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# **GRUAL<br>
CIETY**<br> **Genes, lineages and the neural crest: a speculative review**<br>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 Sensory and sympathetic neurons are generated from the trunk neural crest. The prevailing view has<br>been that these two classes of neurons are derived from a common neural crest-derived progenitor that<br>chooses between neuro Sensory and sympathetic neurons are generated from the trunk neural crest. The prevailing view has<br>been that these two classes of neurons are derived from a common neural crest-derived progenitor that<br>chooses between neuro 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 vi chooses between neuronal fates only after migrating to sites of peripheral ganglion formation. Here I<br>reconsider this view in the light of new molecular and genetic data on the differentiation of sensory and<br>autonomic neur 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 a autonomic neurons. These data raise several paradoxes when taken in the context of classical studies of<br>the timing and spatial patterning of sensory and autonomic ganglion formation. These paradoxes can be<br>most easily reso the timing and spatial patterning of sensory and autonomic ganglion formation. These paradoxe<br>most easily resolved by assuming that the restriction of neural crest cells to either sensory or au<br>lineages occurs at a very ea lineages occurs at a very early stage, either before and/or shortly after they exit the neural tube.<br>**Keywords:** neural crest; neurogenesis; cell lineage; sensory neurons; autonomic neurons

#### **1. INTRODUCTION**

1. **INTRODUCTION**<br>Lineage restriction is the process that accompanies the<br>differentiation of various cell types from a pool of multi-**EXECUTE 1. INTRODUCTION**<br>differentiation of various cell types from a pool of multi-<br>notent stem or progenitor cells (Morrison *et al.* 1997) Lineage restriction is the process that accompanies the differentiation of various cell types from a pool of multi-potent stem or progenitor cells (Morrison *et al.* 1997). Although it is distinct from morphogenesis it is differentiation of various cell types from a pool of multi-<br>potent stem or progenitor cells (Morrison *et al.* 1997).<br>Although it is distinct from morphogenesis, it is often<br>tightly counled to it. The key feature of lineag potent stem or progenitor cells (Morrison *et al.* 1997).<br>Although it is distinct from morphogenesis, it is often<br>tightly coupled to it. The key feature of lineage restriction<br>is the generation of a series of proliferatin 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 developis the generation of a series of proliferating progenitor<br>cells that exhibit gradual restrictions in their develop-<br>mental potentials and/or fates. This type of process is<br>exemplified by haematopoiesis (Morrison *et al.* 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 progenitor mental potentials and/or fates. This type of process is<br>exemplified by haematopoiesis (Morrison *et al.* 1994). It is<br>often employed in situations in which progenitors undergo<br>extensive cell migration or dispersal However, 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 alternativ often employed in situations in which progenitors undergo<br>extensive cell migration or dispersal. However, cellular<br>diversity can also be produced by alternative mechanisms;<br>for example, by generating a field of stationary extensive cell migration or dispersal. However, cellular<br>diversity can also be produced by alternative mechanisms;<br>for example, by generating a field of stationary progeni-<br>tors with different developmental specifications diversity can also be produced by alternative mechanisms;<br>for example, by generating a field of stationary progeni-<br>tors with different developmental specifications imposed<br>by morphogen gradients, as in the spinal cord (Ta for example, by generating a field of stationary progeni-<br>tors with different developmental specifications imposed<br>by morphogen gradients, as in the spinal cord (Tanabe & re<br>Jessell 1996) or by local cell-cell interactions tors with different developmental specifications imposed<br>by morphogen gradients, as in the spinal cord (Tanabe &<br>Jessell 1996), or by local cell-cell interactions as in the<br>*Drasobbila* retina (Wolff *et al*, 1997) 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). Seell 1996), or by local cell–cell interactions as in the *osophila* retina (Wolff *et al.* 1997).<br>The neural crest is similar to the haematopoietic<br>tem in that it generates a diverse array of differentiated

*Drosophila* retina (Wolff *et al.* 1997).<br>The neural crest is similar to the haematopoietic<br>system in that it generates a diverse array of differentiated<br>cell types that are widely dispersed throughout the 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 express in that it generates a diverse array of differentiated<br>cell types that are widely dispersed throughout the<br>embryo (Anderson 1989; Sieber-Blum 1990; Le Douarin *et*<br> $a^{l}$  1991). However, it is unique in that it is 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 generally 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 gener-<br>ates multiple cell types contributing to many differe al. 1991). However, it is unique in that it is the only<br>progenitor population during organogenesis that gener-<br>ates multiple cell types contributing to many different<br>(and often functionally unrelated) tissues located all progenitor population during organogenesis that generates multiple cell types contributing to many different (and often functionally unrelated) tissues located all over ates multiple cell types contributing to many different<br>(and often functionally unrelated) tissues located all over<br>the body. These tissues include the sensory, autonomic<br>and enteric ganglia of the peripheral nervous syste (and often functionally unrelated) tissues located all over<br>the body. These tissues include the sensory, autonomic<br>and enteric ganglia of the peripheral nervous system di<br>(PNS): peripheral nerve fibres: neuroendocrine tiss the body. These tissues include the sensory, autonomic<br>and enteric ganglia of the peripheral nervous system<br>(PNS); peripheral nerve fibres; neuroendocrine tissues<br>such as the medullary secretory cells of the adrenal and and enteric ganglia of the peripheral nervous system<br>(PNS); peripheral nerve fibres; neuroendocrine tissues<br>such as the medullary secretory cells of the adrenal and<br>thyroid glands: the bones of the face; the outflow tracts (PNS); peripheral nerve fibres; neuroendocrine tissues<br>such as the medullary secretory cells of the adrenal and<br>thyroid glands; the bones of the face; the outflow tracts of<br>the heart and smooth muscle walls of the great ve such as the medullary secretory cells of the adrenal and<br>thyroid glands; the bones of the face; the outflow tracts of<br>the heart and smooth muscle walls of the great vessels;<br>and melanocytes in the skin (to name just a subs thyroid glands; the bones of the face; the outflow tracts of<br>the heart and smooth muscle walls of the great vessels;<br>and melanocytes in the skin (to name just a subset) (Le<br>Douarin & Kalcheim 1999) Within some of these tis the heart and smooth muscle walls of the great vessels;<br>and melanocytes in the skin (to name just a subset) (Le<br>Douarin & Kalcheim 1999). Within some of these tissues,<br>moreover, there is further cellular diversity; for exa 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, negliated a sensory ganglia contain glial cells an

upwards of 20 different types of sensory neurons. By upwards of 20 different types of sensory neurons. By<br>contrast, stem cell populations in most other systems<br>contribute differentiated cell types only to a single tissue upwards of 20 different types of sensory neurons. By<br>contrast, stem cell populations in most other systems<br>contribute differentiated cell types only to a single tissue,<br>for example the blood intestinal enithelium or skin contrast, stem cell populations in most other systems<br>contribute differentiated cell types only to a single tissue,<br>for example, the blood, intestinal epithelium or skin<br>(Hall & Watt 1989: Potten & Loeffler 1990) The neur contribute differentiated cell types only to a single tissue,<br>for example, the blood, intestinal epithelium or skin<br>(Hall & Watt 1989; Potten & Loeffler 1990). The neural<br>crest therefore possesses an unusually high degree 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 (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 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 lineage diversification in an extreme form.<br>The diverse locations in which different crest-derived<br>tissues are found, and the broad range of cellular pheno-<br>types produced by the crest, have led to the evolution of The diverse locations in which different crest-derived<br>tissues are found, and the broad range of cellular pheno-<br>types produced by the crest, have led to the evolution of<br>at least two different strategies for generating ce tissues are found, and the broad range of cellular pheno-<br>types produced by the crest, have led to the evolution of<br>at least two different strategies for generating cellular<br>diversity in this system. First, different crest types produced by the crest, have led to the evolution of<br>at least two different strategies for generating cellular<br>diversity in this system. First, different crest derivatives<br>are often generated at different locations al at least two different strategies for generating cellular<br>diversity in this system. First, different crest derivatives<br>are often generated at different locations along the rostro-<br>caudal axis of the spinal cord. For exampl diversity in this system. First, different crest derivatives<br>are often generated at different locations along the rostro-<br>caudal axis of the spinal cord. For example, the bones of are often generated at different locations along the rostro-<br>caudal axis of the spinal cord. For example, the bones of<br>the face are generated from crest cells in the cephalic<br>region enteric and parasympathetic neurons from caudal axis of the spinal cord. For example, the bones of<br>the face are generated from crest cells in the cephalic<br>region, enteric and parasympathetic neurons from the<br>'vagal' region (the posterior rhombencephalon) and the face are generated from crest cells in the cephalic<br>region, enteric and parasympathetic neurons from the<br>'vagal' region (the posterior rhombencephalon) and<br>sympathetic neurons from the trunk region. Second region, enteric and parasympathetic neurons from the<br>'vagal' region (the posterior rhombencephalon) and<br>sympathetic neurons from the trunk region. Second,<br>different derivatives are also generated from crest cells at 'vagal' region (the posterior rhombencephalon) and<br>sympathetic neurons from the trunk region. Second,<br>different derivatives are also generated from crest cells at<br>the same axial level: for example sensory and sympasympathetic 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 different derivatives are also generated from crest cells at the same axial level; for example, sensory and sympathetic neurons, adrenal medullary chromaffin cells, gliand melanocytes are all generated from the thoraco-<br>lumbar regions of the trunk crest (Le Douarin 1980) thetic neurons, adrenal medullary chromaffin cells, g<br>and melanocytes are all generated from the thora<br>lumbar regions of the trunk crest (Le Douarin 1980).<br>These two strategies pose distinct but related develd melanocytes are all generated from the thoracombar regions of the trunk crest (Le Douarin 1980).<br>These two strategies pose distinct but related develop-<br>ental problems. For example, the first (or 'positional

Douarin & Kalcheim 1999). Within some of these tissues, ments employing the chick-quail chimera system. These<br>moreover, there is further cellular diversity; for example, highly informative experiments have indicated that, lumbar regions of the trunk crest (Le Douarin 1980).<br>These two strategies pose distinct but related develop-<br>mental problems. For example, the first (or 'positional These two strategies pose distinct but related develop-<br>mental problems. For example, the first (or 'positional<br>diversification') strategy raises the question of whether<br>neural crest cells at different axial levels are int mental problems. For example, the first (or 'positional<br>diversification') strategy raises the question of whether<br>neural crest cells at different axial levels are intrinsically<br>different in their developmental capacities a diversification') strategy raises the question of whether<br>neural crest cells at different axial levels are intrinsically<br>different in their developmental capacities at the time of<br>emigration from the neural tube or 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 different in their developmental capacities at the time of<br>emigration from the neural tube, or whether they are<br>equivalent but acquire different fates as a consequence of<br>encountering different environments as they migrate emigration from the neural tube, or whether they are<br>equivalent but acquire different fates as a consequence of<br>encountering different environments as they migrate.<br>This question has been addressed by Le Douarin and coequivalent but acquire different fates as a consequence of encountering different environments as they migrate.<br>This question has been addressed by Le Douarin and co-workers using elegant heterotopic transplantation experi encountering different environments as they migrate.<br>This question has been addressed by Le Douarin and co-<br>workers using elegant heterotopic transplantation experi-<br>ments employing the chick-quail chimera system. These This question has been addressed by Le Douarin and co-<br>workers using elegant heterotopic transplantation experi-<br>ments employing the chick–quail chimera system. These<br>highly informative experiments have indicated that, to workers using elegant heterotopic transplantation experiments employing the chick–quail chimera system. These<br>highly informative experiments have indicated that, to a<br>first approximation, axial differences in crest cell fa first approximation, axial differences in crest cell fate are



Figure 1. Possible patterns of segregation of neurogenic and<br>gliogenic lineages in the trunk neural crest. For simplicity,<br>the relationship of the melanocyte lineage to the neuronal Figure 1. Possible patterns of segregation of neurogenic and<br>gliogenic lineages in the trunk neural crest. For simplicity,<br>the relationship of the melanocyte lineage to the neuronal<br>and glial lineages has been omitted, and gliogenic lineages in the trunk neural crest. For simplicity, the relationship of the melanocyte lineage to the neuronal  $\mathbf S$ the relationship of the melanocyte lineage to the and glial lineages has been omitted, and a sing<br>trunk-derived glial cell ( $^{\circ}$ G') is assumed. N<sub>S</sub>, so<br>N<sub>a</sub> autonomic neuron (a) A multipotent cres and glial lineages has been omitted, and a single type of<br>trunk-derived glial cell ('G') is assumed.  $N_S$ , sensory neuron;<br> $N_A$ , autonomic neuron. (*a*) A multipotent crest cell directly<br>generates sensory and autonomic ne trunk-derived glial cell ('G') is assumed. N<sub>S</sub>, sensory neuron;<br>N<sub>A</sub>, autonomic neuron. (a) A multipotent crest cell directly generates sensory and autonomic neurons and glia without generates sensory and autonomic neurons and glia without<br>producing partially restricted intermediates. The choice may<br>be made stochastically, with various probabilities assigned to<br>different lineages: in this case equal p producing partially restricted intermediates. The choice may<br>be made stochastically, with various probabilities assigned to<br>different lineages; in this case equal probabilities ( $p = 0.33$ )<br>have been assigned arbitrarily f producing partially restricted intermediates. The choice may ŏ be made stochastically, with various probabilities assigned t<br>different lineages; in this case equal probabilities ( $p = 0.33$ )<br>have been assigned arbitrarily for purposes of illustration.<br>Alternatively (or in addition), t different lineages; in this case equal probabilities ( $p = 0.33$ )<br>have been assigned arbitrarily for purposes of illustration.<br>Alternatively (or in addition), the choice may be dictated by<br>different instructive signals for have been assigned arbitrarily for purposes of illustrati<br>Alternatively (or in addition), the choice may be dicta<br>different instructive signals for the different lineages<br>(L. L. and L.) (b d) The three lineages are produc ernatively (or in addition), the choice may be dictated by<br>ferent instructive signals for the different lineages<br>,  $I_G$  and  $I_A$ ). (*b-d*) The three lineages are produced via a  $(I<sub>S</sub>, I<sub>G</sub>$  and  $I<sub>A</sub>$ ). (*b-d*) The three lineages are produced via a

environmentally rather than intrinsically determined<br>(although there are a few exceptions) (Le Douarin 1980) environmentally rather than intrinsically determined<br>(although there are a few exceptions) (Le Douarin 1980).<br>However, the heterotopic transplantation of neural tube environmentally rather than intrinsically determined<br>(although there are a few exceptions) (Le Douarin 1980).<br>However, the heterotopic transplantation of neural tube<br>fragments cannot by definition be used to address the (although there are a few exceptions) (Le Douarin 1980).<br>However, the heterotopic transplantation of neural tube<br>fragments cannot, by definition, be used to address the<br>second of the two strategies that is the one in which However, the heterotopic transplantation of neural tube<br>fragments cannot, by definition, be used to address the<br>second of the two strategies, that is the one in which<br>different derivatives are generated from a common axial 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 second of the two strategies, that is the one in which<br>different derivatives are generated from a common axial<br>level of the trunk neural crest. That is the problem on<br>which I will focus the remainder of this discussion 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** ROBLEM OF TRUNK NEURAL CI<br>LINEAGE DIVERSIFICATION

