Roles for miRNAs in Timing Developmental Progression Within Nervous Systems

Jay Z. Parrish, PhD

Department of Biology
University of Washington
Seattle, Washington

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Introduction

Building an organism requires precisely timed execution of countless developmental events. The nervous system, with its highly connected network of diverse cell types, provides a quintessential example of this complexity. Compounding the developmental complexity of the nervous system, the morphological and functional properties of a given neuron change dramatically over time as it differentiates and later maintains differentiation. Neuron differentiation involves numerous distinctive, highly regulated events, including cell migration, specification of an axon and dendrites, axon guidance, elaboration of dendritic fields, and synaptogenesis. Further, some neurons undergo extensive refinement, including synapse stabilization, synaptic homeostasis, maintenance of axonal and dendritic arbors, and local pruning/remodeling, to name a few. The problem of timing developmental events is further complicated by the necessity for compartment (axon/dendrite) and subcompartment (i.e., apical/basal dendrites)–specific programs. How these events are executed in precise order in a given cell, and how they coordinate the development of surrounding tissue, is largely unknown.

miRNAs as Genetic Switches

Studies of developmental timing have been particularly successful in organisms that progress through well-defined developmental stages, such as *Caenorhabditis elegans*, in which the first microRNAs (miRNAs) were identified as heterochronic mutants (Moss, 2007). Loss-of-function mutations in *lin-4* or *let-7* cause global defects in timing, leading to reiteration of early cell division patterns in later stages; in contrast, gain of function of either miRNA causes precocious division patterns. These results demonstrate that miRNAs can function as genetic switches that globally regulate developmental progression. An unresolved question is whether miRNAs similarly function to regulate developmental timing in neurons. The question is difficult to answer, in part because developmental progression in defined neurons has not been extensively documented *in vivo*. However, transplantation and heterochronic co-culture experiments have shown that competency for particular developmental events is time-delimited within a given neuron, in part because neurons develop over extended periods of time in the presence of, and influenced by, diverse populations of neurons and nonneuronal cells.

From a mechanistic perspective, how do miRNA-based genetic switches function? Conventional wisdom holds that miRNAs function in two main capacities: as rheostats, finely tuning the levels of one or a few primary targets whose dose is critically important, and as molecular switches, which regulate entire developmental programs to toggle between two states, a phenomenon referred to as "bistability." In their function as molecular switches, miRNAs often target transcription factors (TFs), allowing the latter's effects to be amplified (Simon, 2010); the interaction between miRNAs and TFs often involves feedback or feed-forward loops (Tsang et al., 2007; Flynt and Lai, 2008; Simon, 2010). miRNAs that function as genetic switches would seem to fit into the molecular switch class, but the site of action of the miRNA is another important factor to consider. For example, miRNAs functioning nonautonomously could tune the expression of a single gene, such as a growth factor, to trigger a developmental transition in the neuron.

miRNAs in Neuronal Development

Observations that miRNAs are broadly expressed in the nervous system provided the first indications that miRNAs play important roles in neuronal development. Although researchers have not described the complement of miRNAs expressed over developmental time for defined classes of neurons *in vivo*, temporally restricted patterns of expression have been documented for many miRNAs (Kosik and Krichevsky, 2005). Additionally, some miRNAs are enriched in dendrites (Kye et al., 2007); given that axons and dendrites of an individual neuron develop at different times, compartment-specific regulation of developmental timing might be possible. Localization of miRNAs to specific domains of dendrites might facilitate temporally uncoupled development of different dendritic compartments, as with the apical and basal arbors of pyramidal neurons. miRNAs expressed in nonneuronal cells might regulate developmental progression of neurons as well.

In their role as heterochronic genes in *C. elegans*, *lin-4*, and *let-7* family miRNAs function globally (Moss, 2007). However, if miRNAs are to function as timers in the developmental progression of neurons, they must show dynamic and/or localized expression: They may be transiently expressed globally or within a neuron, in surrounding cells, or in cells that neurons contact a given time. How is this dynamic expression regulated? This question is still largely unanswered, but one recent study suggests that miRNA catabolism is likely a particularly important component of this regulation. miRNAs appear to turn over at a higher rate in neurons than in other cell types (Krol et al., 2010), and this increased turnover is dependent on activity and age, at least in cultured neurons. These findings suggest that

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miRNA catabolism may be particularly adapted for a certain level of maturity or connectivity. However, additional regulatory mechanisms likely influence miRNA levels in neurons because only a subset of miRNAs appear to be subject to this rapid turnover, and only a subset of neurons display this increased rate of miRNA turnover. Target mRNA levels may also influence miRNA levels in neurons, as target mRNAs can stabilize miRNAs in *C. elegans* cell lysates (Chatterjee and Grosshans, 2009). Thus, the interplay among miRNA expression levels, catabolism, and mRNA target levels likely provides the basis for a highly tuned mechanism for timing the progression of neuronal development.

