Journal of *Developmental Biology*

MDPI

Review

Feedback Regulation of Signaling Pathways for Precise Pre-Placodal Ectoderm Formation in Vertebrate Embryos

Tatsuo Michiue * and Kohei Tsukano

Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1, Komaba, Meguro-ku, Tokyo 153-8902, Japan
* Correspondence: tmichiue@bio.c.u-tokyo.ac.jp

Abstract: Intracellular signaling pathways are essential to establish embryonic patterning, including embryonic axis formation. Ectodermal patterning is also governed by a series of morphogens. Four ectodermal regions are thought to be controlled by morphogen gradients, but some perturbations are expected to occur during dynamic morphogenetic movement. Therefore, a mechanism to define areas precisely and reproducibly in embryos, including feedback regulation of signaling pathways, is necessary. In this review, we outline ectoderm pattern formation and signaling pathways involved in the establishment of the pre-placodal ectoderm (PPE). We also provide an example of feedback regulation of signaling pathways for robust formation of the PPE, showing the importance of this regulation.

Keywords: morphogen; feedback regulation; signaling pathway; ectoderm; placode

Citation: Michiue, T.; Tsukano, K. Feedback Regulation of Signaling Pathways for Precise Pre-Placodal Ectoderm Formation in Vertebrate Embryos. *J. Dev. Biol.* **2022**, *10*, 35. https://doi.org/10.3390/jdb10030035

Academic Editors: Sally A. Moody, Kristin Bruk Artinger and Simon J. Conway

Received: 25 July 2022
Accepted: 24 August 2022
Published: 26 August 2022

1. Introduction

Embryonic patterning is one of the most crucial steps for constructing a complex body shape from a simple egg. The fundamental concept of embryonic fate determination involves localization of signaling molecules inside an egg and differential activation of pathways in each embryonic area, directing localized expression of specific genes. To establish cell fates precisely, strict regulation of signaling strength in each area is essential [1]. There are two types of pattern formation, self-organization and boundary organization [2]. In the "Turing pattern", a primary example of self-organization, it is possible to create a periodic pattern such as a fish skin pigmentation pattern, simply by employing at least two molecules that differ in diffusion rates and activities [3,4]. This model is very simple; it is impossible to form the precise pattern reproducibly. The second is the so-called "French-flag model", by which cells generate a pattern due to the strength of a morphogen gradient. This model allows definition of fixed areas more reproducibly than self-organization. In ectoderm patterning, the principle of boundary formation is adopted.

Ectoderm patterning is established after fertilization in vertebrates. The ectoderm consists of four distinct regions, the neural plate (NP), the neural crest (NC), the pre-placodal ectoderm (PPE, also called the pre-placodal region (PPR)), and the epidermis. Patterning is dependent on positional information provided by several types of signaling molecules secreted from mesendodermal tissues. Major signaling types involved in ectodermal patterning include bone morphogenetic protein (BMP), fibroblast growth factor (FGF), retinoic acid (RA), and Wnt. In addition to the ligands themselves, antagonists of each morphogen also contribute to gradient formation in embryos. For example, in *Xenopus* gastrula, several proteins such as chordin, noggin, and follistatin allow formation of BMP gradients. Both FGF and Wnt signaling are important for anterior–posterior neural patterning. Wnt antagonists (dkk, cer, frzb etc.), secreted from anterior mesendoderm, induce anterior neural structure, including the brain [5,6]. PPE formation requires cooperative actions of BMP, FGF, Wnt, and RA signaling to determine the position of the PPE in naïve ectoderm [7].

The question is whether only the concentration of these molecules enables establishment of the precise ectoderm pattern, because fluctuations of concentration occur, according

to various, unexpected environmental factors, resulting in uncertainty in the region of each tissue. To avoid untenable fluctuations, molecular mechanisms must be able to counter such influences. There are several strategies to establish robustness against noise in embryonic patterning (Figure 1).

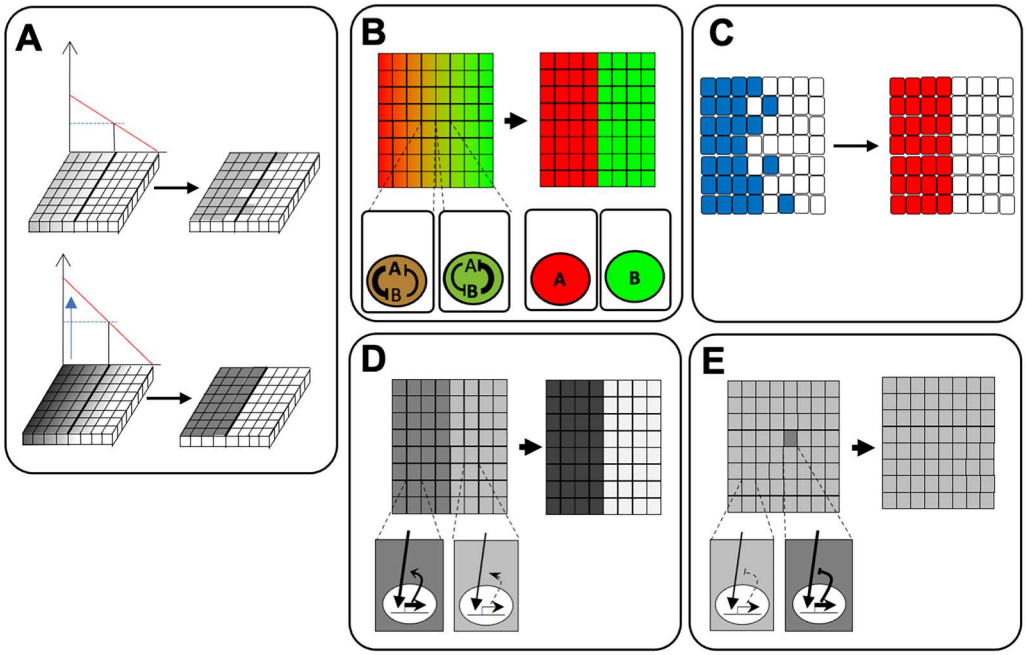

Figure 1. The strategy for robust pattern formation: (**A**) steep gradient formation of a morphogen; (**B**) mutual inhibition of transcription factors; (**C**) cell sorting and clear boundary formation of a tissue; (**D**) positive feedback regulation of morphogen gradients; (**E**) negative feedback regulation of morphogen gradients.

One of these is establishment of steep gradients (Figure 1A). The larger the difference in morphogen concentration among cells, the more easily each cell is able to detect differences in signal levels [8]. Another strategy is mutual inhibition by two transcription factors (Figure 1B). At an early stage, both genes are expressed in the same cells, whereas expression of one of these genes is decreased, resulting in boundary formation between two regions that each express one of these genes. The third strategy is "cell sorting" (Figure 1C). Gathering cells that receive similar levels of morphogen enables a region to absorb (or average) the noise of patterning, e.g., a salt and pepper cell array around the boundary. The fourth strategy is "local" regulation of signaling, including feedback regulation of intracellular signaling pathways, especially in two regions. Positive feedback regulation makes the two regions more discrete, whereas negative feedback enables them to maintain stable levels of signaling against local turbulence of signal intensity (Figure 1D,E) [2]. Among ectodermal regions, the PPE and the NC are narrow; therefore, a system to precisely form them is more critical than in the NP and the epidermis. In this review, we will focus mainly on PPE formation and will discuss the importance of feedback regulation for local control of appropriate signaling.

2. An Outline of PPE Formation

The PPE is a narrow, horseshoe-shaped region induced around the boundary between the neuroectoderm (NE) and the non-neural ectoderm (NNE) during gastrulation [9–11]. The NC is also derived from a boundary region and forms craniofacial structures [12–14]. The model for dividing the PPE and the NC is discussed later.

PPE cells give rise to cranial sensory organs, including lens, olfactory epithelium, inner ear, some of the cranial ganglia, and the anterior pituitary gland [15–20]. In contrast to NC cells, a part of PPE cells remain on the surface of the ectoderm, and after neural tube

closure, various patterns of cell migration occur, according to the subtypes of placode [21]. Olfactory epithelium, lens, and otic cells are mainly rearranged to form their final shape, whereas trigeminal and epibranchial cells migrate and aggregate. Many genes are involved in PPE specification and construct a gene network [22]. *Six1*, the homolog of *sine oculis* (*si*) in *Drosophila*, encodes a homeodomain protein and is uniformly expressed in the PPE [23,24]. Eya1 is a cofactor with Six1 and is also expressed in the PPE [25]. These genes are well utilized as pan-placodal markers. The experiment on both upregulation and downregulation has shown that Six1 is required for the gene regulatory network of PPE formation [26,27]. Many other transcription factors including GATA2, Dlx3/5, FoxI1/3 and AP2 are involved with PPE formation (reviewed in [28]). Nonetheless, the molecular mechanism for segregation of the PPE and the NC is controversial in ectoderm patterning, and there are several models to explain PPE/NC formation (Figure 2).

