

Pheromone Peptides

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ABSTRACT

Pheromones are chemical messages known to elicit changes in behavioral reactions that are followed by physiological alterations in conspecific receiver organisms. Pheromones comprise a wide range of compounds, of varied chemical nature, that range from small-molecule volatile compounds to high-molecular-weight, water-soluble, nonvolatile peptides and proteins. Although volatile pheromone molecules have been studied extensively, much less is known about the chemical nature of the equivalent nonvolatile water-soluble cues. In this chapter, we summarize the state of our knowledge of pheromone peptides and a few proteins, in a variety of species belonging to various phyla and classes.

INTRODUCTION

Pheromones are chemical messages that are secreted from an individual and that elicit a behavioral reaction or an endocrine change in another individual of the same species. In the receiving organism, pheromones can elicit an immediate behavioral reaction, such as alarm, defense, aggregation, attraction, kin and colony recognition, marking of territories and egg-deposition sites, mating behavior, recruitment, and thermoregulation. They also can induce complex behavioral and physiological alterations—such as development of a particular caste, or sexual maturation—via long-term endocrine changes.

Chemical communication among organisms is a highly sophisticated procedure that involves the regulation of many genes in the donor, and initiation of complex processes in the recipient, in which interpretation of the individual chemical constituents or “signals” depends on the particular combination, ratios, concentrations, and order of presentation of signal molecules, and also on the physiological state of the receiver. It is believed that, during the course of evolution, pheromones of different organisms have become very distinctive, to avoid ambiguity and to ensure that the channels of communication through which they exert their activity do not overlap, which causes even greater complexity.

Chemically, pheromones range from small-molecule volatile compounds to high-molecular-weight, water-soluble, nonvolatile peptides and proteins. Usually, small molecules

are used for communication, which requires rapid dispersal of the signal, whereas the larger molecules of less volatile compounds tend to function in attraction and stimulation, for which prolonged exposure is necessary. Although airborne signal molecules have been identified and studied extensively, and are being applied commercially to disrupt mating and to monitor insect pests, much less is known about the chemical nature of the equivalent water-borne cues.

Pheromone peptides are widely distributed throughout the prokaryotic and eukaryotic organisms; they serve to mediate a variety of functions in Gram-positive and Gram-negative bacteria, fungi, ciliates, and in many different animals that belong to the mollusk, annelid, arthropod, and vertebrate (amphibians and mammals) phyla. The very extensive distribution of this means of signaling, among so many, widely diverse organisms, hints at the evolutionary importance of this mode of communication.

Pheromone peptides have been studied for many years, and in several systems, the nature of the peptides, their origins, and their modes of action are quite well understood. In other systems, however, our understanding is still very nascent, and the chemical, molecular, physiological, and biological aspects remain to be explored. Interestingly, in the few years that have passed since the publication of this chapter in the 1st edition of this book, >30 review articles have been published on pheromone peptides in organisms belonging to the bacterial, fungal, and animal kingdoms (most of them on bacterial and mammalian peptide pheromones). A vast amount of knowledge has accumulated on some of these signaling molecules, covering aspects such as: identification of the chemical nature of these peptides; discovery of new peptide pheromones and identification of their biosynthetic pathways, their molecular cloning and their structure/activity relationship (SAR); their inter-molecular interactions; their mechanisms of production and reception, as well as their modes of action at the cellular level; signal transduction pathways that follow peptide pheromone binding to the receptor; intracellular communication at the genome level; and the neural circuitry leading to specific pheromonal outputs and physiological effects. These “signal transduction” and “intracellular communication” studies attracted most

attention from many research groups, and thus emerged as the most studied topic across the various phyla, leaving an overall impression that the field had made immense progress and had moved from “preliminary-discovery” studies that merely documented the involvement of peptide pheromones in conspecific species to studies that focused on in-depth understanding of the molecular and cellular mechanisms that underlie peptide pheromone reception and activity. This chapter presents a short summary of the state of our knowledge on pheromone peptides and a few proteins, in a variety of species. The limited scope of the chapter does not enable a detailed description of each system, and the reader is referred to the references (mostly recent review articles) in each section, for further information. A summary of the peptides mentioned in this chapter is presented in [Table 1](#).

BACTERIAL PHEROMONE PEPTIDES

One of the most complex and diverse systems of pheromone peptides is found in bacteria. Both unicellular organisms and individual cells of metazoans have evolved complex signaling mechanisms by which they respond to the environment and communicate with one another. These mechanisms involve short peptides that can be secreted as unmodified structures or as posttranslationally modified peptides. These peptides are sensed by target organisms via cognate sensors, with each signal molecule initiating a complex multilevel response pathway. There are two basic types of bacterial communication systems: those in which the signal is detected solely by organisms other than the emitter, and those in which the signal is sensed by the emitting organism as well.

