

MINIREVIEW

The Aryl Hydrocarbon Receptor *sans* Xenobiotics: Endogenous Function in Genetic Model Systems

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ABSTRACT

For more than 30 years, the aryl hydrocarbon receptor [Ah receptor (AHR)] has been extensively scrutinized as the cellular receptor for numerous environmental contaminants, including polychlorinated dioxins, dibenzofurans, and biphenyls. Recent evidence argues that this description is incomplete and perhaps myopic. Ah receptor orthologs have been demonstrated to mediate diverse endogenous functions in our close verte-

brate relatives as well as our distant invertebrate ancestors. Moreover, these endogenous functions suggest that xenobiotic toxicity may be best understood in the context of intrinsic AHR physiology. In this literature review, we survey the emerging picture of endogenous AHR biology from work in the vertebrate and invertebrate model systems *Mus musculus*, *Caenorhabditis elegans*, and *Drosophila melanogaster*.

The aryl hydrocarbon receptor [Ah receptor (AHR)] is a founding member of the basic-helix-loop-helix (bHLH)–Per-ARNT-Sim (PAS) superfamily of transcriptional regulators (for a review of PAS biology, see Gu et al., 2000). This protein was originally identified and characterized as a result of its central role in the vertebrate response to many planar aromatic hydrocarbons (summarized in Swanson and Bradfield, 1993). In this capacity, the AHR binds exogenous ligand and transcriptionally activates a battery of enzymes that promote metabolic transformation and excretion of the xenobiotic from the organism (Nebert et al., 2004; Uno et al., 2004; Xu et al., 2005). This AHR-mediated pathway is commonly viewed as an “adaptive” response toward these xenobiotic agents. In addition to adaptive metabolism, the AHR also mediates a spectrum of toxic endpoints in response to high-affinity agonists. The most potent known ligand for the AHR, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is highly resistant to metabolic degradation and elicits numerous AHR-

dependent toxic events, including late stage terata, thymic atrophy, chloracne, tumor promotion, hepatomegaly, cachexia, and death (reviewed in Birnbaum and Tuomisto, 2000; Schecter et al., 2006).

To elucidate the role of the AHR in normal physiology and development, the locus was deleted from the mouse genome (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Mimura et al., 1997). In addition to being refractory to most, if not all, aspects of TCDD toxicity, “*Ahr*-knock-outs” exhibit multiple physiological abnormalities that are independent of xenobiotic exposure (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Mimura et al., 1997). Studies of the mutant mouse models have reproducibly demonstrated that the AHR has an intrinsic role in diverse aspects of mammalian biology that extend beyond (or are entirely independent from) xenobiotic metabolism (Andreola et al., 1997; Fernandez-Salguero et al., 1997; Zaher et al., 1998; Abbott et al., 1999; Benedict et al., 2000, 2003; Lahvis et al., 2000; Thackaberry et al., 2002; Lund et al., 2003, 2005; Vasquez et al., 2003; Corchero et al., 2004; Guo et al., 2004; Baba et al., 2005; Lahvis et al., 2005). Moreover, these studies of null models have led to speculation that aspects of TCDD toxicity may be the result of sus-

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ABBREVIATIONS: AHR, aryl hydrocarbon receptor; Ah, aryl hydrocarbon; bHLH, basic helix-loop-helix; PAS, Per-ARNT-Sim; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; LOF, loss of function; ARNT, aryl hydrocarbon receptor nuclear translocator; DRE, dioxin response element; Hsp90, 90-kDa heat shock protein; RME, ring motor neuron; PNS, peripheral nervous system; da, dendritic arborization; PR, photoreceptor cell; Rh, rhodopsin; LDL, low-density lipoprotein; TGF- β , transforming growth factor- β ; LTBP, latent TGF- β binding protein.

tained interference in these endogenous, physiological pathways mediated by the AHR.

Evolutionary studies have revealed that the *Ahr* gene is highly conserved across both vertebrate and invertebrate species (for a review of AHR diversity and evolution, see Hahn, 2002; Hahn et al., 2006). Furthermore, this research suggests that aryl hydrocarbon metabolism was not involved in the origins of AHR function. Several groups have cloned ancestral AHR orthologs, which either bind no ligand or bind a spectrum of ligands that is qualitatively different from their vertebrate counterparts (Duncan et al., 1998; Powell-Coffman et al., 1998; Butler et al., 2001; Wiesner et al., 2001; Hahn, 2002). If aryl hydrocarbon metabolism is a "recent" adaptation of the vertebrate lineage, what are the original functions of the AHR? How have these functions changed in response to evolutionary adaptation? Are these endogenous functions related to the toxicity of potent xenobiotic agonists such as TCDD?

In an effort to answer these questions, we have summarized what is currently known regarding endogenous function of the vertebrate AHR and its invertebrate orthologs. Forward and reverse genetic studies in *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly), and *Mus musculus* (mouse) have identified essential roles for AHR signaling in multiple aspects of normal physiology, including vascular development, reproduction, neural function, limb patterning, and vision. We propose that a comprehensive review of these phenotypes will foster a broader understanding of AHR signaling in relation to normal physiology as well as TCDD toxicity, which has eluded explanation despite decades of extensive research.

AHR Structure and Mechanism

Most insight into the mechanisms of AHR signaling has been derived from studies in mammalian cells after xenobiotic induction. These studies have indicated that AHR-mediated transcription occurs via two main steps: (1) ligand activation, which facilitates nuclear translocation of the AHR, and (2) ARNT heterodimerization, which facilitates gene transcription (Fig. 1). The latter stage seems to be highly conserved among all species expressing the AHR and its orthologs (Emmons et al., 1999; Prasch et al., 2006; Qin et al., 2006). The role of ligand-binding in ancestral orthologs of the AHR, however, remains uncertain. In mammalian cells, unliganded AHR is primarily found in a cytoplasmic complex, which includes an Hsp90 homodimer and the immunophilin-like Ara9, also known as XAP2 or AIP (the AHR:chaperone complex is reviewed in Petrulis and Perdeu, 2002). Ligand binding increases nuclear shuttling of the receptor via a process presumed to involve structural transformation and/or nuclear localization sequence (NLS) presentation (Ikuta et al., 1998; Henry and Gasiewicz, 2003; Petrulis et al., 2003).

Once in the nucleus, the PAS domain of AHR mediates dimerization with the corresponding PAS domain of a constitutively nuclear protein known as ARNT (aryl hydrocarbon receptor nuclear translocator) (Gu et al., 2000). The bHLH domains present in both AHR and ARNT then facilitate contact with gene-regulatory elements found in DNA, commonly referred to as dioxin response element (DRE) or xenobiotic response element (Murre et al., 1989; Swanson et al.,

1995; Bacsı and Hankinson, 1996; Gu et al., 2000). Several genes that contain functional DRE sequences have been shown to encode enzymes, such as the *Cyp1* family of cytochromes P450, which mediate metabolism of exogenous AHR ligands. The preponderance of AHR-regulated genes, however, has no known role in AHR biology, either xenobiotic or endogenous.

