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CHARACTERIZATION OF DNA PATTERN IN THE SITE OF PERMANENT ATTACHMENT TO THE NUCLEAR MATRIX LOCATED IN THE VICINITY OF REPLICATION ORIGIN

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SUMMARY: The permanent sites of DNA attachment to the nuclear matrix in the domain of chicken α-globin genes originally found in erythrocyte nuclei have also been shown to exist in sperm and cultured fibroblast cells. A primary structure of a 1.7 kb fragment located in 5'-upstream region of chicken α-globin gene domain and containing both replication origin and permanent nuclear matrix attachment site has been determined. It was found to possess homologies with papovaviral replication origins and contain short internal repeats and GC-rich motifs. ©1990 Academic Press, Inc.

The data on the existence of different types of association of DNA with the nuclear matrix have been obtained recently (1,2,3). In functionally active nuclei, most of specific interactions of DNA with the nuclear matrix elements are connected with transcription (1,4). We demonstrated that these interactions were lost in the course of terminal differentiation of chicken erythroid cells which led to inactivation of nuclei of mature erythrocytes (5). At the same time a specific group of DNA associations with nuclear matrix remains in inactive nuclei of mature erythrocytes (5). We designated them as permanent attachment sites. Further studies demonstrated that the permanent attachment sites were located near replication origins on the DNA chain (6).

All these results were obtained on one type of cells, namely chicken erythroid cells. One may ask how ubiquitous are the permanent attachment sites found in erythrocyte cell. The data obtained in the present study demonstrated the presence of similar attachment sites in cells of other lineages. This fact along with the observation that the permanent attachment sites are located near replication origins on the DNA chain (6) made it interesting to study nucleotide sequences involved in formation of permanent sites of DNA attachment to the nuclear matrix.

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In the present work we determined the nucleotide sequence of 1.7 kb DNA fragment from 5'-terminal region of chicken α-globin gene domain which included both replication origin and the permanent site of DNA attachment to the nuclear matrix. Analysis of this sequence allows to reveal several expected features of replication origins

MATERIALS AND METHODS

Cell culture. The primary culture of chicken fibroblasts was obtained using trypsinization. The cells were grown in glass flasks in DMEM with 10% calf serum. Sperm was taken from mature roosters.

Recombinant DNA. A recombinant clone CαG6, and the deletion mutant of clone CαG5 obtained and characterized by Engel and Dodson (7) were used in the present work. The deletion mutant was isolated and characterized in the laboratory of Prof. K. Scherrer.

Isolation of nuclear matrices and DNA attached to the nuclear matrix was carried out using high salt extraction of nuclease-treated nuclei as described (6).

DNA electrophoresis in polyacrylamide and agarose gels, DNA transfer onto nylon filters, ^{32}P labeling of DNA, and hybridization experiments were carried out as described elsewhere (8).

The primary structure of DNA was determined using the Maxam-Gilbert method modified by Chuvpilo (9).

Analysis of nucleotide sequences was carried out using Microgenie (10) computer program.

RESULTS

Estimation of preferential position of DNA attachment to the nuclear matrix within the domain of chicken alpha-globin genes in the nuclei of cultured fibroblasts and mature sperm cells.

The strategy of mapping experiments was similar to that used in our previous work (5,6). Briefly, the restriction fragments of recombinant clones CαG5δ and CαG6 covering different areas of alpha-globin gene domain were separated in agarose gel electrophoresis and transferred onto nylon filters. Nick-translated samples of nuclear matrix-associated DNA (nmDNA) from either cultured fibroblast or mature sperm nuclei were used as hybridization probes. In control experiments, nick-translated total chicken DNA was hybridized to similar filters. In agreement with our previous observations (5,6), total DNA probe hybridized only with those fragments of recombinant DNA that contained repetitive sequences (Fig. 1). In contrast, nmDNA probes from both cultured fibroblast and sperm nuclei also hybridized with a distinct subset of recombinant DNA fragments containing only unique sequences. The strongest hybridization signal was observed with the fragment representing the sequences located ca.3 kb upstream to the π-gene. A weaker hybridization with the fibroblast nmDNA probe was also detected in the fragment including alpha-A gene and upstream sequences. The same areas of chicken alpha-globin gene domain were previously found to be overrepresented in the nmDNA fraction from

