

Roles of *Runx* Genes in Nervous System Development

8

Jae Woong Wang and Stefano Stifani

Abstract

Runx-related (Runx) transcription factors play essential roles during development and adult tissue homeostasis and are responsible for several human diseases. They regulate a variety of biological mechanisms in numerous cell lineages. Recent years have seen significant progress in our understanding of the functions performed by Runx proteins in the developing and postnatal mammalian nervous system. In both central and peripheral nervous systems, Runx1 and Runx3 display remarkably specific expression in mostly non-overlapping groups of postmitotic neurons. In the central nervous system, Runx1 is involved in the development of selected motor neurons controlling neural circuits mediating vital functions such as chewing, swallowing, breathing, and locomotion. In the peripheral nervous system, Runx1 and Runx3 play essential roles during the development of sensory neurons involved in circuits mediating pain, itch, thermal sensation and sense of relative position. Runx1 and Runx3 orchestrate complex gene expression programs controlling neuronal subtype specification and axonal connectivity. Runx1 is also important in the olfactory system, where it regulates the progenitor-to-neuron transition in undifferentiated neural progenitor cells in the olfactory epithelium as well as the proliferation and developmental maturation of specific glial cells termed olfactory ensheathing cells. Moreover, upregulated Runx expression is associated with brain injury and disease. Increasing knowledge of the functions of Runx proteins in the developing and postnatal nervous system is therefore expected to improve our understanding of nervous system development, homeostasis and disease.

J.W. Wang • S. Stifani (✉)
Montreal Neurological Institute, Department of
Neurology and Neurosurgery, McGill University,
Montreal, QC, H3A2B4, Canada
e-mail: stefano.stifani@mcgill.ca

Keywords

Astrocyte • Dorsal root ganglion • Hypoglossal motor neuron • Microglia • Motor neuron somatotopic map formation • Muscle innervation • Nociception • Olfactory ensheathing cells • Olfactory epithelium • Parabrachial nucleus • Proprioception • Runx1 • Runx2 • Runx3 • Sensory neuron development

8.1 Introduction

It was during the study of the expression and function of the *Drosophila runt* gene that it was first recognized that members of the *runt*-related gene family (hereafter collectively referred to as *Runx* unless otherwise indicated) are involved in the regulation of nervous system development. During *Drosophila* embryogenesis, *runt* is expressed in a specific subset of central nervous system (CNS) neurons termed *even-skipped*-expressing lateral (EL) neurons. *In vivo* studies showed that *runt* inactivation causes a selective loss of EL neurons (Duffy et al. 1991). Conversely, ectopic *runt* expression results in the formation of supernumerary EL neurons that can extend axons along the normal trajectory used by these cells (Dormand and Brand 1998). These observations provided the first *in vivo* evidence that *Runx* genes participate in context-restricted mechanisms regulating the specification of defined neuronal subtypes in the developing nervous system and they opened the way to the subsequent investigation of the roles of *Runx* genes in vertebrate neural development. This chapter will review some of the key functions performed by *Runx* genes during mammalian nervous system development, focusing mainly on their roles during the formation of neural circuits mediating somatosensory sensation, motor control, and olfaction.

8.2 Involvement of *Runx* Genes in Peripheral Nervous System Development

Dorsal root ganglion (DRG) sensory neurons in the peripheral nervous system (PNS) mediate somatosensory stimuli such as the sensations of pain (nociception), mechanical pressure (mechanoreception), or relative position (proprioception).

Nociceptive neurons (nociceptors) are connected to the dorsal horn of the spinal cord and cutaneous structures and express neurotrophic factor receptors such as TrkA and Ret. Mechanoreceptive neurons (mechanoreceptors) also project their fibers to the spinal cord dorsal horn and the skin, but express other receptors for neurotrophic factors such as TrkB and TrkC. Proprioceptive neurons (proprioceptors) express TrkC and project to the ventral horn and intermediate zone of the spinal cord, as well as to muscle spindles and Golgi tendon organs (Inoue et al. 2002; Kramer et al. 2006; Yoshikawa et al. 2007; Honma et al. 2010; Abdo et al. 2011; Lallemand and Ernfors, 2012).

Both *Runx1* and *Runx3* are expressed in developing DRG sensory neurons, mostly in non-overlapping patterns. Mouse *Runx1* is preferentially expressed in several nociceptive neurons, including pain-related nociceptors and thermoceptors (Levanon et al. 2002; Chen et al. 2006a; Kramer et al. 2006; Marmigere et al. 2006; Lou et al. 2013, 2015). In contrast, *Runx3* expression marks for the most part proprioceptive neurons (Inoue et al. 2002; Levanon et al. 2002; Chen et al. 2006b; Kramer et al. 2006; Yoshikawa et al. 2007; Inoue et al. 2008; Lallemand and Ernfors, 2012). The next two sections will discuss evidence that Runx1 and Runx3 act during DRG sensory neuron development to regulate the acquisition of specific neuronal subtype identities, defined gene expression profiles, and formation of precise axonal innervations.

8.2.1 Runx1 Involvement in Establishment of Cutaneous Sensory Circuits

Most if not all DRG cutaneous sensory neurons mediating pain, itch and thermal sensation initially express TrkA during embryonic develop-

ment. TrkA^+ DRG sensory neurons also express *Runx1* in developing embryos (Levanon et al. 2002; Chen et al. 2006a; Kramer et al. 2006; Marmigere et al. 2006; Yoshikawa et al. 2007). At perinatal and postnatal stages, DRG expression of *TrkA* and *Runx1* separates, resulting in the appearance of two main groups of sensory neurons characterized by the expression of *TrkA* or *Runx1* (this latter group also expresses *Ret*). TrkA^+ sensory neurons acquire a 'peptidergic' phenotype characterized in part by the expression of the neuropeptide calcitonin-gene-related peptide (CGRP) and specific cell surface proteins. In contrast, Runx1^+ cells become 'non-peptidergic' sensory neurons defined by specific molecular traits and innervation of skin epidermis and hair follicle targets (Chen et al. 2006a; Kramer et al. 2006; Marmigere et al. 2006; Luo et al. 2007; Yoshikawa et al. 2007; Gascon et al. 2010; Yang et al. 2013; Lou et al. 2015). *Runx1* is essential for both the initial separation of TrkA^+ and TrkA^- sensory neuron lineages and the subsequent generation of cutaneous sensory neuron diversity, a process mediated in part by the transient or persistent nature of *Runx1* expression after the separation of *TrkA* and *Runx1* expression (Lou et al. 2015).

During non-peptidergic neuronal fate specification, *Runx1* orchestrates transcriptional mechanisms that directly or indirectly regulate the expression of numerous genes defining the non-peptidergic phenotype. These include, to name only a few, genes encoding cold receptors TRPM8 and TRPA1, heat receptors TRPV1 and TRPV2, several Mrgpr class G protein-coupled receptors, and ATP-gated channels. *Runx1* is also important to restrict the expression of peptidergic genes, including those encoding *TrkA*, CGRP, and mu-class opioid receptor (Chen et al. 2006a; Kramer et al. 2006; Yoshikawa et al. 2007; Liu et al. 2008; Ugarte et al. 2013). *Runx1* is hypothesized to directly regulate *TrkA* expression because it can bind *in vitro* to a *TrkA* minimal enhancer containing putative *Runx* binding sites (Marmigere et al. 2006).

