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Monogenic and monoallelic expression of odorant receptors

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Abbreviations:

Adcy3 (adenylyl cyclase 3)

nATF5 (nuclear activating transcription factor 5 protein isoform)

LBR (lamin B receptor)

ChIP (chromatin immunoprecipitation)

ER (endoplasmic reticulum)

FISH (fluorescent *in situ* hybridization)

LSD1 (lysine-specific demethylase 1)

ORs (odorant receptors)

PSR (post-selection refinement)

RTP (receptor transporting protein)

uORF (upstream Open Reading Frame)

UPR (unfolded protein response)

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Abstract

Odorant receptors (ORs) belong to a large gene family of rhodopsin-like G protein-coupled receptors (GPCRs). The mouse OR gene family is composed of ~1000 OR genes, and the human OR gene family is composed of ~400 OR genes. The OR genes are spread throughout the genome, and can be found in clusters or as solitary genes in almost all chromosomes. These chemosensory GPCRs are expressed in highly specialized cells, the olfactory sensory neurons of the nose. Each one of these neurons expresses a single OR gene out of the complete repertoire of genes. In addition, only one of the two homologous alleles of the chosen OR gene, the maternal or the paternal, is expressed per neuron. Here we review recent findings that help to elucidate the mechanisms underlying monogenic and monoallelic expression of OR genes.

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Introduction

Odorant receptors (ORs) are chemosensory GPCRs that are involved in the detection of a large number of odorants with varied chemical structures (Buck and Axel, 1991). There are around ~1000 mouse OR genes and ~400 human OR genes spread throughout the genome (Godfrey et al., 2004; Malnic et al., 2004; Zhang and Firestein, 2002; Zozulya et al., 2001). Expression of ORs is typically restricted to one major tissue, the olfactory epithelium, although expression in non-olfactory tissues has been increasingly reported (Feldmesser et al., 2006; Flegel et al., 2013). In the olfactory epithelium, the expression of each OR gene is restricted to one out of a series of spatial zones (Miyamichi et al., 2005; Ressler et al., 1993; Vassar et al., 1993). The OR genes are expressed in the olfactory sensory neurons, the major cell type in the olfactory epithelium. Each olfactory sensory neuron expresses a single OR gene in a monoallelical fashion (Chess et al., 1994; Malnic et al., 1999; Serizawa et al., 2000). The OR expressed in a given olfactory neuron is not only responsible for detecting odorants that enter the nasal cavity, but it is also involved in axonal guidance to the brain (Barnea et al., 2004; Feinstein et al., 2004; Wang et al., 1998). Axons of neurons expressing the same OR gene type converge in the same sites in the olfactory bulb of the brain where they form synapses with mitral cells in structures denominated glomeruli (Ressler et al., 1994; Vassar et al., 1994). In this way, a topographical odorant map is formed in the olfactory bulb where each one of the 1000 OR genes is represented. Monogenic and monoallelic expression of the OR genes is therefore required for the formation of this spatial map and odorant discrimination by the olfactory system. In this

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minireview, we focus on the mechanisms underlying regulation of OR gene expression in mammalian olfactory neurons.

Monoallelic expression of OR genes

OR genes show random monoallelic expression; only one of the two homologous alleles of a given OR gene type, the maternal or paternal, is expressed (Chess et al., 1994; Ishii et al., 2001; Serizawa et al., 2000). Considering that there are multiple possible variants of any OR gene type in the population (Mainland et al., 2014), monoallelic expression of OR genes assures singular identity to individual neurons since the expression of two variants of one OR gene in the same neuron could disturb neuronal connectivity to the brain. Notably, transcriptome analysis of single cells obtained from mice of mixed genetic background (C57BL6 X 129P2), where SNPs allow for the discrimination of the two homologous alleles, showed that only one of the alleles is expressed in each analyzed cell (Saraiva et al., 2015). This indicates that control of monoallelic expression of OR genes in olfactory neurons is under tight regulation.

Selection of one of the homologous OR gene alleles for expression is stochastic, and as a result, half of the olfactory neurons will express the maternal allele and half of the neurons will express the paternal allele. While biallelically expressed genes have their two alleles replicated at the same time during the cell cycle, genes that are monoallelically expressed show asynchronous replication, with one of the alleles being replicated earlier during

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S phase than the homologous allele (Singh et al., 2003). Consistent with this, asynchronous replication of OR genes has been observed in different cell types, such as embryonic fibroblasts, embryonic stem cells, and lymphocytes (Alexander et al., 2007; Chess et al., 1994; Singh et al., 2003).

