© 2014. Published by The Company of Biologists Ltd | Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

REVIEW

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 Berghe 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.

Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

*Author for correspondence (gbashaw@mail.med.upenn.edu)

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

DEVELOPMENT

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

Table 1. Relationships between transcription factors and their downstream effectors that regulate axonal guidance and dendritic morphology

Transcription factor	Effector(s)	Effector function	Developmental process	Rescue or suppression data	Direct binding to target	Reference(s)
Vertebrates						
Isl1	EphB1 ↑	Receptor tyrosine kinase	Motor axon guidance (LMC-m)	Yes	No data	Luria et al., 2008
Lhx1	EphA4 ↑	Receptor tyrosine kinase	Motor axon guidance (LMC-l)	No data	No data	Kania and Jessell, 2003
Lhx3 and Lhx4	FGFR1 ↑	Receptor tyrosine kinase	Motor axon guidance (MMC-m)	No data	No data	Shirasaki et al., 2006
Lhx2	Robo3 ↑	Cell-surface receptor	Midline crossing (dI1c)	No data	Yes	Wilson et al., 2008
Lhx2	Robo1 ↓	Cell-surface receptor	Axon guidance (thalamic neurons)	Yes	Yes	Marcos-Mondéjar et al., 2012
	Robo2 ↓	Cell-surface receptor		No data	Yes	Marcos-Mondéjar et al., 2012
Lhx9	Robo3 ↑	Cell-surface receptor	Midline crossing (dI1c)	No data	No data	Wilson et al., 2008
Nkx2.9	Robo2 ↑	Cell-surface receptor	Motor axon guidance (SACMN)	No data	No data	Bravo-Ambrosio et al., 2012
Zic2	EphA4 ↑	Receptor tyrosine kinase	Ipsilateral guidance (dILB)	No data	Yes	Escalante et al., 2013
Zic2	EphB1 ↑	Receptor tyrosine kinase	Ipsilateral guidance (RGCs)	Yes	No data	García-Frigola et al., 2008; Lee et al., 2008
Sim1a and Arnt2	Robo3 ↓	Cell-surface receptor	Lateral position (hypothalamic axons)	Yes	No data	Schweitzer et al., 2013
Fezf2	EphB1 ↑	Receptor tyrosine kinase	Corticospinal axon guidance	No data	Yes	Lodato et al., 2014
Satb2	EphA4 ↑	Receptor tyrosine kinase	Cortical axon guidance (callosal axons)	Yes	No data	Srinivasan et al., 2012
	Unc5c ↑	Cell-surface receptor	Cortical axon guidance	Yes	No data	Srivatsa et al., 2014
	Unc5h3 ↑	Cell-surface receptor	Cortical axon guidance	Yes	No data	Srinivasan et al., 2012
	Dcc ↓	Cell-surface receptor	Cortical axon guidance	Yes	Yes	Srivatsa et al., 2014
Ctip2	Unc5c ↓	Cell-surface receptor	Cortical axon guidance	No data	Yes	Srivatsa et al., 2014
Drosophila						
Eve	Unc-5 ↑	Cell-surface receptor	Motor axon guidance (d-MNs)	Yes	No data	Labrador et al., 2005; Zarin et al., 2014
	Beat1a ↑	Cell-surface receptor				
	Fas2 ↑	Cell-adhesion molecule				
	Nrg ↑	Cell-adhesion molecule				
Zfh1	Unc-5 ↑	Cell-surface receptor	Motor axon guidance (d-MNs)	No data	No data	Zarin et al., 2014
	Beat1a ↑	Cell-surface receptor				
	Fas2 ↑	Cell-adhesion molecule				
Grain	Unc-5 ↑	Cell-surface receptor	Motor axon guidance (d-MNs)	Yes	No data	Zarin et al., 2012; Zarin et al., 2014
Hb9	Robo2 ↑	Cell-surface receptor	Motor axon guidance (v-MNs)	Yes	No data	Santiago et al., 2014
Hb9	Robo3 ↑	Cell-surface receptor	Lateral position (MP1)	Yes	No data	Santiago et al., 2014
Nkx6	Robo2 ↑	Cell-surface receptor	Motor axon guidance (v-MNs)	No data	No data	Santiago et al., 2014
Drosophila						
Atonal	Robo3 ↑	Cell-surface receptor	Lateral position (chordotonal neurons)	No data	No data	Zlatic et al., 2003
Abrupt	Ten-m ↑	Cell-surface receptor	Dendritic morphology (class I da neurons)	No data	Yes	Hattori et al., 2013

Continued

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

Table 1. Continued

Transcription factor	Effector(s)	Effector function	Developmental process	Rescue or suppression data	Direct binding to target	Reference(s)
Drosophila						
Knot	Ten-m ↑	Cell-surface receptor	Dendritic morphology (class IV da neurons)	No data	Yes	Hattori et al., 2013
Lola	Spire ↓	Actin regulator	Dendritic morphology (class I and IV da neurons)	Yes	No data	Ferreira et al., 2014
C. elegans						
MEC-3	HPO-30 ↑	Claudin	Dendritic morphology (PVD neuron)	No data	No data	Smith et al., 2013
AHR-1	HPO-30 ↓	Claudin	Dendritic morphology (AVM neuron)	Yes	No data	Smith et al., 2013
HLH-3	UNC-40 ↑	Cell-surface receptor	Motor axon guidance (HSN)	No data	No data	Doonan et al., 2008

Upwards arrows indicate positive regulation of a target protein; downwards arrows indicate negative regulation.
AHR-1, aryl hydrocarbon receptor related 1; Arnt2, aryl-hydrocarbon receptor nuclear translocator 2; Beat1a, beaten path 1a; Ctip2, COUP-TF interacting protein 2; da, dendritic arborization; Dcc, deleted in colorectal carcinoma; d-MNS, dorsally-projecting motor neurons; Eph, ephrin receptor; Fas2, fasciclin 2; Fezf2, Fez family zinc finger 2; HLH-3, helix-loop-helix; HPO-30, hypersensitive to pore-forming toxin 30; Isl1, islet 1; Lhx, LIM homeobox protein; LMC-m, medial class of lateral motor column; LMC-l, lateral class of lateral motor column; MEC-3, mechanosensory abnormality 3; MMC-m, medial class of medial motor column; Nrg, neuroglian; RGC, retinal ganglion cell; Robo, roundabout; SACMN, spinal accessory motor nerve; Satb2, special AT-rich sequence binding protein 2; Sim1a, single-minded homolog 1; Ten-m, teneurin-m; v-MNS, ventrally-projecting motor neurons; Zfh1, Zn-finger homeodomain 1.

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., 2000; Luria et al., 2008). 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), act downstream of Lhx1 and Isl1. In LMC-l neurons, EphA4 is expressed in a Lhx1-dependent manner, whereas EphB1 is expressed in LMC-m neurons in an Isl1-dependent manner (Kania and Jessell, 2003; Luria et al., 2008). In the limb mesenchyme, ephrin A ligands are enriched ventrally, whereas ephrin B ligands are localized dorsally (Kania and Jessell, 2003; Luria et al., 2008). Overexpression of Lhx1 induces *Epha4* expression in LMC neurons and redirects them dorsally, phenocopying EphA4 overexpression, whereas loss of Lhx1 causes LMC-l axons to misproject ventrally, phenocopying *Epha4* mutants (Eberhart et al., 2002; Helmbacher et al., 2000; Kania and Jessell, 2003). Similarly, overexpression of Isl1 induces *Ephb1* expression and redirects LMC axons ventrally, whereas loss of *Isl1* or EphB function causes LMC-m axons to misproject dorsally (Kania and Jessell, 2003; Luria et al., 2008). Importantly, the *Isl1* loss-of-function phenotype can be rescued by EphB1 overexpression, providing strong evidence that EphB1 acts downstream of Isl1 (Luria et al., 2008).

