The Golden Hamster Aphrodisin Gene
STRUCTURE, EXPRESSION IN PAROTID GLANDS OF FEMALE ANIMALS, AND COMPARISON WITH A SIMILAR MURINE GENE*

Hans-Jürgen Mägert‡, Alexandra Cieslak, Ozan Alkan, Bernhard Lüscher§, Wolfgang Kauffels¶, and Wolf-Georg Forssmann

From the Lower Saxony Institute for Peptide Research, D-30 625 Hannover, Germany and Departments of ‡Biology and §Gynecology and Obstetrics, Hannover Medical School, D-30625 Hannover, Germany

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The so-called lipocalins are a family of extracellular proteins that are known to typically fulfill tasks as transport proteins for small hydrophobic molecules. However, in the last decade, a large diversity has been described concerning their functions, for example as enzymes, immunomodulators, or proteins involved in coloration and pheromone action. Aphrodisin belongs to those lipocalins, which are of significant importance for the pheromonal stimulation of copulatory behavior in male hamsters. We recently succeeded in characterizing the corresponding cDNA and demonstrated the expression of the aphrodisin gene in the vagina, uterus, and Bartholin’s glands of female hamsters. Here we report the structure of the aphrodisin gene and the functionality of its promoter region. We further compare the aphrodisin gene to the related gene for mouse odorant-binding protein 1a, indicating similar functions of their products. As a novelty, we show that the aphrodisin gene, in addition to the above-mentioned tissues, is also expressed in female hamster parotid glands. In contradiction to the results expected, we finally demonstrate that aphrodisin already occurs in vaginal discharge before the female animals reach fertility. These findings may lead to the identification of as yet unknown aphrodisin functions.

Lipocalins are a family of proteins that typically exhibit a molecular mass in the range of ~17–30 kDa. Their main function seems to be the transport of low molecular mass hydrophobic substances such as retinol, progesterone, odorants, and even pheromones within hydrophilic environments. For this purpose, lipocalins exhibit a certain tertiary structure, which is comparable to a coffee filter, containing an apolar binding pocket for specific ligands. This structure is formed by two sets of four antiparallel β-strands arranged in two orthogonally stranded β-sheets (for review, see Ref. 1). Besides their functions as transporter molecules, in the last decade manifold additional tasks have been described, including functions as enzymes (2, 3), gustatory proteins (4, 5), immunomodulators (6), cell regulators (7–9), and proteins involved in the coloration of animals (10). Depending on the existence of all or just a subset of three conserved amino acid stretches, lipocalins are meanwhile divided into subgroups of the so-called kernel and outlier lipocalins (1). Surprisingly, even bacterial lipocalins have recently been discovered as members of the latter subgroup (11, 12).

Another prominent member of the outlier lipocalins is aphrodisin, which was originally isolated from hamster vaginal discharge, where it occurs in relatively high concentrations, such as 100 µg/20 mg of discharge (13, 14). Aphrodisin was demonstrated to be essential for the pheromonal stimulation of copulatory behavior in male hamsters acting via the vomeronasal organ (15), which is located within the nasal septum of a large number of different vertebrates. Although it may be degenerated, humans also seem to possess a functionally active vomeronasal organ (16–18). At the present time it is still not known whether aphrodisin itself or the combination with a low molecular mass ligand is necessary for pheromonal activity (19). However, the ability of purified aphrodisin to modulate the production of the second messenger inositol-1,4,5-triphosphate in membranes of the male hamster’s vomeronasal organ could already be demonstrated (20).

To obtain nucleotide sequence information for the construction of hybridization probes and PCR primers to facilitate the identification of related systems in species other than the hamster, we have cloned and characterized the aphrodisin cDNA. We further demonstrated the high level expression of the corresponding gene in vaginal tissue, several segments of the uterus, and the Bartholin's glands and characterized part of its promoter region (21, 22). In this paper, we report the structure of the entire aphrodisin gene and show the functionality of its promoter. As a novelty, we describe its expression in the parotid glands of female but not of male golden hamsters. In contradiction to the results expected for a copulation-stimulating pheromone, we demonstrate the significant occurrence of aphrodisin in golden hamster vaginal discharge before the female animals reach fertility. In addition, we describe the gene for mouse odorant-binding protein 1a (MMOBP1A), which possibly exhibits a function comparable with that of aphrodisin. These results may in the future allow the discovery of so far unknown functions of aphrodisin, the characterization of related proteins and their genes in mammalian species other than hamster, and the investigation of regulatory mechanisms of the gene.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ225170.

