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Differentiation: the selective potentiation of chromatin domains

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SUMMARY

Potentiation is requisite for the expression of our genome. It is the mechanism of opening chromatin domains to render genes accessible to tissue-specific and ubiquitous transacting-factors that enables transcription. The results presented in this study demonstrate that modulation of stage- and cell-type-specific gene expression during mammalian spermatogenesis involves selective potentiation of testis-expressed genes that reverses their repressive state when present in the spermatogonial stem cell. This directly contrasts hematopoiesis, which acts to selectively restrict lineage potential during differentiation from its permissive stem cell. These results are key to understanding how differentiative pathways are controlled and cellular phenotypes determined. A window to their modulation is presented.

Key words: Potentiation, Chromatin structure, Protamine, Phosphoglycerate kinase, Spermatogenesis, Determination, Commitment

INTRODUCTION

Every step along the pathway toward gene expression, from potentiation of a chromatin domain to the utilization of the functional encoded product presents opportunities for the control of differentiation, growth and development. There is a significant body of evidence that chromatin structure represents the first level of control that directs appropriate tissue-specific expression in higher eukaryotes (reviewed in Felsenfeld, 1996). Prior to the onset of transcription, the chromatin domain that encompasses the gene(s) must open. This potentiative process renders large segments of the genome available to the tissue-specific and ubiquitous transacting-factors that are necessary for appropriate gene expression.

A consensus is now emerging that the opening of a genic domain serves as the cornerstone for the three-tier mechanism of potentiation→initiation→elongation that controls eukaryotic transcription. The first tier, potentiation, i.e., a genic domain adopting a regionally altered open chromatin structure, is a necessary prerequisite, but alone is not sufficient to initiate transcription. It is required to provide the transcriptional transacting-factors with access to specific enhancer and promoter regions of the genome. The second tier, initiation, i.e., the formation of the transcription complex, can then prime the gene for expression. Finally, the third tier, i.e., elongation, yields the gene transcript.

Hematopoiesis has been utilized as a primary model system to study this first tier of transcriptional control. Insight can be gained by considering the contrast displayed between the facultatively potentiated β-globin domain of human chromosome 11 and its co-regulated counterpart, the constitutively potentiated, α-globin domain that is near the telomere of chromosome 16. The α-globin domain comprises the erythroid-specific α-globin gene cluster plus several other genes, including the constitutively expressed 3-methyladenine DNA glycosylase gene (Vickers et al., 1993; Vyas et al., 1995; Flint et al., 1997). Although the erythroid-specific α-globin gene cluster exists in a region of constitutively potentiated euchromatin, it is not obligated to transcription in non-erythroid cells. In contrast, the β-globin domain is in a closed-chromatin conformation in all non-erythroid lineage cells, only potentiated in cells of the erythroid lineage. Accordingly, potentiation enables the interaction of gene-specific *trans*acting factors with their corresponding *cis*-promoter and enhancer elements. It is the necessary obligate for transcription but in itself, not sufficient for transcription.

The role of chromatin structure in mediating somatic cell gene expression is relatively well appreciated when compared to that of germ cells. This is despite the fact that mammalian spermatogenesis provides an excellent model system to address when potentiated domains become committed and how potentiated domains are determined. Spermatogenesis is a complex differentiative pathway marked by the activation of a unique set of testis-specific genes. The expression of these genes is under exacting spatial and temporal control and occurs at discrete stages throughout male gametogenesis. For example, in primary spermatocytes there is a loss of expression of the X-linked phosphoglycerate kinase-1 (P*GK1*) gene at the

onset of X-chromosome inactivation during prophase of meiosis I. This loss of P*GK1* expression is compensated by the initiation of expression of the testis-specific autosomal P*GK2* gene (McCarrey et al., 1992).

