CHAPTER TWELVE

Developing and Regenerating a Sense of Taste

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Abstract

Taste is one of the fundamental senses, and it is essential for our ability to ingest nutritious substances and to detect and avoid potentially toxic ones. Taste buds, which are clusters of neuroepithelial receptor cells, are housed in highly organized structures called taste papillae in the oral cavity. Whereas the overall structure of the taste periphery is conserved in almost all vertebrates examined to date, the anatomical, histological, and cell biological, as well as potentially the molecular details of taste buds in the oral cavity are diverse across species and even among individuals. In mammals, several types of gustatory papillae reside on the tongue in highly ordered arrangements, and the patterning and distribution of the mature papillae depend on coordinated molecular events in embryogenesis. In this review, we highlight new findings in the field of taste development, including how taste buds are patterned and how taste cell fate is regulated. We discuss whether a specialized taste bud stem cell population exists and how extrinsic signals can define which cell lineages are generated. We also address the

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question of whether molecular regulation of taste cell renewal is analogous to that of taste bud development. Finally, we conclude with suggestions for future directions, including the potential influence of the maternal diet and maternal health on the sense of taste *in utero*.

Taste is important for life. It serves as the gateway to substances that enter the body, allowing us to distinguish nutritious food items from potentially toxic ones. Classically, taste buds in the oral cavity, primarily on the tongue, were shown to detect five basic tastes: sour, salty, bitter, sweet, and umami—savory or "deliciousness" in Japanese. More recently, fatty acids and calcium have emerged as potential tastants that can be sensed by taste bud cells (Iwata, Yoshida, & Ninomiya, 2014; Liman, Zhang, & Montell, 2014; Passilly-Degrace et al., 2014; Tordoff, Reed, & Shao, 2008; Tucker, Mattes, & Running, 2014).

Among humans, taste preferences vary widely, and these preferences in turn influence dietary choices, which impact body weight and therefore health (Mennella, 2014). A key question is what underlies this variability. Not surprisingly, it appears that environmental, genetic, and epigenetic mechanisms are at play. In mammals, including humans, the maternal diet during gestation and postnatal lactation is learned by her offspring. In humans, innervated and differentiated taste buds that are presumably functional are evident by 10–13 weeks of development (Bradley & Stern, 1967; Witt & Reutter, 1996, 1998). Throughout gestation, taste stimuli reach the amniotic fluid, which is continually swallowed by the fetus, and following birth, tastes of the maternal diet are evident in breast milk. This exposure heavily influences the dietary choices of offspring as they discover these new tastes (Beauchamp & Mennella, 2009; Mennella, 2014). However, maternal health also impacts the gestational experience, as it results in fetal metabolic programming via presumed epigenetic mechanisms (Dyer & Rosenfeld, 2011), which, in the case of diabetic or obese mothers, can predispose offspring to diabetes and cardiovascular disease. Although conclusive studies regarding alterations in taste sensitivity in this context have not been performed, it is well known that diabetes and obesity affect taste preferences in adults. For example, in diabetic patients, taste responses, especially to sweet, are blunted (Wasalathanthri, Hettiarachchi, & Prathapan, 2014), and obese individuals also have decreased taste sensitivity (Stewart et al., 2010; Stewart, Feinle-Bisset, & Keast, 2011).

The pattern of taste buds is established during embryogenesis, such that the first functional taste bud cells are specified during gestation. Whereas most sensory epithelia, such as hair cells of the inner ear and photoreceptors

