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Cloning and Characterization of SLP3: a Novel Member of the Stomatin Family Expressed by Olfactory Receptor Neurons

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ABSTRACT

The detection of odorants with high sensitivity and specificity utilizes specialized transduction proteins that may be assembled into complexes to afford enhanced speed and efficiency in olfactory neurons. We have used a differential cDNA screening technique to identify novel gene products that display restricted expression within the olfactory epithelium. Here we report the characterization of an olfactory neuronal protein, SLP3, which shares extensive homology with the stomatin family of membrane proteins. Other stomatin family members have been implicated in specific interactions with ion channels and G protein-coupled receptors. The pattern of SLP3 mRNA expression during embryonic development and the subcellular localization of the SLP3 protein in mature olfactory neurons observed here is consistent with a specific role for this protein in the assembly, translocation, or function of the odorant transduction complex in olfactory neurons.

Keywords: olfaction, transduction, stomatin, neuron

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INTRODUCTION

The highly specialized chemosensory neurons of the olfactory system are responsible for the initial detection and discrimination of thousands of odors by the mammalian brain. The sensory neurons are present in a self-renewing neuroepithelium where they are replaced throughout life by new neurons generated from proliferative basal cells (Graziadei and Graziadei 1979). The mature olfactory epithelium (OE), therefore, contains progenitor cells, immature neurons, and mature, fully differentiated receptor neurons. The signal transduction of olfactory stimuli by these neurons is well understood. A large multigene family of G protein-coupled receptors (Buck and Axel 1991) activates a cascade consisting of specialized forms of a $G\alpha$ subunit, an adenylyl cyclase, and a cyclic nucleotide-gated cation channel (Bakalyar and Reed 1990; Dhallan et al. 1990; Jones and Reed 1989).

Many aspects of olfactory neuronal function remain to be elucidated, including the pathways for receptor localization to the membrane and the organization of transduction proteins within the neuronal cilia. In the *Drosophila* visual system, for example, the organization of phototransduction protein complexes has been well studied and involves a scaffolding protein that assembles ion channels, phospholipase CB, and protein kinase C (Tsunoda et al. 1997).

Olfactory bulb ablation induces the rapid degeneration of the mature neurons in the receptor epithelium (Carr and Farbman 1992; Schwob et al. 1992). A robust proliferation of neuronal progenitors within the OE leads to the subsequent epithelial repopula-

tion. Therefore, this neurogenic activity in the OE can generate temporally synchronized populations of mature and immature olfactory neurons suitable for the identification of gene products uniquely present in specific cell types. The selective expression of olfactory transduction components in mature olfactory neurons has been demonstrated by this ablation technique (Bakalyar and Reed 1990; Dhallan et al. 1990; Jones and Reed 1989; Jones et al. 1988).

A differential screening strategy comparing normal and bulbectomized OE led to the isolation of genes expressed by olfactory neurons and the identification of novel RNA transcripts restricted to particular cell populations (Wang et al. 1993). In the present study, we describe an additional characterization of one of these genes, which indicates that it encodes a new member of the stomatin family of plasma membrane-associated proteins. Stomatin was first identified in erythrocytes as a protein (band 7) whose absence correlated with hereditary hemolytic anemia (Stewart et al. 1992; Hiebl–Dirschmied et al. 1991), and it appeared to interact with a novel G protein-coupled receptor in red cells (Mayer et al. 1998). Recently, stomatin has also been shown to be expressed in mechanosensory neurons, where it may interact directly with transduction components, including cation channels (Goodman et al. 2002; Fricke et al. 2000; Mannsfeldt et al. 1999). Other stomatin family members—SLP1, SLP2, and NPHS2 (podocin)—also display selective expression patterns (Boute et al. 2000; Wang and Morrow 2000; Seidel and Prohaska 1998). For instance, robust SLP1 expression appears to be restricted to brain, with low-level expression detectable in heart and skeletal muscle, while podocin expression is restricted to renal glomeruli.

Here, we describe the identification, cloning, and characterization of mouse Stomatin-Like-Protein 3 (SLP3). Expression of the SLP3 mRNA is observed only in olfactory tissue. Within this tissue, it is abundantly expressed in olfactory neurons and displays a late onset of embryonic expression. Analysis of the SLP-deduced amino acid sequence indicates that it shares higher homology with stomatin protein than other members of the stomatin family. The expression pattern and localization to the dendrite and cilia, as well as the involvement of stomatin family members with signal transduction components are consistent with a possible role in the unique transduction pathway utilized by olfactory neurons.

