© 2017. Published by The Company of Biologists Ltd | Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

REVIEW

Epithelial cell behaviours during neurosensory organ formation

Marika Kapsimali[1,2,3,*]

ABSTRACT

Perception of the environment in vertebrates relies on a variety of neurosensory mini-organs. These organs develop via a multi-step process that includes placode induction, cell differentiation, patterning and innervation. Ultimately, cells derived from one or more different tissues assemble to form a specific mini-organ that exhibits a particular structure and function. The initial building blocks of these organs are epithelial cells that undergo rearrangements and interact with neighbouring tissues, such as neural crest-derived mesenchymal cells and sensory neurons, to construct a functional sensory organ. In recent years, advances in *in vivo* imaging methods have allowed direct observation of these epithelial cells, showing that they can be displaced within the epithelium itself via several modes. This Review focuses on the diversity of epithelial cell behaviours that are involved in the formation of small neurosensory organs, using the examples of dental placodes, hair follicles, taste buds, lung neuroendocrine cells and zebrafish lateral line neuromasts to highlight both well-established and newly described modes of epithelial cell motility.

KEY WORDS: Hair follicle placode, Lateral line primordium, Lung neuroendocrine cell body, Molar tooth placode, Slithering, Taste bud

Introduction

In vertebrates, an array of small, epithelium-derived neurosensory organs ensures the perception of environmental conditions. The structure and function of these organs varies: they can be composed of sensory cells innervated by sensory nerve fibres, as occurs in lung neuroendocrine cell bodies (NEBs), they can include support cells, as seen in the case of taste buds and neuromasts of the zebrafish lateral line, or they can include cells that produce protective or isolating substances, as seen in ectodermal appendages such as teeth, hair follicles, scales and feathers. Although they differ in their specific roles and developmental origins, and hence are not classically grouped together, the common feature of these neurosensory organs is that they are initially composed of epithelial cells. Mesenchyme and sensory neurons can provide additional signals, cells or innervation that contribute to induction, differentiation or patterning of the epithelium, thus complementing the capacity of the epithelial cells to construct the organ (i.e. Ahn, 2015; Barlow, 2015; Biggs and Mikkola, 2014; Hardy, 1992; Kapsimali and Barlow, 2013; Lee and Tumbar, 2012; Naveau et al., 2014; Pispa and Thesleff, 2003).

The development of each neurosensory mini-organ relies, first, on a series of events that ultimately results in progenitor cells differentiating into the cell types that compose the mature organ and, second, on cell rearrangements taking place during the transformation of a uniform epithelium to an organ with elaborated structure and function. A plethora of studies have provided insights into the key molecular mechanisms that underlie cell induction and differentiation during the formation of these mini-organs (reviewed by Ahn, 2015; Aman and Piotrowski, 2011; Barlow, 2015; Biggs and Mikkola, 2014; Chitnis et al., 2012; Cutz et al., 2013; Fuchs, 2015; Fuchs and Nowak, 2008; Heller and Fuchs, 2015; Lee and Tumbar, 2012; Montell, 2008). However, it is only during the last few years that methodological improvements have rendered these organs accessible for *in vivo* imaging techniques. These methods have allowed researchers to literally 'follow' cells *in vivo*, providing additional information about cell behaviours during organ formation. Although only some organs have been examined in this way to date, these studies are beginning to provide insights into not only well-established but also newly described modes of epithelial cell displacement, revealing a greater diversity of intraepithelial cell motility than previously appreciated. This Review focuses on the behaviours of epithelial cells during the formation of neurosensory mini-organs, using the examples of the molar tooth placode, hair follicle placode, neuromast primordium, lung neuroendocrine (NE) organs, and taste buds. For the sake of clarity, key terms and phrases are defined in the Glossary (Box 1). In addition, the various types of epithelial cell displacement that have been described to date, and that are referred to throughout the article, are explained in Box 2.

The molar dental placode: a role for migration and a tensile canopy

Mice have three molars and one incisor tooth on each half of the lower and upper jaws. The early steps of tooth development in mammals rely on interactions between oral epithelial and neural crest-derived mesenchymal cells (reviewed by Biggs and Mikkola, 2014; Zhang et al., 2005). Within the mouse mandible epithelium, the first morphological sign of molar tooth formation is the molar placode – a thickening of the tooth-generating epithelium (the dental epithelium) that arises at approximately embryonic day (E) 11.5 (Fig. 1). Through cell proliferation and invagination, this placode grows inward within the mandible, forming a bud (between E12.5 and E13.5) around which mesenchymal cells condense. At E14.5, epithelial cells of the molar bud, distal to the oral surface, undergo further folding and the enamel knot – a cluster of non-dividing epithelial cells – appears. As discussed below, the displacement behaviours exhibited by placodal cells during these events have been analysed in detail.

A number of studies have shown that the fibroblast growth factor (Fgf) and Sonic hedgehog (Shh) signalling pathways play key roles during molar tooth placode formation. Stratification of the molar dental epithelium occurs through cell divisions perpendicular to the basement membrane, generating basal and suprabasal cells, and the abrogation of Fgf receptor signalling (using SU5402) and complementary activation of Fgf signalling (using Fgf10-soaked beads applied to E11.5 mandible slice cultures) has shown that Fgf signalling activity is necessary and sufficient for this epithelial

[1]Institute of Biology of the Ecole Normale Supérieure, IBENS, Paris 75005, France. [2]INSERM U1024, Paris 75005, France. [3]CNRS UMR 8197, Paris 75005, France.

*Author for correspondence (marika.kapsimali@ens.fr)

M.K., 0000-0001-9327-5166

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

Box 1. Glossary

Cell position. Characterized by coordinates along the *x*, *y* and *z* axis.

Cell displacement (or translocation). A change in cell position (and thus coordinates).

Cell motility. The capacity of a cell to be displaced. It implies a variety of morphological and molecular characteristics that enable a cell to change position and is opposite to a stationary state.

Epithelial cell displacement. Can be initiated by introduction of asymmetry in cell-cell adhesion (i.e. reduction of cadherin expression) or when cytoskeletal contractility [constriction of actin filaments (F-actin) by the molecular motor non-muscle myosin II] results in constriction of the cell body (see also Martin and Goldstein, 2014; Sawyer et al., 2010; Panousopoulou and Green, 2016). The cell body translocates and cell displacement takes place when constriction is accompanied by cell membrane protrusion (extension supported by actin filaments, dictating the side of the cell that protrudes, termed the 'front'; the opposite side, termed the 'rear' side, retracts), changes in nuclear shape (Webster et al., 2009), and eventual changes in cell-extracellular matrix adhesion (if the cell is attached to the basement membrane).

Intraepithelial motility/displacement. The corresponding event occurring within a single epithelial tissue. This is to highlight the fact that during mini-organ formation, epithelial cells can be motile within (at the level of) the main epithelial sheet that builds the organ. This is in contrast with the capacity of epithelial cells to exit the epithelium of origin, migrate long distances through the extracellular matrix and form other tissues or organs, for instance as occurs in the mesendoderm during gastrulation or neural crest formation.

Non-motile epithelium. A single or multiple (stratified) layered array of apico-basally polarized cells having regular shape. The basal row of cells secretes components of the basement membrane on which the epithelium is attached. A non-motile epithelium is maintained with minimal energy, as the contact surfaces of adjacent cells are maximal (Farhadifar et al., 2007; Fernandez-Gonzalez and Zallen, 2008; Heller and Fuchs, 2015). The positions of adjacent cells are supported by the cytoskeleton and associated adherens junctions, which are protein complexes including cadherins and catenins (Walck-Shannon and Hardin, 2014). The cytoskeleton-adherens junction network communicates shape changes occurring in one cell to its neighbours (Martin and Goldstein, 2014; Montell, 2008).

stratification (Li et al., 2016). Indeed, various Fgf pathway components are expressed during molar placode formation: Fgf8 and Fgfr2 are broadly expressed in the dental epithelium (Fgfr2 isoform IIIb; Kettunen et al., 1998; Laurikkala et al., 2006; Li et al., 2014; Prochazka et al., 2015), Fgfr1IIIc in the mesenchyme (Kettunen et al., 1998; Li et al., 2014), and Etv4 broadly in the dental epithelium and mesenchyme (Porntaveetus et al., 2011; Prochazka et al., 2015). Invagination of the molar placode, by contrast, relies partially on Shh signalling; inhibition of Smo receptor activity by cyclopamine at E12.5 alters the shape of basal and suprabasal cells and nuclei from elongated to round, suggesting reduced cell motility, and mice with conditional *Shh* deletion in the oral ectoderm show wider and shallower molar buds (Dassule et al., 2000; Li et al., 2016). In line with this, it has been shown that Shh is expressed in the dental epithelium (Bitgood and McMahon, 1995; Dassule and McMahon, 1998; Keränen et al., 1998; Prochazka et al., 2015), and the Smo and Shh targets Ptch1 and Gli1 broadly in the dental epithelium and mesenchyme (Dassule and McMahon, 1998; Hardcastle et al., 1998; Prochazka et al., 2015). Yet, as SU5402 or cyclopamine treatments have tissue-wide (epithelium and mesenchyme) effects, and given that colocalization data of Fgf and Shh signalling components at the single-cell level are not available, it is difficult to assess precisely the direct target cells of Fgf and Shh signalling during molar tooth placodal thickening and invagination.

However, it has been shown that the conditional elimination of Fgf8-expressing epithelial cells (by diptheria toxin A) results in initiation of the molar placode but not further growth of the tooth bud (Prochazka et al., 2015), suggesting that Fgf8-expressing epithelial cells are required for the latter process. In addition, clonal analyses and *in vivo* imaging using a confetti multicolour reporter under the control of $Fgf8^{ires\text{-}cre}$, have shown that Fgf8-expressing cells and their descendants are dispersed in a mosaic manner in the E14.5 molar tooth, rather than retaining adjacent positions in clonal patches, suggesting a complex mode of displacement (Prochazka et al., 2015). *In vivo* imaging has also shown that, at E11.5, Fgf8-expressing cells form a centripetal-oriented structure (a rosette) at the surface of the epithelium (Fig. 1A,B). The centre of this rosette is located at a distance from Shh-expressing epithelial cells located at the level of the molar placode (Prochazka et al., 2015), and it is difficult to judge whether all Fgf8-expressing/derived and Shh-expressing cells are two entirely different and/or distant populations. Nevertheless, the rosette of Fgf8-expressing/derived cells has been shown to migrate anteriorly towards the placode's Shh-expressing cells (Fig. 1B). *In vivo* imaging of the rosette, together with studies of E-cadherin (cadherin 1) and actin localization, suggests that subgroups of cells (mini-rosettes) are present within the Fgf8-expressing/derived cell population. By measuring the track straightness (see Box 2) of Fgf8-expressing/derived cells as they are displaced towards Shh-expressing cells, it was concluded that they have directionality in their displacement and therefore actively migrate.

