Olfactory receptor genes expressed in distinct lineages are sequestered in different nuclear compartments

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The olfactory system translates a vast array of volatile chemicals into diverse odor perceptions and innate behaviors. Odor detection in the mouse nose is mediated by 1,000 different odorant receptors (ORs) and 14 trace amine-associated receptors (TAARs). ORs are used in a combinatorial manner to encode the unique identities of myriad odorants. However, some TAARs appear to be linked to innate responses, raising questions about regulatory mechanisms that might segregate OR and TAAR expression in appropriate subsets of olfactory sensory neurons (OSNs). Here, we report that OSNs that express TAARs comprise at least two subsets that are biased to express TAARs rather than ORs. The two subsets are further biased in *Taar* gene choice and their distribution within the sensory epithelium, with each subset preferentially expressing a subgroup of *Taar* genes within a particular spatial domain in the epithelium. Our studies reveal one mechanism that may regulate the segregation of *Olfr* (OR) and *Taar* expression in different OSNs: the sequestration of *Olfr* and *Taar* genes in different nuclear compartments. Although most *Olfr* genes colocalize near large central heterochromatin aggregates in the OSN nucleus, *Taar* genes are located primarily at the nuclear periphery, coincident with a thin rim of heterochromatin. *Taar*-expressing OSNs show a shift of one *Taar* allele away from the nuclear periphery. Furthermore, examination of hemizygous mice with a single *Taar* allele suggests that the activation of a *Taar* gene is accompanied by an escape from the peripheral repressive heterochromatin environment to a more permissive interior chromatin environment.

olfactory receptor genes | Taar genes | nuclear organization

The mammalian olfactory system possesses enormous discriminatory power (1, 2). It can distinguish a multitude of volatile odorants as having specific odors as well as elicit innate behavioral or physiological responses (3–6).

Mice have 1,000 different odorant receptors (ORs), each expressed by a unique subset of olfactory sensory neurons (OSNs) scattered within one zone of the nasal olfactory epithelium (OE) (7–11). ORs are used in a combinatorial manner to detect odorants, a strategy that explains how myriad odorants can be discriminated (12).

However, the OE also contains 14 trace amine-associated receptors (TAARs), whose expression patterns resemble those of ORs (13). Like ORs, TAARs are evolutionarily conserved in vertebrates, suggesting that they may serve a distinct function. Ligands found thus far for TAARs are volatile amines, including several in mouse or predator urine (5, 14, 15). Ligands for a few mouse, fish, and human TAARs elicit aversive or attractive behaviors in their respective species, hinting at a conserved ability of TAARs to stimulate innate responses of potentially adaptive significance (5, 6, 14, 16, 17).

If this is the case, one might envisage OSNs expressing TAARs as a distinct neuronal subgroup capable of conveying signals to the brain that elicit specific behaviors. Consistent with this idea, some OSNs appear biased to express TAARs. OR-expressing OSNs are thought to randomly select one odorant receptor (*Olfr*) allele for expression, but choose a second *Olfr* allele to express if the first one fails to produce a functional protein (18–20). A high proportion of OSNs that express a mutated *Taar* gene also express a functional *Taar* allele, suggesting similar mechanisms for *Olfr* and *Taar* "gene choice," but one tailored to *Taars* (21, 22).

What regulatory mechanisms dictate TAAR versus OR expression in OSNs? How do OSNs slated to express TAARs avoid expressing ORs when there are about 70 times as many *Olfr* as *Taar* alleles? Another question concerns the regulation of gene expression within the *Taar* family. Whereas *Olfr* genes are found in clusters at multiple loci on most chromosomes (11), *Taar* genes are clustered at a single chromosomal locus (23). Like *Olfr* genes, different *Taar* genes can be expressed in different spatial domains within the OE (13, 21). The differential expression suggests the existence of additional gene regulatory mechanisms that act at the level of the *Taar* gene locus, but remain to be elucidated.

Here, we investigated *Taar* gene choice within the *Taar* locus as well as at the level of nuclear architecture. At the level of the *Taar* locus, we find evidence for fine scale mechanisms that bias *Taar* gene choice to subsets of *Taar* genes expressed in different OE domains. At the level of the nucleus, we find a striking difference between the intranuclear compartments containing *Taar* versus *Olfr* genes. In sharp contrast to *Olfr* genes, which reside primarily in the nuclear interior near large heterochromatin aggregates (24, 25), *Taar* genes preferentially localize to a thin rim of heterochromatin adjacent to the nuclear envelope. Our studies further

Significance

Odorants are detected in the mouse nose by 1,000 different odorant receptors (ORs) and 14 TAARs. Each olfactory sensory neuron (OSN) expresses one receptor allele. While ORs generate diverse odor perceptions, some TAARs appear to be involved in innate responses, raising questions about mechanisms that could segregate ORs and TAARs in functionally distinct OSN subsets. Here, we identify two OSN subsets with different epithelial expression patterns that express different subgroups of TAARs rather than ORs. Our studies show that *Taar* and *Olfr* genes localize in different nuclear compartments, suggesting a physical substrate for their differential regulation. We further find that activation of a *Taar* allele is accompanied by its escape from peripheral repressive heterochromatin to a permissive interior chromatin environment.

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indicate that the activation of a *Taar* allele involves an escape from this peripheral heterochromatin rim to a more interior location, a shift likely to remove the *Taar* allele from a repressive heterochromatin environment to one permissive for *Taar* gene expression.

Results

Differential Regulation of *Taar* Gene Subsets. Similar to *Olfr* genes, different *Taar* genes can be expressed in different OE spatial domains. Some are expressed in only the dorsal or ventral OE and others in both domains. In knockout (KO) mice with a single *Taar* gene replaced by a reporter gene, 50–60% of OSNs expressing the reporter also expressed a functional *Taar* gene (21, 22). Mutation of one dorsal *Taar* (*Taar4*) led to the secondary expression of mostly dorsal *Taars* (21), suggesting that there might be subsets of TAAR OSNs that differ in the *Taar* genes they can express.

