TAK1: Molecular Cloning and Characterization of a New Member of the Nuclear Receptor Superfamily*

Takahisa Hirose, Wataru Fujimoto, Tomoichiro Yamaai, Kwan Hee Kim, Hironori Matsuura, and Anton M. Jetten

Cell Biology Section (T.H., A.M.J)
Laboratory of Pulmonary Pathobiology
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina 27709

Department of Dermatology (W.F., H.M)
Okayama University Medical School and
Department of Oral Anatomy (T.Y.)
Okayama University Dental School
Shikata Okayama, Japan, 700

Department of Genetics and Cell Biology (K.H.K.)
Washington State University
Pullman, Washington, 99164

Using polymerase chain reaction and two degenerate primers whose designs were based on the two best conserved regions of the DNA-binding domain of the nuclear receptor superfamily, we identified and cloned a novel orphan receptor, named TAK1. The open reading frame of TAK1 encodes a protein of 596 amino acid residues. Based on the modular structure and the presence of a DNA-binding domain containing two zinc fingers TAK1 belongs to the steroid/thyroid hormone receptor superfamily. The amino acid sequence of TAK1 is most closely related to the orphan receptor TR2-11. Their overall sequence homology is 64%, with the highest similarity (82%) being observed in the DNA-binding domain. Northern blot analysis using RNA from multiple human tissues showed that a 9.4 kilobase TAK1 transcript was expressed ubiquitously and that the presence of a 2.8 kilobase mRNA was largely restricted to the testis. *In situ* hybridization using sections of rat and mouse testes and Northern blot analysis using RNA from testes of rats at various ages revealed that TAK1 is most abundantly expressed in spermatocytes whereas little expression was observed in other germ cells or somatic cells. *In situ* hybridization using other mouse and rat tissues revealed cell type-specific expression of TAK1 in several tissues. Our observations suggest a role for this putative transcription factor in the regulation of gene expression in specific cell types. In the testis, TAK1 appears to control gene expression during spermatogenesis, particularly during the meiotic phase. (Molecular Endocrinology 8: 1667–1680, 1994)

0888-8810/94$03.00/0
Molecular Endocrinology

INTRODUCTION

The nuclear receptor superfamily comprises a group of ligand-dependent transcription factors that includes the receptors that bind and are activated by retinoids, and steroid and thyroid hormones (1–4). These receptors play a crucial role in the control of animal development, cellular differentiation, and homeostasis. Ligand-activated receptors regulate the expression of target genes by binding *cis*-acting sequences (5, 6). Sequence analysis and functional studies revealed that members of this family share a common modular structure that includes a highly conserved DNA-binding domain and a ligand-binding domain. The DNA-binding domain contains two zinc-finger motifs and mediates the interaction of the receptor with specific DNA sequences (hormone response elements) (7, 8). These response elements consist of either a direct- or inverted-repeat or a palindrome. The ligand-binding domain, in addition to determining ligand-binding specificity, contains a ligand-inducible transactivation function and a heptad-repeat motif implicated in the dimerization function of nuclear receptors (9–12). Based on the sequence and structural similarities with known members of the steroid hormone superfamily, a variety of orphan receptors have been cloned for which the ligand still must be identified (13). In this study, we report the isolation and characterization of a new member of this superfamily, named TAK1, that is relatively highly expressed in several tissues, including testis, kidney, and skeletal muscle.

The cell type-specific expression of TAK1 was further examined in several tissues and in particular the testis. The interstitium and the seminiferous tubules are the two major compartments in the testis (14, 15). The Leydig cell is the key cell type in the interstitium whereas the seminiferous tubules are the site for spermatogenesis and contain the Sertoli cells and germ cells. Spermatogenesis is a unique process of differentiation that occurs in three major phases, a mitotic phase, meiosis, and a postmeiotic phase in which haploid germ cells undergo extensive remodeling to produce spermatozoa (14, 15). The different stages of spermatogenesis are characterized by the expression of distinct patterns of gene expression. The expression of a large number of genes including hsp70.2 (16), *mak* (17), and GAPD-S (18) has been reported to be induced at specific stages of spermatogenesis. It is likely that the stage-specific induction of these genes is under the control of specific transcriptional factors. Several proteins that might regulate gene expression during specific stages of spermatogenesis have been recently described and include the zinc-finger proteins, *Zfp-35* (19) and *CTfin51* (20), and a POU-domain protein Sprm-1 (21). *In situ* hybridization and Northern analyses suggest that the orphan receptor TAK1 is a transcriptional factor that appears to control gene expression, in particular, during the meiotic phase of spermatogenesis. These observations indicate that TAK1 is expressed in a cell type-specific manner and, therefore, is involved in the regulation of specific biological processes.

RESULTS

Cloning of TAK1 cDNA

The polymerase chain reaction (PCR) method was employed to identify new members of the nuclear receptor superfamily in human lymphoblastoma Raji cells. Degenerate primers R-P1 (5′-end primer) and R-P2 (3′-end primer) were designed according to two conserved segments of the DNA-binding domain shared by the retinoid receptors (22–25) and chicken ovalbumin upstream promotor binding transcription factor (COUP-TF) (26). Amplification of a cDNA library prepared from RNA of human lymphoblastoma Raji cells with R-P1 and R-P2 yielded multiple DNA fragments of different sizes. Since the predicted size of products correctly amplified with these primers is about 130 base pairs (bp), we isolated and cloned the amplified products of this size into the TA cloning vector and sequenced the putative DNA-binding domains. Sixty independent clones were sequenced. Most of the sequences were identical to the sequence of the DNA-binding domain of known members of the nuclear receptor family and included retinoic acid receptor-α (RARα) (22, 23), retinoid X receptor-β (RXRβ) (27), thyroid hormone receptor-α (28), NUCI (29), COUP-TFII (30), and the human homolog of Nurr1 (31) (data not shown). However, one fragment constituted a unique sequence not previously described. The amino acid residues predicted by this DNA sequence suggested that this DNA fragment encoded the DNA-binding domain of a novel member of the steroid hormone receptor superfamily which we named TAK1.

With the help of the 5′-rapid amplification of cDNA ends (RACE) method we obtained a longer, 600-bp fragment of the TAK1 sequence that was subsequently used as a probe in screening a Raji cell, a human testis, and a human brain cDNA library (see *Materials and Methods*). No positive clones were obtained from screening a Raji cell cDNA library possibly due to low copy number. Two positive clones (λT7 and λ T10) were obtained from the testis cDNA library after surveying 2×10^5 independent plaques and one clone (λB5) from the brain library after screening 8×10^6 plaques (Fig. 1a). The sequence of these three clones matched each other and that of the amplified 600-bp TAK1 fragment. Because none of these clones included the complete 3′-sequence of the coding region, we rescreened the testis cDNA library with a probe for the 3′-end of the known sequence. Ten positive clones were obtained of which the two clones (λT9 and λT8) containing the largest insert were sequenced. The sequence of the 5′-region of the two clones were identical to each other; however, the clones differed in the length of their 3′-untranslated region (UTR). In clone λT9 the polyadenylation signal [nucleotide (nt) 2184; Fig. 2] was followed 27 nucleotides further by a poly-A tail; clone λT8 had instead of the poly-A tail an extended 3′-UTR (Figs. 1a and 2). To confirm that these different cDNAs constituted the coding sequence of one gene, we designed two primers based on the sequence of the 5′- and 3′-UTRs (Fig. 1a). With these primers we were able to amplify a TAK1 sequence with the expected size of 1.8 kb by reverse transcription (rt)-PCR from human testis and Raji cell mRNA (Fig. 1b).

Analysis of the TAK1 sequence revealed a long open reading frame that starts with a putative initiation codon at nucleotide 241 and ends with a putative stop codon at nucleotide 2029 (Fig. 2). On this basis, TAK1 encodes a protein of 596 amino acid residues with a predicted mol wt of 66 K. Analysis of the proteins synthesized by *in vitro* translation from the full-length coding region of TAK1 cDNA in rabbit reticulocyte lysate revealed that the major protein band migrated at approximately 65 K on sodium dodecylsulfate-polyacrylamide gels (data not shown). Like many other members of this family, the putative DNA-binding domain (amino acid residues 116–182) of TAK1 contains nine cysteine residues, eight of which participate in the formation of two zinc fingers. The carboxyl terminus of the protein may be part of a ligand-binding domain.

Comparison of the amino acid sequence of TAK1 with those of other members of the nuclear receptor family revealed that TAK1 exhibits a 64% identity with the human TR2-11 receptor, another orphan receptor cloned from a human testis cDNA library (32, 33) (Fig. 3). The greatest similarity between TAK1 and TR2-11 occurs in the putative DNA-binding domain (shown as

