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'Runxs and regulations' of sensory and motor neuron subtype differentiation: Implications for hematopoietic development

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Abstract

Runt-related (RUNX) transcription factors are evolutionarily conserved regulators of a number of developmental mechanisms. RUNX proteins often control the balance between proliferation and differentiation and alterations of their functions are associated with different types of cancer and other human pathologies. Moreover, RUNX factors control important steps during the developmental acquisition of mature phenotypes. A number of investigations are beginning to shed light on the involvement of RUNX family members in the development of the nervous system. This review summarizes recent progress in the study of the roles of mammalian RUNX proteins during the differentiation of sensory and motor neurons in the peripheral and central nervous system, respectively. The implications of those findings for RUNX-mediated regulation of hematopoietic development will also be discussed.

Keywords

Acute myeloid leukemia; Differentiation; Dorsal root ganglion; Motor neurons; Nervous system; Proliferation; Runx; Sensory neurons; Spinal cord

Introduction

The *runt*-related gene family comprises a group of evolutionarily conserved genes encoding dual-function DNA-binding transcription factors that play essential roles in the control of lineage-specific gene expression during a variety of developmental mechanisms. For instance, *Drosophila* Runt regulates segmentation, sex determination, and neuronal differentiation during embryogenesis. The three mammalian *runt*-related genes (hereafter designated as *RUNX* when referring to both human and mouse or *Runx* when indicating only mouse genes) play critical roles in developmental events important for hematopoiesis, skeletogenesis, and epithelial development, to name only a few. RUNX family members also play important roles in adult tissue homeostasis and their deregulated activity has been linked to cancer and other diseases. RUNX proteins act as important nuclear effectors of a number of signaling pathways and play essential roles in integrating responses to a variety of extracellular cues [1–6].

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Structure and function of RUNX proteins

Runt-related proteins are characterized by a conserved 128-amino acid motif, termed the Runt domain, which mediates DNA binding (consensus recognition sequence: $^{A}/_{G}CC^{A}/_{G}CA$) as well as heterodimerization. There are four members of this protein family in *Drosophila*, while mammals express three RUNX proteins and most other species have a single Runt-related protein [1–6]. *RUNX* genes encode the α subunit of a transcription factor complex initially termed polyomavirus enhancer-binding protein 2 (PEBP2) or core binding factor (CBF) in mammals [7–9]. The β subunit of this complex, designated PEBP2β or CBFβ (hereafter referred to as CBFβ), has no intrinsic DNA binding capability, shares no structural relatedness to RUNX proteins and binds to the Runt domain of the latter. CBFβ is thought to act by enhancing the affinity of RUNX proteins for DNA as well as protecting them from proteasome-mediated degradation [4–6]. The ability to interact with CBFβ is shared by both invertebrate and vertebrate Runt-related proteins. RUNX factors interact with a variety of other nuclear proteins, including DNA-binding transcription factors as well as transcriptional coactivators or corepressors with no intrinsic DNA-binding ability [1–6]. The capacity of RUNX proteins to engage numerous transcriptional regulators enables them to participate in both transcriptional activation and repression mechanisms, depending on the specific context in which they bind to DNA and the contextual identity of their transcription partners. This versatility can manifest itself even within a single cell, as is the case in the developing *Drosophila* eye, where the Runt family member, Lozenge, can repress or activate separate genes in the same cone cell [10]. As a result, Runt-related proteins have been implicated in the regulation of an increasingly large number of genes in response to a variety of extracellular cues and sit at the crossroad of various signaling pathways [1–6].

Pleiotropic roles of mammalian RUNX proteins during organ development and homeostasis

The three mammalian RUNX proteins, RUNX1 (AML1/PEBP2αB/CBFA2), RUNX2 (AML3/PEBP2αA/CBFA1) and RUNX3 (AML2/PEBP2αC/CBFA3) are expressed in many cell types during development. Their expression persists in adult organs, such as blood, bones and a variety of epithelia, which undergo regeneration from pluripotent stem cells throughout life. RUNX factors play essential functions in regulating the balance between cell proliferation and differentiation, and they control a variety of processes during development and adult tissue homeostasis [1–6]. More specifically, RUNX1 is a critical regulator of fetal and adult hematopoiesis and its malfunction is associated with leukemia, as first demonstrated by the finding that *RUNX1* is frequently targeted by chromosomal translocations associated with acute myeloid leukemia (AML) [2,5,11]. RUNX2 regulates bone development and its haploinsufficiency results in a form of human bone disease termed cleiodocranial dysplasia [12,13]. RUNX3 acts as a tumor suppressor in a number of tissues [2,5] and is required for thymopoiesis [14–16] and gastric system development [17].