TABLE 1 Major Pheromone Peptides or Protein Families in Bacteria, Fungi and the Animal Kingdom

Pheromone Peptide or Protein Family	Organism or Group Studied: (Order/Genus/Species)	Function	Ref.
Bacterial Pheromone Peptides			
Mating pheromones			
cCF10, cAD1, cPD1, cAM373, cOB1	<i>Enterococcus faecalis</i>	Cellular aggregation and plasmid transfer	Dunny and Johnson ¹³ , Thoendel and Horswill ²⁹ , Wardal, Sadowy, and Hryniewicz ³¹
Quorum-sensing peptides			
Gram-positive peptides			
Autoinducing peptide (AIP)	<i>Staphylococcus aureus</i>	Virulence peptides	Thoendel and Horswill ²⁹
Autoinducing peptide (AIP)	<i>Lactobacillus plantarum</i>	Virulence peptides	Thoendel and Horswill ²⁹
Gelatinase biosynthesis activating pheromone (GBAP)	<i>Enterococcus faecalis</i>	Virulence peptides	Thoendel and Horswill ²⁹
PapR	<i>Bacillus cereus</i>	Virulence peptides	Thoendel and Horswill ²⁹
Cytolysin subunit (Cyl ₅)	<i>Enterococcus faecalis</i>	Virulence peptides	Shankar et al. ²⁶
Competence-stimulating peptides (CSP-1, CSP-2)	<i>Streptococcus pneumoniae</i>	Genetic competence	Thoendel and Horswill ²⁹
Competence-stimulating factor (CSF, PhrC)	<i>Bacillus subtilis</i>	Genetic competence	Thoendel and Horswill ²⁹
Competence factor ComX	<i>Bacillus subtilis</i>	Genetic competence	Thoendel and Horswill ²⁹
Nisin	<i>Lactococcus lactis</i>	Production of bacteriocins	Kleerebezem et al. ¹⁸
Subtilin	<i>Bacillus subtilis</i>	Production of bacteriocins	Kleerebezem et al. ¹⁸
Plantaricin A (PlnA) peptides	<i>Lactobacillus plantarum</i>	Production of bacteriocins	Diep et al. ¹¹
BlpC (also termed SpiP) peptides	<i>Streptococcus pneumoniae</i>	Production of bacteriocins	Lyon and Novick ²³
Gram-negative peptides			
Double Gly (G-G) containing peptides	Various bacterial spp.	Quorum sensing	Thoendel and Horswill ²⁹

TABLE 1 Major Pheromone Peptides or Protein Families in Bacteria, Fungi and the Animal Kingdom—*Cont'd*

Pheromone Peptide or Protein Family	Organism or Group Studied: (Order/Genus/Species)	Function	Ref.
Fungal Pheromone Peptides			
α -factor	<i>Saccharomyces cerevisiae</i>	Mating initiation	Naider and Becker ²⁴
a-factor	<i>Saccharomyces cerevisiae</i>	Mating initiation	Anderegg et al. ²
Ciliates Pheromone Peptides			
Er-1, Er-2, Er-7, Er-10, Er-11, Er-20, Er-21	<i>Euplotes</i> spp. and other hypotrichs	Sexual reproduction	Luporini et al. ²²
G3, G4	<i>Euplotes</i> spp. and other hypotrichs	Sexual reproduction	Luporini et al. ²²
Mollusk Pheromone Peptides			
Attractins, enticin, temptin, seductin, Alb-1	<i>Aplysia</i> spp.	Egg laying, mating, aggregation	Cummins, Degnan, and Nagle, ⁸ Cummins et al. ⁹
ILME (Ile-Leu-Met-Glu)	<i>Loligo pealeii</i> , <i>Sepia officinalis</i>	Transport of oocytes in the genital tract	Susswein and Nagle ²⁸
Sperm attractin peptide (SepSAP)	<i>Sepia officinalis</i>	Sperm attraction and facilitation of external fertilization	Susswein and Nagle ²⁸
Annelid Pheromone Peptides			
Nereithione	<i>Nereis succinea</i>	Control of reproductive behavior, mate recognition, and gamete releases	Hardege et al. ¹⁵
Arthropod Pheromone Peptides			
Insect peptides			
Sex peptide (accessory gland protein—Acp70A)	<i>Drosophila melanogaster</i>	Variety of reproductive and postmating functions	Chapman and Davies ⁶
DUP99B	<i>Drosophila melanogaster</i>	Variety of reproductive and postmating functions	Kubli ¹⁹
Pyrokinin/Pheromone biosynthesis activating neuropeptide (PK/PBAN) family	Various moth spp. (see list in Ref. [1] Table 1)	Sex-pheromone biosynthesis	Altstein and Hariton ¹
Crustacean peptides			
Settlement pheromones (e.g. arthropodin)	Barnacles (e.g. <i>Balanus amphitrite</i>)	Colony aggregation and settlement	Forward ¹⁴ , Ziegler and Forward ³⁵
Larval-release pheromones	Various decapod crustaceans (brachyuran crabs)	Synchronization of larval release	Forward ¹⁴ , Ziegler and Forward ³⁵

