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Fingerprinting taste buds: intermediate filaments and their implication for taste bud formation

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Intermediate filaments in taste organs of terrestrial (human and chick) as well as aquatic (*Xenopus laevis*) species were detected using immunohistochemistry and electron microscopy. During development, the potential importance of the interface between the taste bud primordium and non-gustatory, adjacent tissues is evidenced by the distinct immunoreactivity of a subpopulation of taste bud cells for cytokeratins and vimentin. In human foetuses, the selective molecular marker for taste bud primordia, cytokeratin 20, is not detectable prior to the ingrowth of nerve fibres into the epithelium, which supports the hypothesis that nerve fibres are necessary for initiating taste bud development. Another intermediate filament protein, vimentin, occurs in derivatives of mesoderm, but usually not in epithelium. In humans, vimentin immunoreactivity is expressed mainly in border (marginal) epithelial cells of taste bud primordia, while in chick, vimentin expression occurs in most taste bud cells, whereas non-gustatory epithelium is vimentin immunonegative. Our chick data suggest a relationship between the degree of vimentin expression and taste bud cell proliferation especially during the perihatching period. It is suggested that surrounding epithelial cells (human) and mesenchymal cells (chick) may be contributing sources of developing taste buds. The dense perinuclear network of intermediate filaments especially in dark (i.e. non-sensory) taste disc cells of *Xenopus* indicates that vimentin filaments also might be associated with cells of non-gustatory function. These results indicate that the mechanisms of taste bud differentiation from source tissues may differ among vertebrates of different taxa.

Keywords: chick; cytokeratin 20; foetal development; human; taste bud; vimentin

1. INTRODUCTION

Taste buds consist of highly specialized neuroepithelial cells. These cells are organized as clusters of slender bipolar cells which monitor the composition of tastants, for example, in order to protect the organism against ingestion of toxic substances. In vertebrates, taste buds are commonly located in the oral mucosa, and sometimes scattered across the body skin, as, for example, in some scaleless fishes. In higher vertebrates, gustatory information is mediated by branches of the facial (N.VII), glossopharyngeal (N.IX) and vagal nerves (N.X), which project to the nucleus of the solitary tract in the brain stem. Afferent synapses between taste bud cells and nerve terminals occur mainly in the basal part of the taste bud.

Nerve fibres are required to maintain taste buds once the latter are formed and start to function (e.g. Hosley *et al*. 1987), but controversy exists whether nerves are necessary to initiate taste bud development. At present, there are two main hypotheses concerning this issue: (i) the presence of nerve fibres, if not the synaptic contact to local epithelial cells, is a prerequisite for the neurosensory transformation of epithelial cells (Oakley 1998; Witt & Reutter 1996); and (ii) a nerve-independent generation of taste buds, suggested by a series of studies in axolotl (Barlow *et al*. 1996). Moreover, although taste buds appear to develop from local epithelium in the mouse (Stone *et al*. 1995), in birds and mammals the question of cell lineage remains unanswered. For example, in contrast to the olfactory epithelium, it is not definitely clear if basal cells of the taste bud give rise to different subpopulations of specialized cells within the bud, distinguished by their morphological phenotypes and classified diversely among different vertebrate classes (Reutter & Witt 1993).

An intriguing challenge is to obtain data which might indicate an association of taste bud cells with their possible source tissues, especially in the light of developmental and regeneration events. Due to their intraepithelial location, taste bud cells develop within regions which interface with three different compartments (i) adjacent non-gustatory epithelium, (ii) underlying mesenchymal cells, and (iii) nerve fibre terminals. For example, proteins demonstrating the neuroepithelial phenotype of taste buds have been widely used as taste

