

## REVIEW

# Progress and renewal in gustation: new insights into taste bud development

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**ABSTRACT**

The sense of taste, or gustation, is mediated by taste buds, which are housed in specialized taste papillae found in a stereotyped pattern on the surface of the tongue. Each bud, regardless of its location, is a collection of ~100 cells that belong to at least five different functional classes, which transduce sweet, bitter, salt, sour and umami (the taste of glutamate) signals. Taste receptor cells harbor functional similarities to neurons but, like epithelial cells, are rapidly and continuously renewed throughout adult life. Here, I review recent advances in our understanding of how the pattern of taste buds is established in embryos and discuss the cellular and molecular mechanisms governing taste cell turnover. I also highlight how these findings aid our understanding of how and why many cancer therapies result in taste dysfunction.

**KEY WORDS:** Taste buds, Gustatory system, Sonic hedgehog, Wnt/β-catenin, Cell lineage, Molecular genetics, Taste dysfunction, Dysgeusia, Ageusia, Chemotherapy, Stem cells, Lingual organoids

**Introduction**

Gustation, or the sense of taste, allows us to distinguish dangerous from delightful foods. We use taste to select nutritious and delicious foods – those containing essential minerals and with higher calorie content, which are typically salty, sweet and/or savory. By contrast, we reject bitter substances, which are often toxic as many plants and animals have evolved to produce bitter-tasting compounds to avoid being eaten (Reynolds, 2005). When consumed, naturally occurring bitter chemicals can cause nausea or even death (Barlow, 1999; Liener, 1970). The taste system also detects sour stimuli, which can indicate acidic, fermented, or even rotten food (Breslin, 2013; Reed and Knaapila, 2011). By detecting these basic tastes, one can think of taste buds within the oral cavity as gateway chemoreceptors that inform our decision of whether to allow substances already in the mouth to enter our body.

Taste is mediated by multicellular taste buds located primarily on the tongue. Taste bud cells have functional characteristics linking them to neurons; they transduce taste stimuli into electrochemical signals and transmit these signals to sensory nerves (Roper, 2007). However, taste cells are modified epithelial cells and are rapidly renewed (Barlow and Klein, 2015; Feng et al., 2014). Thus, although we consider our sense of taste to be reasonably stable over time, the sensory cells underlying this perceptual stability are constantly turning over. We have little awareness of this dynamism in health, but taste dysfunction is common in disease and injury and occurs as a consequence of cancer therapies (Bartoshuk et al., 1996;

Epstein and Barasch, 2010; Feng et al., 2014; Ovesen et al., 1991; Sandow et al., 2006).

Here, I present basic background on the functional anatomy of the taste system, reviewing the mechanisms governing taste bud development in embryos and how these mechanisms compare to our growing understanding of taste cell renewal in adults. I highlight important new work on the cellular and molecular regulation of taste cell regeneration, which allows for new hypotheses of taste dysfunction, and I introduce the reader to new organoid technology that has allowed the *de novo* generation of functional taste cells from isolated lingual stem cells. For more in-depth discussions, I recommend recent reviews focused on taste bud development (Kapsimali and Barlow, 2013), taste bud innervation (Krimm et al., 2015), and the impacts of age and disease on adult taste bud homeostasis (Feng et al., 2014).

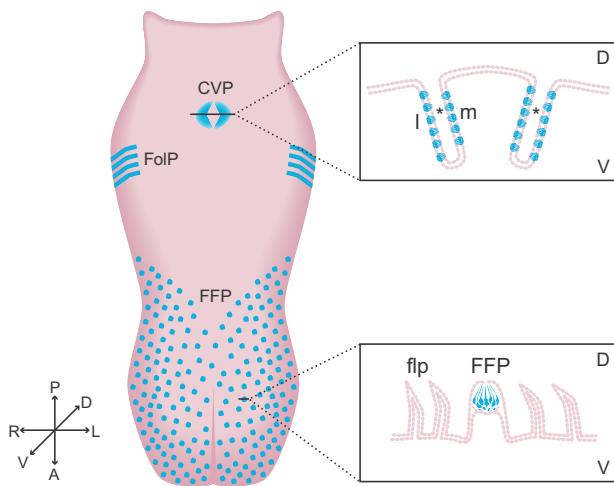
**An overview of the taste system**

Gustation is common to all vertebrates (Kirino et al., 2013; Northcutt, 2004). Broadly, the taste system is composed of multicellular taste buds distributed throughout the oral and pharyngeal cavities. Taste buds are innervated by sensory neurons of the VIIth, IXth and Xth cranial nerve ganglia, whose axons transmit taste information from peripheral taste buds to the hindbrain. In mammals, although some taste buds reside in the soft palate, the majority are situated on the tongue surface and are restricted to specialized taste papillae. In mammals, fungiform papillae (FFP) occupy the anterior two-thirds of the tongue and are distributed among the far more numerous non-taste filiform papillae; the latter form the tough surface of the tongue. Larger, more complex circumvallate papillae (CVP) and foliate taste papillae (FolP) are found in the posterior region of the tongue (Fig. 1). The number and pattern of taste papillae types vary in mammals (Petersen et al., 2011; Reiner et al., 2008; Witt and Miller, 1992), but rodents possess a single midline CVP, bilaterally located FolP, each containing hundreds of taste buds, and anteriorly arrayed FFP, each housing one taste bud (Fig. 1).

In mice, taste buds comprise 60–100 elongated cells belonging to three morphological types (Types I, II and III), and at least five functional types that detect salt, sour, sweet, bitter and umami (savory) (Finger and Simon, 2000; Liman et al., 2014). Type II cells detect sweet, bitter or umami tastes and employ a common G protein-coupled receptor transduction cascade, which involves PLC $\beta$ 2, IP $_3$ R3 (ItpR3) and TrpM5. However, the specific taste quality, i.e. the particular chemical signal, transduced by each Type II cell depends on the taste receptor proteins expressed. These are seven-transmembrane proteins of primarily two classes: those that detect sweet, bitter or umami (T1Rs), and those that transduce bitter compounds (T2Rs). For example, sweet-sensitive Type II cells express T1R2/T1R3 heterodimers, whereas umami-sensitive Type II cells express T1R1/T1R3. Additionally, several metabotropic glutamate receptors are known to function as umami receptors

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**Fig. 1. The locations of taste papillae and taste buds in the rodent tongue.**

Lingual taste buds are housed in distributed fungiform papillae (FFP; blue) in the anterior region of the tongue, which is otherwise covered with mechanosensory filiform papillae (flp in lower inset). Bilateral foliate papillae (FoIP; blue) and a single midline circumvallate papilla (CVP; blue) are located posteriorly in the tongue. Each FFP houses one taste bud, whereas the CVP and FoIP house several hundred taste buds each (depicted for the CVP only). The CVP comprises two epithelial trenches that extend ventrally from the tongue surface (asterisks in upper inset), and taste buds are aligned orthogonal to the trench axes and embedded in both medial (m) and lateral (l) trench epithelia. D, dorsal; V, ventral; A, anterior; P, posterior; R, animal's right; L, animal's left.

