binds specifically to methylated H3 lysine 4. This interaction precedes H3 acetylation by Gcn5 and the subsequent gene activation (Pray-Grant et al., 2005). Thus, the ultimate function of 19S RP in transcription initiation may be to coordinate Chd1 binding by targeting SAGA to the same promoters at which H3 lysine 4 is methylated, which are those that have been monoubiquitylated at H2B lysine 123 by Rad6. Another component of the SAGA complex, the ubiquitin protease Ubp8, has been shown to deubiquitylate histone H2B lysine 123 (Daniel et al., 2004). This modification is also important for gene induction, and hence it is thought that a transient sequence of ubiquitylation followed by deubiquitylation precedes transcription (Henry et al., 2003). Given this evidence, it is tempting to speculate that deubiquitylation of H2B releases 19S RP from the promoter region, which allows association with RNA polymerase II during elongation.

An important consideration to point out is that most of the studies referenced thus far were performed on the *Saccharomyces cerevisiae GAL1-GAL10* gene loci. It remains to be determined whether the proteasome functions similarly in multiple steps of transcription activation in mammalian cells, although it is clear that the proteasome plays a role in regulation of nuclear receptors, including those for estrogen, progesterone, glucocorticoid, and others. In general, nuclear receptors are bound by activating ligands, and following ligand binding they directly interact with chromatin at promoter elements. This interaction is thought to recruit activators and basal transcriptional machinery to the promoter, which induces gene expression. Recent evidence shows that the proteasome tightly controls the exchange rate of ligand bound nuclear receptors at promoters, thereby directly influencing transcriptional output (Kinyamu et al., 2005).

Another important question for future studies is to determine how the proteasomal ATPase activity specifically functions in the targeting of SAGA to the gene promoter. Because the targeting effect was observed even on naked DNA, the work of Lee et al. (2005) indicates that the activity of an ATPase subunit of the proteasome, Sug1, is directed toward SAGA rather than chromatin. Mutations in the ATPase domain of Sug1 decrease the recruitment of the acetyltransferase subunit of SAGA, Gcn5, to the *GAL1-GAL10* promoter. Because of this ATP dependence, the authors postulate that 19S RP loads SAGA onto the promoter in a manner similar to the loading of the Mcm2-7 DNA helicase complex onto replication origins by ORC (origin recognition complex). Clarifying the mechanisms for this process may provide insight into how the proteasome functions in other DNA-related events.

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Hox Genes: The Instructors Working at Motor Pools

Motor neurons are assigned unique subidentities preceding their axon navigation. This ensures proper innervation of muscle targets and is accompanied by a stereotypical clustering of motor neuron cell bodies into "motor pools" within the spinal cord. However, the mechanisms that drive motor neuron diversification have been poorly understood. A new study by Dasen et al. (2005) in this issue of *Cell* shows that a network of *Hox* genes is responsible for instructing motor pool development.

Coordinated body movements are dependent upon the formation of precise connections between specific motor neurons and the muscles they are designated to control. Each motor neuron is restricted to forming connections with several hundred muscle fibers within a single muscle. Although it remains unclear why, motor neurons that innervate the same muscle cluster their cell bodies into "pools" within the spinal cord. Many previous studies have carefully mapped the precise position of individual motor pools using retrograde cell labeling and found that the rostrocaudal and dorsoventral location of these pools are highly conserved from one individual to the next (Landmesser, 2001). In fact, this stereotyped anatomical feature of locomotor neuron organization has greatly facilitated the analysis of motor axon navigation and led to the notion that motor neurons acquire unique intrinsic characteristics that govern their precise pattern of muscle innervation (Landmesser, 2001).

Motor pools typically span two to four spinal cord segments, vary in cell number in relationship to the size of the muscle they innervate, and extensively overlap

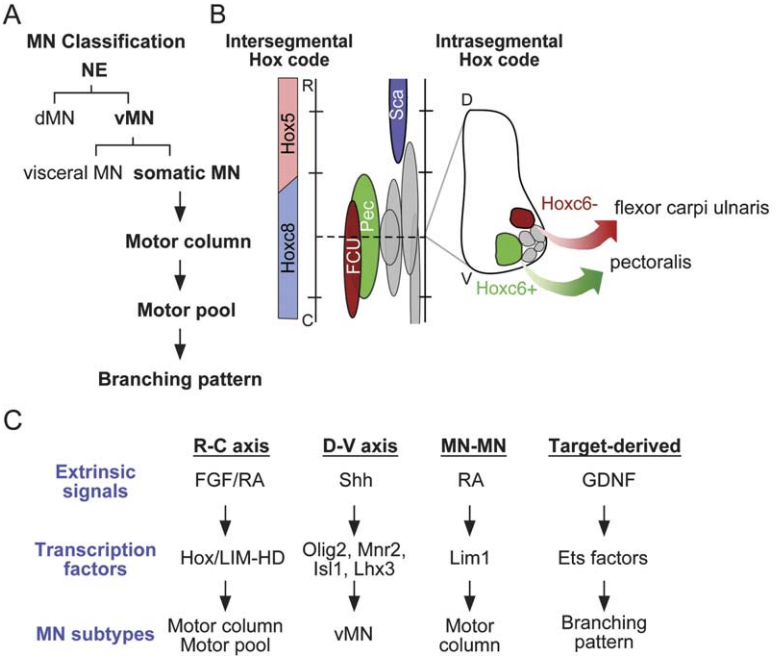

Figure 1. Motor Neuron Subtype Development and the Role of Hox Transcription Factors in Motor Pool Development

