Flexible and dynamic nucleosome fiber in living mammalian cells

Tadasu Nozaki1,2, Kazanari Kaita3, Chan-Gi Pack4, Sachiko Tamura5, Tomomi Tan6, Saera Hihara1, Takehuru Nagai7, Koichi Takahashi2,3, and Kazuhiro Maeshima1,6,*

1Biological Macromolecules Laboratory; Structural Biology Center; National Institute of Genetics; Mishima, Japan; 2Institute for Advanced Biosciences; Keio University; Fujisawa, Japan; 3Laboratory for Biochemical Simulation; RIKEN Quantitative Biology Center; Suita, Japan; 4Cellular Informatics Laboratory; RIKEN; Wako, Japan; 5Cellular Dynamics Program; Marine Biological Laboratory; Woods Hole, MA USA; 6Department of Genetics; School of Life Science; Graduate University for Advanced Studies (Sokendai); Mishima, Japan; 7The Institute of Scientific and Industrial Research; Osaka University; Ibaraki, Japan

*Correspondence to: Kazuhiro Maeshima; Email: kmaeshim@lab.nig.ac.jp

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Genomic DNA is organized three dimensionally within cells as chromatin and is searched and read by various proteins by an unknown mechanism; this mediates diverse cell functions. Recently, several pieces of evidence, including our cryomicroscopy and synchrotron X-ray scattering analyses, have demonstrated that chromatin consists of irregularly folded nucleosome fibers without a 30-nm chromatin fiber (i.e., a polymer-melt-like structure). This melting structure implies a less physically constrained and locally more dynamic state, which may be crucial for protein factors to scan genomic DNA. Using a combined approach of fluorescence correlation spectroscopy, Monte Carlo computer simulations and single nucleosome imaging, we demonstrated the flexible and dynamic nature of the nucleosome fiber in living mammalian cells. We observed local nucleosome fluctuation (~50 nm movement per 30 ms) caused by Brownian motion. Our in vivo-in silico results suggest that local nucleosome dynamics facilitate chromatin accessibility and play a critical role in the scanning of genome information.

Introduction

The long strand of genomic DNA must be organized three dimensionally in cells to scan and read genome information; this mediates various cellular functions. Genomic DNA is wrapped around core histones and forms a nucleosome fiber (bead-on-a-string or 30-nm fiber). The nucleosome has long been assumed to be folded into a 30-nm chromatin fiber and further hierarchical regular structures. Although the higher-order chromatin structure in eukaryotic cells is not fully understood, several lines of evidence, including our recent cryomicroscopy and synchrotron X-ray scattering analyses, have demonstrated that chromatin in cells consists of irregularly folded nucleosome fibers without a 30-nm chromatin fiber, i.e., a polymer-melt-like structure.

Compared with the 30-nm chromatin fiber and further hierarchical regular structures proposed previously, irregular folding of nucleosome fibers, leading to a polymer-melt-like structure, implies a physically constrained state that could be locally dynamic. Nucleosome fibers may be constantly moving and rearranging at the local level, which might be essential for protein factors to scan genomic DNA and locate their target sequences. However, many previous studies, including ours, examined only static structures in fixed cells. Therefore, how can we know the chromatin environment in living mammalian cells, in which many protein molecules are freely mobile? How can proteins access their destinations on chromatin in living cells? To address these fundamental questions, we utilized a combined in vivo-in silico strategy of fluorescence correlation spectroscopy (FCS), single-molecule imaging and Monte Carlo computer simulations. Based on this strategy, we uncovered the unexpected...
local dynamics of individual nucleosomes in living mammalian cells (Fig. 1A)\textsuperscript{23}.

The results obtained here indicate that nucleosome fluctuation increases chromatin accessibility, which is advantageous for many "target searching" biological processes, such as transcription, DNA repair, replication and recombination (Fig. 1A)\textsuperscript{23}.

