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INSIDE

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on and off?

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Sweet News

Odorant Receptor Gene Choice



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CONTENTS

- 2** Editorial
- 3** Odorant Receptor
Gene Choice by
Tim McClintock
- 14** News
- 17** Forth-coming events

Our Cover and Page 10: In situ hybridization in a coronal section of the mouse nasal cavity labels a subset of olfactory sensory neurons expressing odorant receptor Olfr642 (red) in the dorsal zone of the olfactory epithelium. *Image courtesy of W.B. Titlow.*

Image acknowledgement: *Pseudo colouring by Ms Sabine Schmidt, Institute of Neurobiology, University of Ulm, Germany.*

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Graham Bell and Elke Weiler, Co-Editors of ChemoSense

EDITORIAL

Discovery of odorant receptors

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Discovery of odorant receptors (Buck and Axel 1991) unlocked the genetic basis for the sense of smell and opened a new field of exploration to find the molecular mechanisms of olfaction, including the interaction of the odorant with the receptors, and the mechanism for specific expression of a receptor within an olfactory sensory neuron.

Each olfactory sensory neuron expresses only one type of odorant receptor from a repertoire of about 1000 genes. What decides which type of olfactory receptor is expressed? As the sensory neuron develops from progenitor cell to mature olfactory sensory cell, a phase of sequentially expressed odorant receptor genes might occur, a process of "gene switching", before the stable expression of one receptor type takes place in the mature sensory neuron, the process of "gene choice".

Tim McClintock of University of Kentucky, Lexington, describes the research that has hammered out how genes are repressed and de-repressed to ensure the expression of one odorant receptor gene only per sensory cell. Methylation and demethylation of histons, activation of promoters, interaction of enhancers of the same (cis) and other (trans) chromosomes and feedback mechanisms of transcription factors regulate the specific expression.

Once again the science of olfaction is bearing fruit for science as a whole, revealing fundamental mechanisms that can lead to wider understanding of how genes turn off and on within their functional environments, and are selected to change the identity and performance of living cells. Think cell targeting, neural regeneration, think oncogenes...

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Reference: Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65, 175-187

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Odorant Receptor Gene Choice



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When Linda Buck and Richard Axel published the evidence that mammalian odorant receptors (ORs) are a large multigene family of G-protein coupled receptors (Buck and Axel, 1991), which won them the Nobel Prize in Physiology or Medicine in 2004, it caused a flurry of activity directed toward answering two obviously critical questions. One question was, "Which odorants activate which receptors, thereby generating distinct patterns of activity that form the basis for odor discrimination?"

The first step in answering this question was expected to be relatively easy, albeit laborious given the many hundreds of ORs involved; simply express each OR in any of several cultured eukaryotic cell lines, then apply odorant ligands and measure activation of downstream signaling. This heterologous expression strategy has been successful for many G-protein coupled receptors but proved surprisingly difficult in the case of ORs. We eventually learned that in cell types other than mature olfactory sensory neurons (OSNs), ORs have difficulty trafficking to the plasma membrane where they can bind odorants because they tend to be retained in the endoplasmic reticulum (ER) (McClintock

and Sammeta, 2003). Although several creative solutions to this problem have been invented, reviewed by Peterlin and colleagues (Peterlin et al., 2014), progress has been very slow and we are only just beginning to identify the set of receptors that respond *in vivo* to any odorant (McClintock et al., 2014).

The other critical question was, "How does an OSN choose which OR gene to express?" When Richard Axel was asked about OR gene expression control mechanisms at the 2001 Banbury Conference on Olfactory Receptors, he replied, "I don't even know how to think about that problem yet." This answer was not flippant, but rather an accurate reflection of how little was known at that time about the epigenetic control of gene expression in general. For more than a decade, progress in understanding the control of OR gene expression was largely descriptive, the discovery of phenomena associated with OR expression.