‡ To whom correspondence should be addressed: Lower Saxony Institute for Peptide Research, Feodor-Lynen-Strasse 31, D-30 625 Hannover, Germany. Tel.: 49-511-546-6310; Fax: 49-511-546-6102; E-mail: HJ-Maegert@gmx.de.

¶ The abbreviations used are: PCR, polymerase chain reaction; MOBP1A, mouse odorant-binding protein 1a; bp, base pair; kbp, kilobase pair; RT, reverse transcription.
**Oligodeoxynucleotides**—The oligonucleotides listed below were purchased from Perkin-Elmer and used as PCR primers, sequencing primers, and hybridization probes (listed in 5′→3′ direction) (primers used for primer walking reaction are not listed): AP-24, GCTTCTTGACCAGCTCCTTCTTTGCTGCTGCG; MAP-67, TGGAAAATGCGCAAGAAGAATG; MAP-68, CCACTCCGGAGGAACTCGAGAAGAATG; MAP-69, CCCAGTGATTGTGGTTCT; MAP-70, TGCAGTAGCTGAGGAGAACGTTGG; MAP-71, CTCATCCAATTTGTATACCTTTCCTTTC; MAP-72, GAAGAATCTTTTATATGAGGAGGG; MAP-73, TGGCAGTGCCTTGTGGCTGAGG; MAP-74, GAGAAAATGCGCAAGAAGAATG; MAP-75, GGCGGAGGCAATTTTAAAGTACGGC; MAP-76, GTGGAGGAGAACTTAACATTTTATAGTGAGAACGTGG; MAP-77, TGTGCCAAACTTTTGTTACTGC; MAP-78, GCCATGATGGTACAACTTTTTTAT. The oligodeoxyribonucleotides were cloned in pGEM-T vector, and the nucleotide sequence of the 1.5-kbp SstI fragment of the gene as a hybridization probe. A positive 1.5-kbp EcoRI fragment, which seemed to be large enough to overlap with the 5.7-kbp SstI fragment, was subcloned in pBSK. How- ever, the partial sequence determinations did not represent the entire 3′terminal region of the gene. Southern blotting was performed with different restriction fragments of the gene and the oligonucleotide AP-24, which was derived from the 3′ terminal of the cDNA, to position the probe. A positive 1.5-kbp EcoRI fragment, which seemed to be the longest overlapping fragment with the 5.7-kbp SstI fragment, was subcloned in pBSK. However, the partial sequence results obtained indicated that a further gene fragment for the connection of both subfragments mentioned above was still needed. Finally, this fragment was obtained by PCR with primers derived from the 3′ terminus of the 5.7-kbp SstI fragment (AP-42) and the 3′terminal region of the 1.5-kbp EcoRI fragment (AP-44) and DNA of the phage clone as a template. The 2124-bp PCR fragment obtained was subcloned in pGEM-T vector, and the nucleotide sequence of the entire golden hamster aphrodisin gene was assembled by means of the primer walking sequencing strategy with subsequent sequence comparisons.

**RESULTS**

**Gene Analysis**—As already described, a 5.7-kbp SstI fragment of the aphrodisin gene, spanning the region from the 5′terminus down to position 5657 of the data base entry, was cloned in pBSK. To obtain an additional subclone containing the 3′terminal region of the gene, Southern blotting was performed with different restriction fragments of the gene and the oligonucleotide AP-24, which was derived from the 3′ terminal of the cDNA, as a hybridization probe. A positive 1.5-kbp EcoRI fragment, which seemed to be large enough to overlap with the 5.7-kbp SstI fragment, was subcloned in pBSK. However, the partial sequence results obtained indicated that a further gene fragment for the connection of both subfragments mentioned above was still needed. Finally, this fragment was generated by PCR with primers derived from the 3′ terminus of the 5.7-kbp SstI fragment (AP-42) and the 3′terminal region of the 1.5-kbp EcoRI fragment (AP-44) and DNA of the phage clone as a template. The 2124-bp PCR fragment obtained was subcloned in pGEM-T vector, and the nucleotide sequence of the entire golden hamster aphrodisin gene was assembled by means of the primer walking sequencing strategy with subsequent sequence comparisons.

