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The Search for Odorant Receptors

Commentary

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The Question

The first time I thought about olfaction was when I read a 1985 paper from Sol Snyder's group that discussed the unsolved question of how odors are detected in the nose (Pevsner et al., 1985). This paper opened up a fascinating new world for me. It was estimated that humans could perceive 10,000 or more chemicals as having distinct odors. Even more remarkably, subtle changes in an odorous chemical could dramatically change its perceived odor. How could the olfactory system detect such an enormous diversity of chemicals? And how could the nervous system translate this complexity of chemical structures into a multitude of different odor perceptions? To me, this was a monumental problem and a wonderful puzzle. I was hooked.

As a molecular biologist, the logical first question to ask was how the recognition of diverse chemical structures is accomplished in the nose. With this knowledge in hand, one might then be able to explore how sensory information is organized in the nose and the brain to ultimately yield odor perceptions. It seemed obvious from a molecular standpoint that there must be a family of odorant receptors that varied in ligand specificity. It also seemed that olfactory sensory neurons in the nose that detect odorants must express different receptors in order for odorants to elicit different signals in the brain and thereby generate distinct odor perceptions.

The Search

In March 1988, I embarked on a search for odorant receptors; this search would prove arduous, but immensely rewarding. At the time, I had already completed a postdoctoral project in Richard Axel's lab on *Aplysia* neurons. My background was in immunology and I had also been trying to develop a method to identify rearranged genes in the mammalian nervous system, the idea being that such genes might provide insight into its cellular and connectional diversity. I was intrigued by the possibility that gene rearrangement or gene conversion might be involved in the generation of a varied set of odorant receptors or regulate their expression, as with antigen receptors in the immune system. I became obsessed with finding the odorant receptors and stayed on in Richard Axel's lab to look for them.

I first looked for clues as to the molecular nature of the receptors. Odorants were reported to induce GTP-dependent increases in adenylyl cyclase activity in the cilia of olfactory sensory neurons (the apparent site of odorant recognition), suggesting a role for G proteins and cAMP in olfactory transduction (Pace et al., 1985; Sklar et al., 1986). Moreover, the cilia had cyclic nucleotide-gated ion channels, providing a means by which elevated cAMP could alter membrane potential (Nakamura and Gold, 1987). However, odorants were also reported to directly open ion channels in olfactory cilia, suggesting that, like many neurotransmitter receptors, odorant receptors might be ligand-gated ion channels (Vodyanoy and Murphy, 1983; Labarca et al., 1988). Finally, odorants were reported to depolarize other cell types and to even alter the membrane potential of artificial liposomes (Kashiwayanagi and Kurihara, 1984; Nomura and Kurihara, 1987). Thus it was not at all clear what kind of proteins the odorant receptors were or, for that matter, whether they even existed.

I decided to take an unbiased approach with regard to the structure of odorant receptors and to focus on two assumptions: first, odorant receptors would be proteins encoded by a family of related genes and, second, odorant receptors would be selectively expressed by olfactory sensory neurons. I first tried an unconventional approach in which I replica screened an olfactory cDNA library with large amounts of 32p-labeled genomic DNA or brain cDNA. The idea was that clones containing repetitive sequences would be labeled by both probes whereas clones containing members of an olfactory multigene family would be labeled only by the genomic DNA probe. I also tried a cDNA subtraction approach to identify genes selectively expressed in olfactory sensory neurons and, in addition, tried to develop a way of cloning genes that were related, but not identical. These efforts yielded some genes that appeared to be specifically expressed in olfactory sensory neurons, but none belonged to a family, so I set them aside.

The Discovery

Advances in technology often underpin advances in science, and this was indeed the case in our discovery of odorant receptors. The development of the polymerase chain reaction (Saiki et al., 1985), coupled with the discovery of a thermostable DNA polymerase (Saiki et al., 1988) and the development of programmable thermocyclers (Weier and Gray, 1988), revolutionized molecular biological techniques.

In 1989, an olfactory neuron-specific G protein was identified, strengthening the case for a G protein-coupled mechanism of olfactory transduction (Jones and Reed, 1989). In addition, while the sequences of only two types of G protein-coupled receptors (GPCRs) were known in 1986, the number had grown to almost 20 by 1989, and it was evident that the GPCBs all shared limited sequence motifs and a potential seven transmembrane domain structure. That year, it was shown for the first time that degenerate oligonucleotide primers could be used in PCR reactions to uncover new members of protein families, including GPCRs (Libert et al., 1989; Wilks, 1989). I tried using the published GPCR primer pair, but found only a dopamine receptor.

