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Integrative modelling of the genome structure and dynamics in fission yeast

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Abstract

Genome organization in the nucleus is highly structured and dynamic¹. Recent advances in genomic technology have enabled the measurement of genome-wide architecture and locus-specific motion, yielding contact maps and live-cell trajectories^{2,3}. However, these outcomes are derived from different modalities and are not directly comparable, with their quantitative integration being a key challenge. Here we establish a genome-wide live-cell imaging platform in fission yeast *Schizosaccharomyces pombe*, tracking 131 chromosomal loci, along with the spindle pole body (SPB) and nucleolus, to construct a quantitative map of locus dynamics. We integrate these dynamics with contact data⁴ through physics-based polymer modelling of Hi-C data^{5,6}. The resulting model reproduces genome-wide mobility patterns and known architectural features, including centromere and telomere clustering⁷⁻⁹. The model also identifies distinct dynamical regimes: centromere- and telomere-proximal loci relax within ~ 150 s, whereas the remaining loci relax within ~ 70 s. SPB motion exhibits an oscillatory peak near 225 s and $1/f$ fluctuations. We use the model with SPB-directed forcing to show how these low-frequency components propagate through the genome to drive genome-wide chromatin displacements. Together, this predictive physics-based framework integrates genome structure and dynamics to reveal how nuclear mechanical driving forces shape chromosome motion, linking mechanically-driven chromatin responses to genome maintenance and regulation.

Introduction

The eukaryotic genome is packaged into chromatin folded in a hierarchical order within the nucleus^{1,10,11}. If the nucleus could be observed with complete spatiotemporal resolution, it would, in principle, be possible to track the spatial and temporal aspects of genome transactions, such as gene expression, DNA replication, and DNA damage repair, and to reveal how they are linked to chromatin architecture^{2,3}. In practice, however, experimental access is limited and modality-dependent. Fluorescence *in situ* hybridization (FISH) provides single-cell snapshots of nuclear genome organization in fixed cells, from the positioning of specific loci to chromosome-scale arrangements^{12–14}. In addition, chromosome conformation capture approaches coupled with high-throughput sequencing techniques, such as Hi-C¹⁵, yield genome-wide contact maps that report ensemble-averaged three-dimensional (3D) genome features. Furthermore, fluorescent tagging of genomic loci enables live-cell imaging of chromatin motion in living cells^{16,17}. Together, these approaches have established that chromatin is both structured and dynamic, and that its organization is shaped by nuclear geometry and subnuclear positioning, including constraints imposed by intranuclear structures and nuclear boundaries. However, these approaches examine different outcomes, which are often interpreted separately; therefore, it remains challenging to obtain a unified picture of chromatin as a structured yet dynamically fluctuating polymer. Therefore, a key challenge is to establish a quantitative framework that integrates chromatin structure and dynamics across modalities, enabling the interpretation of locus-resolved motion in the context of genome-wide architecture.

Although live-cell imaging makes chromatin dynamics experimentally accessible, quantitative analyses largely focus on a limited number of loci, making it difficult to relate locus-specific motion to genome-wide structural features. In contrast, genome-wide Hi-C contact maps provide a comprehensive view of nuclear organization but do not directly specify how chromatin moves in time. To bridge the gap between these complementary measurements, datasets of genome dynamics comparable in scope to Hi-C and an integrative framework that

can connect structural and dynamical observables are required. Such a framework would also provide a path toward predictive physics-based models that enable systematic *in silico* investigation of how mechanical driving forces and constraints shape genome dynamics at the nuclear scale.

Fission yeast *Schizosaccharomyces pombe* is a genetically tractable model eukaryote whose compact genome and three chromosomes (I–III) make dense genome-wide locus tagging experimentally feasible. In this study, to address the gap between genome-wide 3D genome data and live-cell trajectories, we established a genome-wide live-cell imaging platform in *S. pombe*. Building on a systematic locus-tagging strategy in *S. pombe*, we generated a collection of fluorescently tagged strains targeting 143 genomic loci spaced at ~ 90 kb intervals¹⁸ and established 131 strains in which locus motion could be quantified together with nuclear landmarks, including the spindle pole body (SPB) associated with centromeres and the nucleolus, in the G2 phase. We obtained a comprehensive set of locus-scale dynamical measurements mapped onto genomic coordinates that could be integrated with genome-wide structural information. Next, we integrated two independent observables, genome-wide Hi-C contact data as structural constraints and locus-resolved dynamical readouts, through physics-based polymer modelling of Hi-C data, PHi-C^{5,6}. Finally, we leveraged the PHi-C framework to construct a model that jointly captures chromatin structure and dynamics and enables *in silico* exploration and prediction beyond what is accessible in low-throughput experiments.

Results

Quantitative mapping of chromatin dynamics in fission yeast enabled through genome-wide live-cell imaging. To systematically map chromatin motion across the *S. pombe* genome in living cells, we established a comprehensive live-cell imaging platform based on the *lacO*/LacI–GFP tagging strategy (Fig. 1a). First, we generated a collection of strains, each carrying a single *lacO* array integrated at one of 143 genomic positions at

~90 kb spacing across chromosomes I–III of *S. pombe*, enabling locus-resolved measurements over broad genomic coverage¹⁸ (Supplementary Table 1). To monitor chromosome dynamics within the context of nuclear landmarks, we labelled two prominent reference structures, the SPB and the nucleolus. *S. pombe* centromeres cluster near the SPB and are tethered to the nuclear envelope near the SPB during interphase^{7,9}, forming a characteristic Rabl configuration (Extended Data Fig. 1a); therefore, we tagged the SPB component Sid4 with GFP and used its focus position as a proxy for centromere motion. Simultaneously, we tagged the nucleolar protein Gar2 with mCherry to mark the nucleolus. For subsequent imaging and analysis, we used 131 strains that showed robust dual labelling. This setup enabled us to simultaneously track a single chromosomal locus (LacI–GFP), the SPB-proximal (centromere-proximal) region (Sid4–GFP), and the nucleolus (Gar2–mCherry) in the same living cells¹⁹ (Fig. 1b and Supplementary Video 1).

Using the aforementioned live-cell imaging setup (Fig. 1a,b), we obtained representative 3D trajectories of the SPB, a labelled genomic locus, and the nucleolus centre through time-lapse imaging (Fig. 1c). Consistent with prior observations of microtubule-dependent SPB oscillations in interphase²⁰, Sid4-marked SPBs showed characteristic oscillatory motion, which facilitated visual discrimination of SPB motion from locus trajectories, even within the same GFP channel (Fig. 1c); the frequency components of SPB motion is analysed in detail later. We acquired 3D image stacks every 30 s for 1800 s (61 time points) from a large collection of cells spanning multiple cell cycle stages. Therefore, we assigned cell cycle stages post hoc, and in subsequent analyses, we focused on the G2 phase, the longest stage of the *S. pombe* cell cycle, to characterize interphase chromatin dynamics (Extended Data Fig. 1b). As a result, we obtained a comprehensive G2-phase dataset of chromatin dynamics for 131 genomic loci from a total of 4751 cells (Fig. 1d and Supplementary Table 1).

To quantify locus motion, we used this curated G2-phase dataset to calculate the two-point mean squared displacement (2-point MSD) of the 3D vector from the SPB–centromere system to each locus. A genome-wide heat map of the resulting 2-point MSDs revealed a

clear trend (Fig. 1e): positional fluctuations increased with increasing distance from the centromere, consistent with progressively less constrained motion along chromosome arms. However, although this G2-phase dataset provided broad coverage of locus motion, many regions remained unmeasured (grey), leaving gaps in the genome-wide dynamic landscape.

Construction of a physics-based polymer model consistent with genome-wide chromatin dynamics. To complement the genome-wide 2-point MSD map obtained through live-cell imaging (Fig. 1e) and to infer chromatin dynamics beyond the experimentally sampled loci, we constructed a physics-based polymer model constrained with genome-wide Hi-C contact data using our PHi-C framework^{5,6}. PHi-C estimates an effective interaction parameter matrix (the PHi-C matrix) for an extended bead-spring polymer model through optimization such that the resulting model reproduces the input Hi-C contact matrix (Extended Data Fig. 2a). We applied PHi-C genome-wide to a previously reported interphase Hi-C contact map of *S. pombe*⁴ (>90% of the cells were in the G2 phase) at 25 kb resolution across chromosomes I–III (Fig. 2a and Extended Data Fig. 2b). Importantly, we applied PHi-C jointly to chromosomes I–III instead of modelling each chromosome separately. This genome-wide formulation allowed the PHi-C-inferred model to incorporate inter-chromosomal contacts (including clustering of the three centromeres and prominent telomere clustering among 1L, 1R, 2L, and 2R) observed in previous Hi-C experiments^{8,21}. The reconstructed genome-wide contact map generated from the optimized PHi-C matrix showed excellent agreement with the experimental genome-wide Hi-C contact map (Pearson’s $r = 0.995$; Fig. 2a and Extended Data Fig. 2c), indicating that the PHi-C-inferred interaction landscape faithfully captures genome-wide contact architecture.

Next, to quantitatively connect the PHi-C-inferred model to chromatin dynamics, on the basis of the PHi-C matrix, we derived a theoretical expression for the 2-point MSD between the SPB (used as a proxy for the centromere) and each genomic locus; see Eq. (1) in the Methods section. This expression depends on two physical parameters: the effective bead

diameter σ to set the spatial scale of the coarse-grained polymer and the friction coefficient γ to set the time scale. Here, σ maps bead units to physical length, whereas γ maps model dynamics to physical time, thereby anchoring the otherwise dimensionless coarse-grained polymer model to real spatiotemporal units. To estimate σ and γ , we fit the theoretical 2-point MSD curves to the experimental 2-point MSD measurements for the 131 genomic loci imaged (Fig. 2b and Supplementary Videos 2–4). Results showed that remarkably, the fitted values of σ and γ were broadly consistent across genomic positions and clustered around their genome-wide means (Fig. 2c,d). Therefore, we adopted their mean parameter values ($\sigma = 0.0567 \mu\text{m}$ and $\gamma = 1.26 \times 10^{-7} \text{ kg/s}$) for subsequent analyses.

Using the mean values of σ and γ , we used the PHi-C-inferred model to reconstruct a genome-wide heat map of the 2-point MSD between each locus and the SPB (Fig. 2e), which closely resembled the experimental 2-point MSD map (Fig. 1e). Quantitatively, both reconstructed and experimental 2-point MSDs showed a strong correlation when integrated over lag times from 0 to 900 s (Pearson’s $r = 0.880$; Fig. 2f). We also observed this correlation when analysing the 2-point MSDs as a function of genomic distance from the centromere at individual lag times (Extended Data Fig. 3). Together, these findings demonstrate that PHi-C enables construction of a physics-based polymer model consistent with both the genome-wide Hi-C contact map and the live-cell measurements of the G2-phase chromatin dynamics. Supplementary Video 5 shows a representative simulation displaying time-evolving 3D conformations of chromosomes I–III, along with the corresponding distance map in order to visualize the chromatin dynamics generated using the PHi-C-inferred model.

Structural features and genome-wide mobility patterns predicted using the PHi-C-inferred model. Previous FISH-based observations of interphase chromosomes of *S. pombe* showed characteristic inter-chromosomal organization (centromere and telomere clustering)^{7,8}. The PHi-C framework enables efficient sampling of 3D conformations from the PHi-C-inferred model; therefore, we generated 10^5 structural samples and quantified these hallmark

features in a model-based genome-wide manner.

To assess centromere clustering, we defined the circumradius R as the radius of the circle passing through the three centromeres (cen1–3) of chromosomes I–III (Fig. 3a, inset). The resulting distribution of R was strongly shifted toward smaller values for centromeres (mean $R = 0.261\ \mu\text{m}$) compared to a control distribution for three randomly selected genomic loci (mean $R = 0.544\ \mu\text{m}$; Fig. 3a), indicating tight spatial clustering of the three centromeres in the model. Notably, the mean centromere circumradius was comparable in scale to the optical diffraction limit under typical wide-field imaging conditions, consistent with the frequent appearance of centromeres as a single cluster in imaging-based studies.