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

Since the late 1980s, many investigators in the field produced and do they act selectively or instructively?<br>Since the late 1980s, many investigators in the field<br>(including myself) have favoured the idea that neural<br>crest development proceeds in a manner analogous to 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 (including myself) have favoured the idea that neural<br>crest development proceeds in a manner analogous to<br>that of haematopoiesis, via the generation of progressively<br>restricted intermediates (Anderson 1989: Sieber-Blum crest development proceeds in a manner analogous to<br>that of haematopoiesis, via the generation of progressively<br>restricted intermediates (Anderson 1989; Sieber-Blum<br>1990: Le Douarin et al. 1991). According to this view that of haematopoiesis, via the generation of progressively<br>restricted intermediates (Anderson 1989; Sieber-Blum<br>1990; Le Douarin *et al.* 1991). According to this view<br>many if not all neural crest cells exit the neural tu 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 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-Fra many, if not all, neural crest cells exit the neural tube<br>with the full range of trunk crest potentials (Bronner-<br>Fraser & Fraser 1988, 1989; Fraser & Bronner-Fraser<br>1991) and undergo partial restrictions in these potentia 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-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* 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). Parti-cular patterns of lineage restrictio 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 reproducibl 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). cular patterns of lineage restriction have even been<br>suggested, in which certain fates reproducibly co-segregate<br>from others (Le Douarin *et al.* 1991).<br>There are several aspects of this model, however, that suggested, in which certain fates reproducibly co-segregate

from others (Le Douarin *et al.* 1991).<br>There are several aspects of this model, however, that<br>require critical re-examination, particularly in the light<br>of new data. First, patterns of lineage restriction deduced There are several aspects of this model, however, that<br>require critical re-examination, particularly in the light<br>of new data. First, patterns of lineage restriction deduced<br>by analysing clone compositions in vitro (Le Do require critical re-examination, particularly in the light<br>of new data. First, patterns of lineage restriction deduced<br>by analysing clone compositions *in vitro* (Le Douarin *et al.*) by analysing clone compositions *in vitro* (Le Douarin *et al.*)<br>Figure 1 *(Cont.)* deterministic generation of partially

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restricted intermediates. (*b*) Neuronal and glial lineages segre-Figure 1 (*Cont.*) deterministic generation of partially<br>restricted intermediates. (*b*) Neuronal and glial lineages seg<br>gate before sensory and autonomic lineages.  $P_N$ , neuronal<br>precursor:  $P_N$ , alial precursor (*c*) Se restricted intermediates. (*b*) Neuronal and glial lineages segr<br>gate before sensory and autonomic lineages.  $P_N$ , neuronal<br>precursor;  $P_G$ , glial precursor. (*c*) Sensory and autonomic<br>lineages segregate before neuronal gate before sensory and autonomic lineages.  $P_N$ , neuronal<br>precursor;  $P_G$ , glial precursor. (*c*) Sensory and autonomic<br>lineages segregate before neuronal and glial lineages.  $P_A$ , auto-<br>pomic neuroglial precursor:  $P_A$  precursor;  $P_G$ , glial precursor. (*c*) Sensory and autonomic<br>lineages segregate before neuronal and glial lineages.  $P_A$ , auto-<br>nomic neuroglial precursor;  $P_S$ , sensory neuroglial precursor.<br>(*d*) All glia derive from a lineages segregate before neuronal and glial lineages.  $P_A$ , autonomic neuroglial precursor;  $P_S$ , sensory neuroglial precursor.<br>(*d*) All glia derive from an autonomic-restricted multipotent nomic neuroglial precursor;  $P_s$ , sensory neuroglial precursor.<br>
(*d*) All glia derive from an autonomic-restricted multipotent<br>
precursor ( $P_{AG}$ ), and sensory precursors ( $P_s$ ) are restricted to<br>
a neuronal fate. Other (*d*) All glia derive from an autonomic-restricted multipotent<br>precursor ( $P_{AG}$ ), and sensory precursors ( $P_S$ ) are restricted to<br>a neuronal fate. Other patterns of segregation are possible and<br>are not illustrated precursor  $(P_{AG})$ , and<br>a neuronal fate. Oth<br>are not illustrated.



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Figure 2. Single-cell and cell-population fate mapping of the<br>neural crest (a) Many single pre-migratory crest cells injected Figure 2. Single-cell and cell-population fate mapping of the<br>neural crest. (*a*) Many single pre-migratory crest cells injected<br>intracellularly with the lineage tracer lysinated rhodamine Figure 2. Single-cell and cell-population fate mapping of the<br>neural crest. (a) Many single pre-migratory crest cells injected<br>intracellularly with the lineage tracer lysinated rhodamine<br>dextran produce progeny in all str neural crest. (a) Many single pre-migratory crest cells injected<br>intracellularly with the lineage tracer lysinated rhodamine<br>dextran produce progeny in all structures derived from the<br>trunk neural crest (Bronner-Fraser & F intracellularly with the lineage tracer lysinated rhodamine<br>dextran produce progeny in all structures derived from the<br>trunk neural crest (Bronner-Fraser & Fraser 1991). Mel, dextran produce progeny in all structures derived from the<br>trunk neural crest (Bronner-Fraser & Fraser 1991). Mel,<br>melanocytes; N<sub>S</sub>, sensory neuron; G<sub>S</sub>, sensory glia; G, generic<br>peripheral glia (e g, Schwann cells): N., trunk neural crest (Bronner-Fraser & Fraser 1991). Mel,<br>melanocytes; N<sub>S</sub>, sensory neuron; G<sub>S</sub>, sensory glia; G, generi<br>peripheral glia (e.g. Schwann cells); N<sub>A</sub>, autonomic (sympa-<br>thetic) neuron: G, autonomic glia. The melanocytes; N<sub>S</sub>, sensory neuron; G<sub>S</sub>, sensory glia; G, generic<br>peripheral glia (e.g. Schwann cells); N<sub>A</sub>, autonomic (sympa-<br>thetic) neuron; G<sub>A</sub>, autonomic glia. The green oval represents<br>sensory (dorsal root) ganglio 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 (sympathetic) ganglion. da, dorsal aorta.  $(b)$  Labelling of a population of pre-migratory crest cells within the neural tube<br>by injection of the lipophilic dye DiI results in sequential colo<br>nization of the sympathetic ganglia (SG), peripheral nerve<br>(PN), dorsal root ganglion and epi by injection of the lipophilic dye DiI results in sequential coloby 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<br>(PN), dorsal root ganglion and epiderm mization 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 gen (PN), dorsal root ganglion and epidermis (EPI) (Serbedzija *et al.* 1989, 1990). The results are not inconsistent because the  $\sim$  $1991$ ) are based on the assumption that if different<br>1991) are based on the assumption that if different end-point.

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

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

cell–cell interactions in a colony may affect the outcome<br>of differentiation events. Furthermore, most such studies cell–cell interactions in a colony may affect the outcome<br>of differentiation events. Furthermore, most such studies<br>evaluate colony composition at a single, arbitrary timeof differentiation events. Furthermore, most such studies evaluate colony composition at a single, arbitrary timeof differentiation events. Furthermore, most such studies<br>evaluate colony composition at a single, arbitrary time-<br>point; simple interclonal differences in the kinetics of<br>differentiation of a given cell type could vield a evaluate colony composition at a single, arbitrary time-<br>point; simple interclonal differences in the kinetics of<br>differentiation of a given cell type could yield apparent<br>differences in colony composition at the time of a point; simple interclonal differences in the kinetics of<br>differentiation of a given cell type could yield apparent<br>differences in colony composition at the time of analysis,<br>and therefore lead to false conclusions about fo 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 o differences in colony composition at the time of analysis,<br>and therefore lead to false conclusions about founder cell<br>lineage restrictions. The only rigorous test of develop-<br>mental restriction is to challenge cells with i and therefore lead to false conclusions about founder cell<br>lineage restrictions. The only rigorous test of develop-<br>mental restriction is to challenge cells with instructive<br>signals that promote various crest fates (Shah 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 resis mental restriction is to challenge cells with instructive<br>signals that promote various crest fates (Shah *et al.* 1994,<br>1996) and determine whether the cells are resistant to the<br>effects of such signals (Lo & Anderson 199 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 ident 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 nattern and sequence (if a effects of such signals (Lo & Anderson 1995). Unfortunately, such instructive signals have been identified only<br>recently. Therefore the pattern and sequence (if any) of<br>lineage restrictions particularly in trunk neural cre nately, such instructive signals have been identified only<br>recently. Therefore the pattern and sequence (if any) of<br>lineage restrictions, particularly in trunk neural crest,<br>remains an onen question recently. Therefore the patt<br>lineage restrictions, particu<br>remains an open question.<br>Second the assumption eage restrictions, particularly in trunk neural crest,<br>mains an open question.<br>Second, the assumption that most or all neural crest<br>lls are multipotent and developmentally equivalent at

remains an open question.<br>Second, the assumption that most or all neural crest<br>cells are multipotent and developmentally equivalent at<br>the time they exit the neural tube has been called into Second, the assumption that most or all neural crest<br>cells are multipotent and developmentally equivalent at<br>the time they exit the neural tube has been called into<br>question by single-cell lineage analyses performed on cells are multipotent and developmentally equivalent at<br>the time they exit the neural tube has been called into<br>question by single-cell lineage analyses performed on<br>crest explants in vitm (Henion & Weston 1997) These the time they exit the neural tube has been called into<br>question by single-cell lineage analyses performed on<br>crest explants *in vitro* (Henion & Weston 1997). These<br>studies have revealed evidence of ranid fate restriction crest explants in vitro (Henion & Weston 1997). These would send a negative-feedback signal to equivalent studies have revealed evidence of rapid fate restrictions in precursors to inhibit their sensory differentiation and crest cells very early in emigration. Although restrictions studies have revealed evidence of rapid fate restrictions in<br>crest cells very early in emigration. Although restrictions<br>in fate do not imply restrictions in potential, they<br>certainly raise the possibility that not all cre crest cells very early in emigration. Although restrictions<br>in fate do not imply restrictions in potential, they<br>certainly raise the possibility that not all crest cells are<br>initially equivalent Indeed as described in more 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  $8.6$  such fate restrictions were also observe certainly raise the possibility that not all crest cells are<br>initially equivalent. Indeed, as described in more detail in<br> $\delta$ 6, such fate restrictions were also observed in analogous §6, such fate restrictions were also observed in analogous this is not observed. Rather they have been reported to be<br>lineage studies performed *in vivo* (Bronner-Fraser & colonized sequentially; trunk neural crest cells p  $\S 6$ , such fate restrictions were also observed in analogous<br>lineage studies performed *in vivo* (Bronner-Fraser &<br>Fraser 1991); however, for legitimate reasons the multipo-<br>tency of some neural crest cells was the resul lineage studies performed *in vivo* (Bronner-Fraser & Fraser 1991); however, for legitimate reasons the multipotency of some neural crest cells was the result emphasized.<br>Therefore the question of whether partial restricti Fraser 1991); however, for legitimate reasons the multipotency of some neural crest cells was the result emphasized.<br>Therefore, the question of whether partial restrictions in crest cell notentials occur before or after em tency of some neural crest cells was the result emphasized. gate<br>Therefore, the question of whether partial restrictions in<br>crest cell potentials occur before or after emigration from (S<br>the neural tube remains open. As d Therefore, the question of whether partial restrictions in<br>crest cell potentials occur before or after emigration from<br>the neural tube remains open. As discussed in  $\S 7$ , there<br>are reasons why an early segregation of som crest cell potentials occur before or after emigration from<br>the neural tube remains open. As discussed in  $\S$ 7, there<br>are reasons why an early segregation of some trunk crest the neural tube remains open. As discussed in  $\S$ 7, there are reasons why an early segregation of some trunk crest<br>lineages would be an attractive way to resolve certain<br>paradoxes posed by the assumption of multipotency are reasons why an early segregation of some trunk  $\alpha$  lineages would be an attractive way to resolve cerparadoxes posed by the assumption of multipotency. paradoxes posed by the assumption of multipotency.<br>**3. THE PARADOX OF THE** 

