CMLS, Cell. Mol. Life Sci. 58 (2001) 503–509
1420-682X/01/040503-7 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

The role of perireceptor events in vertebrate olfaction

P. Pelosi

Dipartimento di Chimica e Biotecnologie Agrarie, University of Pisa, Via S. Michele 4, 56124 Pisa (Italy),
Fax +39 050 574235, e-mail: ppelosi@agr.unipi.it

Abstract. The perception of odours and pheromones is mediated by small soluble carrier proteins that belong to the family of lipocalins. Those secreted by the nasal mucosa are called odorant-binding proteins (OBPs) for their binding activity towards volatile compounds. Proteins of similar structure, which we call pheromone-binding proteins (PBPs), help to deliver volatile pheromones in the environment. They are present in high concentration in biological fluids, such as urine, saliva and vaginal discharge, involved in chemical communication between conspecifics. Several subclasses of OBPs have been identified in the same animal species, each best related to a particular group of PBPs. Such similarities, together with anatomical and behavioural evidence, suggest that OBPs may be involved in the perception of pheromones.

Key words. Odorant-binding protein; pheromone-binding protein; urinary proteins; salivary proteins; lipocalins; perireceptor events; vomeronasal organ.

Introduction

Understanding the physiological function of OBPs has been a major problem since their discovery and characterisation. Although at the beginning their role was associated with olfaction, recent data clearly indicate that they may instead be involved in the perception of pheromones, acting in the vomeronasal organ.

The perireceptor space

The task of perceiving stimuli from the external world poses major problems at the interface between the sensory organs and the environment. This is particularly evident for chemical senses, where a physical contact between foreign molecules and receptors of the sensory neurons is necessary in order to trigger the response. In olfaction of air-breathing animals, an additional problem is posed by the danger of dehydration at the air-water interface.

To prevent evaporation of water and at the same time to protect the sensory neuron endings from the environment, different strategies have been adopted in the course of evolution. In air-breathing vertebrates, the olfactory epithelium, with its layer of cilia, protruding from the sensory neurons, is covered by a thick layer of dense mucus. Large glycoproteins bind high quantities of water, thus reducing evaporation; at the same time, the thick mucus matrix offers physical and chemical protection against foreign agents. In insects, a hard cuticular wall prevents evaporation of the aqueous fluid filling the inner space (the sensillar lymph), at the same time allowing diffusion of odorant molecules through tiny pores present all over its surface.

This region around receptor sensory neurons is called ‘perireceptor space’, and ‘perireceptor events’ is the name given to the chemical interactions of odours and pheromones with the soluble macromolecules present in this space [1].

OBPs as major components of the perireceptor space

Both the sensillar lymph of insects and the nasal mucus of vertebrates contain large amounts of small soluble OBPs, that specifically and reversibly bind odours and pheromones [2–6]. The very high concentrations of these proteins in their respective fluids, as well as their rapid turnover, suggest functions important for the individual or for the species.

When OBPs were first isolated [7, 8], it was hypothesised that they could be responsible for recognizing of olfactory stimuli, acting as ‘soluble receptors’, with a

mechanism similar to that used by bacteria to sense sugar molecules [2, 9, 10]. Other proposed functions included OBPs as carriers or scavengers for the hydrophobic molecules of odorants, but definite proof for any of these hypotheses was not provided.

Following the discovery of transmembrane olfactory receptors in vertebrates [11] and their functional expression [12], experimental evidence indicates that olfactory perception does not require the presence of OBPs, as odorant molecules can directly trigger the receptors of the neuronal membrane [13–15]. Recently, behavioural observations, together with biochemical and physiological data, strongly suggest that in vertebrates the target organ of OBPs is not the olfactory epithelium, but the vomeronasal system. Along with such view, then, OBPs would be involved in the perception and recognition of pheromones, rather than of general odorants. In the next paragraphs, experimental data supporting this view are reviewed and discussed.

Pheromone-binding proteins

The strongest support for such a hypothesis is the high similarity of OBPs to structurally related proteins, synthesised by different glands of the body and carrying volatile pheromones. In analogy with the nomenclature employed for insect OBPs, we propose to call these proteins PBPs (pheromone-binding proteins), while leaving the term OBP for those expressed in the nose. Vertebrate PBPs are highly concentrated in biological fluids, such as urine, saliva and vaginal secretion, that are known for their role in chemical communication between conspecifics. Their similarity with OBPs of the nasal epithelium can be very high, with more than 90% identical amino acids.

