PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Genes, lineages and the neural crest: a speculative review

David J. Anderson

Phil. Trans. R. Soc. Lond. B 2000 **355**, 953-964
doi: 10.1098/rstb.2000.0631

THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0631

Genes, lineages and the neural crest: a speculative review

David J. Anderson

Division of Biology 216-76, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125, USA (mancusog@cco.caltech.edu)

Sensory and sympathetic neurons are generated from the trunk neural crest. The prevailing view has been that these two classes of neurons are derived from a common neural crest-derived progenitor that chooses between neuronal fates only after migrating to sites of peripheral ganglion formation. Here I reconsider this view in the light of new molecular and genetic data on the differentiation of sensory and autonomic neurons. These data raise several paradoxes when taken in the context of classical studies of the timing and spatial patterning of sensory and autonomic ganglion formation. These paradoxes can be most easily resolved by assuming that the restriction of neural crest cells to either sensory or autonomic lineages occurs at a very early stage, either before and/or shortly after they exit the neural tube.

Keywords: neural crest; neurogenesis; cell lineage; sensory neurons; autonomic neurons

1. INTRODUCTION

Lineage restriction is the process that accompanies the differentiation of various cell types from a pool of multipotent stem or progenitor cells (Morrison *et al*. 1997). Although it is distinct from morphogenesis, it is often tightly coupled to it. The key feature of lineage restriction is the generation of a series of proliferating progenitor cells that exhibit gradual restrictions in their developmental potentials and/or fates. This type of process is exemplified by haematopoiesis (Morrison *et al*. 1994). It is often employed in situations in which progenitors undergo extensive cell migration or dispersal. However, cellular diversity can also be produced by alternative mechanisms; for example, by generating a field of stationary progenitors with different developmental specifications imposed by morphogen gradients, as in the spinal cord (Tanabe & Jessell 1996), or by local cell–cell interactions as in the *Drosophila* retina (Wolff *et al*. 1997).

The neural crest is similar to the haematopoietic system in that it generates a diverse array of differentiated cell types that are widely dispersed throughout the embryo (Anderson 1989; Sieber-Blum 1990; Le Douarin *et al*. 1991). However, it is unique in that it is the only progenitor population during organogenesis that generates multiple cell types contributing to many different (and often functionally unrelated) tissues located all over the body. These tissues include the sensory, autonomic and enteric ganglia of the peripheral nervous system (PNS); peripheral nerve fibres; neuroendocrine tissues such as the medullary secretory cells of the adrenal and thyroid glands; the bones of the face; the outflow tracts of the heart and smooth muscle walls of the great vessels; and melanocytes in the skin (to name just a subset) (Le Douarin & Kalcheim 1999). Within some of these tissues, moreover, there is further cellular diversity; for example, peripheral sensory ganglia contain glial cells and upwards of 20 different types of sensory neurons. By contrast, stem cell populations in most other systems contribute differentiated cell types only to a single tissue, for example, the blood, intestinal epithelium or skin (Hall & Watt 1989; Potten & Loeffler 1990). The neural crest therefore possesses an unusually high degree of multipotency (as a population), and poses the problem of lineage diversification in an extreme form.

The diverse locations in which different crest-derived tissues are found, and the broad range of cellular phenotypes produced by the crest, have led to the evolution of at least two different strategies for generating cellular diversity in this system. First, different crest derivatives are often generated at different locations along the rostrocaudal axis of the spinal cord. For example, the bones of the face are generated from crest cells in the cephalic region, enteric and parasympathetic neurons from the 'vagal' region (the posterior rhombencephalon) and sympathetic neurons from the trunk region. Second, different derivatives are also generated from crest cells at the same axial level; for example, sensory and sympathetic neurons, adrenal medullary chromaffin cells, glia and melanocytes are all generated from the thoracolumbar regions of the trunk crest (Le Douarin 1980).

These two strategies pose distinct but related developmental problems. For example, the first (or 'positional diversification') strategy raises the question of whether neural crest cells at different axial levels are intrinsically different in their developmental capacities at the time of emigration from the neural tube, or whether they are equivalent but acquire different fates as a consequence of encountering different environments as they migrate. This question has been addressed by Le Douarin and coworkers using elegant heterotopic transplantation experiments employing the chick–quail chimera system. These highly informative experiments have indicated that, to a first approximation, axial differences in crest cell fate are

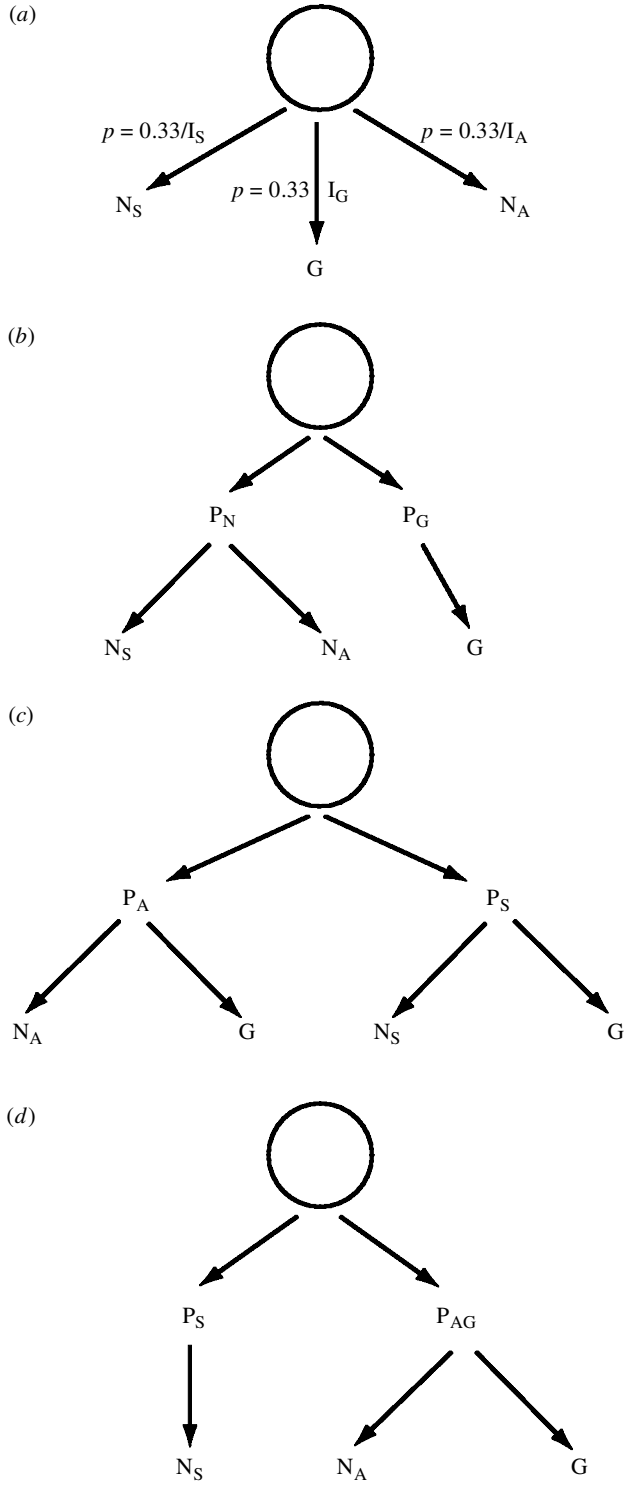

Figure 1. Possible patterns of segregation of neurogenic and gliogenic lineages in the trunk neural crest. For simplicity, the relationship of the melanocyte lineage to the neuronal and glial lineages has been omitted, and a single type of trunk-derived glial cell ('G') is assumed. N_S, sensory neuron; N_A, autonomic neuron. (*a*) A multipotent crest cell directly generates sensory and autonomic neurons and glia without producing partially restricted intermediates. The choice may be made stochastically, with various probabilities assigned to different lineages; in this case equal probabilities ($p = 0.33$) have been assigned arbitrarily for purposes of illustration. Alternatively (or in addition), the choice may be dictated by different instructive signals for the different lineages (I_S, I_G and I_A). (*b–d*) The three lineages are produced via a environmentally rather than intrinsically determined (although there are a few exceptions) (Le Douarin 1980). However, the heterotopic transplantation of neural tube fragments cannot, by definition, be used to address the second of the two strategies, that is the one in which different derivatives are generated from a common axial level of the trunk neural crest. That is the problem on which I will focus the remainder of this discussion.

