平成２４年度採択　文部科学省・生命動態システム科学推進拠点事業
広島大学「核内クロマチン・ライブダイナミクスの数理研究拠点形成」記念シンポジウム

第１回クロマチン動態数理研究拠点国際シンポジウム

講　演　要　旨　集

日時：平成２５年３月１５日（金）～１６日（土）

場所：広島大学大学院理学研究科 大会議室（E203）

15-Mar		
10:00	Prof. Toshimasa Asahara The president of Hiroshima Univ.	Opening Address in Japanese
10:05-10:10	Prof. Manabu Abe Vice dean of the School of Science	Opening Address in Japanese
10:10-10:20	Shin-ichi Tate	Brief introduction to the research project: the Mathmatics on Chromatin Live Dynamics (Japanese)
10:20-11:30	Christoph Cremer	Quantitative lightoptical analysis of nuclear genome organization and its perspectives for numerical modeling of chromatin structure
11:30-13:30	Lunch	
13:30-14:10	Vincent Dion	Live imaging of damaged DNA: implications for genome stability
14:10-14:40	Satoshi Tashiro	Homologous Recombinational Repair in Interchromatin Compartment
14:40-15:10	Yusuke Miyanari	A novel application for uncovering nuclear dynamics
15:10-15:30	Break	
15:30-16:00	Yuichi Togashi	“Minorities” may Rule Reaction-Diffusion Processes in the Cell
16:00-16:30	Akinori Awazu	Theoretical and computational biophysical approaches to analyzing interplays between chromatin dynamics and gene expressions
16:30-17:00	Shin-ichi Tate	Gene regulation in the chromatin context seen from the protein structural dynamics point of view
16-Mar		
9:30-10:15	Yasushi Hiraoka	Live-cell imaging of chromosome dynamics in fission yeast
10:15-11:00	Hiroshi Kimura	Monitoring the dynamics of transcription and epigenome modifications in living cells and organisms
11:00-11:30	Hiroshi Ide	Effects of Covalently Trapped Proteins on DNA Transactions
11:30-13:00	Lunch	
13:00	Post Conference Lecture Course	
	Christoph Cremer	Nanoscale Imaging of Chromatin: Quantitative Fluorescence Microscopy at Molecular Optical Resolution
	Vincent Dion	Chromatin dynamics during DNA repair: implications for human disease

The 1st International Symposium, Mathematics on chromatin dynamics, Hiroshima

Quantitative lightoptical analysis of nuclear genome organization and its perspectives for numerical modeling of chromatin structure

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The existence of chromosome territories (CT) and their distribution in the human cell nucleus poses strong constraints for the topography of given DNA sequences and gene domains and hence also for the induction of specific chromosome aberrations (Cremer T and Cremer C, 2001 and 2006). Computer simulations of large scale nuclear genome structure based on the '1-Mbp Spherical Chromatin Domain Model' allowed to obtain quantitative predictions on CT distribution, and to predict chromosome aberration yields induced by ionizing radiation (Kreth et al. 2004a,b). So far, however, chromatin analysis and modeling was restricted to the Megabasepair level, corresponding to the established resolution limits of far-field light microscopy (ca. 200 nm in the object plane and 600 nm along the optical axis). Recently, various methods of advanced light microscopy/nanoscopy have been developed which made possible to push the spatial resolution of nuclear structures far beyond these limits (Cremer C., 2012; Cremer C and Masters 2013). Quantitative nuclear nanostructure analysis based on structured excitation illumination/structured illumination microscopy (SEI/SIM), and spectrally assigned localization microscopy (SALM) currently allow to resolve fluorescence labeled nuclear structures down to the range of few tens of nanometer in 3D intact cells. Application examples will be presented on the use of SALM/SEI/SIM to measure the size of individual small chromatin domains, of replication units, and of transcription/splicing related structures. Quantitative analyses of the topography of replicating DNA and nascent RNA, RNA polymerase II and histone modifications typical for transcriptionally competent and non-competent chromatin, respectively, support the chromosome territory – interchromatin compartment (CT-IC) model (Markaki et al. 2010 and 2012). Progress in super resolution fluorescence microscopy is steadily narrowing the resolution gap to electron microscopy (Rouquette et al. 2009 and 2010; Hübner et al. 2013) with the added potential of ultrastructural studies of nuclei in living cells. Some perspectives of these novel, quantitative "superresolution" microscopy methods for deciphering the 'chromatin folding code' and for the numerical modeling of chromatin nanostructure and its dynamics will be discussed.

