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Review

Regulation of neural crest cell populations: occurrence, distribution and underlying mechanisms

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KEY WORDS: *neural crest cells, regulation, cell division/death, cell migration, cell fate*

Introduction

Adult organisms have long been known to compensate for injurious elements in their environment. Compensatory growth, wound healing, regeneration and regulation are processes by which organisms heal tissue wounds, regenerate lost body parts such as limbs and tails, or replace lost or damaged cells (Goss, 1964, 1969). Regeneration has been documented in organisms as far removed as vertebrates and zooplankton, but because the most common examples include starfish regenerating arms or lizards regenerating tails, it is often assumed to be restricted to some invertebrate lineages and a few vertebrate classes (e.g., Lange, 1920; Boring *et al.*, 1948-49; Beneski, 1989; Brockes, 1991). Regulation also has been identified in a variety of organisms from urochordates to craniates. However, regulation is an embryonic event that has neither been well defined nor distinguished from

Abbreviations used in this paper: BMP, bone morphogenetic protein; EGF, epidermal growth factor; FGF, fibroblast growth factor, TGFβ, transforming growth factor; AP, aorticopulmonary; MMC, mitomycin C; BrdU, bromodeoxyuridine; BDNF, brain derived neurotrophic factor; SMC, secondary mesenchyme cells; PMC, primary mesenchyme cells.

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wound healing or regeneration. The term 'regulation' historically relates to patterns of early embryonic determination that are classified as either 'autonomous' (mosaic) or 'conditional' (regulative). With autonomous development, embryonic morphogens are distributed early in development, cell fates are fixed, and cells develop independently of their neighbors (see Gilbert, 1997 for an up-to-date summary). In embryos with conditional development, the determination and distribution of morphogens occur later in development and cell fates are dependent upon environmental cues, usually in the form of cell-cell interactions.

Embryos have classically been categorized as conditional or autonomous developers based on how development proceeds after cells are separated during early cleavage stages. Driesch (1883, summarized in Gilbert, 1997) demonstrated that complete sea urchin embryos (conditional developers) could develop from isolated blastomeres at the 8-cell stage. More dramatically, Bellairs (1971) found that two separate embryos formed when the blastoderm from the lizard *Lacerta vivipara* was divided. In contrast, when half of the cells were removed from frog embryos (autonomous developers) as early as the two- or four-cell stage, 'half-neurulae' rather than entire embryos developed (Roux, 1888, summarized in Gilbert, 1997). The ability of cells to produce a complete organism when isolated from one another early in development came to be known as regulation.

Since the classic experiments by Spemann and Mangold (see Gilbert, 1997) the concept of regulation has been broadened to include the ability of embryonic cells to compensate for cell loss, as might occur during defective proliferation or following delayed onset of migration (Bellairs, 1971). Lehman and Youngs (1952, pp. 420-21) defined regulation as the "ability or inability of a part of the pigment primordium to compensate for an experimentally produced deficiency in the amount of neural crest material present in the embryo". Or simply and more generally, regulation is the ability of an embryo to compensate for actions that would otherwise lead to abnormal development (Hall and Hörstadius, 1988). Although these definitions appear to set regulation apart from other developmental events, there is considerable overlap between the terms 'regulation' and 'regeneration' in the literature. This brings to question the level at which 'regulation' and 'regeneration' describe similar phenomena. We suggest these terms do represent distinct events; the challenge is to define how they are distinct. To say the difference lies in regulation being a strictly embryonic event is not adequate; replacement of an already formed embryonic chick limb would be considered regeneration, while replacement of a partial limb field or early limb bud would be regulation. Although regeneration loosely describes replacement of lost cells/tissues, it deviates from regulation at the mechanistic level. Regeneration typically involves a cascade of signals that promote dedifferentiation of cells in the wound site, blastema formation, and outgrowth of new tissue (e.g., Mescher, 1996). Regulation involves compensation for deficiencies in undifferentiated embryonic cell populations, rather than differentiated, organized tissues. Moreover, embryonic cells may be replaced by like cells, or by cells from neighboring tissues. The definition we use for regulation is 'the replacement of lost, undifferentiated embryonic cells by other cells in response to signals received from the surrounding environment'.

Cells that are able to regulate must have particular properties. Of utmost significance is the ability to recognize and respond to changes in the environment, such as occurs with cell-cell signaling. From the moment an egg begins to divide, cell interactions are essential for organizing the embryo, specifying germ layers, tissues, organs and functions, and initiating and maintaining specialized cell states by regulating gene expression. If an important interaction is lost, cells may become competent to receive alternative signals and transform to a different state. The ability of grasshopper ectodermal cells to differentiate into neuroblasts following a change in cell-cell contact, or the transformation of axolotl iris cells into lens cells after the removal of the lens are examples of regulation through cell fate change (Taghert *et al.*, 1984; Gilbert, 1997).

Relatively little is known about regulative potential beyond the gastrula stage. Neural crest cells, embryonic cells unique to craniates, are good candidates for studies of regulation because they (1) exhibit broad potentials during development, (2) are essential for normal vertebrate embryonic development, and (3) are accessible for extirpation experiments from where they originate at the boundary of epidermal and neural ectoderm. Furthermore, several alternative cell populations could regulate for neural crest cell loss. McKee and Ferguson (1984) indicated that many questions regarding regulation for neural crest cell populations remain to be answered. For instance:

1. What triggers compensation?
2. Is the rate of neural crest cell division regulated by density-dependent factors such as number of contacts with adjacent cells?
3. How and why do neural crest cells from other regions change their migration patterns to regulate for cell loss?
4. What factors signal that compensation is complete?

Research also is needed to assess the spatial and temporal patterns of neural crest cell regulation.

The goal of this paper is to review studies relevant to neural crest cell regulation in vertebrates, including the strong contributions made by fate mapping studies. We discuss issues such as how neural crest cells are induced, which neural crest and neighboring cell populations exhibit regulation, what factors influence the extent of regulation, and possible mechanisms of regulation.

Primary players in the induction of major cell populations in neurula stage embryos

To explore how neural crest cells and neighboring cell populations (epidermal ectoderm, neural ectoderm, placodes) compensate for loss, we need to understand how neural crest cells are induced. The contribution of neural crest cells to numerous, diverse embryonic tissues (including skeletal, neural and connective) makes them critical for embryonic survival and development. In fact, it has been argued that these cells comprise a fourth craniate germ layer (Hall, 1998, 1999).

Great strides have been made to refine and expand our understanding of the sequential interactions of gene products required for induction of neural crest and neighboring cell types. During neural tube formation, progenitor cells acquire dorsal or ventral fates. Axial mesodermal cells of the notochord express the secreted protein product of *Sonic Hedgehog (Shh)*, which ventralizes the neural tube (Echelard *et al.*, 1993; Krauss *et al.*, 1993; Bronner-Fraser, 1995; Marcelle *et al.*, 1997). Cells of the ventral neural tube subsequently differentiate into floor plate and motor neurons (Fig. 1).

Dorsalization of the neural tube is more complex, involving specialization of dorsally fated cells into epidermal and neural