RUNX proteins are important regulators of cell proliferation. This function is context-dependent. For instance, RUNX2 inhibits the proliferation of osteoblast progenitor cells and promotes osteoblast differentiation during skeletal development, in part by inducing the cell cycle inhibitor, $p27^{Kip1}$ [18–21]. In contrast, RUNX2 promotes the proliferation of endothelial cells, possibly as a result of its up-regulation during G2/M phases, when it is thought to mediate repression of another cell cycle inhibitor, $p21^{Cip1}$ [21–23]. RUNX1 is also able to repress transcription of the $p21^{Cip1}$ gene, as shown by studies in myeloid and neural progenitor cells [24–26]. In agreement with such a role, RUNX1 delays cell cycle exit in neural progenitor cells [26] and shortens the progression from G1 to S phase of the cell cycle in hematopoietic cells, where it also induces expression of *cyclin D2* and *D3* [25,27]. Moreover, RUNX1 can transform primary embryonic fibroblasts in the absence of p53 [28]. RUNX3 acts as a tumor suppressor

and deletion of the human *RUNX3* gene or hypermethylation of its promoter are associated with several cancers [2,5]. RUNX3 is thought to participate in TGFβ-mediated induction of apoptosis in proliferating epithelial cells [17,29]. The single *C. elegans Runx* gene, termed *rnt-1*, provides another example of the involvement of Runt-related proteins in the regulation of proliferation. *rnt-1* is required for both symmetric and asymmetric divisions of seam cells, a stem cell-like population of self-renewing cells that can give rise to differentiated epidermal cells during larval development [4,6].

The important role of RUNX proteins in regulating the balance between proliferation and differentiation is further demonstrated by the requirement for these factors in the establishment of a variety of differentiated cell fates. RUNX proteins can either delay or promote the mitotic precursor-to-differentiated progeny transition. For instance, Runx1 regulates the precursor-to-neuron transition in the mouse olfactory epithelium and its inactivation leads to premature exit from the cell cycle and accelerated olfactory receptor neuron differentiation [26]. In other contexts, alterations of *RUNX* activity due to mutations causing inactivation, dominant-negative effects, or deregulated activation can block the differentiation of precursor cells, often resulting in their persistent proliferation and the occurrence of pathological conditions such as leukemia (*RUNX1*) or gastric cancer (*RUNX3*) [1–6]. RUNX proteins are also involved in mechanisms that regulate the establishment of specific sublineages. For example, both RUNX1 and RUNX3 are required for the correct segregation of CD4-positive and CD8-positive cell lineages during T lymphocyte development [14–16]. In summary, RUNX proteins have emerged as critical regulators of an increasing number of cell fate choices during both embryonic development and adult organ maintenance.

Runx gene expression in the developing mammalian nervous system

Studies in *Drosophila* provided the first demonstration that Runt-related proteins are important for nervous system development. In the insect embryonic central nervous system (CNS), *runt* is expressed in selected neuronal precursor cells and their progeny, namely a subset of neurons termed *even-skipped*-expressing lateral (EL) neurons [30–32]. *runt* inactivation using a temperature-sensitive allele was shown to cause a selective loss of EL neurons [31,33]. Conversely, ectopic *runt* expression resulted in the formation of supernumerary EL neurons that extended axons along the normal trajectory used by EL neurons [32]. A role for Runt-related proteins in the regulation of axon targeting was further demonstrated by subsequent studies showing that Runt is involved in the control of axon target choices by photoreceptor neurons [34]. Together, these results show that *Drosophila runt* acts to regulate the differentiation of specific neuron subtypes as well as the development of their axonal projections.

The characterization of the neural functions of *Drosophila runt* prompted a number of laboratories to examine the expression of *Runx* genes in the mammalian nervous system [26, 35–45]. Those investigations revealed that *Runx1* and *Runx3* are expressed in the developing mouse CNS and peripheral nervous system (PNS). A few general observations are worth noting. 1) The neural expression patterns of *Runx1* and *Runx3* are for the most part non-overlapping. 2) *Runx1* and *Runx3* expression is restricted to only a few specific neuronal lineages, similar to the expression of *Drosophila runt* in selected neuronal precursors and their progeny [36–45]. 3) *Runx1* is expressed in both mitotic neuronal precursors and post-mitotic neurons, albeit in separate lineages, whereas *Runx3* appears to be exclusively expressed in post-mitotic neurons [26,36–45]. In that regard, *Runx1* expression is more reminiscent of *runt* expression in the *Drosophila* CNS. 4) *Runx1* expression in neuronal precursors is correlated preferentially with cells that are undergoing their final rounds of division before terminal mitosis and differentiation [26]. 5) *Runx1* and *Runx3* are expressed in selected populations of developing neurons after those cells are generated from mitotic precursors and

