

# Lobster olfactory genomics

Timothy S. McClintock,<sup>1,\*</sup> Barry W. Ache,<sup>†,§</sup> and Charles D. Derby<sup>¶</sup>

<sup>\*</sup>Department of Physiology, Cellular and Molecular Neuroscience of Sensory Systems Training Program, University of Kentucky, Lexington, KY 40536-0298, USA; <sup>†</sup>The Whitney Laboratory for Marine Bioscience, University of Florida, St Augustine, FL 32080, USA; <sup>§</sup>Departments of Zoology and Neuroscience, Center for Smell and Taste, McKnight Brain Institute, University of Florida, Gainesville, FL 32610, USA; <sup>¶</sup>Department of Biology, Brains & Behavior Program and Center for Behavioral Neuroscience, Georgia State University, Atlanta, GA 30303, USA

**Synopsis** Lobsters have numerous adaptive specializations of the olfactory system that make them especially suitable model organisms for the study of olfaction. Recent work using genomics and physiological genomics to study the lobster olfactory organ extends the advantages of their use further. A subtracted cDNA library from the mature zone of the olfactory organ and 3 physiological genomics experiments have helped identify numerous functionally interesting genes. These include specific markers of 3 cell types that previously could be discriminated only in anatomical sections, plus a marker of reactive epithelial cells at sites of cellular proliferation for both the normal ongoing replacement of olfactory tissue and the regeneration of damaged olfactory tissue. The approaches were instrumental in the discovery of a new exocrine gland, the aesthetasc tegumental gland, which is linked to grooming and the prevention of fouling of the olfactory aesthetasc setae. They also suggest a previously unknown endocrine or paracrine function performed by auxiliary cells of the olfactory aesthetasc sensory units. Other discoveries include candidates for gene products involved in olfactory transduction, presynaptic modulation of olfactory neuron axons by ionotropic receptors, and neuromodulation of both the olfactory sensory neurons and the interneurons in the olfactory lobe of the brain.

## Introduction

The importance of lobsters as model organisms for the study of olfaction has its roots in the investigation of the chemosensory behavior of crustaceans. The recognition of the significance of olfaction for adaptive behaviors in crustaceans led to an understanding of the odorant chemistry responsible for specific behaviors—most notably simple mixtures of amino acids and nucleotides that trigger food-finding behaviors (Carr and others 1977, 1984). This availability of behaviorally relevant stimuli for crustaceans has enhanced and facilitated both reductionist and integrative approaches. Lobsters, especially the Caribbean spiny lobster *Panulirus argus* (Palinuridae of the Achelata) and the American clawed lobster *Homarus americanus* (Nephropidae of the Homarida), became the primary model organisms for these studies for several reasons. First, lobsters are easily available via commercial fisheries and the agencies that regulate these fisheries. Second, lobsters can be trained by associative conditioning, thereby allowing studies that reveal fundamental principles of the perception of odorant mixtures (reviewed in Derby 2000; Derby and others 2002). Third, the lobster olfactory system

has anatomical and morphological features that facilitate physiological, biochemical, and molecular experiments. The olfactory organ is located at the distal end of the lateral flagellum of the biramous first antennae (Fig. 1). The base structure of the flagellum consists of segments, called annuli, separated by noncalcified arthrodistal cuticle that gives the flagellum flexibility. The most distal annuli contain the olfactory organ, which is composed of an ordered array of olfactory sensory units, each consisting of an olfactory (aesthetasc) seta that is innervated by the dendrites of approximately 300 olfactory sensory neurons (OSNs) and the processes of associated cells (Grünert and Ache 1988). The accessibility of this structure facilitates the collection of samples enriched in olfactory tissue. Dissected preparations of the olfactory organ allow *in situ* patch-clamp electrophysiology of the OSNs. In fact, this was the first use of patch-clamp techniques in tissue sections (Anderson and Ache 1985). The *in situ* patch-clamp approach has contributed to the discovery of hyperpolarizing receptor potentials, modulation of OSN sensitivity by neuroactive compounds, presynaptic inhibition of OSNs, dual olfactory transduction pathways, and regulation of olfactory

From the symposium “Genomic and Proteomic Approaches in Crustacean Biology” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2006, at Orlando, Florida.

