

# The buzz on fly neuronal remodeling

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**Hormone-dependent rewiring of axons and dendrites is a conserved feature of nervous system development and plasticity. During metamorphosis in insects, steroid hormones (the ecdysteroids) and terpenoid hormones (the juvenile hormones) regulate extensive remodeling of the nervous system. These changes retool the nervous system for new behavioral and physiological functions that are required for the adult stage of the life cycle. In honey bees and other highly social insects, hormones also regulate behavioral changes and neuronal plasticity associated with transitions between social caste roles. This review focuses on recent work in fruit flies and honey bees that reveals hormonal and molecular mechanisms underlying metamorphic and caste-dependent neuronal remodeling, with specific emphasis on the role of Krüppel homolog 1.**

## Introduction: hormonal control of neuronal remodeling

Insect metamorphosis is one of the most remarkable and best-understood examples of hormone-dependent developmental plasticity in animals. The process of complete metamorphosis in insects involves the wholesale replacement of larval structures with new adult (imaginal) tissues [1]. Within the nervous system, however, many larval cells are retained in the adult after their extensive remodeling [2–4]. For example, larval dendrites and axons (neurites) are first pruned back before new neurite outgrowth establishes the adult cell morphologies. These morphogenetic events are coordinated by two classes of hormones, steroids (ecdysone) and terpenoids (juvenile hormone [JH]) (Box 1). Steroid hormone-dependent rewiring is also important for normal development and plasticity of vertebrate nervous systems [3,5–7], but the molecular mechanisms underlying these processes remain largely undefined. The highly versatile tools available for hormonal and single-cell genetic manipulation of insect neurons present unique opportunities to examine these mechanisms in fine detail [3,8].

A recent series of parallel studies in the fruit fly *Drosophila melanogaster* and honey bee *Apis mellifera* revealed intriguing similarities in hormonal and molecular mechanisms governing behavioral plasticity in honey bee adults and metamorphic development in *Drosophila*. These studies have centered on signaling by ecdysone and JH and their interactions with specific nuclear proteins, in particular the zinc-finger transcription factor Krüppel homolog 1 (KR-H1). This review discusses the roles of these hormones with KR-H1 in these two model systems.

## Mushroom body growth reflects behavioral maturation

The insect mushroom bodies (MBs) are sites of important cognitive functions, including multimodal sensory integration and associative learning and memory [9]. Their name reflects their structure (Figure 1a), which includes intrinsic neurons, the Kenyon cells, and dense concentrations of nerve-cell processes (neuropils), including the mushroom cap-like calyces and the mushroom stalk-like lobes. In the olfactory system, where their function is best understood, the MBs are thought to be the principal sites of olfactory memory formation. Odors are received by olfactory receptor neurons that project to the antennal lobes to form excitatory synapses with projection neurons (PNs). In turn, the PNs convey this information to Kenyon cell dendrites located in the MB calyces (and to other brain centers). The Kenyon cell axons form the MB lobes and synapse onto targets that remain largely uncharacterized.

The MBs undergo substantial developmental and experience-dependent plasticity. In *Drosophila*, adults raised in more enriched sensory environments have greater MB neuropil volumes [10], which indicate a greater number of synaptic contacts. MB plasticity is also linked to the division of labor in highly eusocial insects (which have overlapping adult generations, cooperative brood care and a sterile worker caste), such as honey bees and some species of carpenter ants and paper wasps [11]. In female queen bees and among workers of more primitively eusocial wasps (in which the worker caste is not anatomically distinct), MB plasticity is linked to social dominance [11].

## Caste differentiation and MB plasticity in the honey bee

In honey bees, workers undergo a caste transition at approximately three weeks of age from in-hive tasks (e.g. nursing of young) to foraging outside the hive for pollen and nectar (Box 2). The ratio of synapse (neuropil) to cell-body (somata) volume in the MBs also changes at this time [12]. For example, the MB synapse to somata volume ratio ('neuropil volume') in foragers is greater than in nurses, which are 7–10 days younger. Given the pivotal role of the MB in associative learning in insects [9], it is thought that these changes might result in enhanced foraging performance. However, this hypothesis has not been tested directly [13].

Experience-independent factors such as age contribute to this MB reorganization [14]. However, in single-cohort colonies in which worker bees are all the same age, the MB neuropil volume remains greater in foragers [14]. Therefore, some of the increase in MB neuropil volume is strongly associated with the transition to the forager caste. Once foraging behavior has been initiated, MB neuropil

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### Box 1. Endocrine regulation of insect development

A large body of work over the past century has revealed how circulating hormones control major developmental transitions in insects [1]. The best-understood examples of this are molting and metamorphosis. Insects undergo repeated molts during their life cycles to accommodate growth and changes in body plan. In insects that undergo incomplete metamorphosis, the immature and adult stages are similar, although functional wings only develop during the molt to the adult. Insects that undergo complete metamorphosis lack adult appendages, wings and eyes as larvae, and many of their larval tissues (including the CNS) are dramatically restructured during metamorphosis. Molting and metamorphosis are triggered and coordinated by circulating steroid hormones, the ecdysteroids (hereafter referred to as ecdysone) [56]. A second class of terpenoid hormones, represented most prominently by juvenile hormone (JH), are 'status quo' hormones that interact with ecdysone to prevent stage transitions during molting (such as from larva to pupa, or from nymph to adult) [68]. Although there are important differences in the actions of these hormones in insects with incomplete versus complete metamorphosis, the general roles of ecdysone and JH in controlling the insect life cycle have been largely conserved during insect evolution [47]. Recent studies have also illustrated how signaling by insulin-like proteins interacts with ecdysone and JH to contribute to size assessment and growth control during molting and metamorphosis. This is an area of intense interest and has been the subject of several recent reviews [69–72].

