Chapter 1

Introduction

1.1 Embryonic origin of the cranial sensory ganglia in vertebrates

In vertebrates, the cranial sensory ganglia have a unique, dual embryonic origin from both neural crest and ectodermal placodes. These two distinct embryonic cell types are responsible for the formation of the entire peripheral nervous system. The cranial ganglia are aggregates of neuronal and glial cells of the sensory component of the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves. Sensory neurons in these ganglia are responsible for relaying information from external and internal stimuli back to the brain to mediate somatosensation (touch, pain, and temperature) of the head region, the special sense of taste, and autonomic visceral sensation of internal organs (such as heart and gut). Not only are these ganglia crucial for the normal function of the nervous system, but they also are an outcome of a collaborative and interactive developmental process between two cell populations.

Neural crest cells arise from the most dorsal aspect of the neural tube in a rostrocaudal progression along almost the entire extent of the body axis. They undergo an epithelial-to-mesenchymal (EMT) transition to detach from the neuroepithelium, migrate along well defined pathways, and give rise to a plethora of derivatives including the craniofacial cartilage and bone, teeth, pigment cells, endocrine cells, smooth muscle, and glia and neurons in all the peripheral ganglia (sensory, sympathetic, and parasympathetic) (Baker, 2005). Grafting experiments of the neural folds (presumptive neural crest) at cranial levels show that in general these cells are not fate restricted to the axial level of their

origin but rather that environmental cues experienced during migration and/or localization have a significant influence on their fate (reviewed in (Baker, 2005; Le Douarin and Kalcheim, 1999). Ectodermal placodes can be divided into two general classes: sense organ and neurogenic. The exception is the adenohypophyseal placode which forms the anterior pituitary gland. First, the sense organ placodes give rise to the paired sense organs (the inner ear, olfactory epithelium, the lens, and the lateral line system in anamniotes) and second, the neurogenic placodes give rise exclusively to sensory neurons in the cranial sensory ganglia (Baker, 2005); however this naming system is not to confuse the point that sense organ placodes also give rise to neurons. The neurogenic placodes are the ophthalmic (OpV) and maxillo-mandibular (MmV) placodes in the trigeminal ganglion, and the geniculate, petrosal, and nodose placodes that give rise to the distal portions of the facial (VII), glossopharyngeal (IX), and vagal (X) ganglia, respectively— generally grouped as the "epibranchial" placodes since they form around the second to the fourth branchial arches. The placodes are discrete regions of the head ectoderm, usually thickened, and found as pairs on both sides of the neural tube. For example, the surface ectoderm from which the trigeminal placodes arise is mostly uniform, except for sporadic spurs or thickenings where cells appear to be delaminating from the surface (D'Amico-Martel and Noden, 1983; Hamburger, 1961) (figures in this thesis). Unlike the neural crest, the placodes represent a diverse and rather heterogeneous population that exhibits varied fate specification processes and morphogenetic movements, which are extensively reviewed elsewhere (Baker, 2005; Baker and Bronner-Fraser, 2001; Begbie and Graham, 2001b; Schlosser, 2006).

The dual origin of the cranial ganglia was historically controversial (Hall, 1999; Hamburger, 1961; Le Douarin and Kalcheim, 1999). Although both the neural crest and placodes were independently discovered over one hundred years ago in the late 19th century— the neural crest by Wilhelm His (His, 1868) in chick embryos and placodes by

