Critical Reviews in Biochemistry and Molecular Biology, 29(3):199–228 (1994)

Odorant-Binding Proteins

Paolo Pelosi

Istituto di Industrie Agrarie, via S. Michele, 4, 56124 Pisa, Italy

Referee: Dr. Frank Margolis, Roche Institute of Molecular Biology, Nutley, NJ 07110

ABSTRACT: Odorant-binding proteins (OBPs) are low-molecular-weight soluble proteins highly concentrated in the nasal mucus of vertebrates and in the sensillar lymph of insects. Their affinity toward odors and pheromones suggests a role in olfactory perception, but their physiological function has not been clearly defined. Several members of this class of proteins have been isolated and characterized both in insects and vertebrates; in most species two or three types of OBPs are expressed in the nasal area. Vertebrates OBPs show significant sequence similarity with a superfamily of soluble carrier proteins called lipocalins. They include some proteins of particular interest that are thought to be involved in the mechanism of releasing and modulating chemical messages with pheromonal activity.

The data on vertebrate OBPs are here reviewed together with the most relevant information on related proteins. Theories and models of the physiological functions of odorant-binding proteins are presented and discussed.

KEY WORDS: olfaction, carrier proteins, pheromone-binding proteins, perireceptor events, chemical communication.

I. INTRODUCTION

The biochemical study of olfaction had been almost completely neglected until the early 1980s, despite the opportunities for basic research to provide exciting and fundamental insights. Olfaction did not pique the interests of biochemists because it could not offer any obvious applications in medicine and because the information available on the olfactory system at the molecular level was so scarce that it was considered too risky to waste time and energy in an area where the rewards of scientific research were still uncertain.

Chemists, however, had been accumulating data on structure-odor relationships (Beets, 1978), using human subjects for defining odor qualities and olfactory thresholds; perfumes and food flavors were the main fields of interest, because of their wide commercial applications; however, they could cover most of the chemical structures that exhibited an odor. Therefore, by the end of the 1970s, there was such a wealth of data in the literature on odor and molecular structure that well-documented working models were accepted on how the olfactory system would interact with different volatile molecules and generate the corresponding odor sensations.

The exceptional power of discrimination of the olfactory system required that receptor proteins should be the peripheral-sensing elements. In the meantime, Amoore had indicated through the study of specific anosmias, which is the odor-blindness to particular classes of volatiles, a way to break the olfactory code. The identification of several types of specific anosmias in the human population and the demonstration that this phenomenon is genetically inherited greatly contributed to a basis for future work in biochemistry and molecular biology of olfaction (Amoore, 1967, 1977; Whissell-Buechy and Amoore, 1973; Amoore and Steinle, 1991).

Strangely enough, while theories of olfaction were still being discussed, the basic mechanisms of bacterial chemotaxis had been characterized and some of the proteins involved had been already

1040-9238/94/$.50

purified (Koshland, 1981). The concept that different forms of chemical communication, from neurotransmitter signaling to vertebrate olfaction and taste, could share the same biochemical mechanisms did not initially seem so obvious until more experimental evidence was provided.

At the beginning two events stimulated biochemical research in olfaction: (1) the identification of odorant-binding proteins (OBPs), which are still the only class of olfactory proteins for which binding of odorants has been experimentally demonstrated (Pelosi et al., 1982), and (2) the discovery that odorants could produce an activation of adenylate cyclase (Kurihara et al., 1972; Minor and Sakina, 1973). This latter observation led to the identification of olfactory G proteins (Pace and Lancet, 1986; Anholt et al., 1987) and, subsequently, to the major breakthrough in the biochemistry of olfaction, the identification of a new family of genes expressed in the olfactory epithelium, coding for putative olfactory receptors (Buck and Axel, 1991).

Thus, in less than 10 years, olfaction has become one of the most active areas of biochemical research and has found a prominent place in the field of the neurosciences. It cannot be overstressed the tremendous contribution given by the pioneering work of Graziadei's laboratory on the development of the olfactory tissue. The discovery that olfactory neurons could regenerate and the clear demonstration of neurons migrating from the olfactory area to the brain during early development demonstrated the olfactory system to be a most interesting model for investigating several aspects of the central nervous system (Graziadei and Dehann, 1973; Graziadei and Levine, 1978; Graziadei and Monti-Graziadei, 1980).

This review summarizes the data so far available on odorant-binding proteins, both in vertebrates and insects, giving also a brief account of other proteins similar to OBPs in their structure or function. Hypotheses are also discussed on the physiological role that OBPs might have in odor perception and discrimination. To help the reader understand the place and the mode of action of OBPs, located between the odor stimuli and the neuronal sensory membrane, two introductory sections are presented, the first on the relationships between odor and chemical structure and the second on the biochemical mechanisms of olfactory transduction, from membrane receptors to ion channels. These latter aspects of olfactory perception have been reviewed recently by Anholt (1993).

While the best effort has been taken to review all the literature related to the central object of this article, the odorant-binding proteins, for other aspects of olfaction only a brief summary is given with a few selected references.

II. CHEMICAL STRUCTURES OF ODORANT MOLECULES

The starting point for a study of the olfactory system is the definition and classification of the stimuli, the odorant molecules. An odorant can be defined as any molecule capable of stimulating the olfactory neurons.

In air-breathing animals, virtually any chemical volatile enough to reach the olfactory epithelium and made up of not more than about 20 major atoms (i.e., excluding hydrogens) can elicit an odor sensation. There are very few exceptions to this rule. In fact, completely odorless volatile chemical compounds are extremely rare.

The volatility of a substance depends on the strength of intermolecular forces; therefore, when strong ionic or polar interactions are present, the volatility is minimal; as a consequence, charged compounds, such as salts and amino acids, are odorless, and those with more than one polar group are, in most cases, weak odorants. The situation is completely different in aquatic animals, where amino acids are among the best odorants.

The hydrophobic character seems to be positively correlated with odor strength not just through the volatility effect; numerous examples show that in homologous series of chemicals, such as straight-chain alcohols or acids, medium-size members, which are more hydrophobic but less volatile than lower members, are also stronger odorants.

Although we can say that nearly all small volatile compounds have an odor, their potencies vary across several orders of magnitude, so that when compared with the strongest odorants at equal concentrations a great number of chemicals can be considered as practically odorless. This

fact has profound implications in food science and perfumery, both when identifying in a complex mixture of volatiles those few components that give the major contributions to the overall odor and when trying to reproduce natural flavors or to create new fragrances. For biochemical research it is of the utmost importance to choose the strongest odorants as probes, being very likely those that would make strong interactions with the olfactory receptors.

A measure of the strength of an odorant is its olfactory threshold, the lowest concentration that can be perceived by a statistically significant sample of the human population. The values of olfactory thresholds depend on the method used, but comparison of data obtained with the same method are satisfactorily significant. Moreover, when comparing values of different orders of magnitude, as is the case of olfactory thresholds, experimental errors become negligible.

Figure 1 reports the structures of some common odorants whose thresholds span a range of about eight orders of magnitude. A few examples are included of extremely strong odorants, such as 2-isobutyl-3-methoxypyrazine, that could indicate a particularly tight and specific binding with

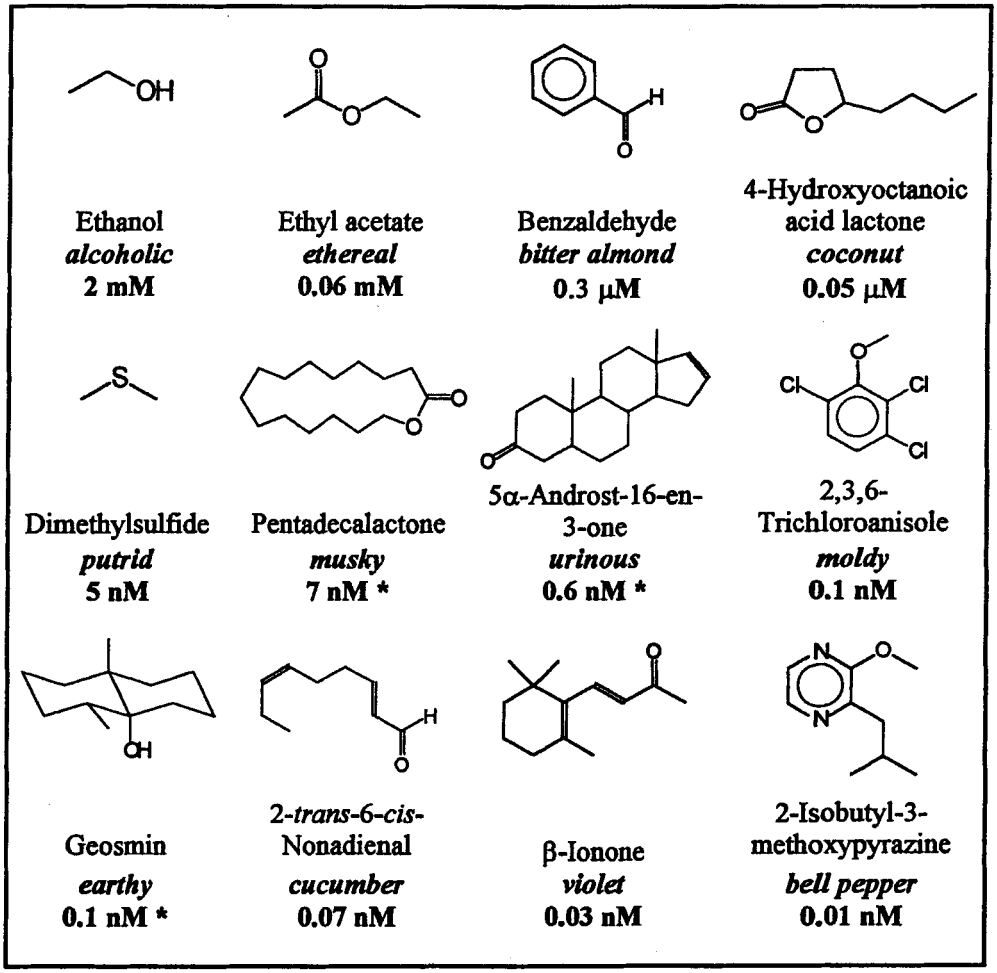

FIGURE 1. Olfactory thresholds of typical odorants. The values indicate the lowest concentrations in water perceivable by human subjects and have been obtained using the method of Guadagni et al., 1963, except for those marked with an asterisk. These have been measured according to Amoore et al. (1968) and can be compared with the other values after dividing by a factor of 6, on the average, as suggested by Amoore and Buttery (1978).

olfactory receptors. These molecules are therefore considered the best biochemical probes when studying the corresponding receptor proteins; in fact, the low-dissociation constants expected would make binding experiments easier to perform and more accurate; moreover, the same probes are likely to be very specific and detect only one or a small number of receptor proteins.

The quality of an odor is far more difficult to describe and measure than its intensity. Attempts to classify different odors have been made since ancient times, but no agreement has been reached on even the number of their classes. Perhaps the most reliable data have been obtained by screening the different types of specific anosmias (Amoore, 1977; Amoore and Forrester, 1976; Amoore et al., 1975, 1976, 1977; Pelosi and Viti, 1978; Pelosi and Pisanelli, 1981). Along with this approach, a number of (about 30) "primary odors" have been suggested; these should match an equivalent number of olfactory receptors, much in the same way as the color vision is based on three primary colors. Under this hypothesis, most of the odor sensations would be generated as a combination of elementary signals produced by the peripheral neurons. On the number of primary odors there is still much debate, especially after the recent discovery of a family of putative olfactory receptors numbering several hundreds of members. Certainly, the number of primary odors cannot be very small, because, in this case, we would be able to break down a complex odor into its components, as we do with colors; on the other hand, it seems unnecessary to have a very large number of receptors, when considering that with only three types of rhodopsins we are able to distinguish hundreds or thousands of different color shades. A number around a few dozen types of basic odors, in agreement with the data of specific anosmia, seems a reasonable hypothesis.

The number of olfactory receptors is also related to their specificities. In principle, we can cover the wide map of molecular structures that are perceived as an odor either with a great number of very specific receptors or with a handful of broadly tuned receptors. Looking at other sensory systems, both approaches can be found in nature: the auditory system makes use of a very large number of narrowly tuned sensors, each responding only to a particular wavelength value; color vision, to the contrary, utilizes just three light-sensitive elements with very broad absorption spectra; this poor specificity at the peripheral level, however, does not prevent a very fine discrimination of the input signals once they have been processed by the brain. So, as far as we know, either solution (or both) can have been adopted in the olfactory system, and the question of the number of primary odors still remains open.

Another major question regards the molecular parameters that can be best correlated with the various odor qualities. The great majority of data available in this area have indicated that stereochemical aspects are more important than functional groups. Thus, several examples can be presented of molecules with different functional groups, but similar size and shape, that also exhibit similar odors. On the other hand, molecules belonging to the same chemical class (i.e., with the same functional group but of different size or shape) usually are also different in their odor quality. Well-known examples of this concept are given in Figure 2.

A more detailed treatment of the relationships between chemical structure and odor is beyond the aim of this review; the relevant literature has been summarized in some excellent books (Beets, 1978; Theimer, 1982; Olhoff, 1990).

In conclusion, it is worth pointing out that olfactory receptor proteins should be able to discriminate odorant molecules on the basis of the same parameters (size and shape) recognized by psychophysical measurements as best related to odor descriptions.

III. BIOCHEMICAL MECHANISMS OF OLFACTORY TRANSDUCTION

The first biochemical work in olfaction was the discovery, about 20 years ago, of the olfactory marker protein (OMP), a soluble protein of 19 kDa expressed uniquely in the olfactory neurons in all the species examined, from salamander to humans (Margolis, 1972; Danciger et al., 1989; Rogers et al., 1987). Although this protein has been thoroughly studied from every point of view, its physiological function still remains unknown.

In the following years, attempts to identify olfactory receptors were made in several labora-

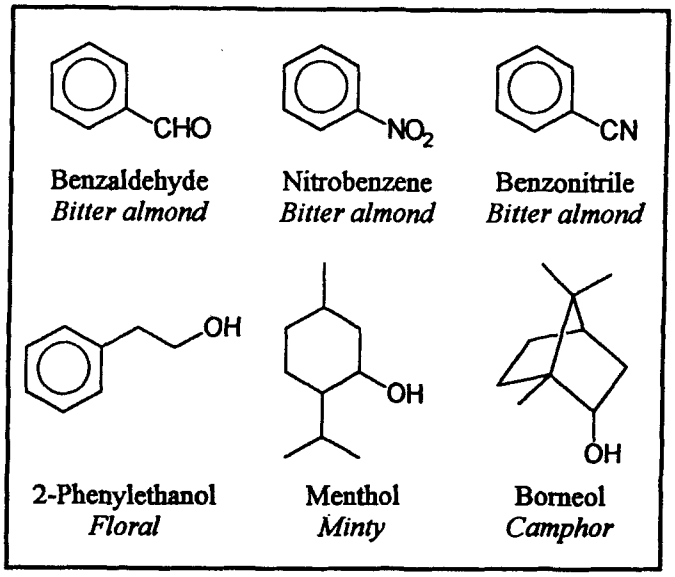

FIGURE 2. Odor and molecular structure. Compounds bearing different functional groups, such as benzaldehyde, nitrobenzene, and benzonitrile, may have similar odors, provided their oriented profiles are similar. On the other hand, molecules belonging to the same chemical class, but different in size or shape, such as the three alcohols reported, can present markedly different odors.

tories by selecting proteins unique to the olfactory area. This approach did not prove successful in the search for membrane-bound receptors for odors, but yielded several candidates, different in their characteristics, that were not further investigated or were later shown to perform other functions. Several techniques and criteria have been adopted, such as binding experiments with radioactive odorants (Cagan and Zeiger, 1978; Rhein and Cagan, 1980; Fesenko et al., 1979, 1983,1987, 1988; Novoselov et al., 1980; Pelosi et al., 1981, 1982; Persuad et al., 1988), affinity chromatography on immobilized odorants (Price 1978; Price and Willey, 1987; Goldberg et al., 1979), and electrophoresis separation of proteins of the olfactory cilia (Chen and Lancet, 1984a, 1984b; Chen et al., 1986).

