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Taste receptor cells arise from local epithelium, not neurogenic ectoderm

(cell lineage/progenitor/mosaic/mouse)

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ABSTRACT **Except for taste bud cells, all sensory receptor cells and neurons have been shown to originate from neurogenic ectoderm (i.e., neural tube, neural crest, or ectodermal placodes). Descriptive studies on taste buds indicate that they, however, may arise from local epithelium. To determine whether taste receptor cells originate from neurogenic ectoderm or from local epithelium, the tongues of X chromosome-inactivation mosaic mice were examined. Results of this analysis show that taste bud cells and their surrounding epithelium always match in terms of the mosaic marker. This suggests that taste cells and epithelial cells arise from a common progenitor and that taste receptor cells originate from local tissue elements. Since taste buds are widespread in the oropharynx, they lie in epithelium derived from both ectoderm and endoderm. Therefore, taste receptor cells can be induced in tissue from two different germ layers. Thus in terms of tissues of origin, taste receptor cells are unlike other cells with neuronal characteristics.**

Taste buds, the sensory end organs that transduce gustatory stimuli into neural signals, consists of 50–150 specialized neuroepithelial cells. These taste cells have characteristics of both neuronal and epithelial cells. Like neurons, they form synapses, store and release neurotransmitters, have voltage-sensitive sodium channels, and are capable of generating receptor and action potentials (1, 2). Like epithelial cells, taste cells have a limited life span and are regularly replaced by a proliferative basal cell population (3–5). All other receptor cells are known to arise from neurogenic ectoderm including the neural tube, neural crest, or ectodermal placodes and, therefore, share the same progenitor population as neurons (6, 7). In contrast, taste buds have been described on the basis of conventional anatomical studies (8–10) as arising from the local epithelium.

Taste buds can be considered analogous to the lateral line organs of fish and amphibians since they contain axonless receptor cells and are organized into compact end organs embedded in the epithelium. Further, both taste buds and lateral line organs are innervated by ganglia containing neurons originating from both neural crest and ectodermal placodes. Lateral line organs arise from migratory cells originating in placodes (11–18) or in both placodes and neural crest (19–21), raising the possibility that taste buds might have a similar embryonic origin. The present experiments were undertaken to test whether taste bud receptor cells, like other receptor cells and neurons, arise from neurogenic ectoderm or instead arise from local epithelium.

To address this question, taste buds and the surrounding lingual epithelium were examined in transgenic X chromosome-inactivation mosaic mice consisting of two populations of cells: one expressing β-galactosidase (β-gal) activity and the other lacking this enzyme. The two populations of cells form as a result of X chromosome inactivation in females hemizygous for a transgenic *Escherichia coli lacZ* marker (22, 23). Before X chromosome inactivation, both X chromosomes are active (24–26) and, therefore, every cell expresses β-gal (Fig. 1). However, as occurs in all mammalian females early in development, each cell independently inactivates one of its two X chromosomes by a random process (27). Thus in hemizygous H253 females, at the completion of X chromosome inactivation, β-gal activity is restricted to approximately half of the cells in the embryo. The inactivation status of each cell is stable and heritable; therefore, further proliferation and differentiation result in a mouse that is mosaic for cells expressing β-gal activity (Fig. 1). This mosaicism can be used to study cell lineage and the cell movements that occur during development. The mosaic mice used in these studies have been employed for studying several features of embryogenesis, including cell dispersion in the cerebral cortex (28).

Previous studies (29) and personal observations (unpublished data) indicate that the lingual epithelium of chimeric mice consists of a patchwork of cell groups derived from each embryo. Although these chimeras were produced by *in vitro* aggregation of two embryos whereas the mosaic mice used in our studies are produced by X chromosome inactivation, the distributions of the two genetically distinct cell populations in both types of mice are expected to be similar (30). Therefore, the patchwork pattern of chimeric lingual epithelium suggests that a similar situation would be found in mosaic animals. If so, the relationship between taste bud progenitors and the local epithelium could be determined by examining taste buds contained entirely within β-gal-positive (blue) or β-gal-negative (red) epithelial patches (Fig. 2). Only taste buds within patches are used in this analysis because at patch borders or in regions of extensive mingling of β-gal-positive and β-gal-negative epithelial cells, the results of an analysis of the taste bud population and its surrounding epithelium would not be informative [i.e., the β-gal phenotype (positive or negative) of taste bud cells would not indicate their embryonic origin]. This is so because the taste bud population would always match the surrounding epithelium since the epithelium contains cells with both β-gal phenotypes. Within patches consisting of epithelial cells with only one β-gal phenotype, however, the question of taste bud progenitor origin can be addressed. If taste bud progenitors derive from the local epithelium, then the color of stained taste bud cells will always match that of the surrounding epithelial patch (Fig. 2*A*). In contrast, if taste bud progenitors migrate into the epithelium during development, then the color of stained cells in the taste

