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Identified Taste Bud Cell Proliferation in the Perihatching Chick

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Abstract

Developing taste buds in the anterior mandibular floor of perihatching chicks were studied by high voltage electron microscopic autoradiography in order to identify proliferating gemmal cell types. Montaged profiles of 29 taste buds in five cases euthanized between embryonic day 21 and posthatching day 2 were analyzed after a single [^{3}H]thymidine injection administered on embryonic day 16, 17 or 18. Results showed that dark cells comprised 55% of identified (n = 900 cells) and 62% of labeled (n = 568 cells) gemmal cells as compared with light, intermediate, basal or perigemmal bud cells. Dark cells had both a greater ($P < 0.05$) number of labeled cells and a greater amount of label (grains/nucleus) than the other four bud cell types, irrespective of injection day. The nuclear area (μm^2) of dark cells was not significantly larger ($P > 0.05$) than that of the other gemmal cell types and therefore cannot account for the greater amount of label in the dark cells. Interestingly, only dark cells showed a positive correlation ($P < 0.003$) between amount of label and nuclear area. Results suggest that, during the perihatching period of robust cell proliferation, dividing dark cells may give rise primarily, but not exclusively, to dark cell progeny.

Introduction

Light microscopic autoradiographic analysis of cell proliferation in developing taste buds of the perihatching chick indicates that DNA synthesis in preparation for mitosis is triggered within 3 days after initial signs of forming gemmal primordia are evident on embryonic day (E) 17 (hatching = 21 days) (Ganchrow *et al.*, 1995). Bud morphogenesis progresses from a predominantly spherical shape, developing below surface epithelium at E17–19, to an ovoid shape which, beginning at E19, traverses the vertical extent of the epithelium and opens to the oral cavity via a pore or pit (Ganchrow and Ganchrow, 1987, 1989; Ganchrow *et al.*, 1993). This shift in gemmal shape is reflected by significant increases in numbers of cells comprising the bud as well as in bud diameter. For example, in the perihatching period it is estimated that 28% of cells in ovoidal buds are labeled after single *in ovo* injections of [^{3}H]thymidine administered in late gestation (Ganchrow *et al.*, 1995). Bud cell proliferation continues into early posthatching, by which time the adult complement of buds and range of gemmal diameters have stabilized in this precocial vertebrate (Saito, 1966; Gentle and Hunter, 1973; Berkhoudt, 1985; Ganchrow and Ganchrow, 1985, 1987, 1989; Ganchrow *et al.*, 1994, 1995).

Taste bud primordia in the chick embyo developing between E17–19 are comprised, at least in part, of earlier-born postmitotic and quiescent (G_0) cells. This is suggested by the absence of immediate gemmal cell proliferation in this time period after single [^{3}H]thymidine injections administered at E15, E16, E17 or E18 (Ganchrow *et al.*, 1995), whereas labeled bud cells are evident at E19 following comparable injections at E2, E4 or E6 (Ganchrow *et al.*, 1997). Thus, in addition to the early embryonic birth of some gemmal primordium cells, a second wave of robust cell proliferation commences after E19 and continues through to the fourth day posthatching (H) (Ganchrow *et al.*, 1994, 1995).

As in other vertebrates, including the limited number of avian species studied (for review see Reutter and Witt, 1993; Sprißler, 1994), the taste bud receptor organ of the precocial chicken contains several gemmal cell types as described

ultrastructurally. Though the same bud cell types are identified at perihatching ages (Ganchrow *et al.*, 1991) and in the adult (Kurosawa *et al.*, 1983; Ganchrow and Ganchrow, unpublished data), whether and in what proportion specific gemmal cell types proliferate during ontogenesis of the initial bud population are not known. This information could pinpoint which bud cell type contributes, perhaps disproportionately, to the formation of developing buds. The present ultrastructural autoradiographic analysis identifies those bud cell types in the perihatching chick which are labeled between E21 and H2 after single [^{3}H]thymidine injections adminstered either at E16, E17 or E18. Anterior mandibular (floor of the mouth) gemmal tissue was examined in those same cases which in previous light microscopic autoradiographic analysis (Ganchrow *et al.*, 1995) had exhibited bud cell poliferation following an equivalent survival period.

