

Preface

The sense of smell has an essential role in locating food, detecting predators, navigating, and communicating social information. Accordingly, the olfactory system has evolved complex repertoires of receptors to face these problems. Although the sense of taste has less far-reaching tasks, they are every bit as essential for the animals well-being, allowing it to reject toxic materials and to select nutritionally valuable food. The last decade has seen a massive advance in understanding the molecular logic of chemosensory information processing, beyond that already achieved in the first few years following Linda Buck's discovery of odorant receptors. Shortly afterwards, the major principles of olfactory representation had been established in mammals as the one neuron/one receptor rule and the convergence of neurons, which express the same receptor, onto individual modules in the olfactory bulb. In recent years, such studies have been extended to lower vertebrates, including fishes and other phyla, i.e., arthropods, worms, and insects, showing both the general validity of these concepts and some exceptions to the rule. In parallel, hallmarks of the molecular logic of taste sensation have been deciphered and found to differ in interesting ways from those of smell sensation. In keeping with the emphasis of the taste system on decision making vs the strength of the olfactory system in complex distinction and recognition tasks, taste receptor cells are specific for taste qualities, not necessarily for single taste receptors, and are linked to stereotyped behavioral outputs. We consider it timely to present the current state of the art in gustatory and olfactory research, as seen by leading researchers in the field. In total 12 contributions are presented, about half of them from each field that cover our current knowledge in mammalian, fish and insect models.

Shi and Zhang start out by presenting an overview of olfactory and gustatory receptor gene families in vertebrates and discuss evolutionary rates, species-specific gene expansions and pseudogenization as factors shaping receptor gene repertoires. Four olfactory receptor families, odorant receptors (ORs), vomeronasal receptors type I (V1Rs), vomeronasal receptors type II (V2Rs), and trace amine-associated receptors (TAARs), first described in mammals, have orthologs in teleost fish. All of them are G protein-coupled receptors (GPCRs). Shi and Zhang describe how ORs are both less numerous and more diverse in teleost fish compared to tetrapods. A loss of the entire V2R repertoire has

occurred at least three times during tetrapod evolution, leading to the complete absence of V2Rs in several species, whereas rodent V2R, as well as V2R-related olfC genes of some fish species, show large scale gene amplification. Extreme variation of family size also characterizes the T2R taste receptor family in vertebrates. Some gene losses, but nearly no gene gains are observed in the small vertebrate T1R repertoire. The authors present the case for adaptive evolution of chemosensory receptor families to reflect changing requirements for chemical senses as species evolve to fill different ecological niches.

Zhang and Firestein discuss the genomics of olfactory receptors and the accuracy of genomic datamining. Through computational analysis of genomic databases, OR repertoires of multiple species were identified, revealing an exceedingly large OR gene family of over 1,000 genes in rodents, and a surprisingly large, unrelated family of about 800 chemosensory receptor genes in nematodes. Evolutionary fluctuation is prominent between different species. Pseudogenization is a leading cause for decreases in repertoire sizes, with pseudogenes representing two-thirds of the OR repertoire in primates such as humans and chimpanzees, and six-sevenths of an avian species, chicken. The characteristics of OR genes were explored through computational and experimental methods, showing a complicated gene structure and particular genomic distribution. Phylogenetically, OR genes may be divided into class I and class II ORs, the latter showing a massive gene expansion in tetrapods compared to teleosts. Class I genes form a single large cluster, but class II genes exist in several clusters, often of closely related OR genes, as well as isolated OR genes. Utilizing high-throughput OR microarrays, expression profiles of the mouse and human OR repertoires were examined, their olfactory functions verified, and their zonal, ectopic and developmental expression determined. Class I genes occupy a particular, and molecularly distinct zone within the olfactory epithelium (dorsalmost, zone 1), and a correspondingly segregated target region in the dorsal olfactory bulb. Variation in human smelling abilities results from different functional OR repertoires, variable expression levels and polymorphisms in the copy number of OR genes.

