In the rat testis, the vasopressin gene is transcribed into precursor RNAs that are processed into a number of mature transcripts. One of these transcripts has a structure identical to that of the hypothalamic RNA that encodes the vasopressin prepropeptide, but is present at such low levels that it can only be detected by the polymerase chain reaction. Other vasopressin-like RNAs are derived from differential splicing events that join transcribed sequences between 3 and 9 kilobases upstream of the hypothalamic transcription start site to exons corresponding to II and III of the hypothalamic-type RNA. Here we describe the sequence of a testis-specific promoter and the exon structure of its transcription unit. We show that an in vitro synthesized RNA corresponding to the longer testicular vasopressin gene-derived transcript is not able to act as a template for protein synthesis in two different cell-free lysates. As attempts to localize the vasopressin-gene derived RNAs to particular cell types in the testis by in situ hybridization have consistently failed, we have used indirect methods. Three different procedures were used to effect germ cell depletion in adult male rats. Acute heat treatment of the testis, chronic ingestion of hydroxyurea, and chronic ingestion of hydroxyurea and copeptin (a 39-amino acid glycopeptide of unknown function), are stored until released into the general circulation in response to appropriate physiological stimuli (3). The neuropeptide vasopressin is synthesized as a prepropeptide (1) in the cell bodies of hypothalamic neurons (1). This precursor is cleaved and processed as it is transported down axons to nerve terminals located in the posterior pituitary (2). Using exon-specific probes, PCR and cDNA sequencing, Foo et al. (16) showed that, whereas the novel, high abundance VP gene-derived RNAs in rat testis lack exon I of the corresponding hypothalamic VP mRNA and thus cannot encode the VP nonapeptide, exons II and III are identical in testis and hypothalamus. Preliminary genomic mapping data suggested that the 5'-end of the testis VP-like RNAs was generated by the splicing of transcribed sequences located many kilobase pairs upstream of the hypothalamic transcriptional start site to the intron I-exon II junction of the hypothalamic form of the VP transcript. Although the testicular VP-like RNAs are developmentally regulated, appearing around late puberty, no function could be ascribed to these molecules because of the lack of convincing open reading frames and the apparent lack of any association with translationally active polyadenylation signals (16). In this report, we address the question of the cellular origin and function of the testis-specific VP gene-derived RNAs. We also describe the structure of the transcription unit encoding these RNAs, including the intron-exon boundaries and the testis-specific VP gene promoter.

Another candidate target of VP is the testis. Evidence has been produced to suggest that VP can reduce local testicular blood flow (5) and is involved in the regulation of Leydig cell steroidogenesis and, hence, male reproductive activity (6-9). However, the physiological significance of these observations has been questioned because the ED50 for VP inhibition of testosterone synthesis (2.5 x 10^-10 M; Refs. 10 and 11) was shown to be 2 orders of magnitude greater than the concentration of VP in the general circulation (2-3 x 10^-12 M; Ref. 12). It has thus been postulated that for VP to have any function in the testis, it must either be sequestered from the general circulation and rendered effective by a local increase in peptide concentration, or it must be synthesized locally. The description of a peptide in rat testicular extracts with the immunologic and chromatographic properties of VP is consistent with both hypotheses (11, 15, 14). However, it was only with the application of technologies that allow for the direct measurement of gene activity that the question of local testicular biosynthesis of VP could be addressed.

An early report described the detection by Northern blotting of an RNA that hybridized to a specific VP probe (15). However, no information was presented at that time to suggest that this transcript was a structurally authentic template for VP prepropeptide biosynthesis. Indeed, it was subsequently shown (16, 17) that these RNAs represent a novel class of VP gene-derived transcripts. Foo et al. (16) could only detect hypothalamic-like VP mRNAs in rat testis by applying the exquisite sensitivity of the polymerase chain reaction. Recently these authentic mRNAs were shown to be present in Leydig cells, where they are probably translated to give authentic VP (18). Whether Leydig cells can synthesize enough biologically active VP to engender a local peptide concentration sufficient to effect the autocrine and paracrine effects postulated (18) has not been determined.