(Chaudhari et al., 2000; Nakashima et al., 2012; Pal Choudhuri et al., 2015). Bitter taste is mediated by a large family of T2R proteins expressed by bitter-sensitive Type II cells (Chandrashekhar et al., 2000; Liman et al., 2014). Type III cells are sour detectors and respond to acidic taste stimuli. Sour is thus an ionic taste and transduced via a proton current, although which transduction protein(s) are responsible remains controversial (Bushman et al., 2015; Chandrashekhar et al., 2009; Huang et al., 2006). Finally, the cell type(s) mediating sodium salt taste remain ambiguous, although transduction clearly involves an epithelial sodium channel, ENaC, as well as other mechanisms (Chandrashekhar et al., 2010; Oka et al., 2013; Roper, 2015).

Type I cells are poorly understood, despite the fact that they make up the majority of cells within each bud (reviewed by Barlow and Klein, 2015). Morphologically, they resemble glia; they have extensive cellular processes that tightly wrap Type II and III cells (Bartel et al., 2006; Miura et al., 2014; Pumplin et al., 1997). Type I cells express membrane-localized NTPDase2 (Entpd2), an ectoATPase that converts ATP to ADP. Type II cells use ATP as a neurotransmitter to signal to sensory nerves (Finger et al., 2005; Vandenbeuch et al., 2015), yet Type II cells lack presynaptic specializations; rather, Type II cells release ATP in a non-vesicular manner (Huang et al., 2007; Romanov et al., 2013, 2007), probably via CALMH1 ion channels (Taruno et al., 2013). Thus, NTPDase2-expressing Type I cells are likely to clear excess ATP released by Type II cells to ensure efficient neurotransmission (Bartel et al., 2006; Finger et al., 2005; Vandenbeuch et al., 2013). Among the taste cell types, only Type III cells form conventional synapses on sensory nerve fibers (Yang et al., 2000, 2004); they release GABA and serotonin, which are likely to be neuromodulators rather than neurotransmitters (Chaudhari, 2014). By contrast, ATP released by Type II cells functions as a bona fide neurotransmitter, as it is required for the transmission of all taste information from taste buds

to sensory nerves (Finger et al., 2005; Vandenbeuch et al., 2015). The source of the ATP required for Type III cell signaling to afferent fibers is not known.

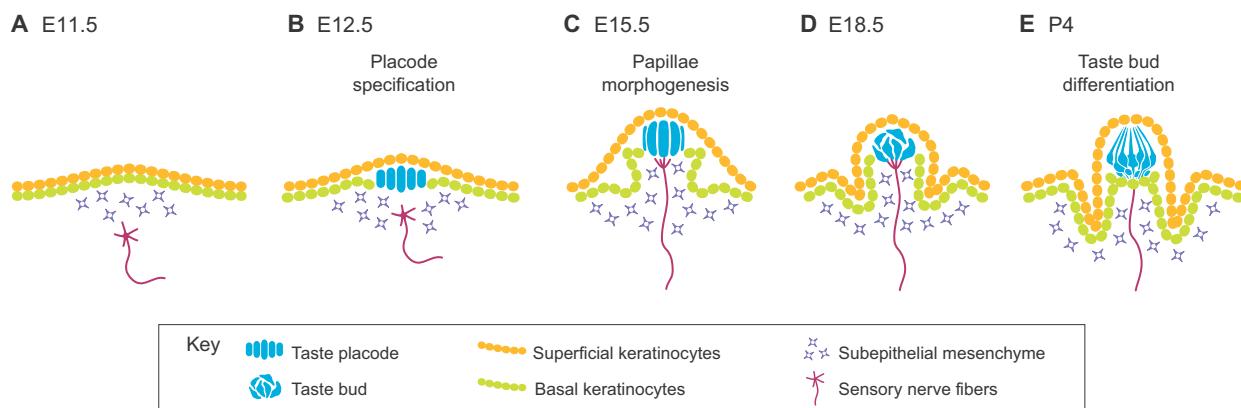
While individual taste cells are thought to be specialists with respect to taste quality, the degree to which transmission of taste information to the CNS is via taste quality-specific ‘labeled lines’ or is integrated in taste buds prior to transmission remains controversial (Barreto et al., 2015; Roper, 2013). Certainly, taste cells communicate among themselves within buds using ATP, adenosine, GABA and serotonin (Chaudhari, 2014; Kinnamon and Finger, 2013). How this intercommunication results in tastes that are cleanly identifiable by the brain is a primary question for the field.

### Taste bud development: from embryo to birth

The development of taste buds and their innervation is well described in rodents (Fig. 2) (reviewed by Kapsimali and Barlow, 2013; Krimm et al., 2015; Mistretta and Liu, 2006). At embryonic day (E) 11 in mice, the tongue rudiment forms and is covered by a homogeneous epithelial bilayer (Fig. 2A). By E12–12.5, taste placodes appear as foci of columnar epithelia in locations destined to become FFP and CVP (Fig. 2B). At E14.5, the epithelium begins invaginating to create taste papillae with distinct mesenchymal cores (Fig. 2C,D). Coincident with morphogenesis, taste nerve fibers reach and then penetrate the epithelium (Krimm et al., 2015). In rodents, most CVP and FFP taste buds do not differentiate fully, i.e. express specific taste cell type markers, until the first postnatal week (Fig. 2E). By contrast, most taste buds of the soft palate are differentiated and presumed functional at birth (El-Sharaby et al., 2001a,b; Harada et al., 2000). Precocious palatal bud differentiation may be required for feeding at birth; as pups suckle, their mother’s milk hits the back of the oral cavity (the soft palate) and thus these buds must be functional to ensure ingestion (El-Sharaby et al., 2001a; Harada et al., 2000).

