



Energy landscape analysis of the development of the chromosome structure across the cell cycle

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During mitosis, there are significant structural changes in chromosomes. We used a maximum entropy approach to invert experimental Hi-C data to generate effective energy landscapes for chromosomal structures at different stages during the cell cycle. Modeled mitotic structures show a hierarchical organization of helices of helices. High-periodicity loops span hundreds of kilobases or less, while the other low-periodicity ones are larger in genomic separation, spanning several megabases. The structural ensembles reveal a progressive decrease in compartmentalization from interphase to mitosis, accompanied by the appearance of a second diagonal in prometaphase, indicating an organized array of loops. While there is a local tendency to form chiral helices, overall, no preferential left-handed or right-handed chirality appears to develop on the time scale of the cell cycle. Chromatin thus appears to be a liquid crystal containing numerous defects that anneal rather slowly.

mitotic chromosome | energy landscape | chromatin dynamics

As cells divide and mature, their chromosomes undergo significant conformational changes. These changes are necessary to ensure the correct segregation of genetic material from parent to daughter cells. A chromosome, while remaining flexible, must be able to be pulled away from its companions by motor assemblies. The transition from interphase, where cells spend most of their life, to mitosis involves, therefore, a coordinated interplay of numerous proteins that orchestrate both local and global structural reorganization. Over four thousand proteins, classified into 28 distinct functional groups, have been associated with mitotic chromosomes (1). However, mitotic chromosome structure formation has been mechanically connected with clarity only to a smaller set of proteins: histones, condensin and cohesin complexes, chromokinesin KIF4A, and DNA topoisomerase II α (2, 3). Still, it is obvious that the detailed mechanism must be rather complex.

Here, we explore a path to understanding the mesoscale aspects of this organization using ideas from energy landscape theory and the theory of symmetry-breaking phase transitions. A variety of structures of the chromosome have been seen to emerge even at the scales accessible to direct imaging. Light microscopy shows that mitotic chromosome structures exhibit some helical coiling (4). The coiled chromatin, called chromonema, can be seen to consist of a rough helix in the chromatid (5). Chromonema occurs in somatic and meiotic chromosomes in metaphase for many organisms. The number of turns that can be made varies with the chromosome size, but typically is in the tens (5). While imaging reveals the overall low-resolution structure, how the large information content of the genome relates to this structural detail is less clear.

The technology of Hi-C gives insights at this finer level (6). DNA–DNA ligation maps reveal changing patterns of contacts in all chromosomes during mitosis in HeLa cells (7). Generally, these maps show a genome-wide decrease in compartmentalization signals in mitotic chromosomes compared to the compartmentalization seen in interphase. Similar patterns have been observed in synchronized Hi-C maps of DT40 cells (8). In addition to the overall weakening of compartmentalization, a second diagonal emerges in the Hi-C maps during prometaphase (8). These features, along with polymer modeling, suggest that mitotic chromosomes are probably organized into an array of consecutive and nonoverlapping loops (7–9). Mechanistic studies suggest condensin II is essential for establishing some kind of helical scaffold, while condensin I may modulate the size and nested arrangement of the chromatin loops within the helix (8). In addition, helicoidal structures on mitotic chromosomes also exhibit Z-loop rearrangements in simulations (10). It has been proposed that the two distinct condensin activities of mediating bridging and stabilizing chromatin loops are responsible for mitotic chromosome compaction (11). The interactions among Structural Maintenance of Chromosomes complexes seem to follow specific rules that enable the transition from interphase to mitosis (12).

Significance

In cell division, chromosomes change folds and assume different shapes during the cell cycle. We combined a maximum entropy approach with polymer physics to model an ensemble of 3D chromosomal structures consistent with Hi-C data during mitosis. Modeled chromosomes show hierarchical helical ordering at two scales: high-periodicity turns at local regions (kilobases) and low-frequency turns spanning larger segments (megabases). We observe no preferential chirality in these helices. This suggests that chromatin in mitotic chromosomes behaves similarly to a liquid crystal.

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In this paper, we use Hi-C data gathered at different points in the cell cycle (8) to infer effective energy landscapes at these points that faithfully reflect these data in an information theoretic approach. While remaining agnostic about biochemical mechanisms, the information theoretic energy landscape framework has previously allowed us to infer chromatin structural ensembles from epigenetic marker information of the interphase (13–16).