Continued

TABLE 1 Major Pheromone Peptides or Protein Families in Bacteria, Fungi and the Animal Kingdom—*Cont'd*

Pheromone Peptide or Protein Family	Organism or Group Studied: (Order/Genus/Species)	Function	Ref.
Vertebrate Pheromone Peptides			
Amphibian peptides			
Sodefrin	<i>Cynops pyrrhogaster</i> ; <i>Triturus carnifex</i>	Female attraction	Woodley ³²
Silefrin	<i>Cynops ensicauda</i>	Female attraction	Woodley ³²
Aonirin	<i>Cynops pyrrhogaster</i>	Female attraction	Woodley ³²
Splendipherin	<i>Litoria splendida</i>	Female attraction	Woodley ³²
Courtship pheromones (PRF, PMF, SPLF)	<i>Plethodon jordani</i>	Shortening of courtship process	Woodley ³²
Leptodactylus aggression-stimulating peptide (LASP)	<i>Leptodactylus fallax</i>	Stimulation of male aggression	King et al. ¹⁷
Mammalian peptides			
Aphrodisin	Rodents	Induction of copulatory behavior	Briand, Trotier, and Pernellet ⁵
Major urinary proteins (MUPs)	Rodents	Communication of individual identity and ownership via scent marks	Touhara ³⁰
Major histocompatibility complex, (MHC,) class I peptides	Rodents	Communicate individual identity and mediate pregnancy blockage or spontaneous abortion	Touhara ³⁰
Tear exocrine-gland-secreting peptide 1 (ESP1)	Rodents	Communication of individual identity and ownership via scent marks	Touhara ³⁰
Vasopressin and oxytocin	Rodents and sheep	Assistance in processing of pheromonal cues associated with social discrimination	Bielsky and Young, ⁴ Higashida et al. ¹⁶

The first type is typified by the mating pheromones of the enterococcal bacteria, e.g. *Enterococcus faecalis*: cCF10 and cAD1, which are hepta- and octapeptides with sequences LVTLVFV and LFSLVLAG, respectively. Among these bacteria, a potential recipient releases a small peptide that is taken up by a potential donor, in which it elicits a plasmid-coded response that leads to cellular aggregation and transfer of the plasmid from donor to recipient. Production of the mating-pheromonal peptide in the donor is dependent on the close proximity of recipient cells. The enterococcal pheromone is processed from a chromosomally encoded lipoprotein precursor. Most strains produce multiple pheromones with mutually differing spectra of activities, but each plasmid responds specifically to its cognate pheromone with

extremely high specificity and sensitivity. Details on the synthesis of the enterococcal mating pheromone molecules, the components of the regulatory machinery that interact specifically with the peptides, the molecular basis for their exquisite sensitivity and specificity, and the regulatory circuits controlling the multiple levels of regulation of intracellular communication have been reviewed recently.^{13,29,31}

The second group of bacterial pheromone peptides comprises the autoinduction or quorum-sensing (QS) systems, which are widespread among bacteria, in which they elicit population-wide responses (cell-cell or cell-environment responses) to low-molecular-weight signaling molecules, depending on cell-population density. The structures of the signaling molecules differ between Gram-negative and

Gram-positive bacteria: in the latter, the mating pheromones and most QS signals are peptides that are sensed by transmembrane receptor components that activate an intracellular response pathway.

