Specification and connectivity of neuronal subtypes in the sensory lineage

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Abstract | During the development of the nervous system, many different types of neuron are produced. As well as forming the correct type of neuron, each must also establish precise connections. Recent findings show that, because of shared gene programmes, neuronal identity is intimately linked to and coordinated with axonal behaviour. Peripheral sensory neurons provide an excellent system in which to study these interactions. This review examines how neuronal diversity is created in the PNS and describes proteins that help to direct the diversity of neuronal subtypes, cell survival, axonal growth and the establishment of central patterns of modality-specific connections.

Neurotrophic tyrosine receptor kinases (Trks). Trk receptors are high-affinity tyrosine kinase receptors for the neurotrophins, with TrkA mainly activated by nerve growth factor, TrkB by brain-derived neurotrophic factor and neurotrophin 4 (NT4), and TrkC by NT3.

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doi:10.1038/nrn2057

The nervous system consists of many different types of neuron. How are all these types formed, and how do their axons establish specific, functional circuits? These fundamental questions remain largely unanswered. Cell fate decisions and phenotypic differentiation are governed by signalling from local organizing centres. This signalling regulates intrinsic transcriptional programmes that, in concert, direct cell fate commitment.

The multipotent trunk neural crest can generate many types of cell, including sensory, sympathetic and enteric neurons of the PNS (FIG. 1a,b). The neural crest is both technically and conceptually an attractive model system in which to investigate how neuronal diversity is created. Under strict spatiotemporal control by signals from the neural tube, neural crest cells (NCCs) delaminate from the neural tube and some migrate ventrally between the dermamyotome and the neural tube to generate the dorsal root ganglia (DRG)[1]. During migration and upon condensation into a ganglion, NCCs are exposed both spatially and temporally to signals from the adjacent somites and spinal cord, which drive the generation of multiple types of sensory neuron in the DRG (FIG. 1b,c). These neurons enable us to sense touch, warm, cold, pain, limb movements and limb spatial position.

Different types of DRG neuron are specialized for the different perceptual modalities. Each functional type of sensory neuron has unique molecular characteristics, contains unique sets of ion channels and responds to unique sets of stimuli. Such types include small diameter neurons with thinly myelinated or unmyelinated axons that either contain or do not contain neuropeptides (such as neurotrophic tyrosine receptor kinase A-containing (TrkA$^+$) peptidergic and TrkA$^-$ non-peptidergic neurons), most of which respond to noxious stimuli (nociceptive neurons) and mediate pain sensations, larger diameter low threshold mechanoreceptive neurons conveying mechanical sensations (for example, touch; these include TrkB$^+$ and/or TrkC$^+$ neurons) and large proprioceptive neurons that sense limb movement and position (TrkC$^+$ neurons). Sensory neurons have stereotypical terminations that generate highly reproducible patterns of connectivity in the spinal cord. The experience of different perceptions depends on where the sensory neurons terminate in the spinal cord (FIG. 1c). The development of the molecular characteristics and receptive properties of sensory neurons must therefore be tightly linked to their central terminations. These properties of sensory neurons make them particularly attractive for studying how neuronal subtypes are created and how subtype-specific gene programmes integrate with the establishment of precise contacts between neurons.

This review focuses on current thinking about how diversity is created in the sensory PNS and how it is coordinated with the establishment of central connectivity. We start at the time of neural crest induction and follow these cells through migration, neurogenesis, subtype specification, axonal growth and the establishment of precise points of contacts in the CNS.

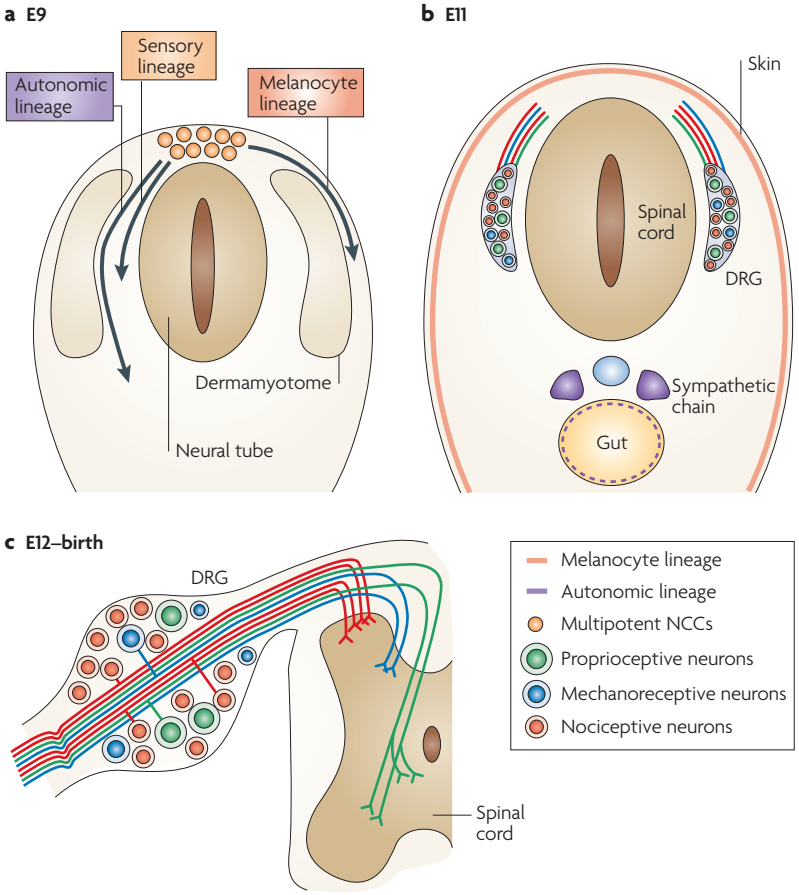

Figure 1 | **Neural crest migration, sensory neurogenesis and the establishment of modality-specific connections. a** | Trunk neural crest cells (NCCs) delaminate from the dorsal neural tube and migrate along a ventral pathway to produce cells of the dorsal root ganglion (DRG; sensory lineage), and sympathetic and enteric neurons (autonomic lineage). Other cells take a dorsolateral pathway, colonizing the skin with pigment cells (melanocytes). **b** | During migration and shortly after coalescing into a DRG, NCCs commit to a sensory and neuronal fate and diversify into principal sensory subtypes such as nociceptive, mechanoreceptive and proprioceptive neurons. **c** | Only after neuronal subtypes are specified are modality-specific central fields of terminations established. Modality-specific terminations are outlined schematically, with nociceptive neurons terminating in the dorsal horn, mechanoreceptors in deeper laminae of the dorsal spinal cord, proprioceptive neurons in the intermediate zone (Golgi tendon organ Ib afferents and muscle spindle Ia afferents) and in the ventral spinal cord horn (muscle spindle Ia afferents). E, embryonic day.

Induction, migration and neurogenesis

The neural crest is found at the border between the ectoderm and the neural plate, and there is evidence that specification occurs as early as gastrulation[2]. Neural crest induction is thought to involve bone morphogenetic protein (BMP) and the signalling factor WNT. BMP is necessary for maintaining WNT expression, and WNT factors are sufficient to induce *de novo* NCCs from the tips of the neural folds[3]. Under the influence of these signals, cells of the dorsal neural tube undergo an epithelial-to-mesenchymal transition into NCCs. This requires a pronounced cytoskeletal reorganization, downregulation of N-cadherin and cadherin 6, and changing the cell adhesion properties to become highly motile[4–6]. The basal lamina around the neural tube dissolves transiently, and NCCs begin to leave the neural tube (between embryonic day (E) 8.5 and E10 in the mouse[7], and stages 11–21 in the chick[8–10]). The NCCs migrate in chain-like structures[11] to form the DRG in a ventral to dorsal order.

