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Mechanisms controlling diversification of olfactory sensory neuron classes

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Abstract

Animals survive in harsh and fluctuating environments using sensory neurons to detect and respond to changes in their surroundings. Olfactory sensory neurons are essential for detecting food, identifying danger, and sensing pheromones. The ability to sense a large repertoire of different types of odors is crucial to distinguish between different situations, and is achieved through neuronal diversity within the olfactory system. Here, we review the developmental mechanisms used to establish diversity of olfactory sensory neurons in various model organisms, including *C. elegans*, *Drosophila*, and vertebrate models. Understanding and comparing how different olfactory neurons develop within the nervous system of different animals can provide insight into how the olfactory system is shaped in humans.

Keywords

G protein-coupled receptor; combinatorial transcription factor code; stochastic neuronal asymmetry; epigenetic regulation; *C. elegans*, *Drosophila*; vertebrate

Introduction

The human brain consists of numerous amounts of different types of neurons, each of which works in concert to provide us with complexity of thought and the ability to sense and react to ever changing environments. Each neuronal type expresses different genes, displays varying morphologies, and forms precisely mapped connections with respective target cells to ensure their unique functions are carried out. Understanding how neurons achieve distinct cell fates during development will provide steps toward treatment of neurodegenerative diseases and insight into cell replacement therapies.

The diversification of neuron classes is especially important in the olfactory system, as olfaction is critical for detecting dangerous signals in the environment, finding sources of food, and sensing pheromones. Loss of sense of smell is an early indicator of several diseases, such as Parkinson's and Alzheimer's disease [1,2].

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Olfactory sensory neurons are located in the olfactory epithelium of animals, and are defined by the types of olfactory receptor genes they express [3,4]. An odorant binds to olfactory receptors that lie in the cilia of their respective olfactory sensory neuron, resulting in depolarization and a signal sent to the olfactory bulb for interpretation. Here, we focus on mechanisms used in the development and differentiation of olfactory sensory neurons in various model organisms, including *C. elegans*, *Drosophila*, and vertebrate models. The olfactory system is a beneficial model to investigate how neuronal diversity develops.

Olfactory system in *Caenorhabditis elegans*

The nematode *Caenorhabditis elegans* has the ability to detect multiple types of odorants, and expresses between 500-1,000 putative seven transmembrane G protein-coupled receptors [5-8]. Three classes of olfactory neurons are responsible for detecting odors in the worm: AWA, AWB, and AWC cells, which send their axons to the nerve ring (the “brain” of the worm). Like other chemosensory neurons in the worm, olfactory neuron classes exist as a bilateral pair, one cell on the left, and one cell on the right side of the body. They are classified by similarities in morphology and synaptic targets. Unlike mammalian olfactory receptor expression, each of the *C. elegans* olfactory neurons expresses several G protein-coupled receptors [5,9]. The neurons extend dendrites to the tip of the nose, where they end in sensory cilia exposed to the environment (Fig. 1A). AWA and AWC neurons are responsible for detecting attractive odorants, while the pair of AWB neurons sense repulsive odors (Fig. 1A) [7,10]. Misexpression of an olfactory receptor for an attractive odorant (normally expressed in AWA neurons) in AWB neurons results in repulsion of the worm to the normally attractive odorant [10]. This indicates that *C. elegans* olfactory neurons are programmed to elicit specific behavioral responses, regardless of the olfactory receptors they express.

The majority of *C. elegans* neurons are derived through stereotypical cell divisions [11]. Although similar in morphology and function, the classes of olfactory neurons are not lineally related. The common ancestors of the pairs of AWA and AWC neurons diverge from one another beginning from the third embryonic cell division. However, the mother cells of AWA and AWC divide in a similar pattern to give rise to the AWAL and AWAR, and AWCL and AWCR cells, respectively. The AWBL and AWBR neurons lineally diverge starting from the second cell division, and their ancestors do not display similar cell division patterns.

