Massachusetts Institute of Technology

8.592: Statistical Physics in Biology
Spring 2018

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Spiralization of the Mitotic Scaffold in Chromosomes

Abstract

Recent studies have shown that mitotic chromosomes organize themselves into loops. Polymer models have been proposed that use the mechanism of loop extrusion to build a mechanistic model of how mitotic chromosomes condense. A crucial element of this model is the presence of a spiral scaffold at the base of the loops. Here we investigate the possibility of spontaneous formation of a spirals for a two dimensional polymer random walk constrained to the surface of a cylinder.

1 Introduction

Reproduction is fundamental to all forms of life. At the cellular level, this involves the division of a single parent cell into two daughter cell. While the mechanistic details may vary between the different branches in the tree of life, all of them involve the replication and reorganization of DNA. In eukaryotes, there are two distinct types of cell division. Mitosis is the type of cell division that occurs in unicellular organisms and in somatic cells of multicellular organisms. Mitosis results in two daughters with identical copies of the genome. In the sex cells of sexually reproducing eukaryotes, a different process known as meiosis occurs in two stages, which results in four daughter cell with only half the normal number of chromosomes.

The eukaryotic cell-cycle is consists of four stages. Three of the phases are collectively known as interphase where cell grows, produces proteins and replicates its DNA in anticipation of cell-division. The remaining Mitotic (M) phase is where the chromosomes condense and segregate themselves into two sets that are pulled apart by the the mitotic spindle. After this, cytokinesis occurs where the cell cytoplasms fission and each section takes a set of the chromosomes with it to form the daughter cells where the process repeats itself. This whole process need to occur with a high degree of accuracy as errors can lead to disorders inhibiting the organism ability to function and survive.

2 Chromosome Conformation Capture Technologies

The overall organization of the chromosomes in the cellular nucleus plays a huge role in the transcriptional activity of the cell. Distal genomic regions may come close together spatially and influence each other's expression levels. The ability to probe the underlying structure of chromosomes is crucial to our understanding of gene expression as well as the process of mitotis. Recently a number of high-throughput chromosome conformation capture technologies have been developed that enable us to study the higher order structure of chromatin and chromosomes [1]. The initial versions were only able to contacts between pairs of points in the genome but subsequent variants such as Hi-C have expanded the capability to being able to observe pairwise contacts across the entire genome [2].

All chromosome conformation capture technologies follow similar protocols (Fig 1). Neighboring chromatin fibers within the nucleus are crosslinked following which a restriction enzyme is used to cut the DNA at particular cites thereby fragmenting the genome. The crosslinked DNA fragments are ligated to each other. Once the crosslinker has been dissolved, what is left is a hybrid DNA fragment where the two ends come from different regions of the genome. These hybrid fragments are sequenced from both ends and each end is mapped to a location in the genome.

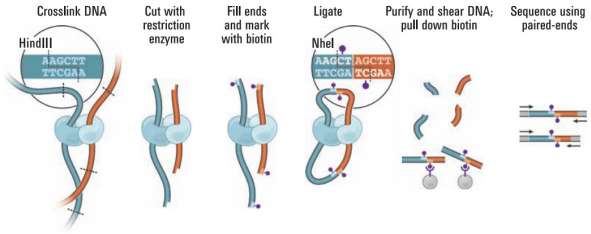

Figure 1: Cartoon representation of the Hi-C protocol. Image was taken from [2].

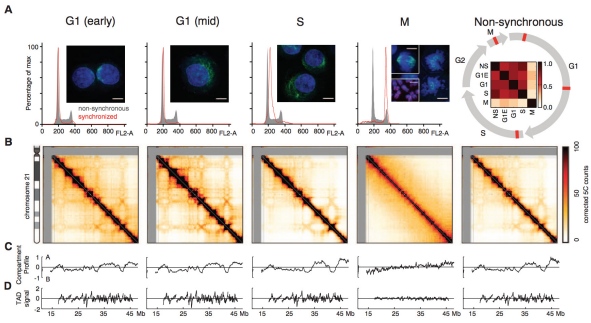

Figure 2: **A** Fluorescence images of cell at different points in the cell cycle. **B** Corresponding HiC heatmaps. **(C,D)** Compartment and TAD signals obtained from HiC. Patterns present in the data during interphase are noticeably absent during mitosis. Image was taken from [3].

