CHAPTER

2

Patterning, morphogenesis, and neurogenesis of zebrafish cranial sensory placodes

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CHAPTER OUTLINE

Abstract

Peripheral sensory organs and ganglia found in the vertebrate head arise during embryonic development from distinct ectodermal thickenings, called cranial sensory placodes (adenohypophyseal, olfactory, lens, trigeminal, epibranchial, and otic). A series of patterning events leads to the establishment of these placodes. Subsequently, these placodes undergo specific morphogenetic movements and cell-type specification in order to shape the final placodal derivatives and to produce differentiated cell types necessary for their function. In this

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chapter, we will focus on recent studies in the zebrafish that have advanced our understanding of cranial sensory placode development. We will summarize the signaling events and their molecular effectors guiding the formation of the so-called preplacodal region, and the subsequent subdivision of this region along the anteroposterior axis that gives rise to specific placode identities as well as those controlling morphogenesis and neurogenesis. Finally, we will highlight the approaches used in zebrafish that have been established to precisely label cell populations, to follow their development, and/or to characterize cell fates within a specific placode.

INTRODUCTION

Cranial placodes arise from the embryonic ectoderm immediately adjacent to the anterior neural plate (recently reviewed by Moody & LaMantia, 2015; Patthey, Schlosser, & Shimeld, 2014; Saint-Jeannet & Moody, 2014; Schlosser, 2005, 2007, 2010, 2014, 2015). While anteriorly, these placodes will ultimately give rise to the adenohypophysis, the olfactory epithelium, and the lens of the eye; more posteriorly the otic placode will generate the inner ear and the statoacoustic ganglion (SAG), and the trigeminal and epibranchial placodes will provide sensory neurons that innervate the skin as well as internal organs in order to transmit information such as heart rate, blood pressure, and visceral distension from the periphery to the brain (Fig. 1). Finally, in aquatic vertebrates, additional placodes have arisen that develop into the anterior and posterior lateral line ganglia and mechanosensory neuromasts of the head and flank that sense water flow.

Cranial placodes give rise to a plethora of different cell types including neuroendocrine cells, sensory neurons, lens fibers, and self-renewing stem cells that will generate the peripheral nervous system of the vertebrate head. As such, cranial placode development provides an attractive model to explore how cell fate is established, and gene regulatory networks controlling cranial placode patterning and specification are beginning to be unraveled. The distinct morphologies of the final placodal derivatives also provide a paradigm for dissecting genetic networks involved in their morphogenesis, including the regulation of epithelial rearrangement, cell adhesion, and migration (Breau & Schneider-Maunoury, 2014, 2015; Grocott, Tambalo, & Streit, 2012; Moody & LaMantia, 2015). Finally, cranial placodes originate adjacent to the neural crest at the neural plate border. As both placodal and crest cells contribute to cranial sensory ganglia, this system also provides a paradigm for understanding how the development of different tissues is coordinated.

At the end of gastrulation, cranial placodes are born from a common ectodermal domain of multipotent progenitors, known as the preplacodal region (PPR) (Fig. 2) (Bailey & Streit, 2006; Bhattacharyya & Bronner-Fraser, 2008; Kwon, Bhat, Sweet, Cornell, & Riley, 2010; Martin & Groves, 2006; Schlosser, 2005, 2010). Using explant cultures in chick embryos, it was demonstrated that placode precursors initially share a common "lenslike" ground state (Bailey,

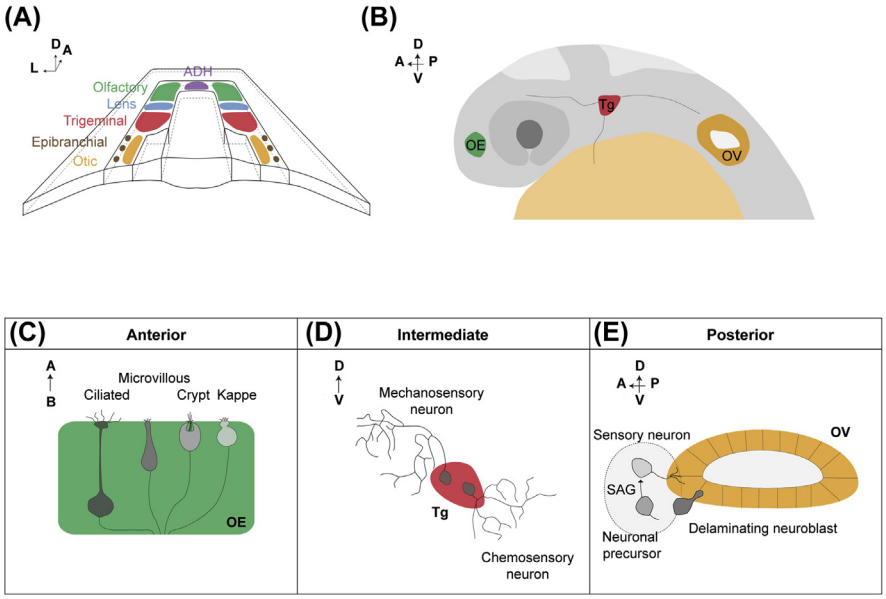

FIGURE 1 Cranial sensory placode development.

(A) Schematic representation of individualized cranial sensory placodes occupying specific positions along the anteroposterior axis during somitogenesis (dorsal view, anterior to the top). (B) Schematic positioning of the anterior olfactory placode (green (gray in print versions)), the intermediate trigeminal placode (red (dark gray in print versions)), and the posterior otic placode (orange (light gray in print versions)) at 24 hpf (hours postfertilization) (lateral view, anterior to the left). *OE*, olfactory epithelium; *Tg*, trigeminal placode, and *OV*, otic vesicle. (C–E) Schemes illustrating olfactory, trigeminal and otic neuronal subtypes. (C) Anterior olfactory sensory neuron (OSN) types. Schematic representation of the four classes of OSNs in the olfactory epithelium: while the cell bodies of ciliated OSNs are located basally, those of microvillous OSNs are positioned at intermediate position and those of crypt and kappe neurons more apically. (D) Intermediate trigeminal mechanosensory and chemosensory neurons. (E) Posterior otic neurogenesis. After specification, neuronal precursors delaminate as neuroblasts from an anterior–ventral position in the otic vesicle and give rise to the eighth cranial or statoacoustic ganglion (SAG).

Bhattacharyya, Bronner-Fraser, & Streit, 2006). During early neurulation, the PPR is subdivided into specialized domains that prefigure the full range of cranial placodes (Breau & Schneider-Maunoury, 2014; Schlosser, 2014). Subsequently, coordinated morphogenetic movements and cell-type specification sculpt the final form of the placodal derivatives and produce differentiated cell types necessary for their function.

Here, we will summarize recently published data primarily concerning the development of a subset of so-called "neurogenic" placodes, focusing on their patterning, morphogenesis, and neurogenesis. We will highlight advances made using the

zebrafish as a model, but will also mention work performed in chick, *Xenopus*, and mouse. Where it might be of particular interest, we will mention specific techniques used to make these advances (schematically depicted in Fig. 4). Finally, we provide two tables summarizing the transgenic zebrafish lines generated (Table 1) and the mutants established (Table 2) to study these processes.

1. SPECIFICATION OF THE PPR AND PATTERNING THE PPR INTO SPECIFIC PLACODES (FIG. 2)

1.1 SIGNALING PATHWAYS ORCHESTRATING PPR SPECIFICATION

Since the beginning of the 20th century, studies have identified a common ectodermal precursor field, the so-called preplacodal region (PPR; also referred to as preplacodal ectoderm or the panplacodal region), which gives rise to the cranial placodes (reviewed elsewhere in Moody & LaMantia, 2015; Patthey et al., 2014; Saint-Jeannet & Moody, 2014; Schlosser, 2005, 2007, 2010, 2014, 2015; Stark, 2014). Initial fate maps and more recent lineage tracing in zebrafish (Dutta et al., 2005; Kozlowski, Murakami, Ho, & Weinberg, 1997; Whitlock & Westerfield, 2000), amphibian and chick (Bhattacharyya, Bailey, Bronner-Fraser, & Streit, 2004; Bhattacharyya & Bronner, 2013; Couly & Le Douarin, 1990, 1987; Pieper, Ahrens, Rink, Peter, & Schlosser, 2012; Streit, 2002; Xu, Dude, & Baker, 2008) embryos have shown that all cranial placodes originate from this domain (Fig. 2). These experiments also illustrate that cells destined to give rise to specific placodes are to some extent intermingled at late gastrula stages, indicating that different cranial placodes arise from partially overlapping domains in the PPR (Bhattacharyya et al., 2004; Dutta et al., 2005; Kozlowski et al., 1997; Whitlock & Westerfield, 2000).