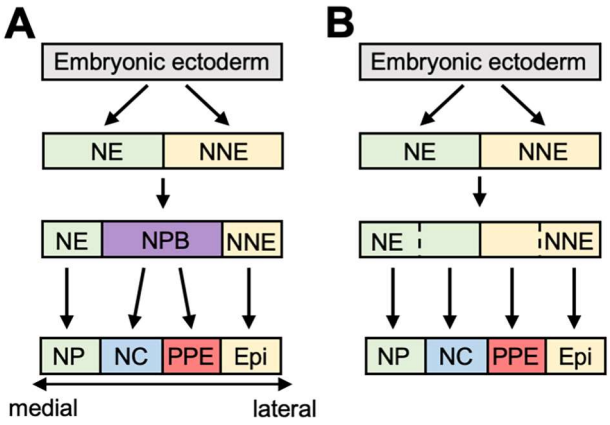

Figure 2. A model of PPE and NC formation: (**A**) the neural plate border (NPB) model; Before division of the NC and the PPE, the NPB region is formed between the neuroectoderm and the non-neural ectoderm; (**B**) binary competence model; The NC is derived from the neuroectoderm, whereas the PPE is from the non-neural ectoderm.

In the "binary competence" model, determination of the neuroectoderm and the non-neural ectoderm occurs during gastrulation, followed by subdivision of the PPE and the epidermis from the non-neural ectoderm, whereas the NC and the NP are derived from the neuroectoderm (Figure 2A). Evidence that supports this model includes the fact that transplantation of NP cells into ventral ectoderm induces Six1, but expression is only seen in the recipient NNE region and not in the donor NP, indicating a difference in competence between the neural and the non-neural ectoderm [6,29]. In addition, Dlx3 plays a role for the formation of differential competence for the PPE [29]. Furthermore, complete inhibition of BMP signaling by dorsomorphin (an antagonist of BMP) at the blastula stage greatly reduced PPE marker expression [30], indicating the importance of at least some BMP signaling at an early stage.

The second model is the "NPB model" (Figure 2B). In this model, the neural plate border (NPB) region is initially induced between the neural and the non-neural ectoderm, followed by subdivision into the NC and the PPE. For NPB formation, several genes are important. *Pax3* and *Zic1* are typical NPB markers. Knockdown of *Zic1* and *Pax3* reduced *Six1* expression, indicating the necessity of both gene functions for PPE formation [31]. The latter study of conserved enhancers revealed that expression of *Pax3* and *Zic1* is regulated by BMP, Wnt, and FGF, and the balance of these signals during the late gastrula stage is essential for *Zic1*/*Pax3* expression [32]. FGF signaling is important for *Pax3* transcription via specific enhancers (called IR2), whereas Wnt signaling positively regulates *zic3* transcription via both E1 and E2 enhancers [32]. *Pax3* expression is positively regulated by itself [33]. Immunostaining with several markers indicates that the PPE and the NC, in addition to the NP, overlap before the neurula stage in chick embryos, supporting this model [34]. Very recently, another model was proposed [35]. The "gradient border model" draws upon both

of the previous models. In this model, the neural plate border is induced, but in this area, cells that express NC or PPE genes are distinct, suggesting that NPB already possesses two regions before neurulation.

In the following section, we will discuss intracellular signaling involved in ectoderm patterning. In this patterning, several signaling pathways, including BMP, FGF, Wnt, and RA, participate, but in this review, we focus mainly on BMP and FGF signaling. On the subject of feedback regulation, we will also discuss the implications of RA signaling.

3. Control of BMP Signaling in PPE Formation

BMP serves important functions in various biological events, including many kinds of organ development in both vertebrates and invertebrates. Interaction of BMP ligands with BMP receptors promotes phosphorylation of the C-terminal serine residue of Smad1, directing it to bind Smad4, and regulating target gene expression (Figure 3) [5,36].

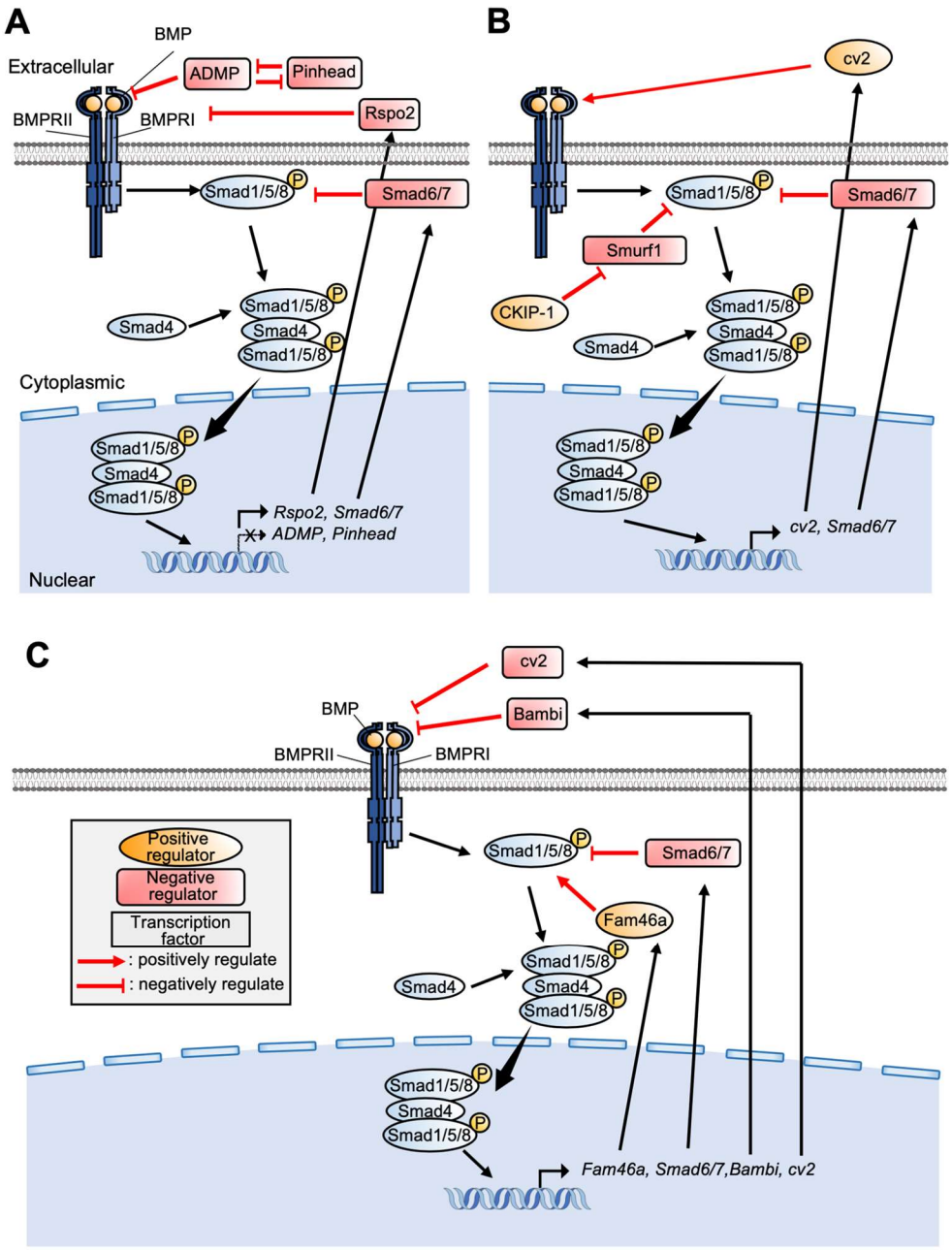

Figure 3. An outline of the BMP signaling pathway and a list of related factors described in this review: Factors involved in DV patterning (**A**), NPB formation (**B**), and PPE formation (**C**) are shown.

A morphogen gradient of BMP signaling is essential to establish embryonic patterning, as in dorsoventral axis formation [37]. Similarly, BMP signaling is crucial for ectoderm patterning. BMP4 and 7 are expressed in NNE, next to the PPE [38–42], whereas BMP antagonists are expressed in mesoderm underlying the PPE or in the PPE itself, contributing to differential control of the BMP level [43,44]. Animal cap experiments indicate that NP gene expression decreases as the dose of BMP increases [45]. Despite the fact that determination of the ectodermal region is crucial for precise body plan formation, the molecular mechanism by which BMP morphogen establishes each region is not still fully understood.

