Fate mapping of mammalian embryonic taste bud progenitors

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Mammalian taste buds have properties of both epithelial and neuronal cells, and are thus developmentally intriguing. Taste buds differentiate at birth within epithelial appendages, termed taste papillae, which arise at mid-gestation as epithelial thickenings or placodes. However, the embryonic relationship between placodes, papillae and adult taste buds has not been defined. Here, using an inducible Cre-lox fate mapping approach with the ShhcreERT2 mouse line, we demonstrate that Shh-expressing embryonic taste placodes are taste bud progenitors, which give rise to at least two different adult taste cell types, but do not contribute to taste papillae. Strikingly, placodally descendant taste cells disappear early in adult life. As placodally derived taste cells are lost, we used Wnt1Cre mice to show that the neural crest does not supply cells to taste buds, either embryonically or postnatally, thus ruling out a mesenchymal contribution to taste buds. Finally, using Bdnf null mice, which lose neurons that innervate taste buds, we demonstrate that Shh-expressing taste bud progenitors are specified and produce differentiated taste cells normally, in the absence of gustatory nerve contact. This resolution of a direct relationship between embryonic taste placodes with adult taste buds, which is independent of mesenchymal contribution and nerve contact, allows us to better define the early development of this important sensory system. These studies further suggest that mammalian taste bud development is very distinct from that of other epithelial appendages.

KEY WORDS: Taste bud development, CreER, Shh, Wnt1Cre, Bdnf, Genetic inducible, Fate mapping, Tamoxifen, Mouse

INTRODUCTION

Taste buds are aggregates of receptor cells, which transduce chemical stimuli into neural signals to mediate the sense of taste. In mammals, lingual taste buds reside within appendages of epithelial placodes, called taste papillae (Mistretta, 1991); fungiform papillae are arrayed on the anterior tongue, whereas foliate papillae and a single circumvallate papilla in rodents reside posteriorly. Taste receptor cells, like neurons, possess voltage-gated channels, generate action potentials, and release neurotransmitter onto post-synaptic nerves (Finger et al., 2005; Roper, 1992). However, unlike neurons or other receptor cell types, taste cells continually regenerate in adults (Beidler, 1953; Beidler and Smallman, 1965; Farbman, 1980; Delay et al., 2012). Furthermore, taste buds differ from other sensory receptors developmentally, arising from local epithelia rather than from neurogenic ectoderm (Barlow and Northcutt, 1995; Stone et al., 1995). Taste buds are thus unique among sensory receptor cells with characteristics of both epithelia and neurons.

Each taste bud comprises a heterogeneous population of ~100 cells belonging to three differentiated cell types (I, II and III) that can be identified via expression of marker proteins. Type I cells, assumed to be support cells, express a specific ectoATPase, NTPDase2 (Bartel et al., 2006). Types II and III are taste receptor cells proper; type II cells express the transmembrane receptors and signaling machinery to transduce sweet, bitter and umami tastes (Clapp et al., 2001; Miyoshi et al., 2001), whereas type III cells are sour detectors (Huang et al., 2006; Huang et al., 2008) and function as relay cells (Yang et al., 2000a; Roper, 2007). These three taste cell types are maintained by continuous renewal from the progenitor population, the characteristics of which remain unclear.

However, birthdating analyses suggest that taste cells arise from either adjacent basal epithelial cells, intragemmal basal cells within taste buds, or edge cells located laterally outside of taste buds proper (Beidler and Smallman, 1965; Delay et al., 1986; Miura et al., 2006; Nakayama et al., 2008; Okubo et al., 2008).

Taste buds differentiate late in embryogenesis, well after the tongue is innervated. This sequence of innervation followed by differentiation has suggested that taste buds are induced by nerves. In axolotls, an aquatic salamander, the taste periphery is organized simply, with taste buds embedded directly in the epithelium (Takeuchi et al., 1997). In a test of the neural induction model using axolotl embryos, taste buds arise without contact by either nerves (Barlow et al., 1996; Stone, 1940) or cranial mesenchyme (Barlow and Northcutt, 1997); rather, specification and patterning of amphibian taste buds is intrinsic to oral epithelium, and occurs early in development via cell-cell signaling (Parker et al., 2004; Barlow, 2001). Analysis of X-inactivation transgenic female mice also supports an epithelial origin of taste buds in mammals (Stone et al., 1995).

