2P1, a novel male mouse cDNA specifically expressed during meiosis

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ABSTRACT **We screened a mouse germinal cell expression library with a probe derived from *Sob1,* a human testis-specific cDNA, and identified *2P1,* a new mouse cDNA. A database search revealed that *2P1* was 91% identical to ORF1 of *E3-3,* a rat gene probably involved in the regulation of alternative splicing. Sequencing showed that *2P1* has a destabilization motif in its 3′-untranslated region. Northern blotting showed strong gene expression in the testis and weak expression in the epididymis, with no signal detected in other tissues. RT-PCR analysis confirmed testis and epididymis expression. *In situ* hybridization revealed that *2P1* mRNA was absent in spermatogonia but expressed in spermatocytes. This last result was confirmed by RT-PCR of FACS isolated primary spermatocytes (pachytene stage). Using RT-PCR, purified spermatids were also shown to express *2P1.***

KEY WORDS: *spermatogenesis, pachytene spermatocyte, mRNA splicing, post-transcriptional regulation, ARE.*

Spermatogenesis is a complex process involving a sequence of stages. This process must have two essential properties if it is to fulfill its function, the translation of proteins with new functions, and very flexible regulation making it possible to switch genes on and off in a precise temporal fashion. There are currently many gaps in our knowledge concerning spermatogenesis. For example, the mechanisms enabling stem cells to undergo meiosis are currently unknown. The identification of genes specifically expressed during the course of male germ cell differentiation may shed light on this process.

A set of genes expressed in postmeiotic cells only has been identified in the mouse. It includes genes encoding specific sperm components (protamines: PRM-1 and PRM-2), transition proteins (TP1 and TP2) (Meistrich, 1989; Shih and Kleene, 1992) and SP-10 (Kurth *et al.,* 1993), as well as genes encoding regulatory proteins such as MSY2, a member of the Y box protein family (Gu *et al.,* 1998). Alternative splicing of the initial transcripts may also generate variants with new functions expressed uniquely in the testis (Williams *et al.,* 1995). One such variant, DNA ligase III, loses its DNA repair function when produced in primary spermatocytes and is thought to be involved in the completion of meiotic recombination (Mackey *et al.,* 1997). Several splicing-dependent changes in function have been identified during the course of spermatogenesis. CREM (cyclic AMP-responsive element modulator) protein

is produced as an antagonist in premeiotic cells but switches to CREMτ, the testis-specific activator form, from the pachytene spermatocyte stage onwards (Foulkes *et al.,* 1992). Conversely, SOX17 protein, which seems to function as a transcriptional activator in premeiotic germ cells, switches to t-SOX17 in postmeiotic germ cells and loses its apparent DNA-binding activity (Kanai *et al.,* 1996).

The original aims of this study was to identify the mouse counterpart of the human *Sob1* gene, which encodes a sperm protein potentially involved in sperm-oocyte binding (Lefevre *et al.,* 1999). We screened a λgt11 mouse germinal cell library with a probe derived from the human *Sob1* cDNA sequence and isolated mouse *Sob1* and *2P1* cDNAs. The *2P1* cDNA displays sequence similarity, over some of its length, to a variant specifically produced from the rat *E3-3* gene by alternative splicing (Hartmann and Stamm, 1997). We also describe the germ cell type pattern of expression of the *2P1* cDNA in the seminiferous tubules. All these observations are in favor of accurately regulated activity during the spermatogenic cycle for *2P1.*

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gcacgagcgcccccggagcccgattctccaaagcctgggattaagggcctggaggctggcaccacaccctgg      72

ccaggtgttcataacaggaccaggtgcgcagctgccccgccctggtccctgggagcccgaataatggctgag     144

aatcccaggaacaggccgcggcagtggcctaaacaatacccttagggcgcccgtactagcaggtgttttttcc    216

ccctcctccttagggactgtcgctggaggcatggggcgggtgggcgacaggtgctgacacacccgcatggg     288

gagacagaggctctgcattggcctactgcgccgttcaaggtgcgcggtgccctccgggacgcaaggggcgct    360
                                                                      M   A      2
gtggctactttgagacagacaactctgtccctacgaccgggtcgttaagcgccgttgcagcc ATG GCT    428

 T   A   L   G   F   R   C   L   F   R   T   R   P   A   P   L   C   R     20
ACT GCT CTA GGA TTT CGC TGC CTT TTC CGA ACG CGA CCT GCG CCG CTC TGT CGG    482

 H   V   D   R   L   W   R   N   P   R   R   G   H   R   L   S   P   A     38
CAC GTC GAC CGG CTG TGG AGG AAC CCG CGT CGG GGA CAC CGG CTT TCT CCG GCG    536