To examine telomere clustering of chromosomes I and II, we grouped the four telomeres (tel1L, tel1R, tel2L and tel2R) into clusters using a threshold distance of 250 nm, chosen to be on the order of the optical diffraction limit (Fig. 3b). Across the 10^5 structural samples, configurations forming two telomere clusters were most prevalent (Fig. 3b), in agreement with previous observations^{7,8}. The model further enabled quantification of the relative frequencies of distinct telomere grouping patterns beyond the overall cluster count (Fig. 3b and Extended Data Fig. 4), providing a more detailed, population-level characterization of telomere organization than is typically accessible using imaging alone. In summary, these findings indicate that the PHi-C-inferred model captures the key structural features of *S. pombe* chromosomal organization.

After validating these structural hallmarks, we used the calibrated PHi-C-inferred model to predict genome-wide mobility patterns over a broad temporal range. We computed $\log_{10} \text{MSD}(t)$ for each 25 kb locus from lag times of 0.1 s to 900 s and visualized the results as a genome-wide heat map (Fig. 3c). Notably, although the mobility clusters were defined post hoc from the full $\text{MSD}(t)$ profiles (Fig. 3d), the heat map revealed a characteristic temporal crossover: although loci in the centromere- and telomere-enriched loci (clusters 2 and 3) had relatively smaller MSDs for short lag times ($t \lesssim 10\text{ s}$) compared to the remaining loci (cluster 1), they reached larger long-time MSD plateaus toward $t = 900\text{ s}$.

(Fig. 3c). This analysis extended the dynamic characterization beyond the experimentally sampled time scales and loci by exploiting the PHi-C-inferred model’s analytical and computational tractability. Clustering of the predicted $\text{MSD}(t)$ profiles revealed three distinct mobility classes (Fig. 3c,d and Extended Data Fig. 5). Notably, the three classes were enriched for specific genomic landmarks: telomere-proximal loci on chromosomes I and II were preferentially assigned to one cluster, centromere-proximal loci to another, and the remaining loci predominantly to the third (Fig. 3c,d), indicating that centromeres and telomeres have distinct dynamic signatures in the model. In addition, the genome-wide MSD heat map highlighted the silent mating-type region (*mat* locus) on chromosome II as a locus with comparatively small short-time MSDs, similar to those observed for the centromere- and telomere-enriched clusters (Fig. 3c). To investigate this, we performed a subclustering analysis within cluster 1, which divided it into two subclasses, 1A and 1B; the *mat* locus was assigned to subclass 1A, whereas the majority of cluster 1 loci belonged to subclass 1B (Extended Data Fig. 5b–d). In the PHi-C-inferred interaction landscape (Extended Data Fig. 2b), the *mat* locus showed comparatively strong attractive interactions with the three centromeres and the telomeres of chromosomes I and II (Extended Data Fig. 2d).

Finally, the predicted $\text{MSD}(t)$ curves were saturated for long times, we quantified a characteristic relaxation time τ for each locus and compared τ across mobility classes. Specifically, for each $\text{MSD}(t)$ curve, we defined the plateau value A for long times with τ as the lag time at which $\text{MSD}(\tau) = A(1 - 1/e)$, as shown in Fig. 3d (filled circles). The relaxation time distributions showed clear class-specific differences (Fig. 3e): the loci in the telomere- and centromere-enriched clusters took a longer time to relax (~ 150 s; mean $\tau = 155$ s and 157 s, respectively), whereas the remaining loci relaxed more rapidly (~ 70 s; mean $\tau = 71.6$ s). Together, these findings define two characteristic time scales of chromatin motion, ~ 150 s for centromere-/telomere-enriched loci and ~ 70 s for the remaining loci. Therefore, the PHi-C-inferred model provides model-grounded quantitative time scales for genome-wide chromatin mobility beyond reproducing known centromere and telomere clustering, estab-

lishing a quantitative basis for interpreting the comprehensive chromatin dynamics dataset.

Oscillatory motion of the SPB and frequency-dependent chromatin response predicted using the PHi-C-inferred model. The relaxation time analysis (Fig. 3) revealed two characteristic chromatin mobility time scales ~ 150 s and ~ 70 s. Therefore, we investigated what physical processes could drive or modulate motion on those time scales in the living cells. One prominent candidate is the oscillatory motion of the SPB²⁰, which is mechanically coupled to clustered centromeres in *S. pombe* and, thus, can act as a periodic driver of chromatin. Notably, in our dataset, the SPB had a mean speed of 16.8 nm/s, which is smaller than the typical 1 s scale displacement of individual nucleosomes inferred from live-cell imaging in human cells: $\text{MSD}(t = 1 \text{ s}) \approx 0.020 \mu\text{m}^2$ (i.e., $\sqrt{\text{MSD}(t = 1 \text{ s})} \sim 141 \text{ nm}$)²². Thus, at short time scales, the directional component of SPB motion can be comparable to, or masked by, stochastic chromatin fluctuations, underscoring the importance of analysing SPB motion over longer time windows, which is enabled by our time-lapse imaging (3D stacks acquired every 30 s for 1800 s). Therefore, we quantified SPB oscillations across the population and investigated how centromere-directed periodic driving forces propagate through chromatin in the calibrated PHi-C-inferred model.

The SPB undergoes directional oscillatory motion¹⁹, which we observed in our live-cell imaging dataset (Fig. 4a–c). To quantify the temporal structure of SPB motion across the population, we computed the power spectral density (PSD) of SPB trajectories for all 4751 G2-phase cells (Fig. 4d). The PSD spectrum displayed three notable features: $1/f$ -like fluctuations (well approximated by $1/f^{0.7}$ for $f \lesssim 1/360$ Hz), where f is the frequency; a local peak at $f = 1/225$ Hz; and substantial attenuation above $f \approx 1/150$ Hz, where the PSD fell to about half of its peak value at $f = 1/225$ Hz (Fig. 4d). The PSD spectrum also revealed a prominent oscillatory component with a period of ~ 225 s (corresponding to $f \approx 1/225$ Hz), consistent with previous reports of SPB oscillations on similar time scales¹⁹. In contrast, oscillatory components with periods shorter than ~ 150 s (i.e., $f \gtrsim 1/150$ Hz)

were comparatively weak, indicating that slow SPB motion dominates the nuclear mechanical driving forces that centromere-proximal chromatin experiences. Notably, these PSD features intersected naturally with the characteristic chromatin relaxation time scales identified in Fig. 3. Results showed that high-frequency SPB components at $f \gtrsim 1/150$ Hz (periods shorter than ~ 150 s) are weak; therefore, SPB motion is unlikely to act as a dominant driving force input on time scales faster than the intrinsic chromatin relaxation times of the centromere-/telomere-enriched regime. Instead, the PSD spectrum suggests that SPB motion is concentrated in the low-frequency region ($f \lesssim 1/150$ Hz), leading to the hypothesis that slow SPB oscillations are the mechanically relevant input for genome-scale deformations.

Next, we analysed how oscillatory driving forces transmitted through the centromere-SPB linkage influence genome-wide chromatin dynamics. A key advantage of the calibrated PHi-C-inferred model is that it provides a physics-based framework to theoretically compute dynamical responses, without the need for additional perturbation experiments.

In the general setting, we considered applying an oscillatory force of angular frequency ω to the m -th bead and quantified the resulting displacement of the n -th bead in the frequency domain. This relation was characterized by a cross-response function $\chi_{nm}(\omega)$, which could be evaluated directly using the PHi-C-inferred model parameters; see Eq. (2) in the Methods section and Extended Data Fig. 6. Following the convention in linear rheology, we obtained an expression for $\chi_{nm}(\omega) = \chi'_{nm}(\omega) - i\chi''_{nm}(\omega)$, where the storage component $\chi'_{nm}(\omega)$ quantifies the elastic-like energy-storing part of the response, while the loss component $\chi''_{nm}(\omega)$ quantifies the viscous/liquid-like energy-dissipating part.

Next, we focused on centromere-directed oscillatory driving forces. We applied an oscillatory force $\mathbf{F}(\omega)$ to the three centromeres and quantified the resulting displacement $\mathbf{r}_n(\omega)$ of each locus n (Fig. 4e). Under the linear response relation $\mathbf{r}_n(\omega) = \chi_n(\omega)\mathbf{F}(\omega)$, we defined $\chi_n(\omega)$ and mapped its storage and loss components, $\chi'(2\pi f)$ and $\chi''(2\pi f)$, respectively, genome-wide as a function of the oscillatory driving force frequency f (Fig. 4f). In these heat maps, the sign indicates the direction of motion relative to the applied force: positive

values (red) correspond to displacements aligned with the oscillatory force direction, whereas negative values (blue) indicate anti-aligned displacements.

As shown in Fig. 4f, at low frequencies, genomic loci near the centromeres showed strong positive responses (red), whereas more distal regions tended to have pronounced negative responses (blue), indicating a global deformation mode with opposite displacements across chromosome arms. In contrast, at $f \gtrsim 1/150$ Hz, appreciable responses were largely confined to centromere-proximal loci, while the remainder of the genome responded only weakly (Fig. 4f), consistent with limited propagation of rapid forces through the chromatin polymer. Notably, at $f \approx 1/225$ Hz, telomere-proximal loci on chromosomes I and II showed positive responses in both storage and loss components (i.e., $\chi' > 0$ and $\chi'' > 0$), indicating coherent displacements in the oscillatory force direction with both elastic-like and dissipative contributions (Fig. 4f).

To assess how efficiently oscillatory inputs propagate without dissipation, we computed the quality factor $Q(f) = |\chi'(2\pi f)/\chi''(2\pi f)|$ (Fig. 4g). Although centromere-proximal loci showed the largest response amplitudes, their low Q values indicated inefficient, strongly dissipative transmission locally. In contrast, more distal regions displayed higher Q values, indicating more efficient propagation of oscillatory inputs along chromosome arms (Fig. 4g). In particular, at frequencies just below $1/225$ Hz, chromosomes I and II showed efficient transmission in their telomere-proximal loci, and chromosome III showed efficient responses across more than half of its length (Fig. 4g).

Together, these findings indicate that the SPB-centromere system forms a mechanical driver that preferentially couples to slow, low-frequency modes of chromatin motion. An oscillatory force at $f \gtrsim 1/150$ Hz has little impact beyond the centromere-proximal region, whereas slow oscillatory inputs, including $1/f$ -like fluctuations and the characteristic peak component at $f \approx 1/225$ Hz, can propagate genome-wide and generate large-scale nuclear displacements predicted using the PHi-C-inferred model (Fig. 4f,g).

Discussion

Integrating genome-wide live-cell dynamics with Hi-C data-constrained polymer

modelling. One key underlying theme of genome biology is that non-random spatial chromosomal organization is tightly linked to diverse DNA transactions, including transcription, replication and genome maintenance^{23,24}. Over the past decades, complementary experimental approaches have helped establish increasingly detailed but inherently partial views of nuclear organization. On the one hand, microscopy-based methods (from FISH to live-cell genomic locus tracking) provide direct access to spatiotemporal chromatin behaviour. On the other hand, chromosome conformation capture (3C)-based methods (e.g., Hi-C) yield genome-wide contact landscapes but, by design, do not directly report the dynamics in living cells²⁵. Recent advances in sparse labelling and single-molecule and super-resolution microscopy now enable tracking of chromatin motion at the nucleosome level in single cells^{1,17,26}. However, with regard to specific genomic loci, live-cell imaging has a few limitations: the number of loci that can be labelled and tracked per cell and strain construction and imaging throughput.

