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FEATURE ARTICLE

Neural Regeneration and the Peripheral Olfactory System

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The peripheral olfactory system is able to recover after injury, i.e., the olfactory epithelium reconstitutes, the olfactory nerve regenerates, and the olfactory bulb is reinnervated, with a facility that is unique within the mammalian nervous system. Cell renewal in the epithelium is directed to replace neurons when they die in normal animals and does so at an accelerated pace after damage to the olfactory nerve. Neurogenesis persists because neuron-competent progenitor cells, including transit amplifying and immediate neuronal precursors, are maintained within the population of globose basal cells. Notwithstanding events in the neuron-depleted epithelium, the death of *both* non-neuronal cells *and* neurons directs multipotent globose basal cell progenitors, to give rise individually to sustentacular cells and horizontal basal cells as well as neurons. Multiple growth factors, including TGF-α, FGF2, BMPs, and TGF-βs, are likely to be central in regulating choice points in epitheliopoiesis. Reinnervation of the bulb is rapid and robust. When the nerve is left undisturbed, i.e., by lesioning the epithelium directly, the projection of the reconstituted epithelium onto the bulb is restored to near-normal with respect to rhinotopy and in the targeting of odorant receptor-defined neuronal classes to small clusters of glomeruli in the bulb. However, at its ultimate level, i.e., the convergence of axons expressing the same odorant receptor onto one or a few glomeruli, specificity is not restored unless a substantial number of fibers of the same type are spared. Rather, odorant receptor-defined subclasses of neurons innervate an excessive number of glomeruli in the rough vicinity of their original glomerular targets. ***Anat Rec (New Anat) 269:33–49, 2002.*** **© 2002 Wiley-Liss, Inc.**

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INTRODUCTION

The olfactory system is unusual among sensory systems in several respects. Most notably, the receptor elements that subserve the olfactory sense are embedded in the olfactory epithelium, which lines a part of the nasal cavity, and are in direct contact with the airborne environment, as they must be to transduce volatile chemical stimuli (Farbman, 1992). Furthermore, the primary sensory cells are bona fide neurons with projections into the central nervous system (CNS) that offer a potential route of transport of infectious agents or other materials from the external environment (Bodian and Howe, 1941; DeLorenzo, 1970; Monath et al., 1983). As a consequence of their relatively unprotected position in the nasal cavity, the cells of the olfactory epithelium can be damaged easily by exposure to toxins, infectious agents, or trauma. Most significantly, the primary olfactory projection, i.e., the olfactory epithelium and its projection by means of the olfactory nerve onto its synaptic target in the CNS, the olfactory bulb, is an exception to the general rule that the nervous system repairs itself only very poorly after injury. Indeed, the remarkable capacity of the olfactory system for recovery after injury helps maintain critical sensory function, despite the system's vulnerability to damage.

Investigative evaluation of regeneration in the primary olfactory projection dates from the middle of the last century and includes the work of Nagahara, which demonstrated regeneration after experimental nerve injury (Nagahara, 1940). Subsequent studies over the next two decades demonstrated the destruction and reconstitution of the epithelium after irrigation with zinc sulfate ($ZnSO_4$), which is directly toxic to the epithelium (Schultz, 1941; Smith, 1951; Schultz, 1960; Mulvaney and Heist, 1971; Matulionis, 1975, 1976; Harding et al., 1978; Burd, 1993; Thompson et al., 2000). Research in this area was invigorated by observations that the epithelium contains neurons of increasing maturity as one proceeds from

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basal to apical in the normal olfactory epithelium, and by data obtained by using the [^{3}H]thymidine technique for assessing cellular proliferation (Andres, 1965; Moulton et al., 1970; Graziadei and Metcalf, 1971; Graziadei, 1973; Moulton, 1974). The latter approach established directly that the olfactory epithelium retains a population of proliferating progenitor cells in the basal layers of the epithelium throughout life, whose daughters can be "chased" apicalward with the passage of time into the neuronal compartment of the epithelium (Graziadei and Graziadei, 1979).

The persistence of progenitor elements throughout adulthood provides a means of accomplishing replacement of olfactory neurons after their experimentally induced destruction by knife cut or lavage with a coagulant toxin such as $ZnSO_4$. Thus, the notion was engendered that the olfactory neuroepithelium undergoes a constitutive, piecemeal turnover of the neuronal population analogous to cellular replacement in other non-neural epithelia, as well as a wholesale reconstitution of that population after lesion as a kind of wound healing (Moulton, 1975; Graziadei and Monti Graziadei, 1978). The scope of the problem further expanded with the discovery of the large family of odorant receptor (OR) genes, the data suggesting selective expression of only one allele of only one OR gene by individual neurons in a spatially restricted manner, and the documentation of the exquisite specificity of axon targeting whereby axons from neurons expressing the same OR converge onto one pair of target glomeruli of the 2000 or so glomeruli in the bulb (Buck and Axel, 1991; Ngai et al., 1993; Ressler et al., 1993, 1994; Vassar et al., 1993, 1994; Chess et al., 1994; Strotmann et al., 1994a,b, 1995a,b, 1996, 2000; Mombaerts et al., 1996). Understanding the extent to which specificity of OR expression and axon targeting reemerge during regeneration is crucial for defining the capacity for recovering sensory function after injury and maintaining perceptual stability during constitutive turnover.

As it has become evident that neural progenitor elements persist and are active in the adult CNS (Gage et al., 1995), albeit to a limited degree, the retention of a robust capacity for repair and replacement in the olfactory system has become less of a biological curiosity and more of an exemplar of a fundamental capacity for adult neurogenesis. Thus, one may hope that a more detailed understanding of the events of epithelial reconstitution will inform our understanding of cell generation in the CNS during development and in maturity and lead to strategies for making replacement of CNS neurons more robust. Accordingly, my purpose is to review what is known regarding the process of epithelial reconstitution in the olfactory system and, to a lesser extent, the reinnervation of the olfactory bulb. My intention is to highlight the cellular and molecular regulation of neuronal regeneration and to outline strategies for future investigation. First, however, it is necessary to describe the constituent cell types and the phenomenology of turnover and regeneration in the olfactory system.

The remarkable capacity of the olfactory system for recovery after injury helps maintain critical sensory function despite the system's vulnerability to damage.

CELLULAR CONSTITUENTS OF THE PERIPHERAL OLFACTORY SYSTEM

The olfactory mucosa, consisting of the olfactory neuroepithelium and the underlying lamina propria, lines the posterodorsal nasal cavity in terrestrial mammals (Farbman, 1992). The epithelium is composed of a limited number of cell types whose somata are arranged in a roughly laminar pattern (Fig. 1). From the apical surface to the basal lamina, they are sustentacular (Sus) cells, mature olfactory sensory neurons (OSNs), immature OSNs, globose basal cells (GBCs), and horizontal basal cells (HBCs). In addition, Bowman's glands/ducts (BG/D) extend from the lamina propria through the epithelium to discharge contents at the apical surface (see Table 1 for a complete list of abbreviations). Finally, the fascicles of the olfactory nerve, in which the axons of the OSNs project to the olfactory bulb accompanied by ensheathing glia, run roughly anterior to posterior in the lamina propria converging progressively to a small number of large bundles that pass through the foramina of the cribriform plate.

The Sus cell is a non-neuronal supporting cell capped by microvilli, rather than the cilia that are typical of columnar respiratory epithelial cells, and contains abundant endoplasmic reticulum (Farbman, 1992). Sus cells express cytokeratins 8 and 18, which are generally found in simple epithelial cells, including those in respiratory epithelium (Schwob et al., 1995). Sus cells express multiple cytochrome P450s and other biotransformation enzymes at levels higher than in liver, suggesting that Sus cells serve a detoxification function (Ding and Coon, 1988; Chen et al., 1992). Sus cells also phagocytose dead neurons when the latter die at a rapid clip (Suzuki et al., 1995, 1996). The low rate of Sus cell proliferation has been interpreted as indicating slow turnover and self-replacement, and/or an accommodation to the slow growth of the olfactory epithelium in rodents (Graziadei and Graziadei, 1979; Weiler and Farbman, 1998). Microvillar cells, a second type of supporting cell, lack the abundant endoplasmic reticulum of Sus cells and express distinct antigens (Carr et al., 1991).

