Histol Histopathol (2019) 34: 503-511

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Abundant proliferating cells within early chicken taste buds indicate a potentially "built-in" progenitor system for taste bud growth during maturation in hatchlings

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Summary. Like other epithelial cells, taste bud cells have a short life span and undergo continuous turnover. An active stem or progenitor cell niche is essential for taste bud formation and maintenance. Early taste bud cells have a life span of ~4 days on average in chicken hatchlings when taste buds grow rapidly and undergo maturation. The average life span is shorter than that of mature taste bud cells of rodents (~10-12 days on average). To better understand the mechanism underlying taste bud growth and homeostasis in chickens, we analyzed the distribution of proliferating cells in different tissue compartments, including taste buds, the surrounding epithelium and the underlying connective tissue in post-hatch (P)1-3 hatchlings and P45 chickens. Unlike rodents, which lack proliferating cells within both early and mature taste buds, chickens possessed abundant proliferating cells within early taste buds. Further, at P45, when taste buds are mature and undergo continuous cell renewal, taste buds also contained proliferating cells, though to a lesser extent. These proliferating cells in early taste buds, indicated by $PCNA^+$ and $BrdU^+$ cells, primarily localized to the basal region of taste buds and were largely unlabeled by the two known molecular markers for chicken taste bud cells (Vimentin and α-Gustducin), suggesting their undifferentiated status. Our data indicate that early chicken taste buds have "built-in" progenitors in order to grow to and maintain their large size and rapid cell turnover in hatchlings.

Key words: Chicken, Taste buds, Development, Proliferation, Progenitor

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DOI: 10-14670/HH-18-055

Introduction

Chicken taste buds emerge at embryonic day 17 (E17), and mature taste buds begin to appear at embryonic day 19 (E19) (Ganchrow and Ganchrow, 1987), enabling response to taste stimuli immediately after hatching (Ganchrow et al., 1990). After hatching, chicken taste buds continue to grow rapidly and undergo further maturation (Ganchrow et al., 1994). Cell recruitment and turnover are essential for taste bud growth and homeostasis during maturation and maintenance. It has been reported that early chicken taste bud cells in hatchlings have a much shorter life span (~4 days on average) than those mature ones of rodents (~10-12 days on average) (Beidler and Smallman, 1965; Ganchrow et al., 1994; Perea-Martinez et al., 2013). Therefore, a more efficient mechanism is required for the development and potentially also maintenance of taste buds in chickens. However, we lack a clear understanding of the distribution of progenitors for chicken taste bud development and maintenance.

Two molecular markers, α-Gustducin and Vimentin, have been used to label chicken taste bud cells (Witt and Kasper, 1999; Witt et al., 2000; Kudo et al., 2010; Venkatesan et al., 2016). Our findings from double labeling with these two markers demonstrated a distinct

but largely overlapping cell population within chicken taste buds (Venkatesan et al., 2016). However, there is a small cell population that is neither labeled by α-Gustducin nor Vimentin. Thus, questions arise: do the α-Gustducin$^+$, Vimentin$^+$, α-Gustducin$^+$ Vimentin$^+$, and α-Gustducin$^-$Vimentin$^-$ taste bud cells represent different taste cell types, or taste cells at different phases of development? What do α-Gustducin-Vimentin$^-$ taste bud cells represent?

In the current study we analyzed the distribution of proliferating cells (PCNA for pan-proliferating cells, BrdU$^+$ for S-phase) and its association with cell markers for different tissue compartments: EpCAM for epithelium, Vimentin for both taste bud and connective tissue cells, and α-Gustducin specifically for taste bud cells. We found that in contrast to the absence of proliferating cells in early mouse taste buds, abundant proliferating cells were observed within early taste buds in chicken hatchlings. Within early chicken taste buds, proliferating cells were primarily in the basal region and largely negative for both α-Gustducin and Vimentin. And proliferating cells were detected, although more infrequently, in post-hatch day 45 chickens, when taste buds are mature and undergo cell renewal. These results suggest that proliferating cells (α-Gustducin$^-$Vimentin$^-$) within early chicken taste buds represent undifferentiated progenitors and that early chicken taste buds are likely dependent on the "built-in" proliferating progenitors to grow to and maintain their large size and rapid cell turnover.

Materials and methods

Animals

Animal use was approved by The University of Georgia Institutional Animal Care and Use Committee and was in compliance with the National Institutes of Health Guidelines for care and use of animals in research.

Newly hatched (P1) female chickens were kind gifts from the Cobb Vantress, Inc, Cleveland Hatchery, Georgia. Chickens were housed and maintained until post-hatching day 5 (P5) in the animal facility with the brood temperature maintained at 35°C and room temperature at 30°C. Chickens were provided with starter feed and water *ad libitum* under a 12-12 hr dark-light cycle. 45-day-old chickens were maintained in the Department of Poultry Science at the University of Georgia.

C57BL/6 wild type mice purchased from Jackson Laboratory (Stock #000664) were bred and maintained in the Animal Facility in the Department of Animal and Dairy Science at the University of Georgia. Newborn mice were harvested on the day of birth (P1).