**Cloning and Characterization of the Golden Hamster Aphrodisin Gene**—Cloning. Southern blotting, subcloning of positive restriction fragments of the genomic golden hamster aphrodisin gene, and sequencing of the gene were accomplished as already described (21). PCR fragments were cloned in pGEM-T vector (Promega). Nucleotide sequences were determined on both strands. In the case of PCR-generated fragments, at least three independent clones were sequenced. Comparison of obtained partial sequences with each other was performed by means of the MacMolly software package (Soflgen, Berlin, Germany). Potential regulatory elements flanking the region of the golden hamster aphrodisin gene and the related MOBP1A gene of the mouse were detected by linearization of the clone with either SstI or XhoI restriction endonuclease and religation. **Cloning Fragments of the MMOBPIA Gene**—High molecular mass mouse genomic DNA was a kind gift from Sigrid Wattler. Three fragments of the mouse MOBP1A gene spanning the end of exon 1 to the beginning of exon 2, the end of exon 2 to the beginning of exon 3, and the end of exon 3 to the beginning of exon 4, were amplified using the primer pairs MAP-66/67, MAP-68/69, and MAP-70/71, respectively, 10 ng of DNA and 200 μM of each nucleotide. The reaction was performed with 2× GeneAmp PCR buffer (Perkin-Elmer) and Taq polymerase (Boehringer Mannheim), and religation. **Gene Expression Analysis**—Golden hamster vaginal discharge and saliva samples were taken by rinsing the vagina or throat with physiological sodium chloride solution using a catheter. The liquid was collected in small reaction tubes by means of a funnel and stored at −20°C. Concentration of the proteins was achieved by precipitation with a 10-fold volume of ethanol and subsequent sedimentation at 4°C. Western blot analysis, RNA extraction, cDNA first strand synthesis, and analytical RT-PCR were performed as described recently (22). The entire aphrodisin gene, spanning the region from the 5′terminus down to position 5657 of the data base entry, was cloned in pBSK. To obtain an additional subclone containing the 3′terminal region of the gene, Southern blotting was performed with different restriction fragments of the gene and the oligonucleotide AP-24, which was derived from the 3′terminal of the cDNA, to position the probe. A positive 1.5-kbp EcoRI fragment, which seemed to be large enough to overlap with the 5.7-kbp SstI fragment, was subcloned in pBSK. However, the partial sequence results obtained indicated that a further gene fragment for the connection of both subfragments mentioned above was still needed. Finally, this fragment was generated by PCR with primers derived from the 3′terminus of the 5.7-kbp SstI fragment (AP-42) and the 3′terminal region of the 1.5-kbp EcoRI fragment (AP-44) and DNA of the phage clone as a template. The 2124-bp PCR fragment obtained was subcloned in pGEM-T vector, and the nucleotide sequence of the entire golden hamster aphrodisin gene was assembled by means of the primer walking sequencing strategy with subsequent sequence comparisons. **By comparison of the nucleotide sequence of the gene with the already known cDNA sequence, the following gene structure was determined:** The entire aphrodisin gene spans a length of >6 kbp excluding the 5′-flanking region. It consists of seven exons, which are split by six introns. With the exception of intron 5, all exon-intron splice junctions agree well with the exon | GTRAG-intron-AG | exon rule (27). Exon 7 does not contain any coding region but just represents the 3′untranslated region (Fig. 1).