In the following sections, the structures of OBPs and PBPs of mammals are reviewed and compared, together with their ligand-binding properties. Some physiological aspects related to these proteins are then examined, and a model of action for OBPs in pheromonal perception is proposed.

OBPs and PBPs

Vertebrate OBPs were discovered at the beginning of the eighties using a ligand-binding approach [7]. Several members of this family were later purified from different species of mammals [16–23]. The frog OBP is the only one isolated in a nonmammalian vertebrate [24]. Of special interest is the expression of three to five types of OBPs in the same animal species. These are not to be considered as isoform, nor individual differences, but as distinct subclasses on the basis of their structural and functional diversity.

Among the PBPs, rat and mouse urinary proteins have been known for a long time [25, 26], but their role in chemical communication had not been suspected, nor were functional data reported. Only after the isolation of OBPs did their structural similarity with these proteins suggest a function of carriers for chemical messengers and prompt functional studies on the isolated proteins [27]. Later, similar proteins were identified in the saliva of mouse [28, 29] and pig [30], as well as in the vaginal discharge of hamster [31, 32].

Structure

OBPs and PBPs of mammals are acidic polypeptides of 17–20 kDa, present in their native state as noncovalent dimers or monomers. Based on their amino acid sequence and three-dimensional structure, they can be assigned to the superfamily of lipocalins. These are small soluble proteins, whose common function seems to be that of carriers for hydrophobic ligands in aqueous biological fluids, although members with different functions, including enzymatic activity, have been reported [33, 34]. A typical representative of this family is the serum retinol-binding protein, which transports vitamin A across the bloodstream [35, 36]. Lipocalins have also been reported in insects [37] and even in bacteria [38].

All lipocalins share a short motif, -G-X-W-, not far from the amino terminus, that is fully conserved. Residue identity between distant members of the lipocalin family can be very small, even below 20%. Despite such wide variability, their tertiary structure is well conserved, with the typical β-barrel motif consisting of eight antiparallel β sheets [33].

Another subclass of OBPs includes sequences similar to the so-called VEG proteins. This name derives from the von Ebner's glands, where they were originally discovered [39, 40]. Intriguingly, the same genes are also expressed in the lachrymal glands [39, 41] and in the prostate [42], and similar proteins have been purified from the nasal epithelium [43, M. Fantacci and P. Pelosi, unpublished]. While it is conceivable that urinary, salivary and vaginal proteins could be involved in chemical communication within the species, the function of VEG proteins is still undefined.

Finally, two lipocalins, called VNS-1 and VNS-2, are specifically expressed in the vomeronasal epithelium of the mouse [44]. They bear significant sequence similarity to OBPs and may be considered as a fourth subclass of these proteins.

Other lipocalins, certainly to be included in the PBP family, are secreted by sweat glands of mammals. One of these, carrying the odorant E-3-methyl-2-hexenoic acid, has been isolated from human armpit secretion [45]: its amino acid sequence identified this protein as apolipoprotein D [46]. The skin glands of the cow also produce a lipocalin, classified as an allergen [47]; however, its sequence homology with urinary proteins may indicate

an additional role of pheromone transport. Another lipocalin, showing significant sequence similarity to urinary proteins, has been isolated from the endometrium of the mare [48].

The number of PBPs in mammals is probably much larger, given the diversity of glands producing chemical markers and messengers. The observation of behaviour has provided and still provides the first clues for identifying the glands and analysing their secretions. Very often it has been observed that the synthesis of volatile chemical signals is associated to the expression of their relative binding proteins.

All OBPs and PBPs share with the other lipocalins the typical three-dimensional folding of the β barrel, a calyx-shaped cavity, made up of eight antiparallel β strands; a short segment of α helix close to the C-terminus completes the structure [34]. So far, the structures of two OBPs, the bovine [49, 50] and the porcine [51], and those of two PBPs, the rat and mouse urinary proteins [52], have been resolved by X-ray crystallography.

The bovine OBP, which is a homodimer in its native state, presents the interesting phenomenon of domain swapping: the C-terminal region of each monomeric unit interacts with the β-barrel domain of the other. This fact probably helps to stabilise the dimeric structure of a protein that lacks cysteine residues. The ligand binding site is located in the core of the β barrel, as clearly demonstrated by the use of a selenium-containing ligand, 2-amino-4-butyl-5-propylselenazole [53].

It has also been observed that there is a gated entrance to the ligand-binding site. Although conformational changes following the uptake of a ligand have not been directly observed, such a model could well account for the unusually long dissociation time (hours) measured for the complex [54], given a thermodynamic constant of the micromolar order. It would also explain the unsuccessful attempts to purify OBPs by affinity chromatography on media derivatised with analogues of odorant molecules [P. Pelosi, unpublished].

