Microrheology for Hi-C Data Reveals the Spectrum of the Dynamic 3D Genome Organization

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ABSTRACT The one-dimensional information of genomic DNA is hierarchically packed inside the eukaryotic cell nucleus and organized in a three-dimensional (3D) space. Genome-wide chromosome conformation capture (Hi-C) methods have uncovered the 3D genome organization and revealed multiscale chromatin domains of compartments and topologically associating domains (TADs). Moreover, single-nucleosome live-cell imaging experiments have revealed the dynamic organization of chromatin domains caused by stochastic thermal fluctuations. However, the mechanism underlying the dynamic regulation of such hierarchical and structural chromatin units within the microscale thermal medium remains unclear. Microrheology is a way to measure dynamic viscoelastic properties coupling between thermal microenvironment and mechanical response. Here, we propose a new, to our knowledge, microrheology for Hi-C data to analyze the dynamic compliance property as a measure of rigidity and flexibility of genomic regions along with the time evolution. Our method allows the conversion of an Hi-C matrix into the spectrum of the dynamic rheological property along the genomic coordinate of a single chromosome. To demonstrate the power of the technique, we analyzed Hi-C data during the neural differentiation of mouse embryonic stem cells. We found that TAD boundaries behave as more rigid nodes than the intra-TAD regions. The spectrum clearly shows the dynamic viscoelasticity of chromatin domain formation at different timescales. Furthermore, we characterized the appearance of synchronous and liquid-like intercompartment interactions in differentiated cells. Together, our microrheology data derived from Hi-C data provide physical insights into the dynamics of the 3D genome organization.

INTRODUCTION

In eukaryotes, the one-dimensional information of genomic DNA is spatiotemporally organized inside the cell nucleus, which is only a few microns in size (1,2). Dynamic orchestration of genomic regulatory elements in three-dimensional (3D) space contributes to proper expression of genes. Genome-wide chromosome conformation capture (Hi-C) and related methods have revealed that chromatin hierarchically forms various sized genomic domains such as topologically associating domains (TADs) at the submegabase scale and A/B compartments at the megabase scale as functional and cooperative units (2–5). These hierarchical folding patterns depend on cell types and states during cell differentiation (6–8). Although Hi-C experiments require fixed cells and Hi-C data make sense in population average, the tracking of single nucleosomes by single-molecule and superresolution live-cell imaging experiments has
revealed the dynamic organization of chromatin domains in single cells (9–12). The dynamic property is just like a “polymer melt” state and reveals liquid-like behavior (13,14). However, the relation of the liquid-like behavior of chromatin to its hierarchical 3D genome organization remains poorly understood.

Thermal fluctuations are dominant in a microscale medium as well as within the cell environment and cause random and stochastic motion of tiny particles, such as Brownian motion. The stochastic dynamics of the Brownian particle can be described by the generalized Langevin equation, which is formulated from micromechanics with the aid of projection methods (15–17). The formalism of microrheology was developed based on the generalized Langevin equation (18); the generalized Stokes-Einstein relation (GSER) allows the calculation of linear viscoelastic quantities from the mean-squared displacement (MSD) of tracer particles in a complex fluid. Microrheology to measure elastic or viscous properties as mechanical responses in a microscale complex fluid has been verified for over two decades (18,19). Besides, bio-microrheology, the study of deformation and flow of biological materials at small length scales, has revealed the nature of the dynamic coupling between cell microenvironment and mechanical response (20–22).

Recently, the quantitative significance of Hi-C contact matrix data was elucidated mathematically by several independent groups (23–25). We developed a polymer modeling and a simulation method called polymer dynamics deciphered from Hi-C data (PHi-C) to decipher Hi-C data into polymer dynamics (25). In the mathematical formalism, we found a one-to-one correspondence between a Hi-C contact matrix and an interaction matrix of the polymer model. Once an optimal interaction matrix of the polymer model to an input Hi-C contact matrix is obtained, the method allows the calculation of not only dynamic information such as the MSD of a modeled genomic region but also conformations in thermal equilibrium, and the suffixes \(x, y, z\) represent the spatial coordinates. Because the equation is linear, we can analytically solve the equation under thermal equilibrium. Therefore, once the interaction matrix \(K\) is given, we can calculate and simulate the dynamics and conformations of the polymer model in thermal equilibrium. The positive values of the matrix \(K\) represent a rheological force, and the model formally resembles the Gaussian network model (27–29). Mathematically, although a negative value of the matrix \(K\) can make the polymer system unstable, the positive semidefiniteness of the Laplacian matrix of \(K\) is a necessary and sufficient condition for the stability of the polymer network model (25). As long as the Laplacian matrix of \(K\) is positive semidefinite, the eigenvalues of the Laplacian matrix are nonnegative, and the stability of the polymer model is ensured (25). Therefore, the negative values are acceptable as repulsive forces in the polymer network model. This assumption is a unique point of our modeling, and different from the recently developed similar polymer modeling (23,24).

