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REVIEW

Scaffold/Matrix Attachment Regions and Intrinsic DNA Curvature

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Abstract—Recent approaches have failed to detect nucleotide sequence motifs in Scaffold/Matrix Attachment Regions (S/MARs). The lack of any known motifs, together with the confirmation that some S/MARs are not associated to any peculiar sequence, indicates that some structural elements, such as DNA curvature, have a role in chromatin organization and on their efficiency in protein binding. Similar to DNA curvature, S/MARs are located close to promoters, replication origins, and multiple nuclear processes like recombination and breakpoint sites. The chromatin structure in these regulatory regions is important to chromosome organization for accurate regulation of nuclear processes. In this article we review the biological importance of the co-localization between bent DNA sites and S/MARs.

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Key words: nuclear matrix, nuclear scaffold, S/MARs, intrinsically bent DNA, *in silico* prediction

SCAFFOLD/MATRIX ATTACHMENT REGIONS (S/MARs)

Nuclear matrix structure. The eukaryotic chromatin fiber is organized into large domains or loops separated through their interaction with a skeleton (scaffold) or the interphase nuclear substructure (nuclear matrix). Due to the dynamic role of this structure the term "nuclear matrix", which implies rigidity, is preferred to the alternative terms [1]. The nuclear matrix is a structural framework formed by a network of proteins that consists of a complex nuclear pore–nuclear lamina, a residual nucleolus, and an internal fibrillar–globular network [2, 3]. The nuclear lamina—a protein meshwork underlying the nuclear membrane [1, 4]—may play an important role in the functional organization of interphase chromatin.

S/MARs features. The specific DNA sequences at the bases of the loops are denominated Matrix Attachment Regions [5] or Scaffold Attachment Regions—S/MARs [6, 7]. The term S/MARs employed in this review is used in accordance with Bode et al. [8]. The length of the DNA loops formed by attachment to the nuclear matrix varies according to the relative S/MAR distance. It has been estimated that these loops range from 5 to 200 kb in size [9]. Two types of S/MARs have been reported: permanent (constitutive), which contain regulatory non-transcribed DNA, and transient (facultative), which contain transcribing and replicating DNA [10, 11]. S/MARs are located more often in non-coding regions of DNA containing putative regulatory elements and binding sites of DNA topoisomerase II [5, 12]. Topoisomerase II is a component of scaffold or matrix isolated by high salt extraction [13, 14]. It has been reported by Iarovaia et al. [15] that loop anchorage sites, which were mapped by topoisomerase II-mediated DNA loop excision protocol, co-localize with weak S/MARs, which have fragile association at the nuclear matrix, indicating that these regions may be an essential but not sufficient element of DNA loop anchorage sites.

Although no obvious characteristics common to nucleotide sequences of S/MARs have been revealed, these regions generally have AT-rich sequences, as the $(AT)_n$ and ATATTT motifs, which are an essential feature for melting under relatively mild conditions [16]. These AT-rich regions are preferentially cleaved/protected by S/MAR-binding proteins [14, 17].

Experimental detection of S/MARs. S/MARs are defined as DNA fragments that can especially bind to isolated nuclear matrices, residual structures produced by histone-depletion of nuclei. There are different methods for isolating S/MARs (e.g., *in vitro* and *in vivo* assay [2, 5, 7, 15, 18]). In the *in vitro* assay, the nuclei are depleted by

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DNase I digestion; histones and other nonhistone proteins are extracted with 2 M NaCl. The nuclear matrices are then incubated with exogenous, end-labeled DNA fragments from the region of interest. Bound DNA fragments are collected by centrifugation, purified, and visualized by agarose gel electrophoresis and autoradiography [5, 18].

The *in vivo* detection separates DNA sequences into matrix associated and non-matrix associated fragments (Fig. 1). In this assay, nuclei are extracted with lithium diiodosalicylate (LIS) and the released DNA is digested with a combination of restriction enzymes. The matrices are recovered by centrifugation and the fraction in relation to DNA matrix bond and non-bond regions are detected by Southern blotting or PCR [2, 7, 16, 19-21] using specific probes and visualized by electrophoresis.

