The type of DNA attachment sites recovered from nuclear matrix depends on isolation procedure used

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Abstract

A large variety of DNA sequences have been described in nuclear matrix attachment regions. It could be most likely a result of the different methods used for their isolation. The idea about how different types of known DNA sequences (strongly attached to the nuclear matrix, weakly attached, or not attached) directly participate in anchoring DNA loops to the nuclear matrices isolated by different experimental procedures was tested in this study. Matrix-attached (M) and matrix-independent or loop (L) fractions as well as nuclear matrices were isolated using extractions of nuclei with 25 mM lithium 3,5-diiodosalicylate (LIS), 2 M NaCl, 0.65 M ammonium sulphate containing buffers followed by DNase I/RNase A digestion, or according to so designated conventional method. Using PCR-based and *in vitro* binding assays it was established that LIS and ammonium sulphate extractions gave similar results for the type of attachment of sequences investigated. The harsh extraction with 2 M NaCl or the conventional procedure led to some rearrangements in the attachment of DNA loops. As a result a big part of matrix attached sequences were found detached in the loop fractions. However, the *in vitro* binding abilities of the MARs to the nuclear matrices isolated by different methods did not change. (*Mol Cell Biochem* **214**: 103–110, 2000)

Key words: nuclear matrix, satellite DNA, MAR, chromosome territories

1. Introduction

The eukaryotic chromatin is organised in a higher-order structure correlating with thousands of discrete topologically constrained loop domains. These loops are fixed at their bases to a network composed of proteins and RNA that is generally referred to as 'nuclear matrix' in interphase or 'chromosomal scaffold' in mitosis [1–3].

Matrix attachment regions (MARs) probably play important role in a wide variety of cellular functions. MARs were mapped to origin of replication to termination sites [4, 5], and to condensed chromatin regions of centromeres and telomeres [6–9]. MARs have also been implicated in a variety of other processes, i.e. as binding sites for activator or repressor proteins, demethylation of DNA [10], chromosomal condensation in the cell cycle [11]. The loop-domain organisation of DNA seems well grounded based on some theoretical con-

siderations and experimental data, however the details are still under investigation and the results obtained are frequently controversial. Various DNA sequences were described at the point of attachment of the loops to the nuclear matrix structures: highly repetitive sequences [7, 9, 12, 13], sequences that are actively transcribed [14–16], or sequences that do not differ in complexity to the total nuclear DNA [17, 18]. This variety could be a result from the different methods used for preparation of nuclear matrices.

It is of particular interest to define which DNA sequences are directly involved in attachment of the loops to the nuclear matrices. In the present study the loop- and matrix-associated DNA fractions were separated using four different methods for nuclear matrix isolation. Such fractions were tested by PCR assay for presence of sequences known as strong, weak, or non-MARs. A comparison to the results obtained by *in vitro* binding assay was made.

2. Materials and methods

2.1. Cells

Human cervical carcinoma *HeLa S3* cell line was used in all experiments. The cells were grown in 1:1 Ham's F12/Dulbecco's modified Eagle's, supplemented with 10% foetal bovine serum (Gibco), and maintained to exponential growth by subculturing in fresh medium every 48 h.

2.2. Isolation and fractionation of nuclei

Unless otherwise noted, all procedures were carried out at 4°C and all buffers contained phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 1 mM. Nuclei were isolated as previously described [19]. The isolated nuclei were washed twice with phosphate-buffered saline (PBS). Nuclei were then extracted (1.5×10^7 nuclei/ml, 10 min at room temperature) using either 25 mM LIS, or 2 M NaCl, or 0.65 M $(\text{NH}_4)_2\text{SO}_4$ in a buffer containing 5 mM HEPES, pH 7.4, 0.25 mM spermidine, 2 mM EDTA, 2 mM KCl and 0.1% digitonin. The histone-depleted nuclear 'halos' were pelleted at 6,000 g for 15 min at 4°C. The resulting soft pellet were carefully washed (twice if nuclei were extracted with 2 M NaCl or 0.65 M $(\text{NH}_4)_2\text{SO}_4$, five times if extracted with LIS) in digestion buffer (20 mM Tris-HCl, pH 7.4, 50 mM KCl, 10 mM MgCl_2 , 0.125 mM spermidine, 0.1% digitonin). The 'halo' DNA was released by digestion with 500 µg/ml DNase I and 100 µg/ml RNase A for 1 h at 4°C. Digestion was stopped by adding EDTA to 20 mM final concentration. After centrifugation at 4°C for 20 min at 10,000 g, the pellet which contained the matrix-associated fraction, was suspended in 3 ml TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). DNA was purified from both fractions using Qiagen® RNA/DNA Kit (Qiagen Ltd., Crawley, West Sussex, UK).