The pig OBP is very similar in its structure [51] to the bovine protein, apart from the obvious absence of the domain swapping, given its monomeric nature. In this case, the C-terminal region is folded back on the main bulk of the protein, owing to the insertion of a glycine residue in position 121. This structure is exceptionally stable to thermal denaturation (up to 80 °C), particularly in the presence of a ligand [55].

The urinary proteins of rat (α2u) and mouse (MUP), both monomers in their native forms, share the same folding with OBPs [52]. In addition, these proteins contain molecules of specific pheromones tightly bound inside the cavity, as described below.

Ligand binding

Odorant-binding proteins were first discovered using ligand-binding assays with 2-isobutyl-3-methoxypyrazine [7], a very powerful green-smelling component of bell peppers that binds to most OBPs with dissociation constants of the micromolar order.

A wide study of binding specificity has been performed only with bovine OBP and pig OBP-I, which could be easily purified in relatively large amounts. The two proteins, which can be assigned to the same subclass on the basis of sequence similarity, also show similar spectra of binding [54, 56–58].

Of all the odorant molecules tested, no one proved to be a particularly strong ligand, with dissociation constants on the order of 0.1–1 μM for the best ligands. A comparison between the chemical structures of good ligands and those of nonligands indicates the former as molecules of medium size, hydrophobic and of planar, rather flat shape, whereas the latter include more hydrophylic compounds and those of round shape. Examples of good ligands are thymol, menthol, 3,7-dimethyloctanol and linear aldehydes of 6–10 carbon atoms. Odorants not exhibiting any appreciable affinity include hydrophilic aromatic compounds, such as 2-phenylethanol and 2-methoxy-3-methylpyrazine, round-shaped compounds, such as camphor and cineol, and charged odorants, such as fatty acids.

Such a broad specificity, together with the rather high dissociation constant measured with general odorants, probably suggests that the physiological ligand for these proteins has not yet been identified. The idea that OBPs could act in the vomeronasal organ supports this idea and indicates the molecules of pheromones as the best putative ligands. Information in this field is still poor and fragmentary.

Binding experiments have also been performed with urinary proteins of rat and mouse [27]. Despite their relatively low sequence similarity with bovine OBP and pig OBP-I, these proteins bind 2-isobutyl-3-methoxypyrazine and several other odorants with similar affinities and specificities. Moreover, MUP, purified from urine as a mixture of isoforms, has been shown to contain at least three tightly bound volatile compounds, 2-sec-butylthiazoline, 2-dehydrobrevicomine and 4-ethylphenol [59, 60], all reported to present pheromonal activity in the mouse. Incidentally, we observe that the first molecule is an analogue of 4-butyl-5-propylthiazole, a derivative that shares with 2-isobutyl-3-methoxypyrazine a strong green odour and a good affinity to OBPs.

The boar lipocalin SAL, sex specifically expressed in the submaxillary glands, is related in its N-terminal sequence to urinary proteins. After purification it retains, as do the MUPs, compounds that are known sex pheromones for the pig, 5α-androst-16-en-3-one and 5α-androst-16-en-3-ol. Moreover, it reversibly binds 2-isobutyl-3-methoxypyrazine with micromolar affinity [30]. The role of saliva

in chemical communication between sexes has been well established, as has that of urine in the mouse.

Although the porcine sex pheromone, 5α-androst-16-en-3-one, showed poor affinity to OBP-I, another protein, OBP-II, also expressed in the nasal tissue, binds the steroid more specifically, whereas it does not bind 2-isobutyl-3-methoxypyrazine. Thus, a complementarity of binding is emerging, while evidence is accumulating on the complexity of pheromones, including, in addition to the sex-related ones, other molecules mediating relationships between mother and young or competing males, as well as stress-releasing pheromones and territorial markers.

Another example is provided by the rat OBPs, where again a microdiversity of these proteins seems to be related to different binding specificities: OBP-I, structurally related to bovine OBP and pig OBP-I, binds 2-isobutyl-3-methoxypyrazine, but not fatty acids, whereas the reverse is true of OBP-II, which belongs to the VEG-like subclass of OBPs [61].

Physiological aspects

Site of production and temporal expression of OBPs

While the structures of OBPs and PBPs have been well characterised, the physiological effects of these proteins are poorly understood. Tissue specificity and regulation of their expression may be suggestive of their function at physiological and ethological levels.

