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Review Article

Migrating into Genomics with the Neural Crest

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Neural crest cells are a fascinating embryonic cell type, unique to vertebrates, which arise within the central nervous system but emigrate soon after its formation and migrate to numerous and sometimes distant locations in the periphery. Following their migratory phase, they differentiate into diverse derivatives ranging from peripheral neurons and glia to skin melanocytes and craniofacial cartilage and bone. The molecular underpinnings underlying initial induction of prospective neural crest cells at the neural plate border to their migration and differentiation have been modeled in the form of a putative gene regulatory network. This review describes experiments performed in my laboratory in the past few years aimed to test and elaborate this gene regulatory network from both an embryonic and evolutionary perspective. The rapid advances in genomic technology in the last decade have greatly expanded our knowledge of important transcriptional inputs and epigenetic influences on neural crest development. The results reveal new players and new connections in the neural crest gene regulatory network and suggest that it has an ancient origin at the base of the vertebrate tree.

1. Introduction

The neural crest is an embryonic cell population characterized by its multipotency, extensive migratory ability, and capacity to form multiple and diverse derivatives [1]. Initially arising within the developing central nervous system (CNS) of vertebrate embryos, these cells depart from the CNS by undergoing an epithelial to mesenchymal transition (EMT) similar to that undertaken by cancer cells during metastasis [2]. Neural crest cells invade the periphery, migrating along characteristic pathways to diverse locations where they differentiate into numerous derivatives.

Just as the CNS is regionalized along the neural axis to form the brain in the anterior portion of the body and the spinal cord in trunk region, the neural crest can also be subdivided into different populations along the body axis [1] which form some overlapping as well as some divergent derivatives (Figure 1). Cranial neural crest cells arise in the head region of the embryo. In the chick embryo, on which this review focuses, they migrate from the forebrain/midbrain as a large swathe of cells which expands like a cobra's hood. At the level of the hindbrain, however, they migrate in segmental streams to populate elements of the facial skeleton, including the upper and lower jaw as well as bones of the neck. Other cranial crest cells contribute to all of the glia and some neurons of cranial sensory ganglia; other neural crest cells form pigment cells. In fact, in vertebrates, all melanocytes of the skin are derived from the neural crest. In the caudal hindbrain region, neural crest cells termed "cardiac" and "vagal" populate the aortic arches and outflow tract of the heart and enteric ganglia of the gut, respectively. In fact, defects in these populations lead to severe birth defects like truncus arteriorosis, where the outflow tract fails to properly undergo septation, and agangliogenesis of the gut, which causes megacolon.

Further caudally, trunk neural crest cells migrate along two major pathways. Some cells migrate ventrally to contribute to dorsal root and sympathetic ganglia of the peripheral nervous system. These form in a segmental pattern, with one pair of ganglia forming bilaterally and aligned with each myotomal segment. These ganglia innervate the skin and various organs to sense touch, temperature and injury as well as to autonomically control internal organs. A second population of neural crest cells migrates dorsolaterally

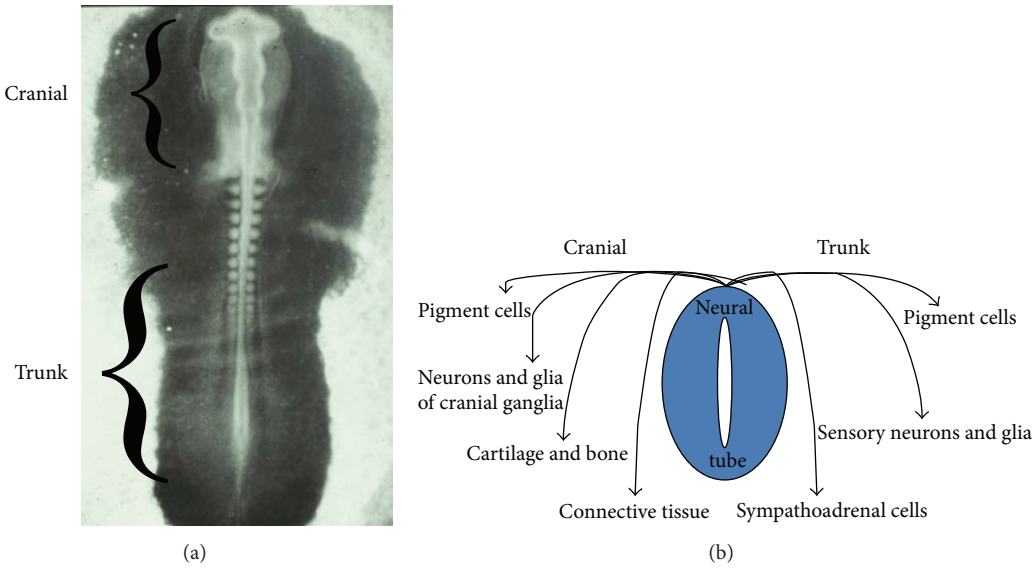

Figure 1: Different populations of neural crest cells along the body axis give rise to different derivatives. (a) A chick embryo at the end of neurulation, in which the neural tube is closed along most of the body axis. Neural crest cells that arise from cranial levels give rise to some derivatives that are distinct from those arising from trunk neural crest population. (b) Schematic diagram summarizing derivatives that arise at cranial versus trunk levels. Both populations contribute to pigment cells, sensory neurons, and glia. However, only cranial neural crest forms cartilage and bone, whereas sympathetic ganglia and the adrenal medulla come uniquely from the trunk neural crest.