However, the discovery of olfactory receptors came only recently, as a consequence of a series of studies that have brought out the main elements of the olfactory transduction cascade.

Thus, the efforts of scientists were concentrated on the intracellular enzymes that were known to be involved in transduction mechanisms of many diverse signaling systems, because their presence could have suggested what the nature of olfactory receptors was likely to be.

The first element of this jigsaw puzzle to be identified was the involvement of an adenylate cyclase with production of the second messenger cAMP on stimulation with odorants (Kurihara et al., 1972; Minor and Sakina, 1973; Menevse et al., 1977; Pace et al., 1985; Shirley et al, 1986; Sklar et al., 1986; Lowe et al., 1989; Ludwig et al., 1990). Experiments performed in several laboratories indicated that the concentration of cyclic AMP in olfactory tissue increased by about 50% after stimulation with several odorants. Such an increase, although significant, was much lower than what had been expected. Only fast kinetic experiments, performed later with preparations of olfactory cilia very elegantly showed that the increase was at least 3 to 4 fold, but the levels decayed very rapidly in a fraction of a second (Breer et al., 1990a). The reason for this rapid decrease was found to be the presence of an active

mechanism for terminating the olfactory signal: this mechanism involves the action of protein kinases, much in the same way as is known to occur in the case of β-adrenergic receptors (Boekhoff and Breer, 1992; Schleicher et al., 1993).

By analogy with neurotransmitter receptor systems, it was suggested that another transduction mechanism, involving production of inositol triphosphate (IP_3) could also take place in olfaction. Experimental data confirmed this hypothesis (Huque and Bruch, 1986; Kalinoski et al., 1992) and indicated that the synthesis of cyclic AMP or of IP_3 could be alternatively stimulated by different odorants (Boekhoff et al., 1990; Breer, 1991; Breer and Boekhoff, 1991).

These results fitted very well with the discovery made in the meantime by several groups that G proteins were involved in olfactory transduction and that a new type of G protein called G_{olf} was highy enriched in olfactory neurons (Pace and Lancet, 1986; Anholt et al., 1987; Jones and Reed, 1989).

This series of findings provided the basis for a well-focused search for the olfactory receptors. Having demonstrated the presence in the olfactory neuron of the enzyme cascade known elsewhere to be coupled to β-adrenergic receptors, it was reasonable to assume that olfactory receptors should be related to the protein superfamily that included β-adrenergic receptors. Thus, a search for membrane-bound proteins unique to the olfactory tissue and belonging to the seven transmembrane region family, known to be coupled to G proteins, led to the discovery of a very large multigene family, assumed to code for the long-sought olfactory receptor proteins (Buck and Axel, 1991).

This discovery stimulated the interest of several groups who dedicated their energies to research programs centered around this family of receptors. As a consequence, in a very short time many interesting papers have been published on the structural and functional characterization of such receptors. The topographical localization of olfactory receptor proteins in the rat (Buck, 1993) has indicated that they are selectively expressed in domains well distinct from each other. On the contrary (Ngai et al., 1993a, 1993b), in catfish there is a complete lack of organization: both results support what electrophysiological measurements had indicated in the past (Shepherd, 1985). Cloning the putative olfactory receptors has also provided the tools for a study of olfaction at the genomic level in humans and eventually correlating biochemistry with the very large number of data on olfactory psychophysics (Lancet et al., 1993). Expression of olfactory receptors has also been successfully reported in heterologous systems (Raming et al., 1993). Finally, a transcription factor, called Olf-1, has been identified and cloned that activates the expression of several olfactory proteins, such as OMP, specific G protein, adenylate cyclase, and a cyclic nucleotide gated ion channel (Kudrycki et al., 1993; Wang and Reed, 1993).

IV. PERIRECEPTOR EVENTS

When odorants enter the nasal cavity two types of processes occur. One is the specific interaction with receptor proteins on the membrane of the olfactory cilia. But, before reaching the neuronal membrane, the odorant molecules have to cross a thick layer of mucus containing high concentrations of several classes of proteins that may interact with the volatile compounds. These processes, which occur in the proximity of the olfactory receptors, have been given the name "perireceptor events" (Getchell et al., 1984).

The olfactory mucus, like other types of protective mucus, is very complex in its composition and several aspects remain to be investigated. Proteins belonging to different classes are very abundant in the mucus and perform different functions (Getchell et al., 1987, 1993, Getchell and Getchell, 1991).

The mucins are large proteins (250 to 1000 kDa) highly glycosylated that give consistency and thickness to the mucus. Apart from these structural proteins, the mucus is rich in antibodies, antibacterial proteins, such as lisozyme, carrier proteins, detoxifying enzymes, and other proteins of yet-unknown function.

While odorant-binding proteins are discussed later in greater detail, a brief description is given

here of some other interesting proteins secreted in the olfactory mucus.

Olfactomedin is a very abundant protein in the olfactory mucus of the frog. It is a glycoprotein of 57 kDa (Snyder et al., 1991; Bal and Anholt, 1993; Yokoe and Anholt, 1993) and is synthesized in sustentacular cells and Bowman's glands. Through disulfide bonds it forms polymers that are the main structural constituents of the mucus matrix. Its association with the olfactory cilia is so tight that, like the enzyme UDP glucuronosyl transferase, was originally thought to be one of the ciliary membrane proteins. This strong binding to ciliary structures could suggest a role for olfactomedin in the mechanism of odor perception, but no further data have been provided to support such hypothesis. It would be desirable to have information concerning odor-binding activity of this protein and its presence in other animal species.

Another glycoprotein of the mucus is vomeromodulin, identified in the rat, where it is secreted by the lateral-nasal glands. It is a polypeptide of about 60 kDa that is glycosylated to produce a mature protein of 70 kDa (Khew-Goodall et al., 1991). It is abundant in the mucus of the vomeronasal organ, but not in the olfactory area. It has been proposed that this protein, like olfactomedin, mediates odor perception, in this case delivering pheromonal odorants to the vomeronasal organ; however, binding data have not been reported so far.

Three more genes have been specifically identified in secretory cells of the olfactory tissue in the rat. They are called RYA3, RY2G5, and RYD5 and encode proteins of 473, 470, and 94 amino acids, respectively. The first two proteins share about 20% of their amino acids with the human neutrophil bactericidal protein, while the third sequence shows nearly 30% similarity with binding proteins for steroids and polychlorinated biphenyls. It has been suggested that these could be carrier proteins for odorant molecules, although no direct evidence of binding activity has been reported (Dear et al., 1991a).

Finally, albumin should be included in the list of mucus proteins. Although this is not a specific protein of the olfactory area, its well known function of carrier for hydrophobic molecules in the serum could also be performed in the olfactory mucus.

The chain of detoxifying enzymes is well expressed in the olfactory area; its activity is comparable or higher than that measured in the liver. Cytochromes P-450 have been described in the olfactory epithelium and specific forms have been cloned (Dahl, 1988; Ding and Coon, 1988, 1990; Ding et al., 1991; Nef et al., 1989). UDP glucuronosyl transferases are among the most abundant proteins that are associated with olfactory membranes; several isoforms have been identified and purified (Longo et al., 1988; Lazard et al., 1990), and their amino acid sequences have been determined through cloning (Lazard et al., 1991). Glutathione transferase has also been shown to be present in the olfactory epithelium and active toward odorant molecules (Rama-Krishna et al., 1992).

The tight association of these enzymes with olfactory membranes has suggested a role in odor perception, in particular in the signal termination process. This hypothesis seems not well grounded on the basis that, like their liver forms, these enzymes are particularly active toward aromatic compounds and would leave a great number of strong aliphatic odorants unaffected. A detoxifying function, on the other hand, seems appropriate and necessary in the proximity of functionally important neurons that are exposed to the external environment.

V. ODORANT-BINDING PROTEINS OF VERTEBRATES

A. History

The term OBPs (odorant-binding protein) refers to a class of small soluble proteins that have been actively studied in the past decade for their property of binding several odorant molecules. They were discovered at the beginning of the 1980s in the search for olfactory receptors using radioactively labeled odorants in ligand-binding experiments (Pelosi et al., 1981, 1982). At the beginning they were called "pyrazine-binding proteins" for their ability to bind 2-isobutyl-3-methoxypyrazine and odorants of related struc-

ture. Later, when odorants of different chemical classes were found to be as good ligands as the pyrazines, the more general name "odorant-binding proteins", as suggested by Pevsner et al. (1986), was preferred.

Although OBPs are the class of soluble olfactory proteins that have been studied most extensively, other proteins have been occasionally reported to present odor-binding activity.

As briefly mentioned at the beginning of Section III, several research groups, attempting to identify olfactory receptors, have chosen the approach of ligand-binding studies, using labeled volatile compounds that were known to be good odorants for the species studied, or at least for humans. Such experiments provided data that were sometimes difficult to reproduce and generally limited to the species used. When the binding proteins were purified, no further characterization followed in most cases, either in terms of structure or site of production.

The first data of this type have been obtained in fishes, where odorants are represented by some amino acids. The availability of radioactive amino acids, as well as the anatomy of the olfactory organ that allowed easy dissection have indicated fishes as the most suitable species for such experiments. Binding activity was found to be associated with olfactory cilia, but a function of the relative proteins in amino acid transport (Brown and Hara, 1981), rather than olfactory recognition, could not be excluded (Cagan and Zeiger, 1978; Rhein and Cagan, 1980; Novoselov et al., 1980; Fesenko et al., 1983).

Using the steroid 5α-androstan-3-one, closely related to the sow sex pheromone 5α-androst-16-en-3-one, binding activity has been described both in porcine and sheep olfactory tissue, but the proteins responsible for such activity were not isolated or further characterized. The main problem associated with this odorant was its extreme hydrophobicity, which accounted for a very high background in the binding experiments and difficulty in reproducibility (Persaud et al., 1988).

Using a different approach, Price managed to purify two proteins from dog olfactory epithelium by affinity chromatography. The affinity gels were prepared by immobilizing *p*-anisic acid or *p*-carboxybenzaldehyde on Sepharose and, in each case, was reported to result in a single protein species from a crude extract of olfactory tissue (Price, 1978; Price and Willey, 1987). Antibodies raised against the anisole-binding protein could reduce the amplitude of the electrophysiological response to several odors (Goldberg et al., 1979), but so could control immunoglobulins. The proteins have not been further characterized, nor was it reported that such data had been reproduced in other laboratories.

Binding data have also been obtained with proteins selected on the basis that they were unique to the olfactory tissue: a protein purified from rat olfactory epithelium was shown to bind the hydrophobic odorants camphor and decanal (Fesenko et al., 1979, 1987, 1988).

Some of these proteins have been proposed as candidates for being the membrane-bound olfactory receptors, but definite proof has not been provided for any of them.

It is interesting to note that all these data originated from searches for the olfactory receptors. The possibility that soluble odorant-binding proteins could exist, as in the system of bacterial chemotaxis, was not even considered.

The same idea guided the experiments that led to the identification of the first OBP. Binding assays were performed with a radioactive sample of 2-isobutyl-3-methoxypyrazine, one of the most potent odorants for humans (Figure 1). The choice of this odorant was based on the assumption that a low olfactory threshold could indicate a strong binding with the specific receptor; it was also assumed, in the absence of experimental data, that strong odorants for humans would also be strong in other species of mammals. Using this probe, a binding protein was found in the nasal mucosa of the cow and other mammals (Pelosi et al., 1981, 1982). Later, this binding protein, which was named OBP, was purified from bovine nasal tissue and chemically characterized (Bignetti et al., 1985, 1987a, 1987b). It is a 19-kDa polypeptide chain that is present in the native state as a homodimer; it binds the labeled 2-isobutyl-3-methoxypyrazine with a dissociation constant of 3 μ*M* and is abundantly present in the nasal mucus. The perfect agreement between these data and those of an independent report published at the same time (Pevsner et al., 1985) gave better confidence and stimulated further research in this field. It was later shown that the bovine OBP is

secreted by tubular-acinar glands underlining the nasal respiratory epithelium, thus definitely ruling out the possibility that this protein could be an olfactory receptor (Avanzini et al., 1987; Pevsner et al., 1986).

At present, several OBPs have been purified from the nasal tissue of different species of vertebrates and characterized to different extents. The soluble nature and the relatively small size of these proteins made structural studies comparatively easy. In spite of the extensive information available for these proteins, their physiological function is not yet clear. However, it is generally assumed that they are in some way involved in the process of olfactory perception.

B. Biochemical Properties

Odorant-binding proteins of vertebrates are soluble proteins of acidic nature (pI around 4 to 5) and small size, with subunit molecular weights around 20 kDa. Several of them have been shown to be present as dimers, but others seem to exist as monomeric species. They are secreted by different glands of the nasal cavity and released in the nasal mucus, where their concentration, although difficult to measure, has been estimated to be very high, possibly reaching millimolar values (Pelosi and Maida, 1990; Pelosi and Garibotti, 1992; Pevsner and Snyder, 1990; Snyder et al., 1988).

They reversibly bind several odorants with dissociation constants in the micromolar range. Their specificity toward various ligand structures is rather poor and seems oriented toward hydrophobic molecules of medium size (Pelosi and Tirindelli, 1989; Pevsner et al., 1990).

The determination of the amino acid sequence indicated that OBPs belong to the large family of carrier proteins called lipocalins, generally involved in the transport of hydrophobic molecules in aqueous media (Pevsner et al., 1988b). The establishment of the three-dimensional structure for the bovine OBP strengthened the similarity with the above class of proteins (Monaco and Zanotti, 1992).

Such structural similarity suggested similar functions for the proteins of the lipocalin family; thus, OBPs would perform a role of nonspecific carriers of the odorant molecules to and from the membrane receptor proteins; this nondiscriminating function was also based on the low specificity of binding, as well as on the fact that, until recently, a single type of OBP was reported to be expressed in each animal species. However, the discovery of several classes of odorant-binding proteins may suggest new hypotheses for the physiological function of OBPs, including a role in odor discrimination.

C. OBPs in Different Species of Vertebrates

The elusive function of OBPs stimulated research aimed at the identification and characterization of these proteins in different species of vertebrates; such information would help to better classify odorant-binding proteins and might provide insights on their physiological role in olfaction.

As soon as the amino acid sequences of the OBPs from bovine (Cavaggioni et al., 1987; Tirindelli et al., 1989) and from rat (Pevsner et al., 1988b) became available, it was clear that they represented a class of poorly conserved proteins, sharing on the average only about 30% of their amino acids. This fact was reflected in the lack of immuno cross-reactivity that precluded the use of antibodies in the search for odorant-binding proteins in other animal species. The use of oligonucleotides as probes for detecting similar sequences was also made difficult by such poor similarity.

