Differential Display Identification of *plunc*, a Novel Gene Expressed in Embryonic Palate, Nasal Epithelium, and Adult Lung*\textsuperscript{*}

Wayde M. Weston‡‡, Elizabeth E. LeClair¶, Wendy Trzyna‡, Kirk M. McHugh‡, Paul Nugent**, Cynthia M. Lafferty‡, Linh Ma‡‡, Rocky S. Tuan†, and Robert M. Greene**

From the ‡Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, the ¶Orthopaedic Research Laboratory, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, the **Department of Biological and Biophysical Sciences, University of Louisville School of Dentistry, Louisville, Kentucky 40292, and the ‡‡Department of Pathology, Allegheny University of the Health Sciences, Philadelphia, Pennsylvania 19102

We have identified a novel gene transcript of approximately 1.1 kilobases in length that is expressed in the presumptive nasal epithelium of the mouse embryo. In situ hybridization analysis shows discrete regions of expression associated with the palate, nasal septum, and nasal conchae. This transcript is also expressed strongly in the trachea and bronchi of the adult lung. Screening of a mouse heart cDNA library yielded several overlapping clones to give a continuous sequence of 1113 bases, containing an open reading frame of 278 codons comprising the complete mRNA. No significant homologies with known genes were observed at the nucleotide level; limited amino acid homology with two salivary gland-specific proteins was noted. A search for functionally significant protein motifs revealed consensus sequences for N-glycosylation, protein kinase C and casein kinase phosphorylation, and a leucine zipper. Additionally, we observed a unique amino acid sequence pattern, consisting of the residues Gly-(Leu/Pro/Gln)-(Pro/Leu)-Leu-Pro-Leu, repeated four times near the amino-terminal portion of the protein with two amino acid residues separating the repeats. Based on these observations, we propose that we have identified a new gene, which we call *plunc* (for palate, lung, and nasal epithelium clone; GenBank\textsuperscript{TM} accession number U69172).

Development of the mammalian palate is a complex process involving the migration and differentiation of neural crest derivatives in the cranial region of the embryo. Palatal structures arise from the first branchial arches, paired swellings of neural crest mesenchyme. The formation of the secondary palate first becomes evident on gestational day (gd)\textsuperscript{1} 12 in the mouse embryo, with the appearance of the palatal shelves, bilateral growths of tissue extending vertically from the roof of the oronasal cavity along either side of the tongue. The palatal shelves continue to grow in this orientation until gd14, when they become reoriented to lie horizontally above the tongue in a process known as elevation. Following elevation, the opposing shelves contact each other and fuse to form a continuous structure separating the oral and nasal cavities. Disruption of the growth and morphological differentiation of these facial primordia through teratological insult, abnormal gene activity or regulation, or a combination of these factors can lead to palatal malformations, which are a relatively common birth defect in humans. (1, 2).

In an effort to identify genes with possible roles in regulating facial development, we have used the differential display method (3, 4) to examine gene expression in the embryonic murine palate. This analysis has led to the identification of a novel gene transcript, expressed peripherally in the palate and quite strongly in the presumptive nasal epithelium of the embryo, with a striking spatially restricted pattern of expression. This transcript also shows strong expression in the adult murine lung. In this study, we describe the identification, expression pattern, and sequence of this novel gene transcript, which we refer to as *plunc* (palate, lung, and nasal epithelium clone).

**EXPERIMENTAL PROCEDURES**

*Animals*—Pregnant females of mouse strain ICR (Ace Animals, Horsham, PA) were killed by cervical dislocation on gd12–14, which represents the period of embryonic palatal morphogenesis. Embryos were removed from the mothers, and palatal tissue was harvested by dissection. Total RNA was prepared by the acid guanidinium thiocyanate-phenol/chloroform extraction method of Chomczynski and Sacchi (5) and quantitated by measurement of optical density at 260 nm.