Materials and methods

Incubation and [^{3}H]thymidine injection

Fertilized eggs (White Leghorn) were incubated at 99.6°F, in saturated humidity, in a rotating forced-draft incubator (Modern Equipment, Inc., Haifa, Israel). Four hours prior to each specified day of thymidine injection, eggs were transferred to a non-rotating incubator (Victoria Incubators, Pavia, Italy) set to identical temperature and humidity conditions as before. The first 24 h of incubation were considered as E1.

Of the 20 embryos utilized in the main study (Ganchrow *et al.*, 1995, table 1), five were selected for the present study. These had been euthanized 5 days after thymidine injection on E16 (n = 2), E17 (n = 1) or E18 (n = 2), when gemmal cell proliferation was most apparent. Briefly, under visual guidance using an operation microscope, 25 µl of a sterile aqueous solution containing 25 µCi (= 0.08 µg) of [^{3}H]thymidine (sp. act. = 72.5 Ci/mmol; New England Nuclear) were injected through a windowed portion of the shell directly into the yolk sac or its vicinity with a Hamilton syringe. The windowed shell piece was re-inserted and secured with tape, and the egg returned to the non-rotating incubator.

Animal preparation and electron microscopic autoradiography

The three hatchlings had self-hatched, and were placed in individually warmed pens and provided with water and feed. All five embryos and hatchlings were euthanized between E21 and H2. All chicks were anesthetized (Nembutal) and their blood was flushed with heparinized 0.05 M sodium cacodylate buffer followed by 0.1 M nonheparinized sodium cacodylate. Transcardial perfusion used 1% paraformaldehyde–2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate (pH 7.3). The excised lower beak was placed in additional fixative, overnight at 4°C.

Dissected anterior mandibular gemmal tissue [innervated by the chorda tympani branch of the facial nerve (Ganchrow *et al.*, 1986)] was bisected by an anteroposterior midline cut. One tissue half was prepared for light microscopic autoradiography (Ganchrow *et al.*, 1995). In preparation for electron microscopy, the other tissue half was osmicated, en-bloc stained in uranyl acetate, dehydrated with graded ethanols, transferred through propylene oxide and embedded in PolyBed 812 (Polysciences)/Araldite 502 (Fullam).

Buds were identified in 2–2.5 µm sections cut from the plastic blocks and the sections re-embedded onto blank PolyBed/Araldite capsules. Serial or near-serial 0.25-µm-thick longitudinal sections were cut on a Reichert Ultracut E ultramicrotome using a Diatome diamond knife and collected on Formvar-coated palladium/copper grids. Grids were coated with Ilford L4 emulsion diluted 1:2 with water, using the loop-and-peg method (Williams, 1985). Grids were exposed in the dark for 5 weeks, developed in Microdol-X, fixed with 25% sodium thiosulfate and washed in distilled water. Subsequently, grids were poststained in alcoholic uranyl acetate followed by triple lead stain (Sato, 1968) and carbon-coated. The sections were examined and photographed (×2000) with a JEOL JEM 1000 high voltage electron microscope (HVEM) at the University of Colorado, Boulder.

Taste bud montaging and gemmal cell typing

Twenty-nine buds were montaged from 179 single, coded photomicrographic profiles (final magnification, ×8250) in the five cases (total number of photomicrographic profiles = 448): E16 injections, 10 buds from two cases; E17 injection, six buds from one case; E18 injections, 13 buds from two cases. Each montage was selected on the basis of its including the greatest longitudinal cross-sectional extent amongst other montages through the same bud. Gemmal cells were cell-typed independently by three of the present authors according to criteria established for hatchling chick bud, dark, light, intermediate, basal and perigemmal cells (Ganchrow *et al.*, 1991; see also adult chicken: Kurosawa *et al.*, 1983). Lack of unanimity in identifying cell types resulted in deleting 20 cells (= 2%) from the analysis. The final analysis was based on 900 gemmal cells: cases 16-1 = 66 cells, 16-2 = 142 cells, 17-2 = 193 cells, 18-7 = 183 cells and 18-8 = 316 cells. Sixty-three percent (568 cells) of these bud cells were considered to be labeled (see below).