For NC formation, various animal models indicate the importance of BMP signaling, although what level of BMP signal is necessary remains controversial. In *Xenopus* embryos, signaling from DLMZ during gastrulation is important, whereas the signal from intermediate mesoderm, as well as adjacent ectoderm is important for maintenance of the NC region, indicating the necessity of stage-dependent inhibition of BMP signaling for NC formation (low BMP level in the early stage, whereas high level in the late stage) [46,47]. On the other hand, positive regulation of BMP signaling is necessary to induce the NC from the neural plate in chick embryos [48,49]. Furthermore, a zebrafish study indicated that intermediate levels of BMP specify a cranial neural crest progenitor [50].

For PPE formation, what function does BMP signaling serve? We need to consider the mechanism along with the binary competence and NPB models described above. According to the NPB model, intermediate levels of BMP signaling during gastrulation and neurulation are necessary for PPE formation, and evidence that supports the NPB model from the point of BMP signaling has been presented. An intermediate level of BMP signaling activity directs PPE induction. In chick embryos, the NPB region shows intermediate intensity of phosphorylated Smad1 protein [51]. A *Xenopus* study using animal cap cells indicated that *Six1* expression is highest with an intermediate dose of noggin or chordin [27,52]. Moreover, *dlx5* and *dlx6* are both expressed in NPB, and the quantitative level of expression was highest in *Xenopus* embryos injected with an intermediate amount of *chordin* (*chd)* mRNA [53]. Another zebrafish study indicated that for PPE formation, a somewhat higher level of BMP signaling is necessary than for the NC [54]. A similar experiment was carried out using zebrafish embryos [55]. In summary, intermediate BMP levels enable induction of NPB/placode gene expression, at least in several experimental systems employing *Xenopus*, zebrafish, and chick embryos.

In the binary state model, it is likely that positive regulation of BMP signaling before gastrulation is important for inducing the PPE, whereas the chick and Xenopus study indicated that attenuation of BMP signaling is necessary at late gastrula/neurula stages to induce the PPE in naïve ectoderm [6,7]. Similarly, using various doses and variable timing of treatments with dorsomorphin, a zebrafish study showed that BMP inhibition at blastula or early gastrula greatly reduced PPE marker expression (*sox3*, *six4* and *pax2*), whereas BMP inhibition at a later stage is important [30]. *Tfap2A/C*, *Fox1i* and *Gata3*, which are necessary to acquire PPE formation competence, are induced by BMP, whereas BMP signaling is not necessary to specify PPE fate after gastrulation [29,30,56]. A chick study also indicated that BMP signaling is required for formation of olfactory and lens placodes [57]. From these studies, it is suggested that during gastrulation BMP promotes PPE formation but subsequently inhibits PPE formation in the non-neural ectoderm.

4. Involvement of FGF Signaling for PPE Formation

Many studies have reported that relevant genes are involved in NPB/NC formation (Figure 4). Anosmin-1(Anos1), an ECM-associated, glycosylated protein directly interacts with FGF ligands and facilitates FGF8-FGFR1 interaction in chick embryo (Figure 4A) [58–61]. *Xenopus Anos1* is expressed downstream of *Pax3* and *Zic1* and contributes to formation of both the NC and the PPE [62]. *Meis3* is also expressed downstream of *Zic3* and *Pax3* and positively regulates *Fgf3* and *Fgf8* (Figure 4A) [63]. *Lrig3*, expressed in the NP and the NC, interacts with FGFR1 and modulates FGF signaling in NC induction and specification (Figure 4B) [64]. For establishment of the NC, the balance of ERK and AKT is important [65].

In the NC state of animal cap cells exhibited by *Foxd3* and *Sox9* expression, the pERK level is high and pAKT is low. Thus, NC formation is inhibited by either ERK inhibition or AKT activation [66].

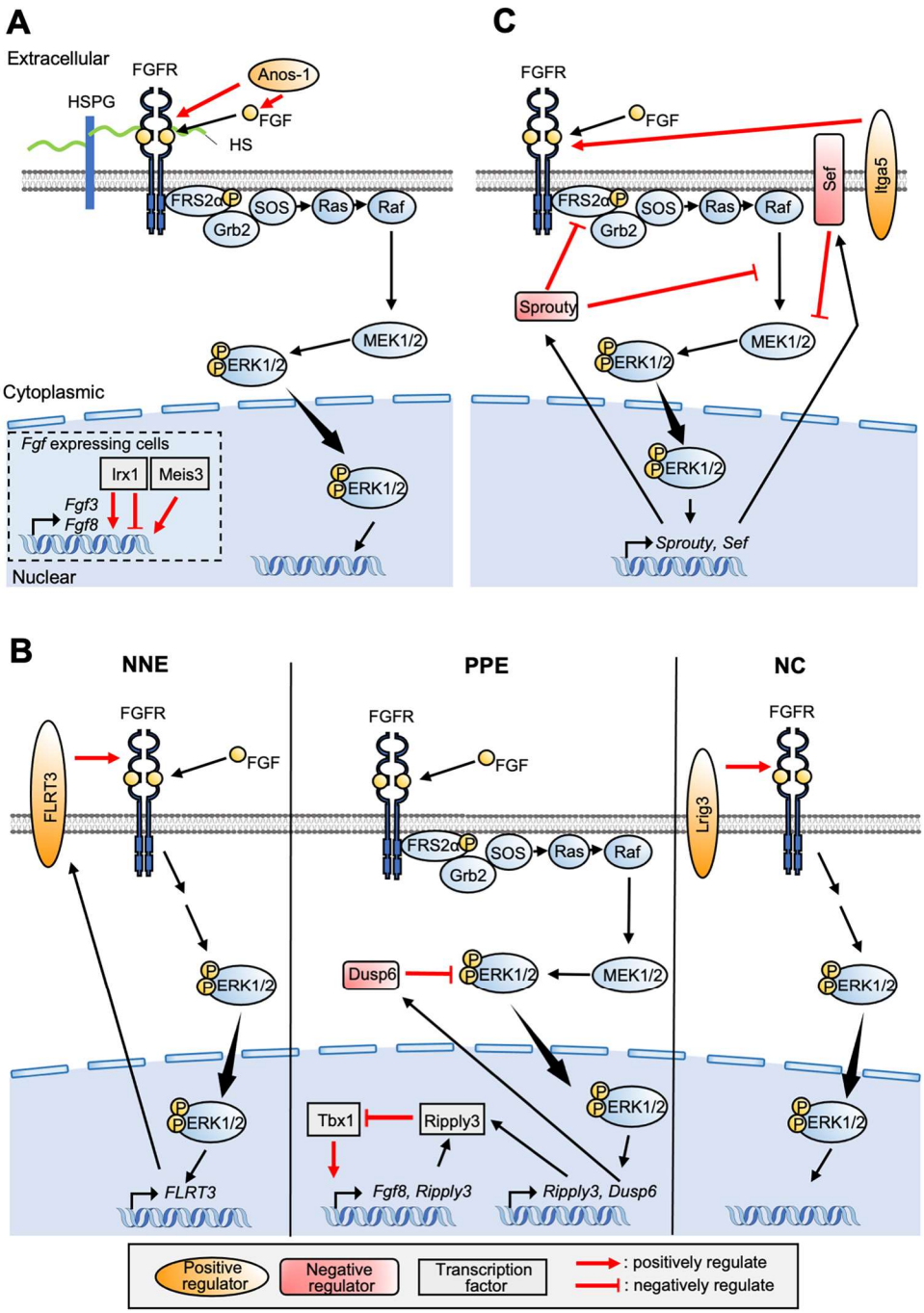

Figure 4. An outline of the FGF signaling pathway and a list of related factors described in this review: Factors involved in NPB (**A**), NC/PPE/NNE (**B**), and posterior placode (**C**) formation are shown. Properties of each factor and their actions on targets are shown by color and by arrows. Factors involved in PPE formation are shown in red or orange.

The importance of FGF signaling for PPE formation has also been shown by a series of studies. In *Xenopus* embryos, *Fgf3*, *Fgf4*, and *Fgf8* are expressed in the dorsolateral marginal zone [67]. In chicken blastula, *Fgf8* is distributed in almost all parts of the epiblast, and expression accumulates in the primitive streak at early gastrula stage [68]. Additionally, *Fgf8* is expressed in the anterior neural ridge, adjacent to the PPE [7,69,70]. In

Xenopus embryos, knockdown of Fgf8 by morpholino anti-sense oligo (MO) decreased *Six1* expression [6,31], and experiments using SU5402 (FGFR inhibitor) also indicated that an FGF signal is required for placode induction [6]. Although FGF signaling is necessary for PPE specification [6,7,31], overactivation of FGF signaling represses a PPE marker gene, *Six1* [27,31]. In addition, slight inhibition of FGF signaling enhances *Six1* expression [52], suggesting that an appropriate level of FGF is required for PPE induction.

