NUCLEAR RECEPTORS — A PERSPECTIVE FROM *DROSOPHILA*

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Abstract | Nuclear receptors are ancient ligand-regulated transcription factors that control key metabolic and developmental pathways. The fruitfly *Drosophila melanogaster* has only 18 nuclear-receptor genes — far fewer than any other genetic model organism and representing all 6 subfamilies of vertebrate receptors. These unique attributes establish the fly as an ideal system for studying the regulation and function of nuclear receptors during development. Here, we review recent breakthroughs in our understanding of *D. melanogaster* nuclear receptors, and interpret these results in light of findings from their evolutionarily conserved vertebrate homologues.

CLADE
A group of organisms that includes common ancestor and all of its descendants, representing a distinct branch on a phylogenetic tree.

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Classical signal-transduction pathways originate with a membrane-bound receptor. On binding its cognate ligand, the receptor initiates a cascade of events in the cytoplasm, eventually affecting specific transcription factors in the nucleus. In contrast to these often complex and convoluted pathways, members of the nuclear-receptor superfamily use an ingenious shortcut to transduce their signals — a strategy that is due to their unique structure. Nuclear receptors harbour a receptor function, DNA-binding capacity and a transcriptional activation function all within the same molecule, allowing a one-step response to a signal that results in direct effects on expression of the target gene.

Members of the nuclear-receptor superfamily are defined by the presence of a highly conserved DNA-binding domain (DBD) and a less conserved C-terminal ligand-binding and dimerization domain (LBD) (BOX 1). Nuclear receptors are ancient proteins that have been found in CLADES as diverse as sponges, echinoderms, tunicates, arthropods and vertebrates, and are therefore believed to be present throughout the Metazoa[1,2]. Phylogenetic analysis of nuclear-receptor genes has revealed six distinct subfamilies, defined by clusters of receptors that share significant sequence conservation between their respective DBDs and LBDs (BOX 2). Well-known vertebrate receptors that are members of some of these subfamilies include the steroid receptors, such as the oestrogen and glucocorticoid receptors (ER and GR), the thyroid hormone receptor (TR), the retinoic acid receptor (RAR), and the retinoid X receptor (RXR). A wide range of small, lipophilic molecules function as nuclear-receptor ligands, including steroids, thyroid hormone, retinoic acid and vitamin D. Not all nuclear receptors, however, have a known natural ligand, and at least some of these so-called orphan receptors can function in a ligand-independent fashion[3,4].

Extensive studies over the past two decades have provided a detailed understanding of the molecular mechanisms by which nuclear receptors convert a hormonal signal into a transcriptional response[3–7]. Steroid hormone receptors, such as GR, reside in the cytoplasm in complexes with chaperone proteins. On binding to hormones, these receptors translocate to the nucleus, where they function as homodimers to regulate the transcription of target genes. Other receptors, such as TR and RAR, function as heterodimers with RXR. Some of these receptors function as repressors in the absence of a ligand, maintaining the silence of target genes through the recruitment of co-repressor complexes (FIG. 1a). On binding to hormones, these receptors recruit co-activators, displace the co-repressors, and thereby induce transcription of the target gene (FIG. 1a). The ability to readily switch the functional state of nuclear receptors by the simple addition of small lipophilic compounds has put these factors in the vanguard of transcriptional regulation studies, providing key insights into the control of eukaryotic gene expression.

Nuclear receptors function as powerful regulators of diverse biological processes, including lipid and glucose homeostasis, detoxification, cellular differentiation and

embryonic development[4,5]. Consistent with these crucial regulatory roles, mutations in nuclear receptors are associated with many common and lethal human disorders, including cancer, diabetes and heart disease[4,5]. As a consequence, extensive efforts by the pharmaceutical industry have focused on modulating nuclear-receptor function by developing new and specific agonists and antagonists, resulting in the production of leading drugs that are currently on the market.

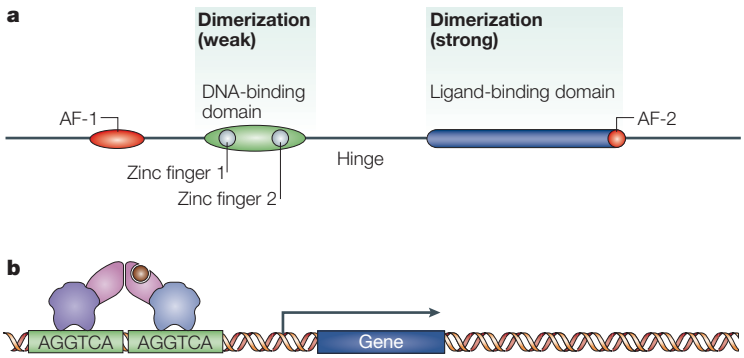
Although studies in vertebrate systems have provided insights into the molecular mechanisms by which nuclear receptors regulate transcription of target genes, much remains to be learned about their biological roles during development. Several unique advantages have pushed the fruitfly, *Drosophila melanogaster*, to the forefront of these studies. In contrast to the complexity of vertebrate hormone signalling pathways, *D. melanogaster* has only two known physiologically active lipophilic hormones, the steroid hormone 20-hydroxyecdysone (20E) and the sesquiterpinoid juvenile hormone (JH). In addition, the fly genome contains only 18 nuclear-receptor genes, as opposed to the 48 genes found in humans, with these fly receptors representing all 6 of the main nuclear-receptor subfamilies (BOX 2). Taken together with the well-established genetic and genomic tools for studying the biology of *D. melanogaster*, this distilled set of fly nuclear-receptor genes and the apparent relative simplicity of insect hormone-signalling pathways define this insect as an ideal model system for characterizing nuclear-receptor function and regulation.

This review focuses on recent genetic studies of the nuclear-receptor superfamily of *D. melanogaster* in the context of their respective mammalian homologues, and identifies common themes and divergent functions. We discuss how maturation is controlled by hormones and nuclear receptors in insects and vertebrates, and describe how studies of 20E signalling in *D. melanogaster* have provided a molecular framework for understanding the genetic regulation of maturation. We discuss the developmental roles of fly nuclear receptors, presenting examples of remarkable functional conservation with their vertebrate counterparts. Recent structural studies of LBDs and DBDs in *D. melanogaster* are discussed in the context of their roles during development. Finally, we survey fly orphan receptors that are likely to have a ligand and describe our current understanding of their possible regulatory functions. These studies show how genetic analysis of nuclear receptors in *D. melanogaster* provides a valuable developmental context for understanding the regulation and function of their vertebrate orthologues. In addition, we identify directions for future research that hold the promise of providing new insights into development, metabolism, longevity and physiology.