Caenorhabditis elegans

The *C. elegans* ortholog of the mammalian AHR, called AHR-1, shares 38% amino acid identity with the human AHR over the N-terminal region that mediates DNA binding, PAS dimerization, ligand binding, and interactions with cytoplasmic chaperones (Powell-Coffman et al., 1998). The nematode genome also contains an ortholog of the mammalian *Arnt*, which was cloned under the locus name *aha-1* (Powell-Coffman et al., 1998). Similar to ARNT, AHA-1 functions as a signaling partner for multiple PAS proteins, including the *C. elegans* homologs of AHR and hypoxia-inducible factor (HIF) 1 α (Powell-Coffman et al., 1998; Jiang et al., 2001). In contrast to what is commonly thought for mammalian ARNT, nuclear localization of AHA-1 depends on PAS heterodimerization (Jiang et al., 2001).

In vitro studies have shown that AHR-1 and AHA-1 share several key biochemical features with their predicted mammalian orthologs. In rabbit reticulocyte lysates, AHR-1: AHA-1 and AHR-1:ARNT form a transcriptionally active complex that binds to canonical DRE sequences (Powell-Coffman et al., 1998). In addition to ARNT dimerization, AHR-1 is also capable of interacting with the mammalian chaperone Hsp90 (Powell-Coffman et al., 1998). The *C. elegans* homolog of Hsp90, however, seems to be dispensable in AHR-1 function (Huang et al., 2004; Qin et al., 2006). Like the vertebrate AHR, a series of AHR-1 deletion mutants has

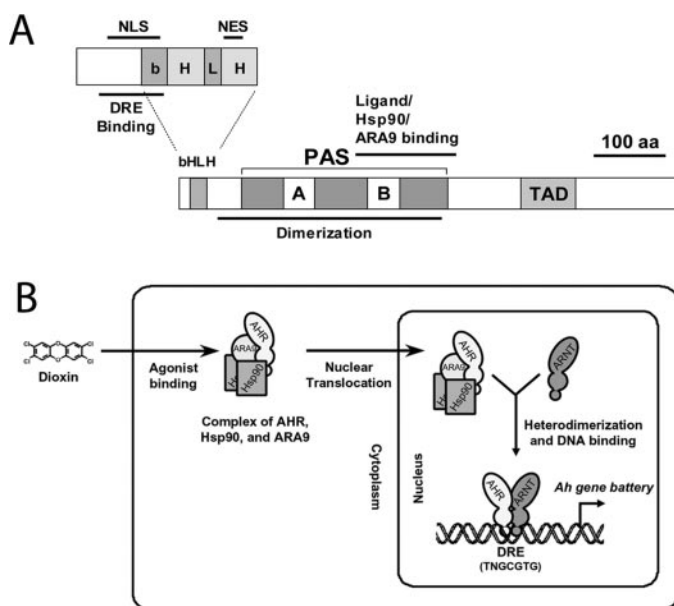


Fig. 1. AHR structure and molecular mechanism. A, domain architecture of the mouse AHR protein. Text indicates key domain regions: NLS, nuclear localization sequence; NES, nuclear export sequence; bHLH, basic helix-loop-helix domain, PAS, Per-ARNT-Sim domain (A and B repeat regions); TAD, transactivation domain. B, mechanism of AHR-mediated gene regulation (see text for details).

indicated that the PAS domain represses nuclear translocation and/or transcriptional activity when expressed in yeast (Powell-Coffman et al., 1998). In mammalian AHR signaling, PAS-mediated repression is ablated via ligand binding and subsequent structural transformation of the receptor; these results suggest that post-translational PAS modification could also be an integral part of AHR-1 signaling. However, AHR-1 does not bind traditional ligand modifiers of the vertebrate PAS domain such as TCDD analogs or β -naphthoflavone (Powell-Coffman et al., 1998; Butler et al., 2001). To date, no ligand agonist of AHR-1—or of any invertebrate AHR homolog—has been identified.

Expression. To elucidate the expression pattern of AHR-1, transgenic worms have been generated with an AHR-1::GFP fusion protein driven by *ahr-1* regulatory elements (Qin and Powell-Coffman, 2004). Fusion protein expression is detectable in the early stages of embryonic development, approximately 260 min after the first cell cleavage (for a searchable review of *C. elegans* development and physiology, see Riddle et al., 1997, available free online). By the first larval stage, 35 cells express observable levels of green fluorescent protein, including blast cells, phasmid socket cells, and numerous neurons belonging to multiple subtypes (Qin and Powell-Coffman, 2004). No function has yet been identified for AHR-1 in the blast or phasmid socket cells. Several neuronal phenotypes, however, have been observed in animals homozygous for loss-of-function (LOF) mutants of AHR-1 (Table 1) (Huang et al., 2004; Qin and Powell-Coffman, 2004; Qin et al., 2006).

Neuron Development. A role for AHR-1 in neuron development was first identified in the GABAergic ring motor (RME) neurons, which innervate the nematode head muscles (White et al., 1976; McIntire et al., 1993). The RME cluster comprises a set of four cells distributed around the neck ring in a symmetrical manner. Although sharing similar neurotransmitter specificity and the same synaptic targets, the RME neurons can be further divided into two subgroups (left/right and dorsal/ventral) based on cell lineage, axon extension, and gene expression—including that of *ahr-1*, which is expressed exclusively in the left/right subset (Hart et al., 1995; Dent et al., 1997; Huang et al., 2004). From a forward genetic screen, it was determined that loss of AHR-1 function results in all four RME cells' adopting the dorsal/ventral phenotype, including axon morphology and gene expression (Huang et al., 2004). Conversely, ectopic expression of *ahr-1* in the dorsal/ventral cells was found to enforce a left/right morphology and gene expression pattern, suggesting that AHR-1 functions as a binary switch to determine RME subtype (Huang et al., 2004).

In addition to RME biology, other investigators have used reverse genetic techniques to determine the broader role of AHR-1 in nematode development (Qin and Powell-Coffman, 2004). In these studies, several neurons from multiple lineages were found to be mislocalized in the adult as a result of faulty cell migration during larval development (for details, see Table 1) (Qin and Powell-Coffman, 2004). Moreover, AHR deficiency was found to dysregulate expression of several key genes found in the pseudo-coelomic neurons that regulate aspects of foraging behavior (Qin and Powell-Coffman, 2004). These initial observations have since been extended to demonstrate an important role for the AHR in oxygen-sensitive aggregation (Qin and Powell-Coffman, 2004; Qin et al., 2006).