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Abbreviations: β-gal, β-galactosidase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A reductase; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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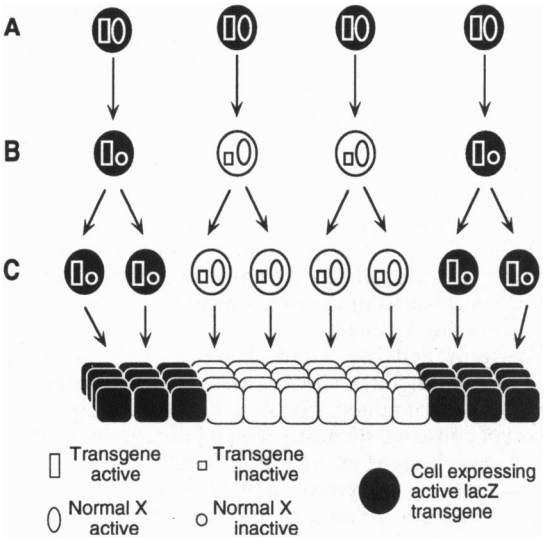

FIG. 1. Production of mosaic tissues by X chromosome inactivation in HMG-CoA–*lacZ* hemizygous females. (*A*) Before X chromosome inactivation, both the X chromosome containing the transgene and the normal X chromosome are active. The β-gal activity is evident in all cells. (*B*) Random X chromosome inactivation occurs, restricting β-gal activity to approximately half of the cell population; this X chromosome-inactivation status is a heritable feature of each cell. (*C*) Cells continue to proliferate and differentiate, producing a mosaic epithelium consisting of blue (β-gal positive) and red (β-gal negative) patches.

bud would be random with respect to the surrounding epithelium. Thus, depending on the number of progenitors contributing to a taste bud, at least half of the taste buds would contain taste cells that do not match the surrounding epithelial patch (Fig. 2*B*).

MATERIALS AND METHODS

X Chromosome-Inactivation Mosaics. Mouse line H253 carries a transgenic X chromosome-linked marker: 14 tandem copies of a 8.9-kb fragment containing the promoter of the mouse housekeeping gene 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, linked to the *E. coli lacZ* gene (31). HMG-CoA reductase is involved in cholesterol synthesis, cholesterol homeostasis, and cell proliferation (32). Thus this enzyme promoter was used to produce ubiquitous β-gal expression in cells of H253 transgenic mice (33).

Since the transgenic marker is linked to the X chromosome, mosaics can be produced. This is accomplished by mating male H253 mice to wild-type females. This results in female progeny that are hemizygous for the *lacZ* marker. Because the transgene is present on only one of the two X chromosomes, X chromosome inactivation results in the random inactivation of the marker in about half the cells of the embryo. In mice, X chromosome inactivation is complete in most ectodermal and mesodermal tissues by 9.5 days post coitum (22) and may occur even earlier (34). By 9.5 days post coitum, the oral (stomodeal) membrane has ruptured, but tongue primordia do not appear until 11 days post coitum (35, 36). These tongue primordia can be identified as two lateral swellings associated with the surface of the mandibular region of the first branchial arch. After X chromosome inactivation, hemizygous females consist of two cell populations: one that expresses β-gal and one that does not. These two cell populations can then be used in cell lineage analyses.

