CHAPTER FOUR

Transcriptional Networks in the Early Development of Sensory–Motor Circuits

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Contents

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Abstract

The emergence of coordinated locomotor behaviors in vertebrates relies on the establishment of selective connections between discrete populations of neurons present in the spinal cord and peripheral nervous system. The assembly of the circuits necessary for movement presumably requires the generation of many unique cell types to accommodate the intricate connections between motor neurons, sensory neurons, interneurons, and muscle. The specification of diverse neuronal subtypes is mediated largely through networks of transcription factors that operate within progenitor and postmitotic cells. Selective patterns of transcription factor expression appear to define the cell-type-specific cellular programs that govern the axonal guidance decisions and synaptic specificities of neurons, and may lay the foundation through which innate motor behaviors are genetically predetermined. Recent studies on the developmental programs that specify two highly diverse neuronal classes—spinal motor neurons and proprioceptive sensory neurons—have provided important insights into the molecular strategies used in the earliest phases of locomotor circuit assembly. This chapter reviews progress toward elucidating the early transcriptional networks that define neuronal identity in the locomotor system, focusing on the pathways controlling the specific connections of motor neurons and sensory neurons in the formation of simple reflex circuits.

1. Introduction

Many organisms are born with a set of innate behaviors that have evolved so that they can confront the challenges imposed by their specific environments. The neural circuits controlling basic motor behaviors such as feeding, breathing, and walking are often functional at the time of birth, independent of any prior interaction with the external world. These genetically hard-wired circuits can be essential for survival by imprinting behaviors such as the predator escape response—a system where sensory input must be tightly linked to motor output. The identification of the substrates for simple and complex innate behaviors has been a major challenge.

There is emerging evidence that stereotyped patterns of movement can be programmed through the actions of a few key regulatory genes, neurons, and microcircuits. In *Drosophila* gender-specific courtship behaviors are specified by a transcription factor encoded by the *fruitless* gene, which is sufficient to interconvert a specific pattern of mating behavior between males and females (Demir and Dickson, 2005). In *Caenorhabditis elegans*, the neural substrates controlling innate patterns of motor behavior are comprised of a relatively small number of anatomically well-defined groups of neurons (Hobert, 2003). Whether similar master gene regulators, or

discrete neural assemblies, function to program the behavioral outputs of the vertebrate motor system remain to be determined. Presumably such a factor, or group of factors, would need to function in distinct classes of interconnected neuronal subtypes that by other criteria might be considered dissimilar.

The problem of defining behaviorally relevant circuits in vertebrate nervous systems is confounded by the shear volume of neurons, and the relative complexity and number of synaptic connections. The spinal cord and hindbrain have provided tractable model systems for defining the neural circuits necessary for basic motor functions such as breathing and walking, and contain the sensory feedback systems that are required for reflex responses and locomotor adaptation (reviewed in Goulding and Pfaff, 2005; Kiehn and Butt, 2003). As a system for studying locomotor behaviors, the spinal cord has an advantage in that the anatomy of the system is relatively well defined and the sensory inputs and motor outputs are accessible and quantifiable. One successful approach to the study of the assembly of locomotor circuits has been to define the embryonic programs that contribute to the identity and connectivity of the cells within the circuit, to try to link control of synaptic specificity with the emergence of a defined behavior.

In this chapter, I review our current understanding of the genetic programs which control the specification of motor neurons and sensory neurons in the vertebrate spinal cord and peripheral nervous system. Emphasis will be placed upon the transcriptional networks which dictate the early identity of these two neuronal classes, and on recent advances that have enriched our understanding of the general principles of circuit assembly. Activity-dependent steps in the wiring of locomotor circuits will not be addressed as this aspect has been the subject of recent reviews (Hanson *et al.*, 2008; Ladle *et al.*, 2007). The potential mechanisms that may contribute to the assembly of sensory–motor circuits will be explored, with a focus on the formation of monosynaptic stretch-reflex circuits, a collection of neural circuits that are critical for coordinated movement.

2. Intrinsic Programs Controlling Neuronal Fate Specification in the Ventral Spinal Cord

In broad terms, the final output of spinal circuit activity is conceptually simple: the activation of specific muscles in the periphery. But in order for basic motor commands to be smoothly executed, spinal circuits must be sufficiently fined tuned to activate only a small subset of the hundreds of unique muscle groups in a specific order. The first and most critical aspect in the formation of these circuits is that motor axons be able to navigate toward

and select their peripheral muscle targets with fidelity and precision. All of the subsequent steps in motor neuron connectivity, such as descending inputs from higher brain centers, local interneuron, and sensory neuron connectivity are constrained by the peripheral connections made by motor neurons. Therefore connection between a motor neuron and the muscle it innervates is a core element in defining synaptic specificity during locomotor circuit assembly.

The problem of establishing appropriate sets of connections between motor neurons and muscles is at its most challenging in the vertebrate limb, which contains dozens of anatomically and functionally distinct muscle groups in most tetrapods (Greene, 1963; Sullivan, 1962). The highly stereotypic nature of developing motor axonal projections within the limb, and the organization of motor neuron cell bodies into topographic maps, led to the idea that motor neurons possess intrinsic identities that define target specificity (Jessell, 2000; Landmesser, 2001). The patterns of motor neuron connectivity in the limb require that a large number of unique identities are generated during development, to accommodate the targeting of each of the approximately 50 muscle groups in the limb. As described below, the diversification of motor neuron subtypes, likely many CNS neurons, appears to be mediated through the integration of two distinct signaling systems that operate along the dorsoventral and rostrocaudal axis of the neural tube.

2.1. Control of neuronal class identity in the ventral spinal cord

Many of the neural circuits which provide the central drive for activation of motor neurons reside within ventral spinal cord. The ventral spinal cord consists of several classes of neurons including motor neurons and multiple types of local circuit interneurons (Goulding and Pfaff, 2005). The identities of these classes are established largely through the actions of the secreted signaling molecule Sonic hedgehog (Shh), which is secreted from the notochord and floor plate and acts in a graded manner to pattern progenitor identities along the dorsoventral axis of the neural tube (Dessaud *et al.*, 2008). A primary function of Shh signaling is to control the expression patterns of homeodomain and helix-loop-helix classes of transcription factors, most of which act as transcriptional repressors (Briscoe *et al.*, 2000; Muhr *et al.*, 2001; Novitch *et al.*, 2001). Two classes of Shh-regulated genes have been defined; class I genes are expressed more dorsally and are repressed by Shh, while class II genes are found ventrally and are activated in response to graded Shh signaling (Briscoe *et al.*, 2000). These initial patterns of expression are subsequently refined through mutually cross-repressive interactions that occur between pairs of transcription factors. As a consequence of these repressor interactions, each domain expresses a

unique transcription factor code that specifies progenitor identities, including motor neuron (pMN) and interneuron (p0, p1, p2, p3) precursors (Fig. 4.1A).

Each cardinal progenitor domain gives rise to specific class of postmitotic neuron characterized by a set of common features such as axonal projection pattern, settling position, synaptic partners, and physiological properties. Like the transcriptional codes present in neural progenitors, postmitotic neuronal classes can also be defined by their unique and combinatorial expression of specific transcription factors. For example, the progenitor domain that gives rise to motor neurons expresses the basic helix-loop-helix (bHLH) protein Olig2 and Nkx6 homeodomain proteins (Briscoe *et al.*, 2000; Novitch *et al.*, 2001). After leaving the cell cycle these newly postmitotic motor neurons are characterized by the expression of a distinct set of homeodomain including factors Hb9, Isl1/2, and Lhx3/4 (Fig. 4.1A) (Tanabe *et al.*, 1998; Tsuchida *et al.*, 1994).

Genetic loss-of-function studies in mouse have established critical roles for these class-specific transcription factors in neuronal subtype specification and have also revealed their essential roles in locomotor circuit assembly and function. In mice lacking the V0 interneuron transcription factor Dbx1, mice fail to form appropriate inhibitory connections between the two sides of the spinal cord and as a consequence the normal alternation of left and right motor outputs of the spinal cord are disrupted (Lanuza *et al.*, 2004). Thus, genetic analyses of class-specific transcription factors have revealed the general mechanisms governing the specification of diverse neuronal classes in the spinal cord, and have also provided important insights into how individual classes of neurons contribute to locomotor circuit function.

2.2. Defining positional identities along the rostrocaudal axis of the spinal cord

Although transcriptional networks mediated through the dorsoventral signaling systems define the identity of several types of neurons, other programs are presumably necessary for the further diversification of neurons within a given class. Classical anatomical and physiological studies indicate that much of the variation of neuronal subtypes in the spinal cord occurs as a function of their position along the rostrocaudal axis. For example, the neuronal circuits responsible for the rhythmic firing of motor neurons during stereotyped behaviors such as walking are located at defined positions along the rostrocaudal axis (Kiehn and Butt, 2003). These specialized limb-controlling circuits (known as central pattern generators—CPGs) likely reflect the programming of neuronal identities at specific rostrocaudal positions.