2. THE PROBLEM OF TRUNK NEURAL CREST LINEAGE DIVERSIFICATION

The problem of how different neural crest derivatives are generated from a common location along the neuraxis can be broken down into several questions. (i) Are different derivatives generated directly from progenitors with a full repertoire of trunk crest fates (figure 1*a*)? Or, does the trunk crest generate partially restricted progenitors with predictable combinations of developmental capacities (e.g. figure 1*b–d*), and if so what are those combinations (Le Douarin *et al.* 1991)? (ii) If partial restrictions are not employed, how do multipotent cells directly generate different derivatives—stochastically (Baroffio & Blot 1992), or in response to instructive signals (Shah *et al.* 1994, 1996)? (iii) If partially restricted progenitors are generated, where and when does this occur? Have some lineages segregated prior to the emigration of crest cells from the neural tube, or do all crest cells emerge from the neuroepithelium with initially equivalent potentials, and only undergo restriction after emigration to the periphery? (iv) How does restriction occur? What are the extracellular signals that regulate the production of restricted progenitors, where are these signals produced and do they act selectively or instructively?

Since the late 1980s, many investigators in the field (including myself) have favoured the idea that neural crest development proceeds in a manner analogous to that of haematopoiesis, via the generation of progressively restricted intermediates (Anderson 1989; Sieber-Blum 1990; Le Douarin *et al.* 1991). According to this view many, if not all, neural crest cells exit the neural tube with the full range of trunk crest potentials (Bronner-Fraser & Fraser 1988, 1989; Fraser & Bronner-Fraser 1991), and undergo partial restrictions in these potentials during or after migration (Duff *et al.* 1991; Sieber-Blum *et al.* 1993; Sextier-Sainte-Claire Deville *et al.* 1994). Particular patterns of lineage restriction have even been suggested, in which certain fates reproducibly co-segregate from others (Le Douarin *et al.* 1991).

There are several aspects of this model, however, that require critical re-examination, particularly in the light of new data. First, patterns of lineage restriction deduced by analysing clone compositions *in vitro* (Le Douarin *et al.*

Figure 1 (*Cont.*) deterministic generation of partially restricted intermediates. (*b*) Neuronal and glial lineages segregate before sensory and autonomic lineages. P_N, neuronal precursor; P_G, glial precursor. (*c*) Sensory and autonomic lineages segregate before neuronal and glial lineages. P_A, autonomic neuroglial precursor; P_S, sensory neuroglial precursor. (*d*) All glia derive from an autonomic-restricted multipotent precursor (P_{AG}), and sensory precursors (P_S) are restricted to a neuronal fate. Other patterns of segregation are possible and are not illustrated.

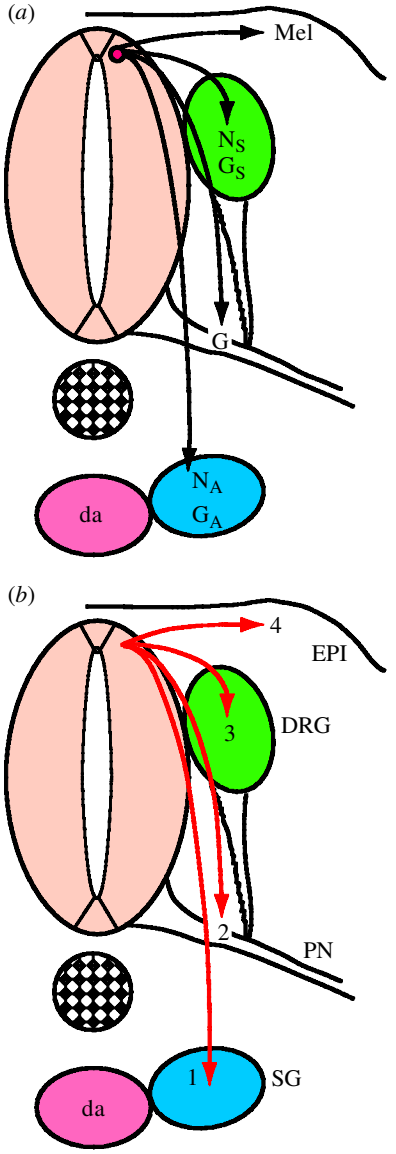

Figure 2. Single-cell and cell-population fate mapping of the neural crest. (*a*) Many single pre-migratory crest cells injected intracellularly with the lineage tracer lysinated rhodamine dextran produce progeny in all structures derived from the trunk neural crest (Bronner-Fraser & Fraser 1991). Mel, melanocytes; N_S, sensory neuron; G_S, sensory glia; G, generic peripheral glia (e.g. Schwann cells); N_A, autonomic (sympathetic) neuron; G_A, autonomic glia. The green oval represents sensory (dorsal root) ganglion, and the blue oval autonomic (sympathetic) ganglion. da, dorsal aorta. (*b*) Labelling of a population of pre-migratory crest cells within the neural tube by injection of the lipophilic dye DiI results in sequential colonization of the sympathetic ganglia (SG), peripheral nerve (PN), dorsal root ganglion and epidermis (EPI) (Serbedzija *et al*. 1989, 1990). The results are not inconsistent because the single-cell injections (*a*) were generally analysed after a late end-point.

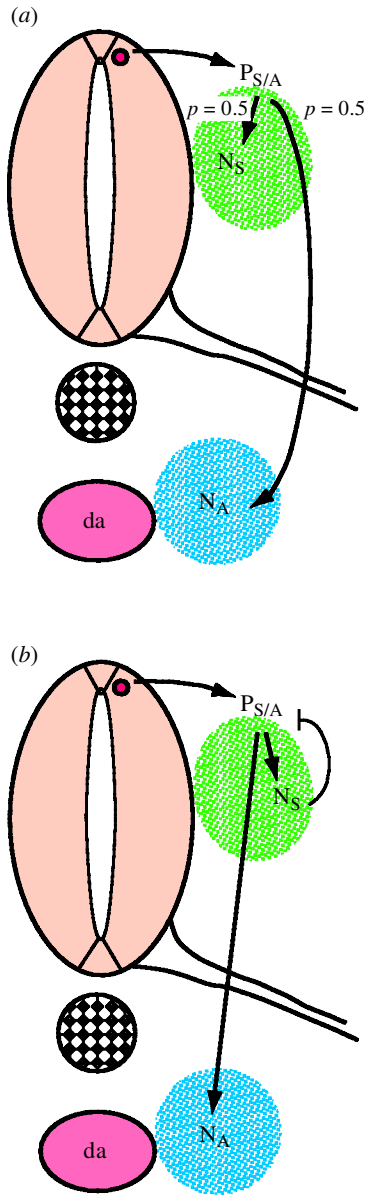

Figure 3. Two alternative mechanisms for generating sympathetic and sensory neurons from a common, equi-competent precursor. These mechanisms explain how sympathetic neurons could be generated from a common precursor ($P_{S/A}$) even if such a precursor encountered sensory-inducing signals (green stippling) before it had a chance to encounter autonomic-inducing signals (blue stippling). Other symbols as in figure 2. (*a*) Stochastic mechanism. The precursor autonomously generates sensory or autonomic neurons according to some fixed probability; in this example equal probabilities ($p = 0.5$) are arbitrarily assigned for purposes of illustration. In this case inducing signals have no influence on the sensory–autonomic decision. (*b*) Negative-feedback mechanism. The common progenitor initially generates sensory neurons in response to sensory-inducing cues, but these cells then produce a negative-feedback signal that prevents further sensory differentiation despite the inducing signals and allows escape of some precursors to the sympathetic primordia. Note that model (*a*) predicts a simultaneous rather than sequential colonization of the two ganglia, which is not observed, and model (*b*) predicts that sensory ganglia should be colonized before sympathetic ganglia, the opposite of what is observed experimentally (Serbedzija *et al*. 1989, 1990).

1991) are based on the assumption that if different founder cells give rise to different subsets of crest derivatives under identical culture conditions, then these founder cells must be intrinsically different. This assumption is questionable, because stochastic differences in the behaviour of equivalent founder cells could have large effects on the ultimate production of cell fates in different colonies. For example, the nature and sequence of early

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

cell–cell interactions in a colony may affect the outcome of differentiation events. Furthermore, most such studies evaluate colony composition at a single, arbitrary time-point; simple interclonal differences in the kinetics of differentiation of a given cell type could yield apparent differences in colony composition at the time of analysis, and therefore lead to false conclusions about founder cell lineage restrictions. The only rigorous test of developmental restriction is to challenge cells with instructive signals that promote various crest fates (Shah *et al*. 1994, 1996) and determine whether the cells are resistant to the effects of such signals (Lo & Anderson 1995). Unfortunately, such instructive signals have been identified only recently. Therefore the pattern and sequence (if any) of lineage restrictions, particularly in trunk neural crest, remains an open question.