METHODS

Isolation of the mouse SLP3 cDNA

In previous experiments, mRNA from normal rat olfactory epithelium and olfactory tissue five days after unilateral olfactory bulb ablation (Carr and Farbman 1992; Schwob et al. 1992) was isolated, labeled, and used in a differential screen of a rat olfactory cDNA library. Briefly, OE tissue was isolated from control rats and rats 5 days following bulb ablation. RNA was then isolated by the Trizol method (Gibco BRL) and purified by oligo dT separation; then ^{32}P-labeled cDNA was generated. Lifts from agar plates with bacterial colonies expressing the normal olfactory cDNA library were then prepared in duplicate. These membranes were hybridized with the ^{32}P-labeled probes from normal or bulbectomized rat OE, and differential screening was performed by directly comparing hybridization patterns. Among the clones identified in this screen was one isolate (50.11) that coded for a protein with significant homology to the human stomatin gene and that recognized a message that expressed at higher levels in normal OE than in neuron-depleted OE.

To identify the mouse homolog of the rat stomatinlike 50.11 cDNA, the rat 50.11 cDNA insert was excised from pGEM with EcoRI, gel-purified, ^{32}P-labeled, and used to screen a mouse olfactory epithelial ΛZAP cDNA phage library. The cDNA insert from strongly hybridizing plaques was excised with M13 helper phage and the resulting pBK–CMV plasmid was purified. The 1862 bp insert was sequenced on both strands and designated stomatin-like-protein 3 (SLP3), based on sequence similarity to mouse stomatin.

Tissue preparation

Timed pregnant CD1 mice (Charles River), with the morning after evening mating designated as embryonic day 0.5 (E0.5), were sacrificed to obtain embryonic tissue of specific developmental age. E15.5 and E17.5 embryos and postnatal day (PD) 2 heads were fixed by immersion in Bouins solution (Sigma). Adult C57BL6J mice were perfused transcardially with cold phosphate buffered saline (PBS) followed by Bouins solution, and olfactory tissue was isolated and postfixed for 1 hour. Tissue was cryoprotected overnight at 4°C in 30% sucrose, 250 mM EDTA, DEPC–PBS, embedded in OCT compound (Miles), and frozen by immersion in liquid nitrogen prior to cryosectioning at 10 μm.

In situ hybridizations

A pBK–CMV plasmid containing 735 bp of the SLP3 coding region and 81 bp of the 5′ untranslated region was used to generate a digoxigenin-labeled riboprobe (Boehringer Mannheim). *In situ* hybridization was performed essentially as described (Tsuchida et al. 1994). Briefly, 10 μm tissue sections, prepared as de-

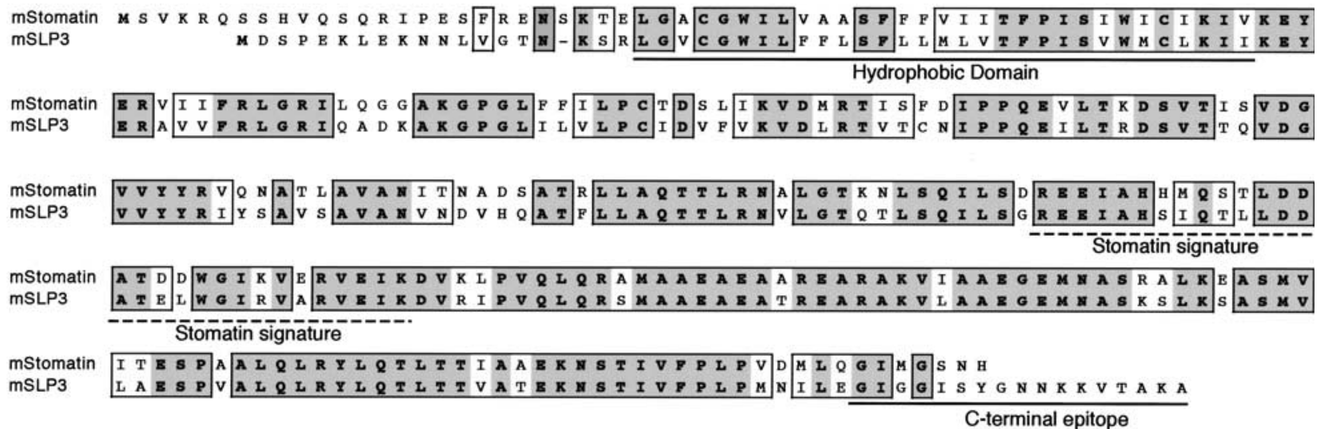

FIG. 1. Alignment of stomatin and the mouse SLP3 (mSLP3) protein. The deduced amino acid sequences of the mouse stomatin and the mSLP3 proteins were aligned by the Clustal algorithm. Identical amino acid residues in the two proteins are shown with dark shading and conservative changes are indicated with light shading. The location of conserved structural motifs, including the hydrophobic domain and the stomatin signature, are indicated by underlines. The carboxyl-terminal epitope of SLP3 used to generate specific antisera is also indicated.

