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Gene potentiation: Forming long-range open chromatin structures

Review Article

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Summary

Gene potentiation is the process of opening a chromatin domain, which in turn renders genes accessible to the various factors requisite for their expression. The formation of an open chromatin structure is central to the establishment of cell fate and tissue-specific gene expression. Both hematopoiesis and spermatogenesis serve as excellent models for examining gene potentiation. Each developmental pathway is governed by a unique differentiative program, which specifies a subset of potentiated genes enabling expression. A discussion of these contrasting potentiative cascades is presented illustrating that cell fate is ultimately determined by the selective opening and closing of gene containing domains. Elucidating the mechanism, which governs these perturbations in chromatin structure, will provide valuable insight into how differentiative decisions are made and whether commitment to a particular phenotype can be modified.

I. Introduction

Many eukaryotic genes are organized into functional chromatin domains. This facilitates their coordinate regulation during development (Reviewed by Dillon and Grosveld, 1993; Vermaak *et al*., 1998). The ability of individual cells to regulate the genes contained within such chromatin domains is key to the establishment of cell fate and tissue-specific gene expression (Reviewed by Bonifer *et al*., 1997). Perturbations in chromatin structure can act both locally to alter the accessibility of *trans*-acting factors to *cis*-regulatory elements and globally to affect the opening and closing of entire chromatin domains (Bodnar *et al*., 1996; Vermaak *et al*., 1998). Gene potentiation is the process of opening a chromatin domain (Choudhary *et al*.,1995), which then renders genes accessible to the various trans-acting factors required for their expression (Reviewed by Higgs, 1998). As such, transcriptionally active genes are found in regions of open chromatin.

Potentiated regions of the genome replicate early in S-phase and are preferentially confined to discreet chromosomal territories within the interphase nucleus (Kurz *et al*., 1996; Lamond *et al*., 1998; Wei et al.,1998). These transcriptionally competent domains exhibit a 10 fold enhanced general nuclease sensitivity (Weintraub and Groudine, 1976). Routinely, increased DNase I sensitivity is used as a diagnostic indicator of gene potentiation. For example, the chicken ovalbumin domain is part of a multigenic, coordinately-expressed locus that exists as a single DNase I-sensitive, potentiated domain in hen oviduct where its members are expressed but remains in a DNase I-insensitive, non-potentiated configuration in all non-expressing cells (Lawson *et al*., 1982). A potentiated chromatin domain may also contain small hypersensitive sequences which are approximately 100 fold more sensitive to DNase I digestion than bulk chromatin (Stalder, 1980). These hypersensitive regions often demarcate sites of interaction between specific effector proteins and *cis*-regulatory elements (Elgin, 1984).

The transition from a closed to an open chromatin conformation is a necessary event, but alone is not sufficient to ensure transcription (Reviewed by Krawetz *et al*.,1999). Consider the contrasting environments of the human β-globin domain on chromosome 11 and its co-regulated family member, the α-globin domain found near the telomere of chromosome 16. Both domains assume a DNase I-sensitive, potentiated conformation in erythroid cells where their respective globin genes are expressed. However, unlike the β-globin domain which forms a DNase I-insensitive, closed configuration in non-erythroid cells, the α-globin domain

remains constitutively potentiated in all cell types (Craddock *et al*., 1995). This can can be reconciles as the α-globin domain lying within a chromosomal region containing several widely expressed genes including the constitutively expressed 3-methlyadenine DNA glycosylase gene (Vyas *et al*., 1992; Vickers *et al*., 1993; Flint *et al*., 1997). Although the α-globin domain remains in a potentiated conformation in non-erythroid cells, the globin genes remain transcriptionally silent because the necessary factors for its expression are lacking. It is also interesting to note that the human β-globin domain exists in a potentiated, open conformation prior to commitment to the erythroid lineage (Jiménez *et al*., 1992) but requires additional elements and factors for appropriate expression (Calzolari *et al*., 1999). Taken together, these data provide supportive evidence that while the formation of a potentiated chromatin domain is a necessary event for transcription, additional levels of control are required to ensure proper spatial and temporal gene expression.