Early evidence demonstrated that each OR gene is expressed in regions, often called zones, of the olfactory epithelium and other evidence soon accumulated that each OSN expresses just one allele of one OR gene (Mombaerts, 2004), so the

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Odorant Receptor Gene Choice

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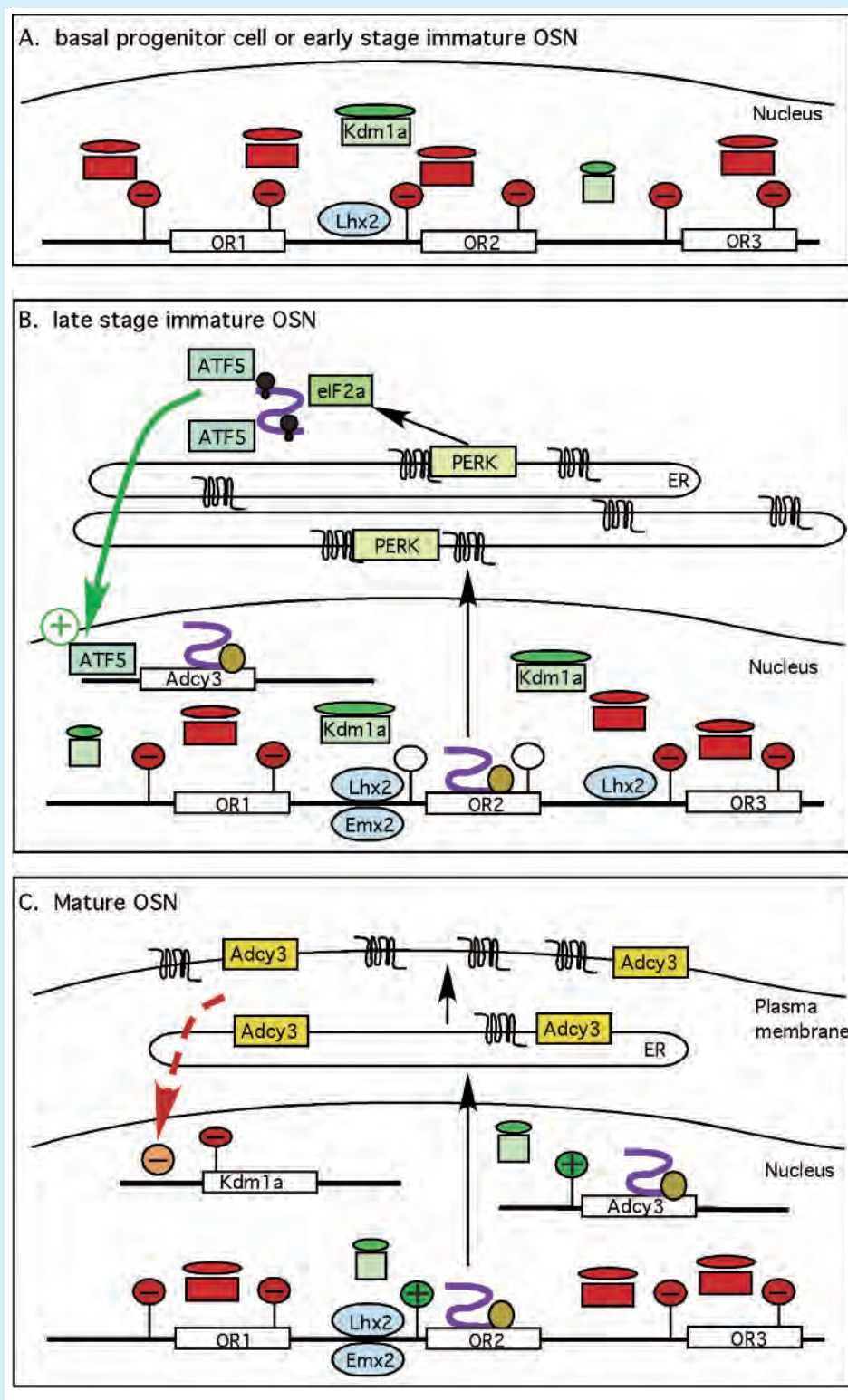