DNA loops and S/MARs can be visualized by *in situ* hybridization (FISH) analysis, indicating the functions

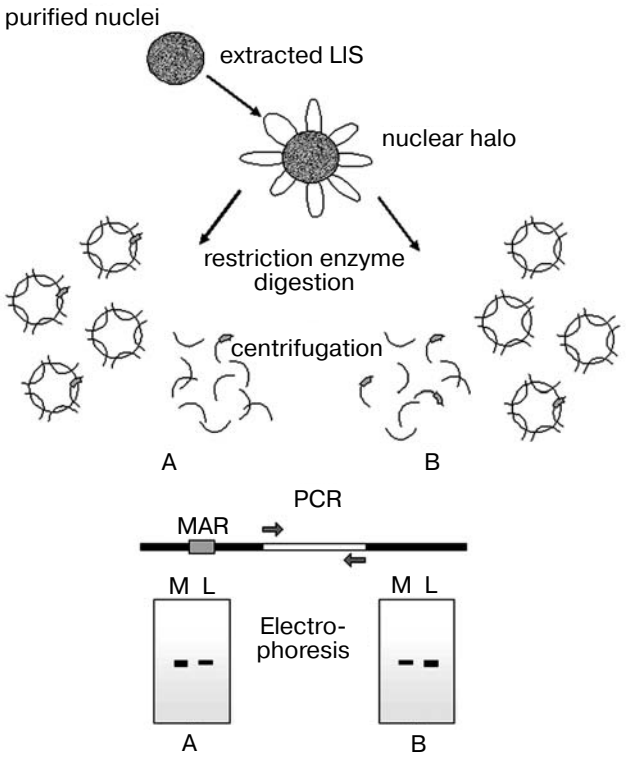

Fig. 1. *In vivo* detection of S/MARs. This assay measures the separation of DNA sequences between associated and non-associated matrix fractions. Purified nuclei are extracted using LIS, and the nuclear "halo" is digested using combinations of restriction enzymes. The matrices are pelleted by centrifugation. The supernatant containing the loops (L) and the pellet containing the matrix-associated fractions (M) are placed in separate tubes. Appropriate PCR primer pairs can be designed to investigate the predicted S/MARs and the products can be visualized by electrophoresis. In some situations, as in transcription control, the nuclear matrix association is dynamic, as in this example: A) there are more DNA regions associated with the matrix than regions not associated; B) there are more DNA regions not associated with the matrix than associated with it. LIS, lithium diiodosalicylate.

and other structural features of these regions [11, 22-24]. Recently, a new proposal for mapping S/MARs in large genomic segments was reported [25] based on microarray technology. Now this methodology, which can substitute the conventional approach for identifying S/MARs, is a powerful tool because of accessible easiness of the sequence of several genomes in biological databanks [26].

Relationship between S/MARs and nuclear processes. Since the discovery of chromosomal DNA loops, the relationship between the organization of DNA into loops and the distribution of genome functional units has been intensively discussed. A variety of nuclear functions, which are supposed to be mediated by S/MARs, have been assigned to the nuclear matrix [8]. It has been suggested that the nuclear matrix may have a role in chromatin organization, as well as replication and gene expression [1]. S/MARs are involved in the modulation of transcription in mammals and plants [27, 28]; they have also been experimentally identified in several defined gene loci, including the chicken lysozyme gene [29], the human interferon-β gene [8], the human β-globin gene [30], the chicken α-globin gene [10, 25, 31], p53 [32], the human protamine gene cluster [33], and the AMPD2 amplicon of Chinese hamster cells, where, despite a defined locus, four S/MARs were identified in intergenic regions [34]. S/MARs may mediate the opening of chromatin in the region of a downstream promoter with subsequent enhancement of transcription [35].