Other testis-specific genes encode for proteins that perform roles unique to spermatogenesis such as the repackaging of the genome. During the pachytene spermatocyte stage, the somatic histone complement is supplemented by several testis-specific histone variants including H1t, TH2A and TH2B (Brock et al., 1980; Bucci et al., 1982; Meistrich et al., 1985). This is followed in stage 7 spermatids by their displacement with the transition proteins that persist until stage 15 spermatids (Heidaran et al., 1988; Morales et al., 1991; Shih and Kleene, 1992; Saunders et al., 1992). Subsequently, the transition proteins are replaced by the small, highly positively charged protamines. The mammalian protamine genes, protamine 1 (P*RM1*), protamine 2 (P*RM2*) transition protein 2 gene (TNP2) exist as a single coordinately expressed multigenic chromatin domain (Choudhary et al., 1995). The members of the P*RM1*→P*RM2*→T*NP2* domain are transcribed in the round spermatid, where their mRNA is stored for translation in the elongating spermatid (Wykes et al., 1995).

The nuclear changes that occur in the developing male gamete correspond to discrete morphological changes during spermatogenesis (Clermont, 1963). This has permitted the development of strategies to isolate relatively pure populations of male germ cells at specific stages along this differentiative pathway. Thus, spermatogenesis presents an ideal model system to elucidate the role of chromatin structure in determining cell fate during differentiation. To address this issue, the potentiative state of several mammalian testis-specific genes was assessed. The data demonstrate that individual testis-specific genes become potentiated or repressed at discrete times during differentiation. This shows that, unlike hematopoiesis, spermatogenesis is a selective potentiative process that opens specific genic domains to reverse the repressive state that is present in the spermatogonial stem cell.

MATERIALS AND METHODS

Preparation of mouse testicular cells by unit gravity sedimentation

Cells were isolated from the testes of mice as described (Kumari et al., 1996). Primitive type A spermatogonia of >85% purity and Sertoli cells of >95% purity were prepared from the testes of 6-day-old mice, while type A and B spermatogonia of >85% and >75% purity, respectively, were prepared from the testes of 8-day-old mice. Testes from 18-day-old mice yielded preleptotene spermatocytes of >85% purity, a mixture of leptotene and zygotene spermatocytes of >85% purity and early pachytene spermatocytes of >90% purity. Late pachytene spermatocytes and round spermatids each of >90% purity, were prepared from the testes of adult mice of approximately 70 days of age.

