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One amino acid change in rat SMR1 polypeptide induces a 1 kDa difference in its apparent molecular mass determined by electrophoretic analysis

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SMR1 is a male-specific, 19 kDa, in vitro translation product of Wistar rat submaxillary glands, which may be the precursor of a small hormone resembling the TRH. In Sprague-Dawley and Fischer rats, instead of SMR1, a male-specific 18 kDa polypeptide may be found. We have cloned the cDNA encoding the 18 kDa polypeptide. We show that the 19 and the 18 kDa polypeptides have the same sequence except for one amino acid change.

Submaxillary gland; Sexual dimorphism; Strain polymorphism; Aberrant electrophoretic mobility

1. INTRODUCTION

The analysis of the in vitro translation patterns of mRNAs prepared from male and female rats had revealed a strong sexual dimorphism in the rat submaxillary glands (SMG) [1]. SMR1 is one of the major in vitro translation products of the male Wistar rats, whose presence is dependent upon androgens [1]. Based on sequence analysis and on expression studies, we have postulated that SMR1 could have a male-specific behavioural role in rats through the release of a small peptide resembling thyrotropin releasing hormone (TRH). Wistar rat SMR1 has an apparent M_r of 19000 [1] by $NaDodSO_4$ gel electrophoresis analysis [2,3]. Curiously, in vitro translation analysis of mRNAs prepared from the SMG of Sprague-Dawley and Fischer strains of rats, did not reveal the presence of SMR1. Instead of SMR1, a male-specific 18 kDa polypeptide (which is present in addition to SMR1 in some Wistar rats) is found. In contrast, an mRNA as abundant as SMR1 mRNA may be detected in male Sprague-Dawley and Fischer rat SMG by Northern blot analysis with an SMR1 cDNA probe.

Because the absence of SMR1 in Sprague-Dawley and Fischer rats would be incompatible with a major role of SMR1 in rats, it was of interest to study whether the 18 kDa polypeptide and SMR1 are related. For instance, they could be encoded by related genes (or by two allelic forms of the same gene) or derive from a same gene by alternative splicing. We have therefore cloned the cDNA corresponding to the 18 kDa polypeptide. We show that both peptides indeed correspond to SMR1 and that the different electrophoretic mobility is only due to one amino acid change.

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2. MATERIALS AND METHODS

Ten-week-old male and female Wistar, Sprague-Dawley and Fischer rats were purchased from Iffa Credo (Lyon, France). RNA preparation, in vitro translation analysis and Northern blot analysis were carried out as previously described [1]. In vitro translation products of mRNA prepared from male SMG were immunoprecipitated using protein A Sepharose (Pharmacia). For PCR amplification, 100 ng of total RNA from male rat SMG were reverse transcribed using an oligodT as primer and AMV reverse transcriptase. The obtained single-stranded cDNA was amplified by PCR, as described [4], using the 2 primers corresponding to the 5′ end and the 3′ end of SMR1 cDNA, respectively (these two primers are separated by two introns in the genomic clones; the PCR product therefore really corresponds to amplification of cDNA sequence). The reaction product was purified and inserted at the *Sma* site of a psp64 vector (Promega Biotec). Five recombinant clones were sequenced using dideoxynucleotide chain termination method [5] applied on double-stranded DNA [6]. In vitro transcription was carried out by the sp6 polymerase after restriction by *PstI* as described in [7].

3. RESULTS AND DISCUSSION

Fig. 1 shows Northern blot analysis of mRNAs prepared from the SMG of different strains of rats. A SMR1-related mRNA can be detected in the three strains. This result contrasts with the absence of SMR1 in the in vitro translation products of mRNAs prepared from Sprague-Dawley and Fischer strains of rats (see