Cremer C. 2012. In: Springer Handbook of Laser and Optics. Editor: Frank Träger. Springer 2012, pp. 1359-1357; Cremer C and Masters BR. 2013. Eur Phys J H: DOI: 10.1140/epjh/e2012-20060-1; Cremer T and Cremer C. 2001. Nat Rev Genet 2: 292-301; Cremer T and Cremer C. 2006. Eur J Histochem 50: 161-176 and 223-272; Hübner et al. 2013. Meth Mol Biol, in press; Kreth et al. 2004a. Biophys J 86: 2803-2812; Kreth et al. 2004b. Cytogenet Genome Res 104: 157-161; Markaki Y et al. 2010. Cold Spring Harb Symp Quant Biol 75: 475-492; Markaki Y et al. 2012. Bioessays 34: 412-426; Rouquette J et al. 2009. Chromosome Res 17: 801-810; Rouquette J et al. 2010. Int Rev Cell Mol Biol 282: 1-90

Live imaging of damaged DNA: implications for genome stability

Vincent Dion, Véronique Kalck, Frank Neumann, Andrew Seeber, Lutz Gehlen, Chihiro Horigome, and Susan M. Gasser

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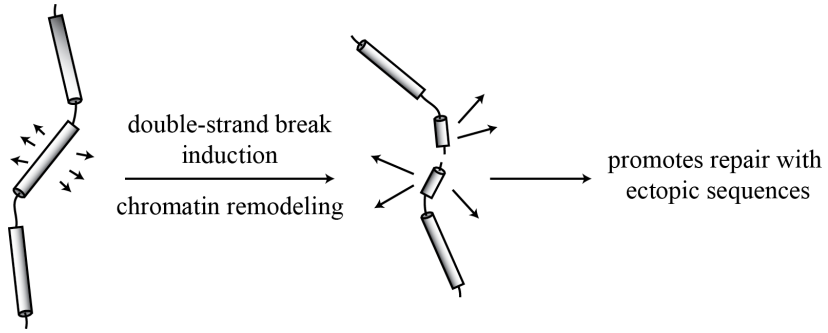
The generation of cancer-causing translocations in the human genome is thought to depend, at least in part, on the mobility of damaged DNA. Unfortunately, the available data in higher eukaryotes is contradictory. Here we take advantage of live cell imaging in budding yeast to address the following questions:

1) Does chromatin mobility change upon DNA damage?
2) What are the factors driving the mobility of damaged and undamaged DNA?
3) What are the implications of chromatin mobility for the repair of DNA breaks?

We find that the induction of a single double-strand break (DSB) increases the mobility of a locus dramatically. Interestingly, this increase in movement is lesion-specific and does not occur at protein-DNA adducts. We find that the enhanced mobility of DSBs require factors involved in DNA damage signaling (i.e., Mec1 and Rad9), homologous recombination (e.g., Rad51), and chromatin remodeling (e.g., INO80 and Rad54). Importantly, the mobility of chromatin correlates with the efficiency of homologous recombination in cases where a repair template is provided on another chromosome. Together these data suggest that mobility is driven by chromatin remodeling enzymes and can promote translocations with distant sequences.



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2) Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. Neumann FR, Dion V, Gehlen LR, Tsai-Pflugfelder M, Schmid R, Taddei A, Gasser SM. Genes Dev. 2012 Feb 15;26(4):369-83.
3) Chromatin Movement in the Maintenance of Genome Stability. Dion V and Gasser SM. Cell, in press.