Second, the assumption that most or all neural crest cells are multipotent and developmentally equivalent at the time they exit the neural tube has been called into question by single-cell lineage analyses performed on crest explants *in vitro* (Henion & Weston 1997). These studies have revealed evidence of rapid fate restrictions in crest cells very early in emigration. Although restrictions in fate do not imply restrictions in potential, they certainly raise the possibility that not all crest cells are initially equivalent. Indeed, as described in more detail in § 6, such fate restrictions were also observed in analogous lineage studies performed *in vivo* (Bronner-Fraser & Fraser 1991); however, for legitimate reasons the multipotency of some neural crest cells was the result emphasized. Therefore, the question of whether partial restrictions in crest cell potentials occur before or after emigration from the neural tube remains open. As discussed in § 7, there are reasons why an early segregation of some trunk crest lineages would be an attractive way to resolve certain paradoxes posed by the assumption of multipotency.

3. THE PARADOX OF THE SENSORY–AUTONOMIC DECISION

Sensory and autonomic neurons constitute the two major branches of the PNS. Although within each branch there are many different neuronal subtypes, sensory and autonomic neurons in a generic sense are distinguished in many ways from one another; for example, sensory neurons project both to the periphery and to the central nervous system (spinal cord), while autonomic neurons project only to the periphery. Therefore the particulars of sensory and autonomic lineage determination is relevant both to the general problem of how neuronal diversity is generated during vertebrate embryogenesis, and to the problem of neural crest lineage restriction.

In the trunk region of the neural crest, the major type of autonomic neuron generated is the sympathetic neuron; as mentioned in § 1, parasympathetic and enteric autonomic neurons are predominantly generated more anteriorly, from the vagal region of the neural crest, as well as posteriorly from the saccral crest (Le Douarin 1980). Sympathetic neurons lie in a metameric chain of ganglia located adjacent to the dorsal aorta, while sensory neurons lie more dorsally in a metameric chain located adjacent to the spinal cord. Neural crest cells that generate sympathetic neurons therefore have to migrate through the region in which sensory neurons are generated before they reach their destination (figure 2).

This geometry poses a paradox; if neural crest cells delaminate from the dorsal neural tube as a uniform population with both sensory and sympathetic potential (figure 2*a*), what prevents all of these cells from differentiating to sensory neurons before they have a chance to migrate more ventrally to the sympathetic anlagen? One possible solution to this problem invokes stochastic (figure 3*a*) or negative-feedback-based mechanisms (figure 3*b*) to allow some multipotent sensory–autonomic progenitors to escape sensory-inducing signals in the dorsal neural tube environment, so that they can continue migrating ventrally to the sympathetic anlagen. In the first case, multipotent crest cells would have a certain probability, $p < 1.0$, of generating sensory neurons in the dorsal root ganglia (DRG) environment; those cells that did not generate sensory neurons would then be free to migrate to the sympathetic primordia (figure 3*a*). In the second case, sensory neurons generated from multipotent precursors would send a negative-feedback signal to equivalent precursors to inhibit their sensory differentiation and allow them to migrate to the sympathetic anlagen.

The problem with these models is that the first mechanism would predict that sensory and sympathetic ganglia are colonized more or less simultaneously, and this is not observed. Rather they have been reported to be colonized sequentially; trunk neural crest cells populate the sympathetic ganglia first, and later the sensory ganglia (Weston 1963; Serbedzija *et al*. 1989). This temporal separation is even more extreme in the mouse (Serbedzija *et al*. 1990) than in the chick. The second model predicts a sequential generation of first sensory and then sympathetic neurons; but this is precisely the opposite of what is observed. In the mouse, not only do trunk neural crest cells migrate to the sympathetic primordia before they form sensory ganglia, they even take different migration routes through the sclerotome to each destination (figure 4*c*). Such a physical separation of migrating sympathetic and sensory progenitors does not support the idea that short-range cell–cell interactions between migrating multipotent neural crest cells determine the segregation of these two neurogenic lineages (figure 3*b*).

4. THE SEQUENTIAL COLONIZATION OF SYMPATHETIC AND SENSORY GANGLIA: DIFFERENT CELLS, DIFFERENT SIGNALS, OR BOTH?

Models to explain the sequential colonization of sympathetic and sensory ganglia by trunk neural crest cells fall into two basic categories: either early- and late-emigrating trunk neural crest cells are multipotent and developmentally equivalent and their environment changes with time (e.g. figure 4*a*,*c*; red circles); or else the cells are intrinsically different (figure 4*b*,*d*; magenta and green circles). In the first category, early-emigrating crest cells fated to generate sympathetic neurons could emerge from the neural tube at a time when sensory neuron-inducing signals (figure 4*a*; green stippling) were not yet present but sympathetic neuron-inducing signals (figure 4*a*; blue stippling) were available. Therefore there would be no instructive signals to divert these cells from their sympathetic fate as they migrated past the dorsal

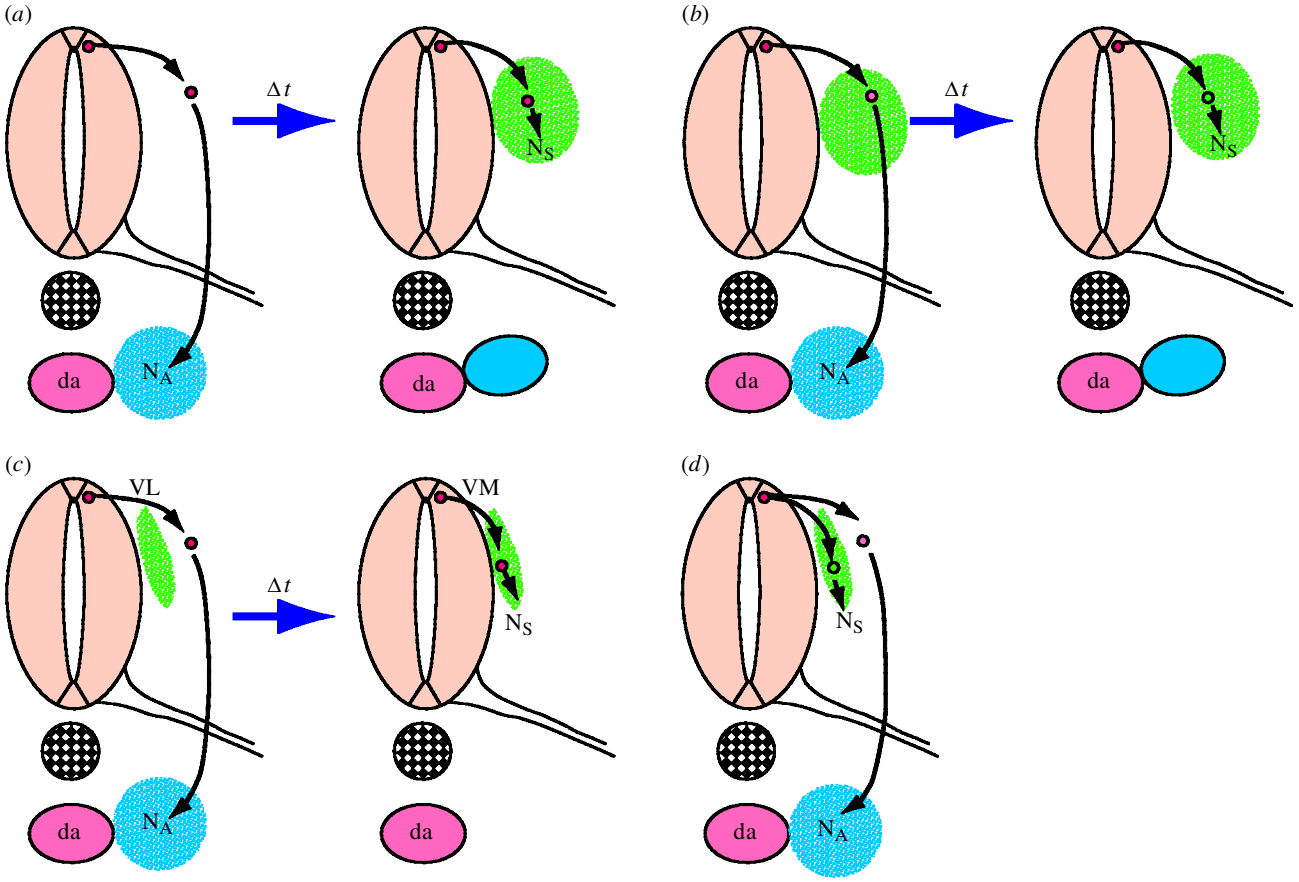

Figure 4. Models to explain the sequential colonization of first sympathetic and then sensory ganglia by trunk neural crest cells. (*a*,*c*) Most or all migratory crest cells are equipotent and equicompetent for sensory and autonomic fates. In (*a*) the inducing signals encountered by the cells change as a function of time (Δt). Early-emigrating cells (left) encounter autonomic (blue stippling) but not sensory-inducing signals; late-emigrating cells (right) encounter sensory-inducing signals (green stippling). In (*c*) both autonomic- and sensory-inducing signals are present early and late, but the migratory pathways available to the cells change as a function of time; the early VL pathway (Serbedzija *et al*. 1990) avoids sensory-inducing signals while the late VM pathway encounters them. Note that this mechanism could operate only in mouse and not chick, for in the latter species separate VL and VM pathways have apparently not been observed (Serbedzija *et al*. 1989). (*b*,*d*) Most migratory crest cells are restricted to either autonomic (magenta circles) or sensory (green circles) fates at the time they delaminate from the neural tube. In (*b*) the two types of progenitors are generated sequentially, although there may be a period of overlap (not illustrated). In (*d*) the progenitors are produced sequentially and in the mouse select different migratory routes that guide them to the sensory or sympathetic ganglia (Serbedzija *et al*. 1990); for simplicity the two progenitor types are shown concurrently.

neural tube and towards the dorsal aorta (figure 4*a*). Subsequent waves of crest cells would then emigrate at a time when sensory-inducing signals were present (figure 4*a*; right). Another version of such a model is one in which early-emigrating crest cells could have available to them only the ventrolateral (VL) migration pathway (figure 4*c*, left), which might avoid short-range sensory-inducing signals (shown by green stippling); the other, ventromedial (VM) migration pathway to the sensory primordia (figure 4*c*, right) would then become available at later times. These types of explanations have tended to be favoured in the literature (Weston & Butler 1966; Serbedzija *et al*. 1989, 1990).