As mentioned, *Runx1* also plays key roles in the further specification of several cutaneous sensory neuronal subtypes, including polymodal

nociceptors, pruriceptors and other neurons associated with pain, as well as specific types of mechanoreceptors (Lou et al. 2013, 2015; Yang et al. 2013). As an example, *Runx1* regulates the development of specific unmyelinated low-threshold mechanoreceptors that persistently express vesicular glutamate transporter 3 (VGLUT3) and mediate pleasant touch and/or pain (Lou et al. 2013). The *Runx1*-dependent transcription factor gene *Zfp521* is required to establish molecular features that define VGLUT3⁺ mechanoreceptors. *Runx1* and ZFP521 work in coordination to determine the molecular phenotype of VGLUT3⁺ mechanoreceptors while suppressing traits typical of other types of sensory neurons such as polymodal nociceptors (Lou et al. 2015). Thus, *Runx1* is a key player in the generation of DRG sensory neuron diversity by regulating various developmental processes leading to the separation of peptidergic vs non-peptidergic phenotypes as well as the specification of selected cutaneous sensory neuron subtypes.

Runx1 is also involved in regulating the connectivity of the DRG sensory neurons in which it is expressed. Non-peptidergic and peptidergic DRG neurons normally send their axons to separate targets in the dorsal spinal cord. In *Runx1*-deficient mice, the majority of nociceptive neurons in which *Runx1* would have been expressed had it not been inactivated send their axons to targets more typical of peptidergic neurons (Chen et al. 2006a; Yoshikawa et al. 2007). Conversely, ectopic *Runx1* expression in DRG neurons is sufficient to cause axons of TrkA^+ neurons to project to layers of the spinal cord normally innervated by non-peptidergic neurons (Kramer et al. 2006). Consistent with these results, *in vitro* studies suggest that *Runx1* might participate in mechanisms promoting axon growth and branching (Marmigere et al. 2006). These results are in agreement with the demonstration that *Drosophila runt* is involved in the control of photoreceptor neuron axonal targeting choices (Kaminker et al. 2002). Loss of mouse *Runx1* function also leads to the selective loss of sensory innervation to the epidermis. In contrast, sensory innervation of 'deep tissues', such as muscle and visceral organs, is not affected

by *Runx1* inactivation, providing further evidence for a role of this gene in genetic programs controlling the differentiation of cutaneous pain pathways (Yang et al. 2013). Given these phenotypic manifestations, it is not surprising that mice lacking *Runx1* in DRG sensory neurons display decreased thermal and mechanical pain perception, underscoring further the key role of *Runx1* in the formation of functional nociceptive circuits (Chen et al. 2006a, b; Abdel Samad et al. 2010).

In summary, *Runx1* acts at multiple levels during the formation of PNS neural circuits mediating pain, thermal, and itch sensations by controlling both sensory neuronal subtype specification and establishment of precise innervations.

8.2.2 *Runx3* Involvement in Formation of Proprioceptive Circuits

During DRG development, *TrkC*⁺ proprioceptive neurons are generated from transiently lived *TrkB*⁺/*TrkC*⁺ cells that also give rise to *TrkB*⁺ mechanoreceptive neurons. *Runx3* expression becomes detectable in *TrkC*⁺ DRG cells at approximately the time when the latter arise from *TrkB*⁺/*TrkC*⁺ precursors (Inoue et al. 2002; Levanon et al. 2002; Kramer et al. 2006). More importantly, *Runx3* inactivation in *Runx3*-deficient mice results in decreased numbers of *TrkC*⁺ cells and also decreased cells expressing Parvalbumin, a protein expressed preferentially in proprioceptive neurons. This phenotype is correlated with a concomitant increase in *TrkB*⁺ neurons (Inoue et al. 2002; Levanon et al. 2002; Kramer et al. 2006; Inoue et al. 2007; Nakamura et al. 2008; Lallemand et al. 2012). The converse situation is observed after ectopic expression of *Runx3* in all developing DRG neurons (Kramer et al. 2006). These findings show that *Runx3* is important for the separation of *TrkC*⁺ and *TrkB*⁺ sensory neuronal lineages.

Runx3 may be directly involved in the extinction of *TrkB* expression in proprioceptive neurons because *Runx3* binds to, and represses transcrip-

tion from, a *TrkB* intronic gene regulatory element containing consensus *Runx*-binding sites (Inoue et al. 2007). Moreover, analysis of DRG and trigeminal ganglion development in mouse embryos lacking the gene *Brn3a* revealed that *Runx3* fails to be activated in *TrkC*⁺ neurons in the absence of *Brn3a* (*Runx1* expression is also greatly attenuated in *TrkA*⁺ nociceptors in *Brn3a*-deficient mice). These changes are accompanied by expanded expression of *TrkB*, followed by the loss of *TrkC* and *TrkA* expression (Dykes et al. 2010, 2011). *Brn3a* binds to a conserved upstream enhancer element within the *Runx3* locus, suggesting that *Runx* factors repress *TrkB* expression downstream of *Brn3a* (Dykes et al. 2010). It should be noted that separate studies suggest that the negative regulation of *TrkB* expression by *Runx3* in sensory neurons mediating touch sensation is indirect and is mediated by another transcription factor, termed *Shox2*, which activates *TrkB* expression and is repressed by *Runx3* (Abdo et al. 2011).

Perturbation of *Runx3* activity is also correlated with proprioceptive neuron axonal targeting defects. *Runx3*-deficient embryos display abnormal projections of proprioceptive DRG neurons to both peripheral and central targets. *TrkC*⁺ proprioceptive neurons fail to establish proper connections in the ventral spinal cord and instead innervate more dorsal positions (Inoue et al. 2002; Chen et al. 2006b; Nakamura et al. 2008). Conversely, ectopic *Runx3* expression in *TrkA*⁺ DRG neurons results in innervation of more ventral sectors of the spinal cord, similar to the targeting of *TrkC*⁺ neurons (Kramer et al. 2006). These observations provide evidence that, similar to *Runx1* participation in nociceptive circuit formation, *Runx3* is important for the connectivity of proprioceptive DRG neurons.

More recent studies have revealed an additional level of involvement of *Runx3* during proprioceptive neuron axonal development. Proprioceptive neurons exhibit different rates of axon extension at different axial levels and these differences are determined by a segmental pattern of *Runx3* levels at different axial positions. *Runx3* is involved in proprioceptive neuron axonal extension at least in part by controlling the

level of expression of genes encoding cytoskeletal proteins involved in axon growth (Lallemend et al. 2012). Taken together, these findings provide evidence that *Runx3* plays important roles in proprioceptive neuron development by regulating both the specification of proprioceptive sensory neurons and the formation of proprioceptive neural circuits.

In summary, *Runx1* and *Runx3* mediate analogous developmental functions during the establishment of pathways mediating either cutaneous sensation or proprioception, ranging from the regulation of specific *Trk* family gene expression and acquisition of cell-type specific transcriptional profiles to the control of neuronal connectivity. It should be mentioned that the presence of certain DRG neurons expressing both *Runx1* and *Runx3* has been observed during embryonic development and after birth. At postnatal stages, some of these *Runx1*⁺/*Runx3*⁺ cells also express *TrkB*, *Ret*, and *TrkC*, suggesting that these proteins are coexpressed in at least a particular group of mechanoreceptive DRG neurons (Nakamura et al. 2008; Yoshikawa et al. 2013). It remains to be determined whether *Runx1* and *Runx3* have overlapping or non-redundant roles in the specific sensory neuronal populations in which they are coexpressed at postnatal stages.