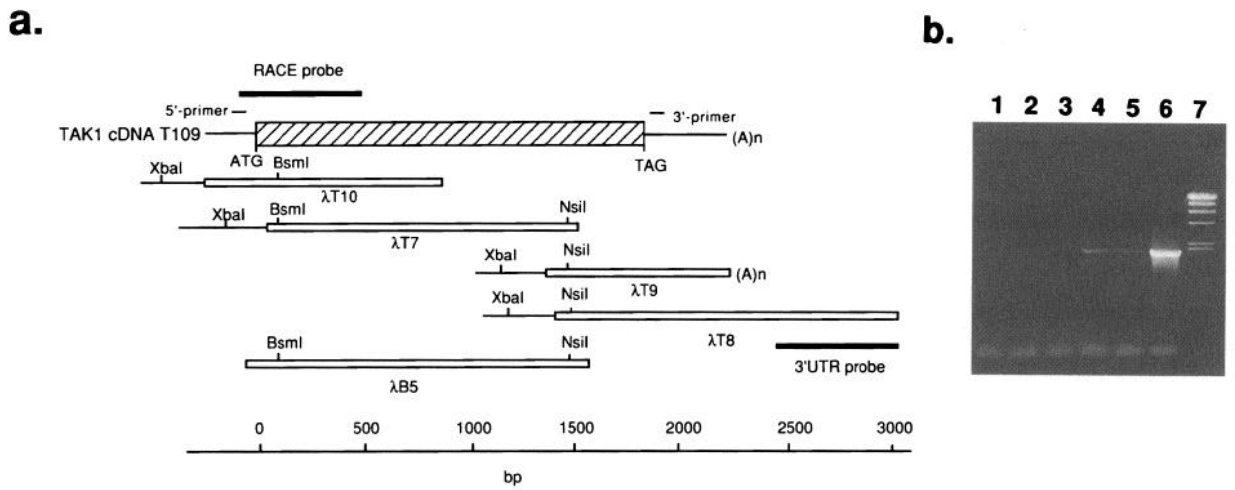

Fig. 1. Molecular Cloning of the Orphan Receptor TAK1

a, cDNA clones representing the complete coding region of TAK1. Strategy used in the construction of cDNA for TAK1. The clones λT7, -8, -9, and -10 were obtained by screening a human testis cDNA library. Clone λB5 was obtained by screening a human brain cDNA library. The plasmid T109 containing the full coding region of TAK1 was constructed from the three overlapping clones λT7, -9, and -10 as described in *Materials and Methods*. The RACE- and 3′-UTR probe (used in Fig. 4b) are indicated in *bold solid lines*. The *hatched box* represents the open reading frame of TAK1; the initiation and stop codons are indicated. *Open box* represents TAK1 inserts in cDNA clones; restriction sites are indicated. b, Amplification of the TAK1 coding region using RNAs from human testis (lane 4), Raji cells (lane 5), and T109 (lane 6) as template. RNA was reverse transcribed with oligo-dT primers and Moloney murine leukemia virus reverse transcriptase (Stratagene). Single stranded DNA was then amplified with the 5′-and 3′-primers shown in Fig. 1a with after cycles: 1-min denaturation at 94 C followed by a 1-min annealing at 65 C and 5-min extension at 72 C for 40 cycles. Control reactions without template (lane 1) or reverse transcriptase (lane 2 for testis RNA, lane 3 for Raji cell RNA) were carried out to rule out template contamination in reaction components or DNA contamination in RNA samples, respectively. λ*Hin*dIII markers are shown in lane 7.

region I in Fig. 3a) where 82% of 66 amino acid residues are identical (Fig. 3b) and in two distinct regions (shown as II and III in Fig. 3a) in the ligand-binding domain. The amino acid residues in regions II and III between TAK1 and TR2-11 are, respectively, 81% and 91% identical (Fig. 3, c and d). Outside these three regions homology between TAK1 and TR2-11 is much less. The N-terminal region and the region between the DNA- and ligand-binding domains exhibit only a 36% and 42% identity, respectively (Fig. 3a). Although the similarity between TAK1 and TR2-11 is substantial, it is much lower than the similarity among members of the retinoic acid receptor family (24). The next best homology was observed between the DNA-binding domains of TAK1 and COUP-TFs (73% identity) and the RXRα receptor (70% identity).

Tissue Distribution of TAK1 mRNA

To study the tissue specificity of TAK1 expression, Northern blot analysis was performed on poly(A)$^+$ RNA from a variety of human tissues. A cDNA probe encoding the full TAK1 coding region (T109 in Fig. 1a) hybridized to a 9.4 kb and a 2.8 kb transcript (Fig. 4a). The 9.4-kb transcript was present in many tissues and was most highly expressed in human kidney, skeletal muscle, and ovary. The presence of the 2.8-kb TAK1 mRNA was largely restricted to the testis (Fig. 4a). The 2.8-kb mRNA was expressed at relatively higher levels than the 9.4-kb transcript. Low levels of the 2.8-kb mRNA were detectable in skeletal muscle. Since the 600-bp RACE product was isolated from a cDNA library prepared from Raji cell mRNA, we examined the expression of TAK1 in these cells. The full-length TAK1 cDNA probe hybridized to a low abundant, 9.4-kb mRNA (Fig. 4d). Hybridization of the tissue blots with probes that only contained the 5′- (nt 211 to 441) or 3′-end of TAK1 (nt 1354 to 2016) or full-length cRNA probe yielded the same results as probing with the full-length cDNA probe (data not shown). When these blots were probed with the 3′-UTR fragment unique for clone λT8 (Fig. 1a), only the 9.4-kb transcript was detected (Fig. 4b). These observations indicate that the 2.8- and 9.4-kb transcripts include the same coding region but are different in the length of their 3′-UTR sequence. The expression of multiple and unique transcripts in the testis is not limited to TAK1 but has been reported for several other genes (34, 35).

Although TAK1 is homologous to TR2-11, the expression pattern and the size of the transcripts between the two receptors are very different. TR2-11 hybridizes to a 2.5-kb mRNA that is highly expressed in the prostate and liver and at a low levels in the testis (32). These observations suggest that these two receptors play different roles.