So far, the main criterion for the identification of new OBPs has been the binding activity to odorants, in particular to the bell pepper odor 2-isobutyl-3-methoxypyrazine. Sequence analysis then, when available, provided further information for correctly classifying the protein. In a few cases similarity of amino acid sequences was the only criterion besides the exclusive presence in the nasal area for assigning newly identified proteins to the class of OBPs.

Table 1 lists the physicochemical characteristics of the OBPs that have been purified from vertebrates. With a single exception, all the vertebrate OBPs so far described have been studied in mammals.

TABLE 1
Odorant-Binding Proteins Purified from the Nasal Tissue of Vertebrates

Protein	Animal species	K_D (μM)	M.W. (kDa)	pI	Sequence	Ref.
bov-**OBP**	Bovine	3.0	2 × 19	4.7	Complete	1–4
rat-**OBP-I**	Rat	20	2 × 18		cDNA	5, 6
rat-**OBP-II**	Rat		23		cDNA	7
rab-**OBP-I**	Rabbit	0.8	2 × 19	4.7	Internal	8
rab-**OBP-II**	Rabbit		22		N-terminal	9
pig-**OBP-I**	Pig	0.5	22	4.2	Internal	8
pig-**OBP-II**	Pig		17		Internal	10
mus-**OBP-I**	Mouse		18 + 19	4.9		10, 11
mus-**OBP-II**	Mouse		21	4.8	N-terminal	10, 11
mus-**OBP-III**	Mouse		22	4.2	N-terminal	10
hys-**OBP-I**	Porcupine	0.1	21–24[a]	4.2–4.6	internal	10, 12
hys-**OBP-II**	Porcupine		18–19[b]	4.3–4.4	internal	10, 12
deer-**OBP-I**	Deer		18	5.1		13
deer-**OBP-II**	Deer		21	4.7		13
cat-**OBP**	Cat		2 × 20	4.5	Internal	10
frog-**BG**	Frog		20		cDNA	14

Note: Binding constants have been measured with tritiated 2-isobutyl-3-methoxypyrazine. (1) Pelosi et al., 1982; (2) Bignetti et al., 1985; (3) Pevsner et al., 1985; (4) Tirindelli et al., 1989; (5) Pevsner et al., 1986b; (6) Pevsner et al., 1988; (7) Dear et al., 1991b; (8) Dal Monte et al., 1991; (9) unpublished data; (10) Felicioli et al., 1993b; (11) Pes et al., 1992; (12) Felicioli et al., 1993a; (13) Ganni et al., 1993; (14) Lee et al., 1987.

([a]) Six isoforms.
([b]) Two isoforms.

The diversity between the members of this class of proteins is not limited to their amino acid sequence but is evident in macroscopic properties, such as their molecular weight and tissue localization. Although the subunit molecular mass is of the same order in all OBPs, around 20 kDa, with not more than 20% variation, in native conditions OBPs have been found to be composed of dimers and monomers. The isoelectric points also differ significantly, although generally within the range 4 to 5.

As for the site of production and accumulation, OBPs are all synthesized within the nasal cavity, but in different glands and areas. Information of this type, which is still very scarce, is fundamental for understanding the physiological role of these soluble proteins. So far, techniques of immunohistochemistry have indicated that the bovine OBP is synthesized in the tubular-acinar glands of the nasal respiratory tissue (Avanzini et al., 1987; Pevsner et al., 1986), while the rat protein is produced by the lateral-nasal glands and postulated to be delivered through narrow ducts at the tip of the nose (Pevsner et al., 1988a). For most of the other known OBPs, the highest concentration was found in the nasal respiratory epithelium. Only the BG protein, which is the frog olfactory protein similar to OBPs, is synthesized in the olfactory epithelium by Bowman's glands (Lee et al., 1987). The fact that OBPs are generally synthesized in a region different than the ciliated olfactory epithelium does not exclude their involvement in odor perception. In several systems proteins are produced in areas different than those where they are postulated to perform their function. An example is the above-cited vomeromodulin, which is also synthesized in the rat by

the lateral-nasal glands to be delivered to the vomeronasal organ, where it is found to be highly concentrated (Khew-Goodall et al., 1991).

Most of the OBPs reversibly bind the odorant 2-isobutyl-3-methoxypyrazine with dissociation constants in the micromolar range. However, the cat-OBP, which has been included in Table 1 on the basis of its sequence similarity with the other OBPs (Felicioli et al., 1993b), does not bind this odorant, while for two more proteins of Table 1 (rat OBP-II and frog BG) binding experiments have not been reported. Their identification as odorant-binding proteins was based, also in these cases, on sequence similarity with other members of this family and on their exclusive occurrence in the nasal area. However, the use of sequence similarity as the sole criterion for assigning a protein to the OBP family could be questioned, as other proteins with the same degree of structural similarity are present in other tissues and perform different functions. This concept is discussed below in more detail.

A particularly interesting result of the recent research on odorant-binding proteins was the discovery of more types of OBPs in the same animal species. This fact opens up the possibility of new hypotheses for the physiological function of OBPs, including a role in the selection and discrimination of different odors at the mucus level.

The presence of a second type of OBP was first reported in the rat, where a cDNA was discovered with significant similarity to the gene coding the synthesis of the already purified OBP. Both OBPs are synthesized in the same glands, but it is not known whether this second member can bind odorants (Dear et al., 1991b).

Shortly after this latter report we isolated more types of OBPs from several animal species, as reported in Table 1. The criterion used was again in all cases (except for the cat OBP) the ability for binding 2-isobutyl-3-methoxypyrazine. In the case of the porcupine (Felicioli et al., 1993a), eight different binding proteins have been purified, belonging, on the basis of partial amino acid sequence, to two different subclasses. It is not clear, at present, whether members of each subclass differ in their primary structure or in their postranslational modifications.

D. Odor-Binding Activity

The binding activity toward odorants should be the main criterion for assigning a protein to the OBP family and distinguishing it from other carrier proteins of similar structure belonging to the lipocalin superfamily, such as retinol-binding proteins and β-lactoglobulin. This concept, while clear in principle, becomes rather confusing when we try to define an odor molecule as distinct from other ligands. In practice, any chemical of molecular weight up to about 300 and volatile enough to reach the olfactory epithelium can be considered an odorant. On the other hand, correlations between binding constants and olfactory properties are difficult to establish as biochemical data can only be obtained with proteins purified from animals, while psychophysical measurements are best done with human subjects.

However, a distinction between OBPs and other proteins of the same superfamily is still feasible on the basis of ligand-binding properties. In fact, OBPs bind a great number of molecular structures, generally hydrophobic in nature, with broad specificity and poor affinities (K_D in the micromolar range), while other proteins of the lipocalin family show higher affinity and selectivity. The retinol-binding protein of the serum, for instance, is known to be very specific for retinol and manifests a nanomolar dissociation constant.

So far, an extensive study of binding specificity has only been performed with the bovine OBP, while some data are also available for the porcine protein.

The bovine OBP has been tested in two independent reports (Pelosi and Tirindelli, 1989; Pevsner et al., 1990) with a great number of odorants belonging to different chemical as well as odor classes. In such experiments 2-isobutyl-3-methoxypyrazine was used as the radioactive ligand, while all the other odorants were used in the nonlabeled form in competitive binding assays. Several odorants have been tested independently in both laboratories: the good agreement between the published data indicates their reliability and reproducibility. Figure 3 lists some representative examples of good and poor ligands for the bovine OBP taken from the above cited

papers. The strongest ligands present dissociation constants between 0.1 and 1 μ*M*, while compounds that do not bind appreciably at concentrations around 100 μ*M* have been classified as nonligands. In addition, other molecules of different structure, such as retinol, benzyl benzoate, and tricyclic musky-smelling compounds have been reported to bind tightly to the bovine OBP. More detailed information is found in the two cited papers. It is worth remembering, when comparing data of different reports, that, unless the actual inhibition constants have been calculated,

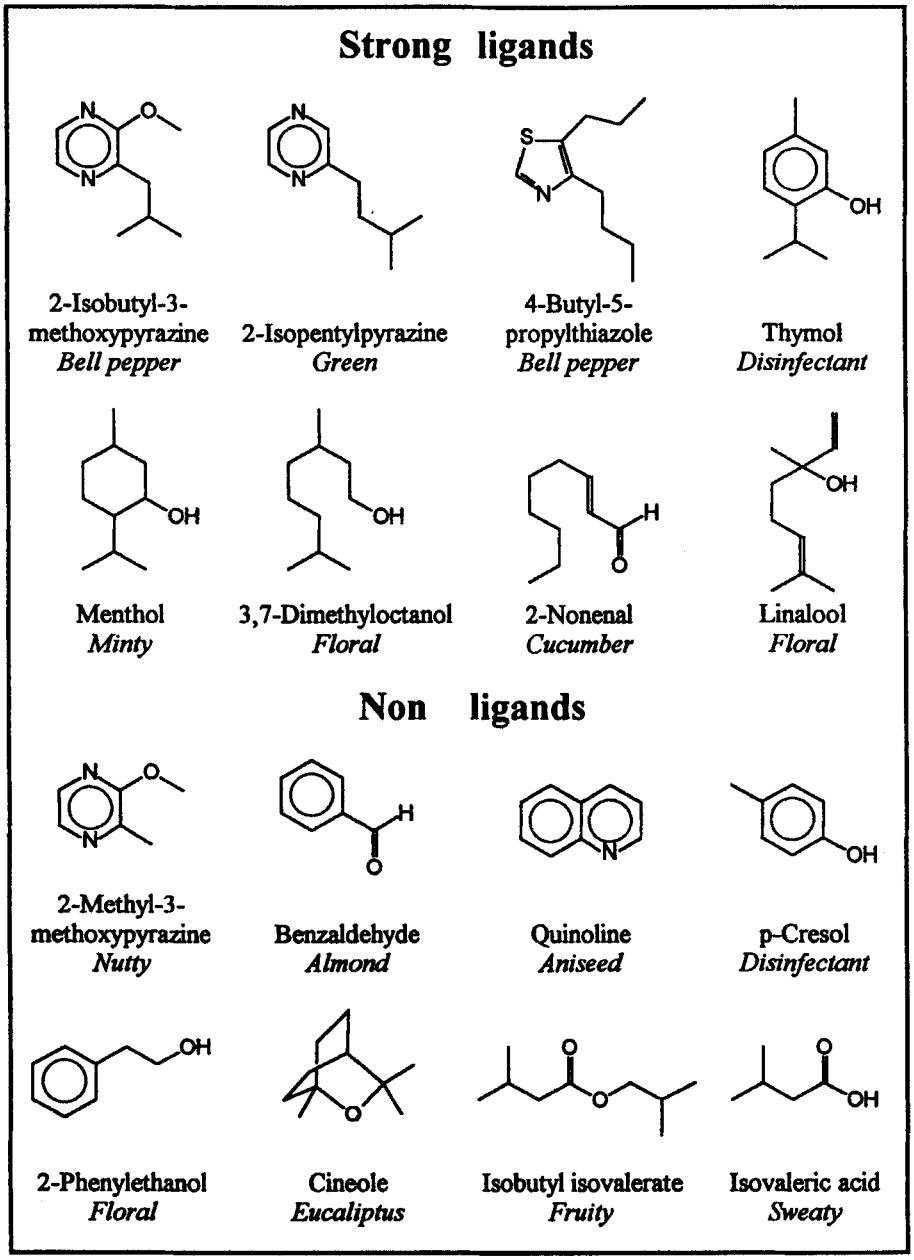

FIGURE 3. Binding of odorants to bovine OBP. The compounds listed as "strong ligands" usually bind with dissociation constants between 0.1 and 1 μ*M*. Those listed as "nonligands" do not show significant binding at the concentration of 100 μ*M*. The data are taken from Pelosi and Tirindelli (1989) and Pevsner et al. (1990).

often the displacement values given depend on the conditions used in the experiments, in particular on the concentration of the protein and of the radioactive ligand.

The results of these binding experiments allow some general conclusions on the specificity of OBP for odor molecules:

- A great number of odorants, different in molecular structure and odor, show some affinity to OBP
- None of the odorants shows very strong affinity for the protein (thymol, which is the best ligand found so far, binds with a dissociation constant of 0.1 μM)
- Compounds belonging to the same chemical class can be good or poor ligands, depending on their hydrophobicity; thus, an increase in the length of the alkyl chain from one to four carbon atoms in the methoxypyrazine derivatives is enough to decrease the binding constant by at least two orders of magnitude; alcohols and phenols are also good ligands (thymol, menthol, etc.) or nonligands (2-phenylethanol, *p*-cresol) according to the extent of their hydrophobic regions
- Stereochemical parameters also seem to be important: compounds of spherical shape, such as cineole and other camphor odorants, although very hydrophobic, did not show any measurable binding
- Some ligands, known to be strong odorants for humans, as are all the compounds reported in Figure 3, do not bind the bovine OBP, even at the concentration of 0.1 m*M*.

These observations indicate the OBP as a protein with a broad but measurable specificity and medium to poor affinity.

The fact that some odorants are not recognized by the protein while others are bound with different degrees of strength indicates a certain capability of discrimination of OBPs and could be the basis for an odor codification system, when more OBPs with different spectra of binding are present and uniformly distributed in the same organism.

Besides these two general reports, other binding studies have focused on selections of structurally related compounds.

In a series of thiazoles fused in position 4 and 5 with substituted aliphatic ring of different size, the relative strength of binding correlated with olfactory properties: the green-smelling compounds were found to be much better ligands for the bovine OBP than the other derivatives of the same series, which at the same time were less hydrophobic and exhibited different odors (Topazzini et al., 1985).

A similar correlation was verified with pyrazine derivatives that present nutty-like or green odor, depending on whether they are substituted with short or long hydrocarbon chains, respectively: the green-smelling pyrazines were found to be good ligands for both the bovine and the porcine OBPs, while no significant binding was measured with the "nutty" lower homologues (Hérent et al., 1994).

A third class of molecules examined included floral and green odorants, all belonging to the classes of 2-tetrahydropyranyl ethers or 2-tetrahydrofuranyl ethers. Most of these compounds were found to be good ligands for both the bovine and the porcine OBP. Interestingly, significant differences were measured with the ligands of these classes that could be related to minor structural variations, such as those between diastereoisomers or *cis/trans* isomers (Dal Monte et al., 1993). These same isomers were also found to have different odors (Anselmi et al., 1992, 1993).

On the basis of the available data, we could try and define the molecular parameters necessary for a tight binding. A certain degree of hydrophobicity seems to be an important requisite, provided some stereochemical parameters are also met. It is clear that more or less planar compounds, such as thymol, pyrazines, and thiazoles, are among the best ligands, in contrast with molecules of round shape, such as camphor-smelling compounds, that have not shown any affinity for the two OBPs tested. On the other hand, the flat shape alone is not enough to make a good ligand: more hydrophylic odorants, such as benzaldehyde, lower pyrazine derivatives and other aromatic compounds, do not present any affinity for either OBP.

Among the best ligands other structures are unrelated to those of the aromatic odorants cited above, being open chain alcohols and aldehydes of 9 to 14 carbon atoms. The strong binding of

these odorants can be rationalized, assuming that, when interacting with OBP, they are present in a bent conformation, reproducing to some extent the shape of the cyclic more rigid ligands. The molecule of 3,7-dimethyloctanol, for instance, can be easily folded in the shapes of menthol or thymol.

