Modeling the binding of H-NS to DNA

van Mastbergen, E.C.; Vreede, J.

DOI
10.1016/j.bpj.2014.11.2948
Publication date
2015
Document Version
Final published version
Published in
Biophysical Journal

substance known as ''chromatin''. In the recent years, more and more evidence has accumulated pointing out chromatin polymorphism and dynamics as a primary mean of control of genome accessibility in time and space, driving the focus on this complex polymer as a critical player in gene regulation. A thorough characterization of chromatin properties would then be a prerequisite step in our understanding of differential gene expression, e.g. ''epigenetics'' in its original definition by Waddington as ''the study of the causal mechanisms by which the genes of the genotypes bring about phenotypic effects''.
We wish here to emphasize some physical characteristics of genome organization in order to provide a more complete framework in which to interpret the control of gene expression. Indeed, as various molecular motors push, pull and twist DNA, transient forces and torques develop within chromatin, with expected consequences on transcription and other DNA metabolism events such as repair or recombination. In addition to discussing some basic mechanical and topological issues, we will also present some recent quantitative and qualitative insights from our lab into chromatin organization and dynamics, including the still controversial role of ions in DNA compaction and the mechanical action of recombinases.
Boulé JB, Mozziconacci J and Lavelle C. (2014). The polymorphism of the chromatin fiber. J Phys Cond Mat (in press).
Lavelle C. (2014). Pack, unpack, bend, twist, pull, push: the physical side of gene expression. Curr Opin Genet Dev 25:74-84.
Huet S, Lavelle C & al. (2014). Relevance and limitations of crowding, fractal, and polymer models to describe nuclear architecture: is a unified picture out of reach? Int Rev Cell Mol Biol 307:443-479.

2718-Pos Board B148
Nucleosome Kinetics and Accesibility of DNA
Jyotsana J. Parmar[1], Dibyendu Das[2], Ranjith Padinhateeri[1].
[1]Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India, [2]Physics Department, Indian Institute of Technology Bombay, Mumbai, India.
Crucial cellular processes like gene regulation, transcription, and replication require access to DNA that is covered with nucleosomes. Many experiments suggest that nucleosome organization and dynamics can significantly influence exposure and accessibility of various locations on the genome. In this work we investigate the kinetics of DNA exposure as a result of nucleosome dynamics. We consider binding and dissociation of nucleosomes taking into account both sequence specificity and ATP-dependent activity, and study accessibility of DNA near different kinds of barriers (e.g. a well-positioned protein or a nucleosome free region near transcription start site). Using analytical calculations and numerical simulations, we find the following results. We show that the timescale of exposure of a DNA site near a barrier can be very diverse and crucially depends on the DNA sequence and the initial nucleosome organization. We show how nucleosome-mediated cooperativity can emerge when multiple transcription factors are binding at nearby locations and we investigate how multi-nucleosome correlations influence the time scale of accessibility as a function of the distance from the barrier. We discuss ramifications of our findings in understanding gene regulation and stochasticity in gene expression.

2719-Pos Board B149
Chromosome-Nuclear Envelope Interactions Have Multiple Effects on Chromosome Folding Dynamics in Simulation
Nicholas A. Kinney[1], Igor V. Sharakhov[2], Alexey V. Onufriev[3].
[1]Genomics, Bioinformatics, and Computational Biology, Virginia Tech, Blacksburg, VA, USA, [2]Entomology, Virginia Tech, Blacksburg, VA, USA, [3]Computer Science, Virginia Tech, Blacksburg, VA, USA.
It is well recognized that the chromosomes of eukaryotes fold into non-random configurations within the nucleus. In humans and fruit flies, chromosomes likely adopt a particular 3D configuration called the fractal globule (FG) which has multiple biologically significant properties. However, the fractal globule is a metastable state which, over time, transitions to a less biologically favorable state called the equilibrium globule. One of the key questions is how the FG state is stabilized in-vivo? We use simulations to study the effects of chromosome-nuclear envelope (Chr-NE) interactions on the dynamics of the fractal globule within a model of Drosophila melanogaster (fruit fly) interphase chromosomes. The computational model represents chromosomes as self-avoiding walks (SAW) bounded by the nuclear envelope (NE). Model parameters such as nucleus size, chromosome persistence length, and chromosome-nuclear envelope interactions are taken directly from experiment. Several key characteristics of the non-equilibrium FG state are monitored during each simulation's progress: chromosome territories, intra-chromosomal interaction probabilities, loci specific diffusion constants, and presence of the Rabl (polarized) chromosome arrangement. Next, we compare the outcomes of simulations which include or exclude Chr-NE interactions. We find that Chr-NE interactions reinforce the non-equilibrium properties such as chromosome territories, high intra-chromosome interaction probabilities, and the Rabl chromosome arrangement. In addition, Chr-NE interactions affect loci specific and averaged chromosomal diffusion. Based on these results we conclude that the presence of Chr-NE interactions may delay the decay of the biologically relevant fractal globule state in vivo.

2720-Pos Board B150
Biophysical Models of Nucleosome Positioning
Razvan V. Chereji, David D. Clark.
NICHD, National Institutes of Health, Bethesda, MD, USA.
A human body contains enough DNA to circle the Earth's Equator more than 2.5 million times. The basic units of DNA packaging are called nucleosomes. Their locations along the chromosomes play an essential role in gene regulation. We study nucleosome positioning in yeast, fly and mouse, and build biophysical models in order to explain the genome-wide nucleosome organization. We show that DNA sequence is not the major cause of the regular arrays of nucleosomes observed in vivo near the transcription start sites (TSS). We construct a minimal model in which nucleosomes are positioned by potential barriers located in the gene promoters, and which accurately reproduces the genome-wide nucleosome occupancy patterns observed over the transcribed regions in living cells. Our statistical mechanics model allows us to study nucleosome phasing against potential barriers and wells [1, 2], sequence-dependent nucleosome affinity [2], nucleosome unwrapping [3], competition between different DNA-binding proteins, and accessibility of transcription factors [4, 5] to target sites which are found in nucleosomal DNA, among others. We also discuss alternative nucleosome positioning mechanisms: nucleosome anchoring [6] and active nucleosome positioning by ATP-dependent remodelers [7].
[1] RV Chereji, D Tolkunov, G Locke, AV Morozov, Phys. Rev. E 83, 050903 (2011)
[2] RV Chereji and AV Morozov, J. Stat. Phys. 144, 379 (2011)
[3] RV Chereji and AV Morozov, Proc. Natl. Acad. Sci. U.S.A. 111, 5236 (2014)
[4] N Petrenko, RV Chereji, MN McClean, AV Morozov, JR Broach Mol. Biol. Cell 24, 2045 (2013)
[5] N Elfving*, RV Chereji*, V Bharatula, S Björklund, AV Morozov, JR Broach, Nucleic Acids Res. 42, 5468 (2014) (* contributed equally)
[6] RV Chereji, AV Morozov, YM Moshkin, in preparation
[7] D Ganguli*, RV Chereji*, J Iben, HA Cole, DJ Clark, Genome Res. (2014) (* contributed equally)

2721-Pos Board B151
Prediction of Chromosome Conformations with Maximum Entropy Principle
Bin Zhang, Peter G. Wolynes.
Rice University, Houston, TX, USA.
The genomes' three-dimensional (3D) organization is crucial in regulating many biological processes, including gene regulation, DNA replication, and cell differentiation. A high-resolution chromosome structure thus will significantly advance our understanding of these important processes. A major step toward building a structural model of the chromosome is the inventions of chromosome conformation capture methods, 5C and Hi-C, that aim at detecting physical contact frequencies between pairs of genomic loci. However, computational approaches to construct 3D structures that are consistent with these experimental contact frequency measurements remain lacking.
We develop a statistically rigorous approach based on maximum entropy principle to determine a least-biased potential energy landscape that reproduces experimentally determined Hi-C contact frequency between genome pairs. The resulting energy landscape supports a knotless chromosome conformation, which has been highly anticipated since complex knotted conformations prohibit the access of gene information for transcription and hinder DNA replication. We further show that the topologically associating domain signal alone also enforces a chromosome structure free of knots. Our results highlight the importance of local interactions in determining the global topology of the chromosome structure. Finally, the derived landscapes for multiple chromosomes support the formation of territories that have long been observed in microscopy experiments. Together with Hi-C experiments, our approach provides a coherent picture of the 3D architecture of the genomes that is consistent with many the available experimental data.

2722-Pos Board B152
Modeling the Binding of H-NS to DNA
Eva C. van Mastbergen, **Jocelyne Vreede**.
van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, Netherlands.
Bacterial chromosomal DNA is organized within a structure called the nucleoid, which is distinctly different from the rest of the cytoplasm. Bacteria have a number of nucleoid-associated proteins that influence the organization of the

nucleoid by bending, wrapping or bridging DNA. The Histone-like Nucleoid Structuring protein H-NS can bridge DNA by binding to two separate DNA duplexes, or shield the DNA by binding to distant sites on the same duplex, depending on external conditions. H-NS occurs in Gram-negative enterobacteria and silences genes involved in bacterial virulence and antibiotic resistance. The current view reflects that the formation of an H-NS - DNA assembly starts with the initial binding of an H-NS dimer to a specific nucleotide sequence, followed by additional H-NS dimers interacting with bound H-NS and binding to adjacent sites on DNA. Several nucleotide sequences have been identified to which H-NS binds strongly. Despite enormous progress in methods aimed at resolving molecular structures, which resulted in resolving the structures of the dimerization domain and the DNA binding domain, it is still impossible to experimentally obtain detailed structural information of the entire complex, whereas dynamic properties are even harder to investigate in experiments. Molecular simulation can complement experiments by modeling the dynamical time evolution of biomolecular systems in atomistic detail. Employing molecular dynamics simulations, we studied the binding mechanism of H-NS to DNA. Our results show that H-NS binds strongly to AT-rich dsDNA in the minor groove. Furthermore, we found that H-NS binds transiently to dsDNA with high GC content, in the major groove. These observations are in excellent agreement with experimental data. By using transition path sampling, we were able to further probe the mechanism of H-NS binding to AT-rich DNA, resulting in the identification of two different modes of interaction.

2723-Pos Board B153

The Binding Landscapes of the H3/H4 and CENP-A/H4 Dimers

Haiqing Zhao[1], Yamini Dalal[2], Garegin A. Papoian[1].
[1]Institute for Physical Science and Technology, University Of Maryland, College Park, MD, USA, [2]Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA.

Centromere protein A (CENP-A) is a centromere-specific variant of histone H3 and shares almost 50% amino acid identity to canonical histone H3. CENP-A is required for packaging the centromere, to facilitate separation of sister chromatids during mitosis. Indeed, significant structural similarities have been reported to exist between CENP-A/H4 dimers and H3/H4 dimers in co-crystals. In this work, we used molecular dynamics simulations to map the binding free energy landscape for the CENP-A/H4 and H3/H4 dimers. The Associated memory, Water mediated, Structure and Energy Model (AWSEM) and umbrella sampling constraints were applied for each simulation system towards obtaining two-dimensional free energy profiles of monomeric protein association and folding. Surprisingly, our calculations revealed significant thermodynamic distinctions between dimerization profiles of CENP-A/H4 and of H3/H4 pairs. Furthermore, we also investigated the actions of various histones chaperones, finding that free energy landscapes of the CENP-A/H4 dimer is significantly remodeled in the presence of its cognate chaperone HJURP. The obtained results are in general agreement with the available experimental data and provide new thermodynamic insights into the mechanisms that form the basis of canonical and histone variant CENP-A nucleosomes assembly in vivo.

2724-Pos Board B154

Topological Polymorphism of Two-Start Nucleosome Fibers: A Stereochemical Analysis

Davood Norouzi, Victor B. Zhurkin.
NCI/NIH, Bethesda, MD, USA.

The spatial organization of nucleosomes in 30-nm fibers remains unknown in detail. To address this issue, we analyzed all stereochemically possible configurations of two-start nucleosome fibers with short DNA linkers L = 13 - 37 bp (nucleosome repeat length NRL = 160 - 184 bp). Four superhelical parameters – inclination of nucleosomes, twist, rise and diameter – uniquely describe a uniform symmetric fiber. The energy of a fiber is defined as the sum of four terms: elastic energy of the linker DNA, steric repulsion, electrostatics and a phenomenological (H4 tail - acidic patch) interaction between two stacked nucleosomes. By optimizing the fiber energy with respect to the superhelical parameters, we found two types of topological transition in fibers (associated with the change in inclination angle): one caused by an abrupt 360° change in the linker DNA twisting, and another caused by over-crossing of the linkers. (The first transition is characterized by change in the DNA linking number, $\Delta Lk = 1$, and the second one by $\Delta Lk = 2$.) To the best of our knowledge, this topological polymorphism of the two-start fibers was not reported in the computations published earlier. Importantly, the optimal configurations of the fibers with linkers L = 10n and 10n+5 bp are topologically different. Our results are consistent with experimental observations, such as the inclination 60-70° (the angle between the nucleosomal disks and the fiber axis), helical rise, diameter and left-handedness of the fibers. In addition, we make several testable predictions, among them existence of different degree of DNA supercoiling in the fibers with L = 10n and 10n+5 bp, different stiffness of the two types of fibers, and a correlation between the local NRL and the level of transcription in different parts of the yeast genome.

2725-Pos Board B155

Multivalent Targeting of Nucleosomes by the BRG1 At-Hook and Bromodmain

Daniel P. Farrell, Katayoun Varzavand, **Catherine A. Musselman**.
Biochemistry, University of Iowa, Iowa City, IA, USA.

Beyond providing an avenue for packaging the genome into the nucleus, chromatin provides elegant mechanisms for the dynamic regulation of the eukaryotic genome. Chromatin structure undergoes dramatic spatial and temporal reorganizations throughout the life cycle of the cell, which require extensive remodeling of the nucleosome, the basic subunit of chromatin. A major mechanism for modulating chromatin structure is through ATP driven nucleosome remodeling. The switching/sucrose non-fermenting (SWI/SNF) chromatin remodeling complex facilitates ATP dependent remodeling of nucleosomes critical in gene regulation. SWI/SNF activity is mediated by one of two possible ATPases, Brahma (BRM) or Brahma related gene 1 (BRG1). The chromatin targeting, occupancy and activity of the complex are positively affected by histone acetylation, and it has been found that this is mediated through the C-terminal Bromodomains (BDs) of BRG1 and BRM. One possible mechanism for this is that BD recognition of acetylated histones targets and/or retains SWI/SNF at chromatin leading to increased activity. This is supported by previous reports of acetylation and BD dependent recruitment and retention of SWI/SNF at gene promoters. However, the BRG1 BD has been shown to have very poor affinity and conflicting specificity for acetylated histone peptides in vitro, calling into question the importance of this interaction. We propose that the nucleosome context is critical for proper activity of the BD in binding acetylated histones, and suggest that an adjacent AT-hook DNA binding domain contributes to BD activity. Here we present our recent results using NMR spectroscopy and TIRFM to investigate the multivalent interaction of the BRG1 AT-hook and BD with nucleosomes, including details of the structural basis by which the AT-hook-BD motif interacts with the 601-mononucleosome, the kinetic and thermodynamic basis of complex formation, as well as the effect of histone acetylation.

2726-Pos Board B156

Quantification of Interphase Chromatin Dynamics in Fission Yeast

Takeshi Sugawara[1], Kenta Masuda[2], Jun-ichi Uewaki[1], Akinori Awazu[1,3], Hiraku Nishimori[1,3], Masaru Ueno[1,2].
[1]Research Center for the Mathematics on Chromatin Live Dynamics(RcMcD), Hiroshima University, Hiroshima, Japan, [2]Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Hiroshima, Japan, [3]Department of Mathematical and Life Sciences, Faculty of Science, Hiroshima University, Hiroshima, Japan.

In mammals and other higher organisms, interphase chromosomes remain separated from each other and compartmentalized into chromosome territories. In yeast, chromosomes adopt a Rabl configuration, with arms extending from centromeres tethered at the spindle pole body(SPB), functionally equivalent to the centrosome, to telomeres at the opposite nuclear envelope. These organizations generally constrain chromatin motion, and contribute to gene positioning inside the nucleus. On the other hand, active genes escape from such physical constraints and are located at active regions such as transcription factories and nuclear pore complexes(NPCs). In spite of extensive studies for these phenomena, how gene loci sustain and change their positioning during the cell cycle still remains unclear. In order to elucidate chromatin dynamics during interphase, we visualized several gene loci and the centromeres (and the SPB) on the fission yeast *S. pombe* chromosomes that show a Rabl configuration, and then tracked the positions of the loci on a longer time scale than conventional one. The statistical analysis of the physical distance from the SPB to each locus suggested that the gene loci show not only restricted diffusion due to the physical constraint of the Rabl configuration, but also a novel dynamic property, that is quite different from simple diffusive behaviors reported so far. In this presentation, we will discuss functional roles that such chromatin dynamics possibly play within the interphase nucleus.

2727-Pos Board B157

Effect of Architecture of Cell Nucleus on the Folding Principles of 3D Genome of Budding Yeast

Gamze Gürsoy, Yun Xu, Jie Liang.
Bioengineering, University of Illinois at Chicago, Chicago, IL, USA.

Architecture of the cell nucleus and the spatial organization of genome are critical for nuclear functions. Single-cell imaging techniques and chromosome conformation capture (3C) based methods provide a wealth of information on the spatial organization of chromosomes. Computational tools for modeling