Homologous Recombinational Repair in Interchromatin Compartment

Satoshi Tashiro[1], Hiroki Shima[2], Hidekazu Suzuki[1], Kazuteru Kono[1], and Thomas Cremer[3]
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[3]Dept Biol. II, LMU)

The territorial organization of interphase chromosomes is now generally accepted as a basic principle of nuclear organization in animals. The chromosome territory-interchromatin compartment (CT-IC) model argues that nuclei are built up from two principal components, chromosome territories and the interchromatin compartment (IC). Several line of evidence suggests that the periphery of a chromatin domain bordering the IC represents the major nuclear sub-compartment for transcription, or co-transcriptional RNA splicing and DNA replication. However, the association of DNA metabolism, including DNA repair, with the higher order nuclear architectures are largely unknown.

Homologous recombination repair (HR) is the accurate repair pathway of DNA double-strand breaks (DSBs) produced by endogenous and exogenous factors like ionizing radiation. A fraction of proteins involved in the HR pathway have been shown to accumulate at sites containing DNA damage to form damage induced nuclear foci. Moreover, damaged chromatin is shown to be mobile after induction of DNA damage in a manner dependent on HR factors. However, the biological significance of the dynamic rearrangements of higher order nuclear architectures around damaged chromatin is still unclear.

RAD51 is a key factor in the HR pathway loaded on single stranded DNA processed around DSBs. In our previous study, we showed that RAD51 forms nuclear foci in S phase and at sites containing DNA damages. Recently, we found that the overexpressed RAD51 is accumulated within bundle-like sub-compartments of IC in human cell nucleus. Single stranded DNA formed at sites containing DNA damage for HR was detected within the sub-compartments. In contrast, the phosphorylated form of histone H2AX around DSBs is located outside of the sub-compartment. These findings support the notion that damaged chromatins are moved into the bundle-like sub-compartment of IC for HR, or HR factory. The molecular mechanism required for the formation of HR factory will be discussed.

A novel application for uncovering nuclear dynamics

Yusuke MIYANARI and Torres-Padilla Maria-Elena
IGBMC, Strasbourg, FRANCE

Abstract;
3D nuclear architecture is emerging as a key player in gene regulation. Although the nuclear organization is drastically restructured during mammalian development, its role remains largely unexplored, possibly due to technical difficulties to study "nuclear dynamics". The live imaging of nuclear architecture is inaccessible by existing approaches such as DNA-FISH and chromosome conformation capture (3C). Only fluorescent-tagged LacO system could be adapted for that, where integration of huge LacO array (~10 kb) into specific genomic region is required, which is laborious and not efficient. To overcome the technical limitation, we developed a novel technique allowing the spatiotemporal organization of target sequences to be monitored in living cells. Given its flexibility, this system provides new approaches for studying nuclear dynamics.

"Minorities" may Rule Reaction-Diffusion Processes in the Cell

Yuichi Togashi
(Graduate School of System Informatics, Kobe University)

Reactions are the basis of a variety of activities of the cell. When we study intracellular processes theoretically or computationally, we often model them as reaction-diffusion systems, typically represented by partial differential equations of chemical concentrations.

Actually, this classical scheme has preconditions that molecules are:

1. *Memoryless:* no internal dynamics; mutually independent reaction events.
2. *Tiny:* no excluded volume; free, normal diffusion.
3. *Many:* no finite-size fluctuations; represented by continuous concentrations.

However, we cannot assume these in the complex intracellular environment. Cells are highly crowded with macromolecules. Among them, there are molecular machines such as enzymes and motors, which often show slow dynamics (milliseconds to seconds). Moreover, many chemical species occur in the order of 1 molecule each per cell, and some species are missing at each moment, as recently shown by Taniguchi *et al*.

