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Hierarchical Control of Sensory Neuron Development by Neurotrophic Factors

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DOI 10.1016/j.neuron.2007.05.023

We are still far from reaching a complete understanding of the molecular mechanisms that control neuronal diversification during nervous system development. In this issue of *Neuron*, Luo et al. bring that goal a step closer by revealing how a hierarchical interaction between two neurotrophic factor systems drives the differentiation, maturation, and extension of peripheral projections in a subclass of sensory neurons that mediate pain perception.

The molecular mechanisms responsible for the specification of distinct sensory modalities are being intensely investigated, not only because of the obvious clinical importance of pain control, but also as a powerful model system of neuronal diversification. Pain perception following harmful mechanical, thermal, and chemical stimuli is mediated by the activation of pain-transducing—i.e., nociceptive—sensory neurons in the dorsal root ganglia (DRG). Nociceptive, mechanoceptive, and proprioceptive sensory neuron subtypes emerge from migratory neural crest cells shortly after neural tube closure and continue to diversify by acquiring distinct functional characteristics long after birth (Hjerling-Leffler et al., 2007). Nociceptors express a diverse collection of ion channels that allow them to transduce external stimuli into electrical activity. The generation of nociceptor cell diversity is believed to be controlled by hierarchical interactions between cell intrinsic and extrinsic signals (Marmigere and Ernfors, 2007). All nociceptive neurons initially express the transcription factor Runx1 and the nerve growth factor (NGF) receptor TrkA, which mediates target-dependent cell survival during the period of programmed cell death. Around birth, and during the first 2 to 3 postnatal weeks, a fraction of nociceptive neurons switch their neurotrophic factor dependence by downregulating expression of TrkA and upregulating that of Ret, a signaling subunit of the receptor complex for members of the GDNF (glial cell line-derived neurotrophic factor) ligand family. $TrkA^+$ and Ret^+ nociceptors are also distinguished by the presence or absence of the neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP)—hence classified as peptidergic or nonpeptidergic subtypes—and believed to mediate inflammatory or neuropathic pain, respectively. While developing nonpeptidergic nociceptors lose TrkA, maintain Runx1, and gain Ret expression, peptidergic sensory neurons retain TrkA but lose Runx1 as they mature (Chen et al., 2006).

The contribution of NGF/TrkA signaling to sensory neuron diversification and maturation has been studied in mice lacking the proapoptotic molecule Bax, a mutation that bypasses the early developmental requirement of neurotrophic support for cell survival.

In the absence of NGF/TrkA signaling, Bax-deficient DRG neurons failed to express multiple phenotypic markers that are characteristic of both peptidergic and nonpeptidergic nociceptive neurons, including CGRP/SP and Ret, respectively (Patel et al., 2000), underlying the importance of NGF and TrkA for the development of the nociceptive sensory phenotype in vivo. A more recent study examined the role of Runx1 in nociceptive development using conditional knockout mice in which specific *Runx1* deletion in premigratory neural crest cells, including the progenitors of DRG neurons, was controlled by a *Wnt1-Cre* transgene (Chen et al., 2006). Runx1 was found to be required for most if not all aspects of the nonpeptidergic nociceptive phenotype, including upregulation of Ret, downregulation of TrkA, expression of a distinct set of ion channels and sensory receptors, and central target selection in the dorsal spinal cord. As a consequence, Runx1 mutants displayed deficits in thermal and neuropathic pain responses, indicating the important role of Runx1 as a master regulator of the nonpeptidergic phenotype in developing nociceptive sensory neurons (Chen et al., 2006). Interestingly, an expansion of the peptidergic fate, characterized by increased numbers of CGRP- and TrkA-expressing neurons, was also observed in the absence of Runx1, leading to the idea that Runx1 may function as a direct repressor of the peptidergic fate associated with a TrkA identity (Chen et al., 2006). Although transcription factors acting as activators and repressors in the same cell lineage are not an exception, a possibility not addressed by previous studies was that other effectors, downstream of Runx1, may be involved in TrkA extinction and neuropeptide gene repression.