What, then, are the signals that promote motility and/or provide direction to Fgf8-expressing/derived cells? When Fgf8 activity is conditionally abrogated in the Fgf8-expressing cell population, cell displacement is severely reduced, and formation of the dental placode arrests, suggesting that Fgf8 activity is somehow involved in cell motility in this context (Prochazka et al., 2015). However, previous studies (MacArthur et al., 1995; Zhang et al., 2006) have shown that Fgf8 activates Fgfr1IIIc, which is expressed in the mesenchyme (Kettunen et al., 1998; Li et al., 2014), and not Fgfr2IIIb, which is expressed in the epithelium (Kettunen et al., 1998; Laurikkala et al., 2006; Li et al., 2014; Prochazka et al., 2015), so it is not clear whether this role of Fgf activity is mediated by epithelial or mesenchymal Fgf receptors. Furthermore, when Fgf8-expressing cells are rendered incapable of responding to Shh signalling (by specific inactivation of the Smo receptor), formation of the molar placode is initially arrested and Fgf8-expressing/derived cell displacement is randomly orientated (Prochazka et al., 2015). However, this phenotype is transient, and the molar tooth germ appears normal by E14.5. Nonetheless, when SmoM2 – a gain-of-function allele – is conditionally expressed in Fgf8-expressing cells, the dental placode expands rostrally and cell motility becomes variable, with subsets of Fgf8-expressing cells showing either increased or decreased displacement compared with wild-type cells, highlighting a role for Shh signalling in the cell displacement process. In line with this, it has been shown that Fgf8-expressing cells are displaced towards Shh-soaked beads placed into the posterior side of the explant (opposite to placodal Shh cells) (Prochazka et al., 2015). In summary, these findings suggest that Shh activity provides directionality to Fgf8-expressing cells in only some contexts. The mechanism through which Shh could act as chemoattractive signal for Fgf8-expressing/derived cells, which are initially localized at a considerable distance from the Shh-expressing cell group, is unclear. It also remains unclear whether all cells of the rosette share the same motility/behaviour, or whether cells of the subgroups (the mini-rosettes), as suggested by

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

Box 2. Types of epithelial cell displacement occurring during organ formation

Various mathematical analyses can be used to characterize modes of cell displacement. For example, mean square displacement (MSD) refers to a statistic describing cell displacement on average, in a given interval of time, squared. If the plot of MSD versus time interval follows a linear trend, the cell moves in a random walk; if it follows an increasing slope, the movement has direction (migration); if the curve reaches a plateau, the motion is confined. Other frequently used measurements include persistence or straightness of the track (i.e. the ratio of the displacement from the initial to the final cell position, to the total length of the path that the cell has travelled) and escape angle (i.e. the angle between two vectors, one drawn from a reference point to the initial cell position, the other from the same reference point to the last cell position) (Beltman et al., 2009; Meijering et al., 2012; Saxton and Jacobson, 1997). In some cases, displacement is defined according to changes in cell neighbours, cell morphology, gene expression and/or molecular localization. As such, different types of cell displacement have been reported for epithelial cells, as briefly described below.

Random cell motility. Cell displacement with no specific pattern (i.e. without direction, by chance), reminiscent of particle diffusion and Brownian motion.

Directed cell motility or migration. Guided cell displacement parameterized by speed.

Confined cell motility. Cell displacement constrained within a region because of obstacles, such as other cells.

Cell intercalation. Intraepithelial displacement that may be mediolateral and/or radial. In the case of mediolateral intercalation, an epithelial layer extends along a given axis to form an elongated structure and cells exchange positions mediolaterally, i.e. orthogonal to the elongation axis. In the case of radial intercalation, cells exchange positions in adjacent layers of a multi-layered epithelium (Walck-Shannon and Hardin, 2014).

Mitosis-associated cell dispersal. The cell body of a pre-mitotic cell translocates into the lumen to divide while maintaining a thin process in contact with the basal membrane. After division, one daughter cell inherits the basal process and retracts from the lumen to acquire a position among the original neighbouring cells, whereas the other integrates within the epithelium a few cell diameters away (Packard et al., 2013).

Slithering. A single epithelial cell passes by neighbouring epithelial cells, makes contact and integrates into a group of cells located several cell diameters away. The cell has directed motility, i.e. it migrates, and this occurs within the epithelium of origin (intraepithelially) (Kuo and Krasnow, 2015; Noguchi et al., 2015; Soulika et al., 2016). It is currently unclear to what extent a slithering cell shares similar molecular characteristics with cohorts of cells undergoing EMT (see below).

Epithelial-mesenchymal transition (EMT). Originally defined as the process by which epithelial cells lose apico-basal polarity, extend filopodia, are transformed to mesenchymal cells with front end-back end polarity forming only transient contacts with neighbours, and are displaced through the extracellular matrix (Hay, 1995). EMT was originally associated with large scale migration, i.e. cohorts of cells being displaced away from the epithelium of origin. Among the most-studied cells undergoing EMT are mesendodermal precursors migrating from the primitive streak to form the respective mesoderm and endoderm, neural crest cells delaminating from the neural tube to form a variety of organs, and cancer cells detaching from tumours and moving into adjacent tissues (Brabletz, 2012; Bronner and Simões-Costa, 2016; Campbell and Casanova, 2016; Diepenbruck and Christofori, 2016; Scarpa and Mayor, 2016; Theveneau et al., 2013).

differences in their expression of E-cadherin and possibly their adherens junctions, behave differently. Related to this, it should be noted that the study conducted by Prochazka et al. (2015) only took into account cells from the upper quartile of vector lengths (longer than 25.177 mm), and eliminated most non-migrating cells, suggesting that there are indeed additional epithelial Fgf8-expressing/derived cells that are motile but do not exhibit directional motility. More detailed kinematics of Fgf8-expressing cells are needed to clarify whether all cells in the rosette migrate cohesively, or if some are displaced in a random or confined mode (see Box 2) relative to others, possibly acting as ‘leaders’.

Further insights into molar dental placode formation have been provided by recent time-lapse imaging of unlabelled molar dental placodal cells combined with either partial incision within the placode or the excision of neighbouring (mesenchymal) cells

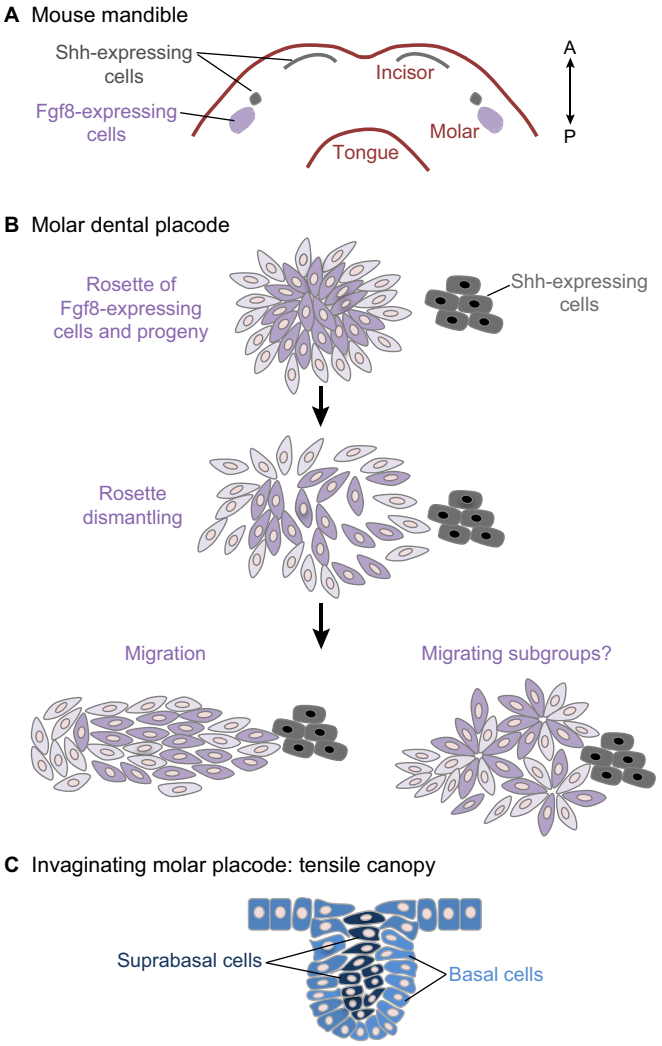

Fig. 1. Epithelial cell behaviours in the molar tooth placode. (A) Schematic oral view of the mouse mandible at approximately E11.5, highlighting Shh-expressing cells and Fgf8-expressing cells that are situated at the positions of the dental epithelium that give rise to incisor placodes and the anterior-most molar placodes. A, anterior; P, posterior. (B) Fgf8-expressing cells and their progeny (purple/light purple) form a rosette-like structure in the oral epithelium, the centre of which (purple cells) is located away from molar placodal Shh-expressing cells (grey). During molar placode morphogenesis, the rosette dismantles and the Fgf8-expressing cells/progeny migrate towards the Shh-expressing cell group. Observations from *in vivo* imaging and cell adhesion molecule expression studies suggest the existence of migrating cell subgroups. Based on data from Prochazka et al. (2015). (C) During molar tooth placode invagination, a tensile canopy is formed by suprabasal cells, which migrate towards the centre of the placode, and basal cell bodies, which remain attached to the basement membrane but elongate and intercalate with suprabasal cells. Additional suprabasal cells are pushed downwards, thereby promoting invagination of the placode. Based on data from Panousopoulou and Green (2016).

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

to examine the forces at play during placode formation (Panousopoulou and Green, 2016). Using this approach, it was shown that molar placode invagination relies on a tensile canopy formed by mechanical coupling of migrating suprabasal cells and elongated, basement membrane-attached basal cells (Fig. 1C). The suprabasal cells were shown to have characteristics of motile cells, such as an ellipsoid nucleus and reduced E-cadherin, and extend apical protrusions and migrate horizontally towards the centre of the placode with a centripetal orientation. This process is driven by endogenous, contractile forces in the suprabasal cells. In the circumference (shoulders) of the placode, an area that mostly shows minor invagination and sustains the placode in its position, basal cells stretch centripetally and actively intercalate with suprabasal cells whilst remaining attached to the basement membrane (see also Box 2). These results show that the invagination of the molar tooth placode relies on a tensile canopy formed by centripetally migrating suprabasal cells and their associated elongated 'shoulder' basal cells. This canopy drives the intercalation of additional suprabasal cells inwards and bends the inner part of the placode (Panousopoulou and Green, 2016). The molecular signals orchestrating this invaginating cell behaviour remain unclear but it is likely that Shh signalling contributes to this process (Li et al., 2016).

