Cloning of a Syrian hamster cDNA related to sexual dimorphism: establishment of a new family of proteins**

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Abstract The clone FHG22, isolated from a female minus male subtracted cDNA library obtained from the sexually dimorphic Syrian hamster Harderian glands (HG) is 440 bp long with a 95 amino acids ORF, and hybridizes to a female HG-specific 0.6 kb mRNA. The FHG22 nucleotide and amino acid sequences are similar to the subunits from prostatein, uteroglobin, major cat allergen Fel dI (chain 1) and mouse salivary androgen binding proteins (subunit α). Therefore I propose that all those polypeptides belong to a common new family. The hamster genome has a single copy of the FHG22 gene, without homologous genes. FHG22 mRNA is also found in male and female parotid (higher levels in females) and submandibular glands, indicating a tissue and sex-dependent control of expression.

Key words: Sexual dimorphism; Subtractive cloning; Harderian gland; Steroid binding; Syrian hamster

1. Introduction

Sexual dimorphism is a phenomenon in which male and female individuals from the same species develop different phenotypes [1] both in reproductive and non-reproductive organs [2] due primarily to the androgen action on particular genes [2,3]. Sometimes sexual dimorphism appears in specific organs, such as the Harderian glands (HG) from Syrian hamsters [4,5]. The HG are endocrine/exocrine structures related to a retinal–pineal–gonadal axis, present in the orbital cavity of most Vertebrates and very well developed in rodents [5]. It has been demonstrated that androgens control the hamster HG sexual dimorphism [4,5], and that they act directly on the HG androgen receptors [6]. Androgen (and steroids) actions on different cell types are usually exerted on the transcription of genes from cell-specific networks [3,7]. Since these networks share some genes, we have used the hamster HG system to isolate genes with sex-specific expression that might also be involved in other cases of sexual differences and/or androgen action. We have applied the subtractive cloning technique [8] to generate cDNA libraries enriched in clones from mRNAs with higher levels in one cell population than in the opposite (male minus female HG or female minus male HG). The hamster cDNA clone FHG22, isolated from female HG, is similar to the subunits from several oligomeric secreted proteins, the functions of which have not yet been clarified: rat prostatein or prostatic steroid binding protein (PSBP) [9], uteroglobin (UG) [10] or Clara cell 10 KDa protein (CC10) [11] from different species, chain 1 of the major cat allergen Fel dI (FdI 1) and α subunit from the mouse salivary androgen binding proteins (msABP α) [12]. Sequence similarities between PSBP and UG [13] and between UG, FdI 1 and msABP α have been reported [11,14], while the genes for PSBP, UG and FdI 1 show a common structure [15,16,17,18,19]. In addition, it has been described that PSBP and msABP bind steroids [9,12,15], while UG/CC10 also binds other ligands [10,20,21]. However, this group of proteins presents a wide tissue distribution and a complex hormonal regulation [10,11,12,22]. FHG22 cloning from a female minus male hamster HG subtracted library provides a new element for the comparison and lead us to propose the establishment of a new gene/protein family.

2. Materials and methods

2.1. Isolation of RNA and poly(A)$^+$ RNA

Syrian hamsters (*Mesocricetus auratus*) were obtained from Charles River (Kingston, NY) and maintained under a controlled photoperiod (14:10 h, light:dark) and temperature (20 ± 2°C). Two males or two females were ether anesthesized and the different organs extracted from both, pooled and homogenized in guanidine isothiocyanate buffer. Total RNA was purified from each sample as described [6,23]. Poly(A)$^+$ RNA from HG was purified from total RNA samples obtained from either five males or five females, after heating at 65°C for 20 min and chromatography through oligo(dT) cellulose Type 3 (Collaborative Research Inc., Bedford, MA) following a standard protocol [23].

