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The Molecular Basis of Craniofacial Placode Development

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Abstract

The sensory organs of the vertebrate head originate from simple ectodermal structures known as cranial placodes. All cranial placodes derive from a common domain adjacent to the neural plate, the pre-placodal region, which is induced at the border of neural and non-neural ectoderm during gastrulation. Induction and specification of the pre-placodal region is regulated by the FGF, BMP, WNT and retinoic acid signaling pathways, and characterized by expression of the EYA and SIX family of transcriptional regulators. Once the pre-placodal region is specified, different combinations of local signaling molecules and placode-specific transcription factors, including competence factors, promote the induction of individual cranial placodes along the neural axis of the head region. In this review, we summarize the steps of cranial placode development and discuss the roles of the main signaling molecules and transcription factors which regulate these steps during placode induction, specification and development.

I. Introduction

Most sensory organs in the vertebrate head originate from simple ectodermal thickenings known as cranial placodes^{1,2}. Together, these sensory organs coordinate with other components of the nervous system to contribute to the proper functioning of the organism in its environment by providing it with sensory information such as vision, hearing and balance, and olfaction. Cranial placodes are formed embryonically by a series of differentiation steps arising at the boundary between neural and non-neural ectoderm. Each step involves the cooperation of distinct signaling pathways and transcription factors which first divide neural and non-neural ectoderm, then promote formation of placodal progenitors and the neural crest, and finally act to induce each placode. In this review, we summarize the current understanding of cranial placode development and discuss the major signaling pathways and transcription factors that play important roles in the development of placodes. We also briefly discuss the role of factors which contribute towards developmental competence of placodal progenitors at different stages of differentiation.

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1. Cranial placodes and their function

Cranial placodes can be divided into anterior, posterior and intermediate groups depending on their place of origin in the developing embryonic head (Figure 1). Anterior cranial placodes include the adeno-hypophyseal, olfactory and lens placodes³. The adeno-hypophyseal placode invaginates from the roof of the mouth to form Rathke's pouch which differentiates into the anterior pituitary and give rise to five types of endocrine hormone-secreting cells⁴. The olfactory placode invaginates to form the olfactory sensory epithelium containing various types of secretory cells and olfactory sensory neurons, while the lens placode invaginates to give rise to the lens vesicle.

The posterior placodes comprise the otic, lateral line placodes and epibranchial placodes that give rise respectively to the inner ear, lateral line organs (in fish and amphibians) and sensory neurons of the geniculate, petrosal and nodose ganglia⁵. The otic placode invaginates and pinches off from surface ectoderm to form the otic vesicle which then differentiates to generate the cochlear and vestibular systems of the inner ear, and the sensory neurons of its associated vestibulocochlear (VIIIth) ganglion. In fish and amphibians, lateral line placodes originate dorsolateral to otic placode and migrate extensively along the body before differentiating into neuromasts containing mechanoreceptors and, in some species, electroreceptors of the lateral line^{6,7}.

Finally, the trigeminal placode develops between the anterior and posterior placodes, giving rise to the sensory neurons of the ophthalmic and maxilla-mandibular divisions of the trigeminal ganglion. With the exception of the adeno-hypophyseal and lens placodes, all other cranial placodes give rise to sensory neurons of their associated sensory structures^{1,2}.

2. The emergence of placodal and neural crest progenitors at the neural plate border

Placode development is a multi-step process whose main features are conserved across all vertebrate groups. It begins at the border between neural and non-neural ectoderm that is induced during early gastrulation as a result of competing interactions between BMP, FGF and WNT signaling. BMP and WNT signaling have been shown to induce non-neural ectoderm while repressing neural differentiation⁸⁻¹⁰, while FGF signaling, in combination with BMP and WNT antagonists, promotes neural induction¹¹. A number of different WNT molecules are expressed in lateral regions of the embryo where they block neural differentiation by inhibiting FGF signaling and instead induce epidermal fate in concert with BMP. WNT signaling is actively inhibited in medial regions of the embryo by secreted WNT inhibitors such as DKK and CERBERUS, which allow FGF signaling to repress BMP expression and activity and promotes neural fate in these cells¹². As neural induction proceeds, non-neural transcription genes such as *Gata2* and *3*, *Foxi1* and *3*, and *Tfap2a/c* are expressed more laterally, while neural genes including *SoxB1*, *Geminin*, *Otx2*, *Gbx2*, and *Sox2/3* are enriched medially, but in an initially overlapping pattern with non-neural genes. This region gradually refines to become two mutually exclusive domains, the neural plate and future epidermis¹³⁻¹⁵ (Figure 2).

