Collective Cell Migration of the Cephalic Neural Crest: The Art of Integrating Information

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Summary: The cephalic neural crest (NC) cells delaminate from the neuroepithelium in large numbers and undergo collective cell migration under the influence of multiple factors including positive and negative taxis, cell–cell interactions mediating cell sorting, cell cooperation, and Contact-Inhibition of Locomotion. The migration has to be tightly regulated to allow NC cells to reach precise locations in order to contribute to various craniofacial structures such as the skeletal and peripheral nervous systems. Several birth defects, syndromes, and malformations are due to improper cephalic NC (CNC) migration, and NC cell migration bears important similarities to cancer cell invasion and metastasis dissemination. Therefore, understanding how CNC cells interpret multiple inputs to achieve directional collective cell migration will shed light on pathological situations where cell migration is involved. genesis 00:1–13, 2011.   © 2011 Wiley-Liss, Inc.

Key words: neural crest cells; collective cell migration; chemotaxis; Contact-Inhibition of Locomotion; craniofacial structures

INTRODUCTION

The neural crest (NC) is a multipotent cell population induced at the neural plate border and located later in the dorsal part of the neural tube. Once specified, the NC cells delaminate from the neuroepithelium and migrate throughout the embryo following well-defined routes to their final locations where they stop and differentiate in a wide range of cell types (Hall, 2008; Le Douarin and Kalcheim, 1999). This review will focus on the cephalic NC (CNC) population, which arises from the diencephalon to the caudal hindbrain. The CNC makes an outstanding contribution to the craniofacial structures, and several syndromes and birth defects are due to improper CNC migration. Understanding how

CNC migration is precisely regulated in time and space gives precious information about NC-related diseases and malformations. In addition, it sheds light on the general mechanisms involved in regulating cell migration in normal and pathological situations. Here, we present a brief overview of the CNC contribution to the craniofacial structures and the main signals regulating CNC migration and discuss how cells can integrate multiple and somehow opposite inputs to achieve directional migration.

CONTRIBUTION OF THE CNC TO THE CRANIOFACIAL STRUCTURES

The CNC makes critical contributions to the cranial nerves (CN) and ganglia, the skeletal system (including teeth and ossicles of the middle ear), the ocular and periocular structures, smooth muscles, and connective tissues of the blood vessels. In addition, it forms the dermis of the head, most of the pigment cells (iris cells are not NC derivatives), and the meninges of the proencephalon as well as influencing striated muscle patterning and the migration of placode-derived neurones (Creuzet *et al.*, 2005; Hall, 2008; Johnston *et al.*, 1979; Le Douarin and Kalcheim, 1999; Noden and Trainor, 2005).

Cephalic Peripheral Nervous System

The CNC produces the glial cells (Schwann cells) of all CN and ganglia except for the CN II, which is ensheathed in the meningeal layers, myelinated by oligodendrocytes, and lies in the central nervous system

(Schoenwolf *et al*., 2009). In addition, CNC cells give rise to neurons of the proximal ganglia of CN VII (facial), IX (glossopharyngeal), and X (vagus). Alongside with ectodermal placodes, they also contribute to neurones of the ciliary (CN III/oculomotor), trigeminal (CN V/trigeminal), and vestibular (CN VIII/vestibulocochlear) ganglia. Distal ganglia of the CN VII (geniculate), IX (petrosal), and X (nodose) as well as the acoustic ganglion contain neurones of placodal origin (summarized in Fig. 1A–C). Therefore, neuronal CNC derivatives are not only involved in controlling eye movements (CN III), biting, chewing, and swallowing (CN V) and facial expressions (CN VII) but also carried sensory information from, for instance, the face, nasal cavity and mouth (CN V), the sense of taste (CN VII and IX), and the sense of balance (CN VIII). Finally, they relay information about the state of the body's organs to the central nervous system through the vagus nerve (CN X) (D'Amico-Martel and Noden, 1983; Hall, 2008; Le Douarin and Kalcheim, 1999; Lee *et al*., 2003; Schoenwolf *et al*., 2009).

Skeletal System: Skull, Tooth, and Ear Ossicles

Studies using amphibians, quail-chick chimeras, and transgenic mice have highlighted the fact that CNC cells contribute to most of the cartilages and bones of the head and neck (Fig. 1D) (Couly *et al*., 1993; Evans and Noden, 2006; Gross and Hanken, 2005; Hall, 2008; Hanken and Gross, 2005; Helms and Schneider, 2003; Helms *et al*., 2005; Le Douarin and Kalcheim, 1999; Minoux and Rijli, 2010; Morriss-Kay, 2001; Trainor, 2005; Trainor and Tam, 1995). Although it is well accepted that all facial skeletal structures are NC-derived, the limit between NC-derived and mesoderm-derived skeletons at the back of the skull and the jaw are still matters of debate. Although studies on avian models classify the parietal bone as a NC derivative, in mammals, it is considered as mesoderm-derived. In addition, mice data support the idea that part of the jaw may be formed by non-NC cells, whereas avian data find it to be entirely NC-derived. A possible explanation for these discrepancies may be that different technical approaches were used. In mice, CNC derivatives were mapped using a mouse model expressing the gene-encoding β-galactosidase under the control of Wnt1 promoter, leaving the possibility that some NC subpopulations may not express Wnt1. In birds, however, the quail-chick chimera system provides a stable means of staining cells and labels all derivatives regardless of the fluctuations of gene expressions among subpopulations. An alternative explanation would be that in mammals some of the NC prerogatives have been transferred to the mesoderm. For a precise account of studies about CNC contribution to the skull in different animal models and discussion about the origin of the parietal bone

