

Role of Topological Exclusion in Formation and Organization of Chromosomal Territories

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(Received December 8, 2010)

Chromosomes of eukaryotic organisms are composed of chromatin loops. Using Monte Carlo simulations we investigate how the topological exclusion between loops belonging to different chromosomes affects chromosome behaviour. We show that in a confined space the topological exclusion limiting catenation between loops belonging to different chromosomes entropically drives the formation of chromosomal territories. The same topological exclusion in a connection with interchromosomal binding via transcription factories explains why actively transcribed genes are found preferentially at the peripheries of their chromosomal territories. This paper is based in part on the results presented in J. Dorier and A. Stasiak, *Nucl. Acids Res.* **37** (2009), 6316 and **38** (2010), 7410.

§1. Introduction

Each somatic cell in a human body contains about 2 meters of DNA crammed within ca. 6 μm -large nuclei. This DNA is divided into ca. 5 cm long linear DNA molecules constituting genetic material within protein-DNA complexes known as chromosomes.³⁾ The structure of chromosomes varies with the stage of the cell cycle. During cell division, where newly replicated chromosomes need to be moved apart to permit their segregation between progeny cells, the chromosomes are extremely condensed having the axial length of about 5 μm . After cell division chromosomes decondense into so-called 30 nm chromatin fibres having a length of about 1 mm per chromosome.⁴⁾ Interestingly, the decondensed chromosomes do not spread over the entire nucleus but form compact territories.^{5),6)} It is poorly understood what underlying mechanisms are responsible for the formation of chromosome territories. Several recent studies suggested an implication of chromatin loops in the formation of chromosome territories.^{7)–10)} We will consider here the possible role of topological exclusion arising from the inability of chromatin fibres to pass through each other. In particular, we will address the question whether the topological exclusion between independent chromatin loops can entropically drives the formation of chromosome territories and whether it can also be responsible for the preferential location of transcriptionally active portions of chromosomes at the peripheries of their chromosome territories.

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§2. Topological exclusion and formation of chromosome territories

After division cells enter interphase stage of cell cycle during which highly condensed mitotic chromosomes decondense and fill entire cell nucleus. Interestingly, the decondensed chromatin fibres of individual chromosomes form territories that are in a direct contact with each other but chromatin fibres belonging to different chromosome territories show only a very limited intermingling with each other. This highly limited extent of intermingling between chromatin fibres from different chromosomes was very puzzling. However, theoretical considerations, simulation and experimental studies of circular polymers revealed that at high concentration, individual ring-forming molecules fold on themselves instead of intermingling with each other.^{11)–15)} Since under the same conditions linear polymers did not show this behaviour and were perfectly intermingling with each other at the equilibrium, it is possible that formation of chromosomal territories could be simply entropy driven if chromatin fibres were forming intrachromosomal loops and DNA topoisomerases were not able to lead to the catenation of loops belonging to different chromosomes.^{1), 15)}

Theoretical and experimental studies revealing the natural tendency of circular polymers to form compact territories were mainly addressing the situation in highly concentrated solutions known as melt state.^{11)–15)} However, chromatin fibres occupy about 10% of nuclear volume¹⁶⁾ and it is questionable whether they form a melt state or a semi-dilute solution. In 3-D lattices the melt state is reached when the lattice elements corresponding to segments of modelled polymer occupy 50% of the maximal capacity of the lattice.^{13), 14)} In off-lattice polymer models it is not yet established what percent of volume occupation corresponds to the melt state. To investigate the effect of topological exclusion in the way that neglects the actual volume occupied by polymers, we decided to study the behaviour of infinitely thin, freely jointed, polygonal chains.¹⁷⁾ Such freely jointed chains are standard coarse-grained models of polymeric chains that undergo thermal fluctuation under conditions where independent segments of polymers neither repulse nor attract each other, which seems to be a good approximation of chromatin behaviour in interphase nuclei.¹⁵⁾ To model the confinement within the nucleus, we placed the polygons into a sphere with a radius that is significantly smaller than the radius of gyration of the polygons in an unconfined state. Using Monte Carlo simulations, we investigate the equilibrium properties of 20 equilateral polygons with 20 segments each, confined within a sphere with a radius corresponding to the length of two segments. To evaluate the effect of topological exclusion, we compared simulations in which the polygons behaved as phantom chains and thus had unrestricted topology with simulations where the individual polygons were constrained to remain unknotted and uncatenated (non-phantom).

