MOLECULAR MECHANISM OF SEQUENCE-DEPENDENT DNA LOOPING

Allocation: Illinois/1.20 Mnh
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EXECUTIVE SUMMARY

The molecular mechanisms underlying regulation of gene expression are central to cell biology but are still not fully understood. Our group uses the Blue Waters supercomputer to determine how the intrinsic properties of DNA affect gene regulation. One mechanism of gene regulation involves the bending of rigid DNA molecules around histone proteins, restricting access to the encoded information. Using molecular dynamics simulations, we elucidated the effect of epigenetic modifications on the bending propensity of DNA molecules. Another mechanism of gene regulation involves restructuring entire collections of genes. Using enhanced sampling methods, we have shown that AT-rich and methylated DNA experience enhanced attraction at physiological conditions, capable of causing large-scale chromosome compaction. Both mechanisms support the notion of "hidden" codes in DNA that contributes to the regulation of gene expression.

INTRODUCTION

Eukaryotic cells store their genetic information in long (millions of base pairs) molecules of DNA. Inside the nucleus, the DNA is wrapped around histone proteins into bundles called nucleosomes, each consisting of approximately 150 base pairs of DNA forming a double-loop around a histone core. At a given stage of a cell's life cycle, only a fraction of a cell's genes are active; the collection of active genes determines the function of a cell. One method of turning genes on and off is through compaction of the DNA, which makes its genetic code inaccessible to the transcription machinery [1,2,3]. Here, we describe two mechanisms that can realize DNA compaction: one that occurs inside a single nucleosome, and another that occurs among multiple nucleosomes. Within a nucleosome, decreasing DNA flexibility increases the likelihood of DNA unwrapping from the nucleosome, exposing the DNA's genetic code. In the case of multiple nucleosomes, attractive interactions between the nucleosomes can modulate the accessibility of multiple DNA fragments. In collaboration with the Ha group (Johns Hopkins University), we demonstrate that flexibility of individual DNA fragments and mutual attraction between several DNA fragments depend on the DNA sequence and can be modulated by chemical modifications of the DNA in the absence of any auxiliary proteins.

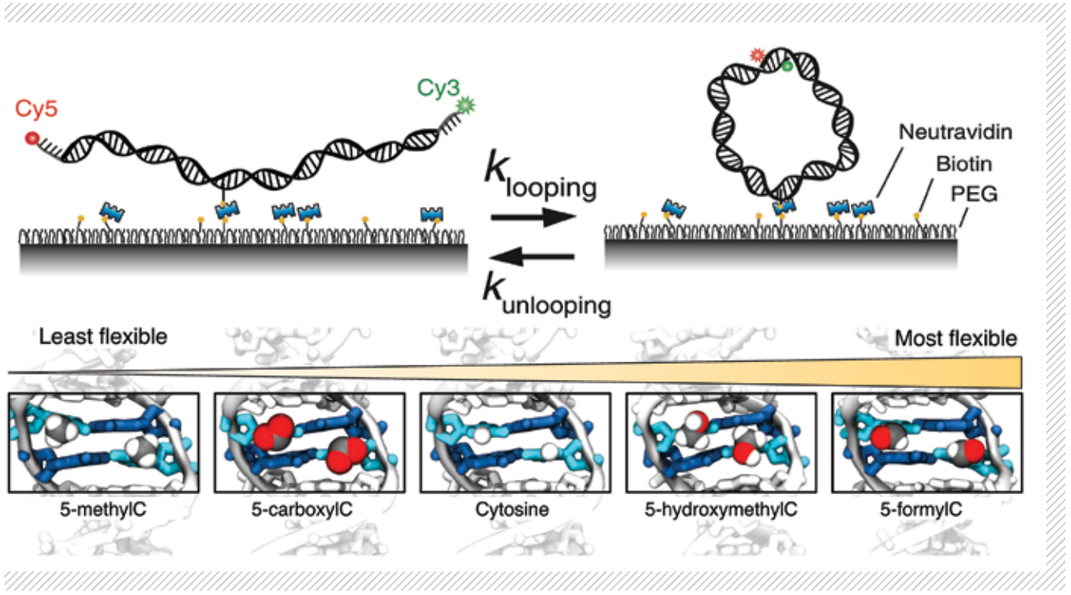


FIGURE 1: Schematic representation of single-molecule DNA cyclization assay. Population of looped DNA is monitored over time using the Fluorescence Resonance Energy Transfer technique. The modified CpG sites are ranked according to their simulated flexibility: 5-formyl C > 5-hydroxymethyl C ≥ 5-carboxyl C ≥ unmodified C > 5-methyl C.

METHODS & RESULTS

In collaboration with the Ha group, we characterized the effect of chemical modifications of cytosine (C) on DNA flexibility. We tested the following four biologically significant modifications: 5-methylcytosine (5-mC), 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC). In both simulation and experiment, the 5-fC modification was found to increase DNA flexibility considerably, 5-hmC to enhance flexibility but to a lesser degree than 5-fC, 5-mC to reduce flexibility, and 5-caC to have no measurable effect. Analysis of the molecular dynamics (MD) trajectories showed that chemical modifications affect DNA flexibility via steric, hydrophobic, and electrostatic mechanisms. The modulation of DNA flexibility increased the mechanical stability of the nucleosome and vice versa, suggesting a gene regulation mechanism where cytosine modifications change the accessibility of nucleosomal DNA through their effects on DNA flexibility. In a separate study performed in collaboration with the Meni Wanunu group (Northeastern University), we have shown that oxidized products of thymine (T) can also enhance the flexibility and hydrophilicity of double-stranded DNA.

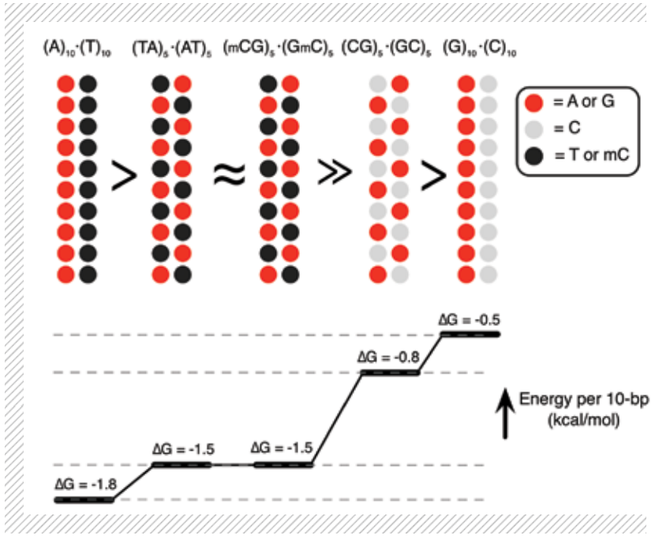


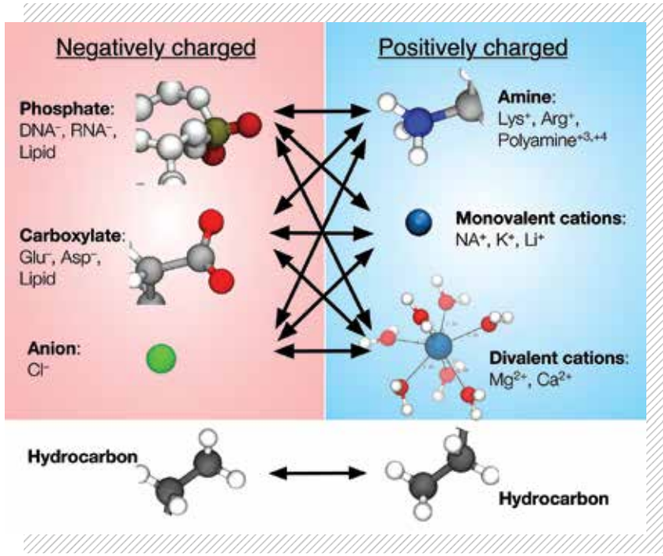
FIGURE 2: The number and spatial arrangement of nucleotides carrying a methyl group (T or mC) determine the interaction free energy of two DNA molecules.

By combining single molecule experiments and MD simulations, we demonstrated a **novel** mechanism of DNA compaction controlled by the methylation pattern of DNA. Prior experiments established a correlation between DNA compaction (high-order chromatin folding) and the nucleotide content: DNA segments containing a higher than average percentage of AT base pairs, a larger than average number of methylated cytosine nucleotides, or both, were found to form more compact folds. One popular explanation of this observation posits the existence of yet unidentified proteins that recognize DNA sequences and their modifications and bring the recognized DNA domains together [4]. Alternatively, the DNA compaction can be induced by direct physical interactions between DNA segments mediated by polyamines, small charged molecules abundant in living cells. To test the latter possibility, we performed a series of free energy calculations at a physiological concentration of spermine, a model polyamine. The computed free energies clearly showed that AT-rich DNA domains attract each other more strongly than GC-rich domains do. Furthermore, methylation of cytosines was found to increase the attraction between two GC-rich domains to the level of two AT-rich domains. Subsequent single molecule experiments confirmed the predictions of our MD simulations.

Our findings suggest a **tantalizing** possibility that polyamine-mediated inter-DNA attraction can play a major role in high-level chromatin folding.

Our inquiry into the molecular mechanism of DNA—DNA interactions found that the standard parameterization of nonbonded interactions in popular MD force fields, CHARMM and AMBER, are not accurate enough to characterize the dependence of the DNA—DNA interactions on the DNA sequence and its modifications. For example, the effective force between two DNA molecules in a 100-mM di-lysine (Lys2+) solution is attractive in both standard CHARMM and AMBER models, whereas the DNA molecules are experimentally known to repel one another at identical conditions. To improve the accuracy of the MD force fields, we reparameterized the strength of amine–phosphate and amine–carboxylate interactions against independent sets of osmotic pressure data. Our extensive validation simulations performed on Blue Waters have shown that our improved parameter set can **significantly enhance** the realism of MD simulations for a broad class of biomolecular systems, including protein folding, protein–DNA interactions, and DNA condensation.

FIGURE 3: Refinement of non-bonded interactions for accurate simulations of inter-molecular forces. The interactions indicated by arrows were reparameterized to reproduce the experimental osmotic pressure data.



WHY BLUE WATERS

Extensive sampling of biomolecular conformations was essential for characterization of DNA flexibility and calculation of inter-DNA forces. Using Blue Waters was essential to achieve the unprecedented accuracy of our simulations that matched and sometimes exceeded state-of-the-art experimental techniques.

NEXT GENERATION WORK

Using Blue Waters, we plan to create a genome-wide map of DNA flexibility that will elucidate the effect of DNA sequence on gene regulation.

PUBLICATIONS AND DATA SETS

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SEQUENCE SIMILARITY NETWORKS FOR THE PROTEIN "UNIVERSE"

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EXECUTIVE SUMMARY

We are devising strategies and tools to facilitate prediction of the *in vitro* activities and *in vivo* metabolic functions of uncharacterized enzymes discovered in genome projects. We used Blue Waters to establish a protocol for generating a library of sequence similarity networks (SSNs) for all Pfam protein families in the UniProt protein sequence databases for dissemination to the scientific community. We have calculated 1) all-by-all Basic Local Alignment Search Tool (BLAST) sequence relationships, 2) statistical analyses of the BLAST results; and 3) merged sets of input sequences based on sequence identity. Based on our experiences, we have defined protocols for the regular (every eight weeks) generation of the library of sequence similarity networks.

INTRODUCTION

The current UniProtKB database contains more than 60M nonredundant sequences. The functions for less than 1% of the entries have been manually curated; the functional annotations for the remaining entries are assigned by automated procedures. As a result, the conservative estimate is that the annotations for at least 50% of the entries are uncertain or incorrect. The majority of the entries are obtained from genome sequencing projects, the rationale being that knowledge of the complete complement of proteins and enzymes encoded by an organism will allow its biological and physiological capabilities to be understood. However, if at least 50% of the proteins and enzymes have uncertain or unknown functions, the considerable investments in genome projects cannot be realized. Because of the very large number of proteins and enzymes for which sequences have or will become available, strategies for predicting their functions must be high throughput and large scale, i.e., computation based.

METHODS & RESULTS

During the past year, we have continued to develop strategies to maximize the usage of RAM to enable the all-by-all sequence comparison using BLAST for the largest Pfam protein families. This has been problematic due to 1) wall time restrictions of 24 hours that recently were increased to 48 hours, and 2) the limited amount of RAM that is available for the input and output (64 GB/node) that makes node usage "inefficient."

We now are exploring the use of DIAMOND, a recently developed alternative to BLAST, for the all-by-all sequence comparisons. We have observed that DIAMOND provides a greater than or equal to 10-fold increase in the rate of all-by-all sequence comparisons relative to BLAST for almost all Pfam protein families. This significant decrease in time "solves" the wall time problem for all Pfam protein families and allows a greater number of nodes to be assigned to the largest Pfam protein families, thereby allowing the all-by-all sequence comparisons to be accomplished. We expect to be able to begin the production phase of this project in which the library of SSNs for all 16,295 Pfam protein families can be updated every eight weeks (with each update of the InterPro protein sequence analysis and classification database).

WHY BLUE WATERS

The project uses an embarrassingly parallel computing model to perform the all-by-all sequence comparison and, in principle, could be run on any cluster of sufficient size. However, because of 1) the scale of the computation (number and sizes of Pfam protein families) and 2) the time sensitivity of the production of the output relative to IntroPro database updates, only a resource at the scale of Blue Waters can perform the job in a reasonable time frame.

NEXT GENERATION WORK

We hope to more efficiently (with respect to RAM and node usage) perform the all-by-all sequence comparisons for all Pfam protein families, and eventually larger clans so that these can be disseminated to the community with each update of the InterPro database.