of the retina, have limited renewal potential, taste cells are remarkable in their ability to turn over rapidly and continuously throughout adult life (Beidler & Smallman, 1965; Farbman, 1980; Feng, Huang, & Wang, 2014; Hamamichi, Asano-Miyoshi, & Emori, 2006; Perea-Martinez, Nagai, & Chaudhari, 2013). Despite regular sensory cell replacement, the sense of taste is remarkably stable throughout life in healthy individuals. However, taste can be distorted or lost in cancer patients when these individuals are treated with chemotherapeutic drugs, and in head and neck cancer patients following targeted radiotherapy (Berteretche et al., 2004; Hong et al., 2009; Ruo Redda & Allis, 2006; Vissink, Jansma, Spijkervet, Burlage, & Coppes, 2003). These treatments are thought to disrupt taste function by diminishing taste bud cell renewal (Nguyen, Reyland, & Barlow, 2012 and references therein). Thus, we hypothesize that both regulation of taste bud development, including patterning and formation of the proper ratio of taste receptor cell types, and taste bud renewal, i.e., generation of functional taste cell types in the proper ratios with the proper timing, underlie variability in taste function and dysfunction.

In this review, we highlight new data in the context of the important open questions in the field rather than providing an exhaustive survey of the literature; for more comprehensive reviews on taste development, regeneration, and function, see Kapsimali & Barlow (2013), Feng et al. (2014), and Liman et al., (2014), respectively.

1. HOW ARE TASTE BUDS PATTERNED?

Taste bud distribution is highly variable across vertebrate species, including in mammals (Jackowiak, 2006 and references therein), fish, amphibians, and birds (Erdoğan & Iwasaki, 2014; Finger, 1997; Northcutt, 2004). In addition, taste bud pattern varies even within a single species, including in humans (Fischer et al., 2013; Miller & Reedy, 1990) and rodents (mouse inbred lines: Reiner et al., 2008; rat: Miller & Preslar, 1975; Tordoff, Alarcon, & Lawler, 2008).

The initial pattern of taste buds on the tongue is evident at mid-gestation (at embryonic day (E) 12.0 in mice), when bilateral rows of columnar epithelial placodes (taste placodes) form in the otherwise cuboidal epithelium of the developing tongue (Farbman, 1965; Mistretta, 1972). Subsequently, placodes undergo morphogenesis into mushroom-shaped (fungiform) taste papillae, which house taste buds that differentiate at birth. Taste placodes express Sonic hedgehog (*Shh*) from the earliest stages of their development,

and expression of *Shh* persists in the apices of papillae through the remainder of embryogenesis (Bitgood & McMahon, 1995; Hall, Hooper, & Finger, 1999; Jung, Oropeza, & Thesleff, 1999). Lineage tracing of the *Shh* + taste placodes, commencing at E12.5 or 13.5, reveals that these cells differentiate directly into the first taste bud cells at birth but do not contribute to the surrounding taste papillae (Thirumangalathu, Harlow, Driskell, Krimm, & Barlow, 2009). Rather, we have proposed that *Shh*-descendent taste bud precursor cells may function as signaling centers to induce adjacent epithelial and mesenchymal cells to form taste papillae (Thirumangalathu et al., 2009).

A number of pathways regulate the initial patterning of taste placodes in rodents, including Wnt/β-catenin (Iwatsuki et al., 2007; Liu et al., 2007), bone morphogenetic proteins (Bmps) (Beites et al., 2009; Zhou, Liu, & Mistretta, 2006), Shh (Hall, Bell, & Finger, 2003; Liu, MacCallum, Edwards, Gaffield, & Mistretta, 2004; Mistretta, Liu, Gaffield, & MacCallum, 2003), epidermal growth factor (Egf) (Liu, Henson, Zhou, D'Silva, & Mistretta, 2008), and fibroblast growth factors (Fgfs) (Kapsimali et al., 2011; Petersen et al., 2011; reviewed in Kapsimali & Barlow, 2013; Table 1). In particular, normal activation of the Wnt/β-catenin pathway

Table 1 Summary of the function of major signaling pathways in embryonic taste bud development