Although chains composed of 20 statistical segments may on first sight seem to have much less statistical segments than eukaryotic chromosomes this is not really the case. The estimations of the statistical segment length of chromatin fibres in vivo range between 170 and 220 nm, while the estimations of linear density of chromatin fibres range between 110–150 bp/nm.¹⁸⁾ Taking the upper range of these values one arrives with one statistical segment of chromatin containing 33,000 bp. As a

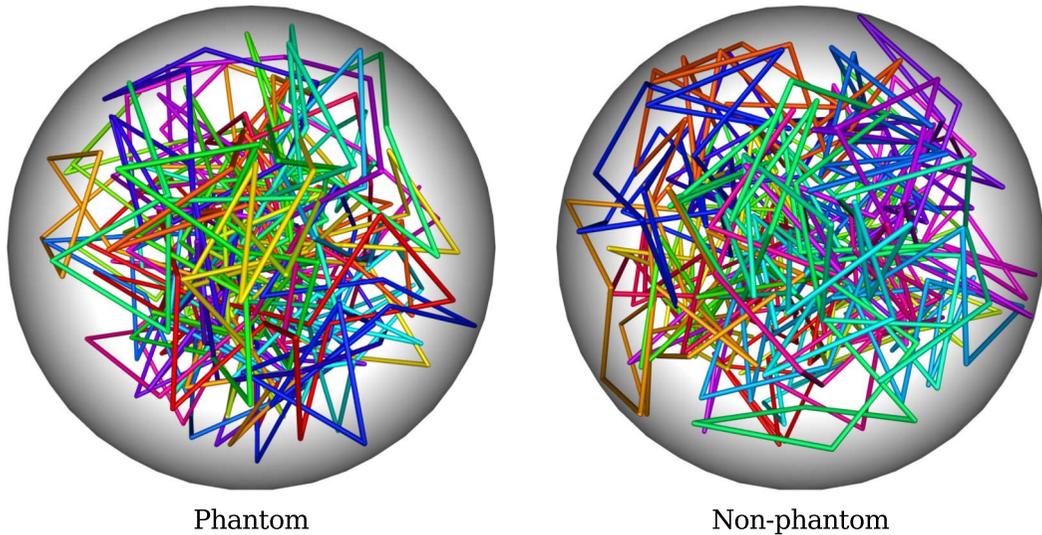


Fig. 1. Representative snapshots obtained during Monte Carlo simulation of 20 freely jointed polygons, each composed of 20 infinitely thin segments, confined within a sphere of radius 2 segment length. Two variations of the model are considered: (left) phantom polygons without topological constraints and (right) non-phantom polygons constrained in an unknotted and uncatenated topology. To help visualize the polygons, each segment is shown as a tube with non-zero radius.

consequence each polygon composed of 20 statistical segments would correspond to 660 kb (which is a good size for an yeast chromosome as these range from 200 to 2200 kb), and all 20 chains would correspond to 13,2 Mb of DNA packed within a sphere with a diameter of about $0.9 \mu\text{m}$. Therefore, our simulation reflects the situation in haploid yeast nuclei that have the diameter of about $1 \mu\text{m}$ and contain 12,5 Mb of DNA. Of course, larger chains would be needed to model decondensed chromosomes of human cells for example.

Figure 1 presents representative snapshots obtained during these simulations. It is well visible that in the case of phantom polygons the centre of the sphere is preferentially occupied and individual polygons are strongly intermingled with each other. In the case of non-phantom polygons the space is filled much more uniformly, which indicates that polygons exclude each other. To gauge the extent of territorialization of individual polygons induced by their mutual topological exclusion, we were inspired by cytological methods used to demarc chromosome territories. Cell biologists use confocal microscopy to analyse optical cross-sections through cell nuclei in which chromatin fibres belonging to different chromosome territories are stained with chromosome specific fluorescence probes.¹⁹⁾ On such cross-sections one can then delineate regions occupied by chromatin fibres belonging to different chromosomes. Following this approach we analysed cross-section planes passing through the centre of the confining sphere. The intersection points of each individual polygon with this plane were enclosed within individual minimal-size convex envelopes (see Fig. 2). These convex envelopes conceptually correspond to cross-sections through chromosomal territories. Figure 3 presents cross-sections of the snapshots shown in Fig. 1.

