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Review

Motor axon guidance in *Drosophila*

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ABSTRACT

The *Drosophila* motor system starts to assemble during embryonic development. It is composed of 30 muscles per abdominal hemisegment and 36 motor neurons assembling into nerve branches to exit the CNS, navigate within the muscle field and finally establish specific connections with their target muscles. Several families of guidance molecules that play a role controlling this process as well as transcriptional regulators that program the behavior of specific motor neuron have been identified. In this review we summarize the role of both groups of molecules in the motor system as well as their relationship where known. It is apparent that partially redundant guidance protein families and membrane molecules with different functional output direct guidance decisions cooperatively. Some distinct transcriptional regulators seem to control guidance of specific nerve branches globally directing the expression of groups of pathfinding molecules in all motor neurons within the same motor branch.

Contents

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1. Introduction

Three decades have passed since the very first molecules involved in axon guidance were discovered and insects have played a very important role in the field. Some of the very first molecules, such as Fasciclins cell adhesion molecules, were first identified in grasshopper [1,2]. Given the similarity among insect embryonic nervous systems and the power of *Drosophila* for molecular genetic analysis and genetic screens the later became the insect model organism of choice [3]. Several screens in *Drosophila* in the early 90's provided a genetic dissection of different axon guidance systems, including the motor system [4]. Since then a vast number of families of cell surface and secreted molecules directing axon guidance and their downstream signaling mechanisms have been identified [5–8].

There are several advantages that have made the *Drosophila* motor system an invaluable resource for understanding axon guidance. In addition to the *Drosophila* genetic tools available, the possibility to identify and label of individual motor neurons and the knowledge of their specific target muscles has brought the possibility of finely understanding motor neuron guidance behavior. In this review we will highlight the major families of guidance molecules that play a role in *Drosophila* motor axon guidance as well as what is known about their regulation.

2. The *Drosophila* motor system

The *Drosophila* embryo motor system is segmented into 3 thoracic and 9 abdominal segments. Each of the abdominal hemisegments A2-A7 has a stereotyped pattern of 30 muscles. The internal muscles are named dorsal acute (DA1-3), dorsal oblique (DO1-5), lateral longitudinal (LL1) ventral lateral (VL1-4) and ventral oblique (VO4-6). External muscles are named dorsal transverse (DT1), lateral oblique (LO1), lateral transverse (LT1-4), Segment border muscle (SBM), ventral transverse 1 (VT1) and ventral acute (VA1-3) [9]. These muscles are innervated by 36 different motor neurons (MNs) that project through three different main branches routes, the Intersegmental nerve (ISN), the segmental nerve (SN) and the transverse nerve (TN). TN MNs run along the segment border, innervate muscle VT1 and also establish connections with the lateral bipolar neuron (LBD), which in turn innervates alary muscles. The ISN innervates internal muscles and can be subdivided into 3 other branches innervating common groups of muscles: ISN dorsal muscles, ISNb ventral muscles (VL1-4) and ISNd a different group of ventral muscles (VO4-6). The SN is composed of 2 branches, SNa innervating LO1, LT1-4 and SMB and the SNc branch innervating VT1 and VA1-3 [10,11]. There are some specific features related to MN cell body position and muscle innervation for bot main branches: The SN innervates external muscles and axons project from cell bodies present in the same segment as the muscles they innervate. The ISN innervates internal muscles and, except RP2 and VUM MNs, axons project from cell bodies positioned in the anteriorly preceding segment [12] (Fig. 1).

3. Molecular mechanisms of axon guidance in *Drosophila* motor neurons

In this section, we will discuss different families of membrane and secreted proteins playing a role in motor axon exit from the central nervous system (CNS) and proper navigation towards their target muscles in the periphery during *Drosophila* embryonic development. We will also highlight the main signaling pathways downstream in the context of motor axon guidance.

3.1. Netrins, Frazzled and Unc-5

Netrins are secreted proteins of around 600 amino acids and are coded by two tandem functionally redundant genes in *Drosophila*, *NetA* and *NetB*. They are expressed in the midline cells of developing CNS, a subset of neurons, dorsal and ventral muscles and longitudinal epidermal patches [13,14]. Netrins signal through two different receptors: Frazzled (Fra) and Unc-5, promoting axonal attraction or repulsions depending on the receptor combination [15–17].

Genetic analysis indicates that *NetA, NetB* double mutant leads to significant defects in motor axon projection of all three MN pools, including the dorsally projecting intersegmental nerve (ISN), the laterally projecting segmental nerve (SN), and the ventrally projecting ISNb [13]. The repulsive Netrin receptor, Unc-5, is expressed in ISN (aCC and RP2 MNs) and SNa motor axons as well as the exit and peripheral glia along them [18,19]. Consistent with its expression pattern, *Unc-5* mutants present guidance phenotypes in both ISN and SNa MNs, where ISN inappropriately crosses the segment boundaries and one or both branches of SNa are missing [18]. Interestingly, ectopic expression of Unc-5 receptor in the CNS neurons elicits repulsion of their axons and cell bodies from the midline [17–20] (Table 1).

On the other hand, the attractive Netrin receptor, Fra, is expressed in aCC and RP2 MNs (ISN) as well as ventrally projecting ISNb MNs (RPs) but not in SNa MNs [18]. In the absence of *fra*, ISN shows wide range of guidance defects, including premature stall, excessive branching, segment boundary crossing and projections beyond it's dorsal muscle targets. Also, the innervation of NetB expressing muscles 6/7 by ISNb MN (RP3) is significantly disrupted in *fra* mutant embryos [13,15,21]. SNa MNs do not show guidance defects in *fra* mutants while severely affected in *NetAB* double mutants, suggesting that Netrin signaling in SNa is exclusively dependent on the repulsive output of Unc-5 [15,18]. Fra's guidance output in different subsets of neurons has been shown to be dependent on several signaling molecules including, Abelson tyrosine kinase (Abl), the guanine nucleotide-exchange factor Trio, and the Abl substrate Enabled (Ena) [22,23]. In addition, targeting of ventral muscles seems mediated through two conserved domains, P1 and P3 in Fra's cytoplasmic domain [24] and the P3 domain is required for Abl signaling [25]. Abl does not seem to require its SH2 domain but rather its C-terminal region [23].

The role of Netrins and their receptors in motor axon guidance could be summarized as mediated through three different mechanisms where the repulsive effect of Netrin present in the midline and epidermal stripes may act through Unc-5 or Unc-5/Fra to promote CNS exit and prevent segment boundary crossing through (1) *Unc-5* and *fra* signaling in dorsal ISN (aCC and RP2) or (2) *Unc-5* signaling in SNa. An attractive Netrin signal in ventral muscles may signal through (3) Fra in ISNb.

3.2. Slit and Robos

The midline screen resulted in the identification of *slit* and *robo* as axon guidance genes controlling the midline crossing in embryonic CNS of *Drosophila* [26–28]. Slit, is a large extracellular matrix protein produced by midline glia in the CNS and detected by Robo

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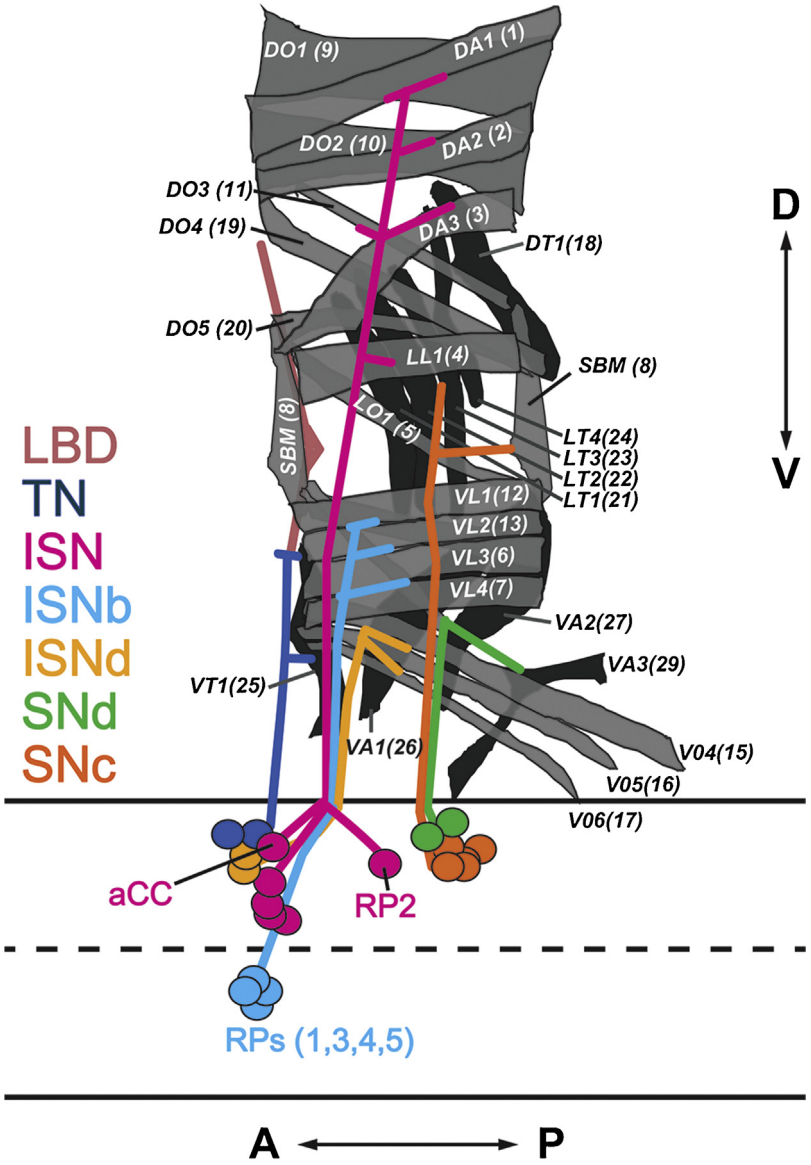

Fig 1. The Drosophila embryonic motor system. The Drosophila embryonic motor system is composed of 30 muscles labeled according to Bate 1993 [9] (First letter: D, dorsal; L, lateral; V, ventral and second letter: O, oblique; A, acute; T, transverse; L, lateral) and from 1 to 30 in brackets. Internal muscles are colored in light grey and external muscles in black. Nerve branches (TN, transverse nerve; ISN intersegmental nerve and its branches b,d and SN segmental nerve branches a and c, LBD, lateral bipolar neuron) are color coded as well as muscle projections and approximate cell body positions for motor neurons of the branch (not all neurons depicted) [10]. Two parallel lines represent the ventral nerve cord and dashed line represents the ventral midline, crossed by RP motor neurons of the ISNb). Orientation is provided by arrows: A, anterior; P, posterior; D, dorsal and V, ventral.

receptors in axonal growth cones [28]. Slit and Robo homologs have been identified in a wide range of animal taxa including vertebrates, planarians, nematodes, and insects [29–32]. In addition to the Robo receptor, critical for midline repulsion and broadly expressed throughout the VNC, there are two more Slit receptors, Robo2 and Robo3, which show diverse expression patterns and have axon guidance functions other than midline repulsion [33–38].

