

Synthetic concatemers as artificial MAR: importance of a particular configuration of short AT-tracts for protein recognition

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Summary

The matrix attachment region (MAR), a class of sequences involved in organization of genomic DNA into looped superhelical domains, is also believed to be important in the regulation of nuclear functions such as DNA replication, transcription, and recombination. The association of MARs to the nuclear matrix is probably mediated by a variety of proteins which selectively bind to MAR. Because of the complex nature of MAR-protein interactions, it is still difficult to tell, from sequence information alone, whether or not a particular DNA region is a MAR. An abundant nuclear protein, termed SP120, is one of the major MAR binding proteins identified so far that selectively binds to AT-rich MARs of different origins *in vitro*. We have recently found that SP120 also binds to a GC-rich concatemer synthesized by random ligation of a short duplex oligonucleotide. Although the result seemed rather paradoxical at first, subsequent experiments with sequence-manipulated concatemers indicated that intactness of short homopolymeric AT-tracts harbored in the concatemer and a particular pattern of their distribution within it are prerequisites for the binding.

I. Introduction

A. Short history

Presence of a long-range organization in nuclear DNA was originally proposed in early studies analyzing the sedimentation behavior of detergent-lysed cells in a solution containing intercalating agents (Cook and Brazell, 1975; Ide et al., 1975). The interphase chromatin DNA appeared to be folded into supercoiled loop domains through its attachment to a subnuclear skeletal structure, which was later isolated and characterized as the nuclear matrix (Berezney and Coffey, 1977) or nuclear scaffold (Mirkovitch et al., 1984). This model acquired further support when characteristic DNA regions were discovered at the attachment sites (Cockerill and Garrard, 1986; Mirkovitch et al., 1984). Such DNA regions, designated SAR or MAR, have been allocated in many cloned genes and implicated in the regulation of gene expression and other nuclear processes (reviewed in Gasser et al., 1989; Zlatanova and van Holde, 1992; Boulikas 1995)

B. Characteristic features of MAR

Comparison of MAR sequences identified thus far revealed some characteristic features. 1) Relatively long DNA region is required for the attachment and very few MARs are shorter than 300 bp. 2) MARs are usually located in noncoding regions. 3) With a few exceptions MARs are rich in adenines (A) and thymines (T); A+T content of a typical AT-rich type MAR is higher than 65%. 4) Regulatory sequences are frequently present in the vicinity of MAR or cohabit with MAR. These include replication origins, transcriptional enhancers and promoters. 5) Other sequence motifs like AT-tracts, DNA unwinding elements, clustered recognition sites for various transcription factors, inverted repeats or palindromes, cleavage consensus for DNA topoisomerase II, curved/kinked DNA motifs, left handed and triple helical structures, and DNase I-hypersensitive sites are frequently associated with MARs (Boulikas, 1995). 6) MARs appear to function beyond

specifically bind to the nuclear matrix prepared from mouse cells.

C. SP120 and other MAR binding proteins

Since MAR sequences are heterogeneous in nature, proteins interacting with MAR can also be heterogeneous. Indeed, several nuclear proteins including ARBP (von Kries et al., 1991), SATB1 (Dickinson et al., 1992), lamin B1 (Ludérus et al., 1992), SAF-A (Romig et al., 1992), nucleolin (Dickinson and Kohwi-Shigematsu, 1995), histone H1 (Izaurralde et al., 1989), and DNA topoisomerase II (Adachi et al., 1989) have already been shown to bind MAR with considerable affinity. We have identified an MAR binding protein, termed SP120, which is associated with the nuclear matrix (Tsutsui et al., 1993). Sequence determination of cDNA for the rat SP120 showed that the protein is a homologue of human hnRNP U protein (Kiledjian & Dreyfuss, 1992), one of the major components of the heterogeneous nuclear RNA-protein complex (hnRNP). U protein had been characterized as an RNA binding protein by UV-induced cross-linking experiments *in vivo* (Dreyfuss et al., 1984) and suggested to be involved in the processing of pre-mRNA (Portman and Dreyfuss, 1994). Therefore, SP120/hnRNP U protein is a multifunctional protein operating in those nuclear processes. Using partial cDNA segments expressed in *E. coli*, a putative MAR binding site of SP120 has been located within the C-terminal tail region of 171 amino acids, designated RG domain, that contains 16 repeats of Arg-Gly dipeptide (Tsutsui et al., unpublished).