Historically, taste bud development has been considered nerve dependent; in-growing nerves induce subsets of cells within taste papillae to become taste buds (reviewed by Kapsimali and Barlow, 2013; Oakley and Witt, 2004). The neural induction model is consistent with three broad sets of findings: (1) innervation precedes taste bud differentiation during development in all vertebrate species examined to date; (2) in adult mammals, intact innervation is required for taste bud maintenance; and (3) denervation of taste papillae in young postnatal rats permanently impairs taste bud differentiation. By contrast, in amphibian embryos, taste bud differentiation is entirely independent of innervation (Barlow et al., 1996) and, in cultured tongue explants from rodent embryos, taste placodes and taste papilla morphogenesis are likewise nerve independent (Farbman and Mbiene, 1991; Mbiene et al., 1997); however, these explants did not survive long enough to permit taste bud differentiation, leaving open the question of whether nerves are required for taste bud differentiation in mammals.

This issue was partially addressed via molecular lineage tracing in mouse. Taste placodes express sonic hedgehog (*Shh*) (Bitgood and McMahon, 1995; Hall et al., 1999; Jung et al., 1999), and cell lineage progression can thus be traced genetically by turning on indelible reporter gene expression in these cells (Buckingham and Meilhac, 2011). Following induction of a tamoxifen-sensitive *Cre* allele driven by the *Shh* promoter (Harfe et al., 2004), *Shh*<sup>+</sup> placode cells gave rise exclusively to differentiated taste cells, but not to taste papilla epithelium, functionally defining *Shh*<sup>+</sup> taste placodes as taste bud precursor cells (Thirumangalathu et al., 2009). Importantly, *Shh*<sup>+</sup> precursor cells develop normally in cultured embryonic



**Fig. 2. The sequence of taste bud and papilla development in mouse embryos.** (A) At E11.5, the tongue rudiment is covered by a homogeneous epithelial bilayer, with basal and superficial keratinocytes, which is immediately above the subepithelial mesenchyme. (B) At E12.5, taste placodes form within the epithelium as clusters of columnar cells. Sensory nerve fibers arrive at taste placodes, but do not penetrate the placode epithelium until E15.5. (C,D) Taste papilla morphogenesis begins at ~E15.5 (C), as the epithelium invaginates into the mesenchyme to form mushroom-shaped papillae with defined mesenchymal cores and immature taste buds situated apically by E18.5 (D). (E) Within a week of birth (here at P4), most FFP house differentiated taste buds. Mouse gestation is 18–19 days.

tongues, and in embryos lacking gustatory innervation immature taste buds are comparable in size and number to those of controls (Hall et al., 2003; Mistretta et al., 2003; Thirumangalathu et al., 2009), leading to the conclusion that, in mammals, the embryonic development of taste buds, as well as papilla morphogenesis, are nerve independent.

If embryonic taste bud development does not require neural signals, what cellular and molecular mechanisms are responsible? Currently, the focus is on processes intrinsic to the tongue. Interactions of epithelium with lingual mesenchyme are not required for taste bud differentiation in amphibians (Barlow and Northcutt, 1997), although they may be required for taste papilla morphogenesis in mouse embryos (Kim et al., 2003). However, molecular genetic manipulations in mice suggest that the specification and patterning of Shh<sup>+</sup> taste placodes are achieved solely by signaling within the epithelium. Wnt/β-catenin is the earliest signal that is both sufficient and required for taste placode formation (Iwatsuki et al., 2007; Liu et al., 2007). Importantly, Wnt/β-catenin activity is required explicitly in the lingual epithelium, as deletion of the canonical Wnt effector β-catenin within the lingual epithelium blocks taste placode formation (Liu et al., 2007). The lingual epithelium is also the source of Wnt ligand(s) responsible for taste placode induction (Zhu et al., 2014); when Wls (Gpr177), which is required for Wnt secretion (Hausmann et al., 2007), is deleted in the oral epithelium, the tongue forms normally but Shh<sup>+</sup> taste precursors and taste papillae are lost, despite continued Wnt production by the lingual mesenchyme.

Shh function in taste placode specification has also been tested, primarily in embryonic tongue explants. When Shh signaling is inhibited *in vitro*, the number and size of Shh<sup>+</sup> placodes increase (Hall et al., 2003; Mistretta et al., 2003). By contrast, excess Shh represses taste placodes (Iwatsuki et al., 2007). Thus, Shh is a negative regulator of taste placode fate. However, Shh is likely to refine rather than induce placode pattern, as Shh is genetically downstream of Wnt/β-catenin (Iwatsuki et al., 2007; Liu et al., 2007).

FGF is likewise implicated in taste placode patterning. The Sprouty genes *Spry1–4* encode negative intracellular regulators of receptor tyrosine kinases, including FGF receptors. Loss of *Spry2* causes duplication of CVP; when *Spry1* and 2 are deleted, three or more CVP form (Petersen et al., 2011). Epithelial *Spry2* primarily regulates the inductive role of mesenchymal *Fgf10*. In *Fgf10*

knockouts, the CVP does not form, while CVP mispatterning in *Spry2*<sup>-/-</sup> embryos is partially rescued in *Fgf10*<sup>+/+</sup> hemizygotes. Interestingly, *Spry2* function appears to be distinct for anterior versus posterior taste papillae, as the number of FFP is reduced, rather than expanded as is the case for the CVP, in *Spry2*<sup>-/-</sup> tongues (Petersen et al., 2011).

Several transcription factors have been implicated in embryonic taste bud development. Principal among these is *Sox2*, which is involved in the development and maintenance of many tissues (Arnold et al., 2011; Sarkar and Hochedlinger, 2013). In mice, *Sox2* is required for the differentiation of taste buds from taste papillae (Okubo et al., 2006). In *Sox2* hypomorphic embryos, taste placodes and papillae form normally but are lost before birth. However, *Sox2* overexpression is not sufficient to induce taste bud differentiation, suggesting that *Sox2* serves a permissive rather than instructive function. In fact, *Sox2* is likely to have broad roles in the lingual epithelium, as both taste placodes and the non-taste epithelium express *Sox2*, albeit at high and low levels, respectively (Nakayama et al., 2015; Okubo et al., 2006). Nonetheless, with respect to taste placodes, *Sox2* is positively regulated by Wnt/β-catenin and *Fgf10/Spry2* signaling (Okubo et al., 2006; Petersen et al., 2011). Whether *Sox2* is regulated by Shh during taste bud development is unknown, although *Sox2* appears to function downstream of Shh during adult taste cell renewal (see below) (Castillo et al., 2014).