The potentiated state of a gene can also be influenced by alterations in the local chromatin environment. For example, many eukaryotic genes are differentially expressed by altering their methylation status. These genes are largely unmethylated in cells where they are transcribed, but fully methylated in all non-expressing cells (Reviewed by Cedar, 1988). Histone acetylation also acts on the local gene environment during the transition from the 30 nm fiber to the more open structure that can be likened to a 10 nm fiber, stabilizing the more relaxed open structure (Reviewed by Davie and Hendzel, 1994). It has also been postulated that DNA methylation patterns may serve to modulate histone acetylation thereby maintaining local chromatin states. Both DNA methylation and histone acetylation render increased accessibility of ubiquitous and tissue-specific *trans*-acting factors to *cis*-regulatory elements, facilitating transcriptional activation (Eden *et al*., 1998).

In addition to enhanced general nuclease sensitivity, transcriptionally active genes are often associated with the nuclear matrix (Ciejek *et al*., 1983). This interaction has been postulated to represent the means by which potentiated chromatin domains are organized within the eukaryotic nucleus (Reviewed by Bode *et al*., 1996). Matrix attachment regions (MARs) have been shown to demarcate the boundary elements of DNase I-sensitive chromatin domains of many eukaryotic loci including the human apolipoprotein-B gene, the chicken lysozyme gene and the human P*RM1*→P*RM2*→T*NP2* multigenic locus (Kalos and Fournier, 1995; Stief *et al*., 1989, Kramer and Krawetz, 1996). MARs can also function as insulators against the position effects of neighboring chromatin (Zlatanova and van Holde, 1992), and have been shown to interact directly with enhancer elements to extend the accessibility of a chromatin domain (Jenuwein *et al*., 1997; Forrester *et al*., 1994). Taken together, these and other observations have led to the suggestion that MARs can be divided into discrete functional classes and may provide a means to tag genic domains for coordinate expression (Kramer and Krawetz, 1996). While it is apparent that gene potentiation involves alterations that affect both the local environment of individual genes and the physical structure and organization of large chromatin domains containing multigene families, the actual mechanism(s) governing this process remain unclear.

II. Models for Examining Gene Potentiation

A. Hematopoiesis

Hematopoiesis is the differentiative pathway by which pluripotent hematopoietic stem cells give rise to the various erythroid, lymphoid and myeloid blood cell lineages. A schematic representation of hematopoiesis is shown in **Figure 1**. This process initiates when a pluripotent hematopoietic stem cell differentiates to form a myeloid or lymphoid stem cell. Both of these multipotent stem cells are capable of self renewal and differentiation. Upon appropriate stimuli, the lymphoid stem cell differentiates to form the committed pre-B and pre-T progenitor cells which in turn differentiate to form mature B and T lymphocytes respectively. In contrast, the myeloid stem cell forms an intermediate stem cell, CFU-GEMM (colony forming unit- granulocyte, erythrocyte, monocyte, megakaryocyte), which differentiates to produce the corresponding unipotent CFU-progenitors for these lineages. Subsequently, these committed precursors, influenced by various cytokines and growth factors, terminally differentiate into their respective mature erythroid, megakaryocytic, monocytic, neutrophilic, eosinophilic and basophilic cell types (Carr and Rodak, 1999).

Hematopoiesis originates in the embryonic yolk sac, temporarily shifts to the fetal liver, and from the fourteenth week of gestation throughout adult life occurs primarily in the bone marrow (Yawata, 1996). Concomitantly, many hematopoietic-specific genes are coordinately regulated and differentially expressed during development (Reviewed by Orkin, 1995). Two of the most extensively characterized examples are the human α and β globin gene clusters which encode the respective globin chains of hemoglobin, the oxygen transport protein found in erythrocytes (Reviewed by Karlson and Nienhuis, 1985). The human β-globin gene cluster is a well-established model for examining the functional role of chromatin in gene regulation. The human β-globin locus is a multigenic domain containing five globin genes arranged in the order of their sequential expression during development (Reviewed by Andrin and Spencer, 1994). The entire β-globin gene cluster exists in a single DNase I-sensitive, open conformation in cells of the erythroid lineage, but remains in a DNase I-insensitive, closed conformation in all non-erythroid cells (Groudine *et al*., 1983). However, the extent of the human β-globin domain remains to be clearly

