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"Runx"ing towards Sensory Differentiation

Somatosensory stimuli are encoded by molecularly and anatomically diverse classes of dorsal root ganglia (DRG) neurons. In this issue of *Neuron*, three papers demonstrate that the Runx transcription factors, Runx1 and Runx3, respectively regulate the molecular identities and spinal terminations of TrkA$^+$ nociceptive neurons and TrkC$^+$ proprioceptive neurons. These findings emphasize the importance of intrinsic genetic programs in generating the diversity of DRG neurons and specifying the circuits into which they incorporate.

Dorsal root ganglia sensory neurons provide an excellent model system for studying the signaling mechanisms that underlie neuronal diversity. No other group of neurons is as well characterized in terms of molecular markers and physiological functions. Two major classes that can be defined from early stages of DRG development are TrkA-expressing/NGF-dependent neurons, many of which have cutaneous targets and transduce pain-producing stimuli (referred to as nociceptors) and TrkC-expressing/NT3-dependent neurons, many of which innervate muscle spindles in the periphery and mediate sense of position (referred to as proprioceptors). These functionally distinct populations have characteristic projection fields in the spinal cord. The axons of nociceptive neurons terminate within the superficial dorsal horn. In contrast, proprioceptive axons project more ventrally to reach targets in the intermediate zone and synapse onto motor neurons in the ventral horn.

Prior to the publication of these three papers in *Neuron*, very little was known about the transcriptional mechanisms that regulate the development of these two classes of sensory neurons. The present studies highlight a pivotal role for Runx transcription factors in cell-autonomously regulating the differentiation of these functionally distinct cell types (Chen et al., 2006a, 2006b; Kramer et al., 2006).

Runx family genes (also referred to as CBFαs) are characterized by the Runt (*Drosophila* run gene) DNA binding domain and heterodimerize with a common cofactor CBFβ (Ito, 2004). In mammals, there are three Runx family genes, Runx1, Runx2, and Runx3. In the mouse immune system, Runx genes have critical roles in the lineage specification of T lymphocytes (Taniuchi et al., 2002). Runt domain factors have received particular attention in the DRG because Runx1 and Runx3 are expressed at high levels in developing sensory neurons (Levanon et al., 2001, 2002). Further, the expression patterns of Runx genes appear to correlate with functional subtypes with Runx1 being expressed by the TrkA$^+$ population and Runx3 being expressed by the TrkC$^+$ population. Two prior studies have additionally suggested that Runx3 is essential for appropriate regulation of spinal axon targeting of proprioceptive TrkC$^+$ DRG neurons (Inoue et al., 2002; Levanon et al., 2002).

At early stages of DRG development, Runx1 is expressed in all TrkA$^+$ neurons (Levanon et al., 2002; Chen et al., 2006a; Kramer et al., 2006). The TrkA$^+$ population undergoes differentiation into a variety of subtypes during mid to late embryonic development and early postnatal life. Two of the most striking changes are appearance of the neuropeptide CGRP in a subset of TrkA$^+$ neurons and the downregulation of TrkA and upregulation of the GDNF receptor, Ret, in another subset (Molliver et al., 1997). Chen et al. (2006a) now demonstrate that expression of Runx1 segregates with this latter population in late embryonic development and early postnatal life.

To address the functions of Runx1 related to nociceptor differentiation, Chen et al. (2006a) generated Runx1$^{f/f}$:Wnt1-Cre$^+$ mice in which Runx1 was ablated in all DRG neurons from the onset of DRG development. Their data show clearly that Runx1 function is essential for the transition from TrkA to Ret in a subset of nociceptive neurons and for repression of CGRP expression probably in this same subset. Further, they demonstrate convincingly that Runx1 is required for the expression of a variety of proteins critical for nociceptor function. Thus, in conditional Runx1 nulls, expression of a number of nociceptor-specific G protein coupled receptors, ATP channels, and TRPV channels is severely attenuated. Regulation of the TRPV channels is particularly important because these are known to be required for appropriate responses to noxious heat (Caterina et al., 2000). Runx1 is thus the first transcription factor identified that is specifically required for the expression of nociceptive markers in DRG neurons.

To further investigate the role of Runx1 in regulating nociceptor differentiation, Chen et al. studied the spinal targeting of nociceptor axons in the Runx1 conditional nulls. In normal adult mice, TrkA$^+$ afferents project to laminae I and IIo of the dorsal horn, whereas Ret$^+$ afferents, which can be labeled by the lectin IB4, project to deeper dorsal laminae (Molliver et al., 1997; Zylka et al., 2005). Chen et al. (2006a) show that loss of Runx1 expression switches the targeting of the IB4$^+$ afferent projection from lamina IIi to the most superficial laminae I/IIo. Thus, spinal axon targeting of nociceptive neurons is regulated by Runx1 in association with regulation of biochemical phenotypes.