PHi-C (https://github.com/soyashinkai/PHi-C) is a simulation tool to decipher Hi-C data based on the mathematical formalism of the polymer network model, in which a normalized contact matrix \(C = (C_{ij})\) is connected in a one-to-one correspondence with the normalized interaction matrix \(K = \frac{1}{\sqrt{C_{ii}}} (K_{ij})\) (25). Here, \(\sigma\) represents the contact distance. The PHi-C optimization procedure allows for extracting an optimal normalized interaction matrix \(K\) from an input-normalized contact matrix \(C\) (Fig. 1). Therefore, we can interpret an input population-averaged Hi-C contact matrix for a single chromosome as interaction parameters of the polymer network model in thermal equilibrium. In practice, as long as the Laplacian matrix of the matrix \(K\) is positive semidefinite, PHi-C simulation to calculate polymer dynamics and conformations returns physically stable results.

In theory, the physical interaction matrix \(K\) includes all information with respect to not only dynamics, but also conformations in thermal equilibrium. The matrix \(K\) can be converted into the Laplacian matrix \(L = D - K\) to characterize the properties of the network, where the degree matrix is defined by \(D = \text{diag}(D_{1}, ..., D_{N})\) and \(D_{i} = \sum_{j=1}^{N} K_{ij}\). Because the Laplacian matrix \(L\) is symmetric, \(L\) is diagonalizable. Furthermore, the \(N\) eigenvalues satisfy \(0 = \lambda_{0} < \lambda_{1} \leq \lambda_{2} \leq ... \leq \lambda_{N-1}\) as long as \(L\) is positive semidefinite and there is an orthogonal matrix \(Q\) such that \(Q^{T}LQ = \text{diag}(\lambda_{0}, \lambda_{1}, ..., \lambda_{N-1})\). Then, the MSD of the \(n\)-th monomer within the modeled single chromosome can be written as
where $t$ represents actual time (25), and the movement of the center-of-mass is eliminated to take into account the dynamic fluctuations within a single chromosome.

The GSER connects the MSD to the complex shear modulus in the Laplace domain (18) with the inertia being neglected, $G(s) = (k_B T / |\nu|) (1 / s^2 \Delta \mathbf{r}^2(s))$, and the complex compliance is defined as the inverse of the complex shear modulus (26),

$$\tilde{J}(s) \equiv \frac{1}{G(s)} \equiv \frac{\pi a}{k_B T} s \langle \Delta \mathbf{r}^2(s) \rangle. \quad (2)$$

Here, we employed the MSD of a modeled genomic monomer derived by PHI-C to consider the dynamic viscoelastic properties of the genome itself within a single chromosome. Calculating the Laplace transformation of Eq. 1, the complex compliance of the $n$-th monomer within a chromosome was expressed by using the eigenvalues $\{\lambda_p\}_{p=1}^{N}$ and the orthogonal matrix Q as

$$\tilde{J}(\sigma; n) = \frac{6\pi a}{k_B T} \sum_{p=1}^{N-1} \frac{Q_{np}^2}{\sigma^2 + \lambda_p}. \quad (3)$$

The matrix one-to-one correspondence between C and $K$ is formally closed as dimensionless quantities in PHI-C, where the two physical quantities $\sigma$ and $\gamma$ are unknown factors. Therefore, our theory cannot directly deal with the above complex compliance with the physical unit Pa$^{-1}$. We should give normalized expressions. In PHI-C, the eigenvalues of the normalized Laplacian matrix $\sum = (\sigma^2 / k_B T) I$, were normalized as $\lambda_p = (\sigma^2 / k_B T) \lambda_p$. Moreover, using the normalized inverse time $\sigma = (\gamma \sigma^2 / k_B T) s$, we could rewrite Eq. 3 as

$$\tilde{J}(\bar{s}; n) = \frac{6\pi a \sigma^2}{k_B T} \sum_{p=1}^{N-1} \frac{Q_{np}^2}{\bar{s} + 3\lambda_p}. \quad (4)$$