Temporal expression and activity of Robo receptors on axonal membranes is tightly regulated through a variety of mechanisms in *Drosophila* [39–44]. Expression of the 3 Robo receptors is very dynamic and at some stage all of them are expressed en different subsets of MNs [27,36,37]. Robo is expressed at early stages of development in aCC and RP2 MNs pioneering the ISN. However, despite its requirement in RP2 MNs to prevent its axons from crossing the midline [45], no gross abnormalities have been described on ISN guidance. Robo2 is expressed in ventrally projecting ISNb MNs and is required for their guidance and innervation of target muscles, in Particular muscles 6 and 7 where *robo2* is also expressed. Interestingly, this function of Robo2 depends on its unique cytoplasmic domain that is absent in Robo and Robo3 receptors [37]. So far, only the role of Robo in midline repulsion has been shown to be dependent on Slit binding [46]. Nevertheless, misexpression of Slit in the muscle field leads to defective innervation of ventral muscles and repulsion of the ISNb [28] that may be mediated through Robo2. Finally, Robo3 is expressed early in development on the aCC MN [36] but no MN defects have been described so far. While Robo processing and signaling has been described in the midline [47–51] it is not clear if the same mechanisms are required in MN guidance (Table 1).

3.3. Semas and Plexins

Semaphorins and Plexins are another group of guidance molecules that are critical for neural circuit formation in both vertebrates and invertebrates [52–55]. In *Drosophila* there are three

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Table 1
Motor axon phenotypes of relevant receptors and ligands.

Gene	Mutation/overexpression	Phenotype	Reference
NetA, NetB.	Double LOF	ISN dorsal segment border crossing	[13,18]
		ISNb lack of innervation of muscle 6 and 7	[13,18]
		SNa stall near the CNS or fasciculate with the ISN	[18,20]
NetB	Muscle GOF	SNa stall near the CNS or fasciculate with the ISN	[13,63]
Unc-5	LOF	ISN dorsal segment border crossing	[18]
		SNa stall near the CNS or fasciculates with the ISN, missing branches	[18]
fra	LOF	ISN dorsal segment border crossing	[15]
		ISNb lack of innervation of muscle 6 and 7	[15,18,21]
slit	Muscle GOF	ISNb stall lack of innervation of muscle 6 and 7	[28]
robo	LOF	RP2 midline crossing	[45]
robo2	LOF	ISNb lack of innervation of muscle 6 and 7	[37]
Sema-1a	LOF	ISNb stall lack of innervation of muscle 6,7,12,13	[61,62]
		SNa stall or missng branches	[61,62]
Sema-1a	Muscle GOF	SNa stall or missng branches	[61]
		ISNb fasciculates with the ISN,lack of muscle innervation	[61]
Sema-2a	LOF	Low penetrance ectopic muscle contact in ISNb, SNa and TN	[63]
Sema-2a	Muscle GOF	TN stalling (suppressed by PlexB LOF)	[58]
Sema-2a, NetA,NetB	Triple LOF	Suppression of ISNb lack of innervation of muscle 6 and 7 from *NetA, NetB* LOF	[63]
NetA, NetB;Sema-2a	Double LOF,Muscle GOF	Enhancement of ISNb lack of innervation of muscle 6 and 7 from NetA, NetB LOF	[63]
PlexA	LOF	ISNb stall lack of innervation of muscle 6,7,11,12	[53,58]
PlexA		SNa abnormal branching	[53,58]
PlexB	LOF	ISNb stall lack of innervation of muscle 6,7,11,12	[58]
		SNa abnormal branching	[58]
Otk	LOF	ISNb stall lack of innervation of muscle 6,7,11,12	[65]
		SNa abnormal branching	[65]
Dlar	LOF	ISN dorsal truncation	[128]
		ISNb fasciculates with the ISN,lack of muscle innervation	[78,128]
		SNd fasciculates with the ISN,lack of muscle innervation	[78,128]
Ptp69D	LOF	ISNb fasciculates with the ISN,lack of muscle innervation	[79,128]
		SNa abnormal branching (low penetrance)	[79,128]
Dlar,Ptp69D	Double LOF	ISN dorsal truncation (stronger than *Dlar*)	[128]
		ISNb defects stronger than Dlar	[128]
Ptp10D	LOF	No phenotype	[80]
Ptp10D,Ptp69D	Double LOF	SNa abnormal branching, stronger than 69D single	[76]
Ptp10D, Dlar, Ptp69D	Triple LOF	ISNb fasciculates with the ISN (stronger than double)	[76]
Ptp99A	LOF	No phenotype	[79,128]
Dlar,Ptp99A	Double LOF	ISN dorsal truncation (stronger than *Dlar*)	[128]
		ISNb fasciculation with ISN suppressed	[128]
Ptp69D, Ptp99A	Double LOF	ISNb fasciculates with the ISN, lack of muscle innervation, stronger than 69D single	[79,128]
		SNa abnormal branching, stronger than *69D* single	[79,128]
Dlar,Ptp69D,Ptp99A	Triple LOF	ISN dorsal truncation (stronger than *doubles*)	[128]
Ptp10D,Dlar,Ptp69D,Ptp99A	Quadruple LOF	ISN dorsal truncation suppressed	[76]
Ptp52F	LOF	SNa stall or missng branches	[80]
Ptp52F, Dlar	Double LOF	SNa stall or missng branches(stronger than *Ptp52F*)	[80]
Ptp52F,Ptp10D	Double LOF	ISN terminal stall phenotypes	[80]
		ISNb stall in ventral muscle field	[80]
		SNa stall or missng branches(stronger than *Ptp52F*)	[80]
Ptp52F, Ptp69D	Double LOF	ISN terminal arbor phenotypes	[80]
		ISNb stall in ventral muscle field	[80]
		SNa stall or missng branches(stronger than *Ptp52F*)	[80]
sdc	LOF	ISNb fasciculates with the ISN (low penetrance)	[83]
sdc,Dlar	Double LOF	ISNb fasciculates with the ISN (stronger than singles)	[83]
sidestep	LOF	ISN terminal stall phenotypes	[87]
		ISNb fasciculates with the ISN,lack of muscle innervation	[87]
		ISNd fails to defasciculate	[87]
		SNa and SNc remain fasciculated missng muscle innervation	[87]
beat Ia	LOF	ISN terminal stall phenotypes	[20,86]
		ISNb fasciculates with the ISN,lack of muscle innervation (suppressed by *Fas2* LOF)	[86]
		ISNd fails to defasciculate	[86]
		SNa and SNc remain fasciculated missng muscle innervation (suppressed by *conn* LOF)	[86]
beat Ic	LOF	TN defasciculation and ventral muscle innervation	[88]
side-VI	LOF	ISNb muscle 12 innervation defects (low penetrance)	[91]
daw	LOF	ISNb stall lack of innervation of muscle 6,7,12,13	[97,98]

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Table 1 (*Continued*)

Gene	Mutation/overexpression	Phenotype	Reference
		SNa stall or missng branches	[97,98]
babo	LOF	ISNb stall lack of innervation of muscle 6,7,12,13	[97,98]
		SNa stall or missng branches	[97,98]
put	LOF	ISNb stall lack of innervation of muscle 6,7,12,13	[97,98]
		SNa stall or missng branches	[97,98]
Fas2	LOF	No phenotype	[20,86]
Fas2	GOF	Increased faciculation preventing ISNb, SNa muscle targetting, ISN misrouting	[102]
Fas2,Sema-1a	Doube LOF	Suppression of ISNb stall present in *Sema-1a* LOF	[62]
conn	LOF	No phenotype	[103]
conn	GOF	Repulsive for ISNb and ISNd axons, attractive homophilic for Sna axons	[103,104]
Sema-1a, Fas2, conn	GOF	SNa axons stall near the CNS or fasciculate with the ISN, stronger than single GOF	[63]
nrg	LOF	ISN dorsal truncation inappropriate contact with trachea	[20,106]
		ISNb stall, lack of innervation of muscle 6 and 7	[106]
		ISNd fails to defasciculate	[106]
		SNa and SNc stal of fail to defasciculatel missng muscle innervation	[106]
nrg,Unc-5	Double LOF	ISN dorsal segment border crossing from *Unc-5* enhanced	[20]
nrg, beat Ia	Double LOF	ISN dorsal truncation enhanced	[20]
Unc-5, beat Ia	Double LOF	ISN dorsal truncation enhanced	[20]
nrg, Unc-5, beat Ia	Triple LOF	ISN dorsal truncation very severe	[20]

LOF: Loss of function, GOF:gain of function.

transmembrane Semaphorins (Sema-1a, Sema-1b, and Sema-5c), two secreted Semaphorins (Sema-2a and Sema-2b), and two Plexins (Plexin A and Plexin B) [56]. Sema-1b and Sema-5c are not expressed in embryonic CNS, and do not seem to have a role in neural development [57]. Plexin B (PlexB) binds and detects the secreted Semas and Plexin A (PlexA) transmembrane Semas [53,58]. Sema-1a acts as a ligand for PlexA but it can also act as a receptor in reverse signaling [59,60].