The mechanisms controlling neural diversification along the rostrocaudal axis of the spinal cord are best understood for motor neurons. While all

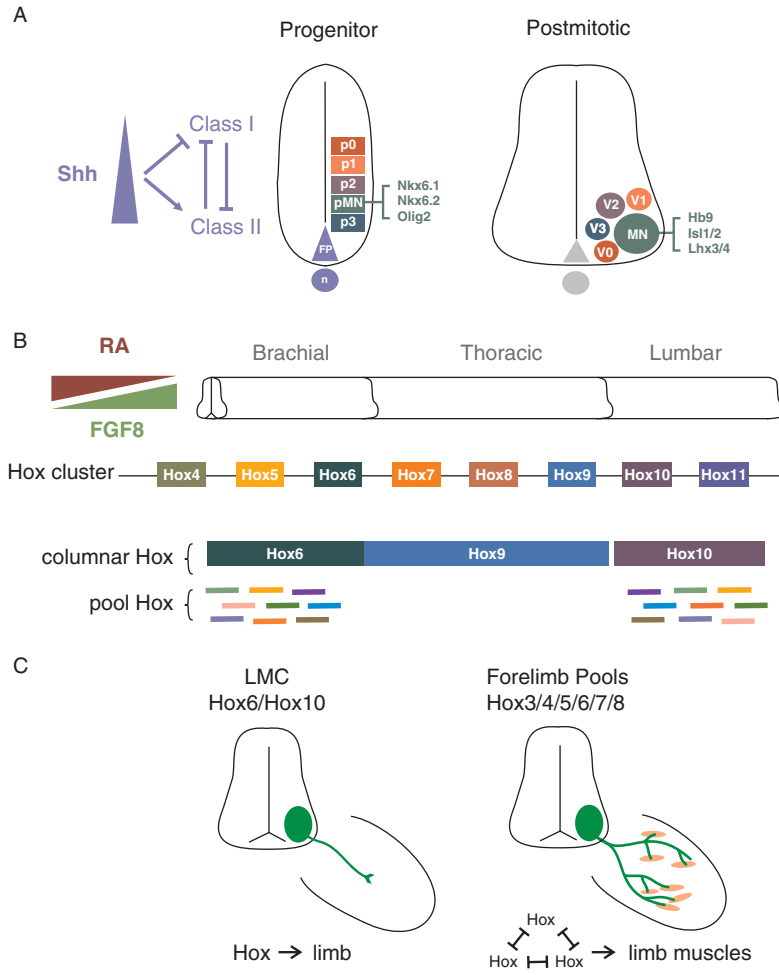

Figure 4.1 Steps in the generation of neuronal subtypes in the ventral spinal cord. (A) Along the dorsoventral axis of the neural tube, several classes of neurons are generated in response to the graded activities of the secreted protein Sonic hedgehog (Shh) which induces the patterned expression of transcription factors in ventral progenitor cells. "Class I" transcription factors are induced by Shh while "Class II" factors are repressed. Selective cross-repressive interactions between these two classes of transcription factors sharpen the boundaries between progenitor domains (see Briscoe *et al.*, 2000). In addition, retinoic acid (RA) from the paraxial mesoderm and fibroblast growth factor (FGF) signaling also influence the pattern of transcription factor expression in neural tube progenitors (not shown). Each of these progenitor domains gives rise to postmitotic neurons, including motor neurons and several classes of inhibitory and excitatory local circuit interneurons. (B) Along the rostrocaudal axis of the neural tube, opposing FGF and RA gradients induce the expression of chromosomally linked *Hox* genes. *Hox* genes located at one end of the cluster are induced more rostrally in response to high levels of RA while genes at the opposite end are expressed caudally

motor neurons are characterized by certain common features, such as their cholinergic mode of neurotransmission, the extension of axons outside the CNS, and expression of class-specific transcription factors, they also acquire intrinsic features which differentiate them from one another. At limb levels, three main levels of motor neuron organization have been defined based on studies of their position within the spinal cord and pattern of connectivity, with each successive step governing a distinct aspect of motor axonal projection pattern (Fig. 4.2). Shortly after leaving the cell cycle, a subset of motor neurons acquire a lateral motor neuron columnar (LMC) identity that directs motor axons toward the limb. Once motor axons reach the base of the limb, the specification of divisional identities within the LMC directs motor axons ventrally or dorsally upon entering the limb mesenchyme. Finally, the specification of a motor pool identity appears to confer motor neurons with the ability to project toward and form precise connections with individual limb muscle targets (Landmesser, 1978a, 2001).

Each of these sequential phases of limb innervation pattern can be linked to a genetic program defined by transcriptional networks which utilize members of the evolutionarily conserved *Hox* gene family. The generation of segmentally restricted motor neuron columnar subtypes are specified by rostrocaudal signaling gradients of fibroblast growth factors (FGFs) and retinoic acid (RA) that establish regional domains of Hox transcription factor expression and activity (Fig. 4.1B) (Bel-Vialar *et al.*, 2002; Liu *et al.*, 2001). The FGF and RA gradients set up an initial pattern of *Hox* expression in motor neurons that is subsequently refined through cross-repressive interactions that occur between specific pairs of Hox proteins (Dasen *et al.*, 2003). As a consequence of these early inductive signals and cross-regulatory interactions, Hox expression patterns segregate with and determine the identity of distinct motor neuron subtypes (Dasen *et al.*, 2003, 2005). For example, at limb levels Hox6 gene paralogs (*Hoxa6* and *Hoxc6*) specify forelimb LMC neuron fate, while Hox10 genes (*Hoxa10*, *Hoxc10*, and *Hoxd10*) specify hindlimb LMC neurons (Dasen *et al.*, 2003; Shah *et al.*, 2004; Wu *et al.*, 2008).

A more intricate transcriptional network, built from nearly two dozen Hox proteins, imposes discrete motor pool identities in LMC neurons

in response to elevated levels of FGF. In motor neurons, Hox patterns are further refined through cross-repressive interactions, giving rise to specific patterns of Hox expression in motor columns and pools. (C) Role of Hox proteins genes in motor neuron innervation patterns in the limb. One set of Hox factors expressed at limb levels of the spinal cord is involved in establishing the lateral motor neuron columnar (LMC) identity which directs motor axons toward the limb. Within this columnar group, an additional network of 8–10 additional Hox factors appear to be involved in the specification of motor pool identities and control programs which direct motor axons to specific muscle targets.

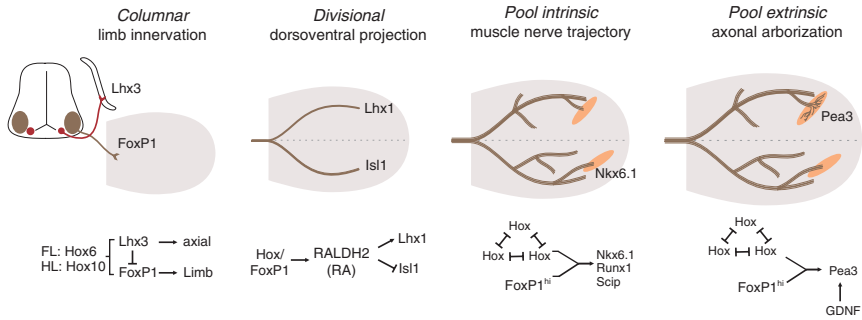

Figure 4.2 Sequential steps in the transcriptional control of limb innervation patterns. Two motor columns are present at limb levels of the spinal cord, one projecting dorsally toward axial muscle the other projecting ventrally into the limb. The LIM homeodomain factor Lhx3 specifies the identity of dorsally projecting neurons, while FoxP1 appears to establish the early limb innervation program. Hox6 proteins specify forelimb (FL) LMC neurons and Hox10 hindlimb (HL) neurons. Upon entering the limb, motor axons follow either a dorsal or ventral trajectory. The dorsoventral decision operates as a consequence of the pattern of LIM homeodomain expression setup by a local source of retinoids, provided by *RALDH2* activity. The guidance of axons to specific muscle is determined by a Hox network operating in the presence of high levels of FoxP1 that establishes the transcriptional identity of motor pools. Late aspects of this program appear to require signaling from the periphery, and these peripheral signals (e.g., GDNF) control expression of ETS transcription factors, such as Pea3. One aspect of Pea3 function in motor neurons is to control muscle-specific patterns of axonal arborization.

(Dasen *et al.*, 2005). Within motor pools Hox proteins appear to act as the primary determinants of motor neuron connectivity since changing the profile of Hox expression in specific pools, through misexpression or *Hox* gene mutation, results in changes in the pattern of muscle innervation (Dasen *et al.*, 2005; Tarchini *et al.*, 2005; Tiret *et al.*, 1998; Wu *et al.*, 2008). As described in the following sections, this core Hox network appears to direct motor innervation patterns by activating a diverse array of downstream transcription factors and cell surface receptors.

2.3. Transcriptional networks in motor neuron columnar and pool identities

Despite evidence that *Hox* genes are critical for motor neuron differentiation, their activities alone cannot account for all aspects of motor neuron diversity. *Hox* genes are global regulators of tissue patterning throughout development (Kmita and Duboule, 2003), and within the locomotor system are expressed by multiple classes of neurons (Belting *et al.*, 1998; Ensini *et al.*, 1998). The ability of *Hox* genes to selectively control columnar, divisional, and pool identities of motor neurons appears to require the

actions of additional transcription factors which act both in parallel and downstream of Hox function.

The network of Hox proteins that drives motor neuron diversification depends on the actions of a single accessory factor, the forkhead class homeodomain protein FoxP1. Genetic inactivation of the *Foxp1* gene in mice erases all of the Hox-dependent steps of LMC motor neurons differentiation and motor neuron identities revert to an ancestral state, consisting of two continuous motor columns (Dasen *et al.*, 2008; Rousso *et al.*, 2008). In *Foxp1* mutants, all known molecular feature of LMC neuron diversification are deteriorated, including the expression of columnar, divisional, and pool-restricted transcription factors and guidance receptors. Remarkably, the overall pattern of axonal projections into limb is well preserved in these mutants although individual motor neurons appear to select projection pathways in the limb in a stochastic manner (Fig. 4.3A). More generally, these observations suggest that the axonal projection pattern observed in the limb may be in part set up by the limb itself, independent of expression of guidance receptors on specific motor axons.

While these studies indicate that FoxP1 is required for Hox actions in motor neurons, it remains unclear whether FoxP1 is a bona fide cofactor for Hox proteins in motor neurons. In addition, how the Hox/FoxP1 network interacts with more globally acting motor neuron determinants such as LIM homeodomain proteins and Hb9, which are required in most motor neurons (Arber *et al.*, 1999; Thaler *et al.*, 1999, 2004), remains to be determined. Since Hox proteins are expressed by interneurons and sensory neurons, it is possible that additional cofactors control Hox-regulated aspects of neuronal identity and connectivity. One possibility is that the Hox network described for motor neurons functions in other classes of spinal neurons to help determine their synaptic specificities.