It is also possible, however, that early- and late-emigrating crest cells are intrinsically different. Indeed, there is evidence that the latest-emigrating trunk neural crest cells, which are fated to generate melanocytes, are restricted in comparison with earlier-emigrating cells (Artinger & Bronner-Fraser 1992). This restriction has been invoked to explain how these cells choose a different, subectodermal migration pathway than earlier VM-migrating cells (Erickson & Goins 1995). Likewise, the fact that trunk crest cells in the mouse take different migratory pathways to the sympathetic and sensory ganglia could indicate that the cells have to be different in order to recognize these distinct migration routes (figure 4*d*). Furthermore, even in the chick where the migration pathways to the DRG and sympathetic ganglia overlap, the sequential generation of sympathetic and then sensory derivatives could also be explained by the sequential emergence of sympathetic-restricted (figure 4*b*; magenta circle) and then sensory-restricted (figure 4*b*; green circle) crest cells. Such explanations have tended to be discounted in the literature, but a closer inspection of this evidence reveals that it does not rigorously exclude the possibility that many trunk crest cells are heterogeneous with respect to sensory and sympathetic potential early in migration.

5. *IN VIVO* TRANSPLANTATION AND *IN VITRO* CULTURE EXPERIMENTS SUPPORTING A LATE SEGREGATION OF SYMPATHETIC AND SENSORY LINEAGES: A RE-EXAMINATION

In vivo heterochronic transplantation has provided one line of evidence that it is the environment of the crest cells that is changing with time, rather than the crest cells themselves. Weston & Butler (1966), using [^{3}H]thymidine to pulse label neural crest cells, and taking advantage of the fact that the migration and differentiation of crest cells occurs in a rostral-to-caudal wave, transplanted neural tube fragments from early (posterior) regions where sympathetic neurons were being generated to late (more anterior) regions where sensory neurons were forming, and vice versa. They found that the neural tube fragments could generate crest cells that populated the type of ganglia appropriate for their site of transplantation in the host, and concluded that the crest cells were the same but their environment was different at different times.

This is not the only interpretation of these data, however. For example, it is possible that the chick trunk crest concurrently generates both sensory- and autonomic-restricted progenitors. The latter may migrate to the sympathetic primordia at early times and differentiate to sympathetic neurons, but may be incorporated into the sensory ganglia at later times where they either remain undifferentiated, or else differentiate to glia. This interpretation is impossible to exclude since no molecular markers were used to distinguish whether the transplanted cells differentiated to neurons or non-neuronal cells. Furthermore, it is consistent with the observation that sensory ganglia contain a population of autonomic-restricted precursors until very late stages of development (Le Lievre *et al*. 1980; Xue *et al*. 1985).

A more recent heterochronic transplantation study of trunk neural tube has, to the contrary, provided evidence that there are axial or temporal differences in the propensity of trunk neural crest cells to generate sympathetic versus sensory neurons (Asamoto *et al*. 1995). This result is most easily explained by separate precursors for sensory and sympathetic neurons existing in different proportions along the trunk rostrocaudal axis. However, because it was a population and not a single-cell study, the experiments could not formally exclude the alternative possibility that the results reflected intrinsic differences in the probability of generating sensory versus sympathetic neurons by a uniform population of bipotent sensory–autonomic progenitors. However, in the context of the other data and arguments presented here (see § 7), the existence of different sensory- and autonomic-restricted subpopulations seems a much more likely explanation for the data.

Cell culture data have also been used to argue against the idea that neural crest cells become restricted to an autonomic fate early in migration. Progenitors expressing both sensory and autonomic markers *in vitro* can be identified among cells from early quail sympathetic ganglia, whereas cells taken from older ganglia exhibit only sympathetic markers under the same culture conditions (Duff *et al*. 1991; Sieber-Blum *et al*. 1993). While such observations demonstrate that there are at least some cells that are not yet fully committed to a sympathetic fate at early stages of gangliogenesis, this lack of restriction was observed *in vitro* and it is possible that the culture conditions promoted the de-differentiation of the cells. Indeed, when the sensory potential of progenitors in early sympathetic ganglia was assessed by *in vivo* transplantation into the crest migration pathway of earlier host embryos, only autonomic and not sensory derivatives were obtained (Le Lievre *et al*. 1980).

6. EVIDENCE FOR FATE-RESTRICTED SENSORY AND SYMPATHETIC PRECURSORS AMONG MIGRATING NEURAL CREST CELLS FROM *IN VIVO* LINEAGE-TRACING EXPERIMENTS

Another line of evidence for a late segregation of the sensory and sympathetic lineages comes from *in vivo* single-cell lineage-tracing experiments. In the initial set of these studies, many individual pre-migratory cells injected in the dorsal neural tube were observed to generate both sensory and sympathetic neurons (Bronner-Fraser & Fraser 1988, 1989). Most of these cells also generated neural tube cells, implying that they were relatively primitive tube-crest progenitors rather than neural crest cells *per se*. Consequently, the fact that the progeny of such cells generated both neuron types after several days of development left open the possibility that these cells sequentially generated both autonomic-restricted, and sensory-restricted precursors, which emigrated as distinct populations from the neural tube. However, in a subsequent experiment, these same authors were able to dye inject a very small number ($n = 17$) of crest cells shortly after their emigration from the neural tube (Fraser & Bronner-Fraser 1991). Four of these 17 neural crest cells generated neurons (as detected by neurofilament antibody staining) in both the sympathetic and sensory ganglia.

These latter data indicate that there are at least some neural crest cells which retain both sensory and autonomic capacities shortly after emigration from the neural tube. Such results would be consistent with the idea that temporal changes in inducing signals (figure 4*a*) and/or migration routes (figure 4*c*), rather than the production of different kinds of neural crest cells, explain the sequential colonization of sympathetic and sensory ganglia. However, the data do not exclude the possibility that other crest cells have made a decision between sensory and autonomic fates before exiting the neural tube. In fact, over 50% (eight out of 17) of the cells marked after exiting the neural tube were fated to generate only sensory and not sympathetic neurons, while almost 25% (four out of 17) conversely generated autonomic (sympathetic or adrenal) but not sensory neurons (Fraser & Bronner-Fraser 1991).

Thus, another way of looking at these data is that the injected migrating crest cells were three times as likely to be restricted to either sensory or sympathetic fates, as they were fated to generate both derivatives. Of course, a unifatent cell is not necessarily unipotent; in theory, all marked cells could have had equivalent potentials and the observed fate restrictions could have simply reflected stochastic variations in what the cells actually did. Nevertheless, the data certainly raise the possibility that the 75% of the marked cells that were fate restricted were

also restricted in their competence or potential. If all of the marked cells had been multifatent, there would be no reason to even consider this possibility—but that was not the result obtained. While it may be tempting to suppose that the figure of 25% represents an underestimate of the proportion of multipotent cells, this remains to be tested. The actual proportion of multipotent versus fate-restricted cells has a significant impact on thinking about the dynamics of the system and the strategies employed to generate lineage diversification.

If there are indeed neural crest cells restricted to generating neurons of the autonomic subset, when and where is this restriction acquired? Strikingly, among three crest cells injected just as they were delaminating from the neural tube, one gave rise to only sympathetic and not sensory derivatives and another conversely to sensory but not sympathetic; the third generated both (Bronner-Fraser & Fraser 1989). It is important to note that these studies were performed at only a single time-point, and an even higher frequency of such fate-restricted progenitors might have been detected had the injections been performed at earlier stages when predominantly sympathetic neurons were being generated. While these observations do not prove that such fate-restricted cells were similarly restricted in their potentials, they provide evidence that such restrictions in potential could occur as early as the time that crest cells are delaminating from the neural tube.

