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Olfactory Marker Protein mRNA Is Found in Axons of Olfactory Receptor Neurons

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The separation between the cell bodies of olfactory receptor neurons in the nasal cavity and their axon terminals in the olfactory bulb make them attractive for studying axonal transport. Although high molecular weight RNAs are generally believed to be excluded from axons of mature neurons, we demonstrate here that mRNA for olfactory marker protein (OMP), an abundant cytoplasmic protein selectively expressed in mature receptor cells, is present in rodent olfactory receptor axons. OMP RNA was detected by *in situ* hybridization at the light microscope level in axons and in terminals. By nuclease protection, the level of OMP RNA in the olfactory bulb was 5–10% of that in the olfactory epithelium where the cell bodies reside. In contrast to axonally transported vasopressin and oxytocin mRNAs, which are deficient in their 3′ polyA tails, axonal OMP RNA fractionated as polyA$^+$. OMP RNA was lost from axons and terminals after deafferentation, suggesting that OMP RNA was synthesized in receptor cell bodies in the epithelium and was transported into axons and terminals in the olfactory bulb. RNA for G_{olf}, a G-protein highly expressed in dendrites of mature olfactory receptor neurons, was not detected in the olfactory bulb. We hypothesize that the immature nature of the cytoskeleton and, specifically, the lack of tightly bundled microtubules allows transport of particular mRNAs in olfactory receptor axons.

[Key words: olfactory marker protein, olfaction, axonal transport, mRNA, in situ hybridization, olfactory epithelium]

Although most proteins are made in the cell body and transported to different subcellular compartments, some proteins are synthesized from RNAs that are themselves sequestered in discrete subcellular sites. Their specific intracellular localization results from the transport of their mRNAs, which occurs prior to translation. In mammalian neurons, the best examples of localized mRNAs are those encoding microtubule-associated protein 2 (MAP2) and the a subunit of calcium/calmodulin kinase type II (CaMKII) (see Steward and Banker, 1992, for review). MAP2 and CaMKII proteins are localized to mature dendrites and are excluded from axons. Dendritic localization of their RNAs has been demonstrated *in vivo* in the cerebral cortex and hippocampus (Garner et al., 1988; Burgin et al., 1990) and in cultured primary neurons (Bruckenstein et al., 1990; Kleiman et al.,1990). RNAs encoding other cytoskeletal proteins such as β tubulin, 68 kDa neurofilament, and MAP5 remained restricted to the cell body (Garner et al., 1988; Tucker et al., 1989; Kleiman et al., 1990).

Because high levels of MAP2 and CaMKII RNA remain in the cell body, these proteins are likely to be translated both in the cell body and at distant sites. A substantial body of evidence suggests that dendritic RNAs are translated: polyribosomes have been observed in dendrites, especially during periods of synaptogenesis (Steward and Levy, 1982; Steward, 1983) and *in vivo* and *in vitro* dendritic elements can incorporate radioactive amino acid precursors into protein (Fass and Steward, 1983; Torre and Steward, 1992). Synaptosomal preparations that include dendritic endings have been shown to synthesize proteins (Autilio et al., 1968; Verity et al., 1980), some of which can be localized to the synaptic plasma membrane and synaptic junctional complex (Rao and Steward, 1991).

Although mRNAs are found in molluscan axons (see Van Minnen, 1994, for review), and tau mRNA has been localized to proximal axon segments in cultured cortical neurons (Litman et al., 1993), in general, mRNAs are not present in mature axons. However, localization of oxytocin, arginine vasopressin (AVP) and tyrosine hydroxylase mRNA has been documented in the axons of hypothalamic paraventricular and supraoptic neurons that terminate in the posterior pituitary. Oxytocin and vasopressin RNAs were detected in the posterior pituitary (Murphy et al., 1989; McCabe et al., 1990) and were lost after transection of the hypothalamo–hypophysial tract (Mohr et al., 1990), suggesting that the RNAs were transported from the cell bodies in the hypothalamus. *In situ* hybridization showed that oxytocin RNA in lactating rats (Jirikowski et al., 1990) and AVP RNA in salt-loaded rats (Trembleau et al., 1994) were intra-axonal. Northern blot analyses of axonal oxytocin and AVP RNAs showed that they became progressively shorter as they moved down the hypothalamo–hypophysial tract (Mohr et al., 1991). Since previous nucleotide sequence analysis had revealed that

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the mRNAs from the hypothalamus and the posterior pituitary were identical (Mohr et al., 1990), the difference in length was attributed to a progressive shortening of the polyA tail.

It was further shown that AVP RNA injected into the hypothalamo–hypophysial tract could ameliorate the diabetic phenotype of Brattleboro rats that lack endogenous AVP (Jirikowski et al., 1992; Maciejewski-Lenoir et al., 1993). Radiolabeled AVP RNA, taken up in axons, was anterogradely transported to the pituitary and was retrogradely carried back to cell bodies in the hypothalamus, where it was presumably translated. Injected polyA$^-$ AVP mRNA was 200-fold more effective than polyA$^+$ RNA (Maciejewski-Lenoir et al., 1993), suggesting that shortening of the polyA tail during transport may have functional relevance. These experiments demonstrate that axonal RNAs can be translated, albeit in the cell body, and that RNAs can be efficiently taken up into axons.

Recently, mRNA for a neurotransmitter biosynthetic enzyme, tyrosine hydroxylase (TH), was found in the same hypothalamo–neurohypophysial tract, suggesting that axonal transport is not restricted to RNAs encoding secretory proteins (Skutella et al., 1994). Like AVP RNA, TH RNA was retrogradely transported back to hypothalamic cell bodies after salt loading. These results prompted us to ask if axonal localization of RNA was restricted to the hypothalamo–neurohypophysial system in mammals.

The rodent olfactory receptor neuron was chosen as a model since it has been used to study axoplasmic protein transport; its cell bodies and terminals are widely separated, and it synthesizes proteins with a highly restricted cellular distribution. We describe studies on the localization of RNA encoding olfactory marker protein (OMP), a 19 kDa, cytoplasmic protein of unknown function whose expression is highly restricted to mature olfactory neurons in many vertebrate species (see Margolis, 1980, for review; Buiakova et al., 1994). In the nasal neuroepithelium, OMP is only found in mature olfactory receptor neurons whose axons terminate in the olfactory bulb (OB). Although low levels of OMP protein have been detected in a few non-olfactory neurons (Baker et al., 1989), there are no OMP-positive cell bodies in the OB (Monti-Graziadei et al., 1980; Baker et al., 1989). Hence, it was surprising that functional OMP RNA could be detected in the olfactory bulb by Northern blot hybridization (Ehrlich et al., 1990) by reverse transcriptase-coupled PCR (Grillo and Margolis, 1990), and by *in vitro* translation followed by immunoprecipitation (Rogers et al., 1987). These earlier studies, however, did not determine where OMP RNA was localized and could not discriminate between two possibilities. Either OMP RNA could be made in OB cells that did not express OMP protein, or OMP RNA reached the OB by axonal transport from receptor cell bodies in the nasal cavity. *In situ* hybridization experiments presented here demonstrate that OMP RNA is found in axons and terminals of the olfactory nerve and is lost after peripheral deafferentation. This strongly suggests that OMP RNA reaches the OB by axonal transport. We further show that the transported OMP RNA is polyadenylated, unlike oxytocin and AVP transcripts found in hypothalamic axons.

