The site for interactions between the nervous system and much of the chemical world is in the olfactory sensory neuron (OSN). Odorant receptor proteins (ORPs) are postulated to mediate these interactions. However, the function of most ORPs has not been demonstrated in vivo or in vitro. For this and other reasons, we created a conditionally immortalized cell line derived from the OSN lineage, which we term odora. Odora cells, under control conditions, are phenotypically similar to the OSN progenitor, the globose basal cell. After differentiation, odora cells more closely resemble OSNs. Differentiated odora cells express neuronal and olfactory markers, including components of the olfactory signal transduction pathway. Unlike other cell lines, they also efficiently target exogenous ORPs to their surface. Strikingly, differentiated odora cells expressing ORPs respond to odors, as measured by an influx of calcium. In particular, cells expressing one ORP demonstrate a specific response to only one type of tested odorant. Odora cells, therefore, are ideal models to examine the genesis and function of olfactory sensory neurons.

Key words: olfactory epithelium; globose basal cell; olfactory sensory neuron; olfactory receptor protein; odorant signal transduction; conditional immortalization

The mature sensory neurons of the mammalian olfactory epithelium (OE) are the primary transducers of odorant signals from the external world to the CNS. Olfactory sensory neurons (OSNs) receive and transmit their stimuli directly: their dendritic termini lie in the nasal cavity, directly exposed to the environment; their axonal termini, unlike those of other sensory neurons, synapse directly onto second-order neurons within the forebrain (Graziadei and Metcalf, 1971; Moulton, 1974; Costanzo and Morrison, 1989). At least partly because of their constant exposure to environmental insults, OSNs die and are replenished throughout life (Farbman, 1990; Crews and Hunter, 1994); this property makes them unlike most other neurons, which, under normal conditions, show limited regeneration in mature mammals (Brustle and McKay, 1996; Weiss et al., 1996; Kuhn et al., 1997; Fawcett and Geller, 1998).

There are three dividing cell types within the mature olfactory epithelium, only one of which gives rise to OSNs (Fig. 1; Caggiano et al., 1994). These are: (1) the horizontal basal cell (HBC), whose nucleus resides in the horizontal cell zone (HCZ); (2) the sustentacular cell (SC), whose nucleus resides in the sustentacular cell zone (SCZ); and (3) the globose basal cell (GBC), whose nucleus resides in the globose cell zone (GCZ). Of these, the GBC is the most prolific in vivo (Graziadei and Monti Graziadei, 1979; Schwartz Levey et al., 1991; Caggiano et al., 1994); for example, retroviral lineage tracing suggests that at least 50% of all cell division occurring just after birth in the rat OE is within the GCZ (Caggiano et al., 1994).

It has been difficult to recapitulate GBC turnover and differentiation in vitro, both in primary cultures (Calof and Chikaraishi, 1989; Pixley, 1992; Mahanthappa and Schwarting, 1993) and in cell lines (Goldstein et al., 1997; Coronas et al., 1997a). In addition, primary culture of OSNs is frequently inefficient and cumbersome (Bozza and Kauer, 1998). Because of these technical obstacles, many questions about the turnover and function of OSNs have remained unanswered. Perhaps the most vexing has been the role of the family of genes encoding putative odorant receptor proteins (ORPs; Buck and Axel, 1991); the lack of robust in vitro models has particularly hindered any direct functional investigations into the coupling by specific members of this family to a physiological odorant response. Although ORPs can be expressed intracellularly via baculoviral infection of insect cells (Raming et al., 1993; Nekrasova et al., 1996), chimeric ORPs can be expressed in heterologous cells (Krautwurst et al., 1998), and at least two receptors have been successfully introduced to OSNs in vivo (Z. Hao et al., 1998; Touhara et al., 1999); functional surface expression of ORPs in intact OSNs has not been achieved in vitro. We, therefore, have created a cell line that we propose will be useful in defining: (1) the role of ORPs in odorant detection; (2) the transduction apparatus within OSNs; (3) signals that influence trafficking of ORPs and other olfactory proteins within OSNs; and (4) factors that influence GBC turnover, differentiation, and maturation.