Sema-1a has been reported to mediate motor axon defasciculation [59] through reverse signaling. Embryos lacking *Sema-1a* show defects in axon pathfinding of ISNb, ISNd and SNa motor nerves [61,62]. Following Sema-1a stimulation its cytoplasmic domain recruits two major antagonistic regulators of the Rho1 GTPase, pebble (Pbl), the activator and RhoGAP p190, the inhibitor. Pbl is a Rho guanine nucleotide exchange factor (GEF) that facilitates the exchange of bound GDP with GTP activating Rho1. Thus, the GEF and GAP activities of Pbl and RhoGAP, respectively play opposing roles in Sema-1a reverse signaling controlling defasciculation of motor axons at specific choice points and target muscle recognition. Pbl triggers axon–axon repulsion and defasciculation activating Rho1, while p190-RhoGAP inhibits Rho1 leading to axonal attraction [59]. *pbl* mutant embryos in *Drosophila* show defasciculation defects in all three motor axon roots (ISN, SNa, ISNb), while RhoGAP p190 mutations only affect the ISNb [59]. Whether Sema-2a and Sema-2b behave as ligands in the motor system in the same way they do in the CNS midline [60] has not been explored yet. However *Sema-2a* mutants present low penetrance ectopic muscle contact in ISNb, SNa and TN branches and its misexpression in muscles presents phenotypes in the TN that can be suppressed by partial elimination of PlexB [58,63].

In addition to its function as a receptor in reverse signaling, Sema-1a acts as a ligand for PlexA in forward signaling and is also required for motor axon defasciculation [64]. *PlexA* mutants present SNa and ISNb guidance defects where these nerves fail to reach their target muscles [53]. Two other components of this signaling complex are transmembrane proteins, Off-track (OTK) that associates with PlexA, and a receptor guanylyl cyclase (Gyc76C). *Otk* mutants present motor axon phenotypes resembling those of *PlexA* and *Sema-1a* [65] consistent with its role as a PlexA coreceptor. *Gyc76C* interacts genetically and physically with the Sema-1a/PlexA complex during motor axon guidance [66,67]. Guanylyl cyclases catalyze the conversion of GTP to cGMP modulating different cellular and physiological processes [68], including axonal and dendritic guidance [69–72]. Sema-1a/PlexA mediated repulsive guidance is also modulated by an extracellular matrix proteoglycan called Perlecan, highly abundant in motor axon trajectories and pathway choice points. Perlecan increases Sema-1a induced dephosphorylation of focal adhesion kinase (FAK) and shows antagonistic relationship with integrin adhesive functions, thereby leading to motor axon defasciculation [64]. MICAL, a cytosolic oxidoreductase belonging to family of flavoprotein monooxygenases interacts with the PlexA and is necessary for Sema-1a mediated cytoskeletal alterations leading to axon repulsion. MICAL interacts with actin, intermediate filaments, and cytoskeletal-associated adaptor proteins [73].

Finally, PlexB is required for motor axon guidance of ISNb and SNa [58]. *PlexB* mutants present strikingly similar phenotypes to those of *PlexA* where ISNb fails to innervate ventral muscles 6, 7, 11, 12 and 13 and SNa stops short of its target muscle 24 indicating that both cooperate on their guidance. In fact, the both interact through their intracellular domains and although *PlexA* can partially substitute for *PlexB*, the opposite does not happen [58]. Despite a high degree of conservation between PlexA and PlexB they can both signal through specific downstream effectors. PlexB interacts with the small GTPase Rac enhancing RhoA signaling, but PlexA does not [73–75]. In addition, PlexB does not interact with MICAL, although it is required downstream for some of its guidance functions [58,60] (Table 1).

3.4. Receptor protein phosphatases and heparan sulfate proteoglycans

The receptor tyrosine phosphatase family (RPTPs) is another group of molecules playing a role in motor axon guidance. There are 8 identified RPTs but only six: DLAR, DPTP69D, DPTP99A, DPTP4E, DPTP10D and DPTP52F with defined roles in axon guidance [76]. These RPTPS contain a variable number of FN-III repeats and can be structurally classified into two groups depending on whether they have one (4E, 10D and 52F) or two (DLAR, 69D and 99A) PTP domains in their cytoplasmic region [77].

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While *Dlar, Ptp69D* and *Ptp52F*, mutants present clear motor axon phenotypes the rest show only mild phenotypes but their function is complementary to the other phosphatases. In *Dlar* mutants ISNb axons do not innervate their target muscles and instead remain fasciculated to the ISN. In addition SNd axons fail to innervate its target muscles 15–17 fasciculating with the ISN and making aberrant contact with lateral or dorsal muscles [78]. *Ptp69D* mutants also present a bypass phenotype on the ISNb and low penetrance defects on the SNa branch. However, these phenotypes are exacerbated in *Ptp69D, Ptp99A* double mutants. *Ptp99A* deficient embryos do not show any obvious phenotype in the motor system [79]. Mutations in *Ptp52F* result in specific defects on the SNa where it fails to bifurcate, has extra branches or stalls at the branching point [80]. *Ptp52F* has low penetrance phenotypes on the ISN where it stops prematurely; this phenotype can be enhanced by further removal of *Ptp10D* and *Ptp69D*, which have no ISN phenotype on their own. *Ptp52F* also seems to be required in combination with *Ptp10D* and *Ptp69D* in ISNb guidance as double mutants present strong stalling phenotypes [80]. Analysis of double, triple and quadruple *Ptp10D*, *Dlar, Ptp69D* and *Ptp52F* mutants reveals a complex interaction among these PTPs: *Ptp10D* is required for SNa outgrowth in combination with *Dlar and Ptp69D. Ptp10D* also works together with *Dlar, Ptp69D* and *Ptp99A* in ISNb defasciculation. Finally, mutations on *Ptp10D* suppress ISN truncation phenotypes of *Dlar, Ptp69D, Ptp99A* mutants [76].

Signaling downstream RPTPs has been thoroughly studied in DLAR and several molecules have been identified. The cytoplasmic tyrosine kinase Abl and its substrate Enabled (Ena) interact with DLAR and both are downstream effectors required for ISNb guidance [81]. Genetic interaction between *Trio*, a GEF for small GTPases, and *Dlar* reveal that both work together in multiple axonal pathways including ISNb [82] (Table 1).

Two Heparan Sulfate Proteoglycans, Syndecan (Sdc) and Dally-like (Dlp) have been identified as ligands for DLAR in the nervous system. Sdc and DLAR bind to each other in vivo. *sdc* and *Dlar* interact genetically in trans presenting ISNb bypass phenotypes, a strong indication of their interaction [83]. In addition Dlp also interacts with LAR and together with Sdc control different aspect of synaptic function [84]. Interactome studies have also detected homophilic interactions for the extracellular domain of DPTP99A, raising the possibility of a trans interaction of this phosphatase in two opposing cellular surfaces [85].

3.5. Beat and Sidestep families in motor axon guidance

Two genes, *sidestep* (*side*) and *beaten path* (*beat,* later renamed *beat Ia*), were identified in the MN screens of the early 90's presenting very similar phenotypes where all branches within the ISN and SN nerves are affected [4,86,87]. Both were just the founding members of large families with 14 *beats* and 8 *sides*. [88,89]. Despite their highly similar phenotypes they were not initially identified as interacting partners [85,90]. Both families belong to the immunoglobulin (Ig) superfamily of proteins. Sides have 5 Ig domains and a fibronectin type III (FN-III) fold in their extracellular region and all seem to be transmembrane proteins [91]. Beat proteins have 2 Ig domains but are more diverse and can interact with membranes through transmembrane domains or glycophosphatidylinositol links [88].

Beat Ia behaves as a receptor present in axons and interacts with Sidestep present in cellular membranes across its path. In *beat Ia* mutants the ISN is often truncated and its ventral branches ISNb and ISNd fail to defasciculate resulting in defective innervation of their ventral muscle targets. The segmental nerve is also affected as SNc remains fasciculated with SNa and their branches do not extend into their muscle domains [86]. The Beat family of proteins comprises14 members and most of them have nervous system expression. Among the *beat I* subgroup *beat Ic* presents defects where the transverse MN defasciculates and occasionally innervates ventral muscles [88]. Among the Beat V group Beat Va and Beat Vb are expressed in aCC and RP2 dorsal MNs suggesting a role for these receptors in ISN guidance [91] (Table 1).

Sidestep is expressed in a highly dynamic pattern in the CNS, PNS and muscles during embryogenesis and functions as a target-derived attractant [87,90]. Side expressing cells down regulate its expression upon contact with Beat-expressing axons [90]. Mutants for *side* present striking similarity with *beat Ia* one and both of them are interacting pairs [85,90]. In addition to Sidestep most of remaining 7 Side proteins have been identified as interactors of Beat family ones and some of them present specific expression in neurons such as Side IV and side VII suggesting they may behave as guidance receptors [91]. Interactome networks have been well characterized and although there is some degree of promiscuity in their interactions it seems restricted within Beat subfamilies. For example Beat vs seem to specifically interact with Side VI [85,91]. Nevertheless, the role of other individual Beat/Side pairs in motor axon guidance has not been studied yet.

3.6. Other guidance molecules

3.6.1. Transforming growth factor-beta family

The TGF-ß superfamily in *Drosophila* is composed of three structurally and functionally distinct subfamilies: Activins, TGF-ßs and Bone Morphogenetic Proteins (BMPs) [92]. They are involved in different functions in the nervous system, including neural induction and patterning, and control of neuronal differentiation and synaptogenesis [93–96]. Two independent studies have shown the requirement of a cell signaling pathway initiated by Activin-like protein Dawdle (Daw) in motor axon guidance [97,98]. Elimination of *daw* results in stalling of the ISNb within the ventral muscle field. SNa is also affected in these mutants stalling or missing branches [97,98]. Daw, is produced in muscle and glia, signaling through a heteromeric receptor complex of Baboon (Babo), the type-I receptor, and Punt (Put), the type-II; both expressed on motor axons. Importantly, *daw* mutations are phenocopied by zygotic mutants of these two receptors [97,98]. A further component of the system is a secreted enzyme, the metalloprotease tolloid-related (Tlr), also called Tolkin (Tok) [98]. Tok processes Daw's pro-domain and enhances signaling mediated by this ligand. Tok is a circulatory enzyme produced in muscles and subset of CNS neurons and is secreted to hemolymph where it acts in a cell non-autonomous manner to process various extracellular matrix components including Daw ligand. Mutations in *tok* also lead to the same ISNb and SNa defects, further confirming the relevance of this whole pathway in motor axon guidance [98,99]. Further metalloproteases are likely to play a role in motor axon guidance as matrix metalloprotease 2 (Mmp2) seems to regulate the BMP signaling pathway trough one of its substrates, faulty attraction (Frac) and both are required for ISNb and SNa guidance [100,101] (Table 1).