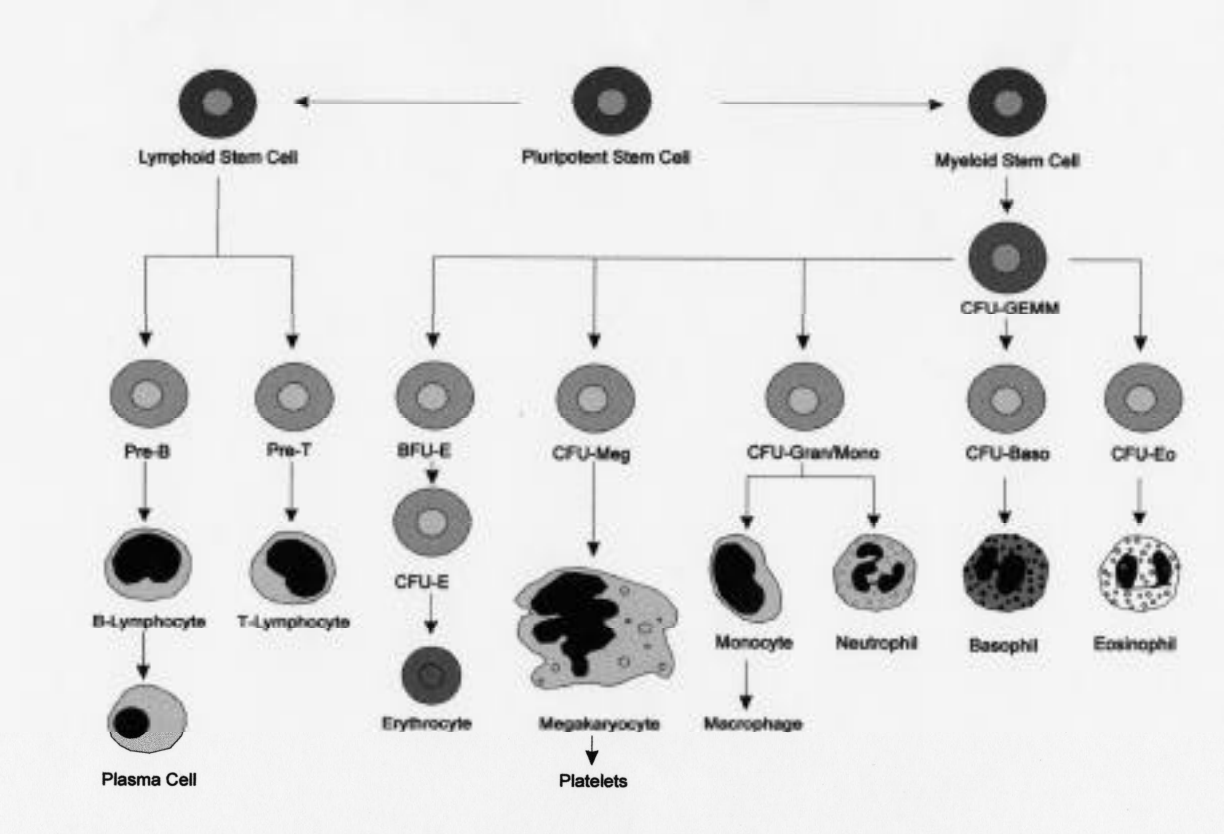

Figure 1 Hematopoiesis: The differentiation and maturation of blood cells. During, hematopoiesis multipotent stem cells, influenced by numerous cytokines and growth factors, divide and differentiate to form committed progenitors for the various erythroid, lymphoid and myeloid blood cell lineages. These unipotent precursors in turn differentiate to produce their respective mature blood cell types. Each step of this differentiative pathway involves the selective restriction of chromatin domains ultimately defining a lineage-specific subset of genes which remains potentiated for expression.