The fourth procedure used for nuclear matrices isolation was so called conventional method [20]. The isolated nuclei were resuspended in digestion buffer (the same as in procedures above) and digested with 500 µg/ml DNase I and 100 µg/ml RNase A for 1 h at 4°C. The digested nuclei were layered on a 1 M sucrose cushion in 100 mM KCl, 10 mM Tris-HCl pH 7.7, and 1.5 mM MgCl_2 and centrifuged at 750 g for 10 min. The supernatant (L-fraction) was transferred to a new tube. The pellet was extracted twice with hypotonic buffer (10 mM Tris-HCl pH 7.2, 0.1 mM MgCl_2 , and 0.1 mM CaCl_2) and then twice with 2 M NaCl, 10 mM Tris-HCl pH 7.7, and 1.5 mM MgCl_2 . The supernatant after each extraction was combined with that received after DNase I/RNase A digestion. The other steps for purification of DNA from both fractions were the same like in the first three methods described.

2.3. PCR-based assay

To identify location of specific sequences in nuclear matrix-bound and/or nuclear matrix-independent fractionated chromatin, PCR-based assay method recently proposed by Kramer and Krawetz [21] was used. Equal amounts of the M- and L-DNA fractions were applied for amplification with corresponding PCR primer pairs (Table 1). Three types of probes were amplified: probes known as strong MARs (probes 1–5), as weak MARs (6–9), and non-MARs (10 and 11). Detailed information concerning the sequences accession numbers and references used is given in Table 1 in the last column.

In all PCR assay experiments the quantity of PCR products was maintained within the linear range (increasing the concentration of template or the number of cycles proportionally increased the signal). Subsequently, aliquots of the PCR products were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide and quantified densitometrically using a scanning densitometer (Bio-Rad, CA, USA). A control ratio between the sum of intensities of M- plus L-fractions and band intensity of PCR-product using total genome DNA as an internal control and the same primer pair as in M- and L-fractions was estimated for each primer pair and DNA-fractions isolated by the different procedures described above. Each time these ratios were about 1 ± 0.023 which confirms the equal efficiency of PCR-amplifications in M- and L-fractions and the total genome DNA. All PCR experiments were carried out in triplicate. Similar results were obtained and summarised graphically.

2.4. In vitro MAR binding assay

The MAR binding assays were performed as described [22]. In brief, the isolated according to different procedures nuclear matrices (see section 2.2) were incubated with ^{32}P -labelled ($[\alpha\text{-}^{32}\text{P}]\text{dCTP}$, 3000 Ci/mmol, Amersham) PCR-amplified probes (Table 1) in presence of competitor *E. coli* DNA. The matrices were pelleted by centrifugation and bound DNA fragments were visualised by agarose gel electrophoresis and autoradiography. These experiments were carried out in triplicate. Similar results were obtained and summarised graphically after densitometrical scanning.

3. Results

It is generally accepted that MARs are several hundred base pairs long. In our previous investigation [15] we have shown that DNA attached to the nuclear matrix recovered after digestion of extracted nuclei with restriction enzyme is in the range between 18.5 and 0.125 kbp. Because such DNA is extracted from matrices, it is referred to, as matrix-associated,

Table 1. Primers, expected PCR products and accession numbers for sequences investigated*