Combinatorial transcription factor codes for neuronal fate specification

All three pairs of olfactory neurons are shown to differentiate using terminal selectors. Terminal selector genes encode transcription factors that recognize a common cis-regulatory element found in multiple fate-determining genes, and are required for terminal differentiation of a particular neuron (Fig. 1B) [12]. Therefore, it is proposed that terminal selectors determine the gene battery (a set of genes that confer a particular property) expressed in a specific neuron. The nuclear hormone receptor ODR-7 is the putative terminal selector for AWA neurons [12,13] (Fig. 1A), as it is required for appropriate expression of several AWA-specific genes essential for the neuron's function. Loss of function mutations

in the *odr-7* gene results in ectopic expression of an AWC-specific gene in AWA neurons. Therefore, in addition to promoting the AWA cell fate, the *odr-7* gene also functions to repress the AWC identity in AWA neurons. The AWB and AWC neurons have been shown to use two transcription factors, which cooperate with one another to act as terminal selectors. In AWB neurons, a LIM homeodomain protein LIM-4 and the highly conserved HMG box transcription factor SOX-2 work together to promote the AWB identity (Fig. 1A). Loss of function mutations in *lim-4* results in loss of expression of AWB-specific genes, and a cell fate transformation of AWB neurons into AWC neurons [14]. A missense mutation in *sox-2* results in a similar cell fate transformation of AWB neurons to an AWC subtype. SOX-2 has been shown to function cooperatively with LIM-4 to promote terminal differentiation of AWB. In AWC neurons, SOX-2 functions cooperatively with the Otx-type homeobox protein CEH-36 for terminal differentiation (Fig. 1A) [15,16].

Intercellular communication-mediated stochastic neuronal asymmetry

Although the pairs of AWA, AWB, and AWC neurons are symmetric at the morphological level, the pair of AWC olfactory neurons displays molecular and functional asymmetries across the left-right axis to increase sensory diversity [17,18]. They are further classified into two subtypes: the default AWC^{OFF} and the induced AWC^{ON}, based on differential expression of putative olfactory receptor genes and distinct functions. The decision for the neurons to adopt the AWC^{OFF} or AWC^{ON} identity is stochastic: in half of the animals, AWC^{ON} is on the left side of the worm, and in the remaining half it is on the right side of the body [17]. Prior to cell-cell communication, both AWC neurons adopt the default AWC^{OFF} state, in which spontaneous calcium influx through voltage-gated calcium channels (the pore-forming $\alpha 1$ subunits UNC-2 N-type or EGL-19 L-type and the regulatory $\alpha 2\delta$ subunit UNC-36) activates a Ca^{2+} /calmodulin dependent protein kinase (CaMKII)-MAP kinase pathway [17,19,20]. Upon UNC-43 (CaMKII) activation, the TIR-1 (Sarm1) adaptor protein assembles a calcium-signaling complex that includes UNC-43 (CaMKII) and NSY-1 MAP kinase kinase kinase (MAPKKK) in a manner dependent on microtubules and the kinesin motor UNC-104 (KIF1A) [18,20-22]. The two AWC neurons communicate with each other and other neurons using calcium signaling in a transient NSY-5 gap junction network during late embryogenesis [22-24], leading to differential calcium levels between the two AWC neurons, to establish AWC asymmetry. The AWC neuron with a relative higher calcium level remains as the AWC^{OFF} subtype. In the future AWC^{ON} subtype, the NSY-5 gap junction protein and NSY-4 claudin like adhesion protein act in parallel to down-regulate calcium signaling [23,25]. Voltage- and calcium-activated SLO-1 and SLO-2 BK potassium channels act redundantly downstream of NSY-5 to antagonize calcium channel-mediated signaling in promoting the AWC^{ON} subtype [26]. In addition, the microRNA *mir-71* acts downstream of NSY-5 gap junctions and NSY-4 claudins to inhibit TIR-1-mediated calcium signaling to promote the AWC^{ON} subtype [27,28]. Together, these steps lead to suppression of the CaMKII-MAPK signaling cascade, and result in expression of AWC^{ON}-specific genes [29,30]. Transcription factors also play a role in establishing AWC asymmetry, with the SOX-2 HMG-box protein promoting the AWC^{ON} identity, and the DIE-1 zinc finger protein promoting the AWC^{OFF} identity [16,29,31]. Each of the AWC subtypes maintains their identities throughout adulthood, using transcriptional regulation, olfactory cyclic guanosine

monophosphate (cGMP) signaling, and transforming growth factor (TGF- β) signaling [17,32,33].

