Regulating olfactory receptor expression: controlling globally, acting locally

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Only one allelle from the odorant receptor gene family is expressed in each neuron. Transgenic expression of an odorant receptor provides new insight into how this process is regulated.

The environmentalists' maxim to "think globally and act locally" may have been adopted long ago by the olfactory system as a strategy to solve a remarkable challenge in the regulation of gene expression. The genomes of mice and humans contain nearly one thousand olfactory receptor (OR) genes, organized into clustered arrays that are distributed on most of the chromosomes. Each of the several million olfactory neurons seems to express only a single member of this large repertoire; this presumably allows each neuron to signal the presence of a specific odorant. Surprisingly, even though each gene is represented by two alleles, only one allele is expressed in any given neuron. This expression pattern raises a paradox: although the transcriptional machinery must act locally on a single allele, it must somehow be regulated at the genome-wide level to ensure that each neuron expresses one and only one receptor. In this issue of *Nature Neuroscience*, Serizawa and colleagues[1] have used genetically altered mice to explore the basis of this extraordinary control of gene expression.

The neurons of the olfactory epithelium bear cilia, which are exposed to the airway and which are thought to be the site of odorant binding and signal transduction. They also project axons to the olfactory bulb of the brain, where they form connections via synaptic structures known as glomeruli; remarkably, all the neurons expressing a single receptor gene converge onto the same target glomerulus. The spatial pattern of receptor gene expression within the olfactory epithelium is highly stereotyped. In mice (the best-studied species), the epithelium is divided into four zones, such that each OR gene is expressed only in one zone. There is a correlation between these zonal distributions and the chromosomal location of the genes: OR genes have been divided into about one hundred subfamilies based on similarities in their coding sequences, and members of a given subfamily tend to be clustered together in the genome and expressed within the same zone of the olfactory epithelium. It is therefore tempting to speculate that at least some aspects of receptor expression derive from transcriptional control at the level of OR gene clusters. Within the appropriate zone, however, each gene is expressed in a small fraction of the neurons, in an apparently random distribution, suggesting that the final selection step occurs through a stochastic process that operates independently in each neuron

Genetic tagging experiments in which β-galactosidase or great fluorescent protein (GFP) reporter genes are introduced into endogenous receptor loci have provided valuable information on the pattern of expression of individual receptors and their projections to the olfactory bulb[2–4]. These studies have also suggested that the OR proteins have an instructive role in directing the convergence of axons to their precise targets in the olfactory bulb. Little is known, however, about the DNA elements that are required for the correct expression of OR genes. In the new study, Serizawa *et al.* have made transgenic mice carrying a 200-kb piece of mouse DNA (cloned as a yeast artificial chromosome or YAC) that includes an OR gene, designated mOR28. By inserting a tag into this gene, they were able to show that it is expressed appropriately, in a stochastic pattern within the appropriate zone. They go on to show, in several independent lines, that when the YAC transgene is truncated to 180kb or less, the expression of mOR28 is abolished or greatly reduced, suggesting that mOR28 is regulated by an element that lies far away from the gene itself. It is interesting to compare these findings with a previous study, in which a much smaller transgene containing 6.7kb upstream of the promoter for a different OR gene (M4) was sufficient to direct tissue-specific, zonally restricted and stochastic expression within the olfactory epithelium[5]. This smaller transgene was expressed correctly in most cases, although one line of mice showed an anomalous pattern in which the gene was expressed in an incorrect zone. One possible explanation for the difference is that M4 may (fortuitously) be the most upstream member of an OR gene cluster, and therefore closer to its corresponding control element than mOR28.

The findings of Serizawa *et al.* have implications not only for the zone-specific expression of OR genes, but also for the stochastic patterns seen within each zone. PCR-based expression studies[6] have shown that each olfactory neuron expresses not only a single gene but also a single allele, which is apparently selected at random. This expression pattern was termed 'allelic exclusion', and was interpreted as suggesting that both OR alleles were selected for expression but that another step insured that one was expressed and the other was silent. This exclusion hypothesis infers a special relationship between the two alleles for a given receptor, which is difficult to explain by known transcriptional mechanisms.

The findings of Serizawa *et al.* suggest an alternative explanation for the exclusion phenomenon. By using lines of mice in which the endogenous mOR28 gene and the transgene copy were labeled with two different tags, they demonstrate that the endogenous, chromosomal OR gene and the transgene copy were almost never expressed in the same cell, even though they were expressed in the same zone and with similar frequencies. In other words, the exclusion process can operate on a gene outside of its normal chromosomal context, indicating that it must depend on local control mechanisms. It seems unlikely that the transgene-expressing neurons express any other (endogenous) OR genes, because their axons all converge on the same glomerulus; if they expressed other OR genes at significant