3. Guidance and Synaptic Specificity of Motor Axons Projecting into the Limb

While there is significant evidence that a Hox/FoxP1-based transcriptional network contributes to the diversity and connectivity of motor neuron subtypes, the specific molecular pathways by which this program contributes to the guidance of motor axons to their muscle targets is not well defined. Nevertheless, the actions of certain Hox proteins can be linked to the ability of motor neurons to innervate specific muscle targets through the control of a diverse repertoire of intermediate factors (Fig. 4.2). One early output of the Hox-based program controlling limb innervation is the establishment of a code of LIM homeodomain protein expression that

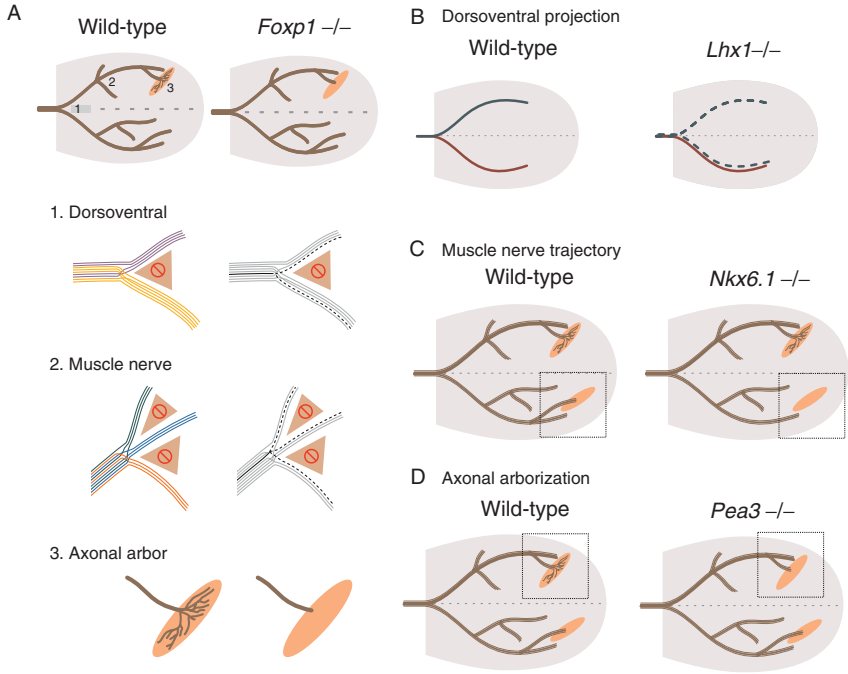

Figure 4.3 Genetic analysis of transcription factors involved in motor axon projections into the limb. (A) In *Foxp1* mutants, the Hox-dependent programs for limb-innervating motor neurons deteriorate, including the loss of pool-specific transcription factors and guidance molecules. The pattern of motor nerve branches is similar in wild-type and *Foxp1* mutant embryos, but the behavior of individual motor axons differs markedly at decision regions encountered in the limb mesenchyme. In *Foxp1* mutants, the axons of motor neurons are still forced to follow permissive pathways in the limb mesenchyme, but deprived of their divisional or pool identity, they select one of the permitted routes in an apparently stochastic manner. "No entry" signs depict zones of the limb mesenchyme from which motor axons are excluded, presumably by virtue of the expression of molecules that are nonpermissive for axon extension. Late aspects of pool differentiation such as muscle-specific axonal arborization patterns are also eroded. (B) In mice mutant for *Lhx1*, which is normally expressed by dorsally projecting lateral LMC motor neurons, the axons that normally project into the dorsal compartment of the limb appear to choose the selection of specific targets in random manner. This randomization is likely due to loss of EphA4 expression which is required to repel this population from ephrins expressed in the ventral limb mesenchyme. (C) At hindlimb levels, the motor nerve branches supplying the tibialis anterior and gracilis posterior muscles in the hindlimb derive from Nkx6.1+ motor pools. In *Nkx6.1* mutants, the axons that normally project to these muscles are rerouted to different muscle targets. (D) In *Pea3* mutants, motor axons from the motor pool expressing Pea3 are able to navigate to their appropriate muscle target. Once they reach their target, however, they are unable to arborize the muscle.

defines the dorsoventral projection choice of motor axon at the base of the limb. While an additional Hox program imparts a second layer of control that specifies the motor neuron pools targeting individual limb muscles.

3.1. LIM homeodomain proteins and axonal trajectories

The cell bodies of motor neurons that inhabit the LMC form a topographic map in the spinal cord such that cell body position is predictive of axonal projection pattern (Sharma and Belmonte, 2001). How the somatotopic organization of motor neurons emerges is best understood for the two divisions of the LMC. These divisional identities are established in a binary manner: motor neurons that settle in the medial half of the LMC innervate ventrally derived limb muscles, whereas motor neurons that settle in the lateral half of the LMC innervate dorsally derived limb muscles (Landmesser, 1978a; Tosney and Landmesser, 1985a,b). At a molecular level, the divisional organization of LMC neurons is revealed in the expression of a set of LIM homeodomain proteins (Tsuchida *et al.*, 1994). Laterally positioned LMC neurons express Lhx1 whereas medially positioned neurons express Isl1. This pattern of LIM homeodomain expression appears to be established through signaling between motor neurons just after they are born and can be linked to the activities of Hox proteins expressed in these subtypes.

At limb levels of the spinal cord, LMC neurons are characterized by expression of the *retinaldehyde dehydrogenase 2 (RALDH2)* gene which encodes an enzyme involved in retinoic acid synthesis. The selective expression of *RALDH2* in LMC neurons is controlled by the activities of Hox proteins expressed at limb levels of the spinal cord: Hox6 paralogs activate *RALDH2* expression at forelimb levels and Hox10 paralogs control its expression at hindlimb levels (Dasen *et al.*, 2003; Shah *et al.*, 2004). This action of Hox factors depends on the presence of accessory factor FoxP1, since in *Foxp1* mutants *RALDH2* is only weakly expressed (Dasen *et al.*, 2008; Rousso *et al.*, 2008). Whether the action of FoxP1 and Hox proteins in the control of *RALDH2* involves cooperative protein–protein interactions, or whether FoxP1 is merely an intermediary factor remains to be determined.

RALDH2 activity in LMC neurons generates a local source of retinoid signaling which appears to be responsible for establishing the pattern of LIM homeodomain expression in lateral and medial LMC neurons (Sockanathan and Jessell, 1998). At this stage, motor neurons can be characterized by a pattern of ''inside–out'' migration where later born lateral LMC neurons migrate past the early born medial LMC neurons. As later born LMC neurons migrate past the early born counterparts, RA provided by medial LMC neurons induces the expression of Lhx1 and specifies a lateral LMC fate. The induction of Lhx1 is also involved in restricting Isl1 expression to medial LMC neurons, thus helping to set up a binary code of LIM

homeodomain expression within these two populations (Kania and Jessell, 2003; Sockanathan and Jessell, 1998).

How do the actions of LIM homeodomain proteins dictate the initial trajectory of motor axons as they enter the limb? LIM homeodomain factors appear to regulate pathway selection through controlling expression of members of the Eph/ephrin signaling pathway. In the dorsally projecting lateral LMC population Lhx1 induces expression of the guidance receptor EphA4, and the presence of this receptor repels these axons from ephrin-A expressing cells in the ventral limb mesenchyme (Kania and Jessell, 2003). Both *EphA4* and *Lhx1* mutants are characterized by defects in dorsoventral projection choice (Helmbacher *et al.*, 2000; Kania *et al.*, 2000), and in *Lhx1* mutants lateral LMC neurons appear to select the dorsal and ventral compartments in a stochastic manner (Fig. 4.3B). Interestingly, in the limb mesenchyme another LIM homeodomain protein, Lmx1b, controls the expression of ephrin-A proteins in the ventral compartment (Kania *et al.*, 2000). Thus, these receptor–ligand interactions appear to be coordinated through actions of LIM homeodomain proteins expressed in neurons and limb mesenchyme.

Recently several additional factors have been implicated in the dorsoventral selection of LMC motor axons including members of the semaphorins/plexin and GDNF/ret signaling pathways (Huber *et al.*, 2005; Kramer *et al.*, 2006a). In mice lacking GDNF/ret signaling, dorsally projecting LMC axons follow an aberrant ventral trajectory, suggesting that this system functions as an instructive guidance signal for LMC neurons (Kramer *et al.*, 2006a). In mice mutant for members of the semaphorin/plexin family, medial and lateral LMC motor axons also display dorsoventral guidance defects, and this phenotype is associated with the defasiculation of axons as they project into the limb. The details of how these signaling pathways interact with one another in the control motor axon guidance decisions are still unclear, and are confounded by the observation that they operate within multiple phases of the limb innervation program. In addition, whether the LIM homeodomain code for dorsoventral trajectories regulates the expression of members of the semaphoring/plexin and GDNF/ret families remains to be determined.

A similar LIM homeodomain-based strategy appears to operate in the regulation of the expression of guidance receptors that control motor neuron innervation of axial musculature. Here the activity of the LIM homeodomain factor Lhx3, which becomes selectively expressed by axial muscle-innervating motor neurons, is sufficient to drive nearly all motor axons to axial muscle at the expense of other pathways (Sharma *et al.*, 2000). This action of Lhx3 appears to be mediated through the regulation of type I FGF receptor expression in motor neurons and FGF cues in the periphery (Shirasaki *et al.*, 2006). The use of LIM homeodomain proteins in motor neuron is conserved in diverse species such as flies and worms (Shirasaki and

Pfaff, 2002), suggesting that the actions of this family represent a highly conserved strategy in the early phases of motor axon guidance.

3.2. Controlling the fine specificity of motor neuron–muscle connectivity

While the regulation of dorsoventral axonal projections in the limb appears to be controlled through LIM homeodomain regulation of Eph receptor expression, significantly less is known about the mechanisms which guide motor axons to their muscle targets. After making their dorsoventral choice in the limb, motor axons follow highly stereotypic pathways along nerve trunks and branches that guide them to the position of newly formed muscle masses (Landmesser, 2001). As with dorsoventral choices, the projection of axons along the anterior–posterior and proximal–distal axes of the limb are aligned with cell body position within the spinal cord. In general, more rostrally positioned motor pools project toward anterior/proximal limb regions while more caudally positioned pools project into posterior/distal regions (Hollyday and Jacobson, 1990; Landmesser, 1978b). Some aspects to the control of this motor neuron–muscle topography can be linked to the actions of Hox-regulated pool-specific transcription factors.