Assaying the state of gene potentiation in mouse cells

The DNase I-sensitivity assays were performed on thawed cells stored in FSB (Frozen Storage Buffer), composed of 50 mM Hepes, pH 7.5, buffer, containing 5 mM MgOAc, 10 mM NaCl plus 25 % glycerol as previously described (Choudhary et al., 1995) using the PCR-based assay (Kramer and Krawetz, 1996, 1997). Briefly, purified testicular cell populations isolated by the Cell-sep protocol were divided into 0.5 ml aliquots of ~1×10^7 cells in FSB, then snap frozen and stored at −70°C. Thawed cells were mixed with one tenth volume of 10× DNase I buffer (500 mM Tris HCl, pH 7.5, buffer, containing 100 mM $MgCl_2$ and 10 mM $CaCl_2$) plus 1% NP-40 to permeabilize the cell membranes. The cells were permeabilized on ice for 5 minutes, then centrifuged at 4°C for 10 minutes at 1,000 ***g***. The cell pellet was subsequently suspended in 500 μl of 1× DNase I buffer, then digested with varying amounts of DNase I, as required by the number of cells and type of cell. The amount of DNase I must be optimized for each type of cell, though all cells utilized in this study required ~0.5 to 2 units of DNase I/million cells. Aliquots of 100 μl were removed after digestion at room temperature for 0, 5, 15, 30 and 60 minutes, then terminated by adjusting the reaction mixture to contain 50 mM EGTA. Upon termination, 3 volumes of binding buffer (50 mM Tris-HCl, pH 8, buffer, containing 10 mM EDTA plus 6 M guanidinium hydrochloride) was added to each sample. Subsequently, 25 μl of Prep-A-Gene matrix (Bio-Rad Laboratories, Hercules, CA) was added to bind the DNA. Matrix-bound DNase I-digested DNAs were then washed as recommended by the manufacturer. The DNA was then eluted from the matrix by incubating the matrix bound DNA with 50 μl of TE for 5 minutes at 37°C, followed by 50 μl deionized nanopure water for 5 minutes at 42°C. The eluents were combined, then the pooled DNA digested overnight at 37°C with *Eco*RI. PCR was then performed on each sample in the linear range of amplification as described (Kramer and Krawetz, 1996, 1997). To achieve linear amplification, the time 0 undigested template was serially diluted and an aliquot of the sample was then subjected to PCR using the DNase I-sensitive control β-actin primer set (AACACCCCAGCCATGTACG : ATGTCACGCACGATTTCCC). Linear amplification was achieved when increasing the amount of template from the diluted sample proportionally increased the strength of the signal. The remaining DNase I-digested samples from all time points were then diluted accordingly. It has been our experience that this template dilution strategy maximizes the number of assays that can be performed from a single set of samples. Specific PCR primers and conditions for each primer pair are available on the World Wide Web at http://compbio.med.wayne.edu/. The primer sets are P*rm*1 (CCAACACCTCCCTCAGTCC : CAGGTGGGTGAAACAGCC); P*rm*2 (CAAGAGGCGTCGGTCAT : CTGGGGAGGCTTAGTGATG); T*np2* (GACCACCAGGACTCAAAGC : AGAGTTCCATTTTCCAC-CC); P*gk*2 (ATTGTCATAATGTACTTGCTTCC : GTTTCCTCCCT-AGGAAGGACTGT); P*gk*1 (TAGTGGCTGAGATGTGGCACAG : GCTCACTTCCTTTCTCAGGCAG); β-globin (ACTCACAACCC-CAGAAACAG : CAACCAGCAGCCTAAAAAG) and β-actin as above. PCR products were resolved on 2% agarose gels, then quantitated using the Bio-Image 60S Whole Band Analyzer system with version 3.3 software. T_{50} was defined as the time at which 50% of the template was digested (Choudhary et al., 1995). The corrected T_{50}, denoted as T_{50C}, was calculated for each primer set using the equation $T_{50C}=T_{50a}$ (A_a/A_s), where A_a is the size of the target amplicon and A_s is the size of the smallest amplicon in the data set (Kramer and Krawetz, 1996, 1997). Differences in DNase I digestion due to the small variations in amplicon size were thus corrected (Kramer and Krawetz, 1997). The amplicons ranged in size from 166 to 265 bp averaging 237 bp. Differences in the levels of digestion between the various cell types not reflective of gene potentiative status were also considered. For each cell type, classification of potentiative status was assigned by comparing the experimental T_{50C} value obtained to the T_{50C} values of the DNase I-sensitive open chromatin control and DNase I-insensitive closed chromatin control. The constitutively expressed DNase I-sensitive control β-actin gene provided the benchmark for genes possessing an open-chromatin conformation. The erythroid-specific DNase I-insensitive control β-globin gene provided the benchmark for genes possessing a closed-chromatin conformation.

RESULTS

Assaying the potentiative state of the genome in spermatogenic cells

A potentiated chromatin domain is operationally defined as the region of the chromosome that displays up to an approximately 10-fold enhanced rate of digestion to exogenously added DNase I. This is believed to represent a global change in the conformation of a chromatin domain as it changes from a 30 nm to a 10 nm fiber to assume the DNase I-sensitive potentiated open-conformation. In comparison, regions of DNase I-hypersensitivity represent those specific sites of transfactor contact that display an approximately 100-fold enhanced rate of digestion to exogenously added DNase I. A PCR-based in vivo nuclease sensitivity assay that distinguishes amongst these rates of digestion was developed as a means to determine the potentiative state of genic domains (Kramer and Krawetz, 1996, 1997). An example of the potentiative status assay is shown in Fig. 1, using both the primitive type A spermatogonia stem cell in which the P*rm*1, P*rm*2, T*np*2 and P*gk*2 genes are not transcribed and the round spermatid cell in which their domains are potentiated and genes transcribed. The amplification of these genomic segments from each time point of digestion is shown in panel A. As the time of digestion of the primitive type A spermatogonia by the exogenously added nuclease increased, the intensity of the P*rm*1, P*rm*2, T*np*2,