Here, a similar procedure yields energy landscapes for each time-point of synchronized Hi-C maps from Gibcus et al. DT40 cell line (8) during the cell cycle. By examining these landscapes and the structural ensembles they produce, we can quantify the competition between phase separation of marked regions and the tendency toward helical structure formation. This competition contributes to the observed structural changes in the chromosome throughout the cell cycle. This data-driven approach generates ensembles of mitotic structures that are organized as helicoidal arrangements which have overall liquid crystalline properties. A prominent issue that emerges is the question of defects in the liquid crystalline order, including how they form and the extent to which they anneal during metaphase.

Results

Maximum Information Theoretic Entropy Energy Landscapes Capture Chromosome Shape Transitions Across the Cell Cycle.

Building on earlier direct maximum entropy inversion of Hi-C data (17–19), we previously were able to build a data-driven energy function for the interphase chromosome based on Hi-C maps called Minimal Chromatin Model (MiChroM) (20). Much in the way that Nuclear magnetic resonance and Fluorescence resonance energy transfer experimental data can be used to infer protein structures (21–29), Hi-C data for chromosomes can be used to construct minimally biased restraint potentials to constrain simulated ensembles so that their average properties conform with observables (15, 19, 30–33).

The data from Hi-C cross-linking experiments result from an ensemble of chromosome conformations across a population of cells, thus it is necessary to include multiple structures to describe the observables (34–37). This contrasts with the situation for globular proteins, where, often, but not always, fluctuations around a single structure are modest when the protein is fully folded.

The maximum entropy approach provides a data-driven method that yields the least biased interpretation of the experimental data (30, 31, 38). MiChroM has been applied successfully to study interphase chromosomes both in human cells across distinct cell types as well as the genome of a zoo of other organisms (16, 39–42), including time-degraded samples (43).

To study the development of mitotic chromosomal structure, we used the synchronized Hi-C data from Gibcus et al. for chromosome 7 of DT40 chicken cells (8) as input. We modeled separately eight time points spanning G2, prophase, and prometaphase (up to 60 min after release from a chemical-induced G2 arrest). Our results describe the chromosome polymer model built at 20 kb resolution. All the simulated chromosomal structural ensembles, whether globular or cylindrical, arise only from pairwise interactions, without any influence from external forces such as lamina or other sources of confinement. The details of the energy function, optimization procedure, and data normalization are described in *Materials and Methods*. While one can also use complete direct inversion because there is less averaging (17, 18), the results from this method may be sensitive to experimental noise.

The MiChroM energy function employed here has fewer parameters than a complete direct inversion because it includes only two interaction terms with parameters learned from the data. The first interaction involves distinct “types” of chromosomal regions labeled A and B, which correspond to the active and inactive areas of the genome in interphase, respectively. The second term [the Ideal Chromosome—IC (17)] is an effective sequence separation–dependent energy. IC is uniform across the chromosome and leads to helical ordering and compaction. This information theoretic interaction is thought to arise from motor activity, such as loop extrusion. Since the Ideal Chromosome potential is a function only of sequence separation (20), it competes with and smooths out the heterogeneity driven by phase separation. These interactions have proved sufficient to explain local rearrangements (18, 42, 44) and lengthwise compaction (15, 41, 45) in many systems, as well as the liquid crystalline ordering of the genome of the *Aedes aegypti* mosquito (40).

Fig. 1 gives a schematic of the modeling and optimization results, showcasing some representative 3D structures as well as documenting that the corresponding Hi-C maps predicted by the landscape ensembles reproduce the input experimental data. Fig. 1*A* includes representative 3D structures from the ensemble of over 200,000 structures used to generate the in silico Hi-C maps shown in Fig. 1*B* (*Upper triangle*). Globular shapes are seen in G2 but persist through early and mid-prophase (2.5, 5, and 7.5 min). The globular shape of the chromosomes seen in these cases is consistent with the interphase ensembles of chromosomal structures observed in other organisms where the genome is usually organized into distinct chromosome territories (39, 45). The initial transition of the simulated ensembles to an overall rod-like chromosome shape occurs between 7.5 and 10 min. This timing coincides with the reported timing of nuclear envelope breakdown (8).

Following the nuclear envelope breakdown, the mixing of the nucleoplasm and the cytoplasm allows for interactions between cytoplasmic macromolecules and chromosomes. These interactions include the increased association of condensin I with chromosomes, facilitating chromosome condensation and segregation. At 15 min, a second diagonal appears in the Hi-C maps (8). This structural rearrangement indicates a decrease in the probability of forming contacts between regions far apart in the genomic sequence, i.e., the ends of the polymer chains now have a lower probability of interacting than in interphase. There is a competition between short- and long-range genomic interactions (39, 46). The intensity of the A–B phase separation decreases (*SI Appendix, Fig. S2*), while the ideal chromosome energy term (*SI Appendix, Fig. S3*), often associated with motor activity, contributes more to local and short-range interactions. Weakening compartmentalization and strengthening of motorization continues for the remaining mitotic time points (Fig. 1*A* and *B*). The peaks in the decay curves shift from occurring at ~3 Mb at 15 min to being at ~6 Mb and ~12 Mb for the 30 and 60 min data, respectively (Fig. 1*C*).