The abbreviations used are: VP, vasopressin; kb, kilobase(s); OT, oxytocin; PCR, polymerase chain reaction.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) L19320-L19323.

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A Testis-specific Promoter in the Rat Vasopressin Gene

MATERIALS AND METHODS

Animals and Animal Procedures

Animal studies were conducted in accord with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats were bred on site or were purchased from Animal Resource Centre (Perth, Australia). Germ cells were depleted using three different methods.

Vitamin A Depletion (19–23)—The normal Chow of 20-day-old male rats (35–40 g) was replaced with a vitamin A-deficient diet (ICN Biochemicals). The age of the rats at the start of the experiment was crucial, since animals given the vitamin A-deficient diet before 20 days of age suffered a high mortality rate, whereas those older than 20 days of age did not consistently show germ cell depletion. Animals continued to be fed normal Chow. Treatment continued for at least 9 weeks, until treated testes weighed less than those of control rats, at which point experimental and control animals were sacrificed and testes were processed for histological examination and RNA extraction. The diet of the remainder of the germ cell-depleted animals was changed to 20% (w/v) powdered rat chow set in 3% (w/v) agar containing 15 mg/ml retinoidic acid. These animals also received an intraperitoneal injection of 7 mg of retinoic acid in 0.1 ml of 100% (v/v) ethanol on the first day of vitamin A supplementation and a second intraperitoneal injection of 3.5 mg of retinoic acid on day 2. Animals were given normal rat chow after 1 week of vitamin A supplementation, after which recovery continued for 5–7 weeks. Following sacrifice by decapitation, animals were processed as described below.

Lower Abdominal Heat Treatment (25)–Male Sprague-Dawley rats weighing 200–250 g were anesthetized by intraperitoneal injection of 0.7 ml of CRC mixture (1 volume of Hypnorm [diazepam, fentanyl citrate, and fluanisone], 1 volume of Dormicum, and 2 volumes of water). The scrotal region of the animals was then submerged in a 42 °C water bath for 30 min. The lower abdomen of control rats was incubated in a 30 °C water bath or were allowed to recover from the anesthetic without any heat treatment. Following 1 or 2 weeks of recovery, animals were sacrificed by decapitation and processed as described below.

Following decapitation, trunk blood was collected into 50 ml Falcon tubes containing 50 μl of heparin (1 vial of Sigma heparin [catalog no. 210.6] was dissolved in 1 ml of 0.9% NaCl). Following centrifugation (4 °C, 3000 rpm, 10 min, Sorvall RT600B), the plasma supernatant was transferred to a 1.5-ml Eppendorf tube and kept frozen at −70 °C until assayed for testosterone (see below). Testes were removed from the sacrificed rats and weighed. The right testis was processed for histological analysis (see below), while the RNA was extracted from left testis (see below).

Histological Analysis

The right testis from each sacrificed animal was collected into Bouin's fixative (75 ml of picric acid, 25 ml of formaldehyde, and 5 ml of glacial acetic acid). Fixed tissue was paraffin-embedded. Sections (4 μm) were stained with periodic acid-Schiff-hematoxylin.

Testosterone Assays

Either extracts of rat plasma were assayed for testosterone using a kit purchased from Amersham Corp.