Restriction of mammalian lingual taste buds to papillae adds a level of complexity to the development of these composite taste organs. In mice, taste organ formation begins at mid-gestation, when focal thickenings called taste placodes arise in the lingual epithelium. Placodes evaginate and develop a mesenchymal core, transforming morphologically into taste papillae (Farbman and Mbiene, 1991). Taste buds differentiate within papillae and begin to express taste cell type-specific markers around birth (Krimm and Barlow, 2008). Because placodes transform into papillae, followed by taste bud differentiation, it has been inferred that taste placodes develop into taste papillae, which in turn produce taste buds from a subset of papillary epithelial cells (Mistretta and Liu, 2006). However, the precise relationship between placodes, papillae and buds has not been tackled experimentally.

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Discerning how these structures are related to one another is particularly important in the context of the role of innervation in mammalian taste development. Taste placodes, and, at least initially, taste papillae develop independently of innervation (Farbman and Mbiene, 1991; Hall et al., 1999; Hall et al., 2003), whereas taste bud differentiation appears to be nerve dependent (Oakley and Witt, 2004). These findings have been obtained primarily from short-term explant culture, where taste placodes and papillae differentiate morphologically and molecularly, despite a lack of innervation (Farbman, 1972; Hall et al., 2003; Mbiene et al., 1997; Miestretta et al., 2003; Nosrat et al., 2001). In particular, Sonic Hedgehog (Shh) is expressed in early taste placodes, and, consistent with neural independence, Shh is expressed in taste papillae that form in cultured lingual explants. However, the fate of the Shh-expressing cells is unknown, and, thus, Shh has been interpreted to be a marker of taste papillae, rather than of taste buds (Hall et al., 1999; Hall et al., 2003; Liu et al., 2004).

To define the lineage relationship of taste placodes to taste papillae and to taste buds, we crossed mice carrying a drug-sensitive Cre recombinase fusion protein under the Shh promoter (Harfe et al., 2004) with R26RlacZ reporter mice (Soriano, 1998), and tracked the fate of taste placode cells in both embryonic and adult taste organs. We show here that Shh-expressing placodes are taste bud progenitors, which give rise exclusively to cells within taste buds but not to taste papillae. Furthermore, Shh-expressing progenitors give rise to at least two differentiated taste cell types as well as to intragemmal basal and adjacent edge cells in adult mice. However, this contribution is transient: within a few months of birth, placodally derived cells are lost from mature taste buds. We demonstrate that the neural crest does not compensate for this loss, and does not contribute to taste buds at any stage. Finally, we show that the specification, patterning and probable early differentiation of taste bud progenitors are not affected by reduced gustatory innervation caused by a null Bdnf mutation.

MATERIALS AND METHODS

Mice
ShhcreERT2 mice (Harfe et al., 2004) were a gift from Clifford Tabin, Harvard Medical School. R26RlacZ (Soriano, 1999) and Wnt1Cre (Danielian et al., 1998) mice were obtained from Trevor Williams (University of Colorado School of Dentistry, USA). Bdnf+/– mice (Jones et al., 1998) were obtained from Thomas Finger (University of Colorado School of Medicine, USA). ShhcreERT2 males are maintained on the C57BL/6 background; the background of all other lines is mixed. Mice and embryos were genotyped as described previously (Soriano, 1999; Harfe et al., 2004; Danielian et al., 1998; Jones et al., 1994), and maintained and sacrificed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Colorado Denver School of Medicine.

