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Genetics

High level of accumulation of a mRNA coding for a precursor-like protein in the submaxillary gland of male rats

(prohormone processing/rodent evolution/androgen regulation/gene duplication/sexual dimorphism)

ISABELLE ROSINSKI-CHUPIN, DIANA TRONIK, AND FRANÇOIS ROUGEON

Unité de Génétique et Biochimie du Développement, Laboratoire associé Centre National de la Recherche Scientifique 361, Département d'Immunologie, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France

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ABSTRACT **NaDodSO$_4$/PAGE analysis of *in vitro* translation products of rat submaxillary gland (SMG) mRNAs has revealed an important sexual dimorphism. Moreover, most of the rat male-specific major translation products differ in size from those translated from male mouse SMG mRNAs. To characterize proteins accumulated in the rat SMG under androgen control, a cDNA library was constructed. Here we report the nucleotide sequence of a 0.7-kilobase mRNA that is 1000–3000 times more abundant in male rats than in female rats. The predicted corresponding protein, SMR1, has a molecular weight of 16,000 and contains a signal peptide for secretion and potential signals for glycosylation. An interesting feature of SMR1 is the presence, in a hydrophilic region, of the tetrapeptide Gln-His-Asn-Pro surrounded by two pairs of basic residues that represent potential cleavage sites for maturation enzymes. In rats, the tissue distribution of the SMR1 mRNA is restricted to the SMG and the prostate. Only very low amounts of SMR1 mRNA can be detected in the SMG of male or female mice. Southern blot analysis indicates the presence of three genes in rats but only one in mice. Hypotheses on the physiological role of SMR1-derived peptides in male rats are discussed.**

A large number of polypeptides with biologically defined properties are synthesized at high levels in the submaxillary gland (SMG) of rodents (1) and particularly of mice. These proteins, including nerve growth factor (NGF), epidermal growth factor (EGF), and renin, share a number of properties. All are synthesized in the same cell type—the granular convoluted tubular (GCT) cells—in response to various hormonal stimuli, in particular to androgens (2). Further, these secretory proteins can be found in the saliva of mice and are synthesized as precursors that become active after posttranslational processing events, possibly involving kallikrein-like proteinases. Some of these kallikrein-like proteinases are also synthesized under androgen-control in the SMG (3).

The biological significance of the accumulation of these polypeptides in the SMG of mice and of their release into the saliva is still unclear. Aggressive behavior in male mice results in the release into the blood of large amounts of submaxillary NGF and renin (4, 5), providing some evidence that these molecules play a physiological role. But surprisingly, these proteins are not detected in the SMG of related species. For instance, renin, which represents 2% of the SMG proteins in wild-type and most inbred male mice (6) is not found in the SMG of rats (7). This suggests that these SMG-synthesized polypeptides could be related to the especially aggressive behavior of male mice.

Attempts to characterize the genes regulated by androgens in the SMG of rats led us to analyze the patterns of *in vitro* translation products directed by the mRNAs of this tissue. As in mice, an important sexual dimorphism was observed in the rat SMG translation products. Moreover, the major polypeptides accumulated in the two species appeared to be very different.

In this work, we present the characterization of a major androgen-regulated, rat-specific mRNA obtained from a cDNA library prepared from male SMG poly(A)$^+$ mRNAs. This mRNA is expressed in a highly tissue-specific manner. Three related genes have been detected in the rat. In contrast, this mRNA is not abundant in the SMG of mice and only one gene is detected. The function of the corresponding polypeptide, SMR1, is not known, but the presence of a tetrapeptide motif surrounded by exposed paired basic residues suggests that it could be processed, and consequently its maturation products may have a physiological role in male rats.*

MATERIALS AND METHODS

Animals and Hormone Treatments. Ten-week-old male and female Wistar rats were purchased from Iffa-Credo (Rhône, France). Androgens were withdrawn by castration and, where indicated, 35 mg of testosterone (Sterandryl Retard, Hoechst-Roussel) was injected intraperitoneally 10 days later. Where indicated, the same dose of testosterone was administered to female rats. Eight-week-old DBA/2 and Swiss mice were obtained from Institut Pasteur.

