The dynamic organization of prokaryotic genomes: DNA bridging and wrapping proteins across the tree of life

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Summary

A skein of cotton yarn with dimensions of approximately 10 by 6 centimeters has a length of 85 meters when completely stretched out. Yarn can be organized in a skein, hank, ball or cake to remain untangled and ready to use for the purpose at hand. In a similar way, the length of prokaryotic genomic DNA (in the order of 2 millimeters) far exceeds the volume of a prokaryotic cell (1-2 by 5-10 micrometers), requiring an even larger degree of compaction while keeping the biological information stored in DNA accessible. Several strategies have evolved to achieve this, one of them being the expression of proteins that bind and organize the DNA. The best-known examples are the histone proteins of eukaryotes, but many other proteins have the same function. They are collectively referred to as nucleoid-associated proteins (NAPs). There are multiple ways in which histones and NAPs can organize DNA. In Chapter 1 we discuss the different possibilities: DNA wrapping, DNA bridging, DNA bending and nucleofilament formation. In each domain of life examples of (nearly) all categories can be found, although the proteins involved are often unrelated. This could be an example of convergent evolution: independent evolution of different proteins that fulfill the same function.

In the model bacterium *Escherichia coli* at least 12 NAPs have been described. The histone-like nucleoid structuring protein (H-NS) organizes the genome and has an effect on the expression of 5-10% of *E. coli*'s genes. H-NS is an example of both a DNA bridging and a nucleofilament forming protein. Several factors play a role in determining which structure is formed by H-NS, such as environmental conditions, protein interaction partners and post translational modifications. In Chapter 2, we review the functional and structural properties of H-NS and its (functional) homologues in other bacteria. We propose that charge distribution in an important characteristic to predict if an H-NS-like protein will react to changes in the environment. If the charge distribution is asymmetrical, and the protein has a certain level of interna flexibility, this is an indication that osmotic strength can be the switch between nucleofilament formation and DNA bridging.

The NAP Rok from *Bacillus subtilis* is one of the proposed H-NS-like proteins, however its charge distribution is different. Instead of an asymmetrical charge distribution, the charges are more spread out and the middle part of the protein is neutral. In Chapter 3, we show that Rok only forms DNA bridges and does only mildly react to environmental changes. This suggests that these conditions are not as important in the regulation of Rok as for other H-NS-like proteins. In search of a mechanism to perturb Rok-DNA bridges, we investigated a smaller variant of Rok, called sRok. This NAP can form both a nucleofilament along the DNA and DNA-DNA bridges and is sensitive to salt

concentration. In a situation where Rok and sRok are together, they form heterodimers and influence each other's behaviour. These results show us the importance of protein partners in the regulation of Rok and other DNA organizing proteins as well.

The other prokaryotic domain of life, the archaea, has its own, sometimes lineage specific, DNA organizing proteins. Archaea were long regarded the third domain of life, but new models show two domains: the bacterial domain and the archaea-eukaryotes domain where eukaryotes evolved from archaea. This is partly reflected in their DNA binding proteins. In eukaryotes, the well-studied histone proteins are the main DNA organizing proteins. Most archaea also express histones, but they generally lack the tails of eukaryotic histones used for gene regulation. Previously, it was found that archaeal histones can wrap DNA in a continuous, rod-like manner. This 'endless' structure is called a hypernucleosome. Practically, the hypernucleosome can be limited in size by several factors such as histone variants, posttranslational modifications, environmental conditions and specific DNA sequences. In Chapter 4, we examined the effect of an artificial high-affinity DNA sequence on the formation of a hypernucleosome by model archaeal histones HMfA and HMfB. We found that the specific DNA sequence is first bound by a tetramer at low protein concentrations, but that this tetramer does not promote hypernucleosome formation. We propose that this is due to a more closed tetrameric conformation of the histones which is not compatible with hypernucleosome formation. Combined with histone variants that are less likely to form a hypernucleosome but do recognize this sequence, this might be a way to mark the start and end of the hypernucleosome.

Some histone variants do not only consist of a histone fold, variants exist with either an N- or C-terminal tail. In Chapter 5, we investigated MJ1647, a histone with a C-terminal tail from *Methanocaldococcus jannaschii*. We found that it has two DNA binding modes: it can wrap DNA like other archaeal histones, but it can also bridge two DNA strands. In both cases, MJ1647 forms tetramers and the behavior is dependent on the C-terminal tail, which we called the tetramerization domain. Due to steric hindrance caused by the C-terminal tail, MJ1647 cannot form a hypernucleosome. It might however act as a roadblock to stop progression of a hypernucleosome.

Following evolution from archaea towards the eukaryotes in the tree of life brings us at the Asgard archaea as the closest relatives to eukaryotes today. This is not only reflected by their high amount of eukaryotic signature proteins, but also the presence of histones with an N-terminal tail. *Heimdallarchaeota* LC_3 encodes 10 histone proteins of which one has such a tail. In Chapter 6, we attempted to produce the eukaryotic-like histone HA and the archaeal-like histone HB, however we were unsuccessful in our

attempts both in *E. coli* and *Pichia pastoris.* We were able to chemically synthesize HB and study its DNA binding behaviour. In contrast to predictions, HB does not seem to form a hypernucleosome.

The results presented in this thesis show that there is great variation in DNA organizing proteins and that proper DNA organization can be achieved in different ways. In Chapter 7, the broader impact of this research is discussed. Prokaryotes play an important part in today's major challenges such as the rise of antibiotic resistant bacteria and the role of methanogenic archaea in climate change. Therefore, it is important to study how prokaryotes regulate their gene expression and how they change its DNA organization according to environmental changes.