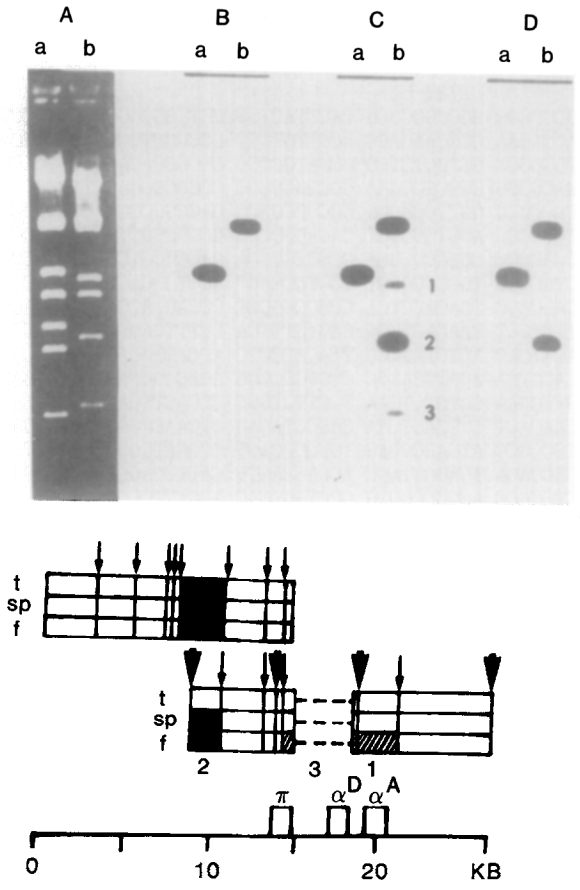

Figure 1. Southern blot hybridization of nmDNA from chicken sperm and cultured fibroblast cells with the cloned fragments of α-globin gene domain.
A - Electrophoretic pattern of restriction fragments of recombinant DNAs used for hybridization with nmDNA. (a), HindIII-treated CαG6δ DNA; (b), HindIII/EcoRI-treated CαG5δ DNA.
B - Hybridization with the total chicken DNA.
C - Hybridization with nmDNA from chicken cultured fibroblast cells.
D - Hybridization with nmDNA from chicken sperm cells.

The maps of recombinant DNA clones are presented below the autoradiograms. The positions of HindIII and EcoRI sites are indicated by small and large arrows, respectively. A dotted line between the two fragments of CαG5δ indicates a deletion.
The black and shaded rectangles indicate fragments which hybridized with total chicken DNA (t), nmDNA from sperm (sp), and cultured fibroblast cells (f) The numerals indicate the restriction fragments of CαG5δ overrepresented in nmDNA fraction as compared to total DNA.

erythrocyte nuclei (5), suggesting that the permanent sites of DNA attachment to nuclear matrix originally found in inactive erythrocyte nuclei actually exist in cells of other lineages.

Analysis of DNA sequences involved in the organization of the permanent site of DNA attachment to the nuclear matrix and replication origin in the 5'-upstream region of chicken alpha-globin gene domain

The 1.7 kb fragment located ca. 3 kb upstream to the π-gene was found to include both replication origin (6) and the permanent site of DNA attachment

Table 1. A nucleotide sequence of the α5HR fragment of chicken α-globin gene domain

