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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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A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition

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Summary

The mammalian olfactory system can recognize and discriminate a large number of different odorant molecules. The detection of chemically distinct odorants presumably results from the association of odorous ligands with specific receptors on olfactory sensory neurons. To address the problem of olfactory perception at a molecular level, we have cloned and characterized 18 different members of an extremely large multigene family that encodes seven transmembrane domain proteins whose expression is restricted to the olfactory epithelium. The members of this novel gene family are likely to encode a diverse family of odorant receptors.

Introduction

In vertebrate sensory systems, peripheral neurons respond to environmental stimuli and transmit these signals to higher sensory centers in the brain where they are processed to allow the discrimination of complex sensory information. The delineation of the peripheral mechanisms by which environmental stimuli are transduced into neural information can provide insight into the logic underlying sensory processing. Our understanding of color vision, for example, emerged only after the observation that the discrimination of hue results from the blending of information from only three classes of photoreceptors (Rushton, 1955, 1965; Wald et al., 1955; Nathans et al., 1986). The basic logic underlying olfactory sensory perception, however, has remained elusive. Mammals possess an olfactory system of enormous discriminatory power (for reviews see Lancet, 1986; Reed, 1990). Humans, for example, are thought to be capable of distinguishing among thousands of distinct odors. The specificity of odor recognition is emphasized by the observation that subtle alterations in the molecular structure of an odorant can lead to profound changes in perceived odor.

How are the diversity and specificity of olfactory perception accomplished? The detection of chemically distinct odorants presumably results from the association of odorous ligands with specific receptors on olfactory neurons, which reside in a specialized epithelium in the nose. Since these receptors have not been identified, it has been difficult to determine how odor discrimination might be achieved. It is possible that olfaction, by analogy with color vision, involves only a few odor receptors, each capable of interaction with multiple odorant molecules. Alternatively, the sense of smell may involve a large number of distinct receptors each capable of associating with one or a small number of odorants. In either case, the brain must distinguish which receptors or which neurons have been activated to allow the discrimination between different odorant stimuli. Insight into the mechanisms underlying olfactory perception is likely to depend upon the isolation of the odorant receptors and the characterization of their diversity, specificity, and patterns of expression.

The primary events in odor detection occur in a specialized olfactory neuroepithelium located in the posterior recesses of the nasal cavity. Three cell types dominate this epithelium (Figure 1A): the olfactory sensory neuron, the sustentacular or supporting cell, and the basal cell, which is a stem cell that generates olfactory neurons throughout life (Moulton and Beidler, 1967; Graziadei and Monti Graziadei, 1979). The olfactory sensory neuron is bipolar; a dendritic process extends to the mucosal surface, where it gives rise to a number of specialized cilia that provide an extensive, receptive surface for the interaction of odors with the cell. The olfactory neuron also gives rise to an axon that projects to the olfactory bulb of the brain, the first relay in the olfactory system. The axons of the olfactory bulb neurons, in turn, project to subcortical and cortical regions where higher-level processing of olfactory information allows the discrimination of odors by the brain.

The initial events in odor discrimination are thought to involve the association of odors with specific receptors on the cilia of olfactory neurons. Selective removal of the cilia results in the loss of olfactory responses (Bronshtein and Minor, 1977). Moreover, in fish, whose olfactory system senses amino acids as odors, the specific binding of amino acids to isolated cilia has been demonstrated (Rhein and Cagan, 1980, 1983). The cilia are also the site of olfactory signal transduction. Exposure of isolated cilia from rat olfactory epithelium to numerous odorants leads to the rapid stimulation of adenylyl cyclase and elevations in cyclic AMP (an elevation in inositol trisphosphate in response to one odorant has also been observed) (Pace et al., 1985; Sklar et al., 1986; Breer et al., 1990; Boekhoff et al., 1990). The activation of adenylyl cyclase is dependent on the presence of GTP and is therefore likely to be mediated by receptor-coupled GTP-binding proteins (G proteins) (Jones and Reed, 1989). Elevations in cyclic AMP, in turn, are thought to elicit depolarization of olfactory neurons by direct activation of a cyclic nucleotide–gated, cation-permeable channel (Nakamura and Gold, 1987; Dhallan et al., 1990). This channel is opened upon binding of cyclic nucleotides to its cytoplasmic domain, and can therefore transduce changes in intracellular levels of cyclic AMP into alterations in the membrane potential.

These observations suggest a pathway for olfactory signal transduction (Figure 1B) in which the binding of odors to specific surface receptors activates specific G proteins. The G proteins then initiate a cascade of intracellular signaling events leading to the generation of an action potential that is propagated along the olfactory sensory axon