

**THE NUCLEAR MATRIX AND CHROMOSOMAL DNA LOOPS: IS  
THEIR ANY CORRELATION BETWEEN PARTITIONING OF THE  
GENOME INTO LOOPS AND FUNCTIONAL DOMAINS?**

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**Abstract:** In this paper we are presenting a critical analysis of the results of experiments aimed to elucidate the principles of the eukaryotic genome structural-functional organization. Although the DNA loops attached to the nuclear matrix (chromosomal scaffold) are frequently considered as independent functional domains, this supposition lack experimental proofs as far as the transcriptional domains are concerned. On the contrary, many observations indicate that organization of chromosomal DNA into loops is directly related to the replicon structure of the genome.

**Key Words:** Nuclear Matrix, Chromosomal DNA Loops, MARs and SARs, Replication Origins, Genomic Domains.

**INTRODUCTION**

The discovery of the nuclear matrix [1] was more than demonstration that the nuclear shape and volume was supported by internal proteinous structure of some kind. The nuclear matrix became a keystone for the modern conception of structural and functional compartmentalization of the eukaryotic cell nucleus. This conception has presently gained a solid experimental support, but it was not easily accepted 25 years ago when scientists were more interested in possibility to reproduce *in vitro* (in a test tube) the DNA replication and transcription using purified enzymes and purified templates. Demonstration of the preferential association of the replicating DNA with the nuclear matrix [2] was, perhaps, the first important result which attracted attention of the specialists in biochemistry and molecular biology to the problem of spatial organization of biosynthetic processes directly involved in the functioning of

eukaryotic genome. Lots of exiting observations related to the above problem has been made since this time. As a matter of fact, a new picture of nuclear spatial compartmentalization has emerged. It is not our actual task to present a comprehensive review of all these findings (For systematic discussion of different aspects of the outlined problem see [3]). In this short article we shall review the experiments aimed to analyse the relationship between the spatial and functional organisation of eukaryotic genome. The researches to be discussed here started with the demonstration that eukaryotic genome is organized into large loops fixed at a high-salt resistant nuclear structure [4, 5] which was called differently (such as "nuclear ghost", "nuclear skeleton", "nucleoid core") but which was in fact identical to the nuclear matrix. The most interesting feature of these loops was, perhaps, their size estimated as 20 to 100 kb (Different approaches used for estimation of the size of chromosomal DNA loops have recently been reviewed [6]). At this size level one could look for a possible correlation between the structural organization of the genome and its partitioning into functional units. Recognition of this possibility have stimulated numerous studies of the specificity of DNA organization into loops. In our laboratory these researches were concentrated on one particular model, the domain of chicken  $\alpha$ -globin genes. Hence, we shall discuss first of all the results obtained with this model, although the review is certainly not restricted to the studies of the above domain. Our own studies using other systems as well as studies of other authors which contributed significantly to the demonstration of specificity of DNA organization into loops will be discussed as well.

### **DNA LOOPS ARE ATTACHED TO THE NUCLEAR MATRIX AT SPECIFIC REGIONS**

Since the discovery of chromosomal DNA loops the question has been discussed about a possible relationship between the organization of DNA into loops and the organization of the genome into functional units. This discussion was stimulated by demonstration that the transcriptional status of large genomic domains is regulated somehow at the level of DNA packaging in chromatin [7]. This suggests that there should be some structural units of chromatin within which the mode of DNA packaging may be changed without affecting the neighbouring regions. In order to check whether DNA loops constitute such structural units, it was necessary to find a way to map the loop borders within characterized genomic areas.

A general procedure for determining approximate positions of different genes within chromosomal DNA loops was developed by Cook and Brazell [8] who reasoned that the estimation of the relative portion of a DNA sequence under study in matrix-bound DNA fractions (operationally defined as "nuclear matrix DNA") of different sizes should show at what distance from the loop anchorage site the DNA sequence under study is located. Indeed, according to the simplest

model of DNA organization in more or less uniform loops any given DNA sequence must be found preferentially in nuclear matrix DNA as long as the average sizes of the nuclear matrix DNA fragments exceed more than twice the distance between this DNA sequence and the loop anchorage site.

Although looking quite simple, the experiment depends completely on the availability of a correct procedure for isolation of what is called "nuclear matrix DNA". Besides, the correct estimation of a distance between a sequence of interest and a loop anchorage site in the above protocol depends on the equal accessibility of the whole DNA loop to a nuclease used for the detachment of DNA from the nuclear matrix. Furthermore, the above nuclease should cut all DNA sequences with an equal probability. As large pieces of chromatin are not soluble in physiological ionic strength, the extraction with a concentrated salt solution was commonly used for disruption of chromatin and elution of cleaved-off DNA. This extraction also assured equal accessibility of DNA to nucleases because of histone removal. The second of the above conditions was usually disregarded, as sequence-specific restriction endonucleases were used for cleavage of distal parts of DNA loops. Consequently, the resolution of the approach was decreased so that it was possible only to discriminate DNA sequences located at or close to the matrix attachment sites from DNA sequences located somewhere in the loops. In experiments of the above type a dependence between the transcriptional status of a gene and its attachment to the nuclear matrix was observed. In other words, active genes were generally found in nuclear matrix DNA, while inactive ones - in cleaved-off DNA fractions (reviewed in [6]).