van Wijhe (van Wijhe, 1883) in shark embryos (in Greek the name “placode” means “plate” or “flat shaped” and was coined by von Kupffer (von Kupffer, 1894)), their common contribution to the cranial ganglia was not accepted until more recently. The debates largely arose from differences in direct observation of embryos from different species and the results of tissue ablation experiments (Hamburger, 1961; Le Douarin and Kalcheim, 1999; Yntema, 1944). Due to the lack of reliable cell marking techniques to follow neural crest and placodal cell lineages, the origin of cranial ganglia was argued as being from neural crest, from placodes, or from both. Direct observations and tissue ablations have several limitations. Ablation experiments cannot definitively demonstrate the fate of the cells removed, because of possible indirect effects leading to the loss of a structure and/or embryonic regulation (compensation by another tissue or regeneneration). Furthermore, it is technically impossible to make any definite conclusions about their fates without molecular or cellular labels, since neural crest and placodal cells are indistinguishable once they have migrated into the mesenchyme. These issues were resolved by the use of radioactive labels, vital dyes, and interspecific grafting techniques that allowed long-term cell marking (such as the quail-chick grafting system (Le Douarin, 1973)). The contribution of neural crest to the cranial ganglia was first clearly shown using radioautographically labeled neural folds (Johnston, 1966) and by quail neural crest grafts (Noden, 1975; Noden, 1978). Ectodermal grafts labeled by Nile blue in salamander embryos (Amblystoma) directly showed placodal contribution to the ganglia (Yntema, 1937). However, it was not until the 1980s that quail-chick chimera labeling was used to firmly establish the two components of all the cranial sensory ganglia and their patterns of distribution (Ayer-Le Lievre and Le Douarin, 1982; D'Amico-Martel and Noden, 1983). Neural crest migration and development into cranial ganglia have also been demonstrated in other vertebrate systems (zebrafish, Xenopus, mouse) reviewed in (Hall, 1999; Le Douarin and Kalcheim, 1999). However, the relationship of neurogenic placodes with

neural crest is still best characterized in chicks. Nonetheless, placodes in the ganglion region have been found across vertebrates— in mammal (Batten, 1957), amphibian (Yntema, 1937), and fish (Nechiporuk et al., 2007); reviewed in (Schlosser, 2005; Schlosser, 2006)— and thus the dual origin of cranial ganglia across vertebrates is widely accepted. Even in humans, systematic characterization of serially sectioned human embryos suggests contributions from cranial neural crest and placodes to cranial ganglia (O'Rahilly and Muller, 2007) similar to that of mouse and chick embryos.

D'Amico-Martel and Noden (1983) grafted presumptive quail neural crest or placodal tissue to replace the homologous tissues in chick embryos. The distribution of these grafted quail cells marked by their unique nuclei (condensed heterochromatin) were examined in the nearly mature ganglia at embryonic day 12 (stage 38). Their results not only confirmed the dual origin of avian cranial ganglia, but also provided a relatively complete picture of the embryonic composition of the maturing ganglion. They show the segregation of neural crest- and placode-derived neurons along the proximal-distal axis; proximal closest to the hindbrain and distal closer to their innervated targets. They further show that all the glia (Schwann and satellite cells) were derived from neural crest. This demonstration in birds provides the currently available study of the contribution of neural crest and placodal cells to the cranial sensory ganglia.

1.2 Neural crest and placode interactions

The most suggestive evidence that interactions between neural crest and placode cells are involved in formation of ganglia has come from tissue ablations in chick embryos. Experiments of ablating either the presumptive neural crest or the placodal tissue were analyzed in the nearly mature trigeminal ganglion at embryonic day 8 (stages 34–35) or

later. Absence of either neural crest or placodes resulted in either dispersed or smaller ganglion (Hamburger, 1961; Lwigale, 2001). However, the effects on cell–cell interactions and ganglion assembly were not examined. Similarly, neural crest ablation in the epibranchial region also suggest a role for neural crest in organizing placode-derived neurons (Begbie and Graham, 2001a). In contrast, neural crest was not involved in induction of the trigeminal and epibranchial placodes (Begbie et al., 1999; Stark et al., 1997). In summary, these ablation experiments suggest an important role of neural crest on integrating and organizing placodal cells in both the trigeminal and epibranchial regions. These interactions appear to occur during ganglion formation but after initial placodal induction. One point to consider, however, about neural crest ablation is that this might yield an incomplete list of neural crest effects, since complete neural crest removal is nearly impossible to achieve. Neural crest cells are highly regenerative (Saldivar et al., 1997; Sechrist et al., 1995) and can also reroute to compensate for loss of neural crest (Kulesa et al., 2005; Saldivar et al., 1997), and therefore assessing the actual extent of neural crest ablation at the time of analysis is important for accurate interpretation of data.