In the spirit of "following the phenotype," this chapter will focus on recent in vivo loss-of-function studies demonstrating that miRNAs function as timers to regulate multiple, distinct aspects of neuronal development. Because several recent reviews have dealt with the roles miRNAs play in dendritic spine morphogenesis, synapse formation, and homeostasis (Schratt, 2009), I will largely omit these topics from my discussion. Additionally, although this chapter focuses on the roles of miRNAs in neuron morphogenesis, it seems likely that miRNAs may regulate transitions in functional properties of neurons, such as changes in ion-channel expression (Okaty et al., 2009). The sections that follow will highlight studies showing that miRNAs can function as timers regulating the acquisition of particular neuronal fates. They do so in part by timing cell cycle progression, regulating postmitotic switches in neuronal and glial growth, and regulating the timing of neuronal remodeling.

Timing of Birth Order: Temporally Regulated Transcription Factors

Neurons derived from a common pool of progenitors often adopt distinct cell fates according to their birth order; thus, the timing of progenitor division is tightly regulated (Kao and Lee, 2010). Birth-dating experiments have established that cortical layers are populated in a temporal sequence (Berry and Rogers, 1965): Cortical neurons in a given layer are born at the same time and share many morphological and functional properties, including layer-specific projection patterns (McConnell, 1988; Kao and Lee, 2010). How timing and birth order are determined is still largely unresolved, but studies in *Drosophila* embryonic neuroblasts (NBs) have defined one paradigm: temporally regulated expression of transcription factors.

Drosophila NBs give rise to different types of motor neurons (MNs) in a stereotyped temporal sequence, and these MNs' temporal identity is determined in part by sequential and transient expression of TFs (Brody and Odenwald, 2005). Temporally regulated TFs appear to regulate the temporal identity of some vertebrate neural progenitors as well. For example, *Ikaros*, a mouse ortholog of *Hunchback* (one of the TFs conferring temporal identity on *Drosophila* NBs), is sufficient to drive early-born cell fates in retinal progenitors (Elliott et al., 2008). And during mammalian cerebral corticogenesis, the TF *Foxg1* regulates the competence of neural precursors by suppressing the generation of the earliest born neurons, Cajal-Retzius cells (Hanashima et al., 2004). One critical question about this paradigm is how the timing of TF accumulation is regulated. Developmental studies using the *Xenopus* retina elegantly illustrate how miRNA-mediated regulation of cell cycle length can time TF accumulation in order to influence neuronal type.

Similar to *Drosophila* NBs, retinal progenitor cells generate different types of neurons (ganglion, horizontal, cone, amacrine, rod, and bipolar) over a conserved time order (Livesey and Cepko, 2001), and different homeobox TFs are important for generation of distinct retinal cell types (Decembrini et al., 2006). How is timely expression of these homeobox TFs achieved? Expression of at least three homeobox TFs involved in fate specification (*XOtx5b*, *Xvsx1*, and *XOtx2*) is posttranscriptionally regulated as follows: Each is transcribed in early progenitors but not translated until later stages, the 3′UTR of each is sufficient to direct time-dependent inhibition of translation, and inactivating *Dicer* in retinal precursors mitigates translation inhibition in these TFs (Decembrini et al., 2008). Thus, it can be seen that miRNAs regulate the timely translation of these TFs. These findings raise an additional question: namely, how timing of miRNA function is regulated in progenitor cells. Cell cycle length of retinal progenitors increases over time (Alexiades and Cepko, 1996), so cell cycle length could contribute to the timing mechanism for miRNA function. Indeed, the expression of *XOtx5b*, *Xvsx1*, and *XOtx2* is linked to cell cycle progression and not to absolute time; deregulating cell cycle length prevents timely translation of these TFs and dissociates birth date from cell fate, generating heterochronic phenotypes (Decembrini et al., 2006). Molecularly, four miRNAs that are specifically expressed (by an unknown mechanism) in early progenitors comprise one portion of this timer by blocking the translation of *XOtx2* and *Xvsx1*, which promote bipolar cell fates in late stage precursors. By extension, miRNAs specific to each stage of retinal (and other) precursors could shape neuronal fates.