MATERIALS AND METHODS

Creation of the odora line. A temperature-sensitive mutant (tsAS8) (Jat et al., 1986) of the SV40 large T antigen (Southern and Berg, 1982) in a retroviral backbone (Cepko et al., 1984) was used to create cell lines from the olfactory epithelium. Postnatal day 3 rats were killed according to protocols approved by Tufts University, the Society for Neuroscience, and the National Institutes of Health. Olfactory epithelia from eight pups...
were dissociated using a method (Hunter et al., 1992) we had developed for dissociation of retinae, and were incubated at 33°C (at which tsA58 should be active) in normal medium (DMEM; Bio-Whittaker, Walkersville, MD) containing 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 100 U/ml penicillin (Irvine Scientific, Santa Ana, CA) and 100 µg/ml streptomycin (Irvine Scientific). During plating, 30 µl of a tsA58-Tag viral concentrate (Jat and Sharp, 1989) were added to each dish, which in turn give rise to mature OSNs in the upper neuron zone (UNZ; arrow). Together, these zones span the width of the epithelium from the basal lamina (BL), which separates the epithelium from the underlying lamina propria in which OSN axons extend in bundles (small arrowheads), to the nasal cavity, in which odorants are presented to OSN cell bodies. Odora cells were grown at 33°C to 90% confluency, and were incubated for 5–7 d in normal medium (see above) supplemented with 10% BCS, 100 µg/ml streptomycin, 100 µg/ml penicillin, 5 µg/ml leupeptin, 5 µg/ml pepstatin, 5 µg/ml aprotinin, 5 µg/ml chymotrypsin, and 100 µg/ml pefabloc. After collection and sonication, two volumes of sample buffer (192 mM Tris-HCl, pH 6.8, containing 9% SDS, 15% glycerol, and 2% 2-mercaptoethanol) were added. Lysates were boiled and separated by electrophoresis on a denaturing 10% polyacrylamide gel, then transferred to Immobilon-P (Millipore, Bedford, MA). The membrane was blocked in 5% (w/v) nonfat milk in 200 mM NaCl, 0.1% Tween 20, 50 mM Tris, pH 7.4 for 1 hr, incubated in primary antibody for 2 hr and peroxidase-conjugated secondary antibody (New England Biolabs, Beverly, MA) for 1 hr. After washing, the membrane was incubated in a chemiluminescent substrate (New England Nuclear, Boston, MA) for 1 min and exposed to x-ray film. Samples were normalized by nucleic acid content in order to load extracts from approximately equal numbers of cells per lane.

RT-PCR. RNA was isolated from dissected adult rat olfactory epithelium and retina using RNAzol B (Biotex) following the manufacturer’s recommendation. For oCNGα, oCNGβ, and rCNGβ4, 0.4 µg of RNA was reverse-transcribed using SuperScript II (Life Technologies) primed with random hexamers following the manufacturer’s recommendations. In addition, for oCNGβ, 100 ng of RNA was similarly reverse-transcribed using a specific oligonucleotide primer (5′-TACATCTCTGGCCAAATGTC-3′). The resultant cDNAs (5% of the randomly primed, or all of the specifically primed) were amplified in PCRs using Platinum Tag High Fidelity (Life Technologies) in 2 mM MgSO4. Oligonucleotides used were: oCNGα, forward, 5′-GTGATCATTTACTGAAATGCTTGTG-3′ and reverse, 5′-ATACAGCTACCACTGCACTGG-3′ (Kingston et al., 1996); oCNGβ, forward, 5′-ACATCGGCTGATGAAAGAGAG-3′ and reverse, 5′-TACATCTCTGGCCAAATGTC-3′ (sequences obtained from the Mombaerts laboratory); and rCNGβ4, forward, 5′-TCTCATGTGGCCAAATGAC-3′ and reverse, 5′-CTG-GTCCACATCGCTGCA-3′ (Sautter et al., 1998). Cycling protocol was: 94°C, 2 min; 35–40 cycles of 94°C, 45 sec; 52°C, 45 sec; 72°C, 2 min; and 10 min at 72°C. Fifty percent of each reaction was analyzed on a 1% agarose/1% NuSieve (FMC Bioproducts, Rockland, ME) gel. The prim-