# **SENSORY±AUTONOMIC DECISION**

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

problem of neural crest lineage restriction.<br>
In the trunk region of the neural crest, the major type<br>
of autonomic neuron generated is the sympathetic<br>
neuron: as mentioned in  $\delta$  l narasymmathetic and enteric In the trunk region of the neural crest, the major type<br>of autonomic neuron generated is the sympathetic<br>neuron; as mentioned in  $\S 1$ , parasympathetic and enteric<br>autonomic neurons are predominantly generated more of autonomic neuron generated is the sympathetic<br>neuron; as mentioned in  $\S$  l, parasympathetic and enteric<br>autonomic neurons are predominantly generated more<br>anteriorly from the vagal region of the neural crest as neuron; as mentioned in  $\S$  l, parasympathetic and enteric<br>autonomic neurons are predominantly generated more<br>anteriorly, from the vagal region of the neural crest, as<br>well as posteriorly from the saccral crest (Le Douari autonomic neurons are predominantly generated more<br>anteriorly, from the vagal region of the neural crest, as<br>well as posteriorly from the saccral crest (Le Douarin<br>1980) Sympathetic neurons lie in a metameric chain of anteriorly, from the vagal region of the neural crest, as<br>well as posteriorly from the saccral crest (Le Douarin<br>1980). Sympathetic neurons lie in a metameric chain of 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 1980). Sympathetic neurons lie in a metameric chain of ganglia located adjacent to the dorsal aorta, while<br>sensory neurons lie more dorsally in a metameric chain<br>located adjacent to the spinal cord. Neural crest cells that ganglia located adjacent to the dorsal aorta, while<br>sensory neurons lie more dorsally in a metameric chain<br>located adjacent to the spinal cord. Neural crest cells that<br>generate sympathetic neurons therefore have to migrate 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 gener-<br>ated before they reach their destination (figure 2) through the region in which sensory neurons are<br>ated before they reach their destination (figure 2).<br>This geometry poses a paradox: if neural cres rough the region in which sensory neurons are gener-<br>ed before they reach their destination (figure 2).<br>This geometry poses a paradox; if neural crest cells<br>laminate from the dorsal neural tube as a uniform

ated before they reach their destination (figure 2).<br>This geometry poses a paradox; if neural crest cells<br>delaminate from the dorsal neural tube as a uniform<br>population with both sensory and sympathetic potential This geometry poses a paradox; if neural crest cells<br>delaminate from the dorsal neural tube as a uniform<br>population with both sensory and sympathetic potential<br>(figure 2*a*) what prevents all of these cells from differendelaminate from the dorsal neural tube as a uniform population with both sensory and sympathetic potential (figure  $2a$ ), what prevents all of these cells from differenpopulation with both sensory and sympathetic potential<br>(figure 2a), what prevents all of these cells from differen-<br>tiating to sensory neurons before they have a chance to<br>migrate more ventrally to the sympathetic anlagen? (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 stochas tiating to sensory neurons before they have a chance to<br>migrate more ventrally to the sympathetic anlagen? One<br>possible solution to this problem invokes stochastic (figure 3*a*)<br>or negative-feedback-based mechanisms (figur migrate more ventrally to the sympathetic anlagen? One<br>possible solution to this problem invokes stochastic (figure 3*a*)<br>or negative-feedback-based mechanisms (figure 3*b*) to<br>allow some multinotent sensory–autonomic prog possible solution to this problem invokes stochastic (figure  $3a$ )<br>or negative-feedback-based mechanisms (figure  $3b$ ) to<br>allow some multipotent sensory-autonomic progenitors to<br>escape sensory-inducing signals in the dors allow some multipotent sensory-autonomic progenitors to escape sensory-inducing signals in the dorsal neural tube allow some multipotent sensory–autonomic progenitors to<br>escape sensory-inducing signals in the dorsal neural tube<br>environment, so that they can continue migrating<br>ventrally to the sympathetic anlagen. In the first case escape sensory-inducing signals in the dorsal neural tube<br>environment, so that they can continue migrating<br>ventrally to the sympathetic anlagen. In the first case,<br>multinotent crest cells would have a certain probability environment, so that they can continue migrating<br>ventrally to the sympathetic anlagen. In the first case,<br>multipotent crest cells would have a certain probability,<br> $h < 10$  of generating sensory neurons in the dorsal root 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 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 migra  $p < 1.0$ , 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 symmethetic primordia (figure  $3q$ ). In the seco 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 the sympathetic primordia (figure 3*a*). In the second case,<br>sensory neurons generated from multipotent precursors<br>would send a negative-feedback signal to equivalent<br>precursors to inhibit their sensory differentiation and sensory neurons generated from multipotent precursors<br>would send a negative-feedback signal to equivalent<br>precursors to inhibit their sensory differentiation and<br>allow them to migrate to the sympathetic anlagen allow them to migrate to the sympathetic anlagen. ecursors to inhibit their sensory differentiation and<br>ow them to migrate to the sympathetic anlagen.<br>The problem with these models is that the first<br>echanism would predict that sensory and sympathetic

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

# **4. THE SEQUENTIAL COLONIZATION OF**<br>SYMPATHETIC AND SENSORY GANGLIA: DIFFERENT<br>CELLS DIFFERENT SICNALS OF BOTH? 4. THE SEQUENTIAL COLONIZATION OF<br>ATHETIC AND SENSORY GANGLIA: DIFFEREI<br>CELLS, DIFFERENT SIGNALS, OR BOTH?

MERTHETIC AND SENSORT GANGLIA. DIFFERENT<br>CELLS, DIFFERENT SIGNALS, OR BOTH?<br>Models to explain the sequential colonization of sympa-<br>etic and sensory ganglia by trunk neural crest cells fall ELLS, DIFFERENT SIGNALS, OR BOTH?<br>Models to explain the sequential colonization of sympa-<br>thetic and sensory ganglia by trunk neural crest cells fall<br>into two basic categories: either early- and late-Models to explain the sequential colonization of sympathetic and sensory ganglia by trunk neural crest cells fall<br>into two basic categories: either early- and late-<br>emigrating trunk neural crest cells are multinotent and thetic and sensory ganglia by trunk neural crest cells fall<br>into two basic categories: either early- and late-<br>emigrating trunk neural crest cells are multipotent and<br>developmentally equivalent and their environment into two basic categories: either early- and late-<br>emigrating trunk neural crest cells are multipotent and<br>developmentally equivalent and their environment<br>changes with time (e.g. figure 4.g.c; red circles); or else the emigrating trunk neural crest cells are multipotent and<br>developmentally equivalent and their environment<br>changes with time (e.g. figure  $4a,c$ ; red circles); or else the<br>cells are intrinsically different (figure  $4b\,d$ ; m developmentally equivalent and their environment<br>changes with time (e.g. figure 4*a*,*c*; red circles); or else the<br>cells are intrinsically different (figure 4*b*,*d*; magenta and changes with time (e.g. figure  $4a,c$ ; red circles); or else the cells are intrinsically different (figure  $4b,d$ ; magenta and green circles). In the first category, early-emigrating crest cells fated to generate symmatheti cells are intrinsically different (figure  $4b$ , $d$ ; 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 se green circles). In the first category, early-emigrating crest<br>cells fated to generate sympathetic neurons could emerge<br>from the neural tube at a time when sensory neuron-<br>inducing signals (figure 4*a*; green stippling) wer cells fated to generate sympathetic neurons could emerge<br>from the neural tube at a time when sensory neuron-<br>inducing signals (figure 4*a*; green stippling) were not yet<br>present but sympathetic neuron-inducing signals from the neural tube at a time when sensory neuron-<br>inducing signals (figure 4*a*; green stippling) were not yet<br>present but sympathetic neuron-inducing signals<br>(figure 4*a*: blue stippling) were available. Therefore ther  $\begin{array}{ll}\n\text{inducing signals (figure 4a; green stippling) were not yet}\n\text{present} & \text{sympathetic} & \text{neuron-inducing} & \text{signals}\n\text{(figure 4a; blue stippling) were available. Therefore there}\n\text{would be no instructive signals to divert these cells from}\n\end{array}$ 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

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Figure 4. Models to explain the sequential colonization of first sympathetic and then sensory ganglia by trunk neural crest<br>cells. (*a*,*c*) Most or all migratory crest cells are equipotent and equicompetent for sensory an Figure 4. Models to explain the sequential colonization of first sympathetic and then sensory ganglia by trunk neural crest<br>cells.  $(a,c)$  Most or all migratory crest cells are equipotent and equicompetent for sensory and a 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  $(\Delta t)$ . Early-emigrating inducing signals encountered by the cells change as a function of time  $(\Delta t)$ . Early-emigrating cells (left) encounter autonomic<br>(blue stippling) but not sensory-inducing signals; late-emigrating cells (right) encounter s (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 t to the cells change as a function of time; the early VL pathway (Serbedzija *et al.* 1990) avoids sensory-inducing signals while the latter VM pathway encounters them. Note that this mechanism could operate only in mouse a to the cells change as a function of time; the early VL pathway (Serbedzija *et al.* 1990) avoids sensory-inducing signals while<br>the late VM pathway encounters them. Note that this mechanism could operate only in mouse and 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). 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 neural tube. In  $(b)$  the two types of progenitors are generated sequentially, although there may be a period of overlap (not illuthe sensory or sympathetic ganglia (Serbedzija *et al.* 1990); for simplicity the two progenitor types are shown concurrently.

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neural tube and towards the dorsal aorta (figure 4*a*).<br>Subsequent waves of crest cells would then emigrate at a neural tube and towards the dorsal aorta (figure 4*a*).<br>Subsequent waves of crest cells would then emigrate at a<br>time when sensory-inducing signals were present (figure neural tube and towards the dorsal aorta (figure 4*a*).<br>Subsequent waves of crest cells would then emigrate at a<br>time when sensory-inducing signals were present (figure<br> $4a$ : right). Another version of such a model is one Subsequent waves of crest cells would then emigrate at a different, subectodermal migration pathway than earlier<br>time when sensory-inducing signals were present (figure VM-migrating cells (Erickson & Goins 1995). Likewise, time when sensory-inducing signals were present (figure  $4a$ ; right). Another version of such a model is one in which early-emigrating crest cells could have available to them only the ventrolateral  $(VI)$  migration pathwa 4a; right). Another version of such a model is one in<br>which early-emigrating crest cells could have available to<br>them only the ventrolateral (VL) migration pathway<br>(figure 4c left) which might avoid short-range sensorywhich early-emigrating crest cells could have available to<br>them only the ventrolateral (VL) migration pathway<br>(figure 4*c*, left), which might avoid short-range sensory-<br>inducing signals (shown by green stippling); the oth them only the ventrolateral (VL) migration pathway (figure  $4\epsilon$ , left), which might avoid short-range sensory-<br>inducing signals (shown by green stippling); the other,<br>ventromedial (VM) migration pathway to the sensory (figure  $4c$ , left), which might avoid short-range sensoryinducing signals (shown by green stippling); the other,<br>ventromedial (VM) migration pathway to the sensory<br>primordia (figure  $4c$ , right) would then become available<br>at later times. These types of explanations have tended ventromedial (VM) migration pathway to the sensory<br>primordia (figure  $4c$ , right) would then become available<br>at later times. These types of explanations have tended to<br>be favoured in the literature (Weston  $\&$  Butler 19 primordia (figure 4c, right) would then become available<br>at later times. These types of explanations have tended to<br>be favoured in the literature (Weston & Butler 1966;<br>Serbedzija et al 1989-1990) at later times. These types of<br>be favoured in the literatur<br>Serbedzija *et al.* 1989, 1990).<br>It is also possible howe favoured in the literature (Weston & Butler 1966; rbedzija *et al.* 1989, 1990).<br>It is also possible, however, that early- and late-<br>pigrating crest cells are intrinsically different. Indeed

Serbedzija *et al.* 1989, 1990).<br>It is also possible, however, that early- and late-<br>emigrating crest cells are intrinsically different. Indeed,<br>there is evidence that the latest-emigrating trunk neural It is also possible, however, that early- and late-<br>emigrating crest cells are intrinsically different. Indeed,<br>there is evidence that the latest-emigrating trunk neural<br>crest cells, which are fated to generate melanocytes emigrating crest cells are intrinsically different. Indeed,<br>there is evidence that the latest-emigrating trunk neural<br>crest cells, which are fated to generate melanocytes, are<br>restricted in comparison with earlier-emigrati there is evidence that the latest-emigrating trunk neural<br>crest cells, which are fated to generate melanocytes, are<br>restricted in comparison with earlier-emigrating cells<br>(Artinger & Bronner-Fraser 1992) This restriction h crest cells, which are fated to generate melanocytes, are<br>restricted in comparison with earlier-emigrating cells<br>(Artinger & Bronner-Fraser 1992). This restriction has (Artinger & Bronner-Fraser 1992). This restriction has *Phil. Trans. R. Soc. Lond.* B (2000)

been invoked to explain how these cells choose a<br>different subectodermal migration pathway than earlier been invoked to explain how these cells choose a<br>different, subectodermal migration pathway than earlier<br>VM-migrating cells (Erickson & Goins 1995) Likewise 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 VM-migrating cells (Erickson & Goins 1995). Likewise, VM-migrating cells (Erickson & Goins 1995). Likewise,<br>the fact that trunk crest cells in the mouse take different<br>migratory pathways to the sympathetic and sensory<br>ganglia could indicate that the cells have to be different the fact that trunk crest cells in the mouse take different<br>migratory pathways to the sympathetic and sensory<br>ganglia could indicate that the cells have to be different<br>in order to recognize these distinct migration routes migratory pathways to the sympathetic and sensory<br>ganglia could indicate that the cells have to be different<br>in order to recognize these distinct migration routes<br> $(\text{four } 4d)$  Eurthermore, even in the chick where the ganglia could indicate that the cells have to be different<br>in order to recognize these distinct migration routes<br>(figure 4*d*). Furthermore, even in the chick where the<br>migration pathways to the DRG and sympathetic ganglia in order to recognize these distinct migration routes<br>(figure  $4d$ ). Furthermore, even in the chick where the<br>migration pathways to the DRG and sympathetic ganglia<br>overlap the sequential generation of sympathetic and (figure  $4d$ ). 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 evoluated by the migration pathways to the DRG and sympathetic ganglia<br>overlap, the sequential generation of sympathetic and<br>then sensory derivatives could also be explained by the<br>sequential emergence of sympathetic-restricted (figure 4b: overlap, the sequential generation of sympathetic and<br>then sensory derivatives could also be explained by the<br>sequential emergence of sympathetic-restricted (figure 4*b*;<br>magenta circle) and then sensory-restricted (figure then sensory derivatives could also be explained by the<br>sequential emergence of sympathetic-restricted (figure 4*b*;<br>magenta circle) and then sensory-restricted (figure 4*b*;<br>green circle) crest cells. Such explanations ha sequential emergence of sympathetic-restricted (figure 4b;<br>magenta circle) and then sensory-restricted (figure 4b;<br>green circle) crest cells. Such explanations have tended to<br>be discounted in the literature, but a closer i magenta circle) and then sensory-restricted (figure  $4b$ ; green circle) crest cells. Such explanations have tended to be discounted in the literature, but a closer inspection of green circle) crest cells. Such explanations have tended to<br>be discounted in the literature, but a closer inspection of<br>this evidence reveals that it does not rigorously exclude<br>the possibility that many trunk crest cells be discounted in the literature, but a closer inspection of<br>this evidence reveals that it does not rigorously exclude<br>the possibility that many trunk crest cells are heteroge-<br>neous with respect to sensory and sympathetic this evidence reveals that it does not rigorously exclude<br>the possibility that many trunk crest cells are heteroge-<br>neous with respect to sensory and sympathetic potential<br>early in migration 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 S. IN VIVO TRANSPLANTATION AND IN VITRO<br>CULTURE EXPERIMENTS SUPPORTING A LATE<br>SEGREGATION OF SYMPATHETIC AND SENSORY LE EXPERIMENTS SUPPORTING A L.<br>ATION OF SYMPATHETIC AND SENS<br>LINEAGES: A RE-EXAMINATION LINEAGES: A RE-EXAMINATION**<br>*In vivo* heterochronic transplantation has provided one

