GENERAL FEATURES OF AUTONOMOUSLY REPLICATING SEQUENCE DARC146

V. V. Lunyak, N. A. Timchenko, L. A. Noskin

DARC146 is the minimal part (146b.p.) of a genomic sequence, which is associated with replicative complex in non-regenerating (G_0)rat liver. The DARC146 fragment can support autonomous plassmid replication in mammalian cells. We have shown that DARC146 sequence is presented in rat genome in at least three copies as minimum, and that arrangements of 5'-flanking regions adjacent to DARC146 are different. Using GMSA and footprint analysis we have shown that AT-rich region of DARC146 contains several binding sites for nuclear proteins: (i) for nuclear factor OCT-1 involved in transcription and replication; (ii) for two different unidentified nuclear factors (binding sites TCTCTTA and CACTTAG). One of features of DARC146 sequence is its ability to form bent DNA in the absence of proteins. Moreover, we identified inverted repeat, which located in area overlapping this region of curved DNA. By means of cross-linking with 3Me-psoralen, we observed that inverted

repeat may exists as a cruciform structure both in vitro and in vivo. It is well known that important regulatory mechanisms may be triggerred by changes in DNA topology. Thus, such structure transition of bent DNA into cruciform could be an event that prevent nucleosome reconstruction. This DNA locus without nucleosomes, apparently, have implications for regulation of initiation of DNA synthesis. Also conversion of bent DNA into cruciform structure essentially changes pattern of DNA-protein interactions in the region studied.