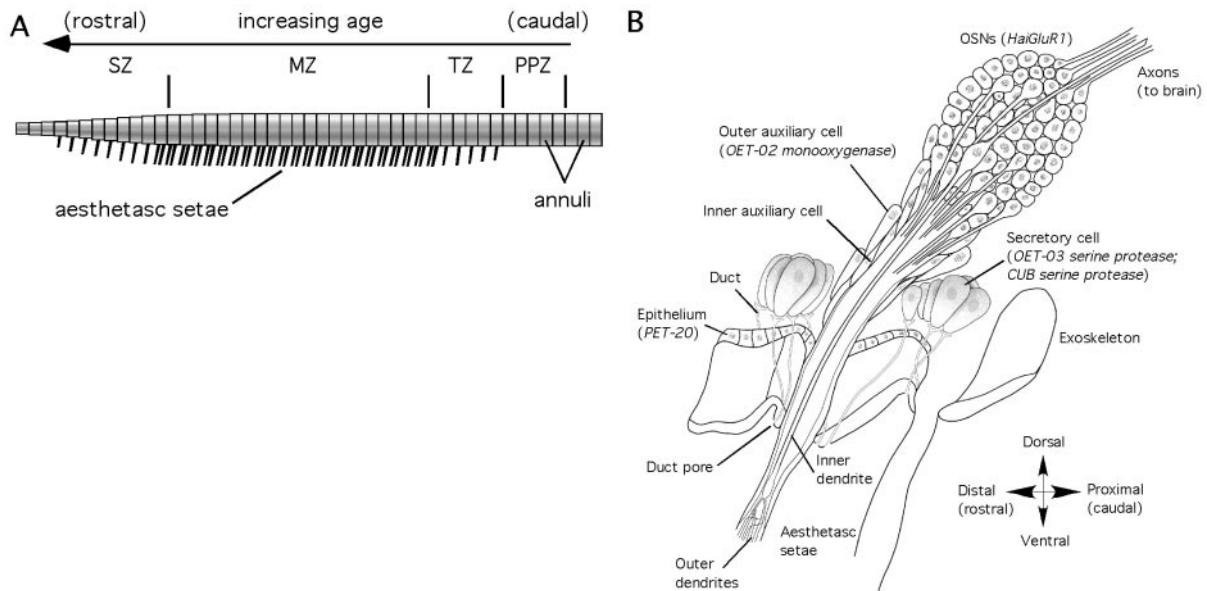
<sup>1</sup> E-mail: mcclint@uky.edu

*Integrative and Comparative Biology*, volume 46, number 6, pp. 940–947

doi:10.1093/icb/icj050

Advance Access publication May 17, 2006

© The Author 2006. Published by Oxford University Press on behalf of the Society for Integrative and Comparative Biology. All rights reserved. For permissions please email: journals.permissions@oxfordjournals.org.



**Fig. 1** Anatomy of the olfactory organ and the aesthetasc sensory unit of lobsters. This organ is highly similar in anatomy and function between *P. argus* and *H. americanus* even though these lobsters are from different phylogenetic families. **(A)** The distal section of the lateral flagellum of the first antennae contains the olfactory organ. Arthrodistal segmentation separates the antenna into annuli and gives it flexibility. Cell proliferation within the epithelium of the proximal proliferation zone (PPZ) gives rise to clusters of cells that form the aesthetasc sensory units. These units mature in the transition zone (TZ), which is characterized by annuli with small numbers of aesthetasc setae. In the mature zone (MZ), a full complement of aesthetasc setae are present and the aesthetasc sensory units function as odor detectors. The decrease in the number of aesthetasc setae in annuli near the tip of the organ marks the senescence zone (SZ) containing old sensory units. Many of the SZ annuli are lost at each molt. **(B)** Anatomy of an aesthetasc sensory unit in *H. americanus*. Each unit consists of 2 layers of auxiliary cells and approximately 300 OSNs that send branching dendrites into a single aesthetasc seta. The OSNs specifically express HaiGluR1 and an  $\alpha$ -tubulin isoform (Hollins and others 2003). The outer auxiliary cells specifically express OET-02, a monoxygenase. Although not specific to inner auxiliary cells, a calcyphosine isoform (OET-17) is undetectable in outer auxiliary cells and serves to distinguish the inner auxiliary cells. Although not part of the olfactory sensory units *per se*, the aesthetasc tegumental glands are found only in association with them and appear to develop along with them. The secretory cells of *H. americanus* aesthetasc tegumental glands specifically express the OET-03 serine protease, whereas those of *P. argus* can be identified by their expression of the CUB serine protease (Stepanyan and others 2005; Schmidt and others 2006). Epithelial cells are the other major cell type in the olfactory organ. They can be identified by expression of exoskeletal proteins and other genes such as the serine protease inhibitor PET-20 (Stoss and others 2004).

transduction by phosphatidylinositols (McClintock and Ache 1989a, 1989b; Ache and Zhainazarov 1995; Zhainazarov and Ache 1997, 1999; Zhainazarov and others 2001; Wachowiak and others 2002). Dissections that collect the aesthetasc setae provide uniquely pure preparations of the OSN outer dendrites that have been used to study olfactory transduction, perireceptor events such as odorant clearance, and odorant binding (Trapido-Rosenthal and others 1987, 1990; Gleeson and others 1992; Olson and others 1992; Boekhoff and others 1994; Olson and Derby 1995; Gentilcore and Derby 1998; Xu and McClintock 1999).