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Timing of Stage-Specific Neuronal Growth Programs

Postmitotic neurons often display stage-specific developmental programs such as the establishment and subsequent maintenance and refinement of dendritic coverage (Parrish et al., 2007a). The dendrites of some neurons provide complete, nonredundant coverage of the receptive field, a phenomenon referred to as "dendritic tiling," which is most likely a way to ensure unambiguous representation of the receptive field (Grueber and Sagasti, 2010). In vertebrates and insects, sensory neurons establish tiling of their receptive field early in development and subsequently maintain it, doing so even as the animal grows in size (Bloomfield and Hitchcock, 1991; Parrish et al., 2007a). In the context of development, this presents two very different growth paradigms:

- Initially, dendrites must outpace the growth of their substrate in order to establish coverage of their territory; and
- Subsequently, dendrite growth must be synchronized with substrate growth to maintain tiling.

Drosophila class IV dendrite arborization neurons illustrate this twofold process. They first tile the larval body wall, establishing tiling at approximately the first/second larval instar transition. They subsequently maintain tiling by growing in precise proportion to their substrate: the underlying body wall epithelium (Parrish et al., 2009). This scaling growth of dendrites is manifest in other sensory neurons as well, including those that do not tile, as well as in insect and mouse MNs (Truman and Reiss, 1988; Li et al., 2005), suggesting that it might be a broadly utilized mode of late-stage dendrite growth. As evidence for this theory, loss-of-function mutations in the miRNA *bantam* cause deregulated late-stage dendrite growth of sensory neurons without affecting axon development or early stages of dendrite development. By contrast, *bantam* activity is both necessary and sufficient in the substrate, epithelial cells to trigger the developmental switch from expansive to scaling growth in sensory neurons.

Several lines of evidence point to this transition as being a bona fide developmental step:

1. The structural plasticity of dendrites changes drastically during this transition (Parrish et al., 2009);
2. Distinct genetic pathways regulate the early-growth and late-growth phases of these dendrites (Emoto et al., 2006; Parrish et al., 2007b); and
3. Sensory neurons undergo extensive changes in gene expression patterns at the time of this transition (C. Kim and J. Parrish, unpublished observations).

Notably, in *bantam* mutants, these dendrites inappropriately retain high levels of structural plasticity and other early larval dendrite growth properties.

How is *bantam* regulating this developmental transition? Although the timing mechanism is unknown, *bantam* activity is significantly upregulated at the first-to-second instar larval transition, so hormonal signals that regulate larval molts could contribute to timing (Parrish et al., 2009). Furthermore, whether *bantam* is transcriptionally induced (similar to the way other miRNAs regulate timing) or posttranscriptionally regulated is unknown. One intriguing finding from these studies is that *bantam* regulates the developmental timing of neurons that switch from expansive to scaling dendrite growth at the first-to-second instar larval transition but not in neurons that undergo growth transitions at other times. These findings suggest that other timers (possibly involving other miRNAs) regulate transitions in neurons developing over different time registers. As for *bantam*'s mechanism of action, clonal analysis suggests that *bantam* likely regulates short-range signals that in turn regulate local dendrite-epithelial interactions. Although many questions remain unanswered about how *bantam* regulates the timing of this developmental transition in sensory neurons, these observations demonstrate that miRNAs can coordinate the growth of neurons and their substrate.

One recent report suggests that, as in the sensory neurons described above, miRNAs can regulate stage-specific growth of glia. Schwann cells (SCs) arise from neural crest cells, migrate to surround groups of peripheral nervous system (PNS) axons, and progressively single out PNS axons in a process known as "radial sorting" (Jessen and Mirsky, 2005; Pereira et al., 2010). Subsequently, an SC attaches to the selected axons and alters its gene expression program, in part by activating the expression of the TF *Krox20*, to facilitate production of myelin. In the absence of *Dicer*, most SCs arrest at the promyelinating stage and fail to form myelin; however, the remaining SCs migrate, sort out, and engage axons, demonstrating that the initial stages of SC development are unaffected. At a molecular level, induction of the master regulator of myelin formation *Krox20* and several myelin proteins was not properly accomplished in *Dicer*$^{-/-}$ SCs. The timing of this transition seems to imply that

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axon-derived (perhaps adhesion-based) signaling might be involved in the miRNA switching on of SC growth.

miRNAs as Regulators of Neuronal Remodeling

Following the development of axonal/dendritic arbors, many neurons refine their arbors. One of the most striking examples of this remodeling occurs during insect metamorphosis, when larval-specific processes are pruned away and subsequently replaced by adult-specific processes. Metamorphosis represents a global developmental transition during which most tissues undergo extensive remodeling. Therefore, perhaps it should come as no surprise that the *Drosophila* homologs of the C. *elegans* heterochronic miRNAs *let-7* and *lin-4* (*let-7* and *mir-125* in *Drosophila*) are required for proper timing of metamorphic events.