D. Aims of this study

Molecular interactions involving MAR at the base of chromatin loops can be quite complex and probably protein-protein interactions would also be important in the stabilization of "anchorage complex". What determines the binding specificity of MARs toward the matrix, therefore, is a difficult question to answer. However, it would be reasonable to assume that relatively small numbers of proteins play a key role to recognize MAR sequences and serve as a nucleation center or a platform for subsequent association of additional proteins. To evaluate this model, sequence motifs recognized by these MAR binding proteins must be nailed down within individual MARs. The proteins listed above are abundant nuclear proteins and expressed ubiquitously except for SATB1 which is specific to thymus. Although consensus motifs for the binding of ARBP (Buhrmester et al., 1995) and SATB1 (Dickinson et al., 1992) have been proposed, those for the other proteins are less characterized.

Before searching for the sequence motifs recognized by SP120, we first tried to identify essential regions within a representative MAR reported previously. In the first part of

this article, relative affinities of subfragments from the mouse Igk MAR are analyzed in an *in vitro* binding reaction with isolated nuclear matrix enriched with SP120. The results suggest an interesting possibility that the MAR activity is elicited through a synergistic interaction of two subdomains separated no farther than 300 bp (Okada et al., 1996). The second part describes experiments with purified SP120 and synthetic concatemers, which suggested that multiple A_n/T_n tracts positioned in a particular configuration are essential for the recognition by SP120.

II. Analysis of subdomain structures in natural MAR

A. Methods for assessment of MAR activity

All the data presented here are based on the *in vitro* binding assay in which a preferential binding of end-labeled MAR fragments to isolated nuclear matrix is measured in the presence of a large excess of unlabeled competitor DNA (Tsutsui et al., 1993). The matrix preparation used here is similar to the "nuclear scaffold" in that histones were extracted with lithium diiodosalicylate after stabilization of nuclei with Cu^{2+} (Tsutsui et al., 1988). SP120 is highly concentrated in the matrix, being a single major MAR binding protein detectable by southwestern blotting. The ^{32}P -labeled DNA fragments bound to the matrix was directly counted in a scintillation counter or analyzed by electrophoresis in 5% polyacrylamide gels after digesting the matrix with proteinase K. The radioactive DNA bands were quantified by densitometric scanning of autoradiograms.

B. The intronic MAR of mouse Igk gene

The MAR localized within the mouse immunoglobulin k gene is a typical example of AT-rich MARs except for its intronic location, consisting of a stretch of DNA (370 bp) with high A+T content (70%). This MAR is interesting in functional aspects since, together with the nearby transcriptional enhancer, it has been shown to be required as a *cis* element for the enhanced expression (Xu et al., 1989), demethylation (Lichtenstein et al., 1994), and somatic hypermutation (Betz et al., 1994) of Igk transgenes.

As shown in **Figure 1**, numerous short stretches of consecutive A's and T's are contained in the MAR. Importance of AT-tracts for protein recognition has been noticed in previous studies (Adachi et al., 1989; Käs et al., 1989). Comparison of sequences for known MARs strongly suggested that these tracts (referred to as A- or T-patches hereafter) are a characteristic landmark for MARs. Another interesting observation is that the occurrence of both patches appears to be essential for the MAR activity since a biased abundance of T-patches, for instance, shows a poor correlation.

Figure 1. Short homopolymeric AT-tracts cluster in the intronic MAR of mouse immunoglobulin κ gene.

Homopolymeric stretches ($n \geq 3$) of adenines (A-patch) and thymines (T-patch) are highlighted by light shade and dark shade, respectively. Cleavage sites for the restriction enzymes used in this study are indicated.

Figure 2. Relative binding affinities of restriction fragments to the nuclear matrix.