During NCC migration, sensory neurogenesis occurs in three successive waves (FIG. 2). Two of these waves have been followed using retroviral tracing of single NCCs labelled in the chick neural tube to clonally examine the lineage of neurons and glia in the DRG[8]; corresponding waves seem also to exist in the mouse[12]. A third wave, which emerges significantly later, was recently described[13]. The cells in the third wave come from the boundary cap, a cellular structure located at the entry and exit points of motor and sensory neurons, at the boundary between the PNS and the CNS[14], and consist of multipotent neural crest stem cells[15,16]. Early migrating NCCs, which represent one-third of the NCCs that delaminate from the spinal cord to produce the DRG, show limited cell division. Each produces, on average, 3.1 neurons, which are restricted to the ventrolateral region of the chick DRG[8] where the large mechanoreceptive and proprioceptive types expressing the neurotrophin receptors TrkB and/or TrkC (TrkB/TrkC) are located[17] (about 4% of the adult neurons in the DRG). The remaining two-thirds of the migrating NCCs generate an average of 35.9 neurons each, with neurogenesis continuing for a longer time[8]. These NCCs produce neurons located both in the dorsomedial region of the DRG, where small neurons that express TrkA reside[17], and in the ventrolateral region[8]. Both types of NCC clone are also equally competent to generate glia[8] (FIG. 2). These observations are consistent with findings that multipotent NCCs delaminate from the neural tube and, during migration, a first wave of neurogenesis occurs[18], producing large TrkB/TrkC neurons. Most NCCs commit to a neuronal fate after migration and DRG condensation. Consistently, these cells produce more progeny by maintaining a continuously high rate of proliferation and by expression of the high-mobility group transcription factor SRY (sex determining region Y) box 10 (SOX10) (REF. 18), which confers multipotency to the neural crest[19] (FIGS 2,3). In a third wave of neurogenesis, the boundary cap cells, which are among the last to be produced from the NCCs (emerging at E11 in the rat and E10.75 in the mouse[13,14,20]), generate about 5% of almost exclusively small TrkA$^+$ DRG neurons in the adult[13]. In addition, the boundary cap cells, like earlier NCCs, also generate peripheral glia[13].

These results show that the specification of sensory neuron subtypes is intimately linked to the timing of delamination of NCCs from the neural tube. However, at present only the fate and not the competence of the NCC precursors that make up the three waves has been addressed.

Sensory neuron specification

Cell specification involves setting into place the transcriptional programmes necessary for a specific path of differentiation, such that the cellular fate is acquired even in the absence of any further extrinsic signals. In the neural crest, neurogenesis and specification to the sensory lineage are linked, and there is also a correlation between proneural transcription factors driving

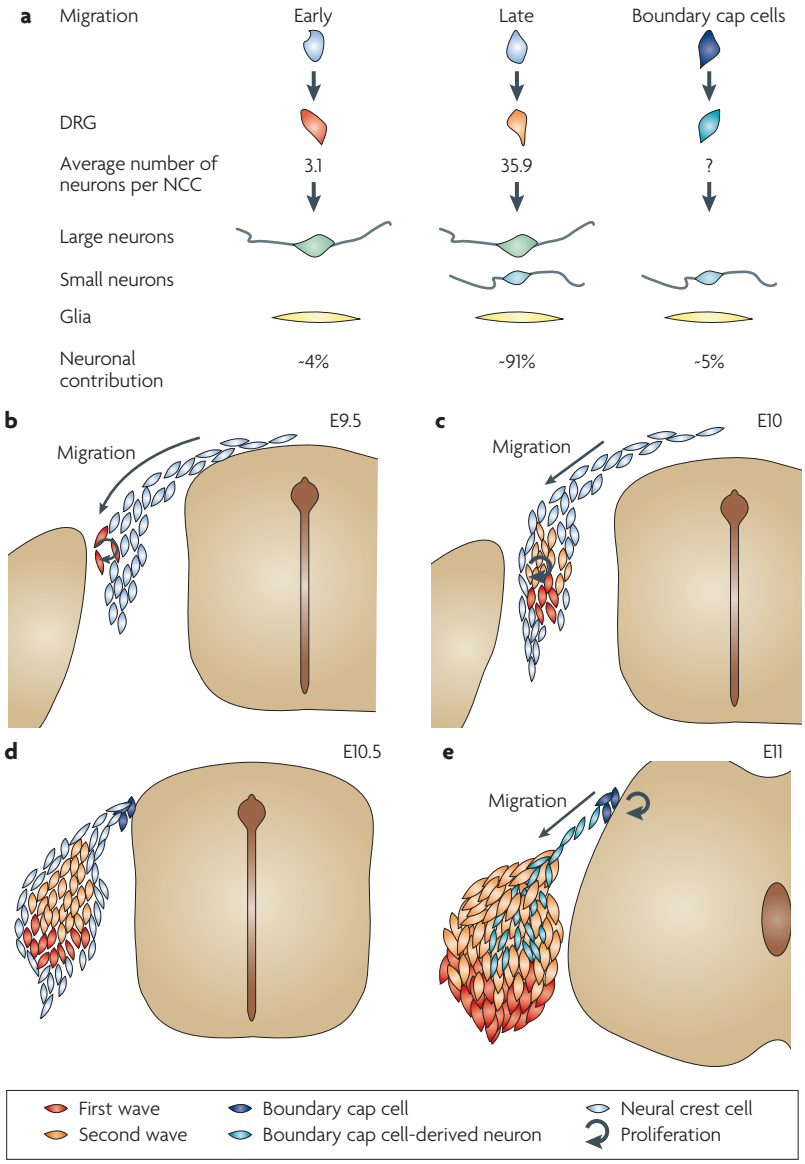

Figure 2 | **Three waves of neurogenesis in the sensory neuron lineage. a** | During development, dorsal root ganglion (DRG) neurons arise from three waves of neurogenesis. In the chick, large proprioceptive neurons are born first, followed by small neurons. Two types of neural crest cell (NCC) with different fates are present. NCCs that produce only large (mechanoreceptive and proprioceptive) neurons expressing neurotrophic tyrosine receptor kinase B (*TrkB*) and/or *TrkC* (an average of 3.1 neurons per NCC) appear only at earlier stages of NCC delamination and migration. NCCs that produce all subtypes of DRG neuron (an average of 35.9 neurons per NCC) are generated throughout NCC migration[8]. As one-third of the migrating NCCs are of the first type and two-thirds are of the second, these two waves of neurogenesis contribute to an estimated 4% (first wave) and 91% (second wave) of the DRG neuronal population. The third wave — boundary cap cells that contribute ~5% of adult mouse DRG neurons — generates mainly TrkA$^+$ small neurons that largely consist of nociceptors[13]. **b** | The first wave of neurogenesis of multipotent NCCs takes place during migration of the NCCs at the level of the rostral part of the dorsal somitic lip[18]. These NCCs have limited cell division and contribute to large (mechanoreceptive and proprioceptive) neurons in the DRG. **c** | The NCCs migrating later to the DRG have a high rate of proliferation. The second wave of neurogenesis occurs in postmigratory cells in the ganglion and results in all subtypes of sensory neuron. **d** | The neural crest-derived multipotent boundary cap cells can be identified at embryonic day (E) 10.5 in the mouse in the dorsal root entry zone of the spinal cord[13,16]. **e** | Starting in late E10, boundary cap cells proliferate and their progenies migrate into the DRG and assume a sensory neuron fate (mainly small TrkA$^+$ neurons). Thickness of curved arrows indicates different rates of proliferation.

neurogenesis in the sequential waves and the appearance of specific sensory subtypes. The basic helix-loop-helix transcription factors neurogenin 1 (NGN1) and neurogenin 2 (NGN2) are required for neurogenesis and the specification of peripheral sensory neurons[12,21–24]. Neurogenins bias NCCs to the sensory as opposed to the autonomic lineage (which arises from expression of the proneural gene mammalian achaete-scute homologue 1 (*Mash1*))[21,25]. NGN2 initiates a first wave of neurogenesis[12] (FIG. 3). *Ngn2* is expressed in a subset of migratory NCCs that are derived from a location close to the dorsal neural tube and continues to be expressed throughout their migration into the coalescing DRG, after which it is largely downregulated[12,21,26]. Early migratory cells that express *Ngn2* at high levels might acquire a neuronal fate during migration before NCCs that express little or no *Ngn2* (REF. 18). *Ngn1* is expressed in most cells within the coalesced DRG, including those expressing little or no *Ngn2* that have not already undergone neurogenesis[18,25] (FIG. 3). NGN1 initiates a second wave of neurogenesis in SOX10$^+$ multipotent cells within the DRG (FIG. 3).