Fate mapping of Shh-expressing taste placodes and lingual mesenchyme
ShhcreERT2 males were crossed with homozygous R26RlacZ females, and double transgenic progeny were assayed for β-galactosidase. Embryos and pups with the R26RlacZ allele served as controls. Midday on the day of an observed plug was considered embryonic day (E) 0.5. Pregnant dams were dosed intra-peritoneally once between E12.5–E14.5 with 4.5 mg tamoxifen (T-564B, Sigma), which was prepared and administered as previously described (Nakamura et al., 2006). Embryos were recovered on desired days of gestation, and staged according to Kaufman (Kaufman, 1999). For fate mapping the lingual mesenchyme, Wnt1cre males were crossed with homozygous R26RlacZ females, and embryos (E13.5) and adults (6 weeks, 4 months) with both alleles were assayed for β-galactosidase.

In situ hybridization, immunofluorescence and β-galactosidase histochemistry
For Shh mRNA expression, wild-type tongues were fixed overnight in 4% PFA, and processed for in situ hybridization as described (Hall et al., 1999) with hybridization of the Shh probe (640 bp, L. Goodrich, Stanford University) and stringency washes at 62°C. For Shh immunodetection, live tongues were cultured in mouse anti-Shh (30 μg/ml, SE1, DSHB) and processed as described previously (Hall et al., 2003).

To analyze Cre-mediated recombination, embryos were harvested at various times after tamoxifen dose and assayed for β-galactosidase (β-gal) by X-gal staining or processed immunofluorescently with guinea pig anti-β-gal (1:1000) (Yee et al., 2003). For whole-mount X-gal, dissected embryonic tongues were fixed briefly in 0.25% glutaraldehyde and stained in X-gal solution (Harfe et al., 2004). Alternatively, embryonic tongues were processed first as whole mounts for anti-Shh immunofluorescence, followed by sectioning and β-gal immunofluorescence.

Tongues from P0 pups were fixed in 0.2% PFA overnight and stained for X-gal after sectioning. Some P0 sections were double labeled with anti-β-gal and rat anti-cytokeratin 8 (CK8, Troma-1; 1:25, DSHB). Light fixation obviated antigen retrieval required when tongues were fixed more strongly, but required less dilute antibody (see below).

To detect innervation in Bdnf–/– and +/+ embryos, E14.5 tongues immunostained with anti-Shh were fixed in 4% paraformaldehyde overnight, sectioned and immunostained with the neurturine marker rabbit anti-Gap43 (1:200; Chemicon; MAB347), followed by goat anti-rabbit Cy3 antibody (1:500; Jackson Immunoresearch; 111-165-006). For detecting CK8 immunoreactivity at E18.5 in wild-type versus Bdnf–/– tongues, embryos were perfused with 4% paraformaldehyde, their tongues embedded in paraffin, sectioned at 8 μm, mounted on slides, dehydrated, rehydrated and treated with proteinase K (antigen retrieval). Sections were incubated with anti-CK8 at 1:100 for 1 hour at 37°C, followed by biotinylated goat anti-rat antiserum and streptavidin-Cy3.

Lineage tracing in adult mice
Postnatal animals at 2 and 6 weeks, and 4 and 7 months with both Cre and R26RlacZ were transcardially perfused with 4% PFA, post-fixed in 4% PFA overnight at 4°C and cryosectioned (12 μm). Sections were double immunostained with guinea pig anti-β-gal and taste cell type-specific rabbit antisera: (1) anti-NTPDase2 (1:1000, a gift from L. G. Lavoie and J. Se’vigny); (2) anti-PLC2 (1:1000, Santa Cruz; sc-206); (3) anti-N-CAM (1:1000, Chemicon; AB5032); or (4) anti-PGP 9.5 (1:100, Abd Serotec, 7863-0504). Wnt1cre;R26RlacZ adult tongue sections were immunostained with a cocktail of NTPDase2, PLC2, and NCAM antiserum to label simultaneously all three taste cell types, and guinea pig anti-β-gal antisera, followed by the appropriate fluorescently conjugated secondary antibodies (1:500, Molecular Probes, Invitrogen).

Image acquisition
Bright-field or multichannel fluorescent images were acquired with an Axiocam CCD camera and Axiosplan fluorescence microscope with Axiovision software (Zeiss, Germany). Z-stack confocal images were acquired at 0.75 μm through 12 μm cryosections using a laser-scanning Olympus Fluoview confocal microscope with Fluoview Software. Images were saved as TIFFs, contrast adjusted and cropped, and figures compiled using Adobe Photoshop CS2.

