A testicular form of hormone-sensitive lipase (HSL\textsubscript{tes}), a triacylglycerol lipase, and cholesteryl esterase, is expressed in male germ cells. Northern blot analysis showed HSL\textsubscript{tes} mRNA expression in early spermatids. Immunolocalization of the protein in human and rodent seminiferous tubules indicated that the highest level of expression occurred in elongated spermatids. We have previously shown that 0.5 kilobase pairs of the human HSL\textsubscript{tes} promoter directs testis-specific expression of a chloramphenicol acetyltransferase reporter gene in transgenic mice and determined regions binding nuclear proteins expressed in testis but not in liver (Blaise, R., Grober, J., Rouet, P., Tavernier, G., Daegelen, D., and Langin, D. (1999) J. Biol. Chem. 274, 9327–9334). Mutation of a SRY/Sox-binding site in one of the regions did not impair in vivo testis-specific expression of the reporter gene. Further transgenic analyses established that 95 base pairs upstream of the transcription start site were sufficient for correct testis expression. In gel retardation assays using early spermatid nuclear extracts, a germ cell-specific DNA-protein interaction was mapped between -46 and -29 base pairs. The DNA binding nuclear protein showed properties of zinc finger transcription factors. Mutation of the region abolished reporter gene activity in transgenic mice, showing that it is necessary for testis expression of HSL\textsubscript{tes}.

Hormone-sensitive lipase (HSL)\textsuperscript{1} hydrolyzes triacylglycerol and, cholesterol and retinyl esters (1, 2). In adipose tissue, HSL catalyzes the rate-limiting step in lipolysis, the catabolic pathway that mobilizes fatty acids from triacylglycerol stored in the lipid droplet. HSL is also expressed in rodent and human testis (3–5). In situ hybridization experiments performed in rat testis showed strong labeling of cells in the adluminal parts of the seminiferous tubules at stages X–XIV and sparsely distributed grains in the basal parts (6). Immunohistochemistry experiments showed an HSL-like immunoreactivity in the adluminal part of the rat seminiferous tubules at stages XIII–VIII (5). The data suggested that rodent HSL mRNA and protein are expressed in haploid germ cells with a lag between mRNA and protein appearance. However, the precise germ cell type(s) expressing HSL was not determined in rat and it was not possible to rule out HSL expression in Sertoli cells. Moreover, the expression of HSL in germ cells of other mammalian species, including man, has not been documented. The effects of HSL gene disruption in mice have recently been reported (7). The most striking feature of the phenotype is male sterility due to oligospermia. Degenerated spermatocytes and spermatids were observed in HSL-deficient testis with a lack of mature spermatozoa. The data clearly demonstrate that HSL is required for spermatogenesis.

The human adipose tissue form of HSL is encoded by 9 exons spanning 11 kb (8). The transcription start site was mapped in a short 5’-noncoding exon located 1.5 kb upstream of the first coding exon (9). The 2.8-kb mRNA encodes a 775-amino acid protein. A specific form of HSL, named HSL\textsubscript{tes}, is expressed in human and rat testis (5). The 3.9-kb human HSL\textsubscript{tes} mRNA encodes a 1076-amino acid protein. HSL\textsubscript{tes} contains a unique NH\textsubscript{2}-terminal domain in addition to the 775 amino acids common to adipocyte and testis HSL. This additional domain is encoded by a testis-specific exon located 15 kb upstream of the first of the 9 exons encoding adipocyte HSL.