Mature OSNs are bipolar in shape: an apical dendrite ends in a knob that elaborates 12 or more cilia splaying out over the surface of the epithelium, and a thin, unmyelinated axon exits the epithelium basally to join with fascicles of the olfactory nerve running to the bulb (Farbman, 1992). Physiological data and analyses of the density of intramembranous particles suggest that sensory transduction occurs in the cilia (Farbman, 1992). The mature neurons are marked by the expression of the olfactory marker protein (OMP), and components of the sensory signal transduction cascade (Monti Graziadei et al., 1977). Deep to the band of mature neurons sit imma-

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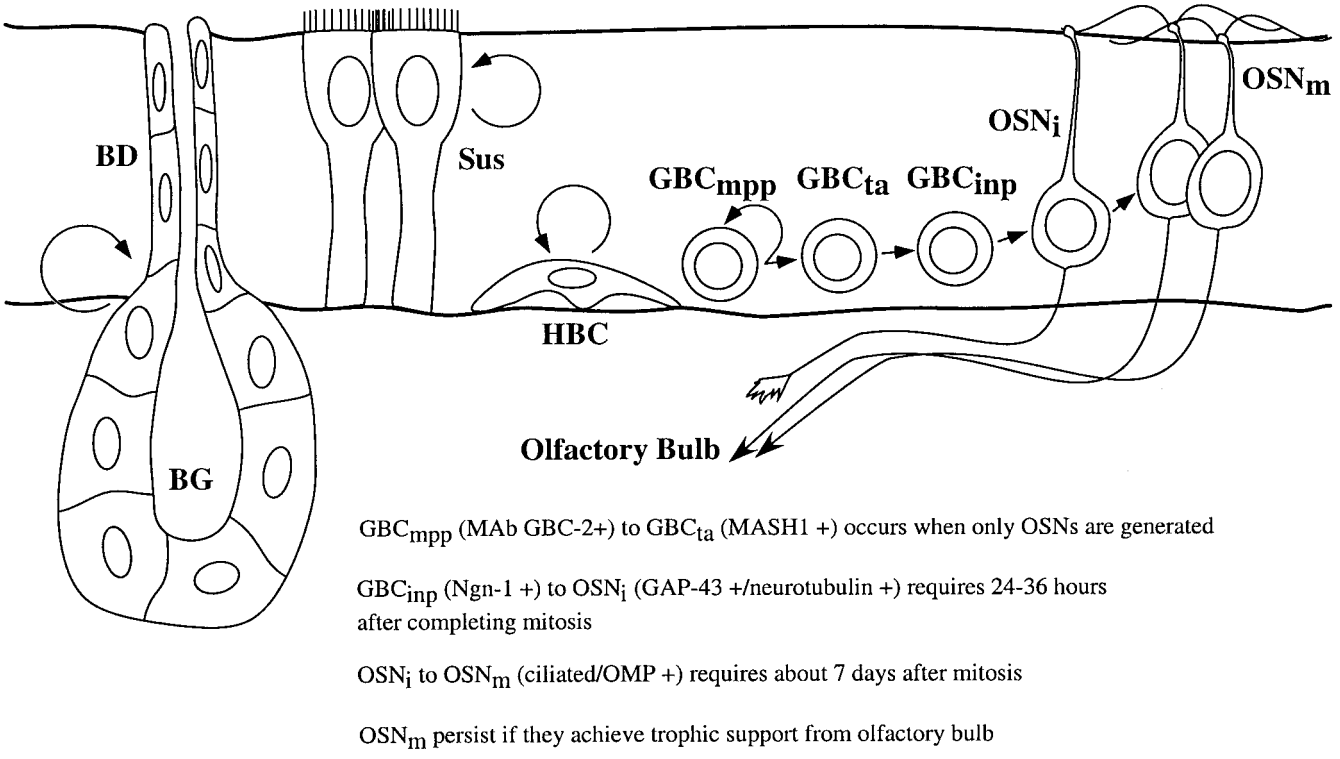

Figure 1. Cellular constituents and progenitor-progeny relationships in the normal olfactory epithelium and after neuronal depletion. Each class of non-neuronal cell resides within its own separate lineage in this context. However, a category of globose basal cell (GBC) that is multipotent is mitotically active even when only neurons are made. Other than general GBC markers, for example, immunoreactivity with the monoclonal antibody GBC-2, no marker is known to identify the class of multipotent progenitors specifically. In contrast, the transit amplifying and immediate neuronal precursor classes express MASH1 and neurogenin-1 (Ngn-1), respectively. BD, Bowman's duct; BG, Bowman's gland; GBC_{mpp}, multipotent progenitor subclass of GBC; GBC_{ta}, transit amplifying subclass of GBC; GBC_{inp}, immediate neuronal precursor subclass of GBC; Sus, sustentacular cell; HBC, horizontal basal cell; OSN_i, immature olfactory sensory neuron (growth-associated protein-43–expressing, nonciliated, axons tipped by growth cones); OSN_m, mature OSN (olfactory marker protein (OMP)-expressing, ciliated, synaptically connected to bulb).

TABLE 1. Abbreviations for cell types, factors, receptors, and reagents

BDNF	Brain-derived neurotrophic factor
BG/D	Bowman gland and duct
BMP	Bone morphogenetic protein
FGF2	Fibroblast growth factor 2
GAP-43	Growth associated protein of 43-kDa molecular weight
GBC	Globose basal cell
HBC	Horizontal basal cell
IGF-1	Insulin-like growth factor 1
IGSF	Immunoglobulin-like superfamily
MeBr	Methyl bromide
NGF	Nerve growth factor
NT-3	Neurotrophin 3
OMP	Olfactory marker protein
OR	Odorant receptor
OSN	Olfactory sensory neuron
Sus	Sustentacular cell
PDGF	Platelet-derived growth factor
TGF-α	Transforming growth factor, alpha
TGF-β	Transforming growth factor, beta

ture OSNs, which express GAP-43 at a high level but not OMP, and have not yet extended cilia (Verhaagen et al., 1989; Meiri et al., 1991; Schwob et al., 1992). The ratio of mature to immature neurons varies through the life of the animal. The epithelium is composed of mainly immature olfactory neurons early in the development of the olfactory system, during the period immediately after a reversible lesion of the olfactory epithelium, and over the long-term as a consequence of the absence of the synaptic target (Verhaagen et al., 1989, 1990; Schwob et al., 1992; Schwob et al., 1995; Loo et al., 1996). Based on these and other data, the time required for a newly born neuron to make the transition to maturity is approximately 1 week after the last mitosis (Miragall and Monti Graziadei, 1982; Schwob et al., 1992).

The basal cells of the epithelium are divided into two categories (Andres, 1965; Graziadei and Graziadei, 1979). GBCs are simple, round cells with scant cytoplasm. Several functionally anonymous markers have been identified that label GBCs, but are not limited to them (Goldstein and Schwob, 1996; Goldstein et al., 1997). GBCs exhibit a high proliferative rate, such that the vast majority of cells labeled

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by the incorporation of thymidine analogues in the normal adult epithelium are GBCs (Schwartz Levey et al., 1991; Huard and Schwob, 1995). By contrast, HBCs appear more specialized. The HBCs form hemidesmosomes with the basal lamina, express cytokeratins 5 and 14 and a carbohydrate moiety recognized by *Griffonia* lectin-like basal cells in other epithelia, overlie small bundles of axons as they exit the epithelium, and proliferate at a low rate (Holbrook et al., 1995).

The final cellular element of the mucosa is the BG/D unit. The acinus, which is composed of cells with abundant secretory granules, resides within the lamina propria, and the channel lined with flattened duct cells extends to the surface (Farbman, 1992). The cells share certain phenotypic characteristics with Sus cells, including the expression of identical cytokeratins, cytochrome P450s, and anonymous markers recognized by monoclonal antibodies (MAbs) raised against the epithelium (e.g., MAbs SUS-1 and SUS-4) (Hempstead and Morgan, 1985; Chen et al., 1992; Schwob et al., 1995; Goldstein and Schwob, 1996). As discussed further below, there is also likely to be a lineage relationship between duct cells and Sus cells, at least during the recovery of the epithelium after direct injury.

Within many of the categories of cells, there is an additional diversity of types. With respect to the OSNs, diversity exists at two levels. First, the epithelium can be divided into zones defined by the subset of ORs from which the OSNs in that zone can select to express (Ressler et al., 1993; Vassar et al., 1993; Strotmann et al., 1995a; Sullivan et al., 1996), and by the differential expression of cell-surface molecules, for example, the immunoglobulin superfamily member variously known as OCAM, RNCAM, NCAM-2 and mamFas II (the latter name intending to indicate the close homology to Fasciclin II in Drosophila) (Mori et al., 1985; Schwob and Gottlieb, 1986; Alenius and Bohm, 1997; Paoloni-Giacobino et al., 1997; Yoshihara et al., 1997; Fang, 2001). Second, within a zone of the epithelium, neurons differ one from their neighbor by their selection of the single OR they express from among the subset available (Ressler et al., 1993; Vassar et al., 1993; Strotmann et al., 1995a; Sullivan et al., 1996). Similarly, Sus and BG/D cells express different biotransformation enzymes depending on epithelial location, as exemplified by differential expression of phenol sulfotransferase G at a high level in dorsomedial epithelium (Miyawaki et al., 1996) and the differential sensitivity of the epithelium to injury by assorted olfactotoxins (Schwob et al., 1994b).

THE PHENOMENA OF OLFACTORY REGENERATION

One of the singular advantages of the study of olfactory regeneration as a model for general neural development is the ease and selectivity with which the system can be lesioned in vivo and the rapidity of its recovery. There are two forms of lesion: (1) direct damage to the epithelium by exposure to one of a variety of toxic agents that damage multiple cell types, and (2) selective neuronal degeneration secondary to axonal damage.