To overcome these limitations in a genetically tractable organism, in this study, we established a genome-spanning live-cell imaging resource in fission yeast *S. pombe*. We constructed a collection of strains containing the markers for the SPB-proximal (centromere-proximal) region and the nucleolus, along with a *lacO* array inserted at one of the 131 genomic loci at ~90 kb spacing across chromosomes I–III of *S. pombe*¹⁸. Within a spatial context of interpretable nuclear landmarks as internal references, this enabled us to take systematic, locus-resolved measurements of interphase chromatin motion at a scale that is unusually large for a locus-tagging strategy. However, even such a large imaging effort samples discrete loci and time windows, leaving substantial genomic intervals unobserved and highlighting the need for a framework based on physical principles to infer genome dynamics beyond the sampled loci and to enable mechanistic interpretation.

In this study, we addressed this gap by integrating the imaging-based chromatin dynamics

map with a genome-wide Hi-C contact map through a physics-based polymer model inferred using our PHi-C framework^{5,6}. A key advantage of this approach is in the explicit physical model of chromosomes obtained, stochastic fluctuations arise naturally and can be analysed quantitatively. Therefore, the PHi-C-inferred 3D chromosome model is inherently dynamic, enabling direct predictions of chromatin motion in addition to genome architecture. We calibrated the model’s physical parameters directly against the experimental 2-point MSDs, simultaneously constraining the interaction landscape with a genome-wide Hi-C contact map, and obtained a dynamical polymer model that is consistent with both genome-wide contacts and the observed chromatin mobility across the genome. Notably, performing PHi-C jointly across chromosomes I–III allowed inter-chromosomal constraints present in the Hi-C map (including centromere clustering and telomere-associated interactions) to be incorporated from the beginning, enabling a unified description of nuclear-scale organization and dynamics. This integrated quantitative framework enables the prediction of chromatin motion beyond the experimentally sampled loci and the connection of genome-wide mobility patterns to underlying physical constraints in the nucleus.

Interpreting genome-wide MSD profiles through a dynamical polymer “digital twin”. By calibrating the PHi-C-inferred model to the experimentally measured 2-point MSDs, we obtained a dynamical representation of the *S. pombe* genome that is consistent with both genome architecture and dynamics in the G2 phase. In this sense, the model functions as a physics-based “digital twin” by providing a quantitative framework to infer chromatin behaviour beyond the genomic loci and time scales that are directly accessible using locus-tagging live-cell imaging.

One advantage of this “digital twin” view is that it enables mechanistic, population-level readouts that are difficult to obtain experimentally. For example, structural sampling allows quantitative decomposition of telomere clustering into distinct grouping patterns (Fig. 3 and Extended Data Fig. 4). In addition, calibrated dynamics allows genome-wide prediction of

locus-specific mobility over a broad temporal range (subseconds to many minutes; Fig. 3c). Importantly, going beyond qualitative descriptions, these predictions translate full MSD(t) profiles into interpretable dynamical quantities.

Clustering of the predicted MSD(t) curves revealed three mobility classes that map naturally onto nuclear landmarks (Fig. 3c,d and Extended Data Fig. 5). Centromere- and telomere-proximal loci form two distinct classes (clusters 2 and 3) characterized by a temporal crossover in which short-time mobility is relatively suppressed ($t \lesssim 10$ s) but long-time movements are larger compared to other loci (Fig. 3c). Each MSD(t) curve was saturated for long times in our framework, so we further extracted a characteristic relaxation time τ as a quantitative time scale for nuclear fluctuations. Notably, the relaxation time distributions could be separated into two regimes: centromere- and telomere-enriched loci relaxed within ~ 150 s, whereas the remaining loci relaxed faster within ~ 70 s (Fig. 3e). Furthermore, although the classes were defined purely from the predicted MSD(t) profiles, constitutive heterochromatin domains at centromeres and telomeres²⁷ were preferentially assigned to cluster 2 and 3, whereas the remaining loci across chromosome arms were predominantly assigned to cluster 1 and appeared more euchromatin-like.

The silent mating-type region (*mat* locus), another constitutive heterochromatin domain positioned at the nuclear envelope^{27–30}, further clarifies this concept. The *mat* locus shares the reduced short-time mobility characteristic of heterochromatin-enriched clusters, forming a distinct subclass within cluster 1 (Extended Data Fig. 5b–d); however, its relaxation time is closer to that of the faster regime ($\tau = 80.5$ s; Supplementary Table 2). In summary, these findings are consistent with the condition under which the *mat* locus experiences short-time confinement, while relaxing more rapidly for longer times. One possible interpretation is that short-time confinement primarily reflects a local chromatin state, whereas long-time relaxation is additionally shaped by the strength and persistence of nuclear landmark associations. Therefore, centromere and telomere heterochromatin may relax slowly because of stronger or more persistent tethering; in contrast, the *mat* locus retains heterochromatin-like short-time

confinement yet relaxes more rapidly in the case of comparatively weaker or more transient nuclear landmark associations. Such locus-specific combinations of short-time confinement and long-time relaxation may be relevant to how heterochromatin maintains stable yet regulatable epigenetic states. In particular, studies have proposed that perinuclear positioning of heterochromatin suppresses histone turnover and promotes epigenetic inheritance³¹; the distinctive long-time relaxation behaviour of the *mat* locus raises the possibility that this locus might tune the stability–plasticity balance by modulating its nuclear positioning and associated histone turnover.

These characteristic time scales raise the question of what cellular processes provide mechanical inputs on comparable time scales and how such inputs propagate through the genome. Therefore, we next examined the oscillatory motion of the SPB and used the PHIC-inferred model to quantify the frequency-dependent chromatin response to centromere-directed oscillatory driving forces (Fig. 4).

SPB oscillations as a geometry-tuned mechanical driver of genome-wide chromatin mobility. Previous live-cell imaging studies have established the interphase motion of the SPB in *S. pombe*. These studies have also simultaneously visualized SPB-associated structures and microtubule dynamics, revealing microtubule-dependent SPB/nuclear movements in living cells²⁰. In meiotic prophase, SPB-led nuclear oscillations (horsetail movement) are a hallmark of fission yeast and are driven by cytoplasmic microtubules, providing a canonical example of how microtubule-generated forces can reorganize nuclear contents over time³².

Beyond these descriptive observations, several studies have also investigated the biological impact of SPB–centromere-associated motion. Centromere-associated motion facilitates the mobility of interphase genomic regions³³. Loss of the microtubule nucleator Mto1 reduces microtubule-dependent chromosomal movement consistently and is associated with impaired DNA repair phenotypes and altered sister-chromatid cohesion³⁴. However, despite

these important advances, the physical principles governing how SPB-driven periodic forces propagate through the chromatin polymer remain largely unexplored using quantitative modelling. It is also unclear which time/frequency components are most effective at deforming the genome. One exception is that SPB motion exhibits a characteristic oscillatory peak with a period of 203–229 s under specific metabolic conditions¹⁹, which is consistent with the pronounced peak near $f = 1/225$ Hz and the strong contribution of low-frequency components that we observed in the PSD spectrum in this study (Fig. 4d).

Therefore, a key goal is to characterize the genome as a mechanically coupled system driven by SPB-centromere-associated motion and to identify the frequency range in which such oscillatory driving forces can generate large-scale chromatin displacements. To this end, we leveraged the PHi-C “digital twin” to compute, on the basis of physical principles, how a force applied at one locus is transmitted as displacement at other loci (Extended Data Fig. 6). In the general setting, this transmission is described using a cross-response function $\chi_{nm}(\omega)$, as shown in Eq. (2) in the Methods section; we focused specifically on centromere-directed forcing by applying an oscillatory force to the three clustered centromeres and mapping the resulting storage and loss components of the genome-wide response (Fig. 4e–g). Results revealed a significant frequency dependence: high-frequency forces at $f \gtrsim 1/150$ Hz are largely confined to centromere-proximal loci, whereas low-frequency driving forces propagate more effectively across chromosome arms (Fig. 4f,g). Together with the relaxation time analysis (Fig. 3), these findings imply that SPB motion is unlikely to be a dominant driver of chromatin fluctuations on the relaxation time scales of ~ 70 s and ~ 150 s. Instead, SPB motion might act primarily through slower, low-frequency mechanical inputs that can propagate over chromosome arms. Notably, at approximately $f = 1/225$ Hz, telomere-proximal loci on chromosomes I and II exhibit positive responses in both storage and loss components (i.e., $\chi' > 0$ and $\chi'' > 0$, respectively), indicating coherent displacements aligned with the centromere-directed forces in both components (Fig. 4f). Consistent with this view, the PSD spectrum shows substantial power in the low-frequency band, with a $1/f^{0.7}$ profile (Fig. 4d),

indicating that SPB motion contains broad, slow components rather than being dominated by a single period. Although the origin of this scaling is beyond the scope of this study, its practical implication is clear: broadband low-frequency driving forces fall precisely in the regime that our response analysis predicts to transmit most effectively through the genome (Fig. 4f,g). Such low-frequency driving forces may therefore shape genome-wide chromatin dynamics not only through a peak component but also by broadening the spectrum of accessible chromosome fluctuations, rather than selectively exciting a single periodic response. Together, these findings show that our quantitative, frequency-domain framework can interpret how SPB motion can be coupled to genome-wide chromatin dynamics.

Importantly, the dominant frequencies of SPB motion may not be universal but can be shaped by cell geometry and microtubule dynamics. In *S. pombe*, SPB motion is mechanically linked to microtubule polymerization dynamics that interact with cell ends, indicating that the effective oscillation period depends on cell size and shape²⁰. Consistent with this view, meiotic nuclear oscillations show clear size dependence that can be captured using a force balance model relating microtubule-generated forces to viscous drag³⁵. These observations support the idea that fission yeast may exploit its rod-shaped geometry to tune SPB-driven mechanical inputs toward low-frequency components, which our response analysis predicts to be most effective at propagating genome-wide.

Finally, the ability of SPB-coupled low-frequency driving forces to mobilize chromatin has potential functional implications. In fission yeast, reduced microtubule-dependent chromosomal motion are correlated with compromised DNA repair phenotypes³⁴, consistent with a model in which large-scale nuclear fluctuations contribute to maintaining genome integrity. More broadly, although diverse eukaryotic systems show increased chromatin mobility in response to DNA damage^{36–38}, the mechanical route by which such mobility is generated is likely shaped by organism-specific nuclear geometry and chromosome architecture. In this regard, the rod-shaped cell morphology and the small number of relatively long chromosomes in *S. pombe* may favour an SPB-driven, low-frequency mechanical strategy that

differs from the mechanisms that organisms with more isotropic cell shapes and/or many shorter chromosomes use.

Conclusion

In this study, we combined comprehensive live-cell locus tracking with Hi-C data-constrained polymer modelling to build a dynamical, physics-based “digital twin” of the *S. pombe* genome. Genome-wide calibration of the PHi-C-inferred model against experimental 2-point MSDs enabled quantitative prediction of chromatin mobility beyond the experimentally sampled loci and time scales; simultaneously, it preserved the contact architecture across chromosomes I–III. This integrated framework reveals nuclear landmark-associated dynamical regimes with characteristic relaxation time scales of ~ 70 s and ~ 150 s and helps obtain a frequency-domain description of how SPB–centromere-directed periodic driving forces propagate through the genome, highlighting the dominant role of low-frequency mechanical inputs in generating large-scale nuclear deformations.

The strategy of integrating an imaging-based genome-wide dynamics map with a genome-wide Hi-C contact map through PHi-C is broadly applicable to other organisms and, in principle, scalable to larger genomes, including human chromosomes. Hi-C contact maps implicitly reflect constraints imposed by nuclear boundaries (e.g., nuclear envelope attachments) and by subnuclear compartments (e.g., the nucleolus); therefore, such models offer a route to interpret genome organization and dynamics at the level of the nucleus as a physical system, rather than chromosomes in isolation. Moreover, the frequency response analysis provides a general framework to incorporate externally imposed or internally generated forces and to connect mechanical driving forces to functional outputs.