Fixation and Histology. Tongues were removed and post-fixed after cardiac perfusion with 4% (wt/vol) paraformaldehyde/0.2% glutaraldehyde in fixing buffer (0.1 M phosphate buffer, pH 7.4/2 mM $MgCl_2$/5 mM EGTA). To view the intact

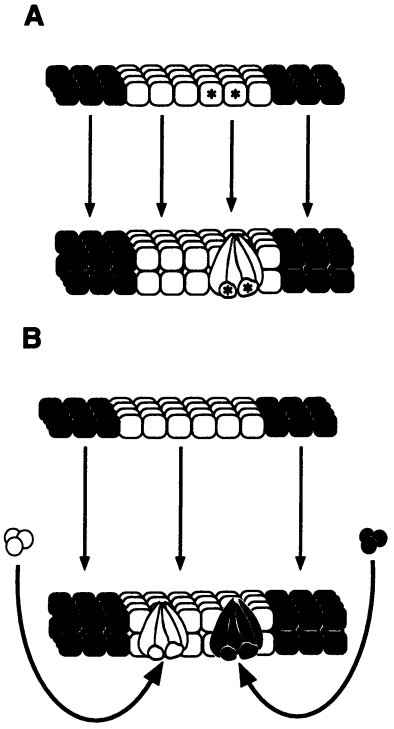

FIG. 2. (*A*) Expected results if the taste bud progenitor(s) are derived from the local epithelium. The taste bud population always will match that of the surrounding epithelial patch in terms of β-gal activity if progenitor(s) arise from local epithelium; blue patches will contain only blue taste buds and red patches will contain only red taste buds. (*B*) Expected results if the taste bud progenitor(s) migrate into the lingual epithelium. Some taste bud cells will not match the surrounding epithelium in terms of β-gal activity if taste cell progenitor(s) migrate into the epithelium; blue patches may contain red taste buds and red patches may contain blue taste buds. If taste buds arise from multiple independent progenitors, then some fraction of the cells in a taste bud would not necessarily match the surrounding epithelium.

lingual surface, fixed tongues were washed in washing buffer (fixing buffer containing 0.01% sodium deoxycholate and 0.02% Nonidet P-40) and incubated overnight at 37°C in a 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal; Sigma) solution [0.1% X-Gal/2 mM $MgCl_2$/5 mM EGTA/0.01% sodium deoxycholate/0.02% Nonidet P-40/5 mM $K_3Fe(CN)_6$/5 mM $K_4Fe(CN)_6$ in 0.1 M phosphate buffer (pH 7.4)]. After rinsing with buffer, whole tongues were viewed with a dissecting microscope.

Fungiform Taste Buds. Fungiform taste buds from five mosaic mice were examined. For two of the mosaics, 50-μm parasagittal sections were collected, incubated in X-Gal overnight, counterstained with neutral red, and then analyzed. Parasagittal serial sections (200 μm) were collected from the tongue tips of the remaining three mosaics. These sections were incubated in X-Gal overnight and examined microscopically for the presence of taste buds. Sections containing taste buds were embedded in plastic, recut at 3 μm, 5 μm, or 15 μm thickness, counterstained with nuclear fast red, and analyzed.

Circumvallate Taste Buds. Circumvallate papillae from five mosaic mice were examined. All sections were cut in the transverse plane, so that both sides of the V-shaped circumvallate crypt, lined by epithelium and taste buds, appeared in each section. Sections (50 μm) were collected from two of the papillae. These sections were incubated in X-Gal overnight, rinsed in buffer, counterstained with neutral red, and analyzed. Serial sections (200 μm) were collected from the remaining three papillae, incubated overnight in X-Gal solution, rinsed with buffer, embedded in plastic, and resectioned

into 3-, 5-, and 8-μm serial sections. These serial sections then were counterstained with nuclear fast red and analyzed.

Criteria for Taste Buds Included in This Study. Only taste buds residing well within patch borders were used in this study. For a fungiform taste bud to be considered, the entire papillar epithelium had to consist of all β-gal-positive (blue) cells or all β-gal-negative (red) cells; taste buds surrounded by papillar epithelium containing both red and blue cells were excluded. For circumvallate taste buds, only taste buds that were at least one taste bud width (about 40 μm) from any patch border were used. These taste buds were completely surrounded by epithelial cells of one color.