Materials and Methods

Animal procedures. Anesthetized, 4-week-old Sprague–Dawley male rats were axotomized by drilling a small bore hole over the left olfactory bulb at a position slightly left of the midline and even with the front of the eyes (Kawano and Margolis, 1982). The axons around the left bulb were cut with a curved syringe needle inserted into the bore hole. Axotomized animals were allowed to recover for 10–14 d. After they were humanely killed, the animals were examined to determine if axotomies were successful. Bilateral deafferentation of mouse olfactory bulbs were performed as described (Kawano and Margolis, 1982). A 25 gauge syringe needle was filed to a blunt tip 3 mm long. The end was inserted into the left naris of an unanesthetized adult CD1 mouse, which was held by hand. The nasal cavity was irrigated with 100 μl of 0.17 M $ZnSO_4$. The animals were kept for 7 d before humanely killed. The olfactory epithelium was histologically examined to determine that deafferentation was successful.

Probe preparation. Single-stranded riboprobes were made according to Melton et al. (1984), using α^{32}P-UTP (New England Nuclear; 800 Ci/mmol) and 1 μg of plasmid DNA. Unincorporated nucleotides were removed on a 1 ml Sephadex G50 column. OMP riboprobe was prepared from a PstI-BamHI fragment (containing nucleotides 185 to 892) of pOMP (Rogers et al., 1987) that had been subcloned into pSP65 and was recloned into Bluescript SKM13- (Stratagene, La Jolla, CA) at the PstI and BamHI sites. The resulting plasmid was linearized with StuI at nucleotide 715, and sense probe was transcribed from the T3 promoter; antisense from the T7 promoter. G_{olf}-Bluescript plasmid (Jones and Reed, 1989) was the generous gift of Dr. Randy Reed (Johns Hopkins School of Medicine) contained a 3.0 EcoRI fragment of G_{olf}. Circular DNA was used for riboprobe preparation; antisense was transcribed from the T7 promoter, sense from the T3 promoter. Riboprobe preparation from the first exon of rat tyrosine hydroxylase (TH) plasmid pAA360 was previously described (Fung et al., 1991).

In situ *hybridization.* Deeply anesthetized adult rats or mice were intracardially perfused; dissected olfactory epithelia and bulbs were subjected to *in situ* hybridization as described (Simmons et al., 1989) using 10 micron frozen sections. Hybridization was performed at 55°C overnight using ^{32}P-labeled riboprobes at 1.25×10^6 cpm per ml. After hybridization, the slides were exposed to NTB-2 (Kodak, Rochester, NY) emulsion diluted 1:1 with water. After the liquid emulsion dried, slides were exposed at −80°C. After developing, sections were stained for 5 min with 5×10^{-5} M Hoechst dye 33258 (Aldrich Chemical, Milwaukee, WI) and rinsed briefly with distilled water before mounting. In all figures except 5*C*, the data are presented as the same field shown both under fluorescence to detect Hoechst-stained nuclei and under dark field to detect autoradiographic grains.

In situ *hybridization combined with immunocytochemistry.* Sections were prepared from 28-month-old Fischer 344 rats and were subjected to *in situ* hybridization using ^{35}S-labeled rat OMP probe and immunocytochemistry to tyrosine hydroxylase as described (Stone et al.1991).

RNA preparation and nuclease protection assays. Total RNA from freshly dissected adult rat olfactory bulbs, epithelia, and liver was prepared using 6 M urea/3 M LiCl/10 m M vanadylribonucleoside complex (Bethesda Research Labs, Grand Island, NY), as described by Fung et al. (1991). When dissecting the olfactory bulb, the cribiform plate remained intact, and only tissue posterior to the plate was removed. PolyA$^+$ and polyA$^-$ RNA was prepared as described by Fung et al. (1991), except a single cycle of purification on oligo-dT cellulose was performed. After one round of selection on oligo-dT cellulose, between 4% and 6% of the total RNA eluted as polyA$^+$. Given that polyA$^+$ RNA represents about 2% of the total RNA in most tissues, this fractionation is a 18–25× enrichment for polyA$^+$ RNA. Nuclease protection assays were performed as described (Fung et al., 1991) using $2–4 \times 10^4$ cpm of TH or OMP antisense probe.

Results

OMP RNA is present in the distal axon segment and terminals of the olfactory nerve in the olfactory bulb

OMP is only expressed in mature sensory neurons in the OE, where it comprises 0.1–1% of the total protein and 0.5% of the mRNA (Rogers et al., 1985). OMP is found throughout the cell including proximal dendritic processes, axons, and terminals (Farbman and Margolis, 1980; Monti-Graziadei et al.,1980). Olfactory receptor neurons are unique in that they are continually generated throughout the life of the animal (for review, see Crews and Hunter, 1994). In mature animals they occupy the upper two-thirds of the sensory or olfactory epithelium (OE) and are absent from adjacent regions of respiratory epithelium which, together with the olfactory epithelium, comprise the lining of the nasal cavity. Receptor neurons arise from an OMP-negative precursor cell located in deep layers of the epithelium