In general, the complex compliance was defined by the Fourier-Laplace transformation, replacing $\bar{s} = i\sigma, J'(\sigma) = J(\bar{s}) = J'(\bar{s}) - iJ''(\bar{s})$, where $\bar{s}, J'$, and $J''$ are the normalized frequency, the storage, and the loss compliances, respectively. Thus, the normalized complex compliance was derived from the result of PHI-C with no reference to physical parameters,
PHI-C simulation to calculate polymer dynamics and conformations

We carried out PHI-C simulations to calculate polymer dynamics of the polymer network model. First, we obtained an optimal normalized interaction matrix \( \mathbf{K} \) by the PHI-C optimization procedure. Then, \( \mathbf{K} \) is converted into the normalized Laplacian matrix \( \mathbf{L} \). Using the eigendecomposition of the matrix \( \mathbf{L} \), the normalized eigenvalues \( \{ \lambda_i \}_{i=0}^{N-1} \) and the orthogonal matrix \( \mathbf{Q} \) are obtained. For a normalized polymer conformation vector \( \mathbf{q}_i \), the normalized interaction matrix \( \mathbf{Q} \) is given:

\[
\mathbf{q}_i = \frac{\mathbf{Q} \mathbf{F}_i}{\mathbf{F}_i^T \mathbf{F}_i} \quad \text{for} \quad i = 1, 2, \ldots, N.
\]

To understand what the values of the complex compliance \( J^* \) reveal along the genomic coordinate, we plotted \( |J^*(\omega)| \) at \( \omega = 10^0 \) to the long timescale \( \omega = 10^{-4} \). The vertical stripe patterns in the spectrum are variable along the chromosome, suggesting different viscoelastic responses depending on the genomic regions of the chromosome. Furthermore, according to changes in the Hi-C patterns during mouse neural differentiation, the spectra of \( |J^*(\omega)| \) also showed different patterns.

To visualize the frequency-dependent viscoelastic response, we plotted the normalized storage and loss compliances, \( J^*(\omega) \) and \( J^*(\omega) \), for all genomic regions (Figs. 2 C and S1 C). The blue bundle of the storage compliances shows a step-like pattern with slopes on the double logarithmic plot, suggesting dynamic and hierarchical viscoelastic responses in the 3D genome organization. Furthermore, the red curves of the loss compliances show convex upward shapes, indicating that the viscous dynamics of a genomic position \( n \) within a chromosome relaxes at the time corresponding to the inverse of the normalized frequency \( \omega \) at the peak.

Taken together, our microrheology method allows the conversion of a hierarchical Hi-C pattern to a dynamic and hierarchical rheological spectrum.

TAD boundaries are more rigid as nodes than intra-TAD sequences

To understand what the values of the complex compliance \( J^* \) reveal along the genomic coordinate, we plotted \( |J^*(\omega)| \) at \( \omega = 10^0 \), \( 10^{-2} \), and \( 10^{-3} \) focusing on the 50- to 100-Mb region of the chromosome 6 in ES cells (Fig. 3 A). According to variously sized triangles corresponding to chromatin domains such as TADs and compartments on the Hi-C contact pattern, the shapes of \( |J^*(\omega)| \) displayed peaks and troughs marked with pink and blue dots, respectively. Our statistical analysis revealed that the troughs in
the $|\tilde{T}(\omega)|$ profile were located near the reported TAD boundaries at each frequency $\omega = 10^{-1}, 10^{-2},$ and $10^{-3}$ for chromosomes 6 and 17 of mouse ES cells, NPCs, and CN cells (Fig. S3; Table S1), suggesting that TAD boundaries are characterized as more rigid nodes than the intra-TAD sequences according to the timescale $\tau = \frac{1}{\omega}$. Therefore, the vertical stripe patterns in the spectrum in Fig. 2B show the variation of peaks and troughs, depending on the frequency.