Pathway	Demonstrated functions	References
Wnt/β-catenin	• Promotes taste fate *in vivo* and *in vitro* • Required for taste fate *in vivo*	Iwatsuki et al. (2007) Liu et al. (2007)
BMP	• Prior to taste placode specification, BMPs promote taste fate • Following placode specification, BMPs repress taste fate *in vitro* • Loss of mesenchymal follistatin promotes taste fate *in vivo*	Zhou et al. (2006) Beites et al. (2009)
SHH	• Shh represses taste fate *in vitro* • Inhibition of Shh *in vitro* expands taste fate	Iwatsuki et al. (2007) Hall et al. (2003), Mistretta et al. (2003)
FGF	• Loss of Spry1/2 promotes taste fate • Loss of mesenchymal FGF10 represses taste fate	Petersen et al. (2011)
EGF	• EGF represses taste fate *in vitro* • Inhibition of EGF promotes taste fate *in vitro*	Liu et al. (2008)

within the developing lingual epithelium is required for formation of taste placodes, whereas ectopic activation of the pathway in the entire epithelium drives differentiation into enlarged *Shh*+ taste bud precursors embedded in oversized fungiform papillae (Liu et al., 2007). Interestingly, Wnt ligands are expressed in both epithelial and mesenchymal compartments in the developing tongue (Iwatsuki et al., 2007; Liu et al., 2007, 2012), leaving open the question of which source(s) of Wnt protein are responsible for taste patterning. Recently, conditional deletion of *Wls* (also known as *Gpr177* or *Evi1*) from the early oral endoderm under the $Shh^{GFP\text{-}Cre}$ allele (Harfe et al., 2004) revealed that epithelial Wnt production is required for taste placode initiation (Zhu et al., 2014). The *Wls* gene encodes an intracellular protein that enables Wnt ligand secretion (Banziger et al., 2006), and deletion of epithelial *Wls*, which causes loss of epithelial Wnt ligand secretion, leads to absence of taste placode formation. One caveat to these studies, however, is that *Shh* is expressed within the oral endoderm commencing at E9.5 (Echelard et al., 1993) and thus loss of *Wls* function using the $Shh^{GFP\text{-}Cre}$ is also induced early with respect to tongue and taste placode formation, which begin at E11.5 (Kaufman, 1999) and E12.0 (Hall et al., 2003), respectively. Therefore, it is formally possible that early epithelial WLS function is required for development of epithelial competence to respond to later Wnt signals from the mesenchyme. Nonetheless, these findings are consistent with experimental data from axolotl (salamander) embryos, where early taste bud specification and patterning are governed by mechanisms intrinsic to the epithelium and independent of oral mesenchyme (Barlow, 2001; Barlow & Northcutt, 1997; Parker, Bell, & Barlow, 2004). Thus, numerous pathways can affect taste bud patterning, and subtle differences in timing, competence to receive the signal, and the strength of the signal may be equally influential.

In addition, there are significant distinctions in patterning of different regions of the tongue as well as patterning of taste papillae in different organisms. One area in which this has been explored is the difference between the small anterior fungiform papillae and the large posterior circumvallate papilla (CVP). Most studies of development and patterning have focused on the fungiform papillae; although there is a large literature concerning physiology, anatomy, and cell biology of the adult CVP, only a limited number of developmental studies have been performed on this organ (see Kapsimali & Barlow, 2013 for review). A number of pathways that are known to regulate fungiform papillae pattern have no reported phenotype in the CVP, including the Shh (Mistretta et al., 2003), Bmp (Beites et al., 2009), and Wnt (Iwatsuki et al., 2007) pathways. In contrast, the Fgf