These two waves of NGN2- and NGN1-mediated neurogenesis might correspond to the two distinct clonal neural crest populations identified in the chick[8,12]. The first, NGN2-mediated wave of neurogenesis can be concluded to produce only TrkB/TrkC mechanoreceptive and proprioceptive neuronal subtypes, that might all arise from a TrkC$^+$ population, whereas the second, NGN1-initiated wave of neurogenesis produces small TrkA as well as large TrkB/TrkC neurons within the DRG (FIG. 3). The conclusion that the NGN1-mediated wave contributes to both small TrkA and large TrkB/TrkC neurons is corroborated by the finding that in *Ngn2*-null mutant mice, NGN1 is sufficient to allow large TrkB/TrkC neurons to develop normally in the DRG[12]. Furthermore, in a chick study, the large clone size that corresponds to the NGN1-mediated wave contributes to both large ventrolateral and small dorsomedial neurons of the DRG[8]. A lineage-tracing study using *Ngn2–Cre* mice provides evidence contrary to the conclusion that the NGN2-mediated wave produces only large TrkB/TrkC neurons; it demonstrates that *Ngn2*-expressing cells also contribute significantly to TrkA$^+$ neurons[25]. Recent data indicate that these neurons could represent the low *Ngn2*-expressing cells escaping the first wave of neurogenesis[18] (FIG. 3), but which express enough Cre to label the lineage in the tracing experiment. This is consistent with findings that NGN2 is unable to compensate for the loss of NGN1 during development, as *Ngn1*-null mutant mice lack small TrkA$^+$ neurons[12]. Co-expression of *Sox10* with graded levels of *Ngn2* in early migratory cells and with *Ngn1* in the condensed DRG indicates that neurogenesis involves a gradual increase of neurogenin activities that is correlated with a loss of *Sox10* expression and multipotency[18,25]. NGN2 and NGN1 specify NCCs to the sensory lineage, and their presence correlates with distinct waves of neurogenesis, but there is no evidence that the different neurogenins specify sensory neurons to distinct subtypes.

Basic helix-loop-helix
Protein structural motif characterized by two α-helices connected by a loop, with one helix containing basic amino acids facilitating DNA binding. This motif is present in certain families of transcription factors.

Proneural gene
Generic name for genes expressed in neural progenitors that promote pan-neuronal differentiation.

WNT1 and WNT3A, which are produced in the dorsal neural tube during neural crest delamination and migration, have important roles in directing NCCs towards the sensory lineage[27,28]. In mice and cultured NCCs with disrupted β-catenin, the intracellular mediator of the canonical WNT signalling pathway, Sox10$^+$, multipotent NCCs fail to express neurogenins and to differentiate into sensory neurons[29,30]. In gain-of-function studies in which a constitutively active form of β-catenin is expressed in the neural crest, NCCs terminate migration prematurely and aggregate into ectopic ganglion-like structures which express *Ngn2* (REF. 31). Similarly, sympathetic neurons of the superior cervical ganglion and trunk sympathetic chain lose expression of the sympathetic neurogenic protein MASH1 and instead produce NGN2. These *in vivo* results indicate that WNT and β-catenin have an instructive role in the specification of the sensory lineage. This conclusion is corroborated by data on WNT signalling in clonal neural crest cultures. In the presence of WNT1, 79.6% of neural crest clones generate BRN3A$^+$ sensory neurons, with 95.4% being sensory neuron-only clones. By contrast, BMP2 induces 90.5% of the clones to adopt an autonomic fate, and again nearly all are autonomic-only clones[31]. The ability of WNT1 and high levels of BMP to direct nearly all NCCs in culture to become either sensory or autonomic neurons indicates that most migrating NCCs are multipotent and can generate both sensory and autonomic neurons. The requirement and sufficiency of WNT signalling for *Ngn2* expression in the migratory neural crest indicates that WNT helps to establish at least the first wave of sensory neurogenesis, which produces large neurons.

Apart from WNT and β-catenin, BMPs are also important for sensory neuron specification. Like WNT1 and WNT3A, they are expressed in the dorsal neural tube during NCC delamination and migration. Although high concentrations of BMP2 promote an autonomic fate in clonal cultures, acting instructively at the expense of

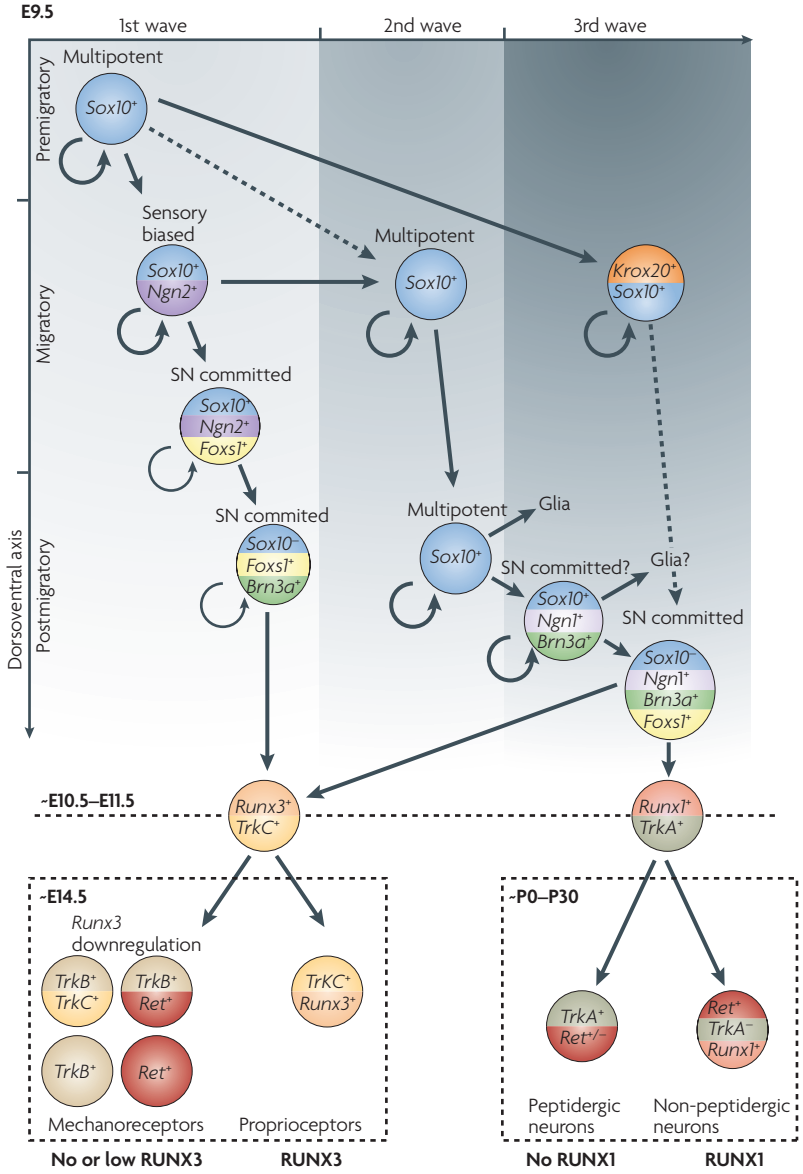

Figure 3 | **Genetic cascades that control neurogenesis and subtype specification.** Distinct genetic cascades control the first two waves of neurogenesis in the dorsal root ganglion (DRG). The vertical axis represents the dorsoventral position and migratory status of the cells during development. In the first wave, SRY (sex determining region Y) box 10-containing (SOX10$^+$) cells migrate and express neurogenin 2 (*Ngn2*), which biases them towards a sensory fate. Cells with high levels of NGN2 subsequently commit to a sensory neuronal fate (SN) as defined by the expression of the forkhead transcription factor *Foxs1* during migration[18]; other cells with low or no NGN2 remain uncommitted (hatched arrow and horizontal arrow to SOX10$^+$ migrating cell). Postmigratory pioneering neurons of the first wave express *Brn3a* and form large proprioceptive and mechanoreceptive neurons expressing runt-related transcription factor 3 (*Runx3*) and neurotrophic tyrosine receptor kinase C (*TrkC*) at early developmental stages[8,12,61,63,65]. In the second wave of neurogenesis, cells are characterized by the continuous expression of *Sox10* throughout migration and in the DRG, where they continue to divide at a high rate[18]. These cells start to express *Foxs1*, *Brn3a* and *Ngn1* in the DRG[18], before they express RUNX factors. They might produce both the TrkA$^+$ and TrkC$^+$ populations of neurons[8] by expressing *Runx1* or *Runx3*, respectively[36,62,65]. The third wave of neurogenesis arises from boundary cap cells expressing *Sox10* and *Krox20*; they contribute mainly to the RUNX1/TrkA population of neurons and to glia[13,16]. The genetic cascade that controls this wave of neurogenesis is unknown. Thickness of curved arrows indicates different rates of proliferation. The level of RUNX proteins helps to control the diversification of sensory neurons into different cell types[61–63] as shown at the bottom of the figure. Cells from the first wave that maintain *Runx3* expression keep TrkC and become proprioceptors, whereas cells that lose or reduce *Runx3* expression make TrkB and TrkC, TrkB alone, RET alone or RET and TrkB mechanoreceptive neurons[61,63]. Among small neurons, maintained *Runx1* expression drives a TrkA$^-$ non-peptidergic phenotype (*TrkA*$^-$/*Ret*$^+$/*Runx1*$^+$ cells), whereas downregulation of *Runx1* allows the cells to acquire a peptidergic phenotype (*TrkA*$^+$/*Ret*$^{+/-}$/*Runx1*$^-$)[62]. E, embryonic day; P, postnatal day.