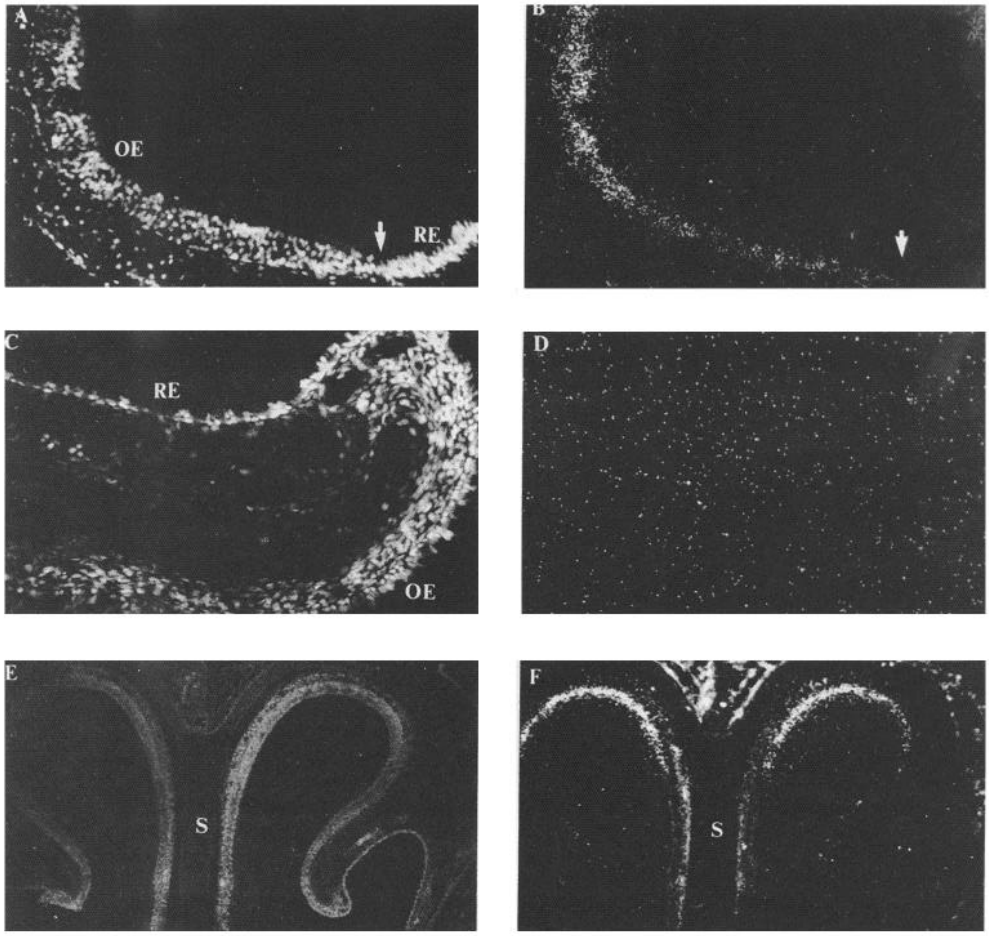

Figure 1. OMP RNA in the olfactory epithelium. Matched fluorescence (*A, C,* and *E*) and dark-field (*B, D,* and *F*) photographs of coronal sections of rat nasal cavities hybridized with OMP riboprobe. *A* and *B*, Adult olfactory epithelium (*OE*) and respiratory epithelium (*RE*) hybridized with antisense OMP. Junction between the OE and RE is marked with an *arrow*. *C* and *D*, Adult OE and RE hybridized with sense OMP probe. *E* and *F*) Postnatal day 3 nasal cavity hybridized with antisense OMP probe. In *F*, some autofluorescence of bone above septum (*S*) is apparent. Exposure times: *B*, 5 d; *D*, 2.5 d; *F*, 2.5 d. Magnification: *A* and *B*, 170×; *C* and *D*, 340×; *E* and *F*, 68×.

near the basement membrane. As they move into superficial layers, they mature into OMP-positive bipolar neurons whose dendrites develop a terminal knob that possesses modified cilia that extend into the nasal cavity and bear proteins specialized to detect odors. Their unmyelinated axons traverse the basement membrane into the lamina propria and fasciculate into bundles that cross into the cranium through perforations in the cribiform plate of the ethmoid bone. The axons spread over the surface of the olfactory bulb (OB) that they penetrate to synapse with second-order neurons in complex glomerular structures that are circumperipheral to the olfactory bulb. The relatively long distance between cell bodies in the nasal cavity and axon terminals on the opposite side of the cribiform plate make these neurons easily amenable to biochemical experiments that require dissection of cell bodies away from terminals.

To validate the specificity of *in situ* hybridization with the rat OMP riboprobe, coronal sections of adult rat olfactory epithelium (OE) were hybridized with antisense OMP probe. As expected, we detected robust hybridization in the outer two-thirds of the sensory portion of the OE, where mature receptor cells reside (Fig. 1*A,B*). The hybridization signal stops at the border between the sensory and respiratory epithelium (RE) (indicated by an arrow) and is absent from the RE. It is also absent from the lamina propria beneath the epithelium. Hybridization to coronal sections of 3-d-old rats (Fig. 1*E,F*) confirms that OMP is present in early postnatal animals and that the expression is largely confined to the dorsal region of the nasal cavity. These data demonstrate that the OMP probe appropriately hybridizes to receptor neurons, the only cells in the OE known to express OMP protein. Similar experiments with mouse OE yielded the same results, confirming that the rat OMP probe cross-hybridizes with mouse OMP RNA (data not shown). Sense strand OMP riboprobe gave no signal with OE sections (Fig. 1*C,D*).

Figures 2, *A* and *B*, and 3*A* demonstrate that OMP hybridization could be detected in the olfactory nerve layer (NL) of the OB. This region contains the distal segments of the olfactory receptor axons. Under higher magnification (Figs. 2*C,D*; 3*B*), the signal can be seen extending into individual glomeruli (G), where the terminals of receptor neurons synapse. In contrast, the next deeper layer of the bulb, the external plexiform layer (EPL), is essentially free of grains. The fact that labeling extends into glomeruli suggests that OMP RNA is present in presynaptic terminals. Figure 3, *C* and *D*, demonstrates that the grains do not cover the TH positive neurons that surround the glomeruli, suggesting that the RNA is restricted to receptor cell terminals in the glomerular neuropil. Sense strand OMP riboprobe gave no signal (Fig. 2*E,F*).

RNA for G_{olf} is not localized in the axons and terminal of receptor neurons in the olfactory bulb

To determine if other RNAs expressed in olfactory receptor neurons were also transported to the bulb, the distribution of G_{olf} RNA was examined. G_{olf} is a Gs-like α subunit that is abundant in the OE. The protein is concentrated in sensory cilia where it is thought to transduce odorant-induced signals from olfactory receptors to activate adenylate cyclase. Like OMP, G_{olf} protein