growth is inhibited in foragers that are kept in small groups in cages separate from the hive and, to a lesser extent, in those blocked from exiting the hive [13]. Thus, at least some of the increases in MB neuropil volume appear to be dependent upon stimuli that are present in the hive and encountered during foraging. The neuropil volume changes are correlated with changes in the branching and length of the Kenyon cell dendrites [14] (Figure 1b). These findings are consistent with studies in mammals

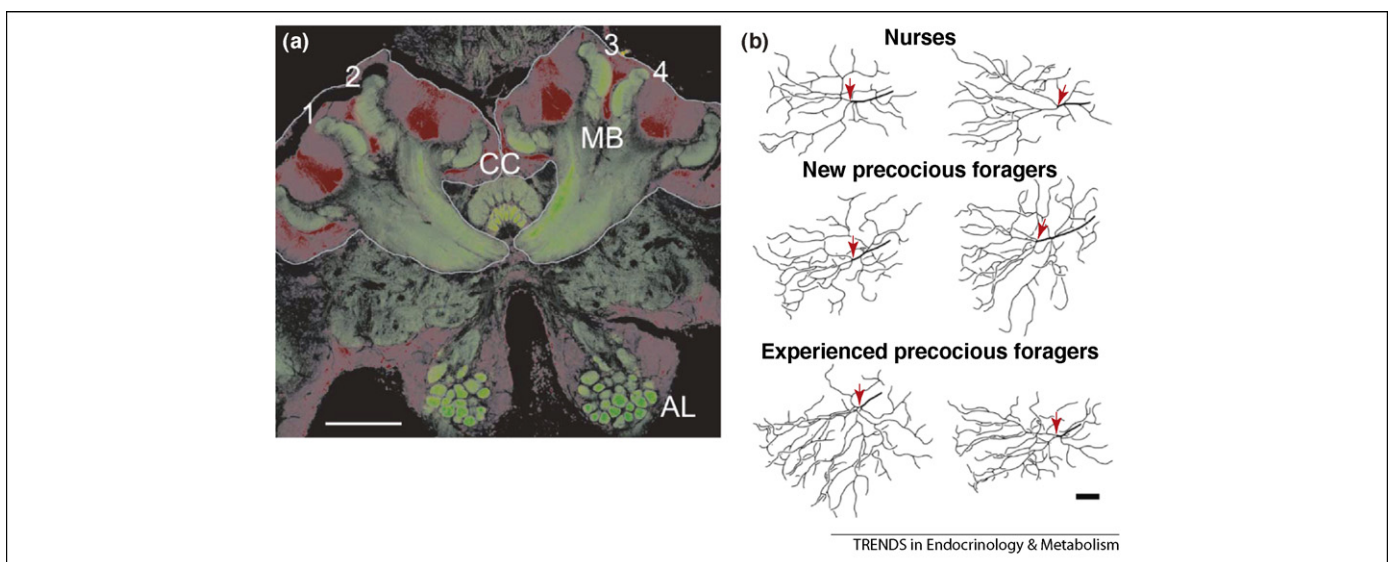
### Box 2. Honey bee caste transitions are flexible

The workers in a honey bee colony are segregated into different castes, with each caste performing different functions [73]. Some workers remain in the hive to care for developing larvae (nurses), handle food, or dispose of dead bees (undertakers). Other workers, the foragers, leave the hive to collect pollen and nectar. Caste roles are not fixed, and workers usually spend the first three weeks of their 4–7-week lives performing in-hive tasks and then transition to their foraging role outside of the hive. Caste transition is developmentally timed, but the timing is flexible and can be accelerated, delayed or even reversed. This plasticity of behavioral castes enables workers to assume new roles according to the changing needs of the hive. An extreme example of honey bee caste plasticity is seen in single-cohort colonies, in which all of the workers start as one-day-old bees. Under these conditions, some of the workers become precocious foragers at four days instead of three weeks, and other workers at the same age become nurses [16]. The queen is an additional and crucial factor in regulating foraging behavior. The presence of a queen bee in a hive elicits immediate changes in worker bee behavior in addition to longer-acting changes in the age-related division of labor. Both of these effects of the queen are mediated largely by pheromones. The best understood of these is queen mandibular pheromone (QMP), a five-chemical blend that is one of at least nine components of a pheromone mixture that attracts workers to attend to the queen [74]. In addition to performing several other social regulatory functions, QMP delays the maturation of workers to the forager caste [75].

showing that enriched environments lead to larger cortical regions and associated increases in the number and length of dendritic branches [15].

### Endocrine regulation of honey bee worker castes and MB plasticity

The development of the forager caste in honey bees is associated with changing hormone levels. Compared to nurses, foragers and precocious foragers display elevated



**Figure 1.** The transition to foraging in honey bees is associated with increases in the ratio of synapse to somata volume in the MBs and in the size of Kenyon cell dendritic arbors. (a) The mushroom bodies (MB, outlined in gray) in the brain of an adult honey bee. The brain was labeled with phalloidin (green) to label neuropil and propidium iodide (red) to label somata. The four MB calyces are indicated (1–4), along with two other prominent brain structures, the antennal lobes (AL) and central complex (CC). The AL are the principal source of olfactory inputs to the MBs. The function of the CC is enigmatic, although it is thought to be a predominantly visual center that contributes to polarization vision and visual pattern recognition [66]. Scale bar, 200  $\mu\text{m}$ . Figure adapted, with permission, from Ref. [67] (Copyright 2004 National Academy of Sciences, U.S.A.). (b) Representative dendritic trees for MB neurons from nurses, new precocious foragers and experienced precocious foragers. The individual neurons were revealed by Golgi impregnation. The main branch point within each dendritic tree is marked with an arrow. Scale bar, 10  $\mu\text{m}$ . Figure adapted, with permission, from Ref. [14].