Isolation and Analysis of RNA

Total cellular RNA was extracted from testis tissues and analyzed by Northern blotting as described (26). The following antisense oligonucleotide Northern hybridization probes were synthesized (Dr. Ben Li, National University of Singapore) and 5’-end-labeled with polynucleotide kinase (New England Biolabs) and 5’-32PITAP (Amersham Corp.). The VP3’ probe corresponds to the sequences encoding the last 16 amino acids of VP (boxes A–D) (16). Primers corresponding to exons A through D of the novel testicular VP RNAs are diagrammed in Fig. 2A; the glyceraldehyde-3-phosphate dehydrogenase probe corresponds to nucleotides 361–404 of the rat cDNA (27). The transferrin cDNA probe pSP65T (28) was the kind gift of Michael D. Griswold and Steven R. Sylvester (Washington State University) and was random primer-labeled. Poly(A) tails were removed from total cellular preparations by hybridization to oligo(dT) followed by digestion with RNase H as described (16). Polyadenylated RNA was isolated from total cellular preparations using the Fast Track system from Invitrogen (San Diego, CA). Primer extension analysis has been described elsewhere (29). Northern blots were quantified by densitometric scanning of linear range autoradiograms (LKB Ultrascan).

Genomic DNA Analysis and Sequencing

Standard restriction mapping and sequencing techniques were employed (30).

Cell-free Translation of in Vitro Transcribed RNAs

The VP-test cDNA was subcloned into the pGem2 vector to make pGem2(VPTest). Full-length double-stranded cDNA copies of the hypothalamic VP and OT RNAs were isolated by polymerase chain reaction amplification of single-stranded cDNA generated by reverse transcription of microdissected rat supraoptic nucleus using oligo(dT) (Pharmacia) as a primer. The following primers were used in the PCR amplification reactions.

VP3’-Sense copies of testicular VP-like RNA were generated from linearized plasmids using SP6 RNA polymerase. Capped transcripts were generated by including 7mG5’pppG3’ (Pharmacia) in the transcription mixture at 0.5 pg/ml. The VP3’ sense RNAs synthesized in either rabbit reticulocyte or wheat germ cell-free translation systems (Promega), both of which were used according to manufacturer's directions.

Sequences described in this paper were obtained from the GenBank database.

NIH-3T3 cells were grown in DMEM and transfected with linearized plasmid DNA by the calcium phosphate procedure. Cells were selected in G418. RNAs were isolated using TRIzol Reagent (Gibco BRL). RNA was analyzed by Northern blotting as described (26). Anti-VP antibodies were purchased from Amersham Corp.
RESULTS

Exon Structure of the Testicular VP-like RNAs—We have previously described the isolation of a cDNA (VPTest) corresponding to one of the VP-related RNAs in rat testis (16). Fig. 1 illustrates the relationship between the exons contained within the VP gene-derived RNAs and the corresponding genomic sequences. Following the localization of 4 exons within genomic fragments, intron-exon junctions were sequenced (Fig. 2B). The location of these exons within the VPTest cDNA is shown in Fig. 2A.

The calculated length of the full-length RNA corresponding to the VPTest cDNA is 958 bases. However, deadenylation of testicular RNA by hybridization with oligo(dT) followed by digestion with RNase H resulted in VP-like RNAs between 650 and 800 bases (Fig. 3A). Comparing these RNAs with RNA synthesized in vitro by T7 RNA polymerase following the subcloning of VPTest into pGem2 markedly illustrates this size difference, even if additional sequences contained within the VPTest RNA, contributed by cloning procedures or vector-derived, are taken into account. In order to investigate the relationship between the exons present in the VPTest cDNA and the VP-like RNAs found in the rat testis, specific oligonucleotide probes corresponding to exons A through D were synthesized and used on Northern blots. All four probes hybridize to the two size classes of VP-like RNA originally identified in the testis by virtue of their containing exon III sequences (16, 17) (Fig. 3A). However, the ratio of the upper and lower size class RNAs was dependent upon the probe used. Exon II or exon III probes gave identical hybridization patterns (16). Similarly, the pattern of VP-like RNAs revealed by exon A and exon III probes is the same (Fig. 3B), with the ratio of the upper to lower transcript classes being 3.39 ± 0.55 (n = 2) and 3.36 ± 0.41 (n = 7), respectively. However, two different exon B probes revealed a more even ratio (Fig. 3B; 0.78 ± 0.22, n = 2, for probe B). Similar patterns were obtained with either of the exon C (0.28 ± 0.08, n = 4) and D (0.64 ± 0.33, n = 4) probes. These data suggest that exon A and exons II and III are represented in all the testicular VP-like RNAs, including the RNA corresponding to the VPTest cDNA, which, although not detectable by Northern blotting, represents a “prototype” testicular VP-like RNA. The smaller RNAs revealed by Northern blotting probably correspond to spliced transcripts containing combinations of 1 or 2 of each of exons B through D. The presence of additional exons not represented in the VPTest prototype cannot be ruled out.