Concerning PBPs, their sex specificity is a clear indication of their role in chemical communication between sexes. In fact, in rat and mouse, urinary proteins are produced only by the male and are under hormonal control [62, 63]. The salivary proteins of the boar are totally absent in the female, as well as in the immature male [30]. Moreover, as reported above, both urinary and salivary proteins carry the specific volatile pheromones as endogenous ligands. Salivary proteins may be involved in sexual communication also in the mouse, as indicated by behavioural data [64]. The role of hamster aphrodisin, given its specific site of production, does not need further comment.

OBPs of the nose are synthesised by several glands located in the respiratory region of the nasal tissue, such as the lateral nasal glands and the glands of the septum [21, 65–70]. The OBP-related lipocalins, VNSP-1 and VNSP-2, previously reported, are synthesised by glands of the vomeronasal organ [44]. Given the anatomy of the VNO, often a narrow cavity, open only at one end, it is reasonable to assume that these proteins perform their function in the same organ. In fact, the mucus covering the epithelium of the VNO is not easily translocated to another sensory tissue, but directly discarded through the mouth. Given the highly significant similarity between VNSPs and OBPs, it is reasonable to put both these types of proteins in the same class and assume that they also share a common role. OBPs in fact may easily reach the vomeronasal cavity, carried in the natural downward flow of nasal mucus towards the opening of the nares, where the entrance of the VNO is usually located. From this point the mucus is pumped inside the vomeronasal organ by the typical behaviour called ‘flemen’, a sort of rythmic contraction of the tip of the nose.

There are not many systematic studies on the temporal expression of OBPs; generally, OBPs are synthesised soon after birth and reach their maximum levels within 2–3 days [68]. This is in contrast with olfactory receptors, whose expression starts during fetal life. During the course of life, however, OBPs are not produced at a constant rate, but only in certain periods and under certain physiological conditions. Particularly interesting is the observation that one of the two vomeronasal OBPs of the mouse, VNSP1, is only expressed in the first weeks of life and at the onset of puberty. The presence of this protein in these periods has been related to the role of olfaction in recognising the mother and finding a partner, respectively [44].

Receptors for PBPs in the vomeronasal organ

The general idea suggested above that OBPs may have their target in the vomeronasal organ finds its stronger support in the evidence, provided in the last few years, that membrane receptors for PBPs are active in the VNO. Such evidence comes from contributions of ethology, physiology, molecular biology and biochemistry, which provided complementary experimental data.

The observation that female mice are attracted by the urine of males first suggested the presence of pheromones in this liquid. In fact, a great number of volatiles were identified with more or less clear pheromonal activity [71, 72]. The presence of binding proteins for these molecules in the same fluid was first related to a function of solubilising these hydrophobic compounds and at the same time providing a system for a gradual release of the same molecules in the environment. The role of the urine, however, is not limited to carrying volatile pheromones. In fact, female mice are also interested in its nonvolatile components, as indicated by their licking behaviour: in this way, female mice send this fluid in the nasal cavity, including the VNO.

Such observations prompted a more detailed investigation of the effect of male urine on female physiology and development [73]. Thus, it was demonstrated that when injected into the nose of young female mice, the urine of their conspecific males accelerates the onset of puberty. The weight of the uterus, taken as a measure of the effect, was on the average twice that of control individuals. The same result was obtained by the use of purified urinary proteins, both when complexed with their pheromonal

ligands and after removal of these small components. The volatile pheromones alone were ineffective, whereas a synthetic peptide, reproducing the first six residues of the urinary protein, was found to produce a result similar to that of the entire protein [73]. These experiments clearly indicate the presence of receptors for PBPs, such as the urinary proteins, in the nasal cavity.

Molecular biology provided another piece of information, however, not directly related to the physiological effect described above. The vomeronasal epithelium expresses two classes of receptors, both belonging to the family of G-protein-coupled seven-transmembrane receptors, but drastically different in their extracellular domain. Whereas receptors of the first class, called V1R, are structurally similar to the previously discovered olfactory receptors [74], those of the second class (V2R) contain a large extracellular domain of about 600 residues at their N-terminus [75–77]. It has been suggested that such a domain could bind polypeptide ligands.

A link between the physiological data and those of molecular biology was recently provided by classical biochemical experiments [78]. Volatile pheromones and urinary proteins were shown both to be active on membranes of the VNO. However, the first ligands stimulated the activity of adenylate cyclase, through a G_o protein, with production of the second-messenger cyclic AMP (cAMP), whereas the proteins had the effect of increasing the concentration of intracellular IP3 through the activation of a G_i protein and the enzyme phospholipase C. Although these experiments do not demonstrate binding between urinary proteins and receptors of the second class, they strongly support such a hypothesis.