RESULTS

Whole mounts of the lingual epithelium of HMG-CoA–*lacZ* mosaic mice, after staining with X-Gal, reveal patches of blue (β-gal positive) and white (β-gal negative) epithelium (Fig. 3). The smallest patches are about 50 μm^2 in area but patches exceeding 1 mm^2 are frequently encountered. Each small patch may represent a single clone of cells (i.e., arising from a single progenitor) and the larger patches may result from the combination of several adjacent clones with similar β-gal activity (37, 38). The patchwork pattern of the lingual epithelium provides a means for addressing the question of the origin of taste bud progenitors as described above.

Two fields of taste buds were examined in this study: (*i*) the anterior tip of the tongue, which contains scattered fungiform papillae, each generally containing one taste bud, and (*ii*) the posterior central region of the tongue, called the circumvallate papilla, that contains hundreds of taste buds embedded in the epithelium lining the V-shaped circumvallate crypt. In both regions, many of the borders between patches of β-gal-positive epithelium and β-gal-negative epithelium were sharp. Virtually no intermingling of blue and red cells occurred within a patch; however, occasionally at patch borders extensive intermingling was evident.

Fungiform taste buds within uniform patches always match the surrounding epithelial cells (Fig. 4*A*). This is especially striking in red (β-gal negative) patches; no blue taste bud cells ever occur in these areas. The first eight β-gal-negative patches that contained fungiform taste buds entirely within the patch borders (all papillar epithelial cells were β-gal-negative) contained taste buds with only β-gal-negative cells. Six of these taste buds were approximately 50 μm from the nearest border; the other two taste buds were approximately 100 μm from the nearest border. These results were obtained from five mosaic mice. The taste buds in blue patches also match the surrounding epithelium, but the presence of a few red cells in a mostly blue taste bud population might be difficult to detect. Therefore, for statistical analysis, only taste buds in red patches were used, although no contrary examples were observed in nine blue areas that contained blue taste buds. No β-gal-positive cells were ever found in the β-gal-negative patches ($P < 0.004$ of this occurring by chance).

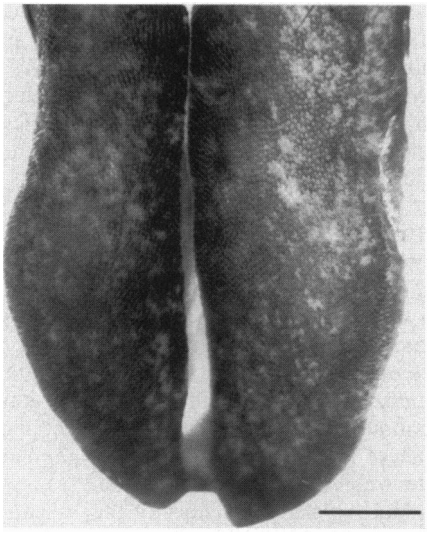

FIG. 3. Tongue of an HMG-CoA–*lacZ* mosaic mouse after staining with X-Gal consists of patches of blue (β-gal positive) and white (β-gal negative) cells. The smallest patches are about 50 μm^2 but patches exceeding 1 mm^2 are frequently encountered. Each small patch may represent a single clone of cells (i.e., arising from a single progenitor) and the larger patches may result from the combination of several adjacent clones with the same β-gal activity (35, 36). (Bar = 1 mm.)

Similarly, the cells of circumvallate taste buds also match the surrounding epithelium. Circumvallate papillae from five mosaic mice were examined. For two of these papillae, one side of the crypt consisted of mostly red (β-gal negative) epithelium and red taste buds (Fig. 4*B*). Specifically, for each section, there were three to seven red taste buds in red epithelial patches and zero to three taste buds containing both red and blue cells in epithelial patches consisting of both red and blue cells. In both animals, the other side of the crypt consisted of mostly mixed (red and blue) epithelium and taste buds. There were five to nine mixed taste buds and zero to two red taste buds on the sides of these crypts. The epithelium lining the circumvallate crypts from two other mice contained primarily blue epithelial cells and taste buds; a few mixed taste buds in mixed regions were also evident. The circumvallate papilla of the fifth mouse consisted primarily of blue epithelium and taste buds. In each circumvallate papilla, taste buds surrounded by blue epithelial cells always contained only blue taste cells while taste buds surrounded by red epithelium contained only red cells. As with fungiform taste buds, this was most obvious within patches of red epithelium in which even a single blue cell would be apparent. No such mismatches were found in a total of 20 circumvallate taste buds contained within red patches.