5´-Bromo-2´-Deoxyuridine (BrdU) administration and tissue processing

BrdU (B5002, Sigma, St. Louis, MO) was prepared in Dulbecco's Phosphate-Buffered Saline (DPBS) at 10 mg/mL and injected intraperitoneally at a single dose of 100 mg/kg. Chickens and mice were harvested 2 hours post-injection.

P1-P5 chickens and newborn mice were decapitated, and P45 chickens were cervically dislocated. Tissues from the palate and the base of the oral cavity containing abundant taste buds (Ganchrow and Ganchrow, 1985) were dissected from P1, P3, P5 and P45 chickens. Tissues from the palate were separated into three pieces: anterior-most maxillary gland opening region, middle palatine papillae region, and posterior region. The whole mouse tongues were dissected from P1 mice.

Collected tissues were embedded in Optimal Cutting Temperature (O.C.T) compound and rapidly frozen. Tissue of the chicken maxillary gland opening region and palatine papillae region of the palate, the base of oral cavity and mouse tongues were sectioned sagittally; the posterior region of the palate was sectioned coronally. All the tissues were sectioned at 5 μm thickness using LEICA cryostat CM1950 and mounted onto charged glass slides.

Immunohistochemistry

The following primary antibodies were used: BrdU (1:400, MCA2060, Hercules, CA), Epithelial Cell Adhesion Molecule markers (EpCAM) (1:200, MBS2027145, Mybioresource Inc, San Diego, CA), α-Gustducin (1:500, serum of rabbit immunized with chicken α-Gustducin, generated by Dr. Shoji Tabata's lab), Keratin 8 (K8) (1:1000, TROMA-I, Developmental Studies Hybridoma Bank, IA), Ki67 (1:200, Abcam 15580, Cambridge, MA), Proliferating Cell Nuclear Antigen (PCNA) (1:500, ab29, Abcam, Cambridge, MA), Vimentin (1:250, Vim3B4, Abcam 28028, Cambridge, MA).

Slides were air-dried for 1 hr at room temperature, then sections were fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer solution (PBS) for 5 minutes, followed by 100% methanol for 15 minutes. After rehydration and rinsing in 0.1 M PBS, nonspecific binding was blocked using 10% normal donkey serum (NDS) and incubated with primary antibodies in a carrier solution of 1% NDS in PBS-X (PBS with 0.3% Triton-X) overnight at 4°C. Slides were rinsed in 0.1 M PBS three times, then incubated in secondary antibodies, i.e., Alexa Fluor 647 conjugated donkey anti-rabbit secondary antibody (1:500, 711-605-152; Jackson Immuno Research Laboratories, West Grove, PA), Alexa Fluor 488 conjugated donkey anti-rat (1:500, 715-545-150, Jackson Immuno Research Laboratories, West Grove, PA), and Alexa Fluor 546 conjugated donkey anti-mouse (1:500, A10036, Life Technologies, Inc., Carlsbad, CA), in carrier solution for 1 hr at room temperature. After rinsing in PBS, cell nuclei were counter-stained with DAPI (200 ng/ml in PBS) for 10 min at room temperature. Slides were then thoroughly rinsed, air-dried and cover-slipped using Prolong® Diamond Antifade mounting medium (P36970, Life Technologies, Inc., Carlsbad, CA).

Photomicroscopy and quantification of proliferating cells within taste buds

All sections were thoroughly examined under a light microscope (EVOS FL, Life Technologies). Representative images of immunosignals were taken using a laser scanning confocal microscope (Zeiss LSM 710). Images were assembled and edited using Photoshop CC 2015.

To quantify the number of proliferating cells within taste buds, 80 taste buds from the base of the oral cavity were analyzed. One representative image containing the largest bud profile in serial sections from each taste bud was used for quantification of labeled cell profiles and co-localization of immunosignals. Taste buds were outlined referring to the α-Gustducin immunosignals and the border of taste buds were set as where α-Gustducin immunosignals reached (see Fig. 3A). $PCNA^+$, $BrdU^+$, α-$Gustducin^+$, and $Vimentin^+$ cells were designated when labeled cells contain an obvious DAPI nucleus. Then criteria for double labeling of BrdU/PCNA with Vimentin/ α-Gustducin is that a $BrdU^+$ or $PCNA^+$ nucleus is immediately and clearly surrounded by Vimentin or α-Gustducin signals. All quantification was carried out manually by the same investigator for consistency among groups using single-plane confocal photomicrographs.

Data analysis

Quantitative analysis was performed for the number of taste buds in the base of the oral cavity, taste bud cell profiles per taste bud profile, and $BrdU^+$ taste bud cell profiles with and without Vimentin and/or α-Gustducin labeling at P3 (n=3). The quantification data is represented as means ± standard deviation (X±SD; n=3).

The percentage of $BrdU^+$ taste bud cell profiles was calculated as the number of $BrdU^+$ cell profiles divided by the number of total cell profiles in a taste bud profile. The percentages of $BrdU^+$ taste bud cell profiles without labeling of taste bud cell markers was calculated as the $BrdU^+$-only taste bud cell profiles divided by the total $BrdU^+$ taste bud cell profiles. The percentages of BrdU+ taste bud cells with labeling of molecular markers for taste bud cells were taken as the taste bud cell profiles co-labeled with α-Gustducin and/or Vimentin immunosignals divided by total $BrdU^+$ taste bud cell profiles.