pathway has been shown to be a critical regulator of CVP development in mice. A balance between Sprouty (Spry) genes and *Fgf10*, which, respectively, antagonize and activate receptor tyrosine kinase signaling, regulates the number of CVPs (Petersen et al., 2011), such that in wild-type mice, only a single CVP forms. Deletion of a single Sprouty family member, *Spry2*, resulted in duplication of the CVP, as a result of an increase in the size of the CV placode progenitor field. Combined deletion of two Sprouty genes in *Spry1*$^{-/-}$;*Spry2*$^{-/-}$ embryos led to the formation of multiple CVPs, demonstrating the redundancy of Sprouty genes in regulating the size of the progenitor field. By contrast, deletion of *Fgf10* led to absence of the CVP, thus identifying FGF10 as an inductive, mesenchyme-derived factor for taste papillae. Recently, the transcription factor Wilms' tumor 1 protein (WT1) was found to have a critical role in CVP development, as deletion of *Wt1* led to failure of CVP development (Gao, Toska, Denmon, Roberts, & Medler, 2014). Several WT1 target genes that are members of canonical signaling pathways were identified, including *Lef1* from the Wnt pathway, *Ptch1* from the Shh pathway, and *Bmp4*. Interestingly, there are also some hints emerging that anterior versus posterior taste papillae may be regulated in opposite ways by specific signaling pathways, such as the Fgf pathway (Petersen et al., 2011). This may be due to origins in different germ layers, as the anterior tongue epithelium is derived from ectoderm, whereas epithelium covering the posterior tongue has an endodermal origin (Adams, 1931; Barlow, 2000; Rothova, Thompson, Lickert, & Tucker, 2012). Of interest, while taste buds are restricted to the oral cavity of most vertebrates, several fishes have evolved external taste buds, including catfishes which have thousands of taste buds distributed in the head and trunk epithelium (Atema, 1971; Landacre, 1907; Northcutt, 2005). Their external location suggests that these taste buds must arise from surface ectoderm (Landacre, 1907), while oral taste buds of fish and amphibians originate primarily from endoderm (Barlow & Northcutt, 1995; Johnston, 1910). Thus, the catfish would be an ideal model to test if and how embryonic origin affects the mode of taste bud development.

2. REGULATION OF TASTE CELL FATE

Taste buds comprise 60 – 100 elongate cells, which are classified into three morphological types and as many as five or more functional categories (Fig. 1) (Feng et al., 2014; Liman et al., 2014). In animal models used in the majority of developmental studies including fish and rodents, taste cells

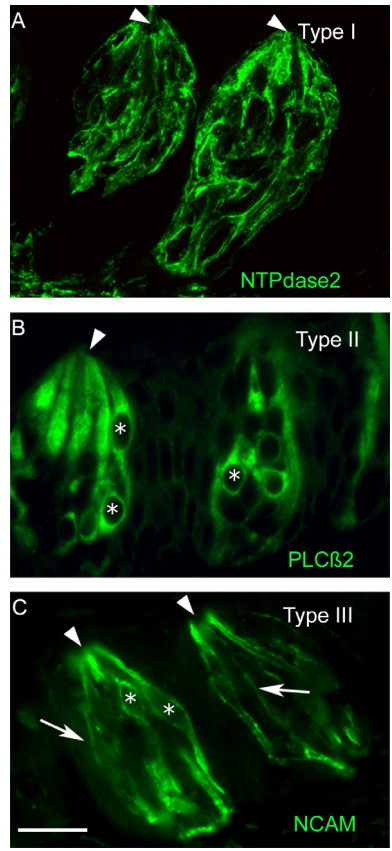

Figure 1 Taste buds comprise three morphological cell types, I, II, and III, which are recognized by their expression of specific marker proteins. (A) Type I cells express NTPdase2, which localizes to cell membranes that tightly wrap other cells within the bud, such that individual NTPDase2+ cells are not discernable (see Miura, Scott, Harada, & Barlow, 2014 for detailed explanation). (B) Type II cells express PLCβ2 in the cytosol, and the protein is excluded from nuclei of PLCβ2+ taste cells (*). (C) NCAM marks the surface of Type III taste cells (*), as well as some nerve fibers extending into buds (arrows). All panels are images of taste buds from the circumvallate papilla of mice. Apical taste pores are indicated with arrowheads. Scale bar is 20 μm.

