Dynamic organization of bacterial chromatin by DNA bridging proteins

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Chapter 7

Summary and Prospects

Bacteria often experience external challenges, such as changes in environmental conditions or attacks by bacteriophages. To cope with these challenges, bacteria need to be able to adapt quickly to the challenges. Key to the survival of bacteria is to be able to adapt to environmental stresses, to acquire new genetic characteristics through horizontal gene transfer to remain competitive and to silence these foreign genes as long as they do not provide any benefits. These mechanisms of adaptation are controlled via the structure of the bacterial genome, which is dynamically compacted and organized.

The Histone-like nucleoid structuring (H-NS) protein is a key regulator of the dynamic bacterial genome. The protein is conserved among enterobacteria and plays a determinant role in the architecture of their nucleoid acting as a global genome organizer and gene regulator. H-NS generally acts as repressor of transcription, silencing the expression of many genes and operons. The gene organization and silencing mechanism of H-NS is determined by its ability to bind and spread across genes. Characteristic of H-NS is the formation of nucleofilaments along the DNA and protein-mediated DNA-DNA bridges. Functional analogs of H-NS have been found in other bacterial species: MvaT in *Pseudomonas* species, Lsr2 in actinomycetes and Rok in *Bacillus* species. These proteins complement *hns*$^-$ phenotypes and have similar DNA-binding properties, despite their lack of sequence homology. These H-NS like proteins are widely distributed in the genome and prefer to bind and repress AT rich DNA acquired through horizontal gene transfer, which makes them bacterial xenogeneic silencers. Structurally, they have overall similar domain organization: the N-terminal domain functions in dimerization and oligomerization, the C-terminal domain binds DNA and the two are connected by a flexible linker. Attributed to their delicate domain arrangement, the four proteins are all capable of bridging DNA duplexes, which is believed to be crucial in gene silencing. The structural and functional characteristics of these four architectural proteins (H-NS, MvaT, Lsr2 and Rok) are summarized in **Chapter 1**.

Stress-responsive regulatory proteins in bacteria, particularly H-NS like proteins, function as gene silencers and as sensors of environmental changes. The bacterial genome operates as an information processing machine, translating environmental cues into altered transcription of specific genes required for adaptation and survival. Key to this process is the dynamic organization of the bacterial genome driven by such cues. The genes targeted by H-NS family proteins are

often regulated by environmental factors such as osmolarity, pH and temperature. H-NS proteins are able to bridge DNA duplexes, which is key to gene regulation and their responding to changing conditions in the environment. Divalent ions have been shown to be able to drive the transition between lateral H-NS-DNA filaments and bridged DNA-H-NS-DNA complexes. This switching between the two types of complexes is believed to be the mechanistic basis of the role of H-NS proteins in bacterial nucleoid organization and transcription regulation. Earlier Molecular Dynamics simulation studies of H-NS, indicated that the switch between the two DNA binding modes involves a change from a half-open (also referred as a closed conformation) to an open conformation driven by $MgCl_2$. However, the molecular basis that governs this phenomenon remains poorly elucidated. In **Chapter 2,** we used an abridged structural version of H-NS, MvaT from *P. aeruginosa*, in which we scrutinized its protomer structural/function relationship in response to changes in the surrounding ionic strength. We have combined integrative structural biology methods and biochemical assays to decipher the structural changes in MvaT that drive the switch between its DNA stiffening and bridging activities under different salt conditions. These structural changes appear to be conserved within the H-NS family of proteins: analysis of the primary sequences of H-NS family members revealed conserved positions of charged residues despite the low sequence identity. This suggests that their osmosensitivity is mediated by the modulation of the electrostatic interactions between their N-terminal and DNA binding domains within their nucleoprotein filaments.

Bacteriophages are among the most abundant and diverse organisms on planet, found wherever bacteria exist, and they represent a constant challenge to bacteria. Bacteria and their associated bacteriophages co-evolve in a continuous battle, developing defensive and offensive mechanisms. The interaction and co-evolution between a bacteriophage and its bacterial host play a key role in the ecological and evolutionary processes of microbial communities. Successful bacteriophage infection requires coping with bacterial resistance systems. Bacterial xenogeneic silencers, H-NS family proteins, play important roles in bacterial phage defense and evolution by silencing incoming genes and genes acquired through horizontal gene transfer. To mitigate the effects of such H-NS-like proteins, bacteriophages encode proteins counteracting gene silencing by H-NS-like proteins. The *Pseudomonas* phage LUZ24 encodes the Mip protein which binds the H-NS