**To investigate the general importance of an aphrodisin-related pheromone system in mammals, we have tried in vain for a long time to clone the corresponding cDNAs and genes from different species using PCR primers and hybridization probes derived from the nucleotide sequence of the hamster. This intention harbors extraordinary difficulties, because proteins of the lipocalin family normally exhibit a high divergence of their amino acid sequences. As a rule, different lipocalins share sequence identities in the range of ~25–35%. Until recently, among the known lipocalins, rat odorant-binding protein (28) showed the highest sequence identity to aphrodisin (40% at the protein level). However, in the past year, two expressed sequence tag data base entries of 5′terminal partial cDNAs from mouse have appeared (accession numbers AA172872 and AA172874), which exhibit sequence identities to aphrodisin of >60% at the nucleotide level and ~50% at the amino acid level. Thus, it is presumed that these sequences represent a murine equivalent of aphrodisin. Recently, another nucleotide sequence entry generated by Mameli and co-workers2 appeared
in the EMBL nucleotide sequence data base. The product of the corresponding gene was designated MMOBP1A. Although the sequence encoding the secretory signal peptide of the precursor was missing, the MMOBP1A cDNA sequence in overlapping regions showed absolute identity to the expressed sequence tag clones mentioned above. Taking together the sequence information of all three clones, a cDNA sequence encoding an entire putative MMOBP1A precursor protein could be assembled. Because of all known lipocalins this protein exhibits the highest sequence identity to aphrodisin at the nucleotide (66%) as well as at the amino acid level (47%), a similar biological function is likely. To obtain further data for the verification of this hypothesis, we decided to compare the structures of the corresponding genes, especially concerning the positions of their introns. For this purpose, we derived MMOBP1A-specific PCR primers (MAP-66–MAP-71) flanking the expected positions of the introns, presuming a distribution nearly identical to that of the aphrodisin gene. At the time we began these experiments, only the expressed sequence tag clones spanning a partial cDNA region including the putative exons 1–4 and the beginning of exon 5 were known. Thus, only primers for the amplification of introns 1–3 could be derived. For amplification of the 5’- and 3’-terminal remnants of the gene, we performed GenomeWalker PCR reactions as described above. From the upstream-directed GenomeWalker PCR we obtained a fragment of ~350 bp including the part of the gene from nucleotide 4 of the translated region up to position ~300 relative to the putative cap site. From the downstream-directed GenomeWalker PCR, we obtained a contig of ~4260 bp spanning the region from nucleotide 44 of exon 4 down to ~1400 bp of the 3’-flanking region of the gene. Inner-positioned, PCR-amplified introns 1–3 as well as introns 4–6 included within the downstream GenomeWalker contig were only roughly sequenced to estimate their size. This means that ambiguous nucleotide positions occurring within the sequence that are generated to a particular low amount by the sequencer have not been precisely determined. On the other hand, exons 1–4 were not sequenced but amplified, using the four primer pairs MAPEX-1/2–MAPEX-7/8, and subsequently analyzed on an agarose gel to determine whether they contain additional introns not known from the aphrodisin gene. Indeed, the sizes of the fragments obtained exactly matched those calculated for exons without additional introns (data not shown). Taken together, the amplification and sequencing of the MMOBP1A gene fragments and comparison with the golden hamster aphrodisin gene revealed an almost identical positioning of the introns (Figs. 1 and 2), verifying the relation of both genes to one another. However, only introns 1–3 and 6 match the sizes of the corresponding aphrodisin gene introns, because introns 4 and 5 show deviations (Fig. 1).

Analysis of the Aphrodisin gene Promoter and Sequence Comparison with the MMOBP1A Promoter—The promoter region of the aphrodisin gene has already been determined by primer extension analysis (21). To verify its functionality in vivo, we performed several promoter-luciferase reporter gene assays with different subfragments of the promoter and a selected number of cell lines. For this purpose, a 2144-bp aphrodisin gene 5’-flanking fragment was amplified by means of the primer OZ-1, derived from the upstream terminus of the 5.-kbp SstI fragment, and the second primer OZ-2, spanning the region from the adenosine nucleotide of the ATG translational start codon up to the position located 23 nucleotides upstream of the ATG start codon and 26 nucleotides downstream from the already determined cap site. The fragment obtained was cloned into the luciferase reporter gene vector pGL2 basic. Two additional clones, containing 1170- and 669-bp subfragments of the 2144-bp fragment, were generated as described above.

Because no cell line that seemed to be ideal for aphrodisin gene promoter analysis (for instance, a hamster vaginal epithelium cell line) was available, we tested the cell lines RK 13 (rat kidney), CRL 6176 (cat salivary glands), and T 84 (human colon carcinoma) for their suitability for aphrodisin gene promoter activity studies. Of these cell lines, only the human T 84 cells gave reproducible results and showed a significant promoter functionality. The results obtained are presented in Fig. 3: the entire aphrodisin gene 5’-flanking fragment of 2144 bp exhibited a promoter activity that is 36-fold above the activity of the basic vector, whereas the 1170- and 669-bp fragments showed 17- and 16-fold activities, respectively. These results strongly indicate the functionality of the aphrodisin gene promoter region in T 84 cells. They further indicate the existence of an activating element within the 1-kbp region, starting ~1150 bp upstream of the cap site.

As another strategy to identify potential regulatory regions within the aphrodisin gene and its 5’-flanking region, we analyzed its nucleotide sequence using the MatInspector program.
The 300-bp 5′-flanking region of the MMOBP1A gene obtained by the above-mentioned genome walking PCR was sequenced precisely on both strands and was analyzed in the same way as the aphrodisin gene promoter. For detection of high probability regulatory elements, the core similarities generally had to be 100%, whereas the matrix similarities had to be at least 90%. In addition, two alternative criteria had to be fulfilled. First, the core sequence of a regulatory element had to occur in the promoter regions of both genes, the golden hamster aphrodisin gene and the MMOBP1A gene. Second, if the first requirement could not be met, the random expectation value for a high probability regulating element within the aphrodisin gene promoter had to be 0.1 at the most.