2.2. Construction of cDNA libraries

Male and female Syrian hamster HG cDNA libraries were prepared starting from 5 μg of poly(A)$^+$ RNA from the corresponding source. cDNA was synthesized using a commercial kit (Pharmacia LKB, Piscataway, NJ), with oligo(dT) as the primer for the reverse transcriptase (EC 2.7.7.49). Purified cDNAs were ligated to a one-hundred molar excess of non self-complementary *Bst*XI adaptors (Invitrogen Corp., San Diego, CA) and then separated from the non-ligated adaptors. 100 ng from each cDNA sample were then ligated to 200 ng of *Bst*XI-cut pcDNAII phagemid (Invitrogen Corp.), the cloning vector. After transformation of *Escherichia coli* XL1-Blue, about 1×10^6 recombinants were found in each cDNA library.

2.3. Construction of subtracted cDNA libraries and isolation of sex-specific cDNA clones

For the preparation of male minus female and female minus male hamster HG subtracted cDNA libraries, single-stranded DNA was initially prepared from amplificated male and female HG cDNA libraries coinfected with the helper phage R408 [8], treated with DNase-free RNase (EC 3.1.27.5) (Boehringer-Mannheim Biochemicals) and puri-

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**This article is dedicated to the memories of Armando Menéndez Peláez and Fernando Prieto Menéndez.

Abbreviations: CC10, Clara cell 10 kDa protein; FdI/FdI 1, major allergen of the domestic cat Fel dI/chain 1; HG, Harderian gland; msABP/msABPα, mouse salivary androgen bindind proteins/subunit α; ORF, open reading frame; PSBP, rat prostatic steroid binding protein or prostatein; UG, uteroglobin.

The nucleotide sequence for the FHG22 cDNA clone has been deposited at the EMBL database with accession no. Z66540.

SSDI 0014-5793(95)01294-X

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            -38     gctcatcctttgctaagtctgaaaacaaactgagcacc    -1

+1   ATG AAG CTG TCC CTG TGT CTT CTG TTG GTC ATC CTG GCT GTT    42
 1   MET Lys Leu Ser Leu Cys Leu Leu Leu Val Ile Leu Ala Val   14

43   CAT TGC TAT GAA GCT AAT GCT GCA AAC GTC TGT CCA GCA GTT    84
15   His Cys Tyr Glu Ala Asn Ala Ala Asn Val Cys Pro Ala Val   28

85   CTT TCT GTA AGC AAA TCT TTC CTA TTT GAC AAG GTG GAG AAA   126
29   Leu Ser Val Ser Lys Ser Phe Leu Phe Asp Lys Val Glu Lys   42

127  TTT GAG GCC TAT CTT CAG ACA TTT AAC GCA CCT CCA GAG GCT   168
43   Phe Glu Ala Tyr Leu Gln Thr Phe Asn Ala Pro Pro Glu Ala   56

169  GTT AAA GCA AAA GTG GAA GTG AAG AAA TGT ATA GAC AGC ACT   210
57   Val Lys Ala Lys Val Glu Val Lys Lys Cys Ile Asp Ser Thr   70

211  CTG AAC TAT TTA GAG AAA ATG GAA ATG GGA AAA ATA CTG GCA   252
71   Leu Asn Tyr Leu Glu Lys Met Glu Met Gly Lys Ile Leu Ala   84

253  GAA GTC GTT GGG TAT TGT AAA GGA ACA GAA AAC tga aac ATG   294
85   Glu Val Val Gly Tyr Cys Lys Gly Thr Glu Asn END  95

255  gct ctt cct ggt ctc cat tgc ttc tca cag ata aac TGA ctt   336

337  tccttgcccaatgtgaaggtttcaacgtcttgcactaataaattactctccttgc   391

392  atgttaaaaaa    402
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Fig. 1. Full-length nucleotide and deduced amino acid sequences from the hamster FHG22 cDNA clone. The nucleotide coding region is in capital letters, with the putative start of translation numbered as +1. The deduced amino acid sequence printed in boldface is also numbered (END: stop codon). The polyadenylation signal AATAAA is underlined. The arrow indicates the hypothetical cleavage site for the signal protease. The origin and end of the second ORF are indicated with underlined capital letters and the separation between codons has been maintained (the deduced amino acid sequence in the one-letter code is MALPGLHCFSQIN).