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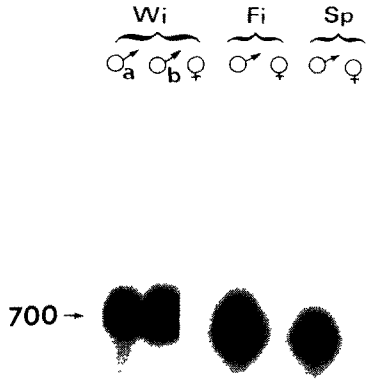
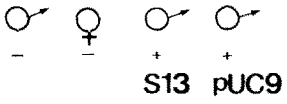
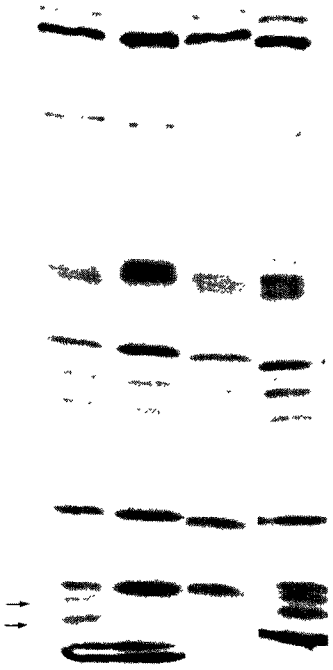


Fig. 1. Northern blot analysis of mRNAs prepared from SMG of Sprague-Dawley (Sp), Fischer (Fi) and Wistar (Wi) rats. One μg of total RNA prepared from SMG of male and female rats was probed with a SMR1 cDNA probe. Wia and Wib designate Wistar rats harbouring only the 19 kDa or both the 19 and the 18 kDa polypeptides, respectively, after in vitro translation.





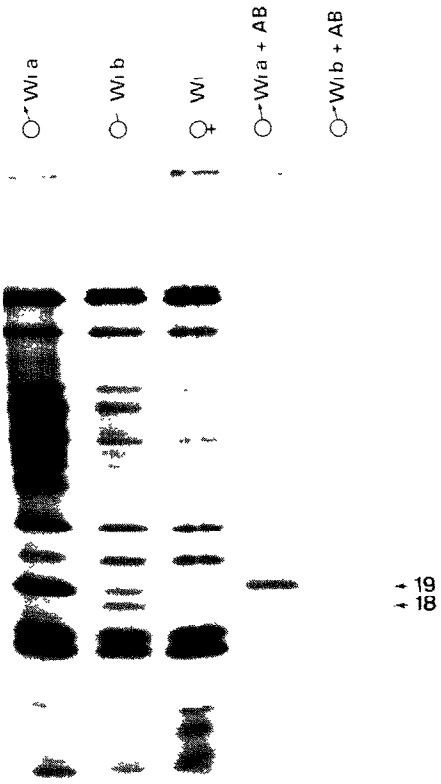


Fig. 3. Immunoprecipitation of the in vitro translation products of mRNAs prepared from Wistar rats. The in vitro translation products of RNAs prepared from SMG of female or male Wistar rats with either the 19 kDa polypeptide only, or both the 19 and the 18 kDa polypeptides, were electrophoresed in a $NaDodSO_4$ 12.5% polyacrylamide gel either directly or after immunoprecipitation with antibodies raised against a synthetic peptide corresponding to amino acids 107–122 of SMR1 sequence.

Fig. 2). As also shown in Fig. 2, in some male Wistar rats both the 18 kDa polypeptide and SMR1 may be simultaneously found.

Hybrid arrested cell free translation analysis [8] was used to determine whether SMR1 and the 18 kDa polypeptide are related. As shown in Fig. 3, translation of the 19 and the 18 kDa polypeptides is inhibited to the same extent by prior hybridization of the mRNAs with SMR1 cDNA. However, the two in vitro translation products react differently towards antibodies raised against a synthetic peptide corresponding to amino acids 107–122 of SMR1 sequence (Fig. 3). Only the 19 kDa polypeptide (SMR1) is efficiently immunoprecipitated by these antibodies.

Fig. 2. Hybrid arrested cell free translation analysis of the 19 and the 18 kDa polypeptides. Five μg of total RNA prepared from SMG of male (encoding both SMR1 and the 18 kDa polypeptides) or female Wistar rats were translated in a reticulocyte cell free system, either directly or after hybridization with 500 ng of the purified SMR1 cDNA insert (S13) or 600 ng of pUC9 DNA. The in vitro translation products were electrophoresed in a $NaDodSO_4$ 12.5% polyacrylamide gel and autoradiographed.