Genes can be located at or near S/MAR elements such as the human rDNA gene, the Chinese hamster ovary *DHFR* gene, the human *c-myc* gene, and the chicken *H5* gene [36]. All S/MARs described by Homberger [37] have been found to be located up or downstream from *Drosophila* genes. It is known that S/MARs occur together with enhancer-like, upstream regulatory elements of four *Drosophila* genes described by Gasser and Laemmli [2]. The nuclear matrix appears to be an attachment region for replication origins in eukaryotes [21, 38-44]. While approximately 40% of *Drosophila*-isolated S/MARs act as ARS elements in yeast [45], multiple S/MARs have demonstrated ARS activities in yeast cells [46]. In humans, S/MARs located within the first intron of the *HPRT* gene and other gene loci have been shown to function as ARS elements in yeast cells [47]. A nuclear matrix attachment region of silkworm rDNA (rS/MAR) was shown to be acting like active replication origins in the chromosome of yeast cells [48]. S/MAR-binding proteins are directly involved in transcription control [49]. The matrix attachment region binding protein, SAFB1, binds to AT-rich sequences with an unwinding propensity, couples chromatin structure, transcription, and mRNA processing, and it may also repress estrogen receptor (ERα)-mediated transactivation.

It has been demonstrated that over expression of SAFB1 results in growth inhibition of human breast cancer [50]. Increased AT content, thought to provide innate

flexibility and curvature, might make DNA more amenable to scaffold/matrix attachment [51]. At present, S/MARs can only be represented as a series of vaguely defined motifs [52, 53]. Thus, the principal determinant in scaffold/matrix attachment appears to be due to some extrinsic epigenetic feature.

It has been reported that hot spot regions of chromosomal recombination, which can lead to chromosomal rearrangements, are usually separated by 20-100 kb and that distance can be correlated with the average size of DNA loops fixed at the nuclear matrix [24] and that DNA topoisomerase II—a component of the nuclear matrix—can mediate illegitimate recombination *in vitro* [54]. Based on these data, it was reported that illegitimate DNA recombination occurs preferentially at DNA nuclear matrix attachment sites [24]. In this work, the hypothesis was proposed that the nuclear matrix plays an important role in determining the positions of recombinant-prone areas in two breakpoint cluster regions present in the *AML-1* and *ETO* genes.

DNA loop attachment regions were found in the human dystrophin gene. A search for S/MARs by *in silico* analysis, in dystrophin intron 49, revealed that junction 1 occurs within an S/MAR [55]. Three out of four replication regions mapped in the dystrophin gene colocalize with loop attachment regions and the major deletion hot spot in human dystrophin gene, mapped by Topo II excision protocol and FISH experiments, is harbored in an attachment region [56]. McNoughton et al. [57] have analyzed deletion breakpoints in intron 7 of the human dystrophin gene, and they proposed that the low representation of matrix attachment regions (S/MARs) in this portion of the gene might expose looped DNA to increased torsional stress and breakage.

In silico ANALYSIS OF S/MARs

S/MARs prediction. After the first introduction of the term nuclear matrix by Berezney and Coffey [58], numerous studies on sequence features of S/MARs have been carried out. Computer analysis of DNA conformation may be a useful tool for examining the matrix attachment regions based in sequence motifs found in S/MARs. There is no consensus sequence that is characteristic of S/MARs, but levels of AT-rich DNA and poly(A) tracts [59], among other properties, have been used to predict S/MARs from genomic sequences [60-63]. The most commonly used S/MAR prediction programs are MarFinder, currently known as MAR-Wiz (http://www.futuresoft.org/), SmarTest (http://www.genomatix.de/), and ChrClass (ftp.bionet.nsc.ru/pub/biology/chrclass/chrclas2.zip). Comparison of ChrClass and MAR-Wiz [61] suggested that the accuracy of S/MAR prediction methods could be improved not only via accumulation of information about S/MAR-specific nucleotide motifs, but also via consideration of the internal heterogeneity of nuclear matrix structures and peculiarities of the localization of the analyzed DNA fragments in various structural–functional regions of genomes. The MAR-Wiz algorithm [60] was based on the statistical occurrence of motifs that are related to DNA secondary structure. The motifs, identified in the vicinity of S/MARs used in the MAR-Wiz program, are A/T and T/G rich sequences, consensus motif, ATC sequence, origin of replication (ORI), curved DNA, kinked DNA, and topoisomerase II sites.