The 20-hydroxyecdysone regulatory cascades

One of the most remarkable hormonally triggered developmental responses in higher organisms is the maturation of a non-reproductive juvenile to a mature adult form. In humans, this is manifested by marked changes that occur during puberty and adolescence,

Box 1 | **Nuclear receptors: clues from their structure**

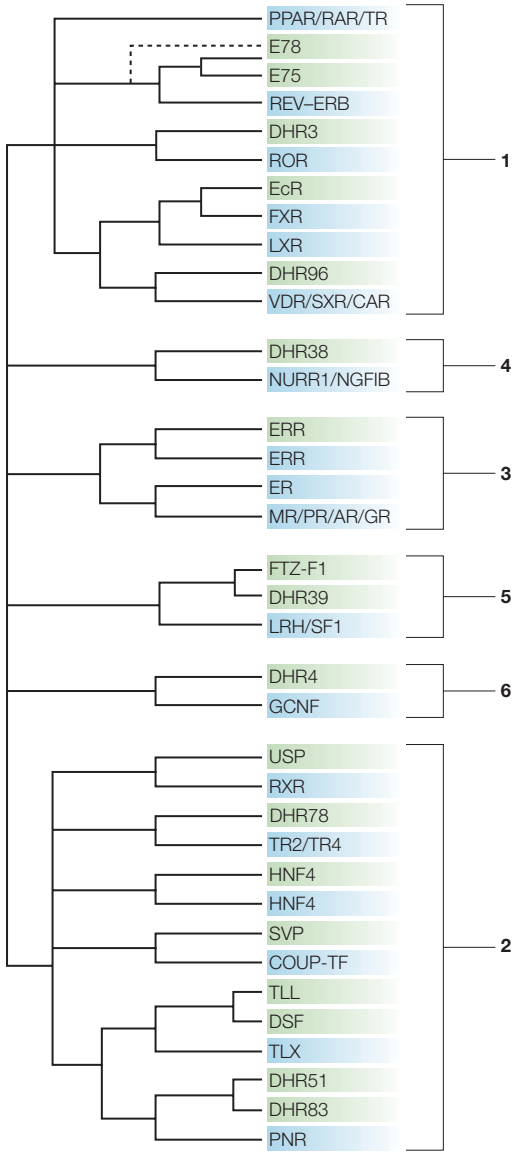


Nuclear receptors are defined by the presence of common structural elements: a highly conserved DNA-binding domain (DBD) and a ligand-binding domain (LBD) are joined by a flexible hinge region (panel a). The DBD comprises two C_4 zinc fingers. The first zinc finger provides DNA-binding specificity through a stretch of five amino acids known as the P-box[143]. The second zinc finger harbours a relatively weak dimerization interface that allows DBDs to dimerize, but only in the presence of a target DNA molecule[144]. The less conserved LBD is located C-terminal to the DBD, and constitutes the principal dimerization interface of this protein family. The LBD allows different receptors to dimerize, thereby vastly expanding the repertoire of potential DNA target sequences and regulatory functions. The LBD comprises 11–13 α-helices that generally form a hydrophobic pocket for the binding of small lipophilic molecules. The activation function 1 (AF-1) domain can act in a ligand-independent fashion and is located at the N-terminus, whereas the AF-2 activation domain is located at the C-terminal end of the LBD and is often ligand-dependent. Detailed structural and molecular studies have shown that, on hormone binding, the LBD switches into an active state by rotating helix 12 into a configuration in which it can recruit transcriptional co-activators through its AF-2 domain[6]. Each nuclear receptor binds to a half site that is derived from, or identical to, the archetypal AGGTCA sequence (panel b). Some nuclear receptors, such as vertebrate steroidogenic factor 1 (SF-1), can function as monomers through a single response element (FIG. 1b). Other nuclear receptors function as homodimers or heterodimers through two half sites, which comprise a functional hormone-response element (panel b). These half sites can be arranged as direct repeats, inverted repeats or everted repeats, and display a dimer-specific spacing that generally ranges from 0–6 bp. The sequence and arrangement of the half sites contributes to receptor binding specificity.

Box 2 | Evolution and genomics of *Drosophila melanogaster* nuclear receptors

The phylogenetic tree shown in the figure depicts the evolutionary relationship between human nuclear receptors (shown in blue) and *Drosophila melanogaster* receptors (shown in green), with the six subfamilies numbered on the right[120]. This tree could also be subdivided into 4 subfamilies, with subfamilies 3, 5, and 6 shown in the figure forming a single MONOPHYLETIC group of their own[1]. Remarkably, both insects and humans encode members of all nuclear receptor subfamilies, despite being separated by ~600–800 million years of evolution, indicating that these subfamilies were established early in metazoan evolution[145]. A systematic nuclear receptor nomenclature has been established on the basis of this subfamily classification, with a number representing the subfamily, a capital letter for the group, and a number for the individual gene[146] (for example, NR1H1 corresponds to the ecdysone receptor, *EcR*; see TABLE 1). A unique and atypical subset of three nuclear receptors is also present in *D. melanogaster*: members of this so-called *knirps* group lack an LBD and have important roles during embryogenesis and neurogenesis. The *knirps* group is not covered in this review because they are unlikely to function as ligand-regulated receptors.

Interestingly, four fly receptors seem to have derived from duplication events that did not occur in the vertebrate lineage (the alternative but less parsimonious model would suggest that the duplicated genes were selectively lost in the vertebrate lineage). These nuclear receptor gene pairs — *E75–E78*, *DHR51–DHR83*, *DHR39–FTZ-F1* and *dissatisfaction* (*dsf*)–*tailless* (*tll*) — are represented by only one orthologous gene in vertebrates, with typically one of the encoded fly receptors being more similar to the vertebrate orthologue than its duplicated counterpart (TABLE 1). Only the *E75–E78* pair has an orthologous set in *Caenorhabditis elegans* (*nhr-85–sex-1*), indicating that this duplication event occurred before nematodes branched off from the arthropod lineage (represented by a dotted line in the figure). According to an alternative phylogenetic analysis, *E78* is most closely related to vertebrate *PPAR*s (peroxisome proliferator-activated receptors)[1].



An interesting feature emerges when we examine the list of nuclear receptors that are unique to vertebrates — that is, those receptors with no convincing orthologue in *D. melanogaster*. This list comprises two-thirds of the known hormone-regulated vertebrate nuclear receptors, representing a range of key endocrinological pathways. They include members of subfamily 1: thryoid hormone receptor (TR), retinoic acid receptor (RAR) and PPAR; as well as members of subfamily 3: oestrogen receptor (ER), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR) and the androgen receptor (AR). The fact that these crucial hormone-regulated receptors have no orthologues in the fly indicates that insects are missing several hormone signalling pathways, although it is still too early to know whether any of the 17 fly orphan nuclear receptors have subsumed some of these vertebrate functions.

The data for the figure are from REF. 2, except for the proposed duplication of the *E75–E78*, *DHR39–FTZ-F1*, *tll–dsf* and *DHR51–DHR83* gene pairs, as described above.

MONOPHYLETIC
A natural clade that includes an ancestral species or gene and all of its descendants.

HOLOMETABOLOUS
Refers to insects that interpose a pupal stage between the final larval stage and the adult.

ECDYSTEROIDS
Insect steroids that are similar in structure to the moulting hormone ecdysone.

PROTHORACIC GLAND
An insect endocrine gland that produces ecdysteroids.

HAEMOLYMPH
Insect blood.

whereas maturation in HOLOMETABOLOUS insects such as *D. melanogaster* occurs during metamorphosis. In both insects and vertebrates, maturation is triggered by rises in hormone titres, and transduced by members of the nuclear-receptor superfamily. These functions are primarily controlled by thyroid hormone and sex steroids in vertebrates, whereas insect maturation is regulated by the steroid hormone 20E (REF. 8). Although relatively little is known about how vertebrate hormones control maturation, molecular and genetic studies in *D. melanogaster* have provided a detailed understanding of the molecular mechanisms by which 20E exerts its effects on development.

