Asian Journal of Andrology (2010) 12: 422–430

www.nature.com/aja

Original Article

POSVP$_{21}$, a major secretory androgen-dependent protein from sand rat seminal vesicles, identified as a transgelin

Naïma Kaci-Ouchfoun[1], Anne Incamps[2], Fatima Hadj-Bekkouche[3], Mohamed Cherif Abbadi[4], Laurent Bellanger[2], Thérèse Gernigon-Spychalowicz[1]

[1]*Laboratory of Arid Areas, Biological Sciences Institute, USTHB, BP 132 El Alia, 16100 Algiers, Algeria*
[2]*Department of biochemistry and nuclear toxicology (SBTN), CEA Marcoule BP 17171. F-30207 Bagnols-sur-Cèze, France*
[3]*Laboratory of Biology and Organisms Physiology, USTHB, BP 132 El Alia, 16100 Algiers, Algeria*
[4]*Department of Immunology, Algerian Pasteur Institute, 16000 Algiers, Algeria*

Abstract

The seminal vesicles of adult sand rat contain a major secretory protein band (MW 21 kDa) designated as *Psammomys obesus* seminal vesicles protein of 21 kDa (POSVP$_{21}$). This protein is abundant in secretions, regulated by androgens and also present in the vaginal plug. POSVP$_{21}$ accounts for over 22.3% of soluble proteins from homogenate during the breeding season, 13.3% during the middle season and 5.3% during the hormonal regression season. It is absent during the non-breeding season. POSVP$_{21}$ is localized in the cytoplasm of epithelial cells and in secretory products in the lumen. It presents an immunological homology with two epididymal proteins with the same molecular weight and a high degree of homology with transgelin from rat (*Rattus norvegicus*).

Asian Journal of Andrology (2010) 12: 422–430. doi: 10.1038/aja.2010.8; published online 19 April 2010.

Keywords: androgen-dependent, *Psammomys obesus*, seminal vesicles, transgelin

1 Introduction

The seminal vesicles present in most adult male mammals secrete a group of proteins that constitute a major portion of seminal plasma [1]. The biological significance of these proteins in mammalian reproductive physiology is currently obscure. Seminal vesicle secretions influence fertility because ablation of the seminal vesicles leads to a great decrease in the fertility of several species of rodents, such as the rat [2] and the mouse [3, 4].

In rodents, the formation of the vaginal plug is often considered to be a major role of the accessory glands. Removal of the seminal vesicles has been shown to alter the motility of uterine sperm, which has the potential to alter sperm transport in the female reproductive tract, thus reducing the chances of fertilization [4].

It is well established that the accumulation of seminal vesicle secretions, which contain a group of proteins that constitute the major protein components of seminal plasma, becomes prominent in the post-pubertal period [5]. Seminal plasma, which facilitates the transport of sperm in the female genital tract, also contains factors

Correspondence to: Dr Naïma Kaci-Ouchfoun, Laboratory of Arid Areas, Biological Sciences Institute, USTHB, BP 132 El Alia, 16100 Algiers, Algeria.
Fax: + 213-2124-7217 E-mail: kacinaima@yahoo.fr
Received: 2 October 2009 Revised: 26 December 2009
Accepted: 2 February 2010 Published online: 19 April 2010

that influence sperm motility [6] and fertility [7, 8]. The rodent has proved to be a good experimental model for the study of this system, and some of the protein components of rat and mouse seminal vesicle secretions have been purified and identified [9, 10].

Furthermore, the seminal vesicles provide the structural proteins of the copulatory plug [11]. In the rat, each of the five most abundant proteins (proteins I–V) of seminal secretion is a structural protein of the copulatory plug [12].

The genetic expression of some of these proteins has been shown to be dependent on the presence of testosterone [13]. The mechanism by which testosterone regulates the expression of androgen-dependent proteins has been investigated; in the mouse, it has been demonstrated that androgens act mainly on Mouse seminal vesicle secretory protein of 99 aminoacids (MSVSP99) gene transcription, and that this transcriptional regulation is exerted by steroid-receptor complexes interacting with enhancer sequences that are adjacent to the gene [14].

Finding the structure and function of these proteins has been attempted in order to understand their roles in seminal vesicle physiology and their effects on gamete activity.

In the seminal vesicles of the Saharian rodent *Psammomys obesus*, one major protein with an apparent molecular weight of 21 kDa, designated $POSVP_{21}$ (i.e., *P. obesus* seminal vesicle protein of 21 kDa), is present in the seminal vesicle homogenate and is found in seminal vesicle secretions and in the vaginal plug during the breeding season. During the non-breeding season, the amount of $POSVP_{21}$ is greatly reduced. The expression of this protein has been shown to be dependent on the presence of testosterone; the expression of the 21-kDa protein is induced by testosterone, and levels of this protein decrease most dramatically after castration [15].

$POSVP_{21}$ is localized in the cytoplasm of the epithelial cells of the seminal vesicles and in the secretory products in the lumen during the breeding season [16].

The purpose of this work was to identify the sequence of $POSVP_{21}$ and elucidate the role of this secretory protein in reproduction.