RNA Extraction and *in Vitro* Translation. RNA was prepared from mouse and rat tissues as described (8). *In vitro* translation of RNAs was performed with the mRNA-dependent reticulocyte lysate translation system (9). The products were analyzed by NaDodSO$_4$/PAGE.

Cloning and Characterization of the SMR1 cDNA. Poly(A)$^+$ RNA prepared from male SMG of Wistar rats was used as template for reverse transcriptase and the cDNAs were converted into double-stranded molecules by using the cDNA synthesis system from Amersham with the protocol given by the manufacturer. The double-stranded cDNA was then inserted in the *Pst* I site of pUC9 by the oligo(dC)-tailing method (10). Host bacteria were transformed and colonies were screened by hybridization with the probes described below. In brief, the mRNAs from SMG of males and females were fractionated in a 5–20% sucrose gradient. Fractions enriched for mRNAs of low molecular weight (which were shown to contain mRNA coding for SMR1 by *in vitro* translation) were ethanol-precipitated and used as template for reverse transcriptase in the presence of [α-^{32}P]dGTP and [α-^{32}P]dCTP. About 3000 recombinant clones were screened on duplicate filters with the radiolabeled cDNA prepared

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Abbreviations: SMG, submaxillary gland(s); NGF, nerve growth factor; EGF, epidermal growth factor; TRH, thyrotropin-releasing hormone; GCT cell, granular convoluted tubular cell.

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04109).

from SMG of male and female rats. The selected clones hybridized strongly with the male cDNA probe but very weakly with the female probe. Recombinant clones were identified by DNA·mRNA hybrid-arrested cell-free translation experiments (11).

Sequencing of SMR1 cDNA. The SMR1 cDNA was digested with several restriction enzymes and the generated fragments were subcloned in the M13 mp9 vector. DNA sequencing was performed by the dideoxy termination method (12). The search for sequence homologies between SMR1 and the proteins of the PSEQUIP library (13) was done with the FASTP program, according to Lipman and Pearson (14).

RNA Analysis. Total RNA was electrophoresed in an agarose/formaldehyde gel, transferred to a nylon membrane, and hybridized with the SMR1 cDNA probe (15). The probe was the full-length cDNA insert labeled by the random priming method (16).

Southern Blot Analysis of Rat and Mouse DNA. Genomic DNA was prepared from liver (mouse) or spleen (rat) and digested with *Eco*RI, *Bam*HI, or *Pst* I. Digested DNA was electrophoresed in a 0.7% agarose gel, transferred to Zeta-Bind membrane (Bio-Rad), and hybridized to the full-length probe or probes described in the text.

RESULTS

***In Vitro* Translation Analysis of mRNAs Prepared from SMG of Rats and Mice.** The patterns of *in vitro* translation of RNAs prepared from SMG of male and female rats were compared (Fig. 1). Several polypeptides (of apparent M_r around 18,000, 19,000, 35,000, and 46,000) were found to be

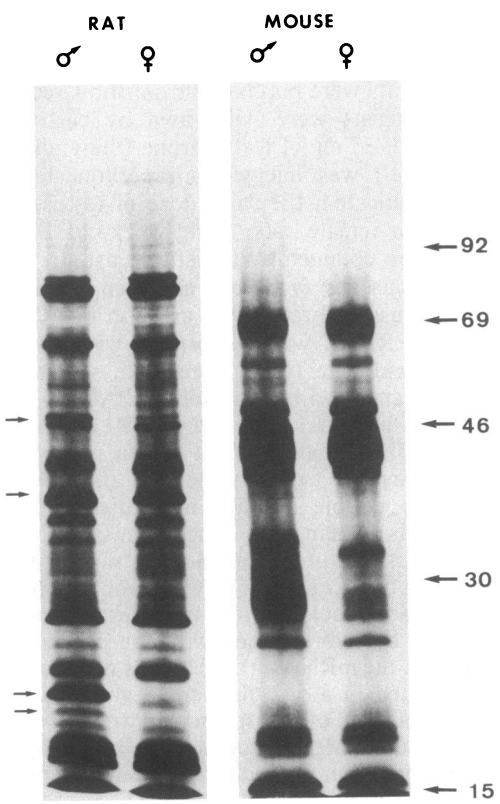

FIG. 1. *In vitro* translation analysis of mRNA prepared from SMG of mice and rats. Five micrograms of total RNA prepared from SMG of male and female mice or rats was translated in a reticulocyte cell-free system in the presence of [^{35}S]methionine. *In vitro* translation products were electrophoresed in a NaDodSO$_4$/12.5% polyacrylamide gel and autoradiographed. Major male rat polypeptides are indicated by arrows at left. Positions of molecular weight markers ($M_r \times 10^{-3}$) are indicated at right.

synthesized in higher amounts from male SMG mRNA than from female SMG mRNA. These data emphasize the existence of sexual dimorphism in the SMG of rats.