7. THE IDENTIFICATION OF AUTONOMIC-INDUCING SIGNALS RAISES NEW QUESTIONS ABOUT THE TIMING OF RESTRICTION TO THE AUTONOMIC LINEAGE

New questions regarding the timing of restriction to the autonomic sublineage have been raised recently by the identification, on the one hand, of autonomic neuron-inducing signals and on the other hand of signals that promote the formation of neural crest cells in the dorsal neural tube. These inducing signals appear to be very similar if not identical; members of the bone morphogenetic protein (BMP) family such as BMP2 and BMP4, produced by the ectoderm and dorsal neural tube, are necessary and sufficient to promote the formation of neural crest cells from the neural tube (Dickinson *et al.* 1995; Liem *et al.* 1995, 1997). The same factors produced in the dorsal aorta appear necessary and sufficient to induce differentiation of autonomic neurons from multipotent neural crest cells *in vivo* and *in vitro* (Reissman *et al.* 1996; Shah *et al.* 1996). So the problem is this: if neural crest cells emerge from the neural tube with equal competence for sensory and autonomic differentiation, why do these cells not immediately differentiate to autonomic neurons dorsally, in response to BMPs secreted by the ectoderm and dorsal neural tube?

There are a number of possible mechanisms that could solve this problem. First, the action of BMPs to induce autonomic neurogenesis in freshly delaminated crest cells may be prevented by other signals in the dorsal environment. These signals could include inhibitors of BMPs, such as noggin, which is present in the roof plate and which could reduce the effective concentration of BMPs below a critical threshold for autonomic neurogenesis; or other signals such as Wnts (which are also present in the roof plate (McMahon *et al.* 1992)) which could qualitatively change the effect of BMPs on emigrating crest cells. This sort of mechanism would be required if freshly delaminated neural crest cells are indeed equally competent to generate both sensory and sympathetic neurons.

It is also possible, however, that freshly delaminated neural crest cells are initially not competent to generate autonomic neurons in response to BMPs, and that this competence is acquired only later as the cells migrate ventrally towards the dorsal aorta. A prediction of this model is that exposure of freshly delaminated neural crest cells to a high concentration of BMPs would not induce autonomic markers in crest cells located dorsolaterally to the neural tube, but only in those cells which had migrated more ventrally. Interestingly, precisely this result was obtained in experiments in which BMP4 levels were artificially increased in chick embryos using a retroviral vector (Reissman *et al.* 1996). Similarly, when BMP2 (or BMP4) is applied to neural tube explants cultured in the absence of surrounding tissues, autonomic markers are induced in those neural crest cells that have migrated farthest from the neural tube, but there is a region of 'non-responding' cells proximal to the explant (Greenwood *et al.* 1999). Taken together, these data suggest that progenitors of sympathetic neurons are initially not competent to respond to autonomic-inducing signals when they first delaminate from the neural tube.

What, if anything, could such a delayed acquisition of autonomic competence tell us about when restriction to an autonomic fate is acquired? Suppose that precursors of sympathetic neurons indeed do not acquire competence to respond to autonomic-inducing signals like BMP2/4 until they have migrated ventrally to the site of DRG formation. Suppose further that all such cells are derived from multipotent progenitors that exit the neural tube with both sensory and autonomic potential. In this case, multipotent neural crest cells would be competent for sensory differentiation before they had acquired competence for autonomic differentiation. This order of competencies is precisely the opposite of what would make sense, given the fact that neural crest cells populate the sympathetic ganglion primordia before they contribute to the sensory ganglia. Moreover, such a model would seem to make the cells even more vulnerable to being diverted to a sensory fate before they had a chance to respond to autonomic-inducing signals. The assumption of equal sensory and autonomic potential by freshly delaminated neural crest cells therefore leads to a paradox when viewed in the light of the new data on autonomic-inducing signals.

It seems far easier to account for the data with a model in which many autonomic precursors are already restricted from a sensory fate almost as soon as they exit the neural tube. Such an early restriction would not only account for the delayed competence of neural crest cells to differentiate to autonomic neurons in response to BMPs, but would also serve to protect these cells from being diverted to a sensory fate before they had a chance to migrate to the sympathetic primordia. Furthermore, this model is consistent with the fact that multipotent neural crest stem cells with autonomic neuronal, glial and

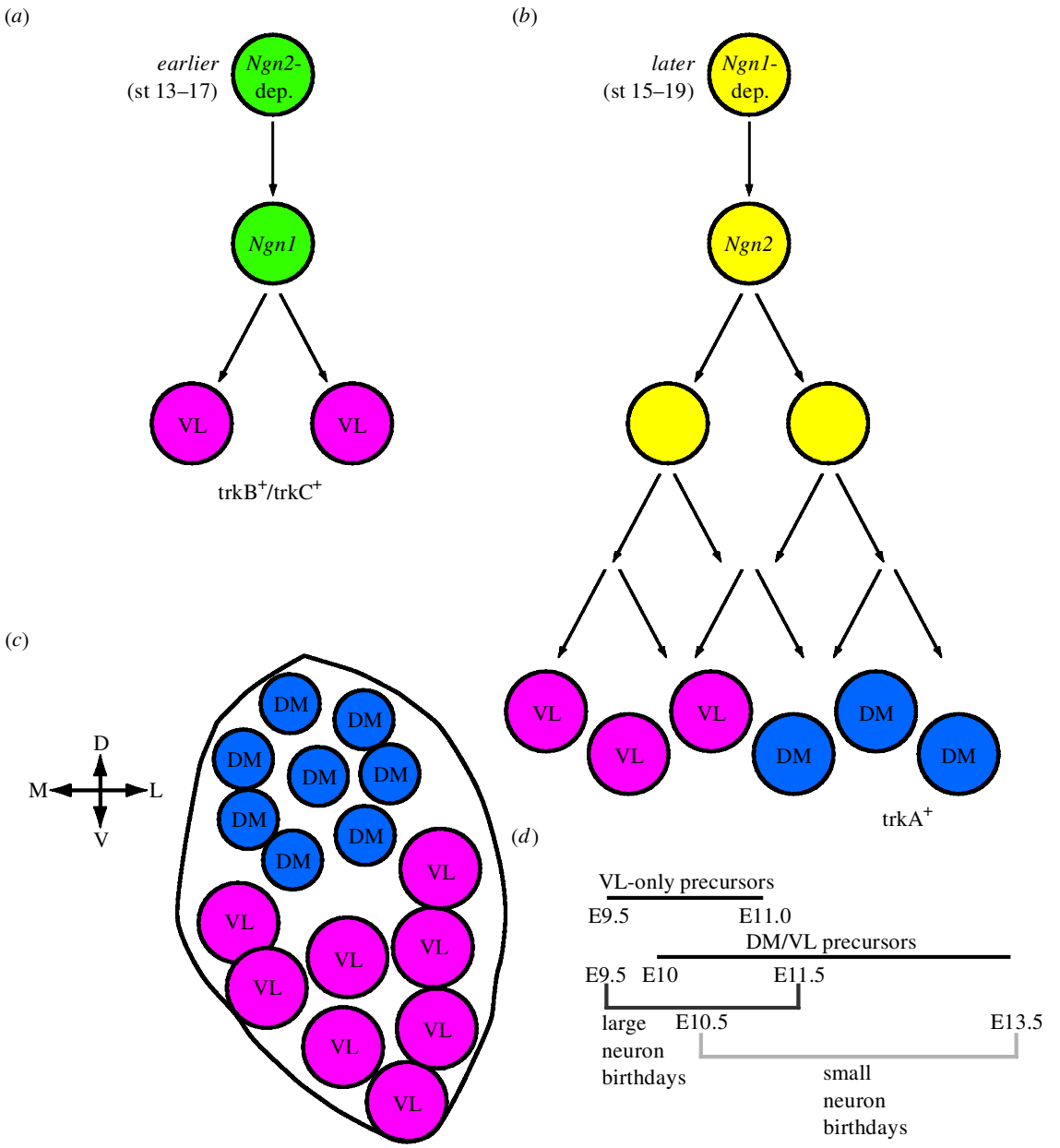

Figure 5. Two genetically and lineally distinct populations of sensory neuron precursors. (*a*,*b*) Summary of results of retroviral lineage-tracing experiments in chick DRG (Frank & Sanes 1991). (*a*) VL-only precursors give rise to early-born (*d*), large-diameter (trkC$^+$ and trkB$^+$) sensory neurons located in the VL region of the ganglia (*c*). This lineage probably corresponds to *Ngn2*-dependent precursors in the mouse (Ma *et al.* 1999). (*b*) DM + VL precursors give rise to both large-diameter (pink) and small-diameter (blue, DM) later-born neurons (*d*), which are located in the DM region of the ganglia (*c*). This lineage probably corresponds to *Ngn1*-dependent (dep.) precursors in the mouse (Ma *et al.* 1999). VL-only precursors are more frequent when marking is performed earlier (St13–17), while DM + VL precursors are seen later (St15–19). VL-only precursors are likely to be restricted to a sensory fate, based on studies of *Ngn2* (Ma *et al.* 1999; Perez *et al.* 1999) and of sensory neuron precursors *in vitro* (Greenwood *et al.* 1999). It is not yet clear whether DM + VL precursors are similarly restricted. (*d*) Summary of birthdating studies of large- and small-diameter sensory neurons (Lawson *et al.* 1974; Lawson & Biscoe 1979).

smooth muscle potential (Stemple & Anderson 1992; Shah *et al.* 1996) have never been observed to differentiate into sensory neurons, either *in vitro* or after transplantation *in vivo* (Morrison *et al.* 1999; White & Anderson 1999; P. M. White and D. J. Anderson, unpublished data). A rigorous test of this conclusion will require the identification of instructive-inducing signals for sensory neurons.