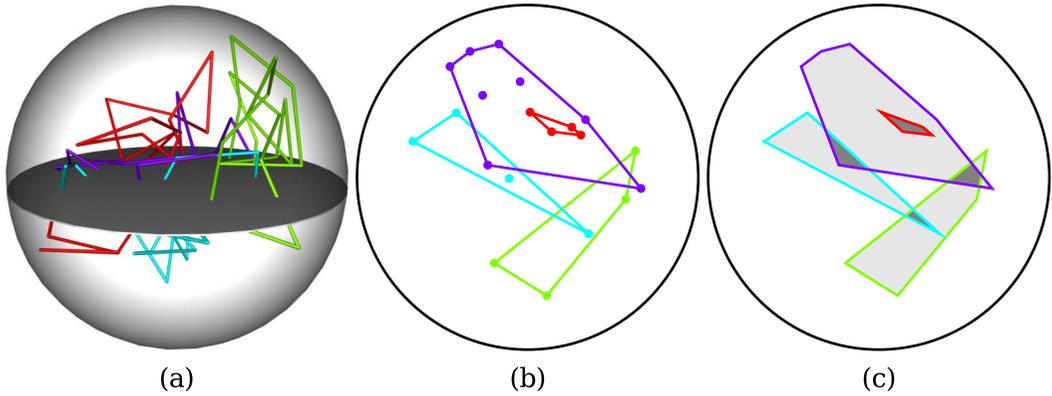


Fig. 2. (color online) To measure the intermingling: (a) We first determine the points of intersection of these polygons with an equatorial plane. (b) For each polygon, we determine the smallest 2D convex envelope enclosing all its intersections points with the equatorial plane. (c) Finally the intermingling is defined as the ratio of the total area of all intersections of the convex envelopes (dark grey region) to the total surface area of all convex envelopes. This ratio may range from 0 to 1 and expresses the extent of the intermingling.

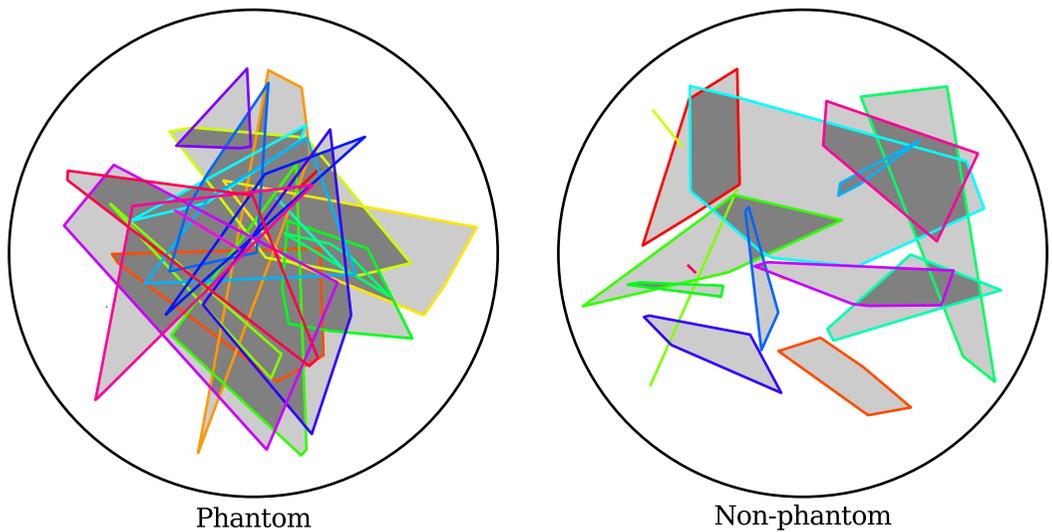


Fig. 3. (color online) Equatorial cross-sections corresponding to the snapshots presented in Fig. 1. Notice strong intermingling in case of phantom polygons (left) and mostly spatially separated convex envelopes in the case of non-phantom polygons (right).