Olfactory system in *Drosophila melanogaster*

Detection of olfactory sensory cues facilitates feeding and social behaviors in the fruit fly, *Drosophila melanogaster* [34]. Adult flies have two olfactory organs, the antenna and the maxillary palp, which arise from the eye-antennal imaginal disc and are covered with sensory hairs called sensilla (Fig. 2A) [35]. Each sensillum contains 1-4 olfactory receptor neurons (ORNs) [34]. Sensilla are classified into three types: basiconic, trichoid, and coeloconic, based on their morphology, regionalization, and the substance they detect. Each sensillum type is further subdivided based on the size, number of ORNs and their stereotyped olfactory receptor repertoire [36].

Unlike mammalian or *C. elegans* G protein-coupled olfactory receptors, *Drosophila* olfactory receptors function as both ligand-gated and cyclic-nucleotide-activated cation channels [37]. *Drosophila* olfactory receptors are seven-transmembrane domain proteins with structural similarity to G protein-coupled receptors but have an inside out transmembrane topology [38]. The *Drosophila* genome contains ~50 olfactory receptor subclasses from 60 gene loci [39]. Like the *C. elegans* olfactory system, multiple olfactory receptors have been identified within a single ORN in *Drosophila* [36,40,41]. However, most ORNs express one unique olfactory receptor and a common co-receptor, Orco (formerly known as Or83b) [42-44]. Highly stereotyped expression of *Drosophila* olfactory receptors suggests that receptor gene choice is deterministic instead of stochastic [45-47]. ORNs expressing the same olfactory receptors send their axons to one of approximately 50 glomeruli in the antennal lobe, where they form synapses with dendrites of projection neurons in a highly stereotypic manner. Projection neurons then send stimuli to higher order processing centers in the mushroom body and protocerebrum [48,49].

Patterning of the eye-antennal imaginal disc that gives rise to ORNs

Drosophila ORNs are derived from the eye-antennal imaginal disc, which is initially patterned by homeotic functions of EGFR and Notch signaling [50]. Then, Homothorax, Extradenticle, and Distal-less induce the antennal expression of multiple genes including spalt, dachshund, and atonal [51-54]. Mutual suppression of wingless and decapentaplegic, induced by hedgehog signaling in the posterior imaginal disc, sets up the dorsal-ventral and proximal-distal boundary [39,55,56].

Combinatorial transcription factor codes for sensilla subtype diversification

Overlaid on top of the initial patterning of the antenna are codes of transcription factors that diversify the *Drosophila* olfactory system. This is analogous to combinatorial transcription factor codes for neuronal subtype specification in *C. elegans* olfactory neuron diversification discussed earlier [57]. Dyad-1 promotes olfactory receptor expression in the maxillary palp, while Oligo-1 represses olfactory receptor expression in the antenna [38]. In addition, several transcription factors also regulate the expression of olfactory receptor genes in the antenna, which dictates the sensilla type lineages and subtype diversification [39].

Dachshund, Rotund, BarH1/H2, Bric-à-brac, and Apterous are part of a gene regulatory network that patterns the antennal disc into seven concentric rings, where these transcription factors work in a combinatorial fashion to diversify ORNs [58]. Engrailed is required for the specification of all three types of sensilla. Lozenge and Amos are required for specifying both basiconic and trichoid sensilla: high expression levels of Lozenge specify basiconic sensilla, while low levels of Lozenge generate trichoid sensilla types. Coeloconic sensilla are specified by the expression of Atonal in its founder cells, while Dachshund specifies basiconic sensilla. Addition of the transcription factor Rotund to preexisting ORN precursor zonal identity increases ORN diversity [59] (Fig. 2C).