Conclusions

Understanding the physiological function of OBPs has been a major problem since their discovery and characterisation. Although at the beginning their role was associated with olfaction, recent data clearly indicate that they may instead be involved in the perception of pheromones, acting in the VNO. The elements of evidence towards this view have been reported above and can be summarised here:

- olfactory receptors functionally expressed in cells not synthesising OBPs are still able to respond to odours;
- OBPs are structurally similar to pheromone-binding proteins of urine, saliva and vaginal discharge;
- OBPs are secreted by glands of the respiratory region of the nasal epithelium; from this area they are translocated to the VNO, but not to the olfactory mucosa; some OBPs are also synthesised in the VNO.

If we assume that OBPs perform their role in the perireceptor space of the VNO, the second unanswered question regards the specific molecular mechanisms where they are involved. The presence of several subclasses of OBPs, as reported in most animal species, suggests a discrimination role of these proteins. This is further supported by the profound structural differences between the sequences of OBPs of the same species, in contrast with the high similarity of each OBP with a member of the PBP family. Thus, pairs of OBP/PBP could have evolved towards the common function of binding a specific ligand. In fact, complementary binding specificities have been measured with OBPs of the same species, as discussed above.

Thus, OBPs could selectively carry different volatile pheromones to the membrane receptors of the VNO. The complex OBP/pheromone could then convey the chemical message to the receptor proteins along with different models:

- the receptor could recognise the complex, but not the ligand alone;
- the ligand could be transferred to the receptor only if assisted by the OBP;
- the ligand could spontaneously dissociate from the complex and bind to the receptor.

Certainly, volatile pheromones are able to activate the enzymatic cascade in the VNO leading to production of cAMP: this observation would exclude the involvement of OBPs, at least for one of the two transduction mechanisms active in the neurons of the VNO.

On the other hand, PBPs certainly interact with membrane receptors, most likely those of class V2R: OBPs of similar structure may well follow the same path and act through the same mechanism; the function of volatile pheromones in this protein/protein interaction, however, is not yet understood.

1 Getchell T. V., Margolis F. L. and Getchell M. L. (1984) Perireceptor and receptor events in vertebrate olfaction. Prog. Neurobiol. **23:** 317–345
2 Pelosi P. (1994) Odorant-binding proteins. Crit. Rev. Biochem. Mol. Biol. **29:** 199–228
3 Pelosi P. (1996) Perireceptor events in olfaction. J. Neurobiol. **30:** 3–19
4 Pelosi P. (1998) Odorant-binding proteins: structural aspects. Ann. N. Y. Acad. Sci. **855:** 281–293
5 Pelosi P. and Maida R. (1995) Odorant-binding proteins in insects. Comp. Biochem. Physiol. **111B:** 503–514
6 Steinbrecht R. A. (1998) Odorant-binding proteins: expression and function. Ann. N. Y. Acad. Sci. **855:** 323–332
7 Pelosi P., Baldaccini N. E. and Pisanelli A. M. (1982) Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. Biochem. J. **201:** 245–248
8 Vogt R. G. and Riddiford L. M. (1981) Pheromone binding and inactivation by moth antennae. Nature **293:** 161–163
9 Bignetti E., Cavaggioni A. and Pelosi P. (1987) An odorant binding protein in cow. In: Discussions in Neurosciences, vol. 4, Sensory Transduction, pp. 37–41, Hudspeth A. J., MacLeish P. R., Margolis F. L. and Wiesel T. N. (eds) FESN, Geneva
10 Prestwich G. D., Du G. and LaForest S. (1995) How is pheromone specificity encoded in proteins? Chem. Senses **20:** 461–469