Sequencing of genomic sequences upstream of exon A (Fig. 2C) revealed divergence from the VPTest sequence at position 23. Sequences between position 1 and 22 of the cDNA sequence were shown not to be represented in the rat VP genomic clone illustrated in Fig. 1, nor could RNAs in testis be detected with a probe corresponding to this region. This and the presence of an XbaI site between positions 13 and 18, which the cloning procedure should have excluded (16), suggests that the 5′ sequences between 1 and 22 are a cloning artifact. Such 5′ end artifacts are common in cDNA clones (32). Primer extension analysis was then used to identify putative
transcriptional start points of the testicular VP-like RNAs. Extension was primed using oligonucleotide A (Fig. 2A), which had previously been shown (Fig. 3B) to hybridize to all of those VP-like RNAs in rat testis detectable by Northern blotting. This primer gave a major extended fragment of 100 bases when in vitro synthesized VPTest RNA was used as a template (Fig. 4, lane 7), corresponding to upstream VPTest and contiguous vector-derived sequences. A minor extended product of approximately 140 bases was observed and may correspond to extension as a consequence of primer annealing which is 58% similar to the sequence between positions 111 and 142 of VPTest, corresponding to extension as a consequence of primer annealing which is 58% similar to the sequence between positions 111 and 142 of VPTest, but specific, extension products were seen up to 100 bases when a series of products were found that comigrated with the probe. A major extended product of 35 bases was derived from a rat testis total RNA template (Fig. 4, lane 4). This band was not seen when total mouse testis (Fig. 4, lane 3) nor total rat liver (Fig. 4, lane 2) RNAs were used as templates. The nonspecific bands produced by the use of total rat liver RNA as a template were lost when poly(A)-selected RNA was used (Fig. 4, compare lanes 2 and 10). Poly(A)-selected rat testis RNA gave the same extended 35-base band as was seen following extension of the total RNA preparation (Fig. 4, compare lanes 8 and 4). Additional minor, but specific, extension products were seen up to around 45 bases, where a series of products were found that comigrated with bands found in the testis mRNAs of the selected fraction (Fig. 4, lane 9). These bands probably correspond to extension from an RNA found in both mouse and rat testis. However, this RNA must be unrelated to VPTest because the use of the full-length cDNA probe has consistently failed to detect any signal in mouse testis RNA preparations.

Cell-free Translation of in Vitro Transcribed VP and VP-like RNAs—Sense RNAs corresponding to the hypothalamic VP and OT nRNAs and both sense and antisense RNAs corresponding to VPTest were synthesized in vitro, then used as templates for translation in rabbit reticulocyte (not shown) or wheat germ (Fig. 5) cell-free systems. The rabbit reticulocyte system readily generated correctly sized proteins when primed with RNAs that encode the hypothalamic VP and OT prepropeptides, but no peptide products were produced when either sense or antisense VPTest transcripts were used as templates (not shown). A small peptide at around 4 kDa was produced in wheat germ lysates by the uncapped VPTest sense template (Fig. 5, lane 2) but was not made when capped RNA was used (Fig. 5, lane 6).

The production of this peptide may be a consequence of nonspecific translation initiation on partially degraded uncapped template (33).

