Nuclear Matrix Interactions within the Sperm Genome*

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Analysis of the haploid-expressed human PRM1 → PRM2 → TNP2 genetic domain has revealed two regions of attachment to the sperm nuclear matrix. These sperm nuclear matrix attachment regions delimit the DNase I-sensitive domain of this haploid-expressed locus. The domain is intimately associated with but not attached to the nuclear matrix. DNase I-sensitive genes within the mature sperm nucleus, such as protamine 1, protamine 2, transition protein 2, α-globin, and β-actin, display this intermediate affinity for the sperm nuclear matrix. This may denote their role in templating the male genome prior to fertilization, thus ensuring the formation of a viable male pronucleus during early embryonic development.

For many years the nuclear matrix received little attention, as it was thought to act merely as a structural element (1). It has now been suggested that the nuclear matrix may play a key role in genome organization and gene potentiation (2). As in the somatic nucleus, chromatin within the male gamete is organized into discrete loops, bound at the base by regions of attachment to the nuclear matrix (3). These loops differ from their somatic counterparts with respect to the packaging of their DNA (4) and their average size. Loops within the sperm nucleus are ∼27 kb¹ in size (5) compared with ∼60 kb in all other types of cells studied to date (6). We have termed these sperm nuclear matrix attachment regions (SMARs) (7). The somatic nuclear matrix has come under intense study, as actively transcribed genes have been shown to be associated with the nuclear matrix (8). Somatic nuclear matrix attachment regions (MARs) have been identified in or near introns (9), enhancers (10), origins of replication (11), and sites of transcription initiation (12), as well as other regulatory elements (9). MARs have also been identified at the ends of the DNase I-sensitive domain in numerous loci (13, 14) and shown to facilitate position-independent gene activity (15). The function of the sperm nuclear matrix is comparatively unknown.

An ∼40-kb region of human chromosome 16p13.13 has recently been sequenced in its entirety and shown to contain the genes for the sperm-specific protamine 1, protamine 2, and transition protein 2 proteins (16). DNase I sensitivity analysis has delineated the boundaries of the domain in the mature spermatozoon, and transgenic analysis has shown that this region of the genome contains all the elements necessary for the appropriate spatial and temporal expression of the genes of this cluster in a position-independent, copy number-dependent manner (17). To characterize structural elements that mediate this response, we have identified regions of genomic interaction with the sperm nuclear matrix. Further, we demonstrate that a specific subset of both haploid-specific and constitutively expressed genes are associated with the mature sperm nuclear matrix. These genes assume an altered structural conformation as evidenced by their increased sensitivity to DNase I. Thus, the mature sperm genome is organized in a specific non-random manner. This could provide the means to template the male genome for ordered protamine replacement immediately subsequent to fertilization.

MATERIALS AND METHODS

Physical characterization of each of the candidate MARs employed nuclei prepared from frozen sperm essentially as described (19). Nuclei were resuspended in 50 mM HEPES, pH 7.5, buffer containing 10 mM NaCl, 5 mM MgOAc, and 25% glycerol, at ∼107/ml, and then used immediately or stored flash frozen at −80°C. DNA halos were prepared from fresh or frozen sperm nuclei as described (5). In brief, sperm nuclei were mixed with an equal volume of 2 M NaCl buffered with 25 mM Tris, pH 7.4, and then pelleted at 4°C for 30 min at 1,600 × g. The pellet was resuspended in 200 μl of 25 mM Tris, pH 7.4, buffer containing 2 M NaCl, and then adjusted to contain 10 mM dithiothreitol. The nuclei were then incubated on ice for 30 min. The resulting halos were centrifuged at 4°C for 30 min at 1,600 × g and then resuspended in 50 mM Tris-HCl, pH 7.5, buffer containing 100 mM NaCl and 10 mM MgCl2. Aliquots were stained with propidium iodide and then visualized by fluorescent illumination using a Leitz DIAPLAN microscope. The remaining halo DNA was subsequently digested with BstXI, EcoRI, HindIII, or SphI for 4 h at 37°C. Successful restriction enzyme digestion was assayed by the inability to amplify across known sites. Following digestion, an equal aliquot of 4 M NaCl was added, and the samples were incubated for an additional 10 min at 37°C. The loop and matrix fractions were then separated by centrifugation for 30 min at 9,000 × g at 4°C. The fractions thus separated were subsequently purified using Prep-A-Gene matrix (Bio-Rad) and then resuspended in deionized water. PCR amplification was performed on both the loop and matrix-associated fractions utilizing primer pairs directed to the PRM1 → PRM2 → TNP2 locus, many of which have been described previously.² PCR was maintained within the linear range of amplification. DNA halos were prepared from HeLa cells essentially as described (21), digested to completion with HindII, and then treated as described above for sperm halos.