As could be expected from the fact that phantom polygons group in the center of the sphere while non-phantom polygons are more equally distributed (Fig. 1), the overlapping regions between territories concentrate in the center of nucleus in the case of phantom polygons and are more equally redistributed within the sphere in the case of non-phantom polygons. It is very well visible that in the case of non-phantom polygons the topological exclusion greatly limits the overlapping of convex envelopes enclosing individual polygons and thus entropically drives their territorialization. To

quantify the reduction of inter-polygonal intermingling by the topological exclusion we have measured the thermal average of a quantity called intermingling¹⁾ that was defined for a given cross-section as the ratio between the total area of overlaps between convex envelopes (dark-grey areas in Fig. 3) and the total area of all convex envelopes. This ratio can range from 0 (no intermingling) to 1 (a complete intermingling). For phantom polygons the intermingling value amounted to 0.667 ± 0.001 but it decreased to 0.361 ± 0.001 for non-phantom polygons. This strong decrease of intermingling when topological constraints are acting suggests that topological exclusion can explain the formation of chromosomal territories.

§3. Topological exclusion and preferential location of transcriptionally active regions within their chromosome territories

During the interphase stage of the cell cycle, cells grow and many genes show high transcriptional activity. These genes serve as templates enabling RNA polymerases to synthesise various types of RNA molecules such as mRNAs that are subsequently translated in ribosomes into freshly synthesized protein molecules. The realization that chromosomes in interphase nuclei form compact chromosome territories has led to the question whether actively transcribed genes occupy specific positions within chromosome territories. Several studies have demonstrated that stretches of chromatin that are rich in active genes locate preferentially at the periphery of their chromosome territories or even protrude from these territories.²⁰⁾ Since genes not belonging to gene rich regions are frequently transcribed within chromosome territories, this helped to establish that RNA polymerases and transcription factors can freely diffuse into chromosomal territories.⁶⁾ Therefore, the peripheral localization of blocks of active genes is not a trivial consequence of a formal possibility that only genes located at the periphery of chromosome territories could have an access to RNA polymerases.

What could then cause that portions of chromosome enriched into genes undergoing transcription are found preferentially at the peripheries of chromosome territories? While considering localization of genes in chromosome territories, one needs to take into account the fact that transcription takes place within transcription factories where about 8 RNA polymerases transcribe a corresponding number of independent genes, bringing them into physical proximity.²¹⁾ The genes that are brought together can be from the same or from different chromosome territories. For a portion of a chromosome that is rich in active genes there is a high chance that some of them will be bound via their transcription factories to active genes from another chromosome territory. To investigate the effect of transcription factories-mediated interchromosomal tethering on positions of transcriptionally active regions within chromosome territories, we performed Monte Carlo simulations where 8 polygonal chains with 40 segments each were tethered to each other by imposing an elastic potential between preselected 8 vertices (one vertex per polygon). This potential was effectively bringing the tethered vertices to a distance smaller than 1/10 of one segment length. In this model the inter-attached vertices corresponded to actively transcribed regions attached together via transcription factories. To be able to es-