and the jaw, the reader will find useful information in the references cited earlier. Apart from its contribution to the skull, the CNC gives rise to ossicles of the middle ear (Gross and Hanken, 2008; Hall, 2008; Le Douarin and Kalcheim, 1999; Le Lievre, 1978) (Fig. 1E), which transmit vibrations from the tympanic membrane to the cochlea. Finally, NC cells also form multiple parts of the teeth (Fig. 1F) including the dentin that is deposited and mineralized by odontoblasts of NC origin (Graveson *et al*., 1997; Lumsden, 1988; Lumsden and Buchanan, 1986; Smith and Hall, 1990).

Neurocristopathies

Syndromes, tumors, and malformations due to incorrect NC development are named neurocristopathies. Because of their implications in a wide variety of craniofacial structures, problems occurring during CNC formation, migration, and differentiation have dramatic consequences on the development of the head. Induction, proliferation, or survival issues usually give rise to dysplasia (abnormal development of an organ or part of the body, including congenital absence) such as seen in Treacher Collins syndrome (Trainor, 2010; Walker and Trainor, 2006), while CNC migration defects lead to malformations. Cleft lip, cleft palate, defects of the anterior chamber of the eye, unusual (or lack of) pigmentation, and abnormal ear development are common features of CNC migration defects that can be found in diseases like the CHARGE association and Waardenburg, DiGeorge, and Goldenhar syndromes (Cohen, 1989, 1990; Hall, 2008; Jones, 1990; Le Douarin and Kalcheim, 1999; Schoenwolf *et al*., 2009; Wurdak *et al*., 2006).

CNC CELL MIGRATION

All along the antero–posterior axis NC cells first separate themselves from the neuroepithelium during the delamination phase and then start migrating following stereotypical routes. Although the general sequence of induction, delamination, and migration is true for all NC cells emerging along the antero–posterior axis, CNC cells bear some specific features that are worth noting. Although trunk NC cells delaminate progressively in a dripping fashion over a long period, CNC cells depart almost all at once (Hall, 2008; Le Douarin and Kalcheim, 1999; Theveneau *et al*., 2007). Therefore, they start migrating as a multilayered cell group, whereas trunk NC cells exhibit a single chainlike organization. The delamination at the cephalic level is regulated by numerous transcription factors including snail2, Lsox5, and ets1, but our understanding of their relationships remains incomplete (del Barrio and Nieto, 2002; Perez-Alcala *et al*., 2004; Taneyhill *et al*., 2007; Theveneau *et al*., 2007). This process involves a change of cadherin expression reducing cell–cell adhesion, which, in chick,
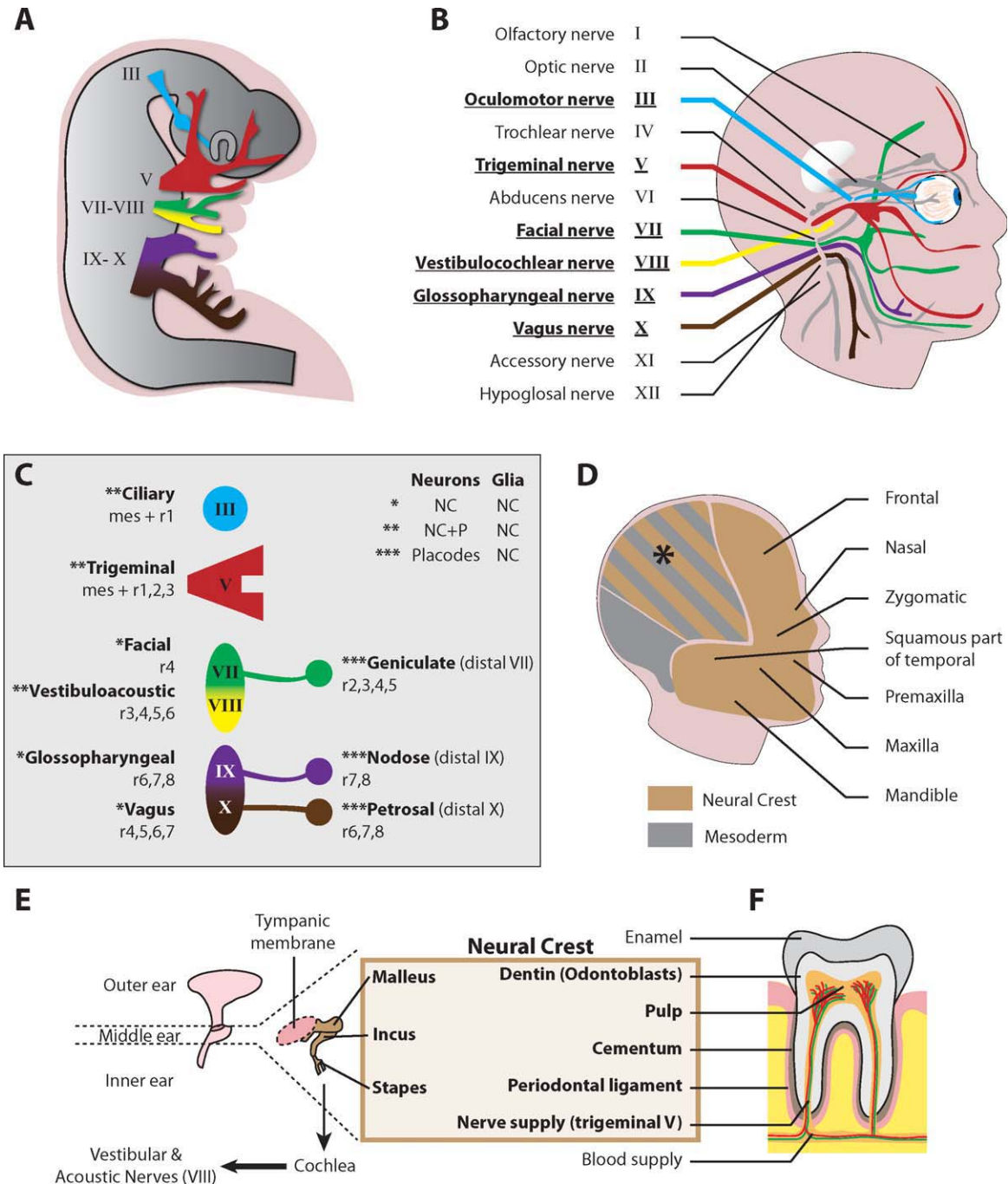
FIG. 1. Neural crest contribution to the craniofacial structures. **A:** Neural crest cells neuronal contribution to cranial PNS shown in a virtual mammalian embryo. **B:** Distribution of the cranial nerves in a human head. Nerves receiving neuronal contribution from the neural crest are in color. The nerves receiving only glial contribution, or no NC contribution (CN II, see text), appear in gray. **C:** Detailed neuronal contribution of neural crest and placodal cells in cranial ganglia III, V, VII, VIII, IX, and X. Origin of the CNC contribution along the antero–posterior axis is indicated under each ganglion. **D:** Human head showing the global repartition of neural crest and mesoderm-derived bones and cartilages. Only the main NC-derived facial elements are labeled. **E:** Neural crest cells form the three ossicles of the middle ear. **F:** Contribution of the neural crest to various parts of the teeth. mes, mesencephalon; r, rhombomere.