The PPR possesses a molecular signature that includes the expression of genes of the Dlx, Six, and Eya transcription factor families during neurulation (Lleras-Forero & Streit, 2012; Pieper, Eagleson, Wosniok, & Schlosser, 2011; Sato, Miyasaka, & Yoshihara, 2005; Schlosser, 2010). Intriguingly, some genes expressed in the PPR are also expressed in the neural crest cells, raising the question of how these two cell populations become distinct from one another and from the surrounding neural and nonneural tissues (recently reviewed by Groves & LaBonne, 2013). It is now known that during gastrulation, interactions between neural and nonneural ectoderm, as well as signals emanating from underlying mesoderm and endoderm induce the formation of an intermediary ectodermal domain, the neural border or NB, that has the competence to give rise to both neural crest cells and PPR (Fig. 2A). The neural border expresses a combination of specific transcription factors that primes the neural border to respond to lineage-specific signals that induce neural crest cell or placode cell fate (reviewed by Groves & LaBonne, 2013; Moody & LaMantia, 2015). We will first focus our attention on the signaling pathways that operate at the neural border.

Table 1 Summary of Transgenic Zebrafish Lines Used to Study Cranial Placode Development

Transgenic Lines	Domain Labeled/Function	References
Tg(BRE:mRFP)	Anterior neural border	Reichert et al. (2013), Wu et al. (2011)
1. *Tg(−4.9sox10:egfp)^{ba}*	**1.** CNC cells give rise to microvillous olfactory neurons. Otic placode	**1.** Saxena et al. (2013)
2. *Tg(sox10(7.2):mrfp)^{vu234}*	**2.** Olfactory sensory precursors do not incorporate CNC lineages in the OPs. *sox10* gene expression is never observed in microvillous olfactory neurons.	**2.** Harden et al. (2012), Torres-Paz and Whitlock (2014)
Tg(six4b:mCherry)=Tg(pTol2six4.1A:mCherry)^{uv87}	Olfactory placode progenitors	Harden et al. (2012)
Tg(pax2a:GFP)^{e1}	Posterior PPR	McCarroll et al. (2012)
Tg(pax2a:CreER^{T2})$^{\#31}$	Recapitulates *pax2a* expression during OEPD development	Hans et al. (2013)
Tg(hsp70l:loxP-DsRed2-loxP-eGFP)	Conditional red to green reporter for PioTrack	Hans et al. (2013)
Tg(hs:gata3(eGFP))	PPR differentiation	Yao et al. (2014)
Tg((hs:foxi1(eGFP))	PPR differentiation	Yao et al. (2014)
Tg(hsp70:tcfΔC-EGFP)	Inhibition Wnt signalling/reduction otic placode	McCarroll et al. (2012)
Tg(hsp70:dkk1-GFP)^{w32}	Wnt antagonist/reduction otic placode	McCarroll et al. (2012)
Tg(hsp70l-dkk1-mCherry)	Proper level of NPB genes (*pax3a* and *zic3*)	Garnett et al. (2012)
Tg(hsp70l-bmp2b)	Proper positioning in NPB of *pax3a* and *zic3*	Garnett et al. (2012)
Tg(hsp70:fgf8a)^{x17}	**1.** Activation PPR competence factor (with bmp antagonist) **2.** Otic neurogenesis	**1.** Kwon et al. (2010) **2.** Kantarci et al. (2015); Vemaraju et al. (2012)
Tg(hsp70:ca-fgfr1)^{pd3}	*pax2a* induction in posterior PPR	McCarroll et al. (2012)
Tg(hsp70l:dnfgfr1-EGFP)^{pd1}	**1.** Otic neurogenesis	**1.** Kantarci et al. (2015), Vemaraju et al. (2012)
	2. Control *pax2a* induction in posterior PPR	**2.** McCarroll et al. (2012)
Tg(hsp70l:dnBmpr-GFP)	Activation PPR competence factor (with *fgf8*)	Kwon et al. (2010)
Tg(hsp70:chordin)	Activation PPR competence factor (with *fgf8*)	Kwon et al. (2010)
Tg(UAS:myc-Notch1a-intra)^{kca3}	Otic neurogenesis	Kantarci et al. (2015)
Tg(hsp70l:gal41.5)^{kca4}	Otic neurogenesis	Kantarci et al. (2015)

Continued

Table 1 Summary of Transgenic Zebrafish Lines Used to Study Cranial Placode Development—cont'd

Transgenic Lines	Domain Labeled/Function	References
Tg(hsp70:fgf8a)^{x17}	**1.** Nonneural ectoderm is competent to express PPR genes in response to FGF plus inhibition of Bmp. **2.** *sox3* shows two distinct responses to FGF signaling	**1.** Kwon et al. (2010) **2.** Padanad and Riley (2011)
Tg(hsp70:fgf3)^{x18}	Nonneural ectoderm is competent to express PPR genes in response to FGF plus inhibition of Bmp.	Kwon et al. (2010)
Tg(hsp70:foxi1)^{x19}	**1.** PPR competence factor. **2.** Maintaining *foxi1* expression after 11 hpf impairs completion of otic development in a cell-autonomous manner.	**1.** Bhat et al. (2013), Kwon et al. (2010) **2.** Padanad et al. (2012)
Tg(hsp70:tfap2a)^{x24}	**1.** PPR specification **2.** Otic neurogenesis	**1.** Bhat et al. (2013) **2.** Kantarci et al. (2015)
Tg(hsp70:gata3)^{x25}	PPR specification	Bhat et al. (2013)
Tg(8.4neurog1:GFP)	EON	Madelaine et al. (2011)
Tg(8.4neurog1:nRFP)	Olfactory neurons	Saxena et al. (2013)
Tg(neurod:EGFP)^{nl1}	Morphogenesis of the trigeminal placode	Bhat and Riley (2011)
Tg(her4:EGFP)^{y83}	Otic neurogenesis	Radosevic, Fargas, and Alsina (2014)
Tg(hsp:cxcl12a)	**1.** Ubiquitous heat-induced misexpression of Cxcl12a/Sdf1 **2.** Trigeminal sensory neurons positioning	**1.** Miyasaka et al. (2007) **2.** Knaut et al. (2005)
Tg(hsp70:slit2-GFP)^{rw015d}	Mimic *robo2* mutant (*ast*): impaired OSN axon pathfinding	Miyasaka et al. (2005)
Tg(OMP^{2k}*:lyn-mRFP)*^{rw035}	Ciliated OSNs	Saxena et al. (2013)
Tg(OMP^{2k}*:gap-YFP)*^{rw032a}	Dynamic of OSN axon projections	Miyasaka et al. (2005)
Tg(OMP^{6k}*:gap-YFP)*^{rw031a}	Dynamic of OSN axon projections	Miyasaka et al. (2005)
Tg(TRPC2$^{4.5k}$*:gap−Venus)*^{rw037}	Microvillar OSNs	Harden et al. (2012), Saxena et al. (2013)
Tg(huc:kaede;p2x3:egfp)	BAPTISM: pan neuronal marker; subset of trigeminal sensory neurons	Caron et al. (2008)
Tg(trpa1b:egfp; huc:kaede)	BAPTISM: pan neuronal marker; subset of trigeminal sensory neurons	Caron et al. (2008)
Tg(brn3c:gap43-GFP)^{s356t}	Otic hair cells	Kantarci et al. (2015)
Tg(bactin2:memb-Cerulean-2A-H2B-Dendra2)^{pw1}	PhOTO-N	Saxena et al. (2013)

CNC, *cranial neural crest;* EON, *early olfactory neurons;* OEPD, *otic-epibranchial progenitor domain;* OP, *olfactory placode;* OSNs, *olfactory sensory neurons;* PPR, *preplacodal region.*