Enter the paper by Luo et al. (2007) in this issue of *Neuron*. These authors set out to investigate the role of Ret signaling in sensory neuron development for which they developed a conditional knockout of the *Ret* gene in DRG precursors driven by the *Wnt1-Cre* transgene. In these mice, Ret expression was lost in DRG neurons as well as myenteric plexus and sympathetic ganglia. Although grossly normal at birth, these mutants developed abdominal distension and progressive weakness, resulting in most mice dying within 3 weeks of age due to enteric aganglionosis. Luo et al. (2007) found that Ret was not required for DRG cell viability but was necessary for normal soma size and extension of cutaneous peripheral projections by nonpeptidergic sensory neurons. In agreement with this, the GDNF family member Neurturin has been found to be expressed at high levels in skin (Golden et al., 1999), and loss of either Neurturin or its cognate coreceptor GFRα2—with which Ret forms a functional signaling complex—results in reduced sensory neuron soma size and peripheral innervation defects similar to those observed by Luo et al. (Heuckeroth et al., 1999; Lindfors et al., 2006). A survey of GFRα family members expressed in DRG neurons made by Luo et al. (2007) identified GFRα2 as the most abundant coreceptor in those cells. Moreover, the authors found that GFRα2 expression was (at least partially) under Ret control, suggesting that Neurturin positively regulates the expression of its own receptor in neurons expressing Ret. Intriguingly, no defect in central projections was observed in conditional *Ret* mutants, indicating that other target-derived signals may control spinal cord innervation and termination by nonpeptidergic sensory neurons.

Importantly, Luo et al. (2007) found that Ret is required for the acquisition of several aspects of the nonpeptidergic phenotype of DRG neurons. Several markers that are characteristic of these neurons were absent in DRG lacking Ret, including the Trp class ion channel TrpA1, and G protein coupled receptors MrgA1, MrgA3, and MrgB4. Other aspects of the nonpeptidergic phenotype, however, were not affected by the absence of Ret, such as expression of TrpC3, TrpV1, MrgD, and the ATP-gated channel P2X3, indicating that Ret signaling controls a subset of the genes that characterize mature nonpeptidergic sensory neurons. Although expression of the cold and menthol receptor TrpM8 was neither affected by the loss of Ret, a recent study has found no overlap between TrpM8 and the nonpeptidergic marker IB4 (Hjerling-Leffler et al., 2007), indicating that this channel may actually be restricted to nociceptive neurons of the peptidergic subclass. Nevertheless, both Ret-dependent and Ret-independent markers, as well as Ret itself, are in turn controlled by Runx1, suggesting that the activity of this transcription factor unleashes divergent signaling cascades that control different aspects of the nonpeptidergic nociceptive phenotype. As Runx1 is also required for TrpM8 expression (Chen et al., 2006), aspects of the peptidergic phenotype may also be controlled by this transcription factor. Whether Ret-independent genes, such as TrpC3, TrpV1, MrgD, and P2X3, are regulated by Runx1 directly or via other sets of extracellular signals remains to be elucidated.

Is TrkA extinction in nonpeptidergic nociceptive neurons Ret-dependent or Ret-independent? Luo et al. finds the answer to be both. By P14, most nonpeptidergic DRG neurons have normally downregulated TrkA expression almost completely, while roughly half of the neurons lacking Ret failed to do so, indicating that Ret signaling is an important step, albeit not the only one, in the postnatal extinction of TrkA expression in nonpeptidergic sensory neurons. More generally, this result suggests that pathways activated downstream of Runx1, rather than Runx1 itself, may act to suppress the expression of genes normally associated with the peptidergic phenotype in sensory neurons.

A final question addressed by Luo et al. (2007) concerns the identity of the signals lying upstream of Runx1 and how these relate to the control of gene expression in nonpeptidergic nociceptors. Because of its early onset during sensory neuron development, NGF/TrkA signaling stood out as a strong candidate for this role. Using double-mutant mice lacking both NGF and Bax the authors could show a drastic—albeit not complete—reduction in Runx1 expression in newborn DRG. This was accompanied