Results

Distinct distribution of proliferating cells within early taste buds in chickens versus mice

To understand the distribution of potential proliferating progenitor cells for early taste bud development and maintenance in chickens, we performed double immunofluorescence labeling. Slides were labeled with proliferating cell marker (PCNA) and either EpCAM, a specific marker for epithelial cells (Fig. 1A), or Vimentin, a taste bud and stromal cell marker of connective tissue (Fig. 1B), or α-Gustducin, a specific taste bud cell marker (Fig. 1C). Results were consistent among the examined stages (P1, P3 and P5) of chicken hatchlings. Therefore, representative images from chicken tissues at one (P3) of the examined stages are shown.

In the epithelium labeled with EpCAM (red, Fig. 1A), abundant $PCNA^+$ cells (green, Fig. 1A) were consistently seen along the chicken oral epithelium that contain early taste buds. Notably, $PCNA^+$ cells were distributed in brightly labeled $EpCAM^+$ epithelial structures that have been demonstrated to be taste buds (Venkatesan et al., 2016). Variance in the intensity of PCNA immunosignals was observed among labeled cells – bright $PCNA^+$ cells were primarily distributed in the basal epithelial layer, whereas weaker signals were found in the suprabasal layer.

$PCNA^+$ cells were also seen in the tissue beneath the $EpCAM^+$ epithelium. To identify the $PCNA^+$ cell type in the tissue underlying the epithelium, a stromal cell marker of connective tissue, Vimentin, was used (red, Fig. 1B). Consistent with previous reports (Witt et al., 2000; Venkatesan et al., 2016), taste buds in the epithelium and underlying connective tissue were labeled with Vimentin (Fig. 1B). Again, $PCNA^+$ cells were also seen within the taste buds labeled with Vimentin (solid arrowheads, Fig. 1B). In the tissue immediately underlying the epithelium, most, if not all, of $PCNA^+$ proliferating cells showed Vimentin immunosignals.

To confirm the distribution of proliferating cells in early chicken taste buds, double immunofluorescence labeling was performed using PCNA and specific taste bud cell marker α-Gustducin (Fig. 1C). As shown by α-Gustducin immunosignals, $PCNA^+$ proliferating cells were abundantly distributed within the taste bud region. $PCNA^+$ proliferating cells, both labeled (solid arrowheads, Fig. 1C) and unlabeled (open arrowheads, Fig. 1C) with α-Gustducin, were observed. Similar to the overall distribution of bright $PCNA^+$ cells in the oral epithelium, bright $PCNA^+$ cells were largely distributed within the basal layer of taste buds. Additionally, $PCNA^+$ proliferating cells were consistently distributed in the basal layer of epithelium outside of taste buds (long arrows, Fig. 1A).

To understand whether the abundance of proliferating cells in early taste buds is species-specific, an analysis was made in post-natal day 1 (P1) mice in parallel. Immunohistochemical labeling with Ki67, a pan-proliferation marker, showed that proliferating cells were only found outside the taste buds labeled by keratin 8 – not within taste buds (Fig. 1D).

Distribution and quantification of S-phase proliferating cells within early taste buds in the base of the oral cavity in chickens

Due to high variance in PCNA expression level, quantification of $PCNA^+$ proliferating cells within early

chicken taste buds was challenging. Instead, BrdU was used to label S-phase proliferating cells via a single intraperitoneal injection. Consistent with distribution of PCNA$^+$ cells within taste buds, BrdU-labeled proliferating cells were abundant within early taste buds and the tissue compartments surrounding them (Fig. 2).