Fluorescence Correlation Spectroscopy (FCS)

Measurements of Interphase Chromatin and Mitotic Chromosomes in Living Cells

To investigate the chromatin environment in living cells, we first employed fluorescence correlation spectroscopy (FCS).\textsuperscript{23} FCS detects the in- and out-motions of fluorescent probe molecules, such as enhanced green fluorescent proteins (EGFPs), in a small detection volume (~0.4 μm in diameter by ~1–2 μm in height), as up- and down-fluorescence intensity fluctuations (Fig. 1B, F.I.). From the intensity fluctuations, we obtained the diffusion coefficient ($D$) for mobile EGFP probe molecules. $D$ shows how far the probe molecules can move in a certain range of time. In the crowded molecular environment, the $D$ of probe molecules becomes much smaller due to the many obstacles that the probes encounter (Fig. 1C).

Since the FCS detection size is much larger than human chromosomes (~0.7 μm),\textsuperscript{25} especially in height, we used Indian Muntjac DM cells.\textsuperscript{26} These cells have giant chromosomes whose size (~2 μm in diameter) is much larger than the detection volume. We obtained three stable DM cell lines coexpressing either the EGFP-monomer, -trimer or -pentamer and H2B-mRFP1.\textsuperscript{23} The three types of EGFP are probe molecules for detecting mobility in the FCS detection volume. The EGFP-trimer (~90 kDa) and -pentamer (~150 kDa) are used to examine the effect of molecular size on diffusion compared with the EGFP-monomer (~30 kDa);\textsuperscript{23,27,28} H2B-mRFP1 is the marker used to conform the chromatin region.\textsuperscript{26,27,28} Using these cell lines optimized for precise FCS measurement, we compared the $D$ values of EGFP probes

![Graphical abstract and FCS measurements in living mammalian cells](image.png)

**Figure 1.** Graphical abstract and FCS measurements in living mammalian cells. (A) Graphical abstract. In the cells, nucleosome fibers (red spheres and lines) are irregularly folded. Nucleosomes fluctuate, and this nucleosome dynamics facilitates chromatin accessibility. Chromatin fluctuation is the basis for scanning genome information. The image was reproduced from reference 23. (B) Schematic diagram of FCS detecting the in-out motion of EGFP molecules (green spheres) in a ~0.1-femtoliter volume (white-out cylinder region in the blue) as fluctuations in fluorescence intensity (shown as a graph). F.I., fluorescence intensity; t, time. The image was modified from reference 23. (C) Cartoon showing that fluorescent proteins (represented as runners) move more slowly in a crowded chromatin environment due to the many obstacles (represented as hurdles).

**Table 1.** Mean $D$ values ($\mu$m$^2$/s) of EGFP-monomers, -trimers and -pentamers in living cells.

| Solution  | Cytoplasm | Interphase chromatin | Mitotic chromosome |
|-----------|-----------|----------------------|-------------------|
| EGFP-monomer | 75.9 ± 2.3 | 23.4 ± 4.3 | 20.6 ± 3.6 | 14.5 ± 1.9 |
| EGFP-trimer  | 43.2 ± 1.4 | 11.2 ± 1.5 | 9.1 ± 1.9 | 6.9 ± 1.6 |
| EGFP-pentamer | 31.6 ± 2.3 | 7.3 ± 1.4 | 6.7 ± 1.1 | 3.6 ± 1.1 |

*The values were reproduced from reference 23.
In Silico Reconstruction of the Chromatin Environment Predicts that Nucleosome Fluctuation Facilitates Chromatin Accessibility

To characterize chromatin accessibility in living cells further, we reconstructed a chromatin environment in silico. To examine the mobility of EGFP-pentamers in the cytoplasm, interphase chromatin and mitotic chromosomes (Table 1). Surprisingly, while the chromosomes showed a highly condensed structure (~0.5 mM at nucleosome concentration), EGFP molecules were able to move inside chromosomes, and the D of mitotic chromosomes was only 30% lower than that of interphase chromatin (~0.05–0.1 mM). Even in the case of the EGFP-trimer and -pentamer, their diffusion profiles appeared to be similar. This suggests that high protein mobility in dense chromosomes can be observed by fluorescent probes of larger size. Our results in Table 1 indicate that interphase chromatin and mitotic chromosomes have considerable chromatin accessibility, implying a novel underlying mechanism, especially in dense chromatin regions.