involves a global shift from type I (N-Cadherin) to type II (Cadherin 6B, 7) cadherin. However, although migrating NC express reduced levels of type I cadherin, these molecules are still present as shown by the expression of N-cadherin in Xenopus, zebrafish, and chick (Piloto and Schilling, 2010; Theveneau et al., 2007, 2010). In addition, CNC cells migrate almost exclusively in close association with the surface ectoderm, at least for the
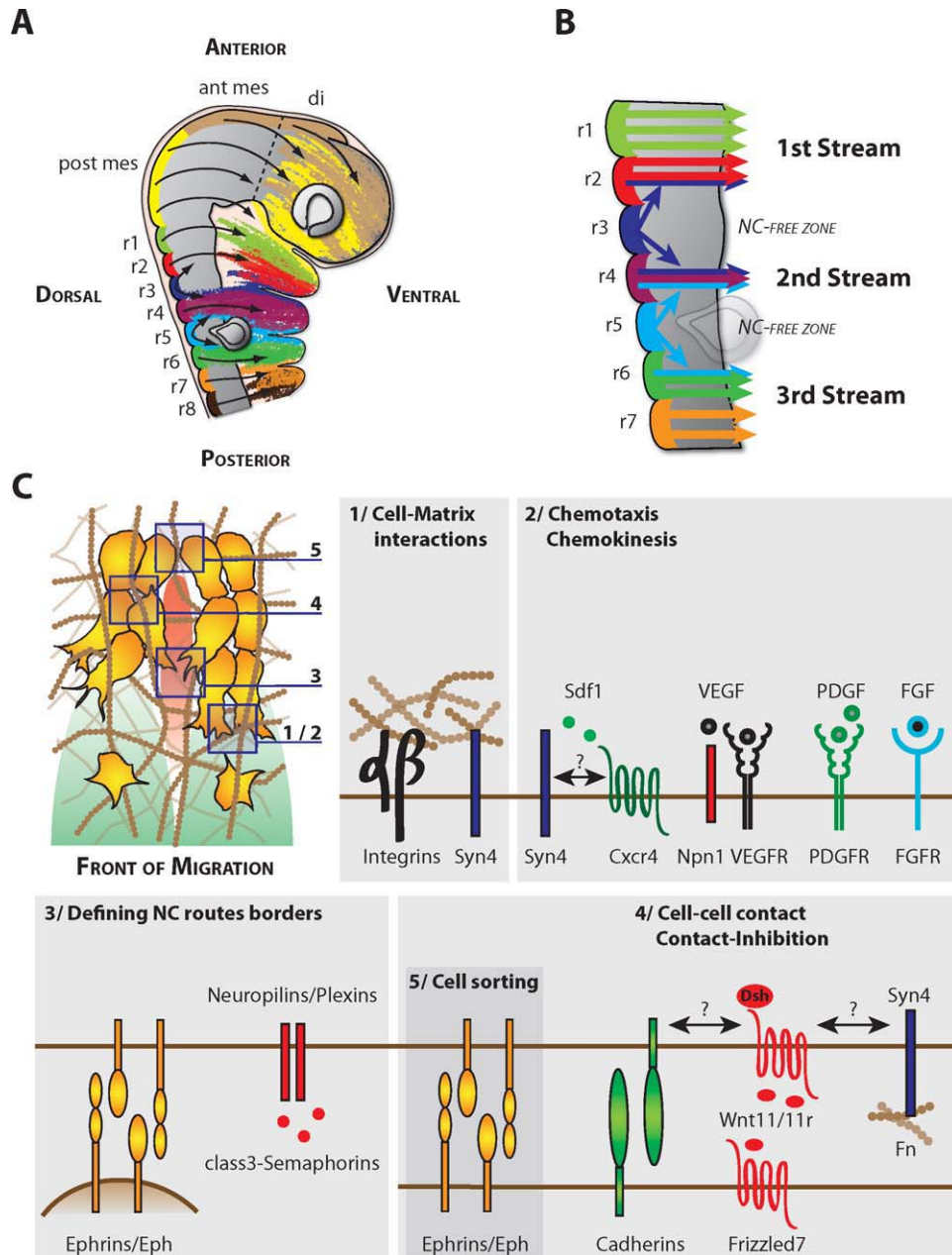
FIG. 2. Molecular control of neural crest migration. **A:** Overview of the cephalic neural crest migration. CNC cells invade their surrounding tissues in register with their original location along the AP axis. **B:** Detailed view of the early phase of CNC migration during which anterior and posterior migration of cells from r3 and r5 give rise to NC-free regions adjacent to the neural tube. **C:** A stream of NC cells migrating through the extracellular matrix. Cells are polarized according to their cell contact. Inhibitors keeping streams separated are shown in red, and attractant or permissive molecules are shown in green. Numbers refer to panels shown hereafter. Overview of the molecules involved in cell-matrix interactions (1), chemotaxis and chemokinesis (2), defining NC routes borders (3), contact-inhibition of locomotion (4), and cell sorting (5) during CNC cells migration. ant, anterior; di, diencephalon; FGF, fibroblast growth factor; Fn, fibronectin; mes, mesencephalon; Npn1, neuropilin-1; PDGF, platelet-derived growth factor; post, posterior; r, rhombomere; Sdf1, stromal cell-derived factor-1; Syn4, syndecan-4; VEGF, vascular endothelium growth factor.