Transection of the Olfactory Nerve

Selective loss of neurons can be accomplished by transection of the large bundles that compose the olfactory nerve as it traverses the cribriform plate. Using a Teflon knife leaves the olfactory bulb mostly intact (although some damage is inevitable with this procedure), preserving the potential for reinnervation (Costanzo, 1984, 1985, 2000; Yee and Costanzo, 1995, 1998; Koster and Costanzo, 1996; Christensen et al., 2001). Alternatively, the olfactory bulb can be removed by aspiration, which both destroys the olfactory axons that are in contact with it and eliminates their synaptic target (Costanzo and Graziadei, 1983; Monti Graziadei, 1983; Schwob et al., 1992). With both manipulations, there is a profound and rapid loss of neurons after their axons are cut due to retrograde degeneration and apoptosis (Monti Graziadei and Graziadei, 1979; Holcomb et al., 1995). All other cell types are spared direct damage, including those immature OSNs whose axons have not yet reached the point where the nerve was lesioned (Fig. 1). In response to the degeneration, there is a selective and substantial increase in the proliferation of GBCs (Schwartz Levey et al., 1991). In the case of olfactory bulbectomy, the epithelium never recovers fully even with long survivals after lesion: there are fewer neurons than normal, because neuronal lifespan is abbreviated in the absence of trophic support from the bulb (Carr and Farbman, 1992, 1993; Schwob et al., 1992). Moreover, most of the OSNs are immature (Monti Graziadei, 1983; Verhaagen et al., 1990; Schwob et al., 1992). Thus, neurons either do not live long enough to achieve maturity, or do not persist for long after making that transition when born in the absence of their target (Schwob et al., 1992). In the case of nerve transection, the recovery in numbers is more nearly complete, but the rate of GBC proliferation and the percentage of OSNs that are immature remain higher than controls (Costanzo, 1984; Christensen et al., 2001). Because areas of the bulb are persistently denervated or at best hypoinnervated, the prevalence of immature OSNs is again a likely indication of accelerated production and death due to the trophic dependence on bulb-derived factors.

Direct Epithelial Lesion

Agents that are directly olfactotoxic produce a lesion that is more complicated (Fig. 2). Nonetheless, direct damage to the epithelium may be more analogous to the type of injury that occurs in a natural setting where pathogens or toxins are airborne. The classic agents, irrigation with $ZnSO_4$ and Triton X-100, kill all cell types in the affected epithelium (Schultz, 1941, 1960; Smith, 1951; Mulvaney, 1971; Matulionis, 1975, 1976; Harding et al., 1978; Burd, 1993). Substantial areas of the mucosa are often spared, presumably due to incomplete or uneven spread of the toxin. In addition, recovery is incomplete, and more severely damaged areas that were previously olfactory, reconstitute as respiratory epithelium. The inhalation of low concentrations of methyl bromide gas (MeBr) has proven to be a more reliable and facile means of reversibly damaging the olfactory epithelium of rodents (Hurtt et al., 1987, 1988;

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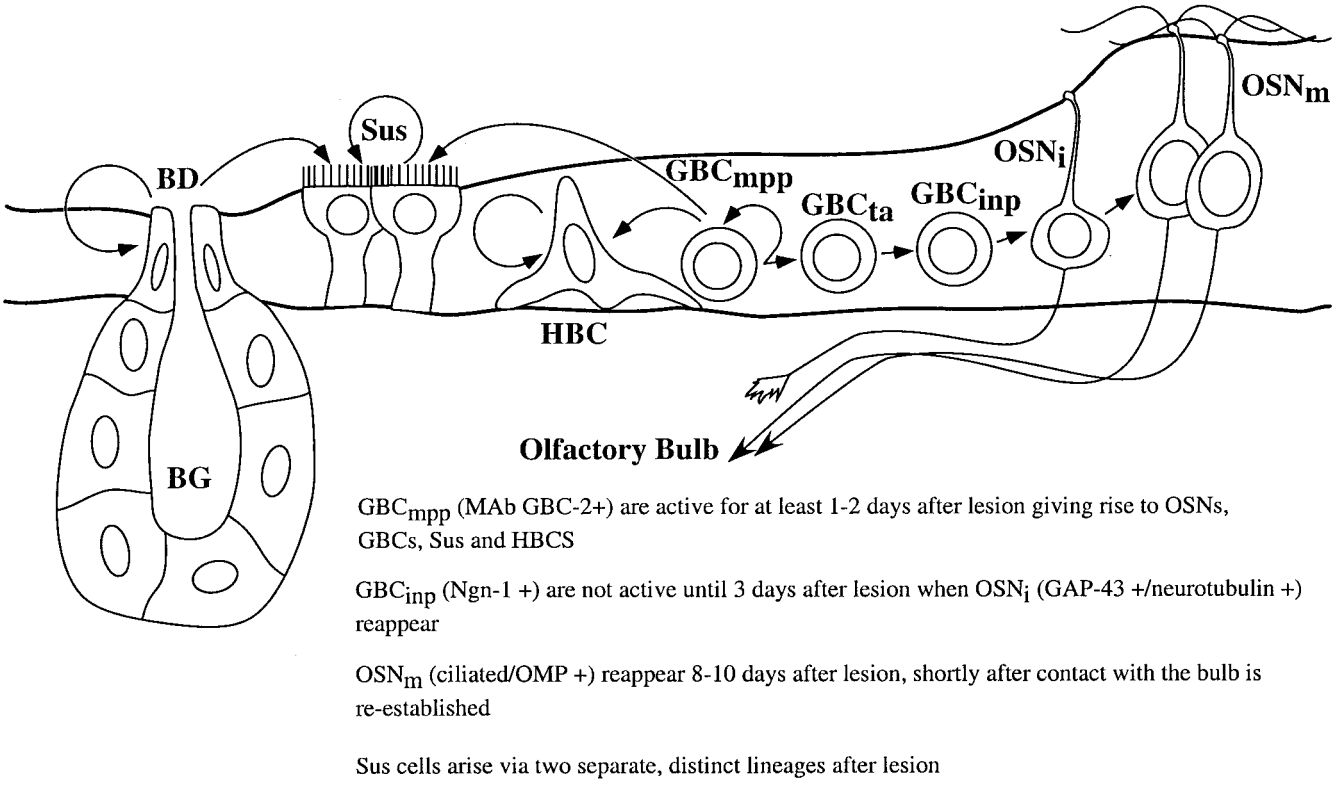

Figure 2. Cellular constituents and progenitor-progeny relationships in the olfactory epithelium after damage to all categories of cells by exposure to methyl bromide (MeBr) or other form of peripheral olfactotoxin. Distinct stages in the reconstitution of the epithelium occur at defined time points after the lesion (symbolized by the progressive stages in neuron generation and the thickening of the epithelium moving toward the right from the center of the figure), allowing them to be studied in relative isolation. Multipotent GBCs are active in giving rise to Sus cells, HBCs, and neurons for at least the first few days after lesion. Neurons do not reappear until 3–4 days after lesion and do not mature until 8–10 days after lesion. HBCs appear reactive in dorsal epithelium. Sus cells also derive from the cells lining the residual Bowman's duct. For abbreviations, see legend to Figure 1.

Schwob et al., 1994b, 1995). The olfactotoxic effects of the gas, which are the sole manifestation of a single multi-hour exposure at levels of 200–400 ppm, are probably due to the generation of free radicals by the cytochrome P450 system, leading to peroxidative damage to sustentacular cells, neurons, ducts, glands, and basal cells (Hallier et al., 1994; Yang et al., 1995). Of these, Sus cells and OSNs are completely eliminated from greater than 95% of the olfactory epithelium, whereas a large percentage of basal cells are spared (Hurtt et al., 1988; Schwob et al., 1995). Despite the severity of the lesion, there is rapid and robust regeneration of OSNs and Sus cells, such that the epithelium closely resembles unlesioned control by 8 weeks after exposure, e.g., the rate of basal cell proliferation, the ratio of mature to immature OSNs, cytochrome P450 levels, and other Sus markers have returned to control levels (Hurtt et al., 1988; Schwob et al., 1995).

With both nerve cut and direct epithelial lesion, the epithelium also recovers with respect to its more global properties. For example, the division of the epithelium into zones defined by differential expression of odorant receptors and of the IgSF member OCAM/mamFas II is restored after the epithelium is reconstituted (Schwob and Youngentob, 1992; Schwob et al., 1994b; Christensen et al., 2001). Indeed, the zonal distribution of OR probe-positive OSNs after recovery from MeBr lesion are indistinguishable from control when assayed by in situ hybridization for each of 8 different ORs (Iwema et al., 1997). Zonality of OR expression is also comparable to control after the partial recovery from the neuron loss caused by bulbectomy (Konzelmann et al., 1998). Likewise, P2 receptor-expressing OSNs are found in the same epithelial zone after recovery from bulb ablation, nerve section, and MeBr exposure (Costanzo, 2000; Iwema and Schwob, 2001).