Examples of QS-induced processes in Gram-positive bacteria include virulence, genetic competence, and production of antimicrobial peptides. Virulence is induced in *Staphylococcus aureus* and *Lactobacillus plantarum* by autoinducing peptides (AIPs)—a 7- to 9- and a 5-residue cyclic peptide, respectively,—and in *E. faecalis* and *Bacillus cereus* by gelatinase biosynthesis activating pheromone (GBAP)—an 11 residue cyclic peptide—and PapR (a heptapeptide with the sequence ADLPFEF²⁹), respectively. Genetic competence is induced in *Streptococcus pneumoniae* and *Bacillus subtilis* by competence-stimulating peptides CSP and competence-stimulating factor (CSF), which are 17 amino acid peptides with the sequence EMRLSKFFRDFILQRKK and a pentapeptide with the sequence ERGMT, respectively.²⁹ *B. subtilis* 168-type strain produces another competence pheromone: a posttranslation modified 10 residue linear peptide containing a farnesyl group, termed Com-X (ADPITRQWGD).²⁹

Production of antimicrobial peptides, known as bacteriocins, also involves a QS mode of regulation. Bacteriocins are divided into Class I posttranslationally modified peptides, often referred to as lantibiotics, and Class II, which are not posttranslationally modified; their production can be regulated in a cell-density-dependent manner by the antimicrobial peptides themselves, as with nisin and subtilin production in *Lactococcus lactis* and *B. subtilis*, respectively, or by signaling molecules that are distinct from the antimicrobial peptides and which include the plantaricin A (PlnA) or BIpC peptides—also termed SpiP peptides—that regulate bacteriocin production in *L. plantarum* and *S. pneumoniae*, respectively.^{11,23} Another example of QS is the regulation of cytolysin production in *E. faecalis*, in which a subunit of the bacteriocin, CylL_s, regulates the transcription of the toxin.²⁶ The mechanisms regulating each of the above QS systems are very complex and differ from one another with respect to secretion and sensing of the autoinducer, and in the regulatory cascade that results from its perception. Detailed reviews on QS peptides in Gram-positive bacteria and their biosynthetic pathways have been published recently (see Refs 11–13, 29 and references therein).

In Gram-negative bacteria, most QS signals are *N*-acyl homoserine lactones, which are internalized by diffusion and bind to an intracellular receptor molecule to activate the response.²⁹ However, recent studies have revealed the existence of signaling peptides in Gram-negative bacteria too. Studies based on an *in silico* strategy to screen the Gram-negative bacterial genome for the presence of leader double-glycine sequences, which are conserved leader motif sequences of many Gram-positive pheromone peptides, have revealed the presence of genes coding for putative peptides that contain such motifs and that also show structural

similarity to bacteriocins and pheromone peptides of Gram-positive bacteria. The role of such peptides in signal transduction, and the mechanisms by which they might exert their activity in Gram-negative bacteria are unclear at present.

FUNGAL PHEROMONE PEPTIDES

Although diverse fungi generate chemical pheromone gradients during mating, the pheromone peptides of yeasts are among the best documented fungal pheromones. Two pheromone peptides that initiate mating in the yeast *Saccharomyces cerevisiae*—the α -factor and the a-factor, which contain 13 and 12 amino acids, respectively, are currently known. The peptides are secreted by donor cells and act on the recipient via G-protein-coupled receptors (GPCRs). The mating pheromones are absolutely indispensable for triggering the mating cycle, and cells that cannot produce these molecules or that lack their cognate receptors are sterile. The α -factor is a tridecapeptide pheromone (WHWLQLKPGQPMY) synthesized constitutively by *S. cerevisiae* mating type α cells, and it acts on *S. cerevisiae* mating type a cells. The peptide was isolated and characterized by Stotzler and Duntze²⁷ and later found to be encoded by two genes: *MFa1* and *MFa2*.²⁰ The a-factor of *S. cerevisiae* is a dodecapeptide pheromone (YIIKGVFWDPA[Cfarnesyl]-OCH₃)² that requires post-translational modification with a farnesyl isoprenoid and carboxymethyl group to enable the expression of its full biological activity. Biosynthesis of these mating pheromones has been studied extensively and used as a model for posttranslational processing, modification, and secretion of mammalian peptide hormones and proteins. The cellular events and signal transduction pathways stimulated by these pheromones were recently found to affect the cell cycle, and they are currently used as a model to study this complex system in eukaryotic cells (for review see Ref. 7). The SAR studies and the extensive biological, biochemical, molecular, and biophysical analyses of the α -factor peptide provided a model of its structure and of its interaction with its receptor (for an overview see Ref. 24). Recently, GPCRs that bind peptide pheromones that mediate mating in filamentous fungi were identified, and their signaling was characterized.²¹ The pheromones, their receptors, the downstream intracellular signal transduction pathway by which the yeast cells respond to the presence of mating peptide pheromone in their vicinity, the importance of chemical gradients in yeast chemotropism, and the peptide's mode of action at the cellular level have been examined thoroughly and are summarized in reviews.^{3,7,21}