Labeled gemmal cells

The determination of a labeled cell in HVEM was not calculated in the same way as that in the light microscopic autoradiographic study (Ganchrow *et al.*, 1995) since

different nuclear emulsions, exposure times and section thicknesses were used. Rather, the following protocol was employed: mean background grains was calculated over non-nuclear areas in intragemmal epithelium, extragemmal epithelium and subepithelium (5 randomly-selected regions/montage × 29 montages = 145 background regions). Background grain counts were obtained by placing a transparent grid over each non-nuclear region and counting the number of grains/16 μm^2 [15.8 ± 0.5 μm^2 (mean ± SEM) = mean nuclear area across bud cell types (see below)]. Mean background value was 0.1 ± 0.0 (SD = 0.30) grains/16 μm^2. A gemmal cell was considered as labeled if the number of grains overlying the nucleus exceeded 2 SD above mean background value, empirically equivalent to at least 1 grain/nucleus. Sampling of the same bud cells in profiles or montages adjacent to those selected for analysis showed that label in these cells was evident in sections separated by up to 2.25 μm, consistent with the effective degree of penetration of electrons (beta particles) through autoradiographic tissue as discussed by Rogers (1973).

Nuclear area of gemmal cells

The nuclear area of bud cells was determined in a subsample (n = 292 cells) of the complete 900 gemmal cell sample. Cells were chosen based on the presence of a complete unobstructed nuclear envelope. In 95% (n = 277 cells) of these cells, nucleoli and/or concentrations of chromatin were identifiable. Cells were scanned [I.T.E. Scanner Model PS-240 X, PowerLook II 350 H4WO (UMAX Data System, Inc., Taiwan)] and their nuclear area (μm2) measured by Image Tool program 'UTHSCSA' for Windows 95.

Statistical analyses

Two-way ANOVA tests compared the amount of label, or nuclear area, amongst bud cell types across incubation day of injection. *A posteriori* multiple comparisons of means were applied by the Tukey–Kramer honest significant difference (HSD) test. Pearson product–moment correlations tested the relation between nuclear area and amount of label as related to gemmal cell type. Statistical significance was set at $P < 0.05$.

Results

Gemmal cell types

Figure 1 is a montage of a longitudinal section through an anterior mandibular taste bud at H2 following a [^{3}H]thymidine injection at E18. Briefly, and mainly based on criteria described previously for thin-sectioned palatal buds in early hatchlings (Ganchrow *et al.*, 1991), five gemmal cell types may be seen: *dark* cells are characterized by irregularly shaped to lobulated nuclei whose densely granular nucleoplasm contains scattered clumps of chromatin, often adhering to the inner leaflet of the nuclear membrane, and at least one nucleolus. The cytoplasm contains a dense filamentous network, often surrounding the nucleus, and small compact mitochondria. Extensions of dark cell cytoplasm partially or completely envelop light and intermediate cells, and nerve fibers. *Light* cell nuclei are large and rounded, and usually contain one nucleolus in a uniformly granular nucleoplasm. The electron-lucent cytoplasm contains sparse perinuclear filaments, plump and pale mitochondria, rough as well as elongated smooth endoplasmic reticulum, and scattered free ribosomes. Clear and dense-cored vesicles in perikaryal cytoplasm sometimes loosely aggregate adjacent to nerve profiles. *Intermediate* cell features include gradations of those described for dark and light cells: nuclei are more irregular and contain increased amounts of clumped chromatin as compared with light cells. Filament bundles often surround the nucleus, as in dark cells, and extend along the length of the cell. Perikaryal cytoplasm contains abundant polyribosomes and free ribosomes. Higher magnification examples of these three gemmal cell types may be seen in Figure 2. *Basal* cells characteristically occupy the proximal region of the taste bud. Nuclei are irregularly shaped and contain clumped chromatin. The cytoplasm is less electron-lucent than that of light or intermediate cells. *Perigemmal* cells are elongate, oriented parallel to the sides of the bud and share desmosomal contacts. Nuclei are ovoidal, often contain clumped chromatin and occupy a large portion of the soma whose cytoplasm is characterized by numerous filaments and free ribosomes.

Gemmal cell labeling

Individual effect analysis in the two-way ANOVA (incubation day of injected thymidine × gemmal cell type) of the amount of bud cell label shows that *bud cell type* [$F(4,885) = 6.34$, $P < 0.0001$] is significant. *A posteriori* comparisons of means indicate that dark cells have a significantly ($P < 0.05$) greater amount of label (2.4 ± 0.1 grains/nucleus) than light, intermediate, perigemmal or basal cells (see Table 1). Neither *day of injection* [mean number of grains/taste bud cell: E16 injection = 2.0 ± 0.2, E17 injection = 1.9 ± 0.2 and E18 injection = 1.8 ± 0.2; $F(2,885) = 0.97$, $P > 0.38$)] nor *interaction* [$F(8,885) = 0.99$, $P > 0.44$] are significant.