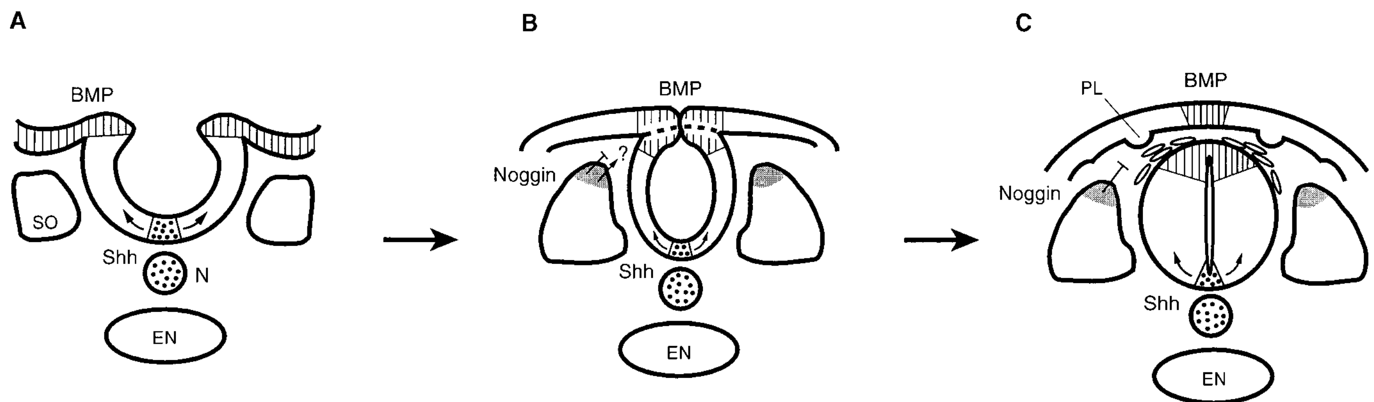

Fig. 1. Generalized model for induction of the ventral and dorsal neural tube, including differentiation of dorsal cells into epidermal ectoderm, neural ectoderm and neural crest – shown as diagrammatic cross sections through neurula stage chick embryos. *Arrows indicate the direction of inducing/inhibiting activity. T-shaped lines represent areas where local molecules block the path of signaling molecules.* (A) *As the neural folds elevate, Shh (Sonic Hedgehog; stippled) is expressed by the notochord and ventral neural tube; inducing activity extends dorsally towards the mid-neural tube. BMP (Bone Morphogenetic Protein; hatched lines) is expressed throughout the ectoderm.* (B) *During closure of the neural tube, Shh expression is maintained ventrally in the developing neural tube and notochord, while BMP expression is now concentrated in the ectoderm at the contact point between the merging neural folds. Noggin (shading), which is expressed in dorso-medial regions of the somites, is thought to inhibit BMP activity (→) and thereby induce neural tissue from dorsal ectoderm, while concurrently preventing BMP from interfering with somite differentiation (→).* (C) *Following closure of the neural folds and separation of epidermal and neural ectoderm, BMP expression is focused in mid-dorsal ectoderm and dorsal neural tube, where it is thought to play a role in inducing differentiation of neural crest cells. EN, endoderm; N, notochord; PL, placode; SO, somite.*

ectoderm through a highly coordinated series of inductions and inhibitions. Neural crest cells are subsequently induced at the boundary of epidermal and neural ectoderm (Northcutt and Gans, 1983, Moury and Jacobson, 1989; Bronner-Fraser, 1995; Dickinson *et al.*, 1995; Liem *et al.*, 1995). Bone Morphogenetic Proteins (BMPs, members of the TGFβ gene family of growth factors – reviewed by Kolodziejczyk and Hall, 1996), namely BMP-4 and 7, promote epidermal ectoderm (Liem *et al.*, 1995, 1997; Tanabe and Jesseu, 1996; La Bonne and Bronner-Fraser, 1998; Marchant *et al.*, 1998). A recent model for neural induction postulates that dorsal ectoderm acquires neural fate by being exposed to a gradient of BMP-binding molecules (Marchant *et al.*, 1998), rather than through a series of reciprocal inductions between epidermal and neural ectoderm (Moury and Jacobson, 1989, 1990). According to the gradient model, BMP specifies epidermis and is then opposed by inhibitory molecules produced by dorsolateral mesoderm, such as Chordin, Follistatin and Noggin (La Bonne and Bronner-Fraser, 1998; Marchant *et al.*, 1998; Nguyen *et al.*, 1998). The inhibitory molecules lower the concentration of BMP, which promotes induction of neural tissue from dorsal ectoderm (reviewed by Tanabe and Jesseu, 1996). Once specified, the dorsal half of the neural tube gives rise to dorsal commissural neurons and roof plate cells and contributes to neural crest cell formation (Fig. 1).

According to Nguyen *et al.*, (1998), the genes *Bmp-2B* and *Swirl* may specify neural crest and placodal cell populations. Placodes are induced in the epidermis adjacent to the neural tube and bordering presumptive neural crest territory (Northcutt, 1996; Stark *et al.*, 1997; Begbie *et al.*, 1999). Two dorsal neural tube markers, *Pax-3* and *Slug*, are thought to distinguish placodes from neural crest cells at later developmental stages because they mark spatially distinct embryonic cell populations and differ in timing of expression (Buxton *et al.*, 1997). *Slug*, a member of the Snail family of zinc-finger transcription factors, is expressed by pre-migratory and migratory neural crest cells, while *Pax-3* appears in more ventrolateral neural tube cells and in ectodermally-derived cells of the ophthalmic trigeminal placode (Nieto *et al.*, 1994; Liem *et al.*, 1995; Buxton *et al.*, 1997; Stark *et al.*, 1997; Sefton *et al.*, 1998). Examples of other factors implicated in neural crest cell induction include genes such as Dorsalin-1 (βTGF family, Basler *et al.*, 1993), *Zic2* (Gli superfamily, Brewster *et al.*, 1998), *Wnt-1* and *Wnt-3a* (Wnt family of secreted glycosylated proteins, Ikeya *et al.*, 1997), and growth factors such as bFGF (basic fibroblast growth factor, Kengaku and Okamoto, 1993; Mayor *et al.*, 1997; La Bonne and Bronner-Fraser, 1998).

Neural crest cell populations and migration

Populations of neural crest cells are referred to by most authors as either cranial, cardiac (vagal), or trunk according to where they originate along the embryonic axis and the derivatives they form (Hall and Hörstadius, 1988) (Fig. 2). Some authors also recognize a sacral neural crest cell population. Except in bony fishes, neural crest cells segregate from the neural epithelium slightly before, during, or following fusion of neural folds, depending on the organism and position along the embryonic axis (Hall, 1999). In bony fishes, neural crest cells segregate from a compact wedge of cells known as the neural keel (Sadaghiani and Vielkind, 1989, 1990; Schmitz *et al.*, 1993; Papan and Campos-Ortega, 1994). Neural crest migration is initiated when cells detach from the neural tube basement membrane (Maclean and Hall, 1987) and is associated with down-regulation of cadherin molecules (e.g., N-cad, cad6B; Nakagawa and Takeichi, 1998). The exact pathways of migration differ along the rostrocaudal axis and among organisms (e.g., Kirby, 1987; Serbedzija *et al.*, 1989, 1992; Lumsden *et al.*, 1991; Epperlein and Löfberg, 1993; Le Douarin and Dupin, 1993). Upon leaving the neural epithelium, populations of neural crest cells become segmentally organized as they encounter signals or physical obstacles along the periphery of the hindbrain (Sechrist *et al.*, 1995), or somites in the trunk (Weston, 1983). Epithelial-mesenchymal interactions are required for the differentiation of

neural crest cells (Weston, 1983; Maclean and Hall, 1987; Hall, 1999); environmental signals from these interactions may be encountered either during migration or at the site of differentiation (Bronner-Fraser, 1995). For more complete reviews on the origin, migration and developmental potentials of neural crest cells see Le Douarin (1982), Weston (1983), Hall and Hörstadius (1988), Bronner-Fraser (1995) and Hall (1999).

Fate-mapping studies provide evidence for neural crest cell regulation

Results from fate-mapping studies provide a major source of information on regulation. Ironically, fate-mapping studies were conducted to discern the origins and developmental potentials of neural crest cell populations, not to investigate whether cells could compensate for loss (Yntema and Hammond, 1945, 1954; and references therein; Newth, 1951, 1956; Langille and Hall, 1988a,b; Moury and Jacobson, 1990). Nonetheless, fate-mapping studies exposed regulation as a significant developmental event, presented mechanisms of regulation for further study, and introduced techniques useful for studying regulation. We revisit fate-mapping results in the context of compensation for surgically removed neural crest cells.

Tracing the origins and developmental potentials of neural crest cells often involved surgically removing or electrocauterizing presumptive neural crest territories and observing which structures were missing or malformed after a period of development (Le Douarin, 1982; Hall and Hörstadius, 1988; Serbedzija *et al.*, 1989). In a series of studies extending over a decade, Yntema and Hammond (1954, and references therein) investigated neural crest cell potential in the chick and the amphibian, *Amblystoma punctatum (=Ambystoma maculatum,* Wake, 1976), by removing portions of the neural fold, including neural crest cells. These extirpations generated depletions and/or reductions in neural-crest-derived tissues such as visceral arch cartilage, sheath cells, and ganglia associated with cranial, cardiac and trunk regions. A major challenge to fate-mapping was determining the length of neural fold tissue extirpation needed to affect cell differentiation without generating severe abnormalities or frequent mortality. In fact, extirpations frequently were extended several segments more posterior than anticipated to create neural-crest-derived deficiencies (Newth, 1951, 1956; Yntema and Hammond, 1954; Hammond and Yntema, 1964). Newth (1956) surmised that such extensive removal of neural fold/crest tissue was necessary to prevent regulation by adjacent cells. Fate-mapping of the head skeleton and anterior trunk in the Japanese medaka, *Oryzias latipes*, (Langille and Hall, 1988a) and the sea lamprey, *Petromyzon marinus,* (Langille and Hall, 1988b) showed that cartilaginous elements of the braincase and branchial arches were missing and/or reduced following removal of neural crest cells. In other cases, neural fold/crest extirpations did not eliminate formation of neural crest cells (Moury and Jacobson 1990, *Ambystoma mexicanum*).