underneath the skin to form melanocytes, just as they do from the cranial neural crest. Interestingly, trunk neural crest cells cannot form all derivatives that are formed by the cranial neural crest. For example, they fail to form cartilage, even if transplanted to the head. Thus, all neural crest cells share some common derivatives (e.g., neurons and melanocytes), whereas other derivatives are unique to a particular axial level.

It is interesting to note that the neural crest is a cell population unique to vertebrate embryos [3]. Even though invertebrates have many differentiated cell types similar to those found in vertebrates, like melanocytes and peripheral sensory neurons, these cell arise from neural crest cells in vertebrates, whereas they have ectodermal and sometimes even an endodermal origin in nonvertebrate chordates and other animals. Therefore, a fascinating question that has engaged this field is why and how were neural crest cells invented in the vertebrate lineage?

Over the past decade, we and others have been trying to understand the molecular rules that guide neural crest cells from their site of origin to differentiate into diverse derivatives. During this time, there has been a sea change in the biological sciences due to rapid improvements in technology and decreasing costs of DNA sequencing together with other tools that have opened up new horizons in genomic analysis. This has facilitated exciting advancements in the field which have propelled studies of the neural crest research from the basic to the "systems" level.

Our laboratory first embarked on genomic studies of the neural crest about a decade ago by formulating a hypothetical gene regulatory network (GRN) that represented a feed forward circuit responsible for explaining how precursor cells are induced to a neural crest cell fate, subsequently undergo the process of EMT, become migratory, and finally differentiate into one of several potential derivatives [4] This neural crest GRN, while rudimentary in its initial formulation, has provided a very useful framework for understanding the process of neural crest formation and testing the role of individual and groups of genes in this process in order to establish direct connections and to understand feedback in the system.

This review aims to provide an overview of the neural crest GRN and how we have been testing it and will continue to do so. Rather than being an overview of the field as a whole, as there are many excellent laboratories world-wide working in this area, this review summarizes recent work emerging from my laboratory over the past decade. I will first provide a description of the ongoing events during avian neural crest formation and then weave in the molecular players underlying those events. Although our initial premise was that the GRN relied on transcriptional events, it is now clear that epigenetic factors also play a critical role in many aspects of neural crest formation. Finally, I will discuss our present speculations regarding how this neural crest GRN may have arisen from an evolutionary perspective.

2. Neural Crest Cells Arise at the Neural Plate Border during Gastrulation

The neural crest arises in the ectoderm concomitant or shortly following neural induction in the gastrula stage embryo. The ectoderm, or top germ layer, will give rise to four distinct cell populations: the neural ectoderm which

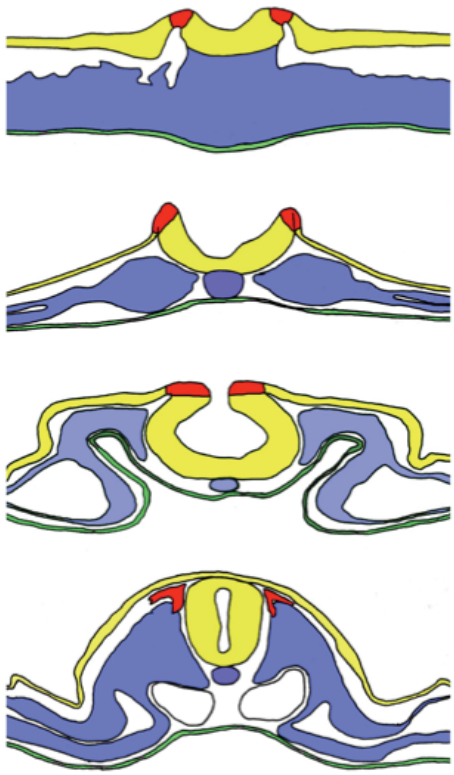

Figure 2: Illustration showing the process of neurulation. The ectoderm (yellow) gives rise to the neural plate in the midline and future epidermis at its lateral edges. Between epidermis and neural plate lies the neural plate border that contains presumptive neural crest cells (red). As the neural plate invaginates to form the neural tube, the neural folds (red) elevate, eventually fusing such that presumptive neural crest cells lie in the dorsal portion of the closed neural tube. Finally, they undergo epithelial to mesenchymal transition, leaving the neural tube and migrating into the periphery.

will form the CNS, the nonneural ectoderm which will give epidermis of the skin, and the neural plate border, which will form neural crest cells and cranial ectodermal placodes. Initially, the ectoderm is a flat sheet of epithelial cells. The cells in the middle region of the ectoderm will form the neural plate. Neural crest precursors arise at the lateral edges of this neural plate at its border with the nonneural ectoderm. Future epidermis lies still more lateral to this.