Similarly, ANOVA of the number of bud cells/cell type that is labeled is significant [$F(4,10) = 5.62$, $P < 0.01$]. *A posteriori* analysis shows that significantly ($P < 0.05$) more dark cells are labeled (≥1 grain/nucleus) than all other bud cell types, irrespective of day of injection (E16, E17 or E18). This may be seen in Table 1, in which dark cells comprise 55% (496/900) of identified bud cell types and 62% (352/568) of identified labeled cells. Moreover, Table 1 also shows that 71% (352/496) of the dark cells were labeled, compared with a mean of 53% for the other four bud cell types [range = 49% (intermediate cells) to 58% (perigemmal cells)]. In general,

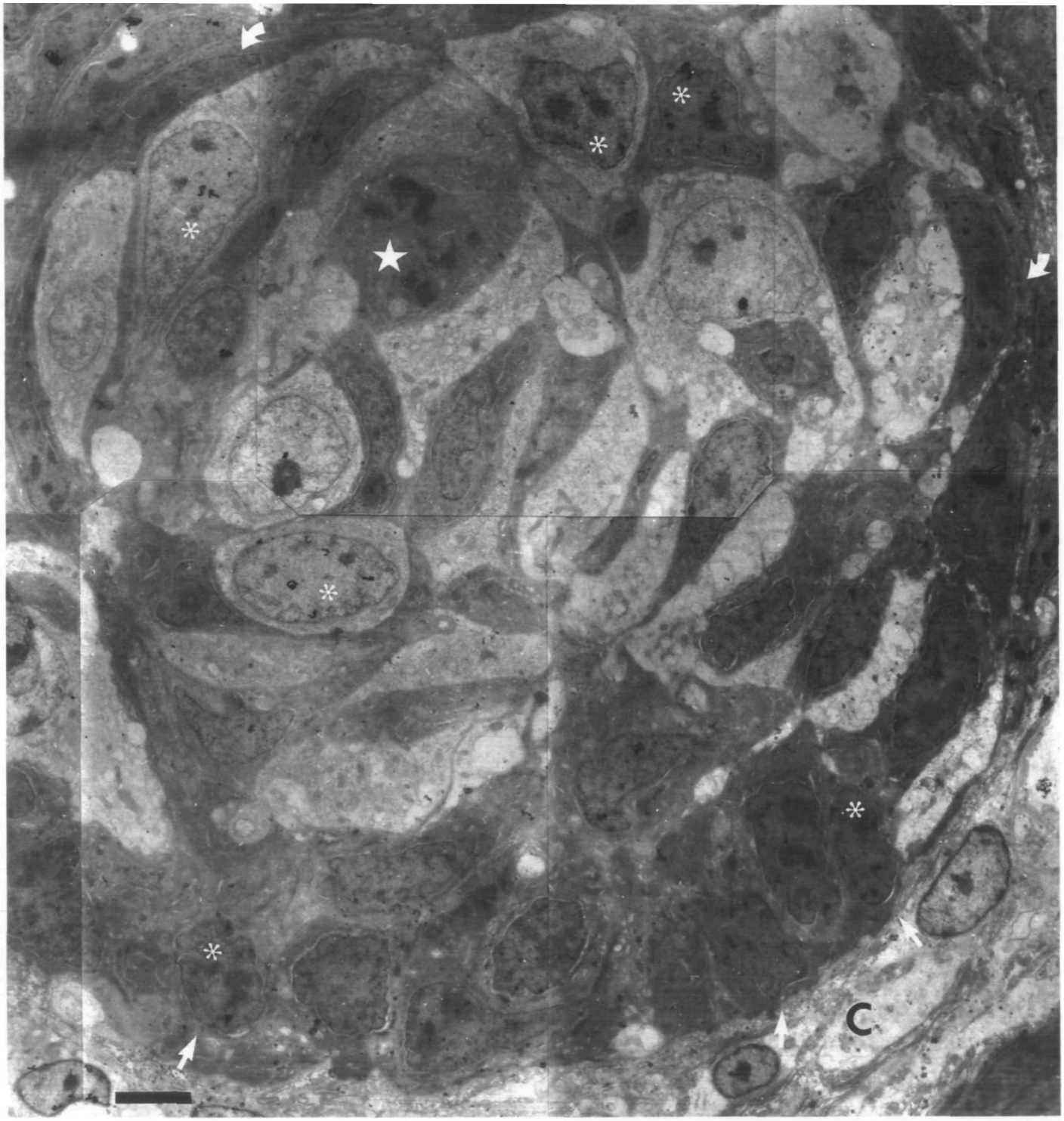

Figure 1 Low magnification montage of an H2 anterior mandibular taste bud in oblique longitudinal section and processed for autoradiography. The surface epithelium is toward the top of the photomicrograph. Basal (straight arrows) and perigemmal (curved arrows) taste bud cells indicate respective distolateral and proximobasal borders of the bud. Asterisks indicate some of the labeled bud cells which were cell-typed by analysis at higher magnification (see text). Star, dividing bud cell; C, connective tissue. Magnification, ×4200. Scale bar, 3 μm.

