Nuclear Matrix Interactions at the Human Protamine Domain

A WORKING MODEL OF POTENTIATION*

Rui Pires Martins‡, G. Charles Ostermeier‡, and Stephen A. Krawetz‡‡¶

Received for publication, August 17, 2004, and in revised form, September 20, 2004
Published, JBC Papers in Press, September 27, 2004, DOI 10.1074/jbc.M409415200

The compact eukaryotic genome must be selectively opened to grant trans-factor access to cis-regulatory elements to overcome the primary barrier to gene transcription. The mechanism that governs the selective opening of chromatin domains (i.e. potentiation) remains poorly understood. In the absence of a well-defined locus control region, the nuclear matrix is considered the primary candidate regulating the opening of the multigenic PRM1 → PRM2 → TNP2 human protamine domain. To directly examine its role, four lines of transgenic mice with different configurations of flanking nuclear matrix attachment regions (MARs) encompassing the protamine domain were created. We show that upon removal of the MARs, the locus becomes subject to position effects. The 3’ MAR alone may be sufficient to protect against silencing. In concert, the MARs bounding this domain likely synergize to regulate the expression of the varied members of this gene cluster. Interestingly, the MARs may convey a selective reproductive advantage, such that constructs bearing both 5’ and 3’ MARs are passed to their offspring with greater frequency. Thus, the MARs bounding the PRM1 → PRM2 → TNP2 protamine domain have many and varied functions.

The final stages of mammalian spermatogenesis are marked by a considerable morphological change, reflective of genomic restructuring mediated by the replacement of the majority of histones with protamines (PRM). These small, basic, arginine-rich proteins compact the genome into the sperm nucleus. This is accomplished in many varied ways throughout the phylogenetic kingdom (reviewed in Ref. 1). For example, mammals, birds, and reptiles utilize a PRM or PRM1-like nuclear protein to repackage their sperm genomes. Compaction can be augmented through the formation of disulfide bonds between adjacent protamines and with the use of a second protamine PRM2 (2). Repackaging of the genome occurs in a stepwise manner with some somatic histones first being replaced by germ cell-specific histone variants (reviewed in Ref. 3). Through a series of DNA strand-breaks (4), supercoiling is lost (5), and some of the histones are displaced by the transition nuclear proteins (TNP1 and TNP2). Finally, the majority of the histones, along with the TNP, are exchanged for protamines.

The human protamine PRM1 → PRM2 → TNP2 gene cluster maps to chromosome 16p13.13 (6). Protamine 1 (PRM1), protamine 2 (PRM2), and transition protein 2 (TNP2) are expressed solely in the testes during a defined stage of spermiogenesis. The specific temporal and spatial pattern of expression of the various members of the PRM1 → PRM2 → TNP2 gene cluster and the ability to isolate purified populations of spermatogenic cells at each stage of differentiation render this system well suited to dissecting the regulatory mechanisms that underlie facultatively expressed genes. This suite of genes resides in a single DNase I-sensitive domain (7) that forms by the late pachytene spermatocyte stage and is maintained in this state in the mature spermatozoa (8, 9). In part, this is believed to reflect the incomplete replacement of the histones with protamines in this region that may be required to initiate repackageing of the male genome to a somatic-like structure upon fertilization (10). The domain is flanked by two haploid-specific nuclear matrix attachment regions (MARs) (11) spanning a distance similar to that of the short sperm chromatin loops (5). The association of these select regions of the genome with the sperm nuclear matrix appears vital to the formation of the male pronucleus (12).

The members of the PRM1 → PRM2 → TNP2 gene cluster are regulated at both the levels of transcription and translation. Both temporal and tissue transcriptional specificity are modulated by the association of various testes-specific factors with their respective promoters (13, 14). Although each member is transcribed at the round spermatid stage, translation is not initiated until the spermatid begins to elongate (reviewed in Ref. 3). This elaborate orchestration of synthesis can only be accomplished once the chromatin domain has formed an open structure (8, 9).