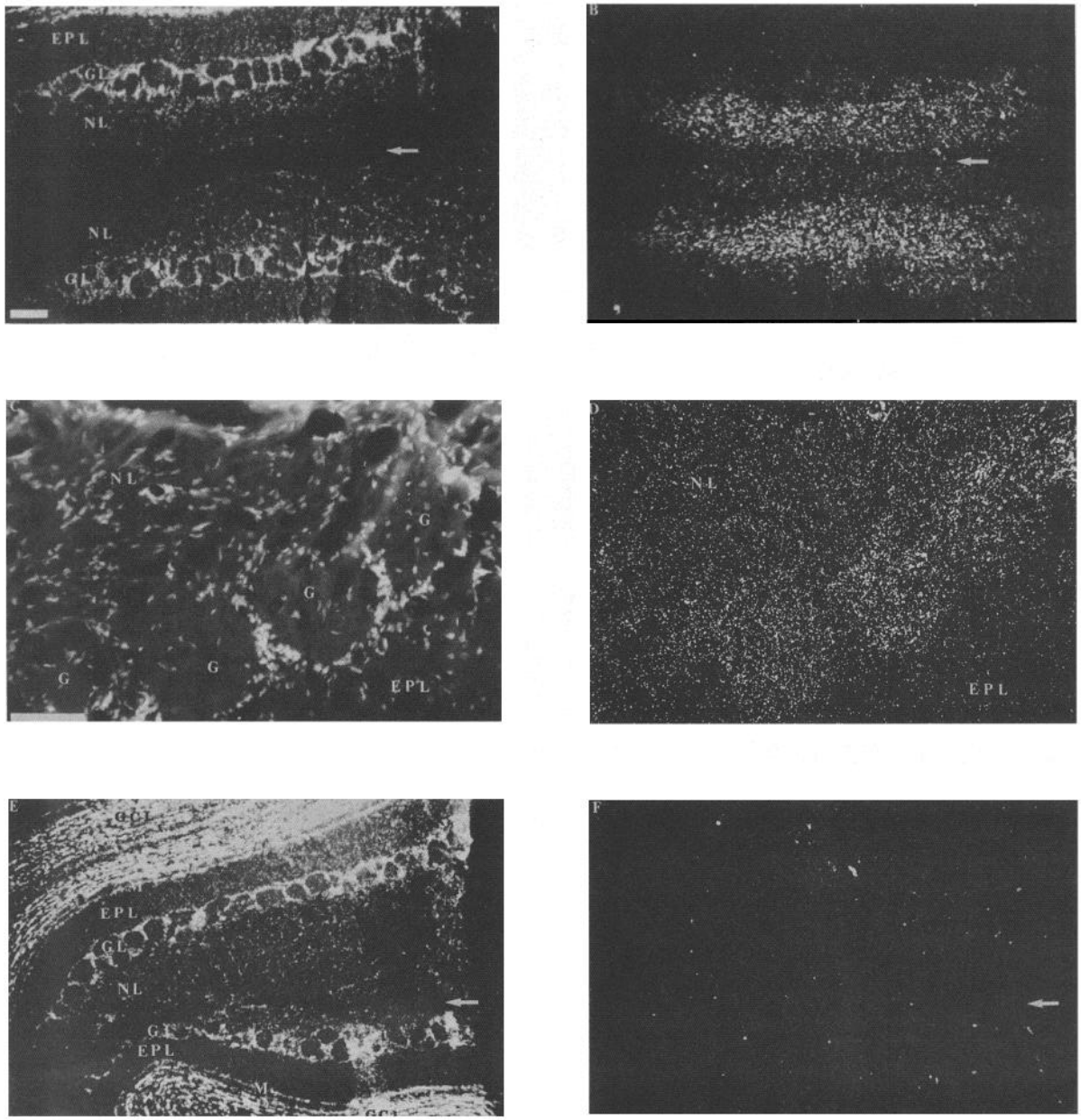

Figure 2. OMP RNA in normal olfactory bulbs. Matched fluorescence (*A, C,* and *E*) and dark-field (*B, D,* and *F*) photographs of coronal sections of rat olfactory bulbs hybridized with OMP riboprobe. *A* and *B*, Hybridization with antisense probe. *Arrow* demarcates the separation between the pair of OBs. *C* and *D*, Higher magnification of the glomerular layer hybridized with antisense probe. *E* and *F*, Hybridization with sense probe. *Arrow* demarcates separation between the pair of OBs. *GCL*, granule cell layer; *EPL*, external plexiform layer; *GL*, glomerular layer; *NL*, olfactory nerve layer; *G*, glomerulus. Exposure times: *B*, 4.5 d; *D*, 4.5 d; *F*, 7 d. Scale bar: *A, B, E,* and *F*, 200 μm; *C* and *D*, 100 μm.

has been shown by immunocytochemistry of the OE to be expressed only in receptor neurons (Jones and Reed, 1989). Figure 4 demonstrates that G_{olf} RNA can be detected by *in situ* hybridization in the neuroepithelium, but cannot be detected in the olfactory bulb (Fig. 5), although its RNA in the OE is at least as abundant as OMP RNA (Jones and Reed, 1989). The low level of silver grains in deeper layers of the bulb (granule cell layer) is likely to be due to cross-hybridization with other Gs RNAs in those regions. Nevertheless, there was no detectable hybridization over the olfactory nerve or glomerular layers even

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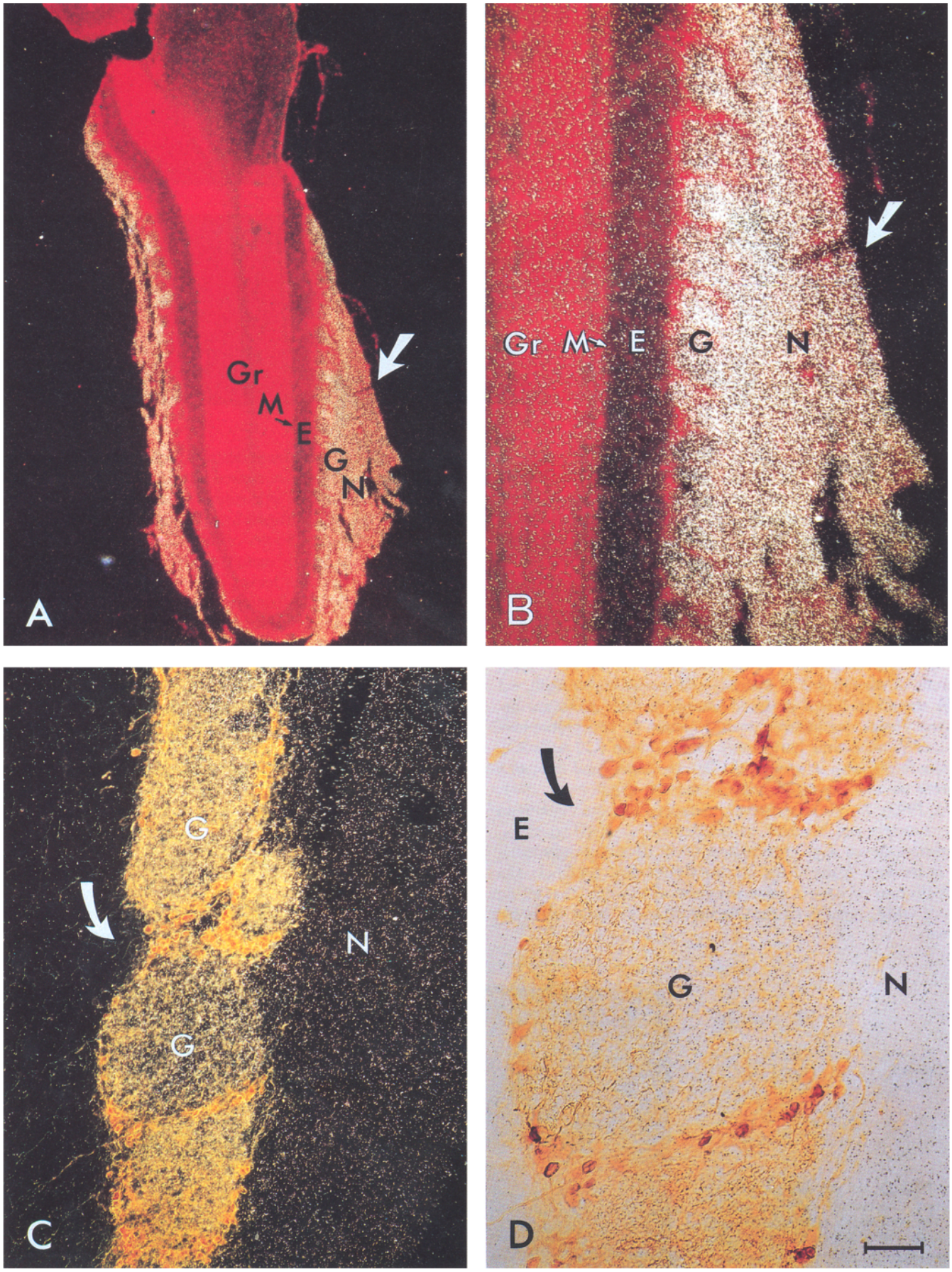
Figure 3. Colocalization of OMP mRNA and TH protein in rat olfactory bulb. Dark-field photographs of OMP mRNA demonstrated by *in situ* hybridization at low (*A*) and high (*B*) magnification. Hybridization signal occurs in fibers in the nerve layer (*N*) and within glomeruli (*G*). *Arrow in A* indicates similar region illustrated at higher magnification (*arrow*) in *B*. The dark-field photograph in *C* shows that TH immunoreactivity surrounds the OMP-labeled glomeruli. The *arrow* indicates the area shown in the bright-field photograph, *D*. As evident in *D*, grains do not occur over TH positive cell bodies or in the external plexiform layer (*E*) to the left of the glomeruli. *M*, mitral cell layer, *Gr*, granule cell layer. Scale bar: 500 μm in *A*; 150 μm in *B*; 50 μm in *C*; 25 μm in *D*. Exposure time for all sections was 14 d.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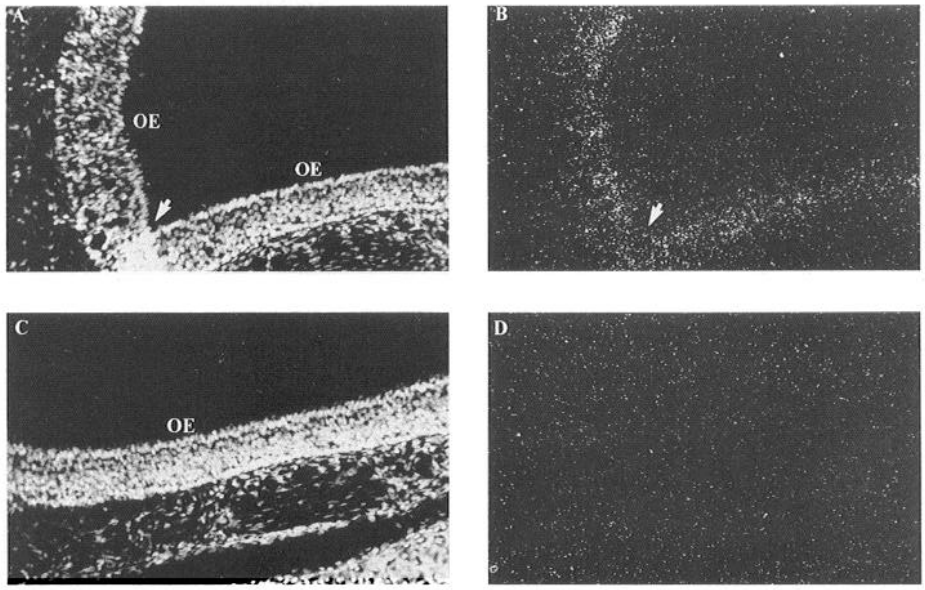