family protein MvaT of *Pseudomonas aeruginosa.* Binding of Mip was earlier proposed to inhibit the silencing of phage LUZ24 DNA by MvaT. However, the mechanism by which Mip modulates MvaT function remained unclear. In **Chapter 3**, we investigated how the DNA binding properties of MvaT are affected by Mip. Also, we defined how Mip interacts with MvaT and how this translates into altered DNA structuring properties. Our studies reveal that Mip interferes with the formation and stability of the bridged MvaT-DNA complex. This effect is due to interaction of Mip with both the dimerization and the DNA-binding domain of MvaT. Based on these observations we propose that binding of Mip promotes the half open - bridging incompetent - state of MvaT, resulting in relief of MvaT-mediated gene silencing.

Rok from Bacillus subtilis is an abundant DNA binding protein similar in function to H-NS-like proteins found in many proteobacteria. Rok binds across the genome with a preference for A/T rich DNA. Such DNA often contains genes of foreign origin that are silenced due to Rok binding. Rok also has been implied in global organization of the B. subtilis genome. However, how Rok binds to DNA and how it represses transcription is unclear. Also, it is unknown whether Rok-mediated gene repression can be induced or relieved following changes in physico-chemical conditions, as noted for H-NS-like proteins. In **Chapter 4**, we investigated the DNA binding properties of Rok and determined the effects of physico-chemical conditions on these properties. We demonstrate that Rok is a DNA bridging protein similar to H-NS like proteins from E. coli (H-NS), Pseudomonas sp. (MvaT) and Mycobacteria (Lsr2). Strikingly, unlike these proteins, the ability of Rok to bridge DNA is not affected by changes in physico-chemical conditions. Not being a direct sensor of such changes sets Rok apart from other H-NS like proteins. It implies the existence of other (protein-mediated) mechanisms to relieve Rok-mediated gene silencing in response to changes in environmental conditions.

In **Chapter 5** and **Chapter 6**, we have described two methods well-suited to the analysis of the interaction between H-NS family proteins: a DNA bridging assay and Acoustic Force Spectroscopy (AFS). These techniques are also helpful in understanding how proteins or molecules interfere with the DNA binding functionality of H-NS family proteins. For instance, the bridging assay can be used as a method to identify proteins or molecules that alter the DNA bridging activity of H-NS proteins which is crucial in gene silencing. Single molecule technique, like AFS, Optical Tweezers

and Magnetic Tweezers, can help in studying mechanisms underlying H-NS mediated effects on gene transcription by RNAP.

Prospects

It is a long journey to understand the (dynamic) chromosome organization of bacteria, however, advances in genome-scale approaches over the past few decades have improved our understanding of bacterial genome folding. Bacterial DNA-bridging proteins play important role in genome organization and gene regulation due to their binding at distinct regions throughout the genome. DNA bridging and modulation of DNA bridging seems to be functionally conserved across species. This provides new opportunities to understand how genomes are dynamically organized by comparative functional and evolutionary analyses of functionally conserved DNA-bridging proteins from different species. A key in understanding genome organization and an important current challenge is to better define local changes in DNA folding in response to environmental challenges and to understand how these affect other nucleic acid transactions within living cells. For instance, biophysical and biochemical techniques have identified structures of H-NS proteins interacting with nucleic acids and have shown how these interactions are modulated by environmental changes *in vitro*, but it is unclear how these affect transcription *in vivo*. Furthermore, although we understand how a single nucleoid-associated protein organizes DNA, it is not obvious how the joint efforts of multiple NAPs combine within cells In addition to the environmental conditions and protein partners, post translational modification can also contribute to modulating the DNA binding properties/functions of bacterial nucleoid-associated proteins. This aspect needs more extensive research which would promote our understanding of the dynamic chromosome organization of bacteria.

Proteins from bacteria and bacteriophages have been shown to be able to interfere with the function of H-NS proteins. Such interference can occur at any of the steps towards assembly of the bridged H-NS-DNA complex i.e. during initial DNA binding, multimerization or the stage of DNA bridging. In general, a better understanding of how viral proteins counteract H-NS proteins is important for design of proteins or molecules countering phage infection. Potential applications of such molecules can be envisioned in biotechnology, where phage infections are a threat to large scale culturing, or in phage therapy where they may aid in enhancing the ability to infect and

kill their host. Moreover, due to their global role in gene regulation in bacteria H-NS-like proteins are potential drug targets to mitigate bacterial infections. Understanding at the molecular mechanistic level how H-NS proteins activity is modulated is key to such developments.