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Correlations between Scaffold/Matrix Attachment Region (S/MAR) Binding Activity and DNA Duplex Destabilization Energy

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Scaffold or matrix-attachment regions (S/MARs) are thought to be involved in the organization of eukaryotic chromosomes and in the regulation of several DNA functions. Their characteristics are conserved between plants and humans, and a variety of biological activities have been associated with them. The identification of S/MARs within genomic sequences has proved to be unexpectedly difficult, as they do not appear to have consensus sequences or sequence motifs associated with them. We have shown that S/MARs do share a characteristic structural property, they have a markedly high predicted propensity to undergo strand separation when placed under negative superhelical tension. This result agrees with experimental observations, that S/MARs contain base-unpairing regions (BURs). Here, we perform a quantitative evaluation of the association between the ease of stress-induced DNA duplex destabilization (SIDD) and S/MAR binding activity. We first use synthetic oligomers to investigate how the arrangement of localized unpairing elements within a base-unpairing region affects S/MAR binding. The organizational properties found in this way are applied to the investigation of correlations between specific measures of stress-induced duplex destabilization and the binding properties of naturally occurring S/MARs. For this purpose, we analyze S/MAR and non-S/MAR elements that have been derived from the human genome or from the tobacco genome. We find that S/MARs exhibit long regions of extensive destabilization. Moreover, quantitative measures of the SIDD attributes of these fragments calculated under uniform conditions are found to correlate very highly ($r^2 > 0.8$) with their experimentally measured S/MAR-binding strengths. These results suggest that duplex destabilization may be involved in the mechanisms by which S/MARs function. They suggest also that SIDD properties may be incorporated into an improved computational strategy to search genomic DNA sequences for sites having the necessary attributes to function as S/MARs, and even to estimate their relative binding strengths.

Keywords: nuclear architecture; nuclear matrix; scaffold-matrix attachment regions (S/MARs); DNA structure; stress-induced duplex destabilization (SIDD)

Introduction

The organization of the eukaryotic nucleus into chromosomal domains is thought to be mediated by a proteinaceous intranuclear framework, called either the nuclear matrix,[1] or the nuclear scaffold.[2] Branched core filaments provide a supporting structure for the formation of DNA loops, and participate in diverse matrix-supported processes

Abbreviations used: S/MAR, scaffold/matrix attachment region; CUE, core-unpairing element; BUR, base-unpairing region; SIDD, stress-induced duplex destabilization.

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involved in DNA replication and transcription, RNA processing and transport,[3,4,5] signal transduction and apoptosis.[6,7] The DNA elements that mediate attachment of chromatin loops to this nuclear scaffold are called scaffold/matrix attachment regions (S/MARs).[8] S/MARs are operationally defined according to the protocols that led to their detection (see below). The elements that are recovered by these procedures have been implicated in a variety of biological activities that are compatible with an affinity for the nuclear matrix.[9] These include the insulation of transgenes from negative effects of the genomic surroundings (insulator function),[10,11] augmentation of transcription rates,[12] long-term maintenance of high transcription levels by counteracting DNA methylation, and the support of histone acetylation,[13,14] enhancer,[15–18] and origin-of-replication functions.[19,20] During their replication, episomes are segregated by S/MARs functioning as maintenance elements.[21] All these activities may be consequences either of the structures of S/MARs or of their interactions with proteins and/or other molecules.

In line with the above activities, S/MARs have been reported to mediate domain opening.[22,23] During embryonic development, this process is accompanied by a regional demethylation activity.[16,17] These data, together with the results from various insulation experiments,[9–11,24,25] strongly implicate S/MARs in defining the boundaries of autonomously regulated chromatin domains. The occupancy of at least some of these boundaries can be regulated dynamically *in vivo*.[26,27] Since these dynamic effects have not been fully characterized, we will examine measures of matrix affinity and associated biological activities that have been observed consistently for all S/MARs.

S/MARs have clearly been shown to have a variety of effects on transcription. They can act *in cis* to increase transcriptional initiation rates,[28,29] even in the absence of an enhancer. This so-called augmentation activity[12] is clearly distinct from prototypical enhancement, since enhancers, but not S/MARs, are active in transient assays.[30,31] The presence of S/MARs has been shown to prevent the ectopic expression of transgenes.[32] In one case, the presence of an S/MAR proved to be indispensable for correct hormonal gene regulation.[33,34] It has been shown that short subsections of a domain border can substitute for the function of an enhancer-associated element.[35]

Several investigations have documented a direct correlation between the matrix binding and transcriptional augmentation activities of S/MARs.[36–38] This suggests that a standard assay of *in vitro* S/MAR binding strength might predict this regulatory S/MAR activity, and perhaps others as well. However, the general augmentation effect of an S/MAR can be modulated or even disrupted by the over-expression of distinct S/MAR-binding proteins.[39] This finding suggests that an intricate interplay may occur among various S/MAR-binding proteins, which could alter constitutive S/MAR-matrix contacts.[40–42]

The relationship between *in vitro* and *in vivo* S/MAR activities

The first studies of the structure/function relationships of S/MARs performed in this laboratory examined the organization of a 14 kb region containing the human interferon β (IFNB1) gene domain that is located at position 9p22 on the short arm of chromosome 9. The transcription unit of IFNB1 is bounded by a strong 7 kb S/MAR upstream, and a strong 5 kb S/MAR downstream (Figure 1(a)). A second, weakly-associating element has been found between the gene and its strong downstream S/MAR.[43] The occupancy of this secondary S/MAR is regulated *in vivo* according to transcriptional activity.

To investigate the structure and transcriptional activity of the native IFNB1 domain, we made a series of transgene constructs and studied their expression in a mouse L host cell line.[31] Next, we created a series of artificial single-S/MAR and double-S/MAR constructs using various reporter systems. These studies again demonstrated that transcriptional augmentation increased as longer S/MAR-like elements were added, and was maximal for constructs having a minidomain structure in which the reporter gene was bracketed on both sides by S/MARs.[31,44]

The properties that confer S/MAR-binding activity have been studied *in vitro* using artificial elements of the appropriate sizes and minimal sequence complexity, whose binding and transcriptional augmentation properties could be determined in parallel. The first study of this kind constructed a series of oligomers of a 166 bp region from the upstream IFNB1 S/MAR.[36] Neither binding nor augmentation was observed for the monomer, but a simultaneous increase in both activities was found with increasing degree of oligomerization (Figure 4). This and other studies indicate that the strength of *in vitro* binding can be used as a qualitative predictor of transcriptional efficiency.

Specific DNA sequences are not associated with S/MAR-binding activity

The prediction of S/MARs from primary DNA sequence data has proven to be unexpectedly difficult. Despite considerable efforts, no single consensus sequence, pattern or motif has been found to be associated with S/MAR-binding activity. A set of six rules has been proposed which, together or alone, have been suggested to contribute to S/MAR function.[45,46] The observations that suggested these rules, which were based on a relatively small sample set of S/MARs, are: (i) sites of matrix attachment share certain AT-rich tracts with homeotic protein recognition sites and origins of replication; (ii) a number of genes