63% (568/900 cells) of identified bud cells were labeled, of which 39% were dark cells.

Since dark cells account for 55% of the identified cells in the overall sample (Table 1), in order to test the possibility that the greater amount of label in these cells might be attributed to random single 'background' grains distributed

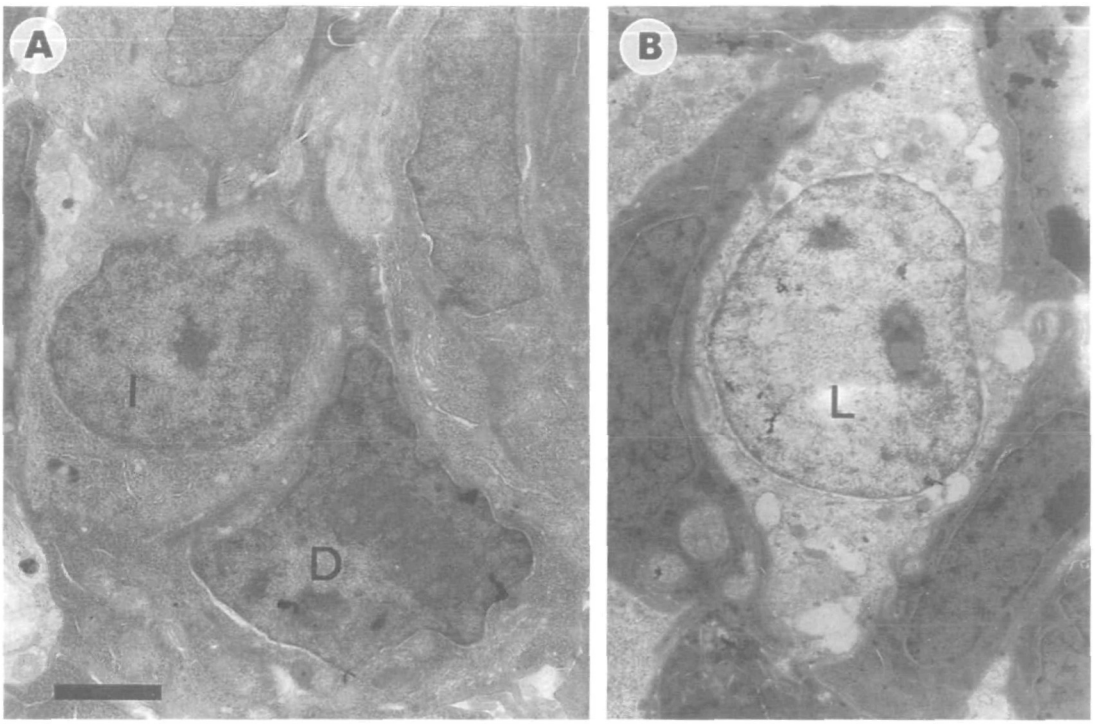

Figure 2 Higher magnification examples of **(A)** a labeled dark (D) and an unlabeled intermediate (I) gemmal cell, and **(B)** a labeled light (L) gemmal cell (see text), 5 days following a single [^{3}H]thymidine injection. Age at euthanasia: (A) E21 and (B) H2. Magnification, ×6900. Scale bar, 2 μm.

Table 1 Number, proportion and amount of label in taste bud cell types of the perihatching chick, 5 days after single [^{3}H]thymidine injection on E16, E17 or E18

	Taste bud cell types					
	Dark	Light	Intermediate	Basal	Perigemmal	Total
Cells	496 (55%)[a]	103 (11%)	109 (12%)	58 (6%)	134 (15%)	900
Labeled cells	352 (62%)[b]	54 (10%)	53 (9%)	31 (5%)	78 (14%)	568 (100%)
% of labeled cell type	71	52	49	53	58	
% of total cells labeled	39	6	6	3	9	63
Amount of label[c]	2.4 ± 0.1[d] (0–14)	1.4 ± 0.2 (0–8)	1.0 ± 0.1 (0–5)	1.2 ± 0.2 (0–5)	1.5 ± 0.2 (0–11)	

[a]Percent of total labeled plus unlabeled cells.