No	Sequences tested	Primers	PCR product, bp	Sequence accession numbers and references
1	IF α 2 upstream MAR3	5'-GGATCCAGCCTGACCTTGTA-3' (S) 5'-TTCTTGTTCCTCCCTGAGG-3' (A)	200	U82705, [16]
2	β -actin	5'-AACACCCAGCCATGTACG-3' (S) 5'-ATGTCACGCACGATTTCCC-3' (A)	254	M10277, [21]
3	c-myc	5'-TGGACACGGAGCGTGAACCT-3' (S) 5'-TCGCTGCTTCCCTCCTCGCT-3' (A)	114	J00889, [32]
4	α 1AT-MAR	5'-CAGATGTTTCAGGTCAATTCC-3' (S) 5'-GAAGCCAAGAACAACCTTAC-3' (A)	82	AF156542, [33]
5	α -satellite DNA	5'-GTGTTGTGTGCATTCATCTCA-3' (S) 5'-GAGATCAACGCACACATCACA-3' (A)	191	AF105153, [30]
6	β -globin	5'-CAACTTCATCCACGTTCCACC-3' (S) 5'-GAAGAGCCAAGGACAGGTAC-3' (A)	268	U01317, [21]
7	SPPR1A intron	5'-GGGAAGTTCATAGCAGAACT-3' (S) 5'-TCTCCGTCATGCCAATTACT-3' (A)	264	L05187, [34]
8	SPPR2A intron	5'-CTCTGTTCTTTGTGCCCTGA-3' (S) 5'-TCTCTGTCCATATGGATACC-3' (A)	178	X53064, [34]
9	MLL (ALL-1) centromeric MAR	5'-GGTTCTTCCTTGTGCTTTTCCC-3' (S) 5'-ACCACACTATCTCTTATCCATAA-3' (A)	200	U04737, [8]
10	IF α 2 non-MAR region	5'-TGGACAGCACTTGTAAGCTC-3' (S) 5'-CTCGTGAGCAGCGAGTAAAC-3' (A)	270	U82705, [16]
11	PRM2	5'-AGGGGTAGAGGCTGCTATGAT-3' (S) 5'-CAGCAGCAAACAGTTCCCTAATAGC-3' (A)	180	U15422, [21]

*S – sense primer; A – antisense primer.

but only a part of this DNA should be directly involved in attachment of DNA loops to the matrix structures [23]. To isolate such DNA, in present study DNase I/RNase A digestions of nuclei or residual 'halo' structures obtained after different extraction procedures were applied. So recovered MAR DNAs are represented short fragments with length about 300 bp in average [7, 19] regardless of isolation method used. The percentage of M- and L-fractions isolated according to different methods of the input DNA is given in Table 2. It is obvious that the sum of both of fractions account for 100% of the input DNA. Hence, there were not losses of DNA sequences during the isolation procedures used.

The relative strength (R) of each individual DNA fragment tested to partition in the matrix fraction was estimated as the band intensity in the matrix divided by the sum of intensities of matrix plus loop fractions ($R = IM/(IM+IL)$) [16]. R-values set for strong MAR fragments represent more than 70% enrichment into the matrix fraction. R-values set for weak MAR fragments represent binding greater than 30%, but less

than 70%. R-values set for non-MAR fragments with negligible binding represent less than 30%.

Firstly, five sequences known as strong MARs (sequences 1–5, Table 1) were tested by PCR-based assay [21] for their presence in M- and L-fractions obtained according to different experimental procedures. The results are shown in Fig. 1, panel A. The five sequences were strong MARs according to LIS and ammonium sulphate extraction methods. However, when 2 M NaCl extraction procedure was used, only α -satellite DNA and α 1AT-MAR remained strongly attached to the nuclear matrix, but their R-values were lower than in the first two cases. The rest sequences turned into weak attachment sites. The situation was much more different when the conventional method for nuclear matrices isolation was used. Only α -satellite DNA was strong MAR (R-value was lower than in LIS and ammonium sulphate extractions), but the others were defined as non-MARs according to the R-value.

A second test was done for *in vitro* binding abilities of these strong MARs to different ways isolated nuclear matrices

Table 2. Percentage of the matrix (M) and loop (L) fractions of the input DNA following different experimental procedures for matrix isolation*

DNA-fraction	LIS	NaCl	(NH ₄) ₂ SO ₄	Conventional procedure
Amount of M-fraction in %	3.40 $\pm$ 0.21	2.18 $\pm$ 0.14	3.32 $\pm$ 0.22	0.18 $\pm$ 0.02
Amount of L-fraction in %	96.52 $\pm$ 1.54	98.01 $\pm$ 1.73	96.71 $\pm$ 1.67	99.85 $\pm$ 2.03

*Mean values of 6 experiments measured by spectrophotometer.

Fig. 1. (A) PCR-based assay for strong MARs carried out with L- and M-fractions isolated by 25 mM LIS (LIS), 2 M NaCl (NaCl), 0.65 M ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ and conventional extraction methods (CM). The positive and negative control experiments with total genome DNA and pUC18 respectively were done with each primer pairs and similar results were received, but only the results with primer pair for IF α 2 upstream MAR3 are shown. (B) *In vitro* binding assay for strong MARs carried out with matrices isolated according to different procedures (as in panel A) and ^{32}P -labelled strong MAR sequences obtained by PCR. Control experiments with pUC18 ^{32}P -labelled fragment were performed.