Next, we tried to visualize the role of the trough positions in the 3D genome organization. Our PHi-C simulation allows for polymer dynamics consistent with the optimized Hi-C matrix. Fig. 3B shows a snapshot of an initial polymer conformation for chromosome 6 in mouse ES cells, where pink and blue dots represent the genomic positions corresponding to the peaks and troughs of $|\tilde{T}(\omega = 10^{-1})|$, respectively. Video S1 shows the dynamic fluctuation of the polymer model within time $\tau = 10$. It seems that, at every time step, the conformation is irregularly folded, and we cannot find distinct chromatin domains. Then, we piled up the pink and blue dots in the dynamics (Fig. 3B; Video S2). We can see that these two color regions are separated and that the blue-labeled trough regions look functional as dynamic boundaries to interfere with interdomain interactions between the pink-labeled intradomain regions.

**Dynamic and hierarchical changes of chromosome rigidity during cell differentiation**

The peaks and troughs in the shape of $|\tilde{T}(\omega)|$ were also observed for chromosome 6 in ES cells, NPCs, and CN cells (Fig. 3C). The number of troughs decreased from the short timescale (high $\omega$) to the long timescale (low $\omega$) (Figs. 3D and S4A). This indicated that dynamic and hierarchical compartmentalization with domain fusions occur in the 3D genome organization according to the time evolution. For example, intra-TAD dynamics is dominant until $\tau = 10^1$, then inter-TAD communications and fusions of TADs arise up to $\tau = 10^2$ in intracompartments, and intercompartment interactions occur over $\tau = 10^3$. Besides, the numbers of troughs for ES cells, NPCs, and CNs were ordered, revealing that the number of chromatin domains as...
chromosome structural units decreases depending on an individual frequency, and the rearrangement occurs during cell differentiation. Furthermore, the average values of $|\mathcal{J}(\bar{\omega})|$ were also ordered until the time $\bar{t} = 10^2$ (Figs. 3 E and S4 B), suggesting that the physical property of chromosomes averagely becomes rigid and less flexible during cell differentiation.

The complex compliance $\mathcal{J}(\bar{\omega})$ is divided into the storage and loss components (Eq. 6), which are not independent of each other in rheological relations (26). We plotted these two compliances within chromosome 6 in ES cells, NPCs, and CN cells at different frequencies (Video S3), relating to the Cole-Cole plot in dielectrics (34). At a fixed frequency $\bar{\omega}$, the scattering pattern should reflect a cooperative rheological response within a chromosome. Therefore, we calculated the correlation between these two compliances on the scatter plots for different frequencies (Fig. 3 F). For ES cells, broad positive correlations were detected in the frequency region $\bar{\omega} = 10^{-3} - 10^{-1}$. A high positive correlation indicates that the elastic and viscous responses described by $\mathcal{J}(\bar{\omega})$ and $\mathcal{J}''(\bar{\omega})$ synchronize within a chromosome to the periodic stress with a frequency $\bar{\omega}$. Therefore, a positive peak of the correlation indicates a characteristic time $\bar{t} = \bar{\omega}^{-1}$ of the synchronous response. For NPCs and CNs, a broad region showing high correlations was not observed. Instead, some peaks were observed around $\bar{\omega} = 10^{-2}$ for NPCs and $\bar{\omega} = 10^{-2}$ and $10^{-3}$ for CNs. Note here that the peaks around $\bar{\omega} = 10^{-4}$ are excepted because the response to the extremely slow fluctuation corresponds to a whole movement of the chromosome. Because we could confirm that the checkerboard pattern corresponding to the A/B compartment organization in Fig. 2 A gradually becomes dense during cell differentiation, the correlations for ES cells, NPCs, and CNs at $\bar{\omega} = 10^{-3}$ are ordered, suggesting that the timescale $\bar{t} = 10^3$ must be related to intercompartment organization. For chromosome 17, we observed similar features of the correlations (Fig. S4 C).