In addition, when the translation products were compared with those obtained with RNAs from the SMG of mice, most of the major polypeptides seemed to be different in the two species. In particular, although sex-related differences could also be observed in mice, they concerned different polypeptides than in rats. The male-specific translation products seen in rats seemed to be absent in mice.

Isolation and Sequence of a cDNA Complementary to a Rat Male-Specific mRNA. To isolate male-specific mRNAs of the SMG of rats, a cDNA library prepared from this tissue was constructed in pUC9. The recombinant clones were screened by a differential screening strategy as described in *Materials and Methods*. The positive clones were characterized by DNA·mRNA hybrid-arrested cell-free translation experiments. One class of recombinant cDNAs abolished the *in vitro* synthesis of a polypeptide of apparent M_r 19,000 (Fig. 2). This polypeptide, named SMR1, is present in the *in vitro* translation products of male rat mRNAs and absent from those of females. The corresponding cDNA was used as a probe in RNA blot experiments. It hybridized to a 700-base mRNA, found in high amounts in the SMG of male rats. This cDNA insert was further characterized.

The sequence of the SMR1 cDNA and the deduced sequence of the protein are shown in Fig. 3. The cDNA insert is 652 bases long excluding the poly(A) tract. The sequence bears one open reading frame of 510 bases. The only ATG that can be used as initiation codon is located 73 bases downstream of the 5′ terminus of the cDNA clone. The 3′ untranslated region (142 bases long) contains the consensus polyadenylylation signal AATAAA (17) 23 bases upstream of the poly(A) tail (bases 625–630). Curiously, the translation stop codon is found within another AATAAA sequence (bases 512–517), which is apparently not a suitable polyadenylylation signal.

The predicted protein is 146 amino acids long, which corresponds to a M_r of ≈16,000. This molecular weight is slightly lower than that determined from the electrophoretic mobility of the *in vitro* translation product (M_r 19,000). Such a discrepancy between the molecular weight predicted from sequence analysis and that determined from electrophoretic mobility has been described for other proteins of the SMG of rats (18) or mice (6, 19).

An exhaustive computer data bank search did not reveal any homology to previously described proteins. SMR1 has a

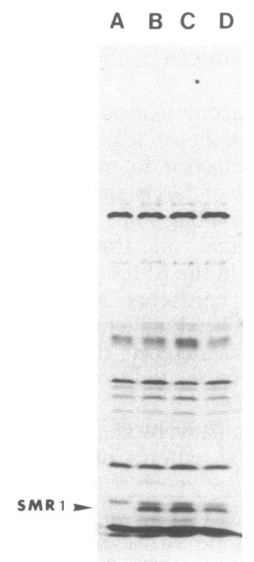

FIG. 2. Hybrid-arrested cell-free translation analysis of SMR1 cDNA clone. Two hundred nanograms of poly(A)$^+$ mRNA prepared from SMG of male rats was translated in a reticulocyte cell-free system after hybridization with 500 ng of purified cDNA insert (lane A), hybridization with the same amount of cDNA insert and further melting of the hybrid (lane B), hybridization with 600 ng of pUC9 DNA (lane C), or without prior hybridization (lane D). The *in vitro* translation products were electrophoresed in a NaDodSO$_4$/12.5% polyacrylamide gel and autoradiographed.