The packaging of chromatin into higher ordered structures in the eukaryotic genome is mediated by DNA-DNA, DNA-histone, protein-protein, and other interactions. These higher ordered structured segments tend to be silent but can, in some cases, permit basal transcriptional activity (15). The transition from a higher ordered conformation to one that is relaxed and amenable to high levels of transcription is termed potentiation (16). This was first observed by increased nuclease sensitivity (17–20) that led to the development of the physical concept of a chromatin domain (19, 21, 22), whereby changes in local chromatin structure must occur before transcription can begin (23–26).

Housekeeping genes are partitioned into chromosomal seg-
Potentiation of the Human Protamine Domain

Table I

Ligation-mediated PCR strategy to produce antisense probes for the ribonuclease protection assay

| Name       | Design | Sequence (5’-3’) | T
|------------|--------|------------------|------|
| PRM1       | fwd    | GGGGATCCCTAGGTCCGCTCTGCTGAATG | 72°C |
|            | rev    | TTTGCTAATGCTCTGCTGATAG       |      |
|            | nested | GAACGCGACGCGAT              |      |
| PRM2       | fwd    | GAACGCGACGCGAT              |      |
|            | rev    | TTTGCTAATGCTCTGCTGATAG       |      |
|            | nested | GAACGCGACGCGAT              |      |
| TNP2       | fwd    | TGGTACTCGGGCGCTGCTGATAC     | 60°C |
|            | rev    | TTTGCTAATGCTCTGCTGATAG       |      |
|            | nested | GAACGCGACGCGAT              |      |
| Prm1       | fwd    | GGGGATCCCTAGGTCCGCTCTGCTGAATG | 72°C |
|            | rev    | TTTGCTAATGCTCTGCTGATAG       |      |
|            | nested | GAACGCGACGCGAT              |      |
| Prm2       | fwd    | GAACGCGACGCGAT              |      |
|            | rev    | TTTGCTAATGCTCTGCTGATAG       |      |
|            | nested | GAACGCGACGCGAT              |      |
| Tnp2       | fwd    | TGGTACTCGGGCGCTGCTGATAC     | 60°C |
|            | rev    | TTTGCTAATGCTCTGCTGATAG       |      |
|            | nested | GAACGCGACGCGAT              |      |

MATERIALS AND METHODS

Constructs and Transgenic Animals—Four constructs bearing different arrangements of MARs about the human PRM1 → PRM2 → TNP2 protamine domain (GenBank™ U15422.1) were generated by restriction endonuclease digestion of cosmid hp3.1 (49). As shown in Fig. 1, this cosmid contains an ~40-kb fragment of human chromosome 16p13.13. Purified DNA was microinjected into fertilized eggs obtained by mating (C57BL/6 × SJL)F1 or C57BL/6 female mice with (C57BL/6 × SJL)F1 male mice essentially as described for single or low copy transgene insertion (50). Transgenes were maintained hemizygous on a C57BL/6 background (Jackson Laboratories, Bar Harbor, ME). Genotype and copy number verification was performed by real time PCR, as described (51).

RNA Isolation and Nuclease Protection—Total RNA was isolated from whole organs by homogenization in 4 ml guanidinium thiocyanate, buffered with 100 mM Tris-HCl, pH 6.5, containing 2% β-mercaptoethanol, 17 mM sarcosyl, using a PRO Scientific 200 homogenizer (PRO Scientific Inc., Oxford, CT). Homogenates were treated with 0.1 volumes of 3.0 M sodium acetate, pH 5.2, and then extracted with 1 volume of pH 4.5, saturated phenol/phenol/sodium dodecyl sulfate solution (50:49:1). The aqueous phase was removed, and then nucleic acids were precipitated following the addition of 1 volume of isopropanol alcohol. The nucleic acid pellet was then suspended in resuspension solution that contained 6 M guanidinium-HCl, buffered with 100 mM Tris-HCl, pH 7.0, containing 20 mM EDTA, 10 mM dithiothreitol. RNA was selectively precipitated overnight at 4 °C following the addition of 0.18 M sodium acetate and 2 M LiBr. The RNA was pelleted by centrifugation and then suspended in the guanidinium-HCl resuspension solution, and purity was verified by spectrophotometry. This was repeated until the A260/A280 ratio of at least 1.6 was obtained. When this was achieved, RNA was precipitated a final time at −20 °C for at least 2 h following the addition of 0.18 M sodium acetate and 0.75 volumes of ethanol. Following precipitation, the RNA was recovered by centrifugation and then suspended in RNAase-free water. RNA integrity was judged by the relative ratio of the 28 and 18 S rRNAs as analyzed by 2% formaldehyde, 1% agarose gel electrophoresis. RNAs with a ratio of 28 to 18 S of at least 1.8 was deemed acceptable.