POU homeodomain
Family of homeodomain transcription factors containing a shared additional conserved sequence adjacent to the homeodomain referred to as POU (bipartite DNA-binding domain) and named after the initials of the founder members: PIT1, OCT1/2 and UNC86.

Zinc finger
Small structural domain found in several DNA interacting proteins organized around a zinc ion, consisting of two antiparallel β-strands and an α-helix.

other fates[32–34], and overexpression of BMP in the pre-migratory neural crest of the chick results in an increase in sympathetic neurons[35], low levels of BMP2 lead to an increase in sensory neurons[24]. So, different neural crest lineages are specified by distinct levels of BMP signalling. Premigratory and early migratory NCCs close to the dorsal tube would be expected to receive high BMP levels together with WNT, and in cultured cells this combination results in the maintenance of *Sox10* expression and the multipotency of cultured NCCs[30].

Sensory neuron subtype specification

Studies using gain- or loss-of-function approaches have shown that the neurogenins can initiate pan-neuronal programmes both *in vivo* and *in vitro*[21,36], but cannot further specify neuronal subtypes in the sensory lineage[25,36]. A starting point for understanding the transcriptional control of sensory subtype diversification is to investigate how expression of the TrkA, TrkB and TrkC neurotrophin receptors is controlled. These receptors are among the earliest known markers for sensory subtypes (BOX 1). They participate in several processes during sensory neuron development, including controlling cell survival during naturally occurring cell death, target innervation and the establishment of functional contacts between cells. They also direct the phenotypic maturation of the cells by regulating peptide and ion channel expression[37–39]. The genetic replacement of *TrkA* by *TrkC* in a knock-in animal led to a switch in fate of some of the small neurons to proprioceptive neurons, as shown by their cytochemical properties and termination patterns in the spinal cord[40]. The expression and consequent activation of Trk receptors are therefore important for turning neuronal progenitors, expressing general sensory neuronal traits initiated by proneural genes, into subclasses of sensory neuron. The *TrkA* promoter is the best characterized of the promoters of the genes for these receptors, and several transcription factors including Kruppel-like factor 7 (KLF7), BRN3A and runt-related transcription factor 1 (RUNX1) can drive its activation[36,41–44].

Foxs1, Brn3a and Klf7 in the sensory lineage. The forkhead transcription factor *Foxs1* is expressed in all of the sensory nervous system regardless of cellular origin and defines the sequential waves of neurogenesis during which NCCs commit to a sensory and neuronal fate[18] (FIG. 3). However, *Foxs1*-null mutant mice exhibit no sensory phenotype[18].

The POU homeodomain transcription factor *Brn3a*[45] is expressed in all sensory neurons[46–50]. Its expression begins early in DRG neurons, coincides with sensory specification and largely with the commitment of the NCCs to a neuronal fate[18,49,50], and is maintained at constant levels throughout life, probably through an auto-regulatory loop[51–53]. Targeted deletion of *Brn3a* results in the death of all subtypes of trigeminal sensory neuron[50,54,55], but neurons in the DRG are apparently not lost[55]. In trigeminal, but not DRG, sensory neurons, BRN3A is required for TrkC expression, as well as for the maintenance, but not the initiation, of TrkA and TrkB expression[54]. Loss of *Brn3a* results in cell death as a consequence of the failure of neurotrophins to sustain cell survival by activating Trk receptors[54]. BRN3A regulates TrkA expression directly by binding to two binding sites in the *TrkA* promoter[43,44]. BRN3A also influences other processes independently of its regulation of Trk receptors, including axonal growth and target innervation[52]. In addition, *in vitro*, BRN3A protects sensory neurons from apoptosis and regulates the expression of apoptotic proteins such as B-cell leukaemia/lymphoma-like 1 (BCLX), BCL2 and B-cell leukaemia/lymphoma 2-associated protein X (BAX)[56–59]. So, although BRN3A is a crucial factor in the sensory nervous system, it is not a key factor in the creation of neuronal diversity.

Expression of the Kruppel-like zinc finger transcription factor *Klf7* coincides with that of *TrkA* in neural crest-derived structures during development, including sensory and sympathetic neurons[41]. Loss of function of *Klf7* in mice leads to a reduction in *TrkA* but not *TrkB* or *TrkC* expression in DRG sensory neurons. The KLF7 binding site in the *TrkA* promoter cooperates with additional DNA binding proteins such as BRN3A[60]

Box 1 | Neurotrophic factors and sensory subtypes

In the vertebrate nervous system, most neuronal populations undergo a process of naturally occurring cell death, which often coincides with the time at which their axons reach their target tissue. At this point, neurons become susceptible to apoptosis and survive only if presented with sufficient neurotrophic factors. Naturally occurring cell death is thereby regulated by the availability of limited concentrations of neurotrophic factors produced by target cells. In the trunk dorsal root ganglia (DRGs), sensory neurons require nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and NT4. The neurotrophin receptors tyrosine receptor kinase A (TrkA), TrkB and TrkC are expressed in subpopulations of DRG sensory neurons[107–109]. The analysis of knockout mice for the neurotrophins and their receptors provided important information on the neurotrophin requirements of neuronal subpopulations in the PNS[39,110]. Different subpopulations of sensory neurons in the DRG depend on different neurotrophins. *NGF*$^{-/-}$ and *TrkA*$^{-/-}$ mice mainly lose the unmyelinated and small myelinated nociceptive DRG neurons, whereas NT3- and TrkC-deficient mice lose the large myelinated neurons that convey limb proprioception, including neurons that innervate Golgi tendon organs and muscle spindles[72]. Slowly adapting mechanoreceptors with Aβ fibres conduction velocity, which innervate specialized Merkel cells in the touch domes of hairy skin, and D-hair afferents, which are believed to terminate on hair follicles, depend on NT3 (REFS 111,112). Approximately 30% of DRG neurons are lost postnatally in mice lacking BDNF[113,114], whereas *NT4*$^{-/-}$ mice show no neuronal deficits[115]. BDNF-dependent neurons probably include myelinated large mechanoreceptive neurons. BDNF also regulates the sensitivity of slowly adapting mechanoreceptors in hairy skin, and in its absence the mechanical sensitivity but not the number of neurons is changed[116]. In *NT4*$^{-/-}$ mice, sensory functions are compromised and innervation of D-hair afferents of hair follicles is lost postnatally[117].

Ia muscle spindle afferent
Proprioceptive sensory afferent innervating the muscle spindles in the periphery and α-motor neuron centrally, distinct from the Ib afferent which innervates the Golgi tendon organs in the periphery.

α-motor neuron
Large motor neuron located in the spinal cord and the brainstem innervating extrafusal muscle fibres of skeletal muscle and responsible for their contraction, distinct from γ-motor neurons that innervate intrafusal muscle fibres.

Ret
Tyrosine kinase receptor activated by the glial cell line-derived neurotrophic factor family of ligand and named after rearranged during transformation because it is mutated in several cancer syndromes.

to drive full *TrkA* transcriptional activity in the trigeminal ganglion[42] and to produce diversity in the sensory lineage. The detailed expression of *Klf7* in the sensory lineage is not known.

***Runx3* and mechanoreceptive and proprioceptive subtypes.** The RUNX family of transcription factors have key roles in the diversification of sensory neurons[36,61–68] (BOX 2; FIG. 3). Preliminary findings indicate that two of the three members, *Runx1* and *Runx3*, are differentially expressed in DRG sensory neurons in a non-redundant manner such that RUNX1 is found in small TrkA$^+$ neurons and RUNX3 in large TrkC$^+$ neurons[65].