**Figure 1.** Major cellular elements of the olfactory epithelium. The olfactory epithelium contains the primary cell in the olfactory pathway, the OSN, which expresses general neuronal markers, including neurotubulin (left panel). In addition, there are several other cell types within the epithelium; the nuclei of all types are present in cell type-specific zones (right panel; arrows are exaggerated). Included are those of the three dividing progenitors (asterisks) that give rise to the cells of the mature olfactory epithelium. Two of these, the horizontal basal cell within the horizontal cell zone (HCZ) and the sustentacular cell within the sustentacular cell zone (SCZ), give rise only to themselves. The third, the globose basal cell within the globose cell zone (GZ), gives rise to itself, as well as to immature OSNs within the middle neuron zone (MNZ; arrow), which in turn give rise to mature OSNs in the upper neuron zone (UNZ; arrow). Together, these zones span the width of the epithelium from the basal lamina (BL), which separates the epithelium from the underlying lamina propria in which OSN axons extend in bundles (small arrowheads), to the nasal cavity, in which odorants are presented to OSN cell bodies.
Table 1. Sources and reactivity of antibodies used in this study (in order of appearance)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Source; Reactivity in OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuritubulin (β-III tubulin)</td>
<td>Mouse monoclonal, “11”</td>
<td>A. Frankfurter; Talamo et al., 1989</td>
</tr>
<tr>
<td>T-antigen</td>
<td>Rabbit polyclonal</td>
<td>D. Hanahan; this report</td>
</tr>
<tr>
<td>Glial fibrillary acidic protein (GFAP)</td>
<td>Rabbit polyclonal</td>
<td>DAKO; Calof and Chikaraishi, 1989</td>
</tr>
<tr>
<td>Sustentacular cell antigen</td>
<td>Mouse monoclonal, “SUS-4”</td>
<td>J.E. Schwob; Goldstein and Schwob, 1996</td>
</tr>
<tr>
<td>Globose basal cell antigen</td>
<td>Mouse monoclonal, “GBC-1”</td>
<td>J.E. Schwob; Goldstein and Schwob, 1996</td>
</tr>
<tr>
<td>Neural cell adhesion molecule (NCAM)</td>
<td>Rabbit polyclonal</td>
<td>Sigma; Caggiano et al., 1994</td>
</tr>
<tr>
<td>Microtubule-associated protein 5 (MAP5)</td>
<td>Mouse monoclonal</td>
<td>Sigma; Coronas et al., 1994</td>
</tr>
<tr>
<td>Growth-associated protein 43 (GAP-43)</td>
<td>Mouse monoclonal</td>
<td>Sigma; Goldstein and Schwob, 1996</td>
</tr>
<tr>
<td>Olfactory G-protein (G&lt;sub&gt;α&lt;/sub&gt;µ)</td>
<td>Rabbit polyclonal</td>
<td>Santa Cruz; Jones and Reed, 1989</td>
</tr>
<tr>
<td>Adenylyl cyclase type III (ACIII)</td>
<td>Rabbit monoclonal</td>
<td>Santa Cruz; Juilfs et al., 1997</td>
</tr>
<tr>
<td>Olfactory cyclic nucleotide-gated channel (oCNGβ)</td>
<td>Mouse monoclonal</td>
<td>K. Zinn; this report</td>
</tr>
<tr>
<td>Olfactory marker protein (OMP)</td>
<td>Goat polyclonal</td>
<td>F.L. Margolis; Margolis, 1972</td>
</tr>
<tr>
<td>Hemagglutinin</td>
<td>Mouse monoclonal</td>
<td>BabCo; this report</td>
</tr>
</tbody>
</table>

Commercial antibody suppliers: DAKO, Carpenteria, CA; Sigma, St. Louis, MO; Santa Cruz Biotechnologies, Santa Cruz, CA; and Berkeley Antibody Company (BabCo), Berkeley, CA.

Table 2. Odorant mixes used in this study

<table>
<thead>
<tr>
<th>Mix A</th>
<th>Mix B</th>
<th>Mix C</th>
<th>Mix D</th>
<th>Mix E</th>
<th>Mix F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geraniol</td>
<td>N-valeric acid</td>
<td>(+)-carvone</td>
<td>Ethyl-N-butylate</td>
<td>Phenyl ethyl alcohol</td>
<td>Lyral</td>
</tr>
<tr>
<td>N-amylic alcohol</td>
<td>N-enanthic acid</td>
<td>Eugenol</td>
<td>Hexitlacate</td>
<td>Eugenol</td>
<td>Phenyl ethyl amine</td>
</tr>
<tr>
<td>N-butyl acetate</td>
<td>N-pelargonic acid</td>
<td>Cinnamaldehyde</td>
<td>Citral</td>
<td>Acetophenone</td>
<td>Vanillin</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>N-heptanol</td>
<td>(-)-limonene</td>
<td>Cineole (1R)+(+)-(α)-pinene</td>
<td>Citral</td>
<td>Isovaleric acid</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>N-hexanol</td>
<td>Citral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetophenone</td>
<td>N-butanol</td>
<td>2-ethyl fenchol</td>
<td>Isobutyraldehyde</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All odorants were at 50 μM. Some mixes were presented simultaneously; these are noted, for example, as “CD”, for a presentation of mixes C and D.
progeny. We chose a temperature-sensitive oncogene in the hope that cells that were proliferating at the permissive temperature of the oncogene would cease proliferation and differentiate when the cells were removed to a nonpermissive temperature.