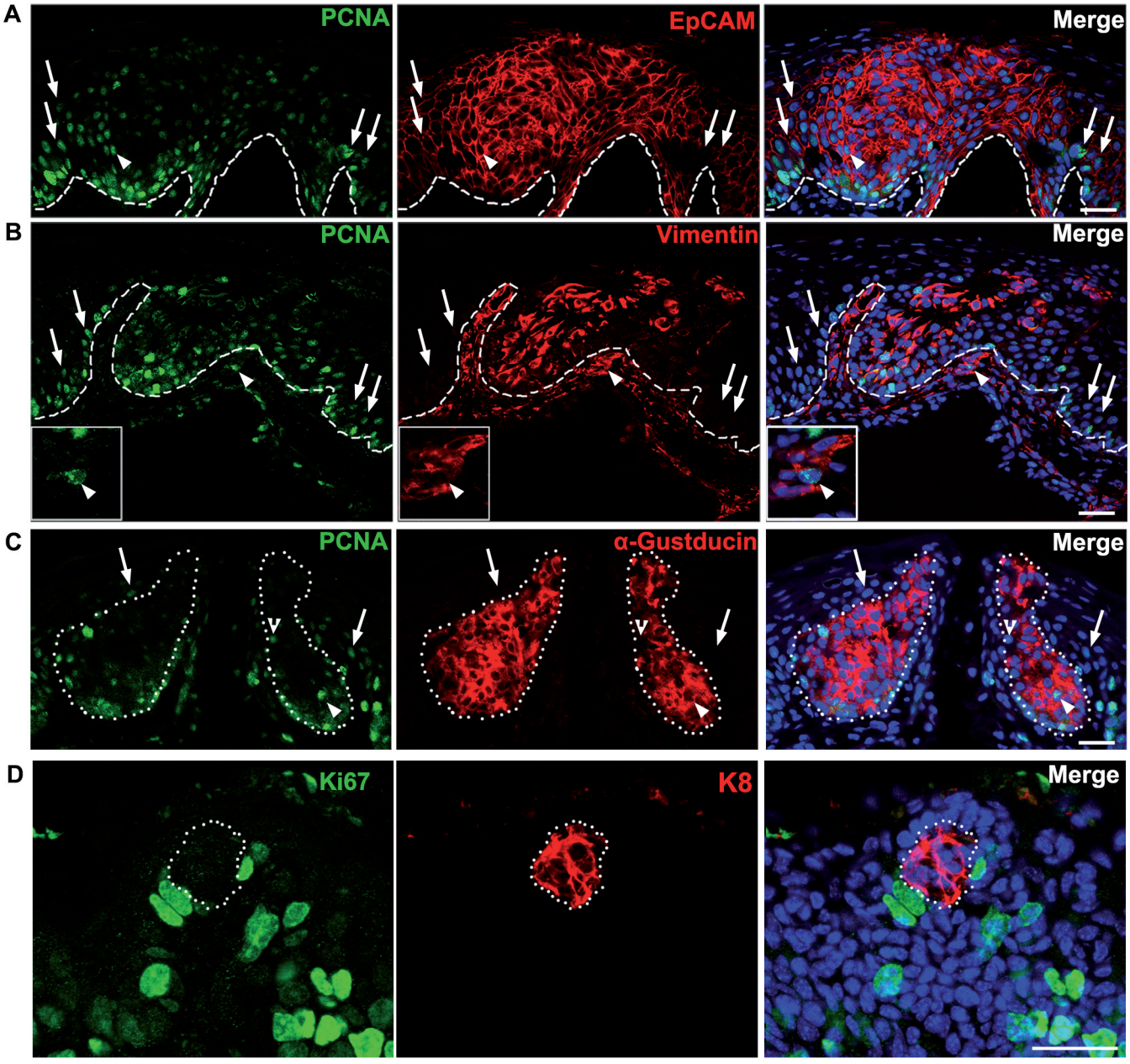

Fig. 1. Representative photomicrographs (single-plane laser scanning confocal) from the tissue sections of the base of oral cavity at P3. **A.** The tissue sections were immunostained with proliferating cell marker PCNA (green) and epithelial cell marker EpCAM (red). Solid arrowheads point to PCNA$^+$ cells in EpCAM$^+$ epithelial cells. Long arrows point to PCNA$^+$ cells in the epithelium outside of the taste bud. **B.** Distribution of PCNA$^+$ (green) cells in the connective tissue labeled with stromal cell marker Vimentin (red). Solid arrowheads point to a representative proliferating cell in the connective tissue immediately beneath the epithelium. Long arrows point to PCNA$^+$ cells in the epithelium outside of the taste bud. Insets show high power images of the cell. **C.** Abundant distribution of PCNA$^+$ (green) proliferating cells in taste buds labeled with specific taste cell marker α-Gustducin (red). Long arrows point to PCNA$^+$ cells in the epithelium outside of the taste bud. **D.** proliferating cells labeled by Ki67 were present surrounding mouse taste bud (K8). Solid arrowheads point to PCNA$^+$ cells that are also α-Gustducin$^+$. Open arrowheads point to PCNA$^+$ cells without α-Gustducin. Dashed lines demarcate epithelium from connective tissue. White dots encircle the taste buds. Scale bars: 20 μm, applies to images in the same row.

Our previous studies showed that Vimentin and α-Gustducin labeled distinct but largely overlapping populations of taste bud cells, and a small proportion of taste bud cells was neither Vimentin$^+$ nor α-Gustducin$^+$ (Venkatesan et al., 2016). To characterize the association of BrdU$^+$ cells with the expression of molecular markers, triple-immunostaining of Vimentin, α-Gustducin, and BrdU was performed. A quantitative analysis was performed for the co-localization of BrdU with either/both Vimentin and α-Gustducin immunosignals. As shown in Fig. 2 and Table 1, 72.42% of BrdU$^+$ cells expressed neither Vimentin nor α-Gustducin (open arrowheads, Fig. 2), while the percentage of BrdU$^+$ cells co-expressing either Vimentin or α-Gustducin is 27.58% (solid arrowheads, Fig. 2). BrdU$^+$ cells were also seen in the surrounding epithelium (long arrows, Fig. 2) and underlying connective tissue (short arrows, Fig. 2). Within taste buds, there were still cells which remained unlabeled by any of the three markers.

To map the distribution of S-phase proliferating cells within taste buds, 80 taste buds from P3 chickens were thoroughly examined in serial sections and 608 BrdU$^+$ cells were found among 7633 taste bud cell profiles from representative images. Taste bud boundaries were drawn according to α-Gustducin signals (Fig. 3A). The relative localization of BrdU$^+$ cells were then plotted in a schematic taste bud (Fig. 3B), displaying that proliferating cells were enriched in the basal area of taste buds. BrdU$^+$ cells were also seen in the side and apical areas of taste buds, corresponding to the distribution of bright PCNA$^+$ cells.