differentiate postnatally (see references in Kapsimali & Barlow (2013)), such that in adults, the different taste cell types occur in proper ratios within each bud (Chaudhari & Roper, 2010; Kim et al., 2003; Ma, Yang, Thomas, & Kinnamon, 2007; Ohtubo & Yoshii, 2010). Generally, the Type I or glial-like cells are most common, followed by Type II sweet/bitter/umami detectors, and least frequent are the Type III sour receptor

cells, although these ratios vary with respect to location in the tongue. For example, in rats and mice, Type III cells are more frequent in taste buds in the posterior CVP than in taste buds of the anterior fungiform papillae (Ma et al., 2007; Ohtubo & Yoshii, 2010). In mice, taste buds in the anterior tongue have more sweet cells per bud, whereas in posterior tongue, taste buds have more bitter cells (Kim et al., 2003; Tizzano et al., 2008). How and when these cell fates are assigned in embryos remains an important open question.

Not surprisingly, in light of its important role in fate decisions in many tissues, Notch function has been implicated in taste cell fate decisions during embryonic development (reviewed in Kapsimali & Barlow, 2013). In mice, Notch pathway genes are expressed in and around the developing CV papilla in the posterior tongue, and this expression begins after the papilla is specified (Seta, Seta, & Barlow, 2003). In late gestation embryos, *Mash1/Ascl1*, which is transcriptionally repressed by Notch signaling (Kageyama & Ohtsuka, 1999), is expressed in small numbers of epithelial cells in locations consistent with that of the first differentiated taste buds at early postnatal stages; this expression pattern suggested a role for *Ascl1* in the differentiation of one or more taste cell types. Indeed, genetic deletion of *Ascl1* results in loss of expression of numerous markers of Type III taste cells, supporting the hypothesis that *Ascl1* is required for Type III cell fate (Kito-Shingaki et al., 2014; Seta, Oda, Kataoka, Toyono, & Toyoshima, 2011). Interestingly, Type III cells are the taste cells most similar to neurons, and *Mash1* is a proneural gene that drives expression of Notch ligands cell autonomously to activate Notch signaling in adjacent cells, but *Mash1* also directs fate cell autonomously while keeping neighbors in a stem cell state (Kageyama, Ohtsuka, Hatakeyama, & Ohsawa, 2005). In addition, the transcription factor *Hes1*, considered a primary Notch target gene (Ohtsuka et al., 1999), has been shown to repress differentiation of Type II taste cells, as excess Type II cells differentiate in $Hes1^{-/-}$ taste papillae (Ota et al., 2009). Notch function in taste cell fate selection is conserved in zebrafish, where it again plays a role in specifying Type II-like versus Type III-like cell fates (Kapsimali et al., 2011).

In addition to components of the Notch pathway, another transcription factor, *Skn1a/Pou2f3* has been shown to be required for differentiation of Type II sweet/bitter/umami cells in adults, as taste buds in adult $Skn1a^{-/-}$ mice lack Type II cells and have excess Type III cells (Matsumoto, Ohmoto, Narukawa, Yoshihara, & Abe, 2011). If and how this transcription factor forms a gene regulatory network with *Ascl1* and/or *Hes1* remains to be

explored. Finally, and intriguingly, genetic control of specification of the most common taste cell fate, the Type I glial-like cell, remains a mystery.

3. HOW CAN WE LINK EMBRYONIC DEVELOPMENT AND ADULT TASTE CELL RENEWAL?

As mentioned above, embryonic *Shh*+ placodes are taste bud precursors, which differentiate into the first taste bud cells. As taste bud cells renew, these first taste cells are ultimately replaced over time. However, embryonic *Shh*+ cells do not contribute to the stem cell pool that enables adult taste cell renewal, as all *Shh*-descendent taste bud cells are lost by 4 months postnatally (Thirumangalathu et al., 2009). Rather, in adults, taste receptor cells are renewed from cytokeratin (K) 14+/K5+ basal keratinocytes adjacent to taste buds (Fig. 2) (Okubo, Clark, & Hogan, 2009). K14+ lingual keratinocytes also give rise to the general epithelium of the tongue, which comprises nontaste filiform papillae (Hume & Potten, 1976; Mistretta, 1972). How K14+/K5+ stem cells are regulated to produce both taste buds and general epithelium is poorly understood. However, this population generates only