Reverse Transcription and Differential mRNA Display—Differential display was performed essentially according to the method described by Liang and Pardee (3) and co-workers (4). Briefly, 2 µg of total RNA from gd13 and gd14 embryonic palate tissues was subjected to reverse transcription using an anchored oligo(dT) primer (dT\textsubscript{12}-GC) to prime the reverse reaction. Following reverse transcription, 2 µl of each reaction was subjected to PCR using the same anchored oligo(dT) primer and an arbitrary 10-base oligonucleotide (5'-CTGATCCATG-3'). 10 µCi of \textsuperscript{32}PdCTP (NEN Life Science Products) was included with the PCR reactions to allow for subsequent visualization of the amplification products by autoradiography. PCR was performed using the following profile: 94 °C for 2 min (1 cycle); 94 °C for 30 s, 40 °C for 2 min, and 72 °C for 30 s (40 cycles); and 72 °C for 5 min (1 cycle). PCR products were then subjected to electrophoresis on a 6% non-denaturing sequencing gel. The gel was dried onto Whatman 3MM paper under vacuum and subjected to autoradiography. As a control for possible DNA contamination, a sample of RNA from gd13 mandible prepared in the same manner as the palate RNA was subjected to PCR without prior reverse transcription. Autoradiographs were examined for bands present exclusively in either gd13 or gd14 palate samples. To be considered as a candidate for further analysis, bands had to be present in both of the duplicate PCR lanes for each sample and could not correspond to any of the bands generated by PCR of the nonreverse transcribed mandible RNA, because any bands in this sample must be derived from contaminating DNA.

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§ Present address: Pulmonary/Metabolism Therapeutic Unit, Smith-Kline Beecham Pharmaceuticals, UP4310, 1250 S. Collegeville Rd., Collegeville, PA 19426.

¶ To whom correspondence should be addressed: Orthopaedic Research Laboratory, Thomas Jefferson University, 501 Curtis Blvd., 1015 Walnut St., Philadelphia, PA 19107. Tel.: 215-955-5503; Fax: 215-955-9159; E-mail: Elizabeth.LeClair@mail.tju.edu.

\textsuperscript{1} The abbreviations used are: gd, gestational day(s); PCR, polymerase chain reaction; PSP, parotid secretory protein.
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Elution of Amplified Fragments from Gels and Reamplification—Bands representing differentially expressed transcripts were cut from the dried gels and transferred to 0.5-ml polystyrene tubes. The gel slices were rehydrated for 10 min in 100 μl of water and boiled for 15 min. The supernatants were transferred to clean 0.5-ml tubes, following which RNA was precipitated by the addition of 5 μl of 3 M potassium acetate (pH 5.2), 2.5 μl of 20 mg/ml glycogen, and 300 μl of absolute ethanol. After an overnight incubation at −20 °C, samples were centrifuged for 20 min in a microcentrifuge at 4 °C to collect the eluted DNA. The eluted bands were then reamplified using PCR to obtain sufficient material for subcloning, using the same primers and amplification profile as the initial reaction. No 32P-labeled nucleotide was included in the reamplification reaction mixtures.

Subcloning of Reamplified cDNA Fragments—The reamplified fragments were subcloned into the pGEM-T vector (Promega) and transformed into competent JM109 Escherichia coli cells. Recombinant plasmids were initially identified by blue/white selection. The presence of inserts in these plasmids was confirmed by removal of the insert by digestion with Ncol and PstI. Plasmids that contained inserts were subjected to sequencing, which was performed by the Thomas Jefferson University DNA Synthesis and Sequencing facility. Sequence analysis was performed using the BLAST sequence analysis program (6).

Northern Blot Analysis—Total RNA from embryonic palate and brain was subjected to electrophoresis on 1% agarose/2.2 M formaldehyde denaturing gels. Following electrophoresis the gels were soaked briefly in water and transferred to nitrocellulose (Schleicher & Schuell) by capillary action using 10 X SSC to effect transfer. RNA was cross-linked to nitrocellulose filters using a Stratagene ultraviolet cross-linker, and the blots were stored at −20 °C until ready for use. For RNA analysis of adult tissues, a commercial multiple tissue blot (CLONTECH number 7762-1) was probed according to the manufacturer’s instructions.

Library Screening—A mouse heart cDNA library (AZAP II adult mouse heart cDNA library, Stratagene, La Jolla, CA) was screened under high stringency conditions using the cDNA probe isolated from the differential display gel. Positive plaques were picked from plates and subjected to secondary and tertiary screenings to ensure purity of the clones obtained from the screening. Positive clones were sequenced by the Thomas Jefferson University DNA Synthesis and Sequencing Facility.