JH titers [16]. Removal of the corpora allata, the endocrine source of JH, delays but does not prevent foraging, and treatment of young bees with the JH mimic (JHM) methoprene accelerates foraging [16–18]. Therefore, JH modifies the transition to foraging by increasing the pace of behavioral development in honey bee workers [18].

These findings led to the hypothesis that the behavioral changes with caste transition depend on JH-mediated reorganization of the MBs. However, removal of the corpora allata in young bees does not alter the growth of the MB neuropil. In addition, as is the case with behavioral maturation, MB neuropil growth occurs in the absence of JH in adults [19]. Neuropil expansion also accompanies the onset of flight in queens and drones, even though JH titers are low in these castes [20]. These findings indicate a more nuanced view of MB reorganization and behavioral maturation in which other experience- and JH-independent factors might more directly govern caste transition [13,20].

Recent evidence indicates that the yolk precursor vitellogenin might be a more proximal signal than JH in the regulation of honey bee foraging. Vitellogenin is produced in the fat body and circulates in the blood (hemolymph), and it constitutes the main hemolymph protein in non-foraging honey bees [21]. In adult honey bee workers, the vitellogenin titer increases for the first 7–10 days and then gradually declines with age [21]. During this period, RNA interference (RNAi) to vitellogenin increases JH levels. Thus, circulating vitellogenin reduces the JH titer [22]. This inhibition is reciprocal; although JH induces vitellogenin synthesis in many adult insects [1], JH only induces vitellogenin expression in honey bees during the late pupal stages [23] and actually inhibits vitellogenin production in adult workers [24]. During the transition to foraging, a change in signals external to the worker might lead to increased JH production [25]. As a result, vitellogenin production is shut off by the rapidly increasing JH titer [24]. Interestingly, RNAi to vitellogenin also accelerates the transition to foraging behavior [26]. Therefore, the loss of vitellogenin might be the signal that triggers foraging onset, perhaps in part through direct or indirect regulation of MB neuropil growth.

#### Functional genomics of foraging ontogeny

To measure changes in gene expression associated with behavioral plasticity in honey bees, genome-wide gene-expression analyses have been performed on individually dissected brains from nurses and foragers [27]. Approximately 40% of all transcripts display changes in abundance associated with the transition to foraging, and single brain expression profiles enable the accurate categorization of nursing or foraging status 95% of the time. Thus, global changes in gene expression in the brains of worker honey bees are strongly associated with naturally occurring transitions to foraging.

Given the role of queen mandibular pheromone (QMP) in delaying the maturation of workers to the forager caste (Box 2), microarray studies have examined changes in gene expression in the brain that correlate with effects of the pheromone. One study identified ~1200 upregulated and 1400 downregulated genes in the brains of young worker bees exposed to QMP [28]. Of these, only ~20 are consistently

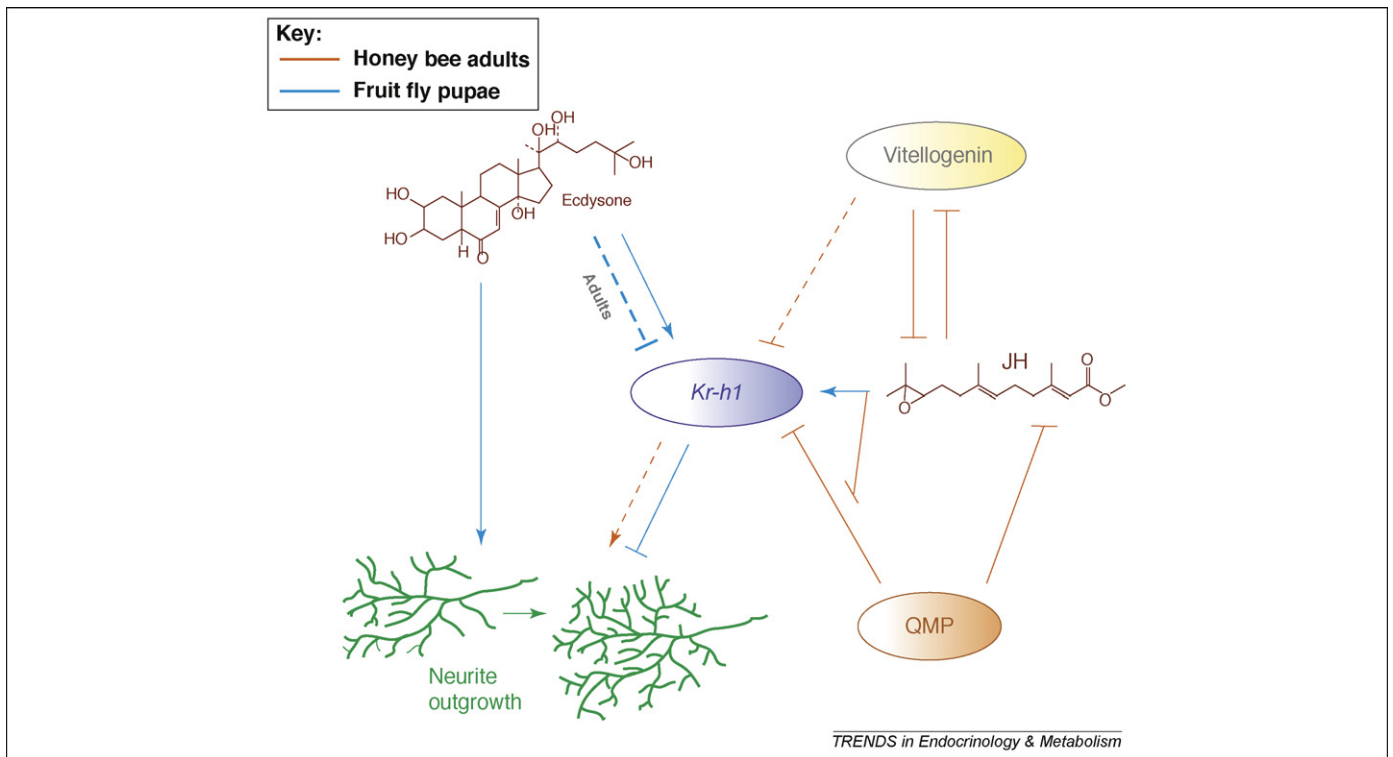
regulated on days 2 to 4 after QMP exposure. If the effects of QMP on gene expression mediate the delay in the transition to foraging, then QMP should repress genes associated with foraging and activate genes associated with nursing. This hypothesis is supported by comparing gene-expression patterns that result from QMP exposure [28] to the patterns observed during the natural transition to foraging [27]. For example, four of the six genes that were consistently downregulated on days 2 to 4 after QMP exposure were on the list of genes expressed preferentially in foragers, and only one of thirteen genes that were consistently upregulated by QMP were found on this list [28]. Thus, QMP might regulate the timing of caste transition from nursing to foraging by regulating the expression of genes that contribute to these behavioral states.