Irx, which encodes Iroquois homeodomain protein, regulates *Fgf8* expression and is involved in NPB specifier-gene expression, including *Msx1*, *Pax3*, and *Zic1* (Figure 4A) [71]. *Irx1* is upregulated by *Six1* and *Eya1*, whereas *Irx1* promotes *Six1* expression in early PPE formation. Irx1 expression overlaps with that of the NPB gene at first, but expression accumulates only in the PPE region. Interestingly, Irx1 changes to suppress *Six1* expression, suggesting a differential stage-dependent role [71].

For specific placode formation from the pan-placodal domain, FGF signaling is necessary [19,72,73]. Mouse KO experiments also indicate essential roles of Fgf3, Fgf8, and Fgf10 in otic placode formation [73,74]. Integrin-α5 (Itga5) is expressed in the PPE, and its knockdown impaired trigeminal, epibranchial, and otic cells (Figure 4C) [75]. In addition, *dlx3/dlx4* negatively regulates *Fgfr1/2* expression, resulting in malformation of otic placode [44].

5. Feedback Regulation of Signaling Pathways for Ectodermal Patterning

As shown above, feedback regulation is a useful way not only to establish discrete areas, but also to maintain levels of intracellular signaling against fluctuations. We will show some examples of feedback regulation in BMP and FGF pathways and their contributions to PPE formation.

5.1. BMP Signaling

Several studies have addressed feedback regulation of BMP signaling in the context of embryonic development. In zebrafish embryos, both Pinhead and ADMP encode BMP-like ligands that promote chd degradation, whereas their transcription is repressed by BMP signaling (Figure 3A) [76–78]. In *Xenopus* ectoderm formation, R-spondins (RSPOs) antagonize BMP signaling by associating with the BMP receptor, affecting dorsoventral patterning. Biochemical analysis indicates that BMP promotes Rspo2 transcription, whereas RSPO protein antagonizes BMP signaling extracellularly, suggesting feedback loop formation (Figure 3A) [79]. Bambi is induced by BMP4, whereas Bambi represses the ligand–receptor complex, indicating negative loop formation (Figure 3C) [80]. This negative feedback regulation extends the dynamic range of BMP signaling because this system enables responses to more intense BMP signaling, contributing to attenuation of morphogen fluctuation in embryos [81]. Actually, in *Xenopus* embryos, the myf5 expression domain induced by intermediate BMP levels is perturbed by Bambi knockdown.

For PPE/NPB formation, there are not many studies that directly demonstrate the contribution of feedback regulation of BMP signaling. In *Xenopus* embryos, expression of *crossveinless2* (*cv2*), which interacts with both chd and BMP, is seen in high BMP regions, although knockdown of *cv2* with cv2 MO increased *vent1* and *cv2* and decreased *Six3* and *chd*, indicating that cv2 participates in a negative feedback loop of BMP signaling (Figure 3C) [82,83]. On the other hand, the zebrafish study indicates that cv2 forms the positive feedback loop by acting as pro-BMP factor and is required for NC induction by locally enhancing BMP activity and regulating the NPB gene network [84,85]. In the PPE region, dlx3 expression domain is outside the cv2 expression domain in the 5-somite stage of zebrafish embryos. *Dlx3b* enhances *bambi-b* in the PPE, suggesting that discrete expression of these genes specifies both the NC and the PPE region [85]. In chick embryos, *casein kinase interacting protein 1* (*CKIP-1*) and *Smurf1*, which encodes a ubiquitin ligase, are both expressed in NPB and establish an intermediate BMP level with *Smurf1* for NC formation. *Smurf1* attenuates BMP signaling via degradation of Smad1/5/8 but also degrades itself. At the same time, CKIP-1 directly interacts with Smurf1, promoting Smurf1 degradation. In

summary, *CKIP-1/Smurf1* double-negative attenuation maintains appropriate BMP signal levels in NPB (Figure 3B) [86].

Our analysis indicates the importance of *Fam46a* in PPE formation. Knockdown of *Fam46a* inhibits PPE-specific gene expression, including *Six1*. Fam46a protein directly interacts with the N-half region of Smad1, including the linker domain, and increases the quantitative level of Smad1. The linker region of Smad1 is phosphorylated by GSK3β, followed by ubiquitination and degradation via the proteosome system; thus, it is suggested that Fam46a upregulates BMP signaling via stabilization of Smad1 protein. Moreover, *Fam46a* transcription is promoted by BMP signaling, indicating formation of a positive feedback loop in BMP signaling (Figure 3C). Notably, activation of BMP signaling by Fam46a is not intense because Fam46a contributes to stabilization of Smad1 but not to direct activation via promotion of C-terminal phosphorylation of Smad1 [87].

5.2. FGF Signaling

For FGF signaling, feedback controls in either NPB/PPE/specific placode formation have been more widely reported than for BMP signaling. Tbx1 and Ripply3 contribute to regulation of PPE gene expression. In detail, Tbx1 facilitates expression of *Fgf8*, *Six1*, *Eya1*, and *Ripply3*. Additionally, Fgf8 promotes *Ripply3* expression. On the other hand, Ripply3 suppresses expression of *Fgf8* and *Tbx1* and forms a negative feedback loop with Fgf8, Ripply3, and Tbx1 (Figure 4B). This feedback loop contributes to the postero–lateral boundary during formation of the PPE by restricting the expressing region of *fgf* [88]. *Fibronectin-leucine-rich transmembrane protein 3* (*FLRT3*) functions as a positive regulator of Ras-MAPK signaling and also promotes ERK phosphorylation. *FLRT3* transcription is upregulated by FGF signaling, suggesting positive feedback formation (Figure 4B) [89,90]. *Xenopus* FLRT3 is co-expressed with *Fgf8* in the anterior neural ridge. From the fact that overactivation of FGF signaling inhibits PPE formation, FLRT3 may play a role in boundary formation outside the PPE region [90,91]. Recently, we showed that *Dual specificity phosphatase 6* (*Dusp6*, also known as *MKP3*) is important to precisely form the PPE region. Dusp6 is a phosphatase that specifically interacts with dual tyrosine and threonine residues of ERK1/2, attenuating Ras/ERK signaling (Figure 4B) [92–95]. Our study showed that *Dusp6* is expressed in the PPE at mid-neurula of *Xenopus* embryos in an FGF signal-dependent manner and is necessary for both NPB and PPE formation by modulating FGF signaling. An experiment combining FGF bead transplantation with Dusp6 knockdown demonstrated the importance of negative feedback control for PPE formation. In this study, it was suggested that stable spatial pattern formation against perturbation of FGF ligands is accomplished by suppressing intracellular signaling activity [96].

Furthermore, several genes involved in FGF signaling contribute to specific placode formation. *Sprouty* (*Spry)* functions as an intracellular negative feedback regulator of FGF signaling in several developmental contexts [97–99]. *Spry* is expressed in an FGF-dependent manner [100,101], and in mouse embryos, conditional knockout of *Spry1* causes defective craniofacial and cardiac development, indicating the importance of NC formation [102]. *Spry1* and *Spry2* are expressed in posterior PPE and participate in otic placode formation by inhibiting FGF signaling [103,104]. *Spry1* and *Spry2* also contribute to epibranchial placode formation and neuronal differentiation [105]. Malformation of otic placode by *Spry1* and *Spry2* knockdown was rescued by haploinsufficiency of *Fgf8* gene function, suggesting the importance of feedback loop-based fine tuning of FGF signaling (Figure 4C) [105]. *Similar expression of fgf* (*Sef*) regulates Ras-MAPK signaling, as well as other types of signaling [61,106]. In both zebrafish and *Xenopus* embryos, *Sef* is expressed in an FGF signaling-dependent manner, whereas FGF target gene expression is suppressed by *Sef* overexpression. Injection with Sef MO expanded the *Fgf8* expression region in the midbrain–hindbrain boundary (MHB) [107]. In chick embryos, *Sef* is expressed in otic placode [108], suggesting negative feedback loop regulation via Sef, at least in otic placode (Figure 4C).