To investigate this question, we compared KO mice with mutant alleles of *Taar5* or *Taar6*, the first expressed in the dorsal OE and the second in both dorsal and ventral OE. In both mutant alleles, the *Taar* coding region was replaced by a lacZ gene encoding beta-galactosidase (β-gal).

To analyze the coexpression of the mutant *Taar* alleles with other *Taar* genes, we costained OE tissue sections with anti–β-gal antibodies and a mix of *Taar* cRNA probes, or probes specific for individual *Taars*, or for the *Taar7* or *Taar8* subfamily, which have five and three members, respectively (Fig. S1). To distinguish expression in dorsal versus ventral OE domains, we used a probe for *Nqo1* (NAD(P)H dehydrogenase, quinone 1), which is selectively expressed in the dorsal domain (26).

In both *Taar5* and *Taar6* heterozygous mutants, about 50–60% of β-gal+ OSNs were colabeled with *Taar* probes (Table S1). The percentage colabeled with the mixed *Taar* probe was similar to the summed percentages of those colabeled with single *Taar* probes, suggesting that individual β-gal+ OSNs expressed only one functional *Taar* gene.

Expression of the mutant *Taar* allele resembled that of the wild-type allele in both mutant strains (Fig. S2). Like *Taar5*, β-gal was expressed almost exclusively in the dorsal *Nqo1*+ domain in *Taar5* mutants. Like *Taar6*, β-gal was expressed in both dorsal and ventral domains in *Taar6* mutants, although the dorsal domain contained a higher percentage of the OSNs expressing β-gal (64.9%) than *Taar6* (23.1%) (Table S2).

We observed a striking difference in the complement of *Taars* coexpressed with *Taar5* versus *Taar6* mutant alleles (Fig. 1). Relative to other OSNs, OSNs expressing the mutant *Taar5* allele showed a similar or increased percentage of cells expressing *Taars* normally expressed in the dorsal OE domain (*Taar2*, *3*, *4*, *5*, *8*, *9*) (21). However, the percentage expressing *Taar6*, which is normally expressed in both dorsal and ventral domains, was reduced ∼7.5-fold. In addition, there was an approximately threefold decrease in cells expressing *Taar7* subfamily members, some of which are normally expressed in only the ventral OE (21). These results suggest that OSNs that express the *Taar5* mutant allele are biased to express *Taar* genes normally expressed in only the dorsal OE domain.

In sharp contrast to *Taar5* mutants, *Taar6* mutants showed little or no bias for the coexpression of particular *Taar* genes with the mutant allele. The only significant difference was a slight decrease in the percentage of *Taar*+ OSNs that expressed *Taar7* subfamily members in the β-gal+ population compared with other OSNs. These results suggest that OSNs expressing the *Taar6* mutant allele either have little preference for the expression of different *Taar* genes or, alternatively, that they comprise at least two distinct subsets, one biased to dorsal *Taars* and the other to ventral *Taars*.

The biased coexpression of the *Taar5* mutant allele with dorsal *Taars* in these studies is similar to that reported for a mutant allele of *Taar4*, another dorsal *Taar* (21). However, our findings differ from another study of the same *Taar5* KO strain that failed to show a preference for coexpression of the mutant *Taar5* allele

