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PERSPECTIVE

spineless provides a little backbone for dendritic morphogenesis

Stephen T. Crews[1,3] and Jay E. Brenman[2]

[1]Department of Biochemistry and Biophysics, Department of Biology, and Program in Molecular Biology and Biotechnology, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599, USA; [2]Department of Cell and Developmental Biology and Neuroscience Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599, USA

I've got my spine, I've got my orange crush.
—REM, "Orange Crush "

In the brain, information processing occurs as synapses relay information through neuronal circuits. Most of these synaptic connections physically form on neuronal dendrites—highly specialized subcellular structures that receive and integrate information. Although most dendrites form connections with other neurons, other dendrites, like those found on sensory neurons, receive information directly from the external environment. Highly elaborate and branched dendrites form expansive surface areas for receiving inputs—a feature that distinguishes neurons as one of the most unique and readily identifiable cell types. However, despite the complexity and dramatic variation among dendritic branching patterns between different neurons, specific neuronal subtypes have remarkably stereotyped dendritic arbors in both vertebrates and invertebrates. This stereotypy suggests a genetic basis, and has led to experimental approaches to identify molecular regulators of dendrite development and morphology. In this article, we discuss new data published by Kim et al. (2006) in this issue of *Genes & Development* on the role of the Spineless (Ss) basic helix–loop–helix-PAS (bHLH-PAS) transcription factor in controlling dendrite morphogenesis, as well as review the contributions of other regulatory proteins.

The *Drosophila* peripheral nervous system (PNS) and dendritic morphology

Both in vitro and in vivo assays have been used to identify genes that affect dendrite development. One attractive system to identify genes that regulate dendrite development is the *Drosophila* PNS. The *Drosophila* PNS contains sensory neurons that are small in number (only 44 per hemisegment), yet they innervate a relatively large epidermal surface area resulting in easily identifiable individual neurons and their dendritic arbors. The development of each sensory organ and constituent neurons has been extensively characterized, and most sensory neurons belong to one of three types. The chordotonal and external sensory organs possess structurally simple neurons containing single dendrites. The multiple dendrite neurons constitute most of the PNS, and include dendritic arborization (da) neurons that display highly branched and elaborate dendrites. The da neurons can be classified into subtypes, each with its own characteristic dendritic tree (Grueber et al. 2002). These are class I, II, III, and IV in order of increasing dendritic complexity (Fig. 1). The class IV da neurons have extensive branching, and more total dendritic branch length than the other three types combined. Class III neurons are unique among da neurons in containing numerous F-actin-rich dendritic filopodia scattered about the dendritic tree. Despite their complex branching, da dendritic morphology is predictable and characteristic. The da neurons possess thermosensory, nociceptive, and rhythmic locomotory functions (Ainsley et al. 2003; Liu et al. 2003; Tracey et al. 2003), and chordotonal and external sensory organs have proprioceptive and mechanoreceptive functions.

Regulation of *Drosophila* dendrite development

Drosophila da dendrite morphogenesis can be divided into two components: an intrinsic program establishing a basic dendritic pattern, and an extrinsic program influenced by support cells (Yamamoto et al. 2006) and competition between dendrites from similar neurons (Grueber and Jan 2004). The phenotypic analyses of a rapidly expanding number of genes that influence dendrite morphogenesis provide evidence that the intrinsic program can be further subdivided into genes that control discrete aspects of dendrite formation. For example, a recent RNA interference (RNAi)-based screen identified 76 transcription factors that influence class I da dendrite structure (Parrish et al. 2006; Tassetto and Gao 2006). These genes could be allocated to three broad groups that control aspects of dendrite patterning: branching, outgrowth (length), and direction. Since all of these genes affect dendrites of the class I ddaD and ddaE neurons, dendritic complexity is likely the consequence of the