These unique advantages of the lobster olfactory system appear to be adaptive specializations of the fundamental properties shared by the olfactory systems of complex metazoans rather than alternative

mechanisms. Shared properties can be detected at both the cellular and molecular levels. OSNs are always bipolar neurons. Lobster OSN outer dendrites are equivalent to the cilia of vertebrate OSNs and are both structurally and functionally similar (Grünert and Ache 1988). They contain variable numbers of microtubules, are nonmotile, and are the sites where odors are detected by odorant receptors. Although the odorant receptors of crustaceans have yet to be identified by molecular cloning and sequencing, evidence indicates that they are G-protein-coupled receptors just as in vertebrates and insects (Fadool and others 1995; Xu and McClintock 1999). The olfactory transduction pathways downstream of these odorant receptors are modulated by phosphatidylinositols in both lobsters and mammals (Zhainazarov and others 2004). The axons of the OSNs terminate

in glomerular neuropil of a specialized region of the brain in crustaceans, insects, mollusks, and vertebrates (Ache and Young 2005). The OSN axons in these glomeruli are the targets of presynaptic inhibition in at least crustaceans and vertebrates (Wachowiak and others 2002).

Another fundamental feature of OSNs is adult neurogenesis. Presumably because they are exposed to damage from external factors, OSNs in long-lived animals such as lobsters experience continuous turnover throughout life. In lobsters, new OSNs originate in the proximal proliferation zone (PPZ), differentiate and mature in the transition zone, function as odor detectors in the mature zone, and senesce in the senescence zone (Fig. 1A) (Steullet and others 2000; Harrison and others 2001). The addition of new aesthetasc-bearing annuli to the proximal end of the organ at each molt shifts older annuli distally in the olfactory organ, with the most distal annuli of the senescence zone being shed at the molt. As in vertebrates, persistent epithelial progenitor cells appear to be the source of new OSNs throughout life.

The advantages of lobsters for studying olfaction and the wealth of information gained about the physiology and biochemistry of the lobster olfactory system raise significant questions about underlying molecular mechanisms. Candidate-gene approaches and homology-cloning methods have produced several successes in identifying lobster genes responsible for olfactory physiology. However, the absence of a sequenced genome and associated functional genomics tools impedes progress toward truly novel discoveries about the function of the lobster olfactory system. To begin to overcome this problem, we have pursued a series of studies that use genomics and physiological genomics approaches to identify genes potentially significant for the functioning of the lobster olfactory system. Herein we review these data and their implications.

## Results and discussion

### Physiological genomics approaches to the olfactory organ

The anatomy of the lobster olfactory organ and the cellular specializations within it lend themselves to physiological genomics approaches designed to identify differentially abundant mRNAs. The organ is compact, and a few functionally important cell types dominate the tissue. The proximal-to-distal age gradient separates developmental stages. This separation reduces the cellular complexity within each compartment of the organ. These features enhance the ability of techniques that compare mRNA abundance to detect

a greater fraction of the mRNAs present and to detect differences in their abundance. We have made use of these advantages in several studies.

In the first of these studies, representational difference analysis (Hubank and Schatz 1994) was used to amplify cDNA fragments from mRNAs more abundant in the olfactory organ than in the brain and second antennae (Hollins and others 2003). The brain served to eliminate common neural mRNAs, and the second antennae suppressed mRNAs common to epithelial cells and nonolfactory types of sensory neurons. This strategy allowed the amplification of cDNAs representing mRNAs expressed specifically in the cell types unique to the olfactory aesthetasc sensory unit.

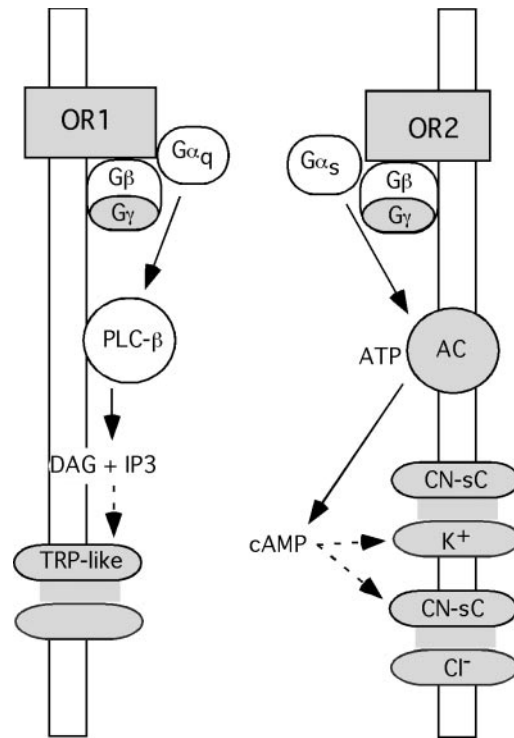
In the second study, the physical separation of different ages of cells in the olfactory organ allowed us to use the same representational difference-analysis technique to investigate mRNAs enriched in the PPZ compared with the mature zone. The most common cells in the PPZ are epithelial cells. Among these epithelial cells must be the cells that act as proliferating progenitors within the OSN cell lineage. As expected, this experiment identified mRNAs expressed primarily by epithelial cells (Stoss and others 2004).