Germ Cell Depletion—We asked if treatments that disrupt spermatogenesis had any effect on the level of the VP-like RNAs. Three treatments were employed. First, 20-day-old male rats were fed with a diet deficient in vitamin A (19-23). Following 9 weeks of treatment, some rats were sacrificed and the diet of the remainder was supplemented with vitamin A to induce recovery of spermatogenesis. Controls received normal rat chow. The second treatment involved the chronic administration of hydroxyurea to adult male rats via their drinking water (19, 24). Controls continued to receive tap water. In the third regime, the scrotal region of anesthetized rats was submerged in a 42 °C water bath for 30 min (25). Controls were anesthetized but not heat-treated, or they were treated at 30 °C. All three treatments resulted in a significant reduction in testicular weight (Table I), and histological analysis revealed...
that this was a result of severe disruption of the structure of seminiferous tubules (Fig. 6). Following vitamin A supplementation of depleted animals, the morphology of the seminiferous tubules gradually adopted a normal appearance. Hydroxyurea treatment and scrotal heat treatment had no effect on plasma testosterone levels, indicating that steroidogenesis in Leydig cells was unaffected by these procedures (Table I). However, plasma testosterone levels were reduced in vitamin A-depleted animals (Table I) but returned to normal following 5 weeks of retinoic acid supplementation. Northern analysis revealed that all three treatments resulted in a marked reduction in the level of the testicular VP-like RNAs (Fig. 7). Retinoic acid supplementation of depleted animals resulted in the reappearance of these RNAs (Fig. 7). The three germ cell depletion treatments also resulted in a reduction in the level of the germ cell-specific 0.9-kb hemiferrin RNA, which is detected by a transferrin cDNA probe (Fig. 7; Ref. 34). However, the 2.6-kb transferrin mRNA, which is expressed in Sertoli cells (35), was enriched in germ cell-depleted animals (Fig. 7), as were the RNAs encoding α-tubulin and glyceraldehyde-3-phosphate dehydrogenase (Fig. 7).

**DISCUSSION**

The neurohypophysyal peptides VP and OT are potential mediators of physiologic function in the testis (11). However, since the dose of VP needed to inhibit gonadotrophin-stimulated testosterone synthesis is 2 orders of magnitude greater than the concentration of VP in the general circulation (10–12), the notion that VP is synthesized locally in the testis has gained in popularity. Such a hypothesis was supported by the description of a peptide in rat testicular extracts with the immunologic and chromatographic properties of VP (11, 13, 14) and by the detection by Northern blotting of an RNA that hybridized to a specific VP probe (15). However, we have shown (16; this report) that this RNA is in fact a family of novel transcripts, which, although derived from the VP gene, do not contain exon I and thus cannot encode the VP nonapeptide.

In this report, we describe the exon structure of the novel VP-like RNAs in the rat testis and address the question of their cellular origin and possible function(s). We have shown that a testicular VP transcript family is generated by differential splicing events that link exons that are many kilobase pairs upstream of the hypothalamic transcription start site to exons II and III of the corresponding hypothalamic VP gene transcript. The testicular VP-like RNAs are unlikely to be messengers. *In vitro* synthesized RNA corresponding to the larger testicular VP-like transcript failed to act as a template for protein synthesis in cell-free lysates. These data are consistent with our earlier demonstration that the testis VP-like transcripts, which do not contain any long open reading frames, were not associated with translationally active polysomes (16). Finally, we show here that the expression of the novel VP-like RNAs in rat testis is closely associated with the integrity of germ cells and ongoing spermatogenesis.