Although the studies described above have focussed on molar teeth, some insights into epithelial motility during placode morphogenesis have also been provided by studies of the developing mouse incisor (Ahtiainen et al., 2016; Sharir and Klein, 2016). At early stages of its formation, the incisor contains a focal group of Shh-expressing cells (Fig. 1A). In contrast to surrounding placodal cells, these cells are non-dividing (i.e. are in the G1 phase of the cell cycle), are present in constant numbers, constantly express Shh (E11.5-E13.5), remain localized close to the oral surface, do not invaginate, and are distinct from the enamel knot (which appears *de novo* at the tip of the mature bud). This early Shh-expressing group initially forms a narrow stripe, but the analysis of cell tracks and angles of displacement has shown that the cells in the lateral part of the cell stripe migrate directionally towards the mandible midline, and medial cells move towards the centre of the cohort, resulting in a condensed cell group. In contrast, the surrounding cells (i.e. those that were originally in the S/G2/M phase of the cell cycle) divide and their progeny contribute to the growing bud (Ahtiainen et al., 2016; Sharir and Klein, 2016), although it should be noted that the motility behaviour of these cells has not yet been described. It is also not clear whether the Shh-expressing cells in the molar placode share similar properties to those of the developing incisor Shh-expressing focal group.

In summary, cell displacement within the mammalian dental epithelium that generates the molar and incisor teeth placodes does not occur only via cell division. Subsets of epithelial cells are actively motile, i.e. they can migrate (e.g. as seen in the case of molar placode cells and the focal group of Shh-expressing cells in the incisor) or intercalate due to endogenous, contractile forces (e.g. the tensile canopy that forms during molar placode invagination).

The hair follicle placode: key roles for migration and compaction

Similar to the dental placode, the formation of the hair follicle placode involves interactions between epithelial and mesenchymal cells. Indeed, the site at which a hair placode forms within the mouse epidermis is dictated by early cues provided by mesenchymal cells. The placodal site is marked by the activity of a molecular network involving the Wnt/β-catenin signalling pathway, its downstream targets Eda-A1 (Eda) ligand and Edar receptor, and the transcription factor NF-κB. Hair primary placodes subsequently transition into hair follicles through downwards growth of the placode towards the dermis, in a process that involves cell proliferation, differentiation, polarization and displacement (Ahn, 2015; Andl et al., 2002; Biggs and Mikkola, 2014; Fuchs, 2007; Hardy, 1992; Zhang et al., 2009).

A recent study examined how cell diversity is generated in the forming hair follicle (Ouspenskaia et al., 2016). It was shown that Wnt signalling is upregulated in basal placodal cells, which divide perpendicular to the apico-basal axis and asymmetrically generate suprabasal daughter cells; the early-generated suprabasal daughter cells are characterized by low Wnt signalling, respond to Shh secreted by the basal parental cell, and become stem cells expressing Sox9, whereas late-generated suprabasal cells give rise to differentiated cells of the hair lineages. Although the role of cell proliferation in the formation of the hair placode itself (i.e. the initial thickening of the epidermal epithelium) has long been debated (Magerl et al., 2001; Mustonen et al., 2004; Schmidt-Ullrich et al., 2006), it was recently shown that most hair follicle placodal cells are in the G1 phase of the cell cycle at the preplacodal and placodal (E13.5-E14.5) stages (Ahtiainen et al., 2014). Furthermore, live imaging of mouse embryonic skin whole-mount explants using Fucci transgenes demonstrated that, within the core of the placodes, many non-dividing cells are motile and their behaviour is tightly linked with changes in contractility and localization of adhesion molecules. For instance, actin is polarized in cells at the edge of the placode, exhibiting an orientation towards the centre of the placode. In contrast, actin remains unpolarized in interplacodal cells. The prevention of actin polymerization or the inhibition of F-actin capping inhibited hair follicle placode formation. When cell motility parameters in placodal and interplacodal epithelial cells were compared in detail, it was shown that placodal cells change neighbours and are displaced along a straighter track, being directed to the centre of the placode. The analysis of escape angles (see Box 2) also showed that placodal cells are directed to the centre of the placode whereas interplacodal cells remain away from it. Furthermore, as epithelial cells accumulated in a restricted space, they became more compact by decreasing their cell volume (Ahtiainen et al., 2014). These findings therefore highlight that migration with an inward direction to the centre of the placode and cell compaction are key processes during hair placode formation (Fig. 2).

The precise molecular mechanisms providing directionality to hair placodal cells have not yet been elucidated, although some insights have recently been obtained. In particular, it was shown that activation of Eda or Wnt canonical signalling results in placodes of increased size. Under these conditions, the number of cells with a placodal fate is increased in interplacodal areas and consequently more cells become motile. This increase in the number of motile cells results in increased variation of cell displacement parameters. For instance, cells with activated Eda are displaced over longer distances, but the straightness of their tracks is reduced (Ahtiainen et al., 2014). Impaired activity of the transcription factor Lhx2 results in compromised proliferation and loss of expression of filamentous actin and activated focal adhesion kinase (FAK; Ptk2), both of which are markers of directed cell displacement, suggesting that Lhx2 is also involved in hair placode growth and morphogenesis (Tomann et al., 2016).

Finally, it should be noted that, macroscopically, the hair follicle placodal epithelium bends downwards, towards the

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

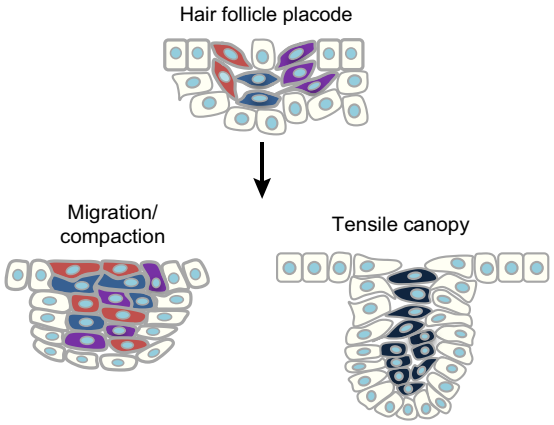

Fig. 2. Epithelial cell motility during hair follicle placode formation. Formation of the hair follicle placode (top panel) relies on the migration of postmitotic placodal cells (multicoloured). In contrast to basal and interplacodal cells (white), the placodal cells actively change neighbours, migrate and compact towards the centre of the placode (lower panel, left) (Ahtiainen et al., 2014). The analysis of cell and nucleus shapes suggests that a suprabasal cell-derived tensile canopy might also be involved in placode formation in this context (lower panel, right; suprabasal cells are shown in dark blue, basal cells in white) (Panousopoulou and Green, 2016); indeed, the two mechanisms could be complementary.

mesenchyme, as occurs in the case of the invaginating molar tooth placode. Although *in vivo* imaging time-lapse studies have not yet been performed, transverse sectioning of the hair follicle placode and observations of cell and nuclear shape suggest that suprabasal and basal epithelial cells form a tensile canopy in the hair follicle placode, reminiscent of that seen in the molar tooth placode (Fig. 2) (Panousopoulou and Green, 2016). Therefore, these ectodermal appendages might utilise common mechanisms of cell topology and displacement to achieve invagination.

The lateral line primordium: migration and differentiation via organized teamwork

The lateral line of aquatic vertebrates is composed of neuromasts – mechanosensory organs consisting of support and hair cells, the latter of which detect the direction of water movement (Chitnis et al., 2012; Nechiporuk and Raible, 2008). The formation of neuromasts has been extensively studied in zebrafish, in particular by imaging the behaviour of neuromast progenitors in the posterior lateral line (pLL) primordium. The pLL primordium consists of an oval-shaped cluster of migrating cells; the cells towards the leading end of this cluster exhibit a mesenchymal-like morphology (this part of the primordium has been referred to as the 'leading region/zone/domain' and the cells with protrusive activity located at the edge of the leading zone as 'leaders'; Haas and Gilmour, 2006; Valentin et al., 2007), whereas cells in the trailing end (which have been referred to as the 'trailing region/zone/domain' or 'followers', corresponding to approximately two thirds of the primordium; Aman and Piotrowski, 2009; Chitnis et al., 2012; Ma and Raible, 2009; Nechiporuk and Raible, 2008; Revenu et al., 2014) are sequentially reorganized to form compact, cohesive epithelial rosettes known as proneuromasts (Fig. 3). The pLL primordium migrates on the surface of the larval body along the horizontal myoseptum from the ear to the tail and deposits the proneuromasts that further differentiate into mature mechanosensory organs.

The displacement of the pLL primordium is a collective, coordinated cell migratory process. Cell displacement and cell polarity during this process have been characterized using vectors that link the positions of the centre of the mass of the nucleus and the centrosome in individual cells with, as a reference point, the centre of the mass of the closest rosette. In this manner, it has been shown that the centrosome is located behind the nucleus in cells within the leading domain, suggesting that they have rear orientation (Fig. 3A). Further along the front-rear axis, a transition zone in which cell organelles become reorganized is present (Revenu et al., 2014). That is, centrosomes become located towards the front of cells, Cadherin 2 molecules are sustained by a dynamic microtubule network to form clusters in the apical plasma membrane, and cell orientation is reversed to allow radial organization of the developing proneuromasts. As progenitor cells differentiate into proneuromasts, and as the centripetal organization of the forming mechanosensory organ is maintained, epithelial adhesion (i.e. apical adherens junctions) becomes more stable and independent of the dynamic microtubule network in the mature deposited organs (Revenu et al., 2014).

