Developmental Biology 419 (2016) 298–310

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Original research article

Patterning of papillae on the mouse tongue: A system for the quantitative assessment of planar cell polarity signaling

Yanshu Wang [a,b], John Williams [a,b], Amir Rattner [a], Shu Wu [c,d], Alexander G. Bassuk [c,d], Andre M. Goffinet [e], Jeremy Nathans [a,b,f,g,*]

[a] Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c] Department of Neurology, University of Iowa, Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA, USA
[d] Department of Pediatrics, University of Iowa, Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA, USA
[e] Institute of Neuroscience, University of Louvain Medical School and WELBIO, B1200 Brussels, Belgium
[f] Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[g] Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form
3 September 2016
Accepted 3 September 2016
Available online 6 September 2016

Keywords:
Planar cell polarity
Epithelial polarity
Papillae
Patterning

ABSTRACT

The dorsal surface of the mouse tongue is covered by ~7000 papillae, asymmetric epithelial protrusions that are precisely oriented to create a stereotyped macroscopic pattern. Within the context of this large-scale pattern, neighboring papillae exhibit a high degree of local order that minimizes the differences in their orientations. We show here that the orientations of lingual papillae are under the control of the core planar cell polarity (PCP) genes *Vangl1*, *Vangl2*, and *Celsr1*. Using *K14-Cre* and *Nkx2.5-Cre* to induce conditional knockout of *Vangl1* and/or *Vangl2* in the tongue epithelium, we observe more severe disruptions to local order among papillae with inactivation of larger numbers of *Vangl* genes, a greater role for *Vangl2* than *Vangl1*, and a more severe phenotype with the *Vangl2 Looptail* (*Lp*) allele than the *Vangl2* null allele, consistent with a dominant negative mode of action of the $Vangl2^{Lp}$ allele. Interestingly, $Celsr1^{-/-}$ tongues show disruption of both local and global order, with many papillae in the anterior tongue showing a reversed orientation. To quantify each of these phenotypes, we have developed and applied three procedures for sampling the orientations of papillae and assessing the degree of order on different spatial scales. The experiments reported here establish the dorsal surface of the mouse tongue as a favorable system for studying PCP control of epithelial patterning.

1. Introduction

The epithelial surfaces of many metazoa exhibit complex sub-cellular, cellular, and/or multi-cellular structures that are arranged in precise and spatially asymmetric patterns. Well known examples of such structures include (1) hairs and bristles on the insect wing and cuticle, (2) scales, feathers, and hair on the skin of reptiles, birds, and mammals, respectively, and (3) the motile cilia that line the trachea, lateral ventricles, and fallopian tubes. These structures exhibit spatial order within the plane of the epithelium and each of them has an axis of asymmetry that is oriented relative to the global asymmetry of the body axes or other large-scale body structures.

The polarity of cellular structure within the plane of an epithelium, referred to as planar cell polarity (PCP), has been analyzed extensively in *Drosophila*, zebrafish, *Xenopus*, and mice (Adler et al., 2002; Goodrich and Strutt, 2011). Pioneering genetic studies in *Drosophila* revealed two inter-related systems – the *Van Gogh* (*Vang*)/*Frizzled* (*Fz*)/*Starry night* (*Stan*) and the *Fat/Dachsous* systems – that utilize cell-surface proteins to transmit polarity information between neighboring cells within an epithelium (Lawrence et al., 2007; Matis and Axelrod, 2013). Each of these "core" PCP genes has multiple homologues in vertebrate genomes. In mice, analyses of the phenotypes produced by conventional and/or conditional knockout of core PCP genes, either singly or in combination, have revealed multiple epithelial structures that utilize PCP signaling. These include hair follicles (*Celsr1*, *Fz6*, *Vangl2*; Guo et al., 2004; Wang et al., 2006a; Devenport and Fuchs, 2008; Ravni et al., 2009; Wang et al., 2010), inner ear sensory hair cells (*Celsr1*, *Dsh*, *Fz3*, *Fz6*, *Vangl2*; Curtin et al., 2003; Montcouquiol et al., 2003; Wang et al., 2005; Wang et al., 2006b), and motile cilia (*Celsr1-3*, *Dsh*, *Vangl1*; Tissir et al., 2010; Vladar et al., 2012; Boutin et al., 2014; Ohata et al., 2014; Shi et al., 2014).

* Corresponding author at: Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA.
E-mail address: jnathans@jhmi.edu (J. Nathans).

http://dx.doi.org/10.1016/j.ydbio.2016.09.004
0012-1606/

The polarized epithelial protrusions that cover the dorsal surface of the tongue, referred to here as lingual papillae (LP), represent the most recent addition to this list. Hua et al. (2014) demonstrated that the orientations of LP are regulated by PCP genes *Fz3* and *Fz6*. Eliminating *Fz3* selectively in the epidermis with a *Keratin14-Cre* transgene ($Fz3^{CKO/-}$;*K14-Cre*; *CKO*, conditional knockout) or eliminating *Fz6* constitutively ($Fz6^{-/-}$) did not alter the LP pattern, but combining both of these genetic alterations ($Fz3^{CKO/-}$;$Fz6^{-/-}$;*K14-Cre*) produced a mild disruption in the local order of LP, primarily in the anterior of the tongue.