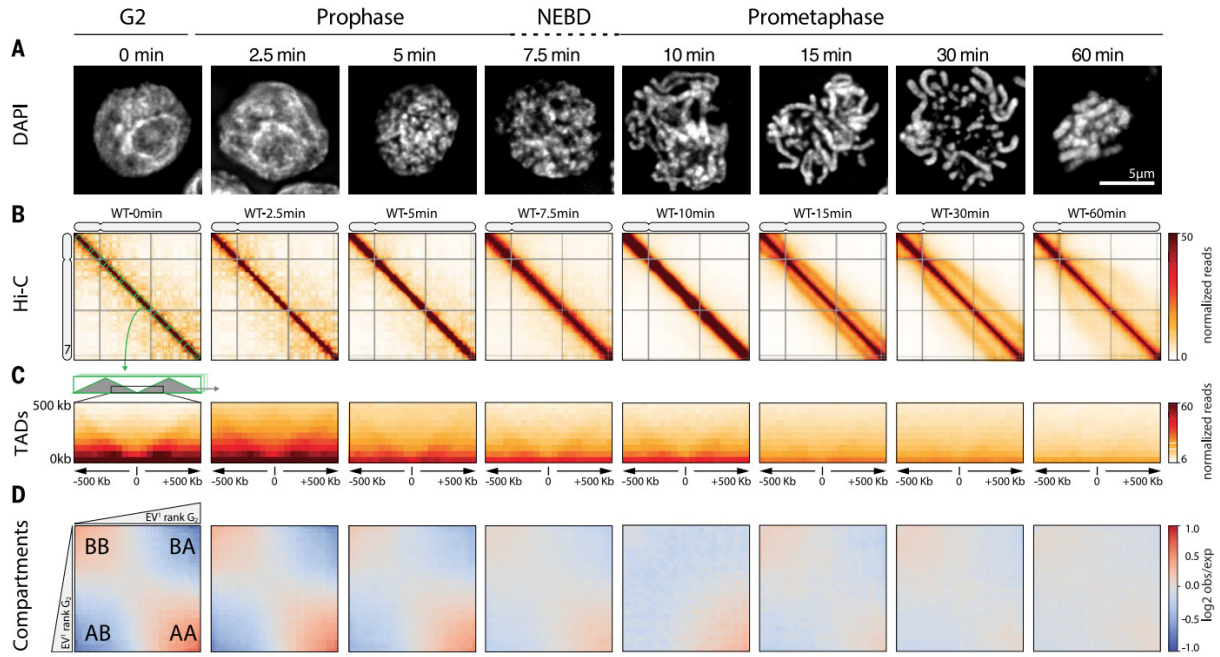

Figure 3: **A** Fluorescence images of cell at different stages of mitosis. **B** Corresponding HiC heatmaps. Prometaphase heatmaps contain a second diagonal. **C** TAD pileups from HiC. These quantify the average behavior of TADs at different stages of Mitotis. **D** Compartment saddleplots from HiC. These quantify the intensity of the checkerboard pattern. Image was taken from [4].

3 Chromosome Organization during Mitosis

A comparative analysis of Hi-C heatmaps between in Interphase and Mitotic cells reveal the reorganization of chromatin across the cell cycle. During interphase, chromosomes display structure at all scales. At short genomic distances, chromatin organizes itself into regions of enhanced enrichment called Topologically Associated Domains (TADs). These are believed to be mediated through the process of loop extrusion. At long distances they organize themselves into compartments characterized by a checkerboard pattern in the HiC maps. The average probability of contact between two loci scales as s^{-1}. In contrast during mitosis, TADs and compartments disappear and contact probability scales as $s^{-0.5}$ (Fig 2) [3]. Polymer simulations of mitotic chromosomes show that a structure organized as loops on a scaffold best recapitulate the features of the data.