The genomic organization suggested, as is often seen when a gene is expressed in somatic tissues and in germ cells, the use of different promoters to govern tissue-specific expression. We recently investigated the molecular mechanisms that control the testis-specific expression of HSL\textsubscript{tes} (10). Transgenic mice were generated with 1.4 and 0.5 kb of the 5’-flanking region of the human HSL\textsubscript{tes}-specific exon linked to the chloramphenicol acetyltransferase (CAT) gene. High levels of CAT activity were measured in testis from different lines of sexually mature transgenic mice. No reporter gene activity was observed in nongonal tissues in males and in all tissues studied in the females. Therefore, the sequences present in the first 0.5 kb of the human HSL\textsubscript{tes} promoter confer germ cell-specific expression. To characterize nuclear protein-DNA interactions in the HSL\textsubscript{tes} promoter, a series of gel retardation assays was performed with oligonucleotides spanning the 0.5-kb region. Four regions bound nuclear proteins expressed in testis but not in liver, an organ that does not express HSL. The most proximal region contained a sequence AACAAAG that bound recombinant Sox proteins. Sox proteins contain a high mobility group

---

\* The work was supported by INSERM and Swedish Medical Research Council Grant 11284 (to C. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed: INSERM Unit 317, Institut Louis Bugnard, Université Paul Sabatier, Hôpital Rangueil, 31403 Toulouse Cedex 4, France, the §GERM-INSERM Unit 435, Campus de Beaulieu, Université de Rennes I, 35042 Rennes Cedex, Bretagne, France, the **Section for Molecular Signaling, Department of Cell and Molecular Biology, Lund University, 22100 Lund, Sweden

---

\* The work was supported by INSERM and Swedish Medical Research Council Grant 11284 (to C. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed: INSERM Unit 317, Institut Louis Bugnard, Université Paul Sabatier, Hôpital Rangueil, 31403 Toulouse Cedex 4, France, the §GERM-INSERM Unit 435, Campus de Beaulieu, Université de Rennes I, 35042 Rennes Cedex, Bretagne, France, the **Section for Molecular Signaling, Department of Cell and Molecular Biology, Lund University, 22100 Lund, Sweden

---

\* The work was supported by INSERM and Swedish Medical Research Council Grant 11284 (to C. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed: INSERM Unit 317, Institut Louis Bugnard, Université Paul Sabatier, Hôpital Rangueil, 31403 Toulouse Cedex 4, France, the §GERM-INSERM Unit 435, Campus de Beaulieu, Université de Rennes I, 35042 Rennes Cedex, Bretagne, France, the **Section for Molecular Signaling, Department of Cell and Molecular Biology, Lund University, 22100 Lund, Sweden

---

\* The work was supported by INSERM and Swedish Medical Research Council Grant 11284 (to C. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed: INSERM Unit 317, Institut Louis Bugnard, Université Paul Sabatier, Hôpital Rangueil, 31403 Toulouse Cedex 4, France, the §GERM-INSERM Unit 435, Campus de Beaulieu, Université de Rennes I, 35042 Rennes Cedex, Bretagne, France, the **Section for Molecular Signaling, Department of Cell and Molecular Biology, Lund University, 22100 Lund, Sweden
DNA-binding domain and are related to the testis-determining factor SRY (11).

In the present study, we determined the germ cell types expressing HSL mRNA and protein and further delimited the region conferring testis specificity in the human HSLtes promoter. Immunohistochemistry experiments performed on rat, mouse, and human testis showed that, in the three species, HSL-like immunoreactivity exhibits a biphasic pattern with a first wave of expression in spermatogonia and primary spermatocytes and, a second wave of expression in elongating and elongated spermatids. The highest expression levels were observed in the latter cells. Northern blot analyses showed that HSLtes mRNA is abundant in early spermatids and in the cytoplasmic fragments of late spermatids and residual bodies. Studies performed on the human HSLtes promoter showed that, although a Sox-like protein present in nuclear extracts from pachytestes spermatocytes binds to the promoter, mutation of the corresponding AACAAAG sequence does not abolish testis-specific CAT expression in transgenic mice. Additional transgenic analyses established that 95 bp upstream of the transcription start site were sufficient for testis-specific expression of the CAT reporter gene. Using early spermatid nuclear extracts and transgenesis, a GT-rich binding region was identified in the 95-bp sequence and shown to be involved in the testis-specific expression of HSL.