A number of studies have shown that the migration of the pLL primordium is driven by a chemokine gradient established along its front-rear axis and generated by the asymmetric availability of the chemokine ligand Cxcl12a, which is localized only in the leading but not the trailing edge (Fig. 3B). Two mechanisms have been proposed to explain this differential chemokine distribution. In the first, it is proposed that Cxcl12a binds to two biochemically distinct receptors, Cxcr4b and Cxcr7b (Ackr3b), that are expressed in the leading and trailing domains, respectively (Fig. 3B), with the latter promoting internalization and degradation of the ligand (Dambly-Chaudière et al., 2007; David et al., 2002; Donà et al., 2013; Valentin et al., 2007). In a second model, it is proposed that the forward migration of the primordium, combined with the binding of Cxcl12a by Cxcr4b, results in a lower concentration of available ligand behind the trailing domain than in the front of the primordium (at the leading edge, where *cxcl12a* is expressed; Streichan et al., 2011). In both cases, it is unclear how the chemokine gradient regulates the migration of the trailing zone. A recent study has attempted to dissect these models (Dalle Nogare et al., 2014). In a primordium with abrogated Cxcr4b activity, the transplantation of a few Cxcr4b cells is sufficient to restore the migration of the primordium, only when the Cxcr4b cells are positioned at the leading edge (as leader cells; Haas and Gilmour, 2006). In addition, when Cxcl12a expression is ectopically induced in the entire primordium, protrusive (mesenchymal-like) cells are localized around the entire leading part of the primordium, yet the trailing cells remain unresponsive (Dalle Nogare et al., 2014). These results favour the following model: Cxcl12a and Cxcr4b interact at the leading edge to drive migration of the primordium, with availability (i.e. a threshold) of Cxcl12a rather than a gradient being necessary for migration; the role of Cxcr7b is to prevent more posteriorly localized Cxcr4b receptors from having access to Cxcl12a, hence preventing activity levels that are sufficient to induce cell protrusions (Dalle Nogare et al., 2014).

Based on these experiments and observations, the dependence of leading and trailing regions on chemokine signalling has also been simulated using agent-based modelling (Dalle Nogare et al., 2014). These simulations could explain the chemokine-dependent behaviour of the leading domain but could not recapitulate the migratory behaviour of trailing cells. Thus, to explore further how leading cells influence the migratory behaviour of trailing cells, laser cell ablation was used to separate the primordium mechanically into three fragments (named the trailing, middle and leading fragments), each containing one developing protoneuromast. *In vivo* imaging demonstrated that, even after

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

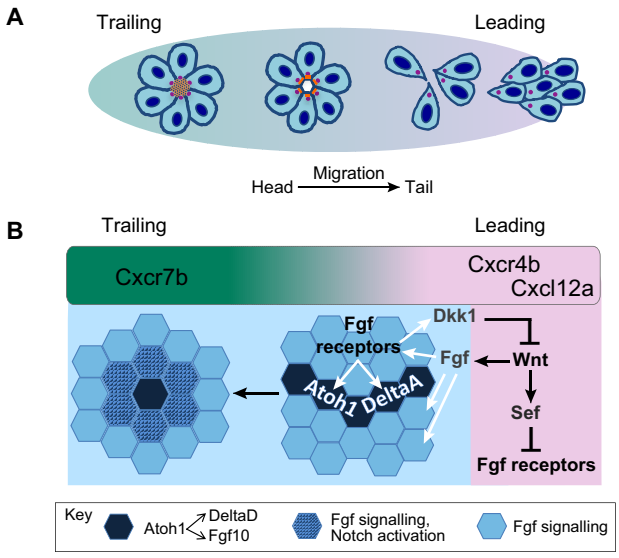

Fig. 3. Epithelial cell behaviours in the migrating pLL primordium. (A) The figure depicts cell orientation and adhesion in the leading zone of the migrating primodium, in the transition zone (as cells assemble into proneuromasts), and in developing proneuromasts (rosettes) at the trailing edge. Apical flattened views of proneuromasts; nuclei are shown in blue, centrosomes in red, apical cell adhesion in orange. The green-pink gradient represents chemokine receptor expression (Cxcr7b and Cxcr4b). (B) The signalling pathways that coordinate migration at the leading edge and centripetal organ formation at the trailing edge are summarized. In brief, at the leading edge of the primordium, Wnt/β-catenin induces the expression of Fgf3, Fgf10 and Sef (an Fgf signalling inhibitor). As a result, Fgf signalling is inhibited by Sef at the leading edge, while Fgf signalling is active in 'follower' cells expressing Fgf receptors but also Dkk1, which restricts Wnt/β-catenin activity in the leading domain. In the trailing zone, Fgf signalling induces the expression of DeltaA and Atoh1a. DeltaA activates Notch in neighbouring cells and restricts Atoh1a expression to a single cell located at the centre of each forming proneuromast. Atoh1a, in turn, induces the expression of DeltaD, which together with DeltaA maintains and stabilizes Atoh1a expression in the central cell through lateral inhibition. In addition, Atoh1a induces Fgf10 expression in the central cell, which signals to adjacent Fgfr1- and Etv4-expressing cells in the trailing region. Cxcr7b expression is inhibited by Wnt/β-catenin in the leading domain (not shown here). Based on published data (Aman and Piotrowski, 2008, 2009, 2011; Chitnis et al., 2012; Dalle Nogare et al., 2014; Haas and Gilmour, 2006; Ma and Raible, 2009; Matsuda and Chitnis, 2010; Revenu et al., 2014).

laser separation, the middle fragment followed the leading fragment, suggesting that an additional signal provided by the leading domain ensures correct migration. By manipulating Fgf signalling, it was further shown that Fgf signalling recapitulates the chemoattractive response of the middle fragment to the leading fragment (Fig. 3B). In addition, Fgf-soaked beads were shown to polarize the migration of an isolated middle fragment. Together, these studies indicate that both chemokine and Fgf signalling are used to guide cell migration in the pLL primordium.

It has also been shown that Wnt/β-catenin induces the expression of Fgf3 and Fgf10, and that of Sef (Il17rd), a membrane-tethered Fgf signalling inhibitor, at the leading domain of the primordium (Fig. 3B) (Aman and Piotrowski, 2008). As a result, Fgf signalling is inhibited by Sef at the leading domain while the Fgf ligands reach and activate Fgf signalling in 'follower' cells, which in turn induce Dkk1 expression to block Wnt/β-catenin activity (Aman and Piotrowski, 2008). Finally, it has also been demonstrated that Wnt/β-catenin signalling inhibits Cxcr7b expression at the leading domain, and thus restricts the expression of this receptor in trailing cells (Aman and Piotrowski, 2008, 2009, 2011; Ma and Raible, 2009).

Collective cell migration in the lateral line must also be tightly coordinated with the regulated deposition of centripetal organs. This latter event is mediated by interactions between Fgf and Notch signalling (Fig. 3B). Fgf signalling initiates the expression of DeltaA and Atoh1a in the trailing zone; DeltaA activates Notch in neighbouring cells and inhibits Atoh1a, restricting its expression to a single cell located at the centre of each forming proneuromast in the trailing edge. Atoh1a, in turn, induces the expression of DeltaD, which, together with DeltaA, maintains and stabilizes Atoh1a expression in the central cell through lateral inhibition. In addition, Atoh1a induces Fgf10 expression in the central cell, which signals to adjacent Fgfr1- and Etv4-expressing cells in the trailing region. This Fgf/Delta/Notch signalling axis is key for regulating cell differentiation in the forming rosettes: the Atoh1-expressing cell differentiates into a hair cell, and the surrounding cells become supporting cells that serve as progenitors (Ma and Raible, 2009; Nechiporuk and Raible, 2008). In this context, the consequences of abrogated Notch signalling are numerous – the number of Atoh1/Fgf ligand-expressing cells increases, the number of Fgfr1/Etv4-expressing cells reduces, the migrating primordium is stalled halfway to the tip of the tail, and rosette formation is initialized but the deposited organs are less cohesive and are fragmented and closely and erratically distributed (Matsuda and Chitnis, 2010) – thus also highlighting a key role for Fgf/Delta/Notch signalling in cell migration.

Finally, in the forming pLL centripetal organs, it was shown that Fgf3 molecules are concentrated in a lumen formed by the apical sides of cohesive trailing cells, and in the adjacent space among protruding sensory kinocilia of those cells. Inhibition of Fgf signalling or lumen disassembly results in delayed deposition of the organs, whereas Fgf3 overexpression accelerates organ deposition (Durdu et al., 2014). Altogether, these findings show that Fgf signalling accounts for the directed motility of follower cells, acts as an attractive signal for proneuromasts, and regulates the deposition and thus patterning of centripetal organs (Chitnis et al., 2012; Dalle Nogare et al., 2014; Durdu et al., 2014; Lecaudey et al., 2008). Importantly, this dissection of the molecular networks that signal during pLL primordium migration has revealed the subtle changes in cell behaviours that are involved during this process. Although the primordium is apparently migrating as a cohesive group, subsets of cells have different properties (i.e. leader cells, cell polarity transition zone, proneuromasts) and respond to distinct signals, thereby ensuring the coordination of cell movement, orientation, differentiation, organ assembly and patterning.

Lung epithelial neuroendocrine mini-organs: slithering until assembling

In the examples discussed so far – the tooth, hair follicle and pLL placodes – entire populations of cells undergo migration within or at the level of the epithelium in which they originate. Their displacement can also be highly coordinated, as in the case of the pLL primordium. However, neuroepithelial cells are also known to acquire mesenchymal characteristics and migrate outside of the epithelium. The most studied example of this occurs in the case of neural crest formation; this process is known as epithelial-to-mesenchymal transition (EMT, see Box 2; Bronner and Simões-Costa, 2016; Nieto et al., 2016; Scarpa and Mayor, 2016). Recently, however, it was found that single developing neuroendocrine epithelial cells in the lung epithelium share displacement characteristics with EMT but do not exit, and instead migrate within, the epithelium. This process is termed 'slithering' (Fig. 4A; Box 2) (Kuo and Krasnow, 2015; Noguchi et al., 2015).

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

Lung neuroendocrine (NE) cells are chemosensory cells of an epithelial origin that sense oxygen among other chemicals. They are located in the bronchial epithelium and are part of the lung stem cell niche. NE cells are particular in their distribution, as they can be solitary or form small or large groups (Fig. 4A) (Cutz et al., 2013). In pathological conditions, their unclustering is associated with increased neuropeptide release and increased immune response (Branchfield et al., 2016). Whereas single cells and small clusters are randomly scattered throughout the bronchial epithelium, large groups, referred to as NEBs, are located in a stereotyped pattern at the branchpoints of the mammalian secondary airway (which excludes the trachea and primary bronchi). NEBs grow in centrifugal orientation, i.e. NE cell differentiation occurs from the proximal to the distal part of the branchpoint (Avadhanam et al., 1997; Hoyt et al., 1990; Noguchi et al., 2015), and they are highly innervated.

Recent studies have examined the molecular and cellular process involved in NE cell and NEB formation in mice. For example, it has been shown that NE cells express Ascl1 and Delta1 (Dll1), and are surrounded by cells (termed SPNC cells) with active Notch signalling (Noguchi et al., 2015). When SPNC cells are selectively eliminated, the number of NE cells remains unaffected, showing that these cells with Notch activity do not contribute to the NE cell fate. However, when Notch signalling is abrogated (via conditional inactivation of the Notch effector Hes1) in the epithelium that generates NE cells, the number of NE cells is dramatically increased and NEB clusters are enlarged, suggesting that Notch/Hes1 signalling restricts NE cell fate in epithelial progenitors through a lateral inhibition mechanism (Noguchi et al., 2015).

