

# Negative Feedback Regulation Ensures the One Neuron–One Receptor Rule in the Mouse Olfactory System

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## Introduction

In the mouse olfactory system, there are ~1500 odorant receptor (OR) genes clustered at ~50 different loci which are scattered among most of the chromosomes (Buck and Axel, 1991; Zhang and Firestein, 2002; Godfrey *et al.*, 2004). Like the antigen receptor genes in lymphocytes, the mammalian OR genes are expressed in a mutually exclusive (Malnic *et al.*, 1999; Serizawa *et al.*, 2000) and monoallelic (Chess *et al.*, 1994; Ishii *et al.*, 2001) manner in olfactory sensory neurons (OSNs). DNA rearrangement has long been thought of as a possible mechanism for the allelic exclusion of the OR genes. However, mice cloned with OSN nuclei excluded the possibility of irreversible gene translocation as a mechanism to activate a single OR gene in each OSN (Eggan *et al.*, 2004; Li *et al.*, 2004). How is it, then, that allelic exclusion is achieved in the olfactory system? Since OSNs expressing a given OR gene converge their axons to a specific set of projection sites (glomeruli) on the olfactory bulb (OB) (Ressler *et al.*, 1994; Vassar *et al.*, 1994; Mombaerts *et al.*, 1996; Feinstein and Mombaerts, 2004), odorous stimuli received in the olfactory epithelium (OE) are converted to a topographic map of activated glomeruli on the OB (Mori *et al.*, 1999). Thus, the one neuron–one receptor rule forms the genetic basis for OR-instructed axonal projection of OSNs. Here, we discuss possible mechanisms that regulate single OR gene expression in each OSN in mouse.

## Positive regulation of the OR gene expression

Three activation mechanisms have been considered for the choice of one member of the multigene family: DNA recombination, which brings a promoter and the enhancer region into close proximity; gene conversion, which transfers a copy of the gene into the expression cassette; and LCR, which interacts with only one promoter site. Irreversible DNA changes, i.e. recombination and gene conversion, have been attractive explanations for the single OR gene expression, because many parallels can be found between the immune and olfactory systems. We have analyzed the nuclei of OSNs expressing the *MOR28* by fluorescent *in situ* hybridization (FISH) (Ishii *et al.*, 2001). Both DNA and RNA FISHs were performed to detect the chromosomal location of the genomic sequence and the transcriptionally active site, respectively. In our experiments, DNA FISH detected two genomic loci in the nuclei for the *MOR28*, one for the maternal and the other for the paternal allele. RNA FISH detected the *MOR28* primary transcripts at only one of the two genomic sites. These experiments not only confirmed the mono-allelic expression of OR, but also excluded a possibility of gene conversion. If it were gene conversion, there should be three DNA FISH sites: no additional genomic site was detected for the transcriptionally active site. Of course DNA changes in nearby regions could not be ruled out in this experiment. More recently, two groups have independently cloned mice with postmitotic OSN nuclei, and found that the cloned mice generated normal repertoire of OSNs and showed no irreversible DNA changes in the OR genes.

Since our FISH analysis did not support the gene translocation models, we searched for the *cis*-acting DNA region that may regulate the single OR gene expression. Using transgenic constructs in yeast artificial chromosomes (YACs), we have studied the OR gene cluster containing the *MOR28* gene (Serizawa *et al.*, 2000). Sequence comparison of the mouse and human genomes revealed a 2-kb homology (H) region 75kb upstream of the murine *MOR28* gene (Serizawa *et al.*, 2003). It was found that the shorter YAC constructs lacking the H region do not express the transgenes. Attachment of the H region DNA to the upstream end restored the expression of all transgenes in the cluster. These results indicated that the H region is a *cis*-acting locus control region (LCR) that activates the *MOR28* cluster.

It is important to ask how the expression of one particular OR gene within the activated cluster is ensured. We propose that the activation complex formed in the LCR interacts with only one promoter site in the OR gene cluster. Such a mechanism has been reported for the mutually exclusive expression of the human visual pigment genes in cone cells (Wang *et al.*, 1999; Smallwood *et al.*, 2002).

## Negative regulation of the OR gene expression

To avoid concurrent expression, the activation processes for the OR gene expression may be relatively slow and rate limiting. However, this would not preclude the possibility of a second OR gene activation in other OR gene clusters. To achieve the mutually-exclusive expression, we assumed that a negative feedback regulation is taking place in OSNs like in the immune system (Martensson *et al.*, 2002). To test this hypothesis, we deleted a coding-sequence of the *MOR28* transgene (*del-MOR28*), and examined whether mutually exclusive expression is violated in the OSNs expressing the *del-MOR28* (Serizawa *et al.*, 2003). We found that the *del-MOR28* transgene permitted the activation of other OR genes in the transgene-expressing OSNs. Similar observations were also made with frameshift mutants, in which nucleotide deletions or additions in the coding regions caused the frame shift and created stop codons in the downstream region. These results suggest that the OR gene product—not mRNA but most likely protein—has a regulatory role in preventing the secondary activation of other OR genes.

## Conclusions

The mammalian OR genes are expressed in a mutually exclusive and monoallelic manner in OSNs. Such unique expression forms the genetic basis for the OR-instructed axonal projection of OSNs to the OB. We have identified the LCR for the mouse OR gene cluster containing *MOR28* (Serizawa *et al.*, 2003). We assume that physical interaction between the LCR and promoter ensures the expression of only one OR gene within the cluster. This model is also attractive in that it reduces the likelihood of a simultaneous activation of two different OR genes, from a probability among ~1200 genes to that

among ~50 loci. Recent transgenic experiments demonstrated an inhibitory role of the OR protein in preventing further activation of other OR genes (Serizawa *et al.*, 2003; Lewcock and Reed, 2004; Shykind *et al.*, 2004). Stochastic activation of an OR gene by LCR and negative feedback regulation by the OR gene product probably ensure the one neuron – one receptor rule in the mammalian olfactory system.

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