**Experimental Procedures**

**Tissue Preparation and Immunohistochemistry**—Mice (8, 16, 24, and 48 days old) and rats (9, 20, 35, 45, and 90 days old) were purchased from Elevage Janvier (Le Genest Saint Isle, France). Human testes were obtained from patients undergoing therapeutic orchidectomy for metastatic prostate carcinoma (protocol approved by the Ethics Committee of the city of Rennes, France). Testes were stored in Bouin’s solution for 24 h (mouse), 72 h (rat), and 48 h (human). The fixed tissues were embedded in paraffin wax. Sections (5 μm thick) were dried overnight at 37 °C, deparaffinized, and rehydrated by decreasing grades of alcohol. Sections were microwaved three times (5 min each) in 0.01 M sodium citrate (pH 6) buffer. Endogenous peroxidase was quenched with 3% H2O2 for 5 min. Sections were incubated with affinity-purified polyclonal anti-rat HSL antibodies (rat and mouse sections) and incubated 15 min at 4 °C. The solution was centrifuged at 11,000 g for 5 min. An equal volume of buffer containing 100 mM HEPES, pH 7.9, 0.7 M NaCl, 1.5 mM MgCl2, 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 2 μg/ml aprotinin, and 6.25% glycerol was added dropwise. The suspension was incubated for 30 min at 4 °C and then centrifuged at 20,000 × g for 30 min at 4 °C. An equal volume of buffer D (20 mM HEPES, pH 7.9, 50 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 2 μg/ml aprotinin, and 6.25% glycerol) was added to the supernatant and aspirated to liquid nitrogen. The protein concentrations of early spermatids and pachytester spermatocyte nuclear extracts ranged between 0.5 and 1 mg/ml (14). Liver and whole testis nuclear extracts were prepared as previously described (10).

**Gel Retardation Assays—Oligonucleotide purification and labeling were performed as described (10).** The positions from the transcription start site and sequences of oligonucleotides were as follows: -250/-216, 5′-CAAGCTTTGTAGAATGAACTAGGGAAGTAAATGCA-3′; -96/-47, 5′-GCCCTAACTGGGAGTGGTATATTATAGAAGAGT-3′; -61/-12, 5′-ATTCTTAACAGGTGGTTGGTGGTGTTGGGCTTTATATAGAAGAGT-3′; -51/-42, 5′-ACGAGAAACTTTTTAGGGAAGTTCTTTTGCCCATTTT-3′; -39/-22, 5′-AAAGAAGATTTTACAGAGACACGTTACAGGTCTAG-3′. Labeled DNA (1 ng at ~100,000 cpm/ng) was incubated in binding buffer (10 mM HEPES, pH 7.9, 60 mM KCl, 1 mM EDTA, 5 mM DTT, 4 mM spermidine, 5 mM MgCl2, 10% glycerol, and 1 μg of poly(di-dC)) (Amersham Pharmacia Biotech) instead of 1 μg of poly(dI-dC). In some experiments with the -61/-12 oligonucleotide, ZnCl2 was added in the binding buffer and 4% Ficoll 400 was used instead of 10% glycerol. DNA-protein complexes were resolved on 6% non-denaturating polyacrylamide/bisacrylamide (29:1) gels at 10 V/cm for 3 h in a 23 mM Tris borate (pH 8), 0.5 mM EDTA migration buffer. Polyacrylamide gels were dried under vacuum and subjected to digital imaging (Molecular Dynamics).

**Transgenic Mice—All constructs were derived from the p0.5HSLtes CAS vector (renamed p515HSLtesCAS in the present study) (10).** Microinjection fragments with 316 and 95 bp of the human HSLtes 5′-flanking region (−316HSLtesCAS and −95HSLtesCAS) were generated by KpnI/BamHI and SstI/BamHI enzymatic digestions, respectively. The correct orientation and human HSLtes 5′-flanking region to 480 and 209 bp (−480HSLtesCAS and −209HSLtesCAS) were generated by PCR with high fidelity pfu DNA polymerase (Stratagene). Mutation of the 5′-AACAAAG-3′ SRY/Sox consensus site in the −p515HSLtesCAS vector into the sequence 5′-CCGCCGTT-3′ (−515mutSoxHSLtesCAS) was obtained by a two-step overlap PCR extension method (15) with high fidelity pfu DNA polymerase. Using the same method, the −209HSLtesCAS mut was mutated into 5′-ATTCTTTGTCT- GTG-3′ (−209mutHSLtesCAS). All PCR constructs were sequenced on ABI PRISM 310 Genetic Analyzer (PerkinElmer Life Sciences).