3.6.2. Cell adhesion molecules

Cell adhesion molecules (CAMS) were the first ones proposed to play a role in axon guidance. They belong to the Ig superfamily of proteins have been generally understood to play a role promoting adhesion through homophilic interactions. Most of the cell adhesion molecule mutants seem to have rather mild phenotypes, if any, in the neuromuscular system. Nevertheless, misexpression studies and compound mutations mutants reveal distinct roles for these molecules. *Fasciclin II* (*Fas2*), for example, lacks any overt neuromuscular phenotype in mutant embryos; however, when misexpressed in neurons it leads to increased fasciculation of virtually all motor nerve branches preventing them from reaching their targets [102]. Compound mutants of *Sema-1a* and *Fas2* reveal

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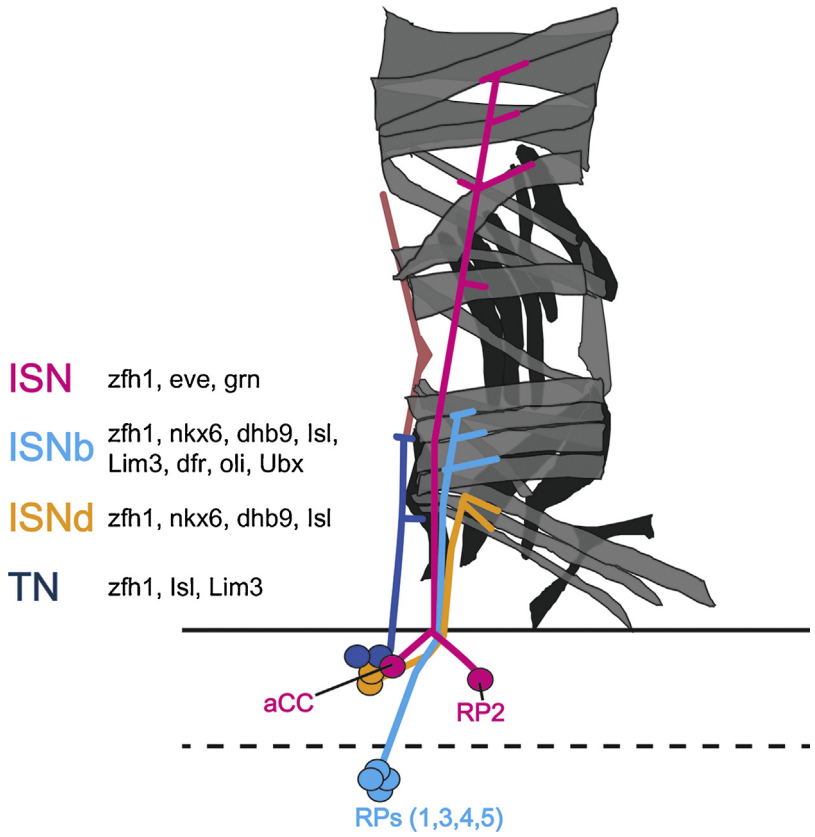

Fig. 2. Expression of transcriptional regulators in the transverse nerve and intersegmental nerve branches. Transcriptional regulators expressed in the transverse nerve (TN), intersegmental nerve (ISN) and branches (b, d). *zinc finger homeodomain 1* (*zfh1*) *even-skipped* (*eve*), *grain* (*grn*), *exex* (hb9), nkx6, *Islet* (*Isl*), Lim3, *Oli* (Olig family), *drifter* (*dfr*) and *Ultrabithorax* (*Ubx*).

opposing roles for both genes as elimination of *Fas2* leads to a suppression of the ISNb phenotype present in *Sema-1a* mutants [62]. Connectin (Conn), another cell adhesion molecule expressed on the SNa and the muscles it innervates does not present any MN defect when its gene is mutated but misexpression studies show that it can work as a homophilic adhesion molecule [103,104]. Triple compound mutants *Sema-1a, Fas2, conn* again reveal the role of cell adhesion molecules as SNa phenotypes present in *Sema-1a* are suppressed in triple mutants [62]. Neuroglian (Nrg) is the Drosophila homolog of the L1 cell adhesion molecule and it is expressed throughout the nervous system including MNs [105]. *nrg* mutants maybe an exception to the rule of lack of motor nerve guidance phenotypes on individual mutants of cell adhesion molecules as they show stalling of ISN and ISNb axons [106] (Table 1).

4. Transcriptional specification of motor neuron guidance

Guidance molecules control how growth cone steer on their path but their expression must be regulated on individual neurons. One of the main levels of regulation is transcriptional. In this chapter we will discuss the different transcriptional regulators that have been show to play a role in motor axon guidance and the pathfinding molecules they may regulate.

While there are transcriptional regulators specifically expressed by subgroups of MNs one is expressed by all of them, Zfh1. Zfh1 is a zinc finger homeobox protein required for proper motor axon guidance of both ISN and SN nerves. In the absence of *zfh1*, ISN and SN MNs fail to exit the CNS or have truncated axons and present severe defects in branching and innervation of target muscles [107]. Interestingly, ectopic expression of *zfh1* in different CNS interneurons redirects their axons away towards the muscle field away from the CNS indicating that it may be regulating CNS exit, a general function shared by all MNs [20,107]. Several guidance genes have been identified downstream *zfh1*, namely *Unc-5*, *beat 1a* and Fas2 [20]. However, these cannot account for all *zfh1* targets as, except *Fas2*, they have restricted expression in MNs. Interestingly *zfh1* does not specify MN identity as all MNs seem to be present in *zfh1* mutants [107].

In addition to Zfh1, the pan-MN transcription factor, Drosophila MNs that exit the CNS and project axons via common routes, share some transcriptional regulators. Unique codes seem be required for specifying the guidance of each distinct MN subclass and the expression of different set of cell surface receptors on them.

4.1. Dorsal ISN motor route

This motor branch is pioneered by the aCC and RP2 MNs and innervates the dorsal muscles of the body wall (Section 2). MNs in this branch express the homeodomain transcription factor Even-skipped (Eve) and the GATA family transcription factor Grain, both of which are necessary for correct motor pathfinding [108–110]. In *eve* mutants aCC and RP2 fail to exit the CNS and innervate their target muscles [108], a phenotype shared by *grain* (*grn)* deficient embryos [110]. There are complex interactions among *zfh1*, *grn* and *eve* that are dependent on individual MNs; and *eve* regulates the expression of *grn* and *zfh1* in aCC but not RP2 [110].

A study of mRNA isolated from aCC and RP2 dorsal projecting MNs (dMNs) identified multiple axon guidance genes that are transcriptionally regulated by *eve* [20]. Genes coding for two cell surface receptors (*Unc-5* and *beat 1a*) and two cell adhesion molecules (*Fas2* and *Neuroglian*, *Nrg*) are among its downstream effectors. *Unc-5*, *beat 1a*, *fas2*, and *nrg* show significant down regulation in

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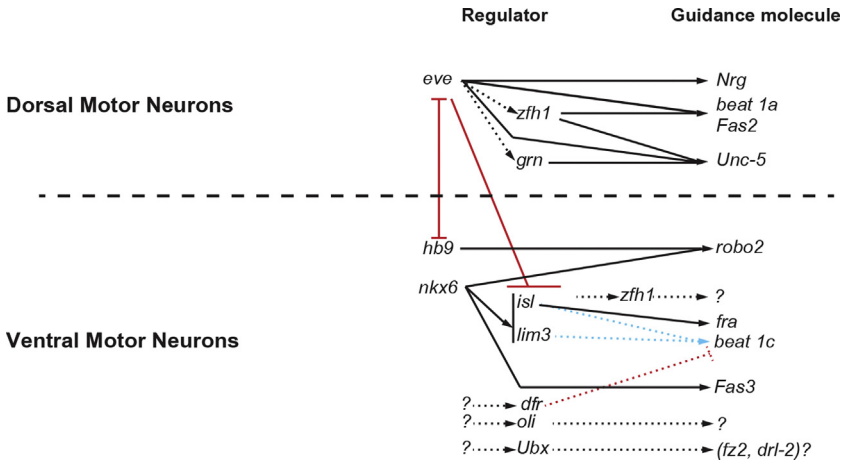

Fig. 3. Transcriptional regulation of guidance in ventral versus dorsal motor neurons. Transcriptional regulators in dorsal and ventral motor neurons and their hierarchy is represented from left to right. Dorsal motor neurons (intersegmental nerve, ISN): *even-skipped* (*eve*) regulates expression of two other transcription factors in dorsal motor neurons, *zinc finger homeodomain 1* (*zfh1*) *even*-and *grain* (*grn)* in aCC but not in RP2 (indicated by doted lines). Different combinations of transcription factors regulate the expression of pathfinding molecules. Only *eve* is required for *Neuroglian* (*Nrg*) expression; both *eve* and *zfh1* for Fasciclin 2 (*Fas2*) and *beaten path 1a* (*beat 1a*) and the three of them for Unc-5 expression. Ventral motor neurons: determinants for these neurons are *nkx6* and *dhb9*. Nkx6 regulates the expression of *Islet* (*Isl*) and *Lim3* but *dhb9* and *nkx6* do not regulate each other. The dorsal determinant *eve* represses *Isl* and *Lim3* and in addition there is a crossrepressive activity between eve and dhb9 indicated by red lines. zfh1 is expressed in all motor neurons, including ventral motor neurons, it is likely downstream *nkx6*, *Lim3* and *Isl* (this uncertainty is signified by doted lines. *drifter* (*dfr*), *Olig* family (*Oli*) and *Ultrabithorax* are also expressed in ventral motor neurons. *nkx6* regulates expression of the cell adhesion molecule *Fasciclin 3* (*Fas3*) and together with *hb9* the expression of the guidance receptor *robo2*. *Isl*, but not *Lim3* regulates the expression of the guidance receptor *frazzled* (*fra*) and both *Lim3* and *Isl* probably regulate *beat 1c* in the transverse nerve and they interact genetically (indicated by cyan dashed lines). *dfr* may represses *beat 1c* expression in the ISNb. *Oli* and *Ubx* are expressed in ventral motor neurons and contribute to its guidance, but the genes they regulate have not been identified in these neurons (indicated by the? sign).

dMNs in the absence of *eve*. Individual mutants for these cell surface molecule leads to milder motor guidance defects compared with *eve* but compound mutants closely resemble its phenotype. Another interesting finding is the different transcriptional regulator requirement for the expression of these genes in dMNs. *grn* and *zfh1* cooperate with *eve* for the expression of these genes in dMNs and, interestingly, also in interneurons where they are misexpressed. *zfh1* is required for *Unc-5*, *beat 1a* and *Fas2* expression in dMNs, while *grn* is only required for *Unc-5* expression [19,20].