(Fig. 1, panel B). The results received were similar (all the sequences were strong MARs) regardless of isolation procedure for nuclear matrices used. All of the MARs tested showed ability for strong attachment to the matrix structures. These two tests were performed in triplicate with a new isolated nuclear matrices and M- and L-fractions each time. The bands received were scanned with densitometer and the results for R-value measured are given in Fig. 2.

Other four sequences (numbers 6–9, Table 1) known as weak MARs was investigated using PCR-based and *in vitro* binding assays (Fig. 3). The four sequences were weak MARs

Fig. 2. (A) Histograms of R-values of strong MARs measured from 3 independent PCR assay experiments: 1 – IF α 2 upstream MAR3/LIS; 2 – IF α 2 upstream MAR3/NaCl; 3 – IF α 2 upstream MAR3/ $(\text{NH}_4)_2\text{SO}_4$; 4 – IF α 2 upstream MAR3/CM; 5 – β -actin/LIS; 6 – β -actin/NaCl; 7 – β -actin/ $(\text{NH}_4)_2\text{SO}_4$; 8 – β -actin/CM; 9 – c-myc/LIS; 10 – c-myc/NaCl; 11 – c-myc/ $(\text{NH}_4)_2\text{SO}_4$; 12 – c-myc/CM; 13 – α 1AT-MAR/LIS; 14 – α 1AT-MAR/NaCl; 15 – α 1AT-MAR/ $(\text{NH}_4)_2\text{SO}_4$; 16 – α 1AT-MAR/CM; 17 – α -satellite DNA/LIS; 18 – α -satellite DNA/NaCl; 19 – α -satellite DNA/ $(\text{NH}_4)_2\text{SO}_4$; 20 – α -satellite DNA/CM. (B) Histograms of R-values of strong MARs measured from 3 independent *in vitro* binding assay experiments. The numbers 1–20 are the same like in panel A.

according to PCR assay (panel A), when LIS and ammonium sulphate extractions of nuclei were used. When 2 M NaCl extraction was used, the MAR in β -globin gene was still

Fig. 3. (A) PCR-based assay for weak MARs carried out with L- and M-fractions isolated as in Fig. 1. The positive and negative control experiments with total genome DNA and pUC18 respectively were done with each primer pairs and similar results were received, but only the results with primer pair for SPPIA intron are shown. (B) *In vitro* binding assay for weak MARs carried out with matrices isolated according to different procedures (as in panel A) and ^{32}P -labelled weak MAR sequences obtained by PCR. Control experiments with pUC18 ^{32}P -labelled fragment were performed.

weak, but the R-value was reduced with 9%. Other three sequences were defined as non-MARs already. The four probes became into non-MARs when conventional method for nuclear matrices isolation was carried out. The results from *in vitro* binding assay (panel B) showed no changes in the *in vitro* binding abilities of these sequences to the nuclear matrices isolated by different extraction procedures (the four sequences were weak MARs). Scanning the bands received in three independent assay experiments assessed the R-values for these four sequences. The results are shown graphically in Fig. 4.

Finally, two sequences known as non-MARs (numbers 10, 11, Table 1) were tested as the first two types of probes by

Fig. 4. (A) Histograms of R-values of weak MARs measured from 3 independent PCR assay experiments: 1 – β -globin/LIS; 2 – β -globin/NaCl; 3 – β -globin/ $(\text{NH}_4)_2\text{SO}_4$; 4 – β -globin/CM; 5 – SPPIA intron/LIS; 6 – SPPIA intron/NaCl; 7 – SPPIA intron/ $(\text{NH}_4)_2\text{SO}_4$; 8 – SPPIA intron/CM; 9 – SPPIA intron/LIS; 10 – SPPIA intron/NaCl; 11 – SPPIA intron/ $(\text{NH}_4)_2\text{SO}_4$; 12 – SPPIA intron/CM; 13 – MLL centromeric MAR/LIS; 14 – MLL centromeric MAR/NaCl; 15 – MLL centromeric MAR/ $(\text{NH}_4)_2\text{SO}_4$; 16 – MLL centromeric MAR/CM. (B) Histograms of R-values of weak MARs measured from 3 independent *in vitro* binding assay experiments. The numbers 1–16 are the same like in panel A.