8.3 Roles of *Runx* Genes in Central Nervous System Development

Motor neurons are specialized cells controlling voluntary and involuntary functions, ranging from the contractile activity of multiple muscle groups to the activity of smooth and cardiac muscle fibers or glands. There are three main classes of motor neurons, termed somatic (innervating muscles that control functions such as speaking, swallowing, breathing, and locomotion), branchial (innervating muscles in the face and upper neck), and visceral (controlling components of the autonomic nervous system such as smooth muscle in the viscera). The next three sections will discuss evidence suggesting that *Runx1* acts during the development of selected motor neu-

rons to regulate the establishment of specific motor neuron identities and axonal innervations. Moreover, they will address the expression of *Runx1*, and other *Runx* genes, in other types of neural cells in the CNS.

8.3.1 *Runx1* Involvement in the Formation of Motor Circuits

Runx1 is expressed in selected types of postmitotic motor neurons, but not their undifferentiated mitotic progenitors. *Runx1*⁺ neurons include visceral and somatic motor neurons in the murine brainstem and cervical spinal cord (Theriault et al. 2004; Stifani et al. 2008; Guizard et al. 2010; Chen et al. 2015; Yoshikawa et al. 2015). *Runx1* also displays a restricted expression in selected motor neuron subtypes in the chick cervical spinal cord (Dasen et al. 2005). The timing of *Runx1* expression does not coincide with motor neuron generation and instead roughly correlates with the time when the *Runx1*⁺ motor neurons are acquiring their specific molecular identities, cell body positions and/or axonal innervations (Stifani et al. 2008; Chen et al. 2015). Consistent with this finding, lack of *Runx1* activity in *Runx1*-deficient mice does not perturb the generation of the somatic motor neurons in which *Runx1* would normally be expressed (Stifani et al. 2008).

The roles of *Runx1* during motor neuron development are best characterized in brainstem somatic motor neurons located in the hypoglossal nucleus (12N). Motor neurons in 12N innervate muscles in the tongue controlling vital functions such as chewing, swallowing, and breathing. Specifically, 12N motor neurons innervate two main tongue muscle groups, anatomically defined as intrinsic or extrinsic based on their origin and location. Extrinsic and intrinsic tongue muscles can be further subdivided into two functional categories: muscles controlling either tongue protrusion ('protrusors') or tongue retraction ('retrusors') (Aldes 1995; Altschuler et al. 1994; McClung and Goldberg 1999, 2000). 12N motor neurons innervating these different tongue mus-

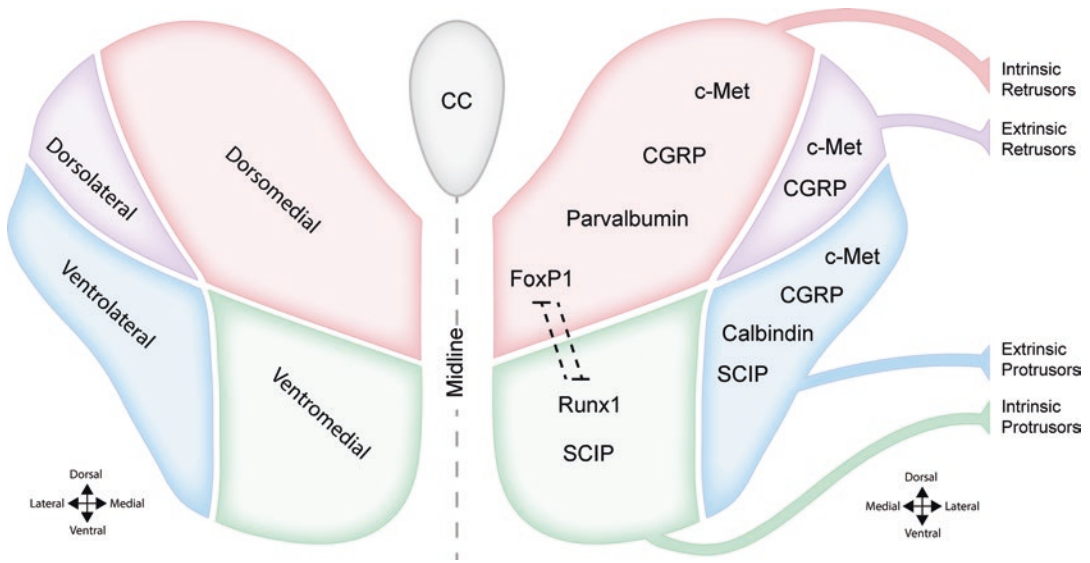


Fig. 8.1 Expression and proposed roles of *Runx1* during hypoglossal motor neuron development. Schematic representation of the restricted expression of *Runx1* in the ventromedial 12N anatomical quadrant at mediocaudal level. *Runx1*⁺ 12N motor neurons coexpress the transcription factor SCIP but do not express the transcription factor FoxP1 nor the calcium-binding proteins Parvalbumin and Calbindin, the neurotransmitter CGRP, or the surface protein c-Met. The combinatorial expression of these proteins defines four separate motor neuron groups whose topology roughly corresponds to different 12N quadrants

associated with innervation of different tongue muscle groups. *Runx1* is thought to act in ventromedial 12N motor neurons to prevent the acquisition of molecular profiles defining dorsomedial and ventrolateral 12N motor neuron phenotypes. *Runx1* and FoxP1 are proposed to cross-repress each other's expression, thereby defining the dorsoventral border of the medial 12N domain. *Runx1* is also involved in mechanisms important for ventromedial 12N motor neuron axonal innervation of intrinsic protrusor muscles in the tongue. Abbreviations: CC central canal

cles are organized into a characteristic 'somatotopic map' in which motor neurons located in the dorsal half of the nucleus generally innervate retrusor muscles, whereas motor neurons located in the ventral part innervate protrusor muscles. Moreover, motor neurons innervating intrinsic muscles are generally located medially, with motor neurons innervating extrinsic muscles found more laterally (summarized in Fig. 8.1) (Aldes 1995; Chibuzo and Cummings 1982; Krammer et al. 1979).

During 12N development, *Runx1* expression is mainly restricted to ventromedial 12N motor neurons characterized by a molecular profile distinct from other 12N motor neurons based on the expression of specific calcium-binding proteins, neurotransmitters, cell surface receptors and transcription factors (Fig. 8.1) (Chen et al. 2015; Yoshikawa et al. 2015). Importantly, the number of motor neurons exhibiting lateral or dorsal 12N

motor neuron molecular traits is decreased when *Runx1* is ectopically expressed in all developing 12N motor neurons. These results suggest that *Runx1* acts to specifically promote a 'ventromedial' 12N motor neuron phenotype at the expense of 'ventrolateral' and 'dorsomedial' 12N motor neuron identities (Chen et al. 2015).

The *Runx1*⁺ ventromedial 12N motor neurons send their axons to intrinsic tongue muscles mediating tongue protrusion (Fig. 8.1) (Chen et al. 2015; Yoshikawa et al. 2015). *In vivo* *Runx1* inactivation results in decreased 12N motor neuron axonal projections, and overall reduction of innervation density, to intrinsic protrusor muscles (Yoshikawa et al. 2015). *Runx1* inactivation also results in decreased 12N expression of the gene *Frizzled3*, which contributes to axonal pathfinding of 12N motor neurons (Hua et al. 2013; Yoshikawa et al. 2015). Together, these observations suggest that transcriptional programs

involving Runx1 are involved in the establishment of motor circuits controlling tongue protrusion.