Antisense RNA probe templates for human PRM1, PRM2, and TNP2 as well as mouse Prm1, Prm2, and Tnp2 were generated by ligase-mediated PCR using Ligation’scribe (Ambion, Austin, TX) as shown in Fig. 1. A eukaryotic 18 S rRNA probe template was purchased from Ambion. Probes were prepared by PCR using Hot Start Taq (Qiagen, La Jolla, CA). A 15-min hot start at 95 °C was followed by 35 cycles of denaturing at 95 °C for 30 s, a 30-s annealing step at 56°C, and then elongation at 72°C for 30 s. A final 10-min extension step at 72 °C for 10 min terminated the reaction.

The primary PCR product was then ligated to a T7 promoter adapter.
Following ligation, the product was subjected to a second round of PCR as above, at an annealing temperature of TA2 (Table I) using the Ambion primer along with the nested primer. Antisense probes, labeled with $^{32}$P-dCTP, were then generated by in vitro transcription using the MAXIscript protocol (Ambion) and then purified by polyacrylamide gel electrophoresis. RNase protection assays were performed with 10 mg of total sample RNA, using the RPAIII kit (Ambion), essentially as recommended by the manufacturer. RNA was hybridized to antisense probes at 48 °C for 16 h. The hybridization mixture containing the hybridized products was then digested with a mixture of RNase A and T1 at a ratio of 1 unit of A/3 units of T1 for 2 h at 37 °C. Protected fragments were then denatured and resolved using a 5% polyacrylamide gel electrophoresis. The protected and resolved products were then visualized by phosphorimaging using a Typhoon 9210 (Amersham Biosciences). Using the probes described, a protected fragment for the human TNP2 message was not detected in any assay, even when 50 mg of total transgenic RNA or 6 mg of normal human testes poly(A)+-enriched RNA obtained from normal males (7) were used (data not shown). Image analysis was then carried out using the Quantity One (Bio-Rad) software suite.

Statistical Analyses—A $\chi^2$ test was applied to binomially distributed data (52). This included assessing whether the transgene was passed with a similar frequency among transgenic lines. A Mann-Whitney test (53) was employed to assess whether the levels of expression of each member of the endogenous and transgenic $\text{PRM1}$, $\text{PRM2}$, and $\text{TNP2}$ protamine locus were similar between transgenic lines.

RESULTS

MARs Convey a Selective Advantage for Transgene Passage—To assess how the MARs of the $\text{PRM1} \rightarrow \text{PRM2} \rightarrow \text{TNP2}$ domain impact expression, a series of transgenic constructs containing one, both, or no-MARs were created. The objective was to produce a series of single copy founders for each transgenic line. This is the most sensitive assay known to reveal position effects in higher ordered eukaryotic systems. The four transgenic constructs, no-MAR, an ~21-kb MluI-HindIII restriction fragment; 3’ MAR only, an ~23.5-kb MluI-EagI restriction fragment; 5’ MAR only, an ~24.5-kb Sail-EcoRV restriction fragment; and 5’ + 3’ MARs, an ~31.5-kb Sail-EagI restriction fragment. b, the mouse protamine domain maps to a syntenic region of chromosome 16A3. Similarly, it is composed of $\text{Prm1}$, $\text{Prm2}$, and $\text{Tnp2}$. The 3’ HSs are marked by downward arrows. Ribonuclease-protected fragments used to evaluate transgene and endogenous gene expression are indicated in both panels by hollow black boxes below the maps.