first part of their migration, whereas trunk NC cells split into several subpopulations migrating deep inside the embryo toward the dorsal aorta through the somites and finally underneath the ectoderm (Kuriyama and

Mayor, 2008; Le Douarin and Kalcheim, 1999). Guidance of collective CNC cell migration involves various molecules, and signaling pathways are summarized in Figure 2 and discussed below.

From a Continuum to Discrete Streams

Despite delaminating continuously from the diencephalon to the caudal hindbrain, the CNC will quickly split into the three following streams (Fig. 2A,B): adjacent to the neural tube from the diencephalon to r2, adjacent to r4, and adjacent to the postotic region from r6 to r8. Crests from r3 and r5 migrate rostrally and caudally to join adjacent streams leaving two NC-free zones opposite to r3 and r5 (Kulesa *et al.*, 2010; Kuriyama and Mayor, 2008; Lumsden and Guthrie, 1991; Lumsden *et al.*, 1991; Sechrist *et al.*, 1993). Several signaling pathways control the separation of the original NC continuum into discrete groups (Figs. 2B,C.3). First of all, molecules of the class 3-semaphorins family (3A, 3F and 3G) and their neuropilin receptors (Npn1 and 2) are involved in keeping NC-free zones adjacent to r3 and r5 in chick, mouse, and zebrafish (Eickholt *et al.*, 1999; Gammill *et al.*, 2007; Osborne *et al.*, 2005; Schwarz *et al.*, 2008; Yu and Moens, 2005). CNC cells express the neuropilin receptors while surrounding tissues express and secrete soluble semaphorin ligands (Fig. 2C, red). Inhibition of the semaphorin signaling causes ectopic NC cells to invade spaces in between the normal streams. In addition, *in vivo* experiments and *in vitro* assays on stripes of semaphorin ligands showed that NC cells preferentially avoid semaphorin-containing regions. In *Xenopus*, CNC cells also express Neuropilins during migration (Koestner *et al.*, 2008), but the role of Semaphorin signaling remains unexplored.

Besides Semaphorin signaling, transmembrane ephrins and Eph receptors also play a role in this process (Figs. 2C.3,C.5). Studies in *Xenopus*, mice, and chick have highlighted a great variability in the combination of the ephrins and Eph expressed by CNC and the head mesoderm, but their functions seem conserved (Adams *et al.*, 2001; Davy *et al.*, 2004; Mellott and Burke, 2008; Smith *et al.*, 1997). Loss- and gain-of-function experiments lead to two main phenotypes: invasion of NC-free zones or misaddressing of NC cells into an unusual stream without abolishing NC-free zones. Therefore, ephrins and Eph seem to play a dual role. They are involved in cell sorting, helping place CNC into a specific stream according to their antero–posterior level of origin, and they also prevent entry into specific areas (Fig. 2C.3,C.5).

Finally, Slit ligands and their Robo receptors (Dickson and Gilestro, 2006; Ypsilanti *et al.*, 2010) have been implicated in regulating trunk NC-cell migration (De Bellard *et al.*, 2003; Jia *et al.*, 2005; Kuriyama and Mayor, 2008) by driving NC cells into the ventromedial pathway. However, at cephalic levels, while Slit/Robo signaling seems to control late events related to ganglia formation (Shiau and Bronner-Fraser, 2009), its putative role in guidance of CNC migration has yet to be assessed.