 D   D   E   L   Y   Q   R   T   R   I   S   L   L   Q   S   E   F   P     56
GAC GAT GAG TTA TAC CAG CGG ACT CGC ATC TCA CTG CTG CAA AGC GAG TTC CCT    590

 Q   A   V   Y   I   D   S   Y   S   S   R   G   F   T   I   C   G   N     74
CAG GCC GTG TAC ATC GAC AGC TAC AGT AGC AGA GGC TTC ACT ATC TGC GGA AAT    644

 R   V   F   G   P   C   V   L   L   P   Q   T   V   V   Q   W   N   V     92
CGC GTG TTT GGC CCT TGC GTG CTG CTC CCA CAG ACG GTG GTC CAG TGG AAC GTG    698

 G   S   H   Q   D   I   T   E   E   S   F   S   L   F   W   M   L   E    110
GGA TCT CAT CAG GAT ATC ACT GAA GAG AGC TTC TCC CTT TTC TGG ATG CTG GAA    752

 P   R   I   E   I   V   V   V   G   T   G   N   K   T   E   R   L   H    128
CCT AGG ATA GAG ATT GTT GTG GTG GGC ACT GGA AAC AAG ACT GAG CGG CTG CAC    806

 P   Q   V   L   Q   A   M   R   Q   R   G   I   A   V   E   V   Q   D    146
CCT CAA GTA CTA CAG GCC ATG AGG CAG CGG GGA ATT GCT GTG GAG GTC CAG GAC    860

 T   P   N   A   C   A   T   F   N   F   L   C   H   E   G   R   V   T    164
ACG CCC AAT GCC TGT GCC ACT TTC AAC TTC CTG TGC CAC GAA GGT CGA GTG ACA    914

 G   A   A   L   I   P   P   P   G   E   T   A   L   A   S   L   G   Q    182
GGA GCT GCC CTC ATT CCC CCT CCT GGA GAG ACT GCA CTG GCT TCT TTG GGT CAA    968

 A   S   E                                                              185
GCC TCA GAA tgacacatcaggaaccatcaaggagggcactgcatgctctcaacgctttctccacactt   1036

gtcatgctcacccaggataataattttactcctcttaaaaaaaaaaaaaaaaaaaa                   1092
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Fig. 1. The nucleotide sequence of the testis-specific *2P1* cDNA and the deduced amino acid sequence of its product. *Genbank accession number AF235017. The nucleotide sequence contains a 422 base pair upstream untranslated segment. The box-like CAAT and GC in the 5'UTR and the destabilization signal in the 3'UTR are underlined.*

Results

cDNA Sequencing

A 1079-nucleotide (nt) cDNA was isolated and named *2P1*. The open reading frame (ORF) region consisted of 555 nt (Fig. 1). The 5'untranslated region (UTR) was 422 nt long. An ATTGG motif that could account for a CCAAT box in its complementary form was observed at position – 118, as well as two GC-rich regions at positions - 107 and - 70 respectively. No TATA box was detected. The 3'-end contained no polyadenylation signal; it had an ATTTA destabilization motif at position + 637. The corresponding protein has a predicted size of 20.72 kDa with an isoelectric point of 8.31.

2P1 Expression

The distribution of *2P1* mRNA was determined by Northern blotting of total RNA from various tissues of mice. The *2P1* transcript was detected as a single band of 1.3 Kb (Fig. 2). It was present in large amounts in the testis, giving a strong signal whereas only a faint band was detected in the epididymis. No signal was detected for any other tissue.

RT-PCR was carried out from the testis and epididymis used as the template. Amplification generated a strong 627 bp band (Fig. 3A). This band corresponded to the expected size according to the primers chosen. *Prm2* was used as a positive control; it was expressed in both tissues.

In situ hybridization was then used to identify cell types containing *2P1* transcripts in sections of mature mouse testis. With an antisense *2P1* probe, we detected a significant signal in primary spermatocytes (pachytene stage step), while spermatogonia were negative (Fig. 4). Hybridization with an ACE antisense probe used as control was detected in haploid cells only, and the *2P1* sense probe acted as a negative control. We found that *2P1* was expressed from stage I in pachytene spermatocytes, with expression persisting until stage XII of the spermatogenic cycle
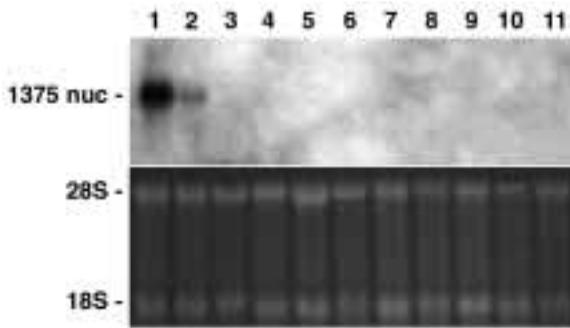
Fig. 2. Northern blot analysis of mRNA extracted from various tissues and probed with the *2P1* cDNA. *We loaded 10 µg RNA per lane: testis (1), epididymis (2), vas deferens (3), seminal vesicle (4), kidney (5), bladder (6), lung (7), liver (8), muscle (9), heart (10), and brain (11).*