Figure 4. G_{olf} RNA in the olfactory epithelium. Matched fluorescence (*A* and *C*) and dark-field (*B* and *D*) photographs of coronal sections of rat nasal cavities hybridized with G_{olf} riboprobes. *A* and *B*, Hybridized with antisense probe. Fold in the olfactory epithelium (*OE*) is noted with an *arrow*. *C* and *D*, Hybridized with sense probe. Exposure for *B* and *D*, 6 d. Magnification, 210×.

at exposure times longer than those used for OMP hybridization in the bulb. Likewise, no hybridization was observed with a NCAM (neural cell adhesion molecule) probe in the outer bulb layers, although signal was detected in receptor neurons and in deep layers of the bulb (data not shown). These data demonstrate that not all mRNAs are localized to axons and terminals of olfactory receptor neurons.

Deafferentation by $ZnSO_4$ lavage or axotomy induces loss of OMP RNA from the olfactory bulb

If OMP RNA is initially transcribed in the nuclei of receptor neurons in the OE and is axonally transported to the bulb, the RNA should be lost if receptor neurons or their axons are destroyed. Hence, we induced deafferentation by two procedures. First, adult mice were intranasally irrigated with 0.17 M $ZnSO_4$, a procedure known to irreversibly destroy mature and immature neurons as well as support cells in the OE (Nadi et al., 1981; Burd, 1993). In our experiments, destruction of the epithelium was complete and bilateral, as determined by histological examination. Seven days after lavage, OMP RNA was undetectable in terminal fields of the olfactory bulb (Fig. 6*A,B*). *In situ* hybridization of control mice irrigated with saline showed no loss of OMP signal from the OE and olfactory bulbs (data not shown).

Because $ZnSO_4$ treatment destroys both sides of the epithelium, unilateral axotomy was used to lesion only one side of the epithelium, leaving the opposite side intact. The drawback of axotomy is that it is difficult to achieve complete lesioning, especially at the medial border of the bulb. Axotomy induces retrograde degeneration of mature receptor neurons whose axons have been lesioned, but leaves other cells in the OE intact. We performed *in situ* hybridization on rats 10 to 14 d after unilateral axotomy. OMP RNA disappeared from the lateral portion of the axotomized bulb and was significantly reduced medially (Fig. 6*C*). The nonaxotomized side retained strong OMP hybridization, both laterally and medially. At higher magnification (Fig. 6*D,E*), it is evident that all OMP signal is lost from the lateral olfactory nerve and glomerular layer, while some residual signal remains on the medial side, probably due to incomplete axotomy. Both methods of deafferentation reduce or ablate OMP hybridization in the olfactory bulb, consistent with the notion that OMP RNA is transported into the bulb from perikarya of receptor neurons in the OE.

Quantification of OMP RNA in the olfactory bulb by nuclease protection

Because of the difficulty in quantifying *in situ* hybridization from different tissues (OE vs OB), we performed solution hy-

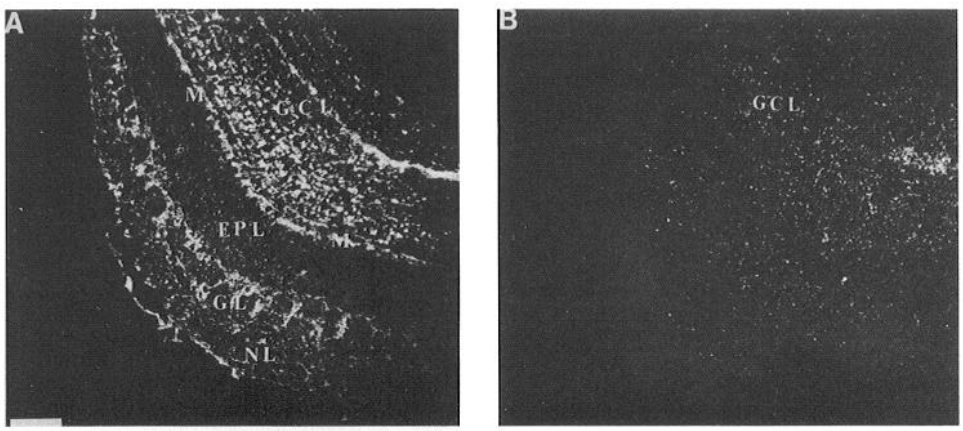

Figure 5. G_{olf} in the olfactory bulb. Matched fluorescence (*A*) and dark-field (*B*) photograph of a coronal section of rat OB hybridized with G_{olf} antisense riboprobe followed by exposure for 7 d. Scale bar, 200 μm.

