Advanced Review

Neural crest specification: tissues, signals, and transcription factors

C.D. Rogers, C.S. Jayasena, S. Nie and M.E. Bronner*

The neural crest is a transient population of multipotent and migratory cells unique to vertebrate embryos. Initially derived from the borders of the neural plate, these cells undergo an epithelial to mesenchymal transition to leave the central nervous system, migrate extensively in the periphery, and differentiate into numerous diverse derivatives. These include but are not limited to craniofacial cartilage, pigment cells, and peripheral neurons and glia. Attractive for their similarities to stem cells and metastatic cancer cells, neural crest cells are a popular model system for studying cell/tissue interactions and signaling factors that influence cell fate decisions and lineage transitions. In this review, we discuss the mechanisms required for neural crest formation in various vertebrate species, focusing on the importance of signaling factors from adjacent tissues and conserved gene regulatory interactions, which are required for induction and specification of the ectodermal tissue that will become neural crest.

How to cite this article:
WIREs Dev Biol 2012, 1:52–68. doi: 10.1002/wdev.8

INTRODUCTION

Neural crest (NC) cells are a unique vertebrate cell type that sets vertebrates apart from their invertebrate relatives. This transitory population of multipotent stem-like cells is first induced in the ectoderm during gastrulation[1] and specified within the neural plate border (NPB), a region of tissue that lies between the presumptive neural plate (NP) and non-neural ectoderm, fated to become the central nervous system, NC, and epidermis. The NPB cells are competent to become both NC and neural tube (NT)[2] cells. After the NT closes, the NC cells leave the NT and migrate throughout the developing embryo to contribute to diverse derivatives. These include not only the sensory and autonomic nervous systems, but also the craniofacial skeleton, smooth muscles, and melanocytes,[3] among other cell types.

Thought to be a unique vertebrate trait, the NC is present in even the most basal vertebrate, the lamprey. Formation of the NC is mediated by a series of regulatory interactions in the form of a gene regulatory network (GRN) that is largely conserved across vertebrates.[4,5] Although NC cells are induced during gastrulation, they only become morphologically recognizable after neurulation, where they manifest in an antero-posterior (AP) fashion first in the head (cranial NC) and proceed caudally to form trunk NC cells.[6] Premigratory NC cells initially reside within the dorsal region of the NT as neuroepithelial cells. Although initially epithelial in nature, they subsequently lose cell–cell adhesion and undergo cytoskeletal changes that result in an epithelial to mesenchymal transition (EMT), allowing them to detach from the epithelial sheet and start migrating in the developing embryo.[7] The abilities of NC cells to migrate extensively and to form diverse cell types are reminiscent of metastatic cancer cells (sharing migration, invasion, and proliferation properties). These characteristics have made them an interesting and well-studied topic for many years.

This review will focus on the molecular events involved in NC induction and specification. Emphasis will be placed on patterning of the presumptive NC region and extracellular signaling required to initiate the NC GRN, as well as the complex interactions that take place between transcription factors (and NC modifiers) to establish the 'transcriptional state' of NC.

*Correspondence to: mbronner@caltech.edu
Department of Biology, California Institute of Technology, Pasadena, CA, USA

GASTRULATION, NEURULATION, AND THE NP BORDER

At the end of gastrulation, interactions between the NP and the non-neural ectoderm lead to the generation of the NPB. The NPB, which elevates to form the neural folds and gives rise to the dorsal NT and NC cells, flanks the NP bilaterally (Figure 2(a)) and expresses genes characteristic of these multi potent border cells, including *Msx1*/2 and *Pax3*/7 (Figure 2(b)).[8,9] In most vertebrates, NC arises from the entire length of the neuraxis with the exception of the most anterior NT, which contributes to the olfactory placode and anterior brain structures. During neurulation, the neural folds elevate, apposing at the dorsal midline to form the NT. Subsequently, NC cells go through an EMT, allowing them to delaminate from the neuroepithelium and to migrate throughout the embryo, beginning at the level of the presumptive midbrain and then proceeding in a rostrocaudal wave.

The induction of NC is a multistep process, starting at the early gastrula stages and continuing until NT closure. Tissue arrangements during gastrulation and neurulation are essential for NP border specification (for an example of early zebrafish embryogenesis go to Zebrafish Movie). During gastrulation, massive cell and tissue migrations lead to the reorganization of the blastula such that cells are given new positions and new neighbors, the outcome being the generation of the three germ layers (Figure 1): the ectoderm (which generates epidermis, NC and placodes, and the central nervous system) covers the surface of the embryo, the endoderm lines the primary gut, while mesoderm lies in between ectoderm and endoderm (Figure 1).

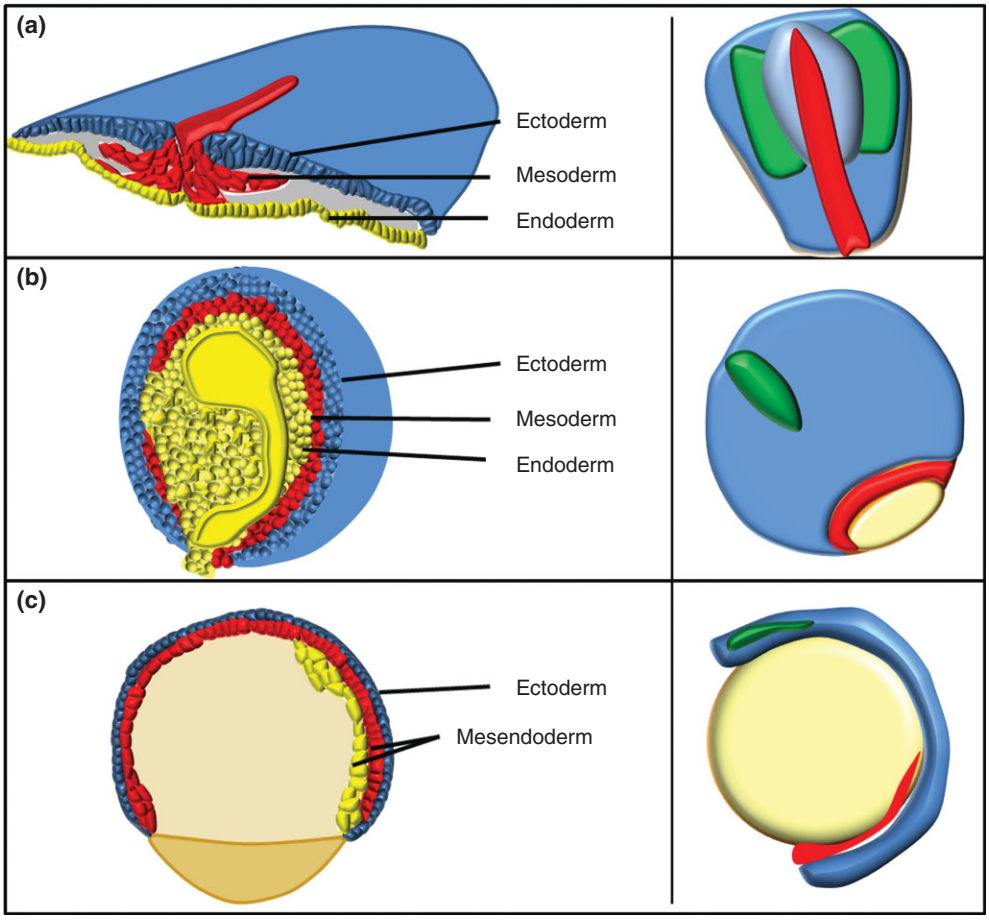

FIGURE 1 | The cellular movements during gastrulation create the necessary germ layers required for neural crest (NC) induction. NC cell gene expression begins in the ectoderm at the end of gastrulation. In chick (a), neural plate border (NPB) markers (green) are expressed surrounding the neural plate (light blue) and the primitive streak. As frog gastrulation begins (b), and during zebrafish epiboly (c), the mesoderm involutes underneath the developing epidermis. These cell movements are required to create the tissues that secrete important factors [bone morphogenetic protein (BMP), fibroblast growth factor (FGF), Wnt] required for presumptive NC cell induction (green). Diagrams are transverse section of gastrulating chick and sagittal view of gastrulating frog and zebrafish (left side) and whole embryos with anterior to the top/left and posterior to the bottom/right (right side). Blue is ectoderm, red is mesoderm, yellow is endoderm, and green is presumptive neural crest.

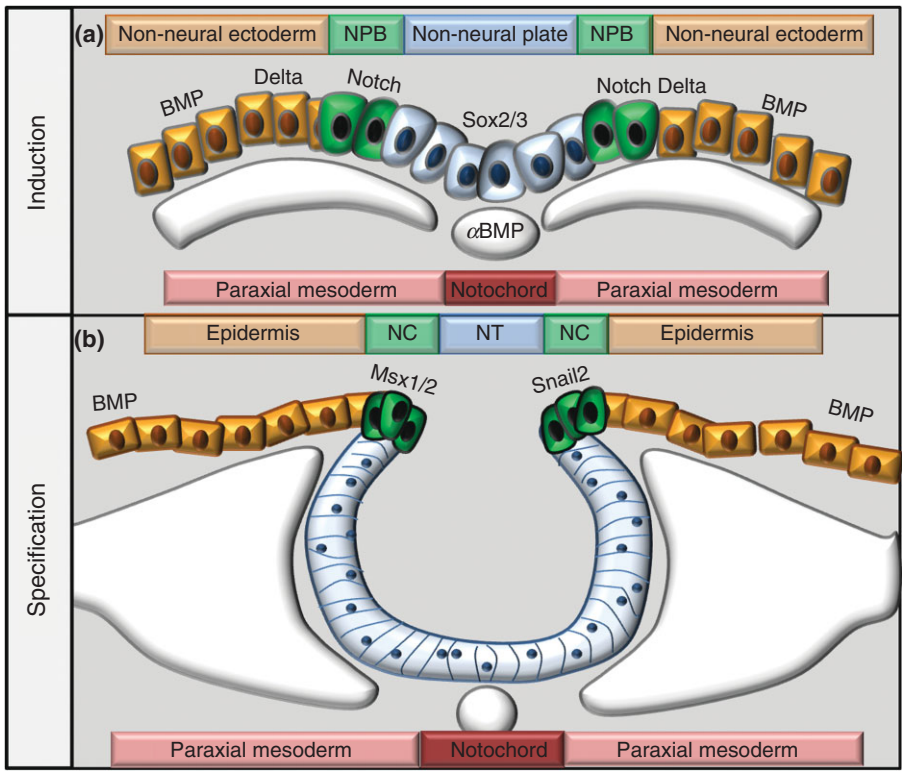

FIGURE 2 | Neurulation and neural crest induction. (a) Secreted factors from the surrounding tissues [bone morphogenetic protein (BMP), fibroblast growth factor (FGF), Wnt] pattern the presumptive neural crest region or NPB. (b) As the neural tube closes, neural crest specification is complete and they begin to express neural crest specifier genes such as *Foxd3*, *Slug*, and *Sox10*.