The process of mitosis can broken down into further sub-phases - Prophase, Prometaphase, Metaphase, Anaphase and Telophase. The boundaries between these phases are demarcated by the onset of particular events. Recent experiments on time resolved data on cells undergoing mitosis revealed variations in chromatin organization between Propase and Prometaphase stages [4]. While Hi-C from Prophase cell had uniform heatmaps and shallow scaling in agreement with earlier mitotic data, the heatmaps in Prometaphase cells show the appearance of a second diagonal and the associated contact probability display non-monotonic behavior at scale of 10 megabases(Mb) (Fig 3).

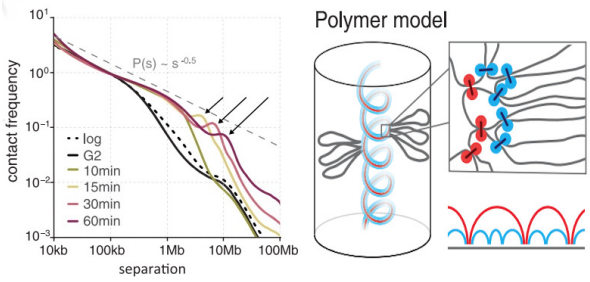

Figure 4: Scalings at different points during mitosis. Polymer model with hierarchical loops and a spiral scaffold describes mitotic chromosome organization. Image was taken from [4].

Polymer simulations done in these studies show that in order to obtain the observed features, DNA needs to be organized into hierarchical loop with the bases of the loops forming a helical scaffold (Fig 4). The hierarchical loops could be generated through the process of loop extrusion using two different types of loop extruding factors. The role of the loop extruding factors are believed to be played by the SMC complexes Condensin I and Condensin II since they associate with mitotic chromatin at different points in the cycle and Yeast Condensin has been shown to extrude loops in vitro. However, the helical scaffold, which is a crucial element of the model, remains unexplained.

4 Random Walks on a Cylinder

There are several mechanisms that could potentially explain the formation of the spiral scaffold. Bad solvent condition could force the condensation of a polymer in certain conformations. Interactions between the loop extruders such as could potentially force them into a spiral conformation [4]. Here we investigate a simple model where loops are considered as monomers of a polymer confined to cylinder. The first stage of the investigation, we explored what external constraints were required in order to have spirals form using steady state. Once this was established, polymer parameters were swept to explore the amount of spiralization that was obtainable.

4.1 External Constraints

Polymer simulations were done using the molecular dynamics package OpenMM. Harmonic potentials were used to confine the polymer to the surface of a cylinder with a radius that is five times the monomer size and the height that is fifty times the monomer size. When linear ordering was imposed by additionally constraining each monomer to occupy a position along the axis of the cylinder (z-direction), we observe the spontaneous formation of spirals once the random walk achieves steady state (Fig 5a). Follow this initial success, we relaxed the external constraints so that the ends of the polymer were tethered to the top and bottom of the cylinder while the rest of it was free to explore the entire surface of cylinder. Upon investigating these equilibrium conformations, we observe that spirals are not longer present. Varying the density and stiffness of the polymer do not yield the desired results (Fig 5b,c).

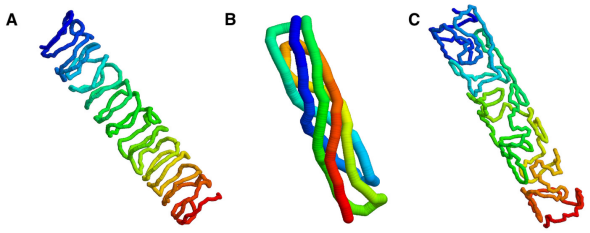

Figure 5: Steady state conformations resulting different external constraints. **A** Obtained by imposing a linear ordering of the individual monomers. **(B, C)** Polymer prefers to align itself along the axial direction especially when they are stiff as is the case in **B** but holds true to a less extent even with minimal stiffness as is shown in **C**.