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AAA CTG ACT GAC CAG AGA GCT TCT GAC CAG CAC ATT TCC CCG CTC AGA AGT TTC     54

                        1
                        Met Lys Ser Leu Tyr Leu Ile Phe Gly Leu Trp Ile
TCC AAG GGG CTA CCA AAG ATG AAG TCA CTG TAT TTG ATC TTT GGC CTG TGG ATC    108

                              20                                    30
Leu Leu Ala Cys Phe Gln Ser Gly Glu Gly Val Arg Gly Pro Arg Arg Gln His
CTT CTA GCA TGC TTC CAG TCA GGT GAG GGT GTC AGA GGC CCA AGA AGA CAA CAT    162

                                    40
Asn Pro Arg Arg Gln Gln Asp Pro Ser Thr Leu Pro His Tyr Leu Gly Leu Gln
AAT CCT AGA AGA CAA CAA GAT CCT TCA ACT CTT CCT CAT TAT CTT GGT CTT CAG    216

     50                                        60
Pro Asp Pro Asn Gly Gly Gln Ile Gly Val Thr Ile Thr Ile Pro Leu Asn Leu
CCT GAT CCC AAT GGT GGA CAA ATA GGA GTA ACA ATC ACT ATA CCC TTA AAT CTT    270

               70                                        80
Gln Pro Pro Arg Val Leu Val Asn Leu Pro Gly Phe Ile Thr Gly Pro Pro Leu
CAA CCA CCT CGT GTT CTT GTT AAT CTT CCC GGT TTT ATC ACT GGA CCA CCA TTG    324

                    90                                        100
Val Val Gln Gly Thr Thr Glu Tyr Gln Tyr Gln Trp Gln Leu Thr Ala Pro Asp
GTT GTA CAA GGT ACC ACT GAA TAT CAA TAT CAG TGG CAG CTA ACT GCT CCA GAC    378

                              110                                   120
Pro Thr Pro Leu Ser Asn Pro Pro Thr Gln Leu His Ser Thr Glu Gln Ala Asn
CCT ACA CCT CTA AGC AAT CCT CCT ACT CAA CTT CAT TCC ACA GAA CAA GCA AAT    432

                                    130
Thr Lys Thr Asp Ala Lys Ile Ser Asn Thr Thr Ala Thr Thr Gln Asn Ser Thr
ACA AAA ACA GAT GCC AAA ATC TCC AAC ACT ACT GCG ACT ACC CAA AAT TCC ACT    486

     140
Asp Ile Phe Glu Gly Gly Gly Lys
GAT ATT TTT GAA GGT GGT GGC AAA TAATAAATTCCTTTTGGCAGTTAGAATAGCATAAATCAA    549

AACACTGTGTAGTTTTGGGCGAAATAATCTTTAAAGGCTTGAGAAACAACCTTTACCCGCATTATAGAAAA    620

TGACAATAAAGAGCTAAGCAGCATTACACAGCAAAAAA                                    658
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FIG. 3. Nucleotide sequence of SMR1 cDNA clone and predicted amino acid sequence of SMR1. The protein sequence is numbered from the first methionine residue. The putative signal peptide is underlined. Potential sites of N-glycosylation are starred. A box surrounds the tetrapeptide and the flanking dibasic sequences. Broken underline indicates the consensus polyadenylylation signal.

relatively high content of glutamine and proline residues but contains no repetitive regions. Thus it does not belong to the family of "proline-rich" or "glutamine-rich" polypeptides (20, 21) that are major proteins of the SMG. Further, the mRNA sequence does not share the 80-base-long sequence characteristic of this family, at its 5′ end. However, similarly to these proteins, SMR1 does not contain any cysteine or methionine residues (except in its signal peptide).

The amino-terminal portion of SMR1 is highly hydrophobic, a characteristic feature of signal peptides of most secretory proteins. Although the amino-terminal sequence of the mature protein has not been directly determined, the signal-peptide cleavage site can be tentatively assigned to be between residues 18 and 19 according to the "−3, −1" rule defined by Von Heijne (22).

The SMR1 protein also presents certain features characteristic of glycoproteins. Two potential N-linked glycosylation sites, Asn-Xaa-Thr (23), are observed at positions 129 and 136. The protein is relatively rich in proline (12%), threonine (12%), and glutamine (9.5%). Several O-linked glycosylation sites might be present in the carboxyl-terminal moiety of SMR1, since regions rich in proline and threonine residues are usually found in heavily O-glycosylated proteins such as mucoproteins and sialoglycoproteins (24).