One of the consequences of ectopic Runx1 expression in 12N motor neurons is the detectable perturbation of the normal pattern of expression of other transcription factors involved in motor neuron development. A notable example is provided by FoxP1, which is a transcription factor that plays important roles in the regulation of spinal motor neuron subtype development (Dasen et al. 2008; Roussio et al. 2008; Palmesino et al. 2010). During 12N development, FoxP1 is expressed in dorsomedial 12N motor neurons neighboring the ventromedial Runx1⁺ cells (Fig. 8.1) (Chen et al. 2015). A number of observations suggest that Runx1 and FoxP1 may transcriptionally repress each other's expression, thereby defining their respective expression domains in the medial part of 12N. In potential agreement with this possibility, forced Runx1 expression in dorsal 12N motor neurons results in a significant decrease in the number of dorsomedial 12N neurons expressing FoxP1 (Chen et al. 2015). These observations raise the possibility that at least one of the functions of Runx1 during 12N development is to prevent FoxP1 expression in the ventral sector of the medial region, thereby contributing to the establishment of separate ventromedial (Runx1⁺/FoxP1⁻) and dorsomedial (FoxP1⁺/Runx1⁻) 12N motor neuron groups. As mentioned, ventromedial 12N motor neurons project to intrinsic protrusor muscles, whereas dorsomedial 12N motor neurons are believed to project to intrinsic retrusors. Thus, it is conceivable that the activities of Runx1 and FoxP1 are involved in the formation of neural circuits controlling either tongue protrusion or retraction, respectively (Fig. 8.1).

Runx1 is expressed in other selected groups of motor neurons, including spinal motor neurons involved in the control of posture and locomotion. For instance, certain Runx1⁺ motor neurons at cervical spinal cord level C1–C4 innervate the *anterior trapezius* muscle. Runx1 expression also marks defined motor neurons at level C4–C5 that innervate the *deltoides* muscle (Stifani et al. 2008). It is possible that, similar to its roles in 12N motor neurons, Runx1 may also be impor-

tant for the acquisition and/or maintenance of specific motor neuron gene expression patterns and axonal connectivity in other somatic motor neuron subtypes.

In summary, the involvement of Runx1 in motor neuron diversity generation and circuit formation shares common themes with the roles of Runx1 during PNS nociceptive neuron development. This situation in turn suggests that Runx1 may perform similar tasks during the formation of additional CNS circuits involving other types of neurons in which Runx1 is expressed.

8.3.2 Runx1 Involvement in Other Central Neural Circuits

In addition to motor neurons, Runx1 is expressed in at least another neuronal population in the rostral brainstem, which was identified as part of the superior lateral subnucleus of the parabrachial nucleus (LPBS) on the basis of several anatomical and molecular properties (Zagami and Stifani 2010). Runx1 expression in these cells is first observed early during brain development and persists into the postnatal brain, similar to the situation observed in sensory and motor neurons. These observations suggest that Runx1 might be involved in mechanisms controlling the differentiation and/or target connectivity of LPBS neurons, as it does in other central and peripheral neurons.

Although the functional significance of the restricted expression of Runx1 in this selected neuronal population remains to be defined, it is worth mentioning that the LPBS has been implicated in the suppression of food intake in response to pain (Gibbs et al. 1973, 1976; Malick et al. 2001). Moreover, thermal and inflammatory noxious stimuli were shown to activate neurons in the LPBS in which Runx1 is expressed (Hermanson et al. 1998; Bester et al. 1995, 1997; Buritova et al. 1998). Taken together with the important roles of Runx1 in sensory nociceptor development and innervation discussed above, these observations raise the possibility that Runx1 might be involved in the formation of functional networks coordinating nociception and regulation of food intake.

8.3.3 *Runx2* Expression in the Central Nervous System

In contrast to *Runx1*, little is known about the involvement of *Runx2* and *Runx3* in the CNS. Previous studies have revealed the presence of *Runx2* transcripts in the adult mouse brain (Takarada and Yoneda 2009). Moreover, *Runx2^{LacZ/+}* knock-in mice display restricted expression of βGalactosidase under the control of the *Runx2* promoter in the postnatal hippocampus and frontal lobe area (Jeong et al. 2008). More recent work has suggested that mouse *Runx2* is expressed under circadian control in specific brain regions including the paraventricular nucleus, olfactory bulb and suprachiasmatic nucleus (Reale et al. 2013). *Runx2* expression is presumed to occur in neuronal cells in these brain areas, although it should be noted that separate studies suggest that at least some of the *Runx2⁺* cells in the brain correspond to glial cells (Takarada and Yoneda 2009). *RUNX2* expression was also detected in the adult human hippocampus, and hippocampal *RUNX2* expression is decreased in bipolar disorder patients (Benes et al. 2007). It will be important to characterize further the brain cells in which *Runx2* is expressed and the functions, and regulation, of *Runx2* in these cells to determine whether or not at least some of the themes uncovered by the analysis of *Runx1* and *Runx3* in central and peripheral neurons also apply to *Runx2*.

8.4 Contribution of *Runx1* to Olfactory System Development

The previous sections have discussed the important roles performed by *Runx1* and *Runx3* in specific subtypes of central and peripheral neurons and have addressed how these functions occur mostly, if not entirely, during postmitotic neuronal development. This section will focus on the olfactory system, which offers a compelling example of the importance of *Runx* proteins in the biology of specific types of undifferentiated mitotic neural cells.

The olfactory system is an evolutionarily ancient sensory system mediating the sense of smell. It has a peripheral component, the olfactory epithelium (OE), located in the nasal cavity, and central elements, the olfactory bulb and olfactory cortex, in the brain. Both the OE and the olfactory bulb host populations of neurons that are constantly regenerated during adult life and thus the olfactory system is one of the few neural tissues with persistent renewal potential (Leinwand and Chalasani 2011; Takeuchi and Sakano 2014; Suzuki and Osumi 2015).

8.4.1 *Runx1* Involvement in Neural Progenitor Cell Proliferation in the Olfactory Epithelium

The developing OE was first identified as one of the sites of most robust *Runx1* expression in the murine nervous system almost two decades ago (Simeone et al. 1995; Levanon et al. 2001). Subsequent work demonstrated that, in contrast to motor and sensory neuronal lineages, *Runx1* is mainly expressed in mitotic olfactory sensory neuron (OSN) progenitor cells located on the basal side of the OE (Theriault et al. 2005). During embryonic development and postnatal life, these cells act as the neural stem/progenitor cell population in the OE, comprising the self-propagating and transit-amplifying pools that drive both development and persistent regeneration of OSNs (Kam et al. 2014; Takeuchi and Sakano 2014). In agreement with the ability of *Runx* proteins to regulate the balance between the undifferentiated and differentiated states in numerous cell lineages, *in vivo* loss-of-function studies showed that *Runx1* acts in OSN progenitor cells to sustain cell proliferation and delay differentiation, thereby contributing to maintenance of the mitotic OSN progenitor pool and regulation of OSN generation. Importantly, forced exogenous *Runx1* expression in primary cultures of self-propagating OE progenitor cells revealed that *Runx1* is sufficient to enhance proliferation in this cellular context (Theriault et al. 2005). Thus, *Runx1* acts to regulate the timing of neuronal differentiation in the OE at least in part

by sustaining the proliferation of OSN progenitor cells.

