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The International Nucleome Consortium

Satoshi Tashiro[1,2,*] and Christian Lanctôt[3,*]

[1]Institute for Radiation Biology and Medicine; Hiroshima University; Minamiku, Hiroshima, Japan; [2]Research Center for the Mathematics on Chromatin Live Dynamics (RcMcD); Hiroshima University; Minamiku, Hiroshima, Japan; [3]Institute of Cellular Biology and Pathology; First Faculty of Medicine; Charles University in Prague; Prague, Czech Republic

The eukaryotic genome adopts in the cell nucleus a 3-dimensional configuration that varies with cell types, developmental stages and environmental condition as well as between normal and pathological states. Understanding genome function will therefore require the elucidation of the structure-function relationship of the cell nucleus as a complex, dynamic biological system, referred to as the nucleome. This exciting and timely task calls for a multi-faceted, interdisciplinary and multi-national effort. We propose the establishment of an International Nucleome Consortium to coordinate this effort worldwide.

Keywords: international consortium, nucleome, 4D genome organization, research themes, scientific collaboration

*Correspondence to: Satoshi Tashiro; Email: ktashiro@hiroshima-u.ac.jp; Christian Lanctôt; Email: christian.lanctot@lf1.cuni.cz

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Background and Rationale

The last 2 decades have seen a genomic revolution. Genomes can now be routinely sequenced, high-throughput sequencing has become a valuable tool in many experimental approaches and sequencing of patients' DNA in a clinical setting is rapidly becoming a reality. This genomic revolution, however, has been a unidimensional one. Chromosome maps, sequences, polymorphism databases, this wealth of information about the linear sequence of the DNA does not take into account cellular context. Yet our genome lives as a 3-dimensional object, intricately folded and packaged in the cell nucleus, structured around nuclear bodies, acted upon by countless force-generating protein complexes and remodeling factors. Ultimately then, understanding how the genome works will require the elucidation of the structure-function relationship of the cell nucleus as a complex, dynamic biological system, hereafter referred to as the nucleome. Developing integrated 4-dimensional maps of genome organization and function is a task much larger in scale and complexity than the sequencing of genomes. However, we believe it is also one of the most exciting and promising challenges now facing biomedical research. With the recent advances that have been made in microscopy, chromatin conformation analysis and modeling, the time is at hand to tackle this challenge in a concerted way and on a global scale. We propose the formation of an International Nucleome Consortium to enhance the achievement of this goal.

In June 2013, a group of scientists from 14 countries met in Mainz, Germany for a conference, "The 4D Nucleome Workshop," to discuss the present state and future directions of this nascent field. This initial meeting was followed by a second 4D Nucleome workshop in December 2014 in Hiroshima, Japan. A consensus was reached during both conferences that, given the complexity and multi-faceted nature of the problem, large-scale collaborations among laboratories with distinct and complementary expertise around the world would be required to solve the nucleome problem. A recommendation to form an International Nucleome Consortium (INC) was strongly supported, first in Mainz and again in Hiroshima.

We are fortunate to be witnessing a convergence of major breakthroughs in microscopy, high-throughput genome conformation analysis and large scale gene expression analysis that now makes the challenging task of deciphering the nucleome feasible. We are also fortunate that the increasing realization of the need to interpret genomic information in its

cellular context is attracting to nucleome research an ever-increasing number of scientists with varied backgrounds, from cell biologists to mathematicians and computer scientists, from geneticists to microscopists and physicists. What is needed at this time is to reinforce the sense of community within the field, to pool expertise, to foster international collaborations, to define "nucleomic" standards and to set common short- and long-term goals. These are precisely the major aims of the International Nucleome Consortium.

Nucleome efforts have commenced on 3 continents. The US. National Institutes of Health launched in December 2014 the 4D Nucleome Initiative linked to the NIH Director's Common Fund. This $120M/5 year granting initiative focuses on the development of technologies to map genomes in space and time with the goals of understanding "... the principles behind the 3-dimensional organization of the nucleus in space and time (the 4th dimension), the role nuclear organization plays in gene expression and cellular function, and how changes in the nuclear organization affect normal development as well as various diseases." (http://commonfund.nih.gov/4Dnucleome/index). Initial steps toward a European 4D Nucleome Initiative have been taken, with close communication with leaders of both the NIH Initiative and those of us behind this article and the International Nucleome Consortium. In Japan, there are several research groups closely related to the nucleome and they are actively engaged in considering how their countries branch of the International Nucleome Consortium project can be developed and funded. These efforts in Japan together with those underway in the US and Europe comprise the International Nucleome Consortium and are envisioned as providing the necessary framework to create links and coordinate efforts among existing and future national or regional initiatives in the field. Given the importance of the research problem and the global array of scientific talent, a cardinal principle of the International Nucleome Consortium is to be inclusive of all approaches and projects that seek to read the genome in 4 dimensions.

The overarching goal of research into the nucleome is to determine the structure-function relationship of the genome within the space of the cell nucleus in physiologically and pathologically relevant biological contexts. To achieve this goal will require the comprehensive description of nuclear components, the identification of their mutual interactions, and the observation of the local and global effects of manipulating them. In view of the tremendous complexity of the nucleome, its embedding in cellular, tissue/organ and organismal contexts, and its known responsiveness to the environment, it is clear that such investigations must be performed at multiple scales of organization, using different biological models and under various conditions. We propose that the major goals of nucleome research should encompass the following 6 general areas.

Generation of Comprehensive, High Resolution Reference Maps of Genome Organization and Nuclear Architecture

The nucleome is shaped by a variety of factors acting in a hierarchical fashion: DNA sequence, DNA methylation, histone modifications, non-coding RNAs, long-range chromatin interactions, replication timing, and nuclear localization. It has become evident that the resulting topology impacts nuclear functions such as transcription, RNA processing, DNA repair and replication.[1] For instance, the position of a genomic segment within the nucleus can affect gene expression levels. The same applies to the position of a gene relative to its own chromosome territory or to nuclear compartments such as the lamina or heterochromatin. At a more local level, the mammalian nucleome is physically partitioned into smaller ~1 Mb domains, which have been proposed to constitute the structural basis for long-range regulation of transcription.[2] Hence, comprehensive topological maps of the nucleome at all levels of organization are needed if we are to understand not only how features of 3D genome folding and nuclear architecture relate to normal cellular functions such as pluripotency, cell fate choices and reprogramming, but also how changes in this folding and architecture can lead to aberrant gene expression patterns and disease phenotypes. It is also crucial to determine where on these 3D maps the various nuclear activities take place.[3]

Determination of the Relationship Between Nucleome Dynamics and Genome Function

The nucleome is a living structure. Its organization differs depending on cell type and developmental stage, and it reacts to changes in cellular metabolism and to environmental stress. A growing number of cases of gene regulation are being linked to specific rearrangements of short- and long-range interactions within the surrounding chromatin.[4] Interestingly, such regulatory contacts have recently been found to occur in the context of fluctuating chromatin structures.[5] With the recent development of single-cell Hi-C and single-cell transcriptomics,[6,7] a systematic analysis of the relationship between dynamic 3D genome folding and gene expression is now at hand. A key goal here is to assess at high spatial and temporal resolution the sequence of changes that occur in the organization of the nucleome during key biological processes such as cellular differentiation, lineage commitment, regeneration or tumorigenesis. The functional importance of these changes will need to be validated, e.g. by targeted manipulation of the known and the yet-to-be identified factors that shape the nucleome.[8,9] While nucleome dynamics is widely acknowledged as an important parameter of nuclear function, a number of questions remain. For instance, are local and global changes in nucleome structure coordinated? On what time scale do such changes occur? What forces are at work? How are they produced and controlled? Answers to these questions lie at the interface between molecular cell biology and physics, an area that can now be explored,[10] for the nucleus thanks to the newly available genome engineering and imaging tools to visualize nucleome dynamics at high temporal and spatial resolution in living cells.

Comparative Analysis of Nucleomes in Evolution, in Model Organisms and Within Tissue Environments

Despite its dynamic nature, the organization of the nucleome surely must obey a number of constraints. These are best identified through comparative evolutionary approaches. In order to determine which features of the 4D nucleome are evolutionarily-conserved, and thus presumably constrained, and which ones are species-specific, a broad range of species will need to be included in nucleome research programs, from unicellular organisms to higher vertebrates. A powerful example of this multi-species approach is the finding that the heterochromatin in the outer rod photoreceptor cells of crepuscular and nocturnal mammals is clustered in the center of the nucleus and that this arrangement leads to an increased efficiency of light channeling through the retina, arguably one of the most striking links between nuclear architecture and cellular function.[11] Fundamental insights will also continue to be gained from the use of established cell lines as well as model organisms including but not limited to *S. cerevisiae, C. elegans, D. melanogaster*. The availability of large collection of mutants and transgenics to investigate nucleome structure and function in these model organisms will continue to prove particularly useful, as will the application of high-throughput RNAi screens to identify key determinants of nucleome structure and function.[12,13] In addition, the nucleome will need to be studied more intensively *in situ* in the context of tissue organization, where complex cell-cell interactions and tissue environment undoubtedly participate in the shaping of cell-type specific nucleomes. Methods for probing the nucleome at the tissue level are now rapidly advancing.

Development of Mathematical Models of Nucleome Structure and Function

Several modeling approaches have been applied to elucidate the general principles that organize the nucleome and regulate its transcriptional output. For instance, 4 dimensional representations of the nucleome have been generated based on generic polymer models, structure-based molecular models and network theory.[14,15] It is obvious that not all aspects of nucleome organization and function can be covered by a single model. Current models are often built at specific space and time scales. The challenge is now for models to incorporate more experimental details and provide overlap between the different levels of nucleome organization in order to accurately represent nucleome behaviors across time and space as well as to properly record key differences and the clues such may offer. It will also be imperative that the generated models are not just self-fulfilling but are focused on making predictions for novel, testable functionalities of the nucleome and that a strong pipeline is created that iteratively tests model-generated predictions by direct experimentation. One of the key requirements in these approaches is that modeling rely on high-quality, well-annotated experimental data sets. To fully exploit the potential of the experimental data generated by chromosome capture-technology methods, imaging of gene loci or any other strategy, public databases to deposit and exchange large amounts of data, as well as high performance supercomputers, are required. Moreover, the close collaboration of mathematicians, physicists and biologists will be essential not only for the establishment but also for the validation of models and predictions.

Development and Implementation of Novel Technologies to Analyze the Nucleome in 4D

Progress in science is often limited by technology. In the case of nucleome research, this notion is best illustrated by the rapid advances that were made possible by the development of chromosome conformation capture techniques in recent years.[16] There is therefore a need to constantly advance the methodological toolbox that is at the disposal of nucleomists. In particular, further improvements in single-cell technologies promise to shed new light on the link between the nucleome and cellular functions. These technological improvements include increased resolution and facile throughput of single-cell Hi-C techniques, development of novel multiplex reporter systems for the simultaneous imaging of multiple components and activities across the nucleome, and implementation of high-performance image analysis algorithms. As in other fields, super-resolution microscopy is proving to be an invaluable tool in nucleome research.[17] Its combination with high-throughput screening will be essential to elucidate the mechanistic basis of nucleome organization and function. It cannot be overstated that the sub-200 nm spatial resolution now afforded by super-resolution light microscopy is precisely in the range where emerging hypotheses about the nucleome are centered. In addition, novel approaches focused on determining force generation or metabolic energy deployment in the nucleus are needed to understand the nucleome at the interface of physics and biology and determine how the mechanics and energetics of this complex system influence its structure and function. Finally, it is necessary in the context of an international research effort to harmonize data formats and set standards for data collection and analysis. The International Nucleome Consortium is the ideal platform to achieve this task, bridging, aligning and integrating the supercomputer powers of the US, Europe and Japan.

Linking Nucleome Alterations to Disease Phenotypes

Several pathological conditions have been related to nucleomic alterations. It has long been recognized that the nuclei of cancer cells display characteristic features of chromatin and nucleoli, collectively known as nuclear anaplasia, that distinguish them from normal nuclei and can be used in the diagnosis and grading of malignancies. In addition to these higher-order changes, chromatin remodeling factors and histone modifiers have been implicated in cancer. Laminopathies, i.e. diseases caused by mutations in the

lamin protein genes, are intriguing examples of pathologies that are associated with defects in the organization of the nucleome.[18] Such changes at the level of chromosomes, chromatin and/or DNA have been suggested to lead to pathological morphological changes of the cell nucleus. However, the biological significance of these changes remains to be clarified. Alteration of genetic information induced by endogenous and exogenous DNA damage in cancer cells has been suggested to lead to the dysregulation of genomic activities including transcription, replication and DNA repair. Recently, chromosomal translocations have been observed in living cells to occur preferentially between genome elements that are in close spatial proximity,[19] highlighting the need to investigate disease-related genome modifications in the context of the cell nucleus. Current and future methods should therefore be applied 1) to understand how nucleome structure and function relate to pathogenesis, especially cancer; and 2) to develop new diagnostic and therapeutic tools.

Conclusion

Being able to read the linear language of genomes is not enough. What is essential is to be able to visualize them in time and space and to functionally characterize them as 4D nucleomes. This constitutes a major enterprise, one which will require a multi-faceted, interdisciplinary and multinational effort over the next decades. We have here articulated the establishment of an International Nucleome Consortium to coordinate this effort. The activities of the Consortium will include the promotion of nucleome research itself, the organization of international workshops and conferences on the 4D nucleome, the setting up of exchange programs between research groups, the creation of platforms to share expertise and reagents, and the pursuit of support for this major cause in the 21st century of biological and biomedical science.

Disclosure of Potential Conflicts of Interest

The authors declare no potential conflicts of interest.

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