[3]**Corresponding author.**
E-MAIL steve_crews@unc.edu; FAX (919) 962-4296.
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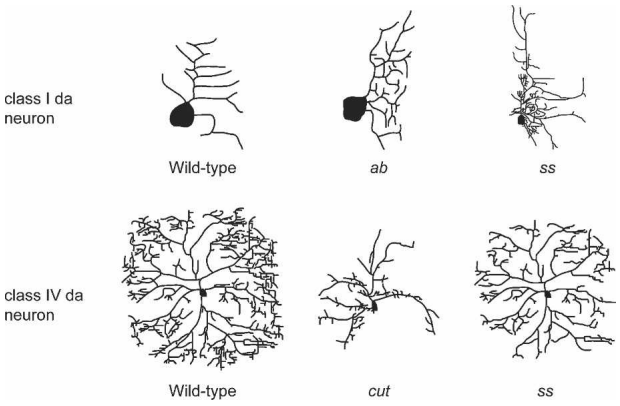

Figure 1. Representative effects of *Drosophila* transcriptional regulators on dendrite morphology. Images represent characteristic class I or class IV da dendrites of wild-type, *ab*, *cut*, or *ss* mutant larvae. Both *ab* and *ss* mutants result in increased complexity of class I da dendrites, and *cut* and *ss* mutants result in decreased complexity of class IV da dendrites. Images are adapted from Grueber et al. (2003a), with permission from Elsevier (© 2003), Sugimura et al. (2004), with permission from Elsevier (© 2004), and Kim et al. (2006).

combined actions of a large number of regulatory genes. In addition, evidence suggests that that the processes of branching and outgrowth are interdependent and require careful coordination (Parrish et al. 2006). Developmental timing can also be important: Mutations in the *sequoia* gene, which encodes a zinc finger protein, result in precocious dendrite extension leading to excessive outgrowth (Brenman et al. 2001). Major questions concern the nature of the effector/target genes that generate the dendritic tree and how the regulatory proteins work together to control their expression.

Mechanistic insight into the transcriptional regulation of dendritic morphology has emerged from detailed analyses of the *abrupt* (*ab*) (Li et al. 2004; Sugimura et al. 2004), *cut* (Grueber et al. 2003a), and *ss* genes (Kim et al. 2006) (see below). Ab is a BTB zinc finger transcription factor that is expressed in class I da neurons, but not class II–IV neurons. Dendrites in wild-type class I neurons are relatively simple, but increase in complexity in *ab* mutants (Fig. 1). Similarly, misexpression of *ab* in class II–IV sensory cells results in a reduction in dendritic complexity. Thus, Ab represents a cell type-specific transcriptional regulator that plays a major role in directing the complexity of that cell's dendritic morphology. In contrast, the *cut* gene also influences dendritic complexity, but is expressed in multiple da classes at different levels. Cut protein levels are lowest or absent in the class I da neuron, which also has the simplest dendritic morphology, and is higher in the more complex da classes. Mutations in *cut*, which encodes a homeobox protein, reduce dendritic complexity of the more complex da dendrites (Fig. 1), and *cut* overexpression increases complexity in classes of low complexity. Thus, Cut is an example of a protein that controls dendritic complexity, but it functions in multiple cell types, and protein levels are critical in regulating complexity. How Cut controls gene expression in a concentration-dependent mode, and what regulates Cut protein levels are key questions.

Regulatory proteins ultimately exert their influence on dendritic morphology by controlling gene expression or function of cell structure proteins. Some of these proteins have been identified, including cytoskeletal regulators such as small GTPases of the Rho family and actin-microtubule cross-linking factors such as Short Stop. However, little is known about the linkage between regulatory protein and effector protein. Another feature of dendritic patterning is how extrinsic interactions between dendrites "tile" dendritic arbors. Tiling was first described in the vertebrate retinal system, and refers to the same neuronal types occupying nonredundant physical space, while different neuronal types can overlap in physical space. In *Drosophila*, da dendrites almost completely cover the outer epidermis, appearing to overlap with each other. Upon careful examination, however, dendrites of the same da class rarely overlap. Experimental analyses indicate that tiling is due to mutual repulsion (Gao et al. 2000; Grueber et al. 2003b; Sugimura et al. 2003). While the molecular basis of this repulsion is largely unknown, this mechanism helps shape dendritic arbors to maximize neural coverage of physical space along the epidermis, while minimizing overlap.