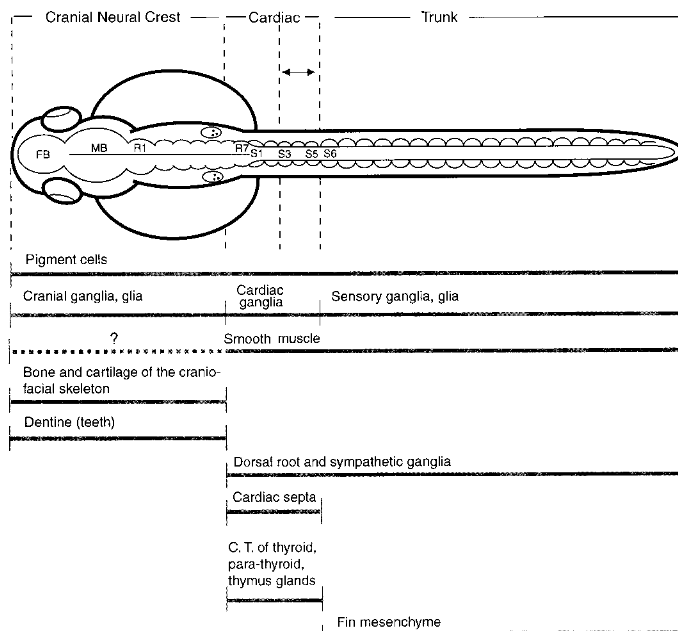

Fig. 2. Locations of cranial, cardiac and trunk neural crest cell populations along the embryonic axis (in this case, a fish) and examples of corresponding derivatives. *Bars demonstrate the regions which give rise to some of the major neural crest cell derivatives during normal development, but do not encompass all possibilities. For example, under experimental conditions, trunk neural crest cells may be able to produce dentine, or cranial neural crest may have the potential to form fin mesenchyme. A transition between cardiac and trunk neural crest populations is thought to occur between somites three and five (? ?); the true boundary remains unclear. C.T., connective tissue; FB, forebrain; MB, midbrain; R1-R7, rhombomeres 1-7; S1-S6, somites 1-6.*

Indeed, because the goal of fate-mapping studies was to identify cellular origins, replacement of cells was a nuisance. Furthermore, neural crest cell contributions vary temporally and spatially along the embryonic axis (Vaglia, zebrafish, submitted). Hammond and Yntema (1964) alluded to this when they found that chicks with neural crest removed at later developmental stages had normal cartilages and neural derivatives. The amount of neural tube and number of presumptive neural crest cells removed also greatly influences results. For example, Newth (1956) contended that inconsistencies in his extirpation experiments reflected differences between surgeries that damaged the spinal cord versus those in which the neural folds were only slightly reduced. Thus, knowing the amount, position and time of neural crest cell removal is imperative to interpreting studies of regulation (Fig. 3).

While fate-mapping has provided evidence for regulation, a plethora of experimental studies have documented the regulative potential of embryonic cells outside the context of fate mapping. In the following sections, regulation for different neural crest cell populations along the embryonic axis will be discussed. Emphasis will be placed on demonstrating (1) that regulation occurs, although the extent of regulation does vary among neural crest cell populations, and (2) that in addition to neural crest cells regulating for ablated neural crest, epidermal, neural and/or placodal ectoderm can be sources of regulating cells.

Regulation for cranial neural crest

Morphological evidence for regulation – the craniofacial skeleton

In general, cranial neural crest cells contribute to connective tissue, cartilage and bone of the craniofacial skeleton, odontoblasts of the teeth, and to neurons and glia of cranial ganglia (Hall and Hörstadius, 1988; Bronner-Fraser, 1995) (Fig. 2). As de-

scribed in the context of fate-mapping, the restoration of normal morphology is evidence of regulation. Although a lack of regulation was implied in early studies of the chick where extensive ablations of cranial neural crest produced facial deformities (Yntema and Hammond, 1954; Hammond and Yntema, 1964), more recently, unilateral and bilateral extirpations of migrating chick mesencephalon neural crest (stages 9-11, Hamburger-Hamilton, 1951) were shown to neither disrupt nor delay facial morphogenesis – skeletal, hypobranchial and neural structures (Noden, 1983). Large regions of cranial neural crest also have been ablated or transplanted in creating quail/chick chimeras without generating the extent of deformities previously reported (McKee and Ferguson, 1984; Scherson *et al.*, 1993; Hunt *et al.*, 1995; Couly *et al.*, 1996; Suzuki and Kirby, 1997) (Table 1). Similarly, unilateral removal of portions of the brain and inclusive neural crest cells in *Ambystoma* reveals considerable regulation (e.g., Detwiler, 1944, 1946, 1947; Harrison, 1947; Birge, 1959). Invariably, there are always exceptions. Sechrist *et al.* (1995) ablated mid- and hindbrain neural folds bilaterally in the chick and observed craniofacial delays along with decreases in crest-derived ganglia and the first branchial arch. Additionally, optimal regulation occurred within a narrower time frame – between somite stages two and five – rather than up to seven somites as demonstrated for unilateral ablations in which the intact side may contribute neural crest cells and facilitate regulation (Scherson *et al.*, 1993). Such experiments are extremely important in revealing how gross abnormalities are generated during development (Johnston, 1975; Johnston and Sulik, 1979; Johnston *et al.*, 1985; Müller and O'Rahilly, 1986).

Neural crest and epidermal, neural and placodal ectoderm as sources of regulating cells

Data on the identity of replacement cell populations is lacking in many studies of regulation. While the obvious candidates to replace ablated cranial neural crest would be other neural crest cells, surprisingly little evidence exists for such regulation of neural crest by neural crest. For instance, Stark *et al.* (1997) showed virtually no regulation of the neural-crest-derived component of the avian trigeminal ganglia following mid- and hindbrain ablations of the dorsal neural tube and adjacent ectoderm. Contrary to Stark *et al.* (1997), Scherson *et al.* (1993) reported that mid-hindbrain ablations had no effect on development of the trigeminal ganglia. Furthermore, neural-crest-derived neuronal axons differentiated normally, and there was no significant difference in the average cell number between operated and control treatments after removal of rhombomeres 4 and 5 (Diaz and Glover, 1996). Thus, the question of whether cranial neural crest can regulate for its own contribution to cranial ganglia remains unresolved (Table 1).

Tissue transplantation and culture experiments reveal that epidermal ectoderm and neural ectoderm/plate may have the potential to produce some neural crest derivatives, thereby presenting alternatives to regulation for cranial neural crest by only pre-existing cranial neural crest. Moury and Jacobson (1989, 1990) transplanted epidermal ectoderm and neural plate from wild type (pigmented) axolotls (stages 14-16) to cranial and trunk regions of host albino axolotls. The resulting production of spinal ganglia from epidermal ectoderm and of melanophores from neural plate suggests that presumptive neural crest cells are not limited to the neural folds. Selleck and Bronner-Fraser (1995) demonstrated that neural plate cultured from chick embryos of stages 6-10 can form pigment cells. Based on the positions of

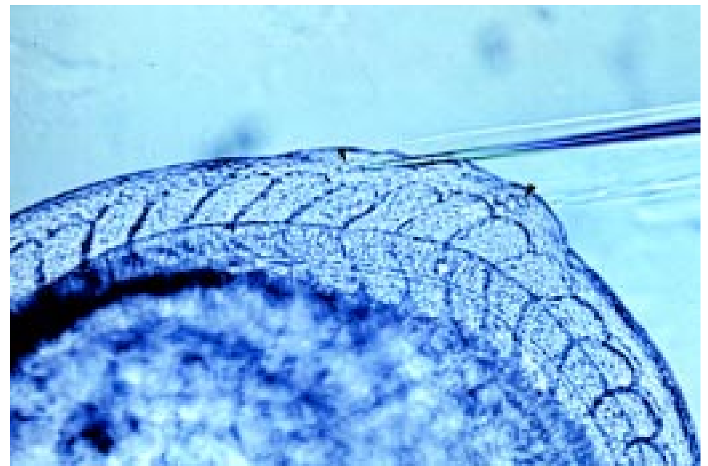

Fig. 3. Lateral view of an 18-somite stage zebrafish embryo demonstrating surgical extirpation of neural crest cells (arrowheads). *Somites are visible as chevron-shaped blocks; the notochord is visible behind the somites. A glass-pulled micropipette has been inserted just beneath the epidermis to remove neural crest cells, in this case, at the level of somites 10 through 13. Rostral is to the left.*

neural crest cells following neural fold injections with DiI, they also interpreted ectoderm as able to produce neural crest. Epidermis cultured alone, however, did not produce neural crest cells. Further study is needed to determine the potential for epidermal and neural ectoderm to form neural crest cells in different organisms at various stages of early development (Table 1).

