Hox proteins as regulators of extracellular matrix interactions during neural crest migration

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Key words: Hox, neural crest, ECM, EMT, Integrin

Abstract

Emerging during embryogenesis, the neural crest are a migratory, transient population of multipotent stem cell that differentiates into various cell types in vertebrates. Neural crest cells arise along the anterior-posterior extent of the neural tube, delaminate and migrate along routes to their final destinations. The factors that orchestrate how neural crest cells undergo delamination and their subsequent sustained migration is not fully understood. This review provides a primer about neural crest epithelial-to-mesenchymal transition (EMT), with a special emphasis on the role of the Extracellular matrix (ECM), cellular effector proteins of EMT, and subsequent migration. We also summarize published findings that link the expression of Hox transcription factors to EMT and ECM modification, thereby implicating Hox factors in regulation of EMT and ECM remodeling during neural crest cell ontogenesis.

1. Introduction: Neural Crest Cell Development

Neural Crest Cells (NCCs) are a migratory and multipotent stem cell population common among vertebrate species. These transient embryonic stem cells arise from the dorsal-most aspect of the neural tube (**Figure 1**)—a transient embryonic structure fated to become the entire central nervous system—and begin extensive and stereotypic migratory paths throughout the developing mesendoderm. (Le Douarin, 1982; Le Douarin and Kalcheim, 1999; Saint-Jeannet, 2016). NCCs contribute to numerous and diversely specialized tissues; including, for example, craniofacial skeleton, corneal endothelium and stroma, auditory skeletal structures, sympathetic and sensory neurons, enteric nervous system, several classes of pigment producing cells across species, as well as many other tissues types(Jasrapuria-Agrawal and Lwigale, 2014; Lapedriza et al., 2014; Matsuo et al., 1995; Prendergast and Raible, 2014). Indeed, the field has come to appreciate that the spectacular diversity of structures formed by NCCs highlights their crucial role in vertebrate development.

NCC ontogenesis occurs in four major stages: specification, delamination, migration, and differentiation (**Figure 1**). NCCs first emerge at the dorsal aspect of the neural tube along the majority of the anterior-posterior (A-P) axis of the elongating embryo in response to a mélange of signaling factors; including, WNTs, BMPs, FGFs, Retinoic Acid, and TGFβ signaling (Conlon, 1995; Goldstein et al., 2005; Hosokawa et al., 2010; Li et al., 2009). At the time of specification, NCCs can be segregated into four major subpopulations which are collinear with their axial region of origin; namely the cranial, vagal, trunk, and sacral (Bronner, 2012; Rothstein et al., 2018) Shortly after their specification, NCCs delaminate from the neural tube and assume mesenchymal character just prior to their emigration, a process known as epithelial-to-mesenchymal transition (EMT)(Taneyhill and Padmanabhan, 2014). Following delamination, NCCs migrate along stereotypic routes throughout the embryo, guided both by NCC-mesenchyme interactions and chemotaxis. The migration paths of NCCs can be predicted by the axial region of origination—the most anterior NCCs migrate rostrally to give rise to structures in the head, while more posterior NCCs give rise to more posteriorly fate structures. Following arrival in the target tissue, NCCs further undergo changes to assume their stably differentiated tissue types.

As NCCs emigrate and migrate throughout the early vertebrate body, NCCs interact with, express, and modify a diversity of extracellular matrix (ECM) constituents. The ECM itself is a diverse and dynamic microenvironment comprised of hydrated scaffold proteins, polysaccharides, and signaling molecules, which fundamentally provide structure and support for cell adhesion and form in tissues (Frantz et al., 2010). Across a number of stem cell niches, the stem cell deposition of and interaction with ECM defines a large number of their most important properties, such as retention of stem state, survival, and onset of differentiation programs (Gattazzo et al., 2014). Undeniably, NCC-ECM interactions are involved in EMT, migration, and differentiation in the embryo, which are summarized here. Further, while tremendous research efforts have been made toward elucidating biomechanical and cellular roles of NCC-ECM interactions, the question of which transcriptional regulators directly regulate the expression of ECM and ECM-interacting components in NCCs remains to be comprehensively addressed. Intriguingly, mounting evidence exists which positions Hox transcription factors as potential modulators of both ECM constituent proteins and cell-surface ECM-interaction components. Thus, we present a primer into the role of the ECM in NCC development, as well as review the potential role of Hox transcription factors as regulators of the NCC-ECM interactions.