Table 2 Summary of Zebrafish Mutants Used to Study Cranial Placode Development

Gene	Mutants	Effect/Phenotypes	References
foxi1	**1.** *foxi1*^{em1} *and foxi1*^{hi3747tg} **2.** *foxi1*^{em1}	**1.** PPR differentiation **2.** Loss of all neuronal OEPD derivatives	**1.** Yao et al. (2014) **2.** Hans et al. (2013)
tfap2a	*mont blanc* *mob*^{m819}	Otic neurogenesis	Kantarci et al. (2015)
pax2a	*pax2a*$^{b593/+}$	Posterior PPR Otic differentiation	McCarroll et al. (2012)
dlx3b, dlx4b	*Df(Chr12:dlx3b,dlx4b,tbx6)* ^{b380}	**1.** *msx1b*, *msx3*, *msx1a* and *dlx3b*, *dlx4b* antagonism at the NB. **2.** Otic and olfactory placodes formation **3.** Trigeminal placode formation **4.** Otic epithelial fates	**1.** Phillips et al. (2006) **2.** Solomon and Fritz (2002) **3.** Kaji and Artinger (2004) **4.** Hans et al. (2013)
msx1b	*Df(Chr01:lef1,msxb)*^{x8}	Reduced size of posterior placodes derivatives	Phillips et al. (2006)
neurog1	*neurog1*^{hi1059Tg}	**1.** Trigeminal sensory ganglia absent at 24 hpf but few neurons at 48 hpf (late born neurons) restricted in their fate. **2.** Delayed EON (reduced number) **3.** Neurog1 controls *her4* expression in otic neurogenic domain	**1.** Caron et al. (2008) **2.** Madelaine et al. (2011) **3.** Radosevic et al. (2014)
eya1	*dog-eared*	Inner ear and lateral line sensory systems	Kozlowski et al. (2005)
cxcr4b	*odysseus*; *cxcr4b*^{t26035} = ody^{JI0049}	**1.** Olfactory placode assembly and sensory axons pathfinding **2.** Trigeminal sensory ganglion assembly	**1.** Miyasaka et al. (2007) **2.** Knaut et al. (2005)
robo2	*astray*, *ast*^{ti272z}	OSNs axons fasciculation	Miyasaka et al. (2005)
n-cadherin, cdh2	*parachute* (*pac*) *glass onion* (*glo*) *pac, glo, ncad, cdh2* *pac*^{tm101B}, *pac*^{fr7} and *pac*$^{paR2.10}$	Morphogenesis Neural tube integrity	Lele et al. (2002)
Integrin-α5	*itga5*^{b926}	Development of posterior placodes	Bhat and Riley (2011)

EON, *early olfactory neurons;* OEPD, *otic-epibranchial progenitor domain;* OSNs, *olfactory sensory neurons;* PPR, *preplacodal region.*

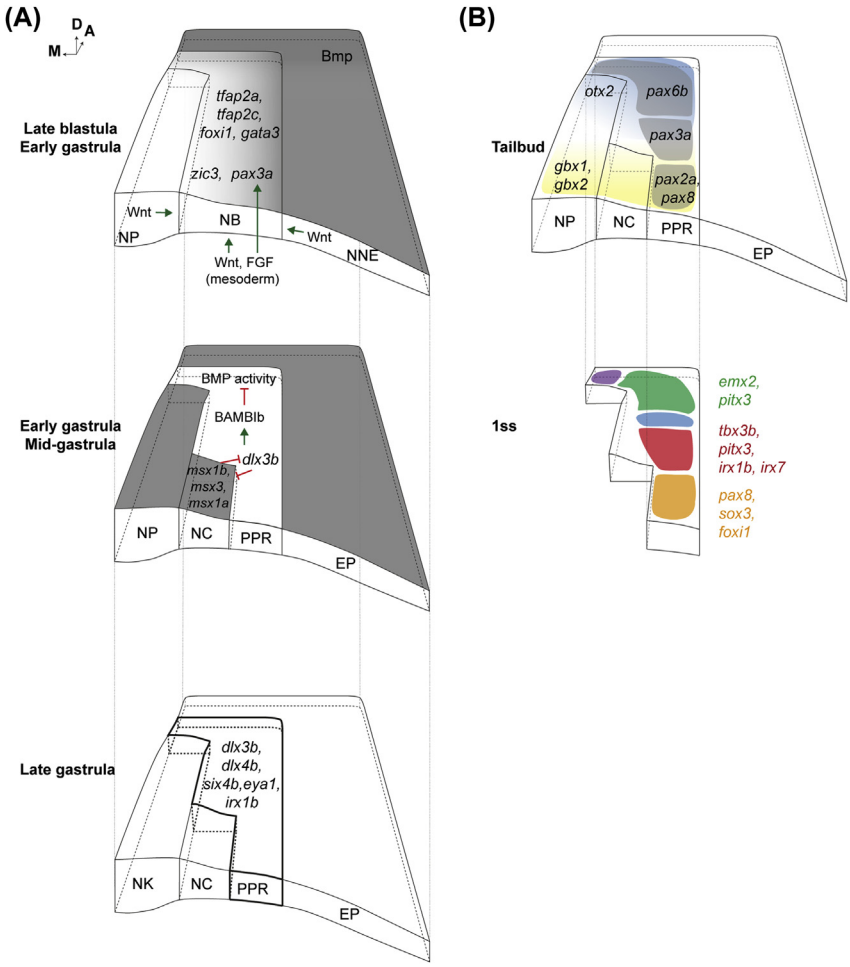

FIGURE 2 Genetic network and key signaling pathways controlling PPR formation and subdivision along the anteroposterior axis.

(A) A gene regulatory network controlling PPR specification during gastrulation. At the late blastula—early gastrula stage, a combined transient activation of FGF signaling with low BMP and Wnt activity are required to induce the expression of the PPR competence factors; *tfap2a*, *tfap2c*, *foxi1*, and *gata3* in the NB. *Zic3* and *pax3a* are expressed in the NB at this stage and are under the control of Wnt and Wnt and FGF, respectively. At the mid-gastrula stage the mutual inhibition between *msx1b*, *msx3*, *msx1a*, and *dlx3b* set up the border between the neural crest domain and the PPR within the NB. Then, *dlx3b* blocks BMP activity through *BAMBIb* to maintain the PPR identity. Finally, at late gastrula stages the PPR signature genes, *dlx3b* and *dlx4b*, *six4b*, *eya1* and *irx1b*, specify this domain (B) Gene network controlling anteroposterior PPR regionalization. At the tailbud stage, the mutual inhibition between rostral *otx2* and caudal *gbx1*, *gbx2* regionalizes the cranial sensory placodes along the anteroposterior axis. Genes specific of the olfactory (green), trigeminal (red), and otic (orange) placode are color coded. *NP*, neural plate; *NB*, neural border; *NNE*, nonneural ectoderm; *EP*, epidermis; *NC*, neural crest cells; *PPR*, preplacodal region; *NK*, neural keel; *1ss*, 1 somite stage; *BMP*, bone morphogenetic protein; *FGF*, fibroblast growth factor. (See color plate)

1.1.1 Fine regulation of bone morphogenetic protein activity during gastrulation defines the neural border

During gastrulation, a gradient of bone morphogenetic protein (BMP) signaling is found across the ectoderm, with the neural border receiving intermediate levels of BMP signaling (Neave, Holder, & Patient, 1997; Nguyen et al., 1998; Schumacher, Hashiguchi, Nguyen, & Mullins, 2011; Tucker, Mintzer, & Mullins, 2008) (Fig. 2A). Recent advances in live imaging have made it possible to visualize the dynamic temporal and spatial regulation of BMP signaling at the NB during ectodermal patterning. Using a BMP reporter *Tg(BMPRE:mRFP)* transgenic zebrafish line that expresses monomeric red fluorescent protein under the control of a BMP response element (Ramel & Hill, 2012; Wu, Ramel, Howell, & Hill, 2011), it was elegantly shown that BMP activity is concentrated in a horseshoe-shaped domain at the NB at the end of gastrulation, which then resolves into two distinct domains, an outer domain abutting the epidermis and an inner domain that corresponds to the PPR and the neural crest, respectively (Reichert, Randall, & Hill, 2013) (Fig. 4A). Bmp2b and Bmp7a are required for establishing the initial BMP^{ON} domain at the NB (Reichert et al., 2013) and have been implicated in promoting neural crest cell fate in several model organisms (reviewed in Stuhlmiller & García-Castro, 2012). Subsequently, PPR specification requires attenuation of BMP signaling (Kwon et al., 2010). This is achieved at least in part via the induction of the BMP antagonist, BMP and activin membrane-bound inhibitor b (BAMBIb), by the PPR-specific transcription factor Dlx3b (Reichert et al., 2013). In parallel, enhanced BMP activity domains in the epidermis and the neural crest are promoted though the BMP-dependent expression of the secreted BMP-binding protein, *crossveinless 2* (*cvl2*, *cv2* or *bmper*, *BMP binding endothelial regulator*) (Reichert et al., 2013). The role of Cvl2 at the NB is controversial, however, because although this factor has been shown to act as a positive regulator of BMP signaling in certain studies (Reichert et al., 2013), its function has also been described as antagonistic to BMP signaling during PPR specification (Esterberg & Fritz, 2009); in the latter example the authors provide evidence that, as for BAMBIb, the transcription factors Dlx3b and Dlx4b regulate *cvl2* expression. The secretion of regulators of BMP signaling from the PPR provides insights into how Dlx factors autonomously establish PPR identity while exerting nonautonomous influence on adjacent neural crest cells. Intriguingly, *dlx3b* and *cvl2* expressions are initially detected in an overlapping domain during gastrulation, but the domains segregate as somitogenesis progress (Reichert et al., 2013). Two possibilities exist for this observation: either one population of cell initially coexpresses these two factors, or they are expressed in specific cell types in a salt-and-pepper manner from the start. It will be of interest in the future to characterize *dlx3b+* and *cvl2+* cell sorting during gastrulation as this might shed more light on the specification of the PPR versus the neural crest cell fates.