RUNX3 is first detected at E10.5 in DRG and cranial ganglia; at E12.5, it is confined to all TrkC$^+$ neurons[64]. *Runx3*$^{-/-}$ mice have severe limb ataxia as a result of disruption of the monosynaptic circuitry between Ia muscle spindle afferents and α-motor neurons and have a loss of *TrkC* expression, a loss of proprioceptive neurons and attenuation of expression of another marker for proprioceptive neurons, parvalbumin[64,66,67]. Blocking both RUNX1 and RUNX3 activities in the chick results in a loss of TrkA$^+$, TrkB$^+$ and TrkC$^+$ neurons[36]. These data indicate that RUNX3 is important for the establishment of an early TrkC$^+$ population from which large TrkB/TrkC neurons transducing mechanical and proprioceptive information arise (FIG. 3). Interestingly, in mice expressing a mutant RUNX3 lacking the carboxy-terminal VWRPY amino acid sequence, which is required for the interaction of RUNX3 with the co-repressor transducing-like enhancer of SPLIT (TLE), no loss of parvalbumin$^+$ neurons is observed[69], indicating that the ability of RUNX3 to tether TLE is not essential for the early development of proprioceptive neurons and suggesting that the early functions of RUNX proteins involve activator rather than repressor activities.

The ability of RUNX3 to repress, for example, *TrkB* expression[63] (see below) was not addressed in the study, although one would predict that consolidation of the proprioceptive phenotype is compromised in these mice because of a failure of *TrkB* downregulation in TrkC$^+$ proprioceptive neurons (see below).

RUNX3 might also be important at later embryonic stages. It seems to participate in the diversification into different subclasses of the early TrkC$^+$ population produced during the first and second waves of neurogenesis, including the TrkC$^+$ proprioceptors and several TrkB/TrkC neuronal subtypes. In the mouse, the expression of *TrkC* precedes that of *TrkB* and *Ret*, and all three proteins are present at E11.5 (REF. 63). Two distinct and transient populations of *TrkB*/*TrkC*- and *TrkB*/*Ret*-expressing DRG neurons exist at early stages. *TrkB*/*TrkC* co-expression drops between E11.5 and E12.5 from 75% to 10% and stops at E14.5 (REFS 63,70). Moreover, at E12, 80% of RET$^+$ neurons co-express *TrkB* but none co-expresses *TrkC*, whereas at E14.5, few RET$^+$ neurons still co-express any of the Trk receptors[63]. At these early stages, *Runx3* is expressed in 85% of TrkC$^+$ neurons, with a lower expression in the double TrkB$^+$/TrkC$^+$ neurons, whereas only 5% of RUNX3$^+$ neurons express *Ret* or *TrkB*[63]. Overexpressing *Runx3* in sensory neurons leads to downregulation of *TrkB* expression in many TrkC$^+$ neurons and Ret$^+$ neurons between E11 and E13.5 and a moderate overall increase in the number of TrkC$^+$ neurons[63]. Conversely, in *Runx3*$^{-/-}$ mice, there is a marked increase in TrkB$^+$ neurons[63]. So, continuous expression of *Runx3* is necessary to consolidate the proprioceptive TrkC$^+$ population of sensory neurons between E11 and E13.5, and its downregulation might be necessary for diversification into mechanoreceptive TrkB/TrkC subtypes (FIG. 3). These changes might take place before target innervation[71,72], indicating that local determinants regulate *Runx3* expression and thereby define many aspects of the mechanoreceptive and proprioceptive phenotypes.

***Runx1* and nociceptive subtypes.** *Runx1* is expressed specifically in an early TrkA$^+$ population during embryogenesis[36,62,65,68], whereas postnatally its expression becomes more restricted to a subtype of nociceptors[62] arising from the TrkA$^+$ population. RUNX1 is important for diversifying the early small diameter TrkA$^+$ population, mostly producing various types of nociceptive neurons (FIG. 3). Interestingly, RUNX1 seems to mediate factors that shape the nociceptive lineage by switching from being a transcriptional activator at earlier stages[36] to acting as a repressor at later embryonic stages[62]. During early sensory neuron development, RUNX1 is necessary for the emergence of the early small diameter TrkA$^+$ population in the chick without affecting large TrkB$^+$/TrkC$^+$ neurons or the expression of pan-neuronal genes, and without RUNX1 and RUNX3 activity all subtypes of sensory neuron die by apoptosis[36] (FIG. 3). RUNX1 activates *TrkA* expression directly at a binding site in the minimal *TrkA* promoter[36]. *Runx1* inactivation in the mouse leads to a loss of TrkA$^+$ trigeminal sensory neurons during neurogenesis, before the period

Box 2 | The RUNX family of transcription factors

The RUNX family of transcription factors is a small family, including RUNX1, RUNX2 and RUNX3. They share a homologous region, the 'RUNT domain', which binds to a DNA consensus core sequence[118–120]. RUNX factors regulate transcription by recruiting classical transcriptional modulators and are key regulators of lineage-specific gene expression in major developmental pathways, including the specification of neuronal cell types[36,61–64,66–68], proliferation of neuronal precursors[121], osteogenesis[122,123], hair shape determination[124] and control of the balance between cell proliferation and apoptosis of the gastric epithelium[125]. RUNX factors have been implicated in several forms of cancer, where they act as both tumour-suppressor genes and dominant oncogenes, according to the cellular context[120]. Because of their multiple facets of action, which mediate transactivation, repression or transcriptional blocking of gene programmes depending on the different partners present in a cell, RUNX factors have more recently been designated as transcriptional organizers[126].

Many isoforms of RUNX factors can be generated by two different promoters and alternative splicing[127–132], and the activities of the products are regulated both translationally and post-translationally[119,127,132–136]. Therefore, the ability of RUNX proteins to differentially regulate transcription[127,137–139] is related to the different protein isoforms and their capacity to interact with other transcriptional regulators[135,136]. Furthermore, RUNX factors are regulated by[84,125,140–151] and part of[136,152–156] most of the classical signalling pathways involved in developmental processes. At the post-translational level, RUNX factor activity and stability are also modulated by phosphorylation[157–159] and acetylation[142,160], which might also modulate the switch from acting as a repressor to an activator[158,161].

