Molecular Genetic Analysis of Mammalian Spermatid Differentiation

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I. Overview of Mammalian Spermatogenesis

Mammalian spermatogenesis is an ongoing developmental process that begins at puberty in humans and shortly after birth in the mouse. Because it is a continuous developmental process that occurs in the adult, it offers unique opportunities to study the regulation and execution of cell differentiation. Spermatogenesis is one of the most dramatic differentiation events that any single cell manifests. The initiation and progression of spermatogenesis are under hormonal regulation that involves both endocrine and paracrine control. As a result of years of work by many expert morphologists, a detailed histological description of the process is available. However, until recently, very little was known about the molecular and cellular events that underlie spermatogenesis. It is our belief that male germ cell development is a relatively undiscovered and underappreciated system in which to study many fundamental questions in cellular and developmental biology, and that it is time to apply the powerful tools of molecular genetics to this important area of biology. This chapter summarizes the major events of spermatogenesis and then reviews recent work on the regulation of translation of the mouse protamine 1 gene during haploid spermatid differentiation.

Spermatogenesis occurs within the seminiferous tubules of the testis. Distributed along the basal lamina of the seminiferous tubules are spermatogonial stem cells that divide approximately once every 8.6 days in the mouse (Oakberg, 1956). Following cell division, one of the daughter cells retains its stem cell identity, while the other progresses through a series of mitoses to generate a clone of descendant spermatogonial cells. Spermatogonial cells derived from each of the mitoses can be distinguished from the previous by their nuclear shape and staining pattern of euchromatin and heterochromatin. One obvious consequence of the mitotic proliferation of spermatogonial cells is an increase in the final output of spermatozoa.
is unknown, however, if there are also obligate differentiation events that occur at each spermatogonial cell division, and that are required for progression of the spermatogonial cells into the later phases of differentiation. Following spermatogonial proliferation, type B spermatogonial cells enter meiosis and differentiate into primary spermatocytes. During the relatively protracted stage of meiosis I, chromosomal homologues pair, recombine, and segregate from one another, thus generating secondary spermatocytes. These cells rapidly segregate their sister chromatids and undergo cytokinesis to generate haploid round spermatids. Differentiation of round spermatids into mature spermatozoa, referred to as spermiogenesis, takes approximately 2 weeks and is one of the most elaborate differentiation events of any mammalian cell.

The mitotic and meiotic proliferation of a single spermatogonial cell generates a clonal population of hundreds of differentiating spermatids (Huckins, 1978; Russell et al., 1991). Because cytokinesis is incomplete at each of the mitotic and meiotic cell divisions, descendants of a single stem cell develop within a syncytium in which cells are connected by intercellular bridges of approximately 1 μm in diameter (Burgos and Fawcett, 1955; Dym and Fawcett, 1971). Because spermatids are genetically distinct, haploid gene expression could result in functional differences between mature spermatozoa, thus resulting in preferential transmission of some alleles over others. It has been proposed that the cytoplasmic bridges allow the passage of various macromolecules between cells, thus ensuring synchronous development of all cells within a clone, and gametic equivalence between haploid spermatids (Dym and Fawcett, 1971; Erickson, 1973). To determine directly if postmeiotic gene products can pass between spermatids, transgenic mice have been generated which express the human growth hormone reporter gene exclusively in haploid spermatids under the control of a spermatid-specific promoter (Braun et al., 1989a). Immunohistological and in situ hybridization analysis of spermatids from hemizygous transgenic has shown that both protein and mRNA can pass between spermatids via the intercellular bridges. Thus, despite the genetic differences between individual cells in a single syncytium, the apparent unrestricted movement of macromolecules between cells guarantees their phenotypic equivalence.

The differentiation of round spermatids into mature spermatozoa requires the synthesis of hundreds of new proteins and the assembly of a unique collection of organelles (Bellvé, 1979; O'Brien and Bellvé, 1980; Hecht, 1986). During this differentiation process, a flagellum is constructed, the acrosomal vesicle is formed, and the nucleus is compacted to approximately one-tenth of its volume (Balhorn, 1982). Subtle changes in spermatid nuclear shape and acrosome morphology, as observed with common stains such as hematoxylin and PAS, respectively, allow very accurate determination of the 12 different stages of the cycle of the seminiferous epithelium with bright-field microscopy. Within these 12 stages of the cycle one can describe 16 steps of spermatid differentiation (Oakberg, 1956; Russell et al., 1990). Because spermatogenesis is initiated approximately every 8.6 days within a given portion of a seminiferous tubule and because spermiogenesis takes approximately 14 days, some sections of a tubule will contain either one or two generations of differentiating spermatids.