ECDYSTEROIDS are produced by the PROTHORACIC GLANDS and released into the HAEMOLYMPH, primarily as α-ecdysone (αE), which is then converted by peripheral tissues to

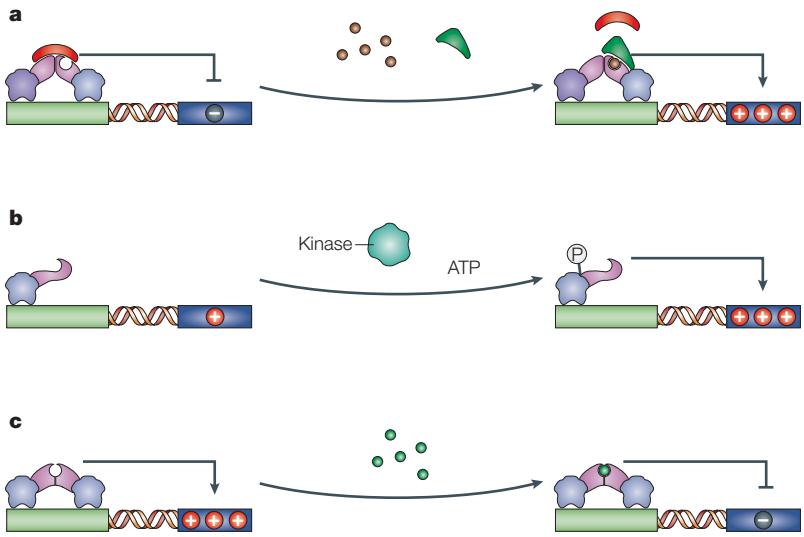


Figure 1 | **Molecular mechanisms of nuclear-receptor regulation.** The diagram illustrates several modes of nuclear-receptor regulation, highlighting the examples mentioned in the text. **a** | A schematic representation of the ligand-mediated switch in thyroid hormone receptor (TR)–retinoid X receptor (RXR) function. In the absence of a ligand (brown circles), the TR–RXR heterodimer (pink and blue shapes) binds co-repressors (red crescent) and actively shuts down target-gene transcription (left). On hormone binding, TR–RXR recruits co-activators (green), and ejects the co-repressors, thereby activating target-gene transcription (right). **b** | A schematic representation of steroidogenic factor 1 (SF-1) regulation by phosphorylation. SF-1 functions as a constitutive activator, binding as a monomer to its response element (left). Phosphorylation by a kinase results in enhancement of its activation function[70] (right). **c** | A schematic representation of inverse agonist function. A homodimer of oestrogen-related receptor-α (ERRa) is depicted, bound to an oestrogen response element (left). ERRa activity is downregulated in response to its inverse agonist[136] (green circles).

the biologically active form, 20E (REF. 9). 20E binds to a heterodimer of two nuclear receptors, the ecdysone receptor (EcR) and ultraspiracle (USP)[10,11], which are orthologues of the vertebrate farnesoid X receptor (FXR) or liver X receptor (LXR), and RXR receptors, respectively. It remains unclear whether any regulatory functions of the FXR and LXR lipid and bile-acid sensors are conserved in EcR. By contrast, USP functions as a crucial heterodimer partner for many fly nuclear receptors, much like its vertebrate counterpart[12].

After the completion of embryogenesis, *D. melanogaster* progresses through three larval stages, or instars, to enter metamorphosis and finally emerge as an adult fly. Each of the transitions between these stages is triggered by a pulse of 20E, defining the hormone as a crucial determinant of developmental timing[13]. The biological processes controlled by ecdysteroids are remarkably diverse and include morphogenetic, apoptotic, physiological, reproductive and behavioural responses. A main goal of current research is to understand how this wide range of effects is achieved in response to the systemic 20E signal. Below, we provide an overview of the biological functions of EcR and USP during development, and discuss their activities in the context of recent studies that have used molecular and biochemical approaches. The reader is directed to two recent review articles for a more detailed overview of EcR and USP functions[14,15].

DOMINANT-NEGATIVE
A mutation in a gene that interferes with the function of its wild-type counterpart.

GERMBAND RETRACTION
A large-scale morphogenetic movement during *Drosophila* gastrulation that results in the repositioning of the future posterior segments at the posterior end of the embryo.

HEAD INVOLUTION
Morphogenetic reorganization of the embryonic head region in *D. melanogaster*.

CUTICLE
A chitinous secretion of epidermal cells that covers the outside of the insect body, providing protection and support.

Embryonic functions of EcR and USP

Ecdysteroid titres peak sharply during mid-embryogenesis, indicating an embryonic role for the hormone[8]. Attempts to study ecdysteroid function during embryogenesis, however, have been hampered by the maternal contribution of ecdysteroids, *EcR* mRNA and EcR protein. Similar to vertebrate steroid receptors, EcR is required for female fertility, preventing the study of its maternal functions during embryogenesis[16,17]. By taking advantage of DOMINANT-NEGATIVE forms of *EcR*, however, a recent study showed that *EcR* is required for two key embryonic morphogenetic movements, GERMBAND RETRACTION and HEAD INVOLUTION[18]. Interestingly, the net effect of these movements is to direct a complete transformation in body plan, from an immature embryo to that of a first instar larva, foreshadowing the remarkable 20E-triggered transformation that will occur later in the life cycle when the larva is transformed into an adult fly during metamorphosis. Curiously, in contrast to the EcR dominant-negative studies, reduction of maternal and zygotic *usp* function results in embryonic lethality with minor CUTICLE defects and no reported defects in morphogenesis[19]. It is possible that partially functional USP protein is still expressed in these mutant embryos[20–22]. Alternatively, an intriguing possibility is that EcR interacts with a distinct dimerization partner during embryogenesis for at least some of its regulatory functions.

Postembryonic functions of EcR and USP

EcR produces three protein isoforms, designated A, B1, and B2, whereas *usp* encodes only one isoform[14,23]. The EcR-A and EcR-B1 isoforms are expressed in distinct sets of tissues during the onset of metamorphosis[23,24], and extensive studies indicate that this distribution contributes to the spatial specificity of 20E responses[25–29]. A biochemical basis for understanding the distinct functions of EcR isoforms arose from *D. melanogaster* cell-culture studies, which showed that EcR-B isoforms function as potent ligand-regulated transcriptional activators through their unique AF-1 domains (BOX 1), but EcR-A functions as a poor transcriptional activator[30,31]. USP, by contrast, does not impart any activation function in this system; rather, it seems to be required primarily for its allosteric effects on EcR, facilitating its DNA- and ligand-binding activities[30]. This is consistent with recent structural studies of the USP LBD, which showed that helix 12 is locked in an inactive conformation[32,33](BOX 1). In addition, the crystal structure of the EcR LBD showed that its ligand-binding pocket is highly flexible and requires dimerization with USP to support a ligand-binding conformation[34], providing a molecular explanation for the observation that EcR is incapable of specific ligand binding on its own — a unique feature of this receptor that distinguishes it from vertebrate RXR heterodimer partners[10,35,36].

Genetic studies have shown that USP can function as a repressor, revealing an intriguing parallel with vertebrate RXR heterodimers that function as repressors in the unliganded state[37]. Removal of USP from wing

IMAGINAL DISC cells results in the premature activation of key 20E-regulated target genes[38]. In addition, some developmental responses that normally take place during early metamorphosis occur precociously in cells that lack *usp* function, indicating that at least some of the metamorphic responses to 20E are implemented through derepression[38]. A similar function has been invoked for USP in adult eye development, as well as in cultured cells, where USP represses some target promoters[22,39]. These studies provide strong support for the idea that USP represses gene activity *in vivo* when ecdysteroid titres are low, preventing premature maturation. It is likely that USP exerts this repressive function in combination with its EcR partner, although a genetic test of this possibility has yet to be reported.

Recent work has shown that ecdysteroids have a role in the adult beyond female fertility. Reduction of either ecdysteroid titres or *EcR* function results in increases in adult longevity[40]. Although changes in steroid hormone titres have been linked to ageing in humans, functions for these changes remain unclear. Interestingly, JH titres have also been linked to the longevity of the adult fly, mediated by insulin signalling[41]. It is possible that *D. melanogaster* will provide a valuable model for understanding the links between endocrine function and the control of ageing in higher organisms, with implications for how similar pathways might be controlled in humans[42].