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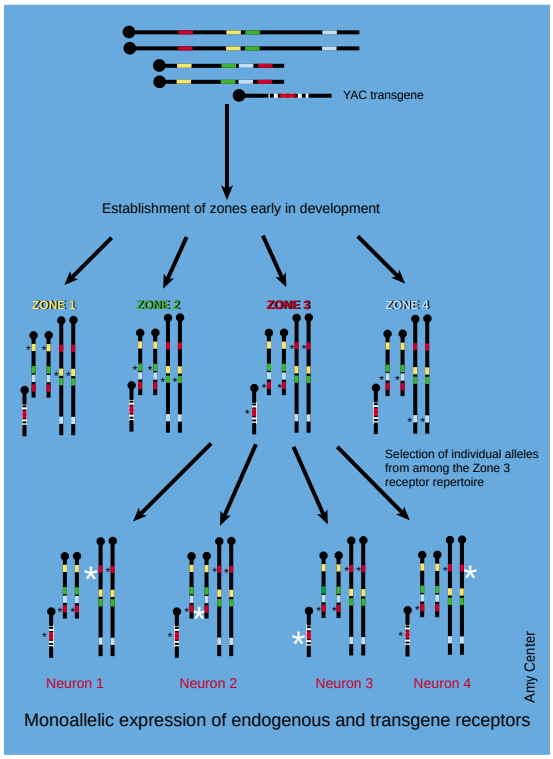

Fig. 1. A model for monoallelic expression of olfactory receptor genes. The colored blocks represent individual or clustered receptor genes expressed in a characteristic zone of the mouse olfactory epithelium according to their colors. The repertoire of zone-specific receptors is indicated by the small asterisks. In a subsequent event during the differentiation of each neuron, one of the large number of receptors appropriate for zone three is chosen in a stochastic manner (large white asterisk), and transcription is initiated from a single allele in each olfactory neuron.

levels, one would expect, based on previous studies[4], to see a more complex pattern of axon targeting.

These findings are difficult to reconcile with the allelic exclusion model, in which the transgene copy of mOR28 would need to suppress the two endogenous copies. They are, however, consistent with a simpler model, (Fig.1) in which one allele is selected from the genomic repertoire consisting of 2000 OR alleles. The observed expression would occur at a single DNA site in the genome and thus lead directly to monoallelic expression.

How could a sensory neuron accomplish the selective expression of a single olfactory receptor allele? Presumably there must be zone-specific transcription factors that insure expression of receptors appropriate for their location within the epithelium, but this still leaves the neuron with the daunting task of selectively expressing one from the roughly 250 genes appropriate for that zone. One possibility is gene conversion or somatic recombination into an expression locus, perhaps through a mechanism analogous to that which determines yeast mating types. However, several observations argue against this possibility. The nucleotide sequences of OR mRNAs can be fully accounted for by DNA at the germline locus—no rearrangement events are required to generate the functional message. Thus, the boundaries defining any conversion or recombination events would have to lie outside of the transcribed region. This, however, is difficult to reconcile with our transgenic experiments[5], in which normal expression was observed even though the transgene lacked any 3′ flanking sequence, which would presumably be a prerequisite for any precise recombination or gene-conversion mechanism.

An alternative model is the following. Receptor expression might begin with a low-probability event in which one allele is selected for potential expression. Once this first allele is made competent for expression, its transcription could then be driven by the same factors that stimulate transcription of other olfactory neuron-specific genes. The expression of a functional OR could result in physiological changes that would prevent further selection events from occurring at other alleles. Such a mechanism would afford a high probability of receptor expression while insuring that only a single functional receptor is expressed in each neuron.

The sites required to establish competence could act on individual receptors or on a locus consisting of several clustered receptors. A locus control region (LCR), perhaps analogous to the one defined at the β-globin cluster, would exert spreading control on adjacent receptor sequences. At the β-globin locus, the LCR is required for setting up a developmental pattern of globin gene expression. In contrast, within OR clusters, an LCR might define the active region, and subsequent steps would be required to generate a single, stable transcription complex at one of the receptors within the cluster. The requirement for a large DNA segment in the experiments by Serizawa *et al.* might therefore reflect the location of LCR with respect to the tagged receptor. It would be interesting to tag two adjacent receptors from the same subfamily and located in the same cluster; if activation works at the level of clusters rather than single alleles, one might expect that alleles in *cis* might be exempt from the exclusion mechanism, whereas alleles in *trans* would be mutually exclusive. Serizawa *et al.* have attempted to address this question by generating mouse lines carrying multi-copy transgenes consisting of a mixture of mOR28-GFP and mOR28-β-galactosidase-containing YACs. These mice displayed mutually exclusive expression of the differentially tagged mOR28 transgenes, even though they apparently formed a tandem array at a single site in the genome. Thus, if LCR activation occurs, it does not extend across the entire 200kb that separates adjacent transgenes within this array.

Although the molecular mechanisms underlying olfactory receptor expression remain mysterious, the model system established by Serizawa holds considerable promise, particularly given the imminent availability of sequences for comparative genomic analysis of human and mouse OR gene clusters. The coordinate control of global gene expression and active regulation at selective sites in the genome is beautifully illustrated through the unique organization and requirements of the olfactory sensory system. It seems likely that similar, as-yet unappreciated, genome-wide control mechanisms will by used in other systems.

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