Diversification of ORN lineages by Notch signaling

The ORNs in a single sensillum are derived from a common sensory organ precursor cell. Cell-cell communication mediated by Notch signaling specifies the founder cells that further divide to generate the Notch-ON versus Notch-OFF specification within a sensillum [60]. Differential Notch activation results from asymmetric segregation of the protein Numb, which antagonizes Notch signaling [61]. In a sensillum, a cluster of ORNs is asymmetrically differentiated into two classes in a manner dependent on differential Notch activity in their sibling precursors. Notch-ON and Notch-OFF specify olfactory receptor expression and axonal targeting of ORNs [62]. The requirement of cell-cell communication for *Drosophila* ORN subtype diversification is reminiscent of the intercellular communication-dependent subtype specification (AWC^{ON} versus AWC^{OFF}) of the *C. elegans* AWC olfactory neurons discussed earlier. However, Notch signaling does not seem to play a role in stochastic specification of AWC subtypes [17].

Epigenetic regulation of olfactory receptor gene expression

Another mechanism regulating olfactory receptor expression in *Drosophila* involves epigenetic regulation by a conserved multiprotein complex, Myb-MuvB (MMB)/dREAM. This DNA-binding complex regulates expression of carbon dioxide olfactory receptor genes (Gr63a/Gr21a) in a specific neuron subtype [63]. The olfactory receptor genes in the antenna show an enrichment of histone methylation similar to what is seen in the mammalian olfactory receptor genes, inferring a repressive state at these loci [63,64]. Myb acts to activate olfactory receptor expression by opposing the repressor activity of a H3K9 histone methyltransferase and changing the chromatin architecture suitable for specific olfactory receptor expression [63]. *Drosophila* may use mechanisms similar to epigenetic regulation of mammalian olfactory receptor choice, discussed later.

Control of axonal targeting of ORNs by combinatorial codes of transmembrane proteins

Axonal targeting of ORNs is controlled by several molecules, including the POU domain transcription factor Acj6, [65], Down syndrome cell adhesion molecule (DSCAM) [66], Robo signaling [67] as well as its downstream signaling molecules Dock and Pak [68], N-cadherin [69], and Toll receptors [70]. Toll receptors function cell autonomously for axon targeting of ORNs and dendrite targeting of projection neurons. Furthermore, Toll receptors play an instructive role in target selection independent of the cytoplasmic domain. Consistent with this observation, canonical downstream molecules of Toll signaling, including dMyD88 (a homolog of *C. elegans* TIR-1 involved in AWC subtype specification),

are not required for target selection [22,70]. In addition to Toll receptors, *Drosophila* transmembrane protein Teneurins act in both ORNs and projection neurons to instruct synaptic partner matching within the antennal lobe through homophilic interactions [71]. Semaphorins also play a role in both ORN axon targeting and projection neuron dendrite targeting [72,73]. Another transmembrane protein Capricious (Caps) also acts to segregate dendrites of Caps-positive and Caps-negative projection neurons to their distinct glomerular targets [74]. Taken together, the combinatorial code of transmembrane proteins expressed in ORNs and projection neurons directs the development of the olfactory circuit in *Drosophila*.

Differences between adult and larval olfactory systems

While *Drosophila* adults have 1,300 ORNs, *Drosophila* larvae possess only 21 ORNs at the mixed dorsal organ dome [75]. In the adult olfactory circuit, 1,300 ORNs express 47 olfactory receptor genes, converge to 43 glomeruli, and subsequently diverge to around 150 projection neurons. The basic structure of the larval olfactory system is similar to the adult one. However, larval ORNs and projection neurons lack cellular redundancy and do not exhibit any convergent or divergent connectivity. Each of the 21 larval ORNs is unique and projects to one of 21 antennal lobe glomeruli, each of which is innervated by a single projection neuron. Each projection neuron sends its axon to one or two of approximately 28 glomeruli in the mushroom body calyx. The difference in olfactory circuits between larvae and adults correlates with differences in their olfactory behavior, since larvae are born near a food source, and therefore do not need to be as motile as adults, which need to avoid predators, find mates, food, and suitable locations to lay eggs [75].