We also quantify the compartmentalization intensity in the ensembles at the different measured time points (Fig. 1*D*). G2-arrested cells display the characteristic pattern of long-range interactions typically found in interphase, indicating the presence of A and B compartments. During early prophase, 5 min after entering mitosis, the compartmentalization signal begins to diminish. Compartments are mostly absent by the late prophase (10 min). At later time points in prometaphase (15, 30, and 60 min), only a very weak, nearly unobservable compartment

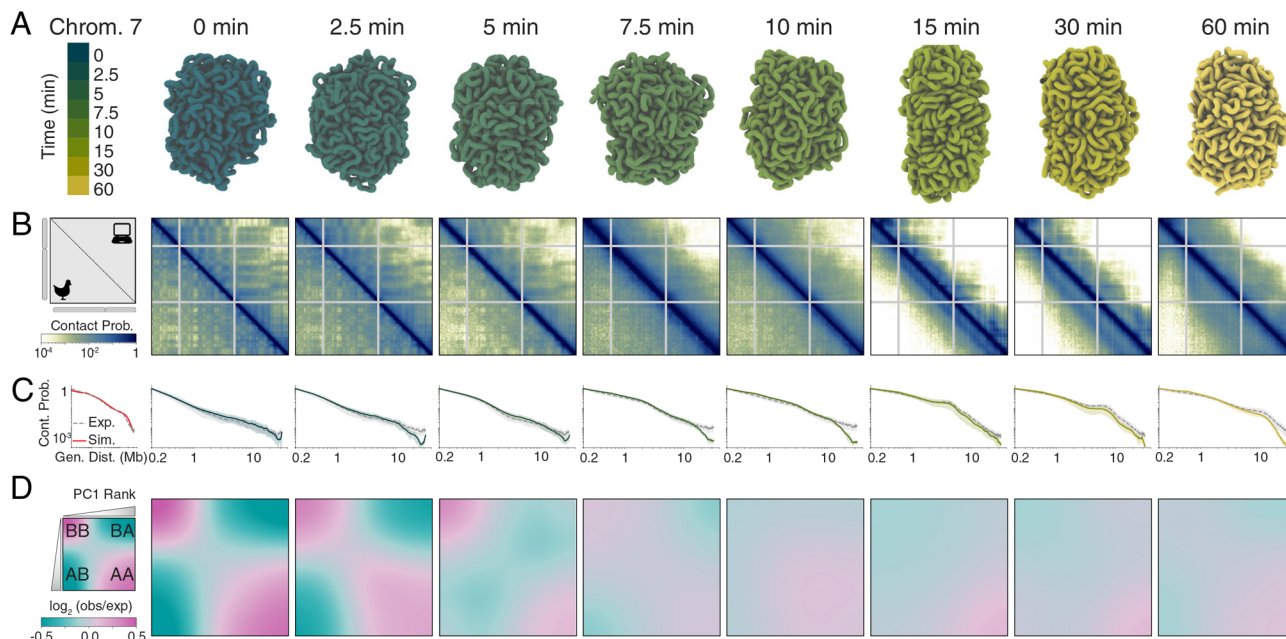


Fig. 1. Modeling chromosome structural changes across the cell cycle. (A) Representative 3D structures of chromosome 7 from DT40 chicken cells at different time points after release from G2 arrest. Structures are shown at information temperature 1.0 and are derived from the average over 3D ensembles of 200,000 structures. Colors represent the time evolution of chromosomes along the cell cycle. (B) Experimental (Lower triangle) and in silico (Upper triangle) Hi-C contact maps for chromosome 7 at different time points. (C) Contact probability as a function of genomic distance curves for chromosome 7 obtained from experimental (dashed gray) and simulated (colored) Hi-C data. (D) Compartmentalization saddle plots showing the A-B phase separation sharpness by averaging interaction frequencies between pairs of 100 kb bins ranked by their G2 eigenvector value.

pattern remained compared to interphase (8). The weakening of the compartmentalization during mitosis is also reflected in the type-to-type energy values. The highest interaction energy ratio value of $(AA+BB)/2AB$ was observed in interphase and decreased as cells progressed through mitosis (SI Appendix, Fig. S2).