8.4.2 *Runx1* Involvement in Proliferation and Developmental Maturation of Olfactory Ensheathing Cells

OSNs located in the OE send their axons to the olfactory bulb in the rostral part of the brain. The axons of OSNs are enveloped by a particular population of glial cells, termed olfactory ensheathing cells (OECs). Residing in both the OE and the olfactory bulb, OECs span the PNS and CNS and perform a remarkable array of functions during olfactory development and regeneration. They accompany and ensheath OSN axons, produce growth factors, cell adhesion molecules and extracellular matrix proteins that promote OSN axon growth and targeting, and act as phagocytic cells that engulf and remove apoptotic olfactory nerve debris (Su and He 2010; Chou et al. 2014; Roet and Verhaagen 2014).

Developmentally mature OECs derive from mitotic precursors that originate peripherally and give rise to different subtypes of differentiated OECs populating the olfactory bulb. *Runx1* is expressed in at least some OEC precursor cells *en route* to the olfactory bulb and the proliferative ability of *Runx1*-expressing OECs is sensitive to *Runx1* dosage. Specifically, decreased *Runx1* levels are correlated with increased numbers of mitotic OECs, with a parallel decrease in the number of more differentiated OECs. In contrast, *Runx1* overexpression results in reduced OEC proliferation (Murthy et al. 2014). Thus, *Runx1* contributes to the development, and possibly regeneration, of the olfactory system by acting, at least in part, to either delay or promote the proliferation-to-differentiation transition in OSN or OEC precursors, respectively.

Runx1 expression is not limited to OEC precursors that are migrating to the olfactory bulb, but persists in a defined subgroup(s) of more developmentally mature OECs. These cells are

specifically located in the inner portion of the olfactory bulb nerve layer (ONL) and exhibit characteristic molecular features, such as the expression of neuropeptide Y. In contrast, *Runx1* expression is not detected in OECs that are located in the outer part of the ONL and express different sets of proteins, including the low-affinity NGF receptor p75NTR (Murthy et al. 2014). The expression of *Runx1* in a particular subtype(s) of developmentally mature OECs with a specific topology and molecular profile is akin to the subtype-restricted expression of *Runx1* in neuronal cells in the CNS and PNS, as discussed above. This observation raises the possibility that *Runx1* may act to regulate specialized glial cell fate acquisition in the olfactory system, similar to its role in the acquisition of specific neuronal identities. Since inner and outer ONL OECs are believed to arise from common precursors, *Runx1* may be involved in the specification of inner ONL OECs by promoting the expression of genes associated with this subtype and/or repressing genes associated with outer ONL OEC phenotype(s).

In summary, these findings suggest that *Runx1* is involved in olfactory system development and regeneration by participating in multiple mechanisms controlling the transition from an immature proliferating state to more a developmentally mature phenotype in both neuronal and glial cell lineages.

8.5 *Runx* Expression in Glial Cells in the Central and Peripheral Nervous Systems

The expression of *Runx1* in OECs is not the only example of *Runx* gene expression in glial cells. Recent studies have provided evidence suggesting that *Runx1* is expressed in Schwann cell progenitors in the PNS (Li et al. 2015). Schwann cells share a number of properties with OECs and are involved in many aspects of peripheral nerve biology, including myelination of axons (a property exhibited by many, but not all, Schwann cells), the secretion of growth factors supporting

nerve development and regeneration, trophic support to neurons, and antigen presentation (Kidd et al. 2013).

A search for active enhancers in myelinating Schwann cells after peripheral nerve injury has recently identified a *Runx2* enhancer bound by c-Jun, a transcription factor required for Schwann cells to support nerve regeneration. These observations suggest that Runx2 might also be involved in Schwann cell biology, possibly by participating in the regulation of genes induced after peripheral nerve injury (Hung et al. 2015). The presence of *Runx2* transcripts was also detected in cultured rat forebrain astrocytes and astrocytic C6 glioma cells (Takarada and Yoneda 2009). Moreover, *RUNX2* is expressed in human gliomas, brain tumors of astrocytic origin (Vladimirova et al. 2008). Although the functional significance of these observations remains to be determined, they raise the possibility that Runx2 might be involved in mechanisms regulating proliferation and/or differentiation along the astrocyte lineage. In contrast to *RUNX2*, *RUNX3* expression is low or absent in glioma due to promoter hypermethylation (Mueller et al. 2007). *RUNX3* mRNA expression was observed in fetal and adult human brain, but whether this expression occurs in astrocytes and/or other glial cells remains to be determined (Mueller et al. 2007).

Together, these observations are suggesting that the involvement of Runx proteins in glial cell biology is not limited to OECs and extends to other glial cells in both the CNS and PNS. In these cells, Runx proteins may act to regulate the expression of genes important for the proliferation-to-differentiation transition and/or the acquisition of defined glial phenotypes. In this regard, it is important to note that Runx1 is upregulated in neurofibroma, a cancer of Schwann cell origin. Impairment of Runx1 activity in Schwann cell progenitor cells delays mouse neurofibroma formation *in vivo* as a result of decreased cell proliferation and increased cell apoptosis, suggesting that Runx1 may play a role in Schwann cell proliferation (Li et al. 2015). Future studies aimed at clarifying Runx expression and function in glial cells in the developing and postnatal CNS and PNS are expected to offer insight into gliogenesis and glial cell functions.

8.6 Conclusions and Perspectives

Much progress has been made in understanding the neural functions of *Runx* genes since the initial observation that *runt* is important for the differentiation of EL neurons in the *Drosophila* CNS. Our current knowledge points to essential roles for Runx1 and Runx3 during the development of specific neural circuits in the mammalian CNS and PNS. Moreover, Runx1 is involved in the development, and possibly regeneration, of the olfactory system, one of the few neural tissues with persistent renewal potential throughout life. In the future, it will be important to characterize further the molecular mechanisms underlying the roles of Runx proteins in the regulation of sensory and motor neuron subtype specific gene expression and axonal targeting choices, including the identity of additional Runx-regulated genes during these events. Moreover, little is known about the upstream mechanisms that establish the exquisite temporal and spatial specificity of *Runx* gene expression in both neuronal and glial cell lineages.

The possible involvement of Runx proteins in the adult CNS and/or PNS also remains to be determined. In the healthy brain, *Runx1* is not detectably expressed in neural stem/progenitor cells capable of supporting the genesis of new neural cells. However, endogenous *Runx1* expression becomes induced in a subpopulation of putative neural stem/progenitor cells after brain injury in adult mice (Logan et al. 2013, 2015). This effect is thought to be mediated at least in part by mechanisms involving FGF signaling, because culturing forebrain neural progenitor cells in the presence of basic FGF (bFGF) induces Runx1 expression, whereas removal of bFGF from the culture medium decreases Runx1 expression (Theriault et al. 2005; Logan et al. 2015). This possibility is also consistent with the observation that bFGF induces human *RUNX1* expression in olfactory neuroblastoma tumors (Nibu et al. 2000).

When expressed in CNS neural stem/progenitor cells as a result of injury or growth factor treatment, Runx1 can participate in mechanisms regulating proliferation and neuronal differentia-

tion (Theriault et al. 2005; Logan et al. 2015), at least in part by enhancing proliferation through repression of cell cycle inhibitory genes such as *p21^{Cip1}* (Theriault et al. 2005). Thus, although Runx1 does not appear to be physiologically expressed in neural stem/progenitor cells in the CNS, it has the potential to participate in neural stem/progenitor cell biology during processes associated with injury and repair of the adult nervous system.