α5HR 1737 bp

GCGGCACGGG GCGGCCCCGG GCCCGGCGCG CACTTACTGG CCTTGGCGGC GGGGTGCTCG
GCGCCGCGCT GGAAGGGGAA GCGGAAGAGC AGCTTGTTGC CGCGGCTGCC CGAGCTCACA
AGGATAACGC TGATGGGGCT GGTGCTCTCG CCCATGCCGC CGCGCCACAG CGAGCACCGG
GCGGGCAACG ACGGACGCGG CTCCGCGGAA GGCGGCCCGG CCCGCGCGAC TTCCGCTTCC
GCGCCTCCGC CGCCGCCGCC GGTTCCCCCG GGCCGCGGCC GAGCGGCGGG GCGGAGCTGC
GGGCACAGCG CTCCCCGGGC AGGTCGCGCT CAGAGGCCGG GCCGCCGCTT CAGCGCCGTG
CCCTCAGTGC GGCCCAGCGC CGTGCCCGCA GCGCTGCCCA CACGCCCTCG GGGTGCCCCA
CGGCTGCTGC TTGCTCCCGG TGCCCGCCGT TCCTCCCAGC ACCTCGCAGT GCAGCCGTGC
CTGAAGTGCA GCCCAGCACC TCACACCTCA GCCCCGGGCT CCCAGTACGA CCAGCAGGTC
ACGTTGGAGT CTCTTGTCCT CAAGACTGCG CAGTGTCTCA CCTTTGAGCC TTGTGCCCCC
CATTCAGCCC AGCACATCAC ACTGTAGCCC TTACACCCTC ACCACAGCAC AGCACCTCAC
GTTCAGGCCC CAGCACGTCA AGATGGAGCC CTGTGCCCCC AGACAGCCAG CATGGAACCA
TCAAATCCTT AGAGTTGGAA GATGTCTGAA TCCTTGTGCC CCCAGTTCAG CCCGGCACCT
CTCACACCCC ACTCAACACT CTTCAGCCAA GAGCCTACAG CTCAACCCAG CACCTCACGC
CACCCAGCAG CACTCCCGCC ATCAGCCCAG TGCCCCCAGT CCGGATCGGT ACCTCTCATG
CCCATGCACA GTGCACCAGA TCAGCCTAGC ACCACTAGTT CATTCCAGCA CCTCACGTGC
CCACAGCCAA CCACTCCAGC ACCCCCGGTG CCCTAGTCAC ACCTCTCCGC TGCCTCAAGG
TTCATTCCCA CCTCTTCCCA CATCCCCTCA CACCCCCTCA TTATTTTCAT GTCTCGCAAT
CTCCTTTGGT CACTTGGAGT CATTCAGTTA TGACAACTCC AGAACTAGAA GCTGCTGGCC
AGCAGCAAGT GCCACAAACT GTGTTCCCCC GGCAGCTCTT CTGGCTCATT TGTCTTATTG
TGTGTCCAGC TGAGATCAGA AAGCTATCGG CAATTATGTC AGAGGATGGC CCAGTTTTTC
ACATAGATTT GTCTGTATTT GATAGCAATA TTTAGTATTT GGTGCTCCGA GTATCCCCAC
TCTGGATTTT TCTCTGCAAG ATTCTTCCCT TGGACTTCAG GCAGAGAAGG GGACTGAAAG
GGAGATGAGC ACCCGCAGTG AGGGCTTAAT CTGCACGGCC ATTCTCTGCA AGGCAGGTGA
TAACAACTGA AGCAAGAGAA GCTGTCATTG AGGGGAGAGA GTTGTTGGTG AGCGATTAAA
GAGCAGTCAC ATTATCACAG CAGAGCATTC ATCGTGGCCC AGTGCTGGGG AGCTACGTTA
GAATTGCCCA GTGTGTCTGC TTCCCAGCAT AACTATGCAT TCTTCAATTA AAAAACTGCA
GGCATGTTTG CCATTTCCAG CTCTCGGAGA TGAGTTAAAG CAAAGCTCTG GAAACCTGCA
AGCTCTCTGA GTGCTAGTAG AATGAAATGA AAGAATAAAG CCAGATATAG ATTCTGC

to the nuclear matrix. In order to gain knowledge on the sequences involved in organization of replication origins and permanent attachment sites we have determined the primary DNA structure of this fragment. The complete nucleotide sequence of the fragment is presented in Table 1.

Computer analysis revealed that the fragment (further referred to as α5HR) had multiple stop-codons in all reading frames. The distribution of AT-pairs in the α5HR is shown in Fig. 2A. It is quite nonrandom: the first part of the insertion (0-500 bp) is very GC-rich (21% AT-pairs), the next part (501-1300 bp) is moderately GC-rich (42% AT-pairs), and the last part (1300-1737 bp) is moderately AT-rich (55% AT-pairs). The last domain has several areas with AT-pair content exceeding 80% (e.g. at positions 1695-1719, 1588-1614). The moderately GC-rich domain of α5HR was found to contain multiple imperfect internal repeats (Fig.2B)

The most important task of the analysis of the nucleotide sequence of α5HR was to find certain patterns which could be involved in organization of putative replication origin. We have found a region with pronounced multiple homology with replication origin of SV 40 and other papovaviruses in positions 205-222, 247-266, 288-314, and 331-354. A large (ca. 200 bp) single-stranded DNA stretch which contains the region with multiple homologies to SV 40 ori can be folded into a hairpin-like secondary structure shown in Fig. 3B.