Role of *spineless* in dendritic morphology

Most of the genes affecting *Drosophila* dendrite morphogenesis were identified in genetic or RNAi screens that used GFP visualization of dendrites to visually assess defects. Kim et al. (2006) carried out an EMS mutagenesis for defects in class I da neurons using a sensory neuron GFP reporter. They isolated an allele of *ss*, which showed increased class I dendritic branching and overgrowth (Fig. 1). The *ss* mutant phenotype is specific for dendritic defects, since cell fate, migration, and axonogenesis are relatively normal. Previous work showed that the *ss* gene encodes a bHLH-PAS transcription factor, and is expressed in sensory cells (Duncan et al. 1998), Sensory cell expression of *ss* is consistent with MARCM (mosaic analysis with a repressible cell marker) clonal analysis that demonstrated that the *ss* dendritic defect was cell autonomous. Besides the increase in dendritic branching, the class I dendrites significantly overlapped, indicating an inability of *ss* mutant cells to repel each other. Thus, *ss* represses dendritic complexity and overgrowth in class I da sensory neurons, and promotes homotypic dendrite–dendrite repulsion.

However, *ss* is expressed in all sensory neurons, not just class I cells. This includes the relatively simple class II neurons and the more complex class III and IV cell types. Class II neurons also show increased branching and overgrowth in *ss* mutants, similar to the class I phenotype. However, in a key departure, complexity of the class III and IV dendritic fields were reduced in *ss* mutants (Fig. 1). Not only were the number and size of branches reduced, but the actin-rich dendritic spikes found on class III dendrites were absent. Strikingly, the

class I, II, and III dendrites resembled each other, with class IV dendrites reduced, but still relatively complex. Levels of Ss protein are comparable among the four da cell classes, suggesting that the quantitative differences in branching are not strictly due to Ss levels. Consistent with this, overexpression of *ss* in class I, II, and III neurons did not alter dendritic morphology. Overexpression of *ss* did result in a reduction of class IV dendritic branching. Interestingly, the tracheal dendrite (td) sensory cell that has a simple bipolar dendrite displayed ectopic branching when *ss* was overexpressed, but was unaffected in *ss* mutants. This indicates that different sensory cells can respond in different ways to varying levels of *ss*. Other mutants that affect dendrite formation act on different cell types in a similar way (e.g., more branching, reduced outgrowth). The unique feature of the *ss* phenotype is that mutant da dendrites tend toward a similar, intermediate state of complexity. This also suggests that Ss could be acting as a molecular scaffold in which other transcription factors may interact. Despite a potential role as a major regulator of dendrite-relevant transcription, Ss does not regulate levels of *ab* or *cut*; however, it could function combinatorially with them.

Since all four classes of da dendrites resemble each other in *ss* mutants, Kim et al. (2006) proposed that the *ss* mutant animal may represent a simple, primitive dendritic ground state. In this hypothesis, an ancestral species had sensory cells with a common dendritic pattern. Ultimately, the appearance of *ss* expression allowed the cells to diversify. This is an intriguing hypothesis, even though the data does not fit perfectly to a mutant ground state for all sensory cells (in *ss* mutants, da class IV is still considerably different from the other classes, and mutant dendrites in es and ch sensory neurons are wild-type, and thus completely different than mutant da dendrites). Nevertheless, the hypothesis is potentially testable, if, for example, a species is identified in which dendritic morphologies are similar among da neurons and *ss* expression is absent. Additional questions regarding Ss are: How does Ss function biochemically? What are the Ss target genes that execute its dendritic program?

Genetic and biochemical functions of Ss and Ahr

Mutants of the *ss* gene were first reported by Calvin Bridges, one of the pioneers of *Drosophila* genetics, in 1923. The original phenotype described a reduction in the size of the large adult bristles (hence the name "*spineless*"). Soon afterward, the *spineless-aristapedia* (ss^a) allele was discovered, and a homeotic phenotype was observed in which the distal segment of the antenna was transformed into a distal leg structure. In addition, most of the tarsal region of the leg was deleted. These results provided strong evidence that *ss* is an important developmental regulatory gene. Much more recently, *ss* was shown to regulate the distribution of different ommatidia responsible for fly color vision (Wernet et al. 2006). Added to these multiple and wide-ranging roles of *ss* is the dendritic phenotype described by Kim et al. (2006).