Data analysis and quantitation
Shh-descendent cells in taste buds or epithelial clones at P0 were counted in pups treated with tamoxifen at E12.5 (n=3) or E14.5 (n=3). To avoid double counting of 30 μm diameter taste buds at P0, labeled cells in every third section were counted (3×12 μm=36 μm intervals). Taste buds with β-gal-immunoreactive (IR) cells at 2 weeks (n=3), 6 weeks (n=4) or 4–7 months (n=3) postnatal were tallied from counts made at every 5th section (5×12 μm=60 μm interval) to avoid overcounting of adult taste buds (50 μm diameter). The average number of β-gal-IR cells per labeled taste bud at each postnatal time point was tallied from 20 randomly selected taste buds per stage across animals. The average number of double-labeled cells for each taste cell marker (anti-NTPDase, anti-PLC82, anti-NCAM or anti-PGP9.5,
and anti-β-gal) was tallied at 6 weeks postnatal in 20 taste buds from each of four animals. Means and standard errors were calculated using Microsoft Excel.

For differences between Bdnf<sup>+/–</sup> and Bdnf<sup>–/–</sup> tongues at E14.5, Shh-IR cell clusters were counted from images of whole tongue, which included the entire dorsal surface. Shh-IR cell clusters were also quantified in serial sections, to include the relatively small number at the ventral tongue tip. The average size of Shh-expressing clusters was obtained from clusters in the middle and tip of the left side of the tongue; each cluster was traced and the area measured (NIH image). T-tests were used to compare the number and mean area of Shh-labeled clusters.

To assess the extent of taste placode innervation at E14.5, serial sections immunostained for both Shh and Gap43 were examined and each Shh-labeled papilla was categorized as innervated – anti-Gap43-IR labeled fibers penetrating Shh-labeled epithelial cells or not innervated – Gap43-IR fibers completely absent, or only reaching the papilla core mesenchyme.

The number of differentiating taste buds at E18.5, identified via CK8 immunoreactivity, was quantified in serial sections. Each taste bud was carefully followed through all sections it occupied, such that each taste bud was counted only once. Comparisons between wild-type and Bdnf<sup>+/–</sup> mice were made using a t-test.

### RESULTS

#### Shh-expressing taste placode cells and their daughters consolidate in the apices of fungiform papillae

The taste placode cells fate mapped in subsequent experiments were targeted by assessing precisely when Shh expression is first restricted to the placodes. As previously reported, Shh mRNA and protein are broadly expressed in the lingual epithelium prior to placode formation, and then focalize to the taste placodes by E12.5 (data not shown) (Hall et al., 1999).

To discern the fate of these Shh-expressing taste placode cells, Cre recombination was activated via tamoxifen at E12.5. When treated embryos were examined at E14.5, β-gal activity was evident in the epithelium of taste placodes (Fig. 1A,D,E, red arrows). Extra-placodal epithelial cells were also labeled (Fig. 1A,D, red arrowheads), and were presumed to be general epithelial cells, which expressed Shh at E12.5 when tamoxifen was injected, but had turned off Shh by E14.5. Consistent with this idea (and see below), in embryos given tamoxifen at E13.5 and examined at E15.5, most labeled cells were limited to taste papillae (Fig. 1B,F, red arrows), with many fewer extra-papillary labeled cells (Fig. 1B,G, red arrowheads). In addition, sectioned material showed that Shh-descendant cells, even at these early stages, contribute only a central population of cells in papillae (Fig. 1F), and that Shh-descendant cells are densely innervated (Fig. 1H); both features suggested that placodes contribute to taste buds, and not papillae. Similar results were obtained when the palatal epithelium of embryos injected 48 hours prior was examined (see Fig. S1 in the supplementary material). Importantly, X-gal reaction product was never observed in embryos lacking Cre (Fig. 1C). Moreover, β-galactosidase activity was routinely observed in positive control tissues where Shh is known to be expressed at E12.5, such as vibrissae and hair follicles (data not shown) (St-Jacques et al., 1998).