Chemotaxis, Chemokinesis: New Findings for Old Ideas

Until very recently, the accepted model for NC cell migration was based on the fact that NC cells were mesenchymal and highly migratory cells facing permissive areas containing extracellular matrix (ECM) and restrictive areas defined by negative cues present in the local environment. Consequently, NC cells would explore their direct surroundings and invade all areas free of inhibitors. Positive taxis attracting NC to specific locations and negative taxis repelling NC from the neuroepithelium were proposed (Erickson, 1985, 1988; Erickson and Olivier, 1983; Sechrist *et al.*, 1994) but remained unsupported or, in the case of negative taxis, were ruled out by experimental data. However, over the last few years, several molecules have been shown as targeting trunk NC cells to specific areas (Druckenbrod and Epstein, 2007; Jiang *et al.*, 2003; Santiago and Erickson, 2002) and more recently attracting or positively influencing CNC migration (Fig. 2C.2). Stromal-cell-derived factor 1 (Sdf1/Cxcl12), a potent chemoattractant for germ cells and lymphocytes, among other cell types (Burger and Kipps, 2006; David *et al.*, 2002; Ganju *et al.*, 1998; Molyneaux *et al.*, 2003; Raz and Mahabaleshwar, 2009; Stebler *et al.*, 2004), was described as an attractant for trunk NC cells invading the hair follicles and required for the formation of the dorsal root ganglia (Belmadani *et al.*, 2005, 2009). Moreover, it has been shown as being required for CNC migration in zebrafish (Olesnicky Killian *et al.*, 2009). In addition, in *Xenopus laevis*, we demonstrated that Sdf1 acts as a strong chemoattractant *in vitro* and that its expression is required for normal migration *in vivo*. Moreover, Sdf1 misexpression leads to ectopic CNC invasion in between CNC streams or induces an early arrest of migration (Theveneau *et al.*, 2010). Sdf1/Cxcr4 axis functions primarily by increasing Rac1 activity, a small Rho GTPase required for lamellipodia formation, and stabilizing cell protrusions, which generate directional movement toward the Sdf1 source. Finally, in chick, expression of Sdf1 and its receptor Cxcr4 suggests that Sdf1 may play a similar role in the CNC, but this remains to be addressed (Rehimi *et al.*, 2008; Yusuf *et al.*, 2005).

Besides Sdf1/Cxcr4 axis, VEGF, PDGF, and FGF-signaling pathways have also been involved in positively regulating CNC migration (Fig. 2C.2). CNC cells in chick, *Xenopus*, zebrafish, and mouse express PDGF receptor α (Ho *et al.*, 1994; Le Douarin and Kalcheim, 1999; Orr-Urtreger and Lonai, 1992; Orr-Urtreger *et al.*, 1992; Schatteman *et al.*, 1992; Takakura *et al.*, 1997). In zebrafish, PDGF signaling attracts some CNC into the oral region while in mice it is believed to be involved in conferring migratory abilities on the cells. Data on PDGF signaling during CNC migration are scarce, and its precise influence on cell motility or directionality remains elusive (for a recent and complete summary of PDGF roles in NC development, see Smith and Tallquist, 2010).

VEGF and FGF signaling are important for CNC homing into the second branchial arch in chick and mouse, respectively (McLennan *et al*., 2010; Trokovic *et al*., 2005). However, whether or not they act as CNC chemoattractants is unclear. Ectopic VEGF leads to a slight change of CNC migration but fails to induce ectopic NC migration, and FGF-based chemoattraction has not been directly tested yet. Furthermore, the homogenous distribution of VEGF and FGF ligands along the pathways of migration makes it difficult to postulate that they give clear directional information. Alternatively, they may promote general random motility (chemokinesis) rather than chemotaxis.

Cell–Cell Interactions: A Touching Story

CNC cells delaminate in large numbers (Hall, 2008; Le Douarin and Kalcheim, 1999; Theveneau *et al*., 2007) and therefore encounter a very high cell density at the beginning of their migration. Despite this situation, the fact that CNC cells may influence each other's behavior because of direct interactions has been globally overlooked, even though the idea of Contact-Inhibition of Locomotion, a process during which two cells collapse their protrusions when contacting each other (Abercrombie and Heaysman, 1953; Mayor and Carmona-Fontaine, 2010), was proposed as a driving force for the migration of trunk NC cells decades ago (Erickson, 1988). Recent observations and experimentations made in chick, *Xenopus*, and *zebrafish* embryos showed that cell–cell contacts between two migratory NC cells have a direct influence on how cells actually move (Carmona-Fontaine *et al*., 2008; Kulesa and Fraser, 2000; Teddy and Kulesa, 2004; Theveneau *et al*., 2010). Original *in vivo* observations in chick by Kulesa and colleagues (Kulesa and Fraser, 2000; Teddy and Kulesa, 2004) showed two types of response after cell–cell interactions. Cells can retract their protrusions upon contact and stop migrating for a while before resuming migration, or, alternatively, a cell can touch another cell located just in front and follow it. In *Xenopus* embryo, we demonstrated that Contact-Inhibition of Locomotion is taking place when two CNC cells are in contact (Carmona-Fontaine *et al*., 2008). Among large groups of CNC cells, Contact-Inhibition of Locomotion prevents the formation of cell protrusions in between the cells (Fig. 3A,B1). However, at the border of a NC cluster, leading cells are free of Contact-Inhibition of Locomotion, and they can form protrusions away from the cluster (Fig. 3B2). At the cell–cell contact, cell protrusions collapse, while new protrusions are formed at the opposite end of the cell. We showed that Contact-Inhibition of Locomotion is dependant on N-Cadherin-mediated cell–cell interaction (Theveneau *et al*., 2010), which triggers the noncanonical Wnt/PCP pathway (Figs. 2C.4 and 3D). This leads to RhoA activation (Carmona-Fontaine *et al*., 2008; Matthews *et al*., 2008) and