In Situ Hybridization of Mouse Tissues—Harvested mouse embryos (gd13–15) and adult mouse tissues were fixed in 4% paraformaldehyde/phosphate-buffered saline (pH 7.4) at 4 °C overnight and processed under RNAse-free conditions for standard paraffin embedding. Serial coronal sections of embryonic heads were used to survey plunc mRNAs expressed during craniofacial development. To detect expression elsewhere during mouse development, embryo bodies were sectioned in both transverse and sagittal orientations. Finally, after our Northern analysis detected high levels of plunc message in the adult mouse lung, the entire thoracic contents of two pregnant female mice were dissected out, fixed in toto, and completely sectioned for in situ analysis.

All sections were processed for RNA hybridization and emulsion autoradiography according to the detailed protocol of Wawersik and Epstein (7). Two overlapping plunc clones (6-1 and 16-3), representing three-quarter-length and full-length cDNAs, respectively, were used to generate sense and antisense 35S-labeled riboprobes. Each probe was synthesized at 1–2 X 106 cpm/ml and diluted to apply 3–5 X 106 cpm/slide in 50 μl of hybridization buffer. After stringent washing, RNase treatment, dehydration, and preliminary autoradiography, the slides were dipped in emulsion and exposed in darkness at 4 °C for 5–7 days before developing. Developed slides were counterstained with hematoxylin and eosin and viewed using sequential bright field and dark field illumination on a Jenaval microscope. All photographs were taken on Ektachrome 64T or 160T color slide film and scanned into Adobe Photoshop 3.0.8.

For analysis of plunc expression in the adult nasopharynx, four heads from 2-month-old mice were perfused with paraformaldehyde/phosphate-buffered saline, decalcified in 0.5 M EDTA for 2–3 weeks at 4 °C, and sliced coronally with a clean razor blade for preparation as whole mounts. Whole mount in situ hybridization was performed with digoxigenin-labeled riboprobes according to Wilkinson (8).

RESULTS

Differential Display Analysis of Gene Expression in the Palate—Total RNA was prepared from palatal tissue dissected from gd13 (prior to palatal shelf elevation) and gd14 (following shelf elevation and fusion) mouse embryos of strain ICR. A portion of a differential display gel comparing gd13 and gd14 RNA is shown in Fig. 1. Comparison of banding patterns revealed several differentially expressed genes. At least two bands were present in the gd13 lanes (Fig. 1, bands 4 and 5) that were not observed in the gd14 lanes, whereas three bands were present on gd14 (Fig. 1, bands 1, 2, and 3) and not gd13. None of these bands appeared in control reactions not subjected to reverse transcription. These bands therefore represent cDNA fragments of up to five genes that show developmental regulation of expression in embryonic palatal tissue between gd13 and gd14. These bands were eluted from the differential display gel and subjected to a second round of PCR, using the same primers as before. Of the five bands identified on the original gel, two (Fig. 1, bands 1 and 2) were reamplified by PCR. The band 1 gene was successfully subcloned into the plasmid vector pGEM-T (Promega).

Confirmation of Differential Expression in the Developing Palate—We confirmed the differential expression of the band 1 gene in the developing palate by Northern blot analysis (Fig. 2A). A radiolabeled band 1 cDNA probe hybridized to a single mRNA that is only weakly expressed in gd13 palate tissue but is strongly expressed in gd14 palate. This message continues to be expressed strongly in the palate on gd15 and gd16 (data not shown). The mobility of this mRNA was approximately that of an endogenous GAPDH transcript (9), allowing us to estimate the size of the band 1 transcript to be approximately 1.2 kilobases.

To determine whether band 1 gene expression was specific to the palate or might be expressed elsewhere in the embryonic head, we included RNA samples prepared from gd13 and gd14 total brain tissue (Fig. 2A). The band 1 probe did not hybridize to any mRNA species expressed in gd13 or gd14 brain RNA. Other embryonic RNAs were not examined.