scribed above, were postfixed with Bouins solution for 5 minutes, rinsed with DEPC–PBS, treated for 10 minutes with proteinase K (20 μg/mL), fixed again in Bouins solution for 5 minutes, and rinsed. Following acetylation for 10 minutes, sections were rinsed and prehybridized for 3 hours at room temperature. Overnight hybridization was performed with 0.5 μg/μL digoxigenin-labeled probe at 65–70°C. Posthybridization washes were performed twice with 0.2× SSC at 65°C. Sections were then incubated with alkaline phosphatase-conjugated antidigoxigenin antibody (Boehringer Mannheim) in 5% normal goat serum for 1 hour at room temperature, developed with NBT/BCIP (Boehringer Mannheim), rinsed, and mounted for microscopy (Aqua-Polymount, Polysciences).

Antibody generation

Peptides corresponding to the 17 C-terminal residues and the 16 N-terminal residues of the deduced mouse SLP3 amino acid sequence (H_2N-GIGGISYGNNKKVTAKA–COOH and MDSPEKLEKNNLVGTN-amide) were synthesized, coupled to bovine serum albumin using glutaraldehyde, and each injected into two rabbits to generate polyclonal antisera as previously described (Davis and Reed 1996). The SLP3 antisera were tested and found to specifically recognize recombinant SLP3 protein when expressed in HEK293 cells. Plasmid containing the CMV promoter directing expression of the full coding region of SLP3 was transfected into HEK293 cells using Lipofectamine (Gibco BRL) per manufacturer's instructions. After 3 days growth, transfected cultures, and control cultures that were not transfected, were fixed with Bouins solution and processed for anti-SLP3 immunochemistry (as described below for immunohistochemistry) to confirm activity of SLP3 antisera. The C-terminal SLP3 antiserum, designated JH3853, was used for the subsequent analysis. Antipeptide sera were aliquoted and stored at −80°C.

Immunohistochemistry

Cyrosections (10 μm), prepared as described above, were postfixed with Bouins solution for 5 minutes, rinsed in 1 × PBS, and blocked for 30 minutes in 10% normal goat serum in 1 × PBS containing 5% nonfat dry milk and 0.1% Triton X-100. The sections were then incubated with SLP3 antiserum (1:1000) for 1 hour at room temperature. Sections were washed 3 times in 1 × PBS with 0.5% BSA, incubated with biotinylated antirabbit IgG, washed again, and incubated with avidin-biotinylated HRP complex (Vectastain Elite ABC kit, Vector Labs). Bound secondary antibody was visualized with 3,3′-diaminobenzidine (DAB), and sections were then rinsed and coverslipped with Aqua-Polymount (Polysciences).

RESULTS

SLP3: a novel member of the stomatin gene family

In previous experiments, mRNA from normal rat olfactory epithelium and olfactory tissue 5 days after unilateral olfactory bulb ablation (Carr and Farbman 1992; Schwob et al. 1992) was isolated, labeled, and used in a differential screen of a rat olfactory cDNA library. The mRNA prepared from OE harvested 5 days following bulbectomy lacks gene products selectively expressed in fully differentiated, mature olfactory neurons. Nitrocellulose filters from agar plates with bacterial colonies containing a normal olfactory cDNA

library were hybridized with the ^{32}P-labeled probes from normal or bulbectomized rat OE, and differential screening was performed by directly comparing hybridization patterns. In addition to the components of the odorant transduction pathway (G_{olf}, type III adenylyl cyclase, the cyclic nucleotide-activated ion channel subunit, CNGA2), several functionally uncharacterized cDNA clones (50.06, 50.08, and 50.11) displayed differential expression levels in these two experimental conditions (Wang et al. 1993). One isolate (50.11) that recognized a 1.8 kb message expressed at higher levels in normal OE than in neuron-depleted OE coded for a 287 amino acid protein with significant homology to the human stomatin gene.