concomitant with phases of post-mitotic developmental maturation including the acquisition of specific sublineages, axonal targeting, and the response to target-derived trophic support [40–45]. These combined observations suggest that *Runx1* and *Runx3* play specific roles in the development of selected neuronal lineages in the mammalian CNS and PNS. In contrast to *Runx1* and *Runx3*, little or no information is available on the expression of *Runx2* during nervous system development. Analysis of *Runx2*$^{LacZ/+}$ knock-in mice has recently shown a restricted activation of β-galactosidase expression in the postnatal brain, namely in the hippocampus and frontal lobe area [46]. In agreement with those results, *RUNX2* expression was detected in the adult human hippocampus. Interestingly, hippocampal *RUNX2* expression is decreased in bipolar disorder patients [47]. The physiological significance of *RUNX2* expression in selected regions of the adult brain remains to be investigated. Here, we shall review our current understanding of the involvement of Runx1 and Runx3 in the development of defined neuronal lineages in the CNS and PNS.

Regulation of sensory neuron subtype development by *Runx1* and *Runx3*

Dorsal root ganglion (DRG) sensory neurons comprise a number of molecularly and morphologically distinct neurons that convey somatosensory stimuli (such as pain producing or mechanical signals, or information about muscle length and tension) to the spinal cord. DRG neurons involved in pain transduction (nociceptive) initially develop as cells expressing the nerve growth factor receptor, TrkA. Nociceptive neurons (‘nociceptors’) generally have peripheral cutaneous targets and send afferent axons to superficial layers of the dorsal region of the spinal cord. Proprioceptive DRG neurons (‘proprioceptors’) mediate sense of position, express a different neurotrophin receptor, TrkC, and depend on neurotrophin-3 for survival. Proprioceptors are connected to peripheral muscle spindles and send afferent axons to spinal cord targets that are located more ventrally than nociceptor targets [48,49]. Work by the Groner [35,36] and Ito [37] laboratories first demonstrated that *Runx1* and *Runx3* have mostly complementary expression patterns during embryonic DRG neuron development. At E16.5, *Runx1* is expressed only in nociceptors while *Runx3* expression is restricted to proprioceptive neurons. This mutual segregation appears to be gradually lost, however, because more recent studies have shown the presence of *Runx1*/*Runx3*-double positive DRG neurons at postnatal stages [50]. These observations suggest mostly non-overlapping, and developmentally dynamic, roles for *Runx1* and *Runx3* during embryonic and postnatal DRG neuron development.

Runx1 expression in nociceptive neuron subtypes in developing dorsal root ganglia

Runx1 is expressed in a complex pattern during embryonic and postnatal development of DRG nociceptive neurons. During mouse embryonic development, *Runx1* expression is first activated in the majority of *TrkA*-positive nociceptors at E12.0–E12.5 in lumbar DRG [36, 40,41]. *Runx1* activation follows the onset of *TrkA* expression (~E11.5 at lumbar levels), suggesting that Runx1 is not involved in the initial activation of *TrkA* [40,41]. Conversely, *Runx1* expression is initially not perturbed in *TrkA*-deficient embryos, showing that the onset of *Runx1* expression does not depend on TrkA signaling [44]. At later developmental stages, however, the absence of NGF-mediated TrkA signaling does result in diminished *Runx1* expression, suggesting a role of TrkA signaling in maintaining *Runx1* expression [51]. The coexpression of *Runx1* and *TrkA* ends by postnatal stages, when *TrkA*/*Runx1*-double-positive neurons are segregated into at least three subgroups. The first type remains *TrkA*-positive and loses *Runx1* expression (‘*Runx1*-transient nociceptors’). The second group loses both *TrkA* and *Runx1* expression, thus also belonging to *Runx1*-transient nociceptors, and most of its components express the MrgprA/B/C class G-protein coupled sensory receptors [52] The third class retains *Runx1*, switches off *TrkA*, and activates *Ret*, the gene encoding the receptor for glial-derived neurotrophic factor (‘*Runx1*-persistent nociceptors’) [40,41,44]. Many, but not

all, of *Runx1*-transient nociceptors acquire a 'peptidergic' phenotype characterized by the expression of the neuropeptide calcitonin-gene-related peptide (CGRP). In contrast, the majority of *Runx1*-persistent nociceptors acquire a 'non-peptidergic' phenotype characterized by the lack of CGRP expression [40,41,43,44]. In summary, the expression pattern of *Runx1* during DRG nociceptor development is dynamic and correlates with the transition from embryonic *TrkA*/*Runx1*-'double-positive' sensory neurons to postnatal neurons expressing *TrkA*, the *Runx1*/*Ret* combination, or none of these genes.

