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Early stages of neural crest ontogeny: formation and regulation of cell delamination

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ABSTRACT Long standing research of the Neural Crest embodies the most fundamental questions of Developmental Biology. Understanding the mechanisms responsible for specification, delamination, migration and phenotypic differentiation of this highly diversifying group of progenitors has been a challenge for many researchers over the years and continues to attract newcomers into the field. Only a few leaps were more significant than the discovery and successful exploitation of the quail-chick model by Nicole Le Douarin and colleagues from the Institute of Embryology at Nogent-sur-Marne. The accurate fate mapping of the neural crest performed at virtually all axial levels was followed by the determination of its developmental potentialities as initially analysed at a population level and then followed by many other significant findings. Altogether, these results paved the way to innumerable questions which brought us from an organismic view to mechanistic approaches. Among them, elucidation of functions played by identified genes is now rapidly underway. Emerging results lead the way back to an integrated understanding of the nature of interactions between the developing neural crest and neighbouring structures. The Nogent Institute thus performed an authentic «tour de force» in bringing the Neural Crest to the forefront of Developmental Biology. The present review is dedicated to the pivotal contributions of Nicole Le Douarin and her collaborators and to unforgettable memories that one of the authors bears from the time spent in the Nogent Institute. We summarize here recent advances in our understanding of early stages of crest ontogeny that comprise specification of epithelial progenitors to a neural crest fate and the onset of neural crest migration. Particular emphasis is given to signaling by BMP and Wnt molecules, to the role of the cell cycle in generating cell movement and to possible interactions between both mechanisms.

KEY WORDS: *BMP, epithelio-mesenchymal conversion, G1/S transition, noggin, rhoB, somite, Wnt*

General introduction

The neural crest is a major cell type arising in the lateral tips of the neural folds during the process of neurulation. Upon fusion of the folds and formation of the neural tube, the neural crest temporarily resides in its dorsal midline being an integral part of the pseudostratified neuroepithelium (Le Douarin and Kalcheim, 1999). The significance of the neural crest stems from the immense variety of derivatives that this discrete group of cells yields during ontogeny. Most of the peripheral nervous system arises from the neural crest to include all the glia of the sensory ganglia, all neurons of the dorsal root ganglia and the majority of neurons of the cranial sensory ganglia. The autonomic nervous system derives entirely from the crest comprising all sympathetic and parasympathetic branches and also the specialized enteric nervous system. Lining along peripheral nerves are the Schwann cells, a subset of peripheral crest-derived glia. In addition to specific neural derivatives, crest cells also develop into distinct endocrine and paraendocrine cells among which the adrenomedullary chromaffin cells are the most studied ones. Notably, all the melanocytes of the body except for the retinal pigment, that provide the vertebrate organism with a rich diversity of colors, also arise from the neural crest. Most strikingly, at cranial levels of the axis, the neural crest gives rise to most of the skeleton and connective tissue of the head, face and neck. So, it appears that during evolution, the neural crest, rather than cells of mesodermal

Abbreviations used in this paper: BMP, bone morphogenetic factor; Brdu, bromodeoxyuridine; cdk, cyclin-dependent kinase; CNS, central nervous system; FGF, fibroblast growth factor; pRB, phosphorylated retinoblastoma; TGF, transforming growth factor.

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origin which normally differentiate into skeletal derivatives, was able to provide a skull to cover and protect the increasing volume of the vertebrate brain (Gans, 1987; Gans and Northcutt, 1983). Furthermore, recent evidence supports the notion that the neural crest is also the source of molecular information that patterns certain aspects of craniofacial development through interactions with adjacent tissues (see for example Couly *et al.,* 2002; Kontges and Lumsden, 1996; Schneider and Helms, 2003).

In this article, we discuss progress in molecular mechanisms that underly early stages of neural crest ontogeny, in particular their specification and onset of migration. The reader is referred to additional reviews that focus on later stages of development which deal with cell migration, phenotypic diversification and differentiation (Anderson, 1999; 2001; Christiansen *et al.,* 2000; Groves and Bronner-Fraser, 1999, Kalcheim, 2000). The discovery of genes that identify prospective neural crest cells still resident in the neuroepithelium made it possible to address the cellular and molecular events that underly the formation of this cell type (see section III). A number of signals have been implicated in the formation of the neural crest, including members of the Wnt, FGF and BMP families. These secreted proteins regulate early expression of transcription factors, cell adhesion molecules, extracellular glycoproteins, etc. which themselves act to stabilize the competence of the epithelium to form neural crest (Knecht and Bronner-Fraser, 2002) and, moreover, some also regulate subsequent developmental events, such as delamination and initiation of migration (Kalcheim, 2000, LaBonne and Bronner-Fraser, 2000).

Although being initially an integral part of the neuroepithelium, presumptive neural crest cells soon segregate from the dorsal midline of the neural tube and adopt a mesenchymal morphology which is compatible with active movement away from their source. A key issue for understanding early neural crest ontogeny is, therefore, elucidating the mechanisms that regulate the separation of these cells from the CNS epithelium. Being basically a process of epithelial-to-mesenchymal conversion, the onset of emigration of crest cells from the CNS primordium is spatiotemporally coordinated with the development of the somites that subsequently serve as substrates for the migrating progenitors. The identity and mechanism of action of molecules that trigger delamination of epithelial pre-migratory neural crest cells is being progressively uncovered (see section IV).

The formation of the neural crest

During neurulation, the embryonic ectoderm becomes subdivided into the neural plate and prospective epidermis. The boundary region between these tissues becomes the neural crest, as defined by expression of a variety of specific markers. These include transcription factors such as *Slug* or *Snail*, *AP-2, Foxd3, PAX3, twist, Sox9, Zic5,* etc. which appear in different species at changing rostrocaudal levels of the axis (see below). Interactions between the epidermal ectoderm and neural plate and contribution of mesodermal signals were found to underly early expression of these crest-specific traits. Evidence obtained primarily in Xenopus embryos suggests that two independent signals mediate these interactions at the various phases of this multistage process, a BMP signal which must be modulated by its inhibitors and separate inputs that can be either a canonical Wnt signal, FGF or retinoic acid. The involvement of these factors and factor combinations was documented and recently reviewed (Kalcheim, 2000, Wu *et al.,* 2003, Knecht and Bronner-Fraser, 2002 and refs. therein). Hence, we shall only consider new data on the possible role of specific transcription factors in neural crest formation.