Transcription factors associated with the Notch pathway also function in taste bud development, primarily with respect to taste cell fate. *Ascl1 (Mash1)*, a proneural gene, which is repressed in cells experiencing active Notch signaling, is required for Type III cell differentiation in mouse embryos and zebrafish larvae (Kapsimali et al., 2011; Kito-Shingaki et al., 2014; Seta et al., 2011). By contrast, *Hes1*, which is induced by active Notch signaling, represses Type II cell differentiation in mice and may function to maintain embryonic taste precursor cells in an undifferentiated state (Ota et al., 2009).

#### Taste bud cell turnover throughout adult life

Taste cells are continually renewed in adults, with an average taste cell lifespan of 10–14 days in rodents (Beidler and Smallman, 1965; Farbman, 1980). Aged taste cells, which are likely to undergo apoptosis (Ueda et al., 2008; Zeng and Oakley, 1999), are

constantly replaced by new cells that enter the taste buds and complete differentiation within 2–3 days of their final division (Cho et al., 1998; Hamamichi et al., 2006; Miura et al., 2006; Nguyen and Barlow, 2010; Perea-Martinez et al., 2013). If taste cells are homogeneous in terms of lifespan, one can estimate that 10% of cells are lost each day via natural attrition, ~20–30% of the cells are new and undergoing differentiation, and the remaining 60–70% are functional taste cells.

Recent studies have provided insights into the cells and factors involved in taste bud renewal (Fig. 3). Using inducible lineage tracing in adult mice, basal keratinocytes expressing cytokeratin 5 (K5, or Krt5) and K14 have been identified as the progenitor population that supplies new cells to taste buds (Gaillard et al., 2015; Okubo et al., 2009). As new cells enter the buds, they initially have an ovoid morphology and reside in the basal compartment. These basal cells are also termed Type IV cells and express *Shh*

commencing 12 h after their final mitosis (Miura et al., 2006, 2001). Inducible lineage tracing has revealed that *Shh*<sup>+</sup> basal cells are postmitotic precursors of all three taste cell types (Fig. 3A), differentiating most frequently as Type I, less commonly as Type II, and least frequently as Type III cells (Miura et al., 2014), reflecting the relative proportions of these cell types in taste buds (Ma et al., 2007; Ohtubo and Yoshii, 2010).

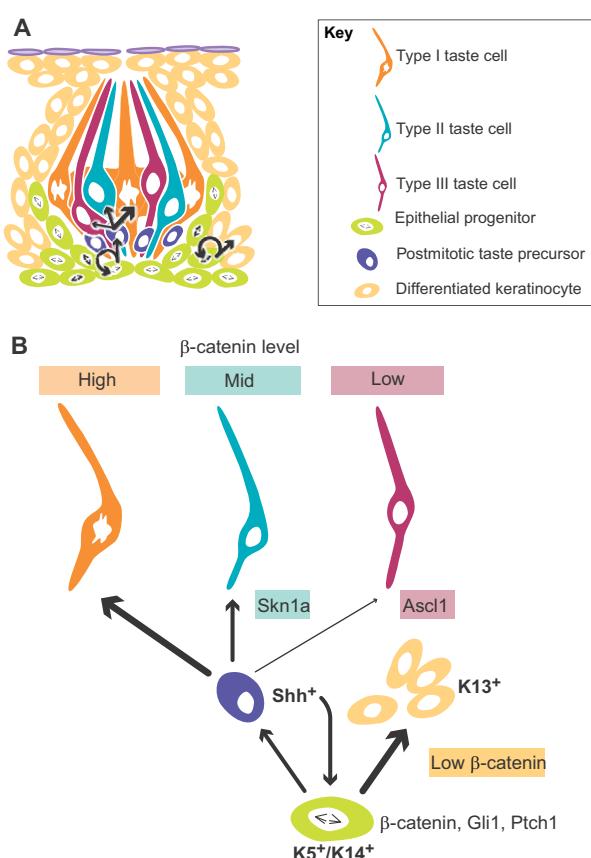
*K5/14*<sup>+</sup> basal keratinocytes also give rise to non-taste epithelium (Fig. 3B), which makes up the majority of the tongue surface and throughout which the taste papillae are distributed (Castillo et al., 2014; Gaillard et al., 2015; Okubo et al., 2009). In contrast to taste cells, non-taste epithelial cells turn over very rapidly – within 5 days in mice (Potten et al., 2002a,b,c). Furthermore, while taste cells live on average 10 days (see above), recent studies suggest that subsets of taste cells have significantly greater (24 days) and significantly shorter (3 days) longevity (Hamamichi et al., 2006; Perea-Martinez et al., 2013). Specifically, the least common Type III cells are the longest lived and the abundant Type I glial-like cells may represent the short-lived population (Perea-Martinez et al., 2013). Interestingly, this large variation in taste cell lifespan was described 50 years ago, when some cells were shown to live up to 3 weeks (Beidler and Smallman, 1965), although only the average lifespan is now cited in textbooks.

Thus, *K5/14*<sup>+</sup> basal keratinocytes give rise to both short-lived, non-taste epithelial cells, as well as a diversity of long-lived taste cell types and the specialized epithelia of taste papillae. In doing so, the progenitor population must accommodate differences in the cell fate, longevity and relative proportions of the different cell types needed and, finally, this renewal process must function seamlessly to provide consistent taste perception.

#### Not all lingual basal keratinocytes are the same

Although *K5/K14*<sup>+</sup> basal keratinocytes are general progenitors for all lingual epithelial cell types, it is clear that these cells comprise a heterogeneous population. The majority of *K5/14*<sup>+</sup> cells appear to be transit amplifying cells, which divide frequently and are rapidly replaced; this interpretation is consistent with the demonstrated high proliferative index of lingual basal keratinocytes (Nguyen et al., 2012; Potten et al., 2002c). A smaller subset are stem cells, and lineage tracing results in sparsely distributed and long-lived epithelial clones (Okubo et al., 2009). These clones can comprise solely filiform papillae/non-taste epithelium, filiform and taste papillae, or taste buds and taste papillae and non-taste epithelia (Okubo et al., 2009). However, initial attempts to generate taste buds from *K5*<sup>+</sup> keratinocytes *in vitro* were unsuccessful, producing only keratinized lingual epithelium (Luo et al., 2009).