In addition, we employed the optimized parameters from chromosome 7 in prometaphase to perform simulations in chromosome 1 of the same cell line. Representative 3D structures and the in silico Hi-C map are presented in SI Appendix, Fig. S9. Similar to what was observed in MiChroM energy function optimization performed for Human Hi-C maps (20), the parameters obtained here are also transferable between chromosomes. Also, even though the energy function was optimized using only Hi-C data, the length of the modeled chromosome 1 in prometaphase is around $8.8\mu\text{m}$, which is consistent with microscopy experimental observations (12) (See SI Appendix for details).

Annealing Simulations Reveal Hierarchical Helical Ordering in Chromosomal Structures. When one studies an ensemble of structures generated by a statistical energy landscape, the local ordering, such as it is, is most clearly visualized by annealing ensemble structures to nearby local energy minima. To find the local minima, we performed annealing simulations leading to minimum energy configurations. The low energy structures highlight the structural tendencies of chromosomes inherent in the maximum entropy-derived energy function (17). These annealing simulations provide insights into how the forces of the optimized energy function act to shape the structures (19, 40). This procedure is analogous to the approach used in protein folding where usually a single well-defined minimum free energy native state is reached through Brownian motion on a funnel-shaped energy landscape following the principle of minimum frustration (47–49). The procedure involves first simulating the chromosome at a high information theoretical temperature, and

then slowly cooling the system by decreasing the temperature at each chromatin dynamics step. Rapid cooling (Quenching) traps the chromosome in a disordered and amorphous state, preventing the polymer chain from relaxing into its potential energy minima. Conversely, slow cooling allows the chromosome to explore different conformations and gradually settle into lower energy states, having lower configurational entropy and allowing the chromosome to form more visibly ordered structures (19, 40). Although the final structures obtained at low temperatures do not fully represent the ensemble at higher information theoretic temperatures (e.g., the simulated ensemble reproducing Hi-C experiments), they can provide insights into the competition between local ordering and long-range phase separation that shapes chromosomal 3D architecture.

Fig. 2A shows representative annealed structures of chromosome 7 at different time points after release from G2 arrest. The structures are colored according to their genomic position, with blue representing the beginning of the chromosome and red representing the end. Similarly to what we see in Fig. 1, the chromosomes change shape from being clearly globular in G2 (0 min) to being rod-like in the mitotic phases. From the 3D structures, one now sees the emergence of rather ordered helices. It is worth mentioning that the helicoidal configurations arise straight from the pairwise interactions learned from Hi-C data, i.e., there is no specific bias in the potential toward forming loops or turns. This helicoidal ordering is an emergent phenomenon, like a phase transition.

To quantify the local structural organization of the chromosome, we calculated the orientation order parameter (OP) that measures the intensity helical twisting (17). The value of the orientation order parameter is obtained from the average of the dot product between two unit vectors connecting beads $[i, i+4]$ and $[j, j+4]$ along the chromosome chain (SI Appendix, Methods). The orientation order parameter oscillates as a function of the