**Shh-descendent cells within taste placodes continue to express Shh, whereas extra-placodal daughter cells cease Shh expression**

Although Shh-descendent cells progressively restrict to placodes, many β-gal-labeled cells were quite close to taste placodes, as well as in extra-placodal epithelium when Cre was induced at E12.5. Labeling of extra-placodal cells suggested that these cells were either: (1) being recruited by and to Shh-expressing placodes, thus contributing to taste organ genesis; or (2) turning off Shh expression and pursuing a lingual epithelial fate. Thus, we examined Shh protein expression in both types of Shh-descendent cells. In placodes, most Shh-descendent cells persisted in expressing Shh (Fig. 2A–C), although some cells at the edge of Shh-descendent clusters (β-gal-IR) ceased to express Shh (Fig. 2A–C, yellow arrowhead). By contrast, all extra-placodal cells indebly marked with β-galactosidase lacked Shh (Fig. 2D,E). We could not determine, however, whether Shh-descendent cells immediately adjacent to placodes are recruited, or cease Shh expression and become epithelial.

**Shh expressing taste placodes contribute to taste buds and not taste papillae at birth**

To identify the postnatal fate of Shh-expressing taste placodes, tongues of embryos treated with tamoxifen at E12.5 (<i>n=3</i>) or E14.5 (<i>n=3</i>) were collected at birth (P0). Irrespective of the timing of tamoxifen treatment, β-gal-expressing cells were confined predominantly to taste papilla apices. Furthermore, labeled cells resembled taste buds; they formed onion-shaped clusters, and...
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Fig. 2. Shh-expressing taste placode cells continue to express Shh, whereas extra-placodal descendants cease Shh expression. (A–C) Tongue cryosections from E15.5 embryos tamoxifen treated at E12.5 and immunostained for β-gal (B; red) and Shh (C; green) show co-labeling in taste placodes (yellow in A; merge). Occasional peripheral cells in placodes were β-gal-IR only (yellow arrowheads). Broken lines indicate epithelial basement membrane. (D–E) Double immunostained tongue cryosections from the same embryo as in A–C show that extra-placodal cells labeled at E12.5 but distant from placodes, and in basal (D) and apical (E) epithelial layers, turned off Shh by E14.5. Scale bars: 20 µm in A–C; 10 µm in D,E.
Taste buds derive exclusively from the epithelium with no contribution from neural crest

Development of taste bud progenitors in embryos which lose gustatory innervation is indistinguishable from that of controls

Several groups have shown that development of taste placodes is initially nerve independent; tongue explants develop taste placodes that evaginate as taste papillae and express Shh (Hall et al., 2003; Liu et al., 2004). However, these cultures do not survive sufficiently

Table 1. Quantitative analysis of Shh-descendent placode cells within postnatal taste buds

<table>
<thead>
<tr>
<th>Postnatal day</th>
<th>0</th>
<th>P14</th>
<th>P42</th>
<th>4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent taste buds with placode descend</td>
<td>85.5±6.24 (n=6 animals)</td>
<td>49.7±7.83 (n=3 animals)</td>
<td>41.7±8.72 (n=4 animals)</td>
<td>1.75±0.5 (n=3 animals)</td>
</tr>
<tr>
<td>Placode-descendent cells per taste bud</td>
<td>5.7±0.27</td>
<td>2.9±0.41</td>
<td>2.2±0.26</td>
<td>–</td>
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* Tamoxifen injection at E12.5.
long to monitor taste bud differentiation. Thus, we examined Shh-expressing taste progenitors within emerging papillae at E14.5 in embryos homozygous null for the gene encoding the neurotrophin BDNF (Fig. 6), which lose gustatory innervation early on (Nosrat et al., 1997; Krimm, 2007). The distribution, total number, and size of Shh-expressing placodes did not differ between Bdnf+/− and Bdnf−/− embryos at E14.5 (Fig. 6A-F), yet the number of innervated placodes was dramatically reduced in the Bdnf−/− embryos (Fig. 6D,G).