The emergence of distinct neural and non-neural ectodermal domains occurs through both positive regulatory feedback within each domain, and inhibitory negative interactions

between the two domains. For example, the zebrafish non-neural genes *tfap2*, *foxi1* and *gata3* require BMP signals for their expression in non-neural ectoderm before gastrulation; however, after definitive neural tissue is induced, their expression become BMP independent due to positive autoregulation¹⁶. In contrast, neural and non-neural genes mutually inhibit each other's expression; for example, overexpression of *dlx*, *gata*, *msx*, *foxi* or *tfap2* repress neural markers such as *sox2*, while inhibition of these genes results in the expansion of *sox2* into the non-neural ectoderm^{16, 17}. As the boundary between neural and non-neural ectoderm becomes distinct, an intermediate border region emerges between these two populations. The neural plate border region is characterized by a distinct set of transcription factors such as members of the *Msx*, *Dlx*, *Pax*, *Gbx* and *Zic* families^{18, 19}.

After establishment of neural, non-neural and border domains in embryonic ectoderm, the border region is further subdivided into populations of neural crest and pre-placodal progenitors^{9, 19-23}. The combined action of FGF signals and inhibition of BMP and WNT signals induces the formation of the pre-placodal domain, while neural crest fate is induced in the presence of FGF, BMP and WNT activity^{13, 14}. It is notable that these two different progenitor populations form in close proximity, one (neural crest) in the presence of WNT and BMP signals, and the other (the pre-placodal domain) requiring inhibition of these signals. This raises the question of how distinct fates can be established in a rapidly changing environment of WNT and BMP signaling. Once again, positive autoregulation of transcription factors within neural crest or pre-placodal territories is used to stabilize their fates, while cross-repression between the two territories helps confine them to distinct regions^{13, 14}. Induction and development of neural crest cells has been studied in great detail over the past two decades and are well documented in recent reviews^{18, 19, 24, 25}. In the remainder of this review, we will focus on the induction of the pre-placodal region and the development of various cranial placodes from this region.

II. Induction and specification of the pre-placodal region

1. Similarities and differences between the pre-placodal region and neural crest

After non-neural and neural territories are established in the early embryo, the neural border region becomes subdivided into future neural crest and a domain of placodal progenitors termed the pre-placodal region²⁶. Several studies suggest that both the pre-placodal region and neural crest are derived from a common progenitor population originating in the neural border region^{9, 21, 22}. However, other studies have proposed an alternative 'binary competence model' which suggests that the pre-placodal region and neural crest derive from two distinct ectodermal regions of varying competence; with the pre-placodal region originating from non-neural ectoderm while neural crest arises from the neural plate^{17, 27}.

Cranial placodes are related to neural crest in several ways, for example, both originate from the neural border region, both are capable of producing migratory cells, and both give rise to glia, sensory and secretory components of the peripheral nervous system. However, the two progenitor populations also differ significantly regarding their origin and timing of induction during embryonic development, as well as their developmental potential. Cranial placodes develop exclusively in the head region from the U-shape pre-placodal domain surrounding the neural plate and are absent from the trunk region²⁸, while neural crest is absent from the

anterior-most part of the head and develops in the posterior part of head and throughout the trunk region²⁹. These two domains are also induced and specified at different times during embryonic development; at least one study suggests that neural crest seems to be induced during late blastula or early gastrula, much earlier than pre-placodal region induction which happens around late gastrula³⁰. Indeed, a recent study in amphibians suggests that neural crest may even preserve some markers of pluripotency as they are specified from pluripotent progenitors in the very early embryo³¹, although this finding has yet to be confirmed in other vertebrate groups. Finally, neural crest cells are developmentally more diverse than cranial placodes: they also form cartilage, bone, smooth muscle, mesenchymal and pigment cells in addition to forming sensory and secretory components of the peripheral nervous system^{2, 25}.

2. Induction of the pre-placodal region

The pre-placodal region is induced around the anterior border of the neural plate during gastrulation and forms various cranial placodes which give rise sensory organs of the head^{2, 26, 32}. Fate mapping and transplantation experiments in various vertebrates revealed that this domain is competent to give rise to all types of placodes at early developmental stages; however, the individual placodal fates become restricted as development proceeds.³³. For example, the chicken trigeminal and otic placodes become committed within 8 hours of the neural folds first appearing¹. This observation led to the concept of a common origin for all placodes, which is further strengthened by the expression in this domain of common transcription factors which play important roles in placode development such as members of the SIX and EYA transcription factor families^{2, 26, 32}. This concept is further supported by the studies showing that cells in any part of the pre-placodal domain are competent to induce an otic placode in response to FGF, while cells outside the pre-placodal domain fail to respond to FGF signaling in this manner³⁴. This demonstrates that the ectoderm must acquire pre-placodal properties to be able to respond to specific local inducing signals for individual placodes.