4.2. ISNb and ISNd motor branches

ISNb and ISNd motor branches share common regulators with the TN therefore all these branches will be discussed together. ISNb MNs are 4 MNs RP1, 3–5 that innervate a group of ventral muscles (Section 2). RPs coexpress the transcription factors Hb9 (Exex), Nkx6, Islet (Isl or Tup), Lim3, Oli (Olig family), Drifter (Dfr) and Ultrabithorax (Ubx). ISNd MN express Islet but do not express Dfr, setting a differential code of transcriptional regulators within these ventral ISN branches (Lim3 and Hb9 expression has not been unequivocally determined in these MNs). TN MNs express Islet and Lim3. These conserved proteins are required for terminal aspects of MN differentiation including axon guidance [111–120] (Fig. 2).

Isl and *Lim3* deficient embryos present phenotypes where ISNb it fails to innervate ventral muscles. In Lim3 mutants ISNb MN are redirected towards ISNd target muscles. Interestingly Lim3 misexpression leads to ISNd redirection towards ISNb ventral muscles likely upregulating effectors responsible for ISNb guidance [111,112]. *hb9* mutants have ventral muscle innervation defects in the ISNb [113,116] and *hb9, isl* compound mutants show stronger effects on ISNb targeting that each single mutant, indicating they work in parallel [21]. In *nkx6* mutants both ISNb and ISNd are defective as they fail to exit the CNS [114]. Reduction of *dfr* together with *lim3* or *isl* leads to defects in ISNb targeting as well but also redirect TN MN towards ventral muscles suggesting they work together in ISNb and TN pathfinding [117]. In *oli* mutants muscles 12 and 13 fail to be innervated by ISNb and TN NM targets ventral muscles and these defects are enhanced in *oli, hb9* double mutants [119]. *Ubx* mutants ISNb presents defects on muscle 12 innervation and is occasionally misrouted making contacts with the TN [120].

The regulatory network among these transcription factors is complex. While *hb9* and *nkx6* do not regulate each other, *nkx6* seems to be upstream all the others. *nkx6* regulates expression of *isl* and *lim3* [114]. *Isl*, *lim3*, *dfr* and *olig* do not seem to be transcriptional targets of each other although they can work in combination [119]. *hb9* does not seem to be downstream *Ubx* as it is clearly detected in *Ubx* mutant RPs [120].

Few targets have been identified downstream these transcriptional regulators. On the ISNb the CAM *Fasciclin 3* (*Fas3*) and *robo2* are regulated in RP MNs by *nkx6* [37,114]. *isl* and *lim3* interact genetically with *beat 1c* in TN MNs targeting ventral muscles rather that the LBD neuron, suggesting it may be downstream these transcriptional regulators [117]. A recent study has shown that *Isl*, but not *Hb9* or *lim3*, is required for expression of *fra*, in an ISNb MN (RP3) which normally projects to ventral muscles 6 and 7. *fra* mRNA is completely missing or significantly decreased in *isl* mutant RP3s. Similar to the *fra* phenotype, lack of *isl* results in innervation defects of muscle 6/7 by RP3 MN and restoring *fra* expression in *isl* mutant RP3 neurons significantly rescues their innervation defects [21]. A parallel function of *hb9* and *nkx6*, but not *Isl*, regulates the expression of *robo2* receptor in RP3. Muscles 6/7 innervation by RP3 is severely affected in *hb9* mutants and to a lesser extent in *robo2* mutants. Furthermore, *robo2* expression is down regulated in *hb9* mutant RP3 neurons. Consistent with this, restoring *robo2* expression in *hb9* mutant RP3 MNs partially rescues innervation defects of muscles 6/7 [37]. *Isl, hb9* double mutants exhibit higher rate of muscle 6/7 innervation defects than either single mutant, similar to embryos lacking both *robo2* and *fra* [37]. *nkx6* also collaborates with *hb9* to regulate *robo2* as removal of one copy of *nkx6* in an *hb9* background leads to a further reduction of *robo2* expression in RP3 neurons and enhances the muscle innervation phenotype seen in *hb9* mutants [37] (Fig. 3).

Ubx mediates the formation of specific neuromuscular connections regulating the expression of Wnt4 repellant cue, a member of the Wnt family of signalling molecules in muscles. In addition to ventral muscles 6,7,12, and 13 *Ubx* is expressed on the ISNb

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RPs [120]. A similar innervation phenotype to *Ubx* is also seen in embryos lacking *Wnt4,* which is highly expressed in MN-13 of wild type embryos [121]. Wnt4 acts as a repellent in muscle 13 for MNs projecting to muscle 12. Lack of *Ubx* results in loss of Wnt4 in abdominal M13 muscles, and ectopic expression of *Ubx* in thoracic muscles activates Wnt4 expression on them [120]. Although *Ubx* target in NMs is not known yet it may be a *Wnt4* receptor such as *frizzled 2* (*fz2*) or the RYK family *derailed-2* (*drl-2*), whose mutants show the same phenotype [121]. In this way *Ubx* may be regulating the guidance receptor and its ligand, a regulation seen by other homeobox transcription factors outside the nervous system [122,123].

Many other guidance genes have been described as putative targets of *isl*, *hb9*, *lim3* and *eve* through high throughput screens using DNA adenine methyltransferase identification (DamID) [124]. Results from these studies suggest a combinatorial regulation as multiple transcription factors interact with putative regulatory regions of individual genes [124,125].

4.3. Dorsoventral determination and transcriptional repression

Interestingly, there are crossrepressive interactions between transcription factors specific for dorsal and ventral projecting MNs. Parallel function of *nkx6* and *hb9* limits *eve* expression to dorsal MNs and panneural misexpression of *eve* represses *hb9* and *nkx6* effectively locking a code for dorsal MNs versus ventral MNs within the ISN branch [108,113–116]. In addition eve misexpression in RP MN represses *lim3* and *Isl* [114]. This function is consistent with Nkx6, Hb9, and Eve functioning as repressors, as they contain corepressor interacting domains required for their rescue of motor axon guidance in *eve* and *hb9* mutants [37,109] (Fig. 3).

The repressor domain of Hb9 is also required for promoting *robo2* expression, perhaps through the suppression of a *robo2* direct inhibitor [37]. In a recent study in where microarray gene expression profiling and the genome-wide DamID were applied in parallel, *hb9* and *nkx6* were shown to exert their effect mainly through the repression of a wide range of target genes, most of which are transcription factor coding genes [126]. It is likely that these dorsal and ventral MN determinants work in this particular fashion to lock the fates of the NMs they specify preventing globally expression of any gene that could trigger a different program.

5. Redundancy and cooperation

A common aspect in the motor system is a relatively low penetrance of phenotypes in mutants for guidance molecules contrasting with strong misexpression effects of the same molecule [127]. With relatively few exceptions Mutants show defects in 20–40% of hemisegments, and many times lower with relatively mild defects affecting a reduced number of branches or muscle contacts. Misexpression studies of single or double combinations of ligands in the muscle field not only revealed functions of these molecules unidentified in mutant studies but started to point out that growth cones respond based on “combinatorial” input of multiple cues [53,62]. The robustness of the neuromuscular system seems to be built in functional redundancy. This is perhaps most obvious when families of molecules and effects of single and compound mutants are studied. For example in the RPTP family (Section 3.4) only 3 of them, *lar, Ptp69D* and *Ptp52F*, present an obvious phenotype in the neuromuscular system. However, it is clear from compound mutants that *Ptp99A* and *Ptp10D* also play a role [76,79,80]. This functional redundancy likely arises from gene duplication as is seen in Beat family where Beats of the same subgroup have very similar expression pattern in individual neurons and bind the same ligand [91]. Obviously, subfunctionalization and expression pattern divergence in occur over time and is responsible for nervous system complexity (Table 1).

The robustness in the motor system does not seem to arise trough functional redundancy but through cooperative functions that result in a similar response. This is clearly exemplified in compound mutant studies of molecules with diverse function (Section 3) like Unc-5, a repulsive receptor, Beat 1a (an attractive receptor/CAM) and two CAMs (Fas2 and Nrg) [20]. Mutants on each one of their genes results in mild or no phenotype in the ISN but double or triple mutants of these genes show more severe defects [20] (Table 1). In addition, their combinatorial restoration in aCC and RP2 cells lacking them all (an *eve* mutant background) results in more efficient rescue than single gene re-expression [20]. Strikingly, combined misexpression of these membrane molecules in CNS local interneurons that do not exit the CNS makes them behave as MN and redirects their axons toward the muscle field. Combines misexpression in this case is critical as exit rarely happens when these genes are misexpressed individually [20]. This study shows that concerted function of attractive and repulsive receptors along with cell adhesion molecules is required to achieve the adequate guidance response indicating that different functions cooperate in the process.

Motor guidance of each individual branch seems to be governed by common regulators on each branch and it is interesting that ISN (dorsal branch) is specified by one regulator, *eve*, that seems to control the global expression of the guidance molecules collaborating in its pathfinding [20]. In a similar way ventral determinants collaborate on the ISNb [21]. While *robo2* and *fra* deficient embryos present deficient innervation of muscles 6/7 from the ISNb, double mutants show a stronger effect, highlighting their cooperative response [21]. This response is again coordinated by the concerted action of the ventral determinants *dhb9* and, likely, *nkx6* (through *Isl*) [21]. Individual MN within the same branch will likely have a slightly different repertory of guidance molecules differentially regulated through transcription as it has been shown for aCC and RP2 [110].

