223.07 *p261*

CELL TYPE SPECIFIC GENETIC MARKERS IN THE ZEBRAFISH OLFACTORY SYSTEM

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The olfactory system of zebrafish constitutes an interesting model system, because it is qualitatively similar but quantitatively reduced compared to that of higher vertebrates. However, layering in the olfactory bulb is less pronounced, making it difficult to identify specific neuronal subsets of the olfactory pathway by their anatomical position or simple morphological appearance. Olfactory nerve layer and glomerular layer are not well separated. Mitral cell bodies are scattered within the glomerular layer and distal border of the inner cell layer. Periglomerular cells are absent, but their juxtaglomerular analogues are intermingled with granule cells and local interneurons within the inner cell layer. To achieve better discrimination between these neuronal subtypes, we focused on cell type specific genetic markers already defined in the mammalian olfactory system: omp for olfactory sensory neurons (Rogers *et al.* 1987), tbr1 for mitral cells and dlx2 for granule cells (Bulfone *et al.* 1998). The dlx2 gene was previously cloned for zebrafish (Akimenko *et al.* 1994) and turned out to label granule cells when analyzed by in situ hybridization. To identify zebrafish homologues of the mammalian omp and tbr1 genes we applied a PCR based homology cloning strategy. PCR fragments were used to screen a zebrafish cDNA library to obtain full-length cDNA clones. Furthermore, a zebrafish genomic cosmid library was screened. So far we obtained a tbr1 PCR fragment and full-length and genomic clones of the omp gene. After establishing the cell type specific expression in each case, we will analyze the corresponding upstream regions by appropriate reporter gene expression.

Akimenko *et al.* 1994 J Neurosci 14: 3475
Bulfone *et al.* 1998 Neuron 21: 1273
Rogers *et al.* 1987 PNAS 84: 1704

223.08 *p262*

DIFFERENTIAL EXPRESSION OF NDF/NEUREGULIN RECEPTORS ERBB-3 AND ERBB-4 DURING DEVELOPMENT OF THE MURINE OLFACTORY BULB

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Biological effects of NDF/neuregulin are mediated by ErbB-3 and ErbB-4, two members of the EGF family of tyrosine kinase receptors. In the adult mouse olfactory bulb, we have previously demonstrated that ErbB-4 immunoreactivity is present in the periglomerular and mitral cell layers: as well as in cells of the rostral extention of the subependymal layer, while ErbB-3 is expressed exclusively in the olfactory nerve layer. We now show that ErbB-4 expression occurs transiently in prenatal stages in the olfactory nerve layer and glomeruli. In the periglomerular and mitral cell layers ErbB-4 immunoreactivity is observed early after birth and starting from day 15, respectively. In the rostral extention of the subependymal layer, alternating expression of the two receptors is observed: during prenatal development ErbB-3 expression slightly decreases to undetectable levels at birth, whereas ErbB-4 expression commenced 15 days afterbirth. ErbB-3 immunoreactivity is found in the olfactory nerve layer at all developmental stages under study. On the whole, our results show that the two NDF/neuregulin receptors differ in spatial and temporal expression patterns. We conclude that the two NDF/neuregulin receptors play distinct, rather than redundant, developmental and physiological roles in the murine olfactory bulb. .

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223.09 *p263*

URINARY COMPOUNDS FROM SEXUALLY RECEPTIVE FEMALE MICE PREFERENTIALLY ACTIVATE AN ANATOMICALLY DISTINCT POPULATION OF NEURONS IN THE MALE MOUSE ACCESSORY OLFACTORY BULB (AOB)

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Chemosensory signals processed by the vomeronasal (VN) system affect a variety of sociosexual behaviors in mammals. These signals are detected by sensory neurons in the VN organ that send axonal projections to the AOB. Differential activation of anatomically distinct areas of the male mouse AOB has been shown after exposure to chemosensory cues associated with hormone-dependent behaviors (1), but the nature of the pheromones contributing to the activation is not known. Here, we investigated the efficacy of hormone dependent urinary compounds to activate the AOB by exposing male mice to urine collected from female mice of various hormonal states and measuring *c-Fos* expression. After 2h exposure to urine from females in late diestrus, proestrus, or estrus stages of their cycles, significantly more cellular activation was observed in the AOB than after similar exposure to urine from early diestrus or ovariectomized females, or distilled water. In all cases, the majority of activated cells were located in the rostral portion of the AOB. Urine from estrous females did not require direct contact to elevate *c-Fos*, while urine from ovariectomized females did. Soiled bedding from estrogen receptor α knockout mice was unable to induce significant *c-Fos* expression. The results indicate that hormone dependent compounds in female urine are discriminated at the level of the AOB. Volatile urinary compounds are sufficient to induce activation if they are derived from estrous females, but recognizing an ovariectomized female requires direct contact. Thus, hormone dependent urinary compounds may act as pheromonal cues that are used by the male to discriminate between sexually receptive and non-receptive females.