In general, a systems-level understanding spanning the extracellular mechanical environment, nuclear organization, and genome regulation is becoming increasingly important. Extracellular matrix stiffness can modulate transcriptional programs via mechanosensitive genomic enhancers³⁹. In this context, tuning the period and amplitude of SPB-driven mo-

tion, for example, by altering the cell size or mechanical conditions, might systematically reshape frequency-dependent chromatin responses predicted using the model. An important next step will be to determine which biological functions are coupled to such changes in chromatin responses to SPB oscillations, such as genome maintenance and gene regulation. More broadly, building physically grounded chromosome models will continue to provide a way to uncover the physical significance of chromatin dynamics within the nucleus and the cell as an integrated system.

Figures

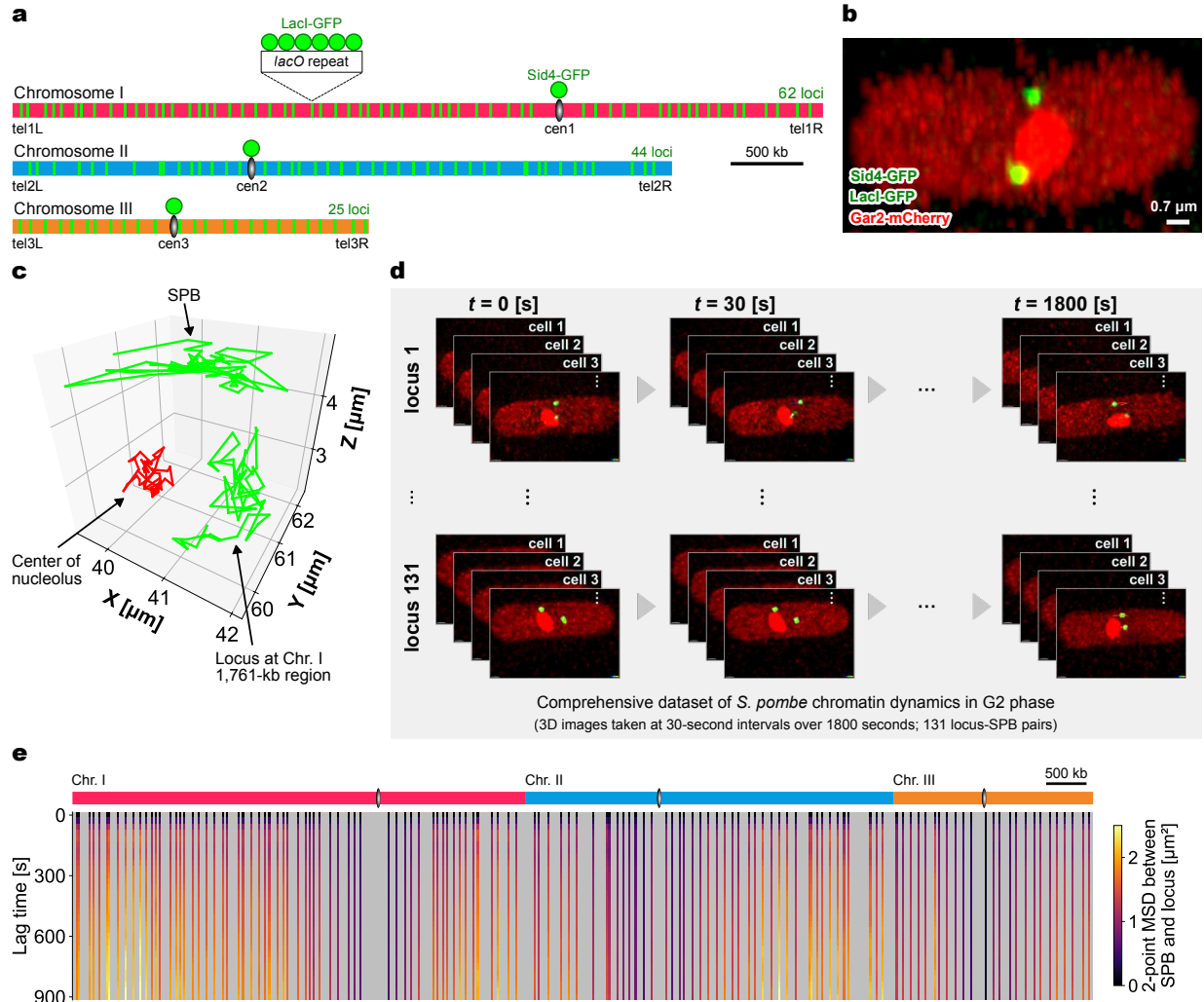


Fig. 1 | Comprehensive live-cell imaging of chromatin dynamics across the *S. pombe* genome.

a, Strategy for genome-wide fluorescent labelling of chromosomal loci. Schematic representations of the three *S. pombe* chromosomes are shown, with target loci (green) and centromeres. Individual cell lines were established for each locus, in which a *lacO* array was inserted at the target site and the loci were visualized by imaging of *lacO*/LacI-GFP, while Sid4-GFP marks the spindle pole body (SPB). **b**, Representative fluorescence image of a living cell showing a single LacI-GFP-labelled locus and Sid4-GFP at the SPB (green), together with Gar2-mCherry, a nucleolar component showing a strong signal in the nucleolus and a weaker

diffuse signal in the cytoplasm (red). **c**, Typical 3D trajectories of the SPB, a labelled locus, and the nucleolus centre obtained from live-cell imaging. **d**, Comprehensive dataset of chromatin dynamics for 131 genomic loci in the G2 phase (3D images acquired every 30 s for 1800 s). **e**, Heat map representation of the two-point mean square displacement (MSD) between each locus and the SPB along the genomic coordinate, revealing genome-wide motion relative to the SPB.

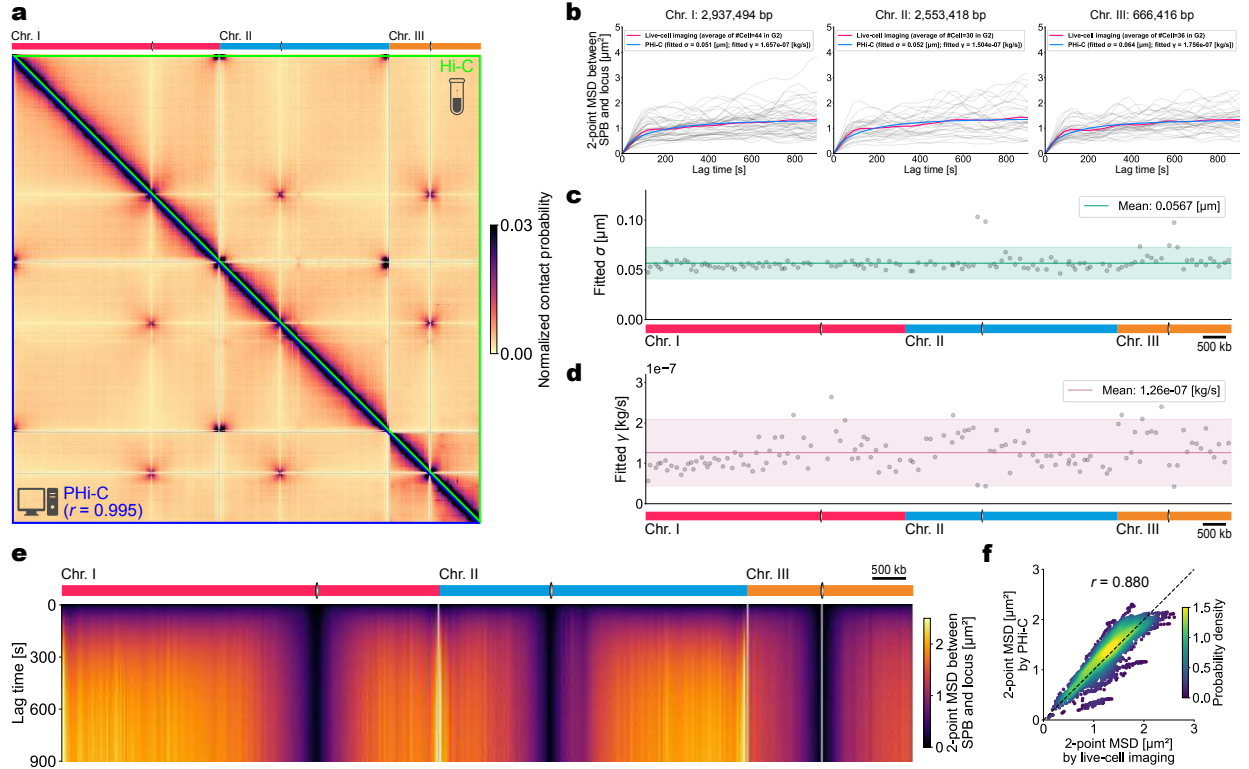


Fig. 2 | Construction of a physics-based polymer model consistent with genome-wide chromatin dynamics. **a**, Comparison between the experimental Hi-C contact map and the reconstructed contact map obtained using PHi-C analysis at 25 kb resolution, showing a high correlation ($r = 0.995$) across the three chromosomes I–III. **b**, Representative fitting of theoretical 2-point MSD curves derived from the PHi-C-inferred model to experimental data for selected loci on chromosomes I–III. The 2-point MSD between the SPB and each locus is plotted against lag time. Grey curves indicate TAMSDs from individual cells, the magenta curve shows the experimental ensemble-averaged TAMSD, and the cyan curve denotes the fitted theoretical curve determined by the parameters σ (μm) and γ (kg/s). **c**, **d**, Fitted values of the model parameters σ (**c**) and γ (**d**) plotted along the genomic coordinates of chromosomes I–III. Each point represents one of the 131 genomic loci. The horizontal line indicates the mean value, and the shaded area denotes the $\pm 2\text{SD}$ range. **e**, Reconstructed genome-wide heat map of the 2-point MSD between each locus and the SPB, obtained using the PHi-C-inferred model with the mean parameter values ($\sigma = 0.0567 \mu\text{m}$

525 and $\gamma = 1.26 \times 10^{-7}$ kg/s). **f**, Correlation between experimental and reconstructed 2-point
526 MSDs. Each point represents a pairwise value of the 2-point MSD using live-cell imaging
527 and the PHi-C-inferred model, integrated over lag times from 0 s to 900 s. The probability
528 density is estimated using Gaussian kernel smoothing, with Pearson's correlation coefficient
529 $r = 0.880$.