Compaction of the spermatid nucleus is accomplished by the replacement of the normal somatic complement of histones by the protamines (Bellvé, 1979; Meistrich, 1993). In the mouse there are two protamine genes (Balhorn, 1989). The protamines are small highly basic proteins that contain short stretches of arginine residues interspersed by cysteine residues. The protamines are believed to bind within the minor groove of the DNA and facilitate the packaging of the DNA into bundles of linear arrays (Balhorn et al., 1984). The protamines do not directly replace the histones. A few days before the synthesis of the protamines a class of basic proteins called the transition proteins are synthesized which partially displace the histones (Meistrich, 1989, 1993). Although the temporal appearance of the transition proteins and protamines during spermiogenesis has been described, relatively little is known about the mechanism of nucleohistone to nucleoprotamine replacement.

II. Regulation of Spermatogenesis

The initiation and maintenance of spermatogenesis is under both endocrine and paracrine control (Bardin et al., 1988; Crowley et al., 1991). The pituitary gland exerts its influence by releasing both leutinizing hormone (LH) and follicle-stimulating hormone (FSH), which act on the steroidogenic Leydig cells that occupy the interstitial space between the seminiferous tubules, and on the Sertoli cells, which are the somatic component of the seminiferous epithelium. LH regulates the production of androgen from the Leydig cell while FSH acts directly on the Sertoli cell. The Sertoli cell performs a critical role in male gametogenesis in providing a structural framework to the seminiferous epithelium, as well as by regulating both the initiation and progression of spermatogenesis (Raj and Dym, 1977; Russell et al., 1987). One such point of regulation is revealed in mice that show testicular feminization (Lyon and Hawkes, 1970). Although these mice are karyotypically 40XY, they appear externally female due to androgen insensitivity resulting from mutations in the androgen receptor (Weiland et al., 1978). These animals have small undescended testes that contain germ cells arrested in meiosis I (Lyon and Hawkes, 1970). Chimera studies have shown that the arrest of sperma-
togenesis is due to the absence of androgen receptors on Sertoli cells and not germ cells, thus demonstrating that Sertoli cells exert at least one level of control on the progression of germ cells through meiosis I (Lyon et al., 1975). The molecular basis for the Sertoli cell-mediated arrest is unknown but is clearly a research area in need of attention. It is also possible that Sertoli cells impinge on the progression of germ cells through later stages of meiosis or even spermiogenesis. For example, it has recently been suggested that the processing of the 3' end of the CREM-t mRNA in pachytene spermatocytes is regulated by FSH, presumably acting via the Sertoli cell (Foulkes et al., 1993). With the generation of a Sertoli tissue culture cell line that will support the transmiotic differentiation of primary spermatocytes into elongating spermatids in vitro, it may now be possible to more rigorously characterize the interaction between Sertoli cells and germ cells (Rassoulzadegan et al., 1993).

III. Translational Control during Spermatogenesis

As in any differentiation program, many genes are expressed at different times during spermatogenesis. The first level of gene control is transcriptional and, as expected, different genes are transcribed at specific times during sperm development. However, in addition to transcriptional control, translational control is also a major form of gene regulation during spermatogenesis. For example, although the two protamine genes are first transcribed a few days after meiosis in Step 5–9 spermatids, they are not translated until up to 1 week later in Step 12 spermatids (Balhorn et al., 1984; Kleene et al., 1984). Because protamine translation initiates after the cessation of global transcription in Step 9 spermatids, there is an obvious need to transcribe the genes required for the later stages of spermiogenesis at an earlier time (Monesi, 1964; Kierszenbaum and Tres, 1975). In addition to translational regulation of genes whose products function after transcription ceases, there are also genes under translational control whose protein products are expressed before transcription ceases. Some of these genes, like the autosomal Pgk-2 gene, are transcribed early in meiosis, yet are not translated until the early round spermatid stage (Geld et al., 1983). It is not obvious why some genes are under translational control during the early stages of spermatogenesis.