INTRINSICALLY BENT DNA

Bent DNA features. DNA molecule can exhibit a high degree of conformational variability. One of the most prominent manifestations of this is the bending of the helix axis. This is observed in two distinct features, intrinsic curvature and flexibility of the DNA. Intrinsic DNA curvature refers to a bending of the DNA helix axis that is the preferred conformation of a particular DNA sequence. DNA flexibility refers to the case with which certain DNA sequence can be bent, for example by being wrapped around a protein. Flexible DNA sequences will not necessary posses any intrinsic curvature in free solution [64]. In this review, only sequence-dependent DNA curvature is taken into account.

The principal sequence feature responsible for intrinsic DNA curvature is generally assumed to be runs of adenines. The occurrence of areas containing blocks of three or more adenine residues (A-tracts) with repetitions of 10 bp or their multiples may promote a curvature in the helix of the DNA [65]. Conversely, when bent motifs are not repeated in the helical turn phase, a superhelix (or anti-bent DNA) is formed [66] (Fig. 2). The extent of DNA curvature may be altered by intrinsic factors, such as length, number of reiterations, and precise position of the A-tracts, or by extrinsic factors, which include the presence of ions such as Cu^{2+}, Mg^{2+}, and polyamines [67, 68].

Models for *in silico* prediction of DNA bending. Three main models have been proposed to describe the details of DNA bending in phased A-tract. The existence of sequence-directed curvature has demanded the further development of theoretical models for DNA structure. The "wedge model" developed by Trifonov and Sussman [69] explains that the bend is due to alterations in the structure of each AA step (two A-tracts) in the DNA sequence. The "junction model" described by Levene and Crothers [70] suggests that intrinsic DNA curvature is only located at the junction between the A-tracts and the rest of the DNA. A third theory called the "mixed sequence model" holds that the bend is caused by a series of slight bends between adjacent bases outside the A-tracts [71]. All the models are useful to predict DNA con-

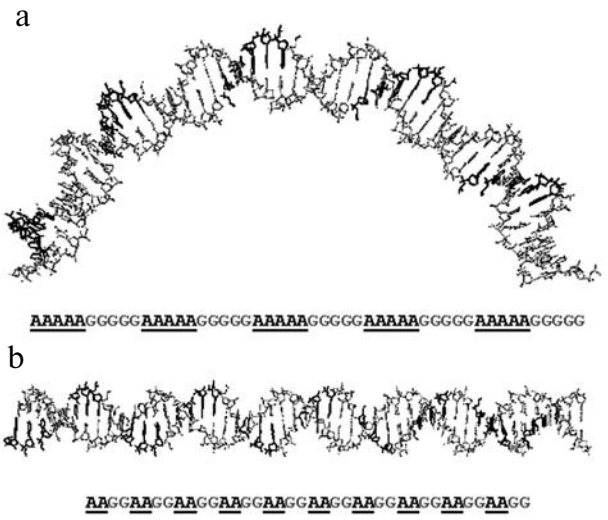

Fig. 2. 2D projection of a three-dimensional path of a hypothetical DNA fragment. a) Tracts of five adenines with repetitions of 10 bp (in phase with the helical repeat) promote a large global curvature. b) Tracts of three adenines with repetitions of 5 bp (out of phase with the helical repeat) promote an anti-bent structure. The A-tracts are bolded and underlined.

formation, though the wedge model seems to be the best [51]. This model assumes that the neighboring base pair stacks are geometrically independent. Bolshoy et al. [72] proposed that, in the wedge model, the DNA axis deflections are described by 16 wedge angles, each comprising roll and tilt components. Several computer programs for curvature analysis were developed using algorithms based on the above, in which the predictions give results that are compatible with electrophoretic data [66].