RA signaling functions cooperatively with FGF signaling. RA nuclear receptor, RARa2, reduced the expression of *Ripply3*, *Tbx1* and *Six1*. As shown above, Ripply3 suppresses

FGF signaling, suggesting that RA and FGF signaling form a negative feedback loop via these genes [109]. Pitx2c is induced by RA and promotes transcription of Fgf8, followed by upregulation of Cyp26c1 (an RA metabolizing enzyme) expression adjacent to the PPE. This negative feedback regulation via both FGF and RA signaling suggests a role in PPE specification [110]. In otic vesicle formation, FGF signaling is required for aldh3 (RA synthesizing enzyme) expression, whereas RA treatment itself downregulates fgf8 expression, resulting in feedback loop formation [111].

6. Conclusions

In this review, we discussed the role of signaling pathways in PPE formation. In particular, we focused on BMP and FGF signaling and showed examples of their feedback regulation in PPE patterning. Signal adjustment is obviously important not only to form clear boundaries, but also to pattern narrow areas robustly. In particular, feedback adjustment contributes to noise suppression, which reduces signal fluctuation, and contributes to robust acquisition of patterns.

7. Future Directions

Further analysis is needed to fully elucidate the mechanisms of formation of the PPE region, the NP, the NC, and the epidermis. In addition, other experimental approaches may be important: one of them is to artificially change the feedback cycle by changing the intron length of a target gene and examining the effect on PPE formation [112]. Furthermore, other principles may need to be considered. One of these is mechanical regulation. Recently, a study using human pluripotent stem cells indicated that NPB fate determination is affected by external forces via changes in BMP signaling [113]. For directional migration of NC cells, a gradient of stiffness in surrounding cells, so-called "durotaxis", is important [114]. Additionally, our studies indicate that there is a difference in cell tension between neural and epidermal ectoderm [115,116]. From these results, it appears that mechanical forces may contribute to form each ectodermal region and to establish their properties. Another point concerns extracellular control of ligand diffusion. Various molecules, including ECM, membrane protein (receptors, etc.), and other cellular processes, including endocytosis, affect ligand diffusion; thus, these mechanisms are also expected to contribute to ectoderm patterning. Other studies report that ECM protein is involved in NC/PPE formation [70]. In addition, anos1, which associates with FGF ligands, also binds to heparan sulfate (HS) [60,117]. By investigating the contributions of these mechanisms to regulation of intracellular signaling, we will better understand the robust and precise system of embryonic pattern formation.

Funding: Preparation of this article was supported in part by JSPS KAKENHI (Grant Number 21K06183 to TM).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We apologize that we could not fully include studies regarding ectoderm patterning. We thank Steven D. Aird for technical editing of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wolpert, L. One Hundred Years of Positional Information. *Trends Genet.* **1996**, *12*, 359–364. [CrossRef]
2. Lander, A.D. Pattern, Growth, and Control. *Cell* **2011**, *144*, 955–969. [CrossRef] [PubMed]
3. Turing, A.M. The Chemical Basis of Morphogenesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1952**, *237*, 37–72. [CrossRef]
4. Kondo, S.; Asal, R. A Reaction-Diffusion Wave on the Skin of the Marine Angelfish Pomacanthus. *Nature* **1995**, *376*, 765–768. [CrossRef]

5. Bier, E.; De Robertis, E.M. BMP Gradients: A Paradigm for Morphogen-Mediated Developmental Patterning. *Science* **2015**, *348*, aaa5838. [CrossRef] [PubMed]
6. Ahrens, K.; Schlosser, G. Tissues and Signals Involved in the Induction of Placodal Six1 Expression in Xenopus Laevis. *Dev. Biol.* **2005**, *288*, 40–59. [CrossRef] [PubMed]
7. Litsiou, A.; Hanson, S.; Streit, A. A Balance of FGF, BMP and WNT Signalling Positions the Future Placode Territory in the Head. *Development* **2005**, *132*, 4051–4062. [CrossRef]
8. Lander, A.D.; Lo, W.-C.; Nie, Q.; Wan, F.Y.M. The Measure of Success: Constraints, Objectives, and Tradeoffs in Morphogen-Mediated Patterning. *Cold Spring Harb. Perspect. Biol.* **2009**, *1*, a002022. [CrossRef]
9. Bhattacharyya, S.; Bronner-Fraser, M. Hierarchy of Regulatory Events in Sensory Placode Development. *Curr. Opin. Genet. Dev.* **2004**, *14*, 520–526. [CrossRef]
10. Streit, A. The Preplacodal Region: An Ectodermal Domain with Multipotential Progenitors That Contribute to Sense Organs and Cranial Sensory Ganglia. *Int. J. Dev. Biol.* **2007**, *51*, 447–461. [CrossRef]
11. Pla, P.; Monsoro-Burq, A.H. The Neural Border: Induction, Specification and Maturation of the Territory That Generates Neural Crest Cells. *Dev. Biol.* **2018**, *444*, S36–S46. [CrossRef] [PubMed]
12. Steventon, B.; Carmona-Fontaine, C.; Mayor, R. Genetic Network during Neural Crest Induction: From Cell Specification to Cell Survival. *Semin. Cell Dev. Biol.* **2005**, *16*, 647–654. [CrossRef] [PubMed]
13. Mayor, R.; Aybar, M.J. Induction and Development of Neural Crest in Xenopus Laevis. *Cell Tissue Res.* **2001**, *305*, 203–209. [CrossRef] [PubMed]
14. Milet, C.; Monsoro-Burq, A.H. Neural Crest Induction at the Neural Plate Border in Vertebrates. *Dev. Biol.* **2012**, *366*, 22–33. [CrossRef] [PubMed]
15. Baker, C.V.H.; Bronner-Fraser, M. Vertebrate Cranial Placodes I. Embryonic Induction. *Dev. Biol.* **2001**, *232*, 1–61. [CrossRef] [PubMed]
16. Schlosser, G. Induction and Specification of Cranial Placodes. *Dev. Biol.* **2006**, *294*, 303–351. [CrossRef]
17. Schlosser, G. Making Senses: Development of Vertebrate Cranial Placodes. In *International Review of Cell and Molecular Biology*; Elsevier Inc.: Amsterdam, The Netherlands, 2010; Volume 283, pp. 129–234.
18. Grocott, T.; Tambalo, M.; Streit, A. The Peripheral Sensory Nervous System in the Vertebrate Head: A Gene Regulatory Perspective. *Dev. Biol.* **2012**, *370*, 3–23. [CrossRef]
19. Saint-Jeannet, J.-P.; Moody, S.A. Establishing the Pre-Placodal Region and Breaking It into Placodes with Distinct Identities. *Dev. Biol.* **2014**, *389*, 13–27. [CrossRef]
20. Singh, S.; Groves, A.K. The Molecular Basis of Craniofacial Placode Development. *Wiley Interdiscip. Rev. Dev. Biol.* **2016**, *5*, 363–376. [CrossRef]
21. Breau, M.A.; Schneider-Maunoury, S. Cranial Placodes: Models for Exploring the Multi-Facets of Cell Adhesion in Epithelial Rearrangement, Collective Migration and Neuronal Movements. *Dev. Biol.* **2015**, *401*, 25–36. [CrossRef]
22. Streit, A. Specification of Sensory Placode Progenitors: Signals and Transcription Factor Networks. *Int. J. Dev. Biol.* **2018**, *62*, 195–205. [CrossRef] [PubMed]
23. Pandur, P.D.; Moody, S.A. Xenopus Six1 Gene Is Expressed in Neurogenic Cranial Placodes and Maintained in the Differentiating Lateral Lines. *Mech. Dev.* **2000**, *96*, 253–257. [CrossRef]
24. Schlosser, G.; Ahrens, K. Molecular Anatomy of Placode Development in Xenopus Laevis. *Dev. Biol.* **2004**, *271*, 439–466. [CrossRef] [PubMed]
25. David, R.; Ahrens, K.; Wedlich, D.; Schlosser, G. Xenopus Eya1 Demarcates All Neurogenic Placodes as Well as Migrating Hypaxial Muscle Precursors. *Mech. Dev.* **2001**, *103*, 189–192. [CrossRef]
26. Maharana, S.K.; Schlosser, G. A Gene Regulatory Network Underlying the Formation of Pre-Placodal Ectoderm in Xenopus Laevis. *BMC Biol.* **2018**, *16*, 79. [CrossRef]
27. Brugmann, S.A.; Pandur, P.D.; Kenyon, K.L.; Pignoni, F.; Moody, S.A. Six1 Promotes a Placodal Fate within the Lateral Neurogenic Ectoderm by Functioning as Both a Transcriptional Activator and Repressor. *Development* **2004**, *131*, 5871–5881. [CrossRef]
28. Schlosser, G. Early Embryonic Specification of Vertebrate Cranial Placodes. *Wiley Interdiscip. Rev. Dev. Biol.* **2014**, *3*, 349–363. [CrossRef]
29. Pieper, M.; Ahrens, K.; Rink, E.; Peter, A.; Schlosser, G. Differential Distribution of Competence for Panplacodal and Neural Crest Induction to Non-Neural and Neural Ectoderm. *Development* **2012**, *139*, 1175–1187. [CrossRef]
30. Kwon, H.-J.; Bhat, N.; Sweet, E.M.; Cornell, R.A.; Riley, B.B. Identification of Early Requirements for Preplacodal Ectoderm and Sensory Organ Development. *PLoS Genet.* **2010**, *6*, e1001133. [CrossRef]
31. Hong, C.-S.; Saint-Jeannet, J.-P. The Activity of Pax3 and Zic1 Regulates Three Distinct Cell Fates at the Neural Plate Border. *Mol. Biol. Cell* **2007**, *18*, 2192–2202. [CrossRef]
32. Garnett, A.T.; Square, T.A.; Medeiros, D.M. BMP, Wnt and FGF Signals Are Integrated through Evolutionarily Conserved Enhancers to Achieve Robust Expression of Pax3 and Zic Genes at the Zebrafish Neural Plate Border. *Development* **2012**, *139*, 4220–4231. [CrossRef] [PubMed]
33. Plouhinec, J.L.; Roche, D.D.; Pegoraro, C.; Figueiredo, A.L.; Maczkowiak, F.; Brunet, L.J.; Milet, C.; Vert, J.P.; Pollet, N.; Harland, R.M.; et al. Pax3 and Zic1 Trigger the Early Neural Crest Gene Regulatory Network by the Direct Activation of Multiple Key Neural Crest Specifiers. *Dev. Biol.* **2014**, *386*, 461–472. [CrossRef] [PubMed]