In the third study, to assess gene expression more broadly in the olfactory organ and to take advantage of the relative cellular simplicity of the mature zone, we initiated a *Homarus* expressed sequence tag (EST) library project (HELP) to sequence cDNAs from the mature zone of the olfactory organ of *H. americanus* (Stepanyan and others 2006). This directionally cloned cDNA library was subtracted to reduce redundancy. A total of 5184 clones were selected for 5' end sequencing. This library contained 2389 distinct sequences, 869 of which had significant similarity to known proteins. Of the remaining sequences, 372 matched ESTs in the National Center for Biotechnology Information dbEST database. More than 1000 clones, therefore, remain novel sequences with no indication as to the function of the proteins they encode. We hypothesize that 4 factors contribute to the relatively large number of novel sequences we detected. First, relatively few crustacean sequences exist in public databases. Second, no crustacean genome sequence is yet available. All currently available genome sequences are from phylogenetically distant organisms, the most closely related being several insects. Third, *H. americanus* occupies a niche that is poorly represented among sequenced genomes and may therefore have genetic adaptations that have gone undiscovered. Fourth, the specialized function of the olfactory organ might contribute to an abundance of novel sequences. Analogies to mammalian olfactory epithelium, which

is renowned for the large number of mRNAs that are more abundant there than in any other tissue, support this prediction (McClintock 2000). To allow us to use this mature zone cDNA library to investigate the differential expression of the mRNAs it represents, we generated a cDNA microarray. Of the 5 subarrays of this microarray, 2 have been used for a preliminary test comparing the olfactory organ with the dactyl of the walking legs, a major contact chemosensory (taste) organ of lobsters. This comparison putatively identified 115 differentially abundant mRNAs whose expression patterns and identities were consistent with several anticipated results. For example, the majority of differentially expressed mRNAs were more abundant in olfactory organ, consistent with the origin of the HELP library. Many of these differentially expressed mRNAs are orthologs of gene products known to be enriched in neurons in other animals, consistent with the fact that OSNs are the dominate cell type in the mature zone of the lobster olfactory organs. In addition, transcripts previously known to be specific to the olfactory organ, such as the olfactory-enriched transcript O3 (OET-03), a serine protease (Hollins and others 2003), were correctly identified as enriched in the mature zone.

### Olfactory transduction

Lobsters have the unique property that excitatory and inhibitory receptor potentials coexist in OSNs and can be simultaneously activated by a mixture of odorant compounds (McClintock and Ache 1989). All OSNs have an excitatory pathway mediated by  $G\alpha_q$  and phospholipase C- $\beta$ , but about half also have an inhibitory pathway mediated by cyclic adenosine 3',5'-monophosphate (cAMP) (Ache and Young 2005). Efforts to clone cDNAs encoding the proteins in these pathways by predicting orthology and performing degenerate PCR have been successful for some components (Fig. 2). These include  $G\alpha_q$ , phospholipase C- $\beta$ ,  $G\beta$ , and  $G\alpha_s$ . Components for which no candidate lobster sequences exist include the odorant receptors,  $G\gamma$ , adenylyl cyclase, and ion channels activated by the transduction biochemistry. However, we recently identified cDNA clones encoding  $G\gamma$ , adenylyl cyclase, and chloride channels among the HELP clones. These cDNAs are now candidates for participation in lobster olfactory transduction. The  $G\gamma$  could be a component of the  $G\alpha_q$  heterotrimeric G-protein that mediates excitatory olfactory transduction, the  $G\alpha_s$  heterotrimeric G-protein that mediates inhibitory olfactory transduction, or both. The adenylyl cyclase is a candidate for producing cAMP in the inhibitory transduction pathway. The 3 chloride channel clones,



**Fig. 2** The dual, opposing olfactory transduction pathways of lobster OSNs depicted in a cross section of an OSN outer dendrite. The excitatory pathway results in depolarization and the inhibitory pathway results in hyperpolarization of the plasma membrane. Odorant mixtures can simultaneously activate both pathways, with different component compounds responsible for activation of each. This phenomenon is common to *P. argus* and *H. americanus*. The open boxes represent components for which cDNAs have been identified and tested. The molecular identities of the shaded proteins have not yet been determined, although candidates for some have recently been identified. AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine 3',5'-monophosphate;  $Cl^-$ , chloride ion; CN-sC, cyclic-nucleotide sensitive ion channel; DAG, diacylglycerol;  $G\alpha$ ,  $G\beta$ , and  $G\gamma$ , heterotrimeric G-protein subunits; IP3, inositol 1,4,5-trisphosphate;  $K^+$ , potassium ion; OR, odorant receptor; PLC- $\beta$ , phospholipase C- $\beta$ ; TRP, transient receptor potential ion channel.

which are most similar to bestrophins or *Clc5* mammalian chloride channels, are candidates to produce part of the inhibitory receptor potential (Doolin and Ache 2005). The excitatory receptor potential is believed to be produced by an ion channel related to the transient receptor potential (TRP) family (Bobkov and Ache 2005). Although we have not yet identified TRP channel sequences in lobsters, we did identify an olfactory organ cDNA similar to *Rga*, a mammalian protein that acts as a chaperone for TRP channels.

