Comparing thyroid and insect hormone signaling

Thomas Flatt,[1,*] Leonid L. Moroz,[†] Marc Tatar,[*] and Andreas Heyland[2,3,†]

*Division of Biology and Medicine, Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA; [†]Whitney Laboratory for Marine Biosciences, 9505 Ocean Shore Boulevard, University of Florida, St Augustine, FL 32080, USA

Synopsis Transitions between different states of development, physiology, and life history are typically mediated by hormones. In insects, metamorphosis and reproductive maturation are regulated by an interaction between the sesquiterpenoid juvenile hormone (JH) and the steroid 20-hydroxy-ecdysone (20E). In vertebrates and some marine invertebrates, the lipophilic thyroid hormones (THs) affect metamorphosis and other life history transitions. Interestingly, when applied to insects, THs can physiologically mimic many facets of JH action, suggesting that the molecular actions of THs and JH/20E might be similar. Here we discuss functional parallels between TH and JH/20E signaling in insects, with a particular focus on the fruit fly, *Drosophila melanogaster*, a genetically and physiologically tractable model system. Comparing the effects of THs with the well defined physiological roles of insect hormones such as JH and 20E in *Drosophila* might provide important insights into hormone function and the evolution of endocrine signaling.

Introduction

Hormones are regulatory signaling molecules that orchestrate numerous aspects of development, physiology, metabolism, and life history, both among and within species (for example, Nijhout 1994; Finch and Rose 1995; Flatt and others 2005; Heyland and others 2005; McCourt and others 2005). Interestingly, there are often profound structural and functional parallels among many lipophilic hormones in distantly related organisms, such as retinoic acid (RA), testosterone (T), estrogen (E), and corticosterone (C) in vertebrates, abscisic acid (ABA) and gibberellin (GA_3) in plants, juvenile hormone (JH) and 20-hydroxy-ecdysone (20E) in insects, and thyroid hormones (THs) in vertebrates and marine invertebrates (for example, Kushiro and others 2003; Wheeler and Nijhout 2003; Heyland and others 2005; McCourt and others 2005; Hodin 2006).

In insects, for instance, JH and 20E interact in regulating a large number of processes, most notably metamorphosis and reproductive maturation, but also behavior, morphology, diapause, stress resistance, immune function, and aging (for example, Riddiford 1993; Nijhout 1994; Dingle and Winchell 1997; Wheeler and Nijhout 2003; Flatt 2004; Flatt and Kawecki 2004; Berger and Dubrovsky 2005; Flatt and others 2005; Tu and others 2006). In plants, ABA regulates multiple aspects of embryonic development, growth, and adult physiology, with the effects reminiscent of JH in insects (for example, Leung and Giraudat 1998; Wheeler and Nijhout 2003; McCourt and others 2005). In vertebrates, and in some marine invertebrates, THs play pleiotropic roles in development, growth, differentiation, and metamorphosis very similar to those of JH/20E in insects (Schneider 1939a; Schneider 1939b; Brent and others 1991; McNabb 1992; Eales 1997; Yen 2001; Heyland and Hodin 2004; Heyland and others 2004; Brown and others 2005; Heyland and Moroz 2005).

These similarities suggest that, despite many important differences, some fundamental aspects of hormone signaling (for example, protein interactions or effects on downstream targets) might be functionally conserved among a wide range of taxa, from plants to animals (for example, Kushiro and others 2003; Wheeler and Nijhout 2003; Heyland and others 2005; McCourt and others 2005). For example, ABA in plants and JH in insects are both sesquiterpenoids derived from the same precursor, farnesyl pyrophosphate (FPP), and when ABA is applied to insects, it mimics the effects of insect hormones (for example, Visscher 1983; cf. McCourt and others 2005). ABA and

From the symposium "Metamorphosis: A Multikingdom Approach" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2006, at Orlando, Florida.

[1] E-mail: Thomas_Flatt@Brown.Edu
[2] E-mail: aheyland@ufl.edu
[3] Present address: Friday Harbor Laboratories, University of Washington, 620 University Road, Friday Harbor, WA 98250, USA

Integrative and Comparative Biology, volume 46, number 6, pp. 777–794
doi:10.1093/icb/icl034
Advance Access publication September 7, 2006

JH both structurally resemble another terpenoid, RA in vertebrates, and RA has JH-like effects in some insects (Nemec and others 1993; also see McCourt and others 2005). Some structural and functional similarities among lipophilic hormones might be explained by the fact that many key animal hormones are derived from external sources such as plants (for example, Heyland and others 2005; Hodin 2006). Insects, for instance, produce ecdysteroids such as 20E from cholesterol/sterol precursors derived from ingested plant and fungal food materials, and it has recently been suggested that JH originated from externally produced compounds in plants, initially ingested by proto-insects (Hodin 2006). Interestingly, plants have recently been shown to be able to synthesize *bona fide* JH, perhaps as an insect deterrent (for example, Bede and others 1999, 2001; Hodin 2006).

Metamorphosis in vertebrates and invertebrates provides a particularly striking example of an endocrine parallelism (Matsuda 1987). In amphibians, THs and prolactin antagonistically regulate metamorphosis (for example, Shi and others 1996; Denver and others 2002; Buchholz and others 2006), whereas 20E and JH have opposing effects in controlling metamorphosis in insects (for example, Riddiford 1993; Nijhout 1994; also see Erezylimaz 2006, papers presented at meetings, for a historical perspective). Remarkably, an increasing body of literature now suggests that THs can mimic many physiological facets of JH action in insects (for example, Davey and Gordon 1996; Eales 1997; Kim and others 1999; Davey 2000a; Davey 2000b; Davey 2004). The notion that THs mimic the action of JH is supported by the fact that the chemical structure of some synthetic JH analogs and JH-like insecticides, such as fenoxycarb, resembles that of THs (for example, Davey and Gordon 1996; Wheeler and Nijhout 2003; Davey 2004). While it is presently unclear whether insects, under natural conditions, produce or exogenously acquire THs, recent evidence indicates that locust follicle cells can take up and process THs (Davey 2004). If insects can indeed synthesize and metabolize THs, these hormones might play an important role in insects (for example, Schneider 1939b; Davey and Gordon 1996; Eales 1997; Kim and others 1999; Davey 2000a; Davey 2000b, 2004). Yet, even if insects cannot endogenously synthesize THs, these compounds might activate endogenous hormonal signaling pathways in insects. Here we discuss the available evidence on TH effects in insects, with a particular focus on the fruit fly, *Drosophila melanogaster*, a model system with well established genetics, genomics, and physiology.

Signaling function of thyroid and insect hormones

Action of THs in vertebrates and marine invertebrates

In all vertebrates examined to date, THs function in governing growth, development, and metabolism (McNabb 1992; Yen 2001). Similarly, in many marine invertebrate phyla, THs and other iodotyrosines regulate development, metamorphosis, and other life history transitions (for example, Eales 1997; Heyland and Hodin 2004; Heyland and others 2004; Heyland and Moroz 2005).

Chemically, THs (iodothyronines) are synthesized from tyrosines and iodine by a process of tyrosine iodination and coupling by thyroglobulins and iodoperoxidases (for example, Eales 1997; Yen 2001). In mammals, THs (T_4 or thyroxine: 3,5,3′,5′-tetraiodothyronine and T_3: 3,5,3′-triiodothyronine) are produced in the thyroid gland, primarily by the action of thyroid peroxidase (TPO) (Eales 1997; Taurog 1999). In the target tissue, deiodinases convert T_4 to T_3, which has a high binding affinity to the thyroid hormone receptors (TRs) and is thought to mediate many physiological actions in vertebrates (for example, McNabb 1992; Yen 2001). T_3 is a lipophilic (hydrophobic) molecule which enters the target cells, either through a process of receptor-mediated endocytosis or through transmembrane diffusion (Di Liegro and others 1987). Inside the cell it can diffuse into the nucleus and bind to TR, which forms a heterodimer with the retinoid X receptor (RXR). This complex directly exerts effects on gene expression (Brent and others 1991; Ribeiro and others 1995). However, inside or outside the cell, T_3 and other THs can also act via nongenomic (that is, nonnuclear hormone receptor-mediated) signaling (Sterling and others 1977; Davis and others 1982; Brent and others 1991; Ribeiro and others 1995; Davis and Davis 1996; Hulbert 2000). These effects are generally more rapid than nuclear effects and can be mediated via membrane receptors. In addition, both nuclear and nonnuclear pathways can also act simultaneously (Davis and Davis 1996; Hulbert 2000).

Action of JH and 20E in insects

Like THs, the 2 major insect hormones, JH, a sesquiterpenoid, and 20E, a steroid, are important regulators of developmental transitions, metamorphosis, and life histories (for example, Riddiford 1993; Nijhout 1994; Dingle and Winchell 1997; Kozlova and Thummel 2000; Wheeler and Nijhout 2003; Flatt and Kawecki 2004; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005; Tu and others 2006). JH is a

product of the mevalonate pathway, whereas 20E is produced from a cholesterol precursor derived from dietary yeast ergosterol or plant sterol (for example, Tobe and Bendena 1999; Gilbert and others 2002; Belles and others 2005; Hodin 2006). The precursor of 20E, 3-dehydro-ecdysone (3dE), is produced by the prothoracic gland in larvae and by the gonads in adult insects, then converted to ecdysone by a hemolymph reductase, and finally converted into 20E by an intracellular 20E monooxygenase in the target tissues (for example, Gäde and others 1997; Kozlova and Thummel 2000; Gilbert and others 2002; Berger and Dubrovsky 2005), while JH is secreted by the *corpora allata* (CA), an endocrine tissue situated close to the brain (for example, Gäde and others 1997; Berger and Dubrovsky 2005; Flatt and others 2005). To date, insects are known to produce at least 8 different forms of JH-like compounds, with JH III being the most common type (for example, Gäde and others 1997; Berger and Dubrovsky 2005; Flatt and others 2005).

Functional 20E signaling typically requires 20E binding to a heterodimer formed by 2 nuclear hormone receptors, the ecdysone receptor (EcR) and the ultraspiracle (USP) protein (for example, Koelle and others 1991; Thomas and others 1993; Yao and others 1993; Hall and Thummel 1998; Berger and Dubrovsky 2005; Dubrovsky 2005; King-Jones and Thummel 2005). However, 20E responses can also be mediated by alternative signaling modes, either involving EcR homodimers (for example, Lezzi and others 1999, 2002; Grebe and others 2003), heterodimers between hormone receptor 38 (DHR38) and USP (Baker and others 2003), or nongenomic actions not mediated by nuclear hormone receptor signaling (for example, Wehling and others 1997; Elmogy and others 2004; Srivastava and others 2005). In addition, some early metamorphic events can be suppressed by the unliganded EcR/USP complex, while moderate levels of 20E will cause a subsequent release of this silencing (Schubiger and Truman 2000; Schubiger and others 2005).