Isolation of cDNA Clones—Preliminary Northern blot examination of band 1 expression in the adult mouse indicated high levels of expression in the heart (data not shown). Although subsequent Northern blots failed to confirm this observation, we obtained several homologous, overlapping clones containing the band 1 sequence from screening a mouse heart cDNA library. Initial screening of the library was accomplished using the band 1 probe isolated from the differential display gel. Two positive clones were obtained from this screen (Fig. 3). These clones were 581 base pairs (clone 6-1) and 783 base pairs (clone 14-1) in length and covered the entire band 1 sequence as well as several hundred 3′-5′ sequences. Because this still left a discrepancy between the length of the cDNA sequence and the apparent length of the mRNA observed on the Northern blots, we performed a second screening of the cDNA library using clone 6-1 as a probe. This screening yielded four more positive clones, including one (16-3) containing an open reading frame that we believe represents the full-length...
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Expression in Adult Tissues—We repeated our Northern analysis of adult mouse tissues using a commercially prepared multiple tissue blot (see "Experimental Procedures"). A 35S-labeled cDNA probe from the clone 6-1 hybridized to a single mRNA species approximately 1.2 kilobases in length expressed in the adult lung only (Fig. 2B). No other tissues examined showed expression of this gene. Based on the embryonic and adult expression patterns, we gave the gene represented by our clones the working name of plunc (for palate and lung clone).

Expression Pattern during Mouse Embryogenesis—Given the temporal expression pattern suggested by the differential display and the embryonic Northern blot, we performed in situ hybridization on several gd14 embryos (Fig. 4). Antisense RNA probes from two plunc clones (6-1 and 16-3) produced identical hybridization patterns. In the embryonic head at this stage plunc is exclusively expressed in parts of the presumptive nasal epithelium, including the lateralmost epithelium on the dorsal aspect of the palatal shelves. In the embryonic body, very weak expression was observed in left and right lobes of the developing thymus. No other embryonic tissues showed any tendency to hybridize with the antisense probes. Sections probed with either of the sense transcripts gave no detectable signals.

Although the developing nasal epithelium is continuous, plunc expression is not. This mRNA species showed multiple segments of strong signal in the presumptive nasal epithelium, separated by gaps where only background levels were detected. These gaps were complex but symmetrical, appearing in the same pattern on each side of the nasal septum. Although the exact configuration of the signal varied depending on the level of section, we observed a consistent expression pattern within embryonic mRNA species observed by Northern blotting.

A, the upper panel shows equal amounts (20 μg) of total RNA isolated from embryonic gd13 palate (lane 1) gd13 brain (lane 2), gd14 brain (lane 3), and gd14 palate (lane 4) probed with the band 1 cDNA probe as described under "Experimental Procedures." The probe hybridizes to an mRNA species approximately 1.2 kilobases in length, based on its mobility compared with a GAPDH loading control (lower panel, same blot reprobed with a murine GAPDH probe). B, the upper panel shows a commercially prepared blot representing a variety of mRNAs from adult mouse tissues (~2 μg mRNA/lane). A 32P-labeled plunc cDNA probe (clone 6-1) shows a single band hybridizing in the lung lane at approximately 1.2 kilobases (kb). The lower panel shows the same blot hybridized with a murine β-actin control probe. Note that the size of the β-actin transcripts depends on the tissue type, and some tissues express more than one form.

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Fig. 3. Schematic representation of the plunc cDNA showing the open reading frame region, the putative 5′- and 3′-untranslated regions (UTR), and the polyadenylated region, as well as representations of the overlapping clones obtained from library screening to yield the full-length plunc cDNA sequence. Solid lines indicate portions of the clones for which only one strand was sequenced; boxes show portions for which double stranded sequence information was obtained.

coding sequence (Fig. 3). Overall, the cDNAs identified by library screening comprise a total overlapping sequence of 1113 base pairs, which is in good agreement with the length of the

expression in the "corner" where the elevated palatal shelf makes a 90-degree angle with the lateral nasal wall. C, more posteriorly, expression is found in discrete segments along the lateral nasal walls, the mid-lateral part of the nasal columella, and the ventrolateral extremities of the columella. In this embryo the palatal shelves are fully elevated and fused. D, plunc is strongly expressed where the lateral nasal walls fuse with the ventrolateral extremities of the nasal columella, forming pared sinuses above (s) and a common nasal passage below: np, common nasal passage; ns, nasal septum; *, palatal shelf; s, sinus cavity; t, nasal turbinate.
our set of embryos (n = 6).