(Fig. 5). No significant difference was observed in the intensity of staining between the various stages after correction for the increase in spermatocyte area during meiosis (P > 0.05). The interstitial tissue, including Leydig cells, was not stained (data not shown).

In order to confirm these results, RT-PCR of FACS isolated pachytene spermatocytes and spermatids was carried out. As for testis and epididymis tissues, amplification generated a strong 627 bp band in pachytene spermatocytes and a slighter one in spermatids (Fig. 3B). As *Prm2* is likely to be expressed only from spermatid stage, *actin* was used as a positive control for sorted cells.

Discussion

We isolated a 1079-nt cDNA, named *2P1*, by screening a mouse male germ cell λgt11 library with *QB6*, a probe derived from *Sob1*, a cDNA coding for a human testis specific protein potentially involved in sperm-oocyte binding (Lefevre *et al.* (1999). *2P1* and *QB6* were found to be 45.5 % identical overall. The segment encompassing nt 303 to 356 of *2P1* was 62.5 % identical to that between nt 369 and 423 of the *Sob1* cDNA. The weak stringency conditions used for the screening of the library probably account for the detection of *2P1*.

Database searches showed that the coding region of *2P1* was 91 % identical to the ORF1 variant of *E3-3*, a rat gene that is likely to be involved in alternative splicing regulation (Hartmann and Stamm, 1997) (GenBank accession number U95160). The *E3-3* gene is itself subject to alternative splicing, giving rise to three variant RNAs. ORF2 and ORF3 generate transcripts with in-frame stop codons, resulting in the production of truncated proteins. The shortest transcript derived from ORF1 thus corresponds to the longest protein. RT-PCR detected the ORF2 and ORF3 variants in almost all tissues tested, except the liver and testis, whereas the testis produced the ORF1 variant. The high degree of similarity between the *2P1* and *E3-3* cDNA sequences indicates that alternative splicing is similar for the mouse and rat genes.

Hartmann and Stamm (1997) compiled six mouse ESTs obtained from tumoral and embryonic tissues. 93% identity was observed comparing the rat and mouse protein sequences corresponding to ORF1.

Furthermore, we found that four recently identified mouse ESTs share 95 to 98 % sequence identity with *2P1*. One of these ESTs, isolated from spermatocytes (GenBank accession number BG101073), corresponds to nt 28 to 623 of *2P1*. Moreover, the RIKEN cDNA Expression Array Database has recently reported the identification of two major cDNAs corresponding to nt 415 to 712 (90 % identity) and nt 881 to 1073 (97 % identity) of the *2P1* cDNA, respectively (Miki *et al.*, 2001).

The presence of a CCAAT box-like in its complementary form ATTGG was detected in the long 5'UTR of *2P1*. We found no evidence of specific regulation except the two GC-rich regions. In return we identified a destabilization motif in the 3' region that may account for precise temporal regulation. Such A/U rich element sequences (ARE) have recently been proved to be responsible for the shortening of mRNA half-life (Powell *et al.*, 2000; Gay and Babajko, 2000).

Northern blot and RT-PCR studies showed strong *2P1* expression in the testis. *In situ* hybridization studies of testis were undertaken to identify the cells that synthetize *2P1* mRNA. No signal was observed in somatic cells while, in the germinal lineage, transcripts were detected in pachytene spermatocytes only. This result was confirmed by RT-PCR of FACScan sorted 4C spermatocytes. The positive result of purified spermatids displayed by RT-PCR suggests that some *2P1* mRNA, that could not be detected by *in situ* hybridization, remain at this stage. The signals observed in epididymis using Northern blots and RT-PCR probably account for a residual expression in spermatozoa. Using flow cytometry, we are currently studying the expression of *2P1* in each germinal cell type.

In summary, *2P1* gene is expressed during spermatogenesis in a stage dependant manner. Furthermore, it displays in its 3'-UTR a destabilization motif that may lead to its transient expression as recently described in several cell types (Paillard *et al.*, 2002; Schaaf and Cidlowski, 2002; Suda *et al.*, 2002). This posttranscriptional regulation pathway has been suggested to account, in association with tranlational regulation, for gene expression in response to hormonal environment or during embryo development (Gay and Babajko, 2000; Ueno and Sagata, 2002).