Examination of the tissue distribution of TAK1 mRNA expression in several mouse tissues (Fig. 4c) showed that the full-length human TAK1 cDNA probe hybridized to a 7.8- and a 2.8-kb transcript. The 7.8 kb was present

```
   1                                                                    GGC
      AAACTGTCATCAGGAGCTTAAATAGGACAGATTTTCACGGCAGTGGATATACCAGCATGGCTATTATCTATGGAGTGTT
      CTCTGCTTCAAATTTGATTACACCGTCAGTGGTTGCCATTGTAGGACCTCAACTCTCTATGTTTGCCAGTGGTTTATTT
      TACAGCATGTACATTGCCGTTTTCATCCAGCCTTTCCCGTGGTCCTTCTACACAGACCTCTCGGCCGGAATCTCCAGGG

 241  ATG ACC AGC CCC TCC CCA CGC ATC CAG ATA ATC TCC ACC GAC TCT GCT GTA GCC TCA CCT
   1  Met Thr Ser Pro Ser Pro Arg Ile Gln Ile Ile Ser Thr Asp Ser Ala Val Ala Ser Pro

 301  CAG CGC ATT CAG ATT GTC ACA GAC CAG CAG ACA GGA CAG AAA ATC CAG ATA GTC ACC GCA
  21  Gln Arg Ile Gln Ile Val Thr Asp Gln Gln Thr Gly Gln Lys Ile Gln Ile Val Thr Ala

 361  GTG GAC GCC TCC GGA TCC CCC AAA CAG CAG TTC ATC CTG ACC AGC CCA GAT GGA GCT GGA  A/B
  41  Val Asp Ala Ser Gly Ser Pro Lys Gln Gln Phe Ile Leu Thr Ser Pro Asp Gly Ala Gly

 421  ACT GGG AAG GTG ATC CTG GCT TCC CCA GAG ACA TCC AGC GCC AAG CAA CTC ATA TTC ACC
  61  Thr Gly Lys Val Ile Leu Ala Ser Pro Glu Thr Ser Ser Ala Lys Gln Leu Ile Phe Thr

 481  ACC TCA GAC AAC CTC GTC CCT GGC AGG ATC CAG ATT GTC ACG GAT TCT GCC TCT GTG GAG
  81  Thr Ser Asp Asn Leu Val Pro Gly Arg Ile Gln Ile Val Thr Asp Ser Ala Ser Val Glu

 541  CGT TTA CTG GGG AAG ACG GAC GTC CAG CGG CCC CAG GTG GTA GAG TAC TGT GTG GTC TGT
 101  Arg Leu Leu Gly Lys Thr Asp Val Gln Arg Pro Gln Val Val Glu Tyr Cys Val Val Cys

 601  GGC GAC AAA GCC TCC GGC CGT CAC TAT GGG GCT GTC AGT TGT GAA GGT TGC AAA GGT TTC
 121  Gly Asp Lys Ala Ser Gly Arg His Tyr Gly Ala Val Ser Cys Glu Gly Cys Lys Gly Phe

 661  TTC AAA AGG AGT GTG AGG AAA AAT TTG ACC TAC AGC TGC CGG AGC AAC CAA GAC TGC ATC   C
 141  Phe Lys Arg Ser Val Arg Lys Asn Leu Thr Tyr Ser Cys Arg Ser Asn Gln Asp Cys Ile

 721  ATC AAT AAA CAT CAC CGG AAC CGC TGT CAG TTT TGC CGG CTG AAA AAA TGC TTA GAG ATG
 161  Ile Asn Lys His His Arg Asn Arg Cys Gln Phe Cys Arg Leu Lys Lys Cys Leu Glu Met

 781  GGC ATG AAA ATG GAA TCT GTG CAG AGT GAA CGG AAG CCC TTC GAT GTG CAA CGG GAG AAA
 181  Gly Met Lys Met Glu Ser Val Gln Ser Glu Arg Lys Pro Phe Asp Val Gln Arg Glu Lys

 841  CCA AGC AAT TGT GCT GCT TCA ACT GAG AAA ATC TAT ATC CGG AAA GAC CTG AGA AGT CCC   D
 201  Pro Ser Asn Cys Ala Ala Ser Thr Glu Lys Ile Tyr Ile Arg Lys Asp Leu Arg Ser Pro

 901  CTG ATA GCT ACT CCC ACG TTT GTG GCA GAC AAA GAT GGA GCA AGA CAA ACA GGT CTT CTT
 221  Leu Ile Ala Thr Pro Thr Phe Val Ala Asp Lys Asp Gly Ala Arg Gln Thr Gly Leu Leu

 961  GAT CCA GGG ATG CTT GTG AAC ATC CAG CAG CCT TTG ATA CGT GAG GAT GGT ACA GTT CTC
 241  Asp Pro Gly Met Leu Val Asn Ile Gln Gln Pro Leu Ile Arg Glu Asp Gly Thr Val Leu

1021  CTG GCC ACG GAT TCT AAG GCT GAA ACA AGC CAG GGA GCT CTG GGC ACA CTG GCA AAT GTA
 261  Leu Ala Thr Asp Ser Lys Ala Glu Thr Ser Gln Gly Ala Leu Gly Thr Leu Ala Asn Val

1081  GTG ACC TCC CTT GCC AAC CTA AGT GAA TCT TTG AAC AAC GGT GAC ACT TCA GAA ATC CAG
 281  Val Thr Ser Leu Ala Asn Leu Ser Glu Ser Leu Asn Asn Gly Asp Thr Ser Glu Ile Gln

1141  CCA GAG GAC CAG TCT GCA AGT GAG ATA ACT CGG GCA TTT GAT ACC TTA GCT AAA GCA CTT
 301  Pro Glu Asp Gln Ser Ala Ser Glu Ile Thr Arg Ala Phe Asp Thr Leu Ala Lys Ala Leu

1201  AAT ACC ACA GAC AGC TCC TCT TCT CCA AGC TTG GCA GAT GGG ATA GAC ACC AGT GGA GGA
 321  Asn Thr Thr Asp Ser Ser Ser Ser Pro Ser Leu Ala Asp Gly Ile Asp Thr Ser Gly Gly

1261  GGG AGC ATC CAC GTC ATC AGC AGA GAC CAG TCG ACA CCC ATC ATT GAG GTT GAA GGC CCC
 341  Gly Ser Ile His Val Ile Ser Arg Asp Gln Ser Thr Pro Ile Ile Glu Val Glu Gly Pro

1321  CTC CTT TCA GAC ACA CAC GTC ACA TTT AAG CTA ACA ATG CCC AGT CCA ATG CCA GAG TAC
 361  Leu Leu Ser Asp Thr His Val Thr Phe Lys Leu Thr Met Pro Ser Pro Met Pro Glu Tyr

1381  CTC AAC GTG CAC TAC ATC TGT GAG TCT GCA TCC CGT CTG CTT TTC CTC TCA ATG CAC TGG
 381  Leu Asn Val His Tyr Ile Cys Glu Ser Ala Ser Arg Leu Leu Phe Leu Ser Met His Trp  E/F

1441  GCT CGG TCA ATC CCA GCC TTT CAG GCA CTT GGG CAG GAC TGC AAC ACC AGC CTT GTG CGG
 401  Ala Arg Ser Ile Pro Ala Phe Gln Ala Leu Gly Gln Asp Cys Asn Thr Ser Leu Val Arg

1501  GCC TGC TGG AAT GAG CTC TTC ACC CTC GGC CTG GCC CAG TGT GCC CAG GTC ATG AGT CTC
 421  Ala Cys Trp Asn Glu Leu Phe Thr Leu Gly Leu Ala Gln Cys Ala Gln Val Met Ser Leu

1561  TCC ACC ATC CTG GCT GCC ATT GTC AAC CAC CTG CAG AAC AGC ATC CAG GAA GAT AAA CTT
 441  Ser Thr Ile Leu Ala Ala Ile Val Asn His Leu Gln Asn Ser Ile Gln Glu Asp Lys Leu

1621  TCT GGT GAC CGG ATA AAG CAA GTC ATG GAG CAC ATC TGG AAG CTG CAG GAG TTC TGT AAC
 461  Ser Gly Asp Arg Ile Lys Gln Val Met Glu His Ile Trp Lys Leu Gln Glu Phe Cys Asn

1681  AGC ATG GCG AAG CTG GAT ATA GAT GGC TAT GAG TAT GCA TAC CTT AAA GCT ATA GTT CTC
 481  Ser Met Ala Lys Leu Asp Ile Asp Gly Tyr Glu Tyr Ala Tyr Leu Lys Ala Ile Val Leu

1741  TTT AGC CCC GAT CAT CCA GGT TTG ACC AGC ACA AGC CAG ATT GAA AAA TTC CAA GAA AAG
 501  Phe Ser Pro Asp His Pro Gly Leu Thr Ser Thr Ser Gln Ile Glu Lys Phe Gln Glu Lys

1801  GCA CAG ATG GAG TTG CAA GAC TAT GTT CAG AAA ACC TAC TCA GAA GAC ACC TAC CGA TTG
 521  Ala Gln Met Glu Leu Gln Asp Tyr Val Gln Lys Thr Tyr Ser Glu Asp Thr Tyr Arg Leu

1861  GCC CGG ATC CTC GTT CGC CTG CCG GCA CTC AGG CTG ATG AGC TCC AAC ATA ACA GAA GAA
 541  Ala Arg Ile Leu Val Arg Leu Pro Ala Leu Arg Leu Met Ser Ser Asn Ile Thr Glu Glu

1921  CTT TTT TTT ACT GGT CTC ATT GGC AAT GTT TCG ATA GAC AGC ATA ATC CCC TAC ATC CTC
 561  Leu Phe Phe Thr Gly Leu Ile Gly Asn Val Ser Ile Asp Ser Ile Ile Pro Tyr Ile Leu

1981  AAG ATG GAG ACA GCA GAG TAT AAT GGC CAG ATC ACC GGA GCC AGT CTA TAG
 581  Lys Met Glu Thr Ala Glu Tyr Asn Gly Gln Ile Thr Gly Ala Ser Leu End

2032  CGCAAACCACACACCTGCCAAGGAGCAACAGAATCCTTCCAGGACCGTTCACATACAAAGAAAAGTAGTGGTATTTTGG
      TATGTGCAAATATTTCCATATGTTAGCCATTTCCTGTCTGGTTTCTCCTTATCTGTTAATCCCAGACAATAGCAATTAA
      AAAGACTAGTAGGATCCTTTCCTGACAT*(A)n

2218  *AAGAAATGTTTTAATGCCTTTTGATGAAGCAGCAGATTTTGGAACAATCTTTTAACTCAATTTGTATTTAGAAATTCT
      CAAAGGGCAAAAAACAAAAAAAAAGGTTTTATAATGTCAGAGACTAGTATTAAAGAAAACTGAAAGAACCTGAGAGAA
      TAGTATGTGTGTATATATATATATAAAAACGTCCTTGGAATTATAGATACTAATTACCAGGGTAATAATATTAGCACT
      TTCTAAGCACTTATCAATGTGTAACGTTAGCAACATCTTGCCTGTGGGCAGGGCAAATGGAAAACTGTGTATGTCTTT
      TGCCGAAATGCTAATGATTTCTGTGAAAACTCACTAGGGTACAGGAGACAATCATTTATGTTTAAGAAAAGAAGGCAT
      CATTCCTAATTGTGTGCACATGTTGTGGAGTTGAGCTGAATTCTGTGAATGGAAACGTGGCTCATTTGCATCATGCAG
      TAAGTGGGAGTGTGGTAGCACAGTCGGTGGTGACCCAGTTCAGAAGCTTCTAGCCATAGAGCAGACACTTGTCAGGTT
      CCCTGACTACTTGGTCCTGGTCTCTGATGGGCATCTGCAGACTCCTCTAGAACCTGGGGTTCTCCTTTGATGACTGGT
      TATCTGAATTGGATTGCAACTAGTCAGACATTAACTGCCAAATGAGATGTACAGTTCCTCCAGCAAGTGAAAACATCC
      AAGTCACATCCGCCGCTCGTGGCAGATGGAGCCTTGTGACCAAAAAGTGCAAGGTGGGCATTTTGAGCTCTTGACAGT
      ACTTCCAATACAATTGGGGGTGCTTGTGTGTTTGTCCAGATGGAGGAGTGTGGCCTTGGAGTGTGAGCGTTACCTTCC
      CAGGGCTTTCCACTCCAAATGCCCCCTTGAAAAGGGCTCGGTTTCTGCAGCTCCATCATAACTGTGAGGCTGGATTGG
      GGTCTGGGCAGGAGCCTGCTGCCGCCTGGCGAGAGTGGTTAGGGCCTGGTAAAGCTGGATGGAAGGTTTCAGGACAAT
      TTCTTCCTGTTGACCTTAAATACCAGAGATTTTAAAAATGTGTATAGACTCAGCATCTCTGTTGGCAAGTCTGTTAAT
3319  GTTAGCAGCACACTGTGCACTTTCGTTGCCCCAGGTGTGTGCCAGCCGACC
```

ubiquitously but was most abundant in kidney, liver, and testis. As shown for human tissues, the 2.8-kb transcript was restricted to the testis; however, the ratio between the level of the two transcripts was quite different in mouse and human testis.

In Situ Analysis of TAK1 Expression in Adult Mouse Testis

To examine whether TAK1 is expressed in a cell type-specific manner, we investigated TAK1 expression in the testis, which is one of the tissues where TAK1 is highly expressed. The mature testis consists of many different cell types, Sertoli cells, Leydig cells, myoid cells, and a variety of germinal cell types. To determine which of these cells express TAK1, we analyzed cross-sections of testes from adult mice by *in situ* hybridization. When sections probed with antisense TAK1 cRNA were examined at low magnification, a ring of silver grains was observed residing in the seminiferous tubules (Fig. 5, a and b). No specific hybridization signal was observed when sections were probed with sense TAK1 cRNA (Fig. 5c). At higher magnification the ring of silver grains was observed to overlie the spermatocytes (Fig. 5, d and e). The number of silver grains associated with spermatogonia, spermatids, spermatozoa, and Sertoli cells was slightly above background levels (average number of silver grains per cell is 3.0, 17.9, 2.4, and 1.8 for spermatogonia, spermatocytes, spermatids, and background, respectively). During spermatogenesis in the adult mouse, germ cell differentiation advances in highly ordered waves along the axis of the seminiferous tubule and each cross-section of a tubule can represent one of the 12 stages of spermatogenesis (15). The observed variation in the intensity of the hybridization signal between tubules (Fig. 5, a and b) appears to be related to the different stages of the spermatogenic cycle in the tubules. The seminiferous tubule on the *left side* in Fig. 5 (panels d and e), corresponding to stage VIII-IX of the spermatogenic cycle, had a strong hybridization signal that was primarily associated with the spermatocytes. The tubule on the *right* (Fig. 5, d and e) with a weak hybridization signal represents stage II-III of the cycle and, in contrast to the stage VIII-IX tubule, contained few primary spermatocytes. After observing many tubules in various stages, we concluded that the expression of TAK1 mRNA is highest around stages VIII-IX and lowest around stages II-III. This expression pattern resembles that of ferT mRNA, which encodes a testis-specific tyrosine kinase (36).

Expression of TAK1 mRNA in Vitamin A-Deficient Rat Testis

Vitamin A deficiency has been reported to block spermatogenesis at an early stage. The seminiferous tubules in the testis of vitamin A-deficient rats contain spermatogonia, a few preleptotene spermatocytes, and Sertoli cells but no mature germ cells (37, 38). We compared the expression of TAK1 mRNA in testes from normal and vitamin A-deficient rats by *in situ* hybridization (Fig. 6). The normal rat testis exhibited a similar pattern of hybridization as was observed in mouse testis (Fig. 6, a and b) but testis of vitamin A-deficient rats did not hybridize to the TAK1 probe (Fig. 6, c and d). (The signal observed in the interstitial region in Fig. 6d is not significant since no silver grains are observed in the same region in Fig. 6c.) In addition, analysis of poly(A)$^+$ RNA isolated from testes of normal and vitamin A-deficient rats by rt-PCR confirmed the absence of TAK1 expression in vitamin A-deficient testis (data not shown). These observations are in agreement with the conclusion that TAK1 is not expressed in spermatogonia but becomes expressed later during the meiotic phase of spermatogenesis.