4.2 Linearly Ordered Random Walks

Focusing on the linearly ordered random walk, we observe that spiral domains forming where the spirals reverse direction between neighboring domains. We find the the domains can be estimated by angular step of the random walk - the points where the step changes signs demarcate the boundary between domains (Fig 6).

We varied the number of monomers (density) and the stiffness of the polymer (persistence length) and observe how the spiralization changes (Fig 7). In the low density regime, no spiral form as the polymer has a lot of room to spread out. As we increase the density, we observed an increase in the average domain size and conformations are forced to become compact. Increasing the stiffness reduced the average domain size.

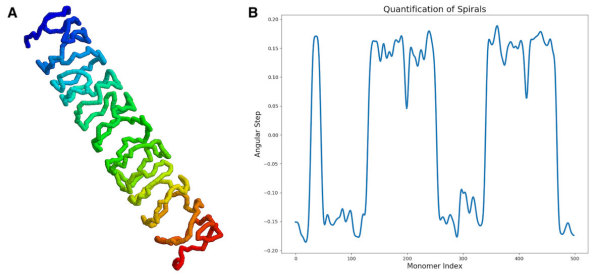

Figure 6: **A** Linearly order steady state conformation and **B** corresponding angular displacement plot. Change in sign of displacement represent boundaries of spiral domains

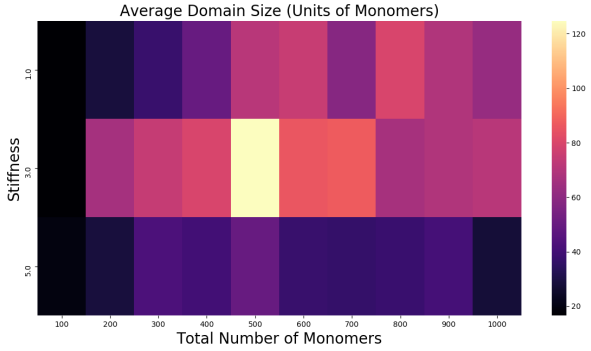

Figure 7: Heatmap representing average domain size for a range of stiffness and monomer number.

5 Discussion

In the simple model address above, we have seen the spontaneous emergence of spirals domains for polymer random walks constrained on the surface of a cylinder. These spiral domains form when the monomers are constrained axially by an external force. Furthermore the chirality of these domains reverses between neighboring domains. The spiral scaffold used in the simulation of mitotic chromosomes consisted of a single macroscopic spiral domain to match the experimental data. However it maybe possible that a certain finite critical domain size may be sufficient to match data. For example, if the average domain size had an angular extent of at least 2π, one may expect an enhancement in contact probability at genomic distance corresponding to the periodicity. However more involved polymer simulations are required to confirm this.

We also observe the behavior that increasing the persistence length of the polymer reduces the average size of

the spiral domains. This behavior is expected due to the fact that orientation of the polymer along the axial direction involves less bending that if it were to oriented along the angular direction. At larger stiffness, such angular orientations become less frequent resulting in the reduction in domain size. This fact also explains why spiralization is not achieved when only the ends of the polymer are tether to either end the cylinder. Even at low stiffness, the polymer prefers to orient itself along the axis to minimize bending energy.

While it remains to be justified as to why a two dimensional random walk on the surface of a cylinder is an accurate model for the condensation of chromosomal loops during mitosis, it is encouraging to see that spiralization can emerge under certain conditions in this simplified model. We hope to continue investigating the question of spiralization to a greater extent in future work.

References

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