At this stage, two major questions arise: when is the protein encoded by the *2P1* transcript synthesized and what is its physiological role? Hartmann *et al.* (1997) isolated *E3-3* cDNA with the yeast two-hybrid system using SRp30c, a protein involved in pre-mRNA splicing, as bait. Their results suggest that E3-3 belongs to the spliceosome protein family. Furthermore, they showed the nuclear location of the protein encoded by ORF1
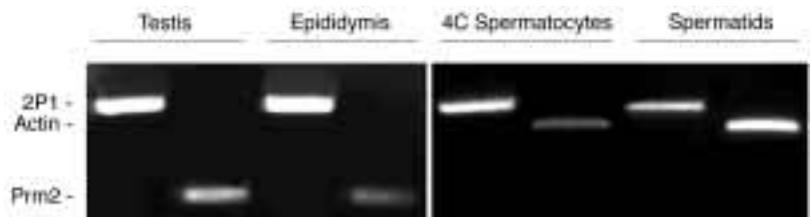
Fig. 3. RT-PCR analysis of *2P1* mRNA in testis and epididymis, and in pachytene spermatocytes and spermatids. *Testis and epididymis* **(A)**, *FACScan isolated 4C spermatocytes and spermatids* **(B).** Protamine 2 (Prm-2) *and* actin *expression were used as controls.*
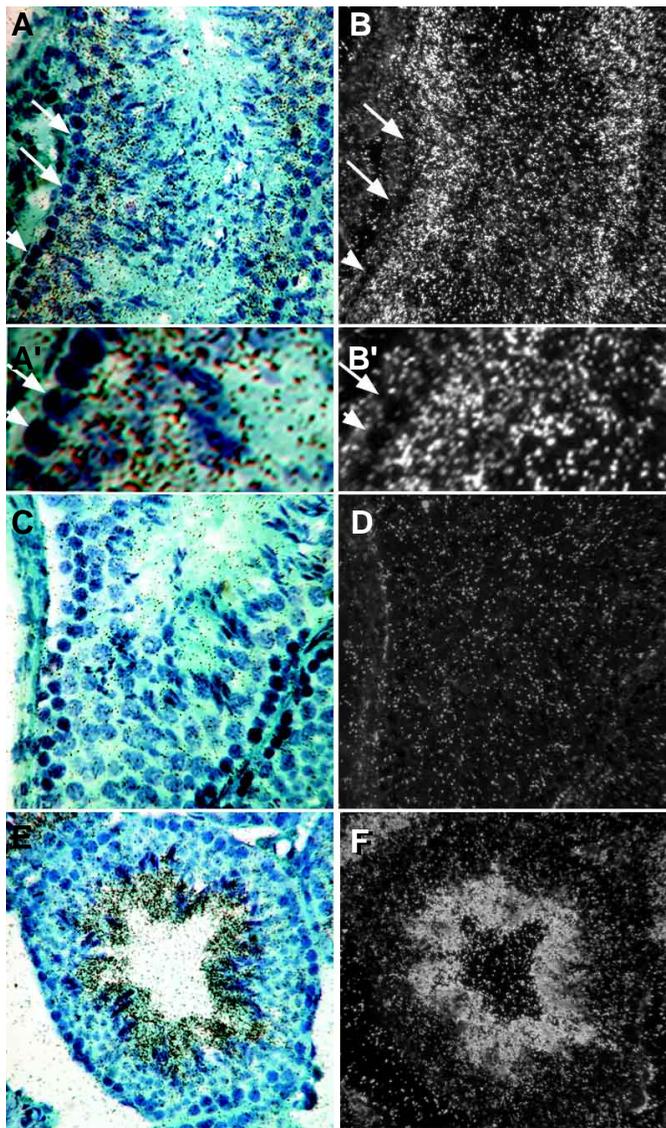
Fig. 4. (Left) *In situ* **hybridization of adult testicular tissue sections.** *Hybridization with the [^{35}S]-UTP labeled antisense* 2P1 *RNA probe* **(A,B, A',B')**. *Hybridization controls were performed with the* 2P1 *sense RNA probe as a negative control* **(C,D)** *or the angiotensin converting enzyme (ACE) antisense RNA probe as a positive control* **(E,F)**. *Arrows in A' and B' point to spermatogonies. Bright field (A, A', C and E) and dark field (B, B', D and F) images of the same sections are shown. Original magnification X 16 in all cases, except A' and B' (X63).*
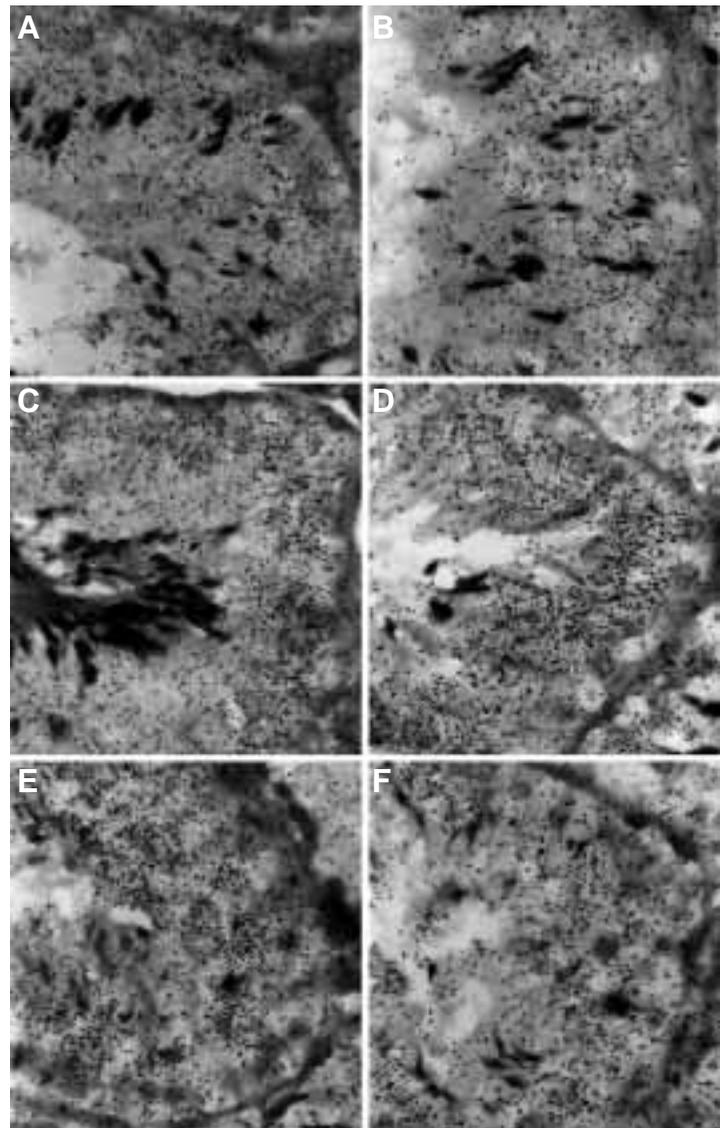
Fig. 5. (Right) *In situ* **hybridization at various stages of the mouse seminiferous tubule cycle.** *Hybridization with the [^{35}S]-UTP labelled antisense* 2P1 *RNA probe. Stages I-II* **(A)**, *III - V* **(B)**, *VII* **(C)**, *VIII and IX* **(D)**, *XI* **(E)** *and XII* **(F)**. *Original magnification X 63 in all cases.*