CELLULAR EVENTS ACCOMPANYING EPITHELIAL REGENERATION

The foregoing demonstrates the capacity of the olfactory epithelium to reconstitute in two very different settings: one where only neurons are needed to restore the epithelium to its prelesion condition, and the other where all cellular populations need to be reconstituted either in whole or in part. Other self-renewing tissues, for example, the hematopoietic system, are confronted with a similar task. Thus, there may be a need to reconstitute whole blood (after hemorrhage) or particular constituents (for example, platelets after their selective depletion by autoimmune destruction). The capacity for full reconstitution of the blood or other renewable tissues depends on the preservation of totipotent stem cells, which are able to give rise ultimately to all types of cells and to self-renew without apparent limitation (Weissman, 2000). At

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subsequent stages, the differentiative and proliferative capacity is progressively restricted as progenitor cells pass through a pluri- or multipotent stage to the point where cells are committed to a particular fate either while continuing to divide and expand the population of derivatives (transit amplifying cells) or producing the final differentiating daughter cells (immediate precursor).

The application of a "poietic" model to the olfactory epithelium has met with some success. The existing data suggest that the population of GBCs harbors both transit amplifying cells committed to a neuronal lineage, and immediate neuronal precursors (Figs. 1, 2). The pulse-chase experiments with thymidine analogues mentioned above first suggested that the population of GBCs harbors the population of immediate neuronal precursors (Graziadei and Graziadei, 1979; Schwartz Levey et al., 1991). Results from studies of neurogenesis in vitro also indicate that some GBCs are immediate neuronal precursors (Calof and Chikaraishi, 1989). Finally, the most direct evidence comes from the use of replication-incompetent, retrovirally derived vectors to define lineage (Caggiano et al., 1994; Schwob et al., 1994a). In these experiments, vector-labeled OSNs are observed in exclusive association with GBCs after direct injection of the vector into the epithelium. Rarely, HBC-only clusters are observed (Caggiano et al., 1994). The timing and spatial pattern of expression of the basic helix-loop-helix transcription factor neurogenin-1 during development suggests that it is expressed by those GBCs that function as immediate neuronal precursors (Cau et al., 1997). In addition, another subset of GBCs, which express the transcription factor MASH1, seem to be transit amplifying cells (Guillemot and Joyner, 1993; Gordon et al., 1995). That functional assignment is based on the expansion of the MASH1 (+) population in advance of the increase in the overall rate of GBC proliferation and upstream of the neuronal differentiation factor neurogenin1 (Gordon et al., 1995; Cau et al., 1997). The latter interpretation fits with the absence of neurons from the olfactory epithelium in *Mash1* knockout mice (Guillemot et al., 1993).

The foregoing data, derived from experimental settings in which only neurons are being generated at a substantial rate, have been interpreted from a somewhat neurocentric viewpoint as indicating that GBCs serve only as *committed* neuronal progenitors (Calof et al., 1998). According to this view, any need to replace Sus cells, HBCs, or BG/D is satisfied by self-renewal wherein existing Sus cells would generate new Sus cells, etc. A stem cell capable of continuing to renew the neuronal population has either been localized with GBCs or with the population of HBCs (Mackay-Sim and Kittel, 1991; Mumm et al., 1996); the assignment of stem cell function to HBCs has been based, in large part, on the identification of cells that are intermediate in appearance between HBCs and GBCs (Graziadei and Monti Graziadei, 1978; Graziadei and Graziadei, 1979).

A slightly more complex view of progenitor cell capacity emerges from the detailed study of the recovery of the epithelium after MeBr lesion (Fig. 2). In this setting, three different strategies—lineage tracing, tissue analysis by using cell-specific markers, and transplantation of marker-selected cell types into the epithelium—all suggest that at least some GBCs and BG/D cells have a broader differentiative capacity than that observed in normal or bulbectomized epithelium. The application of lineage-tracing vectors to the MeBr-lesioned epithelium is facilitated by the fact that the proliferating cells can be infected by means of simple infusion into the nasal cavity after destruction of neurons and sustentacular cells (Huard et al., 1998). In addition, diluting the vector throughout the nasal cavity ensures that only a single progenitor cell is infected productively within a particular area of the epithelium. As a consequence, clusters of vector-labeled cells are clonal in this paradigm. Quite strikingly, the data indicate that single progenitor cells give rise to both neurons and non-neuronal cells, including Sus cells, microvillar cells, and HBCs, in addition to GBCs and neurons. Other clones are composed of Sus cells plus BG/D cells. Finally, some clones encompass only neurons and GBCs, or only Sus cells. The latter data are expected given the kinetic and lineage tracing data in normal or bulbectomized epithelium referenced above and indicate that some progenitor cells are fated to give rise to a single lineage even in the setting of the MeBr-lesioned epithelium. Neurons and GBCs are never observed in the same clone as BG/D cells.

Analysis of marker expression in MeBr-lesioned tissue is also consistent with the lineage results (Goldstein and Schwob, 1996). Antibodies that label GBCs and neurons in the normal epithelium are coexpressed on cells with HBC or Sus cell characteristics during the first few days after MeBr lesion. The most parsimonious interpretation of the data is that both HBCs and Sus cells can be generated by GBCs in a setting in which all cell types are being reconstituted. That is, the immunohistochemical analysis also suggests that some GBCs are multipotent in their differentiative capacity. In addition, Sus and BG/D-specific antibodies label cells that look to be crawling away from the ducts along the surface of the epithelium and are likely to be the source of the lineage association between Sus cells and BG/D cells noted above (Schwob et al., 1995).

Finally, transplantation of labeled cells also confirms the interpretation that GBCs are multipotent in their capacity (Goldstein et al., 1998). In one set of experiments cells were dissociated and harvested from the OE of animals shortly after bulbectomy, labeled ex vivo by infection with a conventional retroviral vector or with a highly complex library of vectors (with a theoretical upper limit of 10^7 sequence-distinct vectors), and then transplanted into the OE of MeBr-lesioned host rats. The high numerical complexity of the vector library ensures that each tag represents a unique infective event, allowing clonality of descendents to be proved. The vast majority of cells that are infectible by the vector, i.e., the vast majority of actively proliferating cells in the bulbectomized epithelium, are GBCs. Clones that include both neurons and the full range of non-neuronal cells are observed as was described above for the lineage-tracing experiments. These data have two implications. First, some GBCs are demonstrably multipotent in this assay.

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Second, some of the GBCs that are actively proliferating in a setting in which only neurons are being replaced are not irreversibly committed to generate neurons alone (Fig. 1). Rather, the data suggest that some of the GBCs in a purely neurogenic epithelium are merely fated to make neurons and are not irreversibly committed to do so. Thus, the decision to make only neurons versus both neurons and non-neuronal cells in neurogenic epithelium is one that is made continually by actively proliferating progenitors. In another set of experiments, GBCs were selectively isolated from bulbectomized epithelium by using a combination of antibody markers and fluorescence-activated cell sorting (FACS) (Chen et al., 2001). After infection with the vector, the GBC preparation is transplanted into the MeBr-lesioned host epithelium. The results are preliminary, but the finding that neurons and Sus cells derive from GBCs in the context of the MeBr-lesioned and recovering epithelium support strongly the interpretation that at least some GBCs are multipotent.

MOLECULAR REGULATION OF OLFACTORY EPITHELIOPOIESIS

The cellular events described above indicate that the progenitor cell population in the olfactory epithelium is subject to regulation at multiple levels. For example, neurogenesis accelerates when a wave of dying OSNs, killed by nerve transection, imposes an increased demand for replacement neurons (Schwartz Levey et al., 1991; Carr and Farbman, 1992, 1993; Schwob et al., 1992). Upstream of a commitment to the selective production of neurons, some GBCs are capable of giving rise to both neurons and non-neuronal cells and do so in the context of an epithelium in which all cell types have been depleted by lesion, as reviewed above. The molecular regulation of the aforementioned cellular events is incompletely understood (Fig. 3).

What we know has come from three approaches: primary cell culture (both dissociated and explant), olfactory-derived cell lines, and transgenic knockout mice. A number of agents increase proliferation of the various populations of epithelial cells, including FGF2, EGF, TGF-α, and PDGF, from among the usual suspects (Calof et al., 1991; Mahanthappa and Schwarting, 1993; Farbman and Buchholz, 1996; Goldstein et al., 1997). Remarkably, OMP also stimulates cell division in explant cultures (Farbman and Ezeh, 2000). Other factors have been shown to promote differentiation and suppress further proliferation, including TGF-βs and BMPs (Mahanthappa and Schwarting, 1993; Shou et al., 1999, 2000). Finally, the neurotrophins NGF, BDNF, and NT-3, or other factors such as IGF-1, may also be crucial for survival and differentiation (Roskams et al., 1996; Pixley et al., 1998). For none of these has the analysis been complete enough to allow us to assert their role unequivocally in the overall cellular economy of the epithelium in vivo.

The decision to make only neurons versus both neurons and non-neuronal cells in regenerating neurogenic epithelium is made continually by actively proliferating progenitors.

Nonetheless, there is sufficient consensus among the published data to justify their examination.