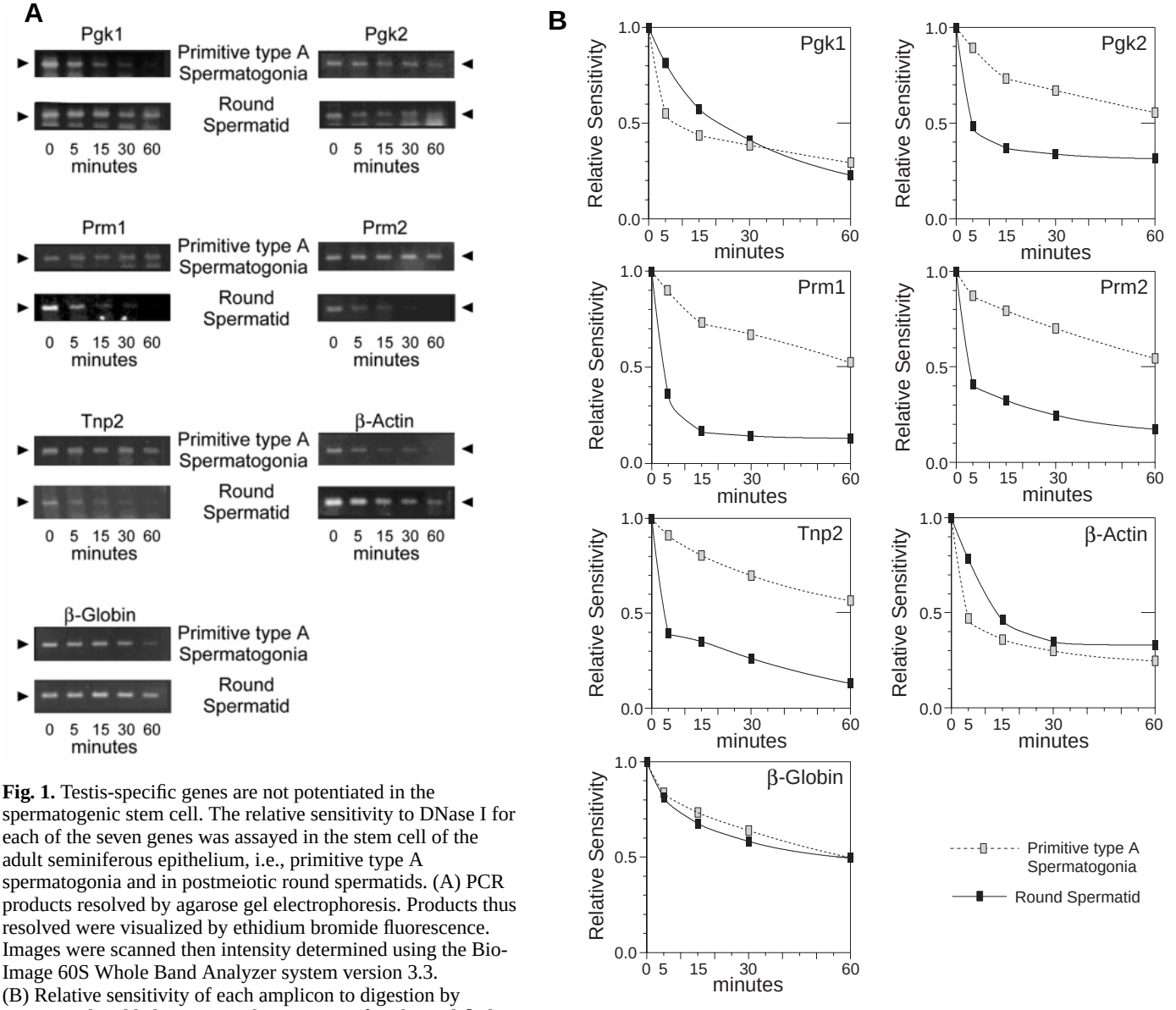

Fig. 1. Testis-specific genes are not potentiated in the spermatogenic stem cell. The relative sensitivity to DNase I for each of the seven genes was assayed in the stem cell of the adult seminiferous epithelium, i.e., primitive type A spermatogonia and in postmeiotic round spermatids. (A) PCR products resolved by agarose gel electrophoresis. Products thus resolved were visualized by ethidium bromide fluorescence. Images were scanned then intensity determined using the Bio-Image 60S Whole Band Analyzer system version 3.3.
(B) Relative sensitivity of each amplicon to digestion by exogenously added DNase I. The intensity of each amplified product was plotted relative to the intensity of the product from the undigested amplicon at t=0 minutes of digestion. Within the stem cell, the testis-specific P*gk2*, P*rm1*, P*rm2* and T*np2* genes are DNase I-insensitive and not potentiated. These genes are potentiated, i.e., DNase I-sensitive in the round spermatid. In contrast, the X-linked P*gk1* gene shows decreased DNase I-sensitivity in round spermatids compared to primitive type A spermatogonia. The non-expressed β-globin gene remains closed relative to the other genes, while the constitutivley expressed β-actin gene is fully potentiated.