by either loss or strong reduction of several characteristic nonpeptidergic markers, including TrpC3, MrgA1, MrgA3, MrgD. However, other markers—such as TrpV1 and P2X3—were only slightly reduced or unaffected by the loss of NGF/TrkA signaling. As both TrpV1 and P2X3 are only partially reduced in DRG lacking Runx1 (Chen et al., 2006), together these data suggest that NGF/TrkA- and Runx1-independent pathways will also contribute to some aspects of the nonpeptidergic phenotype in DRG nociceptors. Interestingly, Luo et al. (2007) found that expression of Runx1 was unaffected in NGF/Bax double mutants at embryonic day 14 (E14), suggesting that NGF/TrkA signaling is not required for the initiation of Runx1 expression but for its maintenance during later stages. Thus, it would appear that Runx1 expression becomes under the control of NGF/TrkA signaling just around the period in which nonpeptidergic nociceptors switch from TrkA to Ret.

The picture that emerges from this and other recent studies on the control of nociceptive neuron diversity is that of a regulatory network of mutually reinforcing feed-forward and negative feedback loops (Figure 1). Switch-like behaviors are not uncommon among signaling networks with this type of configuration. The ability of TrkA signaling to maintain postnatal Runx1 expression in only a subset of nociceptive neurons could explain why peptidergic nociceptors fail to express Ret despite also expressing TrkA; e.g., these cells may lack molecular components required for this activity. Alternatively, or in addition, different levels of TrkA signaling may be reached in the two sensory neuron subclasses, with only the highest level being sufficient for maintaining Runx1. Finally, suppression of Runx1 expression by a component specifically induced in peptidergic neurons could also contribute to reinforcing the segregation of this transcription factor among different nociceptor subclasses in postnatal stages (X in Figure 1). As Runx1 would also appear to be required for some aspects of the peptidergic phenotype (e.g., TrpM8 expression), perhaps acting during earlier developmental stages, an important task for future studies will be to carefully establish the timing of Runx1 extinction in relation to different maturation events in peptidergic nociceptors. Given the requirement of TrkA signaling for postnatal Runx1 expression in nonpeptidergic neurons, another question raised by these data is how Runx1 is maintained in these cells after TrkA expression is extinguished. Luo et al. (2007) show that Ret signaling is not required for this function, at least not by P14 (although it could possibly be so later on), suggesting that another positive feedback loop may reinforce Runx1 expression after nonpeptidergic nociceptors turn off TrkA, either through autoregulation or via other downstream components (Y in Figure 1). The existence of additional components downstream of Runx1 and alongside Ret could be expected by the fact that several differentiation events in nonpeptidergic neurons appear to be Ret-independent. As indicated by Luo et al. (2007), intrinsic transcriptional programs may be responsible for the onset of Runx1 expression during embryonic sensory neuron development, and some of these could also play a role during postnatal stages (Z in Figure 1).

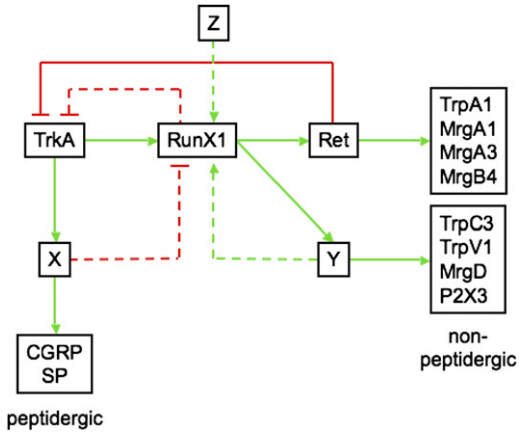

Figure 1. An Emerging Regulatory Network of Nociceptive Sensory Neuron Development

The regulatory connections shown correspond for the most part to those found during postnatal stages and are likely to differ at earlier stages of development (during which Runx1 may also play roles in peptidergic differentiation). Dashed lines and X, Y, and Z denote hypothetical regulatory connections and components, respectively. See text for details.

In summary, the study by Luo et al. (2007) substantially expands our understanding of the mechanisms by which different types of nociceptive neurons emerge during development. Hierarchical interactions between different neurotrophic factor systems may explain how a limited set of neurotrophic signals orchestrates different stages of neuronal development. More generally, this study demonstrates how cell-extrinsic signals, such as those mediated by neurotrophic factors, modulate the function of cell-intrinsic transcription factors to dictate different phenotypic outcomes.

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