To determine whether removal of Bdnf-dependent innervation impacts embryonic taste bud differentiation, we counted developing taste buds in wild type and Bdnf−/− mice using anti-CK8 to mark fusiform taste cells. At E18.5, immature taste buds with numerous CK8-IR cells were evident in both wild-type and mutant tongues (Fig. 6H,I), and their number did not differ significantly between wild-type (59±9.3, n=7) and Bdnf−/− (40±8.4, n=8) mice. However, this last result must be viewed with caution, as the number of CK8-IR taste buds was very variable at this stage even in control embryos. Thus, our data indicate that patterning and specification of Shh-expressing progenitor cells and their descendents, although evident at birth, begin to disappear prior to weaning, and are completely absent in adults. This loss of placodal cells is not compensated for by a neural crest contribution, either embryonically or postnatally. Finally, we show that taste bud progenitors develop in vivo independently of nerve contact, in that specification, patterning and likely initial differentiation of Shh-expressing progenitors cells remains constant, even when BDNF-dependent taste innervation is lost genetically.

**DISCUSSION**

**Shh expressing taste placodes give rise to taste buds and not to taste papillae**

The postnatal fate of embryonic taste placodes has been unclear, obscuring the relationship of these earliest lingual taste structures to adult taste buds and their surrounding papillae (Farbman and Mbiene, 1991). Immature taste buds only become evident in papillary epithelium late in gestation, with fully differentiated taste buds present within 1 week of birth (Mistretta, 1991). This developmental sequence of placodes transforming into papillae, followed by differentiation of taste buds, has suggested that placodes are papilla precursors, which subsequently organize taste bud progenitors within the apical epithelium (Mistretta and Liu, 2006).

We show here, using inducible Cre-lox lineage tracing, that taste placodes do not give rise to papillae, but rather contribute almost exclusively to taste buds. At postnatal stages, embryonic Shh-expressing taste placodes are progenitors for the majority of taste cells, giving rise to both type I and II cells, as well as to intragemmal basal and perigemmal edge cells, although we have yet to identify type III cells as being descended from taste placodes. Unexpectedly, Shh-expressing progenitor cells and their descendents, although evident at birth, begin to disappear prior to weaning, and are completely absent in adults. This loss of placodal cells is not compensated for by a neural crest contribution, either embryonically or postnatally. Finally, we show that taste bud progenitors develop in vivo independently of nerve contact, in that specification, patterning and likely initial differentiation of Shh-expressing progenitors cells remains constant, even when BDNF-dependent taste innervation is lost genetically.

**Taste bud progenitors are specified early, and may organize subsequent papilla morphogenesis**

Taste placodes express several signaling factors, including Shh, Bmp and Wnt/β-catenin, and their expression continues as taste papillae undergo morphogenesis (Hall et al., 1999; Iwatsuki et al., 2007; Jung et al., 1999; Liu et al., 2007). Alteration of each of these pathways during placode formation and papillary morphogenesis in vitro affects the patterning of taste placodes and resultant papillae. For example, both Shh and Bmp inhibit (Hall et al., 2003; Mistretta et al., 2003; Zhou et al., 2006), whereas Wnt/β-catenin promotes placode development, including expression of Shh, in cultured tongue explants (Iwatsuki et al., 2007; Liu et al., 2007). However,
as lingual explants do not survive until taste buds differentiate, this in vitro approach has lead only to the conclusion that these pathways regulate papilla development (Mbiene and Roberts, 2003).

Our results shift this interpretation; early alteration of Shh, Bmp and Wnt/β-catenin signaling in vitro impacts taste bud progenitors directly. In support of this view, in Wnt/β-catenin gain-of-function experiments, mutant embryos examined at E14.5 have many more Shh-expressing taste bud progenitors distributed throughout the lingual epithelium compared with controls. At birth, mutant tongues are completely covered by immature taste buds that express both Shh and some taste cell markers, embedded in enlarged and often fused taste papillae not expressing Shh (Liu et al., 2007). Thus, the increased number of Shh-expressing progenitors in embryonic tongues correlates well with increased taste buds in greatly expanded fungiform papillae observed at birth. Given our new interpretation of early events in taste bud patterning, we speculate that Shh-expressing taste bud progenitors may function as transient signaling centers within the epithelium, which in turn regulate subsequent development of taste buds and papillae. Intriguingly, these taste progenitor cells share a number of similarities with known signaling centers, such as the tooth enamel knot (Obara and Lesot, 2007), and the apical ectodermal ridge of the developing limb (Antalikova et al., 1989). Specifically, taste placodes express a number of secreted signaling factors (see above), are mitotically quiescent during embryogenesis (Farbman and Mbiene, 1991; Mbiene and Roberts, 2003; Zhou et al., 2006) and contribute only transiently to postnatal taste buds (this study).