#### QMP and Krüppel homolog 1

QMP exposure has strong effects on the expression of honey bee *Kr-h1*, a gene orthologous to the *Drosophila Kr-h1* C<sub>2</sub>H<sub>2</sub>-type zinc-finger transcription factor [28–30]. Worker *Kr-h1* expression levels are reduced by ~50% after the exposure of queenless colonies to QMP, and workers also display increased *Kr-h1* expression during the natural transition to foraging [27,28,31]. This relationship between *Kr-h1* and the foraging transition is not strictly age-dependent because precocious foragers show threefold higher *Kr-h1* expression levels than nurses of the same age [31]. Thus, higher *Kr-h1* expression levels are strongly associated with foraging, and this relationship persists even when the timing of caste transition is altered in single-cohort colonies or in younger workers exposed to QMP.

The effects of QMP on the foraging transition and *Kr-h1* expression might be mediated, at least in part, by changes in JH signaling. QMP decreases JH levels in young bees, and JHM treatment at this stage blocks the suppression of *Kr-h1* expression by queen pheromone [31]. JHM alone does not substantially alter *Kr-h1* levels, so the ability of QMP to inhibit *Kr-h1* expression in young bees depends on low circulating JH levels. In the older foragers, however, *Kr-h1* levels (which are already high) are unaffected by QMP. In queenless colonies, some workers begin to lay unfertilized eggs. These ‘laying workers’ have low JH levels, whereas the foragers in the same colonies have high JH levels, and these caste differences arise in the absence of exposure to queen pheromone [31]. However, *Kr-h1* levels are still twofold higher in foragers than in laying workers. Therefore, the maintenance of higher *Kr-h1* levels in foragers is at least partially independent of regulation by QMP exposure or forager responsiveness to the queen pheromone.

Together, these findings indicate a general model for the actions of QMP, JH, vitellogenin and *Kr-h1* in the regulation of honey bee foraging [25,31] (Figure 2). In this model, QMP and vitellogenin inhibit *Kr-h1* and the transition to foraging. During the transition to foraging, the JH titer increases to inhibit vitellogenin expression and the effects of QMP on *Kr-h1* expression. The decline in vitellogenin levels provides the signal to trigger foraging, perhaps in part through relief of *Kr-h1* repression. The model does not define the temporal and causal relationships between changes in JH levels and *Kr-h1* expression, and between



**Figure 2.** Interactions among factors governing MB remodeling. Five factors with roles in MB remodeling in honey bee adults and fruit fly pupae are shown. These include the principal active forms of ecdysone and JH in fruit flies and honey bees (20-hydroxyecdysone and juvenile hormone III, respectively) [1]. The relationships among these hormones, QMP, vitellogenin and *Kr-h1* are indicated by the connecting lines (which are color coded by species). Lines that end with arrowheads illustrate stimulatory interactions, and lines that end in roughly perpendicular bars indicate inhibitory interactions.

*Kr-h1* expression and the transition to foraging. Recent work on the role of *Kr-h1* in hormone signaling and MB remodeling in fruit flies has provided insights into the possible cellular and molecular mechanisms by which these signals are integrated.

### Remodeling the fruit fly brain

The fruit fly is uniquely well suited for the genetic characterization of factors that govern neuronal remodeling through cell-targeted manipulation of gene expression [32] and genetic screening [32,33]. For example, recent genetic studies have demonstrated key roles for glial engulfment, neuron-intrinsic ubiquitin-proteasome activity, transforming growth factor  $\beta$  (TGF- $\beta$ ) signaling and the ecdysone receptor in the reorganization of MB neurons (pruning of larval neurites and outgrowth of adult-specific projections) during metamorphosis [32,34–36]. Some of these systems, such as the ubiquitin-proteasome and TGF- $\beta$  signaling, display extensive evolutionary conservation. Thus, findings in fruit flies might reveal factors controlling neuronal plasticity in higher organisms.

In response to the honey bee studies on *Kr-h1*, the *Drosophila* system recently has been used to examine the contribution of *Kr-h1* to remodeling of MB neurons [37]. Other studies have addressed the role of *Drosophila Kr-h1* in the molecular signaling hierarchy that mediates cellular responses to ecdysone in the epidermis and other tissues [29,38]. Because many core features of the ecdysone response are shared across tissues [39], these studies shed light on *Kr-h1* function specifically in MB remodeling.

