene expression may play an evolutionary role by providing a means of 'sampling' repressed regions of the chromosome during replication. The progress of the replication machinery over the chromosome requires local dismantling of the template chromatin and its reassembly on replicated DNA. This may give rise to a time frame during which the repressed promoter of a cryptic gene or that of a horizontally transferred gene is left unattended. This time frame may be affected by stress, for instance, during the stringent response – a condition that affects replication, transcription, and metabolism (Reviewed in (26)). The binding of RNA Polymerase to such a promoter, and the expression of the repressed unit allows the cell to 'sample' cryptic or horizontally transferred genes. Genes that provide an advantageous characteristic may undergo gradual de-silencing and become incorporated into the transcriptome and proteome. A cross-domain, multi-species study of active and cryptic ABC operons and genes will provide clues to the validity of this hypothesis.

The *Pyrococci* of the Euryarchaeota encode cellobiose/β-glucoside ABC transporters (27–30). The gene cluster is upregulated in *Pyrococcus furiosus* growing in the presence of cellobiose in a medium with limited carbohydrate availability (30). However, in *P. abyssi* and *P. horikoshii* the cellobiose/β-glucoside ABC transporter cluster is not activated under similar conditions, and evidently, the cells are unable to grow in carbohydrate limiting conditions with cellobiose (30). A comparison of the nucleotide sequences, NAP binding profiles, and local three-dimensional architecture of active, cryptic, encrypted (active gene clusters inactivated by mutagenesis), and decrypted (inactive gene clusters activated by mutagenesis) cellobiose/β-glucoside ABC transporter clusters of the *Pyrococci* will enable the investigation of 'gene sampling' in comparable genetic backgrounds. A phylogenetic study of the distribution of orthologous cellobiose/β-glucoside uptake systems and the identification of cryptic and active clusters will allow a step-wise investigation of the role of local chromosome structure in evolution.

In addition to the silencing of transcriptional units mediated by local repressive chromatin, the higher order structure of chromatin assembled from the concerted binding of multiple NAPs may play an additional evolutionary role. NAPs are environmental sensors. The proteins detect and respond to changes in osmolarity, temperature, and pH by modifying their architectural properties, effectively altering three-dimensional chromosome architecture and, hence, regulating genome transactions (31–33). The binding of a diversity of NAPs along the genome and their combined function enables each segment of the chromosome to

respond differently to environmental stimuli. Therefore, the higher-order chromatin structure in the neighbourhood of a transcriptional unit may place a bias on its evolution. For instance, genes encoding elements that alleviate osmotic stress may preferentially be activated when positioned within osmoresponsive chromatin, but may remain cryptic when positioned at pH-sensitive sites. A database of paralogs and orthologs of osmoprotectant ABC transporter operons/genes such as the *proVWX* and *osmYXWV* in which the entries are sorted according to their cryptic/active state, and predicted chromatin environment deduced from NAP binding profiles and transcriptional profiles, will enable validation of this hypothesis.

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