Gene expression is an extreme case. Each type of transcription factor does not exist in a large number of molecules per cell, and DNA copies are even fewer. Thus, interactions between such "minorities" are inevitable, which in general make the system's behavior stochastic and unstable. Indeed, genetically identical cells show diversity, which can be beneficial for adaptation and evolution. On the other hand, cells seem robust against external perturbations. What kinds of mechanisms are required to reconcile these two features? We are now trying to address this question, with close attention to "minority" chemicals in the cell.

We showed that discreteness of molecules may become significant for such "minorities", and drastically change properties of catalytic reaction systems [1]. When "minorities" are involved, not only the numbers but also the states (or individuality) of molecules and their spatio-temporal patterns can affect the behavior of the whole system [2]. The structural dynamics of chromatin may play a crucial role in gene expression, or genetic information processing, also in this way. Our on-going collaboration on the "minority" issues will be also introduced [3].

References

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6) http://www.paradigm-innovation.jp/ ; see also http://www.togashi.tv/lab/

Theoretical and computational biophysical approaches to analyzing interplays between chromatin dynamics and gene expressions

Akinori Awazu[1]

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Recently, along with the progresses of the experimental techniques and investigations, the theoretical and computational methods have also been developed for the analysis of the dynamical and statistical features of living systems in several spatiotemporal scales. In this talk, we introduce some of our recent studies of the micro- and macroscopic biological phenomena as bellows by the following theoretical methods; i) Inferences of models or regulatory networks by the statistical analysis of experimental data, and ii) the constructions and simulations of the phenomenological coarse-grained models of molecular dynamics,

1). Inferences of the signaling and gene network regulating a part of the metabolic network of Arabidopsis thaliana by the statistical analysis of gene expression data in experiments
2). Time series analysis of the trajectory data of individual ants and the constructions of Markov model of the behaviors of ant populations.
3). Analysis of the fluctuations of proteins and DNA based on the normal mode analysis to uncover the contributions of their structures and fluctuations to their functions.
4). Coarse-grained modeling of various molecules to simulate and analyze the influences of the molecular crowding on the enzyme reaction and the signal transduction processes on bio-membranes.

We also discuss the perspectives of the analysis of the chromatin dynamics regulating gene expressions and their feedbacks through the application of the present approaches.

Gene regulation in the chromatin context seen from the protein structural dynamics point of view

Shin-ichi Tate
(Dept. Math. and Life Sciences, Hiroshima University)

Chromatin remodeling is the most essential process in the entire genomic regulation inside nucleus. A variety of protein factors are found to be engaged in the process in a concerted manner. One remarkable structural feature associated with those nuclear protein factors is that they contain high content of the unusual sequences commonly existing in unstructured part of proteins, which part are names as 'intrinsically disordered (ID)' segments. The high content of the ID segments in the nuclear factors evoked the interests in their specific roles in the functional regulations in the context of chromatin.

We have been working on a various types of chromatin associating proteins in addition to the nucleosome itself in primarily focusing on the roles of the ID segments in the proteins [1-5]. In this presentation, we will describe that the two ID segments in the FACT (FAcilitate Chromatin Transcription), a type of chromatin remodeler, can regulate its nucleosome binding activity according to the phosphorylation in an '*ultra-sensitive*' way. We will show how the phosphorylation to the ID parts achieve the characteristic regulation using numerical model and also the coarse grained mechanical model. The present analyses prompt us to use the segment as a probe to see the chromatin dynamic in the cells.

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Live-cell imaging of chromosome dynamics in fission yeast

Yasushi Hiraoka

Graduate School of Frontier Biosciences, Osaka University

We are trying to understand how chromosomes are spatially organized within the nucleus and how they are regulated. We have examined chromosome structures and dynamics in the fission yeast *Schizosaccharomyces pombe* by combination of fluorescence imaging and molecular genetics.