An interesting particularity is the presence in positions 27–28 and 33–34 of paired basic amino acids, Arg-Arg. Such dipeptides represent potential sites of cleavage by processing enzymes (25, 26). They surround a tetrapeptide Gln-His-Asn-Pro. As shown by the diagram of hydrophilicity (Fig. 4), the tetrapeptide and its flanking sequences are located in a hydrophilic environment rendering this region accessible to potential processing enzymes. This structure resembles that found in the precursor protein of the hypothalamic hormone TRH (thyrotropin-releasing hormone), where paired basic amino acids surround the sequence Gln-His-Pro-Gly (28). TRH is generated after cleavage of these Arg-Arg and Lys-Arg bonds. Following excision of the tetrapeptide Gln-

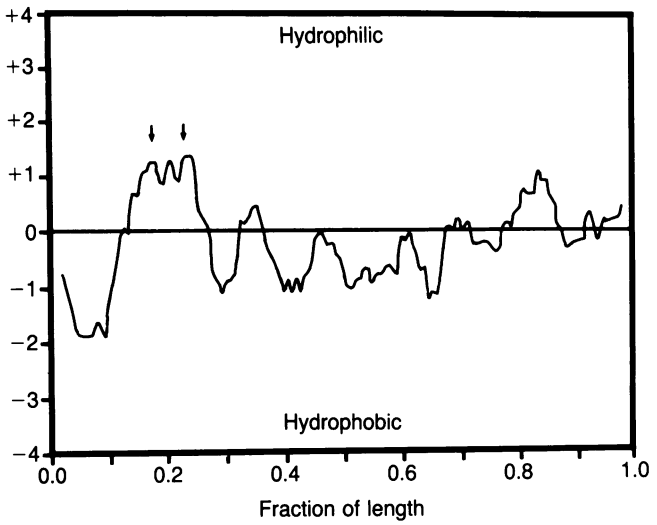

FIG. 4. Hydrophilicity plot of the amino acid sequence of SMR1. Hydrophilicity was calculated by the method of Hopp and Woods (27), with a window size of 7 amino acids. Arrows indicate the position of the tetrapeptide and of its flanking basic sequences.

His-Pro-Gly, amino- and carboxyl-terminal modifications transform it to the biologically active TRH (<Glu-His-Pro-amide, where <Glu is pyroglutamic acid). These modifications include a carboxyl-terminal amidation, involving a carboxyl-terminal amidation enzyme (29), and the cyclization of the glutamine. In the case of SMR1, the first cleavage by one of many candidate proteinases (25) would generate the tetrapeptide Gln-His-Asn-Pro extended by one or two arginine residues at the amino and carboxyl termini. Further processing of these intermediates would then generate <Glu-His-Asn-Pro or <Glu-His-Asn-Pro-Arg, based on the relatively weak activity of the converting carboxypeptidase E (30) on Pro-Arg bonds. The absence of carboxyl-terminal glycine obviates the possibility of carboxyl-terminal amidation.

Androgen Regulation of SMR1 mRNA Accumulation in the SMG of Rats. To study the androgen regulation of SMR1 mRNA accumulation in the SMG of rats, total RNA was prepared from SMG of adult males, males castrated for 20 days, castrated males subjected to androgen treatment, females, and androgen-treated females. These mRNAs were analyzed by gel blot analysis with a ^{32}P-labeled SMR1 probe (Fig. 5*A*). An important difference in SMR1 mRNA accumulation in the SMG of male and female rats was observed (Fig. 5*B*). SMR1 mRNA is accumulated in the SMG of male Wistar rats at a very high level, since as little as 1.5 ng of total RNA was sufficient to give a hybridization signal. In contrast, the level of SMR1 mRNA accumulation in the SMG of female Wistar rats was 1000–3000 times lower than in males.

The amount of SMR1 mRNA was reduced by a factor of 10–20 in castrated males (Fig. 5*A*). Administration of testosterone to these castrated males restored the level of SMR1 mRNA to that found in intact males. Furthermore, administration of testosterone to adult female rats induced SMR1 mRNA accumulation to a level similar to that of males.

