# Molecular and cellular principles of genome folding and genome function

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Prof.dr. R.T. Dame

on the acceptance of his position as professor of

Molecular and Cellular Biochemistry

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*Respected Rector Magnificus, members of the Faculty Board, distinguished colleagues and listeners,*

In this written English summary of my inaugural lecture, I will provide you with a glimpse into my research and describe its relevance for science and society. To this end I will take you on a journey that leads from minor changes only in the structure of a protein to the consequences thereof in terms of the behavior of these bacteria. During this journey we will together make an excursion towards evolution and explore the knowledge that 'simple' single-celled prokaryotic organisms, and simple mechanisms exploited by them, yield on 'complex' eukaryotes, like human. The beginning of this journey is not the protein molecule, but the molecule to which this protein binds, DNA. DNA contains genetic information written in a four letter code consisting of A, T, C and G. These letters stand for the four main different chemical building blocks of DNA. The information contained in DNA is read by the protein RNA polymerase. This protein is responsible for the synthesis of an RNA copy of this information, which in the case of protein coding genes is translated into protein. This process is more elaborate in eukaryotes than in prokaryotes, but the general mechanism is the same. The proteins that I investigate in my group play a role in the organization of DNA as well as in the regulation of processes which take place on the DNA, and by that affect genome function, including regulation of access of RNA polymerase to DNA. I will later return to this point.

For me, personally, the journey starts earlier. As a descendant from a family of artists, where visual and performing arts, were omnipresent, my fascination with chemistry, biology and physics may not seem obvious. My father was a visual artist and also an avid collector and "amateur" researcher fascinated by Nature, life and death, and by cultural history in a very broad sense. He quite disliked the word "amateur" because of its common derogatory connotation; "independent researcher" is perhaps more appropriate. My childhood home was - and is - like a museum, with books, art, collections, biological preparations. This was a particularly beautiful, rich and stimulating environment in which to grow up. Under the influence of my father, and support from my mother, I developed a passion for description, classification and the development of a systematic, in-depth understanding of systems, irrespective of their nature.

Around my tenth birthday I became increasingly engaged with photography, initially for documentation purposes, but later also with its artistic side. That led me naturally into the field of chemistry. I familiarized myself with recipes for the basic chemistry in the field of photography, and developed a strong interest in recipes that were appealing from an artistic perspective. For instance, I was strongly drawn towards chemical recoloring of black and white, metallic silver based, images. This early interest in photography was the motivation to study chemistry in university; I expected to be able to apply my chemical knowledge to the benefit of artistic originality, in a form which I thought I could not develop at an art school. The reality was different: my studies did not bring me closer to artistic photography, but rather distanced me from that. Whereas I did well in my courses of inorganic chemistry, my greatest fascination and passion developed towards biochemistry, in particular application of new and emerging technologies to image molecules, cells and biochemical processes in cells. I was very strongly drawn to visualizing what could not be seen by the naked eye, and had not been seen by others before. I also considered visualization, and the convincing power of the image, a good basis for obtaining a better understanding of biological processes. Later this fascination of the power of image was extended to further techniques and disciplines: anything that for a particular question could bring me closer to an answer. This attitude has allowed me (over the years) to build a broad toolbox spreading across disciplines of chemistry, biology, physics.

The inspiration that I find in various disciplines is at the basis of my research style. In my approach to research I am strongly

question-driven and I do not feel limited by disciplines and methods. This approach has developed under the influence of my advisors as junior researcher, during my PhD and post-doctoral studies; the focus in their groups was on the research question, and if required, the methods for the question to be answered were developed. This approach resonated with the research community consisting of microbiologists, geneticists and biochemists: we were able to find answers to longstanding open questions in the field, naturally leading to new and compelling questions. In the biophysical community we were in a fortunate position to have in house beautiful biological systems which we could interrogate under biologically relevant conditions. Over the last decade I have - together with close colleagues, 'scientific friends' - actively pushed trans-disciplinary interactions through conferences and workshops. The deeper aim for this is to build an integrated research community and establish collaborations to define and address important research questions. Doing research together advances the field at a faster pace. Based on my own interests I have been able to generate bridges between disciplines and research fields. The composition of my own research group is reflective of this interdisciplinary approach, with people having diverse backgrounds and expertise. Collaboration in such environment is not always easy as by background different "languages" are spoken. Importantly, my group members learn to build bridges allowing them to communicate efficiently. In my view openness to different views and learning to benefit from diversity are very important and useful skills for careers both inside and outside the academy. My multidisciplinary and collaborative approach to science is also reflected in my teaching efforts for undergraduate students in biochemistry, molecular biology, biophysics. I emphasize the relevance of quantitative aspects and the importance of exploitation of complementary experimental approaches to be able to understand and describe systems in detail. The same principles apply to my lectures at the master's level, in which I explore chromatin organization from an evolutionary and cross-domain perspective.