Nuclear-receptor genes as 20E targets

Most effects of 20E are mediated through the transcriptional regulatory functions of the EcR–USP receptor, thereby coordinating downstream gene expression. Remarkably, many direct targets of the 20E–EcR–USP complex are themselves members of the nuclear-receptor superfamily. In addition to EcR, six nuclear-receptor genes — *Drosophila* hormone receptor 3 (*DHR3*; or Hr46), *DHR4* (Hr4), *DHR39* (Hr39), *E75*, *E78*, *ftz* transcription factor 1 (*ftz-f1*) — are transcriptionally regulated by 20E and show marked changes in mRNA levels in synchrony with ecdysteroid pulses during development (FIG. 2; TABLE 1). *E75* is induced as a classical PRIMARY-RESPONSE GENE, independent of protein synthesis, *ftz-f1* is repressed by 20E, and *E78*, *DHR3*, *DHR4* and *DHR39* all require 20E-induced protein synthesis for their maximal levels of expression[43–46]. Below, we highlight recent functional studies of these six nuclear-receptor genes and integrate them into 20E-response pathways during development, as well as describing other possible hormone signalling functions for some of these receptors.

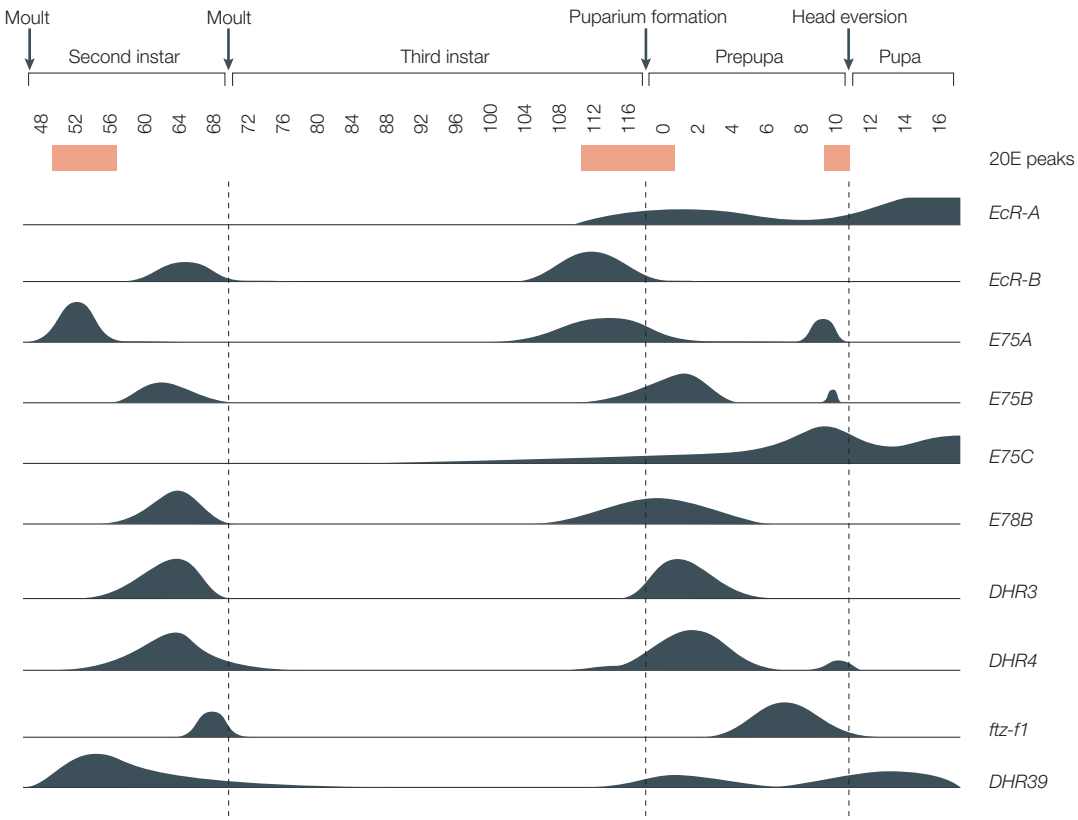


Figure 2 | **Temporal profiles of 20E-regulated nuclear-receptor gene expression.** Nuclear receptor gene-expression patterns are depicted in a schematic form[53]. Individual mRNA isoforms from *EcR*, *E75*, *E78*, *DHR3*, *DHR4*, *ftz-f1*, and *DHR39* are expressed for brief temporal windows during the second larval instar, in late third instar larvae and in prepupae. The main developmental transitions — moults, puparium formation and head eversion — are depicted at the top, along with developmental stage and time in hours after egg laying for larvae, or hours after puparium formation for prepupae and pupae. The approximate times of 20-hydroxyecdysone (20E) titre peaks are also shown[8].

IMAGINAL DISC
An epithelial sheet that gives rise to external adult structures during insect metamorphosis, including the wings, eyes and antennae.

PRIMARY-RESPONSE GENE
A gene that is regulated directly by a transcription factor in response to a signal.

Table 1 | ***Drosophila melanogaster* nuclear receptors**

D. melanogaster receptor (ligand)	Subfamily	Nomenclature	20E-regulated	No. of isoforms (PR/PP/RNA)	Human orthologue (ligand)	DBD/LDB identity
E75	1D/E	NR1D3	+	3/4/4	*REV–ERBA	80/25
E78	1D/E	NR1E1	+	2/3/2	*REV–ERBA	69/23
DHR3	1F	NR1F4	+	1/3/3	*RORB (all *trans* retinoic acid)	76/35
EcR (20-hydroxyecdysone)	1H	NR1H1	+	3/3/5	FXR (chenodeoxycholic acid) *LXR (22(R)-hydroxycholesterol)	72/28 64/37
DHR96	1I/J	NR1J1	(+)	1/1/2§	*VDR (1α, 25-dihydroxyvitamin D_3)	55/20
HNF4	2A	NR2A4	?	1/3/3	*HNF4A	89/61
USP	2B	NR2B4	–	1/1/1	RXRA (9-*cis*-retinoic acid)	84/43
DHR78	2C/D	NR2D1	(+)	1/2/4‡	*TR2	67/23
Tailless	2E	NR2E2	?	1/1/1	TLX	80/34
Dissatisfaction	2E	NR2E4	?	1/1/1	TLX	74/35
DHR83/CG10296	2E	NR2E5	?	0/1/1	PNR	60/20
DHR51/CG16801	2E	NR2E3	?	0/2‡/2‡	PNR	70/47
Seven up	2F	NR2F3	?	1/3/3	*COUP-TF1	89/92
ERR	3B	NR3B4	?	0/2/2	*ERRb (diethylstilbestrol)	88/34
DHR38	4A	NR4A4	–	2/2/2	*NURR1	93/59
FTZ-F1	5A	NR5A3	+	2/2/3§	LRH-1 (phospholipid) SF-1 (phospholipid)	89/35 88/28
DHR39	5A	NR5B1	+	2/2/2	LRH-1 (phospholipid) SF-1 (phospholipid)	62/25 60/26
DHR4	6A	NR6A2	+	1/2‡/2§	GCNF	61/21