In order to further characterize the expression of the rat 50.11 gene and clarify its evolutionary relationship to other family members, we isolated the mouse 50.11 ortholog by screening a mouse OE cDNA library with the rat clone. An 1863 nucleotide mouse cDNA was isolated that shared 84% nucleotide identity to the rat cDNA. The sequence contains an open reading frame coding for 286 residues with only a single amino acid difference ($Q_{215}L$) from the deduced sequence of the rat 50.11 protein. A BLAST sequence search with the mouse 50.11 cDNA coding region revealed that it was a novel member of the stomatin gene family (Fig. 1). Stomatin, or band 7.2b, was first identified as a membrane-associated protein in erythrocytes, and is thought to be involved in cation channel regulation (Gallagher and Forget 1995; Stewart et al. 1992; Hiebl–Dirschmied et al. 1991); however, its definitive function remains to be elucidated (Delaunay et al. 1999; Zhu et al. 1999). The stomatin family includes stomatin-like-protein 1 (SLP1), SLP2, and podocin (Boute et al. 2000; Wang and Morrow 2000; Seidel and Prohaska 1998), as well as homologs in zebrafish, *Caenorhabditis elegans*, *Drosophila*, and plants (Rajaram et al. 1998; Huang et al. 1995). We have designated this newly identified protein stomatin-like-protein 3 (SLP3), in accordance with the published nomenclature for the gene family. The highest sequence homology identified is to mouse stomatin; mouse SLP3 is 62% identical to mouse stomatin at the amino acid level (Fig. 1). DNA sequence searches of the Celera mouse genomic sequence reveals that each of the stomatin family members (stomatin, SLP1, SLP2, SLP3, and podocin) are the products of distinct genes encoded on different mouse chromosomes (chromosomes 2, 9, 13, 3, and 1, respectively). Proteins of the stomatin family share a conserved domain, termed the stomatin signature sequence, that defines this gene family [RX_2(L/I/V)(S/A/N)X_6(L/I/V) DX_2TX_2WG(L/I/V) (K/R/H) (L/I/V) X(K/R)(L/I/V)E(L/I/V)E (K/R)] (Wang and Morrow 2000), which is present in SLP3 from residues 160–189 (Fig. 1). Also, stomatin family members, with the exception of SLP2, contain an NH_2-terminal hydrophobic domain. SLP3 contains this hydrophobic sequence from amino acids 20–48 (Fig. 1). It has been proposed that stomatins are inserted into the plasma membrane in a hairpin fashion at this domain, such that both the NH_2-terminus and COOH-terminus are cytoplasmic (Huang et al. 1995). SLP3 also contains a conserved cysteine at residue 29, shown to be the major palmitoylation site for stomatin (Snyers et al. 1999). Protein structure prediction of SLP3 suggests the presence of a central region rich in B structure and a more distal region rich in α helix, consistent with the structure of other stomatins (Wang and Morrow 2000).

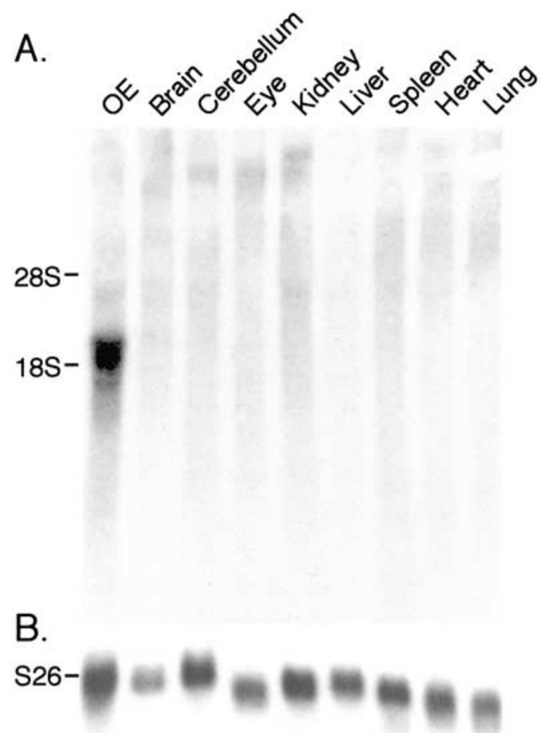

FIG. 2. Tissue-specific expression of SLP3 mRNA. Total RNA isolated from tissues of nine adult mice was fractionated by gel electrophoresis and hybridized with a radiolabeled mSLP3 probe (**A**) and with an S26 probe (**B**) as a positive control for the presence of RNA. **A.** SLP3 signal is restricted to olfactory epithelium (OE) even after longer exposure. The size of the mSLP3 cDNA (1863 bp) agrees well with the observed SLP3 mRNA transcript(1950 bases) based on a comparison to 18S rRNA. **B.** The integrity and abundance of the RNA sample from each tissue was assessed by hybridization with ribosomal protein S26 probe, a broadly expressed mRNA.

Expression of SLP3 is restricted to the olfactory epithelium

We next examined the tissue distribution of SLP3 mRNA. Northern blot analysis was performed with a coding region probe from SLP3 on total RNA from nine tissues of adult mice. A robust signal was detected in OE but not in any of the other tissues