**Results**

**HSL Is Expressed in Different Germ Cell Types—To precisely determine the localization of HSL in testes, we performed light immunohistochemistry on adult mouse, rat, and human testes. Serial adjacent testis sections were labeled with antibodies directed against rat or human HSL. Fig. 1 shows representative sections and a schematic summary of HSL expression in germ cells during the cycle of the seminiferous epithelium. In.
mouse and human, HSL-like immunoreactivity was first observed in the cytoplasms of B spermatogonia and primary spermatocytes. In rat, primary spermatocytes were labeled. In the three species, a second wave of expression was observed in the elongating and elongated spermatids. The staining was intense in the latest steps of spermiogenesis. After spermiation, strong labeling was found in residual bodies in the mouse and rat and residual staining was visible in spermatozoa. Residual bodies are difficult to observe in human testis sections (16). In human testis, weak labeling was also observed in Sertoli cells. The staining of Sertoli cells was clearly visible in tubules devoid of germ cells. To investigate the ontogeny of HSL, immunohistochemistry experiments were also performed in testis from mice (8-, 16-, 24- and 48-day-old) and rats (9-, 20-, 35-, 45-, and 90-day-old) at different ages (data not shown). In rodents, the first wave of spermatogenesis in prepubertal animals is synchronized and the appearance of novel germ cell type can be associated with protein expression. In the rat, HSL-like immunoreactivity appeared at day 20 with weak labeling in pachytene spermatocytes. At day 35, a strong labeling
was observed in elongated spermatids. The results at day 45 were similar to the data in 90-day-old animals (Fig. 1). In the mouse, the appearance of pachytene spermatocytes was associated with a strong labeling at day 16. At day 24, the first early spermatids were not labeled. In 48-day-old animals, high HSL-like immunoreactivity was observed in elongated spermatids. Northern blot analyses using total RNA prepared from elutriated rat germ cells showed that the 3.9-kb HSLtes mRNA is strongly expressed in early spermatids (Fig. 2). No band was visible in other cell types. An abundant expression was also observed in late spermatid cytoplasmic fragments and residual bodies where β-actin mRNA was not detected. Reverse transcription-polymerase chain reaction assay showed that HSL transcripts were weakly expressed in pachytene spermatocytes (data not shown).

The AACAAAG SRY/Sox Consensus Binding Site Is Dispensable for Testis-specific Expression in Transgenic Mice—We recently showed that 0.5 kb of the human HSLtes promoter conferred testis-specific expression in transgenic mice (10). In this region, we characterized an AACAAAG site for Sox proteins that bound a protein expressed in testis but not in liver. Using purified germ cell nuclear extracts, we observed using gel retardation assays that the testis-specific protein binding to the −250/−216 oligonucleotide containing the AACAAAG sequence (10) was more abundant in pachytene spermatocytes than in early spermatids (Fig. 3). Efficient competition of the band was observed with an oligonucleotide containing an AACAT sequence that has been shown to bind members of the Sry/Sox family (17–20). Proteins containing a high mobility group DNA-binding domain such as Sox proteins interact with A-T pairs in the minor groove of the DNA helix (11). The A-T pair selective minor groove DNA ligand distamycin (21, 22) inhibited the testis-specific binding.