In contrast to 20E signaling, the details underlying the molecular action of JH have remained enigmatic (for example, Jones 1995; Davey 2000a; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005; King-Jones and Thummel 2005). One candidate for the JH receptor is the RXR homolog USP (for example, Oro and others 1990, 1992; Yao and others 1992). While RXR forms a heterodimer with TR to transduce TH signaling in vertebrates, USP typically forms a heterodimer with EcR to transduce signaling by 20E, and probably JH, in insects. The model that USP is a JH receptor is supported by the fact that JH can act as a USP ligand and suppress or potentiate 20E-dependent EcR signaling responses (for example, Jones and Sharp 1997; Jones and others 2001; Henrich and others 2003; Xu and others 2003; Maki and others 2004; Berger and Dubrovsky 2005; Fang and others 2005; King-Jones and Thummel 2005). USP's role as a JH receptor is, however, somewhat controversial since USP only shows weak binding affinity for JH (for example, Jones and Sharp 1997; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005; King-Jones and Thummel 2005).

The other candidate for the JH receptor is MET (methoprene-tolerant), a basic helix–loop–helix (bHLH-PAS) transcriptional regulator (reviewed by Wilson 2004; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005). Although MET is not a nuclear hormone receptor, it can specifically bind JH with high affinity, and has been shown to function as a JH-dependent transcription factor (for example, Wilson 2004; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005; Miura and others 2005). However, the fact that *Met* null mutants have normal development makes the MET protein an unlikely candidate for the JH receptor (for example, Flatt and Kawecki 2004; Wilson 2004; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005). While the identity of the JH receptor remains unresolved, it has been shown that JH can function via nongenomic pathways, involving membrane (rather than nuclear) receptors and protein kinase C (PKC) signaling (for example, Yamamoto and others 1988; Sevala and Davey 1989; Davey 2000a; Wheeler and Nijhout 2003; Kethidi and others 2006).

One of the most fundamental aspects of JH and 20E signaling is that both hormones commonly interact to regulate many processes in insect development and physiology: JH often suppresses, but sometimes also potentiates 20E-induced responses (for example, Chihara and Fristrom 1973; Cherbas and others 1989; Berger and others 1992; Farkas and Knopp 1997; Zhou, Hiruma, Jindra and others 1998; Zhou, Hiruma, Shinoda and others 1998; Hiruma and others 1999; Zhou and Riddiford 2002; Henrich and others 2003; Maki and others 2004; Berger and Dubrovsky 2005; Dubrovsky 2005; Fang and others 2005; Flatt and others 2005). Although the details of the interaction are not yet well understood (for example, Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005), recent evidence suggests that JH can antagonize the effects of 20E by promoting the recruitment of corepressors to the EcR/USP complex (Maki and others 2004). Furthermore, JH and 20E can interact synergistically to potentiate the transcription

of a JH esterase reporter gene construct (Fang and others 2005). Both hormones can activate transcription independently, but simultaneous presence of 20E and JH has a greater than additive effect on transcription. Activation by JH alone is through a USP homodimer, while activation by 20E alone is through the 20E/USP heterodimer (Fang and others 2005). However, when both hormones are present, the integration of JH and 20E signals requires the presence of the 20E/USP heterodimer: JH signals through the USP partner of the complex, but can only do so when the EcR partner binds to 20E (Fang and others 2005).

Similarities between thyroid and insect hormone signaling

Several authors have suggested that functional parallels might exist between THs/prolactin and JH/20E in the context of metamorphosis (for example, Matsuda 1987; Cherbas and Cherbas 1996; Davey and Gordon 1996; Shi 1996; Tata 1996; Eales 1997; Kim and others 1999; Davey 2000a, 2000b, 2004; Wheeler and Nijhout 2003). In amphibian metamorphosis, T_3 and T_4 accelerate development and promote various morphogenetic and apoptotic events, while prolactin inhibits such effects (for example, Kaltenbach 1996; Shi 1996; Shi and others 1996; Tata 1996; Buchholz and others 2006). Similarly, in insect metamorphosis, 20E promotes metamorphic events, while JH suppresses these actions of 20E (Riddiford 1993; Nijhout 1994). For example, programmed cell death during *Drosophila* metamorphosis is regulated by 20E (for example, Jiang and others 1997), whereas the massive destruction of larval tissues seen in *Xenopus* metamorphosis is regulated by TH (for example, Shi and Brown 1993; Brown and others 1996), indicating that there might exist similarities in the hormonal regulation of programmed cell death between insects and amphibians. These observations suggest that THs might have similar effects to 20E (for example, Matsuda 1987; Eales 1997; Davey 2004). However, in the sea lamprey (*Petromyzon marinus*), a jawless fish with true metamorphosis (*sensu* Youson 1988), T_3 and T_4 are used as signals suppressing metamorphosis (Youson 2004). In this species, both serum T_3 and T_4 levels drop dramatically before the onset of metamorphosis, and metamorphosis can be experimentally induced by exposing larvae to TH synthesis inhibitors (Manzon and others 2001). Similarly, in insects, metamorphic events are initiated when JH titers are low (for example, Nijhout 1994; Truman and Riddiford 2002), suggesting that the pro-metamorphic effects of JH resemble those of THs (for example, Youson 1997; Davey 2004). However, in contrast to their typically antimetamorphic action, JH and JH-like compounds can induce settlement and metamorphosis in marine annelids (Biggers and Laufer 1999), much like THs which promote metamorphosis in many marine invertebrates (for example, Eales 1997; Heyland and others 2004; Heyland and Moroz 2005).

Similarities between THs and JH/20E might also apply to the hormonal regulation of aging (Tu and others 2006, and references therein). In several grasshopper and butterfly species, ablation of the CA, the glands producing JH, extends life span (see Flatt and others 2005; Tu and others 2006 for a discussion). In some cases, T_4 may have similar pro-aging effects (Vergara and others 2004). Long-lived Snell dwarf mice (mice homozygous for the *Pit1* mutation) have multiple hormonal defects including reduced T_4 production, and treatment of these mice with T_4 restores the extended life span to normal levels. The effects of T_4 resemble the life span reduction seen in long-lived JH-deficient flies treated with the synthetic JH methoprene (cf. Flatt and others 2005; Tu and others 2006).

But how fundamental are the similarities between TH and 20E/JH signaling? Table 1 gives some examples of genes involved in TH signaling in vertebrates and their respective orthologs in *D. melanogaster*. As discussed by Cherbas and Cherbas (1996), 20E acts through a nuclear hormone receptor-mediated signaling cascade similar to the pathways mediating TH, RA, and Vitamin D (VD) signaling in vertebrates. Although insects do not possess any clear TR ortholog (for example, Laudet 1997; King-Jones and Thummel 2005; also see Ollikainen and others 2006, papers presented at meetings), EcR belongs to the same nuclear hormone receptor subfamily as TR (Laudet 1997). Most importantly, however, TR and EcR share a heterodimerization partner protein; TR forms a heterodimer with RXR, while EcR forms a heterodimer with USP, the RXR ortholog in insects (cf. Cherbas and Cherbas 1996; Laudet 1997; King-Jones and Thummel 2005). As shown by Hatzivassiliou and colleagues (1997), USP can mobilize the human TH receptor $T_3R\beta$ to transactivate the human apolipoprotein A-II promoter, suggesting that USP can functionally mimic RXR in vertebrates. Conversely, EcR/RXR (rather than endogenous EcR/USP) heterodimers can transactivate a target promoter when the 20E agonist muristerone is present (Yao and others 1993). Furthermore, mammalian RXR can be activated by the JH agonist methoprene (Harmon and others 1995), and both EcR–USP heterodimers and USP–USP homodimers can transduce JH signals (for example, Maki and others 2004;

Table 1 Examples of vertebrate TH-signaling-related genes and their *Drosophila* orthologs

Gene name in *H. sapiens*	Ortholog in *Drosophila*	GeneIDs Genbank		TH-related function in vertebrates	Putative or Confirmed Function in *Drosophila*
Alien	Alien	9318	34225	Corepressor of TR in the absence of ligand binding	EcR interacting corepressor protein
HOXA7	Antennapedia	3204	40835	N/A	Shares sequence similarity with DNA recognition sequence of vertebrate thyroid transcription factor 1
Ataxin	Ataxin	6311	41883	Putative corepressor protein	Dosage-sensitive regulator of actin filament formation
Chicken ovalbumin upstream promoter (COUP-TF1)	Seven-up (SVP)	7025	41491	TR corepressor	Interaction with rigor mortis, required for ecdysone responses during larval development; interacting with EcR/USP
N/A	CG11063		32351	N/A	TR binding (inferred from sequence similarity)
N/A	CG31453		41013	N/A	TR interactor-like (inferred from electronic annotation)
Deiodinases	N/A	1733, 1734, 1735	N/A	Deiodination of THs	N/A
N/A	Eip75B (ecdysone-inducible protein 75B)		39999	N/A	TR activity (inferred from electronic annotation); similar protein domains as TR
GPHB5 glycoprotein hormone beta 5	GPB5 glycoprotein hormone beta 5	122876	192347	Activates the thyroid-stimulating hormone receptor as heterodimer	N/A
N/A	Lethal(1)G0168		32483	N/A	TR-interacting protein-like (inferred from electronic annotation)
Peroxidasin	Peroxidasin	7837	38326	N/A	Putative TH synthesis enzyme
RXRalpha	USP	6256	31165	Heterodimerization partner of TR	Heterodimerization partner of EcR (ecdysone receptor)
Smart (N-CoR)	SMARTR	9612	32225	Corepressor of TR in the absence of ligand binding	EcR interacting corepressor protein
Sodium iodine symporter (NIS)	N/A	6528	N/A	Iodine transport into thyroid follicle cells	N/A
Thyroglobulin (Tg)	CG2264	7038	36048	TH binding protein	N/A
Thyroxine peroxidase (TPO)	N/A	7173	N/A	TH synthesis enzyme	N/A
Transthyretin	CG30016	7276	246393	TH binding protein	N/A
Thyroid hormone receptors (TRs)	N/A	7067, 7068	N/A	T3 receptor	N/A

Fang and others 2005). Thus, despite the absence of a clear TR ortholog in insects, it remains possible that insects possess proteins functionally equivalent to TR, which would heterodimerize with USP and bind THs (cf. Hatzivassiliou and others 1997). However, systematic receptor-binding studies with THs have so far not been performed in insects.

While TR can transduce TH signaling as a monomer, homodimer, or heterodimer, 20E signaling typically requires a functional EcR/USP heterodimer (cf. Cherbas and Cherbas 1996). It is now becoming increasingly clear, however, that 20E can signal through other routes than EcR/USP. The *Drosophila* DHR38, for instance, heterodimerizes with USP to transduce an atypically broad range of ecdysteroid signals (Baker and others 2003). Remarkably, signaling by DHR38/USP does not require binding of ecdysteroid ligands to either DHR38 or USP; instead, it might occur indirectly through an ecdysteroid-induced cofactor (Baker and others 2003). In addition, EcR

homodimers can transduce 20E signals, at least *in vitro* (Lezzi and others 1999, 2002; Grebe and others 2003), much like TR homodimers are able to mediate TH signals.