The Neurogenic Cascade

Mackay-Sim, Chuah, and colleagues have proposed a sequential model for the molecular regulation of neurogenesis (Mackay-Sim and Chuah, 2000; Newman et al., 2000). Even though not all features of this model are equally well-established, it is a heuristically useful framework for the consideration of the data regarding growth factor control (Fig. 3). In their view, a lineage relationship between HBCs and OSNs remains uncertain but possible. Indeed, there are no direct data in favor of the notion that HBCs are the epithelial stem cells responsible for maintaining neurogenesis in the long term, and some evidence against it (Holbrook et al., 1995). HBCs in semi- or fully dissociated cultures, in explants, and in vivo proliferate in response to growth factors that activate the ErbB family of receptors, including TGF-α and EGF (Mahanthappa and Schwarting, 1993; Farbman and Buchholz, 1996; Ezeh and Farbman, 1998; Farbman and Ezeh, 2000; Getchell et al., 2000). There is evidence that HBCs express the EGF receptor (ErbB-1) (Holbrook et al., 1995; Krishna et al., 1996) and the neu receptor (ErbB-2) (Salehi-Ashtiani and Farbman, 1996; Ezeh and Farbman, 1998). TGF-α is the relevant ligand in vivo; basal cells (not otherwise specified as to type), sustentacular cells, and BGs stain with anti–TGF-α antibodies, whereas EGF has not been demonstrated in the mucosa (Farbman and Buchholz, 1996). In keeping with the data in vitro, insertion of a CK14 promoter-TGF-α transgene to drive overexpression of the growth factor in HBCs causes a multifold, selective increase in proliferation of the HBCs, presumably by means of a paracrine pathway (Getchell et al., 2000). TGF-α is unlikely to be the sole element regulating HBC proliferation; the elimination of TGF-α by genetic recombination does not abolish the already low level of proliferation observed in otherwise normal OE (Getchell et al., 2000). Assaying HBC proliferation after MeBr exposure, because HBCs proliferate in this "natural" setting (Schwob et al., 1995), may provide a more sensitive measure for demonstrating whether TGF-α is an important regulator of HBC behavior in vivo.

A bit more is known about the controls on GBC proliferation and differentiation. Growth factor application to semidissociated and dissociated OE cells in culture demonstrates that FGF2 is a capable of stimulating the proliferation of GBCs (Calof et al., 1991; DeHamer et al., 1994; Mumm et al., 1996; Newman et al., 2000). Most of the cells divide only once or twice in the presence of FGF2, generating a small number of neurons in vitro, i.e., FGF2 appears to drive immediate neuronal precursors. A small percentage of GBCs may respond to FGF2 by undergoing a more pronounced proliferative response, which leads to the formation of larger colonies of neurons (DeHamer et al., 1994). Some slight acceleration of proliferation by

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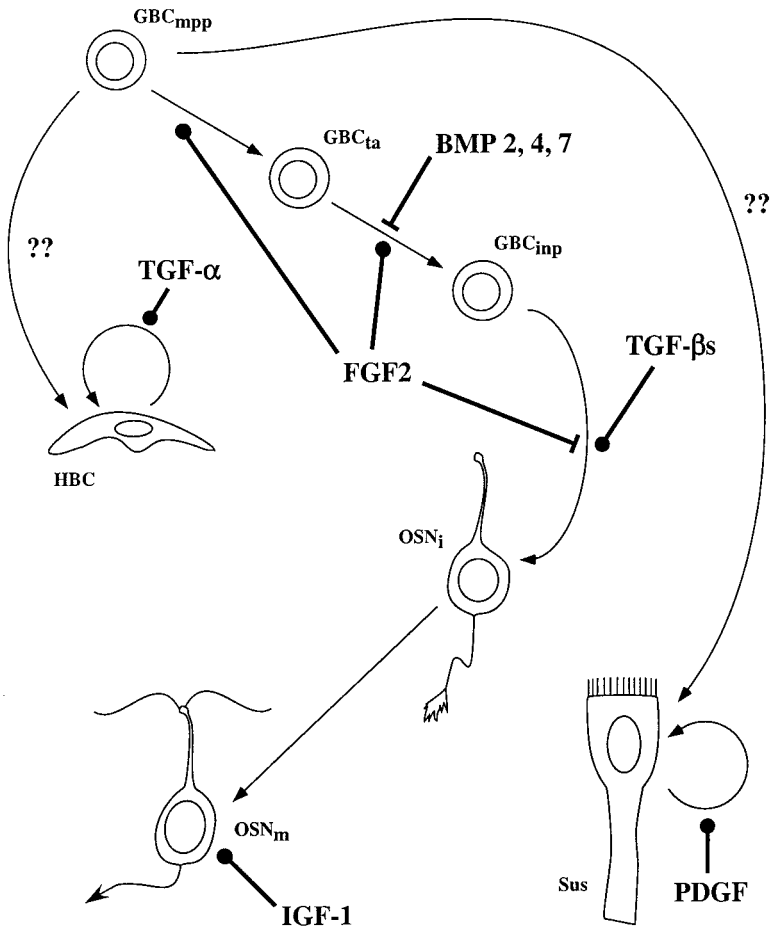

Figure 3. Growth factor regulation on the processes of cellular renewal in the olfactory epithelium apparently depends on similar factors playing similar roles as in other systems. The balance between fibroblast growth factor-2 (FGF-2) and BMP signals seems to be critical for advancing or retarding neurogenesis. Transforming growth factors-β (TGF-βs) stimulate neuronal differentiation in cell lines derived from GBCs. Lines ending in circles designate a stimulatory effect; lines ending in a perpendicular line indicate inhibition. IGF-1, insulin-like growth factor-1; PDGF, platelet-derived growth factor. For other abbreviations, see legend to Figure 1.

FGF2 is also seen with olfactory-derived, spontaneously immortalized cell lines, but to a lesser degree than is observed with a richer medium containing serum and tissue extracts (Goldstein et al., 1997; Ensoli et al., 1998). For the cell lines, the more striking effect is the suppression of neuronal differentiation by FGF2 (Goldstein et al., 1997). Taken together, the data from both suggest that FGF2 may be preparing GBCs to respond to other signals rather than serving as potent mitogen per se. That hypothesis is consistent with the action of FGFs in other differentiating tissues, including muscle (Lathrop et al., 1985; Itoh et al., 1996). Analysis in vivo has been limited to localization of FGF2 and members of the family of FGF-Rs in the OE. FGF2 is found in neurons and sustentacular cells in parts of the epithelium (Goldstein et al., 1997). Members of the FGF-R family that respond to FGF2 have also been found in OE by RT-PCR (DeHamer et al., 1994).

Forms of Negative Feedback on Neurogenesis

Negative regulators of neurogenesis have also been tentatively identified. This line of investigation derives from in vivo phenomena: GBC proliferation accelerates when OSN death is enhanced by nerve transection or bulb ablation, but slows in settings where the neurons are protected and probably longer-lived, i.e., when one side of the nasal cavity is isolated from the environment by naris closure (Farbman, 1990). A more direct demonstration of negative feedback on neurogenesis is the suppression of neuronal colony formation in vitro when cells are cultured with a large excess of differentiated neurons (Mumm et al., 1996). The anti-neurogenic effects of adding neurons to the cultures are mimicked by exogenous BMPs 2, 4, and 7 (Shou et al., 1999, 2000); BMP7 apparently exerts a bimodal effect, stimulating neuronal survival at low concentrations and suppressing neurogenesis at higher levels (Shou et al., 2000). In this setting, BMPs cause the rapid degradation of MASH1 protein (Shou et al., 1999, 2000). It seems that the MASH1-expressing cells die in vitro after the BMP-induced degradation of MASH1 as they do in vivo in animals in which the *Mash1* gene has been disrupted by homologous recombination (Shou et al., 2000). The BMPs are expressed in nasal tissue in vivo; BMPs 4 and 7 are expressed in fetal OE, although cellular localization is incomplete, and all three are found in fibroblasts of the underlying stroma (Shou et al., 2000).

Other members of the TGF-β superfamily, specifically TGF-β1 and TGF-β2, promote neuronal differentiation in semidissociated and dissociated primary cultures of OE and in OE-derived cell lines (Mahanthappa and Schwarting, 1993; Newman et al., 2000). In all of these settings, the expression of neuronal markers, including neuron-specific β-tubulin and NCAM, is markedly up-regulated. In many other tissues, TGF superfamily members have been shown to act similarly. The heterodimer PDGF-AB is effective at preserving the differentiating cells for at least a brief period of time and within a narrow range of concentration (Newman, 2000). Other factors that are expressed in the OE, including BDNF, were ineffective at maintaining long-term neuronal survival in vitro (Holcomb et al., 1995). Indeed, elimination of BDNF, NT-3, or both, by single and double gene knockout does not cause any disruption of OE structure or axon targeting during embryonic development (Nef et al., 2001).