Cells from freshly dissociated perinatal rat OE were infected with a retrovirus carrying a neomycin resistance gene and a temperature-sensitive mutant of an oncogene, large T antigen (tsA58-Tag), driven by the murine moloney leukemia virus long terminal repeat (MMLV-LTR) (Southern and Berg, 1982; Cepko et al., 1984; Jat et al., 1986; Jat and Sharp, 1989). After selection at the permissive temperature (33°C), 18 colonies were isolated; of these, five remained viable after freezing and replating. Of these five, we have chosen one for further study; we now call these odora cells, for olfactory-derived, odorant receptor activatable cells. Surprisingly, the other four lines are qualitatively similar in growth properties, gross morphology, and antigen expression (data not shown), suggesting that they were derived from similar progenitor cells. Thus, one population of progenitors appears to be more easily immortalized.

Characterization of odora cells

Odora cells grow rapidly, attach readily to uncoated glass, and are easily dissociated when grown at 33°C in normal medium (see Materials and Methods). This line, which was originally created in 1995, has now been in continuous culture for 2 years; over that time, the gross characteristics of the cells have not changed. Briefly, the cells achieve an epithelial morphology quickly, only rounding up occasionally (Fig. 2A). After differentiation [shift to the nonpermissive temperature (39°C) and addition of insulin and dopamine; see Materials and Methods], odora cells become somewhat flatter and larger (Fig. 2B); in addition, they begin to attain a bipolar morphology, frequently extending long, sometimes branched, processes over many tens of micrometers (C, D). These processes occasionally contact those from other cells (asterisk) or terminate in a filopodium (arrow). Scale bar, 40 μm.

Figure 2. Morphology of odora cells. Odora cells, immortalized by infection with a temperature-sensitive variant of large T antigen (tsA58-Tag), exhibit an epithelial morphology when grown at 33°C in normal medium (A). Dividing cells, which normally round up from the epithelial sheet, are frequent. In contrast, after differentiation, cells remain largely flattened (B); when sparse, their bipolar morphology is apparent. Differentiated cells occasionally extend long, sometimes branched, processes (arrowheads) over many tens of micrometers (C, D). These processes occasionally contact those from other cells (asterisk) or terminate in a filopodium (arrow). Scale bar, 40 μm.

Figure 3. Odora cells express markers of the olfactory sensory neuron lineage, but not of the other two lineages within the mature olfactory epithelium. T-antigen, the immortalizing oncogene, is expressed in the nuclei of odora cells at the permissive temperature (control), but only infrequently after a shift to the nonpermissive temperature (treated) in nuclei (arrow), and occasionally in perinuclear regions (arrowheads). Markers of glia (GFAP), horizontal basal cells (keratins), and sustentacular cells (SUS-4; data not shown) are not expressed in either condition. Importantly, odora cells express a marker of globose basal cells, GBC-1, under control conditions; this expression decreases dramatically with differentiation, as it does in OSNs. Scale bar, 20 μm.
several neuronal markers, including the microtubule-associated protein MAP5 and the growth-associated protein GAP-43; they also do not appear to assemble neurotubulin into microtubules (Fig. 4). Thus, control odora cells share some characteristics with the undifferentiated OSN progenitor, the GBC. As noted above, our other lines appear qualitatively similar; this suggests that cells within the GBC lineage are more easily immortalized, or more easily cultured, than those within the HBC lineage.

Differentiated odora cells largely do not express nuclear T-antigen (Fig. 3), consistent with their slowing in division (cf. Jat and Sharp, 1989). They also have an antigenic profile similar to that for OSNs: they largely cease expression of GBC-1 (Fig. 3), maintain expression of NCAM (Fig. 4), and begin to express MAP5 and GAP-43 (Fig. 4). In addition, they appear to assemble neurotubulin into discernible microtubules within their somata and processes (Fig. 4). Thus, in many respects, differentiated odora cells are similar to olfactory sensory neurons.