We showed previously that mutation of the AACAAAG sequence abolished Sox protein binding (10). To determine whether the binding site was critical for testis-specific expression of HSL, a CAT construct containing the 0.5-kb 5'-flanking region with a mutation of the AACAAAG sequence was microinjected into mouse oocytes (Fig. 4). Eight independent transgenic lines were obtained (Table I). In 5 out of 8 transgenic lines, high levels of CAT activity were measured in testis from adult mice. Nongonadal tissues showed very low levels of CAT activity (<10 cpm/min/mg of protein). The testis of mice from 3 transgenic lines exhibited background levels of CAT activity, presumably due to the insertion of the transgene at a chromosomal location that suppresses expression.

A 95-bp Promoter Region Is Sufficient to Direct Testis-specific Expression—In the first 0.5 kb of the HSLtes promoter, four regions bound nuclear proteins expressed in testis but not in liver. To determine whether these regions played a role in the testis specific activity of the promoter, CAT constructs with progressive deletions of the 5'-flanking region were used to generate transgenic mice (Fig. 4). Deletion of the first 3 testis-specific binding regions did not abolish CAT activity in transgenic testis (Table II). In agreement with the data obtained with the fragment containing the mutated Sox-binding site, high level of CAT activity was found in testis from transgenic mice produced with a 209-bp 5'-flanking region that does not contain the AACAAAG sequence. Mice harboring a transgenic
construct containing 95 bp upstream of the transcription start site still showed strong testicular CAT activity. In all the lines, CAT activity associated to transgene expression in spermatocytes was detected in epididymis. None of the transgenic lines showed CAT activity above background levels in nongonadal tissues (<10 cpm/min/mg of protein). The different lines were tested for testis expression of the transgenes at day 21 which in prepubertal mice corresponds to the accumulation of pachytene spermatocytes and the appearance of the first early spermatids.

In this paper, we establish the precise cellular localization of HSL in rodent and human seminiferous tubules and show that a short region of the human HSL<sub>tes</sub> promoter confers testis-specific promoter activity. The whole data suggest that the binding of a testis-specific zinc finger transcription factor within the −46/−29 bp region is required for the testis-specific expression of HSL<sub>tes</sub>.

**DISCUSSION**

In this paper, we establish the precise cellular localization of HSL in rodent and human seminiferous tubules and show that a short region of the human HSL<sub>tes</sub> promoter confers testis-specific promoter activity. The whole data suggest that the binding of a testis-specific zinc finger transcription factor within the −46/−29 bp region is required for the testis-specific expression of HSL<sub>tes</sub>.

**TABLE II**

| Construct     | Line | Copy no. | CAT activity |
|---------------|------|----------|--------------|
| −515HSL<sub>tes</sub>CAT | 13   | 1        | 666          |
| −460HSL<sub>tes</sub>CAT | 22   | 2–3      | <10          |
|                 | 32   | 2–3      | 235          |
|                 | 36   | 50       | 1890         |
| −316HSL<sub>tes</sub>CAT | 5    | 1–2      | 402          |
|                 | 18   | 4        | 1264         |
| −209HSL<sub>tes</sub>CAT | 13   | 50       | 900          |
|                 | 16   | 1–2      | 157          |
|                 | 17   | 10       | 223          |
|                 | 33   | 1        | 190          |
| −95HSL<sub>tes</sub>CAT | 24   | 2        | 270          |
|                 | 34   | 4        | 2737         |
|                 | 39   | 10       | 2053         |
|                 | 40   | 8        | 885          |
|                 | 43   | 4        | 1167         |

**DISCUSSION**

In this paper, we establish the precise cellular localization of HSL in rodent and human seminiferous tubules and show that a short region of the human HSL<sub>tes</sub> promoter confers testis-specific promoter activity. The whole data suggest that the binding of a testis-specific zinc finger transcription factor within the −46/−29 bp region is required for the testis-specific expression of HSL<sub>tes</sub>.