Thus, motor axon guidance is an intricate process where master transcriptional determinants seem to globally regulate a shared battery of downstream guidance effectors, which cooperate with one another for proper motor nerve guidance.

References

[1] M.J. Bastiani, A.L. Harrelson, P.M. Snow, C.S. Goodman, Expression of fasciclin I and II glycoproteins on subsets of axon pathways during neuronal development in the grasshopper, Cell 48 (5) (1987) 745–755.
[2] N.H. Patel, P.M. Snow, C.S. Goodman, Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in Drosophila, Cell 48 (6) (1987) 975–988.
[3] J.B. Thomas, M.J. Bastiani, M. Bate, C.S. Goodman, From grasshopper to Drosophila: a common plan for neuronal development, Nature 310 (5974) (1984) 203–207.
[4] D.V. Vactor, H. Sink, D. Fambrough, R. Tsoo, C.S. Goodman, Genes that control neuromuscular specificity in Drosophila, Cell 73 (6) (1993) 1137–1153.
[5] G.J. Bashaw, R. Klein, Signaling from axon guidance receptors, Cold Spring Harbor Perspect. Biol. 2 (5) (2010) a001941.
[6] A.L. Kolodkin, R.J. Pasterkamp, SnapShot: axon guidance II, Cell 153 (3) (2013) 722e1.
[7] A.L. Kolodkin, M. Tessier-Lavigne, Mechanisms and molecules of neuronal wiring: a primer, Cold Spring Harbor Perspect. Biol. 3 (6) (2011).
[8] R.J. Pasterkamp, A.L. Kolodkin, SnapShot: axon guidance, Cell 153 (2) (2013) 494e1–494e2.
[9] M. Bate, The mesoderm and its derivatives., in: M. Bate, A. Martínez-Arias (Eds.), The Development of Drosophila, Cold Spring Harbor, 1993, pp. 1013–1090.
[10] T. Landgraf, G.M. Bossing, The origin, location, and projections of the embryonic abdominal motorneurons of Drosophila, J. Neurosci. 17 (24) (1997) 9642–9655.
[11] C. Schmidt, T. Rickert, O. Bossing, J. Vef, The embryonic central nervous system lineages of Drosophila melanogaster. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm, Dev. Biol. 189 (2) (1997) 186–204.

Please cite this article in press as: A. Arzan Zarin, J.-P. Labrador, Motor axon guidance in *Drosophila*, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2017.11.013

[12] M. Landgraf, V. Jeffrey, M. Fujioka, J.B. Jaynes, M. Bate, Embryonic origins of a motor system: motor dendrites form a myotopic map in Drosophila, PLoS Biol. 1 (2) (2003) E41.
[13] K.J. Mitchell, J.L. Doyle, T. Serafini, T.E. Kennedy, M. Tessier-Lavigne, C.S. Goodman, B.J. Dickson, Genetic analysis of Netrin genes in Drosophila: netrins guide CNS commissural axons and peripheral motor axons, Neuron 17 (2) (1996) 203–215.
[14] R. Harris, L.M. Sabatelli, M.A. Seeger, Guidance cues at the Drosophila CNS midline: identification and characterization of two Drosophila Netrin/UNC-6 homologs, Neuron 17 (2) (1996) 217–228.
[15] L.C. Kolodziej, K.J. Timpe, S.R. Mitchell, C.S. Fried, L.Y. Goodman, Jan, frazzled encodes a Drosophila member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance, Cell 87 (2) (1996) 197–204.
[16] K. Hong, L. Hinck, M. Nishiyama, M.M. Poo, M. Tessier-Lavigne, E. Stein, A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion, Cell 97 (7) (1999) 927–941.
[17] K. Keleman, B.J. Dickson, Short- and long-range repulsion by the Drosophila Unc5 netrin receptor, Neuron 32 (4) (2001) 605–617.
[18] J.P. Labrador, D. O'Keefe, S. Yoshikawa, R.D. McKinnon, J.B. Thomas, G.J. Bashaw, The homeobox transcription factor even-skipped regulates netrin-receptor expression to control dorsal motor-axon projections in Drosophila, Curr. Biol. 15 (15) (2005) 1413–1419.
[19] A.A. Zarin, A.C. Daly, J. Hulsmeier, J. Asadzadeh, J.P. Labrador, A GATA/homeodomain transcriptional code regulates axon guidance through the Unc-5 receptor, Development 139 (10) (2012) 1798–1805.
[20] A.A. Zarin, J. Asadzadeh, K. Hokamp, D. McCartney, L. Yang, G.J. Bashaw, J.P. Labrador, A transcription factor network coordinates attraction, repulsion, and adhesion combinatorially to control motor axon pathway selection, Neuron 81 (6) (2014) 1297–1311.
[21] C. Santiago, G.J. Bashaw, Islet coordinately regulates motor axon guidance and dendrite targeting through the Frazzled/DCC receptor, Cell Rep. 18 (7) (2017) 1646–1659.
[22] D.J. Forsthoefel, E.C. Liebl, P.A. Kolodziej, M.A. Seeger, The Abelson tyrosine kinase, the Trio GEF and Enabled interact with the Netrin receptor Frazzled in Drosophila, Development 132 (8) (2005) 1983–1994.
[23] M.P. O'Donnell, G.J. Bashaw, Distinct functional domains of the Abelson tyrosine kinase control axon guidance responses to Netrin and Slit to regulate the assembly of neural circuits, Development 140 (13) (2013) 2724–2733.
[24] J.N. Dorsten, M.F.A. VanBerkum, Frazzled cytoplasmic P-motifs are differentially required for axon pathway formation in the Drosophila embryonic CNS, Int. J. Dev. Neurosci. 26 (7) (2008) 753–761.
[25] J.N. Dorsten, P.A. Kolodziej, M.F.A. VanBerkum, Frazzled regulation of myosin II activity in the Drosophila embryonic CNS, Dev. Biol. 308 (1) (2007) 120–132.
[26] M. Seeger, G. Tear, D. Ferres-Marco, C.S. Goodman, Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline, Neuron 10 (3) (1993) 409–426.
[27] T. Kidd, K. Brose, K.J. Mitchell, R.D. Fetter, M. Tessier-Lavigne, C.S. Goodman, G. Tear, Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors, Cell 92 (2) (1998) 205–215.
[28] T. Kidd, K.S. Bland, C.S. Goodman, Slit is the midline repellent for the robo receptor in Drosophila, Cell 96 (6) (1999) 785–794.
[29] K. Brose, M. Tessier-Lavigne, Slit proteins: key regulators of axon guidance, axonal branching, and cell migration, Curr. Opin. Neurobiol. 10 (1) (2000) 95–102.
[30] H. Long, C. Sabatier, L. Ma, A. Plump, W. Yuan, D.M. Ornitz, A. Tamada, F. Murakami, C.S. Goodman, M. Tessier-Lavigne, Conserved roles for Slit and Robo proteins in midline commissural axon guidance, Neuron 42 (2) (2004) 213–223.
[31] A. Jaworski, H. Long, M. Tessier-Lavigne, Collaborative and specialized functions of Robo1 and Robo2 in spinal commissural axon guidance, J. Neurosci. 30 (28) (2010) 9445–9453.
[32] T.A. Evans, G.J. Bashaw, Slit/Robo-mediated axon guidance in Tribolium and Drosophila: divergent genetic programs build insect nervous systems, Dev. Biol. 363 (1) (2012) 266–278.
[33] S. Rajagopalan, E. Nicolas, V. Vivancos, J. Berger, B.J. Dickson, Crossing the midline: roles and regulation of Robo receptors, Neuron 28 (3) (2000) 767–777.
[34] S. Rajagopalan, V. Vivancos, E. Nicolas, B.J. Dickson, Selecting a longitudinal pathway: robo receptors specify the lateral position of axons in the Drosophila CNS, Cell 103 (7) (2000) 1033–1045.
[35] J.H. Simpson, K.S. Bland, R.D. Fetter, C.S. Goodman, Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position, Cell 103 (7) (2000) 1019–1032.
[36] J.H. Simpson, T. Kidd, K.S. Bland, C.S. Goodman, Short-range and long-range guidance by slit and its Robo receptors. Robo and Robo2 play distinct roles in midline guidance, Neuron 28 (3) (2000) 753–766.
[37] C. Santiago, J.P. Labrador, G.J. Bashaw, The homeodomain transcription factor Hb9 controls axon guidance in Drosophila through the regulation of Robo receptors, Cell Rep. 7 (1) (2014) 153–165.
[38] T.A. Evans, C. Santiago, E. Arbeille, G.J. Bashaw, Robo2 acts in trans to inhibit Slit-Robo1 repulsion in pre-crossing commissural axons, Elife 4 (2015) e08407.
[39] G. Tear, R. Harris, S. Sutaria, K. Kilomanski, C.S. Goodman, M.A. Seeger, commissureless controls growth cone guidance across the CNS midline in Drosophila and encodes a novel membrane protein, Neuron 16 (3) (1996) 501–514.
[40] T. Kidd, C. Russell, C.S. Goodman, G. Tear, Dosage-sensitive and complementary functions of roundabout and commissureless control axon crossing of the CNS midline, Neuron 20 (1) (1998) 25–33.
[41] K. Keleman, C. Ribeiro, B.J. Dickson, Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo, Nat. Neurosci. 8 (2) (2005) 156–163.
[42] G.F. Gilestro, Redundant mechanisms for regulation of midline crossing in Drosophila, PLoS One 3 (11) (2008) e3798.
[43] T.A. Evans, G.J. Bashaw, Functional diversity of Robo receptor immunoglobulin domains promotes distinct axon guidance decisions, Curr. Biol. 20 (6) (2010) 567–572.
[44] A. Neuhaus-Follini, G.J. Bashaw, Crossing the embryonic midline: molecular mechanisms regulating axon responsiveness at an intermediate target, Wiley Interdiscip. Rev. Dev. Biol 4 (4) (2015) 377–389.
[45] M.J. Murray, P.M. Whitington, Effects of roundabout on growth cone dynamics, filopodial length, and growth cone morphology at the midline and throughout the neuropile, J. Neurosci. 19 (18) (1999) 7901–7912.
[46] H.E. Brown, M.C. Reichert, T.A. Evans, Slit binding via the ig1 domain is essential for midline repulsion by drosophila robo1 but dispensable for receptor expression, localization, and regulation in vivo, G3 (Bethesda) 5 (11) (2015) 2429–2439.
[47] H.A. Coleman, J.P. Labrador, R.K. Chance, G.J. Bashaw, The Adam family metalloprotease Kuzbanian regulates the cleavage of the roundabout receptor to control axon repulsion at the midline, Development 137 (14) (2010) 2417–2426.
[48] G.J. Bashaw, H. Hu, C.D. Nobes, C.S. Goodman, A novel Dbl family RhoGEF promotes Rho-dependent axon attraction to the central nervous system midline in Drosophila and overcomes Robo repulsion, J. Cell Biol. 155 (7) (2001) 1117–1122.
[49] G.J. Bashaw, T. Kidd, D. Murray, T. Pawson, C.S. Goodman, Repulsive axon guidance: abelson and Enabled play opposing roles downstream of the roundabout receptor, Cell 101 (7) (2000) 703–715.
[50] X. Fan, J.P. Labrador, H. Hing, G.J. Bashaw, Slit stimulation recruits Dock and Pak to the roundabout receptor and increases Rac activity to regulate axon repulsion at the CNS midline, Neuron 40 (1) (2003) 113–127.
[51] H. Hu, M. Li, J.P. Labrador, J. McEwen, E.C. Lai, C.S. Goodman, G.J. Bashaw, Cross GTPase-activating protein (CrossGAP)/Vilse links the Roundabout receptor to Rac to regulate midline repulsion, Proc. Natl. Acad. Sci. U. S. A. 102 (12) (2005) 4613–4618.
[52] A.L. Kolodkin, D.J. Matthes, C.S. Goodman, The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules, Cell 75 (7) (1993) 1389–1399.
[53] J.N. Winberg, L. Noordermeer, P.M. Tamagnone, M.K. Comoglio, M. Spriggs, C.S. Tessier-Lavigne, Plexin A is a neuronal semaphorin receptor that controls axon guidance, Cell 95 (7) (1998) 903–916.
[54] L. Tamagnone, S. Artigiani, H. Chen, Z. He, G.I. Ming, H. Song, A. Chedotal, M.L. Winberg, C.S. Goodman, M. Poo, M. Tessier-Lavigne, P.M. Comoglio, Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates, Cell 99 (1) (1999) 71–80.
[55] C. Battistini, L. Tamagnone, Transmembrane semaphorins, forward and reverse signaling: have a look both ways, Cell. Mol. Life Sci. 73 (8) (2016) 1609–1622.
[56] R.J. Pasterkamp, Getting neural circuits into shape with semaphorins, Nat. Rev. Neurosci. 13 (9) (2012) 605–618.
[57] N. Khare, N. Fascetti, S. DaRocha, R. Chiquet-Ehrismann, S. Baumgartner, Expression patterns of two new members of the Semaphorin family in Drosophila suggest early functions during embryogenesis, Mech. Dev. 91 (1–2) (2000) 393–397.
[58] J.C. Ayoob, J.R. Terman, A.L. Kolodkin, Drosophila Plexin B is a Sema-2a receptor required for axon guidance, Development 133 (11) (2006) 2125–2135.
[59] S. Jeong, K. Juhaszova, A.L. Kolodkin, The control of semaphorin-1a-mediated reverse signaling by opposing pebble and RhoGAPp190 functions in drosophila, Neuron 76 (4) (2012) 721–734.
[60] M. Hernandez-Fleming, E.W. Rohrbach, G.J. Bashaw, Sema-1a reverse signaling promotes midline crossing in response to secreted semaphorins, Cell Rep. 18 (1) (2017) 174–184.
[61] H.H. Yu, H.H. Araj, S.A. Ralls, A.L. Kolodkin, The transmembrane Semaphorin Sema I is required in Drosophila for embryonic motor and CNS axon guidance, Neuron 20 (2) (1998) 207–220.
[62] H.H. Yu, A.S. Huang, A.L. Kolodkin, Semaphorin-1a acts in concert with the cell adhesion molecules fasciclin II and connectin to regulate axon fasciculation in Drosophila, Genetics 156 (2) (2000) 723–731.
[63] M.L. Winberg, K.J. Mitchell, C.S. Goodman, Genetic analysis of the mechanisms controlling target selection: complementary and combinatorial functions of netrins, semaphorins, and IgCAMs, Cell 93 (4) (1998) 581–591.
[64] J.Y. Cho, K. Chak, B.J. Andreone, J.R. Wooley, A.L. Kolodkin, The extracellular matrix proteoglycan perlecan facilitates transmembrane semaphorin-mediated repulsive guidance, Genes Dev. 26 (19) (2012) 2222–2235.
[65] M.L. Winberg, L. Tamagnone, J. Bai, P.M. Comoglio, D. Montell, C.S. Goodman, The transmembrane protein off-track associates with Plexins and functions