The notion of functional similarities between TH and JH/20E signaling is also supported by the observation that hormone responsive genes downstream of these pathways have very similar hormone response elements (RE). Hormone RE are short specific sequences of DNA, located in the promoter region of the hormone response genes. Based on the ecdysone response element (EcRE) of the Hsp27 gene in *Drosophila*, Martinez and colleagues (1991) showed that EcRE is almost identical to the response elements of thyroid hormone (TRE), retinoic acid (RRE), and estrogen (ERE). This clearly lends support to the idea that thyroid and insect hormone signaling pathways are structurally and functionally similar.

Other similarities between TH and JH/20E signaling relate to corepressors, and possibly coactivators, of TR/RXR and EcR/USP. For example, JH functions as a USP ligand and is likely to induce recruitment of corepressors with histone deacetylase (HDAC) activity, suppressing EcR-dependent responses (for example, Maki and others 2004). On the other hand, TR and RXR can silence gene expression through the recruitment of corepressors in the absence of hormone ligand (for example, Hörlein and others 1995). For example, in vertebrates, the corepressors N-CoR (nuclear receptor corepressor), SMRT (silencing mediator for retinoid and TRs), and Alien interact with TR to suppress gene expression without hormone binding (for example, Chen and Evans 1995; Hörlein and others 1995; Dressel and others 1999). In *Drosophila*, the EcR interacting corepressor protein SMRTER (SMRT-related EcR interacting factor) is functionally similar to the vertebrate nuclear corepressors SMRT and N-CoR (Tsai and others 1999), and the *Drosophila* ortholog of Alien, which normally binds to EcR, can interact in a hormone-sensitive manner with vertebrate TR (Dressel and others 1999). In mammalian cells, SMRT interacts with Ataxin 1 (Atx1), and Atx1 can also bind *Drosophila* SMRTER, suggesting that TR-interacting corepressors and their related factors are evolutionarily conserved (Tsai and others 2004). Furthermore, TATA-binding protein-associated factors (TAFs) function as coactivators for TR, and *Drosophila* $dTAF_{II}110$ can interact with human TRβ (Petty and others 1996). Taken together, TH and 20E/JH signaling might involve functionally similar or identical corepressor and coactivator proteins.

It is also noteworthy that the chemical structure of some synthetic JH agonists and JH-like insecticides such as fenoxycarb and pyriproxyfen (and similar phenoxyphenyl ethers) strikingly resembles that of THs, supporting the observation that THs can mimic certain aspects of JH action in insects (for example, Davey and Gordon 1996; Davey 2000a; Davey 2000b; Wheeler and Nijhout 2003; Davey 2004). Intriguingly, while the structure of fenoxycarb does not resemble that of JH, it has pervasive JH-like effects (for example, Grenier and Grenier 1993; Davey and Gordon 1996). Whether these compounds or THs can transduce JH-like signals via EcR/USP remains unknown, yet experiments with ecdysteroid-dependent reporter genes in mammalian cells suggest that T_3 cannot induce EcR-dependent gene expression (Christopherson and others 1992).

Most fundamentally, many lipophilic hormones can signal through nongenomic (nonnuclear) pathways. As mentioned above, such nongenomic effects occur for THs (Sterling and others 1977; Davis and others 1982; Brent and others 1991; Ribeiro and others 1995), 20E (Elmogy and others 2004; Srivastava and others 2005), and JH (Yamamoto and others 1988; Sevala and Davey 1989; Biggers and Laufer 1999; Kethidi and others 2006). While the details underlying nongenomic effects are still relatively poorly understood, they might explain why THs can exert hormone-like effects in insects despite the absence of a functional TR ortholog (cf. Wheeler and Nijhout 2003; Davey 2004). It therefore remains an intriguing, yet little explored, possibility that THs, and perhaps some iodotyrosines, can signal through, or interact with, endocrine pathways in insects (for example, Davey and Gordon 1996; Kim and others 1999; Davey 2000b, 2004).

The effects of THs in insects

While invertebrates might not be able to endogenously synthesize THs or related iodinated compounds, many species physiologically respond to iodothyronines (THs), while Scyphozoans and Gorgonian corals appear to react to iodotyrosines or T_4-like compounds (Eales 1997; Kingsley and others 2001). Although recent evidence suggests a critical role of THs, and possibly other iodotyrosines, in echinoderm, molluscan, cnidarian, and urochordate development and metamorphosis (for example, Eales 1997; Heyland and Hodin 2004; Heyland and others 2004; Heyland and Moroz 2005; Heyland and others 2005), insects have received relatively little attention concerning these signaling molecules (cf. Eales 1997; Davey 2004). Here, we discuss the various effects of THs on insect physiology and life history. In particular, we focus on TH effects in the fruit fly, *D. melanogaster*.

The genetics and physiology of endocrine and nuclear hormone receptor signaling in the fly is quite well understood, particularly with respect to 20E and JH (for example, Riddiford 1993; Kozlova and Thummel 2000; Flatt and Kawecki 2004; Wilson 2004; Berger and Dubrovsky 2005; Dubrovsky 2005; Flatt and others 2005; King-Jones and Thummel 2005; Tu and others 2006). The *Drosophila* system, with its set of extensive genetic, genomic, and physiological tools, might thus prove to be an ideal model for examining functional parallels between THs and JH/20E.

Sources of THs and other iodotyrosines in insects

Vertebrates evolved the ability to concentrate iodine in their thyroid gland using highly efficient uptake and concentration mechanisms. In contrast, lower chordates, such as urochordates and cephalochordates, use their endostyle to concentrate iodine (cf. Eales 1997). Using both functional and developmental evidence, a partial homology between the endostyle and the vertebrate thyroid gland has been established (Dunn 1974; Ogasawara 2000). However, various marine organisms with neither an endostyle nor a thyroid gland concentrate and metabolize this element as well (for example, Eales 1997; Heyland and Moroz 2005). For example, food sources (for example, algae) are probably important exogenous sources of THs which can then function as so-called "vitamones" in regulating development, metamorphosis, and settlement in some echinoderm larvae (Eales 1997; Heyland and Moroz 2005).

Despite the relatively low concentration of iodine in terrestrial environments (for example, Mairh 1989), several organs and tissues in insects, including cuticle, brain, nerve cord, and fat body, can concentrate radioactive iodine (for example, Limpel and Caseda 1957; Tong and Chaikoff 1961). Moreover, it has been shown that insects can incorporate the concentrated iodine into scleroproteins (cf. Eales 1997). In particular, follicle cells in locusts (*Locusta migratoria*) can take up, accumulate, and metabolize T_3 when exposed to this compound, probably through receptor-mediated endocytosis, and convert it into a more active but unknown product, possibly T_2 (for example, Davey 2000b).

Yet, 2 important caveats must be kept in mind when thinking about THs in organisms without a thyroid gland, such as insects. First, the ability of many organisms, including insects, to concentrate iodine might be unrelated to THs: iodine is a very "sticky" lipophilic molecule and can bind to various other molecules. Second, despite several attempts, there has not yet been any conclusive demonstration of endogenous TH synthesis in insects (see Eales 1997; Davey 2000b). In particular, great caution has to be used in interpreting data based on TH applications in insects since the observed effects might nonspecific and pharmacological; strong evidence for a role of THs and related compounds in insects would ultimately require the demonstration of deiodinase activity and the chemical characterization of immunoreactive compounds and T_4 derivatives in the insect hemolymph (Davey 2000b).

However, even though insects and other invertebrates might be unable to endogenously synthesize THs and related compounds, these molecules might be taken up from the environment and then function as signaling molecules with physiological effects. As is probably the case for echinoderm larvae (Eales 1997; Heyland and Moroz 2005), some insects might use food as an exogenous source for THs and related iodotyrosines and then employ these molecules to perform physiological functions upon ingestion (Davey 2000b). For instance, T_4 and T_3 can be found in the wheat shoots locusts are feeding on and can be detected in the hemolymph of these insects, suggesting that locusts can ingest and possibly utilize THs (Davey 2000b).

Does *Drosophila* synthesize or metabolize TH-like compounds?

Invertebrates producing or metabolizing TH require peroxidases for either cuticle formation or TH synthesis from tyrosine (for example, Eales 1997). As shown by Wheeler (1950), the fruit fly *Drosophila gibberosa* can incorporate radioactively labeled iodine into the cuticle during the tanning process. In *D. melanogaster*, the enzyme peroxidasin is used in formation of extracellular matrix (ECM) and cuticle (Nelson and others 1994). It is thus possible that flies use this or other peroxidases for synthesizing or processing THs or TH-like compounds. For example, we have recently identified 2 new peroxidasin genes from the mollusc *Aplysia californica* and the sea urchin *Lytechinus variegatus* that might be involved in TH synthesis (Heyland and others 2006). In addition to peroxidasin, *D. melanogaster* might also possess other components for the production and processing of THs or TH-like compounds. For example, flies have a receptor that is structurally closely related to the thyroid-stimulating hormone (TSH) receptor (Hauser and others 1997), and a transthyretin-related protein, a transport protein that might—as in other organisms—bind to and distribute THs (Eneqvist and others 2003).

To test whether *D. melanogaster* larvae contain iodinated tyrosines or synthesize THs from incorporated iodine, we performed thin layer chromatography (TLC) using radioactively labeled iodine (^{125}I). We were able to detect a faint band on the TLC plate which did not correspond to either T_4 or T_3 (Fig. 1). Published data on RF values (retardation factor in planar chromatography, that is, the ratio of the distance traveled by the center of the spot to the distance simultaneously traveled by the mobile phase) of various THs (RF values of T_4 or T_3 are 0.27 and 0.22, respectively; Chanoine and others 1992) suggest that the band seen in *Drosophila* larvae could be T_2 (RF value of 0.15; Pinna and others 1998). Our highly preliminary observation is consistent with previously published data that detected iodinated tyrosines in insects but failed to detect T_4 or T_3 (Tong and Chaikoff 1961). However, it remains possible that the appearance of ^{125}I in a compound running with a RF similar to that of T_2 might result from unspecific binding rather than synthesis since iodine is a "sticky" molecule that can bind to many other molecules. Thus, our interim data will require confirmation by TLC with a T_2 standard and ultimately validation with gas chromatography-mass spectrometry (GC-MS) before we can draw any firm conclusions about the potential occurrence of THs or iodinated tyrosines in the fly.