DISCUSSION

In X chromosome-inactivation transgenic mosaic mice, the β-gal phenotype of both fungiform and circumvallate taste buds invariably matches that of the surrounding epithelium. This suggests that both tissues share a common lineage and that taste bud progenitors are derived from local epithelial cells. Thus taste bud cells are unique among receptors and neurons in terms of arising from embryonic tissues other than neurogenic ectoderm (i.e., neural tube, neural crest, or placodes). Since lingual innervation is required for taste bud development, differentiation of the local epithelium into taste receptor cells appears to involve neural induction (39, 40). Further, the same or similar inductive process is able to affect cells from different embryonic germ layers since different taste buds reside in epithelia derived from ectoderm or endoderm (41–46). For example, the epithelium around fungiform taste buds is ectodermal, whereas the epithelium surrounding pharyngeal taste buds is endodermal. The derivation of particular structures from different germ layers has been suggested previously in the literature. One example is the enamel organ. In *Urodela*, the epithelially derived ameloblasts reportedly arise from ectoderm or endoderm, depending on the location of the future tooth (47, 48). In addition, in the outer skin of *Squalus acanthias*, the enamel layer of placoid scales arises from ectoderm, yet the enamel layer in pharyngeal scales reportedly derives from endoderm (46). The possibility of dual origins for taste buds also has been debated. For example, Landacre (45) suggested that in *Ictalurus* (*Ameiurus*), taste buds in the outer skin are ectodermal in origin, whereas those in the pharynx are derived from endoderm. The conclusion regarding a local origin for taste buds is further