Rac1 inhibition at the cell–cell contact (Theveneau *et al*., 2010). Interfering with Wnt/PCP or N-Cadherin expression/activity, both suppresses Contact-Inhibition of Locomotion and leads to ectopic protrusions in between the cells and a lack of repolarization upon cell collision (Carmona-Fontaine *et al*., 2008; Theveneau *et al*., 2010). We also showed that nonmotile cells (i.e., dividing cells) can be carried passively by their direct motile neighbors (Theveneau *et al*., 2010). Moreover, cells responding to Sdf1 can help cells that do not express Cxcr4 to reach the source of the chemoattractant (Theveneau *et al*., 2010). More surprisingly, we found that cell polarity induced by Contact-Inhibition of Locomotion is required for efficient chemotaxis toward Sdf1. Briefly, cell dissociation completely abolished chemotaxis without interfering with cell motility. On the contrary, a progressive increase in cell density can rescue chemotaxis. Altogether, these data indicate that CNC cells undergo collective cell migration and that cell–cell interactions are critical for cell polarity, cell coordination, and chemotaxis during this process (Carmona-Fontaine *et al*., 2008; Matthews *et al*., 2008; Mayor and Carmona-Fontaine, 2010; Theveneau *et al*., 2010).

Metalloproteinases: The NC Footprint

CNC cells receive information from signals released in their local environment and from direct contact with other NC cells or other cell types, but it is likely that they also have a direct impact on the tissues they invade. Studies in chick, *Xenopus*, and mouse showed that CNC cells express various matrix metalloproteinases [MMP2. 8, 14 (MT1-MPP)] (Cai and Brauer, 2002; Cai *et al*., 2000; Cantemir *et al*., 2004; Duong and Erickson, 2004; Giambernardi *et al*., 2001; Harrison *et al*., 2004; Tomlinson *et al*., 2009) and ADAM proteins (ADAM 9, 13, and 19) (Alfandari *et al*., 1997; Cai *et al*., 1998; Neuner *et al*., 2009). These enzymes are able to degrade a wide range of molecules and have been implicated in invasive behavior in cancer and normal cell migration (Edwards *et al*., 2008; Sternlicht and Werb, 2001). From studies done on other systems, MMPs and ADAMs could be involved in many different ways during CNC migration. By degrading ECM components, they could unravel cryptic binding sites for integrins and therefore promote migration (Giannelli *et al*., 1997; Petitclerc *et al*., 1999). Such a role in matrix remodeling has been proposed in NC cells to explain why NC migration is affected after MMPs or ADAMs loss-of-function (Alfandari *et al*., 2001; Duong and Erickson, 2004), but functional experiments to support this idea are still missing *in vivo*. The local degradation of the ECM could, in addition, release growth factors and signaling molecules that are trapped and would be otherwise inaccessible for NC cells. Such a situation has been previously demonstrated for TGFβ (Imai *et al*., 1997). For
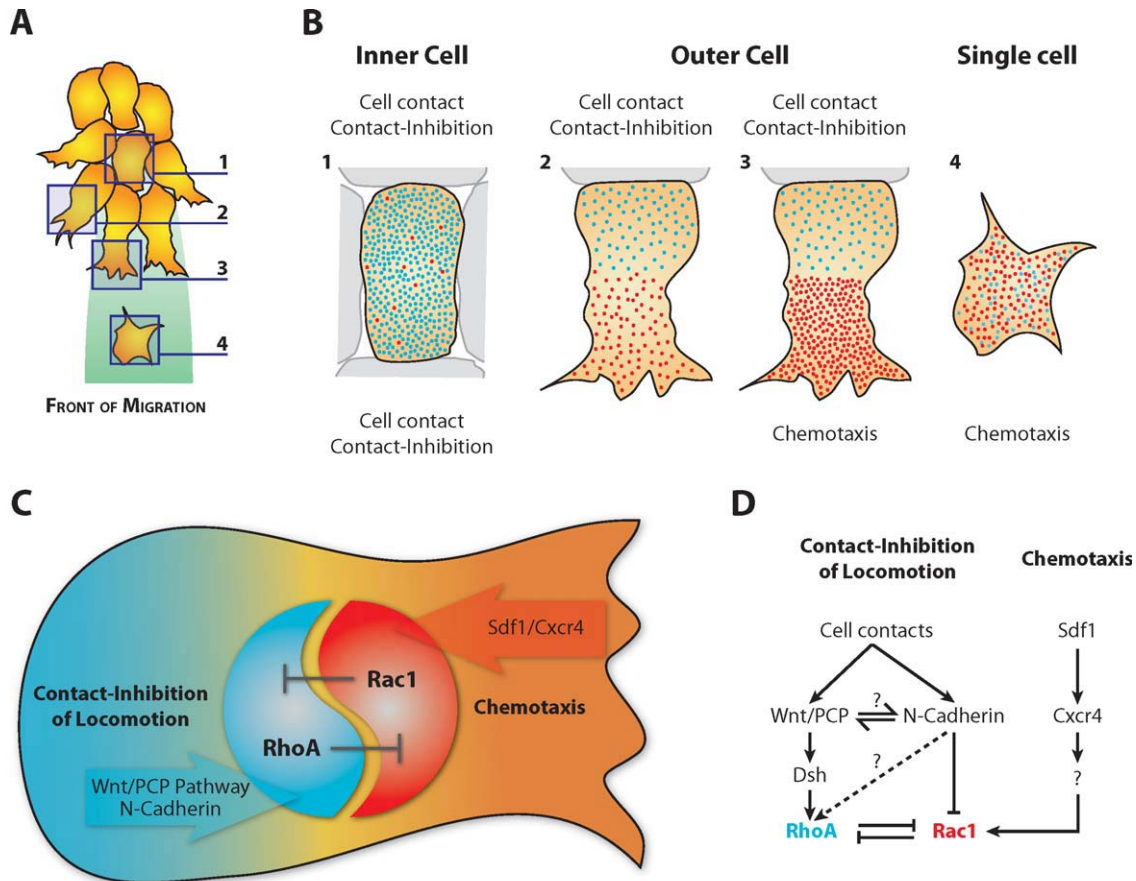
FIG. 3. Contact-inhibition and chemotaxis establish and stabilize cell polarity. **A:** Neural crest cells migrating along a gradient of chemoattractant (shades of green). Numbers refer to cells shown in B. **B:** Distribution of RhoA (blue) and Rac1 (red) activity in CNC that are surrounded by other cells (inner cells; 1), that have a cell contact and a free edge (outer cell; 2, 3), and CNC that have no contact with other cells (single cells; 4). Cell interactions establish cell polarity along a contact (RhoA)—free edge (Rac1) axis. The chemoattractant reinforces contact-dependent polarity by increasing Rac1 activity at the free edge but is unable to polarize single cells. **C:** Antagonistic relationship between contact-inhibition and chemotaxis signaling inside an outer cell through opposite regulations of small RhoGTPases. D: Signalling cascade controlling RhoA and Rac1 activity in Xenopus NC cells [after (Carmona-Fontaine *et al*., 2008; Matthews *et al*., 2008; Theveneau *et al*., 2010; Theveneau and Mayor, 2010)].