Electrophoresis of bent DNA containing fragments. Intrinsically bent DNA containing fragments can be experimentally analyzed by its electrophoretic behavior in polyacrylamide gels [73] and also in capillary electrophoresis, as a free solution [74]. In polyacrylamide gels, the mobilities are determined by sieving effects and in free solution, gel matrix sieving effects do not exist [74]. Curved DNA fragments migrate anomalously and slowly in polyacrylamide gels, compared with normal DNA fragments with the same number of base pairs [73, 75]. This gel forms orderly crossed connections and hinder the passage of "irregular" DNA structures [76]. DNA fragments with a large overall bend at its center migrate slower than that of the same bend located at one or both fragment ends [77, 78] (Fig. 3). The anomalously low mobilities have been attributed to the curvature of the helix backbone because curved DNA molecules have larger cross-sectional areas than normal DNA molecules of the same size and, therefore, they require larger pores to migrate thorough the gel matrix, behaving electrophoretically as through they were larger than their true sizes [73, 79]. The explanation of this fact suggested by Stellwagen et al. [74] is that curved DNA molecules are retarded by polyacrylamide gel fibrils and, as a consequence, move slower than normal molecules because they need larger size pores. Another possible explanation is different mobility in free solution and significant difference in behavior of fragments of normal and curved DNA in polyacrylamide gel with large size pores. In polyacryl-

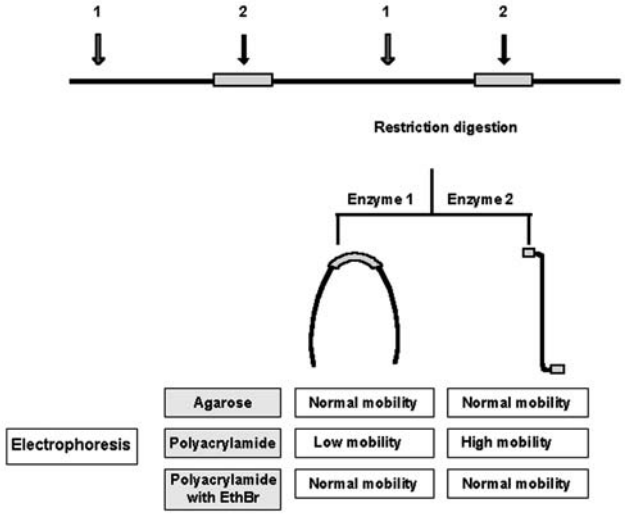

Fig. 3. Influence of the bending position in a DNA fragment on the electrophoretic migration. The map at the top represents a DNA fragment with two bending sites. When cleaved with different restriction enzymes, 1 and 2, restriction fragments can show a centralized bending site or localized in one or both ends.

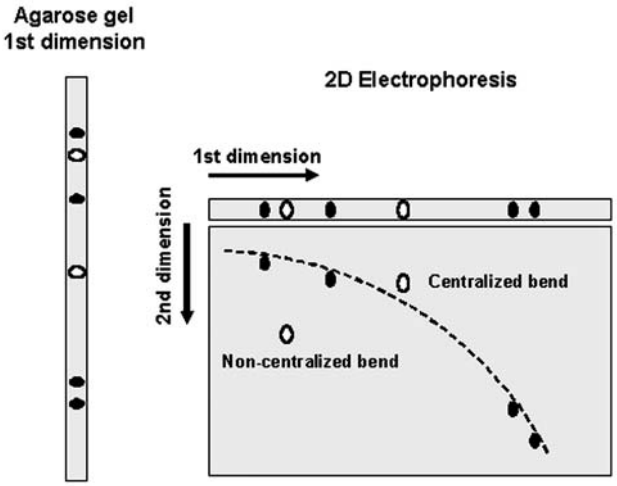

Fig. 4. 2D gel of curved DNA fragments. Fragments (ellipses) are first migrated in agarose gel in the first dimension. Restriction fragments are separated according to their length in the first dimension and only according to their conformation in the second dimension. In the second dimension, fragments with centralized bend migrate slowly and fragments with non-centralized bend migrate more rapidly; they stay above and below the imaginary arc, respectively (white ellipses).

amide gels with intercalating agents such as ethidium bromide and dystamycin [80, 81] all fragments migrated as expected.