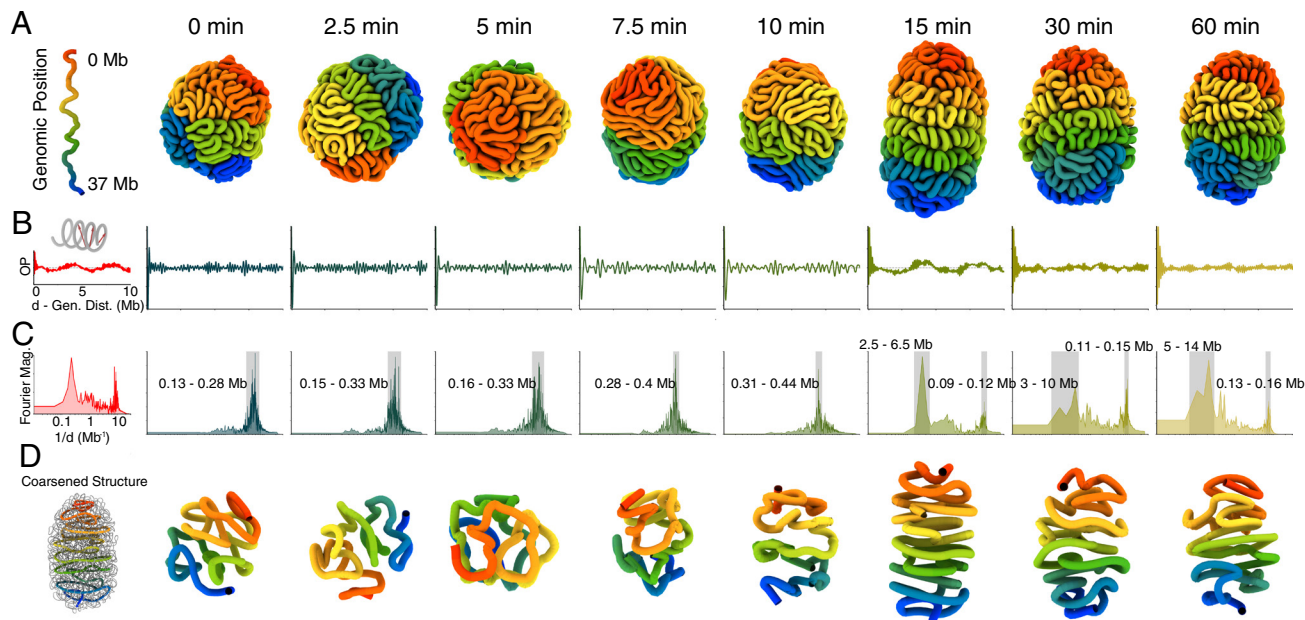


Fig. 2. Structural heterogeneity and helical ordering of chromosome 7 during the cell cycle. (A) Representative quenched structures of chromosome 7 at different time points after release from G2 arrest. Structures are colored according to their genomic position, with blue representing the beginning of the chromosome and red representing the end. (B) Orientation order parameter (OP) as a function of genomic distance for all simulated time points. The Orientation order parameter oscillates as a function of genomic distance, indicating the presence of fibril structures. (C) Fourier spectrum of the Orientation order parameter signals, showing the dominant frequencies of the helical turns in two regimes. The high-periodicity turns are on the order of a few hundred kilobases, while the low-periodicity turns correspond to the second band diagonal observed in the Hi-C maps in the order of megabases. (D) Coarsened structure based on the high-periodicity turns, highlighting the spiral organization of helices of helices in the mitotic chromosome.

genomic separation, indicating the presence of fibril structures in chromosomes that correlate with helical motifs (17, 40). Fig. 2B shows the orientation order parameter as a function of genomic distance for all simulated time points. We see that there is oscillation at all the time points corresponding to the fibril structures related to shorter genomic distances, as mentioned above. In addition to these high-periodicity turns, we see that a “second wave” signal emerges for the mitotic structures, especially at the 15 min time point, where new oscillations corresponding to larger loop sizes (longer spatial periods) become more apparent.

The loops involving shorter genomic distances are on the order of a few hundred kilobases, which is consistent with experimental results (8). High wavenumber turns seen in the simulations are consistent with chromonema structures visualized using oligo-Fluorescence In Situ Hybridization and Sister Chromatid Exchange’s (50). In our simulations at 20 kb resolution, where interactions are modeled using an effective energy function, these helices involving shorter genomic distances are consistent with the combined activity of condensin I and condensin II as described in previous studies (8). During mitosis, condensin II activity is suggested to establish a helical scaffold organization of the chromosomes. Within this scaffold, condensin I forms nested loops, contributing to the progressive compaction of mitotic chromosomes (8). In addition to nested loops, the helical rearrangements sometimes also contain Z-loops, as has been proposed in polymer models (10).

Analysis of the Fourier spectrum also indicates peaks corresponding to longer sequence periods (lower frequencies) for the time points 15, 30, and 60 min (Fig. 2C). These peaks suggest there are also helices involving longer genomic distances in mitotic chromosomes, with wavenumbers on the order of megabases (Mb). Furthermore, these long period peaks correspond to the second diagonal band observed in the Hi-C maps. The average helical turn period increases with time as the cycle

progresses, with observed values of 3 Mb at 15 min, 8 Mb at 30 min, and 12 Mb at 60 min (See *SI Appendix, Figs. S4 and S5* for $T = 1.0$ ensemble analysis). The progressive compaction and shortening of chromosomes can be associated with the helical arrangement of consecutive loops (8, 50). The Fourier transform of the orientation order parameter inferred from the direct inversion of the mitotic chromosome revealed two distinct layers of fibril structures in human mitotic chromosomes, with periodicities of approximately 0.25 Mb and 5 Mb (17, 18). The chromosomes of the *Aedes aegypti* mosquito also show orientation order parameter patterns, exhibiting values in the range of 0.6 Mb and 8 Mb (40). Fig. 2D shows a coarsened structure based on the high period turns representing the chromosome “backbone” (see *Materials and Methods* for details). Mitotic chromosomes show spiral organization of helices of helices in layers much like that seen in liquid-crystals (18, 40).