Transcription factors in neural crest formation

Snail familiy members that include the *Snail* and *Slug* transcription factors are among the earliest markers of neural crest development and therefore their expression is widely used as indication of the appearance of presumptive neural crest. This should be cautiously considered as cells in the dorsal neural folds which express *Slug* have the potential to give rise both to neural tube as well as to crest lineages (Collazo *et al.,* 1993; Selleck and Bronner-Fraser, 1995) and *Slug* transcription is downregulated in the trunk at levels where premigratory crest is still being produced (Sela-Donenefeld and Kalcheim, 1999). The involvement of *Slug* in the production of neural crest cells was first documented in the chick by application of antisense oligonucleotides to *Slug* mRNA which prevented cell emigration perhaps due to an earlier defect in their specification (Nieto *et al.,* 1994). Conversely, overexpression of *Slug* led to an increase in the production of crest cells, an effect that was restricted to cranial levels of the axis (Del Barrio and Nieto, 2002). In the mouse, a swapping between expression of *Slug* and *Snail* in the crest domain took place when compared to avian embryos (Jiang *et al.,* 1998; Sefton *et al.,* 1998), suggesting that Snail functions in the mouse as Slug does in the chick. Functional evidence for a possible role of Snail in crest formation is lacking in mice due to early lethality of the embryos by the time of gastrulation (Carver *et al.,* 2001). Nevertheless, it is clear that, similar to Slug, Snail is at least, able to trigger epithelio-mesenchymal conversions in cultured mammalian epithelial cells (Cano *et al.,* 2000) and the lethality of mice lacking *Snail* gene activity may be due, in fact, to the inability of mesodermal cells to delaminate from the primitive streak. The strongest evidence for an effect of these factors in neural crest formation stems from recent work in Xenopus. Inhibition of Slug function at early stages was shown to prevent the formation of crest progenitors while inhibition at later stages interfered with cell emigration (LaBonne and Bronner-Fraser, 2000). The ability to temporally dissociate between these two sequential events makes the argument more compelling. Aybar *et al.,* (2003) have further elaborated on this question and recently showed that *Snail* is also required for neural crest specification in Xenopus and moreover, is able to induce transcription of *Slug* as well as of additional crest-specific markers both in whole embryos and in animal caps in the absence of accompanying structures. In addition, the effect of a dominant-negative *Snail* could be rescued by overexpressing *Slug* but not vice-versa. Altogether, these results suggest that *Snail* lies upstream of *Slug* in a genetic cascade leading to formation of neural crest cells. Consistent with *Slug* being a direct target of Wnt signaling in Xenopus, a regulatory region in the *Slug* gene promoter was isolated and characterized to bind Lef/β-catenin and to be necessary to drive expression in neural crest cells (Vallin *et al.,* 2001).

In addition to *Slug*, the cascade of *Snail* -induced genes was shown to comprise *Zic5, FoxD3, Twist* and *Ets1* (Aybar *et al.,* 2003). Consistent with the notion that these genes are also part

of the neural crest-producing genetic repertoire, it was shown that *Zic5* overexpression induces neural crest markers at the expense of epidermal ones and loss of function experiments suggested that it is necessary for this process, but not for the induction of anterior neural markers, a property shared by other members of the *Zic* family (Nakata *et al.,* 2000). Surprisingly, at variance with Aybar *et al.,* (2003), this study reported that *Zic5* induces *Snail* and *Slug* transcription in Xenopus and that a dominant negative construct interferes with *Slug* expression (that of *Snail* was not reported), suggesting that *Zic5* lies upstream of the genes so far considered to be the earliest markers of crest development. Hence, the hierarchical relationship between these two genes remains to be clarified.

A family of transcription factors that has been shown to play important functions in cell specification and lineage segregation is the winged-helix or forkhead class (Kaufmann and Knochel, 1996), recently renamed as Fox proteins for the forkhead box (Kaestner *et al.,* 2000). One member of the family was cloned in the chick, *FoxD3* and shown to be expressed in the neural folds and later in early migrating neural crest (Kos *et al.,* 2001, Dottori *et al.,* 2001). Overexpression in the neural tube consistently led to a widening of the HNK-1-positive domain and to an increased number of emigrating cells, suggesting that *FoxD3* biases neuroepithelial progenitors towards a neural crest fate. Consistent with this possibility, Dottori *et al.,* (2001) found that interneuron development is concomitantly supressed. Within the crest lineage itself, *FoxD3* was also found to play a role in the balance between determination of early (neural and glial) versus late (melanocyte) phenotypes in favor of the former (Kos *et al.,* 2001). In Xenopus, *FoxD3*, which was suggested to be a downstream signal of both *Snail* and *Slug* (Aybar *et al.,* 2003), was found to act as a transcriptional repressor, in turn downregulating *Slug* and also *Cadherin 11* as well as its own expression. Likewise, neural crest formation was reduced (Pohl and Knochel, 2001). These results are difficult to interpret in light of a positive effect of *Snail* and"*Slug* on neural crest production and of the timely expression of *FoxD3* in the presumptive neural crest territory. Initial loss of function studies of *FoxD3* revealed no apparent defects. Analysis of additional mutations in this gene are therefore required, as one possibility is that a precise dosage of this factor is required to balance between primary effects on development of the neural plate vis-a-vis the neural plate border leading to crest.

This also appears to be the case for another transcription factor, *Sox9*, whose deletion was shown to cause severe craniofacial malformations. Xenopus *Sox9* is expressed maternally and accumulates shortly after gastrulation at the lateral edges of the neural plate, in the neural crest-forming region. At later stages, it persists in migrating crest cells at cranial regions as they populate the pharyngeal arches. Depletion of *Sox9* using antisense morpholinos caused a loss of neural crest cells, which was also reflected in reduced *Slug* transcription and a compensatory expansion of the neural plate territory (Spokony *et al.,* 2002). Thus, *Sox9* may be part of a cascade involved in neural crest-derived craniofacial development.

Along with *Sox9*, the transcription factor *AP2* α was also implicated in cranial neural crest development as gene targeting of *AP2* α in the mouse resulted in severe craniofacial abnormalities (Schorle *et al.,* 1996, Zhang *et al.,* 1996). *AP2* α is detected in Xenopus already at open neural plate stages where transcripts are abundant mainly in the cranial region but also evident in the prospective trunk neural crest. Like *Sox9* and *Slug*, also *AP2* α is under positive control of Wnt and BMP signaling and is required for neural crest formation. Interference with *AP2* α gene activity reduced transcription of both *Slug* and *Sox9* yet a positive feedback loop was suggested to connect between *AP2* α, *Slug* and *Sox9* that may underly initial formation and later maintenance of the crest phenotype (Luo *et al.,* 2003).