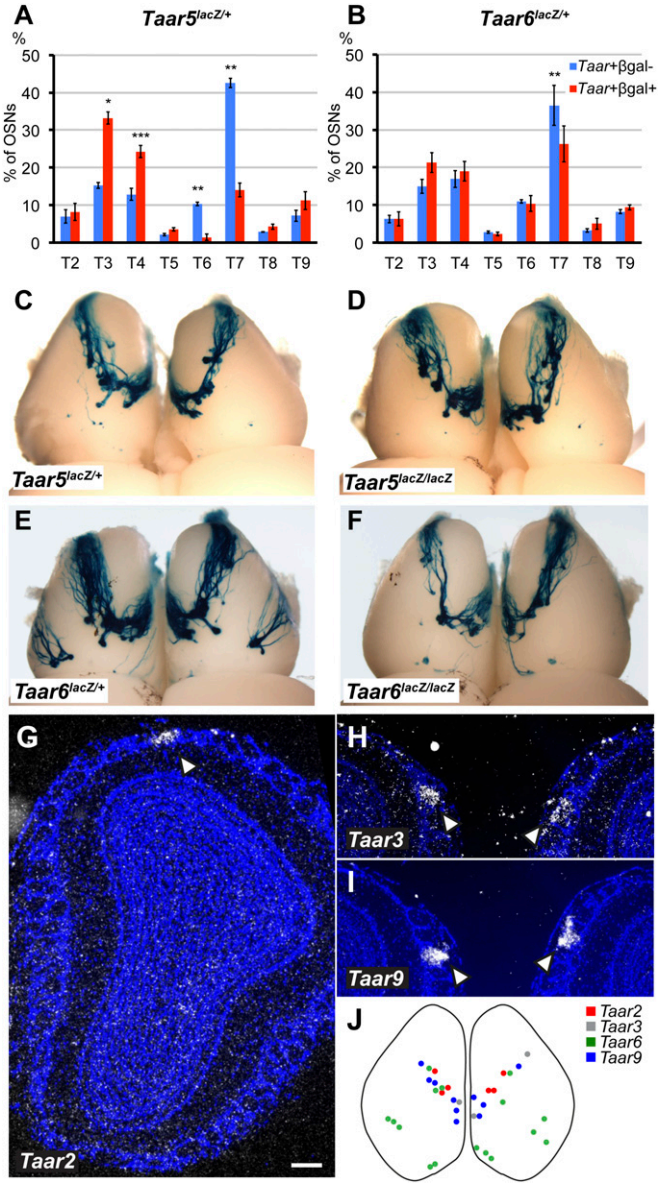

Fig. 1. *Taar* gene choice differs in OSNs expressing mutant alleles of *Taar5* versus *Taar6*. (*A* and *B*) Histograms show the percentages of OSNs expressing different *Taar* genes (T2-T9) in *Taar*+/βgal+ and *Taar*+/βgal− OSNs in $Taar5^{lacZ/+}$ and $Taar6^{lacZ/+}$ mice. The profile of *Taars* coexpressed with the mutant allele differs in *Taar5* versus *Taar6* mutants. Average ± SEM, $n = 4$ per mutant. *P* values were calculated using two-way ANOVA followed by paired t test with Bonferroni correction, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (*C*–*F*) Images of the paired dorsal olfactory bulbs show β-gal+ OSN axons converging in a crescent of glomeruli in $Taar5^{lacZ/+}$ and $Taar6^{lacZ/+}$ mice. $Taar6^{lacZ/+}$, but not $Taar6^{lacZ/lacZ}$, mice, also show more posterior-lateral β-gal+ glomeruli. Anterior is at the top. (*G*–*I*) Darkfield images show rare glomeruli that hybridized to radioactive *Taar2, Taar3, or Taar9* riboprobes in olfactory bulb sections (arrowheads). (Scale bar, 200 μm.) (*J*) A schematic of the dorsal bulb shows the locations of glomeruli that hybridized to four *Taar* probes in three animals.

with other dorsally expressed *Taar* genes (22). One possible explanation for this difference is that we removed the neomycin resistance gene selection cassette present in the original *Taar5* KO allele whereas the other study did not. Previous studies have shown that the presence of such selection cassettes can lead to the abnormal expression of genes with which they are associated (27, 28). Consistent with this explanation, our preliminary experiments showed β-gal+ OSNs in the ventral OE domain of

Taar5 mutant animals before, but not after, removal of the selection cassette (Fig. S3).

The differences we observed in *Taar5* versus *Taar6* mutant animals were reflected in the patterns of glomeruli innervated by β-gal+ OSN axons in the olfactory bulb (Fig. 1 *C–F*). Like OSNs expressing a particular *Olfr*, OSNs expressing a given *Taar* synapse in a few specific glomeruli in the bulb (22, 29). As reported previously for the axons of OSNs expressing *Taar4* or *Taar5* mutant alleles, β-gal+ OSN axons in both *Taar5* and *Taar6* heterozygous mutant mice converged in multiple glomeruli that formed a crescent in the dorsal bulb (Fig. 1 *C* and *E*). However, heterozygous *Taar6* mutants showed additional strongly labeled glomeruli in the more posterior and lateral bulb. These additional glomeruli were absent in homozygous *Taar6* mutants (Fig. 1*F*), suggesting that they were innervated by *Taar6*-expressing OSNs in the heterozygous mutant.

Using radioactive in situ hybridization to label OSN axons containing specific *Taar* mRNAs, we observed glomeruli for four different *Taars* in olfactory bulb sections (Fig. 1 *G–J*). These studies showed similarly located additional posterior-lateral glomeruli for *Taar6*, as also seen using *Taar6* antibodies (22), whereas glomeruli labeled for three other *Taar*s all had more anterior locations in the dorsal olfactory bulb.

Together, these studies suggest that there are subsets of *Taar*-expressing OSNs that are biased to express different *Taar* genes. Moreover, these subsets reflect spatial domain differences in the expression of *Taar* family members. The clear implication is that there are subgroups of *Taar* genes that are coregulated, with differences in *Taar* gene regulation linked to the domain in which the *Taar*-expressing OSN is located.

***Taar* and *Olfr* Genes Are Localized to Different Parts of the Nucleus.** The above results are consistent with the idea that there is a distinct OSN lineage that is biased to express *Taar* rather than *Olfr* genes. In most cells, heterochromatin containing inactive genes is located primarily at the nuclear periphery, adjacent to the nuclear envelope, or in multiple pericentromeric or perinucleolar foci in the nuclear interior. The interior foci are referred to as "chromocenters", because they contain aggregates of DNA and are therefore strongly stained by DAPI (4′,6-diamidino-2-phenylindole dihydrochloride), which binds DNA. However, OSNs have an unusual nuclear architecture in which heterochromatin in the nuclear interior is aggregated at one or a few large chromocenters rather than in multiple smaller chromocenters, as seen in other cell types (24, 25). Previous studies indicate that *Olfr* genes are colocalized at these chromocenters (24, 25). In mutant mice lacking these large foci, *Olfr* genes do not cluster and are abnormally expressed, leading to the proposal that the aggregation of *Olfr* genes governs their monogenic expression (24).

Do *Taar* genes colocalize with *Olfr* genes in OSN chromocenters? To address this question, we used fluorescence in situ hybridization (FISH) with BAC (bacterial artificial chromosome) probes on OE tissue sections to visualize the nuclear locations of *Taar* and *Olfr* genes. *Taar* genes are all clustered at a single locus on chromosome 10 whereas *Olfr* genes are found on most chromosomes and at dozens of different loci (11). We examined five of these *Olfr* gene loci in addition to the *Taar* locus. To visualize the nuclear periphery, we used antibodies against lamin B, which marks the nuclear lamina, a filamentous protein network coating the inner nuclear membrane (30).

As previously reported, the nuclei of OSNs contained one or a few central aggregates of heterochromatin, as indicated by their intense staining with DAPI (Fig. 2 *A–D* and Movie S1). These large chromocenters were not seen to the same extent in "basal cells" at the base of the OE, the location of olfactory stem cells and progenitors, or in the most apical cell layer of supporting (sustentacular) cells adjacent to the nasal lumen. Instead, the basal and sustentacular cells tended to show multiple smaller chromocenters (Fig. 2*A* and Movie S1).

