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Editorial

Peptide pheromones: an overview

Pheromones are chemical messages involved in communication that induces a behavioral reaction or a developmental process among individuals of the same species. Formally, pheromones are divided into two classes: release pheromones and primer pheromones. The first group includes messages that induce an immediate behavioral reaction in the receiver organism such as alarm, defense, aggregation, attraction, kin and colony recognition, marking of territories and egg deposition sites, mating behavior, recruitment and thermoregulation. The primer pheromones, on the other hand, induce complex behavioral and physiological alterations in the receiver (such as development of a particular caste or sexual maturation) via long-term endocrine changes.

Chemical communication among organisms is a highly sophisticated mechanism that involves regulation of many genes in the donor and a complex process in the recipient in which the interpretation of the individual chemical constituent or “signal” depends on the particular combination, ratio, concentration and order of presentation of the signal molecules, as well as 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 by which they exert their activity do not overlap, causing even a higher order of complexity.

The chemical nature of pheromones varies from small-molecule volatile compounds to high-molecular-weight, water-soluble, non-volatile peptides and proteins. Usually, small molecules are used for communication when rapid dispersal of the signal is needed, whereas the larger molecules of less volatile compound tend to function in attraction and stimulation for which prolonged exposure is necessary. While air-borne signal molecules have been studied extensively, identified and are being applied commercially to disrupt mating and to monitor insect pests, much less is known about the chemical nature of the comparable water-borne cues. Recently, it became apparent that some of these signal molecules are peptides and proteins. This special issue of *Peptides* focuses on this group of compounds.

A quick glance at the table of contents clearly indicates that peptide pheromones are widely distributed throughout the prokaryotic and eukaryotic super kingdoms including Gram-positive and Gram-negative bacteria, fungi, mollusks, arthropods and a variety of vertebrates (amphibians, polycheta, gastrocheta and mammals). The great abundance of this route of signaling in such a large number of widely diverse organisms hints at the evolutionary importance of this sort of communication.

Peptide pheromones have been studied for many years, and in several systems, the nature of the peptides, their origins and 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 still have to be explored. The collection of articles presented in the current issue covers the state-of-the-art on peptide pheromones in a variety of species from different orders and phyla, with particular attention to their chemical nature, mechanisms of production, reception and modes of action, as well as the behavioral changes caused in their presence in the recipient organisms.

Interestingly, the most complex and diverse systems of peptide pheromones are 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 the production and release of small molecules that are sensed by organisms with cognate sensors, each setting in motion a more or less complicated response pathway. There are two basic types of bacterial communication systems: those in which the signal is detected solely by organisms other than the producer, and those in which the signal is sensed by the producing organism as well.

The first is typified by the mating pheromones of the enterococci, where a potential recipient releases a small peptide that is taken up by a potential donor, in which it initiates a plasmid-coded response that leads to cellular aggregation and transfer of the plasmid from donor to recipient. The second comprises the auto induction or quorum-sensing (QS) systems, which are widespread among bacteria and which generate population-wide responses (cell–cell or cell–environment

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responses) to low-molecular-weight signaling molecules, dependent on cellular density.

The structures of the signaling molecules differ between Gram-negative and Gram-positive bacteria. In Gram-positive bacteria, the mating pheromones and most QS signals are peptides. They are sensed by *trans*-membrane receptor components of a two-component signal transduction module type (histidine kinase sensor proteins) that activate an intracellular response pathway. 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. However, recent studies have revealed the existence of signaling peptides in Gram-negative bacteria too.

Intercellular signaling in bacteria has, not surprisingly, become an area of intense interest in recent years, and has generated a vast body of literature. This volume includes five articles on peptide pheromone systems: one on mating pheromones, the other four on QS systems. The enterococcal mating pheromones – a unique class of peptides that induce mobility of genetic elements – are reviewed by Chandler and Dunny. This review describes the synthesis of enterococcal pheromones and focuses on the components of the regulatory machinery that interact specifically with the peptides; it emphasizes the molecular basis for the exquisite sensitivity and specificity of these systems. The QS signaling peptides of *Staphylococcus aureus* and other Gram-positive bacteria that are involved in virulence are the subject of the review by Lyon and Novick, whose article focuses mainly on the autoinducing peptides (AIPs), their structure–activity relationship (SAR), the rational design of AIP antagonists and characterization of their processing enzyme and receptor. The cell–cell signaling peptides of *Streptococcus pneumoniae* and *Bacillus subtilis* are also described.