1.1.2 Fibroblast growth factor signaling promotes PPR identity

PPR formation requires fine-tuning of BMP activity levels but also high levels of fibroblast growth factor (FGF) signaling (Ahrens & Schlosser, 2005; Glavic

et al., 2003; Litsiou, Hanson, & Streit, 2005). Remarkably, *cvl2* induced by Dlx3b and Dlx4b promotes competence to respond to FGF signaling in the PRR, mainly through the regulation of expression of FGF receptors and the FGF targets *erm* and *spry4 specification* (Esterberg & Fritz, 2009). Using heat-shock-inducible transgenes and a pharmacological inhibitor to modulate signaling, Kwon et al. (2010) found that FGF combined with BMP attenuation is sufficient to induce PPR markers throughout the nonneural ectoderm in a manner similar to that previously shown in frog and chick embryos (Ahrens & Schlosser, 2005; Litsiou et al., 2005) (Fig. 2A). Finally, FGF and platelet-derived growth factor A (PDGFa), which is expressed dorsally during gastrulation (Kudoh, Concha, Houart, Dawid, & Wilson, 2004; Liu, Korzh, Balasubramaniyan, Ekker, & Ge, 2002), appear to act in a partially redundant manner during PPR specification (Kwon et al., 2010).

1.1.3 Wnt and retinoic acid signaling and the PPR domain

The Wingless integrated protein (Wnt) signaling pathway has been proposed to antagonize PPR specification. Elevated Wnt signaling in chick and *Xenopus* represses PPR markers, whereas secreted anti-Wnt factors expand them (Hong & Saint-Jeannet, 2007; Litsiou et al., 2005). In zebrafish, Wnts and their antagonists are expressed in the anterior neural plate and the underlying mesoendoderm leading to a proposed "low anterior to high posterior" Wnt activity gradient (Cavodeassi, 2014). It has been demonstrated that sets of regulatory elements at two genes required for NB specification (*pax3a* and *zic3*) integrate the activities of Wnt, FGF, and BMP signaling (Garnett, Square, & Medeiros, 2012). Interestingly, however, while these elements drive expression in largely overlapping domains at the NB, they respond to different combinations of BMP, Wnt, and FGF signals; while BMP and Wnt are required for expression of both *pax3a* and *zic3*, FGF signaling is specifically required for proper expression of *pax3a* but not *zic3* (Fig. 2A) (Garnett et al., 2012).

Finally, the signaling cocktail required to induce PPR markers also appears to include retinoic acid (RA). In *Xenopus*, the RA synthesizing enzyme Raldh2 is expressed in the PPR (Chen, Pollet, Niehrs, & Pieler, 2001; Shiotsugu et al., 2004) and RA signaling limits PPR development to the head (Shiotsugu et al., 2004). It, however, remains to be investigated whether these mechanisms are conserved in zebrafish. On the other hand, epistatic analysis of the function of FGF, Wnt, and RA has been performed concerning the patterning of the neural ectoderm in zebrafish along the anteroposterior axis (Kudoh, Wilson, & Dawid, 2002). It would be interesting to use the same tools to determine the epistatic relationship of these signals during neural border formation and PPR and/or neural crest cell fates choice. Furthermore, it is now possible to generate detailed and dynamic spatiotemporal maps of BMP, FGF, Wnt, and RA activity at cellular resolution during early development using transgenic reporter lines and time lapse (for review see Mandal et al., 2013; Moro et al., 2013). These maps should provide precious tools for understanding how signaling pathway activity is integrated at the neural border to induce the PPR fate.

1.2 A GENE REGULATORY NETWORK UNDERLYING PPR SPECIFICATION (FIG. 2A)

The combination of reduced BMP and Wnt signaling and high FGF activity ultimately leads to the expression of specific PPR markers flanking the anterior neural plate. A pair of recent studies has shown that the expression of these PPR markers requires the activity of Tfap2a, Tfap2c, Gata3, and Foxi1, which collectively establish preplacodal competence in the nonneural ectoderm (Bhat, Kwon, & Riley, 2013; Kwon et al., 2010). The combined abrogation of these PPR competence factors using morpholinos results in complete loss of later PPR markers and subsequent defects in the development of all cranial placodes (Kwon et al., 2010). Chemical inhibition of BMP signaling was used to show that the pathway is required only transiently at blastula stages to induce the expression of these competence factors, which then become independent of BMP signaling (Kwon et al., 2010). Using inducible heat-shock transgenes together with transient loss of function of BMP signaling, it was shown that *tfap2a*, *tfap2c*, *gata3*, and *foxi1* form a self-maintaining gene regulatory network, with robustness in this PPR competence network being achieved through mutual cross-activation (Bhat et al., 2013). These factors not only maintain their own expression but also feed back onto the signaling pathways that induced their expression. For instance, both Gata3 and Foxi1 inhibit BMP signaling (Yao et al., 2014).

Genes specifically expressed in the PPR downstream of the competence factors mentioned in the previous paragraph include members of the *Eyes absent* (*Eya*)/*Sine oculis* (*Six*)/*Dachshund* (*Dach*), Distalless (*Dlx*), and *Iroquois* (*Irx*) gene families (Ahrens & Schlosser, 2005; Bhat et al., 2013; Brugmann, Pandur, Kenyon, Pignoni, & Moody, 2004; Glavic et al., 2003; Kwon et al., 2010; Litsiou et al., 2005; Nguyen et al., 1998). In zebrafish, *dlx3b* is the earliest specific marker of the PPR, starting around 8 h postfertilization (hpf) in the nonneural ectoderm and increasing until late gastrulation (Akimenko, Ekker, Wegner, Lin, & Westerfield, 1994; Esterberg & Fritz, 2009; Solomon & Fritz, 2002; Woda, Pastagia, Mercola, & Artinger, 2003). By 10 hpf, *six4b* (Bhattacharyya et al., 2004; Kobayashi, Osanai, Kawakami, & Yamamoto, 2000) and *eya1* are detected in the PPR. Finally, a member of the Iroquois (Irx) homeobox transcription factor family, *irx1b*, is also expressed in the PPR starting around 10 hpf (Lecaudey, Anselme, Dildrop, Rüther, & Schneider-Maunoury, 2005). Using cell transplantation and constitutively active and dominant negative forms of Irx1b both in zebrafish and *Xenopus*, it was shown that this factor is required for PPR specification (Glavic et al., 2003). Likewise, the analyses of *six1b* and *eya1* mutants and morphants in mouse and zebrafish have confirmed their role in the development of cranial placode derivatives. Indeed, the abrogation of the function of these factors leads to defects in multiple sensory organs, and particularly in zebrafish to inner ear malformations (Bricaud & Collazo, 2006; Nica et al., 2006); in the zebrafish *eya1* mutant, defects are found in the inner ear and the lateral line sensory systems (Kozlowski, Whitfield, Hukriede, Lam, & Weinberg, 2005). In mouse, these genes are involved in the formation of many sensory organs, the

adenohypophysis, olfactory epithelium, trigeminal ganglion, inner ear, and epibranchial ganglia (Ikeda, Watanabe, Ohto, & Kawakami, 2002; Laclef, Souil, Demignon, & Maire, 2003; Li et al., 2003; Ozaki, Watanabe, Ikeda, & Kawakami, 2002; Xu et al., 1999; Zheng et al., 2003; Zou, Silvius, Fritzsch, & Xu, 2004).