CILIATES

Ciliates can exhibit both asexual and sexual reproduction. Asexual reproduction occurs by binary fission whereas sexual reproduction involves conjugation, i.e. mating, of two cells that divide to form four new cells. Ciliate sexual mating involves chemical signaling that is mediated by a

complex pheromonal system of small protein pheromones, containing 75 and 130–150 amino acid residues: *Er-1*, *Er-2*, *Er-7*, *Er-10*, *Er-11*, *Er-20*, *Er-21*, G3, and G4. Formation of such mating pairs enables some species to avoid mating between siblings, and promotes mating with foreigners, and thereby retains cell-type-specific signals for distinguishing self from nonself. Most ciliates' pheromones were characterized in *Euplotes* spp. and other hypotrichs. Details of the structures of the various polypeptides, their putative receptors, and their possible modes of interaction and function are detailed in Ref. 22

MOLLUSK PHEROMONE PEPTIDES

Pheromone peptides and proteins have also been implicated in the control of a number of behaviors in mollusks—from egg laying to mate attraction—but only a few have been characterized. Most studies of mollusk pheromone peptides were carried out on *Aplysia*, and the two groups of pheromones that were identified so far were mostly found during these studies. These groups comprise a contact pheromone whose nature has not yet been determined, which triggers a synchronous discharge of neuroendocrine bag cells which, in turn, secrete a family of peptides that promote egg release and mate-attracting pheromones. The latter comprise a family of water-borne pheromonal protein attractants, of which the major is attractin, a 58 residue glycosylated protein that is released from the albumen gland during egg laying and that acts in concert with other water-borne protein pheromones—enticin, temptin, and seductin (which are, respectively, 69-, 103-, and 192-residue proteins found in the albumen gland)—to stimulate mate attraction by recruiting other *Aplysia* to the freshly laid eggs, thereby stimulating other *Aplysia* to increase egg laying and to form mating aggregations (for review see Refs. 8, 9). Recently, another protein—Alb-1, a 101 residue protein—has been identified and cloned from the albumen gland; it is believed to play a pheromonal role, together with the above-mentioned four peptides,⁸ and has been suspected to be associated with factors from non-laying *Aplysia* that might act in conjunction with attractin to stimulate enhanced mate attraction.⁹ Other pheromone peptides are: a tetrapeptide (Ile-Leu-Met-Glu, termed ILME) that is eluted from egg masses of the cephalopod *Sepia*, and that is thought to stimulate the transport of oocytes in the genital tract; and a *Sepia* sperm-attracting peptide, termed SepSAP, that is released from oocytes during egg laying and that facilitates external fertilization by attracting spermatozoa.²⁸ Evidence for the pheromonal role of the above peptides and proteins in various *Aplysia* and *Sepia* species, their expression and localization, biochemical and molecular characterization, structure and SAR, release, inter-molecular interactions and bioactivity is presented in Refs. 8–10.

ANNELID PHEROMONE PEPTIDES

Females of the ragworm, *Nereis succinea* use a tetrapeptide, cysteinyl-glutathione (CSSG) as a mate-recognition and gamete-release pheromone during reproduction. The tetrapeptide pheromone (termed nereithione) controls reproductive behavior and release of gametes. The pheromone is released into water by swimming ripe females, and it induces males to swim faster and to release sperm. The role of peptide-based pheromones in these worms and the time course of their appearance as related to the worms' sexual maturation have recently been reviewed.¹⁵

ARTHROPOD PHEROMONE PEPTIDES

Insects

One of the largest and best documented groups of pheromones comprises those of insects. Chemical cues are major sources of information used by most insects to interpret environmental stimuli; they lead to the initiation of gregarious behavior, formation of aggregations at food sites, dispersal behavior during predator attack, synchronized sexual maturity, mating attraction, etc. However, none of the pheromones that elicit these functions is of a peptidic nature. There are only two examples of peptides (and proteins) being related to pheromonal activity in insects. One involves *Drosophila melanogaster*, in which the peptides act as primer pheromones that cause physiological changes associated with reproductive activity in the fly. The other involves peptides that originate in the central nervous system (CNS) of Lepidopteran species and that stimulate sex-pheromone biosynthesis in female moths.