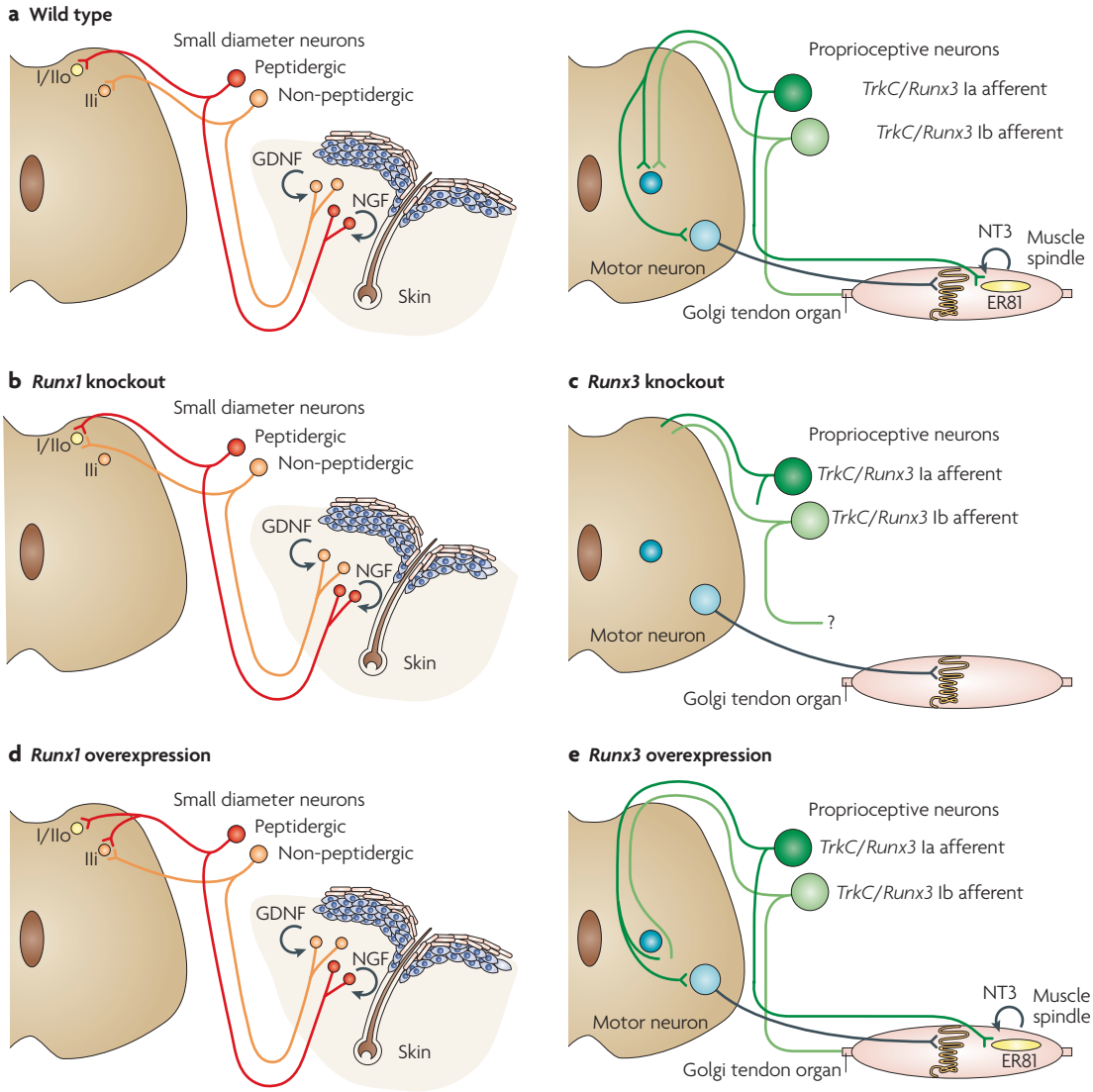

Figure 4 | **Functions of RUNX proteins in the establishment of central projection. a** | In the wild-type mouse, nociceptive neurons terminate in the dorsal layers of the spinal cord with peptidergic (neurotrophic tyrosine receptor kinase A-containing (*TrkA*$^+$)/runt-related transcription factor 1 (*Runx1*$^-$)) neurons in lamina I and the outer layer of lamina II (IIo) and non-peptidergic neurons (*Ret*$^+$/*Runx1*$^+$) in an adjacent, deeper lamina (inner lamina II, IIi)[37,162–165]. Proprioceptive neurons project ventrally in the spinal cord to deeper laminae; the muscle spindle Ia axons terminate in intermediate laminae and on motor neurons in the ventral horn (establishing the stretch reflex circuitry) and Golgi tendon organ Ib axons terminate in intermediate laminae. **b** | In *Runx1*$^{-/-}$ mice, projections of non-peptidergic neurons are redirected to the more superficial lamina I and IIo[62]. **c** | In *Runx3*$^{-/-}$ mice, proprioceptive neurons fail to establish central connections to the intermediate and ventral spinal cord and the neurons are eventually lost (the exact time of cell death is unknown)[64,66]. Peripheral projections necessary for the induction of muscle spindles are also lost at birth. **d** | Overexpression of *Runx1* induces an expansion of peptidergic (TrkA$^+$) axons to deeper layers of the dorsal spinal cord[63]. **e** | Overexpression of *RUNX3* in the chick induces redirection of proprioceptive afferents that normally terminate in the intermediate zone (Ib afferents) into the ventral spinal cord[61]. DRG, dorsal root ganglion; GDNF, glial cell line-derived neurotrophic factor; NGF, nerve growth factor; NT3, neurotrophin 3.

of programmed cell death[68]. Although defects in TrkA$^+$ neurons have not been studied embryonically, adult DRG neurons of conditional *Runx1*$^{-/-}$ mutant mice seem normal[62]. In these conditional *Runx1* mutant mice, the successful elimination of *Runx1* was confirmed to have taken place at E13. However, the TrkA$^+$ and TrkB$^+$/TrkC$^+$ sensory subtypes are, to a large degree, already specified at this time. So, although the early roles of RUNX1 in DRG have been studied in detail in the chick[36], its early function in the mouse remains to be determined. The action of RUNX1 as a gene activator is necessary in the mouse during embryonic stages for the expression of the transient receptor potential (TRP) channel family members TRPM8 (which mediates innocuous sensation to cold and menthol and is predominantly expressed in small TrkA$^+$ peptidergic neurons), and TRPA1 (which

is found in both the small TrkA$^+$ peptidergic and TrkA$^-$ non-peptidergic nociceptive neurons)[62].

RUNX1 is also involved in the further diversification of the TrkA$^+$ neurons into different types of nociceptors during late embryonic and postnatal stages. At these points, RUNX1 seems to act as a repressor. All embryonic small neurons initially express *TrkA*[73]. During postnatal development, some nociceptors stop expressing *TrkA* and begin to express *Ret*[74,75]. So, two populations arise: TrkA$^+$ peptidergic neurons, which maintain *TrkA* expression and express neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P, and TrkA$^-$ non-peptidergic neurons, which do not express neuropeptides but acquire the ability to bind to isolectin B4 (IB4) and switch from *TrkA* to *Ret* expression perinatally[74,75]. The first population has been implicated in the transduction of inflammatory pain and the latter in neuropathic pain. RUNX1 directs several aspects of the diversification of early TrkA$^+$ neurons into these two nociceptive populations. In mice that selectively lack *Runx1* in the PNS, the transition from *TrkA* to *Ret* expression is impaired, resulting in an increase in *TrkA*-expressing neurons and a decrease in *Ret*-expressing neurons[62]. RUNX1, directly or indirectly, is also required for the repression of CGRP, the DRG acid-sensing channel DRASIC and μ-class opioid receptors, as well as the upregulation of several ion channels and seven-transmembrane-domain receptors (such as $P2X_3$, Nav1.9 and MRGPRD) in the non-peptidergic nociceptive population[62]. However, other developmental aspects of this population of nociceptors, such as the acquisition of IB4 binding, are unperturbed[62].

With two known exceptions (the postnatal requirement of RUNX1 for the expression of *TrpA1* in both peptidergic and non-peptidergic neurons, and for the expression of *TrpM8* in peptidergic neurons) it seems that, during postnatal stages, RUNX1 exerts a transcriptional repression of genes associated with the peptidergic phenotype and a transcriptional activation of genes associated with the non-peptidergic identity. RUNX might regulate these genes directly, but the observed phenotypes could also result from the change in afferent central target selection (see below and FIG. 4b) or from the ability of RUNX to control the expression of trophic factor receptors that regulate the ion channel and neuropeptide properties of nociceptors. Consistent with the developmental perturbation of TrkA$^-$ non-peptidergic neurons in conditional *Runx1*$^{-/-}$ mice, these animals show an altered response to thermal sensation, including cold and heat, and deficits in responses to neuropathic pain but not to mechanical stimuli[62]. The ability of RUNX1 to act both as a repressor and an activator in its control of cell diversification draws attention to the importance of cofactors and splice variants for its activities (BOX 2).

Axonal growth

As NCCs undergo neurogenesis and diversify into principal sensory subtypes, DRG axons grow rapidly to the dorsolateral margin of the spinal cord. During sensory neuron diversification, cell intrinsic properties and extrinsic guidance cues participate in the subsequent establishment of modality-specific contacts in the spinal cord, which takes place throughout embryogenesis and into postnatal stages.

Extrinsic factors. Both diffusible and non-diffusible cues contribute to the establishment of connections from DRG neurons in the spinal cord. Semaphorins are secreted or transmembrane proteins that regulate many aspects of development, including axon guidance. The semaphorin SEMA3A acts as a diffusible, chemorepellent, short-range guidance cue[76] for axons of embryonic DRG neurons by interacting with the receptors neuropilin 1 (NRP1) (REF. 77) and plexinA4 or plexinA3 (REF. 78). *Sema3a* is first expressed throughout the spinal cord during the 'waiting period', when axons projecting along several segments have not yet produced axon collaterals that will enter the spinal cord (E3–E6 in the chick and E11–E14 in the mouse). As sensory axons begin to project into the grey matter, *Sema3a* is progressively downregulated in the dorsal spinal cord[76]. This is consistent with gain- and loss-of-function studies that have ascribed SEMA3A and its receptors a crucial role in regulating the extent of the waiting period[79–81].

