Quantified effects of chromosome-nuclear envelope attachments on 3D organization of chromosomes

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Abbreviations: Chr-NE, chromosome-nuclear envelope; CPU(s), central processing unit(s); DAPI, 4’, 6-diamidino-2-phenylindole; Dam, DNA adenine methyltransferase; LADs, Lamina Associated Domains; MSD, mean squared deviation; NE, nuclear envelope; SAWs, self-avoiding walks.

We use a combined experimental and computational approach to study the effects of chromosome-nuclear envelope (Chr-NE) attachments on the 3D genome organization of Drosophila melanogaster (fruit fly) salivary gland nuclei. We consider 3 distinct models: a Null model – without specific Chr-NE attachments, a 15-attachment model – with 15 previously known Chr-NE attachments, and a 48-attachment model – with 15 original and 33 recently identified Chr-NE attachments. The radial densities of chromosomes in the models are compared to the densities observed in 100 experimental images of optically sectioned salivary gland nuclei forming “z-stacks.” Most of the experimental z-stacks support the Chr-NE 48-attachment model suggesting that as many as 48 chromosome loci with appreciable affinity for the NE are necessary to reproduce the experimentally observed distribution of chromosome density in fruit fly nuclei. Next, we investigate if and how the presence and the number of Chr-NE attachments affect several key characteristics of 3D genome organization: chromosome territories and gene-gene contacts. This analysis leads to novel insight about the possible role of Chr-NE attachments in regulating the genome architecture. Specifically, we find that model nuclei with more numerous Chr-NE attachments form more distinct chromosome territories and their chromosomes intertwine less frequently. Intra-chromosome and intra-arm contacts are more common in model nuclei with Chr-NE attachments compared to the Null model (no specific attachments), while inter-chromosome and inter-arm contacts are less common in nuclei with Chr-NE attachments. We demonstrate that Chr-NE attachments increase the specificity of long-range inter-chromosome and inter-arm contacts. The predicted effects of Chr-NE attachments are rationalized by intuitive volume vs. surface accessibility arguments.

Introduction

Experimental studies of polytene nuclei in fruit flies and non-polytene nuclei in other eukaryotes have revealed several common principles of nuclear architecture. Chromosomes fold within the confined nuclear volume to occupy well-defined spatial domains called “territories.” These domains are mutually exclusive in the following sense: although different chromosomes may be in physical contact, they never interweave¹ (as do, for example, strands of DNA in the double-helix). The 3D chromosome folding is manifested in specific intra- and inter-chromosomal interactions. These interactions have been observed microscopically²,³ and, more recently, via cross-linking Hi-C experiments.⁴ The locations of inter-chromosomal contacts correlate with fragile sites where chromosomal breakpoints occur in evolution⁵ and tumorigenesis.⁶ In addition to chromosome-chromosome interactions, there are permanent (centromeric) and statistically high-frequency (but non-permanent) attachments between certain chromosomal loci and the nuclear envelope (NE). Several lines of evidence, including genome-wide mapping studies in Drosophila and human nuclei using the DamID method show that the gene-poor and transcriptionally repressed regions tend to form high-frequency chromosome-nuclear envelope (Chr-NE) attachments.⁷,⁸ Still, the interplay among these principles of 3D nuclear organization is poorly understood. Is the formation of chromosome territories controlled by the Chr-NE attachments, or vice versa? Does the number of Chr-NE attachments influence the patterns of intra- and inter-chromosomal interactions? Is it necessary for computational models to include all of the Chr-NE attachments in order to recapitulate experimental data? Our study aims to address these and several related questions using a combined experimental and computational approach.
Experimental evidence indicates that Chr-NE attachments are present in diverse organisms including fruit fly, yeast, and human. In recognition of this growing body of evidence, many computational studies of genome organization now incorporate the specific sites of Chr-NE attachment as model parameters. Remarkably, these attachments are emerging as key components of 3D genome organization. In yeast, computational studies have considered a range of models differing in the number of attachments they consider. Homogeneous interaction models assume all chromosome sites interact equally with the NE due to the complete presence or absence of attachments at all sites along the chromosome fiber. Heterogeneous interaction models allow affinity for the NE to vary along the chromosome fiber; several models have specifically investigated the effects of chromosome telomere positions at the centromeres and telomeres. These studies in yeast have led to several predictions: the 3D position of a gene can be altered due to the presence of an NE tether positioned within 10 kb; removal of chromosomal tethers at the centromere increases chromosome mobility as quantified by its confinement radius; the presence of Chr-NE tethers affects the distribution of telomere-telomere distances; the position of chromosomes within the nucleus may be altered due to a combination of Chr-NE tethering and volume exclusion; and the distribution of distances between the spindle pole body and the silent mating locus depends on tethering at the telomere. These results have motivated more complicated heterogeneous models to consider more numerous sets of experimentally identified Chr-NE attachments. Although the distribution of telomere-telomere distances did not depend appreciably on the number of attachments, the distance distribution between the spindle pole body and the silent mating locus was unique in the presence of zero, one, or 2 attachments.

In this work, we consider D. melanogaster (fruit fly), which compared to yeast possesses a much more complex set of Chr-NE attachments and a different chromosome organization in interphase. In Drosophila, chromosome interactions decay more gradually with genomic distance compared to yeast, a change that may stem from the underlying difference in the chromosome volume to nucleus volume ratio of these two organisms. In fact, general polymer physics arguments suggest that the conformational state of chromatin across cell types depends on the chromosome to nucleus volume ratio, and thus the basic folding principles may be similar in cells with similar ratios, and different in cell lineages where these ratios differ greatly. Thus, human chromatin organization is expected to have similarities to that of fruit fly (similar chromatin to nucleus volume ratios) but may be quite different from the organization in lower eukaryotes such as yeast, which also has a much simpler genome. In fact, yeast nucleus likely lacks well-defined chromosome territories found in fruit fly or human. Chr-NE attachments are more numerous and predominately correspond to sites of intercalary heterochromatin in Drosophila compared to yeast. Thus, the fruit fly nucleus cannot be adequately modeled with centromere and telomere tethers alone. A seminal experimental work identified 15 chromosome regions frequently in contact with the NE in D. melanogaster polytene chromosomes; most of these contacts were located in regions of intercalary heterochromatin. A follow-up work identified 48 attachment sites, located in regions of heterochromatin or late replication, and possessing properties of euchromatin.