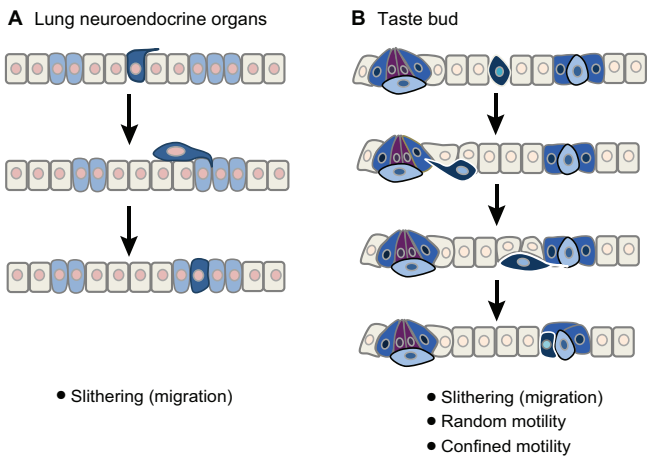

Fig. 4. Single cell motility during neurosensory organ formation. The cartoon shows single cells (dark blue) that slither, i.e. translocate within the epithelium with directed motility, and integrate into developing neurosensory mini-organs. (A) In the case of lung neuroendocrine (NE) organs, a single NE cell (dark blue) slithers, i.e. migrates, makes contact with grouped NE cells of a NEB (light blue), and integrates into the NEB (Kuo and Krasnow, 2015; Noguchi et al., 2015). (B) In the case of taste buds, motile taste bud cells can have random, confined or directed motility relative to the centre of the mass of the organ. In the example shown, two taste bud organs composed of different cells types (light blue, blue, purple cells) are shown, one of which (left) is in a more mature state than the other (right). A differentiating taste bud cell (dark blue) slithers between the two organs, within the epithelium, joins first the taste bud located on the left, then exits, migrates and integrates into the less mature organ on the right (Soulika et al., 2016).

The variability in the distribution of single NE cells and the stereotyped pattern of NEBs raise the question of how NE cells assemble into clusters. Fate mapping has shown that an NE cluster is not the result of clonal proliferation of one or more progenitors specific to a particular cluster. In other words, there is not a link between specific progenitor(s) and NEB clusters, but instead NEBs and mini-clusters arise from multiple founder cells (Kuo and Krasnow, 2015). Apoptosis has also been excluded as a mechanism of NEB spacing (Kuo and Krasnow, 2015). By contrast, using *in vivo* imaging in slice cultures and in air-liquid organ cultures, it has been shown that NE cells are motile (Kuo and Krasnow, 2015; Noguchi et al., 2015). These studies revealed that, as they 'slither', NE progenitors extend long cytoplasmic projections, detach from the basement membrane, take perpendicular positions relative to surrounding epithelial cells, and crawl over and around epithelial cells for tens of microns. This motility mode is not a straightforward process as NE cells can pause, change direction or interact with other NE cells and finally joins a NEB cluster. However, the analysis of cell track straightness has revealed that NEs have directionality to NEBs (Noguchi et al., 2015). As in the case of other motile cells, NE intraepithelial displacement is accompanied by changes in adhesion and cell polarity. For instance, a landmark of non-motile, epithelial-like structure is E-cadherin expression. During NE displacement, E-cadherin expression is downregulated, and is then restored in mature NEB cells, reminiscent of reduced motility. In addition, motile NEs express transcription factors that represent the hallmarks of large-scale EMT, such as Snail (Kuo and Krasnow, 2015).

Despite these insights, and the identification of a novel mode of epithelial cell motility (namely, slithering), several questions still remain unanswered. For example, why do smaller and larger clusters co-exist? What kinds of interactions or factors promote NE pausing and change of direction? What signals underlie directionality and promote the integration of additional NE cells into a NEB? One possible mechanism is through the roundabout receptor (Robo), which is expressed in NEB cells: abrogation of its activity results in non-clustered, dispersed NE cells in the lung epithelium (Branchfield et al., 2016). However, further studies are clearly needed to answer these questions and gain a better understanding of this mode of epithelial cell motility.

Taste bud formation: getting together in many ways

Taste buds are clusters of chemosensory cells that detect different chemical stimuli found in food. They include Type I (support), Type II (taste receptor, sensing bitter, sweet, umami) and Type III [5-hydroxytryptamine (5-HT)/sour and amiloride-insensitive salt sensing] cells (Chandrashekar et al., 2006, 2009, 2010; Chaudhari and Roper, 2010; Lewandowski et al., 2016; Oka et al., 2013; Roper, 2013, 2015). In the majority of vertebrates, taste buds are located in the oropharyngeal cavity and, together with teeth, contribute to food assessment and processing. Several elegant studies, mostly in mice, have shown that Wnt/β-catenin, Shh, Bmp and Fgf signalling are required for taste bud cell specification and patterning (e.g. Barlow, 2015; Barlow and Klein, 2015; Beites et al., 2009; Bloomquist et al., 2015; Gaillard et al., 2015; Iwatsuki et al., 2007; Kapsimali and Barlow, 2013; Kapsimali et al., 2011; Liu et al., 2007, 2013; Petersen et al., 2011; Zhou et al., 2006). A number of more recent studies have also assessed epithelial cell dynamics during taste bud development, showing that individual taste bud cells – like lung NE cells – are motile.

Fate-mapping experiments have shown that taste bud cells derive from ectodermal or endodermal epithelium within the vertebrate oropharynx (reviewed by Barlow, 2015; Kapsimali and Barlow,

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

2013). Taste bud cells differentiate from dividing cells located at specific epithelial sites termed 'taste placodes' but also within the nearby epithelium (Nguyen et al., 2012; Okubo et al., 2009; Perea-Martinez et al., 2013; Ren et al., 2014; Yee et al., 2013). As a result, the cell population of a given taste bud has multiple origins (Gaillard et al., 2015; Stone and Finger, 1994; Stone et al., 2002; Thirumangalathu et al., 2009). The first evidence that developing taste bud cells are motile came from *in vivo* imaging studies of taste bud organoid formation *in vitro* (Aihara et al., 2015). This approach showed that, during taste bud organoid formation, cells within the neck and budding regions of organoids exhibit different motility speeds compared with those in the organoid body region. The kinematics of taste bud organ assembly have also been analysed in developing zebrafish larva. As in other jawed vertebrates, zebrafish taste buds are composed of three molecularly and functionally distinct classes, the Type I, II and III cells, although there is some species adaptation to environmental stimuli (e.g. fish and cats do not sense sweet) (Ishimaru et al., 2005; Kapsimali and Barlow, 2013; Li et al., 2005; Matsumoto et al., 2013; Oike et al., 2007; Yoshida et al., 2007). *In vivo* imaging and subsequent analyses of the displacement of two cell populations, one likely representing an intermediate progenitor pool (expressing Fgf8a) and a second consisting of Type II cells, showed that developing taste bud cells exhibit random, confined or directed modes of motility (see Box 2) relative to the cell closest to the centre of mass of the taste bud and, therefore, show heterogeneity in their mode of displacement (Soulika et al., 2016). In particular, within the early developing oral epithelium, Fgf8a-expressing taste bud cells form organs through confined and/or random displacement. One of the Fgf8a-expressing taste bud cells differentiates into a Type III cell. The resultant taste bud organ can integrate additional cells that originate from the neighbouring epithelium or are even located within a neighbouring organ. The new cells that join the taste bud elongate, extend cytoplasmic processes, pass by other epithelial cells and have directionality, reminiscent of the slithering displacement of lung NE cells. It has also been noted that the slithering taste bud cells make contacts with more than one cell in more than one neighbouring organs that surround them, as if they are 'choosing' the organ into which they will integrate (Fig. 4B) (Soulika et al., 2016).

The physiological significance and the molecular mechanisms underlying the diversity of cell motility during taste bud assembly remain largely unknown. However, it is evident that taste bud cell differentiation might be linked with cell motility mode, as occurs in the case of developing neuromasts. For example, in zebrafish *Ascl1a*$^{-/-}$ larvae, which are devoid of taste bud Type III cells and have slightly increased number of Type II cells, Fgf8a-expressing cells show random or confined but not directed motility mode, and taste bud organs occasionally split into smaller-sized clusters. Therefore, in zebrafish, Ascl1a is required for Type III cell formation, and for the migration and maintenance of Fgf8a-expressing cells in taste buds, suggesting that an attractive signal is provided by Type III cells. Laser ablation of Type III cells has further shown that this cell type is required for the maintenance of Fgf8a-expressing cells by the taste bud. However, none of the motility modes of Type II cells relies on the Ascl1/5-HT/Type III cell, and taste buds do form in *Ascl1a*$^{-/-}$ larvae (Soulika et al., 2016), suggesting that different molecular mechanisms regulate the motility and assembly of Type II cells into taste buds.

Conclusions and open questions

In recent years, *in vivo* imaging has uncovered the remarkable capacity of epithelial cells to be displaced intraepithelially during the development of neurosensory mini-organs (summarized in Table 1). This displacement occurs in different contexts and via different modes: after cell division, as single or grouped cells, in random, confined or directed modes, and by intercalation under the tension caused by a cell canopy (Box 2). The most complete picture of cell motility and the molecular signals that underlie this behaviour come from studies of zebrafish pLL primordium migration, during which cell behaviour shifts progressively from a directionally migrating cell cohort to immotile, compact, centripetally orientated differentiating cell groups. But to what extent are the molecular mechanisms that underlie pLL primordium migration similar to those occurring during the formation of other mini-organs? Does each organ use its own set of mechanisms, modes and rules?

During pLL primordium migration, Fgf signalling activity is required for the migration of follower cells adjacent to the leading domain, and for the formation and deposition of proneuromast rosettes (Chitnis et al., 2012; Dalle Nogare et al., 2014; Donà et al., 2013; Durdu et al., 2014; Lecaudey et al., 2008). Studies have shown that Fgf8-expressing/derived cells also contribute to molar placode formation; these cells likely form rosettes temporarily and show only minor displacement when Fgf8 activity is compromised

Table 1. Types of cell motility and underlying signalling mechanisms observed during the formation of neurosensory mini-organs

	pLL primordium	Molar dental placode	Hair follicle placode	Taste bud	NEB
Cell behaviour					
Migration	+	+	+	+	+
Random motility				+	
Confined motility				+	
Rosette	+	+		+	
Tensile canopy		+	+		
Signalling pathway required for motility					
Atoh1/Ascl1/Delta/Notch (indirect)	Migration, cluster formation			Migration, cluster maintenance	
Cxcl12a, Cxcr4/7	Migration				
Eda/Eda receptor (indirect)			Migration		
Fgf	Migration, organ deposition	Motility			
Robo (indirect?)					Clustering
Shh (indirect?)		Migration			
Wnt (indirect)	Migration		Migration		

Development (2017) 144, 1926-1936 doi:10.1242/dev.148122

(Prochazka et al., 2015). Fgf8a-expressing taste bud cells are also motile (Soulika et al., 2016), and hair follicle placodes express Fgf20, which is required for condensation of the underlying dermal mesenchyme (Haara et al., 2012). Therefore, the timely and spatially restricted regulation of Fgf signalling could be linked with subtle changes in intraepithelial cell motility and might represent a general mechanism that coordinates motility and centripetal organization during neurosensory mini-organ formation.