34. Roellig, D.; Tan-Cabugao, J.; Esaian, S.; Bronner, M.E. Dynamic Transcriptional Signature and Cell Fate Analysis Reveals Plasticity of Individual Neural Plate Border Cells. *eLife* **2017**, *6*, e21620. [CrossRef] [PubMed]
35. Thiery, A.; Buzzi, A.L.; Hamrud, E.; Cheshire, C.; Luscombe, N.; Briscoe, J.; Streit, A. A Gradient Border Model for Cell Fate Decisions at the Neural Plate Border. *bioRxiv* **2022**. [CrossRef]
36. Miyazono, K.; Kamiya, Y.; Morikawa, M. Bone Morphogenetic Protein Receptors and Signal Transduction. *J. Biochem.* **2010**, *147*, 35–51. [CrossRef]
37. Hill, C.S. *Establishment and Interpretation of NODAL and BMP Signaling Gradients in Early Vertebrate Development*, 1st ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2022; Volume 149, ISBN 9780128170977.
38. Fainsod, A.; Steinbeisser, H.; De Robertis, E.M. On the Function of BMP-4 in Patterning the Marginal Zone of the Xenopus Embryo. *EMBO J.* **1994**, *13*, 5015–5025. [CrossRef]
39. Hemmati-Brivanlou, A.; Thomsen, G.H. Ventral Mesodermal Patterning InXenopus Embryos: Expression Patterns and Activities of BMP-2 and BMP-4. *Dev. Genet.* **1995**, *17*, 78–89. [CrossRef]
40. Liem, K.F.; Tremml, G.; Roelink, H.; Jessell, T.M. Dorsal Differentiation of Neural Plate Cells Induced by BMP-Mediated Signals from Epidermal Ectoderm. *Cell* **1995**, *82*, 969–979. [CrossRef]
41. Schmidt, J.E.; Suzuki, A.; Ueno, N.; Kimelman, D. Localized BMP-4 Mediates Dorsal/Ventral Patterning in the Early Xenopus Embryo. *Dev. Biol.* **1995**, *169*, 37–50. [CrossRef]
42. Streit, A.; Stern, C.D. Establishment and Maintenance of the Border of the Neural Plate in the Chick: Involvement of FGF and BMP Activity. *Mech. Dev.* **1999**, *82*, 51–66. [CrossRef]
43. Ogita, J.; Isogai, E.; Sudo, H.; Sakiyama, S.; Nakagawara, A.; Koseki, H. Expression of the Dan Gene during Chicken Embryonic Development. *Mech. Dev.* **2001**, *109*, 363–365. [CrossRef]
44. Esterberg, R.; Fritz, A. Dlx3b/4b Are Required for the Formation of the Preplacodal Region and Otic Placode through Local Modulation of BMP Activity. *Dev. Biol.* **2009**, *325*, 189–199. [CrossRef]
45. Wilson, P.A.; Lagna, G.; Suzuki, A.; Hemmati-Brivanlou, A. Concentration-Dependent Patterning of the Xenopus Ectoderm by BMP4 and Its Signal Transducer Smad1. *Development* **1997**, *124*, 3177–3184. [CrossRef] [PubMed]
46. Marchant, L.; Linker, C.; Ruiz, P.; Guerrero, N.; Mayor, R. The Inductive Properties of Mesoderm Suggest That the Neural Crest Cells Are Specified by a BMP Gradient. *Dev. Biol.* **1998**, *198*, 319–329. [CrossRef]
47. Steventon, B.; Araya, C.; Linker, C.; Kuriyama, S.; Mayor, R. Differential Requirements of BMP and Wnt Signalling during Gastrulation and Neurulation Define Two Steps in Neural Crest Induction. *Development* **2009**, *136*, 771–779. [CrossRef]
48. Selleck, M.A.J.; García-Castro, M.I.; Artinger, K.B.; Bronner-Fraser, M. Effects of Shh and Noggin on Neural Crest Formation Demonstrate That BMP Is Required in the Neural Tube but Not Ectoderm. *Development* **1998**, *125*, 4919–4930. [CrossRef] [PubMed]
49. Endo, Y.; Osumi, N.; Wakamatsu, Y. Bimodal Functions of Notch-Mediated Signaling Are Involved in Neural Crest Formation during Avian Ectoderm Development. *Development* **2002**, *129*, 863–873. [CrossRef]
50. Schumacher, J.A.; Hashiguchi, M.; Nguyen, V.H.; Mullins, M.C. An Intermediate Level of Bmp Signaling Directly Specifies Cranial Neural Crest Progenitor Cells in Zebrafish. *PLoS ONE* **2011**, *6*, e27403. [CrossRef]
51. Faure, S.; De Santa Barbara, P.; Roberts, D.J.; Whitman, M. Endogenous Patterns of BMP Signaling during Early Chick Development. *Dev. Biol.* **2002**, *244*, 44–65. [CrossRef]
52. Watanabe, T.; Kanai, Y.; Matsukawa, S.; Michiue, T. Specific Induction of Cranial Placode Cells from Xenopus Ectoderm by Modulating the Levels of BMP, Wnt, and FGF Signaling. *Genesis* **2015**, *53*, 652–659. [CrossRef]
53. Luo, T.; Matsuo-Takasaki, M.; Lim, J.H.; Sargent, T.D. Differential Regulation of Dlx Gene Expression by a BMP Morphogenetic Gradient. *Int. J. Dev. Biol.* **2001**, *45*, 681–684. [PubMed]
54. Nguyen, V.H.; Schmid, B.; Trout, J.; Connors, S.A.; Ekker, M.; Mullins, M.C. Ventral and Lateral Regions of the Zebrafish Gastrula, Including the Neural Crest Progenitors, Are Established by Abmp2b/SwirlPathway of Genes. *Dev. Biol.* **1998**, *199*, 93–110. [CrossRef] [PubMed]
55. Neave, B.; Holder, N.; Patient, R. A Graded Response to BMP-4 Spatially Coordinates Patterning of the Mesoderm and Ectoderm in the Zebrafish. *Mech. Dev.* **1997**, *62*, 183–195. [CrossRef]
56. Bhat, N.; Kwon, H.-J.; Riley, B.B. A Gene Network That Coordinates Preplacodal Competence and Neural Crest Specification in Zebrafish. *Dev. Biol.* **2013**, *373*, 107–117. [CrossRef] [PubMed]
57. Sjödal, M.; Edlund, T.; Gunhaga, L. Time of Exposure to BMP Signals Plays a Key Role in the Specification of the Olfactory and Lens Placodes Ex Vivo. *Dev. Cell* **2007**, *13*, 141–149. [CrossRef] [PubMed]
58. Hu, Y.; Guimond, S.E.; Travers, P.; Cadman, S.; Hohenester, E.; Tumbull, J.E.; Kim, S.H.; Bouloux, P.M. Novel Mechanisms of Fibroblast Growth Factor Receptor 1 Regulation by Extracellular Matrix Protein Anosmin-1. *J. Biol. Chem.* **2009**, *284*, 29905–29920. [CrossRef]
59. Endo, Y.; Ishiwata-Endo, H.; Yamada, K.M. Extracellular Matrix Protein Anosmin Promotes Neural Crest Formation and Regulates FGF, BMP, and WNT Activities. *Dev. Cell* **2012**, *23*, 305–316. [CrossRef]
60. Hu, Y.; González-Martínez, D.; Kim, S.H.; Bouloux, P.M.G. Cross-Talk of Anosmin-1, the Protein Implicated in X-Linked Kallmann's Syndrome, with Heparan Sulphate and Urokinase-Type Plasminogen Activator. *Biochem. J.* **2004**, *384*, 495–505. [CrossRef]
61. Korsensky, L.; Ron, D. Regulation of FGF Signaling: Recent Insights from Studying Positive and Negative Modulators. *Semin. Cell Dev. Biol.* **2016**, *53*, 101–114. [CrossRef]