Figure 2. Reconstruction of the living chromatin environment using Monte Carlo computer simulation. (A) The nucleosome is represented as a 10-nm red sphere and fixed in a restricted space at a concentration of 0.1 mM (left) or 0.5 mM (right, corresponding to mitotic chromatin) randomly but in a manner that avoids any overlap. The EGFP-pentamer is represented as a 13-nm sphere (green). See also Videos S1 to S3. (B) A simple scheme of the simulation procedure. For the details, see text. (C) Tracing patterns of three 13-nm spheres (EGFP-pentamers) under various conditions. At 0.1 mM of fixed 10-nm spheres (nucleosomes), the 13-nm spheres (EGFP-pentamers) move around freely (left image). However, at 0.5 mM of fixed 10-nm spheres (nucleosomes), the 13-nm spheres (EGFP-pentamers) are unable to move far from their starting points (middle image). In the environment with a fluctuation of 0.5 mM of the 10-nm spheres (nucleosomes), the 13-nm spheres (EGFP-pentamers) move around freely (right image), in contrast to the case of the fixed 10-nm spheres (nucleosomes, middle image). Each 10-nm red sphere (nucleosome) behaves like "a dog on a leash." The leash length is 20 nm. The three different temporal trajectories of the 13-nm spheres (EGFP-pentamers) for 0.2 ms are indicated in blue, green and red. (D) Cartoons showing that a protein (green) is stacked in a confined space of fixed nucleosomes (left), and the protein is able to move freely with fluctuation of the nucleosomes (right).
In these virtual chromatin environments, the simulation procedure was as follows (Fig. 2B): we first included spheres (Step 1 in Fig. 2B) and decided at random the order of the spheres to be moved (Step 2). The first sphere was then moved randomly (Step 3). Its displacement followed the three-dimensional normal distribution with the standard deviation ($SD = \sqrt{\Delta D t}$). We turned to the next sphere (Step 4) and repeated this process a certain number of times. Finally, we traced the movement of the 13-nm spheres (EGFP-pentamer). At 0.1 mM of the nucleosomes, the EGFP-pentamer could move almost freely (Fig. 2C, left; Vide. S1).23 Under the 0.5 mM condition, which corresponded to highly condensed chromatin environment, if the nucleosomes were fixed in space, the EGFP-pentamer could not move far from the starting point (Fig. 2C, middle; Vide. S2).23 They were stacked in a confined space, thus causing a serious mobility problem (Fig. 2D, left). We next performed a simulation with fluctuating nucleosomes. In this model, the nucleosomes were mobile, but their movements were restricted to a certain range, resembling “a dog on a leash” situation. In this dynamic environment, we observed apparent free diffusion of the EGFP-pentamer, even in 0.5 mM nucleosomes (Fig. 2C, right; Vide. S3; Fig. 2D, right). Strikingly, a 10- to 20-nm fluctuation of the nucleosomes was sufficient for the EGFP-pentamers to diffuse freely.23 Furthermore, even at the low concentration (0.1–0.4 mM) of nucleosomes such as interphase chromatin environment, the fluctuation facilitated mobility of the EGFP-pentamers.23 These simulation results suggest that nucleosome fluctuation facilitates chromatin accessibility for the diffusing protein;23 see also references 32 and 33 by Langowski group. Since the nucleosomes in cells, which are linked by linker DNA, fill more space with less freedom, nucleosome fluctuation may be critical for chromatin accessibility.

**Single Nucleosome Imaging in Living Mammalian Cells**

The next obvious question was whether nucleosome fluctuation could be detected in living mammalian cells. For this
Local Nucleosome Fluctuation in Living Mammalian Cells

We recorded nucleosome signals in interphase chromatin and mitotic chromosomes in living DM cells at a video-rate (~30 ms/frame) as a movie. For signal nucleosome tracking, we used PolyParticleTracker software.9 The dots were fitted to an assumed Gaussian point spread function to determine the precise centers of the dots with higher resolution.9 We were able to analyze the behaviors of the nucleosomes over short periods of time, ranging from 0 to 0.18 s.9 Notably, the displacement (movement) distributions of single nucleosomes of interphase and mitotic chromatin appeared similar (~50 nm per 30 ms).9