At that point, I decided to conduct an exhaustive search for GPCRs in the olfactory epithelium by using a number of different degenerate primers in a combinatorial fashion. The idea was that different parts of an olfactory receptor GPCR might be related to different

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non-olfactory GPCRs. After aligning all previously identified GPCRs, I designed a minimal set of 11 degenerate primers that would permit amplification of sequences encoding all known GPCRs. I then used the primers in all 30 possible combinations in PCR reactions with olfactory epithelium cDNA. These reactions yielded a large number of bands (64) in the appropriate size range on agarose gels. I reasoned that if a band contained multiple members of a multigene family, restriction enzymes would cleave the DNA in the band into a large number of fragments whose sizes summed to much more than the size of the undigested DNA. When DNA in each of the 64 bands was reamplified and treated with restriction enzymes, only one met this criterion, #13. When I cloned this PCR product and sequenced five of the clones, I found precisely what we had been looking for. All five encoded proteins were different, but each one showed sequence hallmarks of the GPCR superfamily. Even more importantly, the five shared sequence motifs not seen in other known GPCRs, indicating that they were members of a novel protein family.

Subsequent experiments provided full-length sequences for multiple members of the receptor family. Though they shared sequence motifs not seen in other GPCRs, the receptors were highly variable in sequence, consistent with an ability to recognize odorants with varied structures. Northern blots and cDNA library screens showed that the receptor family was predominantly or exclusively expressed in olfactory sensory neurons. Genomic library screens with a mixed receptor probe (together with nested PCR of clones to assure accuracy) revealed over 100 receptor clones per haploid genome. Given the limited complexity of the probe, this suggested that the olfactory receptor gene family was likely to be composed, at a minimum, of many hundreds of genes.

Analysis of genomic clones showed that the olfactory receptors were encoded by a single exon, excluding the possibility that gene rearrangement or alternative splicing help to generate olfactory receptor diversity. Neither comparisons of the 5′ and 3′ ends of different receptor cDNAs nor Southern blots of DNA from olfactory neurons versus other cell types suggested an involvement of gene rearrangement in the control of receptor gene expression. In addition, comparison of the sequence of one receptor gene and its encoded cDNA revealed no evidence of somatic mutation. Thus, in contrast to the immune system, where both gene rearrangement and somatic mutation are involved in the generation of antigen receptor diversity, it appeared that each olfactory receptor gene faithfully encoded a single receptor protein.

Interestingly, the number of genomic versus cDNA library clones that hybridized to a mixed receptor probe suggested that a single olfactory sensory neuron could not express all olfactory receptor genes. This was consistent with observations that different neurons respond to different odorants (Sicard and Holley, 1984), a presumed requirement for odor discrimination. We finally had a molecular means of exploring the mechanisms underlying olfactory perception. Richard Axel and I published our findings in April 1991 (Buck and Axel, 1991). Shortly thereafter, I moved to Harvard Medical School and established my own lab.

The Next Step and Beyond

The discovery of olfactory receptors provided a set of molecular tools that were subsequently used by many labs to explore the mechanisms underlying odor perception. The ensuing years revealed how information derived from different odorant receptors is organized in the nose (Ressler et al., 1993; Vassar et al., 1993) and its synaptic target, the olfactory bulb (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996), as well as at the next relay in the olfactory system, the olfactory cortex (Zou et al., 2001). It was found that mammals have as many as 1000 different types of odorant receptors (Young et al., 2002; Zhang and Firestein, 2002) and that each olfactory sensory neuron expresses only one type (Malnic et al., 1999). It was also found that each receptor recognizes multiple odorants (Zhao et al., 1998; Krautwurst et al., 1998; Malnic et al., 1999; Touhara et al., 1999; Wetzel et al., 1999), but that different odorants are detected by different combinations of receptors (Malnic et al., 1999). Thus, odorant receptors are used combinatorially to encode odor identities, a scheme that could generate more than a billion different odor codes and therein permit the discrimination of a virtually unlimited number of odorous chemicals (Malnic et al., 1999).

In the past thirteen years since the original description of olfactory receptors was published, it has been a great pleasure for me to see the number of groups working in this area expand and, as a consequence, many new insights gained into the molecular and cellular basis of olfactory perception.

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