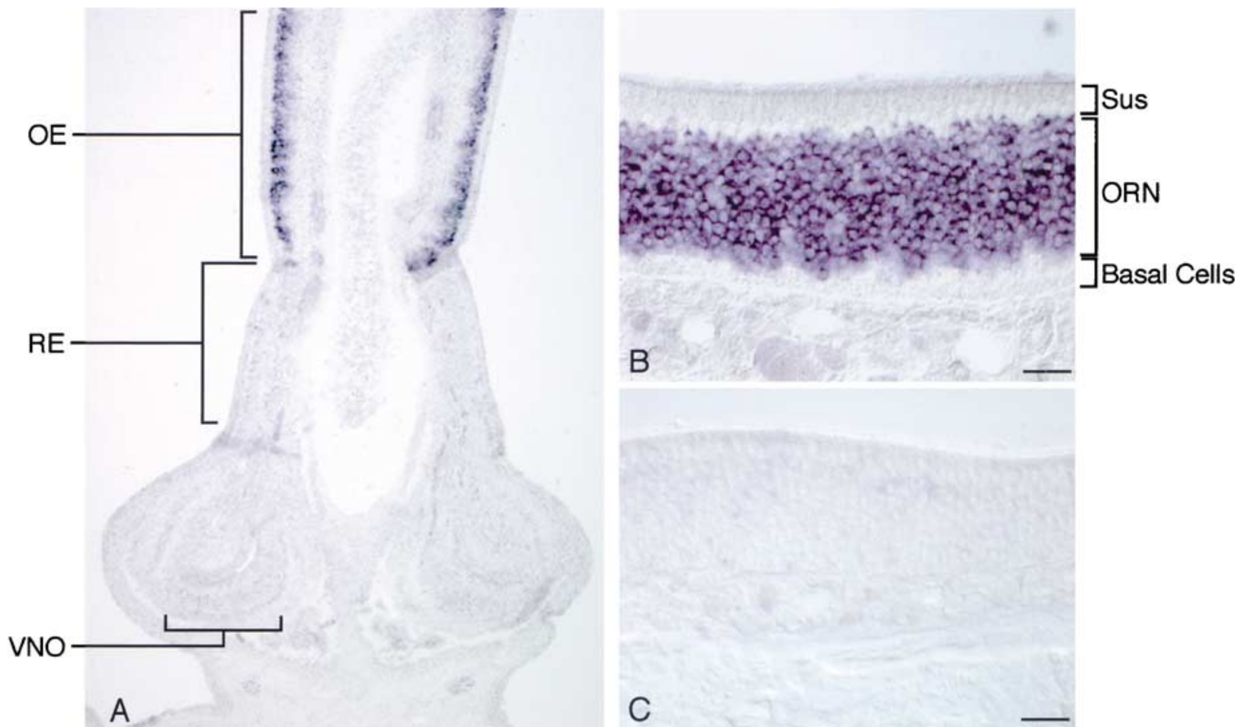

FIG. 3. Expression of SLP3 mRNA in olfactory tissue. Coronal tissue sections from anterior olfactory tissue were hybridized with a digoxygenin-labeled mSLP3 antisense probe (**A**). In this region, olfactory epithelium (OE), nonsensory respiratory epithelium (RE), and the pheromone-sensing vomeronasal organ (VNO) are present along the nasal septum. Signal is restricted to the sensory epithelium containing olfactory neurons. Within the olfactory epithelium (**B**), the SLP3 mRNA is present in the olfactory receptor neurons (ORN) and is absent from sustentacular and microvillar cells (Sus) and globose and horizontal basal cells. Hybridization of sections with a sense control probe (**C**) confirms the specificity of the observed *in situ* signal. (**B**, **C**) Scale bar = 20 μm.