The expression of *let-7* complex (*let-7*C) miRNAs (*mir-100*, *let-7*, and *mir-125*) is upregulated during the first half of metamorphosis in neurons that broadly innervate the adult, including MNs, and in muscle (Sokol et al., 2008). Consistent with the timing of expression, *let-7*C activity appears dispensable for embryonic and larval development (Caygill and Johnston, 2008). On the other hand, l*et-7*C mutants fail to complete the remodeling of the neuromuscular system that normally occurs during metamorphosis: Larval abdominal muscles that should decay during posteclosion maturation fail to completely disappear, adult muscles are smaller than normal, larval-specific neuromuscular junctions (NMJs) are incompletely pruned, and growth of adult-specific NMJs is stunted (Caygill and Johnston, 2008; Sokol et al., 2008). These effects argue that *let-7*C miRNAs regulate the transition from larva to adult in the neuromuscular system.

How does *let-7* regulate developmental progression in the neuromuscular system? The answer to this question has two parts. First, similar to other miRNAs that regulate developmental transitions, *let-7* likely targets a TF, *abrupt* (*ab*), to regulate remodeling of the neuromuscular system (Caygill and Johnston, 2008). As expected for a *let-7* target, Ab protein accumulates in abdominal muscles in the absence of *let-7*, whereas Ab protein is not detectible in MNs of wild-type or *let-7* mutant flies. Although the relative contribution that *let-7* in muscle and MNs makes to the NMJ defects is unknown, reducing the gene dosage of *ab* was sufficient to partially ameliorate the heterochronic phenotypes of *let-7*, *mir-125* mutants. Furthermore, *let-7* overexpression is sufficient to dampen endogenous expression of Ab, albeit in a different tissue. Whether or not Ab levels contribute to the muscle defects of *let-7*C mutants remains to be seen. Altogether, these results suggest that *let-7* regulates developmental timing of the neuromuscular system in *Drosophila*, at least in part, by regulating levels of Ab.

The second component of the answer as to how timing of *let-7* expression is regulated is even less clear at this point. Although *let-7*C miRNAs can be induced by the nuclear hormone ecdysone in *Drosophila* cell culture, the specifics of hormone-dependent expression have been less well defined in vivo. Given that the nuclear hormone receptor DAF12 positively (presence of ligand) and negatively (absence of ligand) regulates *let-7* expression (Bethke et al., 2009) and that *let-7* regulates *daf-12*, providing feedback inhibition of the switch (Hammell et al., 2009), it seems plausible that a hormone-coupled molecular switch might gate *let-7* expression in flies as well.

let-7 and *lin-4* regulate major developmental transitions during C. *elegans* development by regulating stage-specific patterns of cell division. Do they also regulate developmental transitions in postmitotic neurons in C. *elegans*, as in *Drosophila*? Although the question hasn't been directly addressed, one report implies a role for *lin-4* in regulating postmitotic synaptic remodeling in C. *elegans* (Hallam and Jin, 1998). During larval development, six GABAergic MNs remodel their patterns of connectivity during larval development, completely reversing the direction of information flow; *lin-14* is required for this remodeling. In *lin-14* mutants, MNs remodel precociously. Given that *lin-14* is one of the major *lin-4* targets in lineage decisions, including those that give rise to mechanosensory neurons (Mitani et al., 1993), it seems plausible that *lin-4* might function as a developmental switch in this context as well.