Role of Runx1 in the establishment of nociceptive neuron phenotype

Insight into the role of *Runx1* during DRG nociceptor development was provided by a combination of loss- and gain-of-function studies. *Runx1* inactivation in mice causes embryonic death by E12.5 due to a lack of definitive hematopoiesis [53,54]. Because of this situation, Chen et al. [40] examined *Runx1*$^{flox/flox}$;*Wnt1*-Cre mice in which *Runx1* was conditionally inactivated by use of *Wnt1* promoter-driven Cre expression in premigratory neural crest cells, the progenitors of DRG neurons. This approach resulted in *Runx1* inactivation from the onset of DRG development. These mice were viable, thus permitting the study of *Runx1* involvement in DRG neuron differentiation. *Runx1*-deficient nociceptors were correctly generated and survived normally in those knockout mice. However, *TrkA*-positive neurons were increased in numbers in postnatal *Runx1*-deficient animals, concomitant with a significant decrease in the percentage of *Ret*-positive neurons. *Runx1* inactivation was also correlated with a loss/attenuation of the expression of a variety of nociceptive ion channels and receptors, including ATP channels, sodium channels, G protein-coupled receptors, and TRPV channels. Conversely, *Runx1*-deficient nociceptors exhibited a derepression of peptidergic genes such as *CGRP* [40]. In complementary investigations, Kramer et al. [41] performed gain-of-function studies based on the analysis of transgenic mice in which Runx1 was overexpressed or misexpressed in all DRG neurons from early developmental stages. This perturbation was sufficient to cause a suppression of peptidergic differentiation genes like *CGRP*, opposite to the results of the loss-of-function analysis performed by Chen et al. [40]. These findings provided the first demonstration that the separation of *TrkA*-positive and *Ret*-positive nociceptor lineages is perturbed in the absence of Runx1.

Similar results were obtained in subsequent studies in which the effect of *Runx1* inactivation in developing DRG was examined in transgenic *Runx1*-deficient mice in which the embryonic lethality associated with *Runx1* inactivation was rescued by the hematopoietic-specific reactivation of *Runx1* under the control of the *GATA-1* gene hematopoietic regulatory domain [44]. Moreover, Liu et al. [52] showed further that Runx1 is required for non-overlapping expression of two sets of sensory G protein-coupled receptors of the Mrg family in developing nociceptors [52]. More specifically, Runx1 is initially required for the expression of all *Mrg* genes but it becomes a repressor of *MrgA*/*B*/*C* genes during late development. As a result, *MrgA*/*B*/*C* expression persists only in Runx1-negative nociceptors. In contrast, *MrgD* expression becomes restricted to neurons with persistent Runx1 expression [52].

It should be noted that experiments in developing chicken embryos raised the additional possibility that Runx1 might also be important for earlier stages of DRG nociceptive neuron development. Marmigere et al. [43] examined the effects of perturbing Runx1 function during early DRG development by performing *in ovo* electroporation studies in developing chicken embryos. In one line of studies, these authors expressed into premigratory neural crest cells a truncated form of chicken Runx1 ('Runx1d') encoding only the N-terminal 243 amino acids harboring the DNA-binding domain and lacking the C-terminal transcription regulatory domains. This truncated form is equivalent to a short form of human RUNX1 that was shown to have leukemogenic effects and is believed to act as a dominant-negative mutant [2,3,11]. Forced Runx1d expression was correlated with a loss of *TrkA*, but not *TrkC*, expression in

embryonic DRG neurons. In converse experiments, expression of full-length Runx1 induced *TrkA* expression in migratory neural crest cells, without promoting neuronal differentiation [43]. These findings suggest that Runx1 participates in mechanisms that promote/sustain *TrkA* expression in DRG nociceptors at early embryonic stages, prior to its involvement in switching off *TrkA* expression during the separation of peptidergic and non-peptidergic phenotypes at later stages. It is also worth mentioning that Marmigere et al. [43] found that forced expression of Runx1d, as well as siRNA-mediated Runx1 knockdown, was correlated with increased numbers of DRG neurons undergoing cell death, resulting in a significant neuronal loss in the DRG of electroporated embryos compared to controls. This observation contrasts with the lack of increased neuron cell death in the DRG of conditional *Runx1* knockout animals [40] and, more importantly, with the observation that the total number of DRG neurons was increased, rather than decreased, when *Runx1* was inactivated in transgenic *Runx1*-deficient animals selectively rescued by *GATA-1* promoter-driven *Runx1* reactivation [44]. Regardless of this discrepancy, the combined results of those investigations demonstrate that Runx1 is required for the generation of nociceptive neuron subtypes during embryonic and early postnatal phases of DRG development.