fied by repeated precipitations. 50 μg of ssDNA from each sample were sheared by sonication to render an average size of 0.5 kb and biotinylated using biotin aminocaproyl hydrazide (Calbiochem Corp., La Jolla, CA) and glutaraldehyde [8]. Biotinylated ssDNA from each sample was precipitated with 5 μg of intact ssDNA from the sample to be subtracted, resuspended in 10 μl of 0.5 M NaCl, 0.2% SDS, 2 mM EDTA, 50 mM HEPES pH 7.6, covered with mineral oil, heated for 3 min at 95°C and hybridized for three days at 65°C. To remove the common ssDNA clones, the hybridization mixture was diluted 10 times, treated with 10 μg of streptavidin (Calbiochem Corp.) and extracted three times with phenol/chloroform [8]. The aqueous phase material, containing the sample-specific clones, was precipitated and the whole extraction procedure repeated. For the conversion of ssDNA to dsDNA clones, a 17mer complementary to the T7 promoter from pcDNAII was phosphorylated, annealed, extended with Sequenase (EC 2.7.7.7) (USB Corp., Cleveland, OH) and ligated. After transformation, the number of recombinant clones was found to be 2×10^5 for the female minus male library and 5×10^4 for the male minus female library. For the identification of sex-specific cDNA clones, colonies from the subtracted cDNA libraries were inoculated in duplicate nitrocellulose filters, processed for colony hybridization [23] and hybridized either to male or to female total cDNA labelled with [^{32}P]dCTP by using a Random Priming kit (Boehringer-Mannheim Biochemicals). Clones showing differential hybridization to the male and female total cDNAs were used as probes to confirm the differential mRNA expression in Northern blots [6,23] containing male and female hamster HG RNA samples.

2.4. Northern and Southern analysis

RNA samples (30 μg each) from different hamster tissues were hybridized in Northern blots to the FHG22 probe in the presence of 40% formamide at 40°C as previously described [6,23]. The whole FHG22 fragment was used as probe, after labelling with [^{32}P]dCTP by random priming. For Southern analysis, genomic DNA was obtained from liver and HG samples of three hamsters (2 males and 1 female) by using the short protocol from Davis et al. [23]. Genomic DNA (15 μg per sample) was digested with type II restriction endonucleases (EC 3.1.24.4) and electrophoresed on a 0.7% agarose/Tris borate gel. The gel was then depurinated and the DNA transferred to a Duralose UV membrane (Stratagene Cloning Systems, La Jolla, CA) and crosslinked. The membrane was hybridized to the FHG22 probe in the presence of either 40% formamide at 42°C or 30% formamide at 32°C, washed and autoradiographed.

2.5. DNA sequencing and sequence analysis

FHG22 cDNA clones in the vector pcDNAII were sequenced from both ends by the dideoxy chain termination method [24] modified for double-stranded plasmid DNA. The sequence was thoroughly confirmed due to the sequencing of 13 additional FHG22 clones (see section 3.4). Nucleotide or amino acid sequences were compared to the GenBank/EMBL or SwissProt databases by using the FASTA program [25], whereas the sequence comparisons between the different cDNAs and polypeptides were done with the program GAP, both from the University of Wisconsin Package. The amino acid sequences alignment was done by using the program MULTALIN [26].

3. Results and discussion

3.1. Isolation and characterization of the FHG22 cDNA

The cDNA clone FHG22, isolated from a female minus male hamster HG subtracted cDNA library, was found to hybridize to female but not to male hamster HG total [^{32}P]cDNA in duplicate colony hybridization analysis. The FHG22 insert was further purified, labelled with ^{32}P and used as a probe in a Northern blot to estimate the mRNA levels in male and female hamster HG. The mRNA was found to be 0.6 kb long and highly expressed in female but not in male HG (see Fig. 4). Fig. 1 shows the total nucleotide sequence and the amino acid translation of the 285 bp long only plausible ORF, which is preceded by a 5′-untranslated region of 38 bp and followed by an 117 bp long 3′-untranslated region containing an UGA stop codon, an AAUAAA polyadenylation signal and the beginning of a