Ig κ MAR was cut with the enzymes shown on the left side (also abbreviated on the line map. A, AluI; S, SspI; M, MboII; Dd, DdeI; Dr, DraI). Unlabeled internal fragments are omitted from the figure. Fragments with high, low, and negligible ($1\% >$) affinities are represented by filled, hatched, and open bars, respectively. The boxed figures stand for percentages of input fragments bound to the matrix.

C. Ig κ MAR is composed of two elementary subdomains

As the minimal region required for the binding activity of this MAR had not been analyzed, we first compared the binding affinities of several restriction fragments (summarized in **Figure 2**). Under the experimental conditions used here, about 20% of the full length MAR

input is retained on the matrix. Both fragments generated by a cleavage at the unique MboII site show a much reduced, but still significant binding of 5%. This level of binding can be easily overlooked but it has a prime importance as a contri-

Figure 3. Effects of dimerization of MAR fragments.

(a) Self-dimerization of the MboII fragments. (b) Dimerization of the MboII-AluI fragment. pMM22 is a control construct with an extension of nonMAR segment derived from the β lactamase gene.

tribution from elementary local structures, because the binding activity can be largely restored by their self-dimerization (see below). Further fragmentation of the MboII 3'-fragment suggests that the region between the MboII site and the downstream AluI site is required for the residual binding.

Does the large decrease in the affinity after cleavage with MboII simply imply that the whole intact MAR is essential for the high affinity? The experiment shown in **Figure 3a** reveals, however, that this is not the case. Self-dimerization of the 5' half (S) or 3' half (L) of Igk MAR greatly increases their affinity to the matrix. This effect is more evident with the latter half; the dimer fragment (2L) shows even greater affinity than the original MAR.

The binding experiments with restriction fragments (**Figure 2**) suggested that the 3' portion of L fragment is dispensable. When the DNA segment between the MboII

effect is not due to the doubled fragment length since a control experiment with the monomer fragment extended by a nonMAR sequence (A+T=50%) showed no effect.

D. Synergism between the subdomains

The MboII-AluI segment may thus serve as a minimal sequence element that confers MAR-like features upon any DNA, provided that it is duplicated in the same molecule. This possibility can be tested by inserting nonMAR spacers of varying length between the monomer units of the dimer clone (**Figure 4**). As expected, DNA fragments flanked by the duplicated elements are strong MARs as long as they are not separated by more than 300 bp. As the spacer length exceeds 500 bp, however, the binding affinity decreases abruptly and levels off to the monomer level at about 3 kb. Therefore, the synergistic effect does not propagate beyond

It appears that each subdomain is complexed with a subset of matrix proteins and a cooperative interaction between these complexes contributes to the increased affinity. Obviously, the complexes should be positioned within 300 bp for the interaction to occur. This excludes a simple DNA-looping-out mechanism. The bipartite subdomain organization could be a general feature of AT-rich MARs. Similar observations have been reported previously on the *Drosophila* histone gene MAR (Gasser and Laemmli, 1986) and chicken lysozyme 5' MAR (von Kries et al., 1991). This principle may also be applied to the *Drosophila* fushitarazu gene MAR (Tsutsui *et al.*, unpublished).

Figure 4. Attenuation of the synergism between MAR subdomains as a function of increasing distance.

The dimerized MboII-AluI fragments (fat arrows) were separated by insertion of nonMAR spacers derived from plasmid pUC18 (thick lines).

III. Synthetic concatemers as an artificial MAR

A. Initial hints

The frequent occurrence of AT-patches in MARs suggests that they interact directly with MAR binding proteins including SP120. Once the elementary subdomain (about 130 bp) has been identified in the I γ k MAR, next approach would be to compare the affinities of mutated

Figure 5. Sequences of oligonucleotide duplexes for concatemer

subdomain sequences in which AT-patches are disrupted one by one. Before completion of this type of experiments, however, another effective strategy was suggested from a rather serendipitous finding.