Helical Handedness Exhibits Variability in Modeled Mitotic Chromosomes.

Long helices have a handedness. We explored the chirality of the helices formed in the annealed structures on the short and long sequence spatial periods. No chiral bias was included in the energy landscape, so handedness must emerge as a collective phenomenon. A chiral object cannot be rotated or translated to appear identical to its mirror image. The Hi-C maps provide contact frequencies between loci and thus cannot directly convey 3D structural details such as the handedness of helices, turns, or other local rearrangements. The question of whether mitotic chromosomes possess a single handedness seems to be open experimentally. While some studies do suggest opposite handedness in sister chromatids after division (51), other studies suggest there is the same handedness in both (50, 52). We must entertain the thought that the handedness of the overall structure is stochastic.

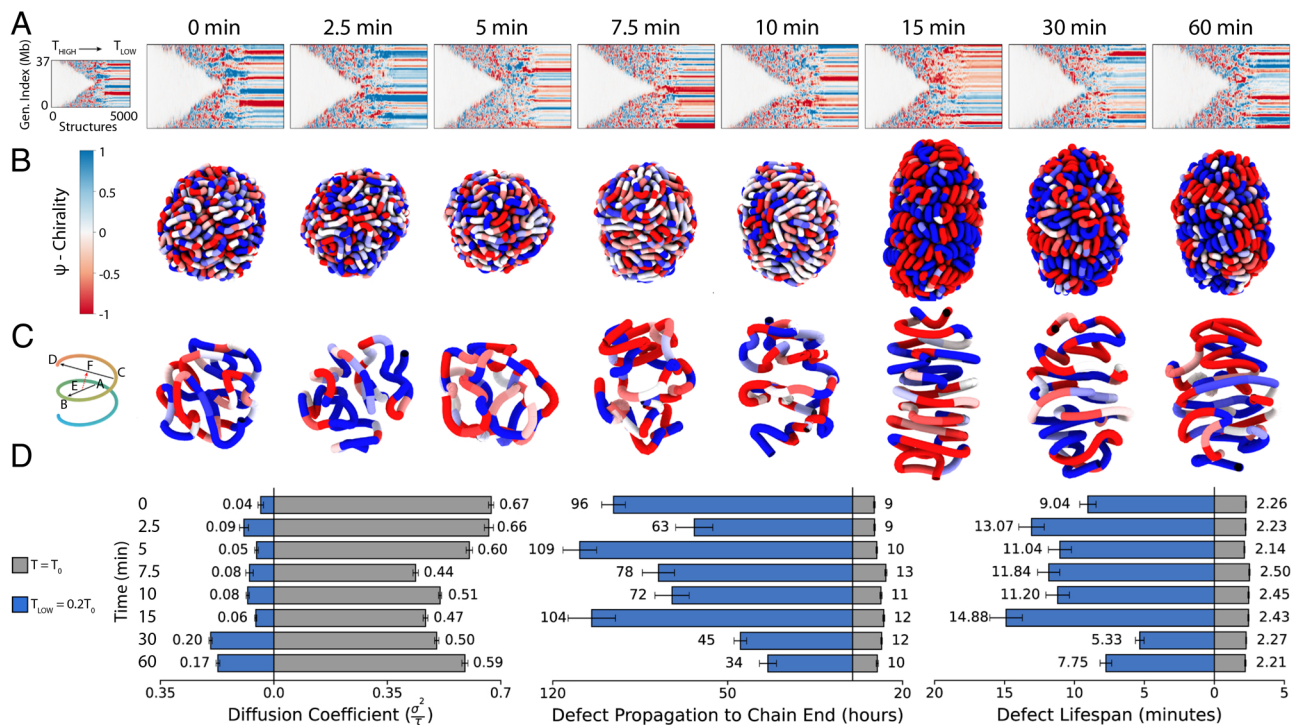


Fig. 3. Chiral symmetry breaking and helical ordering of chromosome 7 during the cell cycle. (A) Local collective chiral variable Ψ along the annealing simulation for different time points across the cell cycle. Blue represents a right-handed twist, and red represents a left-handed twist. (B) Chromosome structures are colored based on their local chirality degree score for high-frequency helices. (C) Chirality for the coarsened chromosome structures, showing global twisting, especially at later time points. (D) Diffusion coefficient, defect propagation time, and defect lifespan analysis for structural ensemble simulated in $T = 0.2$ (blue) and $T = 1.0$ (gray).