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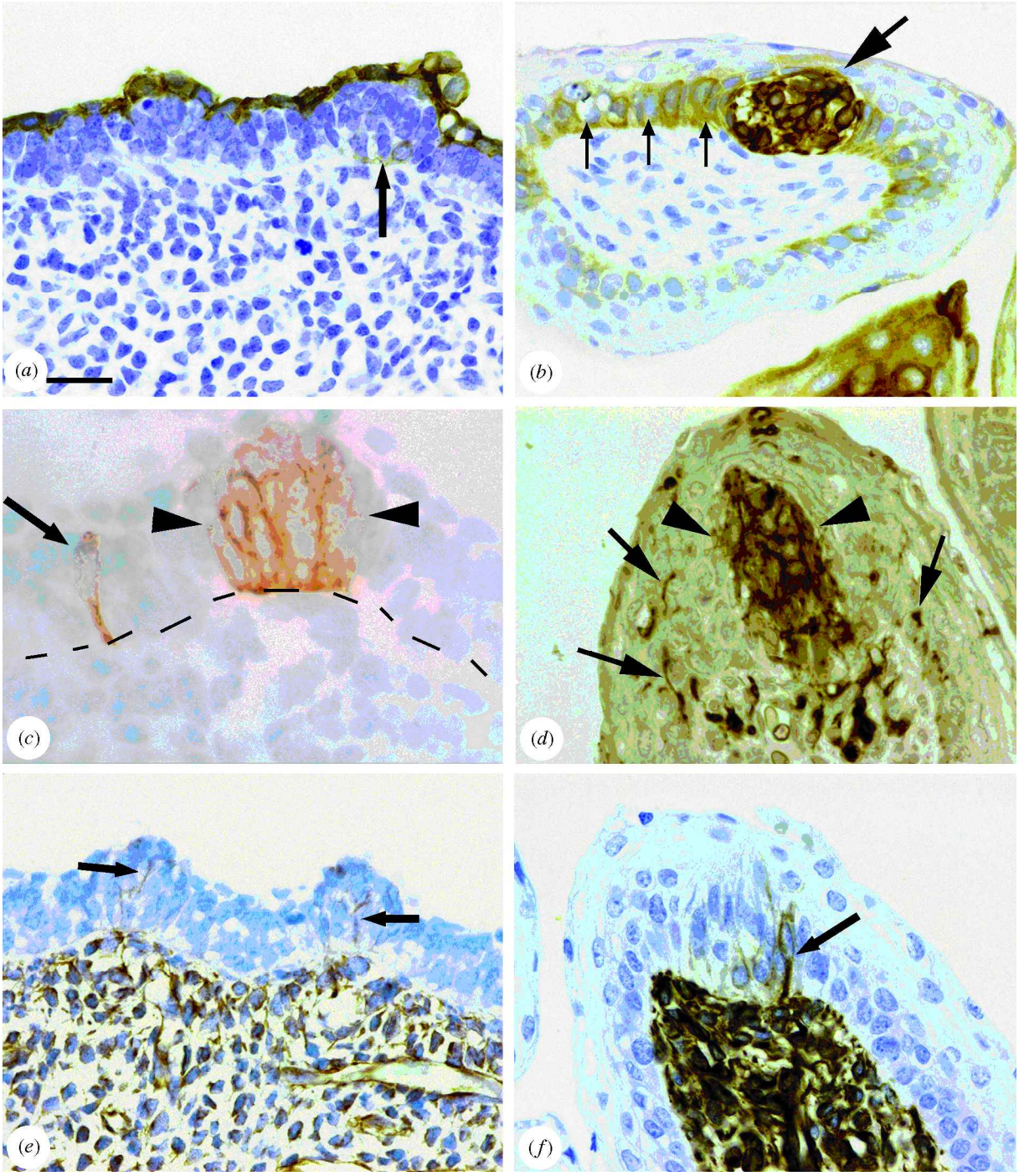

Figure 1. Immunohistochemical detection of intermediate filaments in human foetal taste bud primordia. Haematoxylin counterstain. Paraffin-embedded sections, 6 µm thick. Scale bars, 20 µm (*a*, *b*, *d–f*), and 10 µm (*c*). (*a*) At week eight, CK19 is present in apical cells of both non-gustatory epithelium and the taste bud primordium, as well as in a few basally located taste bud cells (arrow). (*b*) At week 23, CK19 immunoreactivity is restricted to taste buds (arrows) and the basal layer of adjacent epithelium (small arrows). From Witt & Kasper (1999). (*c*) At week eight, CK20 occurs in a subpopulation of taste bud primordial cells (arrowheads) and in an elongated solitary, probably chemosensory, cell (arrow). The dotted line demarcates the basal membrane subjacent a slightly elevated dermal papilla. From Witt & Kasper (1999). (*d*) At week 20, PGP 9.5 is expressed in a taste bud primordium (arrowheads) and some fine nerve terminals (arrows) in surrounding epithelium. Taste bud immunoreactivity is associated with gustatory nerves (chorda tympani) and neuronally transformed intrabud epithelial cells; nerve terminals in surrounding epithelium are probably trigeminal afferents. (*e*) At week eight, vimentin is expressed in taste bud primordia (arrows) and underlying mesenchymal cells (*a* and *e* are adjacent sections). (*f*) At week 20 (same specimen as shown in (*d*)), vimentin immunoreactivity is restricted to marginal cells of the taste bud (arrow). From Witt & Kasper (1999).