Each BAC probe labeled two small foci within the nucleus, marking the two alleles of a gene locus. Using *Olfr* probes, the labeled foci were mostly located within the nucleoplasm and often at or near central chromocenters, as previously reported (Fig. 2 *B–E*) (24, 25). Only one of the five *Olfr* BACs (155M18) labeled foci

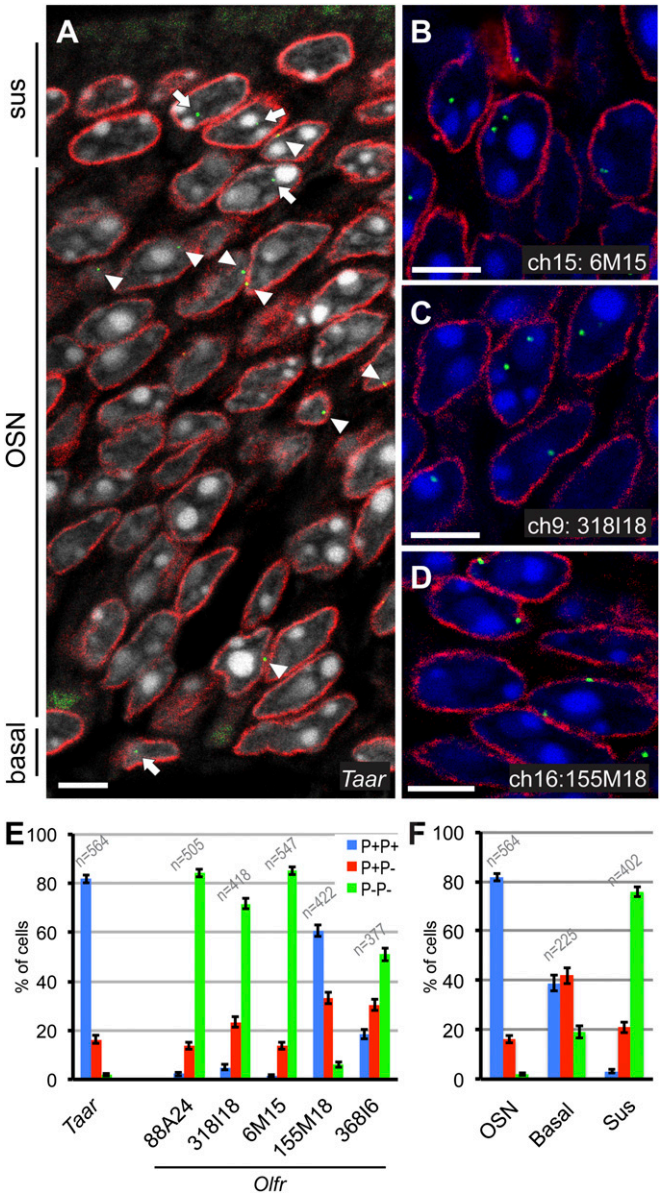

Fig. 2. *Taar* and *Olfr* genes are sequestered in different nuclear compartments. DNA FISH using *Taar* and *Olfr* BAC probes (green) show that *Taar* alleles often overlap with lamin B (red), which marks the lamina at the nuclear periphery (*A* and *E*). Most *Olfr* alleles are instead located in the nuclear interior (*B*, *C*, and *E*), although one preferentially localizes to the lamina (*D* and *E*). The laminar localization of *Taar* alleles seen in OSNs is reduced or absent in OE basal cells and sustentacular (sus) cells (*A* and *F*). Arrowheads indicate *Taar* alleles colocalized with the lamina, and arrows indicate *Taar* alleles in the nuclear interior (*A*). Chromocenters are apparent from their intense DAPI staining (grayscale in A, blue in *B–D*). In histograms showing the locations of *Taar* and *Olfr* alleles (*E* and *F*), P+P+, P+P–, and P–P– indicate cells with, respectively, both, one, and no alleles at the nuclear periphery. *Olfr* BAC reference numbers are indicated (*B–E*). Average ± SEM based on three animals. *n* indicates total number of cells analyzed. (Scale bars, 5 μm.)

preferentially located at the nuclear periphery, with 60.7% of OSNs showing both alleles coincident with the nuclear lamina (Fig. 2 *D* and *E*), as determined by pixel overlap between the two color channels.

In sharp contrast, foci labeled with the *Taar* probe were predominantly located at the nuclear periphery, where they colocalized with lamin B (Fig. 2 *A*, *E*, and *F*). In 81.7% of OSNs, both *Taar* alleles were located at the nuclear lamina (Fig. 2*E*). Moreover, most of the remaining OSNs had one *Taar* allele at the lamina and one allele at a more interior location in the nucleus. Only 4.0% of the *Taar* foci abutted a large chromocenter, and none was seen inside a chromocenter.

Previous studies indicate that a gene can have a preferred location that correlates with its activity, but is not always found at that location, even in the same cell type. For example, the beta globin gene is silent in erythroid progenitors and highly expressed in mature erythroid cells, but only 70% of beta globin alleles localize to the nuclear periphery in the progenitors and 28% of alleles remain there in the mature cells (31). Our results indicate a strong preference for the localization of inactive *Taar* genes to the nuclear periphery.

Notably, other OE cell types did not show the same pattern of *Taar* gene localization as OSNs (Fig. 2 *A* and *F*). In sustentacular cells, the locations of *Taar* alleles were nearly the reverse of what was seen in OSNs, with 75.9% of cells showing both *Taar* alleles in the nuclear interior. *Taar* alleles were also located away from chromocenters, which were smaller and more numerous in sustentacular cells than OSNs. In basal cells, both *Taar* alleles were associated with the lamina in 38.9% of cells, an organization midway between OSNs and sustentacular cells. It is possible that some of the basal cells were stem or progenitor cells that give rise to both OSNs and sustentacular cells whereas others were "precursor" cells already destined to become OSNs.