The notion that a subset of neural crest cells exits the neural tube with the potential to make autonomic neurons and glia but not sensory neurons leaves open the question of whether, conversely, there are neural crest cells which early in migration have sensory but not autonomic potential. Recent studies support this possibility as well.

8. EVIDENCE FOR A DIVIDING PRECURSOR IN THE MAMMALIAN NEURAL CREST THAT IS COMMITTED TO THE SENSORY LINEAGE WITH RESPECT TO AUTONOMIC-INDUCING SIGNALS

Recently, we have provided evidence that the rat neural crest contains a population of precursors that are

committed to a sensory fate with respect to the autonomic-inducing signal, BMP2. (Note that 'commitment' is used operationally here, to indicate that the cell is irreversibly determined for a given fate with respect to a physiological inducer of relevant alternative fates. That does not mean that it is committed with respect to all possible inducing signals, something that can never be excluded.) Sensory neurons, identified by co-expression of the POU homeodomain proteins Brn-3.0 and other markers such as c-ret and peripherin, develop from proliferating neural tube-derived precursors in a fully defined culture medium (Greenwood *et al.* 1999). Under such conditions autonomic neurons, identified by expression of the paired homeodomain protein Phox2a, fail to develop. However, differentiation of autonomic neurons, in numbers vastly exceeding the number of sensory neurons, can be induced by addition of BMP2. This manipulation fails to produce any significant reduction in the number of sensory neurons that differentiate, however (Greenwood *et al.* 1999).

The simplest interpretation of this result is that the sensory and autonomic neurons in these BMP2-treated cultures develop from separate precursors, although more complex explanations involving a common precursor cannot be formally excluded (Greenwood *et al.* 1999). (Unfortunately, this system has thus far resisted efforts to experimentally resolve this question by direct clonal analysis or *in vitro* retroviral lineage tracing.) Nevertheless, if the more parsimonious interpretation is correct, such committed sensory precursors might correspond to the subset of crest cells marked *in vivo* that are fated to generate only sensory neurons (Bronner-Fraser & Fraser 1989, 1991). Our *in vitro* study therefore raises the possibility that some of the sensory fate-restricted cells observed *in vivo* may be restricted in their potential as well.

9. THE EXPRESSION AND FUNCTION OF THE *NEUROGENINS* PROVIDE INDEPENDENT EVIDENCE FOR EARLY SPECIFICATION OF A SENSORY FATE IN A SUBSET OF MIGRATING NEURAL CREST CELLS

An independent line of genetic evidence also suggests that a subset of neural crest cells is at least specified, if not determined, for a sensory fate early in migration. This evidence derives from studies of the expression and function of the *Neurogenins* (*Ngns*), a family of vertebrate proneural genes homologous to the *Drosophila* proneural gene *atonal* (Gradwohl *et al.* 1996; Ma *et al.* 1996; Sommer *et al.* 1996). *Ngns* are essential for the development of neural crest- and placode-derived sensory, but not sympathetic, neurons *in vivo* (Fode *et al.* 1998; Ma *et al.* 1998, 1999). *Ngn2* is expressed very early in neural crest migration, as well as in a subset of cells at the dorsolateral margins of the neural tube, in both mouse (Ma *et al.* 1999) and chick (Perez *et al.* 1999).

Sensory neurogenesis in the DRG is prevented in both single *Ngn2* and double *Ngn2;Ngn1* mutants (Ma *et al.* 1999) while sympathetic ganglia are unaffected. This suggests either that *Ngn2*-expressing neural crest cells only generate sensory and not sympathetic neurons, or that they generate both classes of neurons but neither require nor ultimately express *Ngn2* in the sympathetic lineage. Examination of *Ngn2-lacZ* knockin embryos, in which

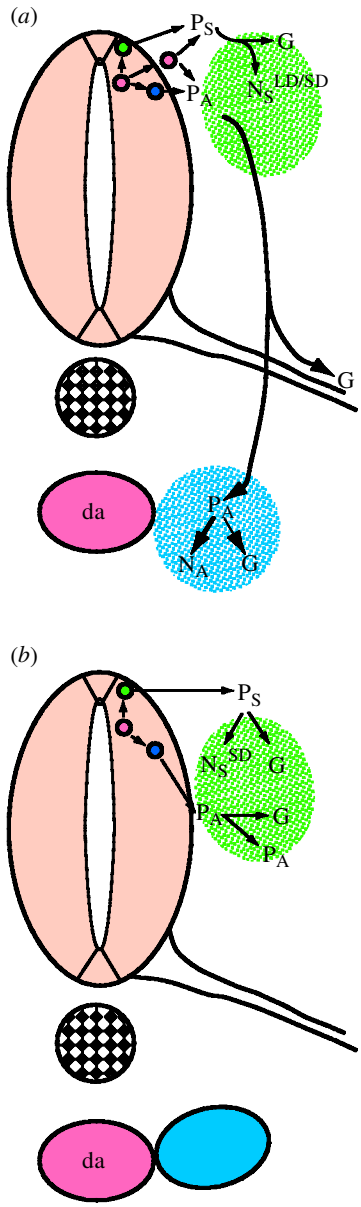

Figure 6. Speculative model for early segregation of sensory and autonomic precursors in the pre-migratory neural crest. (*a*) At early stages of crest migration, it is suggested that many sensory precursors (P_S) and autonomic precursors (P_A) may emerge from the neural tube already restricted to these lineages. However, a proportion of crest cells do not make this decision until after they have emigrated from the neural tube (Fraser & Bronner-Fraser 1991). The early-emigrating sensory precursors give rise primarily to large-diameter sensory neurons (see figure 5), but also to some small-diameter neurons as well (N_SLD/SD). It is assumed that this sensory-restricted precursor also gives rise to glial cells ('G'), but this is hypothetical as well. At this stage of development, autonomic precursors migrate to the sympathetic ganglion anlagen (blue stippling) where they differentiate to sympathetic neurons and glia. (*b*) At later stages, the sensory precursors give rise primarily or exclusively to small-diameter sensory neurons (N_SSD); these precursors may be distinct from those at earlier stages (see figure 5). Autonomic precursors are hypothesized to become incorporated into the DRG at these later stages, explaining the persistence of such precursors in late-stage ganglia (Le Lievre *et al.* 1980; Xue *et al.* 1985).

expression of β-galactosidase perdures for several days in sensory neurons after the endogenous gene is extinguished, has failed to reveal any evidence of *lacZ* expression in sympathetic ganglia (L. Lo, C. Fode, F. Guillemot and D. J. Anderson, unpublished data). While these negative results are not conclusive, they are consistent with the idea that expression of *Ngn2* marks a subset of crest cells fated to generate sensory neurons early in migration.

Gain-of-function studies with the *Ngns* further suggest that by the time neural crest cells express these proneural genes they may be determined as well as specified for a sensory fate. Forced expression of NGNs from a retroviral vector in pre-migratory crest cells *in ovo* biases them to differentiate to sensory neurons in the DRG (Perez *et al.* 1999). Moreover, ectopic expression of NGNs induces the expression of multiple sensory-specific as well as generic neuronal markers, not only in crest-derived glial precursors in peripheral nerve, but surprisingly in the myotome as well (Perez *et al.* 1999). These data suggest that when expressed in certain cell contexts, *Ngns* can bias neural crest cells to a sensory fate. Consistent with this idea, forced expression of *Ngns* in cultured neural tube cells under some conditions promotes not only neuronal differentiation, but expression of the sensory-specific marker Brn-3.0 as well (L. Lo and D. J. Anderson, unpublished data). Taken together, these data suggest that the early expression of *Ngn2* in some migrating crest cells reflects their early determination for a sensory fate. Moreover, the fact that *Ngn2* is also expressed by a subset of cells located at the dorsolateral margins of the neural tube, at the time when neural crest cells are emigrating, could further indicate that the sensory fate is determined in some cells prior to delamination from the neural tube, although this is not yet proven.