**Synchronous and liquid-like intercompartment interactions appear in differentiated cells**

As shown in Eq. 7, the loss tangent is defined as the ratio of the normalized loss compliance $\mathcal{J}''$ to the normalized storage compliance $\mathcal{J}$. Therefore, positive and negative values of the logarithmic loss tangent indicate liquid-like viscosity and solid-like elasticity, with higher and lower energy dissipation, respectively. Here, we asked whether there is
a difference in the liquid-like and solid-like properties between the A and B compartment organization or not. We depicted spectra of the loss tangent $\tan(\delta)$ for chromosome 6 in mouse ES cells (left), NPCs (middle), and CN cells (right) at a 250-kb resolution. Along the genomic coordinate and the logarithmic frequency $\log(\omega)$, a spectrum of $\log(\omega)\tan(\delta)$ is depicted as a heat map. The white arrows for CNs indicate definite “island” regions around $\omega = 10^{-3}$ with negative eigenvectors. (B) The scatter plots between the logarithmic loss tangent and the eigenvalue for $\omega = 10^{-2}$ and $\omega = 10^{-3}$ are shown. For CNs, the black arrow indicates the appearance of the “islands” in (A). To see this figure in color, go online.

**FIGURE 4** (A) Eigenvectors and spectra of the loss tangent $\tan(\delta)$ for chromosome 6 in mouse ES cells (left), NPCs (middle), and CN cells (right) at a 250-kb resolution. Along the genomic coordinate and the logarithmic frequency $\log(\omega)$, a spectrum of $\log(\omega)\tan(\delta)$ is depicted as a heat map. The white arrows for CNs indicate definite “island” regions around $\omega = 10^{-3}$ with negative eigenvectors. (B) The scatter plots between the logarithmic loss tangent and the eigenvalue for $\omega = 10^{-2}$ and $\omega = 10^{-3}$ are shown. For CNs, the black arrow indicates the appearance of the “islands” in (A). To see this figure in color, go online.

In this study, we revealed the rheological information of the dynamic 3D genome organization as a spectrum by integrating the theoretical MSD by PHi-C and the GSER. On the spectrum, the differences in the compliance along the genomic coordinate at a particular timescale revealed the distinct characteristics of the boundaries and insides of chromatin domains; especially, TAD boundaries were found to be more rigid than the intra-TAD regions. During cell differentiation, we quantitatively estimated increasing rigidity of the chromosome, with dynamic organization of chromatin domains with time evolution.

**DISCUSSION**

In this study, we revealed the rheological information of the dynamic 3D genome organization as a spectrum by integrating the theoretical MSD by PHi-C and the GSER. On the spectrum, the differences in the compliance along the genomic coordinate at a particular timescale revealed the distinct characteristics of the boundaries and insides of chromatin domains; especially, TAD boundaries were found to be more rigid than the intra-TAD regions. During cell differentiation, we quantitatively estimated increasing rigidity of the chromosome, with dynamic organization of chromatin domains with time evolution.

Particularly, our microrheology method allows for interpreting static and population-averaged Hi-C matrix data as a dynamic and hierarchical 3D genome picture. Definite boundaries with higher rigidity at a particular timescale $t = \omega^{-1}$ characterize an individual triangle region corresponding to a chromatin domain such as a TAD or a compartment on an Hi-C pattern. In other words, the appearance of a chromatin domain as a functional unit of
Thus, we could characterize the rheological properties of a specific chromosome during cell differentiation, but we could not elucidate a relationship between the physical properties of chromatin and gene expression, i.e., how physical rigidity or flexibility of a specific genomic region including some loci might relate to the gene expression levels. Our findings on TAD boundaries as more rigid nodes would suggest they might be the molecular mechanism regulating the physical rigidity of the boundaries.

In summary, the microrheological spectrum generated from Hi-C data describes the dynamic changes in rigidity and flexibility between individual genomic regions as well as during cell differentiation. To fully understand chromatin domain formation and interdomain interactions, we need to understand chromatin dynamics at different timescales. Our microrheology method opens the possibility of interpreting Hi-C data as information that can reveal the dynamic and hierarchical properties of the 3D genome organization.

SUPPORTING MATERIAL

Supporting Material can be found online at https://doi.org/10.1016/j.bpj.2020.02.020.

AUTHOR CONTRIBUTIONS

S.S., T.S., and S.O. designed the research. S.O. supervised the study. S.S. carried out analytical calculations and PHi-C analysis, as well as analyzing the data. H.M. and I.H. performed TAD boundary analysis. S.S. wrote the article.

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