Taken together, growing evidence points to the involvement of multiple genes in the formation of the neural crest at various levels of the axis. The nature of interactions between these genes awaits to be elucidated in the general context of neural induction, as well as in the actual formation and maintenance of presumptive neural crest. Differences between genetic cascades leading to neural crest specification in the head versus the trunk are already apparent in view of the restricted expression of certain genes to either region {for example *Noelin* (Barenbaum *et al.,* 2000) and *Id2* (Martinsen and Bronner-Fraser, 1998)}. Along this line, most studies dealing with neural crest formation focused so far on the cranial region, so much is still to be done to characterize regional differences as well as common mechanisms.

Notch/Delta signaling in the formation of neural crest

Notch family members are expressed by neural crest cells in rodents (Weinmaster *et al.,* 1991; Williams *et al.,* 1995) and earlier on, both Notch and Delta genes are expressed in avians in the cranial neural plate and epidermal ectoderm (Endo *et al.,* 2002), suggesting they might be involved in neural crest formation. In the latter study, it was suggested that moderate levels of Notch are required for maintenance of ectodermal BMP4 expression, which in turn affects the formation and/or maintenance of the crest phenotype. In zebrafish, impaired *Delta* gene activity caused a reduction in neural crest production but led to formation of supernumerary Rohon-Beard neurons, an effect that appeared restricted to trunk levels of the axis (Cornell and Eisen, 2000). Delta/Notch signals do not appear to directly affect specification of crest cells, but rather indirectly, by regulating levels of *neurogenin 1* which is expressed in Rohon-Beard cells and is required for their differentiation (Cornell and Eisen, 2002 and see below). Hence, the determination of these alternative fates in the zebrafish neural plate border requires Delta/Notch signaling which acts by repressing proneural gene expression, results which are consistent with previous findings (Simpson, 1997; Blader *et al.,* 1997, Ma *et al.,* 1996).

Neural crest formation: does it involve the establishment of resident neural crest stem cells or sequential cell recruitment?

As discussed previously, signaling by BMP and Wnt/FGF mediates initial expression of early neural crest markers, a hallmark that characterizes formation of the presumptive neural crest in the neural plate/ectoderm border which later becomes the dorsal midline of the closed neural tube. In avian embryos at cranial regions of the axis, emigration of specified neural crest cells from the neural primordium lasts approximately 10-15 hours. However, at trunk levels, this process is very prolonged and persists for over two days for any given axial level (Erickson *et al.,* 1992, Reedy *et al.,* 1998, Burstyn-Cohen and Kalcheim, unpublished data). Prolonged departure occurs through sequential waves of emigrating cells that reach the staging area between neural tube

and somites within 4-5 hours following delamination (Burstyn-Cohen and Kalcheim, 2002) and then continue migrating till populating the different crest anlage in a ventral to dorsal direction (reviewed in Le Douarin and Kalcheim, 1999). Moreover, neural crest cells in the trunk delaminate in a synchronous fashion from the neural tube, when they enter the S phase of the cell cycle (Burstyn-Cohen and Kalcheim, 2002 and see next section). In view of the continuous departure of crest cells from their source, the question arises of the mechanisms responsible for replenishment of the dorsal pool of premigratory crest. One possibility is that upon initial specification, a local neural crest stem cell population arises in the dorsal midline which then progressively undergoes asymmetric cell division rendering two daughter cells, one basal and the other apical. In such a case, after completion of mitosis, the basal daughter cells would delaminate upon transition from G1 to S, whereas the apical cells would continue cycling within the epithelium as stem cells to give rise again to one delaminating and one resident stem-like cell, respectively. This mechanism seems unlikely for several reasons. First, once delamination has begun, the proportion of cells in the dorsal midline undergoing DNA synthesis is low (about 25%) when compared to the corresponding values in delaminating cells (80-85%), suggesting this mechanism would cause a very rapid depletion of a pool of putative stem cells in the dorsal midline region if this were the only source of neural crest. Furthermore, this is not compatible with the observations that delamination is still underway by stages HH18-20 in the trunk (Erickson *et al.,* 1992, Reedy *et al.,* 1998). Second, inspite of observing both vertical as well as horizontal cleavage planes in the dorsal midline (our unpublished observations, see also Erickson, 1993), there is no evidence for asymmetric cell division in this area in the sense of one cell remaining as a resident stem cell and its sister undergoing delamination.

An alternative source of premigratory crest could therefore be the neighbouring dorsolateral neural tube cells. It is possible that upon continuous cell withdrawal from the dorsal midline area, laterally localized progenitors are directed towards the center thereby entering the BMP4-expressing zone that triggers their delamination (Sela-Donenfeld and Kalcheim, 1999) following G1/S transition (Burstyn-Cohen and Kalcheim, 2002). Thus, two main models could account for the specification of the premigratory pool of NC cells over time; an early and perhaps single inductive event that leads to the formation of stem-like cells, versus continuous production by relocation of adjacent neuroepithelial cells into the dorsal midline field. The latter would convey the transiting cells with neural crest properties by triggering their delamination. Such a view would be consistent with results of clonal analysis in vivo that showed the existence of common progenitors for both neural tube and neural crest phenotypes (Mujtaba *et al.,* 1998; Sanes, 1989; Bronner-Fraser and Fraser, 1988, 1989, Artinger *et al.,* 1995) thus enabling a change in fate upon relocation. Distinguishing between the above possibilities will be fundamental for understanding the significance of dorsal midline signaling by BMP and Wnt factors in maintenance of the neural crest phenotype until depletion of these cells and subsequent differentiation of dorsal progenitors into spinal interneurons, the mechanisms leading to the segregation of neural crest cells from other CNS lineages and the dynamic histogenesis of the dorsal neural primordium.

Specification of neural crest-derived lineages

From research performed over the past two decades it has become increasingly evident that already at the begining of emigration from the neural tube, the neural crest is composed of heterogeneous populations of cells, some pluripotent and others already restricted to different degrees in their developmental potentials, including precursors commited to one particular fate. These results suggest that environmental signals encountered during migration and homing are likely to operate both by instructive and also by permissive mechanisms on target cells with varying degrees of developmental restriction.