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line of evidence that it is the environment of the crest cells In vivo heterochronic transplantation has provided one<br>line of evidence that it is the environment of the crest cells<br>that is changing with time, rather than the crest cells<br>themselves Weston & Butler (1966) using  $[^{3}H]$ line of evidence that it is the environment of the crest cells<br>that is changing with time, rather than the crest cells<br>themselves. Weston & Butler (1966), using  $[^{3}H]$ thymidine<br>to pulse label neural crest cells, and tak themselves. Weston & Butler (1966), using  $[^{3}H]$ thymidine<br>to pulse label neural crest cells, and taking advantage of themselves. Weston & Butler (1966), using [<sup>3</sup>H]thymidine<br>to pulse label neural crest cells, and taking advantage of<br>the fact that the migration and differentiation of crest cells<br>occurs in a rostral-to-caudal wave transpl to pulse label neural crest cells, and taking advantage of<br>the fact that the migration and differentiation of crest cells<br>occurs in a rostral-to-caudal wave, transplanted neural<br>tube fragments from early (posterior) region the fact that the migration and differentiation of crest cells<br>occurs in a rostral-to-caudal wave, transplanted neural<br>tube fragments from early (posterior) regions where<br>sympathetic neurons were being generated to late (m occurs in a rostral-to-caudal wave, transplanted neural<br>tube fragments from early (posterior) regions where<br>sympathetic neurons were being generated to late (more<br>anterior) regions where sensory neurons were forming sympathetic neurons were being generated to late (more<br> **Theory in anterior)** regions where sensory neurons were forming,<br> **H** and vice versa. They found that the neural tube fragments anterior) regions where sensory neurons were forming, anterior) regions where sensory neurons were forming,<br>and vice versa. They found that the neural tube fragments<br>could generate crest cells that populated the type of<br>ganglia appropriate for their site of transplantation in and vice versa. They found that the neural tube fragments<br>could generate crest cells that populated the type of<br>ganglia appropriate for their site of transplantation in the<br>host and concluded that the crest cells were the could generate crest cells that populated the type of<br>ganglia appropriate for their site of transplantation in the<br>host, and concluded that the crest cells were the same but<br>their environment was different at different tim ganglia appropriate for their site of transplantation<br>host, and concluded that the crest cells were the sai<br>their environment was different at different times.<br>This is not the only interpretation of these host, and concluded that the crest cells were the same but<br>their environment was different at different times.<br>This is not the only interpretation of these data,

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

(Le Lievre *et al.* 1980; Xue *et al.* 1985).<br>A more recent heterochronic transplantation study of<br>trunk neural tube has, to the contrary, provided evidence<br>that there are axial or temporal differences in the propen-A more recent heterochronic transplantation study of A more recent heterochronic transplantation study of<br>trunk neural tube has, to the contrary, provided evidence<br>that there are axial or temporal differences in the propen-<br>sity of trunk neural crest cells to generate sympat trunk neural tube has, to the contrary, provided evidence<br>that there are axial or temporal differences in the propen-<br>sity of trunk neural crest cells to generate sympathetic<br>versus sensory neurons (Asamoto *et al.* 1995) 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 senarate precursors sity of trunk neural crest cells to generate sympathetic<br>versus sensory neurons (Asamoto *et al.* 1995). This result is<br>most easily explained by separate precursors for sensory<br>and sympathetic neurons existing in different versus sensory neurons (Asamoto *et al.* 1995). This result is<br>most easily explained by separate precursors for sensory<br>and sympathetic neurons existing in different proportions<br>along the trunk rostrocaudal axis. However, most easily explained by separate precursors for sensory<br>and sympathetic neurons existing in different proportions<br>along the trunk rostrocaudal axis. However, because it<br>was a population and not a single-cell study, the ex and sympathetic neurons existing in different proportions<br>along the trunk rostrocaudal axis. However, because it<br>was a population and not a single-cell study, the experi-<br>ments could not formally exclude the alternative po 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 i was a population and not a single-cell study, the experility that the results reflected intrinsic differences in the<br>probability of generating sensory versus sympathetic<br>neurons by a uniform population of bipotent sensory–<br>autonomic progenitors. However, in the context of the probability of generating sensory versus sympathetic probability of generating sensory versus sympathetic<br>neurons by a uniform population of bipotent sensory–<br>autonomic progenitors. However, in the context of the<br>other data and arguments presented here (see  $\delta$ 7) the autonomic progenitors. However, in the context of the other data and arguments presented here (see  $\S 7$ ), the existence of different sensory- and autonomic-restricted other data and arguments presented here (see  $\S 7$ ), the other data and arguments presented here (see  $\S$ 7), the existence of different sensory- and autonomic-restricted subpopulations seems a much more likely explanation for the data  $\overline{C}$ existence<br>subpopulat<br>the data.<br>Cell cult populations seems a much more likely explanation for<br>e data.<br>Cell culture data have also been used to argue against<br>e idea that neural crest cells become restricted to an  $\mathbf S$ 

the data.<br>Cell culture data have also been used to argue against<br>the idea that neural crest cells become restricted to an<br>autonomic fate early in migration. Progenitors expressing Cell culture data have also been used to argue against<br>the idea that neural crest cells become restricted to an<br>autonomic fate early in migration. Progenitors expressing<br>both sensory and autonomic markers in vitro can be the idea that neural crest cells become restricted to an autonomic fate early in migration. Progenitors expressing<br>both sensory and autonomic markers *in vitro* can be identi-<br>fied among cells from early quail sympathetic autonomic fate early in migration. Progenitors expressing<br>both sensory and autonomic markers *in vitro* can be identi-<br>fied among cells from early quail sympathetic ganglia,<br>whereas cells taken from older ganglia exhibit o both sensory and autonomic markers *in vitro* can be identi-<br>fied among cells from early quail sympathetic ganglia,<br>whereas cells taken from older ganglia exhibit only<br>sympathetic markers under the same culture conditions fied among cells from early quail sympathetic ganglia, whereas cells taken from older ganglia exhibit only sympathetic markers under the same culture conditions whereas cells taken from older ganglia exhibit only<br>sympathetic markers under the same culture conditions<br>(Duff *et al.* 1991; Sieber-Blum *et al.* 1993). While such<br>observations demonstrate that there are at least some ce 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 vet fully committed to a sympathet (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 *Phil. Trans. R. Soc. Lond.* B (2000)

early stages of gangliogenesis, this lack of restriction was<br>observed *in nitro* and it is possible that the culture condiearly stages of gangliogenesis, this lack of restriction was<br>observed *in vitro* and it is possible that the culture condi-<br>tions promoted the de-differentiation of the cells. Indeed observed *in vitro* and it is possible that the culture conditions promoted the de-differentiation of the cells. Indeed, 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 sympa-<br>thetic ganglia was assessed by *in vivo* tr tions promoted the de-differentiation of the cells. Indeed,<br>when the sensory potential of progenitors in early sympa-<br>thetic ganglia was assessed by *in vivo* transplantation into<br>the crest migration pathway of earlier hos when the sensory potential of progenitors in early sympa-<br>thetic ganglia was assessed by *in vivo* transplantation into<br>the crest migration pathway of earlier host embryos, only<br>autonomic and not sensory derivatives were o thetic 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) the crest migration<br>autonomic and not<br>Lievre *et al*. 1980).

## **6. EVIDENCE FOR FATE-RESTRICTED SENSORY EVIDENCE FOR FATE-RESTRICTED SENSORY<br>AND SYMPATHETIC PRECURSORS AMONG<br>BATING NEURAL CREST CELLS FROM IN VIVO 6. EVIDENCE FOR FATE-RESTRICTED SENSORY<br>AND SYMPATHETIC PRECURSORS AMONG<br>MIGRATING NEURAL CREST CELLS FROM** *IN VIVO***<br>ILINEACE TRACING EXPERIMENTS** SYMPATHETIC PRECURSORS AMONG<br>NG NEURAL CREST CELLS FROM *IN*<br>LINEAGE-TRACING EXPERIMENTS

**LINEAGE-TRACING EXPERIMENTS**<br>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 sensory and sympathetic lineages comes from  $in$  vivo<br>single-cell lineage-tracing experiments. In the initial set<br>of these studies, many individual pre-migratory cells<br>injected in the dorsal neural tube were observed to single-cell lineage-tracing experiments. In the initial set<br>of these studies, many individual pre-migratory cells<br>injected in the dorsal neural tube were observed to<br>generate both sensory and sympathetic neurons (Bronnerof these studies, many individual pre-migratory cells<br>injected in the dorsal neural tube were observed to<br>generate both sensory and sympathetic neurons (Bronner-<br>Fraser & Fraser 1988–1989). Most of these cells also injected in the dorsal neural tube were observed to<br>generate both sensory and sympathetic neurons (Bronner-<br>Fraser & Fraser 1988, 1989). Most of these cells also<br>generated neural tube cells implying that they were relagenerate both sensory and sympathetic neurons (Bronner-<br>Fraser & Fraser 1988, 1989). Most of these cells also<br>generated neural tube cells, implying that they were rela-<br>tively primitive tube-crest progenitors rather than n generated neural tube cells, implying that they were rela-<br>tively primitive tube-crest progenitors rather than neural 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 af tively primitive tube-crest progenitors rather than neural<br>crest cells *per se*. Consequently, the fact that the progeny<br>of such cells generated both neuron types after several<br>days of development left open the possibility crest cells *per se*. Consequently, the fact that the progeny<br>of such cells generated both neuron types after several<br>days of development left open the possibility that these<br>cells sequentially generated both autonomic-res of such cells generated both neuron types after several<br>days of development left open the possibility that these<br>cells sequentially generated both autonomic-restricted, days of development left open the possibility that these<br>cells sequentially generated both autonomic-restricted,<br>and sensory-restricted precursors, which emigrated as<br>distinct populations from the neural tube. However in a cells sequentially generated both autonomic-restricted,<br>and sensory-restricted precursors, which emigrated as<br>distinct populations from the neural tube. However, in a<br>subsequent experiment, these same authors were able to distinct populations from the neural tube. However, in a subsequent experiment, these same authors were able to 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 subsequent experiment, these same authors were able to<br>dye inject a very small number  $(n=17)$  of crest cells<br>shortly after their emigration from the neural tube<br>(Fraser & Bronner-Fraser 1991) Four of these 17 neural dye inject a very small number  $(n = 17)$  of crest cells<br>shortly after their emigration from the neural tube<br>(Fraser & Bronner-Fraser 1991). Four of these 17 neural<br>crest cells generated neurons (as detected by neurofilashortly after their emigration from the neural tube<br>(Fraser & Bronner-Fraser 1991). Four of these 17 neural<br>crest cells generated neurons (as detected by neurofila-(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 crest cells genera<br>ment antibody st<br>sensory ganglia.<br>These latter da ent antibody staining) in both the sympathetic and<br>nsory ganglia.<br>These latter data indicate that there are at least some<br>ural crest cells which retain both sensory and auto-

sensory ganglia.<br>These latter data indicate that there are at least some<br>neural crest cells which retain both sensory and auto-<br>nomic canacities shortly after emigration from the neural These latter data indicate that there are at least some<br>neural crest cells which retain both sensory and auto-<br>nomic capacities shortly after emigration from the neural<br>tube. Such results would be consistent with the idea neural crest cells which retain both sensory and auto-<br>nomic capacities shortly after emigration from the neural<br>tube. Such results would be consistent with the idea that<br>temporal changes in inducing signals (figure 4a) a nomic capacities shortly after emigration from the neural<br>tube. Such results would be consistent with the idea that<br>temporal changes in inducing signals (figure 4*a*) and/or tube. Such results would be consistent with the idea that<br>temporal changes in inducing signals (figure 4*c*) and/or<br>migration routes (figure 4*c*), rather than the production of<br>different kinds of neural crest cells, expla temporal changes in inducing signals (figure  $4a$ ) and/or<br>migration routes (figure  $4c$ ), rather than the production of<br>different kinds of neural crest cells, explain the sequential<br>colonization of sympathetic and sensory migration routes (figure  $4c$ ), rather than the production of<br>different kinds of neural crest cells, explain the sequential<br>colonization of sympathetic and sensory ganglia.<br>However, the data do not exclude the possibility different kinds of neural crest cells, explain the sequential<br>colonization of sympathetic and sensory ganglia.<br>However, the data do not exclude the possibility that<br>other crest cells have made a decision between sensory colonization of sympathetic and sensory ganglia.<br>However, the data do not exclude the possibility that<br>other crest cells have made a decision between sensory<br>and autonomic fates before exiting the neural tube. In However, the data do not exclude the possibility that<br>other crest cells have made a decision between sensory<br>and autonomic fates before exiting the neural tube. In<br>fact over  $50\%$  (eight out of 17) of the cells marked af other crest cells have made a decision between sensory<br>and autonomic fates before exiting the neural tube. In<br>fact, over  $50\%$  (eight out of 17) of the cells marked after 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 symmethetic neurons, while almost  $25\%$ fact, over 50% (eight out of 17) of the cells marked after<br>exiting the neural tube were fated to generate only<br>sensory and not sympathetic neurons, while almost  $25\%$ <br>(four out of 17) conversely generated autonomic (symp exiting the neural tube were fated to generate only<br>sensory and not sympathetic neurons, while almost 25%<br>(four out of 17) conversely generated autonomic (sympa-<br>thetic or adrenal) but not sensory neurons (Fraser & 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). thetic or adrenal) but not sensory neurons (Fraser & etic or adrenal) but not sensory neurons (Fraser &<br>onner-Fraser 1991).<br>Thus, another way of looking at these data is that the<br>ected migrating crest cells were three times as likely to