Within LMC neurons, one critical output of Hox activity is to control the expression of a diverse array of motor pool-specific transcription factors, including members of the Nkx, Runx, POU, and Fox families (Cohen *et al.*, 2005; Dasen *et al.*, 2005; De Marco Garcia and Jessell, 2008; Dou *et al.*, 1997; Stifani *et al.*, 2008). A recent study provides evidence for a role for the Hox-dependent homeodomain transcription factor Nkx6.1 in the establishment of muscle-specific trajectories. While Nkx6 factors are initially expressed and necessary in all motor neuron progenitors (Vallstedt *et al.*, 2001), expression of Nkx6.1 becomes restricted to motor pools at limb levels at the time when motor axons project toward their target muscles. Expression of Nkx6.1 in pools appears to be programmed solely through motor neuron intrinsic transcriptional programs, as ablation of the limb has no affect on its expression in postmitotic neurons (De Marco Garcia and Jessell, 2008). In *Nkx6.1* mutant mice, each of the lumbar LMC pools that normally express Nkx6.1 exhibits early defects in muscle nerve branch formation, and a later reduction in muscle innervation (Fig. 4.3C) (De Marco Garcia and Jessell, 2008). The cell surface molecules which may be controlled by Nkx6.1 and involved in directing axons toward their specified target muscles remain to be identified.

Although the actions of pool-specific transcription factors appear to be required for the specificity of innervation of limb muscles, these factors alone are unlikely to account for all aspects of muscle nerve branch patterns and target selectivity. While several transcription factor families are expressed by motor neuron pools in a manner that is independent of

limb-derived signals, only a handful of factors have been demonstrate to be expressed in specific pools. Whether there exists a single factor for each of the approximately 50 motor pool identities is uncertain. Alternatively, the combinatorial actions of Hox proteins could directly regulate expression of receptors that instruct muscle nerve trajectories without the involvement of intermediary transcription factors. Moreover, since aspects of motor pool identity rely on feedback control from the muscle itself, as well as patterned neural activity in the spinal cord, the cell intrinsic programs likely do not account for every aspect of the limb projection pattern.

3.3. Target-dependent steps in muscle nerve innervation patterns

While many aspects of the Hox/FoxP1 network in LMC neurons emerge in a target-independent manner, expression of certain pool-specific transcription factors relies on the presence signals from the periphery. Expression of the ETS transcription factors Pea3 and Er81 in motor pools depends on limb-derived neurotrophic signals provided by the limb mesoderm and muscle targets (Haase *et al.*, 2002; Lin *et al.*, 1998). These signals appear to be permissive rather than instructive and not all motor neurons are competent in their ability to respond to neurotrophic signals. One peripheral signal, glial-derived neurotrophic factor (GDNF) is expressed by the limb mesenchyme and muscle and appears to instruct the transcriptional identity of forelimb motor pools through regulation of Pea3 expression. In explants of spinal cord treated with GDNF, Pea3 is induced in a pattern approximating the normal number *in vivo* and is confined to the level of the spinal cord which normally expresses Pea3 (Haase *et al.*, 2002). Thus, not all motor neurons are equivalent in their ability to respond to GDNF, and this constrainment appears to be set by the profile of Hox expression in forelimb motor pools (Dasen *et al.*, 2005; Tiret *et al.*, 1998). Additional signals provided through Met signaling appear to enhance the influences of GDNF, through recruitment of additional neurons to the Pea3 motor pools (Helmbacher *et al.*, 2003).

These observations suggest that despite the critical role of Hox factors in motor neuron specification, target-derived cues also contribute to the transcriptional programming of pool fates. Expression of the target-induced factor Pea3 is critical for later aspects of motor pool differentiation such as the clustering of motor neurons into pools and muscle-specific patterns of axonal innervation (Livet *et al.*, 2002; Vrieseling and Arber, 2006). In *Pea3* mutants, motor axons are capable of reaching their appropriate muscle target, but fail to properly arborize the entire length of the muscle (Fig. 4.3D). Thus, motor pool specification appears to unfold in two main phases: an intrinsic phase that confers aspects of motor neuron identity involved in the selection of target muscle connectivity (Landmesser, 2001;

Milner and Landmesser, 1999), and a later phase that operates after motor axons have reached their muscle targets, which is associated with ETS gene expression and the clustering of motor neurons within the LMC (Livet *et al.*, 2002; Price *et al.*, 2002).

4. Control of Sensory Neuron Specification and Connectivity

A major sensory pathway from the body to the central nervous system is mediated through neurons whose cell bodies are located outside the spinal cord within the dorsal root ganglia (DRG). Like motor neurons, DRG sensory neurons are relatively well characterized in terms of their early specification programs and physiological functions (Chen *et al.*, 2003; Marmigere and Ernfors, 2007). However unlike motor neurons, sensory neurons are not organized into discrete columns and pools nor does the position of their cell bodies form topographic maps. In contrast, sensory neurons projecting to a given target appear to be scattered throughout the DRG (Fig. 4.4A). Nevertheless, our understanding of the genetic programs that contribute to the central and peripheral connectivity of sensory neurons has progressed significantly in recent years.

4.1. Early steps in sensory neuron lineage specification

As in the ventral spinal cord, the specification of different classes of sensory neurons appears to have its origins in a set of inductive signaling events operating along the dorsoventral axis of the neural tube. Sensory neurons residing within the DRG are derived from a migratory population of neural crest cells that are specified at the border between neural and non-neural ectoderm in the dorsal neural tube (Sauka-Spengler and Bronner-Fraser, 2008). Graded TGFβ and Wnt signaling from the roof plate and surface ectoderm appear to be essential in specifying the neural crest as a whole, as well as several classes of sensory relay neurons which populate the dorsal half of the spinal cord (Fig. 4.4A). The initial phases of neural crest specification are characterized by the delamination of cells from the neural tube and the expression of early transcription factors common to most neural crest progenitors, such as the HMG transcription factor Sox10 (Kim *et al.*, 2003).

The neural crest derivatives are a remarkably diverse class which, in addition to peripheral sensory ganglia, gives rise to the autonomic nervous system and several non-neuronal cell types (Marmigere and Ernfors, 2007; Sauka-Spengler and Bronner-Fraser, 2008). Recent studies indicate that the sensory lineages of the neural crest are specified in response to high levels of canonical Wnt signaling from the roof plate and surface ectoderm.

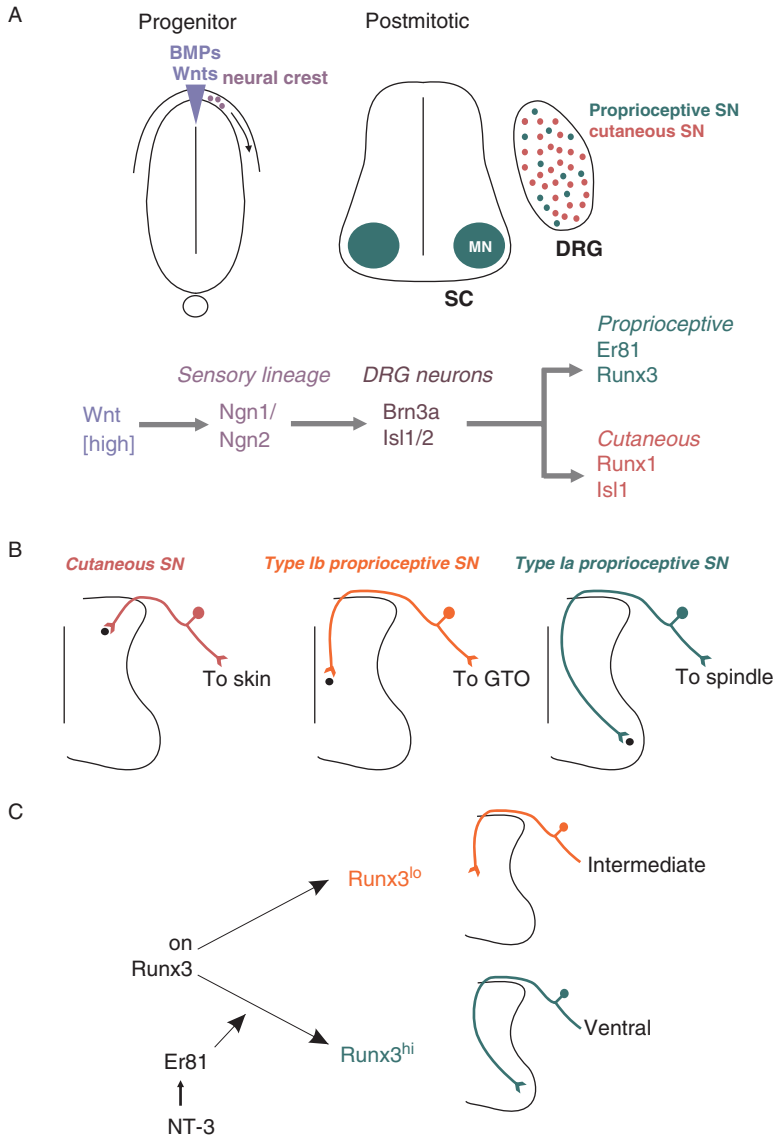

Figure 4.4 Specification of DRG sensory neuron identity and patterns of connectivity. (A) Neural crest cells are specified in response to TGFβ/BMP and Wnt signaling from the roof plate and surface ectoderm. Neural crest cells subsequently give rise to DRG sensory neurons, neurons in the autonomic nervous system, and multiple non-neuronal lineages. The early precursors to the sensory neural (SN) lineage are characterized by the expression of the bHLH factors Ngn1 and Ngn2, likely in response to high levels of canonical Wnt signaling. Postmitotic neurons populating the DRG express the POU domain protein Brn3a and the LIM homeodomain proteins Isl1 and Isl2. These populations segregate into two main classes: cutaneous sensory neurons which express and

Conditional mutation of *β-catenin* (a primary target of canonical Wnt signaling) in neural crest leads to the selective loss of sensory lineages and melanocytes, while other derivatives are relatively spared (Hari *et al.*, 2002). When neural crest cells are forced to express a constitutively active form of β-catenin, sensory neurons are generated at the expense of other neural crest lineages (Lee *et al.*, 2004). Thus, high levels of Wnt/β-catenin activity appear to exert an instructive role in the specification of sensory neurons.