[b]Percent of total labeled cells.

[c]Mean ± SEM grains/nucleus; (range of grains/nucleus).

[d]Tukey–Kramer HSD *a posteriori* analysis: dark versus light, intermediate, basal or perigemmal cells, $P < 0.05$.

disproportionately over dark cell nuclei, the following analysis was carried out: an equivalent number of cells/bud cell type was chosen, based on the total number of cells of that gemmal cell type with the lowest frequency in Table 1, which was 58 basal cells. Fifty-eight cells for each of the other four bud cell types were randomly selected from the entire sample (n = 900 cells) and the number of grains/cell nucleus was recorded in that subsample of 290 cells (58 cells × 5 bud cell types). The mean ± SEM grains/nucleus for each cell type was: dark cells = 2.6 ± 0.4; light cells = 1.3 ± 0.2; intermediate cells = 1.0 ± 0.1; basal cells = 1.2 ± 0.2; and perigemmal cells = 1.3 ± 0.2. These mean values closely match those from the full sample (see Table 1). ANOVA of the amount of bud cell label across bud cell type [$F(4,285)$ =

Table 2 Number, proportion, amount of label and nuclear area in a subsample of taste bud cell types of the perihatching chick

	Taste bud cell types					
	Dark	Light	Intermediate	Basal	Perigemmal	Total
Cells	131 (45%)[a]	49 (17%)	41 (14%)	28 (10%)	43 (15%)	292 (>100%)
Amount of label[b]	2.6 ± 0.2[c] (0–11)	1.8± 0.3 (0–8)	1.3 ± 0.2 (0–5)	1.3 ± 0.3 (0–5)	1.5 ± 0.3 (0–5)	
Nuclear area[d]	16.1 ± 0.8	17.8 ± 1.1[e]	19.1 ± 1.7[e]	11.4 ± 0.9	12.2 ± 0.9	
Correlation[f] (r =)	0.26[g]	0.01	0.08	0.10	0.08	

[a]Percent of total labeled plus unlabeled cells.

[b]Mean ± SEM grains/nucleus; (range of grains/nucleus).

[c]Tukey–Kramer HSD *a posteriori* analysis: dark versus light cells, $P < 0.05$.

[d]Mean ± SEM μm^2.

[e]Tukey–Kramer HSD *a posteriori* analysis: light versus basal cells, $P < 0.001$; light versus perigemmal cells, $P < 0.004$; intermediate versus basal cells, $P < 0.001$; intermediate versus perigemmal cells, $P < 0.006$.

[f]Pearson product–moment correlation of amount of label and nuclear area.

[g]$P = 0.003$.

6.40, $P < 0.0001$] was significant. Tukey–Kramer HSD *a posteriori* analysis again showed significant differences between dark and all other bud cell types: dark versus light cells, $P < 0.01$; dark versus intermediate cells, $P < 0.0001$; dark versus basal cells, $P < 0.001$; and dark versus perigemmal cells, $P < 0.01$.

These results confirm those from the full sample (Table 1), suggesting that random background grains did not contribute to the overall result.

Gemmal cell nuclear area

Individual effect analysis in the two-way ANOVA (incubation day of injected thymidine × gemmal cell type) of nuclear area (μm^2) shows that *bud cell type* [$F(4,277) = 4.21$, $P < 0.003$] is significant. *A posteriori* comparisons of means indicate that the nuclear area of intermediate and light cells is larger than that of basal or perigemmal cells (see Table 2). Analysis showed that the *interaction* effect [$F(8,277) = 2.06$, $P < 0.04$] is also significant, but the effect is not linear and the individual comparisons will not be described here. Consistent with the analysis of amount of label as described in the previous section, analysis showed that the effect of *day of injection* [$F(2,277) = 1.31$, $P > 0.27$] is not significant.