The ephrin A and ephrin B expression patterns in the limb are established by another LIM homeodomain protein, Lmx1b (LIM homeobox transcription factor 1 β), which is restricted to the dorsal limb mesenchyme where it induces ephrin B2 expression and represses the expression of ephrin A ligands (Kania and Jessell, 2003; Luria et al., 2008). Lmx1b also regulates the expression of the guidance molecule netrin in the dorsal limb and is essential for the correct pathfinding of LMC neurons (Kania et al., 2000; Krawchuk and Kania, 2008). Thus, a LIM homeodomain factor in target tissues regulates the expression of molecules that influence the trajectory of motor axons, which also carry out the instructions of a LIM homeodomain code, raising the possibility that these relationships coordinately evolved to ensure the fidelity of axon targeting.

Other cell-surface receptors that regulate LMC guidance include Ret, Gfra1 (glial cell line derived neurotrophic factor family receptor α 1) and neuropilin 2 (Bonanomi et al., 2012; Huber et al., 2005; Kramer et al., 2006). However, it remains to be determined 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., 2003; Palmesino et al., 2010). 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 post-mitotically. 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

 Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

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 (Broihier et al., 2004; Thor and Thomas, 1997; Thor et al., 1999), suggesting a conserved and ancient function for these genes.

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.

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 neuroblast 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).

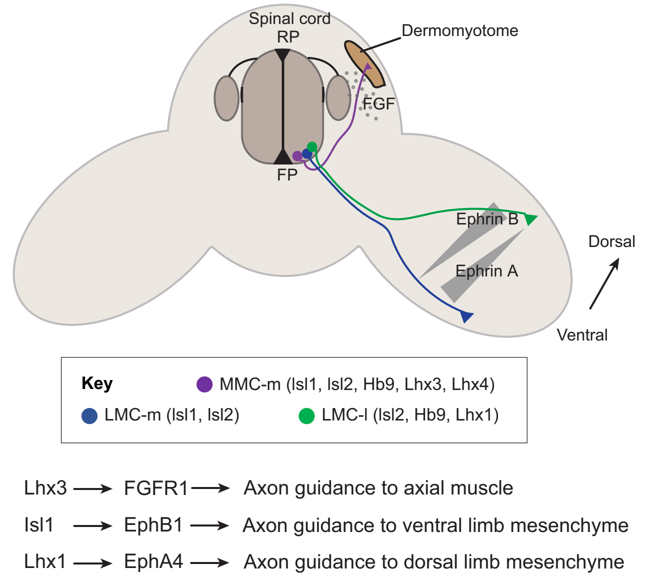

Fig. 1. Downstream effectors of transcription factors that function during vertebrate motor axon guidance. Cross-section of a mouse spinal cord at the limb level. In MMC-m neurons (purple), Lhx3 promotes the expression of the FGF receptor FGFR1 and guides axons to the dermomyotome, which expresses FGF ligands and is attractive to motor axons. In LMC-m neurons (blue), Isl1 directs motor axons into the ventral limb mesenchyme through upregulation of EphB1. In LMC-l neurons (green), Lhx1 promotes EphA4 expression and the selection of a dorsal trajectory into the limb. EphB1 and EphA4 signal repulsion in response to ephrin B and ephrin A ligands, respectively, which are present in the limb mesenchyme. FGF, fibroblast growth factor; FP, floor plate; LMC-l, lateral class of lateral motor column; LMC-m, medial class of lateral motor column; MMC-m, medial class of medial motor column; RP, roof plate.

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 FACs-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 *neuroglian* (*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

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

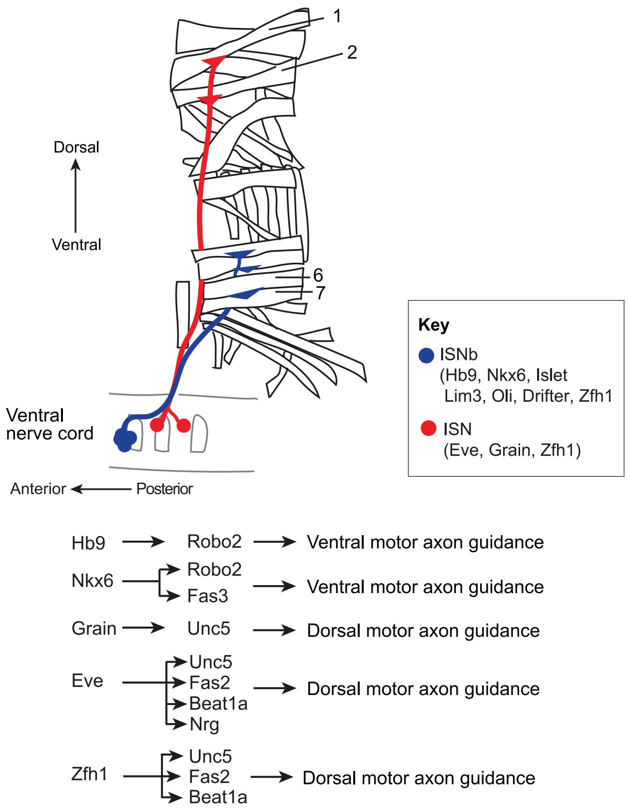

Fig. 2. Downstream effectors of transcription factors that function during *Drosophila* motor axon guidance. A single hemisegment in a filleted late stage 17 *Drosophila* embryo (note that not all motor nerves are shown). In a subset of ISNb motor neurons (blue), Hb9 and Nkx6 promote the expression of Robo2 and Fas3, and direct axons to ventral muscles 6 and 7. In dorsally projecting ISN motor neurons (red), Eve, Grain and Zfh1 regulate guidance by promoting the expression of Unc5, Fas2, Beat1a and Nrg receptors. Beat1a, beaten path 1a; Fas, fasciclin; ISN, intersegmental nerve; ISNb, intersegmental nerve; Nrg, neuroglian; Oli, Olig family; Robo, roundabout; Zfh1, Zn-finger homeodomain 1.

of *unc-5*, *beat1a*, *nrg* and *fas2*, and causes their axons to leave the CNS and assume a motor axon-like trajectory. Co-misexpression of these target genes reproduces this effect. Altogether, these results strongly argue that Unc-5, Beat1a, Nrg and Fas2 act downstream of Eve to regulate motor axon guidance.

Zfh1 and Grain are co-expressed with Eve in dorsally projecting motor neurons, and Zarin et al. found that they also contribute to the expression of *unc-5*, *beat1a* and *fas2* (Zarin et al., 2014). Moreover, ectopic expression of Zfh1 can induce *unc-5*, *beat1a* and *fas2* in interneurons and redirect their axons peripherally; co-expression of Zfh1 and Eve results in an additive effect. Similarly, co-expression of Grain and Eve produces stronger *unc-5* induction than mis-expression of either alone. Although previous studies identified a requirement for Eve in promoting *grain* and *zfh1* expression (Zarin et al., 2012), overexpression of Eve can induce the expression of its targets without inducing *zfh1* or *grain*. In addition, *eve; grain* double mutants have a greater decrease in *unc5* expression than either single mutant (Garces and Thor, 2006; Zarin et al., 2012). Thus, a coherent narrative emerges in which Eve, Grain and Zfh1 function in parallel to promote the expression of a shared set of downstream effectors (Fig. 2). Additional effectors of Eve likely ensure that dorsal motor axons reach their target muscles, as the strongest phenotype produced by triple *unc-5*, *beat1a* and *nrg* mutants does not recapitulate the effect of loss of *eve*. 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 (Broihier and Skeath, 2002; Broihier 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 *hb9* mutants, and *hb9* is required for *robo2* expression in RP3 neurons. Restoring Robo2 activity in *hb9* 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 *hb9* mutant phenotype are enhanced by removing one copy of *nkx6*. There are likely other important targets downstream of these factors, because *robo2* mutants do not have as strong a phenotype as *nkx6* mutants or as *hb9* mutants that lack one copy of *nkx6*. One promising candidate is the cell-adhesion molecule Fasciclin 3 (Fas3). Fas3 protein levels are decreased in *nkx6* mutants, although additional experiments are necessary to determine the functional significance of this change (Broihier 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 *nkx6*. 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 (Fujioka 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 *hb9* 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 *hb9* de-repression (Fujioka et al., 2003). Moreover, *grain* was

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

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 bilaterian 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 by 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 Shirasaki, 2014).

In dI1c 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 dI1 interneurons receive proprioceptive information from sensory neurons and relay it to the brain. After neurogenesis, they segregate into dI1c neurons, which settle at a medial position and are commissural, and into dI1i neurons, which are found more laterally and are ipsilateral. In *Lhx2/Lhx9* double mutants, dI1c axons fail to cross the midline, and *Robo3* mRNA and protein levels are reduced (Wilson et al., 2008). Other dI1 transcription factors are expressed at

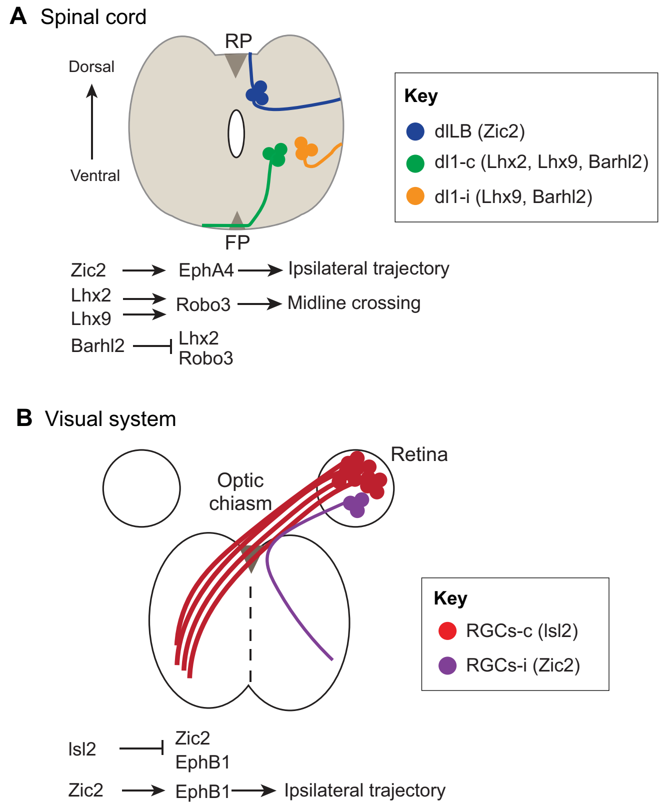

Fig. 3. Downstream effectors of transcription factors that function during midline crossing in the mouse spinal cord and visual system. (A) Cross-section of a mouse spinal cord at E16. In dILB interneurons (blue), Zic2 promotes the selection of an ipsilateral trajectory by upregulating EphA4 expression. In dI1-c interneurons (green), Lhx2 and Lhx9 are required for Robo3 expression and midline crossing. In dI1-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 dI1-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.

normal levels, as are *Dcc* and *Robo1*, and the initial ventral trajectory of dI1c axons is unaffected, indicating that Lhx2 and Lhx9 do not regulate all aspects of dI1c differentiation. The severity of the dI1c 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-Mondéjar et al., 2012). Together, these data strongly argue that Lhx2 and Lhx9 promote midline crossing by activating the expression of *Robo3* in dI1c 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 dI1c and dI1i neurons initially express Lhx2 and Lhx9, Lhx2 is subsequently downregulated in dI1i neurons. In the absence of the Bar-class homeobox gene Barhl2, dI1i neurons ectopically express Lhx2 and Robo3, and aberrantly cross the midline, suggesting that

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

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 dI1i 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

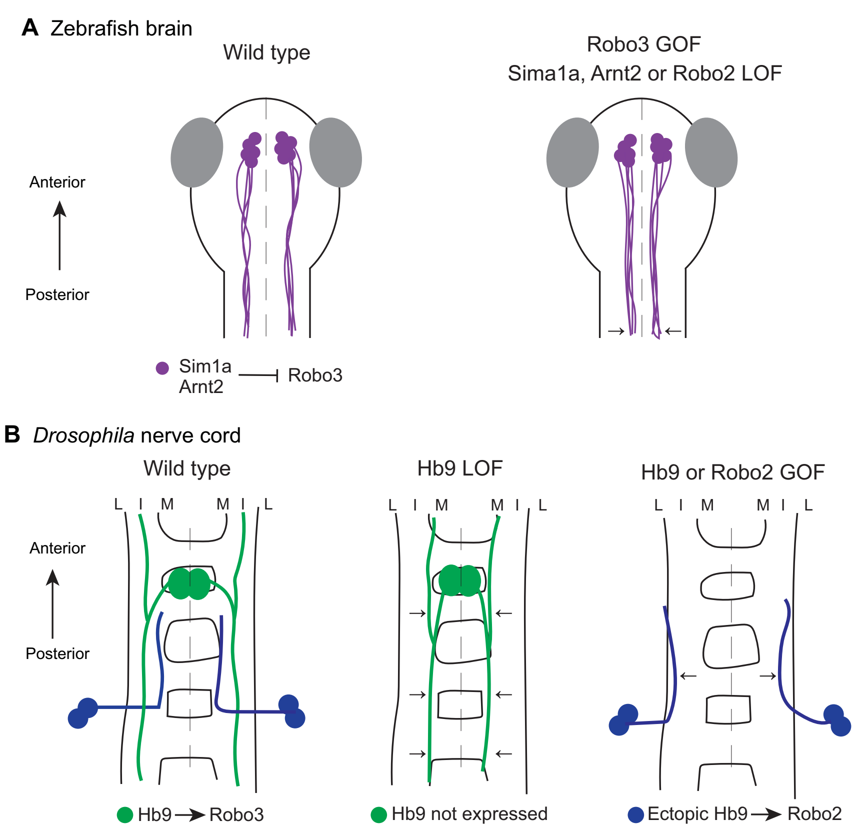

Fig. 4. Transcriptional regulation of lateral position in zebrafish and *Drosophila* embryos. (A) Dorsal view of a 72 hpf zebrafish brain (left panel). The midline is indicated by dashed lines. A subset of dopaminergic neurons (purple) in the hypothalamus expresses Sim1a and sends descending projections into the hindbrain and spinal cord. In the absence of Sim1a (single-minded homolog 1) or Arnt2 (aryl-hydrocarbon receptor nuclear translocator 2), Robo3 is mis-expressed in these neurons and their axons shift closer to the midline (indicated by arrows, right panel). The same phenotype is observed in the case of Robo3 overexpression or Robo2 loss of function. (B) Schematics of late stage 16 *Drosophila* nerve cords. The midline is indicated by dashed lines. In a wild-type embryo (left panel), MP1 neurons (green) express Hb9 and Robo3, and project along an intermediate (I) position within the nerve cord. By contrast, Apterous (Ap) neurons (blue) do not express Hb9, Robo2 or Robo3, and project along a medial (M) trajectory. In the absence of Hb9 (Hb9 loss of function, middle panel), Robo3 expression in MP1 neurons is decreased and their axons shift medially (indicated by arrows). In the case of Hb9 overexpression in Ap neurons (middle panel, Hb9 or Robo gain of function), *robo2* expression is induced and causes axons to shift away from the midline (indicated by arrows) to a more lateral (L) position. GOF, gain of function; LOF, loss of function.

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

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 Sima1a 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 *hb9* mutants can be rescued by restoring Robo3 cell-autonomously. Axons in the lateral-most zone of the nerve cord are also shifted inwards in *hb9* 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 apterous (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).

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

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 Knot. In addition, Lola is required in class I and IV neurons to inhibit the formation of actin-rich protrusions near the cell body. Loss of *lola* results in increased levels of the actin regulator Spire, suggesting that *spire* misregulation may contribute to the *lola* phenotype. Indeed, heterozygosity for *spire* suppresses the ectopic protrusions in *lola* mutant neurons and partially rescues dendritic growth. Moreover, *lola* knockdown in class IV neurons results in a head-turning defect that is characteristic of defective nociception. Strikingly, heterozygosity for *spire* also rescues these behavioral defects. Together, these data suggest that Lola regulates dendrite morphogenesis in part by downregulating *spire*.

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*, although Ab has a greater effect. Accordingly, Ten-m is expressed in both class I and IV da neurons, with higher levels in class I neurons. Ten-m expression is decreased in *ab* mutants, and *ten-m* loss of function disrupts the directionality of class I dendritic branches, reproducing one aspect of the *ab* mutant phenotype. Importantly, the knockdown of *ten-m* in class IV neurons also results in defects in the position of 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.

Transcriptional regulation of dendritic morphology in the *C. elegans* PVD neuron

Recent studies of the *C. elegans* PVD polymodal sensory neuron have emphasized the importance of neural-epidermal interactions during sensory dendrite morphogenesis and have shed light on how transcription factors establish cell type-specific morphologies. PVD neurons are required for the avoidance response to harsh touch, cold and hyperosmolarity (Chatzigeorgious et al. 2010; Way and Chalfie, 1989). During larval development, the two PVD neurons form highly branched dendritic arbors that grow to envelop the animal on each side of the body (Fig. 5B). These arbors exhibit many of the typical features of sensory neurons, including self-avoidance among sister branches and tiling with the functionally related FLP neuron in the head (Smith et al., 2010).

MEC-3 is a LIM homeodomain factor required for the specification of both PVD neurons and light touch neurons (Way and Chalfie, 1989; Zhang et al., 2002). In *mec-3* mutants, the PVD cell body position and axon are normal, but PVD dendrites display dramatic growth defects and fail to initiate secondary branches (Smith et al., 2010; Tsalik et al., 2003). These defects are rescued by PVD-specific expression of MEC-3 (Smith et al., 2013).

A recent study identified *hpo-3/claudin* as 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/claudin*. *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

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

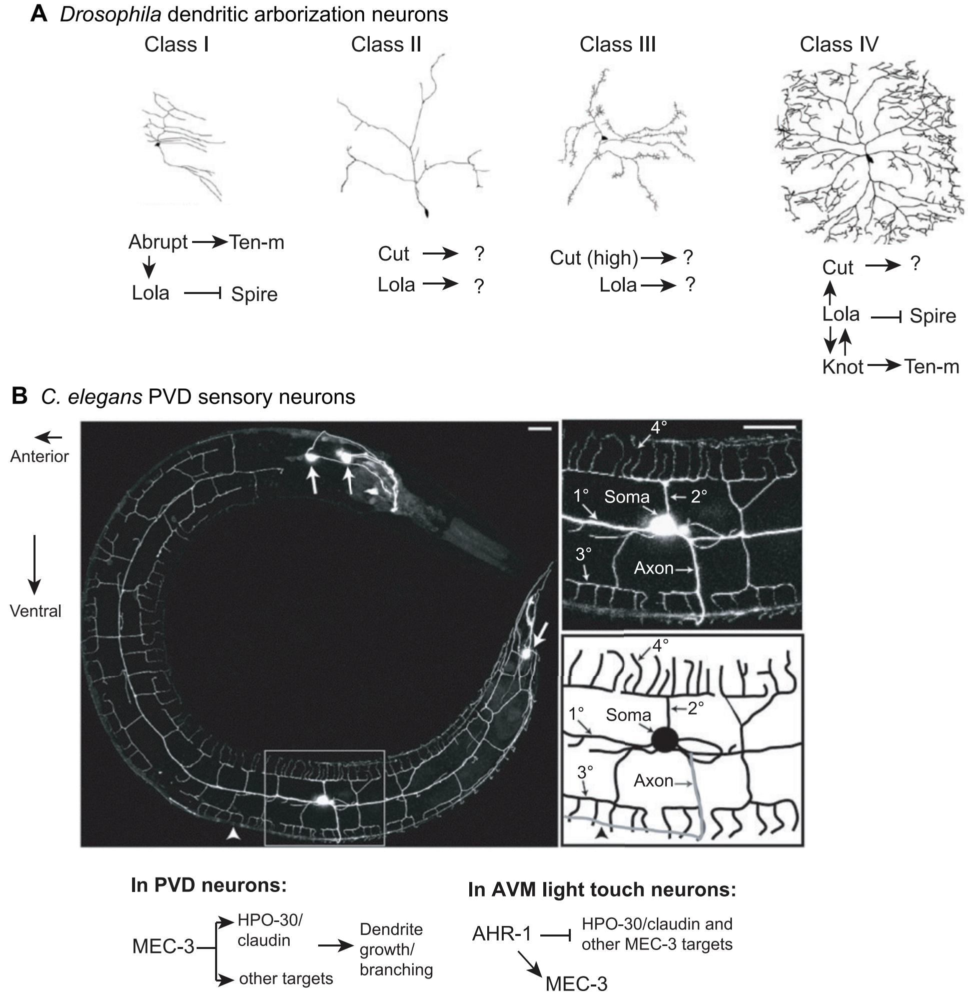

Fig. 5. Transcriptional regulation of dendritic morphology. (A) Camera lucida drawings of the four classes of *Drosophila* dendritic arborization (da) neurons. Adapted, with permission, from Grueber et al. (2003a). In class I da neurons, Abrupt (Ab) regulates morphology in part through upregulation of the cell-surface receptor Teneurin-m (Ten-m) and the transcription factor Lola. Lola promotes class I neuron morphology by repressing the expression of the actin regulator Spire. In class II and III neurons, Lola and Cut act via unknown effectors to regulate dendritic morphology. Knot/Collier is restricted to class IV neurons, where it regulates dendritic morphology in part through Ten-m. Lola and Cut are also required in class IV neurons for dendritic growth, and Lola promotes class IV neuron morphology by repressing *spire*. (B) Image of an adult *C. elegans* expressing a *PVD::GFP* reporter, highlighting the position of the PVD sensory neuron (box). Arrows indicate other neurons that express *PVD::GFP*. The arrowhead denotes the ventral nerve cord. The PVD neuron forms an elaborate dendritic network that wraps around the body; the insets show higher magnification views of the PVD cell body, its axon and its dendritic branches. The LIM homeodomain factor MEC-3 regulates the dendritic morphology of the PVD neuron; MEC-3 in PVD neurons drives the expression of HPO-30/claudin and other genes that promote dendritic growth and branching. However, MEC-3 appears to act in a cell-specific manner; in the AVM light touch neuron, AHR-1 promotes MEC-3 expression but represses the expression of MEC-3 targets that promote PVD morphology, including HPO-30/claudin. Images adapted, with permission, from Smith et al. (2010). Scale bars: 15 µm.

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.,

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

2013). 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 coordinately regulate cell 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 expression of the serotonin transporter *SerT* and for axon guidance, as discussed above (García-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). Isl1, 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 that 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 regulation provides a mechanism to achieve this control (Keleman 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 reactivate developmental programs also presents an important challenge.