Taken together, results from these experiments suggest that interactions between neural crest and placodes may be involved in ganglion formation; however the underlying molecular mechanisms are still unknown. Some questions yet to be addressed include: how do neural crest and placodal cells interact on a cellular level? do they communicate with one another? do their interactions take place during ganglion assembly and are they essential for proper gangliogenesis? Understanding neural crest–placode interactions would be a key to understanding the core piece of this developmental process.

1.3 How peripheral ganglia form

In the developing vertebrate embryo, aggregates of sensory neurons and glia called "sensory ganglia" are present along almost the entire neural tube (presumptive brain and spinal cord), mirroring the earlier pattern of the migratory neural crest streams. As described above, those in the head are called the "cranial sensory ganglia", which form pairwise along the hindbrain at even numbered rhombomeres. There are also an analogous set of sensory ganglia along the trunk neural tube (presumptive spinal cord) called the "dorsal root ganglia", and also a chain of sympathetic ganglia. In contrast to the cranial sensory ganglia, these are entirely derived from the neural crest, which have made studies on trunk ganglia more tractable and easier to interpret. The parasympathetic ganglia also are derived from neural crest which form near or within their target organs throughout the body.

In the trunk, neural crest cells migrate along well defined pathways through the repeated segments of the embryonic trunk called somites. They only migrate through the anterior, not the posterior half of each somitic sclerotome (Bronner-Fraser, 1986; Rickmann et al., 1985). This migration is thought to be guided by signals emanating from the somites with permissive cues in the anterior half and repulsive signals in the posterior half reviewed in (Le Douarin and Kalcheim, 1999). Later, neural crest cells coalesce into dorsal root ganglia adjacent to the neural tube and sympathetic ganglia more ventrally around the dorsal aorta. It has been widely accepted that the discrete migratory streams were the basis for the metameric organization of trunk ganglia formation (Keynes and Stern, 1988; Lallier and Bronner-Fraser, 1988). However, recent results have provided surprising new insights into trunk gangliogenesis that uncouples trunk neural crest migration pattern and the metameric organization of ganglia formation. Genetic evidence from neuropilin 2 and semaphorin 3F mutants show that signaling between this

receptor–ligand pair is critical for normal segmental trunk migration. However, the resulting non-segmental neural crest migration through both halves of the somites did not alter the normal metameric pattern of ganglion formation (Gammill et al., 2006). In vivo time-lapse imaging of trunk neural crest cells forming the sympathetic ganglia show that they initially migrate in segmental streams, but once they have reached the ganglion assembly site, they spread rostrocaudally before later sorting into discrete, metamerically localized ganglia (Kasemeier-Kulesa et al., 2005). These results suggest that signals other than those mediating guidance of neural crest migration are critical for positioning ganglion formation. Whether these signals come from the somites, some other tissue, or within themselves (e.g. cell–cell interactions) is unknown. The aggregation of neural crest cells into sympathetic ganglia appears to involve N-cadherin mediated adhesion (Kasemeier-Kulesa et al., 2006).

In contrast to the trunk ganglia, the position and shape of the cranial sensory ganglia appear to be at least in part regulated by the migration pattern of the cranial neural crest. Loss of semaphorin and neuropilin genes, which caused abnormal migration of neural crest cells in a crest-free zone (between two streams), led to aberrantly interlinked trigeminal and facial ganglia and abnormal positioning of neurons which are presumably at least in part placode-derived (Gammill et al., 2006; Schwarz et al., 2008). These results suggest that proper neural crest migration is important for normal cranial gangliogenesis and that neural crest cells may play an important role in organizing placodal neurons. In zebrafish, it has been suggested that chemokine signaling between trigeminal neurons and the hindbrain plus nearby tissues is involved in properly positioning the trigeminal ganglion (Knaut et al., 2005), but it is unclear if neural crest, placode, or both are affected. Loss of both Msx1 and Msx2 in mouse embryos induces apoptosis in cranial neural crest and results in severely disorganized or reduced neuronal population of the cranial ganglia (presumably due to defects in the placodal component) (Ishii et al., 2005). This suggests

that loss of cranial neural crest may affect placodal ganglion organization. So far, our understanding of cranial gangliogenesis has mostly come from studies of neural crest development. These studies have suggested neural crest play an essential role in cranial gangliogenesis and its possible interactions with placodal neurons. However, the nature of this interaction is uncharacterized, the role of placodal cells is elusive and the underlying molecular mechanisms mediating neural crest–placode signaling are unknown.