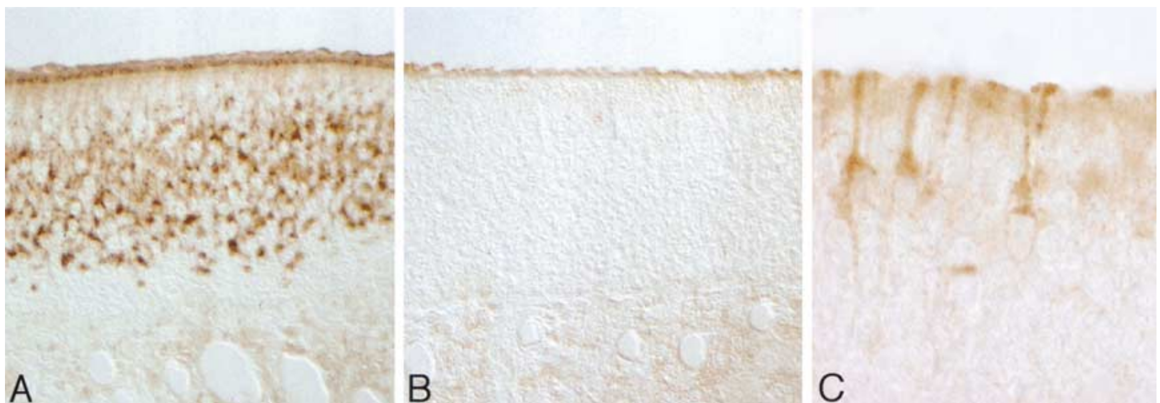

FIG. 4. Localization of SLP3 protein in olfactory epithelium. Sections of olfactory epithelium from adult mice are incubated with antiserum to SLP3 (**A**). Immunoreactivity is observed in the middle region of the epithelium, consistent with expression of SLP3 in mature olfactory neurons. Staining of the luminal surface in the region of the dendritic knobs is evident. Note the absence of signal in the fasciculate axons that pass through the basal lamina on their way to the olfactory bulb. Specific staining is not observed when parallel sections are incubated with preimmune serum (**B**). A high-magnification image of E17.5 olfactory epithelium (**C**) reveals the subcellular location of SLP3 protein. The smaller number of fully differentiated neurons at this time point assists in the visualization of individual cells and their processes. The presence of SLP3 protein apical to the cell nucleus is apparent. Immunoreactivity is also detected in the dendrite and dendritic knob of the olfactory neurons.

examined (Fig. 2). These include tissues known to contain other stomatin family members. The expression of SLP3 was then examined by *in situ* hybridization to coronal sections to the nasal cavity of postnatal day 2 (PD2) mice (Fig. 3A). Signal was restricted to regions containing olfactory neurons and absent from epithelial areas composed of nonsensory respiratory cells. Interestingly, no SLP3 expression was

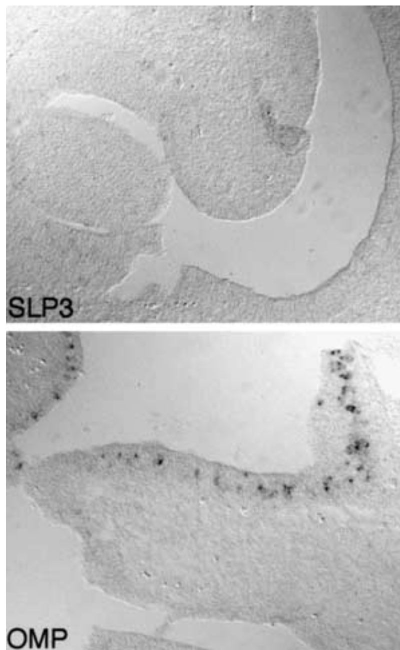

FIG. 5. Late expression of SLP3 during embryonic development. Coronal sections of olfactory epithelium at E15.5 were hybridized with digoxygenin-labeled probes encoding mSLP3 or olfactory marker protein (OMP). At E15.5, no SLP3 mRNA is detected in the olfactory epithelium. At this developmental age, OMP, a mature olfactory neuronal marker, is expressed in scattered neurons throughout the neuronal layer.

detected in the vomeronasal organ containing the cell bodies of the sensory neurons responsible for pheromone detection (Fig. 3A) (Liman 1996). The vomeronasal neurons and olfactory receptor neurons do share expression of other olfactory-enriched genes including olfactory marker protein (OMP) and OE transcription factors (Behrens et al. 2000). We conclude from these data that SLP3 is an olfactory epithelial-specific gene product.

SLP3 expression in olfactory neurons

We next determined the expression pattern of SLP3 mRNA by *in situ* hybridization with a digoxigenin-labeled probe on sections through the nasal fossae of adult mice. SLP3 expression is confined to olfactory neurons that are situated in several layers in the middle region of the adult pseudostratified neuroepithelium (Fig. 3B). The sustentacular and microvillar cells have nuclei located in an apical layer, and two basal populations, globose basal cells (GBCs) and horizontal basal cells (HBCs), situated deep in the epithelium (Graziadei and Graziadei 1979) are not labeled. Additionally, no signal is seen in cells outside of the OE, including Bowman's gland acinar cells. No staining was seen in control hybridization with sense probe (Fig. 3C). We conclude that SLP3 is selectively expressed by olfactory neurons, consistent with the initial data indicating that the SLP3 transcript is present in normal rat OE cDNA but absent when olfactory neurons were depleted.

To examine the expression of SLP3 protein, we generated rabbit antiserum to synthetic peptides corresponding to the N-terminus and C-terminus of the deduced SLP3 amino acid sequence. These peptides were chosen to eliminate cross-reactivity of the SLP3 antiserum with other stomatins. The ability of the antiserum to detect SLP3 protein was demonstrated by expression of recombinant SLP3 in HEK293 cells.