2.1 Signaling molecules involved in induction of the pre-placodal region—As discussed above, a BMP signaling gradient at early stages of gastrulation is proposed to regulate ectodermal patterning. In this model, high levels of BMP activity promote epidermis, intermediate levels and low levels induce neural crest and the pre-placodal region respectively, while still lower BMP activity is required for the formation of the neural plate³⁵⁻³⁸. This is supported by studies which show that inhibition of BMP signaling by dominant-negative receptors or BMP antagonists induces some pre-placodal genes (*Eya2* and *Six4*) and expand the pre-placodal region at the expense of non-neural ectoderm²². Moreover, a number of BMP antagonists that are expressed near the non-neural and neural ectoderm and secreted from underlying mesoderm are important for pre-placodal development^{39, 40}.

BMP signaling has been proposed to regulate zebrafish pre-placodal induction in a two-step model¹⁶, in which BMP is required only transiently during blastula/early gastrula stages to induce pre-placodal competence throughout non-neural ectoderm by promoting the expression of specific competence factors (Tfap2a/c, Gata3 and Foxi1). After this first

induction step, expression of these factors becomes BMP-independent. In the late gastrula, BMP must be actively blocked by BMP antagonists for further induction and specification of the pre-placodal region within this zone of competence¹⁶. Interestingly, different levels of BMP signaling regulate the fate choices between neural crest and pre-placodal cells by regulating different set of genes^{16, 37, 38, 41}. *Gata3* and *foxi1* are induced next to the epidermis in non-neural regions of high BMP activity; whereas *tfap2a/c* is induced by itself close to the neural plate by BMP activity, where it then induces other neural crest genes. Therefore *gata3*, *foxi1* and *tfap2a/c* promote pre-placodal fates from non-neural ectoderm while *tfap2a/c* induces neural crest fates from neural ectoderm in a BMP-dependent manner^{42, 43}.

Several antagonists of both BMP and/or WNT are secreted from cranial mesoderm as well as from the anterior neural plate. Their combined action represses BMP and WNT signaling in the prospective pre-placodal region^{39, 44, 45}. In contrast, lateral and posterior mesoderm express high level of *Wnt8c*²² while *Wnt6* is expressed in trunk ectoderm⁴⁶. Interestingly, activation of WNT signaling in the pre-placodal region by overexpressing *Wnt8c* or active β -catenin attenuates expression of pre-placodal genes (*Eya2*, *Six1* and *Six4*), whereas inhibition of WNT signaling by ectopic expression of WNT antagonists such as Crescent or Frizzled expands the expression domain of these genes²². This indicates that inhibition of BMP and WNT together promotes the induction of pre-placodal genes, while they remain repressed outside the pre-placodal region by active WNT signaling. In this way, expression of placodal genes and therefore the placodes are restricted only to the border of the neural plate in the head. In contrast, high BMP and WNT activity promotes neural crest cell formation along the entire anterior-posterior extent of the body axis (Figure 3).

Unlike BMP and WNT which negatively regulate pre-placodal region induction, FGF signaling promotes the expression of pre-placodal genes^{16, 22}. FGF signals originate from tissues adjacent to the pre-placodal region during development; for example, *Fgf4* and *Fgf8* are expressed in mesoderm in chick, while *fgf8* is expressed in the anterior neural plate in *Xenopus*^{47, 48}. Removing these tissues in chick and *Xenopus* blocks the induction of pre-placodal genes such as *Six1* and *Six4*, whereas cranial mesodermal grafts activate them²⁷. Blocking FGF signaling by using dominant negative FGF receptors or *fgf8* knockdown results in decreased *six1* expression in *Xenopus*^{27, 49}. Simultaneous inhibition of FGF and PDGF signaling with the receptor antagonists SU5402 and AG1295 result in *eya1* down-regulation, but not when either pathway is inhibited individually¹⁶. Finally, in chick, the pre-placodal gene *Eya2* can be ectopically induced in extra embryonic regions by activating FGF signaling in the presence of BMP and WNT inhibitors²².

Retinoic acid (RA) also plays an important role in restricting the posterior boundary of the pre-placodal domain in a manner similar to WNT signaling. *Raldh2*, a RA generating enzyme, and *Cyp26*, a RA degrading enzyme, are expressed in a non-overlapping manner along the anteroposterior axis to create a RA gradient from low (anterior) to high (posterior) which plays an important role in regulating anteroposterior patterning in the developing embryo^{50, 51}. Inhibiting retinoic acid receptor activity causes posterolateral expansion of *Fgf8* and *Fgfr1/2* expression domains, indicating that RA and FGF may interact to restrict the posterior boundary of the pre-placodal region^{52, 53}.

In summary, inhibition of BMP and WNT signals in cranial ectoderm by antagonists in head mesoderm promotes a pre-placodal fate in the presence of FGF, whereas active BMP and WNT signaling induces the neural crest fate at the edge of the neural plate. FGF, WNT and RA signaling play important roles in restricting placode development to the head region, whereas neural crest induction occurs along the entire body axis. In the next section, we describe how combinations of transcription factors induced by these signals are important for placode development.