Studies have shown that the homologous alleles of random autosomal monoallelically expressed genes may show differential epigenetic marks, however, whether the homologous alleles of OR genes are differentially marked is still unclear. Curiously, the Polycomb Group gene Eed, which is required for H3 histone methylation on lysine 27 (leading to the formation of H3K27me3), is involved in OR gene asynchronous replication in embryonic stem cells (Alexander et al., 2007). Eed and other Polycomb group subunits are developmentally regulated in the olfactory epithelium and predominantly expressed in neuronal progenitors (Tietjen et al., 2003). Interestingly, while there is no evidence thus far from chromatin immunoprecipitation (ChIP) experiments that OR genes are marked with H3K27me3 (Magklara et al., 2011), it has recently been shown that this mark is present in genomic regions flanking OR gene enhancers (Markenscoff-Papadimitriou et al., 2014). Whether the enhancers of the homologous OR alleles are asymmetrically labeled with H3K27me3 or other marks remains unknown.

Differential marking of the two homologous alleles, with one of the alleles being marked with permanent repressive heterochromatin marks, and the other one free of these marks and available for transcription, could explain monoallelic

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expression of the OR genes (Figure 1A). Analysis of the position of individual OR genes in the nucleus of olfactory neurons has shown that the two homologous alleles of a given OR gene are frequently segregated to different compartments. While one of the alleles is closely associated with constitutive (stably inactive) heterochromatin blocks (marked with H3K9me3 and H4K20me3), the other one is located further away from these highly repressive blocks, and closer to facultative (reversible) heterochromatin blocks (marked with H3K27me3) or in euchromatin (marked with H3K4me3) (Armelin-Correa et al., 2014a; Armelin-Correa et al., 2014b). Altogether, these results are consistent with a differential epigenetic marking of the OR homologous alleles (Figure 1A).

However, the finding that both homologous alleles of one given OR gene can be sequentially activated in the same olfactory neuron (denominated as 'OR gene switching') indicates that both alleles are transcriptionally competent, and argues against the presence of permanent repressive marks in the excluded allele (Shykind et al., 2004). ChIP experiments have shown that in the olfactory epithelium, but not in liver cells, OR genes are covered with the H3K9me3 and H4K20me3 constitutive histone marks, and therefore are widely repressed, while active OR gene alleles are instead labeled with the H3K4me3 euchromatin histone mark (Magklara et al., 2011). In this context, a model is proposed where all OR genes, including the homologous alleles of each OR gene type, are repressed in constitutive heterochromatin (Figure 1B). During neuronal differentiation, a single OR gene allele is released from this inhibition through demethylation of H3K9me3, leading to the transcription of a single OR

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gene allele per neuron (Lyons et al., 2013). In this case, since only one allele can be activated at a time in the olfactory neuron, monoallelic expression would be achieved. This is probably due to the existence of limiting factors, such as LSD1, an enzyme that catalyzes demethylation of H3K9, required for OR gene activation (Lyons et al., 2013). The involvement of H3K9 methylation in OR gene regulation has been further analyzed using gene targeted mice. Genetic deletion of LSD1 in olfactory neurons results in a complete loss of OR expression, consistent with the requirement of LSD1 for OR gene activation (Lyons et al., 2013). Curiously, genetic deletion of the H3K9 methyl transferases G9a and GLP, which are required for the formation of H3K9me3, results in the expression of only a few OR genes, but not the full repertoire of OR genes as would be expected (Lyons et al., 2014). It is important to note that these same enzymes are also involved in the regulation of additional histone marks, including H3K27 methylation (Lyons and Lomvardas, 2014; Mozzetta et al., 2014), which could cause further modifications in the knock out mice in addition to decreased H3K9 methylation.