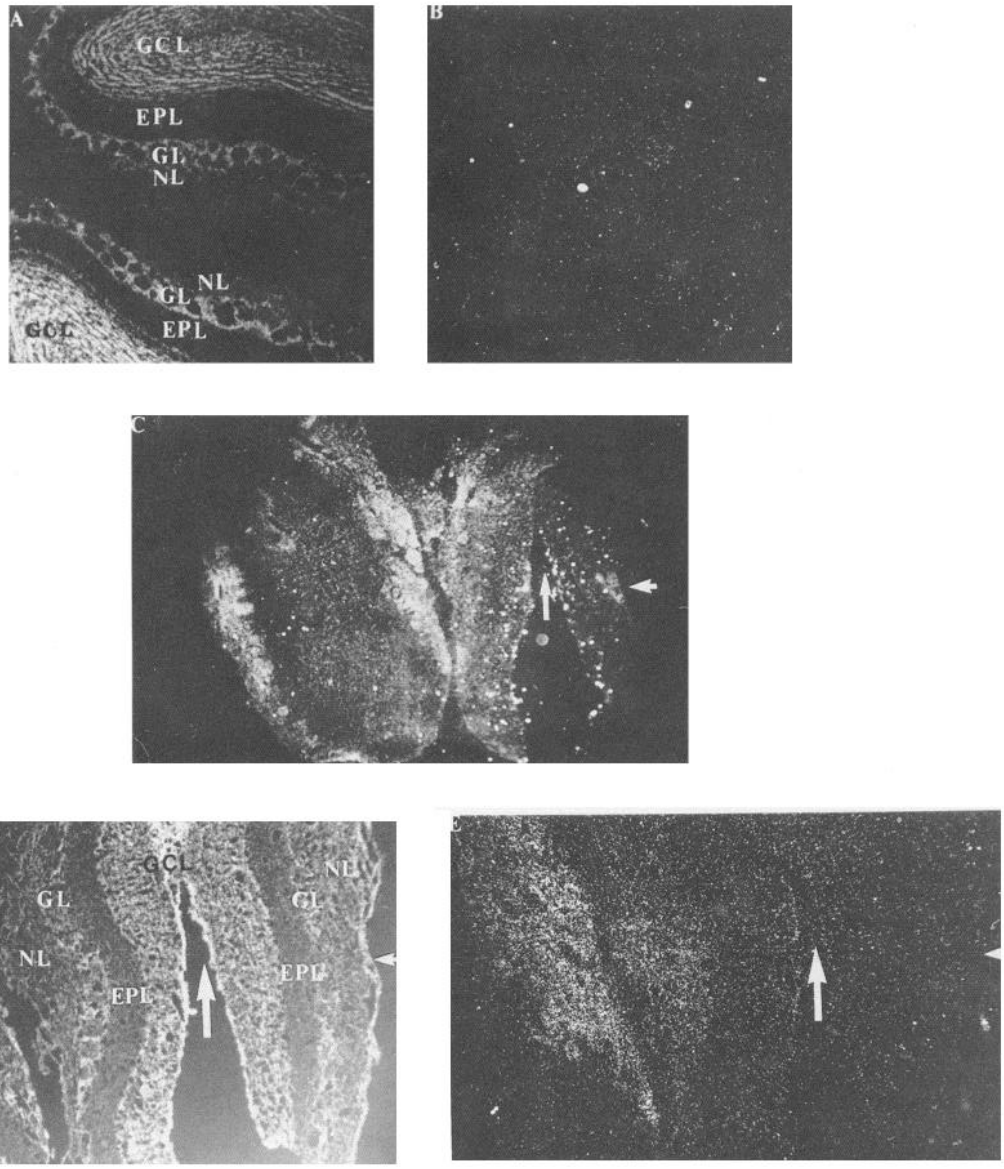

Figure 6. Chemical deafferentation with $ZnSO_4$ and unilateral axotomy induce loss of OMP RNA in the olfactory bulb. Matched fluorescence (*A* and *D*) and dark-field (*B* and *E*) photographs of sections of olfactory bulb hybridized with antisense OMP riboprobe. *A* and B, Mouse olfactory bulbs from $ZnSO_4$-treated animals. Note the lack of hybridization signal. *C*, Low-magnification dark-field photograph of *in situ* hybridization of rat olfactory bulb after axotomy of the right OB. Hybridization is present on both lateral and medial border of intact bulb but is lost on the lateral border and reduced on the medial portion of axotomized bulb. Tear in the axotomized bulb is indicated with an *arrow*. *Arrowhead* marks the lateral edge of the axotomized bulb. *D* and *E*, Another section shown at higher magnification. Tear in the GCL of the axotomized bulb is indicated with *arrow*; lateral edge of axotomized bulb is indicated with an *arrowhead*. *GCL*, granule cell layer; *EPL*, external plexiform layer; *GL*, glomerular layer; *NL*, olfactory nerve layer. Exposure times: *B*, 4 d; *C* and *E*, 5 d. Magnification: *A*, *B*, *D*, and *E*, 70×; *C*, 35×.

bridization to determine the amount of OMP RNA in the olfactory bulb under conditions in which the intensity of hybridization signal is proportional to the amount of RNA in a sample. When OMP riboprobe was annealed to total rat OE or olfactory bulb RNA, a nuclease-protected band at 183 base pair (bp) was detected, the size expected for OMP RNA (Fig. 7). We estimate from the data in Figure 7 and other similar experiments that OMP RNA in the olfactory bulb is about 5–10% the level found in the olfactory epithelium. Similar estimates were obtained from previous Northern blot data (Ehrlich et al., 1990). Our estimate represents a lower limit, since OMP RNA in regions of the nerve not associated with the bulb (e.g., within the cribiform plate) are not included. Nevertheless, it is likely that only a small proportion of the total OMP mRNA is transported. In agreement with the *in situ* hybridization data, RNA extracted from axotomized olfactory bulbs lacked OMP sequences, as did liver and tRNA samples included as negative controls (Fig. 7).

Transported OMP RNA fractionates as polyA$^+$

As axonally transported oxytocin and AVP RNAs were reported to be deficient in their polyA tails, we determined whether OMP RNA in the olfactory bulb was polyadenylated. Total olfactory bulb RNA was fractionated over an affinity oligo-dT cellulose column into polyA$^+$ and polyA$^-$ fractions. PolyA$^+$ and polyA$^-$ RNA from the same preparation and equivalent to the same amount of starting tissue (i.e., four olfactory bulbs) was hybrid-

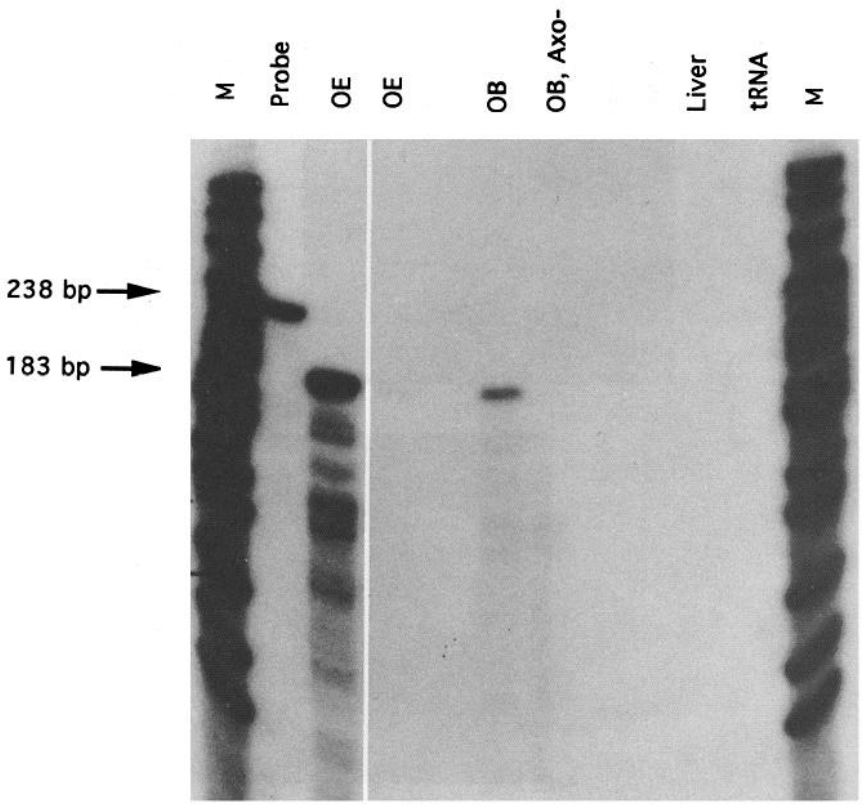

Figure 7. Quantification of OMP RNA by nuclease protection. OMP riboprobe was annealed to rat RNAs and analyzed as described in Material and Methods. The undigested probe is 238 bp, while the size of protected by OMP mRNA is 183 bp. *Lanes 1 and 9,* DNA markers (*M*) of pBR322 DNA digested with MspI; *lane 2,* undigested OMP riboprobe; *lane 3,* 10 μg olfactory epithelium (OE) RNA; *lane 4,* 0.1 μg OE RNA; *lane 5,* 10 μg olfactory bulb (OB) RNA; *lane 6,* 10 μg olfactory bulb RNA after axotomy (OB, axo⁻); *lane 7,* 10 μg liver RNA; *lane 8,* 10 μg *E. coli* tRNA.