We can summarize these results and define the characteristics of good ligands for OBP as molecules of medium size with one polar group, such as hydroxy, carbonyl, or the heteroatom of an heterocycle and an extended hydrophobic area capable of assuming a planar or rather flat conformation.

One important point that has not been adequately investigated is how OBPs from different animal species differ in their binding specificity. Apart from some preliminary experiments performed with only a few odorants (Pelosi et al., 1982; Baldaccini et al., 1986; Pevsner et al., 1986), the only systematic studies have been performed with the porcine OBP (Dal Monte et al., 1993; Hérent et al., 1994) and compared to the data obtained with the bovine protein in the same conditions. The results have so far indicated that the same molecular parameters that define a good ligand for the bovine OBP also apply to the pig protein. Minor differences, up to one order of magnitude, however, have been observed between the two proteins.

More data are necessary before drawing any conclusion or attempting a generalized interpretation of these results. In particular, it would be desirable to measure the binding specificity of different OBPs purified from the same animal species, to test the hypothesis that some odor discrimination could take place at the mucus level.

It would also be interesting to search for other soluble proteins of the nasal mucus with spectra of binding complementary to those of the known OBPs. In such case, the probes to use should be chosen among those odorants that have proved ineffective towards the bovine and porcine OBPs.

E. Amino Acid Sequences

Complete sequence information is limited to four OBPs among those listed in Table 1. For other members, only a partial sequence of 30 to 40 amino acids has been determined, relative to a region starting at or near the amino terminal. The sequences so far available are reported in Figure 4. Amino acids conserved in at least three of the reported sequences are in bold characters and marked with a dot; those conserved in all the sequences are marked with a diamond. Although only four amino acids appear to be fully conserved, similarity between any two members of this class of proteins is much higher, particularly in the region near the amino terminal. Values of similarities, calculated as the percent of identical amino acids for any pair of proteins, are listed in Table 2.

It is clear that OBPs are poorly conserved during evolution; similarity values are in the range of about 10 to 40%, with a single exception observed so far, the mouse OBP-II, that shares nearly 80% of its amino acids with the rat-OBP-I (Pes and Pelosi, 1994). It is also interesting to observe that different OBPs purified from the same animal species do not show higher degrees of similarity than OBPs from different species. These data confirm the presence of distinct classes of OBPs and may indicate different functions within the same organ. However, it is too early to attempt to divide OBPs into classes on the basis of the amino acid sequences available; data on a greater number of proteins would be needed, possibly covering the entire sequences.

As pointed out above, all the odorant-binding proteins so far purified from vertebrates belong to the superfamily of carrier proteins called lipocalins. A representative list of these proteins is reported in Table 3 together with their ligands, where these have been identified.

When comparing the sequences of OBPs with those of the other members of the lipocalin family, such as β-lactoglobulin, retinol-binding proteins, or urinary proteins, we again find a similarity of about 20 to 30%, comparable to the values measured between different OBPs. This fact makes it very difficult to distinguish OBPs from the other members of the lipocalin family solely on the basis of amino acid sequences. Also, the search for conserved motifs in the sequences of OBPs is not very informative. In fact, the main common motif among OBPs is also the

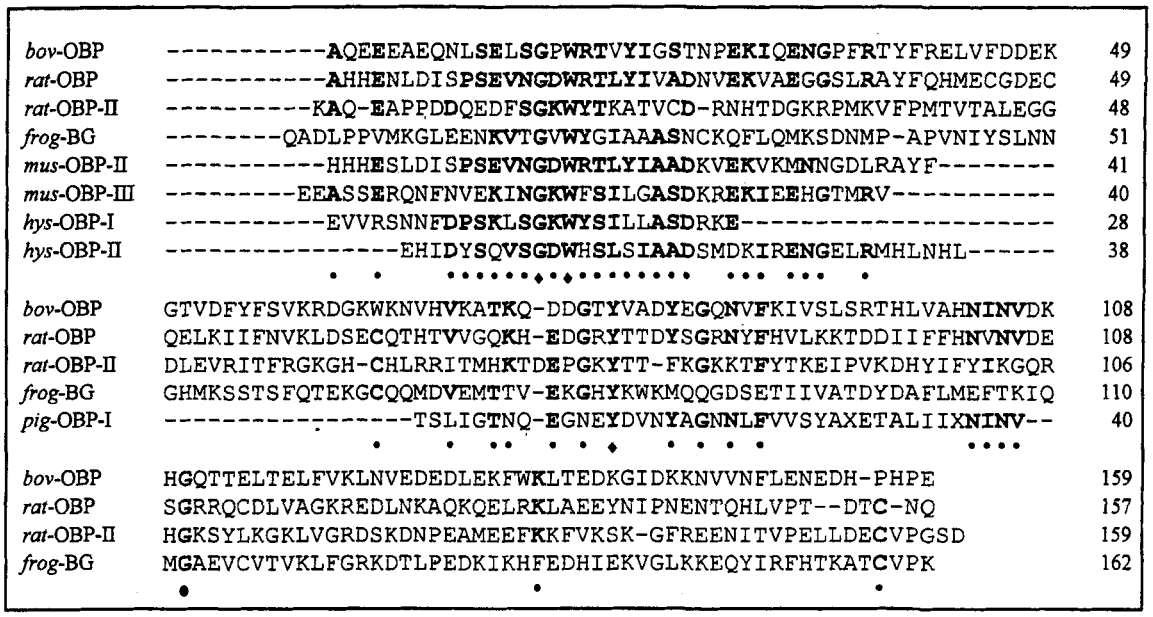

FIGURE 4. Amino acid sequences of vertebrate odorant-binding proteins. The complete sequence of the bovine OBP has been determined by direct sequencing (Tirindelli et al., 1989), while those of the rat OBP (Pevsner et al., 1988), and rat OBP-II (Dear et al., 1991b), as well as that of frog BG (Lee et al., 1987), have been derived from those of the corresponding cDNA. Only the sequences relative to the mature proteins have been reported. For the other OBPs only partial information has been obtained (Felicioli et al., 1993b), either by sequencing the native protein from its amino terminal, as in the case of the two mouse OBPs (mus-OBP-II and mus-OBP-III), or by sequencing a peptide obtained after proteolytic cleavage, as in the case of the pig OBP-I (Pisanelli, Keen, Dal Monte and Pelosi, unpublished results) and the porcupine OBPs (hys-OBP-I and hys-OBP-II). Alignments were made using a computer program based on the method of Higging and Sharp (1989), with manual final adjustments. Bold characters indicate amino acids conserved in at least three of the sequences shown; the relative positions are also marked by a dot (•); a diamond (♦) indicates fully conserved positions.

typical signature of the lipocalins -G-X-W-. This motif is located at about 15 to 20 amino acids from the amino terminal and is present in all the sequences so far reported. Other amino acids are conserved only in some of the members of lipocalins and may help divide these proteins into subclasses; however, clear rules cannot yet be defined for this purpose.

As the amino acid sequence is not a sufficient criterion for assigning a new protein to the OBP family, the members listed in Table 1, for which no binding data are available, can only be tentatively listed as OBPs. These are the cat protein, the second rat OBP, and the frog BG. If we include as a second criterion the site of production of these proteins, then the rat OBP-II, which is produced in the lateral-nasal glands and the frog BG, secreted by the Bowman's glands of the olfactory epithelium, can be assigned with better confidence to the OBP family.

Some members of the lipocalin family are particularly interesting, being involved in chemical communication between sexes in mammals. These are the urinary proteins of rat and mouse and the hamster aphrodisin, which are described with more detail in Section VII.

F. Three-Dimensional Structure

At present, only the three-dimensional structure of the bovine protein has been described among OBPs, but for other members of the lipocalin family the structure has been resolved to a good degree of resolution (Monaco and Zanotti, 1992).

TABLE 2
Percent of Identical Amino Acids between the OBP Sequences Reported in Figure 4

bov-**OBP**								
	rat **OBP-I**							
28.7		*rat* **OBP-II**						
11.9	15.9		*frog*-**BG**					
9.4	10.2	13.2		*mus* **OBP-II**				
39.0	78.0	12.2	12.2		*mus* **OBP-III**			
37.5	22.5	22.5	15.0	26.3		*hys* **OBP-I**		
17.9	21.4	25.0	17.9	28.6	42.9		*hys* **OBP-II**	
23.7	39.5	13.2	10.6	37.1	27.3	34.8		*pig* **OBP-I**
30.0	27.5	15.0	10.0	—	—	—	—	

Note: Values have been calculated limited to the overlapping regions of each pair of sequences. Alignments were basically those reported in Figure 4, with minor adjustments introduced where higher similarity values could be obtained.

The bovine OBP is a homodimer where the two subunits are held together by noncovalent bonds. The structure of the monomeric unit is rather similar to that of other members of the lipocalin family, such as serum retinol-binding protein and β-lactoglobulin. As for these proteins, the bovine OBP is folded into eight antiparallel β-sheets with a single α-helical region located near the carboxy terminal.

It is known that retinol binds to monomeric serum retinol-binding protein by fitting into a hydrophobic pocket located within the interior of the protein. In the urinary proteins of mouse and rat, which are also present in solution as monomers, X-ray crystallography has indicated the presence of pheromone molecules within the interior of the protein (Böcskei et al., 1992). A similar model cannot be easily applied to the structure of bovine OBP, as this protein is extremely compact, precluding the possibility even for a small molecule, like an odorant, to fit inside. On the other hand, the two subunits of OBP when interacting leave a large hydrophobic area, where a ligand could be easily accommodated. The stoichiometry of binding of one molecule of ligand per dimer is in agreement with such model; however, no direct evidence for this hypothesis has yet been provided.

VI. ODORANT-BINDING PROTEINS OF INSECTS

Soluble binding proteins are also found in the olfactory system of insects, although their amino acid sequences do not show any similarity with vertebrate OBPs. Pheromones and general odors are perceived by insects through specialized sensilla of their antennae. The dendrites of individual or pairs of sensory neurons are bathed in a lymph that fills the space within the cuticular wall of the sensillum. This lymph is different and physically separated from the hemolymph. Pheromone-binding proteins (PBPs) and general odorant-binding proteins (GOBPs) are the main constituents of the sensillum lymph, being present in millimolar concentrations (Kaissling and Thorson, 1980; Kaissling, 1986, 1987; Vogt et al., 1990).

Two anatomically distinct systems, sensilla trichodea and sensilla basiconica, are dedicated to the perception of pheromones and general odorants, respectively. The presence of different sen-

TABLE 3
Representative Examples of Proteins Belonging to the Lipocalin Family

Protein	Animal species	Tissue/secretion	Ligand/function	Ref.
Alpha-1-microglobulin (protein HC)	Man	Serum, urine	Porphyrin, retinoids	1, 2
α1-Acid glycoprotein	Man	Liver/serum, urine	Progesterone/ inflammatory response	3
Androgen-dependent epididymal protein	Rat	Epididymal fluid	Sperm maturation	4
Aphrodisin	Hamsters	Vaginal discharge	Aphrodisiac pheromone	5
Apolipoprotein D	Man	Liver, etc./serum	Cholesterol/carrier	6
Crustacyanin	Lobster	Carapace	Astaxanthin	7
Embryo CH21 protein	Chicken	Embryo skeletal tissue	Quiescence	8
Insectacyanin	*Manduca sexta*	Hemolymph	Biliverdin	9
β-Lactoglobulin	Bovine, etc.	Milk	Retinol/carrier	10, 11
Major urinary proteins (MUPs)	Mouse	Salivary, lachrymal glands Liver/saliva, tears, urine	Pheromones	12–16
Pregnancy-associated endometrial globulin	Man	Placenta/amniotic fluid		17
Probasin (PB)	Rat	Prostate		18
Prostaglandin D (PGD) synthetase	Rat, man	Brain		19, 20
Purpurin	Chicken	Retina	Retinol, heparin	21
Retinol-binding protein	Man, etc.	Liver/serum	Retinol/carrier	22, 23
Tear prealbumin	Man	Lacrymal glands/tears	Retinol	24
α2-UG	Rat	Liver/urine		25, 16
VEG protein	Rat, man	Von Ebner's gland		26, 27

Note: (1) Kaumeyer et al., 1986; (2) Mendez et al., 1986; (3) Clark et al., 1984; (4) Brooks et al., 1986; (5) Henzel et al., 1988; (6) Drayna et al., 1986; (7) Keen et al., 1991; (8) Descalzi-Cancedda et al., 1990; (9) Riley et al., 1984; (10) Papiz et al., 1986; (11) Monaco et al., 1987; (12) Finlayson et al., 1965; (13) Shaw et al., 1983; (14) Shahan et al., 1987; (15) Bacchini et al., 1992; (16) Böcskei et al., 1992; (17) Julkunen et al., 1988; (18) Spence et al., 1989 (19) Urade et al., 1989; (20) Nagata et al., 1991; (21) Berman et al., 1987; (22) Zanotti et al., 1993; (23) Newcomer et al., 1984; (24) Redl et al., 1992; (25) Dinh et al., 1965; (26) Schmale et al., 1990; (27) Bläker et al., 1993.

sory organs for these two classes of odorants reflects their chemical diversity.

The sex pheromones of Lepidoptera can be easily distinguished from other odorants, being rather homogeneous in structure: they usually present a linear more or less unsaturated chain of 12 to 20 carbon atoms, with a functional group (alcohol, aldehyde, or ester) in position 1; consequently, most of them are not chiral. A few exceptions to this rule have been described, as in the case of *Lymantria dispar*, whose pheromone is an internal epoxide that can give rise to asymmetric forms. In any case, the stereochemistry plays an important role and *cis/trans* isomerism around a double bond or the correct configuration of asymmetric centers are essential for the recognition of the specific pheromone. The pheromone perception system is therefore very specific and also extremely sensitive when compared with the olfactory organ of vertebrates.

General odorants belong to a very wide range of chemical structures, as, for example, the volatile compounds of plants, and do not differ from the odorants perceived by vertebrates. It seems that the olfactory system for general odorants in insects is much more broadly tuned and less sensitive than that for pheromones, being more similar also in this respect to olfaction in vertebrates.

The first PBP was discovered in the sensillum lymph of the antennae of the large moth *Antheraea polyphemus* (Vogt and Riddiford, 1981), using the specific sex pheromone for this species.

It is a small protein of about 15 kDa and low isoelectric point (4.7), which is extremely abundant in the lymph of sensilla trichodea. These characteristics, common to all the insect odorant-binding proteins so far described, are similar to those of vertebrate OBPs and indicate that olfactory mechanisms in insects and vertebrates might share common features and mechanisms at the perireceptor level.

One report on the binding constant relative to the PBP of *Antheraea polyphemus* indicates a K_D of 60 n*M* (Hemberger and DeKramer, 1987), rather close to the values measured with vertebrate OBPs and general odorants (The K_D for thymol and bovine OBP is 100 n*M*, as reported previously). Unfortunately, binding experiments with insect olfactory proteins have proven very difficult, in contrast with vertebrate OBPs, where such measurements can be obtained with good accuracy and reliability. In particular, experiments performed in solution have always given negative results; binding with radioactive pheromones have only been observed in particular experimental conditions, such as during ion-exchange chromatography or electrophoresis. The reason for this lack of success is not clear; however, it has been hypothesized (Maida et al., 1993) that at the very high concentration measured in the sensillum lymph (10 m*M*), the active binding forms of these proteins could be associated in dimers or oligomers, while at the much lower concentrations usually employed in binding experiments (1 to 10 μ*M*) the proteins could be dissociated into their inactive subunit monomeric forms. In support of this hypothesis, positive results have only been obtained with techniques (ion-exchange chromatography or electrophoresis) that involve dramatic increases in the concentrations of the proteins being analyzed (Vogt and Riddiford, 1981; Klein, 1987; Vogt et al., 1989; Maida et al., 1993).