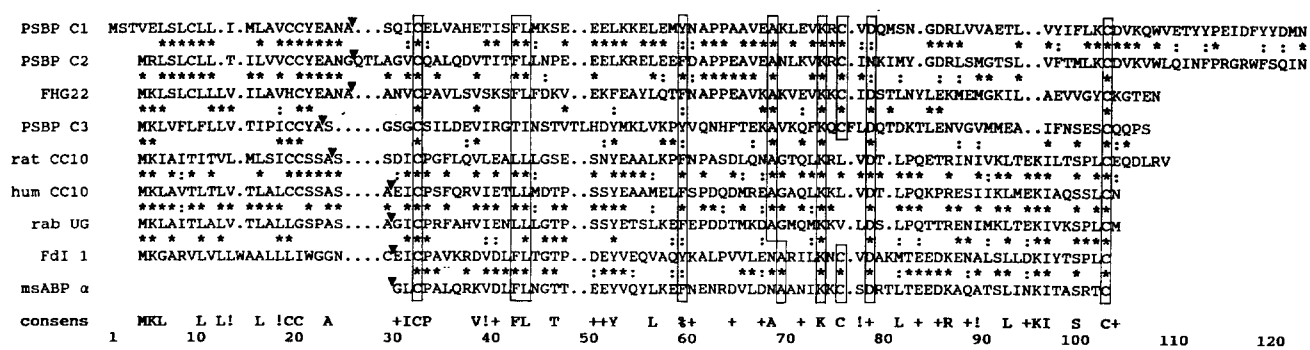

Fig. 2. Amino acid alignment for the sequences from FHG22 and the PSBP, UG/CC10, FdI 1 and msABP α subunits. The program MULTALIN has been applied to these sequences (see references in the text). Gaps have been introduced to optimize the comparison. The numbers refer to the overall consensus sequence, in boldface. Amino acid identities between neighbor sequences are marked '*', and conservative substitutions are marked ':'. Arrows indicate the end of the respective (or putative, for FHG22) signal peptides (see section 3.3). The cysteines in positions 32 and 102 and some other conserved residues have been boxed on the original display. Symbols in the consensus sequence refer to residues aliphatic (!), charged (+) or aromatic (%).

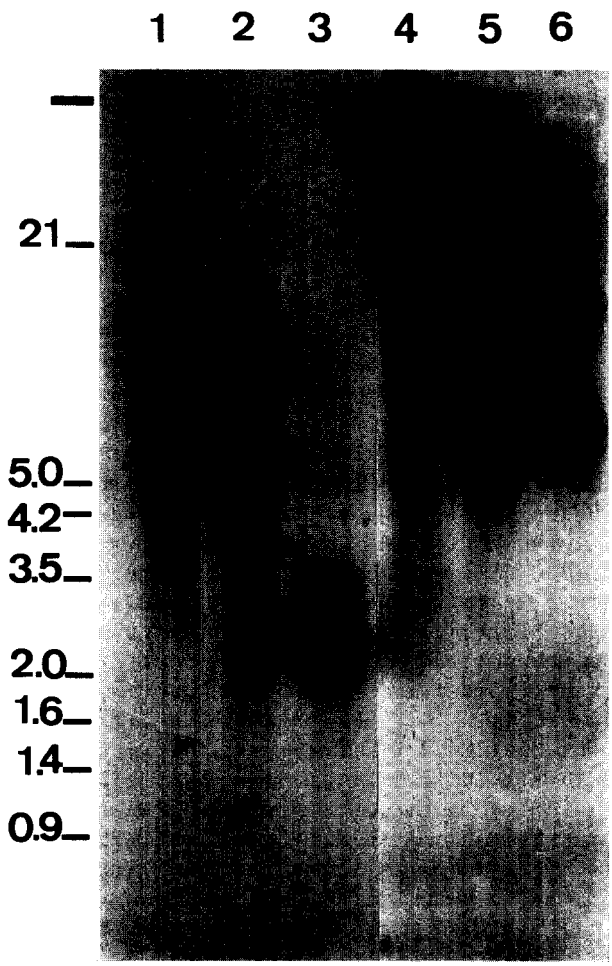

Fig. 3. Hamster genomic DNA hybridization to the FHG22 cDNA. Hamster genomic DNA (15 μg per sample) was treated with the following restriction enzymes: 1 = *Bam*HI; 2 = *Eco*RI; 3 = *Hin*dIII; 4 = *Kpn*I; 5 = *Pst*I; 6 = *Xba*I. A Southern blot was thereafter prepared and hybridized to a FHG22 probe at 42°C in the presence of 40% formamide, washed and autoradiographed. The same pattern was found under low stringency hybridization conditions (see section 3.4). The migration of the DNA markers in kbp is indicated.