ized to OMP riboprobe. As shown in the right panel of Figure 8, 80–90% of the OMP RNA fractionated as polyA⁺. To assess to what extent the small amount of polyA⁻ OMP RNA was due to random degradation and/or incomplete fractionation, we determined what percentage of an endogenous polyA⁺ RNA fractionated as polyA⁻ in the same RNA samples. For this purpose, we used tyrosine hydroxylase RNA, which is a polyA⁺ RNA synthesized by OB juxtaglomerular neurons and is about the

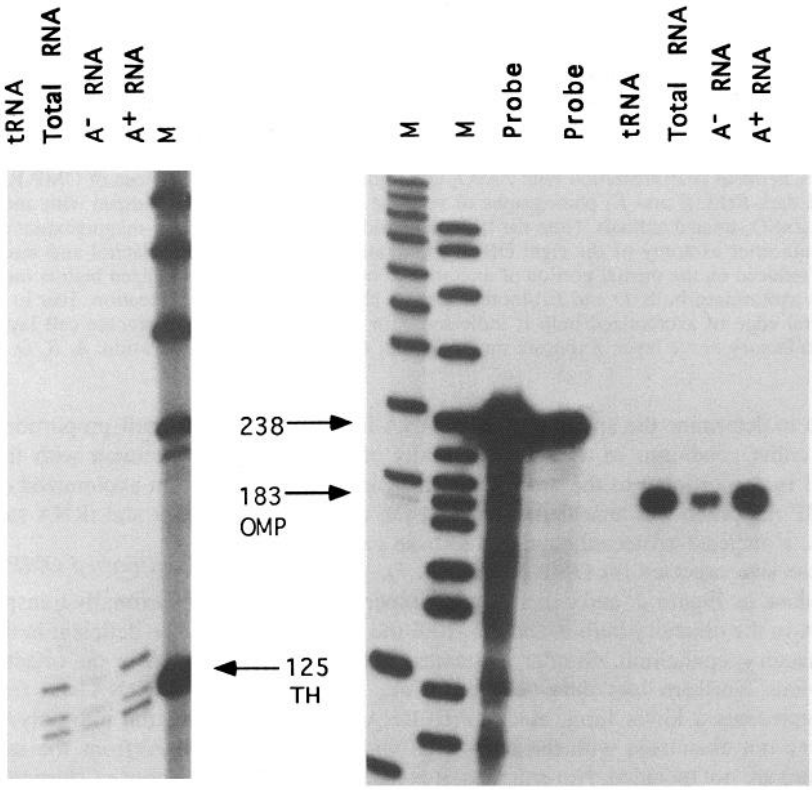

Figure 8. The majority of OMP RNA in the olfactory bulb is polyA⁺. Rat olfactory bulb (OB) RNA was fractionated into polyA⁺ or polyA⁻ fractions on oligo-dT cellulose and annealed with riboprobes for OMP (*right panel*) or tyrosine hydroxylase (*left panel*) and analyzed as described in Materials and Methods. *Left panel, lane 1* (*tRNA*), 50 μg *E. coli* tRNA; *lane 2* (*total RNA*), 35.3 μg of total OB RNA (equivalent to the amount of total RNA from one rat); *lane 3* (*A⁻ RNA*), 64.2 μg of polyA⁻ OB RNA (equivalent to RNA from two rats); *lane 4* (*A⁺ RNA*), 4.1 μg of polyA⁺ OB RNA (equivalent to RNA from two rats); *lane 5* (*M*), DNA markers. The expected size of the hybridized TH mRNA band is 125 bp, which represents the first exon. *Right panel: lanes 1 and 2* (*M*), DNA markers; *lanes 3 and 4* (*probe*), undigested OMP riboprobe; *lane 5* (*tRNA*), 50 μg of *E. coli* tRNA; *lane 6* (*total RNA*), 35.3 μg of total OB RNA; *lane 7* (*A⁻ RNA*), 64.2 μg of polyA⁻ OB RNA; *lane 8* (*A⁺ RNA*), 4.1 μg of polyA⁺ OB RNA.

same size as OMP RNA (TH RNA is 2.0 kb, OMP is 2.3 kb). As seen in the left panel of Figure 8, about the same proportion of TH RNA fractionates as polyA$^-$. These results were obtained from three different RNA preparations. These data suggest that OMP RNA in the bulb is polyA$^+$ and support earlier Northern blot analysis showing that OMP RNA was purified as polyA$^+$ RNAs from the OE and olfactory bulb (Ehrlich et al., 1990; Stone et al., 1991). However, these data do not exclude the possibility that the OMP RNA is partially deadenylated during transport, but enough polyA remains to bind to the affinity column and the reduction in size is too minor to be detected by Northern blots.

Discussion

This report demonstrates that OMP RNA exists in distal segments of rodent olfactory nerve. OMP RNA was detected by *in situ* hybridization using radiolabeled probes and autoradiography at the light microscope level. Although this method lacks sufficient resolution to allow definitive intra-axonal localization, we have combined this with biological evidence to support the contention that OMP is axonally located. Thus, the OMP RNA signal is present in the region of the OB that contains arriving axons and their synaptic terminals and is lost following peripheral deafferentation or olfactory nerve axotomy. These result are similar to those recently described by Vassar et al. (1994) and Ressler et al. (1994), who showed that OMP and/or olfactory receptor RNAs were found in the OB and could be reduced by peripheral deafferentation. Peripheral deafferentation results in degeneration of olfactory axons and their terminals but not associated glia (Burd, 1993). These observations are consistent with biochemical studies demonstrating functional OMP RNA in the OB (Rogers et al., 1987; Ehrlich et al., 1990; Grillo and Margolis, 1990). Although OMP RNA is easily detected in distal nerve segments that surround the OB and in their synaptic terminals in the glomerular layer, we were unable to observe OMP RNA in proximal axon bundles in the lamina propria even at exposures times longer than those used to detect OB signals. We conclude that the level of OMP RNA in proximal axons is below that of the distal segments, suggesting that there may be an increasing proximal to distal gradient of transported OMP RNA. The fact that we could not detect transport of G_{olf} RNA, which is as abundant as OMP RNA in the OE, suggests that not all RNAs are transported. However, a recent report has shown that another RNA, that encoding CGRP (calcitonin gene-related peptide), may be present in the mouse olfactory nerve surrounding the OB during embryonic development (Denis-Donini et al., 1993). Hence, as with dendritic RNAs, specific RNAs such as those encoding OMP, olfactory receptors and CGRP may be targeted for axonal transport.