Korsching presents a comprehensive review of teleost olfactory receptor repertoires focusing on evolutionary history, phylogenomic properties and similarities as well as differences to the corresponding mammalian families. Representatives of all four families, the OR, vomeronasal V1R-related ORA, V2R-related OlfC, and TAAR receptors are found in cartilaginous fish and/or jawless fish, indicating an evolutionary origin before the segregation between cartilaginous and bony fish or cartilaginous and jawless fish, respectively. Gene repertoires of teleost olfactory receptors are smaller in size (OR, ORA), comparable (OlfC), or even larger (TAAR) than the corresponding mammalian gene repertoires, but all teleost families show much larger divergence than their mammalian counterparts. Evolutionary rates vary greatly between families, with evidence for positive selection in teleost OR genes, whereas the *ora* genes are unusually conserved among all teleost species. With one exception, ligands

are not known for any of the four teleost olfactory receptor gene families so far. ORs are expressed stochastically within expression domains, similar to the stochastic expression of mammalian ORs within expression zones. The range of odors relevant to fish is rather well known, and contains amino acids, bile acids, nucleotides, steroid and prostaglandin hormones and metabolites, with different groups of chemicals being processed in different subpopulations of olfactory receptor neurons.

Imai and Sakano describe odorant receptor gene choice and axonal projection in the mouse olfactory system. Each olfactory sensory neuron (OSN or ORN) expresses a single type of odorant receptor (OR) out of about 1,000 different genes. In fact, from the two alleles of an OR gene, only one is chosen for expression (monoallelic expression). Furthermore, the axons of olfactory receptor neurons expressing the same OR converge onto a specific pair of glomeruli in the olfactory bulb. For unknown reasons the mammalian (but not the fish) olfactory bulb contains a duplicated, mirror-symmetrical map of glomeruli, hence the pair of target glomeruli. These two basic principles are fundamental to the peripheral olfactory system, and are regulated by the expressed OR protein itself. Somatic recombination or gene conversion play no role in guiding OR expression, since mice cloned from single olfactory receptor neurons contain the full set of expressed OR genes. Singular OR gene choice is ensured by a two-step mechanism, the first being a stochastic enhancer-promoter interaction with a single enhancer element neighboring a cluster of OR genes. In a second step, negative feedback regulation by OR proteins occurs, which blocks transcription from other clusters of OR genes. In the axonal projection, OR-derived cAMP signals and neuronal activity determine the expression levels of axon guidance/sorting molecules, and thereby direct glomerular positioning and axon sorting.

Rodriguez and Boehm discuss pheromone sensing in mice. Among other strategies mice employ urine investigation as a tool to discriminate between individuals. The authors summarize the available information about the chemical nature of rodent pheromones, their physiological sources, and biological function. Pheromones turn out to belong to structurally diverse classes of chemicals, including peptides secreted by some exocrine lacrimal glands, small volatile molecules and protein fragments present in urine. Most pheromones activate both vomeronasal and main olfactory sensory neurons, contrary to the initial hypothesis of a neat segregation of the main and the accessory or vomeronasal olfactory system. In fact, besides the VR genes, some OR genes are expressed in the vomeronasal organ, and even MHC molecules may play a role in odor detection here. Selective gene-targeting of the main and accessory olfactory systems in mice has shown that both systems can converge and synergize to express the complex array of stereotyped behaviors and hormonal changes triggered by pheromones. Moreover, rodent noses house at least two other distinct chemosensory epithelia: the Gr \ddot{u} neberg ganglion and the septal organ; their functions are currently examined.

Yoshihara presents a molecular genetic dissection of the zebrafish olfactory system. His contribution details the advantages of zebrafish as a vertebrate model system, which includes external fertilization, large clutch sizes, rapid development, transparency of embryos, and the availability of various genetic engineering technologies such as transgenesis, mutagenesis, gene knockdown, and transposon-mediated gene transfer. Yoshihara shows that the ‘one neuron/one receptor rule’ established in mammalian olfaction mostly holds true for zebrafish, and that ‘convergence of axons to target glomeruli’ is preserved as well. Using a transgenic approach the author showed the existence of two segregated neural circuits, one originating from ciliated and the other from microvillous olfactory sensory neurons in the olfactory epithelium to distinct regions of the olfactory bulb. These two segregated pathways are likely to convey different types of olfactory information (e.g. pheromones and odorants) to the higher olfactory centers. The chemotopic odor map present in the olfactory bulb is partially retained in the forebrain, but an integration of different input channels begins to be visible as well. A discussion of the chemical nature of fish odor stimuli is included, together with an evaluation of three zebrafish mutants showing defects in olfactory axonal path-finding and smell-guided behavior.