62. Bae, C.-J.; Hong, C.-S.; Saint-Jeannet, J.-P. Anosmin-1 Is Essential for Neural Crest and Cranial Placodes Formation in Xenopus. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 2257–2263. [CrossRef]
63. Gutkovich, Y.E.; Ofir, R.; Elkouby, Y.M.; Dibner, C.; Gefen, A.; Elias, S.; Frank, D. Xenopus Meis3 Protein Lies at a Nexus Downstream to Zic1 and Pax3 Proteins, Regulating Multiple Cell-Fates during Early Nervous System Development. *Dev. Biol.* **2010**, *338*, 50–62. [CrossRef] [PubMed]
64. Zhao, H.; Tanegashima, K.; Ro, H.; Dawid, I.B. Lrig3 Regulates Neural Crest Formation in Xenopus by Modulating Fgf and Wnt Signaling Pathways. *Development* **2008**, *135*, 1283–1293. [CrossRef] [PubMed]
65. Dinsmore, C.J.; Soriano, P. MAPK and PI3K Signaling: At the Crossroads of Neural Crest Development. *Dev. Biol.* **2018**, *444*, S79–S97. [CrossRef] [PubMed]
66. Geary, L.; LaBonne, C. FGF Mediated MAPK and PI3K/Akt Signals Make Distinct Contributions to Pluripotency and the Establishment of Neural Crest. *eLife* **2018**, *7*, e33845. [CrossRef]
67. Monsoro-Burq, A.H.; Fletcher, R.B.; Harland, R.M. Neural Crest Induction by Paraxial Mesoderm in Xenopus Embryos Requires FGF Signals. *Development* **2003**, *130*, 3111–3124. [CrossRef]
68. Lawson, A.; Colas, J.F.; Schoenwolf, G.C. Classification Scheme for Genes Expressed during Formation and Progression of the Avian Primitive Streak. *Anat. Rec.* **2001**, *262*, 221–226. [CrossRef]
69. Fletcher, R.B.; Baker, J.C.; Harland, R.M. FGF8 Spliceforms Mediate Early Mesoderm and Posterior Neural Tissue Formation in Xenopus. *Development* **2006**, *133*, 1703–1714. [CrossRef]
70. Tereshina, M.B.; Ermakova, G.V.; Ivanova, A.S.; Zaraisky, A.G. Ras-Dva1 Small GTPase Regulates Telencephalon Development in Xenopus Laevis Embryos by Controlling Fgf8 and Agr Signaling at the Anterior Border of the Neural Plate. *Biol. Open* **2014**, *3*, 192–203. [CrossRef]
71. Sullivan, C.H.; Majumdar, H.D.; Neilson, K.M.; Moody, S.A. Six1 and Irx1 Have Reciprocal Interactions during Cranial Placode and Otic Vesicle Formation. *Dev. Biol.* **2019**, *446*, 68–79. [CrossRef]
72. Schimmang, T. Expression and Functions of FGF Ligands during Early Otic Development. *Int. J. Dev. Biol.* **2007**, *51*, 473–481. [CrossRef]
73. Wright, T.J.; Mansour, S.L. Fgf3 and Fgf10 Are Required for Mouse Otic Placode Induction. *Development* **2003**, *130*, 3379–3390. [CrossRef] [PubMed]
74. Domínguez-Frutos, E.; Vendrell, V.; Alvarez, Y.; Zelarayan, L.C.; López-Hernández, I.; Ros, M.; Schimmang, T. Tissue-Specific Requirements for FGF8 during Early Inner Ear Development. *Mech. Dev.* **2009**, *126*, 873–881. [CrossRef] [PubMed]
75. Bhat, N.; Riley, B.B. Integrin-A5 Coordinates Assembly of Posterior Cranial Placodes in Zebrafish and Enhances Fgf-Dependent Regulation of Otic/Epibranchial Cells. *PLoS ONE* **2011**, *6*, e27778. [CrossRef] [PubMed]
76. Yan, Y.; Ning, G.; Li, L.; Liu, J.; Yang, S.; Cao, Y.; Wang, Q. The BMP Ligand Pinhead Together with Admp Supports the Robustness of Embryonic Patterning. *Sci. Adv.* **2019**, *5*, eaau6455. [CrossRef]
77. Moos, M.; Wang, S.; Krinks, M. Anti-Dorsalizing Morphogenetic Protein Is a Novel TGF-Beta Homolog Expressed in the Spemann Organizer. *Development* **1995**, *121*, 4293–4301. [CrossRef]
78. Lele, Z.; Nowak, M.; Hammerschmidt, M. Zebrafish Admp Is Required to Restrict the Size of the Organizer and to Promote Posterior and Ventral Development. *Dev. Dyn.* **2001**, *222*, 681–687. [CrossRef]
79. Lee, H.; Seidl, C.; Sun, R.; Glinka, A.; Niehrs, C. R-Spondins Are BMP Receptor Antagonists in Xenopus Early Embryonic Development. *Nat. Commun.* **2020**, *11*, 1–16. [CrossRef]
80. Onichtchouk, D.; Chen, Y.G.; Dosch, R.; Gawantka, V.; Delius, H.; Massagué, J.; Niehrs, C. Silencing of TGF-β Signalling by the Pseudoreceptor BAMBI. *Nature* **1999**, *401*, 480–485. [CrossRef]
81. Paulsen, M.; Legewie, S.; Eils, R.; Karaulanov, E.; Niehrs, C. Negative Feedback in the Bone Morphogenetic Protein 4 (BMP4) Synexpression Group Governs Its Dynamic Signaling Range and Canalizes Development. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 10202–10207. [CrossRef]
82. Coffinier, C.; Ketpura, N.; Tran, U.; Geissert, D.; De Robertis, E.M. Mouse Crossveinless-2 Is the Vertebrate Homolog of a Drosophila Extracellular Regulator of BMP Signaling. *Mech. Dev.* **2002**, *119*, S179–S184. [CrossRef]
83. Ambrosio, A.L.; Taelman, V.F.; Lee, H.X.; Metzinger, C.A.; Coffinier, C.; De Robertis, E.M. Crossveinless-2 Is a BMP Feedback Inhibitor That Binds Chordin/BMP to Regulate Xenopus Embryonic Patterning. *Dev. Cell* **2008**, *15*, 248–260. [CrossRef] [PubMed]
84. Rentzsch, F.; Zhang, J.; Kramer, C.; Sebald, W.; Hammerschmidt, M. Crossveinless 2 Is an Essential Positive Feedback Regulator of Bmp Signaling during Zebrafish Gastrulation. *Development* **2006**, *133*, 801–811. [CrossRef] [PubMed]
85. Reichert, S.; Randall, R.A.; Hill, C.S. A BMP Regulatory Network Controls Ectodermal Cell Fate Decisions at the Neural Plate Border. *Development* **2013**, *140*, 4435–4444. [CrossRef] [PubMed]
86. Piacentino, M.L.; Bronner, M.E. Intracellular Attenuation of BMP Signaling via CKIP-1/Smurf1 Is Essential during Neural Crest Induction. *PLoS Biol.* **2018**, *16*, e2004425. [CrossRef] [PubMed]
87. Watanabe, T.; Yamamoto, T.; Tsukano, K.; Hirano, S.; Horikawa, A.; Michiue, T. Fam46a Regulates BMP-Dependent Pre-Placodal Ectoderm Differentiation in Xenopus. *Development* **2018**, *145*, dev166710. [CrossRef]
88. Janesick, A.; Shiotsugu, J.; Taketani, M.; Blumberg, B. RIPPLY3 Is a Retinoic Acid-Inducible Repressor Required for Setting the Borders of the Pre-Placodal Ectoderm. *Development* **2012**, *139*, 1213–1224. [CrossRef]
89. Böttcher, R.T.; Pollet, N.; Delius, H.; Niehrs, C. The Transmembrane Protein XFLRT3 Forms a Complex with FGF Receptors and Promotes FGF Signalling. *Nat. Cell Biol.* **2004**, *6*, 38–44. [CrossRef]