Here, we report the partial analysis of the identified peptide sequences of the seminal vesicle secretory protein ($POSVP_{21}$) and two epididymal proteins (P_{21Ep} and P_{21Ed}) of *P. obesus*, all with the same apparent molecular weight of 21 kDa.

2 Materials and methods

2.1 Animals

The sand rat is a diurnal rodent that lives around wadis in the Sahara desert. Adult animals were trapped in the wild in the regions of Béni Abbès (30°07′N, 2°10′W) and M'sila (35°N, 4°E) and killed 24–48 h later to study the proteins secreted by the seminal vesicles and other secretory organs of the male genital tract. Excised tissues were fixed or stored at –80ºC for RNA or protein extraction.

2.2 Extraction of proteins from adult sand rat seminal vesicles and epididymides

In normal adult males, proteins obtained either from secretions or from homogenized tissues were used. Seminal vesicles were homogenized for 30 s at 4ºC in 2 or 4 mL buffer A, in a glass–glass hand-held homogenizer (Braun, Melsungen, Germany). After centrifugation at 12 000 × *g* for 10 min at 4ºC, the supernatant fluid was retained and used as 'homogenate'. The amount of protein in the samples was determined by the micro method procedure of Bradford assay [17].

2.3 Protein gel electrophoresis

One-dimensional electrophoresis was performed under denaturing conditions [18] and carried out on slab gels (140 mm × 120 mm × 1.5 mm) using a Biorad model 220; sodium dodecyl sulphate protein samples (100 μg) were applied to 15% resolving gels with 4.5% stacking gel and run at 20 mA at room temperature until the tracking dye (Bromophenol blue) reached the bottom of the gels. The gels were then stained with 0.25% (w/v) Coomassie blue in an aqueous solution containing 50% (v/v) methanol and 10% (v/v) acetic acid for 45 min at room temperature and destained in a solution without dye. The apparent molecular weights of the proteins were calculated using the mobility of standard proteins as a reference.

2.4 Immunohistochemistry

The seminal vesicles were fixed in a buffered solution of 10% formalin for about 24 h, dehydrated in a graded series of ethanol and embedded in paraffin. Sections were cut at a thickness of 5 μm and mounted on a poly-*L*-lysine-coated glass slide. The sections

were exposed to immunohistochemical staining by an avidin–biotin complex technique (Dako Cytomation, Carpinteria, CA, USA). Endogenous peroxidase was inactivated with 3% hydrogen peroxide (H_2O_2) in H_2O for 10 min. Subsequently, the sections were washed in PBS (pH 7.4) and then incubated with normal goat serum (blocking reagent) (Dako Cytomation) for 45 min, followed by overnight incubation with the primary antibody diluted 1:200 at 4ºC. After washing, the slides were incubated with biotinylated anti-rabbit antiserum (Dako Cytomation) for 20 min. An avidin–biotin–peroxidase complex (Dako Cytomation) was then applied for 20 min. The binding sites of the primary antibody were visualized with diaminobenzidine (DAB) and H_2O_2 solution (0.12 g DAB in 240 mL of PBS, pH 7.4, containing 3% H_2O_2) for 5 min. Slides were contrasted with haematoxylin and mounted with 1,3-diethyl-8-phenylxanthine. As a negative control, samples were incubated with normal goat serum instead of the primary antibody, with the remaining procedures being the same. The images obtained by this technique were taken with a microscope digital camera system.

2.5 Two-dimensional electrophoresis (2-DE)

2-DE was carried out using non-equilibrium pH gradient gel electrophoresis in the first dimension as described by O'Farrell [19]. For the first dimension, 7 cm immobilized pH gradient (IPG) strips (pH 3–10; Amersham Biosciences, Pantin Cedex, France) were rehydrated with 75 μg of protein for 2 h in the solubilization buffer, 1% (w/v) dithiothreitol (DTT) and 1% IPG buffer. Focusing was carried out in a Bio-Rad protean IEF Cell system (Bio-rad, USA) for a total of 80 000 V h. For the second dimension, after reduction and alkylation, the strips were loaded onto a 13.5% (w/v) acrylamide gel using a Bio-Rad Protean 2XI Cell system (Bio-rad, USA). Gels were stained with Coomassie Blue G-250 [20], scanned at 300 dpi on an Umax Scanner (Pharmacia, Bedford, MA, USA) and analysed using Melanie 4.0 Software (GeneBio, Geneva, Switzerland). Three replicate gels of each sample were analysed.

2.6 Immunoblotting

Western blotting of polyacrylamide gels onto nitrocellulose sheets was carried out according to Towbin [21]. After 1-D electrophoresis, proteins were electroblotted onto a polyvinylidene difluoride membrane (Immobilon Psq, Millipore, Bedford, MA, USA) using a semi-dry transfer blotter (Bio-Rad) and visualized by Coomassie Brilliant Blue staining.