Feeding Behavior. Wild isolates of *C. elegans* exhibit either solitary or social (aggregated) feeding behavior on bacterial lawns, depending on natural allelic variation (de Bono and Bargmann, 1998; Rogers et al., 2003). This behavior is determined, in part, by the strain's relative threshold to ambient oxygen (Cheung et al., 2004; Gray et al., 2004). Some strains are more sensitive to oxygen and thus aggregate to reduce exposure, whereas others seek out a more oxygen-rich solitary environment. This oxygen-sensing pathway is expressed in four neurons (AQR, PQR, URXR, and URXL), which directly contact the pseudo-coelomic fluid (Coates and de Bono, 2002). Aggregated feeding is induced via neuron depolarization, which is positively regulated by the oxygen-binding heterodimer of soluble guanylyl cyclases 35 and 36 (GCY-35, GCY-36) and attenuated via the neuropeptide receptor NPR-1 (de Bono and Bargmann, 1998; Cheung et al., 2004; Gray et al., 2004) (see Fig. 2 for details).

Social feeding is highly suppressed in AHR-1 LOF mutants (Qin and Powell-Coffman, 2004; Qin et al., 2006). This behavior can be restored, however, via targeted *ahr-1* expression in the pseudocoelomic sensors URXR and URXL (Qin et al., 2006). In contrast to the other neuronal phenotypes observed in AHR-1 mutants, defects in URX-mediated behavior are due to an ongoing role for AHR-1 in mature cell function—not neuron development or differentiation. Studies using temporal control of *ahr-1* expression via heat shock have demonstrated that developmental expression is not required for wild-type feeding behavior (Qin et al., 2006). Current evidence indicates that AHR-1 controls feeding behavior via indirect transcriptional control of both NPR-1 and several members of the soluble guanylyl cyclase family, including the oxygen-binding heterodimer GCY-35:GCY-36 (Qin and Powell-Coffman, 2004; Qin et al., 2006) (Fig. 2). Overall, these results indicate that AHR-1 mutation leads to widespread defects in the pathways that regulate pseudo-coelomic cell depolarization. The mechanism underlying AHR-1 regu-

TABLE 1

C. elegans: AHR-1 expression and loss of function phenotype

An *ahr-1::gfp* construct driven by the *ahr-1* promoter was used to determine localization of the protein. AHR-1 was detected in 28 neurons, 5 blast cells, and 2 phasmid socket cells. Animals homozygous for AHR-1 LOF mutations exhibit several defects in the development and/or function of these cells (see text for details).

<i>ahr-1</i> Loss-of-Function Phenotype	Cells	Cell type
Failure to express genes required for social feeding behavior	URXR, URXL, AQR?, PQR?	Pseudo-coelomic sensory neuron
Defects in cell pathfinding and axon extension	AVM, SQDR, PLML, PLMR	Mechanosensory neuron
Cell fate/identity	RMEL, RMER	Ring motor neuron
None reported	ALNR/L, PVM, BDUR/L, PLNR/L, PHCR/L, PVWR/L, SDQL, MI, I3, ASKP/R, RIPR/L T.pa, T.ppa, T.ppp, G2, W PHso1, PHso2	Neuron Blast cell Phasmid socket cell

lation of these genes, however, is currently unclear. Although putative DREs are present in the regulatory regions of *gcy-35* and *gcy-36*, mutation of these sequences does not alter dysregulation by AHR-1 LOF, suggesting the involvement of an as-yet unidentified transcriptional intermediate (Qin et al., 2006).

Drosophila melanogaster

The *D. melanogaster* ortholog of the AHR, called Spineless, shares all splicing junctions and 41% amino acid identity with its murine counterpart (Duncan et al., 1998). A higher degree of identity is conserved in the bHLH (71%) and PAS (45%) regions (Duncan et al., 1998). Spineless heterodimerizes with an ARNT ortholog called Tango that, similar to AHA-1, requires concomitant expression of a PAS signaling partner for nuclear localization (Ward et al., 1998; Emmons et al., 1999). Like other invertebrate homologs of AHR, Spineless does not bind prototypical xenobiotic ligands of the vertebrate receptor such as TCDD or β -naphthoflavone (Butler et al., 2001). In vitro coexpression studies have shown that the Spineless:Tango complex induces DRE-mediated transcription in the absence of exogenous ligand in *D. melanogaster* cell lines, indicating that either 1) post-translational modification of Spineless via ligand is not required, or 2) endogenous ligand is ubiquitously produced (Emmons et al., 1999). Consistent with these in vitro observations, ectopic expression of Spineless in the fly has been shown to result in nuclear translocation of Tango independent of tissue/cell type (Emmons et al., 1999).

Expression. *Spineless* is expressed in numerous tissues during embryonic and pupal development (for a searchable review of *D. melanogaster* development, see Gilbert, 2000; available free online). *Spineless* mRNA first appears at the 8th stage of embryonic development just anterior to the cephalic furrow (Duncan et al., 1998). As developmental seg-

mentation proceeds, *ss* is expressed in the maxillary, labial, and mandibular segments in the head, the leg anlagen of the thorax, and the peripheral nervous system in each thoracic and abdominal segment (Duncan et al., 1998). In the pupa, *ss* is expressed in multiple imaginal discs, which develop into the adult leg, antenna, palp, wing, haltere, eye, and genital structures, as well as bristle cell precursors (Duncan et al., 1998; Adachi-Yamada et al., 2005; Wernet et al., 2006).

Homeotic Function. Research into the developmental functions of Spineless has a long history. Calvin Bridges and T. H. Morgan first identified the *ss* locus in 1923, and it has since been recognized as an essential regulator of appendage identity (Bridges and Morgan, 1923; Struhl, 1982; Burgess and Duncan, 1990; McMillan and McGuire, 1992). *Spineless* belongs to a framework of loci, known as homeotic genes, that direct development of each segment into specialized structures. In simplistic terms, homeotic genes function as regulatory switches that direct pluripotent cell clusters into specialized structures, such as the leg or antenna or wing. Mutations to homeotic genes can lead to deletion and/or transformation of appendages to a homologous structure (e.g., a leg developing where an antenna should be). *Spineless* is expressed in many of these developing regions and, in general, predisposes cells toward the antennal fate. Like many homeotic genes, however, the developmental program of *spineless* is highly complex. Recent work has shown that *spineless* functions in a spatio-temporal fashion both upstream (*danlfer*, *danr/hern*, *bric-a-brac*, *BarH1*, and *BarH2*) and downstream (*distal-less*, *homothorax*, *cut*) of multiple homeotic genes, each of which has its own complex expression pattern and regulatory network (Chu et al., 2002; Emerald et al., 2003; Suzanne et al., 2003; Kozu et al., 2006; Emmons et al., 2007). Experimental dysregulation of *spineless*, therefore, has varied consequences that are largely dependent on the strength of mutation or driver of expression. Ectopic expression of *spineless* via the *ptc*-GAL4 driver has been shown to cause transformation of the maxillary palp, rostral membrane, and distal leg to antennal identity (Duncan et al., 1998). Conversely, deletion of *ss* or *tgo* results in a variety of defects, including transformation of the distal antenna to a leg-like structure, truncation of the maxillary palps, deletion of the medial tarsal structures of the leg, and markedly reduced bristle size (Fig. 3) (Struhl, 1982; Lindsley and Zimm, 1992; Duncan et al., 1998; Emmons et al., 1999). Overall, these results indicate that Spineless directs appendages from an epigenetic ground state into a distal antenna identity, but they also suggest a distinct role in the elongation/elaboration of nonantennal structures as well.