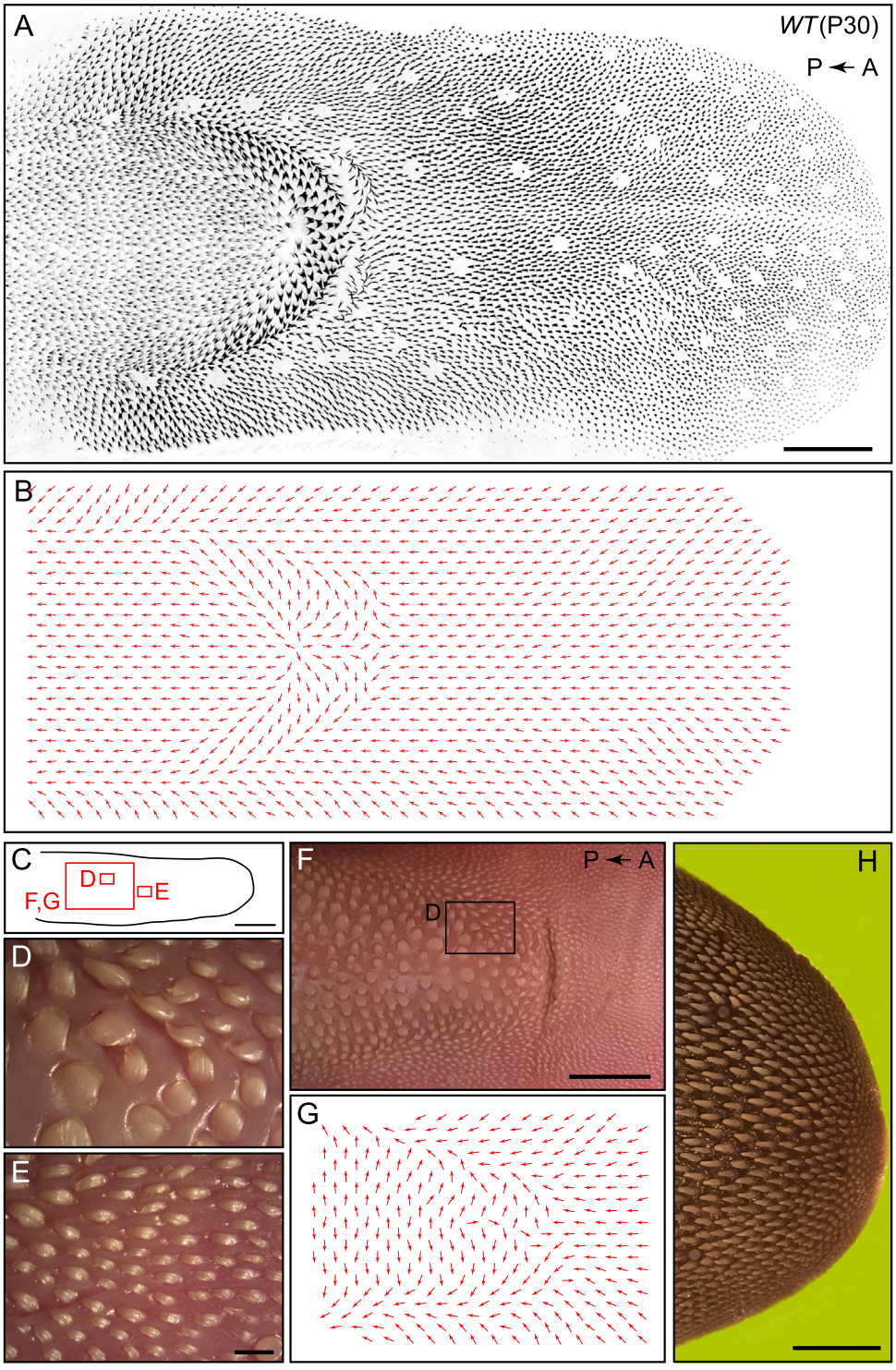

Fig. 1. Similar patterns of LP on the dorsal surface of mouse and bovine tongues. (A) *WT* P30 mouse tongue. Anterior is to the right. The flower region is centered ~65% of the distance from anterior to posterior. Small white circular regions correspond to fungiform papillae (taste buds). A small indentation is characteristically seen at the anterior border of the flower region. A, anterior; P, posterior. (B) Manual scoring of the LP vector field in (A) using a regular grid of vectors (red arrows) and visually interpolating the mean orientation of LP in the immediate neighborhood of each vector. (C–H) Dorsal surface patterns of LP on the adult bovine tongue. In all images, anterior is to the right. (C) Locations of panels (D-G). (D) LP in the bovine equivalent of the flower region. (E) LP anterior to the flower region. (F) The flower region (leftmost 70% of the image) and the adjacent anterior region (rightmost 30% of the image). As in the mouse tongue, the two territories are separated by a transverse indentation. (G) Manual scoring of the LP vector field in (F). (H) Anterior tip of a bovine tongue showing LP oriented from anterior to posterior. Scale bars: (A), 1 mm; (C), 5 cm; (F), 2 cm; (H), 1 cm.