A number of cell/organ-specific transcription factors, notably basic helix-loop-helix (bHLH) proneural factors, also appear to be linked to both cell motility and cell differentiation in their respective cells/organs. In the migrating pLL primordium, for instance, Atoh1 expression is progressively restricted to a single, centrally positioned, rosette cell in the trailing edge, where it induces a cell fate change and Fgf10 expression. Ascl1 and jagged 2 (a Notch ligand) are expressed in NE cells and dental epithelium, respectively (Kuo and Krasnow, 2015; Mitsiadis et al., 2010; Noguchi et al., 2015). In addition, Ascl1a is expressed in Type III taste bud cells and is indirectly, as a transcription factor, required for migration towards and the maintenance of Fgf8a-expressing cells by the taste bud organ (Soulika et al., 2016). Thus, bHLH proneural factor/Notch signalling might regulate not only cell identity but also cell motility and cohesion in these mini-organs. Wnt/β-catenin signalling appears also to be involved indirectly in cell motility. At the leading edge of the pLL primordium, Wnt/β-catenin signalling maintains Cxcl12a activity by inhibiting Cxcr7b expression. During hair placode formation, activation of Wnt/β-catenin results in altered motility parameters of placodal cells, indirectly, by increasing placodal cell number. Therefore, one could speculate that the signalling activities of chemokines could be involved in these Wnt-responsive cell behaviours and could play chemoattractive roles during the assembly of mini-organs more generally (Table 1).

Cell topology is also a key parameter that is likely to influence cell motility, as it may allow or restrict (confine) displacement. In the case of collective cell displacement, as observed during pLL primordium migration and molar placode invagination, the position of a cell within its group and communication with its neighbours are key for its motility and behaviour. For instance, leader cells at the edge of the migrating pLL primordium that guide collective migration are submitted to different topological constraints than follower cells, which adapt their orientation and adhesion to form the centripetally organized organs (Dalle Nogare et al., 2014; Haas and Gilmour, 2006; Revenu et al., 2014). This is achieved mainly via molecular signalling among neighbouring cells. During molar placode invagination, internal placodal forces account for the intercalation of suprabasal cells and basally attached cell bodies. Although it is unclear how tension is maintained in suprabasal cells, cell communication and adhesion linked to an adequate cytoskeletal network are likely to be prerequisites in this case, too. Several hypotheses can be formed about the mechanisms promoting and sustaining intercalation and tension in invaginating cells, including suprabasal/basal cell autonomous signalling activity, an organizing centre that attracts the cells downwards or repulses them from the tensile canopy, or a combination of active migratory suprabasal cell behaviour and confined displacement of either basal membrane-attached cells or a subset of suprabasal cells. In contrast, in the case of slithering cells during NEB and taste bud formation, single cells have radically different motility behaviours compared with their neighbouring, non-migrating, epithelial cells, which appear to be permissive for the slithering behaviour of others. Instead, topological constraints could be imposed by the cells that are already assembled into organs, which somehow block the slithering behaviour and confine newly joining cells. Indeed, the most intriguing characteristic of slithering cells is that their integration into an organ is not straightforward (Noguchi et al., 2015; Soulika et al., 2016). One possibility is that a chemoattractive signalling threshold is required to maintain cells in the organ; if signalling is at insufficient levels, appropriate adhesion is not achieved and cells exit. Alternatively, cells might exit due to failure to acquire appropriate function in the organ, either because of their identity or their failure to be innervated or to functionally communicate with neighbouring cells. As NE cells can initiate small cell lung cancer (Song et al., 2012), it is of interest to dissect not only the molecular mechanisms underlying slithering cell motility but also the topological parameters that might block migration in this context.

Finally, a question arising from these observations of intraepithelial cell displacement is to what extent cell motility can compensate for genetic/cell specification impairment under abnormal conditions. For instance, the activation of Eda and Wnt canonical signalling during hair placode formation still results in placodes, but these exhibit an increased number of migrating cells that show more variability in their displacement parameters compared with wild type, navigating longer distances with less directionality (Ahtiainen et al., 2014). In zebrafish larvae with abrogated Ascl1a activity, taste bud organs form despite the absence of differentiated Type III taste-bud cells, the increase in Type II cells and altered cell motility parameters, i.e. Fgf8a-expressing cells are devoid of directed motility (Soulika et al., 2016). Therefore, modifications in cell specification are accompanied by altered motility behaviour, and this could reflect some flexibility for organ formation. This is perhaps not a surprise: neurosensory mini-organs are exposed to and perceive the frequently changing environment, so some plasticity in their formation would allow adaptation of the organism to changing conditions. It will be interesting to examine in more detail whether this plasticity relies on a combination of similar mechanisms in different sensory organs. Although in its early stages, the study of intraepithelial cell displacement during the formation of sensory mini-organs has contributed to the molecular dissection of known cell motility behaviours (i.e. migration) but has also revealed novel displacement modes at the scale of collective and single-cell behaviour (i.e. tensile canopy intercalation, slithering). Further studies into the organs presented here, but also the *in vivo* imaging of other sensory organs (e.g. the retina; Icha et al., 2016), will lead to a more complete view of intraepithelial cell motility behaviours. Moreover, as discussed here, it appears that intraepithelial cell motility is closely associated with cell differentiation; therefore, it will be necessary to understand both processes in order to obtain a comprehensive view of sensory organ assembly, function and dysfunction.

Acknowledgements

I am particularly grateful to the reviewers of this manuscript, many colleagues at IBENS, ECRO, the zebrafish community for stimulating discussions and support and Patricia Gongal for editing the manuscript. This work is supported by INSERM funding. M.K. is INSERM researcher.

Competing interests

The author declares no competing or financial interests.

Funding

This work was funded by the Institut National de la Santé et de la Recherche Médicale.

References

Ahn, Y. (2015). Signaling in tooth, hair, and mammary placodes. *Curr. Top. Dev. Biol.* **111**, 421-459.

Ahtiainen, L., Lefebvre, S., Ivi, P., Lindfors, H., Renvoisé, E., Shirokova, V., Vartiainen, M. K., Thesleff, I., Mikkola, M. L., Lindfors, P. H. et al. (2014). Directional cell migration, but not proliferation, drives hair placode morphogenesis. *Dev. Cell* **28**, 588-602.

Ahtiainen, L., Uski, I., Thesleff, I. and Mikkola, M. L. (2016). Early epithelial signaling center governs tooth budding morphogenesis. *J. Cell Biol.* **214**, 753-767.

Aihara, E., Mahe, M. M., Schumacher, M. A., Matthis, A. L., Feng, R., Ren, W., Noah, T. K., Matsu-Ura, T., Moore, S. R., Hong, C. I. et al. (2015). Characterization of stem/progenitor cell cycle using murine circumvallate papilla taste bud organoid. *Sci. Rep.* **5**, 17185.

Aman, A. and Piotrowski, T. (2008). Wnt/beta-catenin and Fgf signaling control collective cell migration by restricting chemokine receptor expression. *Dev. Cell* **15**, 749-761.

Aman, A. and Piotrowski, T. (2009). Multiple signaling interactions coordinate collective cell migration of the posterior lateral line primordium. *Cell Adhes. Migr.* **3**, 365-368.

Aman, A. and Piotrowski, T. (2011). Cell-cell signaling interactions coordinate multiple cell behaviors that drive morphogenesis of the lateral line. *Cell Adhes. Migr.* **5**, 499-508.

Andl, T., Reddy, S. T., Gaddapara, T. and Millar, S. E. (2002). WNT signals are required for the initiation of hair follicle development. *Dev. Cell* **2**, 643-653.

Avadhanam, K. P., Plopper, C. G. and Pinkerton, K. E. (1997). Mapping the distribution of neuroepithelial bodies of the rat lung. A whole-mount immunohistochemical approach. *Am. J. Pathol.* **150**, 851-859.

Barlow, L. A. (2015). Progress and renewal in gustation: new insights into taste bud development. *Development* **142**, 3620-3629.

Barlow, L. A. and Klein, O. D. (2015). Developing and regenerating a sense of taste. *Curr. Top. Dev. Biol.* **111**, 401-419.

Beites, C. L., Hollenbeck, P. L. W., Kim, J., Lovell-Badge, R., Lander, A. D. and Calof, A. L. (2009). Follistatin modulates a BMP autoregulatory loop to control the size and patterning of sensory domains in the developing tongue. *Development* **136**, 2187-2197.

Beltman, J. B., Marée, A. F. M. and de Boer, R. J. (2009). Analysing immune cell migration. *Nat. Rev. Immunol.* **9**, 789-798.

Biggs, L. C. and Mikkola, M. L. (2014). Early inductive events in ectodermal appendage morphogenesis. *Semin. Cell Dev. Biol.* **25-26**, 11-21.

Bitgood, M. J. and McMahon, A. P. (1995). HedgehogandBmpGenes are coexpressed at many diverse sites of cell–cell interaction in the mouse embryo. *Dev. Biol.* **172**, 126-138.

Bloomquist, R. F., Parnell, N. F., Phillips, K. A., Fowler, T. E., Yu, T. Y., Sharpe, P. T., Streelman, J. T. and Shubin, N. H. (2015). Coevolutionary patterning of teeth and taste buds. *Proc. Natl. Acad. Sci. USA* **112**, E5954-E5962.

Brabletz, T. (2012). EMT and MET in metastasis: where are the cancer stem cells? *Cancer Cell* **22**, 699-701.

Branchfield, K., Nantie, L., Verheyden, J. M., Sui, P., Wienhold, M. D. and Sun, X. (2016). Pulmonary neuroendocrine cells function as airway sensors to control lung immune response. *Science* **351**, 707-710.

Bronner, M. E. and Simões-Costa, M. (2016). The neural crest migrating into the twenty-first century. *Curr. Top. Dev. Biol.* **116**, 115-134.