P*gk*2 and β-globin genes essentially remained constant (Fig. 1, panel A). This is indicative of these regions of the mouse genome existing in a closed non-potentiated chromatin conformation. These results are in stark contrast to the β-actin and P*gk*1 genes, which are readily digested and thus exist in an open-potentiated chromatin conformation. Fig. 1B shows the corresponding plot of these data from which the time required to achieve 50% digestion (T_{50c}; Choudhary et al., 1995; Kramer and Krawetz, 1997) can be derived. Those regions that exist in a closed non-potentiated conformation exhibited an average T_{50c} ≈97 minutes whereas those regions that exist in an open potentiated chromatin conformation exhibited an average T_{50c} ≈7 minutes.

The potentiative status of these genes in the round spermatid cell, where these haploid-expressed genes are transcribed is also shown. In contrast to that observed in the spermatogonial stem cell and as requisite for expression, the P*rm*1, P*rm*2, T*np*2 and P*gk*2 genes are in an open-potentiated chromatin conformation in the round spermatid. The T_{50c} for this group of genes has changed from an average of ≈97 minutes in the spermatogonial stem cell to an average of ≈6 minutes in the round spermatid. As expected, the constitutively expressed β-actin gene remained in an open-potentiated conformation. Comparatively, the X-inactivated *Pgk1* 1 gene assumed a more closed conformation while the β-globin gene remained in a closed non-potentiated conformation. The recent availability of the PCR-based potentiative assay has provided the means to rapidly assess the potentiative status of any region of the genome when only a limited number of cells are available. This has enabled the examination of potentiative timing during spermatogenesis.

Differential potentiation during mouse spermatogenesis

The results from the above suggested that, unlike the genes of the erythroid lineage, haploid-expressed genes were not potentiated in the stem cell. Accordingly, this set of genes must become potentiated subsequent to the stem cell stage in order to embark upon their differentiative path. To define the exact time during differentiation when the haploid-specific genes first became potentiated required cells from the various stages of differentiation. Cells along the pathway from the spermatogonial stem cell to the round spermatid were purified by unit gravity sedimentation. Primitive type A spermatogonia were isolated from 6-day-old animals, type A and B spermatogonia from 8-day old animals, preleptotene, leptotene/zygotene and early pachytene spermatocytes from 18-day-old animals and round spermatids and pachytene spermatocytes were isolated from 70-day-old mice. Using the PCR-based assay described above, the relative potentiative status of seven genes, i.e., the P*gk1*, P*gk2*, P*rm1*, P*rm2*, T*np2*, β-actin and β-globin genes, were assessed in nine different cell types along this differentiative pathway. The results are summarized in Table 1.

The potentiative status was initially assessed in somatic Sertoli cells since these spermatogenic-expressed genes are not expressed in this cell type yet the Sertoli cell is exposed to the same organ environment. As expected and shown in Table 1, all testis-specific genes (P*gk*2, P*rm*1, P*rm*2 and T*np*2) and the erythroid-specific β-globin gene were not potentiated and existed in a closed-chromatin conformation possessing an average T_{50c} ≈76 minutes. In contrast, the somatic-expressed P*gk*1 gene and the constitutively expressed β-actin gene were in an open-potentiated conformation possessing a T_{50c} of ≈4 and ≈11 minutes respectively. Throughout spermatogenesis, the constitutively expressed β-actin gene remained in a potentiated conformation (Table 1, clear cells), providing an open positive control, whereas the erythroid-specific β-globin gene (only potentiated in cells of the erythroid lineage), remained in a closed non-potentiated conformation (Table 1, shaded cells), providing a negative control. The relative sensitivity of the open β-actin and closed β-globin genes to digestion by exogenously added DNase I was used to define the range of T_{50c} values to classify the potentiative status of each gene within each cell type. This resolved differences in the levels of digestion between the various cell types that were not reflective of changes in gene potentiative status. For example, in preleptotene spermatocytes, the closed β-globin gene possessed a T_{50c} of 40 minutes whereas, in