Proceeding from anterior to posterior through coronal sections of the embryonic head (gd14), plunc is first expressed on the lateral walls of the nasal columella and the ventro-lateral corners of the nasal conchae (Fig. 4A). More posteriorly, these corner patches of expression come to lie on the dorso-lateral presumptive nasal epithelium of the palatal shelves (Fig. 4B). At the same level, another region of plunc expression was detected dorsally in the epithelium of the developing nasal turbinate. Finally, cells expressing plunc mRNA were observed at the ventro-lateral extremities of the nasal columella (Fig. 4C). Thus at least four distinct areas of plunc expression (two on the nasal columella, one on the nasal turbinate, and one on the lateral nasal walls) are found on each side of the nasal septum during this developmental period. The intervening epithelial areas consistently showed no detectable signal.

Although plunc expression is limited to the dorso-lateral epithelium of the developing palatal shelves and is not present at the midline junction, plunc transcripts appear at the site of another important craniofacial fusion. Anteriorly in the face, the ventral part of the nasal columella contacts the elevated palatal shelves to establish the secondary palate and two paired nasal cavities. More posteriorly, however, the columella is free of the secondary palate and fuses instead with the lateral nasal walls, forming a common nasal passage below and two ethmoidal sinuses above. In sections of this posterior region, we observed plunc transcripts in both the lateral nasal wall and the ventral corners of the nasal columella as these areas come together (Fig. 4D). The bands of plunc expression eventually oppose each other and merge as the adjacent epithelia fuse. After fusion, plunc signal is absent from the sinus cavities but continues to be expressed in two small spots on the roof of the common nasal passage (not shown).

Expression Pattern in Adult Tissues—Expression of plunc in the mature mouse nasal epithelium was confirmed by whole mount in situ hybridization with an antisense probe from the 6-1 clone. plunc signal in the mature nasal structures parallels that of the gd14 embryo, consisting of discrete epithelial bands on the exposed surfaces of the nasal columella, turbinates, and common nasal passage (Fig. 5).

Hybridization of ^35^S-labeled probes to adult mouse thoracic sections also confirmed strong plunc expression in the murine respiratory passages and lungs. Unlike the “broken” pattern in the embryonic nasal passages, plunc is uniformly expressed in the entire lining of the adult trachea (Fig. 6A). This continuous expression persists all the way down the tracheal tube and past its division into left and right bronchial passages, each of which shows an unbroken ring of plunc-expressing cells (Fig. 6B). The signal becomes abruptly weaker as these main bronchial passages branch again and ramify throughout the lung lobes (Fig. 6C). In all areas of expression plunc transcripts were found exclusively in the outermost layer of epithelial cells (Fig. 6D). All terminal bronchioles, respiratory bronchioles, and lung alveoli were negative for plunc expression.

As in the embryo, we also detected weak expression of plunc in the adult thymus (not shown). Both left and right lobes of this organ showed small clumps or “islands” of positive cells within a larger, background mass of thymocytes. No other sectioned organs or tissues (including esophagus, heart, major vessels, pericardium, and lung pleura) showed any plunc hybridization signal.

Sequence Analysis of plunc cDNAs—Sequencing of the band 1 cDNA fragment isolated from the differential display gel showed it to be 255 base pairs in length. The sequences of the reverse transcription and PCR primers were faithfully reproduced at the 5’ and 3’ ends of the fragment. A consensus polyadenylation signal sequence beginning 22 bases upstream of the poly(A) tail was also noted.