The 18 canonical *Drosophila melanogaster* nuclear receptors are listed and the ligand for the ecdysone receptor (EcR) is shown in parentheses. Subfamily designation and nuclear-receptor nomenclature is as described[146]. 20-hydroxyecdysone-regulated genes ('20E-regulated' column) are those that are transcriptionally controlled by 20E. (+) indicates a weak response to 20E in organ culture. The number of reported *D. melanogaster* protein isoforms (PR), predicted protein isoforms (PP) or RNA isoforms (RNA) are shown. §Based on size classes detected on northern blots. ‡Insufficient EST or cDNA evidence. The human nuclear receptors that display the highest percentage amino-acid identity with a fly nuclear receptor DNA-binding domain (DBD) and/or ligand-binding domain (LBD) are listed along with a representative ligand, where known. *Additional human family members are equally orthologous to the corresponding fly receptor. The ERRb ligand is not natural and fatty acids are not listed as an HNF4 ligand because it is not clear whether they are freely exchangeable. The final column shows the percentage amino-acid identity between the homologous human and fly DBDs and LBDs. For the DBD comparison, an ~85 amino-acid region was used that encompasses the core DBD (~66 amino acids) and the ~19 amino acid carboxy-terminal extension, which can contribute to DNA-binding specificity. The LBD comparisons relied on structure-based alignments of the entire LBD (helices 1–12) using BLAST and CLUSTALW. COUP-TF1, chicken ovalbumin upstream promoter transcription factor 1; DHR, *Drosophila* hormone receptor; ERR, oestrogen-related receptor; ERRb, oestrogen-related receptor-β; FTZ-F1, Fushi tarazu factor 1; FXR, farnesoid X receptor; GCNF, germ cell nuclear factor; HNF4, hepatocyte nuclear factor 4; LRH-1, liver receptor homolog 1; LXR, liver X receptor; NURR1, NR4A2 (nuclear receptor subfamily 4, group A, member 2) receptor; PNR, photoreceptor-specific nuclear receptor; REV-ERBa, NR1D1 receptor; RORa/b, retinoid-related orphan receptor-α/β; SF-1, steroidogenic factor 1; TLX, tailless homologue; TR2, NR2C1 receptor; USP, ultraspiracle; VDR, vitamin D receptor.

POLYTENE CHROMOSOME
A giant chromosome that is formed by many rounds of DNA replication in the absence of cytokinesis. The replicated DNA molecules are tightly aligned along their length in precise register, creating a transcriptionally active chromosome with a diagnostic banding pattern that is easily visualized through light microscopy.

GERMLINE CLONE
The germline lineage, or the offspring of this lineage, that carries a homozygous mutant allele that was generated by mitotic recombination in a heterozygous female. This use of these clones enables the production of progeny that lack a maternal contribution of the gene in question.

These studies provide a model for understanding how hormones can coordinate the transcription of many nuclear receptors, as well as how crosstalk between these receptors can provide specificity in hormone-response pathways.

EcR–USP targets — E75 and E78. Two *D. melanogaster* orphan nuclear receptors, E75 and E78, are most similar in sequence to the human REV–ERBA receptor (BOX 2; TABLE 1). *E78* mutations have no effects on viability or fertility, although some effects can be seen on larval POLYTENE CHROMOSOME puffing patterns and egg morphology[47,48]. The *E75* gene is a complex genetic locus that encodes at least three protein isoforms from distinct promoters, each of which are given a letter designation and distinguished by unique N-terminal sequences. GERMLINE CLONES of *E75*-null mutants, which are missing all three isoforms, lead to arrest during mid-oogenesis, indicating essential roles in female fertility[16]. An *E75A*-specific null mutation results in a block in larval development arising from reduced ecdysteroid titres[49]. This defines a feed-forward activity for E75A, in which 20E-induced E75A expression directs appropriate 20E titres; this is analogous to feed-forward pathways that have been described for some human nuclear receptors[50]. Interestingly, a recent study has shown that E75 has a haem group in its LBD , and that this provides it with the ability to bind nitric oxide (NO) or carbon monoxide (CO) — small gas molecules that function in stress-response pathways (J. Reinking *et al.*, personal communication). This study raises the fascinating possibility that gas molecules might modulate E75 activity *in vivo*. One possible role for this could be in circadian rhythms, where NO, CO and REV–ERB have crucial functions[42].

EcR–USP targets — DHR3 and DHR4. *DHR3* executes essential functions during embryogenesis, prior to moults occurs and at the onset of metamorphosis[51–54]. Although DHR3 is sufficient to downregulate key 20E-inducible early genes, it is not necessary for this response, indicating that it functions together with other negative regulators to repress the early regulatory response to the hormone at entry into metamorphosis[54].

By contrast, *DHR3* mutants show markedly reduced expression of the bFTZ-F1 orphan nuclear receptor in PREPUPAE, a response that is probably mediated directly through DHR3 binding sites in the *bftz-f1* promoter[55,56]. The ability of DHR3 to activate *bftz-f1* transcription can be suppressed by its binding to E75B, indicating that the precise timing of bFTZ-F1 expression is determined by an interplay between DHR3 accumulation and E75B decay[57]. A null mutation in *E75B*, however, has no effect on the timing of bFTZ-F1 expression, in agreement with the proposal that it might be redundant with *E78B* (REF. 49). Interestingly, the corresponding mammalian orthologues, RAR-related orphan receptor (ROR; DHR3 in *D. melanogaster*) and REV–ERB (E75), can also exert antagonistic functions. The activation of target-gene transcription by RORA can be inhibited by co-expression of REV–ERB, although this interaction seems to occur through binding-site competition rather than heterodimerization[58,59]. Likewise, RORA and REV–ERBA have opposing roles in the control of *Bmal1*, a crucial regulator of the circadian clock in mammals[60,61]. The recent discovery that RORA binds cholesterol derivatives and RORB shows a high affinity for all *trans* retinoic acid supports the possibility that DHR3 is regulated by a ligand in flies[62–64]. However, the relatively low conservation between the LBD of fly DHR3 and that of its human counterparts (RORA: 32% identity, RORB: 35% identity) makes it impossible to predict if such a ligand falls into any of the classes described above.

DHR4 encodes the closest homologue of the vertebrate orphan receptor germ-cell nuclear factor (GCNF), which functions as a transcriptional repressor[37]. The observation that *DHR4* expression closely parallels that of *DHR3* raised the possibility that they might exert common regulatory functions (FIG. 2), a model supported by a recent genetic analysis of the *DHR4* locus. Disruption of *DHR4* function results in two distinct phenotypes: premature PUPARIATION leading to the formation of small animals, and loss of essential functions during early metamorphosis (K.K.-J., J.-P. Charles, G. Lam and C.S.T., unpublished observations). Consistent with a primarily repressive role, *DHR4* is necessary and sufficient to downregulate many genes at the onset of metamorphosis, although it is also required for subsequent *bftz-f1* induction. The parallel functions with DHR3 indicate that these two orphan receptors act together, in a partially redundant manner, to direct the switch from late-larval to prepupal genetic programmes.

EcR–USP targets — FTZ-F1 and DHR39. The *ftz-f1* gene has two promoters that generate distinct protein isoforms with unique N-terminal sequences that are fused to a common LBD. The aFTZ-F1 isoform is maternally deposited and present during early embryogenesis, whereas bFTZ-F1 is expressed during later stages of development[65]. DHR39, a nuclear receptor with high sequence similarity to FTZ-F1, is also expressed during these stages, but typically precedes bFTZ-F1 expression and seems to be downregulated when bFTZ-F1 reaches maximum levels[45,53] (FIG. 2). Both factors bind to identical DNA sequences, indicating a functional overlap at the level of target-gene regulation[66,67]. FTZ-F1 and DHR39 are most closely related to vertebrate liver receptor homologue 1 (LRH-1) and steroidogenic factor 1 (SF-1) (REF. 68). SF-1 activity can be modulated through direct phosphorylation, which seems to mimic the stabilizing effects that normally occur on ligand binding[69,70] (FIG. 1b). Mouse and human SF-1, and human LRH-1, can also bind phosphatidylinositol second messengers, raising the possibility that the fly receptors bind a similar compound, although no biological effect has yet been demonstrated for these compounds on SF-1 or LRH-1 activity[71]. Similar to the *E75–E78* duplication, *ftz-f1* is essential for viability, whereas *DHR39* null mutants are viable and fertile[65,72].