The experiments performed with a photoaffinity label analogue of the pheromone have been more successful; this technique has been applied in the case of *Antheraea polyphemus*, whose pheromone is an acetate; the corresponding diazoacetate, prepared in its tritiated form, was shown to bind efficiently to the relevant PBP and to covalently label the protein after U.V. irradiation (Vogt et al., 1988; Prestwich, 1993). Unfortunately, this approach does not allow an estimation of the binding constant. Moreover, the chemistry involved in the preparation of a radioactive photoaffinity label is rather complex and prevents wide applications of this technique.

Therefore, binding experiments with insect PBPs have been reported for only three species (*A. polyphemus*, *L. dispar*, and *B. mori*), and no data are available for the class of GOBPs. The identification of newly purified proteins from the antennae of other Lepidoptera species as pheromone-binding proteins or general odorant-binding proteins was based on sequence similarity rather than on functional activity. This approach was simplified by the availability of the recent techniques of microsequencing and the powerful tools of molecular biology that can be applied to the minute quantities of protein obtainable from insect antennae.

Odorant-binding proteins have been isolated and studied in *Antheraea polyphemus* (Vogt and Riddiford, 1981; Kaissling et al., 1985; Klein, 1987; Raming et al., 1989), *Antheraea pernyi* (Breer et al., 1990b; Raming et al., 1990; Krieger et al., 1991), *Bombyx mori* (Vogt et al., 1991a; Maida et al., 1993), *Manduca sexta* (Vogt et al., 1991a, 1991b; Gyorgyi et al., 1988), *Lymantria dispar* (Vogt et al., 1989, 1991a), *Heliothis virescens* (Krieger et al., 1993), *Hyalophora cecropia* (Vogt et al., 1991a), and *Orgyia pseudotsugata* (Vogt et al., 1991a).

In most of these species three distinct classes of odorant-binding proteins have been described, either one PBP and two GOBPs or two PBPs and one GOBP, as in the cases of *A. pernyi* and *L. dispar*. The assignment of a newly identified protein to one or another of these classes can be correctly performed on the basis of sequence similarities; in fact, differences between classes are usually much greater that between members of the same class.

For some of these proteins, only N-terminal sequences have been determined, limited to about 30 amino acids, by direct microsequencing (insect OBPs are not blocked at their amino terminal,

unlike most of the vertebrate OBPs). In most cases the protein samples used for sequencing were purified by electroelution from SDS-PAGE gels. The choice of the electrophoretic band to be analyzed was based on a low molecular weight (15 to 16 kDa in denaturing conditions) and, for the PBP candidates, the exclusive presence or a higher abundance (as in the cases of *Bombyx mori* and *Manduca sexta)* in male antennae. The correct choice was then confirmed by a significant sequence similarity with other proteins of the same class. In other cases, PBPs have been identified using techniques of molecular biology, which yielded the complete amino acid sequence.

Molecular weights in denaturing conditions (15 to 16 kDa) are on the average slightly smaller than those of vertebrate OBPs. In native conditions the PBP of *A. polyphemus* is a dimer (DeKramer and Hemberger, 1987), while that of *B. mori* seems to be present as a monomer (Maida et al., 1993). However, the concentration of the protein used in both cases was at least three orders of magnitude lower than that estimated in the sensillum lymph (10 m*M*), where association of higher orders may well take place. Isoelectric points are also rather similar to those of vertebrate OBPs.

The amino acid sequences of PBPs and GOBPs indicate that they are well conserved, at least among most species of Lepidoptera. Several sequences of PBPs are 90% or more similar to one another, while similarity between members of vertebrate OBPs is generally around 30 to 40%. Only the PBPs of *L. dispar* are rather different, sharing only about 30% of their amino acids with the PBPs of other Lepidoptera species.

Such greater similarity between the amino acid sequences of PBPs in contrast to those of vertebrate OBPs could be related to the fact that the corresponding ligands, the specific sex pheromones, are very similar in their chemical structures. The case of *Lymantria dispar*, whose pheromone is markedly different from the other pheromones of Lepidoptera, is consistent with this hypothesis (Vogt et al., 1991a). It is also true that phylogenetic distances between Lepidoptera species are much smaller than those between the species of mammals where OBPs have been studied. It would be useful, in this respect, to have access to the three-dimensional structure of PBPs, with particular reference to the pheromone-binding site. Data of this type should soon be available, because the PBP of *Antheraea polyphemus* has been produced recently in the large amounts needed for structural studies by recombinant techniques and shown to present pheromone-binding activity as the native protein (Prestwich, 1993).

Based on sequence similarities, general odorant-binding proteins of Lepidoptera have been divided in two classes, GOBP-I and GOBP-II. Proteins belonging to the same class are very similar in their sequences (more than 90% identity), while lower similarity (around 50% identity) is observed between the two classes of GOBPs and even lower (about 20 to 50%) with members of the PBP class (Vogt et al., 1991b).

Although GOBPs are believed to mediate the perception of general odors, no binding data have been reported for these proteins that could justify their name; the hypothesis that they could be the equivalent of PBPs for odors other than pheromones was based on their similarity with PBPs (molecular weight, isoelectric point, abundance, amino acid sequence) and their presence in the antennal sensilla of both sexes.

PBPs and GOBPs are not significantly similar to any other known proteins. Based on some chemical characteristics that they share with vertebrate OBPs, we would expect a certain similarity between odorant-binding proteins in insects and vertebrates. In particular GOBPs, although odor-binding experiments have not yet been reported, are supposed to bind general odorants such as terpenoids and other volatile compounds produced by the host plants; these odorants, in fact, can elicit electrophysiological response from the sensilla basiconica, where GOBPs are very likely contained. The same compounds are also strong odorants for vertebrates; consequently, we could expect binding proteins for these chemicals of similar structure in insects and vertebrates; however, it is not excluded that proteins as different in amino acid sequence as the OBPs of insects and those of vertebrates could fold into similar three-dimensional structures. It is also puzzling, on the other hand, the presence in insect hemolymph of a binding protein (insecticyanin of *Manduca sexta*), which is a member of the

lipocalin family and shows significant sequence similarity with vertebrate OBPs (Riley et al., 1984).

If there is no similarity in the amino acid sequence between odorant-binding proteins in insects and vertebrates, we could still expect to find common motifs in their three-dimensional structure, as a case of evolutionary convergence where different precursors have evolved toward a common binding function. Crystals have so far been obtained only with vertebrate OBPs, because of their great abundance; however, the recent availability of recombinant PBP of *Antheraea polyphemus* (Prestwich, 1993) has opened the way to structural studies (X-ray and NMR) that could soon disclose the three-dimensional structure of insect odorant-binding proteins and their binding domains.

VII. PROTEINS SIMILAR TO OBPS IN CHEMICAL COMMUNICATION

The proteins examined so far occur in the olfactory organs and are believed to mediate the perception of odors. However, proteins capable of binding potential odorants are also found in other parts of the body and may be involved in releasing and modulating olfactory messages.

As in the case of olfactory proteins, their odor binding activity does not univocally identify these proteins due to the poor definition of what an "odorant" is, as discussed in Section II. In fact, this term is related to the function of such molecules rather than to their chemical structures. Again, we have to include tissue localization of the binding proteins as one of the discrimination criteria.

Proteins with binding activity to odorants have been discovered in three types of body fluids that are known to have pheromonal activity: urine, vaginal secretion, and saliva. Most of these proteins belong to the lipocalin family and present significant sequence similarity with vertebrate OBPs.

The urinary proteins of mouse and rat had been discovered long before OBPs, but their function remained unknown for many years. Their sequence similarity with OBPs stimulated further research to better characterize these proteins both in terms of structure and function. Their great abundance in the urine of these two species of rodents (up to several milligrams per milliliter) has allowed the purification of the relatively large amounts needed for binding studies and for growing crystals. These proteins, called MUPs (major urinary proteins) in the mouse (Finlayson et al., 1965) and α2-u in the rat (Dinh et al., 1965), are produced in several isoforms by the liver and excreted in the urine. The apparent waste of large quantities of protein in the urine first suggested the idea that they could be involved in some important physiological function; their unique presence in the urine of males was an indication that they could be involved in pheromonal communication.

It has been demonstrated that the urinary proteins of both mouse and rat bind the same odorants recognized by the olfactory OBPs with dissociation constants of the same order of magnitude (Cavaggioni et al., 1990). Later it was reported that the MUPs, when purified from mouse urine, still contain at least two volatile ligands, 2,3-dehydro-*exo*-brevicomin and 2-*sec*-butylthiazoline, that are known to have pheromonal activity in the mouse (Bacchini et al., 1992).

Purified isoforms of MUP and α2-u have been crystalized and their three-dimensional structure has been resolved (Böcskei et al., 1992). The folding of these proteins follows a pattern common to those members of the lipocalin family, such as retinol-binding protein, β-lactoglobulin, and OBP, for which the three-dimensional structure has been elucidated. The X-ray diffraction pattern also indicated the presence of the natural ligand, that had been copurified with the protein bound inside a hydrophobic pocket in the bulk of the structure.

Data on the presence of urinary proteins in other animal species are very scarce. It seems that this phenomenon of excreting such large quantities of proteins in the urine is typical of rodent but certainly has not been observed in all the rodents examined.

Another protein certainly involved in chemical communication between sexes was purified

from the vaginal discharge of the hamster (Henzel et al., 1988; Singer and Macrides, 1990; Singer, 1991). It is called aphrodisin and bears strong similarity to OBPs in molecular weight, isoelectric point, and amino acid sequence. The pheromonal activity of aphrodisin has been clearly demonstrated; binding experiments have not been reported, but recently evidence has been provided that the pheromonal activity is due to low-molecular-weight compounds associated *in vivo* with this protein (Singer and Macrides, 1993). This observation fits well with a carrier role for aphrodisin, common to most members of the lipocalin family. It would be interesting to investigate the presence of aphrodisin-like proteins in other animal species and verify whether this is part of a general mechanism for sexual communication or limited to certain species.

It is worth pointing out that urinary proteins are produced by males and aphrodisin by females. The two mechanisms are therefore complementary and could act alternatively, according to the species, or be present in the same species in a two-way communication system.

The saliva is another recognized medium for chemical communication in some animals. This behavior has been mostly studied in the boar, whose saliva contains steroid molecules with strong pheromonal activity for the sow, 5α-androst-16-en-3-one and related compounds. A protein of 17 kDa has been purified from the saliva of the boar that shows binding activity to the steroid pheromones (Booth and White, 1988). Two isoforms have been separated, whose ratio appears to be related to the pig race. Data on their amino acid sequence, when available, will show if these proteins also belong to the large lipocalin family.

Salivary proteins, on the other hand, similar in their sequences to OBPs and to urinary proteins, are present in the saliva of the mouse and are secreted by several glands of the oral cavity. They have been named "urinary" solely on the basis of their similarity with the proteins of urine. Whether these proteins perform any pheromonal function or act as pheromone carriers has not yet been demonstrated (Shaw et al., 1983; Shahan et al., 1987a, 1987b; Shi et al., 1989a, 1989b).

VIII. THE PHYSIOLOGICAL FUNCTION OF ODORANT-BINDING PROTEINS

The central problem with odorant-binding proteins is the definition of their physiological role. Although a great deal of information has been accumulated during the last few years on the structure of several members of this family, their function is still unknown.

The first question to ask is whether OBPs are involved at all in the process of olfactory perception. Their property of binding odorants is not sufficient in supporting a role in olfactory transduction. In fact, the concept of "odorant" is not related to any particular class of chemicals, but rather to the possibility of stimulating olfactory neurons; several compounds that present an odor are bound by proteins in different tissues that have nothing in common with olfaction. The site of synthesis and translocation of OBPs can give insights in this respect. As far as we know, they are all produced by glands present in the nasal cavity, but only one of them, the frog-BG, which is, incidentally, the only member purified from a nonmammalian species, is known to be secreted by glands of the olfactory epithelium (Lee et al., 1987). For other OBPs the site of production is rather the respiratory part of the nasal epithelium, being the tubular-acinar glands in the cow (Pevsner et al., 1986; Avanzini et al., 1987) and the lateral nasal glands in the rat (Pevsner et al., 1988a). In all the species studied the OBPs are then found in high concentration in the nasal mucus, both in the respiratory and the olfactory areas, but the mechanism of diffusion of these proteins in the mucus layer has not been investigated. On the basis of these data it is not possible to prove that OBPs are involved in the perception of odors, although such hypothesis seems quite reasonable. Better evidence can be found in insects, where the antennal sensilla are very specialized organs whose only function is the detection of odors. In the sensillar lymph PBPs and GOBPs are extremely concentrated, as reported above, and their only function seems very likely to be related to pheromone and odor perception. OBPs share several characteristics with insect odorant-binding proteins and represent their equivalent in vertebrates. On the basis

of this comparison, we can strongly anticipate a role of OBPs in olfaction.

The next problem to be addressed is the functional position of OBPs in the process of olfactory transduction; this question can be broken down into several components:

- Are OBPs essential for odor perception?
- How is the odor quality and intensity of a substance affected by the presence of such large concentrations of OBPs?
- Are odorants complexed by OBPs before or after their interaction with the neuronal membrane?
- Is there any discrimination and codification of odors connected with their interaction with OBPs?

Unfortunately, the available information does not provide any definite answer to these questions. However, hypotheses have been formulated to focus and guide research in this field. Figure 5 shows schematically three models that have been proposed to explain the role of OBPs in olfaction.