instance, Sdf1 and members of the FGF, VEGF, and PDGF families interact directly with Fibronectin (Martino and Hubbell, 2010; Pelletier *et al*., 2000), which can be degraded by MMP2, MT1-MMP, and ADAM13 (Alfandari *et al*., 2001; Gaultier *et al*., 2002; Sternlicht and Werb, 2001). Moreover, MMPs and ADAMs could help in shaping gradients out of homogenous expression patterns. Both MMP2 and MT1-MMP can cut and inactivate Sdf1 (McQuibban *et al*., 2001; Rodriguez *et al*., 2010). *Xenopus* CNC expresses MT1-MMP (Harrison *et al*., 2004; Tomlinson *et al*., 2009). Therefore, cells could digest Sdf1 present in the environment as they migrate, maintaining a sharp gradient at the front of the NC stream and virtually pushing the target as they move forward. Interestingly, some of the signaling pathways involved in CNC cell migration such as Sdf1/CXCR4 or PDGF stimulate MMPs' expression (Busillo and Benovic, 2007; Smith and Tallquist, 2010).

Thus, a complex feedback loop may take place where MMPs and ADAMs could first help NC cells to use the ECM efficiently and access ligands trapped into it and then degrade these molecules to shape gradients. In addition, by degrading molecules, proteinases may eventually get rid of the signals regulating their own expression. Finally, alongside specific changes of cadherin expression mentioned earlier, MMPs and ADAMs may regulate the mesenchymalization and the level of cell–cell interaction of the CNC by degrading Cadherins as it has been proposed for trunk NC cells (Shoval *et al*., 2007). Indeed, MMP2 and ADAM13 can digest N-Cadherin and Cadherin-11, respectively (Covington *et al*., 2005, 2006; Hartland *et al*., 2009; McCusker *et al*., 2009).

Despite MMPs and ADAMs molecules holding great potential as putative key players in CNC migration, we still have a lot to discover about their precise role. The
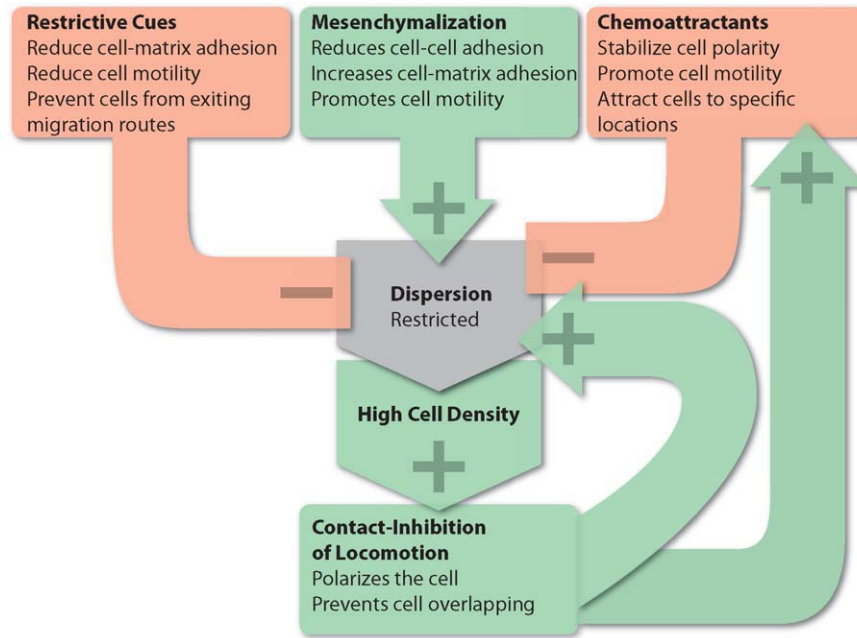
FIG. 4. Integration of multiple signals leading to directional collective migration of the neural crest cells. Cell dispersion promoted by contact-inhibition and epithelium-to-mesenchyme transition is restricted by restrictive cues and chemoattractants. These signals ensure high cell motility, high levels of cell interactions, full response to chemoattractants, and altogether drive directional collective cell migration of the cephalic neural crest cells.