11 Buck L. and Axel R. (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell **66:** 175–187
12 Zhao H., Ivic L., Otaki J. M., Hashimoto M., Mikoshiba K. and Firestein S. (1998) Functional expression of a mammalian odorant receptor. Science **279:** 237–242
13 Breer H., Krieger J., Meinken C., Kiefer H. and Strotmann J. (1998) Expression and functional analysis of olfactory receptors. Ann N. Y. Acad. Sci. **855:** 175–181
14 Krautwurst D., Yau K. W. and Reed R. R. (1998) Identification of ligands for olfactory receptors by functional expression of a receptor library. Cell **95:** 917–926
15 Hatt H., Gisselmann G. and Wetzel C. H. (1999) Cloning, functional expression and characterization of a human olfactory receptor. Cell. Mol. Biol. **45:** 285–291
16 Bignetti E., Cavaggioni A., Pelosi P., Persaud K. C., Sorbi R. T. and Tirindelli R. (1985) Purification and characterization of an odorant binding protein from cow nasal tissue. Eur. J. Biochem. **149:** 227–231
17 Pevsner J., Trifiletti R. R., Strittmatter S. M. and Snyder S. H. (1985) Isolation and characterization of an olfactory receptor protein for odorant pyrazines. Proc. Natl. Acad. Sci. **82**: 3050–3054
18 Dal Monte M., Andreini I., Revoltella R. and Pelosi P. (1991) Purification and characterization of two odorant binding proteins from nasal tissue of rabbit and pig. Comp. Biochem. Physiol. **99B:** 445–451
19 Felicioli A., Ganni M., Garibotti M. and Pelosi P. (1993) Multiple types and forms of odorant-binding proteins in the old-world porcupine *Hystrix cristata*. Comp. Biochem. Physiol. **105B:** 755–784
20 Pes D. and Pelosi P. (1995) Odorant-binding proteins of the mouse. Comp. Biochem. Physiol. **112B:** 471–479
21 Pes D., Mameli M., Andreini I., Krieger J., Weber M., Breer H. et al. (1998) Cloning and expression of odorant-binding proteins Ia and Ib from mouse nasal tissue. Gene **212:** 49–55
22 Garibotti M., Navarrini A., Pisanelli A. M. and Pelosi P. (1997) Three odorant-binding proteins from rabbit nasal mucosa. Chem. Senses **22:** 383–390
23 Paolini S., Scaloni A., Amoresano A., Marchese S., Napolitano E. and Pelosi P. (1998) Amino acid sequence post-translational modifications binding and labelling of porcine odorant-binding protein. Chem. Senses **23:** 689–698
24 Lee H. K., Wells R. G. and Reed R. R. (1987) Isolation of an olfactory cDNA: similarity to retinol binding protein suggests a role in olfaction. Science **253:** 1053–1056
25 Dinh B. L., Tremblay A. and Dufour D. (1965) Immunochemical study of rat urinary proteins: their relation to serum and kidney proteins. J. Immunol. **95:** 574–582
26 Finlayson J. S., Asofsky R., Potter M. and Runner C. C. (1965) Major urinary protein complex of normal mice: origin. Science **149:** 981–982
27 Cavaggioni A., Findlay J. B. C. and Tirindelli R. (1990) Ligand binding characteristics of homologous rat and mouse urinary proteins and pyrazine binding protein of the calf. Comp. Biochem. Physiol. **96B:** 513–520
28 Shaw P. H., Held W. A. and Hastie N. D. (1983) The gene family for major urinary proteins:expression in several secretory tissues of the mouse. Cell **32:** 755–761
29 Shahan K. M., Denaro M., Gilmartin M., Shi Y. and Derman E. (1987) Expression of six mouse major urinary protein genes in the mammary parotid sublingual submaxillary and lachrymal glands and in the liver. Mol. Cell. Biol. **7:** 1947–1954
30 Marchese S., Pes D., Scaloni A., Carbone V. and Pelosi P. (1998). Lipocalins of boar salivary glands binding odours and pheromones. Eur. J. Biochem. **252:** 563–568
31 Henzel W. J., Rodriguez H., Singer A. G., Stults J. T., Macrides F., Agosta W. C. et al. (1988) The primary structure of aphrodisin. J. Biol. Chem. **263:** 16682–16687