2.7 Mass spectrometry analysis

Bands of interest were subjected to tryptic digestion using a procedure previously described [22]. Protein bands were excised from the gel and destained with a solution of 25 mmol L^{-1} ammonium bicarbonate for 30 min and then with a 1:1 (v/v) solution of acetonitrile and 25 mmol L^{-1} ammonium bicarbonate for 30 min. Washes were repeated until the blue colour of Coomassie was removed. Bands were then dried by vacuum centrifugation and rehydrated in 10 mmol L^{-1} DTT in 25 mmol L^{-1} ammonium bicarbonate solution for 1 h at 56ºC. The supernatant was removed and the bands were incubated in the dark for 45 min with 55 mmol L^{-1} iodoacetamide in 25 mmol L^{-1} ammonium bicarbonate. Bands were washed as previously described, dried completely in a Speed Vac Plus (Savant, NY, USA) for 30 min and enzymatically digested overnight using sequencing-grade trypsin (Promega, Madison, WI, USA).

Coomassie Brilliant blue, SDS and salts were removed from the protein sample after passive elution using a $ZipTip_{HPL}$ (hydrophilic interaction chromatography) according to the manufacturer's instructions (Millipore, Bedford, MA, USA). Briefly, the $ZipTip_{HPL}$ was rehydrated in buffer A (H_2O/ACN/AcOH: 50/50/0.1, pH 5.5) and washed with buffer B (H_2O/ACN/AcOH: 10/90/0.1, pH 5.5). Protein eluates were diluted in 200 μL of buffer A and proteins were eluted in 4 μL of H_2O/ACN/TFA (50/50/0.1). NanoLC-MS/MS analyses were performed on an ion trap mass spectrometer (LTQ-Orbitrap-XL, Thermo Fischer, USA) with nano-HPLC Ultimate 3000 (Dionex, France). Peptide sequences were analysed using Mascot software (Matrix Science, London, UK) and the SwissProt database.

2.8 Database searching

MS/MS fragmentation was compared using Mascot software with sequences in the SwissProt database and the NCBInr database.

The reliability of protein identifications was evaluated on the basis of multiple variables, including the Mascot score, the number of peptide matches, mass error (*m/z* accuracy), percent coverage of the matched protein, similarity of experimental and theoretical protein molecular weights (Mr) and isoelectric points (pIs), and degree of phylogenetic divergence of the species from which the sequence was matched.

3 Results

3.1 Isolation and purification of POSVP21

Polyacrylamide gel electrophoretic analysis of soluble proteins from breeding season and non-breeding season sand rat homogenates shows seasonal variations in $POSVP_{21}$ expression. $POSVP_{21}$ is produced in large amounts in autumn and winter (lanes b and c) and production decreases most dramatically in spring and summer (lanes d and e).

3.2 Quantification of POSVP21

$POSVP_{21}$ accounts for 22.3% of the soluble proteins in the homogenate during the breeding season (autumn) (Figure 1 Lane b), 13.3% during the middle season (early spring) (Figure 1 Lane c) and only 5.14% during the hormonal regression season (Figure 1 Lane d) (late spring). It is absent during the non-breeding season (early summer) (Figure 1 Lane e).

This comparative study shows a 9% reduction in the rate of $POSVP_{21}$ production between the early breeding season (autumn) and the middle season (early spring). This reduction is more dramatic (17.2%) between the early breeding season (autumn) and hormonal regression season (late spring) (Figure 1 Lane d)); this rate is reduced to zero during the non-breeding season (Figure 1 Lane e).

The densitometric analysis of electrophoretic profiles showed that during the breeding season, $POSVP_{21}$ accounts for 22% of all soluble proteins in the homogenate (Figure 2a). In secretions, proteins of 21 kDa represent 13% of all soluble proteins (Figure 2b).

Both a comparative study of the proteomic profiles (Figure 2A) and a densitometric analysis of the homogenate and secretions of the seminal vesicles (Figure 2B) reveal that the majority of 21-kDa proteins

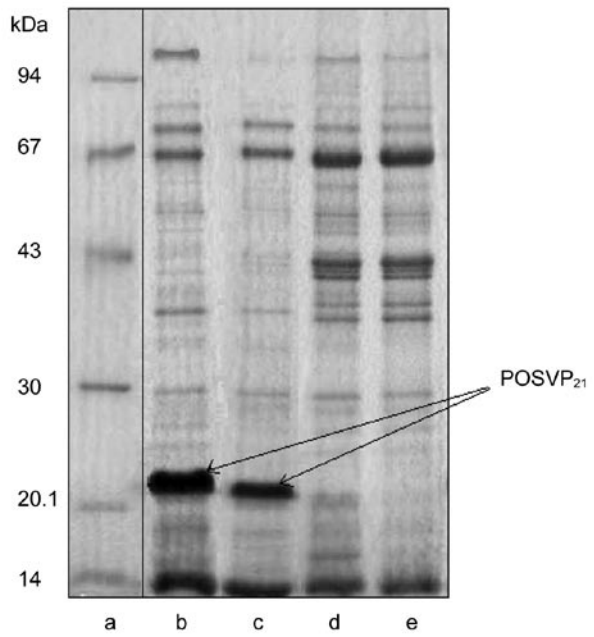

Figure 1. Polyacrylamide gel electrophoretic analysis of soluble proteins from adult sand rat seminal vesicles, including protein components of soluble proteins from homogenate (seminal vesicles and secretions) samples collected in different seasons. A suitable amount of protein was subjected to SDS-PAGE on a 15% slab gel (140 mm × 120 mm × 1.5 mm). Each lane was loaded with 100 μg protein. Proteins were stained with Coomassie blue. The arrows indicate the position of the 21-kDa protein. (Lane a): Protein standards: phosphorylase B (94 kDa); bovine serum albumin (67 kDa); ovalbumin (43 kDa); carbonic anhydrase (30 kDa); soybean trypsin inhibitor (20.1 kDa); a lactalbumin (14 kDa). (Lanes b and c): Proteins from the homogenate of the adult sand rat seminal vesicles from animals caught during the breeding season (early autumn and early spring). (Lane d): Proteins from the homogenate of the adult sand rat seminal vesicles from animals caught during the regression hormonal season (late spring). (Lane e): Proteins from the homogenate of the adult sand rat seminal vesicles from animals caught during the non-breeding season (early summer).