Table 2 shows that the number, proportion and amount of label of gemmal cell types in the subsample ($n = 292$ cells) measured for nuclear area approximate those obtained in the larger sample ($n = 900$ cells) (Table 1). Pearson correlation in the two samples of mean number of grains/cell type/animal is $r = 0.92$. Importantly, it may be seen that, although dark cells in both samples contain the largest amount of label, the nuclear area of the dark cell is not significantly different from that of any other gemmal cell type (cf. Tables 1 and 2). Only dark cells show a significant, and positive, correlation ($r = 0.26$, $P = 0.003$, $n = 131$ cells) between amount of label and nuclear area (Table 2). In order to test whether the latter correlation reflects a greater number of dark cells in the sample, an additional correlation collapsing across all non-dark gemmal cells was performed but was seen to be not significant ($r = 0.06$, $P = 0.454$).

Discussion

Proportions of gemmal cell types

This study shows that within the E21–H2 perihatching period in the chick, i.e. during the first week of initial taste bud development beginning with appearance of the bud primordium at E17 (Ganchrow and Ganchrow, 1987; Ganchrow *et al.*, 1994, 1995), dark cells on the average account for more than half (55%) of the gemmal cell population. Similarly, in early developing buds, cell staining and ultrastructure identify dark cells as the predominant cell type in perihatching quail oral epithelium (Sprißler, 1994), 1-week-old neonatal as well as weanling rat fungiform papilla buds (Farbman, 1965a; Pumplin *et al.*, 1997), and first to mid-trimester fetal monkey fungiform buds (Zahm and Munger, 1983). Dark cells also constitute more than half of the foliate papilla gemmal cell population in adult rabbit (Murray, 1969), and fungiform and circumvallate papilla gemmal cells in the adult rat (Farbman, 1965b, 1980).

In the only other published HVEM autoradiographic study on bud cell proliferation (Delay *et al.*, 1986) known to the present authors, the relative proportion of circumvallate

papilla gemmal cell types in young adult mice was: dark cells, 10% (perigemmal cells were not classified in the report). These results are consistent with the present data in anterior mandibular buds of the perihatching chick, particularly with respect to dark, intermediate and basal cells (see Table 1, row 1). In general, cytoplasmic and nucleoplasmic features characterizing mouse (Delay *et al.*, 1986) and chick (Ganchrow *et al.*, 1991) gemmal cell types are very similar. Species notwithstanding, differences in the percentage of light cells in the two reports may reflect some aspect of cell lineage as studied by Delay and coworkers: at a comparable 5 days survival period after single [^{3}H]thymdine injection in the young adult mouse, no light cells were labeled. However, in the overall 1 h–10 days period studied, light cells made up 10% (25/240 cells) of the labeled bud cells (Delay *et al.*, 1986, table 2), which is consistent with the present report (cf. Table 1).

We do not know whether the proportion of gemmal cell types is comparable in perihatching chicks versus mature chickens. Supplementary chick perihatching data [montages of one anterior mandibular and three palatal buds (unpublished data)] indicate that at E20, dark cells appear to comprise a smaller (37%) and light plus intermediate cells a greater (31%) proportion of the bud cell population than at E21–H2 (cf. Table 1, row 1); also, at H4, when bud diameter has stabilized (Ganchrow *et al.*, 1994), 37% of gemmal cells are dark cells. Possibly, then, the E21–H2 period of gemmal development is characterized by an increase in the proportion of dark cells in the overall as well as in the proliferating portion of the bud cell population (see below).

Gemmal cell proliferation

In the light microscopic autoradiographic portion (Ganchrow *et al.*, 1995) of this study, it was shown that gemmal cell proliferation was not evident until E20 after thymidine injection at E15, E16, E17 or E18, i.e. after a minimal 'delay' of 2 days. This suggests that bud cells may have initially sequestered the thymidine. After E20, proliferation apparently was expressed for ~1 day with an injection on E16 (euthanized at E21), 2 days with one on E17 (euthanized at H1) and 3 days with one on E18 (euthanized at H2). Since day of injection is not significantly related to amount of label (see Results and Ganchrow *et al.*, 1995), it may be that a proportion of the identified labeled gemmal cells are first generation daughter cells; the migration rate of an extragemally labeled cell into the chick bud compartment is estimated to be 9–11 h (Ganchrow *et al.*, 1994). Moreover, since supplementary data collected during the present study show that all bud cell types are labeled already at E20, the case for a single intragemmal stem cell type cannot be supported. This may differ from the adult condition: in young adult mouse circumvallate buds during the first 2 days after [^{3}H]thymidine injection, basal cells comprised 86–100% and dark cells only 3–14% of the labeled bud cells. The proportion of labeled dark cells began to significantly increase after 3 days postinjection. Consistent with the present data (see Table 1), at 5 days postinjection, basal cells accounted for 7% of labeled gemmal cells (Delay *et al.*, 1986, table 2). Similarly, in young adult rabbit foliate buds, only basal cells are labeled within 2 days, while dark cells comprise most of the labeled cells within the first week after thymidine injection (Murray and Murray, 1971).