We next determined the immunohistochemical staining pattern with the C-terminal SLP3 antiserum in olfactory tissues and confirmed the restricted expression to olfactory neurons (Fig. 4A). This pattern corresponds to the *in situ* hybridization signal observed for the unique SLP3 antisense probe. Olfactory neurons elaborate a dendrite protruding to the apical surface of the epithelium, ending in a knob which bears multiple cilia that project into the luminal mucus layer (Graziadei and Graziadei 1979). The initial signal transduction events occur in these dendritic processes. Interestingly, SLP3 protein is preferentially localized to the apical portions of neurons (Fig. 4C). Immunoreactive material in the cell soma is distributed asymmetrically, with intense staining visible in the apical region above the nucleus likely corresponding to the endoplasmic reticulum and Golgi network in these cells. Intense labeling of the dendrite and dendritic knob is evident (Fig. 4C). Remarkably, there is little to no staining of the axons emerging from the basal region of the epithelium, or in the axon fascicles seen in cross section below the basal lamina. The N-terminal SLP3 antibody staining produced the same pattern of staining in olfactory epithelium.

Developmental expression of SLP3

We examined the spatial and temporal pattern of SLP3 expression during mouse development. At the earliest time point examined (E15.5), SLP3 mRNA is not detected (Fig. 5). In contrast, OMP, a well-characterized protein expressed late in the olfactory neuron differentiation pathway and through to correlate well with neuronal maturation (Margolis et al. 1991), is detectable at E15.5 in rat OE, corresponding to about E13.5 in the mouse (Farbman et al. 1994). The signal transduction components G_{olf} and adenylyl cyclase appear at low mRNA levels at E15–16 (Farbman 1994; Dau et al. 1991). The levels increase shortly thereafter at a time coincident with robust extension of sensory cilia. By *in situ* hybridization, SLP3 mRNA is first detectable at embryonic day 17.5

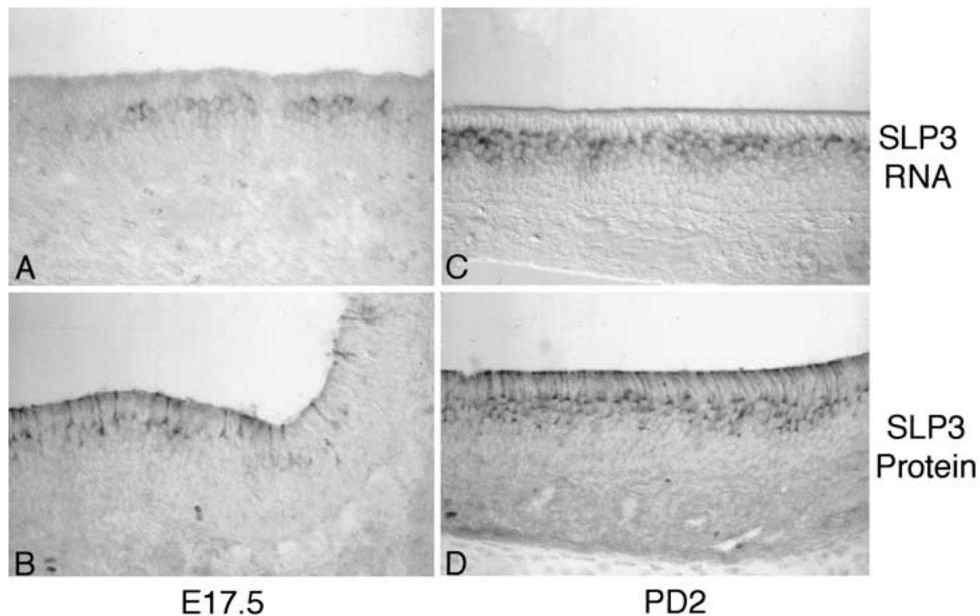

FIG. 6. Developmental expression of SLP3 mRNA and protein. **A.** The SLP mRNA is detected surrounding the nuclei of the most apical sensory neurons at E17.5. **B.** Consistent with this pattern is the expression of SLP3 protein in the dendrites and dendritic knobs of the same cell population. **C.** At postnatal day 2 (PD2), expression of SLP3 mRNA is detected in olfactory neurons located in central regions of the epithelium. **D.** The SLP3 protein is broadly expressed at PD2 in the dendrites, dendritic knobs, and cilia of the olfactory neurons.

(E17.5) in mouse OE (Fig. 6A), quite late in olfactory development. The expression of the SLP3 mRNA corresponds more closely with the appearance of cilia in the mucosa. At PD2 the number of SLP3 mRNA expressing cells has increased substantially (Fig. 6B). Thus, the late embryonic appearance of SLP3 mRNA lags the first appearance of characteristic mature neuronal markers and parallels the maturation of the sensory transduction apparatus in the OE during embryonic development.