Dendrite Diversification. Similar to AHR-1, Spineless seems to have an essential role in neuronal development. In this regard, Spineless has recently been identified as a central regulator of peripheral nervous system (PNS) morphology (Kim et al., 2006). The *D. melanogaster* PNS, replicated in each thoracic and abdominal segment, comprises ≈ 40 neuronal cells containing a range of dendrite complexity from single extensions to highly branched structures (Bodmer and Jan, 1987). The dendritic arborization (*da*) class of PNS neurons displays highly elaborate dendrite structures that innervate the epidermal surface and transmit signals relating to locomotion, thermosensation, and pain (Ainsley et al., 2003; Liu et al., 2003; Tracey et al., 2003). This family of neurons is commonly categorized into four classes based

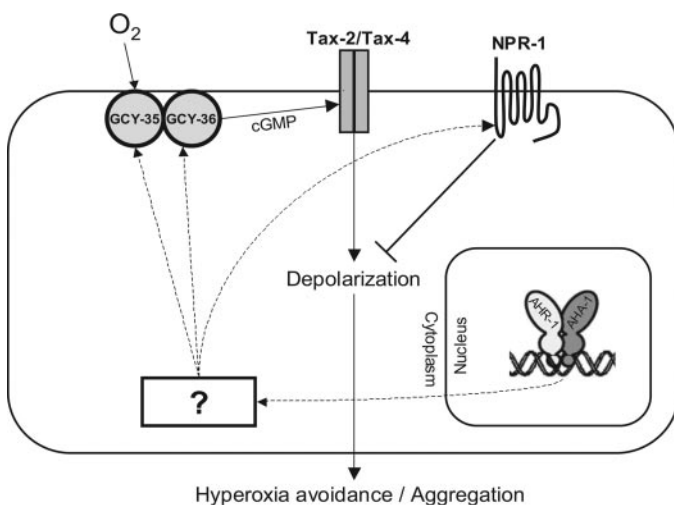


Fig. 2. Molecular mechanism of AHR-1 function in *C. elegans* social feeding behavior. Social feeding is controlled via pseudocoelomic neuron depolarization, which is coordinately regulated by the oxygen-activated GCY-35/36 heterodimer and NPR-1. GCY-35 directly binds environmental oxygen and facilitates production of cGMP, which subsequently activates Tax-2/Tax-4 ion channels. Neuron depolarization is attenuated by the neuropeptide receptor NPR-1. Loss of AHR-1 function results in a marked decrease in expression of the GCY-35/36 heterodimer as well as NPR-1. This phenomenon is hypothesized to occur via dysregulation of an unknown intermediate factor (see text for details).

upon increasing dendrite complexity: class I contains the simplest da neurons, whereas class IV contains the most complex structures (Grueber et al., 2002). The process of dendrite branching is influenced via numerous developmental pathways (Brenman et al., 2001; Grueber et al., 2003; Li et al., 2004; Sugimura et al., 2004; Parrish et al., 2006). The regulatory genes that have been studied in detail have generally been classified as promoters or inhibitors of branching complexity, such as *cut* and *abrupt* (respectively), which are preferentially expressed in opposing neuron classes (Grueber et al., 2003; Li et al., 2004; Sugimura et al., 2004). To date, the effects of *Spineless* on dendrite morphogenesis are unique in that *ss* is equally expressed in all da classes, and LOF results in opposing effects: increased branching in the “simple” class I and II neurons but reduced branching in “complex” class III and IV cells (Kim et al., 2006). Moreover, loss of *ss* expression attenuates class-specific features, such as class III actin spikes, contributing to the idea that *ss* LOF promotes a “generic” da form (Kim et al., 2006). Overall, these results are consistent with the idea that *Spineless* has an essential role in the diversification of neuron morphology and, potentially, function. The mechanism underlying this developmental pathway, including the role of *Tango*, is currently unclear. cursory experiments indicate that *Tango* may be dispensable for normal dendrite morphogenesis (Kim et al., 2006). The possibility that *Spineless* controls PNS branching independent of *Tango*, however, has not been confirmed using more robust methods.

Photoreceptor Development. A recent report has further implicated *Spineless* signaling in sensory cell diversification. In this study, *spineless* expression was demonstrated to be a master regulator of the *D. melanogaster* photoreceptor mosaic required for color vision (Fig. 4) (Wernet et al., 2006). In the fly, vision is achieved via a compound eye composed of ≈ 800 optical units (ommatidia), each of which contains eight photoreceptor cells (PRs) (Gilbert, 2000). The six outer

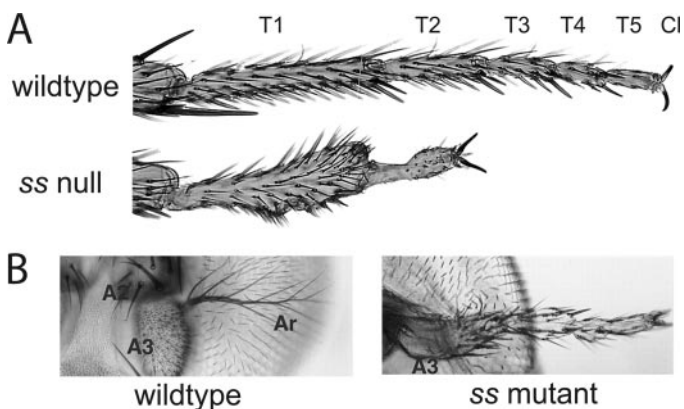


Fig. 3. Homeotic functions of *Spineless*. A, upper, distal region of a wild-type fruit fly leg. Key, T1–T5, 1st–5th tarsal segments; Cl, claw. *Spineless* is transiently expressed in the T2–T4 segments and the distal portion of T1 during the late 2nd to late 3rd instar. Bottom, *Spineless*-null animals exhibit truncated legs as a result of a distal T1 through T4 deletion. B, wild-type fly antenna (left). Key: A2 and A3, 2nd and 3rd antennal segments, respectively; Ar, arista. *Spineless* is expressed in A3 and arista beginning in the late 2nd instar. A weak *ss* LOF allele causes transformation of A3 and arista to a distal leg phenotype, including a well formed claw (right). [Adapted from Duncan DM, Burgess EA, 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. Copyright © 1998 Cold Spring Harbor Laboratory Press. Used with permission.]

PRs (R1–R6) are functionally analogous to the vertebrate rod cells that sense motion in dim light (Miller et al., 1981; Wernet et al., 2006), whereas the two inner cells (R7 and R8) express color-sensitive rhodopsins (Rh) similar to the vertebrate cone cell (Fryxell and Meyerowitz, 1987; Chou et al., 1996; Huber et al., 1997; Papatsenko et al., 1997). The color-sensing R7/R8 photoreceptor pair comes in two “flavors” that are randomly distributed throughout the compound eye. In wild-type animals, the R7 cell in each ommatidium stochastically expresses either *Rhodopsin 3* (*Rh3*) or *Rh4*, which then instructs R8 cells to express *Rh5* or *Rh6*, respectively (Chou et al., 1996, 1999). The resulting mosaic of ommatidia produces a compound eye capable of recognizing a broad spectrum of light. The Rh4/Rh6 rhodopsin combination present in 70% of ommatidia recognizes longer or “yellow” light wavelengths, whereas the remaining ommatidia express Rh3/Rh5 receptors that sense shorter “pale” wavelengths (Feiler et al., 1992).