Fig 1. Speculations on the control of OR gene expression by chromatin modification and feedback signaling. **A.** In early stage immature OSNs and late stage basal progenitor cells of the OSN cell lineage OR genes are dominated by repressive chromatin modifications (negative signs in red circles). This predicts that negative chromatin modifying complexes (red bipartite structures) initially overwhelm the actions of positive chromatin modifying complexes (green bipartite structures) in the OSN cell lineage. **B.** If an OR allele is to be expressed, then by late stage immature OSNs at least one OR allele must be relieved from repression. This would occur if the relative strengths of positive and negative chromatin modifying complexes reach a balance whereby at some low rate the histone lysine demethylase Kdm1a (Lsd1) can completely demethylate H3K9 at the nucleosomes of an OR allele, leading to removal of all the repressive marks at this OR allele and allowing transcription factors to initiate transcription (depicted here by Lhx2 and Emx2 acting on OR2). The OR protein that is subsequently made accumulates in the ER, triggering activation of the PERK arm of the ER stress response. Activated PERK phosphorylates eIF2a, triggering translation of the nuclear form of ATF5, a transcription factor. Nuclear ATF5 stimulates expression of Adcy3, an event associated with the transition of the immature OSN into a mature OSN. **C.** The expression of Adcy3 in the newly differentiated mature OSN, via a mechanism as yet not understood, feeds back to the nucleus and causes suppression of Kdm1a (dashed arrow). Without Kdm1a to help clear repressive marks, other OR genes cannot be expressed, locking in expression of a single OR allele. The OR2 allele is now being transcribed strongly and is marked by the active chromatin modification, H3K4me3 (positive signs in green circles).

Odorant Receptor Gene Choice

continued

phenomenon came to be called 'OR gene choice'. Once executed, OR gene choice appears to be stable for the life of the OSNs. However, during the process of making this choice, immature OSNs have the ability to initiate transcription from more than one OR gene, something they are thought to do sequentially rather than simultaneously, so this process is called OR gene switching (Shykind et al., 2004).

At about the same time as OR gene switching, a third important phenomenon was discovered. Expressed ORs participate in OR gene choice by driving feedback that stabilizes their own expression (Lewcock and Reed, 2004; Nguyen et al., 2007; Serizawa et al., 2003; Shykind et al., 2004). Even with the revelations of these phenomena, a mechanistic understanding of OR gene choice proved elusive until recent work, much of it in a series of papers from the laboratory of Dr. Stavros Lomvardas (University of California at San Francisco, now at Columbia University) revealed a remarkable story of chromatin modification combined with an unexpected feedback signaling pathway. The evidence reveals that the two most critical questions in OR biology are mechanistically linked. The unusual trafficking of OR proteins that has so badly interfered with heterologous expression of ORs by causing retention in the ER is not an accident but instead evolved to permit maturing OSNs to detect the newly expressed OR protein, triggering feedback pathways that are essential for the singularity of a OR expression.

Repressive mechanisms: chromatin modification and its control

Early in the cell lineage that gives rise to OSNs, OR genes acquire repressive chromatin modifications, dimethylation and trimethylation of lysines at position 9 in histone H3 (H3K9me2 and H3K9me3) and trimethylation of position 20 in histone H4 (H4K20me3) (Figure 1A). Histones are the core elements of nucleosomes, the protein macromolecules around which DNA is wound to provide the fundamental structure of chromatin. H3K9me3 and H4K20me3 are commonly found in constitutive heterochromatin, compacted chromatin from which gene expression is essentially nil. By the immature OSN stage where OR gene expression initiates, OR genes are marked by H3K9me2, H3K9me3 and H4K20me3 (Clowney et al., 2012; Magklara et al., 2011). Correspondingly, OR genes tend to be located in the regions of the nucleus occupied solely by heterochromatin, and deletion of the lamin b receptor gene, whose encoded protein helps organize heterochromatin structures in nuclei, interferes with OR gene expression (Clowney et al., 2012). Further analysis suggests that in each OSN a subset of OR alleles are associated with (but not fully contained within) facultative heterochromatin rather than constitutive heterochromatin (Armelin-Correa et al., 2014). This is potentially significant because this location should make these alleles more available for transcription than the alleles buried within constitutive heterochromatin. Taken together, these findings support the interpretation that compaction and localization of OR genes in heterochromatin helps prevent

Odorant Receptor Gene Choice

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OR genes from escaping repression during the life span of an OSN.