During the process of neurulation in avian embryos, the neural plate undergoes invagination, whereby the neural plate border region rises, forming neural folds, which eventually appose, thus transforming the flat neural plate into a closed neural tube, which will become the CNS (Figure 2). The neural folds contain premigratory neural crest cells, which after neural tube closure come to lie within the dorsal portion of the closed neural tube, from which they will subsequently emigrate to commence their long migrations. Because neural crest precursors arise from the neural folds, it was long assumed that they are induced at the time that the neural folds are elevating and closing to form the neural tube. At this time, they initiate expression of bona fide neural crest marker genes like *FoxD3*, *Snail2*, and *Sox10* [5, 6]. That the ectoderm was responsible for inducing the neural crest came from experiments showing that one could juxtapose intermediate regions of the neural tube, which normally only form CNS structures, with nonneural ectoderm, and this would result in the generation of neural crest cells [7–9]. Subsequently, it was shown that BMP [10] and Wnt [11] were the signaling molecules critical for neural crest induction. In particular, we found that Wnt was both necessary and sufficient for neural crest induction.

As a consequence, it was surprising when we discovered that neural crest cells already were specified at a much earlier stage—rather than during neurulation. They had received the necessary signals to autonomously initiate the neural crest program during gastrulation. In looking for early markers of presumptive neural crest cells, we discovered that the transcription factor *Pax7* was expressed at the neural plate border in chick embryos not only when the neural folds are elevating but already at gastrula stages at the border region between neural and nonneural ectoderm. Moreover, *Pax7* loss of function in the chick leads to a loss of neural crest gene expression in the neural folds. Most importantly, when we examined the specification state of the neural plate border (NPB) by dissecting this tissue and explanting it in tissue culture in the absence of additional factors, we discovered that the neural plate border tissue but no other tissue was already specified to execute a neural crest cells fate by the gastrula stage and would express neural crest markers, migrate, and differentiate into appropriate derivatives [12]. This experiment changed our view about the timing of neural crest formation, making it clear that the induction process was already complete by gastrulation stages and that the role of subsequent signaling events was likely for maintenance of this population. This finding raises a conundrum: if the neural crest already was induced by gastrulation, why are neural crest markers not expressed until the time of neural tube closure? The answer to this intriguing question involves new insights gained from studies of epigenetic influences on neural crest development.

3. Elaboration and Testing of the Neural Crest Gene Regulatory Network

With this background information, we can consider different modules that are at play in the neural crest GRN (Figure 3). Given that the process of induction occurs during or before gastrulation, the first step in neural crest formation involves signaling molecules like BMPs, Wnts, FGFs, and perhaps Notch. The data in support of these signaling inputs is cumulative across different vertebrates; indeed, there may be some differences between species as to the location and timing of various signaling processes.

According to the GRN model, these signals initiate transcription of a core group of transcription factors at the neural plate border region. Data from several vertebrates suggests that *Msx1/2*, *Pax3/7*, and *Zic1/2* are components of these

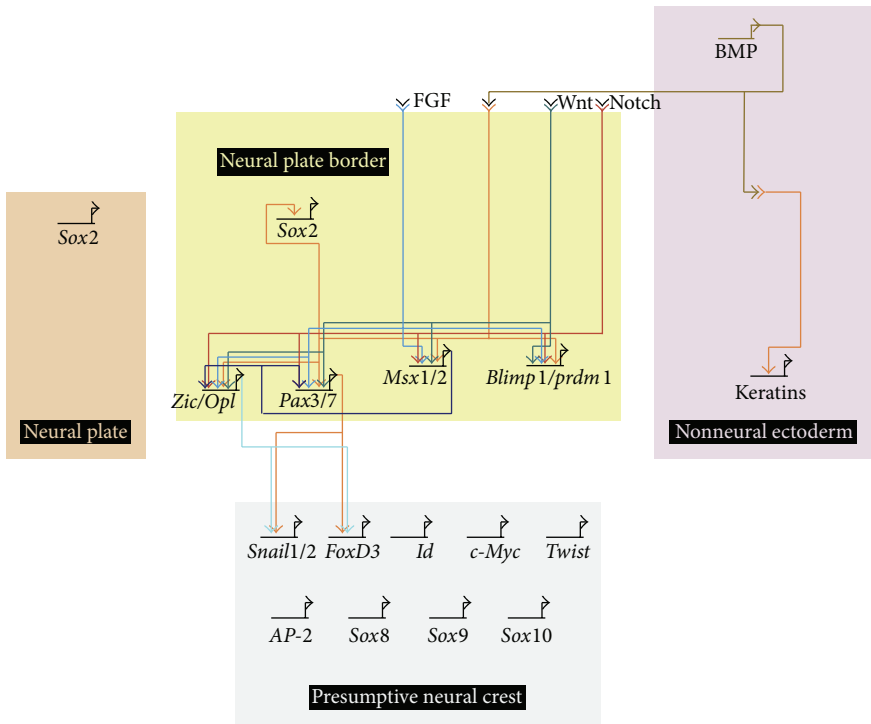

Figure 3: Schematic diagram illustrating inputs in the neural crest gene regulatory network. Signaling molecules like FGFs, Wnts, and BMPs help establish the neural plate border region and are inputs into transcription factors like *Pax7*, *Msx1/2*, and *Zic*. These in turn regulate neural crest specifier gene expression in the presumptive neural crest.