Here, we investigate how fundamental characteristics of the 3D chromosome organization – chromosome territories, chromosome intertwining, and gene-gene contact probability – change under the influence of different sets of Chr-NE attachments. In addition to quantifying these changes, we develop a simple and robust mechanistic understanding of the underlying fundamental reasons for the changes. Specifically, what are the effects of Chr-NE attachments when they are positioned within these regions and coupled with the unique parameters of the Drosophila nucleus? Furthermore, what is the right number of attachments that one needs to include in computational models? To address these issues within a computational model, one needs to vary the number of Chr-NE attachments in the model, including all previously known and recently identified Chr-NE attachments, and verify the outcomes directly against the experiment. These questions are addressed using rigorous definitions of chromosome territories, chromosome intertwining, gene-gene contact probability, and experimental density profiles of chromosomes in the nucleus. Including all of the Chr-NE attachments could be necessary because experimental evidence suggests that they play a significant role in determining 3D genome organization. For instance, live imaging of yeast and fruit fly chromosomes have revealed short time-scale Brownian movements confined within variably sized domains. The movement of chromatin loci depends on their nuclear localization; loci attached to or adjacent to the nuclear periphery are less mobile than other loci. Thus, it is likely that attachments to the NE could constrain chromatin folding and prevent chromosome territories from freely diffusing in the entire nuclear volume. Similar conclusions have been reached in computational studies of yeast where each gene’s genomic separation from the telomeric tether directly affected its spatial distribution. Chr-NE attachments may play a role in non-uniform distribution of chromosomal rearrangements; because of non-random nuclear organization, certain loci are non-randomly closer together in the 3D space than others, and thus are more likely to interact and generate these rearrangements. Genomic studies performed on various organisms have provided evidence for non-uniform distribution of chromosomal rearrangements and breakpoints. Because the possible effects of numerous Chr-NE attachments on chromosome topology are largely unknown, this study aims to provide insights into this important issue.

Polytene chromosomes from D. melanogaster salivary gland nuclei are a well-established model for studying organization and function of the eukaryotic genome. Each polytene chromosome contains approximately 1024 DNA replicas bundled together in parallel; thus the genome organization in a single nucleus becomes visible under a light microscope. This is a critical advantage over “regular” interphase chromosomes because it is possible to obtain detailed spatial information about the position of each individual polytene chromosome. The study of polytene...
chromosomes has significant potential for general understanding of 3D genome organization because recent experiments revealed very similar structural and functional organization of non-polytene and polytene chromosomes in fruit fly. Moreover, the polytene chromosomes are estimated to occupy about a third of the nuclear volume; this chromosome to nuclear volume ratio, which critically affects the overall 3D nuclear architecture, is the same in regular non-polytene nuclei, and is likely similar to the values characterizing human nuclei.

Our current work aims to determine the effect of Chr-NE attachments on the 3D genome organization in D. melanogaster. This work addresses a fundamentally new question compared to our previous work aimed at identifying and characterizing Chr-NE attachments. In total, here we consider 3 computational models of the nucleus; all three models contain the same relevant measured parameters of the polytene nucleus from D. melanogaster and differ only by the number and type of Chr-NE attachments incorporated. (I) the Null model – zero specific Chr-NE attachments; the only model considered in our previous work, which here serves as a reference for determining the effects of Chr-NE attachments. (II) a 15-attachment model, containing the same set of 15 Chr-NE attachments identified in. (III) a 48-attachment model, containing the same set of 48 attachments identified in. We emphasize that Chr-NE attachments are not randomly positioned; rather, the position of attachments in each model has been determined experimentally. Here, the Null model mimics a hypothetical mutant that would completely abolish all Chr-NE interactions, while the 48-attachment model corresponds to the experimentally accessible wild type. The 15-attachment model is an intermediate case. These computational models are in turn used to determine the effect of Chr-NE attachments on the other principles of the 3D genome organization.

Results

The presence of Chr-NE attachments enhances chromosome territories and reduces chromosome intertwining

The five largest chromosome arms of D. melanogaster salivary glands are modeled as beads-on-string and are represented as 5 random self-avoiding walks (SAWs) (Fig. 1). The sixth arm, chromosome 4, is not considered due to its negligible length. Experimental data for the chromosomes and the nucleus become realistic model parameters and constraints imposed during the construction of SAWs. The sampling protocol for generating conformational ensembles has been described previously. Briefly, one bead representing the chromocenter is placed adjacent to the NE at the “north pole” of the nucleus. The arrangement of the 5 initial beads placed around the chromocenter bead is designed to match the relative proportion of chromocenter spatial arrangements seen in experiment. After assigning the chromocenter arrangement, SAWs are constructed using computational efficient Rosenbluth’s algorithm (i.e., SAW chains grow by addition of monomers in a “true” SAW fashion). Complete details of model parameters are described in Text S1 and Table S1. Sampling protocol for generation of computational ensembles is provided in Text S2.

We use recently developed rigorous metrics (briefly summarized in “Material and Methods”) to quantify the effect of Chr-NE attachments on chromosome territories (Fig. 2) and chromosome intertwining (Fig. 3) in our computational models. We can easily predict the effects of Chr-NE attachments by comparing simulated nuclei, which have all or some Chr-NE attachments, against the Null model nuclei (0 attachments). The results are summarized in Figure 4. Overall, the presence of Chr-NE attachments leads to chromosomes that are more territorial and intertwine less frequently. Compared to the Null model, the territory index of the 15- and 48-attachment nuclei ensembles increased by 1.8% and 4.6%, respectively with corresponding p-values of 1.9e-24 and 1.7e-150. The chromosomes in the 15- and 48-attachment nuclei ensembles intertwine 1.6%, and 3.0% less frequently, respectively, with corresponding p-values of 9.1e-9 and 1.8e-30. This also means that the chromosomes become more territorial in the presence of Chr-NE attachments. The statistical significance in

Figure 1. Visual summary of the 3 models of nuclear architecture in D. melanogaster polytene nuclei. Two identified sets of Chr-NE attachments (red) are mapped onto a computational model of D. melanogaster polytene nucleus. The effects of Chr-NE attachments are compared to a previously developed Null model lacking Chr-NE attachments.