**FIG. 1.** Human and mouse protamine domains. a, the human protamine domain maps to chromosome 16p13.13. It is composed of three genes, $\text{PRM1}$, $\text{PRM2}$, and $\text{TNP2}$. Haploid-specific MARs are marked as hatched blocks, and the somatic SOCS1 MAR is marked by a checkered block at the far 3’-end. Exons are represented as black boxes, with HSs marked with downward arrows. The four transgenic constructs are depicted: no-MAR, an ~21-kb MluI-HindIII restriction fragment; 3’ MAR only, an ~23.5-kb MluI-EagI restriction fragment; 5’ MAR only, an ~24.5-kb Sail-EcoRV restriction fragment; and 5’ + 3’ MARs, an ~31.5-kb Sail-EagI restriction fragment. b, the mouse protamine domain maps to a syntenic region of chromosome 16A3. Similarly, it is composed of $\text{Prm1}$, $\text{Prm2}$, and $\text{Tnp2}$. The 3’ HSs are marked by downward arrows. Ribonuclease-protected fragments used to evaluate transgene and endogenous gene expression are indicated in both panels by hollow black boxes below the maps.

| construct | freq. to germ line | founder | sex | freq. of passage | copy number |
|-----------|--------------------|---------|-----|-----------------|-------------|
| NO MAR    | 43%                | F143    | ♂   | 20%             | 2           |
|           |                    | F147    | ♂   | 67%             | 2           |
|           |                    | F155    | ♂   | 25%             | 2           |
| 3’ MAR ONLY | 28%              | F243    | ♂   | 33%             | 1           |
|           |                    | F247    | ♂   | 20%             | 1           |
| 5’ MAR ONLY | 60%              | F329    | ♂   | 20%             | 2           |
|           |                    | F333    | ♂   | 14%             | 2           |
|           |                    | F4003   | ♂   | 40%             | 2           |
| FULL      | 67%                | F4907   | ♂   | 50%             | 2           |
|           |                    | F4908   | ♂   | 75%             | 2           |
|           |                    | F4913   | ♂   | 100%            | 2           |
|           |                    | F4954   | ♂   | 50%             | 2           |
|           |                    | F4975   | ♂   | 100%            | 1           |
|           |                    | F4955   | ♂   | 50%             | 2           |

5’ + 3’ MARs that encompass the $\text{PRM1} \rightarrow \text{PRM2} \rightarrow \text{TNP2}$ region of the human genome are summarized in Fig. 1. The transgenic lines were maintained in a hemizygous state on a C57BL/6 (Jackson Laboratories, Bar Harbor, ME) background. All of the different lines were fertile and showed no obvious abnormal phenotype.

Of the 270 mice born, 31 were transgenic, and 14 achieved germ line transmission. As shown in Table II, single copy transgenic animals were obtained from the 3’ MAR only and 5’ + 3’ MAR lines, whereas low, two-copy transgenic animals were created in all other lines. Surprisingly, each construct yielded transgenic animals with varied efficiency ($p < 0.05$). A
greater number of transgenic animals than expected were produced when the no-MAR construct (27%; 8 of 30) and the 5' MAR only construct (36%; 5 of 14) were injected. In comparison, fewer transgenic animals than expected were produced when the 5' + 3' MAR construct was injected (10%; 11 of 105). Moreover, once created, each construct was passed from the founder to the progeny with different efficiencies ($p < 0.05$). Only the 5' + 3' MAR construct founders passed their transgene at the expected Mendelian frequency (56%; 29 of 52). All others passed the transgene at 50% of the expected efficiency. This suggests that constructs bounded by MARs impart a selective advantage onto the integrated locus, since they exhibit a greater stability when integrated into the genome. This is further evidenced by the long term (at least 6-year) stability and expression of similar PRM1 $\rightarrow$ PRM2 $\rightarrow$ TNP2 transgenic constructs that bear both MARs (7, 54).