aFTZ-F1 has a crucial role during embryonic development — through direct interactions with the FTZ homeodomain protein — in controlling the transcription of key genes that are involved in embryonic pattern formation[73,74]. This interaction is mediated through the FTZ-F1 AF-2 domain, similar to conventional nuclear receptor cofactor recruitment, although in this case the cofactor is itself a fully functional transcription factor[75–77]. Consistent with its expression, *bftz-f1* has essential roles later in development in larval moulting and pupal development[65]. Metamorphosis is initiated by a high-titre 20E pulse at the end of the third larval instar, followed ~10 hours later by a second pulse that triggers adult head eversion, establishing the overall body plan of the adult fly, as well as directing the programmed cell death of obsolete larval tissues such as the salivary glands[13]. bFTZ-F1 is expressed in mid-prepupae and is both necessary and sufficient for appropriate stage-specific responses to the second 20E pulse, acting as a crucial competence factor that dictates the temporal specificity of 20E signalling[46,78]. Therefore, an interplay between several orphan nuclear receptors — E75, DHR3, DHR4, and possibly E78 and DHR39 — all converge on bFTZ-F1 to ensure that responses to the second 20E pulse will be distinct from those of the earlier pulse, directing progression through metamorphosis. These cross-regulatory interactions among fly orphan receptors provide an interesting parallel to studies of regulatory specificity that are conferred by vertebrate nuclear-receptor interactions[79,80]. In addition, they provide crucial insights into the molecular mechanisms by which a steroid signal can be refined into temporally distinct biological responses during development.

Other developmental processes

The remaining fly nuclear-receptor genes provide a range of functions during development, and are discussed below in two groups. We first cover 5 nuclear receptor genes — *DHR51*, *DHR83*, *tailless* (*tll*), *dissatisfaction* (*dsf*) and *seven up* (*svp*) — that seem to be central in neuronal development, in many cases paralleling the functions of their vertebrate counterparts. We then discuss *DHR38* (*Hr38*), *DHR78* (*Hr78*),

PREPUPA
The developmental stage in *Drosophila melanogaster* that follows pupariation and precedes adult head eversion.

PUPARIATION:
Puparium formation. The larval–prepupal transition.

DHR96 (*Hr96*), *Drosophila hepatic nuclear factor 4* (*Hnf4*) and *Drosophila oestrogen related receptor* (*ERR*), describing what is known about their biological functions and speculating on possible ligand-regulated activities.

Brains, eyes and embryos. Subfamily 2 in *D. melanogaster* contains two gene pairs — *tll–dsf* and *DHR51–DHR83* — that seem to be the result of duplication events that occurred after the split between arthropod and vertebrate lineages (BOX 2). Therefore, vertebrate PNR (photoreceptor-specific nuclear receptor) is represented in flies by DHR51 and its less-conserved cousin, DHR83, both of which were only recently discovered as a result of the *D. melanogaster* genome-sequencing project (TABLE 1). Transcripts from both genes are undetectable by northern blot hybridization using total RNA, suggesting that these receptors are expressed in a highly restricted manner[53]. In support of this idea, the *Caenorhabditis elegans* orthologue of DHR51, FAX-1, is expressed in a subset of neurons and is required for axon pathfinding, as well as expression of neuropeptide precursors in specific interneurons[81]. Human PNR expression is restricted to retinal tissue and is required for proper photoreceptor cell fate[82]. Mutations in this gene are associated with an inherited form of retinal degeneration in humans. The P-Box (a stretch of 5 amino acids; BOX 1) of PNR is unique among vertebrate nuclear receptors and is conserved in DHR51 and FAX-1, but not in DHR83, indicating that the two fly PNR orthologues recognize distinct response elements.

Drosophila melanogaster embryogenesis is characterized by the formation of repeated segments along the anterior–posterior axis. The position and identity of these segments is determined by a complex hierarchy of patterning genes. The *tll* gene, a GAP GENE, is expressed in the anterior and posterior terminal poles of the embryo and has a key role in the establishment of these regional subdomains. This is achieved, at least in part, through repression of other gap genes, including *giant*, *krüppel* and *knirps*, and activation of *hunchback* in the posterior of the embryo[83]. Similar to other *D. melanogaster* embryonic patterning genes, *tll* also exerts later functions during development. It is expressed in the developing nervous system, and is required for the development of all protocerebral neuroblasts and the embryonic visual system[84–86].

In another striking example of evolutionary conservation, *Tlx*, the mammalian orthologue of *tll*, is restricted to the forebrain at all stages, and it is strongly expressed in the neuroepithelium of the embryonic brain, in adult neural stem cells and in the OPTIC CUP, where it is required for proper development of the visual system[87–89]. Mice that are homozygous for a targeted mutation in the *Tlx* gene show a range of neuronal defects, including progressive retinal and optic nerve degeneration[90,91]. TLX was recently shown to maintain neural stem cells in an undifferentiated state, consistent with the observation that adult brain cells which have lost *Tlx* function are unable to proliferate and self-renew[87]. Similar to its fly counterpart, TLX functions as a transcriptional repressor in cell culture and mouse *Tlx* mutants show derepression of potential target genes[87].

The DSF receptor is more similar to vertebrate TLX than TLL, and, as with both of these factors, it functions as a repressor[92]. The fly *dsf* gene is expressed in both sexes in a small subset of neurons, and mutations in this gene affect adult sexual behaviour[93]. Mutant males have a defect in the motor neurons that innervate the ventral abdominal muscles, resulting in reduced copulation efficiency. Mutant females have an increased resistance to males during courtship and copulation, and fail to lay mature eggs, primarily because the uterine muscles are not innervated by the required set of motor neurons[93,94]. Interestingly, *Tlx* mutations can lead to aggression in mice, indicating that behavioural functions for this class of nuclear receptors have been conserved through evolution[95]. Considering that *Tlx* mutants also have severe defects in the limbic system, which contributes to libido in humans, it would not come as a surprise if *Tlx* has a role in mammalian sexual behaviour[90,91].

SVP shows the highest degree of sequence conservation of a fly nuclear receptor with its vertebrate orthologue, sharing ~90% identity in the DBD and LBD with chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1). Similar to other members of the TLL group, *svp* has crucial roles in neuronal development. It is required for proper establishment of the embryonic CNS, as well as the development of four of the eight photoreceptor cells that constitute each OMMATIDIUM of the adult eye (R1, R3, R4 and R6) (REFS 96,97). Loss of *svp* function causes a transformation of these cells to an R7 fate and ectopic expression of *svp* in R7 cells alters their identity to an R1 or R6 fate[96,98]. These functions parallel those of COUP-TF1, which is required for neuronal development, axon guidance and spinal nerve growth[99,100]. The fly *svp* gene is also required for the development of non-neuronal tissues, including the FAT BODY[101], the MALPIGHIAN TUBULES[102], and the dorsal vessel of the circulatory system[103,104]. COUP-TF1 functions largely as a repressor, through two distinct mechanisms[37]. First, it competes with several other nuclear receptors, including peroxisome proliferator-activated receptor (PPAR), vitamin D receptor (VDR), TR, and RAR, for binding to a common response element. Second, COUP-TF1 competes with the same set of nuclear receptors for their dimerization partner, RXR, effectively reducing their activity, as they bind poorly to DNA as monomers or homodimers. Similarly, SVP heterodimerizes with the fly RXR orthologue USP, and can compete with EcR–USP for DNA binding in a way that is analogous to that of its vertebrate counterpart[105].

The ligand hopefuls. A wide range of ecdysteroids can be found in insect haemolymph, some of which are present only at specific stages during development. These might be supplemented by phytoecdysteroids and other cholesterol derivatives that can enter the animal through their diet[8,9]. Although most of these compounds are thought to be inactive, increasing evidence indicates that they might contribute to pathways that are

GAP GENE
Genes that direct the development of several contiguous segments in the early fly embryo.