It has been proposed that OBPs could carry odorant molecules to the membrane receptors, thus helping hydrophobic compounds to cross the aqueous barrier of the mucus (Pevsner and Snyder,

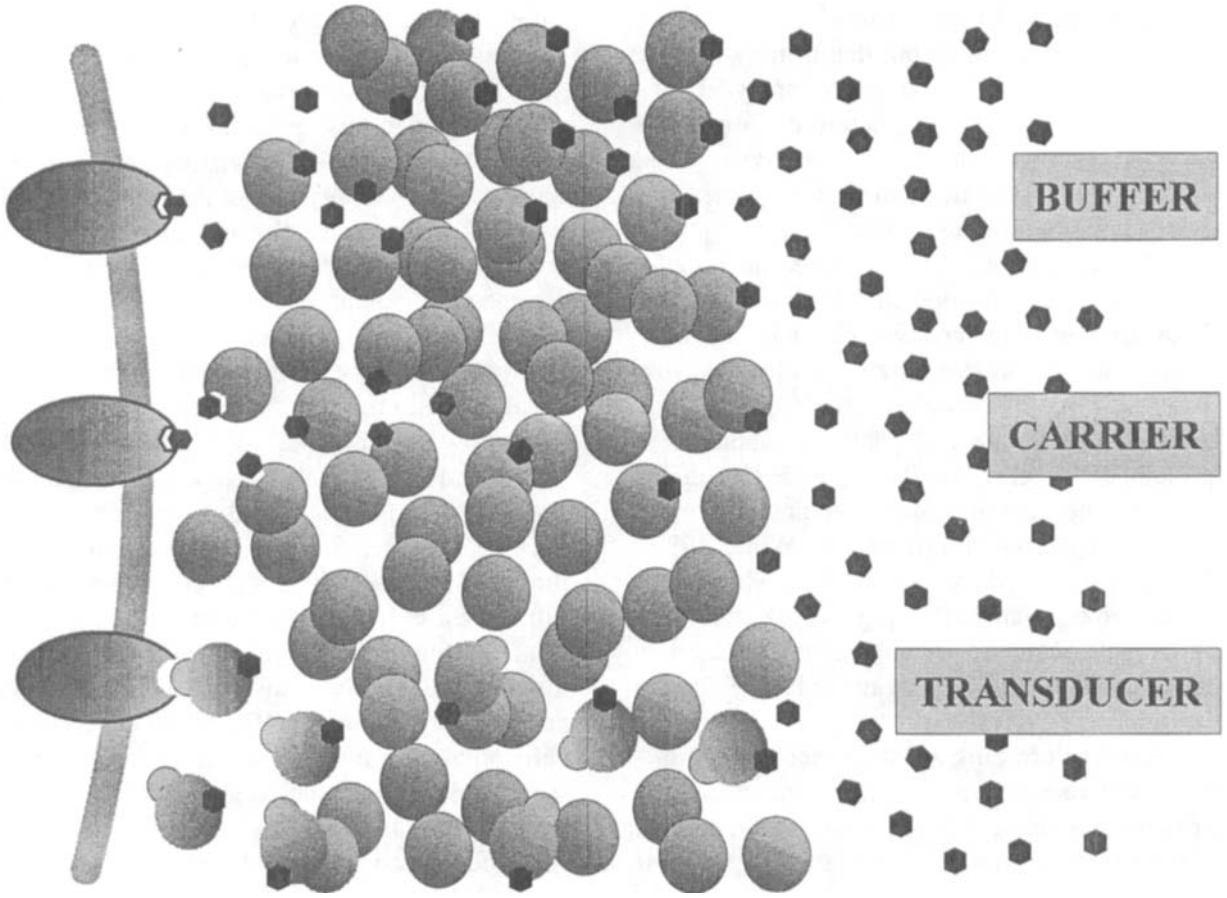

FIGURE 5. Three hypotheses for the physiological functions of OBP. **Buffer:** because of the relatively high values of the dissociation constants, OBP could trap odorants more efficiently at high concentrations, thus narrowing the wide range of stimuli intensities. **Carrier:** OBP could bind odorants and carry them to the membrane of the olfactory neurons, thus helping hydrophobic compounds to cross the aqueous mucus layer; alternatively, OBP could remove odorants from the receptors, contributing to their fast reactivating. **Transducer:** OBPs could selectively bind odorants and then interact, as a complex, with the membrane-bound receptors; according to this model, borrowed from bacterial chemotaxis, OBPs could be involved in the discrimination of different odors.

1990). Alternatively, the OBPs could act after the olfactory receptors have been stimulated and remove the odorant molecules from the receptors' area. Both models rely on the fact that odorants are generally hydrophobic molecules and therefore poorly soluble in the aqueous mucus; the presence of high concentrations of OBPs would thus increase their solubility and favor their partition into the mucus layer, thus representing the first step in the amplification of the olfactory signal. This is certainly true for insect PBPs and their corresponding pheromones: the sex pheromones of Lepidoptera, being generally long hydrocarbon chains with a single functional group have very low solubilities in water, of the micromolar order of magnitude or lower; on the other hand, the concentration of PBP has been estimated to be in the sensillar lymph of *Antheraea polyphemus* around 10 m*M*, four orders of magnitude higher.

For vertebrate olfaction, the situation is different: the concentration of the OBPs in the mucus, although difficult to measure, is certainly lower than 1 m*M*; on the other hand, most odorants can be dissolved in water at concentrations above 10 m*M*; even an increase of the mucus/air partition coefficient, determined by the presence of OBPs, would not produce dramatic differences in the final concentration of odorants in the olfactory area: in fact, water/air partition coefficients are for most odorants of the order of 100 or higher and only in few cases their values are less than 10. On the basis of these data, therefore, a concentration effect by OBPs seems questionable, at least if we consider values averaged over the entire mucus layer. Recent findings that proteins in the mucus are not uniformly distributed but are present in distinct patches (Menco and Farbman, 1992; Getchell et al., 1993) could still support a role of OBPs in concentrating odorants in particular areas of the mucus.

The hypothesis that OBPs could function as active "carriers" to the receptors or away from the receptors is rather difficult to prove. In fact, to provide data in favor of or against each alternative of this model, we need to know kinetic parameters, measured *in vivo*, relative to the interactions between odorants and OBPs. On the contrary, our data are based on *in vitro* experiments performed at the equilibrium and therefore can only measure thermodynamic parameters.

A second hypothesis assigns the OBPs a role of filtering and buffering agents. This assumption is further supported by the poor affinity of OBPs to odorants, much lower than the values generally assumed for olfactory receptors. Therefore, OBPs would be more efficient when high concentrations of odorants enter the nose to trap most of the molecules that would otherwise inactivate the olfactory receptors for a long period. The action of OBPs would be much weaker at low concentration of odorants, with a resulting buffering effect. This model is certainly in agreement with a logarithmic response of the olfactory systems to increasing concentrations of odorants. However, experiments performed with insect antennal sensilla seem to point in the opposite direction. When the sensillum lymph in *A. polyphemus* was replaced with Ringer solution, the electrophysiological response to the specific sex pheromones was greatly reduced; normal sensitivity was then restored by the specific purified PBP, but also by bovine serum albumin (Van den Berg and Ziegelgerber, 1991). These results could indicate that the function of PBP is to concentrate the pheromone in a nonspecific way and consequently increase the sensitivity.

A third model can be borrowed from the study of bacterial chemotaxis. It has been known for a long time that bacteria perceive sugars with the help of soluble proteins that specifically bind these attractants. The complex sugar/protein is then recognized by membrane-bound receptors that in turn activate an enzyme cascade, eventually leading to the chemotactic response (Koshland, 1981; Stewart and Dahlquist, 1987). In a similar fashion, OBPs could specifically bind odorants and convey the relative information (but not the odorants) to the membrane-bound receptors. This model has no better experimental evidence to support it than the others. In any case, it requires that more types of OBPs be present in the same animal species, with different spectra of binding or that heterodimers can form with different specificities.

Until a single type of OBP was known to be present in each animal species, a discriminating role could not be taken into consideration. This is obvious when considering the complexity of

olfactory stimuli in vertebrates, but is also true in the pheromone perception systems of insects. In fact, pheromones are often constituted by two or more chemical substances whose relative concentrations the insect is able to perceive and correctly identify as the species' pheromone.

The discovery of a second class of PBPs as well as of GOBPs in insects, followed by reports of two or more classes of OBPs in vertebrates, provided an expanded basis for theories on the function of odorant-binding proteins. With two or three types of binding proteins it becomes possible to discriminate different ligands; therefore, odor recognition could also occur at the level of soluble binding proteins. The small number of OBPs with broad ligand binding specificity could well codify for a great number of odors, similarly to the color vision, that is based on only three broad spectrum photoreceptors. Moreover, OBPs could be involved in a system for perceiving only certain classes of odors, while other odorants could directly stimulate membrane receptors of olfactory neurons. The presence of two such mechanisms has certainly been demonstrated in bacteria that sense amino acids with membrane receptors and sugars through soluble binding proteins (Mowbray and Cole, 1992).

ACKNOWLEDGMENT

I would like to thank Dr. Krishna C. Persaud for helpful comments and criticism.

National Research Council of Italy, Special Project RAISA, Subproject 4. This is paper no. 1467.

REFERENCES

Amoore, J. E., Specific anosmia: a clue to the olfactory code. *Nature*, **214:**1095–1098 (1967).

Amoore, J. E., Specific anosmia and the concept of primary odors. *Chem. Senses*, **2:**267–281 (1977).

Amoore, J. E. and Buttery, R. G., Partition coefficients and comparative olfactometry. *Chem. Senses*, **3:**57–71 (1978).

Amoore, J. E. and Forrester, L. J., Specific anosmia to trimethylamine: the fishy primary odor. *J. Chem. Ecol.*, **2:**49–56 (1976).

Amoore, J. E. and Steinle, S., A graphic history of specific anosmia, **In:** *Chemical Senses*, Vol. 3, pp. 331–352. (Wysocki, C. J. and Kare, M. R., Eds.) Marcel Dekker, New York (1991).

Amoore, J. E., Forrester, L. J., and Buttery, R. G., Specific anosmia to 1-pyrroline: the spermous primary odor. *J. Chem. Ecol.*, **1:**299–310 (1975).

Amoore, J. E., Forrester, L. J., and Pelosi, P., Specific anosmia to isobutyraldehyde: the malty primary odor. *Chem. Senses*, **2:**17–25 (1976).

Amoore, J. E., Pelosi, P., and Forrester, L. J., Specific anosmia to 5α-androst-16-en-3-one and ω-pentadecalactone: the urinous and musky primary odors. *Chem. Senses*, **2:**401–425 (1977).

Anholt, R. R. H., Molecular neurobiology of olfaction. *Crit. Rev. Neurobiol.*, **7:**1–22 (1993).

Anholt, R. R. H., Mumby, S. M., Stoffers, D. A., Girard, P. R., Kuo, J. F., Gilmann, A. G., and Snyder, S. H., Transduction proteins of olfactory receptor cells, identification of guanine nucleotide binding proteins and protein kinase C. *Biochemistry*, **26:**788–795 (1987).

Anselmi, C., Centini, M., Mariani, M., Sega, A., and Pelosi, P., Odor properties of some tetrahydropyranyl ethers. *J. Agric. Food Chem.*, **40:**853–856 (1992).

Anselmi, C., Centini, M., Mariani, M., Sega, A., and Pelosi, P., Influence of regio- and stereochemistry on the floral odor of THP and THF ethers. *J. Agric. Food Chem.*, **41:**781–784 (1993).

Avanzini, F., Bignetti, E., Bordi, C., Carfagna, G., Cavaggioni, A., Ferrari, G., Sorbi, R. T., and Tirindelli, R., Immunocytochemical localization of pyrazine-binding protein in the cow nasal mucosa. *Cell Tissue Res.*, **247:**461–464 (1987).

Bacchini, A., Gaetani, E., and Cavaggioni, A., Pheromone-binding proteins in the mouse *mus musculus*. *Experientia*, **48:**419–421 (1992).

Bal, R. S. and Anholt, R. R. H., Formation of the extracellular mucous matrix of olfactory neuroepithelium: identification of partially glycosylated and nonglycosydated precursors of olfactomedin. *Biochemistry*, **32:**1047–1053 (1993).

Baldaccini, N. E., Gagliardo, A., Pelosi, P., and Topazzini, A., Occurrence of a pyrazine binding protein in the nasal mucosa of some vertebrates. *Compar. Biochem. Physiol.*, **84B:**249–253 (1986).

Beets, M. G. J., *Structure-Activity Relationships in Human Chemoreception*, Applied Sciences Publishers, Ltd., London (1978).

Berman, P., Gray, P., Chen, E., Keyser, K., Ehrlich, D., Karten, H., LaCorbiere, M., Esch, F., and Schubert, D., Sequence analysis, cellular localization and expression of a neuroretina adhesion and cell survival molecule. *Cell*, **51:**135–142 (1987).

Bignetti, E., Cavaggioni, A., and Pelosi, P., An odorant binding protein in cow. **In:** *Discussions in Neurosciences*, Vol. IV, no. 3. *Sensory Transduction*, pp. 37–41. (Hudspeth, A. J., MacLeish, P. R., Margolis, F. L. and Wiesel, T. N., Eds.) FESN, Geneva (1987a).

Bignetti, E., Cavaggioni, A., Pelosi, P., Persaud, K. C., Sorbi, R. T., and Tirindelli, R., Purification and characterization of an odorant-binding protein from cow nasal tissue. *Eur. J. Biochem.*, **149:**227–231 (1985).

Bignetti, E., Damiani, G., De Negri, P., Ramoni, R., Avanzini, F., Ferrari, G., and Rossi, G. L., Specificity of an immunoaffinity column for odorant-binding protein from bovine nasal mucosa. *Chem. Senses*, **12:**601–608 (1987b).

Bläker, M., Kock, K., Ahlers, C., Buck, F., and Schmale, H., Molecular cloning of human von Ebner's gland protein, a member of the lipocalin superfamily highly expressed in lingual salivary glands. *Biochim. Biophys. Acta*, **1172:**131–137 (1993).

Böcskei, Z., Groom, C. R., Flower, D. R., Wright, C. E., Phillips, E. V., Cavaggioni, A., Findlay, J. B. C., and North, A. C. T., Pheromone binding to two rodent urinary proteins revealed by X-ray crystallography. *Nature*, **360:**186–188 (1992).

Boekhoff, I. and Breer, H., Termination of second messenger signaling in olfaction. *Proc. Natl. Acad. Sci. U.S.A.*, **89:**471–474 (1992).

Boekhoff, I., Tareilus, E., Strottman, J., and Breer, H., Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *EMBO J.*, **9:**2453–2458 (1990).

Booth, W. D. and White, C. A., The isolation, purification and some properties of pheromaxein, the pheromonal steroid-binding protein, in porcine submaxillary glands and saliva. *J. Endocrin.*, **118:**47–57 (1988).

Breer, H., Molecular reaction cascades in olfactory signal transduction. *J. Steroid Biochem. Mol. Biol.*, **39:**621–625 (1991).

Breer, H. and Boekhoff, I., Odorants of the same odor class activate different second messenger pathways. *Chem. Senses*, **16:**19–26 (1991).

Breer, H., Boekhoff, I., and Tareilus, E., Rapid kinetics of second messenger formation in olfactory transduction. *Nature*, **345:**65–68 (1990a).

Breer, H., Krieger, J., and Raming, K., A novel class of binding proteins in the antennae of the silkmoth *Antheraea pernyi*. *Insect Biochem.*, **20:**735–740 (1990b).

Brooks, D. E., Means, A. R., Wright, E. J., Singh, S. P., and Tiver, K. K., Molecular cloning of the cDNA for two major androgen-dependent secretory proteins of 18.5 kilodaltons synthesized by the rat epididymis. *J. Biol. Chem.*, **264:**4956–4961 (1986).

Brown, S. B. and Hara, T. J., Accumulation of chemostimulatory amino acids by a sedimentable fraction isolated from olfactory rosettes of rainbow trout (*Salmo gairdneri*). *Biochim. Biophys. Acta*, **675:**149–162 (1981).

Buck, L., Receptor diversity and spatial patterning in the mammalian olfactory system. **In:** *The Molecular Basis of Smell and Taste Transduction*, pp. 51–64. (Chadwick, D., Marsh, J., and Goode, J., Eds.) Wiley, Chichester.

Buck, L. and Axel, R., A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*, **66:**175–187 (1991).

Cagan, R. H. and Zeiger, W. N., Biochemical studies of olfaction: binding specificity of radioactively labeled stimuli to an isolated olfactory preparation from rainbow trout (*Salmo gairdneri*). *Proc. Natl. Acad. Sci. U.S.A.*, **75:**4679–4683 (1978).

Cavaggioni, A., Findlay, J. B. C., and Tirindelli, R., Ligand binding characteristics of homologous rat and mouse urinary proteins and pyrazine binding protein of the calf. *Comp. Biochem. Physiol.*, **96B:**513–520 (1990).