### *Kr-h1* modulates ecdysone responses essential for *Drosophila* metamorphosis

The *Drosophila Kr-h1* gene produces three mRNA isoforms encoding zinc-finger proteins in the Krüppel-like family of transcription factors [29,30,40]. These factors are known to control diverse aspects of animal development [41]. In embryos, *Kr-h1* transcripts and KR-H1 proteins are found almost exclusively in most, if not all, neurons [38,42–44]. Post-embryonically, KR-H1 expression broadens to most larval and prepupal tissues [29,45,46], including diploid nests of cells (the ‘imaginal discs’) that are set aside in the larva to later form adult structures [47].

Flies homozygous for the *Kr-h1*<sup>1</sup> mutant allele (which eliminates expression of the major isoform and severely attenuates expression of the others) display severe developmental defects at the onset of metamorphosis [29]. The *Kr-h1*<sup>1</sup> mutant phenotype includes failure to complete head eversion, an essential morphogenetic event, and disruption of the programmed cell death of the larval salivary glands. This phenotype is consistent with a disruption in prepupal tissue responses to ecdysone [48]. Extensive work has shown that a small set of transcription factors are directly induced by ecdysone (primary-response genes) to regulate expression of many secondary-response genes that coordinate appropriate cellular responses to the hormone [49,50]. Interestingly, *Kr-h1*<sup>1</sup> mutant prepupae display altered expression of several ecdysone primary-response genes, including *E74A*, *E74B*, *E75B*, *E93* and transcripts encoding two isoforms of the ecdysone receptor, *EcR-B1* and *EcR-B2* [29,46]. *Kr-h1* is itself upregulated by ecdysone by approximately fivefold in cultured salivary

glands from wild-type larvae [29]. This seems to be a primary response to ecdysone because the induction does not require new protein synthesis [49].

#### *Kr-h1 regulates ecdysone-dependent remodeling of MB neurons*

In MB neurons, *Kr-h1* might be actively downregulated to enable neurite outgrowth. Pan-neuronal *Kr-h1* overexpression produces gross defects in axonal pathfinding and reduced, abnormal synapses at neuromuscular junctions in embryos and young larvae [42,45]. *Kr-h1* overexpression specifically in the MBs during initial morphological differentiation in larvae, or during ecdysone-dependent MB remodeling during metamorphosis, also produces abnormal neuronal morphology [37]. In wild-type animals, MB neurons express KR-H1 protein, with levels dropping precipitously during initial morphogenesis and again as ecdysone triggers re-elaboration of pruned neurites during early metamorphosis. In contrast to the effects of *Kr-h1* overexpression, loss of *Kr-h1* in single cell MB clones has no effect on neuron morphology [37]. However, in a separate, dorsal cluster of adult-specific neurons (the 'DC neurons') [51], reduced *Kr-h1* expression dominantly suppresses the delay in neurite morphogenesis caused by loss of a TGF- $\beta$  type-I receptor (*baboon*) [37]. Thus, *Kr-h1* can inhibit neurite morphogenesis.

Genetic mosaic experiments have provided additional evidence that links *Kr-h1* to hormone signaling. The ecdysone receptor is a heterodimer of two nuclear receptors, EcR and ultraspiracle (USP). USP is required for ecdysone signaling in many contexts, including metamorphic remodeling of MB neurons [32]. In *usp* mutant MB clones, KR-H1 expression is markedly reduced [37]. In addition, in *Kr-h1* mutant larvae, many CNS neurons ectopically express EcR-B1. In wild-type larvae, preferential enrichment of EcR-B1 is observed in neurons that are destined to undergo remodeling during early metamorphosis [32,52]. Therefore, KR-H1 might help to prepare the larval CNS for metamorphosis, perhaps in part by delaying neurite morphogenesis when ecdysone levels are high (Figure 2). This model is consistent with microarray studies implicating KR-H1 as an ecdysone primary-response gene [49] and genetic evidence that *Kr-h1* is required for normal prepupal responses to ecdysone [29].

The interactions between KR-H1, EcR, USP and ecdysone signaling are complex. The ecdysone receptor activates or represses transcription of many target genes in the presence of ecdysone, but it also might act as a transcriptional repressor at some promoters in the absence of ligand [53,54]. In fact, in a second class of neurons that undergoes metamorphic remodeling (the thoracic ventral 'Tv' neurons), EcR- and ecdysone-dependent transcriptional activation is required to support pruning of larval neurites, but outgrowth of new adult-specific neurites might require ecdysone binding to its receptor to relieve EcR-dependent transcriptional repression [55]. The regulation and function of KR-H1 is likely to be equally dependent on developmental stage and cellular context. For example, in adult *usp* mutant cells, KR-H1 expression is increased rather than decreased [37]. In *Kr-h1* mutants, some ecdysone-dependent changes in gene expression are advanced (e.g.

induction of *EcR-B1* and *E74B* in mid-prepupae), whereas others are delayed or are largely quantitative (up or down) [29,46]. Other members of the Krüppel-like family of transcription factors act as transcriptional activators or repressors depending on the promoter and interacting cofactors [41]. Therefore, more analysis is needed to identify regulatory interactions and protein partners for KR-H1, EcR and USP to obtain a better understanding of the interplay among these factors and ecdysone signaling during neuronal morphogenesis.

#### *Is KR-H1 a competence factor?*

In *Drosophila*, two successive pulses of ecdysone trigger metamorphosis [56]. The first pulse occurs in late larvae (the 'late larval peak') and directs puparium formation. The second pulse (the 'prepupal peak'), ~10 h later, triggers the prepupal-pupal transition. A handful of genes (e.g. *ftz-f1*, *fork head* and *broad*) have been shown to regulate the ability of tissues ('competence') to display stage-specific responses to the prepupal peak of ecdysone [48,50,57,58]. The mutant phenotypes for some of these genes bear remarkable similarity to those of *Kr-h1* mutant animals. For example, similar to *Kr-h1* mutants [29], larvae bearing mutations in the *ftz-f1* orphan nuclear receptor pupariate normally but display multiple defects at the prepupal-pupal transition, including failure to complete adult head eversion, leg elongation and programmed cell death of the larval salivary glands [48].