The Ss protein belongs to the bHLH-PAS family of transcription factors, a medium-sized group of proteins (11 members in *Drosophila*) that, nevertheless, carry out a wide variety of regulatory functions (Crews 2003). Ss has been shown, both in vitro and in vivo, to dimerize with the Tango (Tgo) bHLH-PAS protein, and together they activate transcription (Emmons et al. 1999) upon binding an asymmetric E-box sequence, GCGTG (Emmons et al. 1999; Kozu et al. 2006). The *ss* gene is highly conserved throughout the animal kingdom, and there is a single gene in *Caenorhabditis elegans*, *Drosophila*, and vertebrates. Somewhat surprisingly, the vertebrate ortholog is the aryl hydrocarbon receptor (Ahr), best known for its role in regulating the metabolism of xenobiotics, including carcinogenic and toxic compounds. Ahr generally requires the binding of small ligands to function, but to date, there is no in vivo evidence that *Drosophila* Ss functions in a ligand-dependent mode.

Kim et al. (2006) propose that Ss functions in a noncanonical mode in sensory cells that does not include dimerization with Tgo. Previous work indicated that the *tgo* gene is expressed in most, if not all cells (Ward et al. 1998). In the absence of a partner bHLH-PAS protein (Ss, Single-minded, Trachealess, Dysfusion, or Similar), Tgo resides in the cytoplasm at low levels. When a partner bHLH-PAS gene is expressed in a cell, it dimerizes with Tgo, and the complex translocates and accumulates in nuclei (Ward et al. 1998). Thus, the observation of nuclear Tgo in a cell is generally an indicator that it is actively functioning there in combination with a partner bHLH-PAS protein. Several lines of evidence indicate that Ss and Tgo commonly interact in vivo. Mosaic analysis of *tgo* mutants indicated that *ss* defects in antennal, bristle, and tarsal development were mimicked by loss of *tgo* function (Emmons et al. 1999), and ectopic transformation of wing tissue to leg/antennal tissue by *ss* requires *tgo* (Adachi-Yamada et al. 2005). In addition, the occurrence of nuclear Tgo in embryonic cephalic cells that also express *ss*, and induction of nuclear Tgo in ectodermal and mesodermal cells in which *ss* was ectopically expressed (Emmons et al. 1999), strongly suggest that Ss commonly partners with Tgo. In contrast, Kim et al. (2006) did not observe *ss*-like dendritic phenotypes in *tgo* mutant sensory neuron clones, or observe nuclear Tgo in wild-type Ss-positive sensory neurons, suggesting that Tgo does not partner with Ss in sensory cells. It was previously observed that *ss* embryonic expression in the limb primordia also did not show detectable nuclear Tgo (Emmons et al. 1999), which fits with the observations of Kim et al. (although this same paper, in contrast to Kim et al., provided some evidence for the occurrence of nuclear Tgo in sensory cells). Cell culture studies implicated Ss and Tgo in controlling the transcription of a gene, *CYP6B1*, that is induced by xanthotoxin, a plant toxin (Brown et al. 2005). Introduction of either *ss* or *tgo* alone influenced *CYP6B1* transcription, and each had different effects on basal and inducible transcription. Consequently, these experiments were interpreted as Ss and Tgo working independently. While intriguing, these results need to be confirmed using genetic and transgenic approaches in vivo.

Recent work has provided additional mechanistic insights into how Ss can function. Analysis of *ss* in eye development indicated that it was regulated in a stochastic manner, and that the levels of Ss protein dictated whether an ommatidium was pale (detects short-wavelength light) or yellow (detects longer-wavelength light) (Wernet et al. 2006). This result indicated that absolute levels of Ss can influence gene regulation. It is unknown whether retinal Ss function utilizes Tgo or operates in a noncanonical manner. It will be interesting to learn more about how *ss* is stochastically regulated, and how different levels of Ss can influence different patterns of retinal transcription. In conclusion, an emerging theme is that Ss employs a variety of biochemical mechanisms to control a multiplicity of developmental and physiological processes.