Just as miRNAs regulate a late-stage developmental switch in the *Drosophila* neuromuscular system, *miR-206* regulates a late aspect of development in the mammalian neuromuscular system (Williams et al., 2009). At birth, muscle fibers in mice are multiply innervated by different MNs, but spurious inputs are developmentally eliminated; those that are spared are strengthened (Sanes and Lichtman, 2001). Subsequently, injured muscle can be re-innervated, but innervated muscle does

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not get multiply innervated. In a mouse model of amyotrophic lateral sclerosis (ALS) (Gurney et al., 1994), expression of the muscle-specific *miR-206* is dramatically upregulated—a consequence of muscle denervation (Williams et al., 2009). Denervation-dependent induction of the TF *MyoD* leads to upregulation of *miR-206* in muscle, where *miR-206* inhibits translation of the histone deacetylase *HDAC4*; this inhibition, in turn, leads to the de-repression of *fibroblast growth factor–binding protein 1* (*FGFBP1*), a secreted factor that potentiates the effects of FGFs during regeneration. Mice that are deficient for *miR-206* form normal neuromuscular synapses during development but fail to regenerate neuromuscular synapses after acute nerve injury. Therefore, in response to environmental signals (denervation), *miR-206* regulates the release of retrograde signals to potentiate a specialized growth program (re-innervation). Similar to some of the examples cited above, *miR-206* is transcriptionally upregulated, providing the basis for timing, and exerts its effect by inhibiting the expression of a transcriptional regulator.

Perspectives

What do these examples tell us? First, that miRNAs can clearly function as switches to regulate developmental progression in the nervous system, including postmitotic developmental transitions. Even within this small number of examples, trends have emerged:

- miRNAs that regulate developmental progression are often transcriptionally regulated, and those functioning nonautonomously have an additional layer of regulation: environmental cues;
- Many miRNAs appear to primarily target TFs, although miRNAs that function nonautonomously likely encounter an additional time lag because they regulate secreted factors; and
- Consistent with the intimate link between miRNAs regulating developmental progression and TFs, recent studies have shown that transcriptional changes accompany functional maturation of some neurons (Tropea et al., 2006; Okaty et al., 2009).

Where do we go from here? A good starting point would be comprehensive identification of the miRNAs expressed by identified neurons over a developmental time course, coupled with characterization of the roles of miRNAs at different developmental time points in identified neurons (perhaps by conditional knockout of *Dicer*). In principle, this would allow us to systematically identify miRNA-regulated developmental transitions and the miRNAs that regulate them.

References

Alexiades MR, Cepko C (1996) Quantitative analysis of proliferation and cell cycle length during development of the rat retina. Dev Dyn 205:293-307.

Berry M, Rogers AW (1965) The migration of neuroblasts in the developing cerebral cortex. J Anat 99:691-709.

Bethke A, Fielenbach N, Wang Z, Mangelsdorf DJ, Antebi A (2009) Nuclear hormone receptor regulation of microRNAs controls developmental progression. Science 324:95-98.

Bloomfield SA, Hitchcock PF (1991) Dendritic arbors of large-field ganglion cells show scaled growth during expansion of the goldfish retina: a study of morphometric and electrotonic properties. J Neurosci 11:910-917.

Brody T, Odenwald WF (2005) Regulation of temporal identities during *Drosophila* neuroblast lineage development. Curr Opin Cell Biol 17:672-675.

Caygill EE, Johnston LA (2008) Temporal regulation of metamorphic processes in *Drosophila* by the *let-7* and *miR-125* heterochronic microRNAs. Curr Biol 18:943-950.

Chatterjee S, Grosshans H (2009) Active turnover modulates mature microRNA activity in *Caenorhabditis elegans*. Nature 461:546-549.

Decembrini S, Andreazzoli M, Vignali R, Barsacchi G, Cremisi F (2006) Timing the generation of distinct retinal cells by homeobox proteins. PLoS Biol 4:e272.

Decembrini S, Andreazzoli M, Barsacchi G, Cremisi F (2008) *Dicer* inactivation causes heterochronic retinogenesis in *Xenopus laevis*. Int J Dev Biol 52:1099-1103.

Elliott J, Jolicoeur C, Ramamurthy V, Cayouette M (2008) Ikaros confers early temporal competence to mouse retinal progenitor cells. Neuron 60:26-39.

Emoto K, Parrish JZ, Jan LY, Jan Y (2006) The tumour suppressor Hippo acts with the NDR kinases in dendritic tiling and maintenance. Nature 443:210-213.

Flynt AS, Lai EC (2008) Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 9:831-842.

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Grueber WB, Sagasti A (2010) Self-avoidance and tiling: mechanisms of dendrite and axon spacing. Cold Spring Harb Perspect Biol 2:a001750.

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX (1994) Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 264:1772-1775.

Hallam SJ, Jin Y (1998) *lin-14* regulates the timing of synaptic remodelling in *Caenorhabditis elegans*. Nature 395:78-82.