Under specific conditions, placodal cells also may regulate for extirpated neural crest cells (Kirby, 1987, 1988a,b). This is not surprising considering the suggestion that neural crest and placodal cells originated from a common precursor tissue (Northcutt and Gans, 1983; Smith *et al.*, 1994b) (Table 1). Placodes arise in the head as ectodermal thickenings composed of columnar cells; for reviews see Le Douarin *et al.* (1986) and Webb and Noden (1993). The columnar cells invaginate and later delaminate following transformation from an epithelial to a mesenchymal cell type (in all but the lens placode). Both neural crest and placodal cells contribute to glia and neurons of sensory ganglia, namely in cranial and cardiac regions (Stark *et al.*, 1997). Supporting cells and proximal neurons of these sensory ganglia are derived from neural crest cells; distal neurons are placodal in origin (Hamburger, 1961; D'Amico-Martel and Noden, 1983). Although placodal cells comprise part of the cranial ganglia, there is no evidence that they can regulate for the loss of cranial neural crest cells. However, as discussed in the following section, placodal cells can regulate for cardiac neural crest. Regulation for ablated placodes is addressed separately later in the paper.

Regulation for cardiac neural crest

Cardiac neural crest extends from the mid-otic placode to the caudal limit of the third somite, rendering it transitional between cranial and trunk neural crest (Kirby *et al.*, 1985; Kuratani *et al.*, 1991; Kirby, 1993) (Fig. 2). Derivatives of cells from this region populate pharyngeal arches 3, 4 and 6, providing neuronal and ectomesenchymal components to the heart, aorta and pulmonary arteries (great vessels) (Le Lièvre and Le Douarin, 1975; Bockman *et al.*, 1987; Kirby, 1988a,b; Kuratani and Kirby, 1991).

Morphological evidence for regulation – ectomesenchymal component

Ectomesenchyme cells provide structural support for the great vessels, contribute connective tissue to the thymus, thyroid and parathyroid glands and are necessary for septation of the conotruncal and aorticopulmonary (AP) regions (Kirby, 1988b). Ablation of cardiac neural crest from between the mid-otic placode and third somite, or between the first and fifth somite, frequently generates abnormalities in the pharyngeal arch vessels, aorta, and pulmonary arteries. This reflects poor regulation for ectomesenchymal-fated cardiac neural crest cells. In the absence of cardiac neural crest, pharyngeal arch arteries become mis-shapen, lose bilateral symmetry, suffer structural integrity and subsequently transform into abnormal adult vessels (Waldo *et al.*, 1996). Hood and Rosenquist (1992) observed that extirpation of cardiac neural crest cells causes abnormal deposition of smooth muscle alpha actin, thereby preventing the myocardiac sheath from retracting from surrounding structures and altering the site of origin of the coronary artery. Abnormalities of the great vessels, commonly called AP septum defects, indicate a cumulative loss of cardiac neural crest. In combination with loss or decreases in thymus, parathyroid, and thyroid tissue, many AP defects characterize medical disorders such as DiGeorge Syndrome (Bockman and Kirby, 1984; Kirby, 1987, 1988b; Nishibatake *et al.*, 1987; Kuratani *et al.*, 1991). The high incidence of abnormalities following extirpation of cardiac neural crest cells indicates that proper timing of cell migration is critical to heart development and that regulation of the ectomesenchymal component is limited (Suzuki and Kirby, 1997).

Placodes and neural crest as sources of regulating cells for neuronal and ectomesenchymal components

The neuronal component of cardiac neural crest consists of supporting cells and neurons of cardiac ganglia (Kirby *et al.*, 1983; Kirby and Stewart, 1983; Kirby, 1988a). Although placodes are defined as head structures, precursor cells from the nodose placode overlap with cardiac neural crest cells. Nodose placodal cells deposit neurons in the vagal (nodose) ganglia of the vagus nerve that provides sensory innervation to the heart and other visceral organs (Kirby, 1993; Harrison *et al.*, 1995). Ablation of cardiac neural crest cells results in nearly complete regulation of vagal ganglia by the nodose placode (Kirby *et al.*, 1985; Kirby, 1987, 1988a,b). But similar to results from cranial neural crest cell extirpations (Stark *et al.*, 1997), the neural crest itself does not appear to regulate for ablated cardiac neural crest, specifically with regard to the neural-crest-derived component of cardiac ganglia. The potential for regulation of cardiac neural crest by epidermal and neural ectoderm is unknown (Table 1).

In the absence of cardiac neural crest, cells from the nodose placode provide ectomesenchyme to outflow vessels of the heart; however, placodal cells cannot reestablish a normally functioning AP septa (Kirby, 1988b). The nodose placode also substitutes cells to initiate elastogenesis of the great vessels after cardiac neural crest ablation. Because the cells cannot propagate, formation of the elastic laminae is inhibited (Rosenquiest *et al.*, 1990). It is not known whether regenerated ectomesenchyme cells are not competent because of a biochemical deficiency, or because they arrive at the neural tube later than the original cells. In either event, these results further indicate that specificity of the ectomesenchymal component of cardiac neural crest is determined by the time of migration (Kirby, 1988b, 1989).

We conclude this section by noting a recent study of Serbedzija *et al.* (1998), where normal hearts developed following unilateral removal of precursor cardiac mesoderm in the chick. Surrounding cells compensated by migrating into the gap from more cranial regions and functioning as heart, rather than head, mesenchyme. This study wonderfully complements what is currently known about regulation of neural crest cells, and is an example of how regulation continues to provide insight to inherent cell plasticity, the origins of embryonic defects and development of other tissues.

Regulation for trunk neural crest

Trunk neural crest contributes to formation of pigment cells, sensory neurons, glia, sympathoadrenal and Schwann cells (Bronner-Fraser, 1995), as well as to formation of the dorsal fin of amphibians and teleost fishes (Hall and Hörstadius, 1988; Smith *et al.*, 1994a; Hall, 1999), and to induction of the dorsal fin fold in amphibians such as *Triturus* and *Ambystoma* (Twitty and Bodenstein, 1941; Bodenstein, 1952) and in lampreys (Newth, 1951, 1956) (Fig. 2). Compared to cranial and cardiac neural crest cell populations, fewer derivatives arise from trunk neural crest. Thus, studies of regulation in this cell population have been limited to analyses of pigment cell and dorsal root ganglia derivatives.

TABLE 1

EVIDENCE FOR REGULATION BY FOUR CELL POPULATIONS IN RESPONSE TO LOSS OF NEURAL CREST OR PLACODAL CELLS FROM CRANIAL, CARDIAC OR TRUNK REGIONS

	CELL TYPE LOST					
	Cranial		Cardiac		Trunk	
Potential replacement cells	NC	PL	NC	PL	NC	PL
Neural Crest	Yes[1] (SK) ?[2] (G)	No[3]	?	Yes (G)	Yes (G, P)	?
Placode*	No	?	Yes (EM; G)	?	?	?
Epi. Ectoderm	?	Yes	?	?	?	?
Neural Ectoderm	?	?	?	?	?	?