An important feature of the Runx1 conditional nulls is that the mice survive postnatally allowing for behavioral studies. A comprehensive behavioral analysis showed that temperature sensitivity was attenuated in Runx1 conditional nulls. Impaired responsiveness to mechanical stimuli in the context of chronic neuropathic pain but not to acute mechanical stimuli was also demonstrated

(Chen et al., 2006a). The results of these behavioral studies further speak to the importance of Runx1 in regulating nociceptor function.

In a complementary gain-of-function approach, Kramer et al. used an elegant strategy to express Runx1 (or Runx3—see below) in all DRG neurons from early developmental stages. They generated mice in which a loxP-STOP-loxP-Runx1 cassette was inserted into the Tau (a neuronal microtubule-associated protein) locus. These mice were then crossed with Islet1-Cre recombinase transgenic mice to remove the STOP cassette and force expression of Runx1 in all DRG and spinal motor neurons (Srinivas et al., 2001).

By analyzing these mice, in which Runx1 is now overexpressed in DRG neurons that normally express Runx1 and ectopically expressed in neurons that do not, Kramer et al. provide direct evidence that expression of Runx1 suppresses CGRP expression in $TrkA^+$ cells. Further, Kramer et al. (2006) found that overexpression of Runx1 drives $TrkA^+$ axons to extend beyond their normal spinal target field into the deeper layers of dorsal horn. These results in Runx1 gain-of-function experiments thus complement the results in the Runx1 loss-of-function experiments reported by Chen et al. (2006a). The studies of the two groups taken together demonstrate that Runx1 regulates the differentiation and circuitry of a distinct nociceptor subset.

Kramer et al. also investigated the role of Runx factors during proprioceptor differentiation. They carefully characterized Runx3 expression patterns in relation to expression of TrkB, TrkC, and Ret at different embryonic stages. Ret is expressed at E11.5 in cells that cannot be confidently classified but may be low-threshold mechanoreceptors. Kramer et al. found that postmitotic $TrkA^-$ DRG neurons can be subdivided into five distinct populations at E11.5: $TrkC^+$, $TrkC^+/TrkB^+$, $TrkB^+$, $TrkB^+/Ret^+$, and Ret^+. The $TrkC^+/TrkB^+$ and $TrkB^+/Ret^+$ subsets are transient and disappear by E14.5. They found that Runx3 is expressed exclusively in $TrkC^+$ but not in $TrkC^+/TrkB^+$, $TrkB^+$, or Ret^+ neurons. Therefore, they propose that downregulation of TrkB is an important step in the subsequent differentiation of $TrkC^+$ proprioceptive neurons that may be regulated by Runx3.

To test this hypothesis, Kramer et al. used the strategy outlined above to force expression of Runx3 in all DRG neurons including those that do not normally express this Runx family member. They demonstrate that expression of Runx3 in all DRG neurons eliminates TrkB expression throughout the DRG and is associated with a significant increase in the number of $TrkC^+$ cells in the Ret^+ population. Kramer et al. next investigated the loss of Runx3 function by using a mouse line in which part of the Runx3 Runt DNA binding domain was deleted, similar to one of the previously reported Runx3 mutants (Inoue et al., 2002). In accordance with their gain-of-function study, Runx3 deficiency lead to a reduction in the $TrkC^+$ population and expression of TrkB in virtually all remaining $TrkC^+$ neurons. The authors conclude that Runx3 consolidates $TrkC^+$ neuron identity, by repressing TrkB (Kramer et al., 2006).

Consistent with an important role for Runx3 in regulating proprioceptor differentiation, prior work has shown that elimination of Runx3 prevents $TrkC^+$ afferents from projecting into the intermediate zone and ventral horn of the spinal cord (Inoue et al., 2002; Levanon et al., 2002). Chen et al. (2006b) have addressed the important issue of whether Runx3 by itself is instructive for directing axon extension into the ventral horn. They transfected Runx3 expression constructs into chick DRG by in ovo electroporation. This technique provides a direct way of assessing the requirement for and instructive abilities of axon growth regulating molecules because axon trajectories of single genetically altered cells can be observed against a backdrop of normal projections of neighboring cells.

Normally, $TrkA^+$ afferents enter the dorsal horn laterally and project to the dorsal horn, whereas $TrkC^+$ afferents enter medially and project to the intermediate zone and ventral horn. Ectopic expression of Runx3 in $TrkA^+$ neurons, in many cases, switched their central axon trajectories from the lateral into the medial area and redirected their axon targeting from dorsal horn to intermediate zone or ventral horn. Conversely, knock down of Runx3 by siRNA in $TrkC^+$ neurons resulted in targeting of their central afferents to the dorsal horn or intermediate zone.