RESULTS AND DISCUSSION

To begin to elucidate the elements necessary to potentiate this domain, candidate regions of sperm nuclear matrix association within the PRM1 → PRM2 → TNP2 biological locus were identified utilizing a computational strategy. Characteristic MAR motifs were gathered from the literature (7, 22) and then expressed as unique sequence patterns as described (18). In this manner, the ∼40-kb sequence containing the PRM1 → PRM2 → TNP2 biological locus was queried for the presence of

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² Primer sequences, PCR conditions, and the PRM1 → PRM2 → TNP2 domain sequence will be made available at the internet address "http://compbio.med.wayne.edu".

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The abbreviations used are: kb, kilobase(s); SMAR, sperm nuclear matrix attachment region; MAR, somatic nuclear matrix attachment region; PCR, polymerase chain reaction; bp, base pair(s).
Various sequence patterns indicative of MARs. Motifs were then weighted according to their expected frequency in a random sequence of the same base composition as that of the sequence queried. A weighted sum was subsequently applied to each region along the locus using a sliding window of 1000 bp with a 100-bp step size. The results are presented graphically in Fig. 1. Regions above a likelihood of 50% were considered as candidates to have strong nuclear matrix binding potential. This computer analysis predicted two SMARs centered at nucleotide positions 8,175 and 34,100 (Fig. 1). These potential SMARs were similar to those previously identified in this locus (7) and were used to guide their physical identification.

DNA “halos” were prepared by extracting sperm nuclei with a high ionic strength reducing buffer (5). This displaced the histones and protamines from the chromatin, while leaving the DNA attached at discrete points to the intact nuclear matrix. The resulting halo structures were then stained with propidium iodide and visualized by fluorescence microscopy as shown in Fig. 2. The intact nuclei stained in a uniform manner, consistent with tightly packaged sperm chromatin, while the halo structures showed a more dispersed pattern of staining. Regions of the sperm chromatin that remained associated with the nuclear matrix possessed a brightly staining center, while the unassociated loop DNA stained dimly. This was manifested as a broad fibrous “halo” of fluorescence surrounding the brightly stained nuclear matrix.

To separate the nuclear matrix-bound and unbound DNA, halos were digested with various restriction endonucleases, separated into their loop (supernatant) and nuclear matrix-bound (pellet) fractions, and purified. PCR primer pairs that span specific regions of the PRM1 → PRM2 → TNP2 domain were delimit the corresponding amplicons within the loop and nuclear matrix fractions. A PCR primer set directed to the β-globin locus was used as a non-matrix-associated control. This same region of the β-globin locus contains a somatic MAR, as shown in HeLa nuclei. Nuclear matrix-bound restriction fragments in which greater than 80% of the amplicon partitioned with the matrix-bound fraction are identified as black boxes. Nuclear matrix-associated fragments are indicated by gray boxes for those amplicons that partitioned (30–70%) within both fractions. Non-matrix-associated fragments are demarcated by open boxes for those amplicons that comprised from 0 to 20% of the matrix fraction. Large restriction fragments that contain the SMARs often showed sterically reduced localization to the matrix fraction. Sites of attachment to the nuclear matrix are denoted as stars. Matrix association for the Sty-digested sample could not be ascertained for the β-globin locus, as there is a Sty site between the β-globin primers.

The 5′ region of attachment to the sperm nuclear matrix was bounded by positions 8,818–9,760, while the corresponding 3′ region was bounded by positions 32,586–33,536. The strong attachment to the nuclear matrix of these ~950-bp regions at the ends of the PRM1 → PRM2 → TNP2 DNase I-sensitive domain suggests the presence of a sequence-dependent MAR-like element. However, these regions do not share extensive similarity.

Regions of intermediate nuclear matrix association are likely...
In accord with the data presented above and that of others, there must be more than one type of association with the nuclear matrix. It is reasonable to assume that there are at least four classes of nuclear matrix association, i.e. regulatory element-associated MARs, somatic boundary elements, haploid boundary elements, and structurally associated elements. Class 1 regulatory element-associated MARs possess an innate ability to be bound by the nuclear matrix as they can be identified by an in vitro competition assay (26). These MARs are not typically situated at the ends of the DNase I-sensitive domain. They have been localized to regions containing enhancers (10), origins of replication (11), and other regulatory elements (9) and may also represent regions where transcriptionally generated supercoiling is relaxed (2). Class 1 MARs likely contain specific consensus sequences recognized by cell-specific nuclear matrix proteins. In fact, the nuclear matrix protein NMP-1, which binds to specific sequences within the histone H4 gene, has recently been shown to be the transcription factor YY1 (27).