Bronner-Fraser 1991).<br>Thus, another way of looking at these data is that the<br>injected migrating crest cells were three times as likely to<br>be restricted to either sensory or sympathetic fates 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 c injected migrating crest cells were three times as likely to<br>be restricted to either sensory or sympathetic fates, as<br>they were fated to generate both derivatives. Of course, a<br>unifatent cell is not necessarily uninotent: be restricted to either sensory or sympathetic fates, as<br>they were fated to generate both derivatives. Of course, a<br>unifatent cell is not necessarily unipotent; in theory, all<br>marked cells could have had equivalent notenti 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 unifatent cell is not necessarily unipotent; in theory, all<br>marked cells could have had equivalent potentials and the<br>observed fate restrictions could have simply reflected<br>stochastic variations in what the cells actually marked cells could have had equivalent potentials and the observed fate restrictions could have simply reflected stochastic variations in what the cells actually did. Never-<br>theless the data certainly raise the possibility observed fate restrictions could have simply reflected<br>stochastic variations in what the cells actually did. Never-<br>theless, the data certainly raise the possibility that the<br>75% of the marked cells that were fate restrict 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<br>the marked cells had been multifatent, there would be no also restricted in their competence or potential. If all of<br>the marked cells had been multifatent, there would be no<br>reason to even consider this possibility—but that was not the marked cells had been multifatent, there would be no reason to even consider this possibility—but that was not the marked cells had been multifatent, there would be no<br>reason to even consider this possibility—but that was not<br>the result obtained. While it may be tempting to suppose<br>that the figure of  $25\%$  represents an underesti reason to even consider this possibility—but that was not<br>the result obtained. While it may be tempting to suppose<br>that the figure of 25% represents an underestimate of the<br>proportion of multipotent cells, this remains to the result obtained. While it may be tempting to suppose<br>that the figure of 25% represents an underestimate of the<br>proportion of multipotent cells, this remains to be tested.<br>The actual proportion of multipotent versus fat that the figure of 25% represents an underestimate of the proportion of multipotent cells, this remains to be tested.<br>The actual proportion of multipotent versus fate-<br>restricted cells has a significant impact on thinking proportion of multipotent cells, this remains to be tested.<br>The actual proportion of multipotent versus fate-<br>restricted cells has a significant impact on thinking about<br>the dynamics of the system and the strategies employ The actual proportion of multipotent versus fate-<br>restricted cells has a significant impact on thinking about<br>the dynamics of the system and the strategies employed to<br>generate lineage diversification 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 If there are indeed neural crest cells restricted to generating neurons of the autonomic subset, when and where is<br>this restriction acquired? Strikingly, among three crest<br>cells injected just as they were delaminating from ating neurons of the autonomic subset, when and where is<br>this restriction acquired? Strikingly, among three crest<br>cells injected just as they were delaminating from the<br>neural tube, one gave rise to only symmathetic and no this restriction acquired? Strikingly, among three crest<br>cells injected just as they were delaminating from the<br>neural tube, one gave rise to only sympathetic and not<br>sensory derivatives and another conversely to sensory b cells injected just as they were delaminating from the<br>neural tube, one gave rise to only sympathetic and not<br>sensory derivatives and another conversely to sensory but<br>not sympathetic: the third generated both (Bronnerneural tube, one gave rise to only sympathetic and not<br>sensory derivatives and another conversely to sensory but<br>not sympathetic; the third generated both (Bronner-<br>Fraser & Fraser 1989) It is important to note that these sensory derivatives and another conversely to sensory but<br>not sympathetic; the third generated both (Bronner-<br>Fraser & Fraser 1989). It is important to note that these<br>studies were performed at only a single time-point, an not sympathetic; the third generated both (Bronner-<br>Fraser & Fraser 1989). It is important to note that these<br>studies were performed at only a single time-point, and<br>an even higher frequency of such fate-restricted progeni Fraser & Fraser 1989). It is important to note that these<br>studies were performed at only a single time-point, and<br>an even higher frequency of such fate-restricted progeni-<br>tors might have been detected had the injections b Sudies were performed at only a single time-point, and were artificially increased in chick embryos using a retro-<br>an even higher frequency of such fate-restricted progeni-<br>tors might have been detected had the injections tors might have been detected had the injections been thetic neurons were being generated.While these observaperformed at earlier stages when predominantly sympa-<br>thetic neurons were being generated. While these observa-<br>tions do not prove that such fate-restricted cells were<br>similarly restricted in their potentials they provide thetic neurons were being generated. While these observations do not prove that such fate-restricted cells were<br>similarly restricted in their potentials, they provide<br>evidence that such restrictions in notential could occu tions do not prove that such fate-restricted cells were<br>similarly restricted in their potentials, they provide<br>evidence that such restrictions in potential could occur as<br>early as the time that crest cells are delaminating similarly restricted in their potentials, they provide 'n<br>evidence that such restrictions in potential could occur as<br>early as the time that crest cells are delaminating from pr<br>the neural tube evidence that such restrictions in potential could occur as<br>early as the time that crest cells are delaminating from<br>the neural tube.

#### **7. THE IDENTIFICATION OF AUTONOMIC-INDUCING SIGNALS REAIST CONSTRAINS ABOUT AUTONOMIC-INDUCING**<br>SIGNALS RAISES NEW QUESTIONS ABOUT THE<br>TIMING OF PESTRICTION TO THE AUTONOMIC THE IDENTIFICATION OF AUTONOMIC-INDUCING<br>SIGNALS RAISES NEW QUESTIONS ABOUT THE<br>TIMING OF RESTRICTION TO THE AUTONOMIC **LINEAGE** TIMING OF RESTRICTION TO THE AUTONOMIC<br>LINEAGE<br>New questions regarding the timing of restriction to the

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New questions regarding the timing of restriction to the<br>
autonomic sublineage have been raised recently by the<br>
identification on the one hand of autonomic neuron-New questions regarding the timing of restriction to the<br>autonomic sublineage have been raised recently by the<br>identification, on the one hand, of autonomic neuron-<br>inducing signals and on the other hand of signals that autonomic sublineage have been raised recently by the<br>identification, on the one hand, of autonomic neuron-<br>inducing signals and on the other hand of signals that<br>promote the formation of neural crest cells in the dorsal identification, on the one hand, of autonomic neuron-<br>inducing signals and on the other hand of signals that<br>promote the formation of neural crest cells in the dorsal<br>neural tube. These inducing signals appear to be very inducing signals and on the other hand of signals that<br>promote the formation of neural crest cells in the dorsal<br>neural tube. These inducing signals appear to be very<br>similar if not identical: members of the bone morphopromote the formation of neural crest cells in the dorsal<br>neural tube. These inducing signals appear to be very<br>similar if not identical; members of the bone morphoneural tube. These inducing signals appear to be very<br>similar if not identical; members of the bone morpho-<br>genetic protein (BMP) family such as BMP2 and BMP4,<br>produced by the ectoderm and dorsal neural tube are 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 genetic protein (BMP) family such as BMP2 and BMP4,<br>produced by the ectoderm and dorsal neural tube, are<br>necessary and sufficient to promote the formation of<br>neural crest cells from the neural tube (Dickinson *et al* 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 produce necessary and sufficient to promote the formation of<br>neural crest cells from the neural tube (Dickinson *et al.*<br>1995; Liem *et al.* 1995, 1997). The same factors produced<br>in the dorsal aorta annear necessary and sufficien 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 1995; Liem *et al.* 1995, 1997). The same factors produced<br>in the dorsal aorta appear necessary and sufficient to<br>induce differentiation of autonomic neurons from multi-<br>potent neural crest cells in viva and in vitra (Rei potent neural crest cells *in vivo* and *in vitro* (Reissman *et al.* induce differentiation of autonomic neurons from multi-<br>potent neural crest cells *in vivo* and *in vitro* (Reissman *et al.*<br>1996; Shah *et al.* 1996). So the problem is this: if neural<br>crest cells emerge from the neural potent 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 compe-<br>tence for sensory and autonomic d 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 aut crest cells emerge from the neural tube with equal competence for sensory and autonomic differentiation, why do<br>these cells not immediately differentiate to autonomic<br>neurons dorsally in response to BMPs secreted by the tence for sensory and autonomic differentiation, why do<br>these cells not immediately differentiate to autonomic<br>neurons dorsally, in response to BMPs secreted by the<br>ectoderm and dorsal neural tube? these cells not immediately differentiate to autonomic<br>neurons dorsally, in response to BMPs secreted by the<br>ectoderm and dorsal neural tube? urons dorsally, in response to BMPs secreted by the<br>toderm and dorsal neural tube?<br>There are a number of possible mechanisms that could<br>we this problem. First, the action of BMPs to induce

ectoderm and dorsal neural tube?<br>There are a number of possible mechanisms that could<br>solve this problem. First, the action of BMPs to induce<br>autonomic neurogenesis in freshly delaminated crest cells There are a number of possible mechanisms that could<br>solve this problem. First, the action of BMPs to induce<br>autonomic neurogenesis in freshly delaminated crest cells<br>may be prevented by other signals in the dorsal environ solve this problem. First, the action of BMPs to induce<br>autonomic neurogenesis in freshly delaminated crest cells<br>may be prevented by other signals in the dorsal environment. These signals could include inhibitors of BMPs, 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 ment. These signals could include inhibitors of BMPs,<br>such as noggin, which is present in the roof plate and<br>which could reduce the effective concentration of BMPs<br>below a critical threshold for autonomic neurogenesis: or such as noggin, which is present in the roof plate and<br>which could reduce the effective concentration of BMPs<br>below a critical threshold for autonomic neurogenesis; or *Phil. Trans. R. Soc. Lond.* B (2000) *Phil. Trans. R. Soc. Lond.* B (2000)

other signals such as Wnts (which are also present in the 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 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 roof plate (McMahon *et al.* 1992)) which could<br>qualitatively change the effect of BMPs on emigrating<br>crest cells. This sort of mechanism would be required if<br>freshly delaminated neural crest cells are indeed equally qualitatively change the effect of BMPs on emigrating<br>crest cells. This sort of mechanism would be required if<br>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 neural crest cells are initially not competent to generate<br>autonomic neurons in response to BMPs, and that this<br>competence is acquired only later as the cells migrate<br>ventrally towards the dorsal aorta. A prediction of thi autonomic neurons in response to BMPs, and that this<br>competence is acquired only later as the cells migrate<br>ventrally towards the dorsal aorta. A prediction of this<br>model is that exposure of freshly delaminated neural cres competence is acquired only later as the cells migrate<br>ventrally towards the dorsal aorta. A prediction of this<br>model is that exposure of freshly delaminated neural crest<br>cells to a high concentration of BMPs would not ind ventrally towards the dorsal aorta. A prediction of this<br>model is that exposure of freshly delaminated neural crest<br>cells to a high concentration of BMPs would not induce<br>autonomic markers in crest cells located dorsolater model is that exposure of freshly delaminated neural crest<br>cells to a high concentration of BMPs would not induce<br>autonomic markers in crest cells located dorsolaterally to<br>the neural tube but only in those cells which had cells to a high concentration of BMPs would not induce<br>autonomic markers in crest cells located dorsolaterally to<br>the neural tube, but only in those cells which had<br>migrated more ventrally Interestingly precisely this autonomic markers in crest cells located dorsolaterally to<br>the neural tube, but only in those cells which had<br>migrated more ventrally. Interestingly, precisely this<br>result was obtained in experiments in which RMP4 levels the neural tube, but only in those cells which had<br>migrated more ventrally. Interestingly, precisely this<br>result was obtained in experiments in which BMP4 levels<br>were artificially increased in chick embryos using a retromigrated more ventrally. Interestingly, precisely this<br>result was obtained in experiments in which BMP4 levels<br>were artificially increased in chick embryos using a retro-<br>viral vector (Reissman et al. 1996). Similarly when result was obtained in experiments in which BMP4 levels were artificially increased in chick embryos using a retro-<br>viral vector (Reissman *et al.* 1996). Similarly, when BMP2<br>(or BMP4) is applied to neural tube explants cultured in<br>the absence of surrounding tissues, autonomi viral 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 (or BMP4) is applied to neural tube explants cultured in<br>the absence of surrounding tissues, autonomic markers<br>are induced in those neural crest cells that have migrated<br>farthest from the neural tube but there is a region are induced in those neural crest cells that have migrated farthest from the neural tube, but there is a region of 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 (Green-wood *et al* 1999) Taken together these data suggest 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 symmethetic neurons are initially not '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 wood *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. progenitors of sympathetic neurons are initially not