Obviously, these results were far from what was expected. Indeed, the relationship between the transcriptional status of a genomic region and positioning of this region close to the basement of a DNA loop could be hardly explained by the simple model of DNA organization into relatively uniform loops anchored to the nuclear matrix. On the other hand, the assumption that all specific DNA interactions with the nuclear matrix are transcription-related [9] also does not explain some experimental observations, for example a correlation between an average size of DNA loops and an average size of replicons in cells of different species [10]. To solve the problem, we have compared the pattern of the chicken  $\alpha$ -globin gene domain interaction with the nuclear matrix in transcriptionally active (erythroblasts) and completely repressed (mature erythrocyte or sperm cells) nuclei [11-13]. While the whole transcriptionally active area was bound to the nuclear matrix in erythroblast nuclei, in erythrocyte and sperm nuclei only two major attachment sites framing the domain from the upstream and the downstream were detected. The same DNA fragments were also attached to the nuclear matrix in cultured chicken fibroblasts [13]. Hence, at least within the domain of chicken  $\alpha$ -globin genes, it was possible to discriminate permanent (preserved in inactive nuclei) and transcription-dependent interactions of DNA with the nuclear matrix. The

generality of this observation was confirmed by comparative analysis of the sequence complexities of nuclear matrix DNA samples prepared from erythroblasts and mature erythrocytes [14]. These experiments demonstrated that in erythroblast nuclei the nuclear matrix DNA constituted a specific subset of unique sequences present in total DNA. This subset included all the sequences present in nuclear matrix DNA of erythrocyte nuclei which comprise about 30% of the unique DNA sequences present in erythroblast nuclear matrix DNA. Consequently, one may conclude that matrix attachment sites in general have specific positions in the genome. Otherwise all unique sequences present in total DNA would be found in the vicinity of matrix attachment sites (i. e. in nuclear matrix DNA). Similar considerations justify a conclusion that erythrocyte nuclear matrix DNA is a subfraction of erythroblast nuclear matrix DNA. Hence, the erythroblast nuclear matrix DNA is composed of two subfractions: DNA fragments permanently attached to nuclear matrix (i.e. erythrocyte nuclear matrix DNA) and DNA fragments interacting with the nuclear matrix in accordance with the functional processes.

The question arise, if it is possible to map the permanent sites of DNA attachment to the nuclear matrix in functionally active nuclei. We believe that now we can give a positive answer to this question. We have developed a new procedure for mapping the DNA loop anchorage regions (LARs) in large genomic areas by topoisomerase II-mediated DNA cleavage at sites of DNA loop anchorage at the nuclear matrix. The procedure is based on previous observations indicating that DNA-topoisomerase II is an integral part of the nuclear matrix and that it introduce double-stranded scissions into DNA in the course of relaxation reaction. In living cells the positions of preferential cleavage sites are determined by the chromatin structure. In particular, DNase I hypersensitive sites constitute preferential targets for DNA cleavage by soluble form of topoisomerase II [15]. We reasoned that the extraction of nuclei with a concentrated salt solution (which does not affect the nuclear matrix (chromosomal scaffold) integrity and does not release DNA loops from the nuclear matrix) must remove soluble topoisomerase II capable of interacting with DNA outside the matrix attachment regions. Hence, in high salt extracted nuclei only the DNA loop anchorage sites (i. e. the parts of the loops which are in contact with the nuclear matrix) should be accessible for cleavage with high salt insoluble topoisomerase II. Consequently, incubation of high salt extracted nuclei (nucleoids) with topoisomerase II-specific drugs should result in the excision of chromosomal DNA loops. On the basis of these considerations we have developed a procedure for excision of chromosomal DNA loops and mapping loop anchorage regions in characterized genomic areas [16, 17]. The topoisomerase II-mediated DNA loop excision protocol has been successfully used for mapping DNA loop anchorage sites in different genomic areas [17-19]. For the present discussion it is important to say that in the upstream area of the chicken domain of  $\alpha$ -globin genes this protocol has

permitted to map the DNA loop anchorage region exactly in the same place where the permanent site of DNA attachment to the nuclear matrix was mapped using the high salt extraction protocol applied to the non-active nuclei [16].