Table 1. Gene potentiation during mouse spermatogenesis

Cell			T_{50C}[4] and standard error (e)[5]													
Type[1]	Age[2]	Purity[3]	Pgk1	e	Pgk2	e	Prm1	e	Prm2	e	Tnp2	e	β-Act	e	β-Glo	e
Sertoli	6	>95%	4	2	76	9	79	9	88	9	73	9	11	3	66	8
6 day A	6	>85%	7	2	100	7	101	7	105	7	96	10	7	2	82	6
8 day A	8	>85%	11	2	83	9	95	10	90	7	75	9	12	2	68	6
8 day B	8	>75%	6.5	1	12	2	42	5	36	4	54	7	6	2	37	4
preLep	18	>85%	5	2	4	2	45	7	45	7	40	6	5	2	40	6
Lep/Zyg	18	>85%	32*	4	5	2	93	7	90	7	94	10	8	2	96	7
ePach	18	>90%	23*	3	7	2	8	2	7	2	7	3	5	2	54	4
lPach	70	>90%	22*	3	6	2	6	2	7	2	6	2	5	2	88	7
Round	70	>90%	21	3	7	3	6	2	6	2	6	2	18	3	85	9

[1]Type; Sertoli, Sertoli cells; 6 day A, primitive type A spermatogonia; 8 day A, type A spermatogonia; 8 day B, type B spermatogonia; preLep, preleptotene spermatocyte; Lep/Zyg, leptotene and zygotene spermatocytes; ePach, early pachytene spermatocytes; lPach, late pachytene spermatocytes; Round, round spermatids.

[2]Age; age in days after birth.

[3]Purity; determined by J.M.

[4]T_{50C}; values determined as described (Kramer and Krawetz, 1996, 1997).

[5]Standard error (e); reported as the square root of the sum of values from all data sets over *n* (*n*=the number of data sets).

Shaded boxes denote genes in a closed non-potentiated conformation; clear boxes denote genes in an open-potentiated conformation.

*Denotes a transitional DNase 1-sensitive state.