delineated. Upstream from the gene cluster is a series of five DNase I-hypersensitive sites (HS1-HS5). Hypersensitive sites 1-4 are commonly referred to as the β-globin locus control region, LCR (Reviewed by Grosveld *et al*., 1993). The importance of the β-globin LCR has been widely demonstrated through the characterization of its individual hypersensitive sites (Reviewed by Dillon and Grosveld, 1993) and by examining the effects of naturally occurring deletions which result in the various β-thalassemias (Van der Ploeg *et al*., 1980; Driscol *et al*., 1989). These de-novo mutations not only lead to reduced levels of gene expression, but have also been demonstrated to alter both the timing of replication of the β-globin locus and its sensitivity to DNase I (Forrester *et al*., 1990). Dissection and characterization of individual hypersensitive sites has shown that the β-globin LCR functions in a tissue-specific manner and is necessary for high level expression of the globin genes. Hypersensitive sites 1-4 act as erythroid-specific enhancers and possess multiple binding sites for various transcription factors (Reviewed by Engel, 1993; Wood, 1996). HS-2 has been shown to be functionally equivalent to the full LCR both by displaying classical enhancer activity in transient transfection assays (Tuan *et al*., 1989) and by its ability to confer high-level, position-independent expression of a transgene in mice (Philipsen *et al*.,1990; Lui *et al*., 1992 and Pawlik *et al*., 1995). HS-3 possesses a dominant chromatin opening and remodeling activity that is separate and distinct from its ability to direct β-globin expression in transgenic mice (Ellis *et al*., 1996). HS-5 is a constitutive site and although it possesses no enhancing activity (Fraser *et al*., 1993), it does contain a matrix attachment region (Jarman *et al*., 1988). In this manner, HS-5 may serve as an insulator against the position effects of neighboring chromatin (Li *et al*., 1994). These observations suggest that the LCR elements act cooperatively to ensure the correct spatial and temporal regulation of the β-globin gene cluster.

The full LCR has been postulated to function synergistically as a holocomplex, to regulate the sequential expression of the β-globin genes through specific HS-promoter interactions (Bresnick *et al*., 1997 and Ellis *et al*., 1996). While several looping models have been proposed for this developmental switching mechanism (Reviewed by

Stamatoyannopoulos and Nienhuis, 1994), others suggest that the temporal regulation of the β-globin genes is LCR-independent, relying solely on promoter proximal elements and gene arrangement (Martin *et al*., 1996). Recent studies examining the organization and temporal regulation of the β-globin genes have further demonstrated that inversion of gene order with respect to the LCR significantly alters their expression (Tanimoto *et al*., 1999).

Although it has been well established that the β-globin LCR plays an essential role in the transcriptional activation and temporal regulation of the β-globin locus, its function in long range chromatin opening is debated. The β-globin LCR was the first element reported to confer tissue-specific, position-independent and copy number-dependent expression of a transgene (Grosveld *et al*., 1987). It has since been held that the β-globin LCR facilitates the creation of an open chromatin environment by altering the topology of the β-globin domain thereby maintaining it in a potentiated configuration (Reviewed by Martin *et al*., 1996). Conversely, others have suggested that while the LCR ensures the high-level, tissue-specific expression of the β-globin genes, it does not function to form or maintain the open chromatin conformation that is necessary for the expression of the locus (Reitman *et al*., 1993; Reik *et al*., 1998; Epner *et al*., 1998). This raises the intriguing possibility that the ability of the β-globin LCR to overcome position effects in transgenic mice may be independent of the mechanism that governs the potentiation of the β-globin domain during development (Reviewed by Higgs, 1998). Interestingly, it has been now shown that the human and mouse β-globin loci reside in a cluster of functional odorant receptor genes (Bulger et al., 1999). The authors postulate that these two overlapping gene families may share some of the same regulatory elements involved in mediating their different expression patterns. Understanding how such multigenic domains are potentiated will provide valuable insight into the functional role of chromatin in the establishment of cell fate and tissue-specific gene expression.