ABBREVIATIONS: Epi, Epidermal; EM, ectomesenchyme; G, ganglia; NC, neural crest; P, pigment; PL, placode; SK, skeleton. [1]'Yes' indicates regulation has been demonstrated in some form, even if only for specific derivatives (in parentheses). [2] '?' indicates evidence for regulation is questionable, needs further testing, or has not been tested. [3]'No' indicates existing studies have not found evidence for regulation. * Placodes contribute to the lateral line system in the trunk

Raible and Eisen (1996) determined that in zebrafish there is regulation for the loss of an early-migrating population of trunk neural crest cells. Typically, the early-migrating cell population produces both non-neuronal derivatives such as pigment cells and glia, and neuronal derivatives such as dorsal root ganglia, while a late-migrating population of cells produces only non-neuronal derivatives (both populations migrate along a medial path between the neural keel and somites). However, if early-migrating cells are ablated, dorsal root ganglia will differentiate from late-migrating trunk neural crest. Interestingly, other studies describe less complete regulation for nerve cells, especially spinal ganglia, than for other derivatives after extirpation of trunk neural fold and crest cells (Detwiler, 1937, 1944; DuShane, 1938; Piatt, 1949; Stefanelli, 1950; Lehman and Youngs, 1952). Following extensive bilateral extirpations of the six most caudal somites in the chick, Yntema and Hammond (1945) observed no regulation of dorsal root ganglia from cranial or caudal spinal levels. After performing a parallel experiment with quail (18-27 somite stage; removing 7-8 segments), Suzuki and Kirby (1997) did not find regenerated dorsal root ganglia but did see normal pigment and feather patterning. Contrary to the greater regulative ability exhibited by neuronal over ectomesenchymal components in the cardiac region, Chibon (1970) also observed more efficient regulation of pigment cells in the trunk compared to other neuronal derivatives such as Rohon Beard cells (Table 1). Regulation for trunk neural-crest-derived ganglia cells is currently being compared to regulation for pigment cells in zebrafish to determine whether this relationship holds true for teleosts (Vaglia, unpublished observation).

The role of cell death in maintaining specific numbers cells should also be mentioned in the context of regulation for trunk neural crest cell derivatives. Carr and Simpson (1978) found that initial cell numbers vary among ganglia which are designed to achieve different sizes, but cell death (apoptosis) controls the final number of neurons in all ganglia. If cell death eliminates 'extra' neurons, then regulation for loss of trunk neural crest cells destined to form ganglia may occur through decreased death. To test this idea, Carr (1984) extirpated trunk neural crest at the levels of somites 20-23 (ganglia 15-17) in the chick. Following surgery, some ganglia were composed of fewer apoptotic cells. Moreover, hypertrophied ganglia and neurons were found around the surgery site, suggesting that trunk neural crest compensates from surrounding regions, especially anterior to the ablation. It was hypothesized that cell removal decreases competition for substances such as nerve growth factor (NGF), leading to a decline in cell death (the sparing neuron theory, Cowan, 1973). This mechanism of regulation could serve as a 'safety net' for problems in the original cell population. Alternatively, it may signify that extra cells are needed initially to create a high density of signaling factor to induce transfer of nerve impulses. Reducing cell death might also eliminate the need for compensation by neural crest cells destined to form other derivatives.

Regulation for placodal cells

Because placodal cells can regulate for ablated cardiac neural crest cells, it is interesting to ask if the reverse situation occurs. Do neural crest cells, or even adjacent epidermal and/or neural ectoderm, regulate for ablated placodal cells? Placodal and neural crest cells originate from closely associated tissues and in close association to one another, and both are mesenchymal populations that contribute to sensory ganglia in the cranial and cardiac regions. Given these close associations, it is appropriate to discuss regulation for placodal cells. In fact, Harrison *et al.* (1995) showed that cardiac neural crest cells regulate for lost placode cells by forming neurons in the nodose ganglion after nodose placode ablation.

Stark *et al.* (1997) found that the opthalmic placodal component of the avian trigeminal ganglia was sometimes reduced but never absent following mid- and hindbrain ablation of the dorsal neural tube and adjacent ectoderm early in development. Because the neural-crest-derived component showed virtually no regulation, regulation could only be occuring from surrounding non-placodal ectoderm (Hamburger, 1961; Stark *et al.*, 1997). Regulation for ablated placodes by other placodal cells would involve both long-range detection of cell loss, and extensive migration. Although the distance over which regulation can operate is unknown, Stone (1924) did demonstrate that ophthalmic placode transplanted into regions of ablated gasserian placode can form normal gasserian ganglia.

Recent molecular data supports the potential for replacement of placodal cells by epidermal ectoderm. While both *Slug* and *Pax-3* genes are expressed by the neural tube, *Pax-3* also appears in surface ectoderm (Buxton *et al.*, 1997). Reexpression of *Pax-3* after hindbrain ablation requires interaction of neural and epidermal ectoderm, but not midline closure of the neural folds as for *Slug*. Thus, *Pax-3* is reexpressed as new placodes invaginate and before neural crest cells appear. The differential expression of *Pax-3* and *Slug* indicates that neural crest cells do not induce generation or migration of placode cells, rendering it likely that ectoderm has some ability to regulate independently of neural crest in the absence of placode cells (Stark *et al.*, 1997).

A note on neural crest cell regulation in mammals

Most studies of mammalian regulation have dealt with the ability of embryos to compensate for cell loss or damage following exposure to a teratogen. Mitomycin C (MMC) is a teratogen known to inhibit cell division and synthesis in developing mouse embryos; lack of cell division during critical phases of development would be expected to cause death or abnormal development (Hall and Hörstadius, 1988). Interestingly, mouse embryos exposed to MMC through injected females exhibited higher mitotic activity than controls, and a remarkable ability to recover (Snow and Tam, 1979). However, female mice exposed to MMC tended to have smaller litters with many runts, and survivors of a litter frequently exhibited motor defects, poor postnatal development and decreased fertility (Snow and Tam, 1979; Tam and Snow, 1981). Although this study is not specific to neural crest cells, a small percentage of embryos exhibited craniofacial neural-crest-derived malformations (e.g., microphthalmia), indicating that the neural crest cell population was affected by MMC. Extensive recovery of embryos following MMC treatments suggests that mammalian neural crest cell populations can regulate.

Mechanisms of regulation

Does regulation recapitulate development?

Having presented examples of regulation and potential sources of replacement cells, we now discuss how neural crest cells regulate. To regulate, cells must be receptive to signals such as transcription and growth factors in their environment. Four possible mechanisms of regulation are (1) decreased cell death, (2) in-

creased cell division, (3) change in cell fate, and/or (4) cell migration. (Fig. 4; Table 2). In surveying these mechanisms, it is intriguing to ask whether regulation for neural crest cells recapitulates their original development? We begin to address this question by comparing what is known about the cellular, molecular and genetic interactions that occur during regulation versus normal development.

TABLE 2

POTENTIAL TECHNIQUES FOR STUDYING REGULATION

Technique	Purpose (s)	Key references
Surgical extirpation	Remove cells	Scherson *et al*., '93 Sechrist *et al*., '95 Saldivar *et al*., '97
Laser ablation	Remove cells	Westerfield, '95 Suzuki and Kirby, '97 Serbedzija *et al*. '98
Tissue grafting: e.g. chick/quail chimera; wild type/albino axolotl	Use naturally labeled cells to trace cell migration	Kirby, '89 Le Douarin and Dupin, 93 Couly *et al*., '96 Moury and Jacobson, '90
Grafting radioisotopically labeled ^{3}H thymidine cells	Trace cell migration	Weston, '63 Johnston, '66
Inject impermeable dye (LRD-lysinated rhodamine)	Trace cell migration	Fraser *et al*., '90
Inject vital lipophilic dye (Dil)	Trace cell migration	Smith *et al*., '94a Baier *et al*., '96 Suzuki and Kirby, '97
Green Fluorescent Protein	Trace cell migration	Gerdes and Kaether, '96
Expose cells to ^{3}H thymidine	Label dividing cells	Noden, '75 Hall and Coffin-Collins, '90
Proliferating cell nuclear antigen (PCNA)	Label dividing cells	Shimotake *et al*., '95
Bromodeoxyruridine (BrdU)	Label dividing cells	Miller and Nowakowski, '88 Zhang *et al*., '97 Pevny *et al*., '98
Nile Blue Sulfate (NBS) Acridine Orange	Vital dyes used to detect cell death	Jeffs *et al*., 92 Graham *et al*., '93
TUNEL (Terminal deoxy-nucleotidly transferase-mediated dUTP nick end label	Detect cell death	Abdelihah *et al*., '96 Frankfurt *el al*., '96 Negoescu *et al*., '97
Clonal cell culture	Investigate potential for cell fate change	Rogers *et al*., '94 Henion and Weston, '97 Sieber-Blum *et al*., '93
Immunostaining	Visualize normal and regulative patterns of expression of neural crest cells and corresponding derivatives	Sadaghiani and Vielkind, '90 Hunt *et al*., '95 Buxton *et al*., '97

Cell death

Decreased cell death as a mechanism of regulation was presented earlier in reference to cranial ganglia. Cell death is important for modeling amniote limbs (Hurle *et al*., 1995; Macias *et al*., 1997; Gañan *et al*., 1998), the hindbrain (Jeffs *et al*., 1992) and trunk (Jeffs and Osmond, 1992). In chick embryos, a combination of BMP-4 signaling and *msx-2* expression elicits apoptosis of cranial neural crest cells in rhombomeres three and five (Graham *et al*., 1993, 1994; Lumsden and Graham, 1996). Addition of exogenous BMP-4 to odd-numbered rhombomeres elevated *msx-2* expression (Graham *et al*., 1994). Furthermore, adenoviral insertion of *msx-2* into even-numbered rhombomeres induced apoptosis among cranial neural crest cells associated with those rhombomeres (Takahashi *et al*., 1998).