Back to the carrier of genetic information, DNA. Since a lot of information is stored in DNA in the form of genes encoding for numerous proteins with different functions, DNA is long. Compared to bacteria, eukaryotic genomes are generally longer, due to a larger number of encoded proteins, and the presence of DNA encoding other functions. A typical human cell has a diameter of a few tens of micrometers. The largest human cells are visible to the naked eye. Bacterial cells are up to a hundred times smaller than human cells and can not be seen by the naked eye. Nevertheless, both types of cells harbor compactly folded DNA molecules with a length, when fully extended, of a few millimeters to a few meters, respectively. A key question relevant to any form of life is: how is the genetic material folded so that it is sufficiently compact to fit into cells or subcellular compartments, and how can it simultaneously be sufficiently accessible, or on demand be made accessible, for dynamic processes, such as transcription by RNA polymerase, to take place?

My fascination for the visualization and quantitation of life was the prelude for a research project on DNA topology and DNA topoisomerases, enzymes regulating DNA compactness, based on classical biochemical methods and atomic force microscopy. Atomic force microscopy made it possible not only to image individual DNA molecules with high resolution and to analyze these quantitatively, but also to also monitor live changes occurring in DNA topology. This fascinating technique is based on the line-by-line scanning of a surface with a biological sample using a fine needle, analogous to the way in which a visually impaired or blind person establishes an image of his or her surroundings using a touch stick. In my group we use this technique to this day. The change of DNA topology by a topoisomerase, similar to the winding of a garden hose, ensures that the DNA, or the hose, effectively occupies less space and can be easily stowed. This is, however, in the case of cells, not yet enough to ensure that the DNA fits inside the cell. There are other specific proteins required for the compact and functional folding of DNA. It has long been

known that cells contain chromosomes. In chromosomes, DNA is in a compact form, bound and wrapped around so-called "histone proteins". This compact structure is referred to as chromatin. It is considered the native form of DNA in eukaryotic cells. It is often pointed out in text books that bacteria do not have this kind of proteins, that, similarly to histone proteins, effectively reduce the volume of DNA and regulate its accessibility to dynamic processes.

That is true. Although bacteria lack histones, they do have proteins with similar function[1]. A part of the research in my group is related to this type of proteins. One of our favorite proteins, H-NS from *Escherichia coli*, has been a subject of investigation by me and my colleagues for more than two decades. Still this protein continues to surprise. A 'boring' protein, with a structural function, and no biocatalytic activity, a protein the only function of which seemed to be to regulate access to DNA and therewith processes such as transcription, turned out to change its structure and function upon changes in physico-chemical conditions[2,3]often mediated by global regulators of transcription. The nucleoid-associated protein H-NS is a key global regulator in Gram-negative bacteria and is believed to be a crucial player in bacterial chromatin organization via its DNA-bridging activity. H-NS activity in vivo is modulated by physico-chemical factors (osmolarity, pH, temperature. Not only have we recently been able to observe these structural changes, we were also able to show that the mode of binding of this protein to DNA changes dependent on conditions in the test tube. Currently, our studies are no longer confined to the test tube. More and more we investigate and test our models in living cells. This is important, because in the test tube DNA and selected proteins are combined in an simple 'physiologically relevant' saline solution, while the composition of a cell is many times more complex. It appears that our models based on test tube knowledge hold in cells; in a recent dissertation[4] we describe for the first time results for a series of related genes in an operon, which are switched on in response to a change in the salt conditions of the living environment. This is a conceptually simple, biologically relevant experiment in which growing bacteria are transferred from 'fresh water' to 'water' with higher salt content. The bacterium then needs to adapt to the new environment, in this case an environment with higher osmotic pressure, by turning specific (sets of) genes 'on' or 'off'. Such adjustments to gene expression are key to survival of free living and commensal bacteria, and crucial for effective infection by pathogenic bacteria. On longer time scales activation of genome regions of foreign, normally silent, DNA contribute to evolution when providing high competitive fitness. How does a change in the structure of a protein and its binding mode mechanistically affect transcription? In our recent studies it was demonstrated that the three-dimensional folding of DNA into a loop structure by the H-NS corresponds with an 'off' state of disruption of this loop yields an 'on' state. Previously, the concept of gene regulation mediated by 3D chromosome structure was only known from 'complex' cells, such as those of humans. We suspect that chromosomal loop formation and dissolution is an important generic mechanism of gene regulation in bacteria. The analogy between prokaryotic and eukaryotic systems illustrates that simple principles, dictated by physical and chemical properties, can be implemented in multiple ways. Functional/conceptual conservation in living systems may be more prominent than anticipated and at least as important as conservation at the level of protein and nucleic acid structure and sequence.