Another QS system comprises the anti-microbial compounds (the lantibiotics nisin and subtilin) produced by *Lactococcus lactis* and *B. subtilis*. This system is described in two articles by Kleerebezem's group: The first is a review article that focuses on the cell-density-dependent autoregulatory circuit of nisin and subtilin biosynthesis; it describes the mechanism by which the lantibiotic molecules themselves exhibit a pheromonotropic function that controls their own biosynthesis (Kleerebezem). The second provides further information on the regulatory mechanisms of subtilin biosynthesis (Kleerebezem et al.)

The possible occurrence of QS peptides in Gram-negative bacteria is discussed in the review by Dirix et al., which describes the employment of an in silico strategy to screen Gram-negative bacterial genome for the presence of a leader double-glycine sequence (a conserved leader motif sequence of many Gram-positive peptide pheromones). The article presents the approach that was used for screening and the discovery of genes carrying such motifs, and discusses whether peptides can play a role in signal transduction in Gram-negative bacteria.

The peptide pheromones of yeasts are among the best-documented pheromones in fungi. Two peptide pheromones, which initiate mating in the yeast *Saccharomyces cerevisiae* – the α-factor and the a-factor (containing 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 (GPCR). Two articles cover the mating pheromones in yeasts. The article by Naider and Becker describes the employment of synthetic, biological, biochemical, molecular and biophysical analyses to generate a model for the structure of the α-factor and its interaction with its receptor. The downstream intracellular signal transduction pathway by which the yeast responds to the presence of peptide mating pheromone in its surrounding is covered in detail by Bradwell.

One of the largest and best-documented groups of pheromones is that of insects. Chemical cues are major sources of the 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 being related to pheromonal activity in insects. One involves *Drosophila melanogaster* in which peptides play a role as primer pheromones that cause physiological changes associated with reproductive activity in the fly; in the second, in Lepidopteran species, peptides originating in the central nervous system stimulate sex pheromone biosynthesis in female moths.

The first system, described in the article by Chapman and Davies, involves proteins and peptides that are transferred together with sperm and that act within and outside the reproductive tract of their female targets, resulting in a wide variety of responses (such as increased oogenesis and ovulation, enhanced sperm storage and decreased female receptivity). The article focuses on the currently known functions of the seminal fluid proteins and peptides of *D. melanogaster*, and on new technologies and approaches (e.g. homologous recombination and RNA interference, along with the use of microarrays and yeast two-hybrid systems) that enables to gain a better insight into the functions of these molecules. The second system, described in the article by Altstein, involves a subesophageal ganglion neuropeptide, termed pheromone biosynthesis-activating neuropeptide (PBAN), which acts via a GPCR mechanism to activate sex pheromone biosynthesis in the pheromone glands of female moths. The article describes the mode of action of PBAN and other related peptides of the pyrokinin (PK)/PBAN family, and addresses possible approaches to the disruption of pheromone production by using rationally designed PK/PBAN antagonists.

Another example of peptide pheromones in arthropods is presented by Rittschof and Cohen, who discuss crustaceans. Crustacean peptide pheromones have been implicated in three different behavioral patterns: gregarious settlement of barnacles (involving settlement pheromones, e.g. arthropodin); shell acquisition by hermit crabs (hermit crab shell pheromones); and synchronization of larval release (larval release pheromones). Based on current knowledge, it is now well established that all known crustacean peptide pheromones are based upon serine protease degradation products that have basic carboxyl termini, which are essential for bioactivity. The settlement pheromones are peptides released from living intact barnacles; the shell acquisition pheromone is released from muscles of dead snails by proteolytic activity, and larval release pheromones are released from the brood. Most of the studies of the above signal molecules were performed with crude extracts or synthetic peptides. The natural peptide pheromones themselves have not yet been identified. This article presents the current state of knowledge of the biological activity and SAR of synthetic settlement pheromone, the larval release pheromone and of a few other peptides (e.g. the hermit crab shell peptide) that serve as a kiromone (interspecific chemical signal).

The first representatives of vertebrate species in the collection are the *Nereidid* worms (Annelida: polychaeta). In these worms, a tetrapeptide pheromone (termed nereithione) controls reproductive behavior and the release of gametes. The pheromone is released into water by swimming ripe females and induces males to increase their swimming speed and to release sperm. The review by Hardege et al. describes the role of peptide-based pheromones in the worms and the time course of their appearance as a function of the worm's sexual maturation.