in transfected A293 cells, that is consistent with a role in gene regulation. Given its high level of similarity to *E3-3*, the presence of a destabilization motif in its 3'UTR region and its specific expression in pachytene spermatocytes, *2P1* may be one transiently expressed gene involved in male meiotic process.

Experimental Procedures

Plasmids and Probes

We screened the Uni-ZAP XR male mouse germ cell expression library (Stratagene, La Jolla, CA, USA) with the *QB6* cDNA probe corresponding to nucleotides 1495 to 2780 of the human cDNA encoding the spermoocyte binding protein SOB1 (Lefevre *et al.*, 1999). *QB6* was obtained from human testis mRNA by 3'RACE using the Marathon cDNA amplifi-

cation kit (Clontech, Palo Alto, CA, USA) with an upstream primer specific for *Sob1* (5'-GATATTGGCAACCTCAGCCTTCCTC-3'), the anchor primer provided by the manufacturer and Taq Gold polymerase (Perkin Elmer, Foster City, CA, USA). The resulting products were inserted into the PCR 2-1 TOPO Vector (Invitrogen, Groningen, the Netherlands). Before screening the library, we showed by Southern blotting that QB6 hybridized to mouse testis DNA (data not shown).

The probe used for Northern blotting was obtained from 10 µg of mouse testis total RNA treated with 10 U of RNase-free DNase. The first-strand cDNA was synthesized with MMLV reverse transcriptase (Life Technologies, Cergy Pontoise, France) and the 627 bp fragment was amplified by PCR with Taq Gold polymerase (Perkin Elmer) and *2P1* specific primers (upstream 5'-GGCTACTGCTCTAGGATTTCGC-3' and downstream 5'-CTGGGTGAGCATGACAAGTGTG-3'). The amplified cDNA was then inserted into the PCR 2-1 TOPO Vector (Invitrogen).