We and others have routinely observed that MAR-containing constructs are directly targeted to nuclear structures (55, 56). They remain stably associated with the nuclear matrix that is intimately involved with both replication and transcription. These unique properties of the nuclear matrix probably facilitate and reflect the observed long term stable integration of MAR-containing constructs.

**MARs Act as Boundary Elements to the PRM1 $\rightarrow$ PRM2 $\rightarrow$ TNP2 Locus**—The spatial pattern of expression of the various members of the PRM1 $\rightarrow$ PRM2 $\rightarrow$ TNP2 gene cluster was assessed by ribonuclease protection. As shown in Fig. 2, in all cases, appropriate tissue-specific expression was recapitulated. Irrespective of transgene copy number, the PRM1 and PRM2 genes were exclusively expressed in testes but not in brain, heart, kidney, liver, and lung. The expression of the endogenous Prm1, Prm2, Tnp2, or 18 S rRNA genes was not altered even when their transgenic orthologs were expressed.

A striking difference in the level of expression of the transgene was noted when founders within the no-MAR construct family were compared. As shown in Fig. 3C, transgenic expression could not be detected in founder F$_4$155, although the transgene was present in the germ line and passed to subsequent generations (Table II). In contrast, founder F$_4$147 (lane B) displayed an opposite effect. In the latter, both the PRM1 and PRM2 transcripts were detected at a significantly elevated level ($p < 0.05$) when compared with animals from the line bearing both MARs (Table III). This lack of expression and appropriate regulation was not observed in any of the other constructs. Without an end region MAR, the PRM1 $\rightarrow$ PRM2 $\rightarrow$ TNP2 locus is subject to the chromosomal context at the site of insertion.

**3' MAR Modifies the Expression of the Locus**—To examine whether a single flanking MAR exhibited a dominant effect, homogenous MAR-containing lines were created that bore either the 5' MAR or the 3' MAR (Fig. 1). The expression of the various members of the protamine domain was then assessed by ribonuclease protection. The expression of the PRM1 and PRM2 transgenes and the endogenous mouse Prm1, Prm2, and Tnp2 genes as well as the 18 S rRNA gene were assessed by ribonuclease protection. The expression of the PRM1 and PRM2 transgenes from animals containing the 5' + 3' MAR construct is shown in lane A, and expression from independent lines that do not contain any MARs is shown in lanes B and C. The no-MAR animals showed position effects, both up-regulation from founder line 147 (lane B) and silencing from founder line 155 (lane C). This confirms that MARs act as boundary elements, shielding the transgene from the effects of chromosomal context at the site of insertion.
Potentiation of the Human Protamine Domain

The relative expression of the transgene compared with the endogenous gene was determined for four animals (F₁,147) and five animals (5' + 3' MAR).

|          | No-MAR            | 5' + 3' MAR |
|----------|-------------------|-------------|
|          | Median (25th, 75th quartile) | Median (25th, 75th quartile) |
|          | %                  | %           |
| PRM1:Prm1 | 20.13              | 4.8*        |
| PRM2:Prm2 | (12.36, 22.19)     | (3.36, 6.13) |
|          | ND                 | (26.54, 50.69) |

* Expression was significantly lower, comparing the no-MAR constructs with 5' + 3' MAR constructs, as determined by the Mann-Whitney test, \( p < 0.05 \).