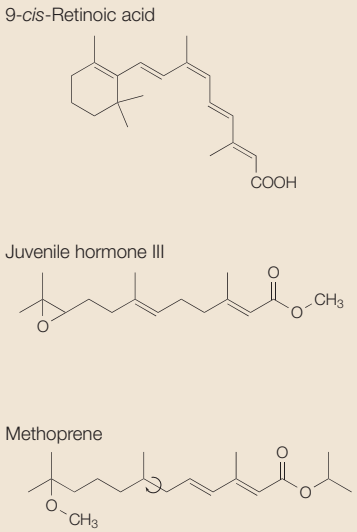
OPTIC CUP
A cup-like depression in the optic vesicle that develops into the sensory (neural) and pigmented layers of the retina.

OMMATIDIUM
The light-gathering and sensory unit of the insect compound eye.

FAT BODY
The principal organ of intermediary metabolism in insects. Similar in function to the vertebrate liver.

MALPIGHIAN TUBULES
The excretory and osmoregulatory organ of insects that opens near the junction of the midgut and hindgut.

Box 3 | Is ultraspiracle a juvenile hormone receptor?



Juvenile hormone (JH) has many vital functions in insect development, including the maintenance of cuticular identity during larval moults, VITELLOGENIN synthesis, ovarian development, caste determination and entry into diapause[147]. JH III is the primary active form in *Drosophila melanogaster*[8]. The structural similarity between JH and the vertebrate retinoid X receptor (RXR) ligand 9-*cis*-retinoic acid, the close phylogenetic relationship between ultraspiracle (USP) and RXR, and the finding that methoprene, a JH analogue, activates RXR[148], raised the possibility that USP might function as a JH receptor. In support of this proposal, USP can bind JH *in vitro*, albeit with low affinity[149]. JH can also induce transcription of a reporter gene in cell culture and this response can be inhibited by dominant-negative forms of USP, although high hormone concentrations are required for these transactivation studies (75–100 μM)[150]. The crystal structure of the USP ligand-binding domain (LBD) revealed that the ligand-binding pocket is filled with a small bacterial-derived phospholipid[32,33]. Theoretical ligand-docking studies using these structures indicate that JH can easily fit inside the *Heliothis virescens* USP ligand-binding pocket, although it shows a low degree of occupancy[151]. This could still support a possible functional interaction between JH and USP, however, because other nuclear receptors bind ligands with relatively low affinity and show partial filling of a large ligand-binding pocket.

Although the available biochemical data indicate that USP might function as a JH receptor, the biological data in *D. melanogaster* are less clear. This is largely because there is no known natural function for JH before adult stages in *D. melanogaster*[8]. Ectopic application of JH on third instar larvae or prepupae causes lethality with defects in abdominal development, indicating that the hormone can at least function at this stage[152,153]. In addition, studies of *usp* mutants provide possible support for a role in JH signalling. Along with the expected defects in 20E signalling, *usp*-mutant third-instar larvae produce an extra larval cuticle as well as their normal pupal cuticle, indicating that larval and pupal programmes are active at the same time[154]. This phenotype is in agreement with JH function *in vivo*, as JH application in classical insect models results in an extra larval moult. It is possible that unliganded USP is required at the end of the third instar to suppress this programme in *D. melanogaster*. Alternatively, JH might normally repress this larval programme at pupariation through the USP receptor.

The stage is set for a detailed analysis of JH function in *D. melanogaster* and a definition of roles for USP in this response. Do *usp* mutations affect vitellogenesis in females or the aberrant effects of ectopic JH application at pupariation? What effect does a *usp* mutation have on the robust and direct induction of *E75A* transcription by JH[155]? What effects would ectopic JH esterase expression, which efficiently degrades JH *in vivo*, have on *D. melanogaster* development? Can JH activate the USP LBD *in vivo* using the GAL4–LBD ligand trap system[140] (FIG. 3)? The next few years should see answers to these and other questions, as well as a clearer understanding of what role, if any, USP has in transducing the JH signal during insect development.

distinct from those regulated by 20E. For example, coordinated changes in ecdysteroid-regulated gene expression occur at several stages in the *D. melanogaster* life cycle at times when the 20E titre is known to be low, raising the question of whether 20E is responsible for these effects[53,106,107]. There is also evidence for a role of hormones that do not have a known receptor. For example, a study in the tobacco hornworm, *Manduca sexta*, has provided evidence that α-ecdysone, the presumed inactive precursor of 20E, drives neuroblast proliferation during early pupal development[108]. Moreover, the key determinant of larval moults in insects, JH, remains without a receptor, although some studies indicate that USP might carry out this function (BOX 3). Below we discuss the remaining *D. melanogaster* nuclear receptors — DHR38, DHR78, DHR96, HNF4 and ERR — and describe how some of them might function in new hormone signalling pathways.

Recent studies of the DHR38 receptor have raised the possibility that it functions as a second ecdysteroid receptor in *D. melanogaster*. Similar to EcR, DHR38 can dimerize with USP or RXR and is expressed widely throughout development[12,109,110]. Interestingly, the DHR38–RXR heterodimer can be efficiently activated by ecdysteroids, including α-ecdysone, 3-Epi-20E and 3-dehydromakisterone A, but only in the presence of an RXR agonist[111]. No activation, however, is seen when a DHR38–USP heterodimer is challenged with ecdysteroids unless an activated form of USP is used, indicating that unidentified USP ligands or other transactivators must be required for this function *in vivo*. The crystal structures of DHR38 and its human orthologue, NURR1 (or NR4A2; nuclear-receptor subfamily 4, group A, member 2), show that they lack a conventional co-activator binding site and a ligand-binding pocket[111,112]. Taken together, these observations indicate that a set of ecdysteroids, which are overlapping but distinct from those that activate EcR–USP, can exert their effects through DHR38 by means of a novel mechanism. Moreover, the indirect regulation of DHR38 activity raises the interesting possibility that other fly and vertebrate orphan receptors might be controlled in

VITELLOGENIN
A protein produced in the fat body of the female insect that is stored in the yolk of oocytes.

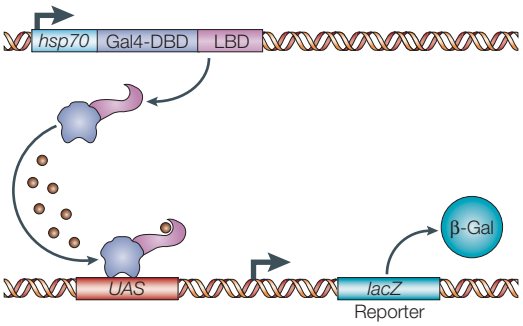


Figure 3 | **The GAL4–LBD ligand-trap system for *in vivo* detection of nuclear receptor ligands.** Fly lines are established that carry two transgenic constructs. One of these, shown at the top of the figure, uses the heat-inducible *hsp70* (heat shock protein 70) promoter to direct the expression of a fusion between the yeast GAL4 DNA-binding domain (DBD; blue) and the ligand-binding domain (LBD) of a nuclear receptor (pink). This fusion protein can interact with ligands and cofactors through its LBD (ligands are shown in brown), as well as a second transgenic insertion that carries an upstream activating sequence (UAS) that is recognized by the GAL4–DBD. In combination with the appropriate ligand and/or cofactor, the GAL4–LBD fusion protein will induce expression of the neighbouring reporter gene. A *lacZ* gene that encodes β-galactosidase (β-Gal) is depicted, although *GFP* could also be used.

a similar way. *DHR38* mutants die at the end of metamorphosis with defects in ecdysteroid-regulated cuticle gene expression, although the crucial role of mouse Nurr1 (or Nr4a2) in midbrain development raises the interesting possibility that its fly orthologue, *DHR38*, might also have neuronal functions[110,113].