B. Spermatogenesis

Spermatogenesis serves as another excellent model for examining gene potentiation because of the vast array of testes-specific genes that are coordinately regulated and expressed in a specific temporal manner during the formation of the male gamete (Reviewed by McCarrey, 1998). This entire differentiative pathway from the uncommitted stem cell to the mature spermatozoa occurs within the testes. The important cellular and molecular events of spermatogenesis are summarized in **Figure 2**. There are three stages to mammalian spermatogenesis: mitosis, meiosis and spermiogenesis (Junqueira *et al*., 1986). During mitosis, primitive type A spermatogonia either actively divide, renewing themselves or differentiate to form the intermediate and type B spermatogonia. Once type A spermatogonia differentiate to form these cell types, they are then committed to the spermatogenic pathway. Type B spermatogonia in turn differentiate to form primary spermatocytes, which subsequently enter meiosis (Reviewed by Dym, 1994). The development of meiotic spermatocytes begins with the primary or pre-leptotene spermatocyte. DNA replication occurs at this stage resulting in a genome content of 4N. After DNA synthesis, chromatin condensation is initiated, signaling the start of meiosis. In humans, approximately 22 out of the 64 days of spermatogenesis are spent in meiosis and the majority of that in prophase I (Reviewed by Willison and Ashworth, 1987). During this process, the chromosomes undergo pairing and the formation of the synaptonemal complex. This is followed by genetic recombination between the homologous pairs. Subsequent to the exchange of genetic material, the first meitotic division occurs resulting in the formation of two secondary spermatocytes. This is quickly followed by meiosis II, reduction division, producing four haploid round spermatids (Gardner and Snustad, 1984). Some of the genes expressed during meiosis include testes-specific variants of somatic cell proteins (Reviewed by Kierzenbaum, 1994). One of the best-characterized examples is P*gk2*, phosphoglycerate kinase 2, and the testes specific isozyme of P*gk1*. P*gk1* is a constitutively expressed, X-linked enzyme that catalyzes the conversion of 1,3 diphosphoglycerate to 3-phosphoglycerate and ATP during glycolysis (Lee *et al*., 1972). However, because it is subject to X-inactivation (Lifschytz and Lyndsay, 1972), the expression of the autosomal, testes-specific P*gk2* gene is initiated to compensate for the reduced levels of this essential enzyme during spermatogenesis (McCarrey *et al*., 1992).

The final stage of spermatogenesis, termed spermiogenesis, is characterized by the morphological differentiation of round spermatids into mature spermatozoa (Clermont and Leblond, 1955). In humans, like other mammals, the round spermatid stage marks the initial expression of the haploid-specific packaging proteins the transition proteins and the protamines (Wykes et al., 1995). These proteins facilitate the remodeling of the chromatin during the morphologic transformation from round spermatid to mature spermatozoa (Reviewed by Wouters-Tyrou, 1998). This process involves the initial disruption of the nucleohistone structure by the transition proteins and their final replacement by the protamines to compact and condense the DNA into a species-specific shaped nucleus (Reviewed by Dadoune, 1995). Subsequent to their expression these genes are again suppressed as part of the genome-wide silencing that yields the transcriptionally quiescent sperm nucleus (Reviewed by Balhorn, 1989; Oliva and Dixon, 1991). Accordingly, many testes-specific genes, including the transition proteins and the protamines, are synthesized relatively early then placed under extensive translational control (Reviewed by Eddy and O'Brien, 1998).