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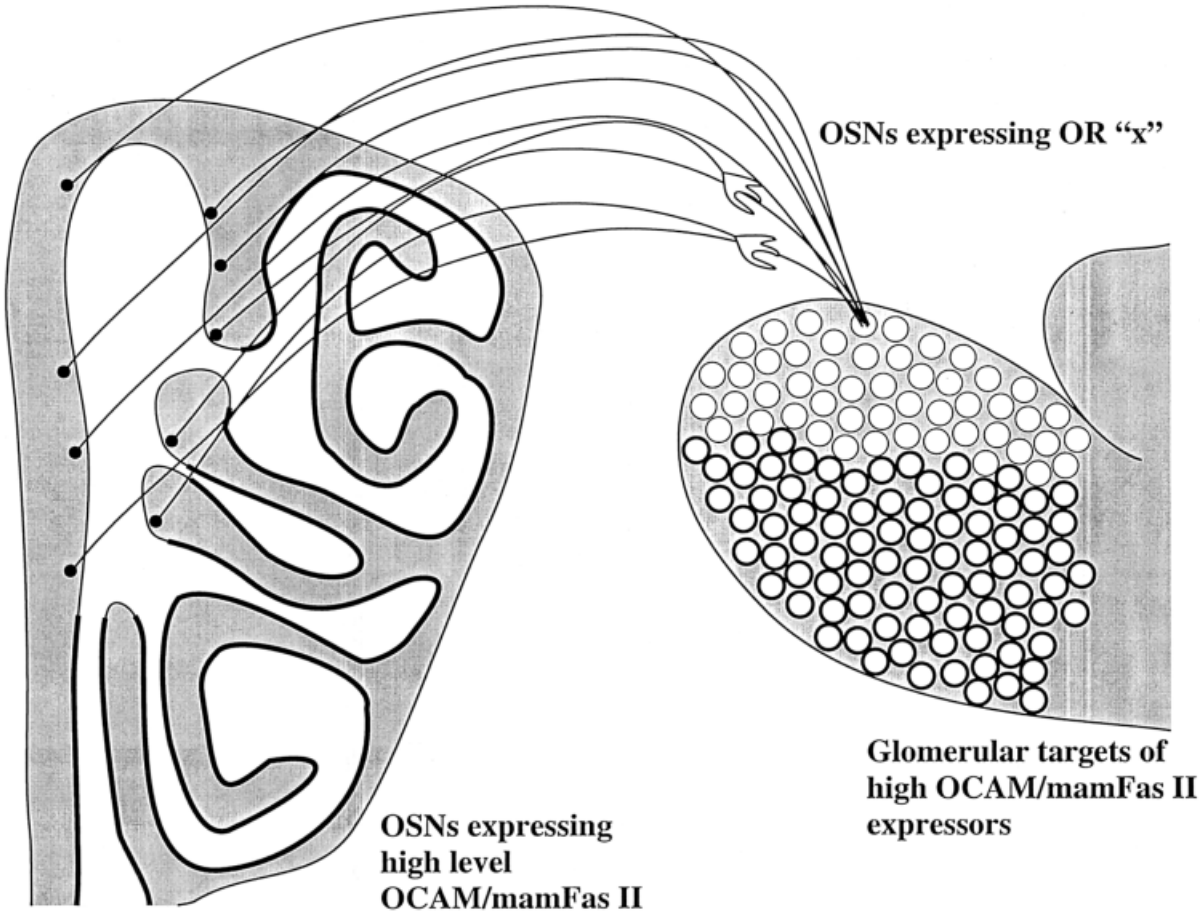

Figure 4. Glomerular targeting during neuronal turnover at a low level. In the primary olfactory projection of "normal" animals, or ones where a substantial population of preexisting neurons and axons survive lesion, newly born neurons are confronted with an environment in which axons that are alike in terms of the odorant receptor (OR) that is expressed can serve to guide later-arriving like-axons (indicated by the symbolic growth cone at their tips). In this setting, glomerular convergence is seemingly preserved throughout life. OSN, olfactory sensory neuron.

LIMITS ON THE REINNERVATION OF THE OLFACTORY BULB AFTER LESION

The capacity for reconstitution of the neuronal population, whether partial or complete, raises the obvious issues of how the olfactory system accomplishes the re-innervation of the adult CNS (not true of other neural systems) and the specificity with which the bulb is reinnervated during the regeneration process. It seems certain that the glial cell environment of the olfactory nerve and the superficial layers of the olfactory bulb is fundamental to the robustness of regeneration. The relationship between axons and ensheathing cells of the olfactory nerve and olfactory glomerular layer, derived from the olfactory placode, is highly reminiscent of that between peripheral axons and Schwann cells at an early stage in their embryonic development (Bunge, 1968; Farbman, 1992). In both settings, the glial cell element ensheathes multiple axons and holds them in juxtaposition, but in the case of the olfactory nerve, each enwrapped fascicle is composed of hundreds of axons (Farbman, 1992). The close apposition of axon to axon likely provides a highly favorable substrate for subsequent growth. The distinct segregation and aggregation of olfactory axons is maintained into the glomerulus, in which central glia are apparently restricted to the periphery (Raisman, 1985; Kasowski et al., 1999). The robustness of olfactory growth is reproduced in part when olfactory glia are transplanted into the lesioned spinal cord fostering both anatomical and functional recovery, or in the dorsal root entry zone promoting the growth of damaged dorsal root fibers back into the CNS (Ramon-Cueto and Nieto-Sampedro, 1994; Li et al., 1997; Raisman, 2001). The molecular features of the olfactory glia responsible for their axonal growth promoting properties remain unknown.

Having reached the bulb, newly innervating axons need to form specific connections within the glomerular layer. The connectivity of olfactory axons with the bulb seems to be specified at a minimum of three levels (Fig. 4). At the broadest level, the subdivision of the epithelium into zones, defined by the pattern of OR expression, is maintained in the projection of olfactory axons onto the bulb, establishing a rhinotopic arrangement between sensory periphery and central target, which may be important for translating the physicochemical interaction of odorant and nasal space (Moulton,

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1976; Schoenfeld et al., 1994; Keyhani et al., 1997). High level expression of the Ig superfamily member OCAM/mamFas II is limited to the ventrolateral zones, allowing projections from dorsomedial and ventrolateral OE to be distinguished on that basis, or by means of more conventional retrograde tract tracing experiments (Mori et al., 1985; Schwob and Gottlieb, 1986; Yoshihara et al., 1997). At its most precise, the convergence of axons elaborated by OSNs that express the same OR onto one or a few glomeruli on the medial and lateral faces of the bulb establishes a kind of receptotopy that depends in some manner on the selection of the OR to be expressed (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998). However, the location of an OR's "target glomerulus" is specified only to the extent that it falls within an array of glomeruli roughly 30–50 in number when compared among conspecifics (Strotmann et al., 2000; Schaefer et al., 2001). Thus, there is an intermediate level of specificity, i.e., targeting to a subarray of contiguous glomeruli, before the final event of glomerular acquisition.

Anatomical and Functional Constancy in the Adult

Is connectivity in the adult maintained with a specificity equivalent to that established during embryonic development? It appears that specific connectivity is maintained at the low rate of turnover that occurs during adult life in a protected laboratory setting, although the issue has been subject to only limited examination (Fig. 4). For example, in aged animals, the glomerular innervation by P2 axons in P2-IRES-tauLacZ mice is roughly equivalent to weanling animals (Costanzo, personal communication; Schwob, unpublished results). The labeled fibers converge and apparently do not wander into aberrant glomeruli, although the incidence of multiple glomeruli at a single bulbar site may be increased. Anatomical constancy correlates well with the usual human experience of perceptual stability throughout life. However, the wholesale replacement of the neuronal population that is elicited by either nerve transection or MeBr lesion is a different task and may be informative regarding the intrinsic capacities for accurate targeting in the adult.

Reinnervation: Nerve Damage vs. Epithelial Lesion

The two paradigms, nerve damage versus epithelial lesion, differ with respect to the condition of the olfactory nerve. With transection, the fascicular structure and blood supply of the nerve is markedly disrupted (Berger, 1971a,b; Graziadei, 1974; Schwob et al., 1994b), which leads to invasion by blood-borne macrophages, judging from events in other traumatically damaged peripheral nerves (Berger, 1971a; Taskinen and Roytta, 1997). With MeBr exposure or other peripheral agent, the nerve is left largely unaffected by the damage to the OE, although there is some glial reaction to the massive wave of axonal degeneration (Schwob et al., 1999). Reinnervation of the bulb occurs rapidly in both settings. By the second-to-third week after either form of lesion, axons have re-entered most areas of the olfactory nerve layer and have grown into underlying glomeruli (Graziadei et al., 1980; Yee and Costanzo, 1995; Schwob et al., 1999; Costanzo, 2000; Christensen et al., 2001).

It seems certain that the glial cell environment of the olfactory nerve and the superficial layers of the olfactory bulb is fundamental to the robustness of regeneration.

In the case of olfactory nerve transection, significant errors occur during the reinnervation of the bulb at all levels at which the projection is organized: rhinotopy, selection of glomerular subarray, and receptotopic glomerular convergence. Areas of the dorsomedial bulb remain permanently denervated or profoundly hypoinnervated, whereas axons from the dorsomedial OE that ought to project there are misdirected into territory usually occupied by fibers from the ventrolateral OE (Christensen et al., 2001). Furthermore, the projection of OR-defined subsets of OSNs is not restored to its prelesion state. For example, P2 fibers converge onto glomeruli but do not target the same area of the bulb as in normal P2 mice, and typically innervate multiple, widely dispersed glomeruli (Costanzo, 2000).