Recent experiments are now suggesting the existence of a functional asymmetry in the homologous OR gene allele, consistent with the model shown in Figure 1A. In these experiments, mice that have a tetracycline-dependent trans activator responsive promoter (tet_o) inserted at the transcriptional start site of the endogenous P2 OR gene were generated by gene targeting so that this modified OR gene can be subjected to conditional activation by tTa, a tetracycline controlled transcription factor that binds to the tet_o and activates transcription. Olfactory neurons from mice that are homozygous for the tet-P2

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modified gene would therefore be expected to show biallelic expression of the P2 OR gene. However, the vast majority of neurons transcribed only one of the two tet-P2 alleles, with biallelic expression observed in only ~3% of the neurons (Fleischmann et al., 2013). Expression from both alleles was more frequently observed in younger olfactory neurons, consistent with previous findings that OR switching occurs in immature olfactory neurons (Shykind et al., 2004) and with the observation that OR gene transcription is more permissive in younger neurons than in mature neurons (Fleischmann et al., 2013). These results indicate that one of the homologous alleles is more likely to be activated than the other is, and suggests that differential marking of alleles, yet to be determined, may exist.

Initiation of OR gene choice

Out of 1000 (Figure 1A) or 2000 (Figure 1B) available OR alleles, each olfactory neuron selects one type of OR gene to express, but the mechanisms involved in the initiation of OR gene choice are not completely understood.

The role played by *cis*-regulatory elements in OR monogenic expression has been largely exploited. Attempts to find specific DNA motifs in OR gene promoters have shown that they share common O/E-like and homeodomain-like binding sites (Clowney et al., 2011; Hoppe et al., 2006; Michaloski et al., 2006; Michaloski et al., 2011). Even though these binding sites are required for normal OR gene expression, and indicate that transcription factors such as Lhx2, Emx2 and Olf-1/EBF are involved in OR gene transcription (Hirota et al., 2007; McIntyre et al., 2008; Rothman et al., 2005), they do not explain the monogenic

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expression of OR genes. It seems however that different combinations of O/E and homeodomain binding sites may increase the probability of an OR gene to be chosen (Vassalli et al., 2011). There are OR enhancers that operate in *cis* and also contain homeodomain-binding sites (Bozza et al., 2009; Fuss et al., 2007; Khan et al., 2011; Nishizumi et al., 2007; Serizawa et al., 2000). Proximity with an OR enhancer leads to increased probability of expression of an OR gene (Khan et al., 2011; Serizawa et al., 2003). For example, the H element, the first OR gene enhancer to be identified, is located 70Kb away from the olfr1507 gene (also known as MOR28), one of the most abundantly expressed ORs in the olfactory epithelium (Serizawa et al., 2000; Serizawa et al., 2003).

The constitutive heterochromatin H3K9me3 and H4K20me3 marks are deposited in OR genes early in the differentiation, before the onset of OR expression (Magklara et al., 2011). ChIP-qPCR experiments from sorted olfactory neurons that express one same given OR gene demonstrated that while the inactive allele is marked with H3K9me3, the active allele is marked with H3K4me3 (Magklara et al., 2011). In a proposed model for the initiation of OR gene choice, specific histone demethylases, including LSD1, and other limiting factors, would de-repress one single OR gene allele, leading to singular OR gene expression (Lyons et al., 2013) (Figure 1B). Accordingly, as mentioned above, genetic disruption of the LSD1 gene before OR gene choice, but not after OR gene choice, leads to widespread loss of OR gene expression and failure of the olfactory neurons to mature (Lyons et al., 2013). Downregulation of LSD1 after OR gene activation is required in order to avoid activation of a second OR gene. Since LSD1 is also involved in the

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demethylation of H3K4me3, downregulation of LSD1 is required to stabilize expression of the activated OR gene (Lyons et al., 2013). A negative feedback mechanism is responsible for the downregulation of LSD1 (discussed below, Figure 2A), and since LSD1 is able to catalyze demethylation of the intermediates H3K9me2 and of H3K4me2, but not of H3K9me3 and H3K4me3, additional demethylases must be also required for OR gene regulation.

Recent single-cell transcriptomics experiments have shown that while mature olfactory sensory neurons express one single OR gene at high levels, immature neurons express low levels of multiple OR genes (Hanchate et al., 2015; Saraiva et al., 2015; Tan et al., 2015). These results indicate that initially a group of OR genes, not a single OR gene, is activated and expressed at low levels, and that singular OR gene expression at high levels is achieved during the differentiation of the olfactory neuron (Figure 1A). Interestingly, the group of OR genes that are initially activated in the same olfactory neuron are localized in different chromosomes and loci, but are expressed in the same or overlapping OR expression zones in the olfactory epithelium (Hanchate et al., 2015). These results suggest that not all OR genes are available for transcription in each olfactory neuron, but only the subset of OR genes expressed in a particular zone. This may help explain the characteristic zonal expression of OR genes.