The sequence of the complete plunc cDNA is shown in Fig. 7. This sequence contains an open reading frame of 834 codons, giving a putative protein product of molecular mass 28,618 Da. Additionally, there is a 55-base pair 5’-untranslated sequence and a 207-base 3’-untranslated sequence. Comparison of the complete sequence with entries in the GenBank™ data base using the BLAST algorithm (6) showed no significant homologies to known sequences at the nucleotide
level. A search of the dBEST database showed 100% identity between *plunc* and three murine expressed sequence tags (GenBank™ accession numbers AA028768, cloned from a gd19 fetal mouse cDNA library and corresponding to *plunc* residues 680–1076; AA763873, cloned from an adult mouse thymus cDNA library and corresponding to *plunc* residues 11–481; and AA880683, cloned from an adult mouse lung cDNA library and corresponding to *plunc* residues 602–1013). The sources of these ESTs are consistent with the expression patterns we have observed for *plunc*. Unfortunately, these sequence tags gave no clues as to the identity of *plunc*, nor did a search of GenBank™ using these ESTs provide any additional significant homologies to known genes. Significant homologies were also detected between *plunc* and two other sequence tags identified by screening of a human olfactory epithelial cell library (GenBank™ accession numbers N27741 and N23239). Again, searching of the GenBank™ data base using these sequences as input did not reveal any further homologies with known sequences.

Significant homologies at the amino acid level were observed with two salivary gland proteins: the von Ebner minor salivary gland protein (GenBank™ accession number U46068) and the parotid secretory protein (PSP) precursor (Refs. 11 and 12; GenBank™ accession number U79414). Comparisons of these amino acid sequences are shown in Fig. 8. The highest degree of homology was seen in the amino-terminal regions of these proteins; of the first 15 amino acids of *plunc* and murine PSP, 12 are identical and 2 show conservative amino acid substitutions. The bovine PSP, BSP30, showed 10 amino acid identities and 2 conservative substitutions in the same region, whereas von Ebner minor salivary gland protein showed 6 identities and 4 conservative substitutions. Interestingly, this region is reported to be part of the PSP signal sequence (11), which is not part of the mature protein. Other homologous regions of the amino acid sequence are characterized by conservative amino acid substitutions more than amino acid identities between the proteins.

Analysis of the predicted amino acid sequence using the PROSITE program (14) found several functionally significant motifs (Fig. 7), including consensus sequences for phosphorylation by protein kinase C (15, 16) and casein kinase II (17), two *N*-glycosylation sites (18, 19), and a leucine zipper (20–22). A portion of the leucine zipper is contained within the region homologous to the PSP signal sequence, so the functional significance of this motif in this protein is questionable.

An interesting and apparently unique repeating sequence pattern is also present in the amino region of this protein. This pattern consists of six amino acids of the sequence Gly-(Leu/Pro/Gln)-(Pro/Leu)-Leu-Pro-Leu and is repeated four times, beginning with residue 23, with two amino acids spacing between the repeated elements. A search of GenBank™ found no such amino acid motifs represented in this data base; whether this repeating sequence is of functional significance or is a coincidental occurrence is presently unknown.

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**Fig. 7. Nucleotide sequence and predicted amino acid sequence of *plunc*.** The repeated Pro-Leu-Pro-Leu motif is shown in italics. Consensus sequences for protein kinase C (SLK) and casein kinase II (SPFVD and SGLD) are indicated by solid underlines. *N*-Glycosylation sequences are indicated by dotted underlines. Leucine residues identified as part of a leucine zipper motif are shown in bold type, as is a polyadenylation consensus sequence.

**Fig. 8. Amino acid sequence homologies between the putative *plunc* gene product, von Ebner minor salivary gland protein, and members of the PSP family.** Homologous sequences were identified by comparing the putative *plunc* protein sequence with proteins in the GenBank™ data base using the BLAST algorithm (6). Identical amino acid residues are indicated by asterisks; conservative amino acid substitutions are indicated by a plus sign. Hyphens indicate gaps introduced into the protein sequences to allow optimal alignment. A shows the region of greatest homology between *plunc*, the von Ebner salivary gland protein, and the PSP family members. B shows the homology of the amino-terminal sequence of the putative *plunc* gene product to the signal sequences of the PSPs and the von Ebner protein.
**DISCUSSION**

*plunc* is a novel gene transcript with a unique spatial and temporal pattern of expression in the embryonic and adult mouse. Expression in the mouse embryo is first noted on gd14 on or about the time of palatal shelf elevation and fusion and is confined to discrete regions of the nasal epithelium. These regions of expression are associated with the dorsal palate, nasal septum, and nasal conchae. The transcript is also expressed strongly in the tracheal and bronchial epithelium of the adult mouse lung.