poly(A)$^+$ tail. The sequences around the possible methionine initiation codon agree with the consensus reported by Kozak (CACCAUGA versus CACCAUGG) [27]. The putative polypeptide has 95 amino acid residues, with a M_r of 10,494. No consensus sequences for post-translational modifications or any other motif have been found. However, the first 21 amino acids could be a signal peptide for protein secretion, by comparison to similar polypeptides (see Fig. 2), and according to the requirements for the cleavage by the signal protease [28]. The hypothetical secretion product would have a M_r of 8,196. A second ORF coding for a 13 amino acids long peptide (MALPGLHCFSQIN), starts in the nucleotide 2923 bp after the UGA stop codon. Since this sequence is very similar to the carboxy-terminal end from the PSBP C2 subunit (see Fig. 2) and both FHG22 polypeptides are in the same frame, the nucleotide sequence was thoroughly checked in different FHG22 clones (see section 3.4) to exclude a sequencing or a reverse transcriptase mistake. Although the second peptide could be translated in vivo [27], the lack of significant homologies after a search in the Swissprot database [25] suggests that it does not play a physiological role. By comparing the sequences from Fig. 2, an appealing hypothesis is that the arrangement of FHG22 could reflect an evolution step by which a stop codon appeared and the last part of the polypeptide was initially separated and then lost.

3.2. *Comparisons of the FHG22 nucleotide and amino acid sequences to those from PSBP, UG, FdI and msABP subunits*

A search in the GenBank/EMBL sequence database revealed high identities between the FHG22 cDNA sequence and those for the rat PSBP C1 and C2 subunits [15] (see Table 1). Since PSBP is androgen-stimulated [29] and almost prostatespecific [22], FHG22 cannot be the cDNA for one of the hamster PSBP subunits, because it was isolated from female HG. As similarities between PSBP and UG [13] and between UG and other polypeptides [14] had been reported, individual comparisons were established between FHG22 and all of them: the rat PSBP C1, C2 and C3 subunits [13,15,30], the subunits of the rabbit, rat and human UG or CC10 [18,31,32], the chain 1 from cat FdI [11] and the mouse msABP α subunit [14]. Table 1 shows the similarity data between FHG22 and each of the mentioned cDNAs/polypeptides. FHG22 nucleotide and amino acid sequences display a lower degree of similarity to the UG/CC10 from several species than those between the rabbit, rat and human homologs [33], indicating that FHG22 is not the hamster UG subunit, and, similarly, suggesting that FHG22 is neither the hamster version of FdI 1 nor msABP α. However, the identity and similarity data imply a relationship between FHG22 and all those genes/polypeptides [11,34], and their belonging to a common new protein family.

3.3. *Primary structure conservation between the FHG22 product and the PSBP, UG, FdI 1 and msABP α polypeptides*

To further investigate and support this relationship, the polypeptide sequences were compared in order to identify the conserved amino acid residues and to generate a consensus sequence (Fig. 2). The polypeptides are roughly between 95 and 110 amino acids long, with signal peptides determined by com-

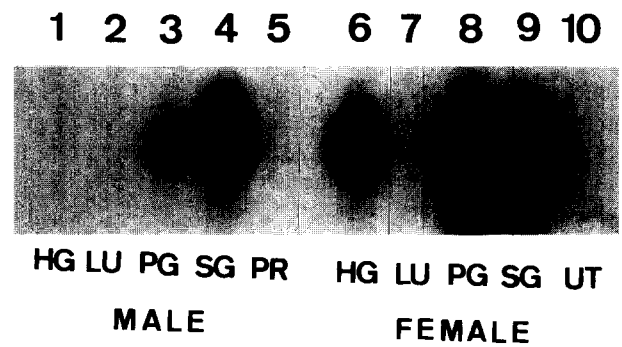

Fig. 4. Distribution of FHG22 mRNA in male and female hamster tissues. A Northern blot with total RNA samples (30 μg each) from male and female tissues was hybridized and processed as explained in Fig. 3. Lanes 1 to 5 = male samples; lanes 6 to 10 = female samples. Abbreviations: HG, Harderian gland; LU, lung; PG, parotid gland; SG, submandibular gland; PR, prostate; UT, uterus. No expression was also found either in male or female brain, liver, kidney, adrenal glands, spleen, heart, small intestine, pancreas and thymus, or in seminal vesicle, testes and ovary.