Finally, it is important to mention that Runx1 can contribute to nervous system development and repair in an additional manner, namely by modulating the activity of microglia, the resident immune cells of the nervous system (Zusso et al. 2012). Microglia survey the nervous system for signs of infection, injury or disease and mediate immune responses during many neuropathological conditions (Ransohoff and El Khoury 2015; Shemer et al. 2015). They derive from primitive myeloid precursors that originate from the yolk sac and colonize the nervous system during embryonic development (Ginhoux et al. 2010; Prinz and Mildner 2011). In the developing brain, microglia are initially proliferative, amoeboid in shape and endowed with phagocytic activity. They mediate several important developmental functions including cell debris phagocytosis, guidance of axons in white matter tracts, and synaptic refinement. As development proceeds, microglia lose their amoeboid morphology and gradually progress to a surveillant, non-phagocytic state characterized by a highly ramified morphology (Schlegelmilch et al. 2010; Prinz and Mildner 2011; Wu et al. 2015). Runx1 is expressed in postnatal forebrain amoeboid microglia and its expression is downregulated as microglia progress to the ramified phenotype. Runx1 inhibits amoeboid microglia proliferation and promotes progression to the ramified state (Zusso et al. 2012). Thus, the involvement of Runx1 in microglia biology during brain development provides an additional example of this protein's ability to regulate proliferation and developmental maturation mechanisms in the developing and postnatal mammalian nervous system. The amoeboid-to-ramified morphological transition of microglia during development is

almost recapitulated in reverse during the process of microglia activation in the adult brain, when surveillant microglia undergo a ramified-to-amoeboid transformation and become phagocytic in response to injury or disease (Ransohoff and El Khoury 2015; Shemer et al. 2015). Runx1 expression is upregulated in microglia following nerve injury in the adult nervous system (Zusso et al. 2012), implicating Runx1 in the regulation of at least certain functions of microglia in the injured/diseased adult nervous system. Runx2 expression has also been observed in microglia (Nakazato et al. 2014), but it is unknown whether Runx1 and Runx2 have overlapping or distinct functions in these cells.

In conclusion, increasing evidence implicates Runx proteins in the development, regeneration, and repair of the nervous system through the regulation of several important mechanisms in neurons, macroglia, and microglia. It is therefore anticipated that increased understanding of the roles of Runx proteins in neural cells and microglia will facilitate the study of nervous system development, homeostasis and disease.

References

- Abdel Samad, O., Liu, Y., Yang, F. C., Kramer, I., Arber, S., & Ma, Q. (2010). Characterization of two Runx1-dependent nociceptor differentiation programs necessary for inflammatory versus neuropathic pain. *Molecular Pain*, 6, 45.
- Abdo, H., Li, L., Lallemand, F., Bachy, I., Xu, X. J., Rice, F. L., & Ernfors, P. (2011). Dependence on the transcription factor Shox2 for specification of sensory neurons conveying discriminative touch. *The European Journal of Neuroscience*, 34, 1529–1541.
- Aldes, L. D. (1995). Subcompartmental organization of the ventral (protruder) compartment in the hypoglossal nucleus of the rat. *The Journal of Comparative Neurology*, 353, 89–108.
- Altschuler, S. M., Bao, X., & Miselis, R. R. (1994). Dendritic architecture of hypoglossal motor neurons projecting to extrinsic tongue musculature in the rat. *The Journal of Comparative Neurology*, 342, 538–550.
- Benes, F. M., Lim, B., Matzilevich, D., Walsh, J. P., Subbaraju, S., & Minns, M. (2007). Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 10164–10169.

- Bester, H., Menendez, L., Besson, J. M., & Bernard, J. F. (1995). Spino (trigemino) parabrachiohypothalamic pathway: Electrophysiological evidence for an involvement in pain processes. *Journal of Neurophysiology*, *73*, 568–585.
- Bester, H., Matsumoto, N., Besson, J. M., & Bernard, J. F. (1997). Further evidence for the involvement of the spinoparabrachial pathway in nociceptive processes: A c-Fos study in the rat. *The Journal of Comparative Neurology*, *383*, 439–458.
- Buritova, J., Besson, J. M., & Bernard, J. F. (1998). Involvement of the spinoparabrachial pathway in inflammatory nociceptive processes: A c-Fos protein study in the awake rat. *The Journal of Comparative Neurology*, *397*, 10–28.
- Chen, C. L., Broom, D. C., Liu, Y., de Nooij, J. C., Li, Z., Cen, C., et al. (2006a). Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron*, *49*, 365–377.
- Chen, A. I., de Nooij, J. C., & Jessell, T. M. (2006b). Graded activity of transcription factor Runx3 specifies the laminar termination pattern of sensory axons in the developing spinal cord. *Neuron*, *49*, 395–408.
- Chen, X., Wang, J. W., Salin-Cantegrel, A., Dali, R., & Stifani, S. (2015, December 19). Transcriptional regulation of mouse hypoglossal motor neuron somatotopic map formation. *Brain Structure and Function*, *221*, 4187–4202. PMID: 26687503.
- Chibuzo, G. A., & Cummings, J. F. (1982). An enzyme tracer study of the organization of the somatic motor center for the innervation of different muscles of the tongue: Evidence for two sources. *The Journal of Comparative Neurology*, *205*, 273–281.
- Chou, R. H., Lu, C. Y., Wei-Lee, F. J. R., Yu, Y. L., & Shyu, W. C. (2014). The potential therapeutic applications of olfactory ensheathing cells in regenerative medicine. *Cell Transplantation*, *23*, 567–571.
- Dasen, J. S., Tice, B. C., Brenner-Morton, S., & Jessell, T. M. (2005). A Hox regulatory network establishes motor neuron pool identity and target muscle connectivity. *Cell*, *123*, 477–491.
- Dasen, J. S., De Camilli, A., Wang, B., Tucker, P. W., & Jessell, T. M. (2008). Hox repertoires for motor neuron diversity and connectivity gated by a single accessory factor, FoxP1. *Cell*, *134*, 304–316.
- Dormand, E. L., & Brand, A. H. (1998). Runt determines cell fates in the *Drosophila* embryonic CNS. *Development*, *125*, 1659–1667.
- Duffy, J. B., Kania, M. A., & Gergen, J. P. (1991). Expression and function of the *Drosophila* gene runt in early stages of neural development. *Development*, *113*, 1223–1230.
- Dykes, I. M., Lanier, J., Eng, S. R., & Turner, E. E. (2010, January 22). Brn3a regulates neuronal subtype specification in the trigeminal ganglion by promoting Runx expression during sensory differentiation. *Neural Development*, *5*, 3.
- Dykes, I. M., Tempest, L., Lee, S. I., & Turner, E. E. (2011). Brn3a and Islet1 act epistatically to regulate the gene expression program of sensory differentiation. *The Journal of Neuroscience*, *31*, 9789–9799.
- Gascon, E., Gaillard, S., Malapert, P., Liu, Y., Rodat-Despoix, L., Samokhvalov, I. M., et al. (2010). Hepatocyte growth factor-Met signaling is required for Runx1 extinction and peptidergic differentiation in primary nociceptive neurons. *The Journal of Neuroscience*, *30*, 12414–12423.
- Gibbs, J., Young, R. C., & Smith, G. P. (1973). Cholecystokinin decreases food intake in rats. *Journal of Comparative and Physiological Psychology*, *84*, 488–495.
- Gibbs, J., Falasco, J. D., & McHugh, P. R. (1976). Cholecystokinin-decreased food intake in rhesus monkeys. *The American Journal of Physiology*, *230*, 15–18.
- Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*, *330*, 841–845.
- Guizard, N., Coupé, P., Stifani, N., Stifani, S., & Collins, D.L. (2010). *Robust 3D reconstruction and mean-shift clustering of motoneurons from serial histological images*. In Proceedings of 5th international conference on Medical Imaging and Augmented Reality (MIAR) (pp. 191–199). 2010. Springer-Verlag Berlin/Heidelberg.
- Hermanson, O., Larhammar, D., & Blomqvist, A. (1998). Preprocholecystokinin mRNA-expressing neurons in the rat parabrachial nucleus: Subnuclear localization, efferent projection, and expression of nociceptive-related intracellular signaling substances. *The Journal of Comparative Neurology*, *400*, 255–270.
- Honma, Y., Kawano, M., Kohsaka, S., & Ogawa, M. (2010). Axonal projections of mechanoreceptive dorsal root ganglion neurons depend on Ret. *Development*, *137*, 2319–2328.
- Hua, Z. L., Smallwood, P. M., & Nathans, J. (2013). Frizzled3 controls axonal development in distinct populations of cranial and spinal motor neurons. *eLife*, *2*, e01482.
- Hung, H. A., Sun, G., Keles, S., & Svaren, J. (2015). Dynamic regulation of Schwann cell enhancers after peripheral nerve injury. *The Journal of Biological Chemistry*, *290*, 6937–6950.
- Inoue, K., Ozaki, S., Shiga, T., Ito, K., Masuda, T., Okado, N., et al. (2002). Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. *Nature Neuroscience*, *5*, 946–954.
- Inoue, I. K., Osato, M., Lee, B., Bae, S. C., & Ito, Y. (2007). The transcription factor Runx3 represses the neurotrophin receptor TrkB during lineage commitment of dorsal root ganglion neurons. *The Journal of Biological Chemistry*, *282*, 24175–24184.
- Inoue, K., Shiga, T., & Ito, Y. (2008). Runx transcription factors in neuronal development. *Neural Development*, *3*, 20.
- Jeong, J. H., Jin, J. S., Kim, H. N., Kang, S. M., Liu, J. C., Lengner, C. J., et al. (2008). Expression of Runx2 tran-