cell markers, e.g. neuron-specific enolase or protein gene product 9.5 (PGP 9.5). During early stages (weeks seven to eight) of human taste bud development, the hyaluronate receptor CD44 has been found exclusively on marginal cells of the taste bud primordium indicating a special role for these cells in cell migration and taste bud differentiation (Witt & Kasper 1998). In spite of the very selective expression of CD44 in human taste buds, this

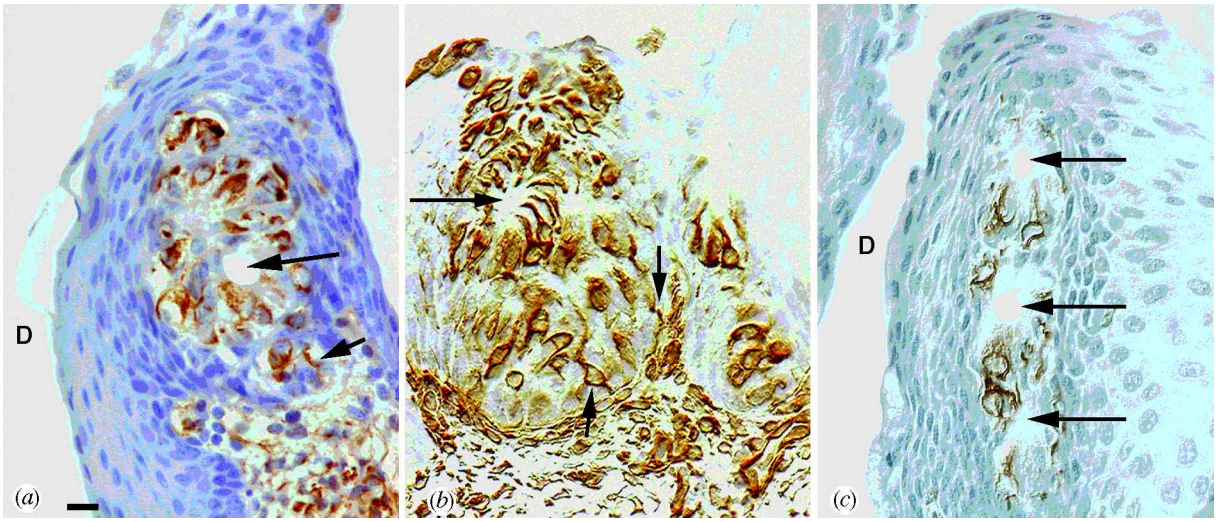

Figure 2. Vimentin immunoreactivity in taste bud primordia in the chick at (*a*) E19, (*b*) H1 and (*c*) H17. Haematoxylin counterstain. Scale bar, 10 μm. Between E17 and hatching, taste buds change from a ball-like to an ovoid shape. Larger arrows indicate intrabud lumina (channels). In (*c*), the lumen is cut through several times. Taste bud cells, some of which are vimentin immunopositive, are arranged in a rosette-like pattern around the channel. Some elongated vimentin-immunoreactive cells appear to contact underlying mesenchyme (shorter arrows in (*a*, *b*)). D, excretory duct of salivary glands.

well-characterized membrane protein occurs in cells that originate from all three germ layers. To distinguish between derivatives from different germ layers, we therefore chose intermediate filaments which comprise a large multigene family that can be subdivided into several classes. The distribution pattern of cytokeratins (CKs) in taste buds is different from that in adjacent non-taste epithelium, especially CK18, 19 and 20 (Witt & Kasper 1999; Zhang & Oakley 1996). In mammalian lingual epithelium, for example, CK20 is restricted exclusively to taste bud cells. If this is a very early protein marker in differentiating taste bud primordial cells, co-labelling studies may test for a direct temporal relationship between nerve fibre invasion and taste bud development.

While CK expression is generally restricted to derivatives of ectoderm and entoderm, another intermediate filament protein, vimentin, occurs in mesodermal derivatives (e.g. fibroblasts, endothelial cells, muscle cells and odontoblasts). In sensory systems, vimentin is expressed in olfactory epithelium, neurons and their projections into the olfactory bulb (Aoki *et al*. 1995; MacDonald *et al*. 1996).