What, if anything, could such a delayed acquisition of autonomic competence tell us about when restriction to What, if anything, could such a delayed acquisition of<br>autonomic competence tell us about when restriction to<br>an autonomic fate is acquired? Suppose that precursors of<br>sympathetic neurons indeed do not acquire competence autonomic competence tell us about when restriction to<br>an autonomic fate is acquired? Suppose that precursors of<br>sympathetic neurons indeed do not acquire competence<br>to respond to autonomic-inducing signals like RMP2/4 an autonomic fate is acquired? Suppose that precursors of<br>sympathetic neurons indeed do not acquire competence<br>to respond to autonomic-inducing signals like BMP2/4<br>until they have migrated ventrally to the site of DRG sympathetic neurons indeed do not acquire competence<br>to respond to autonomic-inducing signals like BMP2/4<br>until they have migrated ventrally to the site of DRG to respond to autonomic-inducing signals like BMP2/4<br>until they have migrated ventrally to the site of DRG<br>formation. Suppose further that all such cells are derived<br>from multipotent progenitors that exit the neural tube until they have migrated ventrally to the site of DRG<br>formation. Suppose further that all such cells are derived<br>from multipotent progenitors that exit the neural tube<br>with both sensory and autonomic potential. In this cas formation. Suppose further that all such cells are derived<br>from multipotent progenitors that exit the neural tube<br>with both sensory and autonomic potential. In this case,<br>multipotent neural crest cells would be connetent f from multipotent progenitors that exit the neural tube<br>with both sensory and autonomic potential. In this case,<br>multipotent neural crest cells would be competent for<br>sensory differentiation before they had acquired compewith both sensory and autonomic potential. In this case,<br>multipotent neural crest cells would be competent for<br>sensory differentiation before they had acquired compe-<br>tence for autonomic differentiation. This order of commultipotent neural crest cells would be competent for<br>sensory differentiation before they had acquired compe-<br>tence for autonomic differentiation. This order of comsensory 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 tence for autonomic differentiation. This order of competencies is precisely the opposite of what would make<br>sense, given the fact that neural crest cells populate the<br>symmethetic ganglion primordia before they contribute petencies is precisely the opposite of what would make<br>sense, given the fact that neural crest cells populate the<br>sympathetic ganglion primordia before they contribute to<br>the sensory ganglia. Moreover such a model would se sense, given the fact that neural crest cells populate the sympathetic ganglion primordia before they contribute to<br>the sensory ganglia. Moreover, such a model would seem<br>to make the cells even more vulnerable to being diverted<br>to a sensory fate before they had a chance to respond the sensory ganglia. Moreover, such a model would seem<br>to make the cells even more vulnerable to being diverted<br>to a sensory fate before they had a chance to respond to<br>autonomic-inducing signals. The assumption of equal to make the cells even more vulnerable to being diverted to a sensory fate before they had a chance to respond to<br>autonomic-inducing signals. The assumption of equal<br>sensory and autonomic potential by freshly delaminated<br>neural crest cells therefore leads to a paradox when autonomic-inducing signals. The assumption of equal<br>sensory and autonomic potential by freshly delaminated<br>neural crest cells therefore leads to a paradox when<br>viewed in the light of the new data on autonomicsensory and autonomic potential by freshly delaminated<br>neural crest cells therefore leads to a paradox when<br>viewed in the light of the new data on autonomic-<br>inducing signals neural crest cells therefore leads to a paradox when<br>viewed in the light of the new data on autonomic-<br>inducing signals.<br>It seems far easier to account for the data with a model viewed in the light of the new data on autonomic-

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

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birthdays<br>Figure 5. Two genetically and lineally distinct populations of sensory neuron precursors.  $(a,b)$  Summary of results of<br>retroviral lineage-tracing experiments in chick DRG. (Frank & Sanes 1991). (*a*) VI-only precu Figure 5. Two genetically and lineally distinct populations of sensory neuron precursors.  $(a,b)$  Summary of results of<br>retroviral lineage-tracing experiments in chick DRG (Frank & Sanes 1991).  $(a)$  VL-only precursors give Figure 5. Two genetically and lineally distinct populations of sensory neuron precursors. (*a*,*b*) Summary of results of<br>retroviral lineage-tracing experiments in chick DRG (Frank & Sanes 1991). (*a*) VL-only precursors retroviral lineage-tracing experiments in chick DRG (Frank & Sanes 1991). (*a*) VL-only precursors give rise to early-born (*d*),<br>large-diameter (trkC<sup>+</sup> and trkB<sup>+</sup>) sensory neurons located in the VL region of the ganglia large-diameter (trkC<sup>+</sup> and trkB<sup>+</sup>) sensory neurons located in the VL region of the ganglia  $(c)$ . This lineage probably corresponds<br>to  $Ngn^2$ -dependent precursors in the mouse (Ma *et al.* 1999). (*b*)  $DM + VL$  precursors g ROYA small-diameter (blue, DM) later-born neurons  $(d)$ , which are located in the DM region of the ganglia  $(c)$ . This lineage probably corresponds to  $NgnI$ -dependent (dep.) precursors in the mouse (Ma *et al.* 1999). VL-only pr small-diameter (blue, DM) later-born neurons (d), which are located in the DM region of the ganglia (c). This lineage probably<br>corresponds to *Ngn1*-dependent (dep.) precursors in the mouse (Ma *et al.* 1999). VL-only pre corresponds to *Ngn1*-dependent (dep.) precursors in the mouse (Ma *et al.* 1999). VL-only precursors are more frequent when<br>marking is performed earlier (St13–17), while DM + VL precursors are seen later (St15–19). VL-onl marking is performed earlier (St13–17), while DM+ VL precursors are seen later (St15–19). VL-only precursors are likely to be<br>restricted to a sensory fate, based on studies of *Ngn2* (Ma *et al.* 1999; Perez *et al.* 1999) 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<br>(Greenwood et al. 1999). It is not yet clear whether DM + VL precursors are similarly r THE studies of large- and small-diameter sensory neurons (Lawson *et al.* 1974; Lawson & Biscoe 1979).<br>
smooth muscle potential (Stemple & Anderson 1992; of whether, conversely, there are neural crest cells which studies of large- and small-diameter sensory neurons (Lawson *et*<br>smooth muscle potential (Stemple & Anderson 1992;<br>Shah *et al* 1996) have never been observed to differ- $\bigcirc$ smooth muscle potential (Stemple & Anderson 1992;<br>Shah *et al.* 1996) have never been observed to differentiate into sensory neurons, either *in vitro* or after  $\mathbf S$ entiate into sensory into sensory neurons, either *in vitro* or after the transportant *into* sensory neurons, either *in vitro* or after transportation *in vitro* (Morrison *et al.* 1999). White *&* **PHILOSOPHICAL**<br>TRANSACTIONS Shah *et al.* 1996) have never been observed to differential. Recent studies support this possibility as well.<br>
transplantation *in vivo* (Morrison *et al.* 1999; White &

entiate into sensory neurons, either *in vitro* or after<br>transplantation *in vivo* (Morrison *et al.* 1999; White &<br>Anderson 1999; P. M. White and D. J. Anderson, unpub-<br>lished data) A rigorous test of this conclusion wil transplantation *in vivo* (Morrison *et al.* 1999; White &<br>Anderson 1999; P. M. White and D. J. Anderson, unpub-<br>lished data). A rigorous test of this conclusion will require<br>the identification of instructive-inducing sig Anderson 1999; P. M. White and D. J. Anderson, unpub-<br>lished data). A rigorous test of this conclusion will require<br>the identification of instructive-inducing signals for<br>sensory neurons lished data). A rigorous test of this conclusion will require<br>the identification of instructive-inducing signals for<br>sensory neurons.<br>The notion that a subset of neural crest cells exits the the identification of instructive-inducing signals for

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<br>early in migration have sensory but not autonomic poten-<br>tial. Recent studies support this possibility as well of whether, conversely, there are neural crest cells<br>early in migration have sensory but not autonomic<br>tial. Recent studies support this possibility as well.

### **8. EVIDENCE FOR A DIVIDING PRECURSOR IN THE MAMMALIAN NEURAL DIVIDING PRECURSOR IN THE MAMMALIAN NEURAL CREST THAT IS COMMITTED<br>TO THE SENSORY LINEACE WITH PESPECT TO EVIDENCE FOR A DIVIDING PRECURSOR IN THE<br>AMMALIAN NEURAL CREST THAT IS COMMITTED<br>TO THE SENSORY LINEAGE WITH RESPECT TO<br>ALITONOMIC INDUCING SICNALS** AN NEURAL CREST THAT IS COMMI<br>SENSORY LINEAGE WITH RESPECT<br>AUTONOMIC-INDUCING SIGNALS **AUTONOMIC-INDUCING SIGNALS**<br>Recently, we have provided evidence that the rat

neural crest contains a population of precursors that are

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committed to a sensory fate with respect to the autonomicinducing signal, BMP2. (Note that `commitment' is used operationally here, to indicate that the cell is irreversibly inducing signal, BMP2. (Note that 'commitment' is used<br>operationally here, to indicate that the cell is irreversibly<br>determined for a given fate with respect to a physiological<br>inducer of relevant alternative fates. That d operationally here, to indicate that the cell is irreversibly<br>determined for a given fate with respect to a physiological<br>inducer of relevant alternative fates. That does not mean<br>that it is committed with respect to all p determined for a given fate with respect to a physiological<br>inducer of relevant alternative fates. That does not mean<br>that it is committed with respect to all possible inducing<br>signals, something that can never be excluded inducer of relevant alternative fates. That does not mean<br>that it is committed with respect to all possible inducing<br>signals, something that can never be excluded.) Sensory<br>neurons identified by co-expression of the POU ho that it is committed with respect to all possible inducing<br>signals, something that can never be excluded.) Sensory<br>neurons, identified by co-expression of the POU homeo-<br>domain proteins Brn-3.0 and other markers such as csignals, something that can never be excluded.) Sensory<br>neurons, identified by co-expression of the POU homeo-<br>domain proteins Brn-3.0 and other markers such as c-ret and peripherin, develop from proliferating neural tubedomain proteins Brn-3.0 and other markers such as c-ret<br>and peripherin, develop from proliferating neural tube-<br>derived precursors in a fully defined culture medium<br>(Greenwood *et al* 1999) Under such conditions autonomic and peripherin, develop from proliferating neural tube-<br>derived precursors in a fully defined culture medium<br>(Greenwood *et al.* 1999). Under such conditions autonomic<br>neurons identified by expression of the paired homeoderived precursors in a fully defined culture medium<br>(Greenwood *et al.* 1999). Under such conditions autonomic<br>neurons, identified by expression of the paired homeo-<br>domain protein Phox<sup>9</sup>a fail to develop However dif-(Greenwood *et al.* 1999). Under such conditions autonomic<br>neurons, identified by expression of the paired homeo-<br>domain protein Phox2a, fail to develop. However, dif-<br>ferentiation of autonomic neurons in numbers vastly neurons, identified by expression of the paired homeo-<br>domain protein Phox2a, fail to develop. However, dif-<br>ferentiation of autonomic neurons, in numbers vastly<br>exceeding the number of sensory neurons, can be domain protein Phox2a, fail to develop. However, dif-<br>  $\Box$  ferentiation of autonomic neurons, in numbers vastly<br>  $\Box$  exceeding the number of sensory neurons, can be ferentiation of autonomic neurons, in numbers vastly<br>exceeding the number of sensory neurons, can be<br>induced by addition of BMP2. This manipulation fails to<br>produce any significant reduction in the number of exceeding the number of sensory neurons, can be<br>induced by addition of BMP2. This manipulation fails to<br>produce any significant reduction in the number of<br>sensory neurons that differentiate however (Greenwood 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) produce any<br> *et al.* 1999).<br> *The simpl* The simplest interpretation of this result is that the simplest interpretation of this result is that the simplest and autonomic neurons in these BMP2-treated

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

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

AN EARLY SPECIFICATION OF A SENSORY FATE IN<br>An independent line of genetic evidence also suggests<br>at a subset of neural crest cells is at least specified if A SUBSET OF MIGRATING NEURAL CREST CELLS<br>
An independent line of genetic evidence also suggests<br>
that a subset of neural crest cells is at least specified, if An independent line of genetic evidence also suggests<br>that a subset of neural crest cells is at least specified, if<br>not determined, for a sensory fate early in migration.<br>This evidence derives from studies of the expressio that a subset of neural crest cells is at least specified, if Figure 6. Speculative model for early segregation of sensory<br>not determined, for a sensory fate early in migration. and autonomic precursors in the pre-migrato mot determined, for a sensory fate early in migration.<br>This evidence derives from studies of the expression and<br>function of the *Neurogenins* (*Ngns*), a family of vertebrate<br>propeural genes homologous to the *Drosphila* p This evidence derives from studies of the expression and<br>function of the *Neurogenins* (*Ngns*), a family of vertebrate<br>proneural genes homologous to the *Drosophila* proneural<br>gene atonal (Gradwobl et al. 1996: Ma et al. function of the *Neurogenins* (*Ngns*), a family of vertebrate<br>proneural genes homologous to the *Drosophila* proneural<br>gene atonal (Gradwohl *et al.* 1996; Ma *et al.* 1996; Sommer<br>et al. 1996). *Nans* are essential for t proneural genes homologous to the *Drosophila* proneural<br>gene atonal (Gradwohl et al. 1996; Ma et al. 1996; Sommer<br>et al. 1996). *Ngns* are essential for the development of<br>neural crest- and placode-derived sensory but not gene *atonal* (Gradwohl *et al.* 1996; Ma *et al.* 1996; Sommer<br>*et al.* 1996). *Ngns* are essential for the development of<br>neural crest- and placode-derived sensory, but not sympa-<br>thetic neurons *in ring* (Eode *et al.* 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). *Nan?* is expressed very early in ne 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 migrathetic, 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 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). tion, as well as in a subset of cell<br>margins of the neural tube, in bo<br>1999) and chick (Perez *et al.* 1999).<br>Sepsory neurogenesis in the DRG