As in the ventral spinal cord, the generation of diverse neural crest derivatives involves combinatorial transcription factor codes in progenitor and postmitotic neurons (Anderson, 1999). The sensory lineage requires the activities of two bHLH proteins, Neurogenin1 (Ngn1) and Neurogenin2 (Ngn2), as in *Ngn1/Ngn2* double-knockout mice the sensory lineages are selectively lost (Ma *et al.*, 1999). Multiple postmitotically expressed transcription factors common to all sensory lineages have also been characterized including the POU domain factor Brn3a, the LIM homeodomain factors Isl1 and Isl2, and the bHLH protein NeuroD (Sauka-Spengler and Bronner-Fraser, 2008). While each of these factors is expressed by all sensory lineages, their activities are often restricted to specific classes. For example, while Brn3a is required in all sensory neurons, Isl1 appears to exert a selective role in the diversification of sensory neurons that innervate the skin (Eng *et al.*, 2001; McEvilly *et al.*, 1996; Sun *et al.*, 2008).

Neurons within the sensory lineage further diversify into two main classes: proprioceptive neurons, which relay information about muscle tension and length, and cutaneous neurons, which relay several sensory modalities from the skin. These distinct sensory neuron classes can be anatomically defined by the termination of their axons within the spinal cord as well as by expression of receptors for neurotrophic factors (Marmigere and Ernfors, 2007). Cutaneous sensory neurons express the neurotrophin receptor TrkA and terminate within the dorsal spinal cord where they synapse with projection relay neurons. Proprioceptive neurons express TrkC, terminate in the intermediate and ventral spinal cord, and form connections with multiple ventral classes including local circuit interneurons, ascending projection neurons, and motor neurons.

require Runx1 and Isl1 function, and proprioceptive neurons that require Runx3 and Er81. (B) Different classes of sensory neurons can be characterized by their peripheral and central projection patterns. Cutaneous neurons project peripherally to skin and centrally to dorsal laminae of spinal cord (SC), type Ib proprioceptives project to Golgi tendon organs (GTOs) and project to the intermediate spinal cord, while type Ia project to muscle spindles and neuron in the ventral spinal cord. (C) Model for the control of central projections of proprioceptive afferents by Runx3. The level of Runx3 activity appears to control the ventral extent of proprioceptive afferents. Elevated levels of Runx3 appear to drive projections more ventrally. Runx3 activity levels may be controlled by interactions with Er81, which may be indirectly regulated through peripheral NT-3 signaling.

The genetic programs which differentiate cutaneous from proprioceptive neurons are not well understood. Both sensory classes are born in sequential manner, with the birth of proprioceptive neurons preceding generation of cutaneous sensory neurons. In addition to the early roles of Ngn1 and Ngn2 in specifying all sensory lineages, these factors also appear to be critically involved in the sequential generation of each of the two major classes. In *Ngn1* mutants, there is a complete absence of TrkA+ cutaneous neurons while proprioceptive neurons are relatively spared (Ma *et al.*, 1999). While in *Ngn2* mutants the early specification of proprioceptive neurons is selectively affected, *Ngn1* appears to be capable of compensating for loss of *Ngn2* in later stages.

Cutaneous and proprioceptive classes are also distinguished from one another by differential expression of two runt-domain transcription factors: Runx1 and Runx3. Runx1 is expressed in all TrkA+ sensory neurons and is required within a subpopulation of these cells for the expression of a variety of genes required for nociceptive (pain) sensation (Chen *et al.*, 2006b). Loss of *Runx1* leads to changes in the normal pattern of target innervation of nociceptive afferents in the dorsal horn, and the subtypes normally dependent on Runx1 function project to more dorsal regions of the spinal cord (Chen *et al.*, 2006b). In complimentary gain-of-function studies, it was also found that forced misexpression of Runx1 forces cutaneous neurons to project into deeper layers of the dorsal horn (Kramer *et al.*, 2006b). Similarly, *in vitro* studies indicate that Runx1 can promote axonal outgrowth in a dose-dependent manner (Marmigere *et al.*, 2006). As described below, Runx3 appears to exert a similar instructive role in the specification and axonal targeting of proprioceptive sensory neurons.

4.2. Genetic control of proprioceptive sensory neuron identity

Because proprioceptive neurons relay information about limb position and the contractile status of muscles to the CNS, their ability to form selective connections with motor neurons and interneurons is critical for coordinated locomotion. Like motor neurons, proprioceptive sensory neurons require a set of postmitotically expressed factors that are shared amongst most subtypes. One aspect of the transcriptional programming of their identities appears to drive the central projections of their axons toward the ventral spinal cord. This program has been shown to be mediated through the coordinated actions of two transcriptions factors, the ETS factor Er81 and Runx3.

In mice Er81 is expressed by virtually all TrkC+ proprioceptive neurons. In *Er81* mutants, the peripheral projections of sensory afferents to muscle appear to be established normally, and early aspects of muscle spindle formation are unaffected (Arber *et al.*, 2000). Centrally however, proprioceptive neurons fail to project to the ventral spinal cord, and the

monosynaptic connections that form between proprioceptive neurons and motor neurons are not established. Electrophysiological studies confirm a loss of the monosynaptic stretch-reflex circuit and *Er81* mutant mice are characterized by severe defects in the coordination of limb movements. Thus, Er81 is required generally for the central connections between sensory neurons and their targets in the ventral spinal cord.

Within the proprioceptive class, sensory neurons further diversify into three major subtypes, termed group Ia, Ib, and II afferents, which differ in their peripheral connections and the extent of their ventral projections (Brown, 1981). Peripherally, group Ia and group II neurons project to muscle spindles while group Ib neurons project to Golgi tendon organs. Centrally, Group Ib afferents do not extend beyond the intermediate spinal cord, and group II afferents project only sparsely into the ventral spinal cord, while group Ia afferents send extensive ventral collateral projections (Fig. 4.4B). Because different laminar zones are populated by distinct classes of projection neurons (Brown, 1981), the selection of sensory axonal termination position has a critical role in establishing precise patterns of sensory connectivity.

How are the distinct termination zones of different proprioceptive afferent subtypes determined? The graded activities of the transcription factor Runx3 appear to exert a central role in the ventral extent of sensory neuron central projection. Like Er81, Runx3 is expressed by all proprioceptive subtypes, and in *Runx3* mutants proprioceptive neurons fail to innervate the ventral spinal cord (Inoue *et al.*, 2002). Gain-of-function studies indicate that Runx3 has a distinct role from Er81 in defining the central projection patterns of proprioceptive afferents. Within the proprioceptive population, the levels of Runx3 vary widely between individual neurons (Chen *et al.*, 2006a). Misexpression studies in the chick suggest that differences in the levels of Runx3 activity in proprioceptive neurons exert a profound influence on axonal projection patterns (Fig. 4.4C). Misexpression or overexpression of Runx3 in sensory neurons that terminate more dorsally can force these populations to project toward the ventral spinal cord. The ability of Runx3 to control the termination zone appears to be specific, as similar studies performed with Er81 overexpression had no effect on ventral projection patterns (Chen *et al.*, 2006a).

These findings suggest a model in which the different termination zones of group Ib, II, and Ia proprioceptive axons are specified by graded Runx3 activity. However, the mechanisms by which sensory neurons acquire different levels of Runx3 remains unclear. Different levels of Runx3 activity could be set by other factors expressed by proprioceptive sensory neurons which elevate the levels of Runx3 expression or modify its transcriptional activities. For example, Er81 could enhance the net concentration Runx3 in Ia afferents, bringing levels in many proprioceptive sensory neurons above a critical threshold that is needed to direct axons into the ventral

spinal cord (Fig. 4.4C). Alternatively, Er81 may enhance Runx3 activity through direct protein interactions on target genes that are dependent on extrinsic or intrinsic cues.

As with motor neurons, aspects of the transcriptional identity of proprioceptive sensory neurons appear to be set by cues in the periphery. Expression of Er81 is controlled by peripheral NT-3 signaling (Patel *et al.*, 2003), and some aspects of the phenotypes observed in *Er81* mutants can be alleviated through over expression of NT-3 in muscle (Li *et al.*, 2006). How the NT-3/Er81 pathway influences the expression levels of Runx3 is not known, although one plausible model is that NT-3 also influences Runx3 expression which in turn may directly or indirectly control the laminar targeting of proprioceptive sensory neurons (Fig. 4.4C). As described below, the use of retrograde signaling by sensory neurons may extend beyond control of laminar targeting, and may define additional aspects of their innervation pattern, such as the synaptic specificity of connections between sensory neurons and motor neurons.

5. Sensory–Motor Circuit Assembly and Function

A relatively simple circuit in the nervous system is the monosynaptic stretch-reflex circuit which fundamentally consists of a motor neuron, a type Ia sensory afferent, and a muscle target (Fig. 4.5A) (Eccles *et al.*, 1957). When a muscle is stretched the activation of mechanoreceptors within muscle spindles leads to the excitation of Ia sensory afferents that synapse with motor neurons that innervate the same muscle. In addition to these direct monosynaptic inputs, proprioceptive neurons also form connections with inhibitory interneurons that are connected to motor neurons that project to functionally antagonistic muscles. This proprioceptive sensory feedback from muscle to neurons in the ventral spinal cord is essential in maintaining body posture and also has a critical role in adaptive responses to changes in the environment (Chen *et al.*, 2003; Dietz, 2002).

The central connections formed between sensory and motor neurons have been shown to be highly selective, as proprioceptive afferents projecting to a given muscle target avoid making monosynaptic connections with motor neurons innervating antagonistic or functionally unrelated muscles (Frank and Mendelson, 1990). Presumably, as Ia afferents reach the ventral spinal cord they must acquire the capacity to select only small subset of their many potential synaptic partners. Remarkably, it has been shown that a single group Ia sensory afferent generates several collateral branches that form synapses with the hundreds of motor neurons within a pool that innervate a homonymous muscle target (Mendell and Henneman, 1968).