In addition to germ-layer generation, gastrulation also provides the major driving force behind axis elongation.

During neurulation, the NP, in the midline of the ectoderm, folds and forms a hollow NT (Figure 2(b)), with the notochord (a mesoderm-derived rod-shaped structure) underlying its ventral-most portion. The same signaling cues required to pattern the NT at different rostrocaudal axial levels are also likely to instruct distinct subpopulations of NC cells[10,11] to take on cranial, vagal, or truncal identities. These differ in their migratory pathways and also contribute to some distinct derivatives. For example, only cranial NC cells normally contribute to bone and cartilage of the facial skeleton. However, some derivatives are shared by all NC populations, including ability to form melanocytes, peripheral neurons, and glia.

Tissue Interactions and NC Induction

The NP border is not only flanked by the presumptive NP and non-neural ectoderm, but also overlays the paraxial mesoderm (Figure 2(a)). Because of their proximity to the prospective NC, each of these tissues has been proposed to act as an NC inducer. Recombination experiments in frog have established that interactions between the NP and non-neural ectoderm are involved in NC formation.[12] In chick and *Xenopus*, grafts of NP explants into the adjacent non-neural ectoderm induced expression of NC markers like *snail2* (formally *slug*) at the boundary, and lineage-tracing studies indicated that *snail2*-positive cells are derived from both the graft and host tissues.[13,14]

The paraxial mesoderm also may be involved in NC induction. In chick, paraxial mesoderm induced formation of melanocytes (a NC derivative) in NP explants,[14] and in *Xenopus*, removal of the presumptive paraxial mesoderm resulted in reduced *Snail2* expression.[15] Also, recombining frog ectodermal explants with paraxial mesoderm was sufficient to activate *snail2* expression and produce melanocytes.[15] Thus, paraxial mesoderm appears to produce some growth factors that can influence NC differentiation. However, paraxial mesoderm may not be critical for NC induction. For example, conditional deletions of either *Tbx6* or *Wnt-3a* in mice caused some paraxial mesoderm loss, yet NC derivatives are generated normally.[16]

At least four distinct signaling pathways have been implicated in patterning the NP border in

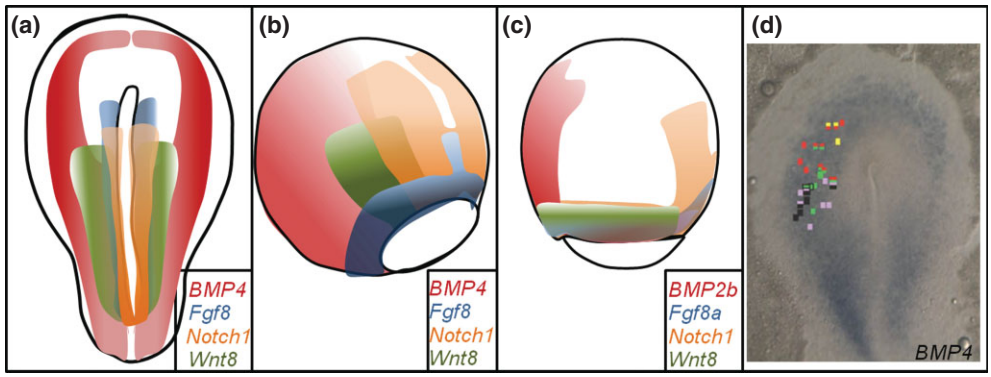

FIGURE 3 | Morphogen expression patterns. (a) In the chicken embryo, *BMP4* is expressed in the ectoderm and neural plate border (NPB), *FGF8,* and *Notch1* are expressed in the mesoderm, and *Wnt8* is expressed in the NPB and the mesoderm. (b) In the *Xenopus* embryo, *BMP4* is expressed in the developing epidermis and mesoderm and *FGF8, Notch1,* and *Wnt8* are expressed in the mesoderm. *Wnt8* is also expressed in the NPB and *Notch1* is expressed in the neural plate. (c) In Zebrafish embryos, *BMP2b* is expressed in the developing epidermis and mesoderm. *FGF8a, Wnt8,* and *Notch1* are expressed in the involuting mesoderm and *Notch1* is also expressed in the neural plate. (d) *In situ* hybridization of *BMP4* in a chick embryo showing that *BMP4* is highly expressed in the presumptive neural crest region at levels of different rostrocaudal fates (Reprinted with permission from Ref 2. Copyright 2009 Elsevier) The differences in expression of these morphogens may explain some of the differences in neural crest (NC) induction between organisms. Embryos are depicted as follows: (a,c) Anterior to the top, posterior to the bottom, dorsal up. (b) Anterior to the top, posterior to the bottom, and dorsal to right. All expression patterns (a–c) were found in Xenbase, ZFIN, and Geisha.

different species. These include bone morphogenetic protein (BMP) from the non-neural ectoderm and paraxial mesoderm, Wingless/Int (Wnt) from the non-neural ectoderm and paraxial mesoderm, fibroblast growth factor (FGF) from the paraxial mesoderm, and the Notch/Delta signaling pathway in the ectoderm (Figure 2(a)). These signaling pathways act in concert to establish competency for NC induction. Details of how these signals are established in the embryos and how downstream transcription factors are activated during NC induction are described below.

SIGNALING EVENTS IN NC INDUCTION

The cellular movements during gastrulation pattern embryonic germ layers, creating new tissue interactions which allow the formation of signaling centers such as the Spemann Organizer in frog, the node in chick or mouse, or the shield in zebrafish. Each of these signaling centers secretes extracellular signaling molecules required for axis specification and organogenesis. Induction and development of the NC require a specific level of signaling by the BMP, Wnt, FGF, retinoic acid, and Notch/Delta pathways. In this section, we describe the roles of each of these extracellular and intercellular signaling molecules in NC induction and specification.

BMP Signaling

BMP is one of the earliest expressed proteins required for NC specification and induction (Figure 3).[17] BMP is a secreted protein of the transforming growth factor-β family that signals through its downstream effectors (Smad proteins) to activate and repress transcription,[18] and is required for dorsal-ventral patterning of the early embryo (Figure 4).[17] *BMP2* and *BMP4* are expressed in the developing mesoderm and ectoderm prior to the onset of NC development in chick, frog, zebrafish, and mouse (Figure 3),[19–21] and in the dorsal NT coincident with premigratory NC cells,[22,23] while *BMP7* is expressed in the NPB of chick and the splanchnic mesoderm underlying the presumptive NC,[24] suggesting that BMP2, BMP4, and BMP7 may each play a role in NC formation in various species.

There are two prevailing models that describe the role of BMP signaling in NC induction. In the first model, moderate levels of BMP signaling are proposed to be required for NC induction. Prior to neural induction, BMP signaling is inhibited in the presumptive NP by BMP antagonists such as Noggin,[25] Chordin,[26] and Follistatin[27,28] that are secreted from the dorsal mesoderm. In addition to molecules required to inhibit BMP for neural induction, recent studies have shown that additional molecules, Tsukushi[29] and SNW1,[30] function extracellularly as antagonists to BMP in frog, chicken, and zebrafish embryos to pattern the embryo and specify NC cells. Experiments using *Xenopus* ectodermal explants[31] and zebrafish embryos[32] demonstrated that NC specification occurs in regions of intermediate BMP signaling levels such as the NPB (Figures 2(a) and 3(d)). However, 'intermediate levels of BMP' is a bit simpler than the

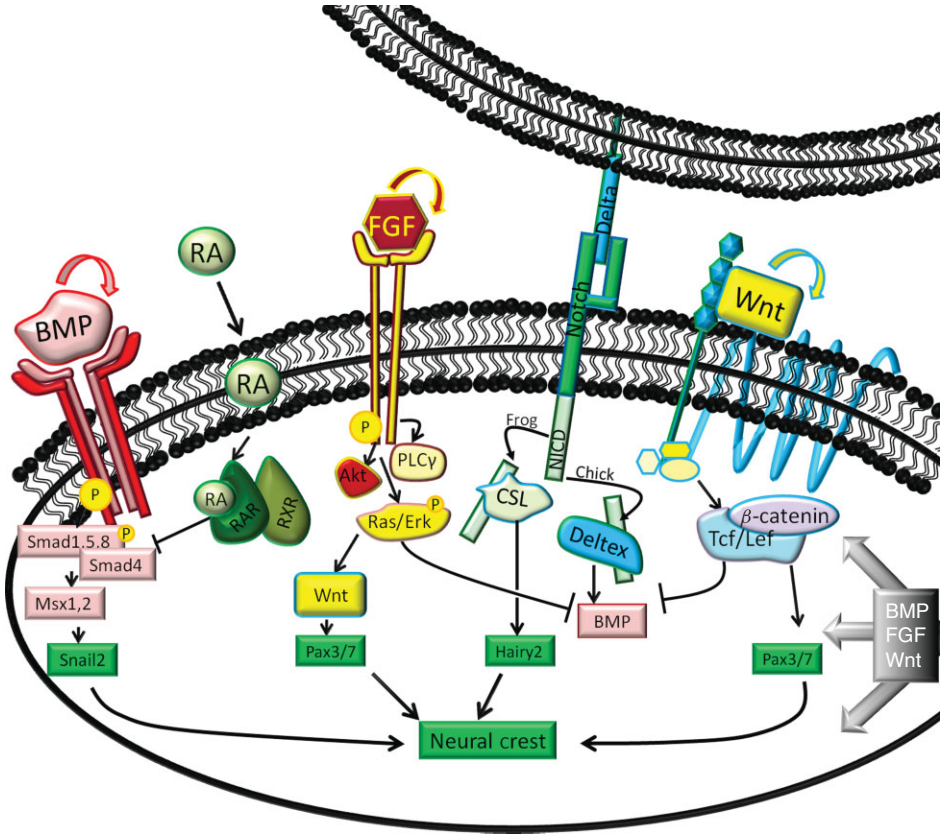

FIGURE 4 | Signaling pathways involved in neural crest induction. From left to right: Bone morphogenetic protein (BMP) activates Smad 1,5,8 proteins that interact with co-Smad to activate transcription of neural plate border (NPB) (*Msx1,2*) and neural crest (NC) specifier (*Snail2*) genes. Retinoic acid (RA) functions as a transcriptional regulator to posteriorize neural tissues, and inhibits BMP signaling and expression of FGF8 and Wnt8. Fibroblast growth factor (FGF) signals through one of its three downstream pathways (Akt, PLCγ, Ras/Erk) to activate expression of Wnt or to inhibit BMP expression indirectly regulating NC development. Notch/Delta interaction activates the Notch intracellular domain (NICD) which then binds to CSL transcription factors to activate expression of the NPB gene *Hairy2* (frog) or activates expression of BMP, indirectly inducing NC (chick). Wnt binds to the frizzled and LRP5/6 receptors which allows for the accumulation of β-catenin in the cell. β-catenin binds to the Wnt effector TCF/LEF to activate NPB gene *Pax3/7,* which activates neural crest genes. Gray arrows indicate the consistent requirement for these signaling pathways throughout neural crest development.

second model that hypothesizes that BMP signaling is required prior to NC induction in the NP border to make the ectodermal cells between the presumptive epidermal and neural tissue competent to respond to additional instructive signals from the Wnt[33] and FGF pathways,[6] which are also required for NC induction.