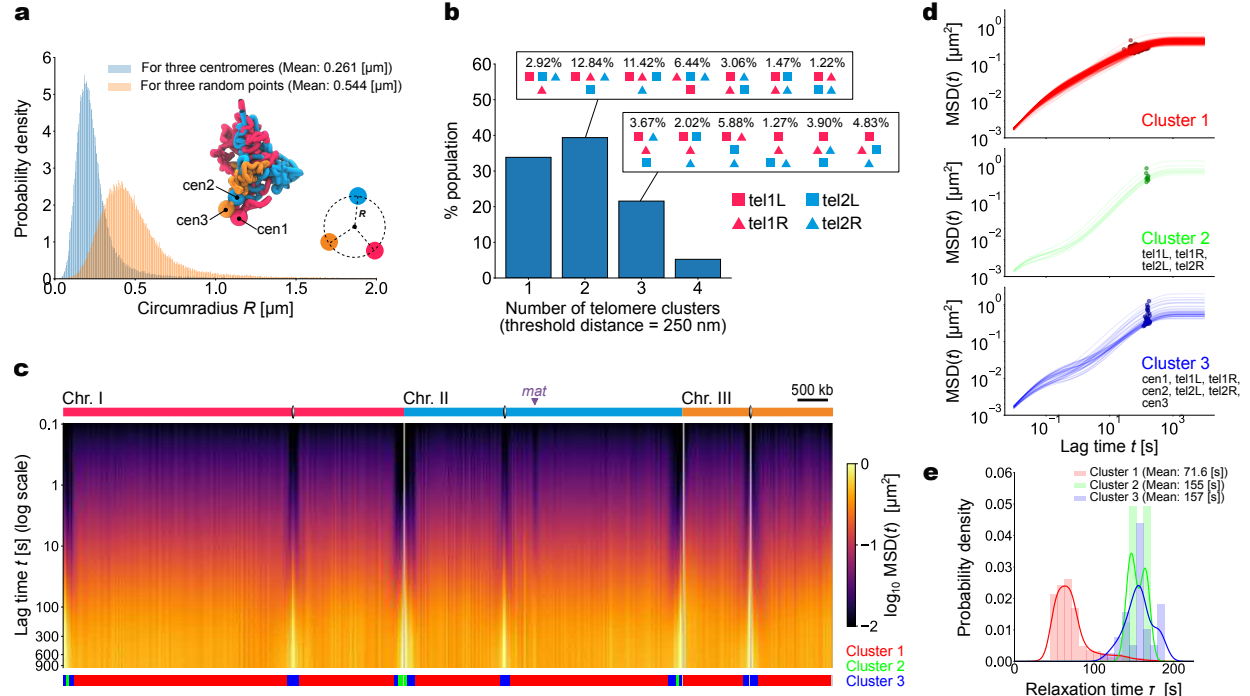


Fig. 3 | Structural features and genome-wide mobility patterns predicted using the calibrated PHi-C-inferred model.

a, Probability distributions of the circumradius R , defined as the radius of the circle passing through centromeres (cen1–3) of chromosomes I–III (inset), computed from 10^5 structural samples generated using PHi-C. The distribution for the centromeres (blue) shows a smaller radius (mean $R = 0.261$ μm) than that obtained by choosing three loci at random (orange; mean $R = 0.544$ μm). **b**, Telomere clustering patterns obtained from 10^5 structural samples. The four telomeres (tel1L, magenta square; tel1R, magenta triangle; tel2L, cyan square; and tel2R, cyan triangle) were grouped into clusters, with a threshold distance of 250 nm. The bar plot shows the population (%) of structures forming 1–4 telomere clusters. Schematic insets illustrate representative telomere groupings for cases with two or three clusters. **c**, Genome-wide heat map of chromatin mobility predicted using PHi-C, showing $\log_{10} \text{MSD}(t)$ for each 25 kb locus along chromosomes I–III (horizontal axis) across lag times from 0.1 s to 900 s (vertical axis, log scale). The coloured strip beneath the heat map denotes the mobility cluster assigned to each locus: cluster 1 (red), cluster 2 (green) and cluster 3 (blue). The

546 genomic position of the *mat* locus is indicated by a triangle. **d**, MSD(t) profiles for the three
547 mobility clusters identified in **c**. For each cluster, MSD curves of all member loci are plotted
548 on log–log axes, with clusters 1, 2 and 3 shown in red, green and blue, respectively. Filled
549 circles mark the MSDs at the relaxation time of each curve. **e**, Probability distributions of
550 relaxation times τ for the three mobility clusters. Kernel density estimate curves are shown
551 for clusters 1, 2 and 3 in red, green and blue, respectively. Mean relaxation times are 71.6 s,
552 155 s and 157 s for clusters 1, 2, and 3, respectively.

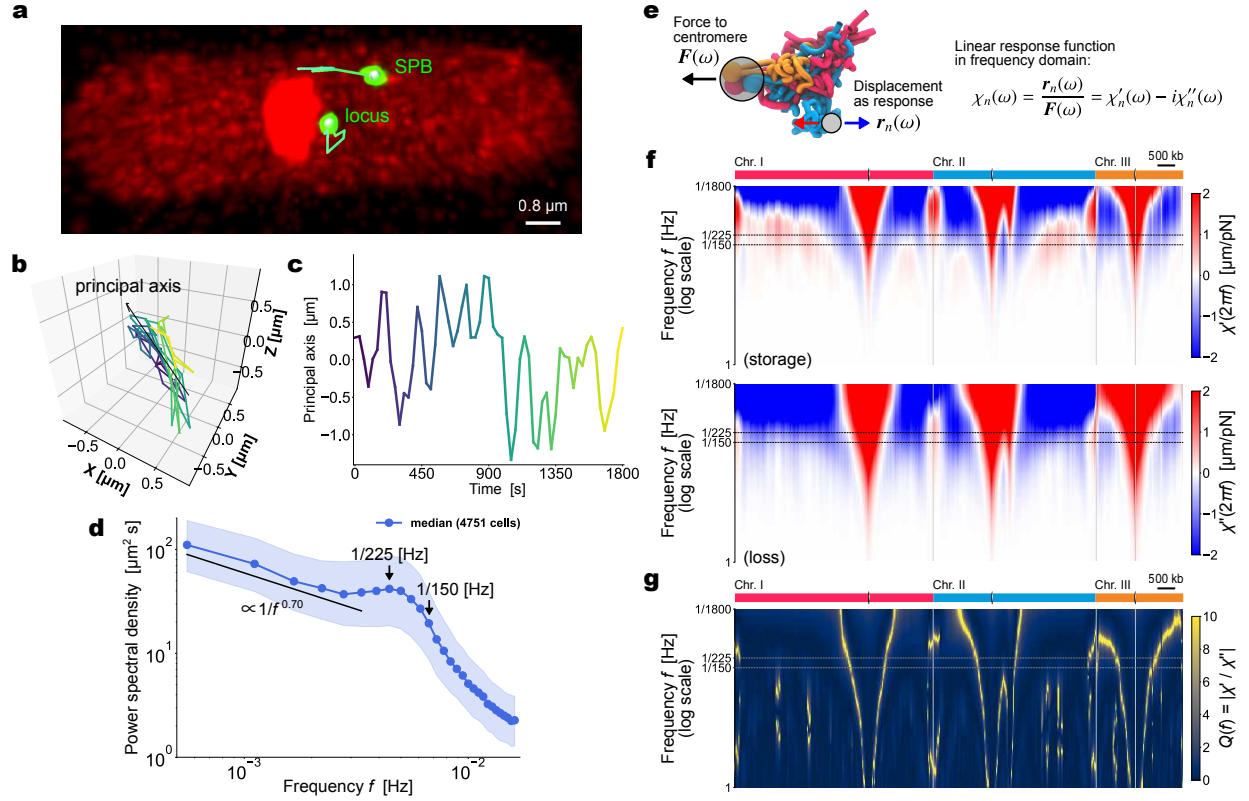


Fig. 4 | Oscillatory SPB motion and frequency-dependent chromatin response predicted using the calibrated PHi-C-inferred model.

a, Representative fluorescence snapshot of a living cell showing the SPB (Sid4-GFP) and a LacI-GFP-labelled locus (green), along with Gar2-mCherry (red). Short-term trajectories of the two green foci highlight the oscillatory motion of the SPB and the locus. Scale bar, 0.8 μm . **b, c**, Typical 3D trajectory of the SPB in a single cell with its principal axis (**b**), and the corresponding time series of SPB displacement projected onto the principal axis (**c**), colour-coded by time (30 s intervals, 61 frames). **d**, Power spectral density (PSD) of SPB motion plotted against frequency f on logarithmic axes. The solid line with circle markers shows the median PSD across 4751 cells, and the filled region indicates the interquartile range (IQR, Q1–Q3). **e**, Schematic illustrating the definition of the frequency-domain linear response function when an oscillatory force is applied to the three centromeres, in which $\chi_n(\omega)$ is decomposed into storage (χ'_n) and loss (χ''_n) components. **f**, Genome-wide linear response of chromatin to oscillatory forces applied at the three centromeres. The storage

568 (upper) and loss (lower) components of the response function, $\chi'(2\pi f)$ and $\chi''(2\pi f)$, are
 569 shown as heat maps across chromosomes I–III as a function of driving force frequency f (log
 570 scale). Horizontal dashed lines mark 1/225 and 1/150 Hz. **g**, Heat map of the quality factor
 571 $Q(f) = |\chi'(2\pi f)/\chi''(2\pi f)|$, quantifying the efficiency with which oscillatory forces applied
 572 at the three centromeres propagate along the genome. Horizontal dashed lines mark 1/225
 573 and 1/150 Hz.

Methods

Strain construction

S. pombe strains (h^{90} *ade6-210 leu1 ura4-D18 lys1 XXX[::ura4⁺-KanR-lacOp] his7⁺::lacI-GFP*), carrying a single *lacO* array inserted at one of the 143 genomic loci (*XXX*), were used to introduce Sid4-GFP and Gar2-mCherry as described previously¹⁹, yielding 131 strains with robust dual labelling (Supplementary Table 1). Briefly, the Sid4-GFP-nat plasmid was linearized using *Stu*I and used to transform the *S. pombe* strains. Subsequently, the Gar2-mCherry-hph plasmid was linearized using *Afl*II and used to transform the resulting *S. pombe* strains.

Live-cell imaging

Cells were grown in YEA medium (0.5% yeast extract, 3% glucose and 40 µg/mL adenine) at 30 °C overnight. A total of 2.5×10^5 cells from a log-phase culture were added to a glass-bottom dish (Iwaki; coated with 5 mg/mL of lectin from *Bandeiraea simplicifolia* BS-I (Sigma)) for live-cell imaging. A stage-top incubator equipped with a lens heater (Tokai Hit) was used to maintain the sample temperature at 30 °C. Each sample was used for a maximum of 3 h of imaging before cells reached a stationary phase. Microscopic images of asynchronous cells were obtained using an iXon3 897 EMCCD camera (Andor) connected to a Yokogawa CSU-W1 spinning-disc scan head (Yokogawa Electric Corporation) and an Olympus IX83 microscope (Olympus) with a UPlanSApo 100× NA 1.4 objective lens (Olympus). Laser illumination was performed at a wavelength of 488 nm for Sid4-GFP and LacI-GFP, and at 561 nm for Gar2-mCherry. Images were captured using MetaMorph software (Molecular Devices). Optical section data (41 focal planes with 0.2 µm spacing, acquired every 30 s; 160 nm/pixel) were collected for 30 min, as shown previously, and we found that the cells could still divide after imaging, suggesting that this imaging condition does not damage cells⁴⁰.

Image processing and 3D spot tracking

Time-lapse sequences were deconvolved using Huygens image analysis software (Scientific Volume Imaging). All deconvolved 3D time-lapse image stacks (STK format), along with the corresponding ND metadata files, have been deposited in the SSBD:repository (ssbd-repos-000493). The dataset is organized by the 131 genomic locus IDs (Supplementary Table 1) and the date of imaging. A video index was assigned as a prefix to each filename. Within each imaging file, individual cells were annotated with a unique cell index, and a cell cycle index based on the morphological criteria shown in Extended Data Fig. 1 was manually assigned: 1, G1/S; 2, early G2; 3, G2; 4, late G2; and 5, other. These annotations were combined to define the final cell ID used in subsequent analyses.

The deconvolved time-lapse sequences for each locus were analysed using IMARIS software (Bitplane). For Sid4-GFP and LacI-GFP, the centre of each fluorescent focus was tracked in 3D over 30 min at 30 s intervals for each strain. For Gar2-mCherry, the geometric centre of the strong fluorescence region was tracked as the 3D position of the nucleolus centre, as described previously¹⁹. The resulting G2-phase trajectories consist of SPB (X,Y,Z), locus (X,Y,Z) and nucleolus-centre (X,Y,Z) coordinates recorded over 61 frames (61×9 values), along with the corresponding cell cycle index; all data are available in the SSBD:repository (ssbd-repos-000493).