The first system involves proteins and peptides that are transferred, together with sperm and seminal fluid, by males; they act within and outside the reproductive tract of their female targets, and elicit a wide variety of short-term and long-term responses such as increased oogenesis and ovulation, decreased female receptivity, enhanced sperm storage, and formation of the mating plug.³³ The very first seminal fluid peptide to be identified was the “sex peptide” (SP) or accessory gland peptide (Acp70A). From the 1960s through the 1980s, the nature of Acp70A was gradually revealed, and the *Acp70A* gene that encodes the 36 amino acid Acp70A peptide was identified (for an overview see Ref. 6 and references therein). In around 2000, another closely related peptide (DUP99B)—a homolog of SP—was revealed.¹⁹ Within females, SP and its closely related homolog DUP99B are thought to elicit postmating responses through a specific GPCR, termed SPR,³⁴ which triggers these responses mainly by modulating neuronal activity in the subsets of the *fruitless* and ventral nerve cord neurons.¹⁹ Detailed SAR studies have associated some of the SP functions with specific parts of the molecules,¹⁹ and

detailed cellular studies uncovered a neuronal mechanism by which SP exerts its control over reproductive behavior.³³ The ongoing study of seminal fluid molecules is revealing that they have an unexpected variety of functions and, furthermore, that some of the genes that encode these molecules show evidence of extremely rapid evolutionary change. These findings suggest that seminal fluid molecules might be strong targets for natural or sexual selection. At present, homologous recombination and RNA interference, along with microarrays and yeast two-hybrid systems, are being used to gain better insight into the functions of these molecules and their receptors. Information on the currently known functions of the seminal fluid proteins and peptides of *D. melanogaster*, and the approaches used to study their role in the fruit fly's reproductive and postmating functions have been published.³³ Interestingly, numerous attempts to identify SP orthologs in other insects revealed their presence in the mosquito *Anopheles gambiae*.

The second system involves a sub-esophageal ganglion neuropeptide—pheromone biosynthesis activating neuro-peptide (PBAN)—that acts via a GPCR mechanism to initiate sex-pheromone biosynthesis in the pheromone glands of female moths. Studies of the regulation of sex-pheromone biosynthesis in moths have revealed that this function can be elicited by additional neuropeptides, all of which share the common C-terminal pentapeptide FXPRL-amide (in which X=S, T, G, or V) and belong to a large family of peptides: the pyrokinin (PK)/PBAN family. In the past two decades extensive studies were carried out on the chemical, cellular, and molecular aspects of PBAN and the other peptides of the PK/PBAN family, aiming to understand the mode of their action on sex-pheromone biosynthesis (for an overview see Ref. 1 and references therein). A detailed review of some of the above topics is presented in the chapter on “The FXPRL-amide Pyrokinin/PBAN Family” by Altstein et al. in the Invertebrate Peptide section of this book.

Crustaceans

Another group of pheromone peptides in arthropods comprises those of the crustaceans. Crustacean pheromone peptides have been implicated in two different behavioral patterns: gregarious settlement of barnacles, which involves “settlement pheromones”; and synchronization of larval release, which involves larval-release pheromones—also termed “pumping pheromones”—which are released from newly hatched embryos and induce stereotypical larval-release behavior in crab (*Rhithropanopeus harrisi*) or lobster (*Panulirus argus*) females. This larval-release behavior involves rapid abdominal extensions and pleopod pumping, which help to break open the egg membrane and results in synchronous release of the larvae. The settlement pheromones are peptides released from intact living barnacles, whereas the larval-release pheromones are released from

the brood. Knowledge of the biological activity and SAR of synthetic settlement pheromones and of larval-release pheromones is quite elusive at present, and most studies were carried out with crude extracts or synthetic analogous peptides, because the natural pheromone peptides have not yet been fully identified. In light of current knowledge, it is well accepted that all known crustacean release pheromone peptides are based on serine protease degradation products that have basic carboxyl termini, which are essential for bioactivity. Recent studies have revealed that the released peptide pheromones are dipeptides and tripeptides that have a neutral amino acid, i.e. Gly, at the amino terminus, a basic amino acid, i.e. Arg, at the carboxy terminus, and a basic-basic dipeptide (Lys-Arg). The current state of knowledge has recently been summarized.^{14,35}