**FIG. 5.** Gel retardation analysis of the −96/+22 region. Lanes 1, 5, and 14, liver nuclear extracts; lanes 2–4, 6–13, and 15–17, early spermatid nuclear extracts. A 100-fold excess of unlabeled −96/+47 oligonucleotide (lanes 3 and 12), of unlabeled −61/−12 oligonucleotide (lanes 7 and 10), of unlabeled −28/+22 oligonucleotide (lanes 13 and 16) and of unrelated unlabeled oligonucleotide from the 0.5-kb HSL<sub>tes</sub> promoter (lanes 4, 8, 11, and 17) were added in competition experiments. Lanes 5 and 9–13, ZnCl<sub>2</sub> was added to a final concentration of 0.5 mM. A testis-specific protein-DNA complex is shown with an arrowhead.

**FIG. 6.** Binding of early spermatid and liver nuclear extracts on the −61/−12 oligonucleotide probe containing a testis-specific protein-DNA binding region. Lanes 1–3, liver nuclear extracts; lanes 4–11, early spermatid nuclear extracts. A 100-fold excess of unlabeled −61/−12 oligonucleotide (lanes 2 and 9), of unrelated unlabeled oligonucleotide from the 0.5-kb HSL<sub>tes</sub> promoter (lanes 3 and 10) and of unlabeled −69/−25 oligonucleotide mutated between nucleotides −49 and −36 (lane 11) were added in competition experiments. ZnCl<sub>2</sub> was added to a final concentration of 0.05 (lane 5), 0.1 (lanes 1–3 and 6), 0.5 (lanes 7 and 9–11), and 1 mM (lane 8). To enhance the specificity of protein-DNA interaction, binding buffer contained 4% Ficoll 400 instead of 10% glycerol. A testis-specific protein-DNA complex is shown with an arrowhead.

**TABLE III**

| Line | Copy no. | CAT activity |
|------|----------|--------------|
| 2    | 70       | <10          |
| 3    | 7        | <10          |
| 19   | 18       | <10          |
| 32   | 18       | <10          |
| 50   | 66       | <10          |
| 52   | 80       | <10          |
| 60   | 35       | <10          |
| 62   | 16       | <10          |
specific expression. Study of HSL expression in male germ cells revealed that the peak of expression occurs during spermatogenesis in elongated spermatids. A similar pattern was found in rat, mouse, and man. In combination with Northern blot analyses of total RNA from isolated rat germ cells (Fig. 2) and the ontogeny of HSLtes mRNA expression in rat and mouse testes (6, 10), the data reveal that HSLtes transcription and translation occur in early spermatids and elongated spermatids, respectively. This feature is characteristic of many genes expressed in haploid germ cells (23). The determination of HSLtes stage-specific expression shown in our immunohistochemistry experiments help to understand the testicular alterations observed in HSL-deficient mice (7). The mice were characterized by severe oligospermia and a reduction from 12 to 5–7 epithelium layers in seminiferous tubules. The lack of elongated spermatids and spermatocytes could be the consequence of the absence of HSL in the preceding germ-cell types, i.e. during elongation of spermatids. The data strongly suggest that the testicular isoform of HSL is necessary for germ cell differentiation during spermiogenesis.

The immunohistochemistry data also show HSL-like immunoreactivity in human spermatogonia B, early spermatocytes, and Sertoli cells. It is possible that the primary spermatocyte and Sertoli cell protein is translated from an HSL mRNA different from the HSLtes mRNA that encodes the 1076-amino acid protein. In support of this hypothesis, two HSL mRNAs are expressed in human testis, the 3.9-kb HSLtes mRNA and an ~3-kb mRNA (5). The 5′-noncoding region of the shorter mRNA differs from previously characterized HSL mRNAs and corresponds to a novel exon. The origin of HSL-like immunoreactivity in rodent pachytenate spermatocytes is more elusive. A single 3.9-kb HSLtes mRNA is expressed in rat and mouse testes (6, 10). The rodent HSLtes promoter may govern two waves of expression, the first in spermatocytes and the second in spermatids.