Recently, another lingual epithelial stem cell population has been identified (Tanaka et al., 2013). These cells express the Polycomb group protein Bmi1 and represent a rare subset of *K5/K14*<sup>+</sup> keratinocytes. Under homeostasis, these cells behave like bona fide stem cells, i.e. they are long lived and slow cycling, and when challenged with tissue irradiation become mitotically active and regenerate injured epithelium. Importantly, however, in control or irradiated tongues Bmi1<sup>+</sup> cells contribute only to non-taste filiform papillae and not to taste buds (Tanaka et al., 2013). This fate restriction is also exhibited *in vitro*, as Bmi1<sup>+</sup> keratinocytes form lingual organoids that generate only keratinized epithelium (Hisha et al., 2013). Intriguingly, Bmi1<sup>+</sup> cells are an important stem population in intestinal crypts; one that is quiescent in homeostasis, but activated when the rapidly renewing stem population is genetically depleted (Tian et al., 2011). It might be that lingual Bmi1<sup>+</sup> keratinocytes also have the potential to generate taste bud



**Fig. 3. Taste cell renewal in adult mice.** (A) The cell types found in adult taste buds. Basal keratinocytes (green) divide asymmetrically to self-renew (curved arrows) and generate daughter cells (straight arrows), which become non-taste cells (differentiated keratinocytes) or contribute to taste buds. Newly generated taste cells are oval shaped and express *Shh* (purple). *Shh*<sup>+</sup> cells are postmitotic and differentiate into each of the three taste cell types (tripartite arrows). Fifty percent of *Shh*<sup>+</sup> cells become Type I cells (thick arrow), while 20–30% differentiate as Type II (intermediate arrow) and <10% as Type III (thin arrow) (Miura et al., 2014). (B) Model of molecular regulation of taste cell renewal (based on Castillo et al., 2014; Gaillard et al., 2015). *K5*<sup>+</sup>/*K14*<sup>+</sup> taste progenitor cells require  $\beta$ -catenin, and different levels of  $\beta$ -catenin within progenitors dictate daughter cell fate. Low  $\beta$ -catenin promotes *K13*<sup>+</sup> keratinocytes, whereas higher levels allow daughter cells to acquire a taste fate, i.e. to become *Shh*<sup>+</sup> taste cell precursors, which in turn differentiate into Type I, II or III cells in response to high, mid or low  $\beta$ -catenin, respectively. *Shh* signaling to *K5*<sup>+</sup>/*K14*<sup>+</sup> progenitors promotes differentiation of all taste cell types.

cells, but the precise type of challenge and/or time frame needed to trigger this response is yet to be discovered.

However, a taste bud stem cell population has now been identified in mice. Using a GFP reporter allele of *Lgr5* (a Wnt target gene that marks distinct stem populations in the intestine and skin; Barker et al., 2007; Jaks et al., 2008; Snippert et al., 2010), Jiang and colleagues found that LGR5 is expressed by epithelial cells at the base of CVP trenches (see Fig. 1). Using lineage tracing, LGR5-GFP cells were shown to replace themselves and produce all three taste cell types and non-taste epithelial cells within the CVP (Yee et al., 2013). Maintenance of CVP taste buds requires intact innervation; if the IXth nerve is crushed, taste buds are lost, but reappear if nerve contact is re-established (Finger and Simon, 2000). Using this experimental approach combined with LGR5 lineage tracing, following re-innervation the re-emerging taste cells were shown to arise from LGR5<sup>+</sup> stem cells (Takeda et al., 2013). LGR5 thus marks an active stem cell population for taste buds in the CVP. However, LGR5 is not expressed in the anterior FFP (Takeda et al., 2013; Yee et al., 2013); instead, LGR6<sup>+</sup> stem cells supply cells to FFP taste buds (Ren et al., 2014).

These findings *in vivo* have now been leveraged to create lingual organoids *in vitro*, which comprise differentiated taste bud cells within a larger non-taste epithelial population (Ren et al., 2014). Lingual organoids have been generated from isolated LGR5-GFP<sup>+</sup> and LGR6-GFP<sup>+</sup> lingual keratinocytes generally following methods developed for intestinal crypts (Ren et al., 2014; Sato et al., 2009; Schuijers and Clevers, 2012). Importantly, these organoids produce all three taste cell types, and these taste cells are functional; like Type II cells isolated from mice, subsets of Type II cells from organoids respond specifically to sweet or bitter compounds with increased intracellular calcium (Ren et al., 2014). By establishing taste cell renewal from stem cells *in vitro*, Jiang and colleagues have paved the way to rapidly and efficiently exploring the molecular and cellular mechanisms that control the generation of mature taste cells.

### The molecular regulation of taste bud cell renewal

As discussed above, molecular studies of embryonic taste bud development have focused on the Shh and canonical Wnt pathways. Emerging data now support a role for both of these pathways, as well as intrinsic factors, in taste cell renewal and differentiation in adults.

### Hedgehog signaling

*Shh* is expressed by Type IV basal cells within adult taste buds, whereas expression of the Shh target genes *Gli1* and *Ptch1* is restricted to taste progenitors outside of buds (Liu et al., 2013; Miura et al., 2001), prompting the hypothesis that Shh<sup>+</sup> cells inside taste buds signal to progenitors to regulate taste cell renewal (Miura et al., 2006). In other renewing epithelia, Hedgehog (Hh) functions as a mitogen or a differentiation factor (Lee and Tumbar, 2012; Takashima and Hartenstein, 2012) or can signal to maintain stem cells (Álvarez-Buylla and Ihrie, 2014; Ferent et al., 2014; Saade et al., 2013). To examine the role of Shh in taste cell renewal, a recent study used inducible genetic models in adult mice to express Shh in K14<sup>+</sup> lingual progenitors (Castillo et al., 2014). Unexpectedly, excess Shh caused the formation of excess and ectopic taste buds interspersed among filiform papillae of the anterior tongue; 1 month after induction, as many as 100 ectopic buds formed in the tip of the tongue, which is far more than the number of endogenous taste buds found in this region (Reiner et al., 2008). These buds contained all three differentiated taste cell types, yet were not housed in FFP, nor were they innervated (Castillo et al.,

2014). These data suggest that Shh is the key signal that directly promotes the entire program of taste bud cell differentiation, at least in non-taste lingual epithelium, and does not act as a mitogen or stem cell maintenance factor. This pro-differentiation function in adults (Fig. 3B) is in direct contrast to the repressive role of Shh in taste placode development (as discussed above). Whether Shh is required for the differentiation of endogenous taste buds residing in FFP, however, remains to be tested directly.