VERTEBRATE PHEROMONE PEPTIDES

Amphibians

In recent years, it has become apparent that although communication among amphibians depends primarily on auditory signals, pheromonal signaling is used by both males and females for mating attraction; it is widespread in salamanders and newts, and may also be important in some frogs and toads. These pheromones are typically peptides and proteins, and several of them have been behaviorally, biochemically and molecularly identified. The best-characterized amphibian pheromones are those that influence male–female interaction. The first pheromone identified in amphibians was the female attractant sex pheromone—sodefrin—of the newt, *Cynops pyrrhogaster*, and a cDNA comparable with the cDNA encoding *Cynops* sodefrin was also isolated from another newt, *Triturus carnifex*. In 2000, a second sex pheromone was isolated from another *Cynops* species, *C. ensicauda* and was termed silefrin. *Cynops* sodefrin and silefrin are decapeptides with amino acid sequences SIPSKDALLK and SILSKDAQLK, respectively, they are secreted from the abdominal glands of males via the cloacae, their synthesis is regulated by prolactin and androgens, and each pheromone attracts only conspecific females. In 2007, a third decapeptide—aoanirin—was isolated from the abdominal gland of the male *C. pyrrhogaster*, which is found in the Nara region of Japan (for review see Ref. 32). Another sex pheromone, isolated from the frog, *Litoria splendida*, is a 25-residue peptide—splendipherin³²—which is produced by a gland in the head of the male during the mating season. Splendipherin attracts conspecific females and has no effect on males or on other species.

In recent years, many studies focused on the courtship pheromones of the salamander. Currently, it is well established that the salamander pheromone is a complex blend of compounds secreted by the male submandibular (mental)

gland. Several such pheromones—plethodontid receptivity factor (PRF), plethodontid modulating factor (PMF), and sodefrin precursor-like factor (SPLF)—have been isolated, molecularly and biochemically characterized, and tested for effects on female behavior. During courtship, salamanders transfer mental-gland pheromones via two main modes: the olfactory mode, in which pheromones from the mental gland of the male reach the female's nasal cavity; and the transdermal mode, in which the male pheromone is deposited directly onto the skin of the female and diffuses into the peripheral vasculature.

Male–male pheromonal signaling has also been reported in amphibians. In 2005, an amphibian pheromonal peptide—leptodactylus aggression-stimulating peptide (LASP)—has been characterized and isolated from male norepinephrine-stimulated skin secretions.¹⁷ LASP exhibited a chemoattractive effect on males, in which it stimulated aggressive behaviors that resulted in the emergence of dominant animals that subsequently attracted females to nesting sites.

Most amphibian pheromones are detected by the vomeronasal organ (VNO) and the main olfactory epithelium (MOE), which form a unique chemosensory system. Chemosensory neurons present in those organs express VNO receptors or olfactory receptors. In recent years, detailed studies were carried out on these pheromones, on the anatomy of the receptive organs, and on the molecular, genomic and physiological characteristics of the chemosensory receptors. To date, no chemosensory ligands have been matched to VNO receptors or olfactory receptors; this indicates the need for further examination of the issue, which could also provide an insight into the nature and evolution of vertebrate pheromonal communication (for detailed review on amphibian pheromones, their receptors and the chemosensory organs, please refer to the review in Ref. 32 and references therein, and to the chapter by Kikuyama in the Amphibian Peptides section of this book).

Mammals: Rodents

Needless to say, the most complex, interesting, but as yet unidentified signaling molecules are those of mammals. All mammals emit chemical cues into the environment, via urine, saliva, and/or divers secreted fluids. So far, only a few mammalian pheromones, especially those of rodents, have been identified, and examination of their chemical nature has revealed a wide diversity of compounds, that range from small organic molecules to large proteins. Several proteinaceous pheromonotropic families are described below; they all play essential roles in sex discrimination and attraction between males and females, and thereby promote mate choice and successful reproduction. Among them are: aphrodisin (for review, see Ref. 5); mouse urinary proteins (MUPs); major histocompatibility complex (MHC) class I peptides; and the tear exocrine-gland-secreting peptide 1

(ESP1) (for a detailed review, see Ref. 30 and references therein).

Aphrodisin is a protein that belongs to the lipocalin family; it is found in hamster vaginal secretions, and when it is detected by the male accessory olfactory system copulatory behavior is induced. At present, it is not clear whether aphrodisin itself performs the pheromonal function or whether it is simply a carrier for hydrophobic small pheromone molecules that have not yet been identified. Studies on this topic focus on the aphrodisin structure, its biological properties, and the associated signal transduction processes in the VNO.⁵

Other members of the lipocalin family are the MUPs, of 19 kD, found in sexually mature male mice, rats, and some other rodents. These proteins are believed to be responsible for the binding and release of low-molecular-weight pheromones, thereby providing a slow-release mechanism for volatile components of scent marks. However, the proteins may function as chemosignaling molecules in their own right, filling one or more roles in communication of individual identity, territorial behavior, and ownership. Male MUPs seem to promote, among other actions, aggressive behavior between males. Other pheromonotropic compounds present in male mouse urine are the MHC class I peptides—SYFPEITHI and AAPDNRETF—which have also been shown to convey information about individual identity and to mediate pregnancy blockage or spontaneous abortion, i.e. the Bruce effect. Recently, a male-specific peptide—ESP1—has been found to be secreted in the tears of the extraorbital lacrimal gland of male mice. This peptide is a member of the ESP gene family and seems to be involved in sexual behavior associated with the conveyance of information on strain and species, similar to the function of MUPs. A few recent reviews^{25,30} summarize the current understanding of the structure and function of urinary and other proteins, and speculate on their roles as supporters or as key participants in the elaboration of the complex chemosensory properties of a rodent scent mark.

