How cells overcome their topological troubles

Compaction and segregation of sister chromatids via active loop extrusion
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Recommended with a Commentary by Helmut Schiessel, Leiden University

Before a cell is able to divide it needs to solve a topological puzzle that seems far too complex to be surmounted: the separation of its two sets of DNA molecules, which—after DNA duplication—form seemingly inseparable tangles. In humans, 46 such tangles need to be resolved, with a total length of 4 meters. Polymer physics does not come to the cell's rescue, as two overlapping polymers only feel a repulsion of about one k_BT, independent of the length of the molecules [1]. An active process involving molecular motors is needed. But how can they distinguish between two identical DNA molecules to pull them apart? This is exactly what happens later, after the well-known mitotic chromosomes have formed, the iconic X-shaped structures where the DNA molecules are already well separated, making it rather straightforward for the spindle apparatus to pull them in opposite directions. But how did the system get to the point of well-separated DNA molecules in the first place?

Goloborodko, Imakaev, Marko and Mirny (GIMM) demonstrate in a large scale computer simulation that just two ingredients are needed to make this seemingly impossible task, separating two identical polymers, straightforward. Maybe even more remarkably, the separated polymers form a structure that looks just like X-shaped mitotic chromosomes. Even though an experimental verification of this mechanism is still missing, after seeing this paper, it appears likely that this is how cells do it. So how does it work?

Before revealing the solution, it is worthwhile to look at two related phenomena where at least some of the same principles seem to be at work. The first concerns the phenomenon of chromosomal territories. Cells in interphase (the normal working state of cells where they produce proteins and grow) have each chromosome (a DNA molecule complexed with proteins) living in its own territory. This can be seen by chromosome painting where one paints each chromosome in a different colour, giving these nuclei the appearance of a multicoloured patchwork quilt [2]. This is in contrast with dense solutions of polymers where molecules overlap strongly, each showing a random walk configuration. So why do chromosomes not mix?

This most likely reflects the fact that chromosomes do not have the time to equilibrate. Starting from their condensed state directly after cell division, the chromosomes expand as they enter interphase. However, it would take about 500 years for human chromosomes to mix [3]. Mixing is hard as each chromatin fiber (the effective polymer formed by a DNA

molecule and proteins) is effectively confined in a tube formed by other fibers. Polymers can free themselves only by a snake-like motion called reptation. Because this process is so slow for larger chromosomes, it has been argued [3] that one does not have to consider the fiber ends and can instead think of such DNA molecules as being closed into rings. As the cell starts from well-separated condensed chromosomes, these different "rings" are non-concatenated. And interestingly, in dense solutions non-concatenated rings do not mix [4], unlike linear polymers. Could the fact that rings do not mix contain a piece of the puzzle of DNA segregation?

The second related phenomenon concerns so-called topologically associated domains (TADs). These domains were discovered with a new powerful experimental method, Hi-C, a type of chromosome conformation capture. First, whole chromosomes are covalently crosslinked. Then, cross-linked DNA stretches are sequenced. This provides information about millions of spatial contacts within a chromosome. Early Hi-C data [5] already suggested that DNA conformations are not consistent with equilibrium polymer physics but might be consistent with the crumpled (or fractal) globule, a non-entangled polymer state that was put forward already long ago as a promising alternative to the heavily entangled equilibrium structures [6] (with strong similarities to the non-concatenated rings mentioned above). Hi-C at higher resolution [7] then revealed TADs, mysterious structures of consecutive DNA stretches (median length 185 kilobase pairs) with a higher than average contact probability. Presumably each TAD achieves this by forming internal loops. But how do they avoid to create loops to DNA outside a given TAD? Moreover, the boundaries of TADs consist of binding sites for certain proteins (called CTCF) with their sequence in convergent orientation. These sites then form the base of the outer loop of such a TAD. Which mechanism could make sure that only those pairs and not any other pairs bind to each other?

A surprisingly simple way to create TADs is the loop extrusion mechanism. In its simplest form, this mechanism has an active motor protein that binds two sites nearby on a chromatin fiber and then extrudes a loop of DNA by having its two bound halves translocate along the fiber in opposite directions. The protein holds together increasingly distant chromosomal sites, enlarging the loop. The spooling of DNA into the loop continues until the loop extruder (which is believed to be cohesin) encounters "stop signs" or "anchors" at the above mentioned inwards pointing CTCF DNA motifs blocking further extrusion. Computer simulations [8, 9] and genome editing [8] (changing the "anchors") support this picture. Note especially that loops created in different TADs are by construction non-concatenated and thus different TADs do not mix, similar to chromosomal territories, but on a much smaller scale.

Non-concatenated rings do not mix and loop extrusion produces non-concatenated loops. With these insights we can return to the original question: How can 4 meters of tangled DNA be separated into 46 mitotic chromosomes? GIMM demonstrate this in a computer experiment. They start from two intertwined polymers, coarse-grained representations of the two copies of identical chromatin fibers. They add the action of loop extruding complexes (in this case believed to be connected pairs of condensin) by binding random pairs of neighbouring monomers together and then extruding loops by moving the condensins along the fiber in opposite directions. Extruding condensins are not allowed to pass through each other and have a finite processivity. A new extruder is added at a random position as soon as a condensin pair falls off a chromosome. Unlike for TADs no anchor sites are present. In addition, the two polymers are connected at their midpoints, mimicking the cohesion of

chromosomes at the centromere. Running such a simulation does not, however, lead to chromosome segregation, as many entanglements cannot be resolved. However, the necessary tool to allow DNA molecules to pass through each other is known to exist inside the nucleus: topoisomerase II. The action of this enzyme is accounted for by GIMM by allowing polymers to pass through each other at a finite energy cost.

With these ingredients in place, the two entangled polymers readily condense and separate into an X-shaped structure that shows an almost eerie resemblance to real mitotic chromosomes. I especially recommend looking at GIMM's video 4, which presents 16 parallel simulations under identical conditions. In each case a perfect X-shaped structure forms. In addition, the mechanism is robust against substantial changes in the various model parameters but breaks down in the absence of topo II enzymes or of excluded volume interactions.

GIMM provided the first demonstration that DNA copies can be segregated using simple local ingredients. It is worthwhile to mention (and GIMM give proper credit) that the whole idea goes back to Kim Nasmyth, a yeast geneticist. In 2001 he wrote [10]: "To those aware of the difficulties of disentangling ropes, the apparent ease with which eukaryotic cells separate their chromatids during mitosis is nothing short of miraculous." And further on: "One possibility is that condensin associates with the bases of small loops or coils of chromatin and enlarges these loops or coils in a processive manner, which ensures that all chromatin within the loop or coil must have been cleanly segregated from all other sequences in the genome." It seems that it took 14 years [8] before physicists became aware of his idea.

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