Hints that Shh may function in normal taste cell renewal come from complaints of taste dysfunction by cancer patients treated with chemotherapies targeting the Hh pathway. Because activating mutations in the pathway are implicated in basal cell carcinoma (BCC), many drugs have been developed to inhibit the Hh signal transducer Smoothened (Smo) (Atwood et al., 2014). These include Vismodegib (LoRusso et al., 2011) and LDE225 (Rodon et al., 2014). Patients on these drugs suffer numerous side effects, and among the most disruptive to patient quality of life is loss or distortion of their sense of taste (ageusia and dysgeusia, respectively) (LoRusso et al., 2011; Rodon et al., 2014). Thus, although these drugs repress the Shh pathway-driven proliferation of tumor cells, we conjecture that, in the context of taste buds, cell differentiation is inhibited. Recent reports of taste bud loss in mouse models are consistent with this interpretation (Kumari et al., 2015a; Yang et al., 2015). Mice treated with either drug have reduced numbers of taste buds within a few weeks, with LDE225 treatment causing a significant reduction. Following treatment with LDE225, innervation remains intact; nerve fibers still respond to mechanical stimulation of the tongue, but taste responses are lost (Kumari et al., 2015a). However, this effect appears reversible. Within a few weeks of drug cessation, taste buds are again evident and nerve responses to tastants applied to the tongue recover (Kumari et al., 2015b). These results are consistent with anecdotal reports from patients who take ‘drug holidays’ from their maintenance Smo-targeted chemotherapy to regain their sense of taste. Together, these data support a model in which Shh is required for taste bud differentiation, yet, importantly, Shh inhibition is unlikely to negatively affect taste progenitor cell survival.

### Wnt/β-catenin signaling

Wnt/β-catenin is both required and sufficient for the induction of embryonic taste placodes (Liu et al., 2007). Additionally, the canonical Wnt pathway is active in adult taste buds; specifically, BATGAL reporter mice (in which β-galactosidase is expressed in the presence of nuclear β-catenin) show β-galactosidase in progenitor cells outside of buds, in Shh<sup>+</sup> taste bud precursor cells, and in subsets of each of the three taste cell types, suggesting that the pathway plays a role in each step of the taste cell lineage (Gaillard and Barlow, 2011). To assess β-catenin function, a recent study conditionally induced the overexpression (OE) of β-catenin in K5<sup>+</sup> basal keratinocytes in adult mice (Gaillard et al., 2015). In both the anterior FFP and posterior CVP, β-catenin OE forced K5<sup>+</sup> cells to differentiate and acquire a taste fate at the expense of K13<sup>+</sup> non-taste epithelium (see Fig. 3). Moreover, these induced taste cells were primarily Type I, with lesser induction of Type II cells; the induction of Type III cells was not observed. To determine whether the role of β-catenin in cell fate selection functions in K5<sup>+</sup> progenitors outside of buds or in Shh<sup>+</sup> precursors once these new cells are in taste buds, β-catenin OE was induced for 8 days in successively generated Shh<sup>+</sup> precursor cells and lineage tracing was used to follow the fate of these precursors. As was the case for β-catenin OE in K5<sup>+</sup> progenitors, excess Type I taste cells were observed in taste buds carrying Shh<sup>+</sup>-descendant cells with

$\beta$ -catenin OE. However,  $\beta$ -catenin OE in Shh<sup>+</sup> precursor cells did not alter fate cell-autonomously, as these cells became Type II or III cells in the proper ratios; rather, the impact of  $\beta$ -catenin OE in Shh<sup>+</sup> cells was to indirectly drive other, later born cells to acquire a Type I cell fate. We posit that this might occur as  $\beta$ -catenin-OE Shh<sup>+</sup> cells within buds signal, by an as yet unidentified pathway, to progenitors outside of buds to influence the fate of incoming daughter cells. Thus,  $\beta$ -catenin functions at the level of lingual progenitors to regulate proliferation and bias cell fate. Our working model is that  $\beta$ -catenin levels, as well as the transcriptional co-factor(s) expressed within each cell population, will dictate proliferative output and taste bud cell fate (Fig. 3B).

#### Intrinsic factors that regulate taste cell fate

Once new cells enter taste buds they undergo cell type-specific differentiation, and recent studies have identified intrinsic factors involved in this process (Fig. 3B). For example, the POU domain transcription factor Skn1a is expressed by, and required for, the generation of Type II cells (Matsumoto et al., 2011). Skn1a may function as a cell fate switch, as its global loss results in taste buds that lack Type II cells but have excess Type III cells. As mentioned above, *Ascl1* is required for the development of Type III cells in embryos, but it is not known if it is required for adult Type III cell differentiation. This is likely, however, because *Ascl1* is expressed by a subset of Shh<sup>+</sup> precursor cells (Miura et al., 2006, 2003) and by differentiated Type III cells (Seta et al., 2006).