Another nuclear receptor that is indirectly linked to ecdysteroid signalling in the fly is DHR78, the orthologue of vertebrate TR2 (NR2C1) and TR4 (NR2C2) (REF. 109). DHR78 binds to a subset of EcR–USP binding sites and can inhibit 20E-induced reporter-gene transcription in cell culture, indicating that DHR78 might function as a repressor through binding-site competition[114]. Consistent with this, TR2 functions as a repressor, although it does so through a silencing domain within the receptor[115]. *DHR78* mutants arrest development during the third larval instar with trachaeal defects, and die shortly after[116]. These trachaeal defects arise during the moults, when 20E directs the replacement of the trachaeal cuticle along with the external cuticle of the animal. A recent study indicates that the lethality of *DHR78* mutants arises from essential functions for this receptor in the trachaea, resulting in death by hypoxia[117]. Interestingly, similar to TR2, the silk moth orthologue BmHR78 is expressed strongly in the testis and can heterodimerize with USP[118], a finding that has yet to be demonstrated for *D. melanogaster*.

No mutations in *DHR96* have yet been reported. However, its close phylogenetic relationship to other ligand-regulated members of subfamily 1, such as EcR, VDR, steroid and xenobiotic receptor (SXR) and constitutive androstane receptor (CAR), indicates that this receptor might be regulated by a ligand[119,120]. Its closest vertebrate relative, VDR, is regulated by 1α,25-dehydroxyvitamin D_3, whereas CAR and SXR bind a range of toxic compounds and function as xenobiotic sensors that control mammalian detoxification responses[121,122]. VDR, SXR and CAR also bind to bile acids and function in the clearance of these toxic cholesterol derivatives[4,121,122]. DHR96 is represented by an orthologous group of three genes in *C. elegans*: *nhr-8*, *nhr-48* and *daf-12*. *nhr-8* mutants are sensitive to colchicine and chloroquine, indicating a role in a xenobiotic pathway[123], whereas DAF-12 controls entry into DIAPAUSE by the formation of so-called dauer larvae, a specialized larval form that allows the animal to survive unfavourable conditions, such as nutrient deprivation or overcrowding. The decision to enter the dauer stage is controlled by a diet-derived cholesterol metabolite[124], which might function as a ligand for DAF-12 (REF. 125). Interestingly, the absence of this diet-derived sterol triggers dauer formation, indicating that the DAF-12 pathway monitors dietary sterol levels and directs diapause under conditions of sterol deprivation. The functions of the vertebrate and *C. elegans* DHR96 homologues therefore indicate two non-exclusive models for DHR96 action: as an insect xenobiotic sensor that controls detoxification pathways, or as a sterol sensor.

Along with DHR38 and SVP, HNF4 is one of the fly nuclear receptors that shares very close sequence identity with its vertebrate orthologue (TABLE 1). During embryogenesis, *Hnf4* is expressed in the midgut, the Malpighian tubules, and possibly the fat body, although evidence for this last possibility is conflicting[101,126]. This expression pattern closely reflects that of its mammalian counterpart, which is expressed in the intestine, kidney and liver[127]. Studies of mouse mutants have shown that HNF4 has an essential role in the visceral endoderm in supporting gastrulation during early embryogenesis[128,129], as well as crucial later functions in hepatocyte differentiation and lipid homeostasis[130]. Human HNF4A is associated with a rare form of type II diabetes called MODY (mature onset of diabetes of the young), and regulates key genes required for glucose transport and metabolism[131]. Determining whether fly *Hnf4* carries out similar roles in *D. melanogaster* awaits the isolation of specific mutations in this gene.

The crystal structure of HNF4G revealed a fatty acid locked in the LBD, resulting in a conformation that is characteristic of a constitutively active receptor[132,133]. The fatty acid seems to function more like a cofactor than a ligand, as it cannot be removed under physiological conditions. However, it remains to be determined whether these molecules can be actively exchanged *in vivo*, thereby modulating HNF4 activity.

Vertebrates encode three ERRs, each of which functions as a constitutive activator[134], with ERRb showing the highest sequence identity with *D. melanogaster* ERR. Although very similar to ER, none of these receptors is known to bind a natural ligand. However, synthetic compounds such as diethylstilbestrol (DES), tamoxifen (TAM) and 4-*OH*-tamoxifen (OHT) can bind with high affinity to ERRb and ERRg and function as inverse agonists, suppressing their constitutive activity in cell

DIAPAUSE
A period of inactivity and cessation of growth or development, accompanied by greatly reduced metabolic activity.

MASS SPECTROMETRY
A method that provides accurate information about the molecular mass and structure of molecules. It can identify and quantify extremely small amounts of drugs, hormones or metabolites.

MICRORNA
Small non-coding RNAs that can control the expression of target mRNAs.

culture[135]. ERRa is unaffected by these compounds, but can be modulated by a recently discovered inverse agonist[136] (FIG. 1c). Similar to ERRa, the constitutive activity of fly ERR is not significantly affected by OHT, TAM or DES[137], although it remains unclear whether fly ERR can respond to the ERRa ligand or whether different compounds might elicit an effect on fly ERR. Mutations in ERRb result in placental defects and consequent early embryonic lethality[138]. As with *Hnf4*, however, the determination of functions for fly *ERR* awaits the isolation of specific mutations in this gene.

Outlook

With the complete family of *D. melanogaster* nuclear-receptor genes in hand, the stage is set for significant advances in our understanding of nuclear-receptor function and hormone action during insect development. In addition, given that vertebrate nuclear receptors have central roles in metabolism, homeostasis and growth regulation, it is likely that future studies of *D. melanogaster* nuclear receptors will provide new insights into insect physiology and endocrinology. The discovery of *DHR51*, *DHR83* and fly *ERR* provides clear directions for future research, along with functional studies of the two other *D. melanogaster* nuclear-receptor genes that have not yet been subjected to genetic analysis: *DHR96* and *Hnf4*. Microarray technology, which has been so effectively directed at studying 20E responses[139], combined with metabolic profiling of wild-type and mutant animals, should provide a broader understanding of nuclear-receptor signalling pathways. In addition, several new technologies and recent discoveries will have a significant impact on understanding receptor regulation and identifying ligands. These include the GAL4–LBD 'ligand-trap' system (FIG. 3). This technique allows the researcher to visualize when and where a particular LBD has been activated in the animal, providing new clues to potential ligands or cofactors[140]. It also provides an effective bioassay to test compounds for their ability to activate a specific receptor[111,140]. This *in vivo* approach, combined with crystallography and MASS SPECTROMETRY, should greatly expand our understanding of potential nuclear receptor ligands[141]. Finally, the discovery of potential MICRORNA binding sites in *EcR*, *DHR3*, *DHR38* and *DHR96* raises the interesting possibility that small regulatory RNAs might contribute to the control of nuclear-receptor signalling pathways[142].

We anticipate that the next 5–10 years will be a time of unprecedented growth for the fly nuclear-receptor field, with exciting new insights into insect development and physiology, and implications for understanding their vertebrate nuclear-receptor counterparts. This research also has significant implications for pest control. Unlike the pharmaceutical industry, which has effectively developed and marketed drugs that modify nuclear-receptor function in humans, little effort has been made to target nuclear-receptor pathways in insects. As agricultural development becomes more crucial to feed a growing world population, and insect-transmitted diseases such as malaria continue to kill millions of people each year, insect population control will become a priority. We can anticipate that nuclear receptors will provide a primary target for these efforts.

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Competing interests statement

The authors declare no competing financial interests.

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