DNA curvature containing fragments can be analyzed by 1D and 2D gels electrophoresis (Fig. 4). Therefore, the use of 1D techniques is limited to situations where DNA fragments, which will be submitted to test, have their lengths already known. In addition, only a few different DNA fragments can be tested in each lane. Two 2D gel electrophoresis methods [82] combine agarose and polyacrylamide electrophoresis and they allow length-independent separation of curved DNA fragments containing A-tracts.

RELATIONSHIP BETWEEN S/MARs AND BENT DNA

S/MARs and bent DNA. S/MARs coincide with non-B-DNA structures, like DNA curvature, and are notable for their AT richness and likely narrowing of the minor groove [83]. Since matrix attachment regions revealed no common pattern of nucleotide sequences, it has been demonstrated that the presence of some structural patterns, such as A-tracts, high thermal instability, and sequence-dependent stable curvature, is common in these regions and could have a role in S/MAR functions. Intrinsically curved DNA has been identified at or near matrix attachment sites [51], although this DNA structure is non-essential for matrix binding.

Sites of intrinsically bent DNA have been shown to co-map with S/MARs [38, 51, 65, 84-90]. Nucleotide sequences of the hitherto reported S/MARs are low in homology [91], and these regions can be distinguished from other DNA regions and classified by DNA conformation [51]. Over the last two decades, the role of intrinsic curvature of DNA in biologically important processes such as chromatin organization, transcription, replication, and multiple nuclear processes like breakpoints and recombination has been well documented [78, 92-100].

Protein binding sites. DNA curvature can be involved in the formation of a DNA–protein complex in the nuclear matrix and in processes of recognition of factors responsible for gene regulation and replication initiation. Intrinsically bent DNA sequences exhibit attenuated binding affinities toward proteins, which may be either structural proteins, such as histone and protamine, or DNA-modifying enzymes, and toward minor groove binder species. Recent studies have described specific S/MAR-binding proteins, which include abundant ubiquitous proteins, such as histone H1 [101], DNA topoisomerase II [11], and other proteins, such as SATBI [102] and SAF-A [103, 104]. The interspecific compatibility between S/MARs and their binding proteins seems to be supported by nucleotide sequences of S/MARs and peptide sequences of binding proteins [51]. It has been reported that intrinsic DNA curvature facilitates the binding of proteins such as DNA topoisomerase I [105] and II [106], several nuclear scaffold proteins [107], high mobility group proteins 1 and 2 (HMG-1/2) [108], and a histone-like nucleoid protein in *Escherichia coli* known as H-NS [109]. The topoisomerase II cleavage site might have bending in *Drosophila* [110]. These structural features of the DNA might contribute to scaffold attachment of the proteins responsible for their functions.

S/MARs and bending DNA can be found at or near the nuclear processes; however, the presence of both structures is not found in papers hitherto reported. The topics below relate some events in which these features have been found.

Transcription process. Intrinsic DNA curvature can be a structural feature of the S/MARs present in gene upstream regions and may participate in the transcription process. Bent DNA sites were found in all S/MAR fragments of *D. melanogaster* genes analyzed by Homberger [37]. Buzas and Boldogkoi [111] demonstrated that restriction fragments of the matrix scaffold attachment region (S/MAR) of the chicken lysozyme gene have intrinsic curvature. In plants, intrinsically curved DNA sequences have been found in the ribosomal RNA genes and interact with HMG-related proteins, and may be involved in the transcription of these genes [112, 113]. Fukuda [90] mapped the S/MAR in the upstream tobacco basic class I chitinase gene (*CHN50*) containing a region of intrinsically curved DNA that binds to nuclear proteins. In this work, with shift assay using permuted fragments containing the bend center in different positions [89], maximum connection of the nuclear proteins to DNA was observed when the bent site was centered in these fragments. Results confirm that the binding of nuclear proteins to S/MARs might be a reflection of some structural feature of the DNA rather than its sequence [90]. Recently, we have found intrinsically bent DNA sites in the promoter region of the *BhC4-1* gene of *Bradysia hygida* [78], in which S/MARs were also detected through Southern blot and PCR analysis (A. Fiorini, G. C. dos Anjos Almeida, L. R. Mikami, J. L. C. Silva, and M. A. Fernandez, unpublished results). In this study, the S/MAR prediction, obtained using MAR-Wiz software, revealed that the multiple bending sites in this promoter region co-localize with the MAR potential peaks (Fig. 5).