While all five gemmal cell types described here are identified and labeled between E20 (Ganchrow and Ganchrow, 1989; Ganchrow *et al.*, 1991, unpublished data) and H2 after a single [^{3}H]thymidine injection, dark cells comprise a significantly greater proportion (62%) of the bud cell population undergoing cell proliferation as compared with light (10%), intermediate (9%), basal (5%) or perigemmal (14%) cells. Interestingly, in contrast to the dark cell, each of the other four cell types forms more consistent proportions of the overall as well as labeled bud cell population (cf. Table 1, rows 1 and 2). A greater proliferation rate in dark versus light circumvallate bud cells also was reported in weanling rat, in which at peak labeling (6.5 days after [^{3}H]thymidine injection) the ratio of dark:light cells increased to 3.5:1 as compared with a minimal ratio of 1.6:1 in the control condition (Farbman, 1980).

Previous light microscopic autoradiographic studies in chick using single [^{3}H]thymidine injections administered on H1 and including hatchlings euthanized at 4 (H5) and 6 (H7) days postinjection (Ganchrow *et al.*, 1994) reported that of two gemmal cell types identified (7 μm sections), the bud cells that were mainly labeled had nuclei that essentially were round, contained clear nucleoplasm, and were found mainly in the central two-thirds of the bud. These general nuclear characteristics and gemmal locations probably include at least the light and intermediate bud cells described here. A second group, of gracile bud cells mainly located in the peripheral one-third of the bud, exhibited an elongate, deeply staining nucleus and were sparsely labeled. These cells could include perigemmal and dark cell types. Also, in the latter study it was shown that the number of labeled bud cells/bud had returned to background levels by H7. Supplementary data (see above) suggest that by H4 dark cells comprise only about one-third, and light and intermediate cells about one-half of gemmal cells. Moreover, taste bud counts have already reached adult values by H1–H2 (Saito, 1966; Gentle and Hunter, 1983; Berkhoudt, 1985; Ganchrow and Ganchrow, 1985, 1987). Therefore, while dark cells comprise a major portion of gemmal cells particularly during the E21–H2 perihatching period of initial bud population development, they may constitute an antecedent source of other cell types or a more stable portion of the gemmal cell population during subsequent periods of bud cell replacement (cf. Delay *et al.*, 1986).

Nuclear area and gemmal cell types: clues to bud cell lineage

Compared with measurements in young adult mouse taste buds (n = 120 cells) (Delay *et al.*, 1986), the nuclear area of dark, light, intermediate and basal cells generally is smaller in the perihatching chick (n = 292 cells). Specifically, the nuclear area of light cells is significantly larger than the other three cell types in the mouse [see also rabbit foliate bud, light cells (Murray, 1986); and adult rat circumvallate bud, light and dark cells (Pumplin *et al.*, 1997)]. In comparison, the nuclear area of chick light and intermediate cells is larger than that of basal and perigemmal but not dark cells. While in the perihatching chick an increase in bud diameter is accommodated by an increase in mean number of gemmal cells/bud until the diameter stabilizes at H4 (Ganchrow *et al.*, 1994, 1995), it is probably the case that differences in nuclear area between the same gemmal cell types in mouse and chicken are species- rather than age-related.

Though the nuclear area of chick light and intermediate cells is significantly larger than that of basal or perigemmal cells, the amount of label (mean ± SEM grains/nucleus) does not differ among the four gemmal cell types. On the other hand, while the dark cell nuclear area also does not differ, a significant (positive) correlation of nuclear area and amount of label occurs only in dark cells, consonant with a significantly greater amount of label as compared with light, intermediate, basal or perigemmal cells. These results may suggest that during the E21–H2 time period, dividing dark cells give rise primarily, but not exclusively, to dark cell progeny. Indeed, the significantly positive correlations of nuclear area between dark and light cells (r = 0.38, P = 0.02), and dark and perigemmal cells (r = 0.35, P = 0.03) suggest parallel development of these gemmal cell types during the E21–H2 period (Ganchrow *et al.*, 1994, 1995).

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