Odora cells also express markers that are more selective for OSNs. These include components of the olfactory signal transduction machinery: the olfactory G-protein (G_{olf}), adenyl cyclase type III (ACIII), the β subunit of the olfactory cyclic nucleotide-gated channel (oCNGβ), and olfactory marker protein (OMP). In tissue sections of the olfactory epithelium, the components of the transduction machinery are located within the dendrites at the lumenal surface, whereas OMP is throughout the somata of OSNs (Fig. 5). In odora cells, all of these markers are expressed to some degree before differentiation (Fig. 5); in this regard, control odora cells appear to be more differentiated than GBCs.

However, the expression of G_{olf} and OMP increases after differentiation. For G_{olf}, the protein level of each expressing cell increases: although the percent of cells that produce immunohistochemically detectable levels of G_{olf} does not change with differentiation (Figs. 5, 6), the overall amount of G_{olf} does increase after differentiation, as assayed by protein transfer (Western) blot (Fig. 6). In contrast, for OMP, this change appears to be in the number of cells that express immunohistochemically detectable amounts of OMP: although some cells produce detectable levels before differentiation, nearly all cells produce detectable OMP after differentiation (Figs. 5, 6). This increase in OMP
The transcript of the retinal rod b least two subunits, oCNG (olfactory cyclic nucleotide-gated channel) (Fig. 5). However, the percentage of cells (right, average ± SD) expressing immunohistochemically detectable levels of G_olf does not increase after treatment, suggesting an increase in G_olf levels in each expressing cell. In contrast, the percent of cells expressing detectable levels of OMP increases, and the percent expressing GBC-1 decreases after differentiation.

Expression coincides with the dramatic decrease in numbers of cells producing detectable levels of GBC-1 after differentiation (Figs. 3, 6).

In addition, the distributions of oCNGb and OMP change after differentiation (Fig. 5). Both shifts are consistent with the differentiation of odora cells to a phenotype more like that of an OSN. Strikingly, the altered distribution of oCNGb after differentiation suggests a novel regulation of subcellular distribution within OE-derived cells that may be partly responsible for localizing olfactory-specific components of OSNs (including, potentially, ORPs; see below) to the plasma membrane.

We have shown that odora cells produce the b subunit of the olfactory cyclic nucleotide-gated channel (Fig. 5). However, the cyclic nucleotide-gated channel in OSNs is thought to consist of at least two subunits, oCNGα (Dhallan et al., 1990) (also known as oCNC1, CNG2, or CNCα3) and oCNGβ (Bradley et al., 1994; Liman and Buck, 1994) (also known as oCNC2, CNG5, or CNCα4). In addition, the olfactory epithelium specifically produces a further potential subunit, rCNGb-t (also known as CNG4.3 or CNCβ1b), which is produced from an alternative transcript of the retinal rod b subunit (Sautter et al., 1998; Bönigk et al., 1999). The exact combination of subunits that is used in native OSNs is not known, but coexpression of all three cDNAs results in a channel similar to that found in OSNs (Sautter et al., 1998; Bönigk et al., 1999), and all three polypeptides are expressed in the OE (Bönigk et al., 1999), suggesting that the native channel is a heterotrimer.

We used RT-PCR to demonstrate the presence of RNAs encoding all of these potential subunits. Using primers for oCNGα that have been shown to amplify that subunit specifically (Kingston et al., 1996), we find that odora cells, like OE (but not retina), produce the oCNGα RNA, and that differentiation of odora cells appears to increase the amount of oCNGα RNA (Fig. 7). Using primers for rCNGβ-t that have similarly been shown to be specific (Sautter et al., 1998), we also find that odora cells, like OE (but not retina), produce the rCNGβ-t RNA (Fig. 6). Finally, using primers for oCNGβ, we find that odora cells, like OE (but not retina) produce the oCNGβ RNA; however, this RNA appears to be in low abundance in odora cells relative to OE, because it is difficult to amplify from odora-derived cDNA that has been randomly primed (requiring additional cycles of amplification), or requires specific priming in order to produce substantial amplification (Fig. 7). The products we amplified were not derived from genomic DNA, as at least two of the primer sets (those for oCNGα and oCNGβ) span introns, and would have resulted in larger products than those we amplified in these reactions. Together, these RT-PCR data suggest that odora cells, like OSNs, produce three potential cyclic nucleotide-gated channel subunits, and are, therefore, likely to assemble an OSN-like channel. Although we have shown immunohistochemically that the oCNGβ protein is present in odora cells and in OSNs (Fig. 5), the precise combinations of proteins that is produced and used in odora cells and in OSNs awaits further characterization of the native channels.