The authors went on to compare the role of Runx3 with that of an ETS family transcription factor ER81, previously identified as a key regulator in proprioceptor circuit formation in spinal cord (Arber et al., 2000). Surprisingly, overexpression of Runx3 did not change ER81 expression, and further, overexpression of ER81 in $TrkA^+$ neurons did not switch their targeting choice from dorsal horn to ventral horn as Runx3 did. Therefore, the authors conclude that Runx3 is the primary determinant for the ventral projection of $TrkC^+$ neurons. They speculate that ER81 is involved in a separate pathway required for ventral projection of $TrkC^+$ afferents, but it is not sufficient alone to redirect trajectories of $TrkA^+$ axons.

The results of Chen et al. (2006b) are generally consistent with the effect of gain and loss of Runx1 function on nociceptor axon targeting in mice (Kramer et al., 2006; Chen et al., 2006a). However, one significant difference emerged between the mouse and chick studies: in the mouse model generated by Kramer et al. (2006), no extension of axons into the intermediate zone or ventral horn could be detected by Runx3 expressing $TrkA^+$ neurons. The authors speculate that the protein expression levels of Runx3 in mouse DRG neurons might be lower than that achieved in the chick and might not reach the critical threshold to redirect axon projections of $TrkA^+$ neurons into the ventral horn.

It is important to point out that the misconnection of the central and peripheral processes might change the extracellular signals acquired by DRG neurons and thereby affect subsequent neuronal differentiation. The authors do not provide any evidence about peripheral connections in these papers. This is an important issue because extracellular cues such as target-derived NGF and activin are not only important for neuronal morphological development and survival but also for the induction of properties like CGRP expression (Ritter et al., 1991; Patel et al., 2000; Hall et al., 2001). Further, it has been reported recently that ablation of NT3/TrkC attenuates ER81 expression in proprioceptive neurons (Patel et al., 2003). It will be important in future work to explore the relationship between phenotype acquisition driven

by intrinsic genetic programs and phenotype determination regulated by extracellular signals. The authors are well positioned to dissect these two paradigms, for example, by using temporally inducible Cre lines to ablate Runx factors in DRG after establishment of the sensory circuitry.

One unresolved question is the role of the Runx binding partner CBFβ. CBFβ does not exhibit DNA binding affinity by itself but rather modulates Runx activities by changing its conformation (Ito, 2004). CBFβ is highly expressed in postnatal DRG and trigeminal ganglia (GNF, 2003). It will be most interesting through genetic analysis to determine the overlap in phenotypic consequences of CBFβ compared to Runx1 and Runx3 ablation in DRG neurons.

In sum, these three studies substantially expand our knowledge about transcriptional regulation of DRG neuronal identity and central axon patterning. They demonstrate that expression of Runx1 and Runx3 consolidate and specify traits of nociceptive and proprioceptive DRG neurons, respectively. The studies of Kramer et al. (2006) and Chen et al. (2006a) agree that Runx factors have critical functions in suppressing markers normally downregulated in specific populations during development. Chen et al. (2006a) further demonstrate a requirement for Runx1 in expression of a variety of proteins that are critical to the normal functioning of nociceptive neurons. Finally, both gain-of-function and loss-of-function studies show that Runx1 and Runx3 regulate sensory axon trajectories in the spinal cord toward appropriate terminal fields.

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A Flashing Line Can Warp Your Mind

Keeping pace with a constantly changing world requires the ability to make predictions about the future on a variety of timescales. A very basic example of this is the ability to predict the future location of a moving object in the brief time that it takes to perceive and respond to that object. In this issue of *Neuron*, experiments by Sundberg, Fallah, and Reynolds reveal a potential neural substrate for making short-range predictions about motion in visual area V4.

We are forever destined to live in the past. Due to neural transmission delays, the sensations we experience are always a fraction of a second behind the events that evoke them. The time differences involved may be slight, yet they represent a distinct disadvantage when dealing with a world full of moving objects, particularly if some of those objects are large, heavy, and rapidly approaching. How the primate visual cortex accurately estimates the position of moving stimuli in the face of neural tarrying is the subject of a current study by Sundberg, Fallah and Reynolds (Sundberg et al., 2006). By performing parallel studies in humans and monkeys, these authors provide perceptual and physiological evidence that a moving target shifts the position of neuronal receptive fields in extrastriate visual area V4, thereby creating a wrinkle in the fabric of visual space. The result is that we perceive not the true position of the target, but its presumed future whereabouts. This kind of short-range prediction allows us to keep a step ahead of our sluggish brains and regain a semblance of simultaneity with reality. In other words, we experience the present by predicting the future of things that happened in the past.

This all works out because inertia causes objects to move in a predictable manner—a tendency codified by Newton's First Law of Motion. But do our brains actually take advantage of this predictability? Some of the first evidence that they do came from the study of eye movements. The primate oculomotor system uses a variety of strategies to keep the image of a moving target on the fovea despite visual-motor processing delays. One such strategy is used when making rapid eye