Neurons versus glia: the role of Notch/Delta

Neural crest cells give rise to neurons and glia in peripheral ganglia at trunk levels of the axis. Notably, these two cell types are sequentially generated with neuronal differentiatiation preceding that of glia. Since Notch signaling inhibits neuronal differentiation in both invertebrates and vertebrates (see for example Coffman *et al.,* 1993, Fortini *et al.,* 1993, Henrique *et al.,* 1997), the notion was tested that Notch ligands are also involved in mutual interactions that restrict the fate among neural crest-derived cells. In avian dorsal root ganglia, *Delta 1* was found to be expressed in young neurons and Notch1 activation in neural crest cultures prevented neuronal differentiation yet permitted that of glial cells (Wakamatsu *et al.,* 2000). Consistently, Morrison *et al.,* (2000) isolated multipotent progenitors from rat embryonic sciatic nerves and cultured them under clonal conditions. Transient activation of Notch in these clonal cultures was sufficient to cause a loss of neurogenic potential accompanied by accelerated glial differentiation. Altogether, these results suggest that intercellular interactions mediated by Notch/Delta activity play a role in the segregation of neuronal versus glial lineages. In vivo evidence for such a switch is still lacking. It would also be important to clarify the possible relationship between Notch/Delta and growth factors implicated in glial differentiation such as the Neuregulins (discussed in Morrison *et al.,* 2000 and Wakamatsu *et al.,* 2000).

Sensory versus autonomic fates: the roles of Neurogenin genes in sensory specification

Another scene in which the problem of lineage segregation has been addressed is the specification of sensory and autonomic lineages, the two major neuronal types of the peripheral nervous system. Back-grafting studies in avian embryos have recognized neural crest-derived cells able to give rise to autonomic but not sensory derivatives. Clonal analysis in vivo and in vitro have identified individual progenitors that can give rise to both neuronal classes, yet, separate sensory neurons were also observed (reviewed in Le Douarin and Kalcheim,1999; Weston,1998).

Cloning of homologues of Drosophila proneural genes in different vertebrate species provided a unique tool to address the molecular basis of segregation between sensory and autonomic lineages. The Neurogenins are specific bHLH transcription factors related to Drosophila atonal (Gradwohl *et al.,* 1996; Ma *et al.,* 1996; McCormick *et al.,* 1996; Sommer *et al.,* 1996). *Neurogenin* was found to precede expression of *NeuroD*. Overexpression of *Neurogenin* in Xenopus embryos stimulated ectopic neurogenesis and induced transcription of *NeuroD* (Ma *et al.,* 1996). These results placed *Neurogenin* upstream of *NeuroD* and suggested that the former is a neuronal determination gene whereas *NeuroD*

is likely to act on cell differentiation. Loss of function experiments were carried out in mice where *Neurogenins 1* and *2* were deleted. Embryos lacking *Neurogenin1* function failed to generate the proximal cranial sensory ganglia that comprise the trigeminal, vestibulo-cochlear, accesory, jugular and superior ganglia (Ma *et al.,* 1998). Complementary to this phenotype, deletion of *Neurogenin 2* resulted in elimination of the distal cranial ganglia including the geniculate and petrosal with no apparent effect on the proximal ones (Fode *et al.,* 1998). Notably, the nodose ganglion, which express both *Neurogenins* was spared in the single mutants of either type, suggesting a mutual compensation of gene activity (Fode *et al.,* 1998). Taken together, these results demonstrated the requirement of the *Neurogenins* in development of cranial sensory ganglia, whether derived from the neural crest, the ectodermal placodes, or both. The question was also raised of the role of Neurogenins in dorsal root ganglia. It was reported that Neurogenin 2 is primarily required for the generation of proprioceptive and mechanoceptive neurons that express trkC+ and trkB+ whereas trkA+ nociceptive afferents depend upon Neurogenin1 function (Ma *et al.,* 1999). Interestingly, lineage tracing of the fate of mouse neural crest cells revealed that *Neurogenin 2*-expressing progenitors were four times more likely than the general crest population to contribute to dorsal root ganglion cells of either neuronal or glial types when compared to sympathetic ganglia (Zirlinger *et al.,* 2002). These data would suggest that expression and function of this gene is associated with at least a bias towards the sensory phenotype. Further evidence in support of the existence of specified sensory progenitors in the mammalian crest was the observation that expression of the *Neurogenins*, of *NeuroD* and of additional sensory markers could not be reversed in a subset of cultured neural crest cells even when challenged with BMP2, a factor that induces autonomic traits (Greenwood *et al.,* 1999). This notion was confirmed in avian embryos where the *Neurogenins* mark subsets of ventrally migrating crest cells. Ectopic expression of these genes biased crest cells to localize to sensory ganglia and even induced sensory marker expression in infected mesodermal tissues such as the dermomyotome (Perez *et al.,* 1999). Similar results were obtained in zebrafish where all peripheral sensory neurons were found to depend on *Neurogenin 1* function (Cornell and Eisen, 2002). Altogether, strong evidence points to a function for the Neurogenins in specification of the sensory fate. The early expression of these genes in subsets of migrating progenitors also suggests these particular cells might be already segregated from the general population to give rise to sensory rather than to autonomic derivatives. Wnt signaling acting via βcatenin and probably operating already in the dorsal neural tube on premigratory crest cells, was reported to act upstream of the Neurogenin cascade to induce the sensory phenotype in mouse embryos (Hari *et al.,* 2002, Lee *et al.,* 2004).

The delamination of neural crest progenitors from the neural tube

The delamination of neural crest cells from the dorsal midline of the neural tube and their migration through neighboring structures represents a unique feature of the neuroepithelium, as CNS counterparts migrate and differentiate within the confines of the neural tube. At the same time, this process of epithelial-mesenchymal conversion of premigratory neural crest cells, is a common feature during normal development of several embryonic structures and also underlies the formation of metastases during tumor progression (reviewed in Thiery, 2003). Hence, investigating delamination of crest progenitors represents a model system for understanding the underlying molecular basis of epithelio-mesenchymal transitions and for evaluating how conserved this process is at various axial levels, across developmental systems and during tumor spreading.