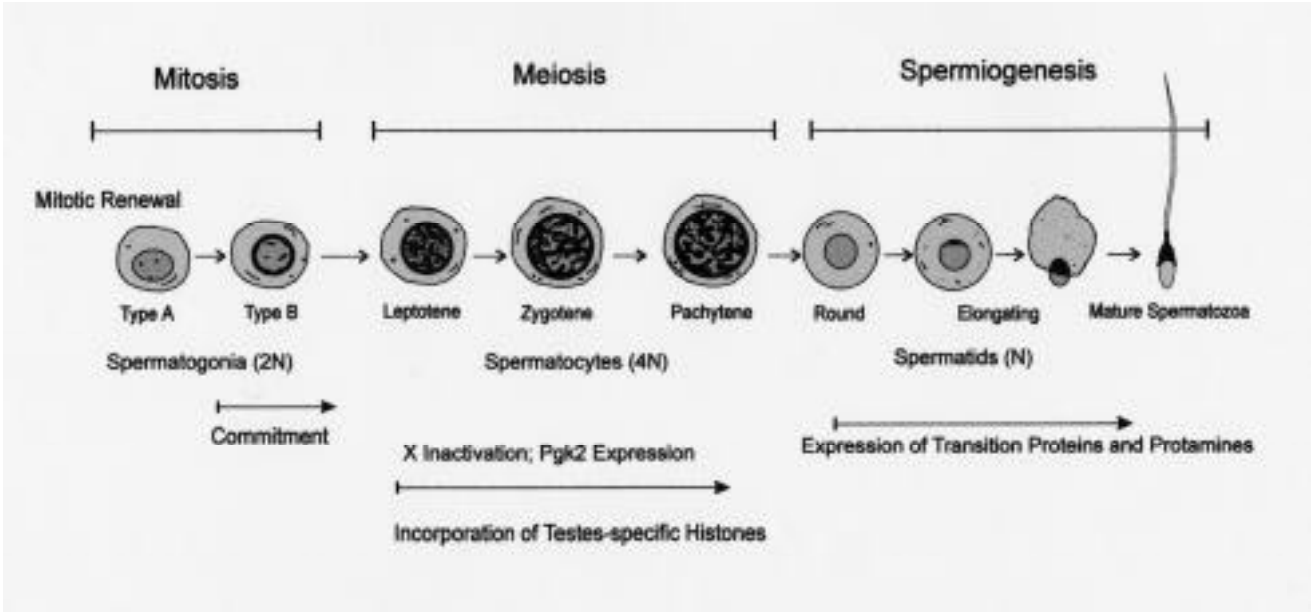

Figure 2 The three stages of human spermatogenesis. Human spermatogenesis is divided into three stages, mitosis, meiosis and spermiogenesis. Some of the important cellular and molecular events are indicated. During mitosis primitive type A spermatogonia either actively divide renewing themselves or differentiate to form type B spermatogonia and thus commit themselves to the spermatogenic pathway. Type B spermatogonia in turn differentiate to form primary or preleptotene spermatocytes which then enter meiosis. Meiosis initiates after DNA replication and is characterized by chromosomal pairing and genetic recombination. Subsequent to the exchange of genetic material, meiotic spermatocytes undergo two successive cell divisions producing four round haploid spermatids. During the final stage, spermiogenesis, these round spermatids then morphologically differentiate to form mature spermatozoa. This process is facilitated by the transition proteins and the protamines which mediate the remodeling of the chromatin during the terminal differentiation of the spermatid nucleus.

Spermatogenesis is a dynamic continuum reflecting the coordinate temporal regulation of genes involved in the formation of functional male gametes. It is an exquisite model for examining the mechanism of gene potentiation.

III. Selective Potentiation of Testes-Specific Domains

It is well established that the DNA in human sperm chromatin is partitioned into both a nucleohistone and nucleoprotamine fraction (Tanphaichutr *et al.*, 1978). This organization is likely to be sequence specific and may serve to designate a particular subset of genes which have a functional role in early development (Gatewood *et al*., 1987). In humans, 85% of the DNA in sperm chromatin is protamine bound, while 15% remains histone bound (Tanphaichutr *et al.*, 1978). The histone bound fraction contains several testes-specific histone variants (Reviewed by Ward, 1994; Donecke *et al*., 1997). This compacts the DNA to a greater extent than its somatic counterparts although histone H1 is absent and histones H3 and H4 are highly acetylated (Gatewood et al., 1990). Since both histone acetylation and the absence of histone H1 are features of active chromatin (Reviewed by Wolffe, 1994), it has been proposed that the histone-associated genes in the human sperm nucleus may be the first genes transcribed after fertilization (Gardiner-Garden *et al*., 1998). Alternatively, these histone bound regions may simply serve as nucleation sites for the replacement of the protamines by the histones upon fertilization for the resumption of the somatic nucleosomal structure.

The transition proteins and the protamines function solely to remodel the chromatin during late spermatogenesis. The P*RM1*→P*RM2*→T*NP2* members of this sperm gene packaging family are clustered into a single 28 kb DNase I-sensitive domain flanked by two regions of marked insensitivity (Choudhary *et al*., 1995). A diagrammatic representation of this domain is shown in **Figure 3**). This biophysical domain has been biologically confirmed. Transgenic mice harboring the human protamine locus express the transgene in a haploid-specific, position-independent and copy-number dependent manner (Choudhary *et al*., 1995; Stewart *et al*., 1999). These data show that this region of the genome contains all of the elements necessary for the appropriate temporal and spatial expression of this suite of genes independent of site of integration.

The ends of the domain are attached to the sperm nuclear matrix (Kramer and Krawetz, 1996). The sequences of attachment at the boundaries of this domain correspond to sperm-specific matrix attachment regions, termed spMARs.