1999) and chick (Perez *et al.* 1999).<br>Sensory neurogenesis in the DRG is prevented in both<br>single *Ngn2* and double *Ngn2;Ngn1* mutants (Ma *et al.*<br>1999) while sympathetic ganglia are unaffected. This Sensory neurogenesis in the DRG is prevented in both<br>single *Ngn2* and double *Ngn2;Ngn1* mutants (Ma *et al.*<br>1999) while sympathetic ganglia are unaffected. This<br>suggests either that  $Nan2$ -expressing neural crest cells 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 o 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 re suggests either that  $Ngn2$ -expressing neural crest cells only<br>generate sensory and not sympathetic neurons, or that<br>they generate both classes of neurons but neither require<br>nor ultimately express  $Ngn2$  in the sympathetic generate sensory and not sympathetic neurons, or that<br>they generate both classes of neurons but neither require<br>nor ultimately express  $Ngn2$  in the sympathetic lineage. Examination of *Ngn2-lac*<sub>Z</sub> knockin embryos, in which Xue et al. 1985).



margins of the neural tube, in both mouse (Ma *et al.* this is hypothetical as well. At this stage of development,<br>1999) and chick (Perez *et al.* 1999).<br>Sensory neurogenesis in the DRG is prevented in both and agen (blue Figure 6. Speculative model for early segregation of sensory Figure 6. Speculative model for early segregation of sensory<br>and autonomic precursors in the pre-migratory neural crest.<br>(a) At early stages of crest migration, it is suggested that Figure 6. Speculative model for early segregation of sensor and autonomic precursors in the pre-migratory neural cres (*a*) At early stages of crest migration, it is suggested that many sensory precursors  $(P_1)$  and auton and autonomic precursors in the pre-migratory neural crest.<br>(a) At early stages of crest migration, it is suggested that<br>many sensory precursors ( $P_s$ ) and autonomic precursors ( $P_A$ )<br>may emerge from the neural tube alrea (*a*) At early stages of crest migration, it is suggested that<br>many sensory precursors  $(P_s)$  and autonomic precursors  $(P_A)$ <br>may emerge from the neural tube already restricted to these<br>lineages. However, a proportion of cr many sensory precursors  $(P_S)$  and autonomic precursors  $(P_A)$ <br>may emerge from the neural tube already restricted to these<br>lineages. However, a proportion of crest cells do not make<br>this decision until after they have emigr may emerge from the neural tube already restricted to these<br>lineages. However, a proportion of crest cells do not make<br>this decision until after they have emigrated from the neural<br>tube (Fraser & Bronner-Fraser 1991) The e lineages. However, a proportion of crest cells do not make<br>this decision until after they have emigrated from the neural<br>tube (Fraser & Bronner-Fraser 1991). The early-emigrating<br>sensory precursors give rise primarily to l this decision until after they have emigrated from the neural<br>tube (Fraser & Bronner-Fraser 1991). The early-emigrating<br>sensory precursors give rise primarily to large-diameter tube (Fraser & Bronner-Fraser 1991). The early-emigrating<br>sensory precursors give rise primarily to large-diameter<br>sensory neurons (see figure 5), but also to some small-diameter<br>neurons as well (N.I.D/SD). It is assumed t sensory precursors give rise primarily to large-diameter<br>sensory neurons (see figure 5), but also to some small-diame<br>neurons as well (N<sub>S</sub>LD/SD). It is assumed that this sensory-<br>restricted precursor also gives rise to g sensory neurons (see figure 5), but also to some small-diam<br>neurons as well  $(N_{\rm s}LD/SD)$ . It is assumed that this sensory<br>restricted precursor also gives rise to glial cells ('G'), but<br>this is hypothetical as well. At thi neurons as well (N<sub>S</sub>LD/SD). It is assumed that this sensory-<br>restricted precursor also gives rise to glial cells ('G'), but restricted precursor also gives rise to glial cells ('G'), but<br>this is hypothetical as well. At this stage of development,<br>autonomic precursors migrate to the sympathetic ganglion<br>anlagen (blue stippling) where they differ this is hypothetical as well. At this stage of developmer<br>autonomic precursors migrate to the sympathetic gang<br>anlagen (blue stippling) where they differentiate to<br>sympathetic neurons and glia (b) At later stages the s sympathetic neurons and glia. (*b*) At later stages, the sensory anlagen (blue stippling) where they differentiate to<br>sympathetic neurons and glia. (b) At later stages, the sensory<br>precursors give rise primarily or exclusively to small-diameter<br>sensory neurons (N-SD); these precursors sympathetic neurons and glia. (b) At later stages, the sensor<br>precursors give rise primarily or exclusively to small-diamet<br>sensory neurons (N<sub>S</sub>SD); these precursors may be distinct<br>from those at earlier stages (see figu precursors give rise primarily or exclusively to small-dia<br>sensory neurons ( $N_SSD$ ); these precursors may be disting<br>from those at earlier stages (see figure 5). Autonomic<br>precursors are hypothesized to become incorporated sensory neurons  $(N_SSD)$ ; these precursors may be distinct<br>from those at earlier stages (see figure 5). Autonomic<br>precursors are hypothesized to become incorporated into the DRG at these later stages, explaining the persistence of such precursors are hypothesized to become incorporated into<br>DRG at these later stages, explaining the persistence of s<br>precursors in late-stage ganglia (Le Lievre *et al.* 1980;<br>Xue *et al.* 1985) DRG at these late:<br>precursors in late-:<br>Xue *et al.* 1985).

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migration. Gain-of-function studies with the *Ngns* further suggest migration.<br>
Gain-of-function studies with the *Ngns* further suggest<br>
that by the time neural crest cells express these proneural<br>
genes they may be determined as well as specified for a Gain-of-function studies with the  $Ngas$  further suggest<br>that by the time neural crest cells express these proneural<br>genes they may be determined as well as specified for a<br>sensory fate. Forced expression of NGNs from a ret that by the time neural crest cells express these proneural<br>genes they may be determined as well as specified for a<br>sensory fate. Forced expression of NGNs from a retroviral<br>vector in pre-migratory crest cells *in an* bias genes they may be determined as well as specified for a<br>sensory fate. Forced expression of NGNs from a retroviral<br>vector in pre-migratory crest cells *in ovo* biases them to<br>differentiate to sensory neurons in the DRG (Per sensory fate. Forced expression of NGNs from a retroviral<br>vector in pre-migratory crest cells *in ovo* biases them to<br>differentiate to sensory neurons in the DRG (Perez *et al.*<br>1999) Moreover ectonic expression of NGNs in 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 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 glia 1999). Moreover, ectopic expression of NGNs induces the expression of multiple sensory-specific as well as generic<br>neuronal markers, not only in crest-derived glial precur-<br>sors in peripheral nerve, but surprisingly in the expression of multiple sensory-specific as well as generic<br>neuronal markers, not only in crest-derived glial precur-<br>sors in peripheral nerve, but surprisingly in the myotome<br>as well (Perez et al. 1999) These data suggest

expression of  $\beta$ -galactosidase perdures for several days in sensory neurons after the endogenous gene is sensory neurons after the endogenous gene

sensory neurons after the endogenous gene is<br>extinguished, has failed to reveal any evidence of  $lac\zeta$ <br>expression in sympathetic ganglia (L. Lo, C. Fode, F.<br>Guillemot and D. I Anderson unpublished data) While extinguished, has failed to reveal any evidence of  $lac\zeta$ <br>expression in sympathetic ganglia (L. Lo, C. Fode, F.<br>Guillemot and D. J. Anderson, unpublished data). While<br>these negative results are not conclusive they are con expression in sympathetic ganglia (L. Lo, C. Fode, F. Guillemot and D. J. Anderson, unpublished data). While<br>these negative results are not conclusive, they are consistent with the idea that expression of  $Nen2$  marks a su Guillemot and D. J. Anderson, unpublished data). While<br>these negative results are not conclusive, they are consis-<br>tent with the idea that expression of *Ngn2* marks a subset<br>of crest cells fated to generate sensory neuron

neuronal markers, not only in crest-derived glial precursors in peripheral nerve, but surprisingly in the myotome<br>as well (Perez *et al.* 1999). These data suggest that when<br>expressed in certain cell contexts. *Now* can bi sors in peripheral nerve, but surprisingly in the myotome<br>as well (Perez *et al.* 1999). These data suggest that when<br>expressed in certain cell contexts, *Ngns* can bias neural<br>crest cells to a sensory fate. Consistent wit as well (Perez *et al.* 1999). These data suggest that when<br>expressed in certain cell contexts,  $Ngns$  can bias neural<br>crest cells to a sensory fate. Consistent with this idea,<br>forced expression of  $Nqns$  in cultured neural 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 crest cells to a sensory fate. Consistent with this idea,<br>forced expression of *Ngns* in cultured neural tube cells<br>under some conditions promotes not only neuronal differ-<br>entiation, but expression of the sensory-specific forced expression of  $Ngns$  in cultured neural tube cells<br>under some conditions promotes not only neuronal differ-<br>entiation, but expression of the sensory-specific marker<br> $Rrn-3.0$  as well (L. Lo and D. L. Anderson, unpubl under some conditions promotes not only neuronal differentiation, but expression of the sensory-specific marker<br>Brn-3.0 as well (L. Lo and D. J. Anderson, unpublished<br>data) Taken together, these data suggest that the early entiation, but expression of the sensory-specific marker<br>Brn-3.0 as well (L. Lo and D. J. Anderson, unpublished<br>data). Taken together, these data suggest that the early 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. Moreo data). Taken together, these data suggest that the early<br>expression of  $Ngn2$  in some migrating crest cells reflects<br>their early determination for a sensory fate. Moreover,<br>the fact that  $Ngn2$  is also expressed by a subset expression of  $Ngn2$  in some migrating crest cells reflects<br>their early determination for a sensory fate. Moreover,<br>the fact that  $Ngn2$  is also expressed by a subset of cells<br>located at the dorsolateral margins of the neur their early determination for a sensory fate. Moreover,<br>the fact that  $Ngn2$  is also expressed by a subset of cells<br>located at the dorsolateral margins of the neural tube, at<br>the time when neural crest cells are emigrating the fact that  $Ngn2$  is also expressed by a subset of cells sensory neurons that differentiate from the apparently located at the dorsolateral margins of the neural tube, at committed precursors in rat neural crest explant fucated at the dorsolateral margins of the neural tube, at<br>the time when neural crest cells are emigrating, could<br>further indicate that the sensory fate is determined in<br>some cells prior to delamination from the neural tub the time when neural crest cells are emigrating, could<br>further indicate that the sensory fate is determined in<br>some cells prior to delamination from the neural tube,<br>although this is not vet proven 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**<br>10. THE MAJORITY OF MUSCLE AFFERENT **THE MAJORITY OF MUSCLE AFFERENT<br>SENSORY NEURONS DERIVE FROM<br>SENETICALLY AND LINEALLY DISTINCT A GENETICALLY OF MUSCLE AFFERENT<br>SENSORY NEURONS DERIVE FROM<br>A GENETICALLY AND LINEALLY DISTINCT<br>BESCURSOR BOBLLATION IN VIIO** SENSORY NEURONS DERIVE FROM<br>¡ENETICALLY AND LINEALLY DISTINCT<br>PRECURSOR POPULATION *IN VIVO*

**EXEMPLE AND LINEALLY DISTINCT<br>PRECURSOR POPULATION IN VIVO**<br>Why should there be a distinct subpopulation of<br>ural crest cells determined for a sensory fate early in **EXECURSUR PUPULATION IN VIVO**<br>Nhy should there be a distinct subpopulation of<br>neural crest cells determined for a sensory fate early in<br>development? The answer may be related to the different Why should there be a distinct subpopulation of<br>neural crest cells determined for a sensory fate early in<br>development? The answer may be related to the different<br>subtypes of sensory neurons that populate the DRG and neural crest cells determined for a sensory fate early in the possibility that the later-differentiating,  $Ngn$ -<br>development? The answer may be related to the different dependent  $VL + DM$  sensory lineage derives from a<br>subtyp development? The answer may be related to the different<br>subtypes of sensory neurons that populate the DRG, and<br>the schedule on which they are generated. In normal<br>development large-diameter muscle afferent (propriosubtypes of sensory neurons that populate the DRG, and<br>the schedule on which they are generated. In normal<br>development, large-diameter muscle afferent (proprio-<br>centive) sensory neurons differentiate before the smallthe schedule on which they are generated. In normal<br>development, large-diameter muscle afferent (proprio-<br>ceptive) sensory neurons differentiate before the small-<br>diameter cutaneous afferent (e.g. pocicentive) neurons development, large-diameter muscle afferent (proprio-<br>ceptive) sensory neurons differentiate before the small-<br>diameter cutaneous afferent (e.g. nociceptive) neurons (equive) sensory neurons differentiate before the small-<br>diameter cutaneous afferent (e.g. nociceptive) neurons<br>(figure 5*d*). In *Ngn2* single knockouts, development of the<br>early differentiating large-diameter muscle affe 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 (M<sub>2</sub> *et al* 1999) By cont (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 ne early differentiating large-diameter muscle afferents is<br>temporarily blocked (Ma *et al.* 1999). By contrast, in *Ngn1*<br>single mutants the majority of these neurons (65–70%)<br>are unaffected while the generation of the smal temporarily blocked (Ma *et al.* 1999). By contrast, in *Ngnl* single mutants the majority of these neurons (65–70%) are unaffected while the generation of the small-diameter cutaneous afferents is almost completely preve single mutants the majority of these neurons  $(65-70%)$ <br>are unaffected while the generation of the small-diameter<br>cutaneous afferents is almost completely prevented (Ma *et*<br> $aI_{1}1999$ ) *al.* 1999).