Past studies suggested that the role of BMP signaling in NC induction may have been overestimated and that BMP signaling was more important for maintenance and migration of NC in frog and mice[6,34] than NC induction. However, recent studies have supported a requirement for BMP signaling in NC induction in both species. Pax3-Cre-BMPR1a mice that lack BMPR1a in their dorsal NTs did not develop NC marked by *Cad6* or *Tcfap2a* in hindbrain or caudal regions and lacked *Sox10*-expressing cells in their trunks,[23] which contrasts with earlier evidence suggesting that loss of BMP signaling has little effect on the induction of NC. These differences may stem from the fact that the original Wnt1-Cre-driven knockdown was induced after NC induction, negating a necessity for BMP. Also, it is possible that knockout of BMP2 alone does not inhibit all BMP signaling pathways involved in NC induction.[34,35] Kwon et al., in zebrafish embryos, proposed that low to moderate levels of BMP signaling are required for specification of NC cells during gastrulation, while specification of the preplacodal region occurs later and requires complete attenuation of BMP signaling via FGF and PDGF signaling.[36] In addition, the requirement for BMP signaling in NC development may be dependent upon a novel regulator of BMP expression in *Xenopus* and zebrafish, SNW1.[30] Although the original model for NC induction in frog and fish proposed that intermediate levels of BMP signaling was sufficient to induce the presumptive NC,[37] the timing and

requirement for BMP signaling in chicken embryos are limited to a specific developmental stage,[38] and in all vertebrate models, FGF and Wnt signaling are also required. Currently, BMP signaling is thought to be necessary for NC induction, but not sufficient.

FGF Signaling

FGF and FGF receptors have been associated with many different aspects of embryonic development such as AP axis patterning and neural development. FGF is a secreted protein that signals through tyrosine kinase receptors (FGFRs). Upon ligand binding, FGFRs dimerize and activate downstream signal transduction pathways such as the Ras/ERK, Akt, or the protein kinase C pathways (Figure 4).[39] Many FGFs are expressed in the correct spatiotemporal patterns to be associated with induction and patterning of the NC. For example, *FGFR4* is expressed in the developing NP in frog,[40] chick[41] and zebrafish,[42] and *FGF8* is expressed in the developing mesoderm that underlies the presumptive NC in *Xenopus*[43] and chick (Figure 3).[44]

Recent evidence in *Xenopus* embryos supports a direct role for FGF signaling through FGFR4 in NC induction. FGFR4 activates Stat3 by phosphorylation allowing it to translocate to the nucleus and induce the expression of NC border genes and NC specifiers, while loss of the FGFR4 signaling-dependent Stat3 prevents NC induction.[45] In addition to a direct role for FGF signaling in frog, FGFs also play an indirect role in NC development. Over-expression of FGF8 or FGF2 concomitant with the inhibition of BMP signaling by Noggin was sufficient to induce NC marked by *snail2* expression[9,46] in *Xenopus,* and inhibition of FGF signaling through dominant-negative FGFR1 inhibited NC marker expression.[47] Over-expression of FGF8 in zebrafish and frog embryos inhibited *BMP2* and *BMP4* gene expression in gastrulae prior to the onset of NC development.[48,49] Furthermore, BMP signaling is inhibited through the phosphorylation and inactivation of its effector protein Smad1, thus allowing for neural and NC induction, signifying that FGF may participate indirectly in NC induction.[50,51] In *Xenopus* embryos, over-expression of FGF8a failed to induce NC cells marked by *snail2* or *sox8* when Wnt8 was blocked,[52] again suggesting an indirect role in NC induction. Taken together, these studies show that FGF signaling is required prior to NC induction, but none have identified a direct molecular connection between FGF and NC that does not occur via BMP or Wnt signaling. Therefore, the role of FGF signaling during NC induction may be indirect; since it functions to pattern *Hox* gene expression via *Cdx* genes,[53] posteriorize the NP,[54] induce paraxial mesoderm,[46,55] inhibit BMP expression and signaling,[49,50] and induce Wnt expression,[52] all of which are required for NC induction and specification.

Wnt Signaling

BMP signaling in the ectoderm, although necessary for NC induction, is not sufficient to induce NC[6] and additional instructive signals from either FGF or Wingless/Int (Wnt) signaling appear to be required. However, recent studies reveal that the role of FGF signaling in NC induction lies in its ability to induce *Wnt8* expression and to moderate BMP signaling and expression. The results suggest that, after moderate levels of BMP signaling creates competent ectoderm, Wnt is the instructive signal required to induce NC. *Wnt1*, *Wnt3*, *Wnt3a*, *Wnt4*, *Wnt8*, *Wnt8b*, and *Wnt10* are expressed in developing neural tissue (Figure 3) at stages that support a potential role for secreted Wnt proteins in NC induction (reviewed in Ref 56). The involvement of Wnt signaling in NC induction has been well documented in several species and it has reiterative roles later during various stages of NC development (reviewed in Ref 7,54,57).

Wnts are secreted ligands that bind extracellularly to receptors, like Frizzled and LRP5/6, to activate signaling either via 'canonical' or 'non-canonical' pathways. In the best-studied canonical pathway, the absence of Wnt leads to the degradation of the co-regulator, β-catenin, by the axin complex with GSK3β. When Wnt is present, Dishevelled is activated by the Wnt receptors, the axin/GSK3β complex is inhibited, and β-catenin translocates to the nucleus to function as a co-activator with the Wnt effector TCF/LEF (Figure 4).[58]

Although multiple Wnts are expressed in the correct spatiotemporal manner to be involved in NC induction, canonical signaling by Wnt8 and Wnt3a appears to be required for NC induction in frog, zebrafish, and lamprey.[52,59–61] Though the Wnt involved in early induction of NC in the chick gastrula is not yet known, later, Wnt6, which is expressed and secreted from the chick non-neural ectoderm, is thought to be important for maintenance of avian NC induction.[7,62] Furthermore, the canonical Wnt/β-catenin pathway is required for *Xenopus* NC induction, although EMT and migration may act through non-canonical signaling (reviewed in Ref 7). Experiments in *Xenopus* ectoderm have shown that Wnt1, Wnt3a, Wnt7b, and Wnt8 were sufficient to induce neural markers in neuralized ectodermal explants and Wnt8 was required for the induction of NC markers by FGF8a.[6,52,63,64] In addition, blocking Wnt signaling using Gsk3β or knocking down signaling by Wnt8, Wnt1, Wnt3, Frizzled3, or Frizzled7 led to a loss of

the NC markers *foxd3* or *snail2*.[52,63,65,66] Recently, downstream effectors of Wnt signaling required for NC induction such as Kermit, Skip, and Gbx2 were identified[66–68] and may help further elucidate the role of Wnt signaling in NC formation.

The cumulative data clearly show that Wnt signaling plays an important role in NC induction in vertebrates. However, more studies are required to determine which specific Wnt pathways are involved in each aspect of NC development. Differential requirements for various Wnts may be due to redundancy or developmental timing differences between organisms.

Retinoic Acid

Whereas FGF and Wnt signaling act in conjunction with moderate levels of BMP signaling to induce NC,[6,69] the role of Retinoic acid (RA) is less clear. RA is a morphogen derived from Vitamin A (retinol) that diffuses through cell membranes to the nucleus and binds to target genes to affect transcription directly (Figure 4).[70] RA is required in early development for AP patterning,[71,72] and RA is required to posteriorize the anterior neural fold (ANF) to allow for NC induction,[54] and is hypothesized to allow NC to form posterior to the ANF. The ANF does not form NC because of inhibition by Wnt antagonists such as Dkk-1.[73] *Xenopus* embryos treated with RA had anteriorly expanded *snail2* expression while loss of RA signaling by injection of a dominant-negative RA receptor led to posterior expansion, supporting a role in patterning of the presumptive NC domain.[54] In addition to the requirement for RA in frog, high doses of Vitamin A in developing mouse embryos caused cranial ganglia and NC abnormalities.[74] Besides functioning as a transcriptional regulator, a recent study in mouse P19 carcinoma cells showed that RA signaling regulated the duration of BMP signaling by reducing Smad1 phosphorylation, thereby causing Smad1 ubiquitinylation and subsequent degradation, which could indirectly affect NC induction and development.[75] Also, RA functions to pattern *Hox* gene expression supporting a role for it in embryonic patterning prior to NC induction.[53]

Notch/Delta Signaling

Notch proteins are membrane-bound extra- and inter/intracellular signaling molecules that confer lateral induction or lateral inhibition to affect cell fate. The intercellular portion of Notch proteins functions as a receptor for the Delta or Jagged/Serrate ligands. Upon ligand binding, the Notch intracellular domain (NICD) is cleaved, translocates to the nucleus, binds to a CSL transcription factor (e.g., Suppressor of Hairless in flies or CBF in vertebrates) and transcribes HES family proteins (Figure 4).[76] In general, cells with activated Notch are maintained in a proliferative state and blocked from differentiation.[77] Notch signaling plays differential roles in NC induction and specification in different species and a unified model for Notch function in this process remains unclear. However, some data suggest that Notch signaling may be involved in NC induction in chick and frog, though there is little evidence to support a similar role in zebrafish or mouse.[76]

In *Xenopus* embryos, both Notch and Delta are expressed in the mesoderm, the presumptive NT and NP border prior to NC induction (Figure 3).[78,79] *Xenopus* embryos over-expressing a constitutively active NICD exhibited a loss of *twist* expression and abnormal branchial arch development.[80] However, activation of Notch signaling following neural and mesodermal specification caused expansion of the NC as marked by *snail2* expression, and concomitant reduction in *BMP4* and *msx1*.[78] This expanded NC domain can be suppressed by over-expressing the BMP signaling target, Msx1. Also, knockdown of Notch signaling via a dominant-negative Su(H) caused a loss of *snail2*. In *Xenopus*, the timing of Notch signaling appears to be critical for the proper development of NC cells and appears to support different functions at different times. If Notch is up or downregulated at the wrong time, NC cells are lost. During NC induction in frogs, Notch lies upstream of *BMP4* expression, and NC induction relies on both Notch and BMP4 signaling (Figure 4).