Peptide pheromones have also been implicated in the control of a number of behaviors in mollusks, both gastropods and cephalopods. A family of water-borne peptide pheromonal attractants (termed attractins) that were found in the gastropod *Aplysia* are released during egg laying and stimulate other *Aplysia* to increase egg laying, to form mating aggregations and to initiate respiratory pumping. A tetrapeptide in the cephalopod *Sepia* (eluted from egg masses) is thought to stimulate transport of oocytes in the genital tract, and a *Sepia* sperm attractin peptide that is released from oocytes during egg laying facilitates external fertilization by attracting spermatozoa. Evidence for the pheromonal role of attractins in various *Aplysia* and *Sepia* species, their origin, SAR and bioactivity are presented by Susswein and Nagle.

In recent years, it has became apparent that although communication in amphibians depends primarily on auditory signals, pheromonal signals are used by both males and females for mating attraction. Many investigators have reported the possible existence of sex pheromones in various amphibian species, but only a few studies have been successful in the isolation and characterization of these substances. Kikuyama and coworkers are amongst the groups that performed these studies, and in two articles (Toyoda et al. and Iwata et al.), they describe the purification, characterization and biological activity of the *Cynops* pheromones (sodefrin and silefrin), the molecular cloning of their cDNA, the processing of the sodefrin precursors and the involvement of endocrine factors (prolactin, androgen, vasotocin) in their secretion and response to their presence. The article discusses the reception mechanisms of the pheromonal signal in the vomeronasal epithelium, the possible involvement of prolactin and estrogen in this process, and the existence of gene duplication and the presence of pheromone variants in view of the significance of peptidic pheromones for reproductive behavior in an aquatic environment.

Needless to say that 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 or diverse secreted fluids. So far, only a few mammalian pheromones, especially of rodents, have been identified, and examination of their chemical nature reveals a wide diversity of compounds ranging from small organic molecules to large proteins. Three proteinacious pheromonotropic families are described in this collection: aphrodisin (Briand et al.), major urinary proteins (MUPs) (Beynon and Hurst), and the neuropeptides vasopressin (AVP) and oxytocin (OT) (Bielsky and Young).

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, it causes induction of copulatory behavior. Currently, 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 been identified yet. In a detailed review, Briand et al. summarizes the current knowledge of the aphrodisin structure, and discusses its biological properties and signal transduction in the vomeronasal organ.

Other members of the lipocalin family are the MUPs of mice, rats and some other rodents. These proteins are thought 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 the communication of individual identity and ownership via scent marks. The review by Beynon and Hurst summarizes the current understanding of the structure and function of these urinary proteins, and speculates about their role as supporters or as key participants in the elaboration of the complex chemosensory properties of a rodent scent mark.

Last but not the least are the neurohypophysial peptides, AVP and OT, that do not serve directly as pheromonal cues in mammalian species, but are rather critical to the mammalian ability to process such cues in an appropriate manner in the olfactory circuit and throughout the brain. In their review, Bielsky and Young present evidence for the roles of OT and AVP in rodent social recognition, and also in offspring recognition in sheep and in mate preference among pair-bonding voles.

In summary, the articles in this special issue present a wide variety of peptide pheromones emphasizing their crucial role, which is associated mostly with mating (or transfer of genetic material). Despite the vast amount of information that is already available to us, the study of peptide pheromones is still nascent and requires further exploitation, and it seems as if we have only just begun to understand some of the languages of a few “inhabitants” of this fascinating world. Understanding the interplay between behavioral and biochemical factors in the deposition and reception of scents, the identification of key signal molecules and elucidation of their chemical nature, and the exploitation of their modes of action and regulation in donor and recipient organisms form a challenge that is still to be faced.

However, even with the limited information in hand, it is 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 is achieved by the existence of multiple genes encoding for peptide variants) make them highly efficient pheromonal candidates. There is no doubt that the sophisticated techniques that have been developed by chemists and biologists will enable us to further determine the chemical nature of peptide pheromones, to dissect their behavioral sequence of events and to study their mechanisms of action at the cellular and molecular level. We hope that the following articles will provide an intellectual exercise, will further 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 hence to practical applications, such as the development of new therapies against bacterial infections or of behavior-modifying compounds for agrochemical, aqua-cultural and medical applications.

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