In a purification protocol for a transcription factor IRF-2 using a DNA-affinity resin, a nuclear protein of 120 kDa copurifies with IRF-2 (Vaughan et al., 1995). Although this is actually a contaminating protein, it shows a strong affinity to the concatemerized recognition sequence for IRF-2 as revealed by southwestern blotting. The sequence shown in **Figure 5a** resides in the promoter region of human histone H4 gene but no MAR has been identified nearby. When the sequence is mutated by an insertion of A/T-pair (Figure 5b), the binding of IRF-2 is abolished whereas the 120 kD protein persists to bind, indicating that this protein has less stringent sequence requirements.

An interesting feature of the IRF-2 recognition sequence is the presence of two AT-patches in a GC-rich context (A+T content of the mutant 26-mer is only 42%). When the mutant duplex is ligated through its sticky ends, two alternative orientations are expected for each monomer. Inverted ligation between neighboring monomer units results in the generation of both A- and T-patches on the same strand. This implies a variegated presentation of AT-patches in the ligation product, which is also a characteristic feature of natural MARs. Based on these observations we suspected that the protein might be SP120.

B. The concatemer interacts with SP120

Before the synthesis of concatemer to test its possible binding with SP120, one modification was made on the mutant sequence (**Figure 5c**). This base change introduces restriction sites only between the monomer units ligated in inverted orientations (**Figure 5d**), enabling easy confirmation of the ligation product and cleavage of concatemers at specific sites. As expected, the concatemer synthesized by ligating the oligomer duplex shown in **Figure 5c** exhibits a strong binding to SP120 on southwestern blots. Poly(dA)-poly(dT), a synthetic duplex DNA frequently used as a competitor for AT-rich MARs, competes the binding effectively, indicating that the concatemer behaves like AT-rich MARs despite its low AT content. When the concatemer is digested with either BglII or FbaI, the binding decreases to a basal level. This is consistent with the predicted importance of both AT-patches in the concatemer binding.

The concatemer immobilized on Sepharose beads selectively binds SP120 in nuclear extracts and can be used as an affinity resin to purify the protein. The specific binding of the concatemer was confirmed by a nitrocellulose filter

AT-patches are shaded. **(a)** recognition motif of IRF-2 (wild type sequence). **(b)** mutant sequence. **(c)** modified mutant. **(d)** structure of a representative concatemer containing all possible orientations of neighboring monomer units (bars). Dark shaded areas in the bar represent AT-patches. Restriction sites created after ligation are also indicated. The upper strand in **(c)** containing T-patches are designated "A", and the complementary lower strand, "B". Using this definition, the concatemer sequence can be described either as AABBA (upper strand) or as BAABB (lower strand).

binding assay with purified SP120. The dissociation constant estimated from these data is in the same order as Ig κ MAR (about 3 nM).

C. Concatemers with defined monomer orientations

Use of synthetic concatemers as an artificial MAR is an extremely powerful strategy to delineate sequence requirements for the recognition by SP120. Ease of obtaining mutated sequences is an obvious advantage. In addition, the GC-rich background of the concatemer should provide a highly informative environment to assess the importance of AT-rich motifs in it. Orientation of monomer units in the concatemer described above are randomized because of the palindromic nature of the overhang or sticky ends. In the following sentences this type of concatemer is referred to as "RC" for random concatemer. It is possible to synthesize other types of concatemers simply by manipulating the overhang sequences; "DC" and "AC" representing direct and alternating orientations, respectively (**Figure 6**). Also here, the terminal sequences were designed so as to introduce junctional restriction sites.

Comparison of affinities of these concatemers to purified SP120, using a filter binding assay or southwestern blotting, gives an unequivocal result; only RC shows a significant binding. It should be emphasized that all the concatemers have identical AT content and the same number of AT-patches. Thus, the result strongly suggests that the binding activity is accounted for by only a subset of RC molecules

with a particular pattern of patch distribution that are contained in the heterogeneous population.

D. Patch mutants

The possible involvement of AT-patches in the protein recognition can be tested more directly with RCs with altered patches. Three categories of mutant sequences were designed and RCs were synthesized by random ligation of mutant oligos (**Figure 7**). In the "patch disruptants", both T₃- and T₄-patches are disrupted by replacing T's with G/C or A. In the "patch convertant", only the T₄-patch is converted to an A₄-patch. All these concatemers show a marginal binding to SP120, indicating that the intactness of AT-patches are indeed required for the binding.