If OMP RNA in the nerve and glomerular layer were not transported via axons, the only local source for RNA would be glia that surround olfactory axons in the olfactory nerve and intermingle with terminals in glomerular neuropil that is devoid of neuronal cell bodies. To our knowledge, OMP has never been localized to glia by immunocytochemistry. Hence, if OMP RNA exists in glia, it is not translated there to any detectable level. It is possible that glial OMP RNA could be imported into adjacent olfactory axons. Precedent for glial–axonal RNA import exists in the squid giant axon. Menichini et al. (1990) recovered labeled tRNA, rRNA, and mRNA in axoplasm when the squid giant axon, free of neuronal cell bodies but with its glial sheath attached, was incubated with tritiated uridine. The authors concluded that glial–axonal RNA transfer was the only possible source for the labeled axoplasmic RNAs. However, the giant axon may be unusual in that proteins are also efficiently and robustly transferred from glia to the axons (Gainer et al., 1977). If OMP RNA is synthesized in glia, synthesis by two different types of glia must be invoked, since the glia that ensheathe the axons in the nerve layer are distinct from those in the glomeruli of the OB proper (Barber and Lindsay, 1982; Vollrath et al., 1985; Pixley, 1992; Doucette, 1993). Most importantly, if OMP RNA is made in glia, this synthesis requires the presence of an intact axon, since deafferentation abolishes the *in situ* hybridization signal and the biochemical detection of OMP RNA by nuclease protection. Thus, the most parsimonious interpretation of our data is that OMP RNA is synthesized in the nuclei of receptor neurons in the OE and is axonally transported to terminals in the OB.

The function of axonal RNAs is unknown. Jirikowski et al. (1990) suggested that axonal oxytocin RNA could represent a storage compartment or be secreted. The *in situ* hybridization signal from OMP RNA extends into glomeruli, where receptor axons terminate on output mitral neurons and juxtaglomerular neurons. This suggests that OMP RNA may be present in presynaptic processes in a position where it could be released. Although oxytocin RNA was found in large granular secretory vesicles in neurohypophysial terminals (Jirikowski et al., 1990), it is unknown whether oxytocin RNA (or any axonal RNA) is secreted by neurons. Possibly, as suggested by Skutella et al. (1994) for TH mRNA in the hypothalamo–neurohypophysial tract, OMP RNA could be retrogradely transported back to receptor cell bodies as a signaling mechanism.

Although OMP RNA extracted from the OB could be translated *in vitro* (Rogers et al., 1987), we have no evidence *in vivo* that OMP protein is synthesized in axons or in terminals. To date, there have been no reports documenting ribosomes in distal segments of mature mammalian axons. In contrast to the lack of protein synthesis in mammalian axons, protein synthesis does occur in the goldfish Mauthner axon (Koening, 1991) and in the squid giant axon (Giuditta et al., 1991; Crispino et al., 1993). However, the experiments in which AVP RNA, injected into axons, ameliorated the diabetic condition of Brattleboro rats, suggests that axonal RNAs can be efficiently translated, presumably after retrograde transport, back into cell bodies (Jirikowski et al., 1992; Maciejewski-Lenoir et al., 1993). Hence, axonal RNAs can be translated, albeit in the perikarya, in mammalian neurons.

OMP RNA has an unusually long 3′ untranslated region: its 3′ untranslated region (1630 bp) is 3.5-fold longer than its protein coding region (486 bp) (Rogers et al., 1987). This is potentially relevant because all known signals that localize RNAs to discrete subcellular sites are found in the 3′ untranslated regions (Singer, 1993). Localization signals are presumed to be bound by unidentified proteins. The resulting ribonucleoprotein complexes, perhaps associated with a small, noncoding RNA called BC1 (Tiedge et al., 1991, 1993), are transported and anchored by microfilaments and/or microtubules (Singer et al., 1989; Yisraeli et al., 1990; Ainger et al., 1993; Bassell et al., 1994). Microtubules are particularly important for RNA transport in glia and neurons. In cultured oligodendrocytes, injected RNA during transport is physically adjacent to microtubules (Ainger et al., 1993). In cultured cortical neurons, the majority of *in situ* hybridization signal from endogenous polyA$^+$ RNA was within 50 nm of a microtubule, and most dendritic localization was lost

after disruption of microtubules with colchicine (Bassell et al., 1994). While the dendritic transport mechanism is microtubule based, part of the selectivity for transport may be exclusionary in nature. Recent experiments by Kleiman, Banker, and Steward (1993) show that RNAs that usually remain in the cell body appear in dendrites when protein synthesis is blocked, suggesting that a rapidly turning-over protein may prevent dendritic transport of nondendritic RNAs.

Recent evidence suggests that microtubules and their associated MAPs may play a second, "exclusionary" role that excludes high molecular weight RNAs from axons. After 1 d in culture, cortical neurons transport polyA$^+$ RNAs into both dendrites and axons (Bassell et al., 1994). In the axon-like process, polyA$^+$ RNA extended throughout the majority of the process. However, after 4 d in culture, RNA was restricted to the most proximal axon segment. Likewise, in cultured hippocampal neurons, newly synthesized RNA was initially transported into both dendritic and axonal processes, but after 7–10 d in culture, it was excluded from axons (Kleiman et al., 1994). Exclusion of RNA from axons coincides with the maturation of the microtubule-based cytoskeleton, particularly the appearance of tightly bundled, microtubules characteristic of mature axons. Bassell et al. (1994) showed that polyA$^+$ RNA was not observed in the vicinity of tightly bundled parallel microtubules, but was preferentially localized near loosely parallel or nonparallel microtubules. Earlier data showed that ribosomes, which were normally excluded from the axons of cultured chick sensory neurons, moved into axons after microtubules were disassembled by nocodazole, griseofulvin, or cold treatment (Baas et al., 1987), supporting the contention that microtubules prevent high molecular weight RNAs from entering axons.

How might these observations impinge on our data showing that OMP mRNA exists in the axons of olfactory receptor neurons? Perhaps because olfactory neurons are generated even in adult animals, their cytoskeleton lacks many components of mature neurons and retains cytoskeletal proteins usually found in immature neurons. For example, receptor neurons do not make neurofilaments (Vollrath et al., 1985) or MAP2, found in mature dendrites, while they continue to express MAP5 and vimentin, which usually disappear during maturation (Schwob et al., 1986; Viereck et al., 1989). Importantly, olfactory receptor axons do not have tau, an axon-specific MAP (Viereck et al., 1989), which has been shown to be responsible for bundling of microtubules *in vitro* (Hirokawa et al., 1988) and *in vivo* (Kanai et al., 1989; Lewis et al., 1989). Hence, it is likely that olfactory receptor axons lack tightly bundled microtubules that exclude mRNAs in most neurons. Electron micrographs from the frog and salamander olfactory nerve support this contention and show that the number of microtubules per axon is 2–5 (Burton, 1984; Burton and Wentz, 1992), too few to allow tight bundling. Therefore, our observations are fully consistent with the hypothesis that compartmentalization of translationally active cytoplasm away from translationally inactive axons is due to the exclusion of mRNA by tightly bundled microtubules organized by tau. Hence, the immature nature of the axonal cytoskeleton in olfactory neurons may resemble that observed in young cultures of primary neurons where polyA$^+$ and newly synthesized RNAs are transported into axons.

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