Developmental Onset of TAK1 Transcription in Rat Testis

We used the rt-PCR technique (Fig. 7, a and b) and Northern blot analysis (Fig. 7c) to determine the precise age at which TAK1 transcription is induced in the rat testis. We used sulfated glycoprotein 1 (SGP-1), which is constitutively expressed in Sertoli cells (39), as an internal control. Analysis of poly(A)$^+$ RNA isolated from testes of rats at ages 5–40 days by rt-PCR showed that TAK1 mRNA was induced as early as day 10, increased 8-fold at day 20. During this period the relative level of SGP-1 mRNA did not change. An increase in the 7.8-kb TAK1 transcript was also observed between day 10 and 20 when RNA was examined by Northern blot analysis. A 2.8-kb transcript was also detected but was expressed at very low levels relative to the 7.8-kb transcript as has been demonstrated for mouse testicular mRNA (Fig. 4b). The time of onset of TAK1 expression correlates with the appearance of spermatocytes (day 18) as the initial wave of spermatogenesis occurs in rat, suggesting that TAK1 mRNA is mainly expressed in spermatocytes.

In Situ Hybridization Analysis of TAK1 Expression in Several Other Tissues

The results above show that, in the testis, TAK1 expression occurs in a cell-type specific manner (Fig. 8). Since

Fig. 2. Nucleotide and Deduced Amino Acid Sequence of TAK1

Nucleotides and amino acids are numbered on the *left side* of the sequence. The putative initiation codon is at nucleotide (nt) 241. An in-frame termination codon in the 5′-UTR is *underlined*. Region C and E/F are *underlined*, and the location of region A-E/F is indicated on the *right side* of the sequence. The termination codon (nt 2029) at the end of TAK1 amino acid sequence is indicated as END. The polyadenylation signal (ATTAAA) is *underscored*. The sequence of the extended 3′-UTR (nt 2218–3360) is derived from clone λT8 (Fig. 1a) and shown at the *bottom*. *Asterisk*, Site of 3′-UTR extension.

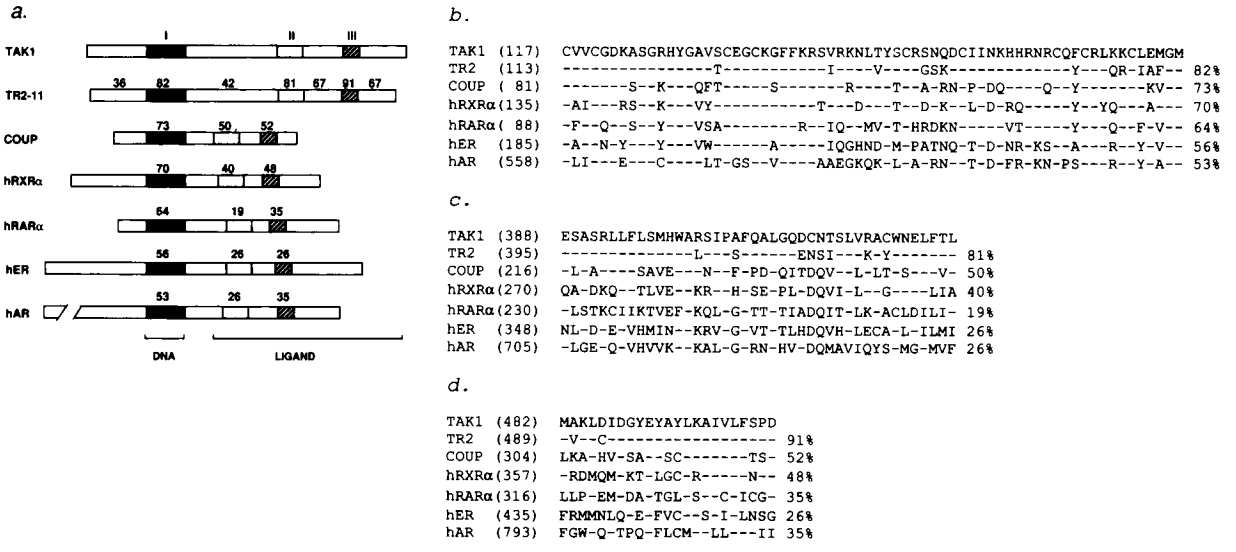

Fig. 3. Schematic Comparison between the Amino Acid Sequence of TAK1 and Several Members of the Steroid Hormone Receptor Superfamily

a, Alignment of amino acid sequences identified three regions with high similarity, shown as I, II, and III. The *numbers above each box* indicate the percentage of identity with TAK1. The DNA and ligand-binding domains are indicated. Specific references are COUP (26), hRXRα (25), hRARα (22, 23), estrogen receptor [hER (63)] and androgen receptor [hAR (64)]. The prefix h denotes human in all cases. Amino acid sequence alignment of DNA-binding domain (region I), region II, and region III of TAK1 and members of the human steroid hormone receptor superfamily are shown in panels b, c, and d, respectively. The *numbers in brackets* represent the positions of amino acid residues in the individual receptors. Amino acids conserved with TAK1 are indicated with a *bar*. The *numbers on the right* of amino acid sequences indicate the percentage of identity with TAK1.

TAK1 is expressed almost ubiquitously as shown by Northern blot analysis (Fig. 4), we examined the cell-type specific expression of TAK1 in several other tissues by *in situ* hybridization. In the brain, TAK1 expression was not uniform but occurred in very distinct regions. In the cerebral cortex, high levels of TAK1 transcripts were detected in the external granular layer (Fig. 8, a and b). In the hippocampus, a strong hybridization signal was observed on the granular layer of the gyrus dentatus and the pyramidal layer of the hippocampus (Fig. 8, c and d). In the cerebellar folia, TAK1 was highly expressed in the granular layer of the cerebellar cortex (Fig. 8, e and f). In mouse spleen TAK1 expression is associated with the red pulp rather than the white pulp (Fig. 8, g and h). In the femoral muscle (musculus biceps femoris), which is one of the tissues where TAK1 is most highly expressed, TAK1 transcripts were highly expressed in the muscle fiber but not in the connective tissue (Fig. 8, i and j). In the rat trigeminal ganglion, a strong hybridization signal was observed in the large neurons (Fig. 8, k and l). In the kidney, where TAK1 is also highly expressed, TAK1 transcripts were present in both the proximal tubules and the glomerulus; however, the most intense hybridization signal was associated with the proximal tubules (data not shown).

DISCUSSION

TAK1 Is a Member of the Steroid/Thyroid Hormone Receptor Superfamily

In this study, we describe the cloning and characterization of a new member of the nuclear receptor superfamily that we named TAK1. Like other members of this superfamily, TAK1 contains a DNA-binding domain that contains two zinc-finger motifs. Recently, members of the nuclear receptor superfamily were classified into four subfamilies with respect to their DNA-binding specificity (40). The amino acid sequence present in the C-terminal region of the first zinc finger (P-box) is the key to this classification. The P-box sequence of TAK1 consists of the amino acid sequence CEGCKG indicating that TAK1 belongs to the group of nuclear receptors comprising the receptors for retinoids, thyroid hormones, and vitamin D, peroxisome proliferator activating receptor, and several other orphan receptors. This may imply that TAK1 recognizes response elements that contain a perfect or imperfect GGTCA motif. Comparison of the TAK1 sequence with that of other nuclear receptors revealed that TAK1 is the most closely related to TR2-11 (33). The TAK1 sequence is 64% identical to that of TR2-11; their DNA-binding domains exhibit an 82% identity. The DNA-binding domain of TAK1 has a 73% and a 70% identity with that of COUP-TF and RXRα, respectively (26, 25). Both TAK1 and TR2-11 have a ligand-binding domain of more than 350 bp, which is relatively long compared with most other receptors of the superfamily. O'Malley and Conneely (41) have suggested that not all orphan receptors necessarily require a ligand for their activation but that some might be activated through posttranslational modifications such as phosphorylation. Recently, it was reported that the orphan receptor Nur77 [also named NGFI-B (42, 43)] may operate by such a mechanism

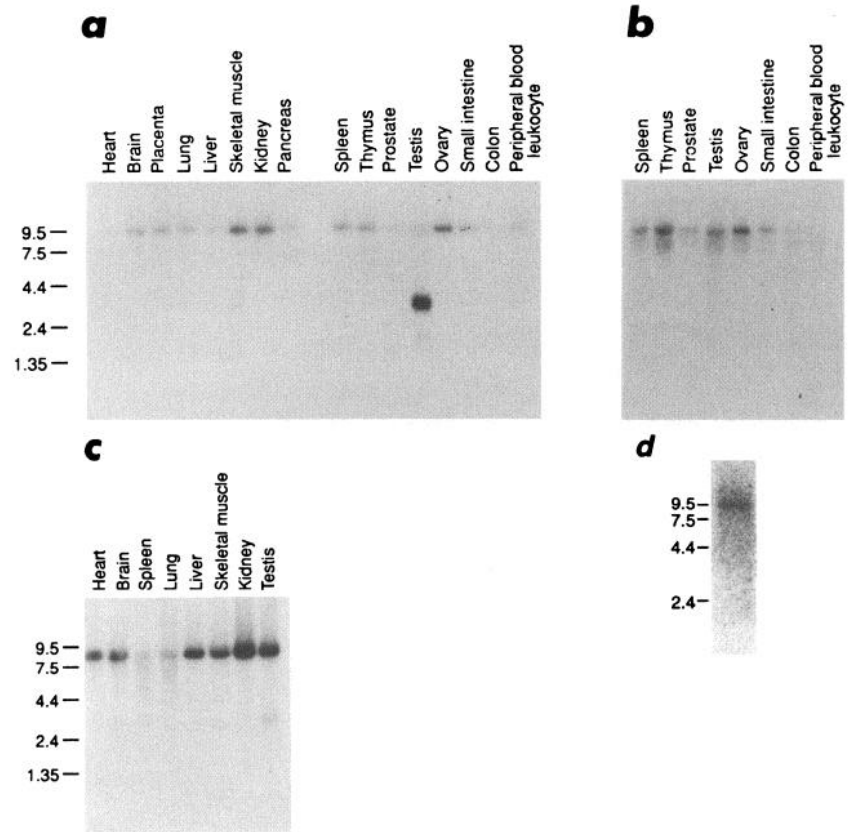

Fig. 4. Tissue Specificity of TAK1 expression

Poly(A)$^+$RNA (2 μg) isolated from different human (a and b) or mouse (c) tissues, or Raji cells (d) were analyzed by Northern blot analysis. a, c and d, The ^{32}P-labeled insert of clone T109 was used as a probe; b, the 3′-UTR unique for clone λT8 (Fig. 1a) was used as a probe. RNA size markers are indicated on the *left side* of the blot. RNA blots (Clontech) used in panels a and b are from different lots. Both probes were labeled with ^{32}P by random priming.

since it can bind the sequence AAAGGTCA and activate promotor activity without ligand binding (44). Whether any transcriptional activating function of TAK1 is dependent on the binding of a specific ligand has yet to be determined. Coexpression of a chimeric receptor containing the DNA-binding domain of the estrogen receptor and the ligand-binding domain of TAK1 with an estrogen-responsive reporter gene in Hela cells has failed thus far to identify a ligand. Several potential ligands were analyzed including 14-hydroxy-4,14-retro-retinol (45).