Studies of nuclear architecture have shown that olfactory sensory neurons have a characteristic nuclear organization that differs from that of other cell types. In the nuclei of eukaryotic cells, heterochromatin is usually localized at the nuclear

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periphery, whereas euchromatin is found in the nuclear interior. Heterochromatin is tethered to the nuclear periphery through interaction with the lamin B receptor (LBR), a nuclear envelope protein that interacts with heterochromatin protein 1 (HP1) and binds to constitutive heterochromatin. Olfactory nuclei show a so called inside-out nuclear architecture: large blocks of heterochromatin are concentrated more centrally in the nucleus, while euchromatin is located more peripherally (Armelin-Correa et al., 2014a; Armelin-Correa et al., 2014b; Clowney et al., 2012; Solovei et al., 2009). LBR is normally absent in olfactory neurons, leading to this inside-out organization of the nucleus (Clowney et al., 2012). In addition, facultative heterochromatin (characterized by the H3K27me3 histone mark), is concentrated in a few domains localized close to the central heterochromatin blocks in the olfactory nucleus (Armelin-Correa et al., 2014a). These facultative heterochromatin blocks seem to be specific to olfactory neurons since they are not found in the other cell types in the olfactory epithelium (Armelin-Correa et al., 2014a; Armelin-Correa et al., 2014b). DNA Fluorescent *In Situ* Hybridization (DNA-FISH) experiments showed that even though the OR genes are spread throughout the genome, in the three-dimensional organization of the olfactory nucleus they are clustered together, and commonly associated with the centrally located repressive heterochromatin compartments (Armelin-Correa et al., 2014a; Clowney et al., 2012).

Aggregation of OR loci in the nucleus could facilitate coordinated regulation of OR genes by locally acting factors, such as LSD1, regulatory RNAs or chromatin remodelers. Accordingly, disruption of the olfactory neuron nuclear

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organization through the ectopic expression of LBR leads to multiple OR gene expression, even though these genes still have repressive H3K9me3 histone marks (Clowney et al., 2012). Hence, these results indicate that the presence of these repressive marks is not sufficient to avoid OR gene transcription, and that the nuclear organization of olfactory neurons is crucial for monogenic OR gene expression.

Recently, ~35 new OR gene specific enhancers have been described (Markenscoff-Papadimitriou et al., 2014). Twelve of these enhancers were validated and shown to be functional by using reporter assays in zebrafish. Circularized Chromosome Conformation Capture sequencing (4C-seq) experiments were performed to identify chromatin regions in close proximity with these enhancers in olfactory nuclei. In these essays, chromatin from olfactory sensory neurons is cross-linked with formaldehyde to link DNA regions in close proximity in the 3D organization of the nucleus. Chromatin is then fragmented using restriction enzymes, and the ends of cross-linked DNA fragments are ligated to form hybrid molecules. The crosslink is then reversed, and using inverse PCR primers adjacent to the restriction sites, a library containing the DNA interactions in olfactory nuclei is constructed and sequenced. The 4 C-seq experiments, together with immuno-DNA-FISH, showed that these OR enhancers interact in *trans* with each other and with an active OR gene (Markenscoff-Papadimitriou et al., 2014). Therefore, it seems that the OR enhancers not only act as *cis* regulatory elements, as demonstrated for the H and P enhancers (Fuss et al., 2007; Nishizumi et al., 2007), but also interact in *trans* with other OR enhancers to generate a transcriptionally

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competent complex that allows for robust OR gene expression. The convergence of multiple enhancers would lead to an increase in the local concentration of binding sites for specific transcription factors near an OR promoter and consequently to higher transcription rates for a singular OR gene. Co-localization of enhancers occurs in euchromatin and outside the repressive OR gene aggregates in the nucleus (Markenscoff-Papadimitriou et al., 2014), consistent with the positions of active OR genes (Armelin-Correa et al., 2014a; Clowney et al., 2012).

The specific OR gene enhancers are enriched in the H3K4me1 and H3K27ac common enhancer histone marks. Interestingly, they are distinguished by the unique distribution of H3K79me3 and H3K27me3 repressive marks, which are missing from the actual enhancer sequence, but are enriched in their flanking sequences (Heinz et al., 2015; Markenscoff-Papadimitriou et al., 2014). The enrichment of H3K27me3 histone marks flanking OR specific enhancers and the aggregation of these regulatory sequences could account for the prominent facultative heterochromatin blocks observed in the olfactory nuclei (Armelin-Correa et al., 2014a). These types of repressive epigenetic modifications could somehow be involved in the regulation of the enhancer network required for OR gene activation. Whether these marks are deposited before or after OR gene choice still needs to be determined.