32 Magert H. J., Hadrys T., Cieslak A., Groger A., Feller S. and Forssmann W. G. (1995) cDNA sequence and expression pattern of the putative pheromone carrier aphrodisin. Proc. Natl. Acad. Sci USA **92:** 2091–2095
33 Sansom C. E., North A. C. T. and Sawyer L. (1994) Structural analysis and classification of lipocalins and related proteins using a profile-search method. Biochim. Biophys. Acta **1208:** 247–255
34 Flower D. R. (1996) The lipocalin protein family: structure and function. Biochem. J. **318:** 1–14
35 Cowan S. W., Newcomer M. E. and Jones T. A. (1990) Crystallographic refinement of human serum retinol binding protein at 2 Å resolution. Proteins **8:** 44–61
36 Monaco H. L., Rizzi M. and Coda A. (1995) Structure of a complex of two plasma proteins: transthyretin and retinol-binding protein. Science **268:** 1039–1041
37 Holden H. M., Rypniewski W. R., Law J. H. and Rayment I. (1987) The molecular structure of insecticyanin from the tobacco hornworm *Manduca sexta* L. at 2.6 Å resolution. EMBO J. **6:** 1565–1570
38 Flower D. R., Sansom C. E., Beck M. E. and Attwood T. K. (1995) The first prokaryotic lipocalins. Trends Biochem Sci. **20:** 498–499
39 Schmale H., Holtgreve-Grez H. and Christiansen H. (1990) Possible role for salivary gland protein in taste reception indicated by homology to lipophilic-ligand carrier proteins. Nature **343:** 366–369
40 Garibotti M., Christiansen H., Schmale H. and Pelosi P. (1995) Porcine VEG proteins and tear prealbumins. Chem. Senses **20:** 69–76
41 Redl B., Holzfeind P. and Lottspeich F. 1992. cDNA cloning and sequencing reveals human tear prealbumin to be a member of the lipophilic-ligand carrier protein superfamily. J. Biol. Chem. **267:** 20282–20287
42 Holzfeind P., Merschak P., Rogatsch H., Culig Z., Feichtinger H. Klocker H. et al. (1996) Expression of the gene for tear lipocalin/von Ebner's gland protein in human prostate. FEBS Lett. **395:** 95–98
43 Scalfari F., Castagna M., Fattori B., Andeini I., Maremmani C. and Pelosi P. (1998) Expression of a lipocalin in human nasal mucosa. Comp. Biochem. Physiol. **118B:** 819–824
44 Miyawaki A., Matsushita F., Ryo Y. and Mikoshiba K. (1994) Possible pheromone-carrier function of two lipocalin proteins in the vomeronasal organ. EMBO J. **13:** 5835–5842
45 Spielman A. I., Zeng X. N., Leyden J. J. and Preti G. (1995) Proteinaceous precursors of human axillary odor: isolation of two novel odor-binding proteins. Experientia **51:** 40–47
46 Zeng C., Spielman A. I., Vowels B. R., Leyden J. J., Biemann K. and Preti G. (1996) A human axillary odorant is carried by apolipoprotein D. Proc. Natl. Acad. Sci. USA **93:** 6626–6630
47 Rouvinen J., Rautiainen J., Virtanen T., Zeiler T., Kauppinen J., Taivainen A. et al. (1999) Probing the molecular basis of allergy. three-dimensional structure of the bovine lipocalin allergen Bos d 2. J. Biol. Chem. **274:** 2337–2343
48 Crossett B., Allen W. R. and Stewart F. (1996) A 19 kDa protein secreted by the endometrium of the mare is a novel member of the lipocalin family. Biochem. J. **320:** 137–143
49 Bianchet M. A., Bains G., Pelosi P., Pevsner J., Snyder S. H., Monaco H. L. et al. (1996) The three dimensional structure of bovine odorant-binding protein and its mechanism of odor recognition. Nat. Struct. Biol. **3:** 934–939
50 Tegoni M., Ramoni R., Bignetti E., Spinelli S. and Cambillau C. (1996) Domain swapping creates a third putative combining site in bovine odorant binding protein dimer. Nat. Struct. Biol. **3:** 863–867
51 Spinelli S., Ramoni R., Grolli S., Bonicel J., Cambillau C. and Tegoni M. (1998) The structure of the monomeric porcine odorant binding protein sheds light on the domain swapping mechanism. Biochemistry **37:** 7913–7918

