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The patch-like pattern of OR37 receptors is formed by turning off gene expression in non-appropriate areas

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ABSTRACT

Olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) gene are generally widely dispersed throughout the olfactory epithelium (OE). In contrast, OSNs expressing any member from the special *OR37* subfamily are concentrated in a small patch in the centre of the OE. To evaluate whether transcription of *OR37* genes is only possible in the patch region, or if they can generally be chosen also in non-appropriate areas, a transgenic approach was employed that permanently labelled all cells which ever transcribed a representative *OR37* gene. It was found that – in addition to cells inside the patch – numerous cells outside were labelled, indicating that they had transcribed the *OR37* gene, but then turned it off while choosing another OR gene. Permanent expression of the *OR37* gene was exclusively maintained in the patch. The results suggest that mechanisms acting downstream of an initial OR gene choice restrict OR37 expression to the patch.

Introduction

The capacity of the mammalian olfactory system to detect a vast array of small volatile compounds is mediated by more than 1000 different isoforms of G-protein coupled odorant receptors (ORs) (Buck and Axel, 1991) which are encoded by the largest gene family in vertebrate genomes (Zhang and Firestein, 2002; Godfrey et al., 2004; Malnic et al., 2004; Mombaerts, 2004a; Young and Trask, 2002). Each of the several million olfactory sensory neurons (OSNs) in the olfactory epithelium (OE) expresses a single gene from this large repertoire; moreover, only one of the two alleles is activated per cell (Chess et al., 1994; Malnic et al., 1999); the resulting receptor type renders the cells selectively responsive to distinct chemical compounds (Touhara et al., 1999; Bozza et al., 2002). Interestingly, OSNs which express a particular OR gene are not randomly dispersed throughout the OE, but restricted to a defined region; most of these OSN populations are arranged in broad, largely overlapping zones (Ressler et al., 1993; Vassar et al., 1993; Strotmann et al., 1994b; Iwema et al., 2004; Miyamichi et al., 2005). In contrast to this predominant zonal patterning, the genes encoding the OR37 receptor types follow a different principle: OSNs expressing these receptors are restricted to a small patch in the centre of the turbinates in all mammalian species that have been investigated so far (Strotmann et al., 1992; Strotmann et al., 1994a; Kubick et al., 1997; Strotmann et al., 1995; Hoppe et al., 2006b).

The molecular mechanisms underlying the expression of OR genes from a restricted repertoire within a defined region of the OE are still largely elusive. Studies using bioinformatic tools revealed that promotors of OR genes which are expressed in the same region of the OE share common DNA motifs, whereas those expressed in different regions contain distinct motif combinations (Hoppe et al., 2006a). It has been discussed that the characteristic topography of expression is determined by transcription factors which bind to these DNA motifs. These factors are themselves thought to be patterned within the OE and thereby ensure expression of only a set of OR genes appropriate for their location within the epithelium (Norlin et al., 2001). In this concept, the capacity to activate a particular OR gene would be limited to a defined zone or the patch, and would not be possible in other regions. However, a series of experiments with mice carrying OR transgenes has recently demonstrated that in some lines the expression of the transgene was shifted from the regular zone to ectopic positions in the epithelium (Nakatani et al., 2003; Rothman et al., 2005; Vassalli et al., 2002); e.g., a gene normally expressed in the most dorsal zone was now found to be expressed ventral in the epithelium. We have recently generated transgenic mouse lines for *mOR37C* (*olfr 157*), a member of the *OR37* subfamily in mouse, using a short transgenic construct and observed a similar phenomenon: whereas in six out of seven mouse lines the special clustered expression pattern was recapitulated by the transgene (Zhang et al., 2007), in one line not only OSNs in the central patch expressed the transgene, but in addition OSNs in other positions in the epithelium. Interestingly, the ectopically positioned cells were not randomly dispersed throughout the epithelium, but in this case arranged in a zonal pattern which is typical for other OR genes. The principles

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underlying such aberrant expression patterns are currently unclear. Since the ectopically positioned OSNs usually were not found randomly dispersed, but displayed patterns typical for other OR genes, it was discussed that the transgenes might have integrated in the vicinity of other OR gene clusters and that these loci exerted a significant influence on the transgene, thereby resulting in a corresponding expression pattern. On the other hand, it was hypothesized that OR transgenes may have integrated into genomic loci that obstruct the full range of OR regulation, thereby preventing the correct pattern to unfold (Shykind et al., 2004).

Altogether, these experiments using the various OR transgenes demonstrated that the machinery necessary to select a particular OR gene for expression obviously is not restricted to the defined area of the OE where the gene is typically found to be expressed, but also present outside that region. It thus seems conceivable that OR genes can be chosen for expression in broader regions of the epithelium, and that unknown mechanisms subsequently restrict their presence to defined regions of the OE. To test this hypothesis for the *OR37* subfamily, we have employed a transgenic approach for *mOR37C*, allowing to visualize all cells that have ever activated this OR gene, also those which have turned it off at a given time.

Results

In order to visualize all cells which ever had turned on the odorant receptor (OR) gene *mOR37C*, we have employed a gene targeting strategy in mice. For this purpose a DNA construct containing an internal ribosome entry site (IRES) driving the translation of the *Cre-recombinase* (*Cre*) was introduced three nucleotides downstream of the *mOR37C* coding sequence by homologous recombination in ES cells. A strain of mice was generated that carries the corresponding mutation (mOR37C-IRES-Cre; short 37CCre*) in which cells that express *mOR37C* now also express *Cre*. These mice were crossed to different strains bearing *Cre*-reporters at the ROSA26 locus, like *lacZ* (R-lacZ) (Soriano, 1999), *EYFP* (R-EYFP) (Srinivas et al., 2001) and *tandemdimerRFP* (R-tdRFP) (Luche et al., 2007). In the resulting double mutant offspring, *Cre* directs loxP-mediated recombination and marker expression. Since this *Cre*-mediated recombination is irreversible, expression of the marker will persist even if *mOR37C* expression is extinguished and therefore serves as a permanent label for all the cells that have chosen the *mOR37C* gene at any time in their life.