- scription factor in non-skeletal tissues, sperm, and brain. *Journal of Cellular Physiology*, 217, 511–517.
- Kam, J. W., Raja, R., & Cloutier, J. F. (2014). Cellular and molecular mechanisms regulating embryonic neurogenesis in the rodent olfactory epithelium. *International Journal of Developmental Neuroscience*, 37, 76–86.
- Kaminker, J. S., Canon, J., Salecker, I., & Banerjee, U. (2002). Control of photoreceptor axon target choice by transcriptional repression of *Runt*. *Nature Neuroscience*, 5, 746–750.
- Kidd, G. J., Ohno, N., & Trapp, B. D. (2013). Biology of Schwann cells. *Handbook of Clinical Neurology*, 115, 55–79.
- Kramer, I., Sigrist, M., de Nooij, J. C., Taniuchi, I., Jessell, T. M., & Arber, S. (2006). A role for *Runx* transcription factor signaling in dorsal root ganglion sensory neuron diversification. *Neuron*, 49, 379–393.
- Krammer, E. B., Rath, T., & Lischka, M. F. (1979). Somatotopic organization of the hypoglossal nucleus: A HRP study in the rat. *Brain Research*, 170, 533–537.
- Lallemend, F., & Ernfors, P. (2012). Molecular interactions underlying the specification of sensory neurons. *Trends in Neurosciences*, 35, 373–381.
- Lallemend, F., Sterzenbach, U., Hadjab-Lallemend, S., Aquino, J. B., Castelo-Branco, G., Sinha, I., et al. (2012). Positional differences of axon growth rates between sensory neurons encoded by *Runx3*. *The EMBO Journal*, 31, 3718–3729.
- Leinwand, S. G., & Chalasani, S. H. (2011). Olfactory networks: From sensation to perception. *Current Opinion in Genetics & Development*, 21, 806–811.
- Levanon, D., Brenner, O., Negreanu, V., Bettoun, D., Woolf, E., Eilam, R., et al. (2001). Spatial and temporal expression pattern of *Runx3* (*Aml2*) and *Runx1* (*Aml1*) indicates non-redundant functions during mouse embryogenesis. *Mechanisms of Development*, 109, 413–417.
- Levanon, D., Bettoun, D., Harris-Cerruti, C., Woolf, E., Negreanu, V., Eilam, R., et al. (2002). The *Runx3* transcription factor regulates development and survival of *TrkC* dorsal root ganglia neurons. *The EMBO Journal*, 21, 3454–3463.
- Li, H., Zhao, X., Yan, X., Jessen, W. J., Kim, M. O., Dombi, E., Liu, P. P., Huang, G., Wu, J. (2015, June 15). *Runx1* contributes to neurofibromatosis type 1 neurofibroma formation. *Oncogene*, 35, 1468–1474. doi:10.1038/onc.2015.207.
- Liu, Y., Yang, F., Okuda, T., Dong, X., Zylka, M. J., Chen, C., et al. (2008). Mechanisms of compartmentalized expression of *Mrg* class G protein-coupled sensory receptors. *The Journal of Neuroscience*, 28, 125–132.
- Logan, T. T., Villapol, S., & Symes, A. J. (2013). TGF- β superfamily gene expression and induction of the *Runx1* transcription factor in adult neurogenic regions after brain injury. *PLoS One*, 8(3), e59250.
- Logan, T. T., Rusnak, M., & Symes, A. J. (2015). *Runx1* promotes proliferation and neuronal differentiation in adult mouse neurosphere cultures. *Stem Cell Research*, 15, 554–564.
- Lou, S., Duan, B., Vong, L., Lowell, B. B., & Ma, Q. (2013). *Runx1* controls terminal morphology and mechanosensitivity of VGLUT3-expressing C-mechanoreceptors. *The Journal of Neuroscience*, 33, 870–882.
- Lou, S., Pan, X., Huang, T., Duan, B., Yang, F. C., Yang, J., et al. (2015). Incoherent feed-forward regulatory loops control segregation of C-mechanoreceptors, nociceptors, and pruriceptors. *The Journal of Neuroscience*, 35, 5317–5329.
- Luo, W., Wickramasinghe, S. R., Savitt, J. M., Griffin, J. W., Dawson, T. M., & Ginty, D. D. (2007). A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpetidergic DRG neurons. *Neuron*, 54, 739–754.
- Malick, A., Jakubowski, M., Elmquist, J. K., Saper, C. B., & Burstein, R. (2001). A neurohistochemical blueprint for pain-induced loss of appetite. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 9930–9935.
- Marmigere, F., Montelius, A., Wegner, M., Groner, Y., Reichardt, L. F., & Ernfors, P. (2006). The *Runx1/AML1* transcription factor selectively regulates development and survival of *TrkA* nociceptive sensory neurons. *Nature Neuroscience*, 9, 180–187.
- McClung, J. R., & Goldberg, S. J. (1999). Organization of motoneurons in the dorsal hypoglossal nucleus that innervate the retrusor muscles of the tongue in the rat. *The Anatomical Record*, 254, 222–230.
- McClung, J. R., & Goldberg, S. J. (2000). Functional anatomy of the hypoglossal innervated muscles of the rat tongue: A model for elongation and protrusion of the mammalian tongue. *The Anatomical Record*, 260, 378–386.
- Mueller, W., Nutt, C. L., Ehrlich, M., Riemenscheider, M. J., von Deimling, A., van den Boom, D., & Louis, D. N. (2007). Downregulation of *RUNX3* and *TES* by hypermethylation in glioblastoma. *Oncogene*, 26, 583–593.
- Murthy, M., Bocking, S., Verginelli, F., & Stifani, S. (2014). Transcription factor *Runx1* inhibits proliferation and promotes developmental maturation in a selected population of inner olfactory nerve layer olfactory ensheathing cells. *Gene*, 540, 191–200.
- Nakamura, S., Senzaki, K., Yoshikawa, M., Nishimura, M., Inoue, K. I., Ito, Y., et al. (2008). Dynamic regulation of the expression of neurotrophin receptors by *Runx3*. *Development*, 135, 1703–17011.
- Nakazato, R., Takarada, T., Watanabe, T., Nguyen, B. T., Ikeno, S., Hinoi, E., & Yoneda, Y. (2014). Constitutive and functional expression of *runt*-related transcription factor-2 by microglial cells. *Neurochemistry International*, 74, 24–35.
- Nibu, K., Li, G., Kaga, K., & Rothstein, J. L. (2000). bFGF induces differentiation and death of olfactory neuroblastoma cells. *Biochemical and Biophysical Research Communications*, 279, 172–180.