Cavaggioni, A., Sorbi, R. T., Keen, J. N., Pappin, D. J., and Findlay, J. B. C., Homology between the pyrazine binding protein from nasal mucosa and major urinary proteins. *FEBS Lett.*, **212:**225–228 (1987).

Chen, Z., and Lancet, D., Polypeptide gp-95. *J. Biol. Chem.*, **259:**1–7 (1984a).

Chen, Z. and Lancet, D., Membrane proteins unique to vertebrate olfactory cilia: candidates for sensory receptor molecules. *Proc. Natl. Acad. Sci.*, **81:**1859–1863 (1984b).

Chen, Z., Pace, U., Ronen, D., and Lancet, D., Polypeptide gp-95: a unique glycoprotein of olfactory cilia with transmembrane receptor properties. *J. Biol. Chem.*, **261:**1299–1305 (1986).

Clark, A. J., Clissold, P. M., Al Shawi, R., Beattie, P., and Bishop, J., Structure of mouse major urinary protein genes: different splicing configurations in the 3′-noncoding region. *EMBO J.*, **3:**1045–1052 (1984).

Dahl, A. R., The effect of cytochrome P-450-dependent metabolism and other enzyme activities on olfaction. **In:** *Molecular Neurobiology of the Olfactory System*, pp. 51–70. (Margolis, F. L. and Getchell, T. V., Eds.) Plenum Press, New York (1988).

Dal Monte, M., Andreini, I., Revoltella, R., and Pelosi, P., Purification and characterization of two odorant binding proteins from nasal tissue of rabbit and pig. *Comp. Biochem. Physiol.*, **99B:**445–451 (1991).

Dal Monte, M., Centini, M., Anselmi, C., and Pelosi, P., Binding of selected odorants to bovine and porcine odorant-binding proteins. *Chem. Senses*, **18:**713–721 (1993).

Danciger, E., Mettling, C., Vidal, M., Morris, M., and Margolis, F. L., Olfactory marker protein gene: its structure and olfactory neuron-specific expression in transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.*, **86:**8565–8569 (1989).

Dear, T. N., Boehm, T., Keverne, E. B., and Rabbitts, T. H., Novel genes for potential ligand-binding proteins in subregions of the olfactory mucosa. *EMBO J.*, **10:**2813–2819 (1991a).

Dear, T. N., Campbell, K., and Rabbitts, T. H., Molecular cloning of putative odorant-binding and odorant-metabolizing proteins. *Biochemistry*, **30:**10376–10382 (1991b).

De Kramer, J. J. and Hemberger, J., The neurobiology of pheromone reception. **In:** *Pheromone Biochemistry*, pp. 433–472. (Prestwich, G. D. and Blomquist, G. L., Eds.) Academic Press, Orlando (1987).

Descalzi-Cancedda, F., Dozin, B., Rossi, F., Molina, F., Cancedda, R., Negri, A., and Ronchi, S., The Ch21

protein, developmentally regulated in chick embryo, belongs to the superfamily of lipophilic molecule carrier proteins. *J. Biol. Chem.*, **265:**19060–19064 (1990).

Ding, X. and Coon, M. J., Purification and characterization of two unique forms of cytochrome P-450 from rabbit nasal microsomes. *Biochemistry*, **27:**8330–8337 (1988).

Ding, X. and Coon, M. J., Immunochemical characterization of multiple forms of cytochrome P-450 in rabbit nasal microsomes and evidence for tissue specific expression of P-450s NMa and NMb. *Mol. Pharmacol.*, **37:**480–496 (1990).

Ding, X., Porter, T. D., Peng, H. M., and Coon, M. J., cDNA and derived amino acid sequence of rabbit nasal cytochrome P-450NM6 (P450IIG1), a unique isozyme possibly involved in olfaction. *Arch. Biochem. Biophys.*, **285:**120 (1991).

Dinh, B. L, Tremblay, A., and Dufour, D., Immunochemical study of rat urinary proteins: their relation to serum and kidney proteins. *J. Immunol.*, **95:**574–582 (1965).

Drayna, D., Fielding, C., McLean, J., Baer, B., Castro, G., Chen, E., Comstock, L., Henzel, W., Kohr, W., Rhee, L., Wion, K., and Lawn, R., Cloning and expression of human apolipoprotein D cDNA. *J. Biol. Chem.*, **261:**16535–16539 (1986).

Felicioli, A., Ganni, M., Garibotti, M., and Pelosi, P., Multiple types and forms of odorant-binding proteins in the old-world porcupine *Hystrix cristata*. *Comp. Biochem. Physiol.*, **105B:**755–784 (1993a).

Felicioli, A., Ferraro, F., Ganni, M., Garibotti, M., Maida, R., Mazza, M., Navarrini, A., Pes, D., Tuccini, A., and Pelosi, P., Multiple classes of odorant-binding proteins: sequence similarities. *Sensors and Biosensors for Food Analysis and Process*, Parma, Italy, Oct. 20–22 (Abstr.), 1993b.

Fesenko, E. E, Novoselov, V. I., and Bystrova, M. F., The subunits of specific odor-binding glycoproteins from rat olfactory epithelium. *FEBS Lett.*, **219:**224–226 (1987).

Fesenko, E. E, Novoselov, V. I., and Bystrova, M. F., Properties of odor-binding glycoproteins from rat olfactory epithelium. *Biochim. Biophys. Acta*, **937:**369–378 (1988).

Fesenko, E. E., Novoselov, V. I., and Krapivinskaya, L. D., Molecular mechanisms of olfactory reception. IV. Some biochemical characteristics of the camphor receptor from rat olfactory epithelium. *Biochim. Biophys. Acta*, **587:**424–433 (1979).

Fesenko, E. E, Novoselov, V. I., and Krapivinskaya, L. D., Molecular mechanisms of odor sensing. VI. Some biochemical characteristics of a possible receptor for amino acids from the olfactory epithelium of the skate Dasyatis pastinaca and carp Cyprinus carpio. *Biochim. Biophys. Acta*, **759:**250–256 (1983).

Finlayson, J. S., Asofsky, R., Potter, M., and Runner, C. C., Major urinary protein complex of normal mice: origin. *Science*, **149:**981–982 (1965).

Ganni, M., Garibotti, M., Pes, D., and Pelosi, P., Odorant-binding proteins from deer nasal mucosa. *38 SIB Conference*, Trieste (Abstr.), 1993.

Getchell, M. L. and Getchell, T. V., Immunohistochemical localization of components of the immune barrier in the olfactory mucosae of salamanders and rats. *Anat. Rec.*, **231:**358–374 (1991).

Getchell, T. V., Margolis, F. L., and Getchell, M. L., Perireceptor and receptor events in vertebrate olfaction. *Prog. Neurobiol.*, **23:**317–345 (1984).

Getchell, M. L., Zielinski, B. S., DeSimone, J. A., and Getchell, T. V., Odorant stimulation of secretory and neural processes in the salamander olfactory mucosa. *J. Comp. Physiol. A.*, 160:155–168 (1987).

Getchell, T. V., Su, Z., and Getchell, M. L., Mucous domains: microchemical heterogeneity in the mucociliary complex of the olfactory epithelium. **In:** *The Molecular Basis of Smell and Taste Transduction*. pp. 27–40. (Chadwick, D., Marsh, J., and Goode, J., Eds.) Wiley, Chichester (1993).

Goldberg, S. J., Turnip, J., and Price, S., Anisole binding protein from olfactory epithelium: evidence for a role in transduction. *Chem. Senses*, **4:**207–214 (1979).

Graziadei, P. P. C. and Dehann, R. S., Neuronal regeneration in frog olfactory system. *Cell Biol.*, **59:**525–530 (1973).

Graziadei, P. P. C., Levine, R. R., and Monti-Graziadei, G. A., Regeneration of olfactory axons and synapse formation in the forebrain after bulbectomy in neonaral mice. *Proc. Natl. Acad. Sci. U.S.A.*, **75:**5230–5234 (1978).

Graziadei, P. P. C. and Monti Graziadei, G. A., Neurogenesis of sensory neurons in the primate olfactory system after section of the fila olfactoria. *Brain Res.*, **186:**289–300 (1980).

Gyorgyi, T. K., Roby-Shemkovitz, A. J., and Lerner, M. R., Characterization and cDNA cloning of the pheromone-binding protein from the tobacco hornworm, Manduca sexta: a tissue-specific developmentally regulated protein. *Proc. Natl. Acad. Sci. U.S.A.*, **85:**9851–9855 (1988).

Henzel, W. J., Rodriguez, H., Singer, A. G., Stults, J. T., Macrides, F., Agosta, W. C., and Niall, H., The primary structure of aphrodisin. *J. Biol. Chem.*, **263:**16682–16687 (1988).

Hérent, M. F., Collin, S., and Pelosi, P., Affinities of nutty and green-smelling compounds to odorant-binding proteins. manuscript in preparation.

Huque, T. and Bruch, R. C., Odorant and guanine nucleotide-stimulated phosphoinositideturnover in olfactory cilia. *Biochem. Biophys. Res. Commun.*, **137:**36 (1986).

Jones, D. T. and Reed, R. R., G-olf: an olfactory neuron specific G-protein involved in odorant signal transduction. *Science*, **244:**790–795 (1989).

Julkunen, M., Seppala, M., and Janne, O. A., Complete amino acid sequence of human placental protein 14: a progesterone-regulated uterine protein homologous to β-lactoglobulins. *Proc. Natl. Acad. Sci. U.S.A.*, **85:**8845–8849 (1988).

Kaissling, K. E., Chemo-electrical transduction in insect olfactory receptors. *Ann. Rev. Neurosci.*, **9:**121–145 (1986).

Kaissling, K. E., *R.H. Wright Lectures on Insect Olfaction*. Simon Fraser Univ. Press, Burnaby, Canada, 1987.

Kaissling, K. E. and Thorson, J. Insect olfactory sensilla: structural, chemical and electrical aspects of the functional organization. **In:** *Receptors for Neurotransmitters, Hormones and Pheromones*, pp. 261–282. (Sattelle, D. B., Hall, M., and Hildebrandt, J. G., Eds.) Elsevier, Amsterdam, 1980.

Kaissling, K. E., Klein, U., De Kramer, J. J., Keil, T. A., Kanaujia, S., and Hemberger, J., Insect olfactory cells: electrophysiological and biochemical studies. **In:** *Molecular Basis of Nerve Activity*, pp. 173–183. (Changeux, J. P., Hucho, F., Maelicke, A., and Neuman, E., Eds.) De Gruyter, Berlin, 1985.

Kalinoski, D. L., Aldinger, S. B., Boyle, A. G., Huque, T., Marecek, J. F., Prestwich, G. D., and Restrepo, D., Characterization of a novel inositol, 1,4,5-triphosphate receptor in isolated olfactory cilia. *Biochem. J.*, **281:**449 (1992).

Kaumeyer, J. F., Polazzi, J. O., and Kotik, M. P., The mRNA for a proteinase inhibitor related to the HI-30 domain of inter-a-trypsin inhibitor also encodes a-1-microglobulin (protein HC). *Nucl. Acids Res.*, **14:**7839–7850 (1986).

Keen, J. N., Caceres, I., Eliopoulos, E. E., Zagalsky, P. F., and Findlay, J. B. C., Complete sequence and model for the A-2 subunit of the carotenoid pigment complex, crustacyanin. *Eur. J. Biochem.*, **197:**407–417 (1991).

Khew-Goodall, Y., Grillo, M., Getchell, M. L., Danho, W., Getchell, T. V., and Margolis, F. L., Vomeromodulin, a putative pheromone transporter: cloning, characterization and cellular localization of a novel glycoprotein of lateral nasal gland. *FASEB J.*, **5:**2976–2982 (1991).

Klein, U., Sensillum lymph proteins from antennal olfactory hairs of the moth *Antheraea polyphemus* (Saturnidae). *Insect Biochem.*, **17:**1193–1204 (1987).

Koshland, D. E., Jr., Biochemistry of sensing and adaptation in a simple bacterial system. *Ann. Rev. Biochem.*, **50:**765–782 (1981).

Krieger, J., Ganble, K., Raming, K., and Breer, H., Odorant binding proteins of *Heliothis virescens*. *Insect Biochem. Mol. Biol.*, **23:**449–456 (1993).

Krieger, J., Raming, K., and Breer, H., Cloning of genomic and complementary DNA encoding insect pheromone binding proteins: evidence for microdiversity. *Biochim. Biophys. Acta*, **1088:**277–284 (1991).

Kudrycki, K., Steinizsak, C., Behn, C., Grillo, M., Acheson, R., and Margolis, F. L., Olf-1 binding site. Characterization of an olfactory neuron specific promoter motif. *Mol. Cell Biol.*, **13:**3002–3014 (1993).

Kurihara, K. and Koyama, N., High activity of adenyl cyclase in olfactory and gustatory organs. *Biochem. Biophys. Res. Commun.*, **48:**30–33 (1972).

Lancet, D., Ben-Arie, N., Cohen, S., Gat, U., Gross-Isseroff, R., Horn-Saban, S., Khen, M., Leharach, H., Natochin, N., North, M., Seidemann, E., and Walker, N., Olfactory receptors: transduction, diversity, human psychophysics and genome analysis. **In:** *The Molecular Basis of Smell and Taste Transduction*. pp. 131–141. (Chadwick, D., Marsh, J., and Goode, J., Eds.) Wiley, Chichester, 1993.

Lazard, D., Tal, N., Rubinstein, M., Khen, M., Lancet, D., and Zupko, K., Identification and biochemical analysis of novel olfactory-specific cytochrome P-450IIA and UDP-glucoronosyl transferase. *Biochemistry*, **29:**7433–7440 (1990).

Lazard, D., Zupko, K., Poria, Y., Nef, P., Lazarovits, J., Horn, S., Khen, M., and Lancet, D., Odorant signal termination by olfactory UDP-glucuronosyl transferase. *Nature*, **349:**790–793 (1991).

Lee, H. K., Wells, R. G., and Reed, R. R., Isolation of an olfactory cDNA: similarity to retinol binding protein suggests a role in olfaction. *Science*, **253:**1053–1056 (1987).

Longo, V., Citti, L., and Gervasi, P. G., Biotransformation enzymes in nasal mucosa and liver of Sprague-Dawley rats. *Toxicol. Lett.*, **44:**289–297 (1988).

Lowe, G., Nakamura, T., and Gold, G. H., Adenylate cyclase mediates olfactory transduction for a wide variety of odorants. *Proc. Natl. Acad. Sci. U.S.A.*, **86:**5641–5645 (1989).

Ludwig, J., Margalit, T., Eisman, E., Lancet, D., and Kaupp, U., Primary structure of cAMP-gated channel from bovine olfactory epithelium. *FEBS Lett.*, **270:**24–29 (1990).

Maida, R., Steinbrecht, R. A., Ziegelberger, G., and Pelosi, P., The pheromone-binding protein of *Bombyx mori:* purification, characterization and immunocytochemical localization. *Insect Biochem. Mol. Biol.*, **23:**243–253 (1993).

Margolis, F. L., A brain protein unique to the olfactory bulb. *Proc. Natl. Acad. Sci. U.S.A.*, **69:**1221–1224 (1972).

Menco, B. Ph. M. and Farbman, A. I., Ultrastructural evidence for multiple mucous domains in frog olfactory epithelium. *Cell Tissue Res.*, **270:**47–56 (1992).