Campbell, K. and Casanova, J. (2016). A common framework for EMT and collective cell migration. *Development* **143**, 4291-4300.

Chandrashekar, J., Hoon, M. A., Ryba, N. J. P. and Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature* **444**, 288-294.

Chandrashekar, J., Yarmolinsky, D., von Buchholtz, L., Oka, Y., Sly, W. S., Ryba, N. J. P. and Zuker, C. S. (2009). The taste of carbonation. *Science* **326**, 443-445.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J. P. and Zuker, C. S. (2010). The cells and peripheral representation of sodium taste in mice. *Nature* **464**, 297-301.

Chaudhari, N. and Roper, S. D. (2010). The cell biology of taste. *J. Cell Biol.* **190**, 285-296.

Chitnis, A. B., Dalle Nogare, D. and Matsuda, M. (2012). Building the posterior lateral line system in zebrafish. *Dev. Neurobiol.* **72**, 234-255.

Cutz, E., Pan, J., Yeger, H., Domnik, N. J. and Fisher, J. T. (2013). Recent advances and contraversies on the role of pulmonary neuroepithelial bodies as airway sensors. *Semin. Cell Dev. Biol.* **24**, 40-50.

Dalle Nogare, D., Somers, K., Rao, S., Matsuda, M., Reichman-Fried, M., Raz, E. and Chitnis, A. B. (2014). Leading and trailing cells cooperate in collective migration of the zebrafish Posterior Lateral Line primordium. *Development* **141**, 3188-3196.

Dambly-Chaudière, C., Cubedo, N. and Ghysen, A. (2007). Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. *BMC Dev. Biol.* **7**, 23.

Dassule, H. R. and McMahon, A. P. (1998). Analysis of epithelial–mesenchymal interactions in the initial morphogenesis of the mammalian tooth. *Dev. Biol.* **202**, 215-227.

Dassule, H. R., Lewis, P., Bei, M., Maas, R. and McMahon, A. P. (2000). Sonic hedgehog regulates growth and morphogenesis of the tooth. *Development* **127**, 4775-4785.

David, N. B., Sapede, D., Saint-Etienne, L., Thisse, C., Thisse, B., Dambly-Chaudiere, C., Rosa, F. M. and Ghysen, A. (2002). Molecular basis of cell migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. *Proc. Natl. Acad. Sci. USA* **99**, 16297-16302.

Diepenbruck, M. and Christofori, G. (2016). Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? *Curr. Opin. Cell Biol.* **43**, 7-13.

Donà, E., Barry, J. D., Valentin, G., Quirin, C., Khmelinskii, A., Kunze, A., Durdu, S., Newton, L. R., Fernandez-Minan, A., Huber, W. et al. (2013). Directional tissue migration through a self-generated chemokine gradient. *Nature* **503**, 285-289.

Durdu, S., Iskar, M., Revenu, C., Schieber, N., Kunze, A., Bork, P., Schwab, Y. and Gilmour, D. (2014). Luminal signalling links cell communication to tissue architecture during organogenesis. *Nature* **515**, 120-124.

Farhadifar, R., Röper, J.-C., Aigouy, B., Eaton, S. and Jülicher, F. (2007). The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. *Curr. Biol.* **17**, 2095-2104.

Fernandez-Gonzalez, R. and Zallen, J. A. (2008). Epithelial organization: may the force be with you. *Curr. Biol.* **18**, R163-R165.

Fuchs, E. (2007). Scratching the surface of skin development. *Nature* **445**, 834-842.

Fuchs, E. (2015). Cell biology: more than skin deep. *J. Cell Biol.* **209**, 629-631.

Fuchs, E. and Nowak, J. A. (2008). Building epithelial tissues from skin stem cells. *Cold Spring Harb. Symp. Quant. Biol.* **73**, 333-350.

Gaillard, D., Xu, M., Liu, F., Millar, S. E. and Barlow, L. A. (2015). beta-catenin signaling biases multipotent lingual epithelial progenitors to differentiate and acquire specific taste cell fates. *PLoS Genet.* **11**, e1005208.

Haara, O., Harjunmaa, E., Lindfors, P. H., Huh, S.-H., Fliniaux, I., Aberg, T., Jernvall, J., Ornitz, D. M., Mikkola, M. L. and Thesleff, I. (2012). Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. *Development* **139**, 3189-3199.

Haas, P. and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev. Cell* **10**, 673-680.

Hardcastle, Z., Mo, R., Hui, C. C. and Sharpe, P. T. (1998). The Shh signalling pathway in tooth development: defects in Gli2 and Gli3 mutants. *Development* **125**, 2803-2811.

Hardy, M. H. (1992). The secret life of the hair follicle. *Trends Genet.* **8**, 55-61.

Hay, E. D. (1995). An overview of epithelio-mesenchymal transformation. *Acta Anat. (Basel)* **154**, 8-20.

Heller, E. and Fuchs, E. (2015). Tissue patterning and cellular mechanics. *J. Cell Biol.* **211**, 219-231.

Hoyt, R. F., McNelly, N. A. and Sorokin, S. P. (1990). Dynamics of neuroepithelial body (NEB) formation in developing hamster lung: light microscopic autoradiography after 3H-thymidine labeling in vivo. *Anat. Rec.* **227**, 340-350.

Icha, J., Kunath, C., Rocha-Martins, M. and Norden, C. (2016). Independent modes of ganglion cell translocation ensure correct lamination of the zebrafish retina. *J. Cell Biol.* **215**, 259-275.

Ishimaru, Y., Okada, S., Naito, H., Nagai, T., Yasuoka, A., Matsumoto, I. and Abe, K. (2005). Two families of candidate taste receptors in fishes. *Mech. Dev.* **122**, 1310-1321.

Iwatsuki, K., Liu, H.-X., Grónder, A., Singer, M. A., Lane, T. F., Grosschedl, R., Mistretta, C. M. and Margolskee, R. F. (2007). Wnt signaling interacts with Shh to regulate taste papilla development. *Proc. Natl. Acad. Sci. USA* **104**, 2253-2258.

Kapsimali, M. and Barlow, L. A. (2013). Developing a sense of taste. *Semin. Cell Dev. Biol.* **24**, 200-209.

Kapsimali, M., Kaushik, A.-L., Gibon, G., Dirian, L., Ernest, S. and Rosa, F. M. (2011). Fgf signaling controls pharyngeal taste bud formation through miR-200 and Delta-Notch activity. *Development* **138**, 3473-3484.

Keränen, S. V. E., Åberg, T., Kettunen, P., Thesleff, I. and Jernvall, J. (1998). Association of developmental regulatory genes with the development of different molar tooth shapes in two species of rodents. *Dev. Genes Evol.* **208**, 477-486.

Kettunen, P., Karavanova, I. and Thesleff, I. (1998). Responsiveness of developing dental tissues to fibroblast growth factors: Expression of splicing alternatives of FGFR1, -2, -3, and of FGFR4; and stimulation of cell proliferation by FGF-2, -4, -8, and -9. *Dev. Genet.* **22**, 374-385.

Kuo, C. S. and Krasnow, M. A. (2015). Formation of a neurosensory organ by epithelial cell slithering. *Cell* **163**, 394-405.

Laurikkala, J., Mikkola, M. L., James, M., Tummers, M., Mills, A. A. and Thesleff, I. (2006). P63 regulates multiple signalling pathways required for ectodermal organogenesis and differentiation. *Development* **133**, 1553-1563.

Lecaudey, V., Cakan-Akdogan, G., Norton, W. H. J. and Gilmour, D. (2008). Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135**, 2695-2705.

Lee, J. and Tumbar, T. (2012). Hairy tale of signaling in hair follicle development and cycling. *Semin. Cell Dev. Biol.* **23**, 906-916.

Lewandowski, X. B. C., Sukumaran, S. K., Margolskee, R. F., Bachmanov, X. A., Lewandowski, B. C., Sukumaran, S. K., Margolskee, R. F., Bachmanov, A. A., Lewandowski, X. B. C., Sukumaran, S. K. et al. (2016). Amiloride-insensitive salt taste is mediated by two populations of type III taste cells with distinct transduction mechanisms. *J. Neurosci.* **36**, 1942-1953.