A balance between BMP and its inhibitor noggin regulates neural crest delamination in the trunk

The onset of neural crest cell migration is a complex morphogenetic process which involves the coordinated action of several categories of molecules (cell adhesion molecules, cytoskeletal components, extracellular matrix macromolecules and transcription factors) upon which environmental signals act (Christiansen *et al.,* 2000, Kalcheim, 2000, Nieto, 2001). The identity of these signals was recently elucidated. In initial studies, Delannet and Duband (1992) reported that added TGFβ1 and 2, stimulated emigration of cultured neural crest cells, possibly by increasing the adhesion of the cells to their substrate at the expense of intercellular interactions. The presence of these factors or that of related molecules in the dorsal neural tube was, however, not investigated, neither was their requirement under physiological conditions.

More recently, Sela-Donenfeld and Kalcheim (1999) reported that in avian embryos over a broad age range (15-40 somites), *BMP4* mRNA is homogeneously distributed along the longitudinal extent of the dorsal neural tube, whereas its specific inhibitor *noggin* exists in the dorsal domain of the tube in a gradient of expression that decreases caudorostrally. This rostralward reduction in signal intensity was found to coincide with the onset of emigration of neural crest cells. Hence it was hypothesized that an interplay between noggin and BMP4 in the dorsal tube generates graded concentrations of the latter that in turn triggers the delamination of neural crest progenitors. Consistent with this suggestion, disruption of the gradient by grafting noggin-producing cells dorsal to the neural tube at levels opposite the segmental plate, inhibited emigration of HNK-1-positive crest cells without affecting expression of *Slug*, either at the mRNA or protein levels, suggesting that BMP4/noggin affect neural crest delamination independently of an earlier effect on cell specification. This notion was further substantiated by the finding that late delamination of crest cells was also inhibited upon noggin treatment of older neural primordia taken from levels opposite epithelial somites, where emigration of crest cells had already started. Hence, specification and delamination of crest cells as induced by BMP signaling are separable processes. In further agreement, Liem *et al.,* (1997) have shown that the competence of neural epithelium to generate neural crest cells in response to BMP is transient. The switch in the ability of BMP4 to generate neural crest cells or to stimulate their delamination may coincide with the downregulation of BMP in the ectoderm and the beginning of its synthesis in the dorsal neural tube. Moreover, a role for BMP2 rather than BMP4 in crest emigration/migration from rhombencephalic areas of mouse embryos was proposed (Kanzler *et al.,* 2000). Notably, a BMP-dependent mechanism for delamination of neural crest cells may not operate along the entire axis of avian embryos since at

mesencephalic levels, the sole inhibition of BMP activity did not prevent crest cells from delaminating (Sela-Donenfeld and Kalcheim, Unpublished results).

As pointed out precedently, the activity of BMP4 along the rostrocaudal axis of the neural tube is modulated by changing levels of *noggin* (Sela-Donenfeld and Kalcheim, 1999). In order to understand the basis underlying regulation of neural crest delamination, it was necessary to clarify what signals help establishing the gradient of production of *noggin* along the neural tube. The temporal coordination between somite dissociation and the onset of neural crest migration, suggested that factors produced by the paraxial mesoderm might regulate the production of *noggin* in the dorsal neural tube. In line with this suggestion, experimental manipulations of the paraxial somitic mesoderm altered the pattern of *noggin* transcription in the dorsal neural tube; deletion of epithelial somites prevented normal downregulation of *noggin*. Furthermore, partial ablation of either the dorsal half or only the dorsomedial portion of epithelial somites was sufficient to maintain high *noggin* expression when compared to the normal downregulation of this gene in the control side. These data suggested that the dorsomedial region of dissociating somites produces an inhibitor of *noggin* transcription in the dorsal neural tube. Consistent with this notion, grafting dissociating somites in the place of the unsegmented mesoderm precociously downregulated expression of *noggin* and triggered premature emigration of neural crest progenitors from the caudal neural tube, an area never releasing mesenchymal cells under normal conditions. Thus, an inhibitory cross-talk exists between the paraxial mesoderm and the neural primordium that is mediated by regulating levels of *noggin* transcription. This interaction controls the timing of neural crest delamination to match the development of the somites into a suitable substrate for subsequent crest migration (Sela-Donenfeld and Kalcheim, 2000). Noteworthy, noggin activity is not restricted to the neural primordium. A dynamic expression of this inhibitory protein was also revealed in the somites, where changing levels of transcription were found to be modulated by BMP itself (Sela-Donenfeld and Kalcheim, 2002), suggesting the existence of a feedbak loop by which BMP controls synthesis of its own inhibitor which in turn modulates ligand activity.

Based on these functional data, it was important to assess whether BMP receptors were correspondingly expressed at the right sites and time. The responses to BMP family members are thought to be mediated by heterotetrameric complexes composed of type II receptors in concert with type I receptors of either class A or B, which transduce preferentially signaling by BMP2/4 or BMP7, respectively (see for example Massague and Chen, 2000). In the developing nervous system, BMP receptors of type IA were already visible in the dorsal neural folds at caudal levels of the neuraxis and later in the dorsal midline of the neural tube where premigratory crest cells reside (Sela-Donenfeld and Kalcheim, 2002). Notably, receptor mRNA signal was still detectable in the delaminating crest cells but was rapidly downregulated to undetectable levels in the migrating progenitors as they moved farther from the tube. At variance, expression of type IB receptors was largely restricted to the mesoderm. Taken together, the observed expression patterns are consistent with a possible role for BMP receptors of type IA, but presumably not for type IB, in mediating the effects of BMP4 on dorsal tube and crest development in avian embryos (Sela-Donenfeld and Kalcheim, 2002).

BMP-dependent genes and neural crest delamination

Genes such as *Slug, FoxD3, PAX3, rhoB, Cad- 6, Msx1* and *2, Wnt 1* and *3a*, etc, are either specifically expressed or become restricted to the dorsal tube from early stages onward, making it in some instances difficult to discriminate between possible roles in specification of the neural crest, subsequent delamination, or both. Experiments had to be designed to inhibit delamination without affecting initial specification of crest cells, hence BMP activity was abrogated following initial expression of these genes. The inhibition of neural crest emigration observed in vivo following noggin treatment was preceded by a partial or total reduction in the expression of *cadherin 6B, rhoB, PAX3, Msx1,2* and *Wnt1*, but not that of *Slug* (see above, Sela-Donenfeld and Kalcheim, 1999, Burstyn-Cohen *et al.,* 2004). Their local downregulation suggests these genes may be part of a molecular cascade triggered by BMP4, that leads to the separation of neural crest cells from the neural tube. This hypothesis requires that the effect of each factor be tested in experimental contexts in which it is possible to dissociate between specification and delamination events.