To study the human HSLtes promoter, we used a combination of transgenic mouse analyses and in vitro DNA-protein binding assays. The use of transgenic mouse technology to assess the activity of testis promoters is necessary because of the lack of suitable male germ cell lines. Furthermore, this approach allows a determination of regulatory elements involved in tissue-specific expression. In a previous study, we showed that 0.5 kb of the 5′-flanking region of the HSLtes promoter governed testis-specific expression of the transgene (10). A systematic analysis of the region using gel retardation assay revealed 4 binding sites for nuclear proteins expressed in testis but not in liver. One of the binding sites located between −232 and −226 bp from the transcription start site contained the consensus motif AAACAAG for Sox proteins. Several lines of evidence support that the binding protein is a member of the Sox family. First, the germ cell-specific binding was competed by an oligonucleotide containing an AACAT sequence with high affinity for Sox proteins (Fig. 3). Second, mutation of the site abolished binding of Sox proteins (10). Third, distamycin, a minor groove DNA ligand (21, 22), competed the binding suggesting that the protein-like Sox proteins contained an high mobility group-binding domain (Fig. 3). Two members of the Sox family, a short form of Sox5 and Sox6 are expressed in early spermatids (18–20, 24). However, the endogenous Sox-like protein binding to the HSLtes promoter is not likely to correspond to Sox5 and Sox6 although recombinant Sox5 and Sox6 readily bind to the HSLtes promoter (10). Antibodies used to demonstrate the binding of Sox5 and Sox6 to the chondrocyte-specific enhancer of the type II collagen gene (24) had no effect on the Sox-like protein binding to the HSLtes promoter. To determine whether the AACAAAG sequence was important for in vivo testis expression of a reporter gene, a CAT construct containing the 0.5-kb region with a mutation of the site was used to produce different lines of transgenic mice. Five of the lines showed strong testis-specific CAT activity (Table I). The data clearly show that, in transgenic testis, the Sox-binding site is not necessary for tissue-specific expression of the human HSLtes promoter. They, however, do not rule out a role for Sox proteins in the regulation of HSLtes promoter activity. The role of most Sox proteins expressed in adult tissues is not known. They could indirectly affect transcription activity by altering chromatin structure. We showed that the high mobility group domain of Sox5 induces a strong bend in DNA through binding to the AACAAAG sequence (10). In pachytenate spermatocytes, the interaction between Sox proteins and DNA minor groove could promote a reorganization of the local chromatin structure preceding the HSLtes gene transcription in early spermatids. Such a pattern of activation has been reported for the pgk2 gene, DNormase I-hypersensitive sites appearing in spermatagonia whereas transcription starts in preleptotene spermatocytes (25).

Analyses of transgenic lines revealed that the first 95 bp of the human HSLtes promoter mediates the in vivo expression of a reporter gene in post-meiotic male germ cells. In all the lines examined, no transgene expression was observed in somatic tissues. In vivo analyses of male germ cell promoters show that testis-specific expression is often conferred by ~100 bp 5′-flanking regions (26–30). However, the transcriptional mechanisms differ between promoters. A subset of them are under the direct control of cAMP-responsive element modulator, e.g. the angiotensin-converting enzyme and protamine 1 promoters (31, 32). Others are activated by cAMP-responsive element modulator τ-independent mechanisms such as the lactate dehydrogenase c, β1.4-galactosyltransferase I, and HSLtes promoters (29, 30). In previous gel retardation analyses, testis and liver nuclear extracts revealed similar binding of nuclear proteins to the −95 bp region (10). To better characterize this region, we used longer oligonucleotides and nuclear extracts from a purified preparation of early spermatids rather than from the whole testis (Fig. 5). Moreover, we tested different binding buffers (data not shown). Most of the protein-DNA interactions observed did not differ between early spermatids and liver. An early spermatid-specific protein-DNA interaction was observed in the region between −46 and −29 bp from the transcription start site that was strongly enhanced by the addition of Zn²⁺ into the binding buffer. Zinc dependence of the binding suggests DNA interaction with zinc finger transcription factors (33). The binding region contains a GT-rich sequence that could bind members of the Sp1 family. It is, however, unlikely that the early spermatid nuclear protein binding to the HSLtes promoter regulatory element is Sp1 because this protein-DNA interaction (shown by an arrowhead on Fig. 5) was not found with liver nuclear extracts and was not competed by an oligonucleotide containing a consensus site GGGC-GGGC-GGGG for Sp1. Of note, an unidentified germ cell nuclear protein different from Sp1 binds to a GC box in the proximal promoter region of lactate dehydrogenase c that confers testis-specific expression (34). Mutation of the GT-rich binding region in transgenic mice abolished the testis-specific expression of the reporter gene, showing that, unlike the SRY/Sox consensus binding site, this region is required for the testis specificity of human HSLtes promoter activity. Further experiments are nec-