It has been hypothesized that cell death may eliminate 'reserve' populations of cells (Takahashi *et al*., 1998). If 'reserve' cells are instructed to die during development, then perhaps they could be instructed to survive in the event that surrounding cell populations fail to develop or migrate properly. For instance, Buxton *et al.* (1997) and Lumsden and Graham (1996) observed a lack of BMP-4 expression in the hindbrain after neural crest cell ablation. Because BMP-4 expression typically corresponds with regions of cell death, disrupted cell-cell signaling may promote survival of cells normally fated to die (Fig. 4). Evidence that changes in cell signaling pathways can alter cell response is found in organisms that retain interdigital webbing, such as ducks, where lack of interdigital apoptosis may result from decreased sensitivity of cells to apoptotic inducing molecules such as BMP-4, and/or from accelerated differentiation (Gañan *et al*., 1998).

Cell division

From results of amphibian neural crest extirpations, Chibon (1970) suggested that one way cells respond to changes in their environment is by modifying mitotic behavior. This is demonstrated when uni- and bilateral extirpation of neural tube and crest cells is followed by increased proliferation among remaining tissues (e.g., Detwiler, 1944; Källen, 1955; McKee and Ferguson, 1984; Couly *et al*., 1996) (Fig. 4). Such increased division following neural crest cell ablations can be detected with bromodeoxyuridine (BrdU; Suzuki and Kirby, 1997) (Fig. 5). Furthermore, BrdU labeling in zebrafish indicates that cell division is elevated at the cranial and caudal extremities of neural crest ablated regions (Vaglia, unpublished observation).

To understand how altering the cellular environment induces change in mitotic behavior, it is necessary to identify the conditions, genes and molecules which control proliferation. Birge (1959) and Couly *et al.* (1996) converged on the idea that there is a relationship between cell population density and division rate. This implies that cell division is maintained by signals functioning within the parameters of a specific cell density. An example can be drawn from *Drosophila*, where a single large cell (Tip cell) controls proliferation of neighboring Malpighian tubule cells. A disruption in signaling to or from the Tip cell could result in uncontrolled cell proliferation (Kerber *et al*., 1998; Skaer, 1989). Although an equivalent to the *Drosophila* Tip cell has not been found among neural crest populations, epithelial cells stimulate proliferation and/or differentiation of neural crest-derived mesenchyme (Hall and Coffin-Collins, 1990; Clouthier *et al*., 1998). Removing cells important for initiating and maintaining proliferation and differentiation (e.g., epithelial

and neural crest cells) might induce proliferation of neighboring cells, similar to how decreased cell death follows a decrease in the number of sensory neurons in fields of developing ganglia (Carr, 1984).

We know little about what factors initiate changes in highly regulated patterns of cell division. Several growth factors, such as EGF and FGF (epidermal and fibroblast growth factors) and NT-3 (Neurotrophin-3) are important to cell proliferation and survival (e.g., Hall and Coffin-Collins, 1990; Stemple and Anderson, 1993). TGFβ increases proliferation of some cell populations while decreasing proliferation of others (Rogers *et al.*, 1994). Other growth factors stimulate proliferation after binding to tyrosine kinase receptors (reviewed by Hall and Ekanayake, 1991; Kolodziejczyk and Hall, 1996). Although not directly applicable to newly emerging or migrating neural crest cells, ET-3 (Endothelin-3) and GDNF (Glial-cell-line-derived neurotrophic factor) stimulate proliferation of vagal neural crest cells before they enter the gut (Hearn *et al.*, 1998). Lastly, it has been suggested that members of the Wnt family of secreted factors signal the mitotic activity of select cell populations such as precursor spinal cord (Dickinson *et al.*, 1995) and neural crest cells (Ikeya *et al.*, 1997). If so, genes of the Wnt family would be good candidates for analyzing differential cell division in control and neural crest-ablated embryos.

Change in cell fate

How embryonic cell populations become restricted during development is important for determining whether cells regulate by changing fate. Migrating neural crest cells are heterogenous, consisting of cells that are pluripotent, have restricted potential, or are already committed to a particular fate (Weston and Butler, 1966; Sieber-Blum and Cohen, 1980; Ito *et al.*, 1993; Le Douarin *et al.*, 1994; Raible and Eisen, 1994; Henion and Weston, 1997; Bronner-Fraser, 1998). Anderson (1989) described the neural crest cell population as a group of multipotent stem cells (self renewing) that are gradually restricted to sublineages, eventually reaching a terminal fate (Hemopoiesis Model) (Fig. 4). The hypothesis that many neural crest progenitors are 'self-renewing' supports the idea that plasticity within neural crest populations contributes to regulation. Thus far, stem cells have been isolated for neurons and glia of the mammalian neural crest (Stemple and Anderson, 1992). As with cell division and migration, differentiation is controlled by environmental cues (Weston, 1970; Anderson, 1989; Collazo *et al.*, 1993; Gershon, 1993; Le Douarin and Dupin, 1993; Bronner-Fraser; 1995; Darland and Le Blanc, 1996; Groves and Anderson, 1996; Baker *et al.*, 1997). The combination of specific signals in specific locations at specific times also accounts for axial differences in neural crest potential that exist prior to migration (Darland and Le Blanc, 1996). For instance, trunk neural crest cells in *Ambystoma mexicanum* cannot form cranial neural crest derivatives such as cartilage or bone (Graveson 1993, 1995).

Interpreting whether cells regulate by changing fate requires knowing their differentiated status prior to regulation. In fact, Harrison, (1937) scoffed at calling cells 'determined' because he concluded there are no good criteria for establishing cell determination. Yet, *in vitro* cell culture has revealed that some neural crest derivatives can 'transdifferentiate', meaning to switch from one terminally differentiated cell state to an alternate cell state. For instance, melanophores, in the presence of guanosine, can convert into xanthophores (Thibaudeau and Holder, 1998); neuronal

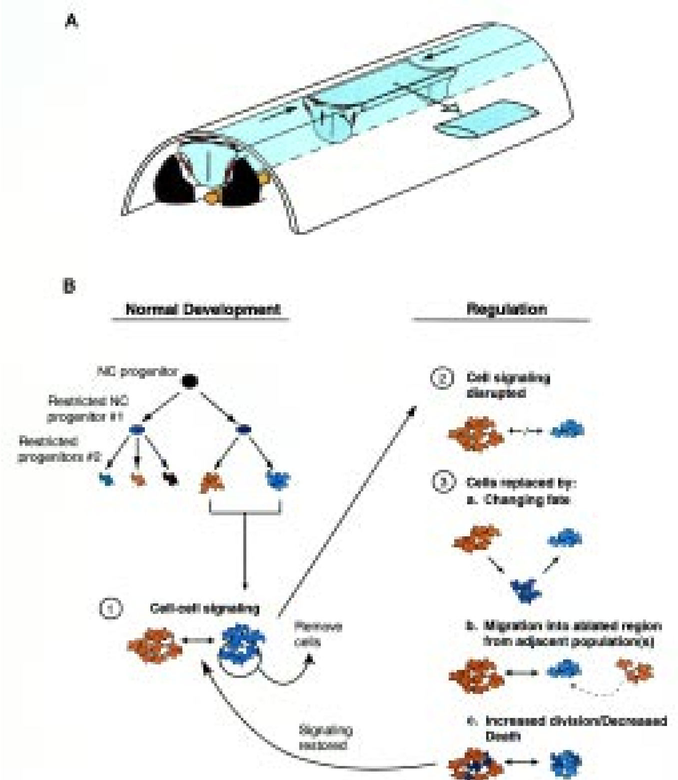

Fig. 4. Potential sources of regulating cells and suggested mechanisms of regulation. (A) *A three-dimensional construction through the trunk of a fish embryo. After removal of trunk neural crest/neural keel cells (bluish-gray arrow), replacement cells may migrate into the extirpated region from anterior, posterior, ventral or lateral locations as shown by the black arrows. These replacement cells may be epidermal or neural, including neighboring neural keel and neural crest cells. Neural crest cells-red; Neural keel-light blue; Notochord-yellow; Somites-dk. red.* **(B)** *During normal development, neural crest (NC) progenitor cells are gradually restricted in potential – shown as restricted NC progenitors #1 and #2. (1) Signaling between closely associated neural crest cell populations both induces and maintains cell differentiation. (2) If a group of undifferentiated neural crest cells is removed, signaling is disrupted, and regulation is initiated. (3) Hypothesized mechanisms of regulation (not mutually inclusive) include: (a) change in fate of closely associated cells – shown as the gray cells that have changed fate from the intact population (yellow), (b) migration of cells into the ablated region, (c) increased cell division and/or decreased cell death (gray) within the population from which cells were removed, or within an alternative (but potentially contributing) population.*