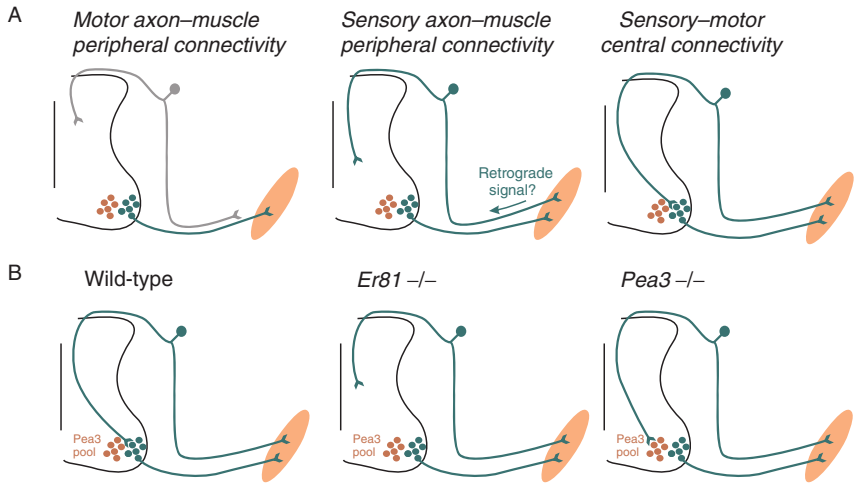

Figure 4.5 Models for the formation of the Ia monosynaptic stretch-reflex circuit. (A) In the initial phases of the formation of the stretch-reflex circuit, motor axons project to specific muscle targets by virtue of their Hox-induced transcriptional identity. In this model, sensory neurons are naïve with respect to their identities, and project along the pathways defined by motor axons. When sensory neurons reach their targets, a retrograde signal may allow them to acquire a muscle-specific identity. The acquisition of a muscle-specific identity by sensory neurons determines their capacity to selectively innervate motor neurons which project to the same muscle target. (B) Role of ETS transcription factors in sensory–motor connectivity. In *Er81* mutants, all proprioceptive sensory neurons fail to reach the ventral spinal cord. In *Pea3* mutants, loss of Pea3 activity in motor neurons leads to inappropriate innervation by other sensory neuron populations.

Because the specificity of connections appears to form in an activity-independent manner (Mendelson and Frank, 1991), the stretch-reflex circuit provides an attractive system to explore the genetic basis of synaptic specificity.

5.1. Early studies on the peripheral and central connectivity of proprioceptive neurons

One potential issue in trying to understand the genetic basis for circuit assembly is whether the principles that apply to one class of neurons are applicable to other classes. Does the concept of a transcription factor-based cell intrinsic program that defines the "muscle-specific" identity of motors neurons apply to the specification and connectivity patterns of sensory neurons? To what extent does the synaptic specificity between sensory neurons and motor neurons rely on cues from the periphery? Developmental studies in chick embryos have provided insights into the novel strategies used by

sensory neurons in the formation of specific peripheral and central connections.

Since both motor and proprioceptive axons project to a variety muscle targets, one question is whether sensory neurons possess independent guidance mechanisms that confer the specificity of their peripheral projections. As motor axons project into the periphery, sensory axons appear to follow the routes pioneered by motor neurons (Gallarda *et al.*, 2008; Wang and Scott, 2000). Studies using chick embryological manipulations suggest that the peripheral projections of proprioceptive neurons are dependent on the routes established by motor axons. When motor neurons are surgically removed at a stage prior to when they project into the limb, sensory neurons are no longer capable of innervating muscle, but instead are diverted toward the targets cutaneous sensory neurons (Landmesser and Honig, 1986; Swanson and Lewis, 1986). A more recent study indicated that when motor neurons were ablated, sensory neurons were able to project to muscle, although some aspects in the specificity of these connections were eroded (Wang and Scott, 1999). These differences in the effects of motor neuron removal may reflect differences in the time period in which the manipulations were performed and hence the developmental potential of sensory neurons at different stages. Nevertheless, these studies suggest that unlike motor neurons, proprioceptive may be initially naïve, and their peripheral target specificities defined by the specificity of motor axon projections.

There is additional evidence that the specificity of the central connections of proprioceptive afferents within the spinal cord is acquired relatively late in development in response to signals provided by the periphery. Evidence in support of this view has come from analysis of the central innervation pattern of proprioceptive afferents in chick embryos grafted with a double-dorsal limb mesenchyme (Wenner and Frank, 1995). In this situation, the peripheral terminals of proprioceptive afferents are forced to innervate inappropriate limb muscles, and there is a corresponding change in central connectivity that matches the new peripheral target (Wenner and Frank, 1995). Aspects of sensory–motor target specificity may therefore be imparted by some specialized property of the muscle group itself, possibly through retrograde signaling to sensory neuron cell body.

These observations have suggested a model in which the identity of motor neurons establishes one fixed element in the stretch-reflex circuit, with a later peripheral influence imposing a matching identity on group Ia proprioceptive afferents, with the consequence that incoming afferent fibers are able to recognize distinct motor neuron pools and select appropriate partners for synapse formation (Fig. 4.5A). How this ''molecular matching'' program is established in unknown but could involve sets of homophilic recognition molecules or complimentary ligand–receptor pairs in pre- and postsynaptic cells.

5.2. Feedback control and molecular matching of sensory and motor neurons

Recent studies exploring the steps that specify motor and sensory neuron subtypes provide evidence that the expression of common transcription factors or their targets define the specificity of connections in the stretch-reflex circuit. Thus far, the most compelling studies have emerged from the analysis of the role of motor neuron identity in the assembly of this circuit. In addition to its role in motor axon arborization patterns, the ETS protein Pea3 also appears to exert a role in the formation of stretch-reflex circuits for specific muscles in the forelimb. In *Pea3* mutants, the motor neurons that have lost Pea3 expression receive input from inappropriate motor pools (Fig. 4.5B) (Vrieseling and Arber, 2006), suggesting that Pea3 is necessary to restrict inappropriate sensory neurons from forming monosynaptic connections. One additional aspect of Pea3 function in sensory–motor connectivity is to configure the pattern of motor neuron dendrites, as in *Pea3* mutants the normal pattern of dendritic arborization is altered. Whether other pool-specific transcription factors exert similar roles in the synaptic specificity in sensory–motor circuits remains to be explored.

What are the possible molecular programs that might control the assembly of the monosynaptic stretch-reflex circuit? Expression of Pea3 in motor neurons is controlled by Hox proteins (Dasen *et al.*, 2005), and Pea3 is necessary for the expression of multiple surface recognition and guidance molecules such as type II cadherins and semaphorins (Livet *et al.*, 2002). Combinatorial expression of semaphorin expression has been suggested to be involved in motor axon guidance decisions in the limb (Cohen *et al.*, 2005), and several type II cadherin family members have been shown to be expressed in both sensory and motor neurons in a limb-dependent manner (Price *et al.*, 2002). In addition, certain *Hox* genes have been shown to be expressed by DRG sensory neurons in a pattern that parallels *Hox* patterns within the spinal cord (Belting *et al.*, 1998), consistent with the hypothesis they may be involved in the matching of sensory and motor neurons. These disparate observations raise the possibility that coordinate expression of *Hox* genes acts in the initial phases of motor and sensory diversification, while intermediate transcription factors, target-dependent programs, and synaptic specificity determinants acting to control the selectivity of sensory–motor connectivity.

Could the concept of a transcription factor-based molecular matching system explain how neural circuits are assembled in other regions of the nervous system? In the developing hindbrain, the *Hoxa2* gene has been shown to regulate the connections formed between motor neurons and sensory neurons and contribute to formation of facial somatotopic maps (Oury *et al.*, 2006). Similarly, the homeodomain protein Phox2b has been shown to function in different classes of neurons involved in the assembly

of autonomic reflex circuits (Dauger *et al.*, 2003). However, the cell-type-specific functions of these genes have not been assessed, nor are the relevant downstream effectors of these transcription factors known. Nevertheless, these observations are consistent with the idea that aspects of synaptic specificity could be determined through expression of common transcription factors within diverse neuronal classes.

6. Conclusions

While our understanding of the early specification programs that control the synaptic specificity of motor and sensory neurons has progressed significantly, the extent to which they provide any insights into the genetic basis of innate motor behaviors remains to be seen. A recent study in *Drosophila* indicates that the activities of individual *Hox* genes can switch the pattern of motor output within embryonic segments and lead to homeotic transformation of larval motility behaviors (Dixit *et al.*, 2008). These observations are consistent with the idea that the activities of single genes, acting in the context of diverse neuronal classes, can reprogram stereotypic patterns of movement. It will be interesting to determine whether these influences of *Hox* genes in the *Drosophila* nervous system are mediated through their activities in motor neurons, sensory neurons, or interneurons, or perhaps all three classes.

Studies of the spinal locomotor system indicate that much the diversification of its resident neuronal classes and subtypes can be linked to the actions of a coherent set of transcription factors expressed in discrete domains along the dorsoventral and rostrocaudal axes. Studies in motor neurons indicate that much of this diversity is mediated through the actions of a single large family of transcription factors encoded by the *Hox* gene clusters. Whether the actions of these factors also influence the specificity of connections between motor neurons, interneurons, and sensory neurons, and contribute to locomotor behaviors, should be an area of exciting investigation in the future.

The control of movement relies on the integration of circuits residing in multiple regions of the nervous system outside the spinal cord, including the hindbrain, cerebellum, basal ganglia, and somatosensory cortex. A more complete understanding of the neural circuitries that specify innate motor behaviors will require a better picture of how these circuits are assembled during development, and how these regions integrate with spinal networks. Recent studies on the developmental programs that specify circuits in the cortex have revealed that pyramidal neurons and interneurons use very similar molecular strategies to drive neuronal differentiation, involving transcriptional networks active in progenitor and postmitotic cells (Fishell,

2007; Molyneaux *et al.*, 2007). Although cortical areas lack expression of the chromosomally arrayed *Hox* genes, these circuits may also use coherent families of transcription factors to control the matching of diverse neuronal classes during synaptogenesis.