As in amphibians, peptide and protein pheromones in rodents—MPUs, MHC peptides, and ESPs—are believed to be recognized by specific transmembrane receptors—members of the VIR and V2R family—that are expressed in the VNO on sensory neurons; however, this issue is still somewhat controversial. Some chemosensory peptidic pheromonal signals, i.e. MHC peptides in rodents are conveyed via MOE. Much of the research on the molecular mechanisms underlying vomeronasal and MOE sensing of peptide pheromones has been conducted with mice and has focused on the structure of the two areas and their receptors, the search for natural ligands, signal processing and signal transduction from the chemosensory organs to the CNS (for review see Refs 25, 30 and references therein).

The VNO in mammals has received a lot of attention within a broader aspect in the past few years, and studies of

this secondary olfactory system yield much new information such as the existence of a large number of VNO receptor genes. This unexpected receptor diversity indicates that there probably is a wide variety of chemosensory signals sensed by these receptors, about which little is known at present and which needs to be further explored. Although peptide pheromones and VNO play a most important role in communication between individuals of mammalian—rodent—and amphibian species, it seems that there was a tendency toward loss of VNO function and elimination of peptide pheromones at this point in evolution, as indicated by the absence of peptide or protein pheromones, and also of the VNO receptor gene families that mediate these cues, in primates, including humans.³⁰ The current consensus is that the VNO is not functional in adult humans. It is possible, however, that pheromone communication does exist in humans and is processed by the receptor neurons in the MOE. This issue also needs further exploration.

Last but not least are the neurohypophysial peptides—vasopressin (AVP) and oxytocin (OT)—that do not serve directly as pheromonal cues in mammalian species, but are rather critical to the mammalian ability to process such cues appropriately in the olfactory circuit and throughout the brain. In a recent review by Higashisa et al.¹⁶ and an earlier review by Bielsky and Young,⁴ the roles of OT and AVP in rodent social interaction, social recognition, pair bonding, and maternal behavior are discussed.

SUMMARY

There is no doubt that the wide variety of pheromone peptides described above highlight their crucial role in many and varied behavioral patterns that are associated mostly, but not exclusively, with courtship and mating, or transfer of genetic material. Despite the vast amount of information that is already available to us, and the immense progress that was made in the past few years the study of pheromone peptides still requires further exploration, and it seems that we have only just begun to understand some of the languages of a few of the “inhabitants” of this fascinating world. As our studies advance along the evolutionary tree we find that the chemical cues responsible for mating become more and more complex, and it is reasonable to assume that social and sexual behavior in vertebrates is too complicated to be controlled solely by the few components or mechanisms so far identified, and that it requires the integration of multiple sensory cues within the neural network of the brain, all of which are still to be explored. Further, comparison among the peptide or protein pheromones that are used for sociosexual communication by the various species reveals no homology, which suggests that each peptide has undergone an independent molecular evolutionary process, and hints at the possibility that there are many more novel peptide pheromones, associated with many and varied species,

that need to be discovered in the future. Understanding of the interplay between behavioral and biochemical factors in the deposition and reception of scents, identification of key signal molecules and elucidation of their chemical nature, and exploitation of their modes of action and regulation in donor and recipient organisms present a challenge that is currently being evaluated in depth, and, as peptide signaling systems continue to be discovered, there is a growing need to understand the details of these communication mechanisms.

In light of current knowledge, it is already obvious that pheromones of a peptidic nature play an important role in species-specific communication, either directly or via neural processing of the olfactory signal. Their high solubility, specificity, and variability, which are achieved through the existence of multiple genes encoding for peptide variants, ensure their highly efficient pheromonal activity. The currently available novel biochemical, molecular and chemical analytical methods and approaches will enable us further to identify new peptide pheromones, to study their biosynthetic pathways, to dissect their sequences of behavioral events, and to study their mechanisms of action in the donors and recipients at the cellular and molecular levels. It is anticipated that such studies will widen our understanding of the extraordinary diversity that surrounds us, and will stimulate further studies that may lead to the unraveling of the chemical signaling mediated by peptides, and thereby lead to practical applications, such as development of new therapies against bacterial infections or of behavior-modifying compounds for agrochemical, aquacultural, and medical applications.

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