Human centromeric DNA is able to form quadruplex helices

The classical view of a chromosome that most of people have in mind is a cross-shaped figure, something like the letter X. Chromosomes look indeed like that when the cell is ready to divide and give rise to two daughter cells, during the stage of the cell cycle known as metaphase. At this stage, the X-shaped chromosome contains two identical copies of the DNA, which must be distributed between the daughter cells in an equitable and precise way. To do this properly, the cell employs a complex machinery called kinetochore. The kinetochore acts like the hand of a robotic arm, recognizing and joining to a specific region of the chromosome, the centromere. Then, the microtubules, interact with the kinetochore and pull from opposite sides to separate the two chromosomes.

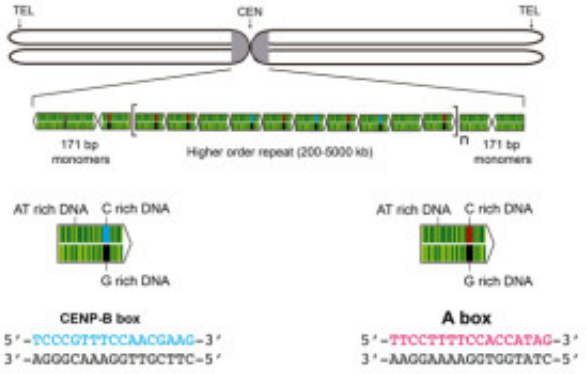

Fig. 1. Structure of the centromeric alpha-satellite DNA showing the position of the A and B boxes. The B box is usually called the CENP-B box because it is the binding site of the centromeric protein CENP-B.

The centromere is easily recognizable in our schematic picture of metaphasic chromosomes: it is the center of the X. When a metaphasic chromosome is observed under the microscope, the centromeres are clearly identified as a constriction between the four arms of the duplicated chromosome. However, everything is much more mysterious when one delve into the structure and composition of the centromere. In fact, the exact sequence of nucleotides that form the centromere has not been elucidated yet. Centromeric DNA is highly repetitive and this is, in fact, what makes its sequencing so difficult. In humans, centromeric DNA is composed by tandem repeats of a 171 base pairs sequence (called Alpha-satellite). Most of those 171 base pairs are formed by adenine and thymine residues. However, there is a small region comprising 17 base pairs of each repeat, where the base pairs are formed mostly by guanine and cytosine nucleotides and that can present two slightly different sequences named A-box and B-Box (Fig. 1). It is known that the C-rich strand of the B-box is able to fold in vitro into a non-canonical structure called i-motif. This peculiar

structure is a four stranded DNA formed by two parallel helices that interact with each other through the formation of intercalated protonated cytosine:cytosine base pairs (Fig. 2). Although this structure was determined long ago, the discovery that the B-box is absent in the Y chromosome, and therefore that it is not common to all the human chromosomes, discouraged further investigation on the role of the i-motif in the centromeric function.

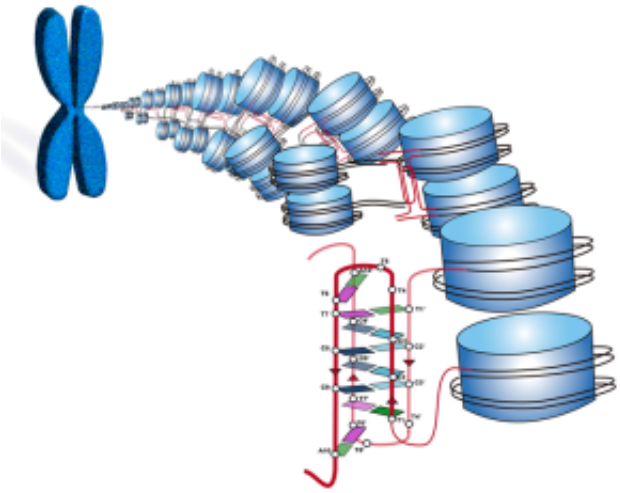

Fig. 2. Artistic view of nucleosome organization at the centromere. The A- or B- boxes of the alpha-satellite DNA (in red) would maintain the structural organization of the centromere by forming dimeric i-motif structures

We now know that the A-box is present in all the human chromosomes, including the Y chromosome. This prompted us to take up the structural study of centromeric sequences. In this paper, we determine the structure of the cytosine-rich strand of the human A-box, and we show that it folds into an i-motif (Fig. 2). Moreover, in a related study, we have found that the C-rich strands of centromeric repeats in Droshophila melanogaster also adopt i-motif structures. The observation that representative centromeric sequences in evolutionary distant species are able to adopt the same non-canonical structure reinforces the hypothesis that this DNA motif may play a role in the structural configuration of the centromere. In the cases studied, whereas the structures are not identical, they all belong to the same family of non-canonical structures. In all cases, the structures are dimers, resulting from the self-recognition of two loops formed by the C-rich strands. Such similarities led us to propose a model in which centromeric i-motifs could serve to connect distant DNA regions (Fig. 2). Interestingly, the A-box and B-box appear in DNA regions located between consecutive nucleosomes in locations that are accessible to form this kind of structural motifs. We postulate that a special packing of the DNA in the centromere could endow it with some unique features, such as an extra resistance against the pulling forces felt by this region of the chromosome during cell division.

All this work has been performed in vitro. Of course, in vivo experiments are necessary to really probe or definitively reject this hypothesis. The challenge now is how to detect i-motif structures in vivo. This is not an easy task, but we are developing new techniques to meet this goal.

Publication

Centromeric Alpha-Satellite DNA Adopts Dimeric i-Motif Structures Capped by AT Hoogsteen Base Pairs.
Garavís M, Escaja N, Gabelica V, Villasante A, González C.
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