2. Neural crest EMT and migration are regulated through ECM interactions

EMT in the NCC is canonically marked by upregulation of the genes *Snail1*, *Snail2* and *Twist1,* which encode for transcription factors that globally regulate EMT. Each of these transcription factors does so by controlling cellular programs and initiate NCC migration by repressing cell-cell adhesion mediating proteins, such as E-cadherin (adherens junctions), Occludins (tight junctions), and Desmoplakin (Desmosomes) (Ohkubo and Ozawa, 2003; Yang et al., 2010; Yook et al., 2006). Additionally, EMT is marked by species-dependent switch in Cadherin composition (Rogers et al., 2018), secretion of Fibronectin and Laminin, and reorganization of actin through expression of Integrins (Henderson and Copp, 1997; Lamouille et al., 2014), all of which coordinate active, directed cellular migration of NCC. Thus, through the loss of tight cell adhesion and promotion of a more mesenchymal cell type, *Snail1*, *Snail2*, and *Twist1* all initiate the dramatic migratory phenotype intrinsic to NCC function.

NCC remodeling of the ECM is a critical aspect of both NCCs undergoing EMT and throughout NCC migration to target tissues. The function of the ECM as a director of NCC migration has recently been thoroughly reviewed (Leonard and Taneyhill, 2020). Building upon this prior summary, a hallmark of NCC migration is the upregulated expression and secretion of ECM proteins, such as the Fibronectin family, which enables a more permissive microenvironment for cell migration(Bilozur and Hay, 1988; Boucaut et al., 1984; Monier-Gavelle and Duband, 1997). NCC interaction with secreted Fibronectin proteins is primarily mediated by the Integrin family of transmembrane proteins, which promote cell adhesion, migration, actin polymerization, cell proliferation, and cell survival(Huttenlocher and Horwitz, 2011; Zeltz and Gullberg, 2016). Antibody blockade of Integrin signaling *in vitro* disrupts NCC ability to interact with fibronectin and inhibits migration (Monier-Gavelle and Duband, 1997; Strachan and Condic, 2003). As such, regulation of Integrin expression is critical to NCC migration. Additionally, not only are ECM constituent proteins secreted by NCC, the existing ECM is proteolytically remodeled before and during NCC migration, typically through secretion of matrix metalloproteinases (MMPs) (Small and Crawford, 2016). Expression of certain MMPs, including ADAM13, MMP14, MMP16, and MMP9, have been shown by either gain or loss of function in *Xenopus* and chick embryos to be crucial for cranial NCC for migration (Alfandari et al., 2001; Garmon et al., 2018; Monsonego-Ornan et al., 2012; Roth et al., 2017). MMPs themselves appear to play even more complex roles in NCC EMT, beyond their catalytic function. Both MMP14 and MMP28 have been observed to have nuclear localization, facilitated through either NCC-centric autocrine or paracrine signaling paradigms, implying a more directed function of these MMPs in EMT progression (Andrieu et al., 2020; Gouignard et al., 2021). Therefore, NCC modulation of ECM by direct contribution, as well as active remodeling, supports a system where differential microenvironmental composition of permissive and non-permissive substrates facilitate NCC migration and EMT.

While NCC do differentially secrete ECM proteins (Duband and Thiery, 1987; Leonard and Taneyhill, 2020; Wang and Astrof, 2016), there is significant production of these components by cells in the migratory environment of the neural tube, ectoderm, and somites (Copp et al., 2011; Crawford et al., 2003; Latimer and Jessen, 2010). Importantly, while Laminins and Fibronectins are expressed by a wide variety of cell types, specific isoforms of each protein are differentially expressed throughout the developing embryo (Copp et al., 2011; Crawford et al., 2003). As Wang and Astrof (2016) demonstrated, the NCC-autonomous expression of Fibronectin is necessary for proper vagal NCC patterning in the heart. Further, antibody staining for Fibronectin expression

shows NCC likely begin secreting Fibronectin following EMT (Newgreen and Thiery, 1980). Laminins, which normally are expressed by cells adjacent to the basal laminae of a tissue, have been shown to play a more important role in NCC differentiation than migration. So, while post-EMT NCC may not upregulate Laminins at the time of EMT, Fibronectin is required as a substrate for correct NCC migration and is expressed cell autonomously and in the surrounding mesenchyme. Cumulatively, these findings highlight the centrality of ECM-NCC interactions in governing appropriate EMT and NCC migration.