Tissue- and Cell Type-Specific Expression of TAK1

Analysis of the tissue-specific expression of TAK1 revealed that TAK1 mRNA is expressed in many tissues but is most highly expressed in the testis, kidney, and skeletal muscle. Furthermore, although TAK1 and TR2-11 appear related, their tissue-specific expression is quite different. The expression of TR2-11 is much more restricted: TR2-11 is most highly expressed in the prostate and liver and present at low levels in the testis. In human and mouse tissues TAK1 exists as two transcripts, 9.4 (7.8 kb in mouse) and 2.8 kb in size. The larger mRNA appears to be present almost ubiquitously whereas the smaller mRNA is largely restricted to the testis. The generation of multiple and unique transcripts in the testis is not limited to TAK1 but has been reported for a number of other genes (34, 35). These transcripts may be generated by alternative splicing, the testis-specific use of alternative promoter or transcription start sites, or the selection of alternative polyadenylation signals (34, 35, 46). The isolation of different TAK1 cDNA clones that differ in the length of their 3′-UTR and the Northern analysis using a probe for the unique 3′-UTR sequence suggest that the proximal polyadenylation signal is preferentially used in human testis whereas a more distal signal is used in other tissues. Other examples of alternative polyadenylation selection have been reported in the testis including aspartate aminotransferase and β1 galactosyl transferase (34, 35). The shorter 3′-UTRs could modify the stability and the translational control of the corresponding mRNAs.

Although the expression of TAK1 is almost ubiquitous, *in situ* hybridization analysis indicates that TAK1 is expressed in a cell type-specific manner. In the testis, TAK1 mRNA is differentially expressed during spermatogenesis. The highest level of TAK1 mRNA is associated with spermatocytes. *In situ* hybridization analy-

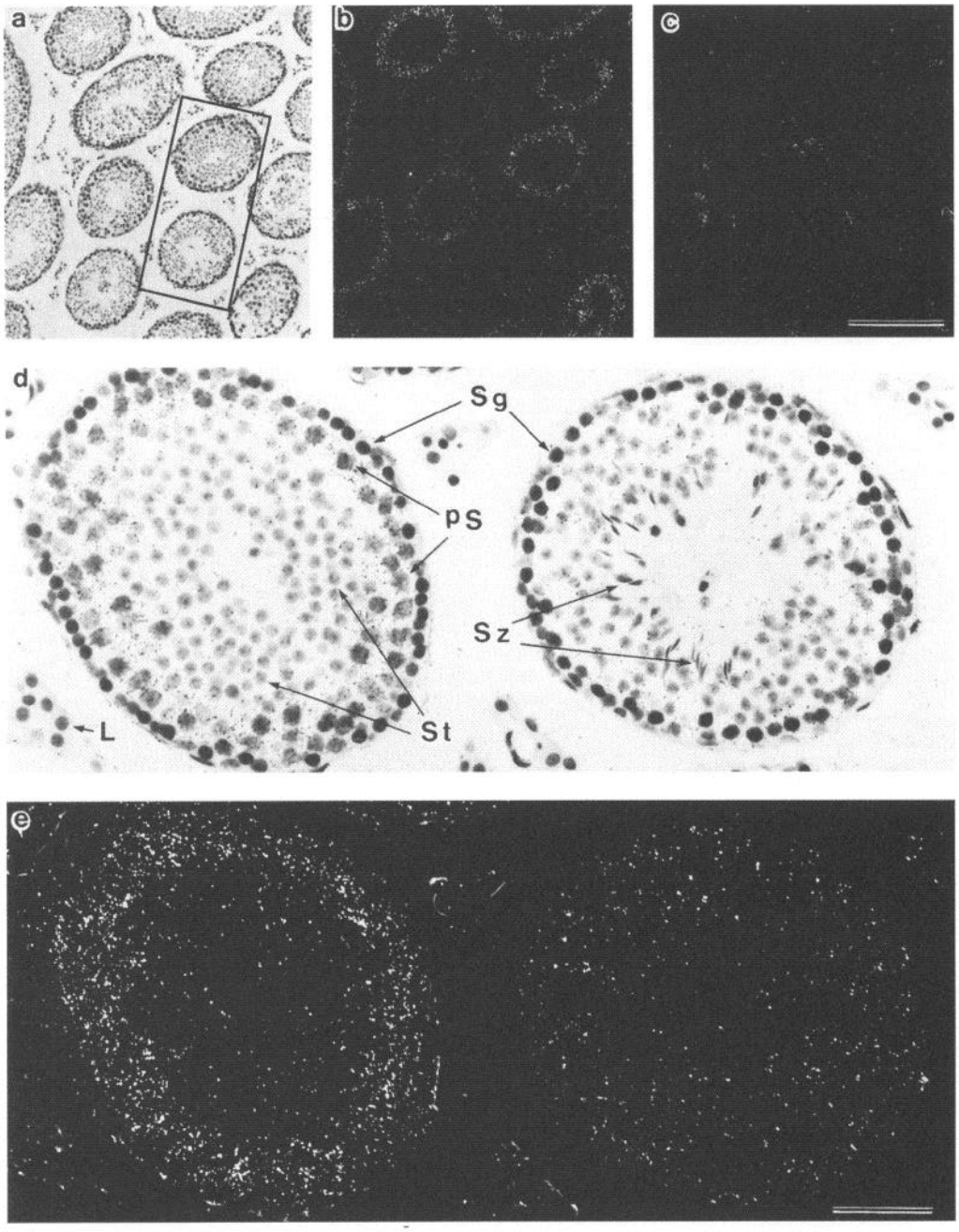

Fig. 5. *In Situ* Localization of TAK1 mRNA in Adult Mouse Seminiferous Tubules

Bright-field (a) and dark-field (b) photomicrographs of a section of mouse testis analyzed by *in situ* hybridization with a radiolabeled antisense TAK1 RNA probe and stained with hematoxylin. c, Dark-field photomicrograph of adjacent section hybridized with a sense TAK1 RNA probe. *Bar*, 200 μm. Higher magnification of the section outlined in panel a is shown in panels d (bright-field) and e (dark-field). *Bar*, 20 μm. L, Leydig cell; Sg, spermatogonia; pS, primary spermatocytes; St, spermatids; Sz, spermatozoa.

sis of vitamin A-deficient rat testis and rt-PCR and Northern blot analysis of mRNA prepared from testes taken from rats at various ages are in agreement with this conclusion. These observations suggest that the expression of TAK1 is dependent on the cell type. This conclusion is supported by the *in situ* hybridization analyses of TAK1 expression in several other tissues. These results show that the distribution of TAK1 transcripts is not uniform but occurs as distinct patterns in the mouse brain, muscle, and spleen and in rat trigeminal ganglion.

In the testis, TAK1 appears to play a role in the regulation of gene expression during the meiotic phase rather than the pre- or postmeiotic phase of spermatogenesis. A number of other genes, including phosphoglycerate kinase (*Pgk-2*) (47), testis-specific tyrosine