Two-point mean square displacement between the SPB and the locus

For each cell, 3D trajectories of the SPB and a LacI-GFP-labelled locus were represented as $\{\mathbf{r}_{\text{SPB}}(t_j)\}_{j=0}^{60}$ and $\{\mathbf{r}_{\text{locus}}(t_j)\}_{j=0}^{60}$ ($t_j = j \Delta t$, $\Delta t = 30$ s). To remove nuclear translation, we computed the relative position vector as $\mathbf{s}_{\text{locus}}(t_j) = \mathbf{r}_{\text{locus}}(t_j) - \mathbf{r}_{\text{SPB}}(t_j)$. For each relative trajectory $\{\mathbf{s}_{\text{locus}}(t_j)\}_{j=0}^{60}$, we calculated the time-averaged mean square displacement

(TAMSD) for lag times $\tau_k = k \Delta t$ ($k = 0, 1, 2, \dots, 30$) using the following equation:

$$\text{TAMSD}_{\text{locus}}(\tau_k) = \frac{1}{61 - k} \sum_{j=0}^{60-k} [\mathbf{s}_{\text{locus}}(t_j + \tau_k) - \mathbf{s}_{\text{locus}}(t_j)]^2.$$

The ensemble average of $\text{TAMSD}_{\text{locus}}(\tau_k)$ over all tracked cells was used as the 2-point MSD between the SPB and the locus, and the MSDs were visualized genome-wide as a heat map (Fig. 1e).

PHi-C analysis

Hi-C preprocessing. Hi-C FASTQ data for wild-type interphase *S. pombe* cells (SRR5227728, SRR5227738, SRR5227739 and SRR5227749) obtained from Kakui *et al.*⁴ were processed following the 4DN Hi-C scripts (<https://github.com/4dn-dcic/docker-4dn-hic/tree/v43/scripts>). Hi-C reads were mapped to the *S. pombe* reference genome (ASM294v2) using **bwa** (v0.7.18), and alignments were handled using **samtools** (v1.21). Valid Hi-C contacts were identified using **pairtools** (v1.1.0) with the subcommands *parse*, *sort*, *dedup*, *split* and *select*, followed by indexing using **pairix** (v0.3.8). The four resulting pairs files (.pairs.gz) were merged, and a genome-wide Hi-C file (.hic) was generated using **juicer-tools** (v1.22.01) with the *pre* command (MAPQ ≥ 30 with genome-wide Knight–Ruiz **GW_KR** normalization).

Optimization of the PHi-C matrix. A contact matrix for PHi-C analysis was generated from the .hic file using **hic-straw** (v1.3.1). Intra- and inter-chromosomal contacts were extracted at 25 kb resolution using **GW_KR** normalization, producing a single square matrix whose size corresponded to the total number of 25 kb genomic bins across the three *S. pombe* chromosomes: chromosome I (5,579,133 bp $\rightarrow$ 224 bins), chromosome II (4,539,804 bp $\rightarrow$ 182 bins), and chromosome III (2,452,883 bp $\rightarrow$ 99 bins); this yielded a 505×505 matrix. To remove poorly mappable genomic regions, such as centromeres, we excluded rows and columns in which $\geq 60\%$ of entries were NaN, resulting in a dense 501×501 contact matrix

that was used as input for PHi-C optimization. Supplementary Table 2 lists the genomic coordinates corresponding to indices $n = 0-504$, including those removed during filtering, with centromeric indices highlighted.

PHi-C optimization was performed using `phic` (v2.0.13), with the *preprocessing* and *optimization* subcommands executed sequentially. The optimized interaction matrix (PHi-C matrix) reproduced the input Hi-C contact map with high fidelity, achieving a Pearson correlation of $r = 0.995$ between the experimental and reconstructed contact maps (Extended Data Fig. 2).

Theoretical computation of the 2-point MSD from the PHi-C-inferred model.

We computed the theoretical 2-point MSD on the basis of the PHi-C-inferred model following the formalism described by Shinkai *et al.*⁵ From the optimized $N \times N$ PHi-C matrix, we constructed the corresponding normalized Laplacian matrix $\bar{\mathbf{L}}$ and diagonalized it to obtain the normalized eigenvalues $0 = \bar{\lambda}_0 < \bar{\lambda}_1 < \dots < \bar{\lambda}_{N-1}$ and the orthogonal matrix $\mathbf{Q}$. Let M denote the set of indices corresponding to centromeric bins. In the model, the centroid of the beads belonging to set M is treated as the position of the SPB. Under these assumptions, the theoretical 2-point MSD between the SPB and the n -th locus can be computed analytically as follows:

$$\text{MSD}_{\text{locus}}(t; n, \text{cen}) = 2\sigma^2 \sum_{p=1}^{N-1} \left(Q_{np} - \frac{1}{|M|} \sum_{m \in M} Q_{mp} \right)^2 \bar{\lambda}_p^{-1} \left(1 - e^{-3 \frac{k_B T}{\gamma \sigma^2} \bar{\lambda}_p t} \right), \quad (1)$$

where σ is the effective polymer segment size (contact distance parameter), γ is the friction coefficient of each bead in the PHi-C-inferred model, k_B is the Boltzmann constant, and T is the temperature (K).

Fitting of model parameters (σ and γ) to the experimental 2-point MSD.

For each of the 131 genomic loci, the experimentally measured 2-point MSD between the SPB and the locus was fitted to the theoretical 2-point MSD function derived from the PHi-C-inferred

666 model (see the previous section). We evaluated the theoretical 2-point MSD at $T = 303$ K,
667 corresponding to the live-cell imaging conditions. Next, nonlinear least-squares fitting was
668 performed using `scipy.optimize.curve_fit` with the following settings: initial parameter
669 values $\sigma = 0.05 \mu\text{m}$ and $\gamma = 1 \times 10^{-7} \text{ kg/s}$, and bounds = $(0, \infty)$ for both parameters. The
670 two parameters σ and γ were simultaneously estimated for each locus.

671 **Structural sampling from the optimized PHi-C-inferred model.** Structural sam-
672 pling was performed using the *sampling* subcommand in `phic` (v2.0.13), generating 10^5 3D
673 configurations of chromosomes I–III of *S. pombe*. Next, the sampled coordinates were scaled
674 using the experimentally determined value $\sigma = 0.0567 \mu\text{m}$ to match the actual physical length
675 scale measured in live-cell imaging. For each sampled structure, we defined the centromere
676 position of each chromosome as the centroid of the bead coordinates corresponding to the
677 centromeric indices in the PHi-C-inferred model. Using the three centromere coordinates (for
678 chromosomes I–III), as well as triplets of beads randomly selected from each chromosome
679 model, we computed the circumradius. For three points A, B and C in 3D space, with side
680 lengths of $a = |\text{BC}|$, $b = |\text{CA}|$, and $c = |\text{AB}|$ and triangle area S , we calculated the circum-
681 radius R from the geometric relation $R = \frac{abc}{4S}$. In addition, we extracted the four telomeres
682 of chromosomes I and II (tel1L, tel1R, tel2L and tel2R) from each sampled structure and
683 quantified telomere clustering by grouping telomeres whose pairwise distances fell below a
684 threshold. The proportions of structures forming 1–4 telomere clusters were computed for
685 threshold distances of 200 nm, 250 nm, and 300 nm.

686 **Genome-wide chromatin mobility analysis.** Using the genome-wide mean values of
687 the fitted parameters obtained from experimental 2-point MSDs ($\sigma = 0.0567 \mu\text{m}$ and $\gamma =$
688 $1.26 \times 10^{-7} \text{ kg/s}$), we computed and predicted the dynamic properties of the three-chromosome
689 physics-based polymer model generated using PHi-C. For each genomic index n in the PHi-
690 C-inferred model, the single-locus mean square displacement $\text{MSD}(t; n)$ was calculated using

the following theoretical expression^{5,41}:

$$\text{MSD}(t; n) = 2\sigma^2 \sum_{p=1}^{N-1} Q_{np}^2 \bar{\lambda}_p^{-1} \left(1 - e^{-3 \frac{k_B T}{\gamma \sigma^2} \bar{\lambda}_p t} \right),$$

where the centre-of-mass mode ($p = 0$) was removed.

To classify the shapes of the $\text{MSD}(t)$ curves, we computed the cosine similarity between all pairs of MSD profiles evaluated on a log-log scale using `cosine_similarity` from `sklearn.metrics.pairwise`. Next, we constructed a cosine distance matrix as $1 - (\text{cosine similarity})$, with diagonal elements set to zero. Finally, we performed hierarchical clustering on this distance matrix using `scipy.cluster.hierarchy.linkage` with `method="average"`, visualized the resulting dendrogram using `scipy.cluster.hierarchy.dendrogram`, and defined three clusters by cutting the dendrogram (Extended Data Fig. 5).

Power spectral analysis of SPB motion

We conducted power spectral analysis of SPB motion using the SPB and nucleolus centre coordinates obtained from 3D time-lapse imaging ($\Delta t = 30$ s, 61 frames). To remove the drift arising during image acquisition, we expressed the SPB coordinates relative to the nucleolus centre at each time point. For each degree of freedom (x , y and z), we constructed a one-dimensional time series, and computed the PSD using the `scipy.signal.welch` function with parameters `fs = 1/30` and `nperseg = 60`. Finally, we summed the PSDs of the three degrees of freedom to obtain the total PSD for each SPB trajectory. This analysis was applied to all G2-phase cells, yielding PSD profiles for 4751 cells.

Frequency-domain response function analysis

The dynamics of the polymer model consisting of N segments in PHi-C are described by the Langevin equation

$$\gamma \frac{d\mathbf{r}(t)}{dt} = -\mathbf{L}\mathbf{r}(t) + \boldsymbol{\xi}(t),$$

712 where $\mathbf{r} = (\mathbf{r}_0, \mathbf{r}_1, \dots, \mathbf{r}_{N-1})$ and $\boldsymbol{\xi} = (\boldsymbol{\xi}_0, \boldsymbol{\xi}_1, \dots, \boldsymbol{\xi}_{N-1})$ denote the position and thermal
 713 noise supervectors, respectively^{5,42}. The matrix $\mathbf{L}$ is the Laplacian matrix derived from the
 714 interaction matrix of the PHi-C-inferred model.

715 When an external force $\mathbf{f}_m(t)$ is applied to the m -th segment, the Langevin equation can
 716 be written using the unit vector $\mathbf{e}_m$ (whose m -th component is 1 and all others are 0) as

$$\gamma \frac{d\mathbf{r}(t)}{dt} = -\mathbf{L}\mathbf{r}(t) + \boldsymbol{\xi}(t) + \mathbf{f}_m(t)\mathbf{e}_m.$$

717 Since the Laplacian matrix $\mathbf{L}$ derived from the PHi-C interaction matrix is a real symmetric
 718 matrix, it admits the spectral decomposition $\mathbf{L} = \mathbf{Q}^\top \boldsymbol{\Lambda} \mathbf{Q}$. Multiplying from the left by $\mathbf{Q}^\top$
 719 and defining the normal coordinates $\mathbf{X} = \mathbf{Q}^\top \mathbf{r}$, we obtain:

$$\gamma \dot{\mathbf{X}}(t) = -\boldsymbol{\Lambda} \mathbf{X}(t) + \mathbf{Q}^\top \boldsymbol{\xi}(t) + \mathbf{f}_m \mathbf{Q}^\top \mathbf{e}_m.$$

720 Since $\boldsymbol{\Lambda} = \text{diag}(\lambda_0, \lambda_1, \dots, \lambda_{N-1})$, the equation of motion decouples into independent equa-
 721 tions for each mode p :

$$\gamma \dot{\mathbf{X}}_p(t) = -\lambda_p \mathbf{X}_p(t) + \boldsymbol{\eta}_p(t) + Q_{mp} \mathbf{f}_m(t),$$

722 where we used $\boldsymbol{\eta}(t) = \mathbf{Q}^\top \boldsymbol{\xi}(t)$ and $(\mathbf{Q}^\top \mathbf{e}_m)_p = Q_{mp}$. Applying the Fourier transform
 723 $\int_{-\infty}^{\infty} dt e^{-i\omega t}$ yields the following relations between the Fourier components:

$$i\gamma\omega \mathbf{X}_p(\omega) = -\lambda_p \mathbf{X}_p(\omega) + \boldsymbol{\eta}_p(\omega) + Q_{mp} \mathbf{f}_m(\omega).$$