3' MAR conveys suppression on the locus

The relative expression of the transgene compared with the endogenous gene was determined for five animals (5' + 3' MAR) and three animals (3' MAR only).

|          | 5' + 3' MAR | 3' MAR only |
|----------|-------------|-------------|
|          | Median (25th, 75th quartile) | Median (25th, 75th quartile) |
|          | %           | %           |
| PRM1:Prm1 | 22.65       | 2.96*       |
| PRM2:Prm2 | (18.30, 45.25) | (2.64, 3.68) |
|          | 19.19*      | (17.08, 24.56) |

* Expression was significantly lower, comparing the 5' + 3' MAR constructs with the 3' MAR only constructs, as determined by the Mann-Whitney test, \( p < 0.05 \).

Two-copy 5' MAR only construct recapitulates expression patterns of the 5' + 3' (flanking) MAR construct

The relative expression of the transgene compared to the endogenous gene was determined for five animals (5' + 3' MAR) and four animals (5' MAR only).

|          | 5' + 3' MAR | 5' MAR only |
|----------|-------------|-------------|
|          | Median (25th, 75th quartile) | Median (25th, 75th quartile) |
|          | %           | %           |
| PRM1:Prm1 | 22.65       | 21.71*      |
| PRM2:Prm2 | (18.30, 45.25) | (2.69, 42.00) |
|          | 45.75*      | (36.72, 54.87) |

* No significant difference was noted when comparing the 5' + 3' MAR constructs to the 5' MAR only constructs, as determined by the Mann-Whitney test, \( p > 0.05 \).

DISCUSSION

Defining the molecular mechanisms that control the temporal and spatial expression of our genes is critical to understanding the physiological mechanisms that drive differentiation. Potentiation (i.e. the opening of chromatin domains) serves as the primary means to guide gene expression. During the mitotic divisions of spermatogenesis, the spermatoozoal nuclear packaging genes of the protamine locus remain in a closed, non-potentiated state. However, during meiosis, the protamine domain is transformed to a potentiated, open chromatin configuration that renders this segment of the genome accessible to the trans-acting factors that are necessary for the expression of the various members of this gene cluster (8, 9). The mechanism by which potentiation is achieved or maintained is not yet known.

It has been difficult to reconcile the observed differences in nuclease sensitivity of potentiated open chromatin domains. Nuclease sensitivity of a potentiated domain can vary from as low as 2.5-fold above background for the ovalbumin-X-Y locus (22), 3-fold for the lysozyme (57) and glyceraldehyde 3-phosphate dehydrogenase (58) loci, and 5-fold for the PRM1 → PRM2 → TNP2 domain in sperm (7) to 10-fold for the PRM1 → PRM2 → TNP2 domain in the round spermatid or pachytene spermatocyte (8). However, recent work describing higher ordered transcriptionally active structures (15, 59) has shown that even 30-nm-like structures can be transcribed. Accordingly, the notion of what constitutes a potentiated domain must be given further consideration.

Open and actively transcribing chromatin is typically marked by a series of N-terminal modifications. They principally include acetylation, demethylation, and/or methylation of specific residues of the H3 and H4 histones (reviewed in Refs. 60 and 61). As exemplified by the Hox gene clusters (62, 63), these modifications are initially localized to promoter regions (64) and then spread throughout the region during periods of high levels of transcription. Whereas these covalent modifications are linked to transcriptionally active chromatin, it remains unclear whether histones or their modification supply the key to opening (i.e. potentiating or maintaining a domain in a configuration that is amenable to transcription) (62, 65–67). This is further supported by our in vivo studies showing that the PRM1 → PRM2 → TNP2 domain remains in a transcriptionally inactive mature spermatoozoa well after the majority of histones are replaced with protamines (10). These observations clearly support the view that histone modification does not constitute the potentiative opening mechanism. Other mechanisms to initiate and/or maintain open, potentiated chromatin configurations must be available to the cell.