Table 1
Nucleotide and amino acid comparison between the FHG22 sequences and those from the PSBP, UG/CC10, FdI 1 and msABP α subunits

	cDNA	Polypeptide	
	% identity	% similarity	% identity
PSBP C1	65.6	60.2	39.8
PSBP C2	69.6	61.3	44.1
PSBP C3	45.5	46.7	19.6
rat CC10	44.4	54.8	21.5
rab UG	42.6	46.7	27.8
hum CC10	40.6	50.5	27.5
msABP α	40.5	44.3	24.3
FdI 1	39.1	40.2	19.6

The GAP program was applied to individual comparisons between the FHG22 nucleotide and amino acid sequences and those for the mentioned polypeptides (see section 3.2 for references).

parison of the nucleotide-deduced sequences (not available for msABP α) to those from the purified mature polypeptides [11,14,15,30,31,32,33,35]. Similarly, the first 21 amino acids in the FHG22 sequence could also be a signal peptide for protein secretion [28]. A strictly conserved motif in all the sequences is the presence of two cysteine residues (positions 32 and 102 in the consensus), located near the amino and carboxy ends of the mature polypeptides and involved in disulfide bridges formation to constitute the oxidized oligomers [9,10,11,12,14,35]. A third cysteine residue is also conserved in the position 75, although it is absent in the UG/CC10 subunits, as well as other residues, some of which have been remarked in Fig. 2. The conservation of these residues supports the idea of a common origin for all the polypeptides.

3.4. Southern blot analysis of the FHG22 gene

Preliminary work by our group suggests that the FHG22 monomer is disulfide bound to itself or to other polypeptides in tissue preparations (J. Alvarez and P. Domínguez, unpublished data). In order to know if the FHG22 oligomer composition was similar to PSBP, a heterotetramer formed by three similar subunits encoded by four homologous genes [17,30], the hamster genome was searched for FHG22-related genes. Fig. 3 shows a Southern blot containing restriction enzymes-treated hamster genomic DNA, hybridized at high stringency (40% formamide, 42°C) to a FHG22 cDNA probe. The simple band pattern found indicates that there is a single copy of the FHG22 gene. The same band pattern appeared under low stringency hybridization conditions (30% formamide, 32°C) (data not shown), indicating that there are not FHG22-homologous genes in the hamster genome. A library screening using the FHG22 probe under low stringency conditions (30% formamide, 38°C) also failed to isolate homologous cDNAs: 13 of them were the full- or partial-length original FHG22 clone when sequenced, whereas another 4 were completely unrelated. The checking of these additional FHG22 clones allowed us to confirm its sequence. The absence of FHG22-homologous genes in the hamster genome suggests either that the FHG22 polypeptide binds to itself to form a homomultimeric protein (as in UG dimers) [10], or that it is associated to a dissimilar subunit(s) and forms a heteromultimeric protein (as in FdI or msABP) [11,12].