These results indicate that *Taar* and *Olfr* genes are largely localized to different compartments within the OSN nucleus. They also indicate that the laminar localization of *Taar* genes seen in OSNs is present to a much lesser degree, or absent, in other OE cell types, and may therefore be specific for OSNs and important for *Taar* gene regulation in OSNs. Consistent with this idea are studies showing that genes can be targeted to different locations or compartments within the nucleus in a tissue-specific or cell-type specific manner that influences their expression (31–33).

LAP2β May Tether Heterochromatin to the OSN Nuclear Periphery. The vast majority of OSNs express ORs rather than TAARs. Thus, most of the *Taar* loci observed at the nuclear periphery in the above experiments were presumably silent and located in a repressive environment. To investigate whether there is repressive heterochromatin at the OSN nuclear periphery, we immunostained OE sections for two histone modifications characteristic of heterochromatin: H4K20me3 and H3K9me3 (34).

Antibodies against both histone modifications strongly labeled the large central chromocenters in OSNs (Fig. 3 *A* and *B*). However, they also showed faint yet distinct labeling at the nuclear periphery, which was costained for lamin B. This outline could also be faintly observed by DAPI staining, indicating that some condensed heterochromatin is present at the periphery.

How might a thin rim of heterochromatin, and likely *Taar* genes, be tethered to the nuclear lamina? Previous studies indicate that OSNs lack the lamin B receptor (LBR), which can anchor heterochromatin to the nuclear lamina (24). Another protein implicated in heterochromatin anchoring to the lamina is lamin A. To investigate whether lamin A might serve this function in OSNs, we immunostained OE sections for lamin A.

These experiments revealed lamin A at the nuclear lamina of OE basal cells, but not OSNs (Fig. 3*C*). Thus, OSNs lack not only LBR, but also lamin A. Previous studies have shown that the loss of both LBR and lamin A can cause an inverted nuclear architecture in cell types with a conventional nuclear architecture, resulting in the appearance of a large central heterochromatin aggregate (35). It is conceivable that the related nuclear architecture of OSNs similarly derives from the lack of both proteins. Consistent with this idea, ectopic expression of LBR in OSNs leads to the disruption of their large central chromocenters and the altered expression of hundreds of genes, including *Olfr* genes (24).

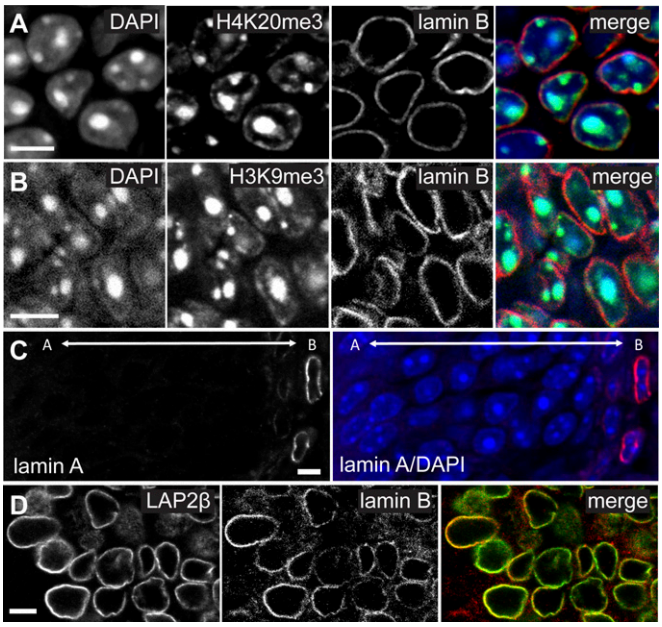

Fig. 3. The OSN nuclear periphery contains heterochromatin and LAP2β, but not lamin A. (*A* and *B*) Immunohistochemistry using antibodies against the heterochromatin marks H4K20me3 and H3K9me3 show a thin rim of staining along the nuclear lamina of OSNs, in addition to the intense staining of internal heterochromatin aggregates characteristic of OSN nuclei. DAPI-staining of heterochromatin is similar. Merged image shows the DAPI, heterochromatin marks, and lamin B pseudocolored in blue, green, and red, respectively. (*C*) Lamin A is seen at the nuclear periphery of OE basal cells, but not OSNs. (*D*) LAP2β, another lamina-associated protein, is present at the OSN nuclear lamina, where it colocalizes with lamin B. A, apical OE; B, basal OE. (Scale bars, 5 μm.)

Immunostaining for other candidate lamina-associated proteins showed that one, LAP2β, is reliably detected at the nuclear lamina of OSNs (Fig. 3*D*). LAP2β has previously been linked to transcriptional repression and the association of chromatin with the nuclear periphery (36, 37). Although we cannot exclude the involvement of other lamina-associated proteins, these results suggest that LAP2β may be responsible for tethering some heterochromatin, and *Taar* genes, to the nuclear lamina.

***Taar* Gene Expression Is Accompanied by a Shift in Nuclear Location.** The above studies indicated that *Taar* and *Olfr* genes largely localize to different nuclear compartments. However, because most OSNs express *Olfrs*, they could not exclude the possibility that the locations of *Taar* genes differ in OSNs expressing *Taar*s.

To explore this question, we examined *Taar4*-IRES-mCherry (T4-mCh or "T4-RFP") mice, which coexpress the fluorescent protein, mCherry with the intact *Taar4* gene (21). OE sections were costained with antibodies against mCherry and a *Taar* BAC FISH probe.