- Palmesino, E., Rousso, D. L., Kao, T.-J., Klar, A., Laufer, E., Uemura, O., et al. (2010). Foxp1 and Lhx1 coordinate motor neuron migration with axon trajectory choice by gating reelin signalling. *PLoS Biology*, *8*(8), e1000446.
- Prinz, M., & Mildner, A. (2011). Microglia in the CNS: Immigrants from another world. *Glia*, *59*, 177–187.
- Ransohoff RM, El Khoury J. 2015. Microglia in health and disease. *Cold Spring Harbor Perspectives in Biology*, *8*(1).
- Reale, M. E., Webb, I. C., Wang, X., Baltazar, R. M., Coolen, L. M., & Lehman, M. N. (2013). The transcription factor Runx2 is under circadian control in the suprachiasmatic nucleus and functions in the control of rhythmic behavior. *PLoS One*, *8*(1), e54317.
- Roet, K. C., & Verhaagen, J. (2014). Understanding the neural repair-promoting properties of olfactory ensheathing cells. *Experimental Neurology*, *261*, 594–609.
- Rousso, D. L., Gaber, Z. B., Wellik, D., Morrisey, E. E., & Novitch, B. G. (2008). Coordinated actions of the forkhead protein Foxp1 and Hox proteins in the columnar organization of spinal motor neurons. *Neuron*, *59*, 226–240.
- Schlegelmilch, T., Henke, K., & Peri, F. (2010). Microglia in the developing brain: From immunity to behavior. *Current Opinion in Neurobiology*, *21*, 1–6.
- Shemer, A., Erny, D., Jung, S., & Prinz, M. (2015). Microglia plasticity during health and disease: An immunological perspective. *Trends in Immunology*, *36*, 614–624.
- Simeone, A., Daga, A., & Calabi, F. (1995). Expression of runt in the mouse embryo. *Developmental Dynamics*, *203*, 61–70.
- Stifani, N., Freitas, A. R. O., Liakhovitskaia, A., Medvinsky, A., Kania, A., & Stifani, S. (2008). Suppression of interneuron programs and maintenance of selected spinal motor neuron fates by the transcription factor AML1/Runx1. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 6451–6456.
- Su, Z., & He, C. (2010). Olfactory ensheathing cells: Biology in neural development and regeneration. *Progress in Neurobiology*, *92*, 517–532.
- Suzuki, J., & Osumi, N. (2015). Neural crest and placode contributions to olfactory development. *Current Topics in Developmental Biology*, *111*, 351–374.
- Takarada, T., & Yoneda, Y. (2009). Transactivation by Runt-related factor-2 of matrix metalloproteinase-13 in astrocytes. *Neuroscience Letters*, *451*, 99–104.
- Takeuchi, H., & Sakano, H. (2014). Neural map formation in the mouse olfactory system. *Cellular and Molecular Life Sciences*, *71*, 3049–3057.
- Therault, F. M., Roy, P., & Stifani, S. (2004). AML1/Runx1 is important for the development of hindbrain cholinergic branchiovisceral motor neurons and selected cranial sensory neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 10343–10348.
- Therault, F. M., Nuthall, H. N., Dong, Z., Lo, R., Barnabe-Heider, F., Miller, F. D., & Stifani, S. (2005). Role for Runx1 in the proliferation and neuronal differentiation of selected progenitor cells in the mammalian nervous system. *The Journal of Neuroscience*, *25*, 2050–2061.
- Ugarte, G. D., Diaz, E., Biscaia, M., Stehberg, J., Montecino, M., & van Zundert, B. (2013). Transcription of the pain-related TRPV1 gene requires Runx1 and C/EBPβ factors. *Journal of Cellular Physiology*, *228*, 860–870.
- Vladimirova, V., Waha, A., Lucknerath, K., Pesheva, P., & Probstmeier, R. (2008). Runx2 is expressed in human glioma cells and mediates the expression of galectin-3. *Journal of Neuroscience Research*, *86*, 2450–2461.
- Wu, Y., Dissing-Olesen, L., MacVicar, B. A., & Stevens, B. (2015). Microglia: Dynamic mediators of synapse development and plasticity. *Trends in Immunology*, *36*(10), 605–613.
- Yang, F. C., Tan, T., Huang, T., Christianson, J., Samad, O. A., Liu, Y., et al. (2013). Genetic control of the segregation of pain-related sensory neurons innervating the cutaneous versus deep tissues. *Cell Reports*, *5*, 1353–1364.
- Yoshikawa, M., Senzaki, K., Yokomizo, T., Takahashi, S., Ozaki, S., & Shiga, T. (2007). Runx1 selectively regulates cell fate specification and axonal projections of dorsal root ganglion neurons. *Developmental Biology*, *303*, 663–674.
- Yoshikawa, M., Murakami, Y., Senzaki, K., Masuda, T., Ozaki, S., Ito, Y., & Shiga, T. (2013). Co-expression of Runx1 and Runx3 in mechanoreceptive dorsal root ganglion neurons. *Developmental Neurobiology*, *73*, 469–479.
- Yoshikawa, M., Hirabayashi, M., Ito, R., Ozaki, S., Aizawa, S., Masuda, T., et al. (2015). Contribution of the Runx1 transcription factor to axonal pathfinding and muscle innervation by hypoglossal motoneurons. *Developmental Neurobiology*, *75*, 1295–1314.
- Zagami, C. J., & Stifani, S. (2010). Molecular characterization of the mouse superior lateral parabrachial nucleus through expression of the transcription factor Runx1. *PLoS One*, *5*(11), e13944.
- Zusso, M., Methot, L., Lo, R., Grenhalgh, A. D., David, S., & Stifani, S. (2012). Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor Runx1. *The Journal of Neuroscience*, *32*, 11285–11298.