Wnt proteins play significant roles in neural crest cell development at different developmental times and in several species (reviewed in Wu *et al.,* 2003). In avian embryos, Wnt 6 is synthesized in the epidermal ectoderm and might mediate crest specification (Garcia-Castro *et al.,* 2002) although a direct link between the two is still lacking. Slightly later, Wnt1 and Wnt3a are present in the dorsal neural tube following initial specification of crest cells (Dickinson *et al.,* 1995). Yet, Wnt3a is intense already opposite the segmental plate while Wnt1 becomes apparent slightly later, opposite epithelial somites and concomitant with BMP relief from noggin inhibition (Burstyn-Cohen *et al.,* 2004). Whereas Wnt1 is likely to be directly regulated by BMPs (Marcelle *et al.,* 1997, Sela-Donenfeld and Kalcheim, 2002, Burstyn-Cohen *et al.,* 2004), the transcription of Wnt3a is not; suggesting that Wnt1 better fits to be a putative candidate in crest delamination (Burstyn-Cohen *et al.,* 2004). Significantly, Wnt signaling through the transmembrane receptor Frizzled is required to modulate the distribution and function of β-catenin (Miller and Moon, 1997). β-catenin, as well as plakoglobin (γ-catenin) associate directly with the highly conserved cytoplasmic domain of cadherins. The so formed cadherin-catenin complex links to the actin filament network via actinin or vinculin (Ozawa *et al.,* 1989; Hinck *et al.,* 1994; Knudsen *et al.,* 1995; Weiss *et al.,* 1998). BMP/Wnt-mediated signals could induce changes in the actin cytoskeleton via rhoB and possible relations between rhoB and cadherin pathways remain to be clarified. A role for rhoB in crest delamination has been already suggested based on inhibition experiments in culture (Liu and Jessell, 1998). A molecular pathway for the activation of Rho by Wnt/frizzled was suggested, which involves the formation of a complex between Rho, dishevelled and Daam1 in the plasma membrane, resulting in the generation of a polarized cytoskeleton (Habas *et al.,* 2001). Thus, the dynamic association of the catenin-cadherin complex and that of rhoB with the cytoskeleton may be essential for regulating cell-cell interactions leading to neural crest delamination. On the other hand, Ikeya *et al.,* (1997) proposed that Wnt signaling might be required for the expansion of a pool

of neural crest cells, a process that could also affect neural crest delamination (see next section). Notably, Rho GTPases could also be effectors of Wnt signals in this pathway as they were shown to affect morphogenesis by interfering with cell proliferation (Wei *et al.,* 2002).

Pax-3 is expressed in both the dorsal neural tube and the adjacent somites (Goulding *et al.,* 1991). The mouse mutation *Splotch* (Russell, 1947) represents a deletion in the gene coding for *Pax-3* (Kessel and Gruss, 1990; Epstein *et al.,* 1991). *Splotch* mutants are characterized by defects in neural tube closure and severe reduction or even absence of certain neural crest derivatives including pigment cells, sympathetic and spinal ganglia, enteric neurons and cardiac structures. These defects were suggested to result from a delay in the onset of neural crest emigration from the neural tube (Moase and Trasler, 1990). Another study found that crest cell emigration (or formation) was severely affected in the vagal and rostral thoracic areas, while virtually no cells emigrated from the tube more caudally, perhaps as a result of aberrant interactions among adjacent neural tube progenitors or between neural crest and somitic cells (Serbedzija and McMahon, 1997). A possible role for *Pax-3* in mediating epithelial-mesenchymal interactions was suggested in other systems (Wiggan *et al.,* 2002) as well as the possibility that *Pax-3* triggers a non-canonical Wnt signaling cascade entailing JNK activation (Wiggan and Hamel, 2002).

The role of *FoxD3* in formation of the neural crest was documented (see section III), yet its possible function in cell delamination remains unclear. Dottori *et al.,* (2001) reported that forced expression of *FoxD3* induced ectopic HNK-1 expression in the lateral part of the neuroepithelium and this event was followed by significant cell delamination. At variance, ectopically induced HNK-1-positive progenitors failed to reveal dispersive behavior according to Kos *et al.,* (2001).

As previously discussed, BMP4 had no effect on the maintenance of *Slug* expression either at the mRNA or protein levels and yet inhibiting BMP prevented crest emigration in the trunk (Sela-Donenfeld and Kalcheim, 1999). This result would indicate that Slug activity is not sufficient for emergence of neural crest cells at least in the trunk region. In support of this notion, it was reported that neural crest cells still leave the neural primordium by stages 18-20 of development at trunk levels of the axis (Erickson *et al.,* 1992, Reedy *et al.,* 1998) a time when *Slug* is not transcribed any longer (Sela-Donenfeld and Kalcheim, 1999, Liu and Jessell, 1998). Furthermore, forced expression of *Slug* enhanced the production and migration of neural crest cells in the head but not in the trunk where *Slug* is exclusively expressed in the pre-migratory population (Del Barrio and Nieto, 2001). Altogether, these results suggest that *Slug* expression in the trunk neuroepithelium may be a hallmark of early forming neural crest but not be instrumental for subsequent cell delamination. A different situation holds for cranial areas, where *Slug* is expressed both in premigratory as well as in the migrating cells (Nieto al, 1994). At this level, Slug activity might affect the progression of crest migration, as shown in Xenopus embryos (Carl *et al.,* 1999), but a direct effect of the Slug protein on delamination of cranial crest cells is still lacking in the chick as neither loss or gain of function experiments discriminated between specification versus epithelio-mesenchymal conversion (Nieto *et al.,* 1994, Del Barrio and Nieto, 2001).

The above results highlight the existence of significant differences in the mechanisms leading to delamination of neural crest cells in cranial as compared to trunk levels of the axis. These are exemplified not only by differences in Slug function and in the duration and intensity of cell delamination between the two areas, but also in differential regional expression of other relevant genes, such as *noggin* (our unpublished results), *AP2* (Schorle *et al.,* 1996, Zhang *et al.,* 1996), etc, in the hierarchical relationship between BMP and Wnt signals in the two areas (Ellies *et al.,* 2000, Marcelle *et al.,* 1997) and in the cell cycle characteristics of delaminating cells (see next section). Interestingly, differences in the role of specific factors in cranial as compared to adjacent vagal levels of the axis were also documented. For instance, deleting the–*zfhx1b* gene, a zinc finger and homeodomain-containing transcription factor that encodes Smad-interacting protein-1, caused arrest of delamination of cranial neural crest cells without impairing their specification and yet resulted in a failure of the actual formation of vagal-level progenitors. The latter led to a phenotype partially resembling the aganglionic megacolon syndome observed in humans carrying a mutation in this gene (Van de Putte *et al.,* 2003). Hence, the upstream trigger/s of the massive and rapid delamination of crest cells in the head remain to be elucidated and the intracellular mechanisms remain to be worked out in all regions.