We must also formally consider, however, that postnatal loss of embryonic taste progenitors may be an artifact of our fate-mapping technique. The R26RLacZ mouse is widely used in developmental studies, owing to ubiquitous expression from the R26 promoter throughout life (Friedrich and Soriano, 1991; Zambrowicz et al., 1997). In studies of thymic development using the Wnt1Cre;R26LacZ mice, neural crest-derived cells were detected in embryonic but not postnatal thymus (Jiang et al., 2000; Yamazaki et al., 2005). In a recent report, however, Wnt1Cre;R26YFP reporter mice were employed, and neural crest-derived mesenchyme persisted in adult thymus (Foster et al., 2008). We employed a cocktail of type I, II and III taste cell markers (red) and β-gal (green), to reveal neural crest cells in taste papilla mesenchyme. In 4-month-old Wnt-1cre;R26LacZ tongue sections immunostained for a cocktail of type I, II and III taste cell markers (red) and β-gal (green), neural crest cells are found only in lingual mesenchyme. (D,F) Blue is Hoechst nuclear counterstain. Arrows indicate the taste pore. Scale bars: 50 μm in A,C,E; 10 μm in B,D,F.

To date, a loss of lacZ transcription has not been reported in epithelial lineage studies (e.g. Berton et al., 2000). Our results, moreover, tend to reject an artificial loss of embryonic taste progenitors postnatally. First, we see no loss of β-gal in neural crest-derived cells of the tongue mesenchyme up to 4 months of age, suggesting that, in lingual tissue immediately adjacent to taste buds,
the lacZ reporter is not silenced. Second, the loss of labeled taste cells in adults follows a comparable time course across animals, already evident at P14 and complete by 4 months, implying a regulated, rather than a stochastic, process.

Are Shh-expressing taste bud progenitors lineage restricted?

Taste buds are a heterogeneous population of three differentiated cell types (I, II and III), which can be identified via expression of distinct proteins: type I glial-like cells; type II sweet, bitter and umami detectors; and type III sour detectors and putative relay cells (Clapp et al., 2001; Clapp et al., 2004; Yang et al., 2000b; Huang et al., 2006; Roper, 2006). The entire taste bud cell population has been estimated to arise embryonically from 7-13 progenitor cells, and then is continually renewed throughout adult life from a proliferative progenitor pool within the papillary epithelium (Stone et al., 2002; Okubo et al., 2008). However, the cell lineage for taste cells generated at any stage is completely unknown. One view is that taste cell types represent separate lineages, which remain distinct throughout the life of each cell (Farbman, 1965). Alternatively, taste cells may have a common lineage, with different cell types representing different stages in the lifespan of a single cell (Delay et al., 1986). More recently, type I cells have been proposed to arise from a dedicated lineage, whereas types II and III have an intermingled relationship, with a subset of type III cells giving rise to type II cells (Miura et al., 2006).

Our fate-mapping studies indicate that Shh-expressing embryonic taste bud progenitors generate both type I and II cells, suggesting that they share a common embryonic lineage. This leaves the issue of type III cell lineage unresolved. We cannot rule out the possibility that type III cells may descend from this same progenitor pool, as we may be looking for a very rare event. Embryonically labeled progenitor cells are steadily lost postnatally, and combined with the low frequency of type III cells within taste buds (Ma et al., 2007), the chance that double-labeled type III taste cells would be detected is very low (<1 double labeled type III cell per animal). We must therefore look at many more experimental animals in order to encounter an example of this lineage relationship. Alternatively, type III cells may have a distinct embryonic origin.