Although *spineless*-null animals exhibit no gross morphological changes to the eye, their ommatidia are composed solely of Rh3/Rh5 (i.e., “pale”) photoreceptors (Fig. 4) (Wernet et al., 2006). Conversely, forced expression of *spineless* induces a 100% Rh4/Rh6 (i.e., “yellow”) eye, indicating that *ss* is both necessary and sufficient for the “yellow” photoreceptor phenotype (Wernet et al., 2006). Temporal regulation of *ss* via heat shock has further revealed that a single burst of *ss* during mid-pupation—similar to *ss* expression during normal development—is sufficient to program adult photoreceptor phenotype (Duncan et al., 1998; Wernet et al., 2006). During normal development, however, an eye-specific enhancer region present in the *ss* promoter confers stochastic *ss* expression and, thus, stochastic cell fate (Wernet et al., 2006). Overall, these findings demonstrate that *Spineless* expression in the *D. melanogaster* eye functions as a binary

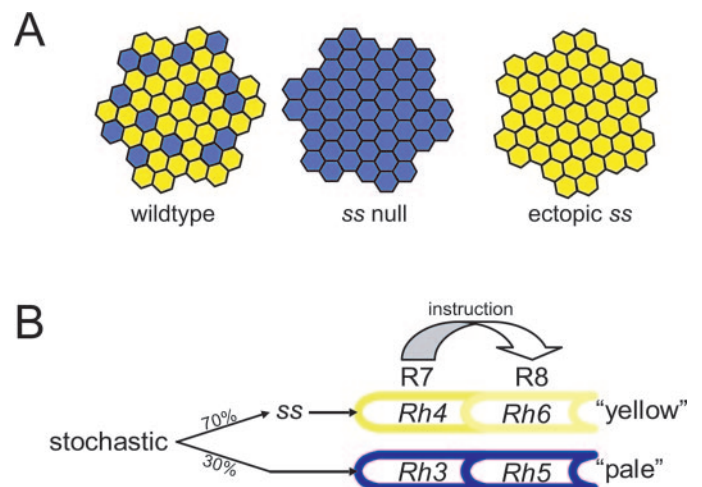


Fig. 4. *Spineless* is a dominant regulator of photoreceptor phenotype. A, in wild-type *D. melanogaster*, the compound eye is composed of a mosaic of ommatidia, which express photoreceptors for either short (“pale”) or long (“yellow”) wavelengths of light (left). Eyes from *ss*-nulls contain only “pale” ommatidia (center), whereas ectopic *ss* induces an all “yellow” phenotype (right). B, during normal development, a stochastic burst of *ss* expression occurs mid-pupation in approximately 70% of all ommatidia. In the presence of *Spineless*, R7 cells adopt a Rhodopsin 4 (Rh4) phenotype and instruct R8 cells to express Rh6 resulting in a receptor pair that recognizes “yellow” wavelengths. In the absence of *Spineless*, R7 and R8 cells express the default Rh3 and Rh5 receptors, respectively, that recognize “pale” light.

(but stochastic) switch, which produces the ommatidial mosaic necessary for color vision.

Mus Musculus

Expression. The aryl hydrocarbon receptor is expressed in numerous murine embryonic and adult tissues. *Ahr* expression can be observed as early as gestational days 10 to 12 in the nasal pit, branchial arches, heart, liver, maxillary prominence, neuroepithelium, and several neurons, including the trigeminal ganglion, spinal ganglia, and posterior branches of spinal nerves (Abbott et al., 1995; Mimura et al., 1997). Given the role of *ahr-1* and *spineless* in neuron development, it is interesting to note that brain/neural expression of mouse *Ahr* largely dissipates as gestation proceeds (Abbott et al., 1995). From gestational days 13.5 to 15.5, the overall range of *Ahr* expression is greatly expanded and can be detected in the developing pituitary, choroid plexus, adrenal gland, palatal shelf, dorsal surface of the tongue, nasal cartilage, olfactory tissues, thymus, lung, liver, bone, muscle, gut, kidney, retina, bladder epithelium, urogenital sinus, and tip of the genital tubercle (Abbott et al., 1995; Jain et al., 1998) (Fig. 5A). In adult 8- to 11-week-old mice, *Ahr* is most highly expressed in the oocyte, epidermis, bladder, lung, digits, vomeronasal organ, liver, trachea, olfactory epithelium, and retina (Su et al., 2002) (Fig. 5B). Xenobiotic studies, however, have demonstrated the presence of functional AHR in countless other tissues and cell types.

Knockout. Three independent laboratories have generated *Ahr*-null mice via excision of either the first (Fernandez-Salguero et al., 1995; Mimura et al., 1997) or second exon of the gene (Schmidt et al., 1996). Although all models share important characteristics, such as resistance to TCDD toxicity, reduced fecundity, portal fibrosis, and smaller livers,

they also seem to differ in some respects, such as lymphoid cell number and age-related lesions (reviewed in Lahvis and Bradfield, 1998). For the purpose of this review, we have focused on the in vivo phenotypes that are shared among all *Ahr*-null constructs. Alterations in cultured cell phenotype have been reviewed elsewhere (Barouki et al., 2007).

Cardiovascular. Perhaps the most overt phenotype of the AHR knockout mouse is a markedly reduced liver size (25–50% smaller than in control mice) (Fernandez-Salguero et al., 1995; Schmidt et al., 1996). Our own studies have indicated that this liver phenotype is the result of defects in the resolution of fetal vascular structure (Lahvis et al., 2000, 2005). In the embryonic vasculature, the flow of blood partially bypasses the liver sinusoids via a shunt known as the ductus venosus (DV), which directly connects the inferior vena cava and the portal vein (Edelstone et al., 1978; Kiserud et al., 2000). During normal development, the DV closes shortly after birth, forcing oxygen- and nutrient-rich blood to migrate through the liver sinusoids. In the absence of AHR signaling, however, the DV remains patent (open) throughout adulthood and is presumed to result in reduced postnatal liver growth via nutrient deprivation (Fig. 6A) (Lahvis et al., 2000, 2005). Follow-up experiments have demonstrated that this physiological role for the AHR is mechanically analogous to xenobiotic activation. DV closure depends on coexpression of ARNT (Walisser et al., 2004b), the nuclear localization/DNA-binding domain of AHR (Bunger et al., 2003), as well as the degree of AHR activation (Walisser et al., 2004a). Recent experiments using a conditional allele of *Ahr* have demonstrated that the DV phenotype fully depends on endothelial and/or hematopoietic, but not hepatocytic, expression of the receptor (Walisser et al., 2005).