Sato and Touhara present a review of the functional anatomy of the insect olfactory system and discuss some remarkable similarities to the vertebrate system despite the evolutionary independent origins. The authors describe the complete olfactory receptor gene repertoire in the fruitfly and compare it to that of several other insect species. As many as 62, 79, 131, 157, 48, and 265 ORs have been identified in *Drosophila*, *Anopheles*, *Aedes*, *Apis*, *Bombyx*, and *Tribolium*, respectively. In adult *Drosophila* about 1,300 olfactory receptor neurons are housed in about 500 sensilla of three different subtypes expressing 40 different ORs, whereas larvae possess just 21 olfactory receptor neurons expressing 25 ORs. The majority of receptor neurons express a single OR (some do express up to three different ORs), together with the ubiquitous OR 83b, which forms a heterodimer with many of the unique ORs and is involved in signal transduction. Even co-expression of OR and a taste receptor are observed for some olfactory sensory neurons. The tuning-curves of olfactory receptor neurons (ORN) are discussed, the most obvious difference to vertebrate ORN being a notable spontaneous activity allowing for activation as well as inhibition as odor response. Unlike vertebrate OR, insect OR form directly gated ion channels for signal transduction. Olfactory-guided behavior is discussed and technical applications in pest control are represented with the example of the insect repellent *N,N*-diethyl-3-methylbenzamide (DEET) and its behavioral and molecular mechanism of action.

Gerber, Stocker, Tanimura and Thum use *Drosophila* to elucidate the generation of behavior from olfactory and gustatory sensation. The functional anatomy of *Drosophila* olfactory receptor neurons is described both for mature flies and larvae, which emerge as simpler model system with fewer olfactory receptors and with attraction and repulsion as easily testable, behavioral outcomes.

Although insect taste cells are neurons, unlike their vertebrate counterparts, their responses can be categorized in the same modalities vertebrates possess, plus an additional sensitivity to water. Gerber et al. give a detailed description of the regulation of distinct behaviors by multiple taste organs distributed over the fly's body. The authors point out that the largest differences between olfaction and gustation do not lie in the peripheral sensation but in the central processing leading to selection of appropriate motor behaviors. Central olfactory pathways in mushroom body and lateral horn, i.e., beyond the antennal lobe, the insect equivalent of the olfactory bulb of vertebrates, are characterized by a certain segregation of pheromone processing vs normal food odors. The role of mushroom body Kenyon cells as coincidence detectors is explored. The existence of a discrete CNS pathway for encoding experience-dependent changes in olfactory behavior is shown. In contrast, gustatory information seems to bypass the brain proper, being received by the subesophageal ganglion, from which premotor commands likely can be triggered directly. A discussion of olfactory and gustatory learning includes conclusions from mutant studies and examines convergence of olfactory and gustatory information in the brain.

Vigues, Dotson and Munger discuss the molecular mechanism of sweet taste in mammals. Due to its distinct hedonic value, and the associated flip-side, over-ingestion of sugars associated with obesity and obesity-related diseases, sweet taste is of large interest to neuroscientists, dieticians and others, including the general public. The sweet taste receptor, a heterodimer of two class C G protein-coupled receptors, T1R2 and T1R3, responds to a vast array of chemically diverse natural and artificial sweeteners. Natural sweeteners come from several chemical classes, including sugars, sugar alcohols, proteins and amino acids, whereas synthetic sweeteners include sulfamates, dipeptides, halogenated sugars and sulfonyl amides. Mammalian species vary strongly in their sweetener preference, similarly, polymorphisms within species lead to large differences in sensitivity to sweeteners. Such polymorphisms in inbred mice strains have in fact led to the molecular identification of the sweet taste receptors. Receptor chimeras have identified the extracellular domain, the cysteine-rich domain and the transmembrane domain as sites of interaction with different sweeteners. Modeling the T1R heterodimer structure has provided evidence for allosteric interactions being involved in sweetener action and allowed further insights in the location of the ligand binding sites.