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downstream of Semaphorin signaling during axon guidance, Neuron 32 (1) (2001) 53–62.
[66] J.C. Ayoob, H.H. Yu, J.R. Terman, A.L. Kolodkin, The Drosophila receptor guanylyl cyclase Gyc76C is required for semaphorin-1a-plexin A-mediated axonal repulsion, J. Neurosci. 24 (30) (2004) 6639–6649.
[67] K. Chak, A.L. Kolodkin, Function of the Drosophila receptor guanylyl cyclase Gyc76C in PlexA-mediated motor axon guidance, Development 141 (1) (2014) 136–147.
[68] S.A. Davies, Signalling via cGMP: lessons from Drosophila, Cell. Signal. 18 (4) (2006) 409–421.
[69] F. Polleux, T. Morrow, A. Ghosh, Semaphorin 3A is a chemoattractant for cortical apical dendrites, Nature 404 (6778) (2000) 567–573.
[70] C. Seidel, G. Bicker, Nitric oxide and cGMP influence axonogenesis of antennal pioneer neurons, Development 127 (21) (2000) 4541–4549.
[71] S.M. Gibbs, A. Becker, R.W. Hardy, J.W. Truman, Soluble guanylate cyclase is required during development for visual system function in Drosophila, J. Neurosci. 21 (19) (2001) 7705–7714.
[72] M. Nishiyama, A. Hoshino, L. Tsai, J.R. Henley, Y. Goshima, M. Tessier-Lavigne, M.M. Poo, K. Hong, Cyclic AMP/GMP-dependent modulation of Ca2+ channels sets the polarity of nerve growth-cone turning, Nature 423 (6943) (2003) 990–995.
[73] J.R. Terman, T. Mao, R.J. Pasterkamp, H.H. Yu, A.L. Kolodkin, MICALs, a family of conserved flavoprotein oxidoreductases, function in plexin-mediated axonal repulsion, Cell 109 (7) (2002) 887–900.
[74] T.F. Hu, C.S. Marton, Plexin B mediates axon guidance in Drosophila by simultaneously inhibiting active Rac and enhancing RhoA signaling, Neuron 32 (1) (2001) 39–51.
[75] H. Driessens, C.D. Hu, A. Nobes, I. Self, C.S. Jordens, A. Goodman, Plexin-B semaphorin receptors interact directly with active Rac and regulate the actin cytoskeleton by activating Rho, Curr. Biol. 11 (5) (2001) 339–344.
[76] Q. Sun, B. Schindelholz, M. Knirr, A. Schmid, K. Zinn, Complex genetic interactions among four receptor tyrosine phosphatases regulate axon guidance in Drosophila, Mol. Cell. Neurosci. 17 (2) (2001) 274–291.
[77] T. Hatzihristidis, N. Desai, A.P. Hutchins, T.-C. Meng, M.L. Tremblay, D. Miranda-Saavedra, A Drosophila-centric view of protein tyrosine phosphatases, FEBS Lett. 589 (9) (2015) 951–966.
[78] N.X. Krueger, D. Van Vactor, H.I. Wan, W.M. Gelbart, C.S. Goodman, H. Saito, The transmembrane tyrosine phosphatase DLAR controls motor axon guidance in Drosophila, Cell 84 (4) (1996) 611–622.
[79] C.J. Desai, J.G. Gindhart, L.S. Goldstein, K. Zinn, Receptor tyrosine phosphatases are required for motor axon guidance in the Drosophila embryo, Cell 84 (4) (1996) 599–609.
[80] M. Schindelholz, R. Knirr, K. Warrior, Regulation of CNS and motor axon guidance in Drosophila by the receptor tyrosine phosphatase DPTP52F, Development (Cambridge, England) 128 (21) (2001) 4371–4382.
[81] Z. Wills, J. Bateman, C.A. Korey, A. Comer, D. Van Vactor, The tyrosine kinase Abl and its substrate enabled collaborate with the receptor phosphatase Dlar to control motor axon guidance, Neuron 22 (2) (1999) 301–312.
[82] J. Bateman, H. Shu, D. Van Vactor, The guanine nucleotide exchange factor trio mediates axonal development in the Drosophila embryo, Neuron 26 (1) (2000) 93–106.
[83] A.N. Fox, K. Zinn, The heparan sulfate proteoglycan syndecan is an in vivo ligand for the Drosophila LAR receptor tyrosine phosphatase, Curr. Biol. 15 (19) (2005) 1701–1711.
[84] K.G. Johnson, A.P. Tenney, A. Ghose, A.M. Duckworth, M.E. Higashi, K. Parfitt, O. Marcu, T.R. Heslip, J.L. Marsh, T.L. Schwarz, J.G. Flanagan, D. Van Vactor, The HSPGs Syndecan and Dallylike bind the receptor phosphatase LAR and exert distinct effects on synaptic development, Neuron 49 (4) (2006) 517–531.
[85] E. Ozkan, R.A. Carrillo, C.L. Eastman, R. Weiszmann, D. Waghray, K.G. Johnson, K. Zinn, S.E. Celniker, K.C. Garcia, An extracellular interactome of immunoglobulin and LRR proteins reveals receptor-ligand networks, Cell 154 (1) (2013) 228–239.
[86] D. Fambrough, C.S. Goodman, The Drosophila beaten path gene encodes a novel secreted protein that regulates defasciculation at motor axon choice points, Cell 87 (6) (1996) 1049–1058.
[87] H. Sink, E.J. Rehm, L. Richstone, Y.M. Bulls, C.S. Goodman, sidestep encodes a target-derived attractant essential for motor axon guidance in Drosophila, Cell 105 (1) (2001) 57–67.
[88] G.C. Pipes, Q. Lin, S.E. Riley, C.S. Goodman, The Beat generation: a multigene family encoding IgSF proteins related to the Beat axon guidance molecule in Drosophila, Development 128 (22) (2001) 4545–4552.
[89] H. Aberle, Searching for guidance cues: follow the Sidestep trail, Fly 3 (4) (2009).
[90] M. Siebert, D. Banovic, B. Goellner, H. Aberle, Drosophila motor axons recognize and follow a Sidestep-labeled substrate pathway to reach their target fields, Genes Dev. 23 (9) (2009) 1052–1062.
[91] H. Li, A. Watson, A. Olechwier, M. Anaya, S.K. Sorooshyari, D.P. Harnett, H.-K.P. Lee, J. Vielmetter, M.A. Fares, K.C. Garcia, E. Özkan, J.-P. Labrador, K. Zinn, Deconstruction of the beaten path-sidestep interaction network provides insights into neuromuscular system development, Elife 6 (2017) e28111.
[92] J. Massague, TGF-beta signal transduction, Annu. Rev. Biochem. 67 (1998) 753–791.
[93] M.F. Mehler, P.C. Mabie, D. Zhang, J.A. Kessler, Bone morphogenetic proteins in the nervous system, Trends Neurosci. 20 (7) (1997) 309–317.
[94] H. Aberle, A.P. Haghighi, R.D. Fetter, B.D. McCabe, T.R. Magalhaes, C.S. Goodman, Wishful thinking encodes a BMP type II receptor that regulates synaptic growth in Drosophila, Neuron 33 (4) (2002) 545–558.
[95] G. Marques, H. Bao, T.E. Haerry, M.J. Shimell, P. Duchek, B. Zhang, M.B. O'Connor, The Drosophila BMP type II receptor Wishful Thinking regulates neuromuscular synapse morphology and function, Neuron 33 (4) (2002) 529–543.
[96] B.D. McCabe, S. Hom, H. Aberle, R.D. Fetter, G. Marques, T.E. Haerry, H. Wan, M.B. O'Connor, C.S. Goodman, A.P. Haghighi, Highwire regulates presynaptic BMP signaling essential for synaptic growth, Neuron 41 (6) (2004) 891–905.
[97] L. Parker, J.E. Ellis, M.Q. Nguyen, K. Arora, The divergent TGF-beta ligand Dawdle utilizes an activin pathway to influence axon guidance in Drosophila, Development 133 (24) (2006) 4981–4991.
[98] M. Serpe, M.B. O'Connor, The metalloprotease tolloid-related and its TGF-beta-like substrate Dawdle regulate Drosophila motoneuron axon guidance, Development 133 (24) (2006) 4969–4979.
[99] F. Meyer, H. Aberle, At the next stop sign turn right: the metalloprotease Tolloid-related 1 controls defasciculation of motor axons in Drosophila, Development 133 (20) (2006) 4035–4044.
[100] C.M. Miller, N. Liu, A. Page-McCaw, H.T. Broihier, Drosophila MMP2 regulates the matrix molecule faulty attraction (Frac) to promote motor axon targeting in Drosophila, J. Neurosci. 31 (14) (2011) 5335–5347.
[101] C.M. Miller, A. Page-McCaw, H.T. Broihier, Matrix metalloproteinases promote motor axon fasciculation in the Drosophila embryo, Development (Cambridge, England) 135 (1) (2008) 95–109.
[102] D.M. Lin, C.S. Goodman, Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance, Neuron 13 (3) (1994) 507–523.
[103] A. Nose, V.B. Mahajan, C.S. Goodman, Connectin: a homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in Drosophila, Cell 70 (4) (1992) 553–567.
[104] A. Nose, T. Umeda, M. Takeichi, Neuromuscular target recognition by a homophilic interaction of connectin cell adhesion molecules in Drosophila, Development (Cambridge, England) 124 (8) (1997) 1433–1441.
[105] A.J. Bieber, P.M. Snow, M. Hortsch, N.H. Patel, J.R. Jacobs, Z.R. Traquina, J. Schilling, C.S. Goodman, Drosophila neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1, Cell 59 (3) (1989) 447–460.
[106] S.G. Hall, A.J. Bieber, Mutations in the Drosophila neuroglian cell adhesion molecule affect motor neuron pathfinding and peripheral nervous system patterning, J. Neurobiol. 32 (3) (1997) 325–340.
[107] M.J. Layden, J.P. Odden, A. Schmid, A. Garces, S. Thor, C.Q. Doe, Zfh1, a somatic motor neuron transcription factor, regulates axon exit from the CNS, Dev. Biol. 291 (2) (2006) 253–263.
[108] M. Landgraf, S. Roy, A. Prokop, K. VijayRaghavan, M. Bate, even-skipped determines the dorsal growth of motor axons in Drosophila, Neuron 22 (1) (1999) 43–52.
[109] M, B.C. Fujioka, M. Lear, G.L. Landgraf, J. Yusibova, K.M. Zhou, N.H. Riley, J.B. Patel, Jaynes, Even-skipped, acting as a repressor, regulates axonal projections in Drosophila, Development 130 (22) (2003) 5385–5400.
[110] A. Garces, S. Thor, Specification of Drosophila aCC motoneuron identity by a genetic cascade involving even-skipped, grain and zfh1, Development 133 (8) (2006) 1445–1455.
[111] S. Thor, J.B. Thomas, The Drosophila islet gene governs axon pathfinding and neurotransmitter identity, Neuron 18 (3) (1997) 397–409.
[112] S. Thor, S.G. Andersson, A. Tomlinson, J.B. Thomas, A LIM-homeodomain combinatorial code for motor-neuron pathway selection, Nature 397 (6714) (1999) 76–80.
[113] H.T. Broihier, J.B. Skeath, Drosophila homeodomain protein dHb9 directs neuronal fate via crossrepressive and cell-nonautonomous mechanisms, Neuron 35 (1) (2002) 39–50.
[114] H.T. Broihier, A. Kuzin, Y. Zhu, W. Odenwald, J.B. Skeath, Drosophila homeodomain protein Nkx6 coordinates motoneuron subtype identity and axonogenesis, Development 131 (21) (2004) 5233–5242.
[115] M.J. Cheesman, T. Layden, C.Q. Von Ohlen, J.S. Doe, Zebrafish and fly Nkx6 proteins have similar CNS expression patterns and regulate motoneuron formation, Development (Cambridge, England) 131 (21) (2004) 5221–5232.
[116] S. Odden, C.Q. Holbrook, Drosophila HB9 is expressed in a subset of motoneurons and interneurons, where it regulates gene expression and axon pathfinding, J. Neurosci. 22 (21) (2002) 9143–9149.
[117] S.J. Certel, S. Thor, Specification of Drosophila motoneuron identity by the combinatorial action of POU and LIM-HD factors, Development (Cambridge, England) 131 (21) (2004) 5429–5439.
[118] M. Landgraf, S. Thor, Development of Drosophila motoneurons: specification and morphology, Semin. Cell Dev. Biol. 17 (1) (2006) 3–11.
[119] J. Oyallon, H. Apitz, I. Miguel-Aliaga, K. Timofeev, L. Ferreira, I. Salecker, Regulation of locomotion and motoneuron trajectory selection and targeting by the Drosophila homolog of Olig family transcription factors, Dev. Biol. 369 (2) (2012) 261–276.
[120] C. Hessinger, G.M. Technau, A. Rogulja-Ortmann, The Drosophila Hox gene Ultrabithorax acts in both muscles and motoneurons to orchestrate formation of specific neuromuscular connections, Development 144 (1) (2017) 139–150.
[121] M. Inaki, S. Yoshikawa, J.B. Thomas, H. Aburatani, A. Nose, Wnt4 is a local repulsive cue that determines synaptic target specificity, Curr. Biol. 17 (18) (2007) 1574–1579.