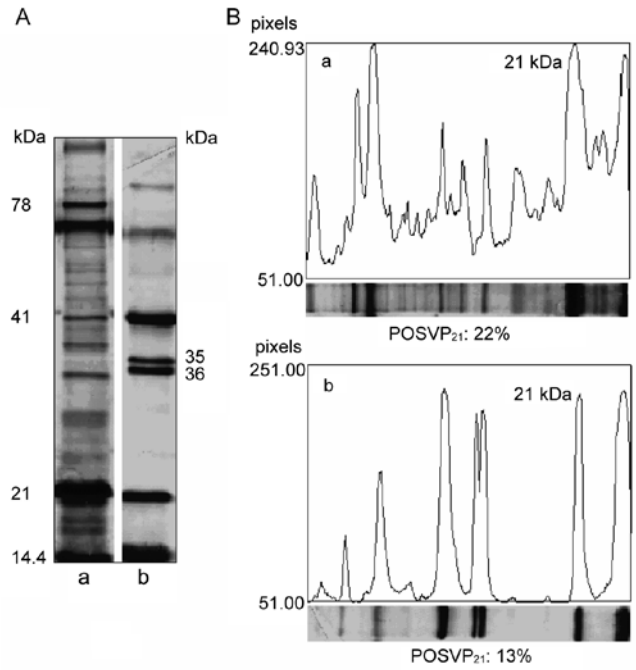

Figure 2. Polyacrylamide gel electrophoretic analysis (A) and densitometric analysis (B) of soluble proteins from adult sand rat seminal vesicles. Protein components of soluble proteins from seminal vesicle homogenate (a) and secretions (b).

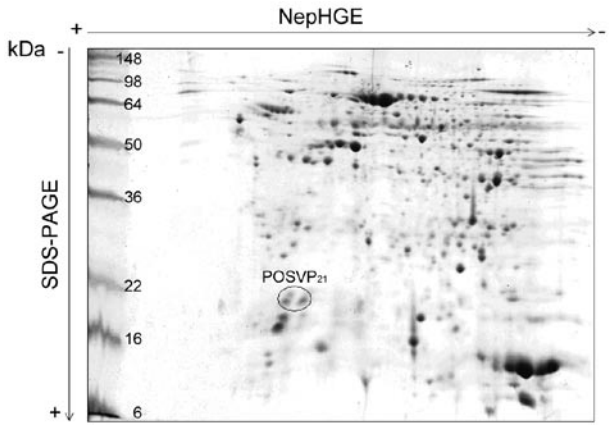

Figure 3. 2-D electrophoretic separation of soluble proteins from sand rat seminal vesicle homogenate during the breeding season. Proteins were stained with Coomassie Blue.

present in the homogenate are secreted (13%).

3.3 Characterization and identification of $POSVP_{21}$, P_{21Ep} and P_{21Ed}

$POSVP_{21}$ seems to be composed of three closely separated spots (Figure 3). The pH values of these spots ranged from 4 to 7.

The comparison of peptide sequences generated by the Mascot software (Swiss-Prot data bank) allowed a partial identification of $POSVP_{21}$.

All of the peptide sequences obtained from $POSVP_{21}$ showed homology with the peptide sequence of the *R. norvegicus* transgelin protein, with 62% homology.

Among these sequences, nine showed homology with the calponin domain (characterizing proteins that can bind actin) of the *R. norvegicus* transgelin protein, with 77% homology. The calponin domain is located between AA24 and AA139 (Figure 4).

Our results suggest that $POSVP_{21}$ is a transgelin protein in the sand rat (*P. obesus*).

3.4 Comparative analysis of $POSVP_{21}$, P_{21Ep} and P_{21Ed}

Before drawing any conclusions regarding the role of $POSVP_{21}$ at the level of the male genital tract in *P. obesus,* an investigation was performed to check whether the epididymal proteins P_{21Ep} (the 21-kDa protein of the proximal epididymis) and P_{21Ed} (the 21-kDa protein of the distal epididymis) would show some other characteristics similar or identical to those of $POSVP_{21}$, in addition to their electrophoretic properties.

Thus, our results suggest that $POSVP_{21}$ is a transgelin protein in the sand rat. The existence of an immunological homology with $POSVP_{21}$ was first investigated, and then structural homologies between these proteins were assessed. For this purpose, the same methods were used.

3.5 Localization of P_{21Ep} and P_{21Ed}

Research to discover a possible immunological homology between P_{21Ep}, P_{21Ed} and $POSVP_{21}$ was performed using anti-$POSVP_{21}$ antibodies with Western blotting and immunohistochemistry.