Role of Runx1 in the establishment of nociceptive neuron circuitry

Studies in both mouse and chicken experimental systems showed that *Runx1* is also important for the lamina-specific innervation pattern of nociceptive afferents in the spinal cord. Chen et al. [40] showed that *Runx1* inactivation is correlated with a perturbation of the targeting choice of *Ret*-positive nociceptor afferent projections. Those axons normally project to deeper dorsal spinal cord lamina (termed lamina 'IIi') than *TrkA*-positive afferents and this situation is perturbed in *Runx1*-deficient animals because those afferents now project to more superficial laminae (I and IIo). These authors showed further that this phenotype, together with the loss of many nociceptive ion channels and receptors, is correlated with an impaired responsiveness of adult mice lacking *Runx1* to thermal, neuropathic and inflammatory pain [40]. A consistent result was obtained in gain-of-function studies in which ectopic Runx1 expression in DRG neurons was shown to be sufficient to drive TrkA-positive axons to deeper layers of the spinal cord [41], as well as in loss-of-function studies utilizing *Runx1*-deficient embryos 'rescued' by the *GATA-1* promoter-mediated reactivation of *Runx1* expression in hematopoietic cells [44]. Although little is currently known about the mechanisms underlying the role of Runx1 in axon targeting, *in vitro* studies by Marmigere et al. [43] suggests that Runx1 might be able to promote axon length and branching. Altogether, these findings underscore an important role of Runx1 in the establishment of nociceptive sensory neuron target connectivity.

Runx3 expression in proprioceptive neurons in developing dorsal root ganglia

Runx3 is not expressed in *TrkA*-positive sensory neurons during embryonic DRG development [36,37]. Several types of *TrkA*-negative DRG neurons are present early in development, including *TrkB*-positive, *TrkC*-positive, and *TrkB*/*TrkC*-double positive. The latter population is transient and disappears by E14.5, resulting in the separation of *TrkC*-positive proprioceptive and *TrkB*-positive mechanoreceptive lineages. The expression of *Runx3* is associated only with *TrkC*-positive neurons and is correlated with the neurotrophin receptor switch that underlies these cell fate choices [36,37,41]. Importantly, recent studies have shown that a *TrkB* intronic gene regulatory element contains putative Runx-binding sites. Runx3 binds to, and represses transcription from, those *TrkB* intron sequences in cultured cells, suggesting that Runx3 negatively regulates *TrkB* expression in developing DRG neurons [55]. Taken together, these results implicate Runx3 in the transcriptional repression of *TrkB* during the establishment of separate *TrkC*-positive and *TrkB*-positive lineages from common precursors. This role is similar to the involvement of Runx1 in the generation of *Runx1*-positive or *TrkA*-positive nociceptors from *Runx1*/*TrkA*-double-positive neurons. Thus, it is possible that different Runx proteins share a common function during DRG neuron development, namely to act in selected

types of bipotential post-mitotic neurons to promote the specification of more specialized phenotypes.

It is worth mentioning that recent studies have shown that the strict correlation between *Runx3* expression and *TrkC*-positive proprioceptive neurons does not apply to postnatal stages, when *Runx3* expression was observed in both *TrkA*-positive and *TrkB*-positive DRG neurons [50]. An overlap of *Runx3* and *Runx1* expression was also observed at postnatal stages, presumably in specific populations of DRG nociceptive neurons [50]. The physiological significance of this observation remains to be defined, but it is possible that changing cellular and developmental contexts might influence the transcriptional functions of Runx3 and modulate its involvement in neurotrophin receptor regulation and sensory neuron function.

Role of Runx3 in the establishment of proprioceptive neuron phenotype and circuitry

Runx3 inactivation in knockout mice causes a loss of DRG *TrkC*-positive proprioceptive cells and increased numbers of *TrkB*-positive neurons [36,37,41,55]. Conversely, gain-of-function studies in which Runx3 was ectopically expressed in all DRG neurons showed a loss of *TrkB* expression and increased numbers of *TrkC*-positive neurons [41]. Together, these studies show that Runx3 promotes the acquisition of the *TrkC*-positive proprioceptive neuron subtype and that this function involves the repression of *TrkB* expression.