How does a single *OR* allele escape repression? Is a single *OR* allele protected from repression, or are repressive chromatin modifications simply removed selectively from one *OR* allele? The *OR* gene switching capabilities of immature OSNs are more consistent with the latter, a de-repression mechanism. Exactly how an *OR* gene locus becomes de-repressed is not fully certain, but H3K9me2, a characteristic feature of facultative heterochromatin, appears to be the key. Prevention of methylation of H3K9 by deletion of the histone methyl transferases Kmt1c (G9a) and Kmt1d (Glp) severely disrupts *OR* gene expression (Lyons et al., 2014). Loss of the histone methyl transferases that methylate H4K20 have no effect (Lyons et al., 2014) consistent with evidence that the state of methylation/demethylation of H4K20 in any cell type simply follows that of H3K9 (Schotta et al., 2004; Tan et al., 2013). The facultative heterochromatin state may be a balance point that could lead either back to repression or onward to expression (Lyons and Lomvardas, 2014; Schotta et al., 2004; Tan et al., 2013).

This balance between enzymatic events that deposit and remove the repressive histone marks at *OR* genes is an appealing mechanistic explanation because if this balance is set to mildly favor repression but still allow the occasional *OR* allele to become free of repressive chromatin modifications, it could allow for both the randomness that is a characteristic feature of *OR*

gene choice and the rarity of initiation of *OR* gene expression that is necessary to lead to expression of a single *OR* allele (Figure 1B). How might this work mechanistically?

Kdm1a (also known as Lsd1) is a histone demethylase capable of demethylating H3K9me2. Kdm1a is strongly expressed in immature OSNs, so it is in the right stage in the OSN cell lineage to act on nucleosomes at *OR* genes. Consistent with this hypothesis, mice lacking Kdm1a have significant deficiencies in *OR* expression (Lyons et al., 2013). Once Kdm1a has demethylated H3K9 in the nucleosomes positioned at an *OR* allele in an OSN, this allele is probably in a state where it is available to be transcribed, at least until repressive chromatin modifications are added back. Eventually, one of these de-repressed *OR* alleles in an immature OSN will be transcribed and *OR* protein will begin to be translated. If this transcribed *OR* allele achieves high levels of expression, then the newly formed *OR* protein is able to trigger feedback that prevents expression of any other *OR* allele, and this is where *OR* gene choice intersects with the ER retention problem that plagues heterologous expression studies of *OR* protein function. Remarkably, this ER retention property is the means for the expressed receptor to signal back to the nucleus. This signaling is not dependent on the normal G-protein signaling pathways that mediate olfactory transduction during odorant stimulation, however. Instead, feedback works through one arm of the ER stress response pathway – the arm that begins with a kinase called

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Odorant Receptor Gene Choice

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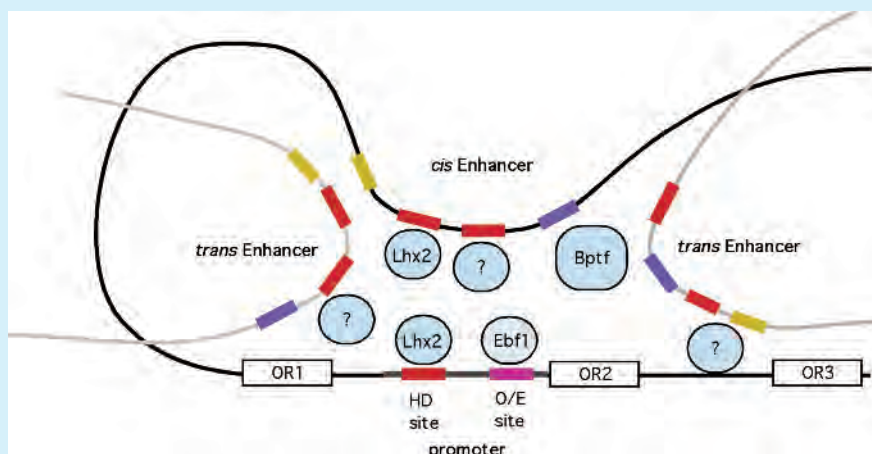