2.2 Transcription factors regulating specification of the pre-placodal region—

The pre-placodal region is uniquely defined by two families of transcriptional regulators, the *Six* and *Eya* gene families. *Six* and *Eya* genes are expressed throughout the pre-placodal domain and later refine to individual placodes, where they play important roles in the development of each placode. *Six* and *Eya* genes were first identified in *Drosophila* as the genes *sine oculis (so)* and *eyes absent (eya)* that regulate compound eye development in combination with *dachshund (dac)* and *eyeless/pax6*⁵⁴. In vertebrates, there are 6 *Six* genes and 4 *Eya* genes based on their sequence homology and conserved domains⁵⁵. *Six* and *Eya* genes are expressed in the anterior neural plate border region that gives rise to all cranial placodes and they continue to be expressed in some of the individual placodes⁵⁶⁻⁵⁹. All SIX proteins contain a C-terminal DNA binding SIX-type homeodomain (HD) and an N-terminal interaction domain known as the SIX Domain (SD) that interacts with other co-factors^{54, 60}. SIX proteins can act as both transcriptional activators and repressors depending on their interacting partners. SIX protein interaction with EYA proteins confers activator activity, whereas interactions with the Groucho co-repressor makes SIX proteins transcriptional repressors^{49, 60}.

SIX proteins play important roles in placodal development by positively regulating the expression of pre-placodal genes while also repressing epidermal and neural crest genes^{49, 58}. In *Xenopus*, overexpression of *six1* at the neural plate border results in expansion of the pre-placodal region at the cost of epidermis and neural crest. Conversely, knockdown of *six1* causes a reduction of the pre-placodal region and expansion of epidermal and neural crest domains as shown by up-regulation of keratin and FoxD3 respectively⁴⁹. In *Xenopus* and chick, expression of constitutively active SIX1 (VP16-SIX1) results in up-regulation of placode genes, whereas expression of a repressor form of SIX1 (EnR-SIX1) causes up-regulation of epidermal and neural crest genes. The activator and repressor activities of SIX1 are attributed to SIX1-EYA2 activator complexes and SIX1-Groucho repressor complexes respectively^{49, 58}. Mutation or removal of *Six* genes cause severe defects in different placodal derivatives, although they do not cause complete loss of particular placodal derivatives, which indicates a functional redundancy between different Six genes⁶¹. For example, *Six1/4* double knockouts show much more severe defects in olfactory placode development than *Six1* knockouts alone⁶¹.

EYA proteins are characterized by an N-terminal transactivation domain and a C-terminal interaction domain known as EYA domain (ED) which also harbors phosphatase activity⁶². Unlike SIX proteins, EYA proteins cannot bind to DNA directly and require interactions with other proteins for their nuclear import and DNA binding⁶³. EYA proteins belong to a family of protein tyrosine phosphatases and act as a transcription co-activator of SIX

proteins⁶². Interactions between the SIX domain of SIX proteins and the EYA domain of EYA proteins facilitates the nuclear import of EYA and SIX complexes^{62, 63}. The phosphatase activity of EYA acts as a molecular switch to turn SIX proteins from repressors into activators by recruiting CREB binding protein (CBP) to SIX target sites⁶². *Eya1* knockout animals show severe defects in inner ear development, in addition to defects in other placodes and organs including kidney and thyroid^{64, 65}. *Eya4* knockout mice also show inner ear defects leading to hearing loss⁶⁶. In humans, *SIX1* and *EYA1* mutations cause Branchio-Oto-Renal syndrome, where affected individuals suffer from branchial and kidney defects in addition to hearing loss⁶⁷⁻⁷⁰.

As discussed in the previous section, several signaling molecules including FGF, WNT, BMP and RA as well as transcription factors including DLX, GATA and IROQUOIS (IRX) play important roles in regulating *Six* and *Eya* expression in the pre-placodal region. *Six1* expression in the pre-placodal region is regulated by a placode-specific enhancer⁵⁹. The *Six1* enhancer is bound directly by DLX5 and MSX1 proteins, which indicates roles of these transcription factors in *Six1* regulation⁵⁹. Other transcription factors like Foxi1, Gata3, Tfp2a/c and Iroquois (Irx) also regulate *six* and *eya* expression during anamniote pre-placodal induction^{16, 17, 71}. By the neural plate stage, EYA and SIX protein expression is established in the pre-placodal region and they maintain their expression by positive autoregulation⁵⁸ and repress neural border genes such as *dlx* and *gata* family members which were initially required for the induction of the neural plate border^{16, 17, 71, 72}. Therefore, similar kinds of positive autoregulation and cross-inhibition which were initially used to establish the non-neural and neural territories of the embryo are employed again for establishing the pre-placodal domain in between the epidermis and neural crest.