Figure 2. The convex hull (shown as green polyhedron) is used to quantify chromosome territories. The convex hull of the green chromosome above may encompass beads belonging to other chromosomes. The ratio of native beads compared to all (including foreign) beads in a hull is the territory index.
the mean territory index and intertwining frequency was tested for each model using the Null model as a Null hypothesis. P-values for the 15- and 48-attachment models support the statistical difference compared to the Null model (Fig. 4).

**Chr-NE attachments affect intra- and inter-chromosomal contacts in opposite ways**

In addition to the global organizational effects of Chr-NE attachments, we predict that more local changes will be induced by introducing Chr-NE attachments into our computational models. We analyzed bead-bead contact probability in our models at 2 progressively increasing resolutions (see “Material and Methods”: whole chromosome (40 Mb) and chromosome arm (20 Mb). Interestingly, Chr-NE attachments did not equally affect all types of chromosome-chromosome contacts. Specifically, intra-chromosome and intra-arm interactions are more common in the Chr-NE attachment models compared to the Null-model, while inter-chromosome and inter-arm interactions are less common in theChr-NE attachment models compared to our Null model (Fig. 5). This observation is consistent with the more territorial chromosomes present in our Chr-NE attachment models (Fig. 4). Overall, the changes in pairwise interaction probability (compared to the Null model) are proportional to the total number of Chr-NE attachments. For example, normalized intra-arm contacts increased by 1.5% in the 15-attachment Chr-NE ensembles compared to the Null model and increased again (by 4.1%) in the 48-attachment Chr-NE ensembles compared to our Null model (Fig. 5). The statistical significance of the difference in the mean number of intra/inter chromosome and intra/inter arm interactions was tested for each model using the Null model as a Null hypothesis. P-values for the 15- and 48-attachment models support the statistical difference compared to the Null model (Fig. 5).

**Chr-NE attachments increase the specificity of long-range inter-arm and inter-chromosome contacts at the highest resolution of the model (0.7Mb)**

We considered bead-bead contact frequencies at the highest resolution (bead size = 0.7 Mb) of our model. Changes in contact probability at bead-bead resolution for each model were compared to the Null model (Fig. 6). The result shows that the
predicted organizational changes brought about by Chr-NE attachments are non-random. For example, the likelihood of specific inter-chromosome and inter-arm contacts may increase due to Chr-NE attachments (2 examples are indicated by black arrows in Fig. 6; the matrix is symmetric) despite the overall decreased probability of inter-chromosome and inter-arm contacts (Fig. 5). In biological terms, our model predicts that chromosomal regions 93D-94E of arm 3R and 52F-53F of arm 2R (top arrow in Fig. 6) interact more frequently in the presence of Chr-NE attachments than expected in the absence of Chr-NE contacts. The rightmost arrow in Figure 6 corresponds to chromosomal regions 31E-32D of arm 2L interacting with regions 94E-96A of arm 3R more frequently in the presence of Chr-NE attachments than expected in the absence of Chr-NE contacts. Interestingly, these hotspots of increased bead-bead interaction correspond to the chromosomal regions that void NE attachments, suggesting that the interacting beads loop into the nuclear interior, which increases the probability of contact.

Experimental radial density of polytene chromosomes changes with the distance from the NE

To date, 2 sets of Chr-NE attachments in D. melanogaster have been identified: a smaller set of 15 "high frequency only" attachments, and a larger set that includes the additional 33 attachments recently inferred from the original experimental data. These sets are enforced in our 15- and 48-attachment Chr-NE ensembles, respectively. Here we performed new experiments to determine which set better represents reality. This determination is important since our simulations clearly suggest that the number of Chr-NE attachments may affect several key parameters of the nuclear architecture (see results above). We collected fluorescence images of optically sectioned fruit fly salivary gland nuclei forming "z-stacks" for an ensemble of nuclei – 100 in this study. The radial density of chromosome in each z-stack image is inferred from fluorescence intensity (see "Material and Methods" for details). Figure 7 shows the average radial density, \( \rho(r) \), of chromosomes over the ensemble of 100 experimental nuclei. The peak density of chromatin occurs near the periphery of the nucleus, approximately 2 \( \mu \)m from the NE. Chromatin density steadily decreases toward the nuclear center (Fig. 7). The integrated density of chromatin within 2 \( \mu \)m of the NE accounts for about 37% of all chromatin in our experimental nuclei. This result is consistent with earlier chromosome tracing experiments, in which approximately 40% of each chromosome arm was located at the nuclear periphery.

The 48-attachment Chr-NE model better represents the real nucleus than does the 15-attachment Chr-NE model or the Null model with no attachments