### Ionotropic receptors and the modulation of OSN axon terminals

GABA and histamine are known to cause presynaptic inhibition of lobster OSN axon terminals in the glomeruli of the olfactory lobe of the brain (Wachowiak and others 2002). Homology-cloning methods previously identified a GABA-A receptor subunit expressed by OSNs (Hollins and McClintock 2000). However, GABA-A receptors are usually heteromeric ion channels, predicting that additional subunit cDNAs remained to be identified. The HELP library contained 2 GABA-A receptor sequences, 1 of which is new. We hypothesize that these 2 cDNAs encode subunits of the GABA-A receptor that mediates GABAergic presynaptic inhibition of OSNs.

In addition to GABA and histamine ionotropic receptors, lobster OSNs also express ionotropic glutamate receptors. This discovery was first made using representational difference analysis to clone olfactory-specific cDNAs. Among the clones was OET-07, an ionotropic glutamate receptor subunit (HaiGluR1) that is specific to OSNs (Hollins and others 2003; Stepanyan and others 2004). Preliminary immunohistochemical results indicate that HaiGluR1 is present in OSN axons entering the olfactory lobe. A second ionotropic GluR subunit, HaiGluR2, was revealed among the HELP library clones. Unfortunately, these 2 subunits fail to produce a functional receptor for glutamate or related compounds when expressed, either alone or in combination, in heterologous cells. The anti-serum against HaiGluR1 provides evidence that the lack of functional expression is due to the absence of expression of the HaiGluR1 protein in the plasma membrane of the human embryonic kidney 293 cell line used for these heterologous expression studies. Sequence conservation, especially of residues known to be important for agonist binding and the ion selectivity of the ion channel pore of ionotropic glutamate receptors, argues that these are *bona fide* ionotropic glutamate receptors. We continue to pursue this hypothesis, suspecting that additional ionotropic glutamate receptor subunit genes exist in lobsters and that their identification will allow us to express functional receptors and thereby confirm the identity of these genes.

### Neuromodulatory genes

In addition to the discovery of ionotropic receptor regulation of the OSN axon terminals, genomics approaches have revealed 2 other potential types of neuromodulation involving the olfactory organ. Among the olfactory-specific mRNAs we discovered is OET-02, a monooxygenase most similar to

mammalian dopamine  $\beta$ -hydroxylase (Hollins and others 2003). OET-02 is expressed only by the outer auxiliary cells that help ensheath the bundle of OSN inner dendrites (Fig. 1B). If this enzyme is an amine monooxygenase, its activity should produce a monoamine such as octopamine. However, evidence is lacking that the lobster olfactory organ produces a monoamine. Although it is possible that OET-02 catalyzes production of an unconventional monoamine whose presence has not yet been tested, we have begun to consider alternatives. The primary alternative is that OET-02 is instead a peptidyl  $\alpha$ -amidating monooxygenase, an enzyme that shares many of the same conserved catalytic residues with amine monooxygenases and helps produce amidated peptide hormones. Whichever function proves correct, the discovery of OET-02 argues that outer auxiliary cells produce a peptide or monoamine hormone. Whether this hormone acts on the OSNs or is instead influenced by olfactory activity to secrete a hormone whose target is elsewhere remains to be determined. Either way, the data begin to suggest that the olfactory aesthetasc sensory units contain an endocrine organ in the form of outer auxiliary cells.

A more direct link to neuromodulation was discovered by the identification of orthologs of orcokinin in the HELP library. Orcokinin is a crustacean neuropeptide hormone with actions on muscle and neural tissues (Skiebe 2003). Immunohistochemistry for orcokinin in crayfish revealed strong staining in the OSN axon terminals of the glomeruli of the olfactory lobe, evidence that orcokinin is expressed by OSNs (Dirksen 2002). We hypothesize that orcokinin released from OSN axons has a modulatory or trophic role on cells in the olfactory lobe of the brain.