III. Regionalization and specification of individual cranial placodes

At neural plate stages, the pre-placodal region and neural plate are divided into two domains along the rostro-caudal axis; an anterior domain expressing *Otx2*, and a posterior domain expressing *Gbx2*⁷³. These two transcription factors mutually repress each other to strengthen the boundaries between prospective placodal domains and establish individual placodal territories⁷³. During this process, *Otx2* regulates the induction of olfactory, lens and trigeminal placodes, while *Gbx2* regulates induction of the otic placode. The regionalization and specification of individual cranial placode is a multi-step process which is regulated by a number of signaling molecules and transcription factors (Figure 4). We will briefly discuss these steps in this section and direct readers to the following reviews for a more detailed discussion^{2, 13, 74, 75}.

1. Induction of adenohipophyseal, olfactory and lens placodes

Induction of the anterior placodes is regulated by four of the major signaling pathways - FGF, BMP, SHH and WNTs. Pax6 is the earliest marker of the anterior placodes and is proposed to be induced by SIX1 in anterior domain⁵⁸. Experiments using cultured explants of chick pre-placodal ectoderm provide evidence for a ground state of competence to form Pax6-expressing lens placode tissue that extends throughout the entire pre-placodal region⁷⁶. This initial propensity to express Pax6 must be repressed by other placode

inducing signals; for example, FGF signaling represses *Pax6* expression during induction of the olfactory placode⁷⁶. *Pax6* and *Dlx5* are co-expressed in the prospective lens and olfactory placodes but as the separation of two placodal fates begins, *Pax6* is transiently down-regulated by FGF8 in the prospective olfactory placode, while *Dlx5* expression is lost from the prospective lens placode^{76,77}. This results in a mutually exclusive expression pattern of *Pax6* and *Dlx5* expression in lens and olfactory placodes respectively, which is important for segregation of the two placodes. Next, BMP signaling regulates the fate choice between lens and olfactory placode, with a short exposure to BMP4 promoting olfactory fate while a more prolonged BMP4 exposure in combination with FGF signaling induces lens fate⁷⁸. Shh signaling regulates various steps of adenohypophyseal development including initial lineage commitment and differentiation while repressing lens and olfactory placodes⁷⁹.

2. Induction of otic and epibranchial placodes

FGF signaling (Fgf3/8 in zebrafish, FGF3/FGF19 in chick and FGF3/10 in mouse) has been proposed to be the main inducer of the posterior placodal domain which give rise to otic and epibranchial placodes⁸⁰. This domain is sometimes referred to as the otic-epibranchial placode domain or OEPD⁵. FGF signaling induces *Pax2* and *Pax8* expression in the OEPD from which otic and epibranchial placodes develop^{5, 81-84}, FGF signaling and WNT inhibition promotes epibranchial placode fate, while induction of the otic placode requires FGF signaling and activation of the WNT pathway^{81, 85}.

As the OEPD is induced, some markers of non-neural ectoderm, such as *Dlx* (*dlx3b/4b* in zebrafish, *dlx3/5* in *Xenopus*, *Dlx5/6* in chick) and *Foxi* (*foxi1* in zebrafish, *Foxi3* in chick and mouse) genes, become restricted to the pre-placodal region and then play roles in induction of the posterior placodes^{5, 86}. Loss of either *dlx3b/4b* or *foxi1* results in smaller otic vesicle formation, while combined loss of both *dlx3b* and *foxi1* causes complete ablation of the otic vesicle and failure of otic placode induction in zebrafish^{87, 88}. Recently, otic expression of chick *Dlx3* was shown to be epigenetically regulated by *Kdm4b* which is also expressed during otic placode specification⁸⁹. Similarly, loss of *Foxi3* results in complete failure of otic placode induction in chick⁹⁰ and mouse^{91, 92}. *Foxi1* and *dlx3b* interact with FGF signaling to regulate *pax8* and *pax2* expression during OEPD induction^{87, 88, 93}. Once the OEPD is induced, *foxi1* as well as FGF signaling must be downregulated towards medial regions of the OEPD to induce the otic placode in the presence of WNT signaling, which positively regulates otic fate and inhibit epibranchial fate⁹⁴. On the other hand, *Foxi1* remains expressed in the lateral region of the OEPD where it induce *pax8* and *sox3* expression to promote epibranchial fate in presence of active FGF and BMP signaling^{94, 95}.

3. Induction of the trigeminal placode

The trigeminal placode develops immediately adjacent to the midbrain and is divided into ophthalmic and maxillomandibular divisions. *Pax3* is the earliest marker of the trigeminal placode and is induced by signals from the brain⁹⁶, which include the combined activity of WNT and FGF signaling⁹⁷. In addition to WNT and FGF, PDGF and Notch signaling also contribute to the induction of ophthalmic placodes^{98, 99}. These signaling molecules also

regulate the later steps of neuronal differentiation during trigeminal placode development^{100, 101}. The induction of *Pax3* in the intermediate pre-placodal region causes *Pax6* suppression, and the consequent mutually exclusive expression of *Pax3* and *Pax6* helps promote the proper regionalization and separation of anterior and intermediate placode territories¹⁰².