Acknowledgements
We thank members of the Bashaw lab for thoughtful discussion and feedback. We apologize to researchers whose work could not be included in this manuscript due to space constraints.

Competing interests
The authors declare no competing financial interests.

Funding
The work in our laboratory is supported by a National Science Foundation graduate research fellowship (C.S.) and by grants from the National Science Foundation and the National Institutes of Health (G.J.B.). Deposited in PMC for release after 12 months.

References
Appel, B., Korzh, V., Glasgow, E., Thor, S., Edlund, T., Dawid, I. B. and Eisen, J. S. (1995). Motoneuron fate specification revealed by patterned LIM homeobox gene expression in embryonic zebrafish. *Development* **121**, 4117-4125.

Bonanomi, D., Chivatakarn, O., Bai, G., Abdesselem, H., Lettieri, K., Marquardt, T., Pierchala, B. A. and Pfaff, S. L. (2012). Ret is a multifunctional coreceptor that integrates diffusible- and contact-axon guidance signals. *Cell* **148**, 568-582.

Bravo-Ambrosio, A., Mastick, G. and Kaprielian, Z. (2012). Motor axon exit from the mammalian spinal cord is controlled by the homeodomain protein Nkx2.9 via Robo-Slit signaling. *Development* **139**, 1435-1446.

Broihier, H. T. and Skeath, J. B. (2002). Drosophila homeodomain protein dHb9 directs neuronal fate via crossrepressive and cell-nonautonomous mechanisms. *Neuron* **35**, 39-50.

Broihier, H. T., Kuzin, A., Zhu, Y., Odenwald, W. and Skeath, J. B. (2004). Drosophila homeodomain protein Nkx6 coordinates motoneuron subtype identity and axonogenesis. *Development* **131**, 5233-5242.

Chatzigeorgiou, M., Yoo, S., Watson, J. D., Lee, W.-H., Spencer, W. C., Kindt, K. S., Hwang, S. W., Miller, D. M., III, Treinin, M., Driscoll, M. et al. (2010). Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. *Nat. Neurosci.* **13**, 861-868.

Chédotal, A. and Rijli, F. M. (2009). Transcriptional regulation of tangential neuronal migration in the developing forebrain. *Curr. Opin. Neurobiol.* **19**, 139-145.

Chen, Z., Gore, B. B., Long, H., Ma, L. and Tessier-Lavigne, M. (2008). Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. *Neuron* **58**, 325-332.

Cho, H.-H., Cargnin, F., Kim, Y., Lee, B., Kwon, R.-J., Nam, H., Shen, R., Barnes, A. P., Lee, J. W., Lee, S. et al. (2014). Isl1 directly controls a cholinergic neuronal identity in the developing forebrain and spinal cord by forming cell type-specific complexes. *PLoS Genet.* **10**, e1004280.

Coonan, J. R., Bartlett, P. F. and Galea, M. P. (2003). Role of EphA4 in defining the position of a motoneuron pool within the spinal cord. *J. Comp. Neurol.* **458**, 98-111.

Corty, M. M., Matthews, B. J. and Grueber, W. B. (2009). Molecules and mechanisms of dendrite development in Drosophila. *Development* **136**, 1049-1061.

Crozatier, M. and Vincent, A. (2008). Control of multidendritic neuron differentiation in Drosophila: the role of Collier. *Dev. Biol.* **315**, 232-242.

Dalla Torre di Sanguinetto, S. A., Dasen, J. S. and Arber, S. (2008). Transcriptional mechanisms controlling motor neuron diversity and connectivity. *Curr. Opin. Neurobiol.* **18**, 36-43.

Dasen, J. S. (2009). *Transcriptional Networks in the Early Development of Sensory-Motor Circuits*, 1st edn. Amsterdam, The Netherlands: Elsevier.

De la Torre-Ubieta, L. and Bonni, A. (2011). Transcriptional regulation of neuronal polarity and morphogenesis in the mammalian brain. *Neuron* **72**, 22-40.

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.

Dickson, B. J. and Zou, Y. (2010). Navigating intermediate targets: the nervous system midline. *Cold Spring Harb. Perspect. Biol.* **2**, a002055.

Dillon, A. K., Fujita, S. C., Matise, M. P., Jarjour, A. A., Kennedy, T. E., Kollmus, H., Arnold, H.-H., Weiner, J. A., Sanes, J. R. and Kaprielian, Z. (2005). Molecular control of spinal accessory motor neuron/axon development in the mouse spinal cord. *J. Neurosci.* **25**, 10119-10130.

Ding, Q., Joshi, P. S., Xie, Z.-h., Xiang, M. and Gan, L. (2012). BARHL2 transcription factor regulates the ipsilateral/contralateral subtype divergence in postmitotic dI1 neurons of the developing spinal cord. *Proc. Natl. Acad. Sci. USA* **109**, 1566-1571.

Dong, X., Liu, O. W., Howell, A. S. and Shen, K. (2013). An extracellular adhesion molecule complex patterns dendritic branching and morphogenesis. *Cell* **155**, 296-307.

Doonan, R., Hatzold, J., Raut, S., Conradt, B. and Alfonso, A. (2008). HLH-3 is a C. elegans Achaete/Scute protein required for differentiation of the hermaphrodite-specific motor neurons. *Mech. Dev.* **125**, 883-893.

Dottori, M., Hartley, L., Galea, M., Paxinos, G., Polizzotto, M., Kilpatrick, T., Bartlett, P. F., Murphy, M., Köntgen, F. and Boyd, A. W. (1998). EphA4 (Sek1) receptor tyrosine kinase is required for the development of the corticospinal tract. *Proc. Natl. Acad. Sci. USA* **95**, 13248-13253.

Dudanova, I., Kao, T.-J., Herrmann, J. E., Zheng, B., Kania, A. and Klein, R. (2012). Genetic evidence for a contribution of EphA:ephrinA reverse signaling to motor axon guidance. *J. Neurosci.* **32**, 5209-5215.

Eberhart, J., Swartz, M. E., Koblar, S. A., Pasquale, E. B. and Krull, C. E. (2002). EphA4 constitutes a population-specific guidance cue for motor neurons. *Dev. Biol.* **247**, 89-101.

Escalante, A., Murillo, B., Morenilla-Palao, C., Klar, A. and Herrera, E. (2013). Zic2-dependent axon midline avoidance controls the formation of major ipsilateral tracts in the CNS. *Neuron* **80**, 1392-1406.

Ferreira, T., Ou, Y., Li, S., Giniger, E. and van Meyel, D. J. (2014). Dendrite architecture organized by transcriptional control of the F-actin nucleator Spire. *Development* **141**, 650-660.

Fu, M. and Zuo, Y. (2011). Experience-dependent structural plasticity in the cortex. *Trends Neurosci.* **34**, 177-187.

Fujioka, M., Lear, B. C., Landgraf, M., Yusibova, G. L., Zhou, J., Riley, K. M., Patel, N. H. and Jaynes, J. B. (2003). Even-skipped, acting as a repressor, regulates axonal projections in Drosophila. *Development* **130**, 5385-5400.

Garces, A. and Thor, S. (2006). Specification of Drosophila aCC motoneuron identity by a genetic cascade involving even-skipped, grain and zfh1. *Development* **133**, 1445-1455.

García-Frigola, C. and Herrera, E. (2010). Zic2 regulates the expression of Sert to modulate eye-specific refinement at the visual targets. *EMBO J.* **29**, 3170-3183.

García-Frigola, C., Carreres, M. I., Vegar, C., Mason, C. and Herrera, E. (2008). Zic2 promotes axonal divergence at the optic chiasm midline by EphB1-dependent and -independent mechanisms. *Development* **135**, 1833-1841.

Ghiretti, A. E. and Paradis, S. (2014). Molecular mechanisms of activity-dependent changes in dendritic morphology: role of RGK proteins. *Trends Neurosci.* **37**, 399-407.

Grueber, W. B., Jan, L. Y. and Jan, Y. N. (2002). Tiling of the Drosophila epidermis by multidendritic sensory neurons. *Development* **129**, 2867-2878.

Grueber, W. B., Jan, L. Y. and Jan, Y. N. (2003a). Different levels of the homeodomain protein cut regulate distinct dendrite branching patterns of Drosophila multidendritic neurons. *Cell* **112**, 805-818.

Grueber, W. B., Ye, B., Moore, A. W., Jan, L. Y. and Jan, Y. N. (2003b). Dendrites of distinct classes of Drosophila sensory neurons show different capacities for homotypic repulsion. *Curr. Biol.* **13**, 618-626.

Hattori, Y., Sugimura, K. and Uemura, T. (2007). Selective expression of Knot/Collier, a transcriptional regulator of the EBF/Olf-1 family, endows the Drosophila sensory system with neuronal class-specific elaborated dendritic patterns. *Genes Cells* **12**, 1011-1022.

Hattori, Y., Usui, T., Satoh, D., Moriyama, S., Shimono, K., Itoh, T., Shirahige, K. and Uemura, T. (2013). Sensory-neuron subtype-specific transcriptional programs controlling dendrite morphogenesis: genome-wide analysis of Abrupt and Knot/Collier. *Dev. Cell* **27**, 530-544.

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* **127**, 3313-3324.

Herrera, E., Brown, L., Aruga, J., Rachel, R. A., Dolen, G., Mikoshiba, K., Brown, S. and Mason, C. A. (2003). Zic2 patterns binocular vision by specifying the uncrossed retinal projection. *Cell* **114**, 545-557.

Hong, W., Mosca, T. J. and Luo, L. (2012). Teneurins instruct synaptic partner matching in an olfactory map. *Nature* **484**, 201-207.

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.

Huberman, A. D., Clandinin, T. R. and Baier, H. (2010). Molecular and cellular mechanisms of lamina-specific axon targeting. *Cold Spring Harb. Perspect. Biol.* **2**, a001743.

Hughes, C. L. and Thomas, J. B. (2007). A sensory feedback circuit coordinates muscle activity in Drosophila. *Mol. Cell. Neurosci.* **35**, 383-396.

Hwang, R. Y., Zhong, L., Xu, Y., Johnson, T., Zhang, F., Deisseroth, K. and Tracey, W. D. (2007). Nociceptive neurons protect Drosophila larvae from parasitoid wasps. *Curr. Biol.* **17**, 2105-2116.

Inamata, Y. and Shirasaki, R. (2014). Dbx1 triggers crucial molecular programs required for midline crossing by midbrain commissural axons. *Development* **141**, 1260-1271.

Iyer, S. C., Wang, D., Iyer, E. P. R., Trunnell, S. A., Meduri, R., Shinwari, R., Sulkowski, M. J. and Cox, D. N. (2012). The RhoGEF trio functions in sculpting class specific dendrite morphogenesis in Drosophila sensory neurons. *PLoS ONE* **7**, e33634.

Jan, Y.-N. and Jan, L. Y. (2010). Branching out: mechanisms of dendritic arborization. *Nat. Rev. Neurosci.* **11**, 316-328.

Jaworski, A. and Tessier-Lavigne, M. (2012). Autocrine/juxtaparacrine regulation of axon fasciculation by Slit-Robo signaling. *Nat. Neurosci.* **15**, 367-369.

Jaworski, A., Long, H. and Tessier-Lavigne, M. (2010). Collaborative and specialized functions of Robo1 and Robo2 in spinal commissural axon guidance. *J. Neurosci.* **30**, 9445-9453.

Jinushi-Nakao, S., Arvind, R., Amikura, R., Kinameri, E., Liu, A. W. and Moore, A. W. (2007). Knot/Collier and Cut control different aspects of dendrite cytoskeleton and synergize to define final arbor shape. *Neuron* **56**, 963-978.

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.

Kao, T.-J. and Kania, A. (2011). Ephrin-mediated cis-attenuation of Eph receptor signaling is essential for spinal motor axon guidance. *Neuron* **71**, 76-91.

Keleman, K., Rajagopalan, S., Cleppien, D., Teis, D., Paiha, K., Huber, L. A., Technau, G. M. and Dickson, B. J. (2002). Comm sorts robo to control axon guidance at the Drosophila midline. *Cell* **110**, 415-427.

Klein, R. (2012). Eph/ephrin signalling during development. *Development* **139**, 4105-4109.

Kolodkin, A. L. and Tessier-Lavigne, M. (2011). Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb. Perspect. Biol.* **3**, a001727.

Komiyama, T., Johnson, W. A., Luo, L. and Jefferis, G. S. X. E. (2003). From lineage to wiring specificity. POU domain transcription factors control precise connections of Drosophila olfactory projection neurons. *Cell* **112**, 157-167.

Kose, H., Rose, D., Zhu, X. and Chiba, A. (1997). Homophilic synaptic target recognition mediated by immunoglobulin-like cell adhesion molecule Fasciclin III. *Development* **124**, 4143-4152.

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

Kratsios, P., Stolfi, A., Levine, M. and Hobert, O. (2012). Coordinated regulation of cholinergic motor neuron traits through a conserved terminal selector gene. *Nat. Neurosci.* **15**, 205-214.

Krawchuk, D. and Kania, A. (2008). Identification of genes controlled by LMX1B in the developing mouse limb bud. *Dev. Dyn.* **237**, 1183-1192.

Kullander, K., Croll, S. D., Zimmer, M., Pan, L., McClain, J., Hughes, V., Zabski, S., DeChiara, T. M., Klein, R., Yancopoulos, G. D. et al. (2001). Ephrin-B3 is the midline barrier that prevents corticospinal tract axons from recrossing, allowing for unilateral motor control. *Genes Dev.* **15**, 877-888.

Kullander, K., Butt, S. J. B., Lebret, J. M., Lundfald, L., Restrepo, C. E., Rydström, A., Klein, R. and Kiehn, O. (2003). Role of EphA4 and EphrinB3 in local neuronal circuits that control walking. *Science* **299**, 1889-1892.

Kwan, K. Y., Sestan, N. and Anton, E. S. (2012). Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. *Development* **139**, 1535-1546.

Labrador, J. P., O'keefe, D., Yoshikawa, S., McKinnon, R. D., Thomas, J. B. and Bashaw, G. J. (2005). The homeobox transcription factor even-skipped regulates netrin-receptor expression to control dorsal motor-axon projections in Drosophila. *Curr. Biol.* **15**, 1413-1419.

Lacin, H., Rusch, J., Yeh, R. T., Fujioka, M., Wilson, B. A., Zhu, Y., Robie, A. A., Mistry, H., Wang, T., Jaynes, J. B. et al. (2014). Genome-wide identification of Drosophila Hb9 targets reveals a pivotal role in directing the transcriptome within eight neuronal lineages, including activation of nitric oxide synthase and Fd59a/Fox-D. *Dev. Biol.* **388**, 117-133.

Landgraf, M. and Thor, S. (2006). Development and structure of motoneurons. *Int. Rev. Neurobiol.* **75**, 33-53.

Landgraf, M., Bossing, T., Technau, G. M. and Bate, M. (1997). The origin, location, and projections of the embryonic abdominal motorneurons of Drosophila. *J. Neurosci.* **17**, 9642-9655.

Landgraf, M., Roy, S., Prokop, A., VijayRaghavan, K. and Bate, M. (1999). even-skipped determines the dorsal growth of motor axons in Drosophila. *Neuron* **22**, 43-52.

Layden, M. J., Odden, J. P., Schmid, A., Garces, A., Thor, S. and Doe, C. Q. (2006). Zfh1, a somatic motor neuron transcription factor, regulates axon exit from the CNS. *Dev. Biol.* **291**, 253-263.

Lee, R., Petros, T. J. and Mason, C. A. (2008). Zic2 regulates retinal ganglion cell axon avoidance of ephrinB2 through inducing expression of the guidance receptor EphB1. *J. Neurosci.* **28**, 5910-5919.

Li, W., Wang, F., Menut, L. and Gao, F.-B. (2004). BTB/POZ-zinc finger protein abrupt suppresses dendritic branching in a neuronal subtype-specific and dosage-dependent manner. *Neuron* **43**, 823-834.

Liu, O. W. and Shen, K. (2012). The transmembrane LRR protein DMA-1 promotes dendrite branching and growth in C. *elegans*. *Nat. Neurosci.* **15**, 57-63.

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.

Lodato, S., Molyneaux, B. J., Zuccaro, E., Goff, L. A., Chen, H.-H., Yuan, W., Meleski, A., Takahashi, E., Mahony, S., Rinn, J. L. et al. (2014). Gene co-regulation by Fezf2 selects neurotransmitter identity and connectivity of corticospinal neurons. *Nat. Neurosci*. **17**, 1046-1054.

Long, H., Sabatier, C., Ma, L., Plump, A., Yuan, W., Ornitz, D. M., Tamada, A., Murakami, F., Goodman, C. S. and Tessier-Lavigne, M. (2004). Conserved roles for Slit and Robo proteins in midline commissural axon guidance. *Neuron* **42**, 213-223.

Luria, V., Krawchuk, D., Jessell, T. M., Laufer, E. and Kania, A. (2008). Specification of motor axon trajectory by Ephrin-B:EphB signaling: symmetrical control of axonal patterning in the developing limb. *Neuron* **60**, 1039-1053.

Marcos-Mondéjar, P., Peregrín, S., Li, J. Y., Carlsson, L., Tole, S. and López-Bendito, G. (2012). The Lhx2 transcription factor controls thalamocortical axonal guidance by specific regulation of Robo1 and Robo2 receptors. *J. Neurosci.* **32**, 4372-4385.

Mazzoni, E. O., Mahony, S., Closser, M., Morrison, C. A., Nedelec, S., Williams, D. J., An, D., Gifford, D. K. and Wichterle, H. (2013). Synergistic binding of transcription factors to cell-specific enhancers programs motor neuron identity. *Nat. Neurosci.* **16**, 1219-1227.

Mosca, T. J., Hong, W., Dani, V. S., Favaloro, V. and Luo, L. (2012). Trans-synaptic Teneurin signalling in neuromuscular synapse organization and target choice. *Nature* **484**, 237-241.

Nagel, J., Delandre, C., Zhang, Y., Förstner, F., Moore, A. W. and Tavosanis, G. (2012). Fascin controls neuronal class-specific dendrite arbor morphology. *Development* **139**, 2999-3009.

Nóbrega-Pereira, S., Kessaris, N., Du, T., Kimura, S., Anderson, S. A. and Marín, O. (2008). Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. *Neuron* **59**, 733-745.

O'Donnell, M., Chance, R. K. and Bashaw, G. J. (2009). Axon growth and guidance: receptor regulation and signal transduction. *Annu. Rev. Neurosci.* **32**, 383-412.

Pabst, O., Rummelies, J., Winter, B. and Arnold, H.-H. (2003). Targeted disruption of the homeobox gene Nkx2.9 reveals a role in development of the spinal accessory nerve. *Development* **130**, 1193-1202.

Paixão, S., Balijepalli, A., Serradj, N., Niu, J., Luo, W., Martin, J. H. and Klein, R. (2013). EphrinB3/EphA4-mediated guidance of ascending and descending spinal tracts. *Neuron* **80**, 1407-1420.

Pak, W., Hindges, R., Lim, Y.-S., Pfaff, S. L. and O'Leary, D. D. M. (2004). Magnitude of binocular vision controlled by islet-2 repression of a genetic program that specifies laterality of retinal axon pathfinding. *Cell* **119**, 567-578.

Palmesino, E., Rousso, D. L., Kao, T.-J., Klar, A., Laufer, E., Uemura, O., Okamoto, H., Novitch, B. G. and Kania, A. (2010). Foxp1 and Lhx1 coordinate motor neuron migration with axon trajectory choice by gating reelin signalling. *PLoS Biol.* **8**, e1000446.

Pfaff, S. L., Mendelsohn, M., Stewart, C. L., Edlund, T. and Jessell, T. M. (1996). Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron – dependent step in interneuron differentiation. *Cell* **84**, 309-320.

Polleux, F., Ince-Dunn, G. and Ghosh, A. (2007). Transcriptional regulation of vertebrate axon guidance and synapse formation. *Nat. Rev. Neurosci.* **8**, 331-340.

Prasad, B. C., Ye, B., Zackhary, R., Schrader, K., Seydoux, G. and Reed, R. R. (1998). unc-3, a gene required for axonal guidance in Caenorhabditis elegans, encodes a member of the O/E family of transcription factors. *Development* **125**, 1561-1568.

Puram, S. V. and Bonni, A. (2013). Cell-intrinsic drivers of dendrite morphogenesis. *Development* **140**, 4657-4671.

Pym, E. C. G., Southall, T. D., Mee, C. J., Brand, A. H. and Baines, R. A. (2006). The homeobox transcription factor Even-skipped regulates acquisition of electrical properties in Drosophila neurons. *Neural Dev.* **1**, 3.

Rajagopalan, S., Vivancos, V., Nicolas, E. and Dickson, B. J. (2000). Selecting a longitudinal pathway: robo receptors specify the lateral position of axons in the Drosophila CNS. *Cell* **103**, 1033-1045.

Sabatier, C., Plump, A. S., Ma, L., Brose, K., Tamada, A., Murakami, F., Lee, E. Y.-H. P. and Tessier-Lavigne, M. (2004). The divergent Robo family protein Rig-1/Robo3 is a negative regulator of slit responsiveness required for midline crossing by commissural axons. *Cell* **117**, 157-169.

Sakai, N. and Kaprielian, Z. (2012). Guidance of longitudinally projecting axons in the developing central nervous system. *Front. Mol. Neurosci.* **5**, 59.

Salzberg, Y., Díaz-Balzac, C. A., Ramirez-Suarez, N. J., Attreed, M., Tecle, E., Desbois, M., Kaprielian, Z. and Bülow, H. E. (2013). Skin-derived cues control arborization of sensory dendrites in Caenorhabditis elegans. *Cell* **155**, 308-320.

Sander, M., Paydar, S., Ericson, J., Briscoe, J., Berber, E., German, M., Jessell, T. M. and Rubenstein, J. L. R. (2000). Ventral neural patterning by Nkx homeobox genes: Nkx6.1 controls somatic motor neuron and ventral interneuron fates. *Genes Dev.* **14**, 2134-2139.

Santiago, C., Labrador, J.-P. and Bashaw, G. J. (2014). The homeodomain transcription factor Hb9 controls axon guidance in Drosophila through the regulation of Robo receptors. *Cell Rep.* **7**, 153-165.

Schweitzer, J., Löhr, H., Bonkowsky, J. L., Hübscher, K. and Driever, W. (2013). Sim1a and Arnt2 contribute to hypothalamo-spinal axon guidance by regulating Robo2 activity via a Robo3-dependent mechanism. *Development* **140**, 93-106.

Serrano-Saiz, E., Poole, R. J., Felton, T., Zhang, F., De La Cruz, E. D. and Hobert, O. (2013). Modular control of glutamatergic neuronal identity in C. elegans by distinct homeodomain proteins. *Cell* **155**, 659-673.

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., 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.

Simpson, J. H., Bland, K. S., Fetter, R. D. and Goodman, C. S. (2000). Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position. *Cell* **103**, 1019-1032.

Smith, C. J., Watson, J. D., Spencer, W. C., O'Brien, T., Cha, B., Albeg, A., Treinin, M. and Miller, D. M. (2010). Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in C. elegans. *Dev. Biol.* **345**, 18-33.

Smith, C. J., O'Brien, T., Chatzigeorgiou, M., Spencer, W. C., Feingold-Link, E., Husson, S. J., Hori, S., Mitani, S., Gottschalk, A., Schafer, W. R. et al. (2013). Sensory neuron fates are distinguished by a transcriptional switch that regulates dendrite branch stabilization. *Neuron* **79**, 266-280.

Spitzweck, B., Brankatschk, M. and Dickson, B. J. (2010). Distinct protein domains and expression patterns confer divergent axon guidance functions for Drosophila Robo receptors. *Cell* **140**, 409-420.

Srinivasan, K., Leone, D. P., Bateson, R. K., Dobreva, G., Kohwi, Y., Kohwi-Shigematsu, T., Grosschedl, R. and McConnell, S. K. (2012). A network of genetic repression and derepression specifies projection fates in the developing neocortex. *Proc. Natl. Acad. Sci. USA* **109**, 19071-19078.

Srivatsa, S., Parthasarathy, S., Britanova, O., Bormuth, I., Donahoo, A.-L., Ackerman, S. L., Richards, L. J. and Tarabykin, V. (2014). Unc5C and DCC act downstream of Ctip2 and Satb2 and contribute to corpus callosum formation. *Nat. Commun.* **5**, 3708.

Sugimura, K., Satoh, D., Estes, P., Crews, S. and Uemura, T. (2004). Development of morphological diversity of dendrites in Drosophila by the BTB-zinc finger protein Abrupt. *Neuron* **43**, 809-822.

Sulkowski, M. J., Iyer, S. C., Kurosawa, M. S., Iyer, E. P. R. and Cox, D. N. (2011). Turtle functions downstream of Cut in differentially regulating class specific dendrite morphogenesis in Drosophila. *PLoS ONE* **6**, e22611.

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.

Thaler, J. P., Lee, S.-K., Jurata, L. W., Gill, G. N. and Pfaff, S. L. (2002). LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. *Cell* **110**, 237-249.

Thor, S. and Thomas, J. B. (1997). The Drosophila islet gene governs axon pathfinding and neurotransmitter identity. *Neuron* **18**, 397-409.

Thor, S. and Thomas, J. B. (2002). Motor neuron specification in worms, flies and mice: conserved and 'lost' mechanisms. *Curr. Opin. Genet. Dev.* **12**, 558-564.

Thor, S., Andersson, S. G. E., Tomlinson, A. and Thomas, J. B. (1999). A LIM-homeodomain combinatorial code for motor- neuron pathway selection. *Nature* **397**, 76-80.

Development (2014) 141, 4667-4680 doi:10.1242/dev.110817

Tsalik, E. L., Niacaris, T., Wenick, A. S., Pau, K., Avery, L. and Hobert, O. (2003). LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. *Dev. Biol.* **263**, 81-102.

Tsubouchi, A., Caldwell, J. C. and Tracey, W. D. (2012). Dendritic filopodia, Ripped Pocket, NOMPC, and NMDARs contribute to the sense of touch in Drosophila larvae. *Curr. Biol.* **22**, 2124-2134.

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.

Van den Berghe, V., Stappers, E., Vandesande, B., Dimidschstein, J., Kroes, R., Francis, A., Conidi, A., Lesage, F., Dries, R., Cazzola, S. et al. (2013). Directed migration of cortical interneurons depends on the cell-autonomous action of Sip1. *Neuron* **77**, 70-82.

Vogt, D., Hunt, R. F., Mandal, S., Sandberg, M., Silberberg, S. N., Nagasawa, T., Yang, Z., Baraban, S. C. and Rubenstein, J. L. R. (2014). Lhx6 directly regulates Arx and CXCR7 to determine cortical interneuron fate and laminar position. *Neuron* **82**, 350-364.

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.

Way, J. C. and Chalfie, M. (1989). The mec-3 gene of Caenorhabditis elegans requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes Dev.* **3**, 1823-1833.

Williams, S. E., Mann, F., Erskine, L., Sakurai, T., Wei, S., Rossi, D. J., Gale, N. W., Holt, C. E., Mason, C. A. and Henkemeyer, M. (2003). Ephrin-B2 and EphB1 mediate retinal axon divergence at the optic chiasm. *Neuron* **39**, 919-935.

Wilson, N. H. and Stoeckli, E. T. (2013). Sonic hedgehog regulates its own receptor on postcrossing commissural axons in a glypican1-dependent manner. *Neuron* **79**, 478-491.

Wilson, S. I., Shafer, B., Lee, K. J. and Dodd, J. (2008). A molecular program for contralateral trajectory: Rig-1 control by LIM homeodomain transcription factors. *Neuron* **59**, 413-424.

Wolfram, V., Southall, T. D., Brand, A. H. and Baines, R. A. (2012). The LIM-homeodomain protein islet dictates motor neuron electrical properties by regulating K(+) channel expression. *Neuron* **75**, 663-674.

Wolfram, V., Southall, T. D., Günay, C., Prinz, A. A., Brand, A. H. and Baines, R. A. (2014). The transcription factors Islet and Lim3 combinatorially regulate ion channel gene expression. *J. Neurosci.* **34**, 2538-2543.

Yan, Z., Zhang, W., He, Y., Gorczyca, D., Xiang, Y., Cheng, L. E., Meltzer, S., Jan, L. Y. and Jan, Y. N. (2013). Drosophila NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature* **493**, 221-225.

Yang, L., Garbe, D. S. and Bashaw, G. J. (2009). A frazzled/DCC-dependent transcriptional switch regulates midline axon guidance. *Science* **324**, 944-947.

Yang, F.-C., Tan, T., Huang, T., Christianson, J., Samad, O. A., Liu, Y., Roberson, D., Davis, B. M. and Ma, Q. (2013). Genetic control of the segregation of pain-related sensory neurons innervating the cutaneous versus deep tissues. *Cell Rep.* **5**, 1353-1364.

Zarin, A. A., Daly, A. C., Hülsmeier, J., Asadzadeh, J. and Labrador, J.-P. (2012). A GATA/homeodomain transcriptional code regulates axon guidance through the Unc-5 receptor. *Development* **139**, 1798-1805.

Zarin, A. A., Asadzadeh, J., Hokamp, K., McCartney, D., Yang, L., Bashaw, G. J. and Labrador, J.-P. (2014). A transcription factor network coordinates attraction, repulsion, and adhesion combinatorially to control motor axon pathway selection. *Neuron* **81**, 1297-1311.

Zhang, Y., Ma, C., Delohery, T., Nasipak, B., Foat, B. C., Bounoutas, A., Bussemaker, H. J., Kim, S. K. and Chalfie, M. (2002). Identification of genes expressed in C. elegans touch receptor neurons. *Nature* **418**, 331-335.

Zhong, L., Hwang, R. Y. and Tracey, W. D. (2010). Pickpocket is a DEG/ENaC protein required for mechanical nociception in Drosophila larvae. *Curr. Biol.* **20**, 429-434.

Zlatic, M., Landgraf, M. and Bate, M. (2003). Genetic specification of axonal arbors: atonal regulates robo3 to position terminal branches in the Drosophila nervous system. *Neuron* **37**, 41-51.