Immunological homology was consolidated by the study of immunolocalization, along with the determination and analysis of the P_{21Ep} and P_{21Ed} peptide sequences.

The immunohistochemical study showed a reactivity of anti-$POSVP_{21}$ antibodies with respect to the P_{21Ep} and P_{21Ed} located in the peripheral area of the epididymal channel (proximal and distal) at the level of the epithelium and the ECM (Figure 5).

The tissue and cellular immunolocalizations of P_{21Ep} and P_{21Ed} consolidate the immunological homology between these two compounds and $POSVP_{21}$.

3.6 Analysis of P_{21Ep} and P_{21Ed} peptide sequences

The determination and analysis of P_{21Ep} and P_{21Ed} peptide sequences showed 41% and 48% homology, respectively, on the peptide sequence of *R. norvegicus* transgelin (Figure 6).

We also noted that all of the peptide sequences identified from P_{21Ep} and P_{21Ed} are found in the $POSVP_{21}$ sequence.

In conclusion, $POSVP_{21}$, P_{21Ep} and P_{21Ed} are immunologically related, and their identified peptide sequences showed a high degree of homology with *R. norvegicus* transgelin.

Our results show that the isolated proteins $POSVP_{21}$, P_{21Ep} and P_{21Ed}, located at the level of the seminal vesicles, the proximal epididymis and the distal epididymis, respectively, seem to correspond to the same protein: the transgelin proteins.

4 Discussion

The analysis of $POSVP_{21}$ peptide sequences reveals a strong homology with the *R. norvegicus* transgelin peptide sequence. Indeed, all of the 14 peptide

```
  1 MANKGPSYGM SREVQSKIEK KYDEELEERL VEWIVMQCGP DVGRPDRGRL  POSVP21
    MANKGPSYGM SREVQSKIEK KYDEELEERL VEWIVMQCGP DVGRPDRGRL  TGLN R.n

 51 GFQVWLKNGV ILSKLVNSLY PEGSKPVKVP ENPPSMVFKQ MEQVAQFLKA
    GFQVWLKNGV ILSKLVNSLY PEGSKPVKVP ENPPSMVFKQ MEQVAQFLKA

101 AEDYGVTKTD MFQTVDLFEG KDMAAVQRTV MALGSLAVTK NDGHYRGDPN
    AEDYGVTKTD MFQTVDLFEG KDMAAVQRTV MALGSLAVTK NDGHYRGDPN

151 WFMKKAQEHK REFTDSQLQE GKHVIGLQMG SNRGASQAGM TGYGRPRQII
    WFMKKAQEHK REFTDSQLQE GKHVIGLQMG SNRGASQAGM TGYGRPRQII

201 S
    S
```

Figure 4. Homologies between the amino-acid sequence from POSVP$_{21}$ fragment and the sequence from rat *R. norvegicus* transgelin. The identical amino acids are presented in bold with other sequences aligned to show homology. *TGLN R.n*: transgelin from *R. norvegicus*.

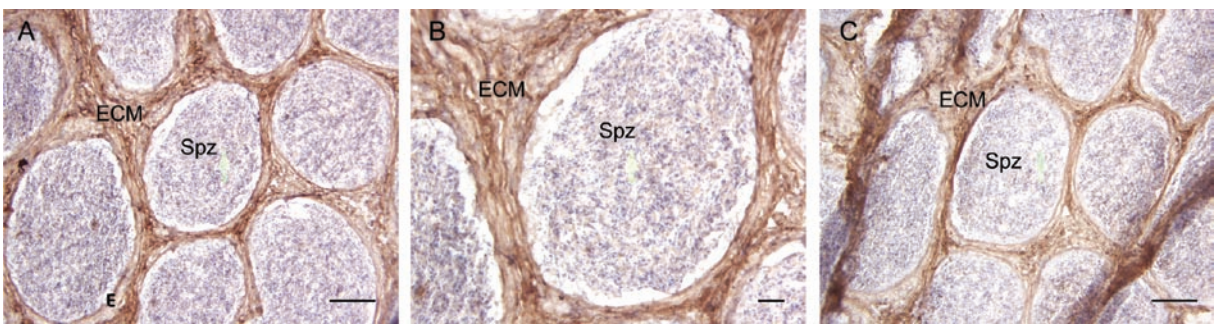

Figure 5. Immunohistochemical localization of P_{21Ep} and P_{21Ed} in various sections of mature sand rat epididymides. (A, B): Proximal epididymis; (C): distal epididymis. The sections were photographed at magnifications of 250 (A); 400 (B); and 250 (C). Spz: spermatozoa; E: epithelium; ECM: extracellular matrix. Scale bars = 20 μm.