Mapping S-phase proliferating cells within early taste buds of the palate in chickens

In chickens, the majority of taste buds are located within the palate and are distributed in distinct

Table 1. S-phase proliferating cell profiles in P3 chickens.

Number of taste buds	Number of taste bud cell profiles per taste bud profile	BrdU$^+$ cell profiles per taste bud profile	BrdU$^+$ taste bud cell profiles labeled with molecular marker(s)	BrdU$^+$ taste bud cell profiles unlabeled with molecular marker
80	115.58±36.01	7.62±2.09 (6.74±0.02)%	50.67±8.02 (27.58±0.12)%	152±73.98 (72.42±0.12)%

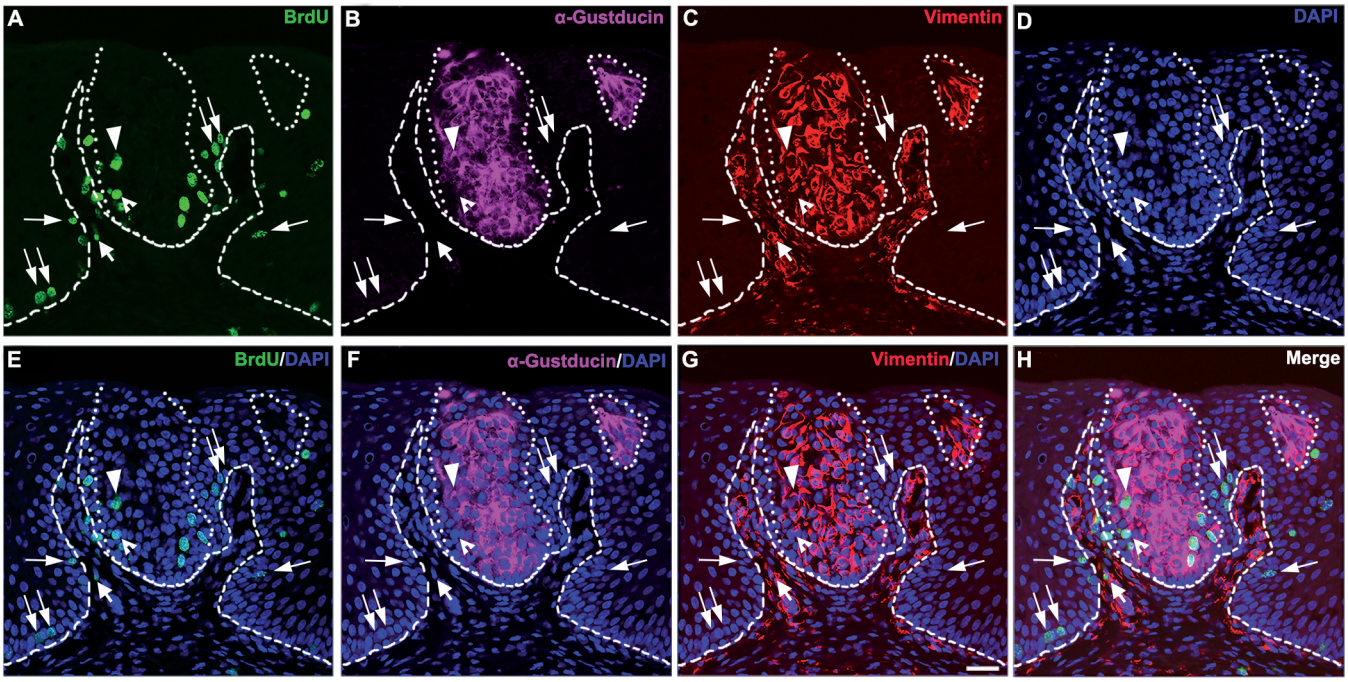

Fig. 2. Single-plane laser scanning confocal photomicrographs of a representative taste bud from tissue sections of the base of oral cavity at P3 **(A-G)**. Tissues were harvested 2 hr after BrdU intraperitoneal injection. Tissue sections were triple-labeled with BrdU (**A**, green), α-Gustducin (**B**, purple), and Vimentin (**C**, red). Open arrowheads point to a BrdU$^+$ cell negative for α-Gustducin and Vimentin. Solid arrowheads point to BrdU$^+$ cells also labeled with α-Gustducin and Vimentin. Long arrows point to BrdU$^+$ cells in the epithelium outside of the taste bud. The short arrow points to a BrdU$^+$ cell in the connective tissue. Dashed lines demarcate epithelium from connective tissue. White dots encircle the taste buds. Scale bar: 20 μm.

distribution patterns in three regions (maxillary gland opening region, mgr; palatine papillae region, ppr; posterior region, pr) (Fig. 4A). $BrdU^+$ cells were mapped in all three regions in serial sections of P3 chicken palates. Similar to the distribution of $BrdU^+$ cells in the base of the oral cavity, labeled cells were seen within early taste buds (solid and open arrowheads, Fig. 4B) as well as surrounding epithelium (long arrows) and connective tissue (short arrows). Although not quantified, $BrdU^+$ cells within taste buds of the palate were equally frequent as those of the base of the oral cavity, indicating that the abundant distribution of $BrdU^+$ cells within taste buds is consistent throughout the oral cavity. Moreover, most $BrdU^+$ taste bud cells were located in the basal and lateral sides of taste buds and neither labeled by α-Gustducin nor Vimentin (open arrowheads, Fig. 4B).