Li, X., Li, W., Wang, H., Cao, J., Maehashi, K., Huang, L., Bachmanov, A. A., Reed, D. R., Legrand-Defretin, V., Beauchamp, G. K. et al. (2005). Pseudogenization of a sweet-receptor gene accounts for cats' indifference toward sugar. *PLoS Genet.* **1**, 0027-0035.
Li, C.-Y., Prochazka, J., Goodwin, A. F. and Klein, O. D. (2014). Fibroblast growth factor signaling in mammalian tooth development. *Odontology* **102**, 1-13.
Li, J., Chatzeli, L., Panousopoulou, E., Tucker, A. S. and Green, J. B. A. (2016). Epithelial stratification and placode invagination are separable functions in early morphogenesis of the molar tooth. *Development* **143**, 670-681.
Liu, F., Thirumangalathu, S., Gallant, N. M., Yang, S. H., Stoick-Cooper, C. L., Reddy, S. T., Andl, T., Taketo, M. M., Dlugosz, A. A., Moon, R. T. et al. (2007). Wnt-β-catenin signaling initiates taste papilla development. *Nat. Genet.* **39**, 106-112.
Liu, H. X., Ermilov, A., Grachtchouk, M., Li, L., Gumucio, D. L., Dlugosz, A. A. and Mistretta, C. M. (2013). Multiple Shh signaling centers participate in fungiform papilla and taste bud formation and maintenance. *Dev. Biol.* **382**, 82-97.
Ma, E. Y. and Raible, D. W. (2009). Signaling pathways regulating zebrafish lateral line development. *Curr. Biol.* **19**, R381-R386.
MacArthur, C. A., Lawshe, A., Xu, J., Santos-Ocampo, S., Heikinheimo, M., Chellaiah, A. T. and Ornitz, D. M. (1995). FGF-8 isoforms activate receptor splice forms that are expressed in mesenchymal regions of mouse development. *Development* **121**, 3603-3613.
Magerl, M., Tobin, D. J., Müller-Röver, S., Hagen, E., Lindner, G., McKay, I. A. and Paus, R. (2001). Patterns of proliferation and apoptosis during murine hair follicle morphogenesis. *J. Invest. Dermatol.* **116**, 947-955.
Martin, A. C. and Goldstein, B. (2014). Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. *Development* **141**, 1987-1998.
Matsuda, M. and Chitnis, A. B. (2010). Atoh1a expression must be restricted by Notch signaling for effective morphogenesis of the posterior lateral line primordium in zebrafish. *Development* **137**, 3477-3487.
Matsumoto, I., Ohmoto, M. and Abe, K. (2013). Functional diversification of taste cells in vertebrates. *Semin. Cell Dev. Biol.* **24**, 210-214.
Meijering, E., Dzyubachyk, O. and Smal, I. (2012). Methods for cell and particle tracking. *Methods Enzymol.* **504**, 183-200.
Mitsiadis, T. A., Graf, D., Luder, H., Gridley, T. and Bluteau, G. (2010). BMPs and FGFs target Notch signalling via jagged 2 to regulate tooth morphogenesis and cytodifferentiation. *Development* **137**, 3025-3035.
Montell, D. J. (2008). Morphogenetic cell movements: diversity from modular mechanical properties. *Science* **322**, 1502-1505.
Mustonen, T., Ilmonen, M., Pummila, M., Kangas, A. T., Laurikkala, J., Jaatinen, R., Pispa, J., Gaide, O., Schneider, P., Thesleff, I. et al. (2004). Ectodysplasin A1 promotes placodal cell fate during early morphogenesis of ectodermal appendages. *Development* **131**, 4907-4919.
Naveau, A., Seidel, K. and Klein, O. D. (2014). Tooth, hair and claw: comparing epithelial stem cell niches of ectodermal appendages. *Exp. Cell Res.* **325**, 96-103.
Nechiporuk, A. and Raible, D. W. (2008). FGF-dependent mechanosensory organ patterning in zebrafish. *Science* **320**, 1774-1777.
Nguyen, H. M., Reyland, M. E. and Barlow, L. A. (2012). Mechanisms of taste bud cell loss after head and neck irradiation. *J. Neurosci.* **32**, 3474-3484.
Nieto, M. A., Huang, R. Y.-J., Jackson, R. A. and Thiery, J. P. (2016). EMT: 2016. *Cell* **166**, 21-45.
Noguchi, M., Sumiyama, K. and Morimoto, M. (2015). Directed migration of pulmonary neuroendocrine cells toward airway branches organizes the stereotypic location of neuroepithelial bodies. *Cell Rep.* **13**, 2679-2686.
Oike, H., Nagai, T., Furuyama, A., Okada, S., Aihara, Y., Ishimaru, Y., Marui, T., Matsumoto, I., Misaka, T. and Abe, K. (2007). Characterization of ligands for fish taste receptors. *J. Neurosci.* **27**, 5584-5592.
Oka, Y., Butnaru, M., von Buchholtz, L., Ryba, N. J. P. and Zuker, C. S. (2013). High salt recruits aversive taste pathways. *Nature* **494**, 472-475.
Okubo, T., Clark, C. and Hogan, B. L. M. (2009). Cell lineage mapping of taste bud cells and keratinocytes in the mouse tongue and soft palate. *Stem Cells* **27**, 442-450.
Ouspenskaia, T., Matos, I., Mertz, A. F., Fiore, V. F. and Fuchs, E. (2016). WNT-SHH antagonism specifies and expands stem cells prior to niche formation. *Cell* **164**, 156-169.
Packard, A., Georgas, K., Michos, O., Riccio, P., Cebrian, C., Combes, A. N., Ju, A., Ferrer-Vaquer, A., Hadjantonakis, A.-K., Zong, H. et al. (2013). Luminal mitosis drives epithelial cell dispersal within the branching ureteric bud. *Dev. Cell* **27**, 319-330.
Panousopoulou, E. and Green, J. B. A. (2016). Invagination of ectodermal placodes is driven by cell intercalation-mediated contraction of the suprabasal tissue canopy. *PLoS Biol.* **14**, e1002405.
Perea-Martinez, I., Nagai, T. and Chaudhari, N. (2013). Functional cell types in taste buds have distinct longevities. *PLoS ONE* **8**, e53399.
Petersen, C. I., Jheon, A. H., Mostowfi, P., Charles, C., Ching, S., Thirumangalathu, S., Barlow, L. A. and Klein, O. D. (2011). FGF signaling regulates the number of posterior taste papillae by controlling progenitor field size. *PLoS Genet.* **7**, e1002098.
Pispa, J. and Thesleff, I. (2003). Mechanisms of ectodermal organogenesis. *Dev. Biol.* **262**, 195-205.
Porntaveetus, T., Otsuka-Tanaka, Y., Albert Basson, M., Moon, A. M., Sharpe, P. T. and Ohazama, A. (2011). Expression of fibroblast growth factors (Fgfs) in murine tooth development. *J. Anat.* **218**, 534-543.
Prochazka, J., Prochazkova, M., Du, W., Spoutil, F., Tureckova, J., Hoch, R., Shimogori, T., Sedlacek, R., Rubenstein, J. L., Wittmann, T. et al. (2015). Migration of founder epithelial cells drives proper molar tooth positioning and morphogenesis. *Dev. Cell* **35**, 713-724.
Ren, W., Lewandowski, B. C., Watson, J., Aihara, E., Iwatsuki, K., Bachmanov, A. A., Margolskee, R. F. and Jiang, P. (2014). Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. *Proc. Natl. Acad. Sci. USA* **111**, 16401-16406.
Revenu, C., Streichan, S., Dona, E., Lecaudey, V., Hufnagel, L. and Gilmour, D. (2014). Quantitative cell polarity imaging defines leader-to-follower transitions during collective migration and the key role of microtubule-dependent adherens junction formation. *Development* **141**, 1282-1291.
Roper, S. D. (2013). Taste buds as peripheral chemosensory processors. *Semin. Cell Dev. Biol.* **24**, 71-79.
Roper, S. D. (2015). The taste of table salt. *Pflugers Arch. Eur. J. Physiol.* **467**, 457-463.
Sawyer, J. M., Harrell, J. R., Shemer, G., Sullivan-Brown, J., Roh-Johnson, M. and Goldstein, B. (2010). Apical constriction: A cell shape change that can drive morphogenesis. *Dev. Biol.* **341**, 5-19.
Saxton, M. J. and Jacobson, K. (1997). Single-particle tracking: applications to membrane dynamics. *Annu. Rev. Biophys. Biomol. Struct.* **26**, 373-399.
Scarpa, E. and Mayor, R. (2016). Collective cell migration in development. *J. Cell Biol.* **212**, 143-155.
Schmidt-Ullrich, R., Tobin, D. J., Lenhard, D., Schneider, P., Paus, R. and Scheidereit, C. (2006). NF-κB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down growth. *Development* **133**, 1045-1057.
Sharir, A. and Klein, O. D. (2016). Watching a deep dive: live imaging provides lessons about tooth invagination. *J. Cell Biol.* **214**, 645-647.
Song, H., Yao, E., Lin, C., Gacayan, R., Chen, M.-H. and Chuang, P.-T. (2012). Functional characterization of pulmonary neuroendocrine cells in lung development, injury, and tumorigenesis. *Proc. Natl. Acad. Sci. USA* **109**, 17531-17536.
Soulika, M., Kaushik, A.-L., Mathieu, B., Lourenço, R., Komisarczuk, A. Z., Romano, S. A., Jouary, A., Lardennois, A., Tissot, N., Okada, S. et al. (2016). Diversity in cell motility reveals the dynamic nature of the formation of zebrafish taste sensory organs. *Development* **143**, 2012-2024.
Stone, L. M. and Finger, T. E. (1994). Mosaic analysis of the embryonic origin of taste buds. *Chem. Senses* **19**, 725-735.
Stone, L. M., Tan, S.-S., Tam, P. P. L. and Finger, T. E. (2002). Analysis of cell lineage relationships in taste buds. *J. Neurosci.* **22**, 4522-4529.
Streichan, S. J., Valentin, G., Gilmour, D. and Hufnagel, L. (2011). Collective cell migration guided by dynamically maintained gradients. *Phys. Biol.* **8**, 045004.
Theveneau, E., Steventon, B., Scarpa, E., Garcia, S., Trepat, X., Streit, A. and Mayor, R. (2013). Chase-and-run between adjacent cell populations promotes directional collective migration. *Nat. Cell Biol.* **15**, 763-772.
Thirumangalathu, S., Harlow, D. E., Driskell, A. L., Krimm, R. F. and Barlow, L. A. (2009). Fate mapping of mammalian embryonic taste bud progenitors. *Development* **136**, 1519-1528.
Tomann, P., Paus, R., Millar, S. E., Scheidereit, C. and Schmidt-Ullrich, R. (2016). LHX2 is a direct NF-κB target gene that promotes primary hair follicle placode down-growth. *Development* **143**, 1512-1522.
Valentin, G., Haas, P. and Gilmour, D. (2007). The chemokine SDF1a coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. *Curr. Biol.* **17**, 1026-1031.
Walck-Shannon, E. and Hardin, J. (2014). Cell intercalation from top to bottom. *Nat. Rev. Mol. Cell Biol.* **15**, 34-48.
Webster, M., Witkin, K. L. and Cohen-Fix, O. (2009). Sizing up the nucleus: nuclear shape, size and nuclear-envelope assembly. *J. Cell Sci.* **122**, 1477-1486.
Yee, K. K., Li, Y., Redding, K. M., Iwatsuki, K., Margolskee, R. F. and Jiang, P. (2013). Lgr5-EGFP marks taste bud stem/progenitor cells in posterior tongue. *Stem Cells* **31**, 992-1000.
Yoshida, Y., Saitoh, K., Aihara, Y., Okada, S., Misaka, T. and Abe, K. (2007). Transient receptor potential channel M5 and phospholipaseC-beta2 colocalizing in zebrafish taste receptor cells. *Neuroreport* **18**, 1517-1520.
Zhang, Y. D., Chen, Z., Song, Y. Q., Liu, C. and Chen, Y. P. (2005). Making a tooth: growth factors, transcription factors, and stem cells. *Cell Res.* **15**, 301-316.
Zhang, X., Ibrahimi, O. A., Olsen, S. K., Umemori, H., Mohammadi, M. and Ornitz, D. M. (2006). Receptor specificity of the fibroblast growth factor family: the complete mammalian FGF family. *J. Biol. Chem.* **281**, 15694-15700.
Zhang, Y., Tomann, P., Andl, T., Gallant, N. M., Huelsken, J., Jerchow, B., Birchmeier, W., Paus, R., Piccolo, S., Mikkola, M. L. et al. (2009). Reciprocal requirements for EDA/EDAR/NF-kappaB and Wnt/beta-catenin signaling pathways in hair follicle induction. *Dev. Cell* **17**, 49-61.
Zhou, Y., Liu, H.-X. and Mistretta, C. M. (2006). Bone morphogenetic proteins and noggin: inhibiting and inducing fungiform taste papilla development. *Dev. Biol.* **297**, 198-213.