dorsal root ganglia cells can form non-neuronal derivatives (Frank and Sanes, 1991; Fraser and Bronner-Fraser, 1991); adrenal chromaffin cells can become sympathetic neurons (Patterson, 1990), and 'pro-cartilage' or 'giant' cells of salamanders can become melanophores (Nice, 1954).

Because the early neural crest population is heterogenous, and because virtually all studies of neural crest cell regulation involve pre-migratory or newly migrating cells, it may be more accurate to say that environmental signals influence neural crest cells that are not terminally differentiated to adopt an alternative fate. In this sense, regulation of neural crest cells may closely resemble regulation of precursor cell types in a variety of invertebrates from

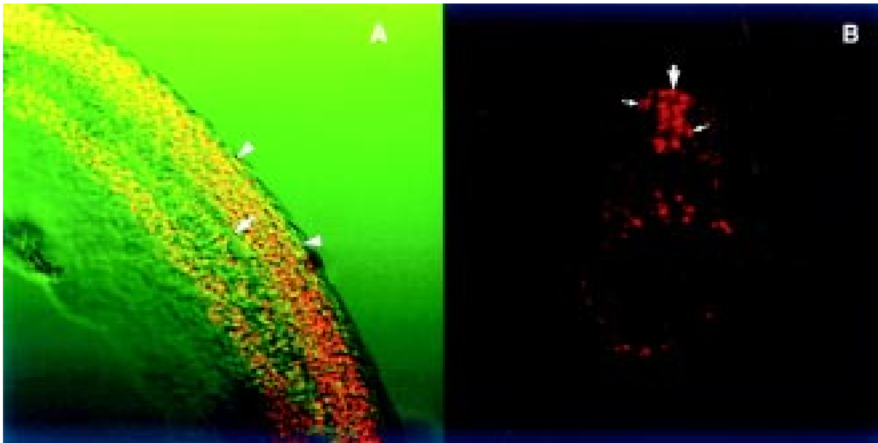

Fig. 5. Examples of bromodeoxyuridine (BrdU) labeling for cell division. (A) *Confocal image showing a lateral view of the trunk of a 19-somite stage zebrafish embryo. Dividing cells have been labeled with BrdU and visualized using anti-BrdU primary antibody and a fluorescent Cy3 secondary antibody (bright red). A distinguishing feature is the notochord (arrow); neural crest cells are indicated by arrowheads. Rostral is to the left.* **(B)** *Example of BrdU staining in a histological cross section through the posterior trunk of a 21-somite stage zebrafish embryo. In this case, the actively dividing neural keel is a distinguishing feature (symbol), and neural crest cells are indicated by arrows. 40x.*

leeches to insects to echinoderms (Taghert *et al.*, 1984; Weisblat and Blair, 1984; Doe and Goodman, 1985; Ettensohn and McClay, 1988; Ettensohn, 1992; Ettensohn and Malinda, 1993). Weisblat and Blair (1984) used the term 'transfate' to describe a form of regulation that occurs in some invertebrates when neighboring cells or alternative cell lines replace cells that have been ablated. The ability to 'transfate' presumes that cells initially have equivalent developmental potential, and that they gradually assume different fates through a series of hierarchical, position-dependent interactions (Weisblat and Blair, 1984; Nishida and Satoh, 1989). There is no regulation by other neuroblasts when neuroblast stem cells are ablated in grasshopper embryos (Taghert *et al.*, 1984; Doe and Goodman, 1985); neuroblasts are replaced by pre-neuroblast cells contained within a cluster of adjacent ectoderm cells.

Use of the term 'transfate' also seems appropriate for regulation of neural crest, where cells at various stages of differentiation may replace those that have been removed. Neural crest cells derivatives can arise from epidermal ectoderm and neural plate cultured in isolation, providing that these tissues had previously interacted (Selleck and Bronner-Fraser, 1995). Cells also can migrate from the spinal cord quite late in development to form sensory ganglion cells and melanocytes (Sharma *et al.*, 1995). These results suggest that epidermis and/or neural tube cells could regulate if exposed to proper signals.

Experiments involving transplantation or 180 degree rotation of rhombomeres also suggest that the fate of neural crest cells can be altered in response to an appropriate signal. In some of these experiments, neural crest cells expressed the *Hox* code corresponding to their original location, yet formed hindbrain derivatives normal to the new location (Couly *et al.*, 1996). Alternatively, neural crest cells formed derivatives corresponding to their origin. This implies the cells were predetermined and unable to alter their fate (Noden, 1988). Thus far it has been difficult to discern distinct trends in the regulatory potential of hindbrain neural crest cells. Neural crest cell fate appears to vary according to which Hox genes are targeted, which and how many rhombomeres are transplanted, and in what direction rhombomeres are transplanted – from cranial to caudal regions or vice versa (Guthrie *et al.*, 1992; Prince and Lumsden, 1994; Grapin-Botton *et al.*, 1995, 1997; Hunt *et al.*, 1995, 1998; Couly *et al.*, 1996; Saldivar *et al.*, 1997). Nonetheless, these experiments provide insight on how timing, position and Hox gene expression affect the regulative ability of neural crest cells.

Determining signals important for altering cell fates

While results from *in vitro* cell culture cannot be entirely extrapolated to the *in vivo* embryonic environment, cell response to different combinations and concentrations of molecules may indicate which signals induce a change in fate, and how such signals are transmitted. To differentiate cells must possess the proper receptors, and contact specific substrates or signals in the right place at the right time. For example, neurotrophin receptors (RTK=receptor tyrosine kinase) expressed by both neuro- and nonneurogenic subpopulations of neural crest cells allow for differential dispersal and response to ligands, growth factors, etc. (reviewed by Weston, 1998). Brain derived neurotrophic factor (BDNF) is an example of a factor that controls migration and differentiation of neural crest cells (Sieber-Blum *et al.*, 1993). A greater proportion of quail neural crest cells differentiated as sensory neurons when cultured with BDNF (Sieber-Blum, 1991). Other molecules capable of diverting or influencing neural crest cell fate include: basic fibroblast growth factor (bFGF), which can induce pigmentation in dorsal root ganglia and cultured peripheral neurons; BMP2 which promotes neuronal differentiation; and TGFβ1 which inhibits pigmentation, but also induces smooth muscle differentiation (Stocker *et al.*, 1991; Rogers *et al.*, 1994; Shah *et al.*, 1996). The cellular and molecular genetics of neural crest lineage determination – including the role of environmental signals such as glucocorticoids, nerve growth factor (NGF) and FGFβ have been reviewed by Stemple and Anderson (1993) and Anderson (1997). The role of growth factors in regulating proliferation and differentiation of neural crest cells (namely cardiac) has been reviewed by Hall and Ekanayake (1991) and Hall (1999).