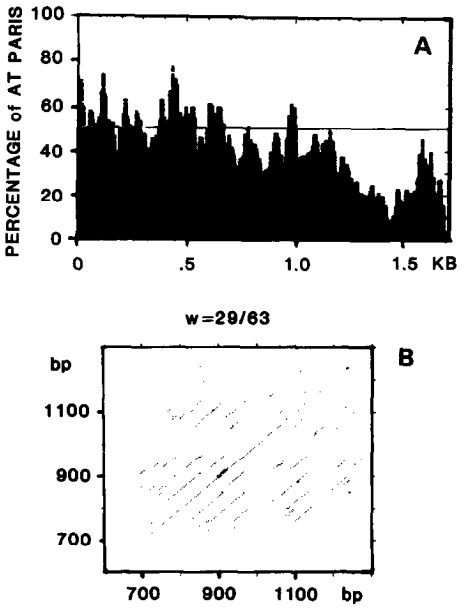

Figure 2. A, distribution of AT-pairs in the α5HR insertion; B, dot matrix homology map of the second domain of α5HR. Frame width is 29/63

The last two domains of α5HR contain a rather long GC-rich inverted repeat GCTGCTGGCCAGCAGC in positions 1130-1146 and binding sites for several sequence specific DNA-binding proteins (Table 2). Indeed, several sequence specific DNA-binding proteins were footprinted in different subfragments of α5HR (Razin et al, submitted).

DISCUSSION

The permanent sites of DNA attachment to the nuclear matrix were originally found in the nuclei of erythroid cells (5). The present study demonstrated that the permanent attachment sites exist in cells of different lineages including sperm cells. Our interest in DNA sequences located in vicinity of the permanent attachment sites is mostly determined by the previous observation that replication origins coincide with or are located in the vicinity of the permanent attachment sites (6). Hence, the study of DNA se-

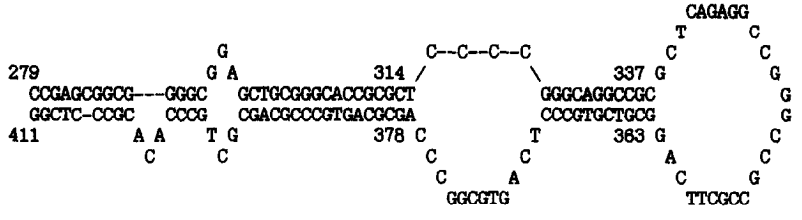

Figure 3. A putative hairpin structure in the GC-rich domain of α5HR.

Table 2. Computer-derived protein-binding sites in the α5HR fragment

Protein factor	Number of matches	Positions
ADR-1	5	1004 1080 1381 1474 1646
ANTP	1	1505
AP-1	2	1010 1181
AP-2	4	15 266 313 512
ATF	3	1086 1226 1600
C/EBP	2	638 1034
CP-1	1	958
CTF	2	1150 1629
DTF-1	1	93
E2	1	252
E4F2	1	1023
EBP20	2	638 1034
GT-2 motif (SV40)	1	940
SP-1 motif	3	9 179 289
Sph motif (SV40)	2	1591 1596
T-antigen binding site	1	333

quences originating from these attachment sites might allow to identify the structures involved in organization of replication origin. We have sequenced the 1.7 kb fragment of chicken alpha-globin gene domain containing both the replication origin and the site of permanent DNA attachment to the nuclear matrix. This DNA fragment has a distinct region with the anticipated features of replication origin. It contains recognition sequences for several proteins which are supposed to be involved in regulation of initiation of replication. The precise position of replication origin within the sequenced DNA fragment is not known yet. One may speculate that it is located in the GC-rich domain

of α5HR because it has very strong homology with SV 40 replication origin and replication origins of other papovaviruses.

It is interesting that a transcriptional enhancer is located near the region of homology to viral ori (Razin et al, J.Mol. Biol., in press). It has been previously suggested that transcriptional enhancers might be involved in regulation of both transcription and replication (11).

The precise position of a matrix attachment site is also unknown. However, several observations indicate that it is located somewhere in the moderately GC-rich domain of α5HR. Recently we have sequenced several short fragments of erythrocyte nmDNA (12). A large share of the sequenced DNA fragments was found to contain multiple imperfect repeats (similar to those observed in the moderately GC-rich domain of α5HR) and possess several strict homologies to this domain of α5HR (13).The existence of the internal repeats in the nmDNA fragments isolated from cells arrested in G1 phase was previously reported (14). Besides, it has been reported that the nuclear matrix attachment sites coincided with the positions of enhancer elements on the DNA chain (15). Further experiments are required to map exact positions of the replication origin and the nuclear matrix attachment site. The experiments of this type are being carried out in our laboratory.

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