The perturbation of DRG neuron development caused by *Runx3* inactivation is correlated with an adult phenotype characterized by uncoordinated gait and abnormal positioning of the legs at rest [36,37]. To characterize this limb ataxia phenotype, both the Groner [36] and Ito [37] laboratories examined the effect of *Runx3* inactivation on the targeting of DRG proprioceptive neuron axons. These cells normally send afferents to intermediate and ventral regions of the spinal cord where they make contacts with motor neurons to establish the stretch reflex circuit that mediates information about muscle length and tension. Both groups found that the motor discoordination defect of *Runx3* null mice is caused by a perturbation of the normal pattern of connectivity that underlies this circuit. More specifically, *TrkC*-positive afferents fail to project to the correct targets in the intermediate/ventral spinal cord in *Runx3* null animals. This defect resembles the proprioceptive phenotype previously observed in *TrkC*-deficient mice [56]. The important role of Runx3 in regulating axonal projections of proprioceptive DRG neurons to spinal motor neurons was confirmed in additional loss- and gain-of-function studies [41,42, 50,55]. More specifically, Chen et al. [42] demonstrated that ectopic expression of Runx3 in *TrkA*-positive DRG neurons biased their axon targeting choices towards more ventral zones of the spinal cord, similar to *TrkC*-positive neurons. Taken together, these results demonstrate an instructive role for Runx3 in directing proprioceptive axon targeting to the spinal cord. Thus, Runx3 also shares with Runx1 the ability to regulate sensory axon targeting choice, in addition to controlling specific neurotrophin receptor expression. Both of these functions are critical to the emergence of specialized sensory neurons during DRG development. However, while Runx1 is required for the expression of a large cohort of ion channels and sensory receptors in nociceptors, it remains to be determined whether Runx3 also controls the specification of sensory modalities in proprioceptors and mechanoceptors, such as the expression of mechanic ion channels.

Regulation of spinal cord motor neuron development by Runx1

Runx1 expression in selected motor neuron subtypes in the developing spinal cord

The spinal cord provides another striking example of the spatial and temporal specificity of *Runx1* expression in the developing nervous system. Mouse *Runx1* expression is restricted to a small number of cells that are present only in the cervical portion of the embryonic spinal cord (cervical levels C1–C8). Within that region, *Runx1* expression is first evident at ~E9.5 in

a small number of post-mitotic spinal accessory column (SAC) motor neurons found at defined ventrolateral positions, but not in their proliferating progenitors [45]. SAC motor neurons innervate branchial arch-derived muscles in the neck and their axons exit the cervical spinal cord through lateral exit points roughly located midway along the dorsoventral axis of the spinal cord [57]. Because of this feature, SAC motor neurons are often referred to as 'dorsally exiting' motor neurons. *Runx1* is expressed in only a fraction of SAC motor neurons, suggesting that it might be involved in the establishment of a specific SAC phenotype(s). Spinal SAC motor neurons are developmentally and functionally related to hindbrain branchial motor neurons, which innervate muscles in the face and jaw [58]. In agreement with this situation, *Runx1* is also expressed in hindbrain branchial motor neurons, where its function is required for the proper embryonic development of those cells [38].

SAC motor neurons represent only a fraction of cervical spinal cord motor neurons. Other types of motor neurons, which have different developmental histories and molecular signatures, send their axons out of the ventral root to innervate somatic forelimb and axial muscles ('ventrally exiting' motor neurons). Following its initial expression in SAC motor neurons at ~E9.5, *Runx1* becomes activated in selected populations of ventrally exiting post-mitotic motor neurons. This occurs at approximately E11.0–E11.5, after the initial generation of those motor neurons and when those cells are presumably starting to make axonal connections with their peripheral targets [45]. Cervical ventrally-exiting motor neurons expressing *Runx1* define restricted groups of neurons that are part of either of two main types of spinal motor neuron columns, termed median motor column (MMC) or lateral motor column (LMC). MMC motor neurons innervate axial muscles throughout most of the spinal cord. In contrast, LMC motor neurons are present only at limb levels, where they innervate muscles in the dorsal or ventral aspects of the limbs [59]. *Runx1*-positive MMC neurons define a group of cells found mostly at cervical levels C3–C6 in the medial portion of the column. Within the LMC, *Runx1* expression is correlated with motor neurons found in both the medial and lateral divisions of the column. Medial LMC (LMCm) and lateral LMC (LMCl) motor neurons innervate muscles in the ventral or dorsal limb, respectively. Thus, in mouse embryos *Runx1*-positive motor neurons define specific groups of cells within MMC and LMC motor column divisions in the cervical spinal cord. A similar, albeit seemingly more restricted, situation was observed in the developing chicken spinal cord, where *Runx1* expression marks a single group, or 'pool', of motor neurons found in the cervical LMCl [60]. In summary, in the neural tube like in DRG, *Runx1* becomes activated in selected post-mitotic neurons that are undergoing developmental maturation toward their terminal phenotype.