Studies on the *Drosophila* epidermis indicate that *Kr-h1* might contribute to competence by mediating some of the effects of JH on ecdysone signaling (Box 1). The cells that form the adult abdominal epidermis (histoblasts) are sensitive to JH. At the onset of metamorphosis, nests of histoblasts proliferate rapidly, displace the cells of the larval epidermis, and then fuse and differentiate to form the adult epidermis and overlying adult cuticle [59]. JH treatments cause developing histoblasts to form a pupal rather than adult cuticle, and evidence from multiple insect species indicates that these actions of JH involve increased expression of ecdysone-induced transcription factors [60]. Although *Drosophila* *Kr-h1* transcripts normally decrease to undetectable levels by ~6 h after puparium formation and remain low until the end of metamorphosis [29,60], JHM treatment promotes re-expression or prolonged expression of *Kr-h1* in the abdominal epidermis and other tissues after pupation. Similar to the effects of lower-dose JHM treatments, overexpression of *Kr-h1* in the histoblasts inhibits or partially disrupts formation of the sensory bristles along the dorsal abdominal midline [60]. Thus, *Kr-h1* seems to function as a general component of the ecdysone and JH signaling pathways controlling metamorphic transitions [37].

#### **Concluding remarks**

The recent work on *Kr-h1* indicates a crucial role for this transcription factor in regulating hormone- and pheromone-controlled developmental plasticity of MB neurons during fruit fly metamorphosis and caste transitions in adult honey bees (Figure 2). In the honey bee component of this model, JH and vitellogenin are mutually inhibitory in adults. While young adult workers remain dedicated to in-

hive tasks, vitellogenin and QMP contribute to maintenance of low JH and *Kr-h1* levels. During the transition to foraging, external signals are thought to tip the balance among these factors, resulting in an increase in circulating JH and inhibition of vitellogenin synthesis. *Kr-h1* might promote neurite growth in the MB, perhaps in response to reduced vitellogenin-dependent inhibition of *Kr-h1* expression.

In fruit fly pupae, however, *Kr-h1* inhibits MB neurite outgrowth. During *Drosophila* metamorphosis, both ecdysone (in the MB) and JH (in several tissues) induce *Kr-h1* expression, and KR-H1 is an important mediator of signaling by both hormones. Thus, ecdysone and JH might act through KR-H1 to delay MB neurite outgrowth until the appropriate stage of metamorphosis. In adult fruit flies, *Kr-h1* expression requires *usp*, a component of the heterodimeric ecdysone receptor. Taken together, these findings indicate that the role of KR-H1 in MB neurite outgrowth switches from growth-inhibitory to growth-stimulatory during the pupal-to-adult transition in both fruit flies and honey bees. However, the molecular mechanisms mediating the regulation of KR-H1 expression and its effects on neurite outgrowth remain undefined, and several key questions remain to be addressed in future studies (Box 3). The answers will provide important insights into the mechanisms that govern developmental plasticity in insect nervous systems.

How broadly applicable is this work to vertebrates? Neuronal remodeling has a crucial role in normal and injury-induced development and refinement of connections in the mammalian CNS [61,62]. However, we are only beginning to understand the molecular mechanisms involved in regulating these morphogenetic events. Krüppel-like transcription factors perform diverse developmental functions in mammals, including the regulation of neuronal differentiation [41,63,64]. The above studies in *Drosophila* and honey bees provide a rationale for examining the involvement of Krüppel-like proteins in hormone-dependent neuronal remodeling in other species. As has been the case for many other aspects of development [65], studies in genetically tractable insect models should provide valuable insights for understanding neuronal plasticity in mammalian systems.

### Box 3. Questions for future research

- Does JH regulate MB morphogenesis in *Drosophila*?
- Where, precisely, does *Kr-h1* fit in the transcriptional hierarchy that mediates ecdysone action, and what are the molecular mechanisms by which JH alters the actions of *Kr-h1* in the ecdysone response?
- To what extent is the role of *Kr-h1* conserved among insect species?
- Does *Kr-h1* alternately inhibit or stimulate neuron morphogenesis, respectively, in insect pupae and adults?
- Is *Kr-h1* primarily regulated by ecdysone in some contexts (correlating with cell type, developmental stage or insect species) and alternatively by other factors (such as JH or vitellogenin) in others? Are some of the roles of *Kr-h1* in development independent of hormone signaling?
- Do proteins orthologous to KR-H1 govern hormone-dependent remodeling in vertebrate nervous systems?