By comparing the sequence of a cDNA clone (VPtest) corresponding to one of the VP-like RNAs in the testis to the corresponding genomic sequences, we have been able to identify four testis-specific exons upstream of the transcriptional start site used in the hypothalamus. Primer extension analysis would suggest that testicular transcription initiates at a heterogeneous group of start sites located approximately 9 kb upstream of the sequences that encode hypothalamic exon I. The family of VP-like RNAs in the testis is probably derived as a result of differential splicing events that link a combination of the testicular exons to exons I1 and I11 of the hypothalamic-type VP transcript. Thus, as the hypothalamic exon I does not contain a splice acceptor sequence, the novel testicular VP gene-derived RNAs exclude hypothalamic exon I, which encodes the VP nonapeptide itself. The sequences of the exon-intron junctions of the four novel testis exons were obtained and compared to consensus and non-consensus sequences of other genes (36). Three of the four 5' donor sequences and all of the 3' acceptor sequences closely resemble the respective prototypes. However, one of the 5' donor sequences (A/B) has a GC pair instead of the "invariant" GT at the first two positions of the intron. This variant has now been documented to occur in at least 26 other 5' splice sites from a number of genes and species (36), and it has been reported (37) that the GC substitution results in a less efficient splicing event.

Repeated attempts to use *in situ* hybridization to localize the
Fig. 6. Germ cell depletion of rat testis. Histological analysis of rat testes following treatments resulting in germ cell depletion. The main picture shows a 40x magnification, and the inset shows 40x magnification. A, untreated adult testis at 400x magnification; B, untreated adult testis at 400x magnification; C, vitamin A depletion for 9 weeks; D-F, gradual recovery of tests of vitamin A-depleted animals following retinoic acid supplementation for 5 (D), 6 (E), and 7 (F) weeks; G and H, effect of scrotal treatment at 30 °C (G) and 42 °C (H). I and J, effect of chronic hydroxyurea treatment (panels I and J represent two independent experiments).

Fig. 7. Germ cell depletion results in the loss of the VP-like RNAs from the rat testis. Testicular RNA was extracted from animals that had been subject to the treatments indicated and analyzed (50 μg) by Northern blotting. Filters were first probed with an oligonucleotide corresponding to VP exon III and were subsequently reprobed with oligonucleotides, corresponding to rat α-tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and a transferrin cDNA, which detects both the hemferin RNA (H) and the transferrin mRNA itself (T). The lanes are as follows. Vitamin A depletion: lane 1, hypothalamic RNA (5 μg); lanes 2-6, testicular RNA from control animals (lane 2), animals depleted of vitamin A (lane 3), and vitamin A-depleted animals supplemented with retinoic acid for 5 (lane 4), 6 (lane 5), and 7 (lane 6) weeks. Vitamin A depletion resulted in the loss of 90% of VP-like RNAs. For scrotal heat treatment, two experiments are shown: no treatment (lane 1), 30 °C treatment (lane 2) and 42 °C treatment (lane 3). Heat treatment resulted in a 50% loss of VP-like RNAs. Chronic hydroxyurea ingestion: control rats (lanes 1 and 8) and individual treated rats (lanes 2-7). Note that there was some variation in the efficacy of the hydroxyurea treatment.
VP gene derived RNAs to particular cell types within the testis have failed to reveal specific signals. This is despite the use of many different tissue fixation and processing protocols and the use of a number of different oligonucleotide and RNA probes, detected using both radioactive and non-radioactive labels. Perhaps as a consequence of its subcellular localization, the RNA is lost from tissue sections or is protected from probe access. We have therefore sought to learn more about the cell-type expression of the VP-like RNAs in the testis using a variety of indirect approaches. We have previously described how EDS treatment of rats, which eliminates Leydig cells, does not affect the VP-like RNAs (16), suggesting a seminiferous tubule location for these transcripts. We now show that three different treatments that result in depletion of germ cells all result in a significant reduction in the level of the VP gene-derived RNAs.