To compare our computational models with the experimentally measured radial density of chromosomes, we first computed the average radial density of chromosome in the Null model (green curve in Fig. 8, top panel), 15-attachment Chr-NE model (red curve in Fig. 8, top panel), and 48-attachment Chr-NE model (blue curve in Fig. 8, top panel). Then for each of the 100 experimental z-stack images, the radial density of chromosomes was computed and compared to that of each model. To quantify the ability of each model to recapitulate the experimental data, we used the mean squared deviation (MSD) between model and
experimental density distributions. The MSD is designed to objectively quantify the difference between the model and experimental curves. The total variation is not used because we are not interested in the arc-length of the chromatin density curves. While several metrics quantifying the difference between 2 curves can be used, we believe that for our purposes the MSD provides a sufficient measure of the difference between the model and experiment. The minimum MSD determines the “best fit” model. The number of best fits indicates how well each model recapitulates the experimental data. In general, comparing orthogonal chromatin density is difficult because both the measured density curves and those present in computational models vary from nucleus to nucleus. We make this problem tractable by comparing the individual experimental z-stacks rather than simply the average of all experimental z-stacks, which would eliminate the intrinsic variability. The population mean may be somewhat different from its constituent parts. Thus, the approach is designed to test how many individual experimental nuclei map to each model. The number of best fits for each model is shown in Figure 8, bottom panel. The 48-attachment Chr-NE model scores the greatest number of best fits, while the Null model and the 15-attachment Chr-NE model score a considerably smaller number of best fits. The fact that not every experimental nucleus has 3D distribution of chromosomes best described by a single model is not surprising: the folding of chromosomes and placement of Chr-NE attachments vary from nucleus to nucleus, in contrast to the folding of small proteins that always assume unique shape in the native compact state. Therefore, the radial density of chromosomes measured in z-stack images varies for individual nuclei. What is important is that the entire ensemble of experimental images favors the 48-attachment Chr-NE model. We conclude that, within the assumptions made by our computational models, the 48-attachment Chr-NE model better represents reality than does the 15-attachment Chr-NE model or the Null model. It is worth mentioning that in contrast to the polytene chromosomes considered here, non-polytene chromosome Chr-NE attachments may number in the hundreds.7,47 These studies used a DamID approach in which DNA adenine methyltransferase (Dam) fused to lamin leaves a stable adenine-methylation “footprint” in vivo at the interaction sites. One DamID study identified 412 Drosophila Lamina Associated Domains (LADs) with a median size of ~90 kb.47 In contrast, the Chr-NE attachment regions in polytene chromosomes correspond to one or few subdivisions on a cytogenetic map,5 which typically span several hundred kb. The difference between polytene and regular interphase chromosome models may stem from the necessary coarse graining of the polytene model. Since each bead represents 0.7 Mb, clusters of LADs along the bundle of polytene strands may be impossible to resolve using a polytene chromosome approach. This seems likely since it has been demonstrated that smoothing of the LAD profile seen in interphase chromosomes (to simulate the microscopy resolution) makes it correspond well to the Chr-NE attachment profile seen in polytene chromosomes.7

The fact that all 48 attachments appear necessary for the closest agreement between model and reality does not completely resolve the still debated number of attachments present in real nuclei, as each model necessarily makes simplifying assumptions. To name a few, the nucleus shape and size, the nucleolus position and size are fixed to their population-average values in our models to improve computational efficiency. It is therefore conceivable that the chromosome density profile and number of best fits may change slightly if these parameters varied as they do in real nuclei. In order to incorporate complete sets of Chr-NE attachments (to the best of our knowledge, for the first time), these compromises simply had to be made to make our models computationally tractable.

**Predicted effects of Chr-NE attachments are rationalized by simple volume vs. surface accessibility arguments**

In what follows, we explain why the relative numbers of intra-chromosome and intra-arm contacts increase due to the “turning on” of the Chr-NE attachments, while inter-chromosome and inter-arm contacts decrease. The demonstration is based on very general volume vs. surface space accessibility arguments. A schematic, intended only to illustrate the concept of the argument for the inter-chromosome (or inter-arm) contacts, is shown in Figure 9. First, consider a centrally located gene represented by the black square. Next, assume that a second gene randomly occupies one of the remaining squares. Four of these possibilities (gray squares) represent locus-locus contact; thus, locus-locus contact probability in this case is 4 out of 15, or ≈0.27 (Fig. 9, left panel). Now consider another limiting case where all the genes are moved to the nuclear periphery due to the turning on of the Chr-NE contacts. In this case, only 2 possibilities (gray squares) represent locus-locus contact; thus, locus-locus contact probability in this case is 2 out of 11, or ≈0.18 (Fig. 9, right panel). The same basic argument is made quantitative in the next paragraph to further rationalize the increases and decreases in chromosomal contact probabilities as
The probability of contact for each interaction type is determined by dividing the contact sub-volume by the accessible volume of the bead. Figure 10 summarizes contact probabilities by interactions type. Predicted derivation for these contact probabilities is given in Text S3, Fig. S1, and Table S2. Note that $P_{\text{bulk}} > P_{\text{attach}} > P_{\text{attach}}$, where variables represent the contact probability of bulk beads with bulk bead, attachment beads with attachment bead, and bulk beads with attachment bead, respectively. Clearly all contacts in the Null model are bulk-bulk contacts. The “turning on” of each Chr-NE attachment essentially replaces bulk-bulk interactions with one of the 2 other interaction types, each having lower contact probability than the bulk-bulk. A total of 15 Chr-NE attachments are “turned on” in our 15-attachment model; therefore, fewer total inter-chromosomal interactions are realized compared to the Null model. The effect is even more pronounced in the 48-attachment Chr-NE model leading to fewer total inter-chromosomal interactions compared to the 15-attachment Chr-NE model. We use these contact probabilities to predict the average number of interactions in each of our 3 models (Null model, 15-attachment model, and 48-attachment model) (Table 1). We arrive at these predictions by multiplying the number of possible interactions of each given type by the contact probability predicted by the volume vs. surface accessibility argument and summing the results. Despite the number of simplifying assumptions made by our volume vs. surface accessibility argument, the resulting predictions are in relatively good agreement with our full SAW simulations. More importantly, our simple accessibility argument predicts a decrease in the number of chromosome-chromosome interactions in our Null model, 15-attachment Chr-NE model, and 48-attachment Chr-NE model, respectively (Table 1). Thus, the effects of Chr-NE attachments as seen in our simulations are most likely determined from the very general volume vs. surface accessibility effects, which are very robust. In the case of intra-chromosomal interactions, we no longer assume that beads are randomly positioned relative to each other due to the linking of beads along the polymer backbone, the effect of the Chr-NE attachments on intra-chromosome contacts is the opposite of the inter-chromosome case.

