Genome Organization as a Source of Chromosome Instability in Cancer

Each human cell has two meters of DNA packed and folded into loops inside of a small nucleus of 10 micrometers of diameter. This extreme compression creates tension in the DNA that is dissipated by an enzyme, Topoisomerase 2 (TOP2). TOP2 constantly cuts and reseal the DNA, relaxing torsions and knots as the cell folds and organizes its genome. Sometimes, TOP2 fails resealing its own breaks and leads to DNA damage, genetic aberrations and cancer. In addition, many effective cancer chemotherapeutic drugs act inhibiting the resealing of TOP2 breaks, but frequently patients treated with these drugs develop secondary cancers years after the treatment. I found that TOP2 breaks where DNA folds to form loops, making these regions vulnerable to genetic aberrations that lead to secondary cancers. The ultimate goal of my research is to understand how DNA organization and folding inside the nucleus impacts the genetic aberrations that drive cancer.

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DNA Organization and Topoisomerase 2

In every human cell DNA is wrapped around proteins called histones to form chromatin. This long complex of DNA and proteins is packed and organized inside of the cell nucleus in small loops. The position and organization of the chromatin within the nucleus is highly regulated and it is important for the correct expression of genes and cell replication and division. The units of organization are chromatin loops, they bring together distant regions in the DNA allowing the interaction between regulatory sequences within the loop. Chromatin loops are formed when ring-shaped protein complexes called cohesin translocate along the chromatin until they encounter a pair of CTCF (CCCTC-binding factor) molecules bound to DNA that stop cohesin. This generates a chromatin loop and the base of the loop, referred as loop anchor, is defined by the binding of CTCF and cohesin. Chromosomes are very long molecules and constant folding and dissociation of loops generates torsions in the chromatin in form of entanglements and knots that could not only block the formation of new loops, but also impair cellular processes such as gene expression, replication and cell division. It would be similar than a pair of headphones in a pants pocket, that keep tying themselves in knots (Fig. 1). Topoisomerase 2 (TOP2) solves this problem in the cell. TOP2 relieves torsions in the DNA by breaking both strands of the DNA (DNA double strand break, DSB) and passing other double DNA helix through the break. As part of its normal catalytic cycle, TOP2 religates the ends of the DSBs and dissociates from the DNA without causing any damage, but TOP2 poisons, such as many chemotherapy drugs, inhibit the ligation step, trapping TOP2 in its cleavage state. These lesions lead to genome instability and oncogenic chromosomal translocations responsible of secondary leukemias following chemotherapy. Also, TOP2 has been implicated in infant leukemia and prostate cancer.

I have developed a methodology called END-seq that allows to locate and quantify DSBs in the genome (Canela A. et al. Mol Cell. 2016). I applied it to map the location of TOP2 breaks in the genome and I found that TOP2 acts at loop anchors, where chromatin fibers folds, and these locations coincide with the breakpoints of chromosomal translocations of infant and secondary leukemias and prostate cancer. This suggests that TOP2 relieves torsions during chromatin organization

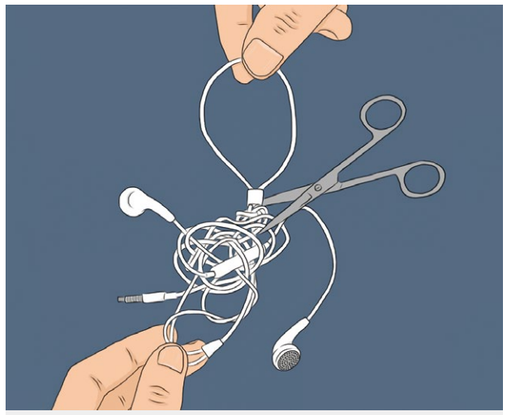

Figure 1

Headphones reflect the 'knotting' problem during genome organization. DNA is channeled through a cohesin complex, represented as a two-way slider. Entangled DNA are converted to tighter knots as is continuously fed through the cohesin complex. The entanglement is resolved by TOP2 acting as enzymatic scissors.
Credit: Ernesto Llamas, Sketching Science

at these locations, making them fragile and vulnerable to chromosomal instability (Fig. 2) (Canela A. et al. Cell. 2017).

Objectives

I am interested in the role of TOP2 organizing the long fibers of chromatin into loops. How TOP2 is recruited and how its activity is regulated? How cells keep their DNA free of entanglements and knots? My hypothesis is that TOP2 works in tight interaction with cohesin and the torsions that are produced during the movement of cohesin and loop formation are rapidly solved by TOP2, acting like a comb. On the other hand, the same breaks that TOP2 produces to relax DNA torsions, sometimes leads to genome instability and cancer. Although TOP2 acts in many locations, only few sites lead to chromosomal rearrangements that produce cancer. I would like to know why these locations are more fragile and lead to cancer and study the basis of their fragility. My hypothesis is that trapped TOP2 in the DNA acts as a road-block for processes that unwind DNA like transcription and replication and this contributes to the generation of genome instability in these locations.

Significance

Recurrent oncogenic translocations are common cancer drivers in hematological malignancies and solid tumors, these translocations locate in regions prone to break. I propose that folding the chromatin inside of the nucleus is a source of fragility by the action of TOP2. Understanding the role of TOP2 in genome organization and chromosomal translocations can be used to avoid DNA breaks and chromosomal translocations and leukemia, helping to make chemotherapy regimens safer by avoiding the development of secondary cancers.

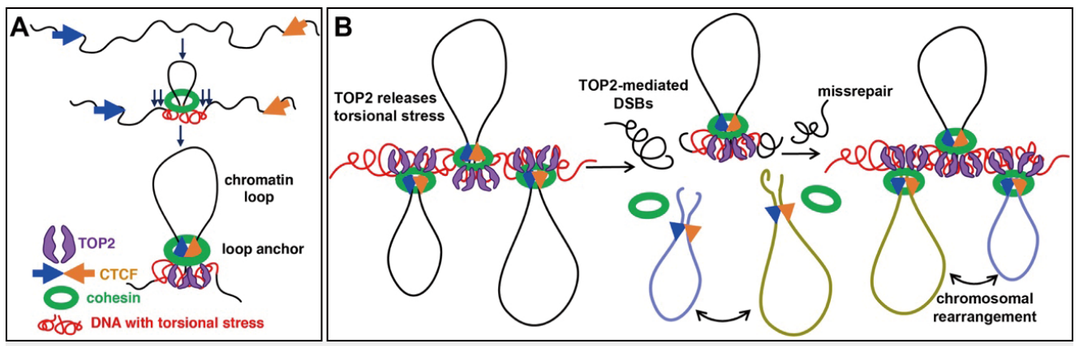

Figure 2

A. TOP2 releases torsional stress at loop anchors. Cohesin is loaded and translocates along the chromatin until it encounters two CTCFs that stop its further progression forming a loop. Torsions generated by cohesin accumulates at the loop anchor and it is released by TOP2.
B. TOP2 activity at loop anchors can lead to permanent breaks that are a source of oncogenic translocations.

References

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- Canela A, Maman Y, Jung S, Wong N, Callen E, Day A, Kieffer-Kwon KR, Pekowska A, Zhang H, Rao SSP, Huang SC, Mckinnon PJ, Aplan PD, Pommier Y, Aiden EL, Casellas R, Nussenzweig A. Genome Organization Drives Chromosome Fragility. *Cell* 170(3) 507-521. Jul 2017