For *in situ* hybridization, the first-strand cDNA was produced by reverse transcription from mouse testis total RNA, then amplified by PCR with Advantage Taq polymerase (Clontech) and upstream and downstream primers containing *Xba*I and *Eco*RI restriction sites (5'-GCTCTAGAATGGCTACTGCTCTAGGATTTCGC-3' and 5'-GGAATTCCTGATGTGTCATTCTGAGGCTTGACC-3' respectively). The amplified DNA was digested with *Xba*I (Boehringer Mannheim, Germany) and *Eco*RI (Gibco BRL, Paisley, Scotland), and inserted into the BlueScript KS vector (Stratagene). Single-stranded sense and antisense *2P1* probes were synthesized with 50 µCi [^{35}S]-UTP (Amersham Pharmacia Biotech Ltd, UK) as described by Sibony *et al.* (1995).

All cDNAs were sequenced on both DNA strands.

cDNA Library Screening and Molecular Cloning

The mouse testis germ cell cDNA library expressed in the Uni-ZAP XR vector containing the BlueScript KS phagemid was used to infect *Escherichia coli* XL1-blue MRF' (Stratagene) and 50,000 plaque-forming units (pfu) were plated on 150-mm NZY 8% top agar plates. The plates were incubated at 37°C for 8 h, and bacteriophage particles were then replicated onto 0.45 µm nitrocellulose filters (Schleicher and Schuell, Dassel, Germany). Membranes were screened with the *QB6* probe radiolabeled with [α^{32}P]-dCTP by random priming with the Rediprime II Kit (Amersham). They were washed once with 3 X SCC 0.1 % SDS at room temperature, then with 2 X SCC 0.1 % SDS, 1 X SSC 0.1 % SDS, and 0.5 SCC 0.1 % SDS at 65°C, finally with 0.5 SCC 0.1 % SDS at room temperature. Positive clones were selected and Phagemid BlueScript was excised *in vivo* from the λ ZAP vector, using the ExAssist helper phage (10^6 pfu) in *Escherichia coli* non suppressing Su⁻ SORL strain (Stratagene). Recombinant phagemids were then purified by alkaline lysis (Sambrook *et al.*, 1989) and digested with *Eco*RI (Gibco BRL). Digestion products were size-fractionated by electrophoresis in a 1% agarose gel. Four positive recombinant phagemid clones were sequenced with the T3 and T7 primers, which bound to opposite sites of the polylinker in the λ Uni-ZAP XR vector. The *2P1* cDNA was sequenced on both strands and the resulting nucleotide sequence is available from GenBank under accession number AF235017.

Primary Spermatocyte and Spermatid Purification

Pachytene spermatocytes (4C) and spermatids were isolated from adult mouse testis, and purified using a dual-laser FACStar Plus (Becton Dickinson, San Jose, CA, USA) according to Kovtun and McMurray (2001). Cells were discriminated by size and DNA content *via* Hoechts 33342 staining. Then they were evaluated for morphology and gene expression (I. Allemand, personal communication).

Northern Blotting

Total RNA was extracted from adult male mouse organs (testis, epididymis, seminal vesicle, vas deferens, kidney, bladder, lung, liver, muscle, heart and brain) by guanidine thiocyanate/phenol-chloroform extraction (Chomczynski and Sacchi, 1987). The RNA was quantified and then treated with 10 U of RNase-free DNase (Boehringer) for 15 min at 37°C. Equal amounts of total RNA (20 µg/lane) were denatured, subjected to electrophoresis in a 1.5% formaldehyde-agarose gel for 4 h at 70 volts and transferred to nitrocellulose membranes (Hybond N, Amersham) by capillary action overnight in 20 X SSC. The RNA was cross-linked to the membrane by exposure to UV light. Membranes were incubated for 4 h at 65°C in prehybridization buffer (50% (w/v) formamide, 0.75 M NaCl, 0.05 M NaH$_2$PO$_4$, 0.5 mM EDTA, 5 X Denhardt's solution, 0.1 mg/ml salmon sperm) to which we added 0.5% SDS. The *2P1* probe was labeled with 50 µCi [^{32}P]-dCTP, using a random priming kit (Amersham) and added to the prehybridization buffer with 0.1% SDS. The membrane was incubated in this mixture for 16 h. Membranes were then washed once with 3 X SSC 0.1% SDS at room temperature, twice with 2 X SSC, 0.1% SDS at 65°C for 15 min and once with 1 X SSC, 0.1% SDS at 65°C for 30 min. Hybridized membranes were placed against X-ray film (Hyperfilm MP, Amersham) at – 80°C with intensifying screens for 48h.