Maintenance and plasticity of olfactory circuit

The transcription factor Fruitless is necessary and sufficient for male sex specific behaviors mediated by olfactory neurons [76]. During development, a putative chromatin modulator Alhambra co-regulates the expression of *fruitless* and the olfactory receptors involved in sex specific behaviors [77]. Furthermore, *fruitless* expression is maintained in the adult through the activity of olfactory receptors, CamK signaling, and chromatin modulatory protein p300/CREB-binding protein to maintain sex specific ORN identity [77].

In addition, Notch signaling is required for structural and functional plasticity of the olfactory glomeruli induced by chronic odor exposure. Chronic exposure of an odorant results in changes in both behavior and the volume of glomeruli [78]. This enlargement of glomeruli is dependent on both canonical and non-canonical Notch signaling [79,80]. Notch is expressed in the adult ORNs and is activated in an odorant specific fashion, mediated by olfactory receptors and the Delta ligand, in a subset of ORNs [81]. The Delta ligand is activated in the projection neurons as a result of neuropeptides released by the ORN through non-canonical Notch signaling and olfactory receptor activation [79].

Vertebrate olfaction

Olfactory acuity varies between different vertebrate species, and even within different individuals of the same species. There are over 1,000 olfactory receptor genes in the mouse genome, each of which is activated by multiple odors [82,83]. This illustrates the large

repertoire of scents the mouse is able to detect. In mammals, the olfactory system is composed of the olfactory bulb and olfactory epithelium. Here, we focus on development of olfactory sensory neurons, which are located within the olfactory epithelium lining the nasal cavity. Olfactory sensory neurons are bipolar, and extend a dendrite to the surface of the olfactory epithelium, where they end in cilia exposed to the environment. Each olfactory sensory neuron expresses an olfactory receptor, which recognizes specific odorants. Upon odorant binding, a signal is triggered that is sent to the olfactory bulb for interpretation via the axons. Each mature olfactory sensory neuron is thought to express a single olfactory receptor from a single allele[3,4].

The olfactory epithelium develops from nasal placodes, which require cooperation of the transcription factors Sox2 and Oct-1 for induction [84]. Olfactory sensory neurons are derived from a stem cell population that give rise to Mash1+ cells, which in turn give rise to intermediate precursor cells. Those cells then divide to give rise to olfactory sensory neurons [85,86].

A central question arises in how each olfactory sensory neuron chooses to express a single receptor gene out of over 1,000 options. There are several proposed mechanisms by which olfactory sensory neurons adopt their identities, each of which occurs through a stochastic choice[87].

Epigenetic regulation of olfactory sensory neuron identity

The first mechanism uses epigenetic regulation and nuclear position as a means to promote particular olfactory neuron identities. Olfactory receptor genes are found in different clusters throughout the genome, and tend to be located in transcriptionally inactive heterochromatic regions of the nucleus [64,88,89]. The silent olfactory receptor genes are marked by H3K9me3, a hallmark of heterochromatin. These alleles appear to be silenced before the choice is made to express a particular olfactory receptor [64]. In contrast, the active olfactory receptor alleles appear to reside in euchromatic (transcriptionally active) regions of the nucleus [88]. The location of the olfactory receptor genes within the nucleus appear to be regulated by lamin b, as ectopic expression of this receptor disrupts the organization of olfactory receptor loci clusters in the nucleus [88]. It is shown that the lysine demethylase LSD1 removes the repressive methylation mark H3K9me3 from a chosen olfactory receptor gene, and changes the status of the locus from transcriptionally inactive to an active state (Fig. 3A) [90].

Locus control elements in olfactory receptor choice

Another, not mutually exclusive model for determining the identity of the olfactory sensory neuron is via cis-regulatory elements. As the olfactory receptor genes appear in clusters, this allows for a common element to regulate genes in a particular cluster. Two such DNA sequences deemed the H and P elements have been identified that regulate olfactory receptor gene expression. They are proposed to act as locus control elements, and make a stochastic decision to physically bind to and activate the promoters of one of the olfactory receptor genes in a cluster (Fig. 3B) [91,92].