Reduced abundance of proliferating cells within chicken taste buds at 45 days post-hatching (P45)

To test whether the abundant distribution of proliferating cells in early chicken taste buds sustains after maturation of taste buds, we characterized proliferating cells of chicken oral epithelium at P45. $PCNA^+$ cells were observed within mature taste buds at P45 (Fig. 5). Though not quantified, the number of $PCNA^+$ cells was far fewer than that of early taste buds at P3 (Fig. 1C).

Discussion

Identifying proliferating progenitor cells is helpful for understanding the formation and maintenance of taste buds. In the present study, we found abundant proliferating cells within early taste buds in chickens, but not in mice. Most $BrdU^+$ cells within early taste buds were not labeled by other molecular markers for taste buds, suggesting their undifferentiated status. The distribution of abundant proliferating cells in early taste buds in chicken hatchlings indicates a unique and potentially "built-in" system in early taste buds for expansion to their large size. Moreover, observation of proliferating cells within mature taste buds at a later stage (P45) of chickens at a low frequency suggests that similar to mice, mature chicken taste buds rely primarily on the surrounding proliferating progenitors for maintenance.

Proliferating cells within early taste buds are abundant in chickens vs sparse in rodents

It is widely accepted that in rodents the basal progenitor cells within mature taste buds are primarily post-mitotic, as Sullivan and colleagues found proliferating cells within taste buds at an extremely low frequency (~0.4 cells per taste bud) in adult mice (Beidler and Smallman, 1965; Okubo et al., 2009; Sullivan et al., 2010; Nguyen et al., 2012). However, whether there are proliferating progenitor cells within early taste buds of rodents has not been characterized. Our data showed that consistent with mature taste buds, early taste buds do not possess proliferating cells. Thus, both formation and maintenance of mouse taste buds rely on the proliferating progenitors in the surrounding tissue compartments, including keratin 14-expressing

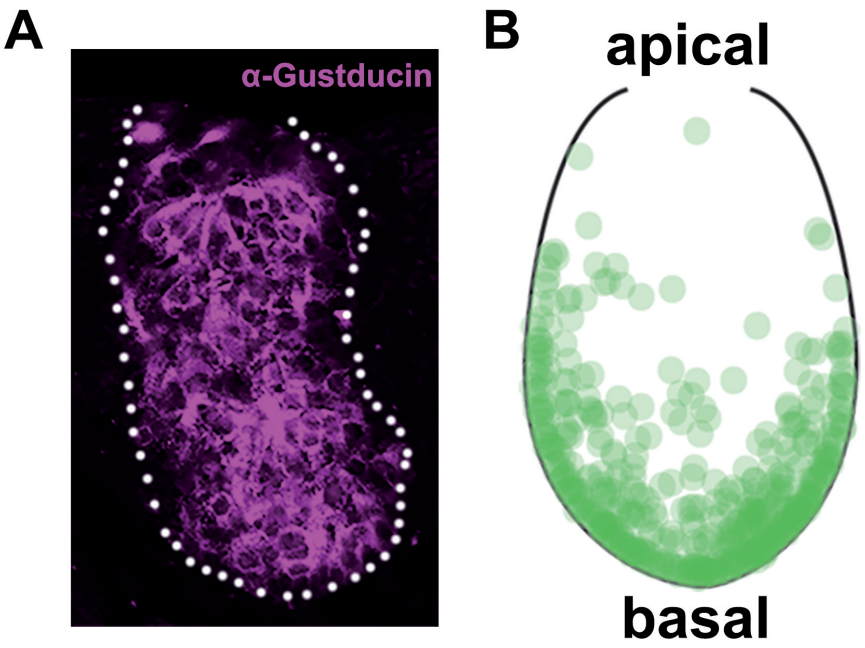

Fig. 3. A. An image of the taste bud from Fig. 2B to illustrate that the border of a taste bud is drawn referring to α-Gustducin signal. **B.** A plotting diagram to illustrate the distribution $BrdU^+$ proliferating cells within a taste bud (outlined by the black line). Each green dot represents a $BrdU^+$ cell nucleus and is plotted in a position relative to the basal and apical sides of a taste bud.