We relate the specific contact probabilities to the following specific volumes: accessible volume for a bead – volume in which a bead is confined (Fig. 10), contact sub-volume for a bead – the small volume within which positioning of nearby beads form bead-bead contacts (cyan in Fig. 10). The three types of pairwise contacts in our models are: attachment-attachment – contacts between two “attachment” beads which form Chr-NE attachment, bulk-bulk – contacts between 2 “bulk” beads which are not attached to the NE (i.e., no constraints), and bulk-attachment – contacts of bulk beads with attachment beads (Fig. 10). The accessible volume of bulk beads and attachment beads clearly differs; bulk beads are found anywhere in the nucleus while attachment beads are only at the periphery. In addition, the contact sub-volume (defined above and indicated with cyan regions) depends on the contact type. To the extent that most beads are randomly positioned in the nucleus relative to each other – a simplifying assumption of this demonstration – we can approximate the probability of contact for each interaction type by dividing its specific volumes: accessible volume for a bead – volume in which a bead is confined (Fig. 10), contact sub-volume for a bead – the small volume within which positioning of nearby beads form bead-bead contacts (cyan in Fig. 10). The three types of pairwise contacts in our models are: attachment-attachment – contacts between two “attachment” beads which form Chr-NE attachment, bulk-bulk – contacts between 2 “bulk” beads which are not attached to the NE (i.e., no constraints), and bulk-attachment – contacts of bulk beads with attachment beads (Fig. 10). The accessible volume of bulk beads and attachment beads clearly differs; bulk beads are found anywhere in the nucleus while attachment beads are only at the periphery. In addition, the contact sub-volume (defined above and indicated with cyan regions) depends on the contact type. To the extent that most beads are randomly positioned in the nucleus relative to each other – a simplifying assumption of this demonstration – we can approximate the probability of contact for each interaction type by dividing its accessible volume by the accessible volume of the bead. Figure 10 summarizes contact probabilities by interactions type. Predicted derivation for these contact probabilities is given in Text S3, Fig. S1, and Table S2. Note that $P_{\text{bulk}} > P_{\text{attach}} > P_{\text{attach}}$, where variables represent the contact probability of bulk beads with bulk bead, attachment beads with attachment bead, and bulk beads with attachment bead, respectively. Clearly all contacts in the Null model are bulk-bulk contacts. The “turning on” of each Chr-NE attachment essentially replaces bulk-bulk interactions with one of the 2 other interaction types, each having lower contact probability than the bulk-bulk. A total of 15 Chr-NE attachments are “turned on” in our 15-attachment model; therefore, fewer total inter-chromosomal interactions are realized compared to the Null model. The effect is even more pronounced in the 48-attachment Chr-NE model leading to fewer total inter-chromosomal interactions compared to the 15-attachmentChr-NE model. We use these contact probabilities to predict the average number of interactions in each of our 3 models (Null model, 15-attachment model, and 48-attachment model) (Table 1). We arrive at these predictions by simply multiplying the number of possible interactions of each given type by the contact probability predicted by the volume vs. surface accessibility argument and summing the results. Despite the number of simplifying assumptions made by our volume vs. surface accessibility argument, the resulting predictions are in relatively good agreement with our full SAW simulations. More importantly, our simple accessibility argument predicts a decrease in the number of chromosome-chromosome interactions in our Null model, 15-attachment Chr-NE model, and 48-attachment Chr-NE model, respectively (Table 1). Thus, the effects of Chr-NE attachments as seen in our simulations are most likely determined from the very general volume vs. surface accessibility effects, which are very robust. In the case of intra-chromosomal interactions, we no longer assume that beads are randomly positioned relative to each other due to the linking of beads along the polymer backbone. As a result, the relative ordering of contact probabilities, $P_{\text{bulk}} > P_{\text{attach}} > P_{\text{attach}}$ changes, with $P_{\text{attach}}$ becoming the largest of the three. That change leads to an increase in the number of intra-arm and intra-chromosome contacts upon the turning on of the Chr-NE attachments (Text S4 and Table S3).

Discussion

Key outcomes

The close integration of experimental data analysis and computational modeling had already led to significant breakthroughs in deciphering some basic principles of the 3D architecture of interphase chromosomes.2,14-16,23,48-50. Despite this progress, the Chr-NE attachments – a potentially critical component of 3D organization – have received limited attention in computational models. In particular, Chr-NE attachments were an essential feature of several recent computational models,14-16,48,51 and their inclusion was necessary to recapitulate experimental results. Our model described here was designed in an...
effort to simulate the effects of Chr-NE attachments on nuclear architecture; it incorporates all known chromosome interactions with the NE in fruit fly. The model makes several key predictions in 2 categories: effect on territories and effect on chromosome-chromosome interactions. Specifically, we show that (1) chromosomes with more numerous NE attachments are more territorial, (2a) intra-arm and intra-chromosome interactions are more common in nuclei with more numerous Chr-NE attachments, (2b) in contrast, inter-arm and inter-chromosome interactions are less common in nuclei with more numerous Chr-NE attachments, and (2c) Chr-NE attachments increase the specificity of long-range inter-arm and inter-chromosome interactions. We show how these conclusions can be rationalized by simple and robust volume vs. surface arguments, which further supports the robustness of the conclusions themselves. Although our biological system is the polytene chromosome of *D. melanogaster*, recent experiments have demonstrated that polytene chromosomes have very similar structural and functional organizations compared to their nonpolytene counterparts.33,34

**Chromosomes with more numerous NE attachments are more territorial**

Chromosome territories have become a focus of a number of experiments5,35,52-56 and computational simulations.57-59. Recently, simulations that addressed the question of territory formation demonstrated that non-specific entropic forces may play a significant role in establishing and maintaining chromosome territories.57-59 However, simulations have also shown that other factors may be involved in addition to the dominant role played by entropic forces. In one study, this entropic effect has been shown to depend on the presence of chromosome loops, which may also arise due to non-specific forces.57 Our simulations suggest that chromosomes in fruit fly are largely territorial regardless of Chr-NE attachments. However, the simulated nuclei with more numerous Chr-NE attachments were consistently more territorial. Therefore, Chr-NE attachments may play a certain role in maintaining the integrity of chromosome territories. This effect may be universal to some extent. Computational studies in yeast reveal that territorial organization of chromosomes is partially governed by chromosome tethering at the centromere.58 The similarity with our results is significant since the composition of Chr-NE attachments differs in fruit fly and yeast. Yeasts have fewer and predominately telomeric and centromeric attachments,17 while *Drosophila* possesses more numerous attachments in regions of intercalary heterochromatin. Despite this progress, the influence of attachment number may be more elusive. In our models, increasing the attachment number from 15 to 48 likely increases the chromosome territory index. In contrast, altering attachment number in yeast models had little effect on the chromosome-chromosome distance measured between telomeres at an experimentally relevant resolution.17 The unique effects of attachment number in yeast and *Drosophila* may be coupled with the unique genome organizations in these two organisms.60 For example, the effect of attachments may change when the chromosome volume to nucleus volume ratio is altered. A combination of higher resolution experimental and computational studies focused on Chr-NE attachments will be needed to address these questions.