cutaneous afferents is almost completely prevented (Ma et al. 1999).<br>This genetic segregation corresponds remarkably well<br>to two types of sensory neuron precursors identified by This genetic segregation corresponds remarkably well This genetic segregation corresponds remarkably well<br>to two types of sensory neuron precursors identified by<br>retroviral lineage analysis in the chick almost ten years<br> $200$  (Frank  $\&$  Sanes 1991). In these studies  $\frac{ln($ to two types of sensory neuron precursors identified by<br>retroviral lineage analysis in the chick almost ten years<br>ago (Frank & Sanes 1991). In these studies, *lac*Z-<br>expressing replication-incompetent retroviruses were<br>inj ago (Frank & Sanes 1991). In these studies,  $lac\zeta$ -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

expression of  $\beta$ -galactosidase perdures for several days in<br>sensory neurons after the endogenous gene is<br>extinguished, has failed to reveal any evidence of *lac* $\zeta$  t<br>expression in sympathetic ganglia (L. Lo. C. Fode tent with the idea that expression of  $Ngn2$  marks a subset correspond to muscle afferents (figure 5*a*,*c*). The other<br>of crest cells fated to generate sensory neurons early in population, which was not labelled until aft characterized by position and morphology. One populacharacterized by position and morphology. One population of precursors, which was labelled only when injections were performed between St13 and St15 produced 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 tion 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 tions were performed between St13 and St15, produced<br>small clones (average three cells per clone) whose neuronal<br>complement consisted exclusively of large-diameter<br>neurons in the VL region of the ganglia which probably small clones (average three cells per clone) whose neuronal<br>complement consisted exclusively of large-diameter<br>neurons in the VL region of the ganglia, which probably<br>correspond to muscle afferents (figure 5*a c*). The oth complement consisted exclusively of large-diameter<br>neurons in the VL region of the ganglia, which probably<br>correspond to muscle afferents (figure 5*a*,*c*). The other<br>nonulation which was not labelled until after St15 and neurons in the VL region of the ganglia, which probably<br>correspond to muscle afferents (figure  $5a,c$ ). The other<br>population, which was not labelled until after St15 and<br>was found un until St19 produced clones ten times la correspond to muscle afferents (figure  $5a,c$ ). The other population, which was not labelled until after St15 and<br>was found up until St19, produced clones ten times larger<br>that contained both VL neurons and small-diameter<br>neurons in the dorsomedial (DM) region of the ganglia was found up until St19, produced clones ten times larger<br>that contained both VL neurons and small-diameter<br>neurons in the dorsomedial (DM) region of the ganglia<br>(figure 5*h c*) which are mostly cutaneous afferents (Frank neurons in the dorsomedial (DM) region of the ganglia (figure  $5b,c$ ), which are mostly cutaneous afferents (Frank neurons in the dorsomedial (DM) region of the ganglia<br>(figure 5*b*,*c*), which are mostly cutaneous afferents (Frank<br>& Sanes 1991). These two lineages correspond remarkably<br>well to the sensory neuron subclasses affected i (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 & Sanes 1991). These two lineages correspond remarkably<br>well to the sensory neuron subclasses affected in the  $Ngn<sup>2</sup>$ <br>and  $Ngn<sup>1</sup>$  single mutants, suggesting that the early VL-<br>only precursor is  $Ngn<sup>2</sup>$  depende well to the sensory neuron subclasses affected in the  $Ngn2$ <br>and  $Ngn1$  single mutants, suggesting that the early VL-<br>only precursor is  $Ngn2$  dependent, and the later VL + DM and *Ngn1* single mutants, suggesting that the early VL-<br>only precursor is *Ngn2* dependent, and the later VL + DM<br>precursor is *Ngn1* dependent (Ma *et al.* 1999) (figure 5*a*,*b*).<br>What does the secrecation of two senso

What does the segregation of two sensory sublineages have to do with the segregation of the sensory and autoprecursor is *NgnI* dependent (Ma *et al.* 1999) (figure 5*a,b*).<br>What does the segregation of two sensory sublineages<br>have to do with the segregation of the sensory and auto-<br>nomic lineages? Although Frank & Sanes (1991) What does the segregation of two sensory sublineages<br>have to do with the segregation of the sensory and auto-<br>nomic lineages? Although Frank & Sanes (1991) did not<br>attemnt to trace the lineage relationship between VI-only have to do with the segregation of the sensory and auto-<br>nomic lineages? Although Frank & Sanes (1991) did not<br>attempt to trace the lineage relationship between VL-only<br>or VI + DM sensory precursors and sympathetic peurons nomic lineages? Although Frank & Sanes (1991) did not<br>attempt to trace the lineage relationship between VL-only<br>or VL+DM sensory precursors and sympathetic neurons,<br>the annarent correspondence of the VL-only precursors 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 or VL + DM sensory precursors and sympathetic neurons,<br>the apparent correspondence of the VL-only precursors<br>to *Ngn2*-dependent precursors provides an indirect link. If<br>VL-only precursors are *Ngn2* dependent then for rea the apparent correspondence of the VL-only precursors<br>to *Ngn2*-dependent precursors provides an indirect link. If<br>VL-only precursors are *Ngn2* dependent, then for reasons<br>discussed earlier (8.9) they are probably fated t 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 symmathetic neurons. It fol 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 an discussed earlier  $(\S 9)$  they are probably fated to generate sensory but not sympathetic neurons. It follows that VL-<br>only precursors probably generate only sensory and not<br>sympathetic neurons. Consistent with this conclusion, the<br>sensory neurons, that differentiate from the apparen only precursors probably generate only sensory and not<br>sympathetic neurons. Consistent with this conclusion, the<br>sensory neurons that differentiate from the apparently<br>committed precursors in rat neural crest explant cultu sympathetic neurons. Consistent with this conclusion, the<br>sensory neurons that differentiate from the apparently<br>committed precursors in rat neural crest explant cultures<br>express a muscle afferent rather than a cutaneous a sensory neurons that differentiate from the apparently committed precursors in rat neural crest explant cultures<br>express a muscle afferent rather than a cutaneous afferent<br>phenotype (Greenwood *et al.* 1999). This suggests that<br>they correspond to the VL-only precursors identif 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 they corresp<br>the chick and<br>the mouse.<br>In summ E chick and the *Ngn2*-dependent precursors identified in<br>
E mouse.<br>
In summary, the foregoing observations make a<br>
counstantial case that the neural crest contains a subset

the mouse.<br>In summary, the foregoing observations make a<br>circumstantial case that the neural crest contains a subset In summary, the foregoing observations make a<br>circumstantial case that the neural crest contains a subset<br>of early-emigrating,  $Ngn2$ -expressing precursors that are<br>restricted to a sensory fate and that these precursors circumstantial case that the neural crest contains a subset<br>of early-emigrating,  $Ngn2$ -expressing precursors that are<br>restricted to a sensory fate, and that these precursors<br>generate, the early-differentiating subclass of of early-emigrating, *Ngn2*-expressing precursors that are<br>restricted to a sensory fate, and that these precursors<br>generate the early-differentiating subclass of muscle<br>afferent sensory neurons That conclusion still leaves restricted to a sensory fate, and that these precursors<br>generate the early-differentiating subclass of muscle<br>afferent sensory neurons. That conclusion still leaves open<br>the possibility that the later-differentiating NonL generate the early-differentiating subclass of muscle<br>afferent sensory neurons. That conclusion still leaves open<br>the possibility that the later-differentiating, *Ngn1*-<br>dependent VL+DM sensory lineage derives from a<br>diffe different subset of migratory neural crest cells, that has dependent VL+DM sensory lineage derives from a<br>different subset of migratory neural crest cells, that has<br>both sensory and autonomic potential. Alternatively,<br>precursors of cutaneous afferent sensory neurons may be different subset of migratory neural crest cells, that has<br>both sensory and autonomic potential. Alternatively,<br>precursors of cutaneous afferent sensory neurons may be<br>distinct from autonomic precursors as well both sensory and autonomic potential.<br>precursors of cutaneous afferent sensory neu<br>distinct from autonomic precursors as well. distinct from autonomic precursors as well.<br>**11. PERSPECTIVE** 

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

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**PHILOSOPHICAL**<br>TRANSACTIONS

**BIOLOGICAL** 

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

eviously in most of the recent review literature about<br>ural crest cell lineage segregation.<br>According to such a model, the sequential timing of the<br>lonization of sympathetic and sensory ganglia (Serbedneural crest cell lineage segregation.<br>According to such a model, the sequential timing of the<br>colonization of sympathetic and sensory ganglia (Serbed-According to such a model, the sequential timing of the colonization of sympathetic and sensory ganglia (Serbed-<br>zija *et al.* 1990) would reflect the sequential emigration of<br>progenitors with first autonomic and then sens colonization of sympathetic and sensory ganglia (Serbed-<br>zija *et al.* 1990) would reflect the sequential emigration of<br>progenitors with first autonomic and then sensory capa-<br>city from the trunk neural tube. Both types of progenitors with first autonomic and then sensory capa-<br>city from the trunk neural tube. Both types of progenitors progenitors with first autonomic and then sensory capa-<br>city from the trunk neural tube. Both types of progenitors<br>could also be generated concurrently, explaining the over-<br>lanning generation of sensory and sympathetic ga city from the trunk neural tube. Both types of progenitors<br>could also be generated concurrently, explaining the over-<br>lapping generation of sensory and sympathetic ganglia in<br>some species (Serbedzija et al. 1989). It is po could also be generated concurrently, explaining the over-<br>lapping generation of sensory and sympathetic ganglia in<br>some species (Serbedzija *et al.* 1989). It is possible that the<br>segregation of the sensory and autonomic Lapping generation of sensory and sympathetic ganglia in<br>La some species (Serbedzija *et al.* 1989). It is possible that the<br>
Segregation of the sensory and autonomic lineages can 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 segregation of the sensory and autonomic lineages can<br>also occur shortly after, rather than before, neural crest<br>cells emigrate from the neural tube (figure 6*a*). This<br>would explain the observation of dual sensory–autonom also occur shortly after, rather than before, neural crest<br>cells emigrate from the neural tube (figure 6*a*). This<br>would explain the observation of dual sensory-autonomic<br>progenitors among the few migrating crest cells mar O cells emigrate from the neural tube (figure 6*a*). This<br>would explain the observation of dual sensory–autonomic<br>progenitors among the few migrating crest cells marked<br>in the single-cell lineage analysis (Fraser & Bronner would explain the observation of dual sensory–autonomic<br>progenitors among the few migrating crest cells marked<br>in the single-cell lineage analysis (Fraser & Bronner-<br>Fraser 1991). However, the idea that most crest cells ha progenitors among the few migrating crest cells marked<br>in the single-cell lineage analysis (Fraser & Bronner-<br>Fraser 1991). However, the idea that most crest cells have<br>made a choice between sensory and autonomic lineages Fraser 1991). However, the idea that most crest cells have made a choice between sensory and autonomic lineages Fraser 1991). However, the idea that most crest cells have<br>made a choice between sensory and autonomic lineages<br>before leaving the neural tube would explain why migra-<br>tory progenitors with both sensory and autonomic fates made a choice between sensory and autonomic lineages<br>before leaving the neural tube would explain why migra-<br>tory progenitors with both sensory and autonomic fates<br>were in the statistical minority in these experiments before leaving the neural tube would explain why mighty progenitors with both sensory and autonomic f<br>were in the statistical minority in these experiments.<br>The notion of an early segregation of the sensory tory progenitors with both sensory and autonomic fates<br>were in the statistical minority in these experiments.<br>The notion of an early segregation of the sensory and

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

cant amount of pre-patterning of pre-migratory trunk neural crest cells occur in the trunk neural tube. There is cant amount of pre-patterning of pre-migratory trunk<br>neural crest cells occur in the trunk neural tube. There is<br>precedent for such pre-patterning in the specification of<br>different subsets of dorsal interneurons (Liem *et* neural crest cells occur in the trunk neural tube. There is<br>precedent for such pre-patterning in the specification of<br>different subsets of dorsal interneurons (Liem *et al.* 1997).<br>In that case, different tupes of interneu precedent for such pre-patterning in the specification of<br>different subsets of dorsal interneurons (Liem *et al.* 1997).<br>In that case, different types of interneurons are generated<br>within a relatively small distance from o different subsets of dorsal interneurons (Liem *et al.* 1997).<br>In that case, different types of interneurons are generated<br>within a relatively small distance from one another<br>according to differences in the concentration In that case, different types of interneurons are generated<br>within a relatively small distance from one another<br>according to differences in the concentration and/or iden-<br>tity of transforming growth factor-8 family morphog within a relatively small distance from one another<br>according to differences in the concentration and/or iden-<br>tity of transforming growth factor- $\beta$  family morphogens<br>produced by the roof plate. It seems reasonable to th according to differences in the concentration and/or identity of transforming growth factor- $\beta$  family morphogens<br>produced by the roof plate. It seems reasonable to think<br>that such a mechanism could be used equally well tity of transforming growth factor- $\beta$  family morphogens<br>produced by the roof plate. It seems reasonable to think<br>that such a mechanism could be used equally well to<br>produce restrictions in the developmental potentials o produced by the roof plate. It seems reasonable to think<br>that such a mechanism could be used equally well to<br>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 I thank past and present members of my laboratory who have<br>contributed data and ideas to our studies of the neural crest.<br>These include Derek Stemple, Nirao Shah, Liching Lo, Amy I thank past and present members of my laboratory who have<br>contributed data and ideas to our studies of the neural crest.<br>These include Derek Stemple, Nirao Shah, Liching Lo, Amy<br>Greenwood Pat White Sherry Perez, Iane John contributed data and ideas to our studies of the neural crest.<br>These include Derek Stemple, Nirao Shah, Liching Lo, Amy<br>Greenwood, Pat White, Sherry Perez, Jane Johnson, Lukas Som-These include Derek Stemple, Nirao Shah, Liching Lo, Amy<br>Greenwood, Pat White, Sherry Perez, Jane Johnson, Lukas Sommer, Joseph Verdi and Qiufu Ma. The perspective presented<br>here is the author's and is not necessarily repr Greenwood, Pat White, Sherry Perez, Jane Johnson, Lukas Sommer, Joseph Verdi and Qiufu Ma. The perspective presented<br>here is the author's and is not necessarily representative of these<br>other individuals I am grateful to Am mer, Joseph Verdi and Qiufu Ma. The perspective presented<br>here is the author's and is not necessarily representative of these<br>other individuals. I am grateful to Amy Greenwood for helpful<br>comments on the manuscript. The au here is the author's and is not necessarily representative of these<br>other individuals. I am grateful to Amy Greenwood for helpful<br>comments on the manuscript. The author is an Investigator of<br>the Howard Hughes Medical Insti other individuals. I am grateful to Amy<br>comments on the manuscript. The auth<br>the Howard Hughes Medical Institute.

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