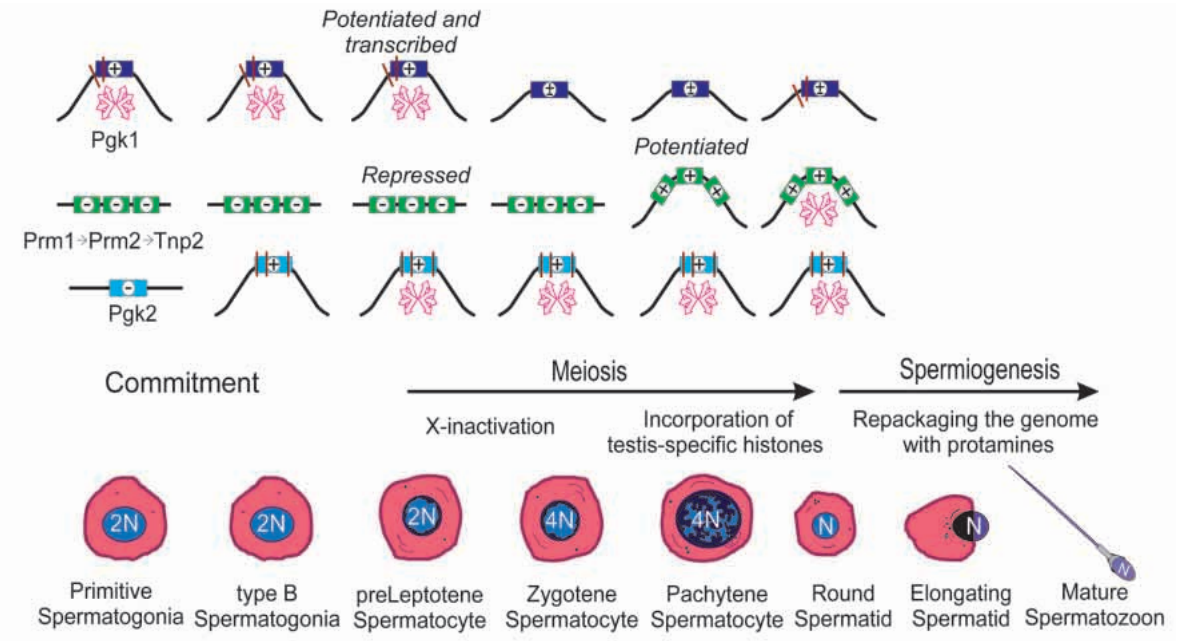

Fig. 2. Potentiation and silencing of individual genes and genic domains occurs at specific stages during spermatogenesis. During the course of spermatogenesis, select genic domains become potentiated, *i.e.* readied for expression, prior to the actual onset of transcription, while others undergo repression in association with transcriptional silencing. Genes on the X chromosome, such as P*gk1*, undergo X-chromosome inactivation, resulting in a decrease in general DNase I-sensitivity and a corresponding decrease in the accessibility of DNase I-hypersensitive sites. Testis-specific genes are differentially potentiated throughout spermatogenesis. Thus, the autosomal P*gk2* gene becomes fully potentiated during the initial stages of meiosis. The haploid-expressed members of the P*rm1*→P*rm2*→T*np2* domain are not potentiated until early in the pachytene spermatocyte stage. Nuclear events occurring during meiosis and spermiogenesis are indicated above the cells. Previously reported DNase I-hypersensitive sites for the P*gk1* and P*gk2* genes are indicated as vertical lines. Fully potentiated domains are indicated by genes that are represented by rectangles with a '+'. Non-potentiated domains are indicated by genes designated as rectangles with a '–'. Genes that appear to be in regions of transitional DNase I-sensitivity are indicated as rectangle with a '±'. Transcription is indicated by 6-headed arrows.

leptotene/zygotene spermatocytes, the T_{50c} increased to 96 minutes. Thus, any preleptotene spermatocyte gene possessing a T_{50c} ≥40 ± e minutes was classified as existing in a closed non-potentiated chromatin conformation while any leptotene/zygotene spermatocyte gene possessing a T_{50c} ≥96 ± e minutes was classified as existing in a closed non-potentiated chromatin conformation. In contrast, the constitutively potentiated DNase I-sensitive β-actin gene possessed a T_{50c} of 5 minutes within preleptotene spermatocytes and 8 minutes within leptotene/zygotene spermatocytes. Accordingly, any preleptotene spermatocyte or leptotene/zygotene spermatocyte gene possessing a T_{50c} of ≤5 ± e minutes or ≤8 ± e minutes, respectively, was classified as existing in an open-potentiated chromatin conformation.

The testis-specific P*gk2*, P*rm1*, P*rm2* and T*np2* genes, which are expressed only in cells of the latter portion of the spermatogenic pathway, existed in a closed-chromatin conformation in primitive type A spermatogonia possessing a T_{50c} of at least 75 minutes. As these cells proceeded along the differentiative pathway to the leptotene/zygotene spermatocyte stage and were subjected to X-chromosome inactivation, the P*gk1* gene assumed a more closed conformation. Just prior to and in conjunction with the inactivation of the P*gk*1 gene, the haploid-expressed P*gk*2 gene assumed a potentiated conformation in the type B spermatogonia. It was now in a readied state to assume the function of the X-inactivated P*gk*1 gene. As spermatogenesis continued the haploid-expressed P*rm1*, P*rm2* and T*np2* genes assumed a potentiated open-chromatin conformation by the pachytene stage of differentiation (Table 1, T_{50c} decreased from an average of ≈63 minutes to a range of ≈7-8 minutes). This clearly demonstrates that spermatogenesis is a process of selective locus potentiation as the genes that initiate expression in spermatocytes or spermatids undergo potentiation subsequent to the stem-cell stage of spermatogenesis where they exist in a restricted state in the spermatogonia.