Tissue Distribution of SMR1 mRNA in Rats. To study the pattern of expression of SMR1 mRNA in rats, total RNA was prepared from kidney, liver, spleen, gut, seminal vesicle, testis, prostate, ovary, and uterus and analyzed by RNA blot hybridization (Fig. 6*A*; liver data not shown). The prostate was the only tissue in which SMR1 mRNA was detected. However, levels in this tissue were about 3000 times lower than in the SMG of male rats. SMR1 mRNA was not detected in either the brain or the pituitary (data not shown).

SMR1-Related mRNA Accumulation in Mice. Although no major male-specific polypeptide having the same molecular

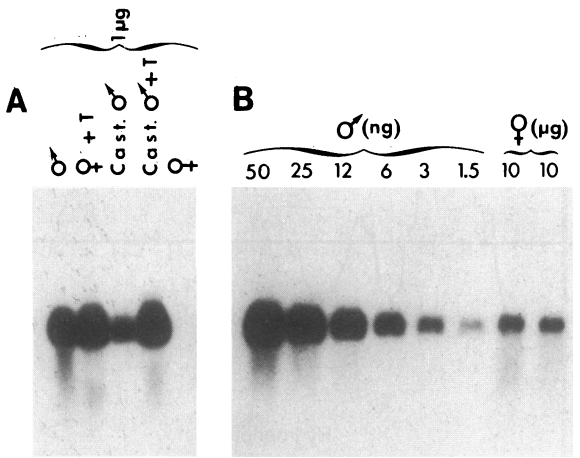

FIG. 5. (*A*) Androgen regulation of SMR1 mRNA accumulation in the SMG of rats. One microgram of total RNA from male, testosterone (T)-treated female, castrated male, castrated and testosterone-treated male, and female rats were electrophoresed in a 1.4% agarose/formaldehyde gel, transferred to a nylon membrane, and hybridized with the SMR1 cDNA probe. Exposure time for the autoradiograph was 2 hr. (*B*) Various amounts (as shown on figure) of SMG RNA of male and female rats were electrophoresed in a 2% agarose/formaldehyde gel, blotted, and hybridized to the SMR1 cDNA probe. The film was exposed for 30 hr.

weight as SMR1 was found in the *in vitro* translation products directed by the mRNAs of mouse SMG, it was of interest to ascertain whether an SMR1-related mRNA is expressed in this tissue. RNAs prepared from SMG of male and female mice were hybridized, after blotting, to the cDNA probe under varying stringency conditions to detect the presence of SMR1 mRNA in mice. A 0.7-kilobase mRNA was detected in the SMG of mice of both sexes. This mRNA was 2000–5000 times less abundant in the SMG of male mice than in that of male rats (Fig. 6*B*). Sex-related differences in SMR1 mRNA accumulation were observed but did not exceed an order of magnitude. The SMG is the only studied tissue where an SMR1 mRNA-related sequence was detected in mice (expression in the prostate of mice has not been evaluated).

Southern Blot Analysis of Genomic Rat and Mouse DNA. To determine the number of SMR1-related genes in rats and in mice, genomic DNAs from Wistar rats and C3H mice, digested with various restriction enzymes were analyzed by Southern blot hybridization with a probe corresponding to the full-length SMR1 cDNA. When a 550-base-pair probe (3′ fragment after *Bam*HI digestion) was used, only three bands were observed with DNA digested with *Eco*RI (Fig. 7). Since these three bands could be due either to three different genes or to the presence of internal *Eco*RI sites (inside introns) in the genomic fragment corresponding to the probe, the probe was subdivided into two shorter fragments. Use of these two fragments (*Bam*HI–*Kpn* I, bases 105–338, and *Ava* II–*Pst* I, bases 314–690) as probes did not reduce the number of hybridizing fragments (data not shown). This indicated that no internal *Eco*RI site was present in the 3′ end of the SMR1 genes. Similar results were obtained after *Pst* I digestion of the genomic DNA (data not shown). Only one band was detected when C3H mouse DNA digested with *Eco*RI (Fig. 7) or *Pst* I was hybridized with the full-length or the 3′ probe. These results are compatible with the presence of three SMR1-related genes in rats but only one in mice.