A

```
  1 MANKGPSYGM SREVQSKIEK KYDEELEERL VEWIVMQCGP DVGRPDRGRL
 51 GFQVWLKNGV ILSKLVNSLY PEGSKPVKVP ENPPSMVFKQ MEQVAQFLKA
101 AEDYGVTKTD MFQTVDLFEG KDMAAVQRTV MALGSLAVTK NDGHYRGDPN
151 WFMKKAQEHK REFTDSQLQE GKHVIGLQMG SNRGASQAGM TGYGRPRQII
201 S
```

B

```
  1 MANKGPSYGM SREVQSKIEK KYDEELEERL VEWIVMQCGP DVGRPDRGRL
 51 GFQVWLKNGV ILSKLVNSLY PEGSKPVKVP ENPPSMVFKQ MEQVAQFLKA
101 AEDYGVTKTD MFQTVDLFEG KDMAAVQRTV MALGSLAVTK NDGHYRGDPN
151 WFMKKAQEHK REFTDSQLQE GKHVIGLQMG SNRGASQAGM TGYGRPRQII
201 S
```

Figure 6. Identified peptide sequences. (A): From P_{21Ep} (the 21-kDa protein of the proximal epididymis); (B): from P_{21Ed} (the 21-kDa protein of the distal epididymis). Amino acids identical to those in *Rattus norvegicus* transgelin are presented in bold, with other sequences aligned to show homology.

sequences identified at the level of $POSVP_{21}$ are also present at the level of the transgelin peptide sequence that covers 62% of the transgelin protein sequence, which accounts for two-thirds of the total sequence of transgelin.

Transgelin is characterized by the presence of a calponin domain or a CH domain (calponin homology domain), which constitutes the actin-binding domain and includes approximately 100 amino acids.

The presence of the calponin domain in the peptide sequence of $POSVP_{21}$ (between AA24 and AA139) suggests that this protein also exhibits actin-binding activity. Indeed, among the sequences identified in $POSVP_{21}$, nine match the calponin domain of *R. norvegicus* transgelin. Calponin and transgelin are known to be expressed in a synchronous way during the cellular differentiation of smooth muscle [23]. The sequence analysis of purified transgelin gives a single amino-acid sequence (KGPSYGMSREV), with a starting signal of 6 pmol. This sequence seems to be unique [24].

In a previous study [16], translation of whole RNA from seminal vesicles revealed one major band of 21 kDa with the same molecular weight. $POSVP_{21}$ mRNA produced the same product (at 21 kDa). This result indicates that the signal peptide involved in the secretion or transport of this protein has a low molecular weight or does not exist, as shown in mouse MVDP [25]. These observations raise the question of the manner in which $POSVP_{21}$ is exported from the epithelial cells to the luminal fluid of seminal vesicles. We hypothesize that $POSVP_{21}$ must be secreted via an apocrine secretion mode, as demonstrated with MVDP [23].

R. norvegicus transgelin shows 96% homology with the human transgelin peptide sequence; however, the amino acids that distinguish it from human transgelin (AA7, AA35, AA36, AA37 and AA 165) are well preserved in the $POSVP_{21}$ peptide sequence. The sequence of $POSVP_{21}$ seems identical to both the *R. norvegicus* transgelin and to the human transgelin.

After characterizing and identifying $POSVP_{21}$, we showed the existence of an immunological parenthood between $POSVP_{21}$ and two epididymal proteins, P_{21Ep} and P_{21Ed}, of identical apparent molecular weight. Similar to $POSVP_{21}$, these proteins are also present in the epithelium and in the ECM of the epididymides. The existence of an immunological parenthood between P_{21Ep}, P_{21Ed} and $POSVP_{21}$ suggests that the proteins P_{21Ep} and P_{21Ed} are identical or similar to the protein $POSVP_{21}$.

$POSVP_{21}$ could thus have two distinct sites of synthesis, one vesicular and one epididymal. The degree of this immunological parenthood was determined when we identified and analyzed P_{21Ep} and P_{21Ed} peptide sequences. The sequence of these peptides also showed, as it did for $POSVP_{21}$, sequence homology with the *R. norvegicus* transgelin (P_{21Ep}: 41%) and (P_{21Ed}: 48%). Therefore, the three 21-kDa proteins found in the seminal vesicle ($POSVP_{21}$) and in the epididymis (P_{21Ep} and P_{21Ed}) of *P. obesus* are related to *R. norvegicus* transgelin.

Transgelin was identified for the first time in 1987 at the level of the smooth muscular cells in the chicken gizzard [26]. It is a 22-kDa protein, known as smooth muscle protein 22 (SM22) [27].

In humans, the transgelin gene was identified and localized at the level of chromosome 11q23.2 [28]. Its expression during embryogenesis constitutes one of the early specific markers of the differentiation of smooth muscular cells [26, 29, 30].

The peptide sequence of SM22α was identified in several mammal species; in mice, this sequence consists of 204 amino acids. Its identity sequence is 98%, 97% and 84% similar to the transgelin peptide sequences of rats, humans and chickens, respectively [29].

SM22α is thought to have a role in contractility, probably by helping organize actin filaments [31]. Transgelin is present only in the smooth muscle cells of many vertebrate adults. It belongs to the family of proteins that bind actin and is involved in its polymerization [29].

Recent studies have shown that the transgelin we have identified in smooth muscles and blood vessels is also found in the fibroblasts and in some epithelia such as the intestinal epithelium, the epithelium of the mammary channel [32], the glomerular epithelium [33] and the prostate epithelium [34].

These data support our conclusion because with *P. obesus* we found transgelin in the epithelium, both in the seminal vesicle and at the level of the epididymis.