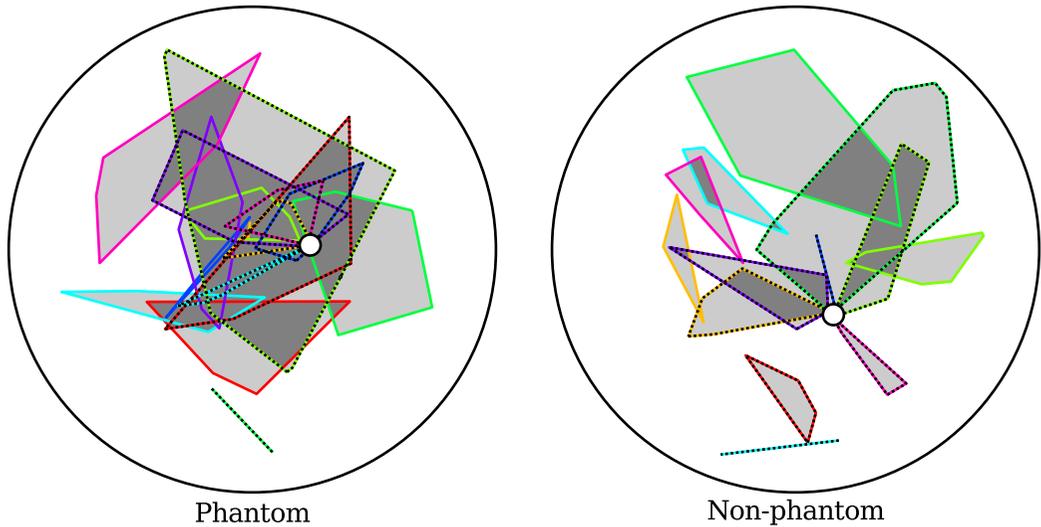


Fig. 4. Equatorial cross-sections of representative snapshots obtained during Monte Carlo simulation of 16 freely jointed polygons, each composed of 40 infinitely thin segments, confined within a sphere of radius 3.6 segment length. Half of the polygons are tethered to each other by imposing an elastic potential between 8 preselected vertices (one per polygon). Convex envelopes of these polygons are shown with striped lines, while the envelopes of the remaining polygons are shown with plain lines. The white circle represents the position of the transcription factory, i.e. the center of mass of the tethered vertices. Two variations of the model are considered: (left) phantom polygons without topological constraints and (right) non-phantom polygons constrained in an unknotted and uncatenated topology.

timate the effect of interchromosomal tethering on the overall shape of chromosome territories we also included into the simulation 8 non-tethered polygonal chains with 40 segments each. To reflect the confinement within the nucleus, we placed all the polygons within a sphere of radius 3.6 segment length. To investigate the effect of topological exclusion on the location of inter-attached regions of polygons, we compared simulations where polygons behaved as phantom with simulations where passages between segments belonging to the same or to different polygons were not allowed and where all polygons were isomorphic to unknotted and uncatenated rings (non-phantom).²⁾

Figure 4 shows cross-sections passing through the centre of the sphere of confinement and through the centre of mass of 8 vertices that were tethered to each other (indicated with a small sphere) for a representative snapshot. As in Fig. 3, we demarked regions occupied by individual polygons with minimal size convex envelopes enclosing all intersection points of a given polygon with the cross-section plane. The convex envelopes enclosing individual tethered polygons are marked with a striped rim. It is striking that in the case of phantom polygons the region of attachment of tethered polygons localizes within regions co-occupied by several tethered polygons, while in the case of non-phantom polygons this region localizes at the peripheries of their respective territories.

To check that the equilibrium position of attached vertices agrees with what

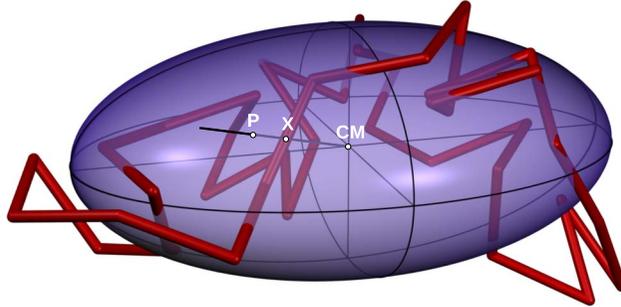


Fig. 5. To determine whether a given point X on a polygon has a peripheral or central position within the territory formed by this polygon, we define the relative radial position as the ratio between the distance X - CM and P - CM . Here CM is the center of mass of the polygon and P is the intersection between the ray X - CM and the characteristic ellipsoid of inertia defined by the given polygon.²²⁾

was observed in the snapshot, more quantitative measurements are needed. Since individual configurations of random polygons are not spherical but ellipsoidal, simple measures like the distance from the centre of mass do not tell us whether a given vertex in a polygon is placed centrally or peripherally as some peripheral positions may be at smaller radial distance than some centrally located positions (see Fig. 5). To normalize for the ellipticity of individual configurations, we calculate for each configuration their characteristic ellipsoids of inertia i.e. ellipsoid that would have the same inertial properties as the polygon it represents if the polygon's mass were equally redistributed along the surface.²²⁾ For a given vertex of a polygon we can now determine its relative location with respect to the ellipsoidal surface using a ray starting from the centre of the ellipsoid and passing through this vertex (see Fig. 5). Along this ray we can measure the distance separating this vertex from the centre of the ellipsoid and the distance from the centre of the ellipsoid till the point of ray's intersection with the surface of the ellipsoid. The ratio between the two distances corresponds to the relative radial distance and it informs us about the position of a given vertex in its territory. The low values indicate central positions and values close to or above 1 indicate peripheral positions.