Using this strategy, potential binding sites for the following transcription factors could be detected within the promoters of both genes: AP-1 (29), Evi-1 (30), GATA-1 (twice) (31), and c-Ets-1(p54) (twice) (32). Furthermore, CAAT boxes in reverse complementary orientation and TATA boxes occur in the expected positions. In addition, putative binding sites for Elk-1 (33, 34) and IRF-1 (35, 36) are represented within the aphrodisin gene 5′-flanking region (Figs. 1 and 4 and Table I).

Expression of the Aphrodisin Gene—The expression of the

**Golden Hamphoridin Gene**

![Fig. 2. Comparison of the cDNA and amino acid sequences of hamster aphrodisin and MMOBP1A. Nucleotides identical in both cDNAs are marked with vertical dashes; agreeing amino acids are marked with black dots above the aphrodisin sequence. The positions of the introns of both genes are indicated; the nucleotides flanking the introns are underlined.](image)

![Fig. 3. Results of the luciferase reporter gene assays with three different aphrodisin promoter fragments in T 84 cells. The relative promoter activities were calculated from three independent assays.](image)

(23). The 300-bp 5′-flanking region of the MMOBP1A gene obtained by the above-mentioned genome walking PCR was sequenced precisely on both strands and was analyzed in the same way as the aphrodisin gene promoter. For detection of high probability regulatory elements, the core similarities generally had to be 100%, whereas the matrix similarities had to be at least 90%. In addition, two alternative criteria had to be fulfilled. First, the core sequence of a regulatory element had to occur in the promoter regions of both genes, the golden hamster aphrodisin gene and the MMOBP1A gene. Second, if the first requirement could not be met, the random expectation value for a high probability regulating element within the aphrodisin gene promoter had to be 0.1 at the most.

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**Expression of the Aphrodisin Gene**—The expression of the
The aphrodisin gene in different segments of the female hamster genital tract has already been demonstrated. Because aphrodisin probably functions as a transporter for low molecular mass hydrophobic pheromone molecules, which act via the vomeronasal organ of the male hamster, additional expression of the gene in several regions of the hamster throat seemed to be likely. We therefore performed Western blot analysis with protein extracts (6 μg of total protein) from the saliva of male and female golden hamsters. Surprisingly, and contrary to our expectations, we obtained signals in the expected size range only from female animals (Fig. 5A). In the saliva of male golden hamsters, which are the recipients of pheromonal signals from the female animals, no aphrodisin-specific signals were detectable. To verify these results at the nucleic acid level, we also performed RT-PCR analysis with total RNA extracted from the parotid glands of female and male hamsters and, as a positive control, from hamster vagina. In this case, we also obtained significant signals only from female hamster parotid glands (Fig. 5B), verifying the results of the Western blot analysis. The corresponding PCR fragments have been reamplified, directly sequenced, and identified as aphrodisin-specific. Unexpectedly, the data obtained clearly indicate an expression of the aphrodisin gene in female golden hamster parotid glands and the secretion of the protein into the female hamster’s saliva.

Another question arising in the context of aphrodisin as a putative pheromone transporter concerns the dependence of gene expression on the stage of fertility of juvenile female hamsters. Provided that aphrodisin functions exclusively as a transporter for sex pheromones, its secretion into female hamster vaginal discharge only would make sense from the time the newborn hamsters reach fertility. To test this hypothesis, we performed Western blot analysis on vaginal discharge from two female animals taken from day 22 to day 43 after birth. Golden hamsters normally reach fertility between days 28 and 40 after birth, whereby both values represent the lowest and highest values, respectively, as known from the literature (37). A comparable high concentration of aphrodisin in vaginal discharge was detectable within all of the analyzed samples (Fig. 6). Thus, secretion of aphrodisin into vaginal discharge does not seem to depend on the point at which female animals reach fertility.