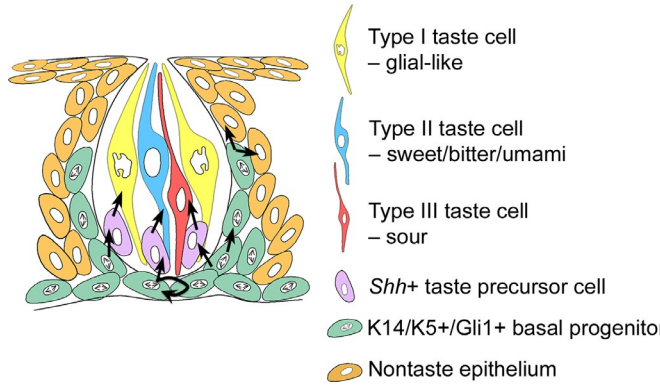

Figure 2 Schematic diagram of taste bud cell types and lineage relationships. Type I (yellow), II (blue), and III (red) taste cells are elongate, postmitotic (open nuclei) cells, which arise from proliferating, K5+/K14+ keratinocytes (green, mitotic nuclei) outside of taste buds. These progenitors also give rise to nontaste epithelial cells (orange). Following their final division, cells destined to become taste cells enter taste buds and become taste bud precursors, which are *Shh*+ ovoid cells (lavender) located in the basal compartment of the taste bud. Shh+ cells are postmitotic, and differentiate into each of the three taste cell types. Black arrows indicate observed lineage relationships among cell types (see text for details), but are not absolute, nor exhaustive.

nontaste epithelium *in vitro* (Luo, Okubo, Randell, & Hogan, 2009), suggesting that either taste bud stem cells represent a rare or distinct population, or that key extrinsic signals are required that were absent under the culture conditions employed.

Intriguingly, other lingual epithelial stem populations that contribute to nontaste filiform papillae, but not to taste buds, have been identified. One gene that has been recently studied is *Bmi1*, which together with K14 and K5 labels cells at the base of the interpapillary pit (Tanaka et al., 2013). These cells were reported to be unipotent stem cells for keratinized epithelial cells but not for taste bud cells. *In vitro* organoids could also be generated from single *Bmi1*-positive cells (Hisha et al., 2013). Similarly, lineage tracing using a $Tcf3^{CreER}$ knock-in mouse model showed that *Tcf3* marks stem cells as well as transient progenitors and cells undergoing active differentiation in the tongue (Howard, Nuguid, Ngole, & Nguyen, 2014).

4. IS THERE A SPECIALIZED TASTE BUD STEM CELL POPULATION, OR ARE EXTRINSIC SIGNALS RESPONSIBLE FOR DEFINING WHICH CELL LINEAGES ARE GENERATED, AND WHEN?

Recently, a new population of lingual stem cells has been identified that gives rise to both epithelium and taste buds in the large CVP situated at the midline of the posterior tongue. These cells express the leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), which is also expressed by the stem cells of the intestine and other organs (Barker et al., 2007; Ng et al., 2014; Plaks et al., 2013). In the CVP of mice carrying an *Lgr5* reporter allele ($Lgr5^{GFP\text{-}CreER}$; Barker et al., 2007), taste buds themselves are not $Lgr5^{GFP}$+; instead, GFP-bright epithelial cells are situated at the deepest portion of the papilla where taste buds are not present, while low expressing $Lgr5^{GFP}$+ basal epithelial cells are found higher up in the papilla and adjacent to taste buds. Lineage tracing revealed that *Lgr5*+ cells give rise all three taste cell types (Takeda et al., 2013; Yee et al., 2013), and that the deep $Lgr5^{GFP}$+ cells are lineage labeled, and persist for months (Yee et al., 2013). Interestingly, both groups reported that *Lgr5* expression was not detectable in the anterior tongue by GFP expression, or by PCR for *Lgr5* transcripts, indicating that this stem cell population is specific to the posterior CVP. This result highlights again the distinction described above between ectodermally derived anterior taste buds and endodermally derived

CVP taste buds, and it is intriguing in that *Lgr5* also marks a key stem cell population in the endodermally derived gut (see Barker, Tan, & Clevers, 2013 for review).