S. pombe provides a striking example of nuclear reorganization during the transition from mitosis to meiosis. During the mitotic cell cycle, centromeres cluster near the spindle pole body (SPB, a centrosome-equivalent structure in fungi) and telomeres locate at the nuclear periphery. In contrast, upon entering meiosis, telomeres become clustered to the SPB, and the nucleus moves back and forth between the cell ends to form an elongated nucleus [1]. During the nuclear movements, telomeres remain clustered at the leading edge of the moving nucleus. The nuclear movements facilitate the paring and subsequent recombination by aligning homologous chromosomes along their entire length [2], and also provides a unique opportunity to examine chromatin structure within a defined orientation of the chromosomes [3].

During meiosis, pairing and recombination of homologous chromosomes are essential for proper chromosome segregation. It has been demonstrated that spatial alignment of chromosomes bundled at the telomeres plays a role in homologous chromosome pairing [2], and that SUN-KASH conserved nuclear membrane proteins move telomeres to bundle chromosomes during the pairing process in meiosis identified [4, 5]. Furthermore, we recently found that RNA transcripts retained on the chromosome play an active role in recognition of homologous chromosomes for pairing in *S. pombe* [6].

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Monitoring the dynamics of transcription and epigenome modifications in living cells and organisms

Hiroshi Kimura

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Histone modification plays a critical role in genome functions such as the epigenetic gene regulation and the maintenance of genome integrity. These modifications change locally and globally during the cell cycle, development and differentiation, and in response to external stimuli. However, it remains largely unknown how these modifications are regulated at single cell levels (1). We have developed a method to visualize histone modifications in living cells by loading the fluorescently labeled antigen-binding fragments (Fabs). This Fab-based live endogenous modification labeling technique has revealed the differential regulation of H3S10 phosphorylation between normal and cancerous cells and the distinct behaviors of H3K9 and H3K27 acetylation in mouse preimplantation embryos produced by in vitro fertilization and somatic cell nuclear transfer (2, 3). We now applied this technique to measure the kinetics of RNA polymerase II, which is assembled into the preinitiation complex as unphosphorylated form, and becomes phosphorylated at Ser5 and Ser2 in the C-terminal domain repeats. By timing the recruitment of these different phosphorylation marks to a gene array upon the stimulation of transcription and by fitting the data to a mathematical model, it was able to differentiate the initiation kinetics from the recruitment and elongation kinetics. Our observations suggested that transcription on the array is quite efficient and that the elongation efficiency is correlated with the preexisting histone H3 acetylation level. I will also present a genetically encoded system to monitor the posttranslational modifications in living cells and organisms.

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Effects of Covalently Trapped Proteins on DNA Transactions

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Chromosomal DNA is associated with various proteins involved in DNA folding and transactions. The association between DNA and proteins is reversible, and when prompted, proteins dissociate from or translocate along the DNA strand, leaving the open nucleotide sequence available for DNA transactions. However, many endogenous and environmental agents covalently trap proteins on DNA, generating DNA-protein cross-links (DPCs) (1). We have been assessing the effects of DPCs on DNA replication, transcription, and repair using *in vitro* and *in vivo* systems.

Our *in vitro* study using replicative helicases has shown that DPCs on the translocating strand but not on the nontranslocating strand impede the progression of the helicases, suggesting that DPCs on the translocating and nontranslocating strands constitute helicase and polymerase blocks, respectively (2). A similar study with T7 RNA polymerase (RNAP) shows that DPCs on the transcribed strand but not on the nontranscribed strand block T7 RNAP. Interestingly, both leading and trailing T7 RNAPs stalled by DPCs become highly error prone and generate mutations in the upstream intact template regions (3).

With respect to repair, DPCs impair the loading of UvrB, a damage-recognition protein involved in bacterial nucleotide excision repair (NER), onto the DPC site in a DPC size-dependent manner (4). In mammalian cells the upper size limit of cross-linked proteins amenable to NER is around 8 kDa, eliminating the role of NER in the repair of DPCs *in vivo* (5). DPCs not repaired by NER are processed by homologous recombination in bacterial and mammalian cells (4,5).

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