Fig 2. Transcription of OR genes depends on the action of two types of transcription factors and on a network of enhancers. OR promoters have two conserved elements. O/E sites, bound by Ebf family transcription factors Ebf1, Ebf2 and Ebf3, are typically located close to the transcriptional start site. At least one homeodomain (HD) site is typically located further upstream. Lhx2, as depicted, is the homeodomain transcription factor most strongly implicated in acting at these sites. Enhancers for OR genes also have homeodomain sites (red) as well as sites for several other transcription factors (other colors) whose identity is not yet proven. The action of an enhancer in cis on the same chromosome (black line) is critical for expression of a subset of OR genes in a nearby OR gene cluster, but additional enhancer elements, including trans interactions between enhancers on different chromosomes (gray lines) that might be linked by the transcription factor Bptf, form enhancer networks that are also important for OR gene expression. Other transcriptional regulators important for stimulating OR gene expression remain to be identified.



cont. pg 8

Odorant Receptor Gene Choice

continued

PERK (Dalton et al., 2013). The accumulation of *OR* protein in the ER of immature OSNs causes activation of PERK (Figure 1B). This triggers translation of ATF5, especially from an alternative start site that produces a nuclear-targeted version of the ATF5 protein. Nuclear ATF5 induces expression of *Adcy3*, the adenylyl cyclase acting downstream of ORs. The expression of *Adcy3*, or perhaps something expressed in concert with it, in return shuts down expression of *Kdm1a* (Figure 1C). Whether this depends on *OR* activation of *Adcy3* to produce cAMP, which is possible because *ORs* tend to have constitutive activity, is as yet unknown but the ability of a G-protein-coupling deficient *OR* mutant to be expressed at relatively normal levels argues that it might not (Imai et al., 2006; Reisert, 2010). The absence of *Kdm1a* should make it nearly impossible for another *OR* gene to transition out of its heterochromatin state and be expressed, thereby locking in expression of one *OR* allele.

Immature OSNs do not express *Adcy3*, but mature OSNs do, so these molecular events must occur at the transition of immature OSNs into mature OSNs; in fact, the events involved in the expression of an *OR* allele appear to be necessary to trigger the developmental transition to maturity. In other words, *OR* gene expression causes an immature OSN to transition into a mature OSN. Mature OSNs also express several of the proteins that help chaperone ORs during membrane trafficking, making it possible for OR proteins to move from the ER to the plasma membrane where they can function as

transducers of odor signals (Saito et al., 2004).

Much remains to be understood about the role of epigenetic repression in *OR* gene choice. Several of the mechanistic elements described above are still unclear and await refinement. The mechanisms currently known also do not provide any insight into the zonality of *OR* gene choice, the restricted expression of each *OR* to specific regions of the olfactory epithelium.

The mechanistic framework described above is derived from experiments using mice, whose OSNs must make choices from a population of ~1,100 functional *OR* genes. Other vertebrates, especially those with fewer receptors, may have evolved distinctly different mechanisms. For example, the feedback that drives the singularity of zebrafish *OR* gene expression depends critically on G-protein activation by ORs, specifically on the $G_{\beta\gamma}$ arm of this pathway (Ferreira et al., 2014). Finally, no matter how compelling the tale of repression and de-repression mechanisms in *OR* gene choice, they are only part of the story.

Active mechanisms: *OR* gene promoters and enhancers.

The mechanisms that result in the singularity of *OR* gene expression depend critically on the repressive mechanisms described above, but they also must involve active mechanisms that drive transcription of whichever *OR* allele is made available. In fact, the balance between the two may be critical for *OR* gene choice.

Odorant Receptor Gene Choice

continued

Unfortunately, we understand much less detail about the mechanisms supporting active transcription of *OR* genes. The epigenetics of active *OR* genes are very difficult to study because only one allele per OSN is in the active state. To date, only one active chromatin modification, H3K4me3, has been demonstrated at an *OR* gene (Magklara et al., 2011). Instead of epigenetics, study of active mechanisms has largely focused on transcription factors and their cis-elements in the promoters of *OR* genes and the enhancers acting at *OR* genes.

Mouse *OR* genes are relatively compact and have short upstream promoter elements. When used in transgenes to drive expression of an *OR*, these promoters are capable of recapitulating zonal, monoallelic expression of transgenic *ORs* at frequencies of expression that often approximate native *OR* genes. *OR* promoters share two conserved cis elements (Figure 2). (1) O/E-like sites bound by the Ebf family of transcription factors (Ebf1, Ebf2, and Ebf3) are common features of genes expressed primarily in OSNs, are thought to be critical for this restricted pattern of expression, and along with the Ebf family transcription factors that bind them, they are important for *OR* expression (Cheng and Reed, 2007; Davis and Reed, 1996; Kudrycki et al., 1993; Vassalli et al., 2011; Wang and Reed, 1993). (2) Homeodomain sites, also found in *OR* gene enhancers, are positive regulators of *OR* gene expression (Khan et al., 2011; Rothman et al., 2005; Vassalli et al., 2011).