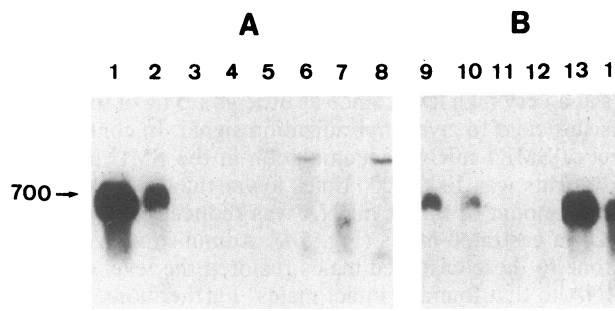

FIG. 6. (*A*) Tissue distribution of SMR1 mRNA in rats. Fifty nanograms of total RNA from SMG of male rat (lane 1) and 30 μg of RNA from prostate (lane 2), seminal vesicle (lane 3), uterus (lane 4), ovary (lane 5), testis (lane 6), gut (lane 7), and kidney (lane 8) were electrophoresed in a 2% agarose/formaldehyde gel, blotted, and hybridized with the SMR1 cDNA probe. Length of SMR1 RNA was estimated to be 700 bases, as indicated. (*B*) RNA blot analysis of SMR1 mRNA in mice. Thirty micrograms of total RNA extracted from SMG of male mice (lane 9), SMG of female mice, kidney of male mice (lane 10), and liver of female mice (lane 12) was electrophoresed together with 50 ng of SMG RNA from male rat (lane 13) and 10 μg of SMG RNA from female rat (lane 14) in a 2% agarose/formaldehyde gel.

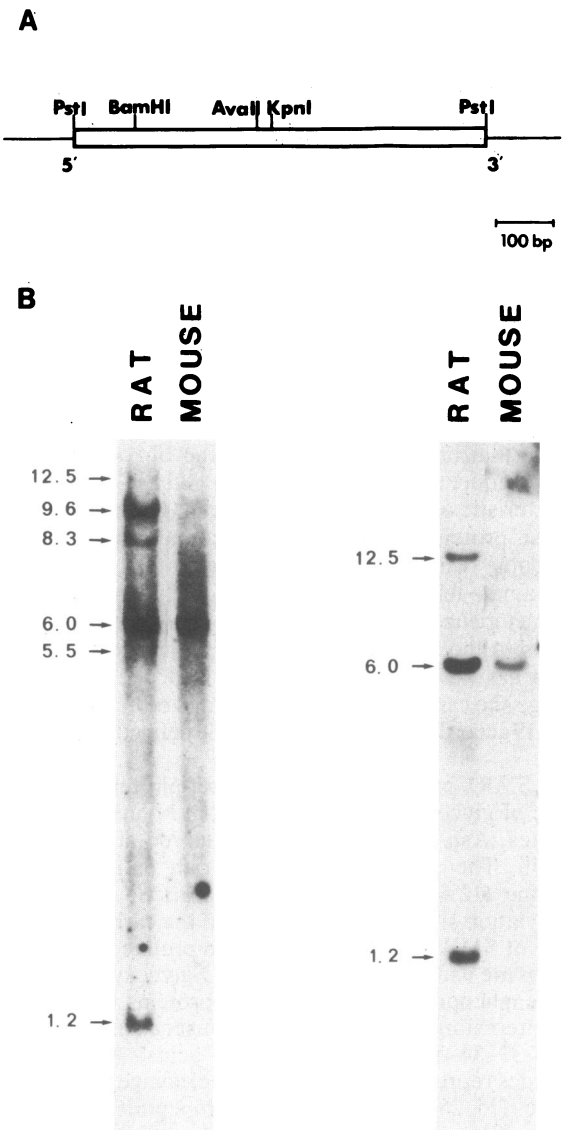

FIG. 7. (*A*) Diagram of SMR1 cDNA. The locations of the restriction endonuclease sites used to obtain the probes for Southern blot analysis are indicated. bp, Base pairs. (*B*) Southern blot analysis of rat and mouse DNA. Ten micrograms of genomic DNA was digested with *Eco*RI, subjected to electrophoresis in a 0.7% agarose gel, and transferred to Zetabind nylon membrane. The filters were hybridized to the full-length cDNA SMR1 probe (*Left*) and to a 3′ *Bam*HI–*Pst* I fragment (*Right*). Size markers are in kilobases.