Please cite this article in press as: A. Arzan Zarin, J.-P. Labrador, Motor axon guidance in *Drosophila*, Semin Cell Dev Biol (2018), https://doi.org/10.1016/j.semcdb.2017.11.013

[122] J. Asadzadeh, N. Neligan, J.J. Canabal-Alvear, A.C. Daly, S.G. Kramer, J.-P. Labrador, The unc-5 receptor is directly regulated by tinman in the developing drosophila dorsal vessel, PLoS One 10 (9) (2015) e0137688.
[123] J. Asadzadeh, N. Neligan, S.G. Kramer, J.-P. Labrador, Tinman regulates NetrinB in the cardioblasts of the drosophila dorsal vessel, PLoS One 11 (2) (2016) e0148526.
[124] V. Wolfram, T. Southall, C. Günay, A.A. Prinz, A.H. Brand, R.A. Baines, The transcription factors islet and lim3 combinatorially regulate ion channel gene expression, J. Neurosci. 34 (7) (2014) 2538–2543.
[125] V. Wolfram, T.D. Southall, A.H. Brand, R.A. Baines, The LIM-Homeodomain protein islet dictates motor neuron electrical properties by regulating K(+) channel expression, Neuron 75 (4) (2012) 663–674.
[126] H. Lacin, J. Rusch, R.T. Yeh, M. Fujioka, B.A. Wilson, Y. Zhu, A.A. Robie, H. Mistry, T. Wang, J.B. Jaynes, J.B. Skeath, 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 (1) (2014) 117–133.
[127] C.S. Goodman, Mechanisms and molecules that control growth cone guidance, Annu. Rev. Neurosci. 19 (1996) 341–377.
[128] C.J. Desai, N.X. Krueger, H. Saito, K. Zinn, Competition and cooperation among receptor tyrosine phosphatases control motoneuron growth cone guidance in Drosophila, Development (Cambridge, England) 124 (10) (1997) 1941–1952.

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