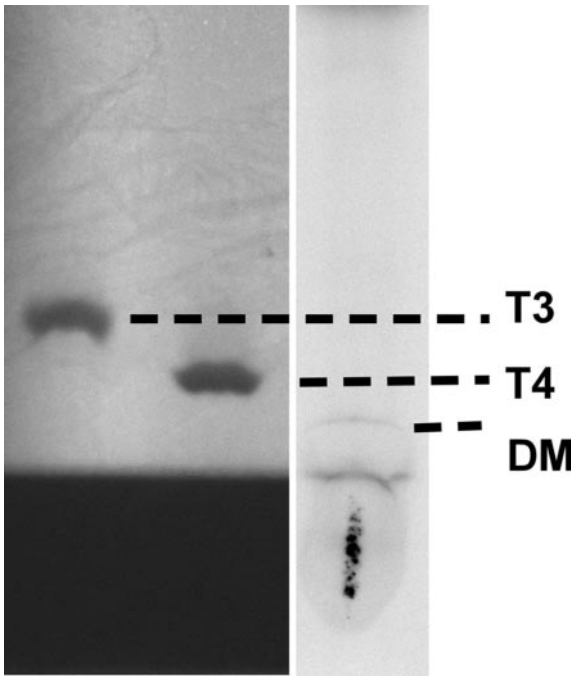

Fig. 1 Does *D. melanogaster* synthesize THs? We performed TLC using radioactively labeled iodine (^{125}I) to test whether *D. melanogaster* larvae can synthesize iodinated tyrosines endogenously. We incubated 200 wild-type larvae per sample in a thin layer of ^{125}I made up in sucrose at 80 000 dpm (decays per minute). The sucrose layer was shallow enough so that larvae could move around without drying out or drowning. After incubating for 12 h we removed larvae from sucrose and washed them in distilled water 5 times until we were unable to detect radioactivity above 30 dpm in solution. All counts were done on a ssMPD instrument (BioTraces, Inc., Herndon, VA) in standard mode. In standard mode, digital signal processing is used to distinguish the ^{125}I decay specific characteristics from those of background events to give a background equivalent to 5 DPM of ^{125}I with ~45% efficiency. We then fixed larvae in 1 ml ice cold 100% MeOH over night. After mixing all samples we centrifuged them at 1980 g for 10 min and collected the supernatant. Then we spiked the samples with 100 μl nonradioactive 10^{-4} M T_4 (thyroxine; Sigma-Aldrich T-1774) and T_3 (3,3′,5-Triiodo-L–thyronine; Sigma: T2877) and concentrated them in a speed-vac to complete dryness. The dry pellet was resolved in 30 μl 0.01 N NaOH. The entire sample was loaded on a TLC plate (Whatman LK5D silica gel 150A with fluorescence marker; Whatman #4851–840) and run for 1.5 h in 2-methylbutanol/ *t*-butyl alcohol/25%NH_3/ acetone (7:14:14:56, vol/vol) solvent. We visualized the cold T_4 and T_3 markers under UV light on a Multi-imager system (BioRadTM Flour-S) and radioactive bands on a PhosphorImager (Molecular Dynamics TM). Overlaying the UV image with the one from the phosphorimager allowed us to compare the radioactive bands with our TH standards. We were unable to detect T_4 or T_3. However, we found evidence for another iodinated tyrosine species in the fly sample (DM, *D. melanogaster*) which might represent T_2 (3,3′-T_2), based on literature data.

THs have pleiotropic effects on insect physiology

In the silk moth, *Bombyx mori*, THs affect a variety of biological processes (cf. Eales 1997, and references therein). When administered to larvae, for example, exogenous T_4 increases the activity of Na^+/K^+ ATPase in silk glands and ovaries, upregulates protein and RNA synthesis, alters the glycogen content of the fat body, elevates the levels of ecdysteroids in the hemolymph, stimulates silk production, increases fecundity, and modifies heart function (for example, Medda and others 1981; Chaudhuri and Medda 1986; Chaudhuri and Medda 1987a; Chaudhuri and Medda 1987b; Thagaraja and others 1991; Chaudhuri and Medda 1992; Thagaraja and others 1993; Chaudhuri and Medda 1994; Reddy and others 1994d, 1996). Similarly, in the silk moth, *Antheraea mylitta*, TH application stimulates the turnover of proteins and amino acids in ovaries, testes, and fat bodies, and alters amino acid profiles in the hemolymph (Reddy and others 1994a; Reddy and others 1994b; Reddy and others 1994c; Reddy and others 1994d). In the adult moth, *Hyalophora cecropia*, T_4 modulates lipid release from the fat body *in vitro* (Bhakthan and Gilbert 1968).

Thus, very much like JH/20E (for example, Flatt and others 2005), THs have remarkably pleiotropic effects on various aspects of physiology (for example, Eales 1997; Davey 2004).

The best-understood example of TH action in insects comes from the pioneering work of Ken Davey and collaborators, showing that THs can mimic certain aspects of JH action (for example, Sevala and Davey 1989, 1995; Davey and Gordon 1996; Kim and others 1999; Davey 2000a; Davey 2000b, 2004). In ovarian follicular cells of insects, JH regulates ovarian patency, that is, the appearance of large spaces between follicular cells, allowing vitellogenin from the hemolymph to access the surface of the oocyte (Sevala and Davey 1989; Davey 1996). This action of JH seems to be mediated by a membrane receptor and an intracellular signaling cascade involving PKC, which activates a JH-sensitive ATPase (Sevala and Davey 1989; Sevala and others 1995). Application of different THs (T_4, T_3, T_2) to ovarian follicular cells of the locust, *L. migratoria*, or the bug, *Rhodnius prolixus*, mimics the action of JH and causes shrinkage of the cells, as do noniodinated phenoxyphenyl compounds such as fenoxycarb, a potent JH mimic (for example, Davey and Gordon 1996; Kim and others 1999). Remarkably, the effects of T_3 can be inhibited by ouabain, an inhibitor of Na^+/K^+ ATPase, which mediates the JH response, by antibodies raised against a membrane-binding protein for JH, and by ethoxyzolamide, which inhibits binding of JH to the membrane receptor (Davey and Gordon 1996; Kim and others 1999). Both JH III and T_3 can bind to membrane preparations of vitellogenic follicles with equal specificity, suggesting that TH and JH action are mediated by the same membrane receptor (Kim and others 1999). Despite the profound similarities between TH and JH action, however, there is no convincing evidence that THs can affect insect metamorphosis in a JH-like manner (Davey 2004). For example, injection of T_3 or T_2 into the last larval stage of *Rhodnius* does not have any JH-like, juvenilizing effects (Davey 2004).

THs might also modulate or mimic 20E function (for example, Eales 1997). As we have discussed above, the EcR and the TR belong to the same subfamily of nuclear hormone receptors, and 20E and TR signaling share many commonalities (for example, Horner and others 1995; Cherbas and Cherbas 1996; Hatzivassiliou and others 1997; Laudet 1997; Dressel and others 1999; Tsai and others 2004; Ollikainen and others 2006, papers presented at meetings). In this respect, it is noteworthy that T_4 fed to larvae of *B. mori* increases the hemolymph levels of ecdysteroids (Thagaraja and others 1991, 1993).

Do THs affect *Drosophila* development and life history?

The effects of THs on the development and life history of the fly were investigated in the 1920s and 1930s (Reznitchenko 1926, 1927; Dobkiewicz 1928; Alpatov 1929; Koller 1932; also see Schneider 1939b). The work by Reznitchenko (1926, 1927) shows that feeding thyroid substance to flies typically does not affect the duration of the larval or pupal period. At high concentrations, however, development was slightly retarded, possibly due to toxic effects (Reznitchenko 1926, 1927). Similarly negative results were obtained by Dobkiewicz (1928), who fed flies dried thyroid gland, but failed to find any effect on development. Koller (1932) exposed flies to chemically pure T_4 and found no effects on development, sex ratio, body size, or crossing-over. However, T_4-fed flies had a slightly reduced egg laying rate, an effect that was attributed to the increased pH of the food medium containing T_4 (Koller 1932). In contrast to the above studies, Alpatov (1929) showed that thyroid feeding can result in larger larvae, yet somewhat smaller adults than in control flies. This was interpreted as TH having an accelerating effect on larval development (Alpatov 1929).

To examine whether THs affect development of *Drosophila* we recently attempted to replicate these classical experiments by exposing eggs and larvae of *D. melanogaster* to T_4, T_3, reverse T_3 (3,3′,5′-Triiodo-L-thyronine), and T_2 (3,5-Diiodo-L-thyronine) and by examining the effects of these compounds on egg-to-adult survival (viability). In a pilot experiment (5 replicate vials per treatment, each with 30 eggs) we used 2 different concentrations for the TH compounds (10^{-6} M and 10^{-8} M) and found that hormone treatment [2-way analysis of variance (ANOVA), $F_{4,34} = 2.15$, $P = 0.095$], concentration ($F_{1,34} = 0.05$, $P = 0.82$), and the interaction between treatment and concentration ($F_{4,34} = 0.10$, $P = 0.98$) did not significantly affect viability (results not shown).

To independently verify these observations we carried out a similar experiment on a larger scale and using additional control treatments (Fig. 2). Specifically, we compared the effects of THs (T_4, T_3, and reverse T_3, all at 10^{-8} M) with various control treatments (no chemicals added to the food medium; L-tyrosine; NaI; NaI plus L-Tyrosine). ANOVA showed that none of the THs or the constituents of THs (L-tyrosine, iodine) had any significant effect on egg-to-adult survival (Fig. 2A) and pupal-to-adult survival (Fig. 2B; details of analysis not shown). Thus, these data, together with our pilot data, strongly suggest that THs or constituents of THs do probably not affect preadult development.

When interpreting the effects of exposing *Drosophila* to THs in the food medium great care must be taken. In particular, insects require large amounts of tyrosine for cuticle formation and tanning (for example, Mitchell and others 1971; Kramer and Hopkins 1987). *Drosophila* must accumulate large stores of tyrosine since, after feeding ceases, the fly forms a puparium, a structure rich in tyrosine that will eventually be shed, causing a loss of tyrosine. Enclosed within the puparium, the fly must molt to a pupa and then ultimately to the adult, with each cuticle and, in particular, the puparium being rich in tyrosine derivatives (for example, Mitchell and others 1971; Kramer and Hopkins 1987). Thus, the effects of iodinated tyrosine derivatives such as THs on preadult viability and development might be confounded by the effects of tyrosine and iodine on cuticle formation and the tanning process itself. For example, it is possible that flies kept under standard culture conditions are short of tyrosine and iodine and feeding THs might simply cure this defect. We therefore examined the effects of L-tyrosine, NaI, NaI plus L-tyrosine, and THs on the proportion of tanned prepupae (Fig. 2C). While visual inspection of the data suggest that NaI, L-tyrosine plus NaI, and T_3 increase the proportion of tanned prepupae, none of the compounds had a statistically significant effect (details of analysis not shown). While it remains possible that iodinated compounds have an effect on tanning (we might not have had sufficient statistical power to resolve these effects), the preliminary observations presented here are consistent with those of most earlier work (Reznitchenko 1926, 1927; Dobkiewicz 1928; Koller 1932) which failed to find clear effects of THs on *Drosophila* development and life history.