Responses to stimuli in odora cells

As differentiated odora cells contain many of the components of the putative signal transduction machinery, we next asked whether these cells can respond to odorants. These cells, unlike many neurons, frequently did not respond (as assayed by changes in intracellular calcium levels; see Materials and Methods) to a potentially depolarizing concentration of potassium (100 mM; Fig. 8). The reasons that they may not always respond to a high concentration of potassium are many; for example, they may not express voltage-activated calcium channels, or they may have an unusually high resting potential. An accurate description of this phenomenon awaits a full characterization of the electrophysiological properties of odora cells.

We have not yet determined whether odora cells express an endogenous ORP; however, because there are hundreds of members of the odorant receptor gene family (Buck and Axel, 1991), the likelihood of testing the cells with an odorant that happened to stimulate their endogenous receptors seems slight. We have used six mixtures of odorants (containing a total of 30 odorants; Table 2), which, because of the broad odorant responsivity in individual cells (Bozza and Kaiser, 1998; Malnic et al., 1999), is likely to stimulate, at least partially, many more than 30 different ORPs. Differentiated odora cells never responded to these mixes (Fig. 8, top; Table 3); although this is consistent with a lack of endogenous ORP expression, it could just as easily reflect our relatively small odorant profile.

Others have shown that the β2-adrenergic receptor can activate the olfactory G-protein (G_olf) (Jones et al., 1990), resulting in a
In this case, the β2-adrenergic receptor to an increase in intracellular calcium, presumably via a stimulation of adenylyl cyclase by G_olf (or G_αs). The resultant increase in cAMP is likely to result in opening of cyclic nucleotide-gated channels within the plasma membrane, as is thought to occur in OSNs.

**Expression and function of ORPs in odora cells**

In marked contrast to the inability of other cell lines to direct ORPs to the plasma membrane (McClintock et al., 1997), odora cells properly and efficiently target exogenous ORPs to their surface in a punctate pattern (Fig. 9, inset). This pattern was seen with both ORP constructs tested (U131, Fig. 9; OR5, data not shown), and was similar to that seen after expression of the β2-adrenergic receptor in odora cells (compare Fig. 8). Odora cells are, therefore, unique in their ability to direct ORPs to their surface. As these ORP-transfected odora cells express (1) an ORP, (2) G_olf (3) ACIII, and (4) subunits of the cyclic nucleotide-gated channel, we reasoned that these cells should respond to stimuli for the exogenous ORP. We have assayed responses by detecting changes in intracellular calcium by ratiometric imaging with fura-2 AM (see Materials and Methods), focusing on those cells that were transfected with the rat ORP, U131. This ORP was isolated in 1997 (McCIntock et al., 1997), but its function has not been previously characterized.

We have used, as noted above, six mixtures of odorants (Table 2) to test for potential odorant responses. As also noted above, the relatively broad responsivity displayed by OSNs (Bozza and Kauer, 1998; Malnic et al., 1999) suggests that our mixes might contain a ligand that would interact with the transfected ORP, particularly since expression of transfected constructs can be quite high.

Differentiated odora cells that had been transiently transfected with an ORP, U131, respond to one of our six mixes of odorants (mix B), but not to the five others (mixes A, C–F; Fig. 9). This increase in intracellular calcium is completely blocked when extracellular calcium is chelated (G. Liu and B. Talamo, personal communication), suggesting that the increase results from calcium influx rather than from release of calcium from intracellular stores. On 18 coverslips, 32% of the cells responded to odorant mix B (Table 3), suggesting that a large fraction (~50%) of the transfected cells (themselves 50–80% of the total number of cells) displayed an odorant response. Those cells that were transfected but did not respond to mix B may express lower levels of transfected ORP, may have been incapable of responding because of changes in other components of the transduction pathway, or may have had inadequate access to the odorant. Nevertheless, after transient transfection with a specific ORP, a relatively large number of cells produced responses to one specific odorant mixture.