In zebrafish embryos, recent evidence has shown that Prdm1a downstream of Notch signaling is required for cell fate specification of NPB cells and acts by promoting them toward a presumptive NC fate[81] at the expense of Rohon-Beard cell fate. However, the cranial but not the trunk NPB phenotype recovers by the 5-somite stage, suggesting a requirement for Notch during trunk NC development. In support of this, loss of Notch signaling via DeltaA,[82] Notch1a,[83] or Mib[84] does not affect cranial NC induction or development, but it does affect trunk NC derivatives. Knockout of the Notch processing protein Presenilin2 decreased melanocytes in the trunk, suggesting a role for Notch signaling in NC differentiation, but not induction.[85] In addition, regulatory studies in zebrafish showed that over-expression of Notch signaling induced ectopic *sox10* expression,[86] a gene required for normal NC development, and that Notch signaling lies upstream of Wnt signaling and is required for differentiation of NC derivatives, again supporting a later role of NC development in zebrafish.[87]

The results from mouse models regarding Notch signaling in NC are ambiguous. Mice over-expressing Notch in epidermal cells had an ectopic accumulation of *Snail2* in the epidermis, interpreted to indicate that Notch may act upstream of NC genes; however, there is no evidence that Notch signaling is required for NC induction,[88] though this may be due to redundancy in Notch signaling factors.[76] In addition, Wnt1-Cre-Rbpj knockout mice lacking Rbpj, which integrates signals from Notch receptors, and Wnt1-Cre-Notch knockout mice develop premature neurogenesis in both the dorsal root and the trigeminal ganglia marked by *NeuroD* expression, both of which have cells derived from the NC.[89,90] However, Nikopoulos et al. showed that the soluble form of Jagged, a ligand for the Notch receptor, is required along with FGF1 to attenuate Notch signaling in cultured rat neural crest stem cells (NCSC) to maintain a proliferative population.[91]

Overall, the data suggest that Notch signaling may be required to maintain a proliferative NC progenitor population and that Notch signaling may lie upstream of some NC specifier genes. However, there is little evidence to support a unique role for Notch in NC induction and specification. The requirement for Notch signaling in NC induction, specification, and differentiation appears to differ depending on stage and tissue type. Clearly, more work is required regarding role of Notch signaling in NC development.

Other Signaling Pathways

Although BMPs, Wnts, and FGFs seem to be the primary players in NC specification, other factors are probably also involved. For example, depleting the Endothelin-A receptor in *Xenopus* inhibited *snail* and *foxd3* expression while expanding the neural domain. This pathway likely acts with other traditional pathways (Wnts, FGFs) to control NC maintenance downstream of Msx.[92] Thus, future work is likely to undercover other influences on NC induction that may synergize and/or influence the known regulators.

THE PUTATIVE NC GRN

Inductive signals (BMP, FGF, and WNT) from surrounding tissues act on the NP border to activate a battery of transcription factors (NP border specifiers) (reviewed in Ref 4), which render this region capable of giving rise to NC and dorsal NT derivatives. The combinatorial expression of NP border specifiers with the above signals then activates another set of transcription factors (NC specifiers), which in turn regulates downstream effectors important for production of bona fide NC cells. This hierarchical activation of transcription factors endows premigratory NC cells in the dorsal NT with the ability to undergo EMT and become migratory. This section of the review will concentrate on formation of premigratory NC in the cranial region, since this has been best described. Studies from multiple organisms (frog, chick, zebrafish, mouse, and lamprey) have provided evidence for the existence of a putative pan-vertebrate cranial neural crest (CNC) GRN that describes the hierarchical interactions that exist between the NP border specifiers and NC specifiers (Figure 5). However, generating a comprehensive GRN that encompasses all NC is a challenge such that this serves as a model and a work in progress that will be constantly updated and improved.

NP Border Specifiers

In addition to the NC, the NP border region can give rise to roof plate cells, dorsal interneurons and sensory neurons like Rohon-Beard cells, and pre-placode ectoderm precursors depending on the temporal and spatial control of signals the border cells receive from the surrounding tissues.[95–104] Comprehensive work in *Xenopus* has demonstrated that the combinatory expression of homeobox transcription factors *ap2, zic1, hairy2, msx1/2, dlx5, pax3/7,* and *gbx2* during early neurulation defines the NPB territory and confers competence onto this region to form NC.[46,94,102,105–108] NP border specifiers are expressed broadly at the NP border, precede the expression of bona fide NC specifiers, and do not generally mark migrating NC.[109] Evidence suggests that AP2, Zic, Msx, Pax3/7, Dlx, and Gbx2 are effectors of BMP, Wnt, and FGF signaling and necessary for NC specifier expression. Furthermore, each factor alone is insufficient to carry out the whole NC specification program,[8,94,104–108,110,111] suggesting that they function in tandem and/or synergistically.

AP2a is an early marker of the NP border in *Xenopus* and in the basal vertebrate (lamprey).[94,112] Chick *AP2* is also localized to the NP border although its expression was not investigated earlier to determine if it precedes expression of other NP border genes.[109] AP2 has a unique dual role during NC development: first during NP border development and secondly during NC specification.[94,109,112] For example, *Xenopus* AP2a is both necessary and sufficient to establish the NPB specifiers (*Hairy2, Msx1*, and *Pax3*) and NC specifiers (*Snail2, Sox9, Sox10*) in neuralized ectodermal explants expressing *Zic1*. In addition, AP2a directly binds to the *Pax3* promoter. *Ap2a, Msx1,* and *Pax3* are immediate-early

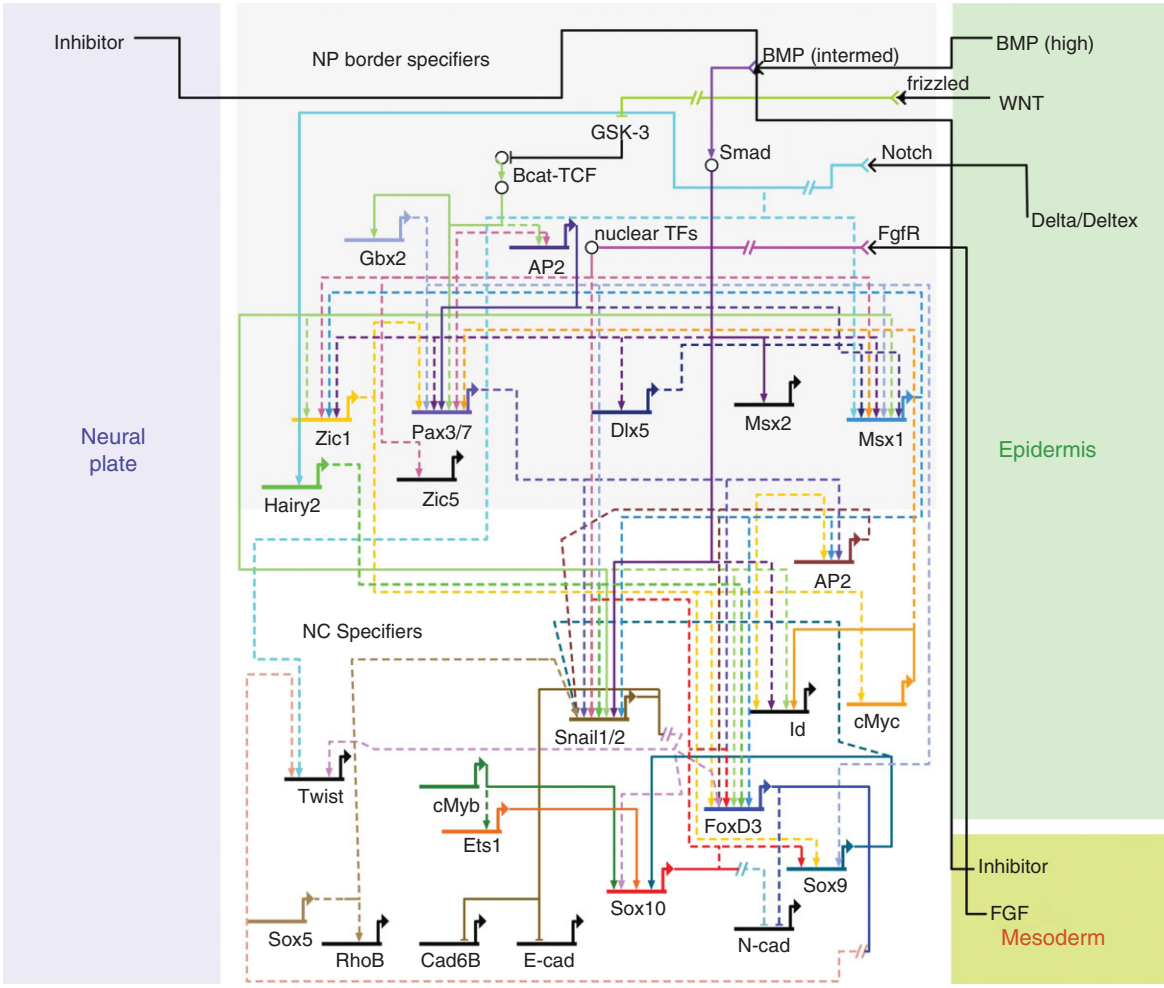

FIGURE 5 | The pan-vertebrate premigratory cranial neural crest gene regulatory network (CNC GRN) model. Generated using Bio Tapestry software[93] and compiled from gene perturbation studies from multiple species.[4] The GRN shows subnetworks of transcription factors during neural plate (NP) border specification (gray) and premigratory neural crest (NC) specification (white). AP2 is represented twice in the network based on recent evidence that it acts as both an NP border specifier and as an NC specifier.[94] Solid lines: direct regulatory interactions based on promoter and *cis*-regulatory analysis. Dashed lines: potential direct interactions. Broken lines: potential interactions. Bubble nodes: protein–protein interactions.

targets of Wnt/β-catenin signaling although TCF/LEF elements were not shown to be harbored within the promoters of these genes.[94]

Gbx2 is expressed at a similar stage as *AP2* during early gastrulation in the ectoderm.[94,105,113] There are many similarities between Gbx2 and AP2 function during NP border specification. (1) Both are targets of Wnt signaling. The *Gbx2* promoter has TCF/LEF regulatory elements and these elements are occupied by β-catenin during NP border specification. (2) Both are upstream of *Pax3* and *Msx1*. (3) Depletion of either AP2 or Gbx2 results in the loss of *Snail2*. (4) Both depend on *Zic1* (which is induced by attenuated BMP levels) to specify NC.[94,105] However unlike *AP2*, *Gbx2* can activate *Foxd3* robustly and appears to be necessary for suppressing border cells from adopting a pre-placode fate (as assayed by *Six1* expression).[105] *AP2* may play a complementary role to *Gbx2* in cooperation with *Zic1* to suppress a neural fate, since depletion of *AP2* leads to expansion of *Sox2*.[94] Thus, *Gbx2* and *Ap2* in combination with *Zic1* are sufficient to induce a majority of the NP border genes and initiate the NC program.