Distal enhancers of *OR* genes are as yet poorly identified, and only a few

have been identified and investigated in detail (Bozza et al., 2009; Markenscoff-Papadimitriou et al., 2014; Serizawa et al., 2003). *OR* gene enhancers are critical only for a subset of *OR* genes in a nearby *OR* gene cluster on the same chromosomes (Figure 2). Deletion of an *OR* gene enhancer significantly reduces expression of a small set of *OR* genes in this neighboring cluster, but other *OR* genes are unaffected, including others in the same cluster (Fuss et al., 2007; Khan et al., 2011; Nishizumi et al., 2007).

Recent work has identified 35 potential *OR* gene enhancers and provided evidence that 12 of them can act as enhancers for *OR* genes (Markenscoff-Papadimitriou et al., 2014). These enhancers have an unusual chromatin modification signature. Like enhancers for many other genes, they show enrichment in DNase I hypersensitivity, H3K4me1 marks, and H3K27ac marks. However, they are also surrounded by regions of chromatin enriched for two repressive chromatin modifications: H3K79me3 and H3K27me3. When three of these enhancers were linked to reporter genes and used to make transgenic mouse strains, these mice showed widespread expression of the reporter in mature olfactory sensory neurons. Chromatin capture techniques reveal that various groupings of these 35 putative enhancers, which are distributed across several chromosomes, can be found in close proximity within mature OSN nuclei much more frequently than chance, evidence that *trans* interactions of enhancers across chromosomes must be occurring. These data suggest that *OR* gene



Odorant Receptor Gene Choice

continued

enhancers might interact and form networks that help regulate *OR* gene expression. One of the protected footprints in these enhancers is a consensus site for the transcription factor *Bptf*, a transcriptional regulator capable of binding multiple types of modified histone tails, and therefore potentially capable of mediating the formation of enhancer networks. In support of this idea, conditional deletion of *Bptf* reduces interactions between the *OR* gene enhancers tested. Whether these enhancer interactions have a role in selecting which *OR* allele is expressed, or whether they simply help drive high levels of transcription of the active *OR* allele is as yet uncertain.

Active mechanisms: transcription factor regulation of *OR* genes

Interestingly, both *OR* gene promoters and *OR* gene enhancers have consensus binding sites used by homeodomain transcription factors (Markenscoff-Papadimitriou et al., 2014; Vassalli et al., 2011), suggesting that homeodomain transcription factors might be doubly important in regulating *OR* gene expression. The conserved cores of *OR* gene enhancers are capable of driving expression of *OR* transgenes, sometimes very robustly, and mutating their homeodomain sites reduces the frequency of expression of the *OR* transgenes.

Which homeodomain transcription factors contribute to *OR* gene expression? Yeast one-hybrid assays using *OR* promoters as bait captured 10 homeodomain transcription factors from olfactory epithelium cDNA expression libraries (Hirota and

Mombaerts, 2004; Hoppe et al., 2003). Of these, *Lhx2* and *Emx2* have expression patterns most consistent with the control of *OR* transcription because they are strongly expressed in immature OSNs and basal progenitor cells but are also expressed at lower levels in mature OSNs. In E18.5 mouse embryos, germline deletion of *Emx2* decreased the frequency of expression of dozens of *OR* genes but also increased the frequency of more than 20 *OR* genes (McIntyre et al., 2008). Germline deletion of *Lhx2* is complicated by the nearly complete loss of mature OSNs but knockout embryos still show evidence of effects on *OR* expression (Hirota and Mombaerts, 2004; Hirota et al., 2007; Kolterud et al., 2004). In addition, *Lhx2* chromatin immunoprecipitation experiments pull down *OR* gene enhancer sequences (Markenscoff-Papadimitriou et al., 2014). A link between homeodomain transcription factor stimulation of *OR* expression and *OR* gene choice is supported by evidence that deletion of *Emx2*, or mutation of homeodomain sites in both *OR* promoters and enhancers, alters the frequencies of *OR* expression rather than the amount of *OR* mRNA per OSN (Khan et al., 2011; McIntyre et al., 2008; Vassalli et al., 2011). These findings suggest that *OR* gene repression and the singularity of *OR* allele de-repression might be sensitive to the effects of positively acting transcription factors on *OR* genes.