To date, an analogous stem cell population remains to be identified for fungiform taste buds. However, our recent data point to a different model, where specialized stem cells may not be required in the anterior tongue, but rather extrinsic signals can drive bipotent K14+ cells toward the taste fate (Castillo et al., 2014). Using an inducible gain-of-function approach, we overexpressed SHH (SHHcKI) in K14+ basal keratinocytes and found that ectopic taste buds form throughout the nontaste epithelium and are interspersed among the endogenous fungiform papillae. Thus, SHH signals can induce cell type-replete taste bud differentiation from K14+ progenitors regardless of location. These results indicate that K14+ keratinocytes are broadly competent to generate taste receptor cells in response to SHH, revealing an unappreciated competency of the anterior tongue epithelium to build taste buds outside of fungiform papillae in the adult tongue. Expansion of taste fate in the lingual epithelium is well documented in the embryonic tongue, as all of the following promote the taste fate in lingual regions where taste buds do not form in controls: increased β-catenin *in vitro* or *in vivo* (Iwatsuki et al., 2007; Liu et al., 2007; Okubo, Pevny, & Hogan, 2006), SHH inhibition *in vitro* (Hall et al., 2003; Liu et al., 2004; Mistretta et al., 2003), or genetic deletion of *Follistatin in vivo* (Beites et al., 2009). Interestingly, manipulation of each of these pathways was reported to have no impact on the development of the posterior CVP (Beites et al., 2009; Iwatsuki et al., 2007; Liu et al., 2004; Mistretta et al., 2003). Likewise, in adult mice, despite SHH overexpression, taste buds in the posterior CV papilla were only mildly affected (D. Castillo, O. Klein, & L. Barlow, unpublished), again suggesting that SHH may function differently in ectodermally derived anterior versus endodermally derived posterior lingual epithelium even in adulthood.

5. IS MOLECULAR REGULATION OF TASTE CELL RENEWAL ANALOGOUS TO THAT OF TASTE BUD DEVELOPMENT?

In contrast to development, during which several pathways have been shown to function in taste bud development, only Shh function has been examined thus far in adults. Specifically, ectopic overexpression of SHH promotes differentiation of cell type-replete taste buds (Castillo et al., 2014).

This protaste function contrasts directly with the taste-repressive function of Shh signaling in development. This difference is likely due to differences in cells receiving SHH signals, as *Shh*+ cells in adult taste buds and in taste placodes appear quite similar (Miura & Barlow, 2010; Miura, Kusakabe, & Harada, 2006; Nakayama et al., 2008): both are specified, immediate postmitotic precursors of differentiated taste bud cells and both are SHH non-responsive, as they do not express *Ptch1* or *Gli1* (Hall et al., 1999; Liu et al., 2013; Miura et al., 2001; Miura, Scott, Harada, & Barlow, 2014; Thirumangalathu et al., 2009). In embryos, established *Shh*+ cells inhibit neighboring *Ptch1* and *Gli1* expressing epithelial cells (Hall et al., 1999) from acquiring a taste fate (Hall et al., 2003; Iwatsuki et al., 2007; Liu et al., 2004; Mistretta et al., 2003). However, in adult epithelium, while postmitotic precursors within taste buds also signal via SHH to surrounding *Gli1*+ and *Ptch1*+ taste papilla epithelial cells (see Fig. 2) (Liu et al., 2013; Miura et al., 2001, 2004), SHH now promotes rather than represses taste fate (Castillo et al., 2014). When this shift in SHH function occurs should shed light on the timing of the transition from initial development to continual taste cell renewal.

