DNA cruciform structure

The formation of cruciforms is strongly dependent on base sequence and requires perfect or imperfect inverted repeats of 6 or more nucleotides in the DNA sequence. Cruciform structures consist of a branch point, a stem and a loop, where the size of the loop is dependent on the length of the gap between inverted repeats (Fig. 1). The AT-rich gap sequences increase the probability of cruciform formation. After its formation, the distant DNA fragments approach. There are two distinct classes of cruciforms. One class of cruciforms, denoted as unfolded, have a square planar conformation characterized by a 4-fold symmetry in which adjacent arms are nearly perpendicular to one another. The second class comprises a folded (or stacked) conformation where the adjacent arms form an acute angle with the main DNA strands (Fig. 1, 2).

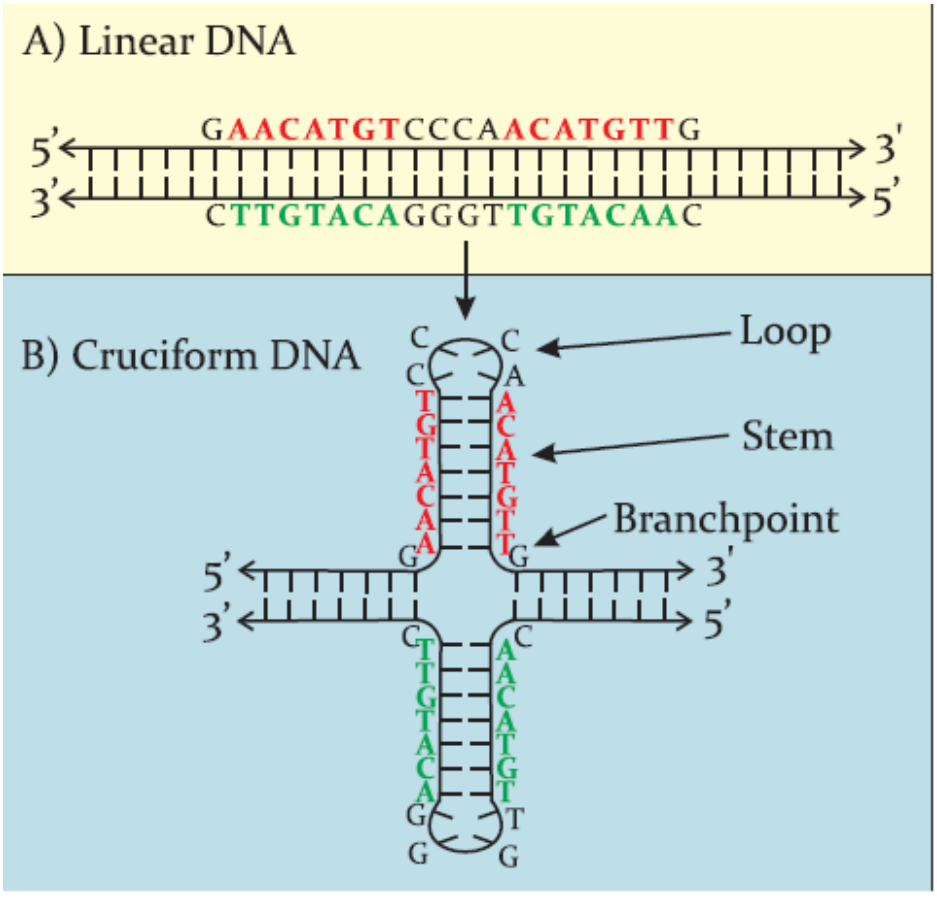

Fig.1 Linear DNA fragment (A) and corresponding cruciform (B).

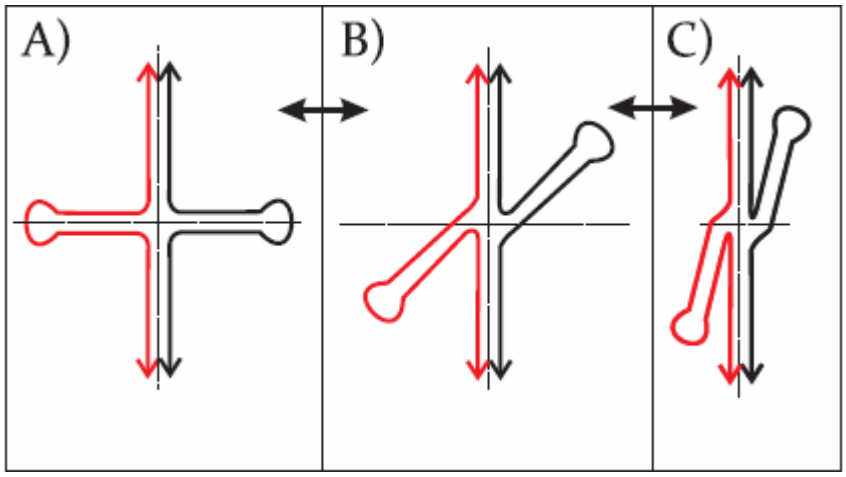

Fig. 2 Different conformations of cruciform DNA.