Whereas I above used the term 'complex' for human cells, this term applies equally well for bacterial cells. There are, however, possibilities to simplify bacterial cells by removing non-essential genes from the genome and to thereby minimize the genome. Several years ago the John Craig Venter Institute, JCVI, took up this challenge. They minimized the genome of Mycoplasma mycoides and thus simplified the cell by removing all sequences not required for viability. The product was *Mycoplasma mycoides* JCVI-syn1.0[5]. This simplification makes this an attractive model organism to obtain a better understanding of individual components in

the context of a living cell. Also detailed whole cell modelling, one of the major challenges in biology, thus becomes more feasible. The modelling of processes in cells and the complex underlying networks are of particular importance when they have predictive value, such that ultimately an intentional perturbation of the system, yields a predefined response. This would yield a level of control that would be of large benefit in (microbial) biotechnology. Interestingly, together with large part of the genome (almost 50%), the majority of genes encoding chromatin proteins have been either removed from this minimal organism, or these genes are expressed at a very low level. This poses the question whether these proteins indeed are fundamentally as important for genome organization and gene regulation as we believe. I currently do not have an answer to that. I can share with you, however, that we, as part of a collective, multidisciplinary initiative to describe these cells in a detailed model, are working on resolving the three-dimensional organization of the 3rd generation JCVI-syn3.0[6] artificial organism. The first results indicate that these cells are indeed simpler than their more 'complete' counterparts as regards to their genomic organization[7]. Possibly this is related to the fact that their genome is almost two times as short as that of wildtype *Mycoplasma mycoides*, ten times as short as that of other bacteria and orders of magnitude smaller than eukaryotic genomes that have been studied

This brings me to my second main line of research, the study of chromatin structure in archaea[8]. What are archaea? Superficially they look like bacteria. They are single-celled organisms and similar in size to bacteria. Archaea are generally known because of their extreme habitats, characterized by high temperature, high pH, high salt concentrations or high pressure. This suggests that these organisms are specifically adapted to an existence under these conditions. There are, however, also many archaeal species that thrive under more moderate conditions. For instance, archaea are also found to be part of the human microbiome, in the gut or on the skin. Pathogenic archaea have, however, not yet been found. Therefore, interest in archaea from a medical perspective is limited. On the other hand, from a fundamental, but also from a biotechnological and evolutionary perspective, there is a lot of attention for these organisms. In the field of biotechnology, for instance, enzymes from extremophilic archaea are interesting because of their robustness. From an evolutionary perspective, archaea are very interesting: according to recent findings, eukaryotes do not form a separate domain of life, but are part of the archaeal branch in the tree of life. This evolutionary closeness is evident in their molecular similarities. The protein machinery involved in transcription, replication and DNA repair in archaea, although somewhat less complex in many cases, is very similar to the machinery with these tasks in eukaryotes. This makes archaea interesting and relevant as model organisms, as 'simplified systems' next to eukaryotes. We are particularly interested in the way in which the genomes of archaea are folded, how genome folding has evolved and whether there are conserved principles of genome folding. In eukaryotes a key role in chromatin organization is attributed to so-called 'histone proteins', which form a protein core around which DNA is wound. The resulting structure contains four pairs of different histone proteins, and is called a nucleosome. Different from bacteria, archaea also express histone proteins. However, archaea lack a nucleus, an organelle characteristic of eukaryotes in which DNA is contained. The archaeal histone proteins also form a protein core around which DNA is wound, but – at least in the case of the histone proteins from the hyperthermophilic model organism *Methanothermus fervidus* – this structure is endless i.e. histone-dimers associate (or dissociate) at the extremities of the spool[9–11]. A mechanism by which growth or shrinkage of the spool is modulated is not known. We refer to the resulting structure as 'hypernucleosome'[12], as its seemingly infinite length clearly sets it apart from the canonical eukaryotic nucleosome, with a histone octamer at its core. The big questions now are how length and stability of hypernucleosomes are modulated, and to what extent hypernucleosome formation is conserved

among archaea. It is expected that stable hypernucleosomes restrict access to the genetic code in the DNA and therewith regulate gene expression. A recent study suggests that genes implied in environmental adaptation are specifically targeted and regulated by binding of histones[13]. Interestingly, this binding behavior and function are analogous to the role of the H-NS protein in bacteria, as described by us. Our preferred model is that modulation of hypernucleosome structure and stability is in part due to expression of histone variants with distinct properties in terms of multimerization and DNA binding. Additionally it is expected that hypernucleosome modulation is mediated by influences on electrostatic protein-protein interactions in the complex by changes in salt conditions, or changes in surface charge as a result of changes in pH.