In the "phase mutants", the two T-patches are either moved toward the center without changing the spacing, or the distance between them are elongated. Distribution pattern of AT-patches in these concatemers is different from that in the wild type RC. Although a decreased binding is observed with these mutants, a significant level of binding persists.

An important conclusion from this experiment is that functional patches are homopolymeric and patches with mixed A's and T's have no contribution to the binding. The decreased affinity of the patch convertants, whose A/T patch ratio is the same as in wild RC, suggests again the distribution pattern of patches is the determinant factor for successful binding.

Figure 6. Synthesis of concatemers with defined monomer orientations.

Complementary oligonucleotide pairs shown in the left column were annealed and ligated. Junctional restriction sites created in the concatemers are shown in shaded boxes and expected restriction fragments are shown in the right column. Structure of the concatemers were confirmed by fragmentation analysis with these enzymes.

individual oligomers were subjected to the filter binding assay, a significant binding was observed with oligomers longer than hexamer but not with shorter ones (dimer to pentamer).

This result suggests that 156 bp is the minimal length to harbor all essential AT-patches supporting the MAR activity. Active hexamer units may be equivalent to the elementary subdomains of Igk MAR, and when they are duplicated in RC, synergistic interaction between them should largely promote the binding. Interestingly, the size of the hexamer is comparable to that of the Igk MAR subdomain (about 130 bp).

In light of available evidence it should be possible to determine the configuration of essential AT-patches that confers MAR activity to RC. One way to do this is to clone the heterogeneous concatemer in a plasmid vector and examine the binding activity of each clone. Subsequent alignment of a battery of sequences for active concatemers should reveal the pattern of essential patches. Although we have made several trials with different cloning systems, no clones with desired inserts have been obtained probably due to the instability of repetitive sequences in host bacteria.

Figure 7. Oligonucleotides used for the synthesis of patch mutants. Only the T-patch strands are shown. Positions of T-patches and altered bases are highlighted.

E. Hexamer is the shortest concatemer with binding activity

Under the ligation conditions used here, the resulting RCs are heterogeneous in size, ranging from 50 bp (dimer) to over 2,000 bp. It is anticipated that longer RC binds more tenaciously to SP120. To determine the minimal size of RC capable of interacting with SP120 with significant affinity,

Therefore, we decided to synthesize all possible hexaconcatemers by a similar strategy as described above. There exist 36 different hexamers theoretically (**Figure 8**). To synthesize all the hexamers, 26 different 26-mer oligonucleotides with complementary overhangs are required. Six sets of complementary oligos are first annealed pairwise and then mixed and ligated. The resulting concatemer is heterogeneous in size but the monomer units are ligated in desired orientations and BamHI sites are introduced at every 6 units. Digestion with BamHI releases a hexamer which can be purified by polyacrylamide gel electrophoresis. Experimental results with hexamers are not shown here since the work along this line is still under way.

F. MAR organization and mode of interaction with proteins

Based on the results presented in this article, I propose here the following principles for MAR organization. 1) MAR activity is exerted by a synergistic interaction between at least two subdomains located no farther than 300 bp apart. 2) Each subdomain contains essential AT-patches positioned with particular intervals. The latter principle was deduced from the binding experiments with concatemers and purified SP120. This could be generalized, however, to any MARs because a similar mode of MAR-protein interaction appears to involve other abundant MAR binding proteins, e.g., histone H1 and topoisomerase II.

It is possible that essential AT-patches are the sites interacting directly with MAR binding proteins. The Arg, Gly-rich C-terminal domain of SP120 may selectively recognize AT-patches by its insertion into the small groove of DNA which is significantly narrowed at homopolymeric AT-tracts. As this type of interaction is usually weak, multiple contacts between MAR and proteins would be essential for a stable complex to form. The complex can be formed either by disordered aggregation of proteins or by organized interaction between protein molecules (**Figure 9**). In the latter case the MAR subdomain is likely to be folded in a particular way. This model is also consistent with the ordered positioning of AT-patches in the subdomain.