In the first series of experiments, whole mount stainings of split heads from 37CCre*/R-lacZ double-heterozygous mice were performed. Numerous lacZ positive cells were visible on the side of the turbinates which is facing the nasal septum (Fig. 1A). The most prominent labelling was visible in the centre of the turbinates, in particular on endoturbinate II. This region represents the central patch, where *mOR37C* is typically expressed, as demonstrated e.g. by our previously generated mouse line (Strotmann et al., 2000) in which the locus for this gene was modified such that cells directly co-express *tau-lacZ* with the receptor (Fig. 1B). The position of the patch was very similar in both lines. Interestingly, in 37CCre*/R-lacZ mice additional labelled cells were detectable outside the central patch (arrowheads in Fig. 1A); they were dispersed over a large area of the epithelium extending from anterior to posterior over all turbinates. A close inspection of their distribution revealed, however, that not all parts of the epithelium contained labelled cells; a region close to the cribriform plate was devoid of staining (arrow in Fig. 1A). LacZ positive cells were symmetrically organized in both nasal cavities and also present on the nasal septum (data not shown). To control for the specificity of the staining, R-lacZ mice which were not crossed to the 37CCre*-line were examined; no labelling was seen in these individuals (data not shown), demonstrating that the β-galactosidase activity was not due to a leaky expression of *lacZ* in the OE, but specifically induced after *mOR37C*-mediated *Cre* expression. These results demonstrated that the *mOR37C* gene was chosen for expression in more cells than those inside the central patch of the OE. The density of cells labelled outside the patch was significantly lower than inside; nevertheless, due to their broad distribution, the cells outside represented a substantial fraction of all labelled cells.

Since whole mount preparations revealed only a view onto the medial side of the turbinates, we next performed analyses on cross sections through the nose. A representative section through the central region of the nose from a 37CCre*/R-EYFP mouse is shown in Fig. 1C; numerous fluorescent cells were visible in the central patch (Fig. 1D). In addition, cells outside the patch were detectable on different turbinates (Figs. 1E, F) confirming the data obtained by whole mount staining. At higher magnification it became clear that cells inside and outside the patch both displayed the morphology typical for OSNs (Fig. 1G). On serial sections through the nasal cavity, labelled cells were visible along the anterior–posterior extension. It emerged, however, that all along this axis, no labelled cells were detectable in the dorsal part of the nasal septum and on the dorsal segments of the turbinates (Figs. 1H, I), indicating that the expression of *mOR37C* might not occur in a specialized region, the so-called dorsal zone of the epithelium. To support this notion we crossed 37CCre*/R-tdRFP animals to MOL2.3-IGITL mice (Conzelmann et al., 2000) in which OSNs co-express GFP together with the class I OR gene *mOR18-2* that is selectively expressed in the dorsal zone. Analyses of serial sections through the nose of triple mutant animals (37CCre*/R-tdRFP/MOL2.3-IGITL) revealed that in those areas of the OE where the *mOR18-2* expressing OSNs were positioned, basically no 37CCre* cells were detectable (Fig. 1K); thus the dorsal zone was indeed excluded from the expression of this particular OR gene.

On sections through the most posterior region of the nose, also the olfactory bulb (OB) was visible; it contained no labelled cells (data not shown). In the anterior region of the nose, the largest part of the nasal cavity is lined with respiratory epithelium; no labelling was found in this tissue, either (data not shown). Surprisingly, however, numerous labelled cells were detectable in the vomeronasal organ (VNO) which is located at the base of the nasal septum (Fig. 2A). Closer inspection of the VNO revealed that the labelled cells were located almost exclusively in the apical layer of the VNO epithelium (Fig. 2B); they all showed the typical morphology of VNO neurons (Fig. 1C). Together, these analyses thus revealed that cells with the *Cre*-induced marker were not only located in the patch, but also ectopically in the OE and even in the VNO. To rule out that this feature was due to the artifactual effect of the neomycin-selectable marker – which is part of the targeting vector (see Supplementary Fig. 1A) – on the regulation of the *mOR37C* gene locus, 37CCre*/R-tdRFP double mutant mice were crossed to mice expressing the *flp-recombinase* (Rodriguez et al., 2000) to remove the FRT-flanked *neo*-gene from the genome. As shown by representative cross sections through the nose of a mouse in which the *neo*-marker had been excised from the genome, ectopically positioned cells were visible in the OE and in the VNO (Supplementary Fig. 2); as observed before, the labelled cells in the OE excluded the dorsal region.

Such a distribution pattern for *mOR37C* expressing cells had never been observed in wildtype mice by *in situ* hybridization, raising the question whether the cells outside the patch in this newly generated 37CCre* mouse line actually contained the mRNA for *mOR37C*. *In situ* hybridization experiments using an *mOR37*-specific antisense riboprobe on cross sections through the nose of 37CCre*/R-EYFP mice stained cells containing the message for *mOR37C* selectively in the typical patch (Figs. 3A, B); outside that region, almost no cells with mRNA for *mOR37C* were visible, indicating that – although cells outside the patch selected *mOR37C* for expression – they did not contain the corresponding mRNA. The molecular basis for this phenomenon is elusive. One possibility could be that cells outside