During transcription, RNA polymerase (RNAP) translocates along DNA and can stall when encountering obstacles such as GC-rich regions or promoter elements. The detection mechanism and regulation of RNAP stalling is a key area of research, as understanding these processes can shed light on the mechanisms of transcriptional regulation.

In the context of the GAL10 gene in yeast, the GAL10 locus in yeast, during activation of the GAL pathway, bacterial toxins such as V. cholerae MARTX toxin are expressed in response to galactose. These toxins modulate the expression of non-coding RNAs whose mechanisms of action are for the most part not understood. However, recent technical advances now allow direct visualization of the synthesis of nascent transcripts from individual genes over time by decorating RNAs with fluorescent proteins. Using the orthogonal RNA-binding MS2 and PP7 bacteriophage coat proteins, we were recently able to tag two regions of the same RNA in two different colors [Coulon et al. 2014, eLife, in press]. Here, we used this technique to visualize simultaneously sense and antisense transcription from the GAL10 locus in yeast, during activation of the GAL pathway.

Using cross-correlation analysis, we uncovered specific temporal windows that show the eukaryotic genome is pervasively transcribed, giving rise to various sorts of non-coding RNAs whose mechanisms of action are for the most part not understood. Recent technological advances now allow direct visualization of the synthesis of nascent transcripts from individual genes over time by decorating RNAs with fluorescent proteins. Using the orthogonal RNA-binding MS2 and PP7 bacteriophage coat proteins, we were recently able to tag two regions of the same RNA in two different colors [Coulon et al. 2014, eLife, in press]. Here, we used this technique to visualize simultaneously sense and antisense transcription from the GAL10 locus in yeast, during activation of the GAL pathway.

Fluorescence fluctuations recorded in both channels at the transcription site reflect the kinetics of transcription on both strands as the GAL10 gene gets activated in response to galactose. We observe transient antisense transcription occurring almost exclusively prior to the appearance of sense transcription. Using cross-correlation analysis, we uncovered specific temporal windows relatively to sense activation where antisense transcription is enriched or depleted - likely reflecting the biochemical mechanisms underlying activation. Once transcription of the GAL10 gene starts, transcripts are produced in bursts separated by periods of inactivity, occasionally leaving the opportunity for antisense transcription to happen. We developed a method for applying fluctuation correlation analysis to non-stationary time traces. This allowed us to isolate the bursting kinetics in the non-steady-state context of a transient response to galactose. By modeling the autocorrelation of a bursting gene, we were able to infer from our data how the elongation rate, burst size and burst frequency of the GAL10 gene are modulated by different doses of galactose. This work shows how in vivo single-molecule methods and fluctuation analysis can reveal unanticipated mechanisms of transcriptional regulation.

We present a simple single-molecule assay for studying transcription and antisense transcription of a Yeast Gene. The mechanism of transcriptional regulation of the GAL10 gene is modulated by different doses of galactose. This work shows how in vivo single-molecule methods and fluctuation analysis can reveal unanticipated mechanisms of transcriptional regulation.
substance known as “chromatin”. In the recent years, more and more evidence has accumulated pointing out chromatin polymorphism and dynamics as a primary controller 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 recombination.


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Nucleosome Kinetics and Accessibility of DNA

Jyotsana J. Parmar1, Dibyendu Das2, Ranjith Padinhaterr2, 1Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India, 2Physics 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.

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Chromosome-Nuclear Envelope Interactions Have Multiple Effects on Chromosome Folding Dynamics in Simulation

Nicholas A. Kinney1, Igor V. Sharakhov2, Alexey V. Onufriev3. 1Genomics, Bioinformatics, and Computational Biology, Virginia Tech, Blacksburg, VA, USA, 2Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India, 3Computer 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 prohibit the access of gene information for transcription and hinder DNA replication, which is distinctly different from the rest of the cytoplasm. Bacteria have a chromosome that is packed within a structure called the nucleoid, which is 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].


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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 invention 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 chromosome structures thus will significantly advance our understanding of these important processes. A major step toward building a structural model of the chromosome is the invention 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 chromosome structure support the formation of territories that have long been observed in microbial and active nucleosome positioning by ATP-dependent remodelers [7].