**Chr-NE attachments affect whole-chromosome and chromosome arm interactions**

Experimental Hi-C data have recently mapped the global chromosome-chromosome interactions of *D. melanogaster* non-polytene nuclei.4 In that study, the pattern of global chromosome-chromosome interactions was characterized by the following trends: an abundance of intra-arm contacts and a paucity of inter-arm contacts. Interestingly, we observe the same pattern (intra arm > inter arm) in all three of our computational models. However, we also show that the ratio of intra-arm/inter-arm contacts progressively increased by 4% and 11% with the addition of 15 and 48 Chr-NE attachments, respectively. This suggests that intra-arm interactions may be more common than inter-arm interactions regardless of Chr-NE attachments, but attachments may increase the ratio of the two. Several other factors may influence global chromosome-chromosome interactions. First, the abundance of intra-arm interactions in *Drosophila* may also be dictated by robust homolog pairing that competes away non-homologous contacts.61 Second, chromosome organization in the yeast nucleus can be explained by the confinement of chromosomes and tethering of chromosome centromere and telomere to the NE.14,15 Finally, non-specific (entropic) forces can drive the self-organization of polymers.56,51,57,62 Thus, Chr-NE attachments likely affect nuclear organization to a degree (see results), but they may not be the dominant factor. However, we do see that the Chr-NE attachments significantly increase intra-arm and intra-chromosome contacts and decrease inter-arm and inter-chromosome contacts. This observation is consistent with the effect of the Chr-NE attachments inducing more territorial organization of chromosomes in our attachment models.

### Table 1. Predicted number of interactions based on volume vs. surface accessibility argument compared to simulation. Although each of our computational models contains the same total of 248*248 possible bead-bead interactions, the types of interactions differ. Since our volume vs. surface accessibility argument predicts a different contact probability for each interaction type, a different number of interactions in each model is expected.

| Model     | Possible bulk-bulk interactions | Possible bulk-attach interactions | Possible attach-attach interactions | Predicted number of interactions from volume-surface argument | Interactions actually seen in simulations |
|-----------|---------------------------------|----------------------------------|------------------------------------|-------------------------------------------------------------|------------------------------------------|
| Null      | 248²                           | 0                                | 0                                  | .03-248² = 1845                                            | 1625                                      |
| 15 attachment | 233²                           | 2-233-15                         | 15²                                | .03-233² + .014-2-233-15 + .019-15² = 1731                | 1591                                      |
| 48 attachment | 200²                           | 2-200-48                         | 48²                                | .03-200² + .014-2-200-48 + .019-48² = 1513               | 1538                                      |

Note: The table shows the predicted number of interactions for different models based on the volume vs. surface accessibility argument compared to simulation. The models are compared by the number of interactions predicted using the volume vs. surface accessibility argument and the actual number of interactions seen in simulations.
Chr-NE attachments increase the specificity of long-range inter-chromosome and inter-arm interactions

Experiments have suggested that actively transcribed genes co-localize in order to share sites of transcription; a result that persists over several genomic-length scales.\textsuperscript{3,4} For example, one study demonstrated frequent 3D co-localization of 2 genes (Hbb-b1 and Eraf) which are separated by $\sim 25$ Mb on mouse chromosome 7.\textsuperscript{4} Another study, focusing on a 2.9 Mb region of \emph{D. melanogaster}, demonstrated that transcription factor co-localization hotspots range in size from 1–5 kb and are separated by 50 kb.\textsuperscript{5} Unlike the experiments, our Null model shows no specificity in inter-arm and inter-chromosome contacts, which are random. However, we do find that specific chromosome regions have the capacity to increase their likelihood to co-localize in the presence of Chr-NE attachments. Thus, the "turning on" of all 48 Chr-NE attachments in our model increases the specificity of inter-arm and inter-chromosome contacts. Likewise, specific long-range chromosome interactions are a key feature of experimental Hi-C contact maps.\textsuperscript{4} We avoid directly comparing the co-localized regions in our 48-attachment Chr-NE model with Hi-C experimental data because specific chromosome-chromosome interactions are not featured as parameters in our computational models. These parameters are absent because specific chromosome-chromosome interactions have not been quantified experimentally in \emph{D. melanogaster} salivary gland nuclei. It’s conceivable that these interactions have a greater impact on chromosome-chromosome contacts than does the presence of Chr-NE attachments and that both factors superimpose their relative influence on chromosome-chromosome interactions. However, we clearly observe chromosome-chromosome interactions stemming from Chr-NE contacts suggesting their effects at higher resolution. Specifically, if the effect of Chr-NE attachments as seen in our models persists at higher resolutions, they may play a role in establishing these long-range chromosome interactions. This possibility will be investigated in a future study using models of non-polytene interphase nuclei. On the other hand, a recent epigenetic-based copolymer model in Drosophila reproduced the chromosome folding inferred from Hi-C data without depending on Chr-NE attachments.\textsuperscript{6} However, the regions of chromatid modeled in that study were on the order of 1 Mb,\textsuperscript{6} and Chr-NE attachments may have limited influence within regions of this size. Indeed, the coarse-grained beads in our model do span one or more subdivisions of the \emph{D. melanogaster} polytene chromosome map. Therefore, the co-localization of beads, and the genes represented by those beads, may be relevant specifically to polytene chromosomes. Interestingly, a recent computational study of gene co-localization demonstrated that simultaneously co-localizing gene pairs depend on a low rate of chromosome intertwining.\textsuperscript{6} This result is consistent with the correlation between the number of Chr-NE attachments and a low rate of chromosome intertwining. We hypothesize that the density of Chr-NE attachments is the source of these localized increases in inter-arm contact frequency. This result shows the importance of considering NE attachments for predicting key details of 3D nuclear architecture.