The discussion of different approaches of mapping the sites of DNA interaction with the nuclear matrix would be incomplete without reviewing of different reassembly approaches which have permitted to identify the so called Matrix Association Regions (MARs)[20] or scaffold attachment regions (SARs) [21]. Cockerill and Garrard have demonstrated that isolated nuclear matrices contain specific affinity sites that can detain a subset of sequences present in total DNA [20]. The number of these affinity sites was estimated as 50000 per nucleus. The DNA sequence elements detained selectively (in the presence of a non-specific competitor) by isolated nuclear matrices were called Matrix Associated Regions or MARs. Binding of these DNA sequences to the nuclear matrices was not tissue- or even species-specific. Recent evidence suggests that an essential feature of MARs is the ability to melt under relatively mild conditions. This ability is probably determined by the presence of (AT)<sub>n</sub> motifs and ATATTT motifs (for review and discussion see [3]).

In an independent, and actually preceding, study Laemmli and collaborators proposed to use for histone removal a weak ionic detergent, lithium diiodosalicylate (LIS) [21]. It was supposed that this extraction produced less artefacts than the commonly used at this time extraction with 2M NaCl or other concentrated salt solutions. However, it turned out that LIS extraction did not release all DNA from the nuclei only if nuclei had been stabilized either by Cu<sup>2+</sup> ions or by incubation at 37°C (for a review see [22]). Furthermore, it turned out that complexes of DNA with the nuclear remnants isolated by LIS-extraction are not stable to reextraction with the same solution. Finally, the matrix (scaffold)-bound DNA fragments (SARs) identified by LIS extraction protocol were identical to MARs mapped by *in vitro* binding approach [20]. Hence, it is most likely that using LIS extraction procedure one can identify DNA interactions with the nuclear matrix that originate as a result of *in vitro* reconstitution at the step of treatment of LIS-extracted nuclei with restriction enzymes [3, 6, 23]. Consequently, the SARs and MARs can be considered as the same genomic elements. The distribution of MARs/SARs in the genome has been intensively studied. They were found to colocalize frequently with regulatory sequences (such as enhancers), recombination hot-spots and the borders of DNase I sensitive genomic domains. At the same time some MARs were mapped within transcriptional units and even within structural genes (for a review see [3]).

For the present discussion the most important question is whether MARs participate (possibly along with other genomic elements) in the formation of DNA loop anchorage sites. Our recent studies give a positive answer to this question. Using the topoisomerase II-mediated DNA loop excision approach we mapped the DNA loop anchorage regions (LARs) within a 500 kb long

fragment of the first chromosome of *Drosophila* where the distribution of SARs had been previously studied by other researchers. Comparison of the two sets of data made it possible to conclude that a fraction of MARs does participate in the organization of loop anchorage regions, although a number of other MARs were located in loop DNA. In the upstream area of chicken domain of  $\alpha$ -globin genes three MARs were mapped [24-26]. Of these only one colocalize with LAR/permanent site of DNA attachment to the nuclear matrix [25, 27]. Hence, it is clear that at best MAR could constitute a part of a loop anchorage region but the presence of a MAR is not sufficient for creation of high salt resistant DNA loop anchorage at the nuclear matrix.

### **IS THERE ANY RELATIONSHIP BETWEEN PARTITIONING OF EUKARYOTIC GENOME INTO FUNCTIONAL DOMAINS AND ORGANIZATION OF CHROMOSOMAL DNA INTO LOOPS?**

Although 20 year ago it was tempting to identify chromosomal DNA loops with individual transcription units (possibly by analogy with loops in "lamp-brush" chromosomes), this hypothesis has gained no experimental support in the following studies and presently can be ruled out. Indeed, even in the chicken  $\alpha$ -globin gene domain, where the gene cluster is flanked by LARs from the upstream and the downstream [16,27], the upstream LAR is nevertheless transcribed both in the globin [28] and in the opposite [29] directions (Fig. 1). The LAR overlaps with the first exon of human *c-myc* gene. In a 500 kb region of *Drosophila melanogaster* first chromosome (see previous section) some of the LARs mapped using topoisomerase II-mediated DNA loop excision fall within transcription units [17]. It should be also mentioned that some known genes have a size that exceeds several times the average size of chromosomal DNA loops (the best example is human dystrophin gene that extends over 2000 kb). Obviously, these genes can not be arranged in a single loop. As far as MARs/SARs are considered, they were also found within genes and even within exons (for discussion see [3, 6]). Although it was demonstrated in functional tests that some MARs (ex. MAR from DNase I HS 5 of the human  $\beta$  globin domain locus control region [30,31] possessed properties of insulators, other MARs did not show any insulating activity in the enhancer-blocking assay [32]. It is even more important that some *bone fide* insulators do not possess properties of MARs [32]. The data concerning the ability of MARs to protect transgenes from the position variegation effect also remain controversial (for discussion see [3]). In known cases of clear colocalisation of MARs with either insulators or (and) domain bordering elements it was not shown by sight-directed mutagenesis that the colocalisation is not hazardous i.e. that the functional activities of MARs and insulators (domain bordering elements) belong to the same DNA sequence motifs. Summarizing, one may conclude that there is no correlation between DNA loops and transcription units.