Mendez, E., Fernandez-Luna, J. L., Grubb, A., and Leyva-Cobian, F., Human protein HC and its IgA complex are inhibitors of neutrophil chemotaxis. *Proc. Natl. Acad. Sci.*, **83:**1472–1475 (1986).

Menevse, A., Dodd, G. H., and Poynder, T. M., Evidence for the specific involvement of cyclic AMP in the olfactory transduction mechanism. *Biochem. Biophys. Res. Commun.*, **77:**671–677 (1977).

Minor, A. V. and Sakina, N. L., The role of cyclic adenosine-3′,5′-monophosphate in olfactory reception. *Neurofiziologiya*, **5:**415–422 (1973).

Monaco, H. L. and Zanotti, G., Three-dimensional structure and active site of three hydrophobic molecule-binding proteins with significant amino acid sequence similarity. *Biopolymers*, **32:**457–465 (1992).

Monaco, H. L., Zanotti, G., Spadon, P., Bolognesi, M., Sawyer, L., and Eliopoulos, E. E., Crystal structure of the trigonal form of bovine b-lactoglobulin and its complex with retinol at 2.5 Å resolution. *J. Mol. Biol.*, **197:**695–706 (1987).

Mowbray, S. L. and Cole, L. B., 1.7 Å X-ray structure of the periplasmic ribose receptor from *Escherichia coli*. *J. Mol. Biol.*, **225:**155–175 (1992).

Nagata, A., Suzuki, Y., Igarashi, M., Eguchi, N., Toh, H., Urade, Y., and Hayaishi, O., Human brain prostaglandin

D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins. *Proc. Natl. Acad. Sci. U.S.A.*, **88**:4020–4024 (1991).

Nef, P., Heldman, J., Lazard, D., Jaye, M., Hanukoglu, I., and Lancet, D., Olfactory-specific cytochrome P-450. *J. Biol. Chem.*, **264**:6780–6785 (1989).

Ngai, J., Chess, A., Dowling, M. M., Necles, N., Macagno, E. R., and Axel, R., Coding of olfactory information: topography of odorant receptor expression in the catfish epithelium. *Cell*, **72**:667–680 (1993a).

Ngai, J., Dowling, M. M., Buck, L., Axel, R., and Chess, A., The family of genes encoding odorant receptors in the channel catfish. *Cell*, **72**:657–666 (1993b).

Novoselov, V. I., Krapiviskaya, L. D., and Fesenko, E. E., Molecular mechanism of odor sensing. V. Some biochemical characteristics of the alanineous receptor from the olfactory epithelium of the skate *Dasyatis pastinaca*. *Chem. Senses*, **5**:195–203 (1980).

Ohloff, G., *Riechstoffe und Geruchssinn.* Springer-Verlag, Berlin, 1990.

Pace, U., Hansky, E., Salomon, Y., and Lancet, D., Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature*, **316**:255–258 (1985).

Pace, U. and Lancet, D., Olfactory GTP-binding protein: signal transducing polypeptide of vertebrate chemosensory neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **83**:4947–4951 (1986).

Papiz, M. Z., Sawyer, L., Eliopoulos, E. E., North, A. C. T., Findlay, J. B. C., Sivaprasadarao, R., Jones, T. A., Newcomer, M. E., and Kraulis, P. J., The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature*, **324**:383–385 (1986).

Pelosi, P., Baldaccini, N. E., and Pisanelli, A. M., Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. *Biochem. J.*, **201**:245–248 (1982).

Pelosi, P. and Garibotti, M., Peripheral aspects of olfaction. *Cytotechnology*, **11**:7–11 (1992).

Pelosi, P. and Maida, R., Odorant binding proteins in vertebrates and insects: similarities and possible common function. *Chem. Senses*, **15**:205–215 (1990).

Pelosi, P. and Pisanelli, A. M., Specific anosmia to 1,8-cineole: the camphor primary odour. *Chem. Senses*, **6**:87–93 (1981).

Pelosi, P., Pisanelli, A. M., Baldaccini, N. E., and Gagliardo, A., Binding of [3H]-2-isobutyl-3-methoxypyrazine to cow olfactory mucosa. *Chem. Senses*, **6**:77–85 (1981).

Pelosi, P. and Tirindelli, R., 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, 1989.

Pelosi, P. and Viti, R., Specific anosmia to l-carvone: the minty primary odour. *Chem. Senses*, **3**:331–337 (1978).

Persaud, K. C., Pelosi, P., and Dodd, G. H., Binding and metabolism of the urinous odorant 5α-androstan-3-one in sheep olfactory mucosa. *Chem. Senses*, **13**:231–245 (1988).

Pes, D., Dal Monte, M., Ganni, M., and Pelosi, P., Isolation of two odorant-binding proteins from Isolation of two odorant-binding proteins from mouse nasal tissue. *Comp. Biochem. Physiol.*, **103B**:1011–1017 (1992).

Pes, D. and Pelosi, P., Odorant-binding proteins of the mouse, manuscript in preparation (1994).

Pevsner, J., Hou, V., Snowman, A. M., and Snyder, S. H., Odorant-binding protein, characterization of ligand binding. *J. Biol. Chem.*, **265**:6118–6125 (1990).

Pevsner, J., Hwang, P. M., Sklar, P. B., Venable, J. C., and Snyder, S. H., Odorant-binding protein and its mRNA are localized to lateral nasal gland implying a carrier function. *Proc. Natl. Acad. Sci. U.S.A.*, **85**:2383–2387 (1988a).

Pevsner, J., Reed, R. R., Feinstein, P. G., and Snyder, S. H., Molecular cloning of odorant-binding protein: member of a ligand carrier family. *Science*, **241**:336–339 (1988b).

Pevsner, J., Sklar, P. B., and Snyder, S. H., Odorant-binding protein: localization to nasal glands and secretions. *Proc. Natl. Acad. Sci. U.S.A.*, **83**:4942–4946 (1986).

Pevsner, J. and Snyder, S. H., Odorant-binding protein: odorant transport function in the vertebrate nasal epithelium. *Chem. Senses*, **15**:217–222 (1990).

Pevsner, J., Trifiletti, R. R., Strittmatter, S. M., and Snyder, S. H., Isolation and characterization of an olfactory receptor protein for odorant pyrazines. *Proc. Natl. Acad. Sci. U.S.A.*, **82**:3050–3054 (1985).

Prestwich, G. D., Bacterial expression and photoaffinity labeling of a pheromone binding protein. *Protein Sci.*, **2**:420–428 (1993).

Price, S., Anisole binding protein from dog olfactory epithelium. *Chem. Senses Fla.*, **3**:51–55 (1978).

Price, S. and Willey, A., Benzaldehyde binding protein from dog olfactory epithelium. *Ann. N.Y. Acad. Sci.*, **510**:561–564 (1987).

Rama-Krishna, N. S., Getchell, M. L., Tate, S. S., Margolis, F. L., and Getchell, T. V., Glutathione and γ-glutamyl transpeptidase are differentially distributed in the olfactory mucosa of rats. *Cell Tissue Res.*, **270**:475–484 (1992).

Raming, K., Krieger, J., and Breer, H., Molecular cloning of an insect pheromone-binding protein. *FEBS Lett.*, **256**:215–218 (1989).

Raming, K., Krieger, J., and Breer, H., Primary structure of a pheromone-binding protein from *Antheraea pernyi*: homologies with other ligand-carrying protein. *Comp. Physiol. B*, **160**:503–509 (1990).

Raming, K., Krieger, J., Strotmann, J., Boekhoff, I., Kubick, S., Baumstark, C., and Breer, H., Cloning and expression of odorant receptors. *Nature*, **361**:353–356 (1993).

Redl, B., Holzfeind, P., and Lottspeich, F., 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 (1992).

Rhein, L. D. and Cagan, R. H., Biochemical studies of olfaction: isolation, characterization, and odorant binding activity of cilia from rainbow trout olfactory ro-

settes. *Proc. Natl. Acad. Sci. U.S.A.*, **77**:4412–4416 (1980).

Riley, C. T., Barbeau, B. K., Keim, P. M., Kezdy, F. J., Heinrikson, R. L., and Law, J. H., The covalent protein structure of insecticyanin, a blue biliprotein from the hemolymph of the tobacco hornworm, *Manduca sexta* L. *J. Biol. Chem.*, **259**:13159–13165 (1984).

Rogers, K. E., Dasgupta, P., Gubler, U., Grillo, M., Khew-Goodall, Y. S., and Margolis, F. L., Molecular cloning and sequencing of cDNA for olfactory marker protein. *Proc. Natl. Acad. Sci. U.S.A.*, **84**:1704–1708 (1987).

Schleicher, S., Boekhoff, I., Arriza, J., Lefkowitz, R. J., and Breer, H., A β-adrenergic receptor kinase-like enzyme is involved in olfactory signal termination. *Proc. Natl. Acad. Sci. U.S.A.*, **90**:1420–1424 (1993).

Shahan, K. M., Denaro, M., Gilmartin, M., Shi, Y., and Derman, E., 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 (1987a).

Shahan, K. M., Gilmartin, M., and Derman, E., Nucleotide sequences of liver, lachrymal and submaxillary gland mouse major urinary protein mRNAs: mosaic structure and construction of panels of gene-specific synthetic oligonucleotide probes. *Mol. Cell. Biol.*, **7**:1938–1946 (1987b).

Shaw, P. H., Held, W. A., and Hastie, N. D., The gene family for major urinary proteins:expression in several secretory tissues of the mouse. *Cell*, **32**:755–761 (1983).

Shepherd, G. M., The olfactory system: the uses of neural space for a non-spatial modality. **In:** *Contemporary Sensory Neurobiology*, pp. 99–114. (Correia, M. and Perachio, A. A., Eds.) Alan R. Liss, New York, 1985.

Shi, Y., Rodriguez, M., Shahan, K., and Derman, E., Subfamily of submaxillary gland-specific Mup genes: chromosomal linkage and sequense comparison with liver-specific Mup genes. *Nucleic Acids Res.*, **17**:6191–6203 (1989a).

Shi, Y., Son, H. J., Shahan, K., Rodriguez, M., Costantini, F., and Derman, E., Silent genes in the mouse major urinary protein gene family. *Proc. Natl. Acad. Sci. U.S.A.*, **86**:4584–4588 (1989b).

Shirley, S. G., Robinson, C. J., Dickinson, K., Aujla, R., and Dodd, G. H., Olfactory adenylate cyclase of the rat: stimulation by odorants and inhibition by Ca^{2+}. *Biochem. J.*, **240**:605–607 (1986).

Singer, A. G., A chemistry of mammalian pheromones. *J. Steroid Biochem. Mol. Biol.*, **39**:627–632 (1991).

Singer, A. G. and Macrides, F., Aphrodisin: pheromone or tranducer?. *Chem. Senses*, **15**:199–203 (1990).

Singer, A. G. and Macrides, F., Composition of an aphrodisiac pheromone. *Chem. Senses*, **18**:630 (1993).

Sklar, P. B., Anholt, R. H., and Snyder, S. H., The odorant sensitive adenylate cyclase olfactory receptor cells: differential stimulation by distinct classes of odorants. *J. Biol. Chem.*, **261**:15538–15543 (1986).

Snyder, D. A., Rivers, A. M., Yokoe, H., Menco, B. Ph. M., and Anholt, R. R. H., Olfactomedin: purification, characterization and localization of a novel olfactory glycoprotein. *Biochemistry*, **30**:9143–9153 (1991).

Snyder, S. H., Sklar, P. B., and Pevsner, J., Molecular mechanisms of olfaction. *J. Biol. Chem.*, **263**:13971–13974 (1988).

Spence, A. M., Sheppard, P. C., Davie, J. R., Matuo, Y., Nishi, N., McKeehan, W. L., Dodd, J. G., and Matusik, R. J., Regulation of a bifunctional mRNA results in synthesis of secreted and nuclear probasin. *Proc. Natl. Acad. Sci. U.S.A.*, **86**:7843–7847 (1989).

Stewart, R. C. and Dahlquist, F.W., Molecular components of bacterial chemotaxis. *Chem. Rev.*, **87**:997–1025 (1987).

Theimer, E. T., *Fragrance Chemistry. The Science of the Sense of Smell*, Academic Press, New York, 1982.

Tirindelli, R., Keen, J. N., Cavaggioni, A., Eliopoulos, E. E., and Findlay, J. B. C., Complete amino acid sequence of pyrazine-binding protein from cow nasal mucosa. *Eur. J. Biochem.*, **185**:569–572 (1989).

Topazzini, A., Pelosi, P., Pasqualetto, P. L., and Baldaccini, N. E., Specificity of a pyrazine binding protein from cow olfactory mucosa. *Chem. Senses*, **10**:45–49 (1985).

Urade, Y., Nagata, A., Suzuki, Y., Fujii, Y., and Hayaishi, O., Primary structure of rat brain prostaglandin D synthetase deduced from cDNA sequence. *J. Biol. Chem.*, **264**:1041–1045 (1989).

Van den Berg, M. J. and Ziegelberger, G., On the function of the pheromone binding protein in the olfactory hairs of *Antheraea polyphemus*. *J. Insect Physiol.*, **37**:79–85 (1991).

Vogt, R. G., Kohne, A. C., Dubnau, J. T., and Prestwich, G. D., Expression of pheromone binding proteins during antennal development in the gypsy moth *Lymantria dispar*. *J. Neurosci.*, **9**:3332–3346 (1989).

Vogt, R. G., Prestwich, G. D., and Lerner, M. R., Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. *J. Neurobiol.*, **22**:74–84 (1991a).

Vogt, R. G., Prestwich, G. D., and Riddiford, L. M., Sex pheromone receptor proteins. Visualization using a radiolabeled photoaffinity analog. *J. Biol. Chem.*, **263**:3952–3959 (1988).

Vogt, R. G. and Riddiford, L. M., Pheromone binding and inactivation by moth antennae. *Nature*, **293**:161–163 (1981).

Vogt, R. G., Rybczynski, R., and Lerner, M. R., The biochemistry of odorant reception and transduction. **In:** *Chemosensory Information Processing, NATO ASI Series H, Vol. 39*, pp. 33–76. (Schild, D., Ed.) Springer-Verlag, Berlin, 1990.

Vogt, R. G., Rybczynski, R., and Lerner, M. R., Molecular cloning and sequencing of general odorant-binding proteins GOBP1 and GOBP2 from the tobacco hawk moth Manduca sexta: comparison with other insect OBPs and

their signal peptides. *J. Neurosci.*, **11**:2972–2984 (1991b).

Wang, M. M. and Reed, R. R., Molecular cloning of the olfactory neuronal transcription factor Olf-1 by genetic selection in yeast. *Nature*, **364**:121–126 (1993).

Whissell-Buechy, D. and Amoore, J. E., Odour-blindness to musk: simple recessive inheritance. *Nature*, **242**:271–273 (1973).

Yokoe, H. and Anholt, R. H., Molecular cloning of olfactomedin, an extracellular matrix protein specific to olfactory neuroepithelium. *Proc. Natl. Acad. Sci.*, **90**:4655–4659 (1993).

Zanotti, G., Ottonello, S., Berni, R., and Monaco, H. L., Crystal structure of the trigonal form of human plasma retinol-binding protein at 2.5 Å resolution. *J. Mol. Biol.*, **230**:613–624 (1993).