From the placodal perspective, the formation of cranial ganglia is poorly understood. Studies on neurogenic placodes have focused on the specification of trigeminal and epibranchial fates, which appear to depend on neural tube–ectoderm interaction involving Wnts and PDGF signaling in the trigeminal OpV region, but may also involve other signaling molecules (Lassiter et al., 2007; McCabe and Bronner-Fraser, 2008; McCabe et al., 2007; Stark et al., 1997). Induction of epibranchial fates is thought to be mediated by pharyngeal endoderm–ectoderm interaction mediated by BMPs (Begbie et al., 1999), as well as with Fgf signaling from the cranial mesoderm (Nechiporuk et al., 2007; Nikaido et al., 2007). At an earlier time point, the different types of placodes can be traced back to the anterior cranial neural plate border in the epiblast (also known as the "preplacodal" or "panplacodal" region), prior to neurulation, and it is thought that all placodal precursors undergo a common "preplacodal" specification before they become separated and diversified (i.e. acquisition of trigeminal and epibranchial fates)(Bailey and Streit, 2006; Streit, 2007). In summary, studies from trunk and cranial regions suggest three essential steps for formation of ganglion after the specification of the precursor cell types: first, migration of precursors to the site of assembly, second, tissue or cell–cell interactions, and third, cellular aggregation into discrete structures.

1.4 Slits and Robos: structure and function

Slits and their cognate receptors Robos (short for "Roundabouts") are evolutionarily conserved signaling molecules found in animals ranging from nematodes and fruit flies to mammals (Chedotal, 2007). The Slit mutant was first described in the classic genetic screen for embryonic patterning defects in *Drosophila* (Nusslein-Volhard et al., 1984). Later Slit and Robo (initially identified in a fly screen for axon commissure defects (Seeger et al., 1993)) were both identified in another screen for mutations in *Drosophila* commissural axon guidance (Hummel et al., 1999). In vertebrates, there are three homologs of Slit (Slit1–3) and four members of the Robo family (Robo1–4), whereas in *Drosophila*, there is a single Slit as well as three Robos (dRobo and dRobo2–3), which arose from independent gene duplication; vertebrate Robo2-3 are not orthologs of dRobo2–3. Slit was shown to be the ligand for Robo receptors based on their dosage-sensitive genetic and biochemical interactions and chemorepulsive function (Brose et al., 1999; Kidd et al., 1999).

Slits are large secreted proteins (about 200 kDa) but may not diffuse far as they have been found associated with the cells that secrete them (Brose et al., 1999; Hu, 1999; Niclou et al., 2000). They share four tandem leucine rich repeats (LRRs), seven (invertebrate) or nine (vertebrate) epidermal growth factor (EGF) like domains, a laminin G domain, and a C-terminal cysteine-rich knot (Chedotal, 2007). Slit2 has been shown to be proteolytically cleaved after the fifth EGF-like domain into 140kDa N-terminal and 60kDa C-terminal fragments in vivo and in vitro, though not all Slit2 are cleaved, since full-length Slit2 was also isolated (Brose et al., 1999; Nguyen-Ba-Charvet et al., 2001; Wang et al., 1999). The N-terminal Slit2 fragment has been shown to retain axon guidance activity (Chen et al., 2001; Nguyen-Ba-Charvet et al., 2001). It is unclear if all Slits undergo proteolytic cleavage; however, this may occur broadly depending on the cellular context.

Cleavage of human Slit3 but not hSlit1 was detected in mammalian CHO cells (Patel et al., 2001), and of *Drosophila* Slit in mammalian cell lines (293T and COS) and *Drosophila* embryo extracts, but not in *Drosophila* S2 cells (Brose et al., 1999).