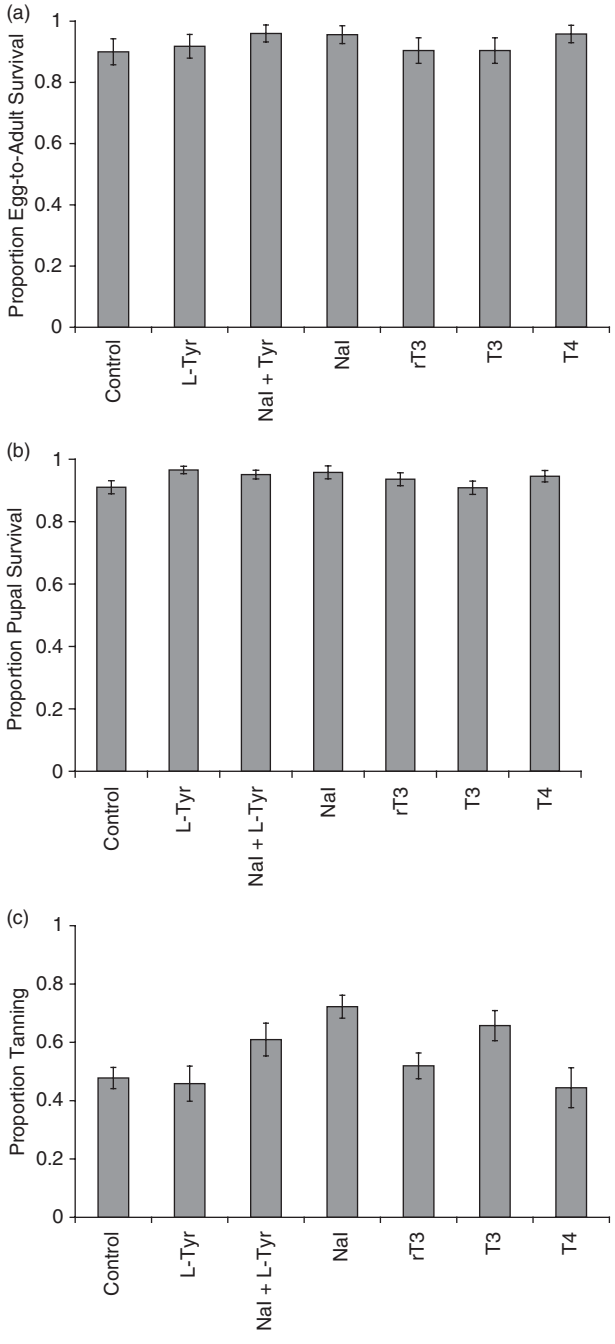

Fig. 2 Effects of tyrosine, iodine, and THs on *D. melanogaster* preadult development and tanning. After an overnight egg lay, freshly laid eggs were allocated to the following experimental treatments (10 replicate vials per treatment, each with 50 eggs): control (no compounds added to standard food medium), T_3, rT_3, and T_4 (all at 10^{-8} M), L-tyrosine (at 2×10^{-6} M), NaI (at 4×10^{-6} M), and NaI combined with L-tyrosine (4×10^{-6} M NaI plus 2×10^{-6} M L-tyrosine). To avoid damage to the different compounds, diluted hormones and other compounds were added to the food medium (standard sucrose–cornmeal–agar–yeast diet; components, respectively, 10.5, 5.0, 0.7, 2.0% with the remainder water) when the temperature of the freshly made food medium was lower than 37°C. (A) Egg-to-adult survival (viability, that is, the proportion of eggs that successfully developed into adults); (B) pupal-to-adult survival (that is, the proportion of pupae that successfully developed into adults); and (C) proportion of tanned prepupape (that is, the ratio of white puparia to brown puparia), at around 120 h after egg laying (AEL) when the puparium is formed.

THs might modulate immunity in *Drosophila*

At metamorphosis, the immune system of many organisms undergoes reorganization (Rollins-Smith 1998; Davidson and Swalla 2002). For example, in ascidians, many innate immunity genes are activated at metamorphosis, representing the programmed maturation of the adult immune system, but probably also serve to mediate phagocytosis and remodeling of tissue (Davidson and Swalla 2002). It is thus likely that metamorphic hormones such as THs, JH/20E, and others

may play a role in the reorganization of the immune system or in its activation. While our understanding of the hormonal regulation of immunity is still limited (for example, Webster and others 2002), it is now clear that, in many organisms, nuclear hormone receptors play an important role in regulating immunity and inflammation (for example, Rollins-Smith and others 1993; Rollins-Smith and Cohen 1996; Rollins-Smith 1998; Beckstead and others 2005; Ogawa and others 2005; Glass and Ogawa 2006). In mammals, for example, immune function is regulated by nuclear hormone receptors such as the peroxisome proliferator-actived receptor (PPAR) and the liver X receptor (LXR), a mammalian homolog of EcR (for example, Glass and Ogawa 2006). In amphibians, THs are required for the development of a functional immune system at metamorphosis (for example, Rollins-Smith and others 1993; Rollins-Smith and Cohen 1996; Rollins-Smith 1998).

In *Drosophila*, 20E upregulates the expression of antimicrobial peptides (AMPs) in third instar larvae, prepupae, and cell culture (Meister and Richards 1996; Dimarcq and others 1997), and recent work suggests that EcR signaling affects the expression of genes involved in immunity at onset of metamorphosis (Beckstead and others 2005). In contrast to 20E, JH suppresses phenoloxidase (PO) levels in the mealworm beetle (*Tenebrio molitor*) and the expression of genes involved in defense and stress response, including several AMPs in *Drosophila* (Rolff and Siva-Jothy 2002; Rantala and others 2003; Flatt and others 2005; Tu and others 2006).

We have thus recently begun to explore whether THs affect innate immunity in *Drosophila*. We found that, in *Drosophila* S2* cells, 20E potentiates the expression of AMPs upon immune challenge, while JH suppresses this effect (T. Flatt, A. Heyland, N. Silverman, M. Tatar, unpublished data). Remarkably, the 20E-induced expression of AMPs can be further potentiated in a dose-dependent manner by THs such as T_4, T_3, and T_2, whereas in the absence of 20E, THs do not affect AMP expression (T. Flatt, A. Heyland, N. Silverman, M. Tatar, unpublished data). Interestingly, it has been reported that THs can synergistically interact with various steroid hormones to potentiate metamorphic actions in amphibians (Denver 1996; Kaltenbach 1996; Tata 1996, and references therein), and our results are clearly reminiscent of such effects. Our preliminary observations thus suggest that THs modulate 20E action, but do not mimic the effects of JH on AMP expression. Since *Drosophila* does not have a clear TR homolog (Laudet 1997; King-Jones and Thummel 2005) and since T_3 does not seem to be a EcR receptor agonist (Christopherson and others 1992), we conjecture that the effects of THs we observed in S2* cells might be nongenomic, a possibility which we are currently testing. Thus, while THs and other tyrosine derivatives might not affect *Drosophila* development (see above), these compounds might nevertheless have hormonal effects in the fly.

THs might affect intermediary metabolism in *Drosophila*

Potential interactions between THs and 20E/JH might not be restricted to AMPs. Interestingly, in vertebrates, THs are known to play an important role in the regulation of the gene encoding malic enzyme, an enzyme involved in glycolysis, converting malate into pyruvate (Dozin and others 1986; Song and others 1988; Petty and others 1990; Desvergne and others 1991; Jeannin and others 1998). In *Drosophila,* this gene is antagonistically regulated by 20E and JH (Farkas and Knopp 1997; Farkas and others 2002). Thus, THs and JH/20E might regulate the expression of various genes in a similar way, and studying these genes might provide important insights into the hormonal regulation of gene expression.

Conclusions

For the past 50 years or so a wealth of experimental data suggest that hormones from a diverse array of species can mimic hormonal effects in other species, yet the mechanisms underlying such "endocrine mimicry" and the cross talk between different hormonal pathways still remain largely unresolved. The highly pleiotropic THs of vertebrates and many invertebrates, often involved in metamorphosis, share many structural and functional similarities with the 2 major metamorphic insect hormones, JH and 20E. Here, we have discussed classical and recent evidence suggesting that (1) insects can incorporate and potentially synthesize and metabolize iodinated tyrosines, (2) THs have pervasive effects on multiple aspects of insect development, physiology, and life history, and (3) some actions of THs mimic those of JH, whereas others resemble those of 20E. While it still remains unclear whether THs are indeed naturally occurring and functionally relevant insect hormones, increasing evidence thus suggests that THs can interact with the hormonal signaling pathways of insects. How can noninsect hormones such as TH mimic the action of insect-specific hormones?

Many lipophilic hormones might represent particularly well-designed "hormone keys" (ligands) that work for a wide variety of "receptor locks" and endocrine pathways, even in organisms that do not

endogenously produce these hormones (Kushiro and others 2003; Wheeler and Nijhout 2003; McCourt and others 2005). For example, it remains possible that THs can bind to nuclear hormone receptors previously unknown to transduce TH signaling. On the other hand, many lipophilic hormones can act through cell surface (nongenomic, nonnuclear) signal-transduction pathways rather than through classical nuclear hormone receptor signaling (for example, Wheeler and Nijhout 2003). Such nongenomic modes of hormone action might include signaling through PKC/phospholipase C (PLC), secondary messengers such as intracellular calcium, intracellular changes in pH, and mitogen-activated protein kinases (MAPK). Since insects do not possess TH receptors, the effects of THs in insects thus might be of nongenomic (nonnuclear) nature.

Addressing the question whether THs play natural, functional roles in insects is important in its own right. Moreover, since THs appear to interact with JH/20E signaling, comparing the effects of these hormones might provide important insights into the so-far enigmatic molecular basis of the interaction between JH and 20E signaling. A potentially useful approach is to investigate the effects of different hormones in model systems, as we have proposed here. The advantage of this strategy is that the actions of endogenous or exogenous compounds with putative endocrine function are mechanistically tractable since they can be evaluated in the context of well established endogenous hormone effects and their signaling pathways, for instance in cell culture systems. One important focus of future studies in *D. melanogaster* should be the analysis of gene expression profiles in response to various hormones and hormone mimics. Similarities in how different hormones affect gene expression may facilitate the search for central components involved in the hormonal response and in the elucidation of its functional relevance.

Acknowledgments

This paper was presented at the symposium "Metamorphosis—A Multi-Kingdom Approach", at the Annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2006, in Orlando, Florida. We thank Jason Hodin, Harold Heatwole, and 2 anonymous referees for helpful comments on the manuscript and the participants of the symposium and the associated sessions for constructive discussion. Our research was supported by the Swiss National Science Foundation (TF and AH), the Roche Research Foundation (TF), the Whitney Laboratories (AH), the National Institute of Health (MT), and the Ellison Medical Foundation (MT). We are grateful to the Society for Integrative and Comparative Biology (SICB), the University of Florida, The Whitney Laboratory for Marine Biosciences, the American Microscopical Society (AMS), and the SICB Division of Evolutionary Developmental Biology (DEDB) for promoting and partially funding the symposium.

Conflict of interest: None declared.

References

Alpatov WW. 1929. The influence of thyroid gland feeding on the acceleration of the growth of larvae of *Drosophila melanogaster*. Proc Natl Acad Sci USA 15:578–80.

Baker KD, Shewchuk LM, Kozlova T, Makishima M, Hassell A, Wisely B, Caravella JA, Lambert MH, Reinking JL, Krause H. 2003. The *Drosophila* orphan nuclear receptor DHR38 mediates an atypical ecdysteroid signaling pathway. Cell 113:731–42.