In addition to the DV phenotype, AHR-null mice exhibit several other less characterized alterations to embryonic,

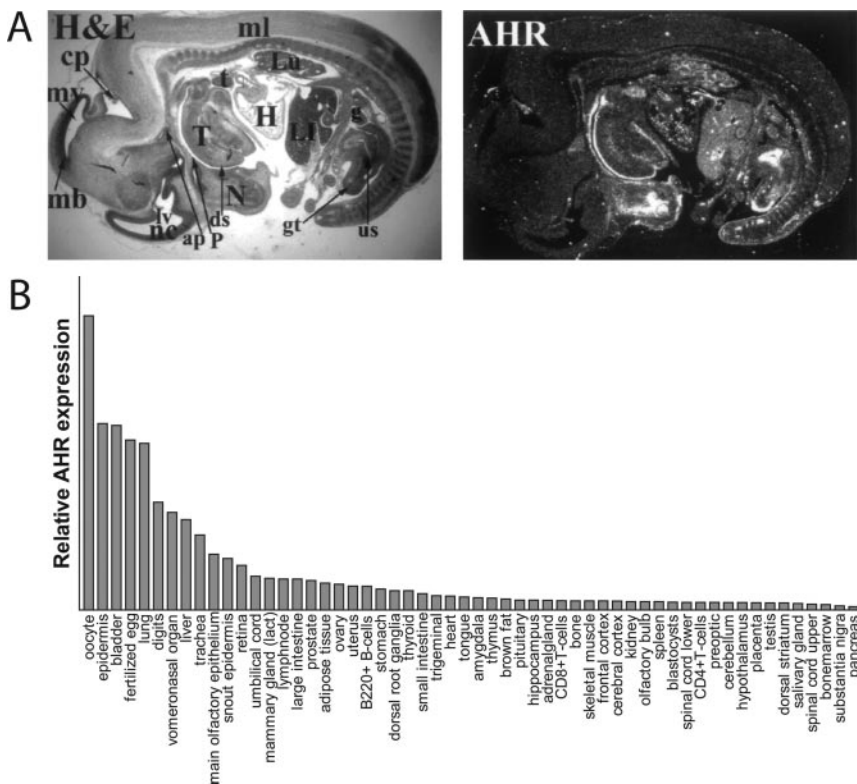


Fig. 5. Fetal and adult expression of murine *Ahr*. A, hematoxylin-eosin stain (left) and corresponding in situ hybridization for *Ahr* mRNA (right) at gestation day 13.5. Text indicates key anatomical features: ne, neuroepithelium; lv, lateral ventricle; mb, mid-brain; mv, mesencephalic vesicle; cp, choroid plexus; ap, anterior pituitary; P, palate; ds, dorsal tongue surface; T, tongue; t, thymus; H, heart; Lu, lung; Li, liver; g, gut; us, urogenital sinus; gt, genital tubercle. [Adapted from Jain S, Maltepe E, Lu MM, Simon C, and Bradfield CA (1998) Expression of ARNT, ARNT2, HIF1, HIF2 and Ah receptor mRNAs in the developing mouse. *Mech Dev* 73:117–123. Copyright © 1998 Elsevier Press. Used with permission.] B, *Ahr* expression in select tissues from 8- to 11-week-old C57BL/6 mice, as measured by microarray hybridization. Data from Su et al. (2002).

neonatal and adult cardiovascular biology. In embryos, a reduction in liver perfusion can be observed as early as gestational day 15.5, indicating that vascular abnormalities precede normal DV closure (Harstad et al., 2006). In neonates, *Ahr*-null mice have abnormalities in the vascular architectures of the kidney (Fig. 6B), liver sinusoid, and eye, including persistence of the embryonic hyaloid artery (Lahvis et al., 2000). In adults, the knockout genotype is linked to cardiac hypertrophy, hypertension, and elevated levels of the potent vasoconstrictors endothelin-1 and angiotensin II (Fernandez-Salguero et al., 1997; Thackaberry et al., 2002; Lund et al., 2003, 2005, 2006; Vasquez et al., 2003). Studies using pharmacological inhibitors of endothelin-1/angiotensin II signaling have suggested that these vasoconstrictors directly contribute to the age-dependent increase in blood pressure and heart size (Lund et al., 2003, 2005, 2006). It is unclear, however, whether these age-related changes are due to a continuing role for the AHR in vascular homeostasis or simply a downstream consequence of the altered liver and/or kidney vasculature.

Several lines of evidence indicate that vascular AHR signaling is mechanistically linked to fluid shear stress. Shear forces are generated via the passage of fluid (i.e., blood) through a constricted space such as the vascular system. This process generates 1) a tangential, frictional force against the inner wall of the blood vessel, which is highly studied because of its direct effects on the vascular endothelium (Resnick et al., 2003), and 2) a velocity gradient within the fluid itself,

which has the capacity to alter serum protein structure and function (Siedlecki et al., 1996; Shankaran et al., 2003; Alexander-Katz et al., 2006). Independent reports have demonstrated that the AHR is highly activated by cellular exposure to fluid shear (Mufti et al., 1995; McMillan and Bradfield, 2007). Moreover, recent evidence indicates that fluid shear stress activates the AHR, at least in part, via a direct effect on serum low-density lipoprotein (LDL) function and/or structure (McMillan and Bradfield, 2007). It is noteworthy that the role of modified LDL in AHR biology does not seem to be limited to shear-induced modification. Conventional methods of LDL modification, such as hypochlorite oxidation, also produce an AHR-activating isoform of this pro-atherogenic macromolecule (McMillan and Bradfield, 2007). In light of these recent data as well as the cardiovascular phenotype of the *Ahr*-null mouse, we speculate that vascular AHR signaling could function as a developmental mechanism to increase blood flow, and thus serum/LDL filtration, through both the liver and kidney.

Fibrosis. In addition to vascular defects, several other pathologic conditions have been observed in the *Ahr*-null liver, including prolonged extramedullary hematopoiesis, fatty metamorphosis, and portal tract fibrosis (Fernandez-Salguero et al., 1995; Schmidt et al., 1996). Of these changes, only portal fibrosis has been characterized in a more detailed fashion. Data suggest that interplay between the AHR, retinoic acid, and TGF- β pathways contribute to this fibrotic phenotype. Studies in cultured cells as well as the liver have shown that AHR deficiency results in increased secretion of active TGF- β , a potent profibrotic peptide (Border and Noble, 1994; Branton and Kopp, 1999), via a post-translational mechanism (Zaher et al., 1998; Santiago-Josefat et al., 2004). This process is likely to involve direct transcriptional regulation of the latent TGF- β binding protein-1 (LTBP-1) (Corchero et al., 2004; Santiago-Josefat et al., 2004; Gomez-Duran et al., 2006) as well as deficiency in retinoid metabolism (Andreola et al., 1997, 2004), which has been shown to increase levels of TGF- β activating enzymes (Kojima and Rifkin, 1993; Okuno et al., 1997). Studies outside the liver have also indicated interaction between the TGF- β and AHR pathways, suggesting that additional endpoints of endogenous biology may also be affected by this relationship (Guo et al., 2004; Thomae et al., 2005).