modules, with *Dlx3/5* playing a role in the adjacent ectoderm. These "neural plate border" genes working cooperatively with the signaling module in turn regulate transcription of another set of transcription factors, the "neural crest specifier" genes that mark the premigratory neural crest population within the dorsal neural tube. This module contains genes like *FoxD3*, *Sox10*, *Snail2*, *Ets-1*, and numerous other factors that are important for initiating EMT, regulating cell migration, and ultimately leading to differentiation to various cell fates. This is accomplished by the neural crest specifier modules' regulation of downstream effector genes, which include not only transcription factors but also structural genes, enzymes, and cytoskeletal components necessary for cell motility. The challenge of elaborating the neural crest GRN is to establish direct connections within this network, expand the number of players, and ultimately understand the entire circuit diagram necessary to generate a neural crest cell.

The first foray into testing the NC GRN was by looking at the role of *Pax7* at the neural plate border [12]. To examine its function in the chick embryo, we adapted techniques frequently used in frog and zebrafish work to introduce morpholino antisense oligonucleotides that inhibit endogenous translation of the target gene. The reason for using chick embryos rather than mice for these experiments was simple: they are much easier to manipulate since they develop outside of the mother; they can be grown ex ovo at gastrula stages and it is possible to manipulate one side of the embryo, leaving the other as an internal control. When we knocked down *Pax7* in this way, we found by in situ hybridization that neural crest specifier genes like *Snail2*, *Sox10*, and *FoxD3* also were lost. This demonstrates directionality in the GRN and that indeed neural plate border genes like *Pax7* are upstream of the neural plate specifier module.

While we originally performed these experiments by performing knockdowns and analyzing many embryos by in situ hybridization, novel methods have now made it possible to do these experiments at a rapid and multiplex level. Using Nanostring analysis, for example, we can monitor changes in hundreds of genes in a single half embryo, which can be compared to the control side [13, 14]. This makes it possible to examine this question at more of a "systems" level.

4. Cis-Regulatory Analysis of Neural Crest Specifier Genes Reveals Direct Inputs and New Players in the Neural Crest GRN

Knockdown of individual genes in the GRN helps to establish order in the network but cannot inform on direct versus indirect interactions. Therefore, we have taken a genome-up view to analyze direct inputs into neural crest specifier

genes. This effort was greatly facilitated by sequencing of the chick genome several years ago, which revealed that the intergenic regions of chick are very compact, ~1/3 the size, of homologous regions in mammals. By aligning multiple species and looking for conserved intergenic regions of noncoding DNA, we can identify putative enhancers due to their high degree of conservation. We can then take advantage of the ease of manipulation of chick embryos to introduce these putative enhancers back into the embryos and test whether they are able to mediate reporter expression in the neural crest. To this end, putative enhancers are cloned upstream of a basal promoter and a GFP or RFP construct. The construct is electroporated into a gastrula stage embryo and examined one day later for reporter expression (Figure 4). This gives a "yes/no" answer within a day of whether the construct bears a neural crest enhancer. The positive constructs are further dissected to reveal a minimal enhancer region that is then examined bioinformatically for putative transcription factor binding sites. These are mutated to see what alters reporter expression. Finally, candidate inputs are tested for their importance in the GRN by morpholino knockdown and chromatin immunoprecipitation.

Using this approach, we have identified neural crest enhancers driving expression of *Sox10*, *FoxD3*, and *Ets-1* in the neural crest [15–18]. Whereas *Ets-1* is expressed only in the cranial neural crest, *FoxD3*, and *Sox10* are expressed in both cranial and trunk axial levels. Our studies of neural crest enhancers have revealed several important things. In the case of both *FoxD3* and *Sox10*, we uncovered distinct enhancers that mediate reporter expression in the cranial versus the trunk neural crest population. Dissection of these enhancers revealed direct inputs into these enhancers. For example, in the case of the cranial Sox10 enhancer, we found that *Sox9*, *c-Myb*, and *Ets-1* were direct inputs. Prior to this analysis, *c-Myb* and *Ets-1* had not been described as neural crest specifier genes. Analysis of the *Ets-1* enhancer revealed that *tfAP2*, *Msx1/2*, and *Pax7* are some of the direct inputs mediating cranial *Ets-1* expression. Despite the fact that there is extensive overlap in the expression patterns driven by enhancers for *FoxD3*, *Sox10*, and *Ets1* enhancers in the cranial region, there is no obvious sequence similarity between them, at least given the current capabilities of bioinformatic analysis.