It should be pointed out finally that other factors like bent structure or inverted repeats might also be involved since these motifs are indeed enriched in the concatemers. We have not done any systematic experiments to test this possibility.

IV. Prediction of MAR from sequence information

As genome projects on human and other species advance, sequence databases are increasingly flooded with uncharacterized genomic sequences. Since MAR is related to many important nuclear functions, it would greatly facilitate the prediction of functional organization of unknown genomic regions if one could predict the precise genomic location of MARs by using computer-assisted search methods. This information may also be useful in gene manipulation technologies including gene therapy. The common strategy adopted in recent works along this line is to search for a variety of sequence patterns that are known to

occur frequently in MARs, e.g., AT- or GT-rich short tracts, curved or kinked DNA motifs, or topoisomerase II cleavage motifs. The statistical significance calculated for observed frequencies of these patterns are then combined to derive a probability function ("MAR potential") which is computed over the region of interest using a sliding window algorithm (Singh et al., 1997). This survey protocol successfully detected known MARs in several genomic regions.

We have applied the MAR organization principle formulated above to predict MARs in various DNA regions containing known MARs and obtained satisfactory results with considerably small incidence of false negatives and false positives. The method also predicted MARs in some genomic regions where presence of MAR had not been assessed experimentally. The MAR activity of candidate regions was confirmed later by binding assays with isolated nuclear matrix (Tsutsui et al., unpublished).

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References

- Adachi, Y., Käs, E., and Laemmli, U.K. (1989) Preferential, cooperative binding of DNA topoisomerase II to scaffold-associated regions. **EMBO J.** 8, 3997-4006.
- Berezney, R., and Coffey, D.S. (1977) Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. **J. Cell Biol.** 73, 616-637.
- Betz, A.G., Milstein, C., González-Fernández, A., Pannell, R., Larson, T., and Neuberger, M.S. (1994) Elements regulating somatic hypermutation of an immunoglobulin kappa gene: critical role for the intron enhancer/matrix attachment region. **Cell** 77, 239-248.
- Boulikas, T. (1995) Chromatin domains and prediction of MAR sequences. **Int. Rev. Cytol.** 162A, 279-388.
- Buhrmester, H., von Kries, J.P., and Strätling, W.H. (1995) Nuclear matrix protein ARBP recognizes a novel DNA sequence motif with high affinity. **Biochemistry** 34, 4108-4117.
- Cockerill, P.N., and Garrard, W.T. (1986) Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. **Cell** 44, 273-282.

Figure 8. List of all possible hexaconcatemers.

Monomers with the alternative orientations are shown by red and yellow bars. T- and A-patches are painted dark blue and green, respectively.

Figure 9. Models for organization of MAR subdomains by interacting proteins. **(a)** Complex formation by disordered aggregation of proteins. **(b)** Formation of an organized complex aided by specific protein-protein interactions. AT-patches are shown by dark disks and proteins by shadowed spheres.