RT-PCR

Total RNA was isolated from 10 µg mouse testis or epididymis tissue (Chomczynski and Sacchi, 1987). Samples were treated with MMLV reverse transcriptase (Life Technologies) at 42°C for 1 h. The cDNA was diluted to 20 µl and 2 µl were used per tube.
Upstream (5'-GGCTACTGCTCTAGGATTTCGC-3') and downstream (5'-CTGGGTGAGCATGACAAGTGTG-3') *2P1* primers were used. Amplification of the mouse *Prm-2* gene was used as a positive control, (upstream 5'-CGCTACCGAATGAGGAGCCCCAGTG-3' and downstream 5'-TTAGTGATGGTGCCTCCTACATTTCC-3'). PCR was then carried out with enzyme Taq Gold (Perkin Elmer) as follows: initial denaturation for 10 min at 94°C followed by 30 cycles of 30 s at 94°C, 45 s at 60°C and 1 min at 72°C, then a final extension at 72°C for 10 min. Total RNA of sorted germinal cells was purified using Rneasy Mini kit according to manufacturer's instruction (Qiagen Gmbh, Hilden, Germany). Reverse transcription was the same as above. The cDNA was diluted to 150 µl and 10 µl were used per tube. Amplification of the mouse *actin* gene was used as a positive control (upstream 5'-ACCCACACTGTGCCCATCTAC-3' and downstream 5'-CTTCATGGTGCTAGGAGCCAG-3'). PCR was carried out with HotStartTaq Polymerase (Qiagen) and conditions were the following: initial denaturation for 15 min at 95°C, then 30 cycles of 30 s at 94°C, 1 min at 60°C and 1 min at 72°C, and a final extension of 10 min at 72°C. For all amplifications, negative controls without the cDNA matrix were included (data not shown).

In Situ *Hybridization*

Prehybridization, hybridization and posthybridization of paraformaldehyde-fixed, paraffin-embedded testis sections (4-6 µm) were carried out as previously described (Sibony *et al.*, 1995). The procedure included a treatment with proteinase K, overnight hybridization at 50°C with 0.7 x 10^6 cpm of labeled probe per section, washing under stringent conditions and RNase A treatment. The dried sections were dipped in Kodak NTB-2 photographic emulsion (IBS INTEGRA Biosciences, Cergy-Pontoise, France) and stored at 4°C for 15 and 21 days in the dark. After photographic processing, sections were counterstained in 0.2 % toluidine blue, and mounted in EUKITT mounting medium (Elvetec Services, Vénissieux, France). Sections were viewed and photographed under dark-field illumination.

The location of the grains was determined by reference to the same section viewed under bright-field illumination. For quantification, sections were examined with a computer-assisted image analysis system, Visilog 4.15 (Noesis, Les Ulis, France), consisting of an IMC 500 camera and an IMC 500 digitizer. The stages of the seminiferous epithelium cycle were identified according to the morphological criteria for mouse spermatogenesis established by Oakberg (1956) and Russell *et al.* (1990). The intensity of the hybridization signal for the various stages of the spermatogenic cycle was quantified and expressed as the percentage of the pixels within a marked area with silver grains.

Statistical Analysis

Analyses were performed with SYSTAT (Wilkinson, 1990). Means were compared by one-way ANOVA and Tukey-Kramer multiple comparison tests. Error estimates are given as standard error of the mean (SEM). Results are expressed as means ± SEM. Data were considered statistically significant if $P < 0.05$.

Sequence Data Analysis

Sequence data were compiled and DNA sequences were aligned with the BLASTN and ALIGN programs.

References

CHOMCZYNSKI, P. and SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159.

FOULKES, N.S., MELLSTROM, B., BENUSIGLIO, E. and SASSONE-CORSI, P. (1992). Developmental switch of CREM function during spermatogenesis: from antagonist to activator. *Nature* 355: 80-84.

GAY, E. and BABAJKO, S. (2000). AUUUA sequences compromise human insulin-like growth factor binding protein-1 mRNA stability. *Biochim. Biophys. Res. Commun.* 267: 509-515.

GU, W., TEKUR, S., REINBOLD, R., EPPIG, J.J., CHOI, Y.C., ZHENG, J.Z., MURRAY, M.T. and HECHT, N.B. (1998). Mammalian male and female germ cells express a germ cell-specific Y-Box protein, MSY2. *Biol. Reprod.* 59: 1266-1274.

HARTMANN, A.M. and S. STAMM, (1997). Molecular cloning of a novel alternatively spliced nuclear protein. *Biochim. Biophys. Acta.* 1353: 224-230.