These experiments revealed a marked difference between the locations of *Taar* genes in OSNs expressing a *Taar* gene versus other OSNs. DAPI staining indicated that the nuclear architecture of *Taar*-expressing OSNs resembles that of other OSNs. However, whereas both *Taar* alleles localized to the nuclear lamina in 81.7% of the total OSN population, this was the case

for only 31.6% of mCherry+ OSNs, which express *Taar4* (Fig. 4 *A* and *B*). Moreover, half of the mCherry+ OSNs had one *Taar* allele at the nuclear lamina and the other at a more interior location. Thus, there is a clear correlation between the status of *Taar* gene expression in OSNs and the location of *Taar* alleles within the nucleus.

Because *Taar* genes are monoallelically expressed, one question raised by these observations concerned the location of the expressed *Taar* allele. To address this question, we crossed the T4-mCh mice with mice in which all OE *Taar* genes are deleted (ΔT2-9 mice) (21). The resulting mice are hemizygous and express *Taar* genes from a single *Taar* locus allele.

In the hemizygous mice, the *Taar* BAC probe labeled a single focus in each cell. In mCherry− OSNs, 85.5% of *Taar* alleles were colocalized with the lamina. In contrast, the single active *Taar* allele colocalized with the lamina in only 41.3% of mCherry+ OSNs (Fig. 4*C*). In other words, 58.7% of OSNs expressing *Taar4* had the active *Taar* allele located away from the lamina, whereas this was true for only 14.5% of other OSNs. Given that the nuclear location of a gene tends to vary somewhat even among cells of the same type, this degree of relocalization from the periphery to the nuclear interior is striking.

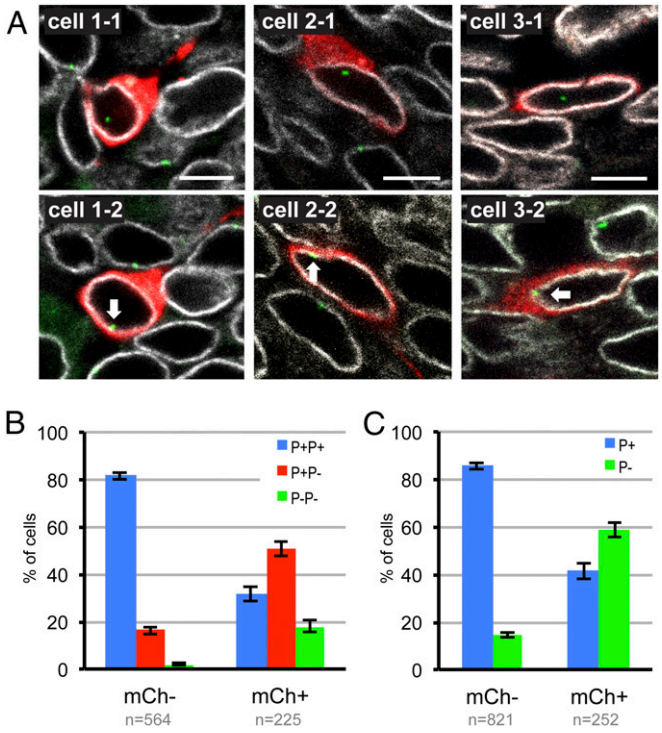

Fig. 4. *Taar* allele activation is accompanied by a shift in nuclear location. The locations of *Taar* alleles were examined in OSNs expressing TAAR4, using *Taar4*-IRES-mCherry mice. (*A*) Different confocal slices through three mCherry+ (red) OSNs show one *Taar* allele (green) located in the nuclear interior (above) and the other colocalized with lamin B (white) at the nuclear periphery (below) (arrows). (*B*) Compared with nearby mCherry- OSNs (mCh−), mCherry+ OSNs (mCh+) show a decrease in the percentage of cells with both *Taar* alleles at the periphery (P+P+) and increases in the percentages of cells with one (P+P−) or both (P−P−) *Taar* alleles in the nuclear interior. (*C*) *Taar* allele localization was scored in *Taar4*-IRES-mCherry × ΔT2-9 hemizygous mice, which only have one *Taar* locus allele. In mCh+ cells, the single *Taar* allele is always active. Compared with nearby mCh− OSNs, mCh+ OSNs show an increase in the percentage of cells with the *Taar* locus located in the nuclear interior (P−). Average ± SEM based on three animals. *n* indicates total number of cells analyzed. (Scale bar, 5 μm.)

Together, these studies indicate that there is a dramatic difference between the nuclear locations of *Taar* versus *Olfr* genes in the general OSN population, with *Olfr* genes largely localized to large aggregates of heterochromatin in the nuclear interior and *Taar* genes primarily localized to a thin rim of heterochromatin at the nuclear periphery. They further indicate that the activation of a *Taar* gene is accompanied by a shift in its location from a repressive heterochromatin environment at the periphery to a more interior location that is likely to be permissive for *Taar* gene expression. This finding is reminiscent of previous observations showing the repositioning of genes away from repressive heterochromatin environments upon, or preceding, their activation (31, 33, 38).

Discussion

The evolutionary conservation of both ORs and TAARs as vertebrate odor detectors has been puzzling, particularly given the large number and combinatorial use of ORs in odor coding. Recent studies indicate that at least some TAARs can elicit innate behaviors (5, 6, 14). This finding suggests a model in which OSNs expressing TAARs versus ORs may be preprogrammed to express those receptor types and to transmit signals through different neural circuits in the brain. This model implies the involvement of distinct regulatory mechanisms in OSNs expressing TAARs versus ORs, but what those mechanisms might be is unknown.