Transient expression of multiple olfactory receptors per neuron in early development

Another mechanism from recent studies has emerged that suggests that multiple olfactory receptor genes are initially transiently expressed during the early development of olfactory sensory neurons [93,94]. The authors suggest that epigenetic regulation later suppresses other olfactory receptor genes, to result in expression of a single olfactory receptor (Fig. 3C) [94].

Olfactory receptors are crucial in axon guidance

Olfactory receptors not only control which odorants the sensory neurons respond to, but are also crucial in guiding the neuron's axon to a target region within the olfactory bulb called a glomerulus [95,96]. In addition to being localized in the cilia of olfactory sensory neurons, olfactory receptors are localized in axons. Neurons expressing the same olfactory receptor gene converge onto the same glomeruli. Deleting a particular olfactory receptor results in expression of another olfactory receptor in the neuron, and misguidance of the axon [97-99]. Swapping an olfactory receptor coding sequence with an alternative receptor sequence leads to targeting of the axon to a different glomerulus. The olfactory receptors regulate axon targeting of the olfactory sensory neurons through cyclic adenosine monophosphate (cAMP) signaling. The G protein-coupled olfactory receptor activates a G protein, which in turn activates adenylyl cyclase type III. Adenylyl cyclase type III then produces cAMP, which subsequently regulates transcription of axon guidance molecules [100]. Mutating a motif of an odorant receptor required for coupling with a G protein (thereby decreasing cAMP levels) resulted in a defect in the ability of the respective olfactory sensory neurons to converge onto their targets, as the axons remained in an anterior portion of the olfactory bulb. Expression of a constitutively active G protein (thereby increasing cAMP levels) resulted in convergence of olfactory sensory axons in a more posterior region of the olfactory bulb [100]. In addition, rescue of the axon guidance defect of the mutant odorant receptor was found when a constitutively active G protein was coexpressed. Partial rescue was observed with expression of constitutively active cAMP-dependent protein kinase (PKA) or constitutively active cAMP responsive element-binding protein (CREB, a transcription factor regulated by PKA) [100]. It was found that neuropilin-1, an axon guidance receptor, was not expressed in olfactory sensory neurons of olfactory receptor mutants where cAMP signals were low [100]. Neuropilin-1 has also been shown to be important for pre-target axon sorting, along with its repulsive ligand Semaphorin-3A [101]. Therefore, olfactory receptors are not only required for sensing odors, but also for connectivity of olfactory sensory neurons.

Maintenance of olfactory sensory neuron identity

It has been suggested that once an olfactory receptor gene has been chosen, the receptor uses feedback mechanisms to maintain the identity of the olfactory sensory neuron and repress the expression of alternative olfactory receptor genes (Fig. 3D) [92]. Once the olfactory receptor is expressed, it triggers the unfolded protein response and the protein kinase Perk, leading to translation of the transcription factor ATF5. ATF5 in turn activates *Adyc3*, an adenylyl cyclase that represses LSD1 (lysine demethylase). Down-regulation of LSD1 ensures that other olfactory receptor genes do not have their repressive methylation marks removed [90,102,103].

Similar to mice, zebrafish have also been shown to express a single olfactory receptor gene per olfactory sensory neuron. A model for how zebrafish achieve this is by first choosing a subfamily of olfactory receptors, and subsequently choosing which particular receptor to express from the chosen subfamily [104]. It is proposed that feedback mechanisms are then used to silence other potential olfactory receptor genes.

Conclusion

Model organisms are extremely useful tools to examine developmental mechanisms. In this review, we examined olfactory neuron diversification in various model organisms, including *C. elegans*, *Drosophila*, and vertebrate models. Although each organism develops olfactory neurons in a different manner, some similarities are observed (Table 1). *C. elegans* and *Drosophila* both express multiple olfactory receptors per neuron, as well as use combinatorial transcription factor codes and cell-cell communication to diversify olfactory neurons. *C. elegans* AWC neurons and vertebrate olfactory sensory neurons each use stochastic mechanisms to establish their cell fates, although the mechanisms of stochasticity differ. The highly conserved transcription factor Sox2 is also used in both *C. elegans* and mouse olfactory development. *Drosophila* and vertebrates are similar in their use of epigenetic regulation in terminal olfactory neuron identity.