PCR based and *in vitro* binding assays (Fig. 5). Both of the sequences were non-MARs regardless of isolation procedure or type of assay used. However, small amounts of both probes

Fig. 5. (A) PCR-based assay for non-MARs carried out with L- and M-fractions isolated as in Fig. 1. The positive and negative control experiments with total genome DNA and pUC18 respectively were done with each primer pairs and similar results were received, but only the results with primer pair for PRM2 are shown. (B) *In vitro* binding assay for non-MARs carried out with matrices isolated according to different procedures (as in panel A) and ³²P-labelled non-MAR sequences obtained by PCR. Control experiments with pUC18 ³²P-labelled fragment were performed.

investigated (3–5%) were found in M-fractions isolated by 2 M NaCl when PCR assay was used. In all other cases presence of these sequences in M-fractions was not found. Results from PCR and *in vitro* binding assays for all of the sequences investigated are summarised in Tables 3 and 4 respectively.

Discussion

Various DNA sequences were described at the point of attachment of DNA loops to the nuclear matrix. This variety could be a result of the different methods for the preparation of nuclear matrices. With respect to this the aim of the present investigation was to define the type of MAR sequences recovered from the nuclear matrices, and their dependence on the isolation procedure used.

In present study M- and L-DNA fractions were isolated according to the four most often used experimental procedures – extractions with 25 mM LIS, 2 M NaCl, or 0.65 M (NH₄)₂SO₄ containing buffers, or conventional method. So recovered MAR DNAs using 500 µg/ml DNase I and 100 µg/ml RNase A digestion for 1 h represented short fragments with length 300 bp in average [7, 15, 19] regardless of the isolation method used. In our previous work [7] we have established that any further increase of enzyme activity or the duration of digestion do not reduce this length. This finding indicates that such DNA fragments are protected against DNase I digestion, and they participate directly in attachment of DNA loops to the nuclear matrix. Two different ways of protection are described: protection by proteins [13, 24] and by complexing with RNA [7].

In the recent study of Craig *et al.* [25] the authors have tested ideas about how genome is arranged into loops by analysing the average loop size over different cytologically resolvable regions of human chromosomes using fluorescent *in situ* hybridisation with loop and attached DNA fractions. They have found that variations in average loop size, along and between chromosomes, measurable at this level of resolution are small but significant and are dependent on the extraction method. This reveals the fundamental differences between the nuclear structure probed by different protocols. The method used by Craig *et al.* [25] is very elegant to investigate the dependence of chromatin loop sizes on the isolation method for residual structures preparation. However, the short length of matrix isolated DNA directly involved in loops anchoring to the nuclear matrices (about 300 bp in average) is not convenient to carry out a fluorescent *in situ* hybridisation. To avoid this drawback in present study PCR amplification of different types DNA sequences (strong MARs, weak MARs, non-MARs) was applied using specific primer pairs, as well as *in vitro* binding assay. These methods allow assessment of the type of DNA, attaching chromo-

Table 3. MAR and non-MAR characteristics of DNA fragments tested by PCR-based assay according to different procedures for nuclear matrix isolation

Sequences tested	Type of the sequences (strong MAR, weak MAR or non-MAR) according to PCR-based assay							
	LIS		NaCl		(NH ₄) ₂ SO ₄		Conventional procedure	
	R value %	Type	R value %	Type	R value %	Type	R value %	Type
IFα2 upstream MAR3	89	Strong MAR	48	Weak MAR	92	Strong MAR	3	Non-MAR
β-actin	82	Strong MAR	60	Weak MAR	87	Strong MAR	5	Non-MAR
c-myc	85	Strong MAR	68	Weak MAR	90	Strong MAR	4	Non-MAR
α1AT-MAR	100	Strong MAR	71	Strong MAR	100	Strong MAR	3	Non-MAR
α-satellite DNA	100	Strong MAR	87	Strong MAR	100	Strong MAR	87	Strong MAR
β-globin	55	Weak MAR	46	Weak MAR	61	Weak MAR	4	Non-MAR
SPPR1A intron	35	Weak MAR	10	Non-MAR	46	Weak MAR	7	Non-MAR
SPPR2A intron	47	Weak MAR	27	Non-MAR	43	Weak MAR	3	Non-MAR
MLL centromeric MAR	34	Weak MAR	8	Non-MAR	40	Weak MAR	8	Non-MAR
IFα2 non-MAR region	0	Non-MAR	3	Non-MAR	0	Non-MAR	0	Non-MAR
PRM2	0	Non-MAR	5	Non-MAR	0	Non-MAR	0	Non-MAR

some loops to the matrix structures isolated according to most often used procedures.