The existence of a limiting number of OR enhancers acting together to induce the activation of an OR gene would preclude simultaneous activation of another OR gene and explain monogenic OR gene expression. It is also possible that in

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each olfactory neuron the specialized transcriptional complex is composed of different combinations of OR genes. For example, only the OR genes that are expressed in the same zone would have access to the transcriptional complex, while those expressed in other zones would be repressed in heterochromatin. In this way, the available OR gene choices would be restricted to a smaller number (McClintock, 2010; Rodriguez-Gil et al.). One intriguing possibility is that the high transcription rate of a given OR allele in a privileged position in the transcriptional complex would lead to the spreading of repressive marks on the neighboring OR alleles, and thereby repress their transcription.

Stabilization of OR gene choice

It is well known that stabilization of the expression of one chosen OR gene requires a feedback signal that is initiated once an intact OR gene is expressed (Lewcock and Reed, 2004; Nguyen et al., 2007; Serizawa et al., 2003; Shykind et al., 2004). Mutant ORs containing a complete coding region that are unable to transduce signal through the G protein are still able to suppress other OR genes, indicating that OR function is not required (Imai et al., 2006; Nguyen et al., 2007). Expression of pseudogenes and mutant OR genes is unstable, probably because they cannot elicit the feedback signal, and consequently the olfactory neuron may “switch” expressed genes until a functional receptor is chosen (Shykind et al., 2004). A model for OR-elicited negative feedback came forth from evidence that translation of the chosen OR gene at high levels in the endoplasmic reticulum (ER) triggers the unfolded protein response (UPR) (Dalton et al., 2013) (Figure 2A). Once UPR is activated, global cell translation is restrained via the phosphorylation of the initiation factor eIF2α by the ER-

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resident kinase PERK. eIF2α phosphorylation specifically increases the translation of mRNAs harboring inhibitory upstream open reading frames (uORFs) within their 5′ untranslated region (UTR) (Hetz et al., 2011). Under normal conditions, only the uORF is translated, but upon ER stress, the uORFs are bypassed and the downstream ORFs are selectively translated. One of these transcripts encodes the nuclear isoform of the Activating Transcription Factor 5 (nATF5), which is then selectively translated, inducing the expression of adenylyl cyclase 3 (Adcy3). Adcy3 expression is followed by ER stress alleviation and LSD1 downregulation, which allows for terminal differentiation of the neuron, and as mentioned above, stabilization of OR choice (Dalton et al., 2013; Lyons et al., 2013). Whether Adcy3 plays a direct role in the suppression of LSD1 remains unclear. Notably, high levels of OR expression may be necessary for activation of UPR (Dalton et al., 2013) (Figure 2B, path 1), and it is likely that only ORs expressed above a certain threshold can elicit a negative feedback.

Prolonged ER-stress may induce apoptotic death of olfactory neurons. Analysis of ATF5 knockout mice showed that ATF5 is required for terminal differentiation and survival of olfactory neurons (Wang et al., 2012), indicating that neurons that do not stably express an OR gene and cannot terminate UPR undergo apoptosis. Interestingly, RNAseq analysis indicates that nATF5 also activates the expression of the OR-specific chaperones RTP1 and RTP2 (Dalton et al., 2013; Saito et al., 2004; Wang et al., 2012). Therefore, analyzing the effects of RTP1 and RTP2 knockout in the olfactory epithelium would be an additional alternative to address the termination of OR-elicited UPR. If OR trafficking to the

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membrane contributes to ER-stress relief, then RTP1/RTP2 knockouts are expected to show a phenotype similar to the Adcy3 knockout: sustained ATF5 and LSD1 expression and possibly increased neuronal apoptosis (Dalton et al., 2013; Watt et al., 2004).