Behrens and Meyerhof discuss mammalian bitter taste perception and summarize our current knowledge. The authors describe results of taste cell and taste fiber responses to tastants, and compare them to the large array of data obtained for heterologously expressed taste receptors. Multiple TAS2R (synonym T2R) genes are coexpressed in individual bitter taste receptor cells, thus creating taste cells with broader agonist spectra than any given receptor responds to. The heterotrimeric G protein composition for bitter taste transduction is given as $G\alpha$ -gustducin, $G\beta 3$, and $G\gamma 13$, with the beta/gamma subunits activating phospholipase $C\beta 2$. $PLC\beta 2$ is an essential molecule in taste signal transduction causing increases in IP_3 levels, which in turn lead to rising

cytosolic calcium concentrations, and eventually to activation of the transient receptor potential channel M5. Structure-function investigations have shed some light on the tuning curves of several TAS2R, which range between highly promiscuous and specific for particular chemical substructures. The genetic variability of taste receptors is explored, which generates a heterogeneous human population that contains tasters and non-tasters for several compounds, phenylthiocarbamide (PTC) being most famous among them.

Passilly-Degrace, Gaillard and Besnard expand the taste world beyond the established categories of sweet, sour, bitter, and salt perception to explore the sensation of lipids. Lipid-rich food is spontaneously preferred by both rodents and humans. Although a necessity in times of food deficiency as the optimal source of energy, lipid-rich food is also preferred in times of affluence, unforeseen as a stable state by Nature. Thus, overconsumption of energy-dense fats is a major cause of obesity in industrialized countries. Fats appear to be sensed as fatty acids that are liberated from triglycerides by lingual lipase. Among three candidates for a long chain fatty acid receptor, the authors make the case for the receptor-like glycoprotein CD36, whereas another candidate, the delayed-rectifying potassium channel Kv1.5, appears less likely to be involved in lipid sensing. A third candidate, the G protein-coupled receptor, GPR120, which is a receptor for unsaturated fatty acids, requires further analysis to confirm its status as lipid sensor. The authors discuss the orosensory mechanisms of fat detection with emphasis on CD36 signal transduction.

In the last chapter, Yasuoka and Abe present a summary of the taste system in fish, which in some species have much higher sensitivity than that of mammals. Taste buds are distributed over nearly the entire body of fish and are innervated by three cranial nerves. The authors discuss the evolution of V2R-related taste receptors (T1Rs) in several fish species including a fish-specific expansion of subfamily T1R2, and the comparatively small fish T2R repertoire, together with molecules involved in signal transduction such as phospholipase C β 2 and the transient receptor potential channel TRPM5. Mutually exclusive expression of T1R and T2R receptors, as well as heterodimer formation within families mimics the situation in mammals. The authors continue to describe the ligand spectra of fish taste receptors, and point out that the fish orthologs of mammalian sweet taste receptors (T1R) recognize amino acids. Despite the differences in agonist spectra, T1R activation appears to be similarly linked to attractive behaviors in both mammals and fishes. Similarly, in both phyla activation of T2R bitter receptors lead to aversion, i.e., rejection of food.

The current knowledge of the genetics, molecular biology, and neurobiology of the several distinct chemosensory systems along with the insight into the molecular architecture of the various chemoreceptor molecules and the functional connectivity of the cells processing chemosensory information summarized in this edition has formed a solid basis for identifying challenging research topics for the period to come. Such challenges will include cracking the neural codes and understanding how chemosensory information triggers behavioral outputs. To this end, new experimental tools need to be developed

such as novel genetically engineered strains of mice, fish and insects, molecules for neuroanatomical tracing, in vivo imaging systems, genetic reporters of neuronal activity, and in silico computation. In this sense, the editors wish that the present book serves researchers who are new in the field as a guide to our current knowledge and inspire those already involved to design future research activities.

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