DNA replication. DNA replication origins can occur in close proximity to (or in association with) the nuclear matrix [36, 114, 115]. However, there are few studies about the presence of DNA curvature in replication origins associated with S/MARs. Segments of bent DNA in the 5′NTS rRNA gene replication origin of *Tetrahymena thermophila* may serve to facilitate initiator protein interactions or the association of the origin with the nuclear matrix [36]. Putative S/MARs were mapped, using

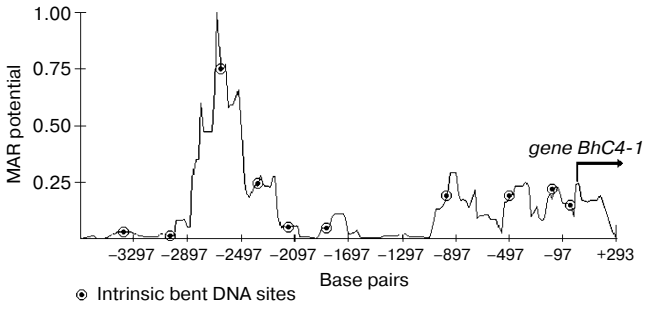

Fig. 5. S/MAR prediction and DNA bending sites. The analyses were performed with a 3990 bp region where 3697 bp is upstream and 293 bp contains part of the *Bradysia hygida BhC4-1* gene transcription unit. The graph indicates the putative S/MARs, which were obtained using MAR-Wiz software. Intrinsic bending DNA sites (points) were previously mapped by Fiorini et al. [78]. The arrow on the graph represents the start site and the direction of *BhC4-1* gene transcription.

MAR-Wiz software, in the *C2-33* gene upstream region of the sciarid *Rhynchosciara americana*, which contains a replication zone [116]. Sequence-derived bends have been found associated with replication origins in the amplified domain of the *AMPD2* gene of the Chinese hamster (Balani et al., manuscript in preparation), in which an S/MAR has also been experimentally identified [34].

Multiple nuclear processes. The relationship between S/MARs and specific nuclear processes was already reported in this review in the previous topic. Here we discuss the association between S/MARs and intrinsic bent DNA sites with other nuclear processes such as DNA recombination. In an analysis of deletion breakpoints in dystrophin intron 49, Nobile [55] reported that one out of five deletions ending in intron 49 was found to occur within an S/MAR. In this paper, it was also shown that there is no common feature sequence across the breakpoints that might facilitate the DNA breakage excepting the sequence TTTAAA that is known to be able to curve the DNA molecule. This sequence was found at or close to the breakpoint junctions 2, 3, and 4. This study suggested that curved DNA may predispose DNA to recombination and/or nuclear processes [60] and therefore to be involved in one of the mechanisms leading to dystrophin [55]. Chromosomal illegitimate recombination junctions in mammals have been reported to be strongly associated with the presence of either bent or anti-bent DNA elements [95], whereas intrinsically bent DNA has also been associated with site-specific recombination in prokaryotes and lower eukaryotes [94].

In conclusion, the presence of structural features such as intrinsically bent DNA and S/MARs within or near regulatory DNA sequences seems necessary to drive the nuclear processes of eukaryotic cells. In addition, DNA curvature can be a structural feature of S/MARs.

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