The available data emphasize the importance of Ebf family transcription factors and homeodomain transcription factors in the active transcription of *OR* genes. However, these are probably not the only factors

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Odorant Receptor Gene Choice

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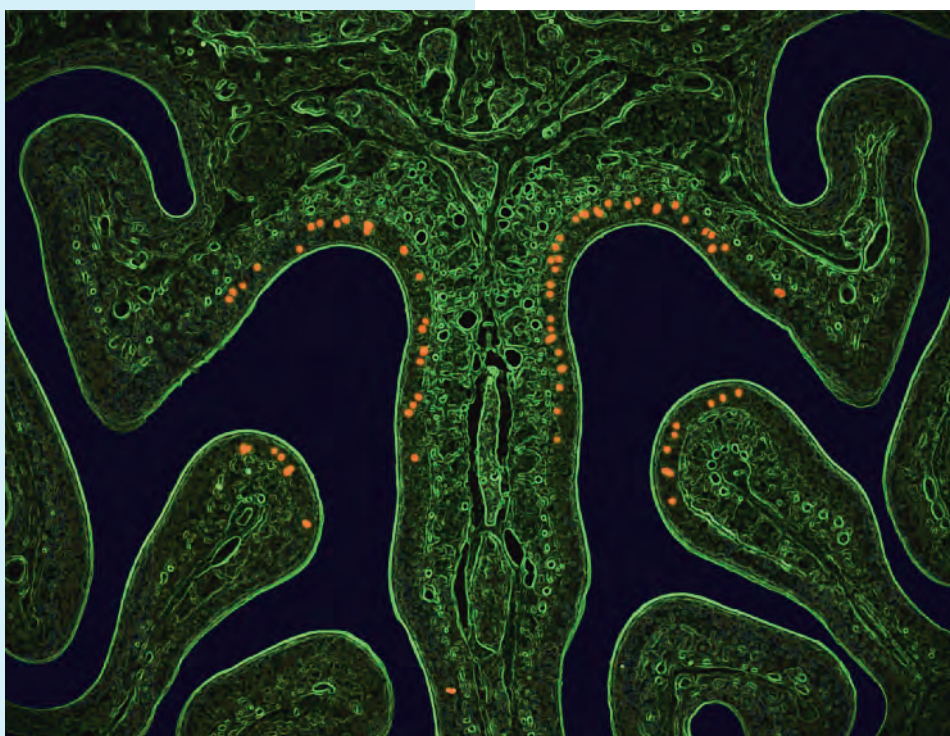
involved in stimulating *OR* gene transcription. Other factors acting at smaller subsets of *OR* genes probably also contribute (Michaloski et al., 2011), perhaps in ways that are critical to other, as yet poorly understood aspects of *OR* gene choice.

Summary

Repressive epigenetic mechanisms limit *OR* gene expression but immature OSNs have mechanisms whereby *OR* alleles become available for expression – perhaps due to rare, random demethylation of H3K9 in the nucleosomes at *OR* alleles.

Transcription of the newly available *OR* allele is driven by transcription factors such as Ebf1-3 and the homeodomain proteins, Lhx2 and Emx2, acting at conserved sites in *OR* gene promoters. Homeodomain transcription factors, especially Lhx2, and other transcriptional regulators probably also help stimulate *OR* gene expression through actions at *OR* gene enhancers. The formation of networks of enhancers, including *trans* interactions between enhancers on different chromosomes, at *OR* alleles may help determine which *OR* allele becomes expressed and may help drive *OR* gene expression sufficiently high to trigger ER stress response signaling. This signal represses Kdm1a, the demethylase that is critical for *OR* gene de-repression, so that no other *OR* allele can become available for expression. This signal also stimulates expression of *Adcy3* and promotes the maturation of the immature OSN into a mature OSN. The epigenetic control of *OR* gene expression through repression and de-repression is a

remarkable story, and as a fundamental mechanism intimately associated with the random differentiation of a neural progenitor cell into multiple neuronal subtypes, one wonders whether it might be a framework for understanding other instances of differentiation of neural subtypes (Lyons and Lomvardas, 2014).