Beckstead RB, Lam G, Thummel CS. 2005. The genomic response to 20-hydroxy-ecdysone at the onset of *Drosophila metamorphosis*. Genome Biol 6:R99.

Bede JC, Goodman WG, Tobe SS. 1999. Developmental distribution of insect juvenile hormone III in the sedge, *Cyperus iria*. Phytochemistry 52:1269–74.

Bede JC, Teal PE, Goodman WG, Tobe SS. 2001. Biosynthetic pathway of insect juvenile hormone III in cell suspension cultures of the sedge *Cyperus iria*. Plant Physiol 127:584–93.

Belles X, Martin D, Piulachs MD. 2005. The mevalonate pathway and the synthesis of juvenile hormone in insects. Annu Rev Entomol 50:181–99.

Berger EM, Dubrovsky EB. 2005. Juvenile hormone—molecular actions and interactions during development of *Drosophila melanogaster*. Vitam Horm 73:175–215.

Berger EM, Goudie K, Klieger L, Berger M, DeCato R. 1992. The juvenile hormone analogue, methoprene, inhibits ecdysterone induction of small heat shock protein gene expression. Dev Biol 151:410–18.

Bhakthan NMG, Gilbert LI. 1968. Effects of some vertebrate hormones on lipid mobilization in the insect fat body. Gen Comp Endocrinol 11:186–97.

Biggers WJ, Laufer H. 1999. Settlement and metamorphosis of *Capitella* larvae induced by juvenile hormone-active compounds is mediated by protein kinase C and ion channels. Biol Bull 196:187–98.

Brent GA, Moore DD, Larsen RP. 1991. Thyroid hormone regulation of gene expression. Annu Rev Physiol 53:17–35.

Brown DD, Cai L, Das B, Marsh-Armstrong N, Schreiber AM, Juste R. 2005. Thyroid hormone controls multiple independent programs required for limb development in *Xenopus laevis* metamorphosis. Proc Natl Acad Sci USA 102:12455–8.

Brown DD, Wang Z, Furlow JD, Kanamori A, Schwartzman RA, Remo BF, Pinder A. 1996. The thyroid hormone-induced tail

resorption program during *Xenopus laevis* metamorphosis. Proc Natl Acad Sci USA 93:1924–9.

Buchholz DR, Paul BD, Fu L, Shi Y-B. 2006. Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog. Gen Comp Endocrinol 145:1–19.

Chanoine JP, Safran M, Farwell AP, Dubord S, Alex S, Stone S, Arthur JR, Braverman LE, Leonard JL. 1992. Effects of selenium deficiency on thyroid hormone economy in rats. Endocrinology 131:1787–92.

Chaudhuri A, Medda AK. 1986. Changes in protein and nucleic acid contents of male gonad of silkworm, *Bombyx mori*, at different developmental stages after thyroxine treatment. Proc Natl Acad Sci India 56:301–6.

Chaudhuri A, Medda AK. 1987a. Thyroxine induced alterations in protein and nucleic acid content of fat body of female silkworm, during different developmental stages. Insect Sci Appl 8:43–8.

Chaudhuri A, Medda AK. 1987b. Effect of thyroxine on protein, RNA and DNA content of ovary of silkworm, *Bombyx mori*, at larval, pupal and adult stages of development and production of eggs. Zool Jahrb Anat 115:85–90.

Chaudhuri A, Medda AK. 1992. Thyroxine induced alterations in glycogen content of fat body of female silkworms *Bombyx mori* (*race nistari*) during larval, pupal and adult stages of development. Ann Entomol 10:17–21.

Chaudhuri A, Medda AK. 1994. Influence of thyroxine on ovarian glycogen of *Bombyx mori* L (*race nistari*) during ontogeny. Insect Sci Appl 14:621–6.

Chen JD, Evans RM. 1995. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454–7.

Cherbas P, Cherbas L. 1996. Molecular aspects of ecdysteroid hormone action. In: Gilbert LI, Tata JR, Atkinson BG, editors. Metamorphosis—postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press. p 175–221.

Cherbas L, Koehler MMD, Cherbas P. 1989. Effects of juvenile hormone on the ecdysone response of *Drosophila* Kc cells. Dev Genet 10:177–88.

Chihara CJ, Fristrom JW. 1973. Effects and interactions of juvenile hormone and β-ecdysone on *Drosophila* imaginal discs cultured *in vitro*. Dev Biol 35:36–46.

Christopherson KS, Mark MR, Bajaj V, Godowski PJ. 1992. Ecdysteroid-dependent regulation of genes in mammalian cells by a *Drosophila* ecdysone receptor and chimeric transactivators. Proc Natl Acad Sci USA 89:6314–18.

Davey KG. 1996. Hormone control of the follicular epithelium during vitellogenin uptake. Invertebr Reprod Develop 30:249–54.

Davey KG. 2000a. The modes and actions of juvenile hormones: some questions we ought to ask. Insect Biochem Mol Biol 30:663–9.

Davey KG. 2000b. Do thyroid hormones function in insects? Insect Biochem Mol Biol 30:877–84.

Davey KG. 2004. Evolutionary aspects of thyroid hormone effects in invertebrates. In: Hall BK, Pearson RD, Müller GB, editors. Environment, development, and evolution. Cambridge, MA: MIT Press. p 279–95.

Davey KG, Gordon DRB. 1996. Fenoxycarb and thyroid hormones have JH-like effects on the follicle cells of *Locusta migratoria in vitro*. Arch Insect Biochem Physiol 32:613–22.

Davidson B, Swalla BJ. 2002. A molecular analysis of ascidian metamorphosis reveals activation of an innate immune response. Development 129:4739–51.

Davis PJ, Davis FB. 1996. Nongenomic actions of thyroid hormone. Thyroid 6:497–504.

Davis FB, Kite JH, Davis PJ, Blas SD. 1982. Thyroid hormone stimulation *in vitro* of red blood cell Ca^{2+}-ATPase activity: interspecies variation. Endocrinology 110:297–8.

Denver RJ. 1996. Neuroendocrine control of amphibian metamorphosis. In: Gilbert LI, Tata JR, Atkinson BG, editors. Metamorphosis—postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press. p 434–64.

Denver RJ, Glennemeier KA, Boorse GC. 2002. Endocrinology of complex life cycles: amphibians. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, Rubin R, editors. Hormones, brain and behavior Volume 2. San Diego: Academic Press. p 469–513.

Desvergne B, Petty KJ, Nikodem VM. 1991. Functional characterization and receptor binding studies of the malic enzyme thyroid hormone response element. J Biol Chem 266:1008–13.

Di Liegro I, Savetteri G, Cestelli A. 1987. Cellular mechanism of action of thyroid hormones. Differentiation 35:165–75.

Dimarcq J-L, Imler J-L, Lanot R, Ezekowitz RAB, Hoffmann JA, Janeway CA, Lagueux M. 1997. Treatment of l(2)mbn *Drosophila* tumorous blood cells with the steroid hormone ecdysone amplifies the inducibility of antimicrobial peptide gene expression. Insect Biochem Mol Biol 27:877–86.

Dingle H, Winchell R. 1997. Juvenile hormone as a mediator of plasticity in insect life histories. Arch Insect Biochem Physiol 35:359–73.

Dobkiewicz L. 1928. Der Einfluss der Schilddrüsenfütterung auf Entwicklung, Wachstum, und Fortpflanzung der Taufliege (*Drosophila melanogaster*). Roux's Arch Entw Mech 113:96–122.

Dozin B, Magnuson MA, Nikodem VM. 1986. Thyroid hormone regulation of malic enzyme synthesis. Dual tissue-specific control. J Biol Chem 261:10290–2.

Dressel U, Thormeyer D, Altincicek B, Paululat A, Eggert M, Schneider S, Tenbaum SP, Renkawitz R, Baniahmad A. 1999. Alien, a highly conserved protein with characteristics of a corepressor for members of the nuclear hormone receptor superfamily. Mol Cell Biol 19:3383–94.

Dubrovsky EB. 2005. Hormonal cross talk in insect development. Trends Endocrinol Metab 16:6–11.

Dunn AD. 1974. Ultrastructural autoradiography and cytochemistry of iodine-binding cells in ascidian endostyle. J Exp Zool 188:103–23.

Eales JG. 1997. Iodine metabolism and thyroid-related functions in organisms lacking thyroid follicles: are thyroid hormones also vitamins? Proc Soc Exp Biol Med 214:302–17.

Elmogy M, Iwami M, Sakurai S. 2004. Presence of membrane ecdysone receptor in the anterior silk gland of the silkworm *Bombyx mori*. Eur J Biochem 271:3171–9.

Eneqvist T, Lundberg E, Nilsson L, Abagyan R, Sauer-Eriksson AE. 2003. The transthyretin-related protein family. Eur J Biochem 270:518–32.

Erezylimaz D. 2006. Imperfect eggs and oviform nymphs: a history of the ideas on the origins of insect metamorphosis. Integr Comp Biol (papers presented at meetings).

Fang F, Xu Y, Jones D, Jones G. 2005. Interactions of ultraspiracle with ecdysone receptor in the transduction of ecdysone- and juvenile hormone-signaling. FEBS J 272:1577–89.

Farkas R, Danis P, Medved'ova L, Mechler BM, Knopp J. 2002. Regulation of cytosolic malate dehydrogenase by juvenile hormone in *Drosophila melanogaster*. Cell Biochem Biophys 37:37–52.

Farkas R, Knopp J. 1997. Ecdysone-modulated response of *Drosophila* cytosolic malate dehydrogenase to juvenile hormone. Arch Insect Biochem Physiol 35:71–83.

Finch CE, Rose MR. 1995. Hormones and the physiological architecture of life history evolution. Q Rev Biol 70:1–52.

Flatt T. 2004. The effects of juvenile hormone on the architecture of life history traits in *Drosophila melanogaster*. Unpublished PhD dissertation, Department of Biology, Unit of Ecology and Evolutionary Biology, University of Fribourg, Switzerland. Fribourg: Imprimerie St Paul.

Flatt T, Kawecki TJ. 2004. Pleiotropic effects of methoprene-tolerant (Met), a gene involved in juvenile hormone metabolism, on life history traits in *Drosophila melanogaster*. Genetica 122:141–60.

Flatt T, Tu MP, Tatar M. 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. Bioessays 27:999–1010.

Gäde G, Hoffmann KH, Spring JH. 1997. Hormonal regulation in insects: facts, gaps, and future directions. Physiol Rev 77:963–1032.

Gilbert LI, Rybczynski R, Warren JT. 2002. Control and biochemical nature of the ecdysteroidogenic pathway. Annu Rev Entomol 47:883–916.

Glass CK, Ogawa S. 2006. Combinatorial roles of nuclear receptors in inflammation and immunity. Nat Rev Immunol 1:44–55.

Grebe M, Przibilla S, Henrich VC, Spindler-Barth M. 2003. Characterization of the ligand binding domain of the ecdysteroid receptor from *Drosophila melanogaster*. Biol Chem 384:105–16.