Intriguingly, odora cells transfected with U131 display odorant desensitization, a phenomenon that has been described in OSNs (Bozza and Kauer, 1998, their references). We routinely observe a diminished response to successive challenges of odorant (Fig. 9), even when those presentations are minutes apart. Remarkably, within a given experiment, there is little difference among individual cells, both in initial amplitude of response and subsequent desensitization. Odora cells present a rich, relatively uniform, system in which to study this phenomenon in detail.

Which of the components of the stimulating mix can elicit a response? When tested individually, only two, the seven-carbon saturated fatty acid (enanthic acid; EA) and nine-carbon satu-
rated fatty acid (pelargonic acid; PA), ever elicited a response in odora cells (Fig. 9; Table 3). Strikingly, the five-carbon saturated fatty acid (valeric acid; VA) and the seven-carbon saturated alcohol (heptanol) gave no measurable response (0 of 225 cells). Of those cells that responded to odorant mix B, 73% responded to EA (Table 3); of those that responded to EA, 29% also responded to PA. The only cells that responded to EA or PA were among those that had responded to odorant mix B; similarly, no cells ever responded to PA that did not respond to EA. Remarkably, as noted earlier with odorant mixtures, the amplitude and duration of responses among cells within each of the groups were consistent within a given experiment, even in the degree of desensitization. A more exhaustive characterization of the response profile awaits additional experiments; nevertheless, it is...
clear that U131 provides odora cells with the capacity to respond to seven- and nine-carbon saturated fatty acids.

**DISCUSSION**

**The nature of the odora progenitor**

The postnatal rodent olfactory epithelium contains three major dividing cell types: sustentacular cell, horizontal basal cell, and globose basal cell. We have previously shown, under the conditions we have studied, that these three cell types appear to be in independent lineages, and that olfactory sensory neurons appear to be exclusively derived from GBCs (Caggiano et al., 1994). Similar results have been reported by others (Schwob et al., 1994). However, these lineal relationships are not absolute: under some experimental conditions, such as exposure to methyl bromide gas, a single, HBC-like progenitor is competent to give rise to all cell types within the olfactory epithelium (Huard et al., 1998). Thus, there is some plasticity within the progenitors which, under most conditions, is dampened.

It is possible that factors present in the resting olfactory epithelium act to repress transdifferentiation among the three lineages. Indeed, within the OSN lineage itself, it is thought that some aspects of the mature OSN phenotype are transcriptionally repressed in the progenitor cells (Tsai and Reed, 1997; Wang et al., 1997). It is conceivable that some of these controls are lost after transfer of cells *ex vivo*. The loss of these controls would, at least in part, explain the difficulty that many laboratories have had in recapitulating OSN generation *in vitro*, and may also partly account for the partially differentiated phenotype that we observe in control odora cells.

Within the usual OSN lineage, i.e., from GBC to OSN, cells move through several stages of development (for review, see Calof et al., 1998). During the initial stages, the cell is still mitotically active; as it progresses through the subsequent stages, mitosis ceases. We could have infected any of these dividing cells within the early stages of OSN production with our retrovirus immortalizing oncogene, thereby obtaining cell lines with a phenotype between GBC and OSN. Our immuno histochemical results with the ODORA line are consistent with our immortalization of a slightly differentiated OSN progenitor.

**Dopamine as a differentiation agent**

Olfactory sensory neurons express D2 dopamine receptor RNA and protein within the olfactory epithelium (Coronas et al., 1997b), as well as at their target sites in the glomeruli of the olfactory bulb (Nickell et al., 1991; Coronas et al., 1997b). One synaptic target of OSNs is the dopaminergic periglomerular cell, which may, therefore, provide the neurotransmitter that acts on D2 receptors expressed by OSNs. It is conceivable that one form of communication between an OSN and its target is the detection of dopamine by presynaptic receptors on OSN terminals. Thus, dopamine is ideally situated as a potential modulator of OSN function. Indeed, dopamine has been shown to modulate adenyl cyclase levels in olfactory epithelium (Mania-Farnell et al., 1993) and to modulate an inwardly rectifying hyperpolarization-activated current in OSNs (Vargas and Lucero, 1999).