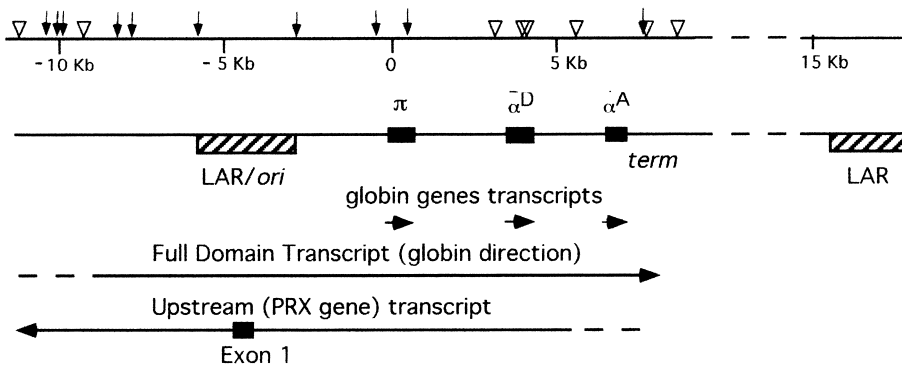


Fig. 1. A scheme showing spatial organisation and transcription pattern of the chicken alpha globin genes domain in cultured erythroid cells. The upper line shows the restriction map of the domain. Recognition sites for Bam HI and Hind III restriction enzymes are shown correspondingly by open triangles and dark arrows. Below the map the positions of globin genes (dark rectangles) and LARs (hatched rectangles) are shown. Positions of the replication origin and the termination site (replicon junction) are indicated in italics (*ori* and *term*). The known transcripts are shown by horizontal arrows. Note that the replication structure of the downstream region of the domain was not analysed. Hence it is not known whether the right-hand LAR colocalise with a replication origin. It is also not known whether all types of transcripts shown in the map are made in a single cell.

Replicons are other functional units of the genome which are characterized by about the same average size as the chromosomal DNA loops. In a pioneering studies carried out by Buongiorno-Nardelli and collaborators [10] it was found that the size of supercoiled loop-domains in the genome of different eukaryotic cells directly correlated with the average size of replicons in these cells. This observation suggested that a specific element of each replicon (and there are actually two such elements, namely a replication origin and a termination site) was attached to the nuclear matrix. Several lines of experimental evidence indicate that the above element is a replication origin. It was shown by Wanka and collaborators that, in synchronised cells, the pulse label incorporated into DNA in the beginning of S phase (i.e. when a large portion of replicons fire simultaneously) remained permanently bound to the nuclear matrix [33]. In contrast, the label incorporated in the middle of S-phase could be easily chased from the nuclear matrix [33], in full agreement with original observations made on non-synchronised cells [2]. These results were interpreted in terms of permanent attachment of replication origins to the nuclear matrix. This supposition was further supported by the results of comparison (in corenaturation experiments) of unique DNA sequences surrounding the

replication origins and permanent sites of DNA attachment to the nuclear matrix [14]. It is also noteworthy that most of the presently mapped mammalian and avian replication origins do colocalize with MARs or loop anchorage regions mapped by other approaches. The list includes the replication origin of the domain of chicken  $\alpha$ -globin genes (Fig. 1, [14]), the replication origin located in the upstream area of the human *c-myc* gene [18], the replication origin located in proximity to the immunoglobulin heavy chain enhancer [34] and replication origins located in non-transcribed spacers of ribosomal genes [16].

There are also some functional evidence indicating that proper spatial organization is essential for replicon function in eukaryotic cell nuclei. Although nobody was yet able to develop a system permitting long-term autonomous replication of plasmids in cells of higher eukaryotes (in contrast to yeast cells), different plasmids can replicate transiently in mammalian and avian cells passing one or even several rounds of replication. In two independent studies it was demonstrated that the ability of plasmids to replicate transiently in cells of higher eukaryotes is enhanced by MAR elements [35] or by fragments derived from permanent sites of DNA attachment to the nuclear matrix [36]. Analysis of distribution of transfected plasmid DNA in the course of nuclear matrix preparation has permitted to conclude that in both cases the effect is due to the targeting of plasmids to the nuclear matrix [35,36].

Summarizing the above data, one may conclude that the organization of chromosomal DNA into looped domains is directly linked to the organization of replication units due to the location of replication origins at or close to the DNA loop anchorage sites.

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