IV. Transgenic Analysis of Sequences Involved in Translational Control

Comparison of the nucleotide sequence of the mouse and human protamine 1 genes reveals that they are more similar in their 3'-untranslated regions than in their coding sequences (Lee et al., 1987; Peschon et al., 1987). Such a surprising conservation in sequence suggests that the 3' UTR may contain important cis-acting regulatory elements for the translational control of Prm-1. To test this idea, gene fusions were constructed between various regions of the Prm-1 gene and a reporter gene (hGH) and assayed in transgenic mice (Braun et al., 1989b). All of the genes contained the promoter for Prm-1 (Peschon et al., 1987) and coding sequence for hGH. Differences between the genes were restricted to their 5'- and 3'-untranslated sequences. Transgenic mice were generated with the genes and assayed for RNA and protein at different stages of spermatogenesis.

Northern blot analysis of RNA isolated from various tissues showed that the genes were transcribed exclusively in the testis. Analysis of RNA isolated from testes of prepubertal animals of different ages and in situ hybridization analysis with a 35S-labeled probe specific for the hGH coding sequence showed that the genes were transcribed exclusively in round spermatids coincident with the onset of transcription of the endogenous Prm-1 gene. Western blot analysis of protein extracts prepared from testes dissected from prepubertal animals of different ages showed that growth hormone was first detected at 32 days of age in animals that contained a transgene with the entire 156 nucleotide (nt) Prm-1 3' UTR. At this age of prepubertal development, the most advanced germ cells have differentiated into elongating spermatids. In contrast, in animals that contained a transgene with the hGH 3' UTR, growth hormone was detected at 28 days of age. Thus, a transgene that contained the 3' UTR of hGH was translated soon after it was transcribed in round spermatids, whereas a transgene that contained the Prm-1 3' UTR was transcribed in round spermatids, but was not translated until several days later in elongating spermatids. Immunohistochemical analysis of prepubertal and adult testis confirmed that growth hormone was not synthesized until the late spermatid stages in animals that contained the Prm-1 3' UTR. Analysis of additional transgenes that lacked the Prm-1 5' UTR showed that sequences in the 5' UTR were not required for translational regulation. In addition, a transgene that contained the 3'-most 62 nts of Prm-1 3' UTR also manifested Prm-1-like translational control, whereas a transgene that contained only the 3'-most 23 nts of Prm-1 3' UTR was translated at the same time as it was transcribed (Braun, 1990). Thus, sequences present in the 3'-most 62 nts of the Prm-1 3' UTR appear to be sufficient to mediate Prm-1-like translational control of the heterologous reporter gene hGH in transgenic mice.

V. Premature Translation of Protamine 1 in Transgenic Mice

To determine if the temporal translation of protamine 1 is important for normal spermiogenesis, we have generated transgenic animals which prematurely translate protamine in round spermatids. To accomplish this,
a transgene was constructed that contained the Prm-1 promoter and protein-coding exons, but lacked its normal 3' untranslated sequence. We substituted the hGH 3' UTR in place of the Prm-1 3' UTR. As expected, transcription of the Prm-hGH 3'UTR transgene was restricted to the testis and expressed in haploid spermatids. Immunolocalization of the protamine 1 protein (P1) with a mouse monoclonal antibody raised against human P1 showed that it was synthesized prematurely in round spermatids and that it was localized to spermatid nuclei, thus confirming the importance of the Prm-1 3' UTR for delayed translation. Premature translation of P1 caused abnormal nuclear shaping beginning in Step 10 spermatids and eventually a complete arrest of spermiation, resulting in sterility in hemizygous transgenic mice. Thus expression of the transgene acts in a dominant fashion, presumably as a result of transfer of P1 between spermatids via the intercellular bridges connecting them. These experiments clearly demonstrate the importance of the proper translational control of Prm-1 for normal spermatogenesis.

VI. Proteins That Bind to the Prm-1 3' UTR in Vitro

Preparation of postmitochondrial supernatants from round spermatids and Northern blot analysis of those supernatants fractionated on sucrose gradients have shown the Prm-1 mRNA sediments as a ribonucleoprotein particle (Kleene et al., 1984). In addition, replacement of the Prm-1 3' UTR with the hGH 3' UTR resulted in premature translation of an hGH reporter gene and a Prm-1 transgene, as described earlier. Together, these results suggest that Prm-1 is normally inhibited in round spermatids and that the mechanism entails protein(s) binding to sequences present in the Prm-1 3' UTR. How the binding of proteins to the 3' end of the mRNA inhibits translation initiation at the 5' end necessitates the identification and purification of such proteins.

We have developed a RNA gel mobility shift assay to identify proteins that bind in a sequence-specific manner to the 3' UTR of Prm-1. In this assay, in vitro-synthesized 32P-radiolabeled RNAs are incubated with protein extracts prepared from various sources, treated with RNase T1 to remove any RNA not bound by protein, treated with heparin to inactivate the RNase T1 and remove low-affinity binding proteins, and fractionated electrophoretically on a low percentage polyacrylamide gel (Koeller et al., 1989). Using this assay we have detected two different sequence-specific RNA-binding proteins present in nonnuclear extracts prepared from whole mouse testes.

The first of these proteins binds to a sequence in the 5'-most region of the Prm-1 3' UTR. The protein is not present in extracts prepared from adult liver, spleen, kidney, heart, or thymus, suggesting that it is restricted in its expression to the testis. Analyses of extracts prepared from mouse mutants blocked at different stages of germ cell development and from purified populations of germ cells by unit gravity sedimentation in bovine serum albumin gradients show that the factor is restricted to germ cells and is present in spermatocytes and round spermatids. Gel mobility shift assays performed with subclones of the Prm-1 3' UTR demonstrate that the protein binds to a region defined by deletion end points that are separated by 22 nts. In addition, RNase T1 mapping studies demonstrate that the protein protects a 16-nt RNA fragment from digestion by RNase T1. As expected, the 16-nt RNase T1-protected fragment is contained within the 22 nts defined by deletion analysis. Ultraviolet light crosslinking experiments using a 39-nt radiolabeled RNA that contains the 5'-most region of the Prm-1 3' UTR generate a doublet of RNA and protein that migrates with apparent molecular masses of 53 and 55 kDa. RNA mobility shift assays performed with various Prm-2 3' UTR RNAs also detect a RNA/protein complex that migrates coincident with the Prm-1 complex, suggesting that the same protein binds to both Prm-1 and Prm-2.

Using the RNA gel mobility shift assay, we have identified a second protein that is present in testis nonnuclear extracts and binds in a sequence-specific manner to the Prm-1 3' UTR. The analysis of subclones defined the region of binding to the 3'-most end of the 3' UTR. We have cloned the gene for the protein that binds to the 3'-most site and describe its binding properties in more detail below.

Kwon and Hecht (1991) have also performed RNA gel mobility shift assays with Prm-1 and Prm-2 3' UTR RNAs. They have described a protein of 18 kDa that binds to a sequence present in both 3' UTRs. They have also described two proteins of 48 and 52 kDa that bind nonspecifically to both Prm-1 and Prm-2 3' UTRs and have shown that the proteins are homologous to the Xenopus germ cell-specific RNA-binding proteins p54/p56 FRG Y2 (Kwon et al., 1993). Neither of the factors that we have described above correspond to the 18-kDa protein or to the p54/p56 Xenopus homologues described by Kwon et al. (1993). The 5'-most activity that we have described binds to a different region of Prm-1 and Prm-2 than does the 18-kDa protein; in addition, it has a combined RNA and protein molecular mass of 53/55 kDa. The 3'-most activity that we have described has been cloned and encodes a protein of 39 kDa.

As described earlier, as little as 62 nts of the Prm-1 3' UTR is sufficient to confer Prm-1-like translational control on the hGH reporter gene in transgenic mice (Braun et al., 1989a). Of the two site-specific RNA-binding activities that we have described for the Prm-1 and Prm-2 3' UTRs, only one of the binding sites is contained within the Prm-1 3'-most 62 nts. The
5'-most binding activity (53/55 kDa) that we have described, as well as the 18-kDa protein that Kwon and Hecht (1991) have described, both bind upstream of the terminal 62 nts that is sufficient for translational control in transgenic mice. Therefore, neither of these sites is absolutely essential for regulating the proper temporal translational control of Prm-1. However, it is possible that there is more than one region of the 3' UTR that can repress translation of Prm-1. Having multiple cis-acting elements might guarantee that even low levels of premature translation of Prm-1 are prevented. Alternatively, one or both of the factors could be involved in other aspects of Prm-1 mRNA metabolism not previously considered. We are currently testing the possibility of redundant cis-acting sequences in transgenic mice.

VII. A Molecular Screen for RNA-Binding Proteins

In an attempt to clone genes directly that encode RNA-binding proteins with specificity for the Prm-1 3' UTR, we screened cDNA expression libraries prepared from pachytene spermatocytes and round spermatids with Prm-1 RNA. In preliminary Northern blotting experiments, we identified seven proteins that hybridized to a 32P-radiolabeled Prm-1 RNA and not to a control RNA, the 3' UTR of hGh, suggesting that such a screen might be successful. cDNA libraries constructed in λgt11 were plated on a lawn of Escherichia coli, the plaques were transferred to nitrocellulose filters, the proteins were denatured in the presence of 6 M guanidine hydrochloride, and the proteins were renatured and hybridized with a digoxigenin RNA corresponding to the 3' UTR of Prm-1. Positive plaques were picked and rescreened with a RNA probe specific for the 3' UTR of the hGh gene. From this positive-negative screen we identified 19 recombinant phages which hybridized to RNA containing the 3' UTR of Prm-1 but not to the 3' UTR of hGh. Characterization of the relatedness of the clones by DNA cross-hybridization analysis indicated that we cloned five different genes. We next describe a summary of our partial characterization of three of these genes. DNA sequence analysis revealed that all three genes encode proteins that share a protein motif that is common to a collection of RNA-binding proteins present in a wide variety of organisms ranging from E. coli to humans. After outlining our current knowledge of all three genes, we present a highly speculative, but testable, model in which we suggest that the products of the three genes may all be involved in a common pathway that is involved in the processing, transport, and sequestration of Prm-1 mRNA.

A. TENR

T enr (Testis Nuclear RNA Binding Protein) was isolated seven times in the primary screen. DNA sequence analysis of the complete cDNA reveals an open reading frame that encodes a protein of 620 amino acids. Included in the final product is one copy of a 65–68 amino acid motif that is also found in the E. coli gene Rnase III, the Drosophila maternal effect gene Staufen, the Xenopus gene rbpu, and the human DAI kinase (Johnston et al., 1992). All of these proteins are thought to bind RNA. Northern analysis of RNA prepared from numerous adult male tissues indicates that Tenr is expressed exclusively in the testis. In situ hybridization analysis of testis sections detects Tenr mRNA in pachytene spermatocytes and round spermatids only. Immunocytochemical analysis of Tenr protein performed with antibodies raised against a maltose-binding protein-T enr (MBP) fusion synthesized in E. coli clearly shows that the protein is present in the nuclei of round spermatids. Thus, Tenr itself is under translational control.

B. SPNR

Spnr (Spermatid Perinuclear RNA Binding Protein) was isolated six times in the primary screen. DNA sequence analysis of the complete cDNA reveals an open reading frame that encodes a protein of 648 amino acids. Included in the protein are two copies of the same RNA-binding motif that is also present in the Tenr protein. Just upstream of the first RNA-binding motif is a region that contains a leucine zipper, suggesting that the protein may either interact with itself or with other proteins. Northern blotting experiments performed with a fusion protein between Spnr and maltose-binding protein show that the Spnr fusion protein binds to a radiolabeled RNA that contains the Prm-1 3' UTR, but not to the hGh 3' UTR or to single-stranded or double-stranded DNA. RNase protection assays indicate that the Spnr gene is transcribed at a relatively high level in the testis and at lower levels in the ovary, brain, and thymus. Immunocytochemical analysis of the Spnr protein performed with antibodies raised against a MBP–Spnr fusion synthesized in E. coli shows that the Spnr protein is first detected in Step 9 spermatids and that it is localized on the ventral cytoplasmic surface of the nuclear envelope opposite the developing acrosome. As spermiogenesis proceeds, the Spnr protein moves caudally to the base of the spermatid nucleus and eventually extends partway along the length of the flagellum. The Spnr protein is not detected in mature spermatozoa.
C. PRBP

PRBP (Protamine RNA Binding Protein) was present two times in the primary screen for Prm-1 RNA-binding proteins. DNA sequence analysis of the complete cDNA reveals an open reading frame that encodes a protein of 365 amino acids. Included in the protein are two copies of the same RNA-binding motif that is also present in Tenr and Spnr. RNA gel retardation experiments performed with a fusion protein constructed between PRBP and MBP demonstrate that the PRBP protein binds to the 3'-most region of the Prm-1 3' UTR. RNA gel mobility shift assays performed with increasing amounts of recombiant MBP-PRBP fusion protein generate additional complexes with slower electrophoretic mobility, suggesting the formation of multimers of RNA and protein.

Comparison of the binding sites for the PRBP and the 3'-most binding activity that is present in the testis protein extracts described in Section VI indicate that they are the same site. To determine if the activity present in testis protein extracts is the product of the PRBP gene, we performed a RNA "supershift" experiment. In this experiment, radiolabeled Prm-1 3' UTR RNA was incubated with testis protein extracts as in the usual gel mobility shift protocol. Following the addition of RNase T1 and heparin, the affinity-purified antibody raised against the MBP-PRBP fusion protein was added to the reaction. Analysis of the complexes present in the reaction revealed the absence of the complex normally found in the reaction and, instead, the presence of a new complex with much reduced mobility. We interpret the new complex as that consisting of the radiolabeled RNA, the endogenous PRBP protein, and the anti-MBP-PRBP antibody.

Immunocytochemistry performed with the affinity-purified antibody revealed that the protein is present in the cytoplasm of pachytene spermatocytes, round spermatids, and early elongating spermatids. Thus, the PRBP protein has a temporal and spatial localization consistent with it having a role in the inhibition of Prm-1 translation. In addition, the PRBP protein binds to a region of the Prm-1 3' UTR shown to be sufficient for translational repression of a reporter gene in transgenic mice.

VIII. A Model

The presence of a common RNA-binding motif in the Tenr, Spnr, and PRBP genes, along with studies of their RNA-binding properties in vitro, suggests that the products of all three genes may bind RNA in vivo. Immunolocalization studies performed with antibodies raised against each protein show that Tenr, Spnr, and PRBP occupy nuclear, perinuclear, and cytoplasmic locations within differentiating spermatids, respectively. We suggest that the products of these three genes may participate in a common molecular pathway involved in the nuclear and cytoplasmic metabolism of Prm-1 mRNA. The product of the Tenr gene may function in either the nuclear processing of Prm-1 mRNA or in its transport to the cytoplasm. The Spnr gene product is localized to the cytoplasmic side of the nuclear envelope and may interact with nuclear pores. If so, the Spnr protein may be involved in "capturing" Prm-1 mRNA as it exits the nucleus and in facilitating the assembly of a translationally inactive Prm-1 mRNP. The product of the PRBP gene binds to the 3'-most region of the Prm-1 3' UTR and is localized to the cytoplasm. We suggest that the PRBP protein is contained within the cytoplasmic Prm-1 mRNP and is directly engaged in preventing its translation. Although this is a highly speculative model for the function of these three gene products, our current data are consistent with the model, and most importantly, the model can be tested with a combination of genetic and biochemical experiments. We look forward to performing these experiments and in contributing to our knowledge of RNA metabolism in general, and to the cellular and molecular basis of mammalian spermatid differentiation.

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Role of the Renin–Angiotensin System in Blood Pressure Regulation and in Human Hypertension: New Insights from Molecular Genetics

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Ever since blood pressure could first be reliably measured, many studies have been carried out on the population distribution and hereditary nature of hypertension. Blood pressure is a quantitative trait that varies continuously throughout the whole population and whose regulation is controlled by a variety of mechanisms that involve several genetic loci and environmental factors. However, little is known about the genes actually involved in human hypertension, about their respective importance in determining blood pressure levels, or about their interaction with other genes and environmental components.

A number of epidemiologic studies have shown that individual blood pressure levels result from both genetic predisposition and environmental factors. The heritable component of blood pressure has been documented in familial and in twin studies, and in studies such as those performed in Montreal, where blood pressure levels were measured in families with natural and adopted children (Annest et al., 1979). The evidence suggests that approximately 30% of the variance of blood pressure is attributable to genetic heritability and 30% to environmental influences (Ward, 1990).

There are no data on the number of genetic loci involved in the regulation of blood pressure, the frequency of deleterious alleles, the mode of transmission, or the quantitative effects of any single allele on blood pressure. The unimodal distribution of blood pressure within each age group and in each sex strongly suggests, but does not definitively prove, that several loci are involved. Because of the likely etiologic heterogeneity of the disease, it is difficult to expect that a single biochemical or DNA genetic marker will help the clinician in the management of most hypertensive patients. However, genetic markers are useful indicators for elucidating the various genetic loci linked to high blood pressure. The genetic approach can disregard a gene as being frequently and importantly implicated.