The discovery of a new gene raises the corresponding question of the function of the gene. Because *plunc* is expressed only at the margins of the palatal shelves, well removed from the sites of shelf contact and fusion, we think it unlikely that *plunc* plays a role in this aspect of facial morphogenesis. *plunc* expression is, however, associated with areas of presumptive fusion of adjacent epithelia as the nasal columella and lateral nasal walls form the sinus cavities and the common nasal passage. It is possible that *plunc* may serve in some regulatory aspect of this morphogenetic event.

Examination of the predicted amino acid sequence of the *plunc* protein indicates that it is a secreted molecule; the related PSP is a major protein component of saliva (11). Thus one alternative hypothesis is that *plunc* is not a morphogenetic factor but a component of nasal or respiratory mucus. The pseudostratified columnar epithelia of the nasal and respiratory passages are provided with scattered, isolated goblet cells, and subepithelial mucous glands that produce a variety of proteinaceous secretions (23). Although *plunc* is abundantly expressed in certain regions of these epithelia, the *in situ* hybridization signal does not specifically match the distribution of either of these cell types. The fact that *plunc* transcripts are absent from the smaller respiratory passages and lung alveoli also makes it unlikely that this gene product is a secretion involved in gas exchange (i.e., not a surfactant).

The potential relationship between the PSPs and *plunc* provides little insight into *plunc* function, because the functions of the PSPs themselves remain unknown. PSP expression in the murine parotid glands is coordinately regulated with salivary amylase (12); whether this indicates that the functions of these proteins are complementary remains to be determined. The reduced expression of the bovine PSP, BSP30, has been linked to resistance to pasture bloat disease in cattle (10). Again, the potential significance of this observation for embryonic development and epithelial cell function is unknown. The strict spatial and temporal regulation of expression of both the PSPs and *plunc*, however, implies that these gene products are important in defining the functions of the expressing tissues.

The onset of *plunc* expression coincides with differentiation of the presumptive nasal epithelium into mature nasal epithelial cells (13). We also note the persistent expression of *plunc* in the adult nasopharynx as well as the fact that sequence tags with high homology to *plunc* have been identified in human olfactory epithelium. These observations lead us to speculate that the association of *plunc* with epithelial cells of the developing palate and nasopharynx may be related to the differentiation and maintenance of an as yet unidentified subpopulation of epithelial cells.

In the embryonic and adult nasopharynx, *plunc* shows a surprisingly discontinuous pattern of expression in a continuous epithelial sheet. Because no obvious histological features distinguish regions of epithelial cells that express *plunc* from those that do not, the basis for this pattern of expression is unknown. These observations suggest that the nasal epithelium, relatively uniform in appearance, may contain functionally distinct regions defined on the basis of differential gene expression. An examination of the molecular regulation of the *plunc* transcript should prove useful in testing this hypothesis.

Unlike the pattern observed in the nasal epithelium, *plunc* expression in the lower respiratory tract is continuous throughout the trachea and upper bronchi. As in the nasopharynx, however, there is no histological demarcation indicating the limit of *plunc* expression in the bronchial epithelium. The distinction between *plunc*-expressing and nonexpressing cells may be present only at the molecular level, and it will be interesting to determine whether the same factors that govern *plunc* expression in the tracheobronchial epithelium also operate in the nasal epithelium.

Sequence analysis of *plunc* also revealed an apparently novel repeating amino acid motif, Leu-Pro-Leu-Pro-Leu. This repeated sequence is likely to represent the amino terminus of the mature protein following removal of the presumptive signal sequence. Because no other known proteins were found to share this motif, its functional significance here remains speculative. A targeted search for other new proteins that may share this sequence element should be useful in determining whether this sequence serves an important role in *plunc* function.

Our goal in undertaking these studies was to identify candidate genes involved in the control of craniofacial morphogenesis. Although *plunc* is unlikely to play a role in this process, the expression pattern of this novel transcript indicates a previously unsuspected level of complexity in the nasal and respiratory epithelium. Determination of the function of *plunc* and the mechanisms underlying its highly restricted pattern of expression represent the next steps in determining the regionally specified functions of these tissues.

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