2. MATERIAL AND METHODS

The immunocytochemical detection of intermediate filaments was carried out using formalin-fixed and paraffin-embedded human foetal tongues which were obtained from legally authorized and spontaneous abortions, from the sixth post-ovulatory week until late gestation. The specimens were collected according to the regulations published in the Declaration of Helsinki (Helsinki 1995). Immunohistochemistry was performed using monoclonal antibodies against CK18, 19 and 20 (Progen, Heidelberg, Germany; dilution 1:20 to 1:100; with pronase pretreatment) (Witt & Kasper 1999). In addition, a monoclonal antibody directed against vimentin (V3B4, Progen; dilution 1:200; pronase pretreatment) was applied in developing human and chick taste buds. In the latter, tissue was selected at representative stages of taste bud development, encompassing early stages of the bud primordium at embryonic day 17 (E17) (hatching = 21 days) and completed organogenesis at post-hatching day 17 (H17) (Ganchrow & Ganchrow 1987; Witt *et al*. 1999). Moreover, taste disc tissue of the aquatic frog, *Xenopus laevis*, was included because of its completely different organ structure and size after metamorphosis (cf. Witt 1993). Since antibodies against intermediate filaments in *Xenopus* were not available, routine transmission electron microscopy was used.

3. RESULTS AND DISCUSSION

CKs in human taste buds appear at very early stages of gestation. For example, as differentiation proceeds, CK18 and CK19 expression become more restricted to taste buds (figure 1*a–c*) and excretory ducts of the Von Ebner gland system. In contrast, CK20 immunoreactivity occurs exclusively in taste bud primordial cells. Exceptionally, at week eight CK20 is also expressed in solitary bipolar cells which are spatially separated from taste buds (figure 1*c*). However, CK20 was not detectable at earlier stages, i.e. prior to neural invasion of epithelium, which begins around weeks seven to eight (Witt & Reutter 1996).

Although the results obtained with CK immunohistochemistry in human tissue demonstrate a highly selective expression of these intermediate filament family proteins within taste bud cells, they do not demonstrate the transformation of epithelial cells into taste organ receptor cells prior to the invasion of nerve fibres. If one focuses on the taste bud's surrounding tissue environment, the interface between prospective taste bud cells and mesenchyme at the organ's base is characterized by an interrupted basal membrane (Witt & Reutter 1996). This could facilitate a variety of cells, nerve fibres, free proteins or simply ions to influence the process of taste bud development. In the developing chick, vimentin occurs in mesenchymal cells, and, surprisingly, in a subpopulation