Chronic dosing of male rats with hydroxyurea resulted in severe germinal cell depletion (Refs. 19 and 24; Fig. 6). Hydroxyurea, which prevents mitosis by inhibiting DNA synthesis, affects only germ cells in the testis. Leydig cells, as judged by gross morphology, relative number, and the continued synthesis of testosterone, are unaffected. Sertoli cells also appear to be unaffected, as would be anticipated because these cells cease division when the animal is 10 days of age. As expected, given the mode of action of the drug, type A spermatagonia are maintained following hydroxyurea treatment, but all cell types derived from the division of this stem cell are depleted. Similarly, scrotal heat treatment has little effect on Leydig cells as judged by morphology and testosterone synthesis. However, Sertoli cells contain lipid droplets, probably as a result of the phagocytosis of degenerating germ cells (25). Again, the germ cells of heat-treated rats were severely depleted, although type A spermatagonia and maturation phase spermatids show less damage (25). Vitamin A depletion, through unknown mechanisms, results in a cessation of spermatogenesis (19–23), which can be reinitiated by retinoic acid supplementation (23). Arrest is at two stages of spermatogenesis: at the preleptotene stage of meiosis and in spermatogonia. Vitamin A depletion thus results in atrophied seminiferous tubules that contain only Sertoli cells, spermatogonia, and a small number of preleptotene spermatocytes. Although the morphology of the interstitium does not change following vitamin A depletion, Leydig cell function is clearly affected as plasma testosterone levels are reduced in these animals. However, the reduction in testosterone is not responsible for germ cell depletion as androgen supplementation does not result in a reinitiation of spermatogenesis (38). That all three treatments resulted in a significant reduction in the level of the VP gene-derived RNAs in rat testis concomitant with varying degrees of germ cell depletion is strongly suggestive of a germ cell ori-

![Fig. 8. VP testis-specific promoter sequence motifs are found in other germ cell promoters. The sequence of the rat testis VP promoter was compared with the promoter sequences of a number of germ cell-specific promoters. Only those motifs found in two other promoters are presented here. A, Alignment of the rat VP testis promoter with a number of germ cell promoters. B, Comparison of CAGGGC motifs from a number of testicular promoters.](image)

which was evident in the parallel reduction in the levels of both the hemiferrin RNA and the VP-like RNAs. Overall, as a consequence of hydroxyurea treatment, the level VP-like RNA was reduced to 29% ± 7% (SE; n = 6) compared to controls. Note that the data presented from the three different experiments cannot be directly compared.
genes with testis-specific RNA variants are the c-abl (45-481, errant transcripts. Transcriptional events might result in the accumulation of ab-
to encode a protein or code for a structurally disabled peptide.
c-mos (49) and pim-1 (47) proto-oncogenes, the Hoxl.4 homeobox gene
riched in germ cell-depleted animals is an additional indica-
polysomes, whereas the transferrin RNA is efficiently trans-
transferrin RNA, which is enriched in Sertoli cells (34). Like
suggested (39) that cells of the testicular germ line are prone
transcripts found in male germ cells. Certain features charac-
lin germ cell promoters, which are located in intron I, are not
more, comparison of the sequence of the testicular
other germ cell promoters are GC-rich and lack TATA boxes,
rather than a “leaky” process. Like the rat
specificity. Many neuropeptide genes encode transcripts that
found between positions -35 and -30 of the rat
motifs are found in the rat farnesyl-pyrophosphate synthetase
gene, upstream of the testicular promoter (Fig. 8B). Similar
in intron 12 of the mouse angiotensin converting enzyme
sequences downstream of +23, which are similar to
intron I sequences downstream of +23, which are similar to the testis VP gene promoter between positions -92 and -53 (Fig. 8A), contain an additional motif (CAGGGC), which is found between positions -35 and -30 of the rat VP testis promoter (Fig. 8A; double-underlined). This motif is found twice in intron 12 of the mouse angiotensin converting enzyme gene, upstream of the testicular promoter (Fig. 8B). Similar motifs are found in the rat farnesyl-pyrophosphate synthetase gene and human acrosin gene germ cell promoters (Fig. 8).

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