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S-6. Guanine-quadruplex-enriched sequences in the tight DNA-protein complexes of barley seedlings

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Recent achievements in studies of the three-dimensional interactions between genes caused significant re-evaluation of the nucleus structure in general. The concept of chromatin domains attached to the nuclear matrix is being revisited, nucleus is described as a set of topologically associating domains. The significance of the tightly bound to DNA proteins (TBP), a protein group that remains attached to DNA after its deproteinisation should be also revisited, as existence of these interactions is in good agreement with the topologically associating domains concept. Aim of the work was to characterize the DNA component of the TBP isolated from barley seedlings. The tight DNA-protein complexes from the first leaves, coleoptiles, and roots of barley seedlings were isolated by means of chromatography on nitrocellulose or exhaustive digestion of DNA with DNase I, cloned in plasmids and sequenced. The BLAST search was performed using sequence databases at NCBI and Ensembl Plants databases. Comparison to MAR/SAR sequences was performed using http://smartdb.bioinf.med.uni-goettingen.de/cgi-bin/SMARtDB/smar.cgi database. The possible DNA curvatures were predicted using DNA Curvature Analysis. Prediction of G qudruplexes (GQ) was performed with aid of R-studion library pqsfinder. CD spectra were recorded on a Chirascan CS/3D spectrometer. About 600 inserts were sequenced. Most DNA fragments associated with TBP were GC-rich. Both fractionation procedures yielded a high proportion of CT-motif sequences presented predominantly by the 16-bp CC(TCTCCC)2TC fragment. BLAST analysis revealed alignment with different barley repeats, however, some clones aligned with both nuclear and chloroplast structural genes. Alignments with MAR/SAR and DNA curvatures were almost absent. Numerous potential quadruplex-forming sites in the TBP-bound sequences were revealed. A set of oligonucleotides containing sites of possible GQs were designed and ordered. Circular dichroism spectroscopy revealed profound changes in spectra when oligonucleotides were incubated with 100 mM KCl. There was either increase of positive band in the area of 260 nm or formation of a positive band at 290 nm. In the former case changes are typical for parallel G-qudruplexes, in the latter – for 3+1 structures. Thus, the DNA component of the tight DNA-protein complexes of barley seedlings are GC-rich and contain numerous guanine quandruplex forming sites, the CC(TCTCCC)2TC fragment being predominant.