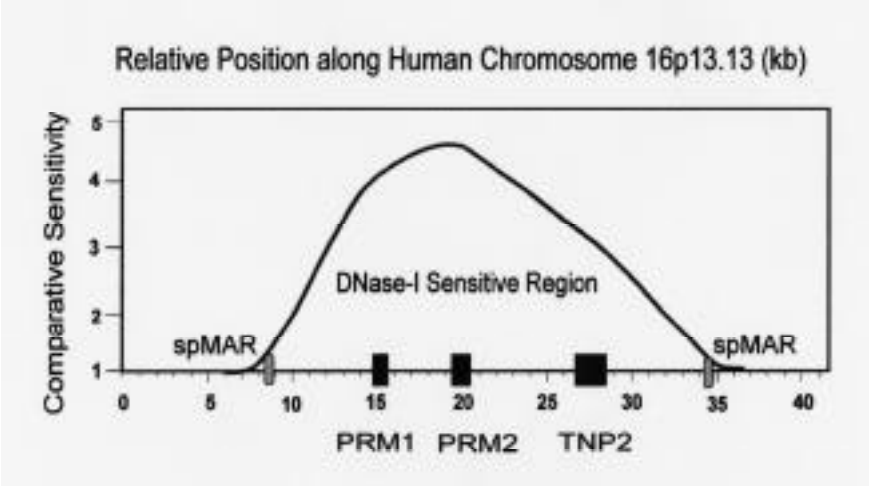

Figure 3: The human P*RM1*→P*RM2*→T*NP2* domain. The human protamine locus is a multigenic domain containing three haploid expressed genes encoding the protamines P*RM1*, P*RM2* and transition protein T*NP2*. This gene cluster exists as a single DNase I-sensitive domain flanked by two regions of matrix attachment. The relative position of the locus along human chromosome 16p13.13 is shown.