A body of evidence is beginning to accumulate suggesting that matrix attachment regions (i.e. MARs) are the likely structural elements that mediate nuclear reorganization (8, 9, 32, 43, 68). Interactions between the genome and the nuclear matrix have been examined in a number of gene systems. For example, specific interactions between distant regulatory elements (29–31) as well as selective attachment to the nuclear matrix (32) through differentiation have been shown to be key in regulating other developmental loci. As observed in T-cell-specifying genes (40, 42, 43), β- and ε-globin genes (40), or estrogen receptor-responsive genes (41), recruitment to the nuclear matrix probably brings each domain into close proximity to chromatin-modifying and tran-
scription-promoting factors. Similarly, the PRM1 → PRM2 → TNP2 domain has been shown to be nuclear matrix-associated in mature spermatozoa (11) and in a potentiated conformation by the late pachytene spermatocyte stage of spermatogenesis (8, 9). It was thus reasonable to pose that a positive relationship must exist between nuclear matrix attachment and potentiation.

To evaluate how MARs regulate the expression of the protamine gene cluster, transgenic mouse lines containing both, one, or neither of the two previously identified domain-flanking spermatozoal MARs (11) were created. Expression analysis showed that in the absence of MARs, the locus was subject to position effects (Table III, Fig. 3). When flanked by both an upstream and downstream MAR, the levels of transgene expression remain unchanged. This is observed among the various lines bearing the 5’ + 3’ MAR constructs and the two-copy 5’ MAR only constructs (Table V). Consistent with previous clinical analyses (69), although tempered, even the single 3’ MAR may provide sufficient shielding over the domain to prevent absolute silencing of the transgene. Alternatively, or in conjunction, the 5’ MAR may modulate the repressive effect of the 3’ MAR so that they synergistically interact to regulate the expression of the various members of the PRM1 → PRM2 → TNP2 domain. Structurally, this could be set in motion by the two haploid-specific MARs tethered to the nuclear matrix. As proposed in Fig. 4, whether through their interaction or through the combined insulative effect of the MARs, the locus is expressed in an autonomous tissue-specific manner. Subsequent to this structural transformation, the domain functionally changes as it brings the genes to the nuclear matrix to promote their association with a series of chromatin-modifying complexes including ATP-dependent chromatin remodelers and histone acetyltransferases (40, 43). These modifications could then be propagated throughout the domain in a manner similar to that seen with other gene clusters (62, 63). Bringing either end of the PRM1 → PRM2 → TNP2 domain to the nuclear matrix presents an opportunity for the upstream and downstream MARs or other neighboring regulatory elements to interact. This would include the elements demarcated by the group of HSs that colocalize with the 3’ MAR and 5’ MAR as well as those encompassing the various promoter regions (70).

Whereas the role of the HSs associated with the PRM1 → PRM2 → TNP2 domain remains to be delineated, they are probably similar to those that colocalize with cis-regulatory regions that demarcate sites of trans-factor contact. Perhaps the best characterized group of HSs are those of the locus control region of the β-globin gene cluster, each serving an enhancer or insulator function (reviewed in Ref. 71) as part of the “active chromatin hub” (29–31). The colocalization of matrix binding sequences with human β-globin HSs (32) and transcribed regions may mediate formation. A series of factors, CTCF (72, 73) and SATB1 (46), that are known to bind to the nuclear matrix have been localized to these regions. To date, the trans-factor(s) associated with the HSs of the human protamine domain have not been isolated, but as we have observed, deletions in the regions containing some of these HSs in the upstream and downstream portions of the locus influenced the patterns of protamine expression. It is certain that their isolation and characterization will provide further insight into the potentiative mechanisim.

Acknowledgments—We acknowledge contributions from Robert Goodrich in animal husbandry and Maggie Van Keuren for preparation of transgenic mice in the Transgenic Animal Model Core of the University of Michigan’s Biomedical Research Core Facilities.
Nuclear Matrix Interactions at the Human Protamine Domain: A WORKING MODEL OF POTENTIATION
Rui Pires Martins, G. Charles Ostermeier and Stephen A. Krawetz

J. Biol. Chem. 2004, 279:51862-51868.
doi: 10.1074/jbc.M409415200 originally published online September 27, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M409415200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 70 references, 34 of which can be accessed free at http://www.jbc.org/content/279/50/51862.full.html#ref-list-1