Vertebrate Ahr

Ahr generally functions as a ligand-dependent transcription factor (for review, see Yao et al. 2003; Fujii-Kuriyama and Mimura 2005). Activity-inducing ligands include polycyclic aromatic hydrocarbons, including the potent carcinogen 3-methylcholanthrene, and halogenated aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin). Unliganded Ahr resides in the cytoplasm in combination with a trio of chaperones. Upon cellular exposure to ligand, the hydrocarbon ligand diffuses into the cell, and binds Ahr, which releases the Ahr from its accessory proteins. Ligand-bound Ahr translocates into the nucleus where it dimerizes with Aryl hydrocarbon nuclear translocator (Arnt), which is the ortholog of *Drosophila* Tgo. Ahr:Arnt bind GCGTG sequences (referred to as xenobiotic-responsive elements, XREs) on target genes, which include members of the *CYP1* family of cytochrome P450 metabolic enzymes and additional genes involved in xenobiotic metabolism.

The major emphasis of work on Ahr is toxicology, and this heavily involves its relationship with dioxin. Dioxin has a high affinity for Ahr, and induces a strong target gene transcriptional response. Dioxin is an industrial byproduct, and is notorious for its contamination of the widely disseminated Vietnam War defoliant "Agent Orange," toxicity testing on "volunteers" at a county prison in Holmesburg, PA, and the poisoning of Ukrainian president, Viktor Yushchenko. Mr. Yushchenko's face is heavily scarred with chloracne, the characteristic external feature of dioxin poisoning. Both fetal and adult exposure to elevated levels of dioxin leads to multiple organ defects, including cleft palate, gonadal atrophy, and lymphoid tissue degeneration (Birnbaum 1995), and increased dioxin and polychlorinated biphenyl levels positively correlate with a variety of neural and other tissue abnormalities (ten Tusscher and Koppe 2004). It is generally thought that these effects are due to activation of Ahr. Nevertheless, despite the intense amount of work devoted to the study of Ahr and the biological effects of its ligands, its natural functions remain murky. Since dioxin and other relevant polycyclic hydrocarbons are recent, man-made chemicals, it is assumed that the evolutionarily relevant xenobiotic role of Ahr is to mediate detoxification of plant toxins. However, there appear to be additional, developmental roles of *Ahr*. Mice mutant for *Ahr* show vascular defects, which can lead to organ size reduction due to insufficient blood flow (Lahvis et al. 2000). Additional work indicated that Ahr negatively regulates TGF-β signaling, leading to increased hepatocyte apoptosis and fibrosis in *Ahr* mutant mice (Zaher et al. 1998). Ahr also interacts with cell cycle proteins in embryonic fibroblasts, and is required for normal proliferation (Elizondo et al. 2000). What is not known is whether *Ahr* plays a role in nervous system development or function, although it is widely expressed in the brain (Petersen et al. 2000).

Important questions regarding Ahr function include discovering endogenous ligands that may control the developmental and physiological functions of Ahr, and whether Ahr can function in ligand-independent, noncanonical modes. Indeed, a number of endogenous substances have been proposed as Ahr ligands, including bilirubin, tryptophan derivatives, and prostaglandins (Fujii-Kuriyama and Mimura 2005). Although their biological functions with respect to Ahr are unknown, their occurrence raises the possibility that cell-type or tissue-specific ligands may differentially affect Ahr function. In another report, Ahr inhibited progression into S phase by displacing components of the E2F transcription complex, and thus repressed E2F-dependent gene transcription (Marlowe et al. 2004). It was proposed that this process was independent of ligand binding, and did not require either Arnt or DNA binding.

C. elegans ahr-1

The *C. elegans Ahr* ortholog, *ahr-1*, is expressed in a large number of neurons (Huang et al. 2004). Four GABAergic *ahr-1*-positive neurons are the RME motorneurons. They innervate head muscles, and control foraging behavior. These cells can be further subdivided into the two lineally related RMEL and RMER neurons, and the two lineally related RMED and RMEV neurons. Mutants in *ahr-1* and *C. elegans aha-1* (the *tgo*/*Arnt* ortholog) show transformations of RMEL/RMER neurons into RMED/RMEV neurons (Huang et al. 2004), indicating that *ahr-1* functions in controlling neuronal cell fate. The *ahr-1* gene is expressed in RMEL and RMER, but not in RMED and RMEV. One important observation is that Ahr-1 does not bind dioxin or other Ahr ligands (Powell-Coffman et al. 1998; Butler et al. 2001). More comprehensive studies (Butler et al. 2001) have shown this to be an important line of demarcation between vertebrate and invertebrate Ahr proteins: All vertebrate Ahr proteins bind dioxin and no invertebrate Ahr proteins do, including *Drosophila*. While providing no positive evidence for the ability of invertebrate Ahr to bind ligand, these results, nevertheless, do not rule out the ability of invertebrate Ahr proteins to bind presently unknown ligands. From an evolutionary perspective, the main value of the work on *C. elegans ahr-1* is that it functions in nervous

system development, potentially a role for current and ancestral *Ahr* genes.