In the case of MeBr-mediated destruction of the OE, the zonal/rhinotopic organization of projection approaches prelesion in the cases for which the degree of damage permits full or nearly full recovery of the epithelium (Fig. 5) (Schwob and Youngentob, 1992). In these experiments, the projection was assessed both by immunostaining by using the anti-OCAM/mamFas II MAb designated RB-8 and by retrograde transport of fluorescently labeled microspheres. However, if the lesion is more severe, which causes some areas of the anterior, ventral, and lateral OE to undergo respiratory metaplasia (i.e., the patchy replacement of olfactory by respiratory epithelium during recovery after injury), then the posterior margin of the bulb remains denervated, indicating that the normal projection to those glomeruli has been redirected to more anterior glomeruli (Schwob et al., 1999).

Failure of Glomerular Targeting

The failure to reinnervate the posterior bulb seems to be the most striking manifestation of a general failure in choosing precisely the original glomerular subarray for reinnervation and in the convergence of like axons, both of which normally maintain the sharply focused receptotopic patterning of the projection (Fig. 5). That interpretation is confirmed by demonstrations of mistargeting of neuronal subsets defined by OR expression (i.e., assaying P2 fibers in mice after MeBr lesion) (Iwema and Schwob, 2001) or by expression of other molecular markers that apparently correlate with OR expression, for example MAb 2A4, which labels neurons projecting to a single pair of glomeruli in the

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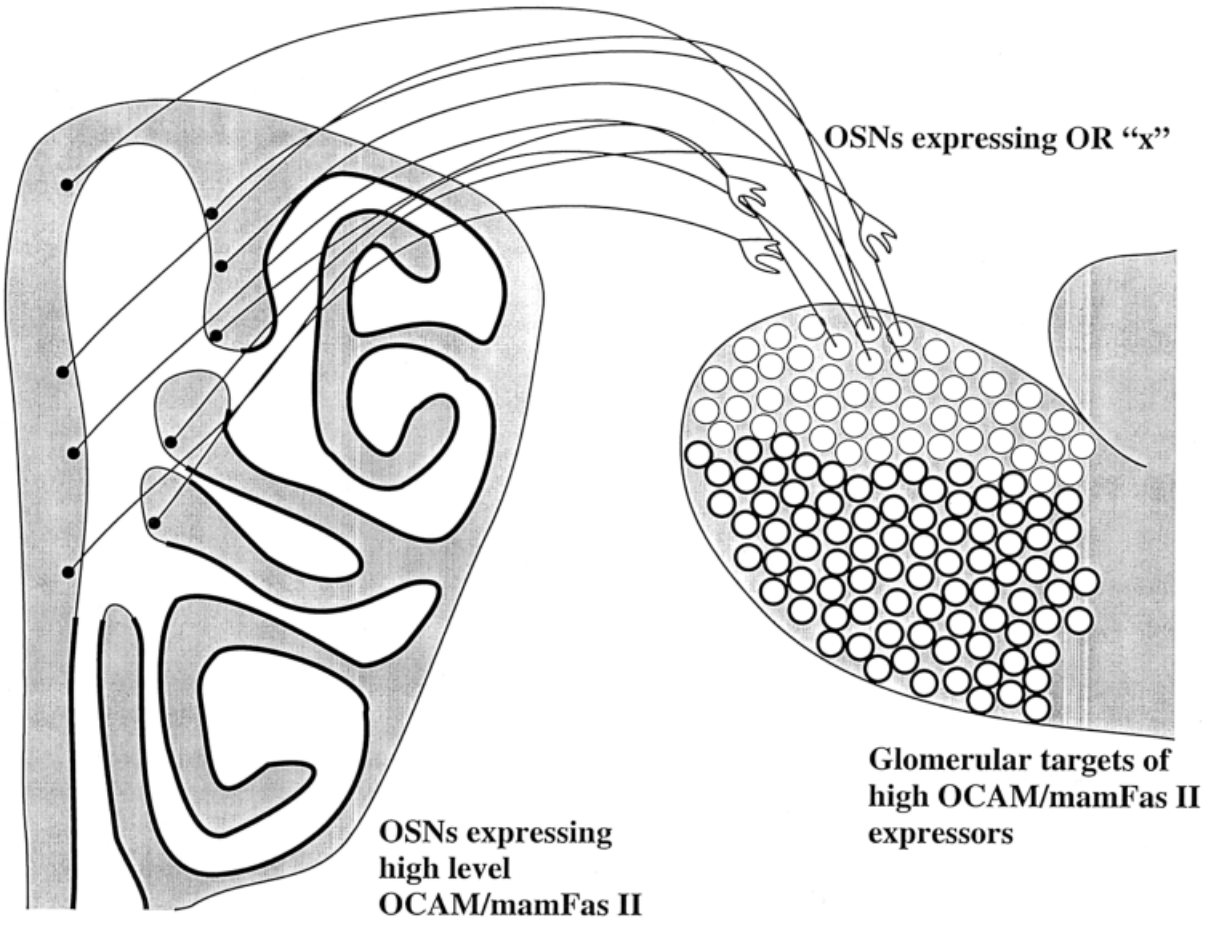

Figure 5. Glomerular targeting following wholesale neuronal turnover. In contrast to the normal setting, wholesale turnover of the neuronal population eliminates the possibility of using preexisting like-fibers as guides for the final stage of glomerular targeting. In the case of MeBr-lesioned animals, as illustrated here, rhinotopy is restored, such that high OCAM/mamFas II-expressing olfactory sensory neurons (OSNs) reinnervate the same area of the bulb, and low OCAM/mamFas II-expressing OSNs reinnervate the complementary area of the bulb, as in normal. In addition, odorant receptor (OR) -defined subsets of neurons return to roughly the same subarray of the glomerular layer of the bulb (consisting of probably 30–50 glomeruli) as in normal. However, the final stage of glomerular convergence is not accomplished, and axons distribute to multiple glomeruli within the subarray. Thus, the mechanisms that achieve targeting to the level of the subarray seem to be maintained throughout adulthood.

normal rat (Carr et al., 1994, 1998), and MAbs 213 and 2C6, which label distinct sets of neurons projecting to largely nonoverlapping groups of necklace glomeruli (Ring et al., 1995, 1997). In these examples, it appears that at least some axons approach the correct glomerular subarray, but the tight convergence onto one or a few grouped glomeruli is seriously distorted (Ring et al., 1995; Carr et al., 1998). Similarly, a return of axons to roughly the right area of the glomerular layer is observed with larger groupings of identified neurons after Triton X-irrigation of the nose of H-OMP-LacZ-6 transgenic mice (Cummings et al., 2000).

The example of the necklace glomeruli is particularly informative, as those glomeruli can be identified from animal to animal solely on the basis of position and structure in conventional histological sections (Ring et al., 1997). In cases for which the lesion is severe, the staining with MAb 2C6 offers a clear demonstration that fibers that would normally target the identifiable glomeruli in the posterior bulb end up in more anterior regions, i.e., the fibers approach the usual target area, defined broadly as the set of glomeruli adjacent or near to the normal necklace, but fall short and do not converge onto a bona fide necklace glomerulus (Ring et al., 1995). In contrast, in animals for which the lesion was less severe, the appropriate necklace glomerulus is reinnervated (Ring et al., 1995). The difference seems to reflect whether a large enough population of neurons is spared, as is the case with the less severe lesion, to offer guidance at the final, glomerular acquisition stage of the reinnervation process. In the absence of the preexisting fibers, as is the case with the more severe lesion, the reinnervating axons do not acquire their prelesion glomerulus.

The selective denervation at the posterior margin of the bulb when the population of innervating neurons is reduced in magnitude due to respiratory metaplasia suggests that a tendency for newly arriving fibers to fill preferentially glomeruli that are close to the cribriform plate and the initial points of contact between olfactory axons and the bulb may play a significant role in the patterning of reinnervation (Schwob et al., 1999). The apparent tendency to fill available

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glomerular space may be an important feature of the reinnervation of the bulb, and may differ from the developing projection. The preference for an unoccupied target may also feature in the restoration of the precise prelesion pattern after targeted ablation of a single OR-defined subset of olfactory neurons by means of application of transgenic technology.

In an elegant experiment from Axel's group, the neurons that express OR P2 are killed by the specific, but transient coexpression of diphtheria toxin in concert with the P2 receptor (Gogos et al., 2000). Replacement P2 neurons are regenerated, at least in part, over a time course of a month (without any preferential production of P2 neurons, apparently). Their axons converge onto glomeruli that are in analogous positions as their wild-type counterparts. The re-emergence of OR-specific targeting is impressive and has been interpreted as an indication that the adult system retains the information needed to restore both zonality and receptotopy (Gogos et al., 2000). However, *only* the P2 specific glomeruli are denervated. To the extent that reinnervating P2 fibers approach their usual glomerular subarray under the control of other guidance mechanisms and then preferentially occupy a vacated glomerulus, the paradigm may not be indicative of receptotopic accuracy, per se.