90. Cho, G.S.; Choi, S.C.; Han, J.K. BMP Signal Attenuates FGF Pathway in Anteroposterior Neural Patterning. *Biochem. Biophys. Res. Commun.* **2013**, *434*, 509–515. [CrossRef]
91. Cho, G.-S.; Park, D.-S.; Choi, S.-C.; Han, J.-K. Tbx2 Regulates Anterior Neural Specification by Repressing FGF Signaling Pathway. *Dev. Biol.* **2017**, *421*, 183–193. [CrossRef]
92. Huang, C.-Y.; Tan, T.-H. DUSPs, to MAP Kinases and Beyond. *Cell Biosci.* **2012**, *2*, 24. [CrossRef]
93. Muhammad, K.A.; Nur, A.A.; Nurul, H.S.; Narazah, M.Y.; Siti, R.A.R. Dual-Specificity Phosphatase 6 (DUSP6): A Review of Its Molecular Characteristics and Clinical Relevance in Cancer. *Cancer Biol. Med.* **2018**, *15*, 14. [CrossRef] [PubMed]
94. Bermudez, O.; Pagès, G.; Gimond, C. The Dual-Specificity MAP Kinase Phosphatases: Critical Roles in Development and Cancer. *Am. J. Physiol. Physiol.* **2010**, *299*, C189–C202. [CrossRef] [PubMed]
95. Gómez, A.R.; López-Varea, A.; Molnar, C.; de la Calle-Mustienes, E.; Ruiz-Gómez, M.; Gómez-Skarmeta, J.L.; de Celis, J.F. Conserved Cross-Interactions in Drosophila and Xenopus between Ras/MAPK Signaling and the Dual-Specificity Phosphatase MKP3. *Dev. Dyn.* **2005**, *232*, 695–708. [CrossRef] [PubMed]
96. Tsukano, K.; Yamamoto, T.; Watanabe, T.; Michiue, T. Xenopus Dusp6 Modulates FGF Signaling to Precisely Pattern Pre-Placodal Ectoderm. *Dev. Biol.* **2022**, *488*, 81–90. [CrossRef] [PubMed]
97. Mason, J.M.; Morrison, D.J.; Basson, M.A.; Licht, J.D. Sprouty Proteins: Multifaceted Negative-Feedback Regulators of Receptor Tyrosine Kinase Signaling. *Trends Cell Biol.* **2006**, *16*, 45–54. [CrossRef] [PubMed]
98. Cabrita, M.A.; Christofori, G. Sprouty Proteins, Masterminds of Receptor Tyrosine Kinase Signaling. *Angiogenesis* **2008**, *11*, 53–62. [CrossRef]
99. Kawazoe, T.; Taniguchi, K. The Sprouty/Spred Family as Tumor Suppressors: Coming of Age. *Cancer Sci.* **2019**, *110*, 1525–1535. [CrossRef]
100. Sasaki, A.; Taketomi, T.; Wakioka, T.; Kato, R.; Yoshimura, A. Identification of a Dominant Negative Mutant of Sprouty That Potentiates Fibroblast Growth Factor-but Not Epidermal Growth Factor-Induced ERK Activation. *J. Biol. Chem.* **2001**, *276*, 36804–36808. [CrossRef]
101. Ozaki, K.; Kadomoto, R.; Asato, K.; Tanimura, S.; Itoh, N.; Kohno, M. Erk Pathway Positively Regulates the Expression of Sprouty Genes. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 1084–1088. [CrossRef]
102. Yang, X.; Kilgallen, S.; Andreeva, V.; Spicer, D.B.; Pinz, I.; Friesel, R. Conditional Expression of Spry1 in Neural Crest Causes Craniofacial and Cardiac Defects. *BMC Dev. Biol.* **2010**, *10*, 48. [CrossRef]
103. Wright, K.D.; Mahoney Rogers, A.A.; Zhang, J.; Shim, K. Cooperative and Independent Functions of FGF and Wnt Signaling during Early Inner Ear Development Organogenesis. *BMC Dev. Biol.* **2015**, *15*, 1–15. [CrossRef] [PubMed]
104. Mahoney Rogers, A.A.; Zhang, J.; Shim, K. Sprouty1 and Sprouty2 Limit Both the Size of the Otic Placode and Hindbrain Wnt8a by Antagonizing FGF Signaling. *Dev. Biol.* **2011**, *353*, 94–104. [CrossRef] [PubMed]
105. Simrick, S.; Lickert, H.; Basson, M.A. Sprouty Genes Are Essential for the Normal Development of Epibranchial Ganglia in the Mouse Embryo. *Dev. Biol.* **2011**, *358*, 147–155. [CrossRef] [PubMed]
106. Yang, R.B.; Ng, C.K.D.; Wasserman, S.M.; Kömüves, L.G.; Gerritsen, M.E.; Topper, J.N. A Novel Interleukin-17 Receptor-like Protein Identified in Human Umbilical Vein Endothelial Cells Antagonizes Basic Fibroblast Growth Factor-Induced Signaling. *J. Biol. Chem.* **2003**, *278*, 33232–33238. [CrossRef] [PubMed]
107. Tsang, M.; Friesel, R.; Kudoh, T.; Dawid, I.B. Identification of Sef, a Novel Modulator of FGF Signalling. *Nat. Cell Biol.* **2002**, *4*, 165–169. [CrossRef]
108. Harduf, H.; Halperin, E.; Reshef, R.; Ron, D. Sef Is Synexpressed with FGFs during Chick Embryogenesis and Its Expression Is Differentially Regulated by FGFs in the Developing Limb. *Dev. Dyn.* **2005**, *233*, 301–312. [CrossRef]
109. Dubey, A.; Yu, J.; Liu, T.; Kane, M.A.; Saint-Jeannet, J.-P. Retinoic Acid Production, Regulation and Containment through Zic1, Pitx2c and Cyp26c1 Control Cranial Placode Specification. *Development* **2021**, *148*, dev193227. [CrossRef]
110. Maier, E.C.; Whitfield, T.T. RA and FGF Signalling Are Required in the Zebrafish Otic Vesicle to Pattern and Maintain Ventral Otic Identities. *PLoS Genet.* **2014**, *10*, e1004858. [CrossRef]
111. Jaurena, M.B.; Juraver-Geslin, H.; Devotta, A.; Saint-Jeannet, J.P. Zic1 Controls Placode Progenitor Formation Non-Cell Autonomously by Regulating Retinoic Acid Production and Transport. *Nat. Commun.* **2015**, *6*, 7476. [CrossRef]
112. Swinburne, I.A.; Miguez, D.G.; Landgraf, D.; Silver, P.A. Intron Length Increases Oscillatory Periods of Gene Expression in Animal Cells. *Genes Dev.* **2008**, *22*, 2342–2346. [CrossRef]
113. Xue, X.; Sun, Y.; Resto-Irizarry, A.M.; Yuan, Y.; Aw Yong, K.M.; Zheng, Y.; Weng, S.; Shao, Y.; Chai, Y.; Studer, L.; et al. Mechanics-Guided Embryonic Patterning of Neuroectoderm Tissue from Human Pluripotent Stem Cells. *Nat. Mater.* **2018**, *17*, 633–641. [CrossRef] [PubMed]
114. Shellard, A.; Mayor, R. Collective Durotaxis along a Self-Generated Stiffness Gradient in Vivo. *Nature* **2021**, *600*, 690–694. [CrossRef] [PubMed]
115. Yamashita, S.; Tsuboi, T.; Ishinabe, N.; Kitaguchi, T.; Michiue, T. Wide and High Resolution Tension Measurement Using FRET in Embryo. *Sci. Rep.* **2016**, *6*, 28535. [CrossRef] [PubMed]

116. Hirano, S.; Yamamoto, T.; Michiue, T. FRET-Based Tension Measurement across Actin-Associated Mechanotransductive Structures Using Lima1. *Int. J. Dev. Biol.* **2018**, *62*, 631–636. [CrossRef] [PubMed]
117. Soussi-Yanicostas, N.; Hardelin, J.P.; Arroyo-Jimenez, M.D.M.; Ardouin, O.; Legouis, R.; Levilliers, J.; Traincard, F.; Betton, J.M.; Cabanié, L.; Petit, C. Initial Characterization of Anosmin-1, a Putative Extracellular Matrix Protein Synthesized by Definite Neuronal Cell Populations in the Central Nervous System. *J. Cell Sci.* **1996**, *109*, 1749–1757. [CrossRef] [PubMed]