Other genes expressed in partially overlapping domains in the neural crest and PPR at the neural border include the muscle segment homeobox genes *msx1b*, *msx3*, and *msx1a*. Combined loss of function of these genes blocks neural crest differentiation, whereas the PPR-derived placodes show elevated levels of apoptosis and are reduced in size (Phillips et al., 2006). As for *dlx* genes, *msx* genes are well-known Bmp targets in vertebrate (Esteves et al., 2014; Feledy et al., 1999; Tribulo, Aybar, Nguyen, Mullins, & Mayor, 2003; Yamamoto, Takagi, & Ueno, 2000). While it remains unclear how *dlx* and *msx* expressing domains segregate after their induction by BMP signaling, it is tempting to speculate that this is achieved through reciprocal inhibition. Indeed, Msx proteins have been described as antagonists of *dlx* genes during development (Suzuki, Ueno, & Hemmati-Brivanlou, 1997, reviewed in Bendall & Abate-Shen, 2000). Furthermore, the loss of function of *msx* genes is able to restore expression of the PPR marker *six4b* in embryos harboring a deletion containing *dlx3b* and *dlx4b* (Solomon & Fritz, 2002), again suggesting an interaction between these two gene families.

Finally, members of the Pax (Krauss, Johansen, Korzh, & Fjose, 1991) and Zic families, in particular *pax3a* and *zic3*, are also expressed during gastrulation at the neural border. As mentioned above, evolutionarily conserved neural borders enhancers associated with these two genes have been characterized that integrate different combinations of FGF, BMP, and Wnt signaling (Garnett et al., 2012).

1.3 ANTEROPOSTERIOR REGIONALIZATION OF THE PPR (FIG. 2B)

Shortly after the establishment of the PPR, gene expression analysis and fate mapping data show that distinct placodal primordia become apparent. This is exemplified by the broad expression of *pax6b*, *pax3a* and *pax2, pax8* in specific domains within the PPR (Pieper et al., 2011). In this section, we will touch on what is known concerning the molecular mechanisms that control anteroposterior patterning within the PPR and mention factors linked with the development of specific placodes.

After gastrulation, *pax* gene expression distinguishes three large placodal primordia along the anteroposterior axis: a large anterior primordium (adenohypophyseal, olfactory, lens), a smaller intermediate primordium (trigeminal placode), and a large posterior primordium (otic, epibranchial, and lateral line). As for the formation of the PPR itself, the establishment of these primordia requires the input of signaling cascades and regional expression of sets of transcription factors but currently less is known about this process in the fish. It is known, however, that subdivision of the PPR happens synchronously with anteroposterior regionalization in the adjacent neural plate, which is characterized by positioning the midbrain–hindbrain boundary (MHB). Toward the end of gastrulation the future MHB is manifested by the border

between the expression of two transcription factors: *otx* (*orthodenticle homeobox*) anteriorly and *gbx* (*gastrulation brain homeobox*) posteriorly (Hibi & Shimizu, 2012; Kiecker & Lumsden, 2012; Raible & Brand, 2004; Rhinn, Lun, Luz, Werner, & Brand, 2005). In the zebrafish, the mutually exclusive expression of *otx2* and *gbx1* is achieved by reciprocal inhibition between these two factors, and with the control of *gbx1* expression requiring the posteriorizing activity of Wnt signaling (Rhinn, Lun, Ahrendt, Geffarth, & Brand, 2009). Interestingly, in chick and *Xenopus*, mutual repression between Gbx and Otx family members not only patterns the neural plate but also the adjacent placodal territory, leading to segregation of otic and trigeminal progenitors (Steventon, Mayor, & Streit, 2012). Similarly in the mouse, Gbx2 is necessary posteriorly for otic vesicle morphogenesis after placode formation (Lin, Cantos, Patente, & Wu, 2005); anteriorly, Otx2 cooperates with Notch signaling to induce lens fate (Ogino, Fisher, & Grainger, 2008). It remains to be addressed whether the Gbx−Otx couple regulates the segregation of the PPR in the zebrafish.

In *Xenopus*, *Gbx2* is among the earliest factors to promote posterior PPR identity and appears to play a dual role (Steventon et al., 2012)—it first represses *otx2* expression and later drives *pax8* and *pax2* expression. These latter factors are critical for normal ear development in zebrafish, chick, and mice (Bouchard, de Caprona, Busslinger, Xu, & Fritzsch, 2010; Burton, Cole, Mulheisen, Chang, & Wu, 2004; Christophorou, Mende, Lleras-Forero, Grocott, & Streit, 2010; Mackereth, Kwak, Fritz, & Riley, 2005; Torres, Gómez-Pardo, Dressler, & Gruss, 1995); while concomitant abrogation of *pax2a*, *pax2b*, and *pax8* function in zebrafish using morpholinos leads to a small otic placode that will ultimately degenerate (Mackereth et al., 2005), overexpression of *pax2a* favors otic at the expense of epibranchial placode differentiation (McCarroll et al., 2012). Intriguingly, the *pax2a*, *pax2b*, and *pax8* loss of function phenotype is reminiscent of the knockdown of *fgf3* activity in *fgf8* (*acerebellar* or *ace*) mutants, and indeed, posterior PPR cells in *Fgf*-deficient embryos fail to express *pax8* and *pax2a* (Phillips, Bolding, & Riley, 2001). FGF3 and FGF8 from the hindbrain and adjacent mesendoderm induce not only *pax* gene expression but also that of *sox3* in preotic cells. In this context, specific responsiveness to FGF requires the competence factor Foxi1, which has, at these stages, become restricted to the otic and epibranchial regions. By 12 hpf, Pax8 in turn induces expression of *fgf24* and represses otic expression of *foxi1*. FGF24 then downregulates otic expression of *sox3* while inducing it in adjacent epibranchial cells (Padanad, Bhat, Guo, & Riley, 2012); FGF10b also participates in the late phase of otic placode induction (Maulding, Padanad, Dong, & Riley, 2014). Finally, Wnt signaling also plays a role in otic placode patterning via regulation of *pax* gene expression (McCarroll et al., 2012). Here, increasing Wnt activity at early somite stages causes *pax2a* upregulation and an enhanced recruitment of cells into otic placode, and blocking Wnt signaling has the opposite effect.

At an intermediate level in the PPR, the chick PDGF receptor beta is expressed in the cranial ectoderm at the time of trigeminal placode formation, while the corresponding ligand is expressed in the adjacent midbrain neural folds. Antagonizing

PDGF signaling abrogates *pax3* expression and impairs trigeminal placode induction (McCabe & Bronner-Fraser, 2009). The functions of PDGF in trigeminal placode induction remain to be elucidated in zebrafish. Two Iroquois transcription factors, *irx1b* and *irx7*, are expressed in zebrafish trigeminal placode and loss of function analysis reveals that *irx7* is required for correct trigeminal placode development (Itoh, Kudoh, Dedekian, Kim, & Chitnis, 2002). Whether this reflects a role in patterning or neurogenesis is unclear, however, as the trigeminal placode is largely composed of sensory neurons and defects in either patterning or neurogenesis would result in similar phenotypes. Another transcription factor expressed in zebrafish trigeminal progenitors is the *T-cell leukemia, homeobox 3b*, *tlx3b* (Langenau et al., 2002), but here again functional requirements for this gene in trigeminal placode remain to be addressed. Finally, the dynamic expression of pitx3 (*pairedlike homeodomain 3*) in the PPR suggests that this gene marks a common step in patterning of the trigeminal placode as well as the pituitary, lens, and olfactory placodes (Zilinski, Shah, Lane, & Jamrich, 2005).

Anteriorly, signals from the mesendoderm are required for lens and olfactory placode formation in both zebrafish and chick embryos (Devos et al., 2002; Lleras-Forero et al., 2013). For example, somatostatin first regulates and then cooperates with nociceptin to control *pax6b* expression in anterior placodal progenitors. Unlike the *gbx−otx* system, this study shows that nociceptin signaling controls anterior placode development without affecting neural plate development. Conversely, FGF signals emanating from the posterior head mesoderm inhibit anterior placode identity (Lleras-Forero et al., 2013). Here, the interplay between two previously mentioned PPR competence factors, Gata3 and Foxi1, and FGF signaling shapes the activity gradient of the pathway along the anteroposterior axis allowing the development of different anterior versus posterior placodal identities (Yao et al., 2014).