Dopamine may also modulate OSN maturation. Others have shown that activation of a D2 receptor in an olfactory epithelium-derived cell line can lead to differentiation of those cells (Coronas et al., 1997a). Similarly, we have shown here that dopamine elicits differentiation in odora cells; we have also found that odora cells express D2 receptors (J. R. Murrell and D. D. Hunter, unpublished observations). Thus, dopamine, derived from targets within the olfactory bulb, and acting on D2 receptors in OSNs, may act as a differentiation signal for some of the final aspects of OSN maturation.

**The cyclic nucleotide-gated channel**

One component of the odorant signal transduction system that is thought to be vital is the cyclic nucleotide-gated channel. Animals in which one subunit of the channel has been disrupted by homologous recombination display anosmia to all tested odorants (Brunet et al., 1996), suggesting that all of these compounds use activation of this channel as part of the signal transduction process. However, the exact nature of the channel as it exists in OSNs is unclear. The olfactory channel consists of at least two subunits, oCNGα (Dhallan et al., 1990; also known as oCNC1, CNG2, or CNCoα3) and oCNGβ (Bradley et al., 1994; Liman and Buck, 1994; also known as oCNC2, CNG5, or CNCe4), and perhaps a third, rCNGβ-t (Sautter et al., 1998; Bönigk et al., 1999; also known as CNG4.3 or CNCe1b). It seems plausible that the native channel is a heterotrimer of all three subunits (Sautter et al., 1998; Bönigk et al., 1999). We have shown that odora cells produce RNAs encoding all three potential subunits of the olfactory cyclic nucleotide-gated channel. Therefore, odora cells are likely to serve as a good model for studying this channel.

Activation of the olfactory cyclic nucleotide-gated channel can lead to an influx of calcium through the OSN membrane, which could subsequently activate a variety of channels within the plasma membrane (for review, see Schild and Restrepo, 1998). Whether a similar cascade exists within odora cells is unclear; however, the influx in calcium we measure in response to odorants suggests that at least some portion of this pathway is operating as it does in OSNs.

**Odorant receptor proteins and odor codes**

We have, for the first time, demonstrated the expression of full-length exogenous odorant receptor proteins in a cell line. The ready availability of large numbers of cells expressing the same odorant receptor will allow for a careful analysis of the odorant response profiles conferred by a given odorant receptor.
protein. Here, we have shown a moderate degree of flexibility, but also a fair amount of selectivity, in the chemical nature of the odorant that stimulates a given odorant receptor protein. Similar results have been obtained in OSNs that were infected with a single odorant receptor (I7, Zhao et al., 1998; MOR23, Touhara et al., 1999), and are consistent with the many reports that the primary reception event is somewhat broadly tuned (Bozza and Kauer, 1998; Malnic et al., 1999; Duchamp-Viret et al., 1999).

Where, then, is odorant selectivity achieved? The bulk of the data generated within the olfactory system in the last several decades has led to the concept that much of the selectivity is achieved via interactions among the neurons of the network within the olfactory bulb (Schild, 1988; Kauer, 1991). An understanding of the relationships among individual odorants and their receptors will, therefore, only lay a partial groundwork for an understanding of odor perception. Nevertheless, it will be important to show carefully whether reproducible response profiles contribute to the combinatorial events that occur within the olfactory bulb and result in odorant discrimination. Such profiles can be more easily generated in the defined, consistent system provided by odora cells.

Summary

Our creation and characterization of odora cells is a major step forward: although other lines have been created (Goldstein et al., 1997; Coronas et al., 1997a), none has been as extensively characterized as this line, none appears to follow the course of differentiation (similar to that of GBCs) displayed by this line, and, in particular, none shows the capacity to respond to odorants when expressing a known ORP that we have shown here. Together, these properties of odora cells should allow for an extensive characterization of the generation and function of odorant-responsive cells, a task that has been limited in the past by the necessity to study OSNs in vivo or in relatively inefficient primary culture systems.

The presence of a family of G-protein-coupled odorant receptors was originally proposed in 1991 (Buck and Axel, 1991), based on the initial identification of a multitude of RNAs that could be infected with a single odorant receptor (I7, Zhao et al., 1998; MOR23, Touhara et al., 1999), and are consistent with the many reports that the primary reception event is somewhat broadly tuned (Bozza and Kauer, 1998; Malnic et al., 1999; Duchamp-Viret et al., 1999).

REFERENCES


Jones DT, Masters SB, Bourne HR, Reed RR (1990) Biochemical characterization of three stimulatory GTP-binding proteins. The large and