Concluding remarks

The discovery that *ss* affects dendritic morphology and the neurodevelopmental role of *C. elegans ahr-1* should encourage researchers to study the role of *Ahr* in vertebrate nervous system development and physiology. *Ahr* is widely expressed throughout the brain, but its normal function is unknown. Administration of dioxin during fetal development (and to a lesser extent, in adults) results in a variety of neurological and cognitive defects, but it is not clear that this reflects a normal function of *Ahr*. Additional vertebrate bHLH-PAS proteins, including Single-minded 1, Single-minded 2, Hypoxia-inducible factor-1α, Endothelial PAS domain protein 1, Neuronal PAS domain protein 3, and NXF are expressed in the brain, and also dimerize with Arnt or Arnt2. Activation of Ahr either normally or by dioxin treatment could disrupt the normal function of these other bHLH-PAS proteins by competing away Arnt/Arnt2. Alternatively, Ahr activation may directly activate or repress transcription of neural target genes, potentially in response to endogenous ligands. In either case, *Ahr* may play a role in nervous system development or function, and have implications for human health issues.

How Ss controls dendritic complexity is a mystery. If Ss does not function with Tgo, it will be important to understand how it accomplishes its transcriptional role. Does it compete transcription factors away from existing transcription complexes, as proposed for the Arnt-independent role of Ahr, or could it act as a non-DNA-binding transcriptional activator? Another issue is: Why doesn't Ss interact with Tgo in sensory neurons? Is *tgo* untranscribed or untranslated in sensory cells, or do additional factors exist that inhibit its interaction with Ss? With regard to how Ss functions differently in different sensory neurons, one possibility, by analogy to the ability of different levels of Ss to influence retinal cell choice (Wernet et al. 2006), is that each class of da neurons has a different amount of Ss protein, and this leads to different dendritic morphologies. This is unlikely to be the whole answer, since antibody staining revealed similar levels of Ss in each cell and *ss* misexpression experiments did not provide evidence for a consistent quantitative effect. Nevertheless, even small quantitative differences may contribute to dendritic differences, as indicated by the effect of misexpression on da class IV and td dendritic morphology. Since activity of Ahr, the vertebrate Ss ortholog, is dependent, in most cases, on ligand binding, Kim et al. (2006) suggest that different ligands acting in different da classes could cause dendritic differences. This is a possibility, but remains just a hunch, since Ss does not bind dioxin-like ligands, nor does its activity generally require ligand binding. An alternative view is that Ss regulates gene expression in combination with different transcription factors or is differentially modified. The identities of these other transcription factors remains unknown. However, it is sobering that >80 transcription factors have already been implicated in controlling some aspect of dendrite morphogenesis. While this complexity of regulation is not surprising given the complexity of sensory cell dendrites, it will be interesting to see whether the uniqueness of *ss* holds up as future work progresses. In the least, it is clear that investigators will have their hands full working out the details of *Drosophila* dendritogenesis. It is particularly important to identify sensory cell target genes of Ss and other transcription factors; ideally, it would be useful to profile the complement of target genes specific for each sensory neuron. Additionally, generating complex dendritic patterns will also require localizing RNAs and proteins at specific defined sites along processes, and understanding how their functions can be modified at these defined sites (Andersen et al. 2005). Connecting molecular genetics with dendrite morphology will require a strong dose of cell biology.

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References

Adachi-Yamada, T., Harumoto, T., Sakurai, K., Ueda, R., Saigo, K., O'Connor, M.B., and Nakato, H. 2005. Wing-to-Leg homeosis by spineless causes apoptosis regulated by Fish-lips, a novel leucine-rich repeat transmembrane protein. *Mol. Cell. Biol.* **25:** 3140–3150.