Interestingly, the majority of OR mRNAs expressed in the olfactory epithelium contains uORFs in their 5' UTR (Michaloski et al., 2006; Shum et al., 2015). In addition, the average expression of OR mRNAs harboring uORFs is significantly higher than those without uORFs (Shum et al., 2015). A positive correlation was also observed between average uORF length and OR mRNA level, as well as between the numbers of uORFs per transcript and OR mRNA level (Shum et al., 2015). Importantly, there was no correlation between any of these parameters and the expression of genes belonging to control and other GPCR groups, suggesting that uORFs positively affect specifically the expression of OR mRNAs. The post-transcriptional regulation of OR mRNAs is largely unknown, and these results may point to a possible mechanism by which expression of the chosen OR is favored in the context of the UPR.

However, a mechanism different from the one involving downregulation of LSD1 (Figure 2A and 2B, path 1) must be responsible for the stabilization of OR gene choice in olfactory neurons that initially express multiple OR genes (Figure 2B, path 2). In this case, all but one OR gene allele will have to be repressed to ensure monogenic OR gene expression. Whether UPR related mechanisms, or

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different types of epigenetic modifications are involved, remains to be determined.

Recently, a post-selection refinement (PSR) mechanism to restore singular OR expression in neurons that co-express two OR genes was described (Abdus-Saboor et al., 2016) (Figure 2B, path 3). In these experiments, forced ectopic expression of the M71 OR gene in mature olfactory neurons suppressed the expression of the previously chosen endogenous OR gene. The efficiency by which the ectopically expressed M71 OR is able to suppress the previously chosen allele is dependent on the expression levels of the ORs; endogenous ORs that are transcribed at higher levels are less efficiently suppressed than endogenous ORs that are expressed at lower levels. These results suggest that a competitive relationship between OR alleles is the basis of the PSR mechanism (Abdus-Saboor et al., 2016). In a physiological context, PSR may occur when the UPR-mediated feedback fails in producing single OR expression. Moreover, PSR or related mechanisms may be involved in the selection and stabilization of the expression of one OR gene in neurons that co-express multiple receptors (Hanchate et al., 2015; Saraiva et al., 2015; Tan et al., 2015) (Figure 2B, path 2). *In situ* hybridization experiments showed that a small number of olfactory neurons in the Septal Organ of newborn mice also express multiple ORs (Tian and Ma, 2008). In this case, the neurons are eliminated through a mechanism that requires neuronal activity induced by sensory input and apoptosis. Alternatively, some of these neurons could escape cell death and achieve singular OR gene expression through PSR.

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The mechanisms through which PSR works to suppress transcription of all but one OR genes are not understood. While UPR mediated stabilization of OR gene choice occurs before the onset of olfactory marker protein (OMP) expression (a marker for mature olfactory neuron), PSR occurs after OMP expression, when LSD1 is no longer expressed. These results indicate that in this case, different mechanisms that do not require down regulation of LSD1, and may or may not require UPR, must exist and must still be uncovered.

Conclusions

Odorant receptors belong to a large rhodopsin-like chemosensory GPCR family and are involved in the detection of a multitude of odorants with diverse chemical structures. While these receptors share several common features with other types of GPCRs, these proteins show characteristic mechanisms of gene regulation that result in the expression of one receptor type at very high levels per sensory neuron. To assure monogenic and monoallelic expression of OR genes, several layers of regulation operate before, during, and after OR choice. This strict regulation is necessary for the formation of a topographical map that is required for odorant discrimination and therefore is essential for the proper functional organization of the olfactory system.

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Wrote or contributed to the writing of the manuscript: Nagai, Armelin-Correa and Malnic.

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Figure legends

Figure 1. Models for monoallelic and monogenic OR gene expression. OR gene expression is regulated during cell differentiation so that individual mature olfactory neurons express only one of the two homologous alleles from a single OR gene type. (A) In one scenario, the homologous OR gene alleles have asymmetric epigenetic marks so that one of them is repressed in constitutive heterochromatin (black genes) and the other one has different epigenetic marks that make it available for selection (white genes). Initially, multiple OR genes are activated at low transcriptional levels (immature olfactory neuron), and as the olfactory neuron differentiates into a mature olfactory neuron, a single OR gene type is expressed at high levels and the remaining OR genes are silenced. (B) In a different scenario, homologous OR gene alleles do not have asymmetrical marks, and are equally available for selection. All of the OR gene alleles are initially repressed in constitutive heterochromatin marks (black genes) and one single OR gene allele is released from repression and stably expressed in the mature olfactory neuron.