Grenier S, Grenier A-M. 1993. Fenoxycarb, a fairly new insect growth regulator: a review of its effects on insects. Ann Appl Biol 122:369–403.

Hall BL, Thummel CS. 1998. The RXR homolog ultraspiracle is an essential component of the *Drosophila* ecdysone receptor. Development 125:4709–17.

Harmon MA, Boehm MF, Heyman RA, Mangelsdorf DJ. 1995. Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. Proc Natl Acad Sci USA 92:6157–60.

Hatzivassiliou E, Cardot P, Zannis VI, Mitsialis SA. 1997. Ultraspiracle, a *Drosophila* retinoic X receptor alpha homologue, can mobilize the human thyroid hormone receptor to transactivate a human promoter. Biochemistry 36:9221–31.

Hauser F, Nothacker HP, Grimmelikhuijzen CJ. 1997. Molecular cloning, genomic organization, and developmental regulation of a novel receptor from *Drosophila melanogaster* structurally related to members of the thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone/choriogonadotropin receptor family from mammals. J Biol Chem 272:1002–10.

Henrich VC, Burns E, Yelverton DP, Christensen E, Weinberger C. 2003. Juvenile hormone potentiates ecdysone receptor-dependent transcription in a mammalian cell culture system. Insect Biochem Mol Biol 33:1239–47.

Heyland A, Hodin J. 2004. Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. Evolution 58:524–38.

Heyland A, Hodin J, Reitzel AM. 2005. Hormone signaling in evolution and development: a non-model system approach. Bioessays 27:64–75.

Heyland A, Moroz LL. 2005. Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. J Exp Biol 208:4355–61.

Heyland A, Price DA, Bodnarova M, Moroz LL. 2006. Thyroid hormone metabolism and thyroid peroxidase function in two non-chordate animals. J Exp Zool B Mol Dev Evol 10.1002/JEZ.B.2003.

Heyland A, Reitzel AM, Hodin J. 2004. Thyroid hormones determine developmental mode in sand dollars (Echinodermata: Echinoidea). Evol Dev 6:382–92.

Hiruma K, Shinoda T, Malone F, Riddiford LM. 1999. Juvenile hormone modulates 20-hydroxyecdysone-inducible ecdysone receptor and ultraspiracle gene expression in the tobacco hornworm, *Manduca sexta*. Dev Genes Evol 209:18–30.

Hodin J. 2006. On the origins of insect hormone signaling. In: Whitman D, Ananthakrishnan TN, editors. Insects and phenotypic plasticity. Enfield: Science Publishers, Inc. (in press).

Hörlein AJ, Näär AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Söderström M, Glass CK and others. 1995. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397–404.

Horner MA, Chen T, Thummel CS. 1995. Ecdysteroid regulation and DNA binding properties of *Drosophila* nuclear hormone receptor superfamily members. Dev Biol 168:490–502.

Hulbert AJ. 2000. Thyroid hormones and their effects: a new perspective. Biol Rev Camb Philos Soc 75:519–631.

Jeannin E, Robyr D, Desvergne B. 1998. Transcriptional regulatory patterns of the myelin basic protein and malic enzyme

genes by the thyroid hormone receptors alpha 1 and beta 1. J Biol Chem 273:24239–48.

Jiang C, Baehrecke EH, Thummel CS. 1997. Steroid regulated programmed cell death during *Drosophila* metamorphosis. Development 124:4673–83.

Jones G. 1995. Molecular mechanisms of action of juvenile hormone. Annu Rev Entomol 40:147–69.

Jones G, Sharp PA. 1997. Ultraspiracle: an invertebrate nuclear receptor for juvenile hormones. Proc Natl Acad Sci USA 94:13499–503.

Jones G, Wozniak M, Chu Y, Dhar S, Jones D. 2001. Juvenile hormone III-dependent conformational changes of the nuclear receptor ultraspiracle. Insect Biochem Mol Biol 32:33–49.

Kaltenbach JC. 1996. *Endocrinology* of amphibian metamorphosis. In: Gilbert LI, Tata JR, Atkinson BG, editors. Metamorphosis—postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press. p 403–31.

Kethidi DR, Li Y, Palli SR. 2006. Protein kinase C mediated phosphorylation blocks juvenile hormone action. Mol Cell Endocrinol 247:127–34.

Kim Y, Davari ED, Sevala V, Davey KG. 1999. Functional binding of a vertebrate hormone, L-3,5,3′-triiodothyronine (T3), on insect follicle cell membranes. Insect Biochem Mol Biol 29:943–50.

King-Jones K, Thummel CS. 2005. Nuclear receptors—a perspective from *Drosophila*. Nat Rev Genet 6:311–23.

Kingsley RJ, Corcoran ML, Krider KL, Kriechbaum KL. 2001. Thyroxine and vitamin D in the gorgonian *Leptogorgia virgulata*. Comp Biochem Physiol A Mol Integr Physiol 129:897–907.

Koelle MR, Talbot WS, Segraves WA, Bender MT, Cherbas P, Hogness DS. 1991. The *Drosophila* EcR gene encodes an ecdysone receptor, a new member of the steroid receptor family. Cell 67:59–77.

Koller PC. 1932. Der Einfluss chemisch reinen Thyroxins auf die Entwicklung von *Drosophila melanogaster*. Rouxs Arch Entw Mech 125:663–72.

Kozlova T, Thummel CS. 2000. Steroid regulation of postembryonic development and reproduction in *Drosophila*. Trends Endocrinol Metab 11:276–80.

Kramer KJ, Hopkins TL. 1987. Tyrosine metabolism for insect cuticle tanning. Arch Insect Biochem Physiol 6:279–301.

Kushiro T, Nambara E, McCourt P. 2003. Hormone evolution: the key to signalling. Nature 422:122–2.

Laudet V. 1997. Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. J Mol Endocrinol 19:207–26.

Leung J, Giraudat J. 1998. Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol 49:199–22.

Lezzi M, Bergman T, Henrich VC, Vögtli M, Frömel C, Grebe M, Przibilla S, Spindler-Barth M. 2002. Ligand-induced heterodimerization between the ligand binding domains of the *Drosophila* ecdysteroid receptor and ultraspiracle. Eur J Biochem 269:3237–45.

Lezzi M, Bergman T, Mouillet JF, Henrich VC. 1999. The ecdysone receptor puzzle. Arch Insect Biochem Physiol 41:99–106.

Limpel LE, Caseda JE. 1957. Iodine metabolism in insects. I. *In vivo* metabolism of radionuclide. J Exp Zool 135:19–27.

Mairh OP, Ramavat BK, Tewari A, Oza RM, Joshi HV. 1989. Seasonal variation, bioaccumulation and prevention of loss of iodine in seaweeds. Phytochemistry 28:3307–10.

Maki A, Sawatsubashi S, Ito S, Shirode Y, Suzuki E, Zhao Y, Yamagata K, Kouzmenko A, Takeyama K-I, Kato S. 2004. Juvenile hormones antagonize ecdysone actions through co-repressor recruitment to EcR/USP heterodimers. Biochem Biophys Res Commun 320:262–7.

Manzon RG, Holmes JA, Youson JH. 2001. Variable effects of goitrogens in inducing precocious metamorphosis in sea lampreys (*Petromyzon marinus*). J Exp Zool 289:290–303.

Martinez E, Givel F, Wahli W. 1991. A common ancestor DNA motif for invertebrate and vertebrate hormone response elements. EMBO J 10:263–8.

Matsuda R. 1987. Animal evolution in changing environments. New York: Wiley.

McCourt P, Lumba S, Tsuchiya Y, Gazzarrini S. 2005. Crosstalk and abscisic acid: the roles of terpenoid hormones in coordinating development. Physiol Plant 123:147–52.

McNabb FMA. 1992. Thyroid hormones. Englewood: Prentice.

Medda AK, Ray AK, Dasgupta AC, Rey CD. 1981. Thyroid hormone actions in magur fish and silk worms. In: Stockigt JR, Nagataki S, editors. Thyroid research VIII. Canberra: Australian Academy of Sciences. p 240–3.

Meister M, Richards G. 1996. Ecdysone and insect immunity: the maturation of the inducibility of the diptericin gene in *Drosophila* larvae. Insect Biochem Mol Biol 26:155–60.

Mitchell KH, Weber-Tracy UM, Schaar G. 1971. Aspects of cuticle formation in *Drosophila melanogaster*. J Exp Zool 176:429–43.

Miura K, Oda M, Makita S, Chinzei Y. 2005. Characterization of the *Drosophila* methoprene-tolerant gene product. Juvenile hormone binding and ligand-dependent gene regulation. FEBS J 272:1169–78.

Nelson RE, Fessler LI, Takagi Y, Blumberg B, Keene DR, Olson PF, Parker CG, Fessler JH. 1994. Peroxidasin: a novel enzyme-matrix protein of *Drosophila* development. EMBO J 13:3438–47.

Nemec V, Kodrik D, Matolin S, Laufer H. 1993. Juvenile hormone-like effects of retinoic acid in insect metamorphosis, embryogenesis and reproduction. J Insect Physiol 39:1083–93.

Nijhout FH. 1994. Insect hormones. Princeton: Princeton University Press.

Ogasawara M. 2000. Overlapping expression of amphioxus homologs of the thyroid transcription factor-1 gene and thyroid peroxidase gene in the endostyle: insight into evolution of the thyroid gland. Dev Genes Evol 210:231–42.

Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, Westin S, Hoffmann A, Subramaniam S, David M, Rosenfeld MG. 2005. others Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. Cell 122:707–21.

Ollikainen N, Chandsawangbhuwana C, Baker M. 2006. Evolution of the thyroid hormone, retinoic acid, ecdysone and liver X receptors. Integr Comp Biol (papers presented at meetings).

Oro AE, McKeown M, Evans RM. 1990. Relationship between the product of the *Drosophila* ultraspiracle locus and the vertebrate retinoid X receptor. Nature 347:298–301.

Oro AE, McKeown M, Evans RM. 1992. The *Drosophila* retinoid X receptor homolog ultraspiracle functions in both female reproduction and eye morphogenesis. Development 115: 449–62.

Petty KJ, Desvergne B, Mitsuhashi T, Nikodem VM. 1990. Identification of a thyroid hormone response element in the malic enzyme gene. J Biol Chem 265:7395–400.

Petty KJ, Krimkevich YI, Thomas D. 1996. A TATA binding protein-associated factor functions as a coactivator for thyroid hormone receptors. Mol Endocrinol 10:1632–45.

Pinna G, Hiedra L, Meinhold H, Eravci M, Prengel H, Brödel O, Gräf K-J, Stoltenburg-Didinger G, Bauer M, Baumgartner A. 1998. 3,3′-Diiodothyronine concentrations in the sera of patients with nonthyroidal illnesses and brain tumors and of healthy subjects during acute stress. J Clin Endocrinol Metab 83:3071–7.

Rantala MJ, Vainikka A, Kortet R. 2003. The role of juvenile hormone in immune function and pheromone production trade-offs: a test of the immunocompetence handicap principle. Proc Roy Soc Lond B 270:2257–61.