3. Integrins as directors of NCC migration and survival

The significance of integrins in NCC migration lies primarily upstream of the formation of focal adhesion complexes, which allow NCC to apply the contractile force necessary to move through the embryo. Integrin binding domains are found on Fibronectins, Laminins, and Collagens. At sites where Integrin heterodimers bind to one of these ECM components, Focal Adhesion Kinase (FAK) is recruited and activated to the intracellular domain of the activated Integrins. FAK further initiates recruitment of accessory proteins and eventually the initiation of actin stress fibers (Huttenlocher and Horwitz, 2011). By regulating the polarity of surface integrin localization, NCC are capable of initiating actin polymerization in a directional manner. Directional and differential actin recruitment applies a force at the cell membrane, propelling the cell forward and enabling migration. While other regulatory and cues are mediated through integrin signaling, their function as mediators of focal adhesion complex formation has been well established in NCC(Breau et al., 2009; Mckeown et al., 2013). As an illustrative example, within the context of the periocular NCCs which give rise to the corneal endothelium and stroma, Integrin α8β1 expressed by NCCs interacts with Nephronectin in the ECM to facilitate migration into the eye (Ma et al., 2022). Inhibition of either component reduced the capacity of NCC to populate the cornea. The failure of periocular NCCs to migrate into the presumptive corneal stroma after Nephronectin-α8β1 abrogation was further shown to be mediated through depletion of focal adhesions. Indeed, considered here as a special case, Integrin mediated interactions with the ECM are fundamental drivers of NCC migration.

Integrin signaling is also known to impact both cell proliferation and survival, but NCC cell proliferation may be governed by a different mechanism (Lawson and Burridge, 2014; Moreno-

Layseca and Streuli, 2014; Pugacheva et al., 2006; Walker and Assoian, 2005). Integrin activation at the cell membrane regulates cell proliferation by inciting a phosphorylation cascade, mediated canonically by Erk signal transduction pathway. Ultimately, the phosphorylation cascade leads to the activation of Cdk4/CyclinD1 complex, initiating G1/S progression during the cell cycle. Interestingly however, NCC-specific depletion of Integrin α4β1 signaling by antibody blockade in chick embryos dramatically increased NCC cell death, but did not decrease the cell proliferation rate as shown by TUNNEL and BrdU assays, respectively (Testaz and Duband, 2001). In partial corroboration of these data, Cre knockout of *Integrin β1* exhibited no change in proliferation rates and did not demonstrate the same NCC-specific cell death in mouse NCC fated for the enteric nervous system(Breau et al., 2006), which differs from Integrin α4β1 functional depletion in chick. Lastly, *Integrin α5β1* null mice showed cranial NCC specific cell death(Goh et al., 1997). As such, loss of Integrin signaling is not sufficient to decrease cell proliferation rates, but may be required in some NCC populations for cell survival. Further work, including analysis of gain of integrin function, is necessary to elucidate the precise role integrins may play specifically in directing NCC proliferation.

Overall, multiple observations—the distinction in ECM composition in various embryonic regions, diversity of integrin heterodimer subtypes (Takada et al., 2007), and regulation of cell surface localization of integrin subtypes—brings to light the possibility of complex regulatory mechanisms which may govern NCC development. Indeed, NCC migration, then, is directed not only by NCC-autonomous expression of integrins, but also by the availability of their local ECM substrate. Additionally, cell survival signaling in migrating NCCs may be facilitated by Integrins. In this way, the Integrin-ECM interaction go beyond facilitating cell adhesion, but specifically directs cell migration and survival in NCCs.

4. Non-integrin ECM interactions with the microenvironment facilitate NCC migration

Beyond Integrin-ECM interactions, NCC migration is guided by additional microenvironmental cues. Protein-Protein interactions on the NCC surface between Eph-Ephrins and Semaphorins-Neuropilins are non-permissive to cranial NCC migration (Davy et al., 2004; Kuriyama and Mayor, 2008; McLennan and Kulesa, 2007). Eph are a family of Tyrosine Kinase Receptors expressed by cranial NCC and detect Ephrins, another class of membrane bound ligand proteins. In the

cranial domain, Ephrins are expressed by the mesenchyme that lies between each cranial NCC stream into their respective pharyngeal arches (PAs), structures which make up much of the lower cranial structures, and restricts the Eph-positive NCC into discrete migratory routes (Santiago and Erickson, 2002). Homozygous knockout of *EphrinB2* leads to mislocalization of mouse cranial NCC, however expression of the extracellular domain is sufficient to rescue proper NCC migration and arch invasion(Adams et al., 2001), clearly demonstrating that *EphrinB2* functions as the required ligand to direct cranial NCC infiltration. Similarly, the Neuropilin and Plexin family of cell surface proteins heterodimerize to mediate NCC migration, where *Npn1* (*Neuropilin 1*) knockdown by siRNA in chicken embryos prevented NCC from migrating completely into the 2nd PA (McLennan and Kulesa, 2007). Semaphorins, the ligand for Neuropilin receptors, are secreted by non-neural crest mesenchyme and are required for proper pattering of certain cranial NCC populations (Lepore et al., 2006). Thus, Semaphorin-Neuropilins and Eph-Ephrin interactions modulate NCC migration by altering the way NCC interact with their microenvironment. Together with Integrin signaling, the layered regulation afforded by these cell surface mechanisms direct NCC migration in a complex manner necessary to give rise to multiple and distinct NCC fates.