In the present studies, we explored this question by investigating receptor gene choice in mice carrying mutant *Taar* alleles and by examining the nuclear organization of *Taar* versus *Olfr* genes. Our findings are consistent with the idea that *Taar* OSNs comprise a distinct OSN lineage. They further suggest that there are subsets of *Taar* OSNs that are biased to express different subgroups of *Taar* genes, and that those subsets underlie the differential expression patterns of *Taars* within the OE. Our studies of the OSN nucleus reveal a dramatic difference in the nuclear organization of *Taar* versus *Olfr* genes. Although *Olfr* genes primarily localize to central heterochromatin aggregates, *Taar* genes localize predominantly to a thin rim of repressive heterochromatin at the nuclear periphery. This finding suggests that the differential regulation of *Taar* versus *Olfr* genes involves their sequestration in distinct chromatin compartments within the nucleus. We further find that *Taar* gene activation is accompanied by a movement away from the periphery, a mechanism that may permit an escape from a repressive environment to one permissive for *Taar* expression.

TAAR OSNs Comprise a Distinct Lineage Containing Subsets. Previous studies of *Olfr* gene expression have suggested that the developing OSN selects a single *Olfr* allele for expression, but will select a second *Olfr* allele to express if the first one chosen fails to produce a functional OR protein (18–20). Our studies are consistent with the operation of similar mechanisms of receptor gene choice in OSNs that express TAARs and ORs. By examining *Taar* gene expression in mice containing mutated *Taar* alleles, we find that about 50–60% of OSNs expressing a mutated *Taar* allele also express another, functional *Taar* allele, as previously reported. Whether the remaining 40–50% of these OSNs instead express an *Olfr* gene is unknown. Previous studies indicate that OSNs expressing a mutant *Taar* allele can coexpress an *Olfr* gene, but a degenerate *Olfr* probe labeled only about 1% of the cells, suggesting that this may be a relatively rare event (21, 22).

Similar studies of *Olfr* genes could not assess whether OSNs that first choose a mutant *Olfr* allele subsequently choose another single *Olfr* allele or multiple *Olfr* alleles for expression. However, due to the biased secondary expression of *Taars* in our studies and the relatively small number of *Taar* versus *Olfr* genes, it was possible to address this question. By examining OSNs that express a mutant *Taar* allele for hybridization to different *Taar*

probes versus a mixed *Taar* probe, we found evidence that only one functional *Taar* allele is coexpressed with the mutant allele per OSN. These results are consistent with a model in which an OSN that selects a nonfunctional *Taar* allele for expression can go on to secondarily express only one additional *Taar* allele.

The above findings clearly indicate that TAAR-expressing OSNs comprise a distinct lineage. However, our results further indicate that there are also TAAR OSN subsets, which are biased to express different subgroups of *Taar* genes. Moreover, these subsets reflect the spatial domains within which individual TAARs are expressed in the OE. OSNs that select a mutated allele of *Taar5*, which is normally expressed in the dorsal domain, preferentially coexpress another *Taar* allele expressed in the same domain. Studies of mice with another mutant dorsal *Taar* (*Taar4*) gave similar results (21). In sharp contrast, we find that OSNs that select a mutated *Taar* gene (*Taar6*) expressed in both the dorsal and ventral domain can go on to secondarily select a *Taar* expressed in either domain. One possible explanation for these findings is that *Taar6* belongs to two different subsets, one dorsal and one ventral, with each subset restricted to select among *Taar* genes appropriate for a single domain. This scenario would suggest the existence of at least two *Taar* OSN subsets: one dorsal and one ventral.

The gene regulatory mechanisms that control the expression of individual *Taar* genes within specific OE spatial domains are unknown, as are those that control the expression of *Olfr* genes in different OE zones. Although there are dozens of *Olfr* gene loci in the genome, *Olfr* genes expressed in the same zone are found at different loci and those found at the same locus can be expressed in different OE zones (39). The clustering of *Taar* genes at a single chromosomal locus should facilitate future studies to explore *cis* regulatory elements and/or epigenetic processes that exert spatial restrictions on *Taar* gene expression that differ across the *Taar* gene locus.

***Taar* and *Olfr* Genes Are Sequestered in Different Nuclear Compartments.** How is the expression of *Taar* and *Olfr* genes segregated in different OSNs? *Taar* genes resemble *Olfr* genes in their singular monoallelic expression in individual neurons as well as the expression of different receptor genes in different OE spatial domains. These similarities suggest, at the very least, the existence of similar molecular mechanisms underlying *Olfr* and *Taar* gene choice and the maintenance of expression of a single receptor gene in the mature OSN. Although one can imagine how an OSN might randomly pick one receptor allele from the total 2,000 *Olfr* and 28 *Taar* alleles, it is more difficult to envision how an OSN that initially chooses a mutated *Taar* allele is subsequently restricted to secondarily choose one of the other 27 *Taar* alleles and not any of the 2,000 *Olfr* alleles.

Our studies reveal one mechanism that may contribute to the segregated expression of *Taar* versus *Olfr* genes in the OSN population: the sequestration of *Olfr* versus *Taar* genes in different compartments within the nucleus.

OSN nuclei show an unusual organization of heterochromatin. In most cell types, silenced genes are found primarily in heterochromatin compartments located at the nuclear periphery or at multiple foci in the nuclear interior. In OSNs, however, heterochromatin is largely concentrated in one or a few large central heterochromatin aggregates. It has been reported that most *Olfr* genes colocalize in or near these large chromocenters (24, 25). Ectopic OSN expression of the lamina binding protein LBR prevents the formation of the large chromocenters and *Olfr* gene clustering, and causes the misexpression of hundreds of genes, including *Olfr* genes.

We find that the nuclear organization of *Taar* genes is strikingly different from that of *Olfr* genes. Using DNA FISH to visualize the nuclear locations of *Taar* and *Olfr* gene loci, *Olfr* gene loci were often found at or near the large central aggregates of heterochromatin. In sharp contrast, *Taar* alleles localized primarily to the nuclear lamina. In 81.7% of OSNs, both *Taar* alleles were found at the periphery and, in the total OSN population, ~90% of *Taar* alleles were localized to the periphery.