DISCUSSION

This work reports the cloning of one of the major rat SMG male-specific mRNAs. The corresponding protein, SMR1, exhibits structural features that are characteristic of the precursors of polypeptide hormones. The analysis of the sequence of this mRNA indicates that SMR1 is synthesized as a preproprotein, with an amino-terminal signal peptide, suggesting that it is a secretory polypeptide. The pro-SMR1 is expected to be about 128 amino acids long. An interesting characteristic is the presence, in an exposed region, of two paired basic residues, representing potential maturation sites (25). Cleavage at these Arg-Arg bonds by a processing enzyme (yet unknown) and further posttranslational modifications would be expected to generate a mixture of tetrapeptide (Gln-His-Asn-Pro), pentapeptide (Gln-His-Asn-Pro-Arg), and their pyroglutamyl derivatives. These structures are reminiscent of that of TRH. This hypothetical precursor–product relationship of SMR1, while consistent with the proposed structure, should be regarded as tentative. It suggests a variety of future experimental tests, including the identification of the maturation products.

A second remarkable property of SMR1 mRNA is its high level of accumulation in the SMG of rats in response to androgen treatment. This strongly suggests (although it has not yet been confirmed by *in situ* hybridization analysis) that SMR1 is synthesized in the GCT cells of the SMG (like EGF, NGF, and renin and other androgen-regulated proteins in the SMG of mice). Moreover, the difference in the level of SMR1 mRNA accumulation in males and females (>3 orders of magnitude) is very high, as compared to that usually observed with other androgen-regulated genes in target organs (kidney, liver, SMG) (3, 31). Due to the relative lack of cell replication during differentiation of the GCT cells (3), the SMG is a biological material much more convenient for the study transcriptional activation of genes by androgens than are secondary sex organs of the male genital tract (prostate, seminal vesicles); therefore, study of the regulation of SMR1 synthesis might be particularly useful for the understanding of the mechanism of action of androgens in target organs.

High levels of SMR1 mRNA are found in the SMG of male rats only. Although the presence of SMR1 in minute amounts in some other tissues cannot yet be excluded, the prostate is the only other organ where SMR1 mRNA has been detected by RNA blot analysis. Levels in this tissue are very low. Since SMR1 may well be enzymatically processed, it is interesting that two kallikrein-like proteinases synthesized under androgen control in the SMG of rats (tonin and a tonin-like enzyme) are also found in the prostate of rats (32).

The high level of SMR1 mRNA accumulation in the SMG seems to be specific to the rat. An SMR1-related mRNA has also been detected in the SMG of mice, but it is >3 orders of magnitude less abundant than in the SMG of male rats. Further, in contrast to what is observed in the SMG of rats, the induction by androgens does not exceed 1 order of magnitude.

The presence of three SMR1-related genes in rats compared to only one in mice, together with the higher level of SMR1 expression in the SMG of rats, is reminiscent of what has been observed with the renin genes in mice. The high level of renin expression in the SMG correlates with a gene duplication event that occurred after mouse/rat species separation (33–35). Complex structural changes in the promoter region, including the insertion of a type-2 *Alu* equivalent element and a transposon-like element (7, 36), seem to be responsible for the differential expression and hormonal control of the renin genes in the SMG of mice. It will be important to determine which of the SMR1-related genes is expressed in the SMG of rats. The comparative study of this gene and of the related gene in mice might provide an insight as to which cis-acting elements are involved in androgen control and specific expression in the SMG. Moreover, it will be of interest to determine whether the cis-regulatory elements of the SMR1 rat gene can be recognized by trans-acting factors in the SMG of mice (for instance, by using transgenic mice).

These results and particularly the high level of induction of the SMR1 gene by androgens suggest that SMR1 may play an important male-specific function in the rat. We propose that SMR1 could be the precursor of a molecule (the tetra- or pentapeptide or the carboxyl-terminal part of SMR1) modulating behavioral patterns in male rats, as has been postulated for mouse SMG NGF and renin (37). The presence of relatively high amounts of renin and NGF in the SMG of mice and of SMR1 in the SMG of rats could represent different adaptive responses to various situations among male rodents.

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