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Odorant Receptor Gene Choice

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Odorant Receptor Gene Choice

continued

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NEWS

Olfactory Transplant Heals Spinal Injury

Transplanted olfactory ensheathing cells recently preceded a patient regaining spinal function after devastating spinal injury. Olfactory ensheathing cells normally enable olfactory sensory axons to grow into the olfactory bulb, and when transplanted into other neural tissue might help guide nerves to reach their target cells. This could be the news the world has been waiting for: neural regeneration of damaged central nervous system tissue. If validated and able to be reproduced safely, the procedure could bring hope and relief to countless people with spinal and brain injuries. More data and evidence is awaited. The whole world is watching!

www.bbc.com/news/health-29645760

Reformed Beer

The religious and political reformation started by Martin Luther also created a tradition of beers named in his honour and brewed outside the established Catholic monasteries. A new limited "fluid gold" production was announced recently.

http://www.swp.de/ulm/lokales/ulm_neu_ulm/Fluessiges-Gold-fuer-den-Neubau;art4329,2278813

www.lutherbier.de



NEWS

SWEET TALK

Hot Chocolate

A war has been raging in German courts over the labelling of chocolate flavour ingredients produced by Ritter Sport, a giant European confectioner with sales in 100 countries and its own cocoa plantations in Nicaragua. Consumer watchdog, Stiftung Warentest claims that Ritter Sport is using an artificial flavour, piperonal, in its "hot chocolate" which Ritter Sport denies, asserting it is naturally extracted from plants. The arguments were heard at several levels of the German court system, through 2014. The final verdict has been recently handed down: Ritter Sport's case was upheld. Compensation is not being pursued.

<http://www.nordbayern.de/ritter-sport-siegt-im-schoko-streit-1.3876742>

Body Painting with Chocolate

The darker and lighter side of chocolate found unbridled expression at ChocolART: in picturesque Tübingen in December 2014. Over 100 exhibitors and 300,000 visitors gathered to celebrate the "king" of confectionery, chocolate, and indulge in some creative ways of using it: chocolate jewellery, hammers, screws, motor cars and yes, body art: which it could be said, had tattoos licked. The 10th festival will be held in Tübingen in December 2015.

<http://www.weihnachtsmarkt-deutschland.de/tuebingen-chocolart.html>

<http://www.chocolart.de/>

Just Call Me Chocolino

A popular chocolate-coated marshmallow sweet, made from chocolate coated sugared eggwhite foam and cotton candy (fairy floss) has been renamed after a competition to find a less politically sensitive name than "Negerkuss" (negro kiss) or "Mohrenkopf" (moor's head). Discussion ranged widely and drew in a number of politicians. "Chocolino" is the new name which it now shares with several other products and businesses, so this is probably not the last word on the subject.

<http://www.swp.de/reutlingen/lokales/reutlingen/Der-Mohrenkopf-heisst-jetzt-CHOCOLino;art5674,2171052>



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Upcoming Events

- 18 – 21 March 2015** Eleventh Göttingen Meeting of the German Neuroscience Society
<http://nwg.glia.mdc-berlin.de/en/conference/>
- 22 – 25 April 2015** AChemS 37th Annual Meeting
 Hyatt Regency Coconut Point, Bonita Springs, Florida
<http://www.achems.org/i4a/pages/index.cfm?pageid=3962>
- 28 June – 1 July 2015** International Symposium on Olfaction and Electronic Nose (ISOEN)
 Dijon, France
www.olfactionsociety.org
- 27 – 31 July 2015** Summer School on Human Olfaction 2015
 Dresden, Germany
thummel@mail.zih.tu-dresden.de
- 23 – 28 August 2015** Australian Neuroscience Society
 Joint meeting with ISN & APSN
<http://www.ans.org.au/>
- 20 – 23 September 2015** Clean Air Society of Australia and New Zealand (CASANZ)
 Melbourne
www.casanz.org.au



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