Consequences of Altered Connectivity

How does the inaccuracy in axon targeting impact on behavioral recovery after injury? Surprisingly little, given the shifting and blurring of the receptotopic map onto the bulb suggested by the existing, albeit limited, analysis in nerve transected and MeBr-lesioned animals. For example, anatomical recovery after knife cut of the olfactory nerve is sufficient to allow the lesioned animal to make simple odorant discriminations (Yee and Costanzo, 1995, 1998). Similarly, MeBr-lesioned and recovered animals retain a fairly complex odorant identification task learned before exposure (Youngentob and Schwob, 1997; Schwob and Youngentob, 2001). In this case, the animals had been trained to associate each of five operant tunnels with a distinct odorant and had learned that task to greater than 90% accuracy; the nature of the analysis allows one to construct a 5 × 5 matrix of odorant stimuli and response alternatives. Lesioned animals rested without testing for 2 months after lesion—a time period sufficient to allow anatomical recovery—require a few more days of testing to return to precriterion levels of performance with respect to percentage correct than control rats merely rested for 2 months without lesion. Nonetheless, the previously lesioned animals do recover to a remarkable degree.

Additional insight into the neural processing of the odorant stimuli can be gleaned from analysis of the pattern of responses across the matrix, both on-diagonal correct ones and off-diagonal errors, by means of multidimensional scaling analysis (Youngentob et al., 1991, 2001; Youngentob, 2001). Indeed, application of multidimensional scaling analysis does reveal a subtle alteration in encoding of olfactory stimuli in the normal vs. regenerated system. When the two groups of rats were compared on the basis of the *pattern* of responses, the control animals were found to cluster tightly together in the multidimensional space in which the patterns are located, whereas the lesioned and recovered animals are at a further remove from the controls and from each other. The analysis indicates that lesioned/recovered animals are processing stimuli in a different manner from controls. The conjunction of the anatomical and behavioral results indicates that the spatial mapping of odorant representation across the glomerular surface is important for the perception of odorant stimuli.

Lineage tracing analyses, cell marker studies, and transplantation experiments all support the assignment of multipotency to some type of globose basal cell.

CONCLUSIONS, UNRESOLVED ISSUES, POTENTIAL STRATEGIES

I have tried to present a broad view of cellular renewal in the olfactory epithelium, emphasizing the need to sustain both neuronal and non-neuronal cell populations in the face of continuing environmental assaults to maintain sensory function. Given the usual form of damage to which the epithelium is subject, analyses limited to neurogenesis are insufficient to understand the biology of the system. Making experimental lesions that damage other cell types in the epithelium as well as neurons reveals unanticipated capacities. The data reviewed here show that the adult epithelium retains cells that are multipotent in their differentiative capacity. Furthermore, cells with the capacity to give rise to nearly all epithelial cell types—OSNs, Sus cells, HBCs, and GBCs—are likely to reside within the broad category of GBCs. Lineage tracing analyses, cell marker studies, and transplantation experiments all support the assignment of multipotency to some type of GBC. That the multipotent stem cells encapsulate a critical capacity is shown by the association between destruction of GBCs and the initiation of respiratory metaplasia after severe, direct injury to the epithelium (Schwob et al., 1995). In addition, the apparent capacity of BD cells to give rise to Sus cells indicates that Sus cells can arise by means of two distinct lineages, which is unusual (Huard et al., 1998).

Unfortunately, we are currently unable to identify which subset of GBCs act as multipotent progenitors. MASH1 (+) GBCs seem to act as transit amplifying cells committed to the neuronal lineage, whereas those expressing neurogenenin1 seem to function as immediate neuronal precursors based on current data (Gordon et al., 1995; Cau et al., 1997). Molecularly anonymous antibody markers, including the ones that we developed (Goldstein and Schwob, 1996; Goldstein et al., 1997; Jang and Schwob, 2001) or antibodies to other cell differentiation antigens, may provide an alternative means of partitioning the broader

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population of GBCs into functionally defined subsets. Fundamental to any attempt to define functional capacity is the nature of the assay to be used, because the definition of commitment, i.e., distinguishing between differentiative *fate* vs. differentiative *capacity*, is an operational one. Transplantation of marked cells into the epithelium of a MeBr-lesioned animal has the potential for offering the donor cells a setting where the all types of cells are being generated at a robust rate, i.e., all progenitors can be and are being activated by the signals needed to direct the full range of differentiative potential. The olfactory transplantation assay is intended to mimic the colony forming unit assay-spleen (of lethally irradiated mice) that revolutionized the study of hematopoiesis, with the added advantage that the host environment is responding to an event that is highly analogous to ordinary life.

The model for epitheliopoiesis presented here differs from the more selectively neurogenic models of other investigators in the field. For example, in one type of assay partially purified dissociated OE cells are placed onto stromal substrates (Mumm et al., 1996). The suppression of neurogenesis by the addition of exogenous neurons or BMPs was referenced above. Although the emphasis was on colonies composed largely of neurons, other colonies were composed of cells with "non-neuronal" phenotypes, including ones that were "epithelial" in appearance. The epithelioid cells do not look like neurons in vivo, nor do they resemble the neurons of simple spindle morphology that have been identified in semidissociated cultures. However, they do resemble ones that express GBC antigens and low levels of neurotubulin and NCAM protein and can be pushed to make the neuronal antigens at higher levels and suppress the GBC antigens by the addition of TGF-βs (Newman et al., 2000). The failure to achieve full neuronal differentiation may reflect the absence of signals in the dissociated cultures that are needed to promote what may be less committed GBCs.

Likewise, the capacity of GBCs to give rise to non-neuronal cells may require cell–cell contacts or other signals, as yet unidentified, that establish a competency state that permits the GBCs to differentiate along a non-neuronal pathway. According to this formulation, neurogenesis may be the default competency of some or all GBCs. However, that component of the GBC population situated upstream of the transit amplifying cell, should they be limited to making neurons under the culture conditions generally in use, is not irreversibly committed to that fate (Goldstein et al., 1998; Huard et al., 1998). The data are not inconsistent with the hypothesis that a broader-than-neuronal capacity has to be elicited in some GBCs by a positive signal in contrast to the alternative, whereby multipotency is actively suppressed and the absence of the purported negative cue is sufficient to release their capacity to make non-neuronal cells.

The goal of understanding how and why regeneration — including reconstitution of the epithelium and reinnervation of the bulb — remains facile in this part of the nervous system and not others is, if not within reach, at least within view.

The foregoing hypothesis may be more consonant with the behavior of other kinds of stem cells. For example, hematopoietic stem cells have the capacity to give rise to other than blood cells, e.g., neurons, when in the corresponding environment, e.g., the brain (Anderson et al., 2001). Clarity will be achieved in the context of the olfactory system only when we are better able to subdivide the population of GBCs and assess their differentiative capacity.

Even when that strategy is fully successful, we will still be left with the difficulties of assessing the cellular and molecular cues that regulate epithelial renewal during normal life and after injury. The standard manipulations—adding growth factors, blocking transduction cascades, knocking-out genes—will all help to clarify the regulatory networks. We may have an advantage in the olfactory system over other neural systems by virtue of the ease with which cells can be put back into the epithelium after lesion (Goldstein et al., 1998). In particular, we have recent preliminary results suggesting that conditionally immortalized cells can be stably integrated into host epithelium after being returned to the animal (Chen et al., 2001). If the results are substantiated, the availability of transplantable cell lines suggests an alternative approach. Namely, it will likely be feasible to manipulate the genome of conditionally immortalized cells in vitro and then assess the consequences for their differentiation after transplantation. In another potentially powerful approach, embryonic stem cells might be manipulated, induced, and then transplanted. By using either of these approaches, we will be able to define the receptive side of the relevant signaling pathways with great facility.

Beyond the question of how and how well the epithelium recovers after injury remains the issue of the mechanisms underlying the accuracy with which the bulb is reinnervated and how errors arise. That a hierarchy of mechanisms culminates in the receptotopic map across the olfactory glomeruli remains the most likely explanation for the establishment of the map during development, its maintenance in adulthood, and its restoration after lesion (to the extent that recovery occurs). The molecular regulation of those events leading up to the final stage of glomerular innervation remains obscure. Candidate molecules and processes include semaphorins, ephrins, and various glycoprotein moieties (Treloar et al., 1996; Whitesides and LaMantia, 1996; Dowsing et al., 1997; Kafitz and Greer, 1997, 1998a; Raabe et al., 1997; Tenne-Brown et al., 1998; Tisay and Key, 1999; Crandall et al., 2000; Schwarting et al., 2000; St John et al., 2000; St John and Key, 2001). Molecular genetic techniques suggest that the OR that is expressed plays a role in the final acquisition of the glomerular target (Singer et al., 1995; Mombaerts et

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al., 1996; Wang et al., 1998). It is likely that the special properties associated with the glia of the olfactory nerve and olfactory nerve layer play a major role in supporting growing axons and may also be crucial in guiding them by some as yet undetermined means (Kafitz and Greer, 1998b, 1999; Bartolomei and Greer, 2000). The reinnervation of the bulb initiated by MeBr lesion may be an ideal in vivo model for most levels of the axon targeting process, because axons return to the appropriate area of the bulb, even though the final stage—i.e., glomerular acquisition—is not accomplished in the absence of preexisting fibers.

Despite the current limitations on our understanding, much has been learned over the recent past about the events and the regulation of the regeneration of the primary olfactory system after injury. The armamentarium that has been and continues to be assembled promises even more rapid advances in the future. The goal of understanding how and why regeneration—including reconstitution of the epithelium and reinnervation of the bulb—remains facile in this part of the nervous system and not others is, if not within reach, at least within view.

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