Overall, however, we have little understanding of the cellular and molecular interactions governing emergence of the different taste cell types. Shh<sup>+</sup> basal cells give rise directly to Type I, II and III cells in proportion to their standing ratios in buds (Miura et al., 2014), but it is not known whether the Shh<sup>+</sup> precursor cell is an obligate step in the differentiation of all taste cells, and if Shh<sup>+</sup> cells fated to become Type II or III cells transiently express Skn1a or *Ascl1*, respectively. In support of this model, *Shh* is expressed by basal cells within 12 h of their final mitosis and then declines, while basal cell expression of *Ascl1* begins at 24 h and persists in Type III cells (Miura et al., 2006). Thus, *Shh* expression may become extinguished in *Ascl1*<sup>+</sup> cells as they become Type III cells (Miura et al., 2006), although it should be noted that some *Ascl1*<sup>+</sup> basal cells may give rise to Type II cells (Miura et al., 2005, 2006). A similar scenario might occur for Shh and Skn1a expression during Type II cell differentiation; Skn1a is expressed by Type II cells and a subset of basal cells (Matsumoto et al., 2011), but it is unknown if Shh<sup>+</sup> cells are Skn1a<sup>+</sup>, or if Skn1a and *Ascl1* are co-expressed in Shh<sup>+</sup> basal cells. Finally, despite the fact that Type I cells comprise ~50% of taste cells (Bartel et al., 2006), there is no information on how Type I cell differentiation is regulated, other than that high  $\beta$ -catenin is sufficient to drive Type I fate (Gaillard et al., 2015). If Shh<sup>+</sup> precursor cells comprise a homogenous population that represents obligate intermediates for all cell types, then intrinsic transcription factors must be differentially regulated within single cells to dictate fate. Alternatively, if Shh marks a heterogeneous precursor population, within which fate-determining transcription factors are already differentially expressed, then extrinsic factors acting early on K5<sup>+</sup> and/or Shh<sup>+</sup> cells will control cell fate. Recent findings support the latter hypothesis; genetic manipulation of  $\beta$ -catenin alters the fate of K5/14<sup>+</sup> progenitors, whereas Shh<sup>+</sup> precursor cell fate is unaffected following cell autonomous manipulation of  $\beta$ -catenin (Gaillard et al., 2015).

#### Future perspectives

In the past decade, we have made significant progress in understanding the cellular and molecular underpinnings of taste bud

development and renewal. These advances have arisen in large part because of the explosion in molecular genetic tools available in mice.

#### The regulation of taste bud development

Interactions among the Shh and canonical Wnt/ $\beta$ -catenin pathways have been most extensively explored during taste bud development, although genes belonging to other key signaling systems are expressed in the developing tongue (reviewed by Kapsimali and Barlow, 2013; Krimm et al., 2015) and thus might play a role. In mouse embryos, Notch pathway genes are expressed in taste placodes, but not until papilla morphogenesis, suggesting a role in cell fate determination and/or differentiation (Seta et al., 2003). In fact, Notch signaling regulates taste cell fate decisions in larval zebrafish (Kapsimali et al., 2011), but to date Notch pathway function(s) in rodent taste bud development remain unexplored.

Additionally, it has become evident that the development of anterior FFP and posterior CVP taste buds may be mediated in part by distinct mechanisms (as discussed above with respect to FGF/Spry signaling). This might be because the CVP arises from endoderm, whereas FFP taste buds are presumed to originate from ectoderm (Rothova et al., 2012). Thus, the tissues giving rise to FFP and CVP buds have undergone significantly different developmental trajectories. The most anterior endoderm lining the posterior oral cavity and pharynx originates in the embryonic organizer at the start of gastrulation (the node in mice; see Solnica-Krezel and Sepich, 2012), which is situated at the presumptive posterior end of the embryo and then undergoes gastrulation movements to arrive at the embryo's anterior (Barlow and Northcutt, 1995; Solnica-Krezel and Sepich, 2012). By contrast, the future oral ectoderm is superficial until the end of gastrulation, and then invaginates to make contact with the oral endoderm to fuse and ultimately generate an oral opening (Barlow, 2000; Dickinson and Sive, 2006). The oral endoderm and ectoderm are thus exposed to very different signals prior to the formation of a contiguous gut tube. Nonetheless, as a consequence, taste buds, which are considered homologous organs, form in both endodermal and ectodermal epithelia. This is particularly interesting as several groups of bony fish possess superficial taste buds, in addition to taste buds within the oral cavity, including zebrafish, which have taste buds distributed on the head (Hansen et al., 2002), and catfish, which are covered with taste buds over their entire surface from head to tail (Atema, 1971; Caprio et al., 1993; Landacre, 1907). These appear to have evolved independently (Northcutt, 2004), so it is unclear if the mechanisms governing the development of these external taste buds are similar to those governing the development of oral taste buds, the latter being common to all vertebrates including zebrafish and catfish.

While there is a long history of the taste map of the tongue, the notion that discrete regions of the tongue are uniquely sensitive to specific tastes has been thoroughly discredited (Chaudhari and Roper, 2010). Rather, taste buds in different regions of the tongue are able to detect all taste qualities, but are relatively more or less sensitive depending on location. For example, in rodents, CVP taste buds are more tuned to bitter, whereas FFP buds are more sensitive to sweet (Spector et al., 1996). Although this could be due to differences in the germ layer of origin, it is more likely that these differences represent functional adaptations reflecting dietary choices of species over evolutionary time.

#### Taste cell renewal

We now have a clearer image of the steps involved in taste cell renewal. Specific cell types have been identified including long-

lived populations that generate taste cells and Shh<sup>+</sup> basal cells that are immediate postmitotic precursors of all three taste cell types. In addition, specific transcription factors have been shown to regulate the differentiation of Type II and III taste cells. With these discoveries in hand, new questions can now be addressed. Key among these is to determine how extrinsic and intrinsic factors coordinate to dictate taste cell fate. Wnt/β-catenin and Shh, both extrinsic signals, regulate cell fate decisions of K5/K14<sup>+</sup> progenitors (Castillo et al., 2014; Gaillard et al., 2015). Are there taste bud-intrinsic signals that likewise influence the differentiation of newly generated Shh<sup>+</sup> basal cells? Notch receptors and their ligands are expressed by subsets of cells in and around taste buds (Seta et al., 2003); might these factors mediate intra-bud signaling to refine cell fate? For example, Type II taste cells are morphologically similar but comprise functional subsets for detecting sweet, bitter or umami tastes (Liman et al., 2014). Thus, one possibility is that Type II fate is selected broadly in new cells generated from progenitors prior to entering buds, and these in turn are induced by local Notch signaling within buds to acquire specific functional fates.