Target muscle innervation by Runx1-positive motor neurons

The pool of *Runx1*-expressing LMCl motor neurons in chicken embryos selectively innervates the forelimb muscle, *scapolohumeralis posterior* [60]. In mice, *Runx1*-positive SAC motor neurons innervate the *anterior trapezius* muscle in the neck, whereas certain *Runx1*-expressing LMC neurons project to the *deltoideus* muscle in the forelimb [45]. Although the precise identity of other muscle innervated by *Runx1*-positive MMC and LMC motor neurons remains to be determined, these combined findings suggest that *Runx1* expression is correlated with specific sets of motor neuron pools, or 'subpools' thereof, that innervate selected muscles. They suggest further that Runx1 might be functionally involved in the axon targeting choices of the motor neurons in which it is expressed, similar to the role of Runx1, as well as Runx3, in regulating the axonal targeting of DRG sensory neurons. This possibility is also in agreement with the demonstrated involvement of *Drosophila* Runt in axonal targeting choices in the insect visual system. In summary, *Runx1* expression is correlated with selected populations of dorsally or ventrally-exiting cervical motor neurons that project to defined muscle types in the upper body.

Role of Runx1 in the development of ventrally exiting motor neurons

Both loss- and gain-of-function studies were performed to investigate the involvement of *Runx1* in spinal motor neuron development. In the former, two separate lines of *Runx1*-deficient mice were utilized. *Runx1* null embryos, which die at ~E12.5, were used to study the effect of *Runx1* inactivation on motor neurogenesis at E9.5–E11.5. To examine later stages, viable *Runx1*-null embryos obtained from a separate line of *Runx1*-deficient mice in which *Runx1* expression was conditionally reactivated in hematopoietic, but not neuronal, cells were also utilized [45]. Equal results were obtained with both lines of mice. More specifically, *Runx1* inactivation did not cause a detectable loss of the SAC, MMC, and LMC motor neurons in which *Runx1* is normally expressed, a result suggesting that Runx1 is not important for motor neuron generation and survival [45]. However, developing motor neurons in which *Runx1* would normally be expressed exhibited decreased expression of both general and specific (ie, MMC vs LMC) motor neuron markers when *Runx1* was inactivated. This situation was correlated with a derepression of specific interneuron genes that would not otherwise be expressed in postmitotic motor neurons [45]. Gain-of-function studies in which Runx1 was exogenously expressed in the developing spinal cord of chicken embryos by *in ovo* electroporation resulted in the converse situation, namely decreased numbers of neurons expressing particular interneuron genes and increased expression of motor neuron-specific genes. Interestingly, the same effect was observed when the leukemogenic fusion protein AML1/ETO was tested [45]. AML1/ETO harbors the DNA-binding domain of human RUNX1 fused to the eight-twenty one protein, a strong transcriptional repressor [11]. ETO replaces the C-terminal transcription activation and repression domains of RUNX1 and is hypothesized to confer to AML1/ETO a constitutive transcription repression activity. Taken together, these results strongly suggest that Runx1 is required in developing motor neurons to sustain specific motor neuron differentiation programs and ensure a persistent suppression of interneuron programs. The latter function is consistent with the essential role of both Runx1 and Runx3 in establishing terminal sensory neuron phenotypes through the repression of genes that mediate alternative differentiation programs. In conclusion, Runx proteins act during neuronal development by regulating the emergence of more restricted lineages from bipotential or multipotential transient post-mitotic precursors.