Reddy KD, Chaudhuri A, Sukumar K. 1994a. Thyroxine effect on protein and nucleic acid turnover of testis and ovary during 5th larval stage of non-diapausing tasar silkworm, *Antheraea mylitta* (Saturniidae: Lepidoptera). Indian J Exp Biol 32:409–12.

Reddy KD, Chaudhuri A, Sukumar K. 1994b. Effect of L-thyroxine–sodium pentahydrate on protein and nucleic acid turnover in fat body during 5th larval stage of non-diapausing tasar silkworm, *Antheraea mylitta* (Saturniidae: Lepidoptera). Indian J Exp Biol 32:413–17.

Reddy KD, Chaudhuri A, Sukumar K. 1994c. L-thyroxine (T4) elevates the free amino acid pool of haemolymph plasnia of tasar silkworm, *Antheraea mylitta* (Saturniidae: Lepidoptera). Horm Metab Res 26:570–3.

Reddy KD, Chaudhuri A, Sukumar K. 1994d. Enrichment of ion specific adenosine triphosphate activities by thyroxine in different tissues of the silkworm, *Bombyx mori* L., during insect development. Insect Biochem Mol Biol 24:243–8.

Reddy KD, Chaudhuri A, Thangavelu K. 1996. Influence of thyroxine on different ion-dependent ATPase activities in fat body of Tasar silkworm, *Antherea mylitta* D. Gen Comp Endocrinol 194:20–8.

Reznitchenko MS. 1926. The influence of the thyroid gland on the development of *Drosophila melanogaster*. Trans Lab Exp Biol Zoopark Moscow 2:198–200 (cited in Schneider 1939b).

Reznitchenko MS. 1927. Zur Frage über die spezifische Wirkung der Schilddrüse und Ca- und K-Ionen auf die Entwicklung von *Drosophila melanogaster*. Trans Lab Exp Biol Zoopark Moscow 3:27–35 (in Russian, with German summary, cited in Schneider 1939b).

Ribeiro RC, Apriletti JW, West BL, Wagner RL, Fletterick RJ, Schaufele F, Baxter JD. 1995. The molecular biology of thyroid hormone action. Ann NY Acad Sci 758:366–89.

Riddiford LM. 1993. Hormones and *Drosophila* development. In: Bate M, Martinez Arias A, editors. The development of *Drosophila melanogaster*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. p 899–939.

Rolff J, Siva-Jothy MT. 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. Proc Natl Acad Sci USA 99:9916–18.

Rollins-Smith LA. 1998. Metamorphosis and the amphibian immune system. Immunol Rev 166:221–30.

Rollins-Smith LA, Cohen N. 1996. Metamorphosis: an immunologically unique period in the life of the frog. In: Gilbert LI, Tata JR, Atkinson BG, editors. Metamorphosis—postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press. p 625–46.

Rollins-Smith LA, Davis AT, Blair PJ. 1993. Effects of thyroid hormone deprivation on immunity in postmetamorphic frogs. Dev Comp Immunol 17:157–64.

Schneider BA. 1939a. Effects of feeding thyroid substance. Q Rev Biol 14:289–310.

Schneider BA. 1939b. Effects of feeding thyroid substance (concluded). Q Rev Biol 14:431–50.

Schubiger M, Carre C, Antoniewski C, Truman JW. 2005. Ligand-dependent de-repression via EcR/USP acts as a gate to coordinate the differentiation of sensory neurons in the *Drosophila* wing. Development 132:5239–48.

Schubiger M, Truman JW. 2000. The RXR ortholog USP suppresses early metamorphic processes in *Drosophila* in the absence of ecdysteroids. Development 127:1151–9.

Sevala VL, Davey KG. 1989. Action of juvenile hormone on the follicle cells of *Rhodnius prolixus*: evidence for a novel regulatory mechanism involving protein kinase C. Experientia 45:355–6.

Sevala VL, Davey KG, Prestwich GD. 1995. Photoaffinity labeling and characterization of a juvenile hormone binding protein in the membranes of follicle cells of *Locusta migratoria*. Insect Biochem Mol Biol 25:267–73.

Shi YB. 1996. Thyroid hormone-regulated early and late genes during amphibian metamorphosis. In: Gilbert LI, Tata JR, Atkinson BG, editors. Metamorphosis—postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press. p 505–38.

Shi YB, Brown DD. 1993. The earliest changes in gene expression in tadpole intestine induced by thyroid hormone. J Biol Chem 268:20312–17.

Shi YB, Wong J, Puzianowska-Kuznicka M, Stolow MA. 1996. Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. Bioessays 18:391–9.

Song MK, Dozin B, Grieco D, Rall JE, Nikodem VM. 1988. Transcriptional activation and stabilization of malic enzyme mRNA precursor by thyroid hormone. J Biol Chem 263:17970–4.

Srivastava DP, Yu EJ, Kennedy K, Chatwin H, Reale V, Hamon M, Smith T, Evans PD. 2005. Rapid, nongenomic responses to ecdysteroids and catecholamines mediated by a novel *Drosophila* G-protein-coupled receptor. J Neurosci 25:6145–55.

Sterling K, Milch PO, Brenner MA, Lazarus JH. 1977. Thyroid hormone action: the mitochondrial pathway. Science 197:996–9.

Tata JR. 1996. Hormonal interplay and thyroid hormone receptor expression during amphibian metamorphosis. In: Gilbert LI, Tata JR, Atkinson BG, editors. Metamorphosis—postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press. p 465–503.

Taurog A. 1999. Molecular evolution of thyroid peroxidase. Biochim 81:557–62.

Thagaraja BS, Kelly TJ, Masler EP, Borkovec AB. 1991. Thyroxine-induced haemolymph protein and ecdysteroid increases in the silkworm, *Bombyx mori* L.: effect on larval growth and silk production. J Insect Physiol 37:153–9.

Thagaraja BS, Kelly TJ, Masler EP, Borkovec AB. 1993. Thyroxine-induced changes in ovarian protein and ecdysteroid increases in the silkworm, *Bombyx mori* L.: effect on ovarian maturation and egg production. Comp Biochem Physiol 104A:247–53.

Thomas HE, Stunnenberg HG, Stewart AF. 1993. Heterodimerization of the *Drosophila* ecdysone receptor with retinoid X receptor and ultraspiracle. Nature 362:471–5.

Tobe SS, Bendena WG. 1999. The regulation of juvenile hormone production in arthropods: functional and evolutionary perspectives. Ann NY Acad Sci 897:300–10.

Tong W, Chaikoff IL. 1961. ^{131}I utilization by the aquarium snail and the cockroach. Biochim Biophys Acta 48:347–51.

Truman JW, Riddiford LM. 2002. Endocrine insights into the evolution of metamorphosis in insects. Annu Rev Entomol 47:467–500.

Tsai CC, Kao HY, Mitzutani A, Banayo E, Rajan H, McKeown M, Evans RM. 2004. Ataxin 1, a SCA1 neurodegenerative disorder protein, is functionally linked to the silencing mediator of retinoid and thyroid hormone receptors. Proc Natl Acad Sci USA 101:4047–52.

Tsai CC, Kao HY, Yao TP, McKeown M, Evans RM. 1999. SMRTER, a *Drosophila* nuclear receptor coregulator, reveals that EcR-mediated repression is critical for development. Mol Cell 4:175–86.

Tu MP, Flatt T, Tatar M. 2006. Juvenile and steroid hormones in *Drosophila melanogaster* longevity. In: Masoro EJ, Austad SN, editors. Handbook of the biology of aging. 6th edition. San Diego: Academic Press (Elsevier). p 415–48.

Vergara M, Smith-Wheelock M, Harper JM, Sigler R, Miller RA. 2004. Hormone-treated snell dwarf mice regain fertility but remain long lived and disease resistant. J Gerontol A Biol Med Sci 59:1244–50.

Visscher NS. 1983. Effects of abscisic acid in animal growth and reproduction. In: Addicott FT, editor. Abscisic acid. New York: Praeger Publishers. p 555–79.

Webster JI, Tonelli L, Sternberg EM. 2002. Neuroendocrine regulation of immunity. Annu Rev Immunol 20:125–63.

Wehling M. 1997. Specific, nongenomic actions of steroid hormones. Annu Rev Physiol 59:365–93.

Wheeler BM. 1950. Halogen metabolism of *Drosophila gibberosa*—I. Iodine metabolism studied by means of ^{131}I. J Exp Zool 115:83–104.

Wheeler DE, Nijhout HF. 2003. A perspective for understanding the modes of juvenile hormone action as a lipid signaling system. Bioessays 25:994–1001.

Wilson TG. 2004. The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: how these compounds kill insects. J Insect Physiol 50:111–21.

Xu Y, Fang F, Chu Y, Jones D, Jones G. 2003. Activation of transcription through the ligand-binding pocket of the orphan nuclear receptor ultraspiracle. Eur J Biochem 269:6026–36.

Yamamoto K, Chadarevian A, Pellegrini M. 1988. Juvenile hormone action mediated in male accessory glands of *Drosophila* by calcium and protein kinase C. Science 239:916–19.

Yao T-P, Forman BM, Jiang Z, Cherbas L, Chen JD, McKeown M, Cherbas P, Evans RM. 1993. Functional ecdysone receptor is the product of EcR and Ultraspiracle genes. Nature 366:476–9.

Yao T-P, Segraves WA, Oro AE, McKeown M, Evans RM. 1992. *Drosophila* ultraspiracle modulates ecdysone receptor function via heterodimer formation. Cell 71:63–72.

Yen PM. 2001. Physiological and molecular basis of thyroid hormone action. Physiol Rev 81:1097–142.

Youson JH. 1988. First metamorphosis. In: Hoar WS, Randall DJ, editors. Fish physiology. Volume XI, Part B. New York: Academic Press. p 135–98.

Youson JH. 1997. Is lamprey metamorphosis regulated by thyroid hormones? Am Zool 37:441–60.

Youson JH. 2004. The impact of environmental and hormonal cues on the evolution of fish metamorphosis. In: Hall BK, Pearson RD, Müller GB, editors. Environment, development, and evolution. Cambridge, MA: MIT Press. p 239–77.

Zhou B, Hiruma K, Jindra M, Shinoda T, Segraves WA, Malone F, Riddiford LM. 1998. Regulation of the transcription factor E75 by 20-hydroxyecdysone and juvenile hormone in the epidermis of the tobacco hornworm, *Manduca sexta*, during larval molting and metamorphosis. Dev Biol 193:127–38.

Zhou B, Hiruma K, Shinoda T, Riddiford LM. 1998. Juvenile hormone prevents ecdysteroid-induced expression of Broad Complex RNAs in the epidermis of the tobacco hornworm, *Manduca sexta*. Dev Biol 203:233–44.

Zhou X, Riddiford LM. 2002. Broad specifies pupal development and mediates the 'status quo' action of juvenile hormone on the pupal-adult transformation in *Drosophila* and *Manduca*. Development 129:2259–69.