Ainsley, J.A., Pettus, J.M., Bosenko, D., Gerstein, C.E., Zinkevich, N., Anderson, M.G., Adams, C.M., Welsh, M.J., and Johnson, W.A. 2003. Enhanced locomotion caused by loss of the *Drosophila* DEG/ENaC protein Pickpocket1. *Curr. Biol.* **13:** 1557–1563.

Andersen, R., Li, Y., Resseguie, M., and Brenman, J.E. 2005. Calcium/calmodulin-dependent protein kinase II alters structural plasticity and cytoskeletal dynamics in *Drosophila*. *J. Neurosci.* **25:** 8878–8888.

Birnbaum, L.S. 1995. Developmental effects of dioxins. *Environ. Health Perspect.* (Suppl 7) **103:** 89–94.

Brenman, J.E., Gao, F.B., Jan, L.Y., and Jan, Y.N. 2001. Sequoia, a tramtrack-related zinc finger protein, functions as a pan-neural regulator for dendrite and axon morphogenesis in *Drosophila*. *Dev. Cell* **1:** 667–677.

Brown, R.P., McDonnell, C.M., Berenbaum, M.R., and Schuler, M.A. 2005. Regulation of an insect cytochrome P450 monooxygenase gene (CYP6B1) by aryl hydrocarbon and xanthotoxin response cascades. *Gene* **358:** 39–52.

Butler, R.A., Kelley, M.L., Powell, W.H., Hahn, M.E., and Van Beneden, R.J. 2001. An aryl hydrocarbon receptor (AHR) homologue from the soft-shell clam, Mya arenaria: Evidence that invertebrate AHR homologues lack 2,3,7,8-tetrachlorodibenzo-p-dioxin and β-naphthoflavone binding. *Gene* **278:** 223–234.

Crews, S.T., ed. 2003. *PAS proteins: Regulators and sensors of development and physiology.* Kluwer, Boston, MA.

Duncan, D.M., Burgess, E.A., and Duncan, I. 1998. Control of distal antennal identity and tarsal development in *Drosophila* by *spineless-aristapedia*, a homolog of the mammalian dioxin receptor. *Genes & Dev.* **12:** 1290–1303.

Elizondo, G., Fernandez-Salguero, P., Sheikh, M.S., Kim, G.Y.,

Fornace, A.J., Lee, K.S., and Gonzalez, F.J. 2000. Altered cell cycle control at the G(2)/M phases in aryl hydrocarbon receptor-null embryo fibroblast. *Mol. Pharmacol.* **57:** 1056–1063.

Emmons, R.B., Duncan, D., Estes, P.A., Kiefel, P., Mosher, J.T., Sonnenfeld, M., Ward, M.P., Duncan, I., and Crews, S.T. 1999. The Spineless-Aristapedia and Tango bHLH-PAS proteins interact to control antennal and tarsal development in *Drosophila*. *Development* **126:** 3937–3945.

Fujii-Kuriyama, Y. and Mimura, J. 2005. Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. *Biochem. Biophys. Res. Commun.* **338:** 311–317.

Gao, F.B., Kohwi, M., Brenman, J.E., Jan, L.Y., and Jan, Y.N. 2000. Control of dendritic field formation in *Drosophila*: The roles of flamingo and competition between homologous neurons. *Neuron* **28:** 91–101.

Grueber, W.B. and Jan, Y.N. 2004. Dendritic development: Lessons from *Drosophila* and related branches. *Curr. Opin. Neurobiol.* **14:** 74–82.

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

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

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

Huang, X., Powell-Coffman, J.A., and Jin, Y. 2004. The AHR-1 aryl hydrocarbon receptor and its co-factor the AHA-1 aryl hydrocarbon receptor nuclear translocator specify GABAergic neuron cell fate in *C. elegans*. *Development* **131:** 819–828.

Kim, M.D., Jan, L.Y., and Jan, Y.N. 2006. The bHLH-PAS protein Spineless is necessary for the diversification of dendrite morphology of *Drosophila* dendritic arborization neurons. *Genes & Dev.* (this issue).