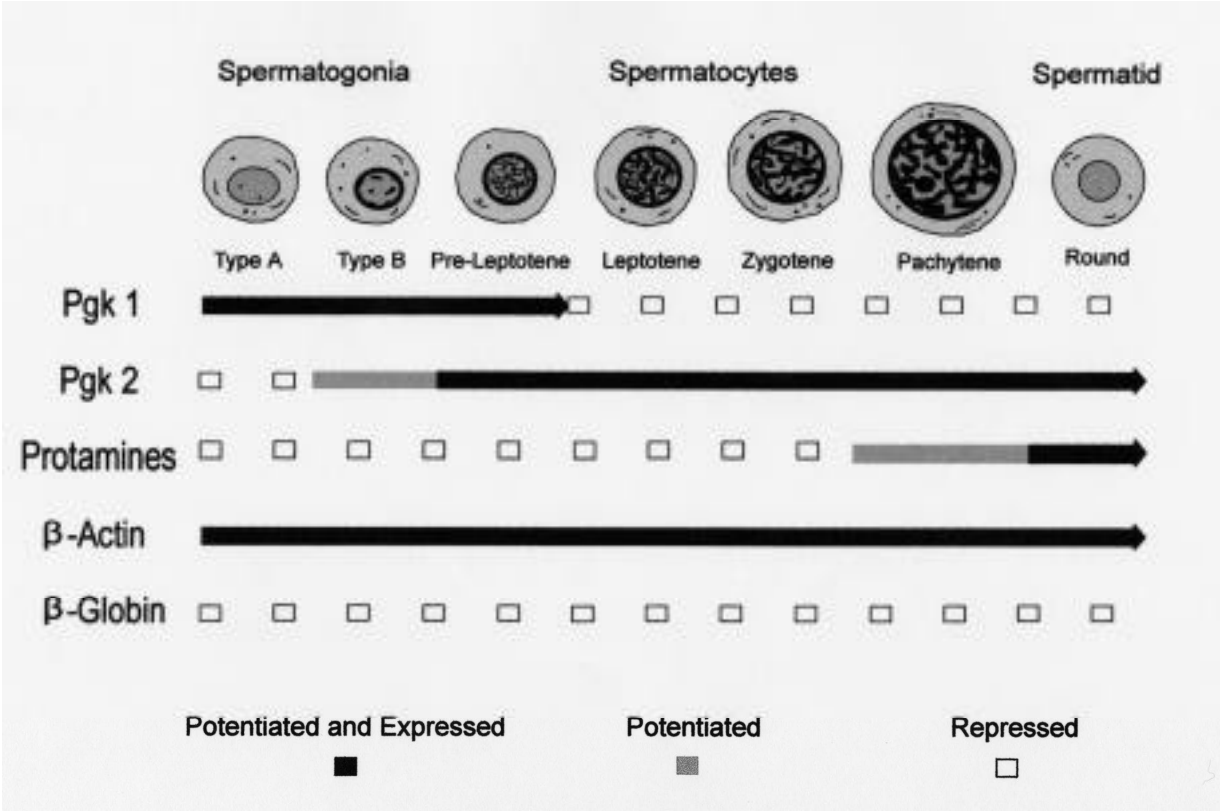

Figure 4: Gene potentiation during mammalian spermatogenesis. The potentiative state of individual genic domains was assessed by DNase I sensitivity in various isolated germ cell populations ranging from Type A spermatogonia to round spermatid. The alterations in the potentiative states of *Pgk1* and the testes-specific P*gk2* and P*rm1*→P*rm2*→T*np2* domains indicates that the temporal regulation of these genes for expression during spermatogenesis is mediated by the selective opening and closing of individual chromatin domains. The constitutively expressed β-actin and erythroid-specific β-globin genes provide positive and negative controls respectively.

Although the functional role of the spMAR elements in gene potentiation remains to be clearly delineated recent evidence suggests that the 3' spMAR of the P*RM1*→P*RM2*→T*NP2* domain is critical for expression of this human locus (Kramer et al., 1997).

The protamine gene cluster remains transcriptionally quiescent until the round spermatid stage when it is then actively transcribed in the final burst of transcription before genome-wide silencing (Fig. 2, Wykes et al., 1995). This is representative of the class of similarly expressed genes that constitute the alternative male haploid genome (Kramer and Krawetz, 1997). It has thus been argued that expression of this alternative genome is controlled by sequestering its members in a closed chromatin conformation until required (Kramer et al., 1998). To directly address this tenet, the potentiative state of the P*rm1*→P*rm2*→T*np2* gene cluster and several other gene loci including P*gk1*, P*gk2*, β-actin and β-globin, was assessed throughout spermatogenesis using purified populations of germ cells (Kramer *et al*., 1998). The results of this study are summarized in **Figure 4**. It was reasoned that since, P*gk1* is a constitutively expressed gene its domain should exist in a potentiated conformation in all cell types. However, P*gk1* is also subject to X-inactivation during spermatogenesis, which is compensated by the expression of the autosomal testes-specific P*gk2*. As the P*gk1* gene is silenced and its chromatin domain is closed, the closed P*gk2* domain opens to enable its transcription through to the round spermatid stage. Similarly, the multigenic P*rm1*→P*rm2*→T*np2* domain remains closed until the pachytene spermatocyte stage when it then assumes an open potentiated chromatin conformation prior to the expression of its members in round spermatids. This demonstrated that the temporal expression of testes-specific genes is ultimately mediated by the selective opening and closing of individual chromatin domains. Interestingly, the physical domain containing the human P*RM1*→P*RM2*→T*NP2* multigenic locus remains in an open conformation in mature spermatozoa (Choudhary *et al.*, 1995).

IV. Prospects

Both hematopoiesis and spermatogenesis serve as excellent model systems for examining gene potentiation. Each developmental process begins with a progenitor stem cell, which, in response to various stimuli, acquires the capacity to differentiate into individual cell types. Commitment to a particular cell fate is a gradual process and is ultimately determined by the selective potentiation of certain genes for expression in conjunction with the repression or silencing of other genes. For example, commitment to a specific hematopoietic pathway involves the selective repression of genes to restrict lineage potential from its multipotent stem cell (Hu *et al.*, 1997). This repressive mechanism is marked by the successive closing of chromatin domains eventually defining a lineage-specific subset of genes that remains potentiated for expression (Jimenez *et al*, 1992). Conversely, spermatogenic differentiation is governed by an expressive mechanism whereby the activation of testes-specific genes is mediated by the selective opening, i.e. potentiation, of individual chromatin domains (Kramer *et al*., 1998). Further characterization of these contrasting potentiative mechanisms which establish cell fate during hematopoiesis and spermatogenesis will provide insight into how differentiation is regulated by chromatin structure and whether commitment to a particular cell fate can be altered (Krawetz *et al*., 1999). The identification of potentiator elements, which facilitate the opening and closing of chromatin domains may be useful to ensure high-level, tissue-specific expression of targeted gene therapeutics.

Acknowledgments

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