Hammell CM, Karp X, Ambros V (2009) A feedback circuit involving let-7-family miRNAs and DAF-12 integrates environmental signals and developmental timing in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 106:18668-18673.

Hanashima C, Li SC, Shen L, Lai E, Fishell G (2004) *Foxg1* suppresses early cortical cell fate. Science 303:56-59.

Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 6:671-682.

Kao C, Lee T (2010) Birth time/order-dependent neuron type specification. Curr Opin Neurobiol 20:14-21.

Kosik KS, Krichevsky AM (2005) The elegance of the microRNAs: a neuronal perspective. Neuron 47:779-782.

Krol J, Busskamp V, Markiewicz I, Stadler MB, Ribi S, Richter J, Duebel J, Bicker S, Fehling HJ, Schübeler D, Oertner TG, Schratt G, Bibel M, Roska B, Filipowicz W (2010) Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. Cell 141:618-631.

Kye M, Liu T, Levy SF, Xu NL, Groves BB, Bonneau R, Lao K, Kosik KS (2007) Somatodendritic microRNAs identified by laser capture and multiplex RT-PCR. RNA 13:1224-1234.

Li, Y, Brewer, D, Burke, RE, and Ascoli, GA (2005) Developmental changes in spinal motoneuron dendrites in neonatal mice. J Comp Neurol 483:304-317.

Livesey FJ, Cepko CL (2001) Vertebrate neural cell-fate determination: lessons from the retina. Nat Rev Neurosci 2:109-118.

McConnell SK (1988) Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation. J Neurosci 8:945-974.

Mitani S, Du H, Hall DH, Driscoll M, Chalfie M (1993) Combinatorial control of touch receptor neuron expression in *Caenorhabditis elegans*. Development 119:773-783.

Moss EG (2007) Heterochronic genes and the nature of developmental time. Curr Biol 17:R425-R434.

Okaty BW, Miller MN, Sugino K, Hempel CM, Nelson SB (2009) Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. J Neurosci 29:7040-7052.

Parrish JZ, Emoto K, Jan LY, Jan YN (2007a) Polycomb genes interact with the tumor suppressor genes *hippo* and *warts* in the maintenance of *Drosophila* sensory neuron dendrites. Genes Dev 21:956-972.

Parrish JZ, Emoto K, Kim MD, Jan YN (2007b) Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields. Annu Rev Neurosci 30:399-423.

Parrish JZ, Xu P, Kim CC, Jan LY, Jan YN (2009) The microRNA *bantam* functions in epithelial cells to regulate scaling growth of dendrite arbors in *Drosophila* sensory neurons. Neuron 63:788-802.

Pereira JA, Baumann R, Norrmén C, Somandin C, Miehe M, Jacob C, Lühmann T, Hall-Bozic H, Mantei N, Meijer D, Suter U (2010). *Dicer* in Schwann cells is required for myelination and axonal integrity. J Neurosci 30:6763-6775.

Sanes JR, Lichtman JW (2001) Induction, assembly, maturation and maintenance of a postsynaptic apparatus. Nat Rev Neurosci 2:791-805.

Schratt G (2009) microRNAs at the synapse. Nat Rev Neurosci 10:842-849.

Simon DJ (2010) Interactions between microRNAs and transcription factors. In: Macro-roles for microRNAs in the life and death of neurons (de Strooper B, Christen Y, eds), pp 19-26. Berlin: Springer.

Sokol NS, Xu P, Jan Y, Ambros V (2008) *Drosophila let-7* microRNA is required for remodeling of the neuromusculature during metamorphosis. Genes Dev 22:1591-1596.

Tropea D, Kreiman G, Lyckman A, Mukherjee S, Yu H, Horng S, Sur M (2006) Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex. Nat Neurosci 9:660-668.

Truman, JW, and Reiss, SE (1988) Hormonal regulation of the shape of identified motoneurons in the moth *Manduca sexta*. J Neurosci 8:765-775.

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Tsang J, Zhu J, van Oudenaarden A (2007) MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. Mol Cell 26:753-767.

Viczian AS, Vignali R, Zuber ME, Barsacchi G, Harris WA (2003) *XOtx5b* and *XOtx2* regulate photoreceptor and bipolar fates in the *Xenopus* retina. Development 130:1281-1294.

Williams AH, Valdez G, Moresi V, Qi X, McAnally J, Elliott JL, Bassel-Duby R, Sanes JR, Olson EN (2009) MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326:1549-1554.

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