### Discovery of the aesthetasc tegumental gland, an exocrine gland in the olfactory organ

Another olfactory-specific mRNA discovered in *H. americanus* was OET-03, a chymotrypsin-like serine protease (Hollins and others 2003; Stepanyan and others 2005). *In situ* hybridization revealed that OET-03 was expressed solely by enigmatic cells occurring in clusters that separate the rows of aesthetasc sensory units. This discovery spurred further investigation of these cells, leading to the accumulation of evidence that they are the secretory cells of an exocrine gland, the aesthetasc tegumental gland. In *H. americanus*, the final confirmation of this by identifying the gland's duct on the surface of the exoskeleton was stymied by technical difficulties. Fortunately, in *P. argus*, an unrelated serine protease (a trypsin-like CUB serine protease) (Levine and others 2001) proved to be

expressed by these same secretory cells, and in this species it was possible to trace the ducts and confirm the exocrine nature of these glands (Schmidt and others 2006). Although it is likely that the aesthetasc tegumental glands of these 2 lobster species are functionally similar, they have anatomical differences. In *P. argus*, the gland has a rosette structure characteristic of many crustacean tegumental glands, whereas in *H. americanus* the gland is not symmetrically organized around a central duct but rather consists of secretory cells that converge ventrally on ducts (Fig. 1B). Whether the 2 lobster species differ in the types of specific proteases their aesthetasc gland secretory cells express or express a common set of proteases has not been determined. Immunohistochemical evidence in both species indicates that neither serine protease is secreted, suggesting that these proteases are involved in the intracellular processing of other proteins critical to the exocrine function of the gland.

The function of the aesthetasc tegumental gland remains to be proven experimentally. However, the available evidence strongly links it to grooming and the prevention of fouling of the highly porous aesthetasc setae (Schmidt and others 2006). Exobiont growth on these setae might impair odor detection and the ability of the lobsters to evaluate many aspects of their environment. Each annulus of the olfactory organ contains an asymmetric seta that extends nearly perpendicular across the rows of aesthetasc setae. The asymmetric setae control grooming behavior (Schmidt and Derby 2005). Prevention of the grooming of the aesthetasc setae causes the aesthetasc tegumental gland to atrophy (M. Schmidt and C.D. Derby unpublished results). This is the strongest evidence to date linking the aesthetasc tegumental gland to the condition of the aesthetasc setae.

### Cell proliferation and neurogenesis in normal and regenerating olfactory organs

The separation of the PPZ, where nascent olfactory aesthetasc sensory units originate, from the regions of the olfactory organ where mature cells reside affords an opportunity to identify mRNAs enriched in the PPZ and possibly involved in cell proliferation and adult neurogenesis. Homology cloning has identified candidate proneural genes expressed in the olfactory organ, including *achaete-scute* and *hairy* homologs (Chien, Schmidt and others 2005; Chien, Liu and others 2005). In addition, we used representational difference analysis to perform this search and discovered 12 mRNAs enriched in the PPZ of *P. argus* (Stoss and others 2004). *In situ* hybridization revealed that these

mRNAs are expressed in epithelial cells, consistent with the fact that epithelial cells dominate in this zone and are the site of genesis of new olfactory sensory units. Because damage to the olfactory organ stimulates proliferation of cells for new olfactory sensory units (Harrison and others 2003), we tested whether the abundance of these mRNAs is sensitive to damage. Only 1 responded—a cDNA named proliferation zone-enriched transcript 15 (PET-15) that has weak similarity to crustin antimicrobial proteins. This response, however, was very robust. More surprising, it was not limited to the damaged ipsilateral olfactory organ. The contralateral undamaged olfactory organ also showed elevated PET-15 mRNA. This contralateral response implies that PET-15 expression responds to a diffusible, hemolymph-borne signal from the site of damage. The response could be detected in both the PPZ and mature zone of the olfactory organ. *In situ* hybridization of undamaged olfactory organs detected PET-15 mRNA only in the PPZ, though real-time quantitative RT-PCR detected small amounts of PET-15 mRNA in the mature zone. Only epithelial cells show labeling, but this labeling is not restricted to the ventral areas where the new aesthetasc sensory units are generated. Instead, epithelial cells throughout the annuli that comprise the PPZ express PET-15. PET-15 expression in damaged mature zone can be detected, but only in epithelial cells close to the blastema at the site of damage. From these data, we conclude that PET-15 specifically marks reactive epithelial cells at locations in the olfactory organ where cellular proliferation among epithelial cells is ongoing. The extent of its expression indicates that PET-15 is certainly not restricted to progenitors of cell types in the olfactory sensory units but instead functions more broadly. Its exact molecular function remains a mystery.

### Summary

Genomics and functional genomics have provided new and novel information about the lobster olfactory system. They led to discoveries that would have been difficult or impossible to reach by other methods. They led to discovery of a new exocrine gland and of specific markers for at least 4 cell types for which no specific markers existed previously. They have identified several interesting genes that are candidates for known functions in the olfactory organ, such as olfactory transduction, presynaptic inhibition, endocrine or paracrine signaling within the olfactory sensory units, and proliferation of new sensory units in normal and damaged olfactory organs. They have also revealed other genes that suggest previously unsuspected functions such as

glutamatergic presynaptic excitation of OSNs. These approaches continue to hold promise for further discoveries as the number of crustacean sequences grows and the tools to take advantage of them, such as our cDNA microarray, continue to be applied to fundamental questions of function and dysfunction.

