

Matrix Attachment Regions (MAR/SAR) as Hotspots of Chromosomal Breakage: Mechanistic Insights with Focus on the *AF9* Gene

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ABSTRACT

Objective: To review current evidence on the structural and functional properties of MAR/SAR sequences and their association with chromosomal breakage, with a specific focus on the *AF9* gene and its relevance to translocation events.

Method: A narrative synthesis of published studies examining MAR/SAR characteristics, chromatin architecture, apoptotic nuclease activity, and breakpoint mapping in MLL, *AF9*, ABL, and other translocation-prone genes.

Results: MAR/SAR elements act as preferential cleavage sites during oxidative stress and apoptosis, where nucleases such as CAD target DNA at chromatin loop bases. In the *AF9* gene, two MAR/SAR regions (SAR1 and SAR2) flank breakpoint cluster regions implicated in MLL-*AF9* fusion formation. Oxidative stress enhances SAR-specific cleavage in nasopharyngeal epithelial cells, while non-SAR breakage is associated with repetitive elements rather than MAR/SAR architecture.

Conclusion: MAR/SAR sequences are essential for chromatin organization but also represent intrinsic fragile sites. Their structural properties help explain susceptibility to chromosomal breakage and the formation of cancer-related translocations.

KEY WORDS

MAR/SAR, Nuclear matrix, Chromatin loops, Genomic instability, Chromosomal breakage, *AF9* gene, MLL-*AF9* translocation

INTRODUCTION TO NUCLEAR ARCHITECTURE AND THE FUNCTIONAL IMPORTANCE OF MAR/SAR ELEMENTS

In eukaryotic cells, the nucleus functions as the central repository for genetic information, housing nearly 3 meters of DNA within a compartment only a few micrometers in diameter. This remarkable level of compaction is achieved through hierarchical chromatin folding, made possible by structural scaffolds such as the nuclear matrix, a filamentous RNA-protein network that forms the architectural backbone for nuclear organization¹. This dynamic scaffold maintains nuclear shape and compartmentalization while ensuring sufficient accessibility for transcription, replication, and chromatin remodeling processes.

At the core of chromatin organization is the loop domain model, wherein segments of chromatin are anchored to the nuclear matrix through specific regulatory DNA sequences known as Matrix Attachment Regions/Scaffold Attachment Regions (MAR/SAR)². These anchor points define the boundaries of chromatin loops and create spatial domains that segregate genes and regulatory elements. MAR/SAR sites also assist in the recruitment of MAR-binding proteins (MAR/SARBP), which modulate chromatin topology and help orchestrate multiple nuclear functions³.

Structurally, MAR/SARs lack strict sequence conservation, but they share distinctive biophysical and structural signatures, including AT-rich compositions, DNA curvature, topoisomerase II cleavage motifs, and TG-rich stretches^{4,5}. These properties facilitate DNA flexibility and unwinding—two features essential for transcriptional activation but which also confer fragility under cellular stress.

FUNCTIONAL ROLES OF MAR/SAR SEQUENCES IN GENE EXPRESSION AND CHROMATIN DYNAMICS

The position of chromatin within the three-dimensional nuclear landscape is closely tied to transcriptional competence. For instance, Croft *et al.* (1999) demonstrated that chromosome 19, situated within the nuclear interior and enriched in MAR/SAR anchors, exhibits elevated transcriptional activity, while chromosome 18, positioned peripherally, shows reduced gene expression⁶. MAR/SAR-mediated chromatin anchoring therefore plays a crucial role in establishing transcriptionally active or repressive domains.

MAR/SARs have been shown to enhance transgene stability, integration efficiency, and expression levels across various organisms, further underscoring their regulatory role in genome function⁷. By maintaining open chromatin domains and preventing silencing, MAR/SAR sequences serve as potent epigenetic elements capable of improving gene expression outcomes.

Beyond normal physiological function, MAR/SAR interactions with the nuclear matrix are altered in disease states, particularly in cancers where nuclear organization is extensively remodeled⁸. Given their involvement in genome topology, it is widely believed that a better understanding of MAR/SAR distribution could illuminate mechanisms of genome instability, although a complete genome-wide MAR/SAR map in humans remains to be established.