### Table I

**Potential regulatory elements of the aphrodisin and MMOBP1A gene promoters identified by the MatInspector program**

The core similarity of all elements had to be 1.0. With the exception of the MMOBP1A Evi-1 site, the matrix similarity had to be at least 0.9.

| Factor, element | Sequence | Position | Matrix similarity | Random expectation | Gene |
|-----------------|----------|----------|-------------------|--------------------|------|
| Elk-1           | GGAA     | -2081    | NA                | 0.06               | Aphrodisin |
| IRF-1           | gaaaaatGAAaa | -1972    | 0.915              | 0.02               | Aphrodisin |
| AP-1            | agTGAACctttta | -281    | 0.916               | 2.45               | Aphrodisin |
| Evi-1           | tggTGAACtta | -290    | 0.901               | Aphrodisin         |      |
| MDS1/Evi-1      | tagAGATac | -230    | 0.603               | MMOBP1A            |      |
| GATA-1          | aacaaGATAgttct | -225    | 0.930               | 2.27               | Aphrodisin |
|                 | atagaGATActct | -231    | 0.927               | MMOBP1A            |      |
|                 | tgtgaGATAgtgag | -144    | 0.947               | Aphrodisin         |      |
|                 | tttaaggGATAgact | -149    | 0.928               | MMOBP1A            |      |
|                 | c-Ets-1 (p54) | taagaGAAAtgct | -204 | 0.932               | 7.63   | Aphrodisin |
|                 | ttaaGAAAtgct | -210    | 0.908               | MMOBP1A            |      |
|                 | aaagaGAAaacac | -108    | 0.941               | Aphrodisin         |      |
|                 | cagcaGAAAgact | -113    | 0.965               | MMOBP1A            |      |
DISCUSSION

One of the main reasons for our decision to characterize the aphrodisin cDNA and gene was the intention to identify and investigate related systems in species other than the hamster. Indeed, sequence comparisons with data base entries led to the discovery of the aphrodisin-related MMOBP1A, the gene structure of which is reported in this paper. As in the case of the aphrodisin gene, it exhibits a seven-exon/six-intron structure. The construction of a phylogenetic tree of several lipocalins based on their amino acid sequences was already performed by Igarashi and co-workers (38). As an alternative, we compared the aphrodisin gene with other lipocalin genes (MMOBP1A, α2UG, βLG, PP14, and RPDS; see Ref. 38) also possessing seven exons under the criterion of their structure. For this purpose, we added the absolute values of size deviations in base pairs of each exon of a given gene from the corresponding exon of the aphrodisin gene. The resulting value was expected to be low for closely related genes and high for genes showing a more distant relation to the aphrodisin gene. Using this strategy, the following relation was obtained in descending order: MMOBP1A > PP14 > βLG > α2UG > RPDS. Despite the fact that, at this time, the structure of the MMOBP1A gene was still not known, the order of relation obtained fully verifies the phylogenetic tree as presented by Igarashi and co-workers (38). Thus, the close relation of MMOBP1A to aphrodisin is demonstrated at the level of sequence identity, as well as at the level of gene structure.

The 290-bp region following the putative cap site of the MMOBP1A gene in the 5' direction also shows a striking sequence identity of 67% to the corresponding region of the aphrodisin gene. This similarity is additionally reflected in the pattern of potential regulatory elements in both gene promoters. As described above, we have identified potential binding sites for the transcription factors Elk-1, IRF-1, AP-1, Evi-1, GATA-1 (twice), and c-Ets-1 (twice) within the 2144-bp 5'-flanking region of the aphrodisin gene (Fig. 4). In the case of the MMOBP1A gene, only a 290-bp fragment of the 5'-flanking region was available. However, within this fragment the putative binding sites for AP-1, Evi-1, GATA-1 (twice), and c-Ets-1 (p54) (twice) as well as the putative TATA and CAAT boxes occur at positions identical to those within the aphrodisin gene. In addition, as in the aphrodisin gene promoter the CAAT element of the MMOBP1A gene exhibits a reverse complementary orientation. These findings indicate the significance of at least a part of the identified elements and comparable mechanisms of regulation of both genes.

Among the corresponding transcription factors possibly interacting with the aphrodisin gene promoter, Evi-1 is the most probable candidate. It functions as a transcriptional repressor (39) but also occurs in an N-terminally elongated form, MDS1/Evi-1, which exhibits a strong activating effect (40). Both forms recognize the same motif containing an AGAT core sequence (see Table I). In mice, besides being found in a few other organs, an Evi-1 gene-specific mRNA was already localized within the uterus and during embryogenesis within regions of that part of
the nasal cavity that later forms the vomeronasal organ (41). On the other hand, receptor genes to test the functionality of the aphrodisin gene in the golden hamster uterus (22). On the other hand, the nasal cavity that later forms the vomeronasal organ (41).

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related systems in other mammals. Although the importance of aphrodisin and related lipocalins for the reproduction of mammals at this time is difficult to predict, future use of such proteins in the breeding of animals is thinkable.

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