## Acknowledgments

*Conflict of interest:* None declared.

## References

- Ache BW, Young JM. 2005. Olfaction: diverse species, conserved principles. *Neuron* 48:417–30.
- Ache BW, Zhainazarov A. 1995. Dual second-messenger pathways in olfactory transduction. *Curr Opin Neurobiol* 5:461–6.
- Anderson PAV, Ache BW. 1985. Voltage- and current-clamp recordings of the receptor potential in olfactory receptor cells *in situ*. *Brain Res* 338:273–80.
- Bobkov YV, Ache BW. 2005. Pharmacological properties and functional role of a TRP-related ion channel in lobster olfactory receptor neurons. *J Neurophysiol* 93:1372–80.
- Boekhoff I, Michel WC, Breer H, Ache BW. 1994. Single odors differentially stimulate dual second messenger pathways in lobster olfactory receptor cells. *J Neurosci* 14:3304–9.
- Carr WES, Blumenthal KM, Netherton JC, III. 1977. Chemoreception in the pigfish, *Orthopristis chrysopterus*: the contribution of amino acids and betaine to stimulation of feeding behavior by various extracts. *Comp Biochem Physiol* 58A:69–73.
- Carr WES, Netherton JC, Milstead ML. 1984. Chemoattractants of the shrimp, *Palaemonetes pugio*: variability in responsiveness and the stimulatory capacity of mixtures containing amino acids, quaternary ammonium compounds, purines and other substances. *Comp Biochem Physiol* 77A:469–74.
- Chien H, Liu H, Schmidt M, Tai PC, Derby CD. 2005. Molecular cloning and characterization of proneural genes in the olfactory organ of spiny lobsters, *Panulirus argus*. *Chem Senses* 30:A91–2.
- Chien H, Schmidt M, Tadesse T, Tai PC, Derby CD. 2005. Molecular cloning and tissue expression patterns of SPLASH, an achaete-scute homolog from the olfactory organ of the spiny lobster, *Panulirus argus*. *Soc Neurosci Abstr* 254:8.
- Derby CD. 2000. Learning from spiny lobsters about chemosensory coding of mixtures. *Physiol Behav* 69:203–9.
- Derby CD, Steullet P, Horner AJ, Cate HS. 2002. The sensory basis for feeding behavior in the Caribbean spiny lobster, *Panulirus argus*. *Mar Freshwater Res* 52:1339–50.
- Dirksen H. 2002. Orcokinin and orcomyotropin in neuronal systems of crayfish: differential distributions and functions of novel partially co-localised peptides in sensory, motor, interneuronal and neurosecretory cells. *Comp Biochem Physiol* 132A:S67.
- Doolin RE, Ache BW. 2005. Cyclic nucleotide signaling mediates an odorant-suppressible chloride conductance in lobster olfactory receptor neurons. *Chem Senses* 30:127–35.
- Fadool DA, Estey SJ, Ache BW. 1995. Evidence that a Gq-protein mediates excitatory odor transduction in lobster olfactory receptor neurons. *Chem Senses* 20:489–98.
- Gentilcore LR, Derby CD. 1998. Complex binding interactions between multicomponent mixtures and odorant receptors in the olfactory organ of the Caribbean spiny lobster, *Panulirus argus*. *Chem Senses* 23:269–81.
- Gleeson RA, Trapido-Rosenthal HG, McDowell LM, Aldrich HC, Carr WES. 1992. Ecto-ATPase/phosphatase activity in the olfactory sensilla of the spiny lobster, *Panulirus argus*: localization and characterization. *Cell Tissue Res* 269:439–45.
- Grünert U, Ache BW. 1988. Ultrastructure of the aesthetasc (olfactory) sensilla of the spiny lobster, *Panulirus argus*. *Cell Tissue Res* 251:95–103.
- Harrison PJH, Cate HS, Steullet P, Derby CD. 2003. Amputation-induced activity of progenitor cells leads to rapid regeneration of olfactory tissue in lobsters. *J Neurobiol* 55:97–114.
- Harrison PJH, Cate HS, Swanson ES, Derby CD. 2001. Postembryonic proliferation in the spiny lobster antennular epithelium: rate of genesis of olfactory receptor neurons is dependent on molt stage. *J Neurobiol* 47:51–66.
- Hollins B, Hardin D, Gimelbrant AA, McClintock TS. 2003. Olfactory-enriched transcripts are cell-specific markers in the lobster olfactory organ. *J Comp Neurol* 455:125–38.
- Hollins B, McClintock TS. 2000. Lobster GABA receptor subunit expressed in neural tissues. *J Neurosci Res* 59:534–41.
- Hubank M, Schatz DG. 1994. Identifying differences in mRNA expression by representational difference analysis of cDNA. *Nucleic Acids Res* 22:5640–8.
- Levine MZ, Harrison PJH, Walthall WW, Tai PC, Derby CD. 2001. A CUB-serine protease in the olfactory organ of the spiny lobster, *Panulirus argus*. *J Neurobiol* 49:277–302.
- McClintock TS. 2000. Molecular Biology of Olfaction. In: Finger TE, Silver WL, Restrepo D, editors. *Neurobiology of Taste and Smell*, 2nd Edition. Chichester, UK: Wiley. p 177–99.
- McClintock TS, Ache BW. 1989a. Histamine directly gates a chloride channel in lobster olfactory receptor neurons. *Proc Natl Acad Sci USA* 86:8137–41.
- McClintock TS, Ache BW. 1989b. Hyperpolarizing receptor potentials in lobster olfactory receptor cells: implications for transduction and mixture suppression. *Chem Senses* 14:637–47.
- Olson KS, Derby CD. 1995. Inhibition of taurine and 5'AMP olfactory receptor sites of the spiny lobster *Panulirus argus* by odorant compounds and mixtures. *J Comp Physiol [A]* 176:527–40.
- Olson KS, Trapido-Rosenthal HG, Derby CD. 1992. Biochemical characterization of independent olfactory receptor sites for 5'-AMP and taurine in the spiny lobster. *Brain Res* 583:262–70.