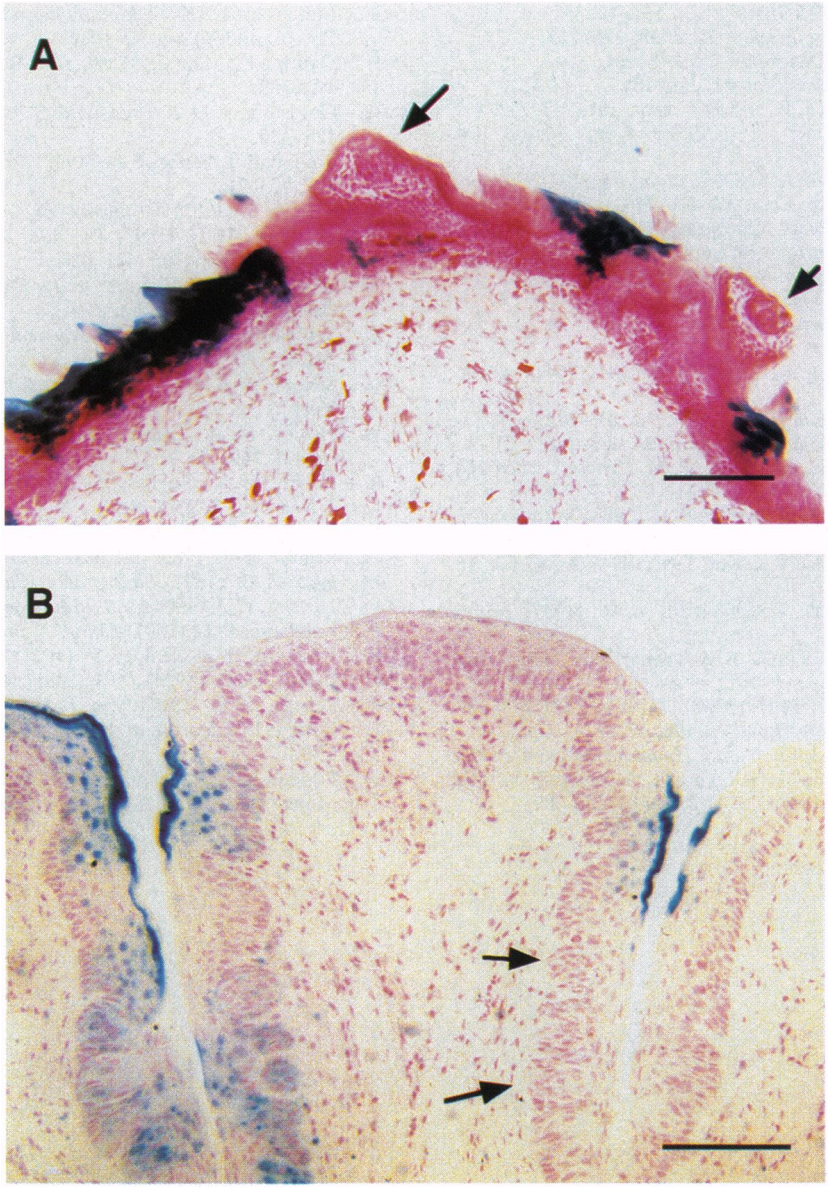

FIG. 4. (*A*) Fungiform taste buds, papillae, and surrounding epithelium from a HMG-CoA–*lacZ* X chromosome-inactivation mosaic mouse. Taste buds (arrows) are located in red (β-gal negative) patches, and like the surrounding epithelium, the taste cells are red. Cryostat section (50 μm) stained with X-Gal to detect β-gal activity (blue) and then counterstained with neutral red. (*B*) Circumvallate papilla from a HMG-CoA–*lacZ* X chromosome-inactivation mosaic mouse. Patches of red (β-gal negative) epithelium contain only red taste buds (arrows); blue patches contain only blue taste buds. Section (5 μm) incubated with X-Gal overnight and then counterstained with nuclear fast red. (Bars = 100 μm.)

supported by preliminary evidence from Barlow and Northcutt (49), indicating that taste buds in the oral cavity of ambystomid salamanders arise from endoderm rather than neurogenic ectoderm. Further study of the developing taste bud population may elucidate factors capable of transforming indifferent epithelium into neuron-like cells. Similarly, the cell lineage relationships between the different morphological cell types within the taste bud (basal, dark, intermediate, and light cells) should be studied.

In addition to showing that taste bud progenitors derive from local epithelium, the current analysis suggests that more than one progenitor gives rise to each taste bud. Taste buds located on patch borders often contain both β-gal-positive and β-gal-negative cells, indicating that a taste bud is a polyclonal structure. This situation is similar to that of lateral line organs where an individual sensory end organ may contain receptor cells originating from different progenitors (29). In contrast, other structures (e.g., intestinal crypts in mice) arise from a single progenitor and are, therefore, clonal (50).

In summary, despite their neuronal characteristics, taste buds likely come from the same progenitor population as the lingual epithelium. Because of the classical subdivision of lingual epithelium regarding its germ layer origin, a common origin of the lingual epithelium and its associated taste bud cells would imply that the capacity to form taste receptor cells is not restricted to either ectoderm or endoderm. Likely, the acquisition of receptor phenotype is the consequence of local tissue interactions.

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1. Roper, S. D. (1992) *J. Neurosci.* **12,** 1127–1134.
2. Roper, S. D. (1989) *Annu. Rev. Neurosci.* **12,** 329–353.
3. Beidler, L. M. & Smallman, R. L. (1965) *J. Cell Biol.* **27,** 263–272.
4. Conger, A. D. & Wells, M. A. (1969) *Radiat. Res.* **37,** 31–49.