Methodological notes

In the pioneering study of genome organization, reconstructions of the 3D chromosome folding were obtained from optically sectioned polytene chromosomes of \emph{D. melanogaster}.\textsuperscript{2} This approach provides a detailed picture of chromosome folding in the nucleus; however, it is typically limited to small ensembles of nuclei. Our approach essentially depends less on complete chromosome reconstruction while depending more on comparison with a computational model. In this study, we compared the radial density of chromosomes from optically sectioned polytene chromosomes to 3 computational models; each of the 3 models has a different pattern of radial chromosome density due to specific Chr-NE attachments. In principle, this strategy could be extended to additional features of chromosomal organization, non-polytene chromosome, and other organisms. In general, our approach involves comparing a Null model to a model that differs in a single organizational feature. In our case, we compare a Null model (which contains no Chr-NE attachments) to models containing 15 or 48 Chr-NE attachments. Comparison with our experiment is then used to support or refute either of these models; we compare the radial density of chromosome in each of our models with experimental z-stack images (see results). Despite the reliance on computation, sophisticated modeling is now possible on desktop computers, which may make this approach feasible for future studies.

Limitations

We acknowledge the limited spatial resolution of our computational models. The bead radius, a key parameter in our models, is determined by the Kuhn length of the polytene chromosomes of \emph{D. melanogaster}, which in turn limits the highest resolution of our computational models to $\sim 0.7$ Mb. Therefore, our model is not suited for determining the effects of Chr-NE attachments at higher resolutions. It is also reiterated that our model is a coarse-grained representation of the polytene chromosomes and that not every conceivable parameter of the nuclear interior was modeled or investigated for robustness. To maintain biological realism, only the parameter values known from the experiment have been included in this study. For example, we did not test what would hypothetically occur if the probability of high-frequency Chr-NE attachments was allowed to vary from its experimental value of 66%; that is, a hypothetical range of NE contact probability was not explored. Likewise, the probability of sub-high frequency contacts was kept fixed at experimental 51%. A future study focused on cell-to-cell variability may explore what happens if any of these parameters vary, extending present work about population-average effects. For that type of work, biologically meaningful ranges for the model parameters, not just their averages, will have to be known. We also recognize that a complete investigation of the effects of Chr-NE attachments in the context of many other potentially relevant chromatin properties has not been made in this work. For example, we have not investigated how changes in chromosome rigidity may alter the predicted effects of Chr-NE attachments. Likewise, we have not made a complete investigation of how the chromosome distribution of Chr-NE attachments may affect the quantified
Observables. These are interesting “what if” questions for several comprehensive future investigations. Also, due to the quasi-equilibrium nature of the models considered, we cannot comment on possible time-dependence of these effects, including variations with cell age. At the same time, we note that all key model parameters (such as chromosome rigidity) currently used in this work are not arbitrary, but are taken directly from experiment. Thus, the evidence presented here demonstrates the importance of the additional recently identified 33 Chr-NE attachments. Chr-NE attachments affect chromosome-chromosome interactions at all resolutions in our computational models, and we hypothesize that at least some of the effects will persist at higher resolutions as well.

**Radial density measurement**

To determine the radial density of chromosomes from z-stack images, we use MATLAB®. Each z-stack image is composed of voxels containing quantitative fluorescent intensity data, which depends on the position of chromosomes in the nucleus. Sobel edge detection is used to detect the NE in each z-stack image. Z-stack images are normalized such that \( \sum f(\mathbf{r})V = 0.3 \). Here \( f(\mathbf{r}) \) is the DAPI fluorescent intensity in each voxel of a z-stack image recorded at 8-bit depth. \( V \) is the volume of each voxel, and 0.3 is the known chromosome volume to nucleus volume ratio. Radial density and fraction of chromosome at the nucleus periphery follow directly from the normalized z-stack images. The radial density of chromosomes measured from each z-stack is compared to that of computational models.

**Modeling approach**

*Bead size and chromosome thickness*

We used a SAW modeling approach to represent the 5 largest chromosome arms of *D. melanogaster* salivary glands as beads-on-string. The diameter of *D. melanogaster* polytene chromosome can range from 3.1–3.2 microns. Our model uses beads with a diameter of 3.1 microns. Each bead represents approximately 0.7 Mb of genomic material. To fully capture the thickness of the chromosome fiber, we place a cylinder of excluded volume around the bond between the nearest neighbor beads. This detail was important for achieving the right nucleus volume to chromosome volume ratio, but was found to have little effect on the scaling of our SAWs in free space.

*Polytene chromosome persistence length*

A 1.5-micron persistence length for *D. melanogaster* salivary gland polytene chromosomes has been inferred from experimental data. A 1.5-micron persistence length means the effective Kuhn length of our model is about twice the persistence length, meeting the condition necessary to build our models as a SAWs. We recognize that recent studies have questioned the standard definitions of persistence length for chains with self-avoidance. Despite the debate, we emphasize that our approach based on the polymer Kuhn length is de rigueur in many current polymer models and that the concept has worked well for several decades.