The PCR assay with strong MARs (Figs 1 and 2; Table 3) showed that the type of MARs depends on the method used for nuclear matrices preparation. Most of these strong MARs became into weak or non-MARs, when harsh 2 M NaCl or conventional method extractions were used. Only α-satellite DNA was strongly attached to the matrices in all isolation procedures tested. Actually, the α-satellite DNA is of particular interest to be investigated, because alphoid DNA is known to be present in the centromeres of all human chromosomes and is presumed to take part in centromere functioning [26, 27]. Recently it was established that deletion of α-satellite DNA in the Y chromosome seems to be responsible for the silencing of the normal centromere [28]. This observation is additional evidence that α-satellite DNA most likely is the functional centromeric DNA in humans. Zhu *et al.* [29] supposed that the repeat (TGGAA)_n could be the functional centromeric sequence in humans since this unit contains a stack of four contiguous guanine residues all of which have

their hydrogen-bonding surface (N²H-N1H-O⁶-N7) exposed to solvent and available for interactions with other bases or ligands. Interestingly, the same functional motif we described in our recent work [9] in mouse γ-satellite DNA as well. According to these important findings, the region of α-satellite DNA amplified in present study [30] contains two TGGAA functional motifs.

Results from the PCR assays summarised in Table 3 confirmed our previous suggestion that using conventional procedure for nuclear matrices isolation only tightly bound and constitutive DNA attachment sites (enriched in satellite DNA) of the chromosomes remain attached to the residual structures [7, 9, 19]. DNA realising the constitutive attachment is bound too strong to the nuclear matrices (R-value from 87–100% depending on isolation procedure). The absence or the small amount of amplification signal in the L-fractions (Figs 1, 2; Table 3) is a sign that the attachments in α-satellite DNA to the nuclear matrix are very frequent. There is a weak signal in L-fraction in the cases of 2 M NaCl and conventional procedure extractions, but it could be suppose that it is due to

Table 4. MAR and non-MAR characteristics of DNA fragments tested by *in vitro* binding assay according to different procedures for nuclear matrix isolation

Sequences tested	Type of the sequences (strong MAR, weak MAR or non-MAR) according to <i>in vitro</i> binding assay							
	LIS		NaCl		(NH ₄) ₂ SO ₄		Conventional procedure	
	R value %	Type	R value %	Type	R value %	Type	R value %	Type
IFα2 upstream MAR3	78	Strong MAR	76	Strong MAR	77	Strong MAR	78	Strong MAR
β-actin	75	Strong MAR	72	Strong MAR	80	Strong MAR	82	Strong MAR
c-myc	80	Strong MAR	82	Strong MAR	79	Strong MAR	85	Strong MAR
α1AT-MAR	79	Strong MAR	89	Strong MAR	82	Strong MAR	81	Strong MAR
α-satellite DNA	100	Strong MAR	100	Strong MAR	100	Strong MAR	100	Strong MAR
β-globin	60	Weak MAR	55	Weak MAR	58	Weak MAR	52	Weak MAR
SPPR1A intron	52	Weak MAR	45	Weak MAR	52	Weak MAR	50	Weak MAR
SPPR2A intron	49	Weak MAR	48	Weak MAR	53	Weak MAR	51	Weak MAR
MLL centromeric MAR	53	Weak MAR	46	Weak MAR	49	Weak MAR	54	Weak MAR
IFα2 non-MAR region	0	Non-MAR	0	Non-MAR	0	Non-MAR	0	Non-MAR
PRM2	0	Non-MAR	0	Non-MAR	0	Non-MAR	0	Non-MAR

the harsh extraction conditions. The data concerning the changes at the MARs strength of attachment (Figs 1–4; Table 3) are in agreement with the results of Ma *et al.* [31] who have shown that 2 M NaCl extraction and combined DNase I/RNase A digestion results disruption of higher order chromosome territory architecture, in contrast to the same ion strength extraction with 0.65 M $(\text{NH}_4)_2\text{SO}_4$.

It is of great interest that the *in vitro* binding abilities of the MARs to nuclear matrix structures isolated by different methods do not change (Figs 1–5; Table 4). One possible explanation of this phenomenon is that the protein composition of nuclear matrices likes to remain almost constant, regardless of method for their isolation used.

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