Reproduction. *Ahr*-null female mice exhibit defects in multiple aspects of reproduction, including conception, litter number, and pup survival (Abbott et al., 1999; Baba et al., 2005). Accordingly, the AHR has been found to have a physiological role in several reproductive tissues, most notably the ovarian follicle. During normal estrous, hormonal signals induce maturation of the granulosa cell layer surrounding the oocyte and trigger release of the follicle into the fallopian tube (Gilbert, 2000). Although the number and morphology of immature, preovulatory follicles is unchanged between wild-type and *Ahr* knockout females, several reports have noted a marked reduction in the number of mature follicles (Benedict et al., 2000, 2003; Baba et al., 2005). Evidence indicates that these defects are not due to upstream changes in the endocrine regulation of ovulatory-stimulating hormones (Baba et al., 2005; Trewin et al., 2007). Rather, data suggest that maturation of *Ahr*-null follicles is disrupted as a result of insufficient synthesis of estradiol within the follicle itself (Baba et al., 2005; Barnett et al., 2007). It has been demon-

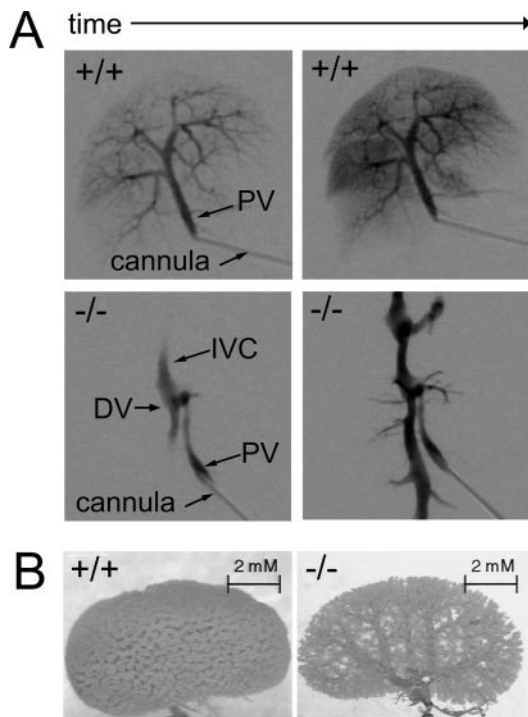


Fig. 6. Vascular phenotype of *Ahr*-null mice. A, time-lapse radiographs of contrast agent entering the liver of a wild-type mouse (top) or *Ahr*-null mouse (bottom). Contrast agent is perfused throughout the branching vessels of the liver in wild-type mice but shunted into the inferior vena cava in null mice via a persistent fetal vascular structure known as the ductus venosus (see text for details). Arrows indicate key features: PV, portal vein; IVC, inferior vena cava; DV, ductus venosus. B, latex corrosion cast of the wild-type (left) and *Ahr*-null (right) renal vasculature. The mechanism underlying decreased vascular density in the null kidney is currently unknown.

strated that the AHR acts synergistically with the orphan nuclear receptor Ad4BP (also known as SF-1) to transiently up-regulate *Cyp19* (aromatase) levels during preovulation (Honda et al., 1993; Baba et al., 2005). The AHR-dependent proestrus peak in *Cyp19* expression is thought to catalyze estradiol production and downstream signaling in the developing follicle, which has been shown to be critical for both release and implantation (Dupont et al., 2000). Moreover, the role of estradiol in the ovarian defects of AHR-null mice has been further strengthened by partial rescue of the null phenotype via exogenous estradiol administration (Baba et al., 2005). It is noteworthy that the *Cyp19* promoters of several vertebrate species, including multiple fish, humans, and mice, contain both AHR and Ad4BP/SF-1 binding sites, suggesting an evolutionarily conserved role for this interaction in ovarian physiology (Kazeto et al., 2001; Tchoudakova et al., 2001; Baba et al., 2005; Nocillado et al., 2007).

Several lines of evidence indicate that the physiological role of the AHR in reproduction extends well beyond oocyte maturation. Fertilization of the oocyte triggers a transient but robust increase in AHR activity, as evidenced by a 100-fold increase in *Cyp1a1* (Dey and Nebert, 1998; Pocar et al., 2004). No physiological role for this phenomenon has yet been demonstrated, although a further relationship with estradiol metabolism would not be unlikely. After implantation, the AHR is strongly expressed in the uterine vasculature and developing tissues between the embryo and dam (Kitajima et al., 2004). The absence of AHR signaling in these tissues has been shown to result in an enlarged placental labyrinth with altered dam-to-pup filtration (Thomae et al., 2004; T. L. Thomae, personal communication). After birth, pups raised by null dams have a significantly lower survival rate than those raised by heterozygotes (Abbott et al., 1999), which may be related to impaired development of the mammary gland (Hushka et al., 1998). Overall, these results indicate that *Ahr* deficiency is deleterious to numerous aspects of reproduction ranging from oocyte maturation to placental development to pup rearing.

Role of Endogenous Ligand(s) in AHR Function. The putative connection between endogenous ligand(s) and the various *ahr*-null phenotypes is a defining question for future study. In mammals, several lines of evidence suggest that intrinsically produced ligands are an integral part of endogenous AHR biology. First, mutant constructs of AHR and ARNT have demonstrated that endogenous signaling is mechanistically analogous to induction via xenobiotic ligand (Bunger et al., 2003; Walisser et al., 2004a,b). It is noteworthy that experiments using AHR hypomorphs have shown that physiological deficiency, as measured by ductus venosus closure, can be rescued via high-affinity xenobiotic ligand (Walisser et al., 2004b). Second, cell culture experiments have demonstrated that AHR activity is markedly increased in the absence of CYP1 metabolism, suggesting that the AHR-regulated *Cyp1* battery actively clears an AHR ligand of either cell or sera origin (Chang and Puga, 1998; Levine-Fridman et al., 2004). Third, several classes of endogenous compounds have been demonstrated to directly modulate AHR activity, including 1) tryptophan metabolites (Heath-Pagliuso et al., 1998; Wei et al., 1998, 1999; Bittinger et al., 2003; Diani-Moore et al., 2006) and other indole-containing structures (Miller, 1997; Adachi et al., 2001; Song et al., 2002; Guengerich et al., 2004; Henry et al., 2006), 2) tetra-

pyroles such as bilirubin and biliverdin (Sinal and Bend, 1997; Phelan et al., 1998), 3) sterols such as 7-ketocholesterol and equilenin (Savouret et al., 2001; Jinno et al., 2006), 4) fatty acid metabolites, including several prostaglandins and lipoxin A4 (Schaldach et al., 1999; Seidel et al., 2001), and 5) the ubiquitous second messenger cAMP (Oesch-Bartlomowicz et al., 2005) (AHR ligand classes were reviewed by Denison and Nagy, 2003). The physiological relevance of these compounds to the abnormalities of the *Ahr*-null mouse, however, is unclear.