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REFERENCES

Anderson, D. J. (1999). Lineages and transcription factors in the specification of vertebrate primary sensory neurons. *Curr. Opin. Neurobiol.* **9,** 517–524.

Arber, S., Han, B., Mendelsohn, M., Smith, M., Jessell, T. M., and Sockanathan, S. (1999). Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. *Neuron* **23,** 659–674.

Arber, S., Ladle, D. R., Lin, J. H., Frank, E., and Jessell, T. M. (2000). ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. *Cell* **101,** 485–498.

Belting, H. G., Shashikant, C. S., and Ruddle, F. H. (1998). Multiple phases of expression and regulation of mouse Hoxc8 during early embryogenesis. *J. Exp. Zool.* **282,** 196–222.

Bel-Vialar, S., Itasaki, N., and Krumlauf, R. (2002). Initiating Hox gene expression: In the early chick neural tube differential sensitivity to FGF and RA signaling subdivides the HoxB genes in two distinct groups. *Development (Cambridge)* **129,** 5103–5115.

Briscoe, J., Pierani, A., Jessell, T. M., and Ericson, J. (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* **101,** 435–445.

Brown, A. G. (1981). "Organization in the Spinal Cord: The Anatomy and Physiology of Identified Neurones." Springer-Verlag, Berlin.

Chen, A. I., de Nooij, J. C., and Jessell, T. M. (2006a). Graded activity of transcription factor Runx3 specifies the laminar termination pattern of sensory axons in the developing spinal cord. *Neuron* **49,** 395–408.

Chen, C. L., Broom, D. C., Liu, Y., de Nooij, J. C., Li, Z., Cen, C., Samad, O. A., Jessell, T. M., Woolf, C. J., and Ma, Q. (2006b). Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron* **49,** 365–377.

Chen, H. H., Hippenmeyer, S., Arber, S., and Frank, E. (2003). Development of the monosynaptic stretch reflex circuit. *Curr. Opin. Neurobiol.* **13,** 96–102.

Cohen, S., Funkelstein, L., Livet, J., Rougon, G., Henderson, C. E., Castellani, V., and Mann, F. (2005). A semaphorin code defines subpopulations of spinal motor neurons during mouse development. *Eur. J. Neurosci.* **21,** 1767–1776.

Dasen, J. S., Liu, J. P., and Jessell, T. M. (2003). Motor neuron columnar fate imposed by sequential phases of Hox-c activity. *Nature* **425,** 926–933.

Dasen, J. S., Tice, B. C., Brenner-Morton, S., and 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., and Jessell, T. M. (2008). Hox repertoires for motor neuron diversity and connectivity gated by a single accessory factor, FoxP1. *Cell* **134,** 304–316.

Dauger, S., Pattyn, A., Lofaso, F., Gaultier, C., Goridis, C., Gallego, J., and Brunet, J. F. (2003). Phox2b controls the development of peripheral chemoreceptors and afferent visceral pathways. *Development (Cambridge)* **130,** 6635–6642.

De Marco Garcia, N. V., and Jessell, T. M. (2008). Early motor neuron pool identity and muscle nerve trajectory defined by postmitotic restrictions in Nkx6.1 activity. *Neuron* **57,** 217–231.

Demir, E., and Dickson, B. J. (2005). Fruitless splicing specifies male courtship behavior in *Drosophila*. *Cell* **121,** 785–794.

Dessaud, E., McMahon, A. P., and Briscoe, J. (2008). Pattern formation in the vertebrate neural tube: A sonic hedgehog morphogen-regulated transcriptional network. *Development (Cambridge)* **135,** 2489–2503.

Dietz, V. (2002). Proprioception and locomotor disorders. *Nat. Rev. Neurosci.* **3,** 781–790.

Dixit, R., Vijayraghavan, K., and Bate, M. (2008). Hox genes and the regulation of movement in *Drosophila*. *Dev. Neurobiol.* **68,** 309–316.

Dou, C., Ye, X., Stewart, C., Lai, E., and Li, S. C. (1997). TWH regulates the development of subsets of spinal cord neurons. *Neuron* **18,** 539–551.

Eccles, J. C., Eccles, R. M., and Lundberg, A. (1957). The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *J. Physiol.* **137,** 22–50.

Eng, S. R., Gratwick, K., Rhee, J. M., Fedtsova, N., Gan, L., and Turner, E. E. (2001). Defects in sensory axon growth precede neuronal death in Brn3a-deficient mice. *J. Neurosci.* **21,** 541–549.

Ensini, M., Tsuchida, T. N., Belting, H. G., and Jessell, T. M. (1998). The control of rostrocaudal pattern in the developing spinal cord: Specification of motor neuron subtype identity is initiated by signals from paraxial mesoderm. *Development (Cambridge)* **125,** 969–982.

Fishell, G. (2007). Perspectives on the developmental origins of cortical interneuron diversity. *Novartis Found. Symp.* **288,** 21–35; discussion 35–44, 96–28.

Frank, E., and Mendelson, B. (1990). Specification of synaptic connections between sensory and motor neurons in the developing spinal cord. *J. Neurobiol.* **21,** 33–50.

Gallarda, B. W., Bonanomi, D., Muller, D., Brown, A., Alaynick, W. A., Andrews, S. E., Lemke, G., Pfaff, S. L., and Marquardt, T. (2008). Segregation of axial motor and sensory pathways via heterotypic trans-axonal signaling. *Science (New York)* **320,** 233–236.

Goulding, M., and Pfaff, S. L. (2005). Development of circuits that generate simple rhythmic behaviors in vertebrates. *Curr. Opin. Neurobiol.* **15,** 14–20.

Greene, E. C. (1963). "Anatomy of the Rat." Hafner Publishing Company, New York.

Haase, G., Dessaud, E., Garces, A., de Bovis, B., Birling, M., Filippi, P., Schmalbruch, H., Arber, S., and deLapeyriere, O. (2002). GDNF acts through PEA3 to regulate cell body positioning and muscle innervation of specific motor neuron pools. *Neuron* **35,** 893–905.

Hanson, M. G., Milner, L. D., and Landmesser, L. T. (2008). Spontaneous rhythmic activity in early chick spinal cord influences distinct motor axon pathfinding decisions. *Brain Res. Rev.* **57,** 77–85.

Hari, L., Brault, V., Kleber, M., Lee, H. Y., Ille, F., Leimeroth, R., Paratore, C., Suter, U., Kemler, R., and Sommer, L. (2002). Lineage-specific requirements of beta-catenin in neural crest development. *J. Cell Biol.* **159,** 867–880.

Helmbacher, F., Schneider-Maunoury, S., Topilko, P., Tiret, L., and Charnay, P. (2000). Targeting of the EphA4 tyrosine kinase receptor affects dorsal/ventral pathfinding of limb motor axons. *Development (Cambridge)* **127,** 3313–3324.

Helmbacher, F., Dessaud, E., Arber, S., deLapeyriere, O., Henderson, C. E., Klein, R., and Maina, F. (2003). Met signaling is required for recruitment of motor neurons to PEA3-positive motor pools. *Neuron* **39,** 767–777.

Hobert, O. (2003). Behavioral plasticity in *C. elegans*: Paradigms, circuits, genes. *J. Neurobiol.* **54,** 203–223.

Hollyday, M., and Jacobson, R. D. (1990). Location of motor pools innervating chick wing. *J. Comp. Neurol.* **302,** 575–588.

Huber, A. B., Kania, A., Tran, T. S., Gu, C., De Marco Garcia, N., Lieberam, I., Johnson, D., Jessell, T. M., Ginty, D. D., and Kolodkin, A. L. (2005). Distinct roles for secreted semaphorin signaling in spinal motor axon guidance. *Neuron* **48,** 949–964.

Inoue, K., Ozaki, S., Shiga, T., Ito, K., Masuda, T., Okado, N., Iseda, T., Kawaguchi, S., Ogawa, M., Bae, S. C., *et al.* (2002). Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. *Nat. Neurosci.* **5,** 946–954.

Jessell, T. M. (2000). Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. *Nat. Rev. Genet.* **1,** 20–29.

Kania, A., and Jessell, T. M. (2003). Topographic motor projections in the limb imposed by LIM homeodomain protein regulation of ephrin-A:EphA interactions. *Neuron* **38,** 581–596.

Kania, A., Johnson, R. L., and Jessell, T. M. (2000). Coordinate roles for LIM homeobox genes in directing the dorsoventral trajectory of motor axons in the vertebrate limb. *Cell* **102,** 161–173.

Kiehn, O., and Butt, S. J. (2003). Physiological, anatomical and genetic identification of CPG neurons in the developing mammalian spinal cord. *Prog. Neurobiol.* **70,** 347–361.

Kim, J., Lo, L., Dormand, E., and Anderson, D. J. (2003). SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. *Neuron* **38,** 17–31.

Kmita, M., and Duboule, D. (2003). Organizing axes in time and space; 25 years of colinear tinkering. *Science (New York)* **301,** 331–333.

Kramer, E. R., Knott, L., Su, F., Dessaud, E., Krull, C. E., Helmbacher, F., and Klein, R. (2006a). Cooperation between GDNF/Ret and ephrinA/EphA4 signals for motor-axon pathway selection in the limb. *Neuron* **50,** 35–47.

Kramer, I., Sigrist, M., de Nooij, J. C., Taniuchi, I., Jessell, T. M., and Arber, S. (2006b). A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. *Neuron* **49,** 379–393.

Ladle, D. R., Pecho-Vrieseling, E., and Arber, S. (2007). Assembly of motor circuits in the spinal cord: Driven to function by genetic and experience-dependent mechanisms. *Neuron* **56,** 270–283.

Landmesser, L. (1978a). The development of motor projection patterns in the chick hind limb. *J. Physiol.* **284,** 391–414.

Landmesser, L. (1978b). The distribution of motoneurones supplying chick hind limb muscles. *J. Physiol.* **284,** 371–389.