**Material and Methods**

*Salivary gland preparation*

The preparation of salivary glands has been described elsewhere. Briefly, salivary glands were dissected in phosphate-buffered saline and then fixed in 3.7% formaldehyde (Fisher Scientific, F79–500) and 1% triton (Fisher Scientific, BP151–100). Glands are removed from the fixative solution after 15 minutes, washed, and stained for 45 minutes in ProLong Gold antifade reagent with 3 μg/ml 4’,6-diamidino-2-phenylindole (DAPI) (Invitrogen, P-36931). Glands are whole mounted on slides, and nuclei are optically sectioned in 312 x 312 x 30 voxel images (z-stacks) with an inverted Zeiss LSM 510 Laser Scanning Microscope (Carl Zeiss MicroImaging, Inc.). The microscope resolution should have little effect on radial density measurements (described below) since each voxel is smaller than the chromosome radius.
attachments belong to our “15-attachment model.” In our previous work, we identified 33 additional Chr-NE attachments present in 51% of experimental nuclei. Here, these Chr-NE attachments are mapped to the corresponding beads in our computational model with the same frequency (0.51) of NE attachment. We thus construct a “48-attachment model” that has the 15 Chr-NE attachments from plus the additional 33 Chr-NE attachments from. These two are novel models in the sense that, for the first time, they incorporate the full complement of previously known and recently identified Chr-NE attachments. We emphasize that the number of attachments differs in each model since they enforce different numbers of Chr-NE attachments: 0, 15, and 48, respectively. The specific sets of Chr-NE attachments and their mapping onto our computational models is provided in Table S1. Our "Null model" contains no Chr-NE attachments and has been considered previously. The 3D genome organization of the full complement of previously known and recently identified 33 Chr-NE attachments from has been utilized in multiple studies in similar contexts. Briefly, we construct our SAWs using an unweighted Rosenbluth algorithm. For short chains, this approach is a good approximation of self-repelling chains that are true equilibrium states of polymers. Although we use a SAW approach, which is equilibrium by construction (to the extent that it approximates self-repelling chains), each of our 3 models contains non-equilibrium features introduced to better represent the experiment. These include the Rabl configuration of chromosomes, right-handed chromosome chirality, and asymmetric chromocenter arrangement. We thoroughly check that the key model conclusions (see results) are robust to the above non-equilibrium modifications introduced into our SAW approach. For robustness checking of the model conclusions, we use an unmodified SAW approach as the reference, which does not introduce (1) Rabl chromosome configuration, (2) right-handed chromosome chirality, or (3) non-random chromocenter arrangement and is equilibrium to the extent that our chain-growing algorithm approximates self-repelling chains (see main text). The same conclusions as presented above are reached with these reference models (Text S5 and Table S4). Additional discussion of these parameters and their effect on the simulation outcomes can be found in a previous work.

Robustness of conclusions to model details

The SAW approach used here to model polytene chromosomes has been utilized in multiple studies in similar contexts. Briefly, we construct our SAWs using an unweighted Rosenbluth algorithm. For short chains, this approach is a good approximation of self-repelling chains that are true equilibrium states of polymers. Although we use a SAW approach, which is equilibrium by construction (to the extent that it approximates self-repelling chains), each of our 3 models contains non-equilibrium features introduced to better represent the experiment. These include the Rabl configuration of chromosomes, right-handed chromosome chirality, and asymmetric chromocenter arrangement. We thoroughly check that the key model conclusions (see results) are robust to the above non-equilibrium modifications introduced into our SAW approach. For robustness checking of the model conclusions, we use an unmodified SAW approach as the reference, which does not introduce (1) Rabl chromosome configuration, (2) right-handed chromosome chirality, or (3) non-random chromocenter arrangement and is equilibrium to the extent that our chain-growing algorithm approximates self-repelling chains (see main text). The same conclusions as presented above are reached with these reference models (Text S5 and Table S4). Additional discussion of these parameters and their effect on the simulation outcomes can be found in a previous work.

Computational simulations of the models

Large conformational ensembles (i.e., snapshots) of nuclei were simulated for each model of Chr-NE attachment: Null model – 3,193 simulated nuclei, 15-attachment model – 3,623 simulated nuclei, and 48-attachment model – 3,477 simulated nuclei. The generating of conformational ensembles was performed on 240 parallel CPUs and took approximately 40,000 CPU hours total. Sampling protocol for generation of computational ensembles is provided (Text S2).

Analysis of the simulated ensembles of nuclei

The simulated ensembles of nuclei are analyzed at 3 spatial resolutions including whole-chromosome resolution, whole-arm resolution, and single-bead resolution (the highest resolution of our model, about 0.7 Mb of linear sequence). To quantify bead-bead interactions (single-bead resolution), we calculate how often specific pairs of beads are in contact in an ensemble of computational nuclei; a contact is defined as 2 microns or less separation between the surfaces of 2 beads. There are 248 beads in a single computational nucleus (including all of the 5 chromosomes); a 248 × 248 contact map has entries for each possible pair of beads. The "(i,j)" entry of this contact map gives the probability that bead "i" and bead "j" in our computational nuclei form a contact. We also compute contact probability at 2 lower resolutions: chromosome arm resolution – bins of beads belonging to the same chromosome arm, whole-chromosome resolution – bins of beads belonging to the same chromosome (i.e., chromosome 2 right and left arms). These interaction types are normalized against the total number of interactions in each model, e.g., the number of intra-arm interactions in our Null model out of all interactions in our Null model. Since our simulations generate equilibrium conformational ensembles of model nuclei, we do not calculate time-dependent observables such as auto-correlation times. Furthermore, we do not consider the proposed molecular clamp forces that may be provided by cohesins, which are thought to play an important role in chromosome territory formation and maintenance. In addition, we do not consider compaction forces provided by condensins or the movement of Chr-NE attachments along the NE surface. Each of these considerations would necessarily require a fully dynamical model capable of describing time-evolution, which would be out of scope of this study. Here, we model mature polytene chromosomes that have reached quasi-equilibrium, with essentially no time-evolution of the relevant experimental time-scales.

Chromosome territories

We quantify chromosome territories by addressing how each chromosome excludes other chromosomes from the volume it occupies in the 3D space. We begin by calculating the convex hull for a single chromosome – this is the minimum volume that includes all the chromosome’s points (bead centers) inside a convex polyhedron. In general, each convex hull contains its own chromosome and may also encompass some beads belonging to other chromosomes. A fully "territorial" chromosome is one whose convex hull does not contain points from any other chromosomes, while a less "territorial" chromosome is one whose convex hull contains some points from other chromosomes (Fig. 2). We define the chromosome territory index as the fraction of points inside a convex hull that belongs to the
chromosome used for its construction. A detailed description of chromosome territories is given in our recent work. 23

Chromosome intertwining

Chromosome intertwining is rigorously assessed by attempting to separate model chromosomes by putative translations in 3D space (Fig. 3). Chromosomes that can be spatially separated by at least one translation are called non-intertwining. We begin by selecting the backbone of 2 chromosomes (excluding centromere) from a model nucleus; the backbone of a model chromosome consists of the line segments connecting the centers of each bead. A direction vector is then chosen to translate the backbone of one model chromosome (Fig. 3). If the two backbones cross during this translation, then a new direction vector is picked. A total of 162 different direction vectors are tested in this manner; the tips of the vectors uniformly cover the surface of a unit sphere, as detailed in our recent work. 23

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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Supplemental Material

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