In addition to *Ptch1* and *Gli1*, *Gli2* is expressed by basal keratinocytes adjacent to taste buds (a pattern similar to that of *Ptch1* and *Gli1*), as well as more broadly throughout the nontaste epithelium (Liu et al., 2013), suggesting that *Gli2* may play a role in taste cell renewal and underlie in part the ability of SHH to induce ectopic taste buds. Indeed, activation of a hyperactive *Gli2* allele, which is oncogenic in skin (Grachtchouk et al., 2011), abolishes fungiform taste buds in adult mice; however, it is unclear if this is due to oncogenic growth or to a more direct effect on lingual epithelial cell fate (Liu et al., 2013).

Finally, in addition to a role in lingual epithelium, SHH signals are received in the mesenchymal compartment of taste papillae of both embryos and adults (Hall et al., 1999; Liu et al., 2013; Miura et al., 2001). In adults, SHH-receiving cells in the mesenchyme have been proposed to comprise a niche for taste bud cell maintenance (Liu et al., 2013), an idea that was suggested initially based on expression of *Bmp4* in the taste papilla mesenchyme (Nguyen & Barlow, 2010). Likewise, in the embryonic tongue, in addition to signaling to adjacent epithelium, *Shh*+ taste placodes signal to the subjacent mesenchymal compartment. This epithelial-to-mesenchymal Shh signaling may function in the extensive morphogenesis of taste papillae, comparable to the role of *Shh* in development of other epithelial appendages,

such as teeth, feather, and hair follicles (Chuong, Patel, Lin, Jung, & Widelitz, 2000; Pispa & Thesleff, 2003), although this remains to be tested. As we mentioned above, taste buds in salamander embryos develop independently of oral mesenchyme. Cultured epithelial explants devoid of mesodermal and neural crest-derived cells develop cell type-replete taste buds (Barlow & Northcutt, 1997), but it is important to note that in axolotls taste buds do not reside in papillae, but rather are embedded in the oral epithelium (Fährmann, 1967; Northcutt, Barlow, Braun, & Catania, 2000; Toyoshima, Miyamoto, & Shimamura, 1987). Lingual taste papillae thus appear to be primarily a mammalian innovation, which may have evolved to prevent taste bud desiccation or protect taste buds from abrasive foodstuffs. Thus, we propose that the primary event in taste development is specification of taste bud precursors, and that papillary development is secondary. Specifically, in both amphibians and mammals, we hypothesize that taste bud precursors are specified by epithelium-intrinsic processes, while in mammals, these taste bud precursors in turn organize adjacent epithelium and mesenchyme to build taste papillae around them (Thirumangalathu et al., 2009). It remains to be determined if the canonical Shh signaling pathway, in addition to its role in taste placode patterning, also guides papilla morphogenesis directly and/or indirectly.

In closing, the overall structure of the taste periphery, which is composed of multicellular taste buds that detect primary tastants in the oral cavity, is conserved in all vertebrates examined to date, except the hagfishes (Braun, 1996, 1998; Finger, 1997), whereas the anatomical, histological, cell biological, and molecular details of taste buds in the oral cavity are quite varied (Barlow, 1999; Jiang et al., 2012; Liman, 2012; Northcutt, 2004). These differences in structure and function have evolved as adaptations to the different diets consumed, but also indicate that this sensory system is quite flexible over evolutionary time scales. In addition, the pattern and cellular makeup of taste buds have been shown to vary within mammalian species, such as humans and mice, and in mouse and zebrafish embryos, taste bud pattern and cell complement are easily manipulated experimentally via drug or genetic perturbations. This raises the question of how impressionable the sense of taste is *in utero*, in terms of exposure to maternal diet and to maternal overall health: can the taste bud array be permanently altered anatomically and at the level of taste cell fate decisions by gestational experience? And how might these changes in the taste periphery impact taste function and dietary selections and ultimately the health of offspring?

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