Kozu, S., Tajiri, R., Tsuji, T., Michiue, T., Saigo, K., and Kojima, T. 2006. Temporal regulation of late expression of Bar homeobox genes during *Drosophila* leg development by Spineless, a homolog of the mammalian dioxin receptor. *Dev. Biol.* **294:** 497–508.

Lahvis, G.P., Lindell, S.L., Thomas, R.S., McCuskey, R.S., Murphy, C., Glover, E., Bentz, M., Southard, J., and Bradfield, C.A. 2000. Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proc. Natl. Acad. Sci.* **97:** 10442–10447.

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

Liu, L., Yermolaieva, O., Johnson, W.A., Abboud, F.M., and Welsh, M.J. 2003. Identification and function of thermosensory neurons in *Drosophila larvae*. *Nat. Neurosci.* **6:** 267–273.

Marlowe, J.L., Knudsen, E.S., Schwemberger, S., and Puga, A. 2004. The aryl hydrocarbon receptor displaces p300 from E2F-dependent promoters and represses S phase-specific gene expression. *J. Biol. Chem.* **279:** 29013–29022.

Parrish, J.Z., Kim, M.D., Jan, L.Y., and Jan, Y.N. 2006. Genome-wide analyses identify transcription factors required for proper morphogenesis of *Drosophila* sensory neuron dendrites. *Genes & Dev.* **20:** 820–835.

Petersen, S.L., Curran, M.A., Marconi, S.A., Carpenter, C.D., Lubbers, L.S., and McAbee, M.D. 2000. Distribution of mRNAs encoding the arylhydrocarbon receptor, arylhydrocarbon receptor nuclear translocator, and arylhydrocarbon receptor nuclear translocator-2 in the rat brain and brainstem. *J. Comp. Neurol.* **427:** 428–439.

Powell-Coffman, J.A., Bradfield, C.A., and Wood, W.B. 1998. *Caenorhabditis elegans* orthologs of the aryl hydrocarbon receptor and its heterodimerization partner the aryl hydrocarbon receptor nuclear translocator. *Proc. Natl. Acad. Sci.* **95:** 2844–2849.

Sugimura, K., Yamamoto, M., Niwa, R., Satoh, D., Goto, S., Taniguchi, M., Hayashi, S., and Uemura, T. 2003. Distinct developmental modes and lesion-induced reactions of dendrites of two classes of *Drosophila* sensory neurons. *J. Neurosci.* **23:** 3752–3760.

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

Tassetto, M. and Gao, F.B. 2006. Transcriptional control of dendritic patterning in *Drosophila* neurons. *Genome Biol.* **7:** 225.

ten Tusscher, G.W. and Koppe, J.G. 2004. Perinatal dioxin exposure and later effects—A review. *Chemosphere* **54:** 1329–1336.

Tracey Jr., W.D., Wilson, R.I., Laurent, G., and Benzer, S. 2003. painless, a *Drosophila* gene essential for nociception. *Cell* **113:** 261–273.

Ward, M.P., Mosher, J.T., and Crews, S.T. 1998. Regulation of *Drosophila* bHLH-PAS protein cellular localization during embryogenesis. *Development* **125:** 1599–1608.

Wernet, M.F., Mazzoni, E.O., Celik, A., Duncan, D.M., Duncan, I., and Desplan, C. 2006. Stochastic spineless expression creates the retinal mosaic for colour vision. *Nature* **440:** 174–180.

Yamamoto, M., Ueda, R., Takahashi, K., Saigo, K., and Uemura, T. 2006. Control of axonal sprouting and dendrite branching by the nrg–ank complex at the neuron–glia interface. *Curr. Biol.* **16:** 1678–1683.

Yao, G., Harstad, E.B., and Bradfield, C.A. 2003. The Ah Receptor. In *PAS proteins: Regulators and sensors of development and physiology* (ed. S.T. Crews), pp. 149–182. Kluwer, Boston, MA.

Zaher, H., Fernandez-Salguero, P.M., Letterio, J., Sheikh, M.S., Fornace Jr., A.J., Roberts, A.B., and Gonzalez, F.J. 1998. The involvement of aryl hydrocarbon receptor in the activation of transforming growth factor-β and apoptosis. *Mol. Pharmacol.* **54:** 313–321.