52 Böcskei Z., Groom C. R., Flower D. R., Wright C. E., Phillips E. V., Cavaggioni A. et al. (1992) Pheromone binding to two rodent urinary proteins revealed by X-ray crystallography. Nature **360:** 186–188
53 Napolitano E. and Pelosi P. (1992) Synthesis of thiazole and selenazole derivatives with affinity for the odorant-binding protein. Bioorg. Med. Chem. Lett. **2:** 1603–1606
54 Pevsner J., Hou V., Snowman A. M. and Snyder S. H. (1990) Odorant-binding protein characterization of ligand binding. J. Biol. Chem. **265:** 6118–6125
55 Paolini S., Tanfani F., Fini C., Bertoli E. and Paolo Pelosi (1999) Porcine odorant-binding protein: structural stability and ligand affinities measured by fourier-transform infrared spectroscopy and fluorescence spectroscopy. Biochim. Biophys. Acta **1431:** 179–188
56 Pelosi P. and Tirindelli R. 1989. Structure/Activity studies and characterization of an odorant-binding protein. In: Chemical Senses. Vol. 1. Receptor events and transduction in taste and olfaction, pp. 207–226, Brand J. G., Teeter J. H., Cagan R. H. and Kare M. R. (eds), Marcel Dekker, New York
57 Dal Monte M., Centini M., Anselmi C. and Pelosi P. (1993) Binding of selected odorants to bovine and porcine odorant-binding proteins. Chem. Senses **18:** 713–721
58 Hérent M. F., Collin S. and Pelosi P. (1995) Affinities of nutty and green-smelling compounds to odorant-binding proteins. Chem. Senses **20:** 601–610
59 Bacchini A., Gaetani E. and Cavaggioni A. (1992) Pheromone-binding proteins in the mouse *Mus musculus*. Experientia **48:** 419–421
60 Robertson D. H. L., Beynon R. J. and Evershed R. P. (1993) Extraction characterisation and binding analysis of two pheromonally active ligands associated with major urinary protein of the house mouse (*Mus musculus).* J. Chem. Ecol. **19:** 1405–1416
61 Loebel D., Marchese S., Krieger J., Pelosi P. and Breer, H. (1998) Subtypes of odorant binding proteins: heterologous expression and assessment of ligand binding. Eur. J. Biochem. **254:** 318–324
62 Clissold P. M., Hainey S. and Bishop J. O. (1984) Messenger RNAs coding for mouse major urinary proteins are differentially induced by testosterone. Biochem. Genet. **22:** 379–387
63 Johnson D., al-Shawi R. and Bishop J. O. (1995) Sexual dimorphism and growth hormone induction of murine pheromone-binding proteins. J. Mol. Endocrinol. **14:** 21–34
64 Marchlewska-Koj A., Pochron E. and Sliwowska A. (1990) Salivary glands and preputial glands of males as source of estrus-stimulating pheromone in female mice. J. Chem. Ecol. **16:** 2817–2822
65 Pevsner J., Sklar P. B. and Snyder S. H. (1986) Odorant-binding protein: localization to nasal glands and secretions. Proc. Natl. Acad. Sci. USA **83:** 4942–4946
66 Pevsner J., Hwang P. M., Sklar P. B., Venable J. C. and Snyder S. H. (1988) Odorant-binding protein and its mRNA are localized to lateral nasal gland implying a carrier function. Proc. Natl. Acad. Sci. USA **85:** 2383–2387
67 Avanzini F., Bignetti E., Bordi C., Carfagna G., Cavaggioni A., Ferrari G. et al. (1987) Immunocytochemical localization of pyrazine-binding protein in the cow nasal mucosa. Cell Tissue Res. **247:** 461–464
68 Krishna N. S., Getchell M. L., Margolis F. L. and Getchell T. V. (1995) Differential expression of vomeromodulin and odorant-binding protein, putative pheromone and odorant transporters, in the developing rat nasal chemosensory mucosae. J. Neurosci. Res. **40:** 54–71
69 Ohno K., Kawasaki Y., Kubo T. and Tohyama M. (1996) Differential expression of odorant-binding protein genes in rat nasal glands: implications for odorant-binding proteinII as a possible pheromone transporter. Neuroscience **71:** 355–366
70 Utsumi M., Ohno K., Kawasaki Y., Tamura M., Kubo T. and Tohyama M. (1999) Expression of major urinary protein genes in the nasal glands associated with general olfaction. J. Neurobiol. **39:** 227–236
71 Novotny M., Harvey S. and Jemiolo B. (1990) Chemistry of male dominance in the house mouse *Mus domesticus*. Experientia **46:** 109–113
72 Jemiolo B., Xie T.M. and Novotny M. (1992) Urine marking in male mice: responses to natural and synthetic chemosignals. Physiol. Behav. **52:** 521–526
73 Mucignat-Caretta C., Caretta A. and Cavaggioni A. (1995) Acceleration of puberty onset in female mice by male urinary proteins. J. Physiol. **486:** 517–522
74 Dulac C. and Axel R. (1995) A novel family of genes encoding putative pheromone receptors in mammals. Cell **83:** 195–206
75 Ryba N.J. and Tirindelli R. (1997) A new multigene family of putative pheromone receptors. Neuron **19:** 371–379
76 Herrada G. and Dulac C. (1997) A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell **90:** 763–773
77 Matsunami H. and Buck L. B. (1997) A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell **90:** 775–784
78 Krieger J., Schmitt A., Lobel D., Gudermann T., Schultz G., Breer H. et al. (1999) Selective activation of G protein subtypes in the vomeronasal organ upon stimulation with urine-derived compounds. J. Biol. Chem. **274:** 4655–4662.