Little is known about how signals are transmitted following neural crest cell loss. However, there is support for position-dependent (spatial) signals for recognizing cell loss in vertebrates (Raible and Eisen, 1996) and in invertebrates (Kimble *et al.*, 1979; Weisblat and Blair, 1984; Doe and Goodman, 1985; Sternberg and Horvitz, 1986; Nishida and Satoh, 1989). As previously mentioned, a population of late-migrating trunk neural crest cells in zebrafish can regulate for the loss of early-migrating trunk neural crest (Raible and Eisen, 1996). Although early- and late-migrating populations of trunk neural crest cells have intrinsically different fates, cell fate can be modified in response to environmental signals. Moreover, late-migrating cells may retain the potential to

produce other derivatives such as dorsal root ganglia, but are usually prevented from this fate by interactions between early- and late-migrating cells. The second scenario wonderfully parallels a situation in sea urchins where interactions (position-dependent signaling) among primary and secondary mesenchyme cells (PMCs and SMCs) are thought to maintain specific cell fates (Ettensohn, 1992). PMCs normally transmit a signal that restricts the fate of SMCs; removing PMCs induces SMCs to revert to a more pluripotent state, and thus express alternative fates. It would be interesting to culture various combinations of neural crest populations at different densities and stages to assess whether position-dependent signals similarly affect neural crest cell fate. Other modes of molecular signaling that might apply to vertebrates include: growth factors (diffusible signals) that repress alternative cell fates; substances that degrade activators or hormones; elements within the extracellular matrix that regulate gene expression, or a cascade of morphogenetic events involving combinations of the above (Ettensohn and McClay, 1988).

Cell migration

Lastly, regulation would not be complete without migration of cells into extirpated regions (Fig. 4). Migration of neural tube and crest cells into 'gaps' – regions devoid of cells – from adjacent locations has been observed using fluorescent DiI. However, almost nothing is known about the signals that govern such detours in normal migratory patterns. Movement of cells into gaps may simply reflect passive migration from regions of high to low concentration, an idea referred to as 'contact inhibition' by Weston (1970). This idea has great appeal when considering that cell migration must work in concert with other mechanisms such as increased cell division.

When regions of neural fold/crest cells are extirpated, replacement neural crest cells may migrate from rostral and/or caudal extremes, ventral neural tube or the contralateral neural fold (Fig. 4). In all cases, additional neural crest cells would need to be generated for regulation to be complete. Such cells could come from either the neural tube or from pre-existing neural crest cells and would involve different developmental mechanisms. The majority of studies contend that cells migrate from locations rostral, caudal, or contralateral (in the case of unilateral neural fold ablation) to the ablation site (Yntema and Hammond, 1945, 1954; Hammond and Yntema, 1947; Lehman, 1951; Newth, 1951, 1956; Sechrist *et al.*, 1995; Couly *et al.*, 1996; Diaz and Glover, 1996; Saldivar *et al.*, 1997). Few studies have observed neural crest cells migrating from the ventral neural tube following mid- and hindbrain ablation. In those that have, labeled cells in the ventro-lateral neural tube were interpreted as originating ventrally (McKee and Ferguson, 1984; Scherson *et al.*, 1993). However, it is plausible that those cells were either neural crest cells that migrated from more peripheral regions, or were mesodermal in origin (Couly *et al.*, 1996). Replacement from the ventral neural tube, which seems to be more the exception than the rule, provides a good scenario to consider why there are differences in regulative ability under virtually identical experimental conditions.

Differences in the origins of replacement cells in seemingly similar studies under similar conditions hint at how position and time of neural crest cell removal affect regulation. Evidence suggests that the location and time of neural crest cell extirpation influence the completeness of regulation and the source(s) of repopulating cells. As discussed, there is little regulation in the cardiac region compared to either the head or trunk, and the extent of trunk neural crest regulation falls short of that for cranial neural crest. Axial variation in regulation may reflect differences in cell proliferation at cranial, cardiac and trunk regions (Scherson *et al.*, 1993). Differences in regulation between specific neural crest cell derivatives demonstrate how the time/stage of neural crest cell extirpation affects regulation. Following trunk neural crest extirpations, dorsal root ganglia were largely missing from the ablated region, yet melanophores migrated into the void and created normal pigmentation (Suzuki and Kirby, 1997). Thus, because the time/stage of extirpation determines the population of neural crest cells removed, differences in regulative potential may be closely associated with the identity of extirpated cells.

Genes and recapitulation of development

Regulation for neural crest cells involves changes in normal cell behavior ranging from altered division and migration to differentiation. For the most part, these changes are in response to gene products such as transcription and growth factors. Therefore, genes thought to play a role in defining and patterning populations of cells such as the neural crest are being sought as markers of regulation. Studies that compare gene expression of corresponding regions in control and neural crest-ablated embryos are needed to interpret whether regulation recapitulates original developmental patterns. Are the genes expressed by neural crest cells and along the neural axis reexpressed in a similar manner following neural crest cell extirpations?

Because it has been well established that *Hox* genes pattern rhombencephalic neural crest derivatives (Hunt *et al.*, 1991, 1995; Grapin-Botton *et al.*, 1995; Couly *et al.*, 1996), members of this gene family are prime candidates for such studies. Genes outside the Hox family would also be useful for studies of regulation. *Krox-20* (Nieto *et al.*, 1995; Saldivar *et al.*, 1997) and *Slug* (Nieto *et al.*, 1994; Sechrist *et al.*, 1995) are expressed by pre-migratory and migratory neural crest. Immediately following mid- and hindbrain extirpation in chicks, Slug expression is absent from the extirpated region. As cranial neural crest cells re-emerge to regulate for the loss, *Slug* is up-regulated, signifying that at least some early developmental pathways are retained (Sechrist *et al.*, 1995). Another gene, *rae28*, alters *Hox* expression and induces defects resembling those derived from neural crest cells. Since *rae28* has a role in regulating *Hox* expression and specifying segmental identity, it may prove useful as a marker of regulation (Takihara *et al.*, 1997).

Summary

Regulation is a significant developmental event because successful cell proliferation and migration are critical to shaping young embryos. Regulation – the replacement of undifferentiated embryonic cells by other cells in response to signals received from the environment – is distinct from wound healing and regeneration. Investigations on regulation of neural crest cells span all vertebrates and have revealed that regulative ability varies both among classes (even species), and spatially and temporally within individuals. In general, there is greatest regulation for cranial neural crest cells, less for trunk, and virtually none for cardiac. Regulation

also appears to be more complete at early embryonic stages. Fate-mapping studies have demonstrated that large regions of neural crest cells must be removed to generate missing or morphologically reduced structures. Recent studies reveal that less extensive neural crest cell extirpations result in normal morphology of cartilaginous and neuronal elements in the head, and normal development of pigmentation in the trunk. Ablation of cardiac neural crest cells frequently generates abnormalities of the heart, great vessels and parasympathetic nerve innervation.

Decreased cell death, increased division, change in fate and altered migration are possible cellular mechanisms of regulation. In most cases, the specific mechanisms of regulation are unknown, but a major premise underlying regulation is that cell potential is greater than cell fate. This concept was born from studies which demonstrated that some cells were able to express alternative fates if transplanted to a new environment.

Among the potential cellular mechanisms for regulation, cell migration has received the most attention. Following ablation of neural crest cells, replacement neural crest cells migrate into gaps, most frequently from anterior/posterior locations. Cells from surrounding epidermal and neural ectoderm may have limited regulative ability, while compensation by cells from the ventral neural tube has been demonstrated to an even lesser extent. Regulation by such non-crest cells would require their transformation into neural crest cells. The potential for regulation of neural crest by placodal cells supports a closer relationship between neural crest and placodal ectoderm than previously recognized. Decreased cell death has been discussed primarily with reference to (1) cranial ganglia that have dual contributions from neural crest and placodal cells and (2) programmed cell death in rhombomeres three and five. Increased cell division in response to neural crest ablation is likely more common than has been reported, but this mechanism is difficult to interpret without a 3-D context for viewing how patterns of division differ from normal. Lastly, changes in cell fate may be the driving factor in regulation of embryonic cells. It has been repeatedly demonstrated that cell potential is greater than cell fate. Once reliable mechanisms for assessing cell potential are established, we may find that fates are commonly altered in response to environmental signals. Regulation is therefore significant both as a basic developmental mechanism and as a mechanism for evolutionary change. The more labile the fate of embryonic cells, the more potential there is for maintaining existing characters and for generating new ones. According to Ettensohn (1992, p. 50), further analysis of such systems might «shed light both on the way evolutionary processes act to modify ontogenetic programs and on the cellular and molecular mechanisms of cell interactions during development». With regard to the neural crest, studies on regulation of this vital population of cells provide insight to the origin of the neural crest, to embryonic repair, and to the source of many craniofacial malformations, heart and other embryonic defects.

On a broader scale, few studies have approached investigations of neural crest deficiencies by looking at both short term compensation and long term developmental and morphogenetic affects of neural crest cell extirpation. In future studies it will be important to compare regulation for similar cell populations among several vertebrate groups. A comparative approach would build a framework for understanding the evolution of an important, but insufficiently understood, property of vertebrate embryos and the evolution of neural crest-derived structures.

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