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Transcription factor mechanisms guiding motor neuron differentiation and diversification

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The embryonic generation of motor neurons is a complex process involving progenitor patterning, fate specification, differentiation, and maturation. Throughout this progression, the differential expression of transcription factors has served as our road map for the eventual cell fate of nascent motor neurons. Recent findings from *in vivo* and *in vitro* models of motor neuron development have expanded our understanding of how transcription factors govern motor neuron identity and their individual regulatory mechanisms. With the advent of next generation sequencing approaches, researchers now have unprecedented access to the gene regulatory dynamics involved in motor neuron development and are uncovering new connections linking neurodevelopment and neurodegenerative disease.

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Introduction

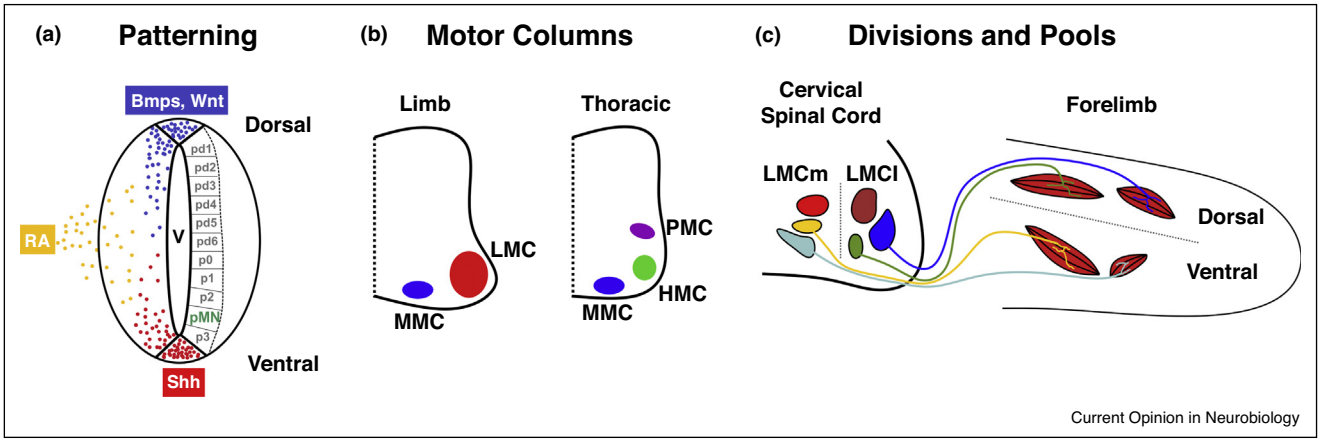
Motor neurons (MNs) are a crucial neuronal subtype responsible for innervating musculature in the periphery and controlling both autonomic and volitional movement. During embryogenesis, combinatorial expression of transcription factors (TFs) guides MN differentiation and diversification [1]. In this review, we survey recent research elucidating the evolutionary origin and broad conservation of these TF programs as well as the DNA-binding mechanics of individual TFs and TF-complexes. We also highlight novel applications of next-generation sequencing technology that have provided valuable genomic and transcriptomic signatures to *in vivo* and *in vitro* derived MNs.

Motor neuron generation

MN generation begins in the embryonic neuroepithelium, wherein opposing gradients of diffusible morphogens (Shh, BMPs, Wnt, RA) pattern proliferating progenitors into discrete domains along the dorsal-ventral body axis. In the ventral spinal cord, MNs are generated from the Olig2+ pMN domain (Figure 1a). Nascent MNs migrate away from the midline and assume positions in distinct motor columns along the rostral-caudal axis that are in register with their target tissues. Motor neurons located within the Medial Motor column (MMC) are found throughout the spinal cord and project to axial muscles. In contrast, Lateral Motor column (LMC) neurons are present at limb levels and innervate target muscles in the forelimb and hindlimb, whereas at thoracic regions, Hypaxial Motor column neurons (HMC) and Preganglionic Motor column neurons (PGC) project to body wall muscles and the sympathetic chain ganglia respectively (Figure 1b). Columnar identity is largely defined by Hox proteins, a class of TFs whose clustered 5′–3′ chromosomal order maps to their topological expression in the rostral caudal axis [2]. Within a motor column, MNs are further segregated into divisions which delineate broad axonal trajectories. For example, the LMC is divided into medial (LMCm) and lateral (LMCl) divisions that target ventral and dorsal muscles in the limb. Located within each division are motor pools that project to discrete muscles within each area (Figure 1c). Divisional identities are defined in part by the expression of FoxP1 and LIM-homeodomain TFs, whereas motor pools can be distinguished by expression of ETS as well as Hox TFs [3]. Importantly, once generated, MNs themselves are also important players in sculpting the final complement of MNs. MNs within the LMCm are the source of local retinoid signaling *via* Raldh2 expression that stimulate LMCl generation [4]. Further, GDE2, a retinoid induced GPI-anchor cleaving enzyme expressed in LMC neurons, non-cell autonomously promotes the generation of specific late-born LMC motor pools [5].