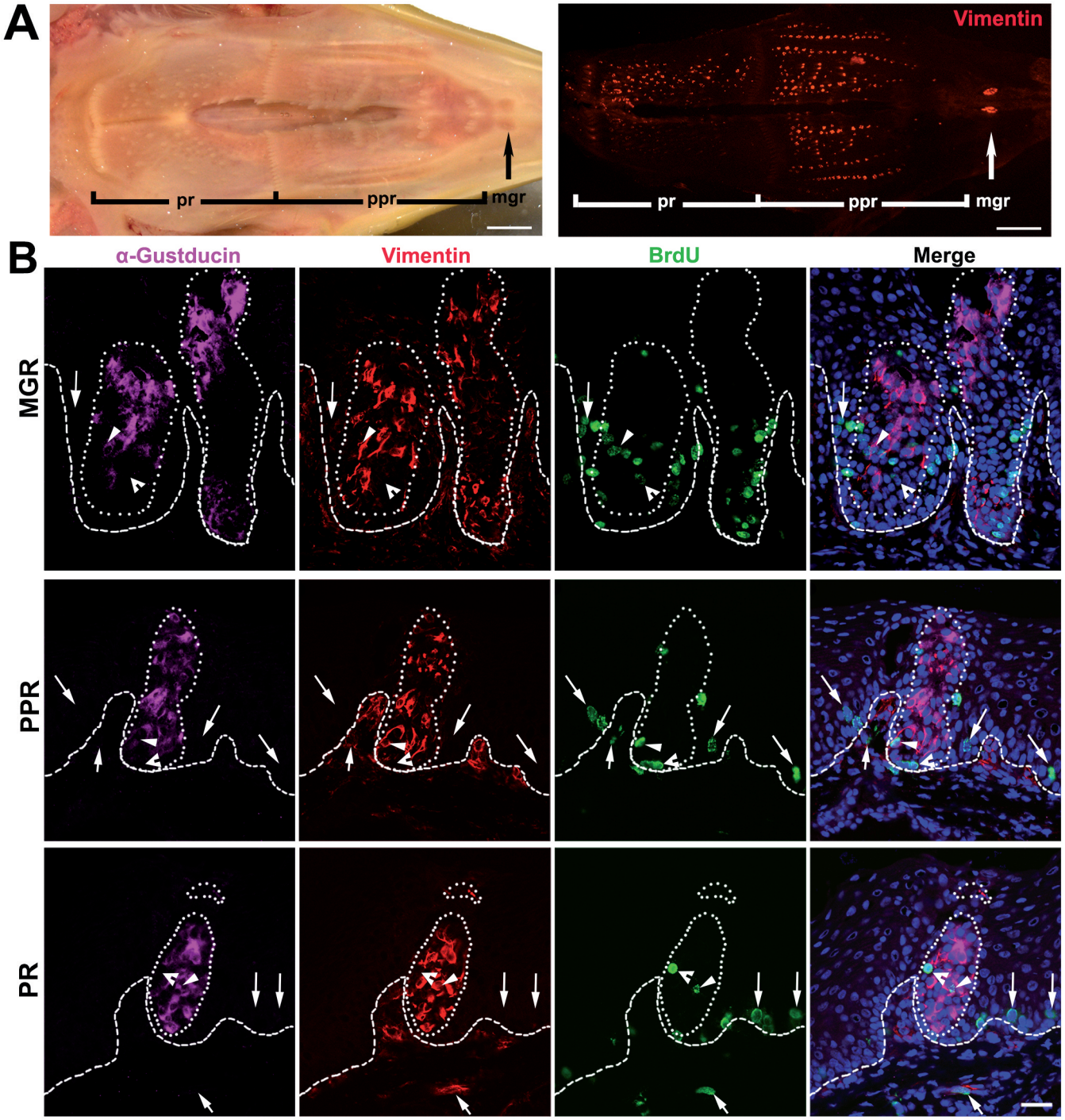

Fig. 4. Distribution of S-phase proliferating cells within taste buds in the palate. **A.** Bright-field image of the palate (left) and fluorescent image of epithelial sheet immunoreacted with Vimentin to illustrate the distribution of taste buds (right). **B.** Single-plane laser scanning confocal photomicrographs of representative taste bud tissue sections from mgr, ppr and pr regions of the palate at P3. Open arrowheads point to a $BrdU^+$ cell negative for α-Gustducin and Vimentin. Solid arrowheads point to $BrdU^+$ cells also labeled with α-Gustducin and/or Vimentin. Long arrows point to $BrdU^+$ cells in the epithelium outside of the taste bud. Short arrows point to a $BrdU^+$ cell in the connective tissue. Dashed lines demarcate epithelium from connective tissue. White dots encircle the taste buds. Scale bars: A, 2 mm; B, 20 μm.

epithelial basal cells that can differentiate into Shh$^+$ immediate progenitors within taste buds (Okubo et al., 2009; Miura et al., 2014) and potentially underlying connective tissues (Boggs et al., 2016).

In chickens, spherical buds emerge at E17 and mature taste bud features (taste pores, elongate shape, etc.) appear at E19. The size of taste buds increases dramatically in the first four days post-hatching (Ganchrow et al., 1994). A pulse-chase experiment on newly hatched chickens displayed that the average life span of chicken taste bud cells is around 4 days (Ganchrow et al., 1994). Here we report a comprehensive analysis regarding where and what the proliferating cells are. Many more proliferating cells were observed within early taste buds in chickens than in mice, i.e., around 7.6 BrdU$^+$ S-phase proliferating cells per taste bud file in chicken, versus zero in mice. Although not quantified, PCNA$^+$ cells were detected at a much higher rate than BrdU$^+$ cells, suggesting the abundance of overall proliferating cells in early chicken taste buds. Considering the large size of chicken taste buds, such an abundant distribution of proliferating progenitors may be necessary in order to meet the need of rapid growth of taste buds in chicken hatchlings.