Robos are structurally more varied—classical vertebrate Robos (Robo1–2) have five immunoglobulin-like (Ig), three fibronectin type III (FN3), a transmembrane, and four conserved cytoplasmic (CC0–3) domains, while Robo3 (Rig-1) and Robo4 (Magic Roundabout) are divergent members. Robo3 has no cytoplasmic domain 1 (CC1) and is implicated as a negative regulator of Slit–Robo signaling, but the underlying mechanism is unclear (Sabatier et al., 2004). Robo4 has only two Ig and two cytoplasmic domains and is expressed specifically in endothelial cells with functions in angiogenesis and vasculogenesis (Bedell et al., 2005; Fujiwara et al., 2006; Huminiecki et al., 2002; Park et al., 2003; Wang et al., 2008). The intracellular tail of Robos has no apparent catalytic activity and, therefore, recruitment of cytosolic proteins is likely required for its function.

Slits can bind to any of the three Robo receptors (Robo1–3) as well as the *Drosophila* Robo with comparable affinity (Brose et al., 1999; Li et al., 1999; Sabatier et al., 2004), but it is not clear whether they bind to Robo4 (Hohenester et al., 2006; Suchting et al., 2005). Recent structure-function studies have localized the region of binding to the leucine rich repeats (LRRs) of Slit and the first two Ig domains of Robo (Chen et al., 2001; Liu et al., 2004). The minimal interaction has been mapped to the second Slit LRR and the first Robo Ig domain, which is conserved in *Drosophila* and mammal (Hohenester et al., 2006; Howitt et al., 2004; Morlot et al., 2007).

While structural studies have clearly established the binding interface of Slit and Robo, the downstream signaling upon Slit activation of Robo remains elusive. Whether this involves oligomerization of either the receptor and/or the ligand, and how and which cytosolic proteins are recruited to the intracellular Robo domains in different cellular contexts, are open questions (Hohenester, 2008). Several cytoplasmic signaling proteins

have been shown to be able to bind to the intracellular conserved Robo domains (CC0–3) such as actin binding protein Ena/VASP (Bashaw et al., 2000), adaptor protein Dock/Nck (Fan et al., 2003), Rho GTPase activating proteins (GAPS) (i.e. Vilse/CrossGAPs and Slit–Robo specific GAPs [srGAPs]) (Hu et al., 2005; Li et al., 2006; Lundstrom et al., 2004; Wong et al., 2001) and the Abelson (Abl) tyrosine kinase (Bashaw et al., 2000)— all major modulators of the actin cytoskeleton which may also have other functions.

Substantial evidence implicates direct interaction of heparan sulfate proteoglycans (HSPGs) with Slit for its function (reviewed in (Hohenester, 2008)). Structural studies show that the LRR2 that binds to Robo also binds to heparin, suggesting a Slit–Robo–HS complex (Fukuhara et al., 2008; Hussain et al., 2006). Enzymatic ablation of heparan sulfate (HS) by Heparinase III (Hu, 2001) decreases affinity of Slit–Robo binding and blocks Slit repulsive activity in vitro, which also occurs when excess HS is applied (Piper et al., 2006). In *Drosophila*, syndecan, a HSPG, has been shown to interact with Slit–Robo genetically in regulating distribution and efficiency of Slit signaling (Johnson et al., 2004; Steigemann et al., 2004). In vertebrates, the role of syndecan with Slit–Robo is not clear, but vertebrate Slit1–2 have been found to bind strongly to the HSPG Glypican-1, likely through its HS chains (Liang et al., 1999; Ronca et al., 2001). The functional relationship of HSPG and Slit–Robo in vivo is not well understood.

