Transcription factors and effectors that regulate neuronal morphology

Celine Santiago and Greg J. Bashaw*

ABSTRACT
Transcription factors establish the tremendous diversity of cell types in the nervous system by regulating the expression of genes that give a cell its morphological and functional properties. Although many studies have identified requirements for specific transcription factors during the different steps of neural circuit assembly, few have identified the downstream effectors by which they control neuronal morphology. In this Review, we highlight recent work that has elucidated the functional relationships between transcription factors and the downstream effectors through which they regulate neural connectivity in multiple model systems, with a focus on axon guidance and dendrite morphogenesis.

KEY WORDS: Transcription factors, Axon guidance, Motor neurons, Midline crossing, Lateral position, Dendritic morphology

Introduction
For a nervous system to function, the cells that compose it must each find their appropriate synaptic partners. The position of a neuron and the shape of its axonal and dendritic extensions are therefore fundamental aspects of its identity. Genetic analyses have confirmed that the initial pattern of neural connections in the embryo is intrinsically specified, and a wealth of studies has identified requirements for specific transcription factors in regulating cell migration, axon guidance, dendritic branching and synaptic partner selection (Chédotal and Rijli, 2009; Dalla Torre di Sanguinetto et al., 2008; Jan and Jan, 2010; Polleux et al., 2007). In parallel, the identification of many guidance receptors and their downstream signaling partners over the past two decades has allowed for a molecular understanding of how neuronal connections are formed (Huberman et al., 2010; Kolodkin and Tessier-Lavigne, 2011; O’Donnell et al., 2009). However, one central challenge that remains is to characterize the relationships between DNA-binding factors and the cell-surface proteins and cytoskeletal modifiers that mediate their effects.

Correlative data identifying targets of transcription factors have accumulated in multiple neurodevelopmental contexts. However, until recently, few studies validated the observed changes in gene expression with experiments to demonstrate the functional relevance of these relationships. Here, we highlight research that places transcription factors upstream of identified cellular effectors in the contexts of axon guidance in the motor system and during midline crossing, as well as during the acquisition of dendritic morphology in sensory neurons (summarized in Table 1). The transcriptional control of cortical neuron migration has recently been reviewed and will therefore not be discussed here (Kwan et al., 2012), although several recent studies have identified cell-surface receptors through which transcription factors regulate neuronal migration and serve as excellent examples of this type of work (Nóbrega-Pereira et al., 2008; van den Bergh et al., 2013; Vogt et al., 2014). In addition, we will not discuss activity-dependent changes in neuronal morphology, but refer readers to Ghiretti and Paradis (2014) and Fu and Zuo (2011) for recent reviews on this topic.

Transcription factors and effectors regulating motor axon guidance
Studies of the embryonic motor systems of invertebrates and vertebrates paved the way for understanding the transcriptional control of axon pathfinding. In mouse, chick, zebrafish, C. elegans and Drosophila, correlations between the transcription factors expressed in motor neurons and the target areas of their axons have been well documented (Appel et al., 1995; Thor and Thomas, 2002; Tsuchida et al., 1994). These correlations are functionally significant, as many of these transcription factors are required for the trajectory of motor axons and can redirect axons to abnormal territories when ectopically expressed. Below, we discuss recent work that has identified downstream effectors of motor neuron transcription factors in vertebrates and Drosophila.

LIM homeodomain transcription factors and their effectors in vertebrate motor axon guidance
In mouse and chick embryos, a transcriptional cascade regulates motor neuron development (reviewed by Dasen, 2009). Motor neuron progenitors, which express the basic helix loop helix (bHLH) transcription factor oligodendrocyte transcription factor 2 (Olig2) and the homeodomain transcription factor NK6 homeobox 1 (Nkx6.1), are generated in the ventral spinal cord in response to sonic hedgehog (Shh) secreted from the notochord and floor plate. The homeodomain transcription factors Hb9 (Mnx1), islet 1 (Isl1), Nkx6.1 and Lhx3 (LIM homeobox protein 3) are initially expressed in all post-mitotic motor neurons whose axons exit the spinal cord ventrally and are required for early events in their development, but their expression patterns subsequently become more restricted (see Box 1). Along the rostrocaudal axis, motor columns are specified by homeobox (Hox) transcription factors, which are themselves activated by gradients of retinoic acid (RA) and fibroblast growth factor (FGF). Limb-specific domains of Hox gene expression further differentiate limb motor neuron pools from each other, allowing them to acquire distinct cell body positions and innervate specific muscles. Finally, once motor axons reach their targets, retrograde signals induce the expression of ETS (E26 transformation specific) transcription factors, which control the final stages of axonal and dendritic arborization and partner matching.

Two examples of transcription factor effectors that act in spinal motor neurons, the Eph receptor tyrosine kinases EphA4 and EphB1, were identified in elegant studies of mouse and chick embryonic lateral motor column (LMC) neurons (Fig. 1). LMC axons fasciculate together as they exit the spinal cord and separate...
Table 1. Relationships between transcription factors and their downstream effectors that regulate axonal guidance and dendritic morphology

<table>
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<tr>
<th>Transcription factor</th>
<th>Effector(s)</th>
<th>Effector function</th>
<th>Developmental process</th>
<th>Rescue or suppression data</th>
<th>Direct binding to target</th>
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<td>Hattori et al., 2013</td>
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Continued
into a dorsal branch and a ventral branch at the base of the limb. Dorsal-ventral pathway selection is controlled by the LIM homeodomain transcription factors Lhx1 and Isl1. Lhx1 and Isl1 are expressed in a mutually exclusive pattern, with Lhx1 restricted to the dorsally projecting LMC-lateral (LMC-l) neurons, and Isl1 to the ventrally projecting LMC-medial (LMC-m) neurons (Kania et al., 2000). Although they can repress each other when overexpressed, there is no indication that Lhx1 and Isl1 establish the expression domains of one another (Kania and Jessell, 2003; Kania et al., 2006). Recent studies indicate that Eph receptors, which are conserved regulators of axon guidance in multiple systems (reviewed by Klein, 2012) and have been shown through in vitro experiments to mediate repulsion in motor axons in response to ephrin ligands (Kao and Kania, 2011) and have downstream effectors of transcription factors in other subsets of motor axons (dissected 1) (Palmesino et al., 2010). As it is not known whether Lhx1 and Isl1 control the expression of these receptors or the neuronal expression of ephrins, which act in motor neurons to control guidance through reverse signaling and cis-inhibition (Bonanomi et al., 2012; Dudanova et al., 2012; Kao and Kania, 2011). Moreover, Lhx1 and Isl1 are required for the mediolateral positioning of LMC cell bodies; although EphA4 regulates the rostrocaudal position of a subset of LMC neurons, Eph receptors do not appear to contribute significantly to mediolateral settling position, suggesting that LIM transcription factors regulate multiple aspects of neuronal morphology through distinct downstream programs (Coonan et al., 2012; Dudanova et al., 2012; Kao and Kania, 2011). Indeed, a recent study found a requirement for Lhx1 in specifying the mediolateral position of LMC-l cell bodies through upregulation of the reelin signaling protein Dab1 (disabled 1) (Palmesino et al., 2010). As it is not known whether Lhx1 and Isl1 directly bind to their target genes, elucidating the mechanism through which these transcription factors regulate their effectors will be a major challenge for future work. Finally, it will also be of high interest to understand how the individual neurons that make up the major motor nerves are differentiated from each other.

### Transcriptional regulation of motor axon guidance in spinal accessory motor neurons

The downstream effectors of transcription factors in other subsets of vertebrate motor neurons are beginning to be identified. Spinal accessory motor neurons (SACMNs) are dorsally exiting neurons found at cervical levels of the spinal cord that innervate neck and back muscles (Dillon et al., 2005). SACMNs are derived from an Nkx2.9+ progenitor domain and retain Nkx2.9 expression postmitotically. In the absence of Nkx2.9, SACMN axons fail to exit the spinal cord (Dillon et al., 2005; Pabst et al., 2003). A recent study

<table>
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<th>Transcription factor</th>
<th>Effector(s)</th>
<th>Effector function</th>
<th>Developmental process</th>
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<th>Direct binding to target</th>
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<td>Spire †</td>
<td>Actin regulator</td>
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<td>HPO-30 †</td>
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<td>Doonan et al., 2008</td>
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Box 1. Vertebrate motor neuron transcription factors act during multiple stages of development

In mice and chick embryos, the homeodomain transcription factors Isl1, Nkx6.1 and Lhx3 are expressed in all post-mitotic motor neurons whose axons exit the spinal cord ventrally and are required for early events in motor neuron development. Subsequently, their expression patterns become more restricted and they play subset-specific roles in regulating axon guidance and target selection. For example, Isl1 is required for the survival and differentiation of spinal motor neurons but then acts at a later stage to control guidance in a subset of limb-innervating lateral motor column (LMC) neurons (Luria et al., 2008; Pfaff et al., 1996). Similarly, Nkx6.1 is required for the differentiation of all spinal motor neurons but is later expressed in a restricted subset of LMC neurons, where it controls muscle target selection independently of its earlier function (De Marco Garcia and Jessell, 2008; Sander et al., 2000; Vallstedt et al., 2001).

Finally, Lhx3 and Lhx4 regulate spinal cord exit in ventrally exiting spinal motor neurons via unknown effectors and then become restricted to medial motor column (MMC-m) neurons, where they regulate guidance to axial muscles, likely through upregulation of the FGFR1 receptor (Sharma et al., 2000; Shirasaki et al., 2006). Interestingly, the Drosophila orthologs of these factors are not required for early events in motor neuron development but do regulate axon guidance in a subset-specific manner (Brothier et al., 2004; Thor and Thomas, 1997; Thor et al., 1999), suggesting a conserved and ancient function for these genes.

Transcription factors and effectors in Drosophila dorsally projecting motor axons

In Drosophila embryos, motor neurons that innervate the body wall muscles required for larval crawling arise from multiple neuromuscular lineages that express distinct combinations of transcription factors (Landgraf et al., 1997). Unlike in vertebrates, there are no known early acting factors that specify a generic motor neuron identity. The zinc-finger homeodomain transcription factor zfh1 is expressed in all motor neurons and regulates axon guidance, but is not required for their survival or differentiation (Layden et al., 2006). There are 36 motor neurons in each hemisegment, forming six major nerves that target different muscle regions. Motor neurons that innervate the dorsalmost muscles of the body wall fasciculate along the intersegmental nerve (ISN) and express the homeodomain transcription factor Even-skipped (Eve) and the GATA family transcription factor Grain (Fig. 2).

Motor neurons that co-express the transcription factors Hb9 (Exex), Nkx6, Islet (Tup), Lim3, Oli (Olig family) and Drifter form the ISNb nerve, which innervates a group of ventral muscles (Fig. 2).

Each of these proteins is required for motor axon guidance in a subset-specific manner (reviewed by Landgraf and Thor, 2006).

found that Nkx2.9 likely regulates spinal cord exit through the Slit receptor roundabout (Robo) 2 (Bravo-Ambrosio et al., 2012). Robo receptors have been well studied in the context of midline crossing, where Robo1 and Robo2 signal repulsion in response to floorplate-derived Slit (Dickson and Zou, 2010; Long et al., 2004). More recently, Robo1 and Robo2 were shown to regulate motor axon pathfinding in spinal motor neurons (Jaworski and Tessier-Lavigne, 2012). Robo2 mutants and Slit1, Slit2 double mutants display SACMN exit defects that resemble those of Nkx2.9 mutants, and Robo2 levels are decreased in the absence of Nkx2.9 (Bravo-Ambrosio et al., 2012). In addition, Slit is enriched at the site of SACMN exit, and Slit treatment causes the outgrowth of SACMN axons in vitro, suggesting that Robo2-Slit interactions may facilitate exit by promoting growth through the Slit-expressing zone. This model would be further confirmed by determining whether the Nkx2.9 mutant phenotype is rescued upon Robo2 overexpression, and how Slit-Robo2 signaling promotes outgrowth in these axons.

Eve and Grain are restricted to motor neurons that innervate dorsal muscles and are required for their correct trajectory (Fujioka et al., 2003; Garces and Thor, 2006; Landgraf et al., 1999). Two recent studies found that Eve and Grain act in part through the Netrin receptor Unc-5 (Labrador et al., 2005; Zarin et al., 2012). unc-5 expression is reduced in the dorsally projecting ISN pioneer neurons RP2 and aCC in the absence of eve or grain, and loss of unc-5 results in stalling of the ISN nerve, similar to the defects observed in eve or grain mutants. Moreover, Unc-5 overexpression partially rescues the CNS exit defects in eve mosaic mutants, as well as ISN stalling in grain mutants, providing strong evidence that Unc5 acts downstream of both Eve and Grain.

A recent genome-wide study of mRNA isolated from FAC-sorted dorsally projecting motor neurons (d-MNs) identified additional downstream effectors of Eve (Zarin et al., 2014). Candidate targets include four cell-surface receptors of the immunoglobulin superfamily (IgSF): unc-5, beat1a (beaten path 1a), fasciclin 2 (fas2) and neuroglan (nrg), all of which are positively regulated by Eve. The authors of this study present a model in which Eve specifies the trajectory of d-MNs through the combinatorial regulation of guidance receptors and adhesion molecules. Although unc-5, beat1a, nrg or fas2 single mutants only weakly phenocopy eve mutants, simultaneous removal of these genes produces an additive phenotype that more closely resembles the loss of eve. Moreover, restoring the expression of the four targets in an eve mutant significantly rescues the CNS exit and dorsal targeting defects, once again in an additive manner. Finally, ectopic expression of eve in a subset of interneurons induces the expression
ectopic expression of Zfh1 can induce the strongest phenotype produced by triple mutants does not recapitulate the effect of loss of either single mutant (Garces and Thor, 2006; Zarin et al., 2012). Nevertheless, by demonstrating a functional connection between upstream regulatory factors and target genes, this study provides insight into how transcriptional regulators exert their activities through a battery of effectors (Zarin et al., 2014).

Transcription factors and effectors in Drosophila ventrally projecting motor neurons

Analysis of another subset of Drosophila motor neurons reveals many of the same principles in action. The RP motor neurons 1, 3, 4 and 5 form the ISNb nerve and innervate four ventral muscles (Fig. 2). They co-express the homeodomain transcription factors Hb9, Nkx6, Lim3 and Islet. Unlike their vertebrate orthologs, these factors are specifically required for terminal aspects of motor neuron differentiation, including axon guidance (Brohier and Skeath, 2002; Brohier et al., 2004; Thor and Thomas, 1997; Thor et al., 1999). For example, Hb9 and Nkx6 function in parallel to promote the expression of Robo2 (Santiago et al., 2014). robo2 mutants lack innervation at muscles 6 and 7, which are normally innervated by the RP3 motor neuron. This resembles the phenotype of hhb mutants, and hhb is required for robo2 expression in RP3 neurons. Restoring Robo2 activity in hhb mutants partially rescues muscle 6/7 innervation. Moreover, Hb9 acts in parallel with Nkx6 to regulate motor axon guidance and robo2 expression, as both aspects of the hhb mutant phenotype are enhanced by removing one copy of nkd. There are likely other important targets downstream of these factors, because robo2 mutants do not have as strong a phenotype as nkd mutants or as hhb mutants that lack one copy of nkd. One promising candidate is the cell-adhesion molecule Fasciclin 3 (Fas3). Fas3 protein levels are decreased in nkd mutants, although additional experiments are necessary to determine the functional significance of this change (Brohier et al., 2004). fas3 mutants do not have motor axon guidance defects (Kose et al., 1997), but perhaps the combined loss of robo2 and fas3 will be similar to the effect of losing nkd. Interestingly, Hb9 regulates the lateral position of axons within the CNS through robo2 and the closely related gene robo3 (Santiago et al., 2014), suggesting that the regulatory relationships identified in motor neurons may be reused in multiple contexts.

One major challenge will be to identify the cis-acting elements to which these transcription factors bind, to allow for a mechanistic understanding of how combinations of transcription factors impinge on common targets. For example, if multiple factors bind to the same site, this might suggest that they form higher-order complexes that affect their target specificities, as was recently shown for Isl1/Lhx3 and Isl1/Phox2a (paired-like homeobox 2a) in cultured cells (Mazzoni et al., 2013; Thaler et al., 2002). In Drosophila d-MNs, Grain might activate unc5 directly, as the unc5 promoter contains consensus GATA sequences, but the relevance of these motifs to the expression pattern of unc5 has not been tested (Zarin et al., 2012). By contrast, Eve and Hb9 likely act as repressors, because their conserved repressor domains are required for rescue of motor axon guidance (Fujioaka et al., 2003; Santiago et al., 2014). A recent microarray performed by Skeath and colleagues identified many transcription factors that are downregulated upon Hb9 and Nkx6 overexpression (Lacin et al., 2014). It will be of great interest to identify the intermediate factors by which Hb9, Nkx6 and Eve promote the expression of their effectors, and to determine whether these intermediate factors bind directly to axon guidance genes. Eve may regulate guidance through Hb9, as hhb is de-repressed in eve mosaic mutants, and rescue experiments suggest a correlation between the extent of motor axon guidance rescue and the extent of hhb de-repression (Fujioaka et al., 2003). Moreover, grain was
identified as a downregulated target of Hb9 and Nkx6 in microarray analyses, and a recent DNA adenine methyltransferase identification (DAM-ID) analysis of the binding sites for Hb9 revealed that it is enriched near the unc5 and fas2 loci (Lacin et al., 2014; Wolfram et al., 2014). Thus, one can propose a model in which Eve represses hb9 in RP2 and aCC to allow for the expression of d-MN genes. In the absence of eve, hb9 is de-repressed in these cells, which might in turn lead to repression of grain, unc5 and fas2, but further experiments will be necessary to confirm this.

Transcription factors and effectors that regulate midline crossing

In bilateral animals, commissural axons cross the midline to innervate targets on the opposite side of the body, allowing for the left-right coordination of sensory input and behavior (reviewed by Dickson and Zou, 2010). In the vertebrate spinal cord, the secreted ligands netrin and Shh promote the extension of axons toward the floor plate by signaling through the Dcc (deleted in colorectal carcinoma) and Boc [bi-regional cell-adhesion molecule-related/downregulated on oncogenes (Cdon) binding protein] receptors, respectively. Midline-derived slit, semaphorin and ephrin proteins engage their respective receptors to ensure that commissural axons do not stall or recross the midline. These repulsive cues are also detected by ipsilateral axons, which never cross the midline. The complement of guidance receptors expressed by growth cones as they approach the midline thus determines whether they will acquire a commissural or ipsilateral trajectory. In particular, recent studies in the spinal cord and in retinal ganglion cells (RGCs) of mice embryos have revealed the importance of the transcriptional regulation of Robo and Eph receptors in this process (Fig. 3).

Transcriptional control of midline crossing through the regulation of Robo3 expression

Robo1 and Robo2 prevent the inappropriate crossing of axons by signaling repulsion in response to Slit secreted from the floor plate (Long et al., 2004). Robo1 and Robo2 mRNA are detected in both commissural and ipsilateral neurons in the spinal cord, suggesting that their transcriptional regulation is not instructive in this system. The divergent Robo family member Robo3 (previously Rig-1) promotes midline crossing by antagonizing Robo1 and Robo2 by an unknown mechanism (Sabatier et al., 2004). In Robo3 mutants, commissural axons are prematurely responsive to Slit and fail to cross the midline. The Robo3 phenotype in the spinal cord is partially rescued by loss of Robo1 and Robo2, suggesting that Robo3 acts in part by inhibiting repulsive Robo signaling (Jaworski et al., 2010; Sabatier et al., 2004). Analyses of the expression pattern of Robo3 in the spinal cord reveal that it is restricted to commissural neurons, and that its mis-expression causes ipsilateral axons to cross the midline ectopically, demonstrating that one key feature of commissural identity involves turning on Robo3 (Chen et al., 2008; Escalante et al., 2013; Inamata and Shinisaki, 2014).

In dl1c interneurons, which are a subset of contralateral interneurons in the dorsal spinal cord, the LIM homeodomain transcription factors Lhx2 and Lhx9 are required for midline crossing and for Robo3 expression (Wilson et al., 2008) (Fig. 3A). The dl1 interneurons receive proprioceptive information from sensory neurons and relay it to the brain. After neurogenesis, they segregate into dl1c neurons, which settle at a medial position and are commissural, and into dl1i neurons, which are found more laterally and are ipsilateral. In Lhx2/Lhx9 double mutants, dl1c axons fail to cross the midline, and Robo3 mRNA and protein levels are reduced (Wilson et al., 2008). Other dl1 transcription factors are expressed at normal levels, as are Dcc and Robo1, and the initial ventral trajectory of dl1c axons is unaffected, indicating that Lhx2 and Lhx9 do not regulate all aspects of dl1c differentiation. The severity of the dl1c midline crossing phenotype in the Lhx2/Lhx9 double mutants resembles that of Robo3 mutants, suggesting that Robo3 is a downstream effector of Lhx2 and Lhx9. Moreover, Lhx2 binds in vitro to a Robo3 genomic fragment containing two LIM homeodomain-binding sites, and chromatin immunoprecipitation (ChiP) experiments found that Lhx2 binds to the Robo3 promoter in spinal cord extracts (Marcos-Mondejar et al., 2012). Together, these data strongly argue that Lhx2 and Lhx9 promote midline crossing by activating the expression of Robo3 in dl1c neurons. Of note, midline crossing and Robo3 expression are not affected in other classes of commissural neurons, implying that multiple programs activate Robo3 in a subset-specific manner. Furthermore, although both dl1c and dl1i neurons initially express Lhx2 and Lhx9, Lhx2 is subsequently downregulated in dl1i neurons. In the absence of the Bar-class homeobox gene Barhl2, dl1i neurons ectopically express Lhx2 and Robo3, and aberrantly cross the midline, suggesting that

**Fig. 3.** Downstream effectors of transcription factors that function during midline crossing in the mouse spinal cord and visual system. (A) Cross-section of a mouse spinal cord at E16. In dlLB interneurons (blue), Zic2 promotes the selection of an ipsilateral trajectory by upregulating EphA4 expression. In dl1-c interneurons (green), Lhx2 and Lhx9 are required for Robo3 expression and midline crossing. In dl1-i interneurons (orange), Barhl2 is required for the repression of Lhx2 and Robo3, and for the maintenance of an ipsilateral trajectory. Barhl2 is also expressed in dl1-c interneurons, where it is not sufficient to repress Lhx2. FP, floor plate; RP, roof plate. (B) Schematic of the mouse visual system at E15.5. In a subset of contralateral retinal ganglion cells (RGCs-c, red), Isl2 is required to repress Zic2 and EphB1 expression, and to promote midline crossing. In ipsilateral RGCs (RGCs-i, purple), Zic2 is required for EphB1 expression and an ipsilateral trajectory. Barhl2, BarH-like 2; EphB1, ephrin receptor B1; Lhx2, LIM homeobox protein 2; Robo, roundabout; Zic2, zinc-finger protein of the cerebellum 2.
downregulation of Lhx2 is crucial for maintaining an ipsilateral trajectory in these cells (Ding et al., 2012).

**Zic2 acting via Eph receptors regulates an ipsilateral trajectory**

Recent studies have demonstrated an instructive role for the zinc homeodomain transcription factor Zic2 (zinc-finger protein of the cerebellum 2) in promoting ipsilateral guidance via the regulation of Eph receptors. In the brain and spinal cord, EphA4 regulates midline crossing by signaling repulsion in response to midline-localized ephrins (Dottori et al., 1998; Kullander et al., 2001). EphA4 mutant mice have a hopping gait caused by ectopic midline crossing of a subset of ventral interneurons that contribute to the central pattern generator (Kullander et al., 2003). EphA4 is also required in a group of dorsal interneurons to prevent crossing at the dorsal midline (Escalante et al., 2013; Paixão et al., 2013). Zic2 is required for EphA4 expression and ipsilateral guidance in dILB neurons, which are distinct from d111 neurons and do not express Barhl2, Lhx2 or Lhx9 (Escalante et al., 2013) (Fig. 3A). ChIP experiments demonstrate that Zic2 binds to the EphA4 promoter in spinal cord extracts. In addition, Zic2 can induce EphA4 and repress Robo3 and Lhx2 when ectopically expressed, suggesting it may regulate midline crossing through multiple effectors, although an endogenous requirement for Zic2 in repressing Robo3 and Lhx2 was not demonstrated. Finally, EphA4 is expressed in many neurons in the brain and spinal cord that do not express Zic2, suggesting that distinct transcription factors act in a subtype-specific manner to activate EphA4, reminiscent of the manner in which Robo3 is regulated.

Zic2 also regulates midline guidance at the optic chiasm by promoting the expression of EphB1 in retinal ganglion cells (García-Frigola et al., 2008; Herrera et al., 2003; Lee et al., 2008). In mice, most retinal ganglion cell (RGC) axons project across the midline to innervate targets on the opposite side of the brain, and a small subset of ipsilateral projections allows for binocular vision (Herrera et al., 2003). EphB1 is exclusively expressed in ipsilateral RGCs and regulates their trajectory by signaling repulsion in response to midline-localized ephrin B2 (Williams et al., 2003). Zic2 is also restricted to ipsilateral RGCs, where it is required for EphB1 expression and for ipsilateral guidance (Fig. 3B). Zic2 overexpression causes an increase in Ephb1 mRNA and a decrease in midline crossing; this phenotype is partially suppressed in an Ephb1 mutant. Interestingly, in a subset of contralateral RGCs, Isl2 promotes midline crossing and is required to repress Zic2 and Ephb1 expression (Pak et al., 2004) (Fig. 3B). Although the direct binding of these transcription factors to their targets has yet to be demonstrated, a model emerges in which a transcriptional repressor specifies the trajectory of one class of retinal axons by restricting the expression of another transcription factor, which itself impinges on an axon guidance receptor, similar to what occurs with Barhl2 and Lhx2 in spinal interneurons (Ding et al., 2012), and with Eve and Hb9 in Drosophila motor neurons (Fujioka et al., 2003).

**Transcriptional control of lateral position through the regulation of Robo receptors**

Both ipsilateral and contralateral axons must correctly position themselves along the mediolateral axis as they form ascending and descending longitudinal projections in the brain and spinal cord. Robo receptors are key regulators of this process (reviewed by Dickson and Zou, 2010; Sakai and Kaprielian, 2012), and recent studies of zebrafish and Drosophila embryos demonstrate the importance of the transcriptional regulation of Robo receptors during the selection of a mediolateral trajectory (Fig. 4).

In a subset of zebrafish hypothalamic neurons that project to the hindbrain and spinal cord, the bHLH-PAS transcription factors Sim1a and Arnt2 regulate lateral position by downregulating Robo3 (Schweitzer et al., 2013). In the absence of either transcription factor, Robo3 is misexpressed and axons shift medially, resembling...
the effect of Robo3 gain of function (Fig. 4A). Loss of Robo2 also results in a medial shift phenotype, and this phenotype is not enhanced by Robo3 overexpression, suggesting that Robo3 acts by inhibiting the activity of Robo2. Furthermore, the Sim1a and Arnt2 phenotypes are partially suppressed in a Robo3 mutant, and knockdown of Sim1a or Arnt2 in a Robo2 mutant does not produce an additive effect, suggesting that Sim1a and Arnt2 act in the same pathway as Robo2 and Robo3 to regulate lateral position in these axons.

In Drosophila embryos, the distinct functions of Robo receptors in regulating lateral position are reflected by their expression patterns: Robo1 is broadly expressed throughout the nerve cord and does not play a role in lateral position; Robo2 is restricted to axons that project to the lateral-most regions and is required for the formation of these tracts; Robo3 is found in the outer two-thirds of the neuropil and regulates the position of axons in intermediate zones (Rajagopalan et al., 2000; Simpson et al., 2000). An elegant study using knock-in alleles demonstrated that the specific requirements for robo2 and robo3 in lateral position are largely due to their transcriptional expression patterns, but how these domains are established in the CNS remained unknown (Spitzweck et al., 2010). However, it was recently shown that Hb9 acts in a subset of neurons to regulate lateral position through robo2 and robo3 (Santiago et al., 2014). In the MP1 neurons, Hb9 is required for robo3 expression and the selection of an intermediate trajectory (Fig. 4B). The medial shift phenotype of MP1 axons in hh9 mutants can be rescued by restoring Robo3 cell-autonomously. Axons in the lateral-most zone of the nerve cord are also shifted inwards in hh9 mutants, and decreased robo2 transcript is observed in a cluster of neurons that may contribute to these pathways. Finally, the mis-expression of Hb9 in apertous (ap) neurons that normally project medially induces robo2 expression and causes a robo2-dependent lateral shift, further reinforcing the connection between Hb9, Robo receptors and lateral position (Fig. 4B).

It is interesting to note that in the zebrafish brain, as in the Drosophila embryo, distinct transcription factors act in a subset-specific manner to regulate lateral position, similar to how midline crossing is regulated in the spinal cord. Sim1a and Arnt2 regulate neuropeptide expression as well as Robo3 expression in zebrafish hypothalamic neurons, raising the intriguing hypothesis that the coordinate regulation of morphology with other terminal aspects of neuronal identity provides an explanation for the modular regulation of axon guidance genes.

Transcription factors and effectors regulating dendritic morphology in sensory neurons

A functional nervous system requires that dendrites grow into the correct target areas and acquire the appropriate morphologies in order to receive and process synaptic input. The staggering diversity in the shapes and sizes of dendritic arbors contributes to the complexity of the nervous system, and is regulated by both intrinsic and extrinsic factors (reviewed by Jan and Jan, 2010; Puram and Bonni, 2013). Below, we discuss recent studies of Drosophila and C. elegans sensory neurons that identify effectors of transcription factors that control dendritic morphology. The transcriptional regulation of dendritic morphology in mammals has recently been reviewed and will therefore not be discussed (de la Torre-Ubieta and Bonni, 2011; Puram and Bonni, 2013).

Transcriptional regulation of morphology in Drosophila dendritic arborization neurons

The dendritic arborization (da) sensory neurons of Drosophila larvae provide a powerful model for understanding the acquisition of dendritic morphology (Corty et al., 2009; Jan and Jan, 2010). These dendrites form a largely two-dimensional array between the body wall muscles and the epidermis. There are four classes of da neurons, which can be distinguished by their transcription factor profile, dendritic morphology and sensory function (see Box 2). The transcription factors Abrupt (Ab), Cut and Knot/Collier are major regulators of da neuron morphology, and several recent studies have identified putative downstream effectors of these factors (Fig. 5A).

In class IV da neurons, the microtubule severing protein Spastin may act downstream of Knot by creating new sites for microtubule growth (Jinushi-Nakao et al., 2007). spastin heterozygotes display class IV da neuron defects that resemble those of knot mutants, including reduced dendritic arbors and decreased branching. Furthermore, spastin is upregulated when Knot is overexpressed, and knocking down spastin suppresses the ectopic branching phenotype caused by Knot mis-expression. However, this model awaits evidence that spastin levels are downregulated in da neurons in the absence of Knot.

The actin-bundling protein Singed/Fascin is required for class III da neuron morphology, and a recent study suggests that its activity may be Cut dependent (Nagel et al., 2012). Fascin is present in the cell bodies of all da neurons, but is not found within the dendrites of class I, II or IV neurons, whereas it is enriched in the filopodial spikes of class III neurons, and is required for their formation. Cut

Box 2. Drosophila dendritic arborization neurons

Dendritic arborization (da) neurons are sensory neurons in Drosophila larvae that allow the animal to move appropriately and react to its environment. Their dendrites form an expansive network between the body wall muscles and the epidermis (Fig. 5). There are four major classes of da neurons, distinguished by their transcription factor profile, dendritic morphology and function.

Class I neurons

Class I neurons are proprioceptive, have the simplest dendritic arbors (Grueber et al., 2002; Hughes and Thomas, 2007) and can be identified by the expression of the BTB/zinc-finger transcription factor Abrupt (Ab), which is both required and sufficient to promote their simple morphology (Li et al., 2004; Sugimura et al., 2004).

Class II neurons

Class II neurons respond to gentle touch, form larger and more-complex arbors than class I neurons (Grueber et al., 2002; Tsubouchi et al., 2012), and express low levels of the homeodomain transcription factor Cut, which is required for their growth (Grueber et al., 2003a).

Class III neurons

Class III neurons respond to gentle touch and form more complex arbors than class I or II neurons (Grueber et al., 2002, 2003b; Tsubouchi et al., 2012; Yan et al., 2013). They can be identified by the presence of actin-rich filopodial spikes along their dendrites and by the highest expression levels of Cut, which is required for filopodia formation and for dendritic growth and branching (Grueber et al., 2003a).

Class IV neurons

Class IV neurons are polymodal nociceptive detectors that are activated by harsh mechanical stimuli and high temperatures (Hwang et al., 2007). They form extensive, space-filling dendritic arbors that display self-avoidance and that do not overlap with dendrites from neighboring class IV neurons (Grueber et al., 2002), and express intermediate levels of Cut, which is required for their normal growth and branching (Grueber et al., 2003a). Expression of the COE (Collier/Olf-1/EBF) transcription factor Knot in da neurons is restricted to the class IV neurons, where it is required for the formation of their complex dendritic arbors (Crozatier and Vincent, 2008; Hattori et al., 2007; Jinushi-Nakao et al., 2007). There is no evidence that Ab, Cut and Knot endogenously regulate the expression levels of one another, although Cut is sufficient to repress ab and promote knot expression when overexpressed (Jinushi-Nakao et al., 2007; Li et al., 2004).
overexpression produces ectopic Fascin-positive filopodia. To determine whether Fascin is a downstream effector of Cut, Nagel et al. overexpressed Cut in fascin mutants and observed reduced ectopic filopodia. As Fascin is expressed in all da neurons, it is unlikely that Cut regulates its expression in a class-specific manner; instead, high levels of Cut might promote Fascin activity indirectly, by inducing the expression of programs that control Fascin subcellular localization in class III neurons.

The guanine nucleotide exchange factor (GEF) Trio may also act downstream of Cut, as it is upregulated by Cut overexpression and its absence produces a similar phenotype to that seen in the absence of Cut (Iyer et al., 2012). Moreover, trio knockdown suppresses the ectopic branching phenotype generated by Cut misexpression, and Trio overexpression partially rescues the branching defects caused by loss of Cut. However, Cut is not endogenously required for Trio expression in da neurons, suggesting that additional factors act redundantly with Cut to regulate trio. Similarly, Cut can induce expression of the cell-surface receptor Turtle, and reducing Turtle levels suppresses the effect of Cut overexpression, but Cut is not required for turtle expression (Sulkowski et al., 2011). Thus, the main transcriptional targets of Cut that mediate its effects on dendritic morphology remain to be identified.

Uemura and colleagues recently undertook an unbiased approach to identify novel targets of Ab and Knot (Hattori et al., 2013). They performed genome-wide DAM-ID analyses for Ab- and Knot-binding sites, as well as gene expression analyses in larvae overexpressing Ab or Knot in da neurons. They cross-referenced these data to identify genes that were bound by either factor, and that responded to changes in Ab or Knot levels. Candidate targets were then validated by examination of their loss-of-function phenotypes.

One shared upregulated target of Ab and Knot that emerged from this analysis was the BTB/POZ transcription factor Lola, and a subsequent study by van Meyel and colleagues demonstrated that Lola controls dendritic morphogenesis through the actin nucleating protein Spire (Ferreira et al., 2014). Lola is expressed in all classes of da neurons, where it is required for dendritic branching and growth. In its absence, Cut and Knot levels are decreased in class IV neurons, possibly explaining how Lola regulates growth in these cells, and suggesting a positive-feedback loop between Lola and Ab and Knot. Candidate targets were then validated by examination of their loss-of-function phenotypes.

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Another target of Ab and Knot identified by Uemura and colleagues that plays a role in regulating dendritic morphology is the cell-surface receptor Teneurin-m (ten-m), which mediates synaptic partner selection in the adult olfactory system and the larval neuromuscular system (Hattori et al., 2013; Hong et al., 2012; Mosca et al., 2012). Both Ab and Knot can upregulate ten-m, and a recent study identified hpo-3/claudin in a downstream effector of MEC-3 in regulating PVD morphology (Smith et al., 2013). Miller and colleagues compared the mRNA profiles of PVD neurons from wild-type animals with those from mec-3 mutants and identified many putative MEC-3 targets, including hpo-30/claunid. hpo-30 is required cell autonomously for the formation of dendritic branches in PVD neurons; in its absence, secondary branches initiate but are not stabilized. The similarity of the loss-of-function phenotypes of hpo-30 and mec-3, as well as the observation that mec-3 is required for the expression of an hpo-30::GFP reporter in PVD neurons, make HPO-30 a likely downstream effector of MEC-3. However, HPO-30 overexpression in mec-3 mutants does not rescue their branching defects, suggesting that additional targets of MEC-3 are required for normal PVD morphology (Fig. 5B).

MEC-3 is also expressed in light touch neurons, which have very simple dendrites (Way and Chalfie, 1989). How does MEC-3 regulate dendritic morphology in a cell-type specific manner? Smith et al. demonstrate that in the AVM light touch neuron, the bHLH transcription factor AHR-1 downregulates MEC-3 targets that promote a PVD morphology, while simultaneously promoting expression of mec-3 itself (Smith et al., 2013). In ahr-1 mutants, the AVM neuron is transformed into a PVD-like neuron both morphologically and functionally; the morphological change is mec-3 dependent. Therefore, the authors hypothesize that AHR-1 is required for their dendrites, demonstrating an endogenous requirement for low levels of Ten-m in these cells. In addition to its expression in da neurons, Ten-m is present in the epidermis in a non-uniform manner, and epidermal-specific ten-m knockdown or overexpression can change the directionality of dendritic projections, suggesting that homophilic Ten-m interactions between neurons and epidermal cells influence dendritic patterning. Uemura and colleagues present a model in which Abrupt ensures that high levels of Ten-m are present in class I da neurons to signal repulsion and direct dendrites posteriorly, whereas Knot promotes low levels of Ten-m in class IV neurons to confer normal dendritic morphology (Hattori et al., 2013).

Additional genetic experiments, such as a rescue of the ab or knot phenotypes, would further strengthen the model, and identifying the factors that regulate epidermal Ten-m expression would shed light on how its expression is coordinately regulated across tissue types.
to repress MEC-3 targets that promote PVD morphology. Indeed, hpo-30/claudin is not expressed in light touch neurons in wild-type animals, but is ectopically expressed in the AVM neuron in ahr-1 mutants. Moreover, in ahr-1;hpo-30 double mutants, the ectopic dendritic branches in the AVM neuron are fully suppressed, further demonstrating a role for HPO-30 as a key regulator of dendritic morphology.

Another essential regulator of PVD morphology is DMA-1, which is a transmembrane receptor expressed in PVD neurons. In its absence, dendritic arbors are greatly reduced (Liu and Shen, 2012). Two recent studies demonstrated that DMA-1 forms a complex in trans with the MNR-1 and L1CAM/SAX-7 receptors expressed in the skin, and that this complex promotes dendritic growth (Dong et al., 2013; Salzberg et al., 2013). The dma-1 phenotype is strikingly similar to the mec-3 and hpo-30 phenotypes. Although dma-1 was not identified as a MEC-3-dependent gene by Smith et al. (2013), it will be interesting to determine whether HPO-30 converges on the same pathway as DMA-1, MNR-1 and SAX-7 to regulate interactions between sensory neurons and epidermal cells that promote dendritic growth and branching.

**Conclusions**

The large-scale datasets generated by recent genome-wide analyses of transcription factor targets provide us with a wealth of information that must now be understood in relation to specific cellular and developmental processes. Studies in invertebrate motor and sensory neurons demonstrate how functional regulatory relationships can be extracted from such data, and will likely be followed by analogous work in other systems. Indeed, in mice, genome-wide datasets for targets of Isl1 and Runx1 (runt-related transcription factor 1) have been generated from embryonic sensory neurons, and these promise to uncover new functional relationships (Sun et al., 2008; Yang et al.,...
In cortical progenitors, a recent analysis of forebrain expressed zinc factor 2 (Fezf2)-regulated genes revealed a role for EphB1 in regulating the trajectory of corticospinal axons (Lodato et al., 2014). It will be of great interest to obtain similar data for cells across the nervous system and to identify the targets that explain how transcription factors regulate neuronal morphology.

As we begin to build a detailed map of the regulatory relationships in diverse model systems (see Table 1), common organizational principles emerge. For example, studies of the transcriptional regulation of midline crossing and lateral position in the CNS revealed that multiple transcription factors control axon guidance in a subset-specific manner. This echoes the modular control of glutamatergic identity in C. elegans, where more than a dozen transcription factors act in a subset-specific manner to activate the expression of eat-4 (the C. elegans ortholog of VGLUT, the vesicular glutamate transporter), which confers glutamatergic identity (Serrano-Saiz et al., 2013). Importantly, these transcription factors also regulate other subset-specific aspects of neuronal identity, such as the expression of neurotransmitter receptors and ion channels. Similarly, the COE-type transcription factor unc-3 was recently identified as a key regulator of cholinergic identity in a group of C. elegans motor neurons, where it also regulates motor axon guidance (Kratsios et al., 2012; Prasad et al., 1998).

Mounting evidence suggests that the co-regulation of multiple aspects of neuronal identity by transcription factors may be a broadly used developmental strategy. The ETS transcription factor Etv4 (previously Pea3) regulates cell body position, axonal arborization and dendritic targeting in a subset of motor neurons, perhaps through regulation of cadherins and semaphorins (Livet et al., 2002; Vrieseling and Arber, 2006). In Drosophila, the POU-domain transcription factor Acj6 (abnormal chemosensory jump 6) regulates both axonal morphology and dendritic guidance in a class of projection neurons in the olfactory lobe, although its effectors in these processes remain unknown (Komiyama et al., 2003). Whether or not specific guidance receptors or cytoskeletal modifiers downstream of transcription factors coordinate regulation of collagen migration, axonal guidance and dendritic morphology remains an unresolved issue.

The co-regulation of genes related to morphology and neural function has also been reported. In the retina, Zic2 is required for the expression of the serotonin transporter SerT and for axon guidance, as discussed above (Garcia-Frigola and Herrera, 2010). Similarly, it was recently shown that Fezf2 regulates both glutamatergic identity and axon guidance in corticospinal neurons (Lodato et al., 2014). IsIl1, a key regulator of motor axon guidance, promotes cholinergic identity in motor neurons and in a subset of forebrain neurons (Cho et al., 2014). Interestingly, Drosophila Islet also coordinately regulates axon guidance with neural function (Thor and Thomas, 1997; Wolfram et al., 2012). Indeed, Baines and colleagues recently demonstrated that Islet specifies the electrical properties of ventrally projecting motor neurons by repressing the expression of the ion channel Shaker, and DAM-ID data for other Drosophila motor neuron transcription factors suggest they may also regulate ion channels and neurotransmitter receptors, in addition to axon guidance genes (Pym et al., 2006; Wolfram et al., 2012, 2014). In Drosophila sensory neurons, Knot is required for the expression of the class IV da neuron gene pickpocket, which encodes a subunit of a Degenerin/epithelial sodium channel family protein that is required for the response to nociceptive touch (Crozatier and Vincent, 2008; Hattori et al., 2007; Zhong and Hwang, 2010). It will be interesting to determine whether Cut is similarly required for the expression of the mechanoreceptor channel NOMPC in class III da neurons (Yan et al., 2013) and to identify the constellation of effectors through which transcription factors regulate different aspects of terminal differentiation across cell types.

Although this Review has focused on the role of transcription factors in specifying the final position and shape of a neuron during embryogenesis, it is important to note that the temporal dynamics of transcriptional regulation are essential for the completion of this process. For example, axons must adjust their responsiveness to extracellular cues as they migrate through the embryo, and transcriptional organizational principles provide a mechanism to achieve this control (Kelemen et al., 2002; Wilson and Stoeckli, 2013). In Drosophila embryos, the Frazzled/DCC receptor is required for induction of the commissureless gene, which encodes a key regulator of midline crossing, suggesting that axon guidance receptors themselves can play a role in regulating changes in gene expression during neural circuit formation (Yang et al., 2009). It remains unknown how Frazzled/DCC signaling impinges on the transcriptional machinery of the cell, and how this response is integrated with the regulatory factors already in place. Unraveling these mechanisms will help us understand how the intrinsic processes that specify fate are elaborated upon as neurons acquire their final morphologies. Finally, activity-dependent changes that occur after embryogenesis can remodel the structure and function of a neuron, and understanding how these mechanisms adjust or reactivates developmental programs also presents an important challenge.

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nintrin-receptor expression to control dorsal motor-axon projections in Drosophila.