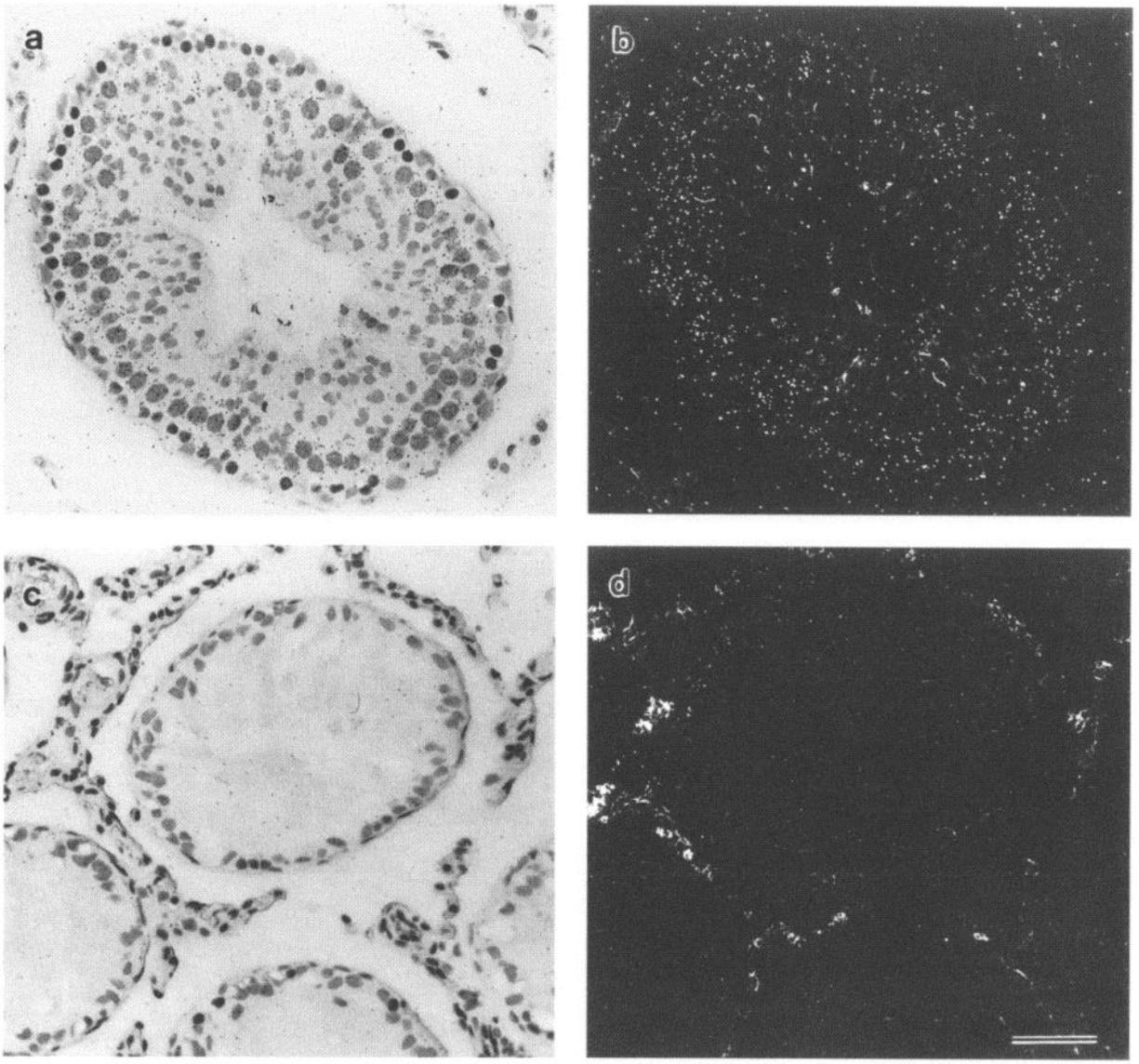

Fig. 6. *In Situ* Localization of TAK1 mRNA in Seminiferous Tubules from Normal and Vitamin A-Deficient Adult Rats

Bright-field (a and c) and dark-field (b and d) photomicrographs of sections of normal (a and b) and vitamin A-deficient (c and d) rat testis analyzed by *in situ* hybridization with a radiolabeled antisense TAK1 RNA probe and stained with hematoxylin. *Bar*, 50 μm.

kinase ferT (36), homeobox gene *Hox-1.4* (48), *meg1* (49), hsp 70.2 (50), and p53 (51), have been reported to be up-regulated during the meiotic phase of spermatogenesis. Some of these genes may be candidates for regulation by TAK1. The genes *Zfy-1, Zfy-2, Zfp-29, Zfp-35*, and *CTfin51* (19, 20, 52–54), encoding proteins with zinc finger motifs, are also induced during meiosis. However, the expression pattern of these genes is different from that of TAK1 since they are expressed both in spermatocytes and spermatids. The recently reported Sprm-1 gene encoding a transcription factor containing a POU-domain is expressed immediately before meiosis and not expressed at later stages (21). This pattern of expression is similar to that of TAK1. Several other members of the nuclear receptor superfamily have been found to be expressed in germ cells. These receptors appear to play a role in the regulation of gene expression at different phases of spermatogenesis. RARα is expressed in germ cells during meiosis (38, 55), and its importance in spermatogenesis was shown by RARα knock-out experiments (56). Recently, we have cloned a highly testis-specific orphan receptor referred to as RTR which is expressed predominantly in round spermatids (56a). Some of the testis-specific genes have been mapped on the Y chromosome and are transcribed only in male germ cells whereas others are also expressed during meiosis in oogenesis (20). Whether TAK1 is expressed in the

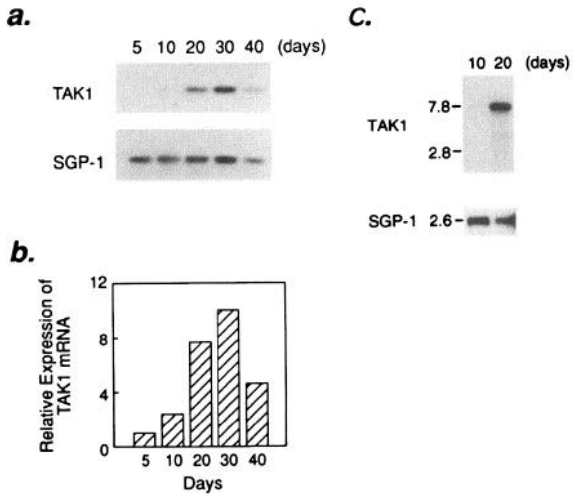

Fig. 7. Developmental Onset of TAK1 mRNA Synthesis in Rat Testis

Poly(A)$^+$ RNA was isolated from testes taken from rats at various ages (days of age denoted *above each lane* or *below each bar*) and was subjected to rt-PCR analysis to determine the relative level of TAK1 mRNA (a). The amplified products were separated on a gel and hybridized to a ^{32}P-end-labeled oligonucleotide probe that is complimentary to a sequence in the middle of the predicted 472-bp amplified product. Relative level of TAK1 mRNA is expressed as the ratio of TAK1 mRNA to SGP-1 mRNA, which was determined with the same method as TAK1 mRNA; the ratio at day 5 was converted to 1 (b). c, Northern blot analysis using 3 μg poly(A)$^+$RNA isolated from testes of 10- and 20-day-old rats. Full-length ^{32}P-labeled TAK1 probe and 480-bp (nt 430–909) SGP-1 probe were used to detect each mRNA (39). This result is representative of similar data from three different experiments.

meiotic phase during oogenesis has yet to be established.

Our results show that TAK1 is a novel member of the nuclear receptor superfamily. This putative transcription factor is likely to play a role in the regulation of gene expression during a variety of developmental processes, homeostasis, cell growth, and differentiation in several tissues.

MATERIALS AND METHODS

PCR Amplification

A set of degenerate primers were designed according to the most highly conserved sequence of the DNA-binding domain of members of the nuclear receptor family (13). The sense strand primer R-P1 was 5′-T-G-(T/C)-G-A-(G/A)-G-G-(G-A-T-C)-T-G-(T/C)-A-A-(G/A)-G-G-(T/C)-T-T-(T/C)-T-T-3′ (CEGCK-GFF). The antisense strand primer R-P2 was 5′-(G/A)-C-A-(T/C)-T-T-C-T-(G/T)-(G/A/T/C)-C-G-(T/C)-C-A-G-T-A-(T/C)-T-G-(G/A)-C-A-3′ (CQYCRL(K/Q)KC). A lymphoblastoma Raji cell cDNA library (Clontech, Palo Alto, CA) and the primers were employed in the amplification reaction with the Amplitaq kit (Perkin-Elmer Cetus, Morrisville, NC) and the DNA thermal cycler (Perkin-Elmer Cetus). We carried out the following amplification cycles: 1 min denaturation at 94 C followed by a 1 min annealing at 50 C and 1 min extension at 72 C for 30 cycles. The amplification products were separated on a 2% low temperature melting grade (LTG) agarose gel (Sigma, St. Louis, MO) and stained with ethidium bromide. The DNA products of 130 bp were isolated from the gel and directly ligated to TA cloning vector (Invitrogen, San Diego, CA). The DNA inserts of 60 clones were analyzed by double strand dideoxy DNA sequencing using Sequenase (US Biochemical, Cleveland, OH).

Anchor PCR

To isolate a greater region of the gene encoding TAK1, we used the 5′-RACE kit (Bethesda Research Laboratories (BRL), Grand Island, NY). Briefly, two sequential antisense primers Sp-1 (5′-ATTGATGATGCAGTCTTGGTTGCT-3′) and Sp-3 (5′-GCGGTTCCGGTGATGTTTATT-3′) were designed from sequence of original TAK1 PCR fragment. Poly(A)$^+$ RNA was isolated from human lymphoblastoma Raji cells and single strand cDNA primed with Sp-3 primer was synthesized from 1 μg mRNA with Superscript reverse transcriptase according to manufacturer's recommendation (BRL). After the degradation of RNA template with RNase H the reaction product was purified with Glass MAX Spin Cartridge (BRL). After denaturation by heating, the products were homopolymerically tailed with deoxy-cytosine triphosphate and terminal deoxytransferase and subsequently amplified by PCR at stringent conditions with the anchor primer poly-(dG) and the Sp-1 primer (57). The amplification products were separated on an 1.2% LTG-agarose gel and subsequently isolated from the gel and ligated to pBluescript plasmid (Stratagene, La Jolla, CA). The DNA inserts were analyzed by single strand dideoxy DNA sequencing using Sequenase.

cDNA Library Screening

λ-Phage cDNA libraries from human brain (Stratagene) and human testis (Clontech) were screened with multiprimed cDNA probe from anchor PCR products with stringent conditions. cDNA inserts from several positive clones were subcloned into the *Eco*RI site of pBluescript. The complete DNA sequences of both strands were determined by the same method as anchor PCR products.

Construction of T109

The plasmid T109 containing the full coding region of TAK1 was constructed from three overlapping clones λT7, -9, and -10 obtained from screening a human testis cDNA library. These clones were subcloned into the *Eco*RI site of the pBluescript SK(−) and named T7, T9, and T10, respectively. To obtain the 2.22-kb cDNA that encoded the full-length coding region of TAK1, the 1.43-kb *Xba*I/*Nsi*I fragment of T7 was inserted into the *Xba*I/*Nsi*I-digested T9 vector yielding a vector named T79. Then the 0.37-kb *Xba*I/*Bsm*I fragment of T10 was inserted into the *Xba*I/*Bsm*I-digested T79 vector yielding the vector T109 (Fig. 1a).

Northern Blot Analysis

Human and mouse multiple tissue Northern blots were purchased from Clontech. The blots were hybridized with a ^{32}P-labeled (Amersham, Arlington Height, IL) full-length TAK1 probe or 3′-UTR as described previously (58).