Although *Drosophila* olfactory receptors differ from those expressed in worms and vertebrates, they display evolutionary organizational and structural parallels. *Drosophila* olfactory receptors function as ion channels rather than G protein-coupled receptors, however they still have a similar seven-transmembrane domain structure to the olfactory receptors found in *C. elegans* and vertebrate olfactory sensory neurons. This poses an interesting question in evolutionary terms of how olfaction may have evolved and where possible branching points are, if any, when they diverged. As animals become larger and more complex, the ORN became more restricted to expressing a single receptor to be more discriminating to an olfactory stimulus. From a simple circuit of six olfactory neurons expressing multiple olfactory receptors per neuron in *C. elegans* to *Drosophila* with a mixed population of both unique receptor/co-receptor ORNs and multiple receptor/co-receptor ORNs to mice expressing only a unique receptor per mature ORN, the mechanism of olfactory receptor choice has become more complex to include epigenetic regulation, monoallelic expression, and presence of an olfactory receptor feedback mechanism where little is known and further studies are warranted. Understanding how olfactory neuron diversification occurs in each of these different model organisms may provide insight into how the human brain generates a tremendous diversity of olfactory neuron fates.

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Fig. 1.
The diversification of *C. elegans* olfactory neurons. **a** Terminal selector genes, morphology, and functions of *C. elegans* olfactory neurons. Anterior is at left and ventral is at bottom. **b** Terminal selector gene(s) determine the gene battery profile to generate olfactory sensory neuron diversity.

Fig. 2. *Drosophila* olfactory system. **a** Schematic of a *Drosophila* head. ANT, antenna; MX, maxillary pulp. **b** Map of three types of sensilla in the *Drosophila* antenna. **c** Rotund expression diversifies three types of antenna sensilla into different subtypes, ac1-4, ab1-10, and at1-4. ac, antennal coeloconics; ab, antennal basiconics; at, antennal trichoids; Rotund+, Rotund-positive ORNs; Rotund-, Rotund-negative ORNs; ++, high levels; +, low levels.

Fig. 3. Mouse olfactory system. **a** Epigenetic regulation of olfactory receptor (OR) gene clusters. Me, methylation marks. Black, transcriptionally active; grey, transcriptionally inactive. **b** Enhancer regulation of olfactory receptors (OR) clusters. **c** During development, immature olfactory neurons may express low levels of multiple ORs and become restricted to one OR when fully developed. ORN, olfactory receptor neuron. **d** Feedback mechanisms from the OR that is expressed are used to repress other ORs from being expressed to generate the one OR in one neuron rule.

Table 1
Comparison of mechanisms of olfactory sensory neuron diversification

	<i>C. elegans</i>	<i>Drosophila</i>	Vertebrate
Olfactory receptors	Putative seven-transmembrane GPCR [8]	Seven-transmembrane ion channel [35,38,42]	Seven-transmembrane GPCR [80]
Model	Stochastic and deterministic [11,17]	Deterministic [44,45]	Stochastic [85]
Sox2 used in olfactory development	Yes [16]	Not determined	Yes [82]
Epigenetic regulation	Not determined	Yes [61]	Yes [62,86-88]
Combinatorial transcription factors	Yes [16]	Yes [57]	Not determined
Olfactory receptor expression	Multiple putative olfactory receptors per neuron [5,9]	Multiple olfactory receptors per neuron [37,43]	Single olfactory receptor per mature neuron [3]
Cell-cell communication used in diversification	Yes (gap junctions, claudins, axon-axon contact) [17,23,25]	Yes (Notch signaling) [49,60]	Not determined
Maintenance of olfactory neuron identity	Transcriptional regulation, olfactory cGMP signaling, TGF- β signaling [17,33,34]	Transcriptional regulation, CamK signaling, chromatin modulator, olfactory receptor activation [75]	Olfactory receptor expression, transcriptional regulation, epigenetic regulation [88,90,98,99]