According to the distribution map of S-phase proliferating cells, most proliferating cells within early taste buds are located at the basal region of taste buds. Proliferating cells were also present in the tissues surrounding chicken taste buds, i.e., epithelium and underlying connective tissue. It is too early to speculate the tissue compartments from which the proliferating taste bud cells originate. Further lineage studies will be necessary to answer this question.

BrdU$^+$ S-phase proliferating cells in chicken taste buds may represent an undifferentiated cell population

Two molecular markers (α-Gustducin and Vimentin) have been found to label a distinct but largely overlapping population of early chicken taste bud cells (Venkatesan et al., 2016). The expression of α-Gustducin indicates a conserved mechanism underlying how gustatory stimuli in oral cavity are transduced among species (Wong et al., 1996). However, there is a taste bud cell population that is unlabeled by α-Gustducin and Vimentin and is yet to be characterized. We speculate that the proliferating cells represent a population of undifferentiated cells in early chicken taste buds. Using triple-staining of BrdU with α-Gustducin and Vimentin, we found that most, if not all, of BrdU$^+$ S-phase proliferating taste bud cells did not express α-Gustducin nor Vimentin, suggesting their undifferentiated status. Notably, some taste bud cells remained unlabeled by any of those markers. It is possible that these cells were proliferating cells in other phases, quiescent precursors, or immature taste bud cells.

In summary, our data indicate that early chicken taste buds have a unique mechanism to meet the high demands for growing up to and maintaining their large size and high cell turnover rate – undifferentiated proliferating progenitors within taste buds. Combined with the other beneficial aspects of chicken taste buds -- many in number (Mozdziak and Petitte, 2004; Roura et al., 2013; Rajapaksha et al., 2016; Venkatesan et al., 2016; Cui et al., 2017), patterned array of distribution (Rajapaksha et al., 2016), genetically and molecularly regulated (Cui et al., 2017), convenience of *in ovo*

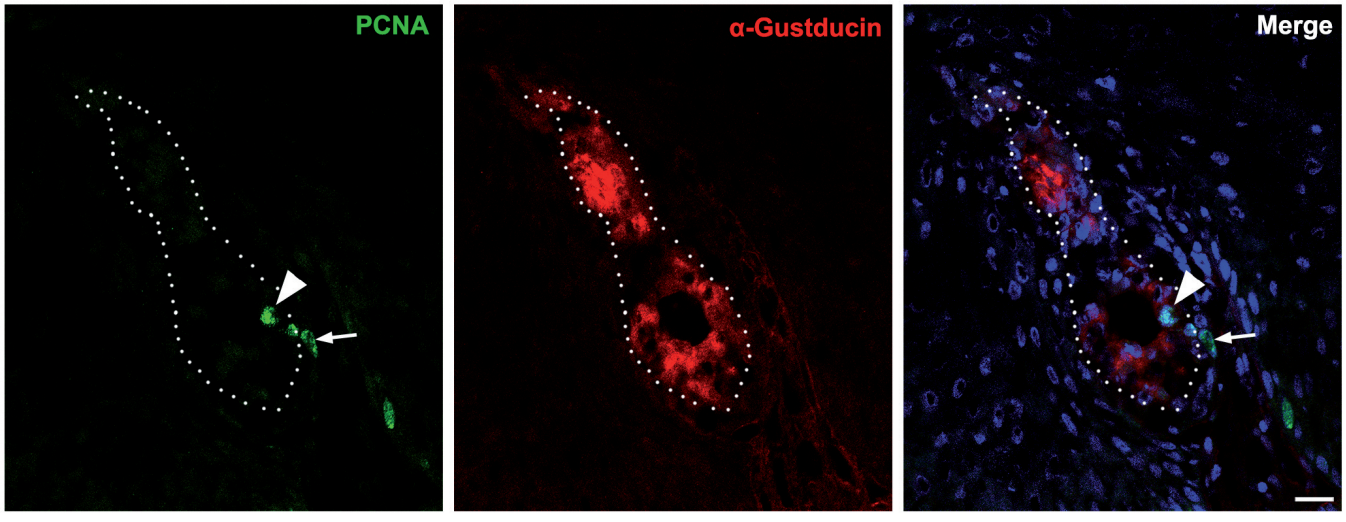

Fig. 5. Single-plane laser scanning confocal photomicrographs of a representative taste bud from tissue sections of the base of oral cavity at P45. Solid arrowheads point to PCNA$^+$ cells also labeled with α-Gustducin. Long arrows point to PCNA$^+$ cells outside the taste bud. White dots encircle the taste bud. Scale bar: 20 μm.

embryo manipulation, rapid development, and high availability (Hughes, 1955; Odani et al., 2009), we believe that the chicken taste organ is an ideal model for studies in organogenesis and regenerative medicine.

Acknowledgements. We give thanks to the staff at Cobb Vantress Cleaveland Hatchery, GA for providing the chickens. We give thanks to Dr. Franklin West for his discussion and feedback, and Dr. Xiaogang Cui for his technical assistance. This study was supported by the National Institutes of Health (grant number R01 DC012308 to HXL) and University of Georgia Start-up fund to HXL.

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Accepted October 31, 2018