To what extent do the histones in archaea resemble the histones from eukaryotes? The most important similarity is the characteristic 'histone fold'. The archaeal histones studied so far only consist of a 'histone fold'. In eukaryotes, however, histones in general have a tail at the N-terminus. This positively charged tail plays a crucial role in affecting surface charge, determining the structure and dynamics of eukaryotic chromatin, and thus in determining gene activity. Some eukaryotic proteins with a 'histone fold' have an extension at the C-terminus. These are not histones, but transcription factors. We have compared hundreds of sequences of archaeal proteins with a "histone fold" with each other and have found that a small fraction of these proteins also harbors an extension at the N-terminus or C-terminus. An important question that arises from these analyses is what is the function of these extensions in archaea, and whether these extensions are the bridge to eukaryotic histones and histone fold- proteins.

We have a fair understanding of how many chromatin proteins operate and also it is becoming more clear how their function is modulated by environmental factors. Up to this point I have talked about direct physico-chemical influences on these proteins, but an important new dimension to our research is the post-translational chemical modification of chromatin proteins in bacteria[14] and archaea. Chemical modification of proteins involved in eukaryotic chromatin organization has been long known. In eukaryotes such modifications affect chromatin organization directly as a consequence of altered physico-chemical properties, or indirectly via recruitment of active chromatin remodelling complexes. In bacteria and archaea modifications of chromatin proteins have also been observed, but no functional meaning has been determined. For instance, it was recently shown that acetylation occurs in histones at amino acid residues that we predicted to influence the stability of the hypernucleosome. Modification of these specific residues constitutes a possible functional alternative for chemical modification of residues in other parts of the histone proteins in eukaryotes. To obtain a detailed mechanistic understanding of the direct impact of chemical modifications via in vitro studies, chromatin proteins with (defined) post-translational modifications are required. This is challenging when considering approaches relying at least in part on genetics. A solution is in chemical peptide synthesis. This synthetic approach is feasible as the proteins of our interest are generally small in size. In addition to employing chemical peptide synthesis for histones that, due to their physico-chemical properties are hard to obtain via heterologous expression in E. coli, we now also use this approach for synthesis of acetylated histone variants. This addition to our toolbox places us in a unique position to unravel the function of chemical modifications of chromatin proteins.

So far have I talked about research aimed at the generation of fundamental knowledge on our systems of interest: chromatin in bacteria and archaea. The emphasis of these studies is on the investigation of structure-function relationships. It is becoming clear that the structures, that we are interested in are not static, but dynamic and are affected and modulated by a variety of external factors. Such fundamental insights

now offer possibilities for artificially influencing, controlling the studied system inspired by the natural mechanisms. This provides opportunities to test and refine our models, but it also offers opportunities in terms of translational applications. I mention this explicitly to underline the societal importance of fundamental research, and to illustrate that applications arise in natural ways, if a strong base of fundamental knowledge is available and combined in creative ways. In the case of one of the proteins that I study, H-NS, we have known for two decades that this protein has special properties in terms of binding to DNA and the formation of loops. A decade ago it became clear that the complex binding properties of H-NS can be modulated by salt conditions. In the last five years we have determined the structural mechanism. This has led to new studies in which we examine how the binding of peptides and small molecules can be used to control the binding properties of H-NS, with potential application as a new type of antibiotics. An other example of an application that is built upon the fundamental knowledge of the properties and structure of H-NS is the use of parts of this protein in a tool for the staining of DNA in chromosomes. This goes beyond our direct field of interest, but has the potential of broad application in the field of eukaryotic cell biology. An other recent example of how fundamental science needs ripening before effective application is the development of mRNA vaccines, which was underway already for years, but gained momentum during the first year of the COVID-19 pandemic.



Our fundamental molecular understanding of the interplay between the cell and its environment, and the role of chromatin therein is for an important part based on test tube experiments, experiments that do not involve living cells, except as a source of purified protein. Testing models based on test tube experiments in living systems is important to support their validity. For that reason the research in my group has over the last years strongly developed towards incorporating technologies that make it possible to directly or indirectly 'see' inside living cells. The initial results are promising, yet also still limited, in the sense that cells are exposed to simple, controlled changes in environment only. The next few years I plan to apply our current toolbox, including the new sequencing and live cell microscopy-based approaches, more and more to studies in a natural environment, focusing on the interaction between bacteria and environment. Directions of interest would include studies of bacteria infected by bacteriophages (bacterial viruses), bacteria incorporated by macrophages, or biofilm formation by bacteria in tissue models. Nevertheless, it will remain of crucial importance to mechanistically support observations in complex contexts with test tube experiments. A thorough molecular mechanistic insight is required to be able to intervene in the system, understand it, and, possibly, to fight bacterial infections. The strength of my research lies in the analysis at different length scales, the interplay between in vitro and in vivo systems and the inspiration which we find in chemistry, biology and physics.

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