The role of the cell cycle in neural crest delamination

Neural crest cells are mitotically active progenitors while residing in the dorsal neural tube and throughout migration. This initially discrete population must expand to reach the final number of cells that populates peripheral ganglia and other derivatives. The first post-mitotic cells appear by the time of gangliogenesis (Kahane and Kalcheim, 1998). Prior to emigration, prospective neural crest progenitors are an integral part of the neuroepithelium and, as such, they undergo interkinetic nuclear migration whereby the position of the cell soma with its nucleus changes upon the phase of the cell cycle (Martin and Langman, 1965, Langman *et al.,* 1966 and refs. therein). Moreover, they reveal similar cell cycle characteristics to laterally located progenitors with a mean generation time of about 8 hours in avian embryos (Langman *et al.,* 1966, Smith and Schoenwolf, 1987,1988 and refs. therein).

Nevertheless, the dorsal area of the neural tube becomes highly distinct from the remaining neuroepithelium when *noggin* is downregulated and consequently, BMP becomes activated at high levels thus triggering crest cells to delaminate. The question was then asked whether neural crest cells randomly emigrate at any phase of the cycle or alternatively, whether there is a preferred phase for delamination. Trunk-level avian neural crest cells were found to emigrate synchronously in the S-phase of the cell cycle (Burstyn-Cohen and Kalcheim, 2002), in support of the latter possibility. The functional significance of the observed synchronous delamination was examined by inhibiting G1-S transition with olomoucine, AG555 or mimosine. All treatments prevented initial delamination of neural crest cells that could be rescued upon drug removal. In contrast, aphidicolin or VM-26, which inhibit the cycle at S and G2 phases, respectively, had no effect. Furthermore, in ovo overexpression of the 15 amino-acid domain of MyoD, which specifically binds to cdk-4/6 and thus prevents G1-S transition, inhibited both Brdu incorporation and NC delamination, but affected neither specification nor survival of the neural progenitors. Likewise,

overexpression of the cyclin-dependent kinase (cdk) inhibitor p27 and of a dominant-negative form of the retinoblastoma- binding E2F-1 transcription factor, prevented both entry into S phase as well as neural crest delamination. These results showed for the first time that the transition between G1 to S is a necessary event for the epithelial-to-mesenchymal conversion of premigratory neural crest cells (Burstyn-Cohen and Kalcheim, 2002).

Roles of the cell cycle in morphogenesis and in the generation of cell movement

Previous studies have already highlighted possible links between specific phases of the cell cycle and generation of cell movement. Short-range interactions between the ganglionic eminence and neocortical epithelium influence interkinetic nuclear migration and cell exit from the primary epithelium (Miyama *et al.,* 2001). Studies on the mechanisms of cell division in Drosophila provided a paradigm for understanding how information that controls stereotypic mitoses is translated into cell movement (Follette and O'Farrell, 1997). Fibronectin substrates induce shortening of the G1 period in migratory neural crest cells (Paglini and Rovasio, 1999). A role for the cell cycle in patterning and morphogenesis during neural development has also been suggested. For instance, lengthening of specific phases of the cycle was found to be associated with bending of the neural plate (Smith and Schoenwolf, 1987, 1988). The laminar fate of cortical neurons was shown to be determined during the S or G2-phases of the final cell cycle (McConnell and Kaznowski, 1991, Ohnuma *et al.,* 2001). The control of cell proliferation is also crucial for the establishment of the correct number of daughter cells and could influence cell fate. The choice between cell cycle progression or exit followed by differentiation is influenced by extrinsic signals operating during G1 (Elshamy *et al.,* 1998). At this point, mitogens stimulate activation and synthesis of pro-cell cycle proteins of the D- and E-type cyclins and their partners, cdk4/6 and cdk2, respectively, the key regulators of the G1 restriction point and the G1-to-S phase transition. In contrast, differentiation signals upregulate cell cycle inhibitory proteins such as p21, p27 or p57 (Ohnuma *et al.,* 2001, Zhang, 1999). The pigmented epithelium modifies the plane of cell division in adjacent retinal progenitors, an event with possible significance in determining cell fate (Cayouette *et al.,* 2001). Regulation of the cell cycle is also intimately linked to cell death. In neuronal cells, apoptosis caused by deprivation of trophic support can be prevented by agents that block G1/S transition (Farinelli and Greene, 1996, Park *et al.,* 1997).

Roles of cell cycle genes in morphogenetic processes

Cell cycle progression is regulated by cdk's that are activated upon interaction and binding to cyclins and inhibited by cdk inhibitors. Cdk's regulate diverse biochemichal pathways while integrating extracellular and intracellular signals, the nature of which can be either mitogenic or growth-inhibitory. Integration of these signals is interpreted by means of cell cycle transitions. The G1/S transition is governed by cdk's coupled to Cyclin D, A and E; while cyclin B-associated cdk's regulate transition between G2 and M phases. Two families of cdk inhibitors negatively regulate the cell cycle: the INK4 family ($p15^{INK4B}$, $p16^{INK4A}$, p18 and p19) bind to and inhibit cdk4/6 and the CIP/KIP family of proteins ($p21^{CIP1}$, $p27^{Kip1}$ and $p57^{Kip2}$) which binds primarily to cyclin E- and cyclin A-bound cdk2 and to cyclin D-bound cdk4/6 with lower affinity (reviewed in Singerland and Pagano, 2000; Coqueret, 2002).

The key regulators of the cell cycle, cdk's and cyclins, are now being "rediscovered" with novel roles that are independent of their classical functions in controlling the cell cycle. Such functions include centrosome formation and DNA replication by cyclin E (reviewed in Winey, 1999), transcriptional control of various genes by cyclin D (reviewed in Coqueret, 2002), muscle and neuronal differentiation as well as acquisition of cell motility by p27 (McAllister *et al.,* 2003; Vernon and Philpott, 2003; Vernon *et al.,* 2003), etc. This association between specific cell cycle genes and cellular functions might reflect the need of the cell to coordinate important events in a timely rather than premature fashion, to avoid malformations or even lethality. Several novel roles of cell cycle proteins will be briefly discussed below, together with their possible relevance to neural crest migration.