724

$$\therefore \mathbf{X}_p(\omega) = \frac{\boldsymbol{\eta}_p(\omega) + Q_{mp} \mathbf{f}_m(\omega)}{\lambda_p + i\gamma\omega}.$$

725 Since the inverse transformation is given by $\mathbf{r} = \mathbf{Q} \mathbf{X}$, the Fourier component of the dis-
 726 placement of the n -th segment becomes $\mathbf{r}_n(\omega) = \sum_{p=0}^{N-1} Q_{np} \mathbf{X}_p(\omega)$. Taking the ensemble
 727 average of both sides and using $\langle \boldsymbol{\eta}_p(\omega) \rangle = 0$, the linear response relationship of the n -th

segment to an external force applied to the m -th segment in the frequency domain is written
 as $\mathbf{r}_n(\omega) = \chi_{nm}(\omega) \mathbf{f}_m(\omega)$. Thus, we obtain the cross-response function as

$$\chi_{nm}(\omega) = \chi'_{nm}(\omega) - i\chi''_{nm}(\omega) = \sum_{p=0}^{N-1} \frac{Q_{np}Q_{mp}}{\lambda_p + i\gamma\omega}. \quad (2)$$

Therefore, the real and imaginary parts of $\chi_{nm}(\omega)$ define the storage and loss response
 functions, respectively, given by

$$\chi'_{nm}(\omega) = \sum_{p=0}^{N-1} \frac{Q_{np}Q_{mp}}{\lambda_p^2 + \gamma^2\omega^2} \lambda_p \quad \text{and} \quad \chi''_{nm}(\omega) = \sum_{p=0}^{N-1} \frac{Q_{np}Q_{mp}}{\lambda_p^2 + \gamma^2\omega^2} \gamma\omega.$$

Using the normalized eigenvalues $\bar{\lambda}_p$ obtained from the PHi-C analysis, we computed the
 physical eigenvalues as $\lambda_p = \frac{3k_{\text{BT}}}{\sigma^2} \bar{\lambda}_p$. To focus on the propagation of forces within the *S.*
pombe three-chromosome system, we removed the contribution of the centre-of-mass mode
 ($p = 0$). Next, we calculated the response function of the n -th segment to an oscillatory force
 applied to the centromeres, $\chi_n(\omega)$ (see Fig. 4e), by averaging the cross-response functions
 over the set M of centromeric indices:

$$\chi_n(\omega) = \frac{1}{|M|} \sum_{m \in M} \chi_{nm}(\omega).$$

Data availability

The datasets are available at ssbd-repos-000493, <https://doi.org/10.24631/ssbd.repos.2026.02.493> in SSBD:repository⁴³.

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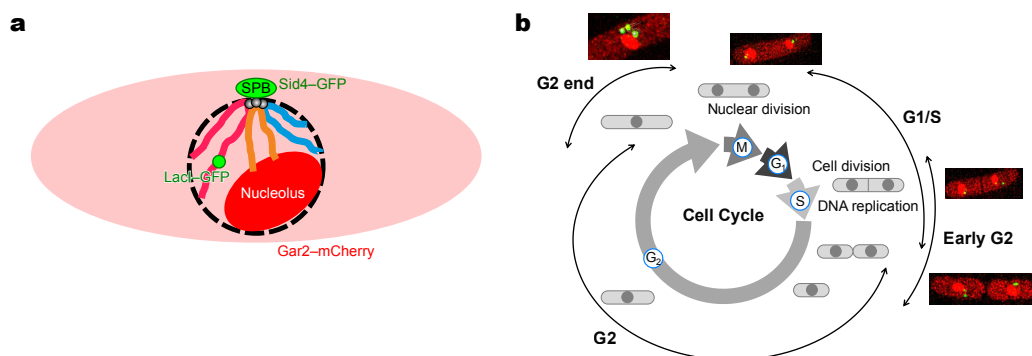
Author contributions

S.T. led the project. S.S., Y.H., A.A., M.U. and S.T. designed the study. M.U., T.H and Y.H. constructed the strains. M.U. conducted imaging experiments. T.N. performed imaging data analysis. S.S., T.N., T.S. and A.A. performed data analysis. S.S., S.H. and S.O. performed PHi-C analysis. S.S. drafted the manuscript. T.N., Y.H. and M.U. contributed to writing the Methods. T.N., Y.H., M.U. and S.T. reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Competing interests

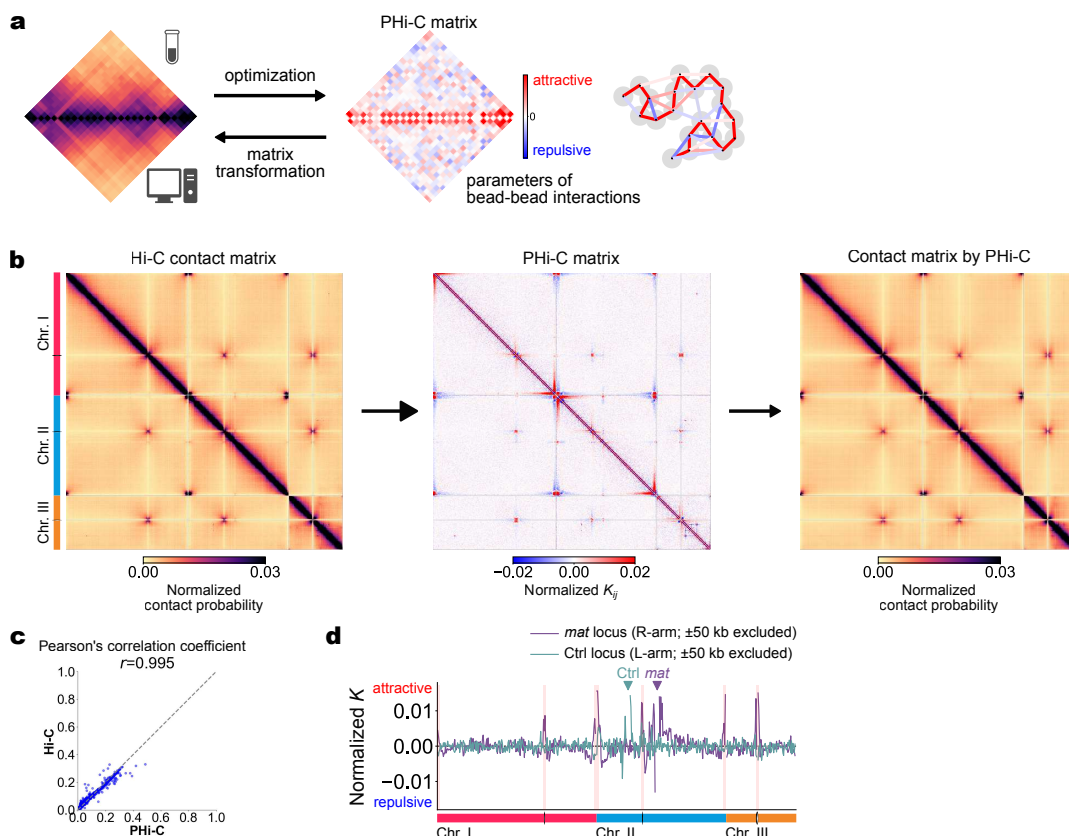
The authors declare no competing interests.

Extended Data Figures



Extended Data Fig. 1 | Nuclear organization and cell cycle staging of *S. pombe* cells for chromatin dynamics analysis.

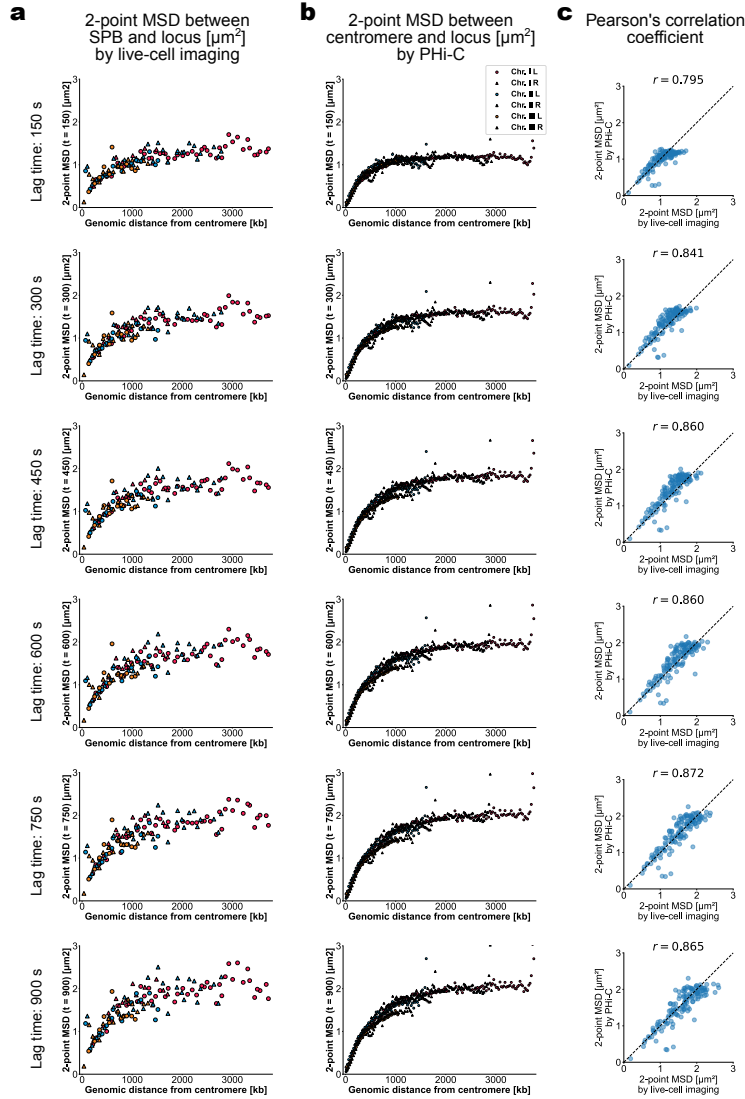
a, Schematic illustration of *S. pombe* chromosome organization in interphase. During interphase, chromosomes are arranged in a Rabl configuration. The schematic also indicates the fluorescence markers used in this study: Sid4-GFP (green) marking the SPB, LacI-GFP (green) labelling the chromosomal locus of interest, and Gar2-mCherry (red) marking the nucleolus, corresponding to the imaging configuration shown in Fig. 1b. **b**, Schematic representation of the *S. pombe* cell cycle with representative fluorescence images of cells at each phase. Before quantitative analyses, cells were visually inspected and assigned to the corresponding stage. We focused on cells in the G2 phase for live-cell imaging and subsequent quantification of chromatin dynamics.



Extended Data Fig. 2 | Overview and validation of PHi-C analysis.

a, Schematic representation of the PHi-C optimization framework. An optimized interaction parameter matrix of the polymer model (PHi-C matrix) was generated from an experimental Hi-C contact map through the PHi-C optimization algorithm that minimizes a cost function, and is finally converted back to a reconstructed contact map. The PHi-C matrix represents attractive and repulsive interactions between segments (beads) of the polymer model. **b**, Workflow of PHi-C analysis showing the transformation from the experimental Hi-C contact map (left) to the optimized PHi-C matrix (middle) and to the reconstructed contact map (right). **c**, Correlation between the experimental Hi-C contact map and the reconstructed contact map obtained using PHi-C analysis, showing high Pearson's correlation ($r = 0.995$). **d**, Genome-wide profiles of the normalized interaction parameter K in the PHi-C matrix for two genomic loci: the chromosome II *mat* locus (R arm) and a symmetric control locus (Ctrl) on the chromosome II L arm at the same genomic distance from the centromere.

910 Regions within ± 50 kb around each locus were excluded. Positive and negative K values
911 indicate attractive and repulsive interactions, respectively. Genomic positions showing strong
912 attractive interactions of the *mat* locus with the three centromeres and the telomeres of
913 chromosomes I and II are highlighted in pink.