MAR/SAR AS HOTSPOTS FOR CHROMOSOMAL FRAGILITY AND REARRANGEMENT

MAR/SAR sequences, while essential for chromatin organization, inherently possess DNA unwinding capabilities, making them more vulnerable to cleavage⁹. This fragility becomes especially evident during conditions that induce apoptosis or oxidative stress. Chromosomal breaks tend to occur preferentially at MAR/SAR regions, as these sites represent the bases of chromatin loops where mechanical strain and nuclease accessibility are highest^{10,11}.

During early apoptosis, the chromatin loop bases are specifically targeted for cleavage. The caspase-activated DNase (CAD), a key nuclease in apoptotic DNA fragmentation, localizes to the nuclear matrix and cleaves DNA at MAR/SAR-anchored sites¹². These events generate high molecular weight (HMW) DNA fragments characteristic of initial apoptotic stages^{13,14}.

Importantly, MAR/SAR regions have been repeatedly implicated in illegitimate recombination and pathological chromosomal rearrangements (Broeker *et al.*, 1996). For example, the MLL gene breakpoint cluster region—a hotspot for leukemia-associated translocations—contains two MAR/SAR sequences¹⁵. Similar MAR/SAR-associated breakpoints have been identified in the ABL and *AF9* genes^{11,16}, demonstrating a strong association between MAR/SAR structure and genomic instability.

THE *AF9* GENE: STRUCTURE, FUNCTION, AND SUSCEPTIBILITY TO REARRANGEMENT

The *AF9* gene, located on chromosome 9p21-22, has drawn significant attention because of its dual relevance in both nasopharyngeal carcinoma (NPC) and acute myeloid leukemia (AML). It is frequently deleted in NPC tissues¹⁷ and is also commonly involved in leukemia-associated chromosomal translocations. Notably, *AF9* often forms a fusion with the MLL gene in the t(9;11)(p22;q23) translocation, a hallmark event in specific AML subtypes^{11,18}. The resulting MLL-*AF9* fusion gene acts as a potent oncogenic driver. Evidence from knock-in mouse models demonstrates that this fusion disrupts normal hematopoietic stem cell differentiation, causing abnormal proliferation of myeloid precursors and ultimately resulting in leukemic transformation^{19,21}.

Within the *AF9* locus, two MAR/SAR regulatory sequences have been experimentally characterized. The first, known as SAR1, is located in intron 4, while the second, SAR2, spans exons 5 through 7¹¹. These regulatory regions are positioned adjacent to two clinically derived breakpoint cluster regions: BCR1, situated in intron 4, and BCR2, located between introns 7 and 8. The physical closeness of the MAR/SAR elements to these breakpoint clusters suggests an inherent structural predisposition to DNA breakage and recombination within this genomic region. This distinctive arrangement makes *AF9* a valuable model for investigating how MAR/SAR-associated chromatin architecture may contribute to genome instability and cancer-related chromosomal rearrangements.

CHROMOSOMAL BREAKAGE PATTERNS WITHIN *AF9* MAR/SAR AND NON-SAR REGIONS

Experimental studies investigating MAR/SAR-mediated fragility in nasopharyngeal epithelial cells have demonstrated that oxidative stress, specifically hydrogen peroxide (H₂O₂), induces caspase-3-dependent cleavage within *AF9* SAR regions¹⁶. These findings support the notion that apoptosis-associated nucleases preferentially target chromatin loop bases anchored by MAR/SAR elements.

Moreover, bile acid treatment has been shown to enhance cleavage frequencies within *AF9* SARs²², further validating their susceptibility to chemical and biochemical stress. Interestingly, analyses of non-SAR regions revealed higher-than-expected cleavage frequencies, though these breakages were not dependent on oxidative stress exposure. This indicates that non-SAR regions contain additional fragile elements—namely, repetitive sequences, which have also been implicated in genomic instability and translocation formation^{10,23}.

CONCLUSION

MAR/SAR sequences serve as critical genomic elements that influence chromatin architecture, gene regulation, and nuclear organization. Their inherent unwinding properties and close association with the nuclear matrix render them particularly susceptible to nuclease-mediated cleavage under apoptotic or stress conditions. The *AF9* gene exemplifies how MAR/SAR-rich regions act as hotspots for chromosomal breakage and rearrangement, contributing to both cancer-associated genomic instability and normal chromatin remodelling processes. A deeper understanding of MAR/SAR distribution, structural characteristics, and interactions with nuclear proteins holds significant promise for elucidating mechanisms underlying chromosomal translocations in human diseases. These insights may ultimately lead to novel therapeutic strategies targeting chromatin architecture to maintain genome stability.

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