We quantify the chirality with an order parameter Ψ (18, 53, 54), defined as $\psi(i) = (\vec{EF} \cdot (\vec{CD} \times \vec{AB})) / (|\vec{EF}| |\vec{CD}| |\vec{AB}|)$ (See Fig. 3C and *SI Appendix* for details). Fig. 3 shows the local collective chiral variable Ψ along the annealing simulation for different time points across the cell cycle. The colors go from blue (right-handed twist) to red (left-handed twist), with negative values indicating left-handed chirality. The plots in Fig. 3A show Ψ for different structures from annealing simulations where, on every step, the temperature decreases. At the beginning of the simulation, the polymer chain starts as a straight line (*Movie S1*), so no twists or turns are observed. Due to thermal fluctuation, no local rearrangements are observed at high temperatures. As the temperature decreases, helices start to become evident, especially at the ends of the chain. At a sufficiently low temperature, the polymer chain maintains its chirality once it is formed for the rest of the simulation.

Fig. 3B and C color the structures based on their local chirality Ψ score. Fig. 3B colors the segments based on the high sequence frequency helices, around 4 to 5 beads or stretches of 80 to 100 kb. Fig. 3C shows the chirality for coarsened structures. One sees that “backbone” twisting event for time points of 15, 30, and 60 min. We observed no overall preferential chirality in our modeled structures. Such organization would also be consistent with the staircase model (8). It is also consistent with the half-turn perverted structures observed in light microscopy (55), which suggest the mitotic chromosomal structures, are organized by layers of helices of helices, but these often change their chirality, resulting in half-turns or perversions in the structures at megabase resolution of turns.

We calculated the persistence and propagation time of defects/perversions. We analyzed the 1D diffusion of a perversion by tracking individual defects along the polymer at different

temperatures (Fig. 3D). The diffusion coefficients for the defects were derived from the mean squared displacement curves (*SI Appendix*, Fig. S6). These estimates yield similar values across different time points independent of the cell cycle stage and chromosome shape for lower temperature simulations ($T = 0.2$). However, for simulation at $T = 1$, there is a decrease in the defect dynamics, especially for time points after 5 min. This is likely associated with increased chromosome condensation.

Based on these 1D diffusion coefficients, we can estimate the time required for a hypothetical defect, originating in the middle of the chain, to propagate to the end of the chain to be resolved. Utilizing timescales from our previous work (56), we calculated that the time to resolve such a defect via passive diffusion to the end would be several hours if there is no additional overall motor activity. Given that mitosis occurs on a similar time scale, chromosome chains might be expected to exhibit a few defects/perversions despite their overall tendency to helical ordering. In the interior of the chain, birth and death of defects occur within minutes (*SI Appendix*, Fig. S7), allowing defects to potentially resolve through annihilation with other defects along the chain, restoring positive or negative chirality. In our simulation, the average lifespan of a defect is on the order of minutes. This suggests that defects which are numerous in interphase could persist into the ensemble of mitotic structures. Events of defects diffusing along the chain, birth, and death of defects are presented in *SI Appendix*, Fig. S8.

Discussion and Conclusions

In this paper, we have used an information theoretic maximum entropy approach to explore the energy landscape of chromosomes as they change during the cell cycle. Starting from the

Hi-C maps from the DT40 chicken cells (8), our model infers effective Minimal Chromatin Model (MiChroM) energy function parameters (20) for cells at different stages, describing two primary contributions: phase separation of distinct chromatin types and the Ideal Chromosome term, which is responsible for compaction and likely arise from motor activity within the polymer chain (15, 19, 20, 45).

Only these two contributions seem to be needed to effectively model chromosome folding mechanisms across different cell cycle phases (18, 41, 45, 57).

Our simulations also show a reduction in the compartmentalization signal from interphase to mitosis. The weakening of A–B phase separation during mitotic chromosome condensation can be partially attributed to the general compaction of chromatin domains that compete with compartmentalization (39, 57, 58). Reducing the spatial distance between active and inactive regions leads to less distinct compartmentalization. Polymer simulations have suggested that loop extrusion can counteract compartmental segregation (46). Furthermore, finer compartmentalization is observed when cohesin is depleted (46).

The coexistence of structural order (helices) and fluidity (A–B phase separation) observed in our models suggests the importance of the liquid-crystalline properties of chromatin during cell cycle rearrangements. A liquid-crystal-like structure seems to be a common characteristic of chromatin organization (39, 40, 59–61).