To address this possibility, we asked whether taste buds receive a cellular contribution from the neural crest, either embryonically or postnatally as the taste placode-descendant cells are lost; perhaps type III cells arise uniquely from neural crest? Although an embryonic neural crest contribution has been excluded in mouse circumvallate papilla epithelium in early embryos (Jitpukdeebodintra et al., 2002), as well as in early development of amphibian taste buds (Barlow and Northcutt, 1995), our studies extend these results. Using the Wnt1Cre line, we confirm extensive neural crest in the lingual mesenchyme (Chai et al., 2000), including that of taste papilla; however, in no case and at no time did we observe neural crest-derived cells within taste buds, let alone in lingual epithelium.

Although not derived from neural crest, type III cells may develop via processes that differs from that of types I and II, perhaps because of their distinct morphology and life history within taste buds. Type III cells are the only taste cell type to form conventional synapses with sensory neurons conveying taste information to the CNS (Roper, 2006; Yang et al., 2000a), and may also be the most long-lived cell within buds. Although the average lifespan of rodent taste cells is 10 days, 4-week-old cells with a type III morphology have been documented in adults (Hamamichi et al., 2006). One final explanation for our failure to label type III cells as placodal descendents is that they may arise from a non-Shh expressing population, which is induced by signals from the taste placodes. This recruitment of type III cells or their progenitors could occur embryonically, or early on in postnatal life. However, they must be recruited before postnatal day 4, when we first observe differentiated type III cells (T. Glover, H. Nguyen and L.A.B., unpublished). In any case, the identity of Type III taste cell progenitors remains unknown.

Neural dependence of taste bud development

In amphibians, development of taste buds can occur without nerves (Barlow et al., 1996). Similarly, cultured lingual explants from rodents form taste placodes and papillae, which express crucial signaling molecules, despite the lack of innervation (Hall et al., 2003; Farbman and Mbiene, 1991; Nosrat et al., 2001; Mistretta et al., 2003). A number of groups have also used an in vivo genetic approach to address this issue. Brain-derived neurotrophic factor (BDNF) is expressed in developing taste papillae (Nosrat and Olson, 1995; Nosrat et al., 1996), and loss of BDNF results in embryonic loss of taste sensory neurons (Jones et al., 1994; Liebl et al., 1997). In tongues of these mutant mice examined 1-2 weeks postnatally, lingual innervation to taste buds is indeed lost, and taste buds and papillae are dramatically reduced (Nosrat et al., 1997). However, we show here that this reported postnatal effect is probably due to a postnatal role of either Bdnf or neural maintenance of taste progenitor cells, rather than to an absolute requirement for either BDNF or nerve contact for taste progenitor induction and initial taste bud differentiation. Specifically, specification and patterning of taste bud progenitors does not differ between wild type and Bdnf−/− mice, despite our finding that most of these progenitors are not successfully innervated during development. Furthermore, 4 days later at birth, taste cells, as defined by CK8 expression, do not differ significantly between control and mutant embryos. However, because of the high variability in CK8-IR taste bud numbers at this later stage in both mutants and controls, it is possible that gustatory innervation may impact taste bud progenitor differentiation at this time. Nonetheless, specification and patterning of mammalian taste bud progenitors, and probably the initial differentiation of taste buds, are independent of both BDNF and gustatory innervation.

The development of mammalian taste buds and papillae is typically considered to be similar to that of other epithelial appendages, such as teeth, hair follicles and feathers, which require extensive interactions between epithelium and mesenchyme (Chuong et al., 2000; Pispas and Theis, 2003). The observation that mammalian taste placodes give rise to taste buds only, and not to papillae, is reminiscent of taste organ formation in axolotls, where taste buds lack papillae and are embedded directly in the lingual epithelium (Takeuchi et al., 1997). Development of axolotl taste organs occurs independently of mesenchyme (Barlow and Northcutt, 1995), and, moreover, has been shown to be an early epithelium-intrinsic process (Parker et al., 2004). Thus, we speculate that mammalian taste progenitors are also specified via an epithelial event, which is probably independent of mesenchyme.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/136/9/1519/DC1
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