IV. The role of transcription factors and pioneer factors in regulating placodal competence

Why do different regions within the pre-placodal domain respond to similar inducing signals, such as FGFs, but differentiate into different placodes? Similarly, why do cells within the pre-placodal region respond to these signals, whereas cells slightly more lateral to the pre-placodal domain remain refractory to induction and differentiate as epidermis¹⁴? One explanation is the presence of transcription factors which provide the competence of ectoderm to respond to specific inducing signals. Such competence may be manifested by the induction of lineage-specific gene expression, or by the regulation of the cellular response towards extracellular signals by regulating surface receptors. For example, in zebrafish, *Tfap2a/c*, *Gata3* and *Foxi1* induce the expression of the pre-placodal genes *dlx*, *eya* and *six* which are important for initiation of placode induction and specification^{16, 17, 71}, by allowing this region to respond to FGF signals⁷⁶. In zebrafish, chick and mice, *Foxi1/3* are necessary for otic and epibranchial placode development by regulating the cellular response towards FGF signaling. *Foxi1/3* mutation or knockdown results in severe defects in otic and epibranchial placodes^{90, 93}, even though the FGF signaling pathway appears to be intact in cells lacking FOXI1/3⁹².

In addition to the mechanisms of competence described above, the transcriptional competence of cells to respond to particular inducing signals is also regulated at the chromatin level by a specific class of transcription factors known as pioneer factors. Pioneer factors typically engage transcriptionally inactive regions of chromatin and allow them to attain a poised state which can be activated as soon as inductive signals are received^{103, 104}. These poised enhancers are characterized by H3K4me1 histone marks which later acquire H3K27ac as an additional histone mark upon gene activation^{105, 106}. Pioneer factors differ from conventional transcription factors in their ability to directly bind compact, DNA-methylated chromatin in the absence of other cofactors and chromatin remodelers. Often, engagement of a silent lineage-specific enhancer by a pioneer factor is accompanied by a progressive recruitment of DNA demethylases and histone-modifying enzymes that replace inactivating histone marks with activating marks during cellular reprogramming and differentiation¹⁰⁷⁻¹⁰⁹.

Interestingly, members of the Forkhead family of transcription factors including FoxA1/2/3, FoxD3, FoxE1, and FoxO have been shown to act as pioneer factors in regulating gene expression^{110, 111}. The winged helix DNA binding domains¹¹² of many FOX proteins have structural similarities to linker H1 histones¹¹³. This DNA binding domain, together with a C-terminal core histone binding domain and N-terminal transactivation domain allows FOXA proteins to bind directly to nucleosomes, open chromatin and promote accessibility

and recruitment of other transcription factors to the DNA. Importantly, binding of FOXA to enhancers results in the recruitment of active histone marks such as H3K4me1/me2 and H3K9ac¹¹⁴.

Fox proteins also play important roles in neural crest and pre-placodal specification; FoxD3 is an important regulator of neural crest specification¹¹⁵ while zebrafish *foxi1* (and its functional homologue in amniotes, *Foxi3*) is important for competence of non-neural ectoderm to respond to pre-placodal inducing signals¹⁶. FOXD3 is already known to act as a pioneer factor in liver and ES cell differentiation¹¹⁶ whereas FOXI1/3 shares several features of pioneer factors such as the ability to bind DNA during mitosis¹¹⁷ and is a close homologue of another pioneer factor, FoxA¹¹⁰. One important feature of pioneer factors is their stage-specific expression, where they begin to be expressed in a naïve, ground or undifferentiated state and keep their target genes in a poised configuration. Once differentiation signals are received, pioneer factors are often down-regulated and their target genes are induced¹⁰⁷. In the case of otic placode induction, *foxi1/3* is initially expressed with other non-neural markers such as *gata3*, *dlx3b*, and *tfap2a/c* in non-neural ectoderm surrounding the future neural plate^{16,71}, and is quickly down-regulated from the otic placode once it is induced^{90,92,118,119}. Moreover, knockdown of *Foxi3* in chick⁹⁰ or *foxi1* in zebrafish^{87,88}, causes a failure of otic placode induction. *Foxi3* mutant mice lack an inner ear and are unable to induce even the earliest markers of the otic placode^{91,92}.

We hypothesize that these competence factors including FOXI1/3 contribute to placode development in two ways - first, by regulating the expression of other co-expressed placodal genes⁷¹, and second, by acting as a pioneer factor, where they remain bound to enhancers of specific placodal genes and keep them poised for activation until the appropriate induction signals are received. Recent evidence supports the idea that the gradual activation of otic-specific genes is accompanied by recruitment of enzymes which create activating histone marks, such as DNA demethylases⁸⁹.