Because we have found transgelin and localized this protein to the epithelium and to the secretion of sand rat seminal vesicles, we suggest that there is a passage through an external medium that, we believe, is involved in the organization of the epithelial cell cytoskeleton. This allows it to maintain the structure and the contractile state of the seminal vesicle.

The specific proteins that we found in the secretions of the seminal vesicles constitute the essential components of the seminal plasma and are involved in com-

plex processes such as the coagulation and liquefaction of seminal plasma [35–37], the formation of a protein layer around spermatozoa leading to some modifications at the level of their thin structure and mobility [38], and the immunosuppressant effect of seminal plasma [39].

Apart from their role in the male genital tract, the secretions of seminal vesicles have a role in the female genital tract, where they mediate stimulation of the motility of smooth muscles [40].

With *P. obesus*, the 21-kDa proteins constitute one major component of the genital female tract coagulum [15]. The importance of coagulum proteins in the progression of spermatozoa along the female genital tract was shown by Cukierski [41]; these proteins also have a role in the stability of spermatozoa chromatin [42]. In the coagulum, the absence of the 21-kDa protein (transgelin) may have an impact on the motility of smooth muscle in the female genital tract, as well as on the mobility of spermatozoa and consequently on fecundity.

5 Conclusion

During the breeding season, *P. obesus* seminal vesicles produce an androgen-dependent protein with an apparent molecular weight of 21 kDa (POSVP$_{21}$).

POSVP$_{21}$ could have two distinct sites of synthesis, one vesicular and one epididymal. A hypothetical function for POSVP$_{21}$ is discussed in terms of the homology between this protein and *R. norvegicus* transgelin.

Further studies on the functional role of POSVP$_{21}$, P21$_{EP}$ and P21$_{Ed}$ would provide a possible relationship between these three proteins and *P. obesus* fertility.

Acknowledgment

We would like to thank F. Hely-Joly and I. Dany for their technical assistance and Dr W. Achwak (Laboratoire d'écologie microbienne de la rhizosphere et des environnements extrêmes [LEMIRe]/DEVM/CEA) for her help in proteomic analysis. We are grateful to E Labbe for assistance with English.

References

1 Mann T. The Biochemistry of Semen and of the Male Reproductive Tract. London: Methuen; 1964.
2 Queen K, Dhavuwala CB, Pierrepoint CJ. The effects of the removal of the various accessory sex glands on the fertility of male rats. J Reprod Fertil 1981; 62: 423–6.
3 Pang SF, Chow PH, Wong TM. The role of the seminal vesicles, coagulating glands and prostate gland on the fertility and fecundity of mice. J Reprod Fertil 1979; 56: 129–32.
4 Peitz B, Olds-Clarke P. Effects of seminal vesicle removal on fertility and uterine sperm motility in the house mouse. Biol Reprod 1986; 35: 608–17.
5 Huang YH, Chu ST, Chen YH. Seminal vesicle autoantigen, a novel phospholipid-binding protein secreted from luminal epithelium of mouse seminal vesicle, exhibits the ability to suppress mouse sperm motility. Biochem J 1999; 343: 241–8.
6 Acott TS, Hoskins DD. Bovine sperm forward motility protein. Partial purification and characterization. J Biol Chem 1978; 253: 6744–50.
7 Killian GJ, Chapman DA, Rogowski LA. Fertility-associated proteins in holstein bull seminal plasma. Biol Reprod 1993; 49: 1202–7.
8 Bellin ME, Oyarzo JN, Hawkins HE, Zhang H, Smith RG, *et al.* Fertility-associated antigen on bull sperm indicates fertility potential. J Anim Sci 1998; 76: 2032–9.
9 Ostrowski MC, Kistler MK, Kistler WS. Purification and cell-free synthesis of a major protein from rat seminal vesicle secretion. J Biol Chem 1979; 254: 383–90.
10 Yu LC, Chen JL, Tsai WB, Chen YH. Primary structure and characterization of androgen-stimulated autoantigen purified from mouse seminal vesicle secretion. Biochem J 1993; 296: 571–6.
11 Williams-Ashman HG. Regulatory features of seminal vesicle development and function. Curr Top Cell Regul 1983; 22: 201–75.
12 Fawell SE, Higgins SJ. Tissue distribution, developmental profile and hormonal regulation of androgen-responsive secretory proteins of rat seminal vesicles studied by immunocytochemistry. Mol Cell Endocrinol 1986; 48: 39–49.
13 Higgins SJ, Füller FM. Effects of testosterone on protein synthesis in rat seminal vesicles analysed by two dimensional gel electrophoresis. Mol Cell Endocrinol 1981; 24: 85–103.
14 Simon AM, Brochard D, Morel L, Veyssiere G, Jean C. The androgen-dependent mouse seminal vesicle secretory protein of 99 aminoacids (MSVSP99): regulation of mRNA and preliminary characterization of the promoter. J Steroid Biochem Mol Biol 1997; 61: 87–95.
15 Gernigon T, Berger M, Lecher P. Seasonal variations in the ultrastructure and production of androgen-dependent proteins in the seminal vesicles of a Saharian rodent (*Psammomys obesus*). J Endocrinol 1994; 142: 37–46.
16 Kaci-Ouchfoun N, Hadj-Bekkouche F, Abbadi MC, Gernigon-Spychalowicz T. Purification, preliminary characterization and immunohistochemical localization of POSVP21 in the sand rat (*Psammomys obesus*) seminal vesicles. Theriogenology 2008; 69: 525–35.
17 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the