Figure 2. Stabilization of OR gene choice. Once the OR genes are activated, a feedback mechanism is elicited that results in the suppression of additional OR genes while maintaining stable expression of the chosen OR gene. (A) A feedback mechanism involving UPR. One single OR gene is de-methylated by LSD1 and activated. Translation of the OR transcript at high levels in the ER triggers UPR through the activation of Perk. As a result, selective translation of

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nATF5 occurs, which induces the expression of adenylyl cyclase 3 (Adcy3). Adcy3 expression leads to ER stress alleviation and LSD1 downregulation, which allows for terminal differentiation of the neuron and stabilization of OR choice. Once the chosen OR allele switches from H3K9 to H3K4 methylation, shutdown of LSD1 is necessary to prevent subsequent repression via its H3K4 demethylation activity. (B) Stabilization of OR gene expression could occur in the following ways: (1) one single OR gene is activated leading to UPR and stabilization of the OR expression, (2) in olfactory neurons that express multiple OR genes, unknown mechanisms lead to stabilization of the expression of a single OR gene in the mature olfactory neuron; or, (3) when a mature olfactory neuron is forced to express a second OR gene at a high transcription rate, a post-selection refinement (PSR) mechanism results in the shutdown of the endogenous OR, ensuring singular OR expression.

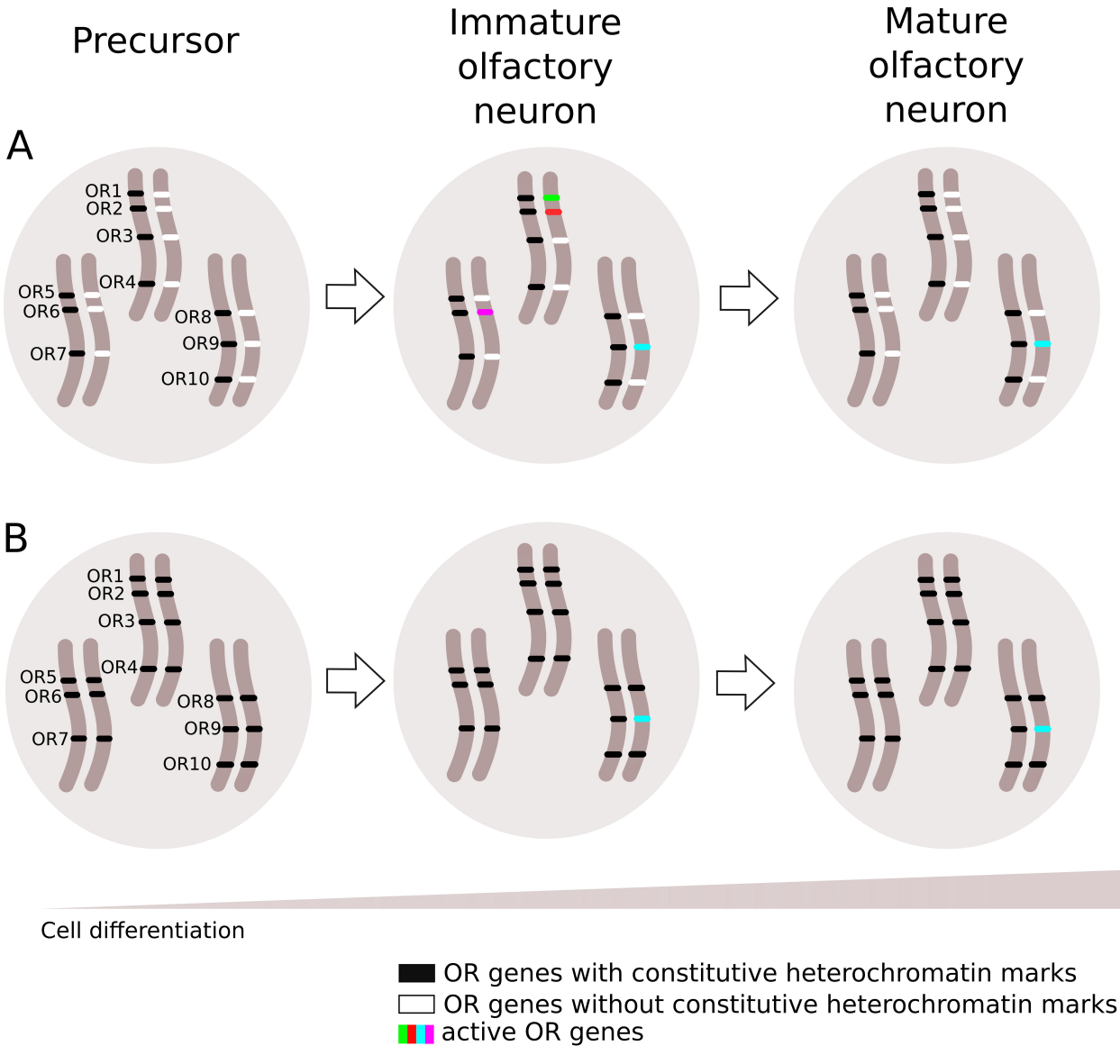
Precursor
Immature olfactory neuron
Mature olfactory neuron
A
OR1
OR2
OR3
OR4
OR5
OR6
OR7
OR8
OR9
OR10
B
OR1
OR2
OR3
OR4
OR5
OR6
OR7
OR8
OR9
OR10
Cell differentiation
OR genes with constitutive heterochromatin marks
OR genes without constitutive heterochromatin marks
active OR genes