DISCUSSION

Temporal control of potentiation

It seems likely that changes in the potentiative state of the genome would be mediated at times of chromatin restructuring, such as condensation, replication or repackaging. For example, to compensate for the loss of P*gk1*, the P*gk2* gene is potentiated in spermatogonia from 8-day-old mice. This corresponds to the final mitotic division prior to the onset of meiosis. Following this final premeiotic replication, the state of the chromatin remains relatively static until the onset of meiosis. Thus, the type B spermatogonia may represent the last cell type before the onset of meiosis in which the chromatin is amenable to domain reorganization leading to the potentiation of the P*gk2* locus to compensate for X-chromosome inactivation. In comparison, expression of the members of the P*rm1*→P*rm2*→T*np2* domain are not required until after meiosis, in the haploid round spermatid. However, as with the

P*gk2* gene in type B spermatogonia, the nuclear processes occurring during the pachytene stage of meiosis, such as the incorporation of the various testis-specific histones (Brock et al., 1980; Bucci et al., 1982; Meistrich et al., 1985) may represent the last opportunity to potentiate the protamine and transition protein 2 locus and other haploid-expressed loci prior to their transcription in postmeiotic cells.

Both hematopoiesis and spermatogenesis initiate their differentiative programs from a stem cell capable of renewal and differentiation. On one hand, the specific sets of genic domains that are potentiated in the hematopoietic stem cell during the 'promiscuous phase of multilineage locus activation' (Hu et al., 1997), are competent to prime several lineage-specific gene programs (Jiménez et al., 1992; Hu et al., 1997). Accordingly, lineage commitment to one of the differentiative pathways like erythropoiesis most likely reflects the closing, i.e., adopting a DNase I-insensitive chromatin conformation, of the domains from the other pathways (Forrester et al., 1986; Jiménez et al., 1992). On the other hand, the spermatogenic stem cell has only one differentiative fate. Differentiation of the spermatogenic lineage occurs only in the adult and is limited to a minority of the progeny cells derived from each spermatogenic stem cell. Accordingly, the potential energetic advantage that may be gained by the hematopoietic stem cell from a generally permissive state may not be beneficial for the spermatogenic stem cell. It may be energetically less favorable to maintain the necessary testis-specific genes in a potentiated open-chromatin conformation in the stem cell, than it is to open these loci upon commitment to differentiation. Further, inappropriate premature expression of members of the P*rm1*→P*rm2*→T*np2* domain has been shown to cause differentiative arrest and cell death (Lee et al., 1995). Thus, maintaining this and other domains in a closed-chromatin conformation in non-expressing cells, presents a key physical barrier that can be exerted to prevent inappropriate expression. To overcome this barrier, spermatogenesis selectively opens individual testis-specific domains for expression as required during differentiation of the gamete.

A model of locus potentiation during differentiation

Fig. 2 shows a diagrammatic representation of the potentiative model of spermatogenesis. It is clear that potentiation of chromatin domains is a dynamic process that mediates the differentiative response by altering chromatin structure. The spermatogonial stem cell can take only one of three paths: renewal, differentiation or degradation (Dym, 1994). Chromosomal replication in the earliest steps of spermatogenesis in the committed type B spermatogonia presents a unique opportunity for chromatin modification during which time the P*gk2* domain becomes potentiated. It is interesting to note that general DNase I-sensitivity in the mouse P*gk2* gene appears coincident to the formation of several DNase I-hypersensitive sites (Kumari et al., 1996) near its transcriptional promoters and enhancers, just prior to the onset of P*gk2* transcription. In contrast, the members of the P*rm1*→P*rm2*→T*np2* domain that are required later during differentiation are not potentiated until a later nuclear event, e.g., the incorporation of the testis-specific histone complement.

Potentiation of chromatin domains can be viewed as an epigentic event. This appears to occur at times of nuclear restructuring such as replication, when the chromatin-bound protein compliment is most readily altered. Whether potentiation is mediated by demethylation, association with the nuclear matrix, histone acetylation and/or the binding of factors to regions of locus control during nuclear reorganization remains to be delineated. It has been shown that transcriptionally active genes are marked to ensure their stable propagation through mitosis (Michelotti et al., 1997). The process of initially potentiating a genic domain may be mediated in a manner similar to marking transcriptionally active genes. Such processes may be mediated by chromatin-remodeling transacting-factors that would most likely bind to regions of locus control, such as LCRs and/or MARs, rather than to individual gene-specific promoters. Replication, decondensation and the unique nuclear events that occur during spermatogenesis, e.g. repackaging with protamines, are examples of chromatin reorganization events during which entire regions of the genome may be marked for repackaging into a potentiated conformation. This is the first step towards the appropriate spatial and temporal expression of the gene(s) contained within a domain.

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