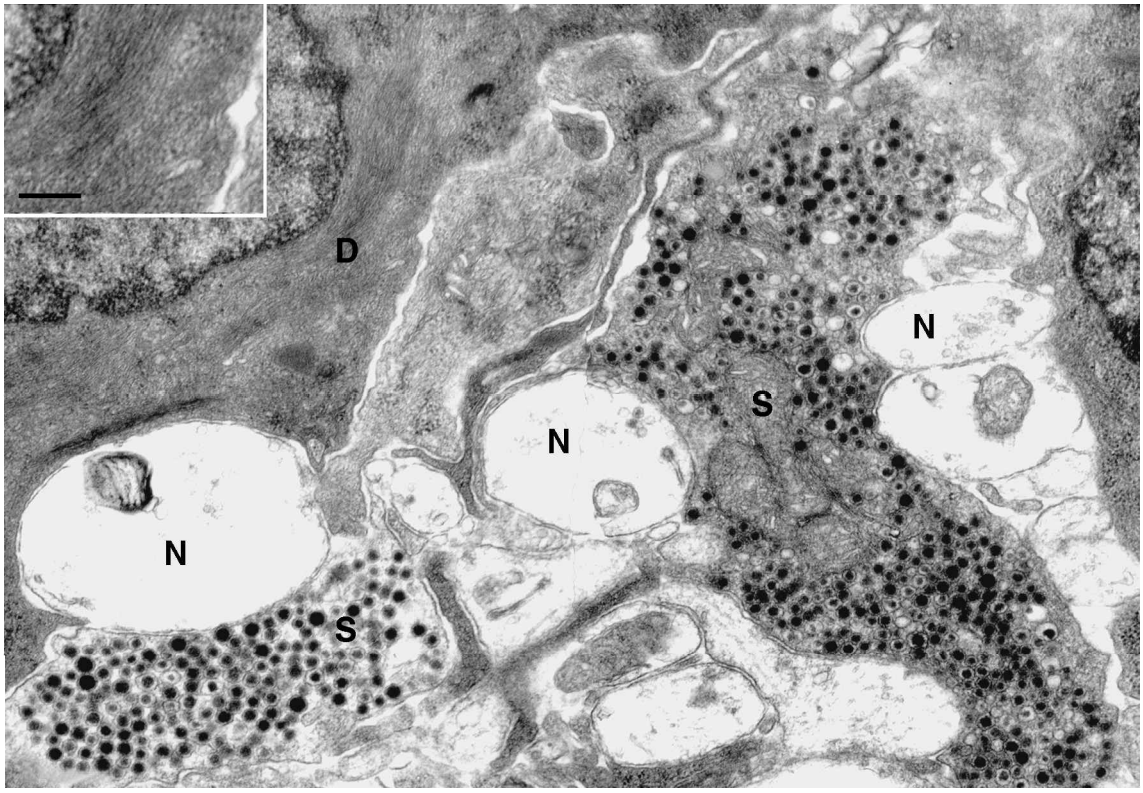

Figure 3. Transmission electron micrograph of the basal region of a *Xenopus* taste disc. Numerous nerve fibre terminals (N) are in close contact with basal processes of sensory cells (S) which are filled with dense-cored vesicles. An electron-dense cell (D) exhibits a dense bundle of parallel-running perinuclear intermediate filaments (see inset enlargement of the area around 'D' in the upper left corner). Scale bar, 200 nm; inset, 100 nm.

of taste bud cells. In humans, modest vimentin expression first occurs in taste bud primordial cells (figure 1*e*), and is later restricted mainly to marginal cells of the bud (figure 1*f*). In chick, an apparent communication with the mesenchymal compartment is indicated by occasionally occurring vimentin-immunopositive cells which appear to 'bridge' the dermal papilla and taste bud primordium (figure 2*a*,*b*).

Comparison of ratios of vimentin-immunoreactivity bud cells across developmental ages was analysed by ANOVA. A posteriori multiple comparisons of means were examined by the Tukey–Kramer honest significant difference (HSD) test. The proportion of vimentin-immunopositive cells in total taste bud cells in the chick varies significantly ($p < 0.05$) as a function of age: the percentage (70%) of immunopositive bud cells peaks at E19 and then declines to about 50% in maturer taste buds (H1 and H17) (Witt *et al*. 1999). Moreover, vimentin expression is more widely distributed compared with that in human taste buds where vimentin-immunopositive cells are fewer and restricted mainly to the taste organ's marginal zone (figure 1*f*). For human taste bud development, this may suggest an interaction with adjacent non-gustatory epithelial cells rather than cells of the mesenchymal compartment.

One of the factors facilitating, or accompanying vimentin expression in taste bud cells may be discontinuities or 'disturbances' in epithelium during development or dedifferentiation. The disrupted basal lamina may provide the possibility for an interaction of early-forming taste bud cells with mesenchyme. Sensory cells in developing olfactory epithelia also express vimentin (Aoki *et al*. 1995), confirming that neuroepithelial chemoreceptive organs share properties observed in other borderline tissue regions in which an active interrelationship between epithelial and mesenchymal compartments is demonstrated by vimentin immunopositivity.

Transdifferentiation from mesenchymal to epithelial properties, and vice versa, is a well-known phenomenon in cell-culture systems (Dumortier *et al*. 1998) and may explain vimentin expression in taste bud cells during proliferation and replacement. In teleostean fishes, replacement of taste bud cells has been shown to occur from adjacent epithelial cells (Raderman-Little 1979). The especially dense distribution of intermediate filaments in electron-dense taste bud cells in fishes (Reutter & Witt 1993) may indicate the presence of vimentin, although immunohistochemical evidence is lacking. The taste disc of *Xenopus* is of particular interest, since the filament distribution in electron-dense taste cells (figure 3) resembles that seen in fishes and chick (Ganchrow *et al*. 1991; Reutter & Witt 1993). Intermediate filaments occurring in Merkel-like basal cells of amphibian taste discs are most probably CKs, since epidermal Merkel cells also are known to possess CK20, suggesting an ectodermal origin (Moll 1993). Moreover, vimentin expression has not been reported in epidermal Merkel cells.

The differences in the extent and location of vimentin expression in early developing taste buds of humans and chicks, however, suggest that mechanisms of differentiation of the same receptor organ vary among vertebrate forms.

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