KANAI, Y., KANAI-AZUMA, M., NOCE, T., SAIDO, T.C., SHIROISHI, T., HAYASHI, Y. and YAZAKI, K. (1996). Identification of two Sox17 messenger RNA isoforms, with and without the high mobility group box region, and their differential expression in spermatogenesis. *J. Cell. Biol.* 133: 667-681.

KOVTUN, I.V. and MCMURRAY, C.T. (2001). Trinucleotide expansion in haploid germ cells by gap repair. *Nature Genetics* 27: 407-411.

KURTH, B.E., WRIGHT, R.M., FLICKINGER, C.J. and HERR, J.C. (1993). Stage-specific detection of mRNA for the sperm antigen SP-10 in human testes. *Anat. Rec.* 236: 619-625.

LEFEVRE, A., DUQUENNE, C., ROUSSEAU-MERCK, M.F., ROGIER, E. and FINAZ, C. (1999). Cloning and characterization of SOB1, a new testis-specific cDNA encoding a human sperm protein probably involved in oocyte recognition. *Biochem. Biophys. Res. Commun.* 259: 60-66.

MACKEY, Z.B., RAMOS, W., LEVIN, D.S., WALTER, C.A., MCCARREY, J.R., and TOMKINSON, A.E. (1997). An alternative splicing event which occurs in mouse pachytene spermatocytes generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. *Mol. Cell. Biol.* 17: 989-998.

MEISTRICH, M.L. (1989). Histone and basic nuclear transitions in mammalian spermatogenesis. In *Histones and other Basic Nuclear Proteins* (ed. Hnilica, L.S., Stein, G.S. and Stein, J.). CRC Press, Broca Raton, pp. 35-55.

MIKI, R., KADOTA, K., BONO, H., MIZUNO, Y., TOMARU, Y., CARNINCI, P., ITOH, M., SHIBATA, K., *et al.* (2001). Delineating developmental and metabolic pathways *in vivo* by expression profiling using the RIKEN set of 18,816 full-length enriched mouse cDNA arrays. *Proc. Natl. Acad. Sci. U.S.A.* 98: 2199-2204.

OAKBERG, E.F. (1956). A description of spermatogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. *Am. J. Anat.* 99: 391-413.

PAILLARD, L., LEGAGNEUX, V., MANIEY, D. and OSBORNE, H.B. (2002). c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding-protein)- dependent pathway. *J. Biol. Chem.* 277: 3232-3235.

POWELL, M.J., THOMPSON, S.A., TONE, Y., WALDMANN, H. and TONE, M. (2000). Posttranscriptional regulation of IL-10 gene expression through sequences in the 3'-untranslated region. *J. Immunol.* 165: 292-296.

RUSSELL, L.D. (1990). Classification and timing of spermatogenesis. In *Histological and histopathological evaluation of the testis* Cache River Press, Clearwater, pp. 44-84.

SAMBROOK, J., FRISCH, E.F. and MANIATIS, T. (1989). *In Molecular cloning: A laboratory manual* (2nd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SCHAAF, M.J. and CIDLOWSKI, J.A. (2002). AUUUA motifs in the 3'UTR of human glucocorticoid receptor alpha and beta mRNA destabilize mRNA and decrease receptor protein expression. *Steroids* 67: 627-636.

SHIH, D.M. and KLEENE, K.C. (1992). A study by *in situ* hybridization of the stage of appearance and disappearance of the transition protein 2 and the mitochondrial capsule seleno-protein mRNAs during spermatogenesis in the mouse. *Mol. Reprod. Dev.* 33: 222-227.

SIBONY, M., COMMO, F., CALLARD, P. and GASC, J.M. (1995). Enhancement of mRNA *in situ* hybridization signal by microwave heating. *Lab. Invest.* 73: 586-591.

SUDA, S., NIBUYA, M., SUDA, H., TAKAMATSU, K., MIYASAKI, T., NOMURA, S. and KAWAI, N. (2002). Potassium channel mRNAs with AU-rich elements and brain specific expression. *Biochem. Biophys. Res. Commun.* 291: 1265-1271.

UENO, S. and SAGATA, N. (2002). Requirement for both EDEN and AUUUA motifs in translational arrest of Mos mRNA upon fertilization of Xenopus eggs. *Dev. Biol.* 250: 156-161.

WILKINSON, L.(1990). In *The system for statistics* (ed. Evanston, I.L.). SYSTAT, Inc. pp. 15-17.

WILLIAMS, T.A., VILLARD, E., PRIGENT, Y., DADOUNE, J.P. and SOUBRIER, F. (1995). A genetic study of angiotensin I-converting enzyme levels in human semen. *Mol. Cell. Endocrinol.* 107: 215-219.