(A) A hierarchy of motor neuron (MN) subtype identities. Neuroepithelial (NE) cells generate motor neurons which project their axons dorsally (dMN) or ventrally (vMN). These two distinctions in motor neurons exist primarily at hindbrain levels, as spinal motor neurons are typically ventral exiting. vMNs are divided into somatic MN or visceral MN based on the nature of their postsynaptic targets. Somatic motor neurons innervate skeletal muscles derived from somites, whereas visceral motor neurons innervate branchial and smooth muscles as well as autonomic neurons. Subsets of MNs form longitudinal columns comprised of multiple motor pools. MNs within motor columns project axons together along the major proximal nerve pathways such as to body wall muscles, dorsal and ventral limb muscles, and axial muscles. Each motor pool contains motor neurons innervating a single muscle group, and some motor pools have more extensive branching patterns than others.

(B) Rostrocaudal (R-C) and dorsoventral (D-V) distribution of brachial *Hox* genes in representative motor pools. The position rostrocaudal of motor pools innervating the scapulohumeralis anterior (Sca, purple), pectoralis (Pec, green) and flexor carpi ulnaris (FCU, red) muscles are determined by Hox5 and Hoxc8. The intrasegmental distinction between FCU and Pec cells depends on the status of Hoxc6 in combination with other transcription factors.

(C) Multiple signaling pathways converge to control MN subtype identity. Extrinsic signals (first row) activate transcription factor programs (second row) respectively for proper MN differentiation. A rostrocaudal (R-C) gradient of FGF and retinoic acid (RA) defines motor columns and motor pools by regulating Hox and LIM homeodomain (HD) factors. A dorsoventral (D-V) gradient of sonic hedgehog (Shh) triggers motor neuron formation. RA secreted from early-born MNs that innervate the ventral limb activates Lim1 expression in later-born MNs to regulate the innervation of the dorsal limb. Target-derived factors such as GDNF activate Ets factors to refine branching patterns of motor axons.

with other motor pools distributed along the rostrocaudal axis (Figures 1A and 1B). Thus, a major challenge in the field of spinal cord development has been to understand (1) how motor pools come to occupy their characteristic positions along the rostrocaudal axis, (2) how diverse motor pool subtypes are specified at a single segmental level, and (3) how the intrinsic properties of these cells are regulated to control muscle connectivity. Grafting studies that rotated the embryonic neural tube along its rostrocaudal axis provided compelling evidence that motor pool identity is defined at very early embryonic stages—perhaps beginning within the ancestral progenitor cells that divide to produce the definitive postmitotic motor neurons (Landmesser, 2001). Thus, surgical rotations of the chick spinal cord at Hamburger Hamiltion stage ~15 (24–28 somite embryos), which precedes lumbar motor neuron birth, resulted in the displacement of motor pools rather than the respecification of their identity. Surprisingly, even if motor neurons project axons from abnormal positions within the embryo following surgical manipulation, they alter their trajectories and in many cases still innervate their appropriate muscles. Taken together, these classical studies have served to establish the general rules for motor neuron diversification and have indicated the existence of intrinsic genetic programs that control motor pool identity within the spinal cord.

In a tour de force, Jessell and colleagues (Dasen et al., 2005) have now examined the expression patterns for all 39 members of the Hox family of homeodomain transcription factors along the rostrocaudal axis of the chick embryo spinal cord and deduced the correlation between motor pool identity and Hox expression. There had been several reasons for suspecting that the Hox family might contribute to the regulation of motor pool identity. First, specific members of the *Hox* gene family have been identified in limb-innervating motor neurons (see Liu et al. [2001]). Second, *Hox* mutants have been found to exhibit defects in motor axon projections consistent with an alteration in their identity (see Dasen et al. [2005]). Third, the broader organization of motor neurons into motor columns is dependent upon *Hox* gene function (Dasen et al., 2003; Figure 1C), suggesting that the subdivision of motor neurons into motor pools might also rely on Hox function. However, testing the hypothesis that *Hox* genes regulate motor pool identity had proven to be a difficult task for several reasons. The extensive use of *Hox* genes for establishing the rostrocaudal pattern of many different tissues has made it difficult to identify cell-autonomous functions using conventional knockout mutants. Furthermore, the complexity of the motor system, difficult anatomy, and the large number of potential *Hox* genes involved had made this a daunting problem to undertake.