Figure 1

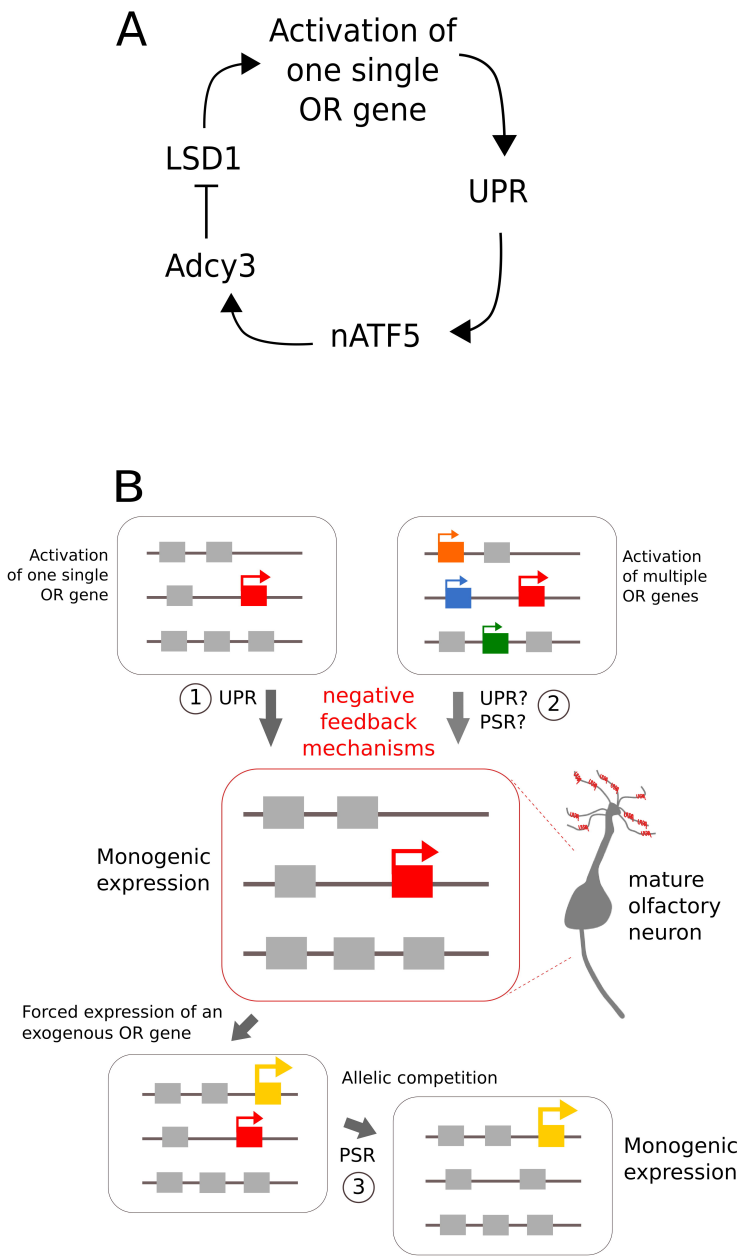
A
Activation of one single OR gene
UPR
nATF5
Adcy3
LSD1
B
Activation of one single OR gene
Activation of multiple OR genes
1 UPR
negative feedback mechanisms
UPR? PSR? 2
Monogenic expression
mature olfactory neuron
Forced expression of an exogenous OR gene
Allelic competition
PSR 3
Monogenic expression

Figure 2