Landmesser, L., and Honig, M. G. (1986). Altered sensory projections in the chick hind limb following the early removal of motoneurons. *Dev. Biol.* **118,** 511–531.

Landmesser, L. T. (2001). The acquisition of motoneuron subtype identity and motor circuit formation. *Int. J. Dev. Neurosci.* **19,** 175–182.

Lanuza, G. M., Gosgnach, S., Pierani, A., Jessell, T. M., and Goulding, M. (2004). Genetic identification of spinal interneurons that coordinate left–right locomotor activity necessary for walking movements. *Neuron* **42,** 375–386.

Lee, H. Y., Kleber, M., Hari, L., Brault, V., Suter, U., Taketo, M. M., Kemler, R., and Sommer, L. (2004). Instructive role of Wnt/beta-catenin in sensory fate specification in neural crest stem cells. *Science (New York)* **303,** 1020–1023.

Li, L. Y., Wang, Z., Sedy, J., Quazi, R., Walro, J. M., Frank, E., and Kucera, J. (2006). Neurotrophin-3 ameliorates sensory–motor deficits in Er81-deficient mice. *Dev. Dyn.* **235,** 3039–3050.

Lin, J. H., Saito, T., Anderson, D. J., Lance-Jones, C., Jessell, T. M., and Arber, S. (1998). Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. *Cell* **95,** 393–407.

Liu, J. P., Laufer, E., and Jessell, T. M. (2001). Assigning the positional identity of spinal motor neurons: Rostrocaudal patterning of Hox-c expression by FGFs, Gdf11, and retinoids. *Neuron* **32,** 997–1012.

Livet, J., Sigrist, M., Stroebel, S., De Paola, V., Price, S. R., Henderson, C. E., Jessell, T. M., and Arber, S. (2002). ETS gene Pea3 controls the central position and terminal arborization of specific motor neuron pools. *Neuron* **35,** 877–892.

Ma, Q., Fode, C., Guillemot, F., and Anderson, D. J. (1999). Neurogenin1 and neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. *Genes Dev.* **13,** 1717–1728.

Marmigere, F., and Ernfors, P. (2007). Specification and connectivity of neuronal subtypes in the sensory lineage. *Nat. Rev. Neurosci.* **8,** 114–127.

Marmigere, F., Montelius, A., Wegner, M., Groner, Y., Reichardt, L. F., and Ernfors, P. (2006). The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons. *Nat. Neurosci.* **9,** 180–187.

McEvilly, R. J., Erkman, L., Luo, L., Sawchenko, P. E., Ryan, A. F., and Rosenfeld, M. G. (1996). Requirement for Brn-3.0 in differentiation and survival of sensory and motor neurons. *Nature* **384,** 574–577.

Mendell, L. M., and Henneman, E. (1968). Terminals of single Ia fibers: Distribution within a pool of 300 homonymous motor neurons. *Science (New York)* **160,** 96–98.

Mendelson, B., and Frank, E. (1991). Specific monosynaptic sensory–motor connections form in the absence of patterned neural activity and motoneuronal cell death. *J. Neurosci.* **11,** 1390–1403.

Milner, L. D., and Landmesser, L. T. (1999). Cholinergic and GABAergic inputs drive patterned spontaneous motoneuron activity before target contact. *J. Neurosci.* **19,** 3007–3022.

Molyneaux, B. J., Arlotta, P., and Macklis, J. D. (2007). Molecular development of corticospinal motor neuron circuitry. *Novartis Found. Symp.* **288,** 3–15; discussion 15–20, 96–18.

Muhr, J., Andersson, E., Persson, M., Jessell, T. M., and Ericson, J. (2001). Groucho-mediated transcriptional repression establishes progenitor cell pattern and neuronal fate in the ventral neural tube. *Cell* **104,** 861–873.

Novitch, B. G., Chen, A. I., and Jessell, T. M. (2001). Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. *Neuron* **31,** 773–789.

Oury, F., Murakami, Y., Renaud, J. S., Pasqualetti, M., Charnay, P., Ren, S. Y., and Rijli, F. M. (2006). Hoxa2- and rhombomere-dependent development of the mouse facial somatosensory map. *Science (New York)* **313,** 1408–1413.

Patel, T. D., Kramer, I., Kucera, J., Niederkofler, V., Jessell, T. M., Arber, S., and Snider, W. D. (2003). Peripheral NT3 signaling is required for ETS protein expression and central patterning of proprioceptive sensory afferents. *Neuron* **38,** 403–416.

Price, S. R., De Marco Garcia, N. V., Ranscht, B., and Jessell, T. M. (2002). Regulation of motor neuron pool sorting by differential expression of type II cadherins. *Cell* **109,** 205–216.

Rousso, D. L., Gaber, Z. B., Wellik, D., Morrisey, E. E., and 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.

Sauka-Spengler, T., and Bronner-Fraser, M. (2008). A gene regulatory network orchestrates neural crest formation. *Nat. Rev.* **9,** 557–568.

Shah, V., Drill, E., and Lance-Jones, C. (2004). Ectopic expression of Hoxd10 in thoracic spinal segments induces motoneurons with a lumbosacral molecular profile and axon projections to the limb. *Dev. Dyn.* **231,** 43–56.

Sharma, K., and Belmonte, J. C. (2001). Development of the limb neuromuscular system. *Curr. Opin. Cell Biol.* **13,** 204–210.

Sharma, K., Leonard, A. E., Lettieri, K., and Pfaff, S. L. (2000). Genetic and epigenetic mechanisms contribute to motor neuron pathfinding. *Nature* **406,** 515–519.

Shirasaki, R., and Pfaff, S. L. (2002). Transcriptional codes and the control of neuronal identity. *Annu. Rev. Neurosci.* **25,** 251–281.

Shirasaki, R., Lewcock, J. W., Lettieri, K., and Pfaff, S. L. (2006). FGF as a target-derived chemoattractant for developing motor axons genetically programmed by the LIM code. *Neuron* **50,** 841–853.

Sockanathan, S., and Jessell, T. M. (1998). Motor neuron-derived retinoid signaling specifies the subtype identity of spinal motor neurons. *Cell* **94,** 503–514.

Stifani, N., Freitas, A. R., Liakhovitskaia, A., Medvinsky, A., Kania, A., and Stifani, S. (2008). Suppression of interneuron programs and maintenance of selected spinal motor neuron fates by the transcription factor AML1/Runx1. *Proc. Natl. Acad. Sci. USA* **105,** 6451–6456.

Sullivan, G. E. (1962). Anatomy and embryology of the wing musculature of the domestic fowl (Gallus). *Aust. J. Zool.* **10,** 458–518.

Sun, Y., Dykes, I. M., Liang, X., Eng, S. R., Evans, S. M., and Turner, E. E. (2008). A central role for Islet1 in sensory neuron development linking sensory and spinal gene regulatory programs. *Nat. Neurosci.* **11,** 1283–1293.

Swanson, G. J., and Lewis, J. (1986). Sensory nerve routes in chick wing buds deprived of motor innervation. *J. Embryol. Exp. Morphol.* **95,** 37–52.

Tanabe, Y., William, C., and Jessell, T. M. (1998). Specification of motor neuron identity by the MNR2 homeodomain protein. *Cell* **95,** 67–80.

Tarchini, B., Huynh, T. H., Cox, G. A., and Duboule, D. (2005). HoxD cluster scanning deletions identify multiple defects leading to paralysis in the mouse mutant Ironside. *Genes Dev.* **19,** 2862–2876.

Thaler, J., Harrison, K., Sharma, K., Lettieri, K., Kehrl, J., and Pfaff, S. L. (1999). Active suppression of interneuron programs within developing motor neurons revealed by analysis of homeodomain factor HB9. *Neuron* **23,** 675–687.

Thaler, J. P., Koo, S. J., Kania, A., Lettieri, K., Andrews, S., Cox, C., Jessell, T. M., and Pfaff, S. L. (2004). A postmitotic role for Isl-class LIM homeodomain proteins in the assignment of visceral spinal motor neuron identity. *Neuron* **41,** 337–350.

Tiret, L., Le Mouellic, H., Maury, M., and Brulet, P. (1998). Increased apoptosis of motoneurons and altered somatotopic maps in the brachial spinal cord of Hoxc-8-deficient mice. *Development (Cambridge)* **125,** 279–291.

Tosney, K. W., and Landmesser, L. T. (1985a). Development of the major pathways for neurite outgrowth in the chick hindlimb. *Dev. Biol.* **109,** 193–214.

Tosney, K. W., and Landmesser, L. T. (1985b). Growth cone morphology and trajectory in the lumbosacral region of the chick embryo. *J. Neurosci.* **5,** 2345–2358.

Tsuchida, T., Ensini, M., Morton, S. B., Baldassare, M., Edlund, T., Jessell, T. M., and Pfaff, S. L. (1994). Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* **79,** 957–970.

Vallstedt, A., Muhr, J., Pattyn, A., Pierani, A., Mendelsohn, M., Sander, M., Jessell, T. M., and Ericson, J. (2001). Different levels of repressor activity assign redundant and specific roles to Nkx6 genes in motor neuron and interneuron specification. *Neuron* **31,** 743–755.

Vrieseling, E., and Arber, S. (2006). Target-induced transcriptional control of dendritic patterning and connectivity in motor neurons by the ETS gene Pea3. *Cell* **127,** 1439–1452.

Wang, G., and Scott, S. A. (1999). Independent development of sensory and motor innervation patterns in embryonic chick hindlimbs. *Dev. Biol.* **208,** 324–336.

Wang, G., and Scott, S. A. (2000). The ''waiting period'' of sensory and motor axons in early chick hindlimb: Its role in axon pathfinding and neuronal maturation. *J. Neurosci.* **20,** 5358–5366.

Wenner, P., and Frank, E. (1995). Peripheral target specification of synaptic connectivity of muscle spindle sensory neurons with spinal motoneurons. *J. Neurosci.* **15,** 8191–8198.

Wu, Y., Wang, G., Scott, S. A., and Capecchi, M. R. (2008). Hoxc10 and Hoxd10 regulate mouse columnar, divisional and motor pool identity of lumbar motoneurons. *Development (Cambridge)* **135,** 171–182.