Interrogation of the cranial and trunk *FoxD3* enhancers provided particularly illuminating results [17]. Separate enhancers mediated the onset of *FoxD3* expression in the cranial versus trunk region. Dissection of the separate enhancers showed that *Pax7* and *Msx1/2* were direct inputs into both enhancers. However, the cranial enhancer required input from *Ets-1*, which is cranial specific, whereas the trunk enhancer required a different transcriptional input from *Zic1*, which was expressed in a gradient that was posteriorly high and anteriorly low. Thus, axial specificity of *FoxD3* expression seems to rely upon location-specific transcriptional inputs coupled with necessary common inputs from neural plate border genes *Pax7* and *Msx1/2*. It is intriguing to speculate that these axial-specific enhancers for neural crest specifier genes may reflect the inherent differences in migratory behavior and/or prospective cell fates between these populations.

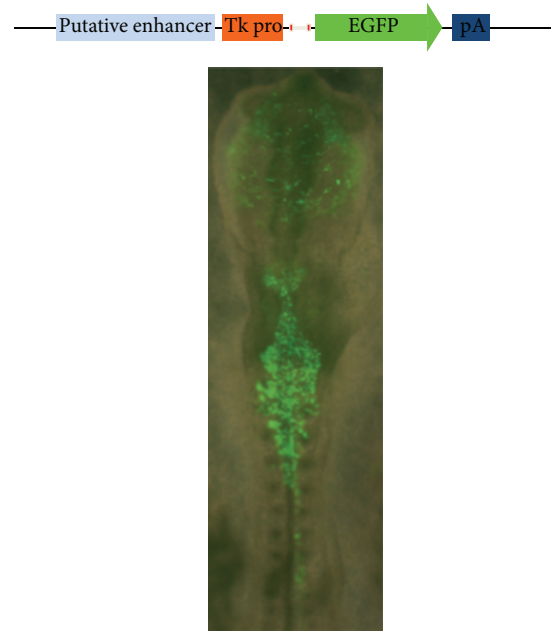

Figure 4: Neural crest cis-regulatory analysis in the chick embryo. Putative enhancers are identified by virtue of the conserved nature of noncoding DNA in intergenic region in proximity to neural crest genes like *FoxD3*, *Sox10*, and so forth. These putative regulatory regions are cloned upstream of a GFP-encoding construct with a basal (TK) promoter. Constructs are then electroporated into gastrula stage chick embryos and analyzed one or more days later. Those with neural crest enhancing activating drive GFP expression in migrating neural crest cells.

5. Transcriptome Analysis Reveals Novel Players in the Neural Crest GRN

Identification of neural crest enhancers not only allows analysis of direct connections in the neural crest GRN but also provides a valuable tool for the isolation of pure populations of neural crest cells at various stages. In a recent study, we have utilized our cranial specific *Sox10* enhancer as a tool to isolate pure populations of migrating cranial neural crest cells for transcriptome analysis. This has been particularly useful for elaborating the numbers of transcription factors known to be expressed in the neural crest, as this analysis has revealed hundreds or additional transcriptional regulators [19]. The most prominent group of upregulated genes were enzymes, which may not be surprising given that neural crest cells are highly motile and metabolically active.

To elaborate connections in the neural crest GRN, we analyzed the effects of knocking down known neural plate border and neural crest specifier genes on a small subset of the novel genes that we identified. The results revealed interesting trends that allowed us to order gene interactions. For example, we found that two genes, *Sox9* and *Ets-1*, had similar effects on all of the downstream genes analyzed. This suggests that these two factors may be high up in the GRN. Loss of other factors (e.g., Sox10 and TFAP2)

affected expression of a smaller subset of downstream genes, suggesting that they may act later at this stage. Thus, the data suggest that different combinations of transcription factors activate distinct neural crest genes with Ets-1 and Sox9 likely working in combination with different regulators (e.g., Pax7, Sox10, and TFAP2) to activate transcription.

6. Epigenetic Inputs into the Neural Crest GRN

In previous as well as ongoing screens to identify novel neural crest genes [19–21], we often find upregulated genes that encode enzymes involved in modifying chromatin or DNA. Examples include lysine demethylases (JmjD2a/KDM4), DNA methyltransferases (DNMTs) 3A and 3B, and histone deacetylases (HDACs), as well as many others. Of particular interest, DNMT3B has been associated with craniofacial anomalies in humans that may be related to defects in the neural crest. For this reason, we began to explore whether and how epigenetic factors might function or modify the neural crest GRN.

Although one might suspect that these epigenetic factors might have rather ubiquitous expression and function, we were surprised to find that, in the early chick embryo, they actually had a surprisingly specific early expression pattern. For example, KDM4 is expressed strongly in the neural plate border region of the chick gastrula [13]. KDM4 is an enzyme that removes methyl groups from trimethylated lysine 9 on histone tails. This is a repressive mark, such that trimethylation of K9 correlates with lack of transcription. Removal of the methyl groups helps open the DNA, correlating with active transcription. When we knocked down KDM4 in the neural plate border region using morpholino antisense oligonucleotides, we found that it very specifically blocked the expression of neural crest specifier genes, most notably *Sox10*, as well as *FoxD3* and *Snail2*. In contrast, loss of KDM4 had no effect on neural genes like *Sox2*. In fact, multiplex Nanostring analysis revealed that it selectively affected neural crest gene expression while leaving other cellular processes intact.

We next used chromatin immunoprecipitation (ChIP) analysis coupled with morpholino knockdown to understand the molecular basis of KDM4's effect on neural crest gene expression, focusing on its effects on the *Sox10* promoter. ChIP analysis revealed that, under normal circumstances, an antibody against H3K9me3 (the repressive trimethylation mark) showed high occupancy of the *Sox10* promoter region at gastrula to neurula stages, but this mark was removed around the time of neural tube closure as *Sox10* expression initiates. Similarly, KDM4's occupancy of the *Sox10* promoter region was initiated in the late gastrula, progressively removing the trimethylation mark. At the time of neural tube closure, KDM4 had completed its function of removing the lysine 9 methylation and was no longer associated with the *Sox10* promoter region [13]. After KDM4 knockdown, however, the *Sox10* promoter retained high occupancy of the H3K9me3 mark that correlates with absence of gene expression and *Sox10*, thus explaining why the *Sox10* promoter was transcriptionally silent on the experimental side of the embryo.

This result helps explain the conundrum posed earlier: if the neural crest is induced during gastrulation, why are neural crest markers not expressed until the time of neural tube closure? The answer lies in epigenetic factors, like KDM4. Our data suggest that neural crest cells are already induced during gastrulation, meaning that they have already received all the necessary signals to be specified as neural crest by that time. Thus, they are poised to become a bona fide neural crest cells and express markers like *Sox10*. However, they cannot execute this program because of the presence of silencing marks. KDM4 appears in the late gastrula and slowly removes the repressive mark that keeps neural crest genes in the "off" state, such that it is completely removed by the time of neural tube closure. At this time, neural crest genes turn on. This suggests that the process of neural crest formation involves a combination of inductive events, mediated by signaling molecules and transcriptional regulators, together with epigenetic factors, which help control the correct time of onset of neural crest gene expression.

In addition to factors that modify chromatin, some epigenetic factors directly modify DNA. For example, DNA methyl transferases (DNMTs) directly methylate the promoter region of genes to inhibit their transcription. There are several DNMTs in the chick genome and most notably, we found that DNMT3A and DNMT3B are expressed by neural crest cells [20]. Interestingly, mutations in human DNMT3B result in craniofacial defects, suggesting a disorder of the neural crest. For these reasons, we examined the function of DNMT3s in neural crest development [14]. At early stages, we found that the DNMT3A was the first paralogs to be expressed—demonstrating strong expression at the neural plate border and later in the closing neural tube. Interesting, it was most strongly expressed in the neural folds and neural crest forming regions. In examining the loss of function phenotype, we found that knockdown of DNMT3A caused a loss of neural crest markers, like *Sox10*, *FoxD3*, and *Snail2*. Although the phenotype resembled that seen after KDM4 knockdown, the mechanism was completely different.

The developing neural tube, which will form the central nervous system (CNS) expresses neural genes like *Sox2* and *Sox3*. These are expressed throughout the CNS with the exception of the dorsal most portion of the neural tube which contains neural crest precursors. After DNMT3A loss of function, we found that *Sox2* and *Sox3* expanded into the dorsal neural tube region, in turn causing shutdown of neural crest genes. Indeed overexpression of Sox2 in the neural crest forming region yielded the same results. We hypothesized that DNMT3A was directly methylating the promoter regions of these genes in the neural crest forming region, resulting in their silencing. ChIP analysis demonstrated that this was correct and that DNTM3A directly methylated the *Sox2* and *Sox3* promoters. These results suggest that DNMT3A acts as molecular switch between CNS and neural crest cell identity.

These two examples nicely illustrate that epigenetic factors like KDMs, DNMTs, and many other factors that modify histones or DNA play an important role in developmental processes like neural crest formation. Together with earlier

experiments showing the important role of transcriptional events in the neural crest gene regulatory network, these findings suggest that a combination of transcriptional and epigenetic factors are likely to control and finely tune the process of neural crest formation.

7. Evolution of the Neural Crest in Vertebrates

Neural crest cells are unique to vertebrate embryos [3], and even the most basal vertebrates possess neural crest cells. This raises the fascinating question of how and why the neural crest may have arisen during vertebrate evolution. Although answers to these questions are not yet in, progress in understanding the evolutionary origin of the neural crest has been made in recent years. In particular, this has come from studies of basal vertebrates, like the sea lamprey, and nonvertebrate chordates, like amphioxus [22] and urochordates [23, 24].

We have examined this fascinating question from a gene regulatory perspective, by examining the regulatory network architecture of the basal vertebrate, lamprey, and the basal chordate, amphioxus (Figure 5). Lampreys are jawless vertebrates called cyclostomes, which together with hagfish, represent the most basal living vertebrates. Indeed, they resemble fossil lamprey that lived over 500 million years ago. Amphioxus, a cephalochordate, is a benthic animal, which has an embryonic body plan that resembles that of vertebrates, with a hollow nerve cord, segmented body, notochord, and gill arches. However, amphioxus lacks neural crest and forebrain structures. It is possible to obtain embryos from both lamprey and amphioxus on a seasonal basis.

By isolating and examining the expression pattern of signaling molecules, neural plate border, and neural crest specifier genes, we observed that the lamprey has nearly all the GRN components present in other vertebrates. We found genes encoding signaling molecules, neural plate border genes, and neural crest specifier genes that were expressed in similar patterns and having similar functions to homologous genes in other vertebrates. In fact, only two exceptions were found. Two genes that served as neural crest specifier genes in jawed vertebrates, *Twist* and *Ets-1*, are only deployed later, as effector genes, in the lamprey neural crest GRN. These results suggest that, except for a few changes, the neural crest GRN is largely conserved to the base of vertebrates. Thus, the network is ancient and has been in place for more than 500 million years [25].

Unlike lamprey, amphioxus lacks neural crest but does have a neural tube that undergoes neurulation somewhat similar to that of vertebrates. In examining the patterns of expression of neural plate border genes in amphioxus, they are highly similar to the patterns seen in vertebrates, suggesting that the proximal portion of the GRN was already in existence in basal chordates. Interestingly, the amphioxus genome possesses homologs of all "neural crest specifier" genes. However, in examining their expression pattern, the results show that they are expressed in other germ layers like mesoderm and endoderm, but absent from the neural folds, from which neural crest cells arise in vertebrates. The one exception is the transcription factor *Snail*, which is expressed in this region [22]. These results raise the intriguing possibility that evolution of the neural crest resulted from cooption of existing genes to the neural plate border/neural fold region. Since jawed vertebrates have undergone two genome-wide duplications, it is possible that duplication and divergence of gene regulatory regions resulted in the emergence of new enhancers that facilitated gene deployment in this region. Cumulative expression of neural crest specifier genes in the neural folds then may have enabled an epithelial to mesenchymal conversion, allowing cells to migrate from the CNS and into the periphery.

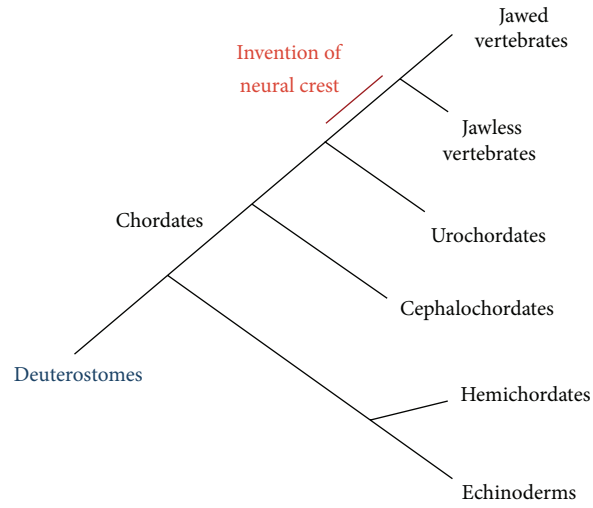

Figure 5: Schematic diagram of the deuterostome phylogenetic tree showing the emergence of neural crest cells in the vertebrate lineage. Whereas nonvertebrate chordates like cephalochordates (e.g., amphioxus) and urochordates (e.g., ascidians) lack neural crest cells, basal jawless vertebrates (e.g., lamprey and hagfish) have neural crest cells.

8. Conclusions

The neural crest is a fascinating cell type due to its multipotency, migratory ability, and contribution to so many diverse derivatives. A question that has fascinated and occupied my laboratory for decades is how these cells form, undergo EMT, and differentiate. Advances that have accompanied the genomic age have greatly increased our knowledge of the transcriptional and epigenetic factors present in the neural crest GRN. It is now abundantly clear that a combination of transcription factors, chromatin, and DNA modifiers act cooperatively to mediate the process of neural crest formation, EMT, and subsequent downstream events. The challenge is to dissect network architecture and to understand how the network has evolved and changed during the advent of vertebrates and during progressive evolution. It is clear that a "big picture" systems level approach will be necessary to

comprehend such complex events. We are still peering at the tip of the iceberg in terms of our knowledge of the neural crest GRN. The novel tools currently available and continuing to be developed make it possible to delve ever deeper into these exciting questions.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

References

[1] N. M. LeDouarin, *The Neural Crest*, Cambridge University Press, New York, 1982.

[2] M. A. Nieto, "Epithelial plasticity: a common theme in embryonic and cancer cells," *Science*, vol. 342, no. 6159, 2013.

[3] C. Gans and R. G. Northcutt, "Neural crest and the origin of vertebrates: a new head," *Science*, vol. 220, no. 4594, pp. 268–274, 1983.