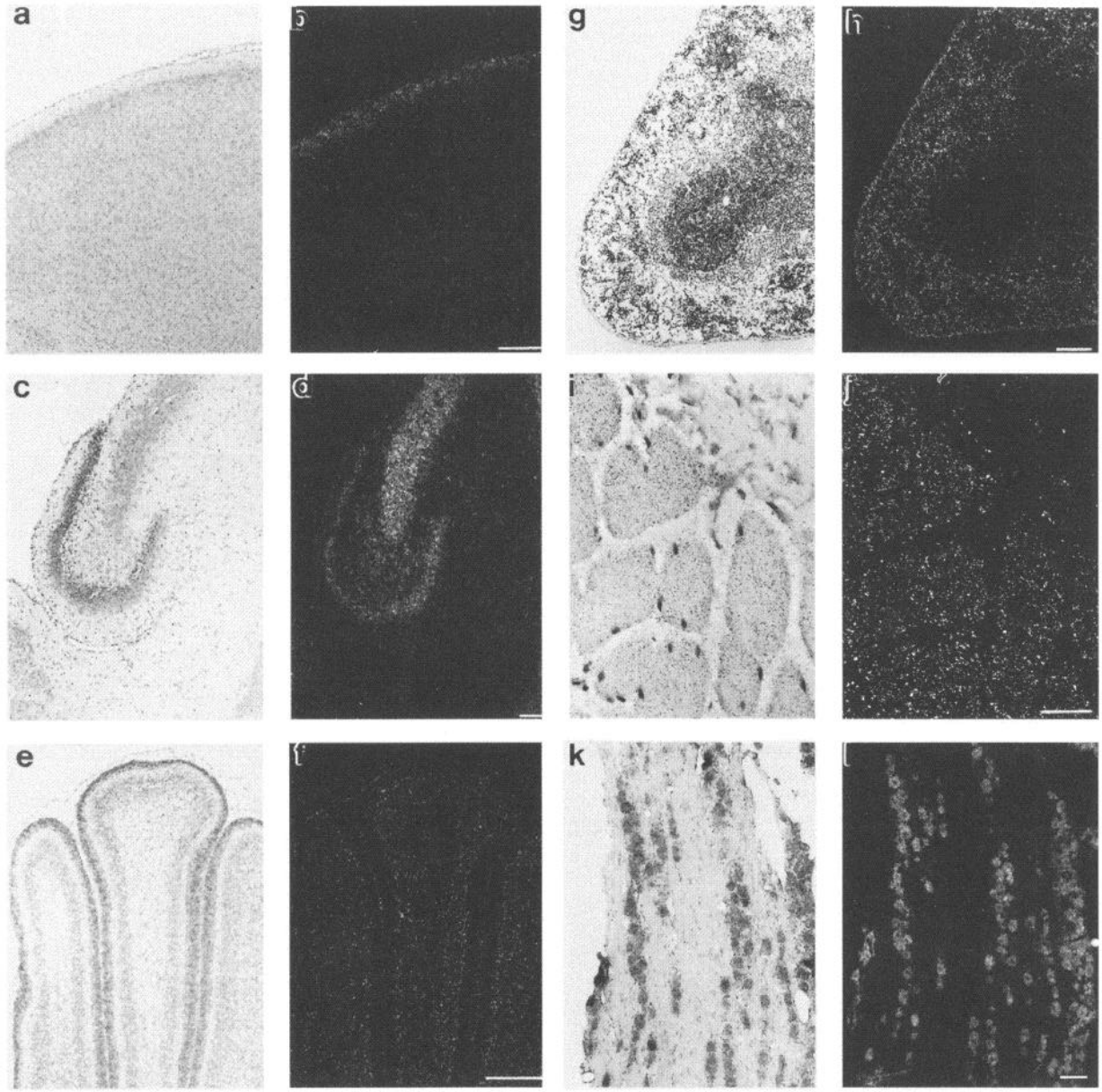

Fig. 8. *In Situ* Localization of TAK1 mRNA in Several Tissues

Bright field (a, c, e, g, i, and k) and dark field (b, d, f, h, j, and l) photomicrographs of different tissue sections analyzed by *in situ* hybridization with a radiolabeled antisense TAK1 RNA probe and stained with hematoxylin. a and b, Mouse cerebral cortex; c and d, mouse Gyrus dentatus and hippocampus; e and f, mouse cerebellar folia; g and h, mouse spleen; i and j, mouse femoral muscle (musculus biceps femoris); k and l, rat trigeminal ganglion. *Bars*, 100 μm except panels i and j (*bar*, 50 μm).

Preparation of Vitamin A-Deficient Rats

Vitamin A-deficient male Sprague-Dawley rats (Laboratory Animal Resource Center at Washington State University, Pullman, WA) were obtained as described previously (37). The animals were examined to verify that the standard criteria for vitamin A deficiency had been met (59, 60). Then they were killed and subjected to *in situ* hybridization experiments.

In Situ Hybridization

Adult (4-month-old) C3H mouse or adult Sprague-Dawley rats (normal and vitamin A-deficient) were fixed by perfusion through the left ventricle with 0.07 M sodium cacodylate-buffered 4% paraformaldehyde, and testes were embedded in Paraplast. Serial sections (5 μm) were obtained, deparaffinized, acetylated, and pretreated with 2× sodium citrate (SSC)-50% formamide-10 mM dithiothreitol at 50 C as described (61). Radiolabeled sense and antisense TAK1 probes (specific activity 5–8 × 10^8 cpm/μg) were generated by *in vitro* transcription from the T3 and T7 RNA polymerase promoter in the presence of uridine 5′-[α-^{35}S]thiotriphosphate (Amersham). Probes were subjected to limited alkaline hydrolysis to reduce the size of transcripts to about 150 nucleotides. After hybridization at 50 C for 15–17 h in a humid chamber, the sections were washed and treated with RNase as described (61). Washes were carried out at high stringency (55 and 65 C); no

differences in the hybridization patterns were observed between these two temperatures. The slides were immersed in NTB2 emulsion (Kodak, Rochester, NY) for autoradiography, exposed for 2 weeks at 4 C, and then developed and counterstained with hematoxylin.

Reverse-Transcriptase PCR

rt-PCR was used to determine the initial appearance of TAK1 mRNA during development according to the method of Murakawa *et al.* (62). Poly(A)$^+$ RNA (1 μg) samples prepared from developmentally staged testes were treated with DNase to eliminate contaminating genomic DNA and were then used as templates for the synthesis of single strand cDNA with Moloney murine leukemia virus reverse transcriptase (Stratagene). Samples were then subjected to PCR amplification with *Taq* polymerase using a kit from Perkin-Elmer Cetus. 5′-TGCCCAGFTCATFAFTCTCTCC-3′ and 5′-GATCTGGCC-ATTA-TACTCTG-3′ delimiting a 472-bp TAK1 fragment were used as a sense and antisense primer, respectively, to initiate amplification. Multiple rounds of PCR (23–35) with specific primer were performed. The amplification products were electrophoresed through 1.2% agarose, and then vacuum blotted to nylon membrane and hybridized with a ^{32}P-labeled (Amersham) oligonucleotide (5′-CCAGGTTTGACCAGCACAAGCC-AGATTGAA-3′) probe that is complimentary to a sequence in the middle of the expected TAK1-amplified product. This sequence is not homologous to the sequence of the TR2-11 orphan receptor. Control reactions without template or reverse transcriptase were carried out for each experiment to rule out template contamination in reaction components or DNA contamination in RNA samples, respectively. To ensure that the relative levels of TAK1 mRNA were based on amplification results obtained from a linear range of PCR, various numbers of cycles of PCR were performed and the relative level of hybridization quantified by Phosphorimaging (Molecular Dynamics, Sunnyvale, CA). These results showed (data not shown) that amplification was consistently exponential at 25 cycles of PCR. To ensure that representations of TAK1 mRNA were based on amplification results from the same amount of template, we amplified SGP-1 mRNA as an internal control. Samples were subjected to 23 cycles of PCR amplification to generate 480 bp SGP-1 fragment using the SGP-1-specific primers 5′-CTCAACCTCTGCCAGTCCC-TT-3′ (upstream primer) and 5′-GGCAGGGA-GGATA-TTCTTGAT-3′ (downstream primer) (39). The PCR-amplified products were processed as decribed above and hybridized with ^{32}P-labeled oligonucleotide probe (5′-CTG-GATGTCAGTCACCAACTTCAT-3′) homologous to the middle region of the expected SGP-1 amplification product. Hybridization signals were quantified by Phosphor-imaging and ratios (TAK1/SGP-1) were calculated.

Acknowledgments

We thank Drs. E. M. Eddy, D. A. O'Brien, B. Sato, and N. A. Saunders for their advice during this study; Drs. E. M. Eddy and D. J. Dix for their comments on the manuscript; and Kyoko Hirose with help in preparing the manuscript.

Received February 25, 1994. Re-revision received August 25, 1994. Accepted August 29, 1994.

Address requests for reprints to: Dr. Anton M. Jetten, Cell Biology Section, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.

* TAK1 was submitted to EMBL/GenBank Database Libraries under accession No. U10990

Note Added in Proof During review of this manuscript, almost the same orphan receptor sequence (3′ UTR sequence was not included) was published by C. Chang *et al.* 1994 in Proc Natl Acad Sci USA 91:6040–6044.

REFERENCES

1. O'Malley BW, Roop DR, Lai EC, Nordstorm JL, Catterall JF, Swaneck GE, Colbert DA, Tsai M-J, Dugaiczyk A, Woo SLC 1979 The ovalbumin gene: structure, organization, transcription and regulation. Recent Prog Horm Res 35:1–42
2. Yamamoto KR 1985 Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 19:209–252
3. Evans RM 1988 The steroid and thyroid receptor superfamily. Science 240:889–895
4. Gronemeyer H 1991 Transcription activation by estrogen and progesterone receptors. Annu Rev Genet 25:89–123
5. Green S, Chambon P 1988 Nuclear receptors enhance our understanding of transcriptional regulation. Trends Genet 4:309–314
6. Beato M 1989 Gene regulation by steroid hormones. Cell 56:335–344
7. Miller J, McLachlan AD, Klug A 1985 Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. EMBO J 4:1609–1614
8. Evans RM, Hollenberg SM 1988 Zinc fingers: guilt by association. Cell 52:1–3
9. Carlstedt-Duke J, Stromstedt PE, Wrange O, Bergman T, Gustafsson JA, Jornvall H 1987 Domain structure of the glucocorticoid receptor protein. Proc Natl Acad Sci USA 84:4437–4440
10. Forman BM, Yang C, Au M, Casanova J, Ghysdael J, Samuels HH 1989 A domain containing leucine-zipper-like motifs mediate novel *in vivo* interactions between the thyroid hormone and retinoic acid receptors. Mol Endocrinol 3:1610–1626
11. Fawell SE, Lees JA, White R, Parker MG 1990 Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. Cell 60:953–962
12. Tasset D, Tora L, Fromental C, Scheer E, Chambon P 1990 Distinct classes of transcriptional activating domains function by different mechanisms. Cell 62:1177–1187
13. Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D 1992 Evolution of the nuclear receptor gene superfamily. EMBO J 11:1003–1013
14. Russell LD, Ettlin RA, Sinha Hikim AP, Clegg ED 1990 Histological and Histopathological Evaluation of the Testis. Cache River Press, Clearwater, FL
15. De Krester DM 1992 The testes. Bailliere's Clinical Endocrinology and Metabolism
16. Rosario MO, Perkins SL, O'Brien DA, Eddy EM 1992 Identification of the gene for the developmentally expressed 70 kDa heat-shock protein (P70) of mouse spermatogenic cells. Dev Biol 150:1–11
17. Matsushime H, Jinno A, Takagi N, Shibuya M 1990 A novel mammalian protein kinase gene (*mak*) is highly expressed in testicular germ cells at and after meiosis. Mol Cell Biol 10:2261–2268
18. Welch JE, Schatte EC, O'Brien DA, Eddy EM 1992 Expression of a glyceraldehyde 3-phosphatase dehydrogenase gene specific to mouse spermatogenic cells. Biol Reprod 746:869–878