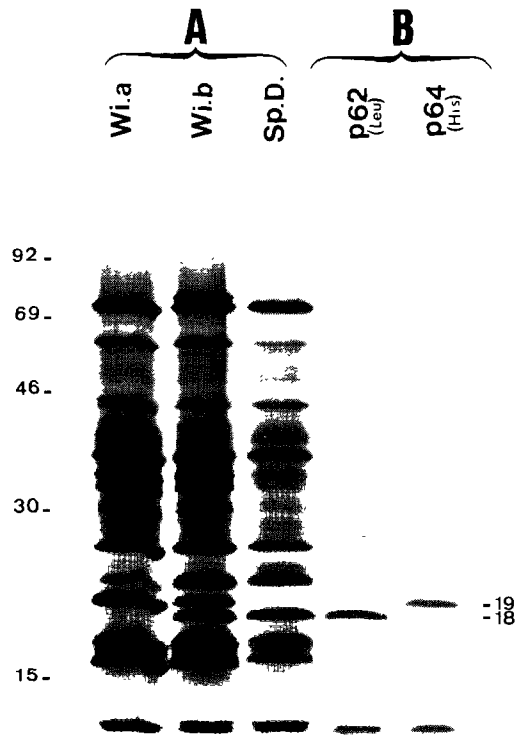


Fig. 4. In vitro transcription and in vitro translation of SMR1 cDNA with the Leu or His codons in position 114. Five μg of p62 or p64 (SMR1 cDNAs with the Leu codon or His codon, respectively, in position) were transcribed as described in section 2. The RNAs were translated in a reticulocyte cell free system and electrophoresed (B) in a $NaDodSO_4$ 12.5% polyacrylamide gel in parallel with the in vitro translation products of SMG RNAs prepared from male Wistar (Wi.a and Wi.b) or Sprague-Dawley (SpD) rats (A).

Sequencing of two SMR1 genomic clones had revealed the presence of either a histidine (CAT codon, identical to the codon found in the cloned cDNA) or a leucine (CTT codon) in position 114 (unpublished data). Since this change affects one of the amino acids of the synthetic peptide used to raise our antibodies, we have investigated the possibility that the 18 kDa polypeptide corresponds to the SMR1 form with a leucine in position 114. In order to clone the corresponding cDNA, polymerase chain reaction was used to amplify cDNAs corresponding to an mRNA preparation from Wistar rats, encoding after in vitro translation both the 18 and 19 kDa polypeptides. The products of the PCR were cloned in a psp64 vector, containing the sP6 promoter. Five different recombinant clones were sequenced. Two of them corresponded to the SMR1 sequence with a leucine in position 114, the three others have a histidine at the same position indicating that the detection of the 18 and the 19 kDa polypeptides is correlated with the presence of the two forms of SMR1 cDNAs. One representative clone of each form was transcribed from the sP6 bacteriophage promoter. The RNAs were translated in a reticulocyte cell free system and the in vitro translation products were analysed by $NaDodSO_4$ polyacrylamide gel electrophoresis. As shown in Fig. 4, the 18 and 19 kDa polypeptides correspond to SMR1 forms with a leucine or a histidine, respectively, in position 114. This demonstrates that SMR1 is present in all studied rat laboratory strains. It is rather surprising that only one amino acid replacement gives rise to a visible change in electrophoretic mobility. SMR1 abnormal electrophoretic mobility may probably be attributed to its relatively high content (12%) in proline residues, since a number of proline-rich proteins also do not migrate according to their molecular mass in $NaDodSO_4$ polyacrylamide gels [9,10].

In some Wistar rats the two forms of SMR1 are translated in the same amounts but each is about half as abundant as the 19 kDa or the 18 kDa present in rats harbouring only a single form. In addition, the chromosomic genes encoding two forms of SMR1 have overlapping restriction patterns. This suggests that the two forms of SMR1 are allelic.

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