#### The relationship between taste bud development and homeostatic renewal

To date, pathways known to regulate taste bud development also control adult taste cell turnover. For example, Wnt/β-catenin promotes both taste bud development and taste cell renewal (Gaillard et al., 2015; Liu et al., 2007). The roles of Shh in taste embryogenesis and adult homeostasis, however, appear to be at odds. In embryos, Shh represses taste fate (Hall et al., 2003; Iwatsuki et al., 2007), whereas in adults it promotes taste bud differentiation (Castillo et al., 2014). This is not surprising, as the Wnt and Hh pathways have temporally and spatially distinct interactions in many tissues, including hair follicles and neural epithelium (Avilés et al., 2013; Lee and Tumbar, 2012). Interestingly, during embryogenesis BMP signaling promotes taste fate during placode specification and then represses taste fate once taste placodes have formed (Zhou et al., 2006). Bmp4 is also expressed in and around adult taste buds

(Nguyen and Barlow, 2010), but it is not known if or how BMP signaling affects taste cell turnover, nor how this pathway interacts with Hh and Wnt/β-catenin.

#### New hypotheses of taste dysfunction

While many drugs cause dysgeusia or ageusia, or actually taste bitter, cancer therapies are notorious for triggering taste loss or alteration. This is entirely predictable, given that most cancer drugs broadly target proliferating cells. In fact, the widely prescribed chemotherapeutic cyclophosphamide, which blocks DNA synthesis, also perturbs taste bud homeostasis and taste function in mice (Mukherjee et al., 2013; Mukherjee and Delay, 2011).

Patients suffering from head and neck cancer usually receive daily radiation over many weeks to reduce tumors, but this treatment invariably has off-target effects including taste loss and permanent taste dysfunction (Box 1). In mice, a single dose of radiation causes a transient interruption in the supply of new taste cells, resulting in a transient reduction in differentiated taste cells a few days later. Thus, these data provide a model for the response and recovery of the taste epithelium to a single radiation injury and, by extrapolation, for how taste function might be interrupted (Nguyen et al., 2012). However, these findings do not address how taste function is permanently altered by repeated head and neck irradiation in patients. Several hypotheses can be considered. First, taste bud progenitors might be permanently reduced in number, resulting in significantly smaller taste buds that are still innervated by a normal or reduced complement of sensory nerves, such that the conveyance of taste information is mismatched with CNS centers that developed and matured when taste buds were normal. In an alternative hypothesis, taste bud stem cells are still impacted but, in this model, stem cells are heterogeneous, and thus when their numbers ultimately recover from a dramatically reduced pool they now represent a subset of the

#### Box 1. Radiotherapy and taste dysfunction

Head and neck cancers comprise ~3% of all cancers diagnosed annually in the USA, with most tumors occurring in the oral cavity. Targeted radiotherapy is standard for these patients, who are treated with 1–2 Gy of radiation daily for 5 weeks, and who almost always suffer a loss or altered sense of taste (ageusia and dysgeusia, respectively). Taste dysfunction reduces appetite and leads to weight loss, diminished quality of life and poorer outcomes (de Castro and Federico, 2006; Ru Redda and Allis, 2006). In rodents, taste function and buds are lost following radiation (Conger and Wells, 1969; Mossman et al., 1979; Yamazaki et al., 2009). Recent work has shown that, in mice, the impact of radiation is primarily on taste progenitor cells (Nguyen et al., 2012). Two days after irradiation, the proliferative index of taste progenitors is reduced by 80% and the flow of new cells into buds is halved. Differentiated taste cells, by contrast, are not reduced until 7 days post-irradiation, as aged taste cells are lost normally but not replaced because of the transient interruption in new cell production. Within a week, taste bud renewal recovers, regenerating the regular complement of taste cells within 2 weeks. Although taste function is regained for most head and neck cancer patients, many find their sense of taste permanently altered (Maes et al., 2002; Mossman and Henkin, 1978); certain foods do not match expectations of what the item should taste like (imagine eating a ripe strawberry and it tasting as sour as lemon). In this case, we have no understanding of what type(s) of long-lasting changes might have occurred in taste cell renewal.

#### Box 2. Wnt/β-catenin, cancer and gustation

Elevated Wnt/β-catenin is implicated in many cancers (Kahn, 2014; Takebe et al., 2015) and chemotherapeutic approaches to target elements of the pathway are thus being pursued. For example, small molecules have been developed to target porcupine, which is required for the secretion of Wnt ligands (Barrott et al., 2011; Biechele et al., 2011). However, as Wnt/β-catenin is also a crucial regulator of taste cell renewal (Gaillard et al., 2015), these new agents may cause significant taste dysfunction.

Another promising chemotherapeutic strategy involves targeting the transcription of discrete subsets of β-catenin target genes using small molecules that interfere with β-catenin and specific transcriptional co-activators, thus inhibiting only one arm of the canonical pathway implicated in a particular cancer and potentially having fewer off-target effects (reviewed by Kahn, 2014). However, since β-catenin has progressive functions in taste cell renewal, and these likewise may be mediated by distinct transcriptional targets, these drugs might have distinct effects on taste cell turnover and lineage, nonetheless distorting taste function.

Finally, because β-catenin promotes taste cell turnover, it might be possible to develop a locally restricted approach to transiently amplify β-catenin signaling in patients with dysgeusia caused by chemotherapies or head and neck irradiation. In addition to causing taste dysfunction (see Box 1), radiation results in oral blistering. Wang and colleagues were able to mitigate blistering in mice by locally activating TGFβ signaling via ectopic expression of Smad7 (Bian et al., 2015; Han et al., 2013). Importantly, this method involved the topical application of a cell-permeant form of Smad7 to the oral epithelium that, when given pre-irradiation, partially prevented radiation-induced mucositis and, when given post-irradiation, improved recovery (Han et al., 2013).

original heterogeneous population and are biased in the types of taste cells produced, resulting in taste buds with distorted cell type ratios. Finally, it is possible that anterior and posterior taste buds might be affected differently and recover differently, again resulting in a mismatch with CNS centers. These hypotheses are not intended to be exhaustive, but rather present testable ideas going forward.

As cancer therapies advance, there are now concerted efforts to target discrete pathways to lessen unwanted effects on healthy tissues. Wnt/β-catenin is overactive in a variety of cancers but is required for many homeostatic functions, so efforts are underway to target specific subsets of effectors of the β-catenin pathway (Box 2). These more specific drugs might also provide insight into Wnt/β-catenin functions in taste cell renewal, allowing us to tease apart the roles of specific downstream effectors in explicit aspects of taste progenitor activity and taste cell differentiation. Given the recent advances in the field and the advent of lingual organoids, these questions can now be addressed more rapidly *in vitro* in concert with informed follow-up studies *in vivo*. I hope this gives the reader a taste of things to come...

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#### Competing interests

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