(1) Kumar *et al.* (1999) J. Neuroscience 19: RC32.

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223.10 *p264*

REGULATED SPLICING OF ODORANT RECEPTOR GENE TRANSCRIPTS IN THE OLFACTORY EPITHELIUM OF THE ZEBRAFISH (DANIO RERIO).

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We have previously cloned a family of odorant receptor molecules in zebrafish, Danio rerio (1). These genes were found to be expressed as two transcriptional variants in the olfactory epithelium. The variants differed with respect to a region immediately preceding the putative open reading frame, that displayed characteristic features of an intron. Most of these introns harbour an additional translation start site, in-frame with the coding region.

As revealed by in situ hybridization, both transcriptional variants are located in the cytosol of sensory neurons. This means that the longer variants enter the cytosol as mature transcripts that are generated by a regulated splicing event. Such regulated splicing of introns preceding the open reading frame and containing in-frame ATG codons also has been reported for other genes (2).

A sequence analysis of both putative start sites shows that both are likely to be used for translation initiation. Thus, two different proteins would be generated from each gene. Alternatively, the regulated splicing of introns could contribute to the mechanisms controlling the rate of odorant receptor gene translation.

(1) Weth *et al.* (1996) Proc. Natl. Acad. Sci. USA 93 : 13321-13326
(2) Kozak (1996) Mammalian Genome 7 : 563-574

223.11 *p265*

CONNECTIONS OF THE OLFACTORY BULB IN A CHONDROSTEAN FISH

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The connectivity of the olfactory bulb have been established in several groups of fishes, but the information of the bulbar projection in chondrosteans is very scarce. We have applied experimental methods using lipophilic carbocyanine tracers to study the connections of the olfactory bulb in Acipenser baeri. All experiments were conducted in accordance with the European guidelines on animal experimentation.

Olfactory bulb efferents course in two extensive and overlapped pathways. In the telencephalon, lateral fibers ran through the lateral telencephalic fascicle giving diffuse terminal fields in the dorsomedial telencephalic lobe. Ventral labeled fibers ran caudally and, some of them cross to the contralateral ventral area. At caudal levels some bulbar projections ran into the diencephalon to the habenula, the rostral and ventral thalamus and the caudal hypothalamus.

Tracer application to the olfactory bulb also revealed a number of bulbopetal neurons from the olfactory epithelium, contralateral olfactory bulb and telencephalon. In the telencephalon we observed sparse labeled neurons in dorso and ventromedial regions both ipsi and contralaterally.

Gnathostomes typically display restricted secondary olfactory projections, however, exceptions are observed in some species of chondrichthyes and a chondrostean that show more extensive olfactory projections, similar to some agnathans. The presence in a chondrostean of important secondary olfactory projections, suggest that such projections are a derived characteristic of bony fishes. Knowledge of the olfactory projections in this primitive ray-finned fish may shed light on the evolution of the olfactory system in fishes and other vertebrates.

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223.12 *p266*

BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO CHEMOSENSORY STIMULATION OF THE LEG OF THE LOCUST, SCHISTOCERCA GREGARIA

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Contact-chemoreception is a vitally important sense, necessary for the detection and selection of appropriate foods, for the selection of mates or oviposition sites, and avoiding harmful chemicals in the environment. Insects have chemosensory receptors scattered over the entire body surface, typically in uniporous sensilla containing groups of 4-12 chemosensory neurones. The behavioural responses of desert locusts to solutions of four behaviourally relevant chemicals (sodium chloride, sucrose, nicotine hydrogen tartrate and lysine glutamate) applied as droplets to the hind tarsus were analysed. All responses following within 1s of stimulation were local leg avoidance reflexes and the probability of eliciting a response increased in a dose-dependent manner with increasing chemical concentration for all of the tested chemicals. Chemical identity, however, critically determined the concentration threshold at which the different chemicals became an effective stimulus. We also analysed the responses of a population of spiking local interneurones and leg motor neurones, parts of the local circuits producing and controlling leg movement, to stimulation with the same chemical solutions. The amplitude and duration of the responses of these interneurones and flexor tibiae motor neurones depended upon chemical identity and concentration applied to the hind leg and paralleled the probability of a behavioural response. The response increased in a dose-dependent manner with chemical concentration and was repeatable for any given concentration of a particular chemical suggesting these neurones may have an important role in organising reflex withdrawal behaviours away from aversive chemical stimuli.