Figures 6 (a) and (b) show distributions of relative radial distances of all vertices and of tethered vertices in phantom and non-phantom polygons within actual territories formed by individual polygons. In the case of phantom polygons the tethered vertices (blue plain line) show more central positions than average vertices in these polygons, whereas the contrary is the case for non-phantom polygons. The more central position of tethered vertices in phantom polygons can be explained by the fact that tethered phantom polygons confined within a small sphere overlap with each other and the region of overlap involves more frequently the central portions of individual polygons than the peripheral portions extending toward the surface of the sphere. In case of non-phantom polygons the more peripheral position of tethered vertices with respect to territories formed by individual polygons is generated by the topological repulsion between the contacting polygons and is expected to be largely

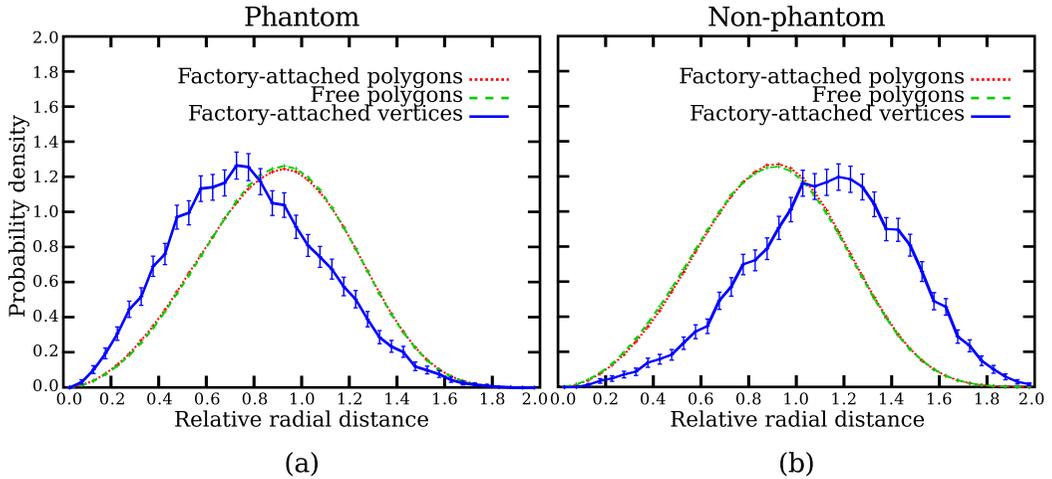


Fig. 6. (color online) Distribution of relative radial distance for: points on free polygons (green dashed lines), points on the polygons attached together (red dotted lines) and for the vertices that were representing points of attachment to a transcription factory (blue plain lines). For red dotted lines and green dashed lines the error bars are smaller than the width of the lines.

independent of the radius of the confining sphere. Only the non-phantom case is consistent with the biological data. On this basis we propose that the topological exclusion between chromatin loops forming chromosomal territories combined with interchromosomal tethering of these loops via transcription factories can explain the preferential location of actively transcribed regions in the peripheries of chromosome territories.

§4. Conclusions

Using simple models we showed that topological exclusion, even in the absence of excluded volume effect, can lead to compaction of chromosome territories. We also showed that the topological exclusion in connection with interchromosomal tethering via transcription factories is sufficient to explain why chromosome regions rich in active genes preferentially locate at the peripheries of their chromosome territories.

Acknowledgements

The computations were performed at the Vital-IT (<http://www.vital-it.ch>) Center for high-performance computing of the Swiss Institute of Bioinformatics. This work was supported in part by Swiss National Science Foundation (31003A-116275 to A.S.).

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