Msx expression is controlled by graded BMP signaling, and *cis*-regulatory analysis has demonstrated a BMP response element within the *Msx2* promoter.[8,114] Msx1 induction of *Snail2, Foxd3*, or *Twist1* requires Pax3, Wnt activity, and BMP antagonists.[8,106] Msx1 is also required for suppressing the NP marker *Sox2*, supporting the possibility that Msx1 acts downstream of AP2.[94,106]

Pax3 and Zic1 can synergistically induce *Snail2* in ventral ectoderm which is normally not competent to form NC. Thus, these two factors are sufficient to

induce ectopic NC.[106] However, distinct thresholds of Pax3 and Zic1 are required for NC formation. Too much Pax3 drives NP border cells to adopt a hatching gland fate and too much Zic1 turns NP border cells into pre-placode ectoderm.[104]

Little is known about the role of Dlx during border specification other than that it is required for positioning of the NPB.[110,115] Grafting of NP cells into non-neural ectoderm, which ectopically expressed a repressor form of *Dlx*, failed to induce the NC specifier or pre-placodal marker, *Snail2* and *Six1,* respectively.[110]

NC Specifiers

A second cohort of transcription factors, Snail2, SoxE, AP2, Twist, cMyc, Id, Foxd3, Ets1, and cMyb, are required to generate migratory NC cells and repress the NP marker *Sox2* (Figure 5). These factors either continue to be expressed in migratory crest (Sox10) and/or are activated at later times and in differentiating derivatives (Sox9, Sox10, Foxd3) (see Ref 4 for a comprehensive review covering the cross-regulatory interactions between NC specifiers). Based on the work in chick and lamprey, NC specifiers can be further subdivided into two distinct phases of NC specification, early and late groups.[112,116] One set of factors acts to maintain the multipotent capacity of these cells, suppressing premature differentiation and suppressing adoption of a neural fate. Another set of factors is required to initiate the EMT program such that these cells become migratory (Table 1).

Work in frog and mouse has shown that cMyc and Id3 are required to maintain NC progenitors and prevent premature differentiation.[119,120,123,124] Id3, a direct target of cMyc, controls the cell cycle to mediate the decision between proliferation and apoptosis, and help bias cells toward an NC lineage and away from NP fate.[123,124] Recently, a non-apoptotic role for p53 was reported in regulating a fine balance between NC progenitor cell maintenance and EMT process by regulating cell proliferation in the chick and mouse embryo.[129] An intriguing possibility is that Id3 may likely cooperate with p53.

Coordinated expression of *Foxd3*, *Snail2*, and *Sox9* during trunk crest induction, delamination, and migration are required in chick and mouse with each factor having a distinct role: *Sox9* expression makes NC responsive to EMT signals, Snail2 is required for the onset of EMT (by directly repressing the expression of *Cad6B*), and finally Foxd3 is required for cell adhesion.[130,131] A second wave of Wnt/β-catenin signaling is required for the induction of *Snail2* downstream of *Pax3* and *Zic1* activation.[106] In support of this, the TCF/LEF binding elements found in the *Snail2* promoter are occupied during NC specification.[105] Furthermore, cooperative interaction between Sox9 and Snail2 is required for *Snail2* maintenance during EMT.[132] Consistent with this, the *Snail2* homolog in zebrafish is upregulated when Sox9b is over-expressed and reduced in Sox9b mutants.[125]

Cis-regulatory analysis of *Sox10* in chick has shown that it is directly regulated by Ets1, cMyb, and Sox9.[118] Ets1 is exclusively expressed in cranial NC and excluded from trunk crest, and thus may influence activation of cranial specific effector genes. The lack of Ets1 in the trunk may account for differences observed in cranial versus trunk NC delamination.[121]

TABLE 1 | Function of NC Specifiers During Premigratory Neural Crest (NC) Development

NC Specifier	Function in Premigratory NC	Reference
AP2	Induction of *Sox9, Snail2*	94,117
cMyb	Induction of *Sox10*	118
cMyc	Cell-cycle control (progenitor maintenance)	119,120
Ets1	Cranial NC EMT	121
Foxd3	EMT	122
Id3	Cell-cycle control (progenitor maintenance)	123,124
Snail2	EMT	122
Sox9	Makes NC progenitors responsive to EMT, NC survival	122,125–127
Sox10	Progenitor maintenance, EMT	126,128

Added Complexity to the GRN

The NC GRN in its current state comprises a hierarchical network of transcription factors. The expression of some transcription factors and signals are reiterated during various stages of NC development and most are not unique to NC. Rather, it is their unique combination at appropriate developmental stages that helps define the NC. However, it would be naïve to assume that the entire process of NC formation is hard-wired. Other cellular processes, such as chromatin remodeling,[133,134] post-transcriptional/translational modification,[126,135,136] and other signaling pathways (apart from Wnt, Notch, BMP, and FGF),[92] act to modulate this network of transcription factors to define the 'transcriptional' state of the NC along its path to becoming a migratory cell. Thus, the future NC GRN model will have to reflect this added complexity (Figure 6).

Much of the data for the putative GRN come from gain and loss of function studies from *Xenopus*,

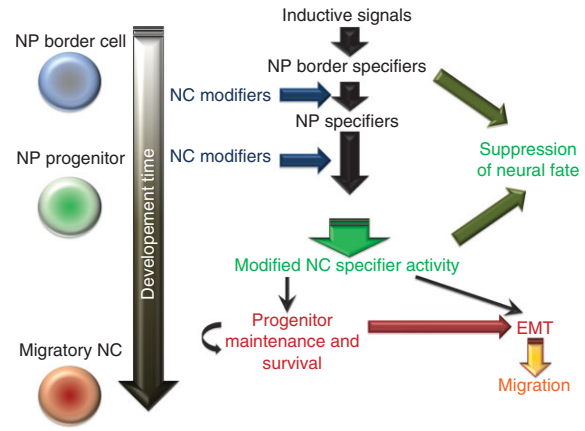

FIGURE 6 | The future premigratory neural crest gene regulatory network (NC GRN). The 'transcriptional state' of NC at any given time can be defined as the sum of interactions between transcription factors and modulation of their activity by neural crest (NC) modifiers (molecules involved in chromatin remodeling, post-transcriptional/translational modification, and other signaling pathways). NC modifiers that act upstream and downstream of NC specifiers (blue arrows) modify the 'transcriptional state' of the NC (green arrow), thereby affecting progenitor maintenance, survival, and EMT. By altering the 'transcriptional state', various NC subpopulations can be generated (e.g., trunk vs cranial NC).

lamprey and chick. However, collating all the data into one pan-vertebrate GRN is proving to be difficult since there are differences in the factors that are required for NC specification between different species. Another key issue is deciphering the temporal relationship between NP border specifiers. Due to inaccessibility or redundancy (mouse or zebrafish) and/or rapid induction of these markers (chick, frog, zebrafish), this has remained elusive. By focusing on *cis*-regulatory analysis of various key promoters, some of these issues can be resolved and these direct inputs can be tested across multiple species.

The current version of the NC network describes formation of the CNC (Figure 5),[4] and the inputs are likely to differ from those at other axial levels. For example, NC from various axial levels differ in their mechanisms of delamination and cell-cycle control as highlighted by the differential expression of Ets1 in CNC.[121] Furthermore, there may be species-specific differences in the NC GRN, as noted by variations in lamprey of two key transcription factors, Ets-1 and Twist, which act in the NC specifier module in higher vertebrates but only later, as effector genes, in the lamprey.[59] The combination of varying sub-GRN interactions between NC specifiers and the expression of varying modifiers of these factors can lead to the diversification of NC subtypes. A future challenge is to generate NC networks that reflect various axial levels of the body across multiple species.

Chromatin Remodeling

The expression of NP border specifiers alone is insufficient to drive cells down the NC lineage. To initiate the NC specifier program in response to extrinsic signals, the accessibility of these factors to various enhancers/promoters requires precise coordination that is mediated by chromatin remodelers. A burgeoning topic is the interplay between chromatin remodeling repressors and activators and how these factors regulate the successive restriction in NC fate within the dorsal NT.[133,137] For instance, the repressive histone mark H3K9me3 is found on the chick *Sox10* promoter during NP border specification but is removed during NC specification. This is mediated by the histone demethylase, JumonjiD2A (JmjD2A), and this places it between NP border specifiers and NC specifiers in the GRN. The *Sox9*, *Foxd3*, and *Snail2* promoters are also likely to harbor repressive marks and also be regulated by JmjD2A.[133]

Work using both *Xenopus* embryos and human NC line has implicated a role for the chromatin remodeling complex containing CHD7 and PBAF during NC specification but not during NP border specification. The CHD7–PBAF complex has been shown to target *Sox9*- and *Twist1*-associated enhancer regions and knockdown of these factors severely diminishes *Snail2*, *Sox9*, and *Twist1*.[134]

Post-Translational Modification

Protein modifications target transcription factors to various subcellular compartments and alter their function. This in turn affects the transcriptional readout of the cell in response to extrinsic signals. SUMOylation and ubiquitination are two common post-translational modifications that can alter the function of transcription factors. For example, the SUMOylation of *Xenopus* SoxE factors (Sox9/Sox10) promotes maintenance of the NC progenitor pool and modulates the ability of SoxE to carry out distinct patterning roles in NC versus otic-placode formation.[126]