RUNX proteins in neurogenesis and hematopoiesis: similarities and differences

RUNX1 and RUNX3 play crucial roles in controlling the development of various immune cells, particularly the specification of T cell lineages. There are a number of similarities and differences in the functions of RUNX proteins during the development of T cells versus sensory neurons. In both cases, RUNX proteins control lineage segregation from bi-potential progenitors. In developing DRG, Runx3 selects a *TrkC*-positive proprioceptor over a *TrkB*-positive mechanoceptor by activating *TrkC* and suppressing *TrkB*. Similarly, Runx1 is required to specify a *Ret*-positive non-peptidergic nociceptor over a *TrkA*-positive peptidergic nociceptor by activating *Ret* and suppressing *TrkA* [36,37,40,41,44,55]. During T cell development, CD4/CD8-double positive precursor cells are segregated into CD4 single positive (CD4SP) helper T cells and CD8SP cytotoxic T cells. Expression of RUNX3 is confined to CD8SP cells, and RUNX3 is required to suppress *CD4* and establish CD8SP cell identity [14,61,62]. *Runx3* inactivation leads to variegated derepression of *CD4* expression in CD8SP cells, and *Runx3*/*Runx1* double mutation causes a complete loss of CD8SP cells [14, 61,62]. In CD8SP cells, the C-terminal Runx repression domain that binds the Groucho/TLE corepressors contributes to *CD4* repression [63,64]. In contrast, this repression domain is dispensable for Runx1-mediated suppression of *TrkA* in sensory neurons (40). In *TrkC*-positive and CD8SP cells, Runx3 directly suppresses *TrkB* and *CD4*, respectively, by binding silencers located in intronic regions [55,65]. Runx1, however, indirectly suppresses *TrkA* expression in

non-peptidergic nociceptors: Runx1 first activates *Ret*, and Ret signaling subsequently acts to suppress *TrkA* [40,51].

RUNX proteins can have distinct activities in regulating the same target genes at different development stages. For example, in CD4/CD8-double negative T cell precursors, as well as in CD8SP cells, Runx1 is able to suppress *CD4* expression [14,61,62]. However, this Runx1-mediated repression is lost in CD4SP cells, despite continuous Runx1 expression in these cells [61,62]. In nociceptors, Runx1 switches from a transcriptional activator to a repressor (in genetic terms) in controlling the expression of a subset of Mrgpr class G-protein coupled receptors [52]. Initially, Runx1 is required for the expression of all 12 *Mrgpr* genes, and some of these receptors, such as MrgprC11 and MrgprD, are coexpressed at early embryonic stages. During postnatal development, Runx1 continues to be a positive regulator of *MrgprD*, but becomes a dominant repressor of *MrgprA*/*B*/*C*. As a result, *MrgprD* expression persists in Runx1-persistent nociceptors but is extinguished in Runx1-transient nociceptors, whereas expression of *MrgprA*/*B*/*C* is suppressed in Runx1-persistent but retained in Runx1-transient nociceptors. These mechanisms lead to non-overlapping expression of two sets of sensory receptors, providing an elegant means of generating sensory neuron diversity [52].

It is interesting to note that Runx1 is competent to activate and repress *MrgprA*/*B*/*C* genes in *MrgprD*-expressing neurons [52]. Removal of the Runx1 C-terminal transcription repression domain in mice causes a derepression of *MrgprA*/*B*/*C* genes in *MrgprD*-expressing neurons. However, this derepression does not occur in *Runx1* null mice, implying that the removal of the repression domain converts Runx1 from a repressor into an active transactivator (in genetic terms) [52]. In other words, *MrgprD*-expressing neurons are likely to contain all necessary coactivators and corepressors for Runx1-mediated activation or repression of *MrgprA*/*B*/*C* genes, but Runx1 transcription repressor activity is normally dominant over its activator function.

It is worth noting that if a Runx1 coactivator were able to compete with a corepressor, this situation could in principle lead to a stochastic mode of gene regulation. Such control mode might provide a plausible solution to a long-standing problem in the immunology field: the stochastic and monoallelic expression of T cell receptors [66]. Under this hypothetical scenario, Runx1 would bind to a promoter and stochastically interact with either a coactivator or a corepressor, resulting in activation of a target gene only in a subset of equally competent cells. The percentage of cells expressing a target gene would be determined by the relative levels of coactivators versus corepressors. An important feature of this control mode is that Runx1 could independently regulate the expression of two alleles of the same gene; accordingly, in some cells Runx1 would activate one allele but suppress the other, leading to a monoallelic expression pattern. With this in mind, it is interesting to note that Runx1 and Runx3 are required for the expression of T cell beta receptors (TCRβ) [61,62,64]. It might therefore be warranted to test if a competition between Runx1 coactivator and corepressor activities could account for the stochastic and partially monoallelic expression of TCRβ [66]. In summary, the combined analysis of the functions of RUNX proteins during neurogenesis and hematopoiesis is facilitating the understanding of how a few RUNX proteins are able to contribute to the generation of the tremendous diversity of both immune and neuronal cells.

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