The D-type cyclins are the first cyclins to be activated during the G1 phase. Following mitogen stimulation, cyclins of type D bind cdk4 or cdk6 and activate their kinase activity to phosphorylate target proteins, including pRB. Hyperphosphorylation of pRB disrupts its interaction with histone deacetylase and histone methylase, facilitating chromatin accessibility to transcription. Phosphorylated pRb also releases the bound transcription factor E2F-1 from repression, thus enabling E2F-1– dependent transcription of genes that are required for transition into S phase, including that of cyclin E (Coqueret, 2002). Unlike the transcription of cyclins E, A and B, that of D-type cyclins, as well their accumulation at a protein level and their cellular localization are largely dependent on extracellular signals such as mitogens and nutrient stimulation (Matsushime *et al.,* 1991). This places D-type cyclins as a putative link between growth inducers and the cell cycle machinery. In addition to this central role, Cyclin D has recently been suggested to control transcription of DNA-binding proteins, that in turn regulate specific target genes. This transcriptional control of cyclin D is probably independent of its cell cycle role as it does not involve cdk4 activation. Furthermore, cyclin D1 is also able to affect the differentiation state of myoblasts through inactivation of MyoD transcription and restricts premature differentiation of intestinal epithelial cells through inhibition of the specific *Beta2/NeuroD* transcription factor (reviewed in Coqueret, 2002).

In light of the precedent findings, it is tempting to speculate that cyclin D1 might also play a role in the ontogeny of neural crest cells. *Cyclin D1* transcription is weak in the neural tube opposite segmental plate levels and becomes gradually prominent at axial levels corresponding to neural crest emigration (Burstyn-Cohen *et al.,* 2004). Along this line, Wnt-dependent transcription of *Cyclin D1* in the dorsal tube (Megason and McMahon, 2002) mediates delamination of crest cells by affecting transcription of genes involved in cell adhesion and in the generation of cell movement (Burstyn-Cohen *et al.,* 2004). Furthermore, the central role of cyclin D1 in the cell cycle, together with its enrichment in the dorsal neural tube, suggests it might be involved in maintaining the balance between neural crest proliferation (G1/S transition) and delamination. Cyclin D1 could affect the continuous recruitment of progenitors to the midline thus ensuring the dorsal tube is not depleted from cells due to extensive crest delamination.

As mentioned above, p27 is a member of the Cip/Kip family of cell cycle inhibitors that negatively regulates cell cycle progression

at G1 (Singerland and Pagano, 2000). Recent work by McAllister *et al.* (2003) suggests a novel cell scattering activity for p27 which is mediated by a motility domain localized to the C-terminus of the molecule. It has been proposed that following HGF/SF stimulation of human hepatocellular carcinoma cells, mediated by the Met receptor, p27 is phosphorylated and exported from the nucleus to the cytoplasm, where it binds F-actin and modulates cytoskeletal rearrangements leading to cell migration. In line with this is the fact that p27-deficient primary fibroblasts failed to migrate, a motility defect that was rescued by introducing into the cells either wild-type p27 or the C-terminal motility domain. Notably, this activity of p27 was reported to be independent of its function in cell cycle inhibition, as the cdk-cyclin binding domain resides in its N-terminus. This scattering ability of p27 may be context-dependent, as mimosine, a drug that stimulates induction of p27 and its translocation into the nuclei of neural crest cells, prevented the emigration of crest progenitors from neural primordia (Burstyn-Cohen and Kalcheim, 2002).

Possible interactions between the BMP cascade and cell cycle genes in controlling neural crest delamination

Having shown that the epithelial to mesenchymal conversion of premigratory neural crest is triggered by a local balance between BMP4 and its inhibitor noggin and that these neural tube-specific events are temporally modulated by an inhibitor of *noggin* transcription produced in the dorsomedial somites (Sela-Donenfeld and Kalcheim, 1999, 2000), an essential question was what is the relationship between the above environmental signals and cell-intrinsic mechanisms such as the requirement for G1/S transition for cell delamination (Burstyn-Cohen and Kalcheim, 2002). One possible link between the two is that BMP4 induces a cascade of secondary signals that influence G1/S transition via activation of cyclin-cdk complexes in dorsal tube progenitors, a process which is in turn translated into parameters of the delamination machinery. In favor of such a content, Panchinsion *et al.,* (2001) have demonstrated that BMP receptors of type 1A, but not 1B, transduce a mitogenic signal in mouse neuroepithelial cells. Notably, the dorsal midline of the avian tube also expresses type 1A receptors at a comparable time in avians (Sela-Donenfeld and Kalcheim, 2002). In addition, *Msx1*, a downstream transcription factor induced by BMP in the dorsal neural tube upregulates *cyclin D1* and cdk4 activity (Hu *et al.,* 2003). Dorsal neural tube-derived BMP4 also stimulates transcription of *Wnt1* (Marcelle *et al.,* 1997, Sela-Donenfeld and Kalcheim, 2002) and the Wnt1-dependent β-catenin/LEF-1 pathway regulates transcription of *cyclin D1* and cell proliferation in a variety of cells (Kioussi *et al.,* 2002; Shtutman *et al.,* 1999; Tetsu and McCormick, 1999) including the avian neuroepithelium (Kubo *et al.,* 2003; Megason and McMahon, 2002 but see Hari *et al.,* 2002). Hence, it is likely that the roles played by environmental signaling such as BMP/noggin and by cell autonomous events such as G1/S transition in delamination of neural crest cells, are part of a single pathway which operates through an intermediate stage that requires Wnt activity. Recent evidence is accumulating in support of this view. Recently, BMP was found to regulate G1/S transition via the canonical pathway of Wnt signaling and inhibition of the latter prevented emigration of neural crest progenitors while downregulating cyclin D1 (Burstyn-Cohen *et al.,* 2004). Whereas the molecular backbone leading to neural crest delamination at trunk levels begins to be clarified, the downstream signals that translate cell cycle parameters into the generation of crest cell movement remain to be investigated.

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Note added in proof

The present review covers studies published until 2003. Many exciting results appeared during 2004 and subsequently, but only few could be added during the final stages of editing this volume. Our apologies to those authors whose work could not be properly addressed.

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