Snail, a highly labile protein, is targeted for ubiquitination by GSK3β in the absence of active Wnt signaling.[138] The SNAG domain is important for Snail1 repressor activity and stability. Disruptions to Snail1 interactions with LSD1, a lysine-specific demethylase 1, that targets H3K4me2 via the SNAG domain likely also targets Snail for degradation by GSK3β.[139] In turn, GSK3β likely regulates levels of the NC specifier c-Myc.[138] In addition, in premigratory NC cells, Snail2, Twist, and Sip1 are post-translationally regulated by the E3 ubiquitin ligase, Partner of Paired during EMT.[140]

Phosphorylation of Sox9 by PKA (cAmp-dependent protein kinase A) has been found

to enhance Sox9 function, and treatment with cAMP/PKA inhibitor prevents Sox9-induced EMT in the quail embryos. The transcriptional activation of Snail2 on the Snail2 promoter is also enhanced by PKA signaling.[132]

Post-Transcriptional Regulation

Whilst an NC GRN results in the generation of a battery of transcripts required to form bona fide NC cells, an underlying network of post-transcriptional regulators [RNA-binding proteins (RBPs) and microRNAs] is likely to influence the stability and compartmentalization of these transcripts and thus cellular responses to extrinsic signals.[141] For example, conditional deletion of *Dicer1* (an enzyme important in microRNA biosynthesis) in NC progenitors resulted in lack of cranial skeletal structures and trunk NC-derived sensory neurons by modulating the Wnt signaling pathway.[136,142] However, although microRNAs are correlated with EMT and the differentiation of NC cell derivatives, there is little known about their role in NC induction or specification. Therefore, post-transcriptional regulation also has an important role in survival, maintenance, and migration of NC progenitors, though the mechanistic details are still unknown. It will be interesting to determine if there is concerted regulation of functionally related mRNA (for instance, those coding for cell adhesion protein versus those coding for transcription factors) by multiple RBPs as has been shown in yeast.[141]

THE BIG PICTURE

NC induction and specification is a complicated process that differs between organisms either based on stage and rapidity of development, gene redundancy or differential gene paralog usage between species. With respect to signals, BMPs are required prior to gastrulation to make ectodermal cells competent to receive instructive signals from the Wnt, Notch (in frog and chick), and RA and FGF pathways (Figures 3 and 4). In addition, there is crosstalk between different pathways to modulate the transcriptional output during NC generation (Figure 4). Wnt, FGF, and RA pathways are required to posteriorize neural tissue to allow for induction, but are also required for instructive signaling to pattern BMP-induced competent cells.[54]

These signals act upstream as well as cooperatively with NPB genes to initiate the NC program (Figure 5). These NPB factors in turn induce NC specifier genes that initiate EMT, turn on cell migration genes, and act upstream of differentiation batteries whereby NC cells choose one of many possible fates. The timing of these events differs between axial levels and organisms, and may be controlled at least in part by epigenetic modifiers of these transcriptional events. The rich and ever-growing NC literature will expand and refine our knowledge of the critical gene regulatory interactions that lead to generation of this fascinating cell type that is a defining feature of the vertebrate embryo (Figure 6).

Current knowledge about NC induction and specification focuses on extracellular signaling, transcription factors, and the GRNs that they regulate. Going forward, it will be important to investigate the state of the methylome during NC specification and to identify novel chromatin remodeling factors involved in regulating the NC GRN. Because transcriptional regulation cannot account for all aspects of NC induction and specification in its complexity, it is important to uncover the molecular mechanisms involved in post-transcriptional and post-translational regulation of mRNAs and proteins. Finally, there is little information about signaling through ion channels and their possible role in regulating NC specification, except for a few studies on the involvement of ions in NC apoptosis[143] and the development of NC-derived cancers.[143,144] Ultimately, although we know many factors involved in NC development, it remains a great challenge to assemble these at a 'systems' level and understand their organization and how one set of factors influences another. As a consequence, this is a topic ripe for discovery and a time during which it will be necessary to synthesize large amounts of information into a clear picture of how NC cells form, migrate, and differentiate.

REFERENCES

1. Basch ML, Bronner-Fraser M, Garcia-Castro MI. Specification of the neural crest occurs during gastrulation and requires Pax7. *Nature* 2006, 441:218–222.

2. Ezin AM, Fraser SE, Bronner-Fraser M. Fate map and morphogenesis of presumptive neural crest and dorsal neural tube. *Dev Biol* 2009, 330:221–236.

3. Knecht AK, Bronner-Fraser M. Induction of the neural crest: a multigene process. *Nat Rev Genet* 2002, 3:453–461.

4. Betancur P, Bronner-Fraser M, Sauka-Spengler T. Assembling neural crest regulatory circuits into a gene regulatory network. *Annu Rev Cell Dev Biol* 2010, 26:581–603.

5. Sauka-Spengler T, Bronner-Fraser M. Insights from a sea lamprey into the evolution of neural crest gene regulatory network. *Biol Bull* 2008, 214:303–314.
6. LaBonne C, Bronner-Fraser M. Neural crest induction in Xenopus: evidence for a two-signal model. *Development* 1998, 125:2403–2414.
7. Sauka-Spengler T, Bronner-Fraser M. A gene regulatory network orchestrates neural crest formation. *Nat Rev Mol Cell Biol* 2008, 9:557–568.
8. Tribulo C, Aybar MJ, Nguyen VH, Mullins MC, Mayor R. Regulation of Msx genes by a Bmp gradient is essential for neural crest specification. *Development* 2003, 130:6441–6452.
9. Mayor R, Morgan R, Sargent MG. Induction of the prospective neural crest of Xenopus. *Development* 1995, 121:767–777.
10. Basch ML, Garcia-Castro MI, Bronner-Fraser M. Molecular mechanisms of neural crest induction. *Birth Defects Res C Embryo Today* 2004, 72:109–123.
11. Fraser SE. Pattern formation in the vertebrate nervous system. *Curr Opin Genet Dev* 1991, 1:217–220.
12. Moury JD, Jacobson AG. Neural fold formation at newly created boundaries between neural plate and epidermis in the axolotl. *Dev Biol* 1989, 133:44–57.
13. Mancilla A, Mayor R. Neural crest formation in Xenopus laevis: mechanisms of Xslug induction. *Dev Biol* 1996, 177:580–589.
14. Selleck MA, Bronner-Fraser M. Origins of the avian neural crest: the role of neural plate-epidermal interactions. *Development* 1995, 121:525–538.
15. Bonstein L, Elias S, Frank D. Paraxial-fated mesoderm is required for neural crest induction in Xenopus embryos. *Dev Biol* 1998, 193:156–168.
16. Yamaguchi TP, Takada S, Yoshikawa Y, Wu N, McMahon AP. T (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev* 1999, 13:3185–3190.
17. Fainsod A, Steinbeisser H, De Robertis EM. On the function of BMP-4 in patterning the marginal zone of the Xenopus embryo. *EMBO J* 1994, 13:5015–5025.
18. von Bubnoff A, Cho KW. Intracellular BMP signaling regulation in vertebrates: pathway or network? *Dev Biol* 2001, 239:1–14.
19. Martinez-Barbera JP, Toresson H, Da Rocha S, Krauss S. Cloning and expression of three members of the zebrafish Bmp family: Bmp2a, Bmp2b and Bmp4. *Gene* 1997, 198:53–59.
20. Knochel S, Dillinger K, Koster M, Knochel W. Structure and expression of Xenopus tropicalis BMP-2 and BMP-4 genes. *Mech Dev* 2001, 109:79–82.
21. Streit A, Stern CD. 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.
22. Monsoro-Burq AH, Duprez D, Watanabe Y, Bontoux M, Vincent C, Brickell P, Le Douarin N. The role of bone morphogenetic proteins in vertebral development. *Development* 1996, 122:3607–3616.
23. Stottmann RW, Klingensmith J. Bone morphogenetic protein signaling is required in the dorsal neural folds before neurulation for the induction of spinal neural crest cells and dorsal neurons. *Dev Dyn* 2011, 240:755–765.
24. Streit A, Lee KJ, Woo I, Roberts C, Jessell TM, Stern CD. Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. *Development* 1998, 125:507–519.
25. Zimmerman LB, De Jesus-Escobar JM, Harland RM. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* 1996, 86:599–606.
26. Piccolo S, Sasai Y, Lu B, De Robertis EM. Dorsoventral patterning in Xenopus: inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* 1996, 86:589–598.
27. Fainsod A, Deissler K, Yelin R, Marom K, Epstein M, Pillemer G, Steinbeisser H, Blum M. The dorsalizing and neural inducing gene follistatin is an antagonist of BMP-4. *Mech Dev* 1997, 63:39–50.
28. Levin M. The roles of activin and follistatin signaling in chick gastrulation. *Int J Dev Biol* 1998, 42:553–559.
29. Kuriyama S, Lupo G, Ohta K, Ohnuma S, Harris WA, Tanaka H. Tsukushi controls ectodermal patterning and neural crest specification in Xenopus by direct regulation of BMP4 and X-delta-1 activity. *Development* 2006, 133:75–88.
30. Wu MY, Ramel MC, Howell M, Hill CS. SNW1 is a critical regulator of spatial BMP activity, neural plate border formation, and neural crest specification in vertebrate embryos. *PLoS Biol* 2011, 9:e1000593.
31. 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.
32. Barth KA, Kishimoto Y, Rohr KB, Seydler C, Schulte-Merker S, Wilson SW. Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* 1999, 126:4977–4987.
33. Bastidas F, De Calisto J, Mayor R. Identification of neural crest competence territory: role of Wnt signaling. *Dev Dyn* 2004, 229:109–117.
34. Correia AC, Costa M, Moraes F, Bom J, Novoa A, Mallo M. Bmp2 is required for migration but not for induction of neural crest cells in the mouse. *Dev Dyn* 2007, 236:2493–2501.
35. Stottmann RW, Choi M, Mishina Y, Meyers EN, Klingensmith J. BMP receptor IA is required in mammalian neural crest cells for development of the cardiac outflow tract and ventricular myocardium. *Development* 2004, 131:2205–2218.