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### References

- 1 Nijhout, H.F. (1994) *Insect Hormones*. Princeton University Press
- 2 Consoulas, C. *et al.* (2000) Behavioral transformations during metamorphosis: remodeling of neural and motor systems. *Brain Res. Bull.* 53, 571–583
- 3 Weeks, J.C. (2003) Thinking globally, acting locally: steroid hormone regulation of the dendritic architecture, synaptic connectivity and death of an individual neuron. *Prog. Neurobiol.* 70, 421–442
- 4 Truman, J.W. (1996) Steroid receptors and nervous system metamorphosis in insects. *Dev. Neurosci.* 18, 87–101
- 5 Morris, J.A. *et al.* (2004) Sexual differentiation of the vertebrate nervous system. *Nat. Neurosci.* 7, 1034–1039
- 6 Theodosios, D.T. (2002) Oxytocin-secreting neurons: a physiological model of morphological neuronal and glial plasticity in the adult hypothalamus. *Front. Neuroendocrinol.* 23, 101–135
- 7 Flanagan-Cato, L.M. (2000) Estrogen-induced remodeling of hypothalamic neural circuitry. *Front. Neuroendocrinol.* 21, 309–329
- 8 Grueber, W.B. and Jan, Y.N. (2004) Dendritic development: lessons from *Drosophila* and related branches. *Curr. Opin. Neurobiol.* 14, 74–82
- 9 Davis, R.L. (2005) Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. *Annu. Rev. Neurosci.* 28, 275–302
- 10 Heisenberg, M. *et al.* (1995) Structural plasticity in the *Drosophila* brain. *J. Neurosci.* 15, 1951–1960
- 11 O'Donnell, S. *et al.* (2007) Developmental and dominance-associated differences in mushroom body structure in the paper wasp *Mischocyttarus mastigophorus*. *Dev. Neurobiol.* 67, 39–46
- 12 Withers, G.S. *et al.* (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature* 364, 238–240
- 13 Ismail, N. *et al.* (2006) Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. *Proc. Natl. Acad. Sci. U. S. A.* 103, 207–211
- 14 Farris, S.M. *et al.* (2001) Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. *J. Neurosci.* 21, 6395–6404
- 15 Nithianantharajah, J. and Hannan, A.J. (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat. Rev. Neurosci.* 7, 697–709
- 16 Robinson, G.E. *et al.* (1989) Hormonal and genetic control of behavioral integration in honey bee colonies. *Science* 246, 109–112
- 17 Schulz, D.J. *et al.* (2002) Juvenile hormone and octopamine in the regulation of division of labor in honey bee colonies. *Horm. Behav.* 42, 222–231
- 18 Sullivan, J.P. *et al.* (2000) Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* 37, 1–14
- 19 Fahrback, S.E. *et al.* (2003) Limits on volume changes in the mushroom bodies of the honey bee brain. *J. Neurobiol.* 57, 141–151
- 20 Fahrback, S.E. *et al.* (1997) Expansion of the neuropil of the mushroom bodies in male honey bees is coincident with initiation of flight. *Neurosci. Lett.* 236, 135–138
- 21 Amdam, G.V. *et al.* (2004) Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Natl. Acad. Sci. U. S. A.* 101, 11350–11355
- 22 Guidugli, K.R. *et al.* (2005) Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett.* 579, 4961–4965
- 23 Barchuk, A.R. *et al.* (2002) Effects of juvenile hormone and ecdysone on the timing of vitellogenin appearance in hemolymph of queen and worker pupae of *Apis mellifera*. *J. Insect Sci.* 2, 1
- 24 Pinto, L.Z. *et al.* (2000) Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *J. Insect Physiol.* 46, 153–160
- 25 Amdam, G.V. and Omholt, S.W. (2003) The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. *J. Theor. Biol.* 223, 451–464
- 26 Nelson, C.M. *et al.* (2007) The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biol.* 5, e62
- 27 Whitfield, C.W. *et al.* (2003) Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302, 296–299