V. Conclusion

Placode development is a multi-step process, with each of these steps being regulated by distinct signaling pathways and transcription factors. These signaling molecules create a local environment of differential expression of various transcription factors which first establish a non-neural and neural boundary followed by formation of the neural border which forms the neural crest and placodes. There are many examples of developmental defects in humans which are caused due to mutation in some of these genes regulating placode development^{69,70}. Understanding the roles of these signaling pathways and various transcription factors during placode induction and development is important for understanding and designing treatment strategies for disease and defects associated with neural crest and placode derived sensory organs.

It remains unclear how the common pre-placodal region becomes competent to respond to similar inductive signaling and still be able to form different placode derivatives during embryonic development²⁶. We suggest that this lineage fate determination and differentiation towards a particular placodal fate may be regulated by pioneer factors¹⁴.

These pioneer factors may be expressed early during induction of the pre-placodal region, where they may be able to bind to the enhancers of lineage-specific genes keeping them poised for transcription by recruiting other co-factors. When lineage-specific inductive differentiation signals become available, they promote the differentiation of the cells towards their respective placodal fates. We hypothesize that FOXI3 may be acting as a pioneer factor in regulating otic placode development as supported by the absence of otic placode induction in *Foxi3* knockout mice^{91, 92}. Pioneer factors are also known to regulate lineage specific gene expression during cellular reprogramming in stem cells¹⁰⁷⁻¹⁰⁹. We suggest that similar kinds of pioneer factor-mediated gene regulation may be operational during regeneration and repair in various sensory neural crest and placode derived sensory organs in which adult stem cells are present^{120, 121}.

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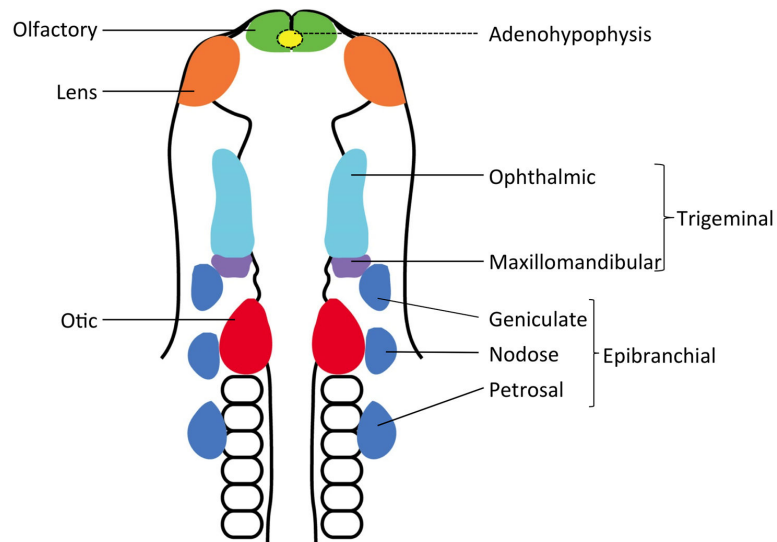


Figure 1. Location of cranial placodes in the embryonic vertebrate head

Schematic representation of various types of cranial placodes in a 10-somite stage chick embryo (modified from Streit, 2004). Individual placodes develop in morphologically distinct domains along the neural tube in the head region. The adenohypophyseal placode develops ventral to the forebrain and is indicated here with a dotted line.

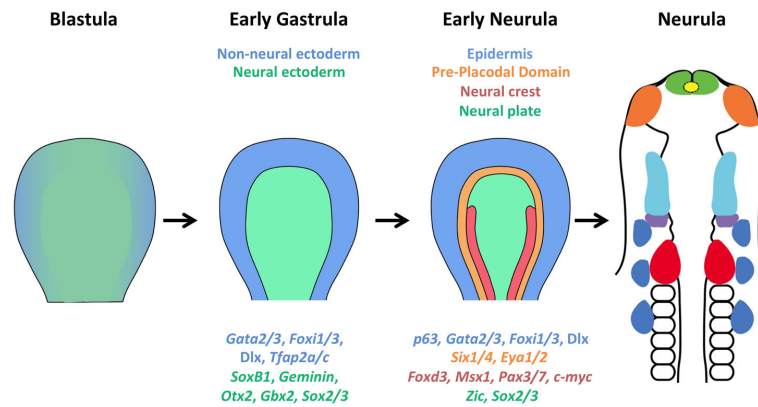


Figure 2. Overview of cranial placode development

Placode development is a multi-step process which first starts with the induction of non-neural (blue) and neural (green) ectoderm from naïve ectoderm. The neural border (not shown) is formed in between neural and non-neural ectoderm, which gives rise to the pre-placodal region (orange) and neural crest (red). The pre-placodal region develops exclusively in the head region as a U-shaped domain surrounding the anterior neural plate and is absent from the trunk region. Neural crest is absent from the anterior-most part of the head and develops in the posterior part of head and throughout the trunk region. Some of the specific genes of each domain are also shown and color coded in accordance to their expression zone. Different cranial placodes are induced from the pre-placodal domain in response to local molecular signaling and specific transcription factors. The diagram represents a simplified and consensus view of a vertebrate embryo, but is closest to amniote embryos such as birds or humans that develop and gastrulate as a disc. Although the precise stages vary considerably in different vertebrate classes, the diagram shows an embryo prior to the onset of gastrulation (“blastula”), after initiation of gastrulation (“early gastrula”), after induction of the neural plate but before the formation of clear neural folds (“early neurula”) and after closure of the neural tube (“neurula”).