However, most of these MARs probably do not act as promoters or enhancers themselves. Instead, proximity of the regulatory element to the matrix-associated region and the nuclear matrix may concentrate all of the diverse elements necessary for transcription. In light of the locus-specific regulatory sequence motifs and the array of cell-specific proteins within the nuclear matrix (28), it is possible that no single consensus sequence for the class 1 MAR will be identified.

Class 2 somatic boundary element MARs are localized to the ends of DNase I-sensitive domains and act as boundary elements in somatic nuclei. They may shield loci against inappropriate potentiation and silencing in multiple types of cells. The MARs of the chicken lysozyme locus that delimit the DNase I-sensitive domain have been shown to mediate position-independent expression (15). It has been suggested that end region MARs may regulate transcription by inducing negative superhelical torsional stress across the domains that they limit (2). A universal consensus sequence for this second class of MAR should become clear as more are identified and sequenced, since many loci possess cell-type-independent end region MARs. The AT-rich MAR may be representative of this class.

The regions of matrix association described above for the haploid-specific PRM1 → PRM2 → TNP2 domain are representative of class 3 haploid boundary element nuclear matrix attachment regions, i.e. SMARs. This report is the first identification of a haploid-specific MAR. Like the class 2 MARs, SMARs seem to act as boundary elements, attaching the ends of chromatin domains to the sperm nuclear matrix. The validation of the computational model suggests that SMAR sequences resemble those of the class 2 somatic boundary element MARs. However, MARs and SMARs are not identical. Unlike class 3 SMARs, class 2 somatic MARs have been shown to be cell type-independent. For example, MARs of three developmentally regulated Drosophila melanogaster genes have been shown to exhibit identical binding profiles regardless of tissue type or developmental stage (10). Further, MARs from the β-globin locus remain constant throughout the induction of terminal differentiation of the erythroid progenitors (29), while MARs of the chicken histone genes have been shown to be retained throughout the cell cycle (30). These differences among the class 3 SMARs and the class 1 and class 2 MARs are highlighted in Figs. 3 and 4. Genes like β-globin that contain a somatic cell type-independent MAR (29) do not partition with the sperm nuclear matrix, and SMARs of the haploid-expressed PRM1 → PRM2 → TNP2 domain do not attach to the HeLa nuclear matrix (Fig. 3). As with the class 2 MARs, identification of a consensus sequence for SMARs will depend upon the identification and sequencing of multiple SMARs.

\(^3\) J. A. Kramer and S. A. Krawetz, unpublished observations.
The intermediate association with the sperm nuclear matrix of those genes that exhibit DNase I sensitivity in mature spermatozoa can be considered to exemplify a class 4 structurally mediated nuclear matrix association. This intermediate affinity for the sperm nuclear matrix may be similar to that mediated nuclear matrix association. This intermediate affinity for the sperm nuclear matrix may be similar to that observed for the somatic expressed β-interferon gene. However, in haploid cells, this cannot be identified using an in vitro competition assay, and it appears that it is not dependent on the presence of a consensus sequence. It is not known if this type of association reflects a structural parameter specific to sperm chromatin.

While the function of MARs has been discussed extensively, the biological role for nuclear matrix attachment and nuclear matrix association within the male haploid genome remains to be clarified. The class 3 end region SMARs, like the class 2 end region MARs discussed above, appear to act as boundary elements. It is not clear whether they shield from position effects, as has been shown for some class 2 MARs (15). Three independent lines of transgenic animals containing SMARs from the PRM1→PRM2→TNP2 locus have been shown to yield copy number-dependent, site of integration-independent expression (25). Whether the human PRM1→PRM2→TNP2 locus contains an locus control region and/or utilizes the SMARs as a means of locus control remains uncertain.

The haploid-specific SMARs and the intermediately associated regions described above represent two of at least four classes of nuclear matrix-associated regions. Further clarification of the classes and functions of various nuclear matrix-associated regions will provide both interesting and enlightening toward the study of the mechanisms of gene potentiation and paternal genome templating.

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