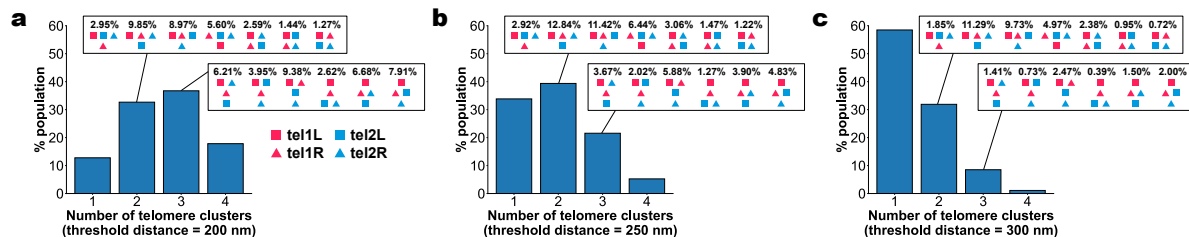


914

915 Extended Data Fig. 3 | Comparison between experimental and PHi-C-derived 916 2-point MSDs across genomic distances and time scales.

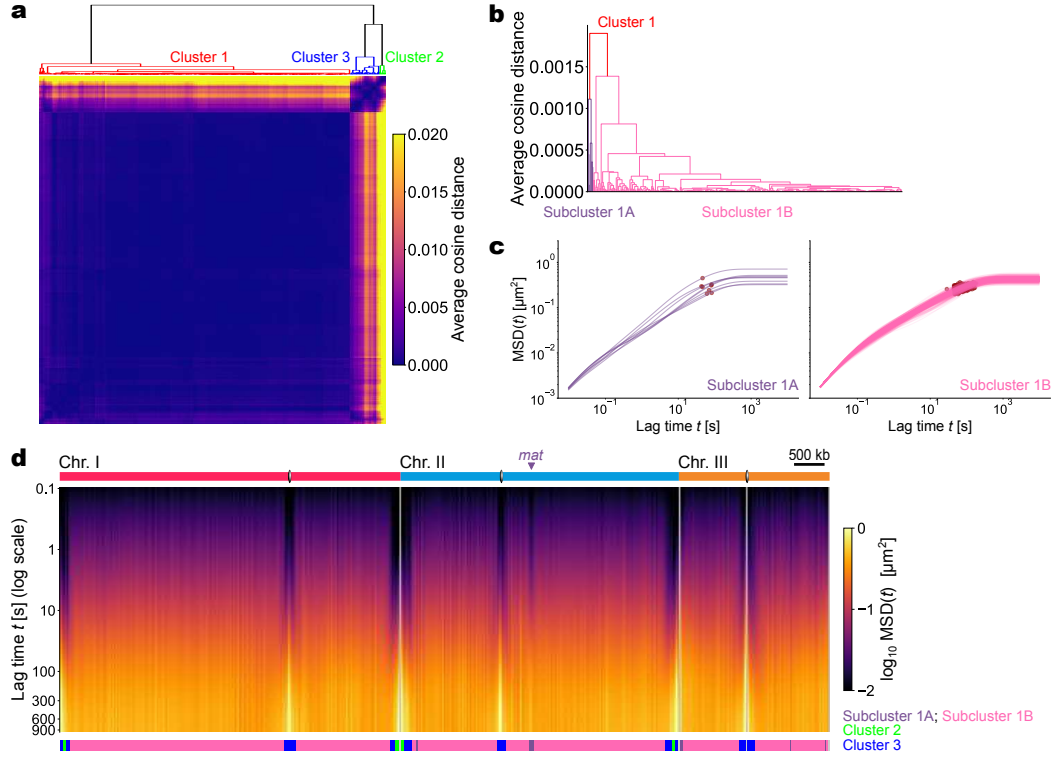
917 **a–c**, 2-point MSDs between the SPB and each locus obtained from live-cell imaging (**a**) and
918 from the PHi-C-inferred model (**b**), as well as their correlations (**c**) at six lag times (150, 300,
919 450, 600, 750, and 900 s). **a**, **b**, The 2-point MSD plotted against genomic distance from the
920 centromere. Data points correspond to loci on the left and right chromosome arms: chromo-
921 some I L (circle, magenta), chromosome I R (triangle, magenta), chromosome II L (circle,
922 cyan), chromosome II R (triangle, cyan), chromosome III L (circle, orange), and chromo-
923 some III R (triangle, orange). **c**, Scatter plots comparing experimental and PHi-C-derived

924 2-point MSDs, with Pearson's correlation coefficients $r = 0.795, 0.841, 0.860, 0.860, 0.872,$
925 and 0.865 for the six lag times, respectively.



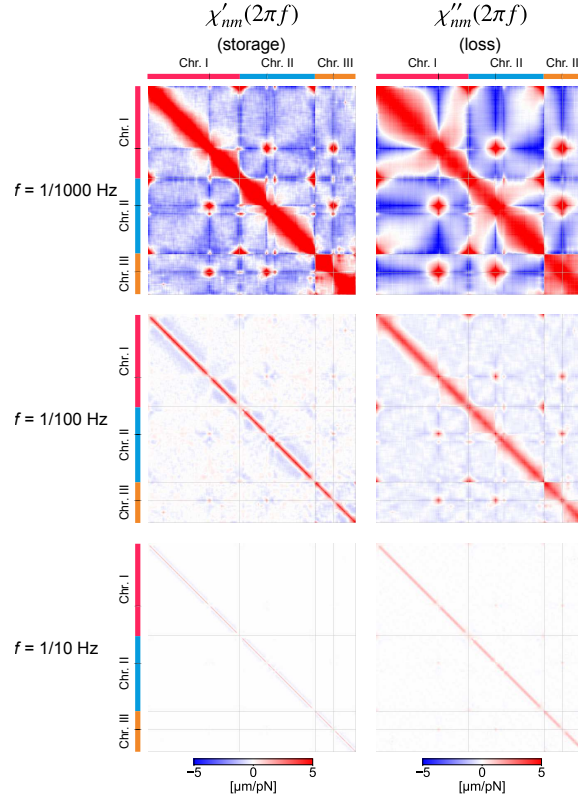
Extended Data Fig. 4 | Quantitative characterization of telomere clustering and chromosome conformation geometry in PHi-C structural samples.

a–c, Population (%) of structures forming 1–4 telomere clusters, calculated from 10^5 structural samples generated using PHi-C, with distance thresholds of 200 nm (**a**), 250 nm (**b**) and 300 nm (**c**). The four telomeres (tel1L, magenta, square; tel1R, magenta, triangle; tel2L, cyan, square; and tel2R, cyan, triangle) were grouped into clusters according to each threshold. Bar plots show the fraction of structures giving rise to different numbers of clusters under the three threshold conditions. Schematic insets indicate representative telomere groupings for cases in which two or three clusters are formed.



Extended Data Fig. 5 | Hierarchical clustering and subclustering of genome-wide MSD(t) profiles across the *S. pombe* genome.

a, Heat map of the average cosine distance between MSD(t) profiles for all loci (25 kb bins), with a dendrogram showing the hierarchical clustering that yields the three mobility clusters used in Fig. 3. **b**, Hierarchical subclustering of mobility cluster 1. The dendrogram shows the hierarchical clustering of loci assigned to cluster 1 on the basis of the cosine distance between MSD(t) profiles on a log–log scale, separating cluster 1 into two subclusters (1A and 1B). **c**, MSD(t) profiles for subclusters 1A and 1B. **d**, Genome-wide mobility heat map with refined cluster annotation. The heat map is shown as in Fig. 3c, but the original cluster 1 band is replaced by the two subclusters 1A and 1B. The genomic position of the *mat* locus is indicated by a triangle.



948

949 **Extended Data Fig. 6 | Genome-wide cross-response functions predicted using**
 950 **the PHi-C-inferred model.**

951 Heat maps showing the cross-response of the chromatin polymer to oscillatory forces applied
 952 at each genomic locus. For three representative driving force frequencies (1/1000, 1/100 and
 953 1/10 Hz; top to bottom), the storage (left; χ'_{nm}) and loss (right; χ''_{nm}) components of the
 954 linear response function are plotted across chromosomes I–III. Each panel shows the response
 955 of all loci to a unit oscillatory force applied sequentially at every 25 kb locus, illustrating
 956 how vibrational perturbations at different frequencies propagate across the genome.

Supplementary Videos and Tables

Supplementary Video 1 | Live-cell imaging of the chromosomal locus and nuclear landmarks in *S. pombe*.

Time-lapse sequence corresponding to Fig. 1b. A cell expressing Sid4-GFP and LacI-GFP (green), along with Gar2-mCherry (red), was imaged at a time interval of 30 s for 61 frames (total duration 1800 s). Sid4-GFP labels the SPB, LacI-GFP marks a single genomic locus, and Gar2-mCherry shows a strong nucleolar signal with weaker diffuse cytoplasmic fluorescence. Scale bar, 0.7 μ m.

Supplementary Video 2 | PHi-C fitting of 2-point MSDs for all loci on chromosome I.

Time-lapse visualization corresponding to the representative fitting shown in Fig. 2b. For each of the 62 loci on chromosome I, grey curves show TAMSDs from individual cells, the magenta curve shows the experimental ensemble-averaged TAMSD, and cyan curve represents the fitted theoretical MSD curve derived from the PHi-C-inferred model.

Supplementary Video 3 | PHi-C fitting of 2-point MSDs for all loci on chromosome II.

Time-lapse visualization of the fitting process for the 44 loci on chromosome II. Grey curves denote TAMSDs from individual cells, the magenta curve shows the ensemble-averaged TAMSD, and the cyan curve represents the corresponding theoretical MSD curve based on the PHi-C-inferred model.

Supplementary Video 4 | PHi-C fitting of 2-point MSDs for all loci on chromosome III.

Time-lapse visualization of the fitting process for the 25 loci on chromosome III. Grey curves represent TAMSDs from individual cells, the magenta curve shows the ensemble-averaged TAMSD, and the cyan curve shows the fitted theoretical MSD curve derived from the PHi-C-inferred model.

Supplementary Video 5 | Dynamical behaviour of the PHi-C-inferred model.

A simulation demonstrating the chromatin dynamics generated using the PHi-C-inferred model with parameters calibrated to experimental MSDs. (Left) Time-evolving 3D configurations of chromosomes I, II, and III (magenta, cyan, and orange, respectively) are shown, with centromeres marked by bead representations and telomeres indicated by yellow beads. (Right) Corresponding pairwise distance map updated at each frame. The elapsed time (0–5 s) is displayed above the distance map.

Supplementary Table 1 | Insertion positions of *lacO* arrays in *lacO*/LacI-GFP.

List of genomic loci at which *lacO* arrays were inserted at one of 143 loci across the genome¹⁸; from them, 131 strains were obtained with the markers for the SPB and the nucleolus. Locus IDs begin with A, B, or C, corresponding to chromosomes I, II, and III, respectively. For each locus, the table reports the genomic position of the insertion (bp), the genomic distance from the centromere (kb), and the number of trajectories analysed during the G2 phase.

Supplementary Table 2 | Genomic bin indices and annotations used for PHi-C analysis.

List of 505 genomic regions (bins) covering the three *S.pombe* chromosomes used in the PHi-C modelling. For each bin, the table reports the bin index, chromosome, and genomic start and end coordinates (bp). Bins that overlap centromeric regions are indicated in the “centromere” column. Bins whose corresponding rows and/or columns in the contact matrix contained $\geq 60\%$ NaN entries and were therefore excluded from the PHi-C analysis are indicated in the “removed” column. The “mat locus” column indicates the bin corresponding to the chromosome II *mat* locus. The “MSD cluster index” column lists the mobility class assigned to each bin from clustering of $\text{MSD}(t)$ profiles (1A, 1B, 2 or 3). The “relaxation time [s]” column reports the relaxation time τ estimated from the analysis in Fig. 3d.

Supplementary Files

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- [SupplementaryVideo5.mp4](#)