3.5. Tissue-specific expression of the FHG22 mRNA

To determine if the FHG22 gene is expressed in other organs than female HG, Northern blots with RNA samples from a variety of tissues were hybridized to a FHG22 probe. A resume with the most interesting tissues is shown in Fig. 4: only parotid and submandibular glands, besides female HG, express the FHG22 mRNA. We have also included lung, uterus and prostate because they are related to the PSBP or UG expression. No expression was detected in male or female adrenal glands, brain, heart, kidney, liver, lung, pancreas, spleen, small intestine or thymus, neither in prostate, seminal vesicle, testes, ovary or uterus (data not shown). The expression in HG is female-specific (lanes 1 and 6), with no detectable FHG22 mRNA in males. Parotid glands also show a sex-dependent expression, with the female samples having the highest mRNA levels from all the tissues studied, although there is also some expression in males (lanes 3 and 8). However, in submandibular glands there are similar mRNA levels both in males and females (lanes 4 and 9). This tissue distribution is different from those for PSBP (mainly prostate) [9,22] and UG/CC10 (lung, uterus, endometrium, prostate, and some tissues with lower expression levels) [10,31]. However, both msABP and FdI have been found in the saliva and in parotid and maxillary glands [11,14], which agrees with the presence of the FHG22 mRNA in parotid and submandibular glands. Like HG, they are exocrine glands, and sexually dimorphic submandibular glands have been described in rat and mouse [36,37,38], and probably in hamster [39,40]. In fact, this tissue distribution supports the experimental approach followed for the sexual differencesrelated gene isolation. The fact that the gene is expressed in a sex-independent manner in submandibular glands whereas is sex-dependent in HG and parotid glands is interesting, and a similar case of tissue- and sex-specific control of expression has been described in rat [41]. In this regard, UG expression also presents a complex hormonal regulation in different tissues [10].

The lack of understanding of the physiological role of the FHG22-related proteins related does not allow us a solid speculation on the function of the putative FHG22 oligomer and its relationship to sexual differences. In this sense, the steroid-binding ability of PSBP, UG/CC10 and msABP [9,10,12,35] might be relevant, if it is demonstrated for the FHG22 protein. However, no homologies have been found to the canonical (but not general) steroid-binding sequence motif [42] in any of these proteins, while it has been found that besides steroids and polychlorinated biphenyls, rabbit UG binds retinoids and probably membrane proteins [21,43] and human CC10 binds calcium [20]. The protein might be secreted both to the saliva and eye secretion and bind different ligands, playing a role similar to the immunomodulatory/protective action described for UG/CC10 in the female genital tract [10,44] and the lungs [31,32]. This role could be related to the HG sexual dimorphism regarding to the pernicious porphyrin accumulation and secretion from female glands [45]. Another possible effect would be the sex or subspecies recognition through grooming and spreading both of the saliva and eye secretion all over the hamster skin, similarly to the presence of the FdI protein in cat skin [11], a role also proposed for msABP [12,14].