SEMA3A is also involved in setting up modality-specific projections in the spinal cord. At slightly later stages of spinal cord innervation by DRG sensory neuron axons, when modality-specific projections are established, the ventral (but not dorsal) spinal cord contains a repulsive factor[82,83], identified as SEMA3A[84], that acts through NRP1 receptors expressed by the sensory neurons[85]. As sensory axons grow into the spinal cord between E6 and E10 in the chick, when *Sema3a* is expressed only in the ventral spinal cord, neurotrophin 3 (NT3)-dependent muscle axons express progressively lower levels of NRP1 and lose their responsiveness to SEMA3A, whereas nerve growth factor (NGF)-dependent axons show the opposite pattern[86]. So, from E7 in the chick and presumably E15 in the mouse, SEMA3A repels NGF-responsive neurons that terminate in the dorsal horn but not NT3 responsive axons, thereby contributing to the correct patterning of sensory projections in the spinal cord[84]. Consistent with this, the elimination of SEMA3A or NRP1 results in misprojection of cutaneous nociceptive CGRP$^+$ and TrkA$^+$ afferents along the spinal cord midline into deeper spinal cord laminae[80,87]. In NRP1 loss-of-function studies in the chick, both TrkA$^+$ and TrkC$^+$ axons showed a premature and misdirected projection, as did TrkA$^+$ axons in mice[79]. It therefore seems that SEMA3A, by acting on NRP1 receptors, prevents cutaneous TrkA$^+$ axons from projecting to deeper laminae, whereas downregulation of NRP1 in TrkC$^+$ neurons might allow proprioceptive afferents to project into the ventral horn. Other short-range guidance cues that attract modality-specific terminations are the cell adhesion molecules axonin 1 and transient axonal glycoprotein for the axons of small neurons, and coagulation factor 11 (F11, also named F3 or contactin) for proprioceptive axons. Perturbation of F11 results in deficits of

longitudinal axonal extension and a failure of proprioceptive axons to extend to the ventral horn, whereas blocking axonin 1 leads to premature entry of the axons of small neurons into the spinal cord (presumably due to the loss of an attractive force), fasciculating longitudinal fibres running along the marginal zone, and projection errors within the dorsal horn in lamina III (REF. 88). So, both longitudinal and rostrocaudal axonal trajectories result from an interplay between attractive and repulsive short-range forces.

Long-range signalling from molecules, including neurotrophic factors[39,89], also helps to establish modality-specific TrkA$^+$ and TrkC$^+$ neuron innervation. Loss of NT3 results in a failure of central TrkC$^+$ proprioceptive neurons to project to the ventral spinal cord and contact motor neurons in the monosynaptic circuit[72,90], and this effect has been proposed to be independent of the role of NT3 in supporting neuronal survival of muscle afferents[91]. To investigate the role of neurotrophic factors for developmental processes occurring after the naturally occurring cell death period, researchers have used a genetic approach exploiting the fact that neuronal death resulting from a lack of trophic factors requires the pro-apoptotic gene *Bax*[92,93]. In *Bax*$^{-/-}$, *NGF*$^{-/-}$;*Bax*$^{-/-}$ or *NT3*$^{-/-}$;*Bax*$^{-/-}$ mice, excessive cell death due to trophic factor deprivation is eliminated[92–94]. In neurons that survive without any NGF–TrkA signalling, there is apparently no deficit in superficial dorsal horn lamina innervation, but there is severely diminished expression of mature neuronal markers, including neuropeptides and ion channels. This shows that NGF signalling not only promotes neuronal survival, but is also required for the full phenotypic maturation of small sensory neurons[92]. In *NT3*$^{-/-}$;*Bax*$^{-/-}$ mice, the central proprioceptive afferents fail to establish monosynaptic connections with motor neurons and instead terminate in the intermediate zone of the spinal cord[93]. Interestingly, in mice in which *TrkC* genetically replaces *TrkA*, some presumptive *TrkA*-expressing neurons change fate and adopt a proprioceptive phenotype, terminating in the ventral instead of the dorsal horn[40]. One factor controlled by NT3 that determines connective patterns in the spinal cord might be the ETS transcription factor ER81. *ER81* is expressed in all proprioceptive sensory neurons; in *ER81*$^{-/-}$ mice, proprioceptive afferents fail to innervate motor neurons and terminate aberrantly in the intermediate zone, as in *NT3*$^{-/-}$;*Bax*$^{-/-}$ mice[95]. *ER81* expression is controlled by NT3 signalling via TrkC signalling[93]. The downstream targets and mechanisms by which ER81 affects central projections are not known.

Intrinsic factors. Recent gain- and loss-of function studies have shown that RUNX proteins are involved in the establishment of neuronal subtype-specific connectivity[61–63] (FIG. 4). These studies did not address the role of RUNX transcription factors in the emergence of the principal neuronal subtypes in the DRG: the early TrkA$^+$ and TrkC$^+$ populations. Instead, the methods used shed light on the role of RUNX proteins later in development in the establishment of terminal patterns of contacts in the spinal cord[61–63].

RUNX1 might be involved in the segregation of terminal fields of TrkA$^+$ peptidergic and TrkA$^-$ non-peptidergic axons in the spinal cord at late embryonic stages. These axons terminate in distinct laminae and have been postulated to mediate inflammatory and neuropathic pain, respectively[37]. TrkA$^-$ non-peptidergic neurons project predominantly to inner lamina II (IIi), whereas the TrkA$^+$ peptidergic neurons project mainly to lamina I and the outer layer of lamina II (IIo) (FIG. 4a). The diversification of the early small diameter TrkA$^+$ population into peptidergic and non-peptidergic neurons is correlated with the downregulation of *Runx1* in the TrkA$^+$ peptidergic population. The continuous expression of *Runx1* in non-peptidergic subtype neurons is accompanied by a downregulation of *TrkA* and the segregation of central terminations into the deeper lamina IIi. Loss of function of *Runx1* in mice results in failure of the non-peptidergic axons to terminate in lamina IIi; instead they terminate in more superficial laminae with the axons from peptidergic neurons[62] (FIG. 4b). Conversely, overexpression of *Runx1* or *Runx3* in mice after sensory neuron specification has taken place results in the aberrant termination of peptidergic axons in lamina IIi[63] (FIG. 4d). RUNX1 therefore seems to be a key determinant for the establishment of distinct central connections by segregating the axon terminations of small peptidergic and non-peptidergic neurons.

Overexpression of *RUNX3* in the chick at stage 26 (E4.5), after the diversification of early TrkA$^+$ and TrkC$^+$ populations is largely complete, does not change the expression of *TrkA* and is not sufficient for the acquisition of *TrkC* or *ER81* expression by this subclass[61]. Overexpression of *RUNX3* leads to some TrkA$^+$ axons, which are normally positioned at the lateral half of the dorsal funiculus, to be redirected to the medial part. In these animals, some TrkA$^+$ axons found at both lateral and medial entry positions show a ventral shift in axon termination to the location of proprioceptive axons. *RUNX3* overexpression also causes Ib proprioceptive axons that normally terminate in the intermediate spinal cord (FIG. 4a) to shift ventrally[61] (FIG. 4e). Using short interfering RNA (siRNA) to reduce RUNX3 in the chick causes proprioceptive axons to shift dorsally so that they terminate predominantly in the dorsal spinal cord[61]. Consistently, targeted deletion of *Runx3* in mice leads to a loss of the proprioceptive axon projections that terminate in the motor horn, a failure to initiate expression of *TrkC* between E10.5 and E11.5, and a marked loss of proprioceptive neurons at E13.5, with the remaining neurons failing to acquire the full expression of proprioceptive neuron markers[64]. In another strain of *Runx3*-null mutant mice, central proprioceptive axons fail to project to the intermediate zone (proprioceptive Ia, Ib and type II neurons) and ventral horn (Ia neurons), peripheral projections do not reach the muscle, and more than half of the myelinated axons are absent from the dorsal horn[66] (FIG. 4c). Interestingly, loss of *Runx3* in mice results in a loss of *ER81* expression[64], presumably because TrkC induces ER81, and RUNX3 is necessary for *TrkC* expression.

ETS transcription factor
One of the largest families of transcription factors that retains a region of conserved sequence, the ETS (E twenty-six) domain forming the winged helix-turn-helix DNA binding domain is composed of three α-helices and a four-stranded β-sheet.

Dorsal funiculus
Division of the white matter in the spinal cord consisting of a bundle of the nerves projecting from the dorsal root ganglion.

Short interfering RNA
(siRNA). Short double-stranded RNA molecules that silence gene expression in a sequence-specific manner by a process termed RNA interference. The 2006 Nobel Prize in Physiology or Medicine was given to the discovery of RNA interference.

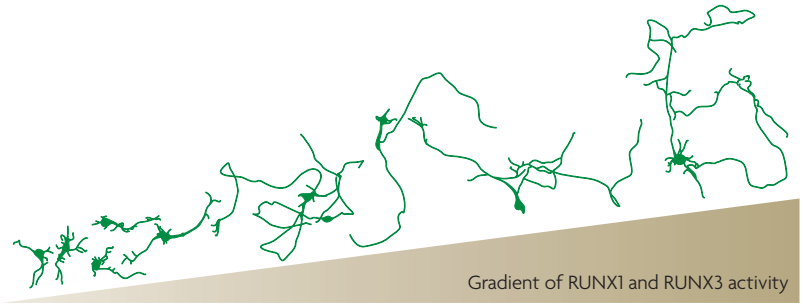

Figure 5 | **Graded RUNX activity controls sensory neuron axonal outgrowth.** Increasing *Runx* expression induces outgrowth both *in vivo*[61,63] and *in vitro*[36] in a dose-dependent manner. This action might be cell-autonomous in part[36].