Another marker of the anterior placode domain is *pitx3* (Zilinski et al., 2005). Knockdown analysis using morpholinos revealed a function for this gene in lens and retina development, without affecting olfactory development (Shi et al., 2005). In *Xenopus*, *Dmrt4* (*Doublesex-relate*d 4) also initially labels the anterior placode domain (Huang, Hong, O'Donnell, & Saint-Jeannet, 2005) and it was shown that reducing Dmrt4 function specifically affects neurogenesis in the olfactory placode. In the teleost Medaka, *dmrt4* is also expressed in the developing olfactory system (Winkler et al., 2004); however, its function in olfactory placode patterning has not been addressed yet. Finally, three members of the *emx* (empty spiracles homeobox) transcription factor family are expressed in the anterior placode domain, and more specifically in the olfactory placode but, here again, functional analyses are lacking (Viktorin, Chiuchitu, Rissler, Varga, & Westerfield, 2009); *Emx2*-knockout mice display smaller olfactory bulbs (Bishop, Garel, Nakagawa, Rubenstein, & O'Leary, 2003). In conclusion, makers of the anterior placode domain in the PPR have mainly been identified based on expression and further functional analysis of these genes will be required in order to understand their role in early anterior placode specification.

2. CRANIAL PLACODE MORPHOGENESIS AND NEUROGENESIS (FIG. 3)

After individualization of specific placodes in the PPR domain, distinct morphogenetic processes will sculpt the final forms of the placodal derivatives (reviewed in Breau & Schneider-Maunoury, 2014; Schlosser, 2010, 2014, 2015). Simultaneously, neurogenesis in certain placodes will produce distinct neuronal subtypes characteristic of the mature placode-derived organs. In this section, we will address the mechanisms underlying morphogenesis and neurogenesis, specifically concentrating on the olfactory, trigeminal, and otic placodes and their derivatives (Fig. 3).

2.1 OLFACTORY PLACODE

The olfactory placode arises from an extended cellular field stretching along the lateral edge of the anterior neural plate (Fig. 3A) (Whitlock, 2004, 2008; Whitlock & Westerfield, 2000 and for reviews see Breau & Schneider-Maunoury, 2014, 2015; Maier, Saxena, Alsina, Bronner, & Whitfield, 2014; Miyasaka et al., 2013). Analysis of early olfactory placode morphogenesis in the zebrafish was first reported 15 years ago (Whitlock & Westerfield, 2000). In this study, fate mapping of cells labeled with fluorescent dextrans in the anterior neural plate indicated that the olfactory placodes develop via anterior convergence of progenitor cells (Whitlock & Westerfield, 2000). More recently, using a *Tg(βeta-actin:GAP-43-GFP)* transgenic line combined with the nuclear marker H2B-RFP, the same laboratory demonstrated the existence of coordinated cells movement within the population of olfactory placode progenitors (Torres-Paz & Whitlock, 2014). A similar live imaging approach showed that cranial neural crest cells associate closely with the forming olfactory placode, suggesting coordination between anterior neural crest migration and olfactory placode condensation (Harden et al., 2012). Finally, live imaging studies using a *Tg(sox10:eGFP)* transgenic line have shown that neural crest cells invade the developing olfactory placode and differentiate into a subclass of olfactory sensory neurons (OSNs) (Saxena, Peng, & Bronner, 2013) (Fig. 4B); laser ablation of *sox10:eGFP* positive cells prevents microvillous neurons formation in the olfactory placode.

The molecular mechanisms driving olfactory placode morphogenesis are beginning to be identified. It is known, for instance, that this process requires the chemokine receptor Cxcr4b, and its ligand Cxcl12a (also known as stromal cell-derived factor 1 or Sdf1). Interfering with Cxcl12a or Cxcr4b signaling, either by misexpression of Cxcl12a or in *odysseus* (ody) embryos carrying mutations in *cxcr4b*, affects olfactory placode condensation (Miyasaka, Knaut, & Yoshihara, 2007). Subsequently, while *cxcr4b* expression persists within the olfactory placode, *cxcl12a* is expressed along the placode−telencephalon border prefiguring the projection route of mature OSNs axons. In keeping with a role for chemokine signaling in the targeting of olfactory projections to the brain, OSN axons frequently fail to exit the olfactory placode and accumulate near the placode−telencephalon border in the absence of Cxcr4b-mediated signaling (Miyasaka et al., 2007). Whether this reflects a role of

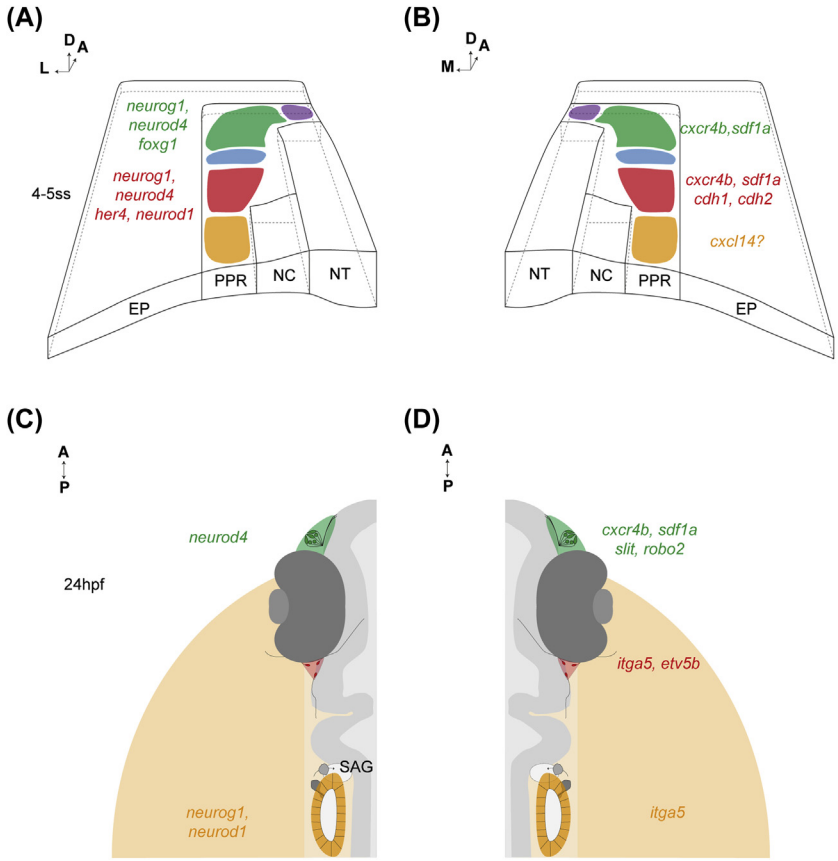

FIGURE 3 Neurogenesis and morphogenesis of olfactory, trigeminal, and otic placodes.

(A, C) Genes essential for neurogenesis of olfactory, trigeminal, and otic placodes at the 4–5 ss (A) and at 24 hpf (C). (B, D) Genes required for morphogenesis of these placodes at the 4–5 ss (B) and at 24 hpf (D). Genes specific of the olfactory (green), trigeminal (red), and otic (orange) placode are color coded. *NT*, neural tube; *EP*, epidermis; *NC*, neural crest cells; *PPR*, preplacodal region; *4–5 ss*, 4–5 somite stages; *hpf*, hours postfertilization. (See color plate)

Cxcr4-Cxcl12 signaling during the guidance of olfactory neuron projects to the olfactory bulb or is a secondary consequence of an earlier morphogenesis defect is not clear. Finally, Robo and Slit, Semaphorin, Ephrin and Netrin signaling have also been implicated in the guidance and fasciculation of olfactory neuron projections (Cutforth et al., 2003; Lakhina et al., 2012; Miyasaka et al., 2005; Schwarting et al., 2000; Taniguchi et al., 2003).

Neurogenesis in the olfactory placodes has been studied in depth in the mouse, and more recently in the zebrafish. In the adult zebrafish, four classes of OSNs have

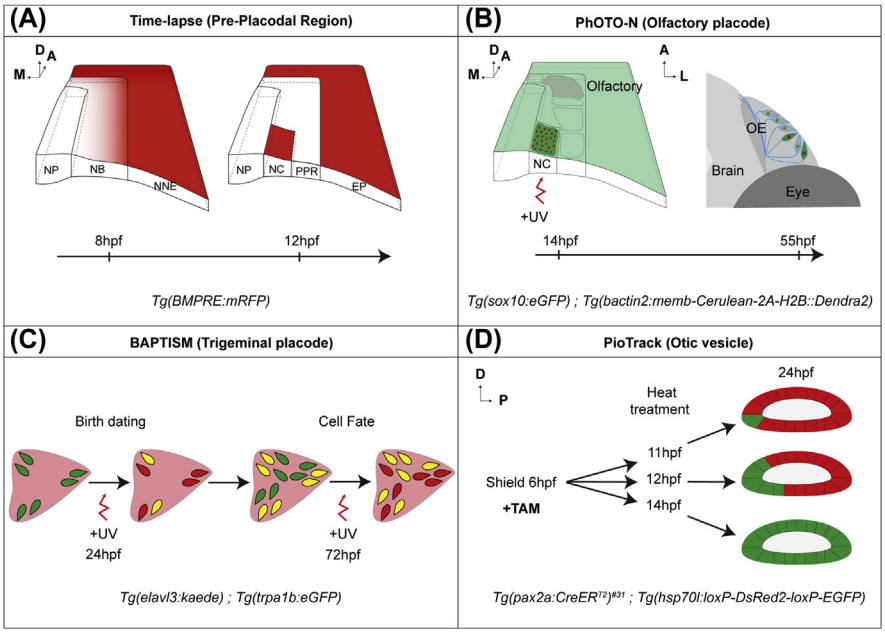

FIGURE 4 Sophisticated methodologies developed in zebrafish to study cranial sensory placodes development.

Examples of cutting of the edge technics elaborated in zebrafish to study cell fate in (A) ectoderm territories, (B) olfactory placode, (C) trigeminal placode, and (D) otic placode. (A) BMP activity reporter during ectodermal patterning (Reichert et al., 2013). (B) Photoconvertible fate mapping with the Photo-N fish (Dempsey, Fraser, & Pantazis, 2012) expressing a nuclear localized version of the highly stable photoconvertible (green to red) protein Dendra2 leading to the intriguing conclusion that NC contribute to olfactory sensory neurons (Saxena et al., 2013). (C) Neuronal birthdate determination using BAPTI (birthdating analysis by photoconverted fluorescent protein tracing in vivo) and neuronal specification using BAPTI combined with subpopulation markers, or BAPTISM, provide evidences that the timing of neurogenesis regulates trigeminal sensory neuron identity (Caron et al., 2008). (D) A Cre-mediated lineage-tracing method called pioneer tracking or PioTrack developed by Hans and collaborators analysing the pax2 positive cells contribution to the otic vesicle during development (Hans et al., 2013). *NP*, neural plate; *NB*, neural border; *NNE*, nonneural ectoderm; *EP*, epidermis; *NC*, neural crest cells; *PPR*, preplacodal region: *OE*, olfactory epithelium; *TAM*, tamoxifen; *UV*, ultraviolet irradiation. (See color plate)

been identified (Ahuja et al., 2014); basally located ciliated and microvillous neurons, and apically located crypt and kappe neurons (Fig. 1C). Each subtype displays specific gene expression profile, and differ both in patterns of innervation and function (Ahuja et al., 2013; Bazáes, Olivares, & Schmachtenberg, 2013; Gayoso,

Castro, Anadón, & Manso, 2012; Hansen & Zielinski, 2005 as a review); the ciliated and microvillous subtypes are predominant and make up at least 85% of the total OSN numbers (Ahuja et al., 2014).

The molecular mechanisms underlying olfactory neurogenesis at embryonic stages are beginning to be unraveled. For instance, it has been shown that a member of the Forkhead family of the winged helix transcription factors, Foxg1 (*forkhead box g1*), is required for patterning, proliferation, differentiation, and cell fate determination of progenitor cells of both the olfactory placodes and the olfactory bulb (Hanashima, Fernandes, Hebert, & Fishell, 2007; Hanashima, Li, Shen, Lai, & Fishell, 2004; Shen, Nam, Song, Moore, & Anderson, 2006); Foxg1 knockout mice ultimately fail to form olfactory structures and a similar phenotype is seen after *foxg1a* knockdown in zebrafish embryos (Duggan, DeMaria, Baudhuin, Stafford, & Ngai, 2008). Studies both in mice and zebrafish suggest that Foxg1 controls olfactory neurogenesis upstream of so-called proneural genes (Duggan et al., 2008), with maintenance of neurogenesis apparently requiring microRNAs of the miR-200 family in both species (Choi et al., 2008). Downstream of Foxg1, genetic analyses in mice have revealed that the sequential activity of members of the Ascl and Atonal families of bHLH proneural transcription factors controls olfactory neurogenesis (Cau, Casarosa, & Guillemot, 2002; Cau, Gradwohl, Fode, & Guillemot, 1997; Guillemot et al., 1993; Nicolay, Doucette, & Nazarali, 2006). Interestingly, while proneural genes are also required in fish, here only Atonal family members appear to be involved. Indeed, we have recently shown that Neurog1 and Neurod4 act in a partially redundant manner during the generation of both early born olfactory neurons (EONs) and mature OSNs (Madelaine, Garric, & Blader, 2011).

Intriguingly, neurogenesis in the olfactory placode in the zebrafish is concomitant with morphogenesis (Madelaine et al., 2011; Whitlock & Westerfield, 1998); early born olfactory neurons leave the cell cycle throughout the period of olfactory placode condensation (Madelaine et al., 2011) and provide the axonal scaffold followed later by OSN projections (Whitlock & Westerfield, 1998). While we now have a growing understanding of the mechanisms controlling either olfactory placode morphogenesis or neurogenesis individually, how these processes are coordinated has yet to be determined.

2.2 TRIGEMINAL PLACODE

The trigeminal placode is composed of neural crest and placodal-derived cells that ultimately form compact ganglia on either side of the head between eye and ear (Fig. 1D) (Davies, 1988; Schlosser, 2014); trigeminal sensory neurons are placodal derived, whereas the glial component of these ganglia is crest derived (Schlosser, 2014). Trigeminal sensory neurons extend peripheral dendrites throughout the skin of the head, to detect mechanical, chemical, and thermal stimuli, and central axons into the hindbrain, to communicate these inputs to the central nervous system (Baker & Bronner-Fraser, 2001; Hamburger, 1961). In zebrafish, by 24 hpf, the trigeminal ganglia are functional and mediate response to mechanical stimulation of the head,

resulting in a highly stereotypic and well-studied escape behavior (Andermann, Ungos, & Raible, 2002; Saint-Amant & Drapeau, 1998).

Studies in zebrafish embryos have shown that trigeminal sensory neurons are born from a dispersed group of progenitors that coalesce into a ganglion during early gastrulation (Knaut, Blader, Strähle, & Schier, 2005). More recently, the dynamics of this process have been studied by live imaging of a *Tg(neurod:eGFP)* transgenic line. In this manner, it was established that *neurod* expressing trigeminal neuronal progenitors were initially scattered at the end of gastrulation but then rapidly converged into a compact placode (Bhat & Riley, 2011). As for the olfactory placode, Knaut et al. (2005) showed that in the trigeminal ganglia, this process requires Cxcr4b and Cxcl12a; *ody* mutant trigeminal ganglia condense poorly and misexpression of the ligand can lead to inappropriate positioning of the ganglia. Interactions between cells and components of the extracellular matrix are involved in these Cxcr4b- and Cxcl12a-dependent morphogenetic movements. For instance, by cell transplantation, it was shown that the adhesion molecules E- and N-cadherin (cdh1 and cdh2, respectively) participate in ganglion assembly (Knaut et al., 2005). Similarly, morpholino knockdown of integrin-α5 leads to trigeminal sensory neuron migration defects (Bhat & Riley, 2011). The same actors described for the trigeminal ganglia morphogenesis have also been implicated in *Xenopus* epibranchial placode coalescence, in a process that requires reciprocal interactions between placodal cells and the neural crest cells (Theveneau et al., 2013). Indeed, neural crest cells chase placodal cells in an Sdf1-dependent manner in a mechanism that also involves Wnt−planar cell polarity and N-cadherin signaling (Theveneau et al., 2013). It will be interesting to determine if Wnt−planar cell polarity is also involved in trigeminal placode morphogenesis in the zebrafish.