As a control, we measured the displacement of fluorescent beads (100 nm in diameter) fixed on a glass surface. The average displacement values were much smaller than those observed in living cells, excluding major contribution of microscopy system drift.9 To exclude the possibility that the detected movement was derived from global motion of the nuclei or chromosomes, we measured centroid movements for many nucleosomes in the same time frame. These values were again much smaller than the movements of individual nucleosomes in living cells.9 Therefore, we concluded that most of the observed displacement was due to the local movement...
movements and the displacement distributions in the $x$-$y$ plane were also shown in figures 3 and 4, respectively. The obtained distribution profile was consistent with our previous results. The results also showed that nucleosome fluctuation was not affected by differences in the fusion position of PA-GFP and the expression promoter.

Second, the displacement data of purified GFP fixed on a glass surface were obtained (fig. 4c and d). The average displacement values were $12.8 \pm 0.2$ nm per 30 ms, which was much smaller than those of

![Figure 5. Local nucleosome fluctuation in living mammalian cells.](image)

(fluctuation) of nucleosomes in living cells. In addition, we found that HeLa cells expressing a low level of PA-GFP-H4 showed considerable nucleosome mobility, suggesting that local nucleosome movement in mammalian cells is a general phenomenon. The McNally group also published single-nucleosome tracking data using H2B-EGFP, which appear to be consistent with our single nucleosome tracking with PA-GFP-H4.

To strengthen our conclusion in reference 23 and for further characterization of nucleosome fluctuation, we performed several additional measurements. First, we measured single H4-PA-GFPs expressed in DM cells. The expression was driven by a different expression promoter (pXY50) from those in the previous study (CDK1 and EF1-α promoters). To show the displacement distribution, we measured approximately 20,000 signal points with single nucleosome tracking with H4-PA-GFP from seven DM cells (figs. 3d, 4a and 4b), instead of the approximately 1000 points previously reported with PA-GFP-H4 in reference 23. Some representative trajectories of the nucleosome movements and the displacement distributions in the $x$-$y$ plane were also shown in figures 3d and 4b, respectively. The obtained distribution profile was consistent with our previous results. The results also showed that nucleosome fluctuation was not affected by differences in the fusion position of PA-GFP and the expression promoter.
H4-PA-GFP observed in living cells (39.0 ± 0.2 nm per 30 ms in Fig. 4A). Then, to evaluate the contribution of whole cell or nuclear movements to the single nucleosome displacements, we again calculated the centroid movements for many nucleosomes in the same time frame (illustrated in Fig. 5A). The centroid movements (3.3 ± 0.1 nm per 30 ms) (Fig. 5B) are much smaller than those in Figure 4A and B, suggesting that the detected nucleosome movement is not derived from the global movement of the cell or nuclei.

To analyze local nucleosome movement in DM cells further, the MSD values (μm²) of nucleosomes in the DM cells and fixed EGFP were plotted with their standard errors (Fig. 5C). The plots for the nucleosomes fitted well with the exponential equation, MSD = 0.022t0.36 (Fig. 5C). The MSD values increased rapidly in a short time and the steepness decreased over time, consistent with previous studies.23 Again, these results support the restricted nucleosome movement model.

It was reported that movements of large chromatin domains tagged with LacO arrays (~100 kb and above) were on the 10 and 100 nm length scale for 30 ms (e.g., ref. 40). We could not exclude the possibility that movements of larger domains might contribute to the local nucleosome fluctuation that we observed. Their relationship would be an intriguing issue for further study.

Local Fluctuation of Nucleosomes is the Basis for Scanning Genome Information

This study revealed local nucleosome fluctuation in living mammalian cells (Fig. 1A). Monte Carlo computer simulations suggested that nucleosome fluctuation facilitates the mobility of diffusing proteins in the environment (Fig. 1A). In addition, such nucleosome fluctuation can expose DNA sequences to the surface of chromatin domains more often, while static regular folding structures, such as 30-nm chromatin fibers, can hide or mask most DNA sequences. It should be emphasized that both facilitation of protein mobility and DNA exposure lead to an increase in chromatin accessibility. This is in good agreement with our finding that right cross-linking of nucleosomes blocked antibody accessibility and targetting in dense chromatin regions.24