36. Kwon HJ, Bhat N, Sweet EM, Cornell RA, Riley BB. Identification of early requirements for preplacodal ectoderm and sensory organ development. *PLoS Genet* 2010, 6:e1001133.
37. Baker CV, Bronner-Fraser M. The origins of the neural crest. Part I: embryonic induction. *Mech Dev* 1997, 69:3–11.
38. Selleck MA, Garcia-Castro MI, Artinger KB, 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.
39. Dorey K, Amaya E. FGF signalling: diverse roles during early vertebrate embryogenesis. *Development* 2010, 137:3731–3742.
40. Golub R, Adelman Z, Clementi J, Weiss R, Bonasera J, Servetnick M. Evolutionarily conserved and divergent expression of members of the FGF receptor family among vertebrate embryos, as revealed by FGFR expression patterns in Xenopus. *Dev Genes Evol* 2000, 210:345–357.
41. Wright TJ, Ladher R, McWhirter J, Murre C, Schoenwolf GC, Mansour SL. Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev Biol* 2004, 269:264–275.
42. Ota S, Tonou-Fujimori N, Nakayama Y, Ito Y, Kawamura A, Yamasu K. FGF receptor gene expression and its regulation by FGF signaling during early zebrafish development. *Genesis* 2010, 48:707–716.
43. Christen B, Slack JM. FGF-8 is associated with anteroposterior patterning and limb regeneration in Xenopus. *Dev Biol* 1997, 192:455–466.
44. Chapman SC, Schubert FR, Schoenwolf GC, Lumsden A. Analysis of spatial and temporal gene expression patterns in blastula and gastrula stage chick embryos. *Dev Biol* 2002, 245:187–199.
45. Nichane M, Ren X, Bellefroid EJ. Self-regulation of Stat3 activity coordinates cell-cycle progression and neural crest specification. *EMBO J* 2010, 29:55–67.
46. Monsoro-Burq A-Hln, Fletcher RB, Harland RM. Neural crest induction by paraxial mesoderm in Xenopus embryos requires FGF signals. *Development* 2003, 130:3111–3124.
47. Mayor R, Guerrero N, Martinez C. Role of FGF and noggin in neural crest induction. *Dev Biol* 1997, 189:1–12.
48. Furthauer M, Thisse C, Thisse B. A role for FGF-8 in the dorsoventral patterning of the zebrafish gastrula. *Development* 1997, 124:4253–4264.
49. Wilson SI, Graziano E, Harland R, Jessell TM, Edlund T. An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. *Curr Biol* 2000, 10:421–429.
50. Pera EM, Ikeda A, Eivers E, De Robertis EM. Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. *Genes Dev* 2003, 17:3023–3028.
51. Schier AF. Axis formation and patterning in zebrafish. *Curr Opin Genet Dev* 2001, 11:393–404.
52. Hong CS, Park BY, Saint-Jeannet JP. Fgf8a induces neural crest indirectly through the activation of Wnt8 in the paraxial mesoderm. *Development* 2008, 135:3903–3910.
53. Bel-Vialar S, Itasaki N, Krumlauf R. Initiating Hox gene expression: in the early chick neural tube differential sensitivity to FGF and RA signaling subdivides the HoxB genes in two distinct groups. *Development* 2002, 129:5103–5115.
54. Villanueva S, Glavic A, Ruiz P, Mayor R. Posteriorization by FGF, Wnt, and retinoic acid is required for neural crest induction. *Dev Biol* 2002, 241:289–301.
55. Kimelman D, Kirschner M. Synergistic induction of mesoderm by FGF and TGF-beta and the identification of an mRNA coding for FGF in the early Xenopus embryo. *Cell* 1987, 51:869–877.
56. Elkouby YM, Frank D. Wnt/β-catenin signaling in vertebrate posterior neural development. 2010. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21452442.
57. LaBonne C, Bronner-Fraser M. Molecular mechanisms of neural crest formation. *Annu Rev Cell Dev Biol* 1999, 15:81–112.
58. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell* 2009, 17:9–26.
59. Sauka-Spengler T, Meulemans D, Jones M, Bronner-Fraser M. Ancient evolutionary origin of the neural crest gene regulatory network. *Dev Cell* 2007, 13:405–420.
60. Lewis JL, Bonner J, Modrell M, Ragland JW, Moon RT, Dorsky RI, Raible DW. Reiterated Wnt signaling during zebrafish neural crest development. *Development* 2004, 131:1299–1308.
61. Dutta S, Dawid IB. Kctd15 inhibits neural crest formation by attenuating Wnt/beta-catenin signaling output. *Development* 2010, 137:3013–3018.
62. Garcia-Castro MI, Marcelle C, Bronner-Fraser M. Ectodermal Wnt function as a neural crest inducer. *Science* 2002, 297:848–851.
63. Saint-Jeannet JP, He X, Varmus HE, Dawid IB. Regulation of dorsal fate in the neuraxis by Wnt-1 and Wnt-3a. *Proc Natl Acad Sci U S A* 1997, 94:13713–13718.
64. Chang C, Hemmati-Brivanlou A. Neural crest induction by Xwnt7B in Xenopus. *Dev Biol* 1998, 194:129–134.
65. Abu-Elmagd M, Garcia-Morales C, Wheeler GN. Frizzled7 mediates canonical Wnt signaling in neural crest induction. *Dev Biol* 2006, 298:285–298.

66. Tan C, Deardorff MA, Saint-Jeannet JP, Yang J, Arzoumanian A, Klein PS. Kermit, a frizzled interacting protein, regulates frizzled 3 signaling in neural crest development. *Development* 2001, 128:3665–3674.

67. Li B, Kuriyama S, Moreno M, Mayor R. The posteriorizing gene Gbx2 is a direct target of Wnt signalling and the earliest factor in neural crest induction. *Development* 2009, 136:3267–3278.

68. Wang Y, Fu Y, Gao L, Zhu G, Liang J, Gao C, Huang B, Fenger U, Niehrs C, Chen YG, et al. Xenopus skip modulates Wnt/beta-catenin signaling and functions in neural crest induction. *J Biol Chem* 2010, 285:10890–10901.

69. Monsoro-Burq AH, Wang E, Harland R. Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during Xenopus neural crest induction. *Dev Cell* 2005, 8:167–178.

70. Duester G. Retinoic acid synthesis and signaling during early organogenesis. *Cell* 2008, 134:921–931.

71. Sive HL, Draper BW, Harland RM, Weintraub H. Identification of a retinoic acid-sensitive period during primary axis formation in Xenopus laevis. *Genes Dev* 1990, 4:932–942.

72. Reijntjes S, Rodaway A, Maden M. The retinoic acid metabolising gene, CYP26B1, patterns the cartilaginous cranial neural crest in zebrafish. *Int J Dev Biol* 2007, 51:351–360.

73. Carmona-Fontaine C, Acuna G, Ellwanger K, Niehrs C, Mayor R. Neural crests are actively precluded from the anterior neural fold by a novel inhibitory mechanism dependent on Dickkopf1 secreted by the prechordal mesoderm. *Dev Biol* 2007, 309:208–221.

74. Mulder GB, Manley N, Grant J, Schmidt K, Zeng W, Eckhoff C, Maggio-Price L. Effects of excess vitamin A on development of cranial neural crest-derived structures: a neonatal and embryologic study. *Teratology* 2000, 62:214–226.

75. Sheng N, Xie Z, Wang C, Bai G, Zhang K, Zhu Q, Song J, Guillemot F, Chen YG, Lin A, et al. Retinoic acid regulates bone morphogenic protein signal duration by promoting the degradation of phosphorylated Smad1. *Proc Natl Acad Sci U S A* 2010, 107:18886–18891.

76. Cornell RA, Eisen JS. Notch in the pathway: the roles of Notch signaling in neural crest development. *Semin Cell Dev Biol* 2005, 16:663–672.

77. Riley BB, Phillips BT. Ringing in the new ear: resolution of cell interactions in otic development. *Dev Biol* 2003, 261:289–312.

78. Glavic A, Silva F, Aybar MJ, Bastidas F, Mayor R. Interplay between Notch signaling and the homeoprotein Xiro1 is required for neural crest induction in Xenopus embryos. *Development* 2004, 131:347–359.

79. Lopez SL, Paganelli AR, Siri MV, Ocana OH, Franco PG, Carrasco AE. Notch activates sonic hedgehog and both are involved in the specification of dorsal midline cell-fates in Xenopus. *Development* 2003, 130:2225–2238.

80. Martinez Arias A, Zecchini V, Brennan K. CSL-independent Notch signalling: a checkpoint in cell fate decisions during development? *Curr Opin Genet Dev* 2002, 12:524–533.

81. Hernandez-Lagunas LPD, Law J, Grant KA, Artinger KB. prdm1a and olig4 act downstream of Notch signaling to regulate cell fate at the neural plate border. *Dev Biol* 2011, 356:496–505.

82. Cornell RA, Eisen JS. Delta signaling mediates segregation of neural crest and spinal sensory neurons from zebrafish lateral neural plate. *Development* 2000, 127:2873–2882.

83. Gray M, Moens CB, Amacher SL, Eisen JS, Beattie CE. Zebrafish deadly seven functions in neurogenesis. *Dev Biol* 2001, 237:306–323.

84. Cornell RA, Eisen JS. Delta/Notch signaling promotes formation of zebrafish neural crest by repressing Neurogenin 1 function. *Development* 2002, 129:2639–2648.

85. Nornes S, Newman M, Wells S, Verdile G, Martins RN, Lardelli M. Independent and cooperative action of Psen2 with Psen1 in zebrafish embryos. *Exp Cell Res* 2009, 315:2791–2801.

86. Dutton JR, Antonellis A, Carney TJ, Rodrigues FS, Pavan WJ, Ward A, Kelsh RN. An evolutionarily conserved intronic region controls the spatiotemporal expression of the transcription factor Sox10. *BMC Dev Biol* 2008, 8:105.

87. Ishitani T, Matsumoto K, Chitnis AB, Itoh M. Nrarp functions to modulate neural-crest-cell differentiation by regulating LEF1 protein stability. *Nat Cell Biol* 2005, 7:1106–1112.

88. Ambler CA, Watt FM. Adult epidermal Notch activity induces dermal accumulation of T cells and neural crest derivatives through upregulation of jagged 1. *Development* 2010, 137:3569–3579.

89. Hu ZL, Shi M, Huang Y, Zheng MH, Pei Z, Chen JY, Han H, Ding YQ. The role of the transcription factor Rbpj in the development of dorsal root ganglia. *Neural Dev* 2011, 6:14.

90. de la Pompa JL, Wakeham A, Correia KM, Samper E, Brown S, Aguilera RJ, Nakano T, Honjo T, Mak TW, Rossant J, et al. Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development* 1997, 124:1139–1148.

91. Nikopoulos GN, Duarte M, Kubu CJ, Bellum S, Friesel R, Maciag T, Prudovsky I, Verdi JM. Soluble Jagged1 attenuates lateral inhibition, allowing for the clonal expansion of neural crest stem cells. *Stem Cells* 2007, 25:3133–3142.

92. Bonano M, Trìbulo C, De Calisto J, Marchant L, Sanchez SS, Mayor R, Aybar MJ. A new role for

the Endothelin-1/Endothelin-A receptor signaling during early neural crest specification. *Dev Biol* 2008, 323:114–129.