- Cook, P.R., and Brazell, I.A. (1975) Supercoils in human DNA. **J. Cell Sci.** 19, 261-279.
- Dickinson, L.A., Joh, T., Kohwi, Y., and Kohwi-Shigematsu, T. (1992) A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. **Cell** 70, 631-645.
- Dickinson, L.A., and Kohwi-Shigematsu, T. (1995) Nucleolin is a matrix attachment region DNA-binding protein that specifically recognizes a region with high base-unpairing potential. **Mol. Cell. Biol.** 15, 456-465.
- Dreyfuss, G., Choi Y.D., and Adam S.A. (1984) Characterization of heterogeneous nuclear RNA-protein complexes in vivo with monoclonal antibodies. **Mol. Cell. Biol.**, 4, 1104-1114.
- Gasser, S.M., and Laemmli, U.K. (1986) The organization of chromatin loops: characterization of a scaffold attachment site. **EMBO J.** 5, 511-518.
- Gasser, S.M., Amati, B.B., Cardenas, M.E., and Hofmann, J.F.-X. (1989) Studies on scaffold attachment sites and their relation to genome function. **Int. Rev. Cytol.** 119, 57-96.
- Ide, T., Nakane, M., Anzai, K., & Andoh, T. (1975) Supercoiled DNA folded by non-histone proteins in cultured mammalian cells. **Nature** 258, 445-447.
- Izaurralde, E., Käs, E., and Laemmli, U.K. (1989) Highly preferential nucleation of histone H1 assembly on scaffold-associated regions. **J. Mol. Biol.** 210, 573-585.
- Käs, E., Izaurralde, E., and Laemmli, U.K. (1989) Specific inhibition of DNA binding to nuclear scaffolds and histone H1 by distamycin. The role of oligo(dA).oligo(dT) tracts. **J. Mol. Biol.** 210, 587-599.
- Kiledjian, M., and Dreyfuss, G. (1992) Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box. **EMBO J.** 11, 2655-2664.
- Lichtenstein, M., Keini, G., Cedar, H., and Bergman, Y. (1994) B cell-specific demethylation: a novel role for the intronic kappa chain enhancer sequence. **Cell** 76, 913-923.
- Ludérus, M.E., de Graaf, A., Mattia, E., den Blaauwen, J.L., Grande, M.A., de Jong, L., and van Driel, R. (1992) Binding of matrix attachment regions to lamin B1. **Cell** 70, 949-959.
- Mirkovitch, J., Mirault, M.-E., and Laemmli, U.K. (1984) Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. **Cell** 39, 223-232.
- Okada, S., Tsutsui, K., Tsutsui, K., Seki, S., and Shohmori, T. (1996) Subdomain structure of the matrix attachment region located within the mouse immunoglobulin κ gene intron. **Biochem. Biophys. Res. Commun.** 222, 472-477.
- Portman, D.S., and Dreyfuss, G. (1994) RNA annealing activities in HeLa nuclei. **EMBO J.** 13, 213-221.
- Romig, H., Fackelmayer, F.O., Renz, A., Ramsperger, U., and Richter, A. (1992) Characterization of SAF-A, a novel nuclear DNA binding protein from HeLa cells with high affinity for nuclear matrix/scaffold attachment DNA elements. **EMBO J.** 11, 3431-3440.
- Singh, G.B., Kramer, J.A., and Krawetz, S.A. (1997) Mathematical model to predict regions of chromatin attachment to the nuclear matrix. **Nucleic Acids Res.** 25, 1419-1425.
- Tsutsui, K., Tsutsui, K., & Muller, M.T. (1988) The nuclear scaffold exhibits DNA-binding sites selective for supercoiled DNA. **J Biol Chem** 263, 7235-7241.
- Tsutsui, K., Tsutsui, K., Okada, S., Watarai, S., Seki, S., Yasuda, T., and Shohmori, T. (1993) Identification and characterization of a nuclear scaffold protein that binds the matrix attachment region DNA. **J. Biol. Chem.** 268, 12886-12894.
- Vaughan, P.S., Aziz, F., van Wijnen, A.J., Wu, S., Harada, H., Taniguchi, T., Soprano, K.J., Stein, J.L., and Stein, G.S. (1995) Activation of a cell-cycle-regulated histone gene by the oncogenic transcription factor IRF-2. **Nature**, 377, 362-365.
- von Kries, J.P., Buhrmester, H., and Strätling, W.H. (1991) A matrix/scaffold attachment region binding protein: identification, purification, and mode of binding. **Cell** 64, 123-135.
- Xu, M., Hammer, R.E., Blasquez, V.C., Jones, S.L., and Garrard, W.T. (1989) Immunoglobulin kappa gene expression after stable integration. II. Role of the intronic MAR and enhancer in transgenic mice. **J. Biol. Chem.** 264, 21190-21195.
- Zlatanova, J.S., and van Holde, K.E. (1992) Chromatin loops and transcriptional regulation. **Crit. Rev. Eukar. Gene Expr.** 2, 211-244.