- 28 Grozinger, C.M. *et al.* (2003) Pheromone-mediated gene expression in the honey bee brain. *Proc. Natl. Acad. Sci. U. S. A.* 100 (Suppl 2), 14519–14525
- 29 Pécasse, F. *et al.* (2000) *Krüppel-homolog*, a stage-specific modulator of the prepupal ecdysone response, is essential for *Drosophila* metamorphosis. *Dev. Biol.* 221, 53–67
- 30 Schuh, R. *et al.* (1986) A conserved family of nuclear proteins containing structural elements of the finger protein encoded by *Krüppel*, a *Drosophila* segmentation gene. *Cell* 47, 1025–1032
- 31 Grozinger, C.M. and Robinson, G.E. (2007) Endocrine modulation of a pheromone-responsive gene in the honey bee brain. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 193, 461–470
- 32 Lee, T. *et al.* (2000) Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in *Drosophila*. *Neuron* 28, 807–818
- 33 Zhao, T. *et al.* (2008) A *Drosophila* gain-of-function screen for candidate genes involved in steroid-dependent neuroendocrine cell remodeling. *Genetics* 178, 883–901
- 34 Awasaki, T. and Ito, K. (2004) Engulfing action of glial cells is required for programmed axon pruning during *Drosophila* metamorphosis. *Curr. Biol.* 14, 668–677
- 35 Zheng, X. *et al.* (2003) TGF- $\beta$  signaling activates steroid hormone receptor expression during neuronal remodeling in the *Drosophila* brain. *Cell* 112, 303–315
- 36 Watts, R.J. *et al.* (2003) Axon pruning during *Drosophila* metamorphosis: evidence for local degeneration and requirement of the ubiquitin-proteasome system. *Neuron* 38, 871–885
- 37 Shi, L. *et al.* (2007) Roles of *Drosophila Krüppel-homolog 1* in neuronal morphogenesis. *Dev. Neurobiol.* 67, 1614–1626
- 38 Beck, Y. *et al.* (2004) *Krüppel-homolog* is essential for the coordination of regulatory gene hierarchies in early *Drosophila* development. *Dev. Biol.* 268, 64–75
- 39 King-Jones, K. and Thummel, C.S. (2005) Nuclear receptors – a perspective from *Drosophila*. *Nat. Rev. Genet.* 6, 311–323
- 40 Benevolenskaya, E.V. *et al.* (2000) *Krüppel homolog (Kr h)* is a dosage-dependent modifier of gene expression in *Drosophila*. *Genet. Res.* 75, 137–142
- 41 Kaczynski, J. *et al.* (2003) Sp1- and *Krüppel*-like transcription factors. *Genome Biol.* 4, 206
- 42 McGovern, V.L. *et al.* (2003) A targeted gain of function screen in the embryonic CNS of *Drosophila*. *Mech. Dev.* 120, 1193–1207
- 43 Brody, T. *et al.* (2002) Identification of novel *Drosophila* neural precursor genes using a differential embryonic head cDNA screen. *Mech. Dev.* 113, 41–59
- 44 Kearney, J.B. *et al.* (2004) Gene expression profiling of the developing *Drosophila* CNS midline cells. *Dev. Biol.* 275, 473–492
- 45 Kraut, R. *et al.* (2001) A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*. *Curr. Biol.* 11, 417–430
- 46 Beck, Y. *et al.* (2005) Dynamic localisation of KR-H during an ecdysone response in *Drosophila*. *Gene Expr. Patterns* 5, 403–409
- 47 Truman, J.W. and Riddiford, L.M. (2002) Endocrine insights into the evolution of metamorphosis in insects. *Annu. Rev. Entomol.* 47, 467–500
- 48 Broadus, J. *et al.* (1999) The *Drosophila*  $\beta$  FTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone. *Mol. Cell* 3, 143–149
- 49 Beckstead, R.B. *et al.* (2005) The genomic response to 20-hydroxyecdysone at the onset of *Drosophila* metamorphosis. *Genome Biol.* 6, R99
- 50 Thummel, C.S. (1996) Flies on steroids – *Drosophila* metamorphosis and the mechanisms of steroid hormone action. *Trends Genet.* 12, 306–310
- 51 Zheng, X. *et al.* (2006) Baboon/dSmad2 TGF- $\beta$  signaling is required during late larval stage for development of adult-specific neurons. *EMBO J.* 25, 615–627
- 52 Truman, J.W. *et al.* (1994) Ecdysone receptor expression in the CNS correlates with stage-specific responses to ecdysteroids during *Drosophila* and *Manduca* development. *Development* 120, 219–234
- 53 Schubiger, M. *et al.* (2005) Ligand-dependent de-repression via EcR/USP acts as a gate to coordinate the differentiation of sensory neurons in the *Drosophila* wing. *Development* 132, 5239–5248
- 54 Tsai, C.C. *et al.* (1999) SMRTER, a *Drosophila* nuclear receptor coregulator, reveals that EcR-mediated repression is critical for development. *Mol. Cell* 4, 175–186
- 55 Brown, H.L. *et al.* (2006) Use of time-lapse imaging and dominant negative receptors to dissect the steroid receptor control of neuronal remodeling in *Drosophila*. *Development* 133, 275–285
- 56 Riddiford, L.M. (1993) Hormones and *Drosophila* development. In *The Development of Drosophila Melanogaster* (Bate, M. and Martinez Arias, A., eds), pp. 899–939, Cold Spring Harbor Laboratory Press
- 57 Cao, C. *et al.* (2007) Fork head controls the timing and tissue selectivity of steroid-induced developmental cell death. *J. Cell Biol.* 176, 843–852
- 58 Zhou, X. and Riddiford, L.M. (2002) Broad specifies pupal development and mediates the 'status quo' action of juvenile hormone on the pupal-adult transformation in *Drosophila* and *Manduca*. *Development* 129, 2259–2269
- 59 Fristrom, D. and Fristrom, J.W. (1993) The metamorphic development of the adult epidermis. In *The Development of Drosophila melanogaster* (Bate, M. and Martinez Arias, A., eds), pp. 843–897, Cold Spring Harbor Laboratory Press
- 60 Minakuchi, C. *et al.* (2008) *Krüppel homolog 1 (Kr-h1)* mediates juvenile hormone action during metamorphosis of *Drosophila melanogaster*. *Mech. Dev.* 125, 91–105
- 61 Bechmann, I. and Nitsch, R. (2000) Involvement of non-neuronal cells in entorhinal-hippocampal reorganization following lesions. *Ann. N. Y. Acad. Sci.* 911, 192–206
- 62 Parrish, J.Z. *et al.* (2007) Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields. *Annu. Rev. Neurosci.* 30, 399–423
- 63 Laub, F. *et al.* (2005) Transcription factor KLF7 is important for neuronal morphogenesis in selected regions of the nervous system. *Mol. Cell Biol.* 25, 5699–5711
- 64 Warming, S. *et al.* (2006) Zfp423 is required for normal cerebellar development. *Mol. Cell Biol.* 26, 6913–6922
- 65 St Johnston, D. (2002) The art and design of genetic screens: *Drosophila melanogaster*. *Nat. Rev. Genet.* 3, 176–188
- 66 Homberg, U. (2008) Evolution of the central complex in the arthropod brain with respect to the visual system. *Arthropod Struct. Dev.* 37, 347–362
- 67 Groh, C. *et al.* (2004) Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4268–4273
- 68 Truman, J.W. and Riddiford, L.M. (1999) The origins of insect metamorphosis. *Nature* 401, 447–452
- 69 Mirth, C.K. and Riddiford, L.M. (2007) Size assessment and growth control: how adult size is determined in insects. *Bioessays* 29, 344–355
- 70 Shingleton, A.W. (2005) Body-size regulation: combining genetics and physiology. *Curr. Biol.* 15, R825–R827
- 71 Edgar, B.A. (2006) How flies get their size: genetics meets physiology. *Nat. Rev. Genet.* 7, 907–916
- 72 Nijhout, H.F. (2003) The control of body size in insects. *Dev. Biol.* 261, 1–9
- 73 Robinson, G.E. *et al.* (1997) Insect societies and the molecular biology of social behavior. *Bioessays* 19, 1099–1108
- 74 Slessor, K.N. *et al.* (2005) Pheromone communication in the honeybee (*Apis mellifera* L.). *J. Chem. Ecol.* 31, 2731–2745
- 75 Robinson, G.E. *et al.* (1998) Queen mandibular gland pheromone influences worker honey bee (*Apis mellifera* L.) foraging ontogeny and juvenile hormone titers. *J. Insect Physiol.* 44, 685–692