93. Longabaugh WJ, Davidson EH, Bolouri H. Visualization, documentation, analysis, and communication of large-scale gene regulatory networks. *Biochim Biophys Acta* 2009, 1789:363–374.
94. de Croze N, Maczkowiak F, Monsoro-Burq AH. Reiterative AP2a activity controls sequential steps in the neural crest gene regulatory network. *Proc Natl Acad Sci U S A* 2011, 108:155–160.
95. Aruga J, Tohmonda T, Homma S, Mikoshiba K. Zic1 promotes the expansion of dorsal neural progenitors in spinal cord by inhibiting neuronal differentiation. *Dev Biol* 2002, 244:329–341.
96. Bang AG, Papalopulu N, Goulding MD, Kintner C. Expression of Pax-3 in the lateral neural plate is dependent on a Wnt-mediated signal from posterior nonaxial mesoderm. *Dev Biol* 1999, 212:366–380.
97. Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J* 1991, 10:1135–1147.
98. Liu Y, Helms AW, Johnson JE. Distinct activities of Msx1 and Msx3 in dorsal neural tube development. *Development* 2004, 131:1017–1028.
99. Mansouri A, Gruss P. Pax3 and Pax7 are expressed in commissural neurons and restrict ventral neuronal identity in the spinal cord. *MechDev* 1998, 78:171–178.
100. McLarren KW, Litsiou A, Streit A. DLX5 positions the neural crest and preplacode region at the border of the neural plate. *Dev Biol* 2003, 259:34–47.
101. Tremblay P, Pituello F, Gruss P. Inhibition of floor plate differentiation by Pax3: evidence from ectopic expression in transgenic mice. *Development* 1996, 122:2555–2567.
102. Woda JM, Pastagia J, Mercola M, Artinger KB. Dlx proteins position the neural plate border and determine adjacent cell fates. *Development* 2003, 130:331–342.
103. 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.
104. 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.
105. Li B, Kuriyama S, Moreno M, Mayor R. The posteriorizing gene Gbx2 is a direct target of Wnt signalling and the earliest factor in neural crest induction. *Development* 2009, 136:3267–3278.
106. Monsoro-Burq A-H, Wang E, Harland R. Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during xenopus neural crest induction. *Dev Cell* 2005, 8:167–178.
107. Sato T, Sasai N, Sasai Y. Neural crest determination by co-activation of Pax3 and Zic1 genes in Xenopus ectoderm. *Development* 2005, 132:2355–2363.
108. Glavic A, Silva F, Aybar MJ, Bastidas F, Mayor R. Interplay between Notch signaling and the homeoprotein Xiro1 is required for neural crest induction in Xenopus embryos. *Development* 2004, 131:347–359.
109. Khudyakov J, Bronner-Fraser M. Comprehensive spatiotemporal analysis of early chick neural crest network genes. *Dev Dyn* 2009, 238:716–723.
110. Feledy JA, Beanan MJ, Sandoval JJ, Goodrich JS, Lim JH, Matsuo-Takasaki M, Sato SM, Sargent TD. Inhibitory patterning of the anterior neural plate in Xenopus by homeodomain factors Dlx3 and Msx1. *Dev Biol* 1999, 212:455–464.
111. Luo T, Matsuo-Takasaki M, Lim JH, Sargent TD. Differential regulation of Dlx gene expression by a BMP morphogenetic gradient. *Int J Dev Biol* 2001, 45:681–684.
112. Nikitina N, Sauka-Spengler T, Bronner-Fraser M. Dissecting early regulatory relationships in the lamprey neural crest gene network. *Proc Natl Acad Sci* 2008, 105:20083–20088.
113. von Bubnoff A, Schmidt JE, Kimelman D. The Xenopus laevis homeobox gene Xgbx-2 is an early marker of anteroposterior patterning in the ectoderm. *Mech Dev* 1996, 54:149–160.
114. Brugger SM, Merrill AE, Torres-Vazquez J, Wu N, Ting M-C, Cho JYM, Dobias SL, Yi SE, Lyons K, Bell JR, et al. A phylogenetically conserved cis-regulatory module in the Msx2 promoter is sufficient for BMP-dependent transcription in murine and Drosophila embryos. *Development* 2004, 131:5153–5165.
115. Phillips BT, Kwon H-J, Melton C, Houghtaling P, Fritz A, Riley BB. Zebrafish msxB, msxC and msxE function together to refine the neural-nonneural border and regulate cranial placodes and neural crest development. *Dev Biol* 2006, 294:376–390.
116. Sauka-Spengler T, Meulemans D, Jones M, Bronner-Fraser M. Ancient evolutionary origin of the neural crest gene regulatory network. *Dev Cell* 2007, 13:405–420.
117. Luo T, Lee YH, Saint-Jeannet JP, Sargent TD. Induction of neural crest in Xenopus by transcription factor AP2alpha. *Proc Natl Acad Sci U S A* 2003, 100:532–537.
118. Betancur P, Bronner-Fraser M, Sauka-Spengler T. Genomic code for Sox10 activation reveals a key regulatory enhancer for cranial neural crest. *Proc Natl Acad Sci U S A* 2010, 107:3570–3575.
119. Wei K, Chen J, Akrami K, Galbraith GC, Lopez IA, Chen F. Neural crest cell deficiency of c-myc causes skull and hearing defects. *Genesis* 2007, 45:382–390.
120. Bellmeyer A, Krase J, Lindgren J, LaBonne C. The protooncogene c-myc is an essential regulator of

neural crest formation in xenopus. *Dev Cell* 2003, 4:827–839.

121. Theveneau E, Duband JL, Altabef M. Ets-1 confers cranial features on neural crest delamination. *PLoS One* 2007, 2:e1142.
122. Cheung M, Chaboissier M-C, Mynett A, Hirst E, Schedl A, Briscoe J. The transcriptional control of trunk neural crest induction, survival, and delamination. *Dev Cell* 2005, 8:179–192.
123. Kee Y, Bronner-Fraser M. To proliferate or to die: role of Id3 in cell cycle progression and survival of neural crest progenitors. *Genes Dev* 2005, 19:744–755.
124. Light W, Vernon AE, Lasorella A, Iavarone A, LaBonne C. Xenopus Id3 is required downstream of Myc for the formation of multipotent neural crest progenitor cells. *Development* 2005, 132:1831–1841.
125. Yan Y-L, Willoughby J, Liu D, Crump JG, Wilson C, Miller CT, Singer A, Kimmel C, Westerfield M, Postlethwait JH. A pair of Sox: distinct and overlapping functions of zebrafish sox9 co-orthologs in craniofacial and pectoral fin development. *Development* 2005, 132:1069–1083.
126. Taylor KM, LaBonne C. SoxE factors function equivalently during neural crest and inner ear development and their activity is regulated by SUMOylation. *Dev Cell* 2005, 9:593–603.
127. Cheung M, Briscoe J. Neural crest development is regulated by the transcription factor Sox9. *Development* 2003, 130:5681–5693.
128. Honore SM, Aybar MJ, Mayor R. Sox10 is required for the early development of the prospective neural crest in Xenopus embryos. *Dev Biol* 2003, 260:79–96.
129. Rinon A, Molchadsky A, Nathan E, Yovel G, Rotter V, Sarig R, Tzahor E. p53 coordinates cranial neural crest cell growth and epithelial-mesenchymal transition/delamination processes. *Development* 2011, 138:1827–1838.
130. Cheung M, Chaboissier MC, Mynett A, Hirst E, Schedl A, Briscoe J. The transcriptional control of trunk neural crest induction, survival, and delamination. *Dev Cell* 2005, 8:179–192.
131. Taneyhill LA, Coles EG, Bronner-Fraser M. Snail2 directly represses cadherin6B during epithelial-to-mesenchymal transitions of the neural crest. *Development* 2007, 134:1481–1490.
132. Sakai D, Suzuki T, Osumi N, Wakamatsu Y. Cooperative action of Sox9, Snail2 and PKA signaling in early neural crest development. *Development* 2006, 133:1323–1333.
133. Strobl-Mazzulla PH, Sauka-Spengler T, Bronner-Fraser M. Histone demethylase JmjD2A regulates neural crest specification. *Dev Cell* 2010, 19:460–468.
134. Bajpai R, Chen DA, Rada-Iglesias A, Zhang J, Xiong Y, Helms J, Chang C-P, Zhao Y, Swigut T, Wysocka J. CHD7 cooperates with PBAF to control multipotent neural crest formation. *Nature* 2010, 463:958–962.
135. Gammill LS, Bronner-Fraser M. Genomic analysis of neural crest induction. *Development* 2002, 129:5731–5741.
136. Huang T, Liu Y, Huang M, Zhao X, Cheng L. Wnt1-cre-mediated conditional loss of Dicer results in malformation of the midbrain and cerebellum and failure of neural crest and dopaminergic differentiation in mice. *J Mol Cell Biol* 2010, 2:152–163.
137. Fraser PE, Sauka-Spengler T. Expression of the polycomb group gene bmi-1 in the early chick embryo. *Gene Expr Patterns* 2004, 5:23–27.
138. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung M-C. Dual regulation of Snail by GSK-3[beta]-mediated phosphorylation in control of epithelial-mesenchymal transition. *Nat Cell Biol* 2004, 6:931–940.
139. Lin Y, Wu Y, Li J, Dong C, Ye X, Chi Y-I, Evers BM, Zhou BP. The SNAG domain of Snail1 functions as a molecular hook for recruiting lysine-specific demethylase 1. *EMBO J* 2010, 29:1803–1816.
140. Lander R, Nordin K, Labonne C. The F-box protein Ppa is a common regulator of core EMT factors Twist, Snail, Slug, and Sip1. *J Cell Biol* 2011, 194:17–25.
141. Keene JD. RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet* 2007, 8: 533–543.
142. Liu Y, Huang T, Zhao X, Cheng L. MicroRNAs modulate the Wnt signaling pathway through targeting its inhibitors. *Biochem Biophys Res Commun* 2011, 408:259–264.
143. Flentke GR, Garic A, Amberger E, Hernandez M, Smith SM. Calcium-mediated repression of beta-catenin and its transcriptional signaling mediates neural crest cell death in an avian model of fetal alcohol syndrome. *Birth Defects Res A Clin Mol Teratol* 2011, 91:591–602.
144. Cohen-Kupiec R, Pasmanik-Chor M, Oron-Karni V, Weil M. Effects of IKAP/hELP1 deficiency on gene expression in differentiating neuroblastoma cells: implications for familial dysautonomia. *PLoS One* 2011, 6:e19147.