However, the effects of RUNX3 on target termination are mediated only partly by TrkC and ER81, as *Runx3*-null mice have a much more severe phenotype than *ER81*- or *TrkC*/*Bax*-null mice, with a complete failure of axon formation in both the intermediate zone and ventral horn.

It is plausible that RUNX transcription factors direct axon projections and a modality-specific termination pattern in the spinal cord indirectly by affecting the responsiveness of DRG sensory neurons to axon guidance cues in the spinal cord. However, RUNX transcription factors might also regulate axonal growth independently of extrinsic cues in a cell intrinsic mechanism related to the propensity of the neurons for axonal growth. The length of axons seems to be directly correlated with the level of *Runx* expression (FIG. 5). *Runx1* overexpression *in vitro* leads to a more than threefold increase in axonal length and a marked increase in branching, and gradual block of RUNX activity with a dominant-negative form of RUNX1 proportionally decreases axonal length[36]. In the chick, overexpression of *RUNX3* in neurons that normally express this factor but terminate in the intermediate zone forces these axons to continue growing all the way to the most ventral part of the spinal cord[61]. Different concentrations of siRNA against *RUNX3* caused a graded reduction of protein levels in the chick DRG, correlated with a graded ventral-to-dorsal shift in the position of axon collateral terminations[61]. So, increasing activity of RUNX1 and RUNX3 correlates with increases in axon length. The effects of RUNX1 on axonal growth *in vitro* are independent of trophic factors and occur in a serum-free defined medium, indicating that RUNX1 and possibly also RUNX3 act cell-autonomously to drive axonal growth.

Coordinating specification and connectivity

The reception of different types of sensory information depends on coordination between the specification of sensory neuron subtypes and central axon target selection. How are the receptive properties of sensory neurons coordinated with their fields of termination? Because neuronal subtypes are produced and then in a coordinated fashion establish precise connections in the nervous system, gene programmes that direct subtype specification are likely also to control connectivity. The growth of DRG axons in the spinal cord to establish modality-specific contacts might therefore be independent of target innervation in the periphery. Cutaneous and muscle afferents show modality-specific projections in the spinal cord after being labelled with the lipophilic neuronal tracer DiI at the earliest stage when they can be labelled separately and allowed to develop *in vitro* in the absence of the peripheral target[96]. The ability of sensory neurons to establish modality-specific connections to the spinal cord might be determined along with neuronal subtype specification.

In the PNS of *Drosophila melanogaster*, the developmental programme that defines the identity of a neuron is shared with the programme that determines the formation of neuronal connectivity, indicating that early specification of neuronal type and position also specifies a cell-autonomous and pre-defined pattern of axonal growth[97]. This genetic programme involves *runt*, the *Runx* orthologue in insects, which has dose-dependent effects on cell specification, axonal growth and the establishment of connectivity[98–102]. Recent results indicate that subtypes of sensory neuron in vertebrates have unique patterns of neurite outgrowth and receptor expression before target innervation, supporting the idea that cell specification and axon outgrowth are similarly coordinated[103,104].

In vertebrates, RUNX transcription factors participate in the establishment, maintenance and diversification of unmyelinated small diameter and large mechanoreceptive and proprioceptive sensory neurons as well as the establishment of central connections. They could therefore be responsible for coordinating cell type specification with modality-specific terminations in the spinal cord. RUNX transcription factors seem to be among the earliest definite markers for unmyelinated small diameter and large mechanoreceptive and proprioceptive neurons. TrkA and TrkC are not upstream of *Runx1* and *Runx3*, as knockout of these genes in mice does not affect *Runx* expression[63]. On the contrary, eliminating RUNX1 and RUNX3 eliminates TrkC and TrkA in the mouse and chick, respectively[36,64]. The number of neurons that express *TrkA* increases continuously from E4 until E6 in the chick, correlating with exit from the cell cycle and birth of the early $TrkA^+$ population of sensory neurons[17]. *Runx1* mRNA is expressed as early as E4 (HH stage 24) in the chick, before *TrkA* is expressed in this population of cells[36]. *TrkC* has been proposed to be expressed before *Runx3* (REF. 63). However, this is based on immunoreactivity studies of RUNX3 and TrkC at E11.5 in the mouse[63]. In the *Runx3*-null mutant mouse, TrkC immunoreactivity is nearly gone at E10.5 and is completely absent at E11.5 (REF. 64). Therefore, RUNX transcription factors are among the earliest markers for sensory subtypes in the DRG, and regulate TrkA and TrkC expression, which, when activated, in turn regulate the expression of neuropeptides and ion channels that determine the receptive properties of sensory neurons. RUNX transcription factors are also necessary for the expression of neuropeptides, G-protein coupled receptors and ion channels, so they might also directly contribute to the receptive properties of sensory neurons[62]. Because expression of RUNX transcription factors affects the terminal fields of contacts

in the spinal cord, sensory neuron subtype specification by RUNX factors predetermines at least some aspects of modality-specific connections in the spinal cord. RUNX factors also continue throughout development to shape central connectivity in parallel with their roles in further neuronal subtype diversification.

Specification, innervation and survival

During development, cell migration and target innervation are synchronized with the susceptibility to cell death in the sensory nervous system. NCCs and early born neurons survive independently of trophic support during subtype specification and during axonal growth to the target tissues of innervation. The acquisition of trophic factor dependency is intrinsically controlled and occurs independently of target innervation, but is related to the time when neurons normally contact their targets *in vivo*. Neurons that innervate nearby targets show slower axonal growth and earlier dependence on trophic factors than neurons with more distant targets[105,106]. Genetic deletion of *Runx1* results in the death of TrkA$^+$ neurons of the trigeminal ganglion at E11.5 (REF. 68), and in *Runx3*-null mutant mice, TrkC$^+$ proprioceptors die, resulting in the loss of half of the myelinated axons in the dorsal horn[64,66]. It is not clear whether the neuronal loss is due to a failure to initiate or maintain *Trk* expression in these mice or whether RUNX factors by themselves control cell survival. A lack of *Trk* expression leads to excessive cell death during the period of naturally occurring cell death, but recent data indicate that RUNX proteins might also support cell survival by a direct, cell-autonomous mechanism. Neurons differentiated from boundary cap neural crest stem cells[16] die after 3 days in culture. Overexpression of *Runx1* leads to full rescue of these newborn neurons. The effect seems to be cell-autonomous, as it is independent of extrinsic signals[36]. RUNX transcription factors might therefore coordinate susceptibility to cell death and trophic factor dependency with cell specification and target innervation.

Conclusion and future directions

Our understanding of the genetic regulation of cell diversification and neuronal connectivity has advanced considerably during recent years. During the three waves of neurogenesis, the fate, function, connectivity and dependency on trophic factors of neurons are largely determined in the sensory lineage. The dynamic *Runx1* and *Runx3* expression in these cell populations sets off a number of events in sensory neuron development including the expression of trophic factor receptors, which in turn shape sensory neuron function, the determination of axonal trajectories and termination fields in the spinal cord. They might also regulate the synchronization of trophic factor dependency with target innervation. The presence of transcription factors that coordinate several key biological processes indicates a new type of logic during the development and wiring of circuits in the nervous system.

The discovery of RUNX transcription factors coordinating several biological processes raises many questions. What are the factors inducing the expression of RUNX and the downstream substrates that execute the effects of its activities? What are the mechanisms by which RUNX transcription factors determine cell type specification? Through what molecular substrates do RUNX factors determine, cell autonomously, central projections? Is the regulation of responsiveness to extrinsic cues also controlled by these factors? Do RUNX factors determine the onset of trophic factor dependency, and if so by which pathways? RUNX transcription factors shape the nervous system by acting as both activators and repressors. To understand how RUNX factors direct sensory neuron development, co-activators and co-repressors need to be identified. The identification of new cofactors for RUNXs, the elucidation of their combinatorial expression patterns and the comprehension of how they confer contextual properties on the newly born sensory neurons will extend our understanding of how sensory diversity is created, and how these gene programmes are coordinated with those building the precise connections of a neuronal circuit.

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Acknowledgments
This work was supported by the Swedish Medical Research Council and the Swedish foundation for strategic research (CEDB grant). We thank J. Hjerling-Leffler for help with the design of figure 1.

Competing interests statement
The authors declare no competing financial interests.

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