Functionally, Slits and Robos are best known for their conserved role in midline axon repulsion, which regulates the crossing of commissural axons and also inhibits crossing of ipsilateral axons in the *Drosophila* ventral nerve cord and the mammalian spinal cord (Dickson and Gilestro, 2006; Kidd et al., 1999; Kidd et al., 1998; Long et al., 2004). In addition to regulating the commissures, they can act as both repulsive (e.g. olfactory bulb, hippocampal, and spinal motor axons) (Bagri et al., 2002; Brose et al., 1999; Hu, 1999; Li et al., 1999; Nguyen Ba-Charvet et al., 1999; Nguyen-Ba-Charvet et al., 2002) and attractive (e.g. on DRG sensory and retinal axons) (Jin et al., 2003; Nguyen-Ba-

Charvet et al., 2001) guidance cues for various types of neurons. They can also promote axon branching and growth, as well as regulate cell migration (Kramer et al., 2001; Nguyen-Ba-Charvet et al., 2004; Wang et al., 1999), including the trunk and vagal neural crest (De Bellard et al., 2003; Jia et al., 2005). Moreover, it has become clear that Slits and Robos have broader roles than axon guidance/branching and cell migration and are not restricted to the nervous system. More recently, in *Drosophila*, novel roles for Slit and Robo have emerged in cell adhesion and morphogenesis of the forming heart tube (MacMullin and Jacobs, 2006; Qian et al., 2005; Santiago-Martinez et al., 2006; Santiago-Martinez et al., 2008). In vertebrates, they have broad expression patterns in diverse tissues including teeth (Loes et al., 2001), otic vesicle (Battisti and Fekete, 2008), lungs (Anselmo et al., 2003), limbs (Vargesson et al., 2001), and kidneys (Piper et al., 2000) and have been implicated in mammary ductal development (Strickland et al., 2006). However, the functions of Slit–Robo in these systems are not well characterized.

Aside from axon and cell migration guidance, new roles for Slit–Robo involved in cell adhesion, cell positioning, and/or other cellular functions are beginning to be realized. Further investigation of Slit–Robo function in tissue morphogenesis and organ development holds the promise of revealing their large range of biological functions and thereby elucidating general principles underlying this signaling pathway during development.

1.5 Cadherins: structure and function

Cadherins are conserved adhesion molecules found throughout the animal kingdom from nematode to mammal and are considered the main facilitators of adhesion between

cells in vertebrates through homophilic binding. For the past few decades, numerous studies have addressed the broad and important roles of cadherins in development and cancer (Gumbiner, 2005; Jeanes et al., 2008; Stemmler, 2008). In addition to adhesion, cadherins have also been implicated in cell signaling, differentiation, and survival (Radice and Takeichi, 2001). To date, over 100 members of the cadherin superfamily have been identified in the animal kingdom (Stemmler, 2008). The best known and most similar subfamilies of these are the classical type I (cadherins 1–4, also respectively named E-, N-, P-, R- cadherins) and type II (e.g. cadherins 5–12). Classical cadherins all have five extracellular cadherin domains (ECs), a single pass transmembrane protein, and two highly conserved intracellular domains—the juxtamembrane domain that binds to p120 catenin (which has been implicated in regulation of cadherin turnover at the cell surface and Rho GTPases that affect the actin cytoskeleton (Perez-Moreno and Fuchs, 2006; Reynolds and Roczniak-Ferguson, 2004)) and at the C-terminus, the binding domain for β-catenin which is indirectly linked to the actin filaments through α-catenin (Radice and Takeichi, 2001). Cadherins are defined by their tandemly arranged extracellular cadherin repeats, but the number of repeats is variable and the intracellular regions differ widely between classical cadherins and the other subfamilies (desmosomal cadherins, protocadherins, atypical cadherins, and the seven-pass transmembrane cadherin-like molecules), which do not bind to β-catenin but interact with plakoglobin and desmoplakin, kinases, and yet to be identified proteins (Gumbiner, 2005; Stemmler, 2008). Therefore, the functions of the non-classical cadherins may be distinct or may include additional roles to cell adhesion.

The expression of some classical cadherins are spatiotemporally regulated during neural crest emigration (Taneyhill, 2008). Functional experiments suggest that switching of cadherin subtypes during neural crest emigration is crucial for changes in cell adhesive properties in order to acquire the ability to migrate (Nakagawa and Takeichi, 1998).

However, the precise function of cadherins once these cells are migrating and later in development as they form the peripheral ganglia remain poorly explored. Similarly, very little is known about their potential role in development of placodal cells, with which neural crest cells interact during cranial ganglia formation.

1.6 Overview

How structures arise in the developing embryo is a fascinating process. Cell–cell interactions are the basic architectural building blocks in multicellular animals. This aspect of development is difficult to tackle because it requires grappling with a multitude of actions, reactions, and changes that are dynamically occurring in two or more different cellular components. The study of cranial gangliogenesis exemplifies such a problem. The origin of cranial sensory ganglia from a combination of two distinct cell populations, neural crest and ectodermal placodes, has intrigued the field for quite some time. Surprisingly, little is known about the nature and function of neural crest–placode interaction. How do they interact and what molecular basis may mediate their interaction? Does this have an important role for ganglion formation? With the advent of new technologies for DNA, vector, and oligos transfer using in vivo electroporation to target specific tissues (such as the presumptive neural crest or the placodal ectoderm) for both gain- and loss-of-function in combinaton with an array of molecular tools, the study of this long-time puzzle is now being elucidated. The chick is arguably the best model system to begin this investigation because of its well characterized embryology, its long history of neural crest and placode studies, and its amenability to embryological manipulation in combination with molecular

perturbation, which makes it an effective and tractable system to dissect the roles of neural crest and ectodermal placodes in vivo.

In my thesis study, the focus was the early development of the cranial sensory ganglia. This represents the time period when neural crest and placodal cells migrate to the ganglion anlage, interact, assemble, and condense into the first recognizable ganglion structure. The later processes after the first sight of a coalesced ganglion: neuronal and glial differentiation, axonal projections to the targets, and neuronal cell type specificities and functions among others, which have been more extensively studied, are reviewed elsewhere (Le Douarin and Kalcheim, 1999). The formation of the trigeminal ganglion was the prime system of study to dissect neural crest–placode interaction during ganglion assembly, due to its large size and unique semi-lunar structural shape which provided easy accessibility and structural analyses of the tissues. In addition, supporting experiments on epibranchial gangliogenesis were also conducted to evaluate the generality of the mechanisms found in our studies.

This thesis presents the results and findings from my study. The first aspect of this work, presented in Chapter 2, is the characterization of neural crest and placode cell–cell association during trigeminal gangliogenesis starting from placodal ingression. Using both cell labeling and molecular markers in successive stages of development, in combination with neural crest–placode cultures and ablation experiments, we show that these cells are in contact and closely intermingled throughout gangliogenesis. These data suggest that bi-directional signaling is likely in place to mediate their reciprocal interaction. We then present our finding that neural crest and placodal cells interact through Slit1–Robo2 signaling which is required for proper assembly of the cells into ganglion. This represents the first molecular lead into the underlying basis for neural crest–placode cell–cell interactions.

The second aspect examines possible mediators of Slit1–Robo2 signaling in driving proper ganglion formation (Chapter 3). We tested the cell–cell adhesion molecule N-cadherin as a possible candidate due to its implicated interaction with Slit–Robo in vitro and its possible role in gangliogenesis. The results show that N-cadherin is expressed by the placode-derived neurons, but not the neural crest, and its function is required for placodal condensation. We further demonstrate that N-cadherin acts downstream of Slit1–Robo2. This suggests a novel mechanism whereby neural crest–placode interaction through Slit–Robo signaling positively regulates N-cadherin mediated placodal cell adhesion.

Finally, the results above pose lingering questions on what then may be mediating adhesion of the other population, the neural crest, and also adhesion between neural crest and placodal neurons. Chapter 4 presents the results showing the role of another cell adhesion molecule Cadherin-7 in cranial neural crest migration and a potential role for Cadherin-7 in mediating neural crest cell aggregation in the trigeminal ganglion. The results raise two possible models for coalescence of trigeminal ganglion: the first is based on homotypic and novel heterotypic interactions between Cadherin-7 and N-cadherin, and the second is based entirely on homotypic cadherin mediated adhesion.