5. Hox genes as potential regulators of NCC migration and proliferation

The broad diversity of ECM constituents and NCC-localized cell surface receptors provides a remarkable regulatory framework to regulate both EMT and migration throughout the vertebrate embryo. The migratory routes chosen by NCC subpopulations are spatially separated along the A-P axis, collinear, at least part, with the expression of Hox transcription factors. This ancient family of genes shares a high degree of conservation in both function and organization between diverse organismal lineages, from flies to fish, from mice to humans (**Figure 2**) (Mallo and Alonso, 2013). Primarily known for their role in establishing the A-P axis, select Hox family members have been shown to play an integral function in tumor metastasis (Hong et al., 2015; Wu et al., 2006), cell proliferation (Krosl et al., 1998; Lee et al., 2015), angiogenesis (Amali et al., 2013; Mace et al., 2005), and pharyngeal arch formation (Trainor and Krumlauf, 2001), among other functions. Hox transcription factors in mammals are organized into discrete paralogous clusters across four chromosomes and are numbered according to their position in the cluster (**Figure 2**). Intriguingly, Hox genes which occur earlier within the chromosome, that is to say with a Hox gene with a lower

number, are expressed more anteriorly, while later genes are expressed more distally from the head.

The close association between the A-P expression of Hox transcription factors and the stereotypic paths chosen by NCCs during their development presents a tantalizing model where the cellular signals which regulate NCC segregation into their terminal cell types may be rooted in combinatorial Hox transcriptional regulation, which has long been under investigation (Parker et al., 2014; Parker et al., 2018; Parker et al., 2019a; Trainor and Krumlauf, 2000; Trainor and Krumlauf, 2001). Particular research efforts have been applied to discerning the role of Hox expression within cranial neural crest, which contribute cell lineages to the lower jaw (Sandell and Trainor, 2006). The earliest Hox expression in vertebrate systems, supported with evidence from mouse, zebrafish, *Xenopus laevis*, and human organoids, is detectable along the level of the hindbrain in the rhombomeres (r), the earliest segment of expression ranging from r2 to r3 depending on species specific contexts (Libby et al., 2021; McNulty et al., 2005; Schilling et al., 2001; Wilkinson et al., 1989). Prior to the rhombomeric Hox expression domains, NCC patterning in the anterior embryo is largely ruled by the non-Hox homeobox transcription factors *Gbx2, Otx1*/*Otx2*, and *Emx1*/*Emx2,* as evidenced by data collected across multiple species (Byrd and Meyers, 2005; Li et al., 2009; Matsuo et al., 1995; Roeseler et al., 2020; Steventon et al., 2012). Within the Hox-positive domain, overlapping and combinatorial expression of Hox genes from different clusters direct specific migration of NCCs into the correct PA. Disruptions in Hox gene expression in facial patterning, such as mutations in *Hoxa2, Hoxb3,* and *Hoxb4*, results in either the fate transformation or abrogation of cranial NCC migration into the PAs (Gendron-Maguire et al., 1993; Kitazawa et al., 2015; Nolte et al., 2019; Simeone et al., 1991). In the case of *Hoxa2* in particular, loss of function in a murine model converts the second-PA fate to mirror that of the first, duplicating jaw and presumptive auditory structures (Gendron-Maguire et al., 1993). Ectopic *Hoxa2* expression in chick conversely abolished first-PA structure, the formation of which requires Hox-negative NCC contribution (Gavalas et al., 2003). Together, these experiments demonstrate the important and conserved role of anteriorly expressed Hox transcription factors in formation of cranial NCC derived tissues.

Due perhaps to the robust nature of Hox-related phenotypes which are manifest at later developmental stages, the functional role of Hox transcription factors in earlier NCC developmental programs, such as during EMT and specification, has been less well

characterized. Aligning with a previously posited hypothesis published in Taniguchi, 2014, below we highlight that Hox regulation on NCCs appears to be mediated at least in part by regulation of reciprocal ECM interactions.