10. THE MAJORITY OF MUSCLE AFFERENT SENSORY NEURONS DERIVE FROM A GENETICALLY AND LINEALLY DISTINCT PRECURSOR POPULATION *IN VIVO*

Why should there be a distinct subpopulation of neural crest cells determined for a sensory fate early in development? The answer may be related to the different subtypes of sensory neurons that populate the DRG, and the schedule on which they are generated. In normal development, large-diameter muscle afferent (proprioceptive) sensory neurons differentiate before the small-diameter cutaneous afferent (e.g. nociceptive) neurons (figure 5*d*). In *Ngn2* single knockouts, development of the early differentiating large-diameter muscle afferents is temporarily blocked (Ma *et al.* 1999). By contrast, in *Ngn1* single mutants the majority of these neurons (65–70%) are unaffected while the generation of the small-diameter cutaneous afferents is almost completely prevented (Ma *et al.* 1999).

This genetic segregation corresponds remarkably well to two types of sensory neuron precursors identified by retroviral lineage analysis in the chick almost ten years ago (Frank & Sanes 1991). In these studies, *lacZ*-expressing replication-incompetent retroviruses were injected into the neural tube so as to infect pre-migratory crest cells, and their clonal progeny in the DRG were characterized by position and morphology. One population of precursors, which was labelled only when injections were performed between St13 and St15, produced small clones (average three cells per clone) whose neuronal complement consisted exclusively of large-diameter neurons in the VL region of the ganglia, which probably correspond to muscle afferents (figure 5*a*,*c*). The other population, which was not labelled until after St15 and was found up until St19, produced clones ten times larger that contained both VL neurons and small-diameter neurons in the dorsomedial (DM) region of the ganglia (figure 5*b*,*c*), which are mostly cutaneous afferents (Frank & Sanes 1991). These two lineages correspond remarkably well to the sensory neuron subclasses affected in the *Ngn2* and *Ngn1* single mutants, suggesting that the early VL-only precursor is *Ngn2* dependent, and the later VL + DM precursor is *Ngn1* dependent (Ma *et al.* 1999) (figure 5*a*,*b*).

What does the segregation of two sensory sublineages have to do with the segregation of the sensory and autonomic lineages? Although Frank & Sanes (1991) did not attempt to trace the lineage relationship between VL-only or VL + DM sensory precursors and sympathetic neurons, the apparent correspondence of the VL-only precursors to *Ngn2*-dependent precursors provides an indirect link. If VL-only precursors are *Ngn2* dependent, then for reasons discussed earlier (§ 9) they are probably fated to generate sensory but not sympathetic neurons. It follows that VL-only precursors probably generate only sensory and not sympathetic neurons. Consistent with this conclusion, the sensory neurons that differentiate from the apparently committed precursors in rat neural crest explant cultures express a muscle afferent rather than a cutaneous afferent phenotype (Greenwood *et al.* 1999). This suggests that they correspond to the VL-only precursors identified in the chick and the *Ngn2*-dependent precursors identified in the mouse.

In summary, the foregoing observations make a circumstantial case that the neural crest contains a subset of early-emigrating, *Ngn2*-expressing precursors that are restricted to a sensory fate, and that these precursors generate the early-differentiating subclass of muscle afferent sensory neurons. That conclusion still leaves open the possibility that the later-differentiating, *Ngn1*-dependent VL + DM sensory lineage derives from a different subset of migratory neural crest cells, that has both sensory and autonomic potential. Alternatively, precursors of cutaneous afferent sensory neurons may be distinct from autonomic precursors as well.

11. PERSPECTIVE

New data challenge the idea that sensory and autonomic neurons invariably arise from a common neural crest progenitor that only becomes restricted to one of these two neurogenic lineages after migrating to the ganglionic primordia (Anderson 1989; Bronner-Fraser 1993; Sieber-Blum *et al.* 1993). These data, when taken in the context of the classical descriptions of trunk neural crest migration and differentiation patterns, suggest a model in which many (although not all) neural crest cells have become restricted to either sensory or autonomic lineages before they delaminate from the neural tube (figure 6). It is important to emphasize that this revised

view of trunk neural crest lineage segregation is still highly speculative and requires more rigorous testing, especially at the single-cell level. The main purpose of this review has been to raise the possibility that this idea should be entertained more seriously than it has been previously in most of the recent review literature about neural crest cell lineage segregation.

According to such a model, the sequential timing of the colonization of sympathetic and sensory ganglia (Serbedzija *et al*. 1990) would reflect the sequential emigration of progenitors with first autonomic and then sensory capacity from the trunk neural tube. Both types of progenitors could also be generated concurrently, explaining the overlapping generation of sensory and sympathetic ganglia in some species (Serbedzija *et al*. 1989). It is possible that the segregation of the sensory and autonomic lineages can also occur shortly after, rather than before, neural crest cells emigrate from the neural tube (figure 6*a*). This would explain the observation of dual sensory–autonomic progenitors among the few migrating crest cells marked in the single-cell lineage analysis (Fraser & Bronner-Fraser 1991). However, the idea that most crest cells have made a choice between sensory and autonomic lineages before leaving the neural tube would explain why migratory progenitors with both sensory and autonomic fates were in the statistical minority in these experiments.

The notion of an early segregation of the sensory and autonomic lineages was raised previously based on the results of 'retro-transplantation' experiments in avian embryos (Le Douarin 1986). However, these experiments dealt only with the developmental potentialities of post-migratory crest cells in sensory and sympathetic ganglia, and not with pre-migratory or early-migrating neural crest cells (Le Lievre *et al*. 1980). Moreover, they did not provide any direct evidence for precursors committed to a sensory fate, only for precursors with autonomic but not sensory capacity. Furthermore, it was not clear whether such autonomic precursors were restricted to a neuronal fate, or had both neuronal and glial capacities. More recent data suggest that these autonomic-restricted precursors are probably self-renewing stem cells with not only neuronal and glial but also smooth muscle or myofibroblast potential (Morrison *et al*. 1999; P. M. White, S. J. Morrison and D. J. Anderson, unpublished data). It is not yet clear whether sensory-restricted precursors also have glial potential; but if that were the case then it would suggest the counter-intuitive idea that neural crest cells choose what type of neuron they will generate before they decide whether to become neurons or glia (figure 1*c*).

If this model is correct, then it requires that a significant amount of pre-patterning of pre-migratory trunk neural crest cells occur in the trunk neural tube. There is precedent for such pre-patterning in the specification of different subsets of dorsal interneurons (Liem *et al*. 1997). In that case, different types of interneurons are generated within a relatively small distance from one another according to differences in the concentration and/or identity of transforming growth factor-β family morphogens produced by the roof plate. It seems reasonable to think that such a mechanism could be used equally well to produce restrictions in the developmental potentials of different subsets of pre-migratory neural crest cells.

I thank past and present members of my laboratory who have contributed data and ideas to our studies of the neural crest. These include Derek Stemple, Nirao Shah, Liching Lo, Amy Greenwood, Pat White, Sherry Perez, Jane Johnson, Lukas Sommer, Joseph Verdi and Qiufu Ma. The perspective presented here is the author's and is not necessarily representative of these other individuals. I am grateful to Amy Greenwood for helpful comments on the manuscript. The author is an Investigator of the Howard Hughes Medical Institute.

REFERENCES

Anderson, D. J. 1989 The neural crest cell lineage problem: neuropoiesis? *Neuron* **3**, 1–12.

Artinger, K. B. & Bronner-Fraser, M. 1992 Partial restriction in the developmental potential of late emigrating avian neural crest cells. *Devl Biol.* **149**, 149–157.

Asamoto, K., Nojyo, Y. & Aoyama, H. 1995 Restriction of the fate of early migrating trunk neural crest in gangliogenesis of avian embryos. *Int. J. Devl Biol.* **39**, 975–984.

Baroffio, A. & Blot, M. 1992 Statistical evidence for a random commitment of pluripotent cephalic neural crest cells. *J. Cell Sci.* **103**, 581–587.

Bronner-Fraser, M. E. 1993 Segregation of cell lineage in the neural crest. *Curr. Opin. Genet. Dev.* **3**, 641–647.

Bronner-Fraser, M. & Fraser, S. E. 1988 Cell lineage analysis shows multipotentiality of some avian neural crest cells. *Nature* **335**, 161–164.

Bronner-Fraser, M. & Fraser, S. E. 1989 Developmental potential of avian trunk neural crest cells *in situ*. *Neuron* **3**, 755–766.

Bronner-Fraser, M. & Fraser, S. E. 1991 Cell lineage analysis of the avian neural crest. *Development* **2** (*Suppl.*), 17–22.

Dickinson, M. E., Selleck, M. A. J., McMahon, A. P. & Bronner-Fraser, M. 1995 Dorsalization of the neural tube by the non-neural ectoderm. *Development* **121**, 2099–2106.