Because of the relationship between AHR gene targets and adaptive metabolism of the receptor's agonists, a causative link between reduced ligand metabolism and the null phenotype cannot be overlooked. Accumulation of a parent compound and/or deficiency of a metabolite could result in the observed null phenotypes. In this context, it is interesting to note that the *Ahr*-null mouse contains increased retinoid levels within the liver, which have been linked to the portal fibrosis phenotype, as well as an increased concentration of AHR-activating serum LDL (Andreola et al., 1997, 2004; McMillan and Bradfield, 2007). However, it remains possible that endogenous agonists do not directly influence the null phenotype but instead determine spatio-temporal expression of downstream AHR gene targets that are required for normal physiology but unrelated to ligand metabolism.

A connection between endogenous ligand and AHR function in invertebrate species is considerably more tenuous. Several studies have shown that invertebrate orthologs of the AHR, including those in the nematode, fruit fly, zebra and blue mussels, and the soft-shell clam, do not bind prototypical xenobiotic ligands; possible interaction with other ligand classes, however, has not been examined (Duncan et al., 1998; Powell-Coffman et al., 1998; Butler et al., 2001; Wiesner et al., 2001; Hahn, 2002). Studies suggest that post-translational modification of the AHR-1 PAS domain, perhaps via ligand binding, could be required for transcriptional activity; modification of Spineless, however, does not seem to be required. Although it is conceivable that one subset of invertebrate AHR orthologs possesses ligand-binding capacity and another does not, this scenario is likely to require an independent gain or loss of ligand function before vertebrate development. Given that the AHR is unique among all PAS superfamily members in its ligand inducibility, this possibility does not seem likely (Gu et al., 2000). Although no conclusion can yet be definitively drawn regarding the role of ligand in invertebrate AHR biology, it should be noted that the body of evidence that supports ligand-independent signaling in *D. melanogaster* is more substantial than the converse proposition in *C. elegans*. Biochemical studies using additional invertebrate homologs may be vital in elucidating the molecular mechanisms underlying ancestral AHR signaling.

Comparative Perspective and Concluding Remarks

What can we learn from the juxtaposition of AHR function in these diverse model systems? Thus far, the most compelling similarities are present in the *Ahr*-deficient phenotypes of *C. elegans* and *D. melanogaster*. The cells/tissues affected by AHR loss of function in these organisms are, with very few exceptions, intimately related to environmental sensation.

Spineless is required for normal development of the antenna and maxillary palp (the two olfactory organs in the fly), the mechanosensory bristle cells, the eye mosaic required for color vision, and the PNS, which relays multiple sensory inputs. A similar role for AHR-1 in *C. elegans* sensation is emerging from studies into nematode feeding behavior and sensory neuron development. In both model systems, AHR seems to function as a diversification mechanism—often as a binary switch—whether directing development from an appendage ground state to the antenna, creating a photoreceptor mosaic, generating two RME neuron subtypes, or diversifying PNS morphogenesis.

The link between invertebrate and murine AHR biology is less clear. Although the liver and kidney, two prominent sites of AHR-mediated vascular diversification, can certainly be viewed as chemosensory organs, such a statement cannot be made regarding the oocyte or the various other reproductive tissues that require endogenous AHR function. Nonetheless, a more comprehensive picture of AHR physiology in the mouse may reveal more overt connections between vertebrate and invertebrate function. Two of the regions that most highly express Ahr in the mouse are the vomeronasal olfactory organ and the retina (Jain et al., 1998; Su et al., 2002), which is markedly similar to *spineless* expression in the fly (Duncan et al., 1998). However, no studies have yet been conducted into AHR function in these tissues. Moreover, the only studies into AHR function with regard to vertebrate neuronal development, a principal theme in invertebrate biology, have been conducted in the framework of xenobiotic toxicity (Hays et al., 2002; Hill et al., 2003; Carvalho and Tillitt, 2004; Powers et al., 2005; Williamson et al., 2005; Hood et al., 2006; Petersen et al., 2006).

In addition to loss-of-function phenotype, another prominent difference between AHR signaling in vertebrate and invertebrate biology seems to be the addition of direct sensory function to its original role in sensory organ development. The vertebrate AHR signaling pathway has clearly developed into an inducible system that mediates the adaptive clearance of a variety of endogenous and exogenous compounds. How could such adaptation occur? Insight may be provided by the recent observation that Spineless regulates basal expression of the insect P450 enzyme CYP6B1, which mediates metabolism of a variety of phytochemicals, via conserved DRE promoter elements (Hung et al., 1996; Li et al., 2002; Petersen et al., 2003; McDonnell et al., 2004; Brown et al., 2005). Substrates of the insect CYP6B family include several dietary agonists of the vertebrate AHR such as furocoumarins (Baumgart et al., 2005), flavones (Ciolino et al., 1999; Reiners et al., 1999), and indole-3-carbinol (Bjeldanes et al., 1991). The addition of ligand inducibility (or expansion of the receptor's ligand repertoire) to AHR function, therefore, could have provided significant advantage over plant defenses. Although such an evolutionary mechanism for the alteration/expansion of AHR function during early vertebrate development bears a certain level of allure, this idea is currently speculative.

The evolutionary chasm between invertebrates and higher mammals may be the most difficult obstacle in comparing murine AHR signaling to that seen in the fly or nematode. Less complex vertebrates such as the zebrafish (*Danio rerio*), however, may better illustrate the similarities, as well as differences, between the pre- and postchordate AHR. Al-

though zebrafish molecular techniques still lag behind the more established model organisms, reverse genetic studies are becoming increasingly more tractable in this organism (Lekven et al., 2000; Nasevicius and Ekker, 2000; Wienholds et al., 2002, 2003). Xenobiotic studies have already established that AHR biology has a dramatic impact on the zebrafish cardiovascular system, neurodevelopment, and oogenesis, although the role of AHR signaling in normal physiology is not yet known (reviewed in Carney et al., 2006b; see also Henry et al., 1997; Tanguay et al., 1999; Andreasen et al., 2002; Hill et al., 2003; Bello et al., 2004; Antkiewicz et al., 2005, 2006; Handley-Goldstone et al., 2005; Carney et al., 2006a; Heiden et al., 2006). The identification of Ahr-deficient phenotypes in the zebrafish is undoubtedly on the horizon.

Highly diverse functions for the AHR have already been established in our most powerful model systems, and more surprises are likely to be in store—in these organisms as well as others. Many of the observations detailed herein were published within the last year, suggesting that we are only now beginning to elucidate the true physiological functions of the protein we know best as the aryl hydrocarbon receptor. With few exceptions, AHR signaling is still a story of disconnected parts: isolated phenotypes and unexplained toxicology. In the coming years, our understanding of AHR biology will inevitably improve. In doing so, these disparate elements of AHR signaling may yet form a cohesive whole.

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