Our studies indicate that OSNs lack not only LBR, but also lamin A, which is thought to be important for the maintenance of a peripheral heterochromatin compartment in the absence of LBR (35). However, we find that OSNs do express LAP2β, another lamina-associated protein that may tether heterochromatin, and *Taar* genes, to the nuclear periphery.

Although it was conceivable that *Taar* alleles might have a different location in *Taar*-expressing neurons, this possibility was excluded by examination of OSNs that coexpress a fluorescent reporter protein (mCherry) with a specific *Taar* gene. Like other OSNs, *Taar*-expressing OSNs had large central chromocenters, but *Taar* alleles were rarely located near the chromocenters.

***Taar* Gene Expression Is Marked by a Shift in Nuclear Localization.** Our studies indicate that the expression of a *Taar* gene is generally accompanied by a movement of the encoding *Taar* locus from the nuclear periphery to a more interior nuclear location. Using mice that coexpress a reporter (mCherry) with a specific *Taar* gene, it was possible to determine the locations of *Taar* genes in *Taar*-expressing OSNs. Strikingly, half of these OSNs showed one *Taar* allele located at the nuclear periphery and the other allele at a more interior location. This disparate localization was only observed in 16.3% of other OSNs, the majority of which showed both *Taar* alleles at the periphery.

Using hemizygous mice with only one functional *Taar* locus, it was further possible to examine the nuclear location of an active *Taar* allele in *Taar*-expressing OSNs. The single active allele had an interior location in 58.7% of *Taar*-expressing OSNs versus only 14.5% of other OSNs. Importantly, these experiments indicate that it is the active *Taar* allele that transitions from the nuclear lamina to a more interior nuclear location in *Taar*-expressing neurons.

These findings suggest that the activation of a *Taar* gene is accompanied, or perhaps preceded, by a shift of the gene away from a repressive heterochromatin environment. Activated *Olfr* genes also show a locational shift, but in this instance away from the large central heterochromatin aggregates (24). Movement of activated genes away from heterochromatin has also been observed in many other instances, often concomitant with differentiation. For example, as the murine β-globin locus becomes activated during erythroid differentiation, it moves away from the periphery (31), as does the *Mash1* locus during neural differentiation of mouse embryonic stem cells (33). In these cases, silenced genes relocate away from a repressive compartment as they are activated and gain access to a transcriptionally permissive environment.

An additional level of spatial regulation is the sequestration of crucial transcription factors in one nuclear compartment and the corresponding gene target in another. For example, during erythroid differentiation, the β-globin locus moves away from the periphery, where it associates with corepressors, into a euchromatic nuclear compartment that contains a transcriptional activator essential for its expression (40). During myogenesis, the relocation of *MyoD* away from the nuclear periphery is accompanied by a switch in core factors from TFIID to the alternate, and exclusively nucleoplasmic, TAF3 (41). It is conceivable that such mechanisms regulate the expression of numerous genes, including *Taar* and *Olfr* genes. By escaping a repressive heterochromatin environment, individual *Taar* or *Olfr* genes may gain access to key transcription factors crucial to their activation.

Materials and Methods

Mice. All procedures using animals were approved by the Fred Hutchinson Cancer Research Center Institutional Animal Care and Use Committee. Adult male and female C57BL/6J mice were obtained from The Jackson Laboratory.

Taar5 and *Taar6* KO mice (Taar5$^{tm1(KOMP)Vlcg}$ and Taar6$^{tm1(KOMP)Vlcg}$ mice) were generated by the trans-NIH Knockout Mouse Project and obtained from the KOMP Repository (www.komp.org). In these mice, the *Taar* coding region is replaced by a lacZ sequence followed by a loxP-flanked selection cassette containing a neomycin resistance gene (neo). To remove the selection cassette, we crossed the mice with EIIa-Cre mice, which carry a Cre recombinase transgene under the control of the adenovirus EIIa promoter [*EIIa-cre* strain (B6.FVB-Tg(EIIa-cre)C5379Lmgd/J) (The Jackson Laboratory (JAX 003724)]. The mice were then backcrossed to C57BL/6J mice, intercrossed, and genotyped to verify the removal of the neo sequence, the presence of the lacZ sequence, and absence of the *Taar5* or *Taar6* coding sequence. *Taar4*-IRES-mCherry and ΔT2-9 mice were kindly provided by Thomas Bozza (21).

RNA In Situ Hybridization with Immunohistochemistry. Fluorescence RNA in situ hybridization was performed as described (13) with minor modifications listed in *SI Materials and Methods*. For β-gal immunohistochemistry, antibodies used were chicken anti–β-gal (Abcam, ab9361) followed by Alexa-488–conjugated goat anti-chicken Ig (Life Technologies).

Radioactive RNA in Situ Hybridization. Radioactive in situ hybridization was performed as described with slight modifications (42). See *SI Materials and Methods* for details.

Whole-Mount X-Gal Staining. Wholemount staining of β-gal+ OSN axons in the olfactory bulb was performed as described (43).

Fluorescence DNA in Situ Hybridization (FISH) and Immuno-FISH. 3D Immuno-FISH was largely performed as published (24, 31, 44) with some modifications. See *SI Materials and Methods* for details.

Image Acquisition. For FISH images, a Zeiss LSM780 confocal microscope running Zeiss Zen 2011 SP2 v.8 software and equipped with a Zeiss Plan-Apochromat 63x/1.40 oil immersion objective was used to collect image stacks (up to 7–10 μm) at 0.25-μm intervals. Image stacks were viewed and scored using either the Zeiss software or ImageJ. FISH spots were deemed to colocalize with the lamina (or chromocenters) if there was pixel overlap between the two channels after appropriate thresholding.

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