- Schmidt M, Chien H, Tadesse T, Johns ME, Derby CD. 2006. Rosette-type tegumental glands associated with aesthetasc sensilla in the olfactory organ of the spiny lobster, *Panulirus argus*. *Cell Tissue Res* in press.
- Schmidt M, Derby CD. 2005. Non-olfactory chemoreceptors in asymmetric setae activate antennular grooming behavior in the Caribbean spiny lobster, *Panulirus argus*. *J Exp Biol* 208:233–48.
- Skiebe P. 2003. Neuropeptides in the crayfish stomatogastric nervous system. *Microsc Res Tech* 60:302–12.
- Stepanyan R, Day K, Urban J, Hardin D, Shetty RS, Derby CD, Ache BW, McClintock TS. 2006. Gene expression and specificity in the mature zone of the lobster olfactory organ. *Physiological Genomics* 13:224–33.
- Stepanyan R, Haley SB, McClintock TS. 2005. Olfactory specific chymotrypsin-like serine protease from the aesthetasc tegumental gland of the lobster, *Homarus americanus*. *Cell Tissue Res* 322:321–30.
- Stepanyan R, Hollins B, Brock SE, McClintock TS. 2004. Primary culture of lobster (*Homarus americanus*) olfactory sensory neurons. *Chem Senses* 29:179–87.
- Stullet P, Cate HS, Derby CD. 2000. A spatiotemporal wave of turnover and functional maturation of olfactory receptor neurons in the spiny lobster, *Panulirus argus*. *J Neurosci* 20:3282–94.
- Stoss TD, Nickell MD, Hardin D, Derby CD, McClintock TS. 2004. Inducible transcript expressed by reactive epithelial cells at sites of olfactory sensory neuron proliferation. *J Neurobiol* 58:355–68.
- Trapido-Rosenthal HG, Carr WES, Gleeson RA. 1987. Biochemistry of an olfactory purinergic system: dephosphorylation of excitatory nucleotides and uptake of adenosine. *J Neurochem* 49:1174–82.
- Trapido-Rosenthal HG, Carr WES, Gleeson RA. 1990. Ectonucleotidase activities associated with the olfactory organ of the spiny lobster. *J Neurochem* 55:88–96.
- Wachowiak M, Cohen LB, Ache BW. 2002. Presynaptic inhibition of olfactory receptor neurons in crustaceans. *Microsc Res Tech* 58:365–75.
- Xu F, McClintock TS. 1999. A lobster phospholipase C-beta that associates with G-proteins in response to odorants. *J Neurosci* 19:4881–8.
- Zhainazarov AB, Ache BW. 1997. Gating and conduction properties of a sodium-activated cation channel from lobster olfactory receptor neurons. *J Membr Biol* 156:173–90.
- Zhainazarov AB, Ache BW. 1999. Effects of phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 4-phosphate on a Na<sup>+</sup>-gated nonselective cation channel. *J Neurosci* 19:2929–37.
- Zhainazarov AB, Doolin R, Herlihy JD, Ache BW. 2001. Odor-stimulated phosphatidylinositol 3-kinase in lobster olfactory receptor cells. *J Neurophysiol* 85:2537–44.
- Zhainazarov AB, Spehr M, Wetzel CH, Hatt H, Ache BW. 2004. Modulation of the olfactory CNG channel by PtdIns(3,4,5)P<sub>3</sub>. *J Membr Biol* 201:51–7.