Recently, we suggested that interphase chromatin forms numerous compact chromatin domains resembling “chromatin liquid drops” in interphase cells.9,10 This chromatin liquid drops view is in line with predictions of the chromosome territory-interchromatin compartment (CT-IC) model.41,42 In the CT-IC model, each CT is built up from a series of interconnected, mega-base-sized chromatin domains, which were originally identified using a pulse labeling as the DNA replication foci.42 Recent high-throughput 3C studies, such as Hi-C and 5C, have also proposed physical packaging of genomic DNA, termed “topologically associating domains”,67,68 “topological domains”67 or “physical domains.”68 Local nucleosome fluctuation would play an especially important role in such compact chromatin domains because the effect of nucleosome fluctuation on the facilitation of chromatin accessibility is more significant in a compact chromatin environment.55 Many biological processes, including transcription, DNA repair, replication and recombination, are based on the dynamic movement of nucleosome fluctuation on the facilitation of chromatin accessibility and/or specific proteins, will be an important factor in their regulation.

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Supplemental Materials
Supplemental materials may be found here:

www.landesbioscience.com/journals/nucleus/article/26053

References
1. Kornberg RD. Chromatin structure: a repeating unit of histones and DNA. Science 1974; 184:868-71. PMID:4825889; http://dx.doi.org/10.1126/sci ence.184.4139.868
2. Woodcock CL, Satre JB, Brandfield S. Structural rearranging units in chromatin I: Evidence for their general occurrence. Exp Cell Res 1976; 97:110-16. PMID:8172708; http://dx.doi.org/10.1016/0014-4827(76)90659-5
3. Olsen AL, Olsen DE. Spherical chromatin unit (± beads). Science 1976; 193:330-5. PMID:4628098
4. Tulk JT, King A. Solenoidal model for superstructure in chromatin. Proc Natl Acad Sci U S A 1976; 73:890-893. PMID:1646661; http://dx.doi.org/10.1073/pnas.73.6.8907
5. Woodcock CL, Fedeu LD, Ratter JF. The higher order structure of chromatin: evidence for a helical fibrous arrangement. J Cell Biol 1986; 99:62-52. PMID:6733853; http://dx.doi.org/10.1083/jcb.99.1.99
6. Fedor J, Manazuru L. A direct approach to the struct ure of subchromatic chromosomes. Cold Spring Harb Symp Quant Biol 1968; 33:35-50. PMID:582805; http://dx.doi.org/10.1101/sqb.1968.033.03.005
7. Belousov AS, Fedor JWC, Agard DA. A three-dimension al approach in mitotic chromosome structure: evidence for a complex functional organization. J Cell Biol 1997; 137:77-92. PMID:1124077; http:// dx.doi.org/10.1083/jcb.137.1.77
8. McDowell AW, Smith JM, Dobrochek J. Cryo electron microscopy of stabilized chromosomes in situ. EMBO J 1986; 5:395-402. PMID:375597
9. Belousov AS, Fedor JWC, Agard DA. A three-dimension al approach in mitotic chromosome structure: evidence for a complex functional organization. J Cell Biol 1997; 137:77-92. PMID:1124077; http:// dx.doi.org/10.1083/jcb.137.1.77
10. McDowell AW, Smith JM, Dobrochek J. Cryo electron microscopy of stabilized chromosomes in situ. EMBO J 1986; 5:395-402. PMID:375597
11. Eltsov M, Maclellan KM, Marusina K, Frangakis AS, Dobrochek J. Analysis of cryo-electron microscopy images does not support the existence of 30-nm chromatin fibers in mitotic chromosomes in situ. Proc Natl Acad Sci U S A 2006; 103:19732-7. PMID:1694512; http://dx.doi.org/10.1073/ pnas.0606077103
12. Marusina K, Hihara S, Eltsov M. Chromatin struct ure: are the X-ray fibres real? Curr Opin Cell Biol 2010, 22:291-7. PMID:20346642; http://dx.doi.org/10.1016/j.ceb.2009.03.002
13. Osawa F, Ching RP, Baeuerle JP. Living without X-ray Chromatin Shines. Trends Biochem Sci 2011, 36:1-6. PMID:21950299; http://dx.doi.org/10.1016/j. tibs.2010.09.002