Two distinct subpopulations of trigeminal neurons are specifically labeled by the expression of *trpa1b* (*transient receptor potential cation channel, subfamily 1, member 1b)* and *p2x3 receptor* (*p2rx3b*, an ATP sensor), which correspond to neurons responsive to chemical irritants (Bandell et al., 2004; Jordt et al., 2004) and neurons involved in the modulation of nociceptive signals (Chen, Gu, & Huang, 1995), respectively. Using a pair of in vivo birthdating techniques (BAPTI and BAPTISM), the temporal dynamics of trigeminal neurogenesis in living zebrafish embryos has been decoded (Fig. 4C) (Caron, Prober, Choy, & Schier, 2008). In this manner, it was demonstrated that early born neurons are competent to form both *trpa1b* and *p2rx3b* expressing neurons, whereas late-born neurons are restricted in their cell-type specification. Neurogenesis in the trigeminal ganglia is affected in zebrafish embryos carrying mutations in *neurog1* (Andermann et al., 2002; Cornell & Eisen, 2002; Golling et al., 2002). While simultaneous knockdown of *neurog1* and *neurod4* results in a fully penetrant reduction of neural marker gene expression at early stages (So et al., 2009; Yeo, Kim, Kim, Huh, & Chitnis, 2007), at later stages a ganglion is generated with a reduced number of neurons in the absence of Neurog1 function alone (Caron et al., 2008). Intriguingly, the residual trigeminal sensory ganglia of *neurog1*-deficient embryos are composed solely of the late-born neuron subtype. Thus, the timing of neurogenesis appears crucial for orchestrating the specification

of fully functional trigeminal sensory ganglia, and cell fate restriction of late-born trigeminal neurons can occur independently of early born neurons (Caron et al., 2008).

2.3 OTIC PLACODE

As in other vertebrates, the zebrafish inner ear is composed of sensory components involved in controlling balance and detecting vibrations. These two functions require so-called sensory patches (known as cristae, saccules, and utricles) that are made of mechanosensory hair cells and surrounding support cells (reviewed in Nicolson, 2005a, 2005b). While a set of three cristae sense the position of the head and angular acceleration by detecting fluid flow through the semicircular canals, the saccule has a more pronounced role in hearing; as for the cristae, the utricle seems devoted to vestibular function. Hair cells in these sensory patches synapse with neurons of the SAG, the axons of which project to nuclei in the hindbrain. While morphogenesis of this highly complex structure has been studied, here we will concentrate only on the initial steps of the process (placode formation and cavitation). Similarly, patterning within the otic vesicle leading to the emergence of the neural (SAG) and nonneural components of the inner ear (hair and support cells) has been intensively studied. Here, however, we will only focus on what is known concerning the formation of the SAG.

As for the olfactory and trigeminal placodes mentioned above, the formation of the otic placode requires coordinated convergence movements within the PPR. Using a *pax2a:GFP* reporter transgene to label the posterior PPR, otic morphogenesis has been investigated in living embryos (Bhat & Riley, 2011). Results from these studies highlight that, unlike the placodes described in previous sections, the convergence of cells of the future otic placode undergo several oriented steps of migration—first medially, then centripetally, and finally anteroposteriorly. Despite this complication, similar molecules appear to be involved. For instance, reducing Itga5 function affects otic placode convergence (Bhat & Riley, 2011); Itga5 appears to works with the FGF target gene *erm* (*etv5b*) in this process. Finally, while the chemokine *cxcl14* is expressed in otic placodes, no role for chemokines signaling has yet been reported during morphogenesis of the ear (Long, Quint, Lin, & Ekker, 2000).

Unlike the olfactory and trigeminal placodes, after convergence of the otic placodes, a lumen develops giving rise to the otic vesicle. Using high-resolution 4D imaging, it has been demonstrated that otic lumenogenesis can be divided into two phases, early lumen assembly (13−17 hpf) and lumen expansion (17−23 hpf) (Hoijman, Rubbini, Colombelli, & Alsina, 2015). Morphogenetic mechanisms driving these phases include active thinning of the epithelium combined with fluid loss from cells of the otic epithelium, which drives expansion of the newly formed lumen. Concomitantly, mitotic rounding during cell division contributes mechanically to the expansion of the lumen by orienting the contraction of the epithelium (Hoijman et al., 2015). The *lethal giant larvae 2* (*lgl2*) gene has been implicated

in controlling lumenogenesis of the otic vesicle and other luminal structures (Tay et al., 2013). How defects that arise in luminogenesis due the loss of function of *lgl2* relate to the two phases described by Hoijman et al. remains an open question.

It is well known that extrinsic signals from surrounding tissues are integrated by cells of the otic vesicle contributing to the complex three-dimensional organization of the organ and the generation of the stereotyped pattern of sensory neuron progenitors, hair cells, and supporting cells at specific positions in the otic vesicle (Bok, Bronner-Fraser, & Wu, 2005; Schneider-Maunoury & Pujades, 2007; Whitfield & Hammond, 2007). The generation of sensory neurons of the future SAG is restricted to an anteromedial subdomain of the otic vesicle in a process that depends on FGF, Notch, BMP, Hedgehog signaling, and RA (Alsina et al., 2004; Haddon, Jiang, Smithers, & Lewis, 1998; Hammond & Whitfield, 2011; Léger & Brand, 2002; Maier & Whitfield, 2014; Millimaki, Sweet, Dhason, & Riley, 2007). Using an inducible labeling of *pax2* expressing cells (PioTrack method), it was elegantly shown that *pax2* positive cells contribute to the neurogenesis domain (Hans, Irmscher, & Brand, 2013) (Fig. 4D). Downstream of these signals, transcription factors such as Tpaf2a, Foxi1, and Tbx1 are required to specify the otic neurogenic territory (Hans et al., 2013; Kantarci, Edlund, Groves, & Riley, 2015; Radosevic, Robert-Moreno, Coolen, Bally-Cuif, & Alsina, 2011). After specification, neuronal precursors delaminate as neuroblasts that will give rise to the SAG in a *neurog1*-dependent fashion (Adamska et al., 2000; Andermann et al., 2002; Bermingham et al., 1999; Haddon & Lewis, 1996; Ma, Chen, del Barco Barrantes, de la Pompa, & Anderson, 1998; Rubel & Fritzsch, 2002). Upon delamination from the otic epithelium, neuroblasts quickly switch from expressing *neurog1* to expressing a second bHLH transcription factor, *neurod*, as well as the homeodomain encoding gene *hmx3a* (Adamska et al., 2000; Andermann et al., 2002; Korzh, Sleptsova, Liao, He, & Gong, 1998). *neurod1* expressing cells comprise a so-called transit-amplifying pool of proliferative progenitors that will differentiate into mature neurons after a limited number of cell divisions (Camarero et al., 2003).

As during the olfactory and trigeminal neurogenesis, there are two waves of otic neurogenesis along the anteroposterior axis within the SAG that correlate with topographic position of the sensory epithelia in the otic vesicle (Kantarci et al., 2015; Sapède & Pujades, 2010; Vemaraju, Kantarci, Padanad, & Riley, 2012). Deciphering the conservation of signaling networks and cell behaviors, underlying these two waves of neuron production among these different placodes provides an interesting challenge for future research.

CONCLUSIONS

Cranial sensory placodes generate key organs required for vertebrates to decode their environment accurately. Since the middle of the 19th century, elegant studies using chick, *Xenopus*, and mice have shed light on the developmental programs that control cranial placode specification and differentiation. More recently, studies

in the zebrafish have highlighted the utility of this model for studying placode development. Thanks to state-of-the-art imaging techniques, important contributions to our understanding of cranial placode development have been made using the zebrafish model (Fig. 4). Indeed, the generation of transgenic zebrafish lines enabling temporally controlled gene overexpression or specific cell types/domain labeling has greatly contributed to the study of signaling pathways and gene regulatory networks controlling trigeminal, olfactory, and otic placode development (Table 1). Similarly, mutant lines generated in classical forward genetic screens or with recently developed genome editing strategies have also helped in understanding placode development (Table 2). This said, specific genetic tools are still lacking to label particular placodal cell types and early ectodermal domains. Hopefully, the advent of Crispr-Cas9 knock-in tools and others will soon provide us with a complete set of approaches for understanding cranial sensory placode development (for reviews Auer, Duroure, De Cian, Concordet, & Del Bene, 2014; Gonzales & Yeh, 2014).

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