Dasen et al. (2005) focused their efforts on the brachial level of the chick spinal cord where wing-innervating motor neurons reside and showed that the scapulohumeralis anterior (Sca) motor pool expresses Hox5, whereas the flexor carpi ulnaris (FCU) and pectoralis

(Pec) pools express Hoxc8 in a reciprocal pattern (Figure 1B). Using in ovo electroporation to target cells within the neural tube, gain- and loss-of-function experiments were performed without disturbing the overall patterning of the embryo. When Hoxc8 expression was blocked using RNAi, Hox5 expression expanded caudally and Runx1$^+$ Sca motor neurons were ectopically formed. Conversely, the misexpression of Hoxc8 repressed Hox5 and promoted the formation of Pea3$^+$ Pec motor neurons at the expense of Sca cells. Furthermore, axon-tracing experiments showed that the ectopically formed motor pools generated by these manipulations innervate the appropriate target muscles.

How do *Hox* genes regulate pool position and identity? The mechanistic underpinnings for Hox function appear to be based on their ability to operate as both transcription activators and repressors in a context-dependent manner. For example, the fusion of Hoxc8 to the engrailed repressor domain prevents it from functioning as an activator. This form of the protein retains its normal ability to repress *Hox5* genes but inhibits the expression of Scip and Pea3 unlike the native form of Hoxc8 (Dasen et al., 2003, 2005). Thus, the repressor function of Hoxc8 excludes Hox5 from the caudal neural tube, thereby contributing to the proper assignment of pool position, whereas the activator function appears to play a more direct role in triggering the expression of pool-specific genes such as Pea3, Scip, and Runx1. These regulatory interactions were also extended to examine how overlapping motor pools such as the Pec and FCU are defined within a single spinal cord segment (Figure 1B). Factors such as Hox4, Hox6, Hox7, and Meis1 were found to define motor pools within a single segment. For example, the intrasegmental pattern of Hoxc6 distinguishes Pec from FCU cells (Figure 1B). Thus, the results from Dasen et al. (2005) suggest that the detailed patterning that takes place to establish motor pool organization not only relies on crossrepressive interactions between specific Hox proteins but also makes use of combinatorial transcription factor codes to create cellular diversity.

From the work of Dasen et al. (2005) and others, a clearer picture is now emerging as to how multiple extrinsic signals, such as sonic hedgehog (Shh) and FGFs, are translated into cell intrinsic transcription factor programs that use crossregulatory interactions to accurately specify motor pool organization. Along the dorsoventral axis of the neural tube, distinct cell types are specified by graded Shh signaling (Jessell, 2000; Figure 1C). At spinal cord levels, cells exposed to the appropriate concentration of Shh activate transcription factor programs such as Olig2, Mnr2, Lhx3, and Isl1 that specify a generic ventral-exiting spinal motor neuron identity (vMN). Likewise, a high-caudal to low-rostral gradient of FGF dictates the rostrocaudal pattern of *Hox* gene expression (Dasen et al., 2003, 2005; Liu et al., 2001). Additional extrinsic signals also appear to contribute to motor neuron diversification. Early born motor neurons that form the medial portion of the lateral motor column synthesize RA, which triggers the expression of Lim1 required for the proper development of late-born motor neurons in the lateral portion of the lateral motor column (Kania et al., 2000; Sockanathan et al., 2003). Furthermore, peripheral signals, such as GDNF, have been found to influence the pattern of Ets transcription factor expression in motor neuron subtypes and drive a program for axon branching (Haase et al., 2002).

What next? The extrinsic signals controlling various aspects of motor neuron subtype identity likely operate at different developmental stages. Thus, a remaining question is to understand how cells respond to information from temporally discontinuous signals. In addition, the combinatorial nature of motor pool specification by transcription factors in the Hox and LIM-homeodomain families, for example, raises the question of how these genes operate in a context-dependent manner. Do these proteins directly interact, synergize, or compete for shared targets? Furthermore, what regulates the activator versus repressor functions of the *Hox* genes?

Each motor pool has a stereotypical size. The authors speculate that the number of motor neurons within a pool could be based on the relative strength of the crossrepressive interactions between *Hox* genes. This attractive idea may explain how cell intrinsic factors could influence the size of neuronal subpopulations and may have broad implications for establishing neuronal diversity. Finally, *Hox* gene manipulations may drive wholesale transformations of rostrocaudal identity within the spinal cord that are analogous to a homeotic transformation (Kmita and Duboule, 2003). Interestingly, many of the *Hox* genes are expressed by both motor neurons and spinal interneurons. Because the interneuronal circuitry at the brachial level that controls the left and right wing musculature—coordinated to move in synchrony—differs from lumbar locomotor circuits that drive the alternating leg movements used for stepping and running, it is possible that the *Hox* genes also control spinal interneuron identity and drive the assembly of distinct intraspinal locomotor circuits found at brachial and lumbar spinal levels (Goulding and Pfaff, 2005).

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