Duff, R. S., Langtimm, C. J., Richardson, M. K. & Sieber-Blum, M. 1991 *In vitro* clonal analysis of progenitor cell patterns in dorsal root and sympathetic ganglia of the quail embryo. *Devl Biol.* **147**, 451–459.

Erickson, C. A. & Goins, T. L. 1995 Avian neural crest cells can migrate in the dorsolateral path only if they are specified as melanocytes. *Development* **121**, 915–924.

Fode, C., Gradwohl, G., Morin, X., Dierich, A., LeMeur, M., Goridis, C. & Guillemot, F. 1998 The bHLH protein NEUROGENIN 2 is a determination factor for epibranchial placode-derived sensory neurons. *Neuron* **20**, 483–494.

Frank, E. & Sanes, J. R. 1991 Lineage of neurons and glia in chick dorsal root ganglia: analysis *in vivo* with a recombinant retrovirus. *Development* **111**, 895–908.

Fraser, S. E. & Bronner-Fraser, M. E. 1991 Migrating neural crest cells in the trunk of the avian embryo are multipotent. *Development* **112**, 913–920.

Gradwohl, G., Fode, C. & Guillemot, F. 1996 Restricted expression of a novel murine *atonal*-related bHLH protein in undifferentiated neural precursors. *Devl Biol.* **180**, 227–241.

Greenwood, A. L., Turner, E. E. & Anderson, D. J. 1999 Identification of dividing, determined sensory neuron precursors in the mammalian neural crest. *Development.* (In the press.)

Hall, P. A. & Watt, F. M. 1989 Stem cells: the generation and maintenance of cellular diversity. *Development* **106**, 619–633.

Henion, P. D. & Weston, J. A. 1997 Timing and pattern of cell fate restrictions in the neural crest lineage. *Development* **124**, 4351–4359.

Lawson, S. N. & Biscoe, T. J. 1979 Development of mouse dorsal root ganglia: an autoradiographic and quantitative study. *J. Neurocytol.* **8**, 265–274.

Lawson, S. N., Caddy, K. W. T. & Biscoe, T. J. 1974 Development of rat dorsal root ganglion neurones: studies of

cell birthdays and changes in mean cell diameters. *Cell Tiss. Res.* **153**, 399–413.
Le Douarin, N. M. 1980 The ontogeny of the neural crest in avian embryo chimeras. *Nature* **286**, 663–669.
Le Douarin, N. M. 1986 Cell line segregation during peripheral nervous system ontogeny. *Science* **231**, 1515–1522.
Le Douarin, N. M. & Kalcheim, C. 1999 *The neural crest*, 2nd edn. Cambridge University Press.
Le Douarin, N., Dulac, C., Dupin, E. & Cameron-Curry, P. 1991 Glial cell lineages in the neural crest. *Glia* **4**, 175–184.
Le Lievre, C. S., Schweizer, G. G., Ziller, C. M. & Le Douarin, N. M. 1980 Restrictions in developmental capabilities in neural crest cell derivatives as tested by *in vivo* transplantation experiments. *Devl Biol.* **77**, 362–378.
Liem Jr, K. F., Tremml, G., Roelink, H. & Jessell, T. M. 1995 Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* **82**, 969–979.
Liem Jr, K. F., Tremmel, G. & Jessell, T. M. 1997 A role for the roof plate and its resident TGF-β-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* **91**, 127–138.
Lo, L.-C. & Anderson, D. J. 1995 Postmigratory neural crest cells expressing c-ret display restricted developmental and proliferative capacities. *Neuron* **15**, 527–539.
McMahon, A. P., Joyner, A. L., Bradley, A. & McMahon, J. A. 1992 The midbrain–hindbrain phenotype of *Wnt-1*$^{-}$/Wnt-1^{-} mice results from stepwise deletion of *engrailed*-expressing cells by 9.5 days postcoitum. *Cell* **69**, 581–595.
Ma, Q., Kintner, C. & Anderson, D. J. 1996 Identification of *neurogenin*, a vertebrate neuronal determination gene. *Cell* **87**, 43–52.
Ma, Q., Chen, Z. F., Barrantes, I. B., de la Pompa, J. L. & Anderson, D. J. 1998 *Neurogenin 1* is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. *Neuron* **20**, 469–482.
Ma, Q., Fode, C., Guillemot, F. & Anderson, D. J. 1999 NEUROGENIN1 and NEUROGENIN2 control two distinct waves of neurogenesis in developing dorsal root ganglia. *Genes Dev.* **13**. (In the press.)
Morrison, S. J., Uchida, N. & Weissman, I. L. 1994 The biology of hematopoietic stem cells. *A. Rev. Cell Devl Biol.* **11**, 35–71.
Morrison, S. J., Shah, N. M. & Anderson, D. J. 1997 Regulatory mechanisms in stem cell biology. *Cell* **88**, 287–298.
Morrison, S. J., White, P. M., Zock, C. & Anderson, D. J. 1999 Prospective identification, isolation by flow cytometry and *in vivo* self-renewal of multipotent mammalian neural crest stem cells. *Cell* **96**, 737–749.
Perez, S. E., Rebelo, S. & Anderson, D. J. 1999 Early specification of sensory neuron fate revealed by expression and function of neurogenins in the chick embryo. *Development* **126**, 1715–1728.
Potten, C. S. & Loeffler, M. 1990 Stem cells: attributes, cycles, spirals, pitfalls and uncertainties: lessons for and from the crypt. *Development* **110**, 1001–1020.
Reissman, E., Ernsberger, U., Francis-West, P. H., Rueger, D., Brickell, P. D. & Rohrer, H. 1996 Involvement of bone morphogenetic protein-4 and bone morphogenetic protein-7 in the differentiation of the adrenergic phenotype in developing sympathetic neurons. *Development* **122**, 2079–2088.
Serbedzija, G. N., Bronner-Fraser, M. & Fraser, S. E. 1989 A vital dye analysis of the timing and pathways of neural crest cell migration. *Development* **106**, 809–819.
Serbedzija, G. N., Fraser, S. E. & Bronner-Fraser, M. 1990 Pathways of trunk neural crest cell migration in the mouse embryo as revealed by vital dye labeling. *Development* **108**, 605–612.
Sextier-Sainte-Claire Deville, F., Ziller, C. & Le Douarin, N. M. 1994 Developmental potentials of enteric neural crest-derived cells in clonal and mass cultures. *Devl Biol.* **163**, 141–151.
Shah, N. M., Marchionni, M. A., Isaacs, I., Stroobant, P. W. & Anderson, D. J. 1994 Glial growth factor restricts mammalian neural crest stem cells to a glial fate. *Cell* **77**, 349–360.
Shah, N. M., Groves, A. & Anderson, D. J. 1996 Alternative neural crest cell fates are instructively promoted by TGF-β superfamily members. *Cell* **85**, 331–343.
Sieber-Blum, M. 1990 Mechanisms of neural crest diversification. In *Comments developmental neurobiology*, pp. 225–249. UK: Gordon and Breach.
Sieber-Blum, M., Ito, K., Richardson, M. K., Langtimm, C. J. & Duff, R. S. 1993 Distribution of pluripotent neural crest cells in the embryo and the role of brain-derived neurotrophic factor in the commitment to the primary sensory neuron lineage. *J. Neurobiol.* **24**, 173–184.
Sommer, L., Ma, Q. & Anderson, D. J. 1996 *neurogenins*, a novel family of *atonal*-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. *Mol. Cell. Neurosci.* **8**, 221–241.
Stemple, D. L. & Anderson, D. J. 1992 Isolation of a stem cell for neurons and glia from the mammalian neural crest. *Cell* **71**, 973–985.
Tanabe, Y. & Jessell, T. M. 1996 Diversity and pattern in the developing spinal cord. *Science* **274**, 1115–1123.
Weston, J. A. 1963 A radiographic analysis of the migration and localization of trunk neural crest cells in the chick. *Devl Biol.* **6**, 279–310.
Weston, J. A. & Butler, S. L. 1966 Temporal factors affecting localization of neural crest cells in the chicken embryo. *Devl Biol.* **14**, 246–266.
White, P. M. & Anderson, D. J. 1999 *In vivo* transplantation of mammalian neural crest cells into chick hosts reveals a new autonomic sublineage restriction. *Development* **126**. (In the press.)
Wolff, T., Martin, K. A., Rubin, G. M. & Zipursky, S. L. 1997 The development of the *Drosophila* visual system. In *Molecular and cellular approaches to neural development* (ed. W. M. Cowan, T. M. Jessell & S. L. Zipursky), pp. 474–508. New York: Oxford University Press.
Xue, Z. G., Smith, J. & Le Douarin, N. M. 1985 Differentiation of catecholaminergic cells in cultures of embryonic avian sensory ganglia. *Proc. Natl Acad. Sci. USA* **82**, 8800–8804.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES