Published in final edited form as:
Histochem Cell Biol. 2012 August ; 138(2): 179–186. doi:10.1007/s00418-012-0999-z.

Formation and migration of neural crest cells in the vertebrate embryo1

Marianne E. Bronner
Division of Biology 139-74, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125, USA

Abstract

The neural crest is a stem cell population, unique to vertebrates, that gives rise to a vast array of derivatives, ranging from peripheral ganglia to the facial skeleton. This population is induced in the early embryo at the border of the neural plate, which will form the central nervous system (CNS). After neural tube closure, neural crest cells depart from the dorsal CNS via an epithelial to mesenchymal transition (EMT), forming a migratory mesenchymal cell type that migrates extensive to diverse locations in the embryo. Using *in vivo* loss-of-function approaches and cis-regulatory analysis coupled with live imaging, we have investigated the gene regulatory network that mediates formation of this fascinating cell type. The results show that a combination of transcriptional inputs and epigenetic modifiers control the timing of onset of neural crest gene expression. This in turn leads to the EMT process that produces this migratory cell population.

Keywords

Neural crest; neural tube; EMT; transcription factor; chromatin; cis-regulatory network

A bit of history: What is the neural crest and how is it identified?

The vertebrate neural crest is a migratory embryonic cell population that is unique to the vertebrate embryo. This cell type was first recognized in the chick embryo over 140 years ago by the embryologist, Wilhelm His (1868), who noticed a band of material lying between the presumptive epidermis and the neural tube. Since then, the neural crest has fascinated biologists, because of its important stem cell properties, broad set of derivatives and extensive migratory abilities.

In the early embryo, the neural crest forms at the border between the neural plate, which will become central nervous system (CNS) and the non-neural ectoderm, the future epidermis. The neural plate invaginates such that the elevating neural folds, containing neural crest precursors, form the leading edges of the closing neural tube (Fig. 1). As a consequence, premigratory neural crest cells initially reside within the neuroepithelium. They subsequently undergo a transition, known as an epithelial to mesenchymal transition (EMT) to delaminate from the neuroepithelium and migrate throughout the embryo. In fact, neural crest cells undergo the most extensive migration of any embryonic cell type in vertebrate embryos. Initially, many neural crest progenitors are multipotent and have stem cell-like properties (Bronner-Fraser and Fraser, 1988; Stemple and Anderson, 1992), but become

[1]Robert Feulgen Lecture presented at the 54th Symposium of the Society for Histochemistry in Vienna, Austria, 5–8 September 2012

Address for correspondence: mbronner@caltech.edu.

progressively restricted to form particular derivatives depending upon the migratory pathways they follow.

Upon reaching their final destinations in the periphery, neural crest cells stop migrating, often reaggregate, and contribute to numerous vertebrate structures. They build much of the head skeleton, form all epidermal pigment cells, and most of the peripheral nervous system. Specific neural crest derivatives include sensory, sympathetic and enteric neurons, glia, melanocytes, smooth muscle, dermis, cardiac septum, connective tissue, facial cartilage and bone, and adrenal chromaffin cells. As a consequence of their broad contributions to diverse structures, defects in neural crest development lead to a variety of birth defects including cleft palate, septal defects of the heart and outflow tract, and Hirschprung's disease, among other debilitating conditions.

Methods for following migrating neural crest cells

Neural crest cells only become recognizable as a separate population upon undergoing EMT and leaving the neural tube. Shortly thereafter, they intermingle with other mesenchymal cells types, making it difficult to follow their migration and differentiation. As a consequence, early studies of the neural crest development relied upon labeling populations of neural crest cells with different markers that allowed their movement to be followed.

In the first half of the 20th century, most studies relied upon performing ablations of the neural folds to determine what was missing and might be attributed to the neural crest. More reliable were grafting experiments, in which the neural folds or neural tube were transplanted between different species of amphibian embryo that differed in cell size or had other identifying characteristics (rev. Hall and Horstadius, 1988). These studies presented a general picture or neural crest cell contributions to several derivatives including melanocytes, craniofacial structures and the peripheral nervous system.

The major breakthrough in studying neural crest development in higher vertebrates came from the pioneering work of LeDouarin (rev. 1982), who discovered that two closely related species of bird, the quail and the chick, were compatible for interspecific grafting but whose cells remained easily distinguishable from one another for long periods of time. Quail cells contain heterochromatin in their nuclei, whereas chick cells are euchromatic. By grafting quail donor neural tubes into chick host embryos, she was able to indelibly mark presumptive neural crest cells and follow not only their emigration from the neural tube but also their long term differentiation into multiple derivatives (Fig. 2). These interspecific grafts were termed "chimeras" from the Greek, meaning fabulous monster.

It was the histological technique developed by Robert Feulgen that made it possible for LeDouarin and those who followed her to analyze the results of these quail-chick chimeras. By staining for DNA, it was possible to recognize the purple dots of condensed heterochromatin that distinguished quail from chick cells. This allowed LeDouarin to comprehensively map the derivatives arising from the neural crest at many axial levels, as well as to challenge the presumptive fate of neural crest populations by grafting them to heterotopic locations (LeDouarin, 1892). Although the advent of a quail-specific antibody has now replaced use of the Feulgen stain, most of what is known about neural crest derivatives comes from data made possible by the method developed by Robert Feulgen.

Quail-chick chimeric data showed that the neural crest is comprised of several populations that differ in their pathways of migration and types of derivatives they form. These populations originate from different levels of the embryonic body axis and are termed: cranial, vagal, trunk and lumbosacral. At cranial levels, some neural crest cells contribute to the cranial sensory ganglia and the parasympathetic ciliary ganglion of the eye, as well as

cartilaginous elements of the facial skeleton. In particular, cranial neural crest cells contribute to the quadrate, Meckel's cartilage and surrounding membrane bones, to the cartilage of the tongue, and to membrane bones of the upper jaw and skull (Noden, 1978; Couly et al., 1992; 1993). Vagal neural crest cells undergo some of the longest migrations of any embryonic cells type, populating the entire gut to form the enteric nervous system. A subset of the vagal crest, termed the cardiac neural crest, migrates to and septates the outflow tract and heart. Trunk neural crest cells form sensory and sympathetic ganglia as well as the adrenal medulla. In addition, neural crest cells from all levels of the body form melanocytes that invade the epidermis and give color to the skin and hair.

More recently, other methods for labeling the neural crest have confirmed the results of the quail-chick chimera performed over 40 years ago. These include: the use of the lipophilic dye, DiI, to label small populations of cells in the neural tube and neural folds (Serbedzija et al., 1989; Kulesa and Fraser, 2000); electroporation or virally-mediated introduction of GFP encoding constructs into the neural tube (McLennan and Kulesa, 2007), etc. A frame from a movie taken after electroporation of GFP into one side of the neural tube to label migrating neural crest cells is shown in figure 3. These methods offer the advantage of allowing one to follow neural crest cell movement in living embryos, allowing time lapse imaging, and have been very useful for analyzing interactions between individual neural crest cells under normal and perturbed conditions.

Gene regulatory network underlying neural crest formation

Neural crest cells form at the border between the neural and non-neural ectoderm. Although they do not become recognizable as a separate population until they depart from the neural tube, it is now clear that neural crest precursors are “induced” at a much earlier stages in all vertebrates examined to date. To explain the temporal sequence of neural crest formation at a system level, we have proposed a neural crest gene regulatory network (NC-GRN) that leads to progressive development of the neural crest (Meulemans and Bronner-Fraser, 2004), leading to production of migratory cells. The data in this putative network derive from information obtained from a number of species, including chick, frog, zebrafish, and mouse. The NC-GRN is a working and testable model, comprised of interacting transcriptional regulators and downstream effector genes that function cooperatively and sequentially to confer neural crest identity.

For hypothetical purposes, the NC-GRN can be divided into distinct phases (Sauka-Spengler et al., 2008). The first involves *inductive signals* (e.g. Wnt, BMP, FGF) that function at the neural plate border to establish the border territory, containing cells with the potential to form neural crest cells. These signals up-regulate a set of genes termed *neural plate border specifier genes* which include transcription factors such as *Msx1/2, Pax3/7*, and *Zic*. These broadly define a region with the ability to form neural crest cells as well as other cell types such as dorsal sensory neurons of the CNS. In the next step, these border genes in turn up-regulate a set of genes that more specifically imbues the border region with the ability to form neural crest cells. These genes are termed *neural crest specifier genes* and include members of the *Snail, FoxD3*, *SoxE* genes, as well as many other factors. Finally, neural crest specifiers turn on specific downstream targets that render them migratory and multipotent. A schematic diagram illustrating the NC-GRN is shown in figure 4. We have performed a variety of screens to identify these effector genes and they are likely to number in the thousands, many of them with still unknown roles in neural crest development (Gammill et al., 2002; Adams et al., 2008).

To better define connections in the NC-GRN, we are using two major approaches: 1) examining the effects on putative downstream targets of loss of neural plate border genes

and/or neural crest specifier genes; and 2) determining direct inputs into neural crest genes by analysis of their regulatory regions to identify enhancer elements.

As an example of the former approach, we examined the function of the neural plate border gene, Pax7, to uncover its role in the NC-GRN. By careful analysis, we found that Pax7 is expressed in the neural plate border at very early stages, prior to gastrulation in the chick (Basch et al., 2006). At this stage, Pax7 is localized in a territory at the border of the presumptive neural plate, which will become the CNS. To test whether these cells were already specified to become neural crest (in other words, whether they had already received signals that would allow them to differentiate even if those signals were subsequently removed), we explanted small pieces of the Pax7-expressing neural plate border region and cultured them in a neutral environment. Despite the fact that they were now outside of their normal embryonic context, they still turned on neural crest markers and differentiated into appropriate derivatives. This suggested that the cells were already ‘specified’ to become neural crest.

We next examined if Pax7 was important for the process of neural crest formation by knocking down Pax7 protein expression in the embryo using antisense morpholino oligonucleotides. After targeting the morpholino to the neural plate border, we found that loss of Pax7 in turn caused loss of several neural crest specifier genes, but not other dorsal neural tube genes (Basch et al., 2006). These experiments showed directionality in the network, i.e. that the neural plate border gene Pax7 was upstream of neural crest specifier genes. However, it also raised some intriguing new questions. Why did neural crest induction occur so early, during gastrulation, and yet neural crest cells only appeared much later, after neurulation? This question will be at least partially answered later in this review. A second important question was whether Pax7 regulates neural crest specifier genes like Sox10 and FoxD3 directly or indirectly. Although directionality is clear, this type of loss-of-function experiment cannot discriminate between direct versus indirect regulatory scenarios.

To identify direct regulatory interactions and identify potential new factors that may play a role in the NC-GRN, we are taking a view from the genome. To this end, we took advantage of the compact nature of the chick genome, which is an optimal distance from mammals to reveal conserved regulatory regions. Our first goal was to identify enhancers responsible for expression of the key neural crest transcription factor, Sox10. We chose Sox10 for initial analysis, not only because it was one of the putative downstream targets of Pax7, but also because it is a key neural crest gene, involved in differentiation of almost every neural crest cell lineage, including neurons and melanocytes. Using our ability to assay putative regulatory regions by electroporation, we found a conserved region of 3.5 kb that was able to recapitulate endogenous Sox10 expression at all axial levels (Betancur et al., 2010a). Interestingly, further dissection of this region revealed two subregions, both of which mediated eGFP expression in neural crest cells but whose patterns were non-overlapping. Whereas Sox10E1 mediated eGFP expression in the vagal and trunk neural crest but not in the head, a small (265bp) region called Sox10E2 mediated expression in the cranial neural crest but not in the trunk.

To find potential inputs, we further examined the Sox10E2 region for putative transcription factor bindings sites. These were mutated to determine which mutations eliminated eGFP expression. This analysis together with chromatin immunoprecipitations demonstrated that Sox9, Ets1 and Myb together were direct inputs into Sox10E2. Furthermore, these factors were critical for expression of endogenous Sox10 expression in the cranial neural crest (Betancur et al, 2010a). Although Sox9 was previously invoked in our first version of the NC-GRN, Ets1 and Myb were ‘new’ and allowed us to expand the NC-GRN (Fig. 4b). Intriguingly, Pax7 was not required for expression of Sox10E2, suggesting that effects of

loss of Pax7 on Sox10 expression are likely to be indirect. This raised the question of what genes might be direct targets of neural plate border genes like Pax7.

Another critical neural crest transcription factor whose expression precedes that of Sox10 is FoxD3. Performing a similar analysis as done for Sox10, we have identified two enhancers (Simoes-Costa et al., 2012), NC1 and NC2, that mediate FoxD3 expression in spatially and temporally distinct manners. Like Sox10E2, NC1 is specific to the cranial neural crest, though its expression pattern is distinct. Detailed regulatory analysis of NC1 reveals that initial expression of FoxD3 in the cranial neural crest is directly mediated by Pax7 and Msx1/2, in combination with the neural crest specifier gene, Ets1. In contrast to NC1, the NC2 enhancer mediates onset of FoxD3 at the vagal/trunk level, and only later in a subset of migrating cranial crest cells that lack NC1. These results not only show that FoxD3 but not Sox10 expression is directly downstream of the neural plate border genes Pax7 and Msx, but also that encoded in the genome is both spatial and temporal information controlling the expression of these critical neural crest transcription factors.

We compared the expression patterns of these two FoxD3 enhancers with the cranial Sox10 enhancer Sox10E2 using time-lapse imaging (Simoes-Costa et al., 2012). The results demonstrate that there is dynamic regulation of multiple enhancers within the cranial neural crest population. The cranial NC1 enhancer is initially restricted to cells in the dorsal midline of the neural tube and its expression precedes that of Sox10E2. NC2 is seen in only a few delaminating/emigrating cranial neural crest cells but later in a majority of the migrating neural crest population where it overlaps with Sox10E2, which appears to label all of the migrating cranial crest population.

The identification of these early neural crest enhancers that turn on concomitant with neural crest EMT has several important uses. First, these enhancers uniquely provide us with the ability to identify direct inputs in the neural crest GRN. Second, they provide an extremely useful tool. As demonstrated above, these enhancers allow the exquisite visualization of dynamic changes in gene expression in living embryos. In addition, rather than encoding GFP variants, these enhancers can be used to drive expression of mRNAs or shRNAs for other transcription factors to enable their ectopic expression in precise populations of neural crest cells at endogenous levels and appropriate times. They also can be used to sort specific cell populations from the embryo. Thus, identification of embryonic enhancers is a valuable tool for studying many aspects of neural crest biology (Betancur et al., 2010b).

Importance of epigenetic modifications in controlling neural crest formation

Although neural crest precursors are induced during gastrulation (Basch et al. 2006) at the border between the neural and non-neural ectoderm (Selleck and Bronner-Fraser, 1995), they only become a distinct cell population after neurulation. Why is this stem cell population induced so early yet pauses for an extensive time prior to forming bona fide neural crest cells? One intriguing possibility is that, like other stem cell populations, the neural crest may be “poised” to differentiate but cannot do so until the appropriate the time due to important controlling factors.

In a screen for genes expressed in newly induced neural crest cells, we identified the histone demethylase, *JumonjiD2a (JmjD2A)* (Strobl-Mazzulla, et al., 2010). Although one might assume that enzymes of this sort would be relatively ubiquitous, we found that JmjD2A was selectively expressed in the developing neural crest, first appearing at the neural plate border during late gastrulation, remaining in the dorsal neural tube and on migrating neural crest

cells. This relatively restricted expression pattern prompted us to study its function in the neural crest.

As with Pax7 above, we tested its role using a morpholino-mediated loss-of-function approach targeting the presumptive neural crest region. The results showed that knocking down JmjD2A had a profound and specific effect on neural crest specifier genes, while eaving other genes including the neural tube gene Sox2 unaffected. In particular, expression of Sox10 was completely lost at early stages (Fig. 5a). Subsequently, neural crest cells failed to migrate but rather seemed “stuck” in the neural tube. Thus, JmjD2A appears to be critical for expression of neural crest genes like Sox10, as well as FoxD3 and Snail2, among others.

The normal role of JmjD2A is to remove a trimethyl mark from lysine 9 on histone H3 (H3K9). Methylation of K9 reflects a repressive mark, meaning that the gene is transcriptionally silent (Fig. 5b). Using a specific antibody to this lysine mark, we examined its association with the Sox10 promoter region at various times. We found a very interesting and precise change in the H3K9 interaction with the Sox10 promoter. Prior to the onset of neural crest EMT, there is strong association of the H3K9 antibody with the Sox10 promoter. However, just as EMT is beginning, this interaction disappears, suggesting a rapid change that correlates with the removal of this repressive mark and the onset of transcription.

What does JmjD2A have to do with this? Using an antibody to JmjD2A, we found that there is strong association with the Sox10 promoter region from the onset of JmjD2A’s expression at late gastrulation until the onset of Sox10 expression. At subsequent stages, JmjD2A no longer occupies the promoter region, suggesting that it leaves once its job is complete. Moreover, when we knock-down JmjD2A with a morpholino, H3K9 remains associated with the promoter region, explaining why Sox10 remains transcriptionally silent after loss of the histone demethylase (Strobl-Mazzullla, et al., 2010).

Taken together, the results suggest that neural crest specifier genes like Sox10 are primed for transcription from early stages and ready to go. However, they are epigenetically silenced by a repressive histone mark. JmjD2A specifically removes this mark, resulting in activation of Sox10 transcription at the appropriate time. This helps to explain the previous results showing that neural crest cells are specified at the early gastrula stage (Basch et al., 2006) but only make migratory neural crest cells after neurulation.

Epigenetic influences on the neural crest epithelial to mesenchymal transition

The epithelial to mesenchymal transition is a process by which epithelial cells are transformed from a tightly adherent sheet into dispersed mesenchymal cells. During EMT, cells undergo adhesive changes, loss of cell polarity and cytoskeletal re-arrangements. As a result, they are endowed both with motility and invasive properties, allowing them to migrate to precise and sometimes distant final sites. EMT occurs reiteratively during development and is important during gastrulation, somite formation and construction of many organs. Furthermore, abnormal EMT in the adult leads to tumor progression and metastasis.

After their induction at gastrulation, elevation and closure of the neural tube, neural crest precursors come to lie in the dorsal aspect of the neural tube. This is the point at which they first become recognizable as bona fide neural crest cells that have migratory and invasive characteristics. These precursors are ready to undergo EMT, leave the neuroepithelium and commence migration as individual mesenchymal cells. In the chick, neural crest cells start

emigrating from the neural tube shortly after it closes and the process of neural crest EMT lasts for approximately one day. Interestingly, single cell lineage analysis of the newly closed neural tube shows that individual precursor cells can give rise to both neural crest and neural tube derivatives (Bronner-Fraser and Fraser, 1988). Thus, controlling the timing of EMT is critical for normal allocation of cells between the central and peripheral nervous systems as well as other crest derivatives.

The transcription factors *Snail2, FoxD3* and *Twist* have been implicated in the control of neural crest emigration as well as during tumor progression, suggesting a link between the two types of EMT. In the neural crest, these factors trigger intracellular responses and guide major cytoskeletal rearrangements, changes in cell junctions and adhesion properties (Sauka Spengler and Bronner-Fraser, 2008). For example, Snail transcription factors have been shown to repress regulatory regions of cadherins: Snail inhibits E-cadherin expression in numerous cancer cells and Snail2 directly represses Cadherin6b in premigratory neural crest cells (Taneyhill et al., 2007). Despite the fact that Snail-mediated repression is well documented in numerous EMTs, the molecular basis of this repression was largely unknown.

One possibility is that Snail mediates the repression of cadherins by recruiting co-repressors. In the case of E-cadherin in tumor cells, it has been proposed by Peinado *et al.* (2004) that there is a co-repressor complex which contains histone deacetylase (HDAC) and Sin3A. Recruitment of HDAC would result in hypoacetylation of histone H3, which is a prerequisite for transcriptional repression.

We identified the chick homologue of human PHD12 (also known as Pf1) and found that it is expressed in the premigratory neural crest (Strobl-Mazzulla and Bronner, 2012). Loss-of-function experiments revealed that knock-down of PHD12 caused an upregulation of two EMT genes, Cad6b and E-cad. This phenotype is very similar to that seen with knockdown of Snail2.

To probe the molecular mechanisms underlying cranial neural crest EMT, we asked whether PHD12 and Snail together might cooperate with the Sin3A-HDAC co-repressor complex during neural crest development. Consistent with this possibility, we found that endogenous PHD12 protein co-immunopreciptates with both Snail2 and Sin3A. To distinguish whether this interaction is direct or indirect, we used bimolecular fluorescence complementation (BiFC), a powerful technique allowing protein interactions to be visualized within living cells. Interestingly, the results show that PHD12 protein directly interacts with Sin3A via its N-terminus. Snail2 does not directly interact with PHD12, but rather interacts with Sin3A. These results not only reveal the mechanisms underlying the binding of PHD12, but also demonstrate that Sin3A forms a molecular bridge within this complex.

As PHD12 and Snail2 appear to interact with the Sin3A repressive complex, containing, which contains a histone deacetylase, we next examined the level of histone acetylation as a possible basis for Cad6b repression. The results reveal a hyperacetylated region on the Cad6b promoter prior NC migration, which was removed after neural crest cells undergo EMT. Taken together, the results suggest a model in which the Cad6b locus loops to bring together two regulatory regions, one located on the promoter, where PHD12 is recruited, and the other on the E-boxes, to which Snail2 binds. Sin3A-HDAC in turn acts as a molecular bridge between PHD12 and Snail2. These results reveal the dual specificity of an epigenetic regulator, PHD12, and a transcription factor, Snail2, that act cooperatively to fine tune the process of neural crest EMT.

Concluding remarks

Our understanding of neural crest development has come a long way since its initial identification by Wilhelm His (1968). We now know that this strand of cells on the dorsal neural tube is a multipotent progenitor cell population that forms a vast number of derivatives. The neural crest give vertebrates their characteristic features, such as the jaws and other bones of the face as well as peripheral ganglia. As a consequence, defects in neural crest development lead to a variety of human birth defects including cleft palate, septal defects of the heart and outflow tract, and Hirschprung's disease.

In its first formulation, our view of the NC-GRN was one that involved primarily transcription inputs that worked in a modular and sequential manner to allow formation, migration and differentiation of neural crest cells. But it is now clear that additional inputs including epigenetic modifiers are also very important for proper neural crest development. Not only must transcriptional inputs be present to activate gene expression, but the DNA must also be in an open and accessible state. Thus, epigenetic regulators and transcription factors act cooperatively to fine tune the precise activation of genes in the neural crest gene regulatory network.

Robert Feulgen would be pleased by his role in catapulting the neural crest into a field that holds major importance in developmental and cell biology. In addition to shedding important new light on the mechanisms underlying normal embryogenesis, analysis of neural crest development is yielding important clues regarding mistakes that may lead to improper development and birth defects.

Acknowledgments

This work was partially supported by NIH grant HD037105 to MEB. I thank Drs. Marcos Simoes-Costa, Shuyi Nie, and Pablo Strobl-Mazzulla for providing figures.

References

Adams M, Gammill L, Bronner-Fraser M. Discovery of transcription factors and other candidate regulators of neural crest development. Dev Dynamics. 2008; 237:1021–1033.

Basch ML, Bronner-Fraser M, Garcia-Castro MI. Specification of the neural crest occurs during gastrulation and requires Pax7. Nature. 2006; 441:218–222. [PubMed: 16688176]

Betancur P, Bronner-Fraser M, Sauka-Spengler T. Genomic code for Sox10, a key regulatory enhancer for cranial neural crest. Proc Natl Acad Sci USA. 2010a; 107:3570–3575. [PubMed: 20139305]

Betancur P, Bronner-Fraser M, Sauka-Spengler T. Assembling neural crest regulatory circuits into a gene regulatory network. Annu Rev Cell Dev Biol. 2010b; 26:581–603. [PubMed: 19575671]

Bronner ME, LeDoaurin NM. Development and evolution of the neural crest: An overview. Dev Biol. 2010; 366:2–9. [PubMed: 22230617]

Bronner-Fraser M, Fraser SE. Cell lineage analysis reveals multipotency of some avian neural crest cells. Nature. 1988; 335:161–164. [PubMed: 2457813]

Couly GF, Coltey PM, Le Douarin NM. The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development. 1992; 114:1–15. [PubMed: 1576952]

Couly GF, Coltey PM, Le Douarin NM. The triple origin of the skull in higher vertebrates: a study in quail-chick chimeras. Development. 1993; 117:409–429. [PubMed: 8330517]

Gammill L, Bronner-Fraser M. A genomic analysis of neural crest induction. Development. 2002; 129:5731–5741. [PubMed: 12421712]

His, W. Untersuchungen uber die erste Anlage des Wirbeltierleibes. Leipzig: Die erste Entwickelung des Hühnchens im Ei. FCW Vogel; 1868.

Hall, BK.; Hörstadius, S. The Neural Crest. Oxford: Oxford Press; 1988.

Le Douarin, N. The Neural Crest. Cambridge: Cambridge University Press; 1982.

McLennan R, Kulesa PM. In vivo analysis reveals a critical role neuropilin-1 in cranial neural crest cell migration in chick. Dev Biol. 2007; 301:227–239. [PubMed: 16959234]

Meulemans D, Bronner-Fraser M. Gene-regulatory interactions in neural crest development and evolution. Dev Cell. 2004; 7:291–299. [PubMed: 15363405]

Noden DM. Spatial integration among cells forming the cranial peripheral nervous system. J Neurobiol. 1993; 24:248–261. [PubMed: 8445390]

Peinado H, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Mol Cell Biol. 2004; 24:306–319. [PubMed: 14673164]

Sauka-Spengler T, Bronner-Fraser M. A gene regulatory network orchestrates neural crest formation. Nat Rev Mol Cell Biol. 2008; 9:557–568. [PubMed: 18523435]

Serbedzija GN, Bronner-Fraser M, Fraser SE. A vital dye analysis of the timing and pathways of avian trunk neural crest cell migration. Development. 1989; 106:809–816. [PubMed: 2562671]

Selleck MAJ, Bronner-Fraser M. Origins of the avian neural crest: the role of neural plate-epidermal interactions. Development. 1995; 121:526–538.

Simoes-Costa M, McKeown S, Sauka-Spengler T, Bronner-Fraser M. Cis-regulatory analysis of FoxD3 expression in the avian neural crest. PLoS Genetics. 2012 in press.

Strobl P, Sauka-Spengler T, Bronner-Fraser M. Histone demethylase JmjD2A regulates neural crest specification. Dev Cell. 2010; 19:460–468. [PubMed: 20833367]

Strobl-Mazzulla P, BronnerFraser M. A PHD12-Snail2 repressive complex epigenetically mediates neural crest epithelial to mesenchymal transition. J Cell Biol. 2012 in press.

Taneyhill L, Coles E, Bronner-Fraser M. Snail2 directly represses cadherin6B during epithelial-to-mesenchymal transitions of the neural crest. Development. 2007; 134:1481–1490. [PubMed: 17344227]

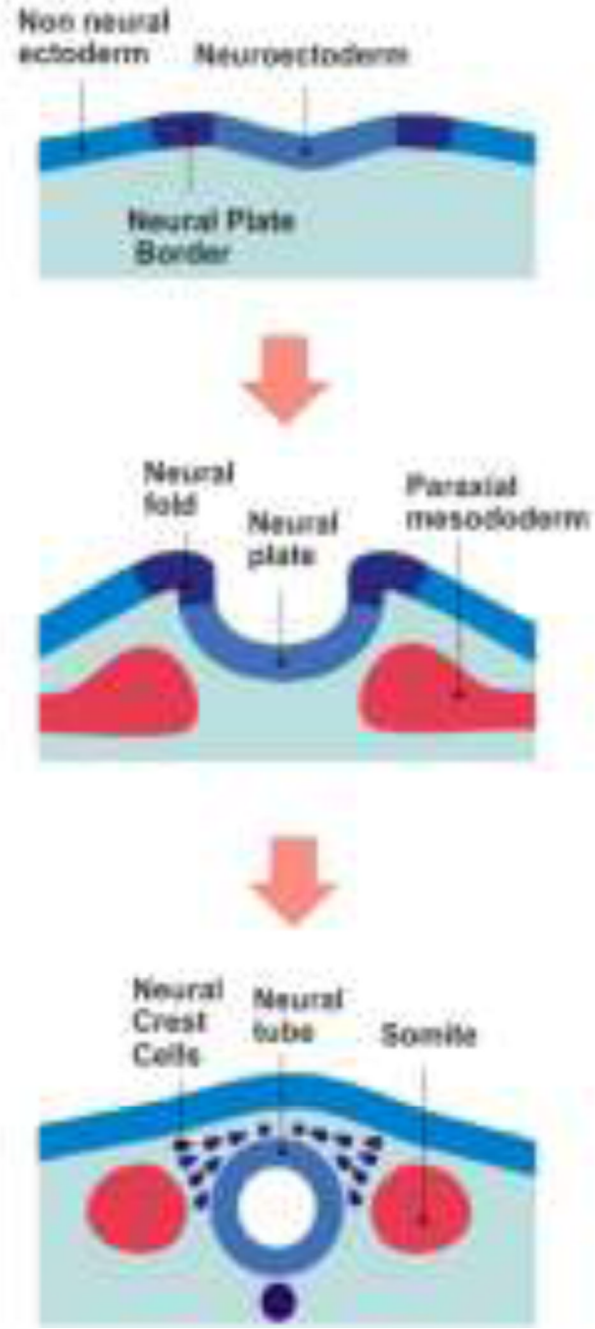

Fig. 1.

Schematic diagram illustrating the process of neural crest formation.

At gastrulation stages (top panel), the neuroectoderm is a flat neural plate (future central nervous system) that apposes the non neural ectoderm (future epidermis). The neural plate border (purple) contains presumptive neural crest cells. As neurulation proceeds (middle panel), the neural folds (purple) have risen, and from the neural plate which will close to form the neural tube. After neural tube closure (bottom panel), neural crest cells undergo an epithelial to mesenchymal transition and migrate from the neural tube into the peripheral as individual mesenchymal cells. At trunk levels illustrated here, they migrate around the

somites with those cells migrating dorsally above the somites forming melanocytes whereas those migrating ventrally forming sensory and sympathetic ganglia.

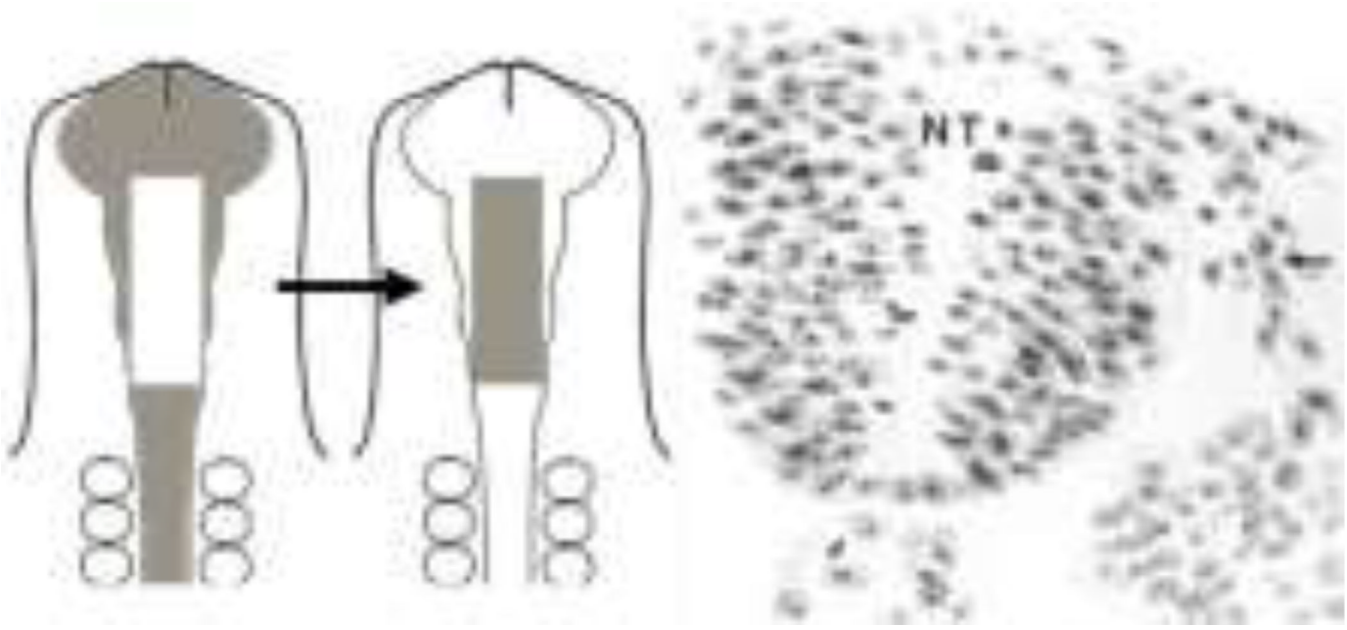

Fig. 2.
Quail-chick chimera. Diagram showing how neural folds from quail (gray) are grafted into a chick host. Section through at embryo showing Feulgen stained quail cells (arrow) migrating into the periphery after grafting into chick host. (adapted from Bronner and LeDouarin, 2012).

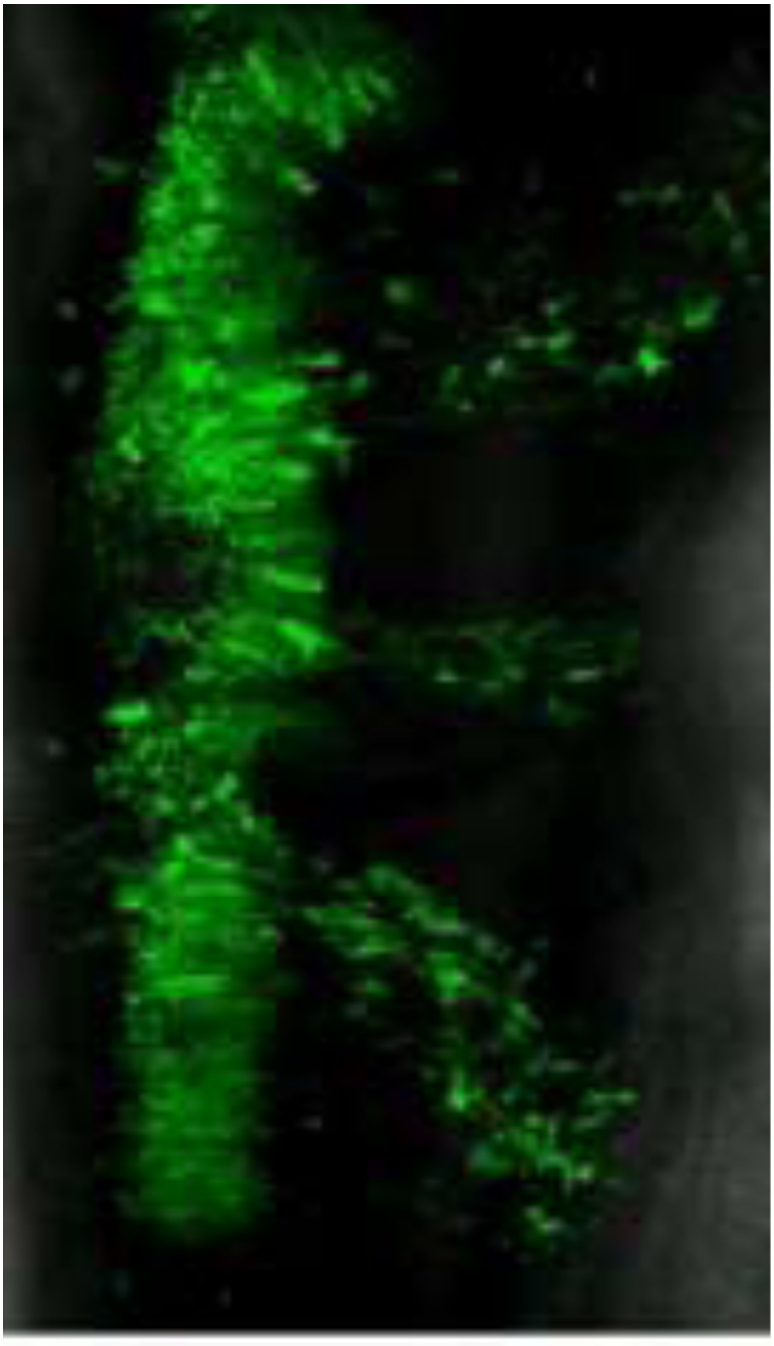

Fig. 3.
A frame of a time lapse moving from an embryo imaged after a construct encoding green fluorescent protein (GFP) was electroporated into the right side of the neural tube. Because neural crest cells are the only cells to migrate from the neural tube, it is possible to follow their migration in real time from the neural tube to the periphery.

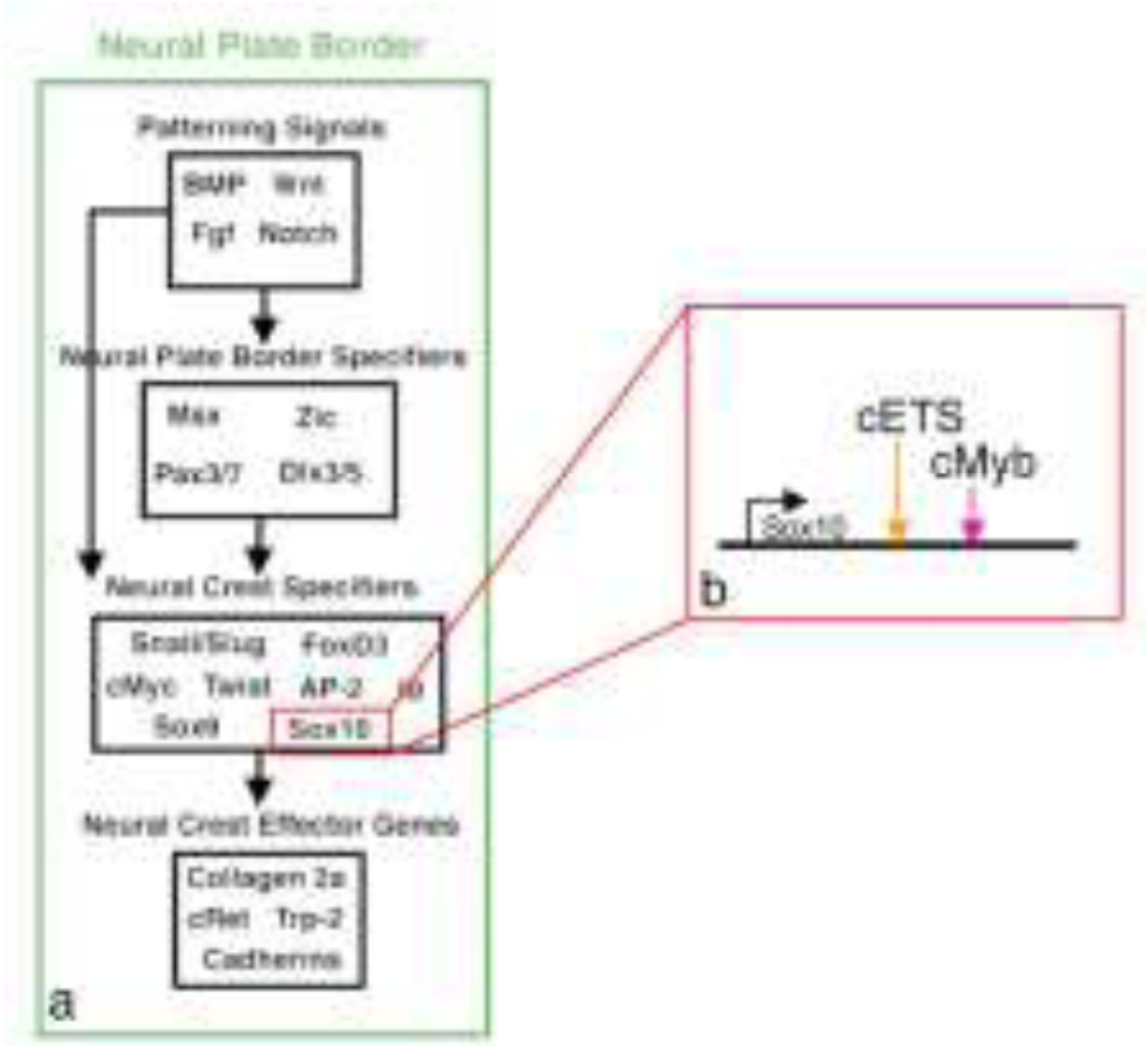

Fig. 4.

(a) A putative gene regulatory network helps to explain the molecular progression of neural crest formation. First, inductive patterning signals, including BMPs, Wnts, and FGFs cooperate to induce the neural plate border containing cells with neural crest potential. These signals in turn up-regulate a suite of genes at the neural plate border, including Pax, Zic, and Msx genes. The neural plate border genes in turn regulate neural crest specifier genes, like Sox10, FoxD3 and Snail2. These help to mediate neural crest EMT by regulating cadherins as well as numerous differentiation genes. (**b**) Cis-regulatory analysis of Sox10 and other genes are helping to expand the NC-GRN by identifying new inputs (e.g. Ets1 and Myb transcription factors) as well as demonstrating direct regulatory interactions. The newly identified neural crest enhancers also serve as useful tools for imaging, and cell type specific over-expression and knock-down.

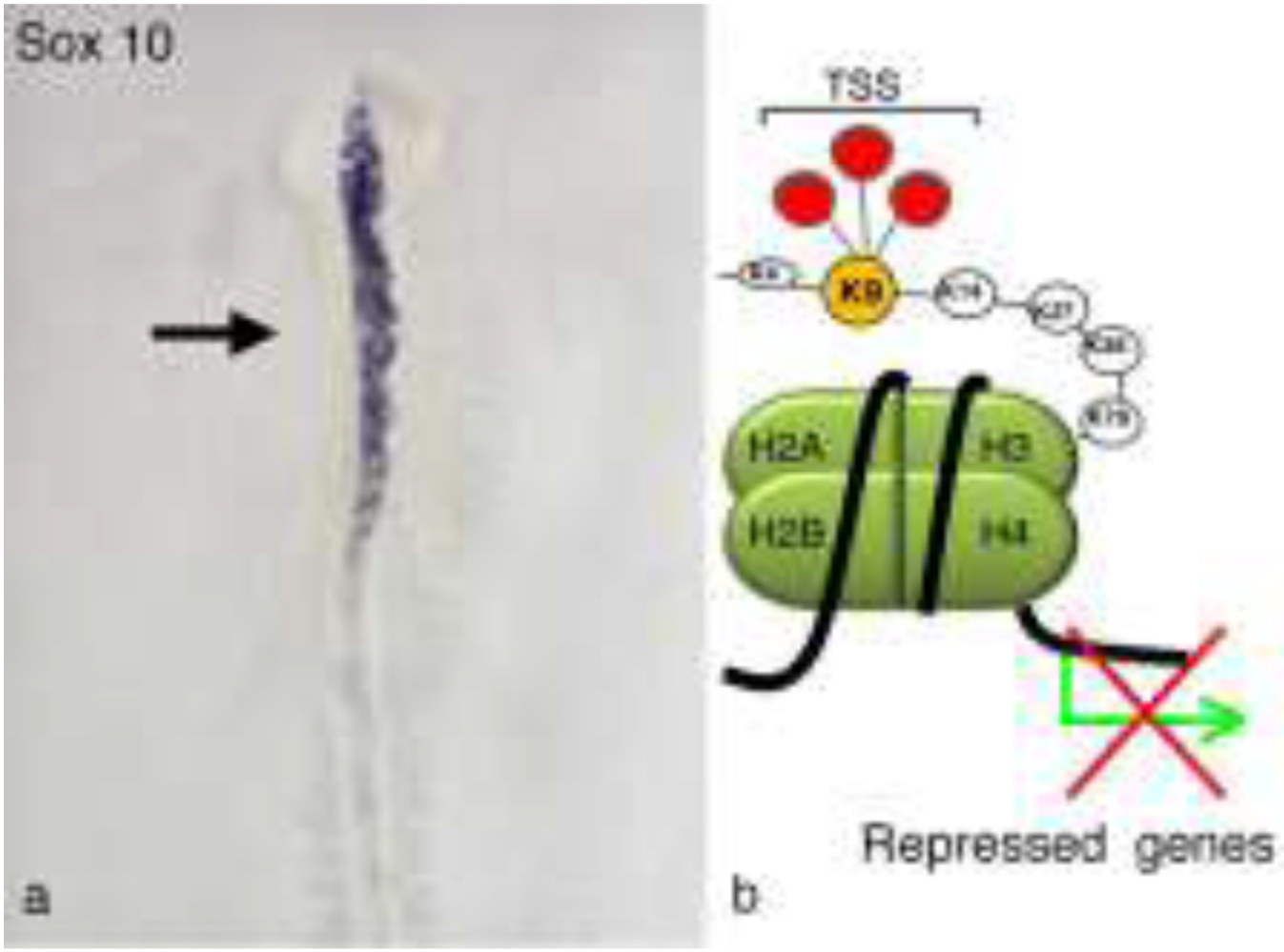

Fig. 5.

(a) Morpholino knock-down of the histone demethylase JmjD2A causes loss of the neural crest specifier gene Sox10 on the electroporated (black arrow) side, whereas there is normal expression on the control, non-electroporated side. **(b)** Schematic diagram shows the repressive state when histone H3 is trimethylated on lysine 9. JmjD2A specifically removes this repressive mark, thereby allowing onset of Sox10 transcription at the appropriate stages. After morpholino-mediated loss of JmjD2A, however, the repressive mark remains on and Sox10 transcription is inhibited. (Data from Strobl-Mazzulla et al., 2010).