19. Cunliffe V, Koopman P, McLaren A, Trowsdale J 1990 A mouse zinc finger gene which is transiently expressed during spermatogenesis. EMBO J 9:197–205
20. Noce N, Fujiwara Y, Sezaki M, Fujimoto H, Higashinakagawa T 1992 Expression of a mouse zinc finger gene in both spermatocytes and oocytes during meiosis. Dev Biol 153:356–367
21. Andersen B, Pearse RV, Schlegel PN, Cichon Z, Schonemann MD, Bardin CW, Rosenfeld MG 1993 Sperm 1: A POU-domain gene transiently expressed immediately before meiosis I in the male germ cell. Proc Natl Acad Sci USA 90:11084–11088
22. Petkovich M, Brand NJ, Krust A, Chambon P 1987 A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330:444–450
23. Giguere V, Ong ES, Segui P, Evans RM 1987 Identification of a receptor for the morphogen retinoic acid. Nature 330:624–629
24. Zelent A, Krust A, Petkovich M, Kastner P, Chambon P 1989 Cloning of murine alpha and beta retinoic acid receptors and a novel receptor gamma predominantly expressed in skin. Nature 339:714–717
25. Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM 1990 Nuclear receptor that identifies a novel retinoic acid response pathway. Nature 345:224–229
26. Wang L-H, Tsai SY, Cook RG, Beattie WG, Tsai MJ, O'Malley BW 1989 COUP transcription factor is a member of the steroid receptor family. Nature 340:156–160
27. Yu VC, Delsert C, Andersen B, Holloway JM, Devary OV, Naar AM, Kim SY, Boutin J-MGlass CK, Rosenfeld MG 1991 RXRβ: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell 67:1251–1266
28. Laudet V, Begue A, Henly-Duthoit C, Joubel A, Martin P, Stehelin D, Saule S 1991 Genomic organization of human thyroid receptor α (c-*erbA*-1) gene. Nucleic Acids Res 19:1105–1112
29. Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA 1992 Identification of new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. Mol Endocrinol 6:1634–1641
30. Wang L-H, Ing NH, Tsai SY, O'Malley BW, Tsai M-J 1991 The COUP-TFs compose a family of functionally related transcription factors. Gene Expression 1:207–216
31. Law SW, Conneely OM, DeMayo FJ, O'Malley BW 1992 Identification of new brain-specific transcription factor, Nurr1. Mol Endocrinol 6:2129–2135
32. Chang C, Kokontis J 1988 Identification of new member of the steroid receptor super-family by cloning and sequence analysis. Biochem Biophys Res Commun 155: 971–977
33. Chang C, Kokonitis J, Satchivi LA, Liao S, Takeda H, Chang Y 1989 Molecular cloning of new human TR2 receptors: a class of steroid receptor with multiple ligand-binding domains. Biochem Biophys Res Commun 165:735–741
34. Toussain C, Bousquet-Lemercier B, Garlatti M, Hanoune J, Barouki R 1994 Testis-specific transcription start site in the aspartate aminotransferase housekeeping gene promoter. J Biol Chem 269:3318–13324
35. Shaper N, Wright W, Shaper J 1990 Murine β1,4-galactosyltransferase: both the amounts and structure of the mRNA are regulated during spermatogenesis. Proc Natl Acad Sci USA 87:791–795
36. Keshet E, Itin A, Fischman K, Nir U 1990 The testis-specific transcript (ferT) of the tyrosine kinase FER is expressed during spermatogenesis in a stage-specific manner. Mol Cell Biol 10:5021–5025
37. Morales C, Griswold MD 1987 Retinol-induced stage synchronization in seminiferous tubules of the rat. Endocrinology 121:432–434
38. Kim KH, Griswold MD 1990 The regulation of retinoic acid receptor mRNA levels during spermatogenesis. Mol Endocrinol 4:1679–1688
39. Collard MW, Sylvester SR, Tsuruta JK, Griswold MD 1988 Biosynthesis and molecular cloning of sulfated glycoprotein 1 secreted by rat Sertoli cells: sequence similarity with 70-kilodalton precursor to sulfatide/GM1 activator. Biochemistry 27:4557–4564
40. Forman BM, Samuels HH 1990 Interaction among a subfamily of nuclear hormone receptors: the regulatory zipper model. Mol Endocrinol 4:1293–1301
41. O'Malley BW, Conneely OM 1992 Orphan receptors: in search of a unifying hypothesis for activation. Mol Endocrinol 6:1359–1361
42. Hazel TG, Nathans D, Lau LF 1988 A gene inducible by serum growth factors encodes a member of the steroid and thyroid hormone receptor superfamily. Proc Natl Acad Sci USA 85:8444–8448
43. Milbrandt J 1988 Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene. Neuron 1:183–188
44. Davis IJ, Hazel TG, Chen R-H, Blenis J, Lau LF 1993 Functional domains and phosphorylation of orphan receptor Nur77. Mol Endocrinol 7:953–964
45. Buck J, Derguini F, Levi E, Nakanishi K, Hammerling U 1992 Intracellular signaling by 14-hydroxy-4,14-retro-retinol. Science 254:1654–1656
46. Eddy EM, Welch JE, O'Brien DA 1993 Gene expression during spermatogenesis. In: de Kretser D (ed) Molecular Biology of the Male Reproductive System. Academic Press Inc, New York, pp 181–231
47. McCarrey JR, Berg WM, Paragioudakis SJ, Zhang PL, Dilworth DD, Arnold BL, Rossi JJ 1992 Differential transcription of *Pgk* genes during spermatogenesis in the mouse. Dev Biol 154:160–168
48. Wolgemuth DJ, Viviano CM, Ginzang-Ginberg E, Frohman MA, Joyner AL, Martin GR 1987 Differential expression of the mouse homeobox-containing gene *Hox-1.4* during male germ cell differentiation and embryonic development. Proc Natl Acad Sci USA 84:5813–5817
49. Don J, Wolgemuth DJ 1992 Identification and characterization of regulated pattern of expression of novel mouse gene, *meg1*, during the meiotic cell cycle. Cell Growth Differ 3:495–505
50. Allen RL, O'Brien DA, Jones CC, Rockett DL, Eddy EM 1988 A novel hsp70-like protein (P70) is present in mouse spermatogenic cells. Mol Cell Biol 8:3260–3266
51. Almon E, Goldfinger N, Kapon A, Schwartz D, Levine AJ, Rotter V 1993 Testicular tissue-specific expression of the p53 suppressor gene. Dev Biol 156:107–116
52. Koopman P, Gubbay J, Collignon J, Lovell-Badge R 1989 Zfy gene expression patterns are not compatible with a primary role in sex determination. Nature 342:940–942
53. Nagamine CM, Chan K, Hake LE, Lau YFC 1990 The two candidate testis-determining Y genes (*Zfy-1* and *Zfy-2*) are differentially expressed in fetal and adult mouse tissues. Genes Dev 4:63–74
54. Denny P, Ashworth A 1991 A zinc finger protein-encoding gene expressed in the post-meiotic phase of spermatogenesis. Gene 106:221–227

55. Eskild W, Ree AH, Levy FO, Jahnsen T, Hansson V 1991 Cellular localization of mRNAs for retinoic acid receptor-α, cellular retinol-binding protein, and cellular retinoic acid-binding protein in rat testis: evidence for germ cell-specific mRNAs. Biol Reprod 44:53–61
56. Lufkin T, Lohnes D, Mark M, Dierich A, Gorry P, Gaub MP, Lemeur M, Chambon P 1993 High postnatal lethality and testis degeneration in retinoic acid receptor α mutant mice. Proc Natl Acad Sci USA 90:7225–7229
56a. Hirose T, O'Brien DA, Jetten AM, RTR: a new member of the nuclear receptor superfamily that is highly expressed in murine testis. Gene, in press
57. Loh EL, Elliot JF, Cwirla S, Larier LL, Davis MM 1989 Polymerase chain reaction with single-sided specificity: analysis of T cell receptor δ. Science 243:217–220
58. Hirose T, Koga M, Kouhara H, Kishimoto S, Matsumoto K, Sato B 1990 Effect of retinoic acid on proliferation of estrogen-responsive transformed murine Leydig cells in serum-free culture. Cancer Res 50:5060–5064
59. Griswold MD, Bishop PD, Kim KH, Ren P, Siiteri KE, Morales C 1989 Function of vitamin A in normal and synchronized seminiferous tubules. Ann NY Acad Sci 564:154–172
60. Ismail N, Molales C, Clermont Y 1990 Role of spermatogonia in the stage-synchronization of the seminiferous epithelium in vitamin-A-deficient rats. Am J Anat 188:57–63
61. Fujimoto W, Marvin KM, George MD, Celli G, Darwiche N, De Luca LM, Jetten AM 1993 Expression of cornifin in squamous differentiating epithelial tissues, including psoriatic and retinoic acid-treated skin. J Invest Dermatol 101:268–274
62. Murakawa GJ, Zaia JA, Spallone PA, Stephens DA, Kaplan BE, Wallace RB, Rossi JJ 1988 Direct detection of HIV-1 RNA from AIDS and ARC patient samples. DNA 7:287–295
63. Green S, Walter P, Kumar V, Krust A, Bornert J-M, Argos P, Chambon P 1986 Human oestrogen receptor cDNA: sequence, expression and homology to v-*erb*-*A*. Nature 320:134–139
64. Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM 1988 Cloning of human androgen receptor complementary DNA and localization to × chromosome. Science 240:327–331