principle of protein-dye-binding. Anal Biochem 1976; 72: 246–54.
18 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–5.
19 O'Farrell PZ, Goodman HM, O'Farrell PH. High resolution two dimensional electrophoresis of basic as well as acidic proteins. Cell 1975; 12: 1133–42.
20 Neuhoff V, Arold N, Taube D, Erhardt W. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. Electrophoresis 1988; 9: 255–62.
21 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of poteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350–4.
22 Sarry JE, Sommemer N, Sauvage FX, Bergoin A, Rossignol M, *et al*. Grape berry biochemistry revisited upon proteomic analysis of the mesocarp. Proteomics 2004; 4: 201–15.
23 Gimona M, Sparrow MP, Strasser P, Herzog M, Small V. Calponin and SM22 isoforms in avian and mammalian smooth muscle absence of phosphorylation in vivo. Eur J Biochem 1992; 205: 1067–75.
24 Shapland C, Hsuan JJ, Totty NF, Lawson D. Purification and properties of transgelin: a transformation and shape sensitive actin-gelling protein. J Cell Biol 1993; 121: 1065–73.
25 Manin M, Lecher P, Martinez A, Tournadre S, Jean C. Exportation of mouse vas deferent protein, a protein without signal peptide from mouse vas deferent epithelium: a model for apocrine secretion. Biol Reprod 1995; 52: 50–62.
26 Lees-Miller JP, Heeley DH, Smillie LB, Kay CM. Isolation and characterization of an abundant and novel 22-kDa protein SM22 from chicken gizzard smooth muscle. J Biol Chem 1987; 262: 298893.
27 Kim S, Lu MM, Ip HS, Pharmacek MS, Clendenin C. A serum response factor-dependant transcriptional regulatory program identifies distinct smooth muscle cell sublineages. Mol Cell Biol 1997; 17: 2266–78.
28 Camoretti-Mercado B, Forsythe SM, Lebeau MM, Espinosa R, Vieira JE, *et al.* Expression and cytogenetic localization of the human SM22 gene (TAGLN). Genomics 1998; 49: 452–7.
29 Li L, Miano JM, Cserjesi P, Olson EN. SM22α, a marker of adult smooth muscle, is expressed in multiple myogenic lineages during embryogenesis. Circ Res 1995; 78: 188–95.
30 Shanahan CM, Weissberg PL, Metcalfe JC. Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. Circ Res 1993; 73: 193–204.
31 Zeidan A, Swärd K, Nordström I, Ekblad E, Zhang JCL, *et al.* Ablation of SM22 alpha decreases contractility and actin contents of mouse vascular smooth muscle. FEBS Lett 2004; 562: 141–6.
32 Schields JM, Rogers-Graham K, Der C. Loss of transgelin in breast and colon tumours and in RIE Cells by ras deregulation of gene expression through raf-independent pathways. J Biol Chem 2002; 277: 9790–9.
33 Ogawa A, Sakatsume M, Wang XZ, Sakamaki Y, Tsubata Y, *et al.* SM22 alpha: The novel phenotype marker of injured epithelial cells in anti-glomerular basement membrane nephritis. Nephron Exp Nephrol 2007; 106: 77–87.
34 Yang Z, Chang YJ, Miyamoto H, Ni J, Niu YJ, *et al*. Transgelin functions as a suppressor via inhibition of ARA54-enhanced androgen receptor transactivation and prostate cancer cell growth. Mol Endocrinol 2007; 21: 343–58.
35 Lilja H. Cell biology of semenogelin. Andrology 1990; 22: 132–41.
36 De Lamirande E, Yoshida K, Yoshiike TM, Iwamoto T, Gagnon C. Semenogelin, the main protein of semen coagulum, inhibits human sperm capacitation by interfering with the superoxide anion generated during this process. J Androl 2001; 22: 672–9.
37 Malm J, Jonsson M, Frohm B, Linse S. Structural properties of semenogelin I. FEBS J 2007; 274: 4503–10.
38 Scheit KH, Kemme M, Aumüller G, Seitz J, Hagendorf G, *et al.* The major protein of bull seminal plasma biosynthesis and biological function. Biosci Rep 1988; 8: 589–608.
39 Wang ZJ, Zhang W. The role of seminal vesicles in male fertility. Zhonghua Nan Ke Xue 2007; 13: 636–8.
40 Clavert A, Gabriel-Robez O, Montagnon D. Physiological role of the seminal vesicle. Prog Reprod Biol Med 1985; 12: 80–94.
41 Cukierski MA, Sina JL, Prahalada S, Robertson RT. Effects of seminal vesicle and coagulating gland ablation on fertility in rats. Reprod Toxicol 1991; 5: 347–52.
42 Richthoff J, Spano M, Giwercman YL, Frohm B, Jepson K, *et al.* The impact of testicular and accessory sex gland functions on sperm chromatin integrity as assessed by the sperm chromatin structure assay (SCSA). Hum Reprod 2002; 17: 3162–9.