We also observed two periodicities of helices in mitotic chromosomes. High-periodicity loops, typically spanning hundreds of kilobases or less, are associated with local chromatin rearrangements, potentially driven by the combined activity of condensin I and condensin II. In contrast, low-periodicity turns span several megabases, related to the formation of a helical scaffold (8). These larger-scale turns are also consistent with the second diagonal in Hi-C maps (8).

The twisting collective variable Ψ indicates no preferential chirality in the formation of helices. Handedness may be stochastic, with equal probabilities for left-handed and right-handed configurations. Alternatively, regulatory mechanisms later in mitosis, potentially involving supercoiling, may influence or establish a preferred handedness. Additionally, the changes in helical handedness observed in our simulations at the megabase resolution can be related to the “perversions” described by Chu et al. (55). It is worthwhile mentioning that DNA/chromatin twisting forces arising from motorized processes like transcription (62, 63) and loop extrusion (64, 65) driving chiral pleconome structures may underlie the chirality observed at longer scales. These twisting forces are typically known to be correlated over tens of kilobases. Whether these forces lead to structural chirality at larger lengthscales is an interesting possibility to explore. We also estimated that a defect in the chromosome structure would take a time longer than the cell cycle to be resolved by diffusion to the end of the polymer chain. This observation suggests some perversions in the mitotic structures are to be expected to remain in such structures if no such bias is imposed.

Materials and Methods

We modeled the chromosomal transition during mitosis using a copolymer energy function similar to the Minimal Chromatin Model (MiChroM), which has been successfully applied to human chromosomes across various cell lines during interphase (13–15, 20, 42). As experimental data for training the parameters, we used the DT40 chicken cell line at different time points from

Gibcus et al. (8). Chromosomes here are modeled at 20 kbp resolution. Initially, we had modeled the chromosomes at 50 kbp resolution in the DT40 chicken cell line (8) consistent with the training procedure earlier employed in the MiChroM original energy function from the Human GM12878 cell line (20, 66) (SI Appendix, Figs. S1 and S10).

MiChroM approach is based on the maximum entropy principle and has two assumptions: 1) chromosome phase separation is related to the annotation of chromatin types (A and B compartments), and 2) the activity of motor proteins affects polymer compaction through ideal chromosome interactions. The energy function of the MiChroM model is defined as

$$U_{\text{MiChroM}}(\vec{r}) = U_{\text{HP}}(\vec{r}) + \sum_{k \geq l} \alpha_{kl} \sum_{\substack{i \in \{\text{Type } k\} \\ j \in \{\text{Type } l\}}} f(r_{ij}) + \sum_{d=3}^{d_{\text{cutoff}}} \gamma(d) \sum_i f(r_{i,i+d}), \quad [1]$$

where $U_{\text{HP}}(\vec{r})$ represents the potential energy of a generic homopolymer details provided in SI Appendix. α_{kl} is the interaction strengths between chromatin types k and l . $\gamma(d)$ is the parameter for ideal chromosome interactions as a function of genomic distance d . $f(r_{ij})$ is the probability function for crosslinking between loci i and j given by $f(r_{ij}) = \frac{1}{2} \left(1 + \tanh \left[\mu(r_c - r_{ij}) \right] \right)$ (see SI Appendix for details) (17, 20).

In our model, chromatin beads are labeled into three types: Type A, Type B, and nonassigned (NA). The sequence of chromatin types along the genome is determined using the first principal component (PC1) from the eigenvector decomposition of the experimental Hi-C correlation matrix (6). We assume that positive PC1 values correspond to Type A chromatin, negative values to Type B, and regions without clear assignment are labeled as NA. The annotations are defined using the Hi-C map in the interphase, and they are kept the same for all other time points of the cell cycle. The Hi-C analysis and PC1 extraction were performed using Open2C collaborative tools: cooler (67) and cooltools (68).

The optimization of interaction parameters α_{kl} and $\gamma(d)$ was performed through an iterative minimization process similar to that employed to develop MiChroM energy function (20). Type-to-type interactions (α_{kl}) values are optimized by minimizing the difference between simulated and experimental contact probabilities for different chromatin type pairs (A–A, A–B, A–NA, B–NA, NA–NA). Ideal Chromosome interactions ($\gamma(d)$) parameters were optimized to consider the dependence of contact probabilities on genomic distance. Simulations were performed with the OpenMiChroM toolkit (69) that uses the OpenMM platform (70) to run Langevin dynamics. The chromatin was modeled at a resolution of 20 kbp per bead. Data analysis utilized OpenMiChroM toolkit that includes CNDBtools in Python 3 (44, 69). The 3D structures of chromosomes were visualized using VMD software (71).

Data, Materials, and Software Availability. All study data are included in the article and/or supporting information.

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