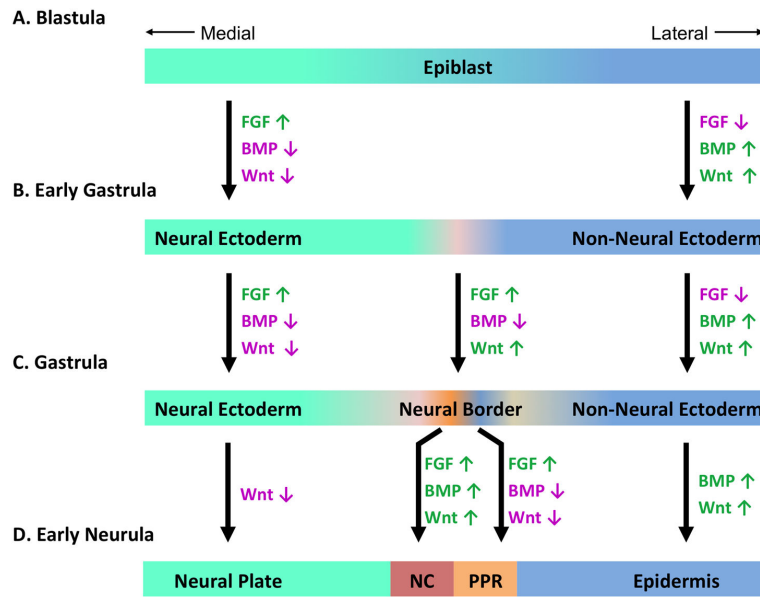


Figure 3. Induction of neural crest and the pre-placodal region

(A) Prior to and during gastrulation, FGF, BMP and WNT signaling from the underlying hypoblast and mesoderm initiates differentiation in naïve ectoderm. (B) High BMP and WNT signaling along with FGF inhibition promotes non-neural ectoderm on the lateral side of the epiblast while high FGF signaling and reduced BMP and WNT signaling induce neural ectoderm formation in the medial side of epiblast in developing embryo. (C) The neural border is induced between non-neural and neural ectoderm. (D) Attenuation of BMP and WNT signaling in presence of FGF induces the pre-placodal region, while high BMP and WNT promote epidermis formation. On other hand, BMP and WNT along with FGF signaling promotes neural crest induction closer to the neural plate. Activation (green) and repression (magenta) of signaling pathways are color coded and the arrows indicate whether a signaling pathway is activated (↑) or inhibited (↓). BMP, Bone Morphogenetic Protein; FGF, Fibroblast Growth Factor; NC, Neural Crest; PPR, Pre-Placodal Region.

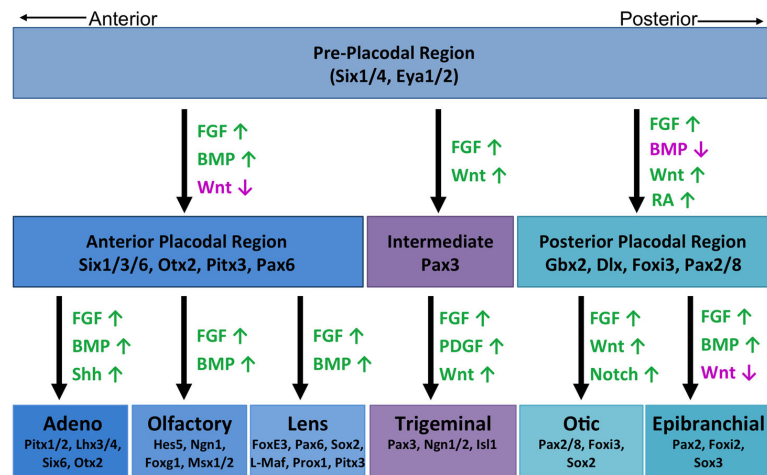


Figure 4. Regionalization and specification of individual cranial placodes from PPR

Schematic depicting the major signaling pathways and some of the important genes involved in regionalization and specification of various cranial placodes from the pre-placodal region. Activation (green) and repression (magenta) of signaling pathways are color coded and the arrows indicate whether a signaling pathway is activated (↑) or inhibited (↓). BMP, Bone Morphogenetic Protein; FGF, Fibroblast Growth Factor; PDGF, Platelet Derived Growth Factor; RA, Retinoic Acid; SHH, Sonic hedgehog.