The early stages of MN development can be effectively modeled *in vitro* using either undifferentiated Embryonic Stem Cells (ESCs) or induced Pluripotent Stem Cells (iPSCs). iPSCs are differentiated cell types that have been reverted to an unspecified progenitor state. iPSCs afford researchers a large array of starting cell types and enable research on human cells without the ethical restraints of collecting fetal tissue [6–8]. These progenitor cells can be subsequently differentiated by applying

Figure 1



Motor neuron organization in the CNS. **(a)** Embryonic progenitors in the ventricular zone are patterned into discrete dorsal-ventral domains by opposing morphogen gradients. Motor neurons are generated from the ventral pMN domain. V = ventricle, Shh = Sonic Hedgehog, RA = retinoic acid. **(b)** Post-mitotic MNs are organized into motor columns that project to muscles in the limbs (LMC), trunk (MMC), intercostal muscles (HMC), or sympathetic ganglia (PMC). **(c)** Medial and lateral divisions of the LMC project to ventral and dorsal limb muscles, respectively. Within these divisions, motor pools innervate specific muscle groups.

exogenous factors that promote neuronal differentiation, including RA, Shh agonists, and Notch antagonists (Induced Differentiation) or by forcing expression of MN TFs such as Lhx1, Isl1, and Ngn2 (Direct Neuronal Programming) (Figure 2a). These reductionist *in vitro* platforms allow for the analysis of MNs in an isolated, controlled condition; and they allow access for transcriptomic analysis at the single cell level.

Motor neuron differentiation: insights from *in vitro* platforms

The basic helix-loop-helix (bHLH) TF Olig2 is crucial for specifying MN progenitors in the pMN domain but whether Olig2 promotes neurogenesis or maintains progenitor character is indeterminate. Olig2 induces Ngn2, another bHLH TF required for neuronal differentiation but Olig2 has also been shown to repress terminal MN homeodomain TFs [9,10]. To gain insight into this question, Sagner and colleagues utilized single-cell transcriptomics to map the gene regulatory networks used by Olig2 during ESC-derived MN generation. Distinct transcriptomic profiles can effectively separate early progenitors, MN progenitors, early MNs, and late MNs. Interestingly, the transition into an early post-mitotic MN is accompanied by an increase in Olig2 expression. Chromatin immunoprecipitation sequencing (ChIPseq) revealed that Olig2 acts as a transcriptional repressor for *Hes1*/*Hes5*, which are canonical Notch target genes that maintain progenitor character by inhibiting proneuronal TFs [11]. These observations lead to a biphasic model for Olig2 function. Initially, progenitors have low levels of Olig2 expression that permits partial *Hes* expression. Upon differentiation, Olig2 expression is significantly heightened, repressing *Hes1*/*Hes5* and disinhibiting the expression of proneuronal TFs [12••]. Underscoring these changes, unbiased statistical analysis of single cell RNAseq data during the differentiation of murine ESCs into MNs also reveals distinct transcriptional states as cells transition through four phases: pluripotency, neural precursors, MN specified progenitors, and MNs [13]. MN differentiation *in vivo* is an asynchronous process, and bulk profiling yields a mélange of cell types in different stages. The single cell resolution of these studies provides a much clearer window into the transcriptional states occupied by differentiating MNs.

Once the correct TFs for terminal MN identity are induced, what are the mechanisms that ensure their expression? Newly born MNs express the LIM-homeodomain proteins Isl1 and Lhx3. They function within a TF complex with nuclear LIM interactor (NLI) to specify MN identity, and these interactions are dependent on key residues within the Lhx3 LIM domain [14,15]. Recent investigations have revealed that the Isl1-Lhx3 complex stabilizes its expression in an autoregulatory manner *via* binding to enhancers adjacent to the *Isl1* and *Lhx3* loci. Further, the Isl1-Lhx3 complex upregulates the expression of LIM only Protein 4 (LMO4). LMO4 works in parallel to block the assembly of an Lhx3-only TF complex, which would misdirect the cell towards an interneuron fate [16]. Importantly, sustained Isl1-Lhx3 expression is not a universal feature of all MNs. For example, HMC and LMC MNs lose expression of Lhx3 as they mature, raising the question how expression of terminal MN identity genes regulated by Isl1-Lhx3 is maintained. Rhee *et al.* performed ChIP-seq from acetylated histone H3 lysine 27 and ATACseq to map genomic regions with an open, accessible chromatin configuration