---

2 H. Laurell, L. Holst, J. Grober, C. Holm, and D. Langin, unpublished observations.

3 R. Blaise and D. Langin, unpublished observations.
essay to establish if the early spermatid nuclear protein binding to the GT-rich sequence in the HSL\textsubscript{los} promoter is a novel zinc finger transcription factor.

To conclude, we have shown that HSL is highly expressed in late spermatids in humans and rodents. A short genomic region of the human HSL\textsubscript{los} promoter confers testis-specific expression in transgenic mice and contains an essential cis-acting element binding an early spermatid-specific nuclear protein.

Acknowledgments—We are grateful to Dr. Michel Raymondjean (CNRS-UPRESA 7079, Paris, France) for insightful discussion, Maxime Fontanié and Stéphanie Lucas (INSERM U317, Toulouse, France) for help with transgenic mice, Dr. Philippe Rouet (INSERM U317, Toulouse, France) for anti-Sox6 and anti-Sox5 antibodies, and Nathalie Melaine for purified help with transgenic mice, Dr. Philippe Rouet (INSERM U317, Toulouse, France) for

REFERENCES

1. Langin, D., Holm, C., and Lafontan, M. (1996) Proc. Nutr. Soc. 55, 93–109
2. Wei, S., Lai, K., Patel, S., Piantedosi, R., Shen, H., Colantuoni, V., Kraemer, F. B., and Blaner, W. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10185–10190
3. Oakberg, E. F. (1956) J. Biol. Chem. 218, 1–9
4. Russell, L. D., Ettlin, R. A., Sinha Hikim, A. P., and Clegg, A. P. (1990) J. Biol. Chem. 265, 14390–14397
5. Palmiter, R. D. (1993) J. Biol. Chem. 268, 14989–14997
6. Stenson Holst, L., Hoffmann, A. M., Muller, H., Sundler, F., Holm, C., and Fredrikson, G. (1996) Genomics 35, 441–447
7. Stenson Holst, L., Langin, D., Mulder, H., Laurell, H., Grober, J., Bergh, A., Mohrenweiser, H. W., Edgren, G., and Holm, C. (1999) J. Biol. Chem. 274, 3365–3372
8. Yang, J., Riley, M., and Thomas, K. (1998) J. Exp. Zool. 282, 12262–12266
9. Westin, G., and Schaffner, W. (1988) Nucleic Acids Res. 16, 5771–5781
10. Yang, J., Riley, M., and Thomas, K. (1998) J. Biol. Chem. 273, 179–187
11. Oakberg, E. F. (1996) Am. J. Anat. 199, 391–409
12. Russell, L. D., Ettlin, R. A., Sinha Hikim, A. P., and Clegg, A. P. (1990) Histological and Histopathological Evaluation of the Testis, Cache River Press, Clearwater, FL
13. Leblond, C. P., and Clermont, Y. (1963) J. Anat. 99, 548–573
14. Dym, M., and Clermont, Y. (1970) Am. J. Anat. 132, 265–282
15. Clermont, Y. (1962) Am. J. Anat. 117, 233–251
16. Sharpe, R. M. (1994) in The Physiology of Reproduction (Knobil, E., and Neil, J. D., eds) pp. 1363–1434, Raven Press, New York

Testis Hormone-sensitive Lipase Promoter

5115