5. Farbman, A. I. (1980) *Cell Tissue Kinet.* **13,** 349–357.
6. Noden, D. M. (1991) *Brain Behav. Evol.* **38,** 190–225.
7. Noden, D. M. (1993) *J. Neurobiol.* **24,** 248–261.
8. Farbman, A. I. (1965) *Dev. Biol.* **11,** 110–135.
9. Bradley, R. M. & Stern, I. B. (1967) *J. Anat.* **101,** 743–752.
10. Farbman, A. I. & Mbiene, J.-P. (1991) *J. Comp. Neurol.* **304,** 172–186.
11. Harrison, R. G. (1904) *Arch. Mikrosk. Anat.* **63,** 35–149.
12. Stone, L. S. (1922) *J. Exp. Zool.* **35,** 421–496.
13. Stone, L. S. (1928) *J. Comp. Neurol.* **45,** 169–190.
14. Stone, L. S. (1931) *Anat. Rec.* **48,** Suppl. 64 (abstr. 136).
15. Stone, L. S. (1933) *J. Comp. Neurol.* **57,** 507–540.
16. Stone, L. S. (1937) *J. Comp. Neurol.* **68,** 83–115.
17. Winklbauer, R. & Hausen, P. (1983) *J. Embryol. Exp. Morphol.* **76,** 265–281.
18. Northcutt, R. G., Catania, K. C. & Criley, B. B. (1994) *J. Comp. Neurol.* **340,** 480–514.
19. Vischer, H. A. (1989) *Brain Behav. Evol.* **33,** 205–222.
20. Vischer, H. A. (1989) *Brain Behav. Evol.* **33,** 223–236.
21. Collazo, A., Fraser, S. E. & Mabee, P. M. (1994) *Science* **264,** 426–429.
22. Tan, S.-S., Williams, E. A. & Tam, P. P. L. (1993) *Nat. Genet.* **3,** 170–174.
23. Tam, P. P. L., Williams, E. A. & Tan, S.-S. (1994) *Dev. Genet.* **15,** 491–503.
24. Adler, D. A., West, J. D. & Chapman, V. M. (1977) *Nature (London)* **267,** 838–839.
25. Epstein, C. J., Smith, S., Travis, B. & Tucker, G. (1978) *Nature (London)* **274,** 500–502.
26. Monk, M. & Harper, M. I. (1979) *Nature (London)* **281,** 311–313.
27. Lyon, M. F. (1961) *Nature (London)* **190,** 372–373.
28. Tan, S.-S. & Breen, S. (1993) *Nature (London)* **362,** 638–640.
29. Kusakabe, M., Yokoyama, M., Sakakura, T., Nomura, T., Hosick, H. L. & Nishizuka, Y. (1988) *J. Cell Biol.* **107,** 257–265.
30. McLaren, A. (1976) *Mammalian Chimaeras* (Cambridge Univ. Press, London), pp. 92–103.
31. Gautier, C., Mehtali, M. & Lathe, R. A. (1989) *Nucleic Acids Res.* **17,** 8389.
32. Goldstein, J. L. & Brown, M. S. (1990) *Nature (London)* **343,** 425–430.
33. Tam, P. P. L. & Tan, S.-S. (1992) *Development (Cambridge, U.K.)* **115,** 703–715.
34. Rastan, S. (1982) *J. Embryol. Exp. Morphol.* **71,** 11–24.
35. Kaufman, M. H. (1992) *The Atlas of Mouse Development* (Academic, London), pp. 421–424.
36. Rugh, R. (1968) *The Mouse: Its Reproduction and Development* (Burgess, Minneapolis).
37. Schmidt, G. H. (1987) *Development (Cambridge, U.K.)* **100,** 535–541.
38. Silvers, W. K. (1979) *The Coat Colors of Mice* (Springer, New York).
39. Hosley, M. A., Hughes, S. E. & Oakley, B. (1987) *J. Comp. Neurol.* **260,** 224–232.
40. Hosley, M. A., Hughes, S. E., Morton, L. L. & Oakley, B. (1987) *J. Neurosci.* **7,** 2075–2080.
41. Leydig, F. (1851) *Z. Wiss. Zool.* **3,** 1–12.
42. Schulze, F. E. (1863) *Z. Wiss. Zool.* **12,** 218–222.
43. Retzius, G. (1892) *Biol. Unters. (Stockholm) N.F.* **4,** 33–36.
44. Herrick, C. J. (1904) *U.S. Fish. Com. Bull. 1902,* 237–272.
45. Landacre, F. L. (1907) *Comp. Neurol. Psychol.* **17,** 1–66.
46. Cook, M. H. & Neal, H. V. (1921) *J. Comp. Neurol.* **33,** 45–63.
47. Adams, A. E. (1924) *J. Exp. Zool.* **40,** 311–379.
48. deBeer, G. R. (1947) *Proc. R. Soc. London B* **134B,** 377–406.
49. Barlow, L. & Northcutt, R. G. (1994) *Chem. Senses* **19,** 438 (abstr. 13).
50. Ponder, B. A. J., Schmidt, G. H., Wilkinson, M. M., Wood, M. J., Monk, M. & Reid, A. (1985) *Nature (London)* **313,** 689–691.