[4] D. Meulemans and M. Bronner-Fraser, "Gene-regulatory interactions in neural crest evolution and development," *Developmental Cell*, vol. 7, no. 3, pp. 291–299, 2004.

[5] T. Sauka-Spengler and M. Bronner-Fraser, "A gene regulatory network orchestrates neural crest formation," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 7, pp. 557–568, 2008.

[6] T. Sauka-Spengler and M. Bronner, "SnapShot: neural crest," *Cell*, vol. 143, no. 3, pp. 486–e1, 2010.

[7] J. D. Moury and A. G. Jacobson, "The origins of neural crest cells in the axolotl," *Developmental Biology*, vol. 141, no. 2, pp. 243–253, 1990.

[8] M. A. J. Selleck and M. Bronner-Fraser, "Origins of the avian neural crest: the role of neural plate-epidermal interactions," *Development*, vol. 121, no. 2, pp. 525–538, 1995.

[9] M. E. Dickinson, M. A. J. Selleck, A. P. McMahon, and M. Bronner-Fraser, "Dorsalization of the neural tube by the non-neural ectoderm," *Development*, vol. 121, no. 7, pp. 2099–2106, 1995.

[10] K. F. Liem Jr., G. Tremml, H. Roelink, and T. M. Jessell, "Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm," *Cell*, vol. 82, no. 6, pp. 969–979, 1995.

[11] M. I. García-Castro, C. Marcelle, and M. Bronner-Fraser, "Ectodermal Wnt function as a neural crest inducer," *Science*, vol. 297, no. 5582, pp. 848–851, 2002.

[12] M. L. Basch, M. Bronner-Fraser, and M. I. García-Castro, "Specification of the neural crest occurs during gastrulation and requires Pax7," *Nature*, vol. 441, no. 7090, pp. 218–222, 2006.

[13] P. H. Strobl-Mazzulla, T. Sauka-Spengler, and M. Bronner-Fraser, "Histone demethylase JmjD2A regulates neural crest specification," *Developmental Cell*, vol. 19, no. 3, pp. 460–468, 2010.

[14] N. Hu, P. Strobl-Mazzulla, T. Sauka-Spengler, and M. E. Bronner, "DNA methyltransferase3A as a molecular switch mediating the neural tube-to-neural crest fate transition," *Genes and Development*, vol. 26, no. 21, pp. 2380–2385, 2012.

[15] P. Betancur, M. Bronner-Fraser, and T. Sauka-Spengler, "Genomic code for Sox10 activation reveals a key regulatory enhancer for cranial neural crest," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 8, pp. 3570–3575, 2010.

[16] P. Betancur, T. Sauka-Spengler, and M. Bronner, "A sox10 enhancer element common to the otic placode and neural crest is activated by tissue-specific paralogs," *Development*, vol. 138, no. 17, pp. 3689–3698, 2011.

[17] M. S. Simões-Costa, S. J. McKeown, J. Tan-Cabugao, T. Sauka-Spengler, and M. E. Bronner, "Dynamic and differential regulation of stem cell factor FoxD3 in the neural crest is encrypted in the genome," *PLoS Genetics*, vol. 8, no. 12, Article ID e1003142, 2012.

[18] M. Barembaum and M. E. Bronner, "Identification and dissection of a key enhancer mediating cranial neural crest specific expression of transcription factor, Ets-1," *Developmental Biology*, vol. 382, no. 2, pp. 567–575.

[19] M. Simoes-Costa and M. E. Bronner, "Insights into neural crest development and evolution from genomic analysis," *Genome Research*, vol. 23, pp. 1069–1080, 2013.

[20] M. S. Adams, L. S. Gammill, and M. Bronner-Fraser, "Discovery of transcription factors and other candidate regulators of neural crest development," *Developmental Dynamics*, vol. 237, no. 4, pp. 1021–1033, 2008.

[21] L. Gammill and M. Bronner-Fraser, "A genomic analysis of neural crest induction," *Development*, vol. 129, pp. 5731–5741, 2002.

[22] J.-K. Yu, D. Meulemans, S. J. McKeown, and M. Bronner-Fraser, "Insights from the amphioxus genome on the origin of vertebrate neural crest," *Genome Research*, vol. 18, no. 7, pp. 1127–1132, 2008.

[23] W. R. Jeffery, A. G. Strickler, and Y. Yamamoto, "Migratory neural crest-like cells form body pigmentation in a urochordate embryo," *Nature*, vol. 431, no. 7009, pp. 696–699, 2004.

[24] P. B. Abitua, E. Wagner, I. A. Navarrete, and M. Levine, "Identification of a rudimentary neural crest in a non-vertebrate chordate," *Nature*, vol. 491, no. 7427, pp. 104–107, 2012.

[25] T. Sauka-Spengler, D. Meulemans, M. Jones, and M. Bronner-Fraser, "Ancient evolutionary origin of the neural crest gene regulatory network," *Developmental Cell*, vol. 13, no. 3, pp. 405–420, 2007.