Cruciform structures are fundamentally important for a wide range of biological processes, including replication, regulation of gene expression, nucleosome structure and recombination. Cruciform structures are targets for many structurall and regulatory proteins, such as histones H1 and H5, topoisomerase IIβ, HMG proteins, HU, p53, and others. A number of DNA-binding proteins, such as the HMGB-box family members, Rad54, BRCA1 protein, as well as PARP-1 polymerase, possess weak sequence specific DNA binding yet bind preferentially to cruciform structures [1]. The mutations and epigenetic modifications that alter the propensity for cruciform formation can have drastic consequences for cellular processes. Thus, it is unsurprising that the dysregulation of cruciform binding proteins is often associated with the pathological processes and diseases.

Primer design.

Primers to the defined fragment of agn^{ts3} sequence of *D. melanogaster limk1* gene (Neurogenetics lab., GenBank number: JX987487) were designed using NCBI PrimerBlast computer resource (http://www.ncbi.nlm.nih.gov/tools/primer-blast). The primer length and and the percentage of GC nucleotides were selected so that the maximal difference of melting temperatures for both primers was 3^{O}C. We used the default selection parameters to provide the allowable value of primers self-complementarity. The designed primers were checked for the absence of non-specific binding sites.

The primer parameters are shown in Table 1.

Strain	Primer direction	Sequence (5'->3')	Length	Tm	GC%	Product length

agn^{ts3}	Forward	AAAGTTCGAATTGCTGTCCC	20	50.89	45.00	119
	Reverse	TGATAGGAAGGTGCTGCATT	20	51.00	45.00	

Table 1. The primer properties: sequence, length, the calculated temperature of melting (Tm), % GC, and product length.

The optimal annealing temperature (52^{O}C) was chosen empirically by polymerase chain reaction (PCR) with *agn*^{ts3} genomic DNA, with 2 °C step variations of annealing temperature around the predicted Tm.

	*The nucleotide sequence of DNA fragment (*5'→3')
***agn*^{ts3}**	AAA GTT CGA ATT GCT GTC CCA GTA TAT CAT AAA AAA ATC ACG GAA TTC GAT TCT TTA TTA TTT ATT ATT ATA GAG TTT TGA ATG CAA TTG GTT TTG GAA AAT GCA GCA CCT TCC TAT CA

Table 1. The nucleotide sequence of agn^{ts3} DNA fragment. The 28 bp A/T rich insertion is shown in red.

DNA isolation

DNA was prepared from *Drosophila* tissues by sodium acetate – ethanol precipitation **(Molecular Cloning: A Laboratory Manual (Third Edition, 2001) by Joseph Sambrook, Peter MacCallum Cancer Institute, Melbourne, Australia; David Russell, University of Texas Southwestern Medical Center, Dallas)** with some modifications. For this 5 adult females were frozen and homogenized in 200 µl buffer for DNA extraction containing 0.5% diethyl pyrocarbonate.

Polymerase chain reaction (PCR).

PCR was performed using the recombinant Taq-polymerase (Beagle, Russia). Touchdown PCR was carried out in thermal cycler AB Veriti (Applied Biosystems, USA) using the following program:

1. Initial denaturation: 94°C (3 min)
2. 2 PCR cycles

1) Denaturation: 94°C, 30 s;
2) Primer annealing: 56°C, 30 s;
3) Extension: 72°C 1 min

3. 2 PCR cycles
   1) Denaturation: 94°C, 30 s;
   2) Primer annealing: 54°C, 30 s;
   3) Extension: 72°C 1 min
4. 30 PCR cycles
   1) Denaturation: 94°C, 30 s;
   2) Primer annealing: 52°C, 30 s;
   3) Extension: 72°C 1 min
5. Final extension 72°C 5 min

The gradual decrease of annealing temperature in touchdown PCR diminish the non-specific primer binding at initial stage, providing the higher quality of product.

PCR products were separated by electrophoresis in 2% agarose (30 min, 120 V, 50 мА, horizontal electrophoresis system Tetra Cell BioRad, Italy). Electrophoresis was carried out for). DNA bands were scaned using Gel Imager system (Lytech, Russia).

PCR purification and DNA preparation for spectrometry

PCR purification was performed using ethanol precipitation. 300 µl of PCR product were mixed with 26 µl $MgCl_2$ (final concentration 10 mM). Probe was mixed with 1165 µl of 96% ethanol, incubated for 30 min at room temperature, and centrifuged 1 hr at $+4^{O}$C (12000 g). Supernatant was discarded, 1 ml of 75% ethanol was added to pellet. After 10 min centrifugation the pellet was dried and diluted in 150 µl of 0.015 M Na-phosphate buffer (pH 7.45). DNA concentration (420-560 µg/ml for different aliquots) was measured using Eppendorf BioPhotometer spectrophotometer (Germany) in Eppendorf UVette cuvette (Germany).