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References

[1] Wilson, J.D., George, F.W. and Griffin, J.E. (1981) Science 211, 1278–1284.
[2] Bardin, C.W. and Catterall, J.F. (1981) Science 211, 1285–1294.
[3] Berger, F.G. and Watson, G. (1989) Annu. Rev. Physiol. 51, 51–65.
[4] Lin, W.L. and Nadakavukaren, M.J. (1979) Cell Tiss. Res. 198, 119–127.
[5] Payne, A.P. (1994) J. Anat. 185, 1–49.
[6] Domínguez, P., Antolín, I., Boga, J.A., Uría, H. and Menéndez-Peláez, A. (1994) Mol. Cell. Endocrinol. 106, 8189.
[7] Yamamoto, K.R. (1985) Ann. Rev. Genet. 19, 209–252.
[8] Liu, A.Y. and Abraham, B.A. (1991) Cancer Res. 51, 4107–4110.
[9] Lea, O.A., Petrusz, P. and French, F.S. (1979) J. Biol. Chem. 254, 6196–6202.
[10] Miele, L., Cordella-Miele, E., Mantile, G., Peri, A. and Mukherjee, A.B. (1994) J. Endocrinol. Invest. 17, 679–692.
[11] Morgenstern, J.P., Griffith, I.J., Brauer, A.W., Rogers, B.L., Bond, J.F., Chapman, M.D. and Kuo, M. (1991) Proc. Natl. Acad. Sci. USA 88, 9690–9694.
[12] Karn, R.C. and Russell, R. (1993) Biochem. Genet. 31, 307–319.
[13] Baker, M.E. (1983) Biochem. Biophys. Res. Commun. 114, 325–330.
[14] Karn, R.C. (1994) Biochem. Genet. 32, 271–277.
[15] Parker, M., Needham, M. and White, R. (1982) Nature 298, 92–94.
[16] Griffith, I.J., Craig, S., Pollock, J., Yu, X., Morgenstern, J.P. and Rogers, B.L. (1992) Gene 113, 263–268.
[17] Parker, M., Hurst, H. and Page, M. (1983) Ann. NY Acad. Sci. 115–124.
[18] Menne, C., Suske, G., Arnemann, J., Wenz, M., Cato, A.C.B. and Beato, M. (1982) Proc. Natl. Acad. Sci. USA 79, 4853–4857.
[19] Bailly, A., Atger, M., Atger, P., Cerbon, M.-A., Alison, M., Vu Haï, M.T., Logeat, F. and Milgrom, E. (1983) J. Biol. Chem. 258, 10384–10389.
[20] Andersson, O., Nordlund-Möller, L., Barnes, H.J. and Lund, J. (1994) J. Biol. Chem. 269, 19081–19087.
[21] López de Haro, M.S., Pérez Martínez, M., García, C. and Nieto, A. (1994) FEBS Lett. 349, 249–251.
[22] Forsgren, B., Bjork, P., Carlstrom, K., Gustafsson, J.-A., Pousette, A. and Hogberg, B. (1979) Proc. Natl. Acad. Sci. USA 76, 3149–3153.
[23] Davis, L.G., Dibner, M.D. and Battey, J.F. (1986) in: Basic Methods in Molecular Biology, Elsevier, New York.
[24] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[25] Lipman, D.J. and Pearson, W.R. (1985) Science 227, 1435–1441.
[26] Corpet, F. (1988) Nucleic Acids Res. 16, 10881–10890.
[27] Kozak, M. (1986) Cell 44, 283–292.
[28] Austen, B.M. (1979) FEBS Lett. 103, 308–313.
[29] Zhang, Y.L. and Parker, M.G. (1985) Mol. Cell. Endocrinol. 43, 151–154.
[30] Parker, M.G., White, R., Hurst, H., Needham, M. and Tilly, R. (1983) J. Biol. Chem. 258, 12–15.
[31] Nordlund-Moller, L., Andersson, O., Ahlgren, R., Schilling, J., Gillner, M., Gustafsson, J.-A. and Lund, J. (1990) J. Biol. Chem. 265, 12690–12693.
[32] Singh, G., Katyal, S.L., Brown, W.E., Phillips, S., Kennedy, A.L., Anthony, J. and Squeglia, N. (1988) Biochim. Biophys. Acta 950, 329–337.
[33] Singh, G., Katyal, S.L., Brown, W.E., Kennedy, A.L., Singh, U. and Wong-Chong, M.-L. (1990) Biochim. Biophys. Acta 1039, 348–355.
[34] Collins, J.F., Coulson, A.F.W. and Lyall, A. (1988) Comput. Appl. Biosci. 4, 67–71.
[35] Tancredi, T., Temussi, P.A. and Beato, M. (1982) Eur. J. Biochem. 122, 101–104.
[36] Pinkstaff, C.A. (1980) Int. Rev. Cytol. 63, 141–261.
[37] Sato, A. and Miyoshi, S. (1988) Anat. Rec. 220, 239–251.
[38] Sato, A., Goto, F. and Miyoshi, S. (1994) Cell Tiss. Res. 277, 407–415.
[39] Shackleford, J.M. and Schneyer, C.A. (1962) Am. J. Anat. 115, 279–308.
[40] Navia, J.M. (1977) in: Animal Models in Dental Research, pp. 225–254, The University of Alabama Press, Alabama.
[41] Myal, Y., Iwasiow, B., Yarmill, A., Harrison, E., Paterson, J.A. and Shiu, R.P.C. (1994) Endocrinology 135, 1605–1610.
[42] Picado-Leonard, J. and Miller, W.L. (1988) Mol. Endocrinol. 2, 1145–1150.
[43] Díaz González, K. and Nieto, A. (1995) FEBS Lett. 361, 255–258.
[44] Mukherjee, D.C., Agrawal, A.K., Manjunath, R. and Mukherjee, A.B. (1983) Science 219, 989–991.
[45] Antolín, I., Uría, H., Tolivia, D., Rodríguez-Colunga, M.J., Rodríguez, C., Kotler, M.L. and MenéndezPeláez, A. (1994) Anat. Rec. 239, 349–359.