As previously noted, ECM interactions are foundational drivers of early NCC development. Importantly for this discussion, expression of several of the ECM modulators and components discussed previously are known to be influenced by *Hox* gene expression. Wu et al. (2006) found that *Hoxb7* overexpression in breast cancer cell lines lead to downregulation of *E-cadherin* and *Claudin* expression, which correlated with increased EMT in tumor cells. Contextualizing this finding with respect to NCCs, suppressed expression of Claudin-1 in chick NCCs is known to increase NCC emigration from the neural tube (Fishwick et al., 2012) and both *Claudin-1* and *Hoxb7* are proximally expressed at early embryonic stages (Bell et al., 2004; Darnell et al., 2007; Simard et al., 2005). Further, *Hoxa2* in chick NCC promotes EMT through the repression of *Cadherin-6b* (Gouti et al., 2011), while mouse *Hoxa1* activates *Cadherin-6b* expression in a transient manner (Inoue et al., 1997). Hox control of Integrin expression is supported by the observation that *Hoxd3* upregulates *Integrin β3* expression in human umbilical vein endothelial cells (Boudreau et al., 1997) which has been shown to increase NCC migratory phenotype (Boudreau et al., 1997; Monier-Gavelle and Duband, 1997). Additionally, Integrin heterodimers *αVβ3* and *α5β1*, are both under direct regulation of *Hoxd3* as identified in a number of cancer types (Boudreau and Varner, 2004).

Reciprocally to direct Hox transcriptional regulation of ECM components, signaling induced by ECM may also impact the expression of Hox genes themselves. *Integrin α5β1* null mice showed a decrease in *Hoxb9* expression along the posterior aspect of the embryo (Goh et al., 1997). Notably, in the same *Integrin α5β1* null mice, anterior expression of *Hoxb-1*, *Hoxb-4*, and *Hoxb-5* were all reportedly similar to wild type mice (Goh et al., 1997). Moreover, both Eph and Neuropilin, whose expression are essential to segregating NCC migration into discrete streams (Kuriyama and Mayor, 2008), are likely to be differentially expressed under combinatorial control by *Hoxd4* and *Hoxb4* expression, at least in part (Prin et al., 2014).

Each of the above-described examples contribute to a model in which Hox transcription factors may directly regulate NCC EMT and migration through transcriptional control of ECM and ECM components. Hence, the role Hox transcription factors play in NCC development may be

intrinsically tied to transcriptional regulation of NCC microenvironmental interactions, which involves both interaction with and modulation of ECM components. Several NCC cell-autonomous regulatory targets have been summarized here, namely those in the Integrin, Ephrin, and Neuropilin families. These data would position Hox genes as potent drivers of early NCC developmental programs, expanding on previous models focused on Hox regulation on later fate acquisition in NCCs.

Beyond EMT and migration, the NCC proliferation rate appears to be at least partially integrin independent (Goh et al., 1997; Testaz and Duband, 2001), which may indicate an alternate mechanism for NCC cell cycle regulation by Hox factors. Supporting a model where *Hox* genes regulate cell cycle progression, *Hoxb7*-driven *ex vivo* cultured tumors were highly proliferative when transplanted into immunodeficient mice (Wu et al., 2006). Furthermore, the hyper-proliferative nature of T47D and MCF7 breast cancer cell lines has also been attributed to altered *Hoxb5* expression, as both gain and loss of function respectively increased or decreased cancer cell proliferation rate (Lee et al., 2015). In fact, a novel binding site for a HoxA9-dependant transcriptional complex was shown to be upstream of the Cyclin-dependent Kinase Inhibitors Cdkn2a/b, the repression of which allows for G1 initiation (Collins et al., 2014). The conserved role of direct Hox activation of cell cycle gene expression is supported by experiments in *C. elegans* involving the *Hox* ortholog *lin-39* as an upstream activator for cdk-4 and cye-1 (cyclin E) (Roiz et al., 2016). Indeed, while the possibility remains for NCC cell cycle control via Integrin signaling, there is a growing body of evidence for direct regulation of expression of cell cycle regulatory genes in NCC by Hox transcription factors.

Because of the nested expression domains of the *Hox* genes in the NCC, a complex "*Hox* code" emerges as a likely mechanism for directing NCC migration towards a correct target tissue. A combination of active *Hox* gene expression modules label various NCC subpopulations along the anterior-posterior axis. Studying these combinations, however, is partially complicated due to functional redundancy of many proximally expressed and true paralogous *Hox* genes (Boucherat et al., 2013; Horan et al., 1995; Hunter and Prince, 2002; Jarinova et al., 2008). Despite this possibility for redundancy, much of what is known regarding *Hox* function is derived from experiments involving loss of a single gene. A chief challenge in determining a mechanism underlying the NCC-ECM-Hox axis will be elucidating not only the impact of individual Hox perturbation on the NCC-ECM interactions, but also, perhaps more importantly, how combinations

of Hox genes work in concert to direct the same interactions. Consideration of combinatorial Hox codes are already shedding refreshing light on other developmental contexts (Alberstat et al., 2022; Parker et al., 2019b; Yamada et al., 2021), which if applied to NCC promises a tantalizing prospect of a mechanism for NCC subtypes selection through discrete activation of ECM-interaction modules.

As reviewed in this manuscript, *Hox* genes can be modeled as regulators of the NCC microenvironment, which includes control of both the contribution to ECM dynamics, as well as the NCC-ECM interactions used during migration. These data, collectively, begin to describe a putative mechanism of action for Hox regulation of cranial NCC (**Figure 3**); combinatorial Hox expression in NCC controls delamination/migration by differentially regulating cell surface receptors and ECM modulation. Putatively, regulation may include the direct transcriptional control of NCC-autonomous ECM interaction molecules expression, such as Integrins and Ephrins. Additionally, NCC cell cycle progression appears to be under direct Hox influence for certain NCC populations. The involvement of Hox regulation on ECM composition is an emerging topic and is an exciting area for further exploration (Akin and Nazarali, 2005; Boudreau and Bissell, 1998; Taniguchi, 2014). Significantly, these results suggest an intrinsic connection between ECM composition, Hox expression, and NCC EMT/migration, which warrants further investigation in other NCC populations beyond the cranial NCC.

Figure 1

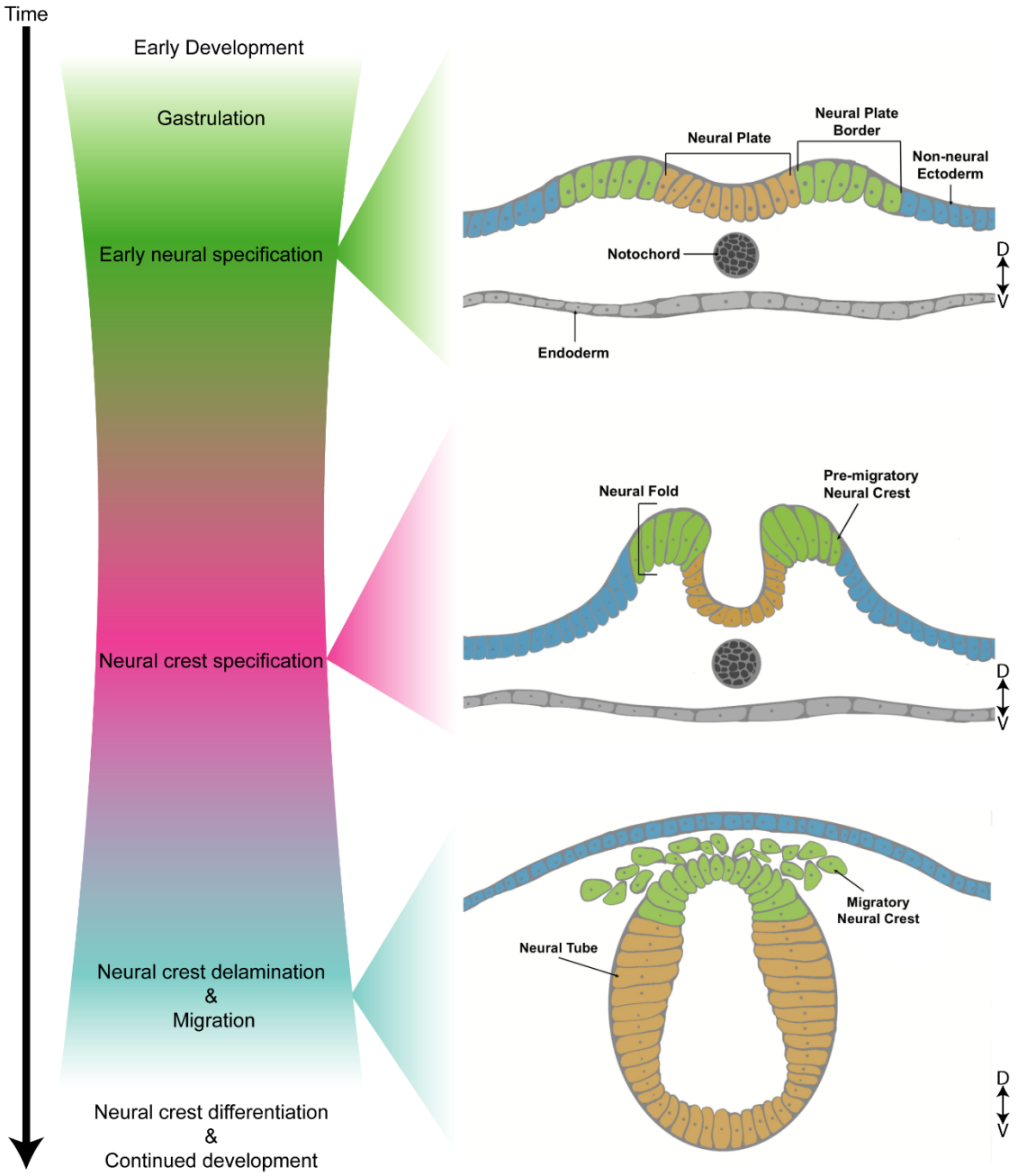

Figure 1. **Generalized schematic of the stages of neural crest cell (NCC) development**. NCCs are first specified following gastrulation in bilateral stripes in border adjacent to the neural plate. These neural plate boarder cells will undergo a dramatic morphogenic rearrangement, which various species to species, to fold inward to and reside toward the dorsal aspect of the neural tube. During and immediately after this morphogenesis, the now specified NCCs will undergo an epithelial-to-mesenchymal transition to complete delamination, and begin stereotypic migratory journeys toward specific tissues throughout the embryo. Upon arriving in this target tissues, NCCs switch on diverse tissue dependent gene regulatory programs to differentiate into a multitude of tissue lineages.

Figure 2

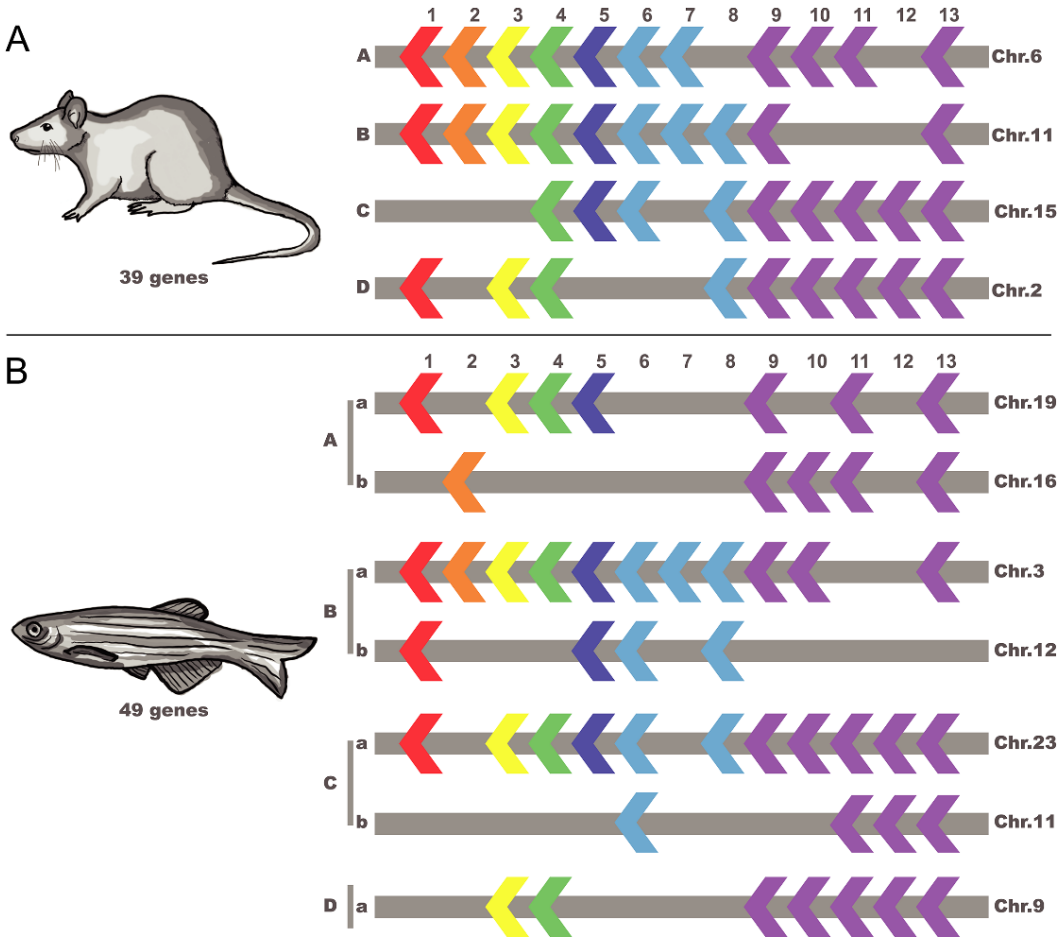

Figure 2. Schematic of the chromosomal organization of murine and zebrafish Hox gene clusters. (A) Hox genes in mice are arranged on four chromosomes with each paralogy group (PG) bearing a label A-D. PGs are defined by sequence similarity. As such, *Hoxa1, Hoxb1,* and *Hoxd1* all are members of PG1. The most 5' (left) Hox genes are expressed more anteriorly. (B) In zebrafish, due to a teleost-specific whole genome duplication event, PGs A-C are duplicated across an additional 3 chromosomes. It should be noted that even with this duplication, there is a high degree of conservation of synteny, order, and representation across both species.

Figure 3

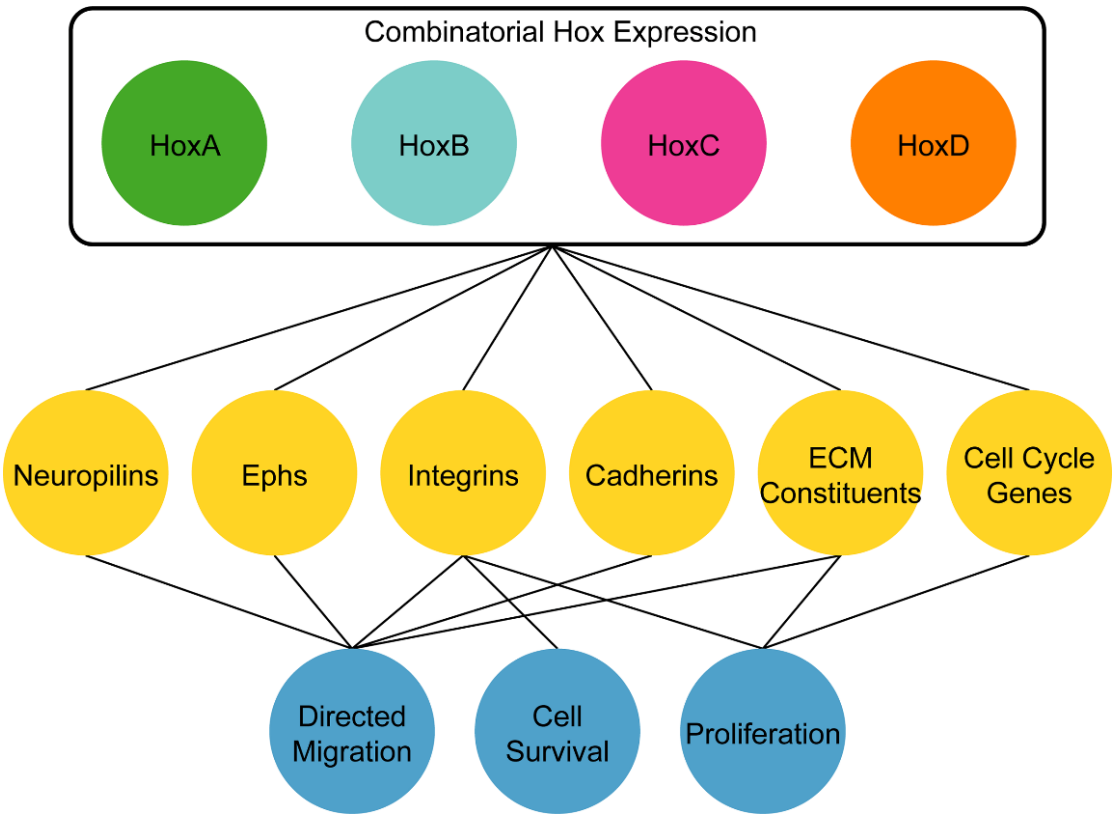

Figure 3. Model of Combinatorial Hox regulation of NCC microenvironment and cell cycle. Different combinations and expression levels of nested Hox genes, for example shown here from paralogy group A-D, leads to different combinations of cell surface protein which allow for differential NCC migratory behavior. The putative combinatorial "Hox code" may work in concert with differentiation gene networks to determine the final cell type contribution for each NCC subpopulation.

Acknowledgements

We acknowledge funding from Cancer Prevention and Research Institute of Texas (CPRIT) Recruitment of First-Time Tenure Track Faculty Members (CPRIT-RR170062), from NSF CAREER Award (1942019) and National Institutes of Health DK124804 awarded to R.A.U. We thank Jessa Westheimer and Ashley Tsang for assistance with figure designs.

Conflict of Interest

R.A.U. is a coauthor of the manuscript and an editor of *Differentiation* and was not involved in the handling of the peer review process of this submission.

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