The expression pattern of SLP3 protein during development is in agreement with the results from *in situ* hybridization. Protein appears to be predominantly localized to the developing dendrites and dendritic knobs (Fig. 6C); this is especially evident upon high-magnification examination of prenatal OE, in which individual SLP3-expressing dendrites at E17.5 can be distinguished easily (Fig. 4C). At this stage, the OE does not yet contain the adult distribution of fully mature neurons, so SLP3-expressing cells are less densely packed within the epithelium. Examination of sections prepared from mice at PD2 indicates that SLP3-expressing neurons are more numerous than at E17.5 and are confined to the most apical layers of the neuronal strata (Fig. 6D). This labeling pattern is more similar to that of OMP at PD2. It is important to note that neurons are continually produced from basal cells in the OE throughout life; there exists a basal-to-apical gradient with newly generated differentiating neurons situated deep in the OE and mature differentiated neurons located in the most apical regions (Graziadei and Graziadei 1979). Thus, at PD2, the expression pattern of SLP3 is consistent with expression in the most mature neurons.

DISCUSSION

We describe here the identification of a novel member of the stomatin gene family termed SLP3. Characterization of SLP3 has revealed that this gene is expressed selectively in the receptor neurons of the olfactory epithelium. Although the definitive functions for stomatin and other stomatin family members have not been established, several studies have implicated these proteins in the localization and organization of cation channels and/or other transduction components (Goodman et al. 2002; Fricket et al. 2000; Mayer et al. 1998). These observations, along with the protein structure, developmental expression pattern, and subcellular localization of SLP3, are consistent with a possible interaction of SLP3 with the transduction apparatus in olfactory neurons.

The SLP3 protein shares 62% identity to stomatin at the amino acid level and is more similar in sequence to stomatin than are other members of this family. SLP3, therefore, may share related functions with stomatin. Several groups have suggested that stomatin may be associated with cation channels di-

rectly or indirectly (Stewart et al. 1992). In *C. elegans*, the stomatin homolog mec-2 is thought to link a mec-4/mec-10 ion channel to the cytoskeleton in mechanosensory neurons (Huang et al. 1995). Rat stomatin and degenerin/epithelial sodium channel proteins, related to mec-4/mec-10, colocalize in trigeminal ganglion and vibrissal follicle-sinus cells (Fricke et al. 2000). Mouse stomatin is expressed in dorsal root ganglia, where it may participate in a mechanotransduction complex (Mannsfeldt et al. 1999). These data suggest that there may be an evolutionarily conserved function for stomatinlike proteins in transduction complexes.

The presence of an abundant stomatinlike protein expressed exclusively in olfactory neurons suggests a specialized function in this tissue. In each of the studies mentioned above, stomatin appears to contribute to the localization or assembly of a transduction complex. The structure and expression pattern of SLP3 suggests that this protein may serve a similar role in olfactory neuronal cells. SLP3 is homologous to stomatin and bears a conserved N-terminal hydrophobic domain and stomatin signature sequence. The subcellular localization of SLP3 within the olfactory neuron corresponds to subcellular organelles where integral membrane proteins, including odorant transduction components, are synthesized, processed, and subsequently translocated to their site of action. Specifically, SLP3 protein is highly enriched in the apical juxtanuclear location containing the Golgi complex where membrane proteins are modified. The presence of SLP3 in the dendrite and dendritic knob parallels the pathway of the odorant transduction components from the Golgi compartment to their eventual insertion into the ciliary membrane. Odorant transduction components colocalized in this region include the G-protein-coupled receptor (Menco et al. 1997), the olfactory G-protein G_{olf}, adenylyl cyclase III, and presumably the olfactory cyclic nucleotide channel (Bakalyar and Reed 1990; Jones and Reed 1989). It is interesting to note that SLP3 expression is not detected in the vomeronasal organ (Fig. 3), a chemosensory neuron that is structurally similar but utilizes distinct transduction components (Behrens et al. 2000).

Although the molecular basis for odorant detection and signal transduction in olfactory neurons is well understood, several aspects of the differentiation and function of olfactory neurons remain poorly defined. For instance, odorant receptors are uniformly unable to be properly inserted into the plasma membrane when expressed in heterologous cells (Gimelbrant et al. 1999). One explanation for this observation is that an unidentified molecular chaperone is required for this process (Dwyer et al. 1998; Krautwurst et al. 1998). Recent work with cell lines suggests that conserved mechanisms involving a membrane-associated ODR-4-like protein may be necessary for odorant receptor trafficking in mammals (Gimelbrant et al. 2001). Interestingly, in erythrocytes, the C-terminus of stomatin interacts with a novel G-protein-coupled receptor (Mayer et al. 1998). The sequence similarity of SLP3 to stomatin suggests that SLP3 might interact similarly with G-protein-coupled odorant receptor proteins in olfactory neurons. The hypothetical structure of stomatinlike proteins and the subcellular localization of SLP3 in the region of olfactory transduction components suggest that SLP3 could directly interact with one or more of the transduction components; however, further experiments on SLP3 function will be necessary to determine if this protein has a role in the facilitation of receptor translocation. The availability of a mouse SLP3 cDNA will facilitate the disruption of this gene by homologous recombination in embryonic stem cells and may allow a direct examination of the role of SLP3 in olfactory neuron function.

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