recent advances in imaging CNC *in vivo* may help to decipher some of their functions during CNC invasion of the surrounding tissues.

The Art of Integrating Information

CNC may seem overwhelmed by information, part of which could also appear as counter productive. So how does it all make sense?

Previous studies in chick on enteric NC cells invading the gut and CNC entering the branchial arches and recent work in *Xenopus* CNC have shown that regulation of cell density and cell–cell interactions are critical aspects for efficient NC-cell migration (Barlow *et al.*, 2008; Kulesa *et al.*, 2008; Simpson *et al.*, 2007; Theveneau *et al.*, 2010). In *Xenopus* CNC, reducing cell number leads to fewer cell interactions, a poor or randomized cell polarity, inefficient chemotaxis, and reduced cell spreading. On the other hand, maintaining strong cell–cell adhesion, which may seems the best way to maintain a high cell density, also disrupts CNC migration (Theveneau *et al.*, 2010). Therefore, it is clear that, even if a proper epithelium-to-mesenchyme transition is required to confer full migratory potential on the NC cells, cell density has to be maintained by other means. In light of recent advances, we can propose a model integrating these multiple inputs into a general control of CNC collective cell migration (see Fig. 4). In this model, epithelium-to-mesenchyme transition and

Contact-Inhibition of Locomotion promote cell dispersion (green, Fig. 4), while restrictive cues preventing cells from exiting normal routes and chemoattractants leading to cell accumulation in precise locations both concur to maintain high cell density (red, Fig. 4). This high cell density ensures high levels of cell interactions, cell polarization, and a full response to chemoattractants, which altogether drive directional collective cell migration of the CNC cells (see Fig. 4).

However, for cells to be able to integrate all this information, common effectors are needed at the molecular levels. Interestingly, some of these events are indeed mediated by the same molecules. Neuropilin-1, for instance, acts as a co-factor for both permissive (VEGF) and restrictive (class 3-Semaphorin) signaling (McLennan and Kulesa, 2007, 2010; McLennan *et al.*, 2010). In addition, N-Cadherin activity has to be lowered to allow mesenchymalization, but not abolished, as it is required to mediate Contact-Inhibition of Locomotion (Theveneau *et al.*, 2010). More importantly, some of these signals, namely, Contact-Inhibition of Locomotion and chemotaxis, regulate common intracellular effectors in opposite manners (see Fig. 3). Indeed, while N-Cadherin/Contact-Inhibition of Locomotion lead to RhoA activation, Rac1 inhibition, and protrusion collapse (Carmona-Fontaine *et al.*, 2008; Matthews *et al.*, 2008; Theveneau *et al.*, 2010), and Sdf1 increases Rac1 activity and stabilizes lamellipodia (Theveneau *et al.*, 2010). Finally, Syndecan-4, being able to interact

with Fibronectin and Sdf1, (Carey, 1997; Charnaux *et al*., 2005; Matthews *et al*., 2008; Pelletier *et al*., 2000) could have a dual role in CNC migration. Associated with fibronectin, Syndecan-4 leads to Rac1 inhibition but could also improve Sdf1 presentation to its receptor (Matthews *et al*., 2008; Theveneau and Mayor, 2010). Altogether, these findings and previous work on cell migration strongly suggest that small GTPases may be molecules of choice to convert a wide range of external inputs into directional information driving collective cell migration (Parsons *et al*., 2010; Ridley *et al*., 2003; Theveneau and Mayor, 2010).

Recent advances make it possible to integrate Contact-Inhibition of Locomotion, EMT, positive, and negative taxis into a global model of CNC behavior during migration (see Fig. 4). However, information on the downstream effectors mediating all these signaling pathways and their putative interconnections are still sketchy. In addition, the proportion of cells able to exhibit Contact-Inhibition of Locomotion or undergo efficient chemotaxis remains unknown. Moreover, recent work on trunk NC cells in the chicken embryo showed that CXCR4-positive and CXCR4-negative subpopulations are driven to different locations where they give rise to different cell types (Kasemeier-Kulesa *et al*., 2010). These data indicate that the migratory NC population is likely to be composed of several subpopulations of specific abilities. It also reinforces the idea proposed for trunk melanocytes that ability to invade a specific region and fate restriction may somehow be linked (Erickson and Goins, 1995). Despite being supported by observations made